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Alginate-Coated Fe_3O_4 Hollow Microspheres for Drug Delivery

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Novel hollow Fe_3O_4 nanoparticles for drug delivery were synthesized via a one-step template-free approach. These nanoparticles were obtained by modifing the Fe_3O_4 nanoparticles with 3-aminopropyltrimethoxy silane, and then grafting alginate onto the surface of amine magnetic. The hollow structure of Fe_3O_4 spheres was characterized by TEM, XRD, and XPS. The *M-H* hysteresis loop indicated that the magnetic spheres exhibit superparamagnetic characteristics at room temperature. Daunorubicin acting as a model drug was loaded into the carrier, and the maximum percent of envelop and load were 28.4% and 14.2% respectively. The drug controlled releasing behaviors of the carriers were compared in different pH media.

Key words: Hollow Fe_3O_4 nanoparticles, 3-Aminopropyltrimethoxy silane, Drug delivery

I. INTRODUCTION

In recent years, nanostructured magnetic materials have attracted great interest because of their unique magnetic properties [1]. Magnetite (Fe_3O_4) is the most important magnetic materials and has been widely used in ferrofluids, catalysts, environmental protection and biomedical [2−4]. Due to the magnetic properties, large surface area and high drug loading efficiency, magnetite hollow nanostructered for drug carries were prepared and tested [5, 6]. Usually, magnetic drug carries comprised magnetic cores and an inorganic or organic shell [7, 8]. Magnetic nanoparticles as drug carriers were used for targeted drug delivery by an external magnet [9, 10].

Alginate is a natural polymer and the sodium salts can be soluble, and it is biocompatible and biodegradable as a drug carrier. It can be easily cross-linked by polyvalent ions, such as calcium, which can keep the biological activity of proteins widely used in cells encapsulation and controlled release of drugs [11−14].

In this work, novel magnetite hollow spheres with a diameter of 400−500 nm and a shell thickness of ∼80 nm have been successfully synthesized. Fe_3O_4 nanoparticles were initially prepared by solvothermal route. Then the magnetic hollow nanoparticles were functionalized with 3-aminopropyltrimethoxy silane (APTMs), and alginate was firstly grafted onto the surface of amine-functionalized magnetic hollow nanospheres. Finally, the alginate coating was immobilized by gelling of Ca^{2+} ions and alginate. The daunorubicin as a model drug was loaded into the carrier and notable sustained drug release was also investigated.

II. EXPERIMENTS

A. Reagents and instruments

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, AR), ethylene glycol (EG), ethylenediamine (EDA), sodium alginate, $CaCl_2$, were from Sinopharm Chemical Reagent Co., Ltd. China. 1-Ethyl-3-3-(dimethylaminopropyl)-carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), were from Yuanju Biotechnology Co., Ltd of Shanghai (China). 3-Aminopropyltrimethoxy silane (APTMs) was purchased from Acros (Belgium). Daunorubicin was obtained from Xingcheng Chemhar Co., Ltd. of Zhejiang, China. Phosphatebuffered saline (PBS, 50 mmol/L, pH=7.4) and the hydrochloric acid buffer (pH=1.8) were prepared by ourselves. All reagents were used as received without further purification.

X-ray powder diffraction (XRD) patterns of the products were obtained with a Japan Rigaku DMax-γA rotation anode X-ray diffractometer equipped with graphite monochromatized Cu Kα radiation (λ=0.154178 nm). Transmission electron microscopy (TEM) photographs were taken on a Hitachi Model H-800 TEM at an accelerating voltage of 200 kV. X-ray photoelectron spectra (XPS) were measured on an ESCA Laboratory MKII instrument with Mg Kα radiation as the exciting source. The UV-Vis spectra were registered by a UV-365 spectrophotometer.

B. Preparation of Fe_3O_4 hollow nanoparticles and amine-functionalized magnetic hollow nanospheres

The Fe_3O_4 hollow nanoparticles were prepared by a one-step template-free approach [15]. Briefly, 5 mmol

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of $FeCl_3{\cdot}6H_2O$ in 37 mL EG was dissolved completely then added 3 mL EDA. The mixture was stirred vigorously until it became homogeneous and then was transferred into 50 mL of Teflon-lined autoclave and reacted at 200 °C for 24 h. The black precipitate was magnetically separated and washed several times with water and ethanol. Finally, the black precipitate was dried in a vacuum oven at 50 °C for 6 h.

For preparation of amine-functionalized magnetic hollow nanospheres, 60 mg of Fe_3O_4 was dispersed in 90 mL absolute ethanol and 3 mL ammonium hydroxide and sonicated for 30 min. After that, about 1 mL of APTMs was added into the solution, and then the solution was mechanically stirred at 25 °C for 8 h. The product was separated by a magnet and washed several times with water and ethanol and then dried under vacuum at 50 °C for 6 h.

C. Fe_3O_4 hollow nanoparticles with alginate

15 mg of EDC·HCl and 18 mg of NHS were dissolved in 10 mL of PBS, then 20 mg amine-functionalized magnetic hollow nanospheres was added and sonicated for 10 min. 50 mL of 0.1% alginate solution was added to the above solution and stirred at room temperature for 24 h. The resulting composites was washed several times with the above PBS and then dispersed in 100 mL 2wt% $CaCl_2$ solution and incubated for 4 h. And then, the product was Fe_3O_4/alginate separated, washed and dried.

D. Drug delivery properties of the hollow microspheres

5 mg of daunorubicin and 10 mg of Fe_3O_4 or Fe_3O_4/alginate hollow microspheres were mixed in 4.00 mL of PBS for 24 h and then magnetically separated. The clear solution was used to calculate the drug loading content: 0.2 mL of the solution was taken, followed by being diluted to 10 mL, and then the drug concentration in the dialyzate was analyzed for monitoring the characteristic 481 nm absorbance peak of daunorubicin. The drug-loaded vehicles were washed three times with PBS (pH=7.4) and then immersed in 10.00 mL PBS or the hydrochloric acid buffer (pH=1.8) at 37 °C, and then the drug concentration in the dialyzate was analyzed by UV-Vis spectrometry in order to detect the drug releasing rate.

III. RESULTS AND DISCUSSION

A. Morphologies of magnetic microspheres

Figure 1 shows the representative TEM images of the prepared Fe_3O_4 hollow nanoparticles, which clearly indicates the hollow nature of the Fe_3O_4 spheres from

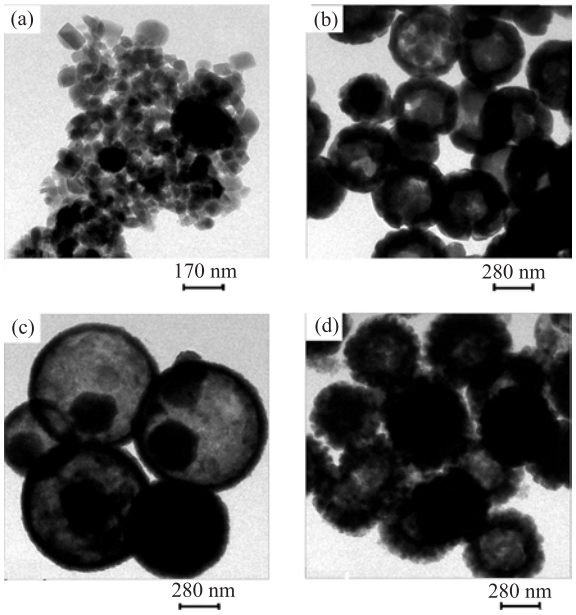

FIG. 1 Representative TEM images of the samples Fe_3O_4 hollow nanoparticles prepared at 200 °C for different reaction times. (a) 12 h, (b) 24 h, (c) 36 h, and (d) 48 h.

the strong contract between the pale center and the dark edges. The average diameter of the samples was 400−500 nm with a wall thickness of 60−70 nm. Figure 2 depicts the TEM images of the Fe_3O_4 hollow spheres (Fig.2(a)) and Fe_3O_4-alginate (Fig.2(b)). Compared with the TEM image of the Fe_3O_4 hollow spheres (Fig.2(a)), the transparency of Fe_3O_4-Alginate (Fig.2(b)) was decreased and the surfaces of the spheres appear filament, indicating that the calcium alginate gel layer has been coated onto the surfaces of the Fe_3O_4 hollow spheres.

B. Compositions of magnetic microspheres

In order to study the crystal structures of the Fe_3O_4 hollow spheres, the samples were subjected to XRD. As shown in Fig.3, all of the diffraction peaks match well with Fe_3O_4 crystal (JCPDS No.75-0033). As magnetite (Fe_3O_4) and maghemite (γ-Fe_3O_3) have similar XRD patterns [16], XPS was then used to identify the composition of the hollow spheres. As shown in Fig.4, the photoelectron peaks at 710.5 and 724.0 eV are the characteristic peaks of $Fe2p_{3/2}$ and $Fe2p_{1/2}$, respectively, which is the mixed oxidation state of Fe in Fe_3O_4 [17]. XRD and XPS experiments demonstrate that the samples are Fe_3O_4 crystal. Figure 4 shows representative XPS spectra of the Fe_3O_4 hollow spheres and amine-functionalized Fe_3O_4. In the spectrum of Fe_3O_4, the main peaks are Fe2p, O1s, and C1s centered at 710.5, 529.9, and 282.6 eV. For amine-functionalized Fe_3O_4, new peaks appear at 102.2 and 399.5 eV, belonging to Si and N from APTM. Therefore, the spectra suggest

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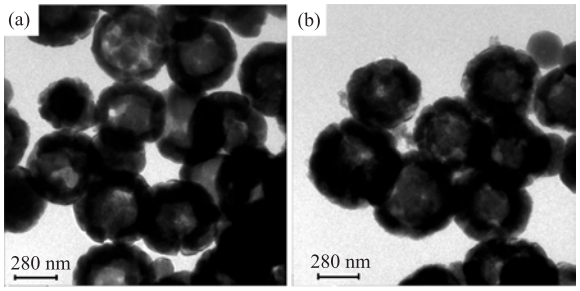

FIG. 2 TEM images of hollow (a) Fe_3O_4 and (b) Fe_3O_4/alginate.

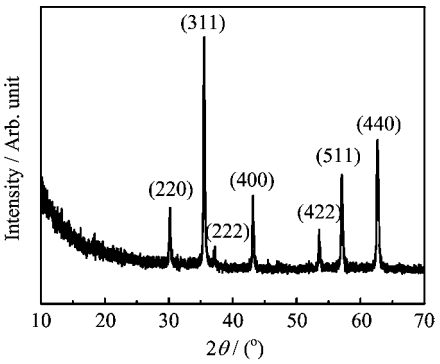

FIG. 3 XRD pattern of hollow Fe_3O_4.

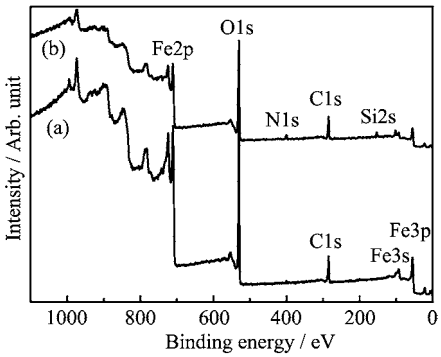

FIG. 4 XPS spectra of (a) the Fe_3O_4 hollow spheres and (b) amine-functionalized Fe_3O_4.

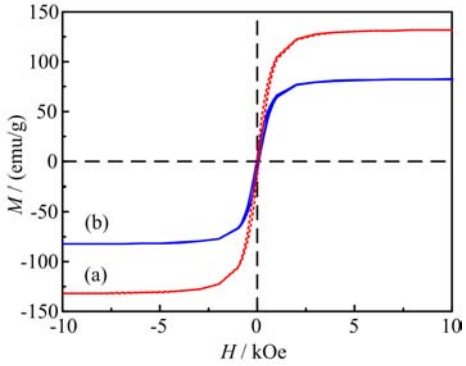

FIG. 5 Magnetization curves of (a) Fe_3O_4 and (b) Fe_3O_4/alginate at room temperature.

that APTM molecules form a layer on the surface of the Fe_3O_4 hollow spheres.

C. Magnetic properties of magnetic microspheres

The magnetic properties of the hollow Fe_3O_4 and Fe_3O_4/alginate were studied via a vibrating sample magnetometer at 300 K by cycling the magnetic field between −10 and 10 kOe. As shown in Fig.5, the hollow Fe_3O_4 shows ferromagnetic behavior with a coercivity (H) of ca. 59 Oe and a remanent magnetization (M) of ca. 7 emu/g. The saturation magnetization of the hollow Fe_3O_4 at 300 K is 112.1 emu/g, which is a high value for hollow spheres, larger than similar structures reported by Yu and co-workers [18]. It implies that the samples have strong response to an external magnet. For Fe_3O_4/alginate, the saturation magnetization is 82.2 emu/g, lower than the value of hollow Fe_3O_4. A large number of alginate coated on the surface of the hollow spheres led that the content of Fe_3O_4 was declined relatively in the sample, so the saturation magnetization of Fe_3O_4/alginate was decreased. It also proved that alginate has been modified on the surface of the Fe_3O_4 hollow spheres successfully.

D. Drug release behaviors of microspheres

Fe_3O_4/alginate hollow microspheres acted as drug carrier, and the maximum percent of envelop and load were 28.4% and 14.2% respectively, whereas Fe_3O_4 hollow microspheres showed much lower value of 14.7% and 7.4% respectively. In comparison with Fe_3O_4 hollow spheres, the percent of envelop and load of Fe_3O_4/alginate hollow microspheres was increased about twice. The results also showed that alginates have been modified successfully on the surface of the Fe_3O_4 hollow spheres. Hydrophilic gel was good for encapsulation of water-soluble drugs; the model drug daunorubicin existed in its carboxylate ions, therefore the enveloping and loading rate were increased.

In this work, two typical pH (1.8 and 7.4) media were used to investigate the controlled releasing from the Fe_3O_4 hollow microspheres and the Fe_3O_4/alginate hollow microspheres [19]. The drug concentration in the dialyzate was analyzed for monitoring the 481 nm absorption peak by UV-Vis spectrometry in order to detect the rate of drug release. The accumulative releasing ω effect of daunorubicin from the two drug delivery systems at different pH values were summarized, as shown in Fig.6.

Figure 6 shows the time dependence of the cumulative release of daunorubicin from the obtained magnetic drug delivery in PBS (pH=7.4) and the hydrochloric acid buffer (pH=1.8) at 37 °C. It can be found that either the Fe_3O_4 hollow microspheres or

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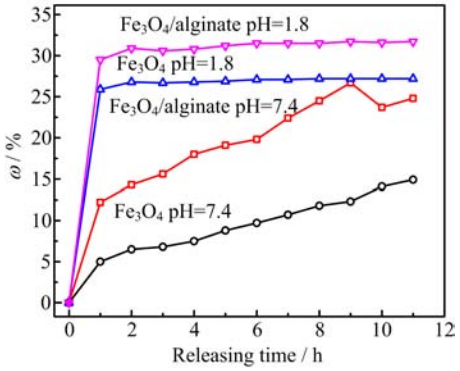

FIG. 6 Accumulative release effect of Fe_3O_4/alginate and hollow Fe_3O_4 at pH=1.8 and pH=7.4.

the Fe_3O_4/alginate hollow microspheres exhibited the faster releasing rate at pH=1.8 than pH=7.4 media. In pH=1.8 media, the releasing rates of the two drug delivery systems reached 25.9% and 29.5% respectively in the first 1 h. However, after the releasing period of 2 h, the cumulative releasing ratios were nearly constant at about 26.8% and 30.9% respectively. It indicated that the two drug vessels had the fast swelling property so they showed the same burst release phenomena in the acidic media [19].

In pH=7.4 media, the drug release rate of the Fe_3O_4 hollow microspheres was faster in the first 1 h. The releasing ratio reached about 12% in the period. It could be speculated that the initial burst of release is ascribed to the rapid release of drugs deposited on the surface. After the releasing period of 8 h, the cumulative releasing ratio was nearly constant at about 26%. It was noted that drug was released slowly from the Fe_3O_4/alginate hollow microspheres, and only 5% drugs were released at the first 1 h. Furthermore, about 15% drugs were released ever after 10 h. The difference of release rate should partly be attributed to the alginate polyelectrolyte shell coated on the microspheres, which could slow down the releasing rate and prolong the releasing time. The biocompatible shell could improve the therapeutic value of various medicines by improving their bioavailability, solubility, and retention time. Therefore, Fe_3O_4/alginate hollow microspheres are expected to be used for the magnetic-targeted sustained release of toxicity drugs.

IV. CONCLUSION

The Fe_3O_4/alginate hollow microspheres acting as a kind of drug delivery systems were prepared. The preparation procedure is simple without any template. In pH=7.4 media, the controlled release of the model drug daunorubicin from the obtained magnetic drug carriers revealed that the alginate polyelectrolyte shell coated on the microspheres improved the loading rate and slowed the releasing rate, especially, improved its biocompatible. The superparamagnetic property ensures that these drug-loaded microspheres were anticipated to be used as an excellent drug releaser by magnetic fields to the target disease area. In addition, the principle demonstrated in this work may also have great effect on preparing novel type of the drug delivery system.

V. ACKNOWLEDGMENTS

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