Colloid Polym Sci (2007) 285:1193–1199
DOI 10.1007/s00396-007-1672-2

ORIGINAL CONTRIBUTION

Polysaccharide-modified iron oxide nanoparticles as an effective magnetic affinity adsorbent for bovine serum albumin

Yuan-Yuan Liang · Li-Ming Zhang · Wei Li · Ru-Fu Chen

Received: 13 November 2006 / Accepted: 8 March 2007 / Published online: 22 March 2007

Abstract The magnetic separation technique based on magnetic iron oxide nanoparticles (MNPs) has potential applications in protein adsorption and purification, enzyme immobilization, cell sorting, nucleic acid detachment, and drug release. However, the naked MNPs are often insufficient for their hydrophilicity, colloidal stability, and further functionalization. To overcome these limitations, chitosan was firstly carboxymethylated and then covalently conjugated on the surface of the MNPs ranging in size from about 5 to 15 nm, which were prepared by co-precipitating iron (II) and iron (III) in alkaline solution and then treating under hydrothermal conditions. It was found that such modification did not result in the phase change of the MNPs, and the resultant modified nanoparticles were still superparamagnetic. In particular, the colloidal stability of MNPs in aqueous suspension was improved after the surface modification. By investigating the adsorption of bovine serum albumin (BSA) on the modified MNPs, it was observed that the adsorption capacity of the BSA on the modified MNPs increased rapidly within several minutes and then reached the maximum value at about 10 min. The adsorption equilibrium isotherm could be fitted well by the Langmuir model. The medium pH affected greatly the adsorption of the BSA. The maximum adsorption of the BSA occurred at the pH value close to the isoelectric point of the BSA, with a saturation adsorption amount of 94.45 mg/g (25 °C). For the BSA feed concentration of 1.017 mg/ml, a high desorption percentage of 91.5% could be achieved under an alkaline condition (pH 9.4).

Keywords Iron oxide nanoparticles · Polysaccharide · Surface modification · Magnetic affinity adsorbent · Bovine serum albumin

Y.-Y. Liang · L.-M. Zhang (✉) · W. Li
Laboratory for Polymer Composite and Functional Materials,
Institute of Optoelectronic and Functional Composite Materials,
School of Chemistry and Chemical Engineering,
Sun Yat-Sen (Zhongshan) University,
Guangzhou 510275, China
e-mail: ceszhlm@mail.sysu.edu.cn

R.-F. Chen
Second Affiliated Hospital, Sun Yat-Sen (Zhongshan) University,
Guangzhou 510102, China

Introduction

As a recent developing biotechnology, the magnetic separation based on magnetic iron oxide nanoparticles (MNPs) has a wide range of applications such as protein adsorption and purification [1, 2], enzyme immobilization [3], cell sorting [4], nucleic acid detachment, and drug release [5, 6]. Different from conventional bioseparation methods, this technique possesses the advantages of rapidity, high efficiency, and cost-effectiveness. It is known [7–11] that MNPs are inherently biocompatible and are amenable to post-synthesis surface modification, which makes them excellent candidates for many important applications. In the absence of any surface coating, MNPs have hydrophobic surfaces with a large surface area to volume ratio. Due to hydrophobic interactions between the particles, these particles are easier to agglomerate and form large clusters, resulting in increased particle size. Ideally, surface modification should provide good colloidal stability, biocompatibility, and specific biorecognition of the particle surface [12]. The applications for the MNPs that are

solely stabilized by either electrical double layers [13] or surfactant monolayers [14] are somewhat limited, as the stability of such dispersions is sensitive to pH and ionic strength [13, 14]. In contrast, polymer-stabilized MNPs can offer enhanced colloidal stability and some unique advantages [15–21]. For example, poly(ethylene glycol) (PEG)-coated MNPs are of particular interest for biomedical applications, as the modified MNPs surfaces are non-immunogenic, non-antigenic, and hydrophilic [22–24]. Moreover, it is possible to prepare the MNPs for the conjugation with targeting agents based on well-developed PEG chemistry [25].

In this work, we developed the polysaccharide-modified MNPs as effective magnetic affinity adsorbent for bovine serum albumin (BSA), an amphiphilic protein. The used polysaccharide is naturally occurring chitosan with many useful features such as hydrophilicity, biocompatibility, biodegradability, antibacterial properties, and remarkable affinity for biomacromolecules [26–28]. For this purpose, chitosan was firstly carboxymethylated and then covalently bound on the surface of MNPs in the presence of the conjugating agent, 1-ethyl-3-(3-dimethyla- minopropyl)carbodiimide hydrochloride. We expect that such surface modification can improve the colloidal stability of MNPs in aqueous suspension and yield new magnetic nano-adsorbent for protein adsorption and purification.

Experimental

Materials

The chitosan with the deacetylation degree of 85.0% and the viscosity-average molecular weight of 350 KD, and the conjugating agent, 1-ethyl-3-(3-dimethyla-minopropyl)carbodiimide hydrochloride (EDC), were purchased from Aldrich in USA. BSA was obtained from Shanghai Biochemical in China. Glutaraldehyde (GLU), iron (II) chloride tetrahydrate (99%), iron (III) chloride hexahydrate (98%) were purchased from Guangzhou Chemical in China. All other chemicals were analytic grade reagents commercially available and used without further purification.

Preparation of MNPs and their surface modification by polysaccharide

The MNPs were prepared by co-precipitating iron (II) and iron (III) in alkaline solution and then treating under hydrothermal conditions [29]. At first, 0.30% aqueous iron (II) chloride tetrahydrate dispersion and 0.85% iron (III) chloride hexahydrate dispersion were thoroughly mixed and added to 8.0 mol/l NaOH under continuous stirring at room temperature. Then, the reaction mixture was heated at 80 °C for 30 min, and the medium pH was maintained at 10 by the addition of aqueous NaOH during the reaction. After removing impurity ions such as chlorides, the resulting magnetic nanoparticles were washed with distilled water and ethanol and dried in a vacuum oven at 70 °C.

The polysaccharide-conjugated MNPs was prepared by the carboxymethylation of chitosan and its covalent conjugation onto the MNPs via EDC. For the carboxymethylation of chitosan [30], 3.0 g chitosan and 15.0 g sodium hydroxide were added into 100 ml of isopropanol/water (80:20 *v*/*v*) mixture at 60 °C to swell and alkalize for 1.0 h. Then, 20 ml of monochloroacetic acid solution (0.75 g/ml in isopropanol) was added into the reaction mixture in drops in 30 min. After reaction for 4.0 h at 60 °C, 200 ml of ethyl alcohol was added to stop the reaction. Finally, the solid was filtered and washed with ethyl alcohol to remove salt and water and dried in vacuum oven at 55 °C. For the covalent conjugation [31], 75 mg of the magnetic nanoparticles were added to 4 ml phosphate buffer (0.2 mol/l Na_2HPO_4–NaH_2PO_4, pH 6.0) containing 25 mg EDC, and then the reaction mixture was sonicated for 10 min. After that, 1.0 ml carboxymethylated chitosan solution (25 mg/ml in phosphate buffer) was added, and the reaction mixture was sonicated for another 1.0 h. The product was recovered from the reaction mixture by a permanent magnet (6,000 G) and then washed with water and ethanol.

To obtain the modified MNPs with a dense polysaccharide coating shell, which may be useful for hindering the irreversible permeation of BSA large molecules on the particle surface and facilitating the desorption of the adsorbed BSA [32], the cross-linking procedure was introduced using glutaraldehyde. One hundred milligram of the chitosan-conjugated MNPs were dispersed in 2 ml ultrapure water and sonicated for 10 min. The formed dispersion was added to 7 ml toluene containing 0.12 g Span 20 as the surfactant under vigorous mechanical stirring, resulting in a water-in-oil dispersion. Then, 0.08 ml glutaraldehyde solution (25 wt%) was added, and the reaction was carried out for 2 h. Finally, the polysaccharide-modified MNPs were obtained by washing with water and ethanol and dried in a vacuum oven at 55 °C.

Characterization methods for the naked and polysaccharide-modified MNPs

Fourier transform infrared (FTIR) spectra were obtained using a Nicolet/Nexus 670 FT-IR spectrometer with a resolution of 4 cm^{-1}. For the naked and polysaccharide-modified MNPs, the size and morphology were observed by a JEM-2010HR transmission electron microscope (TEM, Japan), the crystal structure was measured by a Rigacu D/MAX 2200 VPC diffractometer (Japan) using a monochromatized X-ray beam with nickel-filtered CuKR

radiation, the magnetic property was evaluated using a MPMS Xl-7 magnetic property measurement system (USA) with a maximum magnetic field of 7 T and a sensibility of 10^{-6} emu, the zeta potential in water was measured using a Powereach JS94H microelectrophoresis unit (China), and the stability of aqueous colloidal dispersions was evaluated from the change in relative absorbance of the supernatant with respect to the setting time at a wavelength of 360 nm using a UV-visible spectrophotometer (S52, China).

Absorption and desorption of BSA on polysaccharide-modified MNPs

For the absorption tests, 20 mg of the resultant polysaccharide-modified MNPs were mixed with 6 ml of BSA solution with different concentrations. The pH value of absorption medium was changed in the range of 3.8 to 8.9, adjusted using different buffer systems (0.2 mol/l CH_3COONa–CH_3COOH for pH 3.8, 4.6, 5.6; 0.2 mol/l Na_2HPO_4–NaH_2PO_4 for pH 7.4; 0.05 mol/l Tris–HCl buffer for pH 8.9). All the absorption experiments were conducted at 25 °C in a shaking incubator. At the end of the adsorption, the support was magnetically separated with the surface magnetization of 6,000 G, and the supernatant was analyzed for the BSA concentration at 280 nm using a Spectrum Lab 52 UV spectrophotometer (China).

For the desorption tests, the polysaccharide-modified MNPs absorbed with BSA were mixed with 5 ml of 0.36 mol/l NaH_2PO_4 solution. After incubating at 25 °C for 1 h, the supernatant was collected and analyzed for the eluted BSA amount.

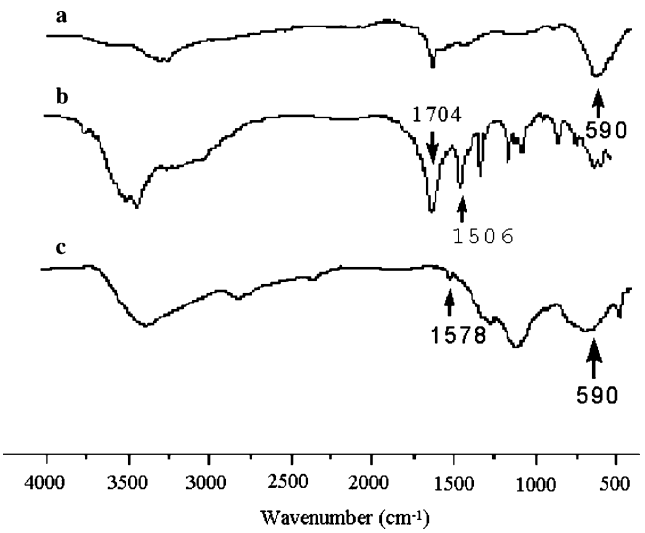

Fig. 1 FTIR spectra of pure MNPs (**a**), carboxymethylated chitosan (**b**), and the modified MNPs (**c**)

Results and discussion

The covalent coating of carboxymethylated chitosan on the surface of MNPs was confirmed by FTIR spectroscopy. As shown in Fig. 1, the spectrum of the modified MNPs shows

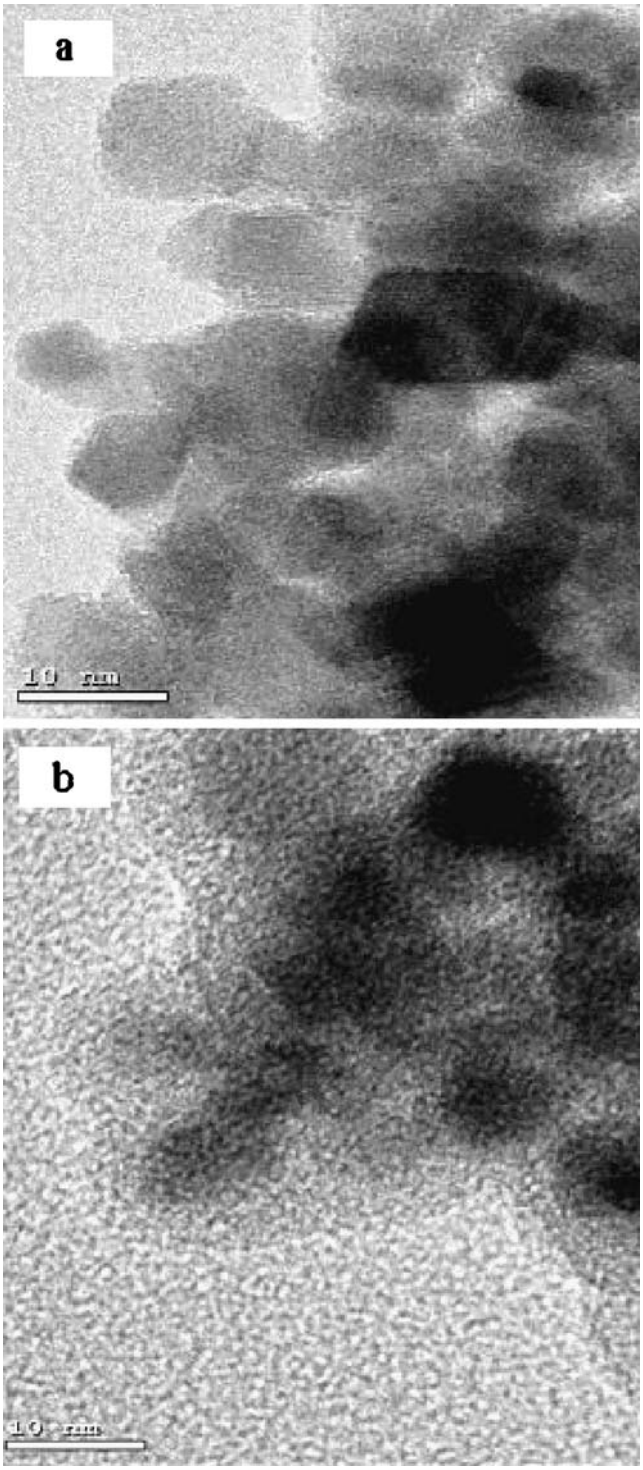

Fig. 2 TEM micrographs of the naked and the polysaccharide-modified MNPs. **a** The naked MNPs; **b** the polysaccharide-modified MNPs

not only the characteristic bands of pure MNPs at 1,578 and 590 cm^{-1}, but also the characteristic peaks of carboxymethylated chitosan at 1,411 and 1,114 cm^{-1}. In particular, the characteristic peaks of carboxymethylated chitosan at 1,704 and 1,506 cm^{-1}, which could be attributed to asymmetric and symmetric stretching vibrations of the COOH, disappeared in the spectrum of the modified MNPs due to the conjugation reaction.

Further evidence for the coating was obtained by scanning electron microscopy observation. Figure 2 shows the typical TEM micrographs of the naked and polysaccharide-modified MNPs. The naked MNPs were found to be nearly rectangular, ranging in size from about 5 to 15 nm with some aggregation (Fig. 2a). In contrast, the modified MNPs became somewhat round and had little aggregation (Fig. 2b). These results may be attributed to the covalent conjugation of carboxymethylated chitosan on the magnetic nanoparticle surfaces and the shielding of hydrophobic interactions between the magnetic nanoparticles due to the existence of hydrophilic polysaccharide coating. To confirm this, the stability of aqueous colloidal dispersions was investigated by a spectroscopy method for the naked and polysaccharide-modified MNPs, respectively. Figure 3 gives the change in relative absorbance with respect to the setting time. As indicated, the relative absorbance has an obvious decrease for aqueous suspension of the naked MNPs. However, there is only a little change in relative absorbance for aqueous suspension of the modified MNPs, which implies an enhanced stability. Thus, our attempt to improve the colloidal stability of MNPs in aqueous suspension by the conjugation of carboxymethylated chitosan on the MNPs has been achieved in this work.

Figure 4 presents the X-ray diffraction patterns for the naked and polysaccharide-modified MNPs. It was found that two samples showed the same characteristic peaks

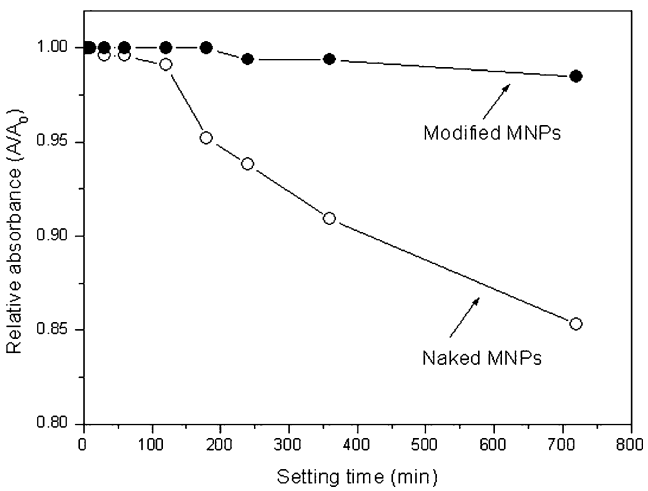

Fig. 3 The change in relative absorbance with respect to the setting time for aqueous suspensions of the naked and polysaccharide-modified MNPs (360 nm, 25 °C)

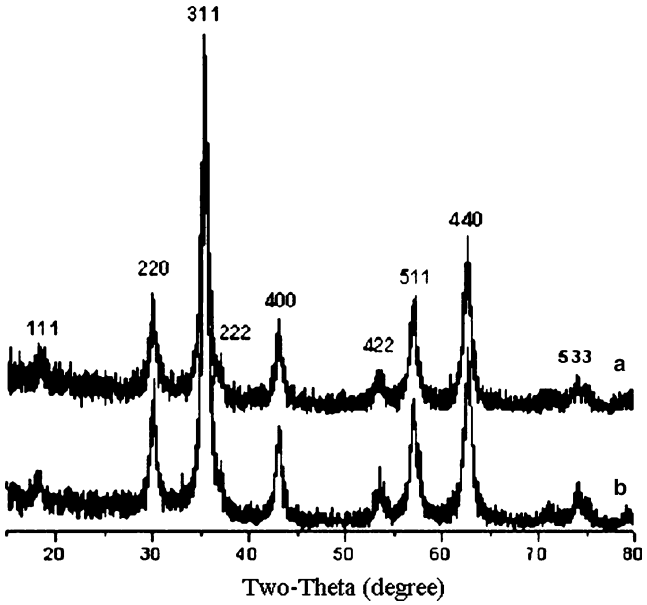

Fig. 4 XRD patterns of the naked and the polysaccharide-modified MNPs. **a** The naked MNPs; **b** the polysaccharide-modified MNPs

located at 2θ=30.1, 35.5, 43.1, 53.4, 57.0 and 62.6°, marked respectively by their indices as (220), (311), (400), (422), (511), and (440), which are consistent with the characteristic peaks for pure Fe_3O_4 with a spinel structure. This fact revealed that the polysaccharide modification did not result in the phase change of Fe_3O_4.

The plots of magnetization versus magnetic field (*M–H* loop) at 300 K for the naked and polysaccharide-modified MNPs are illustrated in Fig. 5. In two cases, the magnetization curve exhibited zero remanence and coercivity, which revealed that the naked and polysaccharide-modified MNPs had both superparamagnetic properties [17, 33]. This

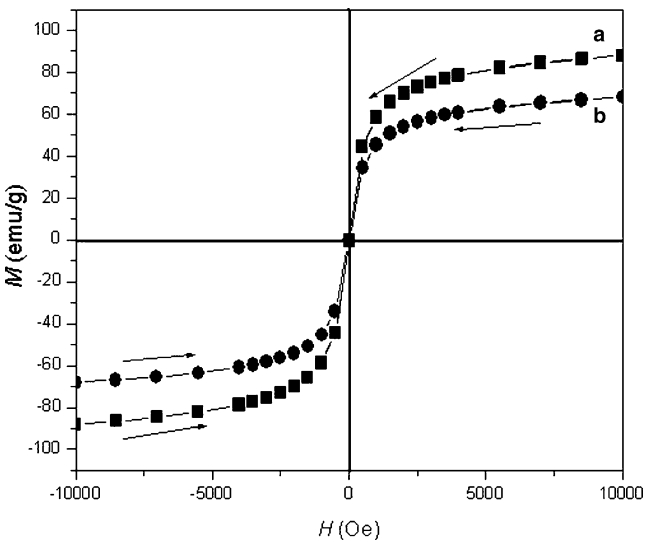

Fig. 5 The magnetization curves of the naked and the polysaccharide-modified MNPs at 300 K. **a** The naked MNPs; **b** the polysaccharide-modified MNPs

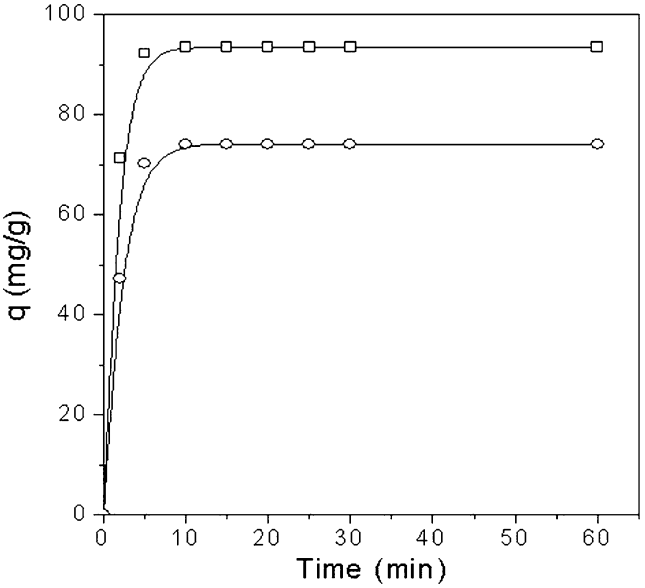

Fig. 6 The adsorption kinetic curves of BSA on the polysaccharide-modified MNPs under various BSA feed concentrations: (*open circle*) 1.012 mg/l; (*open square*) 0.198 mg/l (pH 4.6, 25 °C)

could be attributed to the fact that the magnetic nanoparticles were so small that they may be considered to have a singe magnetic domain [34]. In addition, the polysaccharide-modified MNPs have a lower specific magnetization when compared with the naked MNPs. This might be a result of the conjugation of carboxymethylated chitosan on the magnetic nanoparticle surface, which might quench the magnetic moment. Similar phenomena were also observed by Flesch et al. [35] when they investigated the magnetic properties of poly (ε-caprolactone) (PCL)-modified MNPs

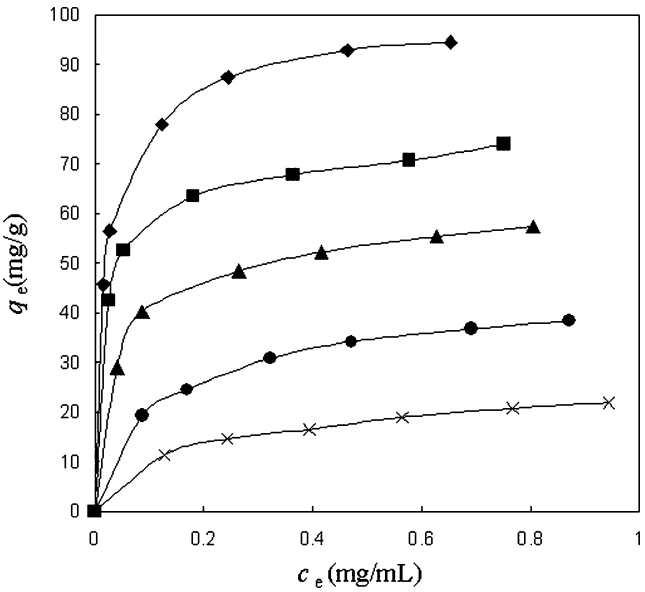

Fig. 7 The adsorption isotherms of BSA on the polysaccharide-modified MNPs in the adsorption media with various pH values: (*filled diamond*) 4.6; (*filled square*) 5.6; (*filled triangle*) 7.4; (*filled circle*) 3.8; (×) 8.9 (25 °C)

Table 1 The Langmuir constants and the correlation coefficients (R^2) for the adsorption isotherms of BSA on the polysaccharide-modified MNPs in the adsorption media with various pH values (25 °C)

Medium pH	q_m (mg/g)	K_L (mg/ml)	R^2
3.8	38.43	0.104	0.988
4.6	94.45	0.018	0.990
5.6	73.94	0.021	0.987
7.4	57.47	0.040	0.993
8.9	21.87	0.145	0.982

and by Kamruzzaman Selim et al. [36] when they carried out the surface modification of MNPs using lactobionic acid (LA). In comparison with these works, however, we obtained the modified MNPs with a higher specific magnetization. Under an applied field of 6,000 Oe, for examples, the specific magnetization were found to have 27, 22, and 64 emu/g for the PCL-modified MNPs, the LA-modified MNPs, and the polysaccharide-modified MNPs in this work, respectively. The large specific magnetization of the modified MNPs makes them very susceptible to magnetic fields and, therefore, makes the solid and liquid phase separate easily.

Figure 6 gives the adsorption kinetic curve of BSA on the polysaccharide-modified MNPs. With the increase of contact time, the adsorption amount of BSA increased rapidly within several minutes and then reached the maximum value at about 10 min regardless of the initial BSA concentration. Tanyolac and Ozdural [37] studied the adsorption behavior of BSA on the polyvinylbutyral-modified magnetic microparticles in the range 125–250 μm and found that 2 h of adsorption time was sufficient for reaching the adsorption equilibrium. In contrast, the

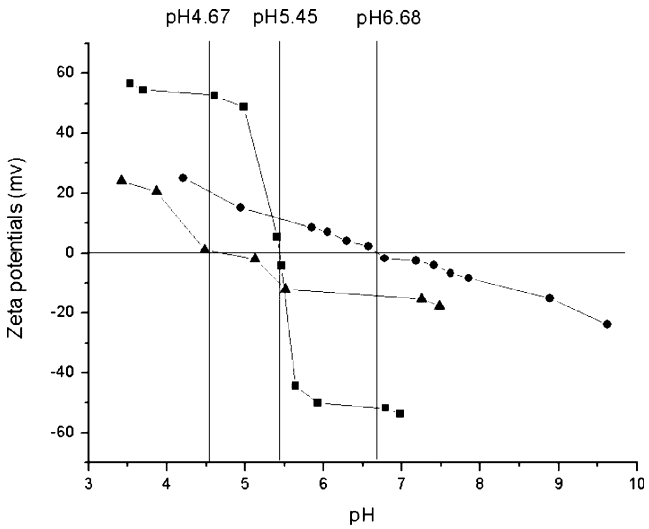

Fig. 8 The zeta potentials of the naked (*filled circle*) and the polysaccharide-modified (*filled square*) MNPs in aqueous suspensions as well as in aqueous BSA solution (*filled triangle*) at different pH values

modified MNPs in this work had a rapid adsorption rate, which might be attributed to the availability of more specific surface area on the modified MNPs.

Figure 7 shows the adsorption isotherms of BSA on the polysaccharide-modified MNPs in the adsorption media with various pH values. The equilibrium data were fitted by the Langmuir isotherm equation, which can be expressed as [38, 39]:

$$C_e/q_e = C_e/q_m + 1/(q_m K_L)$$

where q_e is the equilibrium adsorption amount of BSA on the polysaccharide-modified iron oxide nanoparticles (mg/g), C_e is the equilibrium BSA concentration in solution (mg/ml), q_m is the maximum adsorption amount of BSA on the modified iron oxide nanoparticles (mg/g), and K_L is the Langmuir adsorption constant (ml/mg). The plots of C_e /q_e versus C_e could give straight lines, indicating that the adsorption of BSA on the modified MNPs obeyed the Langmuir adsorption isotherm. Table 1 lists the obtained Langmuir constants and corresponding correlation coefficients.

As shown in Table 1, the pH values affected greatly the maximum adsorption capacity of the modified MNPs. The adsorption capacity increased obviously when the solution pH increased from 3.8 to 4.6 and then decreased continuously with further increase of the solution pH to 8.9. This phenomenon may be attributed to the electrostatic interactions varied with the solution pH. To illustrate this, the zeta potentials of the naked and polysaccharide-modified MNPs in aqueous suspensions as well as in aqueous BSA solution at different pH values were measured (Fig. 8). It was found from Fig. 5 that the naked magnetic nanoparticles, the modified magnetic nanoparticles, and BSA had the isoelectric points (IEP) of 6.68, 5.45, and 4.67, respectively. The modified MNPs and BSA have positive charges below their respective IEP and negative charges above their respective IEP. After the surface modification, the IEP was shifted from 6.68 to 5.45, which also confirmed the conjugation of chitosan with the magnetic nanoparticles and revealed that the polysaccharide-modified iron oxide nanoparticles were positively charged at pH <5.45. Therefore, the electrostatic repulsion between the modified magnetic nanoparticles and the BSA having same charges appeared at pH<4.67 and at pH>5.45, which does not favor the BSA adsorption and results in lower adsorption capacity. In particular, large electrostatic repulsion occurred at the pH of 8.9, leading to lowest adsorption. In the pH range from 4.67 to 5.45, the modified magnetic nanoparticles have a positive charge, while the BSA has a negative charge, which can promote the adsorption of the BSA on the modified MNPs. The maximum adsorption of the BSA occurred at the pH of 4.6, which is close to the IEP of the BSA. This may be due to the fact that the BSA macromolecules have a compact conformational state at the IEP and lead to a minimum intermolecular repulsion [2], which refers to the higher adsorption amount. Similar phenomenon was observed for the adsorption of the BSA on the glass powder coated with polyvinyl alcohol in which the maximum adsorption was obtained at the IEP of BSA [40].

The desorption of the BSA from the polysaccharide-modified MNPs was investigated under alkaline conditions (pH 9.4) due to the fact that high pH is not beneficial for the adsorption of BSA, as observed above. For this purpose, NaH_2PO_4 was used as the desorption agent. The adsorbed and desorbed quantities of BSA at equilibrium for the BSA feed concentration of 1.017 mg/ml were found to be 93.03 and 85.12 mg/g, respectively. In this case, a high desorption percentage of 91.5% was achieved.

Conclusions

Carboxymethylated chitosan was used to modify the MNPs by the covalent conjugation. This surface modification improved obviously the colloidal stability of MNPs in aqueous suspension, and the resultant modified nanoparticles were still superparamagnetic. By investigating the adsorption and desorption of bovine serum albumin on the modified MNPs, it was observed that such materials were characteristic of rapid adsorption rate, large adsorption capacity, and high desorption percentage under certain conditions, showing a potential application as effective magnetic affinity adsorbent for bovine serum albumin.

Acknowledgments This work was supported by NSFC (20273086; 30470476; 20676155), NSFG (5003252; 039184; 6023103), Department of Science and Technology of Guangdong Province (2004B33101003), and NCET Program (NCET-04-0810) in Universities of China.

References

1. Abudiab T, Beitle RR Jr (1998) J Chromatogr A 795:211–215
2. Peng ZG, Hidajat K, Uddin MS (2004) J Colloid Interface Sci 271:277–283
3. Liao MH, Chen DH (2001) Biotechnol Lett 23:1723–1727
4. Ito A, Shinkai M, Honda H, Kobayashi T (2005) J Biosci Bioeng 100:1–11
5. Neuberger T, Schöpf B, Hofmann H, Hofmann M, Rechenberg B (2005) J Magn Magn Mater 293:483–496
6. Gupta AK, Curtis ASG (2004) J Mater Sci Mater Med 15:493–496
7. Perez JM, O'Loughin T, Simeone FJ, Weissleder R, Josephson L (2002) J Am Chem Soc 124:2856–2857
8. Dyal A, Loos K, Noto M, Chang SW, Spagnoli C, Shafi KV, Ulman A, Cowman M, Gross RA (2003) J Am Chem Soc 125:1684–1685
9. Gelinas S, Finch JA, Bohme P (2000) Colloids Surf A 172:103–112

10. Gu HW, Yang ZM, Gao JH, Chang CK, Xu B (2005) J Am Chem Soc 127:34–35
11. Wang DS, He JB, Rosenzweig N, Rosenzweig Z (2004) Nano Lett 4:409–413
12. Gupta AK, Gupta M (2005) Biomaterials 26:3995–4021
13. Bacri JC, Perzynski R, Salin D, Cabuil V, Massart R (1990) J Magn Magn Mater 85:27–32
14. Shen L, Stachowiak A, Fateen SEK, Laibinis PE, Hatton TA (2001) Langmuir 17:288–299
15. Mendenhall GD, Geng Y, Hwang J (1996) J Colloid Interface Sci 184:519–526
16. Lee J, Isobe T, Senna M (1996) J Colloid Interface Sci 177:490–494
17. Harris LA, Goff JD, Carmichael AY, Riffle JS, Harburn JJ, St Pierre TG, Saunders M (2003) Chem Mater 15:1367–1377
18. Si S, Kotal A, Mandal TK, Giri S, Nakamura H, Kohara T (2004) Chem Mater 16:3489–3496
19. Yu S, Chow GM (2004) J Mater Chem 14:2781–2786
20. Kim BS, Qiu JM, Wang JP, Taton TA (2005) Nano Lett 5:1987–1991
21. Ditsch A, Laibinis PE, Wang DIC, Hatton TA (2005) Langmuir 21:6006–6018
22. Zhang Y, Kohler N, Zhang M (2002) Biomaterials 23:1553–1561
23. Kohler N, Fryxell GE, Zhang MA (2004) J Am Chem Soc 126:7206–7211
24. Hu F, Neoh KG, Cen L, Kang ET (2006) Biomacromolecules 7:809–816
25. Veiseh O, Sun C, Gunn J, Kohler N, Gabikian P, Lee D, Bhattarai N, Ellenbogen R, Sze R, Hallahan A, Olson J, Zhang M (2005) Nano Lett 5:1003–1008
26. Martino AD, Sittinger M, Risbud MV (2005) Biomaterials 26:5983–5990
27. Majeti NV, Ravi K (2000) React Funct Polym 46:1–27
28. Wang GH, Zhang LM (2006) J Phys Chem B 110:24864–24868
29. Kim DK, Mikhaylova M, Zhang Y, Muhammed M (2003) Chem Mater 15:1617–1627
30. Chen XG, Park H (2003) Carbohydr Polym 53:355–357
31. Chang YC, Chen DH (2005) J Colloid Interface Sci 283:446–451
32. Haruma K (2000) Prog Polym Sci 25:1171–1210
33. Saravanan P, Alam S, Mathur GN (2003) J Mater Sci Lett 22:1283–1285
34. Huang SH, Liao MH, Chen DH (2003) Biotechnol Prog 19: 1095–1100
35. Flesch C, Bourgeat-Lami E, Mornet S, Duguet E, Delaite C, Dumas P (2005) J Polym Sci A Polym Chem 43:3221–3231
36. Kamruzzaman Selim KM, Ha YS, Kim SJ, Chang Y, Kim TJ, Lee JH, Kang IK (2007) Biomaterials 28:710–716
37. Tanyolac D, Ozdural AR (2001) J Appl Polym Sci 80:707–715
38. Zhang LM, Chen DQ (2002) Colloids Surf A 205:231–236
39. Zhang LM, Zhou YJ, Wang Y (2006) J Chem Technol Biotechnol 81:799–804
40. Bajpai AK (2000) J Appl Polym Sci 78:933–940