



**Prof. Dr. Janko Jamnik,
Director of the
National Institute
of Chemistry,
Slovenia 1964–2014**

The scientific career of Prof. Jamnik began three decades ago as a high school student when he, on his own initiative, contacted a small group that was working on lithium-thionyl chloride batteries in the Faculty of Chemistry and Chemical Technology at the University of Ljubljana. His first project was the development of a methanol/air fuel cell model, with which he entered the popular field of energy storage and conversion that he would never leave for the rest of his career. Before his undergraduate studies in physics he also worked on setting up the first impedance spectrometer and helped to develop an improved Li/SOCl_2 battery. He received Presern's award for his undergraduate research in 1988 and continued his studies of physics, receiving a Master of Science in 1991 and Doctor of Philosophy in 1994. Most of his Ph.D. thesis as well as his post-doctoral research were done at the Max Planck Institute for Solid State Research; however, he also worked as a post-doctoral research associate at Cornell University and Los Alamos National Laboratory. These career experiences distinguished him as a world-class researcher, and his peer-reviewed research papers from this era are still well-regarded as some of the most cited from the solid state chemistry field. His primary focus throughout his career was understanding transport processes in heterogeneous solids in various electrochemical systems, and later in his career he made significant advances in developing state-of-the-art battery systems. He was internationally recognized for his development and understanding of impedance spectrometry,

which is the main focus of more than 30 scientific articles authored or co-authored by him. With the introduction of the transport-reaction equations, he developed analytical solutions with a unique physico-chemical meaning that enabled advances in the understanding of special transport phenomena in solid nanomaterials. He furthered these advances to develop new circuit elements, which enabled the modeling of impedance spectra even by those who are not skilled in the underlying physics behind them. These advances were characteristic of Prof. Jamnik's unique mentality, as he always strove to find a link between theory and practical experiments.

Prof. Jamnik was a highly respected scientist and was an invited speaker at numerous top-tier research universities and international conferences in the solid state field. He had numerous offers to pursue his research career abroad, but he chose the National Institute of Chemistry in Slovenia, where he was appointed group leader of the Materials Chemistry Laboratory in 2000. Interestingly, Prof. Jamnik purposely avoided administrative work as much as he could, dismissing it as "paper-shuffling." This made his application to become the Director of the National Institute of Chemistry all the more surprising. When he was elected director, however, everyone could feel the spirit of the Max-Planck institute. He was aware of the importance of being involved in international research as well as the need for excellent research equipment, and under his leadership the National Institute of

Chemistry has won three centers of excellence awards, the number of publications has increased significantly and today the Institute is recognized internationally as one of the most prominent Slovenian research organizations. In 2007 he received the Zois award for his excellent research accomplishments, which is a testament to his achievements at the Institute.

He was also devoted to mentoring and transferring his knowledge to undergraduate and graduate students. Although Prof. Jamnik was a demanding mentor, he always appreciated intelligence and hard work. He knew and wanted to transfer knowledge into industry, and recognized that all of this required the knowledge of how to motivate and reward the best researchers. Whenever we

discussed Slovenian science policy, he warned us that young people are not given enough opportunities to engage in serious research work. Through his dedication to mentoring, all of his students have gone on to very successful careers in academia or in industry across the world from Chicago to Hong Kong.

Dear friend, Janez. We are very sad writing these lines. You were not just a top researcher, but also a remarkable man, a loving husband and above all, a caring father. Your parents are rightfully proud of you and we understand their immense sorrow at the unreasonable event that has ripped you out of our lives. Janez, we are proud of everything you have achieved. We will never forget you, and you will always remain in our minds and our hearts.

*Prof. Dr. Stane Pejovnik
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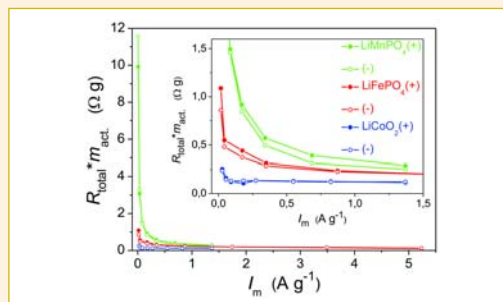
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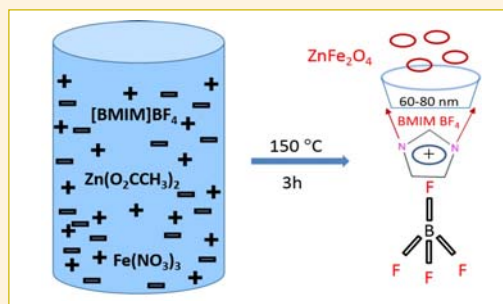


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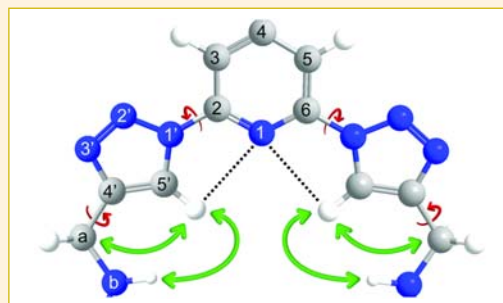


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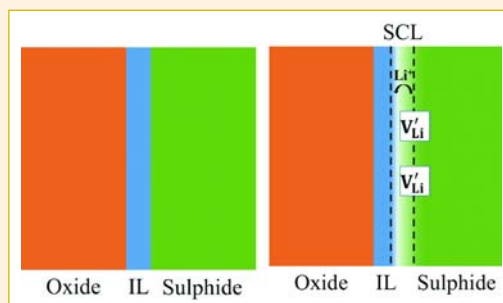


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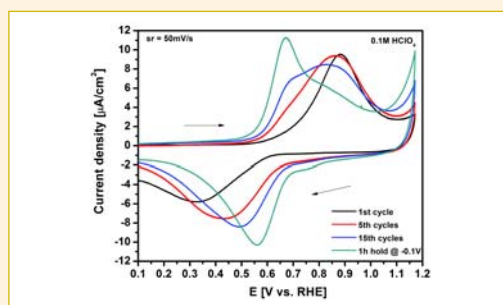


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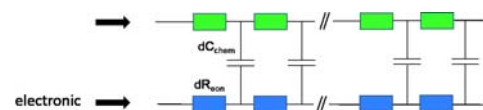
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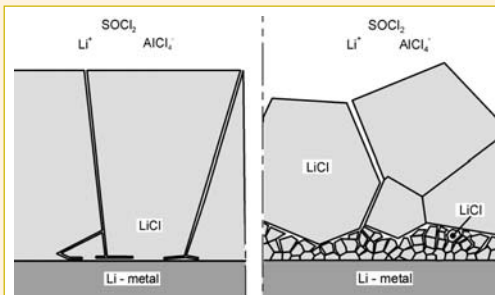
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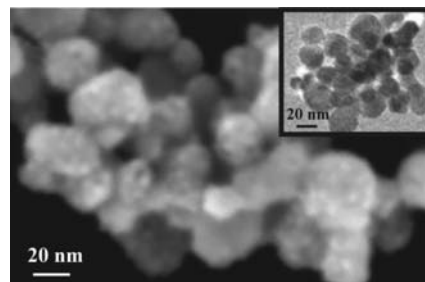
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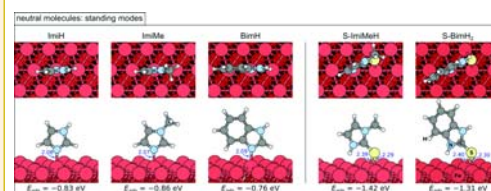
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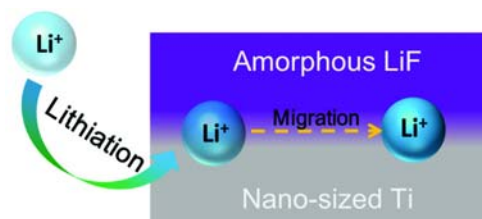
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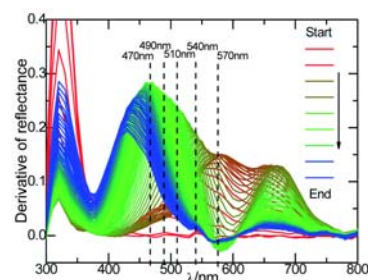


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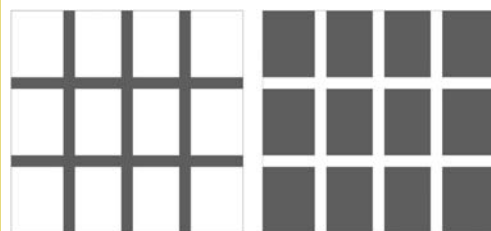


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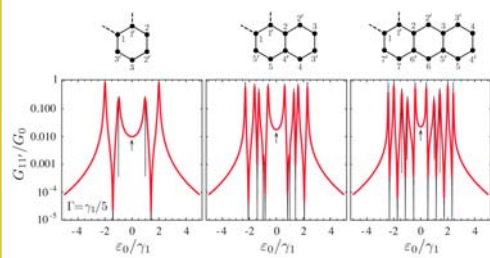


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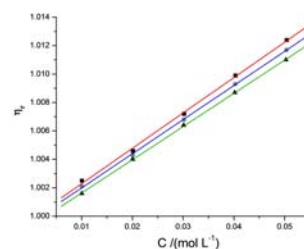


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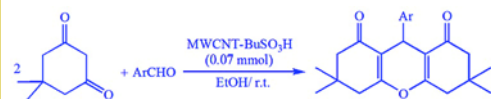


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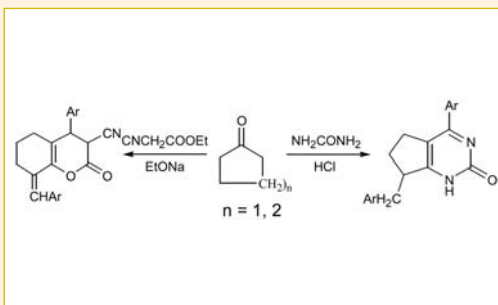


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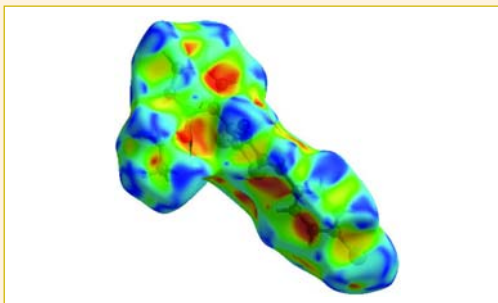


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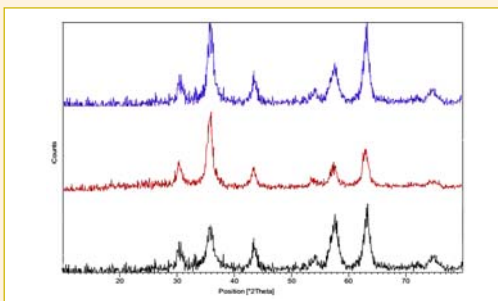


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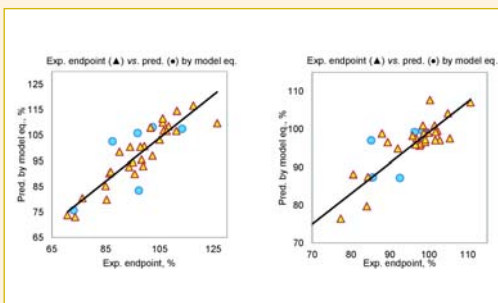


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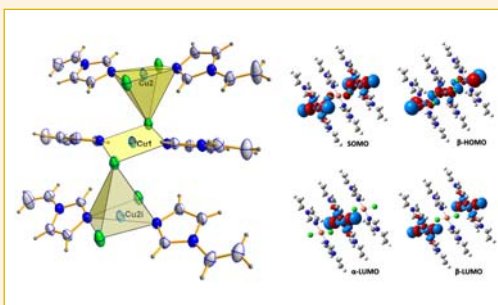


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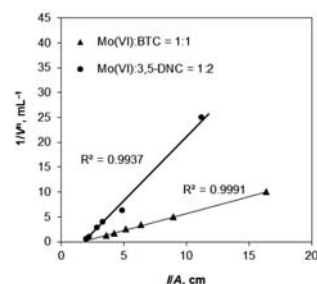


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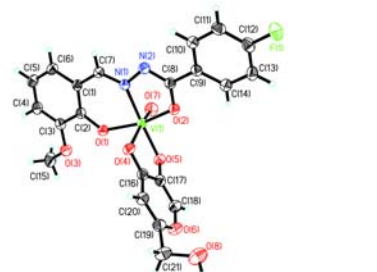


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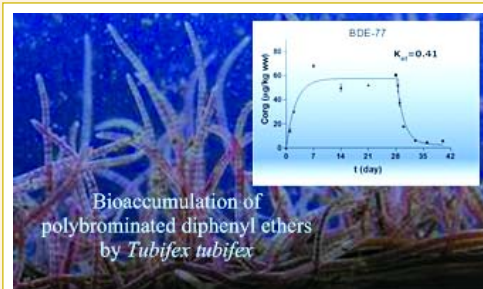


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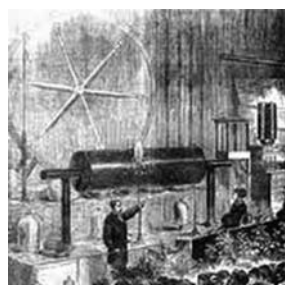


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Stanislav Južnič



Review

Nanomaterials for Electrochemical Energy Storage: the Good and the Bad

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In memory of prof. dr. Janko Jamnik.

Abstract

Abstract: A critical view on the outcome of research in nanomaterials for electrochemical energy storage devices (batteries and supercapacitors) is provided through selected examples. The nano- approach traces back to the early battery research and its benefits realized even before the nano- term was coined. It has enabled important progresses which have translated, for instance, in the possibility of using LiFePO_4 as electrode material. On the other hand, the nano- approach has also been oversold at all levels and hence some examples are also shown on the detrimental side effects of the use of nano-materials which should be taken into account if steady progress is to be made that finally results in practical benefits in energy storage devices.

Keywords : Nanomaterials, electrochemical energy storage, batteries, supercapacitors

1. Introduction

Richard Feynman is considered the father of the nano-revolution, and his 1959 visionary statement « There's plenty of room at the bottom » will remain forever. It took a few years for the “nanomania” to take off and to emerge near the end of the 90's, prior to boost in the early years of the current century. Since then, nanomaterials entered all application domains ranging from medicine to construction with staggering benefits in the field of microelectronics sector.

In 2011 the EU gave a definition for nanomaterials as “A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm.¹ This adage does not greatly differ from the one given by the International Organization for Standardization (ISO): »Material with any external dimension in the nanoscale or ha-

ving internal structure in the nanoscale« with Nanoscale meaning »Size range from approximately 1 nm to 100 nm.«

With such a background, one could naively think that the application of nanomaterials in the energy storage field is unravelling novel cross cutting science which is going to revolutionize the figures of merit for battery and supercapacitor performance in the second millennium. Nonetheless this is not strictly true... The “nano-” prefix has indeed been coined relatively recently and now applied in all fields but nanomaterials are much older than that and some beneficial aspects realized longtime ago. Indeed, dealing with electrochemical energy storage and nickel based batteries (first patents by Jünger in Sweden and Edison in the US around 1900) it soon was established that high surface area Ni(OH)_2 exhibits higher capacity than well-crystallized Ni(OH)_2 . Indeed, it is stated in² that: *The nickel (II) hydroxide used in flat positive electrodes must have a very large active surface area. Investigations have shown that there is a definite relationship*

between the surface area of the nickel material and the capacity. Therefore, when the material is being prepared, the goal must be to obtain the smallest possible particles. It has been shown that a satisfactory nickel (II) hydroxide for flat pocket cells ordinarily consists of crystals in the form of small disks with a height of only 20–60 Å and a width of 100–400 Å. Similarly the field of fuel cells had greatly benefited from nanosized electrode materials.

Early research on nanomaterials science can be traced back to the chemistry of colloids, which does nowadays sound much less fancy! Electrochemical energy storage being ultimately based on an electronic transfer at the atomic scale, one can rigorously state that all electrochemical processes are by definition nano- and thus, foresee a high impact of nanoscience in battery and supercapacitor research. “Real” commercial batteries typically consist of composite electrodes casted on a metal current collector and aside the active materials do also contain additives to enhance the electronic conductivity (typically some sort of carbon black) and a polymer to improve adhesion and mechanical strength. Thus, many types of interfaces between all components exist at the nanometric scale, which may exhibit different properties thus influencing ionic transport and the ultimate device performance.³ Although researchers rapidly caught up with the buzzword *nano* to successfully apply for funding, we must admit that the practical ingress of nanomaterials in today’s commercial Li-ion cells has been limited: only LiFePO₄ and Si electrodes have benefited from it. Supercapacitors have also enjoyed the new nano era with the copious use of nano-textures and nanoarchitected electrodes. High surface area carbons are commonly used as active materials, and the design of the nanosized electrode / electrolyte interface is one the key challenges to improve the electrochemical performance of supercapacitors⁴ (Figure 1).

Overall, in addition to temperature, pressure, and composition, size has provided an extra dimension to both battery and supercapacitor communities for tuning electrochemical device performances. Size does indeed matter: on one side progressive nanosizing brings about decreased ionic diffusion paths and better strain accommodation, on the other, there are also negative sides of the story as the enhanced surface area results in lower packing density while enabling also catalysis, hence promoting undesired side reactions. Within this overall context, the aim of this paper is to give a realistic overview of the benefits of nanomaterials in the field of electrochemical energy storage while at the same time pointing out some the drawbacks through some selected examples as small does not systematically rhyme with better ...

2. Pros: the Importance of Being Small

The size reduction of particles to the nano level leads to a huge increase in the fraction of atoms that reside at its surface while at the same time decreasing the travelling distance for ions from the core to the surface. This has tremendous implications for Li-ion systems in which the charge/discharge rate is controlled by the lithium ions diffusion rate in the electrodes and thus across all existing interfaces and inside the active material particles. Moreover, although frequently omitted, size reduction involves significant changes in surface energy which can have important consequences such as the feasibility to stabilize anatase over rutile.⁵ Similarly, the redox potential values can be substantially affected by nanoeffects.^{6,7}

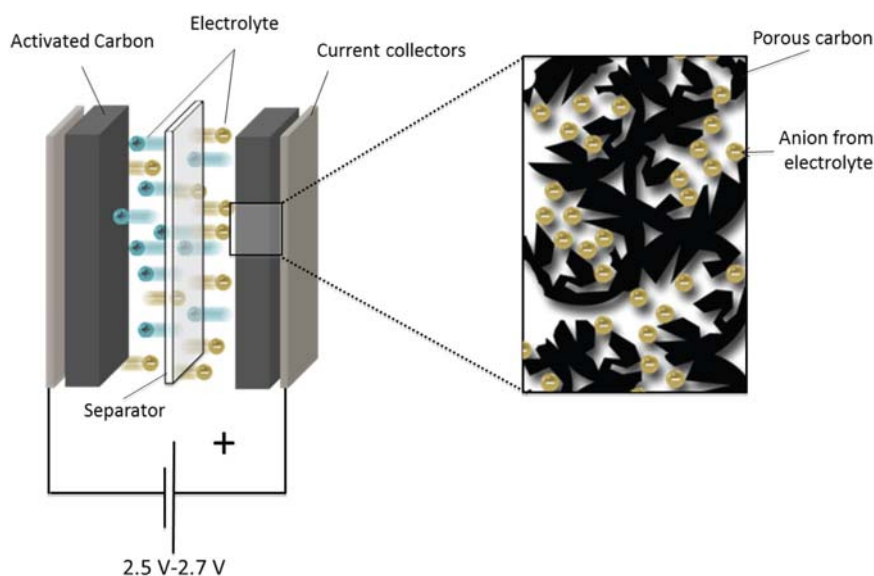


Figure 1: Schematics of a supercapacitor (left) and porous carbon at the positive electrode containing adsorbed anions.

Besides the already mentioned *traditional* example dealing with nickel based batteries, the investigation of nanomaterials in lithium battery research has flourished in the last decade covering not only Li-ion electrode materials but also alternative technologies such as Li/Air or Li/S. A pedagogical example to convey the benefits of Nanomaterials is LiFePO_4 , a mineral that was totally ignored for centuries, and which is now used in commercial batteries for power tools and also electric vehicles. In this case, the material can only be rendered useful if particles are covered by a thin (few nm thickness) conductive carbon coating to enable effective electronic transport.⁸ Once this is achieved, by further playing with the particle size it is feasible to orient the Li-uptake/removal towards an homogeneous single-phase or an heterogeneous two-phase process.⁹ This can be rationalized in terms of the energy associated with the strains induced by volume changes between the lithiated and de-lithiated phases.¹⁰ Of course, the magnitude of such effects is affected by the cycling rate which determines the kinetics of the reaction.¹¹ Moreover, caution has to be exercised because nanomaterials are in most of the cases heavily rich in defects, so that distinguishing between the effect of defects of nano sizing is complex. Nonetheless nanostructuration cannot be taken as the panacea since it failed to deliver comparable results in LiFePO_4 isostructural phases with alternative transition metal such as Mn, Ni or Co instead of Fe, which exhibit higher redox potential and hence could lead to batteries with enhanced energy densities. In fact, in this case, owing to higher redox potentials ($> 4\text{V}$ vs. Li^+/Li for LiMPO_4 when compared to 3.5V for LiFePO_4), having

high surface areas promotes catalytic-driven electrolyte decomposition.

The benefits of downsizing can also be inferred from recent progresses in the Li/S technology which is not commercialized yet but for which prototypes are foreseen in the next coming years. In this case, rather than acting on the active materials, researchers have played on nanoconfinement strategies enlisting amongst others core-shell like approaches or designing yolk-shell like structures. Here the active cathode material is Sulphur, which is unexpensive and highly abundant and reduces upon discharge to form a range of polysulphides (with different degrees of solubility in the electrolyte) to ultimately yield solid Li_2S . Aside the problem of soluble species migrating and contaminating the counter-electrode, the insulating nature of sulfur and Li_2S is an issue for high charge/discharge rates. The introduction of nanostructured mesoporous carbon-sulphur composites as cathodes was a breakthrough in the field¹² since the intimate contact between the insulating sulphur and the conductive carbon framework enabled higher and more reversible capacities while at the same time delaying the diffusion of soluble polysulphides and thus diminishing capacity fading. This has prompted the screening of different types of carbon and the fabrication of a vast spectrum of composite electrodes as the functionalization of carbon was found to be an interesting approach to prevent polysulfide diffusion. More recent approaches may exhibit enhanced complexity such as the use of composites containing graphene to anchor sulfur and confine polysulfides coupled to nano fibrillated cellulose to prevent graphene exfoliation.¹³ Alt-

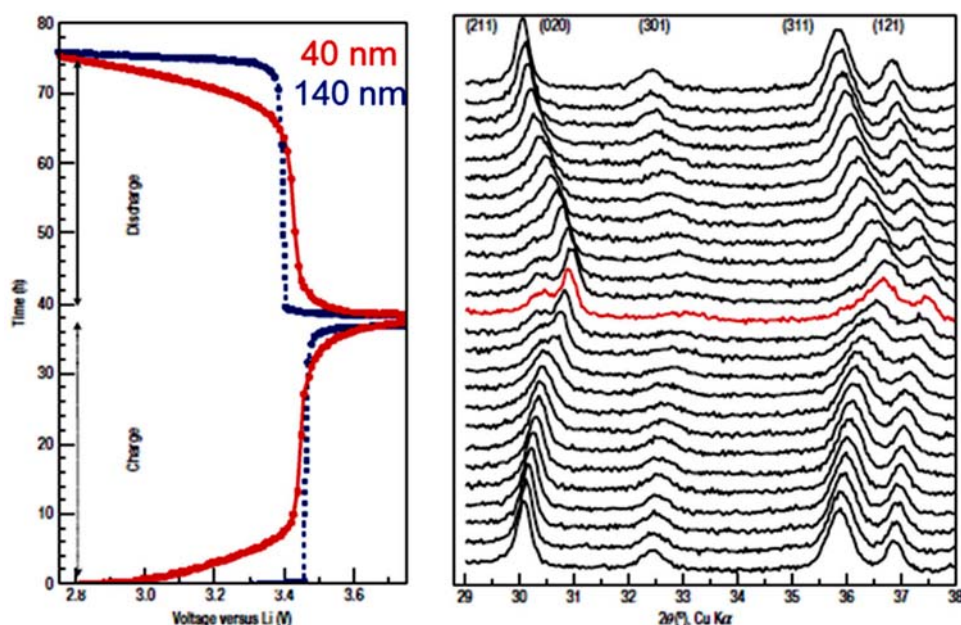


Figure 2. Electrochemical profiles corresponding to LiFePO_4 with different particle size 40 nm (in red) and 140 nm (in blue) at C/40. In situ XRD measurements indicate continuous shift of the diffraction peaks during the 40 nm LiFePO_4 , characteristic of a solid solution redox mechanism. The red pattern corresponds to the fully delithiated phase. (Reprinted from¹¹ with permission)

though elegant, such approaches have the handicap that non-polar entities such as carbon cannot bind to sulfur. Being aware of such an issue, researchers decided to move to more polar supports such as oxides while keeping the confinement approach, hence the use of zeolites or metal organic framework structures (MOF).¹⁴ When instead of insulating phases electronically conducting oxides are used, boosted performance improvements are achieved.¹⁵ Using Ti_4O_7 nanoparticles which strongly bind to thiol-based species sustained capacity retentions (> 2500) could be achieved for electrodes having S loadings greater than 75%. The issue is now to find the way to practically implement such stylish nanomaterials through developing up-scaling synthesis procedures and electrode formulation protocols having high sulfur loadings while decreasing the amount of electrolyte to enable figures of merit beyond the Li-ion technology in terms of practical energy density.¹⁶ Needless to said that for safety reasons, the Li would most likely have to be replaced by an insertion electrode, that would most likely penalize energy density or else develop a strategy to avoid its dendritic growth, which will still take a few years of sustained research.

Nanomaterials can also trigger novel reactivity mechanisms such as conversion reactions as demonstrated in 2000.¹⁷ Until that time all electrode materials used in

Li-ion batteries worked according to a classical insertion/deinsertion process or by electrochemically forming Li alloys. Defying these well-established laws, reversible redox activity was measured for transition metal oxides (MO) that did not have the required structure to enable insertion reactions, and containing metals (M) cannot alloy with Li. The high capacity values achieved ($> 700\text{mAh/g}$ for nearly 100 cycles) were explained by the existence of a reversible conversion reaction: $\text{M}_x\text{O}_y + 2y\text{e}^- + 2y\text{Li}^+ \rightleftharpoons x\text{M}^0 + y\text{Li}_2\text{O}$ forming a composite consisting of a ho-

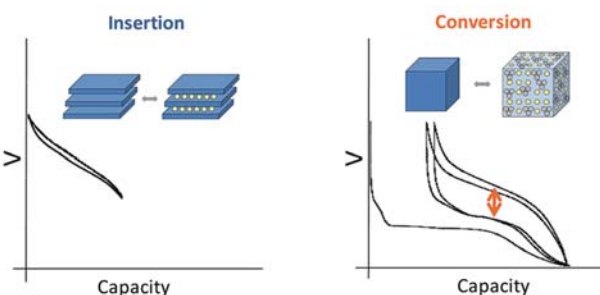


Figure 3. Schematic representation and typical potential vs. capacity profile for conventional insertion and alternative conversion reaction pathways in lithium-ion battery electrodes. (Reprinted from ¹⁸ with permission)

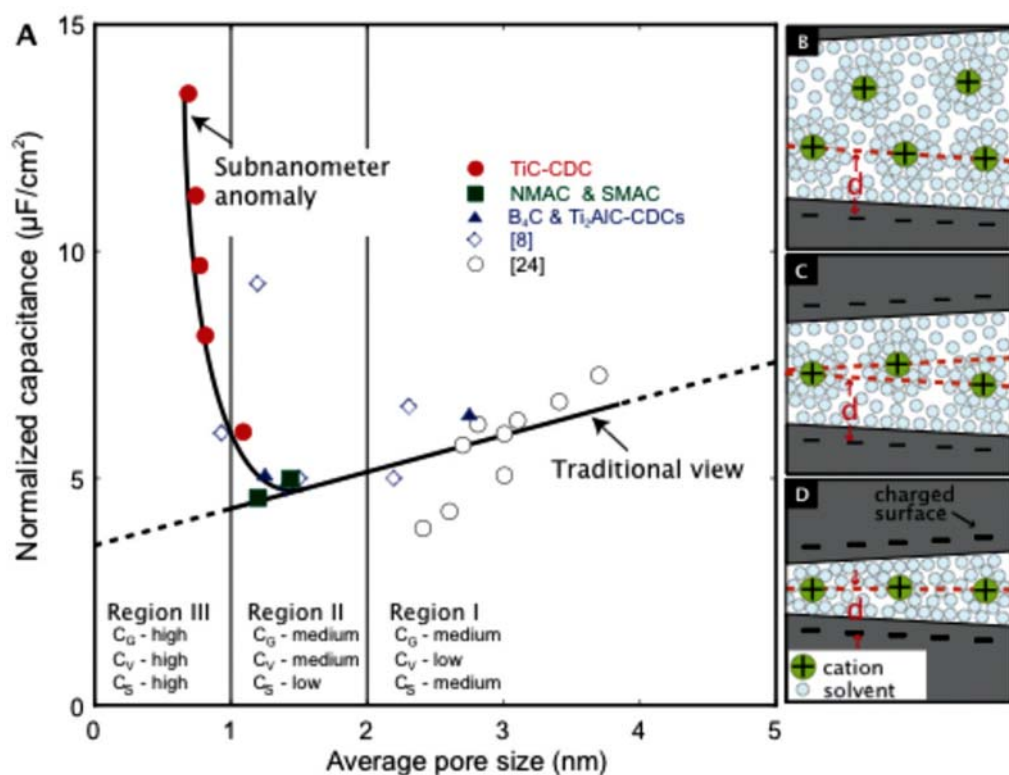


Figure 4: Plot of specific capacitance normalized by BET specific surface area for the carbons in the study and two other studies with identical electrolytes (A). The normalized capacitance decreases with pore size until a critical value is reached. It would be expected that as the pore size becomes large enough to accommodate diffuse charge layers, the capacitance would approach a constant value. CG, CV and CS are gravimetric, volumetric and normalized capacitances, respectively. Illustrations showing solvated ions residing in pores with distance between adjacent pore walls (B) greater than 2 nm, (C), between 1 nm and 2 nm and (D) less than 1 nm illustrate this behavior schematically. (Reprinted from ²⁰ with permission)

mogeneous distribution of metal nano particles in a matrix of Li_2O .

All together, it was rapidly demonstrated that this surprising reactivity, contrary to well-established beliefs, was not specific to oxides, but can also include sulfides, nitrides, fluorides and hydrides. In contrast to the classical insertion reactions that govern the energy stored in the actual Li-ion batteries, and which are limited to $1e^-$ even $0.5e^-$ per 3d metal atom (LiCoO_2), these new conversion reactions can involve $2e^-$ or even more (per 3d metal atom), thus enabling extremely high capacities. Such results have enjoyed a tremendous worldwide resonance but unfortunately batteries based on conversion reaction are not likely to be ever commercialized owing to issues dealing with intrinsic large potential hysteresis resulting in poor energy efficiency.¹⁹

An interesting example of the benefit of nanosize can be found in supercapacitors. Unlike traditional batteries, supercapacitors store the charge electrostatically through reversible adsorption of ions from an electrolyte at the surface of high surface area porous carbons (up to $2,000 \text{ m}^2/\text{g}$) thanks to the presence of sub-nanometer size pores ($< 1 \text{ nm}$). Under polarization, the adsorption of ions in these small, confined carbon nanopores leads to a huge increase in the charge storage capacitance of the carbon (Figure 4).²⁰ Another key result was obtained by studying the ion adsorption in Carbide Derived Carbons (CDC) in solvent-free electrolyte, using neat $\text{EMI}^+\text{TFSI}^-$ solution, where it was we found that the maximum capacitance is obtained when the carbon pore size matches the ions size.²¹ These results ruled out at that time the way charge storage was traditionally described in Electric Double-Layer Capacitor (EDLC) materials, with ions adsorbed on both pore walls. Although the whole mechanism is not still completely understood, recent works have shown that the capacitance increase in carbon nanopores of 1 nm or

below was ascribed to the partial desolvation of the ions when confined in these small pores, as well as a decrease of the approaching distance of the ions to the carbon surface.^{22–24} Despite confinement, ion migration is still fast between various adsorption sites.²⁵ The capacitance increase in carbon pores thus highlights the beneficial attributes of nanosizing the carbon structure. Therefore, as for batteries, the supercapacitor community fell into the development of fancy nanoarchitected objects via multiple synthetic steps, with the global electrode fabrication process being far from practical reality, which is the next challenge the community is facing.

3. Cons: the Other Side of the Coin

Elements forming electrochemical alloys with lithium (such as Si for instance) hold promise to enable high energy density as their electrochemical capacities (e.g. 3589 mAh/g for formation of $\text{Li}_{15}\text{Si}_4$) are well beyond that of graphite (372 mAh/g) which is currently the negative electrode material of choice in almost all commercial Li-ion batteries. Such a high capacity is thus the result of a huge increase in the number of atoms present in each active particle, which creates strains and cracks inducing enhanced reactivity with the electrolyte and thus alloy based materials suffer from severe capacity fading. Materials engineering strategies have been applied to solve the issue, and significant improvements have been recorded through nanostructuration (particles less prone to break upon stress) or the use of nanocomposites with a conducting matrix (e.g. carbon) buffering the volume expansions. In spite of lower tap densities and enhanced reactivity with the electrolyte due to the enhanced surface area coupled to the low operation potential, significant improvements have been made at the laboratory scale although commercialization is restricted to the use of 3–4% silicon as additive in graphite electrodes. Indeed, even after developing methods to produce nanosized Si at the large scale their implementation in commercial electrodes can be penalized due to technological hurdles. Indeed, typical fabrication protocols involve dispersion of the active materials and additives in a solvent (either organic or water) to form slurries that are subsequently tape casted on current collectors. Given the interest in defining greener process and the beneficial effect of using some water soluble species as binders, processing in water seems to be more adapted. Yet, nanosized Si oxidizes under such conditions producing hydrogen which results both in inability to fabricate good quality electrodes and safety risks. Fortunately, alternative strategies based in controlled oxidation of such particles (up to 10 nm) to avoid such effects seem to be successful. Yet, this may also limit the electrochemical capacity and thus, careful design of processing protocols are needed if these processes are ever to be implemented to result in tangible improvements in energy density for Li-ion batteries.

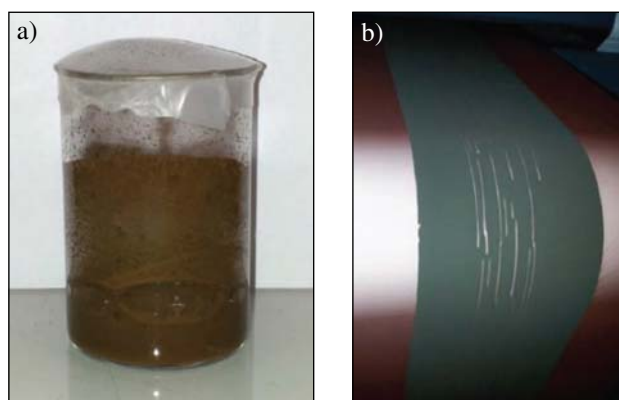


Figure 5. (a) Picture showing the gas (H_2) production during the mixing of a suspension containing Si nanoparticles to prepare an electrode. (b) Resulting Cu-supported casted electrode where bubbles in the slurry have induced a non-uniform coating. Reprinted from²⁶ with permission.

Thus, it is important to state that “nano” does not always mean “better” when it comes to electrodes and scientists should realize that making materials for the sake of it does not bring significant performance improvements but rather serves only to publish irrelevant papers. One key parameter which has to be considered prior to blindly move to nanosizing is the redox potential at which the electrode material is operating. Any attempt to apply the dual nanosizing/nanocoating approach which has been successfully used for LiFePO_4 or even LiFeBO_3 (2.9 V Vs. Li^+/Li) fails for other electrode materials operating at potential greater than 4V, whatever they are LiMPO_4 , Li-NMC, Li-rich NMC or LiMn_2O_4 and its derivative $\text{LiMn}_{2-x}\text{Ni}_x\text{O}_4$. The reason behind, is, as state above, that such materials operate beyond the thermodynamic stability of the electrolyte and thus its catalytic decomposition is promoted by nanosized electrode materials. Coating techniques are presently used to passivate surfaces and enable use of these highly oxidizing materials. A possible alternative option to circumvent these issues consists in developing electrolytes which are more stable against oxidation, a challenge that we have been targeting for decades without real success. In absence of such electrolytes, trying to prepare nanosized Li-NMC or Li-rich NMC in order to bring higher rate capability to the expense of an energy density penalty associated to lower density powders is a dead end route.

4. Conclusion

Through this short discussion illustrated with a few selected examples we have attempted to convey the message that nanomaterials, which can be viewed as “chips off the block” of old materials, can occasionally stand as serious candidates to power the next generation of Li-ion batteries or alternative technologies. This has been demonstrated in the development of LiFePO_4 positive electrodes powering today electric vehicles and Si-based negative electrodes which are on the verge to be implemented. Equally, nanoarchitected electrodes have led to staggering progresses in the development of Li/S technology while supercapacitors have also greatly benefited from electrode nanotexturing. Application-wise, we can just incite researchers to report performances in Wh/l rather than in Wh/kg, the former being more meaningful in practice even if not conveying the full benefits of nanomaterials. Energy conversion devices, enlisting photovoltaics or water splitting have greatly benefited as well from the nano approach and will keep benefiting in the future. However, efficient research towards the implementation of nanomaterials must be cross-disciplinary so as to identify problems and provide viable options. Too frequently, researchers do make nanomaterials for the sake or the beauty of it, as conveyed by beautiful microscopy images which are flooding the literature. Indeed, research on na-

nomaterials has greatly developed because of the great progresses made by microscopists over the last few decades. Has this been good or bad for nanomaterials? The bets are open. In any case, the fact that nanomaterials have obliged experimentalists to push existing analytical technics to their limits and to develop new ones capable of deciphering interfaces has resulted in precious tools for the battery community.

5. Acknowledgement to Prof. J. Jamnik

Besides experimentation, nanomaterials were also viewed as troublemakers to theoreticians within the battery field. Indeed DFT calculations could not be blindly implemented owing to the importance of surface energy or the outcome of the band structure when entering the nanoworld, which is still subject to intense debates, not to mention issues related to true potential. Prof. J. Jamnik had aggressively and energetically attacked these difficult issues at the early stage of ALISTORE by constituting a working group on Theory to address these issues. Owing to his friendly enthusiasm and visionary contributions, he brought the field to a satisfactory level of understanding and this via long lasting and lively passionate discussions that we fully enjoyed and for which we admired his real patience. His last goal was to understand mixed potentials in theoretical terms, Janez, you left too early with the solution that you could not share with us. Fortunately you left countless great memories to all of us that are tied to our lives and will remain forever.

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Povzetek

S pomočjo izbranih primerov, je v članku podan kritičen pogled na raziskave nanomaterialov v napravah za shranjevanje energije (baterije in superkondenzatorji). Nanotehnoški pristop in njegove prednosti so bili v baterijskem svetu poznane že pred monžično uporabo termina nano. Uporaba nanotehnologije je omogočila pomemben razvoj na področju uporabe LiFePO₄ kot elektrodnega materiala. Pomembno pa je tudi omeniti, da se je nemalokrat nanoprstlop prepotencial na vseh nivojih. Od tu izhajajo nekateri opisani primeri, ki kažejo diametralne stranske efekte pri uporabi nanomaterialov. Le-te je potrebno prav tako upoštevati, če želimo narediti korak naprej pri praktičnem razvoju naprav za shranjevanje energije.

Review

How to Study Protein-protein Interactions

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In memory of prof. dr. Janko Jamnik.

Abstract

Physical and functional interactions between molecules in living systems are central to all biological processes. Identification of protein complexes therefore is becoming increasingly important to gain a molecular understanding of cells and organisms. Several powerful methodologies and techniques have been developed to study molecular interactions and thus help elucidate their nature and role in biology as well as potential ways how to interfere with them. All different techniques used in these studies have their strengths and weaknesses and since they are mostly employed in *in vitro* conditions, a single approach can hardly accurately reproduce interactions that happen under physiological conditions. However, complementary usage of as many as possible available techniques can lead to relatively realistic picture of the biological process. Here we describe several proteomic, biophysical and structural tools that help us understand the nature and mechanism of these interactions.

Keywords: Molecular interactions. Yeast two-hybrid, Surface plasmon resonance, Microscale thermophoresis, Quartz crystal microbalance, Structural biology

1. Introduction

Molecular interactions involving proteins are fundamental for all living processes. Understanding of protein complex formation allows description of molecular functions and is, therefore, needed for the basic understanding of cellular processes. Furthermore, this knowledge on molecular interactions is important also for drug discovery and many experimental efforts have been recently invested into developing of small molecules that can affect protein complex formation.^{1–4} Molecular interactions are assessed by multitude of proteomic, biophysical, biochemical and structural methods (Figure 1).^{5,6} Each of these methods has their own advantages and drawbacks and in most cases only a combination of different methods can yield realistic description of molecular interactions that correspond to situation *in vivo*.⁵

Molecular interactions of proteins are diverse and are, according to the affinity, strong or weak.⁷ Strong interactions lead to long-lived protein complexes that can be

assessed by some of the classical biochemical approaches such as size exclusion chromatography or native gel electrophoresis.⁸ Other methods are more appropriate for assessing transient interactions, such as some structural approaches, i.e. nuclear magnetic resonance (NMR) or small angle X-ray scattering (SAXS). Methods also differ by throughput and information content that can be provided (Figure 1). High-throughput methods can report interactions at large scale and can assess interactions globally at the cellular level, but they can be quite hard to perform. While they offer information on interactions at a relatively low resolution, basically just reporting the existence of particular intermolecular interactions, they provide a good and useful basis for further experimentation and analysis of molecular networks within cells or organelles. On the other hand, structural methods provide details at high resolution, all the way to the atomic level, and are thus very detailed and informative and provide essential information for designing molecular therapies aimed at protein complexes as targets. However, these methods are typi-

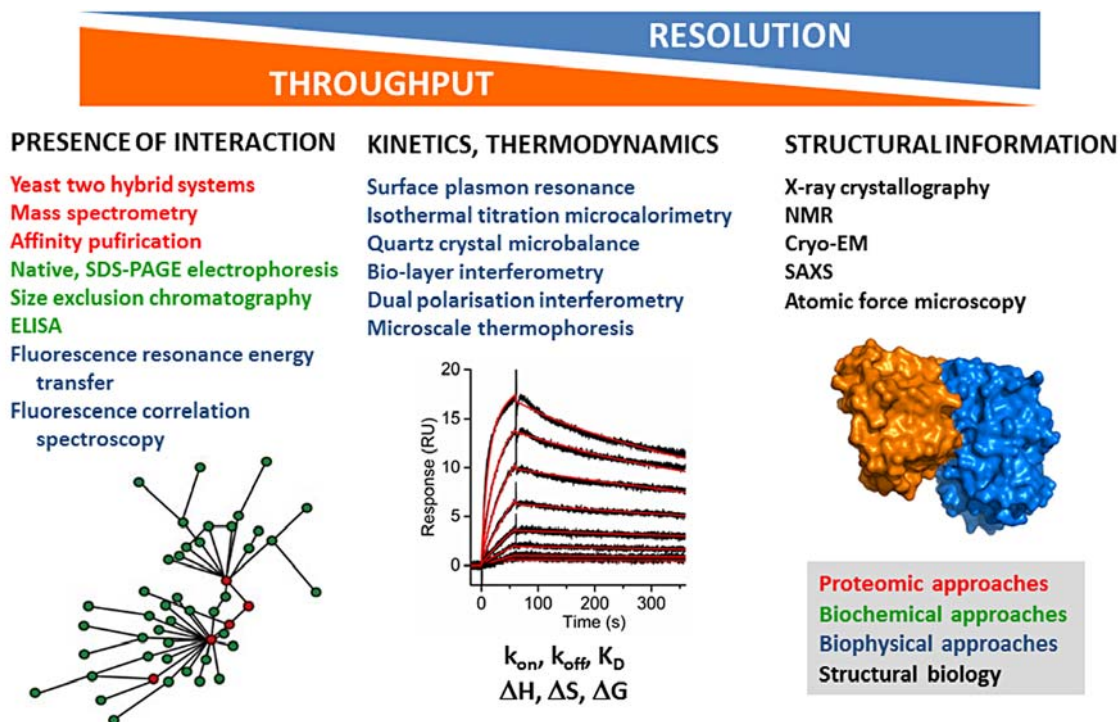


Figure 1. Methods for studying protein-protein interactions by throughput and information content. Some of the most commonly used methods for analysing protein-related interactions are listed. Typical data are presented for each methods assembly.

cally low-throughput, because of the high demands with regards to the quality of the sample and amounts of material needed for structural determination. Biophysical methods are somewhere in the middle. They can provide quantitative information on protein interaction, such as affinity rate constants or thermodynamic parameters from which equilibrium dissociation constant and free energy of binding can be derived (Figure 1).

In this review we will present some of the most commonly used methods for protein-protein interaction characterization with an emphasis on biophysical approaches that are most frequently used due to easier accessibility of the instrumentation. This review does not cover some other approaches that may be used for studying molecular interactions and we would like to highlight some other excellent manuscripts that can direct reader for further information.^{5–7,9}

2. Proteomic Approaches

Proteomic approaches are used to assess molecular networks within cells or cellular compartments. Two most often used are affinity purification followed by mass spectrometry (AP-MS) and yeast two hybrid (Y2H) approaches, which can both assess thousands of interactions in a single study. Analysis of these requires computational approaches and genome-wide mapping.¹⁰ Well-developed databases store these information and are available for

further data-mining in systems biology approaches.⁶ Other high-throughput genetic approaches have become popular in recent years, for example deep sequencing for quantifying protein variants after selection procedure that allow recognition of best binders in protein evolution studies.¹¹

2. 1. Mass Spectrometry Coupled with Tandem-affinity Purification

MS coupled with tandem affinity purification (TAP), TAP-MS, is one of the most effective strategies to isolate and identify protein complexes in a high-throughput manner.⁹ Historically, TAP was developed as a method to purify protein complexes expressed at physiological levels under normal conditions. The method relies on the use of two tags. It involves creation of a fusion of a protein of interest with a designed TAP tag, at the C- or N-end of the protein. TAP tag contains different combinations of two tags, separated with the protease (mostly tobacco etch virus protease, TEV) cleavage site. Various tags can be used, e. g. FLAG-tag, hemagglutinin, poly His, Strep, Myc, glutathione S-transferase, thioredoxin, protein A, protein G, calmodulin binding peptide (CBP), chitin-binding domain, maltose-binding protein, or green fluorescent protein (GFP). Expression is allowed under the control of their endogenous promoters and production at physiological levels following by purification of proteins performed under native conditions. A protein of interest fused to TAP-tag is used as a bait to purify protein

complexes that assemble on the TAP-tagged protein *in vivo*. Subsequently, these complexes are retrieved from the host by breaking the cells and binding to appropriate affinity resin, i.e. IgG matrix if one of the tags is protein A. After washing, TEV protease is introduced to elute the bound material at the TEV protease cleavage site next to protein A tag. This eluate is then incubated with another set of beads that bind the second tag on the fusion protein, for example CBP. This second affinity step is required to remove the TEV protease as well as traces of contaminants remaining after the first affinity step.¹² After washing, the eluate consisted of the protein of interest bound to the interacting partners is then released with ethylene glycol tetraacetic acid (EGTA).¹³

Copurifying proteins from the bound complex can be determined in two complementary ways. Each purified protein preparation is electrophoresed on an SDS polyacrylamide gel, stained with silver, and visible bands removed and identified by trypsin digestion and peptide mass fingerprinting using matrix-assisted laser desorption/ionization–time of flight (MALDI–TOF) MS. In parallel, another aliquot of each purified protein preparation is digested in solution and the peptides are separated and sequenced by data-dependent liquid chromatography tandem mass spectrometry (LC–MS/MS).¹⁴ Machine learning can be used to integrate the mass spectrometry scores and assign probabilities to the protein–protein interactions.¹⁴ A variety of computational scoring pipelines have been developed to identify biologically relevant interactions among a large number of irrelevant interactions in raw TAP–MS data. Data can be characterized into four classes of protein–protein interactions: biologically relevant complexes occur in the cell; physically existing interactions as artefacts of sample preparation that do not occur in the cell (e.g., interaction of proteins from different compartments); interactions involving contaminant proteins; and physically non-existing interactions detected by an error.¹⁵ The results of TAP–MS experiments are networks. Cytoscape is a widely used tool for analyzing and visualizing these networks and a number of databases collect data from various types of protein–protein interaction experiments were launched.¹⁵ TAP–MS was successfully used to identify associated proteins to histones and new sites of post-translational modification,¹⁶ to provide global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*,¹⁴ and to unravel the plant *Arabidopsis* protein cellular machinery complexes.¹⁷

TAP–MS allows determination of protein partners quantitatively *in vivo* without prior knowledge of complex composition. However, the chance for contaminants is reduced significantly, if there is some previous knowledge about interaction available. It is especially good method for testing stable protein interactions. It is considered to be easy to execute, often provides high yields in a throughput manner and has sufficiently low false-negative rate to allow for comprehensive studies of yeast genome.¹⁸ How-

ever, special care should be invested in performing such experiments. Performing biological replicates of purifications is very important for the identification of robust interactions. They should be as different as possible from each other (different harvest date and/or cell clone, different batch of affinity purifications, different times and order for mass spectrometric analysis, etc.). In addition, proper negative controls should always be incorporated in every experiment. By contrast to samples, the controls must be kept as closely linked as possible to the biological samples they are associated with (i.e. harvesting, affinity purification, MS analysis, etc. done in parallel to the experimental sample).¹⁹ There is also a possibility that a tag added to a protein might hinder binding of proteins to their interacting partners and protein expression levels may also be affected. On the other hand, insufficiently exposed tags to the affinity matrices may also result in false results. Moreover, due to several washing rounds, it may not be suitable for identifying transient protein interactions.

2. 2. Yeast Two-hybrid System(s)

Yeast two-hybrid (Y2H) system is a method that allows mapping protein–protein interactions *in vivo*, without a need to break up the cells. The advantage of Y2H system is that it can be carried out without specific equipment and can be automated. Therefore, many proteins can be screened in a high throughput manner against thousands of potentially interacting proteins in a relatively short time.^{20,21} The main weakness is a high number of false positive and false negative identifications. In order to minimize the number of false positive interactions the combination of multiple Y2H vectors and protocols is recommended.²⁰

The interaction between different proteins is conveniently monitored on plates by the activation of reporter gene, which leads to the changed phenotype of yeast colonies. The activation of reporter gene depends on the binding of a transcription factor (TF) onto an upstream activating sequence. The transcription factor consists of two fragments, binding domain (BD) and activating domain (AD) that cannot interact *per se* (Figure 2). The protein of interest is fused to BD and the construct is referred to as the bait protein; the other protein is fused to AD and the construct is referred to as the prey protein. The prey can be a single known protein or a library of proteins. Interaction of bait and prey complete TF and activates the reporter gene. The most efficient is the use of Y2H system on systematic small-scale studies where the screen is performed using specific open reading frames. This kind of Y2H is termed array-based Y2H screening. However, Y2H system is often applied also on a large scale, to large sets of proteins or even whole genomes where the screen is performed using genome or cDNA libraries. This kind of Y2H is termed library-based Y2H screening. The advantage of array-based Y2H screening is the direct identification of interacting protein pairs. Library-based Y2H screening

ning requires identification of individual prey clones and systematic retesting.²²

Y2H systems are available in a variety of different versions, with multiple different host strains, vectors, reporter genes, or protocols (Figure 2). The one-hybrid system enables detection of protein-DNA interactions.²³ There is only one fusion protein constituted by a library, which is linked directly to the BD and AD. The library is selected against the desired target sequence, which is inserted in the promoter region of the reporter gene. The three-hybrid system enables detection of RNA-protein interactions.²⁴ The protein fusion domains cannot interact with each other and a hybrid RNA molecule is essential to connect the two domains. Classical Y2H screen is limited to soluble proteins and cannot be used for membrane proteins. However, in the split-ubiquitin system, two membrane proteins are fused to two different ubiquitin moieties.²⁵ One of them is fused to a TF that can be cleaved off by ubiquitin specific proteases. When bait and prey interact, the two moieties assemble; the ubiquitin is recognized by ubiquitin-specific proteases, which cleave off the TF and reporter gene is transcribed.²⁵ The fluorescent two-hybrid system uses two hybrid proteins that are fused to different fluorescent proteins (GFP, mCherry). Bait protein is fused to the lac repressor (LacI). If bait and prey interact, they bring the fluorescent proteins in close proximity at the binding site of the LacI protein in the host cell genome, which is viewed as colocalization of both fluorescent proteins.²⁶ Enzymatic two-hybrid system uses the detection of enzymatic activity. The example of this ver-

sion of two-hybrid system is KInase Substrate Sensor (KISS), a mammalian two-hybrid system.²⁷ Y2H in combination with next-generation sequencing has become an indispensable tool in analyzing large data sets in proteomics providing unique insights into human proteome and interactions between different proteins.^{28,29}

2. 3. Fluorescence Resonance Energy Transfer

Fluorescence resonance energy transfer (FRET) approach allows identification of molecular pairs at close proximity and is particularly suited for studies employing cells. FRET is a physical phenomenon of energy transfer from an excited donor-fluorophore to an acceptor-fluorophore. The transfer is non radiative and highly dependent on the distance between the two fluorophores. The transfer efficiency is inverse proportional to sixth power of the distance.³⁰ Because of this, effective FRET can be a reliable proof of close proximity of binding partners in living systems. The interacting proteins labelled by either donor and acceptor fluorophores that exhibit effective energy transfer can indicate distance below 10 nm.³¹ The fluorescent excitation-emission properties of an appropriate FRET fluorophore pair must have sufficiently distinct wavelength of their emitted light, which then allows efficient resonant energy transfer.^{32,33} The use of proteins genetically coupled to appropriate fluorescent proteins along with abilities of modern microscopes enable real time micro-imaging of interacting protein

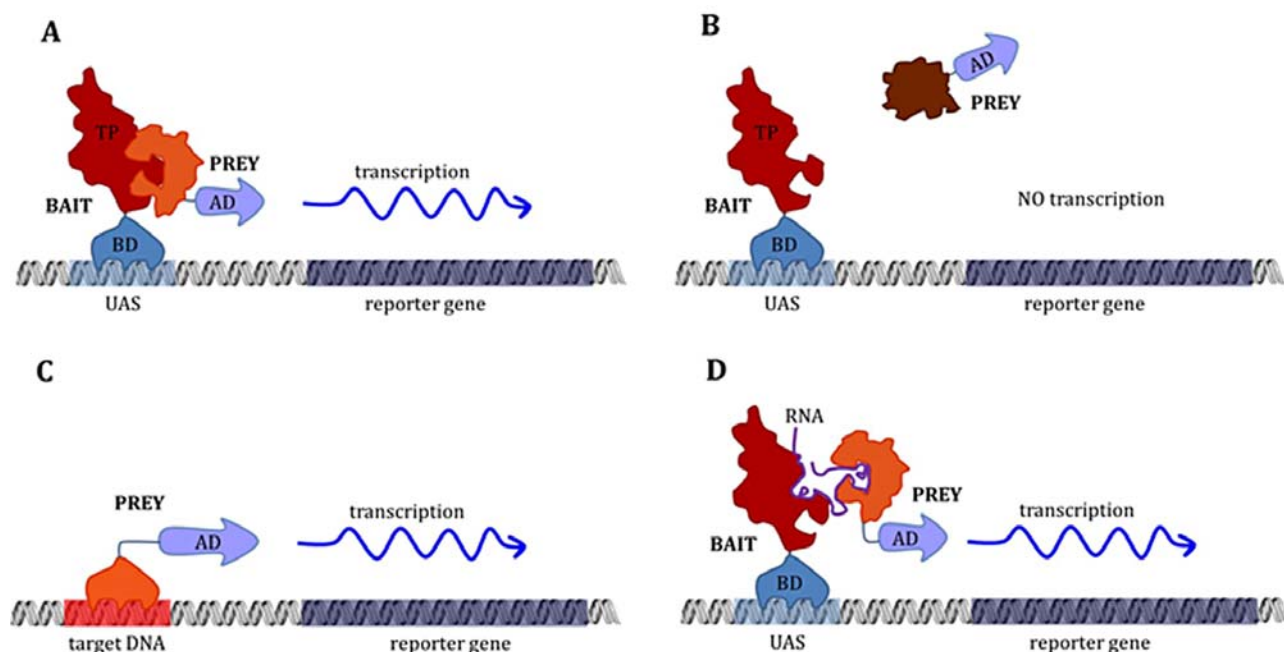


Figure 2. The study of protein-protein interactions using various Y2H systems. Target protein (TP) is fused to DNA-binding domain (BD), forming the bait protein (BAIT). Potential partner protein is fused to transcriptional activation domain (AD), forming the prey protein (PREY). When the two proteins interact (A), the bait recruits the prey to upstream activating sequence (UAS) and transcription of the reporter gene occurs. In the absence of interaction (B), transcription of the reporter gene is not present. Variants of Y2H system: one-hybrid (C) and three-hybrid system (D).

partners in living cells. Ability of monitoring multiple interactions is needed to obtain good spatial and temporal resolution of the cellular processes, and this can be achieved by concomitant application of multiple fluorophores.³³ Natural and genetically modified fluorescent proteins provide for many spectral options that can be used in living cells, however, they have several technical limitations like low stability and low light emission intensity as well as spectral overlapping with cell own auto-fluorescent molecules.³¹ Organic fluorescent dyes with superior properties can be conjugated to active proteins for studying processes in cell or at its surface.^{34,35} FRET imaging is also a powerful approach for identifying protein-lipid and protein-protein interaction in the cell membranes. The lanthanide based chelate fluorophores are another attractive advantage over organic or protein fluorophores. Their long fluorescence life-time enable time resolved imaging and further improving signal to noise ratio, however, lateral diffusion may interfere with results in membrane localization studies.

3. Biophysical Approaches for Studying Molecular Interactions

There is a plethora of biophysical approaches available that are relatively easy to perform and can provide

quantitative data on molecular interactions. Quite a lot of them are optical approaches that exploit some physical phenomena occurring at the surfaces. These methods can therefore be divided by the need to immobilize one of the binding partners on the support, i.e. surface-based approaches, such as surface plasmon resonance (SPR) or enzyme-linked immunosorbent assay (ELISA), or those that can be assessed in solution, i.e. proximity-based assays, such as isothermal titration calorimetry (ITC).⁴ Furthermore, some methods require fluorescence labelling of one of the binding partners, as in microscale thermophoresis (MTS). Besides the availability of the instrumentation, the choice of a method also depends on the amount of the available protein sample of interest and its biochemical and biophysical properties, as well as of the availability and properties of the partner molecules (Table 1). In addition to protein-protein interactions, these biophysical methods can also be used for other binding partners like sugars, lipids, synthetic molecules, ions and others.

3. 1. Surface Plasmon Resonance

Surface plasmon resonance (SPR) has become one of the most important optical-based approaches for studying molecular interactions.^{36–38} Binding of an injected molecule (termed analyte in SPR terminology) to a molecule (termed ligand) immobilized on the surface of a thin

Table 1. Advantages and disadvantages of some of the most commonly used biophysical methods for studying molecular interactions.

Method	Advantages	Disadvantages
Surface plasmon resonance	• Relatively fast • Sensitive • Low amount of sample • Possibility to determine association and dissociation rate constants • Real time monitoring • Label free	• Immobilization of molecules required • High-affinity interactions are not accessible • Difficult to quantify weak binding interactions • Influence of mass transfer effect • High non-specific surface binding of some analytes • Mass-detection limit
Isothermal titration calorimetry	• Label-free • Possibility to determine K_D, stoichiometry and thermodynamics of the binding process • Solution-based • No molecular weight limitation • Minimal assay development	• High amount of a pure sample required • Long preparation time • High solubility of binding partners required • Low to medium throughput • Change in enthalpy upon interaction is a prerequisite • Binding partners need to be soluble in the same buffer system
Quartz crystal microbalance	• Setups that allow measurements of interactions of molecules with exposed binding partners on cells • Allows assessing the physical nature of the adsorbed molecules, i.e. flexibility and thickness of the adsorbed film	• Difficult quantification of results • Immobilization of one of the binding partners on the sensor surface required • Low throughput
Microscale thermophoresis	• Fast • Low sample consumption • Ability to perform measurements in complex samples, such as cell lysates • Possibility of using it label-free	• Modification of molecules with fluorescent probes required • Molecular behavior in thermophoretic field is not well understood • Quenching or photo-bleaching of labels • Labels can affect the interaction of molecules

layer of gold-covered sensor surface (so-called sensor chip) changes the refractive index of the solution and this changes the resonance properties of surface plasmons, which is sensed by the detector. From the experimental data it is possible to derive binding and dissociation rates (kinetics), strength of an interaction (affinity), thermodynamic data, as well as determination of the active concentration of a protein without a need for a calibration curve. SPR is a non-invasive approach that requires small amounts of material and allows measurements in real time. It is relatively fast and does not require labelled molecules (Table 1).

SPR is the gold standard in academic and industrial settings, in which the molecular interactions have to be characterized. The most traditional type of interactions studied using SPR are those between two proteins, aimed to obtain affinity and kinetic data profile for two molecules for basic research or using this technique for medical diagnostics, environmental monitoring and food safety analysis. The ligand is typically covalently attached to the sensor surface by straightforward amine coupling (Figure 3, left panel) or using some other approaches (thiol or aldehyde coupling), which enable more defined orientation on the sensor surface. In addition, surface can be further modified in a way that ligand can be captured exploiting some potential tags on a protein, like His-tag or biotin. After the immobilization the binding and dissociation of an analyte can be followed in real time (Figure 3, right panel). Typically, five concentrations of an analyte are injected over the ligand and obtained binding curves (termed sensorgrams) are fitted to an appropriate binding model. Between each sample injection one or two short regenera-

tion pulses are usually required to clean the sensor chip and prepare it for the next cycle. This step largely depends on dissociation rates.³⁹ Besides proteins it is possible to immobilize various lipid membrane systems and in this way study protein-membrane interactions and even elucidate mechanisms of pore formation for many important molecules.^{40–42} The method is often applied in drug discovery, since the technology has evolved enormously towards high-throughput instrumentation. Analysis of several hundred compounds can be resolved within half a day employing 384 wells microplates along with automated instruments. The first step for this application is usually structure- or ligand-based virtual screening yielding compound library to be tested *in vitro*.^{43,44} The SPR allows also assessing interactions of biomolecules with non-biologic surfaces.

Fast developing field of proteomics brought a need to develop SPR method even further. High-throughput SPR platforms are capable of analyzing large number of analytes in short time, especially by utilizing SPR imaging approach where the multiple interactions can be monitored simultaneously.⁴⁵ The method can be connected with mass spectrometry to analyze unknown bound molecules.⁴⁶ Extremely sensitive detection of femtomolar concentrations of analytes is possible due to development of new types of surfaces and employing ligands with high affinity.⁴⁷ The methodology was further exploited in food safety program by developing biosensors for different types of toxins and artificial residuals in food.^{48,49} Since the first commercial SPR instrument has been launched 25 years ago these instruments became smaller, portable and easier to use with even improved

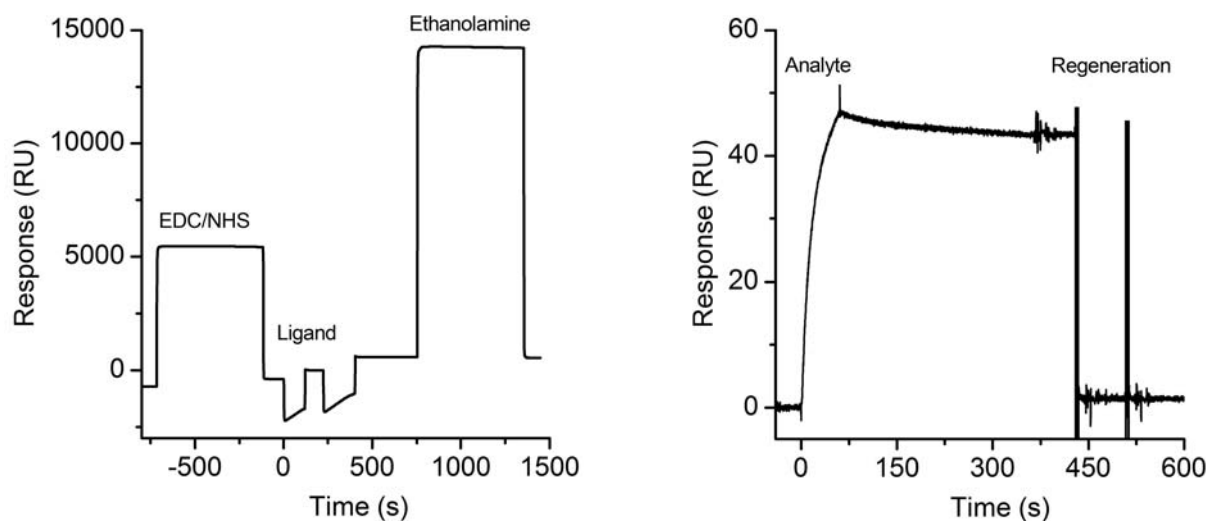


Figure 3. A typical SPR experiment. The left panel shows immobilization of one of the binding partners (ligand) to the surface of the sensor chip. The whole procedure is done through injecting solutions across the sensor chip. At the end of the process the ligand is covalently attached to the surface of the sensor chip, which is visible as the increase of the signal over the baseline value (compare starting signal level with the signal at the end). Right panel shows the typical experiment in which the second interacting molecule (analyte) is injected across the ligand. After the dissociation step, the regeneration procedure prepares the sensor chip surface for the next cycle. EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; NHS, N-hydroxysuccinimide.

sensitivity and overall performance. The LSPR (localized surface plasmon resonance) instruments utilize gold nanoparticles instead of gold covered chips, making the LSPR sensors potentially applicable for an *in situ* detection changing the sensing capability by changing the shape, size, and material composition of the nanoparticles. One of the promising developments is the usage of graphene surfaces which enable large specific sensor surface, long-term stability and immobilization of varieties of biomolecules through covalent, noncovalent or electrostatic interactions.⁵⁰

3. 2. Bio-Layer Interferometry

Bio-Layer Interferometry (BLI) technology is another label-free optical approach suitable for measuring biomolecular interactions in real time. The BLI instrument shines white light onto the sensor surface and the reflected light is influenced by the interference from two surfaces: a layer of immobilized molecule on the biosensor tip, and the reference layer. When the analyte binds to the biosensor tip it causes a shift in the interference pattern.⁵¹ Since BLI detects only binding to the sensor surface, there is almost no interference from the sample buffer so the crude samples can be used with no cleaning step before starting the experiment. Using BLI the affinity and kinetics of various interactions can be determined, such as protein-protein,⁵² protein-nucleic acid⁵³ or binding of proteins to liposomes.⁵⁴

3. 3. Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) is a biophysical technique for measuring the formation and dissociation of molecular complexes. ITC measures the binding equilibrium by determining the heat evolved on association of a ligand with its binding partner. It works by directly measuring the heat that is either released or absorbed during a biomolecular binding event. ITC does not require any labeling of binding partners or immobilization and thus allows measurements of the affinity of binding partners in their native states. During ITC experiment, a complete thermodynamic profile of the molecular interaction can be obtained in a single experiment. Measurement of a heat transfer during binding enables accurate determination of the binding constant (association constant (K_A) in M^{-1} units or dissociation constants (K_A^{-1} or K_D) with M units), the stoichiometry (n), and the enthalpy of binding (ΔH). The free energy (ΔG) and entropy (ΔS) of binding are determined from K_A . The temperature dependence of the ΔH parameter, measured by performing the titration at varying temperatures, describes the heat capacity of binding (ΔC_p).⁵⁵ The ITC instrument is relatively simple. The microcalorimeter contains two cells, a reference cell filled with water, and the sample cell. Both cells are kept at exactly the same temperature. During the measurement, the

ligand is titrated into the sample cell including the binding partner (i.e. protein) in a controlled manner. The heat sensing devices detect temperature difference between the cells when binding occurs and give feedback to the heaters, which compensate for this difference and return the cells to equal temperature. This direct measurement of the heat generated or absorbed when molecules interact and the quantity of heat measured is in direct proportion to the strength of binding.⁵⁵

ITC is used in quantitative studies of a wide variety of biomolecular interactions, directly measuring millimolar to nanomolar affinities, and indirectly nanomolar to picomolar disassociation constants using competitive binding techniques. Besides binding affinities, ITC also elucidates mechanisms of molecular interactions. Information obtained from ITC experiments provides better understanding of structure-function relationships, as well as enables better planning in hit selection and lead optimization in drug design development.⁵⁵ The range of interactions measured is very broad: proteins with small ligands⁵⁶ (Figure 4), protein or peptide interactions with metals and ions,⁵⁷

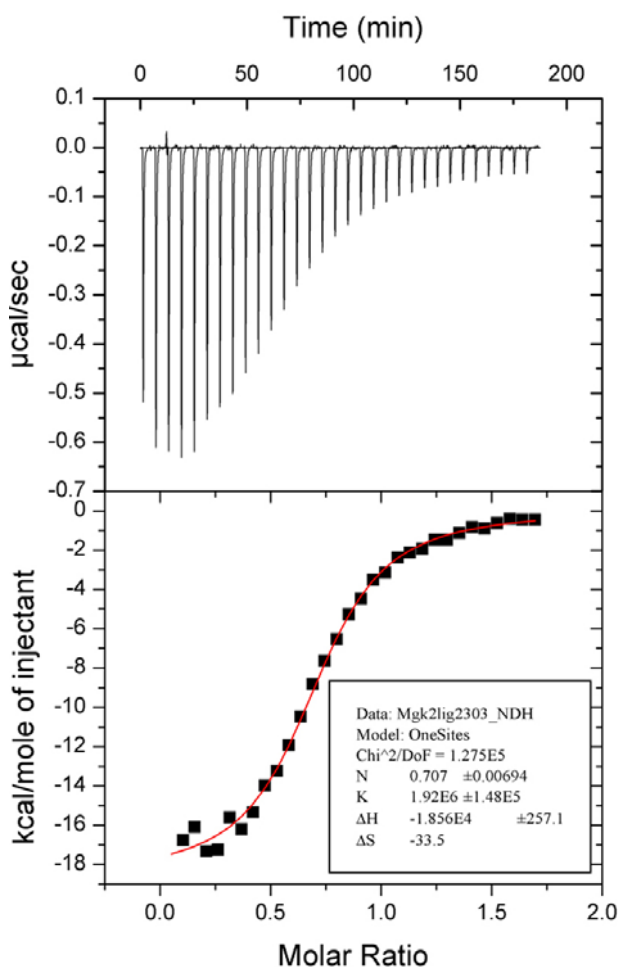


Figure 4. A typical ITC experiment using VP-ITC (MicroCal). Inositol hexakisphosphate kinase 2 was titrated with inositol hexakisphosphate.⁵⁹

protein or peptide interactions with nucleic acids, lipid or membrane interactions, polysaccharide interactions, protein or peptide interactions with polymers and nanoparticles, nucleic acid interactions other than with proteins. In addition, ITC can measure enzyme activity and kinetics, small molecule ?interactions and micelle formation.⁵⁸ While ITC is the best method for accurate quantitative measurements of interactions, one of the main drawbacks is a relatively large amount of sample needed for the experiment, in comparison with other biophysical approaches such as SPR or MST. However, the advent of the upgraded machines requiring significantly lower amounts of samples is gradually overcoming this problem.

3. 4. Quartz Crystal Microbalance

The quartz crystal microbalance (QCM) is a high resolution mass sensor. The sensing mechanism is based on detection of changes in resonant frequency of the piezoelectric crystal resonator. It has been used in various environments, including biological systems.^{60,61} Rigidly deposited mass on the crystal surface results in proportional decrease of resonant frequency thus enabling straightforward analysis of measurements.^{60,61} The binding kinetic is recorded in a flow-through system as a sensograms of real time changes in sensor frequency versus time. Affinity rate constants can be derived from such data. Additionally, dissipation of the signal can be monitored. Less rigid deposits cause more rapid loss-dissipation of crystal oscillatory energy. From the dissipation signal thickness and viscoelastic properties of deposited layer can in addition be derived. This enables further elucidation of changes in the structure of deposited film of material including burst of membrane vesicles on the sensor surface as well as conformational changes of attached proteins.^{62,63} QCM senses mass directly, therefore no labelling of studied material is needed. The sensor surface can be functionalized with capture molecules for specific detection of the selected analyte. Any unspecific binding of mass to the sensor result in biased results. To minimize these artefacts, two channel measurements are generally performed enabling subtraction of unspecific signal. Sensors can be prepared for interaction studies of ions, small molecules or proteins.^{63–65} QCM can be set for detection of viruses and artificial particles or even for binding of cells from suspension.^{61,66,67} Interactions of proteins from complex samples such as culture media or sera can also be measured accurately as the optical properties of samples have no effect on the measurement enabling studies in biologically relevant environments. The method has been frequently used as a means of detection of specific disease related protein markers in serum.⁶⁸ In addition to simple molecular binders the sensor surface can be decorated by complex structures like supported model lipid membranes or cell derived membranes enabling studies of membrane binders like pore-forming proteins and others.^{62,69} QCM can de-

tect protein interactions even if not in close proximity to the surface of the sensor. Multistep binding processes can be successfully monitored in real time. Proteins can be sequentially loaded in a complex structure and the process continuously monitored.⁷⁰ Even adherent cells can be cultured on the sensor surface for testing of interaction with ligands. This allows monitoring of cell surface proteins interactions and physiological responses of cells, like release of micro-vesicle.^{71,72}

3. 5. Microscale Thermophoresis

Although thermophoresis (Ludvig-Soret effect, thermodiffusion) was already described in 19th century, it was only recently developed as a convenient tool for a description of biomolecular characteristics. The thermophoretic behavior of molecules, that is their vectorial diffusion along temperature gradient, is normally present in the nature as for e.g. in the circulation of air or ice.⁷³ While the effect was generally found to be practical for the characterization or separation of some inorganic molecules or polymers,^{74,75} it has first been applied to biomolecular characterization in the last few years. Upon heating the spot of the solution of fluorescently labelled plasmid DNA with infra-red (IR) laser, Braun and Libchaber observed the depletion of fluorescence in the heated area.⁷⁶ The cause for the fluorescence-drop was the movement of labelled molecules along the temperature gradient towards the colder part of the system. The salt-dependent diffusion of DNA along the temperature gradient suggested the new possible approach for the characterization and purification of nucleic acids. Usability of thermophoretic behavior of molecules for their characterization, was further shown with the analysis of aptamer DNA-thrombin interactions.⁷⁷ The DNA is not the only biomolecule that can be applied to thermophoretic gradient for its characterization, as shown by the same group in the analyses of protein-protein and ion-protein binding.⁷⁸ Since nM concentrations and low volumes (μ l-range) of protein and DNA solutions were used in the analyses, the phenomenon was termed microscale thermophoresis (MST).

MST-based instruments track the movement of fluorescent molecules along the applied temperature field (Figure 5). Small volume ($\sim 5 \mu$ l) of fluorescent molecule solution is applied to the glass capillary, which is placed into the instrument. The focused IR laser beam then heats the spot ($\sim 200 \mu$ m) in the capillary for typically 2–6 °C. The IR laser creates the spatial distribution of temperature in the capillary and upon energy absorption, molecules drift usually from (positive thermophoresis) or more rarely towards (negative thermophoresis) heating beam (Figure 5). Since the fluorescence is excited through the same optical element as IR laser, the fluorescence detector then tracks the change in the emission of heated spot. It is possible to analyze and compare the differences in fluorescence before, between and after the heating of the solu-

tion, since molecules possess different patterns of diffusion in the temperature field. There are two possibilities to observe fluorescence of the molecules. They can be labeled by fluorescent probes or their intrinsic fluorescence can be monitored. Fluorescence labelling proved to be most used method in MST, since conventional fluorescent detectors track low (nM, pM) concentrations of the labeled molecule.⁷⁹ In addition, it is possible to track the molecules in complex solutions such as cell lysates or plasma.⁸⁰ But on the other hand labelling with fluorescent tags, chemical dyes or artificial amino acids can influence the properties of the molecules and consequently the corresponding molecular interaction. For this reason “label-free” MST, which analyses the fluorescence emission of natural amino acids such as tryptophan, gives an insight into the behavior of the molecule in the native state.⁸⁰ The potential drawbacks of label-free MST are that the solution of the molecule should be sufficiently pure and due to the lower fluorescence of natural residues the concentration of the molecule used in experiment is often higher compared to the analysis with the labeled molecules.

MST analyses proved to be optimal for investigating molecular interactions. If fluorescent molecule interacts with other parts of the system and interactions affect its mass, surface and/or hydration shell, diffusion of the molecule alters along the thermal gradient. Therefore, by varying the concentration of e.g. ligand in the system and by comparing its influence on thermophoretic behavior of its fluorescently-labelled partner, stoichiometry of the inter-

action can be obtained. The MST showed to have a broad range of sensitivity. It has been shown that is possible to detect from pM to mM affinities of the protein-protein, protein-nucleic acid or nucleic acid-nucleic acid interactions or interactions of biomolecules with ions, lipids or small molecules.⁸⁰ On the other hand also stability of the biomolecules can be analyzed using the same principle.⁸¹ If the environment affects the biomolecule's tertiary structure, its diffusion along the temperature gradient is also altered. Thus the MST behavior of fluorescent molecules can be screened against different salt concentrations, pH, temperature or chemicals that have influence on its structure. Although the method is a novelty in the field of molecular interactions, it quickly showed its potential. Compared to the SPR, analyzing molecules are not attached to the surface and compared to the other methods, particularly ITC, low amounts of samples are used (Table 1). But yet, as thermophoretic behavior of the molecules is still not well understood, interpretation of MST might be quite complex and does not necessarily reflect the behavior of the molecules in natural environment.

3. 6. Molecular Interactions of Nanopores in Lipid Bilayers

A biophysical approach that allows studying interactions of molecules with nanopores is termed planar lipid membranes (PLM), also called black lipid membranes (BLM) approach.^{82,83} BLM are artificial lipid bilayers,

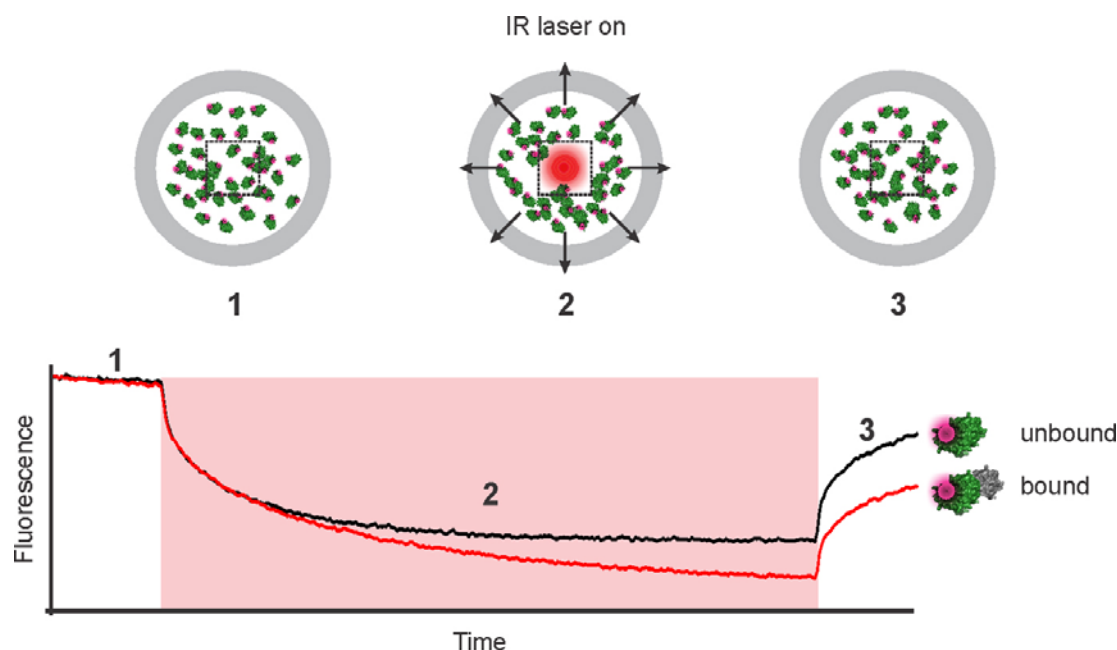


Figure 5. The MST experiment. The solution of molecules (green with magenta dots) is applied to the capillaries (grey circles). Following the initial fluorescence excitation of small fraction of the sample (dashed square) (1), the same part of the capillary is heated with IR laser (2). Upon heating, molecules usually drift away from the heating spot. The drift is observed as a reduction of the fluorescence. After turning the IR laser off, the back-diffusion of the molecules happens and this is detected as an increase of fluorescence (3). The bound and unbound molecules diffuse differently in the thermal field.

enabling studies of properties of membrane active substances (e. g. channel proteins, pore forming proteins, DNA nanostructures) in a well-defined environment. This electrophysiological technique was introduced around 50 years ago and has gained enormous knowledge of biological membranes.⁸² It is used to estimate the pore/channel characteristics such as pore size, ionic selectivity, voltage dependence and transport of molecules through the pore (i.e. sensing).^{82–86} Variable molecules can be detected during passing through the pore and provide the information of the pore geometry or provide the useful kinetic data of the analyte.⁸⁷ It is possible to screen various parameters that affect the pore characteristics, e.g. pH, temperature, salt concentration, or voltage potential.⁸³ Method enables variable interactions studies. It is possible to study the interactions of proteins with lipids and monitor the pore formation. With careful design and chemical modification insertions of DNA nanostructures into lipid bilayer are possible, resulting in artificial ion channel.⁸⁸

BLM is a direct and label-free method that enables high resolution measurements in real time. The set up contains two small chambers (called *cis* and *trans*) separated with an aperture (diameter 50–160 μm), where artificial planar lipid bilayers are formed and act as a capacitor. Chambers are filled with buffer and connected to an electronic system with Ag–AgCl electrodes that permit the application of voltage at one side (usually the *cis* side) in range of tens of mV,⁸³ while the *trans* side is grounded. With current-voltage amplifier we can measure changes in current fluctuations (in range of pico amperes) caused by incorporation of pores into the membrane. Each single pore can be detected as an increase or decrease in the cur-

rent, depending on the sign of voltage, where pore insertion reflects as an step-like current change.⁸³ From ionic current through the membrane (I) and the applied transmembrane potential (V) it is possible to calculate the conductance (G) by simple equation of $G \text{ (nS)} = I \text{ (pA)} / V \text{ (mV)}$.⁸⁹ Usually very low amount of membrane active substances (in range of ato- to nano molar) are needed to reconstitute into the membrane and to enable monitoring of their functional characteristics. Nowadays methods offer parallel high-resolution recordings with automatic bilayer formation and mostly software measurement processing.⁸⁴

In past decades a variable workload from pore sensing to the nanopore-based detector for the DNA or RNA sequencings has been done on α -hemolysin (α HL), an exotoxin from bacterium *Staphylococcus aureus*.^{87,90–92} α HL monomers self-assemble on lipid bilayers to a heptameric pore and form app. 100 Å long channel.⁹⁰ A wide range of molecules have been tested in sensing experiments to gain the data of the concentration and quantification of the analyte (Figure 6).⁸⁷ The pore was also mutated to acquire better DNA bases recognition⁹² and to provide more controllable environment to delivery of ligands.

4. Structural Approaches for Describing Molecular Interactions

4. 1. X-ray Crystallography

Three dimensional structures of molecular interactions at atomic resolution can be measured by X-ray cry-

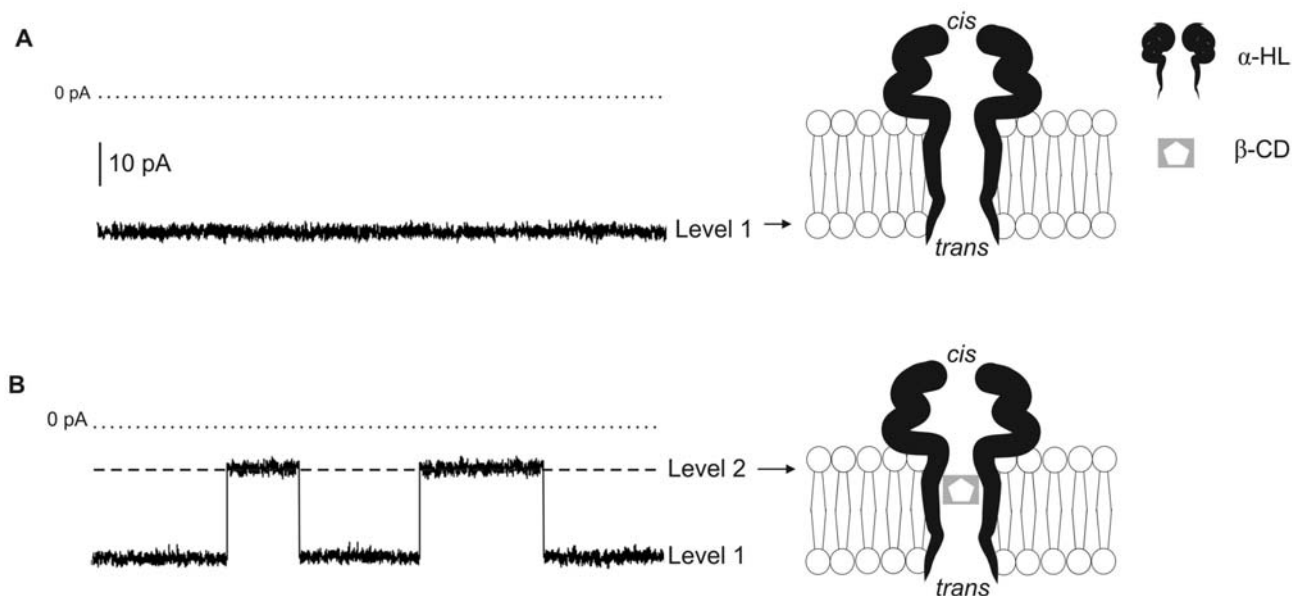


Figure 6. Black lipid membranes recordings of single α -hemolysin (α -HL) pore with β -cyclodextrin (β CD). Currents were recorded at -40 mV (*cis* at ground). α -HL pore was entering from the *cis* side and β CD from the *trans* side. (A) Single α -HL pore constantly open at around -30 pA (level 1); (B) β CD partially blocking the channel at around 10 pA (level 2). Adapted from Gu *et al.*⁹¹ with permission.

stallography, nuclear magnetic resonance spectroscopy (NMR) and with dramatic recent developments also with cryo-electron microscopy (cryo-EM). Of these, X-ray crystallography is the most popular as well as practical, since it can give atomic resolution structural information on a broad range of molecules, namely from small molecules to macromolecules, including proteins, nucleic acids and large cellular complexes, like ribosomes, proteasomes or viruses. Consequently, it has also been a primary method for deducing structural details of molecular interactions. Of the more than 110,000 released entries in the Protein Data Bank, about 90 % were solved by this technique.⁹³ Crystal structure determination involves preparation of protein samples of high purity, homogeneity and stability, crystallization of these molecules, collection of X-ray diffraction data, structure solution, model building, and refinement. The principle of this method is that X-rays scatter on protein electrons as they pass through a protein crystal. The scattered waves interfere with each other, resulting in a diffraction pattern from which the positions of atoms and thus three dimensional structure of proteins is determined (Figure 7). Further analysis of structural features helps understand biological roles and mechanism of action of molecules under study.⁹⁴

However, a care has to be taken when studying macromolecular complexes, since a crystal structure of a complex might not reveal a unique binding interface. Determination of a biological interface from crystal contacts may not be straightforward and unambiguous.⁹⁵ Importantly, macromolecular crystals mostly grow under non-physiological conditions, including high protein concentrations, a wide range of pH values and temperature, high ionic strength, or in the presence of various non-biological compounds that aid crystallization. This can result in intermolecular contacts that are not biologically relevant, or the crystallization of what is expected to be a complex in a solution may not result in the crystal containing all subunits of the complex.⁹⁵

Due to these potentially harsh and non-natural crystallization conditions, complexes between molecules with high affinity have higher chances to actually crystallize as functional complexes, as in the case of proteins Vps29 and Vps35, forming a subcomplex of the retromer cargo-recognition complex with K_D of 350 nM.^{96,97} The same is true for high affinity complexes between proteins and small ligands, as for example tight binding of GMP in the active site of the metallophosphodiesterase MPPED2.⁹⁸ For weaker interactions in high micromolar or even millimolar ranges, combination with NMR and small angle X-ray scattering (SAXS) is a better choice.^{99,100} However, under certain conditions and especially in a high excess of ligands, crystals structure of very low affinity (i.e. millimolar range) complexes can be obtained, like in the case of a mannose binding by pneumolysin.¹⁰¹

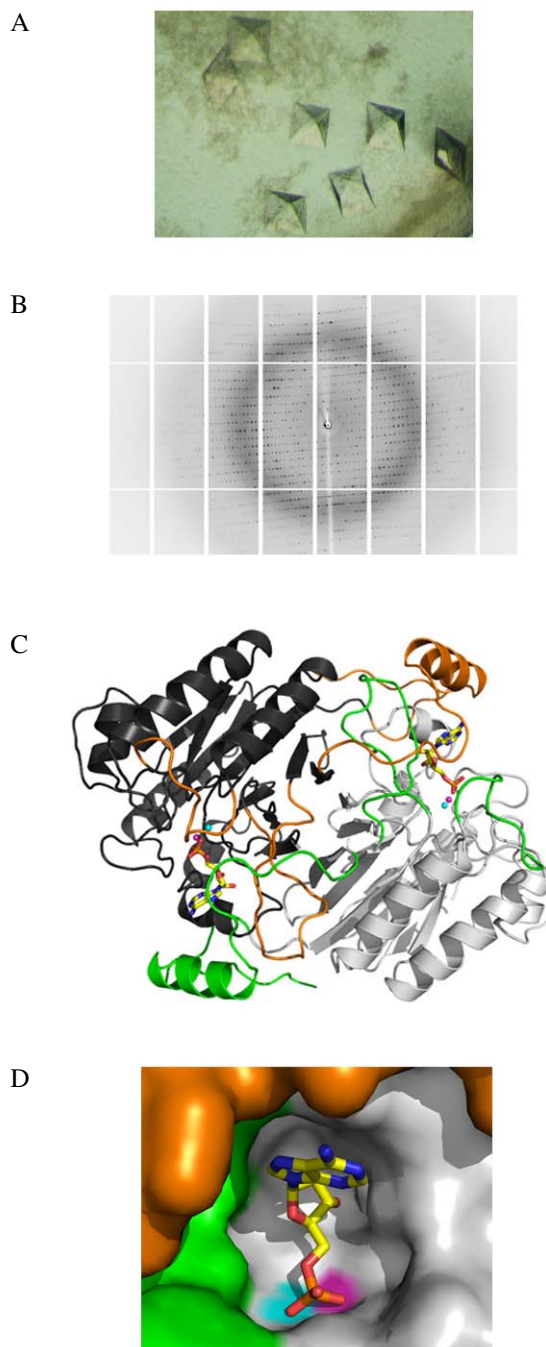


Figure 7. From crystals to structure: (A) Protein crystals. (B) X-ray diffraction data obtained at the synchrotron X-ray source. (C) Crystal structures often reveal details of protein complex with smaller ligands. Here, structure of metallophosphodiesterase Rv0805 homodimer from *Mycobacterium tuberculosis* in complex with AMP is shown.¹⁰² (D) the same as in (C), showing the surface of the active site and the bound AMP molecule in sticks.

4. 2. Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy is the second most powerful and predominant technique used to experimentally determine

three-dimensional structures of biological macromolecules at near atomic resolution, where samples are measured in soluble state. NMR is usually used in cases where no protein crystals can be obtained, and in contrast to crystallography, it also provides information on solution state dynamics. Generally, NMR generates lower resolution structures than X-ray crystallography and is limited to molecular weights below 50 kDa.¹⁰³ However, NMR can be used as a complementary method to X-ray crystallography, representing a great alternative in the case of transient macromolecular complexes, which refuse to crystallize in high quality crystals, or when crystals do not contain the biologically relevant conformation of the proteins. In cases where the interaction is weak ($K_D > 100$ mM), NMR is essentially the only approach that allows the determination of high-resolution structures.⁹⁹

The basis of NMR spectroscopy is the property of many elements to have a nuclear magnetic moment. Stable isotopes of particular importance in biological macromolecules are ^1H , ^{13}C , or ^{15}N . When placed into a static magnetic field (B), the different nuclear spin states of these nuclei become quantized with energies proportional to their projection onto B . The energy difference depends on the type of nucleus, is proportional to field strength of the static magnet, and is dependent on the chemical environment of the nucleus. This energy difference corresponds to electromagnetic radiation in the megahertz range. The transition between these states can be induced by irradiation with a radio-frequency field with characteristic frequencies for each type of nucleus and its chemical environment. The frequency of the NMR signal is extremely sensitive towards changes in covalent bonds, i.e. presence of neighboring groups, as well as to noncovalent bonding found in complexes built by biological macromolecules. Furthermore, transfer of magnetization through bonds or through space results in a characteristic change of the shape and size of the NMR signal and reflects, for example, the bond angle in the case of scalar coupling or spatial distance in the case of dipolar coupling. Various NMR experimental approaches are available to observe these phenomena, and the resulting spectra can provide structural details about the interactions between partner molecules under study.¹⁰⁴

There are several approaches in NMR by which the interaction of biological macromolecules and low molecular weight-ligands can be characterized at an atomic level, using relatively quick and easy ligand-based techniques. These need only small amounts of nonisotope labeled, and thus readily available target macromolecules. As the focus is on the signals stemming only from the ligand, no further NMR information regarding the target is needed. Techniques based on the observation of isotopically labeled biological macromolecules open the possibility to observe interactions of proteins with low-molecular-weight ligands, DNA or other proteins. With these techniques, the structure of high-molecular-weight complexes

can be determined. In this case, the resonance signals of the macromolecule must be identified beforehand.¹⁰⁴ The NMR-based procedures can be roughly subdivided into two groups: (1) observation of the NMR signals of the usually low molecular weight-ligand and its behavior upon binding to the target, and (2) focus on the signals of the usually much higher molecular weight protein or DNA target and the effect of the binding ligand. The former relies on the transfer of magnetization between target and bound ligand giving rise to ligand signals, whereas the latter observes the effect of ligand binding on the chemical shift of the target resonances, thus changing the position of the target NMR signals. One big advantage of NMR measurements is that the experiments are performed in aqueous solutions, that can be relatively close to biological conditions.¹⁰⁴

The available NMR methods for studying interactions are, to name some: intermolecular dipole–dipole relaxation effect, cross-saturation, chemical shift perturbation, dynamics and exchange perturbation, paramagnetic methods, and dipolar orientation. Most of these methods have been used to study complexes with molecular weight of 60 kDa and can be used also for large complexes, up to 1000 kDa.^{99,105,106} Advances in instrumentation have enabled to overcome the classical size-limitation of solution-state NMR and have demonstrated its use in studies of mega-dalton protein complexes, including those containing nucleic acids.^{105,107,108} Furthermore, solid-state NMR (ssNMR) has emerged in the last decade as one of the prominent methods to study the structure of large, poorly soluble molecules, especially of membrane proteins and intrinsically disordered proteins.¹⁰⁵

4. 3. Cryo-electron Microscopy

Cryo-electron microscopy (cryo-EM) is increasingly becoming a mainstream technology for studying the architecture of cells, viruses and protein assemblies at molecular and even atomic resolution. For many years, structure determination of biological macromolecules by cryo-EM was limited to large complexes and low-resolution models. Recent developments in microscope design and imaging hardware, in combination with enhanced image processing and automation, build the crucial basis for further advance of cryo-EM method, which are approaching resolutions obtained by X-ray crystallography, and are becoming applicable also for smaller molecular objects. Experimentation at cryogenic temperatures and averaging of multiple low-dose images are central to modern high-resolution biological electron microscopy.^{93,109}

In Cryo-EM set-up, a frozen protein solution is exposed to a beam of electrons. The electrons scattered by the sample pass through a lens that creates a magnified image on the detector, from which the structure can be deduced.⁹³ Cryo-EM can be divided in several subdisciplines, including cryo-electron tomography, single-particle

cryo-EM, and electron crystallography. These methods can be used singly as well as in hybrid approaches, where the information from cryo-EM is combined with complementary information obtained using X-ray crystallography or NMR.¹⁰⁹ Cryo-electron tomography is emerging as a powerful method to visualize structurally heterogeneous objects (e.g. viruses, tissues, cellular and sub-cellular multimolecular assemblies) at resolutions between ~ 100 Å and ~ 50 Å and reaching up to 20 Å and higher, when applying subvolume averaging.¹⁰⁹ Single-particle cryo-EM is probably the most commonly used variant of cryo-EM. In this case, data from a large number of 2D projection images, featuring identical copies of a protein complex in different orientations, are combined to generate a 3D reconstruction of the structure. Following this, atomic models, available for some or all of the components building the complex are fitted into the density map to provide pseudo-atomic models, which largely extends the information obtained by electron microscopy.¹⁰⁹ Using this approach, resolutions beyond 3 Å can be achieved now, as a combination of a technical development, as well as sample preparation improvement.^{93,109,110} Cryo-electron microscopy of ordered assemblies or electron crystallography allows even higher resolutions due to highly crystalline assemblies, forming two-dimensional crystals or other types of ordered assemblies such as tubular crystals and helical assemblies.¹⁰⁹ This strategy has been extremely effective with membrane proteins that form two-dimensional crystals in the plane of the membrane, and high resolutions, reaching beyond 1.8 Å have been reported.^{109,111} The drawback here is that proteins have to be amenable to form ordered assemblies such as helical or two-dimensional crystals.

Besides using Cryo-EM approach as a method of a choice to study huge and dynamic complexes, molecular machines like ribosomes, viruses and membrane proteins, it can be also used to calculate the structure of a protein that has been flash-frozen in several conformations to deduce the mechanisms by which it works.⁹³ Thus, electron microscopy has the potential to provide both structural and dynamic information of biological assemblies in order to understand the molecular mechanisms of their functions.¹¹²

5. Conclusions

Complementary structural, biophysical, functional and computational methods should be considered in order to correctly describe and interpret macromolecular interactions in biological systems. In many cases this means employing different protein constructs and complementary approaches. These may in addition to those described in this review include SAXS, neutron and light scattering, atomic force microscopy, mass spectrometry and analytical ultracentrifugation, which yield information on the

shape, size and mass of macromolecules. Chemical cross-linking and electron paramagnetic resonance can yield data on proximities of different parts of macromolecules, while circular dichroism informs about the secondary structural content of a protein. Furthermore, mutational analysis of the potential binding interfaces in combination with methods that measure the strength of binding in wild type and in mutated proteins, like ITC, SPR and MST, give further details on correctness of determined interfaces by structural methods, such as X-ray crystallography. Novel approaches, developments in instrumentation and advances in protein recombinant technology will allow better and more rapid description of molecular interactions for many important biological molecules.

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Povzetek

Fizične in funkcionalne interakcije med molekulami imajo osrednjo vlogo pri vseh bioloških procesih v živih sistemih. Identifikacija proteinskih kompleksov postaja za razumevanje celic in organizmov na molekularni ravni vedno bolj pomembna. V zadnjih letih je bilo razvitih več učinkovitih metod in tehnik za raziskave molekulskih interakcij, ki pomagajo osvetliti njihov pomen v biologiji, kot tudi možne načine preprečevanja interakcij med njimi. Vse tehnike, ki so na voljo za te študije, imajo svoje prednosti in slabosti, in ker jih večinoma uporabimo v pogojih *in vitro*, težko z enim samim pristopom učinkovito sledimo vsem interakcijam, ki se zgodijo pri fizioloških pogojih. Z dopolnjujočo uporabo več razpoložljivih tehnik lahko ustvarimo realistično sliko biološkega procesa. V prispevku bomo opisali nekaj proteomskih, biofizikalnih in strukturnih orodij, ki nam pomagajo razumeti naravo in mehanizem teh interakcij.

Review

Chemistry of Metal-organic Frameworks Monitored by Advanced X-ray Diffraction and Scattering Techniques

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In the memory of Janez (Janko) Jamnik.

Abstract

The research on metal-organic frameworks (MOFs) experienced rapid progress in recent years due to their structure diversity and wide range of application opportunities. Continuous progress of X-ray and neutron diffraction methods enables more and more detailed insight into MOF's structural features and significantly contributes to the understanding of their chemistry. Improved instrumentation and data processing in high-resolution X-ray diffraction methods enables the determination of new complex MOF crystal structures in powdered form. By the use of neutron diffraction techniques, a lot of knowledge about the interaction of guest molecules with crystalline framework has been gained in the past few years. Moreover, *in-situ* time-resolved studies by various diffraction and scattering techniques provided comprehensive information about crystallization kinetics, crystal growth mechanism and structural dynamics triggered by external physical or chemical stimuli. The review emphasizes most relevant advanced structural studies of MOFs based on powder X-ray and neutron scattering.

Keywords: Metal-organic frameworks, X-ray diffraction, X-ray scattering, neutron diffraction, neutron scattering

1. Introduction

Metal-organic frameworks (MOFs) are a class of rapidly developing class of nanoporous materials. They are composed of metal-based building units coordinated to organic bridging ligands to form a three-dimensional network with uniform pore system including channels and cages with the openings typically ranging from 3 to 20 Å.^{1–5} In the last two decades, MOFs experienced remarkable progress in the structural engineering, characterization and application due to their enormous structural versatility and unique chemical and physical properties. One of the most important advantageous features of MOF is the possibility to construct its frameworks by combining selected ligand and coordination geometry of metal-based building blocks. In this context, MOF structures with the targeted structural features can be designed.^{6–11} Specific three-dimensional connectivity of inorganic and organic units enables the formation of MOF architectures with

low framework densities (from 0.2 g/cm³), high void volumes (up to 90%) and unprecedented internal specific surface areas (6000 m²/g). Thus MOFs are to a large extent investigated for gas or liquid adsorption and separation.^{12–15} Very intriguing feature of MOF structures represent the presence of coordinately unsaturated metal sites (CUS) which can either be part of the as-synthesized framework or can be generated by post-synthesis modification process. These exposed metal ions act as a Lewis acid sites which are responsible for catalytic activity of MOFs.^{16–18} Unique structural property that MOF frameworks often possess is their flexibility. MOF structures can respond to different external physical or chemical stimuli in a controlled manner.¹⁹ Structure dynamics can be for instance triggered by adsorption/desorption processes, temperature, magnetic field, photochemical or mechanical stimuli. These phenomena enable MOFs implementation in sensor applications as well.

The in-depth studies of MOF's structural features are crucial to enhance the performance in adsorption, separation and catalysis or to provide additional functionalities and thus widening their application opportunities. The diffraction and scattering methods are the most widely used for such purposes and offer different insights into the structure-property relationship of MOFs. Moreover, the increasing demands to understand their crystallization and crystal growth mechanisms which would enable more controllable and rational design, induces the development of *in-situ* diffraction and combined-diffraction techniques.

High-resolution powder X-ray diffraction is most frequently used technique for structure evaluation of MOFs. Even though traditional single-crystal data (SC-XRD) are always preferred for the high-resolution crystal determination of MOFs, the sufficiently large and undamaged single crystals required for such measurements are much more rarely available than the microcrystalline materials. Many improvements in instrumentation and data processing have been achieved to approximate the quality of PXRD data towards SC-XRD structure analysis. Synchrotron radiation with high brilliance, tunable beam energy and flexibility of optical setup that can be available at beam-lines enables better diffraction data which and significantly improves structure analysis of MOFs. Additionally, rapid improvement of detection capabilities improves data acquisition time of high-resolution patterns down to few minutes.^{20–23} This enables not only quick receiving of the data required for crystal structure determination but also detection of short-living metastable phases, monitoring of structure dynamics and performing kinetic studies of MOF formation. Instrumentation progress naturally goes hand in hand with the data processing evolvement as well. There are few recent breakthrough improvements regarding XRPD data processing of MOF materials. For instance, charge-flipping algorithm employed for PXRD structure solution addresses the issue of Bragg reflection overlap by their spherical averaging.²⁴ The advantage over the conventional Le-Bail procedure is that the input of symmetry and chemical composition is not required. Structure difference envelope density map analysis (DED) enables the study of guest molecule inclusion within the MOF crystalline framework.²⁵ The procedure takes into an account the difference between the observed and calculated envelope densities generated from the series of most intense low index reflections. Another, very powerful method for studying materials with limited or no long-range order, which has been increasingly used for total scattering data processing in last decade, is pair distribution function (PDF) analysis.²⁶ The method evaluates the probability of finding two atoms at defined interatomic distances using free Fourier-transform of PXRD data. Developing technique in the field of PXRD methodology, which also needs to be mentioned here, is microdiffraction. Sophisticated optic setup can provide bright and highly focused incident beam with the area below $1\ \mu\text{m}^2$. Such focused beam in-

creases the signal-to-noise ratio of diffraction patterns of micro-sized polycrystalline samples. The setup enables the evaluation of orientation, strain mapping, crystallite orientation or even crystal imperfections.

Small-angle X-ray scattering (SAXS) techniques are more and more used in the field of MOF science. The basics of SAXS differs from the conventional X-ray diffraction from the fact that collimated X-ray beam interacts with the structure species having much larger dimensions than the wavelength of radiation. Scattering angles which are detected on extended sample-to-detector distances are in a narrow region between $0.1\text{--}10^\circ$. SAXS pattern expanded through the $q_{\min}\text{--}q_{\max}$ region provides information about nanoparticle size, shape and porosity. When the scattering angles (or q regions) are expanded out of the mentioned region, other variations of SAXS are used. The measurements in the scattering region below 0.1° is referred as ultra-small-angle X-ray scattering (USAXS), whereas wide-angle X-ray scattering (WAXS) includes data measured above 10° . The development of *in-situ* measurement approaches using micro-beam setup with the beam size down to $10\ \mu\text{m}$ enables the determination of nucleation kinetics of MOFs on sub-millisecond time with the spatial distribution having high statistical accuracy and wide-scale hierarchical structure (inhomogeneity) at local region.²⁷ Grazing-incidence X-ray small angle scattering (GI-SAXS) or more generally grazing-incidence X-ray diffraction (GIXD) also represents an advanced technique which can be used for the studies of MOF thin film growth and their surface properties.

Neutrons radiation is scattered on crystalline materials in a similar manner as X-rays, but give complementary information to XRD due to the different scattering properties. In contrast to XRD, where X-rays scatter on electrons, neutron scattering (NS) or neutron diffraction (ND) techniques are based on interaction on nucleus. Scattering powers are therefore not dependent on Z-values but are sensitive to each isotope. Hydrogen and deuterium atoms possess comparable or even stronger scattering power as heavier atoms. In addition hydrogen has a negative scattering length, whereas deuterium has a positive one. This makes the two isotopes well distinguishable. Information extracted from ND techniques is very useful for MOFs structure-property relationship investigations. In last two decades, MOF community has been intensively devoted to hydrogen adsorption due to MOF's high potential for hydrogen storage. Evaluation of framework-to-hydrogen interaction during H_2 or D_2 adsorption by different neutron diffraction or scattering techniques was performed on numerous MOF systems.^{28–31} Similarly, adsorption processes of other hydrogenous hosting molecules within MOFs were studied as well. Additionally, neutrons scatters on magnetic moments of nucleus, thus ND (particularly small angle neutron scattering or SANS) methods can be used for probing magnetic structure features of MOFs.

Herein, the recent advances on structural-property investigations of rapidly growing field of metal-organic framework science with the focus on advanced elastic scattering and diffraction techniques are overviewed, including studies of crystallization, structural dynamics and guest-host interactions. The emphasis is on the use of advanced powder diffraction and scattering approaches on the selected cases which most significantly contributed to the better knowledge of specific MOF physical-chemical properties.

2. Crystallization and Crystal Growth Studies

The fundamental knowledge of the MOF crystallization mechanisms is highly important in order to optimize the morphological and crystal growth control, as well as the control over the morphology. The studies of MOF crystallization mechanism are mostly focused on the local structure of species which are present in the solutions prior to the appearance of nanocrystals using different spectroscopic techniques (XAS, NMR). On the other hand, the examination of crystal growth in over-all length scale is important as well to build up the whole picture of crystallization process. Naturally, the crystallization mechanisms and the dynamic of the MOF's crystal growth cannot be generalized but are rather specific for each system. Several systems have been thoroughly investigated by means of diffraction techniques to elucidate the mechanisms and crystal growth dynamic of metal-organic

frameworks structures. The recent investigations of crystallization mechanisms on MOFs based on the diffraction techniques are summarized in the Table 1.

Structural nucleation, crystallization and crystal growth was assessed in details for the Zn-methylimidazolate (ZIF-8) using *ex-situ* powder diffraction, selected area electron diffraction (SAED), *in-situ* small-angle and wide-angle scattering (SAXS/WAXS) and time-resolved static light scattering methods.^{32–35} When using Zn-nitrate as metal precursor, the ZIF crystallizes through the metastable semicrystalline-to-crystalline transformation following the Avrami's kinetic regime in the excess of the ligand.³² In the presence of basic Zn-carbonate instead of nitrate precursor however, the coexistence of ZIF-8 nanocrystals and nanosized ZnO wurtzite phase in the early stages of crystallization was proved.³³ Cravillon et al. studied the nucleation at early growth events (in a second timescale) of ZIF-8 and identified the formation and gradual disappearance of pre-nucleation clusters, suggesting their involvement in the formation and growth of nanocrystals (Figure 1a).³⁵ The above mentioned findings were limited to the crystallization mechanisms in methanol. Low et al. suggested different mechanism in the presence of other solvents (water, dimethylformamide, ethanol, etc.) which includes the phase transformation from two-dimensional ZIF-L phase (Figure 1b).³⁶ These examples demonstrate the diversity of crystallization mechanisms which strongly depend on the starting synthesis parameters. Such in-depth studies are often possible only with the use of complementary diffraction techniques.

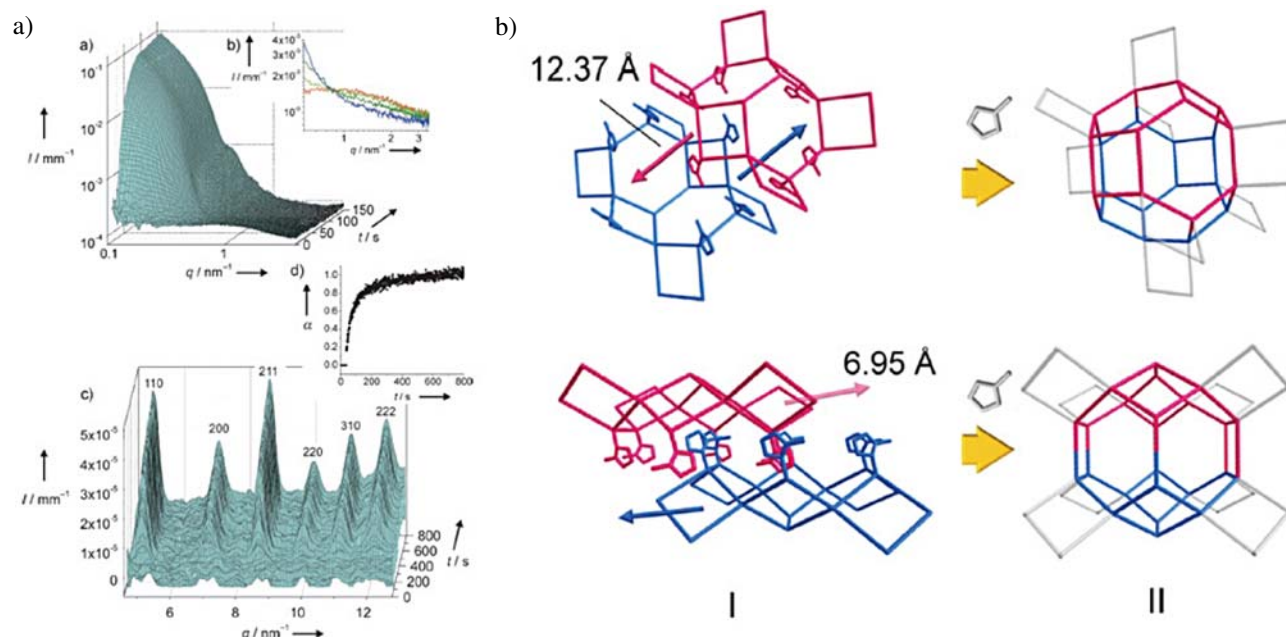


Figure 1. (a) Time-resolved scattering patterns during ZIF-8 nanocrystal formation. Above: SAXS patterns for the first 150 s with the acquisition time interval of 1 s. Inset shows high- q region of selected SAXS patterns originating from the small clusters. Below: WAXS patterns measured between 1 and 800 s with the acquisition time interval of 1 s. Inset shows plot of the extent of crystallization versus time t as produced from the integrated intensity of the 211 reflections in the WAXS patterns.³⁵ (b) Proposed scheme of ZIF-8 formation mechanism from layered phase shown along [100] direction (upper scheme) and [010] direction (lower scheme).³⁶ Reprinted with permission. Copyright American Chemical Society.

Table 1. Recent investigations on MOFs using diffraction and scattering techniques

MOF phase	type of investigation	used technique	key conditions	reference
AEPF-1(Ca)	structure dynamics	TPXRD	solvent removal, 60–120 °C	119
Ag-2-Me-imidazolate	negative thermal expansion	TPXRD		127
Ag ₄ (tpt) ₄	negative thermal expansion	TPXRD		135
Ca(BDC)(DMF)(H ₂ O)	structure dynamics	TPXRD	solvent removal, RT–400 °C	120
Ca,Gd-oxydiacetate	structure determination	PXRD	RT	69
CAU-1(Al)	crystallization	EDXRD	AlCl ₃ /MeOH; 120–145 °C; CH	42,43
			AlCl ₃ /MeOH; 120–145 °C; MW	
CAU-7(Bi)	structure determination	ADT	120 K	73
CPO-27(Mg)	CO ₂ interactions	NPD	20–300 K	94
CPO-27(Ni,Co)	crystallization	EDXRD	THF/H ₂ O; 70–110 °C; CH or	46
	NO interactions	PXRD	MW	95
	H ₂ S interactions	PXRD	RT	96
Cu(bpy) ₂ (OTf) ₂	negative thermal expansion	TPXRD	133–383 K	131
Cu ₄ O(OH) ₂ (Me ₂ trz-pba)	structure dynamics	PXRD	alkanes, alkenes (283–343 K)	115
EMIM[MnBTC]	negative thermal expansion	TPXRD	100–400 K	133
Gd-MOFs	structure determination	HTP-XRD		70
HKUST-1(Cr)	H ₂ interactions	NPD, INS	4 K	84
HKUST-1(Cu)	crystallization	EDXRD	various solvents; 125 °C	47
	structure determination	ADT	77 K	72
	voids	PXRD	RT	81
	H ₂ interactions	NPD, INS	4 K, 50K	85-90
	CH ₄ interactions	NPD	77 K	91
	solvent interactions	SCXRD	RT–200 °C, He flow	92
	solvent structure position	PXRD-DED	synthesized and activated sample	122
HMOF-1(Cd)	negative thermal expansion	PXRD	100–400 K	132
In-imidazole	crystallization	EDXRD	In(OAc) ₃ /DMAA; 120 °C	41
			In(acac) ₃ /Emim-NTf ₂ ; 150 °C	
			In(NO ₃) ₃ /Emim-NTf ₂ ; 160 °C	
In-terephthalate	negative thermal expansion	TPXRD		134
JUC-118(Zn, Co)	structure dynamics	XRPD	different solvent inclusion	121
Li- <i>rho</i> -ZMOF	H ₂ interactions	INS	4 K	99
Li-TPDC	crystallization	EDXRD	LiNO ₃ /DMF; 160 °C	44
MAMS-4(Cu)	solvent structure position	PXRD-DED	synthesized and activated sample	122
MET-1 to -6	structure determination	PXRD-CF	RT	63
MFU-4l	structure determination	ADT	113 K	58
	Xe interactions	XRPD	110 K, 150 K	101
Mg- <i>rho</i> -ZMOF	H ₂ interactions	INS	4 K	99
MIL-47(V)	CO ₂ interactions	XRPD, TPXRD	293–500 K	113
	structure dynamics	QENS	CO ₂ , 230 K	114
	diffusivity	QENS	C ₉ –C ₁₈ alkanes, 300–370K	93
MIL-53(Al)	structure dynamics	XRPD	RT – 500 °C	106
	structure dynamics	NPD	4–77 K, up to 4.5 bar	109
MIL-53(Al)-NH ₂	crystallization	SAXS/WAXS	AlCl ₃ /DMF,H ₂ O; 130 °C	49,50
	structure dynamics	XRPD	various gases (1–30 bar)	107
MIL-53(Cr)	structure dynamics	XRPD	CO ₂ (1–10bar)	106
MIL-53(Fe)	crystallization	EDXRD	FeCl ₃ /DMF,HF; 150 °C	47
	structure dynamics	PXRD	CO ₂ (0–10 bar, RT – 100 °C)	108
	structure dynamics	EDXRD, INS	alcohols	116,117
MIL-53(Sc)	structure dynamics	XRPD	100–623 K, CO ₂ (0–1bar)	103,105
MIL-100(Mn)	crystallization	EDXRD	Mn(NO ₃) ₂ /MeOH	45
MIL-101(Al)-NH ₂	crystallization	SAXS/WAXS	AlCl ₃ /DMF,H ₂ O	49,50
MIL-101(Cr)	Pd inclusion	PXRD, XTS		144
MIL-110(Al)	structure determination	micro-diffraction	RT	68
MMnBTT	specific cation sites	MAD	100 K	136
MOF-5(Zn)	structure determination	SCXRD	RT, guest inclusion	126
	H ₂ interactions	NPD	50 K	98
	CH ₄ interactions	NPD	4 K	100
	negative thermal expansion	NPD, TPXRD, INS	4–600 K	123–126
	Pd inclusion	PXRD		137

MOF phase	type of investigation	used technique	key conditions	reference
MOF-14(Cu)	crystallization	EDXRD	Cu(NO ₃) ₂ /DMF,dioxane, H ₂ O; 110–130 °C	48
	negative thermal expansion	XRPD, NPD	3–400 K	128
MOF-177(Zn)	Pt inclusion	PXRD		138
MOF-205(Zn)	H ₂ interactions	NPD	50 K	98
Ni-poylpyrazolyl MOFs	structure determination	PXRD, TPXRD, EXAFS/XANES	RT	64
NOTT-112(Cu)	H ₂ interactions	NPD	4 K	97
NOTT-202a(In)	structure dynamics	XRPD	CO ₂ , 195K, 0–1 bar	112
NU-125(Cu)	voids	PXRD	RT	80
PCN-11(Cu)	CH ₄ interactions	NPD	4 K	97
PCN-14(Cu)	CH ₄ interactions	NPD	4 K	97
PCN-125(Cu)	voids	PXRD	RT	80
PCN-200(Cu)	CO ₂ structure position	PXRD-DED	CO ₂ loading	122
UiO-66(Hf)	defects	SCDS, AXS		79
UiO-66(Zr)	crystallization	EDXRD	ZrOCl ₂ · 8H ₂ O or ZrCl ₄ /DMF; 70–150 °C	40
	structure determination	ADT		74
	defects	NPD, SCXRD	4K, 100 K	77,78
	solvent structure position	PXRD-DED	synthesized and activated sample	122
UiO-67(Zr)	defects	SCXRD	100 K	78
V-BPDC	structure dynamics	XRPD	CO ₂ , 233 K	111
ZIFs	defects	PXRD, SCDS	RT	76
ZIF-1(Zn)	amorphization	PXRD, XTS	RT – 400 °C	145,146
ZIF-2(Zn)	amorphization	PXRD, XTS	RT – 400 °C	145,146
ZIF-4(Co)	amorphization	NPD, XTS	RT – 400 °C	147
ZIF-4(Zn)	amorphization	NPD, XTS	RT – 400 °C	147
ZIF-7(Zn)	structure determination	RED	90 K	71
ZIF-8(Zn)	crystallization	PXRD, SAED, SAX/WAXS	Zn(NO ₃) ₂ /MeOH; RT	32,34 35
	crystallization	PXRD, SAED	Zn ₃ (CO ₃) ₂ (OH) ₆ /MeOH; RT	33
	crystallization	PXRD, SAED	Zn(NO ₃) ₂ /various solvents; 60 °C	36
	CH ₄ interactions	NPD	4 K	100
	Au inclusion	PXRD		143
	amorphization	XTS, PXRD	RT – 400 °C	148,149
	amorphization	PXRD	0–1.2 GPa	
	amorphization, I ₂ trapping	XTS	ball-milling	150
ZIF-69(Zn)	amorphization, I ₂ trapping	XTS	ball-milling	150
Zn ₂ (BME-bdc) ₂ (dabco)	structure dynamics	XRPD	CO ₂ , 195K	110
Zn-BTP	negative thermal expansion	TPXRD	RT – 200 °C	129
[Zn ₂ (fu-L) ₂ dabco] _n	negative thermal expansion	TPXRD	303–493 K	130
Zn-isonicotinate	negative thermal expansion	TPXRD		134
Zn-pyrazolecarboxylates	vacancies	PXRD	RT	82
Zn-triazolates	structure dynamics	XRPD	H ₂ O, EtOH	118
Zr-fumarate	crystallization	EDXRD	ZrCl ₄ /DMF; 43 °C or ZrCl ₄ /H ₂ O; 120 °C	39

Abbreviations of techniques: ADT – automated diffraction tomography, AXS – anomalous X-ray scattering, EDXRD – energy dispersive X-ray diffraction, INS – inelastic neutron scattering, MAD – multiwavelength anomalous dispersion, NPD – neutron powder diffraction, PXRD – powder X-ray diffraction, PXRD-CF – charge flipping, PXRD-DED – difference envelope density, RED – 3-D rotation electron diffraction, SAED – selective area electron diffraction, SAXS – small-angle X-ray scattering, SCDS – single-crystal diffuse scattering, SCXRD – single-crystal X-ray diffraction, TPXRD – temperature-programmed XRD, WAXS – wide-angle X-ray scattering, XTS – X-ray total scattering; **Abbreviations of chemistry names:** BME-bdc – 2,5-bis(2-methoxyethoxy)-1,4-benzenedicarboxylate, BPDC – biphenyl-4,4'-dicarboxylate, bpy – 4,4'-bipyridine, BTC – benzene-1,3,5-tricarboxylate, BTP – benzenetriphosphonate, BTT – 1,3,5-benzenetristetrazolate, dabco – 1,4-diazabicyclo[2.2.2]octane, DMAA – dimethylacetamide, DMF – N,N'-dimethylformamide, EMIM – 1-ethyl-3-methylimidazolium, Emim-NTf₂ – 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonate)imide, EtOH – ethanol, fu-L – alkoxy functionalized 1,4-benzenedicarboxylate, In(acac)₃ – indium acetylacetonate, In(OAc)₃ – indium acetate, MeOH – methanol, Otf – trifluoromethanesulfonate, TPDC – tiophenedicarboxylate, tpt = 2,4,6-tris(4-pyridyl)-1,3,5-triazine; **Other abbreviations:** CH – conventional heating, MW – microwave heating, RT – room temperature.

The advantage of MOF crystallization is that they can be formed mechanochemically with the absence or with only small amount of solvent. Kinetics and mechanisms of such crystallization obviously significantly differ from the solvothermal processes. However, monitoring of mechanochemical transformations represents a big challenge. A breakthrough in this field was made by the studies of ZIFs crystallization by *in-situ* high-energy X-ray diffraction, where metastable intermediates and interconversions of frameworks were determined.^{37,38} Mechanochemical methods are more and more used even for up-scaled MOF syntheses, due to the energy and environmental efficiency of the process. *In-situ* monitoring of mechanochemical MOFs formation by X-ray diffraction is still relatively unexplored field of diffraction. With more intense development, the technique will certainly help to optimize synthesis conditions in specific environment of many other MOF interesting systems.

One of the most useful tool applied for crystallization investigations is time-resolved *in-situ* energy-dispersive X-ray diffraction (EDXRD), providing the advantage of high intensity white beam X-rays which allow non-destructive penetration through reaction vessels under elevated temperature and autogenous pressures. EDXRD experiments for the purposes of crystallization studies were used for several MOF systems: Zr-fumarate,³⁹ Zr-terephthalate (UiO-66),⁴⁰ In-imidazolate,⁴¹ Al-terephthalates (CAU-1),^{42,43} Li-tiophenedicarboxylate,⁴⁴ MIL-100(Mn)⁴⁵ and CPO-27(Co,Ni).⁴⁶ Additionally, the crystallization investigation using EDXRD is exemplified on the Cu-benzene-1,3,5-tricarboxylate (HKUST-1) under solvothermal conditions.⁴⁷ The structure crystallizes from homogenous DMF/EtOH solutions after no detectable induction period (Figure 2). The monitoring of crystallization at different temperatures

(85–125 °C) enabled the elucidation of typical Avrami kinetic model suggesting that mechanism of crystallization is mostly controlled by the formation of nucleation sites. The trend of increased solvothermal stability with the time of crystallization suggests that HKUST-1 is thermodynamically stable structure. Similar study was performed on Cu-benzenetrisbenzoate (MOF-14) where the kinetics of the crystallization was fitted with Gualtieri model.⁴⁸ In contrast with HKUST-1, the crystallized MOF-14 gradually decomposes to Cu₂O at 130 °C. Even though both systems possess the same local coordination environment and ligand geometry, MOF-14 seem to be less stable indicating that the size of the ligand obviously governs the thermal stability.

Millange et al. used EDXRD technique for the investigation of Fe(III)-terephthalate (MIL-53) crystallization from clear DMF solutions as well.⁴⁷ The formation of MIL-53(Fe) undergoes the formation of intermediate phase (MOF-235). This phase occurs with no induction period and completely transforms to MIL-53 at 150 °C after 30 min and has a longer lifetime at lower temperatures of crystallization (more than 6h at 100 °C). Since both phases do not have any similarities in building unit features, the solid-state rearrangement most likely occurs via dissolving and release of reactive species and final MIL-53 crystallization. With the use of SAXS/WAXS techniques, similar mechanisms of structure rearrangement through MOF-235 phase was found to occur for the crystallization of NH₂-MIL-101(Al) and NH₂-MIL-53(Al) under solvothermal conditions.^{49,50} The stabilization of MOF-253 metastable phase by DMF seems to be essential for the formation of NH₂-MIL-101(Al) which recrystallizes to thermodynamically more stable NH₂-MIL-53(Al) by subsequent dissolution to active species and re-formation. When the reaction takes place in H₂O/DMF mixture,

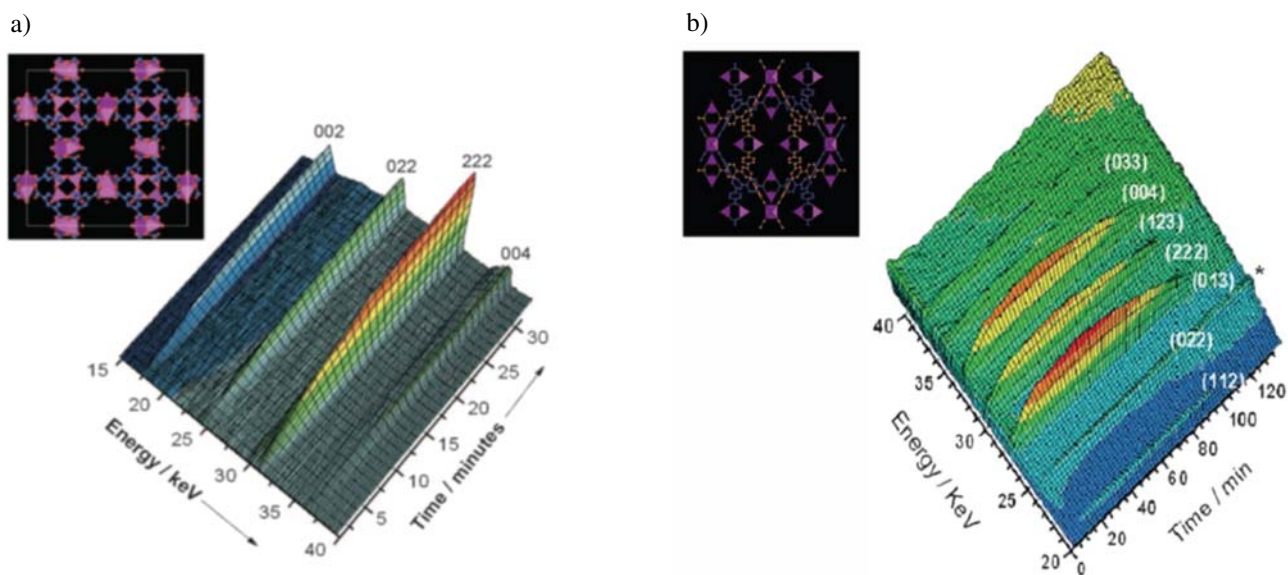


Figure 2. Time-resolved *in-situ* EDXRD data measured during the crystallization of the (a) copper carboxylate HKUST-1 at 125 °C and (b) MOF-14 at 130 °C. Insets: view of the structure of HKUST-1 and MOF-14 with five-coordinate Cu-based units as pink polyhedra.^{47,48} Reprinted with permission. Copyright Royal Society of Chemistry and John Wiley and Sons.

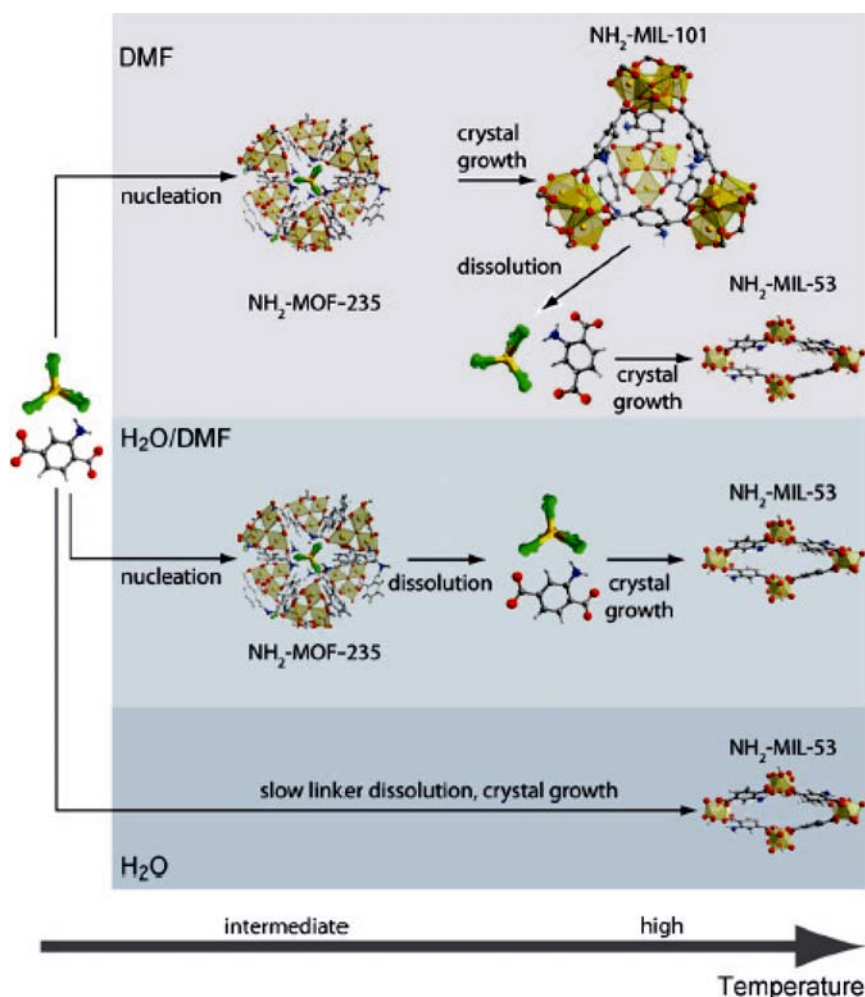


Figure 3. The sequence of events during the crystallization of terephthalate-based MOFs in different media: Low precursor concentrations (DMF); high precursor concentrations ($\text{H}_2\text{O}/\text{DMF}$ or H_2O).⁵⁰ Reprinted with permission. Copyright John Wiley and Sons.

the formation of $\text{NH}_2\text{-MIL-53}(\text{Al})$ undergoes one step re-crystallization from $\text{NH}_2\text{-MOF-235}$ without intermediate MIL-101 phase occurrence (Figure 3).

3. Crystal Structure Determination

The understanding of the details of the MOF crystal structure is a prerequisite for the explanation of its chemical and/or physical behavior and the prediction of their applicable properties. Therefore, the crystal structure determination of the newly synthesized MOF product is the first step before further exploration of its physical or chemical properties. Structure determination from single-crystal data is rather a straightforward process however the dimensions and the quality of the formed MOF crystals are often insufficient for single-crystal X-ray analysis. Therefore, one must rely on the structure determination based on powder X-ray diffraction data, which is much more challenging due to the loss of data caused by peak overlap. Many MOF

structures have been recently determined using the conventional *ab-initio* procedures based on the classical high-resolution synchrotron powder X-ray data with the typical approach of pattern indexing, intensity integration, structure solution and Rietveld refinement.^{51–62} Herein, the more nonconventional strategies and improvement overcoming the challenges of the X-ray powder diffraction structure determination of MOFs will be overviewed.

The improved methodologies of data processing included the use of charge-flipping instead of direct methods to determine the structures 1,2,3-triazolates based on different divalent metal cations.⁶³ With the topological approach, the direct space solution methods providing the information about the rigid components of the structure were successful to solve the structures of nickel(II) polypyrrolyl-based MOFs.⁶⁴ The phenomena of isorectricity of some MOF networks enabled the successful employment of this method to solve the MOF structures with the expanded ligands (IRMOF series).^{65,66} Takashima et al. suggested the possibility of using the conventional X-ray

diffractometer data to explore the structure of isotopical frameworks with modified ligands by the analysis of the obtained electron density maps.⁶⁷

In recent years, the progress regarding the diffraction method instrumentation was made as well. Volkringer et al. used microdiffraction setup with the microfocused beam of 1 μm to determine the crystal structure of the micro-sized Al-benzene-1,3,5-tricarboxylate (MIL-110) with weak scattering factors due to the presence of light elements and very low structure density.⁶⁸ However, successful crystal structure determination based on the data obtained from microdiffraction setups is at present still in large extent limited by the loss of microbeam intensity and the loss of crystallinity under highly focused beams. On the other hand, the crystal structure of bimetallic $[\text{Ca}(\text{H}_2\text{O})_6]_2[\text{CaGd}(\text{oxydiacetate})_3]_2 \cdot 4\text{H}_2\text{O}$ was refined using approach of separate dataset extraction to avoid the loss of crystalline integrity due to the X-ray damaging.⁶⁹ The special data processing for the MOF with heavy atoms and high symmetry produced the model with accuracy comparable with the one obtained from single-crystal-based data. Lau et al. developed a method for high-throughput synchrotron radiation powder X-ray diffraction (HTP-SR-PXRD) data mining (PLUXter) and applied for the generation of library of Gd-based MOFs.⁷⁰

Electron crystallography, combining the electron diffraction and high-resolution TEM imaging offers a promising way to overcome the disadvantages of powder diffraction techniques for the structure solution analysis. However, this approach becomes very challenging for investigations of beam-sensitive materials such as MOFs. Some attempts of solving the MOF structures have been successful by using 3-D rotation electron diffraction (RED) or automated diffraction tomography (ADT). Zn-benzimidazole (ZIF-7) was used as a model structure to proof the feasibility of the RED method on MOFs performed at -90 K to avoid the sample damage.⁷¹ The Zn and N atoms could be positioned using RED data, whereas C atoms were additionally inserted according to geometry of the imidazole ligand. ADT was employed for structure solutions of Cu-benzene-1-3-5-tricarboxylate (HKUST-1),⁷² Zn-BTDD (MFU-4, BTDD = bis(1H-1,2,3-triazolo-[4,5-b],[4,5-i]dibenzo[1,4]dioxin),⁵⁸ Bi-benzenetrisbenzoate (CAU-7)⁷³ and Zr-terephthalate (UiO-66)⁷⁴. These methodologies can only be applied for structure determination of very limited assortment of highly stable MOF systems with the strong support of complementary methods.

4. Structure Defects and Framework Voids

The study of the defects within the crystalline MOF frameworks is important since it can provide important information on crystal growth mechanisms, and their occurrence can significantly influence on MOF's performances

due to the changes in diffusion properties, generation of additional accessible sorption or catalytic sites, establishment of hierarchical architectures, strains, etc. Heterogeneity is often deliberately generated within the MOF structures in order to enhance the sorption or catalytic performances. This can be achieved by using mixed ligands or mixed metal precursors in the starting reaction mixtures or by post-synthesis acid treatment.⁷⁵ The insight on defects within the frameworks by diffraction methods however is limited due to their random occurrence.

Structural disorder within the MOF frameworks has been studied for ZIFs by peak shape analysis of powder diffraction data and single-crystal diffuse scattering.⁷⁶ By high-resolution neutron diffraction it was shown that UiO-66(Zr) framework contains a significant amount of vacancies due to the missing ligands.⁷⁷ The concentration of vacancies can be tuned by using the acetic acid modulator and thus manipulate the porosity properties of the MOF. Study of ligand vacancies were performed on UiO-66(Zr) and UiO-67(Zr) by synchrotron single-crystal X-ray diffraction of as well.⁷⁸ Moreover, with the use of diffuse scattering, electron microscopy, anomalous X-ray diffraction and pair distribution function, Cliffe et al. showed on the case of UiO-66(Hf) that the defect nanoregions within the frameworks do not occur just in random manner, but are correlated between their selves and can even be controlled (Figure 4).⁷⁹ The mesopore voids and defects generated by the metal-ligand-fragment co-assembly approach using ligands with various substituent groups within Cu-based PCN-125, NU-125 and HKUST-1 frameworks was monitored by powder XRD data.^{80,81} The ordered vacancies within Zn-based pyrazole-carboxylates generated by

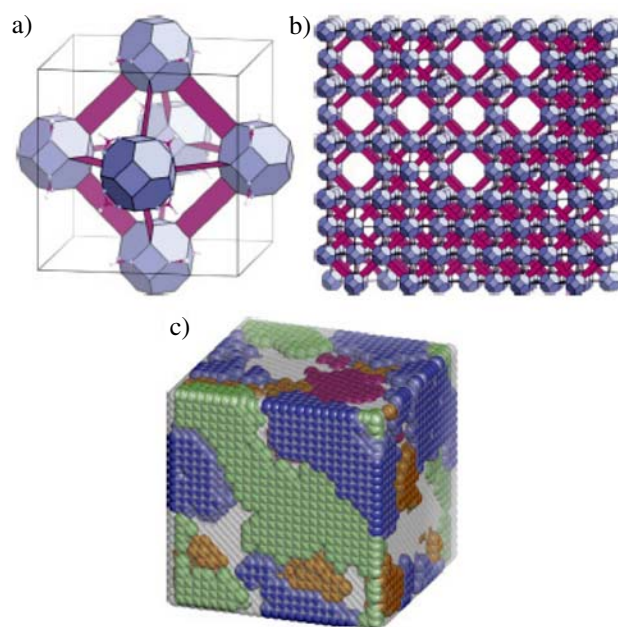


Figure 4: Structural description of defect nanoregions in UiO-66(Hf).⁷⁹ Reprinted with permission. Copyright Nature Publishing Group.

metal and ligand elimination reactions were elucidated by PXRD analysis as well.⁸² As it is indicated by the described examples, the diffraction/scattering methodologies and data processing enable evaluation of inhomogeneity and irregularity within the crystal frameworks only to limited extent, this is, if the imperfection domains still shows some degree of ordering or correlation between them. In opposite cases specific spectroscopic methods are probably more appropriate (e.g. solid-state NMR or XAS).

5. Guest Molecule Interactions

Sorption of molecules (particularly gas molecules) on MOFs is one of the most frequently studied phenome-

na among the MOF community. In terms of suitability for applications, the gas capture efficiency of MOFs is conditioned by their adsorption capacities, adsorption selectivity and the ability of gas storage at mild conditions. Whereas sorption capacity is mainly dependent on the pore properties (dimensions and shape), the selectivity is in large extent governed by host molecule-to-MOF framework interactions. The knowledge about the nature of the sorption sites within the framework provides an understanding of the interactions which are established upon adsorption and it is important to design the materials with optimal adsorption and separation performances.⁸³

HKUST-1 represents a proper platform for crystallographic studies of interactions of guest molecules to framework due to the presence of unsaturated Cu sites. The

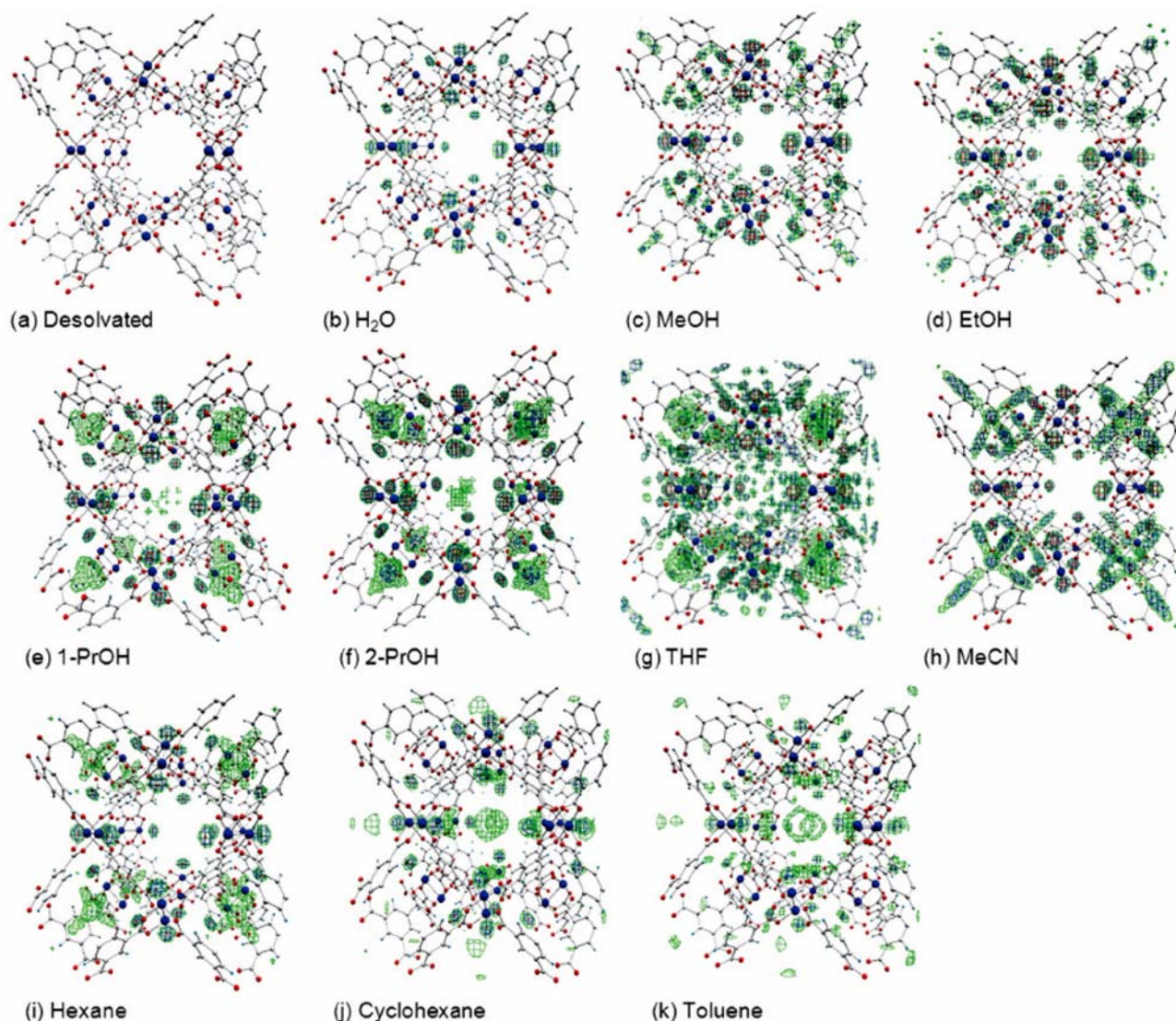


Figure 5: Residual electron density maps for $\text{Cu}_3(\text{BTC})_2(\text{guest})_n \cdot x(\text{guest})$, where guest = none (a), H_2O (b), MeOH (c), EtOH (d), 1-PrOH (e), 2-PrOH (f), THF (g), MeCN (h), hexane (i), cyclohexane (j), and toluene (k). Green = 0.7, blue = 1.1, and red = 2.4 $\text{e}^- \text{\AA}^{-3}$. Coordinated guests have been omitted from the framework model, enabling identification of the guest bound at the Cu site. Shown are Cu (blue), O (red), C (gray), and H (pale blue).⁹¹ Reprinted with permission. Copyright American Chemical Society.

low pressure adsorption of H_2 on Cr-based HKUST-1 material was studied by neutron powder diffraction (NPD) and inelastic neutron scattering (INS).⁸⁴ NPD was performed by deuterium loading of 0.5–3 D_2 per Cr^{2+} site at 4 K. Surprisingly, the binding of D_2 with open Cr^{2+} metal sites preferentially occurs only at higher deuterium loading (from 1.0–1.5 D_2 per Cr^{2+} site), whereas at lower loadings D_2 remains located in the apertures of the octahedral cages. In the case of Cu-based HKUST-1, the progressive filling of nine distinct D_2 sites was found and evaluated by NPD and INS techniques confirming the complexity of the system.^{85–88} The importance of open metal sites for CH_4 adsorption was showed by Getzschmann et al.⁸⁹ CH_4 is adsorbed within HKUST-1 only via two preferential adsorption sites, as it was elucidated by NPD. First type of sites represents open Cu-sites providing strong Coulomb interactions and the second type are defined as ‘pocket sites’ (small cages and the openings to these cages) providing van der Waals interactions with the framework.⁹⁰ The insight on the guest-framework interactions of the variety of solvent molecules incorporated within HKUST-1 was gained using *in-situ* SCXRD (Figure 5).⁹¹ Guests reside in the smallest pores accessible to them. The occupancy of the guest molecules within the pores is governed by competitive guest-guest and hydrogen-bonding interactions.

Hydrophilic guests interact preferentially with the open Cu sites of the framework. The number of coordinated guests is dependent on steric interactions between neighboring bonded guests and guest flexibility. Guest coordination at the Cu sites was found to have a significant effect on the framework structure. Preferred binding sites were investigated for the adsorption of noble gases within HKUST-1 as well.⁹² The diffusion of long-chain alkanes (C_9 – C_{16}) within the MIL-47(V) channels was investigated with the combination of quasi-elastic neutron scattering (QENS) measured at 300–370 K and molecular dynamic simulations (Figure 6). The diffusivities of the hosting molecules are significantly higher in comparison with zeolitic Silicalite-1 system and the diffusivity rates decrease in the non-monotonic manner with the increase of chain length.⁹³

Specific CO_2 adsorption sites were investigated on the rigid MOF-74(Mg) which exhibits one of the highest CO_2 sorption capacities, where the population of a second CO_2 layer was evidenced by NPD.⁹⁴ Preferential binding with open Ni- or Co-sites of biologically active H_2S and NO gases within the Ni-based MOF-74 analogue was determined by XRPD.^{95,96}

Sorption sites for H_2 were investigated by NPD within desolvated Cu-based NOTT-112 materials possessing

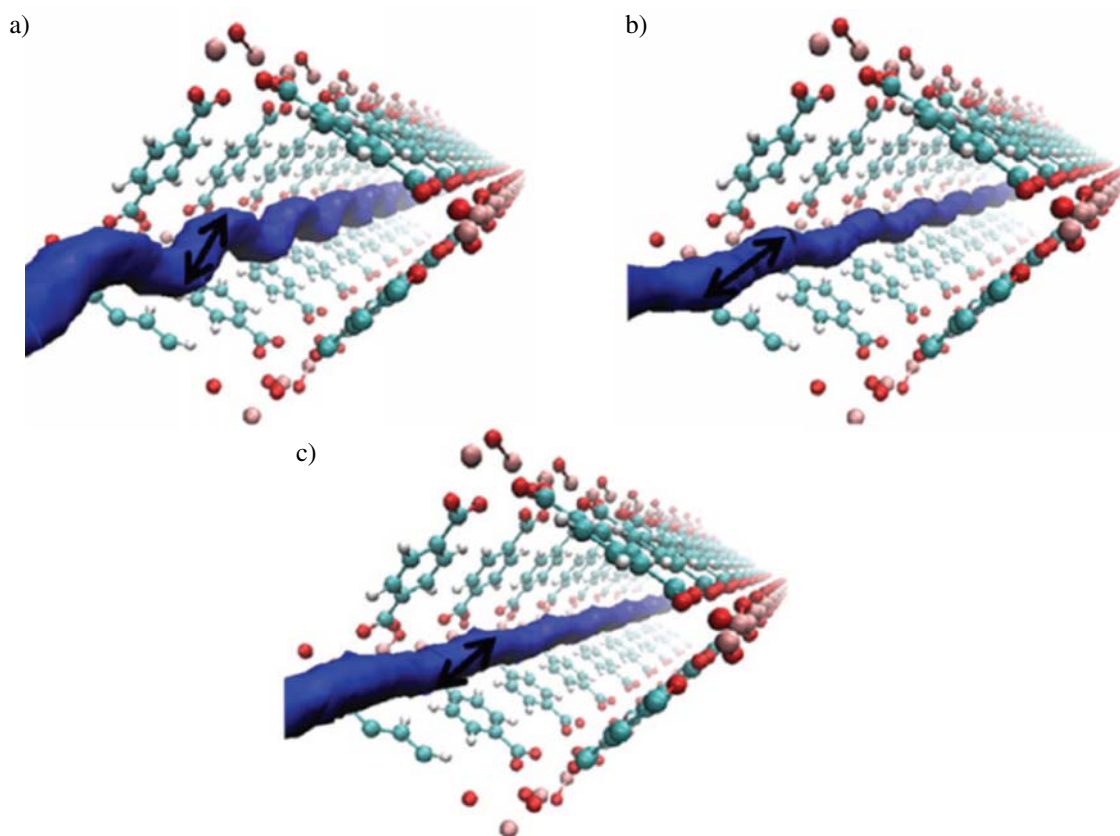


Figure 6: Free-energy isosurface at 2 kJmol⁻¹ deduced from the molecular dynamic calculations within the channels of Mil-47(V) for (a) C_6 -, (b) C_{12} - and (c) C_{18} -chain alkanes.⁹³ Reprinted with permission. Copyright Elsevier.

fcc packed cuboctahedral cages. D₂ establishes a unique preferential binding within the cages which incorporates 12 Cu(II) open metal sites.⁹⁷

Surface adsorption of liquid-like H₂ at 50 K was monitored by NPD. H₂ molecules form a loosely bonded condensed state which is above the critical temperature. Short-range ordering of the H₂ molecule within the pores of MOF-205 was indicated resembling the liquid state in spite of the physical conditions where liquids should not exist.⁹⁸ Inelastic neutron scattering (INS) was used to evaluate the binding sites for H₂ on DMA-rho-, Li-rho- and Mg-rho-ZMOFs.⁹⁹ At low H₂ loadings, all materials show at least 4 specific binding sites for H₂. Slightly stronger metal-to-H₂ interaction was found in the case of Li-rho-ZMOF material in comparison to Mg- analogue due to the more open tetrahedral geometry of Li⁺ versus octahedral environment found around Mg²⁺ and potentially higher electrostatic field in the cavities in the case if Li-rho-ZMOF.

With NPD investigations the CH₄-to-framework primary interactions are associated to the organic ligands and the inorganic oxo-clusters in the cases of ZIF-8(Zn) and MOF-5(Zn) respectively. Methane molecules on these primary sites possess well-defined orientations. With higher methane loading, extra methane molecules populate the secondary sites and are confined in the framework.¹⁰⁰

Preferred adsorption sites for xenon were investigated for MFU-4l material by X-ray powder diffraction measured at 110 and 150 K.¹⁰¹ The reconstruction of the electron density distribution was performed using the maximum entropy method to localize the adsorbed Xe. At 110 K, Xe atoms occupy 8 atoms per large pore, while at 150 K the occupancy descends to 2 atoms per large pore.

6. Structural Dynamic

Dynamics of flexible frameworks are unique feature of MOF structures which can be exploited for various applications such as sensing, separation and adsorption. Structural dynamics can be triggered by external stimuli (inclusion and exchange of guest molecules or by pressure and temperature changes). Adsorption of gases at high pressures in some cases induces structural transition and significantly increases the porosity at certain pressure point (gate opening effect). Some MOFs exhibit extensive flexibility when exposed to a certain type of guest molecules and show reversible structural dynamics upon adsorption/desorption processes (breathing effect). Temperature change is another very common external stimulus that can trigger structural changes. In this case, structural dynamics are usually driven by the removal of solvents or dehydration upon heating. All these processes can be monitored and evaluated by different *in-situ* diffraction studies. Recently, a brief overview of single-crystal X-ray diffraction studies and single-crystal to single-crystal

transformations of porous coordination polymers under various chemical and physical stimuli such as solvent and gas adsorption/desorption/exchange, chemical reaction and temperature change was published by Zhang et al.¹⁰²

It was found that a series of trivalent MIL-53(M³⁺) (M = Fe, Al, Sc, Ga, etc.) analogues possess ability to change their crystal structures markedly in response to other guest-molecule adsorption.^{103–105} The response of flexible MIL-53 frameworks upon guest molecule adsorption was studied by *in-situ* powder XRD on MIL-53(Cr) system where large breathing effect induced by CO₂ employed on MOF at different pressures was observed.¹⁰⁶ Couck et al. studied structural response to the adsorption of several light gases (CH₄, H₂, N₂, C₂H₆, C₃H₈) on NH₂-MIL-53(Al) using *in-situ* XRPD and observed breathing behavior only upon CO₂ sorption process.¹⁰⁷ The breathing behavior was investigated by high-resolution XRD on Fe analogue of MIL-53 as well.¹⁰⁸ CO₂ adsorption on the MIL-53(Fe) as a function of pressure undergoes three steps. Firstly the intermediate phase occurs at room temperature and 2 bars, followed by the transition to the narrow pore formed at 10 bars and finally rearrangement to the large pore form observed at 10 bars as well, but at 220 K. The crystal structures of the corresponding CO₂ loaded materials were successfully determined with the precisely located CO₂ molecules within the pores (Figure 7). MIL-53(Al) with extremely flexible framework was recently studied for D₂ gas adsorption effects by neutron powder diffraction between 4 and 77 K and up to 4.5 bar. Two distinct D₂ sites were found in the fully opened form. The kinetically trapped D₂ was evidenced within the closed MIL-53 channels upon desorption.¹⁰⁹ Recently, the use of *in-situ* powder XRD and quasi-elastic neutron scattering studies revealed the extensive structure response upon CO₂ adsorption on various other MOF systems: [Zn₂(BME-bdc)₂(dabco)]_n (BME-bdc = 2,5-bis(2-methoxyethoxy)-1,4-benzenedicarboxylate, dabco = 1,4-diazabicyclo[2.2.2]octane) where slow adsorption kinetic of CO₂ enabled the identification of the metastable intermediate,¹¹⁰ V-based biphenyl-4,4'-dicarboxylate,¹¹¹ In-based biphenyl-3, 3',5, 5'-tetra-(phenyl-4-carboxylate)¹¹² and MIL-47(V).^{113,114}

The *in-situ* XRD measurements coupled with adsorption equipment was used to perform the experiment of C₄-isomers adsorption (n-butane, isobutane, 1-butene, isobutene) on 3∞[Cu₄(μ₄-O)(μ₂-OH)₂(Me₂trz-pba) (Me₂trz-pba: 4-(3,5-dimethyl-4H-1,2,4-triazol-4-yl)benzoate) at different pressures of and temperatures (283–343 K). The adsorption process is accompanied with phase transition of the material.¹¹⁵

The structural dynamic of MOFs can be induced by inclusion or removal of solvent molecules as well. One of the most known phenomena is an extensive structural response upon water removal and inclusion on MIL-53-type materials. For instance, the removal of water from the Cr and Al materials changes from closed pore form to large

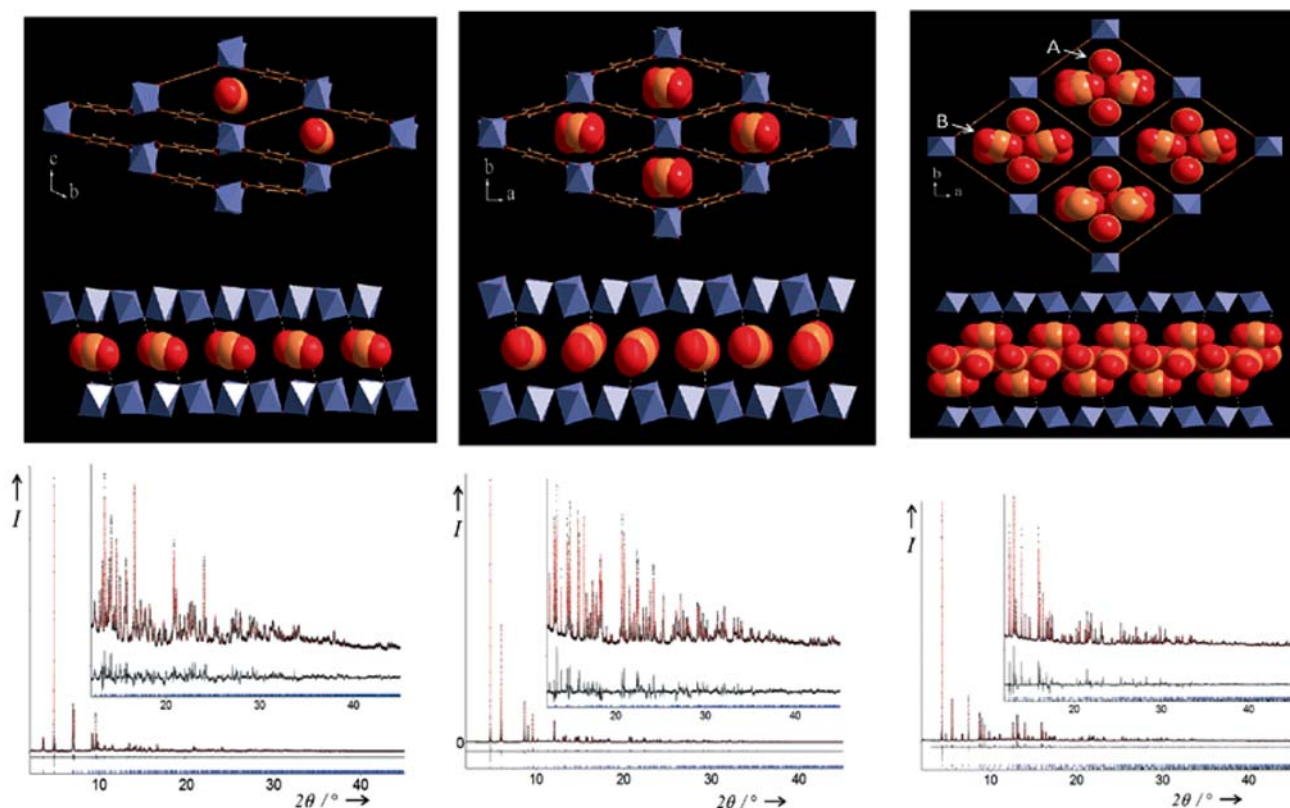


Figure 7. MIL-53(Fe) structure perspective along the 1-D channels (upper images) and perpendicular to the channels (lower images) with determined crystallographic positions of CO₂ molecules within the channels with occupancies of (a) 0.22 CO₂, (b) 0.63 CO₂ and (c) 2.72 CO₂ per formula with corresponding Rietveld plots.¹⁰⁸ Reprinted with permission. Copyright Royal Society of Chemistry.

pore form involving the atomic displacement of >5 Å. However, such breathing behavior was not observed for MIL-53(Fe). Its structure expands in the presence of other solvent molecules. With the use of high-resolution X-ray powder diffraction data, Walton et al. suggested the presence of clusters of methanol located within the MIL-53(Fe) channels with considerable disorientation.¹¹⁶ The further insight on the nature of methanol-to-framework interactions was gained using inelastic neutron diffraction.¹¹⁷ The key reason for the large structure flexibility lies in the motions of terephthalate rings that result in their distortion and rocking motions about the bonds to the carboxylate groups. In the future, similar profound investigations of framework-to-water interactions will be certainly needed the systems with the potential for water sorption applications (heat storage or heat transfer). The structural changes upon guest-adsorption was investigated by *in-situ* powder diffraction on Zn-triazolate system, where high anisotropic structural flexing upon water or ethanol adsorption/desorption could be observed.¹¹⁸ The crystal-to-crystal transformations upon guest removal on Ca-MOFs (AEPF-1) based on the 4,4'-(hexafluoroisopropylidene) bis(benzoic acid) ligand and Ca-terephthalate (Ca(BDC) (DMF)(H₂O) were investigated by XRPD.^{119,120} Structure response upon exposure to different solvents were investigated on pyrene-based JUC-118(Zn,Co).¹²¹ For the struc-

tural evaluation of solvent within the MOF pores the difference envelope density (DED) method employed which is based on the difference of the observed and calculated structure envelope densities.¹²² The case studies of several MOF systems (HKUST-1, UiO-66, MAMS-4, PCN-200) proved that DED can be easily deduced from the routine powder XRD data.

Negative thermal expansion (NTE) is another frequently studied phenomenon which generally occurs in MOFs structures and can be exploited for sensor applications. Exceptionally large linear thermal expansion was monitored over the wide temperature range (4–600 K) by neutron powder diffraction on MOF-5.¹²³ The calculations of first-principles lattice dynamic suggests that rigid-unit modes exhibit certain degree of phonon softening. Multi-temperature XRPD analysis performed over the temperature range between 80 and 500 K confirmed that negative thermal expansion in MOF-5 is caused by local twisting and vibrational movements of carboxylate groups and concerted transverse vibrations of the terephthalate ligand.¹²⁴ Similar experiment was studied under He pressure of 1.7 bar (100–500 K) and 5–150 bar (150–300 K) where the degree of NTE was hampered with the increasing pressure due to the suppressed vibrations of the ligand.¹²⁵ The origin of the NTE phenomenon on MOF-5 was comprehensively studied by inelastic neutron scatter-

ring, variable-temperature X-ray powder diffraction and neutron powder diffraction by Lock et al.¹²⁶ The combination of variable-pressure and variable-temperature single crystal and powder XRD was used to monitor NTE and negative linear compressibility on Ag-2-methylimidazolate.¹²⁷ Extensively large NTE was observed by the combination of the above mentioned techniques for MOF-14 as well.¹²⁸ Negative structure expansion of Zn-1,3,5-benzenetriphosphonate caused by the dehydration is elucidated by *in-situ* XRPD.¹²⁹ The $[\text{Zn}_2(\text{fu-L})_2\text{dabco}]_n$ (fu-L = alkoxy functionalized 1,4-benzenedicarboxylate, dabco = 1,4-diazabicyclo[2.2.2]octane) structure show large anisotropic framework expansion upon heating which can be tuned by mixing differently functionalized linkers to obtain solid solutions of mixed linkers.¹³⁰ Anisotropic thermal expansion was recently studied by XRPD for several other MOF systems: $[\text{Cu}(\text{bpy})_2(\text{OTf})_2]$ (bpy=4,4'-bipyridine, OTf=trifluoromethanesulfonate),¹³¹ HMOF-1 (Cd-meso-tetra(4-pyridyl)porphine),¹³² EMIM[Mn-BTC] (EMIM = 1-ethyl-3-methylimidazolium, BTC = 1,3,5-benzenetricarboxylate),¹³³ indium(III) terephthalate, zinc(II) isonicotinate¹³⁴ and $\text{Ag}_4(\text{tpt})_4\{\delta\text{-}[\text{Mo}_8\text{O}_{26}]\}$ (tpt = 2,4,6-tris(4-pyridyl)-1,3,5-triazine).¹³⁵

7. Catalytic Sites

Metal-organic frameworks have a great potential for heterogenous catalysis processes due to their unique structural features. With the removal of metal-coordinated solvents, the high concentration of unsaturated metal cations, and thus catalytically active sites, can be produced in a controlled manner. Active sites can also be encapsulated within the pores or attached through different post-synthesis modification procedures. In any case, the diffraction techniques offer a valuable tool to gain the information and understanding of functionalities for heterogenous catalysis processes of MOFs.

Multiwavelength anomalous X-ray dispersion (MAD) was used to determine the relative occupation of specific Mn^{2+} metal sites exchanged with different cations (Fe^{2+} , Cu^{2+} , Zn^{2+}) on 1,3,5-benzenetristetrazolate-based MOFs ($\text{M}^{2+}\text{MnBTT}$).¹³⁶ The structure contains two metal sites with C_s and C_{4v} symmetry from which only C_{4v} sites are exchangeable (Figure 8). Refined occupancy difference (ROD) and integrated density difference (IDD) methods were employed to quantify the occupancy of C_{4v} sites. According to MAD analysis, Cu^{2+} and Zn^{2+} are fully exchanged on C_{4v} site whereas Fe^{2+} exchanges in much lower extent (20%).

In order to enhance the catalytic performances of MOFs, various noble-metal nanoparticles were included within the pores of MOF systems. These nanoparticles were usually identified by the conventional X-ray powder diffraction.^{137–143} Recently, the method of high-energy X-ray total scattering (XTS) was employed for the investiga-

tion of low-concentration of nanoparticles within the matrix. The different amounts of Pd nanoparticles were immobilized within the MIL-101(Cr)- NH_2 material for Suzuki-Miyaura catalytic reactions.¹⁴⁴ The distribution of Pd nanoparticles was determined by XTS. The method includes the extracting of pair distribution function (PDF) from Fourier-transformation of the total scattering intensities (including Bragg peaks and diffuse scattering). The obtained PDF describes the statistical distribution of all interatomic distances within the sample.

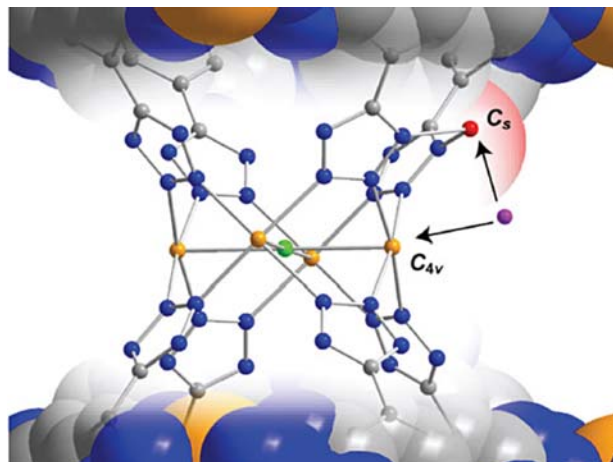


Figure 8. Exchangeable metal sites in M1M2BTT. Orange, red, green, blue, and gray spheres represent the C_{4v} metal site, the partially occupied C_s metal site, Cl, N, and C atoms respectively. The pink sphere represents the substituting cation.¹³⁶ Reprinted with permission. Copyright American Chemical Society.

8. Amorphous MOFs

Amorphous metal organic-frameworks (aMOFs) lack the long-range ordering of building motifs but they still retain their original building blocks which are connected through organic ligands in more or less random manner. The development of aMOFs opened a new chapter in the field of MOF science, offering many exciting application opportunities. Aperiodic structure arrangements result in broad humps in their powder diffraction patterns caused by diffuse scattering.

Various Zn-based aMOFs were prepared from zeolitic imidazolates (ZIF-1, ZIF-2 and ZIF-4) ball-milling and high-temperature decomposition.^{145–146} The analysis of pair distribution function (PDF) calculated from Fourier-transform of X-ray total scattering patterns show that the amorphous structures are indistinguishable from one another. The differences in PDF were found comparing the aMOFs prepared from ZIF-4(Zn) and ZIF-4(Co), due to the different scattering factors of Zn and Co.¹⁴⁷ Pressure induced amorphization of the ZIF-8 was monitored by *in-situ* X-ray powder diffraction.^{148,149} The structure undergoes irreversible transformation to continuous random network

upon compression above 0.34 GPa. The attempt to structurally characterized I₂-trapped aMOFs prepared from ball-milling of ZIF-8 and ZIF-69 was made by collecting X-ray total scattering data using Ag X-ray source ($\lambda = 0.561 \text{ \AA}$).¹⁵⁰ The decrease of the sharp diffraction peak (FSDP) with increased loading of I₂ in both aMOFs suggests retention of internal void structure upon guest inclusion.

9. Conclusion

In recent years progress in scattering and diffraction instrumentation and data processing enabled deeper and more detailed insight into structure determination and properties of MOFs. Better understanding of MOF chemistry consequently contributed to even faster and more intensive development of the structures with the desired properties by rational design. SAXS and SANS methods offered a lot of information about the nucleation kinetics and crystal growth mechanisms of MOFs. Improved high-resolution XRD techniques in combination with new data processing approaches enabled the determination of very complex structures with extremely large unit cell volumes, which previously represented insurmountable obstacles. The investigations of the interaction of guest molecules with crystalline frameworks by neutron diffraction or scattering techniques offered the important insights into preferable sorption sites for specific hosting hydrogenous molecule. Structural dynamics of flexible structures induced by different external physical or chemical stimuli can be more accurately determined by time-resolved diffraction techniques and by employing combinational *in-situ* methods. Topological disorder and structure defects, which found to play important role on different chemical properties of MOFs (i.e. structure stability, catalytic activity) can be recently successfully evaluated by pair distribution (PDF) analysis. Moreover, the effort to improve functionality or to design multifunctional MOF systems induced the investigation on multi-metal MOFs, which were successfully structurally described by MAD and PDF analysis.

With the continuous advance of diffraction and scattering capabilities, the monitoring of MOF's properties will become more and more routine and available to academic and industrial profiles. The future prospects lie on the improvement of instrumentation towards building multi-technique setups, further development of data processing which is prerequisite for successful interpretation of the measured experiments and generating even more powerful and more focused radiation sources. Furthermore, the use of electron diffraction together with electron microscopy imaging (ED, HR-TEM) for the crystal structure-property studies of MOFs is still a scarce area. Successful overcome of beam-damage issues would significantly contribute to easier determination of MOF microstructures.

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Povzetek

Raziskave na kovinsko-organskih poroznih materialih (eng. Metal-organic frameworks, MOFs) so, zaradi njihovih strukturnih raznolikosti in širokega spectra uporabnosti, v zadnjih letih v hitrem vzponu. Stalen napredek rentgenskih in nevtronskih difrakcijskih metod pa omogočajo vse bolj podroben vpogled v strukturne značilnosti MOFov in pomembno prispevajo k razumevanju njihovih kemijskih lastnosti. Izboljšave instrumentacije in procesiranja podatkov visokoločljivostnih difrakcijskih metod omogoča določevanje novih, kompleksnih kristalnih struktur MOFov iz praškovnih analiz. Z uporabo nevtronskih difrakcijskih tehnik je bilo v zadnjem času pridobljenega veliko znanja o interakcijah molekul s kristaliničnimi ogrodji. *In-situ* študije z različnimi tehnikami difrakcije in sipanja omogočajo pridobivanje podrobnih informacij o kinetiki kristalizacije, mehanizmih kristalne rasti in strukturne dinamike pod različnimi fizikalnimi ali kemijskimi vplivi. Pregledni članek povzema novejšo najpomembnejšo napredno strukturno študijo MOFov na osnovi praškovne rentgenske in nevtronske difrakcije.

Scientific paper

Basic Electrochemical Performance of Pure LiMnPO_4 : a Comparison with Selected Conventional Insertion Materials

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This paper is dedicated to Prof. Janko Jamnik, our unforgettable coworker, leader and friend.

Abstract

We compare the basic electrochemical performance of a LiMnPO_4 battery material with the performance of its much more researched olivine counterpart – LiFePO_4 . To get a wider picture, we also included another well understood material, LiCoO_2 . Based on chronopotentiometric (galvanostatic) experiments, we discuss the materials performance in terms of cell energy efficiency and electrode polarization. We propose and justify the use of the “inflection point criterion” for determination of total overpotential (η_{total}). We further demonstrate that the general current-overpotential characteristics can be represented by introducing the total resistance of the cell – R_{total} . We find consistently that whereas in LCO the general current-overpotential characteristics is more or less linear, there is significant deviation from linearity in LiFePO_4 and even bigger in LiMnPO_4 . The phenomenon is discussed in terms of state-of-the art knowledge about phase transformation phenomena in these materials.

Keywords: Insertion battery materials, LiMnPO_4 , non-linearity, activation, efficiency, kinetics

1. Introduction

Soon after their invention, significant differences in the electrochemical performance of different members of the phospho-olivine polyanionic family LiMPO_4 ($M = \text{Fe, Mn, Co, Ni}$) were observed. For example, it was assumed that the iron analogue, LiFePO_4 (LFP), was exhibiting good enough electrochemical performance to be considered as potentially interesting material for low-power battery applications.¹ By contrast, from the manganese analogue, LiMnPO_4 (LMP), basically no lithium could be extracted, neither electrochemically nor chemically.¹ Similar dichotomy persisted for many years, with LMP being able to deliver only a fraction of the reversible theoretical capacity – at low C-rates and with a big potential difference between the charge and discharge curves.^{2–4} After demonstration of much improved performance when used in mixtures with LFP,^{5,6} the interest in this material has grown considerably. In parallel to that, improvements of the performance of pure LMP have also been occasionally

reported. For instance, in 2002 Sony published a reversible capacity of 140 mAh/g for LMP at room temperature (CC-CV cycling protocol with potential window 2.0–4.5 V),⁷ but this achievement could then not be reproduced for many years.

An obvious approach to improving the rate performance of insertion materials seems to be decreasing the particle size.⁸ Drezen et al.⁹ were the first to demonstrate the beneficial effect of particle size minimization on the electrochemistry of LMP. In particular, they used a polyol synthesis approach to prepare nanoparticles of LMP material with a platelet morphology. Particles having a thickness of about 30 nm were subsequently carbon coated in a ballmilling step. A version of this procedure yielded spherical LMP particles with a quite uniform particle size of 25–30 nm coated with relatively thick (~15 nm) carbon layer delivering 145 mAhg⁻¹ at C/20 and giving a stable reversible capacity of 140 mAhg⁻¹ for C/10 C-rate (both at 30 °C using a CC-CV cycling protocol).^{10,11} Oh et al.¹² reported a synthesis of carbon– LiMnPO_4 nanocomposite by

ultrasonic spray pyrolysis followed by ballmilling with carbon. In the case of a $\text{LiMnPO}_4\text{-C}$ material having a native carbon content of ~27 wt.% and 7.5 wt.% of additional carbon black they obtained a reversible capacity of 158 mAhg^{-1} at C/20 (at 25°C using a CC-CV cycling protocol). Choi et al.¹³ synthesized LiMnPO_4 nanoplates *via* a solid-state reaction in molten hydrocarbon. After ball milling with 20 wt.% of conductive carbon, the LMP-based cathode material demonstrated high and stable specific capacity – exceeding 150 mAh/g in initial C/25 cycles and retained ~ 145 mAh/g after pro-longed cycling (CC protocol in voltage range 2.0–4.5 V). Later on, Kang et al.¹⁴ reported that small amounts of Fe and Mg dopants significantly improved the electrochemical power performance of LiMnPO_4 . Rangappa et al.¹⁵ prepared monodisperse nanoparticles of LiMnPO_4 by performing synthesis under supercritical fluid conditions and reported the electrochemical performance of subsequently carbon coated 20 nm sized LMP to be 156 mAh/g (at C/100 and the lower cut off voltage 2 V).

More recently,¹⁶ we introduced a two-step synthesis method which yielded LMP with a primary particle size around ~20–50 nm. In order to keep individual particles sufficiently separated but still in electronic contact we embedded them into pyrolytic carbon, an approach known from many other studies on various insertion materials.^{17–27} Our material delivers an initial reversible capacity of ~ 160 mAhg^{-1} at a C-rate of C/20 at 55°C using a CC cycling protocol and ~ 155 mAhg^{-1} for C/20 C-rate at 25°C using a CC-CV cycling protocol.

Despite the great advances described above, the practical performance of LMP still lags significantly behind that of LFP. In order to understand the critical differences between the two materials, we have performed several systematic sets of experiments on both materials and also on some other well understood insertion materials, such as LiCoO_2 . The most important similarities and differences are shown and discussed in some detail. Quite surprisingly we find that, in fact, the properties of LMP are not essentially different from those of LFP, only the relaxation of charge within the lattice of Li_xMnPO_4 is significantly slower. This, however, implies that if very small particles of LMP (according to our estimation on the order of 10 nm) could be efficiently wired both ionically and electronically, there should be no obstacles for this material to reach its theoretical limitation of capacity, 171 mAh/g , and a high C-rate capability.

2. Experimental

2.1. Active Materials

LiCoO_2 -based cells were prepared using a commercial LiCoO_2 (“cathode powder SC 20”, Merck) with an average particle size of 2–3 μm . $\text{LiFePO}_4\text{-C}$ active material was synthesized according to a citrate precursor

method described in detail elsewhere.²⁸ Briefly, Fe(III) citrate (Aldrich) was dissolved in water at 60°C . Separately, an equimolar aqueous solution of LiH_2PO_4 was prepared from H_3PO_4 (Merck) and Li_3PO_4 (Aldrich). The solutions were mixed together and after 1 h of stirring a rotary evaporator was used for the removal of water (at 60°C under reduced pressure). After thorough drying and subsequent grinding with a mortar and pestle, the obtained greenish xerogel was fired in argon atmosphere for 10 h at 700°C . The heating rate was 10°C/min . This method gives porous LiFePO_4 particles of typical sizes between 5 and 20 μm . All particle surfaces (outer and inner) were essentially covered with a 1–2 nm thick carbon film. The total content of native amorphous carbon was ~3 wt%. The $\text{LiMnPO}_4\text{-C}$ (LMP) active material was synthesized according to the two-step synthesis described in detail elsewhere.¹⁶ In the first step, a homogeneous mixture of reactants without lithium was prepared in a round bottom flask by stirring stoichiometric quantities of manganese acetate (Fluka), citric acid (Sigma-Aldrich), and phosphoric acid (Merck) (the molar ratio of Mn:P:citric acid was 1:1.1:1.5). The pre-dissolved Mn and P precursors and solution of citric acid (each prepared as separate water solution) were mixed together at RT in a flask. The latter was then transferred to a vacuum rotary evaporator with a bath temperature of 60°C . In the first step of drying the pressure was carefully decreased to 60 mbar whereby most of the water was removed forming a viscous sol that was subjected to a sudden pressure decrease to 10 mbar whereby the sol simultaneously expanded to form a voluminous foamy-like sol that was finally dried at 5 mbar for 2 h. The dried sol was thermally treated at 700°C for 1 h in an argon atmosphere. In the second step, the composite from the first step was mixed with a 20% excess of LiOH (Aldrich, the molar ratio Li:Mn = 1.2:1), using planetary ball milling (Retch) for 30 min at 300 rpm. The final $\text{LiMnPO}_4\text{-C}$ material was obtained with additional thermal treatment at 700°C in argon for 12 h.

2.2. Preparation of Electrode Composites and Electrodes

Cathode composites were prepared from the basic active materials (LiCoO_2 , $\text{LiFePO}_4\text{-C}$, $\text{LiMnPO}_4\text{-C}$) to which carbon black (CB, “Printex”) and binder (PTFE) were added to get a final weight ratio of 8:1:1. A mixture consisting from active material, CB and 60% PTFE (Aldrich) in 2-propanol was prepared. The mixture was homogenized by thoroughly mixing in a ball mill (30 min at 300 rpm). After evaporation of the 2-propanol a ductile kneadable composite mass was obtained. The electrodes were prepared by spreading the cathode composite mass onto an aluminum foil current collector that had been roughened using a sandpaper to improve clinging. Circular electrodes with a diameter of 14 mm (1.54

cm²) were cut out. The electrodes were pressed with a force of 5 tons for 1 min in a hydraulic press. The typical loading of active material in electrodes was 3–3.5 mg/cm². Finally, the electrodes were dried for 12 h at 90 °C and stored in an Ar filled glovebox for at least 24 h before use.

2. 3. Cell Preparation and Electrochemical Measurements

The electrochemical characteristics were measured in vacuum-sealed cells (“pouch cells”). Two electrode cells were assembled: the tested working electrode (WE) and a metallic Li counter electrode (CE) (~3 cm²) were placed oppositely over a separator (“Whatman” glass microfibre). The electrolyte used was 1 M LiPF₆ in EC:DEC (1:1 by volume), all received from Aldrich. The galvanostatic measurements were performed using a “VMP3” (Bio-Logic) potentiostat/galvanostat running with EC-Lab[®] software. All the comparative measurements of LiCoO₂, LiFePO₄-C, LiMnPO₄-C materials were conducted at 25 °C.

3. Results and Discussion

The structure and morphology of LMP prepared *via* the two-step synthesis developed in our laboratory were reported previously.¹⁶ Also, degradation processes appearing during various stages of cycling were recently thoroughly examined using a range of techniques.²⁹ Briefly, all diffraction peaks correspond to the olivine type structure with a *Pnma* space group of the orthorhombic crystal system. The Rietveld refinement showed, however, that our LMP had a slightly smaller cell volume – the difference being in the range ~0.1–0.6% compared to the previously reported data if compared to LiMnPO₄ samples obtained in earlier studies,^{10,12,13,30–32} or to our reference sample with bigger crystallites. This deviation still needs to be explained. The estimated particle size from peak broadening is (38 ± 2) nm which matches well with the observation using SEM and TEM.¹⁶ LiMnPO₄ particles are very well dispersed in carbon matrix (14 wt.% of C in LiMnPO₄-C) formed during the first step of the synthesis. The small, well separated but electronically wired particles resulted in very good electrochemistry: a capacity up to 161.5 mAh/g or 94% of the theoretical value (171 mAh/g).

The LFP active material synthesized according to a citrate precursor method²⁸ appears as porous LiFePO₄-C secondary particles of typical sizes between 5 and 20 μm, whereby all particle’s surfaces (outer and inner) are essentially covered with an average 1–2 nm thick carbon film.⁴ The structure and morphology of LFP prepared by this synthesis developed in our laboratory were reported previously.^{4,28} Briefly, all diffraction peaks correspond to the

olivine type structure with a *Pnma* space group of the orthorhombic crystal system. The Rietveld refinement shows, similar to the LMP material, that our LFP with unit cell volume of 290.58 Å³ has a slightly smaller cell volume – on the order of ~0.3% – compared to the experimentally obtained³³ and generally accepted value of 291.4 Å³.³⁴ This deviation could be related to nano-sizing. Unfortunately, a more detailed analysis is rather difficult as the exact primary particle size is difficult to determine for such a porous type of particles.

Although high specific capacities using LMP electrodes have been obtained, several other important electrochemical features have remained poorly understood. Examples of such features are: low power density (rapid decay of capacity above 1C), unusually large voltage hysteresis (~200 mV) between charge and discharge curve already at relatively low rates (C/20) and asymmetry of charge-discharge curve. These issues are systematically addressed in the present paper.

3. 1. Galvanostatic Measurements: Voltage Hysteresis and Energy Efficiency (ε)

Fig. 1a shows measured galvanostatic charge-discharge curves for LMP at different C-rates in the range from C/20 up to 16C at 25 °C. The potential window was 2.7–4.5 V vs. Li using the conventional constant-current “CC” cycling protocol. A comparison to the well-known LFP (Fig. 1b) and LCO (Fig. 1c) electrodes tested under similar conditions shows one particular difference: in LFP and LCO the voltage hysteresis between charge and discharge at low rates is rather small (34 mV and 14 mV for LFP and LCO, respectively, at C/10) but then significantly increases when progressing to higher rates (for example approaching to 400 mV at 5C). By contrast, in LMP the voltage hysteresis is quite large (~200 mV) already at smallest cycling rate (C/20) but then surprisingly remains within tolerable range as the rate increases (eg. ca 550 mV at 4C). Note that all the cells had the same geometric surface area of the electrode (1.5 cm²), the same electrode composition (80 wt.% active material, 10 wt.% carbon black (“Printex”) and 10 wt.% of PTFE binder) and were prepared using the same procedure (1 min of pressing with force of 5 tons in a hydraulic press). The active mass loadings of the electrodes were comparable: 4.3 mg in the case of LiMnPO₄, 5.2 mg in the case of LiFePO₄, and 4.1 mg in the case of LiCoO₂ based electrode.

The unexpectedly good behaviour of LMP at higher rates is even more clearly seen from Figs. 2 (a)–2 (c) which show a comparison of selected galvanostatic cycles at similar current densities that are presented in terms of the mass-normalized current *I_m* (in A per g of active material). The voltage hysteresis of LMP at low rates is distinctly larger than in the case of LFP and LCO. However, with increasing rate, especially above ca. 1C (Fig.

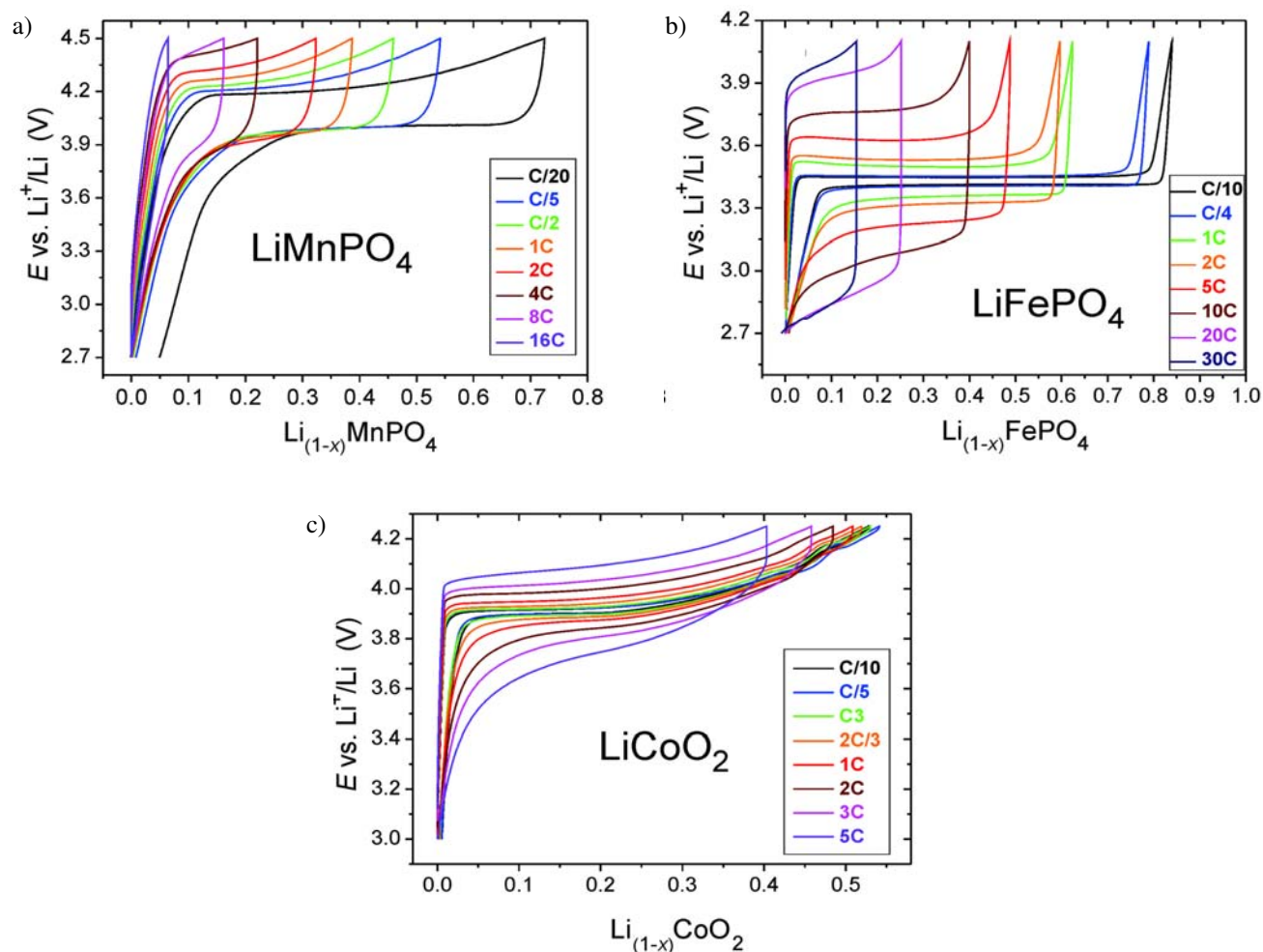


Figure 1. Comparison of a series of galvanostatic cycles measured on different cells at different current densities: a) LiMnPO₄ (from C/20 up to 16C in the potential window 2.7–4.5 V vs. Li), b) LiFePO₄ (from C/10 up to 30C in the potential window 2.7–4.1 V vs. Li) and c) LiCoO₂ (from C/10 up to 5C in the potential window 3.0–4.25 V vs. Li). In all the cases the third cycle measured at certain C-rate is plotted; all the curves were measured at 25 °C and obtained using the conventional constant-current “CC” cycling protocol.

2c), the hysteresis of LMP becomes comparable to the hysteresis for LFP and LCO – which is rather unexpected. Without trying to find a deeper mechanistic reason, we here mainly comment this result in terms of energy efficiency of the various cells, ε . The latter can be defined as follows:

$$\varepsilon = \frac{-\Delta W(\text{discharge})}{\Delta W(\text{charge})} \quad (1),$$

where $\Delta W(\text{charge})$ and $\Delta W(\text{discharge})$ are the total energy changes in the cell during galvanostatic charge and discharge. The total energy that is transferred to/from an electrochemical cell is assumed to be equal to the change of the electrical energy of the cell, ΔW , and is obtained simply by integration:

$$W_{e,2} - W_{e,1} = \Delta W_e = I \int_{t1}^{t2} V(t) dt \quad (2),$$

where indexes 1 and 2 correspond to the start and end of charge (or discharge) process, I is the constant current and $V(t)$ the measured voltage of the cell during charge (or discharge) as a function of time, t . The obtained energy efficiencies (ε) of the cells for sets of measured galvanostatic curves shown on Fig. 1 are shown on Fig. 2d where there are plotted versus normalized current, I_m .

It is generally expected for battery systems that the energy efficiency be reduced when increasing the current density. The results of Fig. 2d are in line with this hypothesis, except in the case of LMP at the lowest tested rate (C/20) where unexpectedly low efficiency (85%) was obtained. This deviation is due to the large coulombic irreversibility of the C/20 galvanostatic cycle (see Fig. 1a), that was measured as the third C/20 cycle in a sequence starting with the “pristine electrode” with the LiMnPO₄-C material in the pristine state. As shown in our recent paper,²⁹ LMP electrodes exhibit a strong irreversibility due to different types of parasitic side reactions that are taking

place during charge at high potentials (especially in the initial cycles).^{11,35} In any case, we may conclude that at low current densities the energy efficiency, ϵ , of LMP is substantially smaller than in the other two active materials, LFP and LCO.

At C/5 rate the LMP based cell has the energy efficiency of 90%, still being much smaller than that of the LCO (98% at C/5) and LFP (97% at C/10) based cells. With increasing current the efficiency of LCO gradually becomes distinctly higher compared to the efficiency of the other two olivine materials. However, there are also pronounced differences in the behaviour of LMP and LFP: quite surprisingly, the rate of efficiency decrease is smaller in the case of LMP, so increasing the current density sufficiently (e.g. above ~ 1.5 A/g), ϵ of LMP becomes even higher than that of LFP.

Based on Fig. 2d one could come to a conclusion that in some aspects LiMnPO_4 exhibits a better electrochemistry performance than LiFePO_4 . This however would

be in contradiction with the known data for conductivity of the two materials at RT,^{3,36} as well as with the experimental data for the lithium diffusion coefficient in these two materials. Specifically, for LFP and LMP the experimentally determined diffusion coefficients for Li range from $\sim 10^{-13}$ to $\sim 10^{-16}$ cm^2s^{-1} and 10^{-16} to 10^{-17} cm^2s^{-1} , respectively.^{22,37–40}

A deeper analysis shows that plots such as that in Fig. 2d need to be interpreted with additional care taking into account various limitations of such an approach. For example, one finds that, at high rates, the energy efficiency obtained using Eq. (1) is strongly affected by the fact that at these conditions the active material cannot be fully charged and discharged. In our specific case this means that the measured average voltage of the cell during charge is artificially reduced and the average voltage during discharge is artificially increased. Consequently, at high C-rates the calculated energy efficiencies using Eq. (1) are overestimated. Conversely, for the low and me-

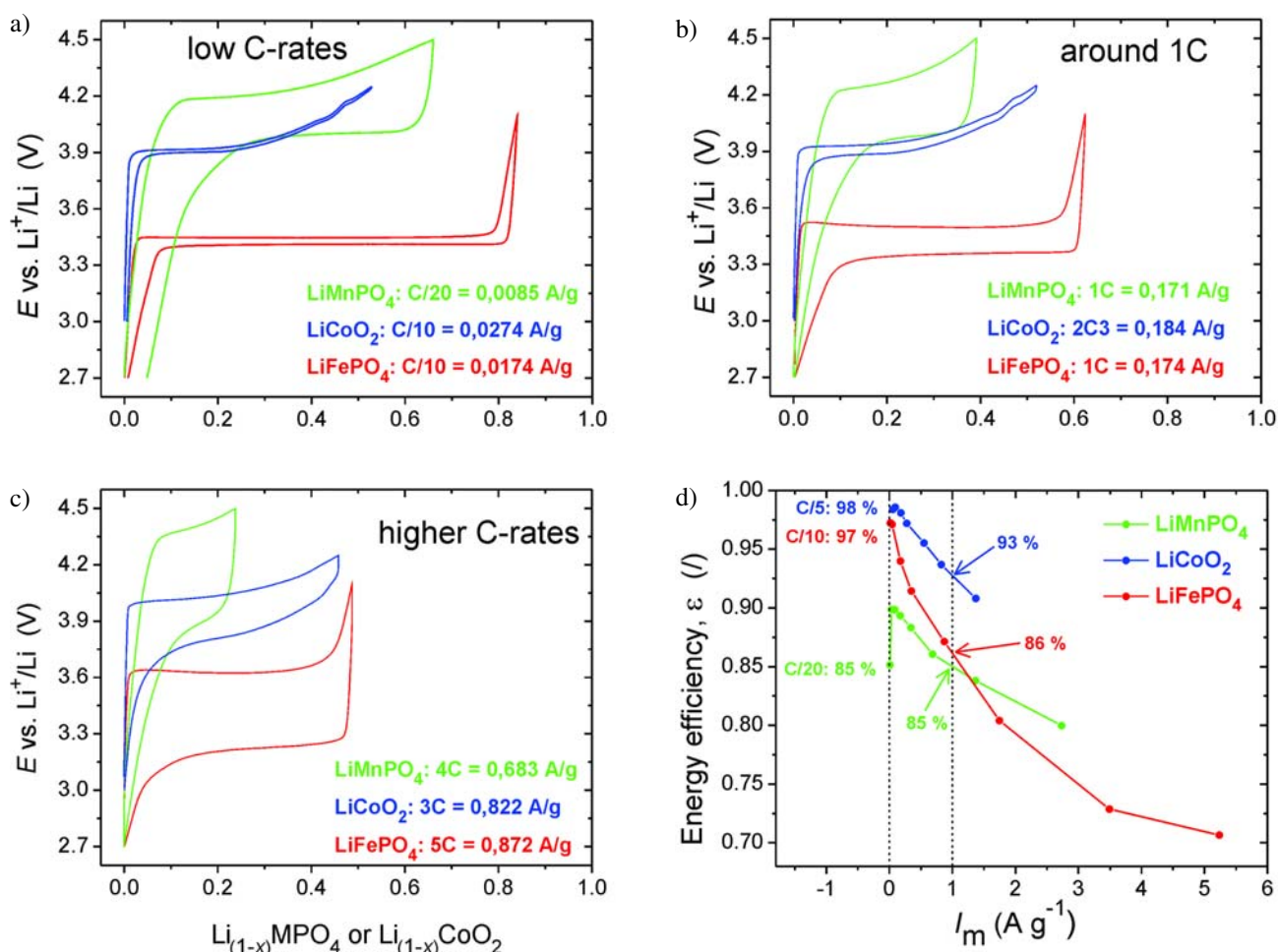


Figure 2. Direct comparison of the shape of selected galvanostatic cycles measured on LiFePO_4 (red line), LiCoO_2 (blue line) and LiMnPO_4 (green line) based electrodes at: a) low, b) medium, and c) higher current densities. The current densities shown are presented in terms of the normalized current, I_m . In all the cases the third cycle at the same C-rate is plotted. d) Energy efficiencies (ϵ) of the cells calculated using Equation (1) and plotted versus normalized current, I_m .

dium C-rates where still a substantial part of total capacity (e.g. > 1/3 of theoretical capacity) is exploited the obtained values of ε are meaningful. Thus in the case of the LMP at 4C, 8C and 16 C (Fig. 1a) and in the case of the LFP at 20C and 30C (Fig. 1b) the obtained values of ε are larger than the real ones (those with the true physical meaning). More realistic values of ε for high C-rates could be obtained by using a wider potential window which, however, would only be possible with a much improved electrolyte that would exhibit excellent ion transport properties together with possessing much wider potential stability window compared to the presently used carbonate-based electrolytes.

3. 2. Current-Overpotential (I- η) Characteristics

In an attempt to gain a more realistic insight into inherent electrochemical performance at different rates we have made additional analyses of the hysteretic behavior of the three materials discussed above. As the hysteresis between the charge and discharge curve varies significantly with the state of charge/discharge (SOC/SOD) (see Figs. 2a–c), an obvious question arises: at which points in the charge and discharge curve should we read out the value of overpotential, η ? One possibility would be to decide for a fixed value of SOC/SOD (e.g. reading the potential at fixed Δx in $\text{Li}_{(1-x)}\text{MnPO}_4$). We have found that choosing, for example $\Delta x = 0.1$, could be quite a good criterion in the case of LFP which exhibits very pronounced potential plateau. However, this criterion is less appropriate for the case of discharge of LCO and for the charge of LMP at lower rates (e.g. C/20) where we do not reach the plateau region yet.

Fig. 3 shows a typical pair of galvanostatic curves of LFP measured at the same current (10C charge/10D discharge) and plotted as a function of time. We can easily observe 3 common regions: I) an initial steep increase/decrease in potential followed by a smooth transition into II) a plateau region with a distinctly “flat” voltage profile that expands into transition to III) a steeper “blocking-like” ending of the galvanostatic curve with progressively increasing/decreasing slope. In LFP all the 3 regions are well expressed both for charge and discharge (see Fig. 1b); in the cases of LMP and LCO region III is not observed in the charge curves due to the fact that either the upper cut-off voltage simply chops away that portion of the curve (as in the case of LMP, see Fig. 1a) or the 2-phase plateau region is further followed by an additional charge-storage mechanism(s) which is/are reflected as additional features complicating the potential profile (as the subsequent transition to a single phase storage and followed by a phase transformation from hexagonal to monoclinic symmetry in the case of LCO,⁴¹ see Fig. 1c).

We can further notice, however, that both curves shown in Fig. 3 have an inflection point (marked with red

dot) that is positioned inside region II. In a galvanostatic curve an inflection point has a physical meaning. Indirectly this is often recognized when authors choose to plot the first derivative of a galvanostatic curve as a function of potential. Generally, the first derivative, dE/dt , at a certain time t from the beginning of the charge/discharge corresponds to the reciprocal of the differential chemical capacitance, $1/C_{chem}$, at time t . If so, one finds that in the case of galvanostatic curve the inflection point corresponds to the global maximum of differential chemical capacitance, C_{chem} . At this particular time, t_{infl} , the potential of cell, E , varies the least with time (or SOC, x). In this sense, this can be seen as a unique point in a discharge/charge curve so we decided to use it as a reference point for evaluation of voltage hysteresis of any material under consideration.

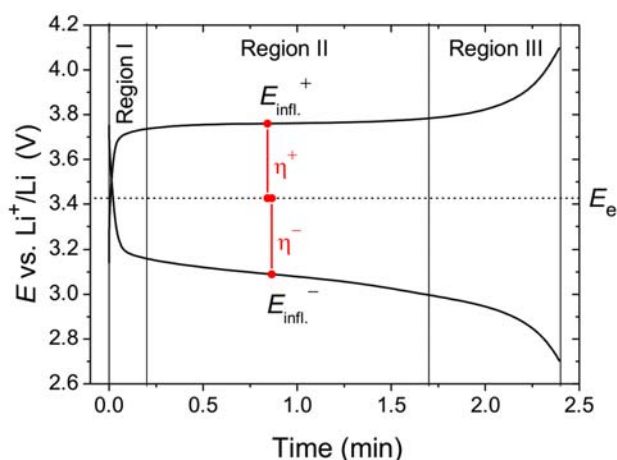


Figure 3. The inflection point criterion for determination of the value of the total overpotential (η_{total}) demonstrated on a typical pair of galvanostatic curves for LFP plotted as a function of time. The charge and discharge rates were the same (10C/10D). The charge/discharge total overpotentials (η_{total}^+ and η_{total}^-) were obtained using Eq. 3. Indicated are the three common regions of a LFP galvanostatic profile.

We further define the total overpotential of the cell, η_{total} , as the difference between the measured voltage of the cell at the inflection point, E_{infl} , and the global equilibrium potential, E_e :

$$\eta_{total} = E_{infl} - E_e \quad (3),$$

where for E_e we took the mean value of potential in a plateau region of a galvanostatic cycle measured at very low current densities. Specifically, for LFP and LMP we took the values of 3.427 V and 4.105 V, respectively, obtained from corresponding $\pm C/1000$ measurements (see also below).⁴² For LCO the selected value was 3.909 V obtained from $\pm C/200$ galvanostatic measurements. In all three cases the measurements were conducted at 25 °C. In Fig. 3 the total overpotentials for the charge and discharge are

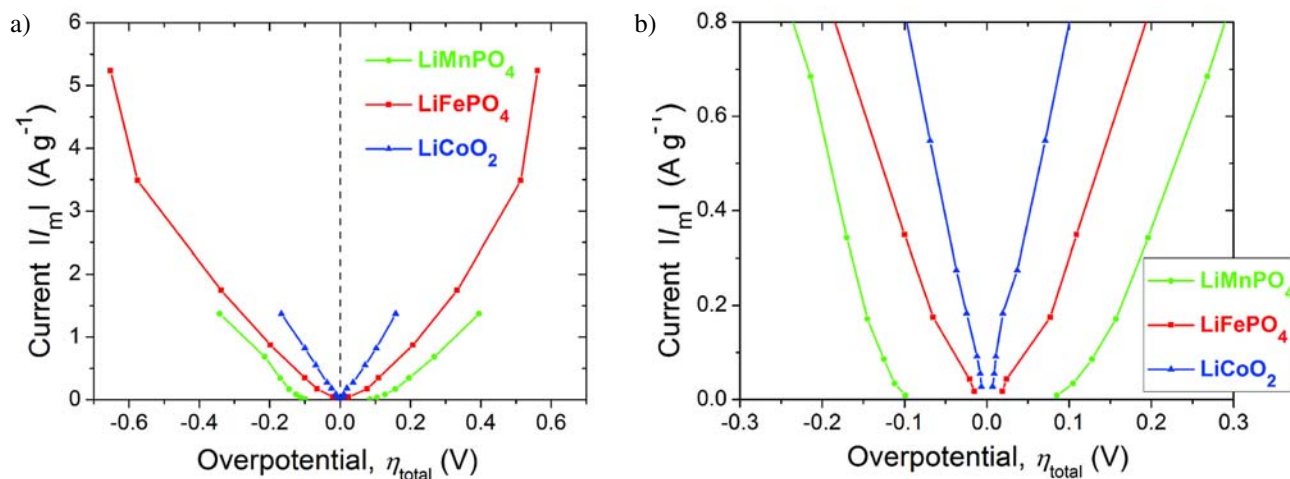


Figure 4. Current-Overpotential ($I_m - \eta_{total}$) characteristics of the compared LMP (green), LFP (red) and LCO (blue) obtained using the inflection point criterion together with Eq. 3. Data in panel (b) are merely a magnification of data in panel (a) for low current values. The data were extracted from the 3 sets of galvanostatic curves shown in Fig. 1.

denoted with “+” and “–” (η_{total}^+ and η_{total}^-), respectively. It is worth noting that the proposed inflection point criterion for reading out the value of overpotential can be employed generally for different Li battery types including the one exhibiting the so called “solid solution” behaviour etc. The results of the analysis of the galvanostatic curves of the compared materials (LMP, LFP, LCO – Fig. 1) using the inflection point criterion are shown on Fig. 4. On the real axis the total overpotential ($-\eta_{total}$) is given in the positive (charge) and negative (discharge) direction. On the ordinate axis the normalized current (in A per g) is displayed.

Fig. 4 reveals that all 3 materials compared in this study exhibit quite symmetric $I_m - \eta_{total}$ characteristics of increasing current with increased overpotential. We find that for all the measured current densities the overpotential (η_{total}) increases in the order: LCO < LFP < LMP. Further we can clearly see that LMP differs from the other two materials in having a much larger hysteresis at the lowest C-rate (close to 200 mV at C/20). A closer inspection of the results shows that the curves for LFP and LMP are bent forming a “U-shape”. In other words – LFP and LMP based cells show a non-linear current-overpotential dependency. Performing further analyses (see below) in which we quantify the resistances of the measured cells, we show that such bending is an inherent property of LFP and LMP (and probably many other insertion materials).

One might argue that the appearance of the non-linear current-overpotential dependency could partly – or even entirely – be due to contribution of the electronic and/or ionic transport (wiring) within the electrode composites. Namely, it is well known that the measured electrochemical performance of Li ion insertion electrodes is strongly affected by the electrode morphology (electrode thickness, porosity and packing density) which has impact

on the course of overpotential curve and, consequently, on the obtained capacity. For the case of LFP based electrodes, this impact was systematically and thoroughly addressed by Lestriez et al.^{43,44} and later effectively demonstrated in a major practical high-rate improvement of $\text{Li}_4\text{Ti}_5\text{O}_{12}$ ⁴⁵ and of LFP⁴⁶ based electrodes.

In the present specific case the effect of different wiring contribution could manifest itself through the higher density (approx. 5 g/cm³) and larger particle size (micron) of LiCoO_2 compared for example to the nanosized and lower density (approx. 3.5 g/cm³) LFP/LMP materials. This means that at the same electrode loading the LiCoO_2 electrode effectively appears (much) thinner than those of olivines. In order to check whether this difference in effective thickness and wiring has any effect on the current-overvoltage curve, we prepared a very thin LFP electrode (0.53 mg of LiFePO_4 per 1.54 cm²). We measured its galvanostatic charge/discharge performance (supplementary Figure S1) using the same conditions as in the case of “standard” LFP electrode (5.2 mg per 1.54 cm²) shown in Figure 1b. The current-overpotential ($I_m - \eta_{total}$) characteristics of the thin and standard LFP electrodes are compared in Figure 5.

As seen from Fig. 5, the current-overpotential characteristics of the “thin” LFP electrode expectedly exhibits comparatively smaller overpotential values across the whole current range (C/10–30C). However, also in the much thinner electrode the deviation from the linear (Ohm’s law like) dependence is clearly detected. As in a (very) thin electrode the electronic and ionic “wiring” contributions are significantly reduced, if not vanishingly small, the persistent observation of non-linear dependence suggests that, at least in the olivines, the origin of the phenomenon is most probably due to their intrinsic bulk properties or, alternatively, due to properties of olivine/electrolyte interface.

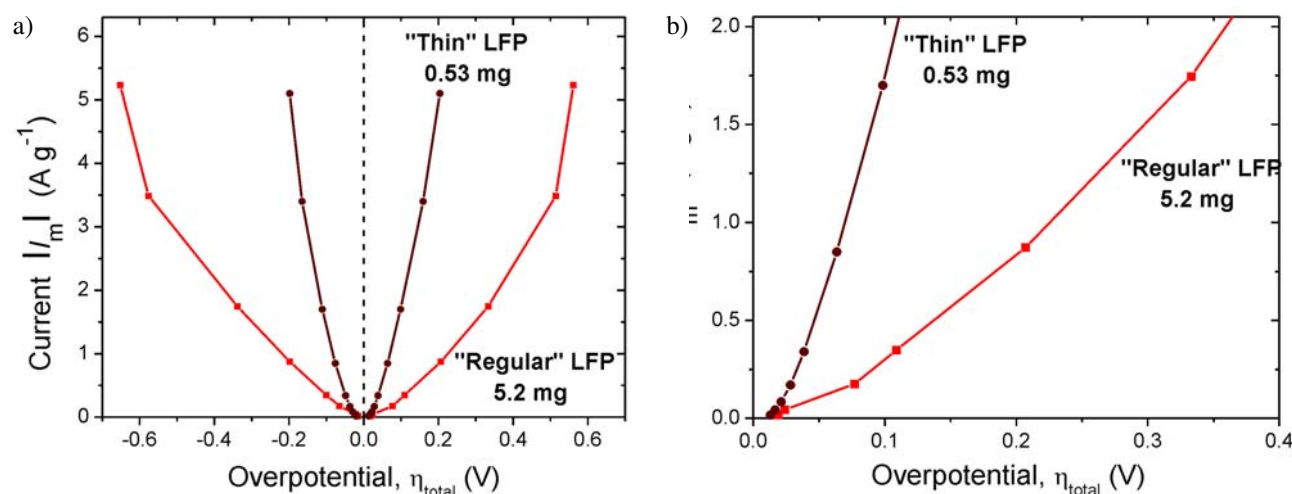


Figure 5. Absolute current-Overpotential ($I_m - \eta_{\text{total}}$) characteristics of the LFP electrode with “regular” thickness (5.2 mg, red, the same as in Fig. 4) and “thin” LFP electrode (0.53 mg, orange) obtained using the inflection point criterion together with Eq. 3. Data in panel (b) are merely a magnification of those in panel (a) for low and medium positive current values. The data for LFP with “regular” thickness was extracted from the set of galvanostatic curves shown in Fig. 1b, while for the “thin” LFP electrode from Fig. S1.

Aside from considering the potential wiring effect, one also needs to address the potential impact of the particle size and morphology of the different cathode materials (powders) used in this study. Namely, particle size can drastically influence the overpotential (and consequently capacity) during Li insertion/extraction, as well known from the very invention of LFP which was initially considered as an “...excellent candidate for the cathode of a low-power...”.¹ Generally, one can roughly say that as we decrease the particle size from micron values towards 100 nm and less, the overvoltage consistently decreases at comparable currents.⁴⁷ Similarly active particle morphology and agglomeration of primary particles in secondary architecture definitely have an effect on the electrochemical performance. Particularly in particles of the two olivine materials with large aspect ratio (e.g. platelet morphology) the effects are strongly manifested due to the existence of preferential transport paths within the volume of the crystallites.

Despite the great impact of particle size and morphology on materials performance, we wish to emphasize once again that in the present work we do not focus on those, otherwise very important, issues but merely want to stress the **occurrence of a strong deviation of the current-overpotential characteristics from the linear dependence** which, to our knowledge, has not been sufficiently and convincingly treated in the literature. In other words, as regards Li ion batteries the data about the current-overpotential characteristics are quite rarely reported and the importance of the obtained results is even more rarely discussed. The aim of this manuscript is to open several directions along which some further progress towards understanding this phenomenon could be expected in the (near) future.

3. 3. Total Resistance of the Cell, R_{total} , and the “Activation” Phenomenon

We define the total resistance of the cell, R_{total} , as follows:

$$R_{\text{total}} = \frac{\eta_{\text{total}}}{I_{\text{meas}}}, \quad (4)$$

where I_{meas} is the measured value of current in galvanostatic experiment. Normalized total resistance of the cell, $R_{\text{total}} m_{\text{act}}$, is obtained simply by multiplying R_{total} with the mass of the active material in the electrode (m_{act}) and has the unit of Ωg .

The total resistances corresponding to the current-overpotential characteristics shown in Fig. 4 are presented in Fig. 6. For the cases of LMP- and LFP-based cells we can observe a very pronounced phenomenon: the total resistance of the cell decreases very much with the increasing current density (or, equivalently, C-rate). In the case of LFP-based electrode we briefly commented on this feature some time ago and termed it an “activation” phenomenon.⁴⁸ Later on, Lestriez et al.⁴³ performed a more systematic analysis of the measured galvanostatic curves of LFP electrodes and confirmed the existence of the phenomenon. They have also observed a similar non-linear characteristic for the case of nano-sized Si-based negative electrode.⁴⁹

However, Fig. 6 also reveals that, in contrast to LMP and LFP, the resistance of the LCO-based electrode is relatively independent of the current density; in other words, the current-overpotential curve (see Fig. 4) is close to linear. One could say that, in a first approximation, the LCO-based cell is linear by nature and follows more or less the Ohm’s law; when measured galvanostatically, a 10 fold increase of current (C-rate) will thus result in a

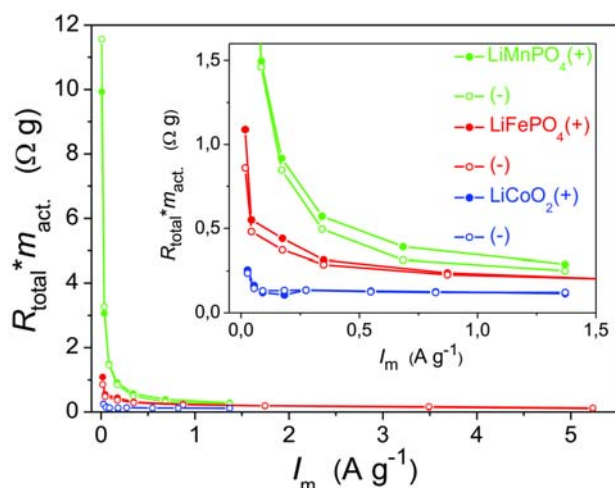


Figure 6. Total resistance of cell, R_{total} , for LMP- (green), LFP- (red) and LCO- (blue) based cells obtained using Eq. 4. The overpotential values were extracted using the inflection point criteria (see also data shown in Fig. 4). In order to compare different electrodes the resistances were mass-normalized $R_{\text{total}} m_{\text{act}}$, where m_{act} is the mass of active material. The obtained values of $R_{\text{total}} m_{\text{act}}$ for charge and discharge are denoted with (+) and (–), respectively.

10-fold increase of overpotential (or voltage hysteresis). In the case of LFP and LMP, however, there is a major deviation from Ohm's law: a 10 fold increase in current (C-rate) will result in much less than 10-fold overpotential increase. It seems that this electrode “activation” at higher rates is even more expressed for LMP.

The origin of electrode “activation” is still poorly understood. It might be correlated to the variable rate of the internal charge redistribution during charge/discharge at different rates,^{50,51} but other options cannot be excluded. For example, it has been shown theoretically and confirmed experimentally that at very low currents active particles tend to phase-transform in a particle-by-particle fashion.^{42,50,52–54} This means that a very small fraction of active material is in fact “active” at any given time during charge/discharge. A more general simulation of porous insertion electrodes has suggested that the fraction of such “active” particles in the electrode scales with the current (charge/discharge rate).⁵⁵ Similarly it has been shown that imposing higher overpotentials during high current rate experiments induces more particles to undergo phase transformations at similar times during charge/discharge.^{56,57} On the experimental level, extensive experimental observation of LiFePO₄ based electrodes using synchrotron Scanning Transmission X-Ray Microscopy (STXM) has confirmed that the fraction of the phase-transforming particles depends on C-rate.⁵¹ It has been suggested that the electrode accommodates the higher current by increasing the active particle population.⁵¹ Finally, *in-situ* XRD studies with high temporal resolution during high-rate galvanostatic cycling have revealed the formation of a non-equilibrium solid solution phase(s), Li_xFePO₄ (0 < x < 1),

where the distribution of solid solution compositions span the entire composition range between two thermodynamic phases, LiFePO₄ and FePO₄.^{58,59} Thus, at high global current densities (e.g. 5C and higher) the fraction of the LFP electrode that reacts simultaneously via nonequilibrium solid solution increases with the increasing C-rate.^{58,59}

In terms of the present results it is important to conclude that any of the mechanisms independently proposed in the above reports could be responsible for the observed current-voltage non-linearity (“activation” phenomenon). Namely, if we assume that the fraction of active particles indeed increases with C-rate (overpotential) then the results of Fig. 6 can be explained straightforwardly. Even more, in this case the curves shown in Fig. 6 could directly serve for estimation of the fraction of “active” particles at any given current (overpotential). Finally, if it is true that the current-voltage characteristic is non-linear due to increasing fraction of active particles, then certainly this is a new, previously unreported origin of current-voltage non-linearity in electrochemistry.

4. Conclusion

We discussed a couple of unusual phenomena in LMP and selected other insertion battery materials. For example, at current rates above ca. 1.5 A/g the energy efficiency of LMP, as calculated according to standard approaches, becomes even slightly higher than that of LFP and gradually approaches to that of LCO. We showed that this may be strongly correlated to the nature of overvoltage-current characteristic. Whereas in LCO this relationship is more or less linear, it deviates from linearity in both olivines. This non-linearity can be seen as an increasing activation of active material with increasing current rates. In LMP the activation is particularly strong which explains its good efficiency and the slowly increasing polarization at higher rates. One reason for the activation phenomenon in certain insertion materials could be the increase of fraction of particles that is actually undergoing phase transformation at given moment. If so, this is a different kind of non-linearity than typically observed in electrochemical systems (the non-linearity related to the well known Butler-Volmer equation). This hypothesis, however, still needs an experimental verification.

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Povzetek

Osnovne elektrokemijske karakteristike baterijskega materiala na osnovi LiMnPO_4 primerjamo s karakteristikami precej bolj raziskanega materiala na osnovi spojine LiFePO_4 z olivinsko strukturo ter prav tako dobro znanega materiala na osnovi LiCoO_2 . Na podlagi obsežnejših kronopotenciometričnih (galvanostatskih) meritev primerjamo energijsko učinkovitost in prenapetost elektrod, narejenih iz omenjenih materialov. V ta namen za odčitavanje celotne prenapetosti, η_{total} , uvedemo in utemeljimo tako imenovani "kriterij točke prevoja". V nadaljevanju pokažemo, da lahko v splošnem tokovno-napetostno karakteristiko insercijskih elektrod predstavimo z enostavnim parametrom – celotno upornostjo elektrode, R_{total} . Medtem ko je tokovno-napetostna karakteristika elektrode na osnovi LiCoO_2 približno linearna, pa pride v primeru LiFePO_4 , in še bolj LiMnPO_4 , do znatnega odstopanja od linearnosti. Pojav razložimo s sklicevanjem na najnovejše ugotovitve o pojavi fazne transformacije v omenjenih baterijskih sistemih.

Scientific paper

Nanostructured ZnFe_2O_4 as Anode Material for Lithium-Ion Batteries: Ionic Liquid-Assisted Synthesis and Performance Evaluation with Special Emphasis on Comparative Metal Dissolution

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In memoriam of Janez (Janko) Jamnik, a brilliant scientist with an exceptional combination of great leadership capabilities and a friendly and kind personality

Abstract

In this work, a ZnFe_2O_4 anode material was successfully synthesized by a novel ionic liquid-assisted synthesis method followed by a carbon coating procedure. The as-prepared ZnFe_2O_4 particles demonstrate a relatively homogeneous particle size distribution with particle diameters ranging from 40 to 80 nm. This material, which is well known to offer an interesting combination of an alloying and conversion mechanism, is capable of accommodating nine equivalents of lithium per unit formula, resulting in a high specific capacity ($\geq 1,000 \text{ mAh g}^{-1}$). The resulting composite anode material displayed a stable capacity of ca. $1,091 \text{ mAh g}^{-1}$ for 190 cycles at a medium de-lithiation potential of 1.7 V and at a charge/discharge rate of 1C. Furthermore, the material displays an excellent high rate capability up to 20C, displaying a reversible capacity of still 216 mAh g^{-1} . Studies on Fe and Zn losses of the ZnFe_2O_4 active material by dissolution in the electrolyte were performed and compared to those of silicon-, germanium- and tin-based high-capacity anode materials. In conclusion, ion dissolution from metal containing anode materials should not be underestimated in view of its impact on the overall cell performance and cycling stability.

Keywords: $\text{ZnFe}_2\text{O}_4/\text{C}$ composite, anode material, ionic liquid, metal dissolution, lithium-ion batteries

1. Introduction

The ever-growing demand for the next-generation lithium-ion batteries (LIBs) with high specific energy (Wh kg^{-1})/ energy density (Wh L^{-1}) as well as high power performance has prompted widespread research to develop novel electrode materials for both the anode and cathode. Currently, commercialized graphite anode materials apparently cannot satisfy the demand of high-energy battery systems due to the relatively low theoretical specific capacity (372 mAh g^{-1}) and poor rate capability, in particular during charge.^{2,3} However, considering that the energy content of a battery cell is the product of capacity and cell voltage, it is difficult to find an anode material other

than carbon, which possesses such a low (and constant) discharge potential as graphite.

Since academic search for alternative anode materials typically focuses on capacity improvements, the first look at novel materials always goes to their gravimetric and volumetric capacities (Figure 1a and 1b). In this respect, Si, Sn, Ge and several types of metal oxides, such as ZnFe_2O_4 , seem to be very promising candidates for the negative electrode. In terms of gravimetric capacity, Si is the most promising material showing the highest storage capability of ca. $3,500 \text{ mAh g}^{-1}$, while for the volumetric capacity also various metal oxides are of strong interest since they display even higher capacity values than Si (Figure 1b), i.e. between ca. $3,000$ and $5,000 \text{ mAh cm}^{-3}$.⁴

However, it has to be considered that the practical volumetric capacity not only depends on the material, but also on the electrode structure and cell design and may change during charge/discharge cycling. Besides the capacity, also the abundance and costs of the anode materials need to be considered (Figure 1c and 1d). In this respect, in particular the high costs and low abundance of Sn and Ge do only allow the use for niche applications. Currently, the research on novel anode materials, considering their electrochemical characteristics, is mainly limited to capacity improvements rather than energy and power density improvements and accordingly, rate capability and not power capability is the measure that counts in most academic reports.

There are mainly three types of anode materials for LIBs according to the different lithiation storage mechanisms: (I) intercalation materials such as graphite⁶ and insertion materials such as $\text{Li}_4\text{Ti}_5\text{O}_{12}$ spinel oxides or TiO_2 anatase,^{7–9} (II) the so called “lithium alloying” materials, such as Si, Sn and Ge forming intermetallic phases with Li,^{10–13} which depending on the used metals have various operating discharge potentials, with Si showing the lowest potential¹¹ and (III) conversion materials, such as transition metal oxides, -sulfides or -nitrides, which store charge

via a conversion reaction. The conversion reaction can be generalized by the equation: $M_aX_b + (b \cdot n) \text{Li}^+ + (b \cdot n) \text{e}^- \rightleftharpoons aM + b\text{Li}_nX$, where M is the transition metal, X is the anion (most commonly oxygen in the case of anode materials) and n is the formal oxidation state of X .^{14,15} Conversion materials, in particular those using light weight metals M , typically show relatively high specific capacities. However, these capacities are usually only available over a large and in average relatively high potential range, negatively affecting the energy of a full LIB cell. As like for lithium alloying host materials,¹⁶ the kinetics of the lithium uptake and release reaction will increase with decreasing particle or grain size of the conversion anode material, most likely resulting in an enhanced performance at high charge/discharge rates, especially for nano- and nanostructured materials. The enhanced surface area, however, will lead to an increased electrolyte decomposition, which may have a negative impact on the long-term cycling stability in LIBs.

Among the conversion materials, the nanostructured binary and ternary metal oxides, such as iron oxides (e.g. hematite $\alpha\text{-Fe}_2\text{O}_3$, spinel Fe_3O_4 and ZnFe_2O_4),^{17–22} manganese oxides (e.g. MnO_2 , Mn_3O_4 and ZnMn_2O_4)^{23–25} and cobalt oxides (e.g. CoO , Co_3O_4 and ZnCo_2O_4)^{26–28} have

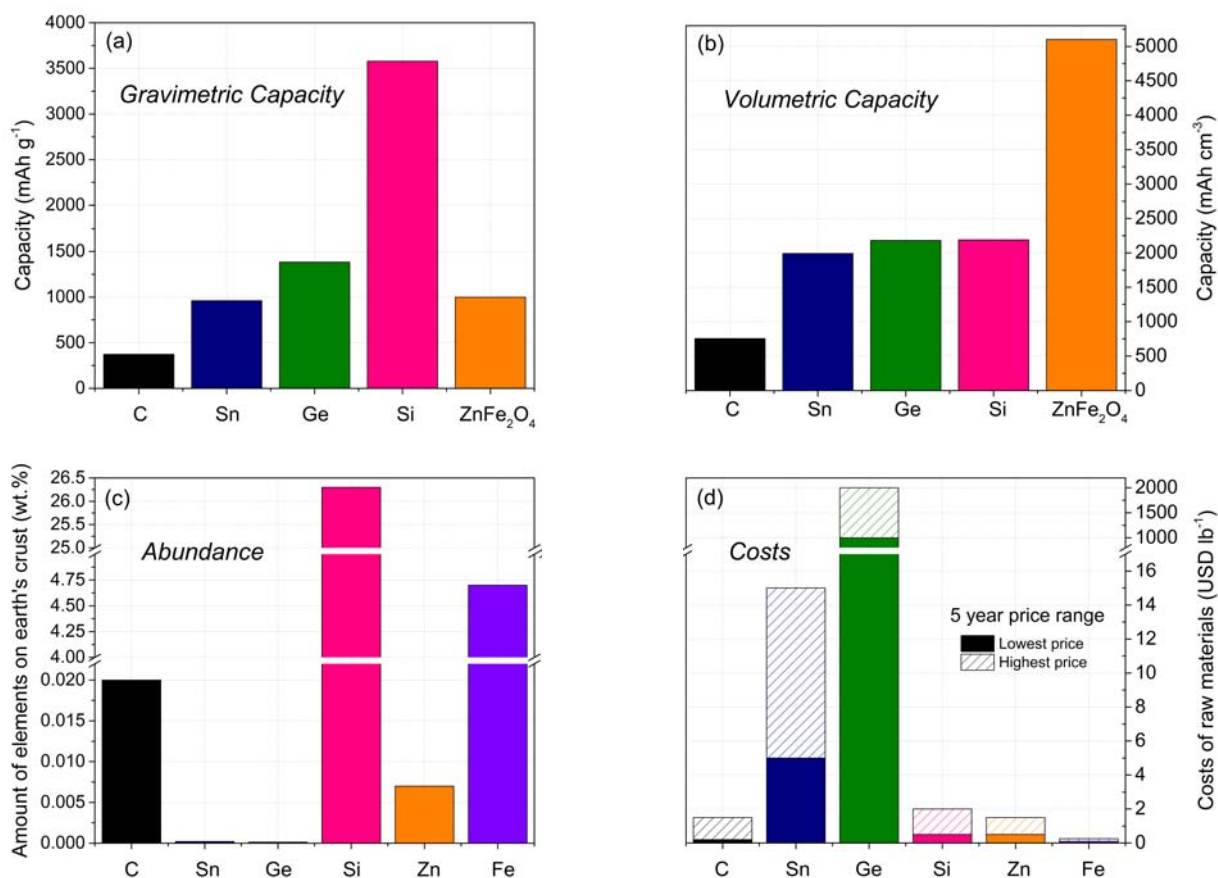


Figure 1. Gravimetric and volumetric lithium storage capacities of different anode materials, abundance of elements (as fraction of earth's crust in wt.%) and costs of elements (approximate 5-year ranges, except for Ge which is a 3-year range, according to⁵).

been extensively studied as alternative anode materials for use in LIBs over the past few years. This is related to the fact that there is a large number of possible new material combinations, which provide high specific capacities ranging from ca. 650 to 1,000 mAh g⁻¹.⁴

Fe₃O₄, for example, gives a theoretical capacity of 926 mAh g⁻¹, considering the complete reversible formation of four Li₂O per formula unit. Low cost, environmentally benign as well as biocompatible iron-based oxides²⁹ have received increasing interest of scientific investigation. However, the reduced iron during the lithiation process is itself not electrochemically active to Li⁺. At contrast, ZnO shows an advantage, because Zn, as the product of ZnO reactions with Li, can reversibly alloy with Li. Thus, the theoretical capacity of these transition metal oxides can be further increased by replacing one iron atom by an element which can reversibly form an alloy

with lithium, such as zinc in addition to the still ongoing conversion reaction.^{30–32} This would result in an enhanced theoretical capacity of ca. 1000 mAh g⁻¹, according to the reversible reaction involving nine lithium ions per formula unit of ZnFe₂O₄.

Chen et al., for the first time, reported the lithium insertion reaction in spinel zinc ferrite using a chemical synthesis approach with n-butyllithium in hexane. However, only about 0.5 Li⁺ per formula unit could be inserted in this host material by n-buli/hexane and, moreover, this reaction was found to be irreversible.³³ Nuli et al. reported the reversible lithium uptake of nanocrystalline thin films of ZnFe₂O₄, which presented a reversible capacity of 560 mAh g⁻¹, corresponding to an uptake of 5 Li per ZnFe₂O₄. This material demonstrated a rather poor cycling performance since only a capacity retention of 78% was obtained after 100 cycles.³⁴ Deng et al.

Table 1. Summary of the synthesis methods for ZnFe₂O₄.

Synthesis method	Example for precursor materials	Synthesis properties
Precipitation method ^{40–42}	ZnCl ₂ ; FeC ₂ O ₄ · 2H ₂ O; conc. HNO ₃ ; CO(NH ₂) ₂ (urea) ⁴⁰	• Simple synthesis route (easy to operate) • Cost-effective preparation • Calcination at high temperature (900 °C) • Agglomerated particles (100–300 nm)
	FeSO ₄ · 7H ₂ O; ZnSO ₄ · 7H ₂ O; Na ₂ C ₂ O ₄ ⁴¹	• Porous structure; • Calcination at high temperature (700 °C) • Big particles (μm range)
Hydrothermal / solvothermal processing ³⁶	(NH ₄) ₂ Fe(SO ₄) · 6H ₂ O; ZnSO ₄ · 7H ₂ O; glucose ³⁶	• Simple synthesis route • Small primary particles (10–20 nm) • Calcination at high temperature (600 °C)
	FeCl ₃ · 6H ₂ O; ZnCl ₂ ; ethylene glycol; PEG-600; urea ³⁵	• Without further calcination at high temperature • Uniform hollow sphere structure • Relatively big particle size (~500 nm)
Refluxing synthesis ⁴³	FeCl ₃ · 6H ₂ O; ZnSO ₄ · 7H ₂ O	• Cost-effective route • Rapid process • Very small particles (~7 nm)
Microemulsion ⁴⁴	Zn(NO ₃) ₂ ; FeSO ₄ ; CTAB; cyclohexane; n-pentanol; H ₂ C ₂ O ₄	• Special morphology (nanorods) • Calcination at high temperature (500 °C)
Polymer pyrolysis ⁴⁵	Zn(NO ₃) ₂ · 6H ₂ O; Fe(NO ₃) ₃ · 9H ₂ O; acrylic acid; (NH ₄) ₂ S ₂ O ₈ ;	• Well distributed particles • Small primary particles (30–70 nm) • Calcination at high temperature (600 °C)
Pulsed laser deposition ³⁴	Targets from zinc and iron powder	• Small particle size (40–100 nm) • High costs • Small scale production technique
Molten salt route ⁴⁶	ZnSO ₄ · H ₂ O; Fe ₂ (SO ₄) ₃ ; LiCl · H ₂ O	• Simple synthesis route (solid state reaction) • High reaction temperature (800 °C); • Big particles (μm range)
Sol-gel method ^{47,48}	Zn(NO ₃) ₂ · 6H ₂ O; Fe(NO ₃) ₃ · 9H ₂ O; Citric acid; Ammonia ⁴⁷	• Calcination at high temperature (600–1000 °C) • Adjustment of pH value necessary
Ionic liquid-assisted synthesis method (this work)	BMIMBF ₄ ; ethylene alcohol; Zn(O ₂ CCH ₃) ₂ ; Fe(NO ₃) ₃	• Simple synthesis route (easy to operate) • Small particle size (40–80 nm) • Calcination at 500 °C

synthesized the monodispersed $\text{ZnFe}_2\text{O}_4/\text{C}$ hollow sphere via a simple solvothermal route. The resulting composite shows a high specific capacity of 911 mAh g^{-1} in the initial de-lithiation process and a (for conversion materials) high capacity retention of 91% after 30 cycles.¹⁹ Ding et al. adopted a polymer pyrolysis method to synthesize a nanostructured ternary transition metal oxide, ZnFe_2O_4 , which showed a high specific capacity and good cycling performance.¹⁸

Hence, considerable efforts have been devoted for the synthesis of ZnFe_2O_4 with a variety of nanostructures, such as hollow spheres,^{35,36} octahedrons,³⁷ nanofibers³⁸ and nanorods.³⁹ However, many of these morphologies were fabricated by high temperature or long reaction time methods. The possible synthesis routes for ZnFe_2O_4 are reviewed in Table 1, including precipitation,^{40–42} hydrothermal/solvothermal,^{35,36} refluxing⁴³, microemulsion,⁴⁴ polymer pyrolysis,⁴⁵ pulsed laser deposition,⁴⁵ molten salt⁴⁶ and sol-gel methods.^{47,48} Among these methods, hydrothermal processing is the most commonly used way to design ZnFe_2O_4 -based anode materials for LIBs. This is due to its simplicity (easy operation), the possibility for large-scale production and relatively low costs of raw material and equipment.

Ionic liquids (ILs), as a new species of reaction media, have been extensively studied due to their unique properties such as low volatility, low flammability, high thermal stability, designable structures, and high ionic conductivity, and depending on the chemistry, also high chemical and electrochemical stability, etc.⁴⁹ Recently, ILs have proved to be an excellent media for inorganic synthesis and they have attracted increasing attention as templates and/or solvents for the preparation of nanostructured materials. ILs demonstrate tunable solvent properties which make them to easily interact with various surface and chemical reaction environments. Moreover, ILs with hydrophobic regions and high directional polarizability can form extended hydrogen bond systems in the liquid state, resulting in a highly structured self-assembly.⁵⁰

In this work, we report a novel synthesis route, i.e., an ionic liquid-assisted synthesis method, for the preparation of ZnFe_2O_4 . The obtained material demonstrates a relatively uniform morphology with fine particles which are composed of ca. 50 nm-sized ZnFe_2O_4 crystallites. Furthermore, sucrose is employed as carbon source to coat the ZnFe_2O_4 with a thin layer so as to enhance the surface electronic conductivity as well as the surface area for use as high-capacity and high-power anode material in LIBs. In addition, studies on the Fe and Zn losses of the ZnFe_2O_4 active material upon storage and cycling were performed, which can be generally related to (1) active particle losses from the electrode and (2) metal ion dissolution in the electrolyte. In this study, metal ion dissolution of ZnFe_2O_4 will be compared to those of other high capacity anode materials including silicon, tin and germanium.

2. Experimental

2. 1. Preparation of $\text{Fe}(\text{NO}_3)_3$

$\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{ H}_2\text{O}$ (Sigma-Aldrich, purity: 98%) was put in a furnace and heated with a rate of 3 min^{-1} to 140°C for 2 hours under argon atmosphere. By this process, the crystal water was removed and $\text{Fe}(\text{NO}_3)_3$ was obtained.

2. 2. Preparation of ZnFe_2O_4 particles

A mixture of 5 mL of 1-butyl-3-methylimidazolium tetrafluoroborate (BMIMBF_4 ; Sigma-Aldrich, purity: 98.5%) and 35 mL of ethylene alcohol (Sigma-Aldrich, purity: 99.8%) was applied as solvent medium. 0.61 g $\text{Zn}(\text{O}_2\text{CCH}_3)_2$ (Sigma-Aldrich, purity: 99.99%) and 2.17 g $\text{Fe}(\text{NO}_3)_3$ were dissolved in the above mentioned solvent by stirring for 24 hours to form a homogeneous solution and then transferred to a 50 mL sized Teflon-lined autoclave at 150°C for 3 hours. The resultant product was collected by centrifugation, alternately washed several times with de-ionized water and ethanol and then dried at 80°C in a vacuum oven. The solid product was finally calcined at 500°C for 2 hours under argon atmosphere.

2. 3. Preparation of $\text{ZnFe}_2\text{O}_4/\text{carbon}$ Composite

0.72 g sucrose (Sigma-Aldrich, purity: 98.5%) was dissolved in 4 mL of de-ionized water and subsequently 1.0 g of as-prepared ZnFe_2O_4 was added under continuous stirring. The obtained mixture was homogenized using planetary ball milling (Vario-Planetary Mill Pulverisette 4, FRITSCH) set at 800 rpm for 2 hours and subsequently dried at 80°C for 12 hours. The dry composite was then heated with a rate of 3°C min^{-1} to 500°C for 4 hours under argon atmosphere. Finally, the obtained powder was grinded manually using an agate mortar for further characterization. The carbon amount of the composite was determined by thermogravimetric analysis (TGA) under oxygen atmosphere. For comparison, commercial ZnFe_2O_4 (Sigma-Aldrich, purity: 99%; hereafter abbreviated as “comm- ZnFe_2O_4 ”) was employed to have a comparison with the self-prepared ZnFe_2O_4 in the electrochemical investigations. The carbon coating of comm- $\text{ZnFe}_2\text{O}_4/\text{carbon}$ was performed in the same way and the carbon content has been identified to be the same as for the self-prepared material.

2. 4. Structure and Morphology Characterization

X-ray diffraction (XRD) measurements were carried out using a Bruker D8 Avance X-ray diffractometer (Bruker AXS GmbH) equipped with a copper target X-ray tube (radiation wavelength: $\lambda = 0.154 \text{ nm}$). The morphology of the samples was observed by a field-emission scanning electron microscope (FESEM, JEOL JSM-7401F).

Thermogravimetric analysis (TGA) was conducted using a TGA Q5000 IR system (TA Instruments). The measurements were carried out in oxygen atmosphere (oxygen flow: 10 mL min⁻¹) in the temperature range of 30 °C to 800 °C with a heating rate of 10 °C min⁻¹.

The BET specific surface area and BJH pore diameter distribution were determined by nitrogen adsorption measurements using an ASAP 2020 (Accelerated Surface Area and Porosimetry Analyzer, Micromeritics GmbH). Before the measurement, the samples were degassed at 120 °C until a static pressure of less than 0.01 Torr (0.0133 mbar) was reached.

2. 5. Electrode Preparation, Cell Assembly and Electrochemical Investigations

Composite electrodes were prepared using a composition of 80 wt.% active material, 10 wt.% of conductive carbon black agent C-nergy Super C65 (Imerys Graphite & Carbon) and 10 wt.% of sodium-carboxymethyl cellulose (Na-CMC, Walocel CRT 2000 PA 12) as binder. Prior to the dispersion of the solid compounds, the binder polymer was dissolved in de-ionized water to obtain a 2.0 wt.% solution. An appropriate amount of Super C65 was added to the binder solution and the mixture was further homogenized by stirring. Afterwards, a high-energy dispersion step (Ultra-Turrax T25, 1 hour, 5,000 rpm) was employed to eliminate agglomerates and to homogenize the mixture. The paste was cast on a copper foil by a standard lab-scale doctor-blade technique. The gap of the doctor-blade was set to 120 µm wet film thickness, leading to an average mass loading of 1.08 mg cm⁻². After casting, the tapes were transferred into an oven and dried in air for 1 hour at 80 °C. Electrodes with a diameter of 12 mm were cut out and a further drying step was performed under an oil-pump vacuum (< 0.1 mbar) at 120 °C for 24 hours.

Electrochemical experiments were performed using CR2032-type coin cells with Celgard 2400 as separator and high-purity metallic lithium foil (Rockwood Lithium) as counter electrode. The electrolyte (UBE Europe GmbH) was 1M LiPF₆ in a mixture of ethylene carbonate (EC) and diethyl carbonate (DEC) (3:7 in weight ratio). The cells were assembled in an argon-filled glove box (Uni-Lab, MBraun) with oxygen and water contents of less than 1 ppm. The electrochemical performance was evaluated on a Maccor 4300 battery test system at 20 °C. The cut-off voltage was 0.01 V for the discharge process (= lithiation) and 3.0 V for the charge process (= de-lithiation). The specific capacity was calculated on the basis of the total composite weight, and the C-rate was calculated with respect to a theoretical capacity of 1,000 mAh g⁻¹ (1C). In the case of the ZnFe₂O₄ composite electrode, the theoretical capacity is ca. 940 mAh g⁻¹ (assuming a reversible capacity of ca. 550 mAh g⁻¹ for the pyrolyzed carbon⁵¹). Cyclic voltammetry (CV; 0.01–3.0 V) was performed

with a scan rate of 0.02 mV s⁻¹ using a VMP multichannel constant voltage-constant current system (Bio-Logic Science Instruments).

In situ XRD analysis of the ZnFe₂O₄ anode material upon galvanostatic lithiation and de-lithiation was performed by using a self-designed *in situ* cell, whose design has been inspired by earlier reports.^{52–54} The electrode stack is electronically insulated from the stainless steel body by a sheet of Mylar foil. The electrode paste was cast on a beryllium (Be) window, which served as both the current collector and “window” for the X-ray beam, to analyze the structure changes during the lithiation and de-lithiation processes of ZnFe₂O₄. The coated Be window was subsequently dried at 80 °C for 30 minutes in air and at 40 °C under vacuum (< 0.1 mbar) for 12 hours. Metallic lithium foil served as the counter electrode. A Whatman® glass fiber (grade GF/D) served as separator, drenched with 500 µL of the electrolyte, 1M LiPF₆ in EC:DEC 3:7 (in weight ratio). The assembled cell was allowed to rest for 6 hours to ensure a sufficient wetting of the electrode. Subsequently, the cell was galvanostatically cycled at a specific charge/discharge current of 0.05C to a complete initial discharge (= lithiation) to 0.01 V, for approximately 10 hours. In parallel, XRD patterns were acquired in an angular range of 20 to 80, with a step size of 0.02239 and a time per step of 0.4 seconds, resulting in a complete scan for every 20 minutes. After discharging, the cell was charged (= de-lithiation) to an upper cut-off voltage of 3 V.

2. 6. Analytical Studies on Metal Ion Dissolution by Total Reflection X-Ray Fluorescence (TXRF)

After electrochemical measurements, the separators were separated from the cells and placed in 1.5 mL Eppendorf reaction vessels. Afterwards, the separator samples were centrifuged at 8500 rpm over 10 minutes using the Galaxy 5D (VWR International Inc.) in order to collect the cycled electrolyte. Thereafter, the electrolyte was diluted 1:10 using a solution containing 1 ppm Germanium (1000 mg/L, Certipur®, Merck) in 2 v% nitric acid (65 v%, Suprapur®, Merck) as internal standard for quantification. The dilution of the acid was carried out using deionized water (18.2 mΩ/cm², 5 ppb TOC, Millipore Corporation). For analysis, a small portion of 5 µL from the diluted electrolyte was transferred onto a pre-siliconized quartz glass carrier (Bruker Corporation) and dried by heat at 55 °C until the solvent was evaporated. For the Total Reflection X-ray Fluorescence (TXRF) measurements, a Picofox S2 system equipped with the Spectra 7.5 software (Bruker Corporation) was used. The conditions for the measurements were set at 1000 seconds irradiation per sample, in order to obtain sharp signals. The X-rays were generated via a Molybdenum source as anode material at voltages of 50 kV and currents of 600 µA leading to exci-

tation energies of 17.5 keV. The X-ray fluorescence of the samples was measured using a silicon-drift detector (SDD).

3. Results and Discussion

3. 1. Structural Characterization of ZnFe_2O_4 and ZnFe_2O_4 /carbon

As schematically illustrated in Figure 2a, the precursors of zinc and iron were dissolved in ethanol, which was accompanied with the formation of M-O bonds. The large cations and anions in 1-butyl-3-methylimidazolium tetrafluoroborate (BMIMBF₄) could act as self-assembling template, in which the long chain surfactants may hinder an increased aggregation rate of the particles. The proposed formation mechanism of ZnFe_2O_4 with the ionic liquid is shown in Figure 2b. The formation of the intermediate product corresponds to an effective aggregation of the MO particles with a highly structured self-assembled ionic liquid due to the hydrogen bonding and/or a π - π stacking mechanism.⁵⁵ After calcination, the desired product of nanostructured ZnFe_2O_4 particles can be obtained.

The ZnFe_2O_4 phase of the prepared ZnFe_2O_4 particles was characterized by powder X-ray diffraction analysis, as shown in Figure 3. The XRD pattern can be indexed to the spinel ZnFe_2O_4 phase (space group $Fd\bar{3}m$, No. 227, PDF card No. 04-008-5691),⁵⁶ and no impurity phase was observed. Meanwhile, the positions of all peaks are in good agreement with commercial ZnFe_2O_4 , which means

it is feasible to obtain ZnFe_2O_4 via an ionic liquid-assisted synthesis method. In the case of the carbon-coated ZnFe_2O_4 composite, no extra peaks were observed, which means the residual carbon is amorphous.

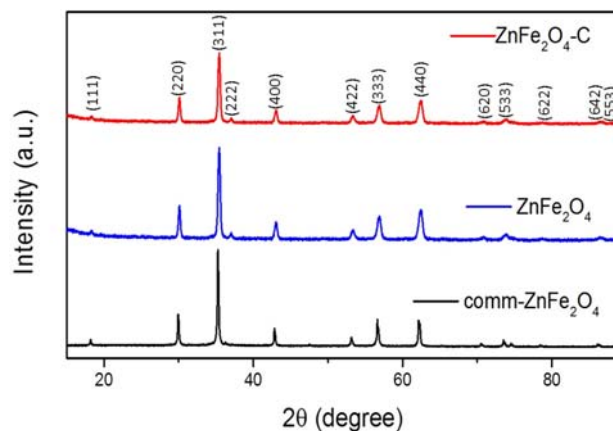


Figure 3. XRD patterns of the self-prepared ZnFe_2O_4 and $\text{ZnFe}_2\text{O}_4/\text{C}$ material and of the commercial ZnFe_2O_4 particles.

The amount of carbon was determined by thermogravimetric analysis, which was carried out under oxygen atmosphere, as illustrated in Figure 4. The weight loss at the beginning can be attributed to absorbed water. From 100 °C to 300 °C, there is a small weight increase (0.7 wt.%), which most likely means that the surface of the composite reacts with the oxygen, eventually for-

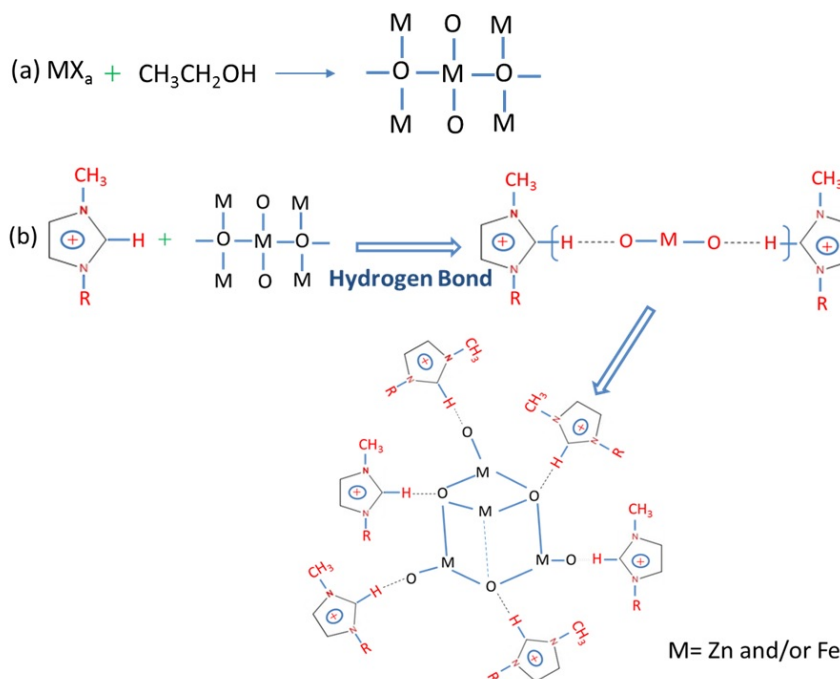


Figure 2. Schematic illustration of the preparation process of the ZnFe_2O_4 material (R = butyl).

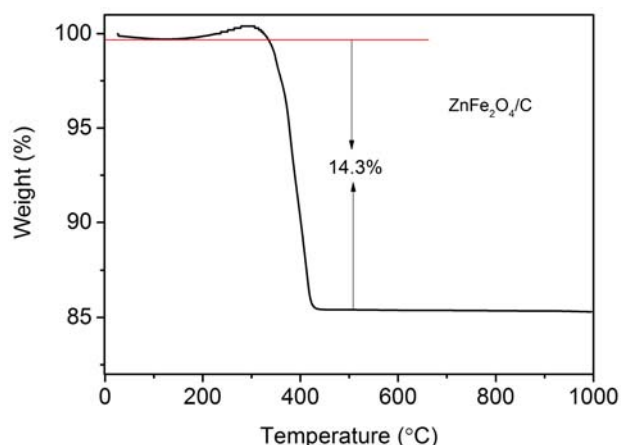


Figure 4. Weight loss curve of the self-prepared $\text{ZnFe}_2\text{O}_4/\text{C}$ composite material under oxygen atmosphere.

ming carbon-oxygen compounds.⁵⁷ The rapid mass loss between 350 °C and 420 °C was caused by the oxidation of carbon. Above 420 °C, almost no more weight loss

was observed, implying the composites are stable. Thus, the mass percentage of carbon in the composite is calculated to 14.3 wt.%.

Figure 5 presents (a) SEM and (b) TEM images of the as-prepared ZnFe_2O_4 material. It can be clearly observed that the ZnFe_2O_4 particles exhibit a spherical-like morphology with particle sizes ranging from 40 to 100 nm, consisting of an agglomeration of some small particles. Nevertheless, in view of the overall particles, the prepared ZnFe_2O_4 material demonstrates a better homogeneity and distribution compared to the commercial ZnFe_2O_4 (Figure S1, see the supporting information).

In order to further determine the chemical composition and distribution of ZnFe_2O_4 , energy dispersive X-ray spectroscopy (EDX) analysis was performed (Figure S2, supporting information). Elemental Zn, Fe and O were detected in the particles, and all the elements are homogeneously distributed. In the case of the commercial ZnFe_2O_4 (see Figure S1), primary particles having sizes ranging from 50 to 100 nm are observed. However, the primary particle demonstrates a strong aggregation tendency,

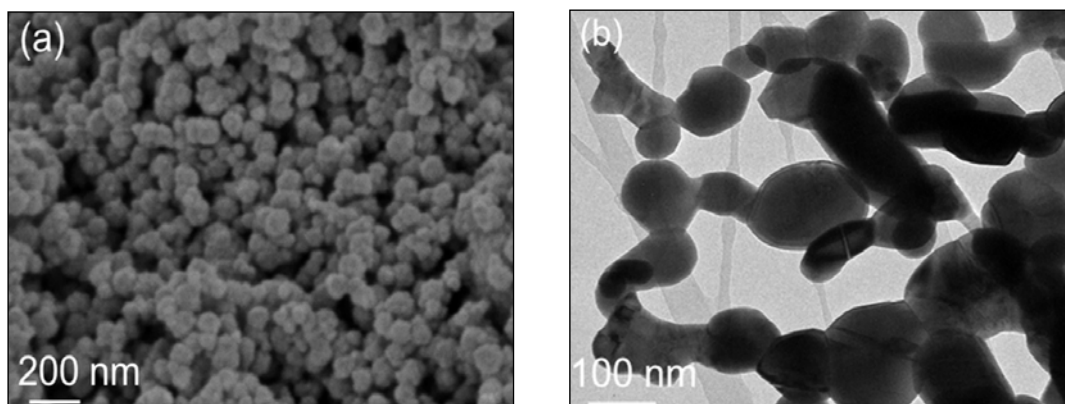


Figure 5. SEM (a) and TEM (b) images of the self-prepared ZnFe_2O_4 particles.

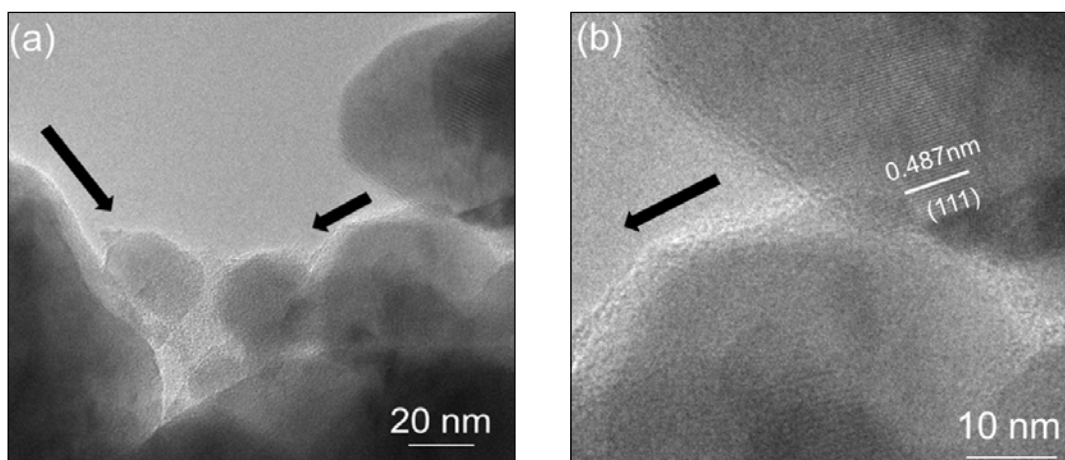


Figure 6. HR-TEM images of the $\text{ZnFe}_2\text{O}_4/\text{C}$ composite.

which will result in increased lithium ion diffusion pathways and lead to poor cycling performance and rate capability.

The conducting surface layer on the poor-conducting active materials often plays an important role on the cycling stability and fast charge-discharge properties. Hence, sucrose was used as carbon source to acquire the carbon coated ZnFe_2O_4 composite (hereafter abbreviated as $\text{ZnFe}_2\text{O}_4/\text{C}$) with the carbon content of 14.3 wt.%. Figure 6 reveals that the carbon layer, covering the surface of ZnFe_2O_4 particles, is about 8 nm in thickness. One can also clearly see the lattice fringes with an interplanar spacing of 0.487 nm, corresponding to the ZnFe_2O_4 (111) plane.

In addition, the BET specific surface area of the prepared ZnFe_2O_4 and the corresponding $\text{ZnFe}_2\text{O}_4/\text{C}$ composite were measured to be $23.8 \text{ m}^2 \text{ g}^{-1}$ and $112.3 \text{ m}^2 \text{ g}^{-1}$, respectively. With regard to commercial ZnFe_2O_4 and carbon-coated ZnFe_2O_4 , the values are $18.6 \text{ m}^2 \text{ g}^{-1}$ and $82.4 \text{ m}^2 \text{ g}^{-1}$, respectively. The increase in the BET surface area can be attributed to the amorphous carbon-coating surrounding the ZnFe_2O_4 particles.^{58,59} The porous and conductive carbon layer is intended to increase the electronic conductivity as well as to offer an easy electrolyte access to the particles, thus resulting in the improvement of the electrochemical performance.

3. 2. Electrochemical Lithiation and de-lithiation of $\text{ZnFe}_2\text{O}_4/\text{carbon}$

Figure 7a displays representative voltage profiles for the discharge/charge cycling of $\text{ZnFe}_2\text{O}_4/\text{C}$ electrodes at a rate of 0.1C in the first cycle (formation cycle) and 0.5C in the following cycles, within the voltage range of 0.01 V to 3 V. The first de-lithiation and lithiation capacities are 995 mAh g^{-1} and $1,327 \text{ mAh g}^{-1}$, respectively, with an initial Coulombic efficiency of 75%. The first irreversible capacity can be attributed to the decomposition of electrolyte to form the solid electrolyte interphase (SEI) on the electrode surface.^{34,40,60} As for many other conversion materials, the discharge curve spans over a wide potential range, in this case 3V, with a medium de-lithiation potential of 1.7 V vs. Li/Li^+ (at 1C), affecting the available energy content in a full cell. In addition, there is a large hysteresis, which will affect the energy efficiency. These effects will be discussed in detail below.

The electrochemical lithiation/de-lithiation characteristics of the ZnFe_2O_4 material were further determined using cyclic voltammetry (CV), as shown in Figure 7b. Upon the initial cathodic sweep, a small peak can be observed at around 0.9 V (section B), followed by a main sharp reduction peak at around 0.7 V (section C). It is proposed that ZnFe_2O_4 could be initially lithiated to $\text{Li}_{0.2}\text{ZnFe}_2\text{O}_4$ or $\text{Li}_{0.5}\text{ZnFe}_2\text{O}_4$ and $\text{Li}_2\text{ZnFe}_2\text{O}_4$, subsequently reduced to Li-Zn , Fe^0 , and Li_2O .^{20,61,62} For the first charge process (= de-lithiation), one broad oxidation peak around 1.6 to 1.7 V

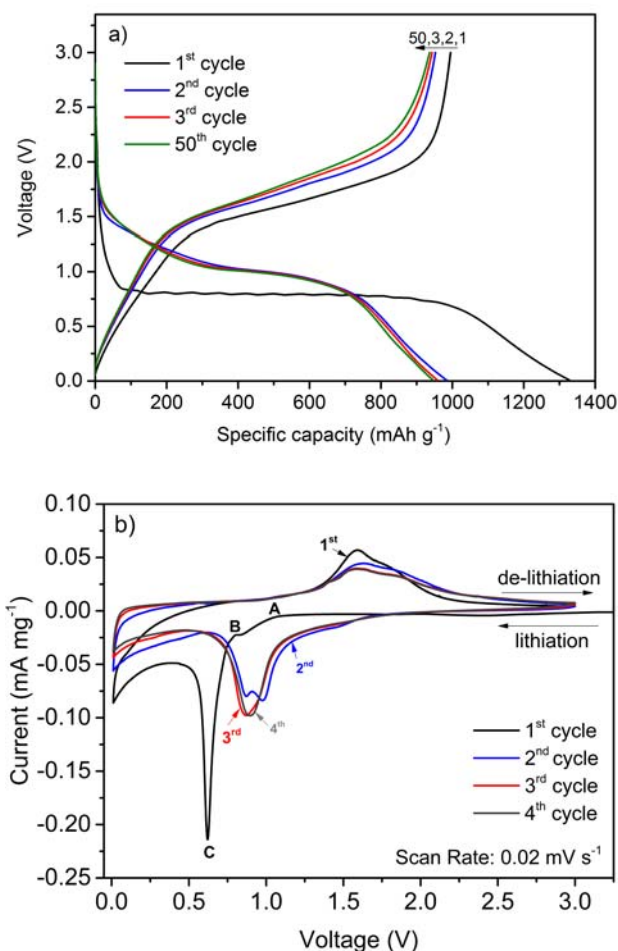


Figure 7. a) Representative voltage vs. specific capacity profiles of the constant current charge/discharge cycling of $\text{ZnFe}_2\text{O}_4/\text{C}$ at 0.1C (1st cycle) and 0.5C (2nd cycle; 3rd cycle and 50th cycle); b) Cyclic voltammetry investigation of the electrode at a scan rate of 0.02 mV s^{-1} displaying cycles 1–4. Voltage limits: 0.01 V and 3 V.

and a shoulder at a higher voltage are observed. In case of the second cathodic scan, the peaks are shifted to a higher voltage and separated into two peaks at around 0.8 and 1.0 V, indicating that the reduction proceeds by a two-step process, while the two broad corresponding anodic peaks appear at 1.6 and 1.9 V. Subsequent scans (3rd and in particular the 4th cycle) show most likely only one reduction peak, which is slightly shifted to lower potentials upon continuous sweeps, and two oxidation peaks, associated with two different oxidation reactions (Figure 7b).

In order to investigate the initial electrochemical reaction of ZnFe_2O_4 with lithium, *in situ* XRD analysis coupled with galvanostatic lithiation and de-lithiation has been carried out. Figure 8a displays the *in situ* XRD patterns, while Figure 8e depicts the corresponding voltage profile of the first electrochemical alloying and de-alloying reaction. For clarity reasons, a more detailed presentation of different scans in the 2θ range from 61 to 63 is given in Figures 7b–d. The numbers of Figure 8e correspond to the

XRD scan numbers and can be correlated to those in Figure 8c and d. The voltage profile illustrated in Figure 8e can be split in three different sections: A, B and C in analogy to the cyclic voltammetry experiment (Figure 8b). As typically occurring during the first discharge (= lithiation process), the voltage rapidly dropped to below 1.0 V. Afterwards, several processes were found to take place concurrently. Within section A, it is assumed that the XRD patterns acquired in this voltage region (scan 1 to 3 in Figure 8b) reveal a shift of the peak to lower 2θ values, indicating an increase of the lattice parameters due to the expansion of the ZnFe_2O_4 host lattice caused by the uptake of lithium within the spinel structure and the concomitant displacement of the Zn^{2+} ions into the octahedral 16c sites.⁶³ This expansion is in good agreement with the JCPDS reference 00-040-116 for $\text{Li}_{0.5}\text{ZnFe}_2\text{O}_4$. However, only a slight shift of the peak is detected in this work, which is even within the error range. Thus, no clear statement can be made. In case of the scans 4 to 17, the intensity of the peak at 62.4° decreases and eventually disappears in scan 17 (Figure 8c). At the same time, a new XRD peak is observed at 61.7° , indicating a phase change of the lithiated sample. Thackeray et al. reported the formation of $\text{Li}_{1.5}\text{Fe}_3\text{O}_4$, obtained during lithium insertion in Fe_3O_4 , which is an intermediate between spinel and rock-salt.⁶³ This agrees very well with the present result of a phase transition from spinel to rock-

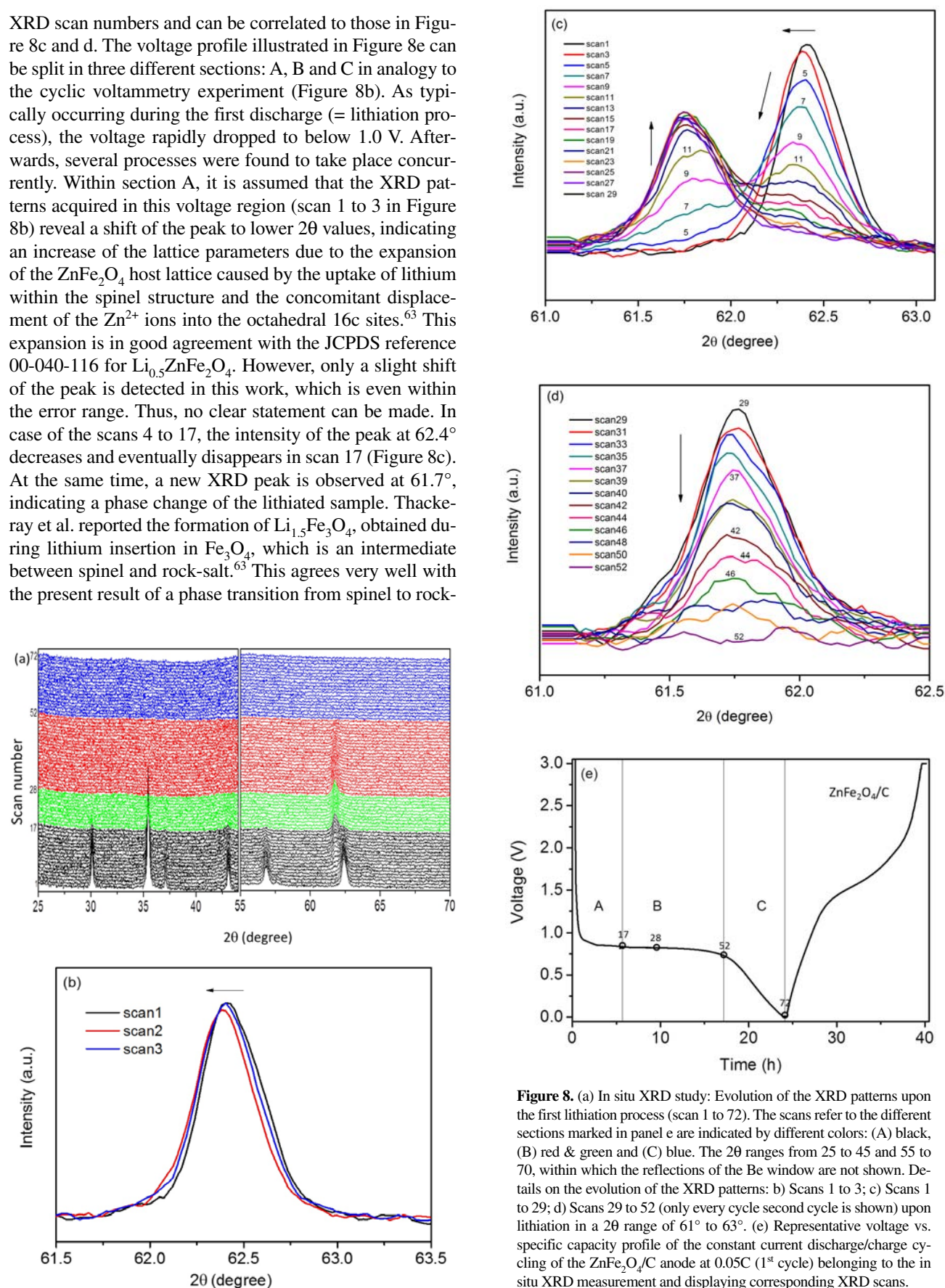
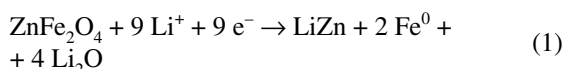


Figure 8. (a) In situ XRD study: Evolution of the XRD patterns upon the first lithiation process (scan 1 to 72). The scans refer to the different sections marked in panel e are indicated by different colors: (A) black, (B) red & green and (C) blue. The 2θ ranges from 25 to 45 and 55 to 70, within which the reflections of the Be window are not shown. Details on the evolution of the XRD patterns: b) Scans 1 to 3; c) Scans 1 to 29; d) Scans 29 to 52 (only every cycle second cycle is shown) upon lithiation in a 2θ range of 61° to 63° . (e) Representative voltage vs. specific capacity profile of the constant current discharge/charge cycling of the $\text{ZnFe}_2\text{O}_4/\text{C}$ anode at 0.05C (1st cycle) belonging to the in situ XRD measurement and displaying corresponding XRD scans.

salt for $\text{Li}_x\text{ZnFe}_2\text{O}_4$. Bresser et al. proposed that the partially lithiated $\text{Li}_x\text{ZnFe}_2\text{O}_4$ was decomposed to Li_2O and a new rock-salt phase of metal oxide, in which all the iron was reduced to Fe^{2+} .²⁰ Upon further lithiation within the main potential plateau (section B in Figure 8e), the newly formed phase shows its highest peak intensity at scan 29. Subsequently, the intensity of the peak decreased with discharge depth, and it completely disappears at scan 52 (at the end of the long plateau), which indicates the complete decomposition to Zn^0 , Fe^0 , and Li_2O (Figure 8d). In case of the final section C (from scan 52 to 72), no XRD features indexed to the particles of Zn and Li-Zn alloy, which is known to generate during this potential region,^{30,32,34,64} could be detected. Thus, we also have faith in the assumption that the fully lithiated phase consisting of LiZn and Fe^0 in an amorphous Li_2O matrix is most likely to be amorphous. In conclusion, the first lithiation reaction can be described by equation (1).



The charge/discharge cycling curves and Coulombic efficiency curves of the $\text{ZnFe}_2\text{O}_4/\text{C}$ composite as well as

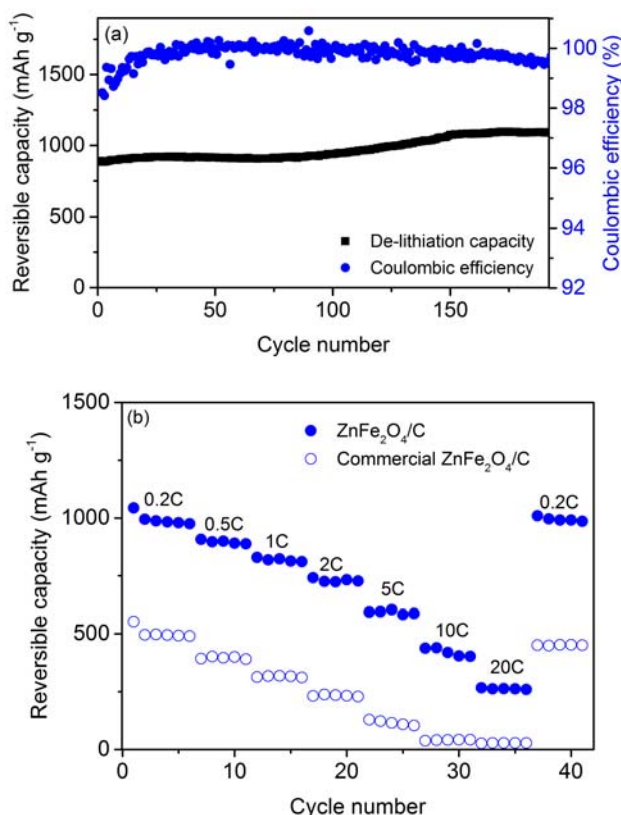


Figure 9. a) De-lithiation capacity curves and coulombic efficiency curves of the constant current cycling of $\text{ZnFe}_2\text{O}_4/\text{C}$ at 0.1C (1st cycle) and 0.5C (following cycles); b) Reversible capacity curves of $\text{ZnFe}_2\text{O}_4/\text{C}$ at different specific currents (C-rate investigation). Cut-off voltages for a) and b): 0.01 V and 3.0 V.

the rate performance of both the commercial $\text{ZnFe}_2\text{O}_4/\text{C}$ and self-prepared $\text{ZnFe}_2\text{O}_4/\text{C}$ composites are illustrated in Figure 9a and b, respectively. The $\text{ZnFe}_2\text{O}_4/\text{C}$ composite shows a superior cycling stability with a high reversible capacity, even up to 190 cycles, followed by slight capacity increase process upon continuous cycling of the electrode, finally stabilizing at around 1,091 mAh g⁻¹. A similar behavior (gaining extra capacity) was also presented for other transition metal oxides^{65,66} to be associated with the (partially) reversible formation of a polymeric layer on the particles surface.²⁰ The excellent cycling stability of ZnFe_2O_4 benefits from its structure, which offers an interesting combination of alloying and conversion mechanisms, and the carbon layer.

In particular, the prepared $\text{ZnFe}_2\text{O}_4/\text{C}$ composite electrode exhibits a superior rate performance, compared to the commercial $\text{ZnFe}_2\text{O}_4/\text{C}$. As displayed in the charge/discharge rate performance investigation in Figure 9b, the reversible capacity decreases from 1,043 mAh g⁻¹ to 216 mAh g⁻¹, when the charge/discharge rate increases in steps from 0.2C to 20C. When the rate finally returns to 0.2C, a capacity of 1,008 mAh g⁻¹ can be recovered. In contrast, the commercial $\text{ZnFe}_2\text{O}_4/\text{C}$ electrode demonstrates lower capacity as well as a dramatic decrease in capacity at high specific currents.

3. 3. Study on Metal Ion Dissolution in the Electrolyte From ZnFe_2O_4 /carbon Electrode and Comparison to Other Anode Active Materials

In general, the mass loss of metals from the electrode and/or active material can be most likely related to two contributions: 1) the active material particles lost contact from the composite electrode during the cycling, e.g. due to an enhanced volume expansion/shrinkage, whereby the detached metal particles can be mainly found close to the separator; 2) metal ions dissolved in the electrolyte, similar to metal dissolution from other conversion materials⁶⁷ and to metal ion dissolution from LIB cathode materials.^{68–70}

In this work, only the latter degradation mechanism, i.e. the metal ion dissolution in the organic solvent-based electrolyte, is studied. Iron and zinc dissolution from ZnFe_2O_4 electrodes during the repeated lithiation/de-lithiation process was confirmed by TXRF studies (see Table 2). The uncycled electrodes (storage for 5 days at 20 C) presented a minor dissolution from the electrodes, i.e. 0.0020% Fe loss and 0.0110% Zn loss, related to the active masses of iron and zinc in the sample. In case of cycled electrodes (up to 100 cycles), iron exhibits a slightly increasing metal ion dissolution of up to 0.0049% after 100 cycles, while the zinc content after cycling is even lower than during storage. Overall, both zinc and iron display only minor metal dissolution from ZnFe_2O_4 /carbon electrodes in comparison to Mn, Ni or Co dissolution from cathode materials such as $\text{LiNi}_{0.33}\text{Mn}_{0.33}\text{Co}_{0.33}\text{O}_2$.

Table 2. Zn and Fe contents (ppm) by metal dissolution in the electrolyte (EC:DEC (3:7), 1M LiPF₆) after cycling or storage of ZnFe₂O₄/C electrodes, detected by the TXRF method. Fe and Zn losses (%) are related to the sample weight. electrolyte solution: 100 μ L; active material of ZnFe₂O₄: 1.55 mg cm⁻², the error for the active material (ZnFe₂O₄) is 0.02 mg cm⁻².

Cycling/storage conditions (at 20 °C)	Fe Content (ppm)	Zn content (ppm)	Fe losses from anode (%)	Zn losses from anode (%)
Storage for 5 days	0.35 $\pm$ 0.04	1.90 $\pm$ 0.03	0.0020 $\pm$ 0.0003	0.0110 $\pm$ 0.0002
1 st cycle	0.39 $\pm$ 0.06	0.14 $\pm$ 0.03	0.0022 $\pm$ 0.0004	0.0008 $\pm$ 0.0002
10 th cycle	0.41 $\pm$ 0.06	–	0.0024 $\pm$ 0.0004	–
50 th cycle	0.22 $\pm$ 0.04	0.06 $\pm$ 0.02	0.0013 $\pm$ 0.0003	0.0004 $\pm$ 0.0002
100 th cycle	0.85 $\pm$ 0.05	0.05 $\pm$ 0.03	0.0049 $\pm$ 0.0003	0.0003 $\pm$ 0.0002

From our studies, there are major differences for the metal ion dissolution in the electrolyte for different high-capacity anode materials, such as Si, Sn and Ge. While silicon does not show dissolution at all, tin and germanium can be found to a certain amount in the electrolyte (see Tables 3 and 4; for experimental details about the materials and electrode composition see supporting information). Ge exhibits a loss of 0.0017% after storage for 5 days, which increases to 0.0142% Ge loss after 100 cycles (Table 2). For the tin-based electrodes, the metal dissolution is even higher, i.e. 0.0053% Sn loss after 5 days of storage and even 0.4200% Sn loss after 100 cycles (Table 3). Therefore, the metal ion dissolution of Sn-based electrodes is in the same order of magnitude as for Mn, Ni or Co dissolution from e.g. LiNi_{0.33}Mn_{0.33}Co_{0.33}O₂-based cat-

hode materials.⁷⁰ However, it has to be kept in mind that metal ion dissolution from the cathode strongly depends on the upper cut-off potential.⁶⁹

In summary, the metal ion dissolution from certain anode active materials, including Sn, Ge and ZnFe₂O₄, should not be underestimated and may have an impact on the overall cell performance and cycling stability. In general, there are several factors which may influence the metal ion dissolution behavior, i.e. the active material properties (particle size, specific surface area, carbon surface coating) and the electrochemical cycling conditions (type of electrolyte, operation potential range, operation temperature, etc.). In case of ZnFe₂O₄, the carbon coating may effectively hinder the metal ion dissolution, while for the Ge and Sn based materials, where no carbon coating was applied, the metal ion dissolution may be enhanced. Further investigations are needed to study systematically the influence of metal ion dissolution on the electrochemical behavior, i.e. to find a correlation to capacity fading and impedance increase.

4. Conclusion

In summary, ZnFe₂O₄ nanoparticles were successfully prepared using a novel ionic liquid-assisted synthesis method. The obtained material demonstrated a homogenous distribution of ZnFe₂O₄ particles, ranging from 40 to 80 nm in particle diameter. After carbon coating, the resulting ZnFe₂O₄/C composite displayed a stable capacity of ca. 1.091 mAh g⁻¹ for 190 cycles at 1C and a high rate capability up to 20C. The great electrochemical performance can be ascribed to the relatively uniform distributed particles, the large BET surface area after carbon coating and highly conductive carbon layer. However, it has to be kept in mind that conversion materials like ZnFe₂O₄ suffer from a large irreversible capacity in the first cycle which is in turn related to a large loss of active lithium from the cathode. Moreover, it has to be considered that ZnFe₂O₄, as like other conversion-type anode materials, displays a relatively poor energy efficiency, which is related to the large voltage hysteresis, i.e. the difference between the lithiation and de-lithiation potential, which also occurs to a lower extent in insertion electrodes.⁷¹ Figure

Table 3. Sn content by metal dissolution in the electrolyte (EC:DEC (3:7), 1M LiPF₆) after cycling or storage of Sn-based electrodes, detected by the TXRF method. Fe and Zn losses (%) are related to the sample weight. electrolyte solution: 100 μ L; active material of Sn: 1.32 mg cm⁻², the error for the active material (Sn) is 0.02 mg cm⁻².

Cycling/storage conditions (at 20 °C)	Sn Content (ppm)	Sn losses from anode (%)
Storage for 5 days	0.80 $\pm$ 0.02	0.0053 $\pm$ 0.0002
1 st cycle	8.16 $\pm$ 0.63	0.0547 $\pm$ 0.0043
10 th cycle	16.25 $\pm$ 0.74	0.1091 $\pm$ 0.0049
50 th cycle	32.16 $\pm$ 0.82	0.2100 $\pm$ 0.0055
100 th cycle	61.37 $\pm$ 0.93	0.4200 $\pm$ 0.0062

Table 4. Ge content by metal dissolution in the electrolyte (EC:DEC (3:7), 1M LiPF₆) after cycling or storage of Ge electrodes, detected by the TXRF method. Fe and Zn losses (%) are related to the sample weight. electrolyte solution: 100 μ L; active material of Ge: 1.51 mg cm⁻²; the error for the active material (Ge) is 0.02 mg cm⁻².

Cycling/storage conditions (at 20 °C)	Ge Content (ppm)	Ge losses from anode (%)
Storage for 5 days	0.30 $\pm$ 0.02	0.0017 $\pm$ 0.0002
1 st cycle	1.02 $\pm$ 0.02	0.0060 $\pm$ 0.0002
10 th cycle	1.51 $\pm$ 0.02	0.0090 $\pm$ 0.0002
50 th cycle	1.90 $\pm$ 0.02	0.0112 $\pm$ 0.0002
100 th cycle	2.42 $\pm$ 0.03	0.0142 $\pm$ 0.0002

10 exemplarily displays the potential vs. capacity (normalized) profiles of the different anode materials, i.e. graphitic carbon, silicon/graphite composite and the $\text{ZnFe}_2\text{O}_4/\text{C}$ composite material. Here, it can be clearly seen that the voltage hysteresis increases from graphite (intercalation mechanism) over Si/C (alloying/intercalation mechanisms) to $\text{ZnFe}_2\text{O}_4/\text{C}$ (conversion and “alloying” mechanism together with intercalation into carbon). Considering that this hysteresis (especially at higher C-rates) will result in significant losses of charge energy and in turn these losses are to a large extent equivalent to heat losses, that heat up the cell and thus will damage heat-sensitive cell components such as the electrolyte, it is questionable, whether conversion electrode materials and other electrode materials with large differences in the charge and discharge potentials will have a relevance in batteries. Future research for conversion materials, like ZnFe_2O_4 , should focus on the topic how to lift the potential of the lithiation process and lower the potential of de-lithiation reaction and thus to increase the energy efficiency.

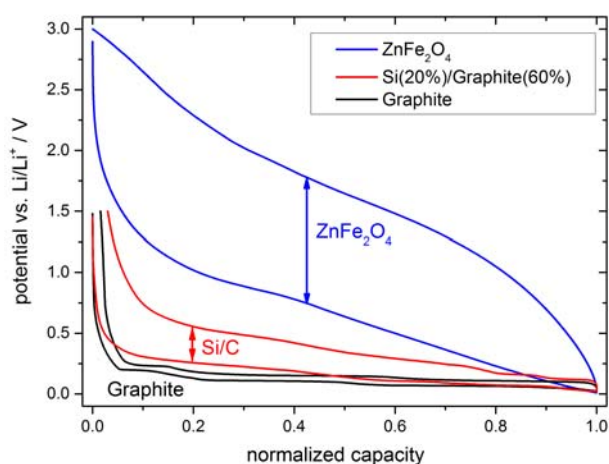


Figure 10. Potential vs. normalized capacity profiles of different anode materials: $\text{ZnFe}_2\text{O}_4/\text{C}$ (0.1C), Si/graphite (0.1C) and graphite (0.1C).

Studies on the Zn and Fe metal ion dissolution process in the electrolyte upon storage and cycling revealed only minor dissolution for both Zn and Fe with increasing cycle number in comparison to Mn, Ni or Co dissolution from cathode materials such as $\text{LiNi}_{0.33}\text{Mn}_{0.33}\text{Co}_{0.33}\text{O}_2$. However, metal ion dissolution cannot be underestimated and may depend on several active material characteristics such as the particle properties or the carbon coating layer, as well as different electrochemical characteristics such as the type of electrolyte or the cycling conditions. All these factors can influence the metal ion dissolution and, in turn, the electrochemical performance, i.e. the cycling stability and capacity fading. Furthermore, we could show that metal ion dissolution is

even more distinct for Sn- and Ge-based anode materials. The metal dissolution of Sn-based electrodes is even in the same order of magnitude as for metal ion dissolution (Mn, Ni, Co) from cathode materials like $\text{LiNi}_{0.33}\text{Mn}_{0.33}\text{Co}_{0.33}\text{O}_2$. A systematic study is necessary in order to find a clear correlation between metal ion dissolution vs. capacity fading and impedance increase.

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Povzetek

To delo opisuje uspešno sintezo z ogljikom prevlečenega anodnega materiala ZnFe_2O_4 s pomočjo ionskih tekočin. Sintetizirani material ZnFe_2O_4 izkazuje ozko porazdelitev delcev v razponu od 40 do 80 nm. Material nudi zanimivo kombinacijo mehanizmov legiranja in konverzije in je zmožen sprejeti devet ekvivalentov litija na enoto formule, kar se odraža v visoki specifični kapaciteti ($\geq 1,000 \text{ mAh g}^{-1}$). Anodni kompozit kaže stabilno kapaciteto, $\sim 1,091 \text{ mAh g}^{-1}$ 190 ciklov, pri srednjem de-litacijskem potencialu 1.7V ter hitrosti polnjenja in praznjenja 1C. Nadalje, material izkazuje odlične lastnosti pri visokih tokovnih obremenitvah (20C) in kaže stabilno reverzibilno kapaciteto 216 mAh g^{-1} . Narejene so bile študije korozije Fe in Zn v aktivnem materialu ZnFe_2O_4 ter primerjane z anodnim materialom z visoko kapaciteto na osnovi silicija, germanija in kositra. Na podlagi rezultatov ugotavljamo, da igra korozija kovin iz anodnega materiala veliko vlogo pri celokupnem odzivu baterije in stabilnosti praznjenja in polnjenja.

Scientific paper

Conformational NMR Study of Bistriazolyl Anion Receptors

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Dedicated to late Professor Janez Jamnik.

Abstract

Conformational features of pyridine- and pyrimidine-based bistriazolyl anion receptors dissolved in acetonitrile- d_3 were assessed by multidimensional, heteronuclear NMR spectroscopy. NOESY correlation signals suggested preorganization of both host molecules in solution in the absence of anions. In addition, only a single set of signals was observed in the ^1H NMR spectra, which suggested a symmetrical conformation of anion receptors or their conformational exchange that is fast on the NMR time-scale. Furthermore, the predominant conformations of the pyridine- and pyrimidine-based anion receptors are preserved upon addition of chloride, bromide, and acetate anions. Chemical shift changes observed upon addition of anions showed that the NH (thio)urea and triazole protons are involved in anion-receptor interactions through hydrogen bonding.

Keywords: Anion receptors, conformational analysis, host–guest systems, NMR spectroscopy

1. Introduction

Small molecules that have appropriate functional groups or binding moieties may serve as sensors for anions, and this has been the focus of intensive research during the last decades.¹ Anions play important roles in biological and chemical processes, and are ingredients of pollutants released in the environment for example; therefore anion sensing remains a topic of interest. Often hydrogen-bonding is involved in the binding event of a potential receptor molecules and an anion, and this is mostly occurring through classical N–H and O–H hydrogen bond donors of receptor molecules. However, recently substantial attention has gone to compounds containing polarized C–H bonds,^{2,3} such as 1,2,3-triazoles^{4,5} due to their large dipole moment (up to 5 D) with the positive end at the 5-hydrogen atom of the heterocycle.^{6#} The ver-

satility of the click reaction⁷ and alternative strategies^{8,9} towards a very wide variety of 1,2,3-triazoles has stimulated our research endeavors. Previously we have described bistriazolyl anion receptors with a central pyridine^{10#} and a central pyrimidine.¹¹

In this study we have analyzed the prospective conformational preorganization and conformational changes of earlier synthesized bistriazolyl anion receptors¹¹ upon addition of various anionic species using NMR techniques. Bistriazolyl anion receptors differ in their middle aromatic ring, compound **1** contains a pyridine and **2** contains a pyrimidine moiety (Figure 1). These two moieties could have a different effect on the preorganization, which is crucial to achieve high binding affinities of receptors. We were intrigued by their conformational features, but unfortunately we were not able to obtain large enough crystals for single-crystal X-ray diffraction. Alternatively,

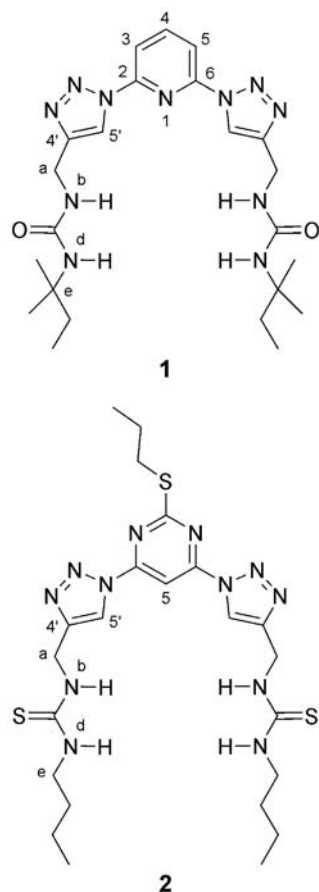


Figure 1. Anion receptors **1** and **2** with atom numbering.

a conformational study was performed by means of NMR spectroscopy. We were encouraged by the observations in our studies on indole anion receptors, which showed different conformational preferences in the absence and in the presence of anions.^{12–14} The conformational preferences of bistriazolyl anion receptors and their complexes with anions were herein correlated with the electronic properties of pyridine, pyrimidine, triazole and (thio)urea moieties with the intention of obtaining new insight and therefore give fresh stimulus to the design of anion-specific receptors.

2. Results and Discussion

The receptors **1**^{10#} and **2**¹¹ were prepared as published previously. The pyridine-based host **1** contains several single bonds, which allow a large rotational flexibility. Nevertheless, conformational preferences along four bonds are expected to influence the binding affinity of the receptor, and involve relative orientations of pyridine and triazole rings as well as preferences along the methylene groups connecting triazole rings and urea moieties (rotations along C-2–N-1' and C-4'–C-a bonds denoted by red

arrows in Figure 2). The conformational features of the pyridine-based receptor were assessed by 2D NOESY experiments (Figure S1 in Supplementary material). Interestingly, the triazole H-5' proton showed no NOESY correlation signals with the pyridine CH protons, which sugge-

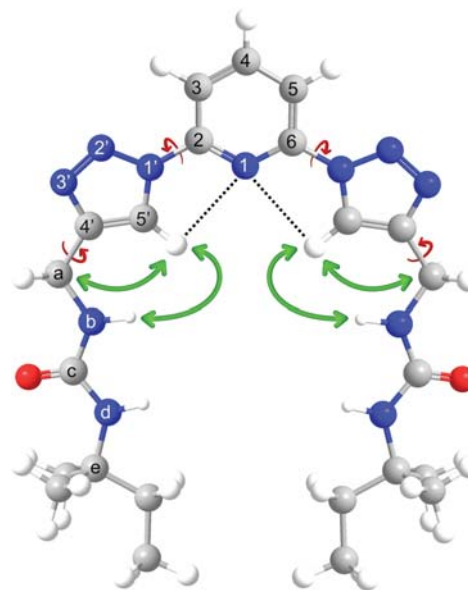


Figure 2. Predominant conformation of **1** in acetonitrile- d_3 at 298 K based on NOESY cross-peaks; the key NOESY correlations are marked with green arrows. Red arrows indicate four main single bonds, which are expected to define the binding affinity of the receptor.

sted a mutual orientation of the two heterocyclic rings in which triazole protons are predisposed for hydrogen bonding with N-1 of pyridine (Figure 2).

In addition, NOESY cross-peaks were observed between triazole H-5' protons and urea moieties (H-b) as well as methylene H-a protons. These NOESY results corroborate preorganization of pyridine-based host **1** as shown in Figure 2. Only a single set of signals was observed for both triazole-urea moieties attached to C-2 and C-6 in the ^1H NMR spectrum, which suggests a symmetrical conformation of both triazole moieties in host **1** or their conformational exchange that is fast on the NMR time-scale.

Interaction of anions with receptor molecules was followed through changes in chemical shifts as concentration of tetrabutylammonium salts was increased. Chemical shift changes observed upon addition of one equivalent of chloride anions clearly corroborate that urea H-b and H-d protons, and triazole H-5' protons are involved in anion-receptor interactions through hydrogen bonding (Figure 3). The preorganized conformation of **1** in the absence of anions is predisposed for anion binding. Additionally, a significant deshielding of triazole H-5' proton of up to 0.6 ppm was observed, while pyridine and aliphatic

tic protons showed only negligible chemical shift changes. Similar chemical shifts changes were observed upon addition of bromide anions, as urea and triazole protons showed moderate deshielding between 0.3 and 0.5 ppm, while the other proton chemical shift changes were insignificant. Major deshielding of urea protons H-b and H-d was observed in the presence of acetate anions ($\Delta\delta$ 1.5 and 1.0 ppm, respectively), whereas triazole H-5' proton showed minor chemical shift change (Figure 3), which could be attributed to the planarity of acetate anions and their predisposition for bidentate interactions with the urea moieties in **1**.

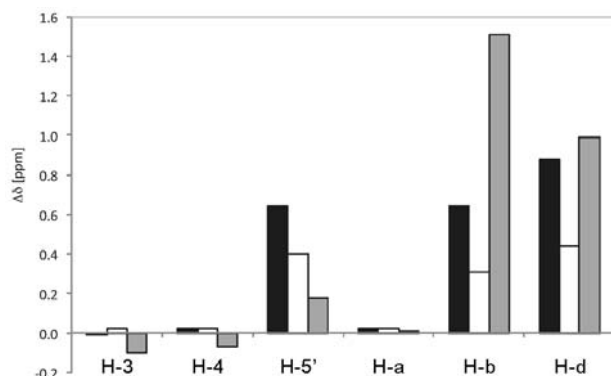


Figure 3. ^1H NMR chemical shift changes, $\Delta\delta = \delta(\text{in the presence of anions}) - \delta(\text{in the absence of anions})$, induced by addition of one equivalent of chloride (■), bromide (□) and acetate (▒) anions to receptor **1**.

2D NOESY spectrum of the **1**- Cl^- complex showed cross-peaks that were observed for the receptor in the absence of anions, which suggested that the predominant conformation of **1** is preserved upon addition of chloride anions (Figure S2). The most significant difference was increase in the volume integral of the correlation signal corresponding to the proximity of the triazole H-5' and urea H-b protons (Figure 4). This observation could indicate that interactions between anions and H-5', H-b and H-d led to a restriction of the rotational degrees of freedom along the C-4'-C-a single bond in **1**. There were no NOESY cross-peaks that would suggest major changes of conformational features for the **1**- Cl^- complex with respect to the conformation of host **1** in the absence of anions. Similarly, an increased volume of the H-5'-H-b NOESY cross-peak was observed upon addition of bromide and acetate anions to **1** (Figure 4), which also suggested that conformational preferences of **1** are preserved upon addition of anions.

A second type of bistriazolyl anion receptors contained a pyrimidine ring. The two electronegative nitrogen atoms in a pyrimidine ring polarize the C-H bond at position 5, resulting in a stronger hydrogen bond donor capacity in comparison with benzene. The good anion binding

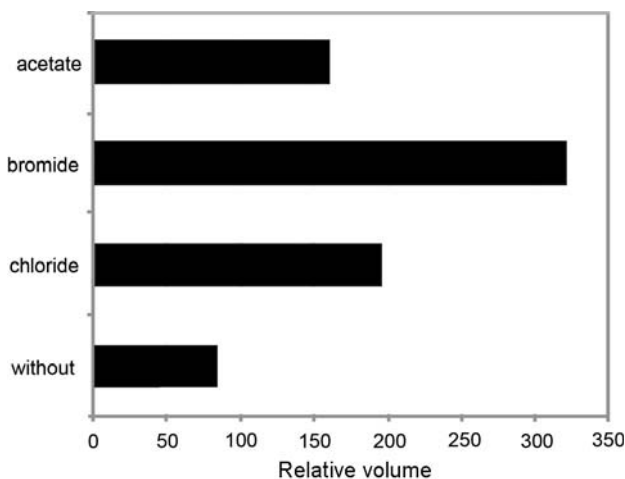


Figure 4. Relative volumes of the H-5'-H-b NOESY cross-peak in **1** in the absence and in the presence of different anions. NOESY spectra (τ_m 200 ms) were acquired in acetonitrile- d_3 at 298 K. The cross-peak between triazole H-5' and methylene H-a protons of **1** in the absence of anions was used as a reference, which was arbitrarily set to 100 units.

properties of pyrimidine together with the high binding affinities of several triazolophanes reported by Flood *et al.*^{15,16} prompted us to synthesize 4,6-bis-(1,2,3-triazol-1-yl)-pyrimidine receptors and analyze their anion recognition abilities. The binding affinities determined for the pyrimidine-based receptors were rather disappointing: the acyclic receptor **2** showed moderate binding affinities (e.g. $\log(K_a) = 2.2$ for chloride and 1.9 for bromide anions, see supplementary material for details) and attempts to improve the binding properties by synthesizing cyclic derivatives were not successful. To find an explanation for these observations, we wanted to take a closer look at the possible conformations of receptor **2**. Unfortunately, crystals for X-ray crystallographic analysis could not be obtained. Alternatively, the conformation of the pyrimidine-based host **2** was further investigated by the use of NMR spectroscopy in solution.

Similar to **1**, the pyrimidine-based host **2** exhibits multiple single bonds, two pyrimidine-triazole linkages and methylene connections between the triazole ring and thiourea moiety that are potentially critical for its anion binding and recognition abilities. The conformational properties of receptor **2** and its complexes with anions were first evaluated with 2D NOESY experiments. Surprisingly, no NOESY cross-peak could be observed between the triazole H-5' protons and the pyrimidine H5 proton. Moreover, a NOESY correlation signal was observed between the triazole protons and the propylsulfanyl group (Figure S3). Accordingly, the pyrimidine-based host **2** is conformationally preorganized in solution in such a way that the pyrimidine H5 proton is oriented away from the triazole protons and thiourea moiety. The NOESY spectra confirmed the spatial proximity between the triazole H-5'

and methylene H-a as well as thiourea H-b protons (schematically presented in Figure 5). Nevertheless, the C-4'–C-a single bonds in **2** that link the triazole rings with the thiourea functionalities allow a quite unrestricted rotational flexibility. Similar NOESY cross-peaks were also observed for receptor **2** in the presence of chloride anions, suggesting that no conformational changes occur upon addition of the anions (Figure S4). A possible conformation, explaining these observations, is depicted in Figure 5.

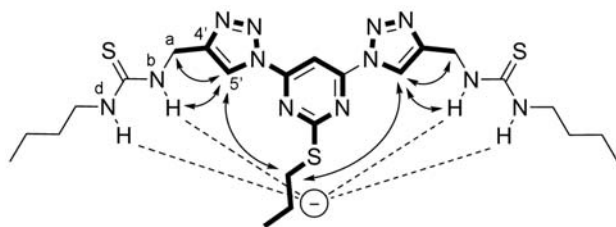


Figure 5. Predominant conformation and position of chloride anion in **2-Cl⁻** complex in acetonitrile-*d*₃ at 298 K based on chemical shift changes and NOESY cross-peaks (τ_m 200 ms). The key NOESY correlations are marked with arrows.

Variable-temperature measurements were performed in order to reduce the rotational flexibility of host molecule. Cooling of the solution containing receptor **2** in the absence of anions down to 233 K resulted in two sets of signals for the thiourea H-b and H-d protons, methylene H-a and H-e protons, as well as triazole H-5' protons in a ratio 3:2, which suggested the presence of two different conformers (Figure 6).

NOESY spectra at 233 K revealed no specific differences between the two species. The strong NOESY correlation signal between the well-resolved methylene

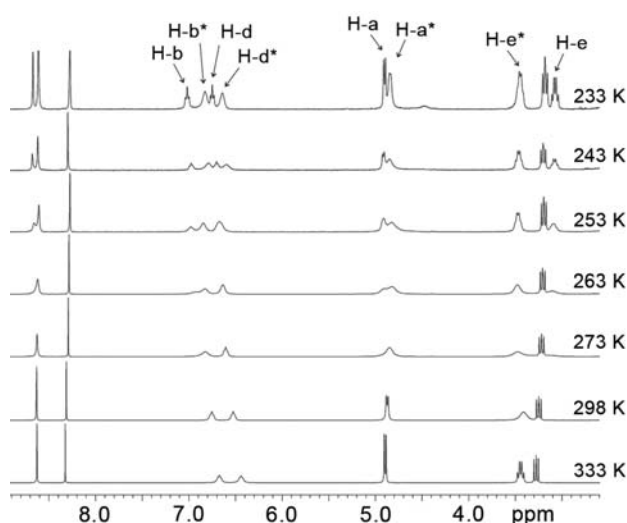


Figure 6. ¹H NMR spectra of **2** in acetonitrile-*d*₃ in the temperature range from 233 to 333 K. Protons of the major species are denoted with asterisk (*).

H-e protons of the butyl chain of the major and minor species suggested a dynamic exchange between the two conformers. The observed two sets of signals at 233 K can be caused by the formation of different aggregates, perhaps monomeric and dimeric complexes. The structural properties of two species observed at 233 K were therefore analyzed by the use of diffusion ordered spectroscopy (DOSY). However, no difference in the translational diffusion coefficients was observed, which indicated that both species are monomeric units ($D = 0.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 233 K). Unfortunately, no unambiguous evaluation of conformational properties of receptor **2** at 233 K could be achieved based on NOESY and DOSY spectra.

3. Conclusion

Multiple single bonds in the bistriazolyl anion receptor **1** with a central pyridine ring allow a fairly unrestricted rotational flexibility. However, 2D NOESY experiments showed only cross-peaks between the triazole and urea protons, and none between the pyridine and triazole protons, which suggested preorganization of the pyridine-based host **1** in such a way that the protons of the triazole ring are predisposed for hydrogen bonding with N-1 of pyridine. In addition, only a single set of signals was observed in the ¹H NMR spectrum, which suggested a symmetrical conformation of host **1** or their conformational exchange that is fast on the NMR time-scale. Chemical shift changes observed upon addition of chloride, bromide, and acetate anions corroborated that NH urea protons and triazole protons are involved in anion-receptor interactions through hydrogen bonding. Furthermore, the correlation signals in 2D NOESY spectra of the anion-receptor complexes confirmed that the predominant conformation of **1** is preserved upon addition one equivalent of various anions. Contrary to our expectations, no NOESY cross-peak could be observed between the triazole H-5' protons and the pyrimidine H-5 proton in pyrimidine-based receptor **2**. Nevertheless, receptor **2** is also preorganized in solution, with the pyrimidine proton oriented away from the triazole protons and thiourea moiety. Similar NOESY correlation signals were observed for receptor **2** in the presence of chloride anions, suggesting that no conformational changes occur upon addition of the anions. Our study demonstrates that combinations of pyridine or pyrimidine rings with triazole moiety offer intriguing scaffolds for the design of novel anion receptors that were preorganized in solution for hydrogen bonding interactions.

4. Experimental

The receptors **1** and **2** were prepared as reported in Supplementary materials (page S2). ¹H NMR and 2D NMR experiments including ¹H–¹³C and heteronuclear

single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) were performed on a DD2 Agilent Technology NMR spectrometer at a frequency of 297.80 MHz and 74.89 MHz for ^1H and ^{13}C NMR, respectively. All data were recorded in acetonitrile- d_3 at 298 K, unless stated otherwise; concentrations of receptors were 2 mM. Chemical shifts are referenced to the residual solvent signal of acetonitrile- d_3 . Individual resonances in the ^1H NMR spectra were assigned on the basis of the chemical shifts, signal integrations, multiplicity, and via ^1H - ^{13}C direct and multi-bond correlation signals in 2D spectra. ^1H - ^1H NOESY spectra were acquired using a mixing time of 200 ms at 298 K. One equivalent of anions were added to anion receptor solution. All anions (chloride, bromide and acetate) were added as tetrabutylammonium salts.

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Povzetek

V prispevku smo s pomočjo večdimenzionalne, heteronuklearne NMR spektroskopije določili konformacijske lastnosti bistriazolilnih anionskih receptorjev dveh tipov, ki vsebujeta substituiran piridinski oziroma pirimidinski obroč. Glede na korelacijske signale v NOESY spektru smo ugotovili, da sta oba tipa anionskih receptorjev v raztopini v odsotnosti anionov predorganizirana. V ^1H NMR spektru smo opazili en sam set signalov za obe triazolil-(tio)sečninski verigi, kar kaže na simetrično konformacijo anionskih receptorjev ali da je njihova konformacijska izmenjava hitra na časovni skali NMR. Prevladujoči konformaciji piridinskih in pirimidinskih bistriazolilnih anionskih receptorjev se po dodatku kloridnih, bromidnih in acetatnih anionov bistveno ne spremenita, torej je konformacija teh receptorjev predisponirana za vezavo anionov. Na podlagi sprememb kemijskih premikov ob dodatku anionov pa smo še pokazali, da so (tio)sečninski NH in triazolni CH protoni ključni za interakcije z anioni preko vodikovih vezi.

Scientific paper

Space Charge Layer Effect in Solid State Ion Conductors and Lithium Batteries: Principle and Perspective

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This paper is dedicated to Prof. Dr. Janko Jamnik, who was my enthusiastic and helpful MPI colleague as well as with high academic level and reputation in science community.

Abstract

The space charge layer (SCL) effects were initially developed to explain the anomalous conductivity enhancement in composite ionic conductors. They were further extended to qualitatively as well as quantitatively understand the interfacial phenomena in many other ionic-conducting systems. Especially in nanometre-scale systems, the SCL effects could be used to manipulate the conductivity and construct artificial conductors. Recently, existence of such effects either at the electrolyte/cathode interface or at the interfaces inside the composite electrode in all solid state lithium batteries (ASSLB) has attracted attention. Therefore, in this article, the principle of SCL on basis of defect chemistry is first presented. The SCL effects on the carrier transport and storage in typical conducting systems are reviewed. For ASSLB, the relevant effects reported so far are also reviewed. Finally, the perspective of interface engineer related to SCL in ASSLB is addressed.

Keywords: Space charge layer, Defect chemistry, Interfacial resistance, All solid state lithium battery

1. Introduction

Space charge layer (SCL) effects correspond to carrier redistribution at space charge regions near a two-phase contact. This concept was first used by Carl Wagner to explain conductivity effects at the interface of two semiconductors.¹ It was tentatively developed to explain the interfacial phenomena in ionic conductor systems by T. Jow and J. B. Wagner.² Later on, J. Maier made fruitful achievement on understanding of SCL effects at various boundary problems, based on quantitatively derived profiles of defect concentrations through consideration of local thermodynamic and electrostatic relationships.³ From then on, SCL effects proved to be of great importance for ion conduction in solids, especially if the interfacial spacing is on the nanometre scale. In the last decades, a large number of instances for space charge effects on the ionic transport in solids have been provided. Variation of not only the magnitude but also the type of conductivity were verified in composites, grain boundaries in polycrystalline

materials and epitaxial heterostructures.^{4–21} Conductivities of various functional materials might be manipulated for fundamental research as well as application. Introduction of interfaces not only led to strong variations in conductivity, but also induced qualitatively change of the type of conductivity.

In recent years, all solid state lithium batteries (ASSLB) have attracted a great deal of attention, since they may provide solution to the safety issue as well as enhancement of energy density compared to the currently commercial rechargeable lithium batteries. The key materials for ASSLB are the solid-state electrolytes and the critical problems are the relevant solid-solid interfaces between the electrolyte and the cathode or inside the composited electrolytes. Though there have already been some discussions on SCL at the sulphide-electrolyte-based batteries,²² understanding of such effect is still in its infancy. In particular for oxide-electrolyte-based batteries, clarification as well as manipulation of SCL at the interfaces of composited electrolytes or electrolyte/cathode is highly demanded.

Therefore, in Section 2, we will address basic knowledge of the SCL effect according to defect redistribution near a two-phase contact. In Section 3, we will present typical examples of SCL effects occurred at various boundaries of functional materials. In Section 4, we will focus on the SCL effects in solid state batteries. The conclusion and outlook will be given finally.

2. Defect Chemistry at Space Charge Layers Near the Two-phase Boundary

At the boundary between the two materials, carrier redistribution in the space charge region is required from the thermodynamical point of view (uniformity of electrochemical potential).²³ The following discussion seems more closely related to a lateral two-phase contact, the basic knowledge is also applied to the interface in the systems including polycrystalline or composited materials.

In the core-space charge model,^{24,25} there are several assumptions: dilute defects on a continuum level, no structural changes up to the interface ($x = 0$), and the same mobility of defects in SCL as in the bulk ($x = \infty$). In the case of mobile carrier j , its concentration enrichment ζ in equilibrium is

$$\zeta_j^{1/z_j}(x) = \left[\frac{c_j(x)}{c_{j,\infty}} \right]^{1/z_j} = \exp \left[-\frac{e[\phi(x) - \phi_\infty]}{k_B T} \right] \quad (1)$$

where c is the carrier concentration of the defect, and ϕ the electrical potential which is determined by the Poisson equation

$$\nabla^2 \phi = -\rho / \varepsilon \varepsilon_0 \quad (2)$$

ρ represents the charge density equal to $\sum_k z_k e c_k$. In the above expressions, ε and ε_0 are the relative permittivity and the dielectric constant of free space, k_B and T are the Boltzmann's constant and the temperature, e is the absolute value of the electronic charge, z_k is the charge number of the defect k .

If all the carriers are mobile in equilibrium, their electrochemical potentials are constant. Then combination of Eq. (1) and (2) leads to the Poisson-Boltzmann relationship in the direction perpendicular to the interface

$$\frac{\partial^2}{\partial x^2} [\phi(x) - \phi_\infty] = -\frac{e}{\varepsilon \varepsilon_0} \sum_k c_{k,\infty} z_k \exp \left[-z_k e \frac{\phi(x) - \phi_\infty}{k_B T} \right] \quad (3)$$

Note that in this case the excess charge is directly given by the species that determine the conductivity. The solution of Eq. (3) for semi-infinite boundary conditions comes to the Gouy-Chapman profile,^{24,26,27} which can be written as

$$\zeta_\pm(x) = \frac{c_\pm(x)}{c_{\pm\infty}} = \left(\frac{1 + \vartheta_\pm \exp(-x/\lambda)}{1 - \vartheta_\pm \exp(-x/\lambda)} \right)^2 \quad (4)$$

where $c_{+\infty} = c_{-\infty} \equiv c_\infty$ because of the electroneutrality in bulk and x being the distance from the interface. As a result, $x = 0$ and $x = \infty$ refer to the interface and the bulk, respectively. λ is the Debye length,

$$\lambda = \left(\frac{\varepsilon \varepsilon_0 k_B T}{2 z^2 e^2 c_\infty} \right)^{1/2} \quad (5)$$

The parameter ϑ (dependent on ζ_0 and hence on c_0) is defined as follows

$$\vartheta_\pm = \frac{\zeta_{\pm 0}^{1/2} - 1}{\zeta_{\pm 0}^{1/2} + 1} = -\vartheta_\mp \quad (6)$$

and represents the degree of influence. For $\vartheta_\pm = 0$, i.e. $\zeta_{\pm 0} = 1$, the defect chemistry in the space charge region is the same as in the bulk. It approaches +1 for maximum enrichment ($\zeta_{\pm 0} \gg 1$) and -1 for maximum depletion ($\zeta_{\pm 0} \ll 1$). Such profiles are shown in Figure 1a.

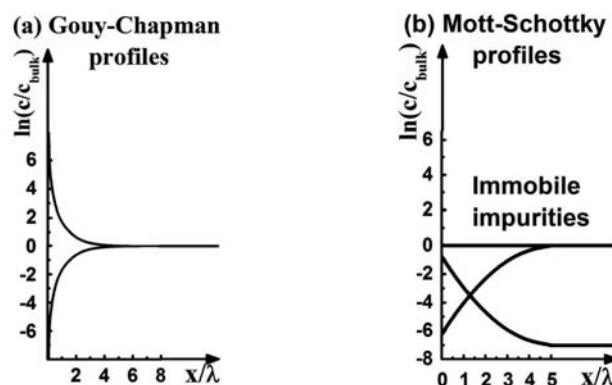


Fig.1. Typical defect profiles in the case of (a) Gouy-Chapman and (b) Mott-Schottky.

While Gouy-Chapman profiles serve as a natural picture for accumulation layers, enrichment effects can also be found in Mott-Schottky situations. Then the conductivity determining carrier has to be a minority carrier with a high mobility in order to surpass the bulk value of the majority carrier significantly. Now, we refer to an extrinsic situation in which the mobile majority species are impurity compensated in the bulk but depleted close to the interfaces. Ideally the impurity profile is assumed to be flat everywhere as shown in Figure 1b. The impurity then is considered to be incorporated during preparation but frozen under operation. In such cases, Eq. (1) is only valid for the mobile carriers. As to Eq. (2), the invariant quantity $c_{imp,\infty}$ dominates the space charge density and hence Poisson's equation reads approximately

$$\frac{\partial^2}{\partial x^2} \phi(x) = \begin{cases} -\frac{z_{imp} e c_{imp, \infty}}{\epsilon \epsilon_0} = const & \text{for } 0 < x \leq \lambda^* \\ 0 & \text{for } x \geq \lambda^* \end{cases} \quad (7)$$

Eq. (7) can be easily integrated with the boundary conditions: $\frac{\partial \phi}{\partial x}|_{x=\lambda^*} = 0$ and $\phi(x=0) = \phi_0$ denoting the define value from the potential in the electroneutral bulk by ϕ_∞ , resulting in a quadratic potential profile,^{28,29}

$$\phi(x) = \frac{-z_{imp} e c_{imp, \infty}}{2 \epsilon \epsilon_0} (x - \lambda^*)^2 \quad (8)$$

Where λ^* is the effective space charge thickness:

$$\lambda^* = \sqrt{\frac{2 \epsilon \epsilon_0}{z_{imp} e c_{imp, \infty}} (\phi_\infty - \phi_0)} \quad (9)$$

that exceeds λ by the factor $\sqrt{\frac{4 z_{imp} e}{k_B T} (\phi_\infty - \phi_0)}$.

Since the typical potential difference is on the order of several 100 mV, this equation indicates that the space charge region in the Mott-Schottky case can be significantly more extended than in the Gouy-Chapman case.

At the point $x = \lambda^*$, ζ_j becomes unity, i.e. the concentration of the mobile carrier reaches the bulk value. When $x \leq \lambda^*$, according to Eq. (1), the defect concentration can be expressed as

$$\zeta_j^{1/z_j}(x) = \left[\frac{c_j(x)}{c_{j, \infty}} \right]^{1/z_j} = \exp\left(\frac{x - \lambda^*}{2\lambda}\right)^2 \quad (10)$$

In the overlap situation (i.e. $\ell < 2\lambda^*$), the electro-neutral bulk does not exist and thus $\phi(\ell/2) \neq \phi_\infty$. Rather in

this case, the preferred boundary conditions are $\frac{\partial \phi}{\partial x}|_{x=\ell/2} = 0$ and $\phi(0) = \phi_0$. Hence, the integration of Eq. (7) yields a potential profile

$$\phi(x) = \frac{-z_{imp} e c_{imp, \infty}}{2 \epsilon \epsilon_0} \left[\left(x - \frac{\ell}{2}\right)^2 - \left(\frac{\ell}{2}\right)^2 \right] + \phi_0 \quad (11)$$

and the defect concentration profile becomes

$$\zeta_j^{1/z_j}(x) = \left[\frac{c_j(x)}{c_{j, \infty}} \right]^{1/z_j} = \exp\left\{ \frac{z_{imp}}{4\lambda^2} \left[\left(x - \frac{\ell}{2}\right)^2 - \left(\frac{\ell}{2}\right)^2 \right] - \frac{e}{k_B T} (\phi_0 - \phi_\infty) \right\} \quad (12)$$

As the mobile majority carrier is depleted, Mott-Schottky analysis mainly applies to resistive boundaries. If however a minority carriers of the same sign as the impurity has a very high mobility, it can well be conceived that its accumulation leads to a conductance increase even though the impurity level is not exceeded. As already pointed out, this demands a high ratio of $u_{minority}/u_{majority}$ (u , carrier mobility).

3. Space Charge Layer Effects on Conductivities at Various Boundaries

Based on the above discussed carrier redistribution at SCL, conductivity anomaly at various boundaries has been quantitatively studies. Briefly speaking, the SCL concept has quantitatively accounted for the composites (Figure 2a), polycrystallines (Figure 2b) and heterostructural multilayers (Figure 2c), the typical examples which are respectively addressed in the following.

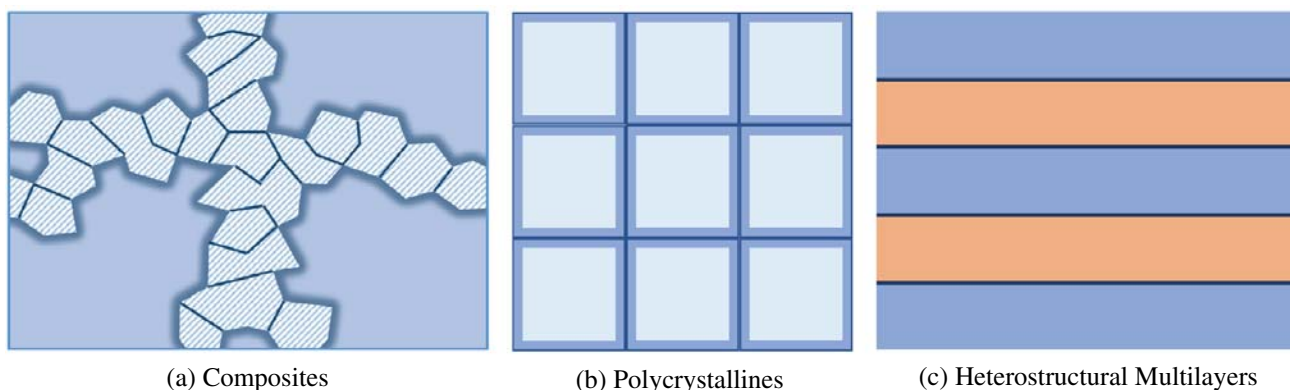


Fig. 2. Typical space charge layer situations of (a) composites; (b) polycrystallines; (c) heterostructural multilayers.

The earliest attractive composite known as “composite electrolytes” or “heterogeneous electrolytes” was the two-phase system $\text{LiI-Al}_2\text{O}_3$, reported by Liang et al. with the ionic conductivity 50 times greater than that of the pure LiI .³⁰ Concerning that the Al_2O_3 particles are insulating, the conductivity enhancement is attributed to the boundary layer phenomena, which can be perfectly explained by the SCL effects. Besides Al_2O_3 similar oxides including SiO_2 , CeO_2 , ZrO_2 and BaTiO_3 were found to be also effective. Since there were a lot of papers on this respect, readers can refer to the literature in Ref. [3]. Further with mesoporous Al_2O_3 as the insulating phase, the enhancement of conductivity can be more effective, which is satisfactorily explained in the framework of the ideal space-charge model.³¹ Here, it is worthwhile mentioning “Soggy Sand Electrolytes”.^{32,33} The total conductivities are highly increased by virtue of the very high conductivities at the edge of the space charge profiles. This work is of significance owing to evidence of heterogeneous doping mechanism as well as improvement of mechanical behaviour for the polymer electrolytes in the field of solid-state lithium batteries.

Besides composite electrolytes, composite electrodes are also worth noting. A novel interfacial storage mechanism for lithium in the nanocrystalline boundaries was elucidated by J. Jamnik and J. Maier.³⁴ Later on, many works both in experiment and theory demonstrated valid of the interfacial storage. Li et al. found the Li storage in TiF_3 and VF_3 , which was attributed to the interfacial effect since neither LiF nor Ti and V could store the Li .¹² Yu et al. prepared LiF , Ti and LiF/Ti composite thin films in the same thickness using pulsed laser deposition. Discharge and charge tests revealed that the LiF and Ti films had negligible capacities, while the LiF/Ti composite film showed significant Li storage capacity, which increased with increasing thickness. These results clearly indicate existence of interfacial storage.^{35,36} In theory, the first principle calculation proved the reasonability of interface storage from the view point of thermodynamics.⁵ Such mechanism is beneficial not only to increase the storage capacity at low potential but also to improve the rate performance.

For polycrystalline systems, there have been many good examples demonstrating the SCL effects at grain boundaries in micrometer scale. The details will not be presented here. Readers can refer to previous reports on ZrO_2 , CeO_2 and SrTiO_3 .^{9–11} With respect to the mesoscopic scale, the nanocrystalline SrTiO_3 gives direct and unambiguous evidence of space charge overlap as characteristic size effect. Owing to the significant extension of depletion zones for the holes, the bulk impedance signal disappears at about 100 nm grain boundary spacing.⁸

Heterojunctions in two-phase systems are particularly advantageous on study of the SCL effects at crystalline interfaces owing to the controllable spacing between the two boundaries. $\text{CaF}_2/\text{BaF}_2$ heterolayers prepared by molecular beam epitaxy serve as a nice demonstration of the potential of nanoionics. They show that ion conducti-

vities both parallel and perpendicular to the interfaces increase with decrease in interfacial spacing. This size effect is attributed to the thermodynamically necessary redistribution of the mobile fluoride ions.^{15,37} On this basis, the striking phenomenon of an upward bending in the effective parallel conductivity as a function of inverse interfacial spacing for low temperatures has been satisfactorily explained by application of a modified Mott–Schottky model for BaF_2 .³⁸ This model was further confirmed by measurements perpendicular to the interfaces that offer complementary information on the more resistive parts. This successful comprehensive modeling of parallel and perpendicular conductivities for the whole parameter range gives a nice picture of ion redistribution at the interfaces and the boundary zones overlap as well as the predicted mesoscopic size effect. This mechanism allows achievement of artificial ionically conducting material with anomalous transport properties.^{37–41}

4. Space charge Layer Effects in All Solid State Lithium Batteries

The SCL effects in ASSLB have been intensively studied by Takada et al. with $\text{Li}_4\text{GeS}_4\text{–Li}_3\text{PS}_4$ (thio-LISICON) as electrolytes and LiCoO_2 as cathodes.^{42–49} Concerning the difference in chemical potential between the sulphide and the oxide, the SCL may form at the two-phase boundary according to requirement of thermodynamic equilibrium. Further concerning that the oxide is more absorptive with Li than the sulphide, the Li ion may transfer from the sulphide to the oxide, as schematically shown in Figure 3a. Electrons of the mix-conducting oxide will eliminate the concentration of Li interstitials near the boundary, extending thickness of SCL at the oxide side. The defect chemistry situation is depicted in Figure 3b. Since the transport property of the interface is determined by the Li interstitial movement, such carrier redistribution may enlarge the interfacial resistance, increasing the polarization and worsening the rate capability. This phenomenon was indeed proved in experiment as well as in theory.²²

To overcome the shortcomings brought by the SCL as mentioned above, an intermediate layer (IL) with sole ionic-conducting property was supposed to be effective. As shown in Figure 3c, with introduction of such a layer between the two phases, the potential difference between the oxide and the sulphide can be greatly eliminated, leading to reduced Li transfer from the oxide side. With negligible electron concentration in the intermediate layer, the depletion region of Li is also reduced, as shown in Figure 3d. Consequently, the interfacial resistance decreases and the rate capability is enhanced. In experiment, $\text{Li}_4\text{Ti}_5\text{O}_{12}$, Li–NdO_3 , LiTaO_3 , Li_2SiO_3 thin layers have been used as the intermediate layer between the sulphide and the oxide, and shown effects to reduce the interfacial resistance and in-

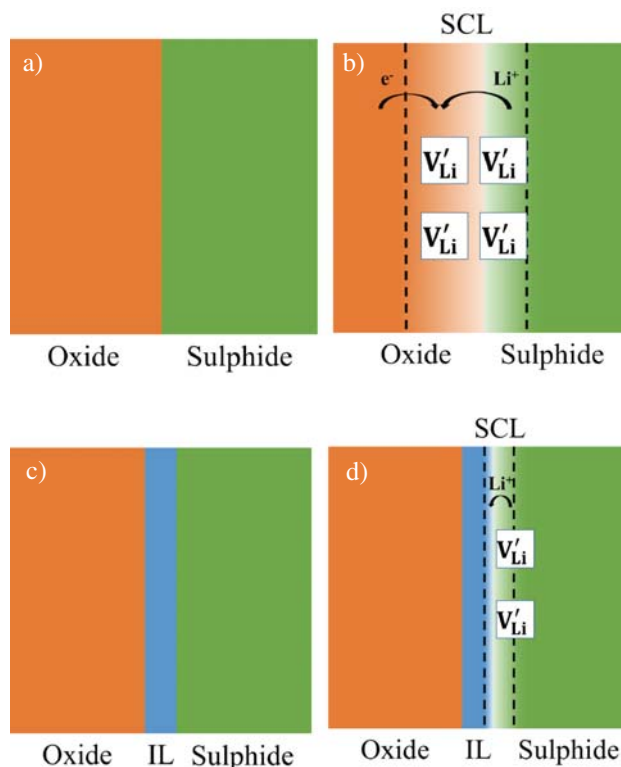


Fig. 3. (a) The two-phase boundary of oxide and sulphide; (b) The defect chemistry situation after the formation of SCL; (c) Introduction of ionic-conducting intermediate layer (IL) between the two phases; (d) The defect chemistry situation after the introduction of the IL.

crease the power density of the ASSLB.^{48–51} Interestingly, the Al-enriched domains formed at the $\text{LiAl}_y\text{Co}_{1-y}\text{O}_2$ surfaces perform the same functionality as the aforementioned intermediate oxide layers, while with more effective influence on reduction of the interfacial resistance and improvement of rate capability.⁵² These results are in agreement with the relevant theoretical calculation.²²

Although not many, discussions on the SCL effect at interfaces between oxides were also reported. Yamada et al. demonstrated that the nonsintered grain boundary resistance of a highly conducting solid electrolyte ($\text{Li}_{1.3}\text{Al}_{0.3}\text{Ti}_{1.7}(\text{PO}_4)_3$) could be suppressed by being coated with poorly conducting solid electrolyte (Li_2SiO_3).⁵³ In addition, Yamada et al. found that at interfaces of solid oxide electrolytes and active materials, lithium-ion transfer across the interfaces occurred, dependent on the potential of the active materials. The Li^+ transfer caused change in the ionic conductivity at the interface and, in some cases, changed in the crystal structure. The Li^+ transfer would be more critical for batteries with high Li^+ conducting solid electrolytes, because the main charge carriers of them are not lithium vacancies but lithium interstitials. The Li^+ transfer from such solid oxide electrolytes to cathodes with high electrode potential leads to depletion of

the charge carriers in the solid electrolytes at the interface and thus increases in the interfacial resistance.^{54,55} With reduction of SCL effect at the grain boundaries, the total conductivity could be increased. Hitosugi et al. reported the surprisingly low electrolyte/electrode interface resistance in thin-film batteries, which is an order of magnitude smaller than that presented in previous reports on ASSLB as well as smaller than that found in liquid electrolyte-based batteries. Such low interface resistance was attributed to the fact that the negative SCL effects at the $\text{Li}_3\text{PO}_{4-x}\text{N}_x/\text{LiCoO}_2$ interface were negligible.⁵⁶

5. Conclusion and Perspective on All Solid State Lithium Batteries

The SCL effects at the interfaces relate to the carrier redistribution near the two-phase boundary, which is led by the SCL potential derived from the requirement of thermodynamic equilibrium at the contact between the two phases. From early experimental phenomena, to qualitative and quantitative understanding, and to interfacial design for manipulation of conductivity by nano technique, many systems including composites, polycrystallines, heterolayer structures have proven important role of the SCL effects in the carrier transport and storage. Especially in research of ASSLB that has attracted much attention recently, the SCL shows the key influence on the interfacial resistance and thus on the rate capability. Though the SCL at the interface between the sulphide electrolyte and the oxide electrode has been deeply discussed, more relevant effects at the interfaces between the oxide electrolyte and the oxide electrode, between the polymer electrolyte and the oxide electrolyte, and between the polymer electrolyte and the oxide electrode are still in infancy. Existence of such effect either at the electrolyte/cathode interface or at the interfaces inside the composites is one of the key issues for ASSLB, which is currently deemed to be a solution to safety as well as enhancement of energy density in the field of rechargeable lithium battery. Therefore, it is clear that study of SCL related to the ASSLB is significant. More researches in this direction can be expected.

6. Acknowledgements

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Povzetek

Efekt prostorsko nabite plasti (SCL) so prvotno razvili za razlago anomalnega zvišanja prevodnosti v kompozitih ionskih prevodnikov. V nadaljevanju so model razširili na razumevanje kvalitativnega in kvantitativnega medfaznega fenomena v drugih ionsko prevodnih sistemih. SCL efekt lahko uporabimo za manipulacijo prevodnosti na sistemih nanometerskih dimenzij, kjer lahko konstruiramo umetne prevodnike. V zadnjem času je obstoj takih efektov na medfazi elektrolit-katoda oz. na medfazah znotraj kompozita v litijevih baterijah s trdnim elektrolitom (ASSLB) pritegnil veliko pozornosti. V zvezi s tem, ta pregledni članek predstavlja prvi pregled SCL principa in njegov vpliv na transport in shranjevanje v tipičnih prevodniških sistemih. Predstavljamo tudi pregled relevantnih efektov za ASSLB baterije. V zaključku obravnavamo še inženirski vidik vezan na SCL in ASSLB sisteme.

Scientific paper

The Synthesis of Diquinone and Dihydroquinone Derivatives of Calix[4]arene and Electrochemical Characterization on Au(111) surface

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In the memory of Janez (Janko) Jamnik, my doctoral advisor who taught me that everything can be done and built if there is a vision and a will.

Abstract

Several new electroactive diquinone and dihydroquinone derivatives of calix[4]arene bearing anchor functional groups were designed, synthesized and characterized. A method for selective protection of the hydroquinone –OH groups with trimethylsilyl groups (TMS) either on lower-rim or on upper-rim was developed. Four selected molecules – with sulfide anchor groups and carboxylic anchor groups – were adsorbed onto Au(111) single crystal surface using *ex-situ* and *in-situ* self-assembly methods. Adsorbed molecules were then electrochemically probed with cyclic voltammetry. All adsorbed molecules showed redox response which changed during cycling. After conditioning CVs stabilized and showed two distinct current peaks for all molecules. Synthesized and electrochemically probed molecules are of interest to: Li-ion batteries (as cathode materials and overcharge protection), beyond Li-ion batteries and redox-flow batteries.

Keywords: Electroactive molecules, calixarene, quinone, hydroquinone, electrochemistry

1. Introduction

World energy consumption is continuously growing with electrical energy being the single largest consumer. Today roughly 68% of electrical energy is generated from the fossil fuels.¹ It is predicted that consumption of electrical energy will double in the next 30 years. Considering the limited natural resources and the environmental impact of the fossil fuels and its combustion products, the alternative sources of electrical energy together with electrochemical energy storage should be taken into account. Batteries, being the main representative of electrochemical energy storage, will play a key role in the future energy consumption cycle.

Over the past decades a tremendous work has been done on the field of the batteries. In particular, Li-ion batteries experienced the biggest boom among them, due to high specific energy (energy per unit weight) and high energy density (energy per unit volume).² However, recently the research was being diverted to the other battery chemistries too, in order to increase energy density and decrease

the cost of the final product.³ One of the revived fields is the use of redox active organic molecules in the battery systems. Advantages of the redox active organic molecules are low cost, high specific capacity, abundance, flexibility, safety, recyclability and sustainability. Up to date, redox active organic molecules were used as: redox shuttles in Li-ion batteries,⁴ active cathode materials,^{5,6} and active anode materials.⁷ Depending on the nature of the system, redox active organic molecules can be: a) dissolved in the organic solvents – redox shuttles and redox-flow batteries,^{8,9} b) grafted to the solid support – solid cathode in Li-ion batteries,^{10–13} or c) free standing cathode material.¹⁴ Redox active organic molecules are chemically divided into:⁶ organosulfur molecules,¹⁵ organic free radical compounds,¹⁶ and carbonyl compounds.¹⁷ The latter, having a quinone/hydroquinone as a key representative, are playing a significant role in electroactivity relevant to biochemistry, medicine and electrochemistry. Quinone derivatives were tested as cathode materials in Li-ion batteries^{10–12,18} and redox-flow batteries.¹⁹ Although it was demonstrated that quinones are promising candidates for

the use in energy storage, the field is still in its infancy. Motivated by above mentioned and the fact that there is a lack of fundamental understanding, we have focused on design of new quinone derivatives and electrochemical characterization.

In the present study, we examined several synthetic routes in order to synthesize quinone derivatives of calix[4]arene which could be then bound to the electrode materials and electrochemically tested. Calix[4]arene,²⁰ which was used as starting compound, is in particularly interesting for electrochemical applications, due to inherently opened cavities in the macrocycle. Cavities are potential sites for sieving cations with small ionic radius such as H⁺ or Li⁺.^{21–23} The latter is relevant to the enhanced accessibility of the active species in the energy conversion and storage systems. The crucial step in synthetic routes was the introduction of anchor functional groups. We have introduced carboxylic and sulfur containing anchor functional group. The latter is advantageous due to strong sulfur – noble metal interaction. Both, “short” anchors where redox active center would be close to the electrode surface and “long” anchors where center is further away from the electrode surface were introduced. By variation of the length we were hoping to investigate electrochemical reaction mechanisms. Successfully synthesized organic molecules were then anchored to the Au(111) single crystal surface using self-assembly protocol²⁴ and electrochemically probed.

2. Results and Discussion

The synthesis scheme is summarized in the Scheme 1. In the first attempt we have tried to introduce “short” sulfur based functional groups to the upper rim of the calix[4]arene macrocycle. We hypothesized that using “short” functional groups would bring electroactive centers of the molecule closer to the electrode material and thus mitigate electron transfer. Following the scheme we started from the basic calix[4]arene which was selectively protected with *n*-propyl groups on sites 26 and 28. Protected product **1b** was then oxidized to quinone derivative **2b** and bromine was introduced to the unoccupied sites. We tried to substitute the bromine on compound **3b** with thioacetyl anchor group but reaction conditions used, did not furnish desired product. We believe that side reaction is occurring on the carbonyl groups of quinone center. To avoid possible side reactions, we reduced **3b** to hydroquinone **4b**. In the next step, we tried to protect hydroxyl groups of hydroquinone with trimethylsilyl groups (TMS). Interestingly, on compound **5b**, upper-rim hydroxyl groups were protected only, while lower-rim stayed intact when using either *N,O*-bis(trimethylsilyl)acetamide (BSA) or trimethylsilyl chloride (TMSCl) (in the presence of triethylamine (TEA) or bis(trimethylsilyl)amine (HMDS)). On the other hand, TMSCl in the

presence of the NaH yielded **6b** where lower-rim OH groups were selectively protected, while upper-rim groups stayed intact. This interesting phenomenon is probably a combination of steric effects and non-covalent interactions of Na⁺ cations with calix[4]arene macrocycle. Despite the fact that we have failed to protect the hydroxyl groups of **4b** with TMS, we have shown new selective synthesis strategy, which can be used in designing new molecules. However, the protection of –OH groups on **4b** was successful when using less bulky *n*-propyl groups, yielding compound **7b₁** or methyl groups yielding **7b₂**. In the next step we introduced thioacetyl anchor groups and obtain compound **8**. The product **8** was then subject to oxidation in order to obtain redox-active quinone centers. Standard oxidation procedures did not furnish desired product, so the synthetic path was abandoned.

Using alternative synthetic path, we targeted the introduction of sulfide anchor functional groups. We again started from calix[4]arene, which was selectively protected with benzyl groups to furnish compound **1a**. Protected compound was then brominated on positions 5 and 17 to isolate product **9a**. In the next step we protected remaining two hydroxyl groups on the lower-rim, which yielded product **10a₁**. Bromine functional groups on upper-rim were then substituted with methylsulfide group using an exchange protocol. The product **11a₁** with –SMe anchor groups was then subject to deprotection of benzyl groups at lower-rim using trimethylsilyl bromide (TMSBr). The attempt to oxidize the deprotected product **12a₁** and synthesize quinone derivative failed. Although the synthesis of the designed molecules did not yield the desired products, the intermediate products could be in the future tested for other applications such as redox shuttles.

After two unsuccessful trials of introducing sulfur anchor groups to the upper-rim of calix[4]arene, we decided to introduce sulfur anchor groups to the lower-rim. Once again we started with calix[4]arene, which was selectively protected with functional groups already bearing sulfur containing anchor groups. We have successfully introduced three different functional groups containing sulfide anchor centers – **1d–g**. All three compounds were then oxidized to final quinone derivative **2d–g** in 10% to 58% yields.

For electrochemical comparison, the molecules with carboxyl functional groups at the lower-rim were also synthesized (Scheme 1). Slightly modified previously published method²⁵ yielded selectively protected calix[4]arene **1c** with protected carboxyl groups. In the next step phenolic units were oxidized to yield the molecule **2c** with quinone redox centers. In order to obtain carboxylic anchor groups, the *tert*-butyl groups were removed from **2c** and molecule **13** was isolated. For electrochemical comparison, a reduced version – hydroquinone **14** was also synthesized from **13**, applying Na₂S₂O₄ as a reducing agent.

Molecules **2f**, **2g**, **13** and **14** were then attached to the Au(111) single crystal surface using *ex-situ* (**2f** and



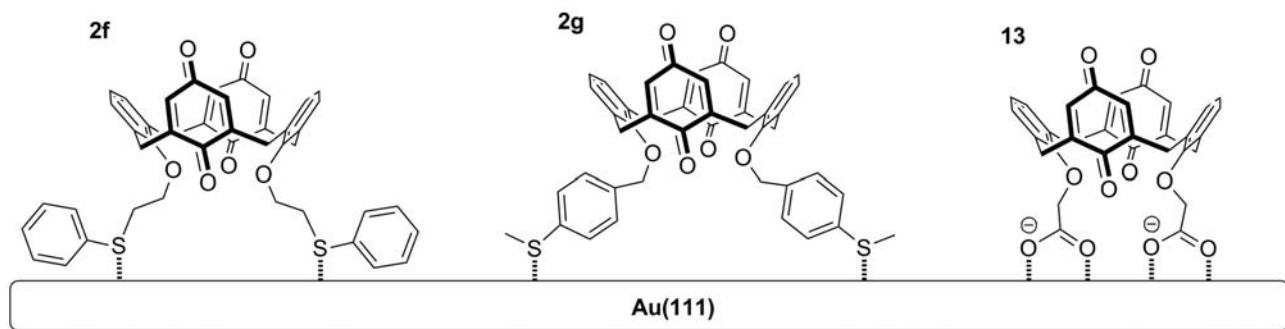


Figure 1. Self-assembled molecules at Au(111) single crystal surface. **2f** and **2g** were attached to the surface using *ex-situ* self-assembly method, **13** was attached using *in-situ* self-assembly method.

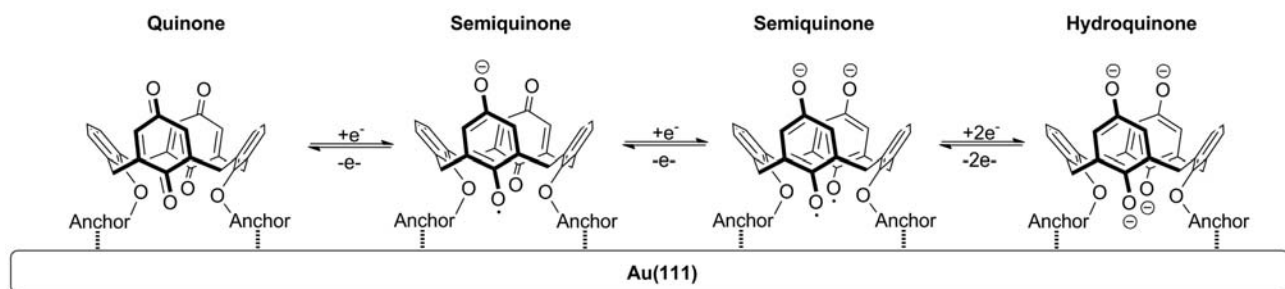
2g) and *in-situ* (**13** and **14**) self-assembly methods (Figure 1). Sulfide anchor groups in **2f** and **2g** were expected to bind to the gold through a dative bond. It was shown previously that sulfur can interact with Au(111) single crystal surfaces by donating the electron pair to the unoccupied Au orbitals.²⁶ On the other hand in the case of the carboxyl anchor groups (**13**, **14**), ion-metal interaction was expected. In this respect, pH of self-assembly system and surface charge of the metal surface are playing significant role, thus *in-situ* self-assembly method was applied. Adsorption of the carboxylates has also been extensively studied over the past decades where several successful grafting methods were shown,²⁷ including adsorption of electroactive anthraquinone-2-carboxylic acid on gold surface.²⁸

To evaluate the electrochemical response of the adsorbed molecules on the Au(111) single crystal surface, cyclic voltammetry (CV) was used. Molecules **2f**, **2g**, **13**, and **14** on Au(111) were tested and their faradaic currents were measured. All chemically modified Au(111) electrodes showed specific faradaic response. Molecule **2f** with long and bulky sulfide anchor groups showed stable redox activity in the potential region from 0.1V to 1.15V vs. reversible hydrogen electrode (RHE). The reaction is quasi-reversible (Figure 2). In the first cycle (Figure 2, black line) the anodic scan shows at least two peak potentials (E_{pa}) at 0.65V and 0.88V while cathodic scan shows three peak potentials (E_{pc}) at 0.77V, 0.56V, and 0.43V. Redox

mechanisms of diquinone derivatives of calix[4]arene in aprotic organic solvents have been studied previously.²⁹ Authors suggest that there are two consecutive one-electron transfers followed by simultaneous concerted two-electron transfer, giving the ionized hydroquinone (Scheme 2). Another study noticed change of the mechanism in the presence of water to concerted four-electron transfer per two quinone units.³⁰ According to above mentioned and recorded CV, we can presume that the mechanism of the **2f** on Au(111) electrode could follow two consecutive one-electron transfers followed by concerted two-electron transfer in the first cycle.

However, after several cycles the shape of the CVs changed dramatically. E_{pa} at 0.88V lost intensity, while E_{pa} at 0.65V substantially increased. Similar was observed for the cathodic scan. E_{pc} at 0.43V diminished while E_{pc} at 0.56V increased. The reasons for the CV changes are unclear. One of the reasons could be, that the molecules on the Au(111) are rearranging and position of the redox centers are, in respect to the electrode, changing. Similar is happening with the molecules **2g** on Au(111) surface.

Scan in the anodic direction reveals single E_{pa} at 0.88V while the reverse scan in the cathodic direction, single peak E_{pc} at 0.33V (Figure 3). After cycling in the defined potential window the E_{pa} shifted to 0.67V and E_{pc} to 0.56V. This was the most pronounced when we held the potential at $-0.1V$ for 1h. The peak potential separation ΔE_p is becoming smaller, thus one can conclude that reac-



Scheme 2. Redox mechanism of electroactive diquinone derivatives of calix[4]arene with anchoring functional groups.

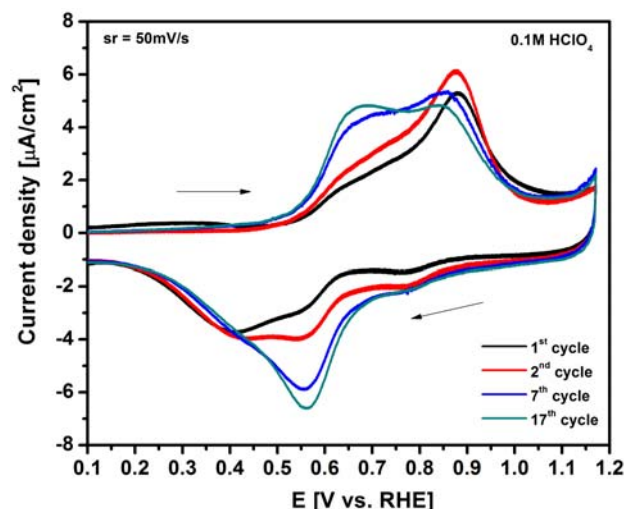


Figure 2. CVs of **2f** bound to Au(111). Black line – 1st cycle, red line – 2nd cycle, blue line – 7th cycle, and green line – 17th cycle. Conditions: 0.1M HClO₄, at room temperature, applying 50 mV/s scan rate. Arrows indicate direction of CV scan.

tion is getting more reversible. As mentioned above, the reason could be; the rearrangement of the self-assembled layer of the molecules on the Au(111) surface and change of the mechanism from two consecutive one-electron transfers followed by simultaneous concerted two-electron transfer to concerted four-electron transfer. In order to confirm that, extensive studies should be done, however, this is beyond the scope of this work.

The electrochemical investigation of the molecule with carboxylate anchor groups **13** on the Au(111) surfaces showed even more pronounced redox changes during cycling (Figure 4). To highlight the response of the redox

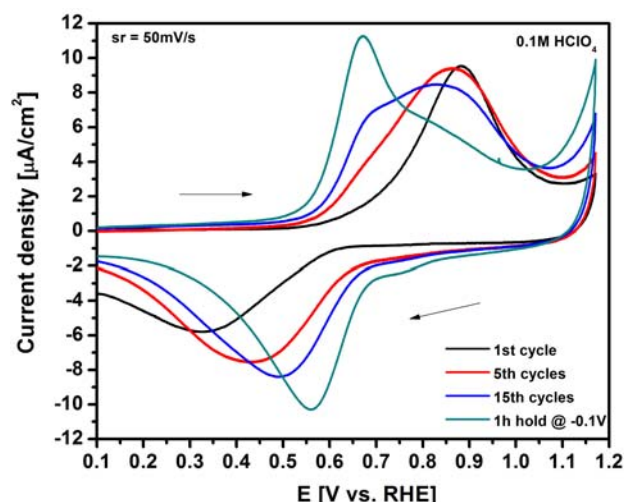


Figure 3. CVs of **2g** bound to Au(111). Black line – 1st cycle, red line – 5th cycle, blue line – 15th cycle, and green line – 1h hold at -0.1V. Conditions: 0.1M HClO₄, at room temperature, applying 50 mV/s scan rate. Arrows indicate direction of CV scan.

electrochemistry of **13** the CV of pure Au(111) is overlaid in the Figure 4. The first scan of **13** on Au(111) exhibited distinct electrochemical response with at least four peaks. Redox processes are reversible with E_{pa} at 0.53V, 0.63V, 0.70V, and 0.88V and E_{pc} at 0.50V, 0.57V, 0.71V, and 0.86V. After the third cycle, CVs stabilized and did not change significantly. However, the difference between first and third cycle is significant. In the third cycle only one E_{pa} at 0.70V can be seen and two E_{pc} at 0.55V, 0.56V. Interestingly, when CV of 3rd cycle of quinone derivative of calix[4]arene **13** was compared to CV of 1st cycle of reduced version – a hydroquinone derivative of calix[4]arene **14** (Figure 5c and 5d), the CVs almost overlapped. One would expect to see overlapping of the CVs in the first cycles since we are probing the same molecule but in different oxidation state. However, the molecules on the surface are immediately reduced when electrode is immersed at lower potential. From above mentioned observation we can deduct that molecule **13** is rearranging on the surface and making more accessible to the protons from the electrolyte which are protonating phenoxide anion of the hydroquinone molecules. Once the film is thermodynamically stable the electrochemical response give the same results for either oxidized quinone **13** or reduced hydroquinone **14**.

To evaluate the possible effect of the anchor groups, conditioned CVs of molecules **2f**, **2g**, **13** and **14** on Au(111) were compared (Figure 5). Surprisingly, all molecules exhibit similar electrochemical response with faradaic currents in anodic scan at E_{pa} – 0.67V and cathodic scan at E_{pc} – 0.56V. Although the ΔE_p (0.11V) of the main electrochemical reaction is the same for all four compounds, there are differences between the CVs which indicate different mechanism either in the anodic scan for

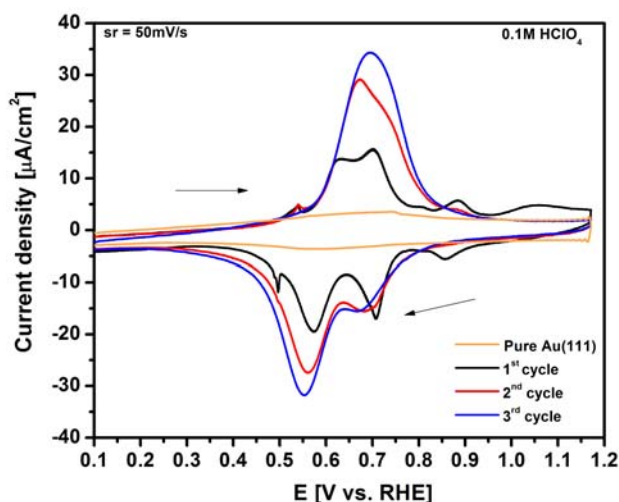


Figure 4. CVs of pure Au(111) (orange line) and **13** bound to Au(111). Black line – 1st cycle, red line – 2nd cycle, and blue line – 3rd cycle. Conditions: 0.1M HClO₄, at room temperature, applying 50 mV/s scan rate. Arrows indicate direction of CV scan.

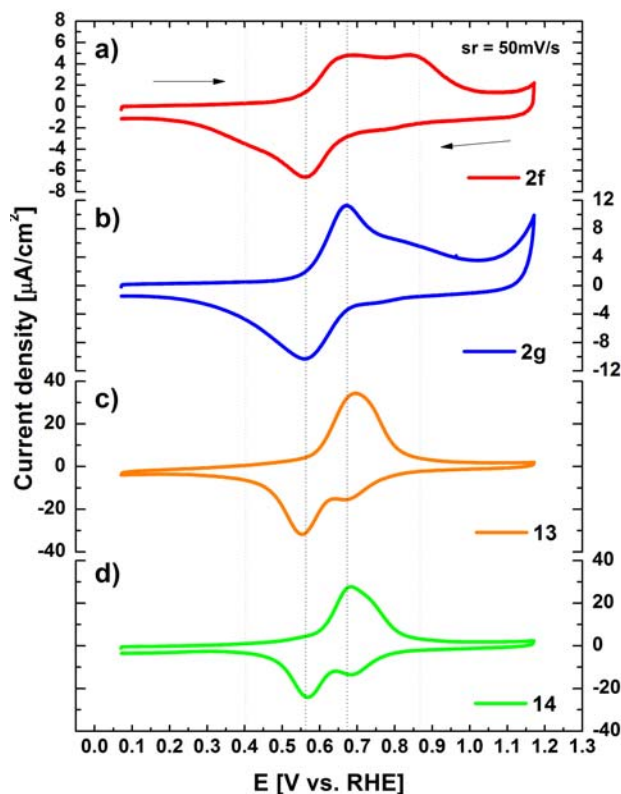


Figure 5. Comparison of stable CVs of quinone and hydroquinone derivatives of calix[4]arene: a) **2f** bound to Au(111), 17th cycle, b) **2g** bound to Au(111), 1h hold at -0.1 V c) **13** bound to Au(111), 3rd cycle, and d) **14** bound to Au(111), 1st cycle. Conditions: 0.1M HClO₄, at room temperature, applying 50 mV/s scan rate. Arrows indicate direction of CV scan.

2f and **2g** or the cathodic scan for **13** and **14**. According to previously published results,³⁰ one would expect that the reduction of all four diquinone compounds would undergo a concerted four-electron reduction in acidic aqueous media. However, the conditioning of the chemically modified Au(111) electrodes shows that all the diquinones; **2f**, **2g**, and **13** bearing different anchor groups exhibit their unique electrochemical response with additional waves. One of the reasons for such behavior could be a specific steric effect of the molecules where one-electron transfer reactions followed by protonation are shielded and thus reaction intermediates are more stable. According to above mentioned we cannot draw any solid conclusions on the effect of the length of the anchor groups.

3. Conclusions

Specifically designed redox-active diquinone derivatives of calix[4]arene bearing various anchor functional groups were synthesized and fully characterized. Designed and synthesized electroactive molecules are particularly interesting for the energy conversion and storage systems

e.g. Li-ion and beyond Li-ion batteries (cathode materials, redox shuttles), and redox-flow batteries. In an attempt to introduce thiol anchor functional groups on the upper-rim of the calix[4]arene macrocycle, we have developed the method where selective protection of the hydroquinone $-OH$ groups either on lower-rim (**6b**) or on upper-rim (**5b**) was achieved. Further, we have successfully synthesized new derivatives of calix[4]arene with 1,4-dimethoxybenzene moieties (**8**) and 1-methoxy-4-(methylthio)benzene moieties (**11a₁** and **12a₁**) that are in particular interest for the non-aqueous redox flow batteries and as an overcharge protection for Li-ion batteries. We have also successfully synthesized diquinone derivatives of calix[4]arene with sulfide (**2e**, **2f**, and **2g**) and carboxylate (**13** and **14**) anchor groups on lower-rim of the macrocycle. Above mentioned molecules could be used as an active cathode component of the Li-ion and beyond Li-ion batteries.

Molecules **2f**, **2g**, **13** and **14** were then attached to the Au(111) single crystal electrode using *ex-situ* and *in-situ* self-assembly methods and electrochemically characterized. All the analyzed molecules gave redox response in 0.1M HClO₄. It was noticed that electrochemical responses are changing during cycling for the molecules **2f**, **2g**, and **13**. The positions of the peak potentials; E_p and E_c are shifting, increasing and decreasing. However, after conditioning the peak potentials; E_p and E_c stabilized and CVs did not change substantially. The conditioned CVs of **2f**, **2g**, **13** and **14** on Au(111) revealed two common peak potentials; E_p at 0.67V and E_c at 0.56V with the peak potential separation $\Delta E_p - 0.11$ V. The latter indicates that main redox mechanism of the different diquinone derivatives of calix[4]arene is similar while the presence of other peak potentials suggest that the full mechanism is complex and specific for the molecules tested.

4. Experimental

4. 1. Synthetic Materials and General Synthetic Procedures

Reactions were performed in dried glassware under Ar atmosphere, unless stated otherwise. Precursor calix[4]arene was prepared according to literature procedures.³¹ Reagent grade tetrahydrofuran (THF) was distilled from Na and sodium benzophenone ketyl. Triethylamine (TEA) and N,N-dimethylformamide (DMF) were distilled over CaH₂. Reagent grade hexanes, CHCl₃, CH₂Cl₂ (DCM), MeOH, and ethyl acetate (EtOAc) were used without further distillation. Acetyl chloride (AcCl) was heated at reflux with PCl₅ and then distilled. *tert*-Butyllithium titrated before use (1.46 M solution in pentane) was obtained from Aldrich. All other commercially available reagents were used as received. Flash column chromatography was performed using 230–400 mesh silica gel from EM Science. Thin layer chromatography was performed using

glass plates pre-coated with silica gel 40 F₂₅₄ purchased from Merck. ¹H spectra were taken at 400 or 300 MHz. ¹³C NMR were recorded on the same instrument at 100.6 or 75.5 MHz, respectively. Proton chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS). Carbon was referenced to CDCl₃ (77.23 ppm), except when specified otherwise. Infrared spectroscopy (IR) spectra were recorded on Nicolet Avatar FTIR instrument. High resolution mass spectrometry (HRMS) was recorded on Q-ToF Premier, Waters-Micromass spectrometer.

General Procedure for synthesis of partially alkylated calix[4]arene (1). Calix[4]arene (3 g, 7.07 mmol) and K₂CO₃ (1.07 g, 7.77 mmol) were suspended in MeCN (50 ml) and alkyl halide (2.15 ml, 14.21 mmol) was added. The reaction suspension was let to stir at the temperature of solvent boiling point, for 24 h. After 24 h MeCN was removed under reduced pressure, the remaining solid dissolved in CHCl₃ (50 ml), and washed with 1M HCl (50 ml) and saturated aqueous solution of NaCl (50 ml). Organic phase was then separated from the aqueous and dried with MgSO₄. Organic solvent was removed under reduced pressure and crude product purified by column chromatography (silica gel) or recrystallization.

General procedure for synthesis of diquinone derivative of calix[4]arene (2). A solution of Ti(NO₃)₃ × 3H₂O (20.97 g, 47.18 mmol) in solvent mixture, MeOH (480 ml) and EtOH (1440 ml) was slowly added to the solution of **1** (4 g, 7.86 mmol) in CHCl₃. Reaction mixture was let to stir for 15 min and then quenched by addition of H₂O (200 ml). 10% aqueous solution of HCl was then added dropwise until precipitate that formed during reaction did not dissolve. By applying reduced pressure, ~80% of the solvents were removed and CH₂Cl₂ (100 ml) and H₂O (50 ml) were added. Organic phase was separated from the aqueous and dried with MgSO₄. Solvents were removed under reduced pressure and remaining solid purified by column chromatography (silica gel) or recrystallization.

General procedure for synthesis of alkoxy-calix[n]arene (7, 10). Compound **7** or **10** (8.50 mmol) was added to the suspension of NaH (1.4 g, 59.50 mmol) in DMF (37 ml) under inert atmosphere. Reaction mixture was let to stir at room temperature for 1 h and then alkyl halide (127.5 mmol) was added dropwise. Reaction mixture was then heated up to 60 °C and let to stir for 24 h. DMF was removed under reduced pressure and remaining solid suspended in CH₂Cl₂ (50 ml). This was shook with saturated solution of NH₄Cl (50 ml). Organic phase was separated from aqueous and dried with MgSO₄. CH₂Cl₂ was removed under reduced pressure and crude product purified by column chromatography or recrystallization.

Cone 26,28-bis(phenylmethoxy)-25,27-dihydroxycalix[4]arene (1a). Following the procedure for synthesis of partially alkylated calix[4]arene; calix[4]arene (3 g, 7.07

mmol), benzyl bromide (1.72 ml, 14.49 mmol), K₂CO₃ (1.11 g, 8.06 mmol) and MeCN (90 ml) were used. Recrystallization from solvent mixture, CHCl₃ – MeOH yields colorless crystalline product (3.56 g, 83% yield). ¹H NMR (300 MHz, CDCl₃): δ 7.82 (s, 2H), 7.65 (m, 4H), 7.36 (m, 6H), 7.05 (d, *J* = 7.5 Hz, 4H), 6.88 (d, *J* = 7.5 Hz, 4H), 6.75–6.63 (m, 4H), 5.06 (s, 4H), 4.31 (d, *J* = 13.1 Hz, 4H), 3.34 (d, *J* = 13.1 Hz, 4H). The spectrum is in accordance with previously published results.³²

Cone 26,28-di-*n*-propoxy-25,27-dihydroxycalix[4]arene (1b). Calix[4]arene (4 g, 9.42 mmol) was dissolved in DMF (87 ml) and BaO (8.41 g, 54.84 mmol), Ba(OH)₂ × 8H₂O (8.95 g, 28.36 mmol), and 1-bromopropane (4.28 ml, 47.11 mmol) were added. Reaction mixture was let to stir over night at 50 °C. After completion of reaction, 10% aqueous solution of HCl was added and extracted with CH₂Cl₂. Organic phase was separated from aqueous and dried with MgSO₄. The solvents were removed under reduced pressure and remaining solid was recrystallized from solvent mixture CHCl₃ – MeOH. Recrystallization yielded colorless crystalline product (3.68 g, 77% yield). ¹H NMR (300 MHz, CDCl₃): δ 8.30 (s, 2H), 7.05 (d, *J* = 7.5 Hz, 4H), 6.91 (d, *J* = 7.5 Hz, 4H), 6.73–6.61 (m, 4H), 4.32 (d, *J* = 12.9 Hz, 4H), 3.97 (t, *J* = 6.3 Hz, 4H), 3.37 (d, *J* = 12.9 Hz, 4H), 2.07 (m, 4H), 1.31 (t, *J* = 7.4 Hz, 6H). The spectrum is in accordance with previously published results.³³

Cone 26,28-bis[(*tert*-butoxycarbonyl)methoxy]-25,27-dihydroxycalix[4]arene (1c). Following the procedure for synthesis of partially alkylated calix[4]arene; calix[4]arene (1 g, 2.36 mmol), *tert*-butyl bromoacetate (0.71 ml, 4.83 mmol), K₂CO₃ (371 mg, 2.69 mmol) and MeCN (30 ml) were used. Recrystallization from solvent mixture, CHCl₃ – MeOH yields colorless crystalline product (1.09 g, 71% yield). ¹H NMR (300 MHz, CDCl₃): δ 7.66 (s, 2H), 7.04 (d, *J* = 7.5 Hz, 4H), 6.88 (d, *J* = 7.5 Hz, 4H), 6.72–6.65 (m, 4H), 4.58 (s, 4H), 4.47 (d, *J* = 13.2 Hz, 4H), 3.37 (d, *J* = 13.2 Hz, 4H), 1.56 (s, 18H). The spectrum is in accordance with previously published results.²⁵

Cone 26,28-bis[2-(methylsulfanyl)ethoxy]-25,27-dihydroxycalix[4]arene (1d). Following the modified procedure (NaI was also used) for synthesis of partially alkylated calix[4]arene; calix[4]arene (2.5 g, 5.89 mmol), 2-chloroethyl methyl sulfide (1.18 ml, 12.07 mmol), K₂CO₃ (928 mg, 6.71 mmol), MeCN (75 ml) and NaI (1.81 g, 12.07 mmol) were used. Column chromatography (CH₂Cl₂ : *n*-hexane 2 : 1) followed by recrystallization from solvent mixture CHCl₃ – MeOH yielded colorless crystalline product (2.48 g, 74% yield). ¹H NMR (300 MHz, CDCl₃): δ 7.56 (s, 2H), 7.07 (d, *J* = 7.5 Hz, 4H), 6.84 (d, *J* = 7.5 Hz, 4H), 6.71 (m, 4H), 4.35 (d, *J* = 13.1 Hz, 4H), 4.15 (t, *J* = 6.7 Hz, 4H), 3.39 (d, *J* = 13.1 Hz, 4H), 3.10 (t, *J* = 6.7 Hz, 4H), 2.29 (s, 6H). ¹³C NMR (75.5

MHz, CDCl_3): Δ 153.5, 152.1, 133.4, 129.3, 128.9, 128.4, 125.7, 119.4, 75.8, 33.9, 31.7, 16.7. HRMS: calcd for $\text{C}_{34}\text{H}_{36}\text{O}_4\text{S}_2 + \text{H}^+$, 573.2133; found, 573.2125.

Cone 26,28-Bis[(benzylsulfanyl)methoxy]-25,27-dihydroxycalix[4]arene (1e). Following the procedure for synthesis of partially alkylated calix[4]arene; calix[4]arene (2 g, 4.71 mmol), bromomethyl benzyl sulfide (1.46 ml, 9.66 mmol), K_2CO_3 (742 mg, 5.37 mmol) and MeCN (60 ml) were used. Column chromatography (CH_2Cl_2 : *n*-hexane 2 : 1) yielded white powder product (1.71 g, 52% yield). IR (KBr): 3372, 3060, 3025, 2925, 1591, 1462 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 7.68 (s, 2H), 7.38–7.24 (m, 10H), 7.09 (d, $J = 7.5$ Hz, 4H), 6.92 (d, $J = 7.5$ Hz, 4H), 6.76 (m, 4H), 5.08 (s, 4H), 4.45 (d, $J = 13.2$ Hz, 4H), 4.05 (s, 4H), 3.42 (d, $J = 13.2$ Hz, 4H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 153.6, 151.9, 137.6, 133.9, 129.8, 129.6, 129.1, 128.6, 127.7, 126.4, 119.7, 116.4, 113.0, 77.9, 36.1, 32.6. HRMS: calcd for $\text{C}_{44}\text{H}_{40}\text{O}_4\text{S}_2 + \text{Na}^+$, 719.2266; found, 719.2289.

Cone 26,28-bis[2-(phenylsulfanyl)ethoxy]-25,27-dihydroxycalix[4]arene (1f). Following the procedure for synthesis of partially alkylated calix[4]arene; calix[4]arene (2 g, 4.71 mmol), 2-bromoethyl phenyl sulfide (1.46 ml, 9.66 mmol), K_2CO_3 (742 mg, 5.37 mmol) and MeCN (60 ml) were used. Column chromatography (CH_2Cl_2 : *n*-hexane 2 : 3) followed by recrystallization from solvent mixture CH_2Cl_2 – MeOH yielded colorless crystalline product (1.3 g, 40% yield). ^1H NMR (300 MHz, CDCl_3): Δ 7.75 (s, 2H), 7.44–7.17 (m, 10H), 7.05 (d, $J = 7.5$ Hz, 4H), 6.85 (d, $J = 7.5$ Hz, 4H), 6.67 (m, 4H), 4.30 (d, $J = 13.1$ Hz, 4H), 4.16 (t, $J = 7.2$ Hz, 4H), 3.56 (t, $J = 7.2$ Hz, 4H), 3.35 (d, $J = 13.1$ Hz, 4H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 153.5, 151.8, 135.6, 133.5, 130.2, 129.5, 129.3, 128.9, 128.7, 128.5, 126.9, 125.8, 119.5, 74.7, 33.3, 31.7. HRMS: calcd for $\text{C}_{44}\text{H}_{40}\text{O}_4\text{S}_2 + \text{Na}^+$, 719.2266; found, 719.2280. The data is in accordance with previously published results.³⁴

Cone 26,28-Bis[(4-methylthio)benzyl]-25,27-dihydroxycalix[4]arene (1g). Following the procedure for synthesis of partially alkylated calix[4]arene; calix[4]arene (1 g, 2.36 mmol), 4-(methylthio)benzyl bromide (1.05 g, 4.83 mmol), K_2CO_3 (371 mg, 2.69 mmol) and MeCN (30 ml) were used. Recrystallization from solvent mixture CH_2Cl_2 – MeOH yielded colorless crystalline product (1.38 g, 84% yield). IR (KBr): 3406, 3022, 2921, 2858, 1595, 1462 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 7.78 (s, 2H), 7.56 (d, $J = 8.3$ Hz, 4H), 7.25 (d, $J = 8.3$ Hz, 4H), 7.04 (d, $J = 7.5$ Hz, 4H), 6.87 (d, $J = 7.5$ Hz, 4H), 6.67 (m, 4H), 5.02 (s, 4H), 4.28 (d, $J = 13.1$ Hz, 4H), 3.33 (d, $J = 13.1$ Hz, 4H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 153.6, 152.2, 138.9, 133.9, 133.6, 129.4, 128.9, 128.4, 128.3, 127.0, 125.8, 119.4, 78.3, 31.8, 16.2. HRMS: calcd for $\text{C}_{44}\text{H}_{40}\text{O}_4\text{S}_2 + \text{Na}^+$, 719.2266; found, 719.2250.

Cone 11,26,23,28-tetraone-25,27-dipropoxycalix[4]arene (2b). Following the procedure for synthesis of diquinone derivative of calix[4]arene; compound **1b** (4 g, 7.86 mmol), $\text{Ti}(\text{NO}_3)_3 \times 3\text{H}_2\text{O}$ (20.97 g, 47.18 mmol), MeOH (480 ml), EtOH (1440 ml), CHCl_3 (400 ml) and H_2O (200 ml) were used. Recrystallization from solvent mixture CH_2Cl_2 – MeOH at 4 °C, yielded orange crystalline product (3.16 g, 75% yield). ^1H NMR (300 MHz, CDCl_3): Δ 6.80 (d, $J = 7.4$ Hz, 4H), 6.63 (m, 6H), 3.81 (d, $J = 12.7$ Hz, 4H), 3.65 (t, $J = 7.1$ Hz, 4H), 3.32 (d, $J = 12.7$ Hz, 4H), 1.81 (m, 4H), 1.00 (t, $J = 7.5$ Hz, 6H). The spectrum is in accordance with previously published results.³⁵

Cone 11,26,23,28-tetraone-25,27-bis[(*tert*-butoxycarbonyl)methoxy]-calix[4]arene (2c). Following the procedure for synthesis of diquinone derivative of calix[4]arene; compound **1c** (500 mg, 0.77 mmol), $\text{Ti}(\text{NO}_3)_3 \times 3\text{H}_2\text{O}$ (2.04 g, 4.60 mmol), MeOH (60 ml), EtOH (180 ml), CHCl_3 (50 ml) and H_2O (25 ml) were used. Column chromatography (CH_2Cl_2 : EtOAc 3 : 1) yielded orange powder product (386 mg, 74% yield). ^1H NMR (300 MHz, CDCl_3): Δ 6.82 (d, $J = 7.4$ Hz, 4H), 6.74 (s, 4H), 6.66 (m, 2H), 4.34 (s, 4H), 3.96 (d, $J = 12.4$ Hz, 4H), 3.36 (d, $J = 12.4$ Hz, 4H), 1.49 (s, 18H). The spectrum is in accordance with previously published results.²⁵

Cone 11,26,23,28-tetraone-25,27-bis[2-(methylsulfanyl)ethoxy]-calix[4]arene (2d). Following the procedure for synthesis of diquinone derivative of calix[4]arene; compound **1d** (50 mg, 0.09 mmol), $\text{Ti}(\text{NO}_3)_3 \times 3\text{H}_2\text{O}$ (233 mg, 0.52 mmol), MeOH (6 ml), EtOH (18 ml), CHCl_3 (5 ml) and H_2O (2.5 ml) were used. Column chromatography (CH_2Cl_2 : EtOAc 10 : 1) yielded orange powder product (5 mg, 10% yield). IR (KBr): 3063, 2921, 2866, 1656, 1459, 1292, 1200 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 6.81 (d, $J = 7.4$ Hz, 4H), 6.65 (m, 6H), 3.90 (m, 8H), 3.33 (d, $J = 12.9$ Hz, 4H), 2.85 (t, $J = 6.5$ Hz, 4H), 2.18 (s, 6H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 188.5, 186.2, 148.4, 132.5, 130.2, 123.9, 93.2, 74.2, 46.0, 34.2, 31.7. HRMS: calcd for $\text{C}_{34}\text{H}_{32}\text{O}_6\text{S}_2 + \text{H}^+$, 601.1719; found, 601.1738.

Cone 11,26,23,28-tetraone-25,27-bis[(benzylsulfanyl)methoxy]-calix[4]arene (2e). Following the procedure for synthesis of diquinone derivative of calix[4]arene; compound **1e** (500 mg, 0.72 mmol), $\text{Ti}(\text{NO}_3)_3 \times 3\text{H}_2\text{O}$ (1.91 g, 4.30 mmol), MeOH (60 ml), EtOH (180 ml), CHCl_3 (50 ml) and H_2O (25 ml) were used. Column chromatography (CH_2Cl_2 : EtOAc 15 : 1) yielded orange powder product (215 mg, 41% yield). IR (KBr): 3060, 3028, 2923, 1653, 1459, 1293 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 7.35–7.15 (m, 10H), 6.79–6.66 (m, 10H), 4.91 (s, 4H), 4.05 (d, $J = 13.0$ Hz, 4H), 3.73 (s, 4H), 3.23 (d, $J = 13.0$ Hz, 4H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 188.9, 187.9, 187.6, 151.4, 148.8, 148.7, 148.1, 137.7, 133.3, 129.5, 129.1, 128.5, 127.0, 126.3, 121.7, 64.5, 34.4, 30.7.

HRMS: calcd for $C_{44}H_{36}O_6S_2 + H^+$, 725.2032; found, 725.2058.

Cone 11,26,23,28-tetraone-25,27-bis[2-(phenylsulfanyl)ethoxy]-calix[4]arene (2f). Following the procedure for synthesis of diquinone derivative of calix[4]arene; compound **1f** (500 mg, 0.72 mmol), $Tl(NO_3)_3 \times 3H_2O$ (1.91 g, 4.30 mmol), MeOH (60 ml), EtOH (180 ml), $CHCl_3$ (50 ml) and H_2O (25 ml) were used. Column chromatography (CH_2Cl_2 : EtOAc 20 : 1) yielded orange powder product (300 mg, 58% yield). IR (KBr): 3056, 2957, 2923, 2868, 1656, 1459, 1288 cm^{-1} . ^1H NMR (300 MHz, $CDCl_3$): Δ 7.35–7.15 (m, 10H), 6.74–6.56 (m, 10H), 3.87–3.83 (m, 8H), 3.25–3.21 (m, 8H). ^{13}C NMR (75.5 MHz, $CDCl_3$): Δ 188.5, 186.1, 155.9, 148.3, 135.6, 132.6, 130.3, 130.1, 129.7, 129.4, 126.9, 124.0, 73.1, 33.9, 31.8. HRMS: calcd for $C_{44}H_{36}O_6S_2 + Na^+$, 747.1851; found, 747.1847.

Cone 11,26,23,28-tetraone-25,27-bis[(4-methylthio)benzyl]-kaliks[4]arene (2g). Following the procedure for synthesis of diquinone derivative of calix[4]arene; compound **1g** (500 mg, 0.72 mmol), $Tl(NO_3)_3 \times 3H_2O$ (1.91 g, 4.30 mmol), MeOH (60 ml), EtOH (180 ml), $CHCl_3$ (50 ml) and H_2O (25 ml) were used. Column chromatography (CH_2Cl_2 : EtOAc 10 : 1) yielded orange powder product (288 mg, 55% yield). IR (KBr): 3026, 2960, 2921, 2859, 1656, 1459, 1293 cm^{-1} . ^1H NMR (300 MHz, $CDCl_3$): Δ 7.24–7.17 (m, 8H), 6.79 (d, $J = 7.5$ Hz, 4H), 6.65 (t, $J = 7.5$ Hz, 2H), 6.48 (s, 4H), 4.75 (s, 4H), 3.65 (d, $J = 12.6$ Hz, 4H), 3.14 (d, $J = 12.6$ Hz, 4H), 2.49 (s, 6H). ^{13}C NMR (75.5 MHz, $CDCl_3$): Δ 188.5, 186.2, 156.1, 148.2, 139.2, 133.7, 132.4, 130.6, 129.9, 129.2, 126.8, 123.9, 76.2, 32.1, 16.0. HRMS: calcd for $C_{44}H_{36}O_6S_2 + Na^+$, 747.1851; found, 747.1879.

Cone 5,17-dibromo-11,26,23,28-tetraone-25,27-dipropoxycalix[4]arene (3b). Compound **2b** (2 g, 3.73 mmol) and *N*-bromosuccinimide NBS (2.26 g, 12.67 mmol) were dissolved in 2-butanone (MEK) (90 ml) and catalytic amount of 48% HBr (60 μ l) was added. The reaction mixture was let to stir at room temperature in a dark place for 24 h. The reaction mixture was quenched by addition of 10% $NaHSO_3$ (aq) (75 ml) and let to stir for 10 min. CH_2Cl_2 (100 ml) and organic phase was separated from aqueous. The latter was extracted with additional CH_2Cl_2 (3 $\times$ 25 ml). Combined organic phases were dried with $MgSO_4$ and then solvents were removed under the reduce pressure. Crude product purified by column chromatography (CH_2Cl_2 : EtOAc 15 : 1) and recrystallized from solvent mixture ($CHCl_3$ – MeOH) to yield orange crystalline product (1.77g, 68% yield). IR (KBr): 2966, 2931, 2876, 1657, 1460, 1294, 1204 cm^{-1} . ^1H NMR (300 MHz, $CDCl_3$): Δ 7.01 (s, 4H), 6.64 (s, 4H), 3.79 (d, $J = 13.3$ Hz, 4H), 3.63 (t, $J = 7.1$ Hz, 4H), 3.28 (d, $J = 13.3$ Hz, 4H), 1.80 (m, 4H), 0.99 (t, $J = 7.4$ Hz, 6H). ^{13}C NMR (75.5

MHz, $CDCl_3$): Δ 188.3, 185.7, 156.1, 147.3, 133.1, 132.4, 116.4, 32.0, 23.9, 10.9. HRMS: calcd for $C_{34}H_{30}O_6Br_2 + H^+$, 693.0487; found, 693.0510.

Cone 5,17-dibromo-11,26,23,28-tetrahydroxy-25,27-dipropoxycalix[4]arene (4b). Compound **3b** (1.15 g, 1.65 mmol) was dissolved in $CHCl_3$ (287 ml) and heated up to 70 $^{\circ}C$. Then $NaHSO_3$ (aq) (2.3 g, 13.2 mmol in 34 ml of H_2O) was added. Reaction mixture was let to stir at boiling point for 1.5 h. Reaction mixture was then cooled down to room temperature and H_2O (20 ml) was added. Organic phase was separated from aqueous and dried with $MgSO_4$. Solvents were removed under reduced pressure and remaining solid recrystallized from solvent mixture ($CHCl_3$ – *n*-hexane) to yield colorless crystalline product (1.12 g, 97% yield). IR (KBr): 3376, 2963, 2928, 2874, 1656, 1460, 1204 cm^{-1} . ^1H NMR (300 MHz, $CDCl_3$): Δ 7.79 (s, 2H), 7.08 (s, 4H), 6.55 (s, 4H), 4.34 (s, 2H) 4.24 (d, $J = 12.9$ Hz, 4H), 3.92 (t, $J = 6.4$ Hz, 4H), 3.24 (d, $J = 12.9$ Hz, 4H), 2.04 (m, 4H), 1.27 (t, $J = 7.4$ Hz, 6H). ^{13}C NMR (75.5 MHz, $CDCl_3$): Δ 151.7, 148.4, 147.4, 135.9, 132.3, 128.6, 118.1, 115.7, 79.1, 31.7, 23.7, 11.2. HRMS: calcd for $C_{34}H_{34}O_6Br_2 + H^+$, 697.0800; found, 697.0816.

Cone 5,17-dibromo-26,28-dihydroxy-11,23-bis[4-(trimethylsiloxy)]-25,27-dipropoxycalix[4]arene (5b). Compound **4b** (200 mg, 0.29 mmol) was suspended in mixture of MeCN (10 ml) and triethylamine (TEA) (0.4 ml, 2.86 mmol). Reaction mixture was then cooled down to –10 $^{\circ}C$ and trimethylsilyl chloride (TMSCl) (0.36 ml, 2.86 mmol) was slowly added. After 10 min of stirring at –10 $^{\circ}C$ a precipitate formed, this was filtrated and washed with MeOH to yield analytically pure product (220 mg, 91% yield). IR (KBr): 3369, 3060, 3023, 2926, 1591, 1464, 1263, 1215 cm^{-1} . ^1H NMR (300 MHz, $CDCl_3$): Δ 7.84 (s, 2H), 7.07 (s, 4H), 6.55 (s, 4H), 4.23 (d, $J = 12.9$ Hz, 4H), 3.91 (t, $J = 6.2$ Hz, 4H), 3.24 (d, $J = 12.9$ Hz, 4H), 2.03 (m, 4H), 1.29 (t, $J = 7.4$ Hz, 6H), 0.24 (s, 18H). ^{13}C NMR (75.5 MHz, $CDCl_3$): Δ 151.5, 148.0, 147.3, 135.8, 132.1, 127.9, 120.3, 117.9, 78.8, 31.6, 23.6, 11.1, 0.3. HRMS: calcd for $C_{40}H_{50}O_6Si_2Br_2 + Na^+$, 863.1410; found, 863.1434.

Cone 5,17-dibromo-11,23-dihydroxy-26,28-bis[4-(trimethylsiloxy)]-25,27-dipropoxycalix[4]arene (6b). NaH (96 mg, 4.01 mmol) was suspended in DMF (7 ml) and **4b** (280 mg, 0.40 mmol) was slowly – in parts – added to the suspension. After completed addition the reaction mixture was cooled down to –10 $^{\circ}C$ and let to stir for 15 min. TMSCl (1.02 ml, 8.02 mmol) was then slowly added to the mixture and let to stir at room temperature for 2 h. After 2 h the DMF was removed under reduced pressure, the remaining solid dissolved in CH_2Cl_2 and shook with H_2O . Organic phase was separated from aqueous, dried with $MgSO_4$ and then solvents removed under reduced pressure. Column chromatography (EtOAc : *n*-hexane 1 :

3) yielded white powder product (80 mg, 24% yield). IR (KBr): 3333, 2961, 2920, 2875, 1598, 1459, 1288, 1252, 1215 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 7.23 (s, 4H), 5.58 (s, 4H), 4.78 (br s, 2H), 4.25 (d, $J = 13.7$ Hz, 4H), 3.89 (m, 4H), 2.97 (d, $J = 13.7$ Hz, 4H), 1.83 (m, 4H), 0.85 (t, $J = 7.4$ Hz, 6H), 0.21 (s, 18H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 157.5, 148.6, 144.8, 139.4, 131.9, 131.7, 115.5, 114.5, 76.6, 32.2, 23.4, 9.9, 0.5. HRMS: calcd for $\text{C}_{40}\text{H}_{50}\text{O}_6\text{Si}_2\text{Br}_2 + \text{NH}_4^+$, 858.1856; found, 858.1888.

Cone 5,17-dibromo-11,23,25,26,27,28-hexapropoxy-calix[4]arene (7b₁). Following the general procedure for synthesis of alkoxy-calix[n]arene; compound **4b** (670 mg, 0.96 mmol), NaH (207 mg, 8.63 mmol), 1-bromopropane (0.79 ml, 8.63 mmol) and DMF (9 ml) were used. Recrystallization from solvent mixture CHCl_3 – MeOH yielded colorless crystalline product (676 mg, 81% yield). IR (KBr): 2962, 2924, 2874, 1597, 1461, 1212 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 6.67 (s, 4H), 6.36 (s, 4H), 4.37 (d, $J = 13.2$ Hz, 4H), 3.84–3.72 (m, 12H), 3.05 (d, $J = 13.2$ Hz, 4H), 1.93–1.84 (m, 8H), 1.75 (m, 4H), 1.05–0.92 (m, 18H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 155.7, 154.3, 151.0, 136.9, 136.0, 130.9, 115.3, 114.8, 70.1, 31.6, 23.7, 23.4, 23.1, 11.0, 10.9, 10.6. HRMS: calcd for $\text{C}_{46}\text{H}_{58}\text{O}_6\text{Br}_2 + \text{Na}^+$, 887.2498; found, 887.2515.

Partial-cone 5,17-dibromo-11,26,23,28-tetramethoxy-25,27-dipropoxycalix[4]arene (7b₂). Following slightly modified (as a solvent mixture of THF – DMF was used) general procedure for synthesis of alkoxy-calix[n]arene; compound **4b** (158 mg, 0.23 mmol), NaH (87 mg, 3.62 mmol), MeI (0.31 ml, 4.98 mmol), THF (5 ml), and DMF (0.5 ml) were used. Recrystallization from solvent mixture CH_2Cl_2 – MeOH yielded colorless crystalline product (150 mg, 88% yield). IR (KBr): 2964, 2925, 2872, 1595, 1464, 1212 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 7.09–6.44 (m, 8H), 4.31–2.99 (m, 24H), 1.91–1.84 (m, 4H), 1.16–1.05 (m, 6H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 155.8, 155.3, 155.2, 155.0, 154.2, 152.9, 152.5, 152.0, 137.2, 136.0, 135.8, 134.0, 133.8, 132.1, 130.8, 130.3, 116.5, 115.5, 115.0, 114.5, 114.3, 76.4, 61.3, 60.2, 59.7, 55.9, 55.8, 36.0, 31.2, 24.3, 24.1, 11.3, 11.2. HRMS: calcd for $\text{C}_{38}\text{H}_{42}\text{O}_6\text{Br}_2 + \text{H}^+$, 753.1426; found, 753.1448.

Partial-cone 5,17-bis(thioacetyl)-11,26,23,28-tetramethoxy-25,27-dipropoxycalix[4]arene (8). Following previously published method;²¹ compound **7b₂** (100 mg, 0.14 mmol), THF (12 ml), *tert*-BuLi (0.51 ml, 1.46 M in pentane, 0.86 mmol), sulfur (28 mg, 0.86 mmol) and AcCl (0.1 ml, 1.44 mmol) were used. Column chromatography (EtOAc : *n*-hexane 1 : 5) yielded white powder product (25 mg, 25% yield). IR (KBr): 2959, 2928, 2874, 2834, 1697, 1601, 1477, 1457, 1234, 1207 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 7.00–6.36 (m, 8H), 4.34–3.04 (m, 24H),

2.25 (s, 3H), 2.13 (s, 3H), 1.89 (m, 4H), 1.05 (m, 6H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 196.1, 156.1, 137.2, 135.4, 134.8, 134.4, 133.8, 116.4, 114.2, 114.0, 76.2, 61.2, 31.0, 30.2, 24.2, 11.1. HRMS: calcd for $\text{C}_{42}\text{H}_{48}\text{O}_8\text{S}_2 + \text{H}^+$, 745.2869; found, 745.2878.

Cone 5,17-dibromo-26,28-bis(phenylmethoxy)-25,27-dihydroxycalix[4]arene (9a). Compound **1a** (3.9 g, 6.45 mmol) was dissolved in CHCl_3 (130 ml) and Br_2 (0.66 ml, 12.96 mmol) solution in CHCl_3 (130 ml) was slowly added in 2 h at 0 °C. The reaction mixture was let to warm up to room temperature and stirred for additional 2 h. The solvent was then removed under reduced pressure and remaining solid recrystallized. Recrystallization from solvent mixture CHCl_3 – MeOH yielded colorless crystalline product (4.65 g, 95% yield). ^1H NMR (300 MHz, CDCl_3): Δ 7.86 (s, 2H), 7.61–6.80 (m, 22H), 5.03 (s, 4H), 4.23 (d, $J = 13.2$ Hz, 4H), 3.27 (d, $J = 13.2$ Hz, 4H). The spectrum is in accordance with previously published results.³⁶

Cone 5,17-dibromo-26,28-bis(phenylmethoxy)-25,27-dipropoxycalix[4]arene (10a₁). Following the general procedure for synthesis of alkoxy-calix[n]arene; compound **9a** (4.91 g, 6.45 mmol), NaH (541 mg, 22.56 mmol), 1-bromopropane (2.05 ml, 22.56 mmol) and DMF (60 ml) were used. Recrystallization from solvent mixture CH_2Cl_2 – MeOH yielded colorless crystalline product (5.01 g, 92% yield). ^1H NMR (300 MHz, CDCl_3): Δ 7.42–7.32 (m, 10H), 7.02 (s, 4H), 6.47–6.38 (m, 6H), 4.82 (s, 4H), 4.33 (d, $J = 13.3$ Hz, 4H), 3.74 (m, 4H), 3.04 (d, $J = 13.3$ Hz, 4H), 1.65 (m, 4H), 0.64 (t, $J = 7.4$ Hz, 6H). The spectrum is in accordance with previously published results.³⁷

Cone 5,17-bis(thioacetyl)-26,28-bis(phenylmethoxy)-25,27-dipropoxycalix[4]arene (11a₁). Following slightly modified (AcCl was replaced by MeI) previously published method;²¹ compound **10a₁** (2 g, 2.36 mmol), THF (96 ml), *tert*-BuLi (16.67 ml, 1.46 M solution in pentane, 28.34 mmol), sulfur (1.06 g, 33.07 mmol) and MeI (2.35 ml, 37.79 mmol) were used. Column chromatography (CH_2Cl_2 : *n*-hexane 2 : 3) and recrystallization from solvent mixture (CHCl_3 – MeOH) yielded colorless crystalline product (780 mg, 42% yield). IR (KBr): 3024, 2961, 2917, 2871, 1582, 1456, 1372, 1212, 1195 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 7.46–7.43 (m, 4H), 7.35–7.32 (m, 6H), 6.92 (s, 4H), 6.37–6.29 (m, 6H), 4.78 (s, 4H), 4.38 (d, $J = 13.2$ Hz, 4H), 3.76 (m, 4H), 3.07 (d, $J = 13.2$ Hz, 4H), 2.44 (s, 6H), 1.64 (m, 4H), 0.60 (t, $J = 7.4$ Hz, 6H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 155.9, 154.7, 137.6, 137.0, 133.4, 130.0, 129.1, 128.2, 128.0, 127.9, 127.8, 122.5, 76.6, 31.1, 22.8, 17.3, 9.5. HRMS: calcd for $\text{C}_{50}\text{H}_{52}\text{O}_4\text{S}_2 + \text{H}^+$, 781.3385; found, 781.3378.

Cone 5,17-bis(thioacetyl)-26,28-dihydroxy-25,27-dipropoxycalix[4]arene (12a₁). Compound **11a₁** (89 mg,

0.11 mmol) was dissolved in CH_2Cl_2 (15 ml) and molecular sieves were added. Mixture was cooled down to $-30\text{ }^\circ\text{C}$ and trimethylsilyl bromide (TMSBr) (0.12 ml, 0.91 mmol) was added and let to stir for 3 h. Reaction mixture was then poured into saturated aqueous NaHCO_3 solution and organic phase was separated. The organic phase was then extracted with H_2O and dried with MgSO_4 . Solvents were removed under reduced pressure and crude product purified by column chromatography (CH_2Cl_2 : n-hexane 1 : 1) followed by recrystallization from solvent mixture (CHCl_3 – MeOH) to yield colorless crystalline product (31 mg, 45% yield). IR (KBr): 3330, 2959, 2921, 2862, 1583, 1464, 1436, 1203 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): Δ 8.33 (s, 2H), 7.02 (d, $J = 7.5$ Hz, 4H), 6.84 (s, 4H), 6.62 (t, $J = 7.5$ Hz, 2H), 4.28 (d, $J = 12.9$ Hz, 4H), 3.95 (t, $J = 6.2$ Hz, 4H), 3.34 (d, $J = 12.9$ Hz, 4H), 2.31 (s, 6H), 2.05 (m, 4H), 1.31 (t, $J = 7.4$ Hz, 6H). ^{13}C NMR (75.5 MHz, CDCl_3): Δ 153.4, 150.0, 134.1, 133.6, 128.6, 127.7, 127.5, 119.2, 78.5, 31.5, 23.5, 16.2, 10.9. HRMS: calcd for $\text{C}_{36}\text{H}_{40}\text{O}_4\text{S}_2 + \text{H}^+$, 601.2446; found, 601.2451.

Cone 11,26,23,28-tetraone-25,27-bis-[(hydroxycarbonyl)methoxy]-calix[4]arene (13). To a solution of **2c** (190 mg, 0.28 mmol) in CH_2Cl_2 (3 ml), trifluoroacetic acid (0.6 ml, 7.53 mmol) was slowly added and then let to stir at room temperature under inert atmosphere for 5 h. Reaction was quenched by addition of cold H_2O ($0\text{ }^\circ\text{C}$). Organic phase was separated from aqueous and dried with MgSO_4 . Solvent was concentrated under reduced pressure and product precipitated as a yellow crystalline substance (105 mg, 66% yield). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, 2.50): Δ 6.83 (s, 4H), 6.75 (d, $J = 7.5$ Hz, 4H), 6.64 (t, $J = 7.5$ Hz, 2H), 4.41 (s, 4H), 3.83 (d, $J = 13.1$ Hz, 4H), 3.33 (d, $J = 13.1$ Hz, 4H). The spectrum is in accordance with previously published results.²⁵

Cone 11,26,23,28-tetrahydroxy-25,27-bis-[(hydroxycarbonyl)methoxy]-calix[4]arene (14). Compound **13** (50 mg, 0.09 mmol) was dissolved in MEK (4 ml) and aqueous solution of $\text{Na}_2\text{S}_2\text{O}_4$ (122 mg, 0.70 mmol in 2 ml of H_2O) was added. Yellow color of reaction mixture disappeared during stirring (1 min). Organic phase was separated from aqueous and dried with MgSO_4 . After addition of CHCl_3 (5 ml) to the dried organic phase, a precipitate formed. This was filtered off using PTFE filter (0.2 μm) and dried. The product was isolated as a pale yellow powder (18 mg, 36% yield). IR (KBr): 3434, 2932, 1739, 1609, 1464, 1440, 1242, 1193 cm^{-1} . ^1H NMR (300 MHz, $\text{DMSO}-d_6$, 2.50): Δ 7.00 (d, $J = 7.6$ Hz, 4H), 6.78 (t, $J = 7.6$ Hz, 2H), 6.47 (s, 4H), 4.68 (s, 4H), 4.36 (d, $J = 12.8$ Hz, 4H), 3.25 (d, $J = 12.8$ Hz, 4H). ^{13}C NMR (75.5 MHz, $\text{DMSO}-d_6$, 39.52): Δ 170.5, 153.1, 149.7, 144.5, 133.9, 129.0, 128.9, 125.0, 114.9, 72.1, 31.0. HRMS: calcd for $\text{C}_{32}\text{H}_{28}\text{O}_{10} + \text{Na}^+$, 595.1580; found, 595.1568.

4. 2. Preparation of Au(111) Substrate.

Au(111) single crystal (MaTecK) was annealed in UHV at $550\text{ }^\circ\text{C}$ after ion bombardment with 1 kV Ar^+ ions at 5×10^{-6} mbar. The cycle was repeated at least two times. Au(111) single crystal was then transferred to a homemade annealing apparatus and annealed at $700\text{ }^\circ\text{C}$ in 3% H_2/Ar atmosphere for 30 min. The surface of the Au(111) single crystal was protected with H_2O droplet (30 μL) and transferred to a vial for self-assembly.

4. 3. Self-assembly

»Ex-situ« method. Au(111) was immersed into $\sim 80\text{ }\mu\text{M}$ solution (solvent mixture CH_2Cl_2 : EtOH 1 : 9) of adsorbate molecules **2f** and **2g** at room temperature for 30 min. Adsorbate solutions; were always freshly prepared. After self-assembly the Au(111) was removed from the solution and washed with EtOH, i-PrOH in Milli-Q H_2O , and dried in Ar atmosphere.

»In-situ« method. The surface of Au(111) single crystal was immersed into an electrochemical cell with 0.1 M HClO_4 with $\sim 80\text{ }\mu\text{M}$ concentrations of **13** and **14**. The system was held at 0.2 V vs Ag/AgCl for 10 min.

4. 4. Electrochemistry

A standard three-compartment electrochemical cell containing 0.1 M HClO_4 (OmniTrace UltraTM from EDM), Au wire as a counter electrode and Ag/AgCl as a reference electrode was used. In each experiment, the electrode was immersed (hanging meniscus technique) at 0.07 V in a solution saturated with Ar and cycled between 0.07 V and 1.17 V versus the reversible hydrogen electrode (RHE). The sweep rate for all measurements was 50 mVs^{-1} . Electrode potentials are given versus the RHE. Autolab potentiostat was used in the electrochemical measurements.

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Povzetek

Opisana je sinteza in karakterizacija novih elektroaktivnih dikinonskih in kinonskih derivatov kaliks[4]arena s sidrnimi funkcionalnimi skupinami. Razvita je bila tudi metoda selektivne zaščite hidrokinonskih –OH skupin s trimetilsililnimi skupinami (TMS) na spodnjem oz. zgornjem obroču makrocikla. Štiri molekule, s sulfidnimi oz. s karboksilnimi sidri so bile adsorbirane na površino monokristala Au(111), z uporabo *ex-situ* in *in-situ* metode samosestavljenosti. Adsorbirane molekule smo testirali s ciklično voltametrijjo (CV). Vse testirane molekule so pokazale redox odziv, ki se je spreminjal med posameznimi cikli. Po kondicioniranju so se CV-ji ustalili in pokazali dva izrazita tokovna vrhova, podobna za vse testirane molekule. Sintetizirane in elektrokemijsko testirane molekule so zanimive za: Li-ionske baterije (kot katodni material oz. kot zaščita prenapetosti), baterije z novimi kemijami in redoks-pretočne baterije.

Scientific paper

The Chemical Capacitance as a Fingerprint of Defect Chemistry in Mixed Conducting Oxides

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Abstract

The oxygen stoichiometry of mixed conducting oxides depends on the oxygen chemical potential and thus on the oxygen partial pressure in the gas phase. Also voltages may change the local oxygen stoichiometry and the amount to which such changes take place is quantified by the chemical capacitance of the sample. Impedance spectroscopy can be used to probe this chemical capacitance. Impedance measurements on different oxides ((La,Sr)FeO_{3-δ} = LSF, Sr(Ti,Fe)O_{3-δ} = STF, and Pb(Zr,Ti)O₃ = PZT) are presented, and demonstrate how the chemical capacitance may affect impedance spectra in different types of electrochemical cells. A quantitative analysis of the spectra is based on generalized equivalent circuits developed for mixed conducting oxides by J. Jamnik and J. Maier. It is discussed how defect chemical information can be deduced from the chemical capacitance.

Keywords: Defect chemistry, impedance spectroscopy, mixed conductors, chemical capacitance

1. Introduction

Many oxides exhibit a significant degree of mixed ion and electron conduction at higher temperatures. Their transport properties are thus usually determined by motion of ionic as well as electronic defects, for example oxygen vacancies and electrons/electron holes. Several mixed conducting oxides such as Sr-doped LaCoO₃ or La-FeO₃ can be employed in cathodes of solid oxide fuel cells.^{1,2} Those materials exhibit high electronic conductivity in air, mostly also at lower temperatures, and substantial ionic conductivity at operation temperatures of several hundred °C. However, also in oxides with applications relying on a high insulation resistance at room temperature, mixed conductivity might become relevant either at high temperatures or upon high field load.^{3–8} Examples for such materials are lead zirconate titanate (PZT) in actuators or BaTiO₃ in capacitors.

Investigation of the electrical and dielectric properties of mixed conducting oxides is often performed by im-

pedance spectroscopy. For a quantitative analysis of the impedance spectra, physical models are required and most models rely on equivalent circuits. Either circuits are simply used to parameterize a spectrum (e.g. two serial RC-elements for describing two semicircles in a complex impedance plane), or specific physical models are developed and mapped by equivalent circuits. The latter is often done on a somewhat intuitive basis. For example, the impedance of a mixed conductor with ion blocking and electron conducting electrodes might be represented by two parallel resistors for ion (R_{ion}) and electron (R_{con}) conduction, the parallel geometrical capacitance of the sample with permittivity ϵ (C_{geo}) and a serial capacitance C_{el} in the ionic rail, reflecting the ion blocking character of the electrodes (Fig. 1). This circuit is indeed able to describe some of the corresponding experiments but fails in other cases.

The transport equations governing mixed ionic electronic conductors (MIECs) were used in numerous papers by J. R. Macdonald to rigorously calculate the impedance

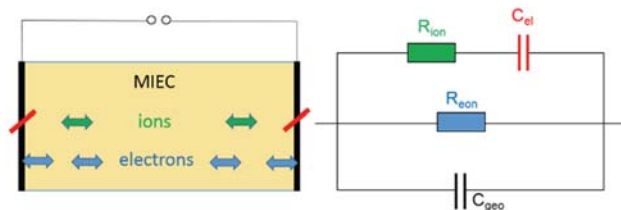


Fig. 1. Sketch of a mixed ionic and electronic conductor (MIEC) with ions blocked at the electrodes and an intuitive (but not exact) equivalent circuit describing such a situation.

of many specific situations.^{9,10} The disadvantage of those numerical calculations, however, is the challenge of intuitively predicting how parameter changes affect spectra shapes and also the difficulty of applying numerical models when quantifying experimental data. Generalized equivalent circuits describing the impedance of mixed conductors were not available until J. Jamnik and J. Maier published several seminal papers on this topic.^{11–13} There, the circuit in Fig. 2a was introduced as the generalized equivalent circuit of a homogeneous mixed conductor. For specific experiments appropriate boundary conditions ha-

ve to be considered via terminal circuit elements to take account of processes such as oxygen exchange at a MIEC surface or ion/electron blocking at an electrode.

The essential additional element compared to the intuitive circuit in Fig. 1 is the chemical capacitance C_{chem} in its differential version (dC_{chem}). This chemical capacitance couples the ionic and electronic rails of charge transport by allowing local changes of stoichiometry and leads to the transmission line-type character of the circuit. It is a measure of the readiness for stoichiometry changes and describes the electroneutral chemical storage of charges, in contrast to an electrostatic capacitor with charge separation. For the sake of simplicity the geometrical sample capacitance C_{geo} , which is in parallel to the entire circuit and describes the dielectric displacement current, is not included here.

This chemical capacitance contains important information on the defect chemistry of oxides and is mainly determined by the concentration of the minority charge carrier. In this publication we first specify the theory of the chemical capacitance in mixed conducting oxides and then examples are given, where the measurement of the chemical capacitance is used to reveal information on de-

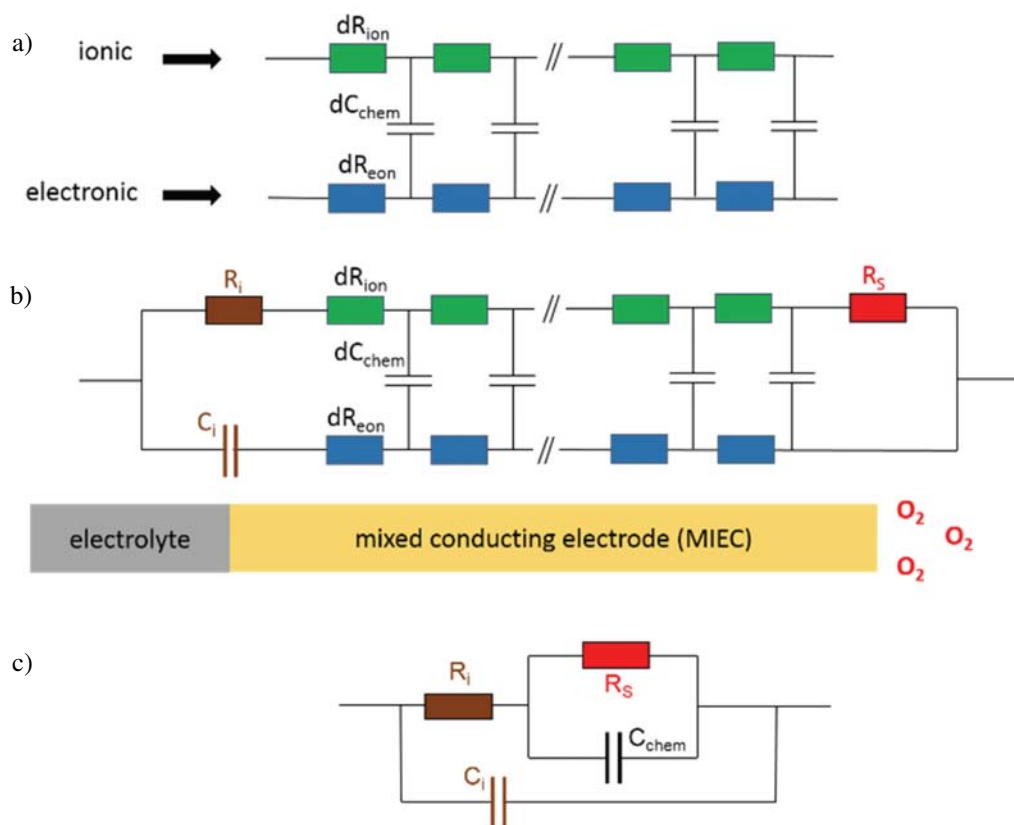


Fig. 2. (a) Transmission line-type equivalent circuit of a mixed conductor with differential chemical capacitances dC_{chem} coupling the ionic and the electronic rails of charge transport. The parallel geometrical sample capacitance (C_{geo}) is not included here. (b) The same circuit including terminal elements for a homogeneous mixed conducting electrode in a solid oxide fuel/electrolysis cell with oxygen exchange at the surface (resistor R_s), an interfacial charge transfer resistance R_i and electron blocking at the MIEC/electrolyte interface (C_i). (c) Simplified circuit resulting from (b) when neglecting the transport resistances R_{ion} and R_{eon} .

fect chemical properties of mixed conducting oxides. Moreover, the generalized MIEC equivalent circuit is applied to deconvolute of ionic and electronic contributions to the total conductivity.

2. The Chemical Capacitance of a Mixed Conducting Oxide

The chemical capacitance of an oxide¹² is defined by

$$C_{\text{chem}} = 4F^2 V n^0 \left(\frac{\partial \mu_{\text{O}}}{\partial c_{\text{O}}} \right)^{-1}. \quad (1)$$

In Eq. (1) F is Faraday's constant, V the sample volume. The key factor is the derivative of the oxygen chemical potential μ_{O} with respect to the normalized oxygen concentration of oxygen c_{O} ; normalization of the concentration is done with respect to the absolute concentration of oxygen sites n^0 . The normalized oxygen concentration c_{O} is directly related to the non-stoichiometry δ of a metal oxide $\text{MO}_{1-\delta}$. The chemical potential of oxygen in an oxide is given by the chemical potentials of oxide ions and electrons via

$$\mu_{\text{O}} = \mu_{\text{O}^{2-}} - 2\mu_{\text{e}^-}. \quad (2)$$

In the following we consider an oxide with oxygen vacancies (V) as dominating ionic defects and electronic defects (eon) either as electrons (e) or holes (h); electron or hole traps are neglected. The oxygen chemical potential can then be expressed as

$$\mu_{\text{O}} = -\mu_{\text{V}} + 2z_{\text{eon}}\mu_{\text{eon}} \quad (3)$$

with $\text{eon} = e$ or h ; z_{eon} denotes the charge number of the electronic defect, e.g. -1 for e .

The variation of the oxygen stoichiometry can be described by

$$dc_{\text{O}} = -dc_{\text{V}} \quad (4)$$

and concentration changes of electronic and ionic defects are coupled by the local electroneutrality, i.e. via

$$dc_{\text{eon}} = -2z_{\text{eon}}dc_{\text{V}} = 2z_{\text{eon}}dc_{\text{O}}. \quad (5)$$

Differentiating Eq. (3) with respect to c_{O} and combining with Eqs. (4) and (5) results in

$$\frac{\partial \mu_{\text{O}}}{\partial c_{\text{O}}} = -\frac{\partial \mu_{\text{V}}}{\partial c_{\text{O}}} + 2z_{\text{eon}} \frac{\partial \mu_{\text{eon}}}{\partial c_{\text{O}}} = \frac{\partial \mu_{\text{V}}}{\partial c_{\text{V}}} + 4 \frac{\partial \mu_{\text{eon}}}{\partial c_{\text{eon}}}. \quad (6)$$

In the case of dilute ionic as well as electronic defects, chemical potentials of all defects d can be expressed as

$$\mu_d = \mu_d^0 + RT \ln c_d \quad (7)$$

with normalized defect concentrations

$$c_d = \frac{n_d}{n^0}. \quad (8)$$

In Eq. (7) μ_d^0 is the standard chemical potential of the defect. Hence, we find

$$\frac{d\mu_d}{dc_d} = \frac{RT}{c_d} \quad (9)$$

and by inserting relation (9) into Eq. (6) we get

$$\frac{\partial \mu_{\text{O}}}{\partial c_{\text{O}}} = RT \left(\frac{1}{c_{\text{V}}} \right) + 4RT \left(\frac{1}{c_{\text{eon}}} \right). \quad (10)$$

We finally obtain from Eqs. (1) and (10)

$$C_{\text{chem}} = \frac{4F^2 V n^0}{RT} \frac{1}{\left(\frac{1}{c_{\text{V}}} + \frac{4}{c_{\text{eon}}} \right)} = \frac{F^2 V}{RT} \frac{1}{\left(\frac{1}{4n_{\text{V}}} + \frac{1}{n_{\text{eon}}} \right)}. \quad (11)$$

In many cases the concentrations of electronic and ionic defects differ strongly and the chemical capacitance is determined by the minority charge carrier concentration (n_{min}), or, more precisely, by the smaller of the two $z_d^2 n_d$ values (z_d = charge number of the defect). This results in the simplified relation

$$C_{\text{chem}} \approx \frac{F^2 V}{RT} z_{\text{min}}^2 n_{\text{min}}. \quad (12)$$

Alternatively, we may also express the chemical capacitance with the so-called thermodynamic factor of oxygen. In general, the thermodynamic factor (f) of particle, defect or component k is defined by

$$f_k = \frac{\partial \ln a_k}{\partial \ln c_k}. \quad (13)$$

The activity a_k is related to the chemical potential μ_k via

$$\mu_k = \mu_k^0 + RT \ln a_k \quad (14)$$

and the standard chemical potential μ_k^0 is constant by definition. Hence, the relation between any chemical potential and oxygen concentration is given by

$$\frac{\partial \mu_{\text{O}}}{\partial c_{\text{O}}} = \frac{\partial \mu_{\text{O}}}{\partial \ln c_{\text{O}}} \frac{\partial \ln c_{\text{O}}}{\partial c_{\text{O}}} = \frac{RT}{c_{\text{O}}} \frac{\partial \ln a_{\text{O}}}{\partial \ln c_{\text{O}}} = \frac{RT}{c_{\text{O}}} f_{\text{O}}. \quad (15)$$

The chemical capacitance of Eq. (1) is thus given by

$$C_{\text{chem}} = \frac{4F^2 V n_{\text{O}}}{RT} \frac{1}{f_{\text{O}}} \quad \text{with} \quad n_{\text{O}} = n^{\text{O}} c_{\text{O}}. \quad (16)$$

In case of our mixed conducting oxide, the thermodynamic factor of oxygen f_{O} can be calculated by Eqs. (15) and (6), resulting in

$$f_{\text{O}} = \frac{c_{\text{O}}}{RT} \left(\frac{\partial \mu_{\text{V}}}{\partial c_{\text{V}}} + 4 \frac{\partial \mu_{\text{e on}}}{\partial c_{\text{e on}}} \right). \quad (17)$$

When thermodynamic factors are introduced also for defects (f_{d}), we get with

$$\frac{\partial \mu_{\text{d}}}{\partial c_{\text{d}}} = \frac{\partial \mu_{\text{d}}}{\partial \ln c_{\text{d}}} \frac{\partial \ln c_{\text{d}}}{\partial c_{\text{d}}} = RT \frac{\partial \ln a_{\text{d}}}{\partial \ln c_{\text{d}}} \frac{1}{c_{\text{d}}} = \frac{RT}{c_{\text{d}}} f_{\text{d}} \quad (18)$$

and Eq. (17) the relation

$$f_{\text{O}} = \frac{c_{\text{O}}}{c_{\text{V}}} f_{\text{V}} + 4 \frac{c_{\text{O}}}{c_{\text{e on}}} f_{\text{e on}}. \quad (19)$$

For diluted defects we have $f_{\text{e on}} = f_{\text{V}} = 1$ and inserting this thermodynamic factor of oxygen into Eq. (1) again results in Eq. (11). For dilute defects in mixed conducting oxides the thermodynamic factor of oxygen is of the order of the inverse normalized concentration of the minority defect and thus often larger than 100. This simple reason of high f_{O} values was already emphasized in Ref. 14.

3. Defect Chemistry and Chemical Capacitances of Polarized Fuel Cell Model Electrodes

Acceptor-doped perovskite type oxides (ABO_3) are the standard materials for cathodes in solid oxide fuel cells. The (relative negative) dopant charge, e.g. of Sr on the A-site of LaBO_3 is charge balanced either by oxygen vacancies or electron holes. The B-cation strongly affects the amount of oxygen vacancies found at operation temperatures of several hundred °C in synthetic air. However, also the oxygen partial pressure $p(\text{O}_2)$ plays an important role and lowering $p(\text{O}_2)$ may strongly increase the oxygen vacancy concentration at the expense of electron holes. This is reflected in so-called Brouwer-diagrams^{15,16} where defect concentrations are plotted versus the oxygen partial pressure, see Fig. 3. As a result of these dependencies, acceptor-doped oxides exhibit high oxygen vacancy concentrations and thus excellent ionic conductivity in reducing atmosphere, but the electronic conductivity might become rather low under such conditions. When using acceptor-doped oxides in anodes of solid oxide fuel cells (see e.g. Refs. 17–20), an additional electron conducting phase might thus be very helpful. A systematic study of the properties of model electrodes with metallic current collec-

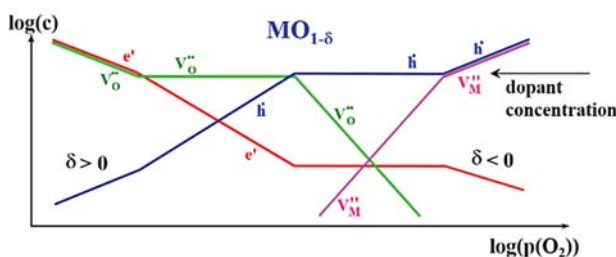


Fig. 3. Sketch of the Brouwer diagram of an acceptor-doped non-stoichiometric oxide $\text{MO}_{1-\delta}$ indicating the partial pressure dependence of all relevant ionic and electronic defects.

tors, based on Sr-doped LaFeO_3 and Fe-doped SrTiO_3 thin films under reducing conditions can be found in Refs. 21,22.

When mixed conductors are used as electrodes in solid oxide fuel cells, the MIEC surface terminal of the equivalent circuit in Fig. 2a has to be modeled by a surface resistance R_s that describes the oxygen exchange. At the MIEC-electrolyte interface, an additional resistance (R_i), representing ion transfer into the electrolyte, is added see Fig. 2b. Moreover, electrons are blocked at the electrode/electrolyte interface, which is taken into account by a capacitor C_i in the electronic rail. However, in many thin film electrodes ionic as well as electronic charge transport across the film is sufficiently fast to warrant the assumption of negligible $R_{\text{e on}}$ and R_{ion} . Hence, only the equivalent circuit shown in Fig. 2c remains from Fig. 2b. This very simple model is indeed often applicable to analyze the impedance of mixed conducting thin film electrodes.²³ It allows to determine the surface-related polarization resistance and the chemical capacitance of the electrode with both R_s and C_{chem} depending on the applied overpotential. The voltage dependence of R_s was hardly investigated so far and is difficult to predict due to the lack of information on the atomistic reaction mechanism of oxygen exchange. Voltage dependent chemical capacitances, on the other hand, should reflect the bulk defect chemical changes upon voltage load.

In most model type thin film electrodes, the oxygen exchange reaction at the surface is rate limiting and leads to the largest part of the electrochemical electrode resistance, i.e. $R_s \gg R_i$. As a consequence, the chemical potential of oxygen (μ_{O}) in the MIEC electrode is directly related to the electrode overpotential via Nernst's equation. Thus, μ_{O} in the electrode can be modified by an applied voltage in the same manner as by changing the outer gas atmosphere and the x-axis of the Brouwer diagram in Fig. 3 can also be scanned by varying the overpotential of the respective electrode. This similarity of changes caused by voltage or oxygen partial pressure was used, for example, in Ref. 24 to interpret impedance data.

The strong relationship between chemical capacitance and defect chemistry, i.e. the decisive role of the minority charge carrier concentration (Eq. (12)),

becomes also clear in the following measurement: $\text{La}_{0.6}\text{Sr}_{0.4}\text{FeO}_{3-\delta}$ (LSF) thin films were deposited on yttria stabilized zirconia (YSZ) single crystals and patterned to circular microelectrodes. A low resistance porous LSF/Pt layer was used as counter electrode. To guarantee a homogeneous electrochemical potential of electrons in the thin film electrode even for very low μ_{O} , a micro-patterned Pt thin film grid was deposited beneath the LSF film (see Fig. 4). Impedance spectra were measured in ambient air for a broad range of bias voltages and always include a large low frequency semicircle in the complex impedance plane (Fig. 4). This arc reflects the polarization resistance due to surface oxygen exchange and the chemical capacitance of the film. Both R_s and C_{chem} depend on the applied voltage.

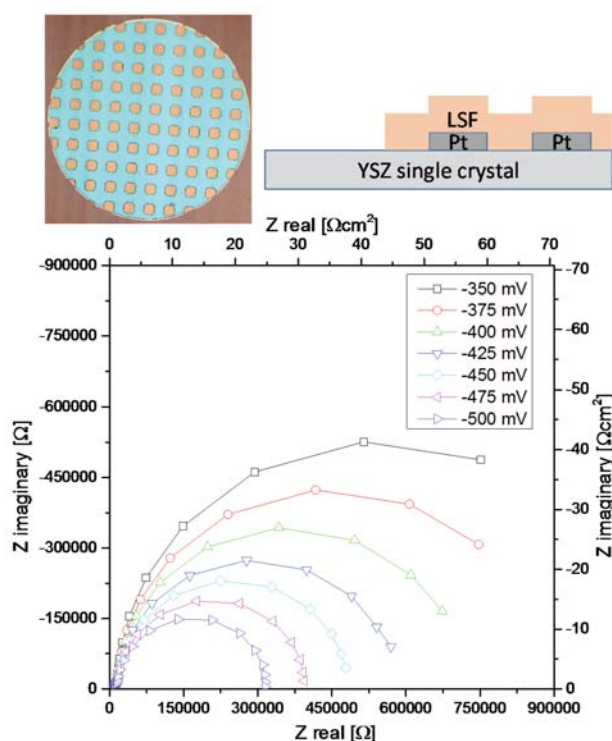


Fig. 4. Impedance spectra measured in air at 600 °C on circular LSF thin film microelectrodes with Pt grid beneath (the photograph of a microelectrode and a sketch of the cross section is also shown); the microelectrodes were deposited on top of an yttria stabilized zirconia single crystal (porous LSF/Pt counter electrode). Depending on the applied cathodic bias voltage the impedance changes.

Chemical capacitances extracted from the main arc are then normalized to the active volume of the thin film electrode and the electrode overpotentials are related to the oxygen partial pressure by Nernst's equation. The resulting capacitance – partial pressure diagram is shown in Fig. 5. From high to low pressures there is first an increase of C_{chem} , then a strong decrease by more than one order of magnitude and finally again an increase. Qualitatively, this very characteristic behavior can be easily understood from

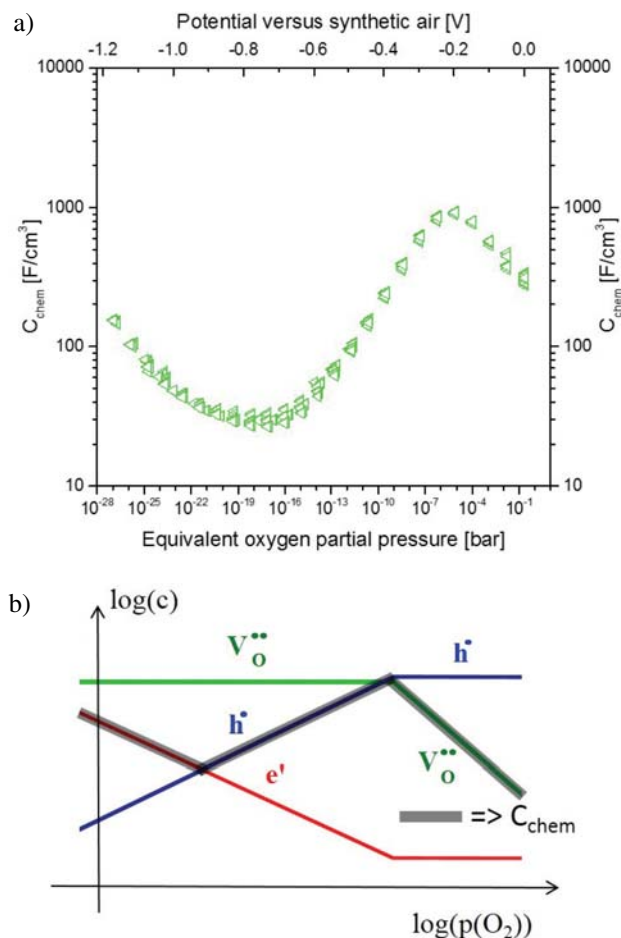


Fig. 5. (a) Chemical capacitances measured at 600 °C in air in dependence of the electrode overpotential (upper abscissa) and the corresponding $p(\text{O}_2)$ (bottom abscissa, calculated by Nernst's equation). C_{chem} is determined from the main arc of the spectra (cf. Fig. 4) and is normalized to the active electrode volume. (b) Sketch of a part of the Brouwer diagram in Fig. 3, indicating the partial pressure dependence of the minority charge carrier concentration which determines C_{chem} ; the decisive charge carrier is reflected by the grey line in parallel to the concentrations.

the concept of chemical capacitances described above, i.e. from Eq. (12). The chemical capacitance reflects the concentration of the minority charge carrier and for high oxygen partial pressures those are the oxygen vacancies. Their concentration and thus C_{chem} increases with decreasing partial pressure until oxygen vacancies become the majority charge carrier counter balancing the Sr dopant, see the detail of the Brouwer diagram sketched in Fig. 5b.

Then, holes (often interpreted as Fe^{4+}) are in minority, though still exhibiting higher concentrations than electrons (Fe^{2+}). In accordance with the Brouwer diagram the hole concentration, i.e. C_{chem} decreases with decreasing $p(\text{O}_2)$ until the intrinsic point of the semiconducting LSF is reached. For even lower $p(\text{O}_2)$ electrons become more important than holes. Accordingly, the relevant minority charge carrier concentration and thus C_{chem} increa-

ses with decreasing partial pressure, again in accordance with the Brouwer diagram. This clearly shows that the chemical capacitance is indeed an excellent indicator of defect chemical changes in materials. However, it has to be emphasized, that only for dilute situations C_{chem} scales linearly with defect concentrations, which is not expected for highly doped LSF. A more detailed analysis of these measurements will be given in a forthcoming paper. A similar analysis of the chemical capacitance, relating defect chemical changes and overpotential as given in Refs. 25,26 for doped ceria.

4. The Role of the Chemical Capacitance in MIECs with Ion Blocking Electrodes

Similar to a Warburg impedance, the MIEC equivalent circuit in Fig. 2 is based on a transmission line. However, in contrast to a Warburg impedance it includes finite resistances in both conduction rails. Still, there are situations in which MIEC circuits include ideal Warburg elements. One example may be found for a MIEC with two ion blocking electrodes. Ion blocking is taken into account by the terminating capacitors C_{el} in the ionic rail (Fig. 6a). An analytical solution (even including further resistive and capacitive terminating elements in the two

rails) is given in Refs. 12 and 27. For chemical capacitances being much larger than the ion blocking capacitor, the analytical expression of the impedance simplifies to

$$Z = R_{\text{eon//ion}} + (R_{\text{eon}} - R_{\text{eon//ion}}) \frac{\tanh\left(\sqrt{\frac{i\omega C_{\text{chem}}(R_{\text{eon}} + R_{\text{ion}})}{4}}\right)}{\sqrt{i\omega C_{\text{chem}}(R_{\text{eon}} + R_{\text{ion}})/4}} \quad (20)$$

The first term in Eq. (20), $R_{\text{eon//ion}}$, refers to the sample resistance relevant for high frequencies R_{hf} and is a parallel connection of the electronic (R_{eon}) and ionic (R_{ion}) resistance:

$$R_{\text{hf}} = R_{\text{eon//ion}} = (R_{\text{eon}}^{-1} + R_{\text{ion}}^{-1})^{-1} \quad (21)$$

The second term corresponds to the analytical expression of a Warburg impedance with resistance R_{w} and fit element T_{w} , i.e. to

$$Z_{\text{w}} = R_{\text{w}} \frac{\tanh(\sqrt{i\omega T_{\text{w}}})}{\sqrt{i\omega T_{\text{w}}}} \quad (22)$$

with

$$T_{\text{w}} = \frac{C_{\text{chem}}(R_{\text{eon}} + R_{\text{ion}})}{4} \quad (23)$$

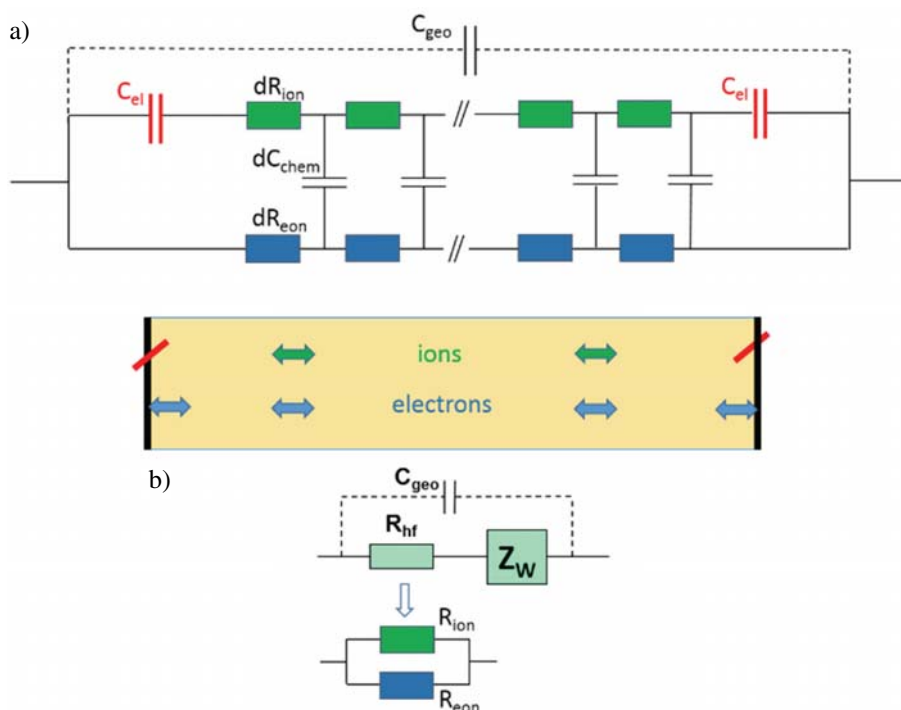


Fig. 6. (a) Equivalent circuit of a mixed conductor (cf. Fig. 2a) with terminals representing ideal ion blocking electrodes with negligible electronic contact resistance, the corresponding situation is sketched beneath. Here, the parallel dielectric displacement current via the geometrical sample capacitance C_{geo} is also taken into account. (b) Simplified equivalent circuit valid for $C_{\text{chem}} \gg C_{\text{el}}$; Z_{w} denotes a finite Warburg impedance. The two serial elements (R_{hf} and Z_{w}) correctly represent the analytical expression of the impedance but are not serial in real space.

Thus, the simple model circuit in Fig. 6b can be introduced to describe such a situation. Please note that the two elements $R_{\text{hf}} = R_{\text{ion/eon}}$ and Z_{W} do no longer represent two serial processes in real space; both include ionic as well as electronic resistances in the entire sample. Still, the corresponding impedance spectra should exhibit the typical shape of a Warburg impedance with 45° slope at high frequencies and semicircle-like shape at low frequencies. Indeed, such spectra can be measured and an example can be found in Ref. 28 for silver telluride.

Here, we show results obtained from 300 nm thin films of $\text{SrTi}_{0.3}\text{Fe}_{0.7}\text{O}_{3-\delta}$ (STF) prepared by pulsed laser deposition on a MgO substrate. STF is a promising mixed conductor for application in SOFC cathodes²⁹ and anodes,²⁰ defect chemical properties were extensively investigated in Ref. 30. In our study, interdigital Pt finger electrodes were deposited on top of these films (see Fig. 7 top) and the impedance was measured in reducing atmosphere ($p(\text{H}_2)/p(\text{H}_2\text{O}) = 1$) at ca. 650 °C. The resulting impedance spectrum is indeed characterized by a resistive high frequency intercept and a Warburg-type impedance, see Fig. 7 bottom, including fit line. The high frequency intercept reflects the total conductivity of the thin film since at such high frequencies both ions and electrons can move ($R_{\text{con/ion}}$); ion blocking is not relevant. At very low frequencies, however, ions are blocked at the electrodes and only electrons contribute. When neglecting additional non-idealities such as an interfacial resistance also for electrons or the two dimensionality of the current lines due to the electrode geometry, the total DC resistance therefore represents the electronic resistance and can be used to calculate the electronic conductivity σ_{eon} . In this specific case $\sigma_{\text{eon}} = 0.037$ S/cm results at ca. 700 °C.

The diameter of the Warburg type impedance indicates, how much the additional (parallel) ion conduction can lower the overall resistance; its resistance R_{W} does not simply correspond to the ionic or electronic resistance or the “ionic minus electronic resistance” but is given by

$$R_{\text{W}} = R_{\text{eon}} - R_{\text{con/ion}} = \frac{R_{\text{eon}}^2}{R_{\text{eon}} + R_{\text{ion}}} \quad (24)$$

From the resistive fit parameters of the circuit in Fig. 6b, i.e. from the high frequency intercept R_{hf} and R_{W} , the ionic resistance can therefore be calculated as

$$R_{\text{ion}} = \frac{(R_{\text{hf}} + R_{\text{W}})R_{\text{hf}}}{R_{\text{W}}} = R_{\text{eon}} \frac{R_{\text{hf}}}{R_{\text{W}}} \quad (25)$$

Here we obtain an ionic conductivity of 0.021 S/cm at ca. 650 °C in reducing atmosphere, which is only slightly lower than the electronic conductivity.

The result of a similar experiment on slightly Nd donor-doped lead zirconate titanate (PZT) with a composition close to the morphotropic phase boundary is shown in Fig. 8a. The impedance was measured between Ag-containing inner electrodes of a PZT multilayer stack (Fig. 8b) consisting of 80 μm PZT layers. Above ca. 600 °C a second feature appears at low frequencies of the impedance spectrum that again resembles a Warburg impedance. An example, measured at 707 °C, is shown in Fig. 8a, together with a fit of the measurement data to the circuit in Fig. 6b (after subtracting an inductive contribution due to wiring). Essentially, the same interpretation as for the STF thin films is possible: At low frequencies only electrons contribute to charge transport (R_{eon}) while at

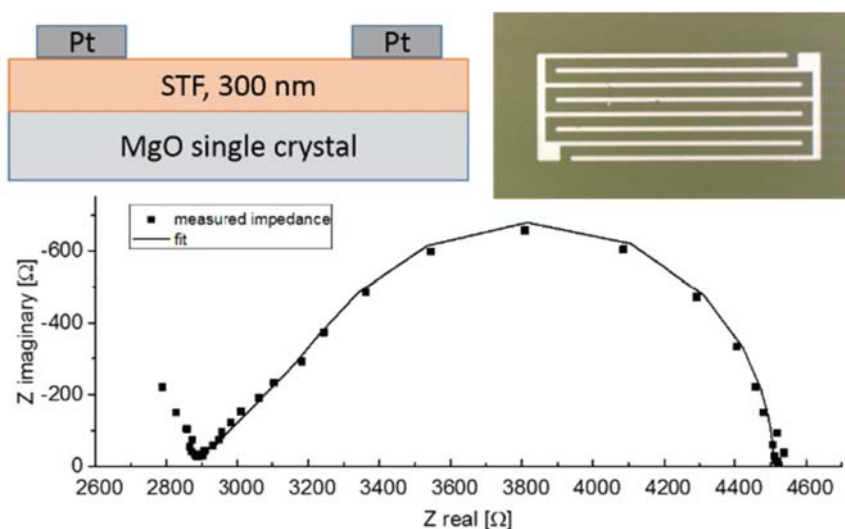


Fig. 7. Impedance spectrum measured at 800 °C between two interdigitating Pt electrodes on a $\text{Sr}(\text{Ti}_{0.3}\text{Fe}_{0.7})\text{O}_{3-\delta}$ (STF) thin film, deposited on an insulating MgO substrate (sketch); the photograph shows a top view of the electrode geometry. The spectrum can be fitted to the circuit in Fig. 6c with a rather ideal finite Warburg element at low frequencies.

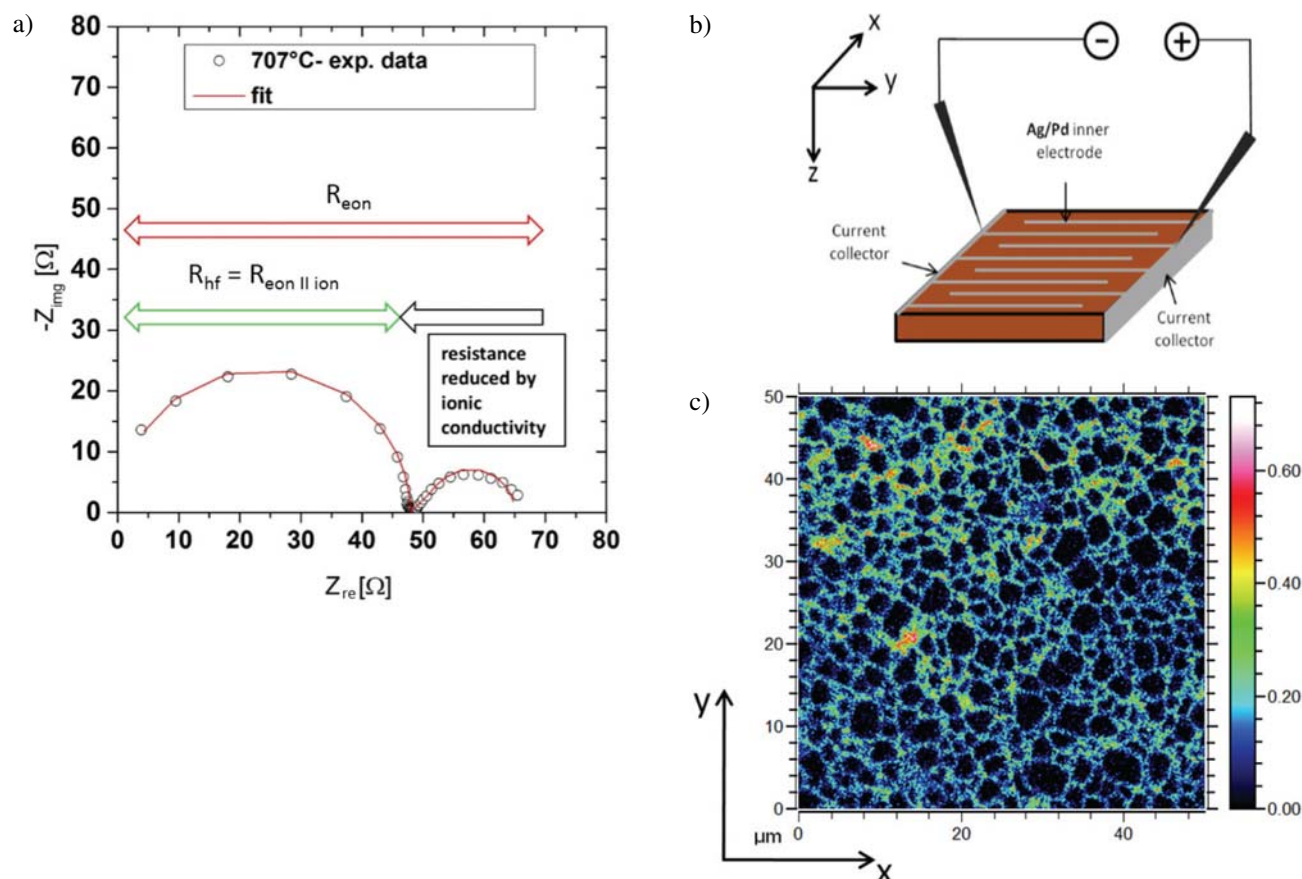


Fig. 8. (a) Impedance spectrum of a PZT multilayer stack piece with interdigitating Ag/Pd electrodes, measured at 707 °C and corrected for wiring inductance. A fit to the simplified circuit in Fig. 6b is also shown. The arrows indicate the meaning of the arcs (diameters) in terms of resistances. (b) Sketch of the sample geometry. (c) ^{18}O distribution image of a PZT polycrystal after exposure to $^{18}\text{O}_2$ at 676 °C for 30 minutes, measured by SIMS. High ^{18}O concentrations at grain boundaries clearly indicate that ion transport is mainly along grain boundaries.

high frequencies ions and electrons can move, leading to the lower R_{hf} resistance. Again the resistances can be used to calculate ionic and electronic conductivities and we obtain $1.6 \cdot 10^{-5}$ S/cm for ions and $4.6 \cdot 10^{-5}$ S/cm for electrons at 707 °C.

In this specific case, a significant ionic conductivity is somewhat surprising since donor dopants are usually assumed to suppress oxygen vacancy formation. Here, the analysis of the chemical capacitance comes into play. According to Eqs. (23) and (24) the chemical capacitance can be determined from the fit parameter T_{w} and the ionic and electronic resistances:

$$C_{\text{chem}} = \frac{4T_{\text{w}}}{R_{\text{eon}} + R_{\text{ion}}} = \frac{4T_{\text{w}}R_{\text{w}}}{(R_{\text{hf}} + R_{\text{w}})^2} \quad (26)$$

Assuming oxygen vacancies as minority charge carriers, we can use Eq. (26) and $C_{\text{chem}} = 4n_{\text{v}}F^2V/RT$ from Eq. (12) to determine the oxygen vacancy concentration. A value of about 10 ppm with respect to oxygen sites in PZT results for the given measurement data. This value is unusually high for a (nominally) donor-do-

ped material and may come from PbO evaporation during sintering, which turns a nominally donor doped PZT into a slightly acceptor doped PZT with hole conduction.³¹

However, here another effect comes into play: Oxygen tracer diffusion measurements with subsequent secondary ion mass spectrometry (SIMS) analysis revealed that the ionic transport and thus the majority of oxygen vacancies is located in or close to grain boundaries (Fig. 8c). When normalizing the measured “effective” ionic conductivity to a grain boundary width of ca. 2 nm we find impressively high oxide ion conductivities along grain boundaries in the range of 10^{-2} S/cm at ca. 700 °C. This is already within the order of magnitude of the best oxide ion conductors. The exact mechanism leading to such a high interfacial ionic conductivity is still under discussion, but there is indication that an accumulation of oxygen vacancies in a space charge layer plays a major role. This also means that most probably the largest contribution to the chemical capacitance comes from this region. More details on these effects can be found in Ref. 32.

5. Conclusions

Two different types of solid state electrochemical cells were shown in which chemical capacitances play an important role. First, the capacitance of mixed conducting thin film electrodes on oxide ion conductors is often a chemical capacitance, while the main resistance is caused by the oxygen exchange reaction at the surface. By applying a voltage, the oxygen chemical potential in the electrode can be varied. Measuring the bias dependent chemical capacitance thus reveals information on the minority charge carrier concentrations. In LSF, three different regimes could be identified with different minority charge carriers dominating the chemical capacitance (oxygen vacancies, holes and electrons, respectively). Second, spectra measured on mixed conducting oxides with ion blocking electrodes may yield Warburg type features in impedance spectra, which again include information on the chemical capacitance. For STF thin films and PZT samples, this type of measurement was used to separate ionic and electronic conductivities. For PZT, the chemical capacitance also allowed an estimate of the oxygen vacancy concentration present despite donor doping. The corresponding ionic conductivity, however, is largely carried by grain boundaries, as shown by oxygen tracer exchange experiments.

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Povzetek

Ker je stehiometrija kisika v mešanih prevodnih oksidih odvisna od kemijskega potenciala kisika, je zato pomemben kisikov parcialen tlak v plinski fazi. Prav tako lahko različne električne napetosti spremenijo lokalno stehiometrijo kisika in intenzivnost pri kateri se take spremembe dogajajo. Spremembe lahko kvantificiramo s kemijsko kapacitivnostjo vzorca, ki jo izmerimo z impedančna spektroskopija. V članku so predstavljene impedančne meritve različnih oksidov ((La,Sr)FeO_{3-δ} = LSF, Sr(Ti,Fe)O_{3-δ} = STF, and Pb(Zr,Ti)O₃ = PZT). Prav tako je na teh materialih pokazano, kako lahko kemijska kapacitivnost vpliva na impedančne spektre različnih elektrokemijskih celic. Kvantitativna analiza spektra temelji na generaliziranih ekvivalentnih vezjih. Za mešane prevodne okside, sta jih razvila J. Jamnik in J. Maier. Opisano je tudi, kako lahko defekt kemijske informacije izpeljemo iz kemijske kapacitivnosti.

Scientific paper

Properties and Structure of the LiCl-films on Lithium Anodes in Liquid Cathodes

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Dedicated to the memory of Janez Jamnik

Abstract

Lithium anodes passivated by LiCl layers in different types of liquid cathodes (catholytes) based on LiAlCl_4 in SOCl_2 or SO_2 have been studied by means of impedance spectroscopy. The impedance spectra have been fitted with two equivalent circuits using a nonlinear least squares fit program. Information about the ionic conductivity and the structure of the layers has been extracted. A new physical description, which is able to explain the circuit parameters, is proposed. It assumes that the LiCl-layer contains a large number of narrow tunnels and cracks filled with liquid catholyte. It is explained why such tunnels probably are formed, and for a typical case it is shown that tunnels associated with most of the LiCl grain boundaries of the fine crystalline layer near the Li surface are requested in order to explain the impedance response. The LiCl production rate and through this, the growth rate of the LiCl-layer, is limited by the electron conductivity of the layer. Micro-calorimetry data parallel with impedance spectra are used for determination of the electron conductivity of the LiCl-layer.

Keywords: Lithium batteries, thionyl chloride, solid interphase

1. Introduction

When lithium metal is exposed to an oxidising liquid like SOCl_2 , a passivating layer of oxidation product, LiCl, will form spontaneously. This layer (which often is called an interphase) and its formation have been extensively investigated in the case of lithium in the $\text{LiAlCl}_4/\text{SOCl}_2$ -solution.^{1–16} Many different features have been described, and often apparently conflicting data were reported. It is generally accepted, however, that the lithium metal is completely covered by a SEI (= solid electrolyte interphase) consisting of LiCl. The SEI layer forms spontaneously on contact between the lithium and the catholyte. Its thickness is determined by the electron tunnelling range, which, according to Peled is 1.5–2.5 nm.² Kazarinov and Bagotzky¹¹ state that the thickness is 1–1.5 nm directly after contact with the catholyte and growing to 5 nm within the first hours thereafter. These numbers seem to be in fair agreement with general quantum mechanical considerations.¹⁷ The thickness is then increased further with time because electrons can tunnel to energetically

favourable defect sites within the film such as dislocations and impurities.¹⁷

Furthermore, the prevailing view seems to be that on top of the compact primary layer, a porous secondary LiCl layer is formed in a later stage, but other views, different from the prevailing one, have been published as discussed below.^{7,12,13,14,15}

Several models of the electrical and/or the microstructural characteristics of the passivating layer have been reported.^{1,2,6,7,13,14,16} Most have been of a qualitative descriptive nature, and even though some mathematical formulations have been attempted, e.g. by presuming that the SEI-resistivity is position dependent,¹⁶ no rigorous treatment of the Li- SOCl_2 -interphase, which was tested extensively against experimental data, has been reported.

The aim of this work is to provide basic information about ionic and electronic conductivity plus structure of the LiCl layers. The electronic conductivity of the layer is derived from parallel measurements of impedance spectra and micro-calorimetry on the same anodes. Further, a dee-

per understanding of the measured impedance spectra of LiCl-interphases has been attempted.

Often, the liquids $\text{LiAlCl}_4/\text{SOCl}_2$ or $\text{LiAlCl}_4/\text{SO}_2$ are referred to as the electrolyte. This may be confusing because the solid LiCl-layer in fact is the electrolyte in these cells. Therefore, in the following the term »catholyte« is used for the liquid solutions. The term »electrolyte« is used for the solid LiCl-layer only.

2. Experimental

2.1. Cell Geometries

Several cell configurations were used. One type of cell had two lithium electrodes in a glass container; size R14 with Teflon lid (Fig. 1). The areas of the lithium electrodes were 23.4 and 9.8 cm², respectively. In the zero polarisation situation this is with respect to impedance equivalent to one electrode having an area of 6.9 cm².

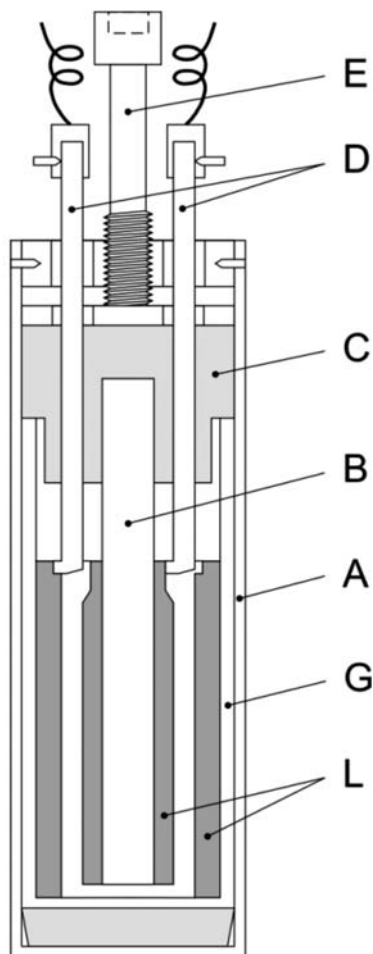


Figure 1. Sketch of the cell with two Li-electrodes (L) in a glass container (G). The cell is assembled in a steel cylinder (A). B is a glass tube supporting the inner Li-electrode, C is a Teflon lid, D points out the SS electrode terminals, and E is a screw which keeps the Teflon lid pressed onto the glass container.

Other types of cells used were 3-electrode cells in glass or in stainless steel (SS) containers, which have been described earlier.¹⁸ The Li reference electrode consists of a 5 mm wide and 1 mm thick Li strip, which was placed vertical between the two current bearing electrodes. The SS-cells included test of measures, which might decrease the passivation rate: (i) adding 1 cm³ of pure SOCl_2 before introducing the catholyte, (ii) adding LiCl-grains to the separator, (iii) pressing a Ni-grid into the Li-surface, and (iv) pressing Ni-fibres into the Li-surface. Electrochemical impedance spectroscopy (EIS) was performed on these cells in order to get input to the understanding of the passivating layers from a variety of Li-anode passivation conditions.

In addition, commercial R14-cells of bobbin type (NIFE) have been measured, which, after 2.5 years, have been regenerated by discharge at 1 Ohm in 15 minutes corresponding to 0.10 Ah = 2% of the total capacity.

Results from all cell types were qualitative in agreement with the description of the LiCl solid electrolyte given below, but only some selected results from the cells listed in Table 1 are reported here.

2.2. Catholytes

Three types of catholytes were studied: 1) The standard catholyte, which in the following is called the A-catholyte, is thionyl chloride with 1.72 molar LiAlCl_4 . Its specific ionic conductivity was calculated to be 0.020 S/cm using the formula given by Berg et al.¹⁹ 2) A more acid catholyte, thionyl chloride with 1.2 molar LiAlCl_4 and 0.6 molar AlCl_3SO_2 , is called the B-catholyte. It was originally introduced by Gabano²⁰ by adding Li_2O to SOCl_2 which by reaction form SO_2 and LiCl. Here, however, it was manufactured by adding SO_2 directly instead of Li_2O . 3) Finally, a catholyte consisting of LiAlCl_4 and SO_2 only was investigated. It is referred to as the SO_2 -catholyte. Gaseous

Table 1. Overview of the cell data. The area of the Li (working) electrodes varies between 20 and 27 cm². The exact value is given where needed.

Cell code	Electrodes, container material	Catholyte type	Other characteristics
G40A	3 Li, glass	A	
3201	2 Li, glass	A	
G24 B	3 Li, glass	B	
SO21	3 Li, glass	SO_2	$\text{SO}_2/\text{Li} = 2$
3303	2 Li, glass	SO_2	$\text{SO}_2/\text{Li} = 4$
602	1 Li + 1 carbon, SS	A	Commercial cell
607	1 Li + 1 carbon, SS	A	Pure SOCl_2 added
610	1 Li + 1 carbon, SS	A	LiCl nuclei added
614	1 Li + 1 carbon, SS	A	Ni-grid on anode
618	1 Li + 1 carbon, SS	A	Ni-fibres on anode
2REG	1 Li + 1 carbon, SS	A	Regen. comm. cell

SO₂ was allowed to react with a mixture of AlCl₃ and LiCl at 0.5 atm. overpressure overnight, finishing to equilibrium at room temperature at 0.1 atm. overpressure. This gives a liquid molar ratio of Li/SO₂ = 3.2. The ionic conductivity is 0.10 S/cm.²¹ Lower Li/SO₂ was prepared by controlling the weight during SO₂ addition.

Table 1 gives an overview of the cells referred to in this paper.

2. 3. Measurement Equipment

The impedance spectra of the cells were measured by a Solatron 1250 Frequency Response Analyzer. The current necessary for the measurements is sufficiently low so that a potentiostat may not be needed. The frequency generator was in this case connected to the cell in series with a capacitor, which was put next to the counter elec-

trode and outside the potential sensing leads, in order to avoid any DC current (i.e. to avoid DC-discharge). In some cases a Solatron 1286 potentiostat was used for the connection to the cells. Measurements were made using a maximum of 2 $\mu\text{A}/\text{cm}^2$ amplitude in the range of 0.1 or 1 Hz to 60 kHz. All measurements were performed at ambient temperature.

The microcalorimeter, which was designed and constructed in-house, is sketched in Fig. 2. In order to facilitate calibration, a 100 k Ω resistor was built into each of the measuring blocks so that a known heat effect can be added e.g. 10 V giving 1 mW. In the calibration mode, an aluminium cylinder which has the same heat capacity as the battery cell is inserted.

During the calorimetric measurements impedance measurements were performed with regular time intervals.

3. Literature Survey

Before the results are presented and discussed a brief summary of the literature is given. Peled and Yamin⁴ have put forward a model in which the Li-anode in SOCl₂ is always covered by a LiCl-layer. Its minimum thickness on freshly immersed Li is 2–4 nm. The spontaneous formation of LiCl crystals on Li₂O-covered Li was observed after 0.5 min at which time their sizes were about 50 nm. These crystals grew with time, and after 24 h the entire Li surface was covered by a layer of crystals of sizes between 100 nm and 500 nm. This layer acts as a SEI. During storage three things happen, (1) the SEI thickness increases, (2) some of the SEI crystals grow preferentially until they are an order of magnitude or more larger than the SEI thickness, and (3) cracks are formed in the SEI. Using a galvanostatic pulse method and the parallel plate capacitor equation for thickness calculations, a minimum thickness of the SEI which was found to be about 30 nm after 1 day, growing to about 60 nm during the next 2–3 days and levelling off at about 100 nm after a month.

Later Peled² added to this picture a second type of passivating film consisting of the compact SEI on top of which a thick, mechanically strong, low porosity secondary layer develops.

Independently, Moshtev, Geronov and Puresheva⁶ presented a similar model (based on similar measurements and calculations) with a thin (15–50 nm) primary compact LiCl film, and on top of this, a thick (1,000–2,000 nm) porous secondary film. The electrical response is described as originating from the primary film only.

Holleck and Brady⁷ also studied freshly exposed Li in LiAlCl₄/SOCl₂ by means of galvanostatic pulses. The interpretations were made using the parallel plate capacitor equation as a first approximation. They proposed a film model consisting of three regions. Region I forms rapidly (in less than 1 h). It has a thickness of 20–40 nm. It

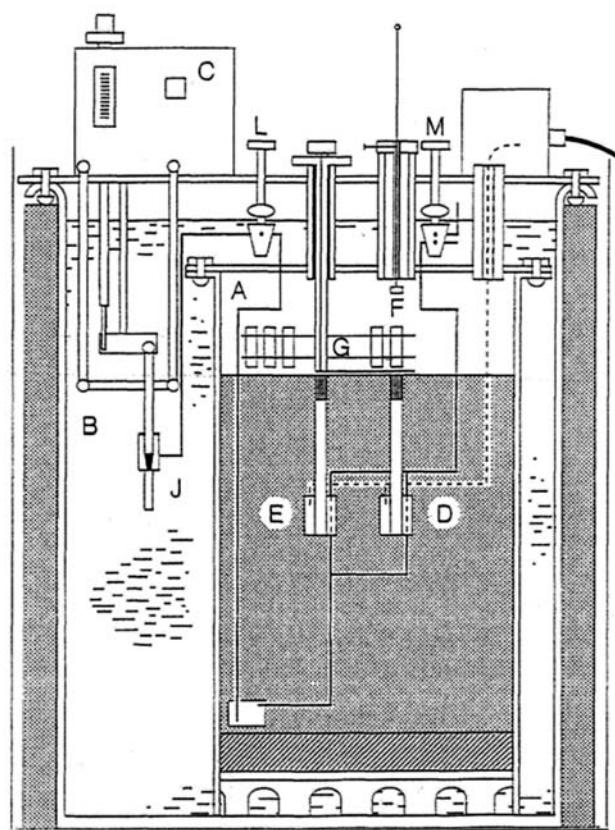


Figure 2. A sketch of the micro-calorimeter used. The calorimeter chamber, A, is submerged in a water bath, B, which is temperature regulated by the thermostat, C. Most of the chamber is filled with polystyrene foam which contains the measuring block, D, and an identical reference block, E. The sample is placed using a magnetic drive, F, on a turntable, G, by which the sample (the cell) is transferred to the measuring block, D, though the magnetic drive after temperature equilibrium is obtained. The two measuring blocks, D and E, are temperature equalized by a water circulatory system consisting of a water injecting pump J and the valves L and M. The valves may be switched so that the tubing system will be filled by air for heat insulation when the apparatus is in measuring mode.

appears to have significant imperfection and some micro-porosity along the grain boundaries. On top of this is the Region II film, which is more ordered and more compact. It grows to 20–60 nm within 20 h. Region III is porous and coarsely crystalline. It is formed by dissolution and recrystallization of the region II film. The paper concludes that the porous film is not detectable by the galvanostatic pulses.

Boyd²⁵ examined the LiCl film by SEM. He found that the film is composed of two layers: A thin layer of small crystals of 2000–5000 nm size (about 10 x bigger than found by Peled and Yamin⁴) on top of the Li surface and a thicker layer of large crystals on top of them. The thickness of the total layer after 14 days was about 11,000 nm with large crystals about 20,000 nm high. Boyd also looked carefully for a thinner dense film with expected thickness in the range around 70 nm (the thickness found by the galvanostatic pulses), but did not find any. The resolution of his SEM was quote: »several hundred angstroms«.

Chenebault, Vallin, Thevenin and Wiart¹² also performed SEM studies and found the thickness of the LiCl film to be about 80,000 nm after 1 month in A-catholyte. The layer thickness and morphology was affected by various additives. No evidence of a thin compact primary layer was observed. The resolution of the SEM was not stated.

By means of impedance spectroscopy several investigators showed that the capacitance was always frequency dependent.^{8,9,13,24,26,27,28} For frequencies in the range of 10–20 kHz, thicknesses in the range of 50–300 nm after 1 day of storage were obtained using the parallel plate capacitor equation.^{8,9,26} Selected data from the literature about LiCl-layers on Li in SOCl₂-based catholytes are given in Table 2.

Based on a number of SEM studies^{1,4,12,25,26} the porosity of the secondary layer is estimated to be no more than a few percent, say max 5% and more probably around 1%. This makes it very difficult to accept that it should not contribute to the electrical response. It should at least block off a lot of surface area of the primary film. Thus, a real discrepancy between the SEM and the electrical parallel-plate-capacitor-model derived thicknesses is seen. Chenebault et al.¹³ have discussed and tried to solve this discrepancy by assuming the existence of few narrow cracks going to the very Li-metal surfaces, and it is discussed to which extent the Li-metal is covered by reaction products if at all covered. (It is, however, beyond doubt that the Li is completely covered by LiCl as it is inconceivable that an overvoltage of 3.68 V (the potential of the SOCl₂ versus Li) could be sustained without immediate reaction). They conclude themselves that the model works only in very special cases.

Gaberscek, Jamnik and Pejovnik have addressed this problem in a series of papers.^{24,16,15,29,30,31,32} It was pointed out that a position dependent resistivity associated with the space charge region might explain the constant phase element (CPE) type of impedance response observed. Further, a model in which the impedance response did not originate from the bulk LiCl at all but only the interface including the space charge region was discussed. A main problem in this model is that it requires a much higher ionic conductivity than the usually found, and in their later paper³² measurements of the Li⁺-conductivity of LiCl grown on Li in SOCl₂ (but measured after removing the catholyte) confirmed the usually found low values, i.e. the impedance response originates in fact to a large extent from the bulk of the layer. A pure interface-response model also has difficulties in explaining the reported sensitivity of the impedance to small mechanical impacts.²⁶

Table 2. Selected literature data describing the properties of the passivating LiCl layer on Li in SOCl₂-based catholytes grown at room temperature. The thicknesses obtained by electrochemical means (impedance spectroscopy or galvanostatic pulses) are compared to results from physical methods SEM, or for one group radioactive Cl-36 (marked with *). The ELCHEM-thicknesses were calculated using the parallel plate capacitor equation. The values marked ** were measured at 25 °C on a film grown at 50 °C after removal of the SOCl₂.

Passivation time, days	Thickness, μm SEM	Thickness, μm ELCHEM	Resist., $\text{k}\Omega\text{cm}^2$	K_{LiCl} , nS/cm	Ref. no.
30	50				1
1	0.1–0.5	0.30	0.05	20	4
30	1–5	.01	1	8	4
1	1–2	0.015–0.05	0.25	8–25	6
1		0.04–0.1	1–8	1–5	7
10		0.08	30	0.03, 1.6	9
1	2				25
14	11				25
10	1–2*	0.05	0.07	100	11
30	80		0.9		12
360	~10	0.6	50	6	28
14		80**	1.510 ³ **	0.2**	32

4. Results and Discussion

4. 1. Impedance Spectroscopy Data

Fig. 3 shows examples of impedance plots for Li-electrodes in A-, B- and SO₂-catholyte, respectively, all measured 1 day after manufacturing. (The experimental details are given in the Figure caption and in Table 1).

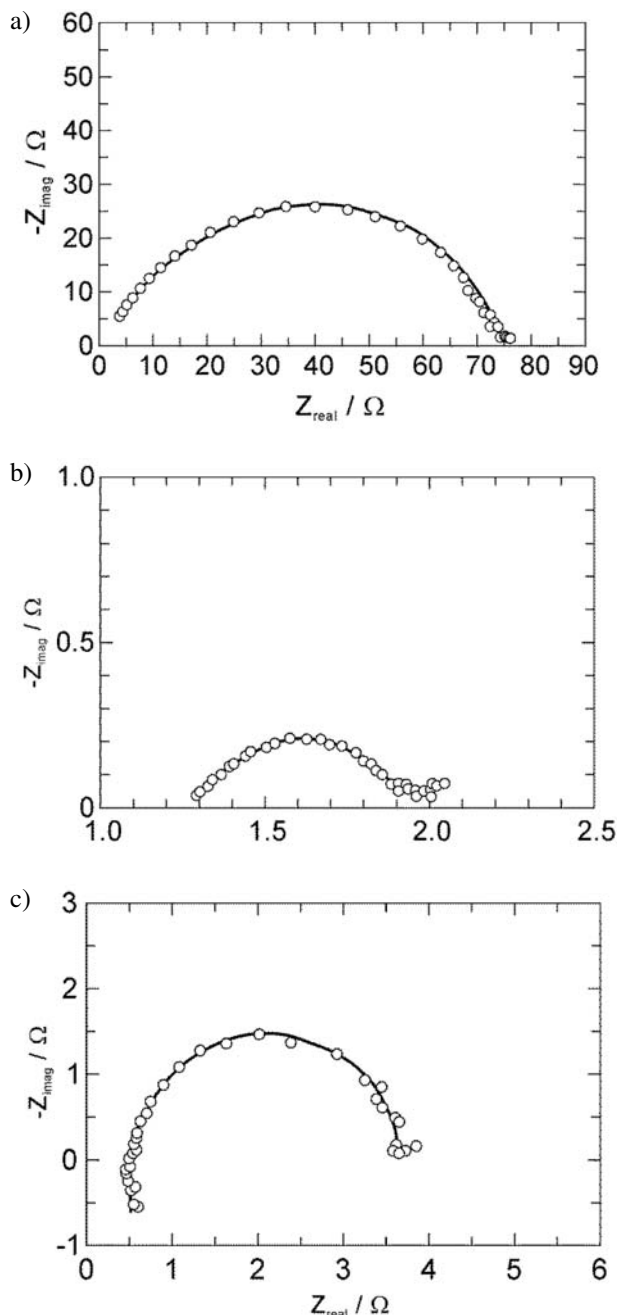


Figure 3. Examples of Impedance spectra measured 1 day after exposure of the Li to the catholyte. a) A-catholyte – cell G40A, b) B-catholyte – cell G24B, and c) SO₂-catholyte – cell SO21. Exposed Li area is 25 cm² in all three cases. For further details, see Table 1 and the text.

When the passivation of say 10 different cells has been monitored over some period by the measurement of say 100 impedance spectra containing each typically 75 data-sets consisting of a frequency, a real part and an imaginary part of the impedance then a huge amount of data is at hand. Therefore, there is a great need to reduce this amount in way so that it can be surveyed, and so that it still contains the significant physical information. In an attempt to do this, the impedance measurements have been fitted to two nominally different (but actually kind of redundant, see below) equivalent circuits using the nonlinear least squares fit program written by Boukamp²²:

Circuit #1: L1 R2 (Q3 R4) (C5 R6) and

Circuit #2: L1 R2 (Q3 (R4 (C5 R6)))

using Boukamp's notation. L denotes inductance, R resistance, C capacitance and Q constant phase angle element (CPE). Often the response of an extra CPE, Q7, is visible at the low frequencies (below 1Hz). The circuits are visualized in Fig. 4. Q is used as the symbol of the CPE.

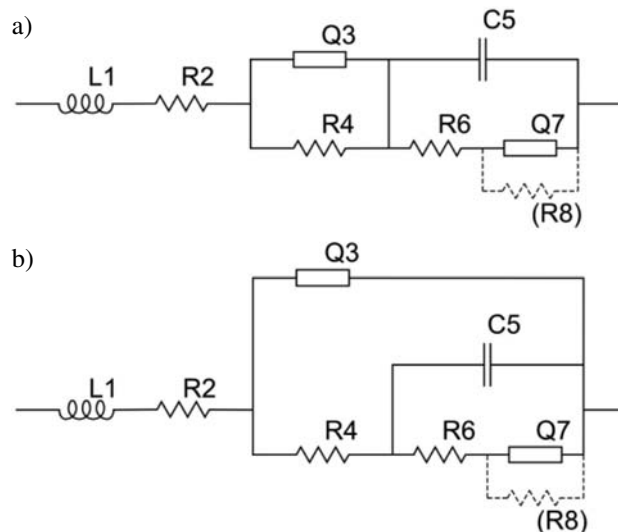


Figure 4. The equivalent circuit used for fitting the impedance spectra. a) circuit #1, b) circuit #2.

It takes two numbers, B and n, to describe the admittance, Y_{CPE} , of a CPE:

$$Y_{CPE} = B \cdot (2 \cdot \pi \cdot f)^n \cdot (\cos(\pi \cdot n/2) + j \cdot \sin(\pi \cdot n/2)) \quad (1)$$

where $j^2 = -1$, and f is the frequency. n is a dimensionless number between 0 and 1, and B must have the dimension Ss^n because the unit of admittance is Siemens (S) and of frequency is s^{-1} . For further details, see e.g. the paper of Boukamp²² and the book of MacDonald²³. A short way of writing (1) is:

$$Y_{CPE} = B(j \cdot 2 \cdot \pi \cdot f)^n \quad (1a)$$

In general, there is no reason to believe that such simple equivalent circuits should reflect the real structure of a passivating film on a metal, but, as the fits are fair, it is believed that the values of the equivalent circuit components will contain the essential physical information about the layer and to some extent the measurement set-up. Thus, L1 is inductance originating from leads and equipment, and R2 is serial resistance which for the main part is associated with the lead and the contact to the Li-electrode and for a small part originates from the resistance in the catholyte between the working and the reference electrode. The other quantities, Q3, R4, C5, R6, and Q7 are all believed to be associated with the SEI and/or the SEI interfaces to the Li and the catholyte.

Q7 is mainly of importance in the frequency range below 1 Hz, and the impedances at the low frequencies are not measured with sufficient accuracy to allow a detailed analysis. Q7 was included in several of the fits in order to improve the accuracy of determination of the other parameters. As the Li-anodes are not blocking electrodes, a resistance, R8, must be there in parallel with Q7, but attempts to use it in the fitting did not give meaningful results, and so it was left out. The high capacitance values in the range of mF/cm^2 associated with Q7 have been observed by other workers.¹⁵ It seems that these »supercapacitor«-values are associated with an electrochemical oxidation/reduction of species inside the LiCl-layer close to the Li-metal surface.

The two equivalent circuits used may give exactly the same impedance response if suitable different R, C, B

and n values are selected for the two circuits, i.e. it is impossible to distinguish between them by means of fitting a single impedance spectrum. Both were tested because one of the different sets of resulting values may be more probable than the other seen in the light of other available information.

In most measurements the contributions to the impedance diagram from Q3-R4 and C5-R6 overlap. This has earlier been interpreted as a single depressed semi-circle^{10,24}, and it should be noted that in many cases it is unclear whether the addition of C5 and R6 gives a better fit or not. In some cases it certainly gives a better fit, and so these two equivalent circuits were tested with the hope that some further understanding would be revealed.

Table 3 gives the circuit #1 parameter values per unit area for selected storage times of the cells in Table 1. Table 4 gives the corresponding values of circuit #2 or values obtained using parts of #2. In several cases only a part of #2 was used in order to check if it gave a significant difference for the values derived from the main part of the impedance curve. In cases where C5, R6 and Q7 are omitted there are no differences between #1 and #2.

Figs. 5 and 6 show values of R4, R6, C3 and C5 as a function of time for the anode of cell G40A using circuit #1 and G24B using circuit #2, respectively. In the case of cells with A-catholyte like G40A, the circuits #1 and #2 do not result in much difference in the parameters, whereas for cells with the acid B-type catholyte quite much lower n3-values are obtained by using #1 than by using #2. As n-values lower than 0.5 are difficult to understand

Table 3. Examples of data derived from fitting the impedance spectra to equivalent circuit #1.

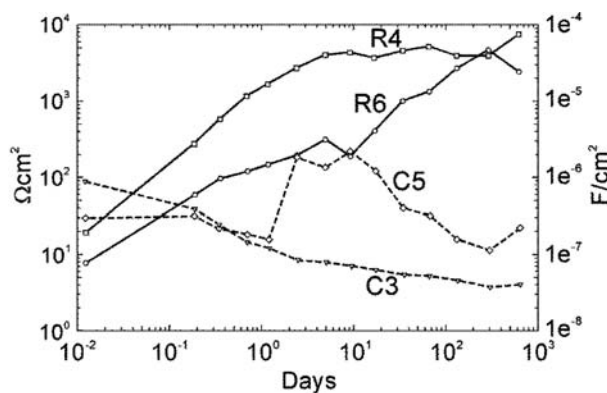
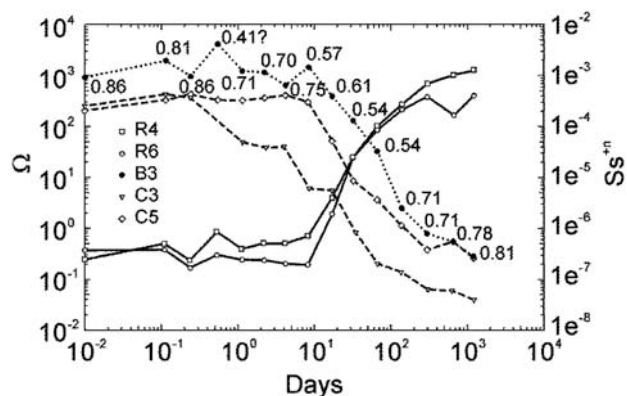
Cell	Time days	B3 $\text{S s}^n/\text{cm}^2$	n3	C3 $\mu\text{F}/\text{cm}^2$	R4 Ωcm^2	C5 $\mu\text{F}/\text{cm}^2$	R6 Ωcm^2
G40A	1	5.3e-7	0.83	0.12	1680	.17	150
	9	5.4e-7	0.76	0.07	4240	2.22	189
3201	1	6.3e-7	0.81	0.11	1310	137	23
	3	4.2e-7	0.82	0.08	2170	22.6	122
G24B	1	4.0e-4	0.48	0.89	14	23.2	6
	8	1.6e-4	0.43	0.07	24	18.9	4
SO21	1	4.94-5	1.00	48.6	4	14.3	74
	10	2.7e-4	0.75	36.6	13	24.0	38
3303	1	5.2e-5	0.80	9.88	28	56.9	8
	8	2.1e-4	0.61	7.14	28	27.8	16
602	1	4.8e-7	0.92	0.22	364	2.22	120
	6	4.1e-7	0.96	0.29	926	0.19	246
607	1	1.6e-5	0.61	0.42	287	0.37	289
	6	1.7e-6	0.86	0.49	425	0.14	144
610	1	8.8e-7	0.91	0.40	285	0.27	68
	6	1.94-6	0.75	0.18	502	0.45	292
614	1	3.9e-6	1.00	3.88	76	0.39	188
	6	3.0e-6	1.00	3.01	236	3.52	192
618	1	1.7e-6	1.00	1.66	162	0.79	61
	6	6.0e-7	1.00	0.60	310	10.25	101
2REG	1	1.6e-5	0.60	1.25	187	395.	152
	8	1.9e-6	0.70	0.11	960	277.	257

Table 4. Examples of data derived from fitting the impedance spectra to equivalent circuit #2 or parts of it.

Cell	Time days	B3 Ss^{n3}/cm^2	n3	C3 $\mu F/cm^2$	R4 Ωcm^2	C5 $\mu F/cm^2$	R6 Ωcm^2
Ge0A	1	5.2e-7	0.80	0.09	1673	1.15	163
	9	5.4e-7	0.76	0.07	4240	2.22	189
3201	1	6.2e-7	0.81	0.11	1286		
	3	4.0e-7	0.82	0.08	487		
G24B	1	4.8e-5	0.71	0.89	10	12.8	6.3
	8	5.7e-4	0.57	0.25	17.8	12.2	5
SO21	1	1.1e-5	1.00	11.2	49.3	12.8	29.5
	10	1.9e-5	0.95	18.9	18	8.7	32.5
3303	1	3.6e-5	0.83	9	36.5		
	8	7.0e-5	0.73	7.7	44.9		
602	1	3.0e-7	1.00	0.30	480		
	6	5.1e-7	0.87	0.16	1139		
607	1	1.1e-6	0.81	1.12	558		
	6	4.5e-6	0.72	0.37	389	0.08	181
610	1	2.6e-7	0.95	1.30	378	0.12	61
	6	8.3e-7	0.81	0.12	298	0.02	658
614	1	3.8e-7	1.00	0.38	231		
	6	4.3e-6	1.00	0.43	371		
618	1	2.0e-6	0.89	0.72	205		
	6	5.6e-6	1.00	0.56	343		
2REG	1	2.7e-6	0.80	0.33	187		
	8	1.7e-6	0.73	0.13	699		

in the context of a physical layer, circuit #2 was used to generate the data shown in for Fig. 6.

The rather irregular course of C5 in Fig. 5 is often seen and is believed to be associated with cracking of the LiCl film. It should also be noted that odd impedance spectra resulting in odd parameter values are occasionally observed like in the case of G24B after 0.5 day (see Fig. 6, the point with the question mark). The cause may be associated with the variance of the impedance with time which will occur during cracking and the early stage of crack healing. If a major cracking happens during the measurement of an impedance spectrum, it may be recognized as a

**Figure 5.** The impedance spectra parameters obtained using circuit #1 as a function of time for cell G40A. C3 was calculated using eq.(8). The values are given in area specific units.**Figure 6.** The impedance spectra parameters obtained using circuit #2 as a function of time for cell G24B. Values are given for the full cell area of 25cm². The figures at the B3 curve are the associated n3-values.

discontinuity in the spectrum whereas the early stages of crack healing are very difficult to identify with certainty and only question marks can be put up.

4. 2. The Test and Failure of the Parallel-plate-capacitor-model

A way of improving or disproving a model is to try and use it as far as possible, analyse the outcome and then maybe modify the model. Therefore, in this section the impedance data will be treated using the parallel-plate-ca-

pacitor-model in spite of the disagreement reported in section 3. As it will be seen it helps in understanding where and how the model fails and the ionic conductivities derived prove to be fair approximations anyway.

4. 2. 1. Assignment of Circuit Components and Data Treatment

As apparent from section 3 it is widely agreed that the outer part of the LiCl layer on Li in SOCl_2 contains porosity and is uneven. Furthermore, a CPE is often associated with porous structures³³ and/or very uneven (fractal) surfaces.³⁴ On this background, Q3 is interpreted as reflecting (together with R4) the secondary layer. R4 is assumed to be the DC ionic resistance through this layer.

It is of general interest to know the size of the capacitance, C3, associated with Q3, especially as it constitutes a dominant part of the frequency response, and it is believed to be of practical importance to the cell performance. For any given frequency, f , the equivalent capacitance is given by

$$C3(f) = B3 \cdot (2 \cdot \pi \cdot f)^{n-1} \sin(n \cdot \pi/2) \quad (2)$$

The main use of C3 in this subsection is for calculation of the »effective« thickness of the layer to which it is associated. From equation (2) this may seem meaningless as one can get any result from 0 towards infinity depending on the frequencies chosen. The given interpretation, however, puts limits on which frequencies are physically meaningful, i.e. it makes no sense if they are above the range where R4 is fully short circuited (say, $|B3 \cdot (j \cdot 2 \cdot \pi \cdot f)^{n3}|^{-1} \ll 0.01 \cdot R4$), or below the very low frequencies where $|B3 \cdot (j \cdot 2 \cdot \pi \cdot f)^{n3}|^{-1} \gg R4$. It seems also clear that it is not possible to give an unambiguous thickness value for a very uneven porous layer. (It may be like describing the height of a mountain chain like the Alps by a single number). So, it might be argued that by selecting the top point of the Q3-R4-arc, an »electrical average« thickness is obtained, and from a performance point of view this may appear relevant. And, as this comes close to what has been done in many other impedance studies of Li-anodes in SOCl_2 , this frequency will be used for calculating C3 and a thickness value. This will not be a constant frequency and it cannot usually be picked directly from the frequencies used for the measurements. Thus, this frequency (also called the peak frequency) has to be calculated from the values of the equivalent circuit fitted to the measured data. This was done by the following procedure where the coordinates (a_0, b_0) and ($a_{\text{peak}}, b_{\text{peak}}$) refer to the centre and the upper most point, respectively, of the depressed semi-circle in a Nyquist diagram:

$$a_{\text{peak}} = R2 + R4/2 \quad (3)$$

$$b_0 = R4/(2 \cdot \tan(n3 \cdot \pi/2)) \quad (4)$$

$$b_{\text{peak}} = b_0 + R4/(2 \cdot \sin(n3 \cdot \pi/2)) \quad (5)$$

From this the imaginary part of the admittance at the peak, is found:

$$Y''_{\text{peak}} = b_{\text{peak}} / ((a_{\text{peak}} - R2)^2 + (b_{\text{peak}})^2) \quad (6)$$

and the frequency of the peak is given by:

$$f_{\text{peak}} = ([Y''_{\text{peak}} / (B3 \cdot \sin(n3 \cdot \pi/2))]^{-n3}) / (2 \cdot \pi) \quad (7)$$

$$C3 = Y''_{\text{peak}} / (2 \cdot \pi \cdot f_{\text{peak}}) \quad (8)$$

Having assigned Q3-R4 to the secondary layer, the only possibilities seen for C5-R6 are that they are associated with either the primary LiCl-layer, or the interfaces of the SEI to Li and/or the catholyte, or both.

Assuming that the Li surface roughness factor is 1, the thicknesses, L_{LiCl} , can be calculated (assuming a parallel plate capacitor):

$$L_{\text{LiCl}} = \epsilon_r \cdot \epsilon_0 / C \quad (9)$$

where the permittivity of vacuum $\epsilon_0 = 8.84 \cdot 10^{-14}$ F/cm and the relative permittivity of LiCl is $\epsilon_r = 10.62$.³⁵ Further, assuming that the distribution and paths of the current lines are independent of frequency, it is possible to calculate the specific ionic conductivity of the layer:

$$K_{\text{LiCl}} = \epsilon_r \cdot \epsilon_0 / (R \cdot C) \quad (10)$$

We are aware that this is an approximation and we do not know the uncertainty. It is, however, believed to give at least a fair estimation of the specific ionic conductivity of the layer, and it is the best approximation that we know.

4. 2. 2. Consequences of the Assignments and Problems Encountered

Two main problems arise as a consequence of the above data treatment. The one may have a solution, but the other has not.

1) The high capacitance problem

Assuming that all C5-R6 belong to the primary layer, ionic conductivities and layer thicknesses can be calculated using the formulas above. Results are shown in Table 5. It shows very low thicknesses in many cases, and the ionic conductivities are unexpectedly low, sometimes lower than the conductivity of the secondary layer (Table 6) which is supposed to be of purer LiCl than the primary layer.²⁶

Table 5. Result derived from C5-R6, the »primary layer«, using circuit #1. The ratio of free to blocked area in the »secondary layer«, $a_{p,sec}$, is calculated assuming that the »true« thickness of the »primary layer« is 3 nm. See text for details.

Cell	Time days	K_{LiCl} S/cm	Thickness nm	$a_{p,sec}$
G40A	1	3.8e-8	56	0.05
	9	2.2e-9	4.2	0.71
3201	1	3.0e-10	0.07	>1
	3	3.4e-10	0.41	>1
G24B	1	7.1e-9	0.40	>1
	8	1.2e-8	0.50	>1
SO21	1	8.8e-10	0.65	>1
	10	1.0e-9	0.39	>1
3303/SO2	1	2.0e-9	0.17	>1
	8	2.0e-9	0.34	>1
602	1	3.5e-9	4.2	0.71
	6	2.0e-8	49	0.06
607	1	8.8e-9	25	0.12
	6	4.8e-8	69	0.04
610	1	5.0e-8	34	0.09
	6	7.1e-9	21	0.14
614	1	1.3e-8	24	0.12
	6	1.4e-9	2.67	>1
618	1	2.0e-8	12	0.25
	6	9.1e-10	0.9	>1
2REG	1	1.6e-11	0.02	>1
	8	1.3e-11	0.03	>1

The problem of very thin layers is especially pronounced in the SO₂-catholytes. It simply does not make physical sense when the calculated sum of the primary and secondary layer thicknesses is below 1 nm and the electron tunnelling distance is about the double or more. Thus, these extremely high values of C5 (and C3) need some other explanation.

Then it seems natural to associate C5-R6 with the Li/LiCl and/or the LiCl/SOCl₂ interfaces. However, just ascribing C5 to compact Helmholtz layers is also problematic. The unit length of the LiCl-grid is 0.514 nm, corresponding to a distance between the planes (1,1,1) of 0.324 nm and a distance between similar atoms of 0.363 nm. According to equation (9) a thickness of 0.324 nm corresponds to a capacity of $C_c = C5 = 29 \mu\text{F}/\text{cm}^2$ of the compact Helmholtz layer. According to the Stern model and derived models it may look as if this value will also be the maximum possible total capacity when the compact Helmholtz layer capacity is added to the diffuse (space charge) layer capacity, C_d , by the formula $1/C5 = 1/C_c + 1/C_d$ (see textbook, e.g. Fried³⁶). This is, however, not believed to be generally valid. It is only valid if the charge transfer resistance $R_t \rightarrow \infty$. If the time constants (i.e. the capacitances and the resistance) of the processes in question are enough different, then the parallel $C_c \cdot R_t$ will appear to be in series connection with the parallel $C_d \cdot R_d$ (R_d is the ionic resistance of the space charge region).

Then the next question is if such high capacitances at all can be associated with the space charge regions. Therefore, as an example, $200 \mu\text{F}/\text{cm}^2$ is inserted into the equation of differential capacity of the diffuse double layer

$$C5 = z \cdot F \cdot (\text{sqr}\{2 \cdot \epsilon_r \cdot \epsilon_0 \cdot c/(R \cdot T)\}) \cdot \cosh(E \cdot z \cdot F/(2 \cdot R \cdot T)) \quad (11)$$

or

$$E = (2 \cdot R \cdot T/(z \cdot F)) \cdot \text{arccosh}(C5/(z \cdot F \cdot \text{sqr}\{2 \cdot \epsilon_r \cdot \epsilon_0 \cdot c/(R \cdot T)\})) \quad (12)$$

which, using a guess of concentration of vacancies of $c = 10^{-7} \text{ mol}/\text{cm}^3$, gives a zeta-potential of 0.32V. An independent measurement of the zeta-potential would be commendable, but the electrode cannot be polarized much without changing its properties (morphology). Taking into account that the total voltage across the LiCl SEI is 3.68V, the 0.32V seems reasonable.

It is recognized that the uncertainty on the determination of the capacitance is rather high, but anyway the size of the capacitances, which must be accounted for in certain cases, seems to be of the order of $100 \mu\text{F}/\text{cm}^2$ or more.

From the data and this discussion it is concluded that the impedance responses of the Li-anode covered with very thin films reflect more than the simple properties of thickness and ion conductivity of the film. Also the interface including the space charge regions are reflected.

2) The secondary layer blocking effect problem

When the secondary layer is not 100% dense, only a maximum value of its LiCl ionic conductivity, $K_{LiCl,max}$, can be calculated using eq.(10) because part of the conduction will take place through catholyte-filled cracks and pores, i.e.

$$K_{LiCl,max} = \epsilon_r \cdot \epsilon_0 / (R4 \cdot C3) \quad (10a)$$

If a pore area ratio is defined as

$$a_{p,sec} = A_{p,sec}/A_{total} \quad (13)$$

where A_{total} is the nominal electrode area, and $A_{p,sec}$ is the effective area available for conduction of ions through the full distance of the LiCl-thickness through catholyte-filled pores, and the conduction paths are assumed to have geometries with parallel walls perpendicular to the Li-surface, then it can be shown that

$$a_{p,sec} < K_{LiCl,max}/K_{lyte} \quad (14)$$

where K_{lyte} is the specific conductivity of the catholyte.

As already mentioned Q3-R4 is assigned to the secondary layer. This means that an apparent thickness, L_{LiCl} , the maximum conductivity, and the maximum pore

area ratio can be calculated from eqs. (9), (10a) and (14), respectively. Table 6 shows the data for the anodes given in Table 1 using the data of Table 3.

Table 6. Results derived from Q3-R4, the »secondary layer«, using circuit #1. The ratio of free to blocked area in the »secondary layer«, $a_{p,sec}$, is calculated using eq.(14).

Cell	Time Days	L_{LiCl} nm	$K_{LiCl,max}$ S/cm	$a_{p,sec}$
G40A	1	76.	4.6e-9	2.3e-7
	9	132.	3.1e-9	1.6e-7
3201	1	82.	6.2e-9	3.1e-7
	3	113.	5.2e-9	2.6e-7
G24B	1	10.6	7.7e-8	3.9e-6
	8	139.	5.7e-7	2.9e-5
SO21	1	0.2	4.8e-9	4.8e-8
	10	0.3	2.0e-9	2.0e-8
3303	1	1.0	3.4e-9	3.4e-8
	8	1.3	4.7e-9	4.7e-8
602	1	44.	1.2e-8	6.0e-7
	6	32.	3.5e-9	1.7e-7
607	1	22.	7.8e-9	3.9e-7
	6	19.	4.5e-9	2.3e-7
610	1	23.	8.2e-9	4.1e-7
	6	52.	1.0e-8	5.2e-7
614	1	2.4	3.2e-9	1.6e-7
	6	3.1	1.3e-9	6.6e-8
	1	5.7	3.5e-9	1.8e-7
	6	16.	5.1e-9	2.5e-7
2REG	1	37.	2.0e-8	9.9e-7
	8	86.	8.9e-9	4.5e-7

From Table 6 it is clear that a model similar to Che-nebault's¹³ implies rather dense secondary layers with a crack cross section area fraction (pore area ratios) generally below 10^{-6} . Holleck and Brady⁷ reported similar densities, but concluded that the porous layer could not be seen by electrochemical means. This conclusion cannot be correct, and when analysing the data of Tables 5 and 6 in detail, this is recognised as a more general problem. In order to illustrate this, it is assumed for a moment that the primary layer thickness, L_{pri} , has a fixed value of 3nm. The fact that it is covered by a secondary layer is expected to block off the surface resulting in a lower measured capacitance, i.e. if no correction is made for this, a too big L_{pri} would be obtained by using eq.(9). In fact a pore area ratio, $a_{p,sec}$ as defined above, might be calculated as the ratio between the true and the apparent thickness of the primary layer:

$$a_{p,sec} = L_{pri,true}/L_{pri,app} = L_{pri,true} \cdot C5/(\epsilon_r \cdot \epsilon_0) \quad (15)$$

If $L_{pri,true} = 3$ nm, all cases (see Table 5) will give $a_{p,sec} > 0.04$ in sharp contrast to the numbers derived from C3-R4 (Table 6). Many values will even become above 1 because of the space charge capacitances resulting in

apparent thicknesses lower than 3 nm. The $a_{p,sec}$ -values of Table 5 infer that the secondary layer is at least quite porous, above 4%, and in most cases non-existent, which is in clear disagreement with SEM-observations. The problem is not eased by proposing that C5-R6 belongs to the interface because the actual active area would be $(a_{p,sec})^{-1}$ times the nominal area. This means that if the $a_{p,sec}$ -values of Table 6 are believed to be correct then the real capacitances per cm^2 should be more than 10^6 times bigger than the »measured« C5-values of Tables 3 and 4 resulting in values up into the range of hundred Farads per cm^2 . This is of course not possible.

In other words, the parallel-plate-capacitor-model has failed and so has in fact the associated concept of a simple double-layer model. And a model with more layers will not help. Another kind of model is needed.

4. 3. Alternative Model Considerations

A complete physical model should explain all aspects of the impedance spectra. Unfortunately, the low frequency data available are too uncertain to allow a further analysis, and so the considerations are restricted to the high frequency (>50Hz) part of the impedance spectrum which, due to the sizes of the involved capacitance, is supposed to reflect the bulk of the layer. The model should then explain the CPE parameters, B3 and n3, and the resistance, R4.

The most direct way of obtaining insight in the structure is by microscopic observations. The SEM studies show that at least the outer parts of the passivating layer are formed by LiCl precipitation from the liquid catholyte because very smooth crystal growth facets are

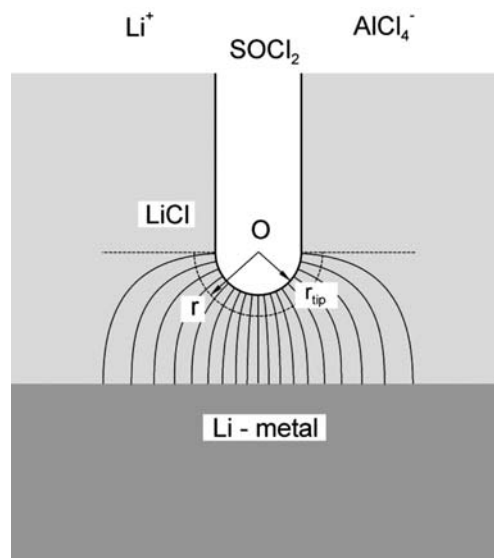


Figure 7. Illustration of the concept behind eqs. (16)–(19). With rotational symmetry it shows a cylindrical hole ending in a semi-sphere, eqs. (16)–(17a). If a cross-section through a crack is imagined then it shows a crack with a tip of semi-cylindrical shape.

seen. This in turn means that differences in radius of curvature of the various surface parts of the LiCl layer, are delivering the driving force for the dissolution and re-precipitation of the LiCl, and thus, it seems probable that catholyte-filled tunnels along the LiCl crystal corners will be left in the layer and possibly some types of crystal faces may have difficulties in growing totally together. It is inherent in such a model that the dimensions of the tunnels in the inner part (towards the Li) of the LiCl have to be in the nanometer range because the crystals themselves are in the range of 100 – 1000 nm size.

Thus, the focus is put on the resistive and capacitive responses of narrow deep holes and cracks as sketched in Fig. 7. If rotational geometry is imagined in Fig. 7 then it is a hole with a half-sphere tip with radius, r_{tip} . If crack geometry is envisaged, Fig. 7 shows a crack width of $2 \cdot r_{tip}$, and the crack tip is a half-cylinder with radius r_{tip} . Making the approximation that the current does not flow »backwards«, the following formulas (see textbook, e.g. Lehner³⁷) may be used for calculating the capacitances and resistances as a function of distance, r , from the hole or crack tip center:

For holes:

$$R(r) = (1/(2 \cdot \pi \cdot K_{LiCl})) \cdot (1/r_{tip} - 1/r) \quad (16)$$

$$R_{max} = 1/(2 \cdot \pi \cdot K_{LiCl} \cdot r_{tip}) \quad (16a)$$

$$C(r) = 2 \cdot \pi \cdot \epsilon_r \cdot \epsilon_0 \cdot (r_{tip}r/(r-r_{tip})) \quad (17)$$

$$C_{min} = 2 \cdot \pi \cdot \epsilon_r \cdot \epsilon_0 \cdot r_{tip} \quad (17a)$$

For cracks per unit length:

$$R(r) = (1/(\pi K_{LiCl})) \ln(r/r_{tip}) \quad (18)$$

$$C(r) = \pi \cdot \epsilon_r \cdot \epsilon_0 \cdot (\ln(r/r_{tip}))^{-1} \quad (19)$$

Here, it should be noted that irrespective of geometry the conductivity is given by eq. (10) which is also obtained by solving eqs. (16) and (17) as well as eqs. (18) and (19) with respect to the conductivity, K_{LiCl} .

If the distance from the crack tip to the Li-metal is used for r in eqs.(16–19) a better approximation is believed to be at hand compared to just assuming a »parallel plate« compact layer. Naturally, if the distance from the tip to the Li is less than or equal to the tip dimensions the parallel plate may locally be a better approximation. Furthermore, it should be noted that eqs.(18) and (19) will also be good approximations for calculating the resistance and capacitance of the LiCl on the Li-side of tunnels parallel to the Li-surface of the type shown in Fig. 8a.

Table 7 gives some examples of the resistances through narrow tunnels and cracks which are filled with catholyte and of the resistance in the LiCl next to the tips of the holes and cracks tips/tunnels parallel to the Li, respectively. It is seen that even though the resistance in catholyte-filled cracks may be relatively small, the resistance of the LiCl at the tip is large also in cases where the distance to the Li is only 10 nm. Tunnels perpendicular to the Li do not lower the layer resistance much unless there are an enormous number of them.

This information may be applied to a typical Li-anode passivated in A-catholyte for about a week. The »SEM-thickness« is about 10,000nm (10 μ m as an average figure, but it is very non-uniform). The measured values are: $R_4 = 7 \text{ k}\Omega \text{ cm}^2$, $B_3 = 2.6 \cdot 10^{-7} \text{ S s}^{-0.8} \text{ cm}^{-2}$, $n_3 = 0.8$

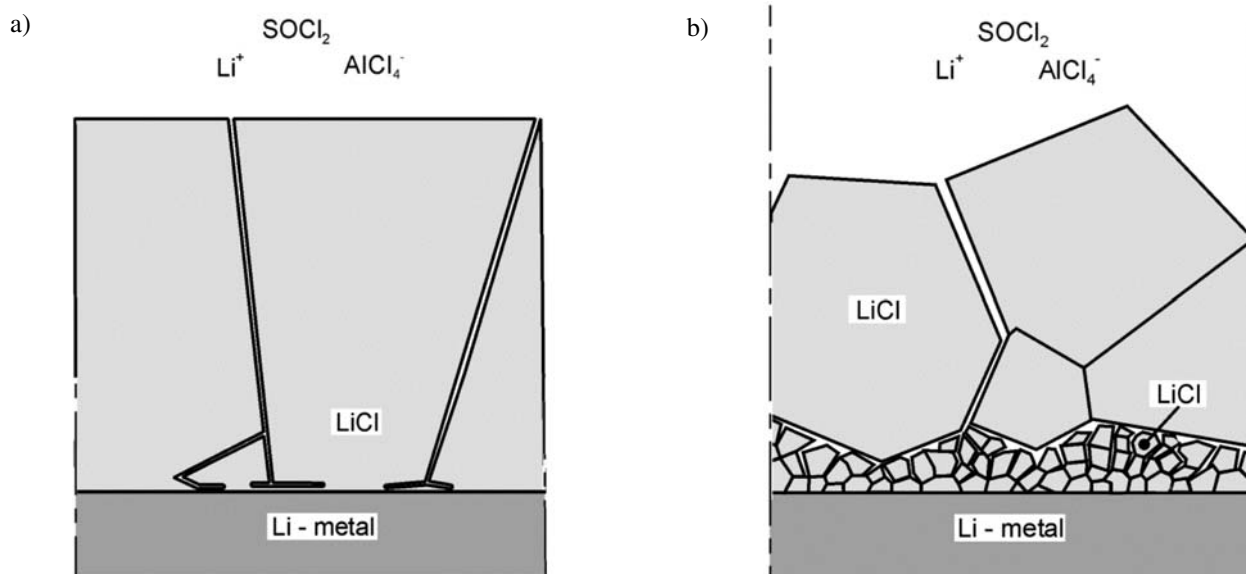


Figure 8. Sketches of possible structures of the LiCl passivating layer. The »simple crack« model (a) cannot explain the CPE type of impedance response. A very branched crack and tunnel system (b) seems necessary, and the small grains towards the Li-metal with bigger grains on top reflects the structures observed by SEM.²⁵

Table 7. Examples of calculated resistance through catholyte filled tunnels and cracks filled with 1.8M $\text{LiAlCl}_4/\text{SOCl}_2$ -catholyte and of the solid LiCl at hole and crack tips.

Through tunnels			Resistance			
Tunnel						
dia., nm			$\Omega/\mu\text{m}$			
10			6.4e8			
100			6.4e6			
1000			6.4e4			
Through 1 cm long cracks						
Crack			Resistance			
width, nm			$\Omega/\mu\text{m}$ depth			
10			5e3			
100			500			
1000			50			
In the LiCl at hole tips. Resistance, R in Ω , and capacitance, C in F.						
Hole diameter, nm						
		10	100		1000	
Distance, nm	R	C	R	C	R	C
10	8.16e13	4.42e-18	4.00e14	7.37e-19	4.00e13	7.37e-19
100	1.16e14	3.05e-18	8.16e12	4.42e-17	4.00e14	7.37e-18
1000	1.21e14	2.96e-18	1.16e13	3.10e-17	8.16e11	4.42e-16
Max/min	1.22e14	2.95e-18	1.22e13	2.95e-17	1.22e12	2.95e-16
In the LiCl at 1 cm long crack tips. Resistance, R in Ω , and capacitance, C in F.						
Crack width, nm						
		10	100		1000	
Distance, nm	R	C	R	C	R	C
10	1.35e8	2.68e-12	3.85e7	9.39e-12	3.85e6	9.39e-11
100	3.73e8	9.69e-12	1.35e8	2.68e-12	3.85e7	9.39e-12
1000	6.49e8	5.56e-13	3.73e8	9.67e-13	1.35e8	2.68e-12

and the layer capacitance $C3 = 51 \text{ nFcm}^{-2}$. This implies a $K_{\text{LiCl}} = 2.6 \cdot 10^{-9} \text{ S/cm}$. The resistance of the layer (10 μm thick) without cracks is then calculated to be 380 kOhm cm^2 and the capacitance 0.94 nF/ cm^2 . This is a real set of data for A-catholyte, selected to compare well with data reported by others.^{1,7,8,9,11,12,27,28} From this, it is possible to make a rough estimate of which lengths of tunnels parallel to the Li are necessary in order to explain the actually observed resistance. If it is assumed that the tunnel

diameter is 10 nm and the distance to the Li surface is 100 nm then it is found that the length necessary to give a resistance of 7000 Ωcm^2 is $5 \cdot 10^4 \text{ cm/cm}^2$. This may be compared to the calculated length of LiCl grain boundaries in one plane parallel to the Li. If the grains are assumed having forms of hexagons of 500 nm size and having all centres in this plane, the grain boundary length is $3.3 \cdot 10^4 \text{ cm/cm}^2$. In reality the grains are irregularly shaped and have a size distribution. The grain corners consti-

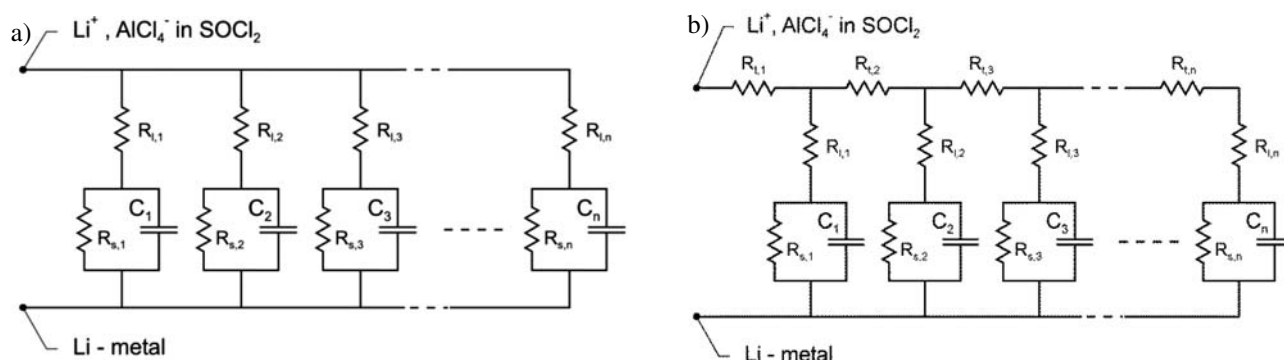


Figure 9. Electrical circuits tested as models of the LiCl layer. Fig. 9a is simulating the »simple crack« model of Fig. 8a, and 9b is another »ladder« type of circuit, which may respond as a CPA over certain frequency ranges dependent on the actual values of resistances and capacitances. Even higher degrees of branching are necessary in order to simulate the observed CPE behaviour pointing to a much branched crack and tunnel system.

tute a three dimensional network which will result in a much longer grain corner length in the layer of small crystals near the Li-surface than obtained by the above simple calculation. Altogether, it seems to suggest that some, not vanishing, fraction of the grain corners and faces are open and catholyte-filled. This indicates a structure of the passivating layer similar to the type shown in Fig. 8b.

Another indication of a much branched crack and tunnel system, as illustrated in Fig. 8b, may be revealed. For this purpose, the simpler structure of Fig. 8a is considered. It might be thought that in a structure with only a few cracks and tunnels, a relative simple equivalent circuit of the kind shown in Fig. 9a could possibly model the LiCl film response, i.e. Q3-R4. This was tried. Naturally, when many circuit parameters are available, it is easy to find values which result in a flat arc of the same size as a measured one. It is, however, only possible to achieve something near to a CPE behaviour if the $R_{s,i} \cdot C_i$ is varying significantly. The consequence of this is that either should the LiCl electrical permittivity or the conductivity decrease towards the Li-surface. None of the possibilities is thought to be likely. The LiCl relative permittivity is about 10 only, so it is not conceivable that it should decrease to 1. The decrease of the conductivity would be in agreement with the position dependent conductivity idea,¹⁶ but this would imply a CPE behaviour in all cases and from Table 3 and 4 it is seen that this is not the case. Finally, the circuit of Fig. 9a does not give a real constant phase angle, i.e. not a real CPE, while the impedance response of the LiCl layer is a real CPE over 4 frequency decades from about 10Hz to 10 kHz. In order to obtain a CPE type of behaviour some kind of branching of resistance and capacitance in a ladder type of circuit is necessary, see e.g. the book of McDonald.²³ Such a kind of circuit is shown in Fig. 9b, and here a depression of the impedance spectrum arc may be achieved over some range of frequency. An analysis show that in this case only an n3-value of 0.5 may be obtained, and in order to get an n3 = 0.75, one more level of branching is necessary, with e.g. Fig 9b type of circuits in the side of the “ladder” and pure capacitances as »steps« (if Fig. 9b is imagined to be a ladder, $R_{t,1}, R_{t,2}, \dots, R_{t,n}$ is constituting the one side of the ladder and $R_{e,1} + R_{s,1} + C_1$ is step no. 1 etc.) The experimental results show that n3 may vary with time and with catholyte composition, and in fact may vary from

anode to anode under nominally identical conditions. This points to a picture with a variable high degree of branching like Fig. 8b, which shows a tunnel system on the boundaries of the small grains next to the Li. This system is covered by big massive (not porous) 10–20 μm grains with a few big cracks between them, reflecting the situation in an A-catholyte after some days. Such a system is believed to form a transmission line network which may account for the varying n3 values seen.

This qualitative model provides an explanation of the course of passivation of Li in the B-catholyte shown in Fig. 6. During the period from the first measurement after 15 min (0.01 day) to about 10 h almost no change in values are seen. This is due to little re-precipitation of LiCl from the acid B-catholyte which can absorb a significant amount of LiCl which it is also able to release again depending on the chemical activity of the LiCl, i.e. the radius of curvature locally on the single LiCl crystals. After this period n3 and C3 start to decrease. This is understood as a result of large crystals being nucleated on the top of the small crystals which were already formed before the first measurement. During the first days after this nucleation, the resistances are not affected very much, but after about 12 days the large crystals start to grow together resulting in a drastic increase of the LiCl-film resistance. After about 200 days this increase levels off meaning that now the large crystal layer are as dense as it possibly will become at all. About the inflection point of the resistance curve n3 reach a value close to 0.5. This is taken as the point where the »transmission lines« consisting of the tunnels beneath the big crystals are longest just before a large part of the tunnel system is blocked off by the big crystals growing into each other.

4. 4. Micro-calorimetry and LiCl Electronic Conductivity

Table 8 shows selected values from the microcalorimetric measurements. The typical range of heat production during the first days is 1 – 10 $\mu\text{W}/\text{cm}^2$.

For the Li-SOCl₂-system having a well defined EMF = 3.68 V, the measured power (heat) can be directly transformed to self discharge current. A source of error is thermal decomposition of thionyl chloride. Frank³⁸ states

Table 8. Microcalorimetry result and the derived electronic conductivities. $K_{\text{ele}} = \varepsilon \cdot \varepsilon_0 / (R_{\text{ele}} \cdot C)$. Min K_{ele} is obtained using C3 data from Table 2, and max K_{ele} is found from $C_{\text{total}} = (1/C3 + 1/C5)^{-1}$ using Table 2 data. $R_{\text{ele}} = \text{EMF}^2/W$, where W is the power density

Cell	Time Days	Power density $\mu\text{W}/\text{cm}^2$	EMF V	$R_{\text{ele}} \text{ M}\Omega\text{cm}^2$	Min. $K_{\text{ele}} \text{ S/cm}$	Max. $K_{\text{ele}} \text{ S/cm}$
3201	1	1.05	3.68	12.9	6.4e-13	1.1e-12
	3	4.82	3.68	2.8	4.0e-12	4.3e-12
3303	1	9.85	3.3	1.1	8.6e-14	9.3e-14
	8	1.93	3.3	5.6	2.4e-14	3.1e-14
2REG	1	11.88	3.68	1.1	3.4e-12	3.4e-14
	8	3.00	3.68	4.5	1.9e-12	1.9e-14

a rate of $\text{EXP}(-0.12-1605/T)\%/ \text{day}$, which at 25 °C gives 1,5%/year, the same level as normal self-discharge rates for aged lithium batteries.

The reaction $4 \text{SOCl}_2 = 2 \text{SO}_2 + \text{S}_2\text{Cl}_2 + 3 \text{Cl}_2$ gives a total enthalpy of -33.3 kcal/mol . For a cell having 19 g thionyl chloride, it is equivalent to a $10 \mu\text{W/cell}$, which normally is much less than the measured effects, but higher rates of decomposition, for example from catalytic reactions, cannot be excluded.

At large self-discharge rates or intentional loads (in the range 5–100 kΩ) there should be a further correction for the entropy-related heat production^{39–41}). The Gibbs-Helmholtz equation may be written as

$$nFE = -Q + nFT(dE/dT) \quad (20)$$

where n = number of electrons, F = Faradays constant, E = EMF, $-Q$ = heat production, T = temperature. The equation may be rewritten as

$$-QI/(nF) = E^2/R - T(E/R)(dE/dT) \quad (21)$$

where R = the discharge resistance. The first term contains the total heat flow including self-discharge and loss from polarization, the next term the heat production in the discharge resistor, and the last term includes Q_E , the heat production of the electrode reaction.

dE/dT is found to be -0.00029 V/K for unused cells (having A-catholyte), -0.00039 V/K for 1% discharged and -0.00044 V/K for 50% discharged cells. As an example, the correction Q_E is $33 \mu\text{W}$ (i.e. $1 \mu\text{W/cm}^2\text{Li}$) at a $10 \text{ k}\Omega$ discharge and $3 \mu\text{W}$ at a $100 \text{ k}\Omega$ discharge. The correction is insignificant at normal self-discharge currents.

So, the electron conductivities given in Table 8 were calculated from the microcalorimetric measurements assuming that the electron conductivity, K_{ele} , is the only factor that limits the self-discharge:

$$K_{\text{ele}} = \varepsilon_r \cdot \varepsilon_0 / (R_{\text{ele}} \cdot C) \quad (10b)$$

and

$$R_{\text{ele}} = \text{EMF}^2/W \quad (22)$$

where W is the heat power density. The C3 values of Table 3 were used. The results are in fair agreement with the values in the range $3 \cdot 10^{-12}$ – $5 \cdot 10^{-13} \text{ S/cm}$ reported in a previous paper [8] in which the electronic conductivity of the LiCl was estimated from the growth rate in certain stages where the parabolic law was followed.

4. 5. General Discussion

The analyses presented show that impedance spectroscopy cannot be used as a reliable method of measuring

the thicknesses of the LiCl-films using the parallel plate capacitor formulas, but the formulas derived for the conductivities are believed to be good approximations because the geometric parameters cancel when the equations are solved with respect to conductivity. There is some uncertainty on the values because of the uncertainty on the capacitances derived from CPEs. The magnitude of this »uncertainty« is dependent on the $n3$ -value. If the previous mentioned example ($R4 = 7 \text{ k}\Omega \text{ cm}^2$, $B3 = 2.6 \cdot 10^{-7} \text{ S s}^{-0.8} \text{ cm}^{-2}$, $n3 = 0.8$) is used, application of the peak frequency of 423 Hz gives $K_{\text{LiCl}} = 2.6 \text{ nS/cm}$ and the frequency of 20 kHz (where the high frequency part of the impedance curve approaches the real axis) gives 5.8 nS/cm . This difference does not reflect any kind of measurement uncertainty. It is because of model limitations, i.e. the »probing« includes different amounts of the LiCl layer with different fractions of catholyte filled porosities according to the model presented above, but as the model is not quantitative the different amounts are not known. It means that the LiCl-conductivities are upper limits, and it points out that the lower frequencies should give the most reliable values, but then there is the problem that at low frequencies other sources than the LiCl layer contribute to the impedance spectrum. The Q3-R4- peak frequency still seems to be no bad choice for the calculation of the conductivities.

A point of general concern in the context of lithium batteries with liquid catholytes is the delayed voltage phenomenon and the explanation of it. The present work does not provide any proof of one or the other interpretation of the delayed voltage effect, but the mechanical cracking and peeling hypothesis is in a way the consequence of the model of Figs. 8b, i.e. the concentrated currents at the crack tips will cause local heating and stress build-up which in turn cause the cracking and peeling. This hypothesis is also in agreement with the observation that a high current for a short time is more effective than a low current for a longer time in effectively removing the delayed voltage. More importantly, it is also in agreement with SEM micrographs of the surfaces (Dey⁴²) showing large extents of cracks, and the fact that delayed voltage experiments are difficult to reproduce. On the other hand the hypothesis does apparently not agree with Delnick,⁹ who finds that during transition, noise is not superimposed on the discharge current. However, each crack only contributes to a minor part of the total current, so that current versus time curves may appear smooth especially when the super-capacitances at the very low frequencies (Q7) are recalled. Such capacitances will certainly be able to smoothen quite big jumps in the Faradaic current.

Further, the presented model (and data) implies that the dissolution and redistribution of LiCl by the catholyte is responsible for the build-up of such a relatively bulky microporous passivating layer. If the LiCl stayed where it was originally formed, the formation of the big blocking crystals would not take place.

5. Conclusions

Analysis of experimental observations has resulted in a qualitative model of the passivating LiCl layer formed on Li in liquid cathodes. The layer is 100% dense towards the Li-metal but otherwise microporous due to numerous tunnels and crevices along the LiCl grain boundaries and some cracks. The structure is changing with time and conditions. It is shown that the double LiCl-layer model and the parallel plate capacitor equation are not applicable, and thus a meaningful thickness of the passivating layer cannot be derived from impedance spectroscopy. In spite of this, impedance spectroscopy is a valuable tool for monitoring and studying the Li passivation as it gives, in a non-destructive manner, a reliable layer resistance and an approximate specific ionic resistivity. Using parallel measurements of impedance spectroscopy and microcalorimetry provide approximate values of the LiCl specific electronic conductivity. This is of special importance for the general description of the layer since the electronic conductivity is controlling the LiCl formation and the self-discharge of the Li-battery.

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Povzetek

Opisana je študija impedančne spektroskopije litijevih anod pasiviranih z LiCl v različnih tekočih katodah (katolitih) na osnovi LiAlCl₄ v SOCl₂ oz. SO₂. Impedančni spektri so bili razloženi s pomočjo dveh ekvivalentnih vezij z uporabo metode nelinearnih namanjših kvadratov. S pomočjo te metode smo ekstrahirali informacije o ionski prevodnosti in strukturi plasti. Predlagali smo nov fizikalni opis, ki razlaga parametre vezja. Domneva se, da plasti LiCl vsebujejo veliko število ozkih tunelov in razpok, ki so napolnjene s tekočim katolitom. Razložili smo tudi, zakaj omenjeni tuneli nastajajo. Na specifičnem primeru smo pokazali, da so tuneli povezani z večino mej med zrni kristalinične plasti LiCl v bližini Li površine ter, da so nujno potrebni pri razlagi impedančnega odziva. Hitrost nastanka plasti LiCl, je omejena z elektronsko prevodnostjo le-te. Podatki mikroklorimetrije so skupaj z impedančnimi spektri služili za določevanje elektronske prevodnosti plasti LiCl.

Scientific paper

Effect of ZnO on the Thermal Degradation Behavior of Poly(Methyl Methacrylate) Nanocomposites

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In the memory of Janez (Janko) Jamnik our dear friend and co-worker.

Abstract

The influence of ZnO nanoparticles on the thermal degradation behavior of poly(methyl methacrylate) (PMMA) was tested using thermoanalytical techniques. The studied materials were investigated using TG, DTA, EGA, XRD, SEM and TEM. The ZnO nanoparticles were synthesized via precipitation by adding LiOH into Zn²⁺ water/ethylene glycol solutions. The ZnO-PMMA nanocomposites were prepared by adding the appropriate amount of ZnO into MMA and subsequent MMA radical polymerization. According to the experimental results and model-free isoconversional activation energy calculations, the addition of ZnO into PMMA played a double role. The ZnO concentrations up to 0.15% stabilized the composite by shifting the degradation interval toward higher temperatures and increasing the apparent activation energy relative to pure PMMA. At higher concentrations, the catalytic effect of ZnO started to prevail and was reflected in the lower temperature intervals of intense PMMA degradation and lower apparent activation energy. The addition of ZnO generally did not change the nature of the PMMA decomposition process.

Keywords: ZnO-PMMA nanocomposites, thermal properties, activation energy, electron microscopy, thermogravimetric analysis (TGA)

1. Introduction

The potential of using nanometer-scale inorganic particles in a polymer matrix has recently been extensively investigated in several applications, e.g. civil and electrical engineering, building and transportation. Such composites offer the potential to create new materials with improved thermal, electrical, mechanical, optical and fire-resistant properties, which arise from the synergies between the components.^{1–5} Among the papers published about this subject, it appears that the majority of the work performed particularly concerns composites of poly(methyl methacrylate) (PMMA) and oxide ceramic filler.^{6–8} PMMA is an optically clear amorphous thermoplastic. It is widely used as a substitute for inorganic glass, because it shows higher impact strength and undergoes ductile rather than brittle fracturing. Compared with inorganic glass, PMMA has some advantages, such as transmission of more light, and has lower density than

silica glass (1.19 and 2.20 g cm⁻³, respectively).⁹ However, PMMA shows poor thermal stability, which restricts it from high-temperature applications. Significant research has been performed in order to improve the thermal stability of PMMA by mixing it with various inorganic fillers. Among inorganic fillers added to PMMA, silica was the most widely investigated.^{10–14} The thermal degradation of such composites was a prime areas of investigation. It was shown that the addition of silica into PMMA improves thermal stability of the composites. This improved thermal stability was explained by the hindered mobility of the polymer chains due to the presence of filler,¹⁵ the ability of silica nanoparticles to trap radicals during PMMA degradation and to act as a gas barrier preventing the degradation products from diffusing out of the composite,¹⁴ or through a hydrogen-bonding interaction between carbonyl groups in PMMA and hydroxyl groups of the silica surface,¹⁶ where hydrogen bonds interrupt the depolymerization of the polymer

chains. Similarly to silica, the addition of Fe_2O_3 and TiO_2 nanoparticles into PMMA also influence the composite thermal stability. The improved thermal stability observed in $\text{Fe}_2\text{O}_3/\text{TiO}_2$ -PMMA composites was the result of two main factors: i) the presence of the nanoparticles restricted the mobility of polymer chains, and ii) adsorption of the PMMA on the oxide surface via methoxycarbonyl groups.^{17,18} Subsequently, it was shown that relatively high additions of TiO_2 nanoparticles into PMMA also play a catalytic role on the polymer thermal degradation.^{19,20} This catalytic effect was explained through the interaction of the methoxy group of the methacrylate with the hydroxyl groups present at the surface of the oxide particles.

In recent years, the addition ZnO into PMMA has also been intensively studied in order to make functional nanocomposites.^{21–27} ZnO is an environmentally friendly, important and attractive semiconducting material. It has drawn enormous research attention due to its distinguished properties in optics, photonics and electronics.²⁸ The combination of these two materials has many potential applications, including antireflection coatings, transparent barrier/protective layers, and as flame-retardant materials. ZnO is also a semiconductor with an optical band gap in the UV region that also makes it useful as an efficient absorber of UV radiation.²⁹ Moreover, ZnO can be simply obtained through wet chemistry, which offers it a potential viable route to achieve uniform dispersion in polymer matrices through solution mixing. In studying the thermal stability of ZnO-PMMA composites, it has been shown that ZnO addition has a profound effect on the thermal degradation of the composite.^{1,30} Liu et al.³¹ prepared PMMA/ZnO nanocomposites through *in situ* polymerization of MMA and organic modified nanoparticles. The thermal stability and UV absorption of the nanocomposites were enhanced as ZnO concentration increased. Similar results were also reported by Demir et al.³² who studied the improved thermal stability of PMA/ZnO nanocomposites compared with the blends of PMMA and ZnO. Liufu et al.³³ also investigated the thermal degradation of polyacrylate/ZnO blends and proposed that the ZnO particles have a role in both stabilization and destabilization depending on the temperature region. Generally, the role of ZnO on the thermal stability of ZnO-PMMA can be explained through the same relatively developed mechanisms, such as the barrier effect, trapping radicals, char formation and catalytic effect, as described for SiO_2 -PMMA or TiO_2 -PMMA composites.^{13,17,34–36}

Since few research reports focused on thermal behavior of ZnO-PMMA composites, the aim of this work was to demonstrate the catalytic effect of ZnO nanoparticles in the path of PMMA thermal degradation. For this purpose, key degradation products were determined and some kinetic aspects were investigated via a model-free method.

2. Material and Methods

2. 1. Used Chemicals

All reagents used for synthesis were of an analytical reagent grade. To avoid hydrolysis upon storage, fresh stock aqueous solutions were prepared from $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (98% Merck), ethylene glycol (99.5% Sigma-Aldrich) and lithium hydroxide (98% Sigma-Aldrich). For *in situ* and *ex situ* coating of ZnO particles, tetraethyl orthosilicate (TEOS) (Sigma-Aldrich), 25% $\text{NH}_3(\text{aq})$ solution (Merck) and absolute EtOH (Sigma-Aldrich) were used. Methyl methacrylate (MMA) (99% Sigma-Aldrich), bis (4-t-butylcyclohexyl)-peroxydicarbonate (P-16) and dilauroyl peroxide (LPO) were used for the preparation of ZnO/PMMA nanocomposites.

2. 2. Synthesis of Spherical ZnO Nanoparticles

Prior to ZnO synthesis, the mother solution was prepared by mixing $\text{Zn}(\text{NO}_3)_2(\text{aq})$, $\text{EG}(\text{aq})$ and $\text{LiOH}(\text{aq})$. In the mother solution, the initial concentrations of Zn^{2+} and Li^+ ions were 0.1 M and the volume ratio of water-to-ethylene glycol was $V(\text{H}_2\text{O}):V(\text{EG}) = 1:5$. The syntheses of ZnO nanoparticles were carried out in a 250 mL flask without stirring at 100 °C, for 2 hours. Firstly, Zn^{2+} and LiOH solutions in 150 mL of mixed solvents were prepared and heated to 100 °C. After the hydrolysis reaction was completed, the resulting white precipitate of ZnO was centrifuged, washed with water four times and dried in air at room temperature or re-dispersed in EtOH. In depth report how preparation conditions influence final ZnO morphology can be found elsewhere.³⁷

2. 3. The Preparation of Silica-coated ZnO Nanoparticles by *ex-situ* Method

Part of the synthesized ZnO nanoparticles was further functionalized by coating them with silica. In a typical *ex-situ* coating process, the dried ZnO was re-dispersed in 10 mL EtOH and 4 mL MQ water, then $\text{NH}_3(\text{aq})$ was added to the re-dispersed ZnO solution, and finally the appropriate quantity of TEOS to reach $C(\text{TEOS}) = 0.048$ M was admixed. The coating process was carried out at room temperature for 1 hour in an ultrasonic bath, as suggested in the literature.³⁸ After the procedure was completed, the final product was purified in EtOH and dried in air at room temperature.

2. 4. Synthesis of PMMA

PMMA (sample A) was prepared by chain growth radical polymerization. Prior to polymerization reaction, the initiators P-16 (0.25 wt.%) and LPO (0.25 wt.%) were added to MMA. The polymerization of MMA was carried out in a water bath at 60 °C for 1 hour. The synthesized

PMMA was poured into glass plates and placed in an oven for 2 hours at 55 °C and subsequently for 10 minutes at 120 °C. Finally, the PMMA sheets were separated from the glass plate moulds.

2. 5. Preparation of ZnO/PMMA Nanocomposites

0.1 wt.% of bare (sample B2) or coated ZnO powders (sample C) were suspended in 5 mL of MMA in a centrifuge tube. The suspensions were sonicated (continuous waves) for 20 minutes prior to the addition of initiators. The polymerization of MMA was conducted as described above. Samples with 0.05%, 0.15% and 0.20% (named sample B1, B3 and B4, respectively) were prepared in the same manner.

2. 6. Products Characterization

The obtained bare or coated ZnO powders were characterized according to their morphological characteristics with a field emission scanning electron microscope (FE-SEM, Zeiss UltraPlus) with the ability to work in STEM mode. TEM observations were carried out in a transmission electron microscope operated at 200 kV (JEM-2100, JEOL) which was equipped with EDX. TEM samples were prepared by dispersing the powders in ethanol, using ultrasonification followed by the deposition of the obtained suspension on carbon-coated copper grids. X-ray powder diffraction analyses (XRD, Siemens D-500 X-ray diffractometer) were carried out with Cu-K α radiation ($\lambda = 1.54 \text{ \AA}$) in the range 2θ between 20° and 70° using an XPert Pro X-ray diffractometer. Thermo-gravimetric decompositions of PMMA or ZnO-PMMA composites were performed using ~50 mg of the sample and a NETZSCH STA 449 F3 set-up with a microbalance having a sensitivity of $\pm 0.1 \text{ g}$ coupled with QMS 403 C system. The experiments were carried out in a constant flow of synthetic air (50 mL/min; Ar-O₂ – 20 vol.% O₂) under non-isothermal conditions (30–600 °C) with heating rates β from 2 K/min to 10 K/min. Partial kinetic analyses of the ZnO/PMMA thermal decomposition were calculated using the non-linear isoconversional method developed by Vyazovkin.³⁹ The isoconversional analysis is based on a model kinetic equation (1):

$$\frac{\partial \alpha}{\partial t} = f(\alpha) k_{\alpha} \exp\left(-\frac{E_a}{RT}\right) \quad (1)$$

where α is conversion degree of the degradation reaction, E_a is the apparent activation energy (J mol^{-1}), k_{α} is the pre-exponential factor (s^{-1}) and $f(\alpha)$ is the reaction model [16]. From the above kinetic model, the linear dependence of $\ln \beta$ vs. $1/T$ may be recognized. Using the Doyle approximation, the slope in the $\ln \beta$ vs. $1/T$ diagram represents a value of $1.052 E_a/R$. The UV-VIS spectra of ZnO/PMMA

nanocomposites were measured on solid plates prepared from pure PMMA, non-coated ZnO and silica-coated ZnO particles into PMMA using the Perkin Elmer Lambda 950 UV-VIS spectrometer in the spectral range between 250 and 800 nm.

3. Results and Discussion

Powder XRD analysis of the prepared nanoparticles (Figure 1) revealed that all major peaks correspond to ZnO zincite (JCPDS 01-079-2205, zinc oxide). ZnO is the only crystalline phase present in the sample. The average crystallite size calculated from the (100) diffraction plane is 14.3 nm.

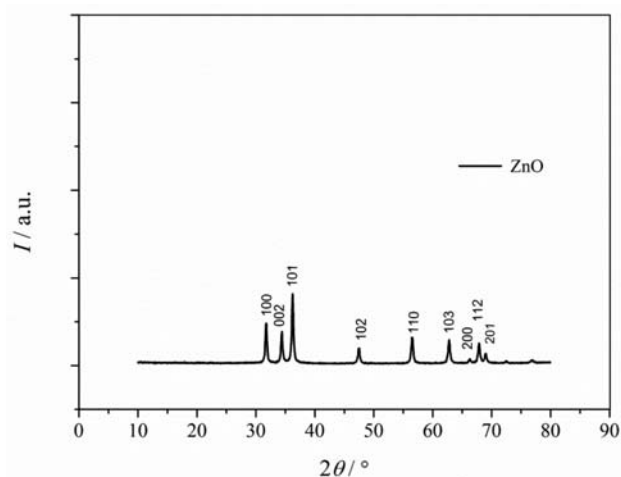


Figure 1. XRD pattern of the prepared ZnO nanoparticles

The morphology of the prepared ZnO nanoparticles (bare or silica-coated) was determined via electron microscopy. SEM and TEM micrographs are presented in Fig. 2. The micrograph reveals spherical ZnO nanoparticles (diameter ~10–20 nm) (Fig. 2a) and ZnO nanoparticles coated with a layer of silica ~8 nm (Fig. 2b). The silica coating had no influence on the morphology of the ZnO nanoparticles.

Introduction of the prepared nanoparticles into PMMA resulted in rather homogenous and uniform dispersion (Fig. 3). Neither areas of highly agglomerated solid particles nor larger areas clear of ZnO addition are observed. Such ZnO-PMMA composites are used for their thermo-oxidative degradation study.

The thermal stability of the pure PMMA or ZnO-PMMA nanocomposites is studied using TG-DTA-QMS experiments (Fig. 4). The basic idea is to show that the addition of ZnO into PMMA alters the thermal decomposition of such composites, either from a chemical point of view or/and with respect to the temperature interval of the

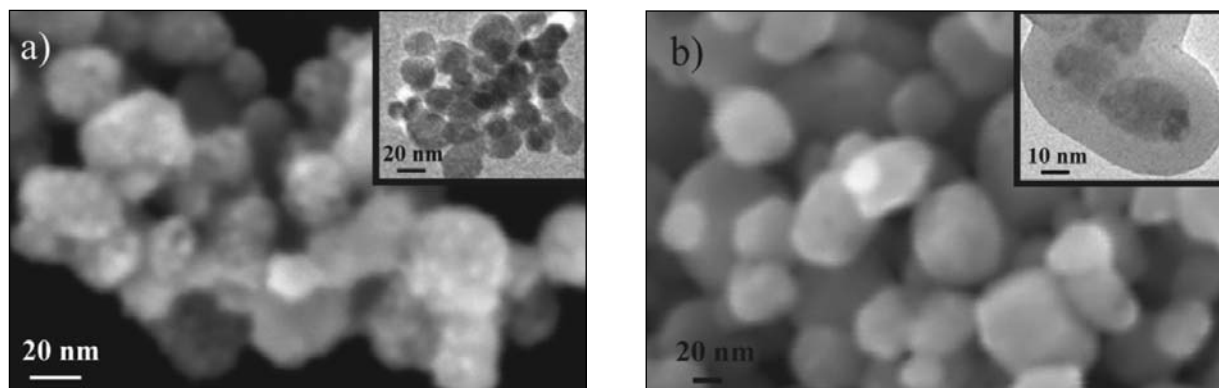


Figure 2. SEM and TEM micrographs of a) bare ZnO and b) silica-coated spherical ZnO nanoparticles

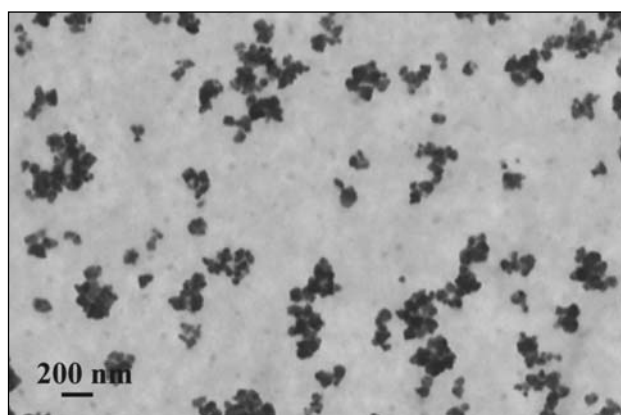


Figure 3. ZnO distribution in PMMA (STEM image)

decomposition. Although the TG-DTA curves describing thermal decomposition of samples A–C are rather similar, there are some distinguishing differences among them. Pure PMMA (sample A) decomposes in one broad interval from ~ 230 °C to ~ 380 °C (Fig. 4a). According to the DTG and DTA curves, at least two steps may be recognized inside this broad interval. The first step at around 300 °C is rather vague while the second step, with its peak temperature ~ 355 °C, may be clearly identified. The chain scission of sample A in synthetic air is practically complete, since less than 0.01% residue remains after the analysis. Volatile products of the decomposition followed by the QMS reveal the presence of H_2O (m/e 18), CO_2 (m/e 44), CO (m/e 28), MeOH (m/e 31), propanoic acid methyl ester (PAME) with a characteristic signal at m/e 88 and methacrylic acid (MA) with characteristic signal m/e 41. All volatile products are evolved continuously throughout the degradation path and their relative intensities are in accordance with the DTG curve.

If bare ZnO nanoparticles are admixed to PMMA (sample B2), the temperature interval of the thermal decomposition does not change significantly with respect to pure PMMA (Figs 4a and 4b). It is shifted to somewhat

higher values but remains inside the range from ~ 240 °C to ~ 400 °C. However, the profiles of the TG curve and, consequently, the DTG and DTA curves change. In the latter case, inside the decomposition interval of ZnO-PMMA, the DTG curve predicts one additional distinctive step. The additional step with peak temperature at ~ 295 °C is reflected in a relatively quick mass change and is not characteristic for the thermal decomposition of pure PMMA without the addition of ZnO. The remaining (main) decomposition steps with peak temperatures of ~ 320 °C and ~ 355 °C are similar to pure PMMA decomposition. All decomposition steps are exothermic and may also be recognized in the DTA curve. Volatile products during thermal decomposition of ZnO-PMMA are generally the same as in the case of pure PMMA. However, the relative intensities of the various detected m/e signals change significantly with temperature if ZnO is added into PMMA. The ZnO addition particularly intensifies signals m/e 88 (PAME) and m/e 41 (MA) during the first decomposition step at ~ 295 °C. Such behavior of ZnO clearly demonstrates its catalytic nature toward PMMA thermal decomposition. It is evident that the addition of ZnO does not change the nature of the decomposition process. Instead, it influences temperature intervals of intense degradation especially during the early stages. From this perspective, ZnO in PMMA behaves similarly to TiO_2 . TiO_2 was also reported to act catalytically for PMMA thermal degradation that is accompanied by MeOH, PAME and MA evolution.¹⁶

To further clarify the role of ZnO during the thermal decomposition of PMMA, silica-coated ZnO nanoparticles were also incorporated into PMMA. As expected from the fact that silica does not catalytically promote the thermal decomposition of PMMA, the sequences of evolved gases for samples C (silica-coated ZnO-PMMA) and A (pure PMMA) are rather similar (Figs 4a and 4c). The coating of ZnO nanoparticles with silica substantially diminish the degradation step at ~ 290 °C. This first degradation step is not negligible, however; it is ascribed to the fact that the minor part of ZnO nanoparticles may be poorly coated.

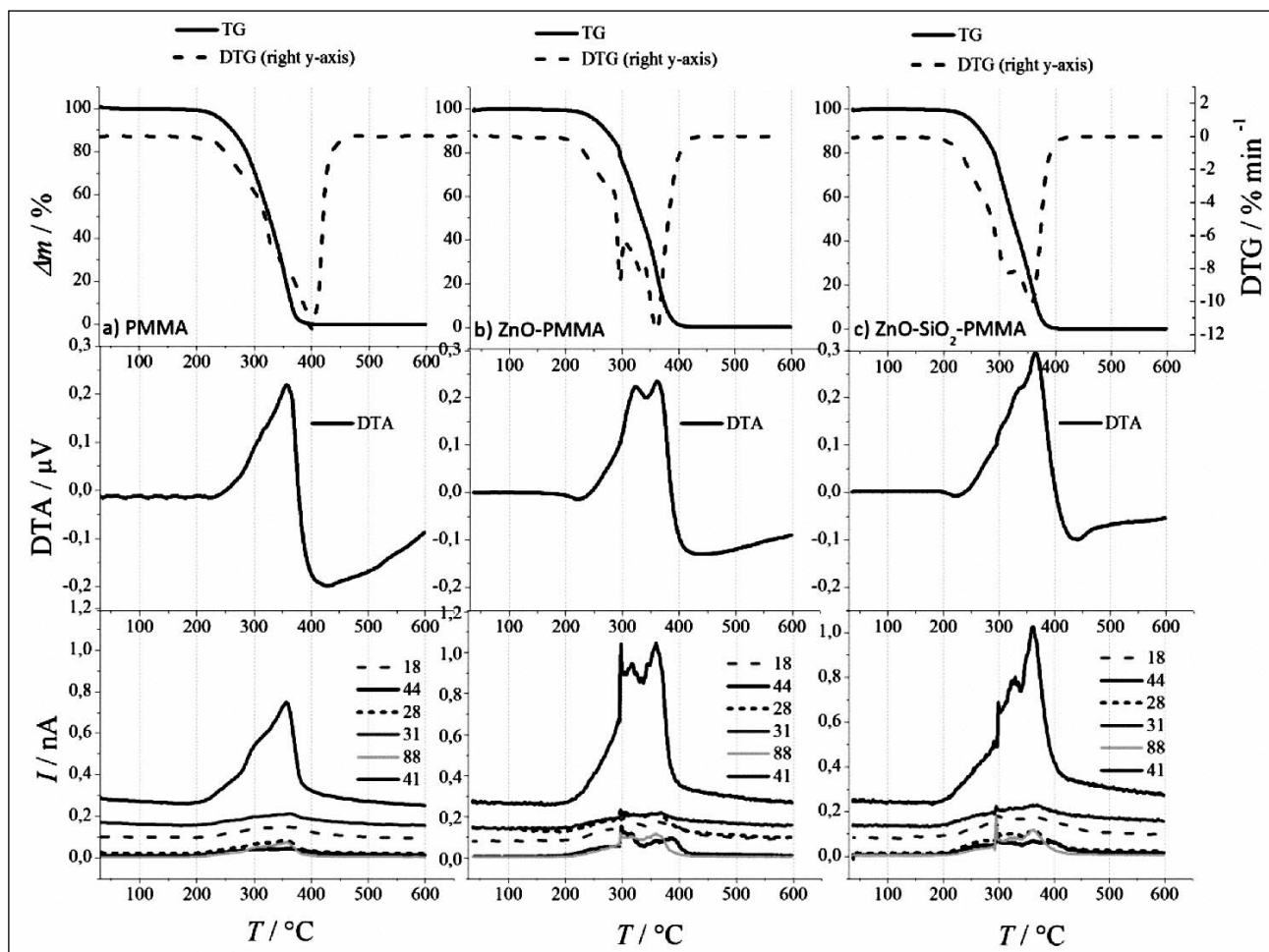


Figure 4. TG-DTG-DTA curves and QMS signals for thermal decomposition of samples A, B2 and C

The detected volatile products during the ZnO-PMMA decomposition suggest acid-base interaction between the ZnO surface and PMMA matrix. This interaction involves acidic electron-accepting OH groups at the ZnO surface and basic electron-donating carbonyl functional groups of PMMA. Such interaction between COO^- of PMMA and metal-oxide surface is accompanied by MeOH elimination and has been proposed for several metal oxides, including Al_2O_3 ,⁴⁰ $\text{Mg}(\text{OH})_2$,⁴¹ TiO_2 ,¹⁹ and ZnO.³³ Subsequently, at the ZnO surface, MA and PAME are generated by random chain scission.

In addition to the catalytic effect of ZnO nanoparticles during the early stages of PMMA thermal degradation, their stabilizing role must also be considered. It is evident from Fig. 5 that the small addition of ZnO or silica-coated ZnO nanoparticles into PMMA shifts the temperature region towards somewhat higher temperatures. Normally, the enhanced thermal stability of such a composite is explained by means of polymer chain mobility restriction due to steric hindrance caused by the presence of solid particles or simply by the fact that metal oxide particles have higher thermal conductivity and greater heat capacity

values than PMMA and thus absorb the heat transmitted from the surroundings and retard the direct thermal impact to the polymer backbone.^{42,43} Such physical causes for the improved thermal stability of the composite are also in accordance with the measured data. Specifically, the temperature regions of PMMA decomposition for samples B2 and C are practically identical. The only difference among these two samples, regarding their thermal stability, arises from already described catalytic behavior of ZnO around 295 °C. Above or below the region of ZnO catalytic activity, both TG curves progress identically, indicating the same impact on PMMA thermal degradation for ZnO and silica-coated ZnO nanoparticles.

Stabilizing or destabilizing effects of metal oxide addition into PMMA on the thermal degradation of the composite should have its reflection in degradation kinetic parameters. In order to determine apparent activation energy E_a of PMMA or PMMA-composite degradation, dynamic weight loss data at various β from the TG analysis were used. The results of E_a vs degree of conversion (α) relationship are shown in Fig. 6. It appears that the addition of 0.1% of solid nanoparticles (bare ZnO or coated

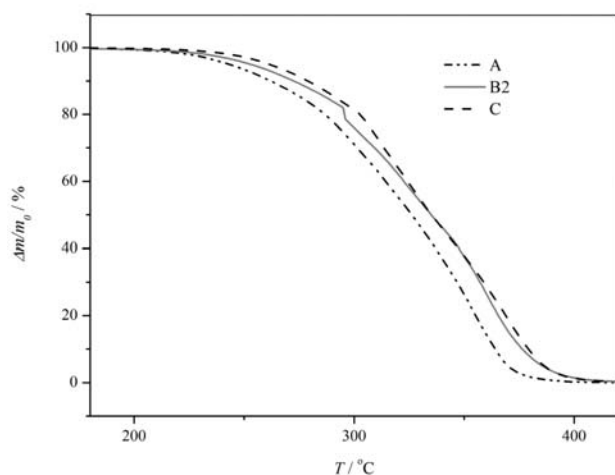


Figure 5. TG-DTG curves for a) filler free PMMA (Sample A), b) PMMA with the addition of nanosized ZnO (Sample B2) and c) PMMA with the addition of silica-coated nanosized ZnO (Sample C)

ZnO) raises E_a of the material's thermal degradation. Such a result is in accordance with the already described stabilizing effect during thermal degradation when solid particles are added into PMMA.^{30–32} However, by comparing the average E_a values for samples A–C, an interesting result is observed. The E_a of pure PMMA was calculated as 145 kJ/mol. If 0.1% bare ZnO is admixed into PMMA, the E_a of thermo-oxidative degradation increases to 155 kJ/mol. In contrast, if 0.1% silica-coated ZnO is added into PMMA, the E_a value further increases to 190 kJ/mol. Rather noticeable differences between the calculated E_a values of samples A–C indicate the double role of ZnO in the ZnO-PMMA composite. In addition to the stabilizing effect that increases the E_a value (comparison between samples A and B2), ZnO also acts catalytically toward PMMA degradation by considerably lowering the E_a value if sample B2 is compared to sample C.

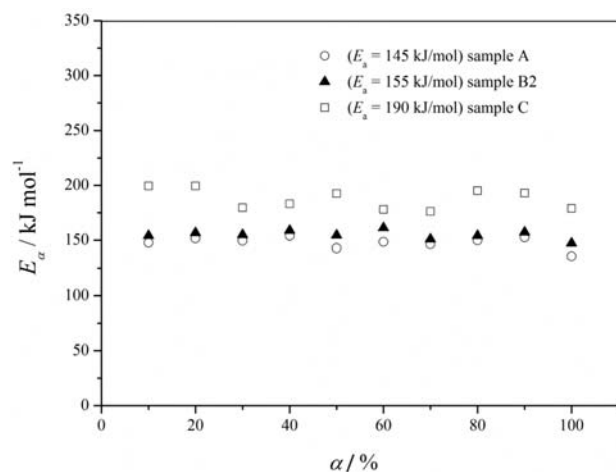


Figure 6. E_a vs α relationship for PMMA or PMMA-composites thermal decomposition

Since the addition of ZnO into PMMA simultaneously stabilizes and destabilizes the thermal stability of PMMA, a critical addition of ZnO was further systematically investigated in order to distinguish between these two opposite effects. For this purpose, a series of ZnO-PMMA composites were prepared with the addition of ZnO 0.05%, 0.10%, 0.15% and 0.20% (samples B1, B2, B3 and B4, respectively) and submitted to further thermal analysis. The TG curves of the B-series ZnO-PMMA composites are shown in Fig. 7. Evidently, any addition of ZnO into PMMA shifts the TG curves to higher decomposition temperature intervals with respect to pure PMMA. Therefore, 0.20% of ZnO in PMMA stabilizes thermal stability of the composite for ~25 °C by shifting the main decomposition peak from ~350 °C (pure PMMA) to ~375 °C (sample B4). Moreover, with increasing amounts of ZnO the additional TG step (at ~295 °C in the case of sample B2) slowly starts to form.

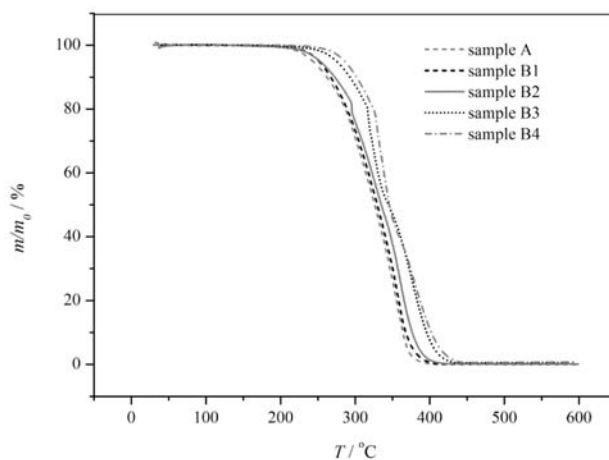


Figure 7. TG curves of PMMA and B-series of ZnO-PMMA composites with various addition of ZnO

When B-series samples are submitted to the Vyazovkin method of partial kinetic analysis, the E_a values are calculated (Fig. 8). It appears that the addition of 0.05% ZnO into PMMA (Sample B1) does not change the average E_a value of the composite thermal degradation relative to pure PMMA. The highest E_a 215 value kJ/mol was determined for sample B3 (0.15% ZnO), while further increased amount of ZnO again slightly lowered the E_a value to 190 kJ/mol (Sample B4). Such behavior may be explained by means of the stabilizing/catalytic effect of ZnO on the thermal stability of PMMA. With relatively small additions of ZnO, its stabilizing effect prevails over the catalytic activity. The smallest addition of ZnO into PMMA apparently represents sufficient steric hindrance for polymer chain mobility; however, the ZnO/PMMA interface simultaneously remains relatively low, not contributing significantly to the composite thermal degradation. The stabilizing effect reaches its

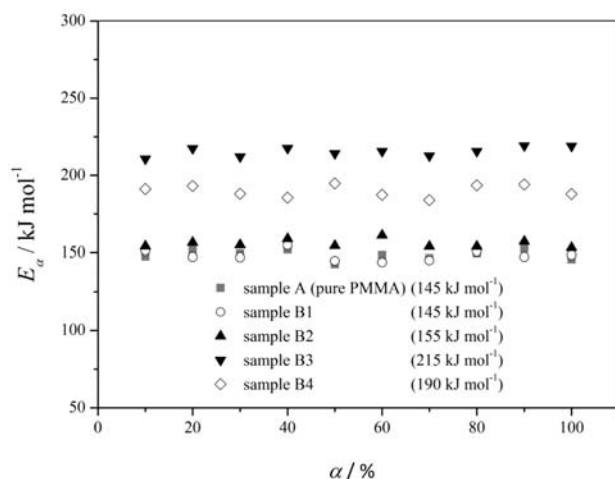


Figure 8. E_a vs α relationship for B-series ZnO-PMMA composites (PMMA is added for the comparison)

peak when 0.15% ZnO is dispersed throughout PMMA. In contrast, the catalytic effect of ZnO becomes significant above 0.15% due to the increased ZnO/PMMA interface.

Another important characteristic of ZnO-PMMA composites is their transparency for UV and visible light. For practical applications final material should absorb UV light but transmit visible light. As shown in Figure 9 pure PMMA (sample A) is transparent for UV and visible light. However, small addition of ZnO into PMMA (sample B2) practically completely absorbs UV light. At the same time the material remains transparent for visible light. Similar effect is achieved also when silica coated ZnO nanoparticles are added into PMMA (sample C). Such material likewise absorbs UV light due to ZnO content, whereas in the region of visible light silica-coated ZnO/PMMA nanocomposite exhibit only slightly lower transmittance than ZnO/PMMA composite.

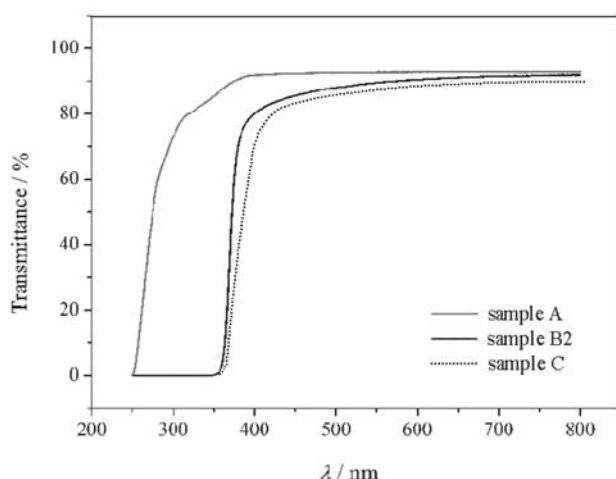


Figure 9. Light transmittance as a function of wavelength of pure PMMA (sample A), nanocomposite ZnO/PMMA (sample B2), silica-coated ZnO/PMMA (sample C)

4. Conclusion

The thermal properties of ZnO-PMMA nanocomposites are investigated via TG-DTA-EGA analyses. The filler-free PMMA thermally decomposes in one broad interval composed of at least two consecutive and partially overlaying steps. Volatile products of the decomposition are determined as H_2O , CO_2 , CO, MeOH, PAME and MA. All volatile products are evolved continuously throughout the degradation path.

By adding ZnO into PMMA, two opposite effects are recognized. Any addition of ZnO into PMMA thermally stabilizes the composite by delaying the occurrence of major polymer cracking and thus shifts the TG curves to higher decomposition temperatures. The stabilizing role of ZnO is explained by means of physical grounds, i.e. steric hindrance caused by ZnO for polymer chain mobility and heat absorption by ZnO through relatively higher thermal conductivity and greater heat capacity. In contrast, during the early stages of PMMA thermal decomposition, ZnO acts also catalytically by intensifying the decomposition rate. Through the detected volatile products during the ZnO/PMMA decomposition, the catalytic acid-base interaction between ZnO surface and PMMA matrix is suggested.

The critical addition of ZnO into PMMA, in which the catalytic effect prevails over the stabilizing effect due to increased ZnO/PMMA interface, is determined between 0.15–0.20% ZnO. Generally, the addition of ZnO does not change the nature of the decomposition process. Instead, it influences temperature intervals of intense PMMA degradation.

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Povzetek

Z uporabo termoanalitskih tehnik je bila določena vloga nanodelcev ZnO pri termični dekompoziciji polimetilmetakrilata (PMMA). Za študij sistema ZnO/PMMA smo uporabili tehnike TG, DTA, EGA, XRD, SEM in TEM. Nanodelci ZnO so bili sintetizirani s precipitacijsko metodo z dodajanjem LiOH v raztopino Zn^{2+} v mešanici topil H_2O in etilen-glikol. Nanokompoziti ZnO/PMMA so bili pripravljene z ustreznim dodatkom ZnO v MMA in kasnejšo radikalno polimerizacijo MMA. Glede na rezultate testov in kinetične izračune aktivacijske energije termičnega razpada PMMA, ima dodatek ZnO v PMMA dvojno vlogo. Dodatek ZnO v PMMA do 0,15% stabilizira kompozit, saj premakne temperaturno okno razpada kompozita k višjim vrednostim in hkrati zviša aktivacijsko energijo termičnega razpada PMMA. Obratno, dodatek ZnO nad 0,15% v ZnO/PMMA kompozitu na razpad PMMA deluje katalitsko, saj zniža aktivacijsko energijo termičnega razpada PMMA in hkrati premakne temperaturno okno razpada kompozita k nižjim vrednostim. Dodatek ZnO ne spremeni mehanizma razpada PMMA.

Scientific paper

Effect of Mercapto and Methyl Groups on the Efficiency of Imidazole and Benzimidazole-based Inhibitors of Iron Corrosion

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Abstract

We report on the combined experimental and computational study of imidazole- and benzimidazole-based corrosion inhibitors containing methyl and/or mercapto groups. Electrochemical measurements and long-term immersion tests were performed on iron in NaCl solution, whilst computational study explicitly addresses the molecular level details of the bonding on iron surface by means of density functional theory calculations (DFT). Experimental data were the basis for the determination of inhibition efficiency and mechanism. Methyl group combined with mercapto group has a beneficial effect on corrosion inhibition at all inhibitor concentrations. The beneficial effect of mercapto group combined with benzene group is not so pronounced as when combined with methyl group. The latter is in stark contrast with the behaviour found previously on copper, where the effect of methyl group was detrimental and that of mercapto and benzene beneficial. Explicit DFT calculations reveal that methyl-group has a small effect on the inhibitor–surface interaction. In contrast, the presence of mercapto group involves the strong S–surface bonding and consequently the adsorption of inhibitors with mercapto group is found to be more exothermic.

Keywords: Iron; corrosion, imidazole inhibitors, polarization resistance, X-ray photoelectron spectroscopy, density functional theory (DFT)

1. Introduction

The inhibition of corrosion using corrosion inhibitors has been for decades one of the most important method of corrosion protection. Inhibitors are usually organic compounds which when added in small concentrations form a surface layer that protects the underlying metal surface from dissolution and, consequently, decreases the corrosion rate. Among the most important inhibitors for copper are benzotriazole and also imidazole-based inhibitors. In this study we test the applicability of several imidazole-type molecules as corrosion inhibitors for iron in NaCl solution.

In our previous publications a combined experimental and computational study on copper was performed.^{1,2}

Electrochemical and immersion study, combined with topography and X-ray photoelectron spectroscopy (XPS) analyses, was carried out in chloride solution with and without the addition of various imidazole derivatives—imidazole (ImiH), 1-methyl-imidazole (ImiMe), benzimidazole (BimH), 2-mercapto-1-methyl-imidazole (SH-ImiMe), and 2-mercaptobenzimidazole (SH-BimH).¹ The skeleton structures of these inhibitors are presented in Fig. 1. Pronounced differences were observed at concentrations ≥ 1 mM. While 1-methyl-imidazole was found to be inferior in activity to imidazole, all other derivatives were superior. At 1 mM concentration the order of inhibition efficiency, IE, is: ImiMe < ImiH < BimH < SH-ImiMe < SH-BimH. The mercapto group and benzene group were shown to have a beneficial effect on corrosion inhibition,

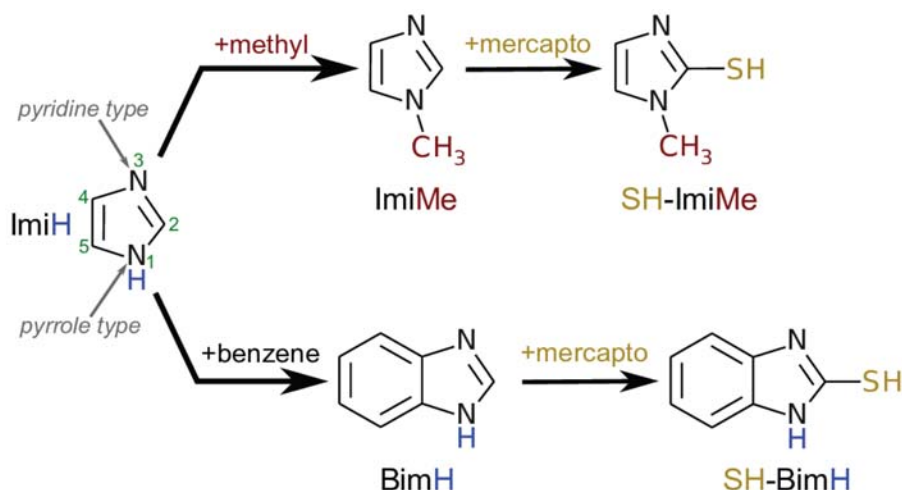


Figure 1. Skeleton structures of imidazole (ImiH), 1-methyl-imidazole (ImiMe), 2-mercapto-1-methyl-imidazole (SH-ImiMe), benzimidazole (BimH), and 2-mercaptobenzimidazole (SH-BimH). Pyridine- and pyrrole-type N atoms and the numbering of atoms are also indicated.

whereas the effect of the methyl group even accelerated the corrosion at 10 mM. The protective ability of BimH, SH-ImiMe and SH-BimH inhibitors is based on the formation of cuprous complexes with species originating from inhibitors, mainly carbon and nitrogen, and sulphur in the case of mercapto compounds.

Experimental study was supplemented by detailed density functional theory (DFT) calculations² to (i) explicitly characterize the interactions between inhibitors and copper surfaces and to (ii) rationalize the experimentally observed trend. We found that mercapto-substituted imidazoles are prone to dissociation upon adsorption (S–H or N–H bond cleavage). They bond stronger to the surface and display weaker tendency to form soluble complexes with hydrated Cu^{2+} ions than non-mercapto imidazoles. By encapsulating these two interactions into a simple model—the first interaction is deemed as beneficial and the second as detrimental—the inhibition efficiency trend was well captured.

Studies on iron are less common than on copper, especially in sodium chloride solution. Bhargava et al. tested different approaches – adding imidazole directly into the 3 % NaCl and direct deposition of inhibitor onto the metal surface prior the exposure to NaCl.³ The former procedure was beneficial which was explained by the interaction of imidazole's π -electron system with Fe such that its aromatic ring is nearly parallel to the metal surface. During direct deposition of inhibitor, however, also a pyridine type of N–Fe interaction is present with imidazole oriented normal to the surface, as indicated by XPS.³ 2-mercaptobenzimidazole (SH-BimH) was proved to be a good corrosion inhibitor for Armco iron in 3 % NaCl at a maximal performance at $5 \times 10^{-3} \text{ mol dm}^{-3}$.⁴ The SH-BimH inhibition mechanism proceeds according to the Langmuir-type adsorption on the active sites and suppressing the dissolution reactions. In slightly acidic solution

more studies have been performed than in neutral chloride solution.^{5–9} Combined electrochemical and computational HOMO/LUMO type study was performed for different imidazole-based inhibitors on iron in 1 M HCl.⁵ The inhibition efficiency increased with increasing electron donating ability being the highest for amino-containing inhibitors. Heterocyclic diazoles were also shown to be good inhibitors for iron in 1 M HCl.⁶ Three types of iron species were identified on the inhibited surface: FeOOH , Fe_2O_3 and Fe_3O_4 . It was suggested that good inhibition agent should form an insoluble complex or surface species with low hydroxide content. Different imidazoline compounds were more strongly adsorbed on steel surface than the amidic compounds.⁷ Another study was performed on carbon steel in 1 M HCl containing different imidazole-based inhibitors showing the following order of inhibition efficiency: $\text{BimH} > \text{ImiH} > \text{ImiMe}$.⁸ SH-BimH was shown to be adsorbed on film-free surface and the formation of oxide was ascribed to post-immersion oxidation.⁹ SH-ImiMe was tested on carbon steels in 1 M HClO_4 and confirmed by XPS analysis to be chemically adsorbed on steel surface.¹⁰

The present study on iron represents a comparative study to that on copper,^{1,2} as it is performed using the same inhibitors—ImiH, ImiMe, BimH, SH-ImiMe, and SH-BimH—under the same experimental conditions. The aim of the study is twofold: (i) to compare the efficiency of inhibitors when used on copper and on iron, and (ii) to reveal the differences in inhibition mechanisms of particular inhibitors when used on copper and on iron. The methodology remains the same: experimental study was performed using electrochemical potentiodynamic measurements and immersion testing in 3 wt.% sodium chloride solution combined with topography and XPS analyses, whilst computational study used DFT calculations to characterize the atomic scale details on the inhibitor-surface

interactions. As a starting point and for the sake of comparison with previous publications of ours^{2,11–14} and those of others,^{15,16} where the bonding of imidazoles with bare metal surfaces was characterized, we choose to elucidate the bonding of the current inhibitor molecules with the bare Fe(110), although the oxidized surface of iron is way more relevant in the context of corrosion under near-neutral pH conditions. Current calculations are therefore more relevant for the reduced patches at the surface, where the oxide film has been breached.¹⁷ Moreover, for obvious modelling reasons we also consider the adsorption at the metal/vacuum interface, although with respect to corrosion and its inhibition, the solid/water interface is far more appropriate. Current calculations are therefore to be taken only as a first crude attempt to address the inhibitor–surface bonding.^a

2. Technical details

2. 1. Experimental Study

2. 1. 1. Materials and Solutions

Corrosion tests were performed on iron metal (99.8%, temper as rolled) supplied by Goodfellow (Cambridge Ltd., UK) in the form of 2 mm thick foil. All iron specimens were cut from the foil in the shape of discs of 15 mm diameter. Using a circulating device the specimens were ground mechanically under a stream of water with successive SiC papers of gradations 500, 800, 1000, 1200, 2400, and 4000. The iron samples were then cleaned with acetone in an ultrasonic bath for three minutes, double-rinsed with distilled water and dried with nitrogen gas.

Samples were immersed in 3 wt.% NaCl aqueous solution with or without the addition of imidazole (ImiH), 1-methyl-imidazole (ImiMe), 2-mercapto-1-methylimidazole (SH-ImiMe), benzimidazole (BimH) or 2-mercaptobenzimidazole (SH-BimH) at various concentrations (0.1, 1, and 10 mM). Skeleton structures of the inhibitors are presented in Fig. 1. All five inhibitors were supplied by Sigma Aldrich (purity for ImiH 99.5%, ImiMe 99%, SH-ImiMe 99%, BimH 98%, and SH-BimH 98%), and NaCl by Carlo Erba (pro analysis). Compounds were used as supplied.

2. 1. 2. Electrochemical Measurements

Measurements were performed in a three-electrode corrosion cell (volume 0.25 L, Autolab, Ecochemie, Netherlands) at 25 °C. A saturated calomel electrode (SCE, 0.242 V with respect to the saturated hydrogen electrode at

25 °C) with a Luggin capillary was used as a reference electrode and carbon rods as the counter-electrode. A specimen embedded in a Teflon holder, with an area of 0.785 cm² exposed to the solution, served as the working electrode.

Measurements were carried out with a PGSTAT-12 Autolab (Metrohm Autolab, Utrecht, Netherlands) potentiostat/galvanostat operated by the NOVA software. Before measuring the polarization resistance (R_p) the iron specimens were allowed to stabilize under open circuit conditions. During that time, the open circuit potential was measured as a function of time. The stabilization process usually took 1 h. The stable, quasi-steady state potential reached at the end of the stabilization period is denoted as the corrosion potential (E_{corr}).

2. 1. 2. 1. Polarization Resistance Measurements

Polarization resistance (R_p) was measured over a potential range of ± 10 mV vs. E_{corr} using a scan rate of 0.1 mV/s. R_p values were deduced from the slope of the fitted potential vs. current density using NOVA software.

Measurements of R_p were repeated at least three times. From the average values the inhibition efficiency (IE), denoted by the symbol η , was calculated using the following equation:

$$\eta = \frac{R_p^{\text{inh}} - R_p^{\text{blank}}}{R_p^{\text{inh}}} \times 100 \quad (1)$$

where R_p^{inh} and R_p^{blank} are the polarization resistances measured with and without addition of inhibitor, respectively. The results are presented as mean value $\pm$ standard deviation.

2. 1. 2. 2. Potentiodynamic Measurements

Potentiodynamic measurements were performed starting at 250 mV negative to E_{corr} , then increased in the anodic direction at a potential scan rate of 1 mV/s.

2. 1. 3. Immersion Test

Iron specimens were hung in 100 mL closed glass vials containing 3 wt.% NaCl or 3 wt.% NaCl with the addition of 1 mM of either ImiH, ImiMe, SH-ImiMe, BimH or SH-BimH. After immersion for one month the specimens were rinsed with deionized water and dried with nitrogen gas.

2. 1. 4. X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) was performed with a TFA Physical Electronics Inc. spectrometer using non- and mono-chromatized Al K_{α} radiation (1486.6 eV) and a hemispherical analyzer. The mono-chromatized radiation used for high-resolution spectra yields a resolution of 0.6 eV, as measured on an Ag 3d_{5/2} peak. These spectra were used to differentiate between va-

^a It should be noted that solid/vacuum interfaces and metallic surfaces are conceptually and structurally simpler than the solid/water interfaces and oxidized surfaces. This is the basic premise behind our choice, which follows the reductionist divide-and-conquer approach, i.e., start with simpler system and elaborate later.

rious species, while those obtained using non-monochromatized radiation were used to quantify the chemical composition. The take-off angle used, defined as the angle of emission relative to the surface plane, was 45°. The energy resolution was 0.5 eV. Survey scan spectra were recorded at a pass energy of 187.9 eV, and individual high-resolution spectra at a pass energy of 23.5 eV with an energy step of 0.1 eV. The diameter of the analysed spot was 400 µm. During the analysis a small shift was observed and compensated by a neutralizer. The values of binding energies were then aligned to carbon peak C 1s at 284.8 eV.

Angle-resolved XPS (AR-XPS) measurements were conducted at take-off angles of 20, 45 and 75° with respect to the surface plane to obtain depth-dependent information on the composition and structure of the layers. The depth of analysis increases with increased angle, in practice up to 10 nm.¹⁸ Thus at small angles (20°) information is provided closer to the surface and, at large angles (75°), closer to the bulk.

2. 1. 5. Surface Topography

Surface morphology was inspected using an optical microscope Olympus BX51. Surface topography was analysed employing a profilometer, model Taylor Hobson, Form Talysurf Series 2 operated by Taylor Hobson Ultra software. The instrument has a lateral resolution of 1 µm and vertical resolution of 5 nm. The surface profile is measured in one direction. Measurements were performed on three locations of each sample using a 1 mm² spot. Data were processed with TalyMap Gold 6.2 software to create 3-D surface topography and to calculate the mean surface roughness (S_a). Corrections were made to exclude general geometrical shape and possible measurement-induced misfits.

2. 2. Computational Details

Spin-polarized DFT calculations were performed in the framework of generalized gradient approximation (GGA) of Perdew-Burke-Ernzerhof (PBE)¹⁹ using the plane-wave pseudo-potential method with ultra-soft pseudo-potentials^{20,21} as implemented in the PWscf code of the Quantum ESPRESSO distribution.²² Kohn-Sham orbitals were expanded in a plane-wave basis set up to a kinetic energy cutoff of 30 Ry (240 Ry for the charge-density cutoff).

Adsorption of molecules was modeled on densely-packed Fe(110) surface, which was modeled by periodic slabs consisting of four atomic layers (the structure of Fe(110) surface is shown in Fig. 2). The bottom layer was constrained to the bulk positions and the in-plane lattice spacing was fixed to the calculated equilibrium Fe bulk lattice parameter of 2.84 Å.^{11,23} All other degrees of freedom were relaxed. Molecules were adsorbed on one side of the slab and the thickness of the vacuum region—the di-

stance between the top of the ad-molecule and the adjacent slab—was set to about 20 Å. Molecular adsorption was modeled at 1/20 ML monolayer (ML) coverage using the (5 0) supercell.^b Dipole correction of Bengtsson²⁴ was applied to cancel an artificial electric field that develops along the direction normal to the slab due to periodic boundary conditions imposed on the electrostatic potential. Brillouin-zone integrations were performed with Gaussian-smearing²⁵ special point technique²⁶ using a smearing parameter of 0.03 Ry and a 2 × 2 × 1 k-point mesh. Molecular graphics were produced by the XCrysDen graphical package.²⁷



Figure 2. Structure of Fe(110) surface. Notice two different spacings between adjacent surface Fe atoms, termed short- and long-bridge. The short-bridge distance is $a_0\sqrt{3}/2$, whereas long-bridge distance is a_0 , where a_0 is the equilibrium bcc lattice parameter of Fe bulk. The angles between two short-bridges and between short-bridge and long-bridge are also indicated.

2. 2. 1. Definitions and Energy Equations

The term “dehydrogenation” will be used as jargon to indicate a reaction analogous to deprotonation ($\text{MolH} \rightarrow \text{Mol}^- + \text{H}^+$), but with a homolytic bond cleavage (this implies that radicals are considered in favour of charged ions), i.e., $\text{MolH} \rightarrow \text{Mol}^\cdot + \text{H}^\cdot$. The labels MolH, Mol[−], and Mol stand for neutral, deprotonated, and “dehydrogenated” inhibitor molecules, respectively (the term “dehydrogenated” designates—in analogy with “dehydrogenation”—a molecule stripped off one H atom);^c the symbol is used to indicate the radical character of isolated dehydrogenated molecule (sometimes Mol[·] will be designated simply as Mol).

The terms “non-mercapto” and “mercapto” designate the molecules without (ImiH, ImiMe, and BimH) or with the mercapto group (SH-ImiMe and SH-BimH), respectively; these shorthand labels for each inhibitor

^b Surface coverage in ML units is defined as the inverse of the number of surface Fe atoms per adsorbed molecule.

^c In contrast with the current usage of the term (removal of H atom), dehydrogenation is instead commonly defined as a reaction where the H_2 is removed from a molecule.

molecule are defined in Fig. 1. It should be noted that the two mercapto molecules can either exist in thiol or thione forms (see Fig. 3); the shorthand labels for thione form of SH-ImiMe and SH-BimH are S-ImiMeH and S-BimH₂, respectively, whereas dehydrogenated mercapto molecules are labeled as S – BimH· and S – BimMe· (or simply S-BimH and S-ImiMe). It should be noted that irrespective of the starting neutral tautomer form (thiol or thione) the resulting dehydrogenated thiolate form is the same.²

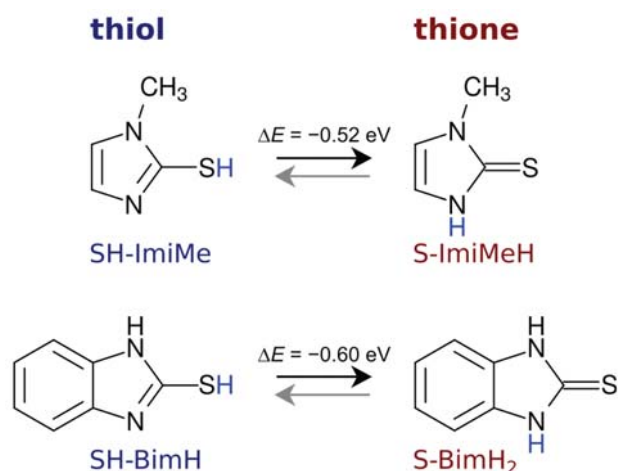


Figure 3. Skeleton structures of thiol and thione tautomers of 2-mercapto-1-methyl-imidazole (top) and 2-mercaptobenzimidazole (bottom). The thiol to thione transformation involves the shifting of blue colored hydrogen atom from the S to the N3 atom. Thiones are more stable than thiols and the corresponding energy differences, calculated at the PBE/plane-wave level of theory, are stated.²

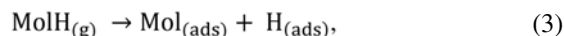
For mercapto molecules only the adsorption of thione tautomers is considered, because they are by about 0.5 eV more stable than thiols (as standalone) and moreover because current calculations indicate that the two thiols dissociate barrier-less or almost so (S–H bond cleavage) on Fe(110) during adsorption.

Dehydrogenated non-mercapto molecules will be designated as Imi, ImiMe^{w/o-H}, and Bim for imidazole, 1-methyl-imidazole, and benzimidazole, respectively. Dehydrogenated molecules will be sometimes designated as Mol_X, where X indicates at which atom the X–H bond was cleaved.

The non-dissociative adsorption energy was calculated as:

$$E_{\text{ads}} = E_{\text{MolH/slab}} - (E_{\text{MolH}} + E_{\text{slab}}), \quad (2)$$

where E_{MolH} , E_{slab} , and $E_{\text{MolH/slab}}$ are the total energies of isolated molecule, bare Fe(110) slab, and molecule/Fe(110) slab system, respectively. Considered molecules can dissociate on iron surface, hence the dissociative chemisorption was also considered, i.e.:



and the respective adsorption energy was calculated as:

$$E_{\text{ads}}^{\text{diss}} = E_{\text{Mol/slab}} + E_{\text{H/slab}} - (E_{\text{MolH}} + 2E_{\text{slab}}) \quad (4)$$

where $E_{\text{Mol/slab}}$ and $E_{\text{H/slab}}$ are the total energies of the Mol/Fe(110) and H/Fe(110) slab systems, respectively. For mercapto molecules, the E_{ads} and $E_{\text{ads}}^{\text{diss}}$ are calculated with respect to isolated thiones as a reference state for the E_{MolH} . Note that Mol–Fe(110) bond is much stronger than indicated by the $E_{\text{ads}}^{\text{diss}}$ because the latter involves the cost for breaking the X–H bond (X = N1 or C2). In particular, the binding energy between the dehydrogenated molecule and the surface can be calculated as:

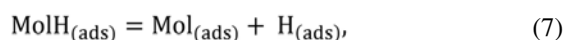
$$E_{\text{b}} = E_{\text{mol/slab}} - (E_{\text{slab}} + E_{\text{Mol}}) \quad (5)$$

where E_{Mol} is total energy of isolated Mol[·] radical. The relation between the E_{b} and $E_{\text{ads}}^{\text{diss}}$ is the following:

$$E_{\text{b}} = E_{\text{ads}}^{\text{diss}} + E_{\text{bond}}^{\text{X-H}} - E_{\text{b}}^{\text{H}}, \quad (6)$$

where E_{b}^{H} is the binding energy of atomic hydrogen onto Fe(110) and $E_{\text{bond}}^{\text{X-H}}$ is the bonding energy of dehydrogenated X–H bond, $E_{\text{bond}}^{\text{X-H}} = E_{\text{MolH}} - (E_{\text{Mol}} + E_{\text{H}})$. Because the $E_{\text{bond}}^{\text{X-H}}$ is significantly more exothermic than E_{b}^{H} also the E_{b} is significantly more exothermic than $E_{\text{ads}}^{\text{diss}}$; the calculated E_{b}^{H} on Fe(110) is –2.94 eV,¹¹ whereas the calculated N–H bonding energies are in range from –3.7 to –4.5 eV and C–H bonding energies are about –5.1 eV for the current molecules.

Dehydrogenation reaction energy (ΔE_{deh}) is calculated by considering the following reaction:



hence:

$$\begin{aligned} \Delta E_{\text{deh}} &= E_{\text{Mol/slab}} + E_{\text{H/slab}} - E_{\text{MolH/slab}} - E_{\text{slab}} \\ &= E_{\text{ads}}^{\text{diss}} - E_{\text{ads}}, \end{aligned} \quad (8)$$

where $E_{\text{ads}}^{\text{diss}}$ is calculated by Eq (4) and E_{ads} by Eq (2).

3. Results and Discussion

3.1. Experimental Results

3.1.1. Experimental Determination of Inhibition Efficiency of Imidazole and Benzimidazole Derivatives

The inhibitory action of the ImiH, ImiMe, SH-ImiMe, BimH, and SH-BimH inhibitors against corrosion of iron in 3 wt.% NaCl solution was analysed by means of

polarization resistance (R_p), by potentiodynamic curves and by one-month immersion testing.

3. 1. 1. Polarization Resistance Measurements

Iron in uninhibited sodium chloride solution exhibited a mean R_p of $797 \Omega \text{ cm}^2$ (Table 1). Polarization resistance was then measured at 0.1, 1 and 10 mM concentrations of inhibitors. In the presence of inhibitor the values increased, for example from $868 \Omega \text{ cm}^2$ for ImiH at 1 mM to a maximum of $2851 \Omega \text{ cm}^2$ for ImiMe at 10 mM (Table 1).

Table 1. Values of polarization resistance, R_p , measured for iron in 3 wt% NaCl solution with and without addition of 0.1, 1 and 10 mM of imidazole (ImiH) and its derivatives: 1-methyl-imidazole (ImiMe), 2-mercapto-1-methyl-imidazole (SH-ImiMe), benzimidazole (BimH), and 2-mercaptobenzimidazole (SH-BimH). Polarization resistance is given as mean value $\pm$ standard deviation. For SH-BimH, the 10 mM concentration could not be prepared due to its low solubility in water.

Solution	Inhibitor concentration (mM)	Polarization resistance ($\Omega \text{ cm}^2$)
NaCl	–	797 ± 56
+ ImiH	0.1	662 ± 41
	1	868 ± 48
	10	2483 ± 302
+ ImiMe	0.1	787 ± 38
	1	2586 ± 64
	10	2766 ± 143
+ SH-ImiMe	0.1	1030 ± 505
	1	1861 ± 255
	10	2851 ± 651
+ BimH	0.1	910 ± 93
	1	1530 ± 791
	10	1340 ± 555
+ SH-BimH	0.1	1107 ± 323
	1	1351 ± 251

Based on the measured R_p values the IE values were calculated according to Eq (1) and are presented in Fig. 4. ImiH showed measurable IE only at concentration of 1 mM, and reached 68% at 10 mM (Fig. 4a). For ImiMe, however, inhibition was high already at 1 mM; the value at 10 mM was similar as for ImiH. When in addition to methyl group, also mercapto group was present in SH-ImiMe, the inhibitor was weakly effective already at 0.1 mM and IE increased linearly with increasing concentration. At 10 mM, similar values were obtained for all three inhibitors (Fig. 4a). This behaviour is different to that observed for copper,¹ where at concentrations above 1 mM the ImiMe acted as corrosion accelerator, and SH-ImiMe achieved 65% IE.

When adding BimH, low but measurable IE of 13% was obtained already the 0.1 mM. IE peaked at 1 mM and then decreased at higher concentration (Fig. 4b). In the case of copper,¹ BimH was the most efficient inhibitor at 10 mM. When using both mercapto-based imidazole inhibitors the IE values were larger already at low inhibitor concentration of 0.1 mM (Fig. 4). For SH-ImiMe, the IE ranged between 23% and 72% (Fig. 4a). SH-BimH behaved similar as BimH and reached smaller IE than SH-ImiMe (Fig. 4b); however, 10 mM solution of SH-BimH could not be prepared due to its low solubility in water ($< 0.1 \text{ g}/100 \text{ mL} = 6.7 \text{ mM}$ at 23.5°C).²⁸

According to the polarisation resistance, the efficiency of inhibitors is as follows. At 0.1 mM only mercapto-based inhibitors are useful as corrosion inhibitors but show relatively low IE of 23 and 28% for SH-ImiMe and SH-BimH, respectively. At 1mM the following order was observed: ImiMe > SH-ImiMe > BimH ~ SH-BimH >> ImiH. Thus, methyl-derivative of imidazole acts as the most efficient corrosion inhibitor at 1 mM. At 10 mM all inhibitors without benzene group show rather similar IE values (between 64 and 78%).

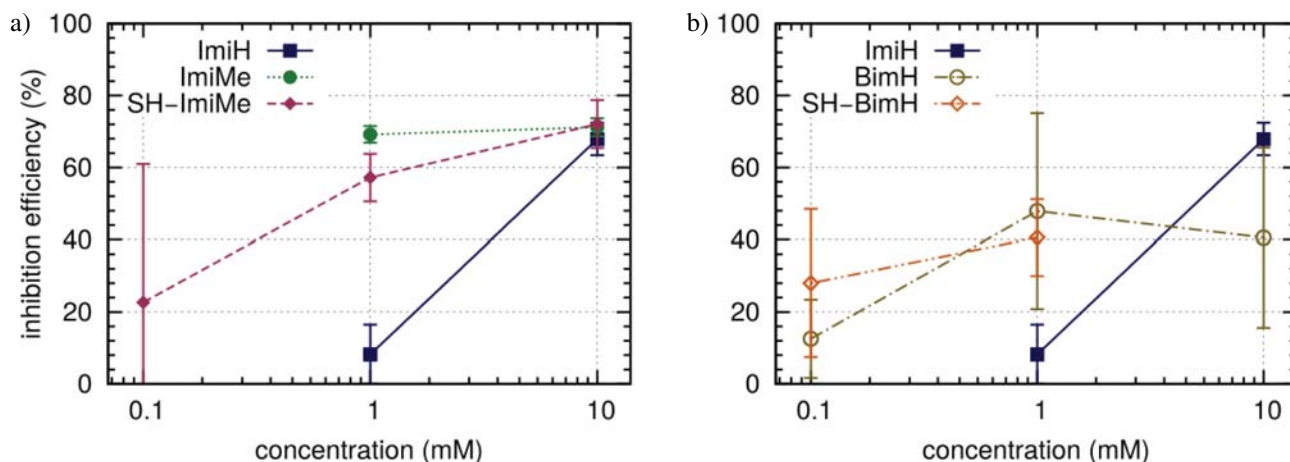


Figure 4. Inhibition efficiency against corrosion of iron in 3 wt.% NaCl solution of imidazole (ImiH) compared to that of (a) 1-methyl-imidazole (ImiMe) and 1-methyl-2-mercapto-imidazole (SH-ImiMe), and (b) benzimidazole (BimH) and 2-mercaptobenzimidazole (SH-BimH) as a function of inhibitor concentrations (0.1, 1, and 10 mM). Lines are drawn to guide the eye. ImiH and ImiMe do not inhibit corrosion at 0.1 mM inhibitor concentration, whereas the solubility of SH-BimH is below 10 mM.

3. 1. 1. 2. Potentiodynamic Polarization Curves

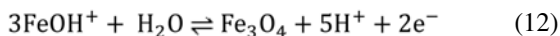
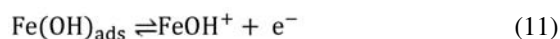
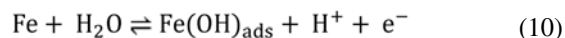
The results of the potentiodynamic measurements obtained for iron in 3 wt.% NaCl solution with and without addition of 0.1, 1, and 10 mM concentrations of ImiH, ImiMe, SH-ImiMe, BimH, and SH-BimH are presented in Fig. 5.

The cathodic reaction in NaCl solution is the reduction of oxygen:



The passivation of iron proceeds in two stages, i.e. a lower oxidation state film of Fe_3O_4 is required, and this film is highly susceptible to chemical dissolution.²⁹ Until the conditions are established whereby the Fe_3O_4 phase can exist on the surface for a reasonable period of time, the $\gamma\text{-Fe}_2\text{O}_3$, which is responsible for full passivation of iron, will not form.²⁹ Therefore, active dissolution of iron will continue. It is generally accepted that the active dissolution of iron occurs via an oxide intermediate, possible $\text{Fe}(\text{OH})_{2,\text{ads}}$ or $\text{Fe}(\text{OH})_2$, which is not a three-dimensional oxide phase.³⁰ At sufficiently high potentials, the conversion of this oxide intermediate into a true three-dimensional passive oxide is favoured over its dissolution.²⁹

The kinetics of dissolution of iron in the active range in the presence of halide ions like chloride ions is largely dominated by competitive adsorption of Cl^- with the OH^- ions:³



At potentials close to E_{corr} the anodic reaction is under mixed charge transfer and mass transport control (rate of movement of iron complex away from the surface to bulk electrolyte) while at higher anodic potentials (above -0.4 V) the anodic reaction is under diffusion control leading to the establishment of current plateau at high current density. Therefore, under the conditions of chloride solution iron is not passivated but continues to dissolve.

Addition of 0.1 mM concentration of either ImiH, ImiMe, SH-ImiMe, BimH, or SH-BimH in 3 wt.% NaCl solution induces a slight decrease in the cathodic current density and a shift of the E_{corr} value to somewhat more positive values (Fig. 5a), indicating increased resistance to general iron corrosion. Curves for all inhibitors are rather similar. The effect of inhibitor type becomes more pronounced at 1 mM concentration (Fig. 5b), especially for SH-ImiMe, BimH and SH-BimH. The current density in the anodic part of the curve is largely reduced at potentials more positive than -0.6 V indicating suppressed iron dissolution. Compared to R_p values obtained in the range around E_{corr} , where ImiMe showed larger values (Fig. 4), mercapto-based inhibitors exhibited better inhibitive ef-

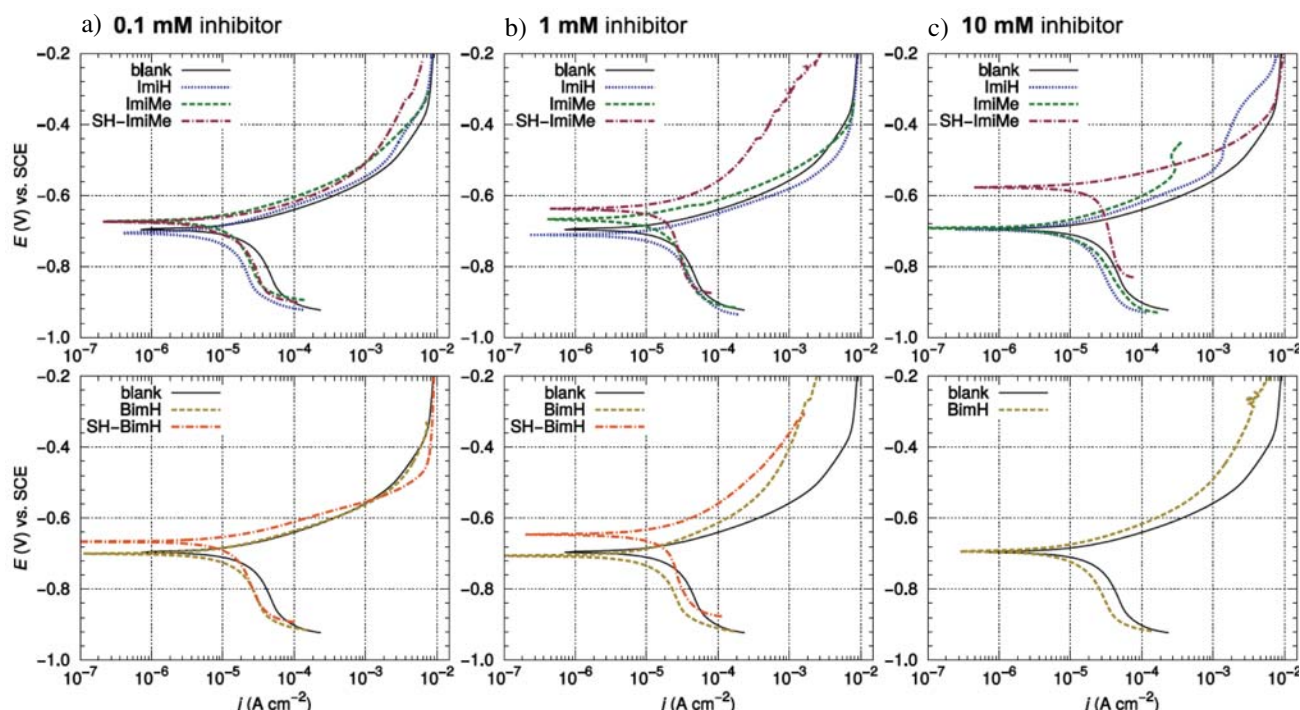


Figure 5. Potentiodynamic curves recorded for iron in 3 wt.% NaCl solution, without and with addition of (a) 0.1 mM, (b) 1 mM, and (c) 10 mM of either imidazole (ImiH), 1-methyl-imidazole (ImiMe), 1-methyl-2-mercapto-imidazole (SH-ImiMe), benzimidazole (BimH), or 2-mercaptobenzimidazole (SH-BimH) inhibitor. Curves for imidazole-based inhibitors (ImiH, ImiMe, SH-ImiMe) are shown in upper panels and those of benzimidazole-based inhibitors (BimH, SH-BimH) in bottom panels. $dE/dt = 1$ mV/s.

fect in the anodic range of the polarization curve. At 10 mM inhibitor concentration a current peak at about -0.5 V followed by a current density plateau appeared in the anodic branch in the presence of ImiH and ImiMe (Fig. 5c). For other inhibitors the increase in concentration does not seem to bring significant improvements in the potentiodynamic curves.

These results indicate that the inhibitors tested on iron act primarily as anodic inhibitors, i.e. affect primarily the anodic reaction. The changes in the cathodic branches are not so pronouncedly affected by the addition of inhibitor.

3. 1. 2. Immersion Test

The efficiency of corrosion inhibitors was further examined by a long-term immersion test. Iron samples were immersed for 30 days in 100 mL solutions of 3 wt.% NaCl containing 1 mM concentrations of either ImiH, ImiMe, SH-ImiMe, BimH, or SH-BimH. The resulting macro- and microscopic images of iron samples rinsed and dried after the immersion tests are shown in Fig. 6. In addition, values of mean surface roughness, S_a , are denoted for each image. Iron samples before and after immersion in uninhibited NaCl solution are taken a control.

The macroscopic images of the samples largely differed: Fe sample in uninhibited solution was covered by an uneven light-grey and dark-green layer, probably corresponding to iron oxides. ImiH sample was dark grey,

whilst ImiMe and BimH samples were light grey, giving a metallic appearance. For uninhibited and ImiMe samples the surface reveals grain structure of the substrate whilst other samples seem to be covered by coating layers. Both mercapto-containing samples were much darker than other samples, the coating appeared thicker, and contained some areas of lighter deposits.

The S_a values, measured after the immersion, revealed large differences between the samples (Fig. 6). For ImiH, ImiMe and SH-BimH the S_a values were smaller than that of control Fe sample immersed in NaCl ($0.61 \mu\text{m}$), whilst for BimH and, especially, SH-ImiMe the values were larger.

3. 1. 3. Chemical Composition and Speciation of Layers

3. 1. 3. 1. Chemical Composition

After immersion in NaCl solution for 30 days, the layer contained 18.2 at.% Fe and 45.4 at.% O, consistent with the formation of an oxide layer (Table 2). Carbon was present in 35.2 at.%, ascribed to adventitious carbon. In our previous study performed on copper,¹ the copper concentration at the surface decreased when inhibitor was present due to the formation of inhibitor layer. In the case of iron, however, the behaviour is different and the concentration of Fe is not decreased in the presence of inhibitor. Similar behaviour was observed for oxygen which

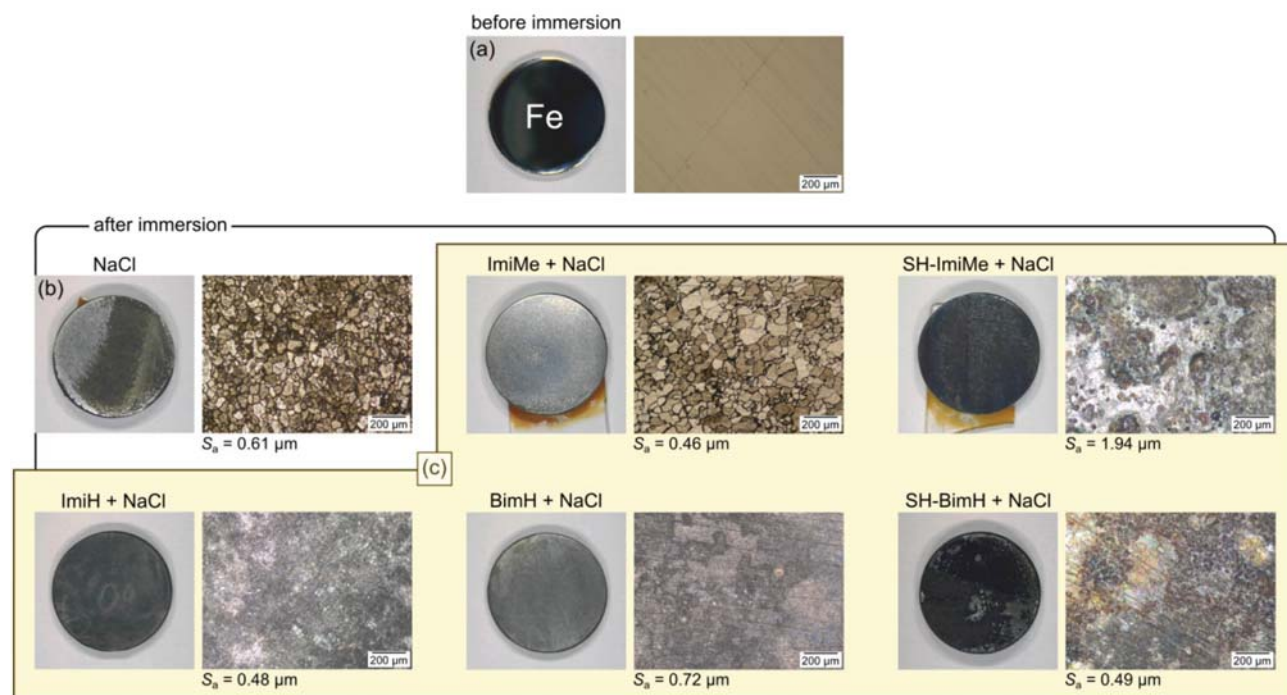


Figure 6. Macroscopic images and optical microscopy images of iron sample (a) before immersion and after 30 days immersion in 3 wt.% NaCl solution (b) without and (c) with the addition of 1 mM of either imidazole (ImiH), 1-methyl-imidazole (ImiMe), benzimidazole (BimH), 1-methyl-2-mercapto-imidazole (SH-ImiMe), or 2-mercaptobenzimidazole (SH-BimH). After immersion the specimens were rinsed with deionized water and dried with stream of nitrogen. The values of mean surface roughness, S_a , as obtained by profilometer, are also stated.

persisted in relatively high concentration at the surface even in the presence of inhibitor. Further, the concentration of carbon in the layer formed on copper in inhibitor solutions reached more than 60 at %; in the case of iron the concentrations remained similar as in uninhibited sample (Table 2). A significant difference between inhibited and uninhibited iron surfaces is the presence of sulphur and nitrogen for mercapto-based compounds which indicates the bonding of the inhibitors to the surface as these elements originate from inhibitor compounds. Again, compared to copper, the concentrations are 2 to 4-times smaller for iron. Another difference exists compared to inhibited copper: relatively small concentration of chlorine was detected at the iron surface only in the presence of BimH; in contrast, chlorine was detected in much higher concentrations (6.8 and 9.3 at%) when Cu sample was immersed in ImiMe and ImiH inhibited solution, respectively, which were less protective than cuprous compounds.¹

Although the general chemical composition of inhibited and uninhibited iron samples is quite similar, chemical speciation based on the high resolution spectra (Figs. 7 and 8) will show that the chemical environment changed in the presence of inhibitor.

3. 1. 3. 2. Chemical Speciation

The chemical composition of the layers formed during 30-day immersion in NaCl solution with and without the addition of inhibitors was further studied by high-resolution spectra aiming to identify the chemical environment of elements and to relate particular chemical species to each other. First the metal peak is considered. The X-ray photoelectron spectra of iron and its oxides is well known.^{6,31} Due to unpaired electrons in the valence band, iron compounds show a complex structure related to the multiplet splitting.³² The center of main Fe 2p_{3/2} peak in metallic iron is located at binding energy, E_b , 706.8 eV, whereas the second peak of lower intensity is located at 711.4 eV. In the case of Fe(II) oxide the multiplet splitting induces the appearance of peaks at 709.8 eV and 711.9 eV. Additionally, Fe(II) oxide shows the shake-up satellite at 715.0–715.5 eV, i.e. ~5.5–6.0 eV above the main peak.

In the case of Fe(III) oxide two peaks at 710.9 eV and 712.7 eV are observed due to multiplet splitting. The shake-up satellite is located ~8.5 eV above the main peak and is thus already in the range of Fe 2p_{1/2} peak. Therefore, several peaks in the Fe 2p XPS spectrum overlay in the range between ~710 eV and ~713 eV making the quantitative analysis complicated and unreliable without using appropriate standards.³¹ High resolution XPS spectra will be therefore addressed in a qualitative manner due to the complexity and large variety of species.

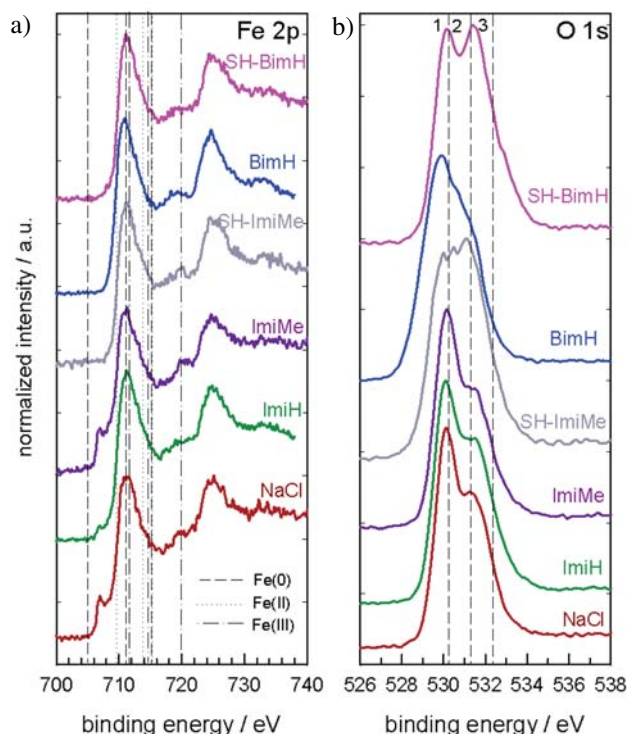


Figure 7: Normalized high resolution (a) Fe 2p and (b) O 1s spectra for layers formed during 30 days of immersion of iron in 3 wt.% NaCl solution with and without the addition of 1 mM imidazole (ImiH), 1-methyl-imidazole (ImiMe), 1-methyl-2-mercapto-imidazole (SH-ImiMe), benzimidazole (BimH) and 2-mercaptobenzimidazole (SH-BimH). The take-off angle is 45. Vertical dotted lines denote the position of peaks of reference compounds: (a) dashed: Fe(0), dotted: Fe(II) oxide, dash-dotted: Fe(III) oxide, (b) 1: O²⁻, 2: OH⁻, 3: H₂O.

Table 2. Chemical composition of the iron surface after 30 days immersion in 3 wt.% NaCl with and without addition of 1 mM imidazole (ImiH) and its derivatives: 1-methyl-imidazole (ImiMe), 2-mercapto-1-methyl-imidazole (SH-ImiMe), benzimidazole (BimH), and 2-mercaptobenzimidazole (SH-BimH). The composition was derived from XPS survey spectra.

Element	Composition (atomic %)					
	NaCl	+ ImiH	+ ImiMe	+ SH-ImiMe	+ BimH	+ SH-BimH
Fe	19.3	20.2	42.9	20.4	30.1	20.6
O	45.4	52.6	18.4	45.0	53.2	44.7
C	35.3	23.7	38.7	25.0	14.9	26.9
Cl	—	—	—	—	1.9	—
N	—	—	—	2.1	—	1.4
S	—	—	—	4.6	—	6.4
Si	—	3.5	—	2.9	—	—

Considering the Fe 2p spectra recorded after immersion (Fig. 7a) several features are observed: (i) peak related to metallic iron at 706.8 eV appears only at the surface of Fe immersed in uninhibited NaCl solution and in solutions inhibited by ImiH and ImiMe. In the latter two samples the layers formed during immersion were obviously not thick enough to prevent the metal signal to be identified, or iron is subjected to continuous dissolution revealing metal sites. For other inhibitors the metal peak could not be identified indicating full coverage of the metal surface by the layer. The most intense peak in the spectra was for all samples located at ~ 711 eV indicating the formation of mixed Fe(II) and Fe(III) oxides. Two satellite peaks are characteristic of iron oxides – at ~ 715 eV for Fe(II) oxide and at ~ 721 eV for Fe(III) oxide. Both peaks are observed in experimental spectra (Fig. 7a).

The peak for oxygen can be resolved into three component peaks, ascribed to oxide, hydroxide and adsorbed water (denoted by lines 1–3 in Fig. 7b). In all the cases there were two peaks: the first located at ~ 530 eV ascribed to iron oxide, and the second at 531.3 eV ascribed to hydroxide component. The intensity of oxygen peak was strong even in the presence of inhibitors (Table 2) proving that the inhibitor layer consists primarily of oxide, presumably mixed with inhibitor species, or that the inhibitor layer formed was not thick enough to hide the presence of underlying oxide.

Carbon C 1s, nitrogen N 1s and sulphur S 2p peaks are important for understanding the chemical composition and environment of layers formed in chloride solutions containing organic inhibitors. In the present work the centre of the C 1s peak was located at 285.0 eV for both inhibitor-free and inhibitor containing solutions (Fig. 8a). This peak is assigned to carbon bonded to carbon or hydrogen (C–C, C–H). In imidazole-based inhibitors other bonds should be taken into account (denoted by lines 1–5 in Fig. 8a): carbon bonded to pyrrole nitrogen (C–N) at 286.1 eV, and carbon bonded to pyridine nitrogen (C=N) at 287.5 eV.¹ Bonding between carbon and oxygen is also possible: the centres of the C–O and C=O peaks are located at mean E_b values of 286.5 eV and 288.4 eV, respectively. In mercapto compounds the C–S should be also taken into account but no specific peak related to C–S bonding was defined in literature.

In inhibitor-free chloride solution the peak at 285.0 eV was narrow indicating that C–C and C–H bonding originating from adventitious carbon is the prevailing bonding in carbon peak. In solutions containing inhibitor the broadening of the carbon peak width occurred (Fig. 8a). The broadening is related to the increasing contribution of different carbon species bonded to other elements, i.e. nitrogen, oxygen and, presumably, sulphur, as described above. Layers formed in the BimH, SH-ImiMe and SH-BimH solutions show another peak at 286.1 eV. As in these layers nitrogen

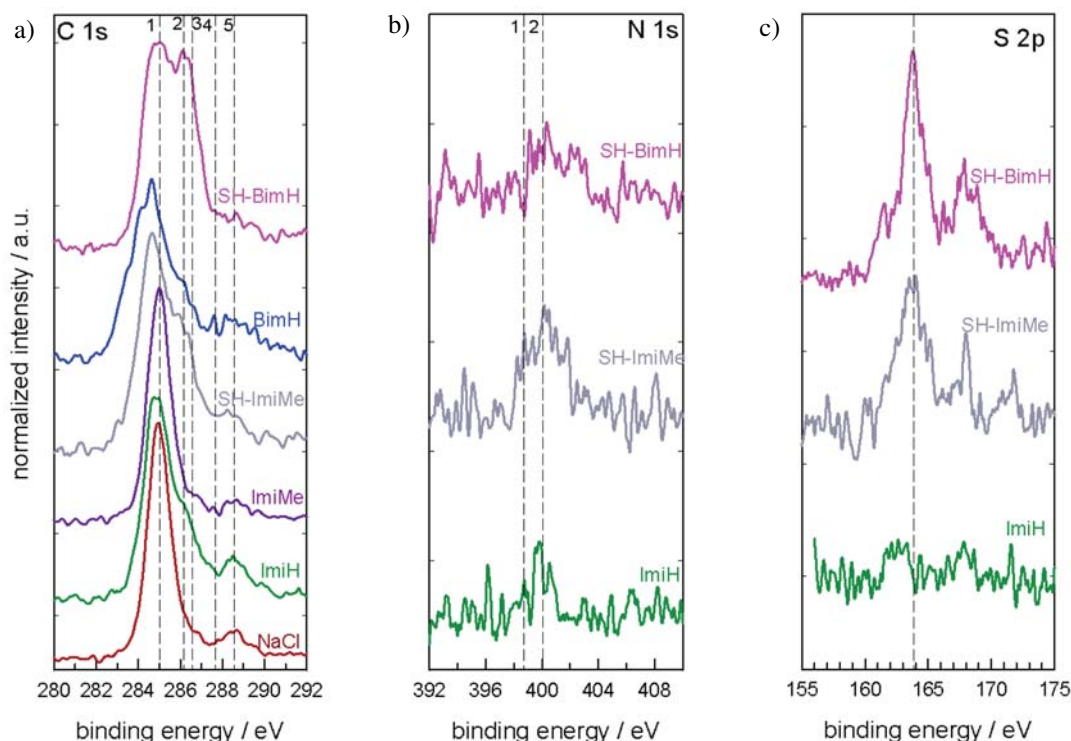


Figure 8: Normalized high resolution (a) C 1s, (b) N 1s and (c) S 2p XPS spectra for layers formed during 30 days of immersion of iron in 3 wt.% NaCl solution with and without the addition of 1 mM imidazole (ImiH), 1-methyl-imidazole (ImiMe), 1-methyl-2-mercapto-imidazole (SH-ImiMe), benzimidazole (BimH) and 2-mercaptobenzimidazole (SH-BimH). The take-off angle is 45. Vertical dotted lines denote the position of peaks of reference compounds: (a) 1: C–C, C–H, 2: C–N (C–S), 3: C–O, 4: C=N, 5: COO[−], (b) 1: C=N–C, 2: C–NH–C, and (c) S 2p_{3/2}/S 2p_{1/2}.

and sulphur are detected (Table 2), this peak can be related to the C–N and presumably C–S bonding.

Nitrogen was present only at the surface of the layer formed in a solution containing mercapto-based imidazoles (Fig. 8b, Table 2); for comparison, spectrum recorded in the layer formed in ImiH solution is given. In the case of copper, nitrogen was identified in much higher concentrations in layers formed in the presence of all inhibitors except ImiH.¹ This is the main difference between inhibited iron and copper. A weak nitrogen 1s peak at iron surface is centred at 400 eV, preceded by a smaller peak at 398.8 eV. The presence of two peaks may be ascribed to the presence of single and double bonds, C–N and C=N.

The sulphur S 2p spectrum comprises S 2p_{3/2} and 2p_{1/2} peaks that differ by only 1.1 eV and are not usually differentiated as two separate peaks. In the layers formed in the presence of mercapto-based inhibitors a single peak was formed, centred at 163.8 eV (Fig. 8c), in agreement with values reported for complexes formed between mercapto compounds and metals between 162.2 eV and 164.4 eV.¹ For comparison, spectrum recorded in the layer formed in ImiH solution is given.

Chlorine peak was detected at low intensity only at the surface exposed to benzimidazole and may be related to adsorption of chloride ions at the surface and/or formation of oxychloride compound with a low chlorine content.

3. 1. 4. Structure of the Layers

Angle-resolved XPS analysis was performed on uninhibited and inhibited iron surfaces. In contrast to copper, where due to intense nitrogen and sulphur XPS peaks such analysis brought about important conclusions regarding the structure of the layer, in the case of iron the com-

parative analysis did not provide sufficient data to postulate a specific structure of the inhibitor layer. Several features were observed: (i) in Fe 2p spectra the metal part at 706.8 eV decreased compared to oxide part at ~711 eV indicating that the latter is enriched at the outer surface of the layer, and (ii) in O 1s spectra the hydroxide part at 531.3 eV also increased compared to oxide part at ~530.0 eV indicating the surfaces is enriched in hydroxide species (results not shown). For C 1s no significant difference were observed depending on the analysing angle, whilst for N 1s and S 2p spectra the intensity of the signal was too low to allow conclusive angle-resolved analysis. Based on these results it can be stated that the layer formed on iron in inhibited solution is mainly iron oxide which contains species originating from inhibitor molecules, i.e. mixed oxide-inhibitor layer.

3. 2. Computational Results

In this section the adsorption bonding of inhibitor molecules to Fe(110), as scrutinized with DFT calculations, is described. For each molecule several different adsorption modes were considered and below only the most stable identified mode per molecule and per adsorption type is considered. Iron surfaces are chemically reactive enough to disturb the molecular π system and to break molecular X–H bonds.^{2,12} Hence, considered molecules can chemisorb to Fe(110) as standing up or lying down in neutral (non-dissociative) or dehydrogenated (dissociative) forms.

3. 2. 1. Adsorption Bonding of Neutral Molecules

In this section the chemisorption bonding of neutral (intact) inhibitor molecules is described. Optimized struc-

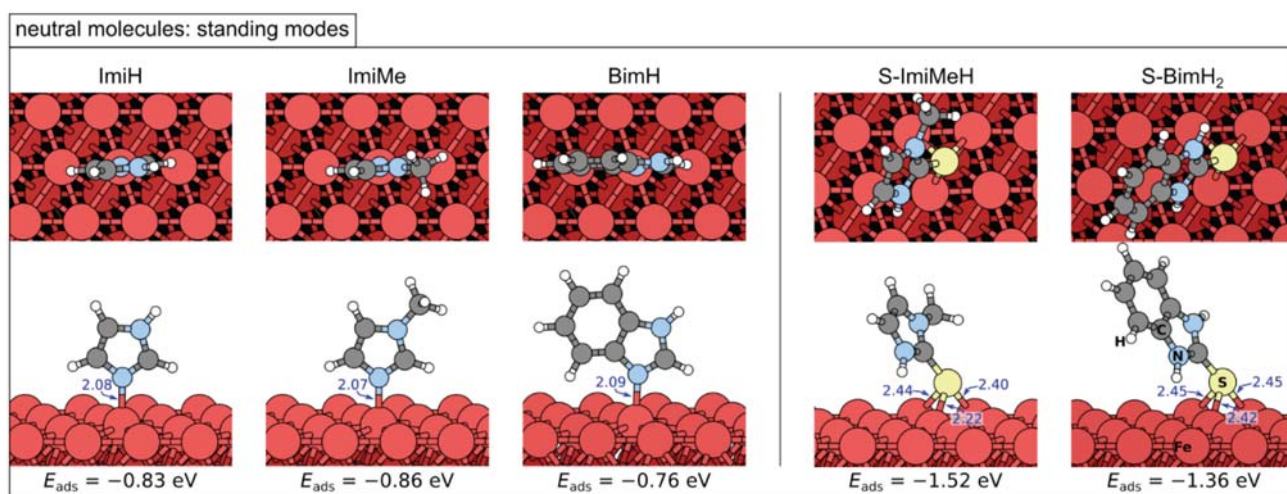


Figure 9. Top and perspective views of optimized standing molecular adsorption modes on Fe(110). From left to right: imidazole (ImiH), 1-methylimidazole (ImiMe), benzimidazole (BimH), and thione forms of 1-methyl-2-mercaptoimidazole (S-ImiMeH) and 2-mercaptoimidazole (S-BimH₂). Adsorption energies and molecule–surface bond lengths (N–Fe and S–Fe), calculated at 1/20 ML coverage, are also stated. Color coding of atoms is the following: H is white, C is gray, N is sky-blue, S is yellow, and Fe is reddish with color becoming darker as going from surface toward the bulk.

tures of the standing and lying adsorption modes are shown in Figs. 9 and 10, respectively.

3. 2. 1. 1. Standing Adsorption Modes

In the standing modes (Fig. 9), the three non-mercapto molecules bond with the pyridine N3 atom on top of a single Fe atom and 1-methyl-imidazole (ImiMe) bonds the strongest among the three molecules, but otherwise the bonding differences are small, being -0.83 , -0.86 , and -0.76 eV for ImiH, ImiMe, and BimH, respectively. Benzimidazole (BimH) thus binds the weakest, despite being the softest, i.e., it has the smallest HOMO–LUMO gap among the three molecules.^{2,33} An opposite behavior was observed for triazoles, where benzotriazole bonds slightly stronger than triazole to Cu(111).³⁴ In accordance with the bonding energy trend the N–Fe molecule–surface bond length follows the BimH (2.09 Å) > ImiH (2.08 Å) > ImiMe (2.07 Å) order, i.e., stronger adsorption bond corresponds to shorter bond length. The analogous bonding trend among the three molecules was also observed on Cu(111) surface and the reason that the BimH binds the weakest was attributed to steric hindrance of the bottom-most H atom of benzene ring, which is too close to the surface.²

The adsorption of mercapto-molecules is considered only in thione tautomer forms, because we find that thiols dissociate (S–H bond cleavage) barrier-less during adsorption. In the standing modes, thiones adsorb via the S atom slightly asymmetrically onto the long-bridge site^d thus bonding to three surface Fe atoms. 2-mercapto-1-methyl-imidazole (S-ImiMeH) binds by about 0.1 eV stronger than 2-mercaptobenzimidazole (S-BimH₂) and correspondingly also the S–Fe bonds of the former are shorter than that of the latter. As for the comparison between the standing mercapto and non-mercapto molecules,

it is evident that the S–surface bonding of mercapto molecules is by about 0.6 eV stronger than the N–surface bonding of non-mercapto molecules.

3. 2. 1. 2. Lying Adsorption Modes

In the lying adsorption modes all the ring C and N atoms form bonds with the surface (Fig. 10). In addition, mercapto-molecules bond also with the S atom, which is adsorbed over the long-bridge site. Similar as for the standing modes, also in the lying modes the mercapto-molecules bond significantly stronger than non-mercapto molecules. 2-mercapto-1-methyl-imidazole again bonds the strongest (-1.60 eV) and 2-mercaptobenzimidazole binds by about 0.1 eV less.

Comparison of adsorption energies of standing and lying modes (cf. Figs. 9 and 10) reveals that for ImiH and ImiMe the two modes are of similar stability ($E_{\text{ads}} \approx -0.85$ eV), but for other molecules the lying modes are more stable. This trend can be, in part, attributed to molecular size. ImiH and ImiMe are the smallest and bond only with the imidazole ring to Fe(110). In addition, BimH bonds also with the benzene ring and thus binds by 0.4 eV stronger than its standing form. Lying mercapto-molecules bind even stronger because they bond also with the reactive S atom to the surface. The N–surface and C–surface bond lengths of the lying molecules are about 2 Å, thus being characteristic of chemisorption.

3. 2. 2. Adsorption Bonding of Dehydrogenated Molecules

Fe is a chemically reactive metal and it was shown that its surfaces can break the N–H and C–H bonds of imidazole. In particular, in the previous publication of one of us,¹² it was shown that the breaking of the C2–H

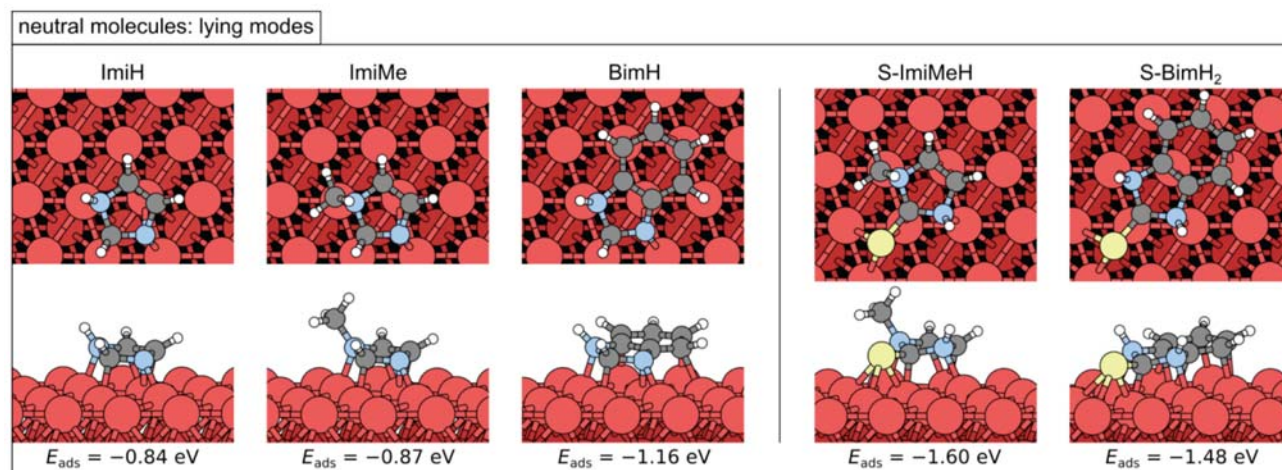


Figure 10. As in Figure 8, but for lying adsorption modes.

^d See Fig. 2 for definition of the short- and long-bridge sites.

bond of imidazole on Fe(100) is particularly easy and it involves the transformation of the standing N3 bonded imidazole to a meta-stable tilted form that bonds with N3+C2 atoms to the surface (the energy barrier for this transformation is 0.3 eV). This transformation is then quickly followed by the cleavage of the C2–H bond (the corresponding energy barrier is only 0.03 eV) resulting into standing C2+N3 bonded dehydrogenated imidazole.¹² This type of dehydrogenated molecules is considered in section 3.2.2.1 below. On the other hand, the cleavage of the N1–H bond of imidazole was found to be less easy and it proceeds from lying imidazole and results in the lying N1 dehydrogenated imidazole: the corresponding energy barrier on Fe(100) is 0.9 eV.¹² This type of dehydrogenated molecules are considered in section 3.2.2.2 below.

3. 2. 2. 1. Standing Dehydrogenated Adsorption Modes

Optimized structures of standing dehydrogenated molecules are displayed in Fig. 11; dissociative chemisorption ($E_{\text{ads}}^{\text{diss}}$) and binding energies (E_{b}) are also stated. Shown non-mercapto molecules are all C2 dehydrogenated (labeled as Mol_{C2}) and bond via C2 and N3 atoms to the surface. Top-view plots reveal that molecular planes are perpendicular to the long-bridge direction with the midpoint of the C2–N3 bond over the midpoint of the long-bridge. Dissociative chemisorption energies are about –2.0 eV for all the three molecules, thus being considerably more exothermic than the non-dissociative E_{ads} , the latter being about –0.8 eV. Dehydrogenation energies, calculated by Eq (8), are thus about –1.2 eV. These molecules, however, bond way stronger to the surface than the $E_{\text{ads}}^{\text{diss}}$ values indicate and the corresponding binding energies, calculated by Eq (5), are about –4.1 eV.

In contrast to non-mercapto molecules, mercapto molecules cannot dehydrogenate at the C2 atom, because they have S instead of H bonded to the C2. But they can dehydrogenate via cleavage of the N3–H bond (note that for S-BimH₂ the N1 and N3 are symmetry equivalent).

The corresponding dissociative chemisorption energies are more exothermic than for non-mercapto molecules, being –2.45 and –2.29 eV for 2-mercapto-1-methylimidazole and 2-mercaptobenzimidazole, respectively. In the dehydrogenated state, the S-ImiMe and S-BimH bond via N3 and S atoms to the surface, with the N3 bonded on top of an Fe atom and with the S located at the short-bridge site thus forming bonds with two bridge Fe atoms. The reason for more exothermic $E_{\text{ads}}^{\text{diss}}$ of mercapto molecules compared to non-mercapto molecules can be, in part, attributed to weaker N3–H bond (about 3.8 eV) compared to the C2–H bond of non-mercapto molecules (about 5.1 eV). Despite the $E_{\text{ads}}^{\text{diss}}$ being more exothermic than for non-mercapto molecules, the S-ImiMe and S-BimH bond weaker to the surface than non-mercapto molecules, and the corresponding binding energies (E_{b}) are about –3.2 eV (to be compared to –4.1 eV for non-mercapto molecules).

3. 2. 2. 2. Lying Dehydrogenated Adsorption Modes

Optimized structures of lying dehydrogenated molecules are shown in Fig. 12; dissociative chemisorption ($E_{\text{ads}}^{\text{diss}}$) and binding energies (E_{b}) are also stated. Shown dehydrogenated molecules have the N–H bond cleaved, which is N1–H for non-mercapto molecules (labeled as Mol_{N1}) and N3–H for mercapto-molecules (for S-BimH₂ the N1 and N3 are symmetry equivalent). Note that ImiMe^{w/o-H}_{N1} cannot exist, because ImiMe does not have any N–H bond.

For all considered molecules, except the S-BimH, the lying dehydrogenated modes are less stable than the

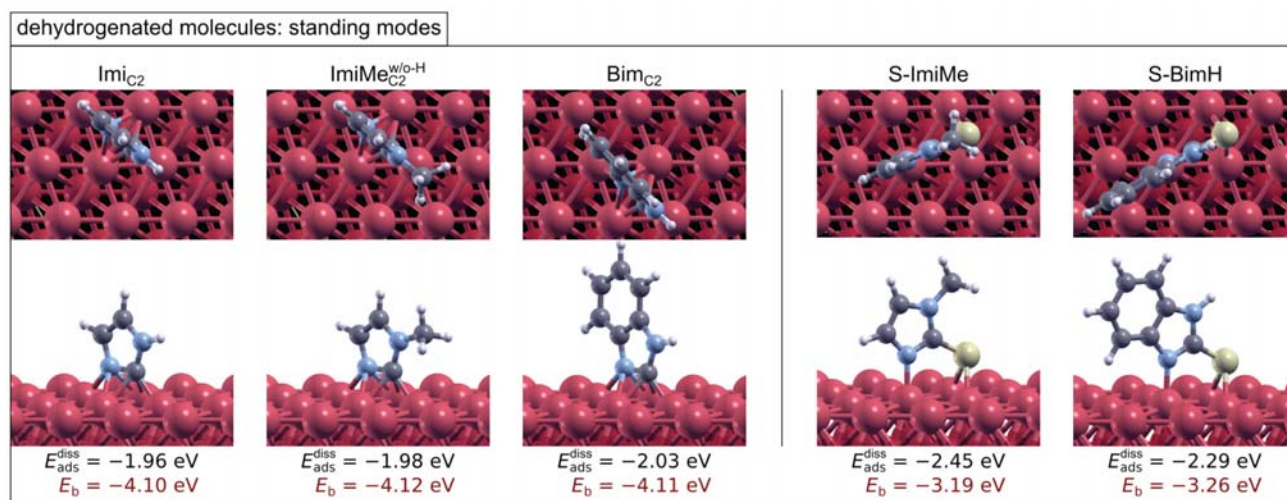


Figure 11. “Dehydrogenated” standing adsorption modes on Fe(110); non-mercapto molecules lack the H at C2 atom, whereas mercapto molecules are in thiolate form (which can be seen as thione lacking the H at N3 or as thiol lacking the H at S). Dissociative adsorption energies ($E_{\text{ads}}^{\text{diss}}$), corresponding to the $\text{Mol}_{(\text{g})} \rightarrow \text{Mol}_{(\text{ads})} + \text{H}_{(\text{ads})}$ process, and the Mol–Fe(110) binding energies (E_{b}) are also stated.

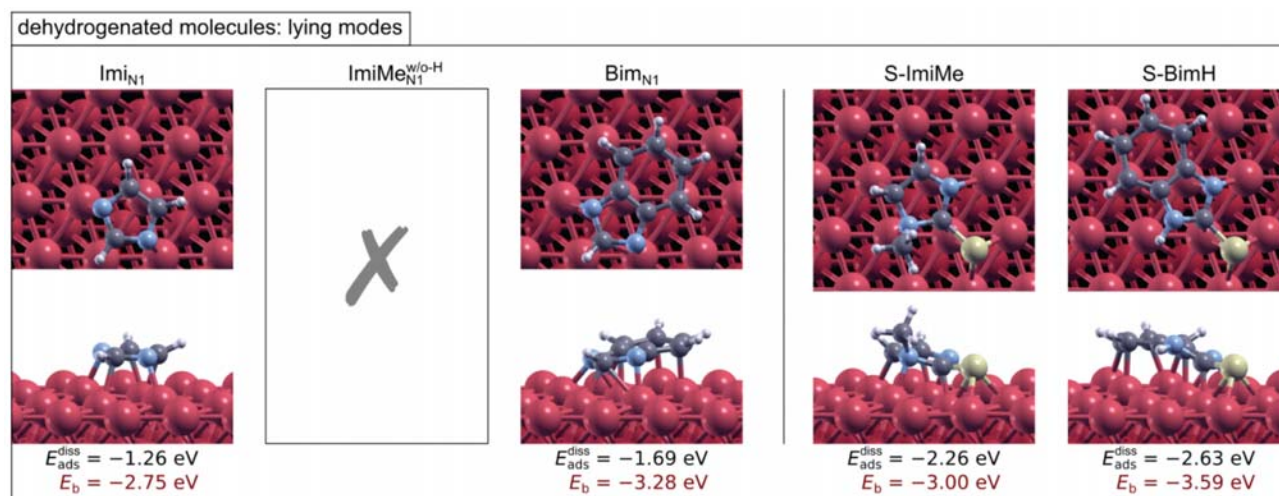


Figure 12. “Dehydrogenated” lying adsorption modes on Fe(110); non-mercapto molecules lack the H at N1 atom, whereas mercapto molecules are in thiolate form.

standing modes. Notable exception is the lying mode of S-BimH, with the $E_{\text{ads}}^{\text{diss}}$ of -2.63 eV , which is the most stable identified adsorption mode among all the currently considered cases (cf. Figs. 9–12).

Comparison of Figs. 10 and 12 reveals that the most stable identified lying modes of imidazole and benzimidazole are analogous between neutral and dehydrogenated molecules, whereas for mercapto molecules the most stable identified lying neutral and dehydrogenated modes are rotated by about 90° around the surface normal with respect to each other, i.e., neutral lying molecules have the S–C bond oriented along the long-bridge direction, whereas dehydrogenated molecules have the S–C bond oriented perpendicular to the long-bridge direction.

4. Conclusions

The effect of mercapto and methyl groups on the adsorption bonding of imidazole and benzimidazole based inhibitors on Fe(110) surface and their efficiency of iron corrosion in 3 wt.% NaCl solution was studied by means of explicit DFT calculations and corrosion experiments, respectively. At 1 mM concentration, the highest inhibition efficiency (69%) was achieved by 1-methyl-imidazole, and the lowest (8%) by imidazole. Other tested inhibitors, 2-mercaptobenzimidazole, benzimidazole and 2-mercapto-1-methyl-imidazole, showed similar efficiencies (41–57%). At 10 mM, the differences between inhibitors were smaller and ranged between 41 and 72%. For 2-mercaptobenzimidazole the solubility below 10 mM prevented it from being tested at this concentration. Inhibitors acted primarily as anodic inhibitors due to the formation of surface layer. Its composition corresponds mainly to oxidized iron, namely a mixture of Fe(II) and Fe(III) oxide and hydroxides, which contains also species

originating from inhibitor molecules, i.e. nitrogen and sulphur. Compared to inhibited copper, the concentration of the latter is much smaller, and, furthermore, is observed only for mercapto-containing inhibitors. In contrast to copper, where mercapto containing derivatives of imidazoles were beneficial for all concentrations tested, their inhibition of iron corrosion was advantageous compared to other derivatives only at low inhibitor concentration. Other inhibitors at 0.1 mM were either inefficient or acted as activators. On the other hand, methyl-derivative of imidazole had a positive impact on inhibition of iron at all concentrations regardless whether imidazole or mercapto-imidazole were used. These results prove that the inhibition effect of given inhibitor is strongly dependent on the metal substrate and that the inhibition efficiency cannot be predicted for inhibitor *per se* without taking into account the characteristics of substrate. This issue is apparently not appreciated in the literature, because majority of computational studies of corrosion inhibitors rely solely on electronic parameters of inhibitor molecules without any consideration of the substrate whatsoever.

DFT calculations reveal that current inhibitor molecules bind stronger to iron than copper surfaces, but this is of no direct relevance to corrosion inhibition. Namely, iron is chemically more reactive than copper, thus it can be reasonably anticipated—on the basis of Hammer-Nørskov chemisorption model³⁵—that it binds, in general, adsorbates stronger than copper. It is thus the relative adsorption bonding strength of inhibitors compared to that of corrosive species such as, chloride, that is relevant.

Current computational results and those obtained previously on copper reveal that the inhibitor–surface bonding, as important as it may be, is not sufficient to explain the trend of corrosion inhibition efficiency of the investigated inhibitors. Namely, the adsorption bonding of investigated inhibitors can be roughly classified into two

types with non-mercapto molecules belonging to one and mercapto molecules to the other type. Non-mercapto molecules display less exothermic adsorption than mercapto molecules and bind through the N-surface bonds (in the lying and/or dehydrogenated mode also via the C-surface bonds), whereas mercapto molecules bond in addition also via the S-surface bonds. On this basis one can infer that mercapto inhibitors should be superior to non-mercapto inhibitors, which is correct only at low inhibitor concentration. Adsorption characteristics of non-mercapto molecules display insignificant differences between them (with the exception of lying benzimidazole on iron, which binds stronger), yet the experimentally observed inhibition efficiency differ significantly between them. In this respect the effect of methyl group is the most interesting and non intuitive, because on both copper and iron surfaces, 1-methyl-imidazole display similar adsorption characteristics as the imidazole, yet on copper the presence of methyl group is disadvantageous or even detrimental, whereas on iron it is beneficial.

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Povzetek

V tej študiji smo s kombinacijo eksperimentalnih in računskih metod obravnavali imidazolne in benzimidazolne inhibitorje, ki vsebujejo metilno in/ali merkapt skupino. Eksperimentalni del študije obsega elektrokemijske meritve in enomesečni potopitveni test v 3% raztopini NaCl, računski del pa eksplicitno obravnava podrobnosti vezave inhibitorja na površino železa na molekularnem nivoju z uporabo teorije gostotnega funkcionala (DFT). Eksperimentalni podatki, dopolnjeni s 3D topografijo in rentgensko fotoelektronsko spektroskopijo, so bili osnova za izračun inhibicijske učinkovitosti in mehanizma inhibicije. Merkapt skupina v kombinaciji s metilno skupino ima pozitiven vpliv na inhibicijo korozije pri vseh testiranih koncentracijah. Pozitiven vpliv merkapt skupine v kombinaciji z benzenskim obročem pa ni tako izražen kot v kombinaciji z metilno skupino. Opaženo vedenje je v nasprotju s tistim, ki smo ga zasledili pri bakru, kjer je bil vpliv metilne skupine škodljiv, pri čemer je 1-metil-imidazol celo pospeševal korozijo pri koncentraciji 10 mM. Po drugi strani pa je bil vpliv merkapt skupine in benzenskega obroča vedno pozitiven. DFT izračuni so razkrili, da ima metilna skupina majhen vpliv na interakcije inhibitor–površina Fe, saj ni direktno vključena v vezavo molekula–površina. Nasprotno, prisotnost merkapt skupine povzroči nov tip interakcij molekula–površina, ki vključuje močno vez S–površina, posledično je adsorpcija inhibitorja preko merkapt skupine zato bolj eksotermna.

Scientific paper

Transport of External Lithium Along Phase Boundary in LiF-Ti Nanocomposite Thin Films

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This paper is dedicated to the memory of Prof. Janez Jamnik and his pioneer research on space charge layer effect in energy storage materials

Abstract

The electronic and ionic conductivity of the LiF-Ti nanocomposite films prepared by the co-sputtering have been investigated by the method of impedance spectrum (IS), current-voltage curves (IV) and isothermal transient ionic current (ITIC) measurements. It is found that the ionic conductivity of the obtained LiF-Ti nanocomposite film is very low. After electrochemical and chemical lithiation, ionic conductivities of the lithiated composite films are increased to be 10^{-3} and 10^{-4} S/cm separately. This phenomenon indicates that the phase boundary between LiF and Ti could be the ionic conducting channels for external lithium in the LiF-Ti nanocomposite. Our results suggest a new strategy to design ionic or mixed ionic conductor.

Keywords: LiF-Ti, nanocomposite, thin film, chemical lithiation, ITIC, ionic transportation

1. Introduction

Transport behaviours of ions in solid can be classified into four types:^{1–7} 1) motion of ions through vacancy or interstitial sites in a defected host lattice; 2) diffusion of ions in the free space through the segment wriggle of a polymer; 3) diffusion of ions on the surface of a solid; 4) diffusion of the ions along or across the interfaces between grains or phase. The later one has attracted wide attention.^{8–12} Nearly in all investigated heterogeneous polycrystalline or multiple phase composite or heterogeneous junctions systems, at least one phase at the side of the interface is an ionic conductor. The charge carriers come from at least one phase and ions transport along or across the phase boundaries influenced by the space charge layer effect.³

In searching for new reversible electrode materials for Li-storage, conversion type materials have attracted

wide attention. It is found that lithium could electrochemically extracted from converted M-Li₂O (M = Fe, Co, Ni, Cu) nanocomposites.¹³ This means that chemical bonding between Li and O can be broken and lithium ions should be mobile in such nanocomposite.

Later in 2003, the reversible lithium storage was also observed in the LiF-Ti nanocomposite. Besides the conversion reaction, it is also noticed that extra lithium can be stored reversibly at the low voltage range after the formation of LiF-Ti nanocomposite.¹⁴ Jamnik and Maier explained such heterogeneous storage phenomenon as the interfacial charging.^{15,16}

In previous reports on lithium storage through conversion reaction, the nonaqueous electrolyte was used in the test cells.^{14,18} Consequently, the formation of the solid electrolyte interphase (SEI) is not avoidable. Therefore, the interfacial charging and the SEI evolve within the similar

low voltage range, at least for the initial charge-discharge cycles. The SEI is covered on the formed LiX-M nanocomposite. Experimentally, the transport properties of the lithium ions and electrons in the phase boundaries of the LiX-M nanocomposite and the SEI film are difficult to be measured separately. One valuable *in situ* TEM investigations on the reaction of Li with FeF_2 in a solid cell showed that FeF_2 can be fully lithiated and converted into the LiF-Fe nanocomposite.¹⁷ Accordingly, Wang *et al* suggested that the massive interface formed between nanoscaled solid phases provides pathways for ionic transport during the conversion process. Since either LiF or Li_2O or M is not lithium ion conductors, it is quite reasonable that lithium should diffuse through the phase boundaries of the LiX-M nanocomposites, although direct evidences are absent.

In addition, one thing is still unclear: for a given LiX-M nanocomposite, whether are the lithium at the lattice site in the LiX or the extra lithium inserted from the outside of the composite mobile in the nanocomposite? In order to clarify above issues, in this work, the LiF-Ti nanocomposite thin film electrodes are prepared and lithiated either by electrochemical or chemical method. Therefore, the ionic and electronic conductivity of the LiF-Ti nanocomposites with and without external lithium can be measured and compared.

2. Experimental

2. 1. Preparation of the Substrate Ti Electrodes for Measuring Conductivity

The parallel electrodes were usually used in thin film conductivity measurement.^{11–12} A 100 μm strip of photoresist was spin coated on the quartz substrate. The titanium electrodes were deposited by sputtering on the substrate. Then immersed the substrate into acetone solution for a while, the photoresist will be peeled off and the parallel Ti electrodes with a 100 μm gap were prepared, as

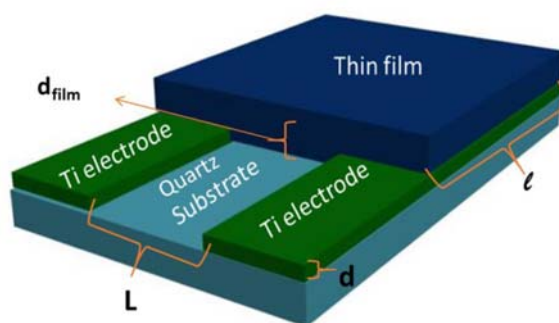


Figure 1. The scheme of the bottom parallel electrodes for transportation measurement

Table 1. Dimensions of the parallel electrode device for transportation measurement

Dimension	Value
Thickness of thin film: d_{film}	2.5 μm or appr.300 nm
Length of thin film: l	4–7 mm
Thickness of Ti electrode: d	500 nm
Gap of two Ti electrodes: L	100 μm

shown in Figure 1. Table 1 shows the dimensions of the parallel electrode device for transportation measurement

The conductivity is obtained by formula:

$$\sigma = \frac{L}{l \cdot d} \cdot \frac{1}{R} = k \cdot \frac{1}{R}, \text{ in which } \sigma \text{ has unit S/cm, } R \text{ has unit } \Omega,$$

k is a constant value depends on the device dimension, here we take it as 520 cm^{-1} .

2. 2. Preparation of the LiF-Ti Nanocomposite Thin Film

Yu *et al.* reported the LiF-Ti nanocomposite thin film prepared by PLD (pulse laser deposition).¹⁸ In this study,

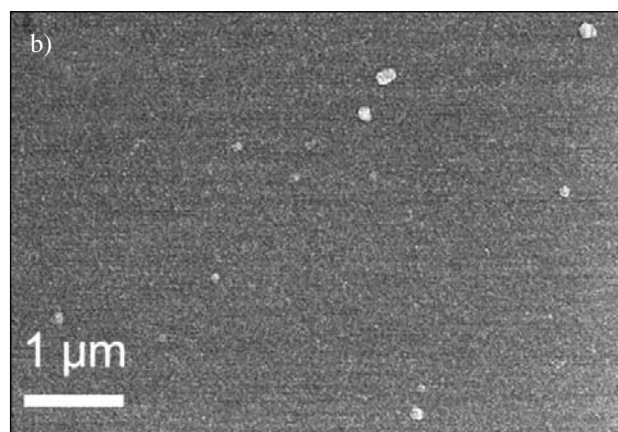
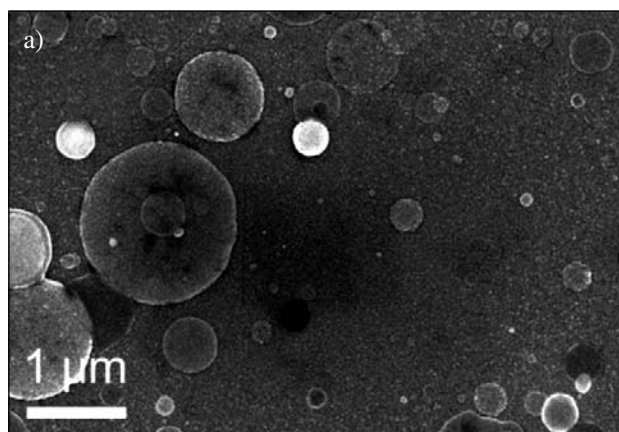


Figure 2. the surface morphology (by FE-SEM) of LiF-Ti nanocomposite thin films, prepared by (a) PLD and (b) co-sputtering

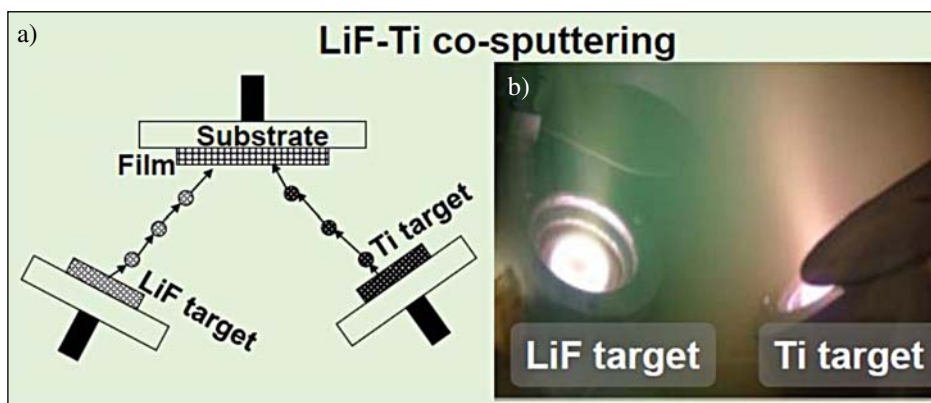


Figure 3. (a) the scheme and (b) photograph of the co-sputtering

we also repeated the PLD method to deposit the LiF-Ti thin film. As shown in Figure 2a, the particles of the thin film was not deposited homogeneously as reported previously,¹⁸ the size of some particles were as large as 1 μm . In order to improve the homogeneity of the thin film, the LiF and Ti components in the thin film were deposited by the RF-sputtering (Radio Frequency sputtering) and DC-sputtering (Direct Current Sputtering) respectively. As shown in Figure

2b, the LiF-Ti nanocomposite thin film prepared by the sputtering is much flatter and more homogeneous than the one prepared by PLD. In this work, the samples tested were all prepared by the co-sputtering method, Figure 3 shows the scheme and photograph of co-sputtering method.

Figures 4 (a–c) show the cross-section images and element analysis of the co-sputtered thin film by FE-SEM and Energy-dispersive elemental analysis (EDX). It can

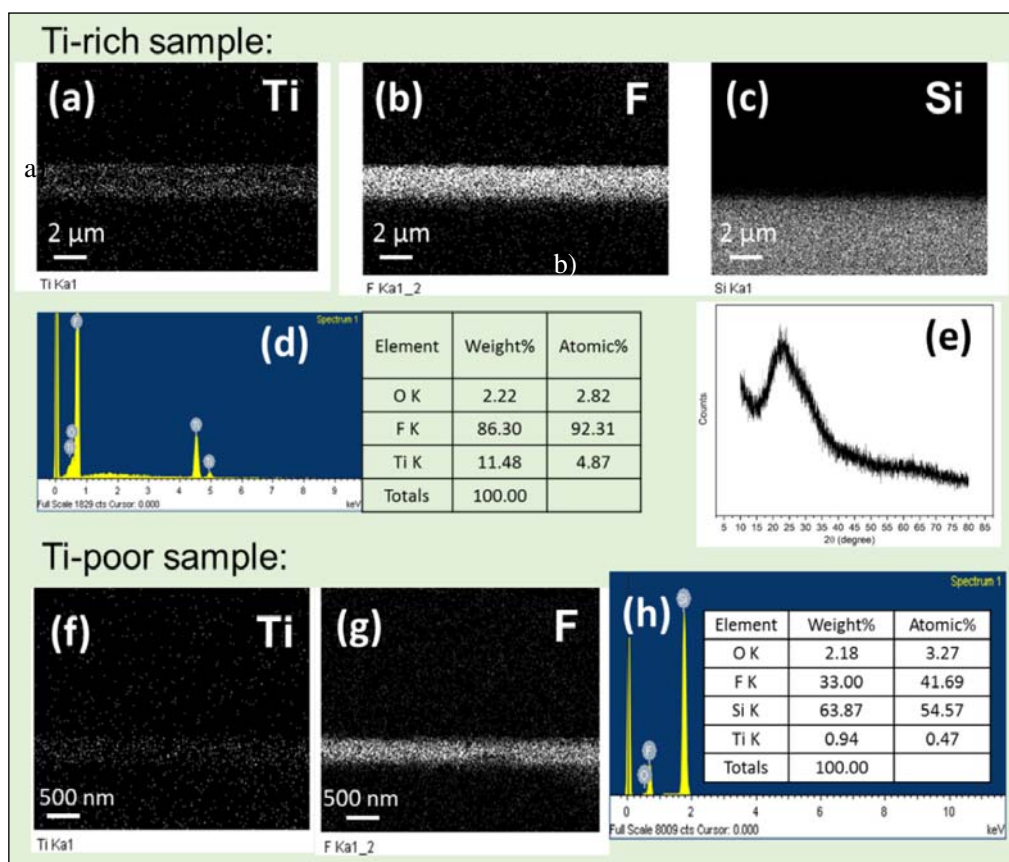


Figure 4. Element mapping (a, b, c: Ti-rich sample, f, g: Ti-poor sample), EDX (d: Ti-rich sample, h: Ti-poor sample) and XRD pattern (e: Ti-rich sample) of the co-sputtered LiF-Ti nanocomposite thin film

be seen that the film is flat and the distribution of Ti and F elements is quite homogeneously. Atomic ratio of F to Ti is 19:1 roughly. No XRD peaks from LiF and Ti is found in Figure 4e, indicating that the composite thin film is composed of the nano-sized particles and the grain sizes of which are much smaller than that in our previous report.¹⁸ This sample is Ti-rich LiF-Ti composite. As the comparison, another sample of Ti-poor LiF-Ti nanocomposite is also prepared, and the atomic ratio of F to Ti is about 88:1 as shown in Figure 4h. Figures 4f and 4g show the cross-section element analysis of the Ti-poor sample.

2. 3. Electrochemical Test and Electrochemical Lithiation

The as prepared LiF-Ti nanocomposite thin film was immersed into a small electrochemical cell filled with organic electrolyte (1M LiPF₆ dissolved in ethylene carbo-

nate (EC) and dimethyl carbonate (DMC), 1:1 in volume). The lithium foil was also immersed into the electrolyte as the counter electrode of the cell. When the cell is charged firstly, the charging capacity is less than 0.4 μ Ah, as shown in Figure 5a. While the cell is discharged firstly, then the capacity is above 40 μ Ah and the charging capacity is about 20 μ Ah. As seen in Figure 5b, there is a significant irreversible capacity. It could be related to the formation of the SEI since the voltage range of the cell is already lower than 1.0 V. Since the existence of the SEI cannot be excluded, the electrode prepared by electrochemical lithiation in nonaqueous electrolyte is not very reliable for investigating the conductivity.

2. 4. Chemical Lithiation

In order to eliminate the possible influence from the SEI, the prepared nanocomposite thin film was chemi-

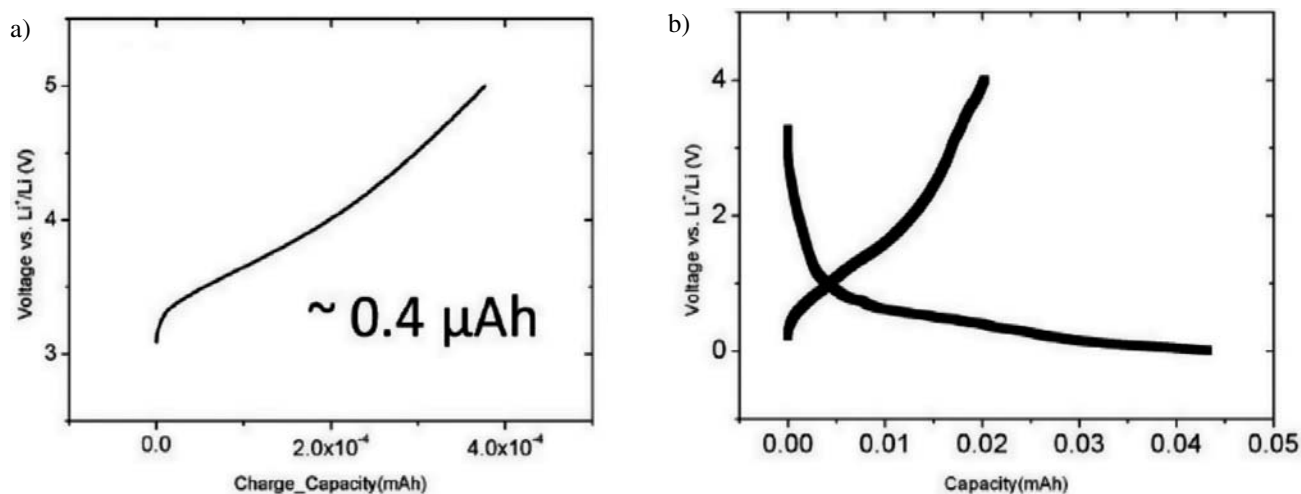


Figure 5. The first charge curve of the Li/LiF-Ti cell and the first discharge-charge curves of the Li/LiF-Ti nanocomposite cell at room temperature in 1M LiPF₆ dissolved in EC: DMC, 1:1 in volume). LiF:Ti = 19:1 for atomic ratio.

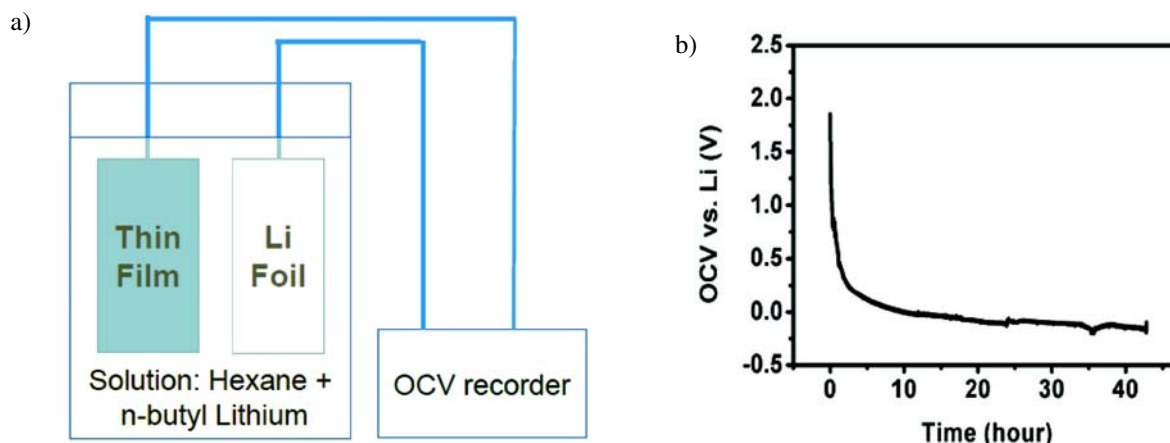


Figure 6. (a) The scheme of the chemical lithiation process and the tracking method for monitoring the lithiation level. (b) The recorded voltage of the cell during chemical lithiation.

cally lithiated by immersing the thin film into the 20 mL hexane solution with 20 μL n-butyl lithium (*i.e.* 1% V/V). This chemical lithiation method has been known for long time.¹⁹ In order to track chemical lithiation level, a new cell was designed as shown in Figure 6a. The LiF-Ti thin film electrode was put into a cell with metal lithium electrode. Once the n-butyl lithium solution was dropped into the cell, the open circuit voltage (OCV) between the LiF-Ti electrode and Li was recorded. Since the OCV of the LiF-Ti electrode is only related to the content of inserted lithium, the record of the OCV can be used to track the chemical lithiation level, as shown in Figure 6b. After about 40 hours, the voltage deviation between lithium metal electrode and the LiF-Ti electrode remains constant, which means that the thin film electrode is fully lithiated.

2. 5. IV, IS, ITIC Measurement

The electronic and ionic transport properties in the as prepared thin film and the electrochemically or chemically lithiated thin films were measured by the method of I–V curve (current-voltage curve), IS (impedance spectrum) and ITIC (isothermal transient ionic current). When the LiF-Ti thin film was lithiated electrochemically or chemically, it was taken out from the organic liquid electrolyte (1M LiPF₆ dissolved in EC: DMC, 1:1 in volume) or hexane solution with n-butyl lithium respectively. Then the sample was dried at RT under vacuum overnight. The dried and lithiated sample was sealed into a glass container and taken out from the glovebox for the following analysis. IV test was conducted by Agilent 4156c precision semiconductor parameter analyzer, with the scanning voltage ranging from –1 V to 1 V, and the scanning step set to 0.01 V. IS test was conducted by IM6ex (Zahner, Germany), frequency range for test was between 1MHz and 0.1 Hz, the amplitude of voltage was 10 mV, and the IS result was fitted by Zview. ITIC test was conducted by Agilent 4156c, the applied voltage bias between the two electrodes was 100 mV. The parallel electrodes device as described in Figure 1 was used for all of these tests, and the tests were conducted at room temperature.

The electronic conductivity of the thin film at room temperature (RT) can be obtained from the I–V curve. The total conductivity (including electronic conductivity and ionic conductivity) at RT can be obtained from IS measurement. As for the ITIC measurement,^{20–23} a constant voltage is applied across the thin film and the current response was measured using the Agilent 4156C. Since the electrode in this work was the deposited Ti thin film, it blocks the transport of the lithium ions. If the applied voltage is U and the distance between the two Ti electrodes is L , the relationship between the current response and the time could be described as follows:

$$J(t) = \frac{\sigma_{dc,e}U}{L} + \frac{\sigma_{dc,Li^+}U}{L} \exp\left(-\frac{\mu_{Li^+}U}{L^2}t\right) \quad (1)$$

Where $J(t)$ is the current density, t is the time that voltage applies on the sample, $\sigma_{dc,e}$ is the electronic conductivity, σ_{dc,Li^+} is the ionic conductivity, μ_{Li^+} is the mobility of the lithium ion. There are two items in the right side of the equation. The first item is related to the current density of electronic current. This item could be obtained by the classical ohm's law. The second item is related to the current density of ionic current. In our case of LiF/Ti, the lithium ion transportation will contribute to this item. Since the ion blocking electrode (*i.e.* Ti film electrode) was used during measurement, the lithium ion transportation will not remain as a constant. As the lithium ions move from one electrode to the other electrode and aggregated on the electrode. The ionic current density will obey the ohm's law at the beginning, but then decrease and at last disappear. The rate of ionic current decrease is related to the mobility of lithium ion μ_{Li^+} , the factor of describing

ionic current decreasing could be written as $\exp\left(-\frac{\mu_{Li^+}U}{L^2}t\right)$.

The detailed derivation of this formula could be found in literatures.^{20–23} The electronic conductivity, ionic conductivity and mobility of lithium ion could be calculated by fitting the current response with the formula.

3. Results and Discussion

The co-sputtered LiF-Ti nanocomposite thin film before and after electrochemical lithiation show different current-voltage feature in Figure 7a. The IV curve of the pristine LiF-Ti thin film shows a linear IV curve within ± 1 V range, a typical ohmic behaviour. The conductivity is calculated from IV curves and increases from 3.6×10^{-3} S/cm to 7.3×10^{-3} S/cm after electrochemical lithiation. As mentioned above, the electrochemical lithiation may lead to the coverage of the insulating SEI film. Our conductivity measurement is performed based on the configuration in Figure 1. The SEI film layer, which is an electronic insulating and ionic conductor in many cases,²⁴ may cover the top of the LiF-Ti film. The SEI film can be regarded as in parallel with the deposited LiF-Ti film in view of electrode configuration and equivalent circuit. Therefore, the insulating SEI may have negligible effect on the measuring of the electronic conductivity.

Since LiF and Ti cannot accommodate extra inserted lithium within the lattice, the inserted lithium should stay at the phase boundaries between LiF and Ti in a charge separation way with the positive and negative charged carriers separated into counter sides of interfaces. Only when enough amount lithium is accumulated in the LiF-Ti composite, the state of lithium is approaching neutral state of lithium atom and the voltage of the Li/LiF-Ti cell is closed to 0 mV. Above this voltage, lithium should stay in a charge separation way. Both electrons and ions could be

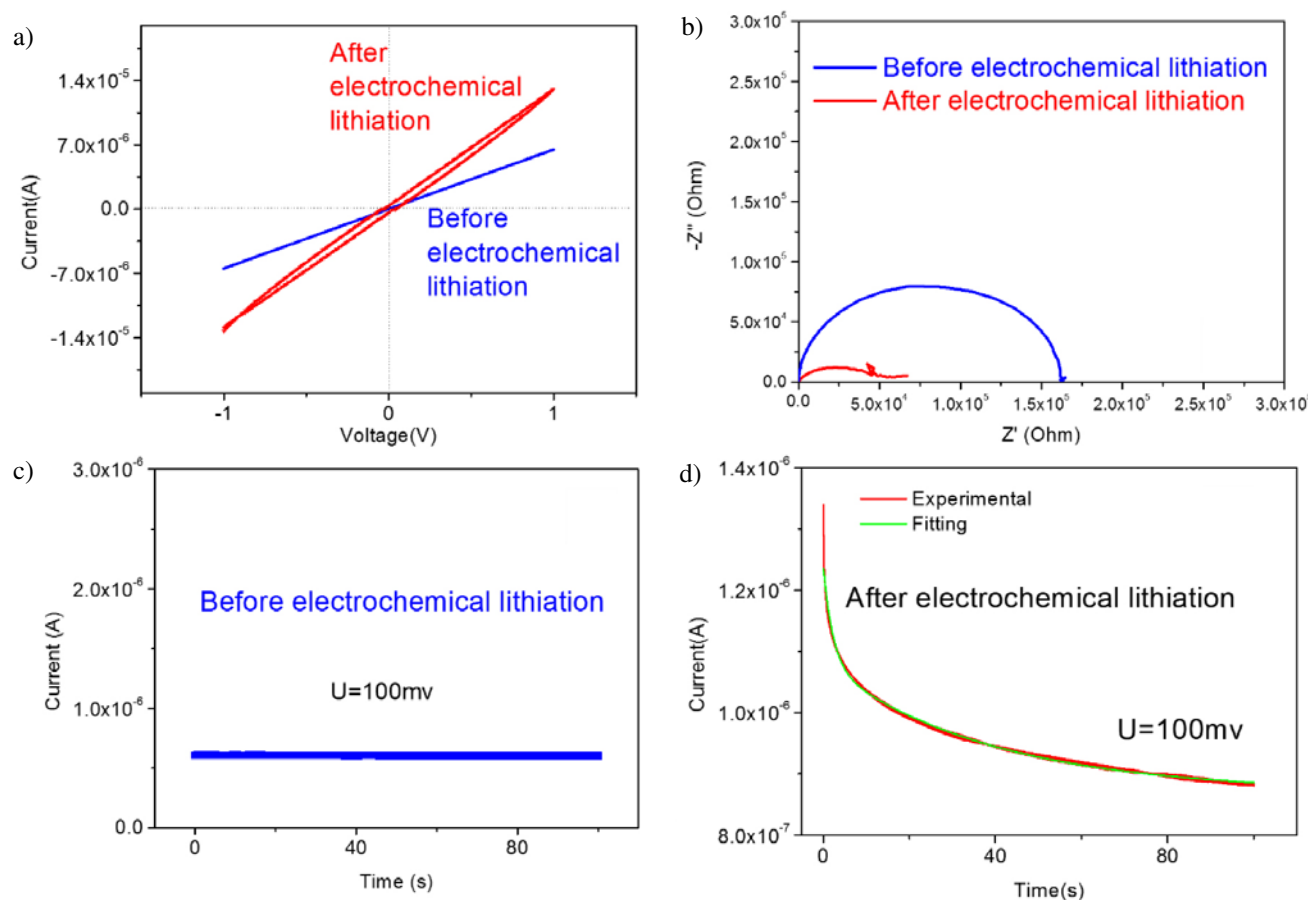


Figure 7. (a) IV curves, (b) IS curves, (c, d) ITIC curves of the pristine LiF-Ti (LiF:Ti = 19:1) electrode and the Ti-rich LiF-Ti electrode after electrochemical lithiation (*i.e.* Li/LiF-Ti cell is discharged to 5 mV).

mobile. IV measuring cannot distinguish the contribution from electronic conductivity and ionic conductivity. One fact should be noted. After the electrochemical lithiation, IV curves show a symmetrical hysteresis loop, implying a kind of reversible polarization behaviour. The appearance of the loop is quite reasonably caused by ionic transport behaviour in the electrochemically lithiated LiF-Ti nanocomposite in an ion block system. Since the deviation of the loop is not significant, the ionic conductivity should be lower compared to electronic conductivity. In addition, the absence of the loop in the pristine LiF-Ti nanocomposite thin film means that its ionic conductivity could be negligible.

Impedance spectrum of the pristine LiF-Ti composite thin film shows a feature of a semi-circle. This indicates that the LiF-Ti composite does not act as a metal. Ti should exist in the composite as isolated domains, as supported by element mapping in Figure 4. The electrical behaviour of this composite likes a typical composite capacitor. After electrochemical lithiation, the semicircle is compressed and the diameter decreases significantly. The measured total conductivity increases from $3.2 \times 10^{-3}\text{ S/cm}$ to $1 \times 10^{-2}\text{ S/cm}$ as shown in Table 2. The tendency consists with IV measurement.

ITIC analysis can provide information of the electronic and ionic conductivity. As shown in Figure 7c and 7d, ITIC curves are quite different before and after electrochemical lithiation. Figure 7d shows typical ionic transport behaviour. Electronic conductivity of the pristine LiF-Ti thin film is $3.12 \times 10^{-3}\text{ S/cm}$ and the ionic conductivity is negligible. Total conductivity is determined by the electronic conductivity. After electrochemical lithiation, electronic conductivity is increased to $4.5 \times 10^{-3}\text{ S/cm}$ and ionic conductivity is as high as $3.3 \times 10^{-3}\text{ S/cm}$. Total conductivity is $7.8 \times 10^{-3}\text{ S/cm}$. The transference number of t_{e^-} and t_{Li^+} is 0.58 and 0.42.

All results from IV, IS and ITIC confirms that electrochemically lithiated LiF-Ti nanocomposite thin film shows higher electronic and ionic conductivity compared than pristine LiF-Ti thin film. As mentioned above, the influence of the SEI could be negligible but not very clear in above measurements. Therefore, chemically lithiated samples are also measured.

In order to emphasize the effect of the lithiation, the ratio of LiF to Ti increase further to form a Ti-poor nanocomposite. This time, the sample composition changes from original 19:1 form LiF: Ti to a Ti-poor composite (LiF: Ti = 88:1).

The transport behaviours of chemically lithiated LiF-Ti nanocomposite are similar as Figure 7. Pristine LiF-Ti (88:1) composite thin film show a very high resistance in Figure 8a, 8c and 8e. This is reasonable since the ratio of Ti is decreased significantly.

After chemical lithiation, the IV curve shows a very significant loop. The impedance spectrum shows a linear slope and the ITIC curve shows an exponential decay as Figure 8(f). All features indicate that both electronic and ionic conductivity are improved significantly. The related data are listed in Table 2.

It can be seen from Table 2 that the electronic conductivity is determined to the ratio of Ti in the composite.

This is reasonable in view of percolation and tunneling effect of Ti grains. It is quite clear that pristine LiF-Ti thin films have poor ionic conductivity. The ionic conductivity of LiF single crystal at room temperature is 1×10^{-19} S/cm²⁵. The amorphous thin film of LiF shows an ionic conductivity of 1×10^{-10} S/cm²⁶. Recently, Li *et al* reported that the LiF/MO (Al₂O₃, SiO₂, TiO₂) heterogeneous bilayer thin film shows enhanced ionic conductivity due to the space charge effect and interfacial disorder.^{27, 28} In our case of LiF-Ti, the existence of the second phase Ti did not show a measurable enhancement on the ionic conductivity of LiF. In the view of the second phase, Ti was the metal in nano size, it provides no lattice site or lattice

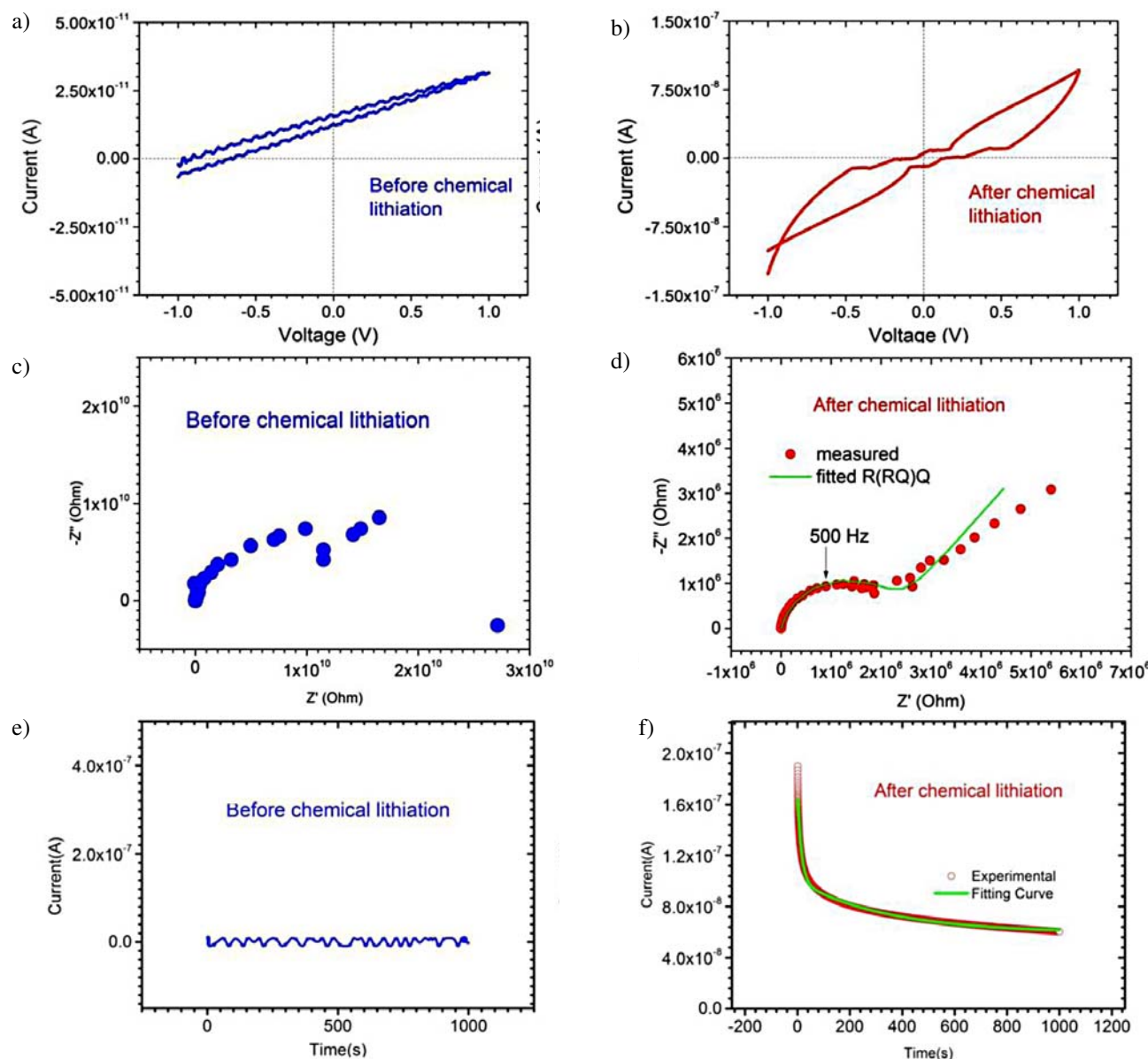


Figure 8. The IV (a, b), IS (c, d) and ITIC (e, f) measurement results of the LiF-Ti (LiF:Ti = 88:1) nanocomposite thin film at room temperature before and after chemical lithiation. The pristine Ti-poor LiF-Ti sample shows a relative low electric conductivity due to the Ti deficiency. Thus the signal of IV curve was affected by noises.

Table 2. RT conductivity of the LiF-Ti nanocomposite before and after electrochemical and chemical lithiation (measured by IV, IS and ITIC).

Measuring method	IV	IS	ITIC			
Conductivity	σ_{e-}	σ_{total_IS}	σ_{e-}	σ_{Li+}	μ_{Li+}	$\sigma_{total\_ITIC} = \sigma_{e-} + \sigma_{Li+}$
Unit	S/cm	S/cm	S/cm	S/cm	cm ² /V s	S/cm
<i>Ti-rich LiF-Ti sample (LiF:Ti=19:1)</i>						
Pristine LiF-Ti	3.6×10^{-3}	3.2×10^{-3}	3.1×10^{-3}	Very low	Very low	3.1×10^{-3}
Electrochemically lithiated LiF-Ti	7.3×10^{-3}	1×10^{-2}	4.5×10^{-3}	3.3×10^{-3}	2.4×10^{-4}	7.8×10^{-3}
<i>Ti-poor LiF-Ti sample (LiF:Ti=88:1)</i>						
Pristine LiF-Ti	5.0×10^{-9}	5.0×10^{-8}	Very low	Very low	Very low	Very low
Chemically lithiated LiF-Ti	3.0×10^{-5}	2.6×10^{-4}	6.8×10^{-5}	1.3×10^{-4}	4.0×10^{-5}	2.0×10^{-4}

vacancies for the storage and transportation of lithium. Moreover, the sputtered LiF was totally amorphous according to the X-ray analysis. As mentioned above, LiF phase cannot provide lattice sites for the storage and interior channels for the transportation of lithium. Therefore, the only possible ionic transport channels are the phase boundary. In our case of LiF-Ti, the lithium from the external source may stay and migrate along the phase boundary between the LiF and Ti.

Therefore, in spite of poor ionic conductivity of LiF-Ti nanocomposite thin film, it is no doubt that both electrochemically and chemically lithiated thin films show much enhanced ionic conductivity and electronic conductivity. The enhancement mechanism is related to the external lithium stayed at the phase boundaries in the LiF-Ti nanocomposite in a form of charge separation. Unfortunately, the local structure of the lithiated LiF-Ti nanocomposite is still not known due to the difficulty in characterization.

Currently, exploring superionic conductor as solid electrolyte and enhancing ionic transport property of the electrode and the interface are two very important targets for developing advanced lithium batteries. Most of efforts are focused on tuning the intrinsic ionic transport properties of the host lattice. Not the first time but from a new viewpoint, our finding emphasizes that tuning phase boundary by introducing the second phase and considering the transport of external ions could be important and new strategies, needing comprehensive investigations.

4. Summary

The electronic conductivity and ionic conductivity of the LiF-Ti nanocomposite thin film before and after electrochemical and chemical lithiation are measured using IV, IS and ITIC methods. It is found that the electronic conductivity of the LiF-Ti film is determined by the ratio of Ti and can be enhanced after the insertion of lithium. The ionic conductivity of pristine LiF-Ti nanocomposite is poor and no significant space charge enhancement effect is observed. Electrochemical and chemical lithiation can improve the ionic conductivity of the LiF-Ti nanocomposite

thin films to a level of 1×10^{-3} S/cm at room temperature. This finding indicates that even when both phases of LiF and Ti are not ionic conductor and cannot accommodate extra lithium, external lithium at phase boundaries can lead to the high ionic and electronic conductivity. It means that phase boundary composed of ionic insulating phases can be also utilized as fast ionic channels.

5. Acknowledgement

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Povzetek

S pomočjo različnih metod, kot so: impedančna spektroskopija (IS), tokovno-napetostnih krivulj (IV) in izotermičnih prehodnih tokov (ITIC), smo merili elektronsko in ionsko prevodnost Li–F–Ti naokompozitnih filmov pripravljenih s ko-pršenjem. Ugotovili smo, da je ionska prevodnost Li–F–Ti naokompozitnih filmov zelo nizka. Po elektrokemijski in kemijski litiaciji so ionske prevodnosti narasle na 10^{-3} in 10^{-4} S/cm. Omenjeni fenomen kaže na možnost tvorbe, za zunanji litij, ionsko prevodnih kanalov na faznih mejah med zrni LiF in Ti v nanokompozitu. S pomočjo naših rezultatov predlagamo novo strategijo, kako krojiti ionske in mešano-ionske prevodnike.

Scientific paper

Sulphured Polyacrylonitrile Composite Analysed by *in operando* UV-Visible Spectroscopy and 4-electrode Swagelok Cell

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In the memory of Janez (Janko) Jamnik, respected scientist, excellent coworker and leader and friend we will never forget

Abstract

The electrochemical characteristics of sulfurized polyacrylonitrile composite (PAN/S) cathodes were compared with the commonly used carbon/S-based composite material. The difference in the working mechanism of these composites was examined. Analytical investigations were performed on both kinds of cathode electrode composites by using two reliable analytical techniques, *in-situ* UV-Visible spectroscopy and a four-electrode Swagelok cell. This study differentiates the working mechanisms of PAN/S composites from conventional elemental sulphur/carbon composite and also sheds light on factors that could be responsible for capacity fading in the case of PAN/S composites.

Keywords: Polyacrylonitrile, sulphur, batteries, *operando*, UV-Vis

1. Introduction

Sulphur (S) is considered as a potential cathode material because of its high theoretical capacity 1672 mAh g⁻¹ and energy density 2500 Wh kg⁻¹. The high capacity is based on the conversion reaction of elemental sulphur to form the lithium sulphide (Li₂S) by reversibly incorporating two electrons per S atom. This is an order of magnitude higher compared to current commercial lithium ion technology. In addition to this, lithium-sulphur (Li-S) batteries have many advantages; S is a waste material of the petroleum industry, which makes it an inexpensive raw material; Li-S can be operated under wide range of temperatures; systems using it are considered safe and environmentally friendly.^{1–4}

Despite the advantages mentioned above, the system does have major shortcomings that prevent it from being commercialised. Sulphur is a highly insulating element and needs conductive electron additives, such as carbon black, to make it feasible as an electrode material. That decreases the practical energy density that

can be obtained from the system. Additionally, the intermediate lithium polysulphides formed during battery cycling are soluble in the electrolyte and can diffuse or migrate to the negative electrode, which induces the so-called shuttle mechanism within the electrochemical cell. The electrochemical conversion of sulphur to Li₂S involves repetitive dissolution and deposition of reactive species, which passivates both electrodes, leading to a significant increase in internal resistance of the battery. Furthermore, there has been a lack of understanding of the system, which has many complicated equilibrium species and radicals arising due to the dissolution of lithium polysulfides in the liquid electrolyte. All these factors have led to underperforming Li-S batteries.^{5–7}

To overcome these obstacles, recent efforts have been focused on the reduction of the adverse effects caused by the shuttling of polysulphides by developing new types of S cathodes.^{8–9} The most common type of cathodes in use today include carbon nanotubes/S composites,¹⁰ graphene/S composites,^{11–15} conductive polymer/S

composites,^{16–22} porous oxide additive composites,²³ and nanostructured Li_2S cathodes.²⁴ The basic electrochemical reaction taking place in most of these carbon/S based electrode composites is the same, i.e. elemental S is reduced to lithium polysulphides of different chain lengths during the discharge followed with a precipitation of Li_2S as end discharge product. While charging, the reverse process of discharge takes place, in which most of the lithium polysulphides are oxidised back to elemental sulphur.

A different mechanism was proposed for the poly(acrylonitrile)/sulphur (PAN/S) based cathode materials which are typically synthesised by heating a mixture of polyacrylonitrile (PAN) and S at moderate temperatures.^{21,22,25} This class of materials has been widely investigated due to their advantages in terms of high capacity, cycling stability and compatibility with conventional electrolytes used in Li-ion batteries.^{21,22,25–27} However, the synthesis process, the material structure, and the working principle of these cathode composites remain under debate and need further understanding.^{28–31}

In this study, we analyse the PAN/S composite via the use of analytical techniques, such as operando mode UV-Vis spectroscopy and 4-electrode modified Swagelok cell. We compare electrochemical behaviour with results obtained from the cathode composites in which sulphur is impregnated in the pores of carbon black particles. Both aforementioned analytical tools were recently used for studying mechanisms in the different Li–S battery systems.^{32–34}

With both techniques, we can detect soluble polysulphides responsible for the shuttle lock system in the case of using carbon black/sulphur composite, while we prove the absence of formation of polysulphides in the case of PAN/S composites.

2. Experimental

a) Preparation of composites

The polyacrylonitrile (PAN) and sulphur were obtained from Sigma-Aldrich. A mixture of PAN and sulphur in a weight.% ratio of 30:70 was ball milled for 30 minutes at 300 rpm and heat treated in a ceramic crucible in a quartz tube at 300 °C under argon atmosphere for 6 hours. After cooling to room temperature, a sample with a black colour was recovered. In the same way, carbon black (Vulcan) and sulphur were taken in a weight ratio of 60:40, respectively, and ball milled for 30mins at 300 rpm. The ball milled mixture was heated to 155 °C under argon atmosphere for 6 hours. Both composites were checked using CHNS elemental analysis. The results of the PAN/S composite showed that sample contains C: 38.73 wt.%, S: 40.25 wt.%, and N: 14.67 wt.%; the remainder is hydrogen. The carbon/S composite showed 40 wt.% of sulphur in the composite.

b) Preparation of electrode and electrochemical characterization:

Electrodes from the composites were prepared by making a slurry of composite, polytetrafluoroethylene (Sigma-Aldrich) and carbon black (PRINTEX XE2) in a mass ratio of 80:10:10 in isopropanol solvent. The slurry was then cast on the surface of aluminium foil, using the doctor blade technique. Dried electrodes were used to assemble a coffee bag batteries in an argon-filled glove box, where 1 M Lithium Bis(Trifluoromethanesulfonyl)Imide (LiTFSI) in sulfolane was used as an electrolyte and pure lithium metal was used as an anode electrode. The sulphur loading was 1.0–1.5 mg/cm² among the electrodes. Electrodes were separated by a glass fiber separator, and the amount of electrolyte used in all the batteries was normalized per active mass; it was 60 mL per mg of S. Galvanostatic cycling was performed at room temperature by using a Bio-Logic VMP3 instrument with a current density of 167.5 mA g^{−1} in the potential window between 3 V and 1.5 V for carbon/S composites and 3V–1 V for the PAN/S composite electrode. The coulombic efficiency was calculated as a ratio between discharge capacity and charge capacity obtained from the previous charge.

c) Operando mode measurements

In the case of UV-Vis measurements, electrodes prepared for the electrochemical characterisation were used. The battery assembly, electrochemical and UV-Vis measurements were carried out in the manner as explained by Patel et al.³²

The composites from PAN/S and carbon/S along with carbon black (Printex XE2) were taken in a 90:10 wt.% ratio and mixed to obtain powdered electrode materials. A controlled amount of the powder was taken in the 4-electrode Swagelok cell and the battery was assembled and cycled as explained by Dominko et al.³³ The quantity of electrolyte was quantified to 60 μL per mg of S in the cathode composite.

3. Results and Discussion

Figure 1 shows the thermogravimetric analysis of PAN, carbon/S and PAN/S samples performed in air atmosphere. All three samples can be clearly differentiated from each other in terms of their weight loss. The PAN polymer shows a degradation process in the temperature range of 270–600 °C with more than 60 wt.% loss. The carbon/S sample shows 40 wt.% loss that corresponds to elemental S in the sample. Meanwhile, the PAN/S sample did not show a similar kind of weight loss, as CHNS analysis indicated a presence of 40 wt.% S in the sample. The PAN/S shows slow degradation process at a relatively higher temperature of above 300 °C with less than 8 wt.% loss even after reaching

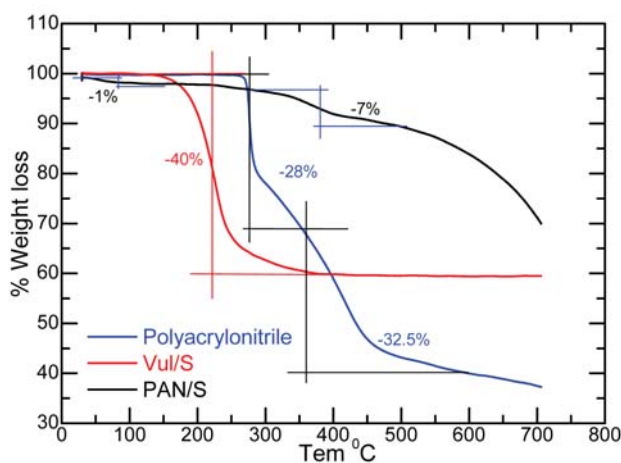
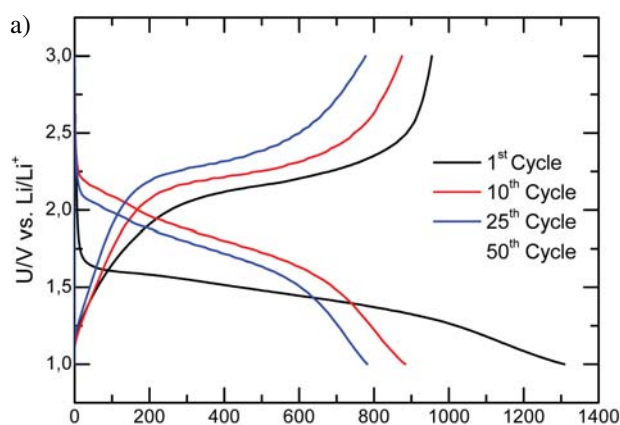


Figure 1. TGA plots of PAN (blue), carbon/S (red) and PAN/S composite (black).

700 °C, which indicates that the structure of the composite was quite stable and complex. It must be remembered that no posttreatment of the PAN/S composite in order to remove excesses of the elemental S was performed. Further proof for the absence of elemental S in the PAN/S composite can be found in the voltage profiles of the battery (Figure 2a) where a typical two plateaus characteristic for sulphur conversion into polysulphides and Li_2S is not observed.

The PAN/S composite starts to discharge at a voltage of around 1.8 V in the first cycle and the voltage plateau shifts to 2.1 V in the following cycles. The presented electrochemical behaviour is different in comparison to conventional Li–S battery behaviour obtained from composite containing 40 wt.% of elemental S, which starts to discharge at 2.4 V for the first plateau, followed by the second discharge plateau at approximately 2.0 V (Figure 2b). These first sets of measurement suggest the absence of elemental S in the PAN/S sample.



The results of the galvanostatic cycling for both composites are shown in Figure 3. The performance of PAN/S composite outperforms the carbon/S composite in terms of capacity retention, while the capacity fading was similar in both cases. Reasons for constant capacity fading can be diverse. One potential reason is a non-stable lithium surface exposed to the electrolyte or cathode stability in terms of wiring. Out from shape of the galvanostatic curves, a different mechanism of sulphur conversion can be expected. For that purpose, we used two operando mode analytical tools developed recently in our laboratory.^{31–33}

At first, in-situ UV-Vis spectroscopy was applied on both composites. As is known, when lithium polysulphides are formed during the battery operation, they tend to dissolve in the electrolyte and then diffuse into the separator of the battery. This indicates that they can be detected in the separator of the battery, as they have different colours depending upon the chain length of the polysulphide. The colour of the polysulphides ranges from dark red for long chain polysulphides to green for medium

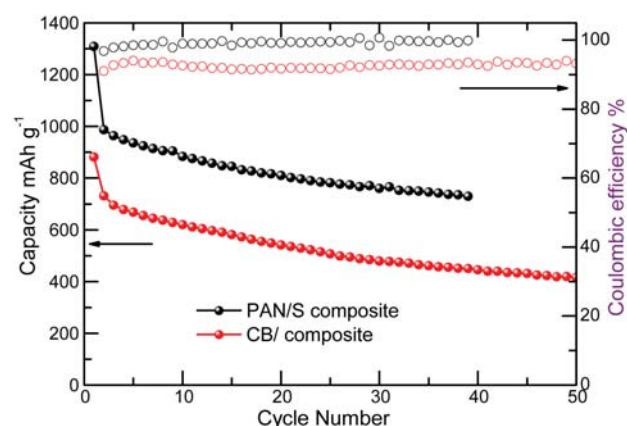


Figure 3. Discharge capacity (left axis) and coulombic efficiency (right axis) of PAN/S and carbon/S based composites for 50 cycles.

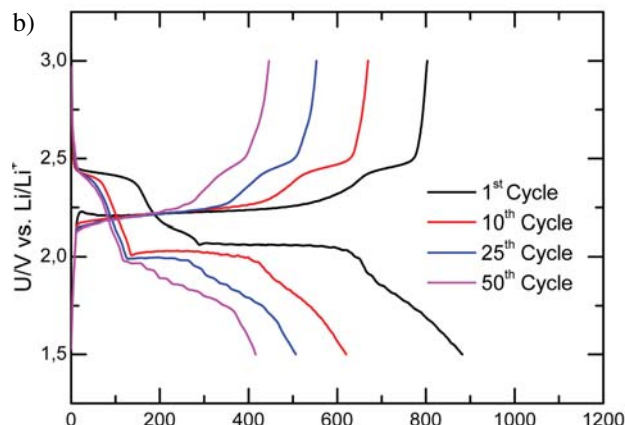


Figure 2. a) Discharge/charge capacity of the elemental PAN/S based composite in the selected cycles and b) Discharge/charge capacity of carbon/S composite in the selected cycles.

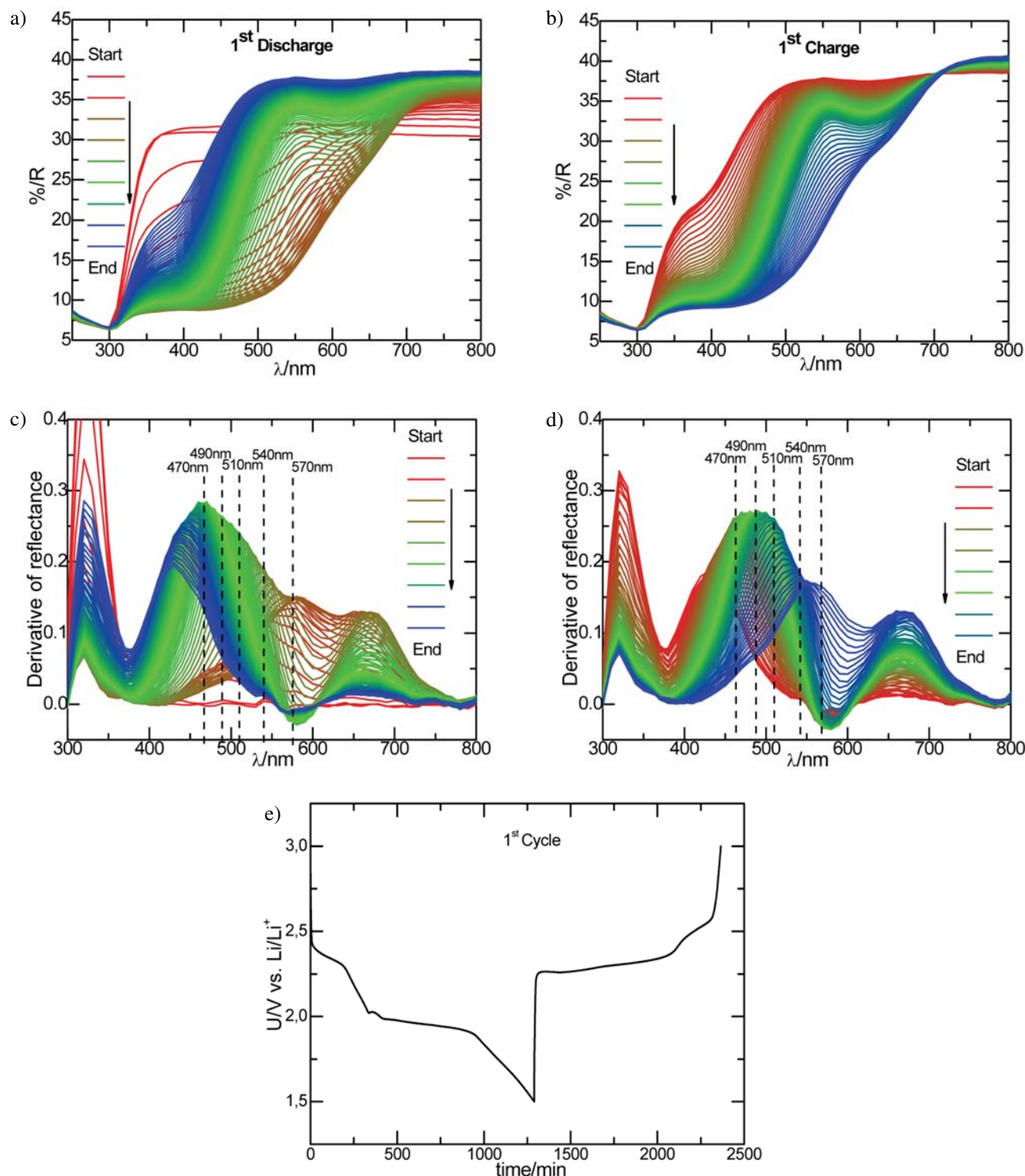


Figure 4. a, b) Obtained UV-Vis spectra from discharge and charge cycle of carbon/S composite. c, d) The first derivative of the obtained spectra during discharge and charge cycle showing shifts in the wavelength. e) The electrochemical curve of in-situ UV-VIS measurement of carbon/S composite.

and short chain polysulphides.³² By using this as a platform, the in-situ UV-VIS measurements were conducted. The obtained spectra indicate that when the carbon/S composite was used, the formation of lithium polysulphides of different chain lengths took place at different po-

tentials of battery operation. In other words, during discharge the long chain polysulphides were formed at higher voltage plateau and they appear at longer wave numbers on the UV-Vis spectra. The medium and short chain polysulphides were formed at the lower voltage plateau and

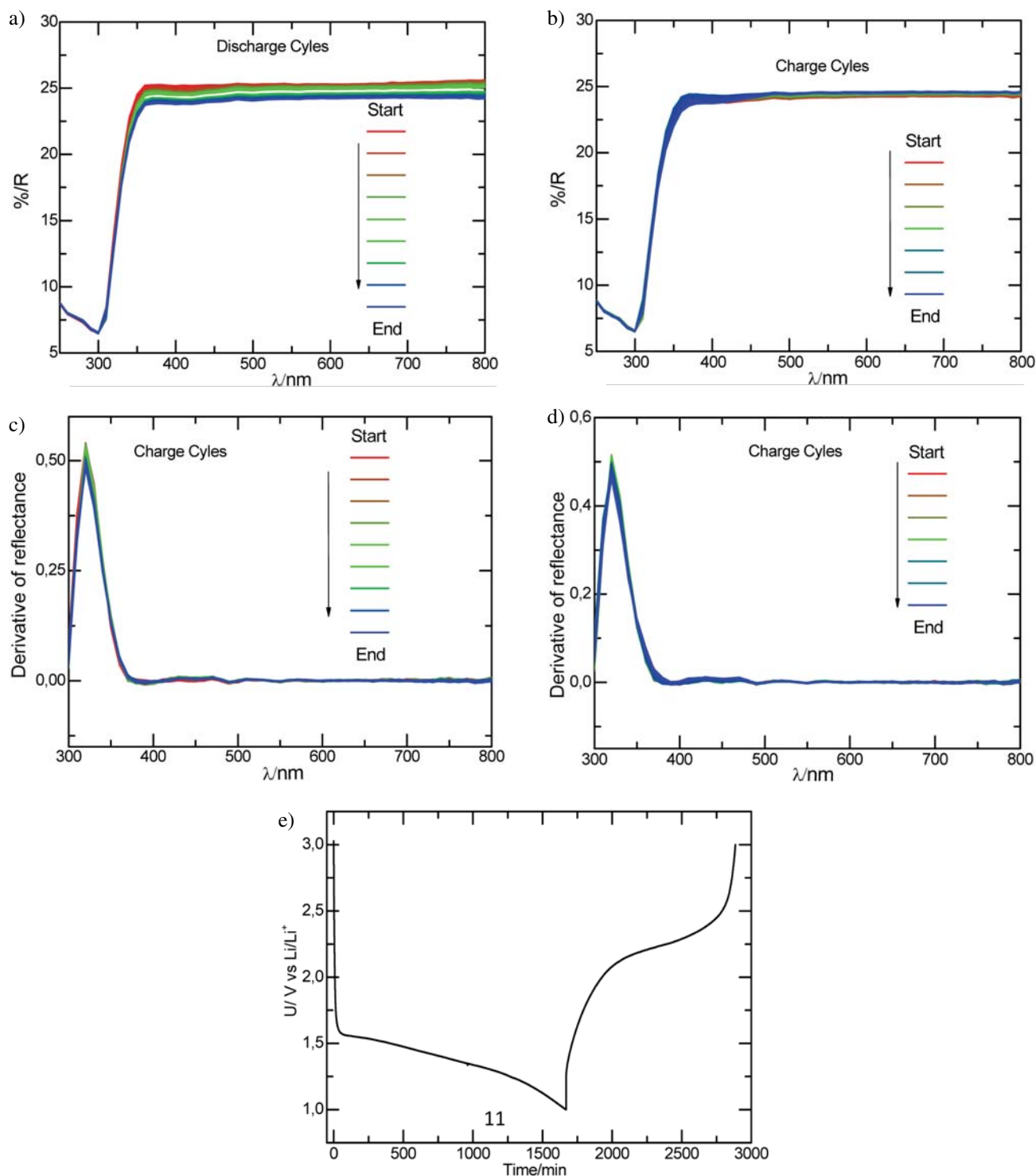


Figure 5. a, b) Obtained UV-Vis spectra from discharge and charge cycle of PAN/S composite. c, d) The first derivative of the obtained spectra during discharge and charge cycle showing shifts in the wavelength. e) The electrochemical curve of in-situ UV-VIS measurement of PAN/S composite.

appeared at shorter wave numbers in the UV-Vis spectra. While charging the battery the reverse process of discharge i.e. short chain polysulphides were formed at the beginning of the charge followed by the mid-chain and long chain polysulphides towards the end of the charge. Figure

4a and 4b shows the obtained UV-Vis spectra during discharge and charge of the first cycle. Figure 4c and 4d shows the first derivative of the obtained spectra during discharge and charge of the elemental carbon/S composite. The first derivative of the in-situ measurements clear-

ly show that the long chain polysulphides appeared at 570 nm, mid-chain polysulphides appeared in 510–550nm and short chain polysulphides at 430nm, which was a normal observation for the elemental S based composites.

However, the interesting part arises from the in-situ UV–Vis measurements on the PAN/S composite that clearly shows the absence of any lithium polysulphides. Figure 5a and 5b shows the obtained UV–Vis spectra during discharge and charge of the first cycle. Figure 5c and

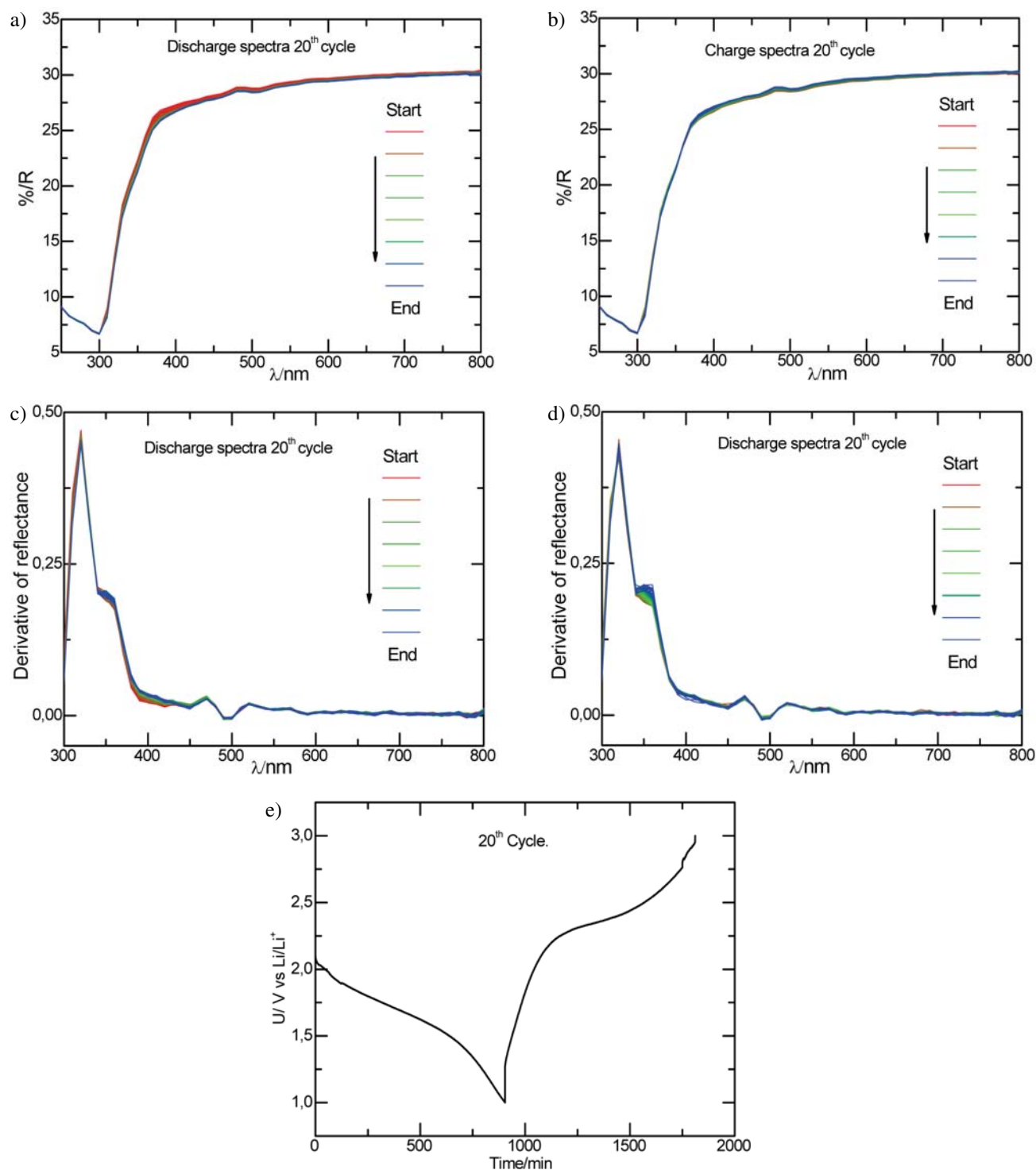


Figure 6. a, b) Obtained UV-Vis spectra from discharge and charge cycle of PAN/S composite on the 20th cycle. c, d) The first derivative of the obtained spectra during discharge and charge cycle showing shifts in the wavelength. e) The 20th cycle electrochemical curve of in-situ UV-VIS measurement of PAN/S composite.

5d shows the first derivative of the spectra during discharge and charge of PAN/S composites. The scanned spectra are stable; there is no change in the absorbance position suggesting the absence of any coloured species in the electrolyte. Knowing that the polysulphides are coloured in solvents, we can exclude the formation of soluble polysulphides during the reduction and oxidation process in the battery composed of PAN/S composite. Even in the case of the first derivative there was no trace of polysulphides that could show the shift in wave number to higher wavelengths. This confirms that there was no elemental S in the composite and consequently no soluble polysulphides that can be detected in the separator of the battery.

However, Fanous et al.³⁰ proposed that after the removal of any remaining elemental S in the PAN/S composites via an extraction with toluene their analysis suggested that S is exclusively covalently bound to carbon and not to nitrogen in the composite. They also proposed a chemical structure for PAN/S composite, which consists of a conjugated π -system. It was mentioned that the discharge profile of a cell prepared from LiTFSI in 1,3-dioxolane (DOL) and dimethoxyethane (DME) changes after the first cycle. They indicated that the voltage shifts from 1.8 V to 2.15 V in the following cycles was due to the formation of elemental S in

the electrode. It is expected that the reduction of S leads to the formation of polysulphides and a continuous diffusion of polysulphides, as in the case of a normal Li–S cell. In order to verify whether elemental S is, in fact, formed during the battery cycling, which could lead to lithium polysulphide formation, we measured a UV-Visible spectroscopy after 19th cycles, when the voltage plateau of the discharge curve was shifted to 2.1 V. All the parameters were kept the same while measuring the UV-Visible spectroscopy of 20th cycle, as done for the first cycle. The obtained spectra during discharge and charge for the 20th cycle are shown in Figures 6a and 6b and their derivatives are presented in Figures 6c and 6d. The small difference between the measured spectra and related derivatives suggests the change of the colour in the separator, which is probably connected with a degradation process in the cell. However, due to the absence of strong colouration we can exclude the formation of elemental sulphur in the formation cycles since this should be observed as a formation of polysulphides which would give a characteristic colour in the separator.

To confirm these findings, additional analysis by using a modified 4-electrode Swagelok cell was performed. In addition to the standard Swagelok configuration, this cell has two additional perpendicular electrodes (wires)

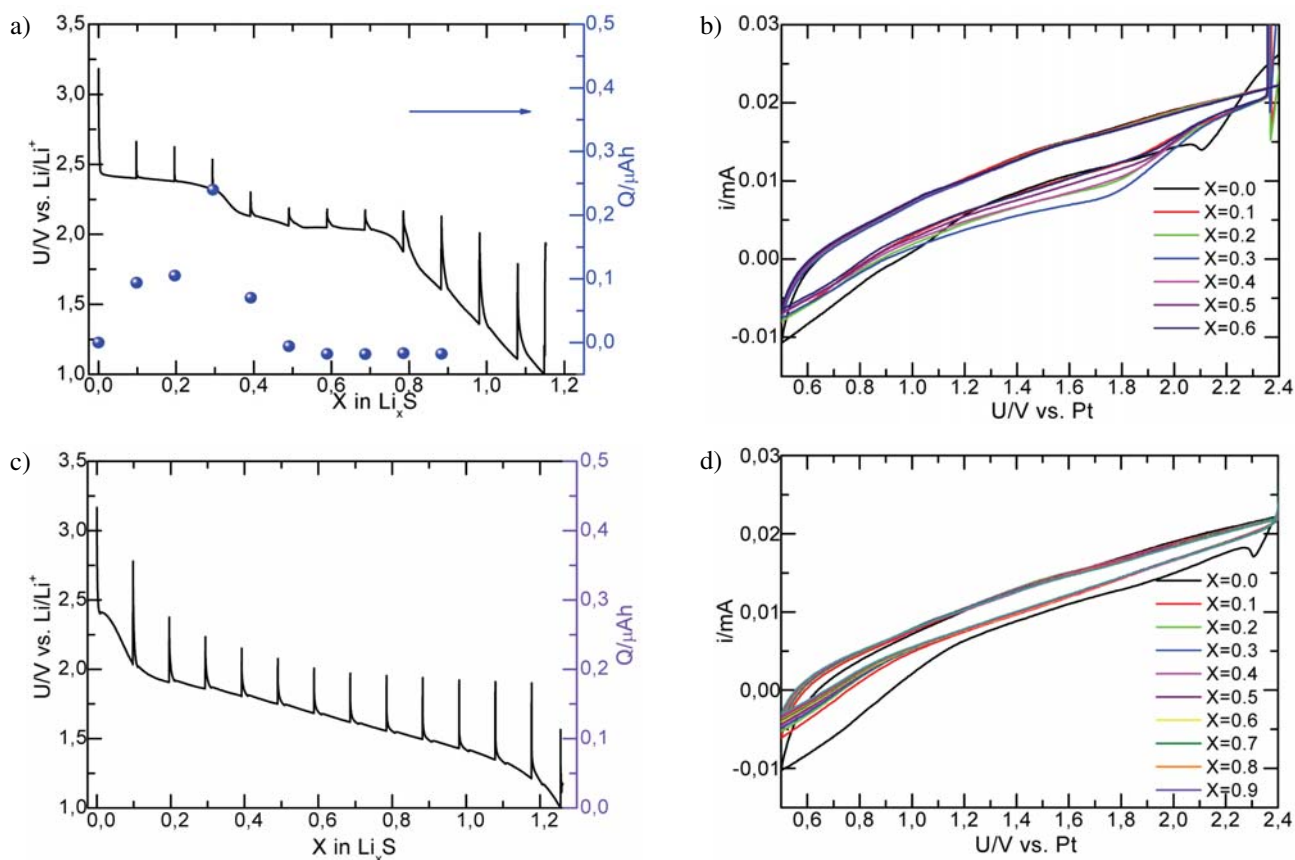


Figure 7. a) Electrochemical behaviour during the first reduction of carbon/S composite and cumulative charge obtained by the integration of individual CV scans. b) Corresponding CV scans measured for each $\Delta x = 0.1$ change of composition in Li_xS. c) Electrochemical behaviour during the first reduction of PAN/S composite. d) Corresponding CV scans measured for each $\Delta x = 0.1$ change of composition.

placed between two separators. Soluble polysulphides that are diffusing into the electrolyte are reduced on the stainless steel wire and the cumulative charge in the potential range between 2.25 V and 1.5 V versus platinum is used for the quantitative analysis.³² At first, we measured the elemental carbon/S based composite and lithium with the electrolyte solution of 1 M LiTFSI in sulfolane. Figure 7a (solid line) shows the discharge curve of the first cycle, and the corresponding CVs measured during the battery relaxation are shown in Figure 7b. A reduction peak at $U_p \approx 1.8$ V versus platinum is observed in almost every CV measurement. An integration of this peak in the 2.25 V–1.5 V range gives the cumulative charge which is plotted in Figure 7a (blue spheres). This result shows that soluble polysulphides are formed at the beginning of the discharge. The partial cumulative charge associated with soluble polysulphides increases at the beginning, reaches the maximum at a nominal composition of $Li_{0.3}S$, and then decreases, due to the disappearance of long chain polysulphides and formation of short chain polysulphides.

Then we measured the PAN/S-based composite in the same way as done for the carbon/S composite. Figure 7c (solid line) shows the discharge curve of the first cycle, and the corresponding CVs measured during the battery relaxation are shown in Figure 7d. As shown in Figure 7d we do not see any reduction peak at any voltage versus platinum and we do not have the reduction peak at all in the CV measurements. This result confirms our prediction that there are no soluble polysulphides formed, neither at the beginning of the discharge nor at lower voltages. The partial cumulative charge for the PAN/S composite was not calculated as no reaction peak in the CV cycles was observed.

The electrochemical measurements, TGA, and analytical observations indicate that the studied PAN/S composites did not contain any elemental S in the initial state nor was there any elemental S formation taking place during cycling. When PAN was heated to 300 °C along with elemental S, it led to the dehydration of PAN by S. The highly polar CN functional groups in the PAN cyclized to form a thermally stable heterocyclic compound that was conductive in nature. The capacity from the PAN/S composite may be derived from lithium insertion into the anionic conjugated backbone in a polymer network, as is known to occur in electrically conducting polymers.³⁷ One of the reasons for the observed capacity fading can be due to the degradation of the PAN/S host matrix and this can influence colouration of the electrolyte.

4. Conclusion

In this work, we successfully differentiate and demonstrate the process taking place in a PAN/S composite by comparing it with a carbon/S-based composite. Our analytical tools help us to obtain insight of the Li–S bat-

tery composites and understand the mechanisms inside the cell. The information provided by the in-situ analytical tools along with other experimental methods used in our work provide convincing evidence for the absence of elemental S in PAN/S composite initially or in the intermediate states of cycling. Needless to say that further experiments have to be done in this direction to understand PAN/S composite, as it is a promising composite for next generation batteries.

5. Acknowledgements

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Povzetek

Litij žveplovi akumulatorji predstavljajo novo generacijo akumulatorjev, kjer je poleg višje energijske gostote pričakovati tudi nižjo ceno. Ena izmed večjih težav, ki preprečuje njihovo komercializacijo, so topni polisulfidi, ki pospešijo degradacijo in upad kapacitete akumulatorja. Žveplo kovalentno vezano v organsko matriko (npr. v poliakrilonitril) izkazuje podobne elektrokemijske lastnosti in je eden izmed alternativnih katodnih materialov za litij žveplove akumulatorje. V tem članku smo primerjali elektrokemijske karakteristike kovalentno vezanega in prostega žvepla ter mehanizem delovanja, ki smo ga preučevali s pomočjo dveh in-situ analiznih metod. Uporaba in-situ UV-Vis spektroskopije in štiri elektrodne Swagelok celice pokaže, da v primeru kovalentno vezanega žvepla nimamo težav s topnimi polisulfidi.

Scientific paper

Electrochemical Circuit Elements

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In memory of prof. dr. Janko Jamnik.

Abstract

The vast majority of electrochemical processes can be modelled by resistors and capacitors. These will then be, in addition to usual circuit elements, electrochemical and chemical resistors or chemical capacitors. The paper shows the significance of understanding these parameters and their connections in given systems for a variety of timely scientific examples. This rationale mirrors one of the intellectual facets, if not the most important one, of Janko Jamnik's scientific work.

Keywords: Equivalent circuit, chemical capacitor, electrochemical resistor, transport, batteries, fuel cells

1. Introduction

This contribution is a tribute to Janko Jamnik's outstanding comprehension of electrochemical processes. In many joint papers we set out the concept of analyzing processes in terms of electrochemical equivalent circuits, by not only including electrical but also chemical and electrochemical circuit elements.^{1–5} Although there have been earlier treatments,^{6–10} the above-mentioned publications provide a significant step forward in terms of interpretation and understanding.

The set-out conception allows one to intuitively, but rather precisely though, tackle diverse problems such as stoichiometric polarization, Maxwell-Wagner polarization, battery storage, chemical diffusion in heterogeneous systems, or surface kinetics of oxygen incorporation in oxides.

A central role is played by the archetypical chemical capacitance that allows for a profound understanding of solid state processes whenever concentration changes are involved. The term has already been introduced by Pelton.¹¹ Here a more appropriate thermodynamic access will be given. For this purpose we consider a binary solid (generalization is trivial), where the composition of mobile component is regulated by fixing the chemical potential of component 1 μ_1 (e.g. by outer partial pressure), while the rigid component 2 is characterized by a given mole number n_2 . The other control parameters are total pressure (p) and temperature (T). (Note that it is possible to vary the partial pressure of component 1 at constant total pressure.)

re.) One then refers to a $\mu_1 N_2 p T$ -ensemble for which the characteristic potential will be

$$\Gamma = U - TS + pV - \mu_1 n_1 \quad (1)$$

with the consequence that

$$\begin{aligned} d\Gamma &= Vdp - SdT + \mu_2 dn_2 - n_1 d\mu_1 \\ &= Vdp - SdT - n_1 d\mu_1 \equiv \sum_i \ell_i d\lambda_i. \end{aligned} \quad (2)$$

(As the generalized Gibbs-Duhem equation leads, for homogeneous systems, to $U = TS - pV + \mu_1 n_1 + \mu_2 n_2$, one finds $\Gamma = \mu_2 n_2$. For a one-component system, it results that $\Gamma = 0$, corresponding to the characteristics of the “intensive ensemble” referred to.¹²)

We can now define generalized capacities via $\frac{\partial^2 \Gamma}{\partial \lambda_i^2}$, namely the thermal capacitances (specific heat $C^T \equiv T \frac{\partial S}{\partial T} = -T \frac{\partial^2 \Gamma}{\partial T^2}$), the mechanical capacitance (compressibility $\chi \equiv -\frac{1}{V} \frac{\partial V}{\partial p} = -\frac{1}{V} \frac{\partial^2 \Gamma}{\partial p^2}$) and the chemical capacitance, viz. $C_1^\delta \equiv \frac{\partial n_1}{\partial \mu_1} = -\frac{\partial^2 \Gamma}{\partial \mu_1^2}$. The sign of these three quantities is decisive in a thermodynamic stability analysis.¹³ One realizes that the definition is also analogous to the electrical capacitance $C^q = \frac{\partial q}{\partial \phi}$ (q : charge, ϕ : electric potential) that

becomes relevant if one also includes electrical effects. In view of the significance of the electrochemical potential $\tilde{\mu} = \mu + zF\phi$ one is tempted to define a generalized electrochemical capacitance $\tilde{C}_1^\delta = \frac{\partial n_1}{\partial \tilde{\mu}_1}$. Yet as shown in Ref.

[5] this is only meaningful in very special cases. In general C^δ and C^q refer to different microscopic processes. In contrast, the introduction of an electrochemical resistor R^δ (in the quasi-one dimensional case defined via $\nabla \tilde{\mu}/j$), however, is generally meaningful owing to current density j being proportional to $\sigma \nabla \tilde{\mu} = \sigma \nabla \mu + zF\sigma \nabla \phi$, i.e., owing to referring to the very same transport process caused by both concentration and electrical potential gradients (σ : conductivity). Let us consider a few selected examples.

2. Chemical Diffusion

A comparatively simple situation is chemical diffusion in the bulk, e.g. describing the variation of oxygen stoichiometry on varying the outside oxygen partial pressure. The diffusion coefficient describing that variation is

$$D^\delta \propto \left(\frac{\sigma_{\text{ion}} \sigma_{\text{eon}}}{\sigma} \right) \left(\frac{\partial \mu_{\text{O}}}{\partial c_{\text{O}}} \cdot \frac{1}{RT} \right). \quad (3)$$

For a simple planar geometry (L : thickness, A : area) the first factor can be rewritten as inverse chemical resistor

$$R^\delta = \frac{\sigma}{\sigma_{\text{ion}} \sigma_{\text{eon}}} \frac{L}{A}, \quad (4)$$

resulting from a series switching of ionic and electronic resistors, as in a logical sense both ions and electrons are needed.¹⁴

The second factor in Eq. (3) can be rewritten as

$\left(\frac{\partial \ln a_{\text{O}}}{\partial \ln c_{\text{O}}} \frac{1}{c_{\text{O}}} \right)$, where a_{O} is the oxygen activity and $\frac{\partial \ln a_{\text{O}}}{\partial \ln c_{\text{O}}}$ is referred to as thermodynamic factor, often erroneously termed an enhancement factor. In fact the second factor represents an inverse chemical capacitance¹⁵

$$C^\delta = A \cdot L \frac{\partial c_{\text{O}}}{\partial \mu_{\text{O}}} \frac{RT}{F^2}, \quad (5)$$

expressing the storage effect, here occurring in the course of bulk diffusion. The term enhancement factor stems from the incorrect conception of comparing D^δ with the tracer diffusion coefficient D^* . Yet, chemical diffusion refers to the defects only, and a closer look yields under di-

lute conditions $\left(\frac{1}{c_{\text{ion}}} + \frac{1}{c_{\text{eon}}} \right)$ for the second term, with c_{ion} ,

c_{eon} being the respective defect concentrations. Tracer diffusion, however, involves the total ionic ensemble and has hence a much higher chemical capacitance, thus D^* must be much smaller owing to the low fraction of defects. The fair comparison is with the defect diffusion coefficients. The analogously defined thermodynamic factor of the defects is now close to unity and in most cases even reflects a depression factor.¹⁶

The product $R^\delta \cdot C^\delta$ yields the time constant τ^δ as L^2/D^δ , which is well-known from diffusion kinetics. (Depending on the boundary conditions, the relation between τ^δ and L^2/D^δ includes numerical constants.) At any rate τ^δ is proportional to L^2 as both R^δ and C^δ are proportional to L . This is very different from the electric analogue where for quasi-1D transport (along L) $R \propto L$ but $C \propto 1/L$ with $\tau = RC$ being independent of L . Table I gives thickness dependences of various time constants for transport along L for various degrees of nano-structuring: nano-plates of thickness L (dimensionality of nano-structuring $d = 1$), nano-rods of cross section $L \times L$ (i.e. $d = 2$) and nanodots of dimension $L \times L \times L$ (i.e. $d = 3$), showing that the results for τ_e are independent of d .¹⁷

Table I: Dimensionality (d) dependent exponents (n) of the explicit thickness (L) dependence of resistors, capacitors, relaxation times for various electrochemical mechanisms (ϵ).¹⁷ The interface process is in series with bulk process, i.e. transport across the boundary is considered.

mechanism ϵ	R_ϵ	C_ϵ	τ_ϵ
dielectric response	$2-d$	$d-2$	0
diffusion controlled bulk storage	$2-d$	d	2
interfacial rate controlled bulk storage	$1-d$	d	1
interfacial storage (semi-infinite) [#]	$1-d$	$d-1$	0

[#] In the finite size regime the situation is non-autonomous as it also depends on the neighboring phases. Reprinted with permission from the American Chemical Society, Copyright 2013.¹⁷

The generalization of the chemical diffusion concept to internal reactions includes so-called differential trapping factors.¹⁸ This generalization nicely explains why D^δ in Fe-doped SrTiO₃ is orders of magnitude smaller than expected according to the then established theory. The reason is the coupling of internal redox reactions to the electronic ensemble. The same happens for Y-doped ZrO₂, where both ionic and electronic conductivities are insensitive to additional redox active impurities.¹⁹ An intuitive understanding again is enabled by the concept of chemical capacitance. In ZrO₂ the internal valence changes do not vary R^δ but increase the chemical capacitance (increase of reservoir).

3. Interfacial Kinetics

The chemical capacitance concept became most influential as far as the surface kinetic analysis is concerned. Here R^δ refers to the reaction rate of the rate-limiting surface step, but C^δ is given by the uptake in the bulk as long as the sample is not extremely thin. The corresponding equivalent circuit is now used in labs all over the world to analyze surface kinetics via impedance spectroscopy (cf. Fig. 1) (cf. Ref.²⁰ for a recent example of interest). In most cases of interest, the decisive resistive term refers to the surface kinetics, while the capacitive terms refer to the bulk capacity

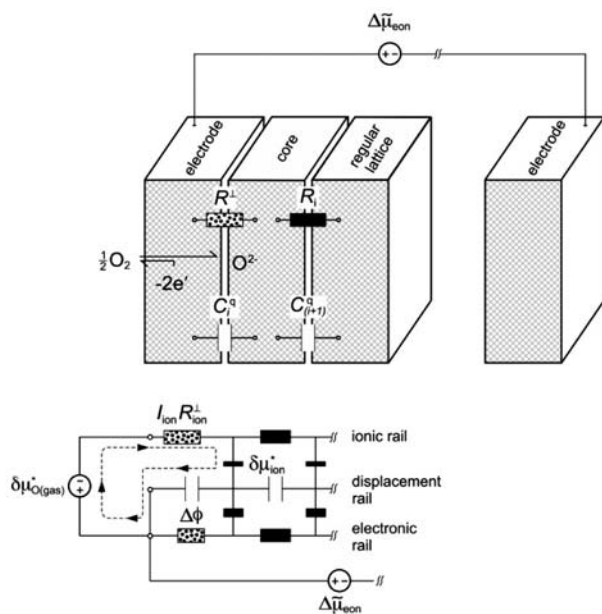


Figure 1. Equivalent circuit of the electrode/electrolyte impedance under gas exchange. Reprinted by permission of the PCCP Owner Societies.⁵

A further highly relevant example is mass transport across a grain boundary. Then R^δ refers to the transfer resistance of the neutral mass, typically controlled by the structure of the grain boundary core or by space charge effects owing to depletion. Well investigated are grain boundaries in SrTiO₃, where both necessary defects, the oxygen as well as electronic defects are depleted and hence R^δ is substantial. Again C^δ refers to the bulk if one can ignore storage of oxygen in the grain boundary itself. The detailed treatment of transport across space charge zones is rather involved.¹

The full power of these considerations unfolds in heterogeneous systems (Fig. 2).

If we refer to a polycrystal in which grain boundary diffusion is very fast so that transport from there to the grains of size ℓ occurs on a different time scale, both R^δ and C^δ in the effective D_m^δ are due to bulk,¹⁶ and hence

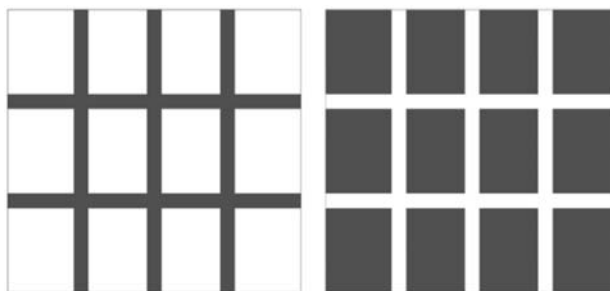


Figure 2. Diffusion through a ceramic with highly permeable grain boundaries (left) or scarcely permeable grain boundaries (right). The black and white zones refer to the local permeabilities.

$$D_m^\delta = \frac{L^2}{\ell^2} D_{\text{bulk}}^\delta \propto \frac{L^2}{\ell^2} \frac{1}{R_{\text{bulk}}^\delta C_{\text{bulk}}^\delta}. \quad (6)$$

Such a situation is, e.g., met in the case of chemical diffusion in donor-doped SrTiO₃. In the opposite case that the grain boundaries of width d_{gb} are hardly permeable and transport through them is very sluggish when compared to the bulk, a case briefly addressed above, it holds for not too small grains.^{16,21}

$$D_m^\delta \propto \frac{\ell}{d_{\text{gb}}} \frac{1}{R_{\text{gb}}^\delta C_{\text{bulk}}^\delta}. \quad (7)$$

Even though redistribution in the grain interior is locally fast, grain size matters for the time behavior, as the chemical diffusion is the more sluggish, the more component mass the grain-interior is able to take up.

In fact generally the diffusion rate is not only determined by the rates at which the carriers would move in the steady state (R^δ) but also by how much mass is absorbed (C^δ). This is analogous to thermal diffusion, where the speed of the temperature variation not only depends on the thermal conductivity (conduction of heat) but also on the heat capacity (absorption of heat).

At the moment we are considering chemical diffusion along interfaces where again deconvolution into R^δ and C^δ is highly beneficial. It seems that chemical diffusion in job-sharing composites along the boundaries is extremely fast not only due to the expectedly low R^δ but also due to a very low C^δ . Here one meets one of the cases in which C^δ represents truly an electrochemical capacitance.²²

4. Impedance of Mixed Conductor

A long-standing impedance problem became transparent by the use of equivalent circuits.²³ Imagine a purely ionic conductor between electrodes that are only reversible for electrons. Evidently, the material should behave as a capacitor with vertical line (90°) in the impedance plot. Now let us tackle this problem from the viewpoint of

a mixed conductor with vanishing electronic conductivity. For a mixed conductor one expects a Warburg increase (45°) bending in a semi-circular behavior and an intercept yielding the electronic resistance. If one nullifies the electronic contribution (i.e. pure ion conductor) an inconsistency occurs as we would obtain an infinitely extended Warburg line and not a vertical one.

This inconsistency is shown to be due to the neglect of space charge polarization that also must occur. The generalized approach is characterized by the counter-play of space charge and chemical bulk capacitances depending on the charge carrier concentration rather than the conductivities (Fig. 3).²³

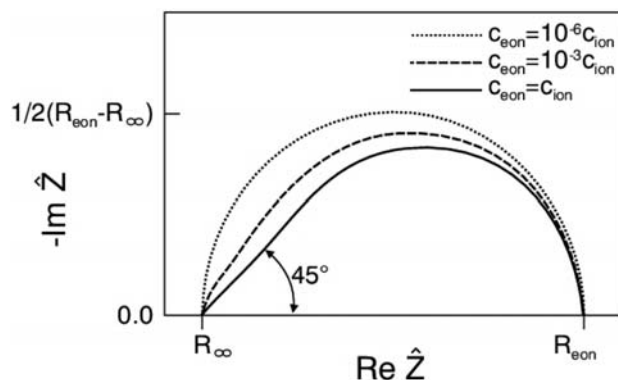


Figure 3. Normalized impedances for ion blockage. On variation of the defect concentration the response changes from Warburg to a pure semicircular behavior. Reprinted with permission from Elsevier.²³

Other contributions to be mentioned in that context are (i) the polarization behavior of a polycrystalline material in which the grain boundaries as well as electrodes can lead to a stoichiometric polarization²⁴ and (ii) the establishing of a penetration impedance method with the potential of identifying buried interfaces.²⁵

5. System Impedance and Relevance of Morphology

As already mentioned, electrochemical processes of interest can be modelled by R 's and C 's if we refer to electrical, chemical and electrochemical circuit elements. Figure 4 indicates that size dependencies can occur through size dependencies of the effective materials constants, e.g., overall conductivities of a composite (e.g. through space charge effects), but also through the path dependence itself ($n = 2$ for chemical diffusion, see above). This points towards the necessity for electrochemically integrated circuits for high performance electrode design in Li-batteries.¹⁷

Figure 5 shows the equivalent circuit of a battery as given by Jamnik. It clearly exhibits that both the ionic path ($\text{Li}^+(\text{Li}) \rightarrow \text{Li}^+$ (electrolyte) $\rightarrow \text{Li}^+$ (counter electro-

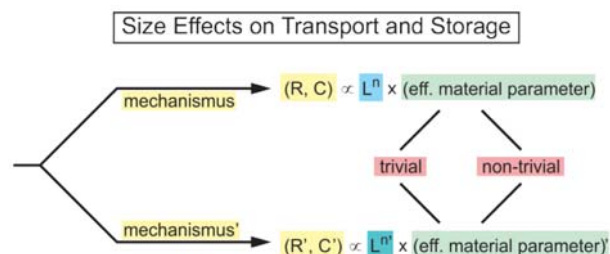


Figure 4. Explicit and implicit size dependence of resistive and capacitive elements. The explicit size dependence (L^n) reflects the direct geometrical influence (cf. Table 1), the implicit size dependence mirrors the dependence of the effective materials parameter on the interfacial density in the case of a heterogeneous object.¹⁷ Tuning the parameters by size effects can even result in a switching-over to an alternative mechanism. Reprinted with permission from the American Chemical Society, Copyright 2013.¹⁷

de) $\rightarrow$ || current collector) and the electronic path (e^- (current collector) $\rightarrow e^-$ (electrode) $\rightarrow$ || (electrolyte)) are stopped by chemical capacitors.

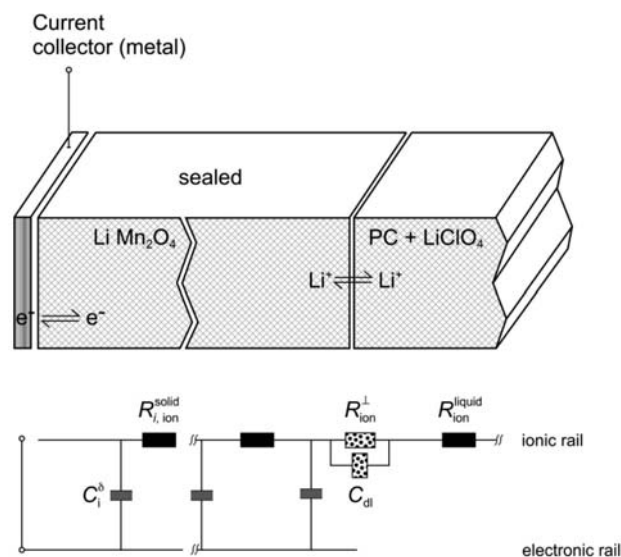


Figure 5. Scheme and equivalent circuit of a lithium battery (cathode side). Reprinted by permission of the PCCP Owner Societies.⁵

This equivalent circuit can describe the mass and charge variation upon discharge/charge in quite a detail. Solid state electrochemistry is full of such interwoven electrical and chemical problems and in such cases Jamnik's contributions are of invaluable worth and we miss him as a competent discussion partner.

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Povzetek

Večino elektrokemijskih procesov lahko modelno predstavimo z upori in kondenzatorji. Ti bodo nato poleg običajnih elementov vezja, elektrokemijski in kemijski upori ali kemijski kondenzatorji. V članku je prikazan pomen razumevanja teh parametrov in njihovih povezav v določenih sistemih, za različne aktualne znanstvene primere. To načelo se zrcali v enem izmed intelektualnih pristopov, ki so znanstveno delo, morda celo najpomembnejše, Janka Jamnika.

Scientific paper

Linear Conductances of Gated Graphene Structures with Selected Connectivity

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In memory of prof. dr. Janko Jamnik.

Abstract

The ratio of conductances through carbon-ring based molecules are calculated for various positions of source-drain electrode leads on the molecule. These ratios are usually integers the so-called magic numbers. We find that deviations of the magic number ratios are either zero or quadratic in ratios of tight-binding model parameters.

Keywords: Conductance, Landauer formalism, quantum dots, interference effects

1. Introduction

Charge transport through nanostructures represents a challenge from the experimental point of view as well as for theoretical approaches.¹ Most of experimental work so far has been done for semiconducting structures² as promising candidates for tailoring various electronic devices, such as single electron transistors³ and charge or spin quantum bits.⁴ One of the advantages of semiconductor technology is its versatility in formatting structures on demand, with reliable and reproducible gating and connectivity to external leads. A disadvantage of these structures is their relatively large spatial extent limiting functional operation to low temperatures. For possible applications to sensors or quantum information processing devices room-temperature operation is desired, which demands reduction of device size leading to larger energy scale. Molecules connected to metallic leads therefore represent ideal candidates for devices where phase-coherent transport between attached electrodes is required at moderate temperatures.^{5,6}

In general, nanostructures exhibit an extremely rich spectrum of quantum phenomena. In particular, in conductance measurements strong electron-electron interaction leads to Coulomb blockade,⁷ the Kondo effect,⁸ various spin dependent anomalies^{9,10} or instabilities due to vi-

brational degrees of freedom, where different electron-phonon interactions can play an important role.^{11,12} Due to large coherence lengths in clean structures, interference effects also play an essential role in transport through the nano-device, as was recently studied experimentally even at room temperature.^{13–21} Small graphene based nanostructures are well defined since they are nearly defect-free which enables reproducible conductance measurements exhibiting subtle interference effects.^{22–24}

Here we concentrate on “magic” ratios, found recently in connectivity driven electrical conductance of graphene-like aromatic molecules.²⁵ Theoretical analysis of experiments using mechanically controlled break junctions to measure electrical conductance of such molecules reveals specific ratios between different connectivity geometry of external leads. These magic ratios appear in the regime of particle-hole symmetrically filled molecules, where the chemical potential is located at the HOMO-LUMO mid-gap. Numerical analysis has been performed for a tight-binding approximation of a molecule weakly coupled to charge reservoirs connected to the graphene molecule via linear chains referred to as source and drain leads. In this paper we analyze the stability of magic ratios with respect to changes of the coupling to the leads and also due to changing the potential of top gates which in turn change the electron occupation of the molecule.

2. Model and Methods

We consider polycyclic aromatic hydrocarbon-like graphene structures, coupled to two metallic electrodes via source and drain leads. This is shown schematically in Figure 1 for the case of a benzene molecule.

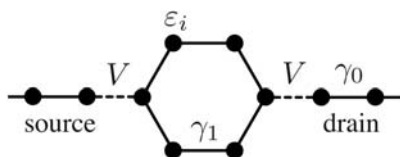


Figure 1. A benzene-like structure (molecule) attached to the leads.

To model such a system we adopt the effective Hamiltonian

$$H = H_{\text{molecule}} + H_{\text{leads}} + H_{\text{coupling}}, \quad (1)$$

where the molecule is described in terms of a tight-binding Hamiltonian

$$H_{\text{molecule}} = \sum_{i,\sigma} \varepsilon_i n_{i,\sigma} - \sum_{i,j,\sigma} \gamma_{ji} c_{j,\sigma}^\dagger c_{i,\sigma}. \quad (2)$$

Here i and j run over the sites of the molecule, i.e., the p_z orbitals on each of the carbon atoms, $c_{i,\sigma}^\dagger$ and $c_{i,\sigma}$ are the electron creation and annihilation operator, respectively, for site i and spin σ , and $n_{i,\sigma} = c_{i,\sigma}^\dagger c_{i,\sigma}$ is the electron number operator. ε_i are the on-site energies controlled by the top gate voltage with energy zero being the Fermi-energy in the leads in the limit of zero source-drain bias. They may also be influenced by the electrodes attached to the leads. To be specific in what follows, we assign a uniform on-site energy ε_0 to all molecular sites, which includes the effect of the top gate voltage. However, we allow on-site energies on the two sites where the leads are attached to the molecule to take a different value of ε_i . γ_{ji} are hopping integrals for which we take a value depending only on the distance on a lattice between the two sites, i.e., $\gamma_{ji} = \gamma_1$ if atoms i and j are nearest neighbors, $\gamma_{ji} = \gamma_2$ if atoms i and j are next nearest neighbors, etc. Taking into account that next nearest neighbor hopping integrals in graphene are at least an order of magnitude smaller than nearest neighbor hopping integrals,²⁶ in what follows we neglect all but nearest neighbor hopping integrals γ_1 .

The leads, modeled as chains with sites connected by nearest neighbor hopping integrals γ_0 , have a Hamiltonian

$$H_{\text{leads}} = \sum_{\alpha,i,\sigma} \varepsilon_{\alpha,i} n_{\alpha,i,\sigma} - \gamma_0 \sum_{\alpha,i,\sigma} c_{\alpha,i+1,\sigma}^\dagger c_{\alpha,i,\sigma} + \text{H.c.} \quad (3)$$

Here $\alpha \in \{\text{s,d}\}$ labels the source and drain leads, respectively, and i runs over the sites of a lead. $c_{\alpha,i,\sigma}^\dagger$, $c_{\alpha,i,\sigma}$ and $n_{\alpha,i,\sigma} = c_{\alpha,i,\sigma}^\dagger c_{\alpha,i,\sigma}$ are the electron creation, annihilation operator and the electron number operator for lead sites, respectively.

The couplings between leads and the molecule are

$$H_{\text{coupling}} = -V \sum_{\alpha,\sigma} c_{\alpha,1,\sigma}^\dagger c_{i_\alpha,\sigma} + \text{H.c.} \quad (4)$$

Here V is the hopping integral between the lead site closest to the molecule and the molecular site i_α to which lead α is attached.

The electron-electron and the electron-phonon interactions are not considered here – the systems are not in the Coulomb blockade regime. However, some interesting features due to the electron correlations in benzene were found recently.^{27,28}

In the absence of many-body effects, the conductance of such a molecule, i.e., the proportionality coefficient between the current through the molecule and the voltage V_{sd} applied between the source and the drain electrode, is, in the limit of vanishing V_{sd} given by the Landauer-Büttiker formula,^{29,30}

$$G = G_0 \int \mathcal{T}(\varepsilon) \left(-\frac{\partial f(\varepsilon)}{\partial \varepsilon} \right) d\varepsilon, \quad (5)$$

where $G_0 = 2e^2/h$ is the conductance quantum, with e and h being the electron charge and the Planck constant, respectively. $\mathcal{T}(\varepsilon)$ is the transmission probability through the molecule at energy ε . $f(\varepsilon) = (\exp \frac{\varepsilon - \mu}{k_B T} + 1)^{-1}$ is the equilibrium Fermi function of the leads with μ and T being their chemical potential and temperature, respectively, and k_B being the Boltzmann constant. For the sake of convenience we set the chemical potential to the middle of the band in the leads and vanishing on-site energies in the leads, $\mu = \varepsilon_{\alpha,i} = 0$.

To calculate the transmission probability $\mathcal{T}(\varepsilon)$ we need to find the scattering eigenstate $|\psi\rangle$ of the Hamiltonian for an electron with energy ε and spin σ , incoming from the source electrode,

$$H|\psi\rangle = \varepsilon|\psi\rangle. \quad (6)$$

We expand such an eigenstate in the local basis states of the molecule $c_{j,\sigma}^\dagger|0\rangle$ and the leads $c_{\alpha,j,\sigma}^\dagger|0\rangle$

$$|\psi\rangle = \sum_j \psi_j c_{j,\sigma}^\dagger|0\rangle + \sum_{\alpha,j} \psi_{\alpha,j} c_{\alpha,j,\sigma}^\dagger|0\rangle. \quad (7)$$

The wavefunction in the source electrode is a linear combination of an incoming and a reflected plane wave, $\psi_{\text{s},j} = e^{-ikj} + re^{ikj}$, while the wavefunction in the drain elec-

trode consists of a transmitted wave, $\psi_{d,j} = te^{ikj}$. The wave-vector k can be calculated from the dispersion relation of the leads, $\varepsilon = -2\gamma_0 \cos k$. r and t are the reflection and the transmission amplitude, respectively. The transmission probability is $\mathcal{T}(\varepsilon) = |t|^2$.

Here we demonstrate the method of calculating the transmission probability $\mathcal{T}(\varepsilon)$ for the case of the simplest possible molecule, namely a single site with the on-site energy of ε_0 coupled to two leads. The Schrödinger equation for such a system reads as a set of linear equations for t , r and ψ_0

$$\begin{aligned} -\gamma_0 (e^{-2ik} + re^{2ik}) - V\psi_0 &= \varepsilon (e^{-ik} + re^{ik}), \\ -V(e^{-ik} + re^{ik}) + \varepsilon_0\psi_0 - Vte^{ik} &= \varepsilon\psi_0, \\ -V\psi_0 - \gamma_0 te^{2ik} &= \varepsilon te^{ik}. \end{aligned} \quad (8)$$

By eliminating r and ψ_0 we find the transmission amplitude,

$$t = \frac{i\frac{2V^2}{\gamma_0} \sin k}{\varepsilon - \varepsilon_0 + \frac{2V^2}{\gamma_0} e^{ik}}. \quad (9)$$

A Breit-Wigner resonance of width $\Gamma_0 = \Gamma_s + \Gamma_d$, where $\Gamma_s = \Gamma_d = \frac{2V^2}{\gamma_0}$ are the partial widths due to coupling to the source and the drain lead, respectively, forms at energy ε_0 in the transmission probability in the wide band limit where $\gamma_0 \gg \varepsilon$, $\varepsilon_0 \Gamma_0$,

$$\mathcal{T}(\varepsilon) = \frac{\left(\frac{\Gamma_0}{2}\right)^2}{(\varepsilon - \varepsilon_0)^2 + \left(\frac{\Gamma_0}{2}\right)^2}. \quad (10)$$

Generalization to more general molecules with arbitrary topology is straightforward. In the limit of weak coupling to the leads, $\Gamma = \frac{2V^2}{\gamma_0} \gg \gamma_1$, the transmission probability consists of similar resonances, positioned at eigenenergies of the molecule.

3. Results

As shown in Figure 2(a–c), sites of graphene-like molecules we consider in this work form a bipartite lattice, i.e. they break up into two sublattices in such a way that unprimed sites 1, 2, 3 ..., forming one sublattice, are connected only to primed sites 1', 2', 3' ..., forming the other sublattice. The Hamiltonian of such a system possesses the particle-hole symmetry.³¹ In molecules considered here, this symmetry is actually weakly broken due to next nearest neighbor hopping integrals γ_2 . Since, as discussed in Section 2, $\gamma_2/\gamma_1 \ll 1$ for structures considered in this work, we neglect such terms in what follows. Therefore, the conductance as a function of the top gate voltage ε_0 is even with respect to the particle-hole symmetric point $\varepsilon_0 = 0$, where the Fermi energy of the leads coincides with the center of the HOMO-LUMO gap. This is shown in Figure 2 where the zero temperature and room temperature conductances are plotted as a function of top gate voltage for benzene, naphthalene and anthracene molecules for a particular choice of sites to which electrodes are attached. The zero temperature conductance curves consist of a set of resonances, each corresponding to a molecular level being at the Fermi energy of the electrodes. The width of a resonance measures the coupling of the molecular level to the leads. Note that some of the re-

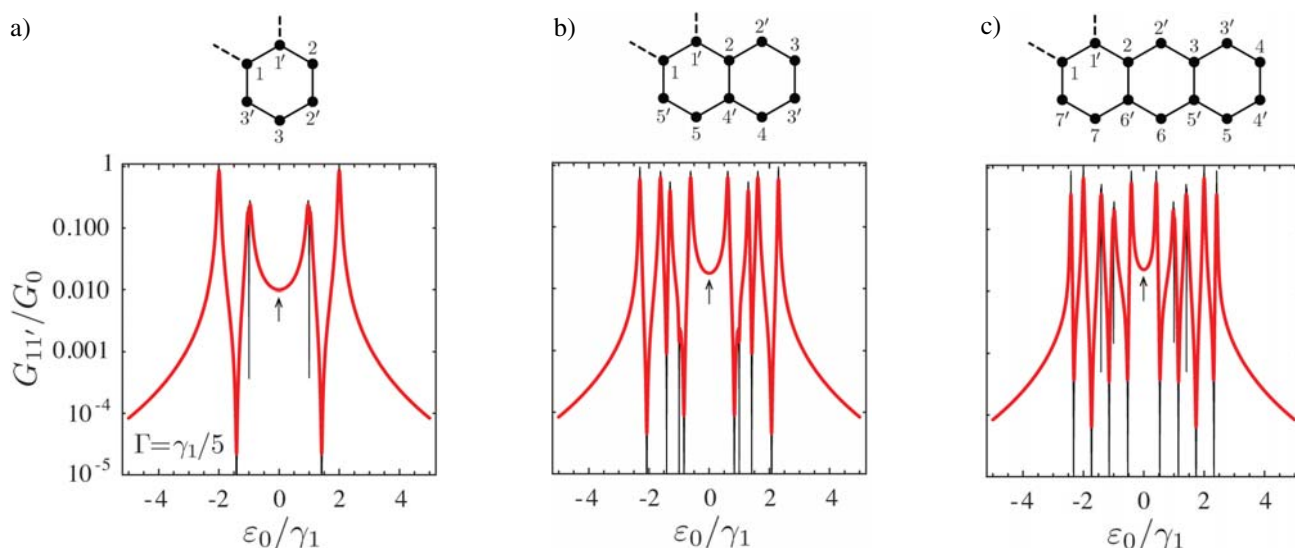


Figure 2. Conductance as a function of top gate voltage of the (a) benzene, (b) naphthalene, and (c) anthracene molecule when one electrode is connected to site 1 and the other is connected to site 1' of the molecule, at $T = 0$ (black lines) and at the room temperature (red lines). The coupling of an electrode to the molecular site is $\Gamma = \gamma_1/5$. Arrows indicate the position of the center of the HOMO-LUMO gap.

sonances are split due to degeneracy of molecular orbitals. At room temperature, i.e., $T = 300 \text{ K} \sim 0.01 \gamma_1$, with $\gamma = 2.5 \text{ eV}$ as appropriate for graphene,³² thermal broadening only slightly lowers the peak heights, increases their widths and broadens minima. Within the HOMO-LUMO gap the effect of finite temperature is negligible at room temperature. As we will concentrate on the vicinity of the center of the HOMO-LUMO gap in the rest of this work, the calculations will be done at zero temperature in what follows.

We now study the dependence of the conductance on the on-site energy ε_0 incorporating the top gate voltage and the coupling Γ of a lead to a molecular site, for different combinations of molecular sites to which the electrodes are attached. Let us first discuss the situation at the particle-hole symmetric point (indicated by arrows in Figure 2), when the coupling to the leads Γ is weak.²⁵ If the leads are connected to two sites in the same sublattice the conductance is zero due to destructive interference. On the other hand, if the leads are attached to two sites in distinct sublattices, a “magic integer” can be associated with such a system. The ratio of conductances of two such systems is the so-called “magic ratio” which is a square of the ratio of the corresponding magic integers.

To prove this, we follow Ref. 25. Provided the molecule is weakly coupled to the leads, all the on-site energies of the molecular sites are equal and the Fermi energy of the leads coincides with the center of the HOMO-LUMO gap, the conductance is proportional to the absolute square of the Green’s function of an isolated molecule between sites to which the source and drain leads are attached $G_{i_d i_s}(0)$. The Green’s function is determined by the molecular Hamiltonian, $G(0) = (0 - H_{\text{molecule}})^{-1}$. If only nearest neighbor hopping is included in the Hamiltonian and all the corresponding hopping integrals are equal, such a Hamiltonian can be written in a block form: the diagonal blocks representing Hamiltonians of each sublattice are zero while the off-diagonal blocks are proportional to the adja-

cency matrix of the graph of the molecule. The inverse of the adjacency matrix, multiplied by its determinant, is a matrix containing integer matrix elements, the “magic integers”. Comparing conductances of the same molecule with different connectivities, the ratio of those conductances is equal to the square of the ratio of the corresponding magic integers, provided the coupling to the leads is the same for both connectivities.

In Figure 3 we show how magic ratios evolve with the coupling Γ increasing both at the particle hole symmetric point and away from it at $\varepsilon_0 = \gamma_1/5$, which is still within the HOMO-LUMO gap of all the molecules considered in this work. At the particle-hole symmetric point a magic ratio, provided its weak coupling value is different from one, starts to deviate from its weak coupling value when Γ becomes of the order of γ_1 . A magic ratio increases with Γ if its weak coupling value is less than one and it decreases with Γ if its weak coupling value is larger than one. At the particle-hole symmetry point $\varepsilon_0 = 0$ the deviation of magic ratios greater than one is quadratic in Γ for $\Gamma \ll \gamma_1$. Away from the center of the HOMO-LUMO gap magic ratios deviate from a square of the ratio of magic integers even in the weak coupling limit. The deviation is again quadratic in ε_0 for $|\varepsilon_0| \ll \gamma_1$. At $\varepsilon_0 \neq 0$ a molecule conducts even if electrodes are attached to sites in the same sublattice. Compared to the conductance when electrodes are attached to sites in different sublattices it is smaller by a factor of $(\varepsilon_0/\gamma_1)^2$ for $|\varepsilon_0| \ll \gamma_1$.

An electrode may shift the on-site energy at the molecular site to which it is attached. This turns out to be another cause of deviation of a magic ratio from a square of the ratio of the magic integers. Figure 4 displays that the departure of on-site energies ε_1 at these molecular sites from on-site energies $\varepsilon_0 = 0$ at other molecular sites causes a magic ratio to increase quadratically with ε_1 if it is larger than one for $\varepsilon_1 = 0$. A magic ratio is independent of ε_1 if it is equal to one for $\varepsilon_1 = 0$.

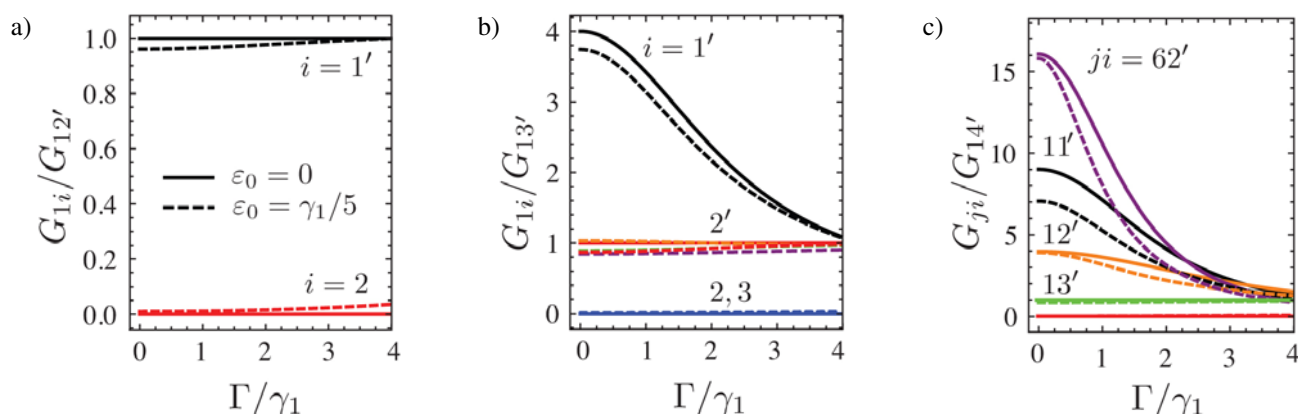


Figure 3. Magic ratios at $T = 0$ of the (a) benzene, (b) naphthalene, and (c) anthracene molecule at the particle-hole symmetric point (full lines) and for $\varepsilon_0 = \gamma_1/5$ (dashed lines) as a function of the coupling Γ of an electrode to a molecular site. Molecular sites to which electrodes are attached are indicated next to each curve. The other combination of electrode attachment sites appearing in the conductance ratio corresponds to the most distant sites of a particular lattice.

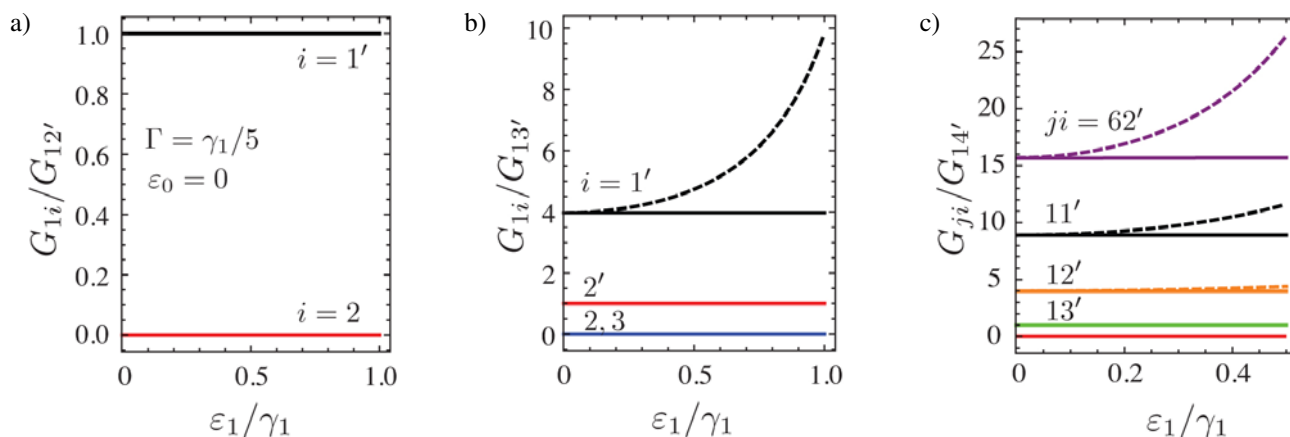


Figure 4. Magic ratios at $T = 0$ of the (a) benzene, (b) naphthalene, and (c) anthracene molecule when the on-site energies ε_i at molecular sites where the electrodes are attached differ from those at other molecular sites where $\varepsilon_0 = 0$ (dashed lines). Full lines show magic ratios for $\varepsilon_i = 0$. Here $\Gamma = \gamma_1/5$.

4. Conclusions and Outlook

In conclusion, we have calculated the ratios of conductances of graphene-like structures for different combinations of sites to which leads are attached away from the regime where those ratios can be expressed in terms of magic integers. The deviations were due to top gate voltage pushing on-site energies ε_0 away from the center of the HOMO-LUMO gap, due to the coupling to leads Γ being non-negligible and due to the electrodes causing on-site energies ε_i on atoms to which leads are attached to deviate from on-site energies on other atoms. The deviation from the ratio given by magic integers was found to become important when those parameters become of the order of the hopping integral γ_1 of the molecule. For small values of those parameters, the deviation was found to increase proportionally to $(\varepsilon_0/\gamma_1)^2$, $(\Gamma/\gamma_1)^2$ or $(\varepsilon_i/\gamma_1)^2$. Furthermore, if the top gate voltage is non-zero, the molecule conducts even when both leads are attached to sites in the same sublattice, which for other perturbations is not the case. What remains to be done is to study the robustness of magic ratios to Coulomb interaction and to perturbations breaking the particle hole symmetry, i.e., the second neighbor hopping within the molecule.

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Povzetek

Razmerja prevodnosti skozi molekule, sestavljene iz obročev ogljikovih atomov, smo izračunali za različne konfiguracije priključkov elektrod med izvorom in ponorom. Običajno so ta razmerja cela števila, t.i., magična števila. Ugotovili smo, da s spreminjanjem parametrov v okviru modela tesne vezi ostanejo magična števila ali nespremenjena, ali pa so od parametrov odvisna kvadratično.

Scientific paper

Interactions of L-Aspartic Acid with Aqueous Solution of 1,2-Propanediol at Different Temperatures: A Volumetric, Compressibility and Viscometric Approach

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Abstract

The volumetric, acoustic and viscometric methods are used for investigating the interactions of L-aspartic acid (Asp) in aqueous solution of 1,2-propanediol (PD) over a temperature of (298.15, 303.15 and 308.15) K at atmospheric pressure. Using the experimental results, the apparent molar volume, V_ϕ , limiting apparent molar volume, V_ϕ^0 , the slope, S_v , and partial molar volume of transfer, $V_{\phi, tr}^0$, have been calculated from density data. The apparent molar isentropic compressibility, $K_{\phi, s}^0$, limiting apparent molar isentropic compressibility, $K_{\phi, s}^0$, its slope, S_k , and partial molar compressibility of transfer, $K_{\phi, s, tr}^0$, have been calculated from speed of sound data. These values are also used for calculating the number of water molecules hydrated, n_H , to the Asp. The viscosity data has also been used to determine relative viscosity η_r , viscosity B-coefficients, temperature derivative of B-coefficients, dB/dT and viscosity B-coefficients of transfer, B_{tr} . The calculated parameters have been discussed in terms of various solute–solute and solute–solvent interactions prevailing in these solutions. Further, a detailed insight into the physicochemical interactions between Asp and aqueous PD, e.g., ion-hydrophilic and hydrophilic–hydrophilic interactions along with the structure-making tendency have been retrieved through the perusal of these calculated parameters.

Keywords: L-aspartic acid, Apparent molar volume, 1, 2-Propanediol, Transport properties

1. Introduction

The physicochemical and thermodynamic properties of amino acids are of utmost interest, as these biomolecules are the building blocks of all living organisms which provide valuable information leading to a better understanding of proteins. Since proteins are large complex molecules, their direct study of interactions become difficult. Therefore, the feasible approach is to investigate interactions of the model compounds of proteins, e.g., amino acids, in aqueous and mixed-aqueous solutions.^{1–6} The choice of water for preparing mixed solvent stems from its important and unique role in determining the structure and stability of protein. Its presence also gives rise to hydrophobic forces, which are of prime importance in stabilizing native globular structure of protein.^{7,8} Binary solvents, representing a concentrated mixture of a co-sol-

vent and water as the principal solvent, are of special interest from both the biological and the physico-chemical view points.^{9–11} The added co-solvent may exert a stabilizing or destabilizing influence or neutral effect¹² on the native conformation of a protein.

It is known that polyhydric alcohols increase the thermal stability of proteins or reduce the extent of their denaturation by other substances.^{13,14} The alkane diols have wide range of applications in pharmacology and cosmetic industry, however, they are not components of living organisms, but they act as a vehicle for pharmaceuticals or cosmetics when introduced into living organisms.¹⁵ Thus, the properties of amino acids in aqueous-polyols solutions are essential in understanding the chemistry of biological systems.^{16–18}

The literature survey reveals physical and thermodynamic studies on Asp (abbreviated as Asp) and inor-

ganic salts as a function of temperature^{19,20} but no report seems to be available on thermodynamic studies of Asp and organic solvents. However, the effect of dielectric constant on protonation equilibria of Asp in one of the alkane diol-water mixtures has been reported recently by Rani et al.²¹ In order to gather information about thermodynamic and transport properties of Asp in aqueous and aqueous-1, 2-propanediol (the latter being abbreviated as PD) solvent, we are using volumetric, compressibility and viscosity approaches at different temperatures.

2. Experimental

Asp and PD with mass fraction purity >99% obtained from Sigma Aldrich were used as such without further purification. Freshly prepared triply distilled with specific conductance less than $1 \times 10^{-6} \text{ S cm}^{-1}$ was used for the preparation of solutions in which 0.5M hydrochloric acid was added to increase the solubility.²¹ The aqueous solution of PD was used as solvent to prepare solutions of Asp of different molal concentrations. All the solutions were prepared with care and stored in special airtight bottles to avoid the exposure of solutions to air and evaporation. The solutions were prepared by weighing on an electronic single pan five digit Mettler Balance (Model AE-240) with an accuracy of $\pm 0.01 \text{ mg}$. The densities of the solutions were automatically measured, using an Anton Paar DMA 5000M densimeter. A density check or an air/water adjustment was performed at 20°C with triply distilled, degassed water, and with dry air at atmospheric pressure. As the density is extremely sensitive to temperature, it was controlled to $\pm 1 \times 10^{-3} \text{ K}$ by built-in Peltier device. The sensitivity of the instrument corresponds to a precision in density measurements of $1 \times 10^{-3} \text{ kg m}^{-3}$. The uncertainty of the density estimates was found to be within $\pm 5 \times 10^{-2} \text{ kg m}^{-3}$. The speeds of sound in the solutions were measured using a single-crystal variable-path multi-frequency ultrasonic interferometer (M-82, Mittal Enterprises) having stainless steel sample cell (with digital micrometer) operating at fixed frequency of 4 MHz. The uncertainty in speeds of sound measurements was found to be within $\pm 0.5 \text{ m s}^{-1}$. The temperature of the sample solutions was maintained to an accuracy of $\pm 0.01 \text{ K}$ in an electronically controlled thermostatic water bath (Model: TIC-4000N, Thermotech, India). The viscosities of the solutions were measured by using Ubbelohde viscometer. The viscometer containing the test liquid was allowed to stand for about 30 minutes in a thermostatic water bath so that the thermal fluctuations in viscometer were minimized. The time of flow was recorded with a digital stopwatch with an accuracy of $\pm 0.01 \text{ s}$. The accuracy of viscosity measurements was found to be $\pm 1 \times 10^{-6} \text{ Pa s}$.

3. Results and Discussion

3. 1. Apparent Molar Volume

The experimental values of densities of Asp in aqueous and 5%, 10%, 15% and 20% aqueous solutions of PD, measured at 298.15, 303.15 and 308.15 K, were used to calculate apparent molar volume (V_ϕ) of the solutions using Eq. (1).

$$V_\phi = (M/\rho) - [(\rho - \rho_0) 1000/\text{m}\rho\rho_0] \quad (1)$$

where m is the molality (mol kg^{-1}) of the solution, M is the molar mass of the solute (kg mol^{-1}), and ρ_0 and ρ are the densities (kg m^{-3}) of the solvent and solution, respectively.

The values of densities and apparent molar volume at various temperatures are reported in Table 1, while the curve for latter is presented in Figure 1. The results reveal that the apparent molar volume increases with increase in PD concentration as well as temperature. This may be attributed to the increase in solvation (release of some solvent molecules from loose solvation layers of the solute in solution) of Asp at higher temperature as well as at higher concentration of PD due to strong attractive interactions.²² The high value of apparent molar volume for Asp in different concentrations of solvent (5% PD to 20% PD) as compared to their values in aqueous solution suggests that ion-hydrophilic and hydrophilic-hydrophilic interactions dominate the ion-hydrophobic and hydrophobic-hydrophobic interactions.

3. 1. 1. Limiting Apparent Molar Volume

The limiting apparent molar volume (V_ϕ^0), also known as partial molar volume, has been obtained by employing the least square fitting of apparent molar volume values to the linear Eq. (2).

$$V_\phi = V_\phi^0 + S_v m \quad (2)$$

where S_v , the experimental slope, indicates the nature of the solute-solute interactions, while V_ϕ^0 shows the presence of solute-solvent interactions. The values of S_v , V_ϕ^0 along with their standard errors, reported in Table 2, show less positive behavior for S_v , thus, suggesting that solute-solute interactions are weak. On the contrary, the values of V_ϕ^0 are positive and show an increasing behaviour with increase in concentration of PD as well as increase in temperature, thus, indicates dominance of solute-solvent interaction in the system. Our values of V_ϕ^0 of Asp in aqueous solution at 298.15 K and 308.15 K resemble to the values reported by Banipal et al.¹⁹ which is shown in parenthesis in Table 2, whereas the data at 303.15 K could not be found in the literature. The value at 298.15 K is also in close agreement with the values obtained from the work of Millero et al.²³ and Mishra and Ahluwalia.²⁴

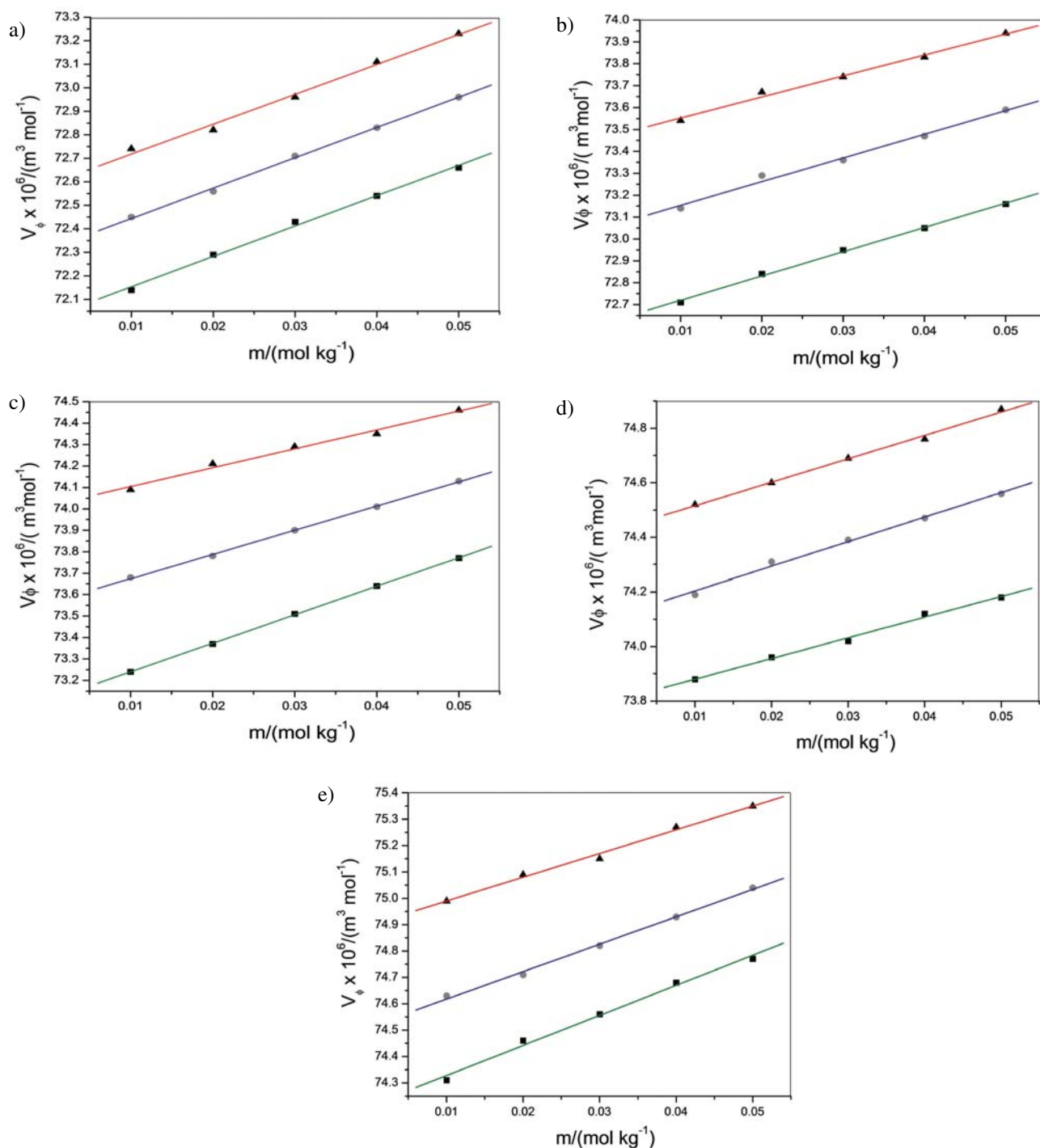


Figure 1. Variation of apparent molar volume versus molality of Asp in aqueous and aqueous + PD solutions: (a) water, (b) 5% PD, (c) 10% PD, (d) 15% PD and (e) 20% PD at temperatures, $T/K = 298.15$ (■); 303.15 (●); 308.15 (▲).

3. 1. 2. Partial molar Volume of Transfer

The partial molar volume of transfer, $V_{\phi, \text{tr}}^0$, of Asp from aqueous to aqueous PD solution has been calculated using Eq. (3).

$$V_{\phi, \text{tr}}^0 = V_{\phi}^0(\text{aq} - \text{PD solution}) - V_{\phi}^0(\text{aq}) \quad (3)$$

where $V_{\phi}^0(\text{aq})$ is the limiting apparent molar volume of Asp in aqueous medium (Table 2). The V_{ϕ}^0 of an amino acid can be considered as²⁵:

$$V_{\phi}^0 = V_{\text{vw}} + V_{\text{void}} + V_{\text{shrinkage}} \quad (4)$$

where $V_{\text{shrinkage}}$ is shrinkage in volume due to solvent-sol-

Table 1. Densities, ρ , and apparent molar volumes, V_ϕ , of solutions of Asp in aqueous and aqueous-PD (5, 10, 15 and 20%, PD w/w in water) as function of molality, m , of Asp at different temperatures.

$m/(\text{mol kg}^{-1})$	$\rho/(\text{kg m}^{-3})$			$V_\phi \times 10^6/(\text{m}^3 \text{mol}^{-1})$		
	298.15 K	303.15 K	308.15 K	298.15 K	303.15 K	308.15 K
Asp in aqueous						
0.00	1005.148	1003.655	1002.002			
0.01	1005.756	1004.260	1002.605	72.14	72.45	72.74
0.02	1006.361	1004.863	1003.205	72.29	72.56	72.82
0.03	1006.963	1005.461	1003.801	72.43	72.71	72.96
0.04	1007.560	1006.056	1004.393	72.54	72.83	73.11
0.05	1008.155	1006.648	1004.983	72.66	72.96	73.23
Asp in 5% aqueous-PD						
0.00	1008.396	1006.824	1005.024			
0.01	1008.998	1007.422	1005.618	72.71	73.14	73.54
0.02	1009.597	1008.016	1006.209	72.84	73.29	73.67
0.03	1010.193	1008.609	1006.798	72.95	73.36	73.74
0.04	1010.786	1009.198	1007.384	73.05	73.47	73.83
0.05	1011.376	1009.783	1007.967	73.16	73.59	73.94
Asp in 10% aqueous-PD						
0.00	1011.533	1009.832	1008.014			
0.01	1012.129	1010.424	1008.602	73.24	73.68	74.09
0.02	1012.722	1011.013	1009.187	73.37	73.78	74.21
0.03	1013.311	1011.599	1009.770	73.51	73.90	74.29
0.04	1013.897	1012.182	1010.351	73.64	74.01	74.35
0.05	1014.479	1012.761	1010.928	73.77	74.13	74.46
Asp in 15% aqueous-PD						
0.00	1015.324	1013.487	1011.458			
0.01	1015.913	1014.073	1012.041	73.88	74.19	74.52
0.02	1016.500	1014.656	1012.622	73.96	74.31	74.60
0.03	1017.085	1015.237	1013.200	74.02	74.39	74.69
0.04	1017.666	1015.816	1013.776	74.12	74.47	74.76
0.05	1018.246	1016.391	1014.348	74.18	74.56	74.87
Asp in 20% aqueous-PD						
0.00	1018.722	1016.786	1014.614			
0.01	1019.306	1017.367	1015.192	74.31	74.63	74.99
0.02	1019.886	1017.946	1015.767	74.46	74.71	75.09
0.03	1020.464	1018.521	1016.340	74.56	74.82	75.15
0.04	1021.038	1019.093	1016.909	74.68	74.93	75.27
0.05	1021.610	1019.662	1017.476	74.77	75.04	75.35

Table 2. Limiting apparent molar volumes, V_ϕ^0 , experimental slopes, S_v , and standard deviations of linear regression (in parenthesis) of Asp in aqueous and aqueous solution of PD at different temperatures.

System	$V_\phi^0 \times 10^6/(\text{m}^3 \text{mol}^{-1})$			$S_v \times 10^6/(\text{m}^3 \text{mol}^{-2} \text{kg})$		
	298.15 K	303.15 K	308.15 K	298.15 K	303.15 K	308.15 K
Asp in aqueous	72.02 (± 0.015) 72.30 (± 0.040) ¹⁹	72.31 (± 0.009)	72.59 (± 0.225) 73.23 (± 0.020) ¹⁹	12.9 (± 0.470)	12.9 (± 0.300)	12.7 (± 0.680)
Asp in 5% aqueous-PD	72.60 (± 0.009)	73.04 (± 0.020)	73.45 (± 0.016)	11.1 (± 0.302)	10.8 (± 0.621)	9.6 (± 0.503)
Asp in 10% aqueous-PD	73.10 (± 0.003)	73.56 (± 0.006)	74.01 (± 0.018)	13.3 (± 0.101)	11.3 (± 0.191)	8.8 (± 0.565)
Asp in 15% aqueous-PD	73.80 (± 0.010)	74.11 (± 0.013)	74.43 (± 0.010)	7.6 (± 0.326)	9.0 (± 0.416)	8.6 (± 0.326)
Asp in 20% aqueous-PD	74.21 (± 0.018)	74.51 (± 0.011)	74.90 (± 0.014)	11.4 (± 0.565)	10.4 (± 0.346)	9.0 (± 0.447)

vent interactions, V_{void} is contribution due to voids and V_{vw} is van der Waals volume. The contribution of first two terms in Eq. (4) is believed to be approximately same in water and also in mixed aqueous solutions. Hence, the positive volume of transfer for Asp may be rationalized in

terms of a decrease in the volume of shrinkage. This means few water molecules in the vicinity of the Asp may be released to the bulk water in presence of PD. In general, the types of interactions occurring between Asp and PD may be classified as follows:^{26,27}

- ionic-hydrophilic interactions between NH_3^+ and COO^- head groups of Asp and OH group of PD
- hydrophilic-hydrophilic interactions between CH_2COOH group of Asp and OH group of PD
- ionic-hydrophobic interactions between NH_3^+ and COO^- head groups of Asp and methyl group of PD
- hydrophilic-hydrophobic interactions between OH group of PD and CH_2 group of Asp, and

- hydrophobic-hydrophobic interactions between CH_2 group of Asp and the methyl group of PD.

The positive values of partial molar volume of transfer indicate that interactions of types (a) and (b), mentioned above, predominates due to reduction in electrostriction effect and enhancement of overall structure of water. Furthermore, the values of partial molar volume of transfer increase with increase in concentration of PD in the solution as shown in Table 3. This may be due to greater ionic-hydrophilic and hydrophilic-hydrophilic group interactions with increased concentration of PD. The explanation lies in the fact that the OH group of PD is highly polar; it has a strong electron-pair donating ability which is capable of forming $\text{HO}\cdots\text{HOOC}$ hydrogen bond with the side group of Asp. With the increasing concentration of PD, the probability of forming hydrogen bond increases and, hence, favoring the ionic-hydrophilic

Table 3. Partial molar volumes of transfer, $V_{\phi, \text{tr}}^0 \times 10^6 / (\text{m}^3 \text{mol}^{-1})$, of Asp in aqueous solution of PD at different temperatures.

System	298.15 K	303.15 K	308.15 K
Asp in 5% aqueous-PD	0.58	0.73	0.86
Asp in 10% aqueous-PD	1.08	1.25	1.42
Asp in 15% aqueous-PD	1.78	1.80	1.84
Asp in 20% aqueous-PD	2.19	2.20	2.31

Table 4. Speeds of sound, u , and apparent molar isentropic compressibilities, $K_{\phi, s}$, of Asp in aqueous and aqueous-PD (5, 10, 15 and 20%, PD w/w in water) as function of molality, m , of Asp at different temperatures.

$m / (\text{mol kg}^{-1})$	$u / (\text{m s}^{-1})$			$K_{\phi, s} \times 10^{15} / (\text{m}^3 \text{mol}^{-1} \text{Pa}^{-1})$		
	298.15 K	303.15 K	308.15 K	298.15 K	303.15 K	308.15 K
Asp in aqueous						
0.00	1497.76	1510.50	1520.80			
0.01	1498.29	1511.03	1521.33	−25.82	−25.17	−24.54
0.02	1498.81	1511.55	1521.86	−25.47	−24.66	−24.13
0.03	1499.32	1512.05	1522.35	−25.05	−24.04	−23.52
0.04	1499.82	1512.54	1522.85	−24.59	−23.54	−23.14
0.05	1500.30	1513.02	1523.32	−24.04	−23.07	−22.52
Asp in 5% aqueous-PD						
0.00	1524.62	1533.33	1543.52			
0.01	1525.14	1533.85	1544.04	−23.05	−22.46	−21.84
0.02	1525.65	1534.36	1544.55	−22.69	−21.99	−21.44
0.03	1526.15	1534.85	1545.04	−22.27	−21.49	−20.91
0.04	1526.64	1535.33	1545.52	−21.89	−21.03	−20.45
0.05	1527.12	1535.79	1545.98	−21.51	−20.46	−19.93
Asp in 10% aqueous-PD						
0.00	1562.03	1568.23	1573.04			
0.01	1562.55	1568.74	1573.55	−20.50	−19.67	−19.22
0.02	1563.06	1569.24	1574.04	−20.15	−19.27	−18.67
0.03	1563.56	1569.72	1574.52	−19.78	−18.73	−18.23
0.04	1564.04	1570.19	1574.98	−19.27	−18.29	−17.74
0.05	1564.52	1570.65	1575.43	−18.92	−17.87	−17.30
Asp in 15% aqueous-PD						
0.00	1586.35	1590.26	1594.38			
0.01	1586.86	1590.75	1594.86	−18.18	−17.05	−16.31
0.02	1587.35	1591.23	1595.32	−17.65	−16.64	−15.78
0.03	1587.83	1591.69	1595.77	−17.26	−16.19	−15.37
0.04	1588.29	1592.13	1596.20	−16.77	−15.67	−14.89
0.05	1588.73	1592.56	1596.61	−16.34	−15.23	−14.36
Asp in 20% aqueous-PD						
0.00	1610.27	1613.13	1616.44			
0.01	1610.75	1613.59	1616.89	−15.27	−14.20	−13.63
0.02	1611.21	1614.03	1617.33	−14.71	−13.67	−13.27
0.03	1611.66	1614.46	1617.75	−14.33	−13.28	−12.83
0.04	1612.10	1614.87	1618.16	−13.96	−12.81	−12.40
0.05	1612.52	1615.28	1618.56	−13.52	−12.46	−12.05

interaction between COOH of Asp and OH group of PD and hydrophilic–hydrophilic interaction between CH_2COOH of Asp and OH group of PD which results in the large partial molar volume of transfer of Asp in PD solution.

3. 2. Apparent Molar Isentropic Compressibility

The experimental values of speed of sound of solution of Asp in aqueous and 5%, 10%, 15% and 20% aqueous solutions of PD have also measured at aforementioned

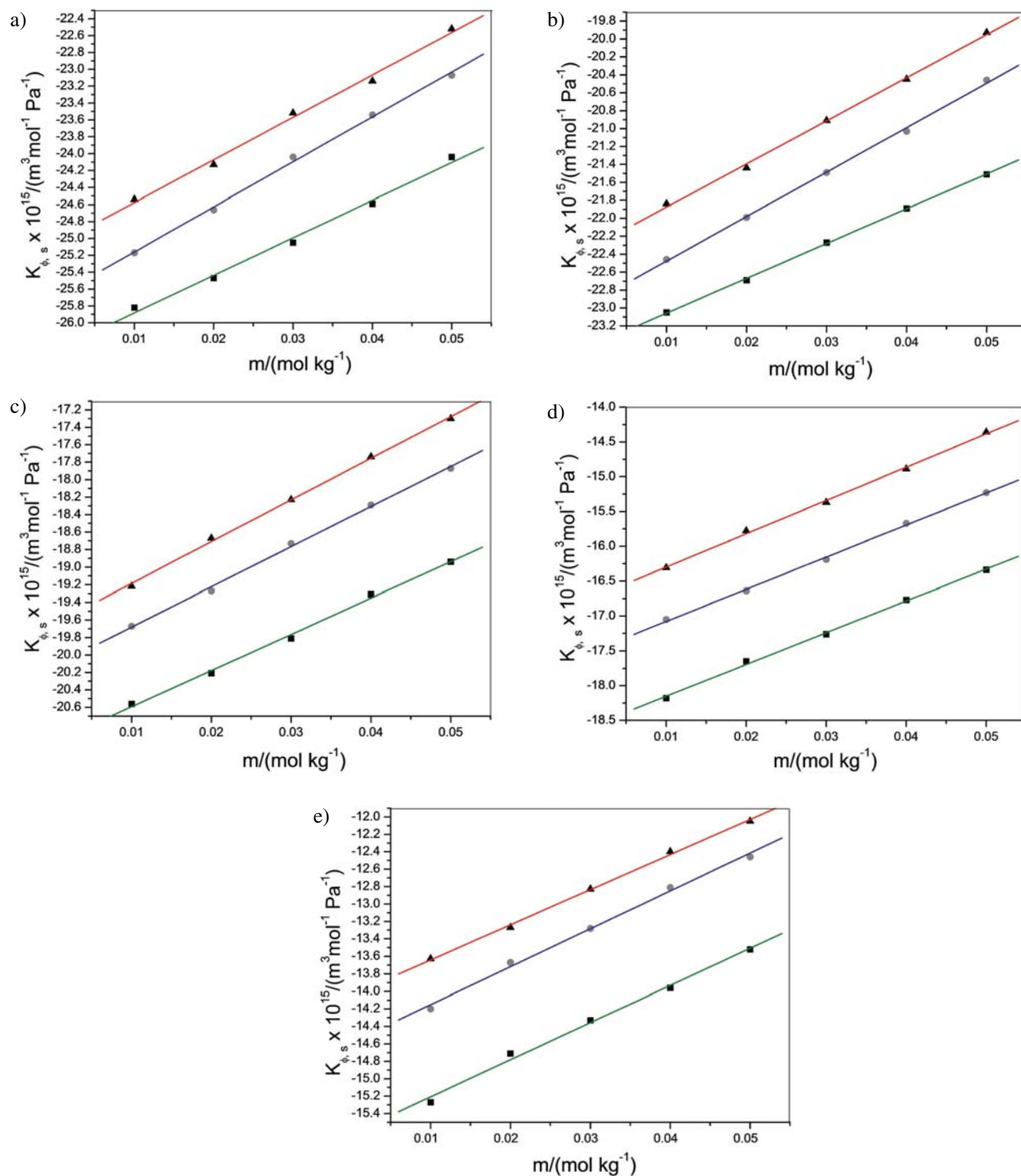


Figure 2. Variation of apparent molar isentropic compressibility versus molality of Asp in aqueous and aqueous + PD solutions: (a) water, (b) 5% PD, (c) 10% PD, (d) 15% PD and (e) 20% PD at temperatures, $T/\text{K} = 298.15$ (■); 303.15 (●); 308.15 (▲).

temperatures in order to evaluate apparent molar isentropic compressibilities, $K_{\phi,s}$, using Eq. (5).

$$K_{\phi,s} = (Mk_s/\rho) - [(k_s^0\rho - k_s\rho_0)/m\rho\rho_0] \quad (5)$$

where k_s and k_s^0 are isentropic compressibilities of solution and solvent, respectively, calculated using speed of sound (u) relation:

$$k_s = 1/(u^2\rho) \quad (6)$$

The plot between $K_{\phi,s}$ and molality (Fig. 2) are found to be linear at each temperature. The magnitude of negative values of $K_{\phi,s}$ decreases both with increase in temperature as well as concentration of PD in aqueous medium. The negative $K_{\phi,s}$ values shows that water molecules around solute are less compressible than those in the bulk which is attributed to strong attractive interactions.^{28,29}

3. 2. 1. Limiting Apparent Molar Isentropic Compressibility

The variation of apparent molar isentropic compressibility with the molality can be adequately represented by Eq. (7).

$$K_{\phi,s} = K_{\phi,s}^0 + S_k m \quad (7)$$

where $K_{\phi,s}^0$ is limiting apparent molar isentropic compressibility, which is also referred to as partial molar isentropic compressibility, and is a measure of solute-solvent interaction. S_k is the experimental slope; an indicative of solute-solute interactions. The values of $K_{\phi,s}^0$, S_k together with their standard errors are shown in Table 5.

The computed values of $K_{\phi,s}^0$ which come out to be negative, become less negative with increase in concentration as well as temperature. The negative values of $K_{\phi,s}^0$ (loss of compressibility of medium) indicate that the water molecules surrounding the amino acids would present greater resistance to compression than bulk. With increase in temperature, the values become less negative which means that electrostriction interaction between amino acids and water molecules are suppressed due to formation of ion pairs between ions of PD and Asp, and some

water molecules are released to bulk. In other words, the more negative values of $K_{\phi,s}^0$ reflect strong solute-solvent interactions attributed to electrostatic interactions between Asp and solvent which makes the solution rather incompressible. An irregular trend of S_k is governed by number of effects which also suggest the presence of solute-solute interactions in the system.

3. 2. 2. Partial Molar Compressibility of Transfer

The partial molar compressibility of transfer ($K_{\phi,s,tr}^0$) of Asp from aqueous to aqueous PD solutions has been calculated using the following relation.

$$K_{\phi,s,tr}^0 = K_{\phi,s}^0(\text{aq-PD solution}) - K_{\phi,s}^0(\text{aq}) \quad (8)$$

where $K_{\phi,s}^0(\text{aq})$ is the limiting apparent molar isentropic compressibility of Asp in aqueous medium (Table 5).

The values of partial molar compressibility of transfer $K_{\phi,s,tr}^0$, as reported in Table 6, are positive which increase with increase in concentration of PD. The positive values indicate the dominance of the charged end groups NH_3^+ and COO^- . The interactions between PD and the zwitterionic center of Asp increase with increasing PD concentration due to structure-making tendency of the ions and decrease in electrostriction. As a result, the electrostricted water is much less compressible than bulk water giving rise to a large decrease in the compressibility with increase in the PD concentration. Thus the $K_{\phi,s}$ values are negative and $K_{\phi,s,tr}^0$ values are positive for Asp.

It is important to mention here that on addition of Asp to the co-solute there is decrease in the pH of solution, and the side chains of the acidic amino acids remain fully deprotonated.¹⁹ Thus, the amino acid studied mainly exists in zwitterionic form in co-solute solutions and their side chains remain fully deprotonated due to hydrolysis. Thus, deprotonated amino acid interacts strongly with aqueous solvent resulting in increase in apparent molar volume, V_ϕ which in turn leads to increase in limiting apparent molar volume, V_ϕ^0 . A similar increasing trend is shown by all other investigated thermodynamic properties.

It is well known that the acid group on the side chain of Asp undergoes ionization in water to small extent, i.e., 5 to 8%, to give a small fraction of L-aspartate ions along with their unionized form. The values reported in litera-

Table 5. Limiting apparent molar isentropic compressibilities, $K_{\phi,s}^0$, experimental slopes, S_k , and standard deviations of linear regression (in parenthesis) of Asp in aqueous and aqueous solution of PD at different temperatures.

System	$K_{\phi,s}^0 \times 10^{15}/(\text{m}^3 \text{mol}^{-1} \text{Pa}^{-1})$			$S_k \times 10^{15}/(\text{m}^3 \text{mol}^{-2} \text{Pa}^{-1} \text{kg})$		
	298.15 K	303.15 K	308.15 K	298.15 K	303.15 K	308.15 K
Asp in aqueous	-26.32 (± 0.071)	-25.70 (± 0.047)	-25.08 (± 0.072)	44.4 (± 2.157)	53.2 (± 1.446)	50.3 (± 2.184)
Asp in 5% aqueous-PD	-23.44 (± 0.015)	-22.97 (± 0.034)	-22.35 (± 0.038)	38.8 (± 0.461)	49.6 (± 1.263)	48.1 (± 1.170)
Asp in 10% aqueous-PD	-21.00 (± 0.046)	-20.14 (± 0.040)	-19.66 (± 0.035)	41.4 (± 1.385)	45.8 (± 1.194)	47.7 (± 1.063)
Asp in 15% aqueous-PD	-18.60 (± 0.036)	-17.54 (± 0.033)	-16.78 (± 0.037)	45.6 (± 1.101)	46.1 (± 1.018)	47.9 (± 1.135)
Asp in 20% aqueous-PD	-15.63 (± 0.063)	-14.58 (± 0.054)	-14.04 (± 0.031)	42.5 (± 1.900)	43.4 (± 1.650)	40.3 (± 0.936)

Table 6. Partial molar compressibilities of transfer, $K_{\phi}^0 \times 10^{15}/(\text{m}^3 \text{mol}^{-1} \text{Pa}^{-1})$, of Asp in aqueous solution of PD at different temperatures.

System	298.15 K	303.15 K	308.15 K
Asp in 5% aqueous-PD	2.88	2.72	2.73
Asp in 10% aqueous-PD	5.32	5.55	5.42
Asp in 15% aqueous-PD	7.72	8.16	8.30
Asp in 20% aqueous-PD	9.55	11.11	11.04

ture for limiting apparent molar volume, V_{ϕ}^0 , of aqueous solution of Asp³⁰ at 298.15 K is 74.78 when ionization of Asp is considered. Since we have not considered the ionization correction, our value of limiting apparent molar volume comes out to be 72.02 which is in close agreement with literature value of 72.30 evaluated without taking into account the ionization correction factor.¹⁹ It is also not-

ed that the value for this system at 298.15 K reported by Jolicoeur et.al.³¹ ($V_{\phi}^0 = 71.79$) is also in excellent agreement with our value. The contributions due to ionization or protonation of side chain groups were considered negligible in the concentration ranges investigated. The zwitterionic structure (terminal-NH₂, COOH groups) is assumed predominant in this amino acid.

3. 3. Viscosity

The viscosities of Asp in aqueous and 5%, 10%, 15% and 20% aqueous solutions of PD were also measured at $T = 298.15, 303.15$ and 308.15 K (Table 7). The variation of relative viscosity which is calculated as the ratio of viscosity of solution, η , and corresponding solvent, η_0 , i.e., $\eta_r = \eta/\eta_0$, (Table 7) can be represented by³² Jones–Dole equation:

Table 7. Viscosities, η , and relative viscosities, η_r , of Asp in aqueous and aqueous-PD (5, 10, 15 and 20%, PD w/w in water) as function of molarity, C , of Asp at different temperatures.

C /(mol L ⁻¹)	η × 10 ³ /(Pa s)	η _r	C /(mol L ⁻¹)	η × 10 ³ /(Pa s)	η _r	C /(mol L ⁻¹)	η × 10 ³ /(Pa s)	η _r
298.15 K			303.15 K			308.15 K		
Asp in aqueous								
0.00000	0.9163		0.00000	0.8241		0.00000	0.7412	
0.01005	0.9186	1.0025	0.01004	0.8259	1.0021	0.01002	0.7424	1.0016
0.02011	0.9206	1.0046	0.02009	0.8277	1.0043	0.02005	0.7442	1.0040
0.03019	0.9229	1.0072	0.03016	0.8297	1.0068	0.03011	0.7460	1.0064
0.04030	0.9254	1.0099	0.04024	0.8318	1.0093	0.04017	0.7477	1.0087
0.05040	0.9277	1.0124	0.05036	0.8338	1.0117	0.05024	0.7494	1.0110
Asp in 5% aqueous-PD								
0.00000	0.9957		0.00000	0.9221		0.00000	0.8286	
0.01008	0.9990	1.0033	0.01007	0.9248	1.0029	0.01005	0.8306	1.0024
0.02019	1.0016	1.0059	0.02016	0.9272	1.0055	0.02005	0.8329	1.0051
0.03030	1.0046	1.0089	0.03025	0.9299	1.0084	0.03011	0.8352	1.0079
0.04043	1.0074	1.0117	0.04036	0.9324	1.0111	0.04017	0.8374	1.0105
0.05056	1.0103	1.0146	0.05051	0.9350	1.0139	0.05024	0.8396	1.0132
Asp in 10% aqueous-PD								
0.00000	1.0623		0.00000	0.9999		0.00000	0.9352	
0.01012	1.0664	1.0038	0.01011	1.0032	1.0033	0.01008	0.9380	1.0029
0.02025	1.0695	1.0067	0.02023	1.0062	1.0063	0.02018	0.9408	1.0059
0.03039	1.0731	1.0101	0.03036	1.0094	1.0095	0.03029	0.9436	1.0089
0.04055	1.0766	1.0134	0.04051	1.0126	1.0127	0.04041	0.9466	1.0121
0.05072	1.0800	1.0166	0.05069	1.0158	1.0159	0.05054	0.9495	1.0152
Asp in 15% aqueous-PD								
0.00000	1.2434		0.00000	1.1548		0.00000	1.0593	
0.01016	1.2489	1.0044	0.01014	1.1595	1.0040	0.01012	1.0064	1.0036
0.02032	1.2530	1.0077	0.02029	1.1633	1.0073	0.02025	1.0667	1.0069
0.03051	1.2575	1.0113	0.03045	1.1672	1.0107	0.03039	1.0700	1.0101
0.04070	1.2620	1.0149	0.04063	1.1714	1.0143	0.04055	1.0740	1.0138
0.05091	1.2660	1.0181	0.05084	1.1749	1.0174	0.05071	1.0770	1.0167
Asp in 20% aqueous-PD								
0.00000	1.4053		0.00000	1.3067		0.00000	1.2156	
0.01019	1.4125	1.0051	0.01017	1.3129	1.0047	0.01015	1.2209	1.0043
0.02039	1.4174	1.0086	0.02036	1.3175	1.0082	0.02031	1.2250	1.0077
0.03061	1.4232	1.0127	0.03057	1.3226	1.0121	0.03049	1.2296	1.0115
0.04084	1.4284	1.0164	0.04078	1.3274	1.0158	0.04067	1.2341	1.0152
0.05108	1.4344	1.0207	0.05103	1.3300	1.0178	0.05087	1.2391	1.0193

$$\eta_r = \eta/\eta_0 = 1 + AC^{1/2} + BC \quad (9)$$

where A and B are the Falkenhagen and viscosity B-coefficients, respectively. The former specifies interactions between solute-solute^{33,34} while the later is a measure of

structural modification induced by solute-solvent interaction.^{35,36} For non-electrolytes, A is negligible³⁵ and Jones–Dole equation reduces to:

$$\eta_r = \eta/\eta_0 = 1 + BC \quad (10)$$

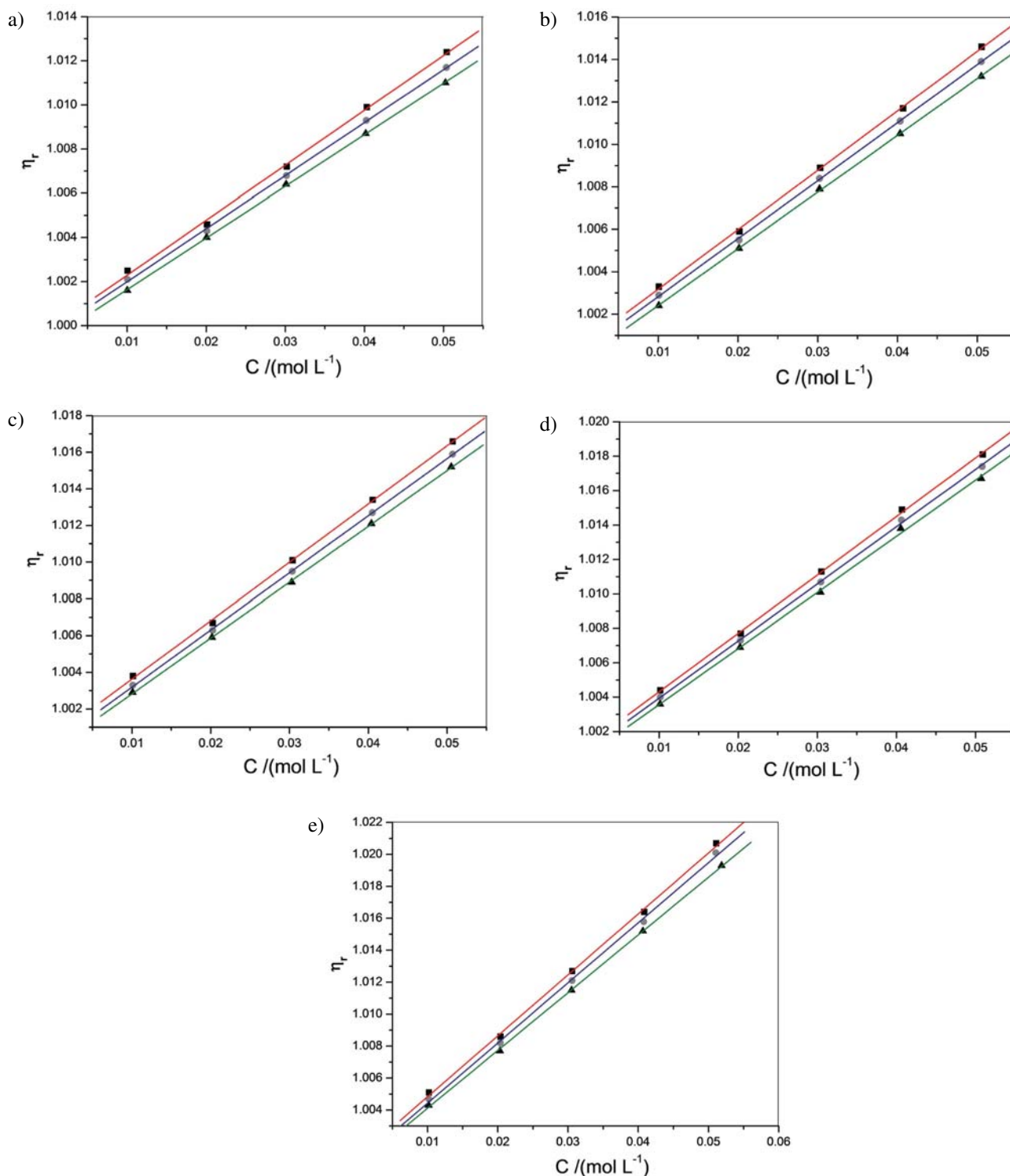


Figure 3. Variation of relative viscosity versus molarity of Asp in aqueous and aqueous + PD solutions: (a) water, (b) 5% PD, (c) 10% PD, (d) 15% PD and (e) 20% PD at temperatures, T/K = 298.15 (■); 303.15 (●); 308.15 (▲).

where C is the molarity obtained from molality by using our density values.

The values of B-coefficients, obtained using a plot between η_r and concentration (Table 8) by least squares analysis, are found to be linear at all concentrations and temperatures. The positive B-coefficients values, which increase with increasing concentration of PD, also indicate a structure to allow the co-solute (PD) to act on solvent.⁴ The values of B-coefficients increase (i) when the water is replaced by PD, i.e., PD act as water structure-maker by H-bonding, and (ii) with increasing concentration of PD. The strong interaction immobilizes the solvent molecules, present obstruction to viscous flow of solution, thus, increasing the viscosity.³⁷

The temperature derivative of B-coefficients (dB/dT) and its sign is useful in establishing structure-making or structure-breaking ability of the solute in solvent. In general, the dB/dT is negative for structure-maker and positive for structure-breaker solutes in solution.^{34,38} The dB/dT values reported in Table 8 are negative which indicate that this amino acid, i.e., Asp act as structure-maker in aqueous-PD solvent.

3.3.1. Viscosity B-coefficients of Transfer

The viscosity B-coefficients of transfer, B_{tr} , of Asp from aqueous to aqueous PD solutions was calculated using the following relation:

$$B_{tr} = B(\text{aq-PD solution}) - B(\text{aq}) \quad (11)$$

where $B(\text{aq})$ is the viscosity B-coefficients of Asp in water (Table 8). The values of B_{tr} are reported in Table 9 which shows that viscosity B-coefficients of transfer increase with increase in concentration of PD. Hence, the aforementioned transport parameters show similar trends based

on the impact of hydrolysis reaction and dissociation constant of Asp as observed in the thermodynamic properties.

3.4. Hydration Number

The hydration number, n_H ,^{39–41} can be calculated from methods based on volume, compressibility and second temperature derivative of the partial molar volume or partial molar compressibility data. From volumetric properties (Table 10), the hydration number of an amino acid can be estimated from the electrostriction partial molar volume $V_{\phi(\text{elect})}^0$ by the following relation:²³

$$n_H = V_{\phi(\text{elect})}^0 / (V_{\phi(e)}^0 - V_{\phi(b)}^0) \quad (12)$$

where $V_{\phi(e)}^0$ is the molar volume of electrostricted water and $V_{\phi(b)}^0$ is the molar volume of the bulk. The values of $(V_{\phi(e)}^0 - V_{\phi(b)}^0)$ are (–3.3, –3.7 and –4.0) $\text{cm}^3 \text{mol}^{-1}$ at $T = (298.15, 303.15, \text{ and } 308.15) \text{ K}$.⁴² The electrostriction partial molar volume, $V_{\phi(e)}^0$ can be estimated from the experimental measured V_{ϕ}^0 values by the following equation:

$$V_{\phi(\text{elect})}^0 = V_{\phi(\text{amino acid})}^0 - V_{\phi(\text{int})}^0 \quad (13)$$

where $V_{\phi(\text{int})}^0$ is the intrinsic partial molar volume of the amino acid. The $V_{\phi(\text{int})}^0$ is made up of two terms: the van der waals volume, $V_{\phi(w)}^0$, and the volume due to packing effects, $V_{\phi(P)}^0$, i.e.,

$$V_{\phi(\text{int})}^0 = V_{\phi(w)}^0 + V_{\phi(P)}^0 \quad (14)$$

The intrinsic volume can be calculated from several methods. One such approach is to estimate the intrinsic volume from crystal molar volume:

$$V_{\phi(\text{crystal})}^0 = \text{mol. wt.}/d_{\text{crystal}} \quad (15)$$

Table 8. Viscosity B-coefficients and temperature coefficients, dB/dT, of Asp in aqueous and aqueous solution of PD different temperatures.

System	Viscosity B-coefficients			dB/dT
	298.15K	303.15K	308.15K	
Asp in aqueous	0.248 (± 0.005)	0.240 (± 0.003)	0.233 (± 0.001)	–0.0015
Asp in 5% aqueous PD	0.280 (± 0.003)	0.273 (± 0.002)	0.267 (± 0.001)	–0.0013
Asp in 10% aqueous PD	0.318 (± 0.004)	0.311 (± 0.002)	0.304 (± 0.002)	–0.0014
Asp in 15% aqueous PD	0.340 (± 0.004)	0.332 (± 0.004)	0.326 (± 0.006)	–0.0014
Asp in 20% aqueous PD	0.381 (± 0.007)	0.376 (± 0.007)	0.361 (± 0.003)	–0.0020

Table 9. Viscosity B-coefficients of transfer, B_{tr} , of Asp in aqueous solution of PD at different temperatures.

System	298.15 K	303.15 K	308.15 K
Asp in 5% aqueous-PD	0.032	0.033	0.034
Asp in 10% aqueous-PD	0.070	0.071	0.071
Asp in 15% aqueous-PD	0.092	0.092	0.093
Asp in 20% aqueous-PD	0.133	0.136	0.128

where d_{crystal} is the density of dry amino acid. The packing density for molecules in organic crystals is about 0.7. The packing density for random packing spheres is 0.634. By making the appropriate corrections for packing densities (ρ), the intrinsic partial molar volume of the amino acid can be calculated as:

$$V_{\phi(\text{int})}^0 = (0.7/0.634) V_{\phi(\text{crystal})}^0 \quad (16)$$

The values of $V_{\phi(\text{crystal})}^0$ is determined from the work of Berlin and Pallansch⁴³ and thus the value of $V_{\phi(\text{elect})}^0$ can be calculated.

The n_H values (volumetric method) for Asp in aqueous-PD solutions, given in Table 10, shows that the hydration number of Asp in PD solution is less than that in water and decrease with increasing concentration of PD which again indicates that the increase in solute-solvent interactions reduces the electrostriction effect of amino acids. It also suggests that PD has a dehydration effect on the Asp, i.e., water molecules are replaced by PD molecules with increasing concentration of PD in solution.

From compressibility data, the number of water molecules hydrated to the amino acid was calculated by using the method given by Millero et al.,²³

$$n_H = -K_{\phi, s(\text{elect})}^0 / (V_{\phi(b)}^0 \times K_{\phi, s(b)}^0) \quad (17)$$

where $K_{\phi, s(b)}^0$ is compressibility of bulk water. Value of $V_{\phi(b)}^0 \times K_{\phi, s(b)}^0$ is $\approx 0.81 \times 10^{-5} \text{ m}^3 \text{ mol}^{-1} \text{ GPa}^{-1}$ (or, $8.1 \times 10^{-13} \text{ m}^3 \text{ mol}^{-1} \text{ Pa}^{-1}$). The electrostriction partial molar compressibility, $V_{\phi(\text{elect})}^0$, can be calculated from experimentally measured values of $K_{\phi, s(\text{amino acid})}^0$ using the following equation:

$$K_{\phi, s(\text{elect})}^0 = K_{\phi, s(\text{amino acid})}^0 - K_{\phi, s(\text{int})}^0 \quad (18)$$

where $K_{\phi, s(\text{int})}^0$ is $K_{\phi, s(\text{isomer})}^0$ and its value for Asp is $3 \times 10^{-6} \text{ m}^3 \text{ mol}^{-1} \text{ GPa}^{-1}$ (or, $3 \times 10^{-15} \text{ m}^3 \text{ mol}^{-1} \text{ Pa}^{-1}$).²³ Since the value of $K_{\phi, s(\text{int})}^0$ is less than $5 \times 10^{-6} \text{ m}^3 \text{ mol}^{-1} \text{ GPa}^{-1}$ (or, $5 \times 10^{-15} \text{ m}^3 \text{ mol}^{-1} \text{ Pa}^{-1}$) for ionic crystals and many organic solutes in water, we can assume $K_{\phi, s(\text{int})}^0 = 0$. Therefore, Eq. (18) becomes

$$K_{\phi, s(\text{elect})}^0 = K_{\phi, s(\text{amino acid})}^0 \quad (19)$$

The values of n_H estimated from Eq. (17) (Table 10) show similar behavior as discussed in volume data.

It is pertinent to mention here that when Asp is dissolved in water there is a strong interaction between ions of Asp and water molecules. The small water molecules are surrounded by the ions of Asp which result in electrostriction of water molecules. In other words, there is collapse of structure of water molecules. On addition of PD to this solution, there is strong interaction between ions of Asp and PD solvent which results in decrease in electrostriction effect. Thus, large number of water molecules of solute is associated with solvent molecules which increase the interactions among the ions. The electrostricted water becomes more like bulk water which has an open structure. Thus, Asp acts as structure maker in presence of PD solvent. A similar conclusion is evident from the compressibility as well as viscosity data.

4. Conclusion

Density, speed of sound and viscosity of Asp in aqueous and aqueous PD solvent (5%, 10%, 15% and 20% of PD in water) measured at different temperature have been reported in this study. From the experimental data, various parameters like apparent molar volume, limiting apparent molar volume, the corresponding slope, partial molar volume of transfer; apparent molar isentropic compressibility, limiting apparent molar isentropic compressibility, its slope, partial molar compressibility of transfer; relative viscosity, viscosity B-coefficients, temperature derivative of B-coefficients, viscosity B-coefficients of transfer, and hydration number have been calculated. The result indicates the existence of strong solute-solvent interactions i.e. ionic-hydrophilic and hydrophilic-hydrophilic interactions. The extent of interactions increases with an increase in the molar mass of Asp and an increase in the concentration of PD solution. The negative value of temperature derivative of B-coefficients (dB/dT) indicate that this amino acid, i.e., Asp act as structure-maker in aqueous-PD solvent. The hydration numbers n_H of the amino acids under investigation decrease with temperature as well as with increasing concentration of PD and this shows that both of these factors cause a dehydration effect on the amino acids.

Table 10. Hydration number, n_H , calculated from volume and compressibility data of Asp in aqueous and aqueous solution of PD at different temperatures.

System	Volume data			Compressibility data		
	298.15 K	303.15 K	308.15 K	298.15 K	303.15 K	308.15 K
Asp in aqueous	5.40	4.86	4.30	3.24	3.17	3.09
Asp in 5% aqueous-PD	5.21	4.66	4.09	2.90	2.83	2.75
Asp in 10% aqueous-PD	5.06	4.51	3.95	2.60	2.48	2.42
Asp in 15% aqueous-PD	4.85	4.36	3.84	2.30	2.16	2.07
Asp in 20% aqueous-PD	4.73	4.25	3.73	1.93	1.80	1.73

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Povzetek

Z meritvami gostote, viskoznosti in hitrosti zvoka v raztopinah smo proučevali interakcije L-asparaginske kisline (Asp) v mešanicah 1,2-propandiola (PD) in vode pri treh temperaturah (298.15, 303.15 in 308.15) K pri atmosferskem tlaku. Iz eksperimentalnih podatkov za gostote raztopin smo določili navidezni molski volumen V_ϕ , vrednosti limitnega molskega volumna, V_ϕ^0 , ter parcialni molski volumen prenosa, $V_{\phi, tr}^0$. Iz izmerjenih vrednosti hitrosti zvoka smo izračunali navidezno molsko kompresibilnost, $K_{\phi, tr, s}$, ter parcialno molsko kompresibilnost prenosa partial, $K_{\phi, s, tr}^0$. S pomočjo dobljenih vrednosti smo ocenili število vodnih molekul v hidratnem plašču amino kisline, n_H . Podatke za viskoznost smo uporabili pri izračunu relativne viskoznosti, η_r , viskoznostnih B-koeficientov, njihovih odvodov po temperaturi, dB/dT , ter B-koeficientov prenosa, B_{tr} . Dobljene vrednosti smo uporabili za oceno prevladujočih interakcij v proučevanih raztopinah.

Scientific paper

Carbon Nanotube-Supported Butyl 1-Sulfonic Acid Groups as a Novel and Environmentally Compatible Catalyst for the Synthesis of 1,8-Dioxo-octahydroxanthenes

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Abstract

A novel multiwalled carbon nanotube catalyst with $-\text{SO}_3\text{H}$ functional groups was easily prepared from its starting materials and used as an efficient heterogeneous catalyst for one-pot Knoevenagel condensation, Michael addition, and cyclodehydration of 5,5-dimethyl-1,3-cyclohexanedione (dimedone) with various aromatic aldehydes. Using this method 1,8-dioxo-octahydroxanthenes were obtained in excellent yields at room temperature. The present method is superior in terms of reaction temperature, reaction time, easy work-up, high yields, and ease of recovery of catalyst.

Keywords: Nanocatalyst; Heterogeneous catalysis; 1,8-Dioxo-octahydroxanthene; Aldehyde; 5,5-Dimethyl-1,3-cyclohexanedione

1. Introduction

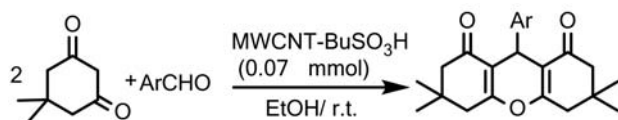
Recently, carbon nanotubes (CNTs) have been considered as good supports for homogeneous and heterogeneous catalysts.^{1–3} When compared to other commonly used supports in heterogeneous catalysis, CNTs present the advantage of extraordinary electrical, thermal, and mechanical strength characteristics, resistance to chemical attack in acidic and basic media, high surface areas, and low cost. They are cylindrically shaped and their surface can be modified with various functional groups, which can be used as building blocks for covalent and noncovalent attachment of catalytic active species.

There is a widespread interest in the synthesis of xanthene derivatives owing to their diverse range of biological and therapeutic properties, such as anti-inflammatory,⁴ antiviral,⁵ and anticancer activities.⁶ Also, they were used as antagonists for the paralyzing action of zoxazolamine,⁷ fluorescent markers for the visualization of biomolecules,⁸ and photostable laser dyes.⁹ Among various derivatives of xanthene, 1,8-dioxo-octahydroxanthenes have aroused considerable interest. Synthesis of 1,8-dioxo-octahydroxanthenes is generally achieved by the con-

densation of dimedone with aldehydes. Several types of catalysts were introduced previously for this reaction, such as $\text{NaHSO}_4\text{-SiO}_2$ or silica chloride,¹⁰ polyphosphoric acid- SiO_2 ,¹¹ $\text{In}(\text{OTf})_3$,¹² H_2SO_4 ,¹³ InCl_3 or P_2O_5 ,¹⁴ ceric ammonium nitrate (CAN) under ultrasound irradiation,¹⁵ succinimide-*N*-sulfonic acid,¹⁶ CaCl_2 ,¹⁷ Fe_3O_4 nanoparticles,¹⁸ CAN supported HY-zeolite,¹⁹ $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ -Imid- $\text{H}_3\text{PMo}_{12}\text{O}_{40}$ nanoparticles,²⁰ piperidine,²¹ Mg-Al hydro-talcite,²² thiourea dioxide,²³ and ZnO nanoparticles.²⁴ Although these methods are suitable for certain synthetic conditions, there exist some drawbacks, such as low yields, high reaction temperature, long reaction times, tedious work-up, the formation of 2,2'-aryl-methylene bis(3-hydroxy-2-cyclohexene-1-one) derivatives due to competitive side reactions, and the use of unrecyclable, hazardous or difficult to handle catalysts. In view of this, utilizing eco-friendly and green catalysts for this useful reaction is in demand.

In a continuation of our recent work on synthesis and application of heterogeneous catalysts in organic reactions,^{25–28} herein we now report the synthesis of multiwalled carbon nanotube-supported butyl 1-sulfonic acid groups (MWCNT- BuSO_3H) from the reaction of the salt

form hydroxyl functionalized multiwalled carbon nanotube (MWCNT–OH) with 1,4-butane sultone followed by the reaction with HCl. MWCNT–BuSO₃H was used as a heterogeneous catalyst for one-pot Knoevenagel condensation, Michael addition, and cyclodehydration of dimedone with various aromatic aldehydes at room temperature (Scheme 1).



Scheme 1. Synthesis of 1,8-dioxo-octahydroxanthenes using MWCNT–BuSO₃H.

2. Experimental

2. 1. Materials and Methods

Chemicals were either prepared in our laboratory or were purchased from Merck and Fluka. Reaction monitoring and purity determination of the products were accomplished by GLC or TLC on silica-gel polygram SILG/UV₂₅₄ plates. Gas chromatography was recorded on Shimadzu GC 14-A. IR spectra were obtained by a Shimadzu model 8300 FT-IR spectrophotometer. ¹H NMR spectra were recorded on 400 MHz spectrometer in CDCl₃. The Leco sulfur analyzer was used for the measurement of sulfur in the catalyst. TGA was carried out on a Stanton Redcraft STA-780 with a 20 °C/min heating rate. SEM and TEM images were taken with a Hitachi S-3400N scanning electron microscope and a Philips CM10 transmission electron microscope, respectively. Melting points were determined on a Fisher–Jones melting-point apparatus.

2. 2. Synthesis of Multiwalled Carbon Nanotubes

MWCNT and MWCNT–OH were prepared as reported in our previous work.¹

2. 3. Synthesis of MWCNT–BuSO₃H

In a round bottomed flask (50 mL) equipped with a reflux condenser was added 1 g of the MWCNT–OH to an aqueous solution of sodium hydroxide (1 M, 10 mL) and the mixture was stirred at 60 °C for 12 h, filtered, washed with distilled water (20 mL), and dried at 80 °C overnight to give MWCNT–ONa. Then, 1,4-butane sultone (1.5 mL) was added to the obtained solid and the mixture was stirred at 100 °C for 24 h, filtered, washed with distilled water (20 mL), and dried at 80 °C overnight to give MWCNT–OBuSO₃Na. Afterwards, HCl (3 M, 10 mL) was added to MWCNT–OBuSO₃Na and the mixture was stirred at room temperature for 2 h, filtered, washed with distilled

water (20 mL), and dried at 80 °C overnight to give MWCNT–BuSO₃H.

2. 4. General Procedure for 1,8-Dioxo-octahydroxanthene Synthesis

A mixture of an aldehyde (2 mmol), dimedone (0.28 g, 2 mmol), MWCNT–BuSO₃H (0.066 g, 0.07 mmol), and ethanol (3 mL) was stirred for an appropriate time at room temperature. After completion of the reaction (monitored by TLC), the catalyst was filtered off and washed with ethanol (2 × 10 mL). Then, the filtrate was concentrated on a rotary evaporator under reduced pressure and the crude product recrystallized from ethanol. All products are known compounds and were identified by comparison of their physical and spectral data with those of the authentic samples.

2. 5. Representative Spectral Data of Some of the Obtained Compounds

3,3,6,6-Tetramethyl-9-phenyl-1,8-dioxo-octahydroxanthene¹⁴ (Table 1, entry 1). ¹H NMR (400 MHz, CDCl₃) δ 1.01 (s, 6H, 2 CH₃), 1.14 (s, 6H, 2 CH₃), 2.19–2.48 (m, 8H, 4 CH₂), 4.80 (s, 1H, CH), 6.95–7.22 (m, 5H, ArH). IR (KBr) ν 2960, 2950, 1663, 1490, 1390, 1250, 850 cm^{−1}.

9-(4'-Nitrophenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8-(2H)-dione¹⁴ (Table 1, entry 11). ¹H NMR (400 MHz, CDCl₃) δ 1.05 (s, 6H, 2 CH₃), 1.16 (s, 6H, 2 CH₃), 2.20–2.50 (m, 8H, 4 CH₂), 4.88 (s, 1H, CH), 7.52–7.60 (d, 2H, ArH), 8.09–8.14 (d, 2H, ArH). IR (KBr) ν 2966, 2930, 2870, 1730, 1670, 1600, 1350, 1192, 860 cm^{−1}.

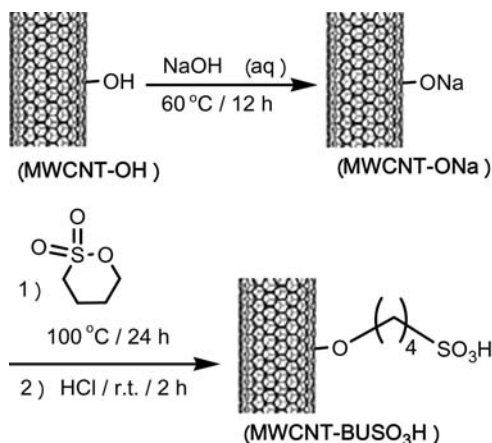
9-(4-Methoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione¹² (Table 1, entry 4). ¹H NMR (400 MHz, CDCl₃) δ 1.06 (s, 6H, 2 CH₃), 1.14 (s, 6H, 2 CH₃), 2.19–2.48 (m, 8H, 4 CH₂), 3.80 (s, 3H, OCH₃), 4.72 (s, 1H, CH), 6.83–6.91 (d, 2H, ArH), 7.20–7.23 (d, 2H, ArH). IR (KBr) ν 2960, 2948, 1668, 1200, 1190, 795 cm^{−1}.

3. Results and Discussion

3. 1. Preparation of MWCNT–BuSO₃H

A chemical vapour deposition (CVD) method was used for the synthesis of MWCNT.¹ In order to develop hydroxyl groups on the MWCNT surface, the carbon nanomaterials were submitted to a heat treatment in a synthetic air flow (10 mL/min) at 500 °C for 2 h.¹

The synthetic routes for the MWCNT–BuSO₃H are shown in Scheme 2. At the first stage, MWCNT–OH was treated with NaOH to form the MWCNT–ONa. In the second step, MWCNT–BuSO₃H was prepared from the reac-



Scheme 2. Preparation procedure to MWCNT-BuSO₃H.

tion of MWCNT-ONa with 1,4-butane sultone followed by the reaction with HCl. The resulting black solid was analyzed by elemental analysis to quantify the percentage loading of the sulfonic acid groups by measuring the sulfur content, giving 0.98 mmol sulfonic acid moiety per gram. The acidic sites loading in MWCNT-BuSO₃H obtained by means of acid-base titration was found to be 1.05 mmol/g.²⁵

3. 2. Characterization of MWCNT-BuSO₃H

FT-IR spectra of the MWCNT-OH and MWCNT-BuSO₃H are presented in Figure 1. As can be seen in the spectrum of MWCNT-BuSO₃H new peaks appeared at 1120, 1150, 1190, and 1230 cm⁻¹, which can be assigned to S=O stretching vibration.^{25,26}

The thermogravimetric analyses (TGA) of MWCNTs, before and after the functionalization processes, are provided in Figure 2. The TGA curves of MWCNT-OH and

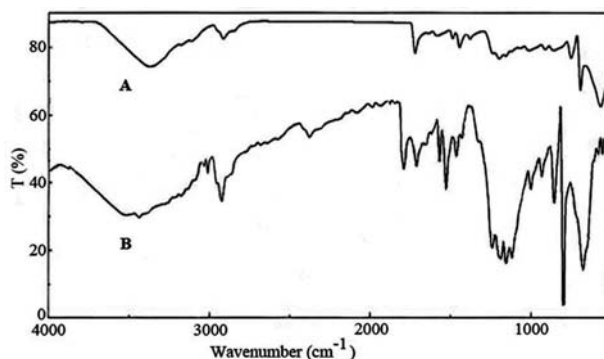


Figure 1. FT-IR spectra of MWCNT-OH (A) and MWCNT-BuSO₃H (B).

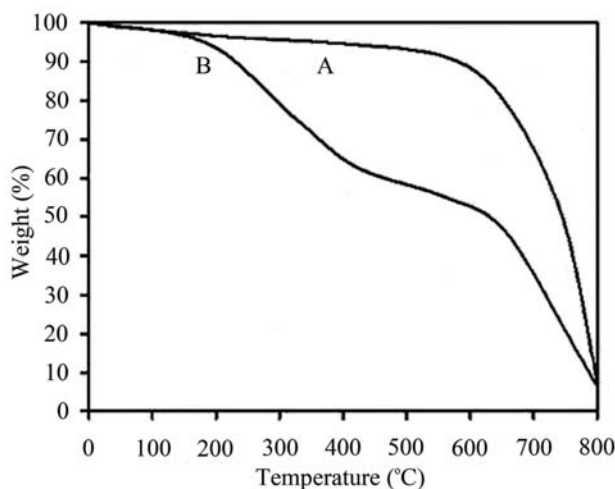


Figure 2. TGA curves of MWCNT-OH (A) and MWCNT-BuSO₃H (B).

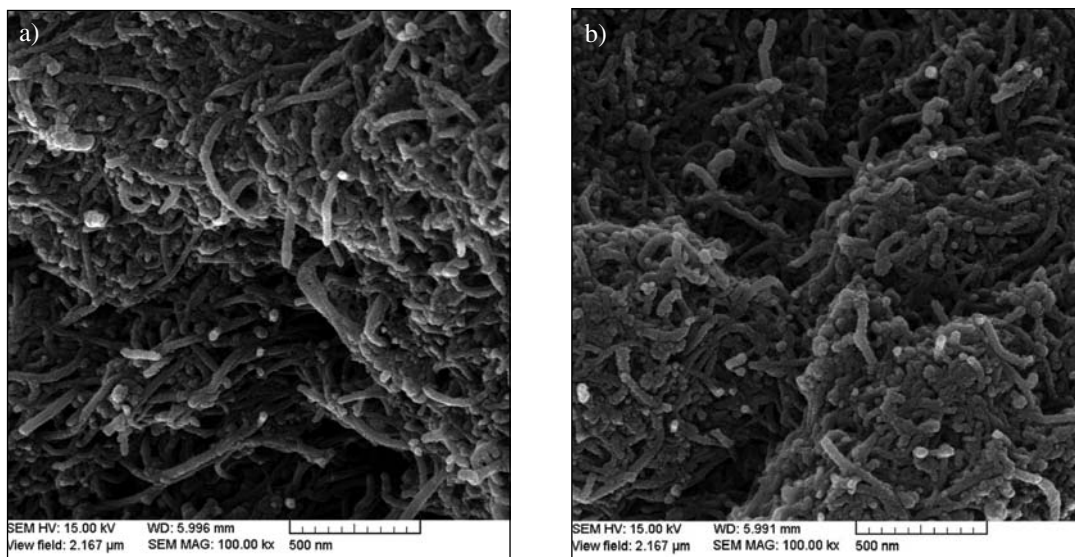


Figure 3. SEM photographs of MWCNT-OH (a) and MWCNT-BuSO₃H (b).

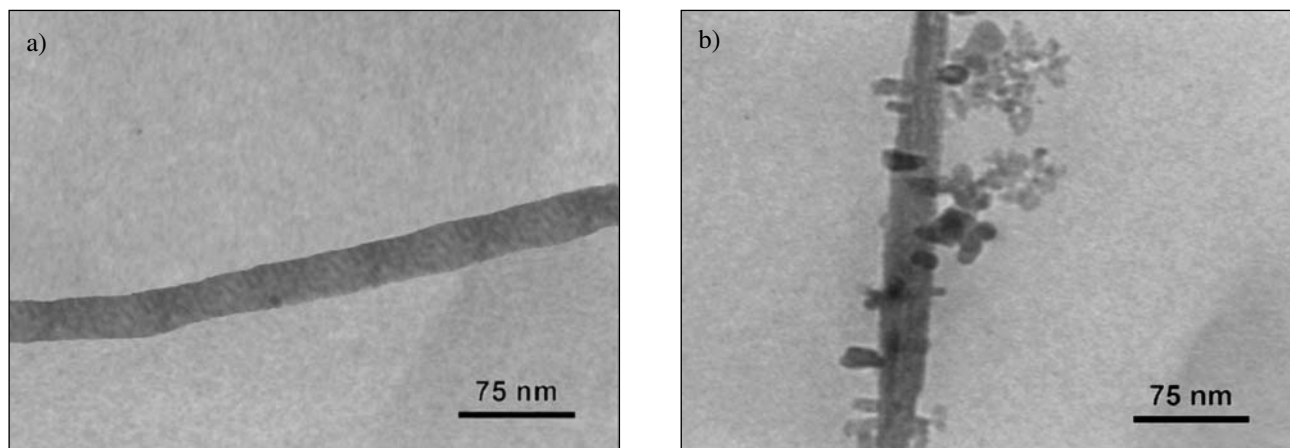


Figure 4. TEM photographs of MWCNT–OH (a) and MWCNT–BuSO₃H (b).

MWCNT–BuSO₃H displayed a weight loss around 100 °C which is corresponding to the loss of the physically adsorbed water. In the case of MWCNT–BuSO₃H, the second weight loss started at about 180 °C and is mainly assigned to the decomposition of the alky-sulfonic acid groups. In TGA curves of MWCNT–OH and MWCNT–BuSO₃H the last weight losses at about 570–640 °C were likely due to the degradation of MWCNTs.

An attempt was made to investigate the morphology of the MWCNTs using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). From SEM photographs of MWCNT–OH and MWCNT–BuSO₃H (Figure 3), it is obvious that the MWCNTs are well distributed and no amorphous carbon is detected. In the TEM photograph of MWCNT–BuSO₃H (Figure 4 (B)), it can be seen that the CNTs do not suffer damage after the functionalization and anion-exchange processes and that there are small particles affixed on the surface of MWCNT due to functionalization processes.

3. 3. Catalytic Activity of MWCNT–BuSO₃H

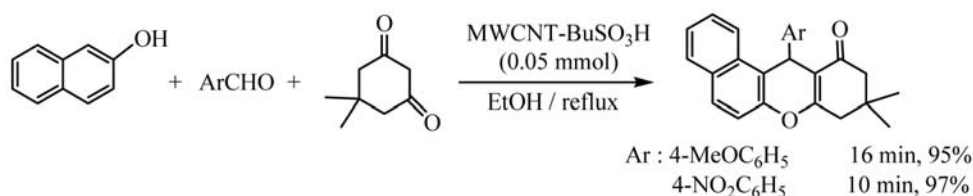
In order to explore the catalytic activity of MWCNT–BuSO₃H, we studied the synthesis of 1,8-dioxo-octahydroxanthenes by the reaction of aldehydes with dimedone. Initially, to optimize the reaction conditions, we tried to convert benzaldehyde to 3,3,6,6-tetramethyl-9-phenyl-1,8-dioxo-octahydroxanthene with dimedone at different conditions and various molar ratios of substrates. The best results were obtained at room temperature and a molar ratio of benzaldehyde:dimedone:MWCNT–BuSO₃H of 1:2:0.07. Then, under optimal conditions, a wide variety of substituted benzaldehydes (containing both electron withdrawing and donating groups) and 1-naphthaldehyde were treated with dimedone to give the corresponding products in high to excellent yields (Table 1, entries 1–13). Acid sensitive substrates, such as thiophene-2-carbaldehyde and cinnamaldehyde gave the cor-

responding products without generation of polymeric by-products under the present reaction conditions (entries 14,15). In the case of substituted benzaldehydes, the 2-substituted isomer (entries 8,9,12) was less reactive than the 4-substituted isomer, probably due to the increased steric hindrance. It is noteworthy that no competitive side reactions such as the formation of 2,2'-aryl-methylene bis(3-hydroxy-2-cyclohexene-1-one) derivatives were observed in these transformations.^{10,17}

To the best of our knowledge synthesis of 1,8-dioxo-octahydroxanthenes from the reaction of aldehydes with dimedone at room temperature is rare. Most of the reported methods need high temperatures or the use of an additional energy (ultrasound or microwave).^{29,30}

Following these results, we further investigated the potential of MWCNT–BuSO₃H for the synthesis of tetrahydrobenzo[*a*]xanthen-11-ones through condensation of aldehydes, dimedone, and 2-naphthol at room temperature with ethanol as the solvent. We observed that tetrahydrobenzo[*a*]xanthen-11-ones were obtained in moderate yields after long reaction times. However, when the reactions were carried out in refluxing ethanol the desired products were obtained in high yields at very short reaction times in the presence of 0.05 mmol of catalyst (Scheme 3). In comparison with the other catalysts employed for the synthesis of tetrahydrobenzo[*a*]xanthen-11-ones,^{31,32} MWCNT–BuSO₃H showed a higher catalytic activity in terms of shorter reaction time and higher yields.

As shown in Table 1 (entries 10,11), the aromatic aldehydes with electron withdrawing groups reacted very well at faster rate compared with aromatic aldehydes substituted with electron releasing groups. This observation can be rationalized on the basis the mechanistic details of the reaction (Scheme 4). The aldehyde is first activated by MWCNT–BuSO₃H. Nucleophilic addition of dimedone to the activated aldehyde followed by the loss of H₂O generates intermediate I, which is further activated by MW-



Scheme 3. Synthesis of tetrahydrobenzo[*a*]xanthen-11-ones using MWCNT–BuSO₃H.

CNT–BuSO₃H. Then, the 1,4-nucleophilic addition of a second molecule of dimedone on the activated intermediate I, in the Michael addition fashion, affords the intermediate II, which undergoes intramolecular cyclodehydration to give the 1,8-dioxo-octahydroxanthene. The electron withdrawing groups present on the aromatic al-

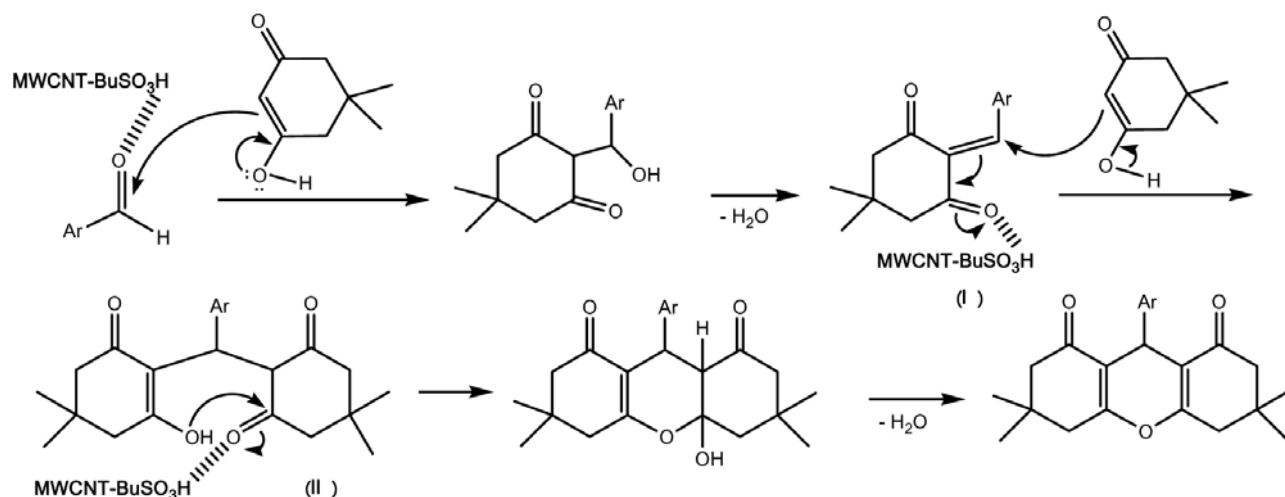
dehyde in the intermediate I increase the rate of 1,4-nucleophilic addition reaction because the alkene LUMO is at lower energy in their presence compared with the aldehydes possessing electron donating groups.³³

The reusability of the MWCNT–BuSO₃H was also determined. MWCNT–BuSO₃H recovered after the reac-

Table 1: Synthesis of 1,8-dioxo-octahydroxanthenes.

Entry	Aldehyde	Time (min)	Yield (%) ^{a,b}	mp (°C) (lit.) ^{ref.}
1	Benzaldehyde	30	95	201–203 (204–205) ¹⁴
2	4-Methylbenzaldehyde	35	94	210–215 (213–215) ¹⁴
3	4-Isopropylbenzaldehyde	36	95	201–203 (203–206) ¹⁸
4	4-Methoxybenzaldehyde	37	96	240–243 (242–244) ¹²
5	3-Methoxybenzaldehyde	36	93	163–165 (162–165) ¹¹
6	4-Hydroxybenzaldehyde	40	93	243–245 (249–251) ¹⁴
7	4-Chlorobenzaldehyde	30	95	228–231 (231–233) ²⁰
8	2-Chlorobenzaldehyde	35	91	226–228 (224–226) ²³
9	2,4-Dichlorobenzaldehyde	35	92	249–253 (248–250) ¹¹
10	4-Cyanobenzaldehyde	26	95	222–225 (218–220) ²³
11	4-Nitrobenzaldehyde	25	96	225–227 (223–224) ¹⁴
12	2-Nitrobenzaldehyde	28	91	260–263 (259–261) ¹²
13	1-Naphthaldehyde	40	95	235–237 (232–234) ¹²
14	Thiophene-2-carbaldehyde	35	94	162–164 (161–162) ¹⁴
15	Cinnamaldehyde	36	93	179–181 (177–178) ²²

^a Isolated yield, ^b All products are known compounds and were identified by comparison of their melting points and ¹H NMR and FT-IR data with those of the authentic samples.



Scheme 4. Suggested mechanism for the preparation of 1,8-dioxo-octahydroxanthenes.

tion can be washed with EtOH and used again at least six times without any noticeable loss of catalytic activity (Figure 5).

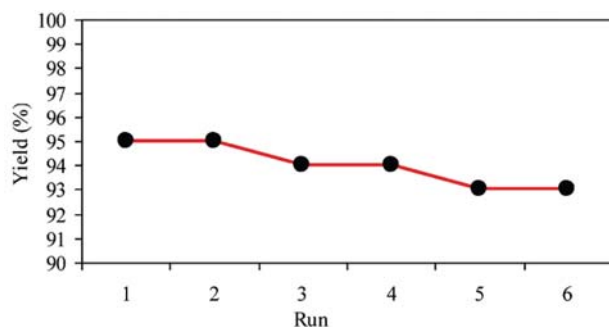


Figure 5. Recyclability of MWCNT-BuSO₃H (0.07 mmol) in the reaction of benzaldehyde (1 mmol) with dimedone (2 mmol) at room temperature. Reaction time 30 min.

To show the merit of the present work in comparison with the other results reported in the literature, we compared results of MWCNT-BuSO₃H with selected previously known protocols in the synthesis of 1,8-dioxo-octahydroxanthenes (Table 2). As can be seen in addition to having the general advantages attributed to the solid catalysts, MWCNT-BuSO₃H has a good efficiency compared to many of other reported catalysts in the synthesis of 1,8-dioxo-octahydroxanthenes.

4. Conclusion

In conclusion, we synthesized a novel multiwalled carbon nanotube catalyst with -SO₃H functional groups.

This reusable heterogeneous acid catalyst preserved high catalytic activity for the synthesis of 1,8-dioxo-octahydroxanthenes. Easy preparation and handling of the catalyst, easy workup, high selectivity, excellent yields, short reaction times, and mild reaction conditions are the obvious advantages of the present method.

5. Acknowledgement

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Table 2: Comparison of the efficiencies of a number of different reported catalysts with that of MWCNT-BuSO₃H in the reaction of benzaldehyde with dimedone.

Entry	Reaction conditions	Time (min)	Yield (%) ^a
1	Silica chloride, MeCN, reflux	360	93 ¹⁰
2	Polyphosphoric acid-SiO ₂ , neat, reflux	30	92 ¹¹
3	In(OTf) ₃ , toluene, reflux	240	85 ¹²
4	H ₂ SO ₄ , water, 70–80 °C	120	90 ¹³
5	InCl ₃ , solvent-free, 100 °C	36	83 ¹⁴
6	CAN under ultrasound irradiation, 2-propanol, 50 °C	35	98 ¹⁵
7	Succinimide- <i>N</i> -sulfonic acid, solvent-free, 80 °C	35	92 ¹⁶
8	CaCl ₂ , DMSO, 85–90 °C	240	85 ¹⁷
9	Fe ₃ O ₄ nanoparticles, solvent-free, 100 °C	30	89 ¹⁸
10	CAN supported HY-zeolite, solvent-free, 80 °C	90	88 ¹⁹
11	Fe ₃ O ₄ @SiO ₂ -Imid-H ₃ PMo ₁₂ O ₄₀ , EtOH, reflux	150	82 ²⁰
12	Mg-Al hydrotalcite, H ₂ O or EtOH, reflux	180	85 ²²
13	Thiourea dioxide, H ₂ O, 50–60 °C	45	96 ²³
14	ZnO, H ₂ O, reflux	20	94 ²⁴
15	MWCNT-BuSO ₃ H, EtOH, r.t.	30	95

^aIsolated yield.

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Povzetek

Iz ustreznih izhodnih snovi smo enostavno pripravili nov katalizator sestavljen iz večstenskih ogljikovih nanocevk z $-\text{SO}_3\text{H}$ funkcionalnimi skupinami. Uporabili smo ga kot učinkovit heterogeni katalizator za enolončno Knoevenaglovo kondenzacijo, Michaelovo adicijo in ciklodehidracijo 5,5-dimetil-1,3-cikloheksandiona (dimeđona) z različnimi aromatskimi aldehidi. S pomočjo te metode smo z odličnimi izkoristki pri sobni temperaturi pripravili serijo 1,8-diokso-oktahidroksantenov. Predstavljena metoda je boljša od že znanih glede na mnoge reakcijske parametre: reakcijsko temperaturo, reakcijski čas, postopek izolacije, izkoristek in ponovno uporabo katalizatorja.

Scientific paper

Heteroannellation of Cyclic Ketones: Synthesis, Characterization and Antitumor Evaluation of Some Condensed Azine Derivatives

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Abstract

A series of pyrimidine and thiazine derivatives was synthesized by one-pot reaction of cyclopentanone with a mixture of an aromatic aldehyde, namely *o*-anisaldehyde, and different ureas, namely urea, guanidine and thiourea, respectively. Furthermore, cycloaddition reaction of active methylene reagents, namely acetyl acetone, malononitrile, ethyl cyanoacetate, cyanoacetamide and *N*-phenyl cyanoacetamide with 2,6-bis(2-methoxybenzylidene)cyclohexanone afforded chromene and quinoline derivatives in basic medium. The antitumor evaluation of some new compounds against three human cell lines, namely MCF-7, NCI-H460 and SF-268 showed significant and moderate activity compared with the positive control doxorubicin.

Keywords: Cyclopentapyrimidine, Thiazolopyrimidine, Quinazoline, Chromene, Antitumor activity

1. Introduction

The azines have been reported to have antibacterial,^{1,2} analgesic,³ antitubercular,^{4,5} anti-inflammatory,^{6,7} antioxidant,^{8,9} and antiviral activities.^{11–14} 2-Oxo-1,2-dihydropyridine-3-carbonitrile derivatives were reported as inhibitors of the oncogenic serine/threonine kinase^{15,16} and for the treatment of the congestive heart failure.^{17,18}

Cycloalkanones, such as cyclopentanone and cyclohexanone, react cleanly with urea or thiourea and aromatic aldehydes to give three families of fused heterobicyclic, benzylidene heterobicyclic, and spiro heterotricyclic pyrimidines as key intermediates for the preparations of many biologically active compounds.^{19–28} The modification, however, is still able to maintain the active moiety of the compound.

In view of these observations and due to our recent interest in developing novel multicomponent reactions (MCRs) for heterocyclic synthesis via dipolar intermediates,^{29–39} we report herein the synthesis of some new derivatives of condensed pyrimidines of cycloalkanone and aldehyde bearing ortho effect with nitrogen nucleophiles and preliminarily evaluate their anticancer properties.

Furthermore, reaction of 2,6-bis(2-methoxybenzylidene)cyclohexanone (**6**) with different cyano nucleophi-

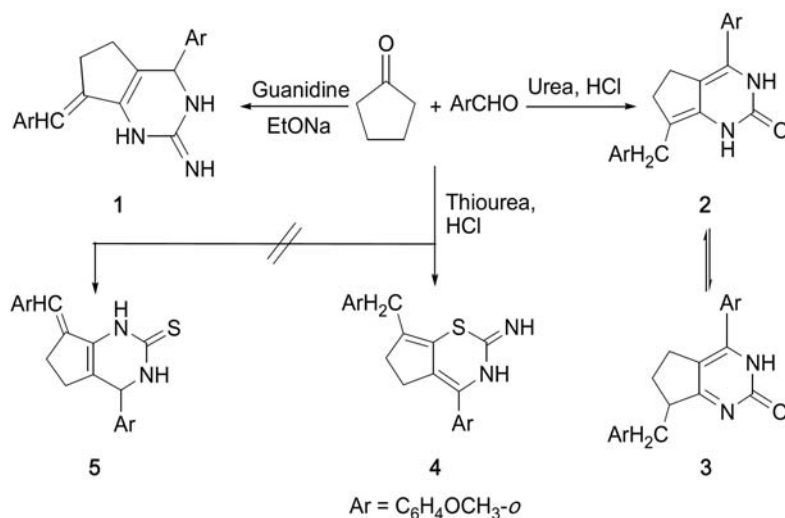
les yielded chromene and quinoline derivatives of promising antitumor activity.

2. Results and Discussion

2.1. Chemistry

The goal of this work was to study the possibility of azine synthesis by [3+3] cycloaddition of α,β -unsaturated systems to diverse nucleophiles, to afford condensed pyrimidine and pyridine ring systems. These compounds are readily available in high yields under the conditions of both acidic and basic catalysis. Thus, one-pot three component reaction of *o*-anisaldehyde, guanidine sulphate and cyclopentanone in a basic medium resulted in a Michael-type adduct that was identified as the cyclic product **1** (Scheme 1).

The ¹H NMR spectrum of **1** exhibited three singlets at δ 10.25–8.60 (D₂O exchangeable) corresponding to the guanidine protons and a singlet at 5.65 ppm belonging to the CH methylenic group. ¹³C NMR of **1** was in agreement with the expected structure that can exist in equilibrium with its non isolable tautomers. On the other hand, acid induced [3+3] cycloaddition of cyclopentanone, anisaldehyde and urea afforded cyclopentapyrimidine derivati-



Scheme 1. One pot synthesis of cyclopenta[*d*]pyrimidines **1**, **2**, **3** and cyclopenta[*e*][1,3]thiazine **4** derivatives.

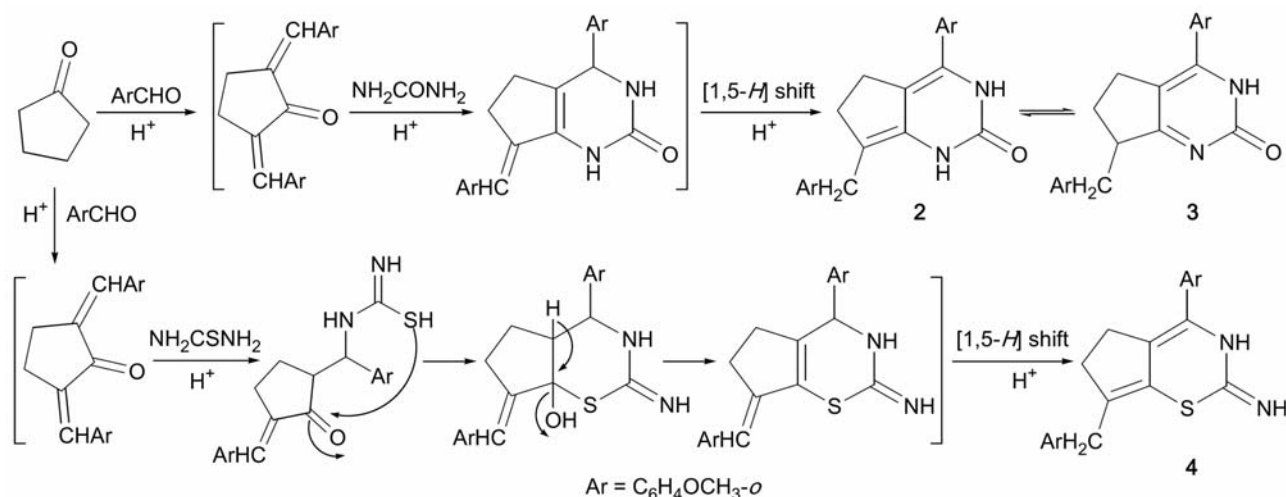
ves **2** and **3** (in ratio 1:1) as shown in Scheme 1. The structures of the latter products were established on the basis of analytical and spectral data. Thus, the ¹H NMR spectrum of **2** showed two singlets at δ 11.81 and 9.98 (D₂O exchangeable) corresponding to the two NH groups and a singlet at δ 3.80 ppm indicating CH₂ benzylic group. The ¹H NMR spectrum of **3** showed a multiplet at δ 5.40, a triplet at 3.75 and a multiplet at δ 3.64–2.65 ppm corresponding to CH methylenic, CH₂ benzylic groups and CH₂ of cyclopentane, respectively.

The three-components Biginelli-like reaction of *o*-anisaldehyde, cyclopentanone and thiourea in an acidic medium resulted in heterocyclization potentiated by the more reactive SH than NH group (*i.e.* kinetic product)⁴⁰ affording thiazine derivative **4** and none of the pyrimidine derivative **5** was obtained (Scheme 1). The structure of **4** was established from its analytical and spectral data.

Thus, the ¹H NMR spectrum of **4** showed two singlets at δ 10.00 and 9.94 (D₂O exchangeable) corresponding to two NH groups and a singlet at 3.91 ppm indicating CH₂ of benzyl group.

Formation of the pyrimidinones **2**, **3** and thiazine **4** from cyclopentanone, *o*-anisaldehyde, urea and/or thiourea presumably proceeds via the formation of acyclic Michael-type adducts of 2,5-bis(2-methoxybenzylidene)cyclopentanone, followed by the heterocyclization and a series of hydrogen shifts with the subsequent isomerization in the case of urea cycloaddition as shown in Scheme 2.

Furthermore, synthesis of pyrimidine thione **7** was achieved via a base induced [3+3] cycloaddition of thiourea and α,β-unsaturated system **6** as shown in Scheme 3. ¹H NMR spectrum of **7** showed two singlets at δ 9.13 and 8.69 corresponding to NH groups and a singlet at δ 5.18 ppm corresponding to the CH methylenic proton. Com-



Scheme 2. Postulated mechanism for the formation of cyclopenta[*d*]pyrimidin-2-ones **2**, **3** and cyclopenta[*e*][1,3]thiazin-2(3*H*)-imine **4** derivatives.

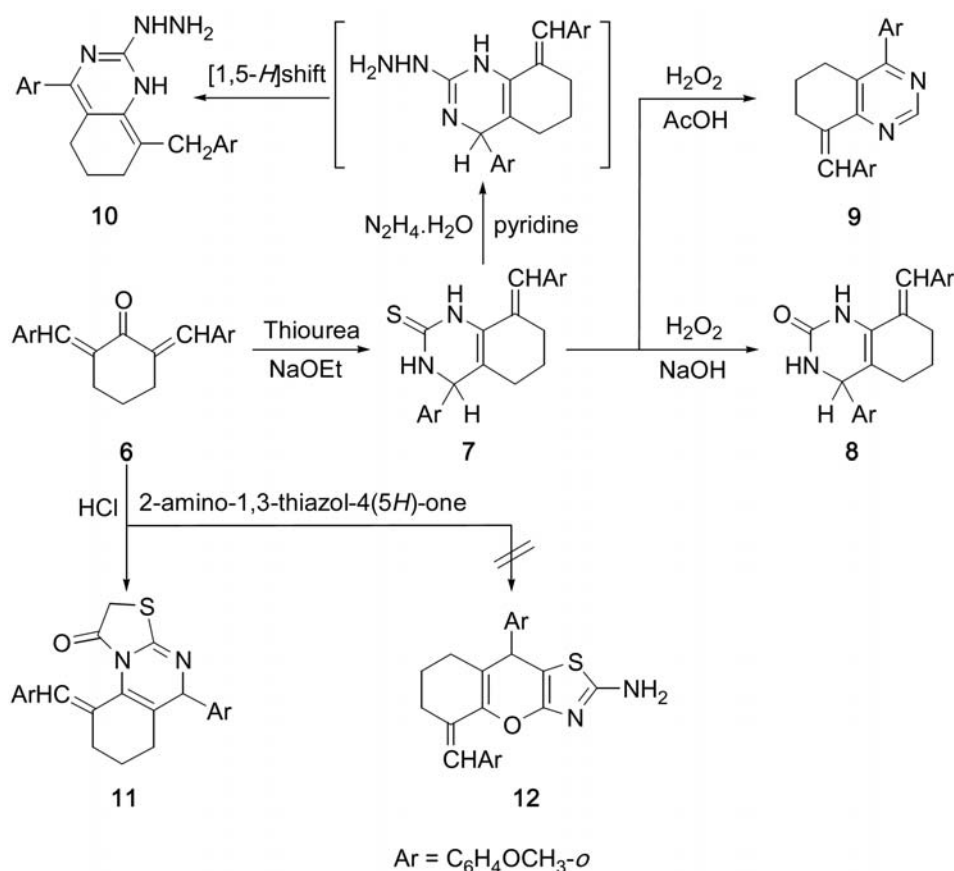
pound **7** was reacted with H_2O_2 in the presence of NaOH to produce the oxidized product that was identified as the pyrimidinone **8**. Whereas using H_2O_2 in acetic acid as the oxidizing agent resulted dehydrogenation, in addition to the desulfurization, afforded the quinazoline derivative of type **9**. Also, the pyrimidine thione **7** was allowed to react with hydrazine hydrate in dry pyridine resulting in the hydrazinolysis in addition to the basic isomerization producing the final product **10** (Scheme 3).

The structures of the latter products were established on the basis of analytical and spectral data. The IR spectrum of **8** revealed a peak at 1671 cm^{-1} of the carbonyl group and ^1H NMR spectrum showed a singlet at δ 8.07 ppm corresponding to the NH group. ^1H NMR spectrum of **9** showed a multiplet at δ 8.20–6.96 ppm corresponding to the aromatic and ethylenic protons. The ^1H NMR of the hydrazino derivative **10** showed two singlets at δ 9.13 and 8.68 (D_2O exchangeable) corresponding to NH groups, a singlet at 5.18 (D_2O exchangeable) belonging to the NH_2 group and a singlet at 3.84 ppm indicating CH_2 benzylic protons.

Curiously, α,β -unsaturated system of the type **6** underwent intermolecular cycloaddition with 2-amino-1,3-thiazol-4(5*H*)-one to produce thiazolopyrimidine derivative **11** potentiated by the high nucleophilicity of the ring

nitrogen than the enolic tautomer of thiazolone, therefore none of the chromenothiazole **12** was obtained (Scheme 3). The analytical and spectral data were consistent with the proposed structure. Thus, the IR spectrum of **11** revealed a peak at 1696 cm^{-1} of the carbonyl group and the ^1H NMR spectrum showed double doublet at δ 4.14 corresponding to the CH_2 group of thiazole, a singlet at δ 4.50 indicating CH methylenic and a multiplet at δ 7.95–6.93 ppm corresponding to Ar-H and CH ethylenic group.

Upon the reaction of *o*-anisalcyclohexanone **6** with acetyl acetone (AcAc) a cycloaddition took place forming chromene derivative, which in turn underwent a hydrogen shift giving the final product **13**. None of the naphthalene derivative **14** was obtained due to the enolic tautomer of the intermediate adduct facilitating the attack of the enolic OH to the acetyl carbonyl under the reaction conditions to produce the desired chromene **13** (Scheme 4). The analytical and spectral data were consistent with the proposed structure. Thus, the IR spectrum of **13** revealed a peak at 1660 cm^{-1} of the carbonyl group and the ^1H NMR spectrum showed a singlet at δ 3.88 indicating the CH_2 benzylic group, a singlet at δ 2.49 corresponding to the acetyl protons and a singlet at δ 2.46 ppm belonging to methyl protons.



Scheme 3. The synthetic route for cycloaddition of α,β -unsaturated cyclic ketone.

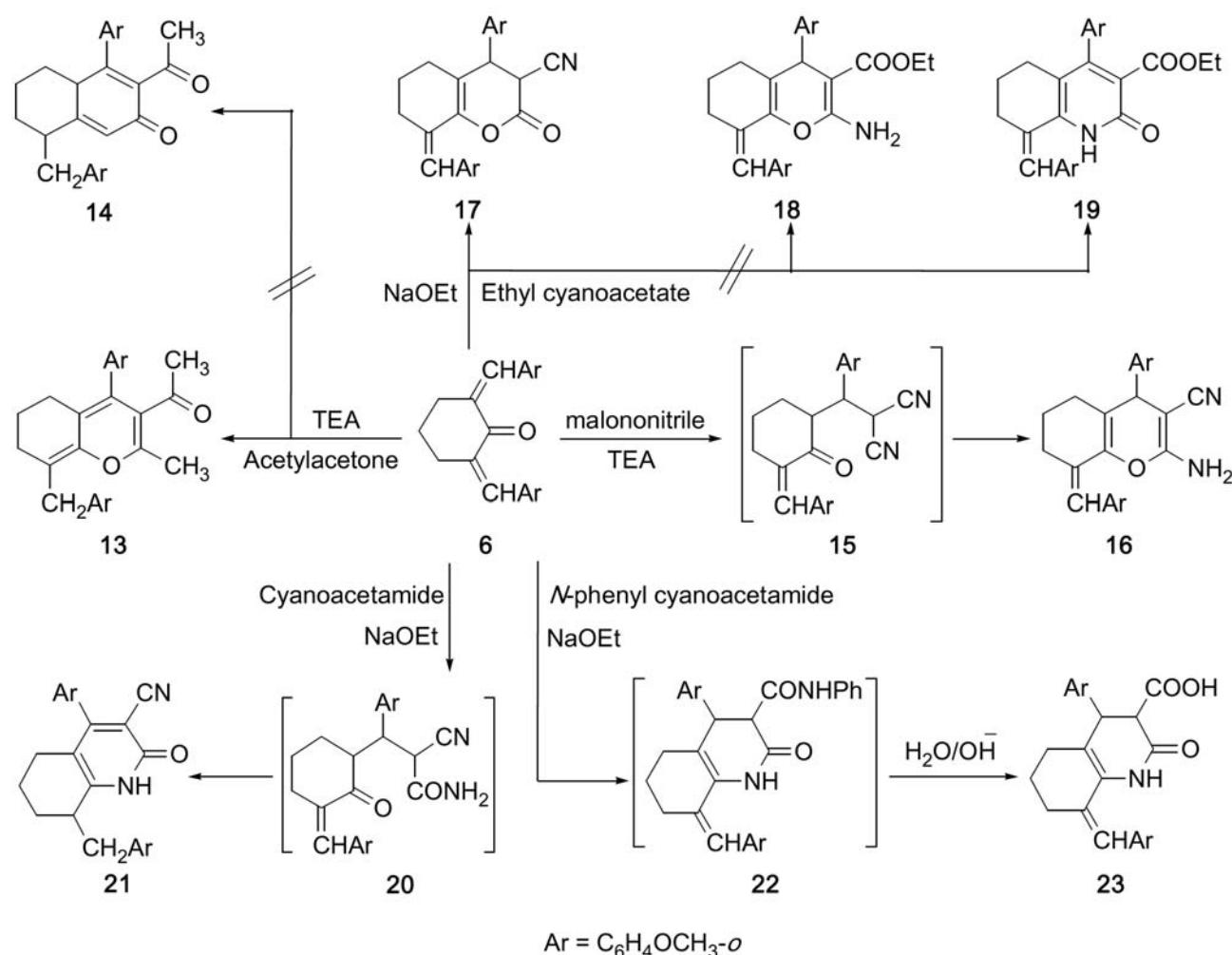
The high yield of α,β -unsaturated system of the type **6** encouraged us to study their further reactivity towards cyanomethylene reagents. Thus, malononitrile added its nucleophilic carbon to the electrophilic carbon of **6** producing acyclic Michael-type adduct **15** that intramolecularly cyclizes producing chromene-3-carbonitrile of the type **16**. While, α,β -unsaturated system **6** when allowed to react with ethyl cyanoacetate afforded chromene-3-carbonitrile of the type **17**. None of the products **18** and **19** were obtained. Concerning the proposed mechanism, we expected that attack of the enolic OH to the ester carbonyl, which is more electrophilic than the cyano carbon, leads to the formation of chromene-3-carbonitrile **17** (Scheme 4). The analytical and spectral data of the obtained products were in agreement with the assigned structures. Thus, the ^1H NMR spectrum of **17** (as an example) showed beside the expected signals of the cyclohexane moiety, two singlets at δ 3.83 and 3.78 ppm corresponding to the two CH groups, a multiplet at δ 7.80–6.97 ppm including the aromatic protons with CH ethylenic groups and the IR spectrum exhibited peaks at

2197 and 1674 cm^{-1} of the cyano and carbonyl groups, respectively.

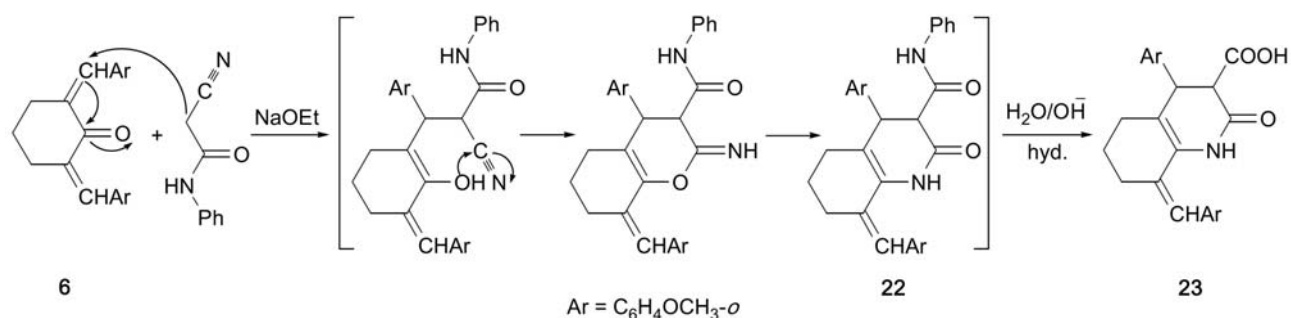
Also, cyanoacetamide produced the Michael-type adduct **20** upon its reaction with ketonic compound **6** followed by basic isomerization giving the final quinoline product **21**. The IR spectrum of **21** revealed a peak at 2223 cm^{-1} of the CN group and the ^1H NMR spectrum showed a singlet at δ 12.05 according to NH group and doublet at δ 3.71 ppm indicating the Ar-CH₂ protons.

Finally, reaction of 2-cyano-*N*-phenylacetamide with the chalcone **6** in a basic medium afforded the intermediate product **22** which in turn underwent basic hydrolysis producing quinoline derivative **23** (Scheme 4). This reaction presumably proceeds via Michael addition followed by an intramolecular cyclization and subsequent Dimroth rearrangement affording **22** which in turn underwent basic hydrolysis producing quinoline derivative **23** (Scheme 5). The analytical and spectral data were consistent with the proposed structure.

Thus, the IR spectrum of **23** revealed peaks at 3432 for the acidic OH (broad) and $1707\text{--}1628\text{ cm}^{-1}$ characteri-



Scheme 4. Condensation reactions of α,β -unsaturated cyclic ketones with active methylene reagents.



Scheme 5. Mechanism for the formation of product 23.

stic for the carbonyl groups. The ¹H NMR spectrum showed a multiplet at δ 7.56–6.88 corresponding to the Ar-H and CH ethylenic, a singlet at δ 9.52 (D₂O exchangeable) indicating the NH group and a singlet at δ 12.11 ppm belonging to the carboxylic proton, in addition to the expected signals of the cyclohexane moiety.

3. 2. Antitumor Activity

2. 2. 1. Tumor Cell Growth Assay

The effects of compounds **1**, **13**, **16**, **17** and/or **21** on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth.^{41,42} Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Winooski, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50% (GI₅₀), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere.⁴³ Doxorubicin was used as a positive control and tested in the same manner. For our newly synthesized products we selected the three

cancer cell lines: the breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) as our compounds are electron rich systems substituted with electronegative groups and many reports from previous work used such cell lines together with the use of doxorubicin which was showed to be the best positive control against the three cell lines (Table 1).

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI₅₀) after a continuous exposure of 48 h and show means ± SEM of three independent experiments performed in duplicate.

2. 2. 2. Structure Activity Relationship (SAR)

The compound **16** with –CN substitution at C-3 position of chromene ring and –NH₂ substitution at C-2 position exhibited potent antitumor activity in MCF-7, NCI-H460 and significant effect in SF-268. Also, compound **17** with –CN substitution at C-3 position of chromen-2-one ring exhibited potent antitumor activity in SF-268, NCI-H460 and significant effect in MCF-7. However, compound **13** with –COCH₃ substitution at C-3 position of chromene moiety as well as –CH₃ substitution at C-2 position showed significant effect in MCF-7 and moderate activity in both NCI-H460 and SF-268. On the other hand, 2-oxoquinolinecarbonitrile **21** with –CN substitution at C-3 position was the lowest in both. Comparing the antitumor activity of the tested compounds and their analogous described in the literature,^{37–38} it is obvious that the highest cytotoxicity might be attributed to the presence of

Table 1. Effect of compounds **1**, **13**, **16**, **17** and **21** on the growth of three human tumor cell lines

Compound	GI ₅₀ (μM) (% growth)		
	MCF-7	NCI-H460	SF-268
1	20.23 ± 4.50	18.28 ± 4.21	42.62 ± 4.80
13	14.27 ± 6.07	18.15 ± 4.05	20.27 ± 2.40
16	4.16 ± 1.09	7.25 ± 1.30	12.80 ± 3.90
17	13.48 ± 4.22	6.09 ± 1.88	4.62 ± 1.12
21	22.31 ± 3.40	18.29 ± 2.40	28.11 ± 10.30
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

the cyanoaminochromene and cyanochromen-2-one moiety bearing 2-CH₃OC₆H₄ group.

3. Experimental

3.1. Chemistry

All melting points were determined using a Stuart melting point apparatus by the open capillary tube method and are uncorrected. IR spectra were recorded on a Perkin–Elmer model 1600 FT-IR instrument as KBr pellets. ¹H and ¹³C NMR spectra were recorded on a Varian 300 MHz in DMSO-*d*₆ as solvent, using TMS as internal standard and chemical shifts are expressed as δ ppm. Antitumor activity and elemental analyses were performed by the Micro Analytical Center, Cairo University, Egypt. The starting material **6** was prepared as described in the literature.⁴⁴ The progress of the reaction and the purity of the compounds were routinely monitored on TLC by pre-coated aluminum silica gel 60F₂₅₄ thin layer plates obtained from Merck (Germany) eluting with petroleum ether/ethyl acetate. The yields of all products were not optimized. All reagents used were obtained from commercial sources. All solvents were of analytical grade and used without further purification.

7-(2-Methoxybenzylidene)-4-(2-methoxyphenyl)-1,3,4,5,6,7-hexahydro-2*H*-cyclopenta[*d*]pyrimidin-2-imine (1)

A mixture of *o*-anisaldehyde (2.72 g, 0.02 mol), cyclopentanone (0.8 g, 0.01 mol) and guanidine sulphate (1.57 g, 0.01 mol) in 50 mL ethoxide solution [prepared by dissolving Na (0.92 g, 0.04 mol) in 50 mL absolute ethanol] was heated under reflux for 5 h. The reaction mixture was cooled, poured onto crushed ice and neutralized with acetic acid. The separated solid was filtered off, dried and recrystallized from acetic acid.

Yield: 78%; m.p.: 258–260 °C; IR (KBr, cm⁻¹): 3434 (NH), 2925, 2856 (CH aliphatic), 1635 (C=N); ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.25, 9.85, 8.60 (s, 3H, 3NH), 7.82–6.90 (m, 9H, Ar-H + CH ethylenic), 5.65 (s, 1H, Ar-CH), 3.85, 3.78 (s, 6H, 2OCH₃), 3.17–2.85 (m, 4H, CH₂ cyclopentane); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 26.59, 28.28, 28.92, 29.32, 51.49, 55.78, 55.86, 55.98, 56.21, 111.44, 111.61, 111.84, 112.06, 115.96, 116.23, 118.35, 118.80, 119.35, 119.55, 119.63, 119.66, 120.09, 120.36, 120.57, 120.74, 120.84, 121.00, 121.36, 122.92, 124.07, 125.52, 125.87, 127.42, 128.12, 128.27, 128.45, 128.61, 128.97, 129.09, 129.64, 129.91, 129.97, 130.25, 130.45, 131.43, 132.37, 133.19, 136.92, 138.24, 138.90, 140.29, 152.77, 156.66, 156.72, 156.84, 156.95, 157.87, 157.95, 160.79, 160.83, 161.00, 161.28, 171.40, 195.79. Anal. Calcd. for C₂₂H₂₃N₃O₂ (361.43): C, 73.11; H, 6.41; N, 11.63. Found: C, 73.05; H, 6.17; N, 11.56.

General Procedure for the Synthesis of Compounds 2, 3 and 4

A mixture of *o*-anisaldehyde (2.72 g, 0.02 mol), cyclopentanone (0.8 g, 0.01 mol) with 0.60 g urea and/or 0.76 g thiourea (0.01 mol), and conc. HCl (0.03 mol) in ethanol (30 mL) was heated under reflux for 5 h. The reaction mixture was cooled and poured into ice cold water. The precipitated solid was filtered off, dried and recrystallized from the proper solvent to give the products **2**, **3** and **4**, respectively.

7-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-1,3,5,6-tetrahydro-2*H*-cyclopenta[*d*]pyrimidin-2-one (2). Yield: 40% from benzene; m.p.: 240–242 °C; IR (KBr, cm⁻¹): 3414 (NH), 2924, 2854 (CH aliphatic), 1626 (C=O); ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.81, 9.98 (s, 2H, 2NH), 7.86–6.92 (m, 8H, Ar-H), 3.87, 3.82 (s, 6H, 2OCH₃), 3.80 (s, 2H, Ar-CH₂), 3.04, 2.62 (m, 4H, 2CH₂ cyclopentane). Anal. Calcd. for C₂₂H₂₂N₂O₃ (362.42): C, 72.91; H, 6.12; N, 7.73. Found: C, 73.22; H, 5.82; N, 7.33.

7-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-1,5,6,7-tetrahydro-2*H*-cyclopenta[*d*]pyrimidin-2-one (3). Yield: 45% from methanol; m.p.: 200–202 °C; IR (KBr, cm⁻¹): 3408 (NH), 3076 (CH aromatic), 2930, 2854 (CH aliphatic) 1646 (C=O amide); ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.95 (s, 1H, NH), 7.87–6.80 (m, 8H, Ar-H), 5.40 (m, 1H, Ar-CH), 3.87, 3.82 (s, 6H, 2OCH₃), 3.75 (t, 2H, *J* = 10.2 Hz, Ar-CH₂), 3.64–2.65 (m, 4H, 2CH₂ cyclopentane). Anal. Calcd. for C₂₂H₂₂N₂O₃ (362.42): C, 72.91; H, 6.12; N, 7.73. Found: C, 72.63; H, 6.00; N, 7.45.

7-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-5,6-dihydrocyclopenta[*e*][1,3]-thiazin-2(3*H*)-imine (4). Yield: 79% from aqueous methanol; m.p.: 220–222 °C; IR (KBr, cm⁻¹): 3383, 3205 (NH), 3066 (CH aromatic), 2925, 2857 (CH aliphatic), 1592 (C=N); ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.00, 9.94 (s, 2H, 2NH), 7.54–6.46 (m, 8H, Ar-H), 3.91 (s, 2H, Ar-CH₂), 3.87, 3.74 (s, 6H, 2OCH₃), 3.17–2.73 (m, 4H, 2CH₂ cyclopentane). Anal. Calcd. for C₂₂H₂₂N₂O₂S (378.48): C, 69.81; H, 5.86; N, 7.40. Found: C, 70.12; H, 5.78; N, 7.14.

Synthesis of 4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-3,4,5,6,7,8-hexahydro-2(1*H*)-quinazolinethione (7)

A mixture of compound **6** (3.34 g, 0.01 mol), thiourea (0.76 g, 0.01 mol) and sodium ethoxide (0.02 mol) [prepared of sodium (0.46 g) dissolved in absolute ethanol (20 mL)] in absolute ethanol (30 mL) was heated under reflux for 4 h. The solid product obtained upon cooling was poured onto crushed ice and acidified with acetic acid, filtered off, dried and recrystallized from acetic acid.

Yield: 85%; m.p.: 165–167 °C; IR (KBr, cm⁻¹): 3404, 3247 (NH), 3063 (CH aromatic), 2933, 2832 (CH aliphatic), 1655 (C=N); 1594 (C=C), 1243 (C=S); ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.14, 8.70 (s, 2H, 2NH), 7.31–6.89 (m, 9H, Ar-H + CH ethylenic), 5.19 (s, 1H,

Ar-CH), 3.81, 3.79 (s, 6H, 2OCH₃), 2.50–1.46 (m, 6H, CH₂ cyclohexane). ¹³C-NMR (75 MHz, DMSO-*d*₆): δ 22.25 (CH₂), 26.11 (CH₂), 26.63 (CH₂), 52.46 (N-C-C), 55.17 (OCH₃), 55.56 (OCH₃), 110.81, 111.24, 113.72, 119.00, 119.78, 120.81, 125.51, 127.53, 127.56, 128.32, 128.98, 130.14, 130.67 (N-C=C), 156.00 (O-C=C), 156.88 (O-C=C), 174.48 (C=S). Anal. Calcd. for C₂₃H₂₄N₂O₂S (392.51): C, 70.38; H, 6.16; N, 7.14. Found: C, 70.03; H, 5.86; N, 6.83.

Synthesis of 4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-3,4,5,6,7,8-hexahydro-2(1H)-quinazolinone (8)

A mixture of **7** (3.92 g, 0.01 mol) and sodium hydroxide (0.40 g, 0.01 mol) was dissolved in DMF (30 mL). To this solution, H₂O₂ (0.02 mol) was added drop wise with stirring at r.t. for 2 h. The reaction mixture was neutralized by HCl, and the precipitated solid was filtered off, dried and recrystallized from methanol.

Yield: 89%; m.p.: 180–182 °C; IR (KBr, cm⁻¹): 3407 (OH enolic); 3336, 3235 (NH), 3111, 3067 (CH aromatic), 2947, 2878 (CH aliphatic), 1671 (C=O), 1594 (C=N); ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.07 (s, 2H, 2NH), 7.28–6.82 (m, 9H, Ar-H + CH ethylenic), 5.19 (s, 1H, Ar-CH), 3.80, 3.78 (s, 6H, 2OCH₃), 2.49–1.49 (m, 6H, CH₂ cyclohexane). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 22.45 (CH₂), 25.93 (CH₂), 26.64 (CH₂), 52.35 (N-C-C), 55.71 (OCH₃), 55.56 (OCH₃), 110.83, 110.98, 111.24, 118.99, 119.75, 120.74, 125.87, 127.36, 127.56, 128.13, 128.62, 128.97, 130.68, 131.58 (N-C=C), 153.73 (C=O), 156.20 (O-C=C), 156.90 (O-C=C). Anal. Calcd. for C₂₃H₂₄N₂O₃ (376.44): C, 73.38; H, 6.43; N, 7.44. Found: C, 73.03; H, 6.53; N, 7.63.

Synthesis of 8-(2-Methoxybenzylidene)-4-(2-methoxyphenyl)-5,6,7,8-tetrahydroquinazoline (9)

To a solution of **7** (3.92 g, 0.01 mol) in acetic acid (20 mL), H₂O₂ (0.02 mol) was added drop wise at r.t. with stirring. Furthermore, the reaction mixture was stirred at r.t. for 3 h. The separated solid was collected by filtration, washed with water, dried and recrystallized from methanol.

Yield: 65%; m.p.: 136–138 °C; IR (KBr, cm⁻¹): 2924, 2856 (aliphatic CH), 1600 (C=N); ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.20–6.96 (m, 10H, Ar-H + CH ethylenic), 3.81, 3.72 (s, 6H, 2OCH₃), 2.72–0.74 (m, 6H, CH₂ cyclohexane). Anal. Calcd. for C₂₃H₂₂N₂O₂ (358.43): C, 77.07; H, 6.19; N, 7.82. Found: C, 76.79; H, 5.98; N, 7.59.

Synthesis of 2-Hydrazino-8-(2-methoxybenzyl)-4-(2-methoxyphenyl)-1,5,6,7-tetrahydroquinazoline (10)

A mixture of **7** (3.92 g, 0.01 mol) and hydrazine hydrate (0.015 mol) in pyridine (20 mL) was refluxed for 5 h. The reaction mixture was cooled and neutralized with dilute HCl. The separated solid was filtered off, dried and recrystallized from methanol.

Yield: 54%; m.p.: 130–132 °C; IR (KBr, cm⁻¹): 3400–3264 (NH, NH₂), 2926–2856 (CH aliphatic); ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.13, 8.68 (s, 2H, 2NH, D₂O exchangeable), 7.32–6.89 (m, 8H, Ar-H), 5.18 (s, 2H, NH₂, D₂O exchangeable), 3.84 (s, 2H, Ar-CH₂), 3.81, 3.78 (s, 6H, 2OCH₃), 2.45–1.05 (m, 6H, CH₂ cyclohexane); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 22.77 (CH₂), 26.64 (CH₂), 27.11 (CH₂), 42.64 (Ar-CH₂), 52.91, 55.57 (OCH₃), 56.07 (OCH₃), 111.31, 111.75, 114.31, 119.55, 120.29, 121.33, 126.00, 128.02, 128.05, 128.85, 129.46, 129.52, 130.66, 131.20 (C-NHNH₂), 156.47 (Ar-C), 157.38 (Ar-C), 174.96 (C=N). Anal. Calcd. for C₂₃H₂₆N₄O₂ (390.48): C, 70.75; H, 6.71; N, 14.35. Found: C, 70.51; H, 6.91; N, 14.63.

Synthesis of 5-(2-Methoxyphenyl)-9-[(2-methoxyphenyl)methylidene]-6,7,8,9-tetrahydro-5H-[1,3]thiazolo[3,2-a]quinazolin-1(2H)-one (11)

A mixture of chalcone **6** (3.34 g, 0.01 mol), 2-amino-1,3-thiazol-4(5H)-one (1.16 g, 0.01 mol) and conc. HCl (1.5 mL) in ethanol (30 mL) was refluxed for 5 h. The reaction mixture was left to cool at room temperature. The precipitated solid was filtered off, dried and recrystallized from acetic acid.

Yield 63%; m.p.: > 360 °C; IR (KBr, cm⁻¹): 3411 (OH enolic), 2927–2859 (CH aliphatic), 1696 (C=O), 1618 (C=N); ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.95–6.93 (m, 9H, Ar-H + CH ethylenic), 4.50 (s, 1H, Ar-CH), 4.14 (d, 2H, *J* = 0.6 Hz, CH₂ of thiazole), 3.83, 3.80 (s, 6H, 2OCH₃), 2.86–1.70 (m, 6H, CH₂ cyclohexane). Anal. Calcd. for C₂₅H₂₄N₂O₃S (432.53): C, 69.42; H, 5.59; N, 6.48. Found: C, 69.12; H, 5.45; N, 6.64.

General Procedure for the Synthesis of Chromene Derivatives 13 and 16

A mixture of **6** (3.34 g, 0.01 mol), acetyl acetone and/or malononitrile (0.01 mol) and a few drops of TEA in dimethyl formamide (30 mL) was heated under reflux for 20 h. The solid product obtained upon cooling, poured into ice cold water and acidified by acetic acid, filtered off, dried, and recrystallized from the proper solvent gave compounds **13** and **16**, respectively.

1-[8-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-2-methyl-6,7-dihydro-5H-chromen-3-yl]-1-ethanone (13). Yield: 69% from aqueous methanol; m.p.: 170–173 °C; IR (KBr, cm⁻¹): 3064 (CH aromatic), 2925, 2851 (CH aliphatic), 1660 (C=O), 1600 (C=C); ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.38–6.64 (m, 8H, Ar-H), 3.88 (s, 2H, CH₂ benzylic), 3.84, 3.71 (s, 6H, 2OCH₃), 2.49 (s, 3H, COCH₃), 2.46 (s, 3H, CH₃), 2.79–1.23 (m, 6H, CH₂ cyclohexane). Anal. Calcd. for C₂₇H₂₈O₄ (416.50): C, 77.86; H, 6.78. Found: C, 77.58; H, 6.67.

2-Amino-4-(2-methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-5,6,7,8-tetrahydro-4H-chromene-3-car-

bonitrile (16). Yield: 73% from methanol; m.p.: 280–282 °C; IR (KBr, cm^{-1}): 3340–3223 (NH_2), 3089 (CH aromatic), 2935 (CH aliphatic), 2205 (CN), 1664 ($\text{C}=\text{N}$), 1593 ($\text{C}=\text{C}$); ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.00 (s, 2H, NH_2), 7.43–6.20 (m, 9H, Ar-H + CH ethylenic), 4.08 (s, 1H, Ar-CH), 3.78, 3.76 (s, 6H, 2OCH_3), 2.82–1.50 (m, 6H, CH_2 cyclohexane); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 28.38 (CH_2), 28.99 (CH_2), 32.96 (CH_2), 33.95 (Ar-CH), 34.54, 55.79, 77.18, 85.81, 111.64, 113.72, 114.40, 118.33, 120.91, 124.90, 126.21, 126.49, 128.56, 128.78, 131.04, 156.31 (Ar-C), 158.27 ($\text{C}-\text{NH}_2$), 164.49 (Ar-C). Anal. Calcd. for $\text{C}_{25}\text{H}_{24}\text{N}_2\text{O}_3$ (400.46): C, 74.98; H, 6.04; N, 7.00. Found: C, 74.69; H, 5.95; N, 6.74.

General Procedure for the Synthesis of Compounds 17, 21 and 23

A mixture of chalcone **6** (3.34 g, 0.01 mol), ethyl cyanoacetate, cyanoacetamide and/or *N*-phenyl cyanoacetamide (0.01 mol) and sodium ethoxide (0.02 mol) [prepared of 0.46 g sodium dissolved in ethanol absolute (20 mL)] in ethanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled, poured into ice cold water and neutralized with acetic acid. The precipitated solid was filtered off, dried to give crude material of **17**, **21** and **22**, respectively. The crude product **22** in 20 mL aqueous NaOH (10%) was heated under reflux for 1 h. The resultant solution was cooled, diluted with ice cold water and acidified with HCl. The precipitated solid was filtered off, dried to give compound **23**.

4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-2-oxo-3,4,5,6,7,8-hexahydro-2H-chromene-3-carbonitrile (17). Yield: 78% from methanol; m.p.: 148–150 °C; IR (KBr, cm^{-1}): 3432 (OH enolic), 3055 (CH aromatic), 2927–2846 (CH aliphatic), 2197 (CN), 1674 ($\text{C}=\text{O}$), 1594 ($\text{C}=\text{C}$); ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 7.80–6.97 (m, 9H, Ar-H + CH ethylenic), 3.83, 3.78 (dd, 2H, $J = 9.0$; 6.6 Hz, 2CH), 3.77, 3.72 (s, 6H, 2OCH_3), 2.79–1.56 (m, 6H, CH_2 cyclohexane). Anal. Calcd. for $\text{C}_{25}\text{H}_{23}\text{NO}_4$ (401.54): C, 74.79; H, 5.77; N, 3.49. Found: C, 74.47; H, 5.47; N, 3.14.

8-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-2-oxo-1,2,5,6,7,8-hexahydro-3-quinolinecarbonitrile (21). Yield: 75% from acetic acid; m.p.: 265–267 °C; IR (KBr, cm^{-1}): 3468 (NH), 3011 (CH aromatic), 2932, 2837 (CH aliphatic), 2223 (CN), 1635 ($\text{C}=\text{O}$); ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 9.82 (s, 1H, NH), 7.59–6.83 (m, 8H, Ar-H), 4.31 (d, 2H, $J = 4.2$ Hz, Ar- CH_2), 3.82, 3.72 (s, 6H, 2OCH_3), 2.45–1.55 (m, 7H, CH cyclohexane). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 22.13 (CH_2), 25.06 (CH_2), 26.48 (CH_2), 55.40 (OCH_3), 55.65 (OCH_3), 111.13, 111.72, 115.91, 119.97, 120.76, 124.19, 124.69, 127.40, 128.80, 129.68, 130.10, 130.87, 155.20 ($\text{O}-\text{C}=\text{C}$), 157.33 ($\text{O}-\text{C}=\text{C}$), 160.18 ($\text{C}=\text{O}$). Anal. Calcd. for $\text{C}_{25}\text{H}_{24}\text{N}_2\text{O}_3$

(400.47): C, 74.98; H, 6.04; N, 7.00. Found: C, 75.33; H, 5.95; N, 6.78.

4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-2-oxo-1,2,3,4,5,6,7,8-octahydro-3-quinolinecarboxylic acid (23). Yield: 67% from benzene; m.p.: 238–240 °C; IR (KBr, cm^{-1}): 3432 (OH broad), 2924, 2854 (CH aliphatic), 1707, 1628 ($\text{C}=\text{O}$); ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 12.11 (s, 1H, OH), 9.52 (s, 1H, NH), 7.56–6.88 (m, 9H, Ar-H + CH ethylenic), 4.92 (d, 1H, $J = 3$ Hz, CH-CO), 3.77 (d, 1H, $J = 4$ Hz, Ar-CH), 3.74, 3.70 (s, 6H, 2OCH_3), 2.73–1.23 (m, 6H, CH_2 cyclohexane). Anal. Calcd. for $\text{C}_{25}\text{H}_{25}\text{NO}_5$ (419.47): C, 71.58; H, 6.01; N, 3.34. Found: C, 71.93; H, 5.86; N, 3.66.

3. 2. Antitumor Activity Tests

Reagents: Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures: Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7, XF498, colon; A549, ovarian; HCT15, stomach; was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), NCI-H460, SF-268 and normal fibroblast cells (WI 38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as a monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μM), at 37 °C in a humidified atmosphere containing 5% CO_2 . Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7, NCI-H460 and SF-268 and 0.75×10^4 cells/mL followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

4. Conclusion

A series of novel condensed pyrimidine, pyran and pyridine derivatives were synthesized and assayed for their antitumor activity against three human cell lines namely MCF-7, NCI-H460 and SF-268. The activity comparison and the structure correlation of the tested compounds had shown that these potencies paralleled the electron withdrawing powers of the substituent groups. Hence, the higher cytotoxicity of compounds **14** and **15** was attributed to the presence of the electronegative cyano group.

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6. Supplementary Material

Copies of the IR, ¹H, ¹³C NMR spectra and antitumor evaluations of the new compounds are available on the Journal's website.

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Povzetek

Z »one-pot« reakcijo med ciklopentanonom, ustreznim aromatskimi aldehidom (*o*-anisaldehyd) in različnimi sečninami (sečnina, gvanidin, tiosečnina) smo sintetizirali serijo pirimidinskih in tiazinskih derivatov. Pri cikloadiciji reagentov, ki vsebujejo aktivno metilensko skupino (acetil aceton, malononitril, etil cianoacetat, cianoacetamid in *N*-fenil cianoacetamid), z 2,6-bis(2-metoksibenziliden)cikloheksanonom pod bazičnimi pogoji nastanejo kromenski in kinolinski derivati. Za nekatere nove spojine smo preučili tudi njihove antitumorne lastnosti proti trem človeškim rakastim celičnim linijam, in sicer MCF-7, NCI-H460 in SF-268, ter ugotovili zmerno dobre aktivnosti glede na pozitivno kontrolo doksorubicin.

Scientific paper

Crystal Structure, Hirshfeld Surface Analysis and Computational Studies of Thiazolidin-4-one derivative: (Z)-5-(4-Chlorobenzylidene)-3-(2-ethoxyphenyl)-2-thioxothiazolidin-4-one

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Abstract

The title compound (Z)-5-(4-chlorobenzylidene)-3-(2-ethoxyphenyl)-2-thioxothiazolidin-4-one (CBBTZ) was characterized by X-ray single crystal diffraction, ¹H NMR and ¹³C NMR spectra. Theoretical investigations were carried out using HF and DFT levels of theory at 6-31G(d,p) basis set. The X-ray structure is compared with that computed. The calculated geometrical parameters are in good agreement with those determined by X-ray diffraction. The dihedral angle between the two benzene rings is 16.89(5)° indicating that the structure is non planar. The molecule exhibits intra- and intermolecular contacts of type C–H...O, C–H...S and C–H...Cl. The intercontacts in the crystal structure are explored using Hirshfeld surfaces analysis method.

Keywords: Structure, thiazolidin-4-one, theoretical calculations, intermolecular interactions, Hirshfeld surface

1. Introduction

Heterocyclic compounds containing five membered rings with nitrogen, sulfur, and oxygen atoms have been investigated since a long time for their important properties. Several theoretical and experimental investigations were performed to determine the absolute molecular configuration of organic compounds containing five-membered heterocyclic derivatives.^{1–3} Among these types of compounds, 4-thiazolidinones have been shown to have various significant activities. Furthermore, thiazolidinone derivatives have been developed into useful materials for a variety of applications, such as of biological interest, nonlinear optical field and photovoltaic cells.^{4–10} Organic photovoltaic compounds (OPVs) have attracted significant attention as low-cost alternatives to conventional semiconductor photovoltaic devices.^{11–13} Consequently, (Z)-5-(4-chlorobenzylidene)-3-(2-ethoxyphenyl)-2-thioxothiazolidin-4-one (CBBTZ) is a

interesting member of the above-mentioned molecules containing delocalized π electrons with donor and acceptor groups. Appropriate electron donor and acceptor groups and π -conjugated system allow the CBBTZ to exhibit the asymmetric electronic distribution which leads to an increased charge transfer.

Recently, some studies have been carried out on CBBTZ. The optical, electrochemical and X-ray photoelectron spectroscopy (XPS) characterization of CBBTZ has been explored and CBBTZ thin films with electronic properties were also studied as an exciton blocking layer in CuPc/C60 heterojunction solar cells.^{14,15}

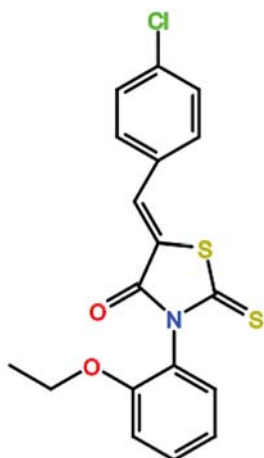
In this context, and in continuation of our works on thiazolidinones molecules, this study was aimed to report the structural properties and intermolecular interactions of CBBTZ.^{1,16}

2. Experiment and Computational Methods

2.1. Experimental

Synthesis, Spectral Data and Spectral Analysis of (Z)-5-(4-Chlorobenzylidene)-3-(2-ethoxyphenyl)-2-thioxothiazolidin-4-one (CBBTZ)

Synthesis and spectral data (IR, ^1H NMR, ^{13}C NMR) of CBBTZ are reported in previous work.¹⁶ The structure of CBBTZ is presented in Scheme 1.



Scheme 1. (Z)-5-(4-Chlorobenzylidene)-3-(2-ethoxyphenyl)-2-thioxothiazolidin-4-one (CBBTZ)

2.2. X-Ray Structure Determination

X-Ray diffraction study was done on single crystal diffractometer Kappa CCD Nonius. X-Ray data have been measured using graphite monochromated MoK α radiation ($\lambda = 0.71073 \text{ \AA}$) at ambient temperature. The program SHELXS-97 was used to solve the structure by direct methods.¹⁷ Then, full-matrix least-squares refinement using SHELXL-97 revealed the final structure.¹⁸ Hydrogen atoms were located in their calculated positions. Figure 1 shows the structure of CBBTZ along with the atomic labeling using ORTEP visualization program.¹⁹ For highlighting intra- and intermolecular interactions, Hirshfeld Surface analyses were performed and fingerprint plots were drawn using Crystal Explorer.²⁰ Crystallographic details and refinement data are summarized in Table 1.

2.3. Theoretical Approach

Throughout this study, Gaussian 03 software²¹ and Gauss-View program²² have been used to perform molecular modelling. B3LYP^{23,24} and HF²⁵ methods were used to optimize the molecular structure of the title compound in the ground state using the 6-31G(d,p) basis

Table 1. Crystallographic details and refinement data

Empirical formula	$\text{C}_{18}\text{H}_{14}\text{ClNO}_2\text{S}_2$
CCDC reference no.	1044524
Molecular weight	375.87
Crystal size (mm)	$0.19 \times 0.14 \times 0.11$
Temperature (K)	295(2)
Crystal system, space group	Triclinic, $P\bar{1}$
Unit cell dimensions	
a (Å)	9.2171(19)
b (Å)	8.4612(7)
c (Å)	11.935(3)
α (°)	101.623(11)
β (°)	90.89(2)
γ (°)	118.148(9)
Wavelength (Å)	0.71073
Volume (Å ³)	797.3(3)
Z , calculated density (mg/m ³)	2/1.566
$F(000)$	388
Reflections collected/unique	4080/2591
Parameters	219
Goodness of fit on F^2	0.945
Final R indices	
R_1	0.0562
wR_2	0.1331
R indices (all data)	
R_1	0.0997
wR_2	0.1683

set.^{26,27} The spatial coordinate positions of the title compound, as obtained from X-ray structural investigation, were used as initial coordinates for the theoretical calculations.

3. Results and Discussion

3.1. Description of the Crystal Structure

Selected experimental geometrical parameters are summarized in Tables 2, 3 and 4. The molecular structure with atomic labelling (thermal ellipsoids are drawn at 50% probability) is depicted in Fig. 1. The thioxothiazolidine ring is essentially planar. The full molecule has a Z configuration about the C7=C8 double bond (Figure 1). This Z configuration of CBBTZ crystal is stabilized by intramolecular hydrogen bonds C–H $\cdots$ O and C–H $\cdots$ S. The CC bond lengths in the phenyl rings have average value of 1.38 Å obtained by X-ray diffraction and calculated mean values of 1.40 Å and 1.39 Å obtained with B3LYP and HF, respectively. The double bond of C7=C8 is characterized by the experimental distance of 1.332(8) Å. The thiazole ring contains two C–S bonds, namely S1–C8 [1.729(7) Å] and S1–C10 [1.717(7) Å]. C9–O2 distance shows a typical double bond character with bond length of 1.198(7) Å. The bond lengths are consistent with previous phenyl ring-containing studies.¹ In the thiazole moiety formed by C8, C9, C10, N1 and S1

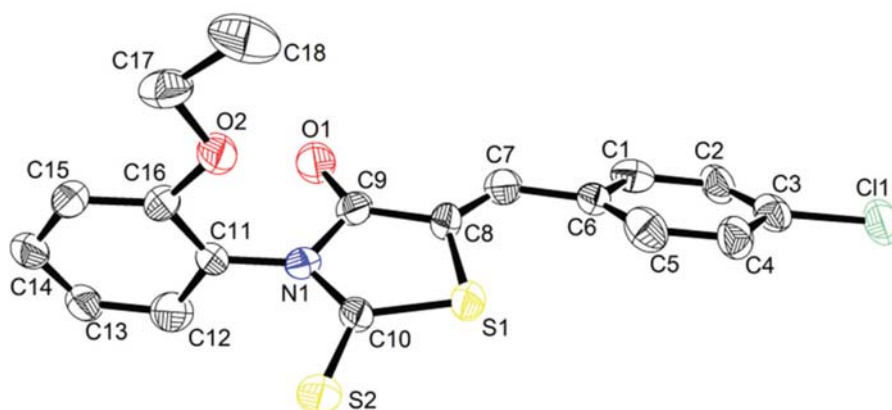


Figure 1. Structure of CBBTZ with atomic labeling scheme (ellipsoids are drawn at 50% probability). For clarity, the hydrogen atoms are omitted.

atoms, the average value of bond angles is $108(5)^\circ$. In addition, delocalization of the π electrons in CBBTZ is confirmed by C–C–C, C–N–C and C–C–N bond angles which are around 120° . The torsion angle between the two benzene (C1–C6) and (C11–C16) rings is $16.89(5)^\circ$. The ethoxyphenyl group is twisted slightly, with a C9–N1–C11–C12 torsion angle of $82.9(12)^\circ$. The two moieties chlorobenzene and thioxothiazolidinone are nearly planar according to the dihedral angle C5–C6–C7–C8 of $16.4(18)^\circ$ (X-ray diffraction) and from 172.5° to 179.5° (theoretical calculation). As mentioned in our previous work,¹⁶ the chirality of this kind of compounds is highlighted by the value of the dihedral angle formed by the heterocyclic ring and the aryl bound at the nitrogen atom. In the present study, this angle is 95.8° . As we can easily see from the above results, there is a good correlation between the experimental and theoretical structural results. The observed differences are due to the fact that experimental results belong to the solid phase, while theoretical calculations belong to the

gas phase of isolated molecules. The molecular packing in the crystal structure of CBBTZ is stabilized by intermolecular interactions forming a three-dimensional network (Figure 2).

3. 2. DFT and HF Optimized Geometry

Figure 3 depicts the calculated molecular structure of CBBTZ using the B3LYP/6-31G(d,p) level of theory. Theoretical geometric parameters by HF and DFT levels of theory using 6-31G(d,p) basis set are given in Tables 2, 3 and 4 together with the experimental ones. The theoretical structural results of CBBTZ have a little different values compared with corresponding experimental results. Thus, bond length values of the thiazole ring N1–C9 and N1–C10 are 1.411 and 1.377 Å computed with B3LYP, with respect to the X-ray results 1.379(8) and 1.330(7) Å, respectively. In the same context, calculated distances S1–C8 (1.765 Å) and S1–C10 (1.763 Å) are comparable to the experimental values (1.729(7) and 1.716(7) Å). The C–C distances in the two aromatic cycles vary from 1.380(8) to 1.401(8) Å compared to the theoretical values which vary from 1.389 to 1.415 Å. According to the above results, deviations of 0.01 Å for bond lengths and 3° for bond and torsion angles are found between experimental

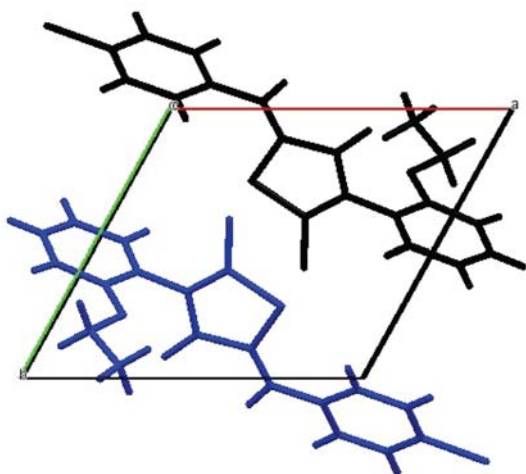


Figure 2. A perspective view of the crystal packing in the unit cell. View along the *c* axis.

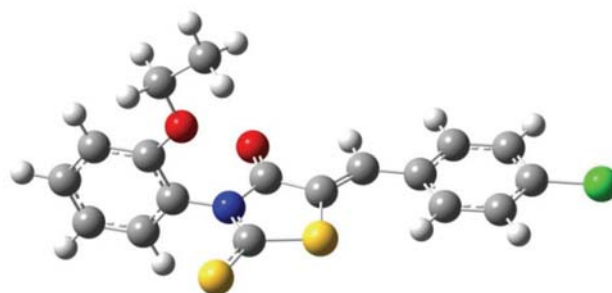


Figure 3. Theoretical crystal structure of CBBTZ with B3LYP/6-31G(d,p) level.

and theoretical geometries. This can be explained by considering that the theoretical calculations were carried out in a gaseous phase, whereas the X-ray diffraction study was performed on the compound in the solid form. Figure 4 compares the calculated structure to that obtained by X-ray diffraction.

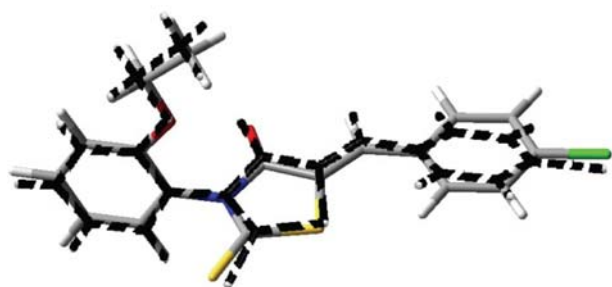


Figure 4. Atom-by-atom superimposition of the structures calculated (solid line) over the X-ray structure (dashed line) for CBBTZ.

Table 2. Experimental and calculated bond lengths

Bond distances (Å)	X-ray	6-31G(d,p)	
		HF	DFT
S1C8	1.729(7)	1.775	1.765
S1C10	1.716(7)	1.745	1.763
S2C10	1.600(7)	1.634	1.642
O1C9	1.198(7)	1.209	1.213
O2C16	1.356(7)	1.340	1.357
O2C17	1.404(12)	1.411	1.431
N1C9	1.379(8)	1.395	1.411
N1C10	1.330(7)	1.354	1.377
N1C11	1.403(7)	1.432	1.436
C7C8	1.332(8)	1.336	1.352
C8C9	1.467(8)	1.486	1.482

Table 3. Experimental and calculated bond angles

Bond angles (°)	X-ray	6-31G(d,p)	
		HF	DFT
S1C10N1	123.0(5)	110.19	109.31
S1C8C7	124.0(6)	120.31	119.60
S1C8C9	111.3(5)	108.47	109.81
S2C10N1	120.8(5)	127.33	127.30
S2C10S1	116.2(4)	122.46	123.38
O1C9N1	113.3(7)	122.19	121.87
O1C9C8	132.4(8)	127.54	127.91
N1C9C8	114.2(6)	110.25	110.21
C8S1C10	85.6(3)	93.10	93.40
C9N1C10	105.7(6)	117.93	118.08
C9N1C11	123.8(6)	119.45	119.73
C10N1C11	130.4(7)	122.29	122.04
C7C8C9	124.1(7)	120.20	119.47

Table 4. Experimental and calculated dihedral angles

Torsion angles (°)	X-ray	6-31G(d,p)	
		HF	DFT
S1C8C9O1	179.8(10)	178.9	179.5
S1C8C9N1	−3.1(10)	−1.8	−0.9
S1C8C7C6	−9.2(15)	−1.9	−0.2
S1C10N1C9	3.5(10)	−2.2	−1.6
S1C10N1C11	−172.4(7)	−175.6	−177.1
S2C10S1C8	177.1(6)	−179.6	−179.5
S2C10N1C9	−178.3(7)	178.2	178.8
S2C10N1C11	5.8(13)	4.8	3.2
O1C9N1C10	177.6(9)	−178.1	−178.8
O1C9N1C11	−6.1(13)	−4.4	−3.2
O1C9C8C7	−8.8(18)	−2.4	−0.7
N1C9C8C7	168.3(9)	176.7	178.7
N1C10S1C8	−4.6(8)	3.1	1.8
C6C7C8C9	−179.5(9)	1.5	0.2
C10S1C8C7	−167.5(9)	−178.1	−179.6
C10S1C8C9	3.8(7)	0.6	0.1
C10N1C9C8	−0.1(11)	2.6	1.7
C11N1C9C8	176.2(7)	176.3	177.3
C9N1C11C12	−82.9(12)	−84.7	−86.9
C10N1C11C12	92.4(12)	88.6	88.5
C9N1C11C16	96.5(11)	94.4	95.4
C10N1C11C16	−88.2(12)	−90.2	−89.6

3. 3. Intermolecular H-Bonds

C–H...O, C–H...S and C–H...Cl intra- and intermolecular interactions are present in the crystal structure. These interactions are responsible for the stability of the crystal structure. C atoms, namely C2, C4, C5, C7, C14 and C18 act as donors and O atoms, namely C2, C4, C5, C7, C14 and C18 act as acceptors. H-Bond interactions are presented in Table 5. Figure 5 shows C7H7...O1 H-bond in the crystal.

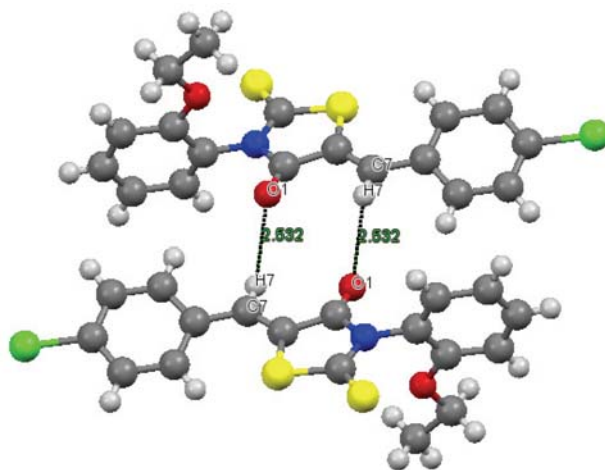


Figure 5. C–H...O, C–H...S and C–H...Cl H-bond in the crystal.

Table 5. C–H...O, C–H...S and C–H...Cl H-bonds in CBBTZ crystal.

DH...A	DH	H...A	D...A	DH...A
C5H5...S1	0.93	2.48	3.082 (8)	122.5
C2H2...S1 ⁱ	0.93	2.96	3.647 (11)	131.9
C2H2...Cl1 ⁱⁱ	0.93	2.83	3.313 (11)	113.1
C4H4...O2 ⁱⁱⁱ	0.93	2.77	3.575 (13)	145.3
C7H7...O1 ^{iv}	0.93	2.53	3.110(13)	134.5
C14H14...S1 ^v	0.93	2.98	3.354 (14)	105.4
C18H18B...S1 ^{vi}	0.96	2.92	3.747 (13)	144.7

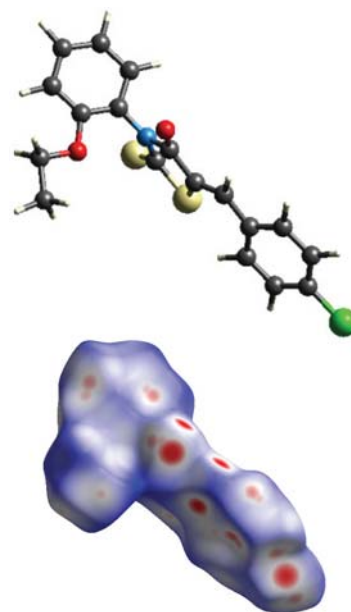
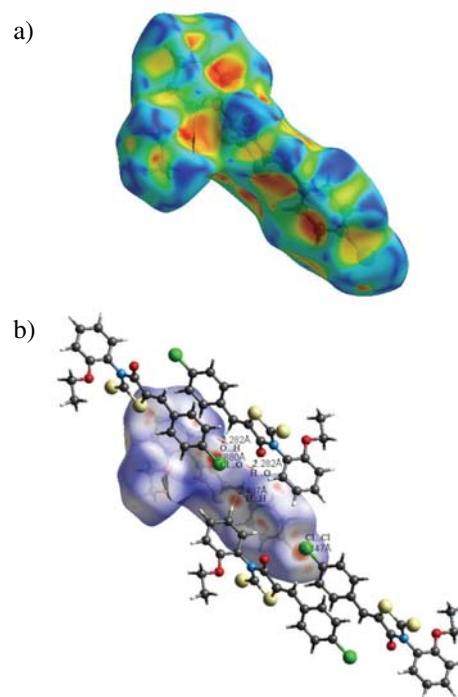
Symmetry codes:

(i) $-x+1, -y, -z$; (ii) $-x, -y-1, -z$; (iii) $x-1, +y, +z$;(iv) $-x+2, -y, -z$; (v) $x+1, +y, +z$; (vi) $-x+2, -y, -z+1$.

3. 4. Hirshfeld Surface Analysis

Hirshfeld surface (HS) analysis represents a unique approach towards an understanding of different interactions in the crystal structure and is a necessary tool in crystal engineering. In addition to the HS analysis, the fingerprint plots also provide some useful quantitative information about the individual contribution of each intermolecular interaction in the crystal packing. The intra- and intermolecular interactions of CBBTZ crystal are quantified using HS analysis. The three-dimensional HS generated for structure of CBBTZ crystal is presented in Fig. 6. The red contacts highlight the intermolecular interactions with distances closer than the sum of the van der Waals radii, while white indicates contacts near the van der Waals separation, and blue depicts longer contacts.²⁸ Figure 7 shows Hirshfeld surfaces mapped for CBBTZ compound with the shape index property (a) and with d_{norm} selected intermolecular contacts (b). The full fingerprint plot for the CBBTZ crystal and the contribution of each type of interaction to the total HS are presented in Figure 8 displaying surfaces that were mapped over d_{norm} (0.242 to 1.414).

As seen in Figure 7, the deep red colour indicates hydrogen-bonding contacts. For example, a deep red spot indicated the presence of a CH...O H-bond (between H7 and O1). The other colour spots are observed due to the presence of other close contacts, such as H...H, C...H, S...H, O...H and Cl...H. The fingerprint plots of CBBTZ are dominated by H...H and C...H contacts. The remaining area of the fingerprint plot is occupied by C...C (3.2%), Cl...C (2.9%), Cl...O (2%), S...S (1.5%), C...S (0.9%), Cl...Cl (0.8%), C...O (0.6%), Cl...N (0.2%), Cl...S (0.1%) and O...O (0.1%) contact regions. The molecular stacking, in spite of having a considerable energetic stabilization, contributes much less [C...C (3.2%)] towards the crystal packing. The H...H contacts, which are prominent in the molecular packing, appear as the scattered points along with double broad peaks in the middle of the region of the fingerprint plot. The positions of the peaks marked with (2) in Fig. 8(a), are at $d_e = d_i = 1.2$ and $1.0 \text{ \AA}$, and the percent contribution is 28.4%. The contribution from C...H

**Figure 6.** View of the HS for CBBTZ molecule.**Figure 7.** HS mapped for CBBTZ compound with (a) the shape index property (b) d_{norm} selected intermolecular contacts.

contacts (24.4% of the HS) results in a symmetrical pair of wings, see Fig. 8(c). The prominent spikes at $d_e = d_i = 1.8 \text{ \AA}$ are due to S...H contacts. These contributions are highlighted in the fingerprint plot, Fig. 8(d). For the title compound, H...O contacts, which are attributed to CH...O H-bond interactions, occur as two sharp symmetric spikes in the two dimensional fingerprint map. The presence of

these long spikes (indicated by (1) in Fig. 8) is characteristic of strong hydrogen bonds. The intermolecular $O\cdots H$ and $H\cdots O$ contacts, Fig. 8(a and e) and Fig. 9, provide contribution of 8.3% to the HS of the CBBTZ crystal. Figure 8(f) shows the contribution of $Cl\cdots H$ intermolecular contacts to the HS.

The quantitative results of the HS analysis for the CBBTZ crystal are presented in Fig. 9 which gives a de-

tailed quantitative analysis of all intra- and intermolecular contacts contributing to the HS.

4. Conclusion

A novel thiazolidinone derivate, (Z)-5-(4-chlorobenzylidene)-3-(2-ethoxyphenyl)-2-thioxothiazolidin-4-

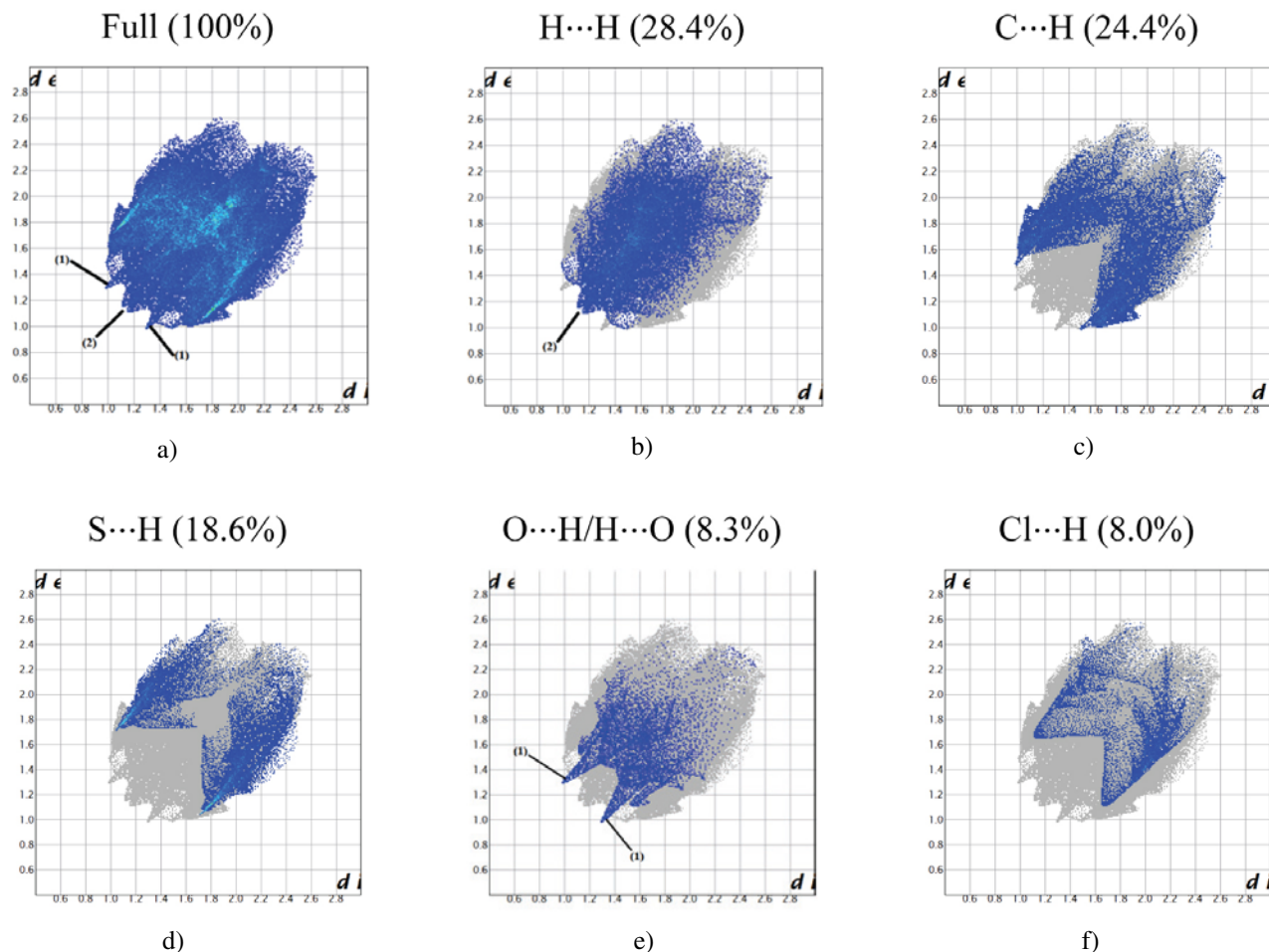


Figure 8. The 2D fingerprint plots showing the percentage contribution of the individual types of interaction to the total HS area.

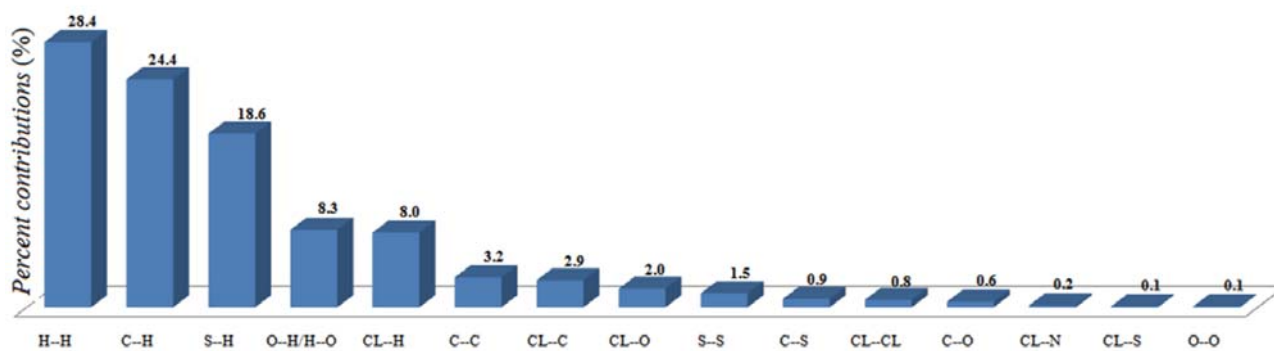


Figure 9. Quantitative results of different intra- and intermolecular interactions contributing to the HS.

one (CBBTZ) has been investigated for the first time. Its structural properties have been examined by theoretical calculations using HF and DFT methods and X-ray diffraction technique. CBBTZ crystallizes in the triclinic system with the space group P-1. Obtained results indicate that the theoretical calculations can reproduce the experimental results. In the crystal packing, the molecules are connected by intra- and intermolecular H-bonds of the type C–H...O, C–H...S and C–H...Cl. In general, a good agreement was observed between the calculated geometrical parameters (with B3LYP) and that of reported similar derivatives. All the calculated data and experimental results of the studied molecule are useful in the application in fundamental research in chemistry and photovoltaic cells in the future. Finally, HS analysis and fingerprint plots are a unique way for understanding the contribution of individual types of interactions within the crystal structure. More theoretical calculations can be performed on this compound to assess other properties especially in the photovoltaic field.

5. Supplementary Material

Crystallographic data for the structure reported in this article have been deposited with Cambridge Crystallographic Data Center, CCDC 1044524. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 IEZ, UK. Facsimile (44) 01223 336 033, E-mail: deposit@ccdc.cam.ac.uk or <http://www.ccdc.com.ac.uk/deposit>.

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Povzetek

Naslovno spojino (Z)-5-(4-klorobenziliden)-3-(2-etoksifenil)-2-tioksotiazolidin-4-on (CBBTZ) smo karakterizirali z rentgensko difrakcijo na monokristalu ter ^1H in ^{13}C NMR spektri. Teoretične izračune smo izvedli s pomočjo teorije na nivoju HF in DFT z uporabo 6-31G(d,p) baznega seta. Rentgensko strukturo smo primerjali z izračunano in ugotovili, da se izračunani geomterijski parametri dobro ujemajo s tistimi, ki smo jih dobili z rentgensko difrakcijo. Dihedralni kot med dvema benzenovima obročema je $16.89(5)^\circ$, kar nakazuje, da struktura ni planarna. Molekula izkazuje tudi intra- in intermolekularne kontakte, npr. $\text{C-H}\cdots\text{O}$, $\text{C-H}\cdots\text{S}$ in $\text{C-H}\cdots\text{Cl}$. Interakcije v kristalni strukturi smo raziskali s pomočjo metode Hirshfeldovih površin.

Scientific paper

$\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ Nanocomposite as a Robust and Effective Catalyst for the One-pot Synthesis of Polysubstituted Dihydropyridines

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Abstract

A practical, simple and efficient method for the synthesis of polysubstituted dihydropyridines was described via multi-component reactions of aldehydes, arylamines, dimethyl acetylenedicarboxylate and malononitrile/ethyl acetoacetate in the presence of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanocomposites. The present methodology provides a novel and efficient method for the synthesis of dihydropyridine derivatives with some advantages, such as excellent yields, short reaction times, recoverability and low catalyst loading. The nanomagnetic catalyst could be readily recovered using an external magnet and reused several times without any significant loss in activity. The catalyst was fully characterized by FT-IR, SEM, XRD, EDX and VSM analysis.

Keywords: $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, magnetite, multi-component reaction, nanocatalyst, dihydropyridine.

1. Introduction

Substituted dihydropyridone derivatives are an important class of nitrogen heterocyclic compounds due to a variety of biological and pharmacological activities¹ such as: antihypertension,² antioxidant,^{3,4} anticancer⁵ and anti-tumor activity.⁶ However, there are many methods for the synthesis of dihydropyridines. Among various dihydropyridine structures, some of them have medicinal properties such as: nicardipine **1** and nifedipine **2**,⁷ isradipine **3** and niguldipine **4**,⁸ which exhibit dihydropyridine moiety (Figure 1).

Consequently, synthesis of highly functionalized dihydropyridine derivatives, with the aim of developing new drug molecules has been an active area of research. Recently, much attention has been paid to the development of new methodologies for the preparation of dihydropyridines. The main synthetic routes for the preparation of substituted dihydropyridines are Hantzsch method via the cyclocondensation of an aldehyde, β -ketoester and ammonia,⁹ regioselective [4+2] cycloaddition of 1-aryl-4-phenyl-1-azadienes and allenic esters for the synthesis of *N*-aryl-1,4-DHPs,¹⁰ and a multi-component reaction of alkyl amines, ethyl propiolate, and benzal-

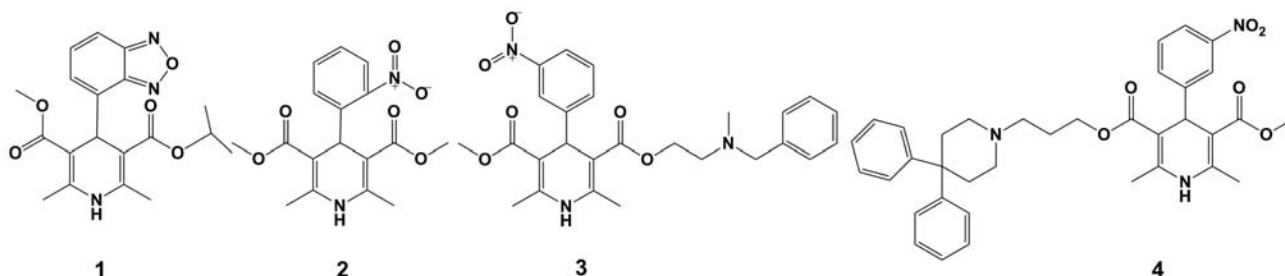


Figure 1. Some biologically important dihydropyridine derivatives

dehydes for the construction of *N*-alkyl-1,4-DHPs,¹¹ and other methods.

Recently, a few methods have been reported for the syntheses of polysubstituted dihydropyridines via four-component reactions of aldehydes, arylamines, acetylenedicarboxylates and malononitrile/ethyl acetoacetate using Et_3N ,¹² NaOH ,¹³ $(\text{NH}_4)_2\text{HPO}_4$.¹⁴

In the modern science, one of the growing and important fields is nanotechnology. Because of different physical and chemical properties of nano-sized catalysts compared to bulk material, they attract interests in different researcher areas.¹⁵ Since the particles are small in size, the surface area exposed to the reactant is maximized so allowing more reactions to occur at the same time, hence the process is accelerated.¹⁶

Magnetic nanoparticles show a great potential as catalysts because of their large surface area and the large ratio of atoms available at the surface to perform the chemical transformation of substrates.^{17,18} However, the bare Fe_3O_4 nanoparticles have high reactivity and easily undergo degradation upon direct exposure to certain environments, leading to poor stability and dispersity. Therefore, the surface of magnetic nanoparticles should be modified to improve the dispersity and biocompatibility, which could significantly facilitate its utilization.

$\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanocomposites are one of the most important supported magnetite nanostructures which have received great attention due to their significant properties and potential applications in various fields.¹⁹ This kind of catalyst is eco-friendly, non-toxic, non-volatile and can be recycled several times without loss of activity

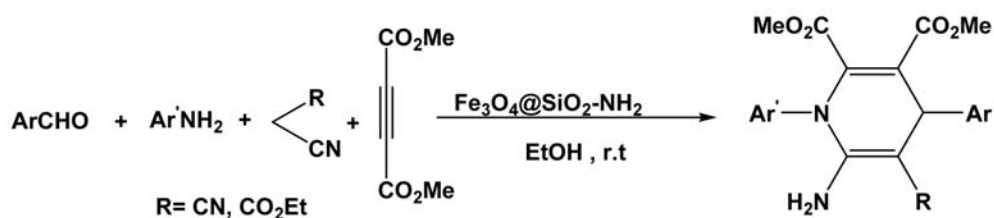
in the reaction. The use of these nanoparticles follows the principles of green chemistry.

Recently, functionalized Fe_3O_4 nanocatalysts were applied as a robust and effective catalyst in many organic reactions, such as: Knoevenagel condensation and Michael addition,²⁰ Suzuki and Heck cross-coupling,²¹ asymmetric aldol reaction,²² Suzuki coupling,²³ asymmetric hydrogenation of aromatic ketones,²⁴ acetalization reaction,²⁵ reduction of nitro aromatic compounds,²⁶ cyanosilylation of carbonyl compounds,²⁷ Henry reaction,²⁸ enantioselective direct-addition of terminal alkynes to imines.²⁹

In continuation of the progress of the synthetic approach to the synthesis of heterocyclic compounds using reusable nanocatalysts and multi-component reactions,^{30–34} herein we report a simple, efficient, mild and practical method for the synthesis of polysubstituted dihydropyridines via a four-component coupling reaction in the presence of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanocomposite as a green and environmentally benign nanocatalyst in ethanol as solvent at room temperature (Scheme 1).

2. Results and Discussion

The chemical purity of the samples as well as their stoichiometry was tested by energy dispersive X-ray spectroscopy (EDX) studies. The EDX spectrum given in Figure 2a shows the presence of Fe and O as the only elementary components of Fe_3O_4 NPs. EDX spectrum of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ in Figure 2b shows the elemental composition of core-shell nanocomposite to be Fe, Si and O. EDX



Scheme 1. Synthesis of polysubstituted dihydropyridines catalyzed by $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanocomposite

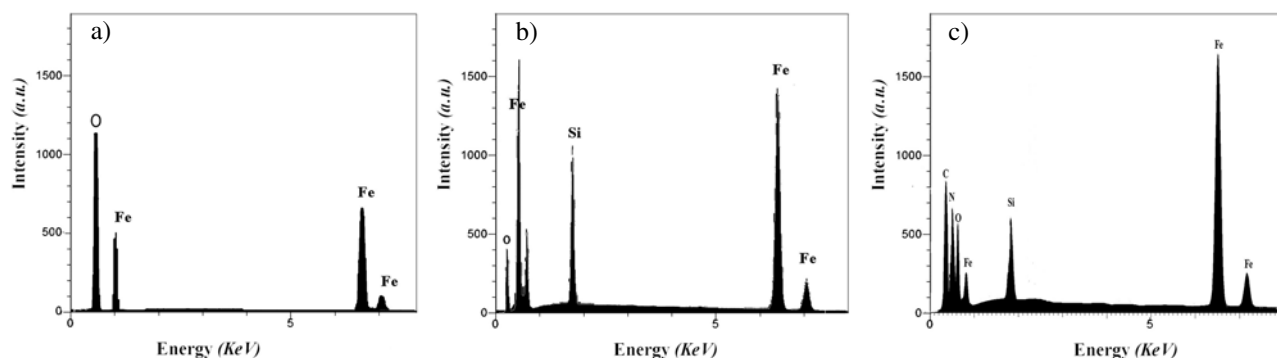


Figure 2. The EDX spectra of Fe_3O_4 (a), $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (b) and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ (c)

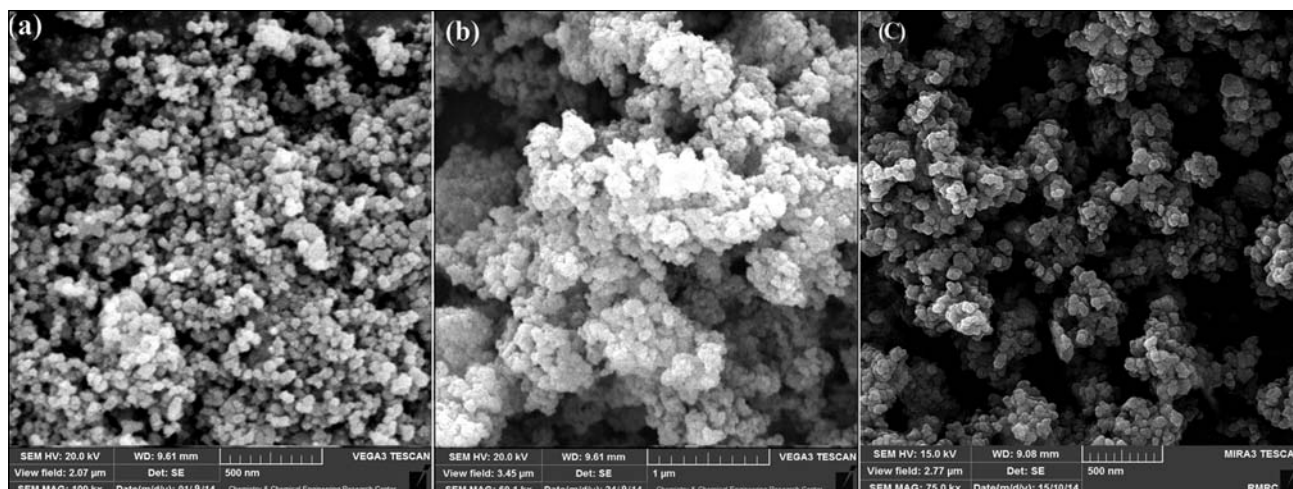


Figure 3. The SEM images of Fe₃O₄ (a), Fe₃O₄@SiO₂ (b) and Fe₃O₄@SiO₂-NH₂ (c)

spectrum of Fe₃O₄@SiO₂-NH₂ in Figure 2c shows the elemental composition of amino-functionalization silica-coated magnetite nanocomposite with core-shell structure to be Fe, Si, C, O and N.

Scanning electron microscopy (SEM) images of the prepared nanostructures are shown in Figure 3. Figure 3a shows that the Fe₃O₄ nanoparticles are cubic in shape with an average size about 15 nm. Figure 3b (Fe₃O₄@SiO₂) and Figure 3c (Fe₃O₄@SiO₂-NH₂) show that both are apparently of similar shape, but approximate size of amino-functionalization silica-coated magnetite nanocomposite

is more than *r* of the Fe₃O₄@SiO₂ core-shell nanocomposite.

The structures of Fe₃O₄ (a), Fe₃O₄@SiO₂ (b) and Fe₃O₄@SiO₂-NH₂ (c) were analyzed by X-ray diffraction (XRD) spectroscopy (Figure 4). XRD diagram of the bare Fe₃O₄ NPs displayed patterns consistent with the patterns of spinel ferrites (Figure 4a). The same peaks were observed in both of the Fe₃O₄@SiO₂ (Figure 4b) and Fe₃O₄@SiO₂-NH₂ (Figure 4c) XRD patterns, indicating retention of the crystalline spinel ferrite core structure during the coating process. The average MNPs core diame-

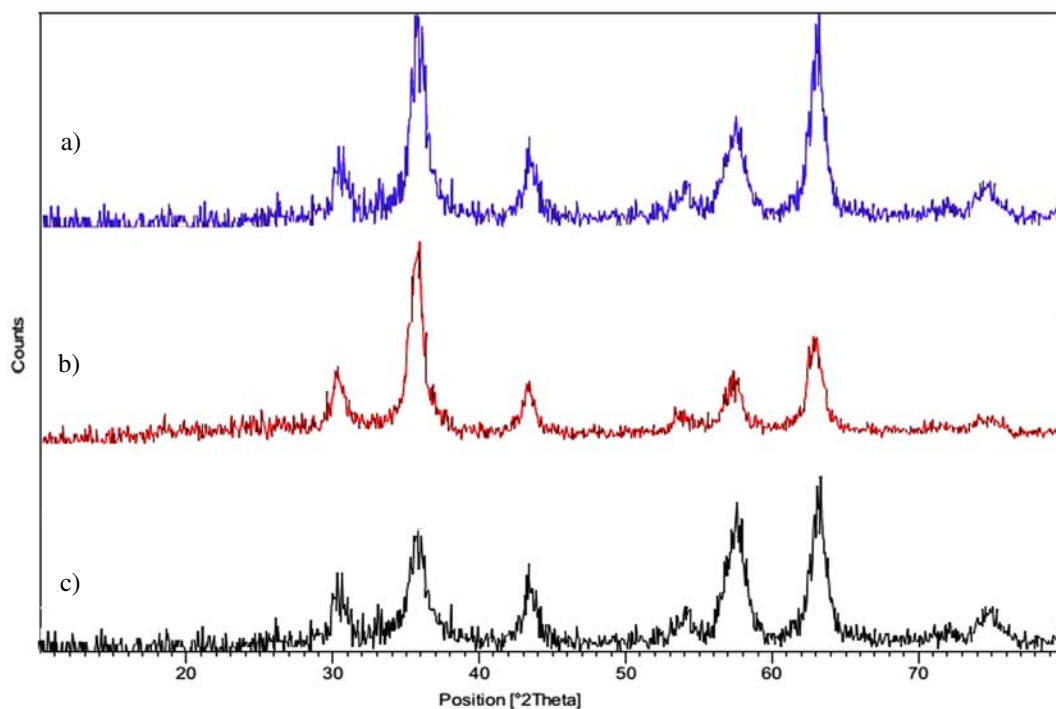


Figure 4. X-ray diffraction for Fe₃O₄ (a), Fe₃O₄@SiO₂ (b) and Fe₃O₄@SiO₂-NH₂ (c)

ters of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ were calculated to be about 18, 25 and 40 nm, respectively, from the XRD results by Scherrer's equation, ($D = K\lambda/\beta\cos\theta$), where β FWHM (full-width at half-maximum or half-width) is in radian and θ is the position of the maximum of diffraction peak, K is the so-called shape factor, which usually takes a value of about 0.9, and λ is the X-ray wavelength (1.5406 Å for Cu K α).

The FT-IR spectra of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanostructures are shown in Figure 5. For the bare magnetic nanoparticle (Figure 5a) the vibration band at 567 cm^{-1} is the typical IR absorbance induced by structure Fe–O vibration. In the case of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanocomposite (Figure 5b), the band at 1072 cm^{-1} is corresponding to Si–O–Si antisymmetric stretching vibrations, being indicative of the existence of SiO_2 on the nanoparticles. $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ can be ascribed to the stretching and deformation vibrations of SiO_2 , reflecting the coating of amino group on the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ core-shell nanocomposite surfaces. Successful aminopropyl functionalization of the silica layer on $\text{Fe}_3\text{O}_4@\text{SiO}_2$ was also evidenced by the absorption at 1478 cm^{-1} attributed to bending vibrations of amino

groups. The absorption peaks in the region $2800\text{--}3025\text{ cm}^{-1}$ were associated with the stretching vibration of methylene groups of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ (Figure 5c). The results verified the formation of a silica shell on the Fe_3O_4 surface and the amino-functionalization of the silica shell.

The magnetic properties of the uncoated magnetic iron oxide (Fe_3O_4), $\text{Fe}_3\text{O}_4@\text{SiO}_2$, and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ were measured by vibrating sample magnetometer, VSM, at room temperature (Figure 6). The hysteresis loops that are characteristic of superparamagnetic behavior can be clearly observed for all the nanostructures. Superparamagnetism is the responsiveness to an applied magnetic field without retaining any magnetism after removal of the applied magnetic field. From M versus H curves, the saturation magnetization value (M_s) of uncoated Fe_3O_4 NPs was found to be 47.12 emu g^{-1} . For $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, the magnetizations obtained at the same field were 41.23 and 32.42 emu g^{-1} , respectively, lower than that of uncoated Fe_3O_4 . These results indicated that the magnetization of Fe_3O_4 decreased considerably with the increase of SiO_2 and aminopropyl groups. This is mainly attributed to the existence of nonmagnetic materials on the surface of the nanoparticles.

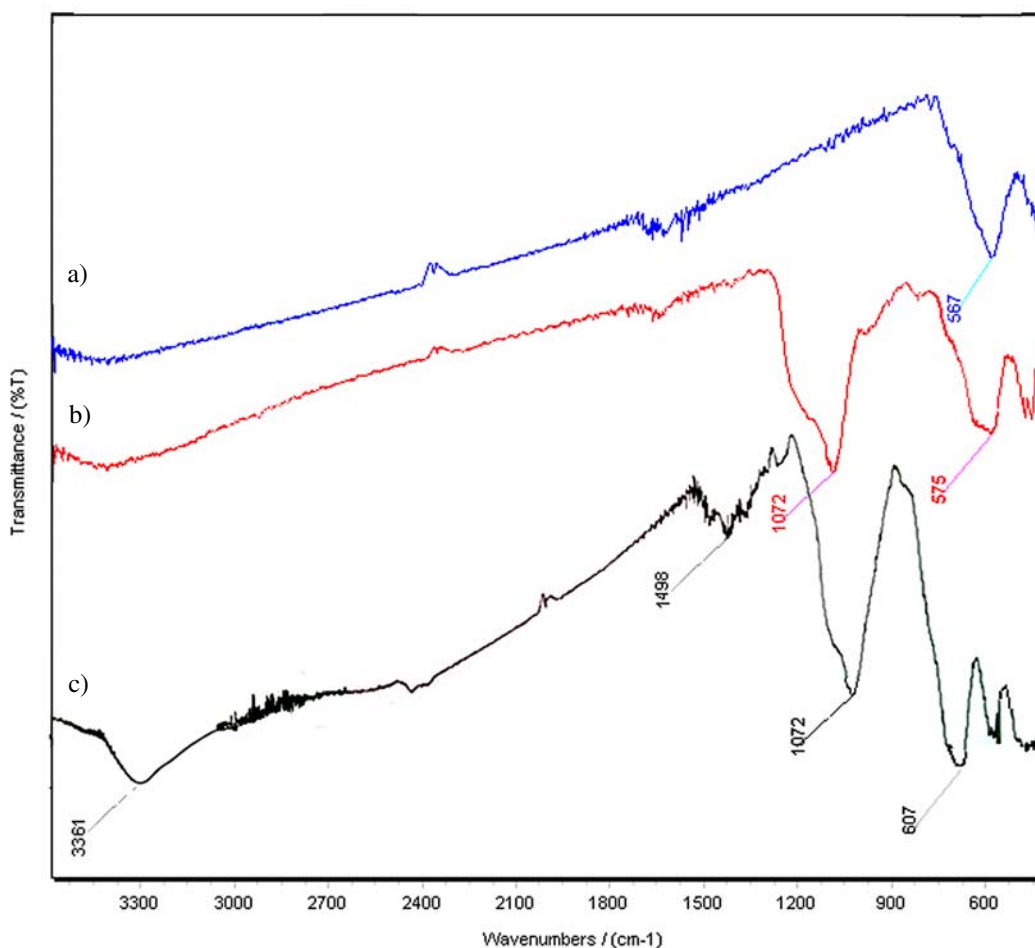


Figure 5. The comparative FT-IR spectra of Fe_3O_4 (a), $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (b) and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ (c)

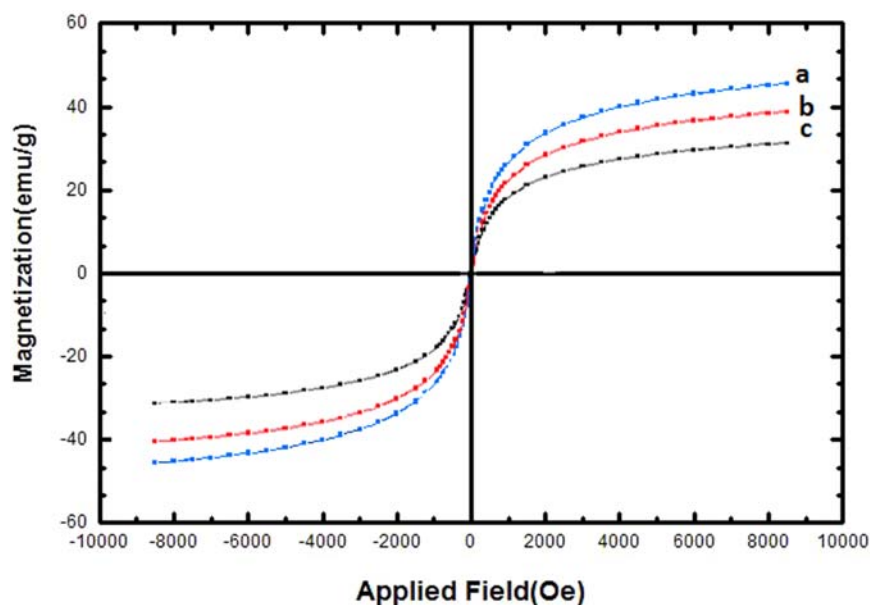


Figure 6. VSM magnetization curves of Fe_3O_4 (a), $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (b) and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ (c)

We decided to optimize the four-component reaction of benzaldehyde, aniline, dimethyl acetylenedicarboxylate and malononitrile as a model study. The reaction conditions were optimized on the base of solvents, catalysts and different temperatures. The product of this model reaction is dimethyl-6-amino-5-cyano-4-(4-cyanophenyl)-1-phenyl-1,4-dihydropyridine-2,3-dicarboxylate (**5a**) (Scheme 2).

Firstly, to obtain the best reaction conditions for the synthesis of polysubstituted dihydropyridine **5a**, the model study was carried out in the presence of various catalytic systems including homogenous and heterogeneous catalysts. As can be seen from Table 1, among the various catalysts, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ core-shell nanostructures were found to be the most effective catalyst for the synthesis of polysubstituted dihydropyridine **5a** at room temperature.

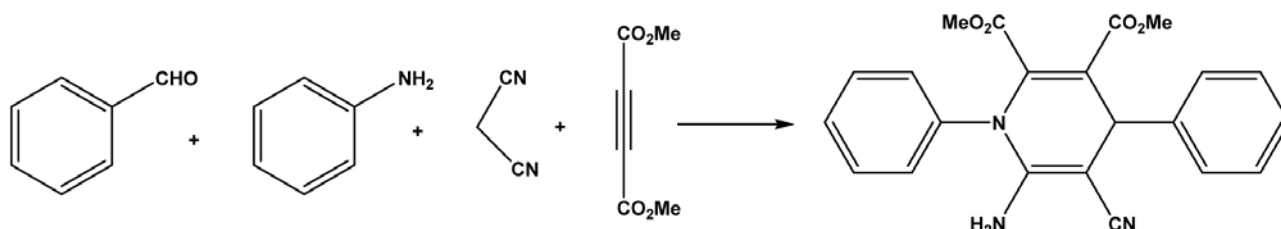
To find the optimized amount of the catalyst, the preparation of dihydropyridine **5a** was carried out using different amounts of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ as the catalyst (0.002, 0.005, 0.01, 0.02, 0.03 g). The best result was obtained by using 0.01 g of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanocomposites at room temperature.

In continuation of this research, to select the appropriate solvent, various solvents such as, dichloromethane, DMF, water, toluene and ethanol were used in the model reaction in the presence of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ at room

Table 1. Yields and reaction times for the preparation of 1,4-dihydropyridine **5a** in the presence of various catalysts.^a

Entry	Catalyst	Time (h)	Yield (%) ^b
1	None	10	–
2	NaOH	7	75
3	Et_3N	8	78
4	pyridine	10	70
5	CaO NPs	6	78
6	MgO NPs	5.5	75
7	ZnO NPs	12	55
8	Fe_3O_4 NPs	8	66
9	$\text{Fe}_3\text{O}_4@\text{SiO}_2$	6	72
10	$\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$	4	94

^a Reaction conditions: benzaldehyde (1 mmol), malononitrile (1 mmol) aniline (1 mmol) and acetylenedicarboxylate (1 mmol) in ethanol (10 mL) at r.t. ^b Isolated yield.



Scheme 2. The model reaction for the synthesis of dihydropyridine **5a**

temperature. As shown in Table 2, we found that ethanol is the most efficient solvent for the synthesis of polysubstituted dihydropyridine **5a**, giving the product in 94% yield (Table 2, entry 6).

Table 2. The effect of solvents/reaction times on the yield of dihydropyridine **5a**

Entry	Solvent	Time (h)	Yield (%) ^b
1	Solvent Free	6	35
2	Dichloromethane	10	52
3	DMF	6	68
4	Water	8	65
5	Toluene	12	45
6	Ethanol	4	94

^a Reaction conditions: benzaldehyde (1 mmol), malononitrile (1 mmol) aniline (1 mmol) and acetylenedicarboxylate (1 mmol) in various solvents (10 mL) at r.t. in the presence of Fe₃O₄@SiO₂-NH₂ (0.01 g). ^b Isolated yield.

The substrate scope of this protocol for the synthesis of a variety of polysubstituted dihydropyridines was studied next by applying various amines and aldehydes to the reaction (Scheme 1 and Table 3). As shown in Table 3, aniline derivatives, including those bearing electron-donating or electron-withdrawing as well as sterically demanding substituents, reacted very well to afford the desired products **5a–t** in excellent yields over short reaction times. Also, various arylamines with electron-releasing groups such

as methoxy and methyl groups reacted in short reaction times and giving products with higher yields than those with electron-withdrawing groups such as NO₂ and Cl.

In addition, aryl aldehydes with electron-withdrawing groups such as NO₂, Cl and Br reacted very smoothly to produce highly functionalized dihydropyridines in relatively short reaction times in comparison with aryl aldehydes bearing electron-donating groups, but sterically hindered aldehydes reacted more slowly compared to unhindered aldehydes.

As the results in Table 3 show, Fe₃O₄@SiO₂-NH₂ proved to be a useful nanomagnetic heterogeneous acid nanocatalyst for green synthesis of polysubstituted dihydropyridines in excellent yields.

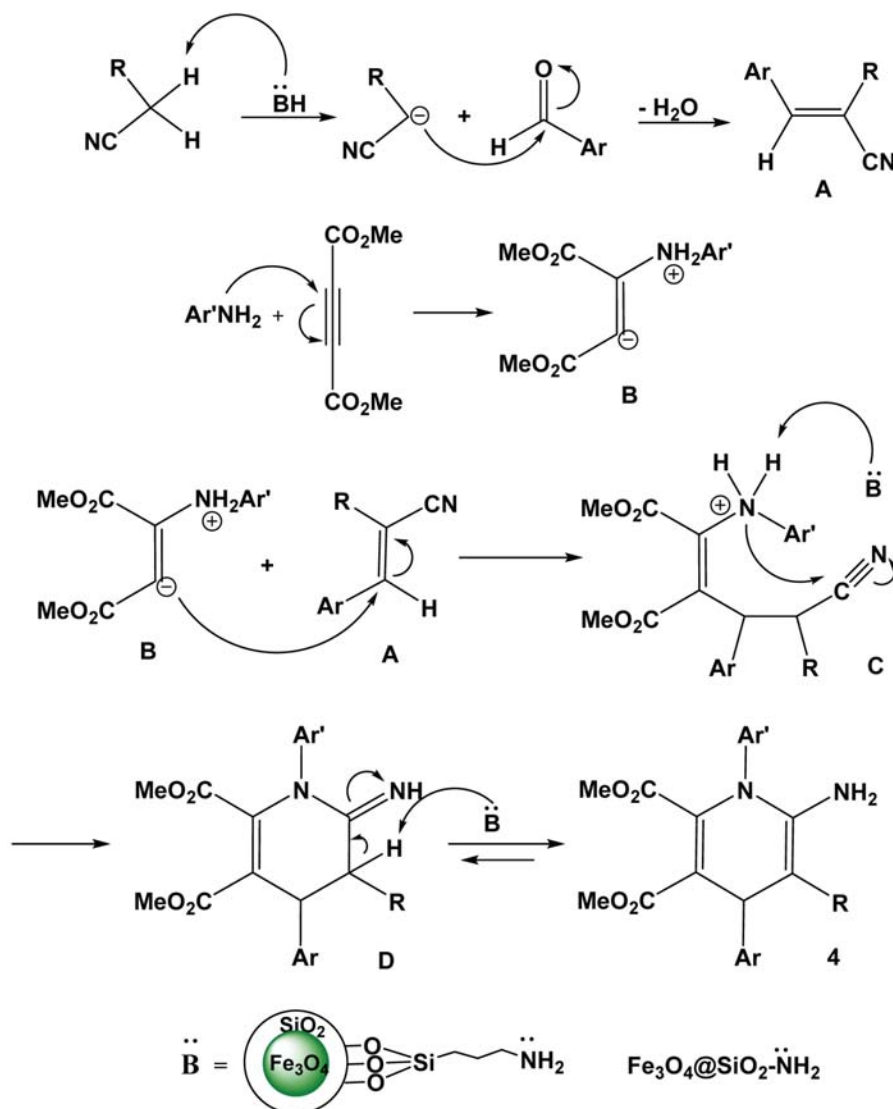
A plausible mechanism for the preparation of highly functionalized dihydropyridines using Fe₃O₄@SiO₂-NH₂ NPs is shown in Scheme 4, given on the basis of our experimental results together with some literature data.^{12–14}

It is likely that NH₂ groups on the surfaces of nanoparticles act as a Brønsted base and cause the dehydrogenation of substrates. First, the Knoevenagel condensation of malononitrile/ethyl acetoacetate is suggested to give the intermediate **A**. Then, a nucleophilic attack of arylamine to dimethyl acetylenedicarboxylate leads to the formation of intermediate **B**. Michael addition of intermediate **B** to **A** forms the intermediate **C** which undergoes an intramolecular cyclization which is catalyzed by Brønsted basic (–NH₂) functionalized Fe₃O₄ core-shell nanoparticles. In the last step, the intermediate **D** is tautomerized to the product **4**.

Table 3. Yields and reaction times for the preparation of dihydropyridines **5a–t** using Fe₃O₄@SiO₂-NH₂ nanocomposite^a

Entry	Ar	Ar'	R	Product	Time (h)	Yield (%) ^b	m.p. (°C)	Lit. m.p (°C)
1	H	H	CN	5a	4	94	162–164	(161–163) ¹³
2	H	4-Me	CN	5b	4	96	165–167	(165–167) ¹³
3	4-Cl	4-Me	CN	5c	3.5	97	184–186	(186–188) ¹³
4	4-Br	4-Me	CN	5d	3.5	95	186–188	(185–187) ¹³
5	3-Cl	4-Me	CN	5e	7	90	181–183	(180–182) ¹³
6	3-NO ₂	4-Me	CN	5f	5	90	210–212	(212–214) ¹³
7	4-OMe	4-OMe	CN	5g	4.5	88	162–163	(159–161) ¹³
8	4-Cl	4-Cl	CN	5h	4	95	130–132	(130–132) ¹³
9	4-OMe	4-Me	CN	5i	4	92	167–169	(168–170) ¹³
10	3-NO ₂	4-OMe	CN	5j	4	90	185–187	(184–186) ¹³
11	3-NO ₂	4-Cl	CN	5k	5	86	195–197	(195–197) ¹³
12	4-Br	4-Cl	CN	5l	4	91	164–166	(163–165) ¹³
13	3-NO ₂	4-Me	COOEt	5m	4.5	88	172–174	–
14	3-NO ₂	4-OMe	COOEt	5n	4	88	178–180	–
15	4-Cl	H	CN	5o	3.5	94	138–140	–
16	4-NO ₂	4-Cl	CN	5p	4	93	185–187	–
17	4-Cl	3-NO ₂	CN	5q	5	95	157–159	–
18	4-Me	4-Cl	CN	5r	4.5	91	191–193	–
19	4-CH(CH ₃) ₂	4-Cl	CN	5s	5	86	195–197	–
20	4-CN	4-Cl	CN	5t	4	94	164–166	–

^a Reaction conditions: aldehyde (1 mmol), malononitrile/ethylcyanoacetate (1 mmol), aromatic amine (1 mmol) and acetylenedicarboxylate (1 mmol) in ethanol (10 mL) for various times in the presence of Fe₃O₄@SiO₂-NH₂. ^b Isolated yields.



Scheme 3. The proposed mechanism for the synthesis of dihydropyridines by $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ NPs

3. Experimental

3.1. General

Chemicals were purchased from the Sigma-Aldrich and Merck in high purity. All of the materials were of commercial reagent grade and were used without further purification. All melting points are uncorrected and were determined in capillary tube on Boetius melting point microscope. NMR spectra were obtained on a Bruker DRX-400 MHz spectrometer (^1H NMR at 400 Hz, ^{13}C NMR at 100 Hz) with CDCl_3 as the solvent, using TMS as an internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. FT-IR spectrum was recorded on Magna-IR, spectrometer 550. The elemental analyses (C, H, N) were obtained from a Carlo Erba Model EA 1108 analyzer. Powder X-ray diffraction (XRD)

was carried out on a Philips diffractometer of X'pert Company with monochromatic $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). Microscopic morphology of the products was visualized by SEM (LEO 1455VP). The mass spectra were recorded on a Joel D-30 instrument at an ionization potential of 70 eV. Magnetic properties were obtained on a BHV-55 vibrating sample magnetometer (VSM) made by MDK, I. R. Iran. The compositional analysis was done by energy dispersive analysis of X-ray (EDX, Kevex, Delta Class I).

3.2. Preparation of Fe_3O_4 Nanoparticles

Fe_3O_4 NPs were prepared according to a procedure previously reported by Hu et al.³⁵ using the chemical coprecipitation method. Typically, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (2.7 g) and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (1.0 g) were dissolved in aqueous HC-

l (100 mL, 1.2 mM) in an ultrasonic bath (30 min). Then, aqueous NaOH (150 mL, 1.25 M) was added under vigorous stirring and a black precipitate was immediately formed. The resulting solution was heated at 80 °C with rapid mechanical stirring under N₂ atmosphere (2 h). The black products were centrifuged, filtered off and washed with deionized water and ethanol several times, and finally dried at 60 °C for 12 h.

3. 3. Preparation of Fe₃O₄@SiO₂ Nanoparticles

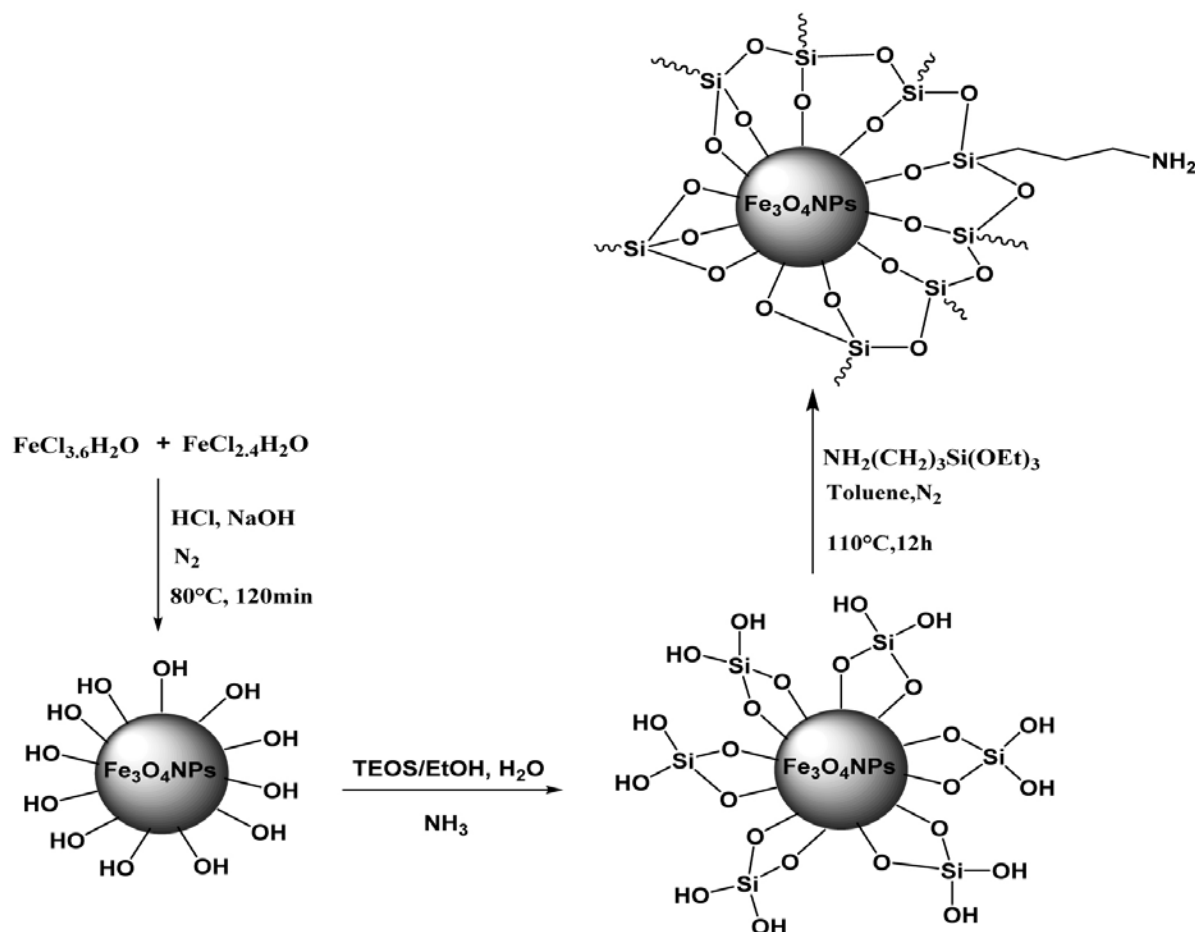
Fe₃O₄@SiO₂ core-shell nanoparticles were prepared via modified Stöber sol-gel process.³⁶ 30 mg as-prepared Fe₃O₄ submicrospheres were ultrasonically dispersed in a solution containing 160 mL ethanol, 40 mL water and 10 mL concentrated ammonia (28 wt%). Then, 0.4 mL TEOS was added dropwise to the solution under sonication, followed by mechanical stirring for 3 h at room temperature. Subsequently, the resulting particles were separated using a magnet and washed with deionized water and ethanol. This step was repeated several times before drying at 60 °C for 12 h.

3. 4. Preparation of Fe₃O₄@SiO₂-NH₂

Fe₃O₄@SiO₂-NH₂ MNPs were prepared according to a previously reported procedure by Jiahong Wang et al.³⁷ Amino-functionalized Fe₃O₄@SiO₂ nanocomposite was prepared by surface functionalization of Fe₃O₄@SiO₂ nanocomposite using (3-aminopropyl)triethoxysilane (APTES). 2 g of Fe₃O₄@SiO₂ nanocomposite and 50 mL of toluene were added to a 250 mL three-necked flask and then ultrasonically dispersed for 15 min. 4 mL of APTES (Sigma) was then added into the flask, and the mixture was refluxed at 110 °C with continuous stirring for 12 h under a nitrogen flow (40 mL/min). The resulting functionalized Fe₃O₄@SiO₂ was gathered by filtration followed by washing with ethanol and acetone several times and drying at 50 °C under vacuum for 12 h. The materials obtained are referred to as Fe₃O₄@SiO₂-NH₂ nanocomposite (Scheme 2).

3. 5. General Procedure for the Synthesis of 1,4-Dihydropyridine Derivatives 5a–t

A solution of aldehyde (1 mmol), malononitrile/ethylcyanoacetate (1 mmol) and Fe₃O₄@SiO₂-NH₂



Scheme 4. Preparation of amino functionalized silica-coated magnetite nanocomposites

NPs (0.01 g) were stirred in 5 mL of ethanol at room temperature. Then, a solution of aromatic amine (1 mmol) and acetylenedicarboxylate (1 mmol) in 5 mL ethanol was added to it. The resulting mixture was stirred until the reaction was completed as indicated by thin-layer chromatography (TLC), then the catalyst was separated by an external magnet, the solid obtained was filtered and washed well with cold ethanol. The crude product was crystallized from hot ethanol to afford the pure product in high yield.

All of the products were characterized and identified with m.p., ^1H , ^{13}C NMR and FT-IR spectroscopy techniques. Spectral data of the new products are given below.

5-Ethyl-2,3-dimethyl-6-amino-4-(3-nitrophenyl)-1-(p-tolyl)-1,4-dihydropyridine-2,3,5-tricarboxylate (5m).

Yellow solid; m.p. 172–174°C; ^1H NMR (400 MHz, CDCl_3) δ 1.23 (t, 3H, CH_3), 3.46 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.07 (q, 2H, CH_2), 5.09 (s, 1H, CH), 6.27 (2H, NH_2), 7.02 (d, 2H), 7.35 (d, 2H), 7.45 (t, 1H), 7.74 (d, 1H), 8.07 (d, 1H), 8.33 (d, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 14.4, 21.3, 37.2, 52.0, 52.6, 55.6, 59.6, 106.4, 121.4, 123.1, 128.8, 130.1, 130.7, 132.1, 134.0, 142.4, 148.3, 149.3, 151.6, 163.7, 165.8, 169.1. FT-IR (KBr) ν 3422, 3375, 3182, 2960, 2184, 1752, 1701, 1653, 1572, 1526, 1423, 1349, 1250, 1217, 1108, 1050, 970, 930, 872, 809, 783 cm^{-1} ; MS (EI) m/z 495.16 (M^+); Anal. Calcd. For $\text{C}_{25}\text{H}_{25}\text{N}_3\text{O}_8$: C 60.60, H 5.09, N 8.48. Found: C 60.69, H 5.01, N 8.44.

5-Ethyl-2,3-dimethyl-6-amino-1-(4-methoxyphenyl)-4-(3-nitrophenyl)-1,4-dihydropyridine-2,3,5-tricarboxylate (5n).

Yellow solid; m.p. 178–180°C; ^1H NMR (400 MHz, CDCl_3) δ 1.23 (t, 3H, CH_3), 2.42 (s, 3H, CH_3), 3.45 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 4.06 (q, 2H, CH_2), 5.10 (s, 1H, CH), 6.26 (2H, NH_2), 7.27 (d, 2H), 7.32 (d, 2H), 7.46 (t, 1H), 7.72 (d, 1H), 8.05 (d, 1H), 8.33 (s, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 14.4, 37.2, 52.0, 52.6, 55.6, 59.5, 106.3, 115.1, 121.4, 123.0, 126.9, 128.8, 131.7, 134.0, 142.6, 148.3, 149.4, 151.8, 160.7, 163.7, 165.8, 169.1. FT-IR (KBr) ν 3421, 3375, 3182, 2962, 2184, 1752, 1702, 1653, 1572, 1526, 1423, 1349, 1250, 1217, 1108, 1050, 970, 930, 872, 809, 783 cm^{-1} ; MS (EI) m/z 511.16 (M^+); Anal. Calcd. For $\text{C}_{25}\text{H}_{25}\text{N}_3\text{O}_9$: C 58.71, H 4.93, N 8.22. Found: C 58.83, H 4.85, N 8.19.

Dimethyl-6-amino-4-(4-chlorophenyl)-5-cyano-1-phenyl-1,4-dihydropyridine-2,3-dicarboxylate (5o).

Yellow solid; m.p. 138–140°C; ^1H NMR (400 MHz, CDCl_3) δ 3.44 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 4.10 (s, 2H, NH_2), 4.67 (s, 1H, CH), 7.27–7.36 (m, 6H), 7.51 (d, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 38.2, 52.7, 52.8, 60.9, 105.3, 120.1, 128.2, 128.5, 128.8, 129.0, 130.6, 131.2, 133.0, 135.4, 137.2, 142.5, 149.1, 151.4, 163.7, 165.8. FT-IR (KBr) ν 3465, 3360, 3058, 2950, 2180,

1746, 1707, 1651, 1573, 1526, 1414, 1353, 1249, 1222, 1108, 1017, 971, 928, 833, 809, 784 cm^{-1} ; MS (EI) m/z 423.10 (M^+); Anal. Calcd. For $\text{C}_{22}\text{H}_{18}\text{ClN}_3\text{O}_4$: C 62.34, H 4.28, N 9.91. Found: C 62.29, H 4.24, N 9.98.

Dimethyl-6-amino-1-(4-chlorophenyl)-5-cyano-4-(4-nitrophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (5p).

Yellow solid; m.p. 185–187°C; ^1H NMR (400 MHz, CDCl_3) δ 3.52 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 4.14 (s, 2H, NH_2), 4.81 (s, 1H, CH), 7.30 (d, 2H), 7.52 (t, 4H), 8.27 (d, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 20.5, 38.2, 52.7, 52.8, 61.7, 105.6, 121.9, 128.2, 128.6, 129.0, 129.3, 131.2, 131.7, 134.0, 135.6, 137.0, 141.5, 150.2, 151.8, 163.1, 168.5. FT-IR (KBr) ν 3449, 3354, 3055, 2951, 2186, 1744, 1710, 1651, 1575, 1519, 1421, 1346, 1229, 1226, 1111, 1014, 971, 930, 866, 823, 762 cm^{-1} ; MS (EI) m/z 468.08 (M^+); Anal. Calcd. For $\text{C}_{22}\text{H}_{17}\text{ClN}_4\text{O}_6$: C 56.36, H 3.65, N 11.95. Found: C 56.39, H 3.58, N 11.90.

Dimethyl-6-amino-4-(4-chlorophenyl)-5-cyano-1-(3-nitrophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (5q).

Yellow solid; m.p. 157–159°C; ^1H NMR (400 MHz, CDCl_3) δ 3.42 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 4.07 (s, 2H, NH_2), 4.67 (s, 1H, CH), 7.28 (d, 2H), 7.37 (d, 2H), 7.72 (s, 2H), 8.23 (s, 2H), 7.38 (d, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 38.1, 52.3, 53.0, 63.9, 106.2, 119.7, 125.5, 128.4, 129.1, 131.0, 133.2, 136.4, 136.5, 141.0, 142.7, 148.7, 149.1, 163.2, 165.3. FT-IR (KBr) ν 3454, 3346, 3054, 2952, 2182, 1741, 1714, 1650, 1573, 1525, 1420, 1346, 1227, 1225, 1118, 1014, 973, 930, 864, 823, 762 cm^{-1} ; MS (EI) m/z 468.08 (M^+); Anal. Calcd. For $\text{C}_{22}\text{H}_{17}\text{ClN}_4\text{O}_6$: C 56.36, H 3.65, N 11.95. Found: C 56.31, H 3.67, N 11.97.

Dimethyl-6-amino-1-(4-chlorophenyl)-5-cyano-4-(p-tolyl)-1,4-dihydropyridine-2,3-dicarboxylate (5r).

Yellow solid; m.p. 191–193°C; ^1H NMR (400 MHz, CDCl_3) δ 2.35 (s, 3H, CH_3), 3.50 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 4.01 (s, 2H, NH_2), 4.63 (s, 1H, CH), 7.17–7.46 (m, 6H), 7.48 (d, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 21.1, 38.0, 52.1, 52.8, 63.7, 105.8, 120.2, 126.8, 129.6, 130.2, 131.6, 131.6, 133.7, 136.7, 136.9, 141.3, 141.6, 149.1, 163.2, 165.7. FT-IR (KBr) ν 3454, 3318, 3054, 2951, 2187, 1744, 1711, 1653, 1574, 1525, 1416, 1353, 1227, 1223, 1110, 1017, 972, 931, 864, 823, 762 cm^{-1} ; MS (EI) m/z 437.11 (M^+); Anal. Calcd. For $\text{C}_{23}\text{H}_{20}\text{ClN}_3\text{O}_4$: C 63.09, H 4.60, N 9.60. Found: C 63.19, H 4.58, N 9.54.

Dimethyl-6-amino-5-cyano-4-(4-isopropylphenyl)-1-(p-tolyl)-1,4-dihydropyridine-2,3-dicarboxylate (5s).

Yellow solid; m.p. 195–197°C; ^1H NMR (400 MHz, CDCl_3) δ 1.37 (s, 9H, CH_3), 3.51 (s, 3H, OCH_3), 3.62 (s,

3H, OCH₃), 4.00 (s, 2H, NH₂), 4.64 (s, 1H, CH), 7.21–7.27 (m, 4H), 7.31 (d, 2H), 7.48 (d, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 23.9, 33.7, 37.9, 52.1, 52.7, 63.7, 106.0, 120.3, 126.8, 126.9, 130.2, 131.6, 133.7, 136.7, 141.2, 141.8, 147.7, 149.2, 163.5, 165.7. FT-IR (KBr) ν 3466, 3327, 3056, 2957, 2184, 1746, 1709, 1652, 1577, 1521, 1415, 1353, 1227, 1223, 1154, 1017, 972, 929, 864, 818, 770 cm⁻¹; MS (EI) *m/z* 445.20 (M⁺); Anal. Calcd. For C₂₆H₂₇N₃O₄: C 70.09, H 6.11, N 9.43. Found: C 70.18, H 6.04, N 9.40.

Dimethyl-6-amino-1-(4-chlorophenyl)-5-cyano-4-(4-cyanophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (5t).

Yellow solid; m.p. 164–166°C; ¹H NMR (400 MHz, CDCl₃) δ 3.52 (s, 3H, OCH₃), 3.60 (s, 3H, OCH₃), 4.12 (s, 2H, NH₂), 4.74 (s, 1H, CH), 7.29 (t, 2H), 7.48 (q, 4H), 7.49 (d, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 38.8, 52.2, 52.9, 62.1, 104.4, 111.1, 118.7, 119.7, 127.8, 130.3, 131.5, 132.8, 133.1, 137.2, 142.2, 149.5, 163.0, 165.1. FT-IR (KBr) ν 3467, 3337, 3059, 2951, 2185, 1746, 1709, 1652, 1575, 1521, 1415, 1353, 1227, 1223, 1152, 1017, 971, 929, 869, 820, 775 cm⁻¹; MS (EI) *m/z* 448.09 (M⁺); Anal. Calcd. For C₂₃H₁₇ClN₄O₄: C 61.55, H 3.82, N 12.48. Found: C 61.51, H 3.86, N 12.45.

3. 6. Catalyst Recovery

After completion of the reaction, the catalyst was separated using an external magnet and then was washed three to four times with chloroform and ethyl acetate and then dried at 50 °C for 10 h. The separated catalyst was used for six cycles with a slightly decreased activity as shown in Table 4.

Table 4. Reusability of the Fe₃O₄@SiO₂-NH₂ nanocomposite

Yield (%)					
First	Second	Third	Fourth	Fifth	Sixth
94	93	91	90	88	87

4. Conclusions

In summary, here we describe an efficient method for the synthesis of polysubstituted dihydropyridines through a one-pot four-component reaction of aldehydes, aryl amines, dimethyl acetylenedicarboxylate and malononitrile/ethyl acetoacetate using Fe₃O₄@SiO₂-NH₂ nanocomposites at room temperature. This method offers several advantages including high yields, short reaction times, simple work-up procedure, mild and green reaction conditions, ease of separation, recyclability and reusing of the magnetic nanocatalyst.

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Povzetek

Opisujemo praktično, enostavno in učinkovito metodo za sintezo polisubstituiranih dihidropiridinov s pomočjo večkomponentne reakcije med aldehidi, arilamini, dimetil acetilendikarboksilatoma in malononitrilom/etil acetoacetatom v prisotnosti $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanokompozitov. Predstavljena metodologija je nov in učinkovit način sinteze dihidropiridinskih derivatov, ki prinaša kar nekaj prednosti: odlične izkoristke, kratke reakcijske čase, ponovno uporabo in majhno potrebno množino katalizatorja. Nanomagnetni katalizator lahko namreč po reakciji zlahka izoliramo z uporabo zunanjega magneta in uporabimo večkrat brez posebne izgube učinkovitosti. Katalizator smo popolnoma karakterizirali z analizami FT-IR, SEM, XRD, EDX in VSM.

Scientific paper

Synthesis of 6-*N-R*-Tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones and Their Anticancer Activity

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Abstract

Chemical compounds with tetrazole ring are very interesting systems that can be valuable in pharmaceutical and clinical applications, especially as anticancer agents. In this work, novel 6-*N-R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones were synthesized. A large set of IR, LC-, EI-MS, ¹H, ¹³C NMR and elemental analysis data were collected and evaluated for their structures and purity. Details of synthesis, namely the *N*-alkylation, are discussed, including reactions with secondary and tertiary amides. Four new synthesized compounds (**2.7**, **3.2**, **5.2**, **5.3**) were tested *in vitro* for anticancer activity at 10 μM against 60 cell lines of nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. Further synthesis of substances within the series of substituted tetrazolo[1,5-*c*]quinazoline systems will be attempted to develop improved compounds with better anticancer activity.

Keywords: Anticancer activity; 6-*N-R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones; organic synthesis

1. Introduction

Tetrazole ring is a very interesting system and chemical compounds with this ring find diverse biological, pharmaceutical, and clinical applications, despite its absence in nature.¹ Many highly effective agents have active pharmaceutical ingredients containing the tetrazole ring. During relatively short period of time many such compounds have appeared in the world of pharmaceutical market. Thus, among the drugs with tetrazole ring and agents under trials are the following compounds: hypotensive (Losartan), antimicrobial (Cefamandol), antifungal (TAK-456), anti-inflammatory (Figure 1, a), antiviral (5-CIT-EP), antihistaminic (Tazanoplast, Planlukast), cytostatic (Figure 1, b), central nervous system influence (Corazolum), and others (Figure 1).^{2–5} Also, the anticancer activity of tetrazol was recently reported.^{6–7} Thus, platinum(II) complexes (Figure 1, c) with the general formulae

cis-[PtCl₂(DMSO)L], where ligands are a Schiff base or hydrazone are derived from tetrazolo[1,5-*a*]quinoline-4-carboxaldehyde (Figure 1).⁸ Moreover, fibrinolytic and bronchodilating activities of such compounds were claimed by several US patents.^{9,10} Our latest investigations of substituted condensed tetrazolo[1,5-*c*]quinazolines have also proved their potential as pharmaceutical agents, namely anticancer (*N*-(benzo[*d*]thiazol-2-yl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)acetamides against cells of melanoma), antimicrobial (1-(2,5-dimethoxyphenyl)-2-(tetrazolo[1,5-*c*]quinazolin-5-ylthio)ethanone against *Staphylococcus aureus*), antifungal (5-(3-chloropropylthio)tetrazolo[1,5-*c*]quinazoline against *Candida albicans*), and bioluminescence inhibition properties.^{11,12}

So, tetrazolo[1,5-*c*]quinazolines are of undoubting interest and valuable objects for further research. In this work, as a logical continuation of our previous investigations a range of 6-*N-R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-

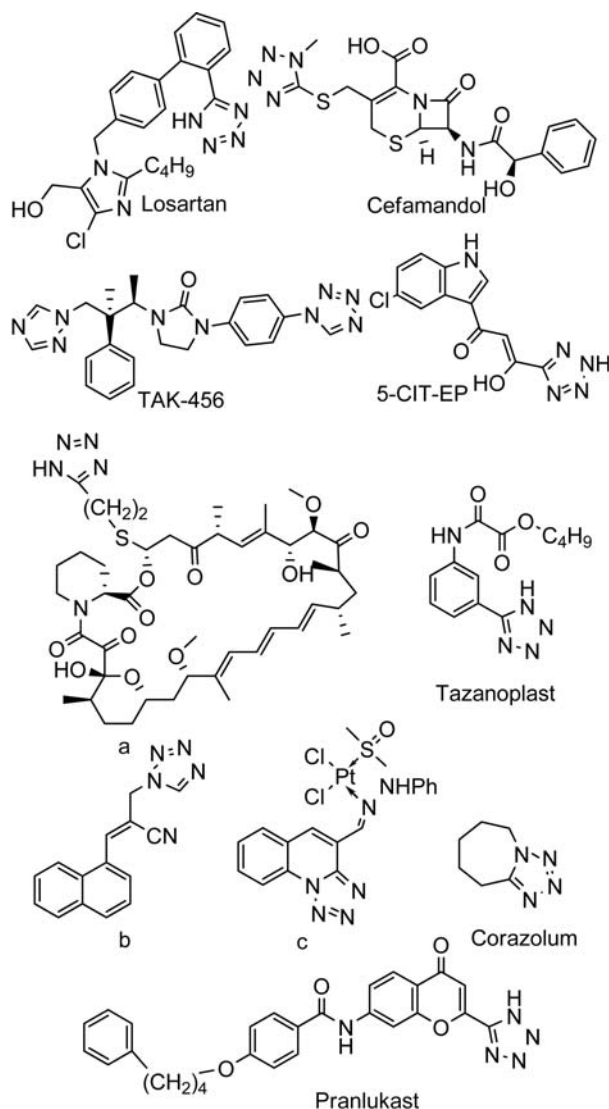


Figure 1. Structures of known tetrazole ring containing drugs available on the market and related agents in clinical trials

ones was synthesized. Thus, the aim of this work was the study of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one *N*-alkylation with subsequent investigation of the 6-*N-R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones for their anticancer activity.

2. Experimental Section

2.1. Chemistry

2.1.1. General Methods

Melting points were determined in open capillary tubes in a «Stuart SMP30» apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using the ELEMENTAR vario EL cube analyzer. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-

IR spectrometer using a module ATR eco ZnSe. ^1H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded at a Varian-Mercury 400 and Bruker Avance DRX-500 spectrometers with SiMe_4 as internal standard in $\text{DMSO}-d_6$ solution. LC–MS were recorded using chromatography/mass spectrometric system which consists of high-performed liquid chromatograph «Agilent 1100 Series» equipped with diode-matrix and mass-selective detector «Agilent LC/MSD SL» (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV.

2.2. Pharmacology

2.2.1. Anticancer Assay for Preliminary *in vitro* Testing

From the newly synthesized compounds 4 substances, namely 2.7, 3.2, 5.2, 5.3 were selected by the NCI Developmental Therapeutic Program for *in vitro* cell line screening to investigate their anticancer activity. The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. Initially, a single high concentration was used (10 μM) in the full NCI 60-cell panel. In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Then test substances were added to the plate and the culture was incubated for further 48 h. End point determinations were made with a protein binding dye, sulforhodamine B. Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells (Table 1).

General Procedure for the Synthesis of 6-*N-R*-Tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones.

To a solution of 0.9 g (4.8 mmol) of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) in DMF 0.17 g (4.8 mmol) of sodium hydride (60% oil suspension) was added. After 5–10 min, when all hydrogen has been released, the appropriate halogen derivative was added (4.8 mmol). The mixture was refluxed for 2 h and cooled down. Then DMF was evaporated under vacuum and water was added to form the precipitate. It was filtered, washed with water, dried and crystallized from a mixture of propane-2-ol : water (1:1).

Tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**)^{13,14}

Yield 95.6%; mp 295–297°C; IR (cm^{-1}): 3147, 3114, 3085, 3047, 2974, 2916, 2849, 2755, 2709, 1746, 1714, 1660, 1625, 1587, 1547, 1515, 1483, 1463, 1442, 1430, 1415, 1388, 1342, 1304, 1256, 1202, 1167, 1157, 1114, 1088, 1025, 995, 969, 896, 880, 810, 783, 757, 742, 731, 709, 698, 675, 658, 623. ^1H NMR: δ (ppm) 12.68 (s, 1H, NH), 8.27 (d, $J = 7.8$ Hz, 1H, H-10), 7.71 (t, $J = 7.7$ Hz, 1H, H-9), 7.50 (d, $J = 8.2$ Hz, 1H, H-7), 7.42 (t, $J = 7.5$

Hz, 1H, H-8). LC-MS: m/z 187 $[M+H]^+$. Anal. Calcd. for $C_8H_5N_5O$: C, 51.34; H, 2.69; N, 37.42. Found: C, 51.38; H, 2.63; N, 37.46.

6-Methyltetrazolo[1,5-*c*]quinazolin-5(6H)-one (2.1)

Yield 57.0%; mp 224–226 °C; IR (cm^{-1}): 1726, 1650, 1620, 1588, 1555, 1518, 1489, 1454, 1423, 1398, 1340, 1315, 1299, 1259, 1235, 1173, 1130, 1108, 1087, 1042, 1013, 1001, 965, 877, 787, 761, 731, 710, 674, 633. ^1H NMR: δ (ppm) 8.39 (d, $J = 7.6$ Hz, 1H, H-10), 7.87 (t, $J = 7.5$ Hz, 1H, H-9), 7.71 (d, $J = 8.5$ Hz, 1H, H-7), 7.54 (t, $J = 7.5$ Hz, 1H, H-8), 3.80 (s, 3H, CH_3). EI-MS: m/z ($I\%$ rel.) 201 (100, M^+), 173 (50.2), 172 (42.7), 145 (15.8), 144 (88.2), 130 (30.1), 129 (32.4), 106 (11.5), 105 (21.2), 104 (55.5), 103 (38.1), 102 (84.3), 78 (29.4), 77 (90.7), 76 (71.8), 75 (45.1), 74 (45.1), 74 (17.0), 71 (15.3), 70 (10.6), 69 (20.0), 65 (10.3), 50 (26.6), 43 (44.2), 42 (23.1), 41 (28.7), 40 (30.9). LC-MS: m/z 202 $[M+H]^+$. Anal. Calcd. for $C_9H_7N_5O$: C, 53.73; H, 3.51; N, 34.81. Found: C, 53.71; H, 3.55; N, 34.78.

6-Ethyltetrazolo[1,5-*c*]quinazolin-5(6H)-one (2.2)

Yield 36.8%; mp 214–216 °C; IR (cm^{-1}): 2992, 2916, 2849, 1730, 1714, 1620, 1586, 1554, 1487, 1459, 1447, 1402, 1383, 1370, 1354, 1322, 1296, 1264, 1239, 1216, 1171, 1130, 1106, 1085, 1045, 1021, 970, 859, 781, 757, 732, 710, 671, 628, 608. ^1H NMR: δ (ppm) 8.40 (d, $J = 7.4$ Hz, 1H, H-10), 7.86 (t, $J = 7.5$ Hz, 1H, H-9), 7.74 (d, $J = 8.3$ Hz, 1H, H-7), 7.53 (t, $J = 7.1$ Hz, 1H, H-8), 4.43 (d, $J = 6.7$ Hz, 2H, $NHCH_2$), 1.41 (t, $J = 6.5$ Hz, 3H, CH_3). Anal. Calcd. for $C_{10}H_9N_5O$: C, 55.81; H, 4.22; N, 32.54. Found: C, 55.84; H, 4.20; N, 32.56.

6-Benzyltetrazolo[1,5-*c*]quinazolin-5(6H)-one (2.3)

Yield 78.1%; mp 203–205 °C; IR (cm^{-1}): 2953, 2918, 2851, 1718, 1619, 1589, 1580, 1556, 1487, 1455, 1400, 1371, 1352, 1324, 1295, 1259, 1227, 1188, 1171, 1161, 1107, 1096, 1050, 1005, 974, 960, 853, 807, 785, 756, 732, 701, 672, 643, 619. ^1H NMR: δ (ppm) 8.41 (d, $J = 7.5$ Hz, 1H, H-10), 7.73 (t, $J = 7.7$ Hz, 1H, H-9), 7.50 (dd, $J = 13.9, 7.6$ Hz, 2H, Bz-3,5), 7.41 (d, $J = 6.9$ Hz, 2H, H-7,8), 7.36–7.22 (m, 3H, Bz-2,4,6), 5.61 (s, 2H, NCH_2). LC-MS: m/z 278 $[M+H]^+$. Anal. Calcd. for $C_{15}H_{11}N_5O$: C, 64.97; H, 4.00; N, 25.26. Found: C, 64.94; H, 4.04; N, 25.22.

6-Phenethyltetrazolo[1,5-*c*]quinazolin-5(6H)-one (2.4)

Yield 63.0%; mp 207–209 °C; IR (cm^{-1}): 2937, 2924, 1739, 1679, 1622, 1591, 1555, 1531, 1511, 1489, 1455, 1360, 1326, 1294, 1275, 1251, 1229, 1204, 1183, 1155, 1107, 1095, 1083, 1050, 1034, 1010, 997, 967, 917, 888, 870, 852, 828, 774, 750, 733, 704, 671, 656. ^1H NMR: δ (ppm) 8.42 (d, $J = 7.2$ Hz, 1H, H-10), 7.87 (t, $J = 7.8$ Hz, 1H, H-9), 7.79 (d, $J = 8.2$ Hz, 1H, H-7), 7.54 (t, $J = 6.9$ Hz, 1H, H-8), 7.38 (d, $J = 6.5$ Hz, 2H, Ph-2,6), 7.31 (t, 2H, Ph-3,5), 7.23 (d, $J = 6.8$ Hz, 1H, Ph-4), 4.55 (t, $J = 7.6$ Hz,

2H, NCH_2), 3.08 (t, $J = 7.4$ Hz, 2H, NCH_2CH_2). LC-MS: m/z 292 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.50; N, 24.04. Found: C, 65.95; H, 4.53; N, 24.01.

2-(5-Oxotetrazolo[1,5-*c*]quinazolin-6(5H)-yl)acetonitrile (2.5)

Yield 88.4%; mp 230–235 °C; IR (cm^{-1}): 3117, 3075, 3006, 2917, 2849, 1729, 1620, 1588, 1556, 1485, 1463, 1422, 1400, 1359, 1350, 1317, 1297, 1263, 1239, 1200, 1173, 1107, 1094, 1057, 1024, 1010, 983, 959, 920, 855, 779, 756, 734, 712, 686, 673. ^1H NMR: δ (ppm) 8.43 (d, $J = 7.7$ Hz, 1H, H-10), 7.95 (t, $J = 7.9$ Hz, 1H, H-9), 7.85 (d, $J = 8.5$ Hz, 1H, H-7), 7.61 (t, $J = 7.4$ Hz, 1H, H-8), 5.54 (s, 2H, CH_2). LC-MS: m/z 227 $[M+H]^+$. Anal. Calcd. for $C_{10}H_6N_6O$: C, 53.10; H, 2.67; N, 37.15. Found: C, 53.14; H, 2.65; N, 37.18.

6-(2-Oxo-2-phenylethyl)tetrazolo[1,5-*c*]quinazolin-5(6H)-one (2.6)

Yield 95.6%; mp 199–201 °C; IR (cm^{-1}): 2919, 2850, 1747, 1696, 1623, 1593, 1557, 1531, 1487, 1465, 1449, 1400, 1377, 1340, 1296, 1259, 1227, 1196, 1174, 1130, 1111, 1098, 1076, 1055, 1026, 997, 970, 835, 812, 750, 728, 708, 687, 670, 632. ^1H NMR: δ (ppm) 8.46 (d, $J = 7.6$ Hz, 1H, H-10), 8.20 (d, $J = 7.5$ Hz, 2H, Ph-2,6), 7.78 (t, $J = 7.8$ Hz, 1H, H-9), 7.72 (d, $J = 6.9$ Hz, 1H, H-7), 7.59 (m, 4H, Ph-3,4,5, H-8), 6.04 (s, 2H, NCH_2). EI-MS: m/z ($I\%$ rel.) 305 (26.6, M^+), 277 (43.7), 207 (13.4), 129 (33.2), 118 (21.2), 117 (20.4), 116 (23.3), 92 (18.4), 91 (64.8), 90 (100), 89 (51.2), 88 (11.9), 65 (29.4), 64 (25.7), 63 (48.0), 62 (28.5), 57 (23.8), 55 (11.7), 52 (17.6), 51 (54.9), 50 (16.2). LC-MS: m/z 306 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{11}N_5O_2$: C, 62.95; H, 3.63; N, 22.94. Found: C, 62.99; H, 3.60; N, 22.97.

6-(2-Oxo-2-(*p*-tolyl)ethyl)tetrazolo[1,5-*c*]quinazolin-5(6H)-one (2.7)

Yield 64.5%; mp 220–222 °C; IR (cm^{-1}): 3045, 2997, 2957, 2916, 2847, 1732, 1684, 1621, 1604, 1589, 1557, 1530, 1488, 1470, 1432, 1354, 1318, 1295, 1264, 1233, 1202, 1185, 1124, 1106, 1091, 1057, 1035, 998, 972, 887, 879, 870, 861, 839, 829, 813, 782, 774, 753, 731, 704, 670, 654, 624. ^1H NMR: δ (ppm) 8.46 (d, $J = 7.5$ Hz, 1H, H-10), 8.10 (d, $J = 6.8$ Hz, 2H, Ph-2, 6), 7.80 (t, $J = 7.4$ Hz, 1H, H-9), 7.58 (d, $J = 7.8$ Hz, 2H, H-7, 8), 7.43 (d, $J = 7.1$ Hz, 2H, Ph-3, 5), 6.01 (s, 2H, NCH_2), 3.17 (s, 3H, CH_3). LC-MS: m/z 320 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{13}N_5O_2$: C, 63.94; H, 4.10; N, 21.93. Found: C, 63.90; H, 4.16; N, 21.88.

Methyl 2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5H)-yl)acetate (3.1)

Yield 14.5%; mp 194–196 °C; IR (cm^{-1}): 2953, 2918, 2851, 1718, 1619, 1589, 1580, 1556, 1487, 1455, 1400, 1371, 1351, 1324, 1295, 1259, 1227, 1188, 1171, 1161, 1107, 1096, 1050, 1005, 974, 960, 853, 807, 785, 756,

732, 701, 672, 643, 619. ^1H NMR: δ (ppm) 8.43 (d, J = 7.5 Hz, 1H, H-10), 7.85 (t, J = 7.8 Hz, 1H, H-9), 7.64 (d, J = 8.6 Hz, 1H, H-7), 7.57 (t, J = 7.4 Hz, 1H, H-8), 5.22 (s, 2H, NCH_2), 3.80 (s, 3H, CH_3). Anal. Calcd. for $\text{C}_{11}\text{H}_9\text{N}_5\text{O}_3$: C, 50.97; H, 3.50; N, 27.02. Found: C, 50.95; H, 3.54; N, 27.01.

Ethyl 2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5H)-yl)acetate (3.2)

Yield 54.4%; mp 172–174 °C; IR (cm^{-1}): 2984, 2918, 1730, 1621, 1588, 1558, 1488, 1467, 1444, 1428, 1377, 1354, 1297, 1268, 1223, 1202, 1107, 1093, 1057, 1032, 1018, 990, 958, 887, 854, 814, 772, 752, 726, 707, 683, 672, 647. ^1H NMR: δ (ppm) 8.44 (d, J = 7.7 Hz, 1H, H-10), 7.88 (t, J = 7.8 Hz, 1H, H-9), 7.67 (d, J = 8.5 Hz, 1H, H-7), 7.59 (t, J = 7.2 Hz, 1H, H-8), 5.22 (s, 2H, NCH_2), 4.27 (dd, J = 13.1, 6.2 Hz, 2H, OCH_2), 1.32 (t, J = 6.9 Hz, 2H, CH_3). ^{13}C NMR: δ 167.38 (s, CO), 150.01 (s, C-5), 142.93 (s, C-6a), 137.83 (s, C-1a), 134.68 (s, C-8), 125.68 (s, C-9), 125.09 (s, C-10), 116.20 (s, C-7), 108.19 (s, C-10a), 61.75 (s, OCH_2), 45.59 (s, NCH_2), 14.07 (s, CH_3). LC-MS: m/z 275 $[\text{M}+\text{H}]^+$. Anal. Calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_5\text{O}_3$: C, 52.75; H, 4.06; N, 25.63. Found: C, 52.71; H, 4.09; N, 25.60.

Propyl 2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5H)-yl)acetate (3.3)

Yield 72.5%; mp 108–110 °C; IR (cm^{-1}): 3128, 3060, 3019, 2959, 2918, 2873, 2849, 1730, 1620, 1589, 1557, 1519, 1487, 1466, 1426, 1395, 1351, 1298, 1267, 1220, 1199, 1106, 1093, 1058, 1029, 1010, 991, 958, 934, 876, 841, 827, 749, 707, 671, 648. ^1H NMR: δ (ppm) 8.44 (d, J = 7.6 Hz, 1H, H-10), 7.85 (t, J = 7.7 Hz, 1H, H-9), 7.62 (d, J = 8.5 Hz, 1H, H-7), 7.57 (t, J = 7.4 Hz, 1H, H-8), 5.20 (s, 2H, NCH_2), 4.19 (t, J = 6.5 Hz, 2H, OCH_2), 1.38 (dd, J = 14.7, 7.3 Hz, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 0.93 (t, J = 7.2 Hz, 3H, $\text{OCH}_2\text{CH}_2\text{CH}_3$). Anal. Calcd. for $\text{C}_{13}\text{H}_{13}\text{N}_5\text{O}_3$: C, 54.35; H, 4.56; N, 24.38. Found: C, 54.37; H, 4.54; N, 24.41.

2-((2-(1H-tetrazol-5-yl)phenyl)amino)acetic acid (4.1)

Yield 75.8%; mp 260–270 °C; IR (cm^{-1}): 3546, 3356, 3191, 2890, 2582, 1908, 1616, 1575, 1564, 1517, 1480, 1436, 1408, 1307, 1281, 1257, 1166, 1095, 1052, 984, 938, 845, 746, 721, 703, 665. ^1H NMR: δ (ppm) 8.22–7.93 (br.s, 1H, NHtetraz.), 7.80 (d, J = 7.6 Hz, 1H, Ph-3), 7.30 (t, J = 7.6 Hz, 1H, Ph-4), 6.72 (t, J = 7.4 Hz, 1H, Ph-5), 6.66 (d, J = 8.3 Hz, 1H, Ph-6), 4.01 (s, 2H, NCH_2). LC-MS: m/z 220 $[\text{M}+\text{H}]^+$. Anal. Calcd. for $\text{C}_{10}\text{H}_7\text{N}_5\text{O}_3$: C, 49.31; H, 4.14; N, 31.95. Found: C, 49.34; H, 4.11; N, 31.99.

***N*-(2-methoxyphenyl)-*N*-(2-((2-methoxyphenyl)amino)-2-oxoethyl)-2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5H)-yl)acetamide (5.1)**

Yield 72.2%; mp 184–186 °C; IR (cm^{-1}): 3324, 3004,

2953, 2919, 2850, 1771, 1710, 1683, 1600, 1539, 1486, 1453, 1417, 1379, 1360, 1321, 1290, 1264, 1253, 1237, 1199, 1179, 1159, 1080, 1037, 996, 970, 950, 907, 864, 841, 827, 815, 799, 784, 747, 723, 698, 682, 668, 644, 621. ^1H NMR: δ (ppm) 10.42 (s, 1H, NH), 8.26 (d, J = 7.0 Hz, 1H, H-10), 7.64 (dd, J = 13.1, 5.0 Hz, 3H, H-7,8,9), 7.32–7.23 (m, 2H, Ph'-3, Ph-3), 7.17 (t, J = 8.1 Hz, 1H, Ph'-4), 7.05 (d, J = 7.8 Hz, 1H, Ph'-6), 6.97–6.83 (m, 3H, Ph'-5, Ph-4,5), 6.60 (d, J = 6.8 Hz, 1H, Ph-6), 5.66 (s, 2H, NCH_2CO), 4.46 (s, 2H, NCH_2CONH), 3.77 (s, 1H, Ph'- OCH_3), 3.75 (s, 1H, Ph- OCH_3). EI-MS: m/z ($\%_{\text{rel.}}$) 513 (33.0, M^+), 335 (51.9), 309 (10.9), 308 (32.7), 307 (26.3), 178 (17.9), 150 (34.0), 149 (100), 148 (17.2), 147 (15.5), 123 (10.1), 119 (19.9), 92 (12.9), 91 (18.9), 88 (10.2), 86 (39.6), 84 (41.2), 57 (18.1), 55 (10.7). LC-MS: m/z 513 $[\text{M}+\text{H}]^+$. Anal. Calcd. for $\text{C}_{26}\text{H}_{17}\text{F}_6\text{N}_7\text{O}_3$: C, 60.81; H, 4.51; N, 19.09. Found: C, 60.85; H, 4.47; N, 19.12.

***N*-(2-Oxo-2-((4-(trifluoromethyl)phenyl)amino)ethyl)-2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5H)-yl)-*N*-(4-(trifluoromethyl)phenyl)acetamide (5.2)**

Yield 51.2%; mp 249–251 °C; IR (cm^{-1}): 3304, 3213, 3166, 3141, 3081, 3014, 2921, 2851, 1777, 1716, 1704, 1609, 1550, 1491, 1441, 1416, 1386, 1358, 1328, 1285, 1258, 1230, 1181, 1162, 1113, 1067, 1044, 1017, 1005, 992, 968, 951, 868, 851, 834, 805, 785, 748, 738, 726, 690, 674, 660, 643, 624. ^1H NMR: δ (ppm) 10.95 (s, 1H, NH), 8.29 (d, J = 7.4 Hz, 1H, H-10), 7.80 (t, J = 7.4 Hz, 1H, H-9), 7.73 (br.s, 4H, Ph-2,3,5,6), 7.70–7.64 (m, 4H, Ph'-2,3,5,6), 7.59 (d, J = 8.2 Hz, 1H, H-7), 7.49 (dd, J = 15.5, 7.9 Hz, 1H, H-8), 5.82 (s, 2H, NCH_2CO), 4.56 (s, 2H, NCH_2CONH). LC-MS: m/z 590 $[\text{M}+\text{H}]^+$. Anal. Calcd. for $\text{C}_{26}\text{H}_{17}\text{F}_6\text{N}_7\text{O}_3$: C, 52.98; H, 2.91; N, 16.63. Found: C, 52.93; H, 2.95; N, 16.60.

***N*-(4-Fluorobenzyl)-*N*-(2-((4-fluorobenzyl)amino)-2-oxoethyl)-2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5H)-yl)acetamide (5.3)**

Yield 54.4%; mp 152–154 °C; IR (cm^{-1}): 3296, 1770, 1737, 1705, 1659, 1621, 1604, 1556, 1520, 1487, 1455, 1433, 1417, 1381, 1369, 1352, 1296, 1258, 1217, 1203, 1154, 1095, 1044, 1005, 995, 959, 917, 851, 829, 782, 770, 757, 744, 732, 711, 680, 659, 641, 617. ^1H NMR: δ (ppm) 8.86 (br.s, 1H, NH), 8.19 (d, J = 7.2 Hz, 1H, H-10), 7.62 (m, 3H, H-9,8,7), 7.35 (m, 4H, Ph-2,3,5,6), 7.09 (m, J = 8.4 Hz, 4H, Ph'-2,3,5,6), 5.43 (s, 2H, $\text{NCH}_2\text{C(O)N}$), 4.59 (s, 2H, $\text{NCH}_2\text{C(O)NH}$), 4.39 (s, 2H NCH_2Ph), 4.35 (d, J = 5.0 Hz, 2H, NHCH_2Ph). EI-MS: m/z ($\%_{\text{rel.}}$) 517 (3.2, M^+), 337 (26.6), 165 (20.5), 150 (30.8), 137 (11.8), 136 (19.7), 131 (18.0), 122 (21.4), 110 (47.0), 109 (100), 107 (17.5), 105 (11.5), 104 (14.2), 103 (30.6), 89 (10.9), 86 (14.9), 84 (20.4), 83 (47.0), 77 (16.9), 76 (13.3), 75 (19.6), 51 (17.8). LC-MS: m/z 518 $[\text{M}+\text{H}]^+$. Anal. Calcd. for $\text{C}_{26}\text{H}_{21}\text{F}_2\text{N}_7\text{O}_3$: C, 60.35; H, 4.09; N, 18.95. Found: C, 60.39; H, 4.05; N, 18.98.

***N*-(2-Oxo-2-((4-(trifluoromethyl)benzyl)amino)ethyl)-2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5*H*)-yl)-*N*-(4-(trifluoromethyl)benzyl)acetamide (5.4)**

Yield 31.1%; mp 166–168 °C; IR (cm⁻¹): 3293, 2916, 1774, 1708, 1671, 1621, 1558, 1486, 1452, 1439, 1422, 1412, 1374, 1324, 1260, 1203, 1178, 1158, 1114, 1080, 1067, 1044, 1019, 953, 925, 817, 784, 762, 744, 735, 712, 679, 635, 613. ¹H NMR: δ (ppm) 8.99 (br.s, 1H, NH), 8.20 (d, *J* = 6.8 Hz, 1H, H-10), 7.63 (m, 7H, H-7,8,9, Ph-2,3,5,6), 7.52 (m, 4H, Ph'-2,3,5,6), 5.52 (m, 2H, NCH₂C(O)N), 4.70 (s, 2H, NCH₂C(O)NH), 4.46 (d, *J* = 4.9 Hz, 2H, NHCH₂Ph'), 4.42 (s, 2H, NCH₂Ph). LC-MS: *m/z* 590 [M+H]⁺. Anal. Calcd. for C₂₈H₂₁F₆N₇O₃: C, 54.46; H, 3.43; N, 15.88. Found: C, 54.41; H, 3.40; N, 15.85.

6-(2-Morpholino-2-oxoethyl)tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (6.1)

Yield 73.3%; mp 217–219 °C; IR (cm⁻¹): 2981, 2917, 2868, 2848, 1733, 1665, 1620, 1587, 1556, 1485, 1454, 1422, 1399, 1365, 1344, 1329, 1314, 1299, 1269, 1261, 1226, 1213, 1198, 1163, 1120, 1104, 1091, 1060, 1037, 1024, 1008, 987, 949, 908, 872, 842, 801, 780, 760, 730, 709, 669, 621. ¹H NMR: δ (ppm) 8.43 (d, *J* = 7.5 Hz, 1H, H-10), 7.91 (t, *J* = 7.5 Hz, 1H, H-9), 7.63 (d, *J* = 8.7 Hz, 1H, H-7), 7.59 (t, *J* = 7.4 Hz, 1H, H-8), 5.38 (s, 2H, NCH₂), 3.75 (s, 2H, H-3 morph), 3.71 (s, *J* = 3.1 Hz, 2H, H-5 morph), 3.63 (s, 2H, H-2 morph), 3.48 (s, 2H, H-6 morph). EI-MS: *m/z* (I%_{rel}) 314 (1.4, M⁺), 286 (17.9), 228 (49.8), 172 (26.0), 131 (15.1), 130 (26.6), 129 (57.4), 127 (19.1), 103 (43.9), 102 (49.0), 99 (18.9), 77 (19.2), 76 (18.5), 75 (16.4), 70 (100). LC-MS: *m/z* 315 [M+H]⁺. Anal. Calcd. for C₁₄H₁₄N₆O₃: C, 53.50; H, 4.49; N, 26.74. Found: C, 53.53; H, 4.45; N, 26.77.

6-(2-(4-(2-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (6.2)

Yield 96.8%; mp 247–249 °C; IR (cm⁻¹): 2995, 2963, 2917, 2849, 1727, 1656, 1618, 1589, 1557, 1504, 1485, 1463, 1447, 1435, 1397, 1377, 1359, 1341, 1329, 1294, 1279, 1262, 1235, 1213, 1199, 1166, 1149, 1106, 1091, 1057, 1039, 1025, 1009, 994, 960, 937, 927, 912, 803, 779, 755, 730, 670, 616. ¹H NMR: δ (ppm) 8.44 (d, *J* = 7.8 Hz, 1H, H-10), 7.91 (t, *J* = 7.9 Hz, 1H, H-9), 7.65 (d, *J* = 8.4 Hz, 1H, H-7), 7.60 (t, *J* = 7.3 Hz, 1H, H-8), 7.21–7.15 (m, 2H, Ph-5,6), 7.12 (t, *J* = 8.2 Hz, 1H, Ph-4), 7.04 (dd, *J* = 12.3, 5.9 Hz, 1H, Ph-3), 5.43 (s, 2H, NCH₂), 3.87 (s, 2H, ppz-3), 3.67 (s, 2H, ppz-5), 3.21 (s, 2H, ppz-2), 3.05 (s, 2H, ppz-6). ¹³C NMR: δ (ppm) 164.20 (s, CO ppz), 150.38 (s, CF), 143.30 (s, C-5), 140.09 (s, Ph-1), 138.74 (s, C-6), 135.00 (s, C-1a), 125.90 (s, C-9), 125.38 (s, Ph-5), 125.24 (s, C-8), 123.49 (s, C-7), 120.14 (s, C-10), 116.95 (s, Ph-6), 116.65 (s, Ph-4), 116.48 (s, Ph-3), 108.42 (s, C-10a), 50.97 (s, C-3 ppz), 50.49 (s, C-5 ppz), 46.02 (s, NCH₂), 44.97 (s, C-2, ppz), 42.31 (s, C6 ppz). LC-MS: *m/z* 408 [M+H]⁺. Anal. Calcd. for C₂₀H₁₈FN₇O₂: C, 58.96; H, 4.45; N, 24.07. Found: C, 58.98; H, 4.42; N, 24.09.

6-(2-(3,5-Diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-2-oxoethyl)tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (6.3)

Yield 87.2%; mp 248–250 °C; IR (cm⁻¹): 2932, 1734, 1675, 1622, 1592, 1558, 1487, 1455, 1445, 1398, 1385, 1356, 1302, 1261, 1195, 1173, 1161, 1143, 1106, 1092, 1060, 1042, 1023, 1012, 955, 884, 864, 788, 776, 752, 731, 694, 672, 659, 644, 618. ¹H NMR: δ (ppm) 8.41 (d, *J* = 7.6 Hz, 1H, H-10), 7.92–7.82 (m, 2H, H-9,7), 7.78 (t, *J* = 7.6 Hz, 1H, H-8), 7.56–7.44 (m, 5H, 3-Ph(2-6)-5pyr.), 7.35–7.20 (m, 5H, 5-Ph(2-6)-5pyr.), 5.76–5.51 (m, 3H, NCH₂, pyr.-5), 3.98 (dd, *J* = 17.9, 11.8 Hz, 1H, pyr.-4), 3.22 (dd, *J* = 17.9, 4.4 Hz, 1H, pyr.-4). LC-MS: *m/z* 450 [M+H]⁺. Anal. Calcd. for C₂₅H₁₉N₇O₂: C, 66.81; H, 4.26; N, 21.81. Found: C, 66.85; H, 4.22; N, 21.85.

6-(2-Oxo-2-(3-phenyl-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethyl)tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (6.4)

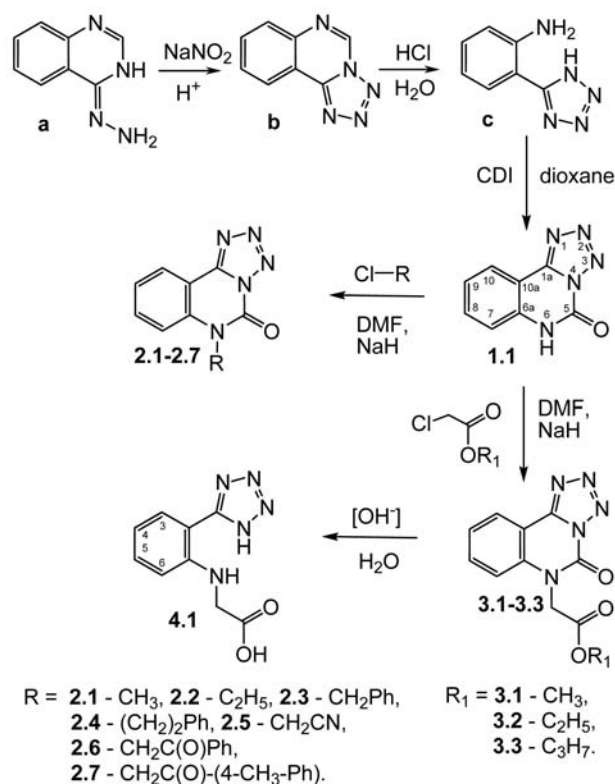
Yield 91.5%; mp 264–265 °C; IR (cm⁻¹): 2918, 2850, 1738, 1669, 1621, 1589, 1556, 1525, 1487, 1462, 1419, 1357, 1344, 1306, 1261, 1196, 1172, 1142, 1105, 1094, 1083, 1059, 1036, 1020, 1010, 996, 964, 954, 878, 863, 839, 787, 774, 753, 725, 699, 673, 643, 612. ¹H NMR: δ (ppm) 8.41 (d, *J* = 7.3 Hz, 1H, H-10), 7.78 (t, *J* = 8.1 Hz, 1H, H-9), 7.64 (s, 1H, H-7), 7.52 (s, 2H, Ph-2,6), 7.45 (s, 1H, H-8), 7.31 (d, *J* = 6.1 Hz, 2H, thioph.-4, pyr.-5), 7.26 (s, 3H, Ph-3,4,5), 7.13 (s, 1H, thioph.-3), 5.63 (s, 2H, NCH₂), 5.53 (s, 1H, thioph.-2), 4.99 (m, 1H, pyr.-4), 3.22 (d, *J* = 16.1 Hz, 1H, pyr.-4). LC-MS: *m/z* 456 [M+H]⁺. Anal. Calcd. for C₂₃H₁₇N₇O₂S: C, 60.65; H, 3.76; N, 21.53. Found: C, 60.62; H, 3.79; N, 21.51.

3. Results and Discussion

3.1. Chemistry

The tetrazolo[1,5-*c*]quinazoline synthesis was described in detail in our previous works.^{11,12} 5-(2'-Aminophenyl)-1*H*-tetrazole (Scheme 1, **c**) was cyclized with carbonyldiimidazole with formation of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**), which was used as the starting compound for further modifications at position 6. *N*-Alkylation of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) was carried out using chloro derivatives, namely substances **2.1–2.7** (Cl-R) and **3.1–3.3** (Cl-R₁) (Scheme 1).

To find the most efficient way of synthesis, various reaction conditions were explored. At first, tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) was dissolved in DMF with equimolar amount of sodium hydride. The corresponding halogen derivative was added only after all hydrogen has been released. The resulting mixture was refluxed for 2 h. Alternatively, the reaction was performed by the addition of potassium carbonate in DMF or sodium bicarbonate in dioxane. The best yields and purity of derivatives **2** were observed in the presence of sodium hydride. This method was chosen as the primary one.

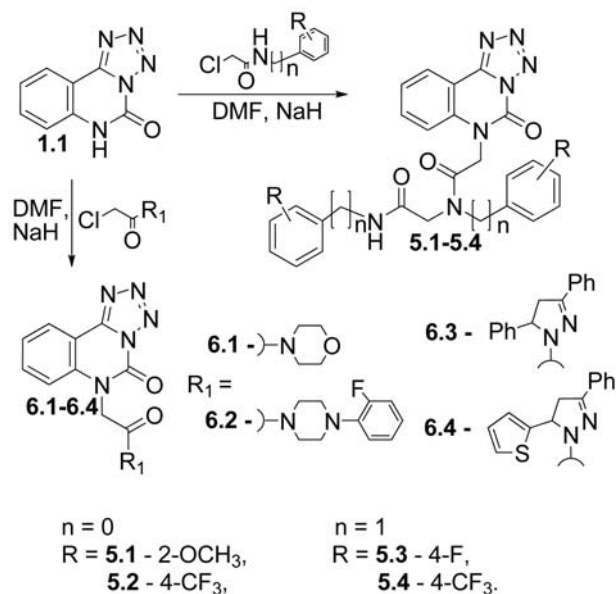


Scheme 1. Synthesis of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) and its *N*-substituted derivatives

The next step was the synthesis of acetamides. Firstly 2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5*H*)-yl)acetic acid should be obtained with further aminolysis. The direct alkylation of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) with chloroacetic acid has not resulted in the desirable product. Thus, an alkaline hydrolysis of 2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5*H*)-yl)acetate esters **3.1–3.3** was necessary (Scheme 1). However, the cleavage of quinazoline cycle was observed, and the product of the cleavage turned out to be 2-((2-(1*H*-tetrazol-5-yl)phenyl)amino)acetic acid (**4.1**) (see further discussion on spectral data).

Then, *N*-alkylation of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) with chloroacetamides was used to synthesize amides **5.1–5.4**. The reaction was quite interesting, since the products obtained were disubstituted compounds **5.1–5.4**. In this reaction NH proton of acetamide was acting as a competitive acid moiety, which results in the alkylation of the quinazolin-5(6*H*)-one NH group, and of acetamide NH group of intermediate alkylated product (Scheme 2). This was confirmed by LC-MS and ¹H NMR spectra of the synthesized compounds with intensive peaks of molecular ions with a mass of two acetamide residues. Alkylation with tertiary amides **6.1–6.4** has not revealed any unexpected products (Scheme 2).

The identity of the synthesized compounds was confirmed by IR, LC-, EI-MS, ¹H, ¹³C NMR, and elemental



Scheme 2. Alkylation of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) with secondary and tertiary amines

analysis. LC-MS of the synthesized compounds in a »soft« ionization (chemical ionization at atmospheric pressure) allowed to register the molecular ion peak [M+1] in high intensity. For compound **4.1** the ion with molecular weight of 220 was observed, confirming cleavage of the quinazoline ring.

In the ¹H NMR spectra of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones the clear splitting of aromatic quinazoline protons' signals was observed. Thus, H-10 signal can be found at a range of 8.19–8.46 ppm, H-9 at 7.71–7.95 ppm, H-7 at 7.50–7.85 ppm, and H-8 at 7.42–7.61 ppm. The signals for these protons for some compounds were overlapping with each other (**2.3**, **2.7**) or with other aromatic substituents protons (**5.4**). At the same time, for compound **4.1** the diamagnetic shift of aromatic protons was observed, obviously due to the absence of electron-deficient tetrazoloquinazoline system. Thus, Ph-3 signal was registered at 7.80 ppm as a doublet, Ph-4 at 7.30 ppm as a triplet, Ph-5 at 6.72 ppm as a triplet, and Ph-6 as a doublet at 6.66 ppm. Besides that, the NH tetrazole proton was detected as a broad singlet at 8.22–7.93 ppm.

The signal of the NCH₂ group can be used as a confirmation of *N*-alkylation. For compounds **2.1–2.7** it was registered as a two-proton singlet at 5.54–3.80 ppm, except for compound **2.4**, where it was as a two-proton triplet at 4.55 ppm. Due to electron acceptor influence of 2-oxo-2-phenylethyl and 2-oxo-2-(*p*-tolyl)ethyl moiety the signal of NCH₂ group was observed in the weak field at 6.04–6.01 ppm for substances **2.6** and **2.7**. Esters **3.1–3.3** displayed two-proton singlet signal at 5.20–5.22 ppm. For 2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5*H*)-yl)acetic acid the signal of NCH₂ group was detected in the stronger field at 4.01 ppm. Moreover, for acetamides **5.1–5.4** and

tertiary amides **6.1–6.4** the signal of NCH_2 group was also shifted to the weak field and observed at 5.82–5.38 ppm as a two-proton singlet. Only for compound **6.3** the NCH_2 signal was overlapped with H-5 of pyrazol and registered as a multiplet at 5.76–5.51 ppm. The signal of NH proton of phenylacetamides **5.1** and **5.2** was located as a singlet at 10.42–10.95 ppm, whereas for benzylacetamides **5.3** and **5.4** as an unsplitted triplet at 8.86–8.99 ppm. All alkyl groups are located in the strong field.

As for the IR spectra, the main tetrazolo[1,5-*c*]quinazoline ring and C–H deformations were detected at 1623–1485 cm^{-1} and at 917–608 cm^{-1} . Azo fragments had stretchings at 1604–1400 cm^{-1} . Moderate absorptions caused by cyclic N–C(=O)–N stretching were overlapped with ester and amide carbonyl vibrations. Vibrations of the $\nu_{\text{C=O}}$ in esters **3.1–3.3** were found at 1746–1730 cm^{-1} . Wide stretchings of C–O–C appeared at 1250–1188 cm^{-1} . Vibrations of ν_{OH} of **4.1** were found at 3546–3191 cm^{-1} and δOH at 938–845 cm^{-1} . Secondary amides **5.1–5.4** had two stretching signals of the N–H group: strong at 3324–3296 cm^{-1} and mild at 3330–3070 cm^{-1} ; and deformations at 1550–1520 cm^{-1} . The carbonyl stretchings we-

re overlapped at 1683–1621 cm^{-1} for secondary and tertiary amides fragments in the substances **5.1–5.4**, and were at 1669–1618 cm^{-1} for substances **6.1–6.4**.

In EI-MS spectra the characteristic peaks of M^{+} were observed, with different intensity, depending on the moiety of synthesized compounds (**2.1** m/z 201 (100%), **2.6** m/z 305 (26.6%), **5.1** m/z 513 (33.0%), **5.3** m/z 517 (3.2%), **6.1** m/z 314 (1.4%)). The first step of M^{+} fragmentation was associated with N1–N2 and N3–N4 of tetrazole fragment cleavages (**2.1** m/z 173 (50.2%), **2.6** m/z 277 (43.7%), **6.1** m/z 286 (7.9%)). Whereas for compounds **5.1** and **5.3** the fragmentation of molecular ion was caused by the concurrent processes: tetrazole ring destruction and α -scission of the second acetamide moiety (**5.1** m/z 335 (51.9%) and **5.3** m/z 337 (26.6%)).

3. 2. Anticancer Assay for Preliminary *in vitro* Testing

Among all newly synthesized compounds substances **2.7**, **3.2**, **5.2**, **5.3** were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program for

Table 1. Percentage of *in vitro* tumor cell lines growth at 10 μM exposed to 6-*N-R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones (**2.7**, **3.2**, **5.2**, **5.3**)

Cmpd.	Mean	Range	Most sensitive cell lines ^a
2.7	103.29	81.02–127.01	94.69 (HOP-62/nscLC), 87.49 (HOP-92/nscLC), 89.53 (NCI-H226/nscLC), 99.73 (NCI-H23/nscLC), 97.44 (NCI-H322M/nscLC), 97.56 (SF-539/CNSC), 93.50 (SNB-75/CNSC), 92.85 (U251/CNSC), 97.21 (LOX IMVI/M), 97.99 (SK-MEL-5/M), 92.55 (SK-OV-3/OV), 99.52 (SN12C/RC), 81.02 (UO-31/RC) , 91.05 (PC-3/PC), 94.11 (MCF7/BC), 91.80 (MDA-MB-231/ATCC/BC), 97.43 (HS 578T/BC), 98.86 (BT-549/BC), 96.83 (T-47D/BC).
3.2	101.85	85.49–118.12	97.93 (RPMI-8226/L), 92.84 (A549/ATCC/nscLC), 93.56 (HOP-62/nscLC), 88.43 (HOP-92/nscLC), 96.75 (NCI-H226/nscLC), 94.74 (NCI-H322M/nscLC), 98.04 (HCT-116/ColC), 96.07 (SNB-75/CNSC), 86.66 (U251/CNSC), 98.42 (M14/M), 95.12 (SK-MEL-5/M), 85.49 (UACC-257/M) , 89.08 (OVCAR-8/OV), 97.26 (CAKI-1/RC), 99.65 (SN12C/RC), 89.59 (UO-31/RC), 98.76 (PC-3/PC), 96.76 (MCF7/BC), 99.85 (HS 578T/BC), 98.24 (BT-549/BC), 97.86 (T-47D/BC).
5.2	102.25	84.19–128.99	89.75 (A549/ATCC/nscLC), 97.40 (HOP-62/nscLC), 93.88 (HOP-92/nscLC), 93.84 (NCI-H226/nscLC), 90.72 (NCI-H23/nscLC), 86.55 (NCI-H522/nscLC), 99.58 (COLO 205/ColC), 93.31 (SF-539/CNSC), 98.98 (SNB-75/CNSC), 88.56 (U251/CNSC), 97.77 (SK-MEL-2/M), 95.35 (SK-MEL-5/M), 86.93 (UACC-257/M), 88.58 (OVCAR-5/OV), 84.93 (OVCAR-8/OV), 97.41 (SK-OV-3/OV), 97.74 (786-0/RC), 84.19 (UO-31/RC) , 97.38 (PC-3/PC), 97.16 (HS 578T/BC), 97.66 (BT-549/BC), 93.67 (T-47D/BC).
5.3	95.34	65.64–115.96	85.49 (CCRF-CEM/L), 90.41 (HL-60(TB)/L), 88.03 (K-562/L), 88.98 (MOLT-4/L), 65.64 (RPMI-8226/L) , 94.18 (SR/L), 93.21 (A549/ATCC/nscLC), 94.52 (HOP-62/nscLC), 84.56 (HOP-92/nscLC), 78.93 (NCI-H226/nscLC), 91.43 (NCI-H322M/nscLC), 90.50 (HCT-116/ColC), 97.47 (HCT-15/ColC), 92.86 (SF-295/CNSC), 97.98 (SNB-19/CNSC), 88.72 (SNB-75/CNSC), 90.50 (U251/CNSC), 98.39 (LOX IMVI/M), 95.76 (M14/M), 78.17 (SK-MEL-5/M), 94.08 (UACC-257/M), 98.92 (UACC-62/M), 98.00 (OVCAR-4/OV), 78.84 (OVCAR-8/OV), 82.75 (NCI/ADR-RES/OV), 95.22 (786-0/RC), 77.20 (ACHN/RC), 94.36 (CAKI-1/RC), 92.08 (RXF 393/RC), 66.45 (UO-31/RC) , 81.19 (PC-3/PC), 96.59 (MCF7/BC), 91.55 (BT-549/BC), 70.81 (T-47D/BC), 99.64 (MDA-MB-468/BC).

^a L – leukemia, nscLC – non-small cell lung cancer, ColC – colon cancer, CNSC – CNS cancer, M – melanoma, OV – ovarian cancer, RC – renal cancer, PC – prostate cancer, BC – breast cancer, bold values – the most sensitive ones.

the *in vitro* cell line screening to investigate their anticancer activity^{15,16} (Table 1).

The most sensitive cell line turned out to be UO-31 of renal cancer. It should be mentioned that *N*-(4-fluorobenzyl)-*N*-(2-((4-fluorobenzyl)amino)-2-oxoethyl)-2-(5-oxotetrazolo[1,5-*c*]quinazolin-6(5*H*)-yl)acetamide (**5.3**) had the highest inhibition at 33.55%. Besides, this compound also negatively influenced RPMI-8226 of leukemia cell line, displaying inhibition at 34.36%.

4. Conclusions

Due to their unique characteristics, compounds with tetrazole ring have been used in pharmaceutical and clinical applications especially as anticancer agents. In this work, novel 6-*N-R*-tetrazolo[1,5-*c*]quinazolin-5(6*H*)-ones were synthesized. *N*-Alkylation reaction of tetrazolo[1,5-*c*]quinazolin-5(6*H*)-one (**1.1**) with various chloro-derivatives was performed under various reaction conditions. As the best option a reflux in DMF with an equimolar amount of sodium hydride was selected. Spectral data confirm molecular structures of investigated compounds. These investigations will be continued for other activities and core structures.

5. References

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Povzetek

Spojine, ki vsebujejo tetrazolski obroč, so zelo zanimivi sistemi s pomembnimi farmacevtskimi in kliničnimi uporabi, zlasti kot učinkovine proti raku. V tem članku predstavljamo sintezo novih 6-*N-R*-tetrazolo[1,5-*c*]kinazolin-5(6*H*)-onov, katerih strukturo in čistost smo ugotovili s pomočjo zbranih IR, LC-, EI-MS, ¹H in ¹³C NMR podatkov ter rezultatov elementarnih analiz. Opisujemo podrobnosti sinteze, torej *N*-alkiliranja, vključno z reakcijami s sekundarnimi in terciarnimi amidi. Štiri nove pripravljene spojine (**2.7**, **3.2**, **5.2**, **5.3**) smo *in vitro* testirali za protirakavo učinkovitost pri 10 μM na 60 celičnih linij devetih vrst rakov: levkemije in melanoma ter raka pljuč, debelega črevesja, centralnega živčnega sistema, jajčnika, ledvice, prostate in dojke. V prihodnosti bomo poizkušali izvesti še dodatne sinteze spojin iz serije tetrazolo[1,5-*c*]kinazolinskih sistemov z namenom izboljšanja protirakave učinkovitosti.

Scientific paper

Synthesis, Characterization and DFT-Based Investigation of a Novel Trinuclear Singly-Chloro-Bridged Copper(II)-1-Vinylimidazole Complex

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Abstract

A novel trinuclear copper(II) complex $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$ with monodentate 1-vinylimidazole (1-Vim) and chloro ligands has been prepared and experimentally characterized by elemental analysis, thermogravimetry (TGA, DTG, DTA), X-ray single crystal diffractometry, TOF-MS and FT-IR spectroscopies. The electronic and structural properties of the complex were further investigated by DFT/TD-DFT methods. Density functional hybrid method (B3LYP) was applied throughout the calculations. The calculated UV-Vis results based on TD-DFT approach were simulated and compared with experimental spectrum. Based on the data obtained, DFT calculations have been found in reasonable accordance with experimental data.

Keywords: 1-vinylimidazole, trinuclear copper(II), singly-chloro-bridged, DFT/TD-DFT

1. Introduction

Molecular behaviours of polynuclear transition metal complexes have been an indispensable subject of numerous researches in coordination chemistry because of their multifarious electronic, magnetic and optical properties.^{1–3} These prominent features essentially stem from the existence and the adjacent positions of two or more metallic centers in the same molecular unit. Among these, polynuclear copper(II) species^{4–8} are the most intriguing and investigated ones due to their plain structural properties, ability to mimic active sites in certain copper containing proteins and easy to rationalize and diverse magnetic properties as the simplest examples of magnetic exchange interactions.^{9–14} From another standpoint, the selection or design of the organic ligands are of crucial importance on self-assembly of metal ions owing to their requisite functional roles as transmitters of close metal-metal communications other than they are the backbones of these polynuclear aggregates.^{15–17} Generally, the use of

polydentate ligands that can present different coordination behaviours according to nature of the metal ions is straightforward for aforementioned versatilities. In case of our study reported herein, a novel mono-chloro-bridged trinuclear complex consisting of two discrete metal(II) centers connected through simple bridging chloro ligands and coordinated with monodentate 1-vinylimidazole ligands and monodentate chloro ligands were prepared and fully characterized. The interesting topology of the the complex was probed with X-ray single-crystal data. Qualitative picture of spectroscopic and structural properties of the complex beyond the experimental data were further investigated in the framework of density functional theory (DFT) and its time-dependent extension (TD-DFT). It is well known from the literature that DFT methods, especially the latter introduced hybrid ones that predict almost all molecular properties from simple organic molecules to more complex systems such as transition metal complexes are superior to other wave-function based electron correlation methods in preference. This is

first because they present same or near accuracy results and second, require much more affordable computation time compared to other multi-determinantal approaches that are still applicable only to small/moderate molecules within current computational facilities.^{18–20}

2. Experimental

2. 1. Synthesis of the Complex

0.855 g (5.00 mmol) $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 40 mL of water:ethanol (3:1) mixture and to this solution which was heated up to 70 °C, 0.941 g (10.0 mmol) of 1-vinylimidazole was gradually added with constant stirring at the same temperature. The obtained mixture was stirred for half a hour, filtered off and left undisturbed for crystallization. After 30 days needle-shaped dark green crystals of X-ray quality were obtained from the solution. Yield: 48%. Anal. Calcd.(%): C 37.21, N 17.36, H 3.72. Found: C 37.12, N 16.82, H 3.25. IR(KBr, cm^{-1}): 3143–3117, $\nu(\text{C-H})_{\text{vinyl}}$; 3023, $\nu(\text{C-H})_{\text{arom.}}$; 1645, $\nu(\text{C=C})$; 1503, $\nu(\text{C=N})$ (See Figure S1 in supporting information).

2. 2. Physical Measurements

All the reactants and solvents were obtained from commercial suppliers and used without further purification. FT-IR spectrum was recorded on a BRUKER 2000 spectrometer as KBr slice. UV-Vis spectrum was measured with a UNICAM UV2 spectrometer in 200–800 nm range in methanolic solution. ESI mass spectrum was recorded with Agilent LC/MS-TOF spectrometer using methanolic solution. Elemental analysis was performed with a Costech ECS 4010 CHNS Elemental analyzer. Simultaneous thermal analyses were conducted by using a SIIO-Exstar 6000 Thermal analyzer within 35–1000 °C temperature range, by a heating rate of 10 °C/min. and in static nitrogen atmosphere.

Intensity data were collected using a STOE IPDS 2 diffractometer with graphite monochromated Mo K_α radiation ($\lambda = 0.71073 \text{ \AA}$) at 293(2) K. The structure was solved by direct methods²¹ and refined with full-matrix least-squares procedure on F^2 using SHELXL97.²² All non-hydrogen atoms were refined anisotropically. Hydrogens bonded to carbon atoms were positioned geometrically and refined with a riding model with U_{iso} 1.2 times that of attached carbon atoms. The positions of water hydrogens were found by difference fourier map and refined isotropically.

2. 3. Computational Procedure

All computations reported herein were carried out using Gaussian 03W suit of program²³ under C_i constrained symmetry within unrestricted formalism. Spin-doub-

let gas-phase B3LYP optimizations of the complex starting from experimental X-ray geometry was performed employing triple-zeta 6-311G(d) basis set for Cu atoms, double-zeta 6-31G(d) basis set for H, C and N atoms and 6-31G+(d) basis set for more diffuse chlorine atoms. Vibrational frequency analysis was calculated at the optimized structure at the same level of theory to ensure that the final geometry is a local minimum having no imaginary frequency.

TD-DFT excited state calculation was performed at the geometry optimized structure using the flexible LANL2DZ basis set for all atoms considering the large size of the system.

Investigation of natural charge distributions, population analysis of valence core orbitals and non-covalent energetic stabilizations by second order perturbation theory analysis of Fock matrix were carried out using natural bond orbital (NBO) analysis at the UB3LYP optimized structure by NBO version 3.1 implemented in Gaussian 03W package.

The percentage molecular orbital contributions (MOCs) from atoms and groups to the related molecular orbitals (MOs), were extracted from single point energy (SPE) calculation of the optimized structure using *VMO-des* software.²⁴

3. Results and Discussion

3. 1. Crystal Structure

A perspective plot with the atom numbering scheme of the complex is shown in Figure 1 and the crystallographic data are given in Table 1. The crystal consists of neutral trimeric units and the symmetric unit of the network consists of two geometrically different copper(II) centers one of which ($\text{Cu}2$ and $\text{Cu}2^i$ i: $1-x, -y, 1-z$) is distorted square-pyramidal and the other ($\text{Cu}1$) is distorted square-planar geometry as shown in Figure 2. In the trimer unit, the central copper(II) ion located on inversion center possessing a quasi-octahedral coordination geometry in which the two singly-bridging chloro ligands ($\text{Cl}1, \text{Cl}1^i$) together with two N atoms ($\text{N}1, \text{N}1^i$) of monodentate 1-vim molecules are located on square plane. The two monodentate chloro ligands ($\text{Cl}2, \text{Cl}2^i$) weakly coordinated and reside in the apical positions. Symmetry related copper(II) terminal has more common penta-coordination with the two monodentate chloro ligands ($\text{Cl}2, \text{Cl}3$), two N atoms ($\text{N}3, \text{N}5$) of 1-vim molecules of basal plane and one axially coordinated mono-bridging chloro ligand ($\text{Cl}1$). The coordination number 5 for copper(II) ions usually presents either a square pyramidal (SP) or trigonal-bipyramidal (TBP) geometry (or an intermediate geometry). The Addison distortion index, τ used for the evaluation of the distortion of coordination geometry from TBP to SP was calculated as 0.187 and clearly suggests a distorted SP extreme for terminal copper(II) ions in the tri-

mer unit^{25,26} ($\tau = \alpha - \beta / 60$, where α and β correspond to two angles showing tendency to linearity). The τ -values of square-based-pyramidal and trigonal-bipyramidal extremes are 0 and 1, respectively.²⁷ In one aspect, as the quasi-octahedral geometry of Cu1 ion is considered, the long Cu1...Cl2 distance (3.189(7) Å) indicates Jahn-Teller distortion by elongation through coordination of chlorine linkers to terminal copper(II) ions. But in another, bond valence analysis^{28,29} more clearly substantiates the preponderance of square-planar geometry. In basal plane, Cu2–Cl2 distance (2.339(7) Å) is longer than that of Cu2–Cl3 (2.299(7) Å) and approves the weak coordination of Cl2 ligand to the central copper(II) ion to form a quasi-octahedron around Cu1. The terminal copper(II) ions displaces 0.131 Å from the basal plane upon coordination to bridging chloride ions.

The two mono-bridging chloro ligands bond in apical position to the terminal copper(II) ions and in equatorial position to the central copper(II) ion. Through these connections, copper(II) centers are arranged in a zig-zag fashion in the trimer. The bridging bond lengths Cu1–Cl1 and Cu2–Cl1 are 2.315(7) Å and 2.825(8) Å, respectively, while corresponding bridging angle Cu1–Cl1–Cu2 is 101.92(2)°. The small bridging angle gives rise to a shorter Cu1–Cu2 separation (4.005 Å) and probably to the formation of a monomeric structure rather than a polymeric one as compared to the similar molecular structure repor-

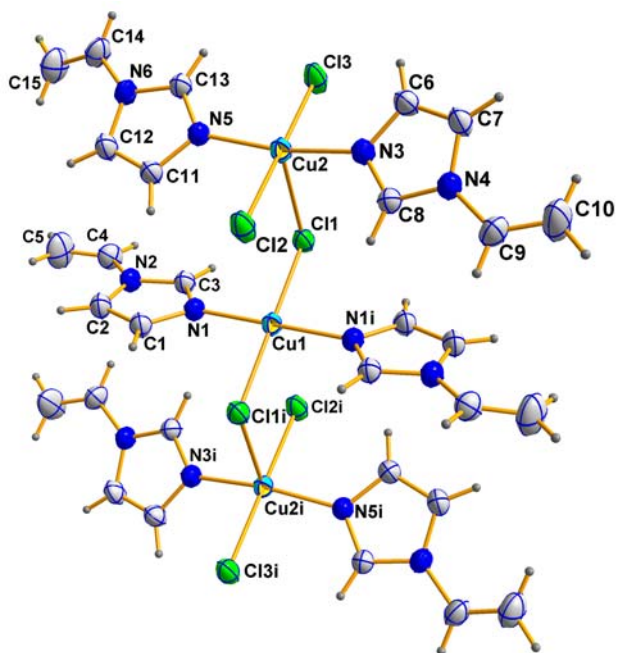


Figure 1. Molecular structure of $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$ with atom labeling scheme.

Table 1. Crystallographic data for $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$

Chemical formula	$\text{C}_{30}\text{H}_{36}\text{Cl}_6\text{N}_{12}\text{Cu}_3$
Formula weight	968.03
Temperature (K)	293(2) K
Wave length (Å)	0.71073 Å
Crystal class	Triclinic
Space group	$P - 1$
Unit cell dimensions	
a (Å)	8.7544(8)
b (Å)	11.0084(10)
c (Å)	11.8464(10)
α	62.551(6)
β	70.781(7)
γ	70.947(7)
V (Å ³)	935.65(14)
Z	1
Density calculated (mg/m ³)	1.718
Absorption coefficient (mm ⁻¹)	2.16
$F(000)$	489
Reflections collected	7572
Independent reflections	4571 [R(int) = 0.0375]
Reflections measured (>2 σ)	3724
GOF	1.056
final R indices [I>2 σ (I)]	$R1 = 0.0348, wR2 = 0.0858$ (all data) $R1 = 0.0306, wR2 = 0.0806$
Largest difference peak and hole (e Å ⁻³)	0.281 and -0.598

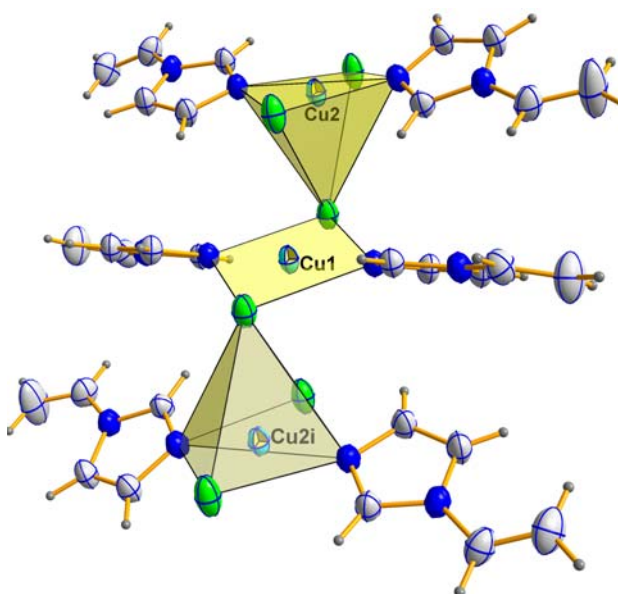


Figure 2. Two different coordination polyhedra around two different copper(II) centers

ted by Ray et al.³⁰ This type of copper(II) complexes incorporating only one bridging-chloro ligands are not common and hence are of special interest.^{31–33} None of intramolecular H-bonds were detected in the trimer. But the weak intermolecular non-classical C12–H12...Cl3($x - 1, y, -z$) interactions together with C4–H4...Cg1($x, 1 + y, z$) 3.00 Å, C8–H8...Cg2 2.81 Å and C11–H11...Cg2($1 - x,$

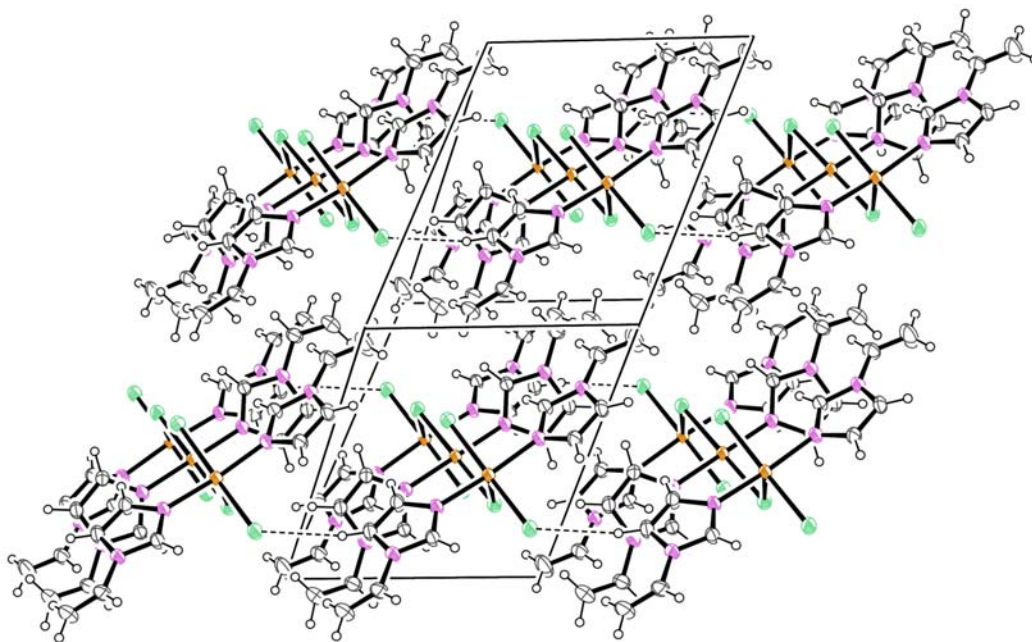


Figure 3. Crystal packing diagram of $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$ down to c -axis

$-y, 1 - z)$ 2.69 Å stacking interaction propagate the molecule along the c -axis as shown in crystal packing diagram in Figure 3.

3. 2. TOF-MS Spectroscopy

Successive MS measurements of the compound sample demonstrated almost same fragmentation products. The most abundant signal at $m/z = 991.5$ $[\text{Na} + \text{complex}]$ in TOF-MS spectrum of the complex in Figure 4 is related to sodiated adduct of the trinuclear structure since the spectrum was taken in positive ion mode. Designation of other significant peaks is not possible due to probably different stable adducts and fragments in solution medium. Nevertheless, the expected

sodium adduct of the complex is the most abundant one among them (Figure 4).

3. 3. Thermogravimetry

The thermogram of the complex comprising simultaneous TG and DTA curves is depicted in Figure 5. Endothermic DTA signal observed immediate before decomposition onset denotes to melting phenomenon at c.a 137 °C. After the completion of the melting, the ligands endothermically release between 146–262 °C and 262–932 °C. The mismatch between the observed (84.20%) and calculated (75.36% for CuO final product, and 80.33% for Cu residue) total mass losses is only attributable to additional carbon remains with either two final products.

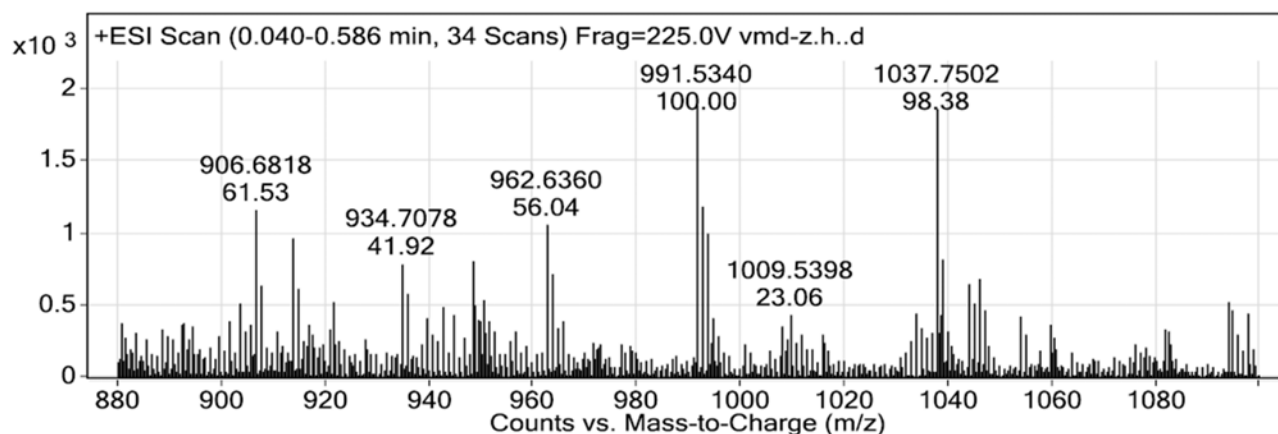


Figure 4. TOF-MS spectrum of $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$

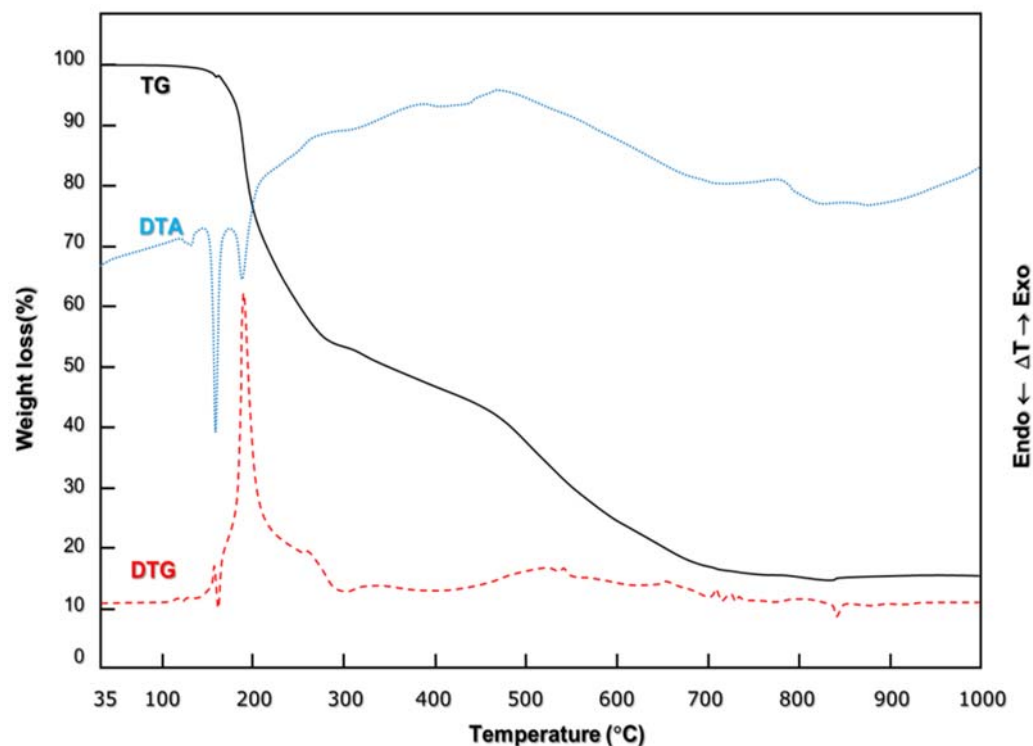


Figure 5. Simultaneous TG, DTG and DTA curves of $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$

3. 4. Geometry Optimization

The ground state geometry of the complex was optimized on spin unrestricted doublet state applying tight SCF procedure. The main geometrical parameters related

to optimized structure are listed in Table 2 together with experimental ones. Calculated MOCs from atoms and group were given in Table 3 and frontier molecular orbitals were depicted in Figure 6. The calculated SOMO (singly occupied molecular orbital (from unrestricted

Table 2. Selected experimental and calculated bond lengths and angles of $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$

Bond	Bond lengths (Å)		Angle	Bond angles (°)	
	Exp.	Calc.		Exp.	Calc.
Cu1–Cu2*	4.005	4.078	N1–Cu1–Cl1	90.14(5)	90.21
Cu1–Cl1	2.315(7)	2.445	N1 ⁱ –Cu1–Cl1	89.86(5)	89.79
Cu1–N1	1.978(16)	1.972	N1–Cu1–Cl2	88.75(5)	85.85
Cu1–Cl2	3.189(7)	3.193	N1 ⁱ –Cu1–Cl2	91.25(5)	94.15
Cu2–Cl2	2.339(7)	2.449	Cl1–Cu1–Cl2	79.35(3)	81.75
Cu2–Cl3	2.299(7)	2.405	Cl1–Cu1–Cl2 ⁱ	100.64(2)	98.25
Cu2–N3	1.993(17)	1.979	Cu1–Cl1–Cu2	101.92(2)	98.70
Cu2–N5	1.990(17)	1.987	Cu1–Cl2–Cu2	91.53(2)	91.61
Cu2–Cl1	2.825(8)	2.915	N3–Cu2–N5	166.92(7)	178.43
			N3–Cu2–Cl2	89.84(5)	89.75
			N3–Cu2–Cl3	89.41(5)	88.98
			N3–Cu2–Cl1	96.59(5)	90.19
			N5–Cu2–Cl2	90.40(5)	90.93
			N5–Cu2–Cl3	89.95(5)	90.03
			N5–Cu2–Cl1	96.48(5)	91.25
			Cl1–Cu2–Cl2	87.19(2)	87.75

ⁱ: 1 – x, –y, 1 – z

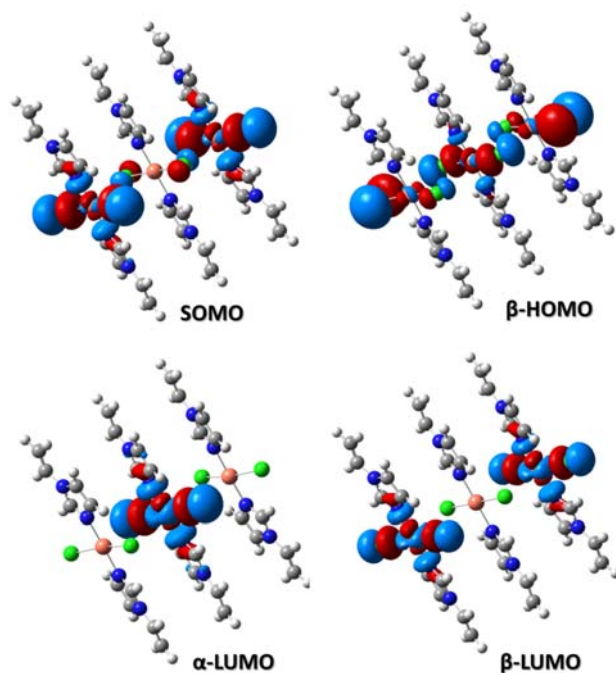
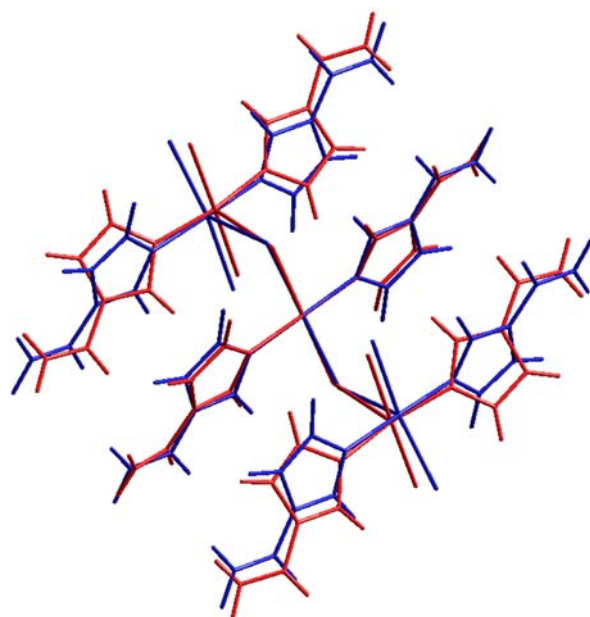
*: The Cu1...Cu2 separation being rather than a bond is given together with other important geometrical parameters for uniformity of the data.

Table 3. Calculated MOCs from atoms and groups

MO	S(α)	H		H-1		H-2		L	L+1		L+2	
		α	β	α	β	α	β	β	α	β	α	β
E (eV)	-6.03	6.070	6.038	-6.147	6.83	6.164	6.108	2.786	2.898	2.785	1.350	1.351
Cu1	0.7	0.0	3.0	2.6	0.0	0.0	6.6	0.0	54.9	0.0	0.1	0.1
Cu2	8.5	9.6	3.0	2.5	3.2	2.6	1.9	25.9	0.0	26.6	0.0	0.0
Cl1	3.8	0.6	7.2	3.8	7.8	15.7	21.5	0.2	11.0	0.1	0.1	0.1
Cl2	15.2	11.8	2.8	6.5	2.8	7.7	15.2	4.7	0.1	5.2	0.1	0.1
Cl3	11.3	12.3	31.3	31.1	32.1	19.0	3.1	5.8	0.1	6.3	0.0	0.0
1-Vim(1)	0.2	0.1	1.1	0.3	0.5	0.7	3.0	0.0	9.0	0.4	13.4	13.5
1-Vim(2)	5.0	6.3	0.9	1.5	1.2	1.7	0.4	4.6	0.3	4.7	3.2	3.1
1-Vim(3)	4.7	5.8	1.0	1.6	1.0	1.6	0.7	4.4	0.2	4.6	3.1	3.0

S, SOMO; H, HOMO; H-1, HOMO-1, etc. L, LUMO; L+1, LUMO+1, etc. the numbering of 1-Vim molecules was made by concerning preceding number of atoms involved α spin LUMO is considered as SOMO and so not given in the table again

doublet calculation))–LUMO gap is 3.132 eV. Of the distributions of MOs, β -spin LUMO and LUMO+1 are mainly localized on copper(II) ions (%51.8 for β -LUMO and %53.2 for β -LUMO+1) while α -spin LUMO is composed mainly of central copper(II) ion (%54.9). The very low contributions from metal ions to occupied MOs indirectly drawn attention to a predominant coulombic interaction between metal centers and coordinated atoms. Superimposition of experimental and gas-phase optimized structures was presented in Figure 7. In general, there is a pleasant consistence between optimized and X-ray geometries according to the results and as expected, the general tendency of gas-phase optimizations in favour of somewhat extending the bond distances was introduced.

**Figure 6.** Frontier Molecular orbitals of $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$ **Figure 7.** Superimposition of the X-ray (red) and Optimized (blue) geometries of $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$

3. 5. NBO Analysis and TD-DFT Calculation

Valence core electron populations of the molecule are presented in Table 4. The calculated charges of central metal ions are 0.66 and 0.70 respectively and lower than the oxidation state +2 and indicates the total charge donations from ligands to Cu1 and Cu2 ions equals. The nearest Cl3 atom to metal ion hold the least negative charges (−0.74) as expected. The bridging Cl1 and so-called monodentate Cl2 have almost even charges due to their similar positions between two metals. Consequently, the natural charges of the ligands and metals are comparatively expedient to their corresponding distances among each other.

Table 4. Valence core electron populations from NBO analysis

	Atom	Cu1	Cu2	Cl1	Cl2	Cl3	N1	N3	N5
	Charge	1.34	1.30	−0.75	−0.74	−0.77	−0.63	−0.63	−0.62
Population	2s	—	—	—	—	—	1.37	1.38	1.37
	3s	—	—	1.97	1.98	1.97	—	—	—
	2p	—	—	6.00	6.00	6.00	4.20	4.23	4.22
	3p(total)	6.00	6.00	5.78	5.76	5.79	—	—	—
	3dxy	1.88	1.86	—	—	—	—	—	—
	3dxz	1.98	1.98	—	—	—	—	—	—
	3dyz	1.69	1.72	—	—	—	—	—	—
	3dx ² −y ²	1.99	1.99	—	—	—	—	—	—
	3dz ²	1.74	1.73	—	—	—	—	—	—
	3d(total)	9.28	9.29	—	—	—	—	—	—
	4s	0.37	0.39	—	—	—	—	—	—

Selected non-covalent interactions and corresponding second order energies are listed in Table 5. The presence of natural bond orbitals between metal centers and coordinated atoms according to NBO results indicate also the covalency of corresponding bonds in addition to coulomb type interactions except for the longest and the weakest two Cu2–Cl1 and Cu2–Cl2 bonds incorporating none of natural bond orbitals. Therefore, no remarkable contribution to the second order energy lowering comes from these weak covalent bonds as understood from Table 5.

Table 5. Selected second orders energies from NBO analysis

Donor	Acceptor	E ⁽²⁾ (kcal/mol)
Cu1–Cl1(BD)	Cu1–N6(BD*)	0.70
Cl1(LP)	Cu1–N1i(BD*)	2.40
Cl1(LP)	Cu2(LP*)	5.66
Cu2–Cl3(BD)	Cu2–N3(BD*)	0.94
Cu2–N3(BD)	N4–C8(BD*)	3.10
C6(LP)	N4–C7(BD*)	180.48
Cl2(LP)	Cu2–Cl3(BD*)	23.38
Cu2–Cl3(BD)	Cu2–N3(BD)	0.94
Cl3(LP)	Cu2–Cl2(BD*)	3.52
Cu1–N1(BD)	C1–H1(BD*)	0.54

LP: a lone pair valence orbital, BD: 2-center bond orbital, BD*: 2-center antibond orbital, LP*: empty valence orbitals

The first 100 vertical excitations were analyzed by TD-DFT approach with B3LYP/LANL2DZ level of theory. Due to the large size of the system, the intra-ligand transitions under 298 nm were not calculated and not assigned. Only a single low intensity transition at 777 nm ($A: 0.174$) in the experimental spectrum (see Figure S2 in supporting information) is already assumed as an orbitally-forbidden d-d transition in view of electrostatic theory. From TD-DFT results, this transition can be assigned to the calculated one at 774 nm ($f = 0.0010$) or the other at 716 nm ($f = 0.0032$) both of which is qualitatively assigned

as $\pi \rightarrow \pi/d$ (β -HOMO $\rightarrow$ β -LUMO) transition by full population analysis of ground state MOs. Since none of transitions with non-zero oscillator strength in 600–800 nm region was found in TD-DFT aside from the aforementioned transitions (excitations under 298 nm not participate within 100 calculated ones and were not be able to involve because of scratch file size limitation of the program on 32 bit operating system).

4. Conclusions

Successfully prepared a novel singly-chloro-bridged trinuclear monomeric $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$ complex has been structurally characterized by X-ray crystallography. The revealed X-ray structure clearly shows that the molecule consists of two different copper(II) coordination sites, a distorted square-pyramidal terminal copper(II) ions and either distorted (Jahn-Teller elongated) octahedral or square-planar central copper(II) ion. Since copper(II) ion within significant amount of its complex compounds is mostly prone to give these coordination geometries. Interestingly, single chlorine atom in the trimer connects two copper(II) centers in the complex otherwise two chlorine atoms participate in bridging coordination in most of the other reported chlorine-bridged polynuclear species.^{34,35} The comprehensive computational studies were executed by the most efficient Hybrid-density functional method (DFT-B3LYP) in a tolerable computation time despite the large size of the molecule. The data obtained from DFT, TD-DFT and NBO analysis successfully represented the experimental trends.

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Povzetek

Pripravili smo nov trijedrni bakrov(II) kompleks $[\text{Cu}_3(\mu\text{-Cl})_2\text{Cl}_4(1\text{-Vim})_6]$ z enoveznimi 1-vinilimidazolnimi (1-Vim) in klorido ligandi in ga okarakterizirali z elementno analizo, termogravimetrijo (TGA, DTG, DTA), monokristalno rentgensko difrakcijo, TOF-MS in FT-IR spektroskopijo. Elektronske in strukturne lastnosti kompleksa smo nadalje proučevali z DFT/TD-DFT metodami. Pri izračunih smo uporabili hibridno metodo gostotnostnega funkcionala (B3LYP). Izračunane UV-Vis spektre na osnovi TD-DFT pristopa smo primerjali z eksperimentalnimi podatki. DFT izračuni so v skladu z eksperimentalnimi opažanji.

Scientific paper

Study on the Complex Equilibria of Molybdenum(VI) with 3,5-Dinitrocatechol and Ditetrazolium Salt

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Abstract

The complex formed between an anionic chelate of Mo(VI)–3,5-dinitrocatechol (3,5-DNC) with the cation of 3,3'-(3,3'-dimethoxy-4,4'-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (Blue Tetrazolium Chloride, BTC) in the liquid-liquid extraction system Mo(VI)–3,5-DNC–BTC–H₂O–CHCl₃ was studied. The optimum conditions for the complex formation and extraction of the ion-associated complex were established by spectrophotometry. The molar ratio of the reagents was determined by independent methods. The validity of Beer's law was checked and some analytical characteristics were calculated. The association process in aqueous phase and the extraction equilibria were investigated and quantitatively characterized. The following key constants of the processes were calculated: association constant, distribution constant, extraction constant and recovery factor. Based on this, a reaction scheme, a general formula and a structure of the complex were suggested.

Keywords: Molybdenum, extraction equilibria, ion-associated chelate, UV-Vis spectroscopy

1. Introduction

The molybdenum is the only second row transition metal essential from biochemical point of view. It stimulates the synthesis of nucleic acids and proteins. In the biological systems, the molybdenum forms complexes with the carboxylic or hydroxide groups of tyrosine and serine. The most important utilization of the molybdenum atom in the living organisms is as a metal hetero-atom at the active site in certain enzymes, e.g. xanthine oxidase, aldehyde oxidase, sulfite oxidase, nitrate reductase, dimethyl sulfoxide reductase.^{1–4}

The molybdenum occurs in various oxidation states, coordination numbers, geometries and its chemistry is among the most complex of the transition elements.^{1,5,6} Molybdenum(VI) forms complexes with various natural organic ligands, such as polyphenols and

their functional derivatives, polyhydroxycarboxylic acids, aminopolycarboxylic acids, hydroxamic acids, amines (primary, secondary and tertiary), 8-hydroxyquinoline and its derivatives, aldehyde hydrazones, oximes, β -diketones, fluorones, hydroxyazodyes, biomolecules (chitosan, chitin, D-glucosamine, L-alanine, L-phenylalanine).^{7–16}

Molybdenum(VI) gives colored chelates with aromatic compounds, containing two or more hydroxyl groups in *o*-position relative to each other. The colored anionic chelates of molybdenum(VI) form ion-associated complexes with bulky organic cations, like methyltriethylammonium, cetylpyridinium, cetyltrimethylammonium, tetraphenylammonium.^{7,17–20} The structure and properties of tetrazolium salts determine their ability to form ion-associated complexes.²¹ The bulky hydrophobic organic substituents in the molecules of the tetrazolium

salts increase the extractability of the ion-associated complexes. The presence of a quaternary nitrogen atom in the molecules of the tetrazolium salts determines the ability to form ionic associates in aqueous phase without protonation, as opposed to the amines. Tetrazolium salts are used as reagents for the preparation of various ion-associated complexes of metals, e.g. W(VI), Ge(IV), Tl(III), Nb(V), V(V), Ga(III), Co(II).^{22–26}

The extraction spectrophotometry is a relatively simple and inexpensive method for preparation and characterization of new complex compounds as well as for their application in the chemical analysis.^{27–31} The liquid-liquid extraction is a part of the chemistry of the solutions and the coordination compounds. It is applied to study the processes of complex formation and the extraction equilibria. This present work aims to study the extraction equilibria for complex formation between the anionic chelate of Mo(VI)–3,5-dinitrocatechol (3,5-DNC) and the cation of 3,3'-(3,3'-Dimethoxy-4,4'-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (Blue Tetrazolium Chloride, BTC) by spectrophotometry.

2. Experimental

2. 1. Reagents and Apparatus

Na₂MoO₄ · 2H₂O (Fluka AG, p.a.). An aqueous 1.04 × 10^{–2} mol L^{–1} solution was prepared.

3,5-Dinitrocatechol (3,5-DNC) (Sigma–Aldrich, p.a.). 3,5-DNC was dissolved in CHCl₃ to give a 1.0 × 10^{–3} mol L^{–1} solution.

3,3'-(3,3'-Dimethoxy-4,4'-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (Blue Tetrazolium Chloride, BTC); (Sigma–Aldrich, p.a.). An aqueous 2.0 × 10^{–3} mol L^{–1} solution was prepared.

H₂SO₄ (95–97% for analysis, Merck). A 5 mol L^{–1} solution was prepared. The concentration of H₂SO₄ was determined titrimetrically.

A Camspec M508 spectrophotometer (UK), equipped with 10 mm path length cells, was employed for reading the absorbance values.

The organic solvent, CHCl₃, was additionally distilled.

2. 2. Procedure for Establishment of the Optimum Conditions for Complex Formation

Aliquots of the solution of Mo(VI), BTC and H₂SO₄ were introduced into 250 cm³ separatory funnels. The resulting solutions were diluted with distilled water to a total volume of 10 cm³. A required volume of a chloroform solution of 3,5-DNC was added and the organic phase was adjusted to a volume of 10 cm³ with chloroform. The funnels were shaken for a fixed time (up to 180 s). A portion

of the organic extract was filtered through a filter paper into a cell and the absorbance was read against a blank. The blank extraction was performed in the same manner in the absence of molybdenum.

2. 3. Procedure for Determination of the Distribution Constant

The distribution constant (K_D) was determined by the equation (1), where A_1 and A_3 are the absorbances (measured against blanks) obtained after a single and triple extraction, respectively.

$$K_D = A_1 / (A_3 - A_1) \quad (1)$$

The single extraction and the first stage of the triple extraction were performed under the optimum conditions for complex formation (Table 1, column 1). The organic layers were transferred into 25 cm³ calibrated flasks and the flask from the single extraction was brought to volume with chloroform. The second stage of the triple extraction was performed by adding 7 cm³ of chloroform to the aqueous phase that remained after the first stage. After extraction, the obtained extract was added to that of the first stage of the triple extraction. The third stage of the triple extraction was performed in the same manner as for the second stage and the extract was added to those the first two stages. The volume of the flask was brought to the mark with chloroform. The calibrated flasks were shaken before the spectrophotometric measurements.³²

3. Results and Discussion

3. 1. Absorption Spectra, Effect of Acidity of the Aqueous Phase and Shaking Time

The colored anionic chelate of molybdenum (VI)–3,5-DNC was extracted in chloroform in the presence of the bulky hydrophobic ditetrazolium cation. The absorption spectrum of the extract of the ion-associated complex Mo(VI)–3,5-DNC–BTC in CHCl₃ is characterized by an absorption maximum in the visible range ($\lambda_{\max}$ = 405 nm, Figure 1).

The acidity of the aqueous phase has a substantial effect on the extraction equilibrium. The maximum and constant extraction of the ion-associated complex is achieved in strongly acidic solution of (0.2–1.6) mol L^{–1} H₂SO₄. The further experiments were performed with 0.4 mol L^{–1} H₂SO₄. The carried out experiment showed that the extraction equilibrium cannot be achieved within less than 30 s. The prolonged shaking does not have an impact on the absorbance. The next experiments were performed for 1 min.

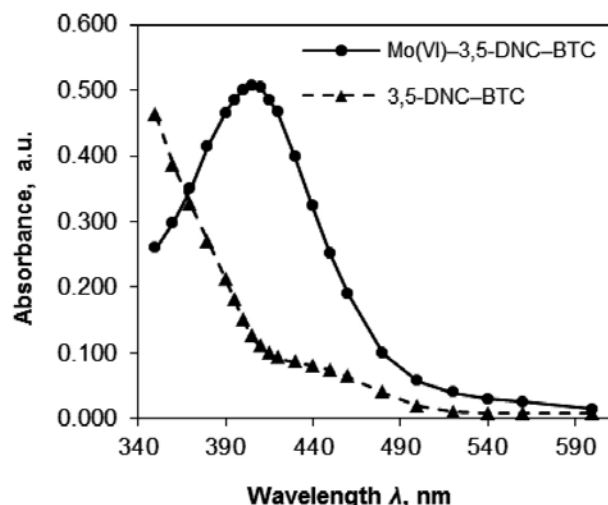


Figure 1. Absorption spectra of the complex Mo(VI)–3,5-DNC–BTC and of the blank sample 3,5-DNC–BTC in CHCl_3 . $C_{\text{Mo(VI)}} = 3.12 \times 10^{-5} \text{ mol L}^{-1}$; $C_{3,5\text{-DNC}} = 2.0 \times 10^{-4} \text{ mol L}^{-1}$; $C_{\text{BTC}} = 2.4 \times 10^{-4} \text{ mol L}^{-1}$; $C_{\text{H}_2\text{SO}_4} = 4.0 \times 10^{-1} \text{ mol L}^{-1}$; $\lambda = 405 \text{ nm}$; $\tau = 1 \text{ min}$

3. 2. Effect of Reagents' Concentrations

The reagents' concentrations are the most important factor influencing the extraction equilibria. The chelate formation of Mo(VI)–3,5-DNC requires 4.8-fold excess of 3,5-DNC ($\geq 1.5 \times 10^{-4} \text{ mol L}^{-1}$). For maximum association and extraction the amount of BTC should not be lower than 3.5-fold excess of BTC ($\geq 2.2 \times 10^{-4} \text{ mol L}^{-1}$).

The optimum extraction-spectrophotometric conditions for the chelate formation and the extraction of the ion-associated complex Mo(VI)–3,5-DNC–BTC are summarized in Table 1, column 1.

3. 3. Beer's Law, Apparent Molar Absorptivity and other Analytical Characteristics

For establishment of the range of adherence to Beer's law, i.e. the linear relationship between the molybdenum concentration in the aqueous phase ($C_{\text{Mo(VI)}}$, $\mu\text{g cm}^{-3}$) and the absorbance of the ion-association complex

in the organic phase after extraction regression analysis under the optimum conditions for complex formation was used. The equation of a straight line was found to be $Y = 0.1547 X + 0.0123$ with a correlation coefficient squared 0.9969. Under the optimum conditions for complex formation, the linearity is observed for concentrations up to $7.48 \mu\text{g cm}^{-3}$ Mo(VI). Further analytical characteristics, e.g. apparent molar absorptivity ϵ' , Sandell's sensitivity, limit of detection and limit of quantification, are shown in Table 1, column 2.

3. 4. Molar Ratios of the Complex, Reaction Scheme and Suggested General Formula

The straight-line method of Asmus, the mobile equilibrium method and the method of continuous variations were applied to prove the molar ratios Mo(VI):3,5-DNC and Mo(VI):BTC.³³ The results from the application of the independent methods are shown in Figure 2, Figure 3 and Figure 4, respectively.

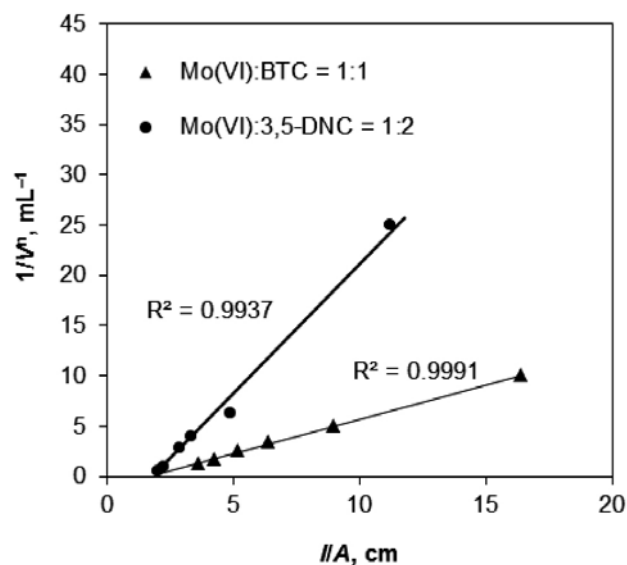


Figure 2. Determination of the molar ratio (n) by the method of Asmus. $C_{\text{Mo(VI)}} = 3.12 \times 10^{-5} \text{ mol L}^{-1}$; $C_{\text{H}_2\text{SO}_4} = 4.0 \times 10^{-1} \text{ mol L}^{-1}$; $\lambda = 405 \text{ nm}$; $\tau = 1 \text{ min}$ ● Mo(VI):3,5-DNC, $C_{\text{BTC}} = 2.4 \times 10^{-4} \text{ mol L}^{-1}$, ▲ Mo(VI):BTC, $C_{3,5\text{-DNC}} = 2.0 \times 10^{-4} \text{ mol L}^{-1}$

Table 1. Optimum extraction-spectrophotometric conditions and analytical characteristics of the system $\text{M(VI)}\text{--}3,5\text{-DNC--BTC--H}_2\text{O--CHCl}_3$

Optimum conditions	Analytical characteristic
Absorption maximum (λ_{max}) 405 nm	Apparent molar absorptivity (ϵ') (1.551 ± 0.078) $\times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$
Volume of the aqueous phase 10 cm^3	True molar absorptivity (ϵ) (1.609 ± 0.062) $\times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$
Volume of the organic phase 10 cm^3	Sandell's sensitivity (SS) 6.19 ng cm^{-2}
Concentration of H_2SO_4 in the aqueous phase $4.0 \times 10^{-1} \text{ mol L}^{-1}$	Adherence to Beer's law up to 7.48 $\mu\text{g cm}^{-3}$
Shaking time (τ) 1 min	Relative standard deviation (RSD) 2.50%
Concentration of 3,5-DNC $2.0 \times 10^{-4} \text{ mol L}^{-1}$	Limit of detection (LOD) 0.46 $\mu\text{g cm}^{-3}$
Concentration of BTC $2.4 \times 10^{-4} \text{ mol L}^{-1}$	Limit of quantification (LOQ) 1.52 $\mu\text{g cm}^{-3}$

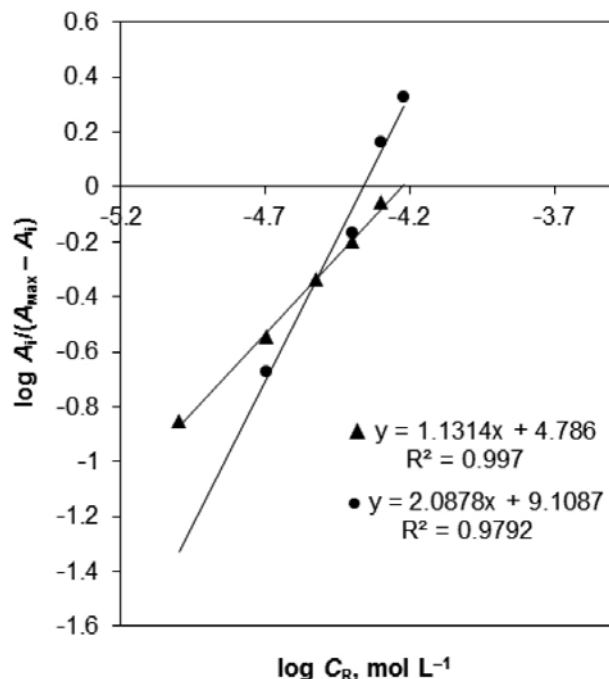


Figure 3. Straight lines by the mobile equilibrium method for determination of the molar ratios (n) Mo(VI):3,5-DTC and Mo(VI):BTC $C_{\text{Mo(VI)}} = 3.12 \times 10^{-5} \text{ mol L}^{-1}$; $C_{\text{H}_2\text{SO}_4} = 4.0 \times 10^{-1} \text{ mol L}^{-1}$; $\lambda = 405 \text{ nm}$; $\tau = 1 \text{ min}$ • Mo(VI):3,5-DNC, $C_{\text{BTC}} = 2.4 \times 10^{-4} \text{ mol L}^{-1}$; $n = 2$; ▲ Mo(VI):BTC, $C_{\text{3,5-DNC}} = 2.0 \times 10^{-4} \text{ mol L}^{-1}$; $n = 1$

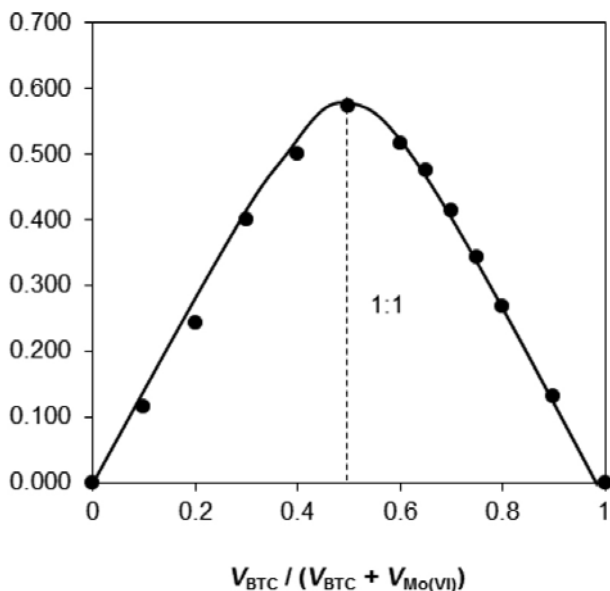
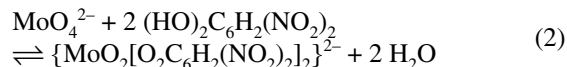


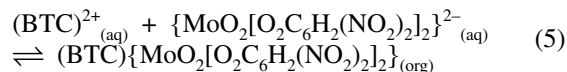
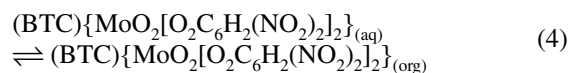
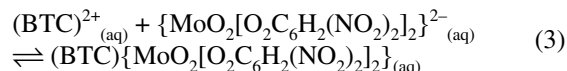
Figure 4. Determination of the molar ratio (n) Mo(VI):BTC by the method of continuous variations $C_{\text{Mo(VI)}} + C_{\text{BTC}} = 1.04 \times 10^{-4} \text{ mol L}^{-1}$; $C_{\text{3,5-DNC}} = 2.0 \times 10^{-4} \text{ mol L}^{-1}$; $C_{\text{H}_2\text{SO}_4} = 4.0 \times 10^{-1} \text{ mol L}^{-1}$; $\lambda = 405 \text{ nm}$; $\tau = 1 \text{ min}$

Based on the performed studies, it could be concluded that Mo(VI), 3,5-DNC and BTC interact in molar ratio 1:2:1. The carried out experiments showed that the

complex formation and the extraction of the ion-associated complex occurred in strongly acidic solution. Under these conditions, the complex formation of anionic chelate Mo(VI)–3,5-DNC is given by the equation (2):



Having in mind the molar ratio indicated above and the reaction of chelate formation of Mo(VI)–3,5-DNC, it can be suggested that the formation of the ion-associate in the aqueous phase, its distribution between the aqueous and the organic phase and its extraction in chloroform can be given by the following equations (3–5).



Therefore, the ion-associated chelate of Mo(VI)–3,5-DNC with TV can be represented by the general formula $(\text{BTC})\{\text{MoO}_2[\text{O}_2\text{C}_6\text{H}_2(\text{NO}_2)_2]_2\}$.

3. 5. Extraction Equilibria, True Molar Absorptivity, Recovery Factor and Structure of the Complex

The association process in aqueous phase and the extraction equilibria were investigated and quantitatively

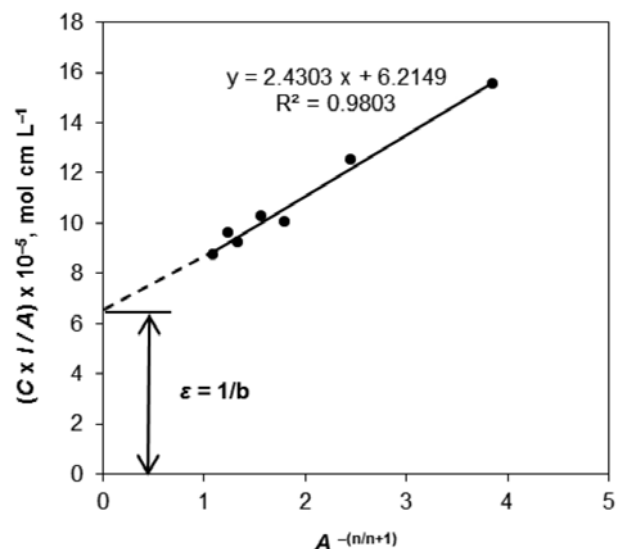


Figure 5. Dependency of $(C \times l / A)$ on $A^{-n/(n+1)}$ (method of Komar–Tolmachev) $C_{\text{Mo(VI)}} = C_{\text{BTC}} = C$, mol L^{-1} ; $C_{\text{3,5-DNC}} = 2.0 \times 10^{-4} \text{ mol L}^{-1}$; A – absorbance; l – cell thickness, $l = 1 \text{ cm}$; $n = 1$

characterized by the following key constants: association constant, distribution constant, extraction constant and recovery factor.

The association constant β was determined by two independent methods: Komar–Tolmachev method and Holme–Langmyhr method.^{33,34} The true molar absorptivity ε was determined by the method of Komar–Tolmachev (Figure 5).

The association constant β was calculated by the equation (6):³³

$$\beta = (l/n)^n / [\varepsilon (\text{tg } \alpha)^{n+1}] \quad (6)$$

where l is the cuvette thickness ($l = 1$ cm); n – the molar ratio between the components independently determined (e.g. by the mobile equilibrium method, the straight-line method of Asmus or the method of continuous variations) ($n = 1$), ε – the true molar absorptivity.

The distribution constant K_D was determined from the equation (1) and the recovery factor – from equation (7)

$$R\% = 100 K_D / (K_D + 1) \quad (7)$$

The extraction constant K_{ex} was calculated by two independent methods:

$$(i) \quad \log K_{\text{ex}} = \log K_D + \log \beta \quad (8)$$

where β was determined by the method of Komar–Tolmachev.

$$(ii) \quad \text{the method of Likussar-Boltz}^{35}$$

The method uses the data from the method of continuous variations (Figure 5). The extraction constant K_{ex} was calculated by the equation of Likussar–Boltz for molar ratio 1:1 (equation (9)):

$$\log K_{\text{ex}} = 0,3010 - \log K + \log Y_{\text{max}} - 2 \log (1 - Y_{\text{max}}) \quad (9)$$

where K is the total concentration of reagents – ($K = C_{\text{Mo(VI)}} + C_{\text{BTC}} = 1.04 \times 10^{-4} \text{ mol L}^{-1}$); Y_{max} and $(1 - Y_{\text{max}})$ are determined from the additionally plotted normalized absorption curve ($Y_{\text{max}} = 0.739$; $(1 - Y_{\text{max}}) = 0.261$).

The values of the equilibrium constants and the recovery factor are presented in Table 2. The analysis of the results obtained showed that sufficiently stable ion-associated complex was formed in the aqueous phase and it was quantitatively extracted into the organic phase with high sensitivity.

The results obtained by the independent methods are statistically dissimilar and confirm the proposed scheme of the process of complex formation of the ion-associate in the aqueous phase, its distribution between the aqueous and the organic phase and its extraction in chloroform.

Table 2. Values of the equilibrium constants and the recovery factor

Equilibrium constant and recovery factor	Value
Equilibrium (equation 3) – Association constant β	$\log \beta = (5.02 \pm 0.90)^a$
$\beta = (\text{BTC})\{\text{MoO}_2[\text{O}_2\text{C}_6\text{H}_2(\text{NO}_2)_2]_{2(\text{aq})}\} / \{[(\text{BTC})^{2+}]_{(\text{aq})}\{[\text{MoO}_2[\text{O}_2\text{C}_6\text{H}_2(\text{NO}_2)_2]_{2-}]_{(\text{aq})}\}^2\}$	$\log \beta = (4.79 \pm 0.84)^b$
Equilibrium (equation 4) – Distribution constant K_D	$\log K_D = (0.50 \pm 0.04)^c$
$K_D = \{(\text{BTC})\{\text{MoO}_2[\text{O}_2\text{C}_6\text{H}_2(\text{NO}_2)_2]_{2(\text{org})}\} / \{(\text{BTC})\{\text{MoO}_2[\text{O}_2\text{C}_6\text{H}_2(\text{NO}_2)_2]_{2(\text{aq})}\}\}$	$\log K_{\text{ex}} = (5.52 \pm 0.94)^d$
Equilibrium (equation 5) – Extraction constant K_{ex}	$\log K_{\text{ex}} = (5.32 \pm 0.01)^e$
$K_{\text{ex}} = \{(\text{BTC})\{\text{MoO}_2[\text{O}_2\text{C}_6\text{H}_2(\text{NO}_2)_2]_{2(\text{org})}\} / \{[(\text{BTC})^{2+}]_{(\text{aq})}\{[\text{MoO}_2[\text{O}_2\text{C}_6\text{H}_2(\text{NO}_2)_2]_{2-}]_{(\text{aq})}\}^2\}$	$R = (76.02 \pm 0.71)\%^f$
Recovery factor $R\%$	

^a Calculated by Komar–Tolmachev method (equation 6); ^b Calculated by Holme–Langmyhr method; ^c Calculated by equation (1); ^d Calculated by equation (8), where β is determined by the Komar–Tolmachev method; ^e Calculated by Likussar–Boltz method (equation (9)); ^f Calculated by the equation (7).

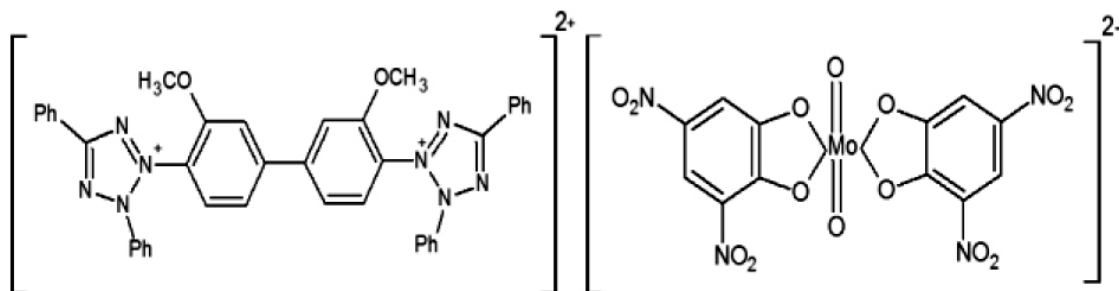


Figure 6. Structure of the complex Mo(VI)–3,5-DNC–BTC

Based on this, the proposed structure of the ion-associated complex is represented in Figure 6.

4. Conclusion

The solvent extraction of an ion-associated complex formed between the anionic chelate of Mo(VI)–3,5-Dinitrocatechol (3,5-DNC) with the cation of the ditetrazolium salt, Blue Tetrazolium Chloride (BTC) was studied by spectrophotometry. The processes of the chelate formation and extraction of the ion-associated complex Mo(VI)–3,5-DNC–BTC into chloroform were investigated. The optimum conditions for the association in aqueous phase and extraction of the ion-associated complex were established. The equilibrium constants and analytical characteristics needed for the quantitative assessment of the extraction equilibrium were calculated, i.e. the association constant (β), the distribution constant (K_D), the extraction constant (K_{ex}), the recovery factor (R), the apparent molar absorptivity (ϵ'), the true molar absorptivity (ϵ), the limit of detection (LOD), the limit of quantification (LOQ) and the Sandell's sensitivity (SS). The molar ratio of reagents determined by independent methods showed that the ion-associated chelate complex of Mo(VI)–3,5-DNC with BTC could be represented by the general formula $(BTC)\{MoO_2[O_2C_6H_2(NO_2)_2]_2\}$. A reaction scheme and a structure of the complex were suggested.

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Povzetek

Tvorba kompleksa med anionskim kelatnim kompleksom Mo(VI)–3,5-dinitrokatehol (3,5-DNC) in kationom 3,3'-(3,3'-dimetoksi-4,4'-bifenilen)bis(2,5-difenil-2*H*-tetrazolijev klorid) (Blue Tetrazolium Chloride, BTC) v tekočina–tekočina ekstrakcijskem sistemu Mo(VI)–3,5-DNC–BTC–H₂O–CHCl₃ je bila proučevana. Optimalni pogoji za tvorbo in ekstrakcijo kompleksa ionskega asociata so bili določeni spektrofotometrično. Molsko razmerje reagentov je bilo določeno z neodvisnimi metodami. Preverjena je bila veljavnost Beerovega zakona ter izračunane nekatere analzne karakteristike. Asociacijski proces v vodni fazi in ekstrakcijsko ravnotežje je bilo proučeno in kvantitativno okarakterizirano. Sledeče najpomembnejše konstante procesov so bile izračunane: asociacijska konstanta, distribucijska konstanta, ekstrakcijska konstanta in izkoristek ekstrakcije. Na podlagi dobljenih podatkov je predlagana reakcijska shema, splošna formula in struktura kompleksa.

Scientific paper

Simultaneous GC-MS Determination of Free and Bound Phenolic Acids in Slovenian Red Wines and Chemometric Characterization

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Abstract

Several phenolic acids (PAs), caffeic, vanillic, syringic, *p*-coumaric and ferulic acid, found in Slovenian red wines were studied using gas chromatography and mass spectrometry. For isolation of the PAs from wine samples, solid phase extraction using hydrophilic modified styrene – HLB cartridges was used. The bound PAs were extracted after basic hydrolysis and *o*-coumaric acid was used as the internal standard. The developed method was validated and the linear concentration range for all analytes was from 1 to 100 mg L⁻¹ with correlation coefficients above 0.999. We show that the method is repeatable (RSD<2%), recoveries were above 96%, and LOD and LOQ values were acceptable. In all of the wine samples tested, caffeic and *p*-coumaric acid were determined to be the predominant PAs (17–72 mg L⁻¹), while other compounds were found in lower concentrations. Principal Component Analysis and Cluster Analysis were used to study differences between wines related towards varieties and Slovenian wine regions. The results demonstrate that variety has more influence on PAs content than wine regions in Slovenian red wines.

Keywords: Phenolic acids, Slovenian red wines, gas chromatography, mass spectrometry, PCA, CLU

1. Introduction

Antioxidant activity of plant materials and natural products has received a great deal of interest over the past years both in the public and scientific communities.^{1–4} Generally, it is believed that consumption of plant phenolics decreases the risk of diseases related to oxidative stress.⁵

Wine, as a complex matrix containing several hundreds of different chemical compounds,^{6,7} presents an analytical challenge, especially for identification and quantification of compounds in low concentrations. The chemical composition of red wines includes minerals, vitamins, proteins, sugars and phenolic compounds, among them PAs. Red wines are considered to have more protective function than white or rosé wines, because of their higher content in antioxidant substances released from the grape skin and seeds.⁸ The total amount of polyphenols in red wines has been estimated in the range from 2000 to 6000 mg L⁻¹.⁹ Polyphenols are usually responsible for wi-

ne colour and contribute to the bitter flavour of wine.¹⁰ From the literature it is known that lactic acid bacteria (LAB) are responsible for the occurrence of malolactic fermentation (MLF), a secondary fermentation which is considered to be beneficial in most red wines.¹¹ The phenolic acids content of grapes and wines can positively or negatively affect the rate of MLF.¹² For example, gallic acid at low concentrations has stimulatory effects on the growth and malolactic activity of LAB.¹³ On the other hand, some phenolic acids, especially those from the hydroxycinnamic class, delayed the conclusion of the malolactic fermentation by these bacteria.¹⁴ Hydroxycinnamic acids (particularly *p*-coumaric acid) are also known to inhibit growth of a variety of microorganisms including wine-spoilage strains of *L. collinoides*, *L. brevis* and *L. hilgardii*.¹⁵

Although Slovenia is a small country, its wine production has a significant role in the economy. Altogether, 22,000 hectares of vineyard area is divided among three

major regions (Drava Valley-Podravje, Lower Sava Valley-Posavje and the Littoral-Primorska) with further division into sub-regions.^{16,17} The Slovenian Littoral is Slovenia's most widely known and prominent wine region of both white and red wines. Slovenian vineyards are planted with different vine varieties, including Merlot, Cabernet Sauvignon, Chardonnay, Pinot Noir, Syrah, Barbera, and many others.

PAs are present in their free forms or as glycosylated and esterified derivatives.^{18–20} Acidic, basic and enzymatic hydrolysis are the most commonly used methods for the extraction of PAs from natural materials.^{21–25} From the scientific literature it is obvious that the most commonly used techniques for the determination of PAs are high-performance liquid chromatography (HPLC) with UV or DAD detection or liquid chromatography coupled with mass spectrometry (LC-MS).^{26–29} Because of the longer sample preparation process for analysis, using gas chromatography with mass spectrometry (GC-MS) in analysis of phenolic compounds is relatively rare, but in comparison with the other methods mentioned, GC-MS offers several advantages, including complete and high-resolution separation, sensitive detection, unambiguous identification and quantitation of a wide range of phenolics (including all isomers) in one chromatographic run.^{30–32}

The aim of our study was to develop a simple and quantitative extraction method of selected PAs to ensure clean extracts in order to obtain a much more sensitive, selective and accurate GC-MS method for identification and quantitation of both free and bound PAs in red wine samples. For extraction of target compounds from the wine samples, solid-phase extraction (SPE) using hydrophilic modified styrene (HLB) cartridges was used. The bound PAs were determined after basic hydrolysis using NaOH in the presence of L-ascorbic acid and EDTA as stabilizers. The applicability of the developed method was tested on Slovenian red wines. Statistical and chemometric analyses were performed and the wines were classified.

2. Experimental

2.1. Chemicals

All reagents and solvents used were minimally of analytical purity. Standard compounds, *trans*-caffeic acid (99%), vanillic acid (97%), syringic acid (97%), *trans*-*p*-coumaric acid (98%), *trans*-*o*-coumaric acid (98%) and *trans*-ferulic acid (98%) and solvents, tetrahydrofuran-THF (99.5%) and pyridine (99.9%), were supplied by Merck (Germany). Derivatization reagent *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), HPLC-grade methanol (MeOH) and sodium hydroxide-NaOH (99%) were purchased from Sigma (USA). GC-grade toluene (99.5%) and hydrochloric acid-HCl (36.5%) were purchased from Carlo Erba (Italy). Dichloromethane-DCM was purchased from JT Baker (Germany), L-ascorbic acid (99.7%) was purchased

from Alkaloid (Macedonia) and EDTA was purchased from Kemika (Croatia). The water used was obtained from a Milli-Q water purification system.

2.2. Preparation of Standard Solutions and Calibration Curves

Standard stock solutions of caffeic acid, vanillic acid, syringic acid, *p*-coumaric acid and ferulic acid, as well as of *o*-coumaric acid (ISTD) were prepared by accurately weighing 10 mg of each into a 10 ml volumetric flask, and then dissolving in THF. Five calibration standard solutions were prepared by combining various volumes of PAs stock solutions with 50 μ l of ISTD in a 50 mL conical glass flask. Each solution was derivatized by treating it with 100 μ L of MSTFA and 50 μ L of pyridine for 1 h at 80 °C in a sand bath. After derivatization was finished, TMS derivatives were quantitatively transferred to 1 mL flasks and filled up to the mark with toluene. Five calibration standard solutions in concentration range from 1 to 100 mg L⁻¹ were injected in triplicates. The calibration curves were constructed by linear regression of the peak-area ratio of individual PA standard to the ISTD (*y*), versus the concentration (mg L⁻¹) (*x*).

2.3. GC-MS Instrumentation and Working Conditions

TMS derivatives of PAs were analyzed with a Varian 3900 gas chromatograph (GC), coupled to MS/MS Saturn 2100 ion trap mass spectrometer. GC separation was performed using a Varian capillary column VF-5ms CP8944 (30 m $\times$ 0.25 mm, with the stationary phase 0.25 μ m). 1 μ L of the sample was injected in split mode (split ratio 1:10). Carrier gas was He (6.0 UHP) at a flow rate of 1.0 mL min⁻¹. The initial oven temperature was 40 °C, held for 1 min, and then the temperature was raised to 320 °C at a rate of 10 °C min⁻¹, and finally, held for 3 min. The total run time was 32 min. The injection-port and transfer-line were set to 250 °C and 170 °C, respectively. Mass spectra were recorded in SCAN or SIM mode in a range from 50 to 650 *m/z* using electron ionization energy at 70 eV. Peak identification was done by comparing retention times (*t_R*) and spectral properties with those of standard compounds or by library matching from NIST MS library containing the mass spectra of TMS derivatives of PAs.

2.4. Validation Parameters for the GC-MS Method

The method was validated for linearity, precision as repeatability, limit of detection (LOD) and limit of quantitation (LOQ). For linearity determination, all calibration curves were constructed using the internal standard method. The curves were fitted to linear least-squares regression. The precision was evaluated through the within-day

(WD) and between-days (BD) repeatability, and expressed as relative standard deviation (RSD). The limit of detection (LOD) was calculated using the equation $(3.3 + s_y)/b_1$ and the limit of quantitation (LOQ) was calculated from the equation $(10 + s_y)/b_1$ (where s_y is standard deviation of linear regression and b_1 is slope of the calibration line).³³

2. 5. Wine Samples

The developed method was tested using Slovenian red wine samples. Twelve red wines from different Slovenian wineries and different varieties were purchased from local supermarkets. All the tested wine samples originated from four vintages (2011–2015). **Table 1** shows the varieties, wineries, year of production and percentage of alcohol. Wines were stored in a refrigerator at the temperature of +4 °C until analyzed.

2. 6. Preparation of the Wine Samples and Optimization of the Extraction Procedure

2. 6. 1. Extraction of Free PAs

A standard solution of PAs mixture (in a concentration of 1000 mg L⁻¹) was prepared in MeOH. Solutions of PAs mixture in synthetic wine (hydroalcoholic solution of 5 g L⁻¹ tartaric acid, 12% of ethanol, and pH 3.2),³⁴ were prepared by pipetting 30 and 100 µL of standard solution, respectively, in a 10 mL volumetric flask, and diluted with synthetic wine up to the mark. 1 mL of each solution was transferred into a 50 mL conical flask, spiked with 50 µL of ISTD (1000 mg L⁻¹), diluted with 1 mL of ultra-pure water and acidified with 6 M HCl to a pH value of 2. Prepared samples were added to pre-conditioned HLB Supelco-

co® SPE cartridges (3 mL, 60 mg stationary phase made from hydrophilic modified styrene). A schematic procedure of the sample extraction is shown in **Table 2**.

The free PAs fraction was eluted with 2 × 2 mL of THF. The eluate was collected and dried in a rotary evaporator (at 40 °C) to absolute dryness. Then the sample was derivatized by adding 100 µL of MSTFA and 50 µL piridine, heated at 80 °C for 1 h, diluted with toluene, and analyzed by GC-MS. The analyses were carried out in triplicate. The accuracy of the extraction process was determined through the recovery value in % of the PAs.

Table 2. Sample extraction by SPE (using HLB Supelco® cartridges).

Sample extraction by SPE	
1.	pre-washing of cartridge with 2 × 2 mL DCM
2.	column conditioning: 2 × 2 mL of MeOH and 2 × 2 mL acidified water (pH = 1–2)
3.	sample application: 2 mL of the acidified sample
4.	column washing: 2 × 2 mL ultra-pure water
5.	elution: 2 × 2 mL THF

For the determination of free PAs in selected red wines, the samples were prepared according to the same procedure. 1 mL of homogenized wine sample was spiked with 50 µL of ISTD, diluted with 1 mL of ultra-pure water and acidified with 6 M HCl to a pH value of 2, followed by the previously described steps.

2. 6. 2. Alkaline Hydrolysis of PAs

The stability of the compounds and their recovery percentage after alkaline hydrolysis was first determined

Table 1. Characteristics of the analyzed wine samples.

Sample code	Variety	Variety code	Winery	Wine region	Year of production	% alcohol*
SW1	Cabernet Sauvignon	1	„Vina Koper“	Primorska	2014	13
SW2	Modra Frankinja	2	„Stari Hram“	Posavje	2014	10.5
SW3	Cabernet-Sauvignon	1	„Vipava“	Primorska	2014	11
SW4	Modri Pinot (Pinot noir)	2	Štajerska Slovenia-Ptuj	Podravje	2011	12.5
SW5	Cabernet Sauvignon	1	Goriška Brda	Primorska	2013	12.5
SW6	Refošk	3	Srednje Škofije	Primorska	2014	11
SW7	Refošk	3	„Vina Koper“	Primorska	2014	12.5
SW8	Modra Frankinja	2	Štajerska Slovenia-Ptuj	Podravje	2011	11.5
SW9	Modri Pinot (Pinot Noir)	2	„Vipava“	Primorska	2013	12
SW10	Portugalka	3	Bela Krajina	Posavje	2015	11
SW11	Cabernet Merlot	1	Jeruzalem-Ormož	Podravje	2013	12.5
SW12	Metliška Črnina	2	Bela Krajina	Posavje	2012	11.5

* According to the declaration on the wine bottle.

with the standard compounds and later an optimized procedure was used on the real wine samples. Standard solution of PAs mixture (at a concentration of 1000 mg L⁻¹) was prepared in MeOH. Solutions of PAs mixture in synthetic wine were prepared by pipetting 30 and 100 µL of standard solution, respectively, into the 10 mL volumetric flask, and diluted with the synthetic wine up to the mark. 1 mL of each solution was transferred into a 50 mL conical flask, spiked with 50 µL of ISTD, and exposed to alkaline hydrolysis, according to the previously described method with some modifications.³⁵ 1 mL of the spiked synthetic wine was treated by adding 9 mL of 2 M NaOH (which contained 1% L-ascorbic acid and 10 mM EDTA as stabilizers) for 2 h at room temperature. Then the sample was acidified to pH 2 using 6 M HCl, and PAs were extracted with SPE HLB cartridges. The whole procedure with alkaline hydrolysis was repeated also without stabilizers.

2. 7. Quantitation of PAs

The contents of free and total PAs were determined from the corresponding calibration curves using the ISTD method, taking into account the recovery of the extraction procedure. PAs from the cinnamic group exist in *trans*- and *cis*-forms, both found in plants. *Trans*-forms of PAs are naturally predominant isomers. Therefore, for quantitative determination, the peak areas of the *trans*- and *cis*-

forms of caffeic acid, *p*-coumaric acid and ferulic acid were summed.

2. 8. Statistical Analysis

Chemometrical data analysis was carried out in order to discover any statistically or other significant differences between the samples grouped according to two categorical variables – wine variety and wine region. Microsoft Excel was used for the data preparation and result outputs. Statistical data treatment was performed using SPSS Statistics version 22.

3. Results and Discussion

Our study tested isolation and quantitative determination of five target PAs (caffeic acid, vanillic acid, syringic acid, *p*-coumaric acid and ferulic acid) in red wine samples using the GC-MS method. All GC-MS SCAN parameters for trimethylsilylated standard compounds, together with their retention times (*t_R*) and characteristic fragment ions, are listed in **Table 3**.

Linear regression analysis proved that the responses for all of the investigated compounds were linear over the tested concentration range (1–100 mg L⁻¹), and correlation coefficients (*r*²) were above 0.999. The results of the regression analysis and calibration data are shown in **Table 4**. Table 4

Table 3. Retention times and fragmentation parameters for trimethylsilylated PAs obtained after trimethylsilylation using the ion-trap mass detector.

Compound	<i>t_R</i>	Characteristic fragmentation ions <i>m/z</i> (relative intensity %)
<i>cis</i> - <i>o</i> -Coumaric acid	16.65	147(100), 293, 308
Vanillic acid	17.70	253, 267, 282, 297(100), 312
<i>cis</i> - <i>p</i> -Coumaric acid	17.94	219, 249, 293 (100), 308
<i>trans</i> - <i>o</i> -Coumaric acid	18.18	147, 219, 293 (100), 308, 381
Syringic acid	19.11	298, 312, 328, 342 (100)
<i>cis</i> -Ferulic acid	19.32	249, 293, 308, 323, 338(100)
<i>trans</i> - <i>p</i> -Coumaric acid	19.49	219, 250, 293 (100), 308, 381
<i>cis</i> -Caffeic acid	19.99	219, 381, 396 (100), 397
<i>trans</i> -Ferulic acid	20.95	249, 293, 323, 338 (100)
<i>trans</i> -Caffeic acid	21.38	73, 219, 381, 396 (100)

Table 4. Validation parameters for investigated PAs.

PA	Linear correlation	<i>r</i> ²	¹ WD-RSD	² BD-RSD	LOD*	LOQ*
Vanillic acid	y = 0.0477x + 0.0771	0.9999	0.11	0.72	0.05	0.09
Syringic acid	y = 0.0231x + 0.0921	0.9999	0.95	1.81	0.06	0.12
<i>p</i> -Coumaric acid	y = 0.0398x + 0.0555	0.9996	0.38	1.47	0.06	0.13
Caffeic acid	y = 0.0558x + 0.0986	0.9999	1.36	1.97	0.07	0.15
Ferulic acid	y = 0.0324x + 0.0718	0.9996	1.01	1.81	0.03	0.09

¹ Within-day PA/ISTD peak-area ratio repeatability of individual PAs at the concentration 10 mg L⁻¹, expressed as %RSD. ² Between-days PA/ISTD peak-area ratio repeatability of individual PAs at the concentration 10 mg L⁻¹, expressed as %RSD. * LOD and LOQ are in mg L⁻¹.

also shows the within-day (WD) and between-days (BD) repeatability expressed as relative standard deviation (RSD), and it gives RSD below 2% in all cases. The determined values of LODs and LOQs for all selected PAs are also shown in Table 4.

From the literature it is well known that anthocyanin-type pigments can cause great interference in the chromatographic separation and identification of non-anthocyanin phenolic compounds.³⁶ In our study, anthocyanins were successfully removed using HLB cartridges. Another ad-

vantage of HLB cartridges over conventional C18 columns in the separation of phenolic compounds are that more polar interferences (e.g. sugars) can be eliminated with water without losing analytes, higher sensitivity, good repeatability, reproducibility, and high percentages of recovery were reported by Perez-Magarino et al., 2008.³⁷

Accuracy of SPE in determining free PAs was evaluated by spiking a synthetic wine with the standard solution at two different concentrations levels (30 and 100 mg L⁻¹). The recovery of free PAs ranged from 93% to 114%

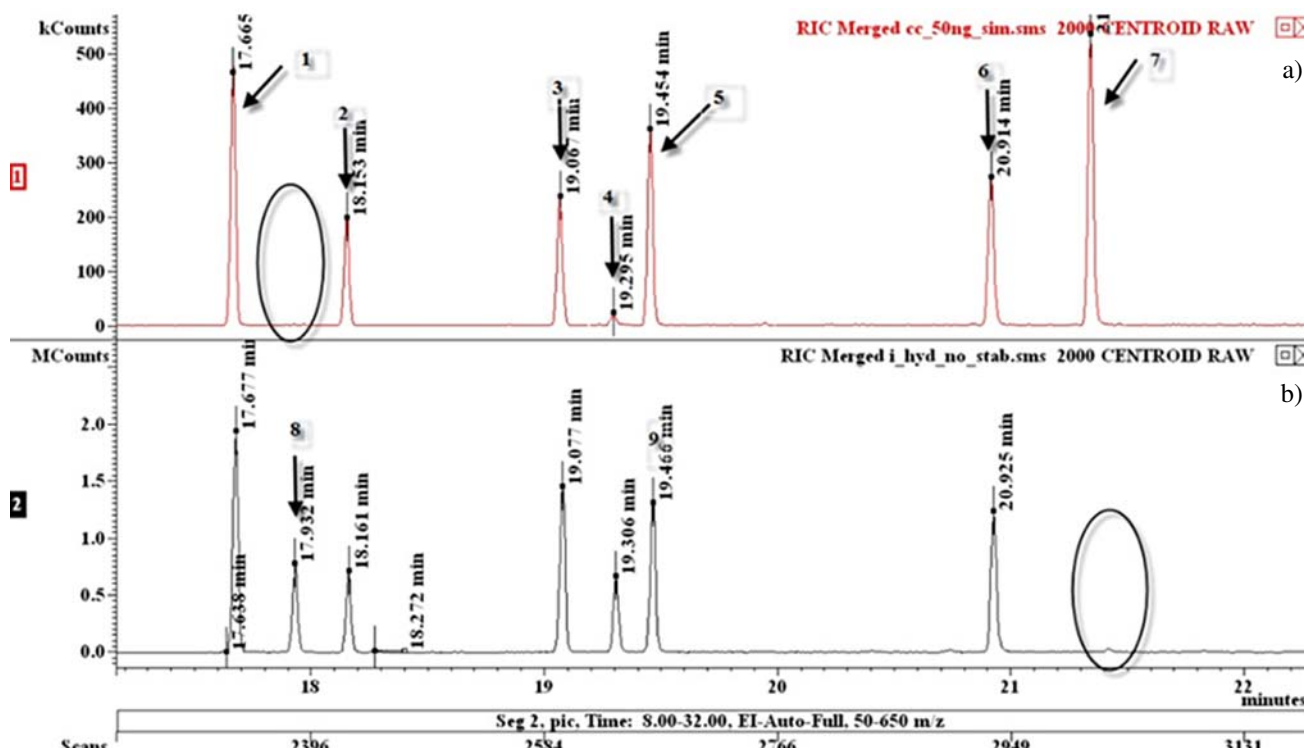


Figure 1. Chromatograms of standard solutions after; **a)** hydrolysis in presence of stabilizer (1. vanillic acid; 2. *trans-o*-coumaric acid; 3. syringic acid; 4. *cis*-ferulic acid; 5. *trans-p*-coumaric acid; 6. *trans*-ferulic acid; 7. *trans*-caffeic acid; 8. *cis-p*-coumaric acid; *cis*-caffeic acid (minimal peak at *t*R 19.99 min); **b)** hydrolysis without stabilizer (1. vanillic acid; 2. *trans-o*-coumaric acid; 3. syringic acid; 4. *cis*-ferulic acid; 5. *trans-p*-coumaric acid; 6. *trans*-ferulic acid; 7. *trans*-caffeic acid (missing peak); 8. *cis-p*-coumaric acid).

Table 5. Determination of the method accuracy expressed as recovery (%).

Recovery of extraction procedure (%)					
Phenolic acid	Free PAs		Bound PAs		
			In the presence of stabilizer	Without of a stabilizer	
	Concentration (mg L ⁻¹)				
	30	100	30	100	100
Vanillic acid	105.5	98.9	114.2	105	113.7
Syringic acid	106.8	93.6	107.5	101	105.4
<i>p</i> -Coumaric acid	101.7	106	110.5	103	135.1
Ferulic acid	94.5	96	97.4	104.4	133.7
Caffeic acid	102.7	106	105.9	96.32	NQ ^a

^a NQ-not quantified. Concentration (mg L⁻¹) <LOQ (for details see Table 4.).

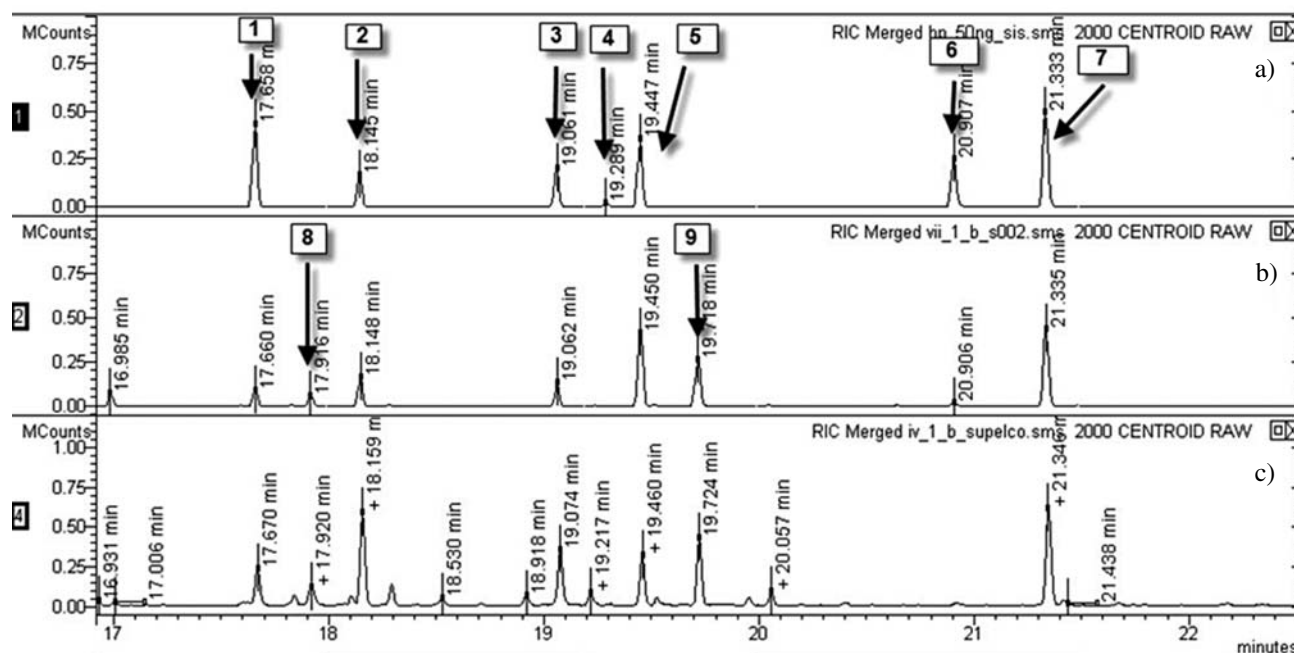


Figure 2. Typical chromatograms of selected PAs recorded in: **a)** SIM mode of standard mixture; **b)** SIM mode of red wine extract and **c)** SCAN mode of red wine extract (1. vanillic acid; 2. *o*-coumaric acid; 3. syringic acid; 4. *cis*-ferulic acid; 5. *trans*-*p*-coumaric acid; 6. *trans*-ferulic acid; 7. *trans*-caffeic acid; 8. *cis*-*p*-coumaric acid; 9. L-ascorbic acid (stabilizer)).

Table 6. Content (mg L⁻¹) of free and total PAs in Slovenian red wines.

Wine code	Form	Phenolic acid ^a				
		Vanillic acid	Syringic acid	<i>p</i> -Coumaric acid	Ferulic acid	Caffeic acid
SW1	Free	NQ ^b	3.7 ± 0.5	7.7 ± 0.2	ND ^c	3.2 ± 0.3
	Total	3.8 ± 0.4	19.8 ± 0.4	32.6 ± 0.3	ND	17.1 ± 0.3
SW2	Free	1.0 ± 0.1	3.2 ± 0.3	0.7 ± 0.1	NQ	5.4 ± 0.9
	Total	14.5 ± 2.5	29.8 ± 3.1	28.1 ± 2.2	0.1 ± 0	49.7 ± 3.2
SW3	Free	NQ	NQ	NQ	NQ	NQ
	Total	5.4 ± 1.7	14.3 ± 1.42	37.5 ± 2.1	ND	17.8 ± 0.4
SW4	Free	2.1 ± 0.2	10.7 ± 0.2	1.9 ± 0.1	ND	11.8 ± 1.2
	Total	12.6 ± 1.9	25.9 ± 0.5	31.0 ± 1.1	ND	44.5 ± 2.0
SW5	Free	0.9 ± 0.1	5.1 ± 0.1	1.6 ± 0.1	ND	4.0 ± 0.3
	Total	7.3 ± 0.6	21.9 ± 2.6	48.4 ± 0.9	ND	29.3 ± 0.5
SW6	Free	1.6 ± 0.1	4.7 ± 0.4	0.7 ± 0.1	NQ	4.8 ± 0.1
	Total	9.5 ± 1.0	25.2 ± 2.8	42.8 ± 3.6	2.5 ± 0.8	41.0 ± 0.1
SW7	Free	2.5 ± 0.4	3.4 ± 0.7	1.4 ± 0.1	NQ	3.6 ± 0.4
	Total	10.3 ± 1.4	29.9 ± 3.0	71.9 ± 0.2	4.9 ± 0.1	48.1 ± 1.1
SW8	Free	3.9 ± 0.8	4.4 ± 0.4	2.1 ± 0.2	NQ	3.5 ± 0.4
	Total	14.6 ± 1.0	20.0 ± 0.1	9.5 ± 0.7	NQ	36.9 ± 0.5
SW9	Free	5.1 ± 0.1	5.6 ± 0.2	NQ	ND	0.7 ± 0.0
	Total	10.9 ± 0.6	19.7 ± 0.3	20.9 ± 0.1	NQ	38.4 ± 0.6
SW10	Free	4.9 ± 0.3	7.7 ± 0.3	NQ	NQ	NQ
	Total	7.7 ± 0.8	29.5 ± 2.2	54.2 ± 1.2	0.7 ± 0.1	64.0 ± 0.6
SW11	Free	2.0 ± 0.1	4.6 ± 0.3	2.9 ± 0.1	ND	4.1 ± 0.0
	Total	11.8 ± 0.3	29.7 ± 2.5	63.1 ± 0.9	ND	33.7 ± 0.3
SW12	Free	6.0 ± 0.6	10.3 ± 0.1	NQ	NQ	NQ
	Total	14.6 ± 0.2	27.6 ± 1.1	47.2 ± 0.6	0.2 ± 0.01	64.4 ± 0.6

^a Each value is the mean (mg L⁻¹) of three independent replicates ± standard deviation.

^b NQ-not quantified. Concentration (mg L⁻¹)<LOQ (for details see Table 4.).

^c ND-not detected. Concentration (mg L⁻¹)<LOD (for details see Table 4.).

(Table 5). These results are in agreement with the results reported by other authors.^{38,39} The recovery of the standard compounds after alkaline hydrolysis (without or with stabilizers) extracted by SPE were also determined (Table 5). The results prove that hydrolysis without stabilizers (L-ascorbic acid and EDTA) led to a complete loss of caffeic acid³⁵ and promoted isomerization of *trans-p*-coumaric acid to its *cis*-form (Figure 1). For all other investigated compounds, the recoveries were above 96%.

The developed method was then used for the determination of selected PAs in twelve Slovenian red wine samples. Figure 2c presents a typical chromatogram of wine extract.

Contents of five different PAs present in red wine samples are shown in Table 6 (mean value $\pm$ sd). From these results it can be concluded that caffeic acid and *p*-coumaric acid are the most important of total PAs, with contents ranging from 17 to 72 mg L⁻¹. In all of the wines investigated, ferulic acid is present at the lowest concentration level. It was measured only in wines from the Posavje region and in two samples from the Primorska region. It is also worth to mention that red wine sample SW12 represents a mixture of red wine varieties Modra Frankinja, Žametovka, Portugalka and Šentlovrentka and therefore is a very specific red wine sample. This fact was confirmed by the obtained results (Table 6) as the contents of all investigated PAs in the sample SW12 were comparable with contents in the samples SW2 and SW10, both of them belonging to varieties Modra Frankinja and Portugalka, respectively. The largest proportion of PAs was present in bound form.

3. 1. Statistical Analysis

Exploratory data analysis was performed using the SPSS program. In the first step we searched for outliers but no outliers were found in the dataset. Departures from the normal distribution were demonstrated by the Q-Q plots and tested with the Kolmogorov-Smirnov test. Significance value for all tested variables was above 0.05, which indicates normal distribution of data. The Pearson correlation test (0.01 and 0.05 significance levels) was used to determine any inter-relation between two variables. Statistically significant correlations were found only between caffeic acid and syringic acid at the 0.01 level (0.744), and between caffeic acid and vanillic acid at the 0.05 level (0.592).

3. 1. 1. Principal Component Analysis (PCA)

Principal component analysis (PCA) is an unsupervised multidimensional method used for reducing the number of variables along with preserving the information contained in the data table. Projection of the wines on the first two principal components (accounting for 79.8% of the total data variability) demonstrates a clear separation

according to the wine variety (Figure 3.). The first principal component (PC1) explained 53.7% of the variation between the samples, and the second (PC2) explained 26.1% of the variation. Wines from the Cabernet-Sauvignon variety (group 1 on the biplot) were separated from the other samples, and formed a group in the positive part of PC2, while the Modri Pinot variety (group 2 on the biplot) formed a group in the negative part of PC2.

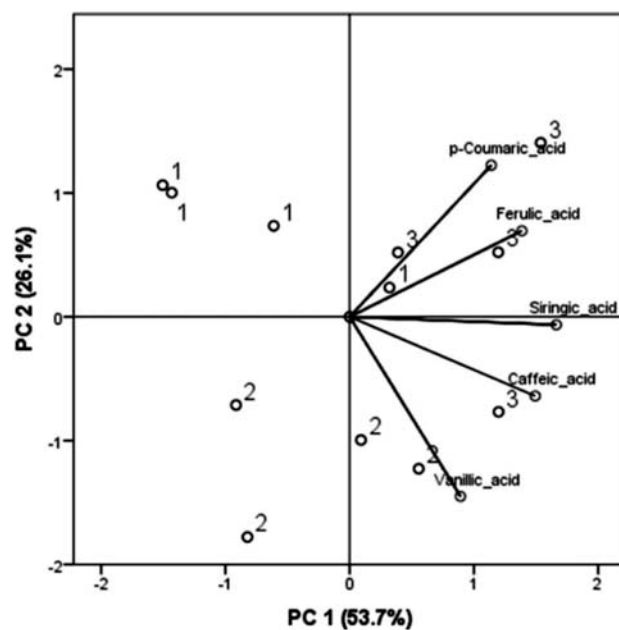


Figure 3. PCA biplot displaying the position of wine samples and descriptors in the plane PC 2 vs. PC 1 for twelve Slovenian red wines. The objects were labelled by the wine variety.

3. 1. 2. Cluster Analysis (CLU)

Cluster analysis (CLU) is one of the unsupervised multidimensional procedures that involve measuring the distances or similarities between the objects (or variables) to be clustered.⁴⁰ In the present work, agglomerative hierarchical cluster (AHC) analysis was performed in order to classify the wines tested according to variety type or wine region. Dissimilarities between the samples were determined based on the squared Euclidean distance, and the objects were clustered using Ward's method. A CLU dendrogram is presented in Figure 4. and suggests three groups of clusters. The first cluster group consisted of wine marked as SW2, SW4, SW8 and SW9. All of these wines belong to the Modri Pinot and Modra Frankinja varieties. Samples marked as SW1, SW3, SW5 and SW6 comprised the second group of wines. Three of these wine samples belong to the Cabernet-Sauvignon variety, and sample SW6 belongs to the Refošk variety. All were produced in the Littoral region. Samples marked as SW7, SW10, SW11 and SW12 comprise the third cluster. These results are in accordance with those observed using PCA,

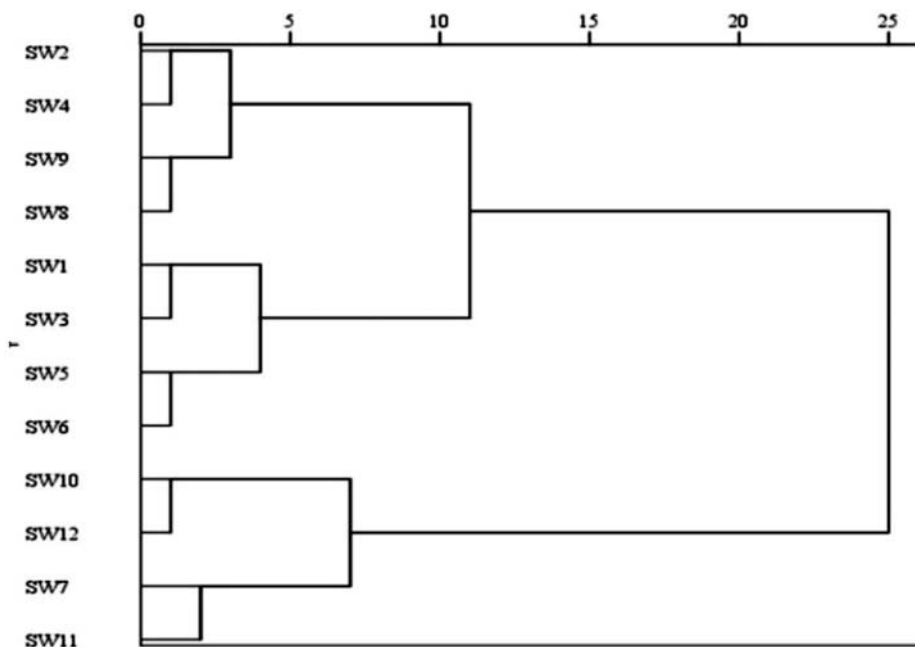


Figure 4. Dendrogram constructed with minimum linkage method for twelve Slovenian red wines.

and confirm that variety has more influence than wine regions on the content of PAs in Slovenian red wines.

4. Conclusions

A simple SPE extraction technique for the isolation of PAs from red wine samples was introduced. The SPE technique allows good separation of target compounds and almost complete removal of matrix influence. A GC-MS method for the identification and quantitative determination of five selected phenolic acids, together with their isomers, was developed. The method was validated with linearity, precision as repeatability, limit of detection and limit of quantitation. The GC-MS method proposed in this study was successfully applied to characterization of Slovenian red wines according to PAs content. From these results it can be concluded that caffeic acid and *p*-coumaric acid are the most important part of total PAs, with contents varying from 17 to 72 mg L⁻¹. The highest contents of all of the PAs investigated were found in wine samples from the Posavje wine region (SW2, SW10 and SW12). Among those wines exist the correlation in the variety. Namely, sample SW12 (Metliška črnina) is a specific wine variety consisting of Modra Frankinja (SW2), Portugalka (SW10), Žametovka and Šentlovrentka varieties. The concentrations of free and bound PAs in Slovenian red wines were between 0.03–11.8 mg L⁻¹ and 3.8–71.9 mg L⁻¹, respectively. In accordance with the data obtained using statistical analysis and chemometric methods (PCA and CLU), it can be concluded that variety has more influence on the PA content of Slove-

nian red wines, but the influence of wine region cannot be completely ignored.

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Povzetek

Izbrane fenolne kisline: kavno, vanilinsko, siringinsko, kumarinsko in ferulno smo v različnih rdečih slovenskih vinih analizirali z uporabo GC-MS. Za izolacijo smo uporabljali ekstrakcijski postopek na HLB SPE kolonicah, ki vsebuje hidrofilno modificiran stiren. Pri delu smo vezane fenolne kisline hidrolizirali, kot interni standard pa smo uporabljali *ortho*-kumarinsko kislino. Razvito metodo smo validirali: linearno koncentracijsko območje fenolnih kislin je med 1 in 100 mg L⁻¹, korelacijski koeficienti so bili nad 0,999. Potrdili smo dobro ponovljivost, RSD je znašal pod 2%, izkoristke nad 96% in sprejemljive vrednosti LOD ter LOQ. Ugotovili smo, da sta v vzorcih slovenskih vin prevladujoči fenolni spojini kavna in *para*-kumarna kislina (17–72 mg L⁻¹), medtem ko ostale spojine najdemo v nižjem koncentracijskem območju. Metodo glavnih osi (PCA) in analizo klastrov (CLU) smo uporabili za študij podobnosti in razlik med vzorci glede vsebnosti in deležev fenolnih kislin. Potrdili smo korelacijo in večji vpliv sorte grozdja kot vinorodne dežele oziroma regije, iz katere vzorci vin izvirajo.

4-Fluoro-*N'*-(2-hydroxy-3-methoxybenzylidene)benzohydrazide and its Oxidovanadium(V) Complex: Syntheses, Crystal Structures and Insulin-enhancing Activity

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Abstract

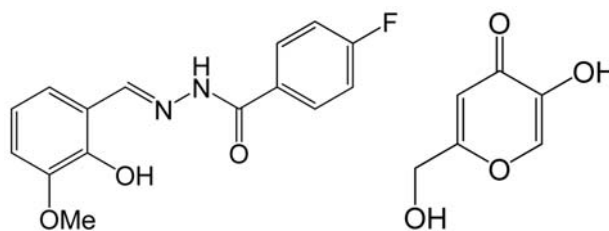
A hydrated hydrazone compound, 4-fluoro-*N'*-(2-hydroxy-3-methoxybenzylidene)benzohydrazide monohydrate ($H_2L \cdot H_2O$), was prepared and characterized by elemental analysis, HRMS, IR, UV-Vis and ^1H NMR spectroscopy. Reaction of H_2L , kojic acid (5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one; Hka) and $VO(acac)_2$ in methanol afforded a novel oxidovanadium(V) complex, $[VO(ka)L]$. The complex was characterized by elemental analysis, IR, UV-Vis and ^1H NMR spectroscopy. Thermal analysis was also performed. Structures of H_2L and the complex were further confirmed by single crystal structural X-ray diffraction. The vanadium complex is the first structurally characterized vanadium complex of kojic acid. Insulin-mimetic tests on C2C12 muscle cells indicate that the complex significantly stimulated cell glucose utilization with cytotoxicity at 0.11 g L^{-1} .

Keywords: Oxidovanadium complex, Kojic acid, Hydrazone, Crystal structure, Insulin-enhancing activity

1. Introduction

Since 1980s, inorganic vanadium salts and vanadium complexes with various ligands have been reported to possess potent pharmacological effects of insulin-mimetic activity.^{1–4} Studies indicated that vanadium compounds improve not only hyperglycemia in human subjects and animal models of type I diabetes but also glucose homeostasis in type II diabetes.^{5,6} However, the inorganic vanadium salts are considered as less active and more toxic. In order to reduce the side effects of inorganic vanadium salts, vanadium complexes with various organic ligands have received particular attention and demonstrated to be effective.^{7–9} Among the complexes, bis(maltolato)oxovanadium(IV) (BMOV),¹⁰ synthesized by simple metathesis of vanadyl sulfate trihydrate and maltol (3-hydroxy-2-methyl-4-pyrone), has important and interesting insulin-enhancing activity.^{11,12} Yet, there are some side effects of BMOV, principally diarrhea.¹³ Schiff bases play important role in biological chemistry. Several vanadium complexes derived from Schiff bases have shown to nor-

malize blood glucose level with high efficiency and low toxicity, even at low concentration.^{14,15} Schiff bases with hydrazone type are of particular interest due to their biological properties.^{16–20} In recent years, a number of vanadium complexes were prepared from tridentate hydrazones, because of their excellent coordination ability and facilitate preparation. In view of the increasing importance of vanadium complexes with hydrazone type Schiff bases, we report herein the synthesis, characterization, and insulin-enhancing activity of a novel oxidovanadium(V) complex with the hydrazone compound 4-fluoro-*N'*-(2-hy-



Scheme 1. H_2L and Hka

droxy-3-methoxybenzylidene)benzohydrazide monohydrate ($\text{H}_2\text{L} \cdot \text{H}_2\text{O}$; Scheme 1) and maltol analogous compound kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one; Hka; Scheme 1). The vanadium complex is the first structurally characterized vanadium complex of kojic acid.

2. Experimental

2.1. Materials and Measurements

Starting material, reagents and solvents were purchased from commercial suppliers and used as received. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were recorded on a Jasco FT/IR-4000 spectrometer as KBr pellets in the 4000–400 cm^{-1} region. Electronic spectra were recorded on a Lambda 10 spectrometer. Absorbance was recorded on a Bio-Tek model ELx800 96-well plate reader. HRMS data was obtained with ESI (electrospray ionization) mode. ^1H NMR data were recorded on Bruker 300 MHz spectrometer. X-ray diffraction was carried out on a Bruker SMART 1000 CCD diffractometer.

2.2. Preparation of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$

To a methanolic solution (20 mL) of 3-methoxysalicylaldehyde (0.152 g, 1.00 mmol) was added a methanolic solution (20 mL) of 4-fluorobenzohydrazide (0.154 g, 1.00 mmol) with stirring. The mixture was stirred for 10 min at room temperature and filtered. Upon keeping the filtrate in air for a few days, colorless block-shaped crystals of the compound, suitable for X-ray crystal structure determination, were formed at the bottom of the vessel on slow evaporation of the solvent. The crystals were isolated, washed with MeOH and dried in a vacuum desiccator containing anhydrous CaCl_2 . Yield: 91%. Analysis: Calcd. for $\text{C}_{15}\text{H}_{15}\text{FN}_2\text{O}_4$: C, 58.82; H, 4.94; N, 9.15%. Found: C, 58.69; H, 5.03; N, 9.06%. IR data (KBr, cm^{-1}): 3550 m, 3418 w, 3210 w, 1652 s, 1609 s, 1507 w, 1470 m, 1368 w, 1325 w, 1282 m, 1241 s, 1162 w, 1103 w, 1069 w, 965 w, 893 w, 853 m, 764 w, 730 m, 668 w, 637 w, 610 w, 502 w. UV-Vis [methanol, λ/nm ($\text{d}/\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$): 298 (21,220), 338 (5,130). HRMS (ESI): m/z calcd for $\text{C}_{15}\text{H}_{14}\text{FN}_2\text{O}_3$ [$\text{M} + 1$] $^+$ 289.0983; found: 289.0986. ^1H NMR (300 MHz, d^6 -DMSO): δ 12.11 (s, 1H, NH), 10.93 (s, 1H, OH), 8.67 (s, 1H, CH=N), 8.03 (d, 2H, ArH), 7.41 (d, 2H, ArH), 7.18 (d, 1H, ArH), 7.06 (d, 1H, ArH), 6.89 (t, 1H, ArH), 3.84 (s, 3H, CH_3).

2.3. Preparation of the Complex

A methanolic solution (30 mL) of $\text{VO}(\text{acac})_2$ (0.27 g, 1.0 mmol) was added to a methanolic solution (20 mL) of H_2L (0.288 g, 1.00 mmol) and kojic acid (0.142 g, 1.00 mmol) with stirring. The mixture was stirred at room tem-

perature for 30 min to give deep brown solution. The resulting solution was allowed to stand in air for a few days until three quarters of the solvent was evaporated. Brown block-shaped single crystals of the complex suitable for X-ray single crystal diffraction were formed at the bottom of the vessel. The crystals were isolated by filtration, washed three times with cold methanol and dried in a vacuum desiccator containing anhydrous CaCl_2 . Yield: 62%. Analysis: Calcd. for $\text{C}_{21}\text{H}_{16}\text{FN}_2\text{O}_8\text{V}$: C, 51.03; H, 3.26; N, 5.67%. Found: C, 50.86; H, 3.35; N, 5.76%. IR data (KBr, cm^{-1}): 3481 m, 1600 s, 1560 m, 1504 m, 1449 m, 1341 m, 1264 s, 1223 s, 1153 w, 1097 w, 1036 w, 972 s, 915 w, 863 m, 736 m, 650 w, 595 w, 558 w, 511 w, 434 w. UV-Vis [acetonitrile, λ/nm ($\text{d}/\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$): 215 (23,530), 280 (19,220), 354 (7,285), 460 (4,175). ^1H NMR (300 MHz, d^6 -DMSO): δ 9.22 (s, 1H, ArH), 8.65 (s, 1H, CH=N), 7.92 (d, 2H, ArH), 7.45 (d, 2H, ArH), 7.30 (d, 1H, ArH), 7.06 (d, 1H, ArH), 6.65 (m, 1H, ArH), 5.84 (s, 1H, ArH), 4.46 (s, 2H, CH_2), 3.81 (s, 3H, CH_3).

2.4. X-ray Crystallography

Diffraction intensities for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex were collected at 298(2) K using a Bruker SMART 1000 CCD area-detector diffractometer with Mo $\text{K}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and Cu $\text{K}\alpha$ radiation ($\lambda = 1.54178 \text{ \AA}$) for the complex. The crystals were mounted on the top of thin glass fibers. The collected data were re-

Table 1 Crystallographical and experimental data for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex

	$\text{H}_2\text{L} \cdot \text{H}_2\text{O}$	The complex
Formula	$\text{C}_{15}\text{H}_{15}\text{FN}_2\text{O}_4$	$\text{C}_{21}\text{H}_{16}\text{FN}_2\text{O}_8\text{V}$
Mr	306.29	494.30
Crystal size/ mm^3	$0.20 \times 0.18 \times 0.13$	$0.33 \times 0.30 \times 0.28$
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$Pbca$
$a/\text{\AA}$	4.7985(4)	8.6357(3)
$b/\text{\AA}$	13.0361(11)	14.3372(5)
$c/\text{\AA}$	22.8054(17)	32.6037(12)
$V/\text{\AA}^3$	1426.6(2)	4036.7(2)
Z	4	8
$D_c/(\text{g cm}^{-3})$	1.426	1.627
μ/mm^{-1}	0.113	4.692
$F(000)$	640	2016
Measured reflections	2533	4008
Observed reflections	2165	3262
$[I \geq 2\sigma(I)]$		
Data/restraints/parameters	2533/4/210	4008/0/300
Goodness-of-fit on F^2	1.055	1.080
R_1, wR_2 [$I \geq 2\sigma(I)$] ^a	0.0323, 0.0697	0.0485, 0.1199
R_1, wR_2 (all data) ^a	0.0441, 0.0742	0.0615, 0.1271
Large diff. peak and hole ($e \text{ \AA}^{-3}$)	0.114 and -0.127	0.809 and -0.421

^a $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$, $wR_2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$.

duced with SAINT,²¹ and multi-scan absorption correction was performed using SADABS.²² Structures of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex were solved by direct methods, and refined against F^2 by full-matrix least-squares methods using SHELXTL.²³ All non-hydrogen atoms were refined anisotropically. The amino and water hydrogen atoms in $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ were located from a difference Fourier map and refined isotropically, with N–H and O–H distances restrained to 0.86(2) and 0.82(2) Å, respectively. The remaining hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Crystallographic data for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex are summarized in Table 1.

2. 5. Cell Culture and Viable Cell Counts

The biological assay was determined according to the literature method.¹⁴ In general, C2C12 mouse skeletal muscle cells were cultured in Dulbecco modified Eagle's medium with 4 mmol L⁻¹ *L*-glutamine adjusted to contain 1.5 g L⁻¹ Na₂CO₃, 4.5 g L⁻¹ glucose, and 10% fetal bovine serum in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C. C2C12 cells were sub-cultured in log phase to 70% confluence and seeded at a density of 5000 cells per well into 96-well culture plates. To limit batch-to-batch variation, cell subcultures were limited to 10 passages. After three days culture myotube formation was induced by replacing the fetal bovine serum in the medium with 10% horse serum. All experiments were done in five days when more than 75% of the cells were differentiated morphologically. The cells were suspended in a trypan blue (0.1% w/w) phosphate buffered saline solution and the ratio of stained to non-stained cells was determined after 5 min of incubation time. Viable cell counts were performed using a hemocytometer.

2. 6. Glucose Uptake Determination

Three hours prior to glucose uptake, cells were incubated in glucose and serum-free media. On the 5th day, the medium was removed and replaced with 50 mL Dulbecco modified Eagle's medium without phenol red, which was supplemented with 8.0 mmol L⁻¹ glucose and 0.1% bovine serum albumin containing either the complex at concentration of 0.10 g L⁻¹ or the positive control, insulin, or metformin at 1.0 mmol L⁻¹. The plate was then incubated for 2 h at 37 °C and 5% CO₂. After incubation, 4.0 mL media was removed from each well and transferred to a new 96-well plate to which 196 mL deionized water was added in each well. A total of 50 mL of this diluted medium was transferred to a new 96-well plate and 50 mL of the prepared glucose assay reagent was added per well and incubated for 30 min at 37 °C. Absorbance was taken at 570 nm on a 96-well plate reader. The glucose concentration per well was calculated from a standard curve.

Glucose utilization was determined by subtracting the glucose concentration left in the medium of the relevant wells following incubation to media not exposed to cells during incubation. All assays were performed in triplicate to minimize the error.

2. 7. Cytotoxicity Assay

MTT (3-(4,5-Dimethylthiazo)-2-yl)-2,5-diphenyltetrazolium bromide) was dissolved in phosphate-buffered saline without phenol red at a concentration of 2.0 g L⁻¹. Dulbecco modified Eagle's medium in the 96-well plate was refreshed with 200 mL of fresh media followed by addition of 50 mL of MTT solution to each well. The plate was wrapped in aluminium foil to prevent light and incubated at 37 °C for 4 h, after which the media with MTT was removed and replaced with 200 mL DMSO and 25 mL Sorensen's glycine buffer. Absorbance was read at 570 nm in a plate reader.

3. Results and Discussion

3. 1. General

The hydrazone compound $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ was readily prepared by the condensation reaction of 3-methoxysalicylaldehyde with 4-fluorobenzohydrazide in methanol. Facile reaction of VO(acac)₂ with H_2L and kojic acid in methanol afforded the oxidovanadium(V) complex. Crystals of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex are stable in air at room temperature. Elemental analyses are in good agreement with the chemical formulae proposed for the compounds.

3. 2. Structure Description of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$

Fig. 1 gives perspective view of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ together with the atomic labeling system. The compound contains a hydrazone molecule and a water molecule. The hydrazone molecule adopts *E* configuration with respect to the methylenide unit, which is isostructural with the chlorosubstituted compound, 4-chloro-*N'*-(2-hydroxy-3-methoxybenzylidene)benzohydrazide.²⁴ The length of the C(7)–N(1) methylenide bond (1.286(2) Å) confirms it as a typical double bond (Table 2). The shorter length of the C(8)–N(2) bond (1.345(2) Å) and the longer length of the C(8)–O(2) bond (1.227(2) Å) for the –C(O)–NH– unit than usual, suggest the presence of conjugation effect in the molecule. The bond lengths in the compound are within normal values.^{20,25,26} The dihedral angle between the two benzene rings is 14.8(3)°. In the crystal structure of the compound, hydrazone molecules are linked by water molecules through intermolecular O–H...O and N–H...O hydrogen bonds, to form two-dimensional sheets along *ab* plane (Table 3, Fig. 2). There is no obvious $\pi \cdots \pi$ interactions along *a* axis.

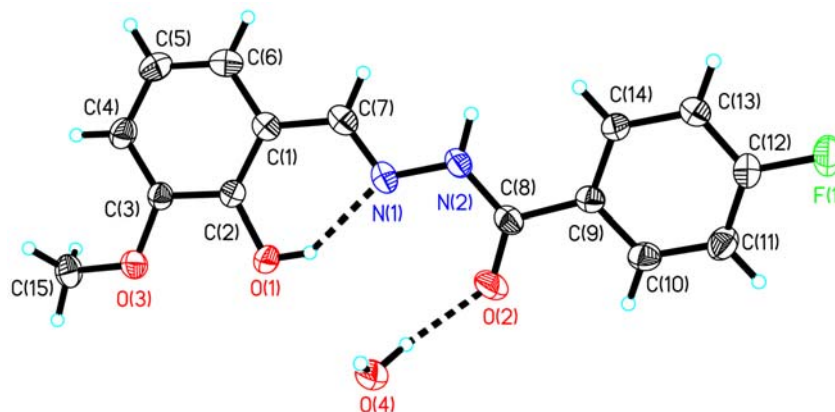


Figure 1. Molecular structure of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

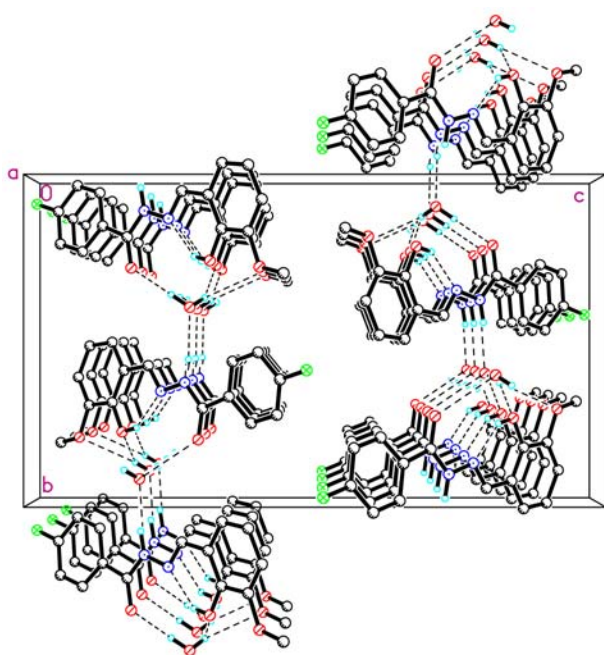


Figure 2. The crystal packing of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$, viewed along the *a* axis. Hydrogen bonds are shown as dashed lines.

3. 3. Structure Description of the Complex

Fig. 3 gives perspective view of the complex together with the atomic labeling system. The V atom in the complex is in an octahedral coordination, with the phenolate O, imino N, and enolate O atoms of the hydrazone ligand, and the deprotonated hydroxyl O atom of the kojic acid ligand defining the equatorial plane, and with one oxido O and the carbonyl O atom of the deprotonated kojic acid ligand locating at the axial positions. The V atom deviates from the least-squares plane defined by the equatorial atoms by 0.290(1) Å. The coordinative bond lengths in the complex are similar to those observed in vanadium complexes with hydrazone ligands.^{27–31} Distortion of the octahedral coordi-

nation can be observed from the coordinative bond angles (Table 2), ranging from 74.84(8) to 103.54(8)° for the perpendicular angles, and from 153.05(9) to 174.94(10)° for the diagonal angles. The dihedral angle between the two benzene rings of the hydrazone ligand is 13.1(3)°. Upon coordination, the C(7)–N(1), N(1)–N(2) and C(8)–O(2) bonds of the complex are longer than those of the free hydrazone, while the N(2)–C(8) bond of the complex is shorter than that of the free hydrazone. This is caused by the tautomerization of the carbonyl form of the hydrazone ligand to the enolate form. In the crystal structure of the complex, adjacent complex molecules are linked through intermolecular O–H...O hydrogen bonds to form an infinite chain propagating along a axis (Table 3, Fig. 4). There are short π ... π interactions along a axis (Table 4).

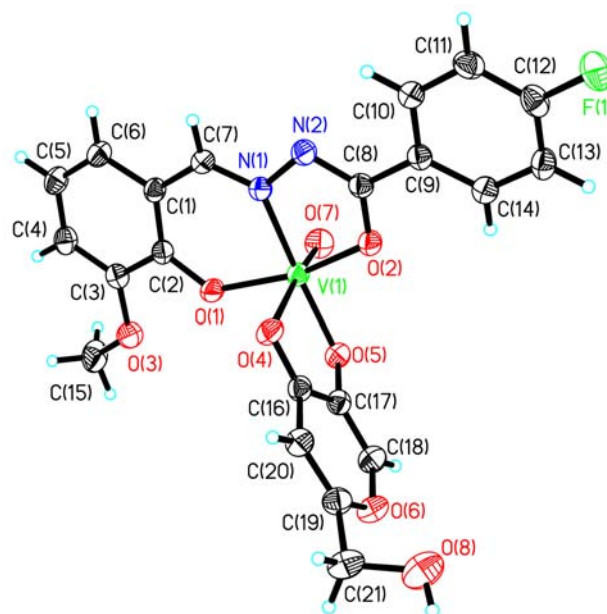


Figure 3. Molecular structure of the complex. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

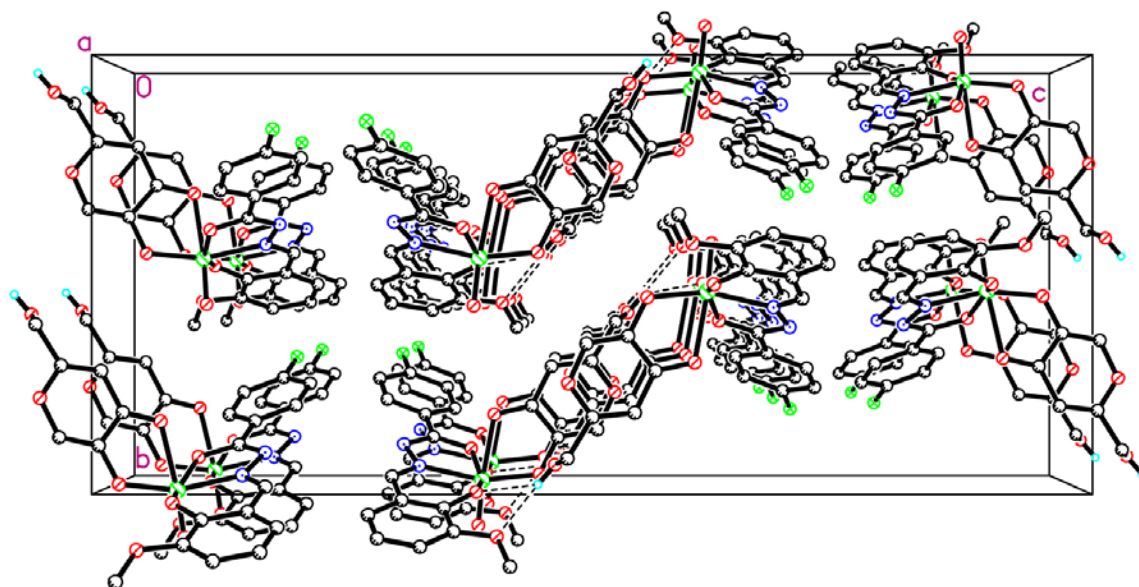


Figure 4. The crystal packing of the complex, viewed along the *a* axis. Hydrogen bonds are shown as dashed lines.

Table 2. Selected bond lengths (Å) and angles (°) for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex

$\text{H}_2\text{L} \cdot \text{H}_2\text{O}$			
C(7)–N(1)	1.285(2)	N(1)–N(2)	1.3797(19)
N(2)–C(8)	1.345(2)	C(8)–O(2)	1.227(2)
The complex			
C(7)–N(1)	1.295(3)	N(1)–N(2)	1.394(3)
N(2)–C(8)	1.294(3)	C(8)–O(2)	1.314(3)
C(16)–O(4)	1.250(3)	C(17)–O(5)	1.350(3)
V(1)–O(1)	1.845(2)	V(1)–O(2)	1.941(2)
V(1)–O(5)	1.870(2)	V(1)–O(7)	1.580(2)
V(1)–O(4)	2.282(2)	V(1)–N(1)	2.084(2)
O(7)–V(1)–O(1)	99.52(11)	O(7)–V(1)–O(5)	97.49(10)
O(1)–V(1)–O(5)	103.54(8)	O(7)–V(1)–O(2)	99.72(11)
O(1)–V(1)–O(2)	153.05(9)	O(5)–V(1)–O(2)	92.45(8)
O(7)–V(1)–N(1)	98.18(11)	O(1)–V(1)–N(1)	83.89(8)
O(5)–V(1)–N(1)	161.27(9)	O(2)–V(1)–N(1)	74.84(8)
O(7)–V(1)–O(4)	174.94(10)	O(1)–V(1)–O(4)	81.97(9)
O(5)–V(1)–O(4)	77.44(7)	O(2)–V(1)–O(4)	80.51(8)
N(1)–V(1)–O(4)	86.77(8)		

Table 3. Hydrogen bond lengths (Å) and bond angles (°) for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex

<i>D</i> –H... <i>A</i>	<i>d</i> (<i>D</i> –H)	<i>d</i> (H... <i>A</i>)	<i>d</i> (<i>D</i> ... <i>A</i>)	Angle (<i>D</i> –H... <i>A</i>)
$\text{H}_2\text{L} \cdot \text{H}_2\text{O}$				
O(1)–H(1)...N(1)	0.82	1.96	2.672(2)	145
O(4)–H(4A)...O(2)	0.83(1)	1.89(1)	2.708(2)	169(2)
N(2)–H(2)...O(4) ⁱ	0.87(1)	2.02(1)	2.882(2)	170(2)
O(4)–H(4B)...O(1) ⁱⁱ	0.82(1)	2.27(1)	3.002(2)	149(2)
O(4)–H(4B)...O(3) ⁱⁱ	0.82(1)	2.47(2)	3.148(2)	142(2)
The complex				
O(8)–H(8)...O(1) ⁱⁱⁱ	0.82	2.44	3.025(4)	130(2)
O(8)–H(8)...O(3) ⁱⁱⁱ	0.82	2.15	2.923(4)	157(2)

Symmetry codes: (i) $1 - x, -1/2 + y, 1/2 - z$; (ii) $1 + x, y, z$; (iii) $-1/2 + x, 3/2 - y, 1 - z$.

Table 4 Parameters between the planes for the complex

Cg	Cg...Cg distance (Å)	Dihedral angle (°)	Perpendicular distance of Cg(I) on Cg(J) (Å)	Perpendicular distance of Cg(J) on Cg(I) (Å)	β (°)	γ (°)
Cg(1)–Cg(2)	4.787(3)	77.26	1.559	2.979	51.52	71.00
Cg(1)–Cg(3) ^{iv}	4.684(3)	9.72	3.589	3.543	40.85	39.98
Cg(2)–Cg(2) ^v	4.549(3)	44.14	3.898	4.105	25.54	31.03
Cg(3)–Cg(4) ^{vi}	3.805(3)	13.24	3.475	3.657	16.05	24.04
Cg(4)–Cg(3) ^{vii}	4.871(3)	52.73	1.567	4.479	23.13	71.23

Symmetry codes: (iv) $1 + x, y, z$; (v) $-1/2 + x, 1/2 - y, -z$; (vi) $-1 + x, y, z$; (vii) $3/2 - x, -1/2 + y, z$.

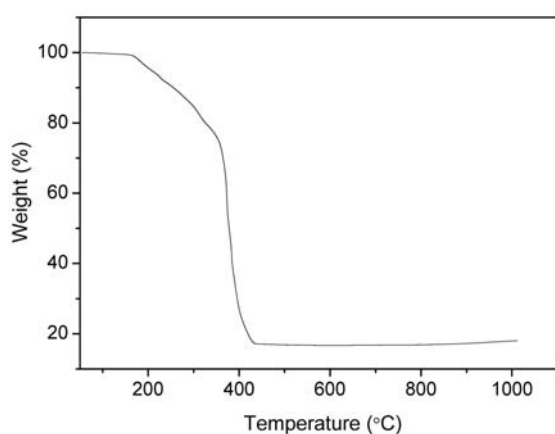
3. 4. IR and UV–Vis Spectra

The medium and broad absorption centered at 3550 and 3418 cm^{-1} in the spectrum of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and 3481 cm^{-1} in the spectrum of the complex substantiates the presence of O–H groups. The sharp band indicative of the N–H vibration of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ is located at 3210 cm^{-1} , and the intense band indicative of the C=O vibration is located at 1652 cm^{-1} in the spectrum of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$, which are absent in the complex, indicating the enolisation of the amide group and subsequent proton replacement by the V atom. The strong absorption bands at 1609 cm^{-1} for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and 1600 cm^{-1} for the complex are assigned to the azomethine $\nu(\text{C}=\text{N})$.³² The typical absorption at 972 cm^{-1} of the complex can be assigned to the V=O vibration.³³

The UV–Vis spectra of $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ and the complex were recorded in $10^{-5} \text{ mol} \cdot \text{L}^{-1}$ in methanol and acetonitrile, respectively, in the range 200–600 nm. The complex shows band centered at 354 nm and weak band at 460 nm. The weak band is attributed to intramolecular charge transfer transitions from the p_π orbital on the nitrogen and oxygen to the empty d orbitals of the metal.³⁴ The intense bands observed at 280 nm for the complex and 298 nm for $\text{H}_2\text{L} \cdot \text{H}_2\text{O}$ are assigned to intraligand $\pi-\pi^*$ transitions.³⁴

3. 5. Thermal Stability

Thermal analysis was conducted to examine the stability of the complex (Fig. 5). The complex

**Figure 5.** TG-DTA curves of the complex.

decomposed from 160 °C and completed at 430 °C, with the final product of V_2O_5 . The total observed weight loss of 82.3%, corresponding to the total organic part of the complex, is in accordance with the calculated value of 81.6%.

3. 6. Glucose Uptake in the Presence of the Complex

Glucose level is a key diagnostic parameter for many metabolic disorders. Biovision glucose assay kit provides direct measurement of glucose in various biological samples. The glucose enzyme mix specifically oxidizes glucose to generate a product, which reacts with a dye to generate color. The generated color is proportional to the glucose amount. The method is rapid, simple, sensitive, and suitable for high throughput.¹⁴ The insulin-like activity of vanadium compounds is usually related to their ability to lower the blood glucose level by activating the glucose transport into the cell of the peripheral tissues. In this study, we have investigated the *in vitro* glucose uptake by C2C12 muscle cells following exposure to the complex. The results are given in Table 5.

Insulin-mimetic test on C2C12 muscle cells indicates that the complex significantly stimulated cell glucose utilization with cytotoxicity at 0.11 g L^{-1} . In general, the insulin enhancing activity of the complex is similar to the reference drugs Insulin and Metformin. So, it is a promising vanadium-based insulin-like material.

Table 5. Glucose uptake in C2C12 cell line results^b

Compound	Percentage in glucose utilization
DMSO	100
The complex	127 ± 8
Insulin	141 ± 15
Metformin	146 ± 13

^b The results show the uptake of glucose from the culture media containing 8.0 mmol L^{-1} glucose by C2C12 cells over one h. C2C12 cells were pre-exposed to the compounds, in glucose and serum-free media for 3 h before the glucose uptake experiments. Basal glucose uptake for solvent vehicle only (DMSO) is represented as 100% and the subsequent increase or decrease induced by the compounds is reflected as $\pm 100\%$.

4. Conclusion

A new hydrazone compound, 4-fluoro-*N'*-(2-hydroxy-3-methoxybenzylidene)benzohydrazide monohydrate, and a new oxidovanadium(V) complex with the hydrazone and kojic acid as ligands were prepared and characterized. The vanadium complex is the first structurally characterized vanadium complex of kojic acid. Insulin-mimetic tests on C2C12 muscle cells indicate that the complex significantly stimulated cell glucose utilization with cytotoxicity at 0.11 g L⁻¹.

5. Supplementary Information

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre (CCDC-1477846 for H₂L · H₂O and 1477845 for the complex). Copy of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or www: <http://www.ccdc.cam.ac.uk>).

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Povzetek

Sintetizirali smo 4-fluoro-*N'*-(2-hidroksi-3-metoksibenziliden)benzohidrazid monohidrat ($H_2L \cdot H_2O$) in ga okarakterizirali z elementno analizo, HRMS, IR, UV-Vis in ^1H NMR spektroskopijo. Pri reakciji H_2L , kojične kisline (5-hidroksi-2-(hidroksimetil)-4*H*-piran-4-on; Hka) in $VO(acac)_2$ v metanolu nastane nov oksidovanadijev(V) kompleks, $[VO(ka)L]$. Kompleks smo okarakterizirali z elementno analizo, IR, UV-Vis in ^1H NMR spektroskopijo. Izvedli smo tudi termično analizo. Strukturi H_2L in kompleksa sta bili dodatno potrjeni z monokristalno rentgensko analizo. Vanadijev kompleks je prvi strukturno okarakteriziran vanadijev kompleks s kojično kislino. Inzulinomimetični test na C2C12 mišičnih celicah je pokazal, da kompleks opazno stimulira presnovo glukoze s citotoksičnostjo pri 0.11 g L^{-1} .

Scientific paper

Bioaccumulation of Polybrominated Diphenyl Ethers by *Tubifex Tubifex*

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Abstract

The selective uptake of polybrominated diphenyl ethers (PBDEs) by oligochaetes makes it possible to assess the bioaccumulation of individual congeners in commercial mixtures. Twenty-one congeners from three BDE commercial mixtures (TBDE-71, TBDE-79 and TBDE-83R) and as individual congeners (BDE-77, BDE-126, BDE-198 and BDE-204) were tested on *Tubifex tubifex* in accordance with the OECD TG 315 “Bioaccumulation in Sediment-Dwelling Benthic Oligochaetes”. All the congeners that were spiked in the sediment were detected at the end of the uptake phase and at the end of the experiment. The bioaccumulation factor (BAF), the kinetic bioaccumulation factor (BAF_K) and the biota-sediment accumulation factor (BSAF) were calculated, and indicate a high bioaccumulation potential for tri- to hexa-BDEs and a lower bioaccumulation potential for hepta- to deca-BDEs. The penta-homologues BDE-99 and BDE-100 showed the highest BSAFs of 4.84 and 5.85 (BAFs of 7.34 and 9.01), while the nona- and deca-BDEs exhibit bioaccumulation in up to one-order-lower concentrations. The change in the bioaccumulation potential between the group of tri- to hexa-BDEs and hepta- to deca-BDEs correlated with the generally accepted molecular-mass threshold for the molecular transition through biological membranes (700 g/mol).

Keywords: Bioaccumulation; polybrominated diphenyl ethers, BSAF; *Tubifex tubifex*

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants for a large number of synthetic applications, such as building materials, furnishing textiles, and electronic equipment, in order to reduce the risk of fire.¹ However, in 2003 the production and use of PBDEs was restricted in many parts of the world because of environmental problems and risks to human health.

PBDEs have a structure consisting of two benzene rings connected by an ether bond. The bromines substituted at positions 1 to 10 allow, in theory, 209 congeners.² PBDEs are categorized by their degree of bromination, where the term homologue is used to refer to a group of PBDEs with the same number of bromines (PBDEs containing five bromine atoms are, for example, referred to as

penta-BDEs). Based on the number of bromine substituents, there are 10 homologous groups of PBDEs (monobrominated through deca-brominated homologues).³ Historically, three major PBDE products have been commercially available on the global market, i.e., the penta-, octa- and deca-BDEs.⁴ The commercial products are not pure substances; rather they are mixtures of congeners. For instance, the commercial product penta-BDE is a mixture of a tri-, tetra-, penta- and hexa-BDE, whereas octa-BDE consists of hexa-, hepta-, octa- and nona-BDE. In contrast to the commercial penta- and octa-BDEs products, commercial deca-BDE is a relatively pure mixture, composed predominantly of deca-BDE.⁵ PBDEs are insoluble substances with moderate-to-high lipophilicity. Some of the physical and chemical properties of PBDEs are shown in Table 1.

Table 1. PBDE homologues: molecular mass, log octanol-water partition coefficient ($\log K_{OW}$) molecular size ($D_{max,ave}$ -maximum average diameter) and estimated enthalpy change for the phase transition of the dissolved compound from octanol to water (ΔH_{OW}).

PBDE homologues	Congener	Molecular mass (g/mol)	Log K_{OW}	$D_{max,ave}$ (nm) ^f	ΔH_{OW} (kJ/mol) ⁶
tri	BDE-28	406.89	5.94 ^{a,7} ; 5.53 ^{a,8} ; 5.48–5.58 ^{a,9}	1.45	15
tetra	BDE-47	485.79	6.81 ^{a,7} ; 6.11 ^{a,8} ; 5.87–6.16 ^{a,9}		20
	BDE-51	485.79			
	BDE-66/42	485.79			
	BDE-77	485.79		1.45	
penta	BDE-99	564.68	7.32 ^{a,7} ; 6.61 ^{a,8} ; 6.64–6.97 ^{a,9}	1.45	20
	BDE-100	564.70	6.51 ^{a,8}		
	BDE-119	564.70			
	BDE-126	564.70		1.44	
hexa	BDE-153	643.62	7.90 ^{a,7} ; 7.13 ^{a,8} ; 6.86–7.93 ^{a,9}	1.45	20
	BDE-154	643.62	7.82 ^{a,8}		
hepta	BDE-180	722.50	8.27 ^{a,7} ; 7.49 ^{b,8}	1.45	25
	BDE-183	722.50		1.45	
octa	BDE-197	801.30			25
	BDE-198	801.30			
	BDE-203	801.47	7.90 ^{b,8}		
	BDE-204	801.47		1.46	
nona	BDE-206	880.27	8.30 ^{b,8}	1.47	25
	BDE-207	880.27		1.41	
	BDE-208	880.27		1.47	
deca	BDE-209	959.17	8.70 ^{b,8} ; 9.97 ^{a,9}	1.45	25

^a measured ^b calculated ^f calculated with OASIS¹⁰

In line with the intelligent testing strategy¹¹ to make environmental risk assessments of large numbers of chemicals more efficient and to reduce the number of tests on vertebrates such as fish and amphibians, the aquatic annelids have become a frequently used test species.¹² The oligochaeta species *Tubifex tubifex* has proven to be a good model organism to replace aquatic vertebrate species such as fish when assessing the bio-accumulative properties of substances.¹³ In contrast to fish, marine polychaeta and freshwater oligochaeta present a surrogate for sediment-dwelling organisms that allows a relevant assessment of the impact of chemicals on the lower part of the food chain. These organisms are exposed to pollutants by an uptake from pore water, the ingestion of detritus, and through skin contact with contaminants bound to the sediment particles.¹⁴ Therefore, sediment-dwelling organisms represent a worst-case scenario for bioaccumulation effects.¹⁵ Experimental results indicated that the combined exposure of fish to the lipophilic chemicals in water and contaminated oligochaete *T. tubifex* as a food source leads to a significantly greater bioaccumulation than an exposure to water only.¹⁶

The purpose of the study was to generate reliable experimental data on bioaccumulation in oligochaetes for the relevant PBDE congeners in accordance with the standardized test method. The performance and test results fulfill the quality criteria for an environmental risk assessment in the legal frameworks for chemicals (Regulation (EC) No. 1907/2006; REACH). The bioaccumulation fac-

tor (BAF), kinetic bioaccumulation factor (BAF_K), and the biota-sediment accumulation factor (BSAF) were calculated based on the measured concentrations of congeners in the sediment and tested organisms.

Twenty-one BDE congeners were selected in accordance with their analytical possibilities, REACH relevance, structural representativeness and the relevance based on their toxicity and physicochemical properties. The dataset obtained in this study was then extended by predicting the bioconcentration (BCF) and biomagnification factors.¹⁸ In addition, the available data indicated that the BAF values can subsequently be used to calculate the toxicity endpoints, either using experimentally obtained critical body burdens (CBBs) for the various PBDEs, or by using QSAR approaches for predicting the CBBs¹⁹. This study was conducted as part of the CADASTER project (Case Studies on the Development and Application of In-Silico Techniques for Environmental Hazard and Risk assessment) (<http://www.cadaster.eu/>).

2. Materials and Methods

A 28-day test of bioaccumulation with *T. tubifex* was performed with selected PBDEs to determine the bioaccumulation of substances according to OECD TG 315 "Bioaccumulation in Sediment-Dwelling Benthic Oligochaetes".²⁰ The tested worms were exposed using many routes for the uptake, including direct contact, ingestion

of contaminated sediment particles, porewater and overlying water. However, the main endpoint of the test according to OECD TG 315 was the bioaccumulation factor (BAF) as the ratio between the concentration of the contaminant in the tested animal and the concentration of the contaminant in the sediment. The design of the test does not allow us to consider other routes of exposure.

2. 1. Test Compounds

Three BDE commercial mixtures (penta-DE-71, octa-DE-79, and deca-DE-83R) and four individual congeners (BDE-77, BDE-126, BDE-198, and BDE-204) were purchased from Wellington Laboratories, Canada. The supplier certified each mixture and compound with a Certificate of Analysis (CofA) and guaranteed a minimum chemical purity of 98%. A labeled internal standard solution (Catalogue number: EO-5277) and a labeled injection internal standard solution (Catalogue number: EO-5275) were purchased from Cambridge Isotope Laboratories, Andover, USA. Penta-DE-71 is a mixture of approximately 28% BDE-47, 43% BDE-99, 8% BDE-100, 6% BDE-153, and 4% BDE-154. BDE-49 and BDE-66/42 account for approximately 1%.²¹ The main constituents of the octa-DE-79 are the congeners BDE-183 (44%) and BDE-153 (14%), followed by BDE-154 (2%). The nona- and deca-analogues are present in concentrations below 1%.²² In contrast to the penta-DE-71 and octa-DE-79, the commercial mixture deca-DE-83R consists predominantly of deca-BDE (97–98%) and a smaller fraction of nona-BDE (0.3–3%).²³

The solvents dichloromethane, hexane and toluene (ENVISOLV for the analysis of dioxins, furans and PCB-s) were purchased from Sigma Aldrich, Germany. The anhydrous sodium sulphate and potassium hydroxide were purchased from J. T. Baker, Deventer, Netherlands. The silica gel (SiO₂, mesh 60) and the concentrated sulfuric acid were purchased from Sigma Aldrich, Germany.

2. 2. Preparation of Sediment

Artificial sediment was prepared as recommended by OECD TG 315.²⁰ The peat content was 2% of the dry weight so as to correspond to the average level of organic content in natural sediment. The sediment consisted of

quartz sand (obtained from Termit Domžale, Slovenia), kaolinite clay (obtained from Pika, Ljubljana, Slovenia), and finely ground sphagnum peat (from gardening stores). As a food source we added finely ground (particle size ≤ 0.5 mm) leaves of stinging nettle (*Urtica sp.*). To achieve good mixing of the constituents, de-ionized water was added (conductivity < 10 µS/cm), representing 46% of the total volume of the mixture. The percentage of the dry constituents in the artificial sediment is given in Table 2. In order to determine the dry weight, the sediment was weighed after the excess water was decanted. The sediment was dried at 105 °C for 2 hours and weighed again. The ratio of the dry weight to the wet weight for the sediment was 0.7. The total organic carbon (TOC) was measured and expressed in terms of the sediment's wet and dry weights.

The artificial sediment was spiked with a BDE mixture by coating the quartz-sand fraction. A BDE solution was prepared to provide a final total concentration of 35–70 µg per gram of wet sediment. The quartz-sand fraction of the sediment was soaked with this solution in a shallow glass vessel. After the solvent had evaporated, the quartz sand was mixed with the other constituents of the sediment. The test-substance concentrations for the whole sediment were more than 10 times lower than the LOECs for burrowing activity.²⁵ The spiked sediment was then added to the test chamber and rotated on a wheel at 4 rpm for 5 days to allow partitioning of the test substance between the sediment and the aqueous phase.

2. 3. Culture of Test Organisms

A permanent single-species culture of the tubificid oligochaetes *T. tubifex* was obtained from a fish-food supplier and was cultured over several years at 14 ± 2 °C and a light regime of approximately 250 lx for 16 hours per day. The stock culture was maintained on artificial sediment, with tap water flowing through the system. The oligochaetes were fed on aquarium cyprinid food twice a week. The cultures of the oligochaetes were kept in flat containers of 50–100 L with a height of 25 cm. The containers were loaded with a layer of wet artificial sediment to a depth of approximately 4 cm, which made possible the natural burrowing behavior of the oligochaetes. A tap-water flow of 3 L/hour formed a layer approximately 8 cm abo-

Table 2. Percentage of dry constituents for the artificial sediment²⁰

Constituent	Characteristics	weight percent of dry sediment (%)
Peat	Ground sphagnum peat	2
Quartz sand	Grain size: particles 0.05–0.200 mm	66
	Grain size: particles 0.180–0.350 mm	10
Kaolinite clay	Kaolinite content >30 %	22
Food source	Powdered leaves of stinging nettle (<i>Urtica sp.</i>)	0.4
CaCO ₃	CaCO ₃ , pulverised, chemically pure	0.1

ve the sediment. The water body was gently aerated using an aquarium air stone.

2. 4. Performance of the Test

The bioaccumulation experiments were carried out in a temperate chamber. The replicate test vessels (700 mL glass cup) were incubated at 20 ± 2 °C. Each of the test vessels contained a 2-cm layer of spiked artificial sediment (150 g), 1.2 g of oligochaetes and 500 mL of tap water. The maximum load of oligochaetes was 1–2 individuals/cm² of sediment surface. Control chambers were each loaded with uncontaminated sediment and 1.2 g of oligochaetes. For each sampling, two replicate test chambers were assembled. The animals were kept under artificial daylight for 16 hours per day.

Before the test, adult oligochaetes of the same age class (10–12 weeks) were collected from the culture by sieving the sediment through a 1-mm mesh that retained adult individuals. The animals were weighed and transferred to the pre-weighed replicate test chambers. An acclimation period of 4 days was required, since the temperature conditions of the test were different to the conditions in the culturing vessel. No reproduction was observed during the test. For the determination of the BDE concentration in the sediment, oligochaetes and water, sampling intervals were set at 0, 1, 2, 7, 14, 21 days, and on day 28, when the uptake phase was terminated. During each sampling two replicate test vessels and the two control vessels were removed from the incubation chamber. The temperature, dissolved oxygen and pH of the overlying water were measured in the test and control vessels. The controls were re-incubated, while 200 mL of overlying water, approximately 10g of wet sediment and the oligochaetes were removed from each of the replicates for analytical purposes.

The experiments to determine the time course of the elimination kinetics were conducted immediately after the uptake phase. The remaining replicate test chambers were removed from the incubation box on sampling days 29, 30, 33, 36 and 40, and processed as described above. After the rinsing, the oligochaetes were weighed and inserted into pre-weighed test chambers containing uncontaminated artificial sediment and tap water. All subsequent procedures were performed according to the methods used during the uptake phase.

Four experimental trials were conducted on the BDE commercial mixtures and on selected individual congeners. In the first and second experiments, the penta-BDE commercial mixture (DE-71) of 10 congeners and the octa-BDE commercial mixture (DE-79) of eight congeners were tested, respectively. The third experiment involved the individual congeners BDE-77 and BDE-126. The last experiment was performed on the individual congeners BDE-198 and BDE-204, together with the deca-BDE (DE-83R) commercial mixture of four congeners. No toxic response was observed during the test.

2. 5. Sample Preparation

The sediment samples were centrifuged at 2640 xG-force for 5 min at room temperature. Centrifuged sediment samples of approximately 5 g (the exact weight was recorded) were spiked with an internal standard mixture containing ¹³C₁₂-labeled isomers. A two-step, ultrasound extraction was performed, first with a mixture of toluene:acetone (10ml:30ml), and second with a mixture of toluene:acetone (30ml:10ml). The extracts were combined and dried over anhydrous sodium sulfate. A clean-up of the extract was performed on a mixed column (layers: silica gel/sulfuric acid, silica gel/KOH and silica gel). Sample extracts were concentrated prior to the GC/MS analysis to 40 µl and transferred to an auto-sampler vial.

The lipid content of the worms was determined according to the Weibull-Stoldt²⁶ method in the batch prior to and after the test. For a determination of the dry weight the oligochaetes were weighed after excess water had been removed by gently touching the animals against the edge of the holding dish. The animals were dried at 105 °C for 2 hours and weighed again.

Prior to the analysis, the oligochaetes were rinsed with tap water in a petri dish to remove the sediment particles. Then they were weighed and stored at minus 20 ± 2 °C for at least one night. Next day, 400 pg of an internal standard mixture containing ¹³C₁₂-labeled isomers was added to the oligochaetes (approx. 1 g) in a test tube and mixed with anhydrous sodium sulfate (3 g). A two-step, ultrasonic extraction was performed with dichloromethane (2 × 10 ml). The extracts were combined and dried over anhydrous sodium sulfate. A clean up of the extract was performed on a mixed column (layers: silica gel/sulfuric acid, silica gel/KOH and silica gel). Sample extracts were concentrated prior to the GC/MS analysis to 40 µl and transferred to an auto-sampler vial.

Water samples (200 ml) were spiked with an internal standard mixture containing ¹³C₁₂-labeled isomers. A two-step, liquid-liquid extraction was performed with dichloromethane (2 × 40 ml). The extracts were combined and dried over anhydrous sodium sulfate. A clean up of the extracts was performed on a mixed column (layers: silica gel/sulfuric acid, silica gel/KOH and silica gel). The sample extracts were concentrated prior to the GC/MS analysis to 40 µl and transferred to an auto-sampler vial.

2. 6. Analytical Method

The concentrations of the BDEs in the sediment, *T. tubifex* and water were determined by high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS).

The GC-HRMS was performed with a HP 6890 GC (Hewlett-Packard, Palo Alto, CA, USA) coupled to a Finnigan MAT 95XP (Finnigan, Bremen, Germany) high-resolution mass spectrometer. The GC separation was performed

on a Zebron ZB-5HT INFERNO column (Phenomenex), 15 m × 0.25 mm I.D. with a film thickness of 0.10 µm.

An aliquot (2 µL) of sample extract was injected into the GC system in pulsed splitless mode at 250 °C. The mass spectrometer operated in the electron impact ionization mode using selected ion monitoring (SIM) at a minimum resolution of 8000. The samples were analyzed for the BDE concentrations using the isotope-dilution or internal-standard method based on the US EPA 1614 protocol. In addition to daily sensitivity and relative response factor (RRF) checks, the mean RRF was regularly re-evaluated for each congener.

2. 7. Determination of BAF

In the four series of tests the BAF was calculated as the ratio of the concentration of the test substance in the test organism, C_{org} , and the sediment, C_{sed} , at steady state (Equation 1).

$$BAF = \frac{C_{org}}{C_{sed}} \quad (1)$$

To describe the time course of the uptake of the BDEs with a one-compartment model, equation 2 was used:

$$C_{org} = \frac{k_{up}}{k_{el}} * C_{sed} (1 - e^{-k_{el} * t}) \quad (2)$$

where k_{up} is the uptake-rate constant in the tissue (g sediment * kg⁻¹ organism * day⁻¹) and k_{el} is the elimination-rate constant (d⁻¹) at time point (t) of the uptake phase.

When a steady state is achieved during the uptake phase, equation 2 can be simplified (Equation 3):

$$C_{org} = \frac{k_{up}}{k_{el}} * C_{sed} \quad (3)$$

When equilibrium is not achieved during 28 days, the bioaccumulation factor can also be calculated as the ratio of the uptake- and elimination-rate constants assuming first-order kinetics (Equation 4).

$$BAF_K = \frac{k_{up}}{k_{el}} \quad (4)$$

For an interspecies comparison of bioaccumulation, the Biota-Sediment Accumulation Factor (BSAF) as a normalized version of the BAF should be used^{27,28}. The BSAF is the lipid-normalized concentration of a test substance in the test organism divided by the organic-carbon-normalized concentration of the substance in the sediment at steady state, calculated as follows (Equation 5):

$$BSAF = BAF * \frac{f_{oc}}{f_{lip}} \quad (5)$$

When the test oligochaetes are transferred from a contaminated sediment-water system to a system free of the test substance, the accumulated substance can be eliminated from the animal's body. If data points plotted against time indicate a constant exponential decline of the test substance's concentration in the animals, a one-compartment model can be used to describe the time course of the elimination (Equation 6):

$$C_{org}(t) = C_{org} * e^{-k_{el} * t} \quad (6)$$

$C_{org}(t)$ is the average concentration in the oligochaetes on day t of the elimination phase and C_{org} is the average concentration in the oligochaetes at steady state on day 28 of the uptake phase.

The equations presented here are taken from OECD TG 315.²⁰ GraphPad Prism 5.04 was used to calculate the BAF and BAF_K values.

3. Results

No mortality among the tested oligochaetes, as well as no deviation in burrowing activity in comparison to the control, was observed in the four series of tests. Thus, the validity criteria according to the OECD TG 315²⁰ were met. The relative standard deviation (RSD) for the BAF in the test replicates was generally below 30%, which is acceptable for this type of study. The bioaccumulation kinetics during 28 days of uptake and 12 days of elimination of the BDEs was plotted against the time course. The congeners BDE-153, BDE-154, and BDE-183 were present in different concentrations in both the penta-DE-71 and octa-DE-79 mixtures. Similarly, the deca-DE-83R and the octa-DE-79 commercial mixtures contained the congeners BDE-207 and BDE-209. The uptake of the congeners BDE 153 and BDE 154, and congeners BDE 197 to BDE 209 did not reach a steady-state plateau within the exposure period of 28 days. As the absence of a steady state apparently leads to an underestimation of the bioaccumulation potential of the substances,²⁹ the kinetic bioaccumulation factor (BAF_K) was calculated.

The elimination-rate constant (k_{el}) in the uptake phase proved to be a reliable indicator of a steady-state plateau in the uptake phase. When the k_{el} was higher, equal, or approaching a value of 0.1, the steady-state plateau was achieved in the timeframe of the test and the calculation of the BAF was a reliable estimation of the bioaccumulation. A significantly lower k_{el} than the value of 0.1 indicated that the theoretical steady-state plateau would be reached way beyond the time frame of the test. In this case, the BAF_K was more reliable for an estimation of the bioaccumulation potential.

When the elimination-rate constant (k_{el}) was approaching 1, the term $e^{-k_{el} * t}$ was approaching 0 and, con-

sequently, the BAF become similar or equal to the BAF_K . The BAF was lower than the kinetic value when k_{el} was approaching 0 and the term $e^{-k_{el} \cdot t}$ was approaching 1. Within the timeframe of the test the steady-state plateau was not reached for the hexa, nona, and deca congeners. Three characteristic graphs of the uptake and elimination of the BDE congeners in relation to the k_{el} are shown in Figure 1. In the time course of the bioaccumulation of BDE-51 and BDE-100 (test 1; technical mixture DE-71) the k_{el} was ≥ 0.1 and the steady-state plateaus were reached for both congeners. The BDE-203 (test 2; technical mixture DE-79) shows an example when the k_{el} is < 0.1 , and the steady state could be expected to be way beyond the timeframe of the test. The mean values of the concentrations measured for the oligochaetes in each of the two replicate test chambers were plotted, with error bars representing the corresponding standard deviation (SD).

The measured lipid content of the tested animals was 1.9 ± 0.2 mg/kg and did not vary significantly during the test, nor between the batches from different test series. The dry-to-wet weight ratio for the tested *T. tubifex* was 0.131. The total organic carbon (TOC) content was 1.25 ± 0.1 mg for the wet weight and 1.73 ± 0.06 mg/kg for the sediment dry weight. The biota-sediment accumulation factor (BSAF) calculation for the congeners that reached the steady-state bioaccumulation plateau was based on the BAF. When the elimination constant rate (k_{el}) was lower than 0.1 the BSAF was calculated based on the BAF_K . Results for the bioaccumulation of the tested BDEs based on the wet weight of sediment and the tested animals are given in Table 3.

According to OECD TG 315, the test is terminated when either 10% of the concentration measured in the oligochaetes on day 28 of the uptake phase is reached, or after a maximum duration of 10 days of depuration. Exceed-

Table 3. The concentration of the BDE in *T. tubifex* and in the spiked sediment on day 28 and the bioaccumulation factor BAF, kinetic bioaccumulation factor BAF_K and sediment-biota normalized accumulation factor (BSAF) presented with a standard deviation.

Commercial mixture/ Congeners	<i>T. tubifex</i> at day 28-uptake	Sediment at day 28-uptake	Bioaccumulation		
	C_{org} (µg/kg ww)	C_{sed} (µg/kg ww)	BAF	BAF_K	BSAF
DE-71					
BDE-28	1.20 ± 0.15	0.18 ± 0.03	6.57 ± 0.36	6.58 ± 0.39	4.34 ± 0.60
BDE-47	77.00 ± 9.22	11.60 ± 0.99	6.64 ± 1.05	5.55 ± 0.54	4.38 ± 0.55
BDE-51	0.93 ± 0.25	0.13 ± 0.10	6.91 ± 0.28	7.84 ± 0.49	4.56 ± 1.92
BDE-66/42	4.11 ± 0.61	0.59 ± 0.11	6.98 ± 0.45	7.73 ± 0.76	4.61 ± 0.79
BDE-99	87.94 ± 0.48	11.98 ± 2.02	7.34 ± 0.60	5.34 ± 0.03	4.84 ± 0.37
BDE-100	46.01 ± 8.56	5.11 ± 1.06	9.01 ± 2.53	10.47 ± 0.20	5.95 ± 1.24
BDE-119	0.38 ± 0.04	0.06 ± 0.02	6.54 ± 0.44	9.16 ± 0.11	4.31 ± 0.82
BDE-153	9.73 ± 1.18	2.56 ± 0.63	3.80 ± 0.62	4.64 ± 0.58	3.06 ± 0.38^a
BDE-154	13.85 ± 2.24	2.43 ± 0.31	5.71 ± 0.98	8.01 ± 1.60	5.29 ± 1.06^a
BDE-183	0.05 ± 0.01	0.15 ± 0.01	0.36 ± 0.16	0.82 ± 0.10	0.24 ± 0.01
DE-79					
BDE-153	15.00 ± 0.20	4.60 ± 0.97	3.30 ± 0.66	4.07 ± 0.24	2.69 ± 0.16^a
BDE-154	3.36 ± 0.12	0.73 ± 0.22	4.83 ± 1.54	5.75 ± 0.56	3.80 ± 0.37^a
BDE-180	0.21 ± 0.40	0.53 ± 0.83	0.39 ± 0.04	0.46 ± 0.03	0.26 ± 0.03
BDE-183	7.13 ± 0.01	13.91 ± 0.04	0.51 ± 0.05	0.47 ± 0.05	0.34 ± 0.07
BDE-197	7.93 ± 0.93	16.35 ± 1.79	0.49 ± 0.11	0.59 ± 0.06	0.39 ± 0.04^a
BDE-203	2.68 ± 0.56	3.41 ± 0.21	0.79 ± 0.23	0.98 ± 0.19	0.65 ± 0.12^a
BDE-207	1.88 ± 0.73	12.49 ± 0.17	0.15 ± 0.07	0.19 ± 0.03	0.13 ± 0.02^a
BDE-209	0.38 ± 0.15	1.46 ± 0.01	0.26 ± 0.02	–	–
Individual congeners					
BDE-77	60.31 ± 1.31	11.20 ± 3.47	5.39 ± 0.12	5.14 ± 0.25	3.55 ± 1.11
BDE-126	45.25 ± 0.84	7.95 ± 2.54	5.69 ± 0.11	5.75 ± 0.60	3.75 ± 1.20
DE-83R and individual congeners BDE-198, BDE-204					
BDE-198	19.38 ± 0.34	10.13 ± 0.98	1.92 ± 0.15	2.03 ± 0.89	1.34 ± 0.59^a
BDE-204	12.47 ± 0.65	9.68 ± 0.82	1.29 ± 0.04	2.97 ± 1.93	1.71 ± 0.93^a
BDE-206	0.73 ± 0.01	1.01 ± 0.03	0.72 ± 0.16	1.18 ± 0.68	0.78 ± 0.44^a
BDE-207	0.89 ± 0.08	0.91 ± 0.14	0.97 ± 0.06	4.91 ± 16.40	1.41 ± 1.68^a
BDE-208	0.56 ± 0.03	0.33 ± 0.26	1.67 ± 0.19	4.74 ± 6.10	2.14 ± 1.64^a
BDE-209	16.89 ± 0.70	24.79 ± 0.73	0.68 ± 0.03	0.86 ± 0.28	0.93 ± 0.57^a

^a BSAF is calculated based on BAF_K

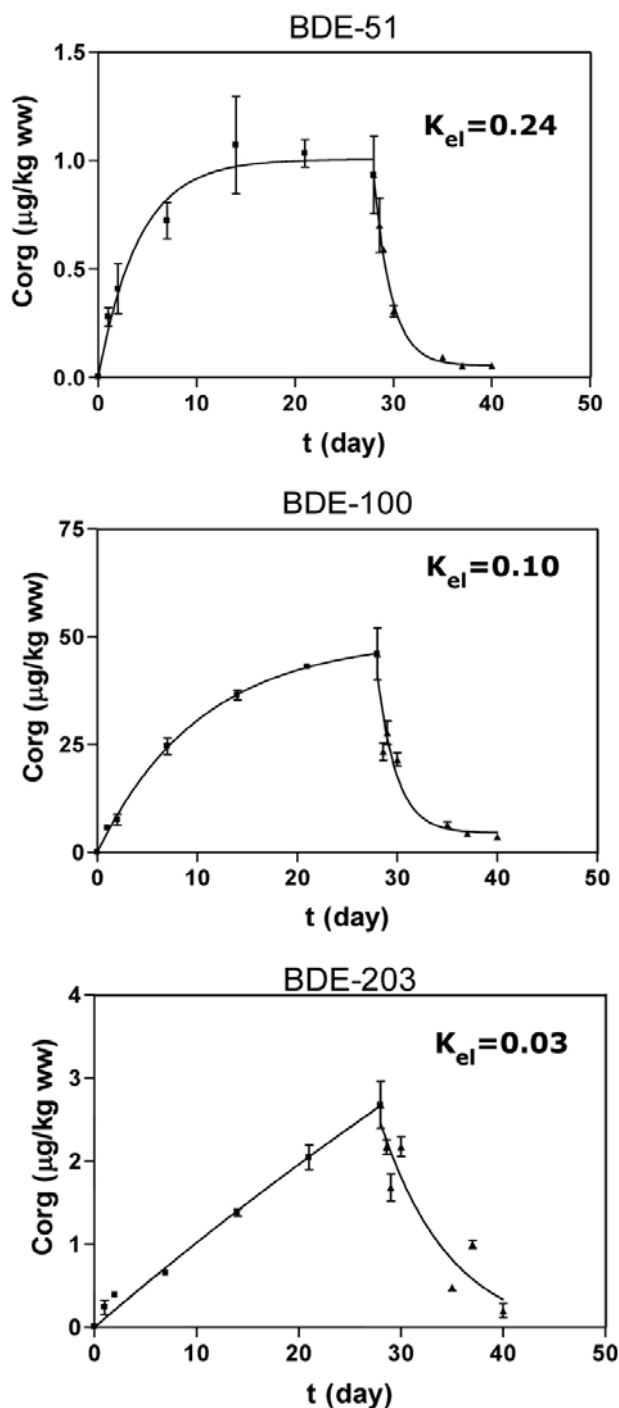


Figure 1. Time courses of the uptake and elimination of three PBDE congeners in *T. tubifex* in relation to k_{el} . $k_{el} \geq 0.1$ indicates that the congeners reach a steady-state plateau in the test animal. Filled squares with error bars represent the measured concentrations on the sampling day.

4. Discussion

The testing of the bioaccumulation of commercial mixtures of PBDEs combined with individual congeners was possible due to the selective uptake of PBDE congeners by the oligochaetes.^{30,31} The sediment was spiked with 21 congeners from three BDE commercial mixtures (TBDE-71, TBDE-79 and TBDE-83R) and with individual congeners (BDE-077, BDE-126, BDE-198, and BDE-204). All of the spiked congeners were detected in increasing concentrations in the test animals during and at the end of the test. These results lead to the conclusion that the accumulated BDEs undergo only a little or no transformation in the oligochaetes, which is particularly relevant in studies of the lower trophic levels of aquatic as well as of terrestrial ecosystems. The difference between the bioaccumulation of BDEs in sediment-dwelling oligochaetes (*T. tubifex*) and in fish on the higher trophic level was demonstrated in a water/sediment microcosm system by Tian et al.³² The simple digestive tract and metabolic system of the oligochaetes mean a relatively low metabolic capacity to debrominate BDEs. At the end of 80 days of exposure to the tri-, tetra-, penta-, hexa-, hepta-, and nona-BDE homologues, all 11 monitored BDE congeners could be quantified in worms, while only 5 of them were found in fish fillet and fish liver.

The results of the tests on oligochaete *T. tubifex* presented in Table 3 and Figure 2 show distinct differences in bioaccumulation for the two groups of BDE homologues (“bioaccumulation gap”). The BSAF for tri- to hexa-BDEs varied between 5.95 and 2.69 (on average 4.34) and in the hepta- to deca-homologues between 2.14 and 0.13 (on average 0.95). Similar conclusions were drawn by Tian et al.³² In the 80-day microcosm study the BSAF for tri- to hepta-BDEs varied between 5.89 and 1.09 (on average 3.46), while a distinctive bioaccumulation gap appeared between the octa- (BSAF of 0.99) and nona- to deca-homologues (BSAF in the range 0.05–0.01, on average 0.3).

Several studies describe the differences in bioaccumulation between the tri- to hexa- and the hepta- to deca-BDE homologues in sediment-dwelling or soil-dwelling annelids. For instance, the oligochaete *Lumbricus variegatus* exposed to a sediment spiked with BDEs accumulated tetra-, penta-, and hexa-BDEs, but the uptake of the deca congener BDE-209 was.³³ A study on earth worms (*Eisenia fetida*) in soil spiked with the commercial BDE products DE-71 and DE-79 brought a similar conclusion. The uptake of the congeners BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 was ten times higher than the uptake of the congener BDE-183.³¹ Nevertheless, in the same study it was suggested that even large molecules can be taken up by oligochaetes from the soil. The bioaccumulation of BDEs by the marine polychaete *Nereis virens* showed the predominance of penta-BDE-47, BDE-99 and BDE-100 followed by hexa-BDE-153 and BDE-154 in

ding the 10% threshold was observed for BDE 198 and BDE 209. After 10 days of the elimination phase these congeners were still present in the amounts 18.8% and 23.5% of the concentration measured on day 28, respectively.

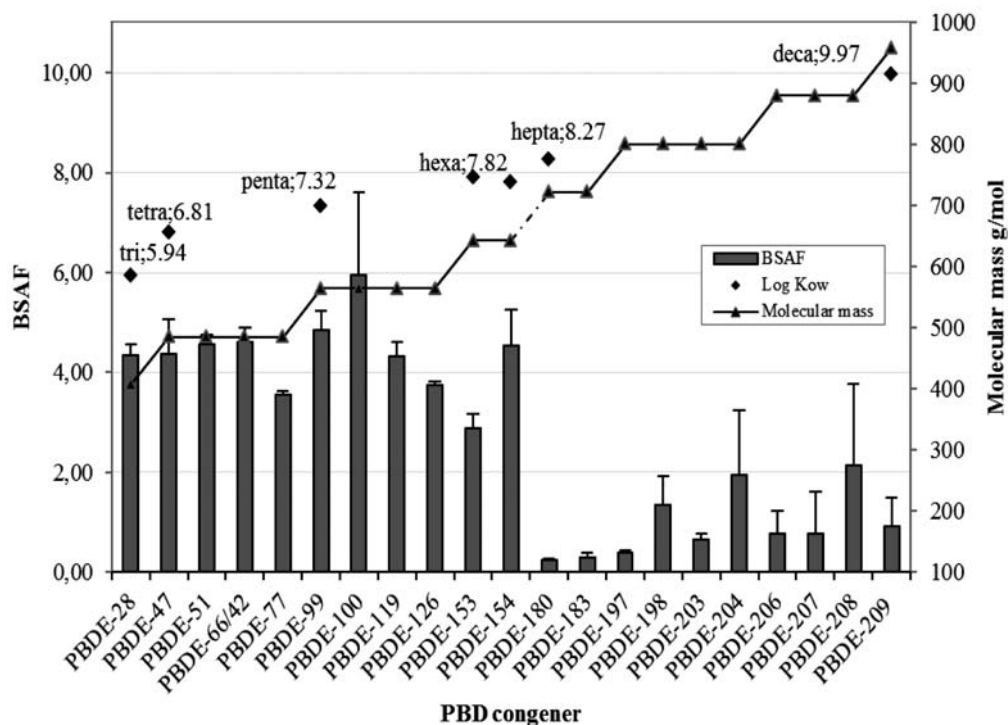


Figure 2. The biota-sediment assessment factor (BSAF) of tri- to deca-homologues of the BDEs in relation to the molecular mass and the measured log K_{ow} . The dotted line indicates the bioaccumulation gap between the hexa- and hepta-homologues.

spiked artificial sediment. The accumulation of congeners with higher bromination was limited in a spiked matrix, while nearly no accumulation was registered in estuarine sediment.³⁰

The change in the bioaccumulation potential related to the increasing bromination of BDEs can be explained by the impact of the molecular weight, lipophilicity, enthalpy change in the transition between media and probably by the size of the molecules, which precludes large congeners from crossing the cell membranes in organisms. On the other hand, the bioavailability of extremely lipophilic substances generally decreases because of the stronger bond with the organic matter in the sediment and the lower solubility in water, digestive fluid and body surface.^{34,35}

Large organic molecules cannot passively diffuse through the phospholipid bilayer of the cell membrane.³¹ Therefore, the cross-membrane transport of molecules such as BDEs is replaced by exocytosis and endocytosis. Dimitrov³⁶ justified the existence of a transition point for the change in the mechanism of the uptake of chemicals. The threshold value of 1.5 nm for the size of the molecules is very comparable with the cell-membrane architecture.

The test according to OECD TG 315 for testing bioaccumulation in sediment-dwelling benthic oligochaetes is not designed to study the bioavailability of bio-accumulative substances. It is, therefore, difficult to assess the impact of lipophilicity on the bioaccumulation potential of tri- to hexa-congeners and the low bioavailability of hepta-

to deca-congeners due to a strong bond to the artificial sediment particles. The comparison of the bioaccumulation dynamics of BDE congeners with the size of the molecules did not help to explain the biodynamics of the BDE congeners either. The values for the average maximum diameter ($D_{max, ave.}$) calculated with the program OASIS (Table 1) for the range of tri- to deca-BDE molecules are close to the threshold value of 1.5 nm and vary between 1.44 and 1.47 nm.¹⁰ The size of the molecules generally did not correlate with increasing bromination or with the bioaccumulation potential of the BDEs.

The change in bioaccumulation potential between the hexa- and hepta-BDEs in our study correlates with the molecular-mass threshold for penetration through the cell membrane and the estimated enthalpy change. Molecular mass is used as a good descriptor for the upper limits of the pharmacological activity of molecules and it is commonly assumed that a low bio-accumulative potential is related to compounds having molecular weights close to or above 700 g/mol.^{10,37} The threshold molecular mass of 700 g/mol is between hexa-BDEs (molecular mass of 643.62 g/mol) and hepta-BDEs (molecular mass of 722.50 g/mol), and so is the bioaccumulation gap (Figure 2).

A possible explanation for the bioaccumulation gap could be based on the thermodynamic properties of BDEs. The proposed enthalpy change for the phase transition of the dissolved compound from octanol to water (ΔH_{ow}) is assumed to be 15, 20, and 25 kJ/mol for the tri-, tetra- to hexa- and hepta- to deca-homologues, respectively.⁶ The

decreased bioaccumulation of hepta- to deca- in regard to the tri- and tetra- to hexa-BDEs can be interpreted with the larger energy input that is needed for higher brominated BDEs in the transition between media; in our case between the organic matter in the sediment (dissolved organic carbon) and the digestive fluid.

In our study the elimination-rate constant for the hexa- and octa- to deca-BDEs approaches 0, which indicates that the time to achieve a steady-state plateau in the oligochaetes is way beyond the time frame of 28 days. The hepta-homologues (BDE-180 and BDE-183), however, did reach the steady-state plateau in the tested oligochaetes. In the study we could not explain the reason for such behaviour of the congeners. Kraaij¹⁵ showed a relation between the long contact time and the bioavailability of lipophilic chemicals in sediment. The BSAF for PCB congeners (PCB 138, PCB 153, and PCB 156) in *T. tubifex* exposed to the spiked sediment was slowly increasing over nearly 1000 days. Tubificidae have a relatively long life span of more than 3 years,³⁸ therefore, a slow and steady uptake of octa-, nona-, and deca-BDEs can, similar to PCBs, last for several months if not for the whole of their life cycle.

5. Conclusion

The freshwater oligochaete *T. Tubifex* selectively accumulated 21 congeners from three commercial mixtures (TBDE-71, TBDE-79 and TBDE-83R) and a mixture of four BDE individual congeners (BDE-077, BDE-126, BDE-198 and BDE-204), which were spiked with artificial sediment. The increasing concentrations of congeners in the test animals during the test indicated that the accumulated BDEs undergo only a little or no transformation in oligochaetes. The bioaccumulation factor (BAF), the kinetic bioaccumulation factor (BAF_k) and the normalized biota sediment accumulation factor (BSAF) were determined in four series of tests, indicating a high bioaccumulation potential for the tri- to hexa-BDEs, whereas the bioaccumulation of the hepta- to deca-BDEs was considerably lower. A comparison of our results with the data on bioaccumulation in other species of annelids shows a similar bioaccumulation gap between these two groups of BDEs. Although the test according to OECD TG 315 “Bioaccumulation in Sediment-Dwelling Benthic Oligochaetes”²⁰ is not designed to study the bioavailability and bioaccumulation dynamics of tested substances, we come to the conclusion that the molecular mass and enthalpy change for the phase transition from octanol to water correlates with the change of the bioaccumulation potential between the tri- to hexa- and hepta- to deca-homologues.

We can confirm that annelids such as terrestrial and freshwater oligochaetes and marine polychaetes are reliable surrogate species that reflect the bioavailability of sediment-associated lipophilic substances.

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Povzetek

Maloščetinci selektivno privzemajo polibromirane difenil etre (PBDE), kar omogoča vrednotenje bioakumulacije posameznih kongerjev kot tudi njihovih komercialnih mešanic. Na maloščetincih vrste *Tubifex tubifex* smo v skladu s tehničnim navodilom OECD 315 "Bioaccumulation in Sediment-Dwelling Benthic Oligochaetes" testirali 21 BDE kongerjev iz treh komercialnih mešanic (TBDE-71, TBDE-79 in TBDE-83R) ter kot posamezne kongenerje (BDE-77, BDE-126, BDE-198 in BDE-204). Vse kongenerje, ki smo jih vmešali v sediment, smo v organizmih zaznali na kocu privzemne faze kot tudi ob koncu poskusa. Izračunali smo bioakumulacijski faktor (BAF), kinetični bioakumulacijski faktor (BAF_k) ter normalizirani bioakumulacijski faktor (BSAF) ter ugotovili visok bioakumulacijski potencial za tri do heksa BDE in nižji bioakumulacijski potencial za hepta- do deka-BDE. Penta homologa BDE-99 in BDE-100 sta izkazala najvišje vrednosti BSAF in sicer 4,84 in 5,85 ob vrednostih BAF 7,34 oziroma 9,01, medtem, ko je bila bioakumulacija nona in deka BDE kongerjev približno desetkrat nižja. Ta sprememba bioakumulacijskega potenciala med skupinama tri do heksa BDE in hepta- do deka-BDE se ujema s splošno sprejeto mejo 700 g/mol, ki velja za molekulska maso ob prehodu bioloških membran.

DRUŠTVENE VESTI IN DRUGE AKTIVNOSTI

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Prvi Teslovi koraki v kemijske vede

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Povzetek

Prvič je predstavljen popoln popis Teslovih srednješolskih profesorjev. Poudarjene so odlike njegovih učiteljev kemijskih in sorodnih ved. Analizirane so knjige o kemiji, ki jih je bral srednješolec Tesla, in s kemijo povezana objavljena dela njegovih predavateljev. Še posebej je izpostavljeno raziskovanje spektroskopije, ki se je razbohotilo po Kirchhoffovih in Bunsenovih začetkih v Teslovi zgodnji mladosti in je močno vplivalo na raziskovanja Teslovega najljubšega profesorja Martina Sekulića.

Ključne besede: Nikola Tesla, Rakovac pri Karlovcu, Martin Sekulić. Gustav Kirchhoff, Antoine Cesar Becquerel, Zgodovina pouka kemije in elektrotehnike.

Slika 1: Teslov razrednik profesor tehniškega risanja visoki Franz Kreminger levo zgoraj, ob njem sivolasi profesor geografije-zgodovine in tujih jezikov Christoph Nieper ki je prišel iz slovaških Kremnic in kmalu odšel v Trst, sivolasi učitelj petja Vinzenz Knapp, profesor geografije-zgodovine in nemščine Franz Sehr, profesor hrvaščine pisec slovnice Josef Vitanović, **profesor kemije francoščine in zgodovine-geografije v prvih treh letih Teslovega študija v Rakovcu bradati Emanuel Kregez**, Tesli nič kaj ljub profesor prostoročnega risanja še bolj bradati Karl Pallasman in prezgodaj umrli fizik-matematik Moritz Antulić desno zgoraj na posnetku iz januarja 1868, poldrugo leto pred Teslovim vpisom. Spodaj profesor geometrije, tehniškega risanja in strojništva Sekulić levo spodaj, profesor kmetijstva in naravoslovja Christian Lechleitner in katoliški veroučitelj Nikolaus Živković. Ime psička na levi zaenkrat še ni znano, čeravno v tej smeri potekajo intenzivna raziskovanja.



Prva profesorja kemije Nikole Tesle sta bila Ivan Jamnicki in Emanuel Kregez. Jamnicki je Teslo učil na gospiški nižji realki in nato znova v zadnjem letniku višje realke v Rakovcu ob Karlovcu po Emanuelovemu odhodu v Vinkovce 12. 9. 1872.¹ Oba sta vodila nadvse dobro opremljen kemijski laboratorij, saj je pripomočke neposredno financirala vojaška uprava v Zagrebu.

Kregez je kemijo predaval po učbeniku Friedricha Hinterbergerja (1826–1875),² zvedavi Tesla pa si je poma-

gal tudi z bogato založeno knjižnico v kateri so leta 1869/70 nabavili Časopis za analitično kemijo ustanovljen leta 1862 pod taktirko nekdanjega Liebigovega asistenta Carla Remigiusa Freseniusa (1818–1897) kot vodilnega strokovnjaka za kemijsko analizo katerega sistem je obveljal za celo stoletje. Prav tako ni bil od muh O. Erdmannov Časopis za praktično kemijo, ki je pod tem imenom začel izhajati že leta 1834 in ga je Tesla prav tako bral že v svojem prvem letu 1869/70 v Rakovcu. Leta



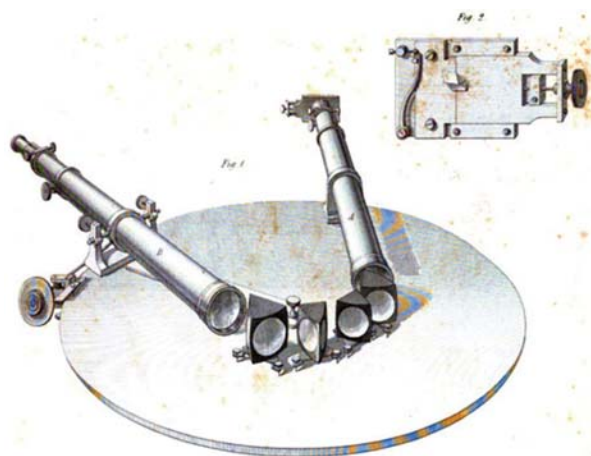
Slika 2: Teslovo maturitetno spričevalo izdano ducat let po maturi za njegovo ameriško zaposlitev pri Edisonu. Sestavil ga je Teslov nekdanji sošolec profesor kemije v Rakovcu Ivan Bielić, vendar je pri tem zagrešil pomoto. Zapisalo se mu je, da je Tesla obiskoval nižjo realko v Gospiću med letoma 1867/68–1869/70, višjo realko pa v Rakovcu od 1870/71 do 1872/73, kar je skupno le 6 let. Bržkone pomota in je v Gospiću začel študirati eno leto prej, tako da je bil leta 1869/70 že v Rakovcu.

1871/72 je Teslov šolski knjižničar Kreminger nabavil Kemijo A. Streckerja (1822–1871) in Henrija Victorja Regnaulta (1810–1878), naslednje leto pa v Teslovem maturitetnem letu še Hinterbergerjevo Kemijsko Tehniko in Kemijo Karla Augusta Neumanna (1771–1866)³ Regnault je s svojimi natančnimi meritvami specifičnih toplot omogočil prevlado mehanske teorije teorije topote, čeravno so mu pomembni spisi zgoreli med boji za Pariško komuno, ki so zavdali tudi njegovemu sinu. Strecker je bil med najpomembnejšimi predhodniki periodnega sistema Mendeleejeva.⁴ V predzadnjem letu Teslovega študija je dr. Kugler podaril dijaški knjižnici realke Rakovac Kemijo

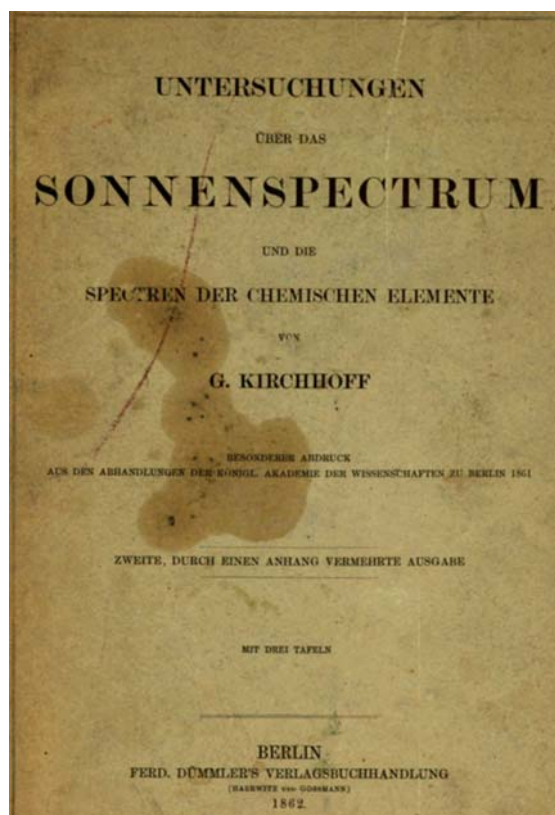
dunajskega univerzitetnega profesorja kemije in botanike med letoma 1707–1838 Josepha Franza barona Jacquina (1766–1839), ki je med prvimi sprejel nov Lavoisierjev pristop in Daltonovo-Berzeliusovo atomistično kemijo.⁵ Radodarni Kugler je dodal še prve tri zvezke Poljudnega naravoslovja Antoine Cesarja Becquerela (1788–1878) iz družine slovitih francoskih kemikov in raziskovalcev radioaktivnosti.⁶ Becquerel je začel pregled razvoja kemije podobno kot Teslov sošolec kemik Prica leta 1883. Kemija se je ločila od fizike v poznem 18. stoletju, kjer sta imela v mislih delo Lavoisierja. Becquerel je še posebej pohvalil vakuumske raziskave Torricellija in Otta Guericka. Obravnaval je razvoj do 15. stoletja preden je opisal najnovejša odkritja.⁷ Galilej je raziskal težnost, Torricelli je odkril zračni tlak, Descartes pa je Pascalu dal idejo Puy de Dome tlačnih poskusov za merjenje višin hribov. Zgodovinsko skico so sklenili raziskovalci loma svetlobe.⁸ V uvodu druge knjige je Becquerel podal za Teslo zelo zanimiv oris teorije svetlobnih delcev in valov od Bacona, Descartesa in Newtona do Thomasa Younga, Brewsterja in Fraunhoferja.⁹ Na koncu drugega zvezka je posebno skrb namenil teoriji molekul Ampèra in Marca Antoina Gaudina (1804 Saintes–1880 Pariz). Becquerel je zaključil knjigo s kristalografsko-atomistično teorijo. Ampère je brez dokončnega dokaza predstavil molekule različnih stvari v posameznih geometrijskih oblikah piramide, kocke in podobnih. V kemični sestavi se molekule vežejo na druge glede na danosti svoje prostorske geometrije. Več atomov gradi molekulo, pravilna prostorska geometrija molekul pa omogoča pravilne oblike kristalov.¹⁰ Keplerjevim podobne špekulacije te vrste so navdihnile Teslo za vpis na graške politehniške kemijske študije.

V Teslovem maturitetnem zaključnem letu 1872/73 je njegov razrednik Franc Kreminger kot knjižničar realke Rakovac kupil 81 knjig, vključno s številnimi pripomočki za pouk meteorologije, matematike in kemije. Med njimi je bila Graham-Ottova Kemija, katere del je Kreminger nabavil že v preteklem letu. Thomas Graham (1805–1869) je bil profesor kemije v Glasgowu in Londonu kjer je končno nadomestil Herschla kot *Master of Mint* na prestižnem položaju svoj čas dodeljenemu neprekosljivemu Newtonu. Seveda upravičeno, saj sta leta 1847 v knjigi, ki so jo uporabljali Tesla in njegovi učitelji, Graham in Otto prva objavila tabelo izomorfni kemijskih elementov in z njim tlakovala pot D. Mendeleejeva v periodni sistem iz let 1869–1871.¹¹

Ob Graham-Ottovi knjigi je knjižničar Kreminger nabavil še Kirchhoffovo Spektroskopijo.¹² Strokovnjak za spektroskopijo nekdanji Bunsenvov učenec Henry E. Roscoe FRS (1833–1915) je prevedel leta 1861 v Heidelbergu napisano Kirchhoffovo delo, ki je bilo objavljeno v Berlinu leta 1862. Bunsen in Kirchhoff sta ustanovila povsem novo vejo kemijske znanosti z uporabo opazovanje spektralnih črt in s kvantitativno analizo. Kirchhoff je navaljal stališča Škota Sira Davida Brewsterja o obliki posebnih temnih spektralnih črt v spektru zahajajočega Sonca, ki ned-



Slika 3: Kirchhoffov spektrometer na koncu prve Kirchhoffove knjige, ki jo je Tesla bral v Rakovcu.



Slika 3: Kirchhoffov spektrometer na koncu prve Kirchhoffove knjige, ki jo je Tesla bral v Rakovcu.

vomno pripadajo vplivom zemeljske atmosfere.¹³ Pri nastanku osončja je Kirchhoff upošteval Laplaceovo meglično hipotezo,¹⁴ Aragu pa so se Sončne pege zdele dovolj ohlajene za morebitne prebivalce.¹⁵ Slike sončnega spektra in spektroskopa s prizmo je Kirchhoff postavil na konec knjige. Roscoe prevedel še leta 1862 v Heidelbergu napisano in naslednje leto v Berlinu objavljeno Kirchhoffovo delo pod enakim naslovom. Kirchhoff je primerjal spektre Sonca s

Leopold Mözburger

15 Nov 1711 Gradec
14 Oct 1726 Dunaj
1731 Dunaj

1743 Gradec; prof. mat.
Bet. 1744–1746 Linz; prof. fil. in mat.

Bet. 1747–1748 Gradec; prefekt astronomskega observatorija
Bet. 1749–1754 Dunaj
Terezijanišče; prof. mat. prefekt muzeja

Karel Dollenc

07 Oct 1703 Gradec
14 Oct 1726 Gradec
1725 Dunaj

Bet. 1737–1741 Dunaj; 1737–41 prof. hum. in fil.
Bet. 1742–1744 Gradec; profesor fizike, promotor Regnaullovih del

12 Oct 1751 Buda no Ogrskem

Peter Halloy

25 Apr 1707 Namur v Belgiji
28 Oct 1722 Trenčin na Slovaškem

1727 Gradec 1730 Dunaj

1739 Trnava; profesor matematike
1740 Dunaj; profesor matematike

1742 Gradec; profesor fizike; 1741 prof. logike, 1743 metafizike

Bet. 1744–1745 Gradec; profesor matematike
1746 Gradec; profesor spec. Astron. & Muzeja Matematika spovednik konvikta

Bet. 1747–1748 Dunaj Terezijanišče; profesor matematike eksperimentalne fizike spovednik

1749 Gradec; prof. spec. astron. & muzeja matematike
Bet. 1750–1765 Gradec; Profesor repetitorov matematike

Bet. 1766–1773 Maribor; superior, prefekt šole

23 Jul 1789 Maribor

Josef Führer

18 Mar 1722 Bratislava
17 Oct 1754 Dunaj

1743 Gradec
Bet. 1755–1757 Dunaj Torozijanišče; prefekt filozofov

Bet. 1758–1761 Košice; prof. fil. mat. katehet
Bet. 1762–1773 Trnava; prof. rep. mat. prof. mat. vodja kongregacij

21 Jan 1774 Bratislava

Josephus Szerdahlyi

27 Sep 1727 Vathensis
1750 Košice

Bet. 1752–1763 Gradec
1761 Košice; prof. mat.

Bet. 1762–1763 Čiž; prof. mat.
Bet. 1764–1765 Buda no Ogrskem; prof. fil.

Josef Ildefons Karvančič

1744 13.11.1780 32/33 annorum po zapisniku seje akademije Zagreb, Kukec 38/40 annorum, znal latinsko, italijansko, hrvaško, Kukec pa latinsko in nemško

pavlinec
Bet. 1776–1798 Zagreb; profesor matematike
Bet. 1805–1819 Senj; opat kot bivši pavlin in zagrebaški meščan

1819 Senj

Antun Kukec

12 Nov 1738 Ptuj
18 Oct 1756 Dunaj

Bet. 1760–1761 Košice

1763 Trnava

Bet. 1771–1773 Zagreb; profesor hebrejščine, matematike, grščina

Bet. 1773–1797 Zagreb; profesor fizike

18 Oct 1796 Zagreb

Jozef Wolfstein

29 Jun 1773 Kartovac
1794 Zagreb

1796 Pavia

Bet. 1797–1803 Osijek; profesor matematike

Bet. 1803–1818 Košice; profesor matematike

Bet. 1818–1848 Pošta; profesor višje matematike

19 Apr 1859 Budimpešta

1819 Senj

1819 Senj

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Slika 5: Teslovi akademski predniki glede na njegov študij spektroskopije pri Sekuliću; prikazani so vse do zadnjega mariborskega rektorja Halloya. Sekulić je študiral pri vodilnih ogrskih raziskovalcih dinama in še posebej pri izumitelju dinama benediktincu Jedliku, tako da je Tesli najnovejša spoznanja ponudil tako rekoč iz prve roke.

spektri žarnic plinov opazovanih skozi teleskop po lomu na več prizmah. Kirchhoff je dokazal, da svetle črte posameznih kemijskih elementov sovpadajo s temnimi programi sončnega spektra, ki kažejo pečat plinastih snovi v atmosferi Sonca. Kirchhoffov študent Karl Hofmann je nadaljeval študij spektrov plinov, ker se je Kirchhoff popolnoma posvetil raziskovanju spektrov Sonca.¹⁶ Večji del drugega zvezka je vseboval tabele izmerjenih podatkov s spektrom na kon-



Slika 6: Teslovi akademski predniki glede na njegov študij kemije v Rakovcu.

cu. Nabavo Kirchhoffove knjige je nedvomno naročil Teslov profesor Sekulić, ki mu je Tesla neformalno pomagal pri pripravi poskusov. Sekulić je obenem nabavil laboratorijsko opremo za lastne spektroskopske meritve, ki jih je začel že oktobra 1870, ko je imel izjemno priložnost tudi na naših zemljepisnih širinah opazovati severni sij. Svoje ugotovitve je tudi s Teslovo mladostno pomočjo objavil v tisti čas najbolj eminentnih kemijskih in fizikalnih revijah. Z njimi si je pridobil velik mednarodni sloves in članstvo v Jugoslovanski akademiji znanosti in umetnosti v Zagrebu.¹⁷ Roscoe je moral biti Sekuliću nadvse pri srcu, saj so leta 1872/73 ob njegovem prevodu Kirchhoffa nabavili še prevod Roscoeve *Lehrbuch der Chemie*.

Preglednica 1: Teslovi profesorji na realki v Rakovcu

Področje	4. letnik 1869/70 profesorjeve ure na teden	5. letnik 1870/71	6. letnik 1871/72	7. letnik 1872/73	Matura 1872/73: člani izpitne komisije
Teslov razrednik	Moritz Antolić	Löffler? (5. razred 1872/73)	Loys Möstl poučeval neobezno modelatrstvo za 10 študentov	Franz Kreminger (27. 10. 1835 – 23. 12. 1915 Dunaj)	Zapisnikar Kremiger, 1874 na Ljubljanski realki
Matematika	Moritz Antolić 6–7	Löffler trigonometrija stereometrija po Wiegandu 4–5	Josef Angelo Spinca (odšel na realko Pančevo) ali Sekulić: Diplomatska enačba, enačbe višjih redov, vrste, konvergenca divergenca, kombinacijski račun, binomski teorem, stereometrija, sferična trigonometrija 3	Šoštarić, Löffler, Kreminger ali Sekulić	
Geografija in zgodovina	Löffler 4	Emanuel Kregez? 3	Stefan Vargić 3	Mio Brašnić ali Stefan Vargić 4	Živko Vukasović (1829 Beravci južno od Đakova-1874), zoolog, entomolog, redni član JAZU, šolski inšpektor Vojne Krajine, doštudiral pravo na Dunaju in biologijo v Gradcu
Verouk	Nikolaus Živković 2	2	2	Nikolaus Živković 2	Živković nekdanji kaplan v Crkvenom-bioku, škofijski svetovalec 1868
Nemški jezik	Christian Nieper (prej v Kremnicah, leta 1870 v Trstu) 3–4	3	3 ??	Mio Brašnić ali Jamnicky († 1881) 2	Brašnić (v Vinkovcih 1874–1883)
Francoski jezik			Emanuel Kregez 3	Peter Tomić 4 ur 1. semester/ Šoštarić 3 ur 2. semester	Petar Tomić (1839 Zabok v Zagorju–1918) doktoriral v Gradcu 1874
Hrvaški jezik	Josef Vitanović (* 1844 Osijek; † 1909) Zagreb, 1870 Osijek, na Realki Zagreb objavil Hrvaško slovnico 1872 in 1880	2–3	Stefan Vargić 2, 2 profesor katoliškega verouka kateht Josef Jagunić po 25. 3. 1873	Stefan Vargić 3	Jagunić (* 1831, † 1891), nekdanji kaplan v Dubovcu, Rakovac 1873–1875, Vinkovci, 1882 profesor Gimnazije Karlovac

Opisna geometrija	Franz Kreminger projekcije, piramida 3	Franz Kreminger po Schnedersu polieder, preseki, krivulje, projekcije, piramida 4	Josef Angelo Spinca: ukrivljene površine, Tangentne projekcije 3	Franz Kreminger Centralne projekcije 3	
Naravoslovje	Sigmund Šoštarić Šišman von Letovanić (†14/11/1874 Karlovac) zoologija sistematika po Giebelu 1 ura, (Botanična ekskurzija v Ozaljšu)	Botanika po Billu 2	Sistematika rastlin 2	Šoštarić Kristalografija 3	Ravnatelj Šoštarić
Fizika	/	Löffler, Kregez, ali Sekulić, značilnosti teles, trdnine ali kapljevine po Kunzeu 3	Löffler, Kregez, ali Sekulić Toplota elektrika magnetizem statika trdnin, kapljev plinov 4	Löffler in Sekulić izmenoma: Dinamika, valovi, akustika, optika, teorije optike in toplote, matematična geografija in astronomija 4	Sekulić
Kemija	Emanuel Kregez (1872 v Vinkovcih, upokojen pred 1882) Tehniška in anorganska po Hinterbergerju 3 ure	Emanuel Kregez Tehniška in organska po Hinterbergerju 3	Emanuel Kregez težke kovine, ogljikove spojine 3	Ivan Jamnický Ogljikove spojine 2	Jamnický
Kmetijstvo	Sigmund Šoštarić von Letovanić 2 s praktičnim delom	Kmetijstvo sadjarstvo vinogradništvo 2 s praktičnim delom	/	/	
Telovadba	Sekulić neobvezna gimnastika proti plačilu 50 fl	Sekulić?	Löffler za 160 študentov 4	Löffler 2	Löffler
Prostoročno risanje	Carl Pallasman 4	komPLICIRANI ornamenti, glava, roka, noga 4	Loys Möstl Biste, akti, dvojna kreda in svinec, gips-ornamenti 4	Gjuro Fridrich (tudi po 1883) Akti in naravni ornamenti 4	Gjuro Fridrich
Strojništvo			Sekulić 4	Sekulić	

Naprave za poskuse

Leta 1866/67 je predstojnik fizikalnega kabineta Moritz Antolić pridobil Woltmannov merilec toka, v Teslovem četrtem razredu leta 1869/70 pa termo-multiplikator (termoelektrični) Macedonio Mellonija (1798–1854) s svetilko in zaslonom, termo-element za temeljne poskuse, Frickovo valovno napravo in zbirko pripomočkov za dokaz Mariotevega zakona vključno s pipo za izpust. Löffler dobil za svoja meteorološka opazovanja Brusottinov aerometer (higrometer) iz Milana¹⁸ kot darilo feldmaršala poročnika Antona viteza Mollinary von Monte Pastello (* 8/9 oktobra 1820 kot Anton Mollinary v Titelu v Bački; † 26. oktobra 1904 v Vili Soave pri Comu v Lombardiji) ob obisku šole. Leta 1870 je bil Mollinary zagrebški generalni poveljnik Hrvaško-slavonske Krajine. Podarjeni aerometer je bil vreden 300 frankov v zlatu, njegov izumitelj pa je bil Ferdinand Brusotti (1837–1899 Pavia), ki je izpopolnjeval elektrostatične naprave in dobil zlato izumiteljsko medaljo na razstavi v Aleksandriji leta 1870, zasnoval pa je tudi novo vrsto vlagomera.

Leta 1871/72 Sekulić za fizikalni kabinet dobil majhen parni-motor, Dubasquevo votlo prizmo, teleskop z mikrometrom, dve stekleni zrcali, role papirja za telegraf. Nadaljnjih 95 fl je porabil za popravilo naprav in potrošni

material, medtem ko je Kregeza potrošni material v kemijskem laboratoriju stal 141 fl, leta 1869/70 pa le 40 fl.¹⁹

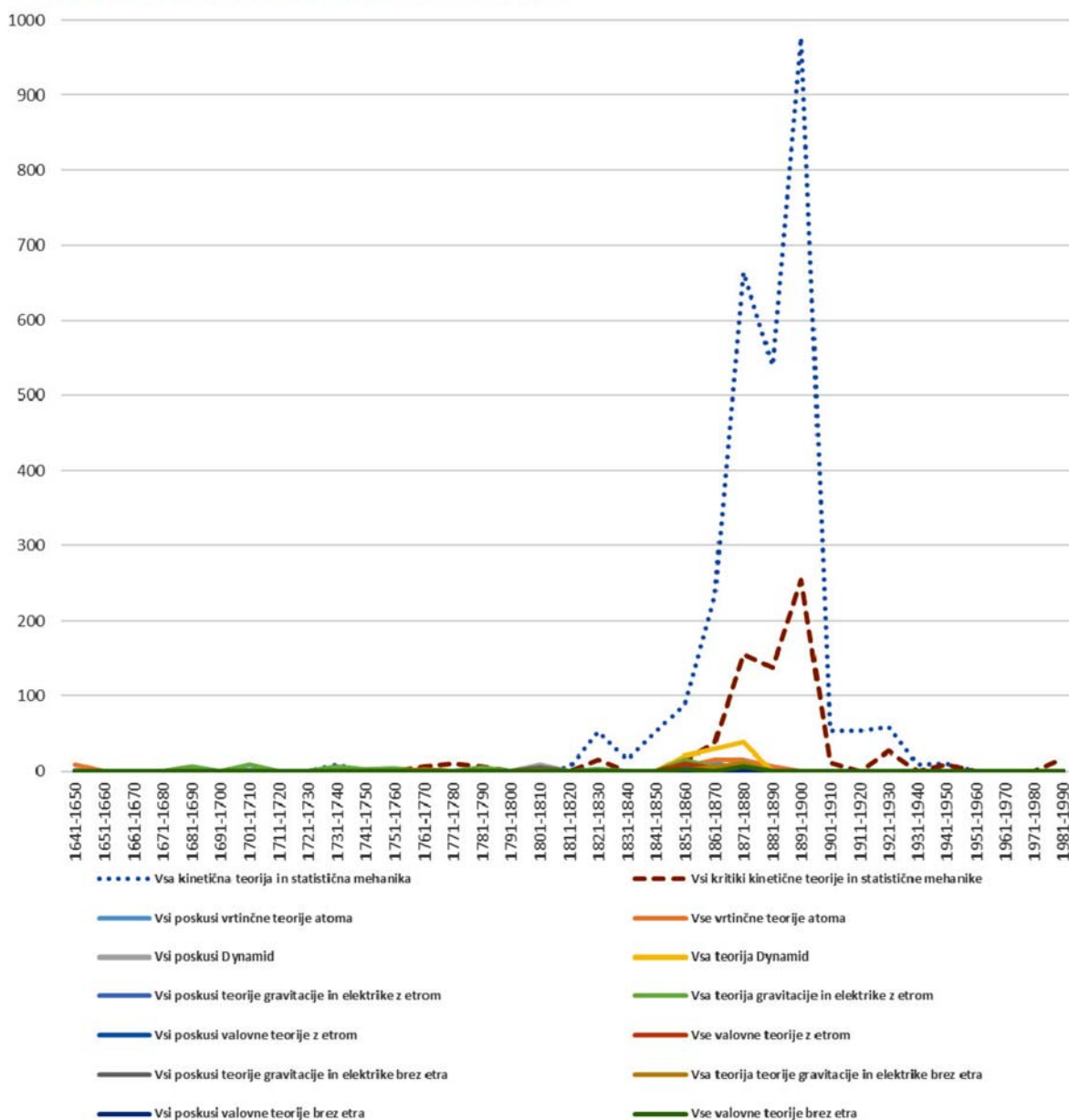
Leta 1871/72 je bilo 579 instrumentov v Sekulićevev fizikalnem kabinetu, leta 1880/81 pa 277 aparatov in 25 instrumentov. Leta 1880/81 je imela naravoslovna zbirka 7000 primerkov, 20 instrumentov in 125 slik, matematično-geometrijska zbirka 181 modelov in 101 instrumentov. Kemijski laboratorij Jamnickega († 1881) je štel 111 aparatov in 87 instrumentov. Za prostoročno risanje so imeli 1500 predlogov in 201 modelov, študentov knjižnica imela okoli 1700 knjig, profesorska knjižnica pa 2400.²⁰

V Teslovem zaključnem letniku leta 1872/73 je Sekulić za realko v Rakovcu pridobil pet fizikalnih eksperimentalnih naprav, vključno z Grovejevim elementom s platinastimi elementi površine 23 inčev, Brehetovim kovinskim termometrom, kleščami, kladivom in žago.²¹

Teslove srednješolske zamisli in znanja

Šolske spretnosti mladostnik včasih sprejme za vedno. Inovativni duhovi jih raje spremenijo, tako kot so to počeli Galilej, Tesla ali Einstein. Včasih spremembe razmeroma

Kinetična atomistika s statistično mehaniko in njuni kritiki



Slika 7: Raziskovanja atomov in etra s Teslovo kritiko statistične mehanike ob vztrajanju pri etru za brezžični prenos energij na predavanju članom Ameriškega instituta elektroinženirjev na univerzi Kolumbija v New Yorku 29. 5. 1891. Tesla je s svojo vizijo pozneje ostal osamljen.

urno povzročijo preobrate v osnovnem toku raziskovanja kot v primeru Galileja ali Einsteina. Ob drugih priložnostih kaže še čakati, da se spremembe uveljavijo, denimo pri Teslovih vizijah etra, ki ga je njegov učitelj Sekulić že opustil.

Einsteinova posebna teorija relativnosti je oblikovala novo fiziko, vendar je njegova splošna teorija še vedno predmet različni razlag. Enako velja za Teslov brezžični prenos energije energijskih žarkov. Možno je, da jih je Tesla podedoval od Sekulića, gotovo pa je vmes veliko Teslovih zamisli. Zato spodnja tabela ponuja več idej, ki jih je Tesla spoznal med šolanjem. Tesla je ljubosumno

čuval domnevo, da bi lahko njegov eter prenašal velike energije na dolge razdalje, čeravno njegove vizije ni sprejelo akademsko občestvo. Moderna astrofizika s časovnimi stroji, kvantnimi skoki in črvinami Boltzmannovega zeta Ludwiga Flamma zasnovanimi med Prvo Svetovno vojno leta 1916, Johna Archibalda Wheelera (1957) in njegovega mormonskega študenta Kipa Stephena Thorneja (* 1940) znova predpostavljajo Teslov prenos znatnih energij-mas na velike razdalje. Teorija v ozadju skoraj meji na znanstveno fantastiko in ni enaka Teslovim vizijam, vendar se v nekem globljem smislu nedvomno nave-

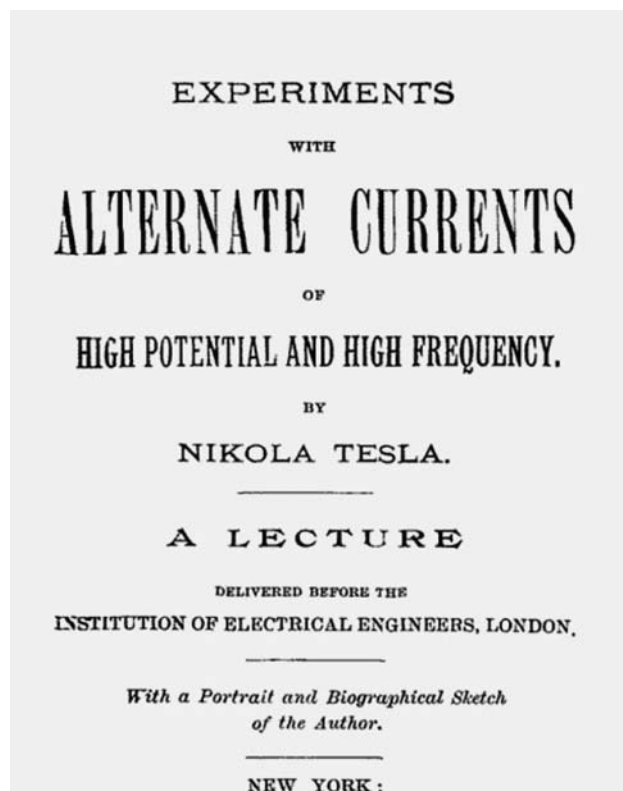
Preglednica 2: Tesli znane ideje

Izumitelji in ideje	Zagovornik	Nasprotnik
Clausiusova kinetična teorija z izogibanjem etru	Sekulić, 1874, 110, 121, 147, 151; ²² Prica, 1883 ²³	Karl Robida
Tyndalova kinetična teorija z izogibanjem etru	Sekulić, 1874, 110; Pisko-Hessler, 1866; ²⁴ Stefan	Robida
Eter	Beer, 1853; ²⁵ Tesla po letu 1891	Sekulić, 1874, 111; Šubic 1862; ²⁶ Michelson-Morley 1876; Kunzek ignoriral eter leta 1851; Einstein 1905
Energija Voltne baterije sorazmerne proizvedeni toploti proti afiniteti nepovezani s toploto kemijske reakcije, temveč odvisni od maksimalnega dela reverzibilne reakcije	Martin Sekulić 1878; Hans Peter Jørgen Julius Thomsen (1826–1909) na univerzi Kopenhagen leta 1854; teorija afinitete Marcellina Berthelota (1827–1907) leta 1864	Nekdanji rektor munchske politehnike Wilhelm von Beetz (1822–1886), direktor dunajskega fizikalno-kemijskega Instituta Franz Serafin Exner (1849–1929), Hermann Helmholtz v Berlinu 1882; Jožef Stefan pri Dunajski akademiji 21. 6. 1878; Wiedemannovi <i>Annalen der Physik</i>
Širjenje plinov: Davyjeva rotacija	Humphry Davy	Sekulić, 1874, 135
Širjenje plinov: Joule-Tyndalova translacija	Sekulić, 1874, 135 z modifikacijami; Tobias Gruber	Erasmus Darwin; Humphry Davy
Dve zvrsti elektrike v delcih	Grothius, De le Rive, Berzelius, Fechner; Sekulić, 1874, 140 odvisno od smeri vrtenja	Magnus, Ampère
Ena sama vrsta elektrike v delcih	Magnus, Ampère za anione in katione	Sekulić, 1874, 140 odvisno od smeri vrtenja
Stefan-Boltzmannova kinetična teorija	Štrkljević, 1877; ²⁷ Stefanova optika v Poggendorffovih <i>Annalen</i> 1865 (Pisko-Hessler, 1866, 1170); Helmholtzeva ohranitev energije v <i>Mlada Srbadija</i> 1872	Šubic, 1874 ²⁸
Regnaultove termodinamične meritve	Sekulić v <i>Jahresbericht</i> 1875, 3–15, 16–25, 34, 41–42, 44, 50, 54; Prica 1883, 5, 7, 16; Zeuner, 160, 5; ²⁹ Pisko-Hessler, 1866, 1074, 1369, 1373	
Enoten zakon teorije vsega	Bošković 1758; Le Sage; Luka Lavtar 1872; Ampère; Sekulić 1874, 152; Gell Man	Moffrat ³⁰
Enotna sila in teorije vsega	Königov odboj 1857	Privlak in odboj med molekulami (Kunzek, 1851; Kunzek, 1850) ³¹
Atomi	Molekule in pore imajo različno število in velikost v različnih telesih, kar dela razliko v gostotah (Kunzek, 1851, 12); Ampèrova in Gaudinova teorija molekul (Becquerel, 1845, 2: 220–222, 224); Sekulić; Ettingshausen; Stefan	Mach, Zeuner?
Elektromagnetno delovanje na razdaljo	December 1856 Riessovo pismo Faradayu v podporo stari statični teoriji elektrike (Reiss, 1867). ³²	Faradayevo pismo Riessu 1856 dovoljuje delovanje na daljavo le indukciji in težnosti (Riess, 1867, 43)
Praktično takojšnje delovanje elektromagnetizma	Faraday 1856 (Riess, 1867, 43)	Riess 1856 (Riess, 1867, 43); Boltzmann; Klemenčič
Bunsen-Kirchhoffova spektralna analiza Aprila 1860	Helmholtz; Ignjat Bartulić, 1870; ³³ Sekulić	
Siemens-Halske kazalčni-telegraf	Galle, Zetzsche, 1870 ³⁴	Slabyjeva AEG
Dunajska Telegrafaska Konferenca 1868	Galle, Zetzsche, 1870, 368–370	ZDA telegrafi se ne pridružijo Konferenci
Edisonova telegrafija in žarnice	Zetzsche, 1877; ³⁵ Tivadar Puskás v Budimpešti	Napeti odnosi Tesla-Edison v 1890ih
Tehniško nikoli dokazan brezžični prenos energije	Tesla 1891, 1935, in 12/5/1938; Teslovi oboževalci 21. stoletja	Einsteinova Relativnost in Bohrova kvantna mehanika
Tehniško nikoli dokazane vizije energijskih žarkov	Tesla 1891, 1935, 1938; časovni stroji, kvantni skoki, črvine Flamma, Wheelerja, Thorna ³⁶	Teorija relativnosti in kvantna mehanika 20. stoletja

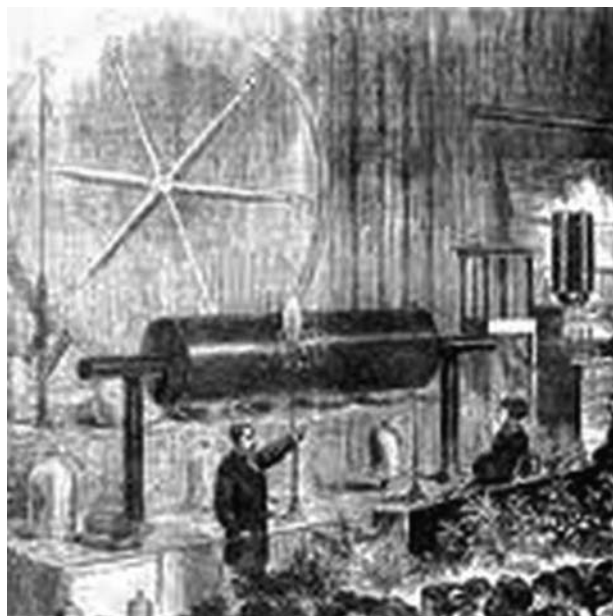
zuje nanje. Čeprav so Flammove ideje dozorele v aktivnih časih Tesle ob rušenju Teslovega stolpa, jih je Tesla bržkone spregledal. Kot Boltzmann v svojih zadnjih letih, tudi podobno neprilagodljivi Tesla ni upošteval vseh znanstvenih novosti po svojem polomu s stolpom, medtem ko je Einstein v svojih poznih letih na uglajen način še vedno spodbujal obojetne ideje svojih dopisovalcev po vsem svetu.

Zaključek

Teslov profesor Sekulić je spadal med vodilne raziskovalce spektroskopija svoje dobe, svoje ideje pa je objavljaj tako pri zagrebški akademiji, kot v vodilnih nemških revijah. Nadarjenega Teslo si je izbral za demonstratorja, kar je Tesli omogočilo prve korake v svet eksperimentov. Sekulić je za svoja raziskovanja naročal najnovejše naprave in knjige, ki jih premladi Tesla morda ni vedno docela razumel. So ga pa spodbijale k nadaljnjemu snovanju. Po upokojitvi in H. Helmholtzevi kritiki leta 1882 Sekulić ni več objavljaj znanstvenih razprav. Sekulić je skušal dokazati, da je energija kemijske reakcije sorazmerna proizvedeni toploti,³⁷ morda po



Slika 8: Gradivo za Teslovo predavanje v Londonu za Ustanovo elektroinženirjev in Kraljevo Družbo februarja leta 1892 pod naslovom »Poskusi z izmeničnimi tokovi visokih napetosti in velikih frekvenc«, tik preden ga je novica o materini bolezni zvalila nazaj v domovino.



Slika 9: Tesla predava v Londonu leta 1892.

vзору na Zakon o ohranitvi energije sprejet v njegovih deških letih. Tako je podprl teorijo svojega prijatelja Hansa Petra Jørgena Juliusa Thomsena (1826–1909) z univerze v Kopenhagnu objavljene leta 1854 in potrjevalne desetletje pozneje s teorijo Marcellina Berthelota (1827–1907). Vendar je bil Zakon o ohranitvi energije že zunaj poglobitnih raziskav, ki so se veliko bolj vrtele okoli entropije 2. zakona. Tako se Sekulićeve domneve niso skladale z rezultati meritev nekdanjega direktorja Münchenske Politehnike Wilhelm von Beetz (1822–1886), Stefanovega študenta direktorja prej Loschmidtovega dunajskega fizikalno-kemijskega inštituta Franza Serafina Exnera (1849–1929) in Hermann Helmholtza v Berlinu leta 1882. Helmholtz je pojasnil, da afiniteta ni povezana s toploto kemijske reakcije, temveč z največjim delom reverzibilne reakcije. V tem času je bil Tesla že dodobra zasidran v elektrotehniki, Sekulića pa je znova srečal desetletje pozneje v Zagrebu. Sekulić je bil na Teslov razred gotovo izjemno ponosen, saj je kar polovica Teslovih sošolcev študirala kemijsko-fizikalne vede na Dunaju in Gradcu, le Tesli pa se je študij ponesrečil. Teslove ideje so namreč potrebovale ameriških potrditev pod niagarskimi slapovi za sprejem v njegovem domačem evropskem okolju.

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Abstract

Tesla's First Footsteps into Chemical Sciences

For the first time a complete list of Tesla's High School professors is provided. The excellency of his lecturers of chemistry and related sciences is put into the limelight. The books and articles about chemistry which Tesla read in his High School times are focused together with the related published works of his teachers. The pioneering spectroscopic research of Kirchhoff and Bunsen began in Tesla's early youth and had a considerable impact on Tesla's professors, especially on Martin Sekulić.

Keywords: Nikola Tesla, Rakovac by Karlovac, Martin Sekulić. Gustav Kirchhoff, Antoine Cesar Becquerel, History of Education in Chemistry and Electro-technics.

48. Mednarodna kemijska olimpijada 2016 v Gruziji

Tekst in foto: Andrej Godec

Letošnja mednarodna kemijska olimpijada je potekala od 23. julija do 1. avgusta v Tbilisiju, Gruzija. Na olimpijadi je sodelovalo 264 dijakov iz 73 držav.

Vsako državo zastopa ekipa, sestavljena iz največ štirih dijakov in dveh mentorjev. Slovenijo so letos zastopali Uroš Prešern in Tristan Kovačič (oba gimnazija Novo mesto), ter Vid Kermelj in Martin Rihtaršič (oba gimnazija Škofja Loka). Uroš je osvojil srebrno medaljo, Tristan, Vid in Martin pa bronaste. Vsem štirim iskrene čestitke!

Mentorja ekipe sta bila dr. Andrej Godec in dr. Darko Dolenc, oba UL, FKKT.

jila. Gruzija je kot druga na svetu (prva je bila Armenija) že v prvi polovici 4. stoletja sprejela krščanstvo kot državno vero. Danes je več kot 83 % prebivalcev pravoslavne vere, okrog 10 % pa je muslimanov.

Nekje iz tega obdobja so tudi zametki gruzijske pisave, ki je res nekaj posebnega, če upoštevamo, da ima država danes okrog 4,5 milijona prebivalcev. Med vplivi na pisavo omenimo grščino, vendar boste včasih kljub poznavanju grških črk presenečeni, saj imajo v gruzijščini čisto drugo obliko in tudi drugačen zvok. Še bolj zabavno pa je, da je včasih črka, ki izgleda kot soglasnik, v resnici samoglasnik.



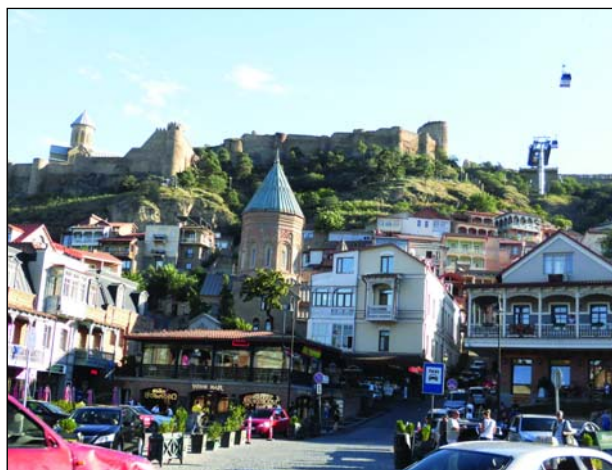
Slovenska ekipa na kemijski olimpijadi 2016: z leve strani Martin, Tristan, Vid, Uroš, Darko in Andrej.

Za organizacijo letošnje olimpijade je bil sicer najprej izbran Pakistan; po pritožbah in varnostnih pomislekih predvsem predstavnikov večjih zahodnih držav pa je Pakistan odpovedal. Na srečo je vskočila Gruzija, kateri je uspel neverjeten podvig. V štirih mesecih je ob strokovni pomoči Madžarov in Rusov organizirala in tudi odlično izpeljala olimpijado.

Gruzija (საქართველო, transliteracija je Sakartvelo), je zaradi svoje lege na stičišču svetov, Evrope in Azije, zelo bogata z zgodovino. Tod so šle vse večje vojske tega sveta, nobena pa ni Gruzije v celoti in za dolgo osvo-

Današnja Gruzija je nastala iz starodavnih kraljestev Kolkheti in Kavkaške Iberije. Na tem področju naj bi se nahajalo tudi zlato runo, ki so ga iskali in ukradli Jazon in Argonavti. Na begu pred zasledovalci so prišli vse do Nauportusa (današnje Vrhniko).

Zlato obdobje za to državo je bilo enajsto in dvanajsto stoletje, ko sta vladala kralj David IV (Graditelj) in še posebej njegova vnukinja kraljica Tamar. To je bil čas kulturne renesance in začetkov organizirane izobrazbe, še danes pa lahko občudujemo mnoge cerkve s freskami iz tega obdobja.



Na sliki levo je kvevri, glinen vrč za pridelavo vina. Na desni je staro mestno jedro Tbilisija.

Med pomembnimi Gruzijci pa je tudi Ioseb Besarionis Jughashvili (Stalin), pred in po drugi svetovni vojni 25 let voditelj sovjetske zveze in tiran, ki je dal umoriti veliko svojih sodelavcev in jih na milijone poslal v sibska delovna taborišča. Kljub vsemu so njegov kip v Goriju, kjer se je rodil, odstranili šele leta 2010.

Gruzija, ki je bila del sovjetske zveze, je leta 1991 razglasila neodvisnost, vendar je imela tudi kasneje probleme z Rusijo, vključno s kratko vojno leta 2008. Danes je Gruzija turistično zelo ambiciozna država. Ponosni so na svojo zgodovino, na hrano, ki je nekoliko podobna turški z večjim poudarkom na mesu, in še posebej na svoje vino. Pridelava vina je na tem področju posebna in stara že več kot osem tisočletij; posebnost je to, da natočijo mošt skupaj z grozdniimi kožicami v velike keramične vrče (*kvevri*, ქვევრი), ki jih nato zakopljejo v zemljo in pustijo tam več mesecev. Vina imajo zato posebno barvo in okus. Tipični kvevri ima prostornino tam do 1000 L, obstajajo pa desetkrat večji. Tudi pri nas so danes v modi t. im. oranžna vina, kjer gre za daljši stik mošta in kožic.

Gruzija se nahaja ob obali Črnega morja, meji pa na Rusijo, Turčijo, Armenijo in Azerbejdžan. Bruto domači proizvod znaša malo več kot 9000 evrov na prebivalca. Glavno mesto Tbilisi (orig.: თბილისი, včasih Tiflis, »toplo mesto« zaradi žvepljenih vrelcev) se razteza med hribi na obeh bregovih reke Mtkvari. Staro je več kot tisoč petsto let, danes pa ima okrog 1,5 milijona prebivalcev. Odlikuje ga zelo lepo staro mestno jedro, impozantne pa so tudi lesene hiše, zgrajene na navpičnih skalah nad reko. Zanimiv je tudi metro sistem, kjer gredo tiri za vlake večkrat pod reko, in so zato metro postaje zelo globoke.

Tbilisi je bil gostitelj letošnje kemijske olimpijade. Na otvoritveni slovesnosti nas je nagovoril minister za znanost in izobraževanje Aleksandre Jejelava, videli pa smo tudi nekaj gruzijskih značilnih plesov in poslušali polifonično petje zgodnjekrščanskega izvora.

Po pravilih olimpijade organizator takoj po otvoritvi loči dijake od mentorjev. Dijaki imajo sicer dva tekmovalna dneva, ostalih sedem pa je nabitih z raznimi aktivnostmi in izleti. Urnik mentorjev pa je ravno obraten; večino



Levo je samostan Jvari pri Mtskheti. Desno je laboratorijski pult tekmovalca.

časa porabimo za usklajevanje tekstov nalog, prevajanje in na koncu ocenjevanje.

Mentorji smo takoj po otvoritvi odšli na ogled laboratorijev, kjer bodo dijaki opravljali eksperimentalni del tekmovanja. Laboratorije je dala na voljo kmetijska univerza v Tbilisiju, delovni pult dijaka pa lahko vidite na spodnji sliki.

Eksperimentalni del tekmovanja je bil letos sestavljen iz treh nalog, za katere so imeli 5 ur časa. Prva je bila identifikacija 5 raztopin neznanh spojin. Dijaki so imeli na voljo le raztopino HNO_3 , raztopino NaOH , heksan in vodne raztopine 10 čistih spojin: AgNO_3 , $\text{Al}_2(\text{SO}_4)_3$, $\text{Ba}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, KI , KIO_3 , Na_2CO_3 , Na_2SO_3 , MgCl_2 , in NH_3 .

Druga praktična naloga je bila določitev koncentracije fluorida in klorida v mineralnih vodah, s katerimi je Gruzija bogato obdarjena. Fluorid so določali kolorimetrično, klorid pa s titracijo po Volhardovi metodi.

Tretja praktična naloga je bila določitev dišav in arom. Dijaki so dobili 8 vzorcev neznanh organskih spojin, ki se v industriji uporabljajo kot dišave. Z uporabo različnih testov (test s KMnO_4 , cerijevim(IV) nitratom, 2,4-dinitrofenilhidrazinom, in železovim(III) hidroksamatom) so morali identificirati neznane spojine, in napisati enačbe reakcij.

Dva dni kasneje smo usklajevali in prevajali še teoretične naloge. Teh je bilo osem, dijaki pa so imeli na voljo 5 ur časa za reševanje.

Prva teoretična naloga je bila na temo stabilnosti spojin NF_3 , NHF_2 in NH_2F , ter tetrafluoroamonijevih soli, ki so bile predmet raziskav zaradi možne uporabe kot trdna raketna goriva; to je zato, ker se iz njih pri segrevanju sproščata NF_3 in F_2 .

Druga naloga je bila v zvezi z rdečim bakrovim(I) oksidom. To je eden od prvih materialov, ki so ga uporabili v elektroniki. Danes je ponovno zanimiv, ker bi lahko bil netoksična in poceni komponenta v sončnih celicah. Dijaki so morali določiti kristalno strukturo tega oksida, in z uporabo termodinamskih podatkov (standardnih tvorbenih entalpij in entropij) ugotoviti temperaturno območje, kjer bi bili baker ali njegovi oksidi termodinamsko stabilni.

	$\Delta_f H^\circ / \text{kJ mol}^{-1}$	$S^\circ / \text{J mol}^{-1} \text{K}^{-1}$
Cu(s)	0	65
$\text{O}_2(\text{g})$	0	244
CuO(s)	-156	103
$\text{Cu}_2\text{O(s)}$	-170	180

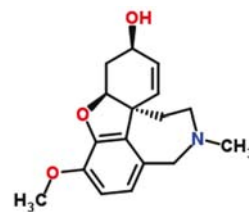
Tretja naloga je bila določanje joda v soleh, s katerimi jodirajo kuhinjsko sol. Jodat določajo z jodometrično titracijo, jodid pa s potenciometrično (argentometrično) titracijo, ki pa je občutljiva na prisotnost klorida. Za oba primera so morali dijaki izračunati ustrezne koncentracije. Bolj praktična analizična metoda, ki ni občutljiva na prisotnost klorida, je reakcija H_3AsO_3 s Ce(IV) , ki daje v kisli raztopini Ce(III) , in je močno katalizirana z

jodidom. Za to reakcijo so morali dijaki iz spektrofotometričnih meritev določiti hitrost in delne rede glede na posamezne snovi.

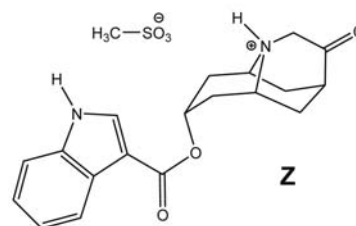
Četrta naloga je bila uporaba kinetičnih študij za analizo vode. 1,4-dioksan, znan pod imenom dioksan ($\text{C}_4\text{H}_8\text{O}_2$), je industrijsko topilo in stranski produkt, ter hud onesnaževalec vode. Lahko pa ga oksidiramo v netoksično obliko, če uporabimo oksidante kot so peroksodisulfat, ozon ali vodikov peroksid. Dijaki so morali na osnovi mehanizma teh reakcij določiti hitrost razpada dioksana. Razen tega so morali izračunati še hitrost hidrolize lizin acetilsalicilata, ki se pod trgovskim imenom Aspegic uporablja kot sredstvo proti bolečinam in proti vnetju, ter aspirina. Hidroliza je odvisna od pogojev, naprimer pH, in morali so izračunati še vpliv pH na potek reakcije.

Peta naloga je bila na temo barvil: pred 5500 leti so v antičnem Egiptu odkrili način za sintezo modrega pigmenta, ki je danes znan pod imenom egipčansko modro. Približno 2000 let kasneje so na Kitajskem znali pripravljati podoben pigment, ki mu danes pravimo kitajsko modro. Pigmenta imata podobno strukturo, vendar različno elementno sestavo. Dijaki so morali na osnovi podatkov o sintezi določiti formuli obeh pigmentov.

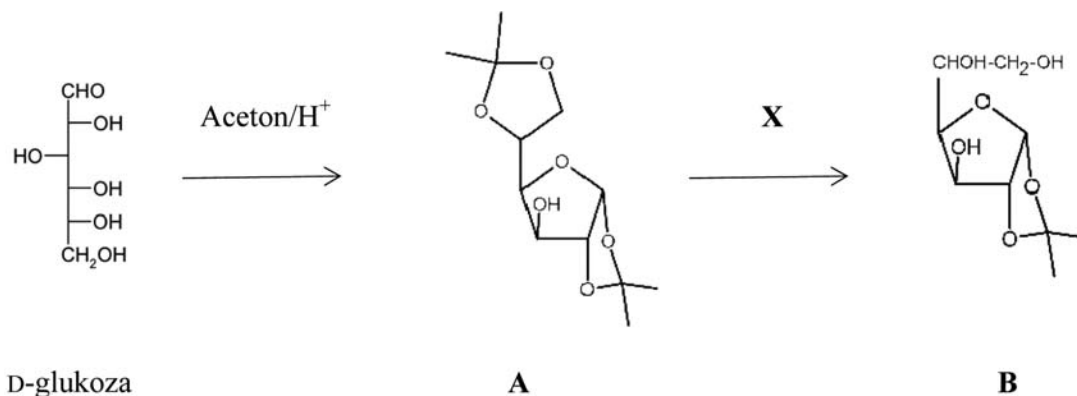
Šesta naloga je bila na temo bolezni in zdravil zanje: danes še ne poznamo zdravila za Alzheimerjevo bolezen, vendar pa obstajajo zdravila, s katerimi blažimo nevrodegenerativne spremembe. Med temi so inhibitorji acetilholinesteraze, primer je galantamin. To spojino lahko izoliramo iz kavkaške rastline, avtohtone v Gruziji. Večje količine te spojine, uporabne za zdravljenje, lahko pridobimo z industrijsko sintezo. Dijaki so morali predlagati reagentne in opisati vmesne produkte pri industrijski sintezi galantamina.



Sedma naloga je obravnavala sintezo *dolasetron mezilata* (Z), zdravilne učinkovine, ki se trži pod imenom *Anzemet* in se uporablja za lajšanje postoperativne slabosti in bruhanja. V tej nalogi so morali dijaki določiti empirične formule posameznih vmesnih produktov, in narisati njihove strukture. Razen tega so morali pokazati še dobro poznavanje stereokemije.



Osmi naloga je bila na temo eksotičnega, vendar biološko pomembnega sladkornega analoga, ki ga lahko pripravimo iz D-glukoze na naslednji način: najprej segregamo D-glukozo z acetonom v prisotnosti nekaj kapljic močne kisline, pri čemer nastane diacetonid **A**. Tega lahko selektivno delno hidroliziramo v **B**.



Dijaki so morali odgovarjati na vprašanja o stereo-kemiji navedenih spojin. Razen tega so morali predvideti produkte reakcij spojine B z izbranimi reagenti v specifičnih pogojih.

Naloge so bile v splošnem težke in obsežne. Pokrivalo so vsa področja kemije, poudarek pa je na fizikalni in organski kemiji. Več o olimpijadah najdete v naši spletni učilnici Kemljub (<http://skupnost.sio.si/login/index.php>), kjer bodo objavljene tudi rešitve nalog.

Najboljši dosežek na letošnji olimpijadi je bil skoraj 97 % (dijak iz Romunije), naš najboljši Uroš Prešern pa je

dosegel malo pod 71 %, kar je pomenilo odličen rezultat, ki mu je prinesel srebrno medaljo. Veseli smo predvsem, da naši dijaki tudi v praktičnem delu dosegajo vedno boljše rezultate.

Več informacij dobite na spletni strani olimpijade: <http://www.icho2016.chemistry.ge/>.

Priprave na olimpijado potekajo na Fakulteti za kemijo in kemijsko tehnologijo v Ljubljani. Letos so pri pripravi sodelovale naslednje sodelavke in sodelavci: dr. Helena Prosen, dr. Alojz Demšar, dr. Darko Dolenc in dr. Andrej Godec. Vsem se najlepše zahvaljujemo.

Pri organizaciji udeležbe na olimpijadi sodelujemo z Zvezo za tehnično kulturo Slovenije, ki se ji prav tako iskreno zahvaljujemo, kot tudi Slovensku kemijskemu društvu, kjer je sedež Odbora za pripravo kemijske olimpijade.

KOLEDAR VAŽNEJŠIH ZNANSTVENIH SREČANJ S PODROČJA KEMIJE IN KEMIJSKE TEHNOLOGIJE

SCIENTIFIC MEETINGS – CHEMISTRY AND CHEMICAL ENGINEERING

October 2016

3 – 5	INTERNATIONAL SYMPOSIUM ON APPLIED CHEMISTRY (ISAC) 2016 Tangerang, Indonesia Information: http://situs.opi.lipi.go.id/isac2016
3 – 6	16 TH INTERNATIONAL NUTRITION & DIAGNOSTICS CONFERENCE Prague, Czech Republic Information: http://www.indc.cz/en/conference/conference-venue/
4 – 7	IUPAC-PSK40 CONFERENCE ON ADVANCED POLYMERIC MATERIALS Jeju, South Korea Information: www.psk40.orgs
7 – 9	JOINT INTERNATIONAL CONFERENCE OF THE HELLENIC CRYSTALLOGRAPHIC ASSOCIATION AND THE HELLENIC SOCIETY FOR COMPUTATIONAL BIOLOGY AND BIOINFORMATICS – HeCrA-HSCBB16 Athens, Greece Information: https://sites.google.com/site/hecrashscbb16/
9 – 12	XXVII INTERAMERICAN CONGRESS OF CHEMICAL ENGINEERING Cuzco City, Peru Information: http://ciiq.pe/
9 – 12	SPICA 2016 – 16 TH INTERNATIONAL SYMPOSIUM ON PREPARATIVE AND INDUSTRIAL CHROMATOGRAPHY AND ALLIED TECHNIQUES Vienna, Austria Information: www.spica2016.org
11 – 13	INTERNATIONAL CONFERENCE AND EXHIBITION FOR FILTRATION AND SEPARATION TECHNOLOGY Cologne, Germany Information: http://www.filtech.de/
12 – 13	DYNAMICS OF EVOLVING FLUID INTERFACES – DEFI 2016 Lyon, France Information: http://www.rs-defi2016.com/
12 – 14	2 ND INTERNATIONAL EPNOE JUNIOR SCIENTISTS MEETING Sophia-Antipolis, France Information: http://chem-soc.si/novice/2nd-international-epnoe-junior-scientists-meeting
14 – 19	12 TH IUPAC INTERNATIONAL CONFERENCE ON NOVEL MATERIALS AND THEIR SYNTHESIS (NMS-XII) Changsha, China Information: http://www.nms-iupac.org/present/
16 – 19	GREEN SOLVENTS CONFERENCE Kiel, Germany Information: http://dechema.de/en/gsc2016.html

- 16 – 19 3RD INTERNATIONAL CONFERENCE ON BIOINSPIRED AND BIOBASED CHEMISTRY & MATERIALS
Nice, France
Information: <http://www.nice2016-conference.com>
- 16 – 19 CSChe 2016 – 66TH CANADIAN CHEMICAL ENGINEERING CONFERENCE
Quebec, Canada
Information: <http://www.csche2016.ca/>
- 17 – 19 INTERNATIONAL CONFERENCE ON APPLIED CRYSTALLOGRAPHY
Houston, United States
Information: <http://www.iucr.org/calendar/events/topics/general/international-conference-on-applied-crystallography>
- 17 – 19 ORGANIC PROCESS RESEARCH & DEVELOPMENT
Prague, Czech Republic
Information: <https://www.scientificupdate.co.uk/index.php/conferences/conferences-and-workshops.html>
- 18 – 19 2016 EUROPEAN INVERSE GAS CHROMATOGRAPHY (iGC) SYMPOSIUM
London, United Kingdom
Information: <http://surfacemeasurementsystems.com/2016-european-igc-symposium/>
- 18 – 19 INTERNATIONAL CONFERENCE ON APPLIED SCIENCE ENGINEERING AND TECHNOLOGY (ICASET-16)
Chittoor, India
Information: <http://www.icaset.in/>
- 20 – 23 36TH AUSTRALASIAN POLYMER SYMPOSIUM
Lorne, Victoria Australia
Information: <http://www.36aps.org.au/>
- 21 – 23 CONGRESS OF CHEMISTS AND CHEMICAL ENGINEERS OF BOSNIA AND HERZEGOVINA
Sarajevo, Bosnia and Herzegovina
Information: <http://www.pmf.unsa.ba/hemija/kongres/index.php/en>
- 24 – 26 17TH INTERNATIONAL BIOTECHNOLOGY SYMPOSIUM AND EXHIBITION (IBS 2016)
Melbourne, Australia
Information: www.ibs2016.org
- 24 – 26 CHEMICAL RESEARCH IN FLANDERS – CRF
Blankenberge, Belgium
Information: http://www.efce.info/Chemical+Research+in+Flanders+_+CRF-p-20000670.html

November 2016

- 3 – 5 PROCESS INTENSIFICATION COLLOQUIUM 2016
Izmir, Turkey
Information: <http://pic2016.org/>
- 8 – 10 TeQ – CHEMICAL AND PROCESS TECHNOLOGY 2016
Rio de Janeiro, Brazil
Information: <http://tecnologiaquimica.com.br/en/>

December 2016

- 11 – 16 21ST IUPAC INTERNATIONAL CONFERENCE ON ORGANIC SYNTHESIS (ICOS 21)
Mumbai, India
Information: www.chem.iitb.ac.in/icos21/

2017**February 2017**

- 22 – 24 INTERNATIONAL CONFERENCE ON CATALYSIS AND CHEMICAL
ENGINEERING
Baltimore, USA
Information: <https://unitedscientificgroup.com/conferences/catalysis/index>

April 2017

- 10 – 13 ELECTROSTATICS 2017
Frankfurt am Main, Germany
Information: <http://www.dechema.de/en/electrostatics2017.html>
- 19 – 22 25TH CROATIAN MEETING OF CHEMISTS AND CHEMICAL ENGINEERS
Porec, Croatia
Information: <http://25hskiki.org/en/homepage/>

May 2017

- 7 – 11 SETAC EUROPE 27TH ANNUAL MEETING
Brussels, Belgium
Information: <http://www.setac.org/events/EventDetails.aspx?id=683532&group=>
- 18 – 21 29TH EUROPEAN SYMPOSIUM ON APPLIED THERMODYNAMICS
Bucharest, Romania
Information: <http://jetc2017.hu/>
- 21 – 25 12TH ADVANCED POLYMERS VIA MACROMOLECULAR ENGINEERING
(APME 2017)
Ghent, Belgium
Information: <http://www.ldorganisation.com/apme2017>
- 23 – 25 14TH JOINT EUROPEAN THERMODYNAMICS CONFERENCE 2017
Budapest, Hungary
Information: <http://jetc2017.hu/>
- 25 – 27 MaCKiE-2017 – INTERNATIONAL CONFERENCE ON MATHEMATICS IN CHEMICAL
KINETICS AND ENGINEERING (MaCKiE)
Budapest, Hungary
Information: <http://www.mackie-workshops.com/>

June 2017

- 6 – 10 8TH INTERNATIONAL SYMPOSIUM ON MACRO- AND SUPRAMOLECULAR
ARCHITECTURES AND MATERIALS
Sochi, Russian Federation
Information: www.mam-17.org
- 11 – 16 COLLOQUIUM SPECTROSCOPICUM INTERNATIONALE XL (CSI-XL)
Pisa, Italy
Information: www.csi-conference.org
- 13 – 15 V INTERNATIONAL SYMPOSIUM ON RELIABLE FLOW OF PARTICULATE SOLIDS
Skien, Norway
Information: <http://www.relpowflo.no/>
- 18 – 22 ICCE 2017 – 16TH EUCHEMS INTERNATIONAL CONFERENCE ON CHEMISTRY AND
THE ENVIRONMENT
Oslo, Norway
Information: <http://www.icce2017.org/>

- 19 – 21 6TH EUROPEAN DRYING CONFERENCE
Liège, Belgium
Information: <http://efce.info/EuroDrying+2017.html>
- 19 – 23 9TH INTERNATIONAL SYMPOSIUM ON MOLECULAR MOBILITY AND ORDER IN POLYMER SYSTEMS
Saint-Petersburg, Russian Federation
Information: <https://iupac.org/event/mmops2017/>
- 25 – 29 INTERNATIONAL SYMPOSIA ON ORGANOMETALLIC CHEMISTRY DIRECTED TOWARDS ORGANIC SYNTHESIS (OMCOS 19)
Jeju Island, Republic Of Korea
Information: <https://iupac.org/event/omcos-19/>

July 2017

- 2 – 5 4TH EUCHEMS INORGANIC CHEMISTRY CONFERENCE – EICC-4
Copenhagen, Denmark
Information: <http://www.euchems.eu/events/4th-euchems-inorganic-chemistry-conference-eicc-4/>
- 2 – 6 INTERNATIONAL SYMPOSIUM ON MACROCYCLIC AND SUPRAMOLECULAR CHEMISTRY IN CONJUNCTION WITH ISACS: CHALLENGES IN ORGANIC MATERIALS & SUPRAMOLECULAR CHEMISTRY
Cambridge, United Kingdom
Information: <http://www.rsc.org/events/detail/17933/international-symposium-on-macrocyclic-and-supramolecular-chemistry-in-conjunction-with-isacs-challenges-in-organic-materials-and-supramolecular-chemistry>
- 2 – 7 16TH EUROPEAN POLYMER CONGRESS
Lyon, France
Information: <http://www.europolyfed.org/home>
- 7 – 10 10TH INTERNATIONAL SYMPOSIUM ON CATALYSIS IN MULTIPHASE REACTORS (CAMURE-10) & 9TH INTERNATIONAL SYMPOSIUM ON MULTIFUNCTIONAL REACTORS (ISMR-9)
Tsingtao (Qingdao), PR China
Information: <http://camure2017.csp.escience.cn/dct/page/1>
- 9 – 13 EuCOMC 2017 – 22ND EUROPEAN CONFERENCE ON ORGANOMETALLIC CHEMISTRY
Amsterdam, The Netherlands
Information: <http://www.eucomc2017.amsterdam/>
- 9 – 14 46TH IUPAC WORLD CHEMISTRY CONGRESS (IUPAC-2017)
São Paulo, Brazil
Information: www.IUPAC2017.org
- 23 – 29 RACI CENTENARY CONGRESS
Melbourne, Australia
Information: <http://www.racicongress.com>

August 2017

- 13 – 17 SE2017 – 200 YEARS OF SELENIUM RESEARCH
Stockholm, Sweden
Information: <http://se2017.se/>
- 20 – 23 GLS-13 – 13TH INTERNATIONAL CONFERENCE ON GAS-LIQUID AND GAS-LIQUID-SOLID REACTOR ENGINEERING (GLS-13)
Brussels, Belgium
Information: <http://www.gls13.com/>

- 28 – Sept. 1 EuroAnalysis 2017
Stockholm, Sweden
Information: <http://euroanalysis2017.se/>
- 28 – Sept. 2 11TH ICHC – 11TH INTERNATIONAL CONFERENCE ON THE HISTORY OF CHEMISTRY
Trondheim, Norway
Information: <http://www.ntnu.edu/11ichc>

September 2017

- 3 – 6 3RD EuGSC – 3RD EuCheMS CONGRESS ON GREEN AND SUSTAINABLE CHEMISTRY
York, UK
Information: <http://www.euchems.eu/events/3rd-eugsc-3rd-euchems-congress-on-green-adn-sustainable-chemistry/>
- 3 – 8 21ST EUROPEAN CONFERENCE ON THERMOPHYSICAL PROPERTIES
Graz, Austria
Information: <http://ectp2017.tugraz.at/>
- 5 – 8 THERMODYNAMICS 2017
Edinburgh, UK
Information: <http://www.thermodynamics2017.efconference.co.uk/>

October 2017

- 1 – 5 EPIC 2017 – 6TH EUROPEAN PROCESS INTENSIFICATION CONFERENCE 2017
Barcelona, Spain
Information: <http://www.wcce10.org/index.php/en/>
- 1 – 5 WCCE10 – 10TH WORLD CONGRESS OF CHEMICAL ENGINEERING
INCORPORATING THE 11TH EUROPEAN CONGRESS OF CHEMICAL ENGINEERING
(ECCE11)
Barcelona, Spain
Information: <http://www.wcce10.org/index.php/en/>
- 1 – 5 4TH EUROPEAN CONGRESS OF APPLIED BIOTECHNOLOGY – ECAB3
Barcelona, Spain
Information: <http://www.wcce10.org/index.php/en/>

November 2017

- 5 – 9 HPLC 2017 – THE 46TH INTERNATIONAL SYMPOSIUM ON HIGH PERFORMANCE
LIQUID PHASE SEPARATIONS AND RELATED TECHNIQUES
Jeju Island, Republic Of Korea
Information: <http://www.hplc2017-jeju.org>

2018

June 2018

- 4 – 6 IIS PRAGUE 2018 – 13TH INTERNATIONAL SYMPOSIUM ON THE SYNTHESIS AND
APPLICATIONS OF ISOTOPES AND ISOTOPICALLY LABELLED COMPOUNDS
Prague, Czech Republic
Information: <http://www.iis-prague2018.cz/>

September 2018

- 16 – 19 DISTILLATION & ABSORPTION CONFERENCE 2018
Firenze, Italy
Information: <http://www.aidic.it/da2018/>

Acta Chimica Slovenica

Author Guidelines

Submissions

Submission to ACSi is made with the implicit understanding that neither the manuscript nor the essence of its content has been published in whole or in part and that it is not being considered for publication elsewhere. All the listed authors should have agreed on the content and the corresponding (submitting) author is responsible for having ensured that this agreement has been reached. The acceptance of an article is based entirely on its scientific merit, as judged by peer review. There are no page charges for publishing articles in ACSi.

Submission material

Typical submission consists of:

- full manuscript (Word file, with title, authors, abstract, keywords, figures and tables embedded, and references);
- supplementary files:
 - **Statement of novelty** (Word file),
 - **List of suggested reviewers** (Word file),
 - ZIP file containing **graphics** (figures, illustrations, images, photographs),
 - **Graphical abstract** (single graphics file),
 - **Proposed cover picture** (optional, single graphics file),
 - **Appendices** (optional, Word files, graphics files).

Submission process

Submission process consists of 5 steps. Before submission, authors should go through the checklist at the bottom of these guidelines page and prepare for submission:

Step 1: Starting the submission

- Choose one of the journal sections.
- Confirm all the requirements of the **checklist**.
- Additional plain text comments for the editor can be provided in the relevant text field.

Step 2: Upload submission

- Upload full manuscript in the form of a Word file (with title, authors, abstract, keywords, figures and tables embedded, and references).

Step 3: Enter metadata

- First name, last name, contact email and affiliation for all authors, in relevant order, must be provided. Corresponding author has to be selected. Full postal address and phone number of the corresponding author has to be provided.
- **Title and abstract** must be provided in plain text.
- Keywords must be provided (max. 6, separated by semicolons).

- Data about contributors and supporting agencies may be entered.
- **References** in plain text must be provided in the relevant text field.

Step 4: Upload supplementary files

- **Statement of novelty** in a Word file must be uploaded
- **List of suggested reviewers** with at least three reviewers must be uploaded as a Word file.
- All **graphics** have to be uploaded in a single ZIP file. Graphics should be named Figure 1.jpg, Figure 2.eps, etc.
- **Graphical abstract image** must be uploaded separately.
- **Proposed cover picture** (optional) should be uploaded separately.
- Any additional **appendices** (optional) to the paper may be uploaded. Appendices may be published as a supplementary material to the paper, if accepted.
- For each uploaded file the author is asked for additional metadata which may be provided. Depending of the type of the file please provide the relevant title (Statement of novelty, List of suggested reviewers, Figures, Graphical abstract, Proposed cover picture, Appendix).

Step 5: Confirmation

- Final confirmation is required.

Article Types

Review articles are welcome in any area of chemistry and may cover a wider or a more specialized area, if a high impact is expected. Manuscripts normally should not exceed 40 pages of one column format (letter size 12, 33 lines per page). Authors should consult the ACSi editor prior to preparation of a review article.

Scientific articles should have the following structure:

1. Title (max. 150 characters),
2. Authors and affiliations,
3. Abstract (max. 1000 characters),
4. Keywords (max. 6),
5. Introduction,
6. Experimental (Results and Discussion),
7. Results and Discussion (Experimental),
8. Conclusions,
9. Acknowledgements (if any),
10. References.

The sections should be arranged in the sequence generally accepted for publications in the respective fields. Scientific articles should report significant

and innovative achievements and exhibit a high level of originality.

Short communications generally follow the same order of sections, but should be short (max. 2500 words) and report a significant aspect of research work meriting separate publication.

Technical articles report applications of an already described innovation. Typically, technical articles are not based on new experiments.

Preparation of Submissions

Text of the submitted articles must be prepared with Word for Windows. Normal style set to single column, 1.5 line spacing, and 12 pt Times New Roman font is recommended. Line numbering (continuous, for the whole document) must be enabled to simplify the reviewing process. For any other format, please consult the editor. Articles should be written preferably in English. Correct spelling and grammar are the sole responsibility of the author(s). Papers should be written in a concise and succinct manner. The authors shall respect the ISO 80000 standard, and IUPAC Green Book rules on the names and symbols of quantities and units. The Syst me International d'Unit s (SI) must be used for all dimensional quantities.

Graphics (figures, graphs, illustrations, digital images, photographs) should be inserted in the text where appropriate. The captions should be self-explanatory. Lettering should be readable (suggested 8 point Arial font) with equal size in all figures. Use common programs such as Word Excel to prepare figures (graphs) and ChemDraw to prepare structures in their final size (8 cm for single column width or 17 cm for double column width) so that neither reduction nor enlargement is required. In **graphs**, only the graph area determined by both axes should be in the frame, while a frame around the whole graph should be omitted. The graph area should be white. The legend should be inside the graph area. The style of all graphs should be the same. **Figures and illustrations** should be of sufficient quality for the printed version, i.e. 300 dpi minimum. **Digital images and photographs** should be of high quality (minimum 250 dpi resolution). On submission, figures should be of good enough resolution to be assessed by the referees, ideally as JPEGs. High-resolution figures (in JPEG, TIFF, or EPS format) might be required if the paper is accepted for publication.

Tables should be prepared in the Word file of the paper as usual Word tables. The captions should be above the table and self-explanatory.

References should be numbered and ordered sequentially as they appear in the text, likewise methods, tables, figure captions. When cited in the text, reference numbers should be superscripted, following punctuation marks. It is the sole respon-

sibility of authors to cite articles that have been submitted to a journal or were in print at the time of submission to ACSi. Formatting of references to published work should follow the journal style; please also consult a recent issue:

1. J. W. Smith, A. G. White, *Acta Chim. Slov.* **2008**, 55, 1055–1059.
2. M. F. Kemmere, T. F. Keurentjes, in: S. P. Nunes, K. V. Peinemann (Ed.): *Membrane Technology in the Chemical Industry*, Wiley-VCH, Weinheim, Germany, **2008**, pp. 229–255.
3. J. Levec, Arrangement and process for oxidizing an aqueous medium, US Patent Number 5,928,521, date of patent July 27, **1999**.
4. L. A. Bursill, J. M. Thomas, in: R. Sersale, C. Collela, R. Aiello (Eds.), *Recent Progress Report and Discussions: 5th International Zeolite Conference*, Naples, Italy, 1980, Gianini, Naples, **1981**, pp. 25–30.
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NOMENKLATURA ANORGANSKE KEMIJE

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Obseg: 367 str.

Kemijska nomenklatura oz. poimenovanje kemijskih elementov in spojin je potrebno zato, da se vsi, ki jih uporabljajo, med seboj lahko sporazumevajo. Najpomembnejše pri tem je, da je poimenovanje spojin enotno in enoznačno, saj mora biti zagotovljeno, da si pod določenim imenom vsi predstavljajo isto kemijsko spojino.

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spreminjala, kadar je bilo to potrebno zaradi jasnosti ali možnosti različnih razumevanj.

Pred nami je tako v letu 2008 prevod »Nomenclature of Inorganic Chemistry, IUPAC Recommendations 2005« v slovenskem jeziku, le tri leta po izidu izvirnika. Zadnja slovenska nomenklatura anorganske kemije je bila izdana leta 1986, njen obseg pa je bil 86 strani (brez preglednic). Nova izdaja prevoda obsega skoraj 400 strani strokovno izjemno zahtevnega teksta. Slovenski prevod je pripravil Andrej Šmalc, z recenzijo in z nekaterimi dodatnimi dejavnostmi v zvezi s pripravo za tisk pa mu je pomagal Primož Šegedin. Za obsežno in strokovno korektno opravljeno delo se obema iskreno zahvaljujem.

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Priročnik je rezultat dela strokovnjakov Fakultete za farmacijo in Fakultete za kemijo in kemijsko tehnologijo. Podatki so zbrani iz različnih virov, ki so bili dosegljivi v strokovni literaturi, na spletnih straneh, v uradnih listih in drugih sprejemljivih publikacijah.

Ker je takšen način obravnave nevarnih kemikalij pripravljen v slovenščini, je knjiga pomemben prispevek uresničevanju nacionalnega programa o kemijski varnosti.

Avtorji knjige so Prof. Dr. Aleš Krbavčič, Prof. Dr. Aleš Obreza, Prof. Dr. Marija Sollner-Dolenc, Prof. Dr. Branko Stanovnik in Mag. Milan Škrlić.

Vsebinsko priročnik zajema opise blizu 800 kemikalij, IUPAC kemijski nomenklaturni sistem za organske in neorganske spojine, opis svetovnega usklajenega sistema za razvrščanje in označevanje kemikalij (GHS), mednarodni sistem merskih enot, pregled aktivnih snovi in preparatov za zaščito rastlin registriranih v RS in osnovne farmakološko toksikološke lastnosti nekaterih kemijskih funkcionalnih skupin.

Priročnik predstavlja monografije nevarnih kemikalij, opisuje njihove kemijske in fizikalne lastnosti, praktično uporabo ter njihov vpliv na žive organizme in okolje. Namenjena je strokovnjakom, ki delujejo na področju kemije, farmacije, veterine, agronomije pa tudi poslovnim osebam, ki se ukvarjajo s proizvodnjo in prometom z nevarnimi kemikalijami ter nadzirajo njihov promet.

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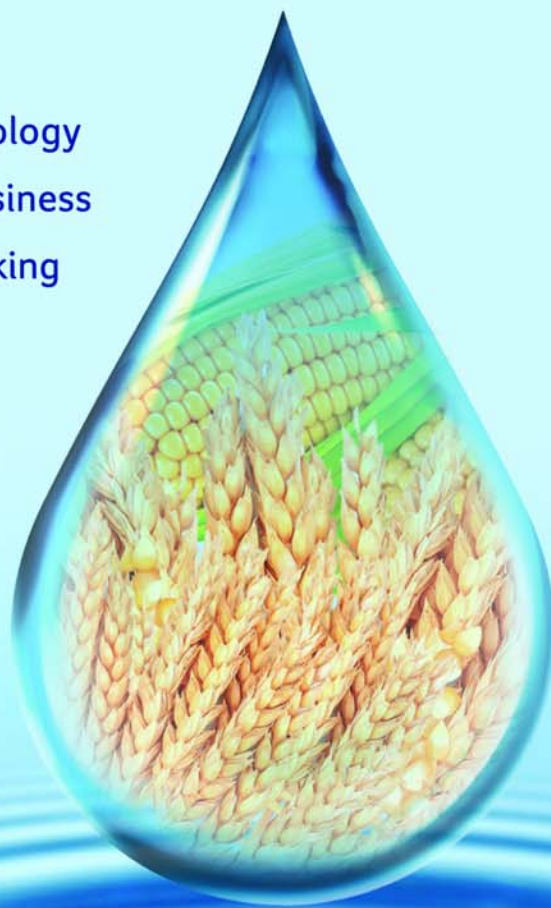
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