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MAGNETIC COBALT FERRITE NANOPARTICLES: SYNTHESIS AND SURFACE FUNCTIONALIZATION WITH NATURALLY SMALL PEPTIDE

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The aim of the present study was to prepare inorganic-organic hybrid material for biomedical potential application. The cobalt-ferrite ($CoFe_2O_4$) magnetic nanoparticles (MNPs) obtained by sol-gel method and treated at 200 °C were immobilized in a small peptide. Covalent and non-covalent attachment of proteins with MNPs provides access to functional hybrid systems with applications in biotechnology, medicine and catalysis. The crystalline phases, morphology and chemical composition of the particles were characterized by GC-MS, NMR, TEM/EDX, BET and FT-IR.

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1. Introduction

Magnetic nanoparticles (MNPs) have an additional advantage of being easily manipulated by permanent magnets or electromagnets, independent of normal microfluidic or biological processes. A variety of nanoparticles (NPs) with various shapes such as spheres, nanotubes, nano-horns and nano-cages, made of different materials, from organic dendrimers, liposomes, gold, carbon, semiconductors, silicon to iron oxide, have already been fabricated and explored in many scientific fields, including chemistry, material sciences, physics, medicine and electronics [1].

Advances in nanotechnology play an important role in designing nanomaterials with specific functional properties that can address the shortcomings in the area of diagnostics and therapeutics. The potential of nanomaterials has sparked enormous interest in the drug industry and has envisaged several applications, as can be evidenced by the exponential growth of activities in this field. The advantages of the nanoparticles are mainly due to their nanoscale size and large surface area with the ability to get functionalized with targeting ligands, therapeutic moieties and biomolecules [2].

The phase structure and microstructure of the nanoparticles determine their physical properties. Nanoparticle syntheses utilizing biomimetic approaches have advanced in recent years. Peptides, with their ability to influence inorganic crystal growth, are a topic of great interest. The peptide influences the phase as well as the microstructure and therefore, the magnetic properties of the particles [3].

The chemical coating of these nanoparticles may also to be linked to molecules compatible, that specifically targeting a area such as an organ, a disease or a particular biological system [4].

In the last decade, magnetic NPs are used in bio-applications, including magnetic bio-separation and detection of biological entities (cell, protein, nucleic acids, enzyme, bacterial, virus, etc.), clinic diagnosis and therapy (such as MRI (magnetic resonance image) and MFH (magnetic fluid hyperthermia)), targeted drug delivery and biological labels [5].

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Nanotechnology presents very promising characteristics for its application in the biomedicine area. By now the most advanced application of nanoparticles in medicine is the use of iron oxide nanoparticles embedded in biocompatible polymers as magnetic resonance imaging (MRI) contrast agents. Until now have been studied various synthesis techniques for the preparation of $CoFe_2O_4$ nanoparticles, such as co precipitation [6], hydrothermal [7] micro emulsion [8], but the principal difficulty of these methods is that the obtained nanoparticles are agglomerated, having limited control over dimensional distribution, thus restricting their applications [9]. For early detection of tumors by MRI were used iron oxide bond with various types of ligands such as proteins, peptides and small molecules demonstrate active targeting of tumors via specific molecular recognition[10]. Bio-sensing strategies based on magnetic nanoparticles (MNPs) have recently received considerable attention.

The chemical synthesis of multimaterial nanocrystal heterostructures combining sections of oxide, metal and semiconductor materials in a single multifunctional nanoscale object represents a challenging research direction along which nanochemistry research is investing substantial efforts [11]. Multi-functional nanomaterials possessing fluorescent and magnetic properties may be used in a number of biomedical applications in nanobiotechnology, such as bioimaging, bio- and chemo-sensing, cell tracking and sorting, bioseparation, drug delivery and therapy systems in nanomedicine [12]. The therapeutic applications of oxide and hybrid nanostructures strongly depend on their physicochemical properties such as permeability, stability, morphology (size, shape and functionality) and biocompatibility. These physicochemical properties are dictated by the types, structures and orientations of the materials that comprise the oxide and hybrid nanostructures [13]. The bio-functionalization of monodisperse magnetic nanoparticles (NPs) of size 10-20 nm is of great interest as it would enable the ultra-sensitive magnetic detection of both proteins and nucleic acids. Given their extremely small size and high magnetization, such nanoparticles could also be used to bind and transport proteins, nucleic acids, and other biomolecules through microfluidic networks and, following introduction into a living organism, they could provide a means of monitoring and influencing cellular processes [14].

The present study investigates a new and easily synthetic route of preparation of inorganic-organic hybrid material with potential application in biomedicine. A proper characterization methodology was developed for this hybrid material.

2. Materials and Methods

All used reagents are analytical grade. Iron (III) nitrate hexahydrate, cobalt (II) nitrate hexahydrate, ammonium hydroxide and polyvinyl alcohol, triethylamine, dichloromethane and *N,N'*dicyclohexylcarbodiimide were purchased from Merck. Amino acids were acquired from Applichem and Alfa Aesar (USA).

2.1. Peptide synthesis:

Boc-protected dipeptide (Boc-Ser-Val-OMe) was obtained from valine methyl ester hydrochloride, *N*-BOC-*L* serine, triethylamine and *N, N'*dicyclohexylcarbodiimide in a molar ratio of 1:1:1.1:1.1. The *N-tert*-butyloxycarbonyl group was removed using 50% TFA/ dichloromethane. The dipeptide was afforded in 75.8 % yield.

2.2. Nanoparticles synthesis and functionalization

Spinel cobalt ferrites were prepared by sol-gel method using iron and cobalt nitrates as precursors. The preparation protocol included the following steps: (**1**) dissolution of metal nitrates in bi-distilled water; (**2**) addition of polyvinyl alcohol (PVA) to first solution for obtain a colloid; (**3**) increase pH to about 8 by addition of NH_4OH solution; (**4**) stirring at 80°C; (**5**) drying the gel at 140°C; (**6**) and finally the dried gel was treated at 200°C. The solid product thus obtained was incorporated in dipeptide in mass ratio 1:1 and 1:4. The mixture was dissolved in dichloromethane and ultrasonic for one hour at 40°C, in an ultrasonic bath equipped with thermostat and timer.

The hybrid material obtained was characterized using following methods: GC-MS. TEM/EDX, and BET analysis.

2.3. Materials Characterization

Qualitative analysis of dipeptide was performed on a GC-MS 7890A-5975C system (Agilent Germany) using the EZ: faast GC-MS free amino acids kit and ZB-AAA GC column (Phenomenex, Torrance, CA, USA). The used analysis conditions were the standard conditions written on the kit.

GC-MS separation conditions: the standard analysis conditions were the instructions from the kit: Oven: 30ºC (hold 1 min) to 40ºC at 30ºC/min (hold 10 min) to 360ºC (hold 1 min); Equilibration time: 1 min; Injection: split 1: 15; 250ºC; 2µL; Carrier Gas: Helium 1.1mL/min; 110ºC; Inlet pressure: 5.824 kPa/min; Detector: MS; Mode: Scan Transfer Line Temperature: 250ºC; Analyzer Type: Electron Energy: 70eV.

^{1}H NMR spectra were recorded on a Varian Mercury 300 spectrometer operating at 299.97 MHz.

The surface morphology of the materials obtained was observed using a transmission electron microscope (TECNAI, F30 G2) with linear resolution 1 Å and a punctual resolution of 1.4 Å and elemental analysis was performed with Energy Dispersive X Ray (EDX) spectrum.

The nanomagnetic compound and the hybrid material specific surface area (BET) were determined by Brunauer-Emmet-Teller (BET) method, based on adsorption/desorption isotherms of nitrogen at 77 K obtained with NOVA 2200 apparatus. The pore size distribution (PSD) was calculated from the adsorption isotherms using BJH (Barrett-Joyner- Halenda) method [15].

The Fourier transformed infrared spectrum was recorded in KBr pellet on a Bruker FT/IR-Vertex 70 instrument (resolution 4 cm^{-1}) in spectral range 4000-400 cm^{-1} (32 scans).

3. Results and Discussions

3.1 GC-MS analysis

As a first step were prepared the hybrid material precursors: MNPs and the dipeptide. The synthesis of the dipeptide was provided by a conventional solution method, according to a procedure previously described by our research team [17-19]. For the synthesis of peptide was selected two amino acids that are found in natural products, especially in medicinal herbs, namely: *L*-serine and *L*-valine. The formation of the dipeptide was investigation by GC-MS method. The obtained chromatograms are shown in the Figure 1.

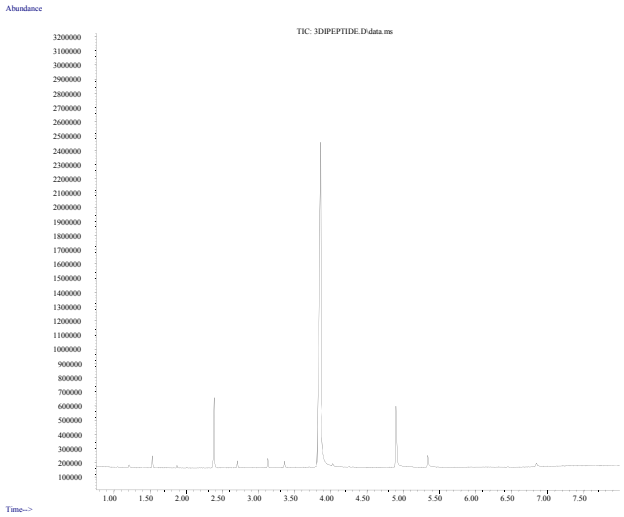

Fig. 1. GC-MS chromatogram for dipeptide

The mass spectra of the component from the GC-MS chromatogram was compared with the spectra from the NIST/NBS spectral database, and was identified the presence of dipeptide *L*-serin-*L*-valine.

3.2 H-NMR analysis

^{1}H-NMR (D_2O, δ, ppm): 1.16 d, 2.39m, 4.19t, 4.32m, 4.42m, 4.54m, 4.71s, 6.51s, 7,46s, 8.75s. The assigned ^{1}H NMR signals confirmed the serin-valine formation. The assigned ^{1}H NMR signals demonstrate the serin-valine formation.

3.3 TEM/EDX analysis

MNPs synthesis was carried out by sol-gel method. This approach present the advantage that by imposition of certain values of the reaction parameters (pH, temperature, etc) it can be controlled the size (at about 30 nm) and shape of spinel cobalt ferrites nanoparticules [20-24].

The morphology of obtained hybrid material was evaluated by comparative analyzing of inorganic material before and after the functionalization.

In Figures 2 (a) and 2 (b) are shown TEM images of the $CoFe_2O_4$ nanoparticles before assembly and after with dipeptide assembly, demonstrating their structures from the nanoscale level up to the aggregate particles.

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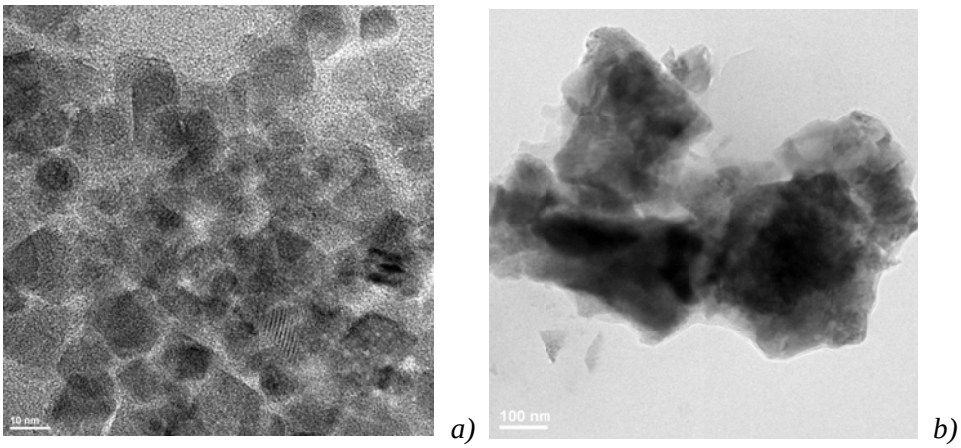


a) *b)*

Fig. 2. TEM images for $CoFe_2O_4$ (a) and $CoFe_2O_4$/dipeptide (b)

Also can be seen the TEM image for the $CoFe_2O_4$/dipeptide where are showed structures that covers matrix of the nanoparticle, and suppose that belong of the dipeptide structures assembled. These lamellar self-assembled structures are similar to the βCD crystals described previously in the literature [16], confirming the presence of the dipeptide in the ferrite matrix.

The EDX spectrum presents the elemental composition of the sample in which Co, Fe, O_2 are the majority elements and currently Cu in the sample is due to grid – support (Figure 3).

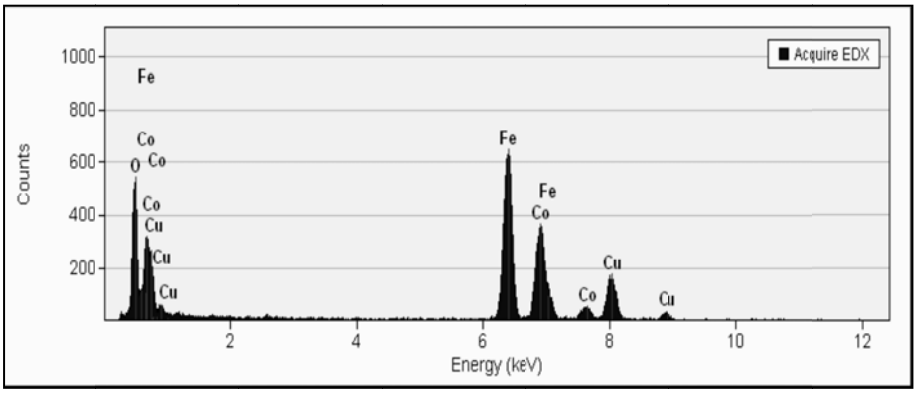


Fig. 3. EDX spectrum for $CoFe_2O_4$

3.4 BET analysis

Many properties of nanoparticles are improved with reducing size, so it is very important to determine their surface area.

The isotherm data obtained in partial pressure range of 0.05 to 0.3 (**Figure 4**) is plugged into the Langmuir adsorption isotherm, to obtain the BET plot.

Table 1. BET results for $CoFe_2O_4$ and $CoFe_2O_4$/dipeptide

BET $CoFe_2O_4$		
p/p*	cm^3/g STP	1/x[(p*/p)-1]
0.0577	0.313	0.1956
0.1128	0.3766	0.3378
0.1755	0.3949	0.539
0.238	0.3935	0.7937
0.3005	0.3677	1.1683

BET $CoFe_2O_4$ /dipeptide		
p/p*	cm^3/g STP	1/x[(p^*/p)-1]
0.0577	1.3601	0.04505
0.1126	2.1129	0.04601
0.1758	2.5434	0.08385
0.238	2.7805	0.11233
0.3006	2.9192	0.14723

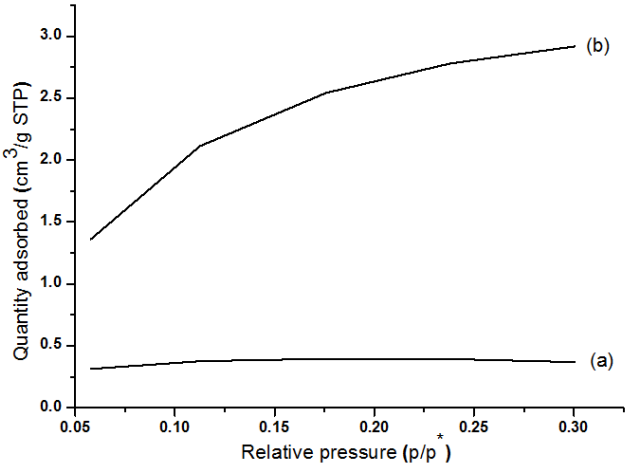
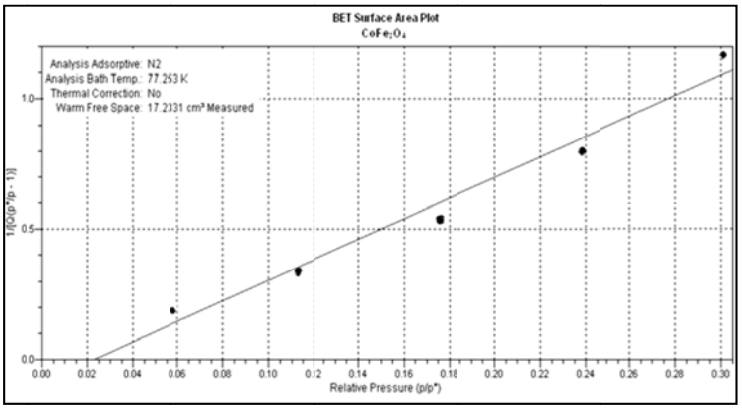
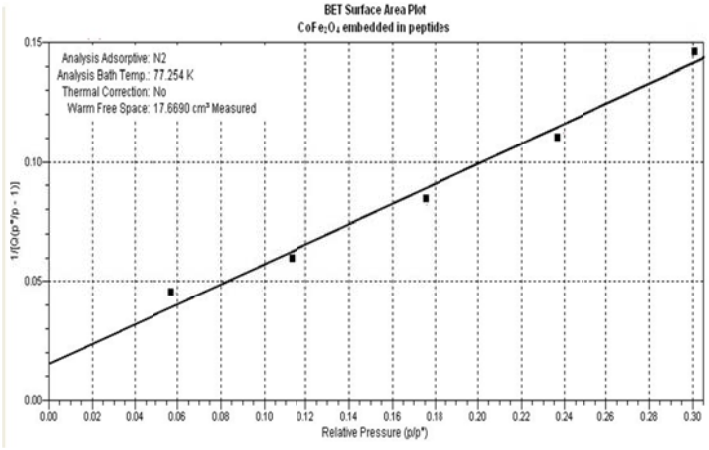


Fig. 4. The BET isotherms of the $CoFe_2O_4$ (a) and $CoFe_2O_4$/dipeptide (b)

In figure 5 is presented BET plot of IRMOF-13 using points collected at the pressure range 0.05 to 0.3 by the equation used to determine the surface area.



(a)



(b)

Fig. 5. BET plot using points collected at the pressure range 0.05 to 0.3 by the BET equation used to determine the surface area for $CoFe_2O_4$ (a) and $CoFe_2O_4$/dipeptide (b)

BET surface area and pore volume analysis were examined to confirm the surface modification. Figure 5 shows nitrogen adsorption isotherms of $CoFe_2O_4$ nanoparticle (a) before assembly and (b) after assembly of the dipeptide at 77°K, with corresponding pore-size distribution calculated by BJH method from desorption. Before assembly, the cobalt ferrite nanoparticles have a BET surface area of 1.1301m^2/g and warm free space 17.2331 measured. After dipeptide assembly, the values are 9.9801m^2/g and warm free space 17.6690 measured. From the result of BET analysis, we can find that the BET surface area increase after the layer-by-lay assembly.

Confirmation of MNPs functionalization was carried out also by FTIR spectroscopy. In this regard was recorded IR spectrum for hybrid material (figure 7) and for comparison, are included also spectres of precursors. Investigation of IR spectra dates of final products showed presence of wavelength characteristic to Fe – O bonds at approximate 567 cm^{-1}, C=O bond at 1617 cm^{-1}, amide bond at 1354 cm^{-1}, CN stretching at 1472 cm^{-1}. The strong signal at 1587 cm^{-1} is attributed amide band from dipeptide. The peak at 3400 cm^{-1} corresponds for hydroxyl group –OH. According to these results the functionalization of MNPs with targeting dipeptide was successfully accomplished.

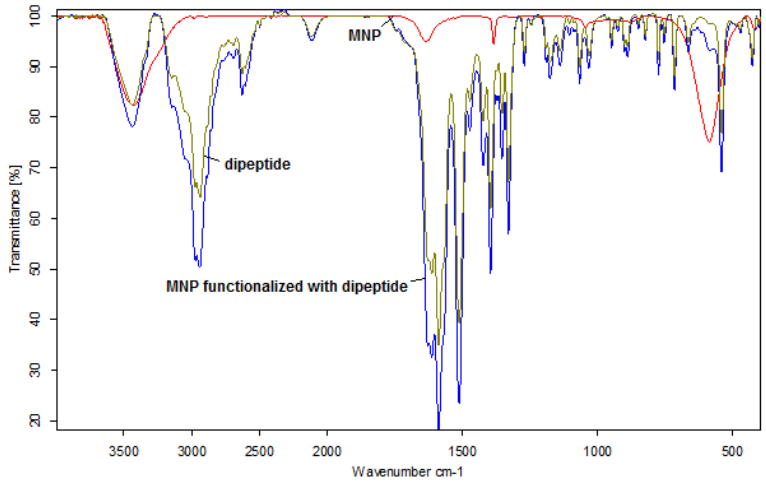


Fig. 6. Overlapping IR spectra for hybrid inorganic-organic material and precursors

4. Conclusions

Hybrid magnetic nanoparticles based on cobalt ferrite and serin-valine were prepared through a simple, effective method. The chemical structure of organic compound was evaluated by GC-MS and NMR analysis. Investigation of the specific surface of hybrid material crystalline phases, morphology and chemical composition of the final compound and precursors proved the confirmed obtaining of the hybrid system. The final product present interesting potential application in biomedicine, due to the fact that shows both features of magnetic nanoparticles and total synthesis of a natural dipeptide. Obtaining of inorganic-organic hybrid material was proven by TEM/EDAX, FT-IR and BET analysis.

This MNPs functionalization with a naturally small peptide can be considered as just a first step in design of new inorganic –organic hybrid materials with interesting features for development of new and improved nanotechniques especially for medical area.

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