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Full Length Article

Effect of the surface treatment on the structural, morphological, magnetic and biological properties of MFe_2O_4 iron spinels (M = Cu, Ni, Co, Mn and Fe)

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ABSTRACT

This research proposes to obtain iron spinels MFe_2O_4, and their hybrids $MFe_2O_4@SiO_2$ and $MFe_2O_4@SiO_2$/chitosan (M = Cu, Ni, Co, Mn e Fe) from the surface modification with (3-aminopropyl)trimethoxysilane agent and functionalization with chitosan, and then evaluate them as to their structure, morphology, magnetism and cytotoxicity, towards obtaining biocompatible hybrids for biological applications. The samples were analyzed by XRD, FTIR, SEM, TEM, magnetic measurements and in vitro cytotoxicity. XRD results showed that all samples presented the formation of the desired spinel major phase, with a crystallite size between 12 and 45 nm. According to the morphology and FTIR spectra of the samples, it was possible to confirm the formation of the hybrids. All samples presented soft magnetic material characteristics, exception given to the iron spinels $CoFe_2O_4$, which showed characteristic of hard magnetic material. The best results of cell viability were obtained by the $CoFe_2O_4$, $MnFe_2O_4$ e Fe_3O_4 MNPs and their hybrids.

1. Introduction

In recent years, more and more the nanotechnology has been widely used throughout the medicine, drug delivery systems, therapeutic agent for hyperthermia, optical, electronics, chemical and mechanical applications. Magnetic nanoparticles (MNPs) of general formula MFe_2O_4 (M = Fe, Mn, Co, Ni, Zn, Cu, Ni-Zn, Mn-Zn, for example.) are in the forefront of research fields. Not only because of their outstanding properties such as nanometric size, large surface area to volume ratio, superparamagnetic behavior, and high saturation magnetization, but also in virtue of that their electrical and magnetic properties can be effectively engineered by varying the chemical composition of M^{2+} ion [1–4]. MFe_2O_4 MNPs have shown great potential for many important applications, ranging from information storage, electromagnetic devices to medical diagnostics, protein and cell separations, immunoassay, medium for targeted drug delivery, magnetic resonance imaging (MRI) and sensors, biosensors and other detection systems [5–10]. Furthermore, MNPs have been used to enhance the sensitivity and the stability of sensors and biosensors for the detection of several analytes in clinical, food and environmental applications. Sensing strategies based on MNPs offer advantages in terms of analytical figures of merit, such as enhanced sensitivity, low limit of detection (LOD), high signal-to-noise ratio, and shorter time of analysis than non-MNP-based strategies [11,12]. In sensing applications, MNPs are used through direct application of tagged supports to the sensor, being integrated into the transducer materials, and/or dispersion of the MNPs in the sample followed by their attraction by an external magnetic field onto the active detection surface of the (bio)sensor [13].

For biomedical applications, MNPs should be non-toxic materials or adequately coated with substances that assure their stability, biocompatibility and prevent non-specific reactions with the physiological medium. Since small particles tend to form agglomerates due to attractive van der Waals forces, then, it is necessary to coat magnetic nanoparticles with a protective layer such as polymer [14], and carbon

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[15] in order to obtain stable particles.

Surface modification of the metal oxide nanoparticles (NPs) with organic compounds, such as silane coupling agents, has been employed to overcome this problem and reduce the particle surface energy and decrease their tendency to agglomerate [16,17]. These silane agents are organosilicon compounds that have two different functional groups, including one that reacts with organic materials and one that reacts with inorganic materials (MNPs). The general formula for a silane coupling agent typically shows the two classes of functionality is ($X–(CH_2)_n–Si–R_3$). The X groups represent the functional organic, for example: amino, methacryl, epoxy, etc., and the R represents hydrolyzable functional group such as methoxy, ethoxy and etc. [18,19].

The hydroxyl group (–OH) commonly exists on the surface of metal oxide NPs that is used as a reaction site [20]. The coupling process can be accomplished via the chemical reaction between the trialkoxy groups of silane coupling agent and the hydroxyl groups on the NP surface. These silane groups can have different organic moieties at one end of the silane coupling agent and a functional group at the other for further conjugation with other molecules. The surface modifications of metal oxide NPs by silane coupling agents (in this research, (3-aminopropyl)trimethoxysilane, APTMS) improve the compatibility of NP surface (hydrophilic) with a hydrophobic polymer surface (in this research, chitosan). Also increases the dispersion stability in organic media [21,22]. PEG, amino, vinyl and phenyl are some of the popular functional groups at the other end of these silane coupling agents.

Protective coatings not only stabilize nanoparticles, but can also be used for further functionalization [23]. When the surfaces of magnetic nanoparticles functionalized to create substrate specific surfaces, the targeted molecules can be easily captured. Thus, magnetic separation, which does not need pretreatment operations (such as centrifugation and/or filtration), can be used as a convenient and rapid technique for the separation and purification of biomolecules, such as proteins, nucleic acids, and enzymes [6,24].

Due to the excellent film-forming ability, high permeability, and mechanical strength, nontoxicity, biocompatibility, low cost and availability chitosan has been found to be an interesting biopolymer for biomolecules for biosensor applications. Chitosan is a natural poly-cationic polymer, which is non-toxic, hydrophilic, biocompatible, biodegradable, and anti-bacterial. It is composed of D-glucosamine and N-acetyl-D-glucosamine linked by b-(1.4)-glycosidic bonds, and thus has one free amino group and two free hydroxyl groups in the repeating unit. Chitosan and its derivatives have been widely used in many biomedical fields [25–29].

Therefore, the objective of this work is to produce MNPs of iron spinels by combustion reaction, to prepare its contact surface by means of a functionalization with the silane agent (3-aminopropyl)trimethoxysilane and with the addition of chitosan, to then evaluate them for its magnetic feature and cytotoxic behavior via route in vitro, aiming a possible biomedical application.

2. Material and methods

For the synthesis of MNPs, the following reagents were used: $Cu(NO_3)_2·3H_2O$ (241.60 g/mol), $Ni(NO_3)_2·6H_2O$ (290.81 g/mol), $Co(NO_3)_2·6H_2O$ (291.03 g/mol), $Mn(NO_3)_2·4H_2O$ (251.01 g/mol), $Fe(NO_3)_3·9H_2O$ (404.0 g/mol) and $CO(NH_2)_2$ (60.06 g/mol). All reagents of 99% purity.

The process of synthesis of the MNPs by combustion reaction involved a mixture of metal ions (nitrates) as oxidizing reagents, and the fuel (urea) as a reducing agent. In order to carry out the combustion reaction, the ratio of the initial mixture of each reactant was calculated according to the valences of the reactive elements established by the stoichiometry of the reaction according to the concepts of the chemistry of propellants and explosives [30]. The chemical reaction proposed to synthesize the spinel ferrites MFe_2O_4 (M = Cu, Ni, Co, Mn or Fe) by combustion reaction followed the scheme of the Eq. (1):

$$C_1\text{Metal Nitrates} + C_2\text{Urea} \xrightarrow{\Delta} P_1MFe_2O_4 + P_2\text{gases} \quad (1)$$

where C_1, C_2, P_1 and P_2 are the stoichiometric coefficients of the reaction responsible for the molar balance of the chemical components. According to Jain et al. [30], the gases generated in the reactions are N_2, H_2O and CO_2.

The reactions were performed in a stainless steel conical container designed to produce nanomaterials with capacity of 10 g/batch [31,32], and subjected to direct heating in a ceramic base with spiral electrical resistance, used as an external heat source, with a maximum temperature of approximately 350 °C, until it reached the autoignition (combustion). The reactions happened under atmospheric pressure, in a chapel with industrial exhaust capacity and with a gas washing system. All reactions showed maximum duration time less than 4 min, with maximum flame temperature ranging from approximately 700–1000 °C.

As product of the reaction, ferrites with the following chemical composition were produced: $CuFe_2O_4$, $NiFe_2O_4$, $CoFe_2O_4$, $MnFe_2O_4$ e $FeFe_2O_4$. The different compositions were named: FCu, FNi, FCo, FMn and FM, respectively. All compositions were deagglomerated in mortar, and sieved in 325 mesh (45 µm ABNT).

Then, the MNPs underwent a surface treatment involving a process of silanization with the (3-aminopropyl)trimethoxysilane agent [$H_2N(CH_2)_3Si(OCH_3)_3$] of 97% purity via reflux method [33,34] (Step I), followed by a chitosan functionalization process (Step II).

Step I: in the silanization process, for each composition of MNPs it was used 5 g previously oven dried at 100 °C; 5 mL of the (3-aminopropyl)trimethoxysilane agent and 50 mL of anhydrous toluene $C_6H_5C(O)OH$. For mixing, a 250 mL round-bottomed flask was placed on a heating mantle to 100 °C and coupled to the reflux system for a period of 72 h. After this process, the solution was transferred to test tubes for centrifugation in a baby model FANEM equipment with a speed of 500 rpm/30 min in order to promote the sedimentation and separation of the MNPs. Afterwards, the product was subjected to a washing process using CH_3CH_2OH ethyl alcohol for better removal of toluene still present, followed by the drying process in FANEM Model 315 at 150 °C/12 h. In this step, the silanized MNPs were obtained, which were designated by: FCuS, FNiS, FCoS, FMnS and FMS, respectively.

Step II: for the functionalization process of the MNPs, 1 g of chitosan was dissolved in 50 mL of acetic acid (1% v/v) to form a solution. Then, 5 g of silanized MNPs were added to the solution, which was kept under mechanical stirring for a period of 2 h at 50 °C. After this period, 1 mol/L sodium hydroxide solution was added per 1 h for complete removal of acid residues. After the alkaline reaction, the solution was washed with distilled water to remove excess sodium hydroxide and centrifuged for final sample separation, which was oven dried at a temperature of approximately 50 °C for a period of 24 h. After functionalization with the chitosan the samples were designated by: FCuSQ, FNiSQ, FCoSQ, FMnSQ and FMSQ, respectively.

The MNPs were characterized by X-ray diffraction (XRD) in a Shimadzu diffractometer (model LAB 6000) with scanning in the region of 15–75° 2Θ, Cu Kα radiation (λ = 1.5418 Å). The crystallinity was determined from the ratio between the integrated area of the peak relative to the crystalline phase and the area related to the amorphous fraction. The crystallite size was calculated from the X-ray enlargement line for the main peak by deconvolution of the secondary diffraction line of the polycrystalline cerium (used as standard) by the Scherrer equation [35].

FTIR spectra were obtained using a Perkin Elmer 400 FT-IR/FT-NIR spectrometer, between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1} and 20 scans. The morphology of particle agglomerates was investigated by scanning electron microscopy (SEM) using a Philips XL30 FEG microscope and by transmission electron microscopy (TEM) using