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Low toxicity superparamagnetic magnetite nanoparticles: One-pot facile green synthesis for biological applications

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ABSTRACT

Superparamagnetic magnetite nanoparticles have been synthesized by a highly reproducible polyvinyl alcohol (PVA)-based modified sol-gel process using water as the only solvent. The synthesis method has proven to be effective, time and cost saving and environmental friendly, resulting in PVA-coated magnetite nanoparticles as direct product from the synthesis, without any special atmosphere or further thermal treatment. X-ray diffraction and transmission electron microscopy revealed that the biocompatible PVA-coating prevents the nanoparticle agglomeration, giving rise to spherical crystals with sizes of 6.8 nm (as-cast) and 9.5 nm (heat treated) with great control over size and shape with narrow size distribution. Complementary compositional and magnetic characterizations were employed in order to study the surface chemistry and magnetic behavior of the samples, respectively. Cytotoxicity endpoints including no observed adverse effect concentration (NOAEC), 50% lethal concentration (LC50) and total lethal concentration (TLC) of the tested materials on cell viability were determined after 3, 24 and 48 h of exposure. The PVA coating improved the biocompatibility of the synthesized magnetite nanoparticles showing good cell viability and low cytotoxicity effects on the MTT assay performed on BHK cells. Preliminary assessment of nanoparticles *in vivo* effects, performed after 48 h on Balb/c mice, exposed to a range of different sub-lethal doses, showed their capacity to penetrate in liver and kidneys with no significant morphological alterations in both organs.

1. Introduction

The application of superparamagnetic iron oxide nanoparticles (SPIONs) in magnetic resonance imaging [1], guided drug and gene delivery [2,3], cancer therapy with magnetic hyperthermia [4,5], magnetic guided bottom-up tissue engineering [6], bioseparation and others [7], has become an important tool in scientific research, mainly due to the integration between therapy and diagnosis, the so-called theragnostics [8,9], and the possibility to design biosensors and therapeutic devices. Furthermore, SPIONs are renowned for their chemical stability and low toxicity. There are several established SPIONs synthesis methods, including co-precipitation, microemulsion, sol-gel, hydrothermal and thermal decomposition [10–12], each of those displaying advantages as well as drawbacks [13]. In order to improve its dispersibility and chemical stability as well as enhance its biocompatibility and reduce the toxic effects, it becomes necessary coating the SPIONs with a biocompatible material. Many coating materials have been used for its surface modification including polymers, proteins and silica. In this way, the polyvinyl alcohol (PVA) based sol-gel process arises as an attractive method. The PVA aqueous solution forms a gel-like substrate containing OH-groups in their composition which can form hydrogen bonds with the molecules of the desired solvent. This promotes statistic allocation of hydrated cations in the structure of the gel preventing separate crystallization of their compounds during the drying process [14]. Although PVA-coating has excellent barrier and mechanical properties, it has a low water stability that restricts its potential use in some applications. Therefore, to overcome this problem, PVA-based materials are often treated to ensure its structural integrity, including freeze-thaw crystallization, heat treatment, UV radiation, chemical agents and radical production [15,16]. As stated above, there are several chemical methods used in the production of magnetite nanoparticles, but

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most of them require expensive reagents and controlled atmosphere with Argon or Nitrogen. In the present work, superparamagnetic magnetite nanoparticles were synthesized by a PVA-based modified sol-gel process using cheap reagents and water as the only solvent, with no need of any special atmosphere, further coating or thermal treatment. The synthesized nanoparticles were characterized by thermal analysis (TGA and DTA), X-ray powder diffractometry (XRD), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and magnetic measurements (magnetization versus temperature and versus applied magnetic field). *In vitro* assays were performed to test the cytotoxicity of the coated and uncoated SPIONs. Preliminary assessment of SPIONs *in vivo* effects was performed with the aim to obtain the dose-response curves for qualitative parameters of morphological status in liver and kidneys of mice exposed to a range of sub-lethal doses. Also, analyses of the NPs in these tissues, urine and feces will indicate aspects of biodistribution and elimination (in course).

2. Experimental

2.1. Materials

Iron(III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$; ACS reagent) (Vetec, Brazil) and polyvinyl alcohol (PVA; MW 146000-186000 99+% hydrolyzed) (Sigma-Aldrich, USA) were used as the starting materials.

2.2. Chemical synthesis

Magnetite nanoparticles were synthesized by a modified sol-gel method [17]. Briefly, aqueous diluted PVA (10% w/v) and saturated ferric nitrate solutions were separately prepared and then mixed at Fe:PVA monomeric unit ratio of 1:18. The solution was maintained at room temperature under vigorous stirring for 2 h and then heated, under the same stirring, until total water evaporation. The final product (black fluffy material) was smashed and kept at 423 K for 1 h in order to cross-link the PVA. After the synthesis, two different samples were obtained: i) the as-cast material was manually ground to a black powder in an agate mortar and then diluted in distilled water and sonicated for 20 min. After this step, the magnetic powder was collected by means of a strong permanent magnet and washed several times with ethanol and distilled water. This sample was labeled as SPION-PVA (PVA-coated sample); ii) the as-cast material was heat treated in a furnace at 573 K for 1 h and after this step, the sample was ground to a black powder in an agate mortar and labeled as SPION-300 (uncoated sample).

2.3. Thermal analysis

The thermogravimetric analysis (TGA) was performed on the sample SPION-PVA, on a NETZSCH STA 409 PC LUXX in Ar atmosphere with controlled flow of 30 mL /min, from room temperature to 1473 K at a sweep rate of 5 K/min.

2.4. Microstructure

2.4.1. X-ray analysis

The crystalline microstructure of the samples was analyzed using a PANalytical X'Pert powder diffractometer in a Bragg-Brentano configuration with Cu-Kα_1 and Kα_2 radiation plus monochromator. The X-ray powder diffractogram were fitted by means of the Le Beil method (profile matching mode) [18], which is implemented within the Fullprof software [19]. A Voigt function, in the Thompson-Cox-Hastings approximation [20], has been chosen as analytical function for fitting diffraction profiles. Automatic background point selection was performed and used during the refinement procedure. A LaB_6 sample was used as an external standard reference material (SRM), in order to remove instrumental contribution to the diffraction profiles. Once diffraction profiles are fitted, two methods were used for microstructure analysis:

- *The Willianson-Hall method*:
  The classical Williamson-Hall method (WH) [21] relates the integral breath values of the peaks in reciprocal units (β^*) as a function of the diffraction vector (Q) after correction for instrumental broadening so that:

$$\beta^* = \frac{1}{< D >} + 2\epsilon Q. \quad (1)$$

  The method does not give information about the nature of the structural defects in the studied samples, but it allows evaluating microstrains (ϵ) and average crystallite size values ($<D>$) from the slope and the intercept of Eq. (1) respectively.
- *The Warren-Averbach (WA) method*:
  WinFit freeware [22] was used for fitting diffraction peaks with a Pearson VII analytical profile function. Multiple order profile analysis was performed using the first and second order reflections (220) and (440) of the magnetite phase taking into account instrumental broadening as well. The selection of these reflections fulfills the requirement of no overlap between neighboring diffraction peaks, except the overlap of the $K_{\alpha 1}$ and $K_{\alpha 2}$ components of the chosen peaks. It has been also considered during the extraction of the physical line profile. The background contribution was treated as in the WH method.

  The Warren-Averbach analysis [23] is based on a deconvolution Fourier-transform method in order to determine the intrinsic physical line profile, followed by the Fourier method for the evaluation of lattice imperfections. In this method the Fourier coefficients A(L) of the intrinsic physical line profile can be represented as the product of two terms: size and strain coefficients $A^S(L)A^D(s,L)$, that are numerically calculated, where L is the column length of orthogonal diffracting planes and s is a variable expressed in reciprocal unit.

2.4.2. TEM analysis

A small amount of powder was dispersed in tetrahydropyran (oxane) and sonicated for 15 min at room temperature. This solution was dropped in an ultrathin carbon film/holey carbon cooper grid (Ted Pella, Inc.). A conventional transmission electron microscopy (TEM-FEG) was performed in a JEOL 2100F at an accelerating voltage of 200 kV.

2.5. Composition

The composition and surface chemistry of the samples were analyzed by X-ray photoelectron spectroscopy (XPS). The samples were firstly ground with acetone in an agate mortar, followed by ultrasonic acetone dipping, magnetic separation, acetone washing and finally vacuum drying. The samples were, then, deposited in copper tape and analyzed in a SPECS PHOIBOS 100/150 spectrometer with a 150 mm hemispherical analyzer at X-ray energy of 1486.6 eV from a polychromatic Al Kα radiation in 30° of take-off angle, Epass of 30.0 eV, energy step of 0.5 eV for the survey spectrum and energy step of 0.02 eV for the high resolution spectra over each element peak. The calibration energy used was of C 1s with binding energy at 284.6 eV. The fittings of the envelope peaks of C 1s, O 1s and Fe 2p measures by high resolution were determined by the CASA-XPS software and NIST XPS database. For the Fe 2p fit, some restrictions were imposed to the CASA XPS software: the higher energy Fe $2p_{\frac{1}{2}}$ peak has to be the half of Fe $2p_{\frac{3}{2}}$ peak area ($A\,2p_{\frac{3}{2}}/A\,2p_{\frac{1}{2}} = 0.5$) and their peak shift was kept at 13.6 eV ($\Delta\,Fe(2p_{\frac{3}{2}} - 2p_{\frac{1}{2}}) = 13.6$ eV) [24]. The Fe satellite