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Formation and characterization of biobased magnetic nanoparticles double coated with dextran and chitosan by layer-by-layer deposition

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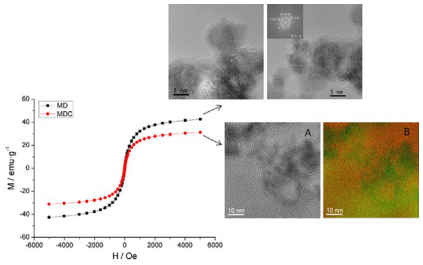
HIGHLIGHTS

- Biobased particles can be successfully achieved through layer-by-layer deposition.
- The first layer deposited determines the size and stability of the systems.
- Both magnetic nanoparticles presented similar colloidal stability.
- Both polymers are suitable for alternate layer-by-layer coating.
- The double coating process did not shield the magnetization of the nanoparticles.

GRAPHICAL ABSTRACT



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ABSTRACT

Magnetic nanoparticles double coated with different concentrations of dextran sulfate or reduced dextran and chitosan solutions were formed by layer-by-layer deposition and characterized by physicochemical techniques. Magnetic particles based on iron oxides presented superparamagnetic behavior, which indicates that they could be used in the future in magnetic driven therapies. The first layer of dextran sulfate on magnetic nanoparticles gave spherical nanoparticles with hydrodynamic diameters ranging from 45 nm to 88 nm and ζ-potential ranging from −45 mV to −52 mV depending on the concentration of dextran sulfate, and with hydrodynamic diameters of 77 nm and ζ-potential around 42 mV when the polymer coating was reduced dextran. The second layer was of chitosan and gave nanoparticles with hydrodynamic diameters ranging from 230 nm to 250 nm. The coating with this polymer at concentrations between 5 mg/mL and 10 mg/mL shifted the ζ-potential from negative to high positive values. The resulting colloidal systems were stable over time for a period 60 days. The content of iron, dextran sulfate and chitosan in the final double coated colloidal system were estimated in 47%, 9% and 34% using termogravimetric analysis. Dextran sulfate and chitosan layers could be visualized by microscopic techniques.

1. Introduction

Magnetic nanoparticles (MNPs) are important materials that offer several opportunities in chemistry, physics and biology. Colloidal suspensions of these particles, the so-called ferrofluids, have been used in numerous biological fields including diagnostics, drug targeting, molecular biology and cell purification. Iron oxides are the main constituents of magnetic particles although pure transition metals such as cobalt and nickel are also employed.

Abbreviations: MNP, magnetic nanoparticle; DXS, dextran sulfate; DXS-R, reduced dextran sulfate; CHI, chitosan; DLS, dynamic light scattering; TGA, thermogravimetric analysis; Cryo-TEM, cryogenic transmission electron microscopy; HRTEM, high resolution transmission electron microscopy; EFTEM, Energy filtered transmission electron microscopy.

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One of the most interesting features of the magnetic particles is that their movements can be deliberately controlled by time-modulated magnetic fields, which can penetrate human tissues without impediment. Consequently, magnetic nanoparticles can be used to deliver packages, such as anticancer drugs or radionuclide atoms, to a targeted area of the human body and can be applied in dynamic methods of cancer treatment, such as hyperthermia [1,2]. Alternatively, they are also useful to enhance the contrast in magnetic resonance imaging [3].

The miniaturization of iron oxide from the bulk (macroscale) to nanoscale is important because the properties of these materials depend strongly of their dimensions. Some of the medical applications of magnetic nanoparticles require that such nanoparticles possess superparamagnetic properties at room temperature. This property is displayed in particles of the order of tens of nanometers or less (10–15 nm for magnetite). Superparamagnetic particles are nonmagnetic in the absence of an external magnetic field, but they do develop a mean magnetic moment in an external magnetic field [4]. With particles with this size, thermal energy prevents efficiently the nanoparticles from sedimentation in a gravitational field or from the agglomeration produced by the dipole interaction due to the existence of a single magnetic domain in particles. However, thermal energy does not prevent coagulation produced by the van der Waals forces that induce strong short-range isotropic interactions [5]. To overcome this problem the inorganic nanoparticle core must be coated with surfactants, polymers, polyelectrolytes, block copolymers or inorganic materials [6]. In general, electrostatic repulsion or steric repulsion can be used to disperse nanoparticles and keep them in a stable colloidal state.

For biomedical applications *in vivo*, MNPs must present additional properties: they must be water dispersible, non-toxic, non-immunogenic, biocompatible and resistant to the biodegradation when exposed to the biological system. Iron oxide nanoparticles such as magnetite (Fe_3O_4) or its oxidized form maghemite (γ-Fe_2O_3) are by far the most commonly employed nanoparticles for biomedical applications. Highly magnetic materials such as cobalt and nickel are susceptible to oxidation and are toxic [7]. To achieve the biocompatibility, hydrodispersibility and resistance to biodegradation, MNPs must to have a suitable size and be coated with non-immunogenic, biocompatible materials [8]. In this way, the hydrodynamic size of the nanoparticle (combined size of the core and coating) should remain small. A size of 10–100 nm is optimal for *in vivo* delivery, as nanoparticles escape rapid renal clearance (<10 nm) and sequestering by the clearance organs (i.e., liver, kidneys, and spleen). The coating must be a polymer that reduces the interactions between nanoparticles and plasma proteins and avoids the action of the mononuclear phagocytic system (MPS). If the above requisites are satisfied, particles can remain in circulation after injection and to pass through the capillary systems of organs and tissues, avoiding vessel embolism. In this way, nanoparticles that are able to bypass biological barriers, achieve a more prolonged blood half-life and improve the likelihood of reaching target cells [9]. Dextran (DXS) is one of the most used polymers to coat magnetic nanoparticles. This polymer offers an attractive combination of solubility, low toxicity, biocompatibility, absence of accumulation in the MPS and improved circulation time in blood [10,11]. Moreover, DXS is used as plasma expander and presents a high affinity to iron oxides [12]. Other promising material is chitosan (CHI), a natural polymer carrying positive charge that presents low toxicity and biodegradability. Biocompatible and mucoadhesive properties of CHI have received substantial attention in novel adhesive systems [13,14]. Researchers have used derivatives of CHI to coach liposomes in order to enhance the absorption into intestinal epithelial cells [15,16].

MNPs can be used for cancer therapy. Following intravenous delivery of MNPs, an external magnetic field is used to concentrate MNPs at a specific target site; this procedure has been well tolerated in cancer patients [4,17]. But, for an effective cancer therapy, the MNPs must distribute throughout the tumor, be taken up by tumor cells, and localize to their intracellular site of action. Experiments reveal that cationic nanoparticles have better cell uptake but poor distribution throughout the tumor, while anionic nanoparticles distribute very well in the tumor but have poor cellular uptake [18]. Thus, an effective coating strategy should combine the better distribution of anionic nanoparticles in the tumor with the high cell uptake associated with cationic nanoparticles [9].

Layer-by-layer assembly is a promised methodology that uses electrostatic interaction to fabricate polyelectrolyte multilayers on substrates. The layer-by-layer assembling process creates a monolayer of adsorbed polymers at each assembly step. Therefore, it is possible to construct alternating biological activities of the layers in the assembly, as reported by [19]. These authors have achieved alternative anti and pro-coagulation activity of human blood using layer-by-layer coating of quartz crystal microbalance electrodes.

As we understand, the development of efficient biobased magnetic nanoparticles combining the cationic and anionic features of DXS and CHI may be useful to improve cell uptake and distribution of MNPs in cancer therapies. In the present work, we have prepared and characterized biobased magnetic nanoparticles double coated with DXS and CHI polymers by layer-by-layer methodology. Some authors have claimed that the higher stability of MNPs is obtained when they are coated with the reduced form of dextran (DXS-R) (Jarret et al., 2007, [20]). For this reason, we have included in our study samples coated with DXS and with DXS-R in order to compare both results.

The obtained biobased MNPs were characterized regarding their size, morphology, charge, stability, iron and polymer content and magnetic properties. Our results show that it is possible to obtain MNPs with alternate bioactive layers. This finding would be useful in several biomedical applications.

2. Materials and methods

2.1. Materials

A strong neodymium–iron–boron ($Nd_2Fe_{12}B$) magnet (1.2 T) was obtained from Halde GAC (Barcelona, Spain). Iron (II) chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) and iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) were purchased from Sigma–Aldrich (St. Louis, MO). Dextran sulfate sodium salt (molecular weight, 5000 kDa) was obtained from Fluka (Buchs, Switzerland), Chitosan chloride (Protasan UP CL 118, molecular weight < 200 kDa) was purchased from Nova Matrix (Oslo, Norway), ammonium hydroxide (NH_4OH, 25%) was from Panreac (Barcelona, Spain). Deionized Millipore Milli-Q water was used in all experiments.

2.2. Preparation of magnetite cores

Magnetite cores were prepared by coprecipitation of iron salts according to Berger et al. [21]. The method is based on the stoichiometric mixture of Fe^{2+} and Fe^{3+} in aqueous media, the coprecipitation of the corresponding hydroxides [$Fe(OH)_2$ and $Fe(OH)_3$] upon the addition of a strong alkali (NH_4OH), and the relatively fast aging of those hydroxides under vigorous stirring to form magnetite:

$$2FeCl_3 + FeCl_2 + 4H_2O + 8NH_3 \rightarrow Fe_3O_4 + 8NH_4^+ + 8Cl^- \quad (1)$$