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Synthesis of aligned hematite nanoparticles on chitosan–alginate films

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ABSTRACT

Iron oxide nanoparticles are being viewed with interest owing to the great potential they have in the biomedical applications like MRI contrast enhancement, targeted drug delivery, hyperthermia and recently in magnetic separation of cancer cells from the body. Templated synthesis has been considered ideal for synthesis of iron oxide nanoparticles as particles are attracted magnetically, in addition to usual flocculation through van der Waals attraction. Biological templates are attractive owing to their biocompatibility and the attractive porosity and surface chemistry that nature provides. Polysaccharides like chitosan and alginate have been employed in the synthesis of a polyion complex, which provided the active-binding sites for iron(II) ions in solution to bind. The natural organization of chitosan and alginate into a porous film has been exploited to synthesize spherical iron oxide nanoparticles through careful calcination of the iron(II) conjugate film. Our experiments indicate that the formed nanoparticles are highly crystalline, confirm to the hematite structure and have a superparamagnetic response with a low coercivity of 116 Oe. Particles thus synthesized were highly monodisperse with hydrodynamic diameter of 1.8 nm. The symmetric porosity of the film translates into the synthesis of well-aligned nanoparticles of iron oxide. Compared to synthesis in solution, the film-assisted synthesis offered a greater degree of control over the particle size distribution pattern, with the chitosan–alginate template providing the needed spatial separation to prevent the aggregation due to magnetostatic coupling. Such hematite nanoparticles can either be used directly or converted to paramagnetic magnetite by reduction. Zeta potential measurements indicate highly stable nanoparticles, which can therefore be conjugated to cationic liposomes carrying drugs and magnetically guided to target sites.

1. Introduction

Templating is one of the most frequently used methods of synthesizing materials with structural units ranging from nanometers to micrometers. Aqueous gel like lyotropic liquid crystal with extensive hydrogen bonding and nanoscale hydrophilic compartments can be employed for direct templating of nanoscale feature [1]. In this, the use of biological materials as templates is gaining importance. Biotemplating takes advantage of the structural stability and specificity of biological systems to create novel materials [2]. Many researchers have exploited biological media for the synthesis of nanoparticles and this include DNA [3], virus [4], protein [5], engineered bacteriophage [6], butterfly wings [7], chaperonin [8] and even natural fibers like spidersilk [9].

Synthesis of well-structured, monodisperse nanostructures of iron oxide has been of tremendous interest. Such nanostructures are extensively used in magnetic materials [10], as photocatalyst [11], in sensors [12] and medical applications such as hyperthermia, targeted drug delivery, magnetic resonance imaging, etc. [13]. Advancement in the use of magnetic particles for biomedical applications depends on the synthetic methods which have a higher control on size, size distribution, magnetic properties and particle surface characteristics [14]. Below 15 nm, iron oxide nanoparticles tend to behave like superparamagnetic material. Choosing the right materials for synthesis of iron oxide nanoparticles is crucial as by varying the processing conditions various forms of iron oxides such as Fe_3O_4, γ-Fe_2O_3 and α-Fe_2O_3 can be synthesized. Precipitation of the aqueous Fe^{2+}/Fe^{3+} solution [15] or in situ generated Fe^{3+} solution [16] results in Fe_3O_4 and γ-Fe_2O_3, while high temperature reaction results in α-Fe_2O_3 [17]. Ferromagnetic particles can also be synthesized through the hematite route owing to the higher stability of hematite and then subsequently reduced to magnetite [18]. Highly aligned nanoparticles of magnetite have been synthesized employing trypsin as a template [19]. Chitosan has served as a template for the in situ synthesis of magnetite nanoparticles and as coating for γ-Fe_2O_3 [20]. We have reported the synthesis of hematite nanoparticles in the size range of 100–200 nm on alginate [21], chitosan and starch [22] templates. Coupling of iron oxide nanoparticles with gold [23] and silica [24] in a core–shell model has offered added advantages in biological applications. Encapsulating iron oxide nanoparticles within a cationic liposome for targeted delivery of drugs has been reported [25]. Such liposomes

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carrying iron oxide nanoparticles find extensive applications in magnetic resonance imaging [26]. Recently the use of iron oxide nanoparticles in photonic applications have also been reported [27].

In the absence of surface coatings, magnetic iron oxide particles have hydrophobic surfaces with large surface area to volume ratio. Hydrophobic interactions coupled with magnetic interactions result in aggregation, making coating of the synthesized nanoparticles inevitable. This however results in a decrease in magnetic properties of iron oxide nanoparticles. A significant number of work has resulted in a decrease in magnetic properties owing to the encapsulation of the synthesized nanoparticles with coating agents like dextran [28], oleic acid [29], etc. Further to maintain the magnetic properties several authors have employed cobalt ferrites [30], the biocompatibility of which is yet to be proven [31].

In medical applications, magnetic nanoparticles are physiologically well tolerated. However, the particles with large hydrophobic surfaces are coated with plasma components and rapidly removed from circulation, where as particles that are more hydrophilic can resist coating process and are cleared slowly [32]. The background of this work is to provide iron oxide with such coating at the time of synthesis that the crystalline character of the oxide is retained, without affecting the properties of the resultant oxide. Further, the route employed should be facile and easily adoptable to all forms of iron oxide. There is also a need to choose such a template, which while serving the purpose of coating agent, would also be biocompatible. Polysaccharides then seem to be the obvious choice, owing to their excellent biocompatibility, drug carrying ability, lower cost and easier application. Direct use of polysaccharides in solution provided with particles above 100 nm [21,22]. This is possibly because the spatial separation was not maintained at higher temperatures. A constricted movement of iron(II) ions in a two-dimensional matrix would provide for the needed spatial separation and the size of the nanoparticles would be limited by the porosity. We have chosen to continue to work with chitosan and alginate. The utility of these templates are exemplified from the fact they are universally acceptable for synthesis of various metal oxide nanoparticles. For instance, gels of polyguluronate-rich sodium alginate and biopolymer like chitosan have been employed for the facile synthesis of type II, high-T_c superconductors with precise control over morphology and size [33,34].

The addition of chitosan to alginate endows alginate with a positive surface charge and also prolongs the time that the active ingredients contact with the epithelium and enhance absorption via the para-cellular transport pathway through the tight junctions [35]. Alginate-based chitosan hybrid polymer fiber has considerable potential as a desirable biomaterial scaffold for tendon and ligament tissue engineering [36] and as protein carriers [37].

In the present paper we have employed chitosan–alginate polyion complex, which at appropriate ratio forms a film with naturally formed porosity and placement of $-COO^-$ groups for binding to Fe(II) ions in solution. We have demonstrated in this paper the synthesis of hematite nanoparticles as it is the most stable form of iron oxide and can be reduced to magnetite as well. Further, the uniqueness of the choice of template and the methodology permits the facile direct synthesis of magnetite nanoparticles by precipitation. The iron oxide bound chitosan–alginate templates can then be used directly after removal of unbound ions, owing to their remarkable biocompatibility. In this work, the Fe(II)-bound films were calcined at controlled temperatures for complete removal of the template and formation of hematite nanoparticles of defined size and shape. The iron oxide nanoparticles thus synthesized have been characterized for the size distribution, charge and magnetic properties. The absence of any residual material has also been confirmed through infra red spectroscopy. The mechanism of formation of hematite nanoparticles has been investigated through thermo-gravimetric analysis (TGA) as well. The methodology can also be replicated for the synthesis of other nano-oxides.

2. Materials and methods

2.1. Materials

Ferrous sulfate AR, ACS ($Fe_2SO_4{\cdot}7H_2O$), chitosan and alginate where purchased from M/s. Aldrich USA. These reagents were used directly as received without further purification. The mean molecular mass (M) of chitosan was determined on a capillary viscometer (Ubbelohde, USA) using equation $\eta = KM^{\alpha}$, where η is the intrinsic viscosity, K and $^{\alpha}$ are coefficients equal to 1.38×10^{-4} and 0.85, respectively [38]. The molecular mass was estimated as 6 kDa.

2.2. Film fabrication

In this work, chitosan–alginate film was prepared by solution casting and solvent evaporation technique. 2 wt% solution of chitosan (in 2% v/v acetic acid) and 2 wt% of sodium alginate were prepared individually and mixed in the ratio 1:1, respectively. The mixture was then stirred for a period of half-an-hour and filtered to remove undissolved matter. A bubble free solution was cast on to a clean glass plate and evaporated to dryness in atmosphere at room temperature to obtain a dense porous film. Reaction between chitosan and alginate in solution (under appropriate ratios) has led to their assembly as a film. This film served as template. The active sites on alginate and chitosan served as sites for adsorption of Fe(II).

Film obtained was immersed for 30 s in 0.02 M ferrous sulfate solution made acidic with sulfuric acid to maintain a pH of 2 and subsequently air dried. Subsequent treatment of the film in a high temperature furnace (600–1000 °C) resulted in the complete conversion of Fe(II) to Fe(III), and its binding to the chitosan–alginate film, resulting in defined shapes and structures based on the porosity of the film. Further increase in temperature resulted in removal of template.

2.3. Characterization

2.3.1. Transmission electron microscopy

The transmission electron microscopy (TEM) micrographs, selected area electron diffraction (SADP) pattern and high-resolution transmission electron micrographs (HRTEM) were obtained with a JEOL 3010 field emission electron microscope with an accelerating voltage of 300 kV. The samples were analyzed by preparing a dilute solution made in propanol, drop casted on a carbon coated copper grid, followed by drying the sample at ambient conditions before it is attached to the sample holder on the microscope.

2.3.2. Contact angle measurements

The contact angle measurements were carried out by sessile drop method using a goniometer. The volume of the liquid droplets was kept constant at 1 μL.

2.3.3. Thermo-gravimetric analysis

Thermo-gravimetric analysis was performed using a universal V 3.9 A TA instrument. These studies used ramp setting of 10 °C/min from 32 to 1000 °C. Samples were run as prepared under nitrogen gas at 60 mL/min.

2.3.4. Fourier transform infrared spectroscopy

The infrared (IR) spectrum was recorded on a PerkinElmer RX-1 model FTIR spectrophotometer. Infrared spectroscopic measure-