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Maghemite and poly-DL-alanine based core–shell multifunctional nanohybrids for environmental protection and biomedicine applications

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ABSTRACT

This paper deals with the synthesis of two nanohybrid materials based on maghemite (γ-Fe_2O_3) and poly-DL-alanine using a two-step procedure consisting of maghemite nanoparticles synthesis by microemulsion method and nanohybrids obtaining by coating of maghemite nanoparticles with poly-DL-alanine biopolymer in two different molar ratios (H1:5 and H1:15). The maghemite and their corresponding nanohybrids were characterized by X-ray diffraction, Fourier transform infrared spectroscopy, X-ray photoemission spectroscopy, Mössbauer spectroscopy, Transmission electron microscopy, High resolution transmission electron microscopy with selected area electron diffraction and Atomic absorption spectroscopy. The two nanohybrids under the investigation have the average particle sizes of 22 nm and 23 nm. The Fourier transform infrared spectroscopy spectra and X-ray photoemission spectroscopy data indicate the existence of some interactions between the maghemite nanoparticles and poly-DL-alanine shell. The saturation magnetization values for maghemite and the two nanohybrids determined by a Vibrating Sample Magnetometer correspond to a typical superparamagnetic behavior suitable for applying in biomedical field. Also, with respect of biomedical application the biological activity of maghemite and its corresponding nanohybrids was investigated on healthy human cells (PBMC) and cancerous cells (HeLa). Furthermore, in order to support the multifunctionality of the γ-Fe_2O_3 sample and nanohybrids we also investigated their wastewater treatment properties by measuring the removal efficiency of heavy metal Cd (II) ions.

1. Introduction

Magnetic core–shell nanohybrids consisting of inorganic and organic components are very interesting due to the combination of several important properties which gives them the possibility of applying in many fields. An example of this type of magnetic hybrids is represented by magnetic oxide core nanoparticles coated by polymeric shells which have the potential to be used in fields as: environmental protection and biomedicine [1–3]. Magnetic nanohybrids may exhibit special magnetic properties, such as superparamagnetism, suitable for various biomedical applications that involve the use of an external magnetic field, such as magnetic resonance imaging (MRI), magnetically controlled transport of pharmaceuticals and localized hyperthermia. As regarding de environmental protection applications of the magnetic materials are currently investigated the priority pollutants removal from wastewater such as heavy metals which have as major source of pollution the disposal of effluents from the industries like battery manufactures, painting, paper, electroplating, metal finishing [4]. The required characteristic for using magnetic nanoparticles as adsorbents for removal heavy metals removal from wastewater is the diameter <50 nm [5].

Various chemical methods can be used to synthesize maghemite nanoparticles with the required characteristics for use in the biomedical field, but these have to provide the following main

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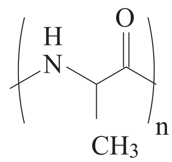

Fig. 1. Structural formula of poly-DL-alanine.

challenges: the selection of the optimum preparation condition for obtaining nanometric and monodisperse magnetic oxide particles, finding a reproducible procedure which can be applied in the pharmaceutical industry and using of a cost-efficient method [6–8]. From various synthesis methods microemulsion has proved to be a very efficient route to prepare nanosized and monodisperse nanoparticles [9].

The coating of the magnetic oxide nanoparticles with biopolymers brings many advantages such as: it prevents the agglomeration by providing a steric barrier, it avoids the opsonization and their clearance by reticuloendothelial system (RES) from the human organism before the therapeutic action is achieved. In addition, the polymeric coatings compared with inorganic coatings provide biocompatibility to magnetic oxide nanoparticles, the possibility to link on the target zone within the human body and attachment on the nanoparticles surface of various drugs. A variety of natural and synthetic polymers have been evaluated for using as coatings of the magnetic nanoparticles such as: dextran, sodium alginate, chitosan [10,11].

Polyaminoacids, including poly-DL-alanine, are biodegradable polyanionic materials having very low toxicity and immunogenicity and a wide range of applications from release agents in agriculture to water treatments, paint additives, cosmetics, metal adsorbents, and surfactants. Polyaminoacids have clinical applications being used as components for diagnostics, dialysis membrane, artificial skin, orthopedic implants, drug delivery systems and carriers for therapeutic protein conjugates [12].

Until now poly-DL-alanine polymer has not been used for inorganic nanoparticles biocompatibilization (Fig. 1). This articles reports a simple and economical synthesis procedure of core–shell multifunctional nanohybrids based on γ-Fe_2O_3 nanoparticles and poly-DL-alanine: synthesis of the maghemite nanoparticles by microemulsion method in the presence of a soft template followed by their coating with poly-DL-alanine. These hybrids were primarily characterized by X-ray diffraction (XRD) and Mössbauer spectroscopy providing information about the crystalline and polymeric phases. The morphological characterization reveals the core–shell nanostructure and the nanometric sizes of the two synthesized nanohybrids. The magnetic properties were provided from the experimental measurements of the first magnetization curve and the major hysteresis cycles. The results show the superparamagnetic behavior of the maghemite and its corresponding hybrid nanoparticles, which is different from the ferrimagnetic-type magnetization of the "bulk" sample. The biological activity was evaluated on human cervical carcinoma cells (HeLa) and peripheral blood mononuclear cells (PMBC) of a healthy donor. The results of the magnetic properties along with those of biologic activity are very important characteristics for the purpose of application in the biomedical field. The influence of the amounts of maghemite and poly-DL-alanine used in synthesis upon the characteristics of the core–shell nanohybrids finally obtained (core and shell sizes, magnetic properties) was also studied. For sustaining the multifunctionality of prepared nanopowders was done the investigation of environmental protection application in terms the heavy metal ion Cd (II) removal efficiency was investigated.

2. Experimental

2.1. Synthesis of core–shell magnetic nanohybrids based on γ-Fe_2O_3 and poly-DL-alanine

All chemicals were supplied from commercial suppliers (Sigma-Aldrich Co.) and were used without any further purification.

The synthesis of maghemite was done by microemulsion method using ferric chloride ($FeCl_2 \cdot 6H_2O$), sodium dodecyl sulfate (SDS), n-butanol, ammonia solution (NH_3) 25% as raw materials. A transparent mixture was obtained from SDS, water and n-butanol starting from a molar ratio of 1:2:5. Prior adding NH_3 solution to achieve the pH value of 10, the $FeCl_2 \cdot 6H_2O$ 0.5 M solution was added into the mixture. The precipitate obtained after 2 h of stirring the reaction mixture at room temperature was separated by centrifugation and washed several times with water and ethanol and finally kept at 105 °C for 3 h. The two nanohybrids were obtained starting from two molar ratios (1:5 or 1:15) between maghemite nanopowder and poly-DL-alanine biopolymer. The maghemite powder was added gradually to 15 mL of poly-DL-alanine 0.5 M solution to prepare nanohybrid noted H1:5 or 50 mL of poly-DL-alanine 0.5 M solution for obtaining nanohybrid noted H1:15 and stirred at room temperature for 6 h. The hybrid particles were separated by centrifugation and dried at 90 °C for 2 h.

2.2. Core–shell magnetic nanohybrids based on γ-Fe_2O_3 and poly-DL-alanine characterization

X-ray diffraction was done in order to characterize the crystalline structure, using a X- ray analytical diffractometer, X'PERT PRO MPD with Cu-Kα radiation (λ = 0.15418 nm). The maghemite content in the two nanohybrids was determined by using ANALYTIK JENA Atomic Absorption Spectrophotometer Contra AA7. The ^{57}Fe Mössbauer spectra were recorded at room temperature using a ^{57}Co(Rh) source and a standard spectrometer in constant acceleration mode. α-iron foil was utilized for the spectrometer calibration. The computer fit was performed in the hypothesis of Lorenzian shape of the absorption lines. X-ray photoemission spectroscopy (FTIR) spectra were registered on a Bruker Tensor 27 spectrometer using 32 scans at 4 cm^{-1} resolution in the 400–4000 cm^{-1} range. The X-ray Photoelectron Spectroscopy (XPS) was done on a K-Alpha instrument from Thermo Scientific, monochromated AlK_α source (1486.6 eV) at a bass pressure of 2×10^{-9} mbar. The pass energy for the survey spectra was 200 eV and 20 eV for high resolution. The transmission electron microscopy images (TEM) were obtained on FEI Tecnai ™G^2F30 S-TWIN with energy dispersive X-ray spectrometer. The magnetic curves (first magnetization curve and hysteresis loop) at 24 °C (297 K) for the maghemite and hybrids samples were measured by a vibrating sample magnetometer (VSM 7304 LakeShore USA).

2.2.1. Cell culture

Peripheral blood mononuclear cells (PBMC) were separated from the whole heparinized blood of a healthy volunteer and cultured as described previously [13]. Briefly, blood was diluted with PBS (1:1) and layered on Histopaque solution. After centrifugation, interface cells were collected and washed three times with PBS. After counting, cells were resuspended in nutrient medium. Nutrient medium was RPMI medium supplemented with 10% fetal calf serum (FCS), glutamine (2 mM), β-mercaptoethanol (50 μM), penicillin (100 IU mL^{-1}) and streptomycin (100 μg mL^{-1}). Cells were seeded (200,000 cells per well) in 96-well plates in nutrient medium with phytohemagglutinin (PHA) (5 μg mL^{-1}) and different concentrations of nanoparticles. Cells were incubated for 72 h at 37 °C in a humidified atmosphere with 5% CO_2.