Short communication

Polypyrrole Layers Electrodeposited from TRIS Buffer Solution as Matrix of Tyrosinase Biosensor

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Abstract

A novel supporting electrolyte – tris(hydroxymethyl)aminomethane hydrochloride (TRIS) buffer solution was used to obtain polypyrrole (PPy) layers based on the anodic oxidation of pyrrole. Influence of some parameters: temperature, monomer and supporting electrolyte concentration, polarization potential and hydrodynamic conditions on electrodeposition process were investigated. Obtained layers were preliminary tested in construction of an amperometric biosensor based on enzyme tyrosinase, which response was tested using catechol as a substrate.

Keywords: Biosensor, tyrosinase, polypyrrole, electropolymerization, catechol determination

1. Introduction

According to the IUPAC recommendation biosensor is defined as an integrated receptor-transducer device, which is capable of providing selective quantitative or semi-quantitative analytical information using a biological recognition element.[1] Immobilization of bioreceptor onto matrix plays an important role for biosensor application and stability. Recently, the immobilization of biocatalysis in electropolymerized conducting polymers has made considerable progress.[2] Conducting polymers can be defined as polymers with long-range conjugation. Polypyrrole (PPy) seems to be potentially very attractive for the bioelement immobilization, it is biologically compatible and can be easily deposited onto electrodes of different shape and size at room temperature from the aqueous solutions. The electrochemical approach for preparing PPy layers is very versatile and provides facile vary the film properties by a simple modification of the electrolysis conditions.

In this work application of TRIS buffer solution as the supporting electrolyte for electrodeposition of PPy layers is proposed. Influence of some critical parameters on electrodeposition process was investigated. Obtained under the optimized conditions PPy layers were preliminary tested as matrix for immobilization of tyrosinase in amperometric biosensor for determination of phenolic compounds. Tyrosinase catalyses the oxidation of monophenols and o-diphenols to corresponding o-quinones. Quinones can be electrochemically reduced to allow low potential detection of phenolic compounds.[3]

2. Experimental

2. 1. Chemicals and Reagents

Mushroom tyrosinase (5370 U/mg) and pyrrole (98%), were purchased from Sigma (USA); buffer TRIS – tris(hydroxymethyl)aminomethane hydrochloride- was from Fluka (Switzerland); ammonium chloride (Suprapur) was bought from Merck (Germany); potassium chloride, disodium tetraborate and catechol (all analytical grade) were from POCh (Poland). Pyrrole was cleaned prior to use by passing through a neutral Al_2O_3-column to remove any colored components. 0.3 μm alumina (Buehler Micropolish, USA) was used for polishing surface of working electrode. Solutions were prepared using double-distilled water and, if necessary, deareated using high purity nitrogen (99.996%).

2. 2. Apparatus

EMU (WUT, Poland) was employed for electrochemical measurements. Potential of working electrode, WE, (vs. reference electrode, RE) was additionally controlled using Digital Multimeter V553 (Meratronik, Poland). A three electrode system was used consisted of platinum or glassy carbon disc WE, saturated silver/silver chloride RE (Mtm-anko, Poland) and platinum auxiliary electrode

(AE). Before each experiment the WE was polished onto glass plate using alumina suspension, rinsed with double distilled water and methanol.

2. 3. Electropolymerization of the Monomer

Anodic oxidative polymerization of pyrrole was carried out in mixtures of monomer and TRIS that were deoxygenated using high purity nitrogen. Polymer layers were deposited using two techniques: potentiostatic or potentiodynamic. In potentiostatic experiments, two steps were applied: 300 mV for 120 s and 800 mV for 400 s. The polymerization process occurred only in the second step. However, it was noticed (based on charge used) that the employment of the first step improved the repeatability of electrodeposition process. During this step, impurities presented on electrode surface or in buffer solution were probably oxidized. In potentiodynamic method, cyclic voltammetry technique was used. The WE potential was changed between 0 and 1000 mV.

2. 4. Construction of the Tyrosinase Biosensor

Immobilization of tyrosinase was performed by a physical adsorption of enzyme onto electropolymerized polypyrrole layer (Electrode I) and by an incorporation of enzyme into growing polymer layer (Electrode II). For physical adsorption 10 μL of enzyme solution (6.7 mg ml^{-1}) was dipped onto a polymer-modified electrode formed from unstirred 0.1 mol L^{-1} solution of Py using the potentiostatic technique. In order to entrap tyrosinase into growing polypyrrole layers the cyclic voltammetry or a pulse technique were employed. For the pulse method the WE potential was maintained for 10 s at 300 mV and then changed to 800 mV for 1 s. These changes were repeated 20 times. In both cases of potentiodynamic experiments, electrolyzed solutions were 0.1 mol L^{-1} pyrrole in 0.05 mol L^{-1} TRIS to which 50 μL of the enzyme solution (the same as for Electrode I) was added.

2. 5. Evaluation of Biosensor Response

Chronoamperometric curves (WE potential set at 50 mV) were recorded for different catechol concentration using 0.1 mol L^{-1} phosphate buffer solution (pH = 6) as the supporting electrolyte.

3. Results and Discussion

3. 1. Electrodeposition Process

3. 1. 1. Effect of Monomer Concentration

Monomer concentration influence was examined by potentiodynamic technique using Pt electrode. For each monomer concentration (0.01, 0.1, 0.2, 0.3 and 0.4 mol L^{-1}), ten successive voltammograms were recorded with scan rate 0.5 V s^{-1}. It was noticed that for the fixed WE potential, current corresponding to the pyrrole electrooxidation was linearly proportional to the monomer concentration (Fig. 1).

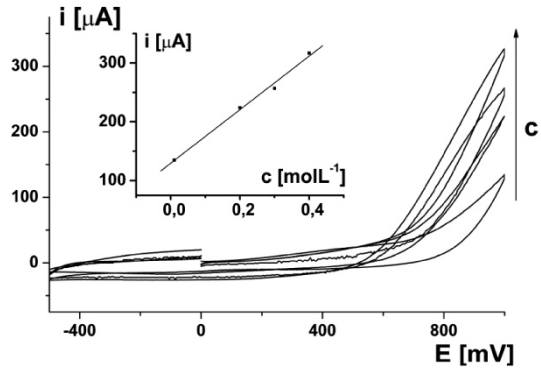

Fig 1: Effect of monomer concentration on electrodeposition process. Voltammograms (first cycle, 0.5 V s^{-1}) obtained on Pt electrode in 0.05 mol L^{-1} TRIS. Inset: relationship between current (for 1000 mV) and Py concentration.

This linear dependence suggested that at employed scan rate the oxidation process was diffusion-limited. For the lower scan rates the dependence current vs. square root of scan rate was non-linear. Moreover, after each cycle the increase of current corresponding to the oxidation of previously reduced polymer layer as well as the increase of peak current for oxidation of the monomer were observed (data not shown). This proves that in each cycle an additional amount of polymer was deposited onto the electrode.

3. 1. 2. Effect of the Supporting Electrolyte Concentration

Formation of the polypyrrole layer is associated with incorporation of supporting electrolyte anions (A^-) into growing positive charged polymer. In order to study the influence of electrolyte concentration on electropolymerization, potentiostatic electrodeposition of polymer onto Pt electrode was performed. Pyrrole concentration was set at 0.1 mol L^{-1} and TRIS concentration was changed from 0.001 mol L^{-1} to 0.1 mol L^{-1}. Because the enzyme's denaturation probability rose with electrolyte concentration, 0.1 mol L^{-1} was chosen as its highest value. Good linear relationship between the consumed charge (obtained after integration of area under chronoamperometric curves) and the supporting electrolyte concentration was obtained ($Q = 1002.2\ c + 11.0$; $R^2 = 0.9927$ for Q in mC and c in mol L^{-1}). Since the WE potential for each TRIS concentration was the same (ohmic drop in electrolyte was compensated) it could be concluded that the

incorporation of anions into growing layer was easier in solution with higher amount of anions and the whole process ran faster.

3. 1. 3. Effect of the Polarization Potential

Potentiostatic technique (see 2.3) was used to study influence of the WE potential on electrodeposition process. Results of experiments (charge vs. WE potential) are presented in Fig. 2. It is seen that increasing the potential of WE caused the rise of the electrodeposition rate, but this dependence is non-linear. This phenomenon could be explained as follows: the higher the WE potential was, the higher was the anodic reaction rate constant, but in the same time the higher were the rate of overoxidation process and probability of nucleophilic attack of water molecules and anions on formed radical cations.[4] These side reactions disturb the main process of the electrodeposition of polymer layers. It can be observed (data not shown) that for the lower potentials of WE (ex. 800 mV), where the overoxidation process seems to be negligible, there existed linear dependence between the consumed charge and the polarization time.

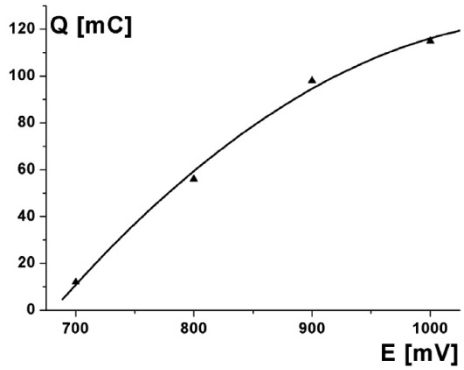

Fig. 2: Charge used during electrodeposition of PPy layer from 0.1 mol L^{-1} Py in 0.05 mol L^{-1} TRIS as a function of platinum WE potential. Polarization time was 400 s.

3. 1. 4. Effect of the Temperature

The effect of temperature on electrodeposition process was evaluated using potentiodynamic electrodeposition technique. The relationship of current vs. temperature (Fig. 3) exhibits increase of the rate of the electrooxidation process when temperature was enlarged. This could be explained by rise of two factors: monomer diffusion coefficient and increasing reaction rate constants.

3. 1. 5. Effect of Stirring

According to the mechanism described by Diaz at al.[5] pyrrole oxidative deposition begins by electron transfer (E) (with formation of radical cation) followed by a succession of chemical reaction (C) and electron transfer reactions, which can be described as $E(CE)_n$. Because the overall process takes place near the electrode surface, stirring of solution has great influence on it. This effect is shown in Fig. 4, where the amperometric curves obtained for different stirring rate were presented. It was found that electropolymerization process was possible only in non-stirred solutions or in solution stirred with low rate. This fact could be explained by formation of very reactive radical cations. Intensive stirring could reduce their concentration near the electrode surface and decrease the chance of coupling reaction between two radicals. In non-stirred solution, the high radical concentration near the electrode could be maintained by the continual diffusion of monomer towards the electrode.

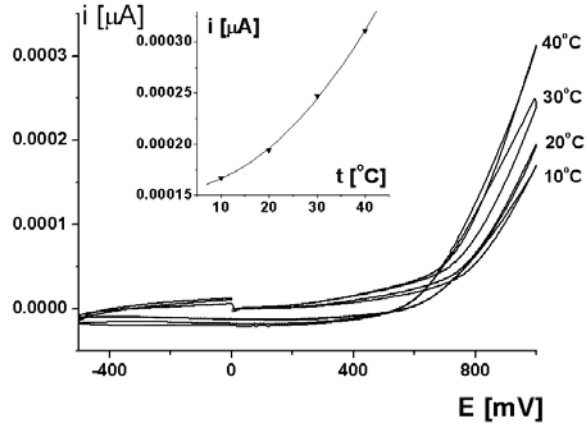

Fig. 3: Effect of temperature on electrodeposition process. Voltammograms for first cycle obtained in solution contains 0.1 mol L^{-1} Py and 0.05 mol L^{-1} TRIS; scan rate 0.5 V s^{-1}. Inset: relationship between current (at 1000 mV) and temperature.

3. 2. Biosensor Response

3. 2. 1. Electrode I (Physical Adsorption of Enzyme)

A typical amperometric electrode response is presented in Fig. 5. It was found that successive additions of catechol solution caused increase of current corresponding to quinone reduction.

Estimated sensitivity of the proposed biosensor for catechol was equal to 35 *nA* μmol^{-1} L^{-1} and the response time ($t_{95\%}$) was about 20 s. The obtained sensitivity appeared higher then is offered by other biosensors: constructed by covalent tyrosinase immobilization onto copolymer poly(N-3-aminopropyl pyrrole-co-pyrrole) film (3.46 *nA* μmol^{-1} L^{-1}),[6] based on 1,6-heksanedithiol and nano-Au self-assembled monolayers (3.94 *nA* μmol^{-1} L^{-1}),[7] and based on Os-complex functionalized polymer (6.10 *nA* μmol^{-1} L^{-1}).[8]

The constructed biosensor exhibited poor stability. Most of biomolecules were lost from the electrode surface

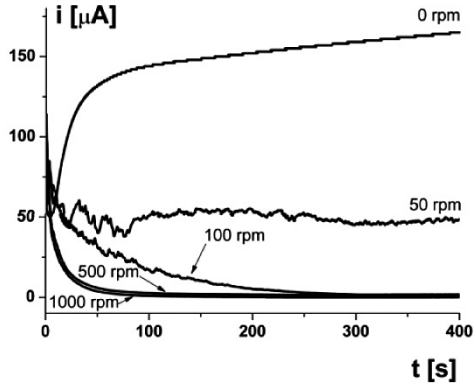

Fig. 4: Influence of stirring on electrodeposition process. Chronoamperograms at the Pt working electrode recorded for 0.1 mol L^{-1} pyrrole solution in 0.05 mol L^{-1} TRIS; polarization potential: 800 mV.

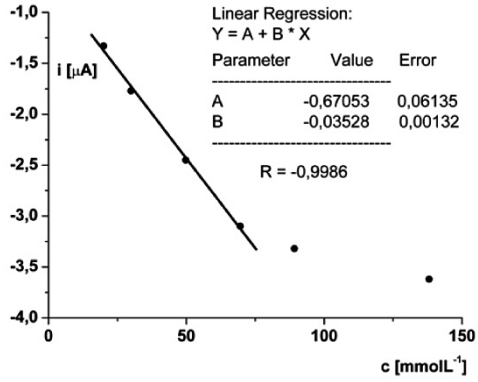

Fig. 5: Electrode I response for catechol in stirred 0.1 mol L^{-1} phosphate buffer solution (pH = 6); polarization potential: 50 mV.

after few measurements. It was detected as decrease of the electrode response and color changes of tested solutions (from colorless to deep brown) caused by the enzymatic reaction products.

3. 2. 2. Electrode II (the enzyme entrapped in polypyrrole layer)

In this case entrapment of the enzyme in polypyrrole layer was performed but obtained electrode gave no significant response in the catechol solutions of different concentrations irrespective of method of polymer deposition: pulse or cyclic voltammetry technique. This suggested that either the enzyme was not incorporated onto growing layer due to their high hydrophobicity or the enzyme was incorporated in denaturated form. It was also probably that analyte (catechol) transport to enzyme active site was disturbed by the structure of polymer film.

4. Conclusions

Electrochemical deposition of the PPy layers from TRIS buffer solution was performed. The influence of some critical parameters (temperature, monomer and supporting electrolyte concentration, polarization potential and hydrodynamic conditions) on electrodeposition process was investigated. It was found that mechanically stable polymer layers could be obtained on electrochemical way using this supporting electrolyte. Based on promising results of preliminary experiments, especially the value of biosensor sensitivity, polypyrrole layers electrodeposited from TRIS could be employed for construction of tyrosinase biosensor. Further research will be focused on an improvement of biosensor performance.

5. References

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Povzetek

Da bi z anodno oksidacijo pirola iz raztopine dobili polipirol (PPy) smo uporabili elektrolit tris(hidroksimetil)aminometan hidroklorid (TRIS). Preučili smo vpliv temperature, koncentracije monomera in elektrolita, polarizacijskega potenciala in hidrodinamskih pogojev na proces elektrodepozicije. Dobljene sloje PPy smo preizkusili pri izdelavi amperometričnega biosenzorja temelječega na encimu tirozinazi. Odziv dobljenega senzorja smo preizkusili z uporabo katehola kot substrata.