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Facile synthesis of high-magnetization γ-Fe_2O_3/alginate/silica microspheres for isolation of plasma DNA

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ABSTRACT

A novel process combining emulsification with sol–gel method is described to synthesize γ-Fe_2O_3/alginate/silica microspheres. The synthesis procedure consists of two steps: (1) synthesis of magnetic alginate microspheres via an emulsification technique; (2) in situ synthesis of magnetic silica microspheres by the hydrolysis of TEOS in the presence of ammonia solution. The as-synthesized composite microspheres with a typical average diameter of 4.4 μm were spherical and superparamagnetic. Moreover, they contained up to 31.7 wt% maghemite with a saturation magnetization of 15.1 emu g^{-1}. The application of these magnetic microspheres as adsorbents for isolation of plasma DNA has also been studied.

1. Introduction

Superparamagnetic microspheres consisting of magnetic nanoparticles (NPs) (e.g. γ-Fe_2O_3 or Fe_3O_4) encapsulated in polymer and/or inorganic oxide matrix have attracted intense attention in recent years due to their potential applications in biomedical fields such as cell separation [1], magnetic resonance imaging [2], drug delivery systems [3], and hyperthermia [4]. As specifically required in biological and biomedical applications, high magnetic sensitivity, biocompatibility, hydrophilic character, high stability against aggregation and versatility in surface modification are crucial [5]. So far, a lot of procedures have been developed to prepare such magnetic microspheres [6–9]. Among a variety of coating materials mentioned above, silica is most commonly used, providing magnetic microspheres with several benefits such as good hydrophilic character and high stability against aggregation. Moreover, its versatile surface functionality would allow for bioseparation, biolabeling and drug delivery. Recently, several approaches have been proposed for the synthesis of superparamagnetic silica microspheres [10–14]. Xu et al. described a new process to obtain monodispersed, nanoscale, superparamagnetic Fe_3O_4/polystyrene/silica spheres with 80 wt% of magnetite which exhibit a much higher saturation magnetization [15]. Nevertheless, these composite particles described above have some drawbacks, such as time-consuming preparation, complicated isolation and purification, low stability and so on, which restrict their further applications. Therefore, it is of fundamental importance to develop convenient, economic and efficient methods for the preparation of superparamagnetic microspheres with a high fraction of magnetic nanoparticles and hydrophilic surfaces.

Alginate, a naturally occurring polysaccharide, has attracted intense attention as an important class of biomaterial in recent years because of its unique properties including inexpensiveness, relatively inert hydrogel environment within the matrix, a mild room-temperature encapsulation process and biocompatibility. Furthermore, alginate can be ionically crosslinked in the presence of divalent cations such as Ca^{2+}, which has been extensively investigated for many biomedical applications including tissue engineering, drug delivery vehicles, and cell transplantation matrices [16–20]. It is well known that ionically crosslinked alginate gels dissolve in PBS buffer or biological environments due to the loss of divalent crosslinking cations into the surrounding medium, which is a critical aspect to control for further applications. Very recently, nanoorganized multilayer coatings involving chitosan/alginate, polylysine/alginate, polyethyleneimine/alginate and other polyelectrolyte coatings have been applied on alginate hydrogels using the layer-by-layer self-assembly technique in order to enhance the

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overall stability of the hydrogels in biological environments [19,21–23].

Herein, we describe a novel process combining emulsification with sol–gel method for the synthesis of magnetic silica microspheres, which may serve multiple purposes including stabilizing alginate hydrogels against dissolution in biological environments and providing versatility in surface modification, as well as making the structures useful for biological and biomedical applications. The synthesis procedure consists of two steps: (1) synthesis of magnetic alginate microspheres via an emulsification technique; (2) in situ synthesis of magnetic silica microspheres by the hydrolysis of TEOS in the presence of ammonia solution. The morphology, structure, and magnetic properties of the as-synthesized γ-Fe_2O_3/alginate/silica microspheres were characterized by transmission electron microscope, X-ray diffraction, thermogravimetric analysis and vibrating sample magnetometer. Furthermore, the utility of such magnetic microspheres is demonstrated by isolation of plasma DNA.

2. Materials and methods

2.1. Materials

Sodium alginate (1 wt%, viscosity $\geq$ 0.2 Pas, 20 °C), sorbitan trioleate (SPAN 85), polyoxyethylene sorbitan trioleate (TWEEN 85), tetraethoxysilane (TEOS), ferric chloride hexahydrate, ferrous sulfate heptahydrate, ammonia solution and calcium chloride were all purchased from Shanghai chemicals Co. Ltd. meso-2,3-dimercaptosuccinic acid (DMSA) was obtained from Sigma–Aldrich. Agarose (Biological grade) was purchased from Shanghai Haoyuan Co. Ltd. All chemicals were used as received without further treatment. PCR primers were synthesized by Takara. Other reagents used in DNA isolation and analysis were of analytical grade.

2.2. Synthesis of magnetic alginate microspheres (MAMs)

γ-Fe_2O_3 NPs, used as magnetic cores, were prepared by chemical coprecipitation. The stable ferrofluid was obtained via surface modification with DMSA according to our previous procedure [24,25]. The synthesis of MAMs was a modification of the conventional emulsification technique [21,26]. Briefly, 50 g aqueous solution of DMSA-coated γ-Fe_2O_3 NPs (200 mg) and sodium alginate (500 mg) was dispersed in 75 g isooctane containing 1.696 g SPAN 85, ultrasonicating and stirring for 10 min. Then, a solution of 5 g isooctane containing 0.904 g TWEEN 85 was added to the emulsion under stirring and ultrasonication at the same power for 20 min. After that, another 30 min stirring was proceeded to achieve stable water-in-oil emulsion droplet. Subsequently, 20 mL of aqueous solution containing 10 wt% of calcium chloride was added to form ionic crosslinks. Finally, the products were washed with water by magnetic decantation for four times and redispersed into water at room-temperature.

2.3. Synthesis of silica-functionalized magnetic alginate microspheres (MAMs@SiO_2)

The suspensions of the MAMs used for synthesis of MAMs@SiO_2 were prepared at a maghemite concentration of 0.84 mg/mL in 20 mL of aqueous solution. In a typical process, to the obtained suspension was added 0.3 mL of ammonia aqueous solution under stirring. After 5 min, 20 μL TEOS was added and the mixture was allowed to stand at room-temperature for 3 h. The resulting composite microspheres were collected by magnetic separation and washed several times with water.

2.4. Isolation of plasma DNA using MAMs@SiO_2

2 mL of vein blood was centrifuged at 1800 rpm for 10 min at room-temperature. The supernatant was added to a 1.5 mL microcentrifuge tube, and was centrifuged at 17000 rpm for another 10 min at 4 °C. Then a 200 μL aliquot of the supernatant (plasma) was aspirated and stored at −70 °C. In a typical experiment for the isolation of DNA from plasma, 100 μL of lysis buffer (0.1 M Tris–HCl, 0.05 M EDTA, and 0.5 M NaCl, pH 8.0) were added to the plasma and left at room-temperature for 10 min. Subsequently, 10 μL of magnetic microspheres (maghemite concentration: 1.674 mg/mL) and 150 μL of binding buffer (20% PEG_{8000}–2 M NaCl) were added and DNA binding to the microspheres completed by 5 min of incubation at room-temperature. After the magnetic separation of the microspheres, the supernatant was removed. The magnetic microspheres were washed with 80% 2-propanol, followed by 70% ethanol. The immobilized DNA was eluted from the magnetic microspheres by 30 μL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.8) and was incubated at 50 °C for 5 min. A 5 μL aliquot of the eluted DNA was used as DNA matrix in PCR amplification. PCR was performed in a 25 μL reaction volume containing 10 μL of PCR buffer, 400 μM of dNTPs, 200 nM of primers 5′-GGACCTGACTGACCTCATGAA-3′ and 5′-CTTAATGTCACGCACGATTTCC-3′, and Taq-polymerase (0.025 U/L). The amplification was carried out in one cycles of 300 s at 95 °C; 30 s at 94 °C; 30 s at 58 °C; 45 cycles of 40 s at 72 °C. PCR products were detected using gel electrophoresis on 1% agarose.

2.5. Characterization

The size and morphology of the mcirospheres were characterized by transmission electron microscope (TEM, JEOL, JEM-2000EX). The samples were prepared by dropping 6 μL of solution on the carbon-coated copper grids and allowing the solution to dry in the air. Energy-dispersive spectroscopy (EDS) spectrum was collected from the core-shell regions. The samples were prepared by depositing a drop of solution on the aluminium substrate. The shape of the samples was determined by an inverted optical microscope (Zeiss, Axioscop200). Particle size distribution was measured by quasi-elastic light scattering (Malvern Mastesizer 2000).The surface charge of the products were investigated through a ζ-potential analyzer (Beckman Coulter, Delsa 440SX). Powder X-ray diffraction (XRD, Rigaku, D/MaxRA, λ=1.5405 $\times$ 10^{-10} m, CuKα) and selected-area electron diffraction (SAED) were used to determined the crystal structure of the products. Thermogravimetric analysis (TGA) was performed on a PerkinElmer Pyris-1 series thermalanalysis system under a flowing nitrogen atmosphere at a scan rate of 10 °C/min from 100 °C to 700 °C. Magnetic properties were determined with vibrating sample magnetometer (VSM, Lakeshore 7407) at room-temperature in a field up to 5 kOe. The PCR reaction mixture was amplified on a thermal cycler (ABI 7500, Applied Biosystems). Agarose gel electrophoresis was carried out using a 3000 Xi power supply (Bio-Rad).

3. Results and discussion

3.1. Synthesis of MAMs and MAMs@SiO_2

Scheme 1 illustrates the preparation procedure. Firstly, aqueous solution containing alginate and DMSA-coated γ-Fe_2O_3 NPs was added to isooctane with surfactants SPAN 85 and TWEEN 85. The mixture was treated ultrasonically to obtain water-in-oil emulsion. Secondly, calcium ions were added to the emulsion to form MAMs. Finally, the MAMs were coated with a silica layer via the hydrolysis and condensation of TEOS onto the surface of the microspheres. It is worthy mentioning that the presence of DMSA-coated γ-