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Synthesis of biocompatible hybrid magnetic hollow spheres based on encapsulation strategy

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ABSTRACT

A kind of novel magnetic hollow spheres was prepared by encapsulating magnetofluid into polymeric hollow spheres. Polymeric hollow nanospheres were constructed by self-assembly of rod-coil complexes, in which the rod-like segments were formed by inclusion of α-cyclodextrins (α-CD) and grafting poly(ethylene glycol) (PEG) chains of chitosan-*graft*-PEG (CS-*g*-PEG). Structural characteristics of CS-*g*-PEG/α-CD hollow spheres were investigated in detail by NMR, XRD, TEM, etc. Furthermore, those hollow spheres showed a pH responsive property which induced a considerable change of their radius. Magnetofluid was physically entrapped into the empty domain while hollow spheres were formed, it was found that the hollow spheres can encapsulate large quantities of magnetofluid and the encapsulated magnetofluid still possess magnetic responsiveness properties. We expect that this strategy may be served as a novel and more straightforward approach to obtain magnetic hollow spheres for biomedical application.

1. Introduction

In recent years, the construction of magnetic nanostructures has become a particularly important research field and being attracted growing interest (Arruebo et al., 2006; Nam, Thaxton, & Mirkin, 2003; Yu et al., 2006). Due to their advantages, such as magnetic properties, chemical stability, biocompatibility and low toxicity, magnetic nanocrystals have been intensively studied not only for fundamental scientific interest but also potential applications in biomedical fields, especially in the field of targeted drug delivery (Bráhler et al., 2006; Cao, Zhu, Ma, Li, & Zhang, 2008; Gupta & Gupta, 2005; Hogemann, Ntziachristos, Josephson, & Weissleder, 2002; Hu et al., 2006; Willner & Katz, 2003; Zhao, Kircher, Josephson, & Weissleder, 2002). Pure magnetic nanoparticles are not very useful in practical applications since they are prone to aggregate and rapidly biodegradate when they are directly exposed to a biological system. To overcome these limitations, the magnetic nanoparticles are usually used in the form of core–shell structures or composite nanoparticles (Bruce et al., 2004; Liu, Ma, Xing, & Liu, 2004; Santra et al., 2001). As a kind of ideal material for target drug delivery investigations manipulated by external magnetic fields, nanosized hybrid hollow spheres with magnetic component has attracted much attention owing to their special magnetic properties and great potential for encapsulation of large quantities of guest molecules within the "empty" core domain (Caruso, Spasova, Susha, Giersig, & Caruso, 2001; Pedro, Teresita, & Carlos, 2001; Zhang, Li, Tang, & Ren, 2006).

Currently, the core-template-based strategy is widely used for the synthesis of hybrid magnetic micro or submicron hollow spheres by coating the magnetic components on the polymer particles (Imhof, 2001; Lin, Chen, Wang, & Chen, 2011; Shiho & Kawahashi, 2000; Tissot, Reymond, Lefebvre, & Bourgeat-Lami, 2002; Zhong, Yin, Gates, & Xia, 2000) or layer-by-layer deposition (Caruso, Caruso, & Mohwald, 1998; Caruso et al., 2001; Kawahashi & Matijevic, 1990). One advantage of such an approach is that thickness of the coating layers is controllable. Although it is successful in the preparation of hollow magnetic spheres, core-template-based approach still faces limitations including the selection of core composition and nanosized core templates. Furthermore, the core must be removed to create the hollow center. Hence, a core-template-free strategy for the production of hollow hybrid magnetic hollow spheres is of particular interest (Ding, Hu, Jiang, Zhang, & Yang, 2004; Ding, Hu, Zhang, Chen, & Jiang, 2006; Wong, Cha, Choi, Deming, & Stucky, 2002). However,

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recently established core-template-free strategies to prepare hollow hybrid magnetic hollow spheres need harshly physical or chemical procedures which cannot favor to biomedical application.

As well known, hollow spherical aggregates itself have great potentials for encapsulation of large quantities of guest molecules or large sized guests within the "empty" core domain (Bergbreiter, 1999; Cao, Dong, O'Rorke, Wang, & Pandit, 2011; Meier, 2000). Magnetofluid, which has significant superparamagnetism and appropriate size for encapsulation (<10 nm), is a kind of ideal material for constructing magnetic hollow spheres by encapsulation strategy. Therefore, for magnetic target biomedical application, we can try combine delivery and controlled release in one system through filling the hollow core with magnetofluid and desirable drugs. We envision that the strategy of encapsulating magnetofluid into polymer hollow spheres rather than coating magnetic nanoparticles on hollow spheres may be served as a novel and more straightforward approach to obtain magnetic hollow spheres for biomedical application.

In our group, the efforts have been devoted to utilizing rigid/coil system to construct hollow spheres, in which the rod-like blocks are formed by inclusion of the host and guest molecules, the coil-like blocks are always natural polysaccharides and other biocompatible materials. Because of the biocompatible and biodegradable properties, the application of such hollow spheres for enzyme encapsulation and gene delivery was further investigated in detail (Ha et al., 2010, 2011; Li et al., 2011; Meng et al., 2010). In this work presented herein, chitosan (CS) was employed as the matrix of the hollow structure (Wang, Luo, Shao, & Zhou, 2010). We use chitosan-*graft*-PEG (CS-*g*-PEG) as the guest polymer for self-assembly inclusion and the hollow spheres could be formed by inclusion CS-*g*-PEG and α-cyclodextrin (α-CD), in which the rod-like blocks are formed by self-assembly α-CD with poly (ethylene glycol) (PEG). It was found that those hollow spheres showed pH responsive properties which inducing a considerable change of their radius. Furthermore, a kind of much simpler and more straightforward method was applied to construct magnetic hollow spheres, by which entrapping magnetofluid into CS-*g*-PEG/α-CD hollow spheres directly. The obtained magnetic hollow sphere still has magnetic responsiveness and has no effect on the superparamagnetism of magnetofluid which indicates a commendable potential in drug delivery therapeutics.

2. Experimental methods

2.1. Materials and instrument

Chitosan muriate (degree of deacetylation was ≥85%) was purchased from Shandong AK Biotech, Ltd. mPEG (M_W = 2000), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiamide (EDC), morpholinoethane sulfonic acid (MES) and N-hydroxysuccinimide (NHS) were procured from Sigma–Aldrich (Shanghai, China). α-CD was purchased from TCI Co., Ltd., Tokyo. Hydrophilic magnetofluid was a gift sample obtained from State Key Laboratory of Polymer Materials Engineering, Sichuan University (Chengdu, China). Other reagents were analytical pure and used directly without further purification.

^{1}H NMR was measured on an Avance Bruker-600 spectrometer. The chemical shifts of ^{1}H NMR are expressed in parts per million downfield relative to the internal tetramethylsilane (δ = 0 ppm). IR spectra were recorded on a Perkin Elmer spectrum one FT-IR spectrometer using KBr discs in the range of 400–4000 cm^{-1} region. The crystalline changes in the hollow nanospheres were confirmed by X-ray diffraction measurements, which were performed by using Cu Kα irradiation with PHILP X'Pert MPD (20 kV; 35 mA; 2°/min). The centrifugations were taken on TGL-20M, Saite Centrifuge Co., Shanghai, China. The absorbance value of the solution was recorded on APL-UV-2000 spectrophotometer, Shanghai, China. The TEM observations were performed by JEOL JEM-100CX at an accelerating voltage of 80 kV. The dynamic light scattering (DLS) was measured by BI-9000AT, BI-200SM, Brookhaven Instruments Co., USA.

2.2. Synthesis of CS-g-PEG

2.2.1. Brief procedures

1 g poly(ethylene glycol) methyl ether and 0.5 g NaBr was dissolved in 40 ml water, then 5 ml NaClO and 3 ml TEMPO solution (0.001 mg/ml)were added and a pH was adjusted to 3. The reaction mixture was stirred at ice bath under N_2 for 3 h. Then the reaction solution was extracted three times with chloroform, the organic phases were combined and dried over anhydrous Na_2SO_4. The Na_2SO_4 was filtered, the solvent was evaporated, and the oily residue was dried under vacuum at room temperature to obtain PEG-COOH. **PEG—COOH**: ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 3.27 (s; O**CH$_3$**), 4.04 ppm (s; C**H$_2$**COOH), 3.40–3.67 (m; O**CH$_2$CH$_2$**). FT-IR (KBr): 3444 (—OH), 2886 (C—H), 1747 (COOH), 842–1280 (C—O—C) cm^{-1}.

A 5% (w/w) PEG—COOH solution was prepared in a buffer solution of 0.1 M MES and 0.5 M NaCl, and the pH was adjusted to 6. The molar ratio of EDC:NHS:COO^- was 1:0.5:1. A series of sample of NHS and EDC were added to a 2% (w/w) PEG—COOH solution to activate the carboxylic acid groups on the PEG—COOH. The solution was agitated for 10 h to obtain a homogeneous solution followed by the addition of 2% chitosan solution dissolved in MES buffer at pH 4. The solution was dialysed by bag filter (MWCO: 8000–14,000) at 37 °C for 36 h, the solvent was evaporated, washed with acetone 6 times at 60 °C and dried under vacuum at room temperature. **CS-g-PEG**: ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 3.05 (**H-2** of sugar), 3.24 (s, O**CH$_3$**), 3.43–3.78 (m, O**CH$_2$CH$_2$**), 4.10 (s, **CH$_2$**COOH), 4.75 ppm (**H-1** of sugar). FT-IR (KBr): 3434 (—OH), 2893 (C—H), 1620 (carbonyl), 843–1244 (C—O—C of PEG) cm^{-1}.

2.3. Preparation of self-assembly CS-g-PEG/α-CD hollow spheres

The novel building block of rigid chains of α-CD/PEG was found to be able to self-assemble easily into hollow spheres when PEG-branched chitosan solutions were added dropwise to the α-CD solutions in water. Brief procedures: a series of 1 ml branched chitosan solutions in different degree of PEG substitution were added dropwise into 3 ml α-CD [5% (w/v)] solutions. After stirring for 4 h, the solution turned to muddy and slightly blue, which indicated the formation of hollow spheres. Then the hollow spheres were collected by centrifugation at 16,000 rpm and lyophilized for the further utilization.

2.4. Size distribution of self-assembled hollow spheres

The size and size distribution of CS-*g*-PEG/α-CD self-assembled hollow spheres in water were determined using dynamic laser light-scattering (DLS) with a digital auto correlator at a scattering angle of 90°, a wavelength of 533 nm and a temperature of 25 °C. To investigate the relationship between the degree of PEG substitution and the size of hollow spheres, a series of CS-*g*-PEG/α-CD self-assembled hollow spheres in water with different PEG graft density (DS) of CS-*g*-PEG were prepared. Furthermore, in order to confirm whether the pH value can affect the size of the CS-*g*-PEG/α-CD self-assembled hollow spheres or not, a series of samples with different pH value were prepared.