

SYNTHESIS OF MICRO-COMPOSITE BEADS WITH MAGNETIC NANO-PARTICLES EMBEDDED IN POROUS CaCO₃ MATRIX

SINTEZA MIKROKOMPOZITNIH KROGLIC Z MAGNETNIMI NANODELCI V POROZNI MATRIKI CaCO₃

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A method for synthesis of soft magnetic microbeads is presented. The microbeads are made from magnetic nanoparticles dispersed in CaCO₃ (calcium carbonate) matrix. The composite beads are almost perfectly spherical with a diameter of few micrometers. The majority of the composite beads consists of a porous CaCO₃ matrix. Magnetic nanoparticles with a size of about 10-15 nm are made of Fe₂O₃. They are captured inside the pores of CaCO₃ matrix during its formation. CaCO₃ matrix is formed by crystallization from saturated solution of sodium carbonate and calcium chloride. The composite beads are coated with a layer of functionalized polymer. The magnetic microbeads were characterized by SEM and XPS. Different functional groups were detected by XPS measurements including SO₃⁻, NH₃⁺, NH₂, CO₃²⁻ and OH groups. The results indicate that the iron oxide particles are absent on the surface and that the polymer coating serves as a good biocompatible film.

Keywords: composite, surface characterization, XPS, functionalization, Fe nanoparticles, microbeads

V tem prispevku predstavljamo metodo za sintezo delcev, ki so sestavljeni iz kompozita magnetnih nanodelcev v matrici iz kalcijevega karbonata (CaCO₃). Velikost kompozitnih delcev, ki so popolnoma sferične oblike, je nekaj mikrometrov. Ključni del ogrodja kompozitnega delca sestavlja zelo porozna matrica CaCO₃. Magnetni nanodelci velikosti približno 10-15 nm so narejeni iz Fe₂O₃. Ti nanodelci se med tvorjenjem matrice CaCO₃ ujamejo v notranjost por. CaCO₃ matrica se tvori pri kristalizaciji nasičene raztopine natrijevega karbonata in kalcijevega klorida. Tako nastali kompozit je nato oplášen še s plastjo funkcionaliziranega polimera. Sintetizirane magnetne kompozitne delce smo analizirali z metodama SEM in XPS. Kot je razvidno iz XPS meritev, je prišlo na površini do nastanka različnih funkcionalnih skupin kot so SO₃⁻, NH₃⁺, NH₂, CO₃²⁻ in OH skupine. Rezultati dokazujejo, da na površini mikrodelcev ne najdemo nanodelcev, ki so skriti znotraj por, ter da lahko polimerna prevleka, ki prekriva mikrodelce, služi kot dobra biokompatibilna podlaga za nadaljnjo vezavo bioloških substanc.

Ključne besede: kompozit, površinska karakterizacija, XPS, funkcionalizacija, Fe nanodelci, mikrokroglice

1 INTRODUCTION

Superparamagnetic nanoparticles have found potential application in various diagnostic tests and assays as substrates or supports for immunologically based reactions. They can be also used in therapeutic purposes for the transportation of active substances to specific targets.¹⁻⁴ Magnetic material for nanoparticles used in medicine is usually maghemite γ -Fe₂O₃ which is a non-toxic material.⁵ Application of external magnetic field enables easy handling and distant manipulation with magnetic nanoparticles, or separation of nanoparticles from liquids. Magnetic nanoparticles must express their magnetic properties only in the presence of an external magnetic field but otherwise they must have no magnetic polarization which would otherwise cause their agglomeration. They must be well dispersed in a liquid medium. To fulfil these requirements they must be small enough (below approx. 15 nm) meaning that particles are single magnetic domains. On the other hand, in some applications they must also have sufficiently large specific surface, which provides enough binding sites. In such cases usually polymer metal composites are used.⁶

Before application in diagnostic tests and assays the surface properties of magnetic nanoparticles or their composites must be improved by coating them with a layer of specific functionalized material to which specific molecules (e.g. proteins, antibodies) are linked. Usually they are first coated with a layer of polymer or silica and then functionalized with specific functional groups depending on particular application. Numerous methods for synthesis of metal oxide nanoparticles have been elaborated. Although advanced plasma treatments based on application of non-equilibrium processes enable synthesis of particles with interesting properties,⁷⁻¹⁰ classical chemical methods are still popular since they often allow for uniform size distribution in quantities of industrial importance. These groups provide necessary binding sites for further covalent attachment of proteins (e.g. streptavidin) for capturing of molecules of interest.

In this paper we present a method for synthesis of magnetic composites consisting of CaCO₃ porous matrix with captured magnetic nanoparticles. The composite is coated with a layer of functionalized polymer. The

magnetic composites were characterized by SEM and XPS methods.

2 EXPERIMENTAL

2.1 Synthesis of magnetic nanoparticles

The synthesis of superparamagnetic maghemite nanoparticles (γ -Fe₂O₃) has been carried out via a controlled chemical co-precipitation approach.¹¹⁻¹³ Prior to synthesis an aqueous mixture of ferric and ferrous salts and sodium hydroxide as an alkali source were prepared separately as stock solutions. In this method, the corresponding metal hydroxides were precipitated during the reaction between the alkaline precipitating reagent and the mixture of metal salts. The metal hydroxides were subsequently oxidized in air resulting in the formation of the γ -Fe₂O₃ spinel product.

Superparamagnetic γ -Fe₂O₃ nanoparticles obtained after the synthesis can not be directly dispersed in a liquid medium to obtain stable colloids. In this regard, it is necessary to chemically modify the particle surface in order to prevent the particles from aggregation and subsequent sedimentation. The surface of the prepared γ -Fe₂O₃ nanoparticles were stabilized electrostatically, and sterically with amino (-NH₂) and hydroxyl (-OH) alkyl groups.

Prepared samples were characterized using X-ray diffractometry (XRD) and transmission electron microscopy (TEM). Specific surface area of powders was determined by BET method (Brunauer, Emmett and Teller).¹⁴ A specific magnetization of the prepared samples was also measured using a vibrating sample magnetometer (VSM). These results have been already published and can be found in Ref¹².

2.2 Synthesis of magnetic composite microbeads

2.2.1 Materials

Polymer materials poly(sodium 4-styrenesulfonate) (PSS) and poly(alianin hydro chloride) (PAH), were received from Aldrich (Germany). Ethylene-diamine-tetraacetic acid trisodium salt (EDTA), calcium chloride (CaCl₂) and sodium carbonate (Na₂CO₃) were received from Sigma (Germany). HCl and NaOH were purchased from Riedel-de Haën and Sigma, respectively. Stabilized suspensions of iron oxide (Fe₂O₃) nanoparticles with average diameter 10 nm ± 2 nm and specific surface of 90 m²g⁻¹ were prepared as described in a previous paragraph.

2.2.2 Microparticles fabrication

Polyelectrolyte microcapsules were self-assembled on narrow size CaCO₃ microparticles that were synthesized through recrystallization from 0.33 M CaCl₂ and Na₂CO₃ saturated solutions. The following chemical reaction occurred: Na₂CO₃ + CaCl₂ → CaCO₃ + 2NaCl.

At room temperature the equal volumes of solutions were rapidly mixed and agitated for 30 s. Mixture was left for 15 min to form porous spherical-like microparticles. Synthesized microparticles were four times washed with water. Iron oxide nanoparticles were encapsulated in the porous microparticles by addition to one of saturated solutions on volume ratio 1,33 % of final solution to create magnetic CaCO₃ microparticles.

2.2.3 Polyelectrolyte deposition

The 1 gL⁻¹ polyelectrolyte solutions were prepared in 0.25 M NaCl. Magnetic CaCO₃ particles were suspended in PAH (polyallylaminehydrochloride) and PPS (polystyrenesulfonate) solutions, following the 15 min adsorption time during which the particles were continuously shaken. The pH value of polyelectrolyte solution was adjusted to 6.5. Following the adsorption procedure, the particles were rinsed using three centrifugal washings with 5 mM NaCl and subsequently re-suspended in different polyelectrolyte solutions. This sequential process was repeated, until a layer-by-layer polyelectrolyte film of eight bi-layers was assembled onto the magnetic microbeads. The resulting microcapsule suspension with encapsulated iron oxide nanoparticles was stored in water at 4 °C.

2.3 Characterization of composite particles with XPS

Composite microbeads were also analyzed by XPS (X-ray Photoelectron Spectroscopy) to see their chemical composition. Samples were analyzed with an XPS instrument TFA XPS Physical Electronics. Since XPS analyzes are performed in vacuum, a drop of solution containing microbeads was put on Si slice and dried. Such sample was then placed in XPS chamber and excited with X-rays with a monochromatic Al K_{α1,2} radiation at 1486.6 eV. The photoelectrons were detected with a hemispherical analyzer positioned at an angle of 45° with respect to the normal to the sample surface. Survey-scan spectra were made at a pass energy of 187.85 eV and 0.4 eV energy step. High-resolution spectra of C1s, N1s and S2p were made at a pass energy of 23.5 eV and 0.1 eV energy step. The concentration of elements was determined by using MultiPak v7.3.1 software from Physical Electronics, which was supplied with the spectrometer.¹⁵ The high-resolution spectra were fitted using the same software. The curves were fitted with symmetrical Gauss-Lorentz functions. A Shirley-type background subtraction was used. Both the relative peak positions and the relative peak widths (FWHM) were fixed in the curve fitting process.

3 RESULTS AND DISCUSSION

The schematic structure of magnetic composite microbeads which were prepared according to our procedure is shown in **Figure 1**. In **Figure 2** is shown the chemical structure of the polymers which were used for

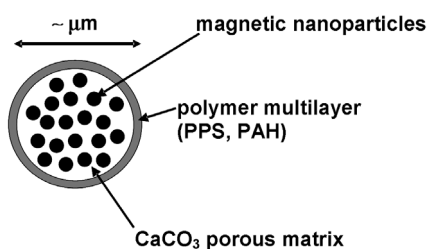


Figure 1: Schematic composition of magnetic microbeads

Slika 1: Shema sestave magnetnih mikrodelcev

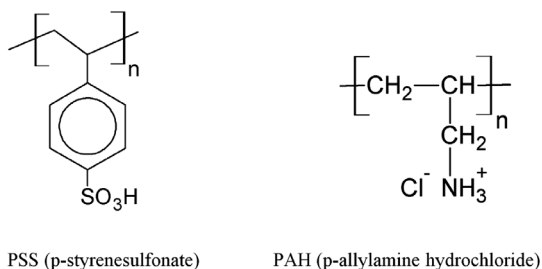


Figure 2: Chemical structure of PPS and PAH polymer

Slika 2: Kemična struktura polimerov PPS in PAH

final coating the magnetic composite microbeads. We have used different polymers like PAH (polyallylaminehydrochloride) and PPS (polystyrenesulfonate) which were deposited in a multilayered structure. The whole polymer thickness is estimated to several nanometers. This will be discussed later in the text. SEM image of the magnetic composite microbeads is shown in **Figure 3**. We can see that composite microbeads are almost perfectly spherical. Their size is about 5 micrometers. The surface chemical composition of the magnetic composite microbeads was studied by XPS (X-ray photoelectron spectroscopy) in order to prove the presence of functionalized polymer coatings on the surface of magnetic composites. XPS is very powerful method for studying surface chemical composition of different materials especially organic samples^{16–22} as well as other materials including micro and nanomaterials or powders like in our case.^{23–25} In **Figure 4** is shown survey XPS spectrum of the composite microbeads. Concentration of

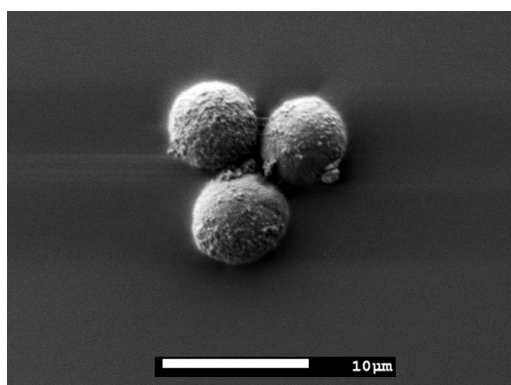


Figure 3: SEM image of the magnetic composite microbeads

Slika 3: Posnetek magnetnih mikrodelcev

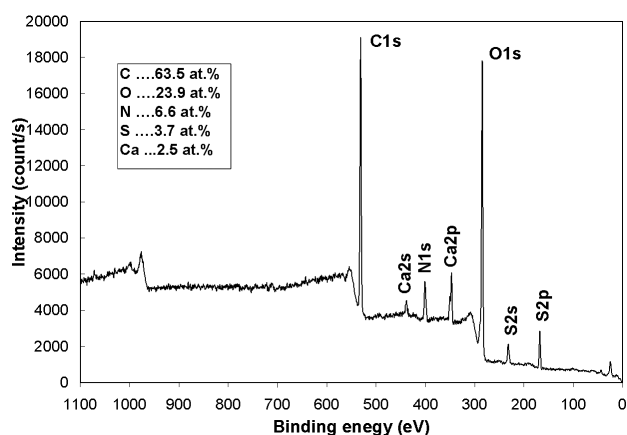


Figure 4: XPS survey spectrum of the magnetic composite microbeads

Slika 4: Pregledni XPS-spektri magnetnih mikrodelcev

the elements is shown as well. Elements like nitrogen, sulphur, carbon, and oxygen originates from the polymer shell. We can observe also calcium Ca from CaCO_3 matrix. Therefore, some of the oxygen and carbon are coming also from calcium matrix. We can not see any traces of iron meaning that iron oxide nanoparticles are hidden well inside the composite microbeads. A prove that composite microbeads really contain magnetic nanoparticles was mobility of these composite microbeads which was observed under the influence of external magnetic field.

Observation of calcium in the survey spectrum needs further discussion. Since a detection depth of XPS method is few nanometers only we can conclude that the polymer thickness must be of the same order. If it was thicker, we could not see calcium. By tilting the sample and changing the angle between electron analyzer and the sample's surface plane we can little vary the detection depth. But unfortunately, this works well only for perfectly flat samples. In our case the surface is rough and furthermore the samples are spherical. Therefore, we could not observe any difference in the surface composition during rotation of the sample.

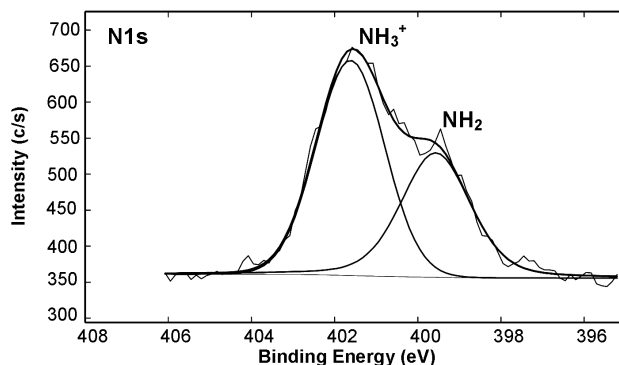


Figure 5: XPS high resolution spectrum of nitrogen measured at the surface of magnetic composite microbeads with polymer shell

Slika 5: Energijsko visoko ločljivi XPS-spektri dušika, izmerjeni na površini magnetnih mikrodelcev s polimerno prevleko

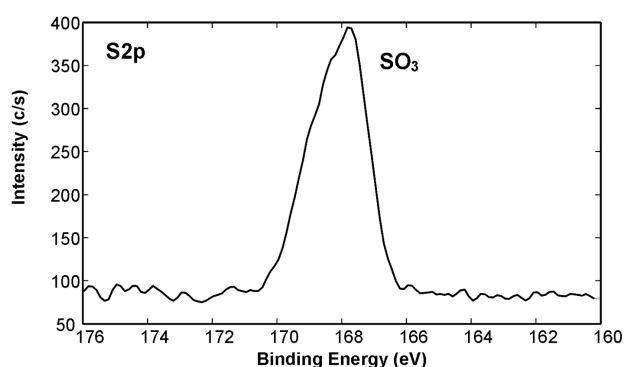


Figure 6: XPS high resolution spectrum of sulphur measured at the surface of magnetic composite microbeads with polymer shell

Slika 6: Energijsko visoko ločljivi XPS-spektri žvepla, izmerjeni na površini magnetnih mikrododelcev s polimerno prevleko

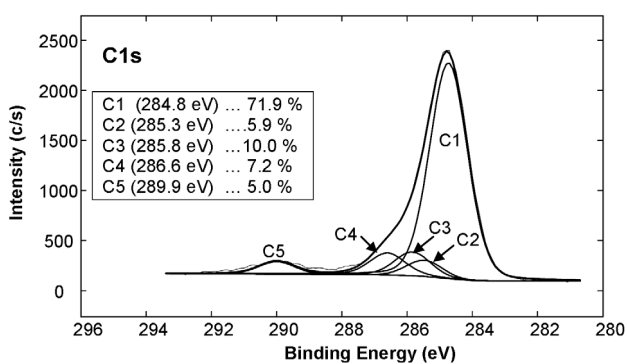


Figure 7: XPS high resolution spectrum of carbon measured at the surface of magnetic composite microbeads with polymer shell. Sub-peak C1 is attributed to C-C bonding, C2 to C-S bonding, C3 to C-N bonding, C4 to C-O bonding and C5 to O=C-O bonding.

Slika 7: Energijsko visoko ločljivi XPS-spektri ogljika, izmerjeni na površini magnetnih mikrododelcev s polimerno prevleko. Spektri so bili razstavljeni na vrhove C1–C5, ki pomenijo različne vezi ogljikovih atomov: C1 pripada C-C vezavi ogljika, C2 pripada C-S vezavi, C3 pripada C-N vezavi, C4 pripada C-O vezavi ter C5 pripada vezavi ogljika v O=C-O skupini.

High resolution spectra of nitrogen N1s, sulphur S2p and carbon C1s which are shown in **Figures 5–7** can give further information about the surface functionalization. As shown in **Figure 5** nitrogen peak consists of two peaks located at the binding energy of about 399.8 eV and 401.7 eV. These two peaks correspond to NH_2 and NH_3^+ groups. NH_3^+ groups are expected for PAH polymer according to its chemical structure shown in **Figure 2**. During the formation of this polymer layer also NH_2 groups were formed. Sulphur peak is shown in **Figure 6**. It is located at a binding energy of about 168 eV and it is attributed to SO_3 groups originating from polymer PPS. Very interesting is a carbon peak shown in **Figure 7**. Carbon peak consists of many subpeaks indicating different binding of carbon atoms. The major peak marked as C1 is due to C-C (aliphatic) and $-\text{C}=\text{C}$ (aromatic) bonds. Other small peaks are contributed to C-S bonds (C2), C-N bonds (C3), C-O bonds (C4) and

carbonate bonding CO_3^{2-} (C5). Bonds like C-S and C-N originate from PPS and PAH polymers respectively, while CO_3^{2-} groups are from CaCO_3 matrix. These means, that a mixture of both polymers was successfully deposited on the outer surface of magnetic composite microbeads. These functional groups can enable further binding of specific molecules like proteins.

4 CONCLUSION

We have developed a method for synthesis of magnetic composite microbeads consisting of CaCO_3 matrix with magnetic nanoparticles and outer polymer shell. The composite magnetic microbeads were found to be almost perfectly spherical and their size was about few micrometers. The surface composition of the particles was analyzed by XPS. Elements like carbon, oxygen, nitrogen and sulphur were found on the surface. Calcium from CaCO_3 matrix was found as well meaning that the polymer shell has a thickness of about several nanometers. No iron was detected in XPS spectra. This means that magnetic nanoparticles are hidden well inside the CaCO_3 matrix. XPS measurements showed also formation of different functional groups like SO_3^- , NH_3^+ , NH_2 , CO_3^{2-} and OH groups. Groups like SO_3^- originate from PPS polymer, NH_3^+ and NH_2 are from PAH polymer and CO_3^{2-} groups are from CaCO_3 matrix. We can conclude that a mixture of both polymers was successfully deposited onto the outer surface of magnetic composite microbeads. These functional groups can enable further binding of specific molecules like proteins used in biomedical application. Furthermore, polymer coating not just provides surface functional groups needed for good biocompatibility, but it also prevents aggregation of microbeads.

Acknowledgement

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5 REFERENCES

- V. P. Torchilin, Nanoparticulates as Drug Carriers, Imperial College Press, London, 2006
- M. Konerack, P. Kopcansky, M. Timko, C.N. Ramchand, J. Magn. Mater., 252 (2002), 409–411
- P. A. Gupta, C. T. Hung, Life Sci., 44 (1989), 175–186
- G. T. Hermanson, Bioconjugate Techniques, Academic Press, Amsterdam, 2008, 582
- U. Häfeli, W. Schüt, J. Teller, M. Zborowsky, Scientific and clinical application of magnetic carriers, Plenum Press, New York, 1997
- L. C. Santa Maria, M. A. S. Costa, F. A. M. Santos, S. H. Wang, M. R. Silva, Mat. Lett., 60 (2006), 270–273
- M. Mozetic, Mater.Tehnol. 44 (2010) 4, 165–171

- ⁸ M. Mozetic, J. Phys. D Appl. Phys. 44 (2011) 17, 174028-1-174028-9
- ⁹ I. Arcon, M. Mozetic, A. Kodre, Vacuum 80 (2005) 1–3, 178–183
- ¹⁰ M. Mozetic, Vacuum (2012), doi:10.1016/j.vacuum.2011.02.017
- ¹¹ S. Čampelj, D. Makovec, M. Bele, M. Drogenik, J. Jamnik, Mater. Tehnol., 41 (2007) 2, 103–107
- ¹² A. Kosak, D. Makovec, A. Znidarsic, M. Drogenik, Mater. Tehnol., 39 (2005) 1/2, 37–41
- ¹³ D. Makovec, A. Kosak, A. Znidarsic, M. Drogenik, J. Magn. Magn. Mater., 289 (2005), 32–35
- ¹⁴ S. Brunauer, P. H. Emmett, E. Teller, J. Am. Chem. Soc., 60 (1938), 309–319
- ¹⁵ J. F. Moulder, W. F. Stickle, P. E. Sobol, K. D. Bomben, Ed: J. Chastain, R. C. King, Handbook of X-ray photoelectron spectroscopy, Physical Electronics: Minesota, 1995
- ¹⁶ A. Vesel, M. Mozetic, S. Strnad, K. Stana-Kleinschek, N. Hauptman, Z. Persin, Vacuum, 84 (2010) 1, 79–82
- ¹⁷ M. Gorjanc, V. Bukosek, M. Gorenssek, M. Mozetic, Tex. Res. J., 80 (2010) 20, 2204–2213
- ¹⁸ A. Vesel, M. Mozetic, A. Zalar, Vacuum, 82 (2008) 2, 248–251
- ¹⁹ T. Vrlic, D. Debarnot, M. Mozetic, A. Vesel, J. Kovac, A. Coudreuse, G. Legeay, F. Poncin-Epaillard, J. Colloid. Interface Sci., 362 (2011) 2, 300–310
- ²⁰ A. Vesel, M. Mozetic, A. Zalar, Surf. Interface Anal., 40 (2008) 3–4, 661–663
- ²¹ I. Junkar, U. Cvelbar, A. Vesel, N. Hauptman, M. Mozetic, Plasma Processes Polym., 6 (2009) 10, 667–675
- ²² A. Vesel, M. Mozetic, A. Hladnik, J. Dolenc, J. Zule, S. Milosevic, N. Krstulovic, M. Klanjek-Gunde, N. Hauptman, J. Phys. D: Appl. Phys., 40 (2007) 12, 3689–3696
- ²³ J. Kovac, P. Panjan, A. Zalar, Vacuum, 82 (2007) 2, 150–153
- ²⁴ G. Drazic, S. Novak, J. Kovac, Imaging Microsc., 8 (2006) 3, 36–37
- ²⁵ M. Remskar, J. Kovac, M. Virsek, M. Mrak, A. Jesih, A. Seabaugh, Adv. Funct. Mater., 17 (2007), 1974–1978