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Synthesis and characterization of near-infrared fluorescent and magnetic iron zero-valent nanoparticles

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ABSTRACT

Polyethylene glycol coated iron nanoparticles were synthesized by a microemulsion method, modified and functionalized. The polymer coating has a crucial role, preventing the iron oxidation and allowing the functionalization of the particles. The nanoparticles were characterized and their magnetic properties studied. A photochemical study of the iron nanoparticles conjugated with a near-infrared fluorescent dye, Alexa Fluor 660, confirmed that the fluorescent dye is attached to the nanoparticles and retains its fluorescent properties. The bioimages in red and near-infrared (NIR) region are favourable due to its minimum photodamage and deep tissue penetration. The nanoparticles obtained in this study present a good magnetic and fluorescent properties being of particular importance for potential applications in bioscience.

1. Introduction

A broad range of nanosized inorganic particles, including magnetic nanoparticles and quantum dots, have been extensively investigated because of their unique optical, electrical and magnetic properties [1–5]. Moreover, magnetic iron oxide colloids have been successfully used as magnetic resonance imaging (MRI) contrast agents and for cancer hyperthermia therapy [6–9].

The shape, size and size distribution of the magnetic materials are the key factors in determining their chemical and physical properties. Thus, the development of size and shape-controlled magnetic materials is crucial for their application [3,9]. So far, the most widely used and studied magnetic material is iron oxide, in the form of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3). Elemental iron has a significantly higher magnetic moment than its oxides. Moreover, elemental iron is the most useful among the ferromagnetic elements; it has the highest magnetic moment at room temperature (218 emu g^{-1} in bulk), and a Curie temperature which is high enough for the majority of practical applications. However, obtaining Fe nanoparticles, relatively free of oxide (usually Fe_3O_4), is still a challenge, to a large extent, not overcome [10–13].

Besides the properties of the metallic core, the coating of the nanoparticles could determinate or improve the uses of this kind of materials. For example, functionalized magnetic nanoparticles have been employed for site-specific drug delivery [14] or treatment waterwaste [15,16]. The variety of potential coating materials is continuously increasing with the development of new polymeric materials. However, polyethylene glycol (PEG) could be considered one of the most suitable polymer coatings for nanoparticles designed to be used in biomedicine. PEG is a water-soluble polymer with a low toxicity and antibiofouling properties that make it an appropriate candidate for several bioscience related applications [17,18]. PEG chains attached to a nanoparticle surface exhibit a rapid chain motion, this could contribute to the good physiological properties of the PEGylated nanoparticles [19] for imagining and therapy application. Also, successful studies haven been devoted to PEG-PLA coated nanoparticles for drug delivery [20,21]. PEG grafted onto the surface of nanoparticles provides steric stabilization that competes with the destabilizing effects of Van der Waals and magnetic attraction energies. Thus, there is a growing demand for improved methods for the synthesis and characterization of polyethylene glycol (PEG) derivatives [22–25]. Especially, polyethylene glycols (PEGs) of long polymeric chains have found significant applications in the structure stabilization [26–28].

Finally, the polymeric coatings of the nanoparticles could be conjugated with antibodies or fluorescent dyes adding different

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properties to the system [29–31]. That is, fluorescent-magnetic nanoparticles could be designed as an all-in-one diagnostic and therapeutic tool, able to visualize and simultaneously treat various diseases.

Fluorescence imaging is one of the most powerful techniques for monitoring biomolecules in living systems. Compared with fluorescent imaging in the visible region, biological imaging in red and near-infrared (NIR) region is favourable due to its minimum photodamage, deep tissue penetration, and minimum background autofluorescence caused by biomolecules in living systems. Therefore, chromophores with emission in red or near-infrared region have been paid increasing attention in recent years [32,33].

However, there is a specific difficulty in the preparation of fluorescent magnetic nanoparticles due to the risk of quenching of the fluorophore on the particle surface by the magnetic core. This problem could be solved by coating the magnetic core with a stable isolating shell prior to the introduction of the fluorescent molecule or by attaching an appropriate spacer to the fluorophore. Most fluorescent magnetic nanoparticles thus have a core-shell structure.

Several studies have been devoted to develop iron oxide nanoparticles conjugated with fluorescent dyes, in order to obtain dual-responsive nanoparticles, with magnetic and fluorescent response [31]. Often, the methods are time consuming due to the many synthetic steps or the fact that gold or silica precoating is required to protect the iron oxide nanoparticles previous to their functionalization [34–36]. Also, there is a significant lack on studies about iron nanoparticles functionalized with fluorophores [37]. The aim of this work is to synthesize iron nanoparticles coated with a PEG-derivative and functionalized with a fluorescent dye. The iron core of the nanoparticles will provide higher magnetization saturation than iron oxides, the PEG not only protects the metallic core but also adds interesting properties to biologically related applications. The selected fluorescent dye, imaging in red and near-infrared, is highly adequate for an application in medicine owing to its low photodamage. So, the obtained nanoparticles could be highly promising materials for combined MR/Optical imaging applications.

2. Materials and methods

2.1. Chemicals

All chemicals were reagent grade and used without purification. Ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), sodium borohydride ($NaBH_4$) and cyclohexan solvent were purchased from Sigma–Aldrich. Methanol and chloroform were purchased from Panreac and Lab-Scan, respectively. Polyethylene glycol (PEG) of 1000 g mol^{-1} molecular weight and methoxy polyethylene glycol (mPEG) of 2000 g mol^{-1} molecular weight were obtained from Sigma–Aldrich. Deionized Millipore Milli-Q water was used in all experiments. Alexa Fluor® 660 Protein Labeling Kit was purchased from Invitrogen.

2.2. Synthesis of iron nanoparticles

The preparation of PEG-stabilized nanoscale zero-valent iron nanoparticles was carried out via a controlled microemulsion method. The microemulsion synthetic methodology makes use of a biphasic heterogeneous solution of water-in-oil in which iron precursors are stirred. Water droplets are used as nucleation sites for the formation of nanoparticles, often in the presence of surfactant molecules dispersed in the oil, essentially forming micelles.

The reactions were carried out at room temperature using a single micellar system (sample FePEG-04) and two micellar systems (sample FePEG-02). The procedure followed in the first case is described here.

A surfactant solution prepared by dissolving 31.5 g of polyethylene glycol in 105 mL of cyclohexane was maintained under stirring and degassed for 10 min under N_2 atmosphere. Next, 6 mL of 0.33 M $FeCl_2{\cdot}4H_2O$ were added to the surfactant solution, stirred and degassed for 10 min. Metal particles were formed inside the reverse micelles via reduction of the metal salt using an excess of $NaBH_4$ (6 mL, 1.76 M). After a few minutes, the reaction was quenched by adding 50 mL of chloroform and 50 mL of methanol. The black precipitate was recovered with a permanent magnet, washed several times with methanol and dried under vacuum.

The same procedure was carried out in the synthesis performed by two micellar systems with the only difference that the reducing agent ($NaBH_4$), was added in aqueous solution instead of in solid form. This solution, when added to flask reaction, will result the second micellar system. Definitely, the method involves mixing two microemulsions: one containing the metal salt and the other the reducing agent; due to collision and coalescence of the droplets the reactants are brought into contact and react to form the nanoparticles.

Polyethylene glycol methyl ether (mPEG) shows greater versatility in functionalization, which increases the potential applications of nanoparticles. Specifically, this will be the derivative chosen to functionalize nanoparticles. The syntheses with this surfactant were carried out at room temperature using a single-micellar system, 0.40 g of iron salt, 0.20 g of the reducing agent, 105 cm^3 of cyclohexane and 6.0 g of water. The concentration of surfactant in this system was 0.095 M.

2.3. Functionalization of nanoparticles and labelling with fluorescent dye

The incorporation of the fluorescent molecule to the nanoparticles consists of several steps. Firstly, functionalized nanoparticles are synthesized and then the fluorophore is anchored. After that the labelled nanoparticles must be purified to take out the excess dye by size-exclusion chromatography.

2.3.1. Modification of mPEG

Polyethylene glycol methyl ether (mPEG) of molecular weight 2000 g mol^{-1} was firstly treated to obtain the aldehyde-derivative by oxidation of the hydroxyl end groups by dimethylsulfoxide (DMSO) and acetic anhydride at room temperature. Then the m-PEG-amine was obtained by the method described by Harris et al. [38], via reduction of the aldehyde groups using sodium cyanoborohydride in methanol at room temperature.

2.3.2. Synthesis of nanoparticles with mPEG-NH_2 and PEG

The synthesis of nanoparticles was performed by the method previously described for one micellar system. Owing to the small amount of material fluorescent necessary, the appropriate amount of mPEG-NH_2 was used, and the rest was PEG surfactant, as already shown, to provide adequate protection to the nanoparticles.

The surfactant consisted of a mixture of 7.5 g of PEG and 217 mg of mPEG-NH_2, amounts required to have a total surfactant concentration of 0.30 M.

2.3.3. Labelling of nanoparticles

The interaction of metal nanoparticles with fluorophores near its surface affects the intensity of their emission being critical the distance between the fluorophore and the surface of the nanoparticle so that the fluorescence is quenched when the distance is too short. For this study Alexa Fluor 660 was used. This is a succinimidyl ester of Alexa Fluor which exhibits bright fluorescence and high photostability characteristics allowing us to