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A comparative physicochemical, morphological and magnetic study of silane-functionalized superparamagnetic iron oxide nanoparticles prepared by alkaline coprecipitation

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ABSTRACT

The characterization of synthetic superparamagnetic iron oxide nanoparticle (SPION) surfaces prior to functionalization is an essential step in the prediction of their successful functionalization, and in uncovering issues that may influence their selection as magnetically targeted drug delivery vehicles (prodrugs). Here, three differently functionalized magnetite (Fe_3O_4) SPIONs are considered. All were identically prepared by the alkaline coprecipitation of Fe^{2+} and Fe^{3+} salts. We use X-ray photoelectron spectroscopy, electron microscopy, time-of-flight SIMS, FTIR spectroscopy and magnetic measurements to characterize their chemical, morphological and magnetic properties, in order to aid in determining how their surfaces differ from those prepared by $Fe(CO)_5$ decomposition, which we have already studied, and in assessing their potential use as drug delivery carriers.

1. Introduction

Advances in the technology of nanoparticles used in drug delivery applications herald a promising future for their use in medicine. Currently many research teams are investigating targeted drug delivery for the treatment of cancers, (Davydov et al., 2014) and pulmonary, (Pham et al., 2015) cardiovascular (Rao et al., 2014) and infectious diseases, (Davydov et al., 2014; Pham et al., 2015; Rao et al., 2014; Mbeh et al., 2012) among others. Our group, for example, is interested in nanocarriers that act against bacteria and are capable of preventing biofilm formation on implant surfaces, because nosocomial infections represent a huge health problem around the world: just in Canada, a quarter of a million cases of associated infections are reported annually. This fact results in 8500–12,000 deaths, and the rate is rising(Healthcare-associated Infections, 2009). Infections caused by *Staphylococcus epidermidis* and *Staphylococcus aureus* are the most common, and are present in 70–90% of reported cases of implant-related infections (Dickinson and Bisno, 1989). Our approach to the prevention of the bacterial adhesion is to develop functional coatings that are capable of destroying adhering bacteria (Nablo et al., 2005). Antibiotics, (Trampuz and Widmerm, 2001; Wilcox et al., 2001) silver ions (Yamanaka et al., 2005) and nitric oxide (NO), (Nablo et al., 2005) among others, have all shown antibacterial properties, reducing the amount of bacterial adhesion. Various vehicles have been proposed for use as prodrugs; these include nanoparticles, (França et al., 2013; Zhang et al., 2012) micelles (Zhang et al., 2012) and polymers (França et al., 2013; Zhang et al., 2012).

We propose to use functionalized SPIONs to target the infected site. SPIONs are considered to be excellent candidates for drug delivery, (França et al., 2013) since they can be directed in vivo to the specific target sites using external magnetic fields, when their diameters are smaller than 100 nm (Frankel et al., 1997; Felfoul et al., 2010; Brosseau et al., 2003).

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On attaining the target, they function as advanced medical tools. As examples, they may be used as prodrugs, (França et al., 2013; Zhang et al., 2012; Felfoul et al., 2010; Afkhami et al., 2011) as contrast agents, (Felfoul et al., 2010; Afkhami et al., 2011) in hyperthermia (Felfoul et al., 2010; Afkhami et al., 2011; Liu et al., 2012) and in cancer treatment (Afkhami et al., 2011; Liu et al., 2012; Sawant et al., 2009). They possess low cytotoxicity and high biocompatibility, (Mbeh et al., 2012) and have been accepted by the United States Food and Drug Administration (FDA), both as prodrugs and for clinical MRI applications (Gupta and Wells, 2004; Tassa et al., 2011). In vivo, SPIONs are converted to ferric ions, which are assimilated into the biological iron storage pool, as erythrocytes, indicating their acceptable use in humans (Li and Chen, 2011).

In a previous paper, we considered SPIONs manufactured by the thermal decomposition of $Fe(CO)_5$, whose mechanism is obscure: the nominally zero-valent Fe must oxidize to Fe^{2+} and Fe^{3+}, in the correct 1:2 ratio, before obtaining O, and forming Fe_3O_4 SPIONs. Their surfaces were found to be heavily contaminated during fabrication, (França et al., 2013) and they were found to have batch-to-batch variations of the surface chemistry, extensive enough to remove them from consideration as prodrugs. In fact, the general reproducibility of nanoparticle surface chemistry may presently be unfeasible. This presents a serious challenge for new biomedical applications, with their safety-related issues (protein corona formation, and the resultant bio- and hemocompatibilties) that depend on the control of the chemical and physical properties of the surfaces.

In the present paper, we characterize SPIONs made in a more conventional manner: the alkaline coprecipitation of Fe^{2+} and Fe^{3+}, in the correct 1:2 ratio; the precipitate loses water and forms Fe_3O_4 on annealing. SPIONs fabricated in this manner have been the subjects of several recent papers (Forge et al., 2008; Bridot et al., 2013). It is our purpose to thoroughly characterize them, to determine to what extent their surfaces differ from those made by the thermal decomposition of $Fe(CO)_5$, which we previously studied, (França et al., 2013) to what extent their synthesis is reproducible, and, through this, if they can be considered as potential candidates for use as prodrugs to target the infected sites.

2. Materials and methods

2.1. Synthesis

2.1.1. Bare SPIONs

Bare SPIONs were synthesized by the alkaline coprecipitation of iron salts in diethylene glycol (DEG), according to a published protocol (Forge et al., 2008; Bridot et al., 2013). Briefly, a mixture of $FeCl_2{\cdot}4H_2O$ (45 mmol; 8.9 g) and $FeCl_3$ (45% solution; 37 mmol; 9.1 ml) in DEG (250 ml) was heated under N_2 at 170 °C, with stirring. After 15 min, solid NaOH (15 g) was added. After stirring for 1 h at 170 °C, the solution was cooled and the magnetic particles were isolated by magnetic decantation (B_0 = 0. 5 T). The black precipitate was washed five times with aqueous HNO_3 (200 ml, 1 M) and the SPIONs were dispersed in deionized water, sonicated (45 min), and centrifuged (16,500 × G; 45 min) to remove aggregates (Eq. (1)).

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O \quad (1)$$

2.1.2. Positive SPIONs

TPED [*N*-[3-(trimethoxysilyl)propyl] ethylenediamine] (50 mmol; 10.8 ml) was slowly added to an aqueous suspension of bare SPIONs (200 ml; [Fe] = 25 mM) at 50 °C and the mixture was heated at reflux for 2 h. On cooling, the suspension was purified by membrane filtration (membrane cut-off: 30 kDa), and centrifuged (16,500 × g; 45 min).

2.1.3. Negative SPIONs

Bare SPIONs were treated with TEPSA [3-(triethoxysilyl) propyl succinic anhydride] in organic medium, as previously described (Bridot et al., 2013; Stanicki et al., 2014). Briefly, TEPSA (25 mmol; 7.1 ml) was slowly added to a suspension of SPIONs in dimethylformamide (50 ml; [Fe] = 100 mM). Water was then added (4.3 ml), followed by an aqueous solution of $(CH_3)_4NOH$ (1 M; 2.5 mmol; 2.5 ml) at room temperature while stirring. After heating at 100 °C for 24 h under continuous stirring, the SPIONs were collected by pouring the suspension into an acetone/diethyl ether mixture, followed by magnetic decantation. After washing with acetone, the black precipitate was dispersed in water and purified by membrane filtration (membrane cut-off: 30 kDa) before centrifuging (16,500 × g; 45 min).

2.2. Characterizations

2.2.1. Transmission electron microscopy (TEM)

Photomicrographs were obtained by bright-field imaging, using a JEM-2100F electron microscope, at beam energy of 200 keV. The elemental analysis was carried out by energy dispersive spectroscopy (EDS). Selected area electronic diffraction (SAED) was used to obtain crystal diffraction patterns. All the samples were diluted in water before preparation, and sonicated for 3 min to disperse the SPIONs. One drop of each sample was spread onto a copper grid and covered with a microscope cover glass until dry.

2.2.2. Vibrating sample magnetometry

Vibrating sample magnetometry was used to obtain the magnetization vs. magnetic field (M vs H) loop at room temperature, from H = 0–2 T, with a measurement precision of 1×10^{-6} emu. Measurements were determined on a known quantity of sample. Drops of each sample were spread onto a 10 mm × 10 mm piece of cleaned glass, and dried, and the weight of each sample was obtained before the measurement.

2.2.3. Transmission IR spectroscopy

Transmission IR spectra were obtained, in the range 400–4000 cm^{-1}, using a Thermo Scientific Nicolet 6700 Fourier transform IR spectrometer, at a resolution of 4 cm^{-1}; 96 co-additions were used to increase S/N. The samples were deposited by placing a drop on a diamond plate, and drying before adding the next drop; spectra were obtained after depositing three drops.

2.2.4. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was performed with a VG ESCALAB 3 MK II (Thermo VG Scientific), using non-monochromated Al $K\alpha$ X-rays ($h\nu$ = 1486.6 eV) at an instrument resolution of 0.85 eV and a perpendicular take-off angle. The analysis chamber pressure was < 10^{-9} Torr. Following Shirley background removal, the component peaks were separated by the VG Avantage software. The energy was calibrated by setting the C1s C—C peaks of all but the negative SPIONs to 285 eV; the energy of the negative SPIONs was calibrated by setting the more prominent C—Si peak to 284.1 eV. FWHM values were those previously established in our laboratory. Drops were deposited onto highly oriented pyrolytic graphite (HOPG) and permitted to dry.

2.2.5. Time-of-flight SIMS

ToF-SIMS was carried out on an ION-TOF TOF-SIMS IV mass spectrometer, with a mono-isotopic Bi^+ beam, generated by a liquid metal gun mounted on the instrument. The beam current was 1.5 pA. Drops of the samples were deposited onto a silicon substrate, covering an area greater than 2 mm × 2 mm, and permitted to dry. Positive ion spectra were calibrated using H^+, $H_2{}^+$, and $C_xH_y{}^+$