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Separation of lysozyme using superparamagnetic carboxymethyl chitosan nanoparticles

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ABSTRACT

Functionalized Fe_3O_4 nanoparticles conjugated with polyethylene glycol (PEG) and carboxymethyl chitosan (CM-CTS) were developed and used as a novel magnetic absorbing carrier for the separation and purification of lysozyme from the aqueous solution and chicken egg white, respectively. The morphology of magnetic CM-CTS nanoparticles was observed by transmission electron microscope (TEM). It was found that the diameter of superparamagnetic carboxymethyl chitosan nanoparticles (Fe_3O_4 (PEG + CM-CTS)) was about 15 nm, and could easily aggregate by a magnet when suspending in the aqueous solution. The adsorption capacity of lysozyme onto the superparamagnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles was determined by changing the medium pH, temperature, ionic strength and the concentration of lysozyme. The maximum adsorption loading reached 256.4 mg/g. Due to the small diameter, the adsorption equilibrium of lysozyme onto the nanoparticles reached very quickly within 20 min. The adsorption equilibrium of lysozyme onto the superparamagnetic nanoparticles fitted well with the Langmuir model. The nanoparticles were stable when subjected to six repeated adsorption–elution cycles. Separation and purification were monitored by determining the lysozyme activity using *Micrococcus lysodeikticus* as substrate. The lysozyme was purified from chicken egg white in a single step had higher purity, as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Considering that the superparamagnetic nanoparticles possess the advantages of high efficiency, cost-effectiveness and excellent binding of a larger amount of lysozyme and easier separation from the reaction system, thus this type of superparamagnetic nanoparticles would bring advantages to the conventional separation techniques of lysozyme from chicken egg white.

1. Introduction

Lysozyme (E.C.3.2.1.17), which has bactericidal and bacteriostatic properties, is often used in food technology and pharmacological technology such as to be used in eye drops and wound healing creams [1], as a food preservative to inhibit growth of spoilage organisms in foods and spoilage lactic acid bacteria in wine during alcoholic fermentation [2–4]. In recent years, the potential use of lysozyme as an anticancer drug [5] and in the treatment of HIV infection has also been discussed [6,7]. Nowadays, lysozyme is widely used and greatly demanded based on the importance.

Practically applied lysozyme is mostly separated from chicken egg white for adequate supplies of raw materials and higher activity. However, a large number of non-aimed proteins make the lysozyme purification and separation from chicken egg white challenging, since the content of lysozyme is about 3.5% of total protein in chicken egg white [8]. The classical method adopted for lysozyme purification is a combination of conventional processes, such as precipitation, centrifugation, dialysis, ultrafiltration, and chromatography, which are generally complicated, time-consuming, and expensive for large-scale production [9]. To solve these problems, magnetic separation technology has been introduced and widely investigated, because it can be used to selectively recover aimed proteins from mixture systems in an easy and rapid way. The advantages of this technology are as following: (1) its separating rate is rapid because of the large volume-to-surface area ratio; (2) it causes significant reduction in the operation cost; and (3) no expensive equipment was needed, it facilitates simple separation and recovery through a magnet [10–12].

Combination of organic and inorganic components in a single particle at the nano-sized level has attracted considerable attention because of the wide potential applications in many fields [13–16]. The natural polysaccharide – carboxymethyl chitosan (CM-CTS) was favored to decorate the magnetite nanoparticles

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because of excellent features such as hydrophilicity, biocompatibility, biodegradability and remarkable affinity for many bio-macromolecules [17,18]. CM-CTS has been demonstrated having good tolerable pH and good solubility, which may be due to the abundant of –COOH and $–NH_2$ groups in it. Furthermore, CM-CTS can act as an ion-exchange material for carboxyl groups, but few reports can be found about its application for ion-exchange adsorbing medium because of its water-soluble nature. So in order to take the advantage of CM-CTS meanwhile overcome its shortcomings, using CM-CTS to modify the magnetic Fe_3O_4 nanoparticles has attracted researchers' attention [19,20].

The goal of this study is to prepare magnetite nanoparticles for efficient separation of lysozyme from chicken egg white. In this paper, a new kind of functionalized Fe_3O_4 nanoparticles conjugated with CM-CTS were explored by chemical co-precipitation method. Lysozyme adsorption properties onto the magnetic nanoparticles from aqueous solutions were investigated at different experimental conditions (medium pH, temperature, ionic strength and the concentration of lysozyme). Reusability of the adsorbents was also tested. Finally, the nanoparticles were used for purification of lysozyme from chicken egg white. The purity of the eluted lysozyme was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and the activity of the purified lysozyme was measured using *Micrococcus lysodeikticus* as a substrate. This kind of nanoparticle which has a cation exchange adsorbing medium possess better stability, good magnetic response, and specific adsorption of lysozyme.

2. Experimental

2.1. Materials

The sources of the chemicals are as follows: carboxymethyl chitosan (CM-CTS), lysozyme (chicken egg white, E.C.3.2.1.17, activity 20,000 units/mg protein), lyophilized *M. lysodeikticus* cells, iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), polyethylene glycol (PEG, M_W = 6000), 25% ammonia solution ($NH_3{\cdot}H_2O$), Fresh chicken eggs were bought from local market. All the other chemicals were of analytical reagent grade used without further purification and the water used in all experiments was prepared in a three-stage purification system and had an electrical resistivity of 18.2 MΩ cm^{-1} (highly pure water).

2.2. Preparation of superparamagnetic carboxymethyl chitosan nanoparticles

Functionalized superparamagnetic nanoparticles which is short as Fe_3O_4 (PEG + CM-CTS) were prepared by chemical coprecipitating iron(II) and iron(III) in alkaline solution followed by treating under hydrothermal conditions in the presence of CM-CTS. The whole chemical reaction was under nitrogen atmosphere.

Firstly, $FeSO_4{\cdot}7H_2O$ and $FeCl_3{\cdot}6H_2O$ (molar ratio was 3:2) were dissolved in water–ethanol solutions (v/v 5:1) at the concentration of 0.03 M iron ions, a certain amount of PEG6000 was added under continuous stirring. Chemical precipitation was achieved by adding $NH_3{\cdot}H_2O$ solution (25%) into the above solution at 60 °C for 10 min, during the reaction process, the medium pH was at about 10 by the addition of aqueous $NH_3{\cdot}H_2O$ during the reaction.

Secondly, 5% CM-CTS was added dropwise to the above reaction mixture over 10 min. After incubation for 60 min at 80 °C, the magnetite nanoparticles were precipitated with permanent magnet at room temperature, and rinsed with distilled water for six times to remove unreacted chemicals.

Finally, a black precipitate (magnetite) was obtained by freeze-drying for about 48 h. For comparison, we prepared the naked Fe_3O_4 nanoparticles by the same method only in the absence of PEG6000 and CM-CTS.

Fourier Transform Infrared (FTIR, Nicolte Nexus, Thermo Electrin Corporation) uses infrared radiation to determine the chemical functionalities present in a sample. The freeze-dried samples were grounded with KBr powder (3:100) and the mixtures were made into pellets under high pressure. The sample was scanned from 4000 to 400 cm^{-1}. Shape and morphology analysis of naked Fe_3O_4 and Fe_3O_4 (PEG + CM-CTS) nanoparticles was examined by transmission electron microscope (TEM, JEOL JEM-2100 (HR)). Samples were prepared by placing two drops of nanoparticle suspension onto a carbon-coated copper grid, followed by drying at room temperature. Zeta-potential of Fe_3O_4 (PEG + CM-CTS) was assessed by Zeta-sizer Nano Series (Nano-ZS, Malvern Instruments Ltd., UK) at room temperature, pH was adjusted using 0.1 M HCl and 0.1 M NaOH solutions. Three measurements for each sample were performed.

2.3. Lysozyme adsorption studies from aqueous solution

10 mg of magnetite Fe_3O_4 (PEG + CM-CTS) nanoparticles and 4 mL of lysozyme (0.5–4 mg/mL) buffer solution (20 mM, pH 4.0–11.0) were added into a 5 mL of centrifugal pipe, the mixed suspension was shaken in a thermostated shaker (200 rpm) for a certain time which the adsorption had reached equilibrium, samples were withdrawn at suitable time intervals. The supernatant obtained was used to determine the content of un-immobilized lysozyme. Effects of the pH of medium, temperature, ionic strength and the lysozyme concentration on the adsorption capacity were studied. Changing the initial lysozyme concentration, adsorption model was studied. The concentration of lysozyme was measured at 280 nm by using UV/Vis spectrophotometer (MAPADA, Shanghai, China, Model 1100). Each experiment was performed in three times for quality control and statistical purposes. The amount of lysozyme adsorbed onto the magnetite nanoparticles was calculated by using the following equation:

$$q = \frac{V(Co - Ce)}{m} \quad (1)$$

where q is the amount of lysozyme adsorbed onto the magnetite nanoparticles (mg/g); Co and Ce are the concentrations of the lysozyme in the initial solution and in the supernatant phase after adsorption, respectively (mg/mL); V is the volume of the lysozyme solution (mL); m is the mass of the magnetite nanoparticles (g).

2.4. Stability of magnetite nanoparticles in repeated use

To determine the reusability of the magnetite Fe_3O_4 (PEG + CM-CTS) nanoparticles, the adsorption and desorption cycle was repeated six times by using the same batch nanoparticles. The lysozyme elution was performed in buffer solution at pH 5.0 of 20 mM containing 0.5 M NaCl and stirred continuously (at stringing rate 200 rpm) for 1 h at room temperature. The final lysozyme concentration in the elution medium was measured at 280 nm by using UV/Vis spectrophotometer. The elution ratio was calculated by using the following expression:

$$\text{elution ratio}(\%) = \frac{\text{amount of lysozyme eluted}}{\text{amount of lysozyme adsorbed on magnetite nanoparticles}} \times 100 \quad (2)$$

2.5. Lysozyme purification from chicken egg white

Chicken egg white was separated from fresh eggs and diluted to 50% (v/v) with buffer solution (20 mM, pH 7.0–10.0). The diluted egg white was mechanical agitation in an icebath for 6 h, and then centrifuged at 4 °C at 10,000 rpm for 30 min. The supernatant fluid was used as source of lysozyme.

Purification of lysozyme from chicken egg white solution was studied with 10 mg of magnetite Fe_3O_4 (PEG + CM-CTS) nanoparticles and 40 mL of different pH of diluted chicken egg-white solution. The adsorption experiments were conducted at 37 °C for 1 h while continuous stirring. The elution of lysozyme from magnetite nanoparticles was performed with buffer solution (20 mM, pH 5.0) containing 0.5 M NaCl. The natural diluted chicken egg white solution, the supernatant solution after absorption and the elution solution were examined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) with 12% separating gel, 6% stacking gels and stained with brilliant R250 which could analyze the component of the protein solution according to the different molecular weights of proteins.

After purification of lysozyme from chicken egg solution, the activity of lysozyme was also detected. The definition of the lysozyme activity was the quantity that makes the absorbance of a certain concentration of *M. lysodeikticus* solution at 450 nm decreases 0.001/min [21]. The activity was calculated with following equation:

$$U = \frac{A_{450}}{0.001 \times m} \quad (3)$$

where U (U/mg) is the activity units contained in 1 mg lysozyme, A_{450} is the decrease of absorbance at 450 nm per minute, and m is the mass of lysozyme (mg) added in the reaction solution.

3. Results and discussion

3.1. Properties of superparamagnetic nanoparticles

The biocompatible and biodegradable CM-CTS coating not only endowed the magnetic nanoparticles water-soluble properties but also allowed the magnetic nanoparticles to be bio-conjugated with enzyme molecules by its functional group. In this study, surface modification of naked Fe_3O_4 magnetite nanoparticles by CM-CTS and subsequent separation of lysozyme from aqueous solution and chicken egg white solution were studied.

The presence of both –COOH and –OH groups of CM-CTS will induce the formation of surface complexes between Fe_3O_4 and CM-CTS. To confirm the binding of CM-CTS, FTIR spectra of CM-CTS, naked Fe_3O_4 nanoparticles, and Fe_3O_4 (PEG + CM-CTS) nanoparticles were examined (Fig. 1). As seen in the spectrum, with two

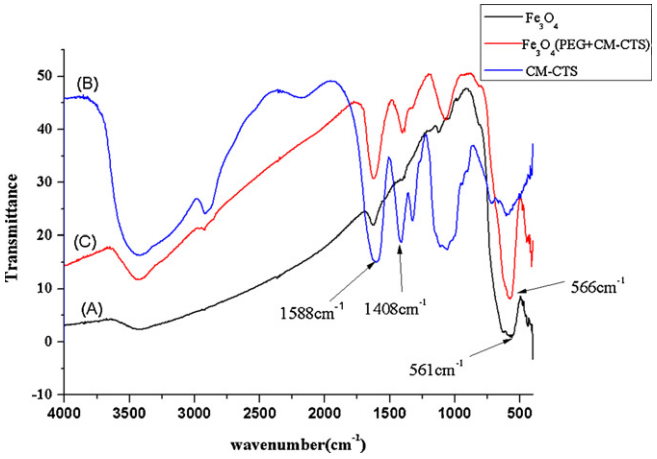

Fig. 1. FTIR spectrum of (A) naked Fe_3O_4, (B) CM-CTS and (C) Fe_3O_4 (PEG+CM-CTS) nanoparticles

main characteristic peaks of the amino and carboxyl groups in CM-CTS at 1408 cm^{-1} and 1588 cm^{-1} were observed in the spectrum of magnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles, revealing that carboxymethyl chitosan was indeed bound on the surface of Fe_3O_4 nanoparticles. The FTIR spectrum also exhibited that: the presence of CM-CTS did shift IR vibrations of Fe_3O_4 but did not alter them very much (the band of Fe–O shifted from 561 to 566 cm^{-1}). Besides, no new bands were found in the spectra of Fe_3O_4 (CM-CTS). So we can conclude that CM-CTS was successfully coated on the surface of the Fe_3O_4.

Typical TEM micrographs of naked Fe_3O_4 before and after coating with CM-CTS are shown in Fig. 2. It can be seen that both naked Fe_3O_4 (A) and Fe_3O_4 (PEG + CM-CTS) (B) nanoparticles are spherical in shape with an average size of about 15 nm. It is known that magnetic particles less than about 25 nm will exhibit super paramagnetism [22]. Therefore, the prepared Fe_3O_4 (PEG + CM-CTS) nanoparticles have superparamagnetic properties. The dispersing behavior of Fe_3O_4 (PEG + CM-CTS) nanoparticles has obviously been improved in comparison with that of naked Fe_3O_4 which might be related to the coated CM-CTS enhancing the repulsion of magnetite particles. With this improved dispersibility, the Fe_3O_4 (PEG + CM-CTS) nanoparticles which suspended in the solution

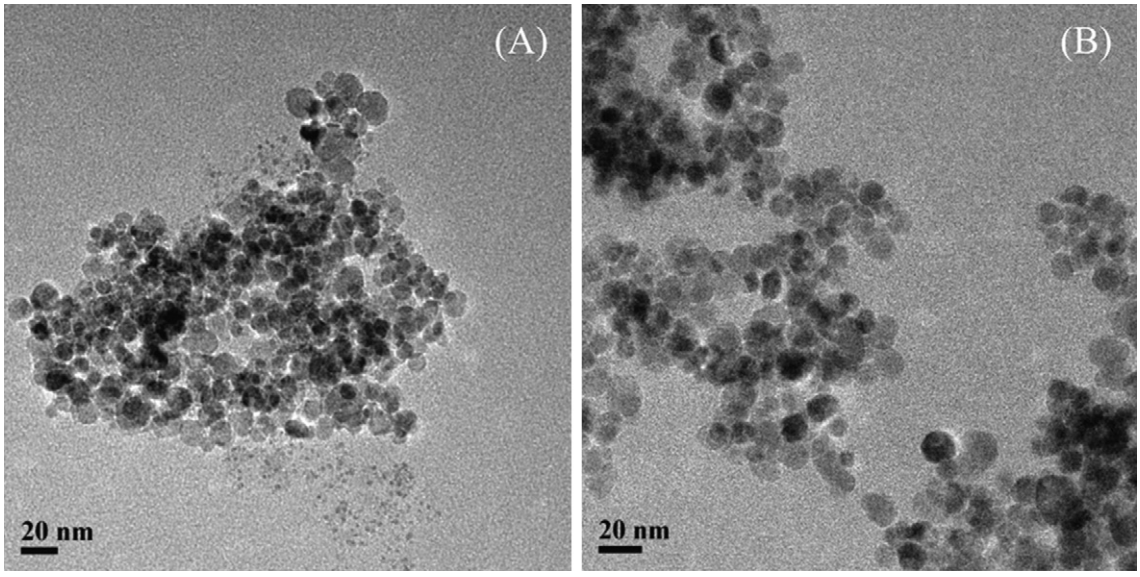

Fig. 2. Size distribution of naked Fe_3O_4 (A) and Fe_3O_4 (PEG+CM-CTS) nanoparticles (B)

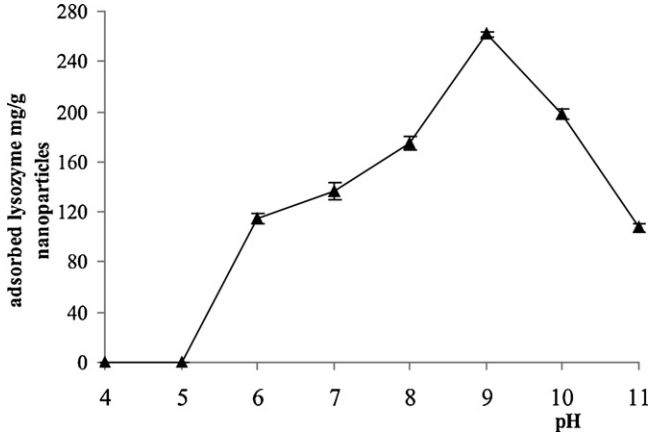

Fig. 3. Effects of the pH on lysozyme adsorption on the superparamagnetic nanoparticles (initial concentration of lysozyme: 1.0 mg/mL, temperature: 37 °C; ionic strength: 0M)

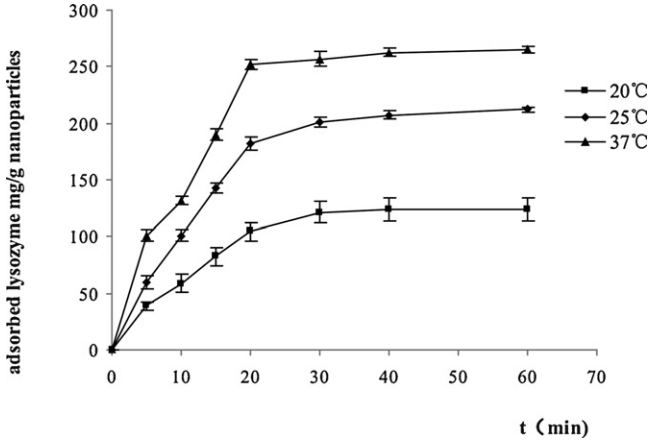

Fig. 4. Effects of temperature on lysozyme adsorption on the superparamagnetic nanoparticles initial concentration of lysozyme: 1.0g/mL; pH: 9.0; ionic strength: 0M)

were aggregated quickly when a magnet was near the bottle, so it indicated that the nanoparticles could easily and quickly separate from the solution by magnet.

3.2. Adsorption of lysozyme from aqueous solution

3.2.1. Effect of the pH

The zeta potential of the Fe_3O_4 (PEG + CM-CTS) superparamagnetic nanoparticles was measured to calculate its isoelectric point (pI), it was about 5.1. The result reveals that the Fe_3O_4 (PEG + CM-CTS) nanoparticles were positively charged at pH < 5.1 and negatively charged at pH > 5.1. Meanwhile, because proteins are amphoteric, the type (positive or negative) and number of charges on the surface of a protein will vary with the pH of the medium. As the pI value of lysozyme is 11.2 as reported [23], so lysozyme molecules would be cationic at pH < 11.2 and anionic at pH > 11.2.

The effect of pH on the amount of lysozyme adsorbed on the Fe_3O_4 (PEG + CM-CTS) superparamagnetic nanoparticles is shown in Fig. 3. The maximum lysozyme adsorption was obtained at pH 9.0, with a clear decrease at lower and higher pH values, especially, no lysozyme adsorption was observed when medium pH equal to 4 and 5. These results indicate that the pH of the medium has a crucial effect on the adsorption of lysozyme, and there is a preferential interaction between lysozyme and magnetite nanoparticles at pH 9.0.

According to the result we can conclude that there is mainly electrostatic interaction between lysozyme and nanoparticles. When the pH values were at 4–5, both nanoparticles and lysozyme have a positive charge; therefore, the electrostatic repulsion does not favor the adsorption of lysozyme on magnetic nanoparticles. When pH values equal to 6–11, superparamagnetic nanoparticles are negatively charged because of the carboxyl groups in the CM-CTS macromolecular chains while lysozyme is positively charged (pH < pI), the larger the charge of the lysozyme and superparamagnetic nanoparticles, the higher the adsorption loading will be achieved, when pH was equal to 9, the lysozyme and nanoparticles both achieved the larger charge, so under this condition, the superparamagnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles have the maximum absorption capacity.

3.2.2. Effect of temperature

The effect of temperature on lysozyme absorption from aqueous solution is clearly shown in Fig. 4: the adsorption of lysozyme onto tested Fe_3O_4 (PEG + CM-CTS) nanoparticles significantly increased with increasing temperature from 20 to 37 °C, the maximum adsorption capacity of the superparamagnetic nanoparticles for lysozyme increased from 124 mg/g to 265 mg/g. This indicated that the increase in temperature was favorable to lysozyme adsorption onto the nanoparticles. This could be attributed to the chemical interaction between the nanoparticles and the lysozyme molecules as the temperature increased or may be because the natural logarithm of the adsorption capacity was linearly related to the reciprocal of the absolute temperature [24].

3.2.3. Effect of ionic strength

The effects of ionic strength on protein interaction with surfaces of adsorbing medium is very crucial which have been widely investigated for over several decades; the research proved that if the dominant force was electrostatic attraction between adsorbent and adsorbate, the ionic strength had a large effect on the adsorption capacity [25]. In this paper, the effect of ionic strength on the lysozyme adsorption onto Fe_3O_4 (PEG + CM-CTS) nanoparticles was investigated by the addition of NaCl. The ionic strength (NaCl concentration) was varied between 0, 0.2 and 1.0 M as displayed in Fig. 5. The adsorption capacities of magnetic nanoparticles to lysozyme were reduced significantly with increasing salt concentration from 0 to 1.0 M. Our results imply the decrease in lysozyme adsorption capacity of the magnetite nanoparticles with increasing ionic strength, which indicates that lysozyme adsorption by the

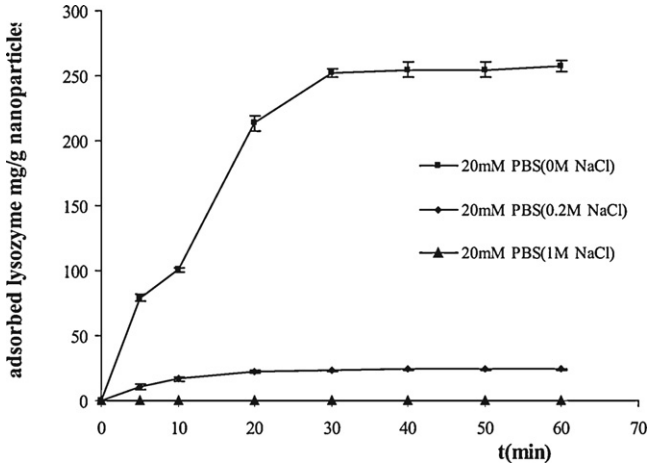

Fig. 5. Effects of ionic strength on lysozyme adsorption on the superparamagnetic nanoparticles (initial concentration of lysozyme: 1.0 mg/mL; pH: 9.0; temperature: 37 °C;)

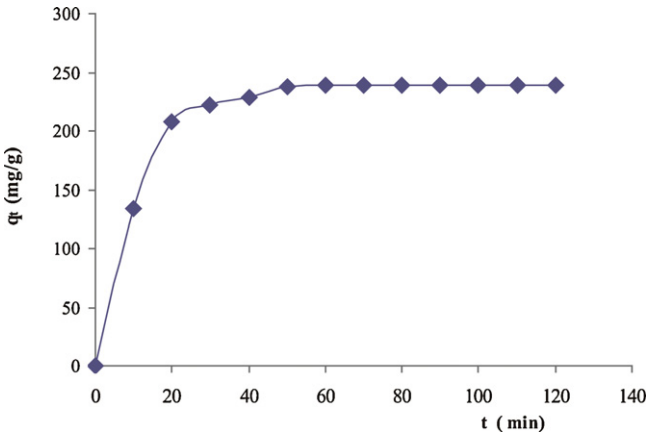

Fig. 6. Effect of reaction time to the adsorbing process (initial concentration oflysozyme: 1.0 mg/mL; pH: 9.0; temperature: 37 °C; ionic strength: 0M)

Fe_3O_4 (PEG + CM-CTS) nanoparticles is mainly through electrostatic interactions. Rusremier, Killmann and R. Blanco. once reported that with increasing electrolyte concentration in the medium the surface charges are screened [26,27]. In addition, protein solubility generally decreases with increasing salt concentration (salt-out effect), this also favors the formation of protein aggregates, leading to the decrease in protein transmission, based on the result of the our experiment and previously reported research finding, we can arrive at a conclusion that it is disadvantage for lysozyme absorption in high ionic strength.

3.2.4. Kinetic properties

With regard to further adsorption characteristics of lysozyme onto the surface of Fe_3O_4 (PEG + CM-CTS) nanoparticles, the effect of contact time on the amount of lysozyme adsorbed was examined using 10 mg of Fe_3O_4 (PEG + CM-CTS) nanoparticles and 4 mL of lysozyme solution with initial concentration of 1.0 mg/mL, the adsorption kinetic is studied as shown in Fig. 6. Since the nanoparticles have very small diameter and large specific surface area, it can quickly reach the adsorption equilibrium within 20 min, so the efficient adsorption of lysozyme onto superparamagnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles was promising.

The adsorption time data obtained were treated in the form of pseudo first- and pseudo second-order kinetic models. The pseudo first-order model is expressed as following [28]

$$\frac{1}{q_t} = \frac{k_1}{q_e t} + \frac{1}{q_e} \tag{4}$$

where k_1 is the pseudo-first-order rate constant (min^{-1}) of adsorption; q_e is the maximum adsorption capacity for pseudo-first-order (mg/g); q_t is the amounts of lysozyme adsorbed at time t (min) (mg/g).

The pseudo-second-order model is expressed as shown in equation (5) [29]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e{}^2} + \left(\frac{1}{q_e}\right) t \tag{5}$$

where k_2 is the pseudo-second-order rate constant (min^{-1}) of adsorption.

By plotting $1/q_t$ was versus $1/t$ according to pseudo-first-order model, a straight line is obtained in Fig. 7A. Fig. 7B is obtained by plotting t/q_t versus t. Related parameters are shown in Table 1. From Table 1 we can see that the correlation coefficients for the pseudo-second order kinetic model are higher than those of the pseudo-first order kinetic model, these results suggest that the pseudo-second-order equation fitted well with the experimental data and

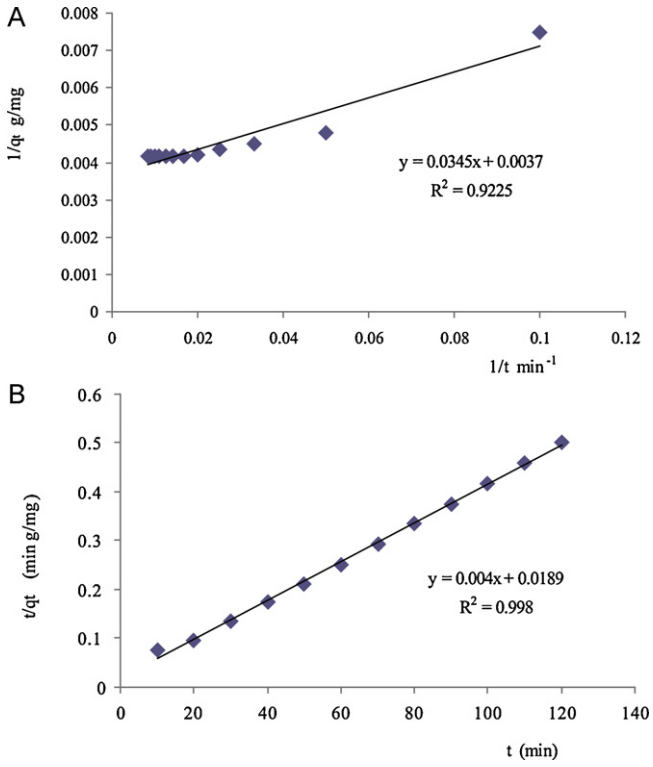

Fig. 7. kinetics of the adsorption of lysozyme by superparamagnetic nanoparticles. A: Pseudo first-order kinetics, the linear plot of 1/qt vs. 1/t; B: Pseudo second-order kinetics, the linear plot of t/qt vs. t.

could be used to describe the adsorption kinetics of lysozyme onto superparamagnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles.

3.2.5. Adsorption model

Using 10 mg of superparamagnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles and 4 mL of lysozyme solution with different initial concentrations, adsorption model was studied. As the relationship between the adsorption capacity and the initial concentration of lysozyme is presented in Fig. 8. The result indicated the increase in lysozyme adsorption capacity with increasing the initial concentration of lysozyme, but the increase was slowed down when the original lysozyme solution concentration was higher than 1 mg/mL, and tends to maximum adsorption. So absorption of lysozyme onto superparamagnetic nanoparticles basically complies with Langmuir model for which equation can be expressed as following:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m k} \tag{6}$$

where C_e (mg/mL) and q_e (mg/g) are lysozyme concentration in the aqueous solution and the absorbed lysozyme on the nanoparticles at equilibrium, respectively. q_m is the maximum adsorption and k is the adsorption constant.

In this work the equilibrium concentration and adsorption capacity have the expression as follows: $C_e/q_e = 0.0039C_e + 0.0011$, $q_m = 1/0.0039 = 256.4$ mg/g, $k = 1/256.4 \times 0.0011 = 3.545$.

The related coefficient is 0.9929; high R^2 values indicate that the model describes well the adsorption behavior. The maximum equi-

Table 1
The kinetic parameters of first and second-order model.

Model	Rate constant	Maximum adsorption (mg/g)	Coefficient R^2
First-order model	9.51	270.3	0.9471
Second-order	7.88×10^{-4}	250	0.998

Table 2
Comparison of the maximum adsorption capacities of the lysozyme onto various adsorbents.

Adsorbents	pH	Ionic strength	Maximum adsorption capacity	Reference
Chitosan/carboxymethyl cellulose blend membranes	9.2	100 mM sodium borate buffer salt	240.0 mg/g	24
RG 19 immobilized membrane	7	50 mM phosphate buffer salt	60.8 mg/mL	23
Lysozyme-imprinted poly(HEMA-MAH) [Lys-MIP] particles	7.4	20 mM morpholinopropane sulfonic acid	12.1 mg/g	28
Magnetic poly(2-hydroxyethyl methacrylate) mPHEMA beads carrying Cibacron Blue F3GA	7	100 mM phosphate buffer salt	342 mg/g	29
L-Tryptophan immobilized magnetic poly(glycidyl methacrylate)	7	50 mM phosphate buffer salt	259.6 mg/g	30
Poly(2-hydroxyethyl methacrylate) poly(HEMA) beads having methacryloylamido-phenylalanine (MAPA) ligand	10.0	0.75 M Na_2SO_4	114.3 mg/g	31

librium adsorption capacity was calculated to be 256.4 mg/g which was close to that of pseudo-second-order kinetic equation and considerably higher than some other materials published before which is shown in Table 2.

3.2.6. Elution of adsorbed lysozyme and stability of superparamagnetic nanoparticles in repeated use

The lysozyme-loaded superparamagnetic nanoparticles were placed within the elution medium containing 0.5 M NaCl at PBS (pH 5.0, 20 mM). The result showed that >99% of the adsorbed lysozyme was removed when 0.5 M NaCl which is a suitable desorption agent for lysozyme desorbed from nanoparticles was used as an elution agent, and no remarkable reduction in the adsorption capacity during six repeated adsorption–desorption operations.

3.3. Purification of lysozyme from chicken egg white solution

Using Fe_3O_4 (PEG + CM-CTS) nanoparticles to purification of lysozyme from chicken egg white which was very simple and efficient. The purity of the lysozyme desorbed from nanoparticles was determined by SDS–PAGE electrophoresis. Non-reducing SDS–PAGE of fractions (Fig. 9) clearly show that: in the natural chicken egg white solution, five major proteins were detected, through adsorption and desorption, an amount of lysozyme were detected in the elution, and eluted fractions had high purity since only one band was detected, meanwhile, electrophoretic band was darker than the other when the pH of chicken egg white equal to 9.0, which means the best pH of purification of lysozyme from chicken egg white was 9.0, and about 85.2% of lysozyme can be extracted in purification of lysozyme from chicken egg white solution. Furthermore, the lysozyme activity was well maintained after purification from chicken egg white, about 90.2% of total activity could be retained. The experimental results showed that this type of superparamagnetic nanoparticles with high efficiency and cost-effectiveness would bring advantages to the conventional separation techniques of lysozyme from chicken egg white.

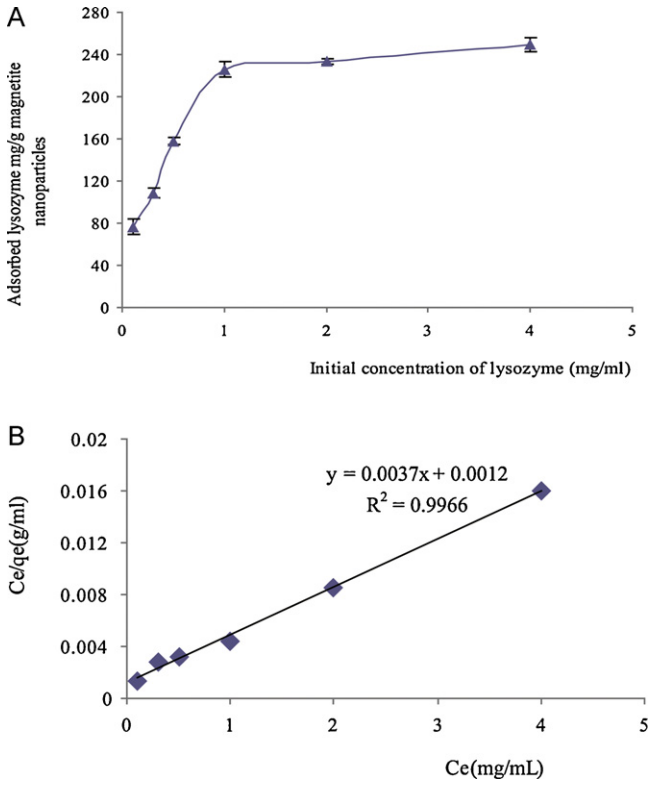

Fig. 8. Effect of lysozyme initial concentration on lysozyme adsorption on the superparamagnetic nanoparticles (pH: 9.0; temperature: 37 °C; ionic strength: 0M)

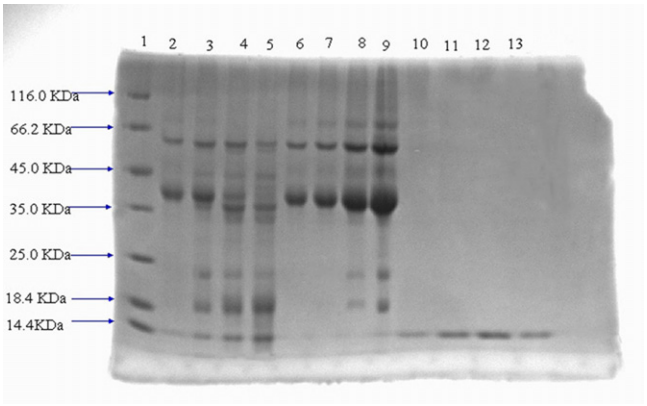

Fig. 9. The purification result of magnetite nanoparticles to lysozyme from chicken egg white solution lane1, biomarker (sigma), lane 2-5, different pH(7.0, 8.0, 9.0, 10.0) of natural chicken egg white solution; lane 6-9, the supernatants after adsorption; 10-13, eluted samples; 5 μL samples were applied to lane 2-5, 10 μL samples were applied to lane 1 and lane 6-13.

4. Conclusion

Using natural polysaccharide – carboxymethyl chitosan (CM-CTS), we have developed a highly efficient and low-cost superparamagnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles, and then separation and purification of lysozyme from aqueous solution and chicken egg white. The superparamagnetic nanoparticles had stability, repeatability and high adsorption capacity for lysozyme. The purified lysozyme had satisfying activity and purity. The purification process avoiding expensive equipment makes routine lysozyme production feasible. The conjugated CM-CTS with magnetic nanoparticles will make lysozyme purification much more convenient and cost effective. It is anticipated that such superparamagnetic Fe_3O_4 (PEG + CM-CTS) nanoparticles may have great

potential to be used in large-scale purification of lysozyme from chicken egg white.

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