POLAR SHELL MAGNETIC NANOSTRUCTURED SYSTEMS FOR HETEROGENEOUS NANOPHASE REACTIONS

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Core-shell type magnetic nanoparticles based on magnetite and para-aminobenzoic acid (PABA) were synthesised by coprecipitation method and characterized. A linker system, glutaraldehyde, was grafted on the surface and subsequently functionalized with free amino groups provided by para-phenylenediamine. The dispersed systems were tested in preliminary reaction with a N-terminal protected amino acids using the principle of solid phase synthesis through the Fmoc strategy. The obtained materials were characterized by X-ray diffraction, FT-IR, DLS and TEM.

Keywords: magnetic nanoparticles, core/shell, linker, Fmoc, glutaraldehyde, solid phase synthesis.

1. Introduction

Magnetic nanoparticles (MPs) or ferrite can be defined as magnetic materials composed of oxides containing ferric ions as the main constituent. They are resistant, biocompatible and inexpensive materials that can be removed by simply magnetic separation from the reaction medium [1]. MPs present several advantages, such as low toxicity [2], small size, high surface-to-volume ratio [3], good biocompatibility [4], high thermal stability, high corrosion resistance and low prices [5]. Neat MPs contain hundreds of unique atoms that can interact magnetically and have an extremely high surface energy, therefore, in the usual preparation kits, they tend to undergo uncontrolled aggregation. To be magnetically stabilized, bounce forces must be present to counteract the related attractions. Particle stability can be greatly enhanced by the presence of a coating layer, thereby facilitating the formation of dispersions of MPs [6].

Several techniques may be involved in the synthesis of MPs: sol-gel, co-precipitation, microemulsion, laser pyrolysis, hydrothermal technique, microwave

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irradiation, sonolysis and biological synthesis [7,8]. Chemical co-precipitation is a quick, simple, inexpensive method and allows the control of the size and morphology of nanoparticles [9] to obtain nanoparticles of the desired size and shape [10]. The size, shape, crystallinity and polydispersity are all dependent on reaction conditions: the type of salt used (eg chlorides, sulfates, nitrates, perchlorates, etc.), raw material concentration, Fe^{2+}/Fe^{3+} ionic strength, as well as other reaction parameters (e.g., stirring rate, drop speed of the basic solution). These parameters are particularly important for obtaining high-quality nanoparticles [11, 12, 13].

The concept of "solid-phase" was introduced by Robert Bruce Merrifield in 1963, revolutionizing the process of peptides synthesis, performing the reaction on a polymeric solid support [14]. Since then, this method has been widely used in any field where organic synthesis is involved. In comparison to classical synthesis, solid-phase peptide synthesis (SPPS) offers great advantages in terms of efficiency, applicability and purification processes [15], but presents the disadvantage of using of a large amount of organic solvents [16]. At the moment, the process is automated, and reagents are commercially available.

Solid-phase peptide synthesis is based on the sequential coupling of side chain protected α-amino acids, two strategies being frequently involved: *"Fmoc synthesis strategy"*, where protection can be achieved with the Fmoc (fluorenylmethyloxycarbonyl) group or *"Boc synthesis strategy"*, where the protection is carried out with the Boc group (tert-butyloxycarbonyl). Using these strategies, the desired peptide sequence is synthesized from the C-terminus at the N-terminal end [17], this being opposite to the way the proteins are synthesized in nature [18].

As an alternative to the polymeric support, a synthesis using MPs as base for SPPS was reported, with good stability in reaction conditions, and no modification of the magnetic properties of the particles throughout the solid-phase synthesis [19].

This paper describes a new approach to solid-phase synthesis, namely *Nanostructured Support Synthesis* (NSS), using core-shell PABA-magnetite MPs, functionalized with a linker and reactive amino groups able to bond Fmoc-protected amino acids. The designed NSS method is readily available, allows an improved control of the reaction due to a larger active surface, the easier access of the reagents to the reaction center and provides a facile separation of the product from the reaction medium.

2. Experimental

2.1. Materials and methods of characterization

All materials used to perform this experiment are of analytical purity and were purchased from Sigma-Aldrich: ferric chloride ($FeCl_3$), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), sodium hydroxide (NaOH), *para*-aminobenzoic acid (PABA), glutaraldehyde (GA), *para*-Phenylenediamine (PPD), N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), N,N-diisopropylethylamine (DIPEA), dimethylformamide (DMF), Fmoc-glycine-OH, piperidine. The solutions were obtained and washing were performed using ultrapure water 18.2 MΩ*cm.

FT-IR spectra were recorded using a Zn-Se window m-ATR mounted on a Thermo Nicolet 6700 spectrometer.

X-Ray analysis were performed with X-ray powder diffraction patterns using a Shimadzu XRD 6000 diffractometer, using Cu Kα (1.5406 Å) at 30 mA and 40 kV. The 2θ range was 10–80° and the scan rate 1° min^{-1}.

Particle size distribution analysis was performed by the Dynamic Light Scattering (DLS) technique using Zetasizer Nano ZS (Malvern Instruments, Malvern, United Kingdom). The mean size and polydispersity index of nanoparticles in the dispersion were measured at a spreading angle of 90° and at a temperature of 25°C. The average diameter of the diameters and the polydispersity index are presented as the average of three measurements.

TEM analyses were performed on a Tecnai G2 F30 S-TWIN high-resolution transmission electron microscope (HR-TEM) equipped with energy dispersive spectroscopy (EDS) as well as a selected area electron diffraction detector (SAED). The microscope was operated in transmission mode at 300 kV; TEM point resolution was 2 Å and line resolution was 1 Å.

2.2. Synthesis of nanostructured system and nanoparticle reaction

The synthesis of core-shell magnetite nanoparticles (Fe_3O_4-PABA) was accomplished by the co-precipitation method using a 1,3:1 molar ratio of aqueous solution of divalent iron ($FeSO_4x7H_2O$) and trivalent iron ($FeCl_3$) and a basic solution consisting of NaOH and PABA. For this purpose, two solutions were prepared: *Solution I* containing the Fe^{2+} and Fe^{3+} ions from 0.7 g $FeCl_3$ and 1.5 g $FeSO_4x7H_2O$ in 300 mL H_2O and *Solution II* (the basic shell) consisting of 3 g NaOH and 3 g PABA in 200 mL H_2O. Solution II was added dropwise over Solution I under magnetic stirring (700 rpm) at room temperature, for 1 hr. The mixture was subjected to magnetic separation using a NdFeB magnet, with decantation of supernatant, followed by repeated washes with water of the remaining immobilized MPs, until a 6.62 pH value of supernatant was achieved. The resulted nanoparticles have been dispersed in 200 mL water using the sonication method.

Functionalized Fe_3O_4-PABA-GA nanoparticles were obtained by dropwise addition of 2 mL 5% aqueous solution of glutaraldehyde over 100 mL of a diluted (~0.07%) water dispersion of Fe_3O_4/PABA nanoparticles at room temperature under sonication and for 30 minutes, followed by repeated washes with water and magnetic separation until no presence of glutaraldehyde was detected by UV (280nm) [20]. The resulted functionalized nanoparticles have been dispersed in 200 mL water using the sonication method.

Amino grafted Fe_3O_4-PABA-GA-PPD nanoparticles were prepared by dropwise addition of 30 mL of 17% aqueous solution of PPD and added over 100 mL of Fe_3O_4-PABA-GA dispersion, under sonication at room temperature for 30 minutes, followed by repeated washes and magnetic separation. The resulted amino-grafted nanoparticles have been dispersed in 200 mL water using the sonication method.

Coupling of the Fmoc protected amino acid was performed by introducing 3 mL of Fe_3O_4-PABA-GA-PPD dispersion into a Falcon tube with a mixture of: 7.6 mg HBTU + 2.7 mg HOBt + 0.1 mL DIPEA + 1 mL DMF + 5.9 mg of Fmoc-glycine-OH. The obtained mixture was maintained at room temperature for 1 hour, using the shaker. Nanoparticle separation was performed in a magnetic field, then two washes with 2 mL of DMF for 10 minutes with controlled shaking at the shaker, were done.

Deprotection of the coupled amino acid was performed with 5 mL solution of DMF/piperidine=4/1 for 15 minutes with controlled shaking at the shaker.

Kaiser Test was performed according to literature [21].

3. Results and discussions

3.1. Synthesis of nanostructured system and nanoparticle reaction

An ambident linker, labile under the final cleavage conditions, was used, compatible with all the synthesis steps. For this purpose, glutaraldehyde linker was chosen because of readily coupling reaction with amino groups of PABA shell, providing also a reactive group at a sufficiently large distance from the surface of the nanoparticle to position the next reaction center in the turbulence mass transfer area similar with classical solid-phase synthesis. A reactive -NH_2 group at the end of the linker system, capable of efficiently binding N-terminal blocked amino acids, was obtained by grafting *para*-phenylenediamine (Fig. 1).

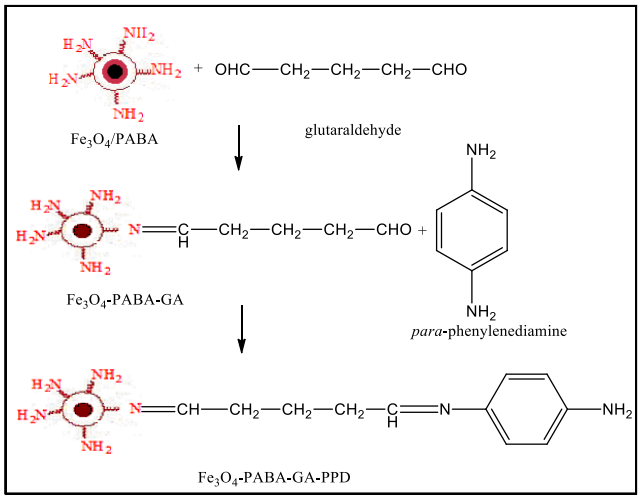

Fig. 1. The final core-shell-linker nanostructured system

In a preliminary assay of the attachment ability of the newly designed nanostructured solid phase support, Fe_3O_4-PABA-GA-PPD, the coupling of the Fmoc protected amino acid (Fmoc-Gly-OH, Fmoc blocked amino acid at the N-terminus) was achieved (Fig. 2), following sequence of reactions:

- Coupling of Fmoc blocked amino acid
- Magnetic separation and washing
- For the qualitative determination of the amino acid attachment to the nanostructured system, a color test (Kaiser Test) was performed. A negative test was obtained (the solution did not change its color) confirming that the coupling reaction took place.
- Deprotection of the coupled amino acid
- Magnetic separation and washing
- Final Kaiser Test to highlight the N-terminal end deprotection by detecting the color change of the solution in dark brown. This certifies the deprotection of the amine groups.

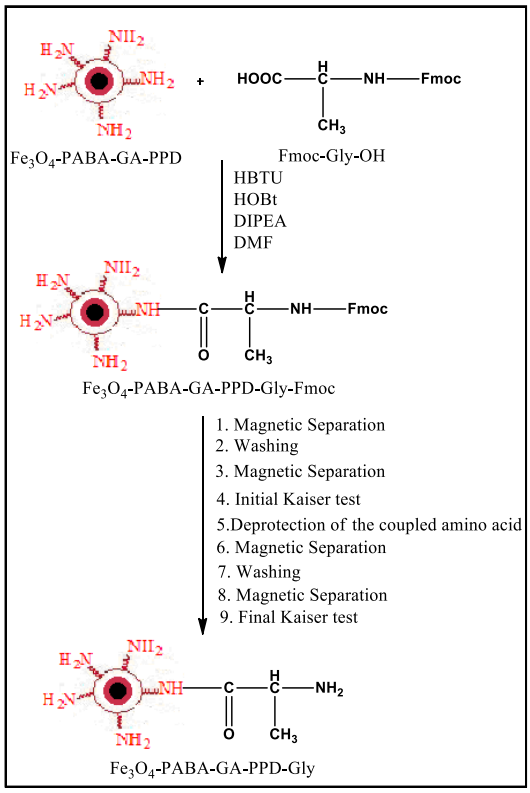

Fig. 2. Sequence of reactions for amino acid binding to the nanostructured system

3.2. Characterization of the new magnetic nanostructured system

The synthesized core–shell nanoparticles Fe_3O_4-PABA were investigated in order to establish the structure, dimension, stability and morphology.

According to DLS, the obtained nanoparticles present an average hydrodynamic diameter of 49 nm good uniformity proved by the obtained polydispersity index value of 0.173 and very good stability in dispersion according to the determined value of the zeta potential + 48.9 mV (Fig. 3). The obtained dimensions and polydispersity are in good agreement with other previously synthesized core-shell Fe_3O_4/salicylic acid nanoparticles [22, 23]. However, replacing salicylic acid with PABA improved the stability of the nanostructured system by increasing the zeta potential value with 30%. We may explain the increased stability taking into account a better solvatation provided by PABA as compared to salycilic acid sustained by the value of pKa - 2.41 for PABA and 2.98 for salycilic acid [24] and the difference in dipole moment: 2.8 D for PABA and 2.65 for salicylic [25].

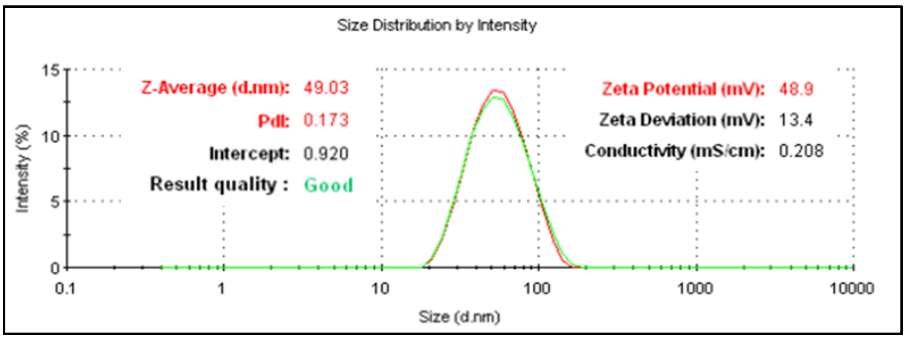

Fig. 3. DLS analysis of magnetite nanoparticles with PABA shell

XRD pattern was obtained for the synthesized Fe_3O_4-PABA and the identified peaks confirm the specific crystalline spinel structure of magnetite (JCPDS 79-0417 and 75-0449). (Fig. 4)

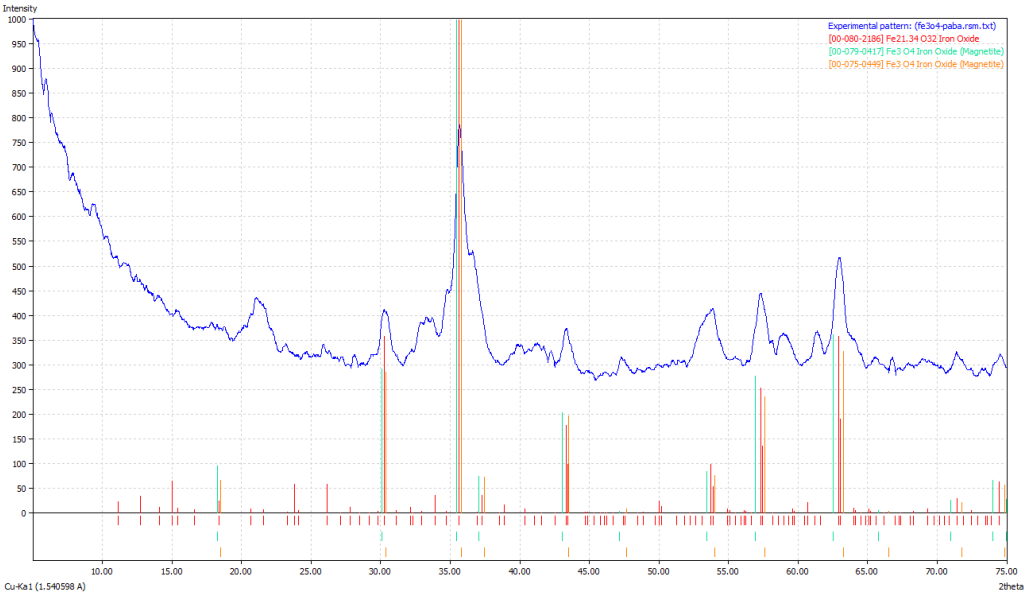

Fig. 4. Diffractogram for magnetite nanoparticles with PABA shell

Morphology of Fe_3O_4-PABA was determined by TEM. The obtained micrograph confirms the presence of nanoparticles and the size of the ferrite core below 30 nm (Fig. 5).

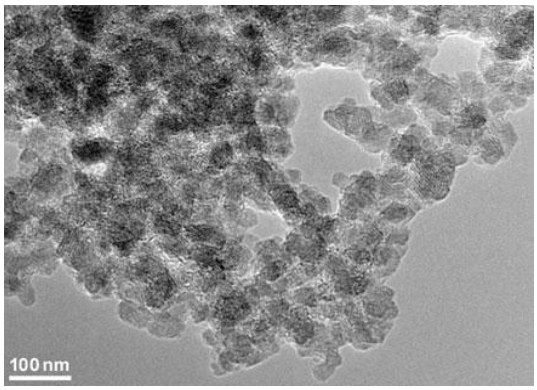

Fig. 5. TEM image for Fe_3O_4 / PABA

The PABA shell of the obtained Fe_3O_4-PABA nanoparticles was confirmed in the FT-IR spectrum by the presence of the N-H stretching band at 3160 cm^{-1} and aromatic C-C skeleton stretching vibrations (1427-1535 cm^{-1}). The characteristic band for -NH_2 is much widened due to the vibrational interaction given by the nanoparticle proximity. The carboxylate group specific bands are not observed due to the direct interaction of the -COO- group with the crystalline network ions. The presence of para substituted aromatic ring of PABA is also confirmed by the C_{Ar}-H deformation vibrations at and in the range of 787 and -884 cm^{-1} (also broadened due to the proximity of the nanoparticle). The magnetite characteristic band attributed to vibration of the Fe-O functional group at 561 cm^{-1} a band was also identified (Fig. 6).

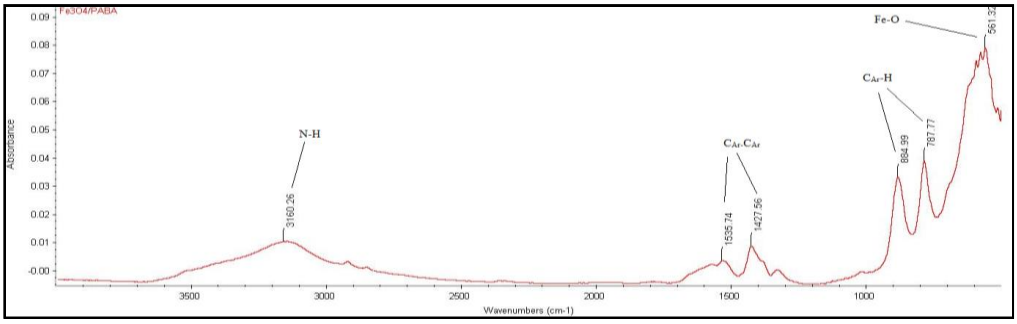

Fig. 6. FT-IR spectra of the Fe_3O_4/PABA system

The presence of the grafted glutaraldehyde in Fe_3O_4-PABA-GA and the subsequent attachment of PPD in Fe_3O_4-PABA-GA-PPD nanoparticles were confirmed by FT-IR spectrometry. Thus, the attachment of GA is proved by the characteristic stretching absorptions for -CH=N moiety at 1630cm^{-1} and unreacted formyl group -CH=O at 1718 cm^{-1}. Reaction with PPD resulted in increased band

intensity for CH=N moiety corroborated with the disappearance of the characteristic band for aldehyde. Attachment of PPD is also confirmed by the appearance absorptions for terminal -NH_2 groups at 3372 and 3298 cm^{-1} (Fig. 7).

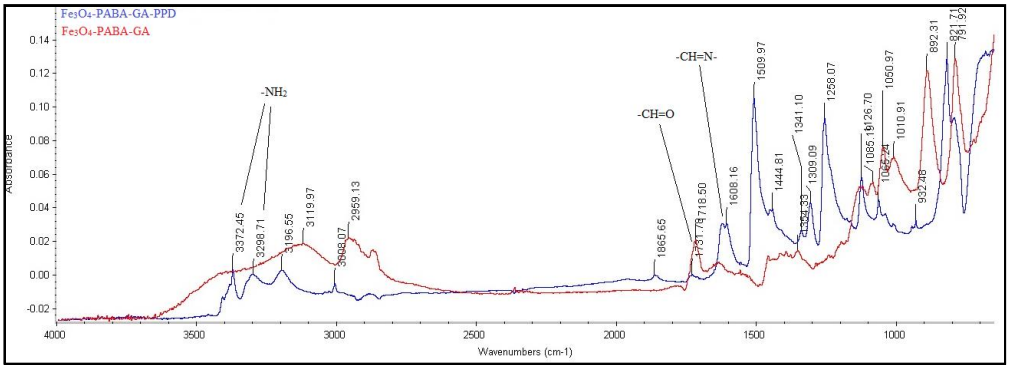

Fig. 7. Comparative FT-IR spectrum: Fe_3O_4-PABA-GA and Fe_3O_4-PABA-GA-PPD

The ability of the newly designed Fe_3O_4-PABA-GA-PPD to act as solid phase nanostructured system was also confirmed by FT-IR spectroscopy. Thus the successful attachment of Fmoc–glycine followed by deprotection was proved by the appearance of the characteristic stretching absorptions: amide II at 1579 cm^{-1} and -NH_2 at 3332 cm^{-1} (Fig. 8).

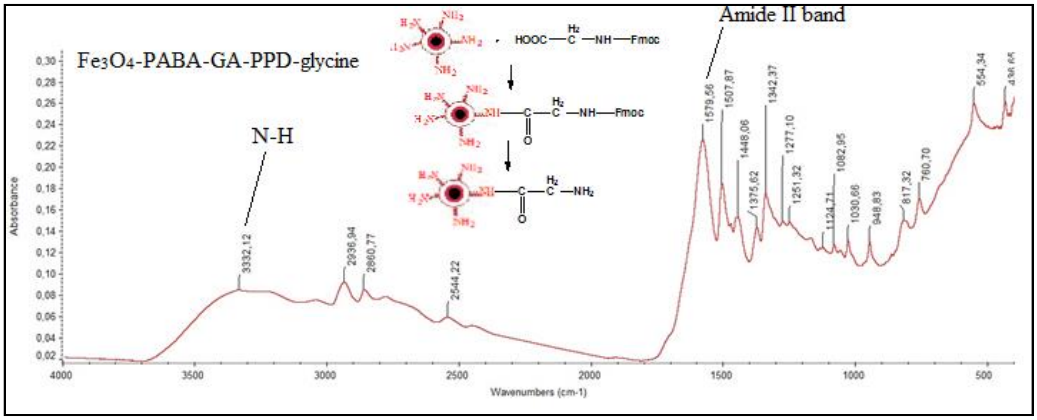

Fig. 8. FT-IR spectrum for Fe_3O_4-PABA-GA-PPD-glycine after the deprotection step

4. Conclusions

A new magnetic support system was designed using core–shell PABA-Fe_3O_4 nanoparticles and functionalized with a newly designed linker system with free -NH_2 group. This was achieved by grafting glutaraldehyde on PABA and

subsequent binding of *para*-phenylenediamine leading to the corresponding imine.

This nanostructured magnetic support system represents a new approach to solid support synthesis, and this ability was tested in a successful coupling with a Fmoc protected amino acid. The crystalline structure of the magnetite was demonstrated by XRD, and their morphology and size was determined by DLS and TEM.

The capacity of the free -NH_2 group of the core-shell-linker final nanostructure system to react in a nanoparticle dispersed system with a N-terminal Fmoc-protected amino acid was highlighted. The intermediate and final materials were characterized by FT-IR, demonstrating coupling of the proposed molecules in the desired sequence.

R E F E R E N C E S

[1]. *E. Nehling, L. Motte, E. Guénin,* Nano-organocatalysts synthesis: Boc vs Fmoc protection, Catalysis Today, **vol.** 208, 2013, pp. 90-96.

[2]. *L. Yu, G. Hao, J. Gu, S. Zhou, N. Zhang, W. Jiang*, Fe_3O_4/PS magnetic nanoparticles: synthesis, characterization and their application as sorbents of oil from waste water, Journal of Magnetism and Magnetic Materials, **vol**. 394, 2015, pp. 14–21.

[3]. *V. F. Cardoso, D. Miranda, G. Botelho, G. Minas, S. Lanceros-Méndez,* Highly effective clean-up of magnetic nanoparticles using microfluidic technology, Sensors and Actuators B, **vol.** 255, 2018, pp. 2384–2391.

[4]. *E. Cho, M. N. Tahir, J. M. Choi, H. Kim, J.-H. Yu, S. Jung,* Novel magnetic nanoparticles coated by benzene- and β-cyclodextrin-bearing dextran, and the sorption of polycyclic aromatic hydrocarbon, Carbohydrate Polymers, vol. 133, 2015, pp. 221–228.

[5]. *S. Amiri, H. Shokrollahi*, The role of cobalt ferrite magnetic nanoparticles in medical science, Materials Science and Engineering C, **vol**. 33, 2013, pp. 1–8.

[6]. *N. A. Frey, S. Sun*, Magnetic nanoparticles for information storage applications; in: inorganic nanoparticles-synthesis, Applications and perspectives (C. Altavilla, E. Ciliberto Eds.), CRC Press, 2011.

[7]. *V.* Angamuthu, *D.-F. Tai,* Asymmetric reactions of chiral organo-magnetic nanoparticles, Applied Catalysis A: General, **vol**. 506, 2015, pp. 254–260.

[8]. *I. L. Ardelean, D. Ficai, A. Ficai, G. Nechifor, D. Dragu, C. Bleotu*, Synthesis and characterization of new magnetite nanoparticles by using the different amino acids such as stabilizing agents, U.P.B. Scientific Bulletin, Series B, **vol.** 80, 2018, pp. 33-46

[9]. *G. Schmid*, Nanoparticles: From theory to application, Wiley-VCH Verlag, Weinheim, 2010.

[10]. *G.G. Nedelcu, A. Nastro, L. Filippelli, M. Cazacu, M. Iacob, C. Oliviero Rossi, A. Popa, D. Toloman, M. Dobromir, F. Iacomi*, Structural characterization of copolymer embeddedmagnetic nanoparticles, Applied Surface Science, **vol**. 352, 2015, pp. 109-116.

[11]. *W. Wu, Q. He, C. Jiang*, Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies, Nanoscale Research Letter, **vol**. 3, 2008, pp. 397–415.

[12]. *S. S. Staniland, A. Rawlings, J. Bramble, J. Tolosa, O. Wilson, J. C. García-Martínez, C. Binns*, Nanomagnetism: fundamentals and applications-Novel methods for the synthesis of magnetic nanoparticles, Frontiers of Nanoscience, **vol**.6, no. 3, 2014, pp. 85-88.

[13]. *A. M. Abu-Dief, S. M. Abdel-Fatah*, Development and functionalization of magnetic nanoparticles as powerful and green catalysts for organic synthesis, Beni-Suef University Journal of Basic and Applied Sciences, **vol.** 7, 2018, pp. 55-67

[14]. *T. Kimmerlin, D. Seebach*, 100 years of peptide synthesis: ligation methods for peptide and protein synthesis with applications to β-peptide assemblies, Journal of Peptide Research, **vol**. 65, 2005, pp. 229–260.

[15]. *K. Hojo, H. Ichikawa, Y. Fukumori, K. Kawasaki*, Development of a method for the solid-phase peptide synthesis in water, International Journal of Peptide Research and Therapeutics, **vol**. 14, 2008, pp. 373–380.

[16]. *K. Hojo, N. Shinozaki, A. Hara, M. Onishi, Y. Fukumori, H. Ichikawa*, Aqueous microwave-assisted solid-phase peptide synthesis using Fmoc strategy. II. Racemization studies and water based synthesis of cysteine containing peptides, Protein & Peptide Letters, **vol**. 20, 2013, pp. 1122-1128.

[17]. *M. D'Hondt, N. Bracke, L. Taevernier, B. Gevaert, F. Verbeke, E. Wynendaele, B. De Spiegeleer*, Related impurities in peptide medicines, Journal of Pharmaceutical and Biomedical Analysis, **vol**. 101, 2014, pp. 2–30.

[18]. *M.V. Rodnina, M. Beringer, P. Bieling*, Ten remarks on peptide bond formation on the ribosome, Biochemical Society Transactions, **vol**. 33, 2005, pp. 493-498.

[19]. *K. Norén, M. Kempe*, Multilayered Magnetic Nanoparticles as a support in solid-phase peptide synthesis, International Journal of Peptide Research and Therapeutics, **vol**. 15, 2009, pp. 287–292.

[20]. *J. Kawahara, T. Ohmori, T. Ohkubo, S. Hattori, M. Kawamura*, The structure of glutaraldehyde in aqueous solution determined by ultraviolet absorption and light scattering, Analytical Biochemistry, **vol**. 201, 1992, pp. 94-98.

[21]. *E.Kaiser, R.L.Colescott, C.D.Bossinger, P.I.Cook*, Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides, Analytical Biochemistry, **vol**. 34, no. 2, 1970, pp. 595-598.

[22]. *I. Mîndrilã. S.A. Buteicã, D.E. Mihaiescu, G. Badea, A. Fudulu, D. N. Mãrgãritescu*, Fe_3O_4/salicylic acid nanoparticles versatility in magnetic mediated vascular nanoblockage, Journal of Nanoparticle Research, **vol**. 18, no. 10, 2016, pp.1-10.

[23]. *D.E. Mihaiescu, A.S. Buteicã, J. Neamţu, D. Istrati, I. Mîndrilã*, Fe_3O_4/Salicylic acid nanoparticles behavior on chick CAM vasculature, Journal of Nanoparticle Research, **vol**. 15, 2013, pp. 1-10.

[24]. *D. Koval, V. Kašička, I. Zusková*, Investigation of the effect of ionic strength of Tris-acetate background electrolyte on electrophoretic mobilities of mono-, di-, and trivalent organic anions by capillary electrophoresis, Electrophoresis, **vol.** 26, 2005, pp. 3221–3231

[25]. *O. Rubio-Pons, Y. Luo*, The permanent dipole moment of gas-phase para-amino benzoic acid revisited, Journal of Chemical Physics, **vol.** 121, 2004, pp. 157-151