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Fabrication and Characterization of Chitosan-Poly(acrylic acid) Magnetic Nanospheres

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Iron oxide nanoparticles modified with meso-2,3-dimercaptosuccinic acid (DMSA), were encapsulated with a natural polymer with abundant primary amino groups, chitosan (CS), to improve their biocompatibility. Characterization was performed using Fourier transform infrared spectroscopy, thermogravimetirc analysis, transmission electron microscope analyses, vibrating sample magnetometer, zeta potential analyzer. It was found that highly ordered spherical aggregates with a mean diameter of 98 nm are obtained. The formation of aggregates depended on cross-linking of acrylic acid (AA) monomers and CS. These biodegradable CS-PAA polymer magnetic nanospheres might be useful for various biomedical applications.

Keywords: Chitosan, Acrylic Acid, Magnetic Nanospheres.

1. INTRODUCTION

Maghemite (γ-Fe_2O_3) or magnetite (Fe_3O_4) nanoparticles have potential to bring dramatic improvements to biomedical science fields.[1] Many applications require maghemite nanoparticles (MNPs) with large magnetization to achieve facile manipulation of the particles and supersensitivity for magnetic detection. The nonmagnetic matrix not only prevents the magnetic components from aggregating into large, but also provides a useful platform for surface modification. It is also incorporate to other types of probes into the matrix to form multifunctional colloidal nanoparticles.[2] In addition to their small sizes, comparable to biological functional molecules, the ability to tune the nanoparticles properties through controlling their size, shape, and composition makes these materials useful and optimized for the biomedical purposes, such as cell labeling,[3] separation,[4] tissue repair, drug delivery,[5] magnetic resonance imaging,[7] hyperthermia.[8, 9]

Chitosan (CS) is a transformed polysaccharide obtained from deacetylation of natural chitin, which is one of the important natural polymers constituting the shells of crustaceans and the cell walls of many fungi. CS has been extensively investigated for several decades for clinical use, drug delivery systems, solid polyelectrolytes, molecular separation, food packaging films, artificial skin, bone substitutes, water engineering, and so on due to its excellent properties such as biocompatibility, biodegradability, nontoxicity, and adsorption properties. Many approaches have been developed to prepare the chitosan beads including water in oil method,[10] emulsion-droplet coalescence technique,[11] chemical cross-linking[12] et al. However, several researches report the fabrication of chitosan magnetic nanosphere with small size and multifunctional molecules.[13]

We selected chitosan (CS), which bears amino groups, as the cationic polymer and acrylic acid (AA), which bears acid groups, as the anionic monomer. The CS and AA polymer–monomer pair and γ-Fe_2O_3 nanoparticles stabilized by DMSA were mixed and formed nanospheres loaded with γ-Fe_2O_3 nanoparticles. One advantage of such an approach is that the nanospheres are obtained spontaneously under very mild conditions without the need of high temperature, organic solvent, surfactant and some other special experimental technology. Meantime, the nanospheres have small particle size and positive surface charges, which are favorable for the applications due to the interaction with negative charged biological membranes and site-specific targeting *in vivo*.

2. EXPERIMENTAL DETAILS

2.1. Materials

Chitosan with deacetylation degree (DD) of 90% and molecular weights (Mw) of 200 kD was obtained from Yuhuan Ocean Biochemical Co. Ltd. (China, Zhejiang). Potassium persulfate ($K_2S_2O_8$) was recrystallized from distilled water. Acrylic acid (AA) (Shanghai Lingfeng Chemical Company) was distilled under reduced pressure

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in nitrogen atmosphere. All other reagents were of analytical grade and used as received without further purification.

2.2. Synthesis of DMSA-Coated MNPs

Fe_3O_4 nanoparticles were synthesized by chemical coprecipitation of Molday,[14] In typical synthesis, a solution of mixture of $FeCl_3$ and $FeSO_4$ (molar ratio 2:1) was prepared under N_2 protecting, then, enough ammonia aqueous solution was dropped into it with violently stirring. After 30 minutes, the resulting Fe_3O_4 nanoparticles were washed immediately with distilled water for 5 times by magnetic separation. The final magnetite nanoparticles were dispersed in distilled water with pH 3.0, and oxidized into more stable MNPs (γ-Fe_2O_3) by air at the temperature of 90 °C. After that, MNPs were coated with meso-2, 3-dimercaptosuccinic acid (DMSA). One finally obtained an aqueous sol of MNPs, which was stable in a large pH range (from 3 to 11), in suitable ionic strength and in various buffers.[15]

2.3. Preparation of CS–PAA Nanospheres by Polymerization

The preparation procedure is schematically illustrated in Scheme 1. The CS–PAA polymer magnetic microapheres were prepared by adding 10 mL of a DMSA-coated magnetite nanoparticle suspension to 25 mL of 1% (v/v) acetic acid solution of CS (0.5 g). The reaction mixture was stirred for 30 min. Then AA with a stoichiometric molar ratio of 1:1.5 (glucosamine unit/acid) was added to CS solution. Polymerization was then initiated by $K_2S_2O_8$ at 80 °C under a nitrogen stream and mechanical stirring. The concentration of $K_2S_2O_8$ in the reaction system is about 3 mmol. Afterwards, 0.5 mL of glutaraldehyde, a bifunctional crosslinker, was added to the reaction system at 40 °C to crosslink chitosan selectively. The resultant suspension was washed immediately with distilled water for 3 times by magnetic separation.

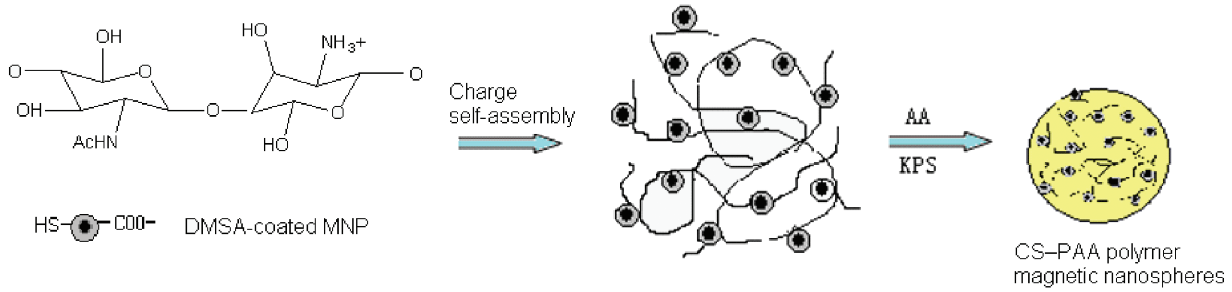
2.4. Characterization of CS–PAA Nanospheres

The mean particle size and zeta potential of the spheres were determined by photon correlation spectroscopy (PCS) on a N4 PLUS submicron analyzer (Beckmann-Coulter, USA) at a scattering angle of 90° and Zeta Potential Analyzer (BECKMAN, Delsa 440SX, USA), respectively. All the measurements were repeated three times. The particle size and morphology of the samples was determined by transmission electronic microscopy (TEM, JEOL, JEM-200EX,). The magnetic measurements were carried out with a Vibrating Sample Magnetometer (VSM, Lakeshore 7407). Fourier transform infrared (FTIR) spectroscopy measurements were performed on a Bruker Fourier transform spectrometer model VECTOR22 using KBr pressed discs.

3. RESULTS AND DISCUSSION

The preparation procedure of CS–PAA polymer magnetic nanospheres was illustrated in Scheme 1. First, the DMSA first forms a stable coating through its carboxylic chelate bonding and further stabilization of the ligand shells is attained through intermolecular disulfide cross-linkages between the ligands under ambient conditions.[16] DMSA-coated MNPs suspension was suspended in CS solution. After sufficient adsorption between carboxyl groups from MNPs and positively charged amino groups of CS. The polymerization of AA was initiated by $K_2S_2O_8$. When the polymerization of AA reached a certain level, the inter- and intra-molecular linkages occurred between carboxyl groups from PAA and positively charged amino groups of CS. These linkages could make the macromolecular chains of CS rolling up, which was responsible for the formation of the gelation of the CS solution. As the polymerization time extended, the amount of PAA in the solution increased, the CS–PAA polymer magnetic nanospheres were formed.[17]

The size and morphology of the CS–PAA polymer magnetic nanospheres were investigated by PCS in aqueous solution and by TEM in the solid state. The mean size of CS–PAA polymer magnetic nanospheres was measured to be 160.8 ± 26 nm by PCS and the zeta potential to be 28.7 ± 2.6 mV by Zeta Potential Analyzer at pH 4.0. Figure 1 shows representative TEM images of the synthetic materials. After the addition of DMSA coated MNPs (10 nm in size) (Fig. 1(a)) into an aqueous solution of CS–AA followed by polymerization of AA and selective



Scheme 1. Illustration of preparation of CS–PAA polymer magnetic nanospheres.

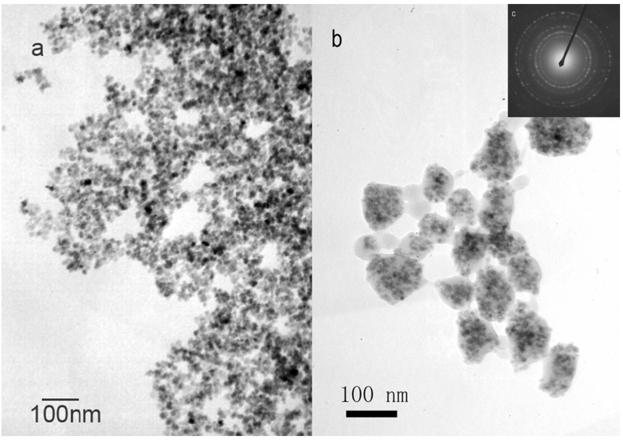


Fig. 1. TEM photograph of (a) DMSA-coated MNPs, (b) CS–PAA polymer magnetic nanospheres, (c) electron-diffraction pattern of nanospheres.

cross-linking of CS at the end of polymerization, spheres with incorporated MNPs were formed (Fig. 1(b)). The average diameter of such a structure is 98 ± 21 nm, smaller than that determined by PCS in aqueous solution as a result of the sample being dry for TEM analysis. The electron-diffraction pattern recorded from these spheres confirms that magnetite nanoparticles were loaded into the nanospheres. A salient feature of Figure 1(c) is that these nanospheres have an intense dark circle within the shells of the spheres and dark spots at the surface of some spheres, which suggests that the distribution of the γ-Fe_2O_3 nanoparticles is not concentrated in the core of the spheres, which corresponds well to the size of γ-Fe_2O_3 nanoparticles used.

The magnetic property of the CS–PAA polymer magnetic nanospheres was measured by VSM. Figure 2 shows the magnetization as a function of an external field at ambient temperature. The magnetic parameter such as saturation magnetization (Ms) was about 16.5 emu/g, which represented a magnetic content of 32.9% by comparing the value of pure MNPs to be 50.2 emu/g. The result was very close to the loading content of magnetite was about 36% by theromgravimetric analysis.

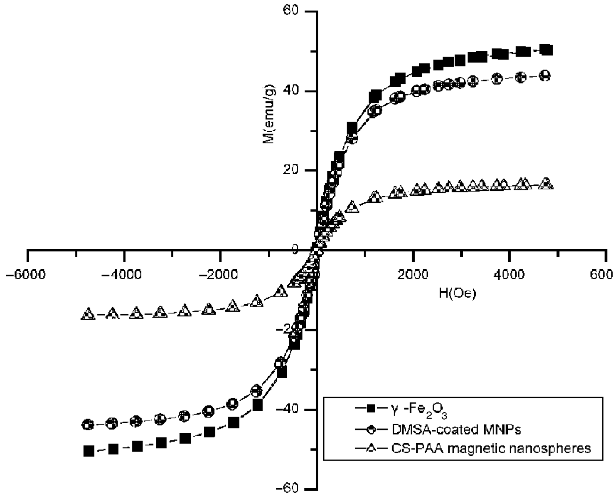


Fig. 2. Magnetization versus applied magnetic field for γ-Fe_2O_3, DMSA-coated MNPs, CS–PAA polymer magnetic nanospheres.

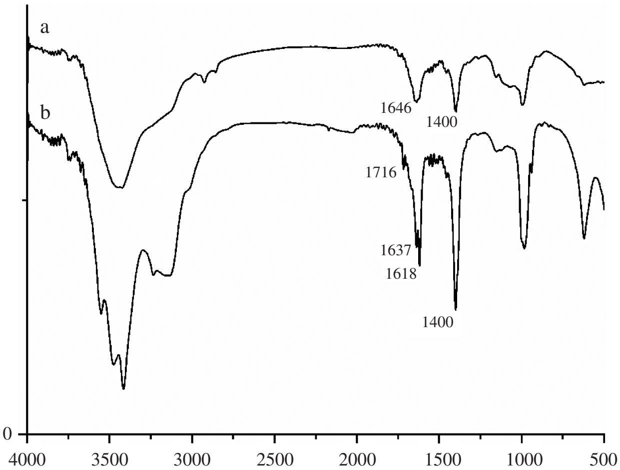


Fig. 3. FTIR spectra of (a) CS and (b) CS–PAA polymer magnetic nanospheres.

Figure 3 showed FTIR spectra of CS and CS–PAA polymer magnetic particles. For the IR spectrum of CS (Fig. 3(a)), the characteristic absorption bands appeared at 1646 cm^{-1} (amide I), 1400 cm^{-1} (amide II). In the spectrum of CS–PAA polymer magnetic particles (Fig. 3(b)), the characteristic absorption bands at 1716 and 1618 cm^{-1}, which can be assigned to the absorption peaks of the carboxyl groups of PAA (the absorption peak of carboxyl groups in pure PAA appears at 1740 cm^{-1}),[18] and the NH_3^+ absorption of CS, respectively, were observed. Furthermore, the stronger absorption peaks at 1400 cm^{-1} could be assigned to symmetric stretching vibrations of COO^- and amide anion groups. These results indicated that the carboxylic groups of PAA were dissociated into COO^- groups, which complexed with protonated amino groups of CS through electrostatic interaction to form the polyelectrolyte complex during the polymerization.

4. CONCLUSIONS

In conclusion, we report the preparation of CS–PAA polymer magnetic nanospheres by polymerizing acrylic acid into chitosan template. We examined the CS–PAA polymer magnetic nanospheres by TEM, PCS, Zeta Potential Analyzer, VSM and FTIR. The nanospheres are obtained spontaneously under very mild conditions without the need of high temperature, organic solvent, surfactant and some other special experimental technology. These biodegradable CS–PAA polymer magnetic nanospheres have small particle size and positive surface charges, which might be useful for various biomedical applications.

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