

# SYNTHESIS COMPARISON AND CHARACTERIZATION OF CHITOSAN-COATED MAGNETIC NANOPARTICLES PREPARED WITH DIFFERENT METHODS

## PRIMERJAVA POSTOPKOV IN KARAKTERIZACIJA MAGNETNIH NANODELCEV, PREVLEČENIH S HITOZANOM

Gordana Hojnik Podrepšek, Željko Knez, Maja Leitgeb

University of Maribor, Faculty of Chemistry and Chemical Engineering, Laboratory for Separation Processes and Product Design, Smetanova ul. 17, 2000 Maribor, Slovenia  
maja.leitgeb@um.si

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In this study, magnetic maghemite nanoparticles were prepared with the coprecipitation method, due to its simplicity and productivity. Thereafter, chitosan-coated magnetic nanoparticles were synthesized with three different methods, the micro-emulsion process, the suspension cross-linking technique and the covalent binding. Subsequently, a comparison of the used methods was done using various analyses such as Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetry (TGA), differential scanning calorimetry (DSC), vibrating-sample magnetometry (VSM) and dynamic light scattering (DLS). The characterization results from Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analysis (TGA) indicated a successful binding of chitosan on the magnetic nanoparticles. SEM pictures showed that spherical structured particles with an increased particle size were obtained as the chitosan layer around the particles was increased. Considering that the magnetic-separation technique has the advantages of rapidity, high efficiency, cost-effectiveness and lack of negative effect on the biological activity, these carriers may be applied in enzyme immobilization.

Keywords: magnetic nanoparticles, chitosan, surface functionalization

V prispevku je opisana enostavna priprava magnetnih nanodelcev, prevlečenih s hitozanom. Postopek je potekal v dveh stopnjah. V prvi smo sintetizirali magnetne nanodelce s koprecipitacijo železovih ionov. V drugi smo nanodelce prevlekli s hitozanom, da bi preprečili aglomeracijo, z uporabo treh različnih postopkov: z metodo mikroemulzije, metodo zamreženja in s kovalentno vezavo. Karakterizacija tako pripravljenih nanodelcev je bila izvedena s Fourierjevo transformacijsko infrardečo spektroskopijo (FTIR), z vrstično elektronsko mikroskopijo (SEM), s termogravimetrično analizo (TGA), z diferencialno dinamično kalorimetrijo (DSC), z analizo vibracijskega magnetometra (VSM) in dinamičnim sipanjem laserske svetlobe (DLS). Rezultati analiz FTIR in TGA so potrdili vezavo hitozana na magnetne nanodelce, medtem ko je bila oblika in debelina sloja hitozana določena s SEM-analizo. Ker ima tehnika magnetnih nanodelcev veliko prednosti pri ločevanju, cenejši proizvodnji in nima negativnih učinkov na biološko aktivnost, se lahko potencialno uporablja pri encimski imobilizaciji.

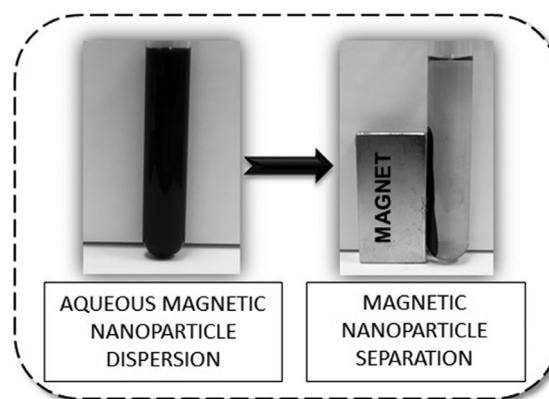
Ključne besede: magnetni nanodelci, hitozan, površinska funkcionalizacija

## 1 INTRODUCTION

Recently, magnetic nanoparticles such as maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ) have attracted a great deal of attention due to their unique, controllable sizes, shapes, other physical properties, compositions and also for their wide applications in biomedicine, biotechnology, engineering, material science and environmental areas<sup>1-3</sup>. They have a magnetic response and can be manipulated with an external magnetic-field gradient (**Figure 1**).

Chitosan, a deacetylated derivative of chitin, is a polysaccharide with both a hydroxyl and an amine group in its structure. In addition, it is non-toxic, biocompatible, biodegradable, and anti-bacterial<sup>4</sup>. It is insoluble in water, but becomes soluble and positively charged in acidic media. Nowadays, the preparation of chitosan-modified magnetic nanoparticles are of great interest<sup>5,6</sup>. In addition, this polymer has been used successfully to colloidally stabilize magnetic nanoparticles and has also been used as a matrix for enzyme immobilization since it has numerous amino groups that can interact with enzyme<sup>7,8</sup>. The amino groups are responsible for the distinct

characteristics attributed to this basic polymer. Therefore, the characterization of chitosan is extremely important with respect to the structure-property relationship, defining a possible industrial application<sup>9</sup>.



**Figure 1:** Magnetic-property illustration of the maghemite nanoparticles dispersed in water

**Slika 1:** Prikaz magnetnih lastnosti nanodelcev maghemita, dispergirana v vodi

## 2 EXPERIMENTS

### 2.1 Materials

Iron (II) chloride tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ), iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) and acetic acid were supplied from Merck, (Germany). Chitosan (CTS, MMW, the degree of deacetylation was 75–85 %), glutaraldehyde (GA) and Span-80 were obtained from Sigma-Aldrich. Ammonia was purchased from Carlo Erba and paraffin from Kreiger. All the solutions were prepared with Milli-Q water.

### 2.2 Apparatus and procedures

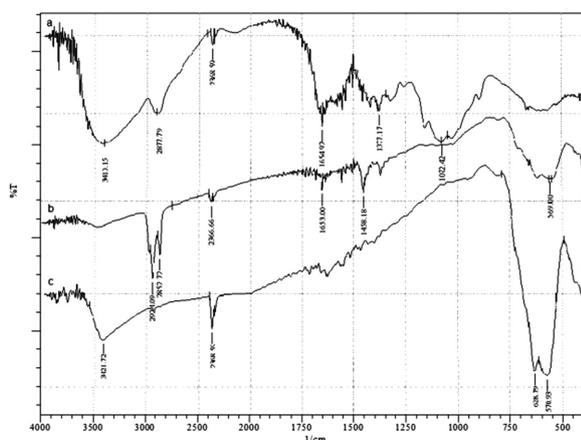
Maghemite nanoparticles were synthesized by coprecipitating  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  ions in the presence of ammonium. A functionalization of chitosan was carried out with three different methods: the micro-emulsion process<sup>10</sup>, the suspension cross-linking technique<sup>11</sup> and the covalent binding of chitosan<sup>12</sup>. These methods differ with respect to the chitosan concentration, the presence and concentration of the acetic acid solution, the glutaraldehyde concentration, the synthesis temperature, the pH of the medium and the time of the synthesis.

The properties and structures of non-functionalized magnetic iron-oxide nanoparticles and chitosan-functionalized magnetic maghemite nanoparticles were characterized with Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), dynamic light scattering (DLS), vibrating-sample magnetometry (VSM) and potentiometric titration.

## 3 RESULTS

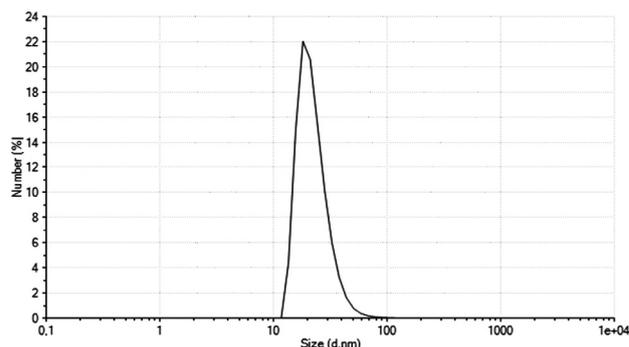
### 3.1 Characterization of chitosan-coated magnetic nanoparticles

The FTIR spectra of chitosan, chitosan-coated maghemite and maghemite nanoparticles are shown in



**Figure 2:** FTIR spectra of: a) chitosan, b) maghemite coated with chitosan and c) maghemite

**Slika 2:** FTIR-spektri: a) hitozan, b) maghemit z nanosom hitozana in c) maghemit



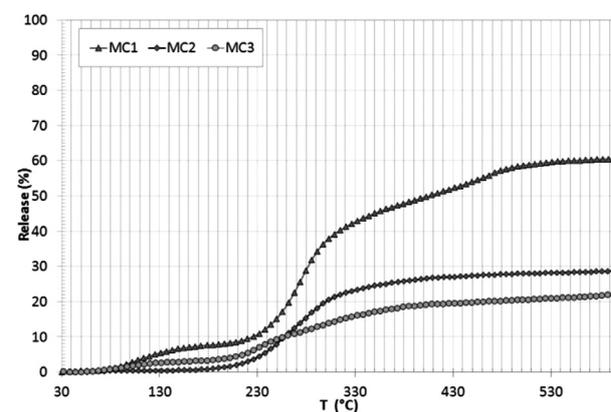
**Figure 3:** Particle-size distribution of non-functionalized magnetic nanoparticles

**Slika 3:** Razporeditev velikosti nefunkcionaliziranih magnetnih nanodelcev

**Figure 2a to 2c.** Undoubtedly, the FTIR spectra of chitosan showed a broader band at  $3410\text{ cm}^{-1}$ , which was attributed to the hydroxyl (OH) stretching as reported in<sup>6</sup>. For the IR spectra of chitosan, the characteristic absorption bands appeared at  $1654\text{ cm}^{-1}$  which can be assigned to the N-H bending vibrations, and at  $1377\text{ cm}^{-1}$  assigned to the C-O stretching of the primary alcohol group in chitosan. For the magnetic maghemite nanoparticles, the peaks at  $570\text{ cm}^{-1}$  and  $628\text{ cm}^{-1}$  were related to the Fe-O group<sup>10</sup>. However, the adsorption of chitosan on the surface of the magnetic iron-oxide nanoparticles was confirmed with the FTIR analysis.

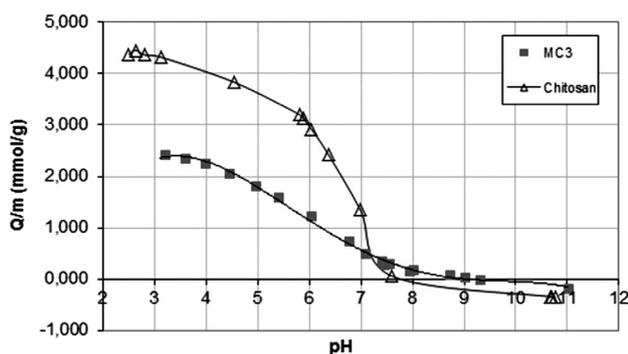
**Figure 3** shows the size distribution of the magnetic nanoparticles determined with DLS in an aqueous solution with the mean diameter of 22.8 nm.

The results of the TGA characterization of the maghemite nanoparticles coated with chitosan obtained with three different methods were used for an estimation of the amount of the chitosan coating on the maghemite



**Figure 4:** Weight-loss curve of chitosan by sample; (MC1) maghemite particles coated with chitosan with the micro-emulsion process, (MC2) maghemite nanoparticles coated with chitosan with the suspension cross-linking technique and (MC3) maghemite nanoparticles coated with chitosan with the covalent-binding method

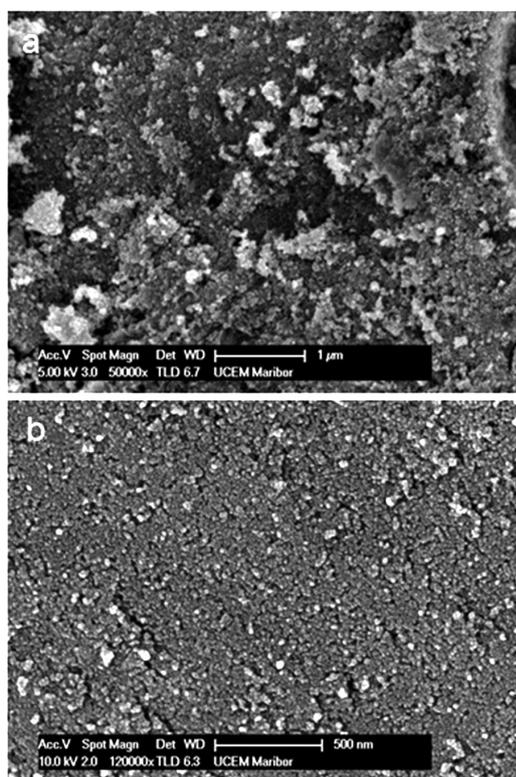
**Slika 4:** Krivulje zmanjšanja mase hitozana v vzorcih; (MC1) delci maghemita, pokriti s hitozanom s postopkom mikroemulzije, (MC2) delci maghemita, pokriti z metodo zamreženja in (MC3) maghemitni nanodelci, pokriti s hitozanom z metodo kovalentne vezave



**Figure 5:** Charging isotherms of pure chitosan and MC3 nanoparticles  
**Slika 5:** Izoterme naboja čistega hitozana in MC3-nanodelcev

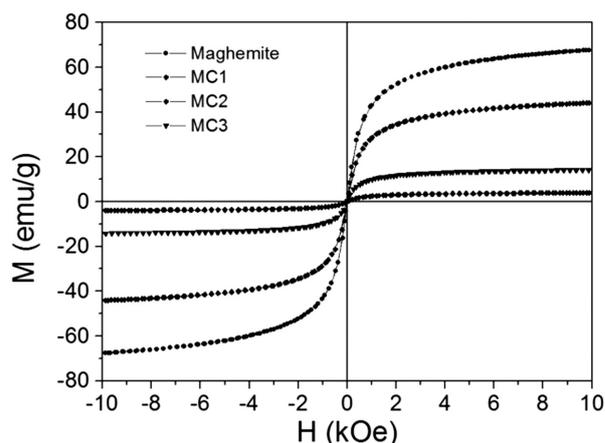
nanoparticles. The actual weight loss of pure maghemite nanoparticles was subtracted from the actual weight loss of maghemite nanoparticles coated with chitosan to get the mass loss of chitosan in percentages for MC1, MC2 and MC3, presented in **Figure 4**. The mass loss of chitosan for MC3 (22.2 %) is lower than for MC2 (28.8 %) and MC1 (60.7 %).

The presence of the amino-group amount was studied for pure chitosan and chitosan-functionalized magnetic nanoparticles, using a potentiometric titration. The resulting charging isotherms  $QV/m_f$  versus pH are pre-



**Figure 6:** SEM images of: a) magnetic maghemite nanoparticles and b) maghemite nanoparticles, coated with chitosan with the covalent-binding method (MC3)

**Slika 6:** SEM-posnetka: a) magnetnih nanodelcev maghemita in b) nanodelcev maghemita, pokritih s hitozanom s kovalentno metodo vezanja (MC3)



**Figure 7:** Hysteresis loops of the maghemite and chitosan-functionalized maghemite nanoparticles

**Slika 7:** Histerezna zanka maghemita in s hitozanom funkcionaliziranih nanodelcev maghemita

sented in **Figure 5**. The figure contains the titration data for pure chitosan and the MC3 sample. The amount of amino group in free chitosan was 4.22 mmol/g, while in sample MC3 it was 2.48 mmol/g.

Typical SEM micrographs for maghemite nanoparticles and chitosan-coated maghemite nanoparticles are shown in **Figure 6**, presenting maghemite nanoparticles (a) and maghemite nanoparticles, coated with chitosan with the covalent-binding method (b). **Figure 6** reveals that the maghemite particles, coated with chitosan (MC3) have spherical shapes and a size range of 50–100 nm.

**Figure 7** shows the magnetization curves for the maghemite nanoparticles, revealing superparamagnetic properties. The magnetization of the micro- and nanoparticles, coated with chitosan with the micro-emulsion process was found to be 3 emu/g, for the suspension cross-linking technique it was 40 emu/g and for the covalent-binding method it was 20 emu/g. These values were compared to the value for the uncoated maghemite nanoparticles. Therefore, it can be concluded that a chitosan-surface functionalization of the maghemite nanoparticles was achieved.

## 4 CONCLUSION

In this paper, chitosan-coated maghemite nanoparticles were synthesized with the micro-emulsion process, the suspension cross-linking technique and covalent binding of chitosan. The samples exhibited clear differences in the saturation magnetization, which can be ascribed mainly to different chemical compositions and magnetic moments of Fe. The chitosan-coated maghemite nanoparticles appeared in granules with the average sizes of 40–350  $\mu\text{m}$  after the micro-emulsion process, 400 nm after the suspension cross-linking technique and 50–100 nm after the covalent binding of chitosan. The size of the nanoparticles was increased with an increase

in the concentration of chitosan and decreased with an increase in the cross-linker concentration. We found that the magnetic nanoparticles, coated with chitosan with the covalent-binding method (MC3) are suitable for practical applications due to their sufficiently high values of amino groups and nanosized particles. The maghemite nanoparticles, functionalized with chitosan, have a potential to be used in assisted drug-delivery systems, cell/enzyme immobilization, separation processes, medical diagnosis and therapy and many other industrial applications.

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