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Macroporous chitosan layer coated on non-porous silica gel as a support for metal chelate affinity chromatographic adsorbent

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Abstract

A new immobilized metal affinity chromatography (IMAC) matrix was prepared by coordinating Cu^{2+} with cross-linked chitosan coated on non-porous silica gel (Cu–CTS–SiO_2). Macroporous structure could be formed on the coated layer by imprinting polyethylene glycol (PEG) in chitosan film. The surface morphology changes on Cu–CTS–SiO_2 bead prepared in different condition were confirmed by scanning electron microscopy (SEM). Effects of chitosan and PEG content in coating solution, the molecular mass of PEG on the surface macropore formation and adsorption capacity of bovine serum albumin (BSA) were investigated. Results indicated that coating solution with 2% chitosan and 10% PEG 20000 was optimal. Batch experiments were also conducted for elucidating the optimal pH, the adsorption isotherm and adsorption kinetics of BSA. Adsorption isotherm of trypsin on the same adsorbent was also performed. Results showed that the support itself had low non-specific interaction with both BSA and trypsin. The maximum adsorption capacity for BSA and trypsin on the prepared IMAC adsorbent could reach 192 mg and 5000 IU, respectively calculated by every gram of chitosan. The binding and eluting condition for BSA were tested on column filled with the adsorbent. Crude BSA sample could be purified on the IMAC column.

Keywords: Immobilized metal affinity chromatography; Copper ion; Macroporous chitosan; PEG imprinting; Adsorption; Bovine serum albumin

1. Introduction

Over the past 28 years since its first introduction, immobilized metal affinity chromatography (IMAC) has become a widespread analytical and preparative separation method for therapeutic proteins, peptides, nucleic acids, hormones, and enzymes [1–11]. In IMAC technique, separation methods utilize metal ions as pseudo-biospecific ligands for protein separation especially in His-tagged protein [4–6,12]. Up to now, the most commonly used chromatographic matrices for IMAC were various polysaccharide or synthetic polymers coupled with organic ligands for metal chelating and the most often used ligands were iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA) [13,14]. These kinds of IMAC adsorbent were relatively expensive, and might be suffered from slow releasing of the covalent bond chelators off the matrix during purification step [13]. This might limit their application in purification of products especially for food or pharmaceutical purposes. So, coating a non-toxic and metal chelating polymer on suitable support would be a new choice for IMAC [15].

Chitosan was the N-deacetylated products of chitin manufactured from shrimp or crab shells. It was inexpensive, non-toxic, hydrophilic, biocompatible, and biodegradable. Chitosan could provide an excellent binding capacity with heavy metal ions since it had active primary amino groups in the polyaminoglucosan chain, which could provide sufficient amount of chelating sites for metal ions [16,17]. When it was used as matrix for IMAC, the step of covalent coupling of the chelating ligands could be totally eliminated. However, pure chitosan bead had some disadvantages such as unsatisfied mechanical properties, severe shrinkage, deformation after drying, soluble in acidic conditions, and com-

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pressible at high operating pressure [18]. Several works including ours had been made to overcome these disadvantages by coating chitosan on porous silica gel (CTS–SiO_2) followed by cross-linking and other steps [19–21] for preparing affinity chromatographic matrix. Silica gel itself had been widely used in many chromatographic adsorbent, but when it was used as an affinity chromatographic support, two aspects should be considered. First, the non-specific interaction between the gel surface and bimolecular should be minimized. Second, surface porous structure should be maintained and controlled since the original porous structure of the gel might disappear or totally be changed after the coating step. Non-specific interaction force between the silica gel and analytes include dipolar interaction, hydrogen bond and hydrophobic interaction force mainly caused by surface silanol groups [22,23]. Inhibition of the non-specific interaction by coating a high dense cross-linked chitosan layer on porous silica gel beads had been reported [24]. However, when the high ratio cross-linked chitosan was coated on porous silica gel, the surface porous structure might be changed and surface area was deceased. Since macropores on the gel surface plays an important role in accommodating the accessibility of the protein adsorption [25], it was an alternative to use non-porous silica gel as a support of affinity adsorbent for eliminating the non-specific interaction even if some of the silica gel surface were exposed. Meanwhile, the surface porous structure for specific binding the target protein could be maintained by coating a chitosan layer with macroporous structure.

In this paper, a new IMAC adsorbent was prepared by coating chitosan–PEG solution on non-porous silica gel followed by the steps of chitosan deposition, PEG removing, crosslinking and copper ion loading. The non-porous silica gel not only acted as a rigid support but also had little non-specific interaction between its residue exposed gel surface and protein. In order to maintain the surface porous structure which was needed in affinity binding for target protein, a macroporous structure of the coated chitosan layer was obtained by PEG molecular imprinting and removing method. Suitable pore size could be designed to achieve the maximum adsorption for given protein through changing the molecular mass and content of PEG in coating solution.

Since bovine serum albumin (BSA) had copper ion binding sites in its amino acid sequence (PIR search, http://pir.georgetown.edu/), it was used as a model protein for investigating the adsorption capacity of the beads with different surface porosity through batch experimental methods. The new IMAC adsorbent had the quality of high specific binding capacity for metal binding proteins such as BSA and trypsin, low non-specific interaction, stable in wide pH range, and rigidity at high pressure. Column experiments were also performed filled with the adsorbent. BSA in phosphate buffer solution could be bound on the column and eluted by imidazole solution. SDS–PAGE verified that a crude BSA sample could be purified after one column purification step. The adsorbent might be applied in the separation of metal binding biological macromolecules especially in His-tagged protein.

2. Experimental

2.1. Preparation of silica gel beads coated with macroporous chitosan layer (CTS–SiO_2)

Solutions with different contents of chitosan (supplied by Potuo Biomedical Corp., China) and polyethylene glycol (PEG) were prepared by dissolving various amounts of chitosan and PEG in 1 M acetic acids solution. Non-porous silica gel (75–100 um) was added into the solution and immersed overnight before drying in vacuum condition. The chitosan-coated gel was suspended in dimethylsulphoxide (DMSO) and stirred vigorously in a flask. After dissipation, NaOH was added until pH reached above 10.0. Continued to stir the mixture for 30 min in order to remove the imprinted PEG and form macropores on the bead surface. Filtered the solution and the solid was re-suspended in DMSO containing 0.1 M NaOH and 1 M epichlorohydrin at 60 °C for 8 h. After thoroughly washed with pure water, the cross-linked matrix with some active epoxy groups was shaken in a 0.85 M ammonia solution at 60 °C for 4 h for recovering some amino groups lost in cross-linking step [26]. Then, the martrix was washed with pure water to remove the residual ammonia.

2.2. Preparation of IMAC matrix (Cu–CTS–SiO_2) by coordinatering Cu^{2+} with CTS–SiO_2

One gram of the CTS–SiO_2 were mixed with 50 mL of aqueous solutions containing 0.1 M Cu^{2+} at constant pH of 5.0 (adjusted with HCl and NaOH), which was the optimum pH for Cu^{2+} chelating at room temperature. The flask was stirred magnetically at 100 rpm for 1 h (sufficient to reach equilibrium). The concentration of the Cu^{2+} ions in the resulting solution was determined with a flame atomic adsorption spectrometer (So1 AAR S2, Thermo Electron Corporation, UK). The amount of adsorbed Cu^{2+} was calculated by using the concentration of the Cu^{2+} ions in the initial solution and in the equilibrium.

The copper ion immobilized blue-colored bead was filled in a glass column and washed with sufficient amount of PBS solution to remove the unbound Cu^{2+}. Finally, the Cu–CTS–SiO_2 affinity adsorbent was obtained.

2.3. Surface morphology of the adsorbent bead

Scanning electron micrographs of Cu–CTS–SiO_2 was done by gold plating the dried powder of the adsorbent and scanning it on a SIRION (FEI, USA) scanning electron microscopy.

2.4. BSA adsorption experiment in different pH

BSA (purchased from Shanghai Shengong Bioengineering Corp., China) was dissolved in 15 mM PBS plus 0.1 M NaCl with pH ranging from 4.0 to 8.0, respectively. Bead of SiO_2, CTS–SiO_2 and Cu–CTS–SiO_2 was incubated individually with above BSA solution and shaken in a vibrator for 120 min. After equilibration, the amount of the BSA in the each supernatant was analyzed. The adsorption capacity of BSA in different condition was calculated by following equation:

$$Q = \frac{(C_0 - C_e)V}{m_a} \quad (1)$$

C_0 and C_e represent initial and equilibration concentration or activity of protein, V is the volume of the solution and m_a the weight of adsorbent.

2.5. Binding kinetics of BSA

Binding kinetics of BSA to Cu–CTS–SiO_2 was performed by adding 150 uL BSA (9 mM) into 0.5 g Cu–CTS–SiO_2 bead immersed in 2 mL PBS buffer (pH 6.0). The content of BSA in the interval supernatant was measured.

2.6. Adsorption isotherm of BSA and trypsin

Adsorption isotherm of BSA and trypsin (BBI, 250 IU mg^{-1}) was determined by equilibrating different concentration of BSA and trypsin with 0.5 g Cu–CTS–SiO_2 in 15 mM PBS (pH 6.0 and 7.8, respectively) for 100 min at room temperature. After equilibration, the amount of BSA and trypsin bound was calculated by Eq. (1). The amount of the trypsin was expressed in specific enzyme activity.

2.7. Estimation of protein

The amount of the protein was measured by the method of Bradford [27].

2.8. Determination of the activity of trypsin

The activity of trypsin was determined at 25 °C using BAPAN as substrate. A suitable amount of trypsin solution was added to 3.0 mL 50 mM Tris–HCl buffer containing 10 mM $CaCl_2$, pH 8.5. Then 15 ul of 0.15 M *N*-benzoyl-arginine–*p*-nitroanilide (BAPAN) in DMSO solution were added to the mixture. One unit (IU) of trypsin was defined as 0.001 increase in optical density at 410 nm min^{-1}. UV1200 UV–vis spectrophotometer (Shimadzu Corp., Japan) was used to monitor the optical absorption.

2.9. Column chromatography

Packed a 1.0 cm × 20 cm (bed volume 10 mL) column with Cu–CTS–SiO_2 and equilibrated with the PBS binding buffer (pH 6). Seven milligrams of BSA in 10 mL solution was loaded on the column by peristaltic pump. The flow rate was controlled at 0.5 mL min^{-1}. The column was washed with binding buffer for two columns volume at the same flow rate. Elution was carried out with 0.1 M imidazole in PBS at flow rate of 1 mL min^{-1}. The amount of protein in each eluted fraction was monitored with the method of Bradford after dialyzing to remove the imidazole.

2.10. SDS–PAGE [28]

SDS–PAGE was conducted on DDY-12 power supply and DYCZ-24D vertical electrophoresis slab (Liuyi Instrumental Corp., China). A loading of 200 ng protein per lane was applied to a 10% gel. Electrophoresis was carried out at a constant current of 20 mA. The gel was silver-stained [29].

2.11. Regeneration of Cu–CTS–SiO_2

The copper ion in Cu–CTS–SiO_2 could be stripped with 0.05 M HCl, the chelating ability of the CTS–SiO_2 matrix could be recovered by washing with 0.05 M NaOH followed by pure water washing to remove the residue alkaline. After this step, the Cu^{2+} can be chelated again and the column was regenerated.

3. Results and discussion

3.1. Cu^{2+} adsorption on CTS–SiO_2

Cu^{2+} adsorption capacity on the CTS–SiO_2 prepared in different condition was shown in Table 1. Adsorption capacity of Cu^{2+} on CTS–SiO_2 was significantly increased with the increasing of chitosan content coated on the silica gel. Since it was the chitosan that could coordinate with the Cu^{2+}, the higher amount of chitosan was coated on the adsorbent, the higher amount of Cu^{2+} could be adsorbed. Table 1 also indicated that the PEG content and its molecule mass did not affect Cu^{2+} adsorption capacity significantly when chitosan concentration was same in the coating solution. PEG in this experiment only affected the porous structure. It might affect the adsorption of protein but it could not affect the adsorption behavior of small metal ion, which could access different size of pores. In overall, the amount of immobilized Cu^{2+} was significantly higher than the previous reported value of other IMAC materials [24]. The high Cu^{2+} binding capacity could be ascribed to our unique chitosan coating, deposition, crosslinking and amino group recovering method. In the preparing procedure, the dried chitosan-soaked silica gel was dispersed in DMSO solution and chitosan was deposited on silica gel surface through phase inversion method, which could prevent diffusion of chitosan into liquid phase. This method might acquire a relative thick chitosan layer comparing with other method [24]. In the process of crosslinking, epichlorohydrin reacted with both amino and hydroxyl

Table 1
Effect of coating condition on the Cu^{2+} adsorption capacity of adsorbent

Chitosan content (percentage in silica gel) (%)	PEG and its molecular mass (percentage in coating solution, %)	Cu^{2+} adsorption capacity (mmol L^{-1} wet bead)
Silica gel (control)	–	0.02
2	PEG 10000 (10)	65.0
2	PEG 20000 (10)	60.2
2	PEG 20000 (25)	52.0
5	PEG 10000 (10)	82.4
5	PEG 20000 (10)	80.7
5	PEG 20000 (25)	76.6

groups in chitosan molecules to improve the stability of chitosan film. As a result, the primary amino groups on chitosan layer lost in two ways: crosslinking and activation by epoxy group. Through the second way the lost could be recovered after amination of the epoxy groups with ammonia [26]. High amount of metal ion loading is important in IMAC materials, which could lead to a high capacity of protein adsorption.

3.2. Effect of pH on the BSA adsorption capacity

The principle of IMAC suggested that the adsorption of protein to the adsorbent was governed by the coordination of metals with electron-donor ligands exposed on the surface of the proteins [2,7]. Interaction between BSA and immobilized copper on the surface of Cu–CTS–SiO_2 in different pH was investigated in 15 mM PBS + 0.1 M NaCl with pH 4.0, 5.0, 6.0, 7.0, 7.5. Significant difference in binding capacity could be found in Fig. 1. Results showed that in acidic condition, the capability of protein coordination with the copper ion was decreased with the lowing of pH. This tendency may be caused by the protonation of electron donor group in amino acid residues, leading to decreasing coordination ability with metal ion. The maximal adsorption capacity was reached at pH 6.0. But when the pH went more higher than 6.0, the adsorptive capacity was decreased remarkably. It might be explained that the phosphate ion in buffer might compete with the protein to coordinate with copper ion. When the pH value was high, the percentage of $HPO_4{}^2$ and $PO_4{}^{3-}$ in buffer was increased. These two kinds of ions possessed a relative higher coordinating ability compared with $H_2PO_4{}^-$ and would competed with protein for metal binding. This phenomenon well supported the theory that coordination was the driving force

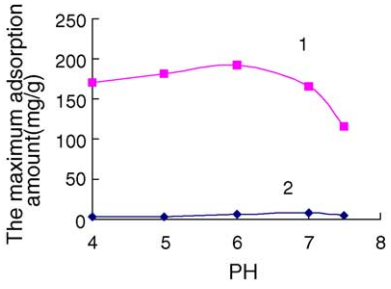

Fig. 1. BSA adsorption capacity on Cu–CTS–SiO_2 (curve 1) and CTS–SiO_2 (curve 2) as a function of the pH.

in IMAC. In the following experiments, 15 mM PBS (pH 6.0) containing 0.1 M NaCl act as the binding buffer. Fig. 1 also showed that the CTS–SiO_2 matrix almost did not adsorb BSA in the whole experimental pH range. This result further proved that the BSA was bound on the adsorbent surface through specific coordination force and the non-specific interaction could be almost neglected.

3.3. Effect of the adsorbent preparing condition on the BSA adsorption capacity and the morphology of the adsorbent

The results of BSA adsorption capacity on Cu–CTS–SiO_2 prepared in different condition were shown in Fig. 2 compared with bare silica gel. In the CTS–SiO_2 preparation step, PEG was used as the porogen according the method described by Yang [26]. It was extracted into the liquid phase to form a macroporous chitosan layer before chitosan was cross-linked by epichlorohydrin. Generally, the surface porosity was controlled by cross-linking ratio. However, in this paper, the concentration of epichlorohydrin was so high that the imprinted PEG content and its molecular size would dominate the macropores formation on the bead surface. The maximum BSA adsorption capacity of adsorbent prepared in different condition was shown in Fig. 2. It could be seen that the highest adsorption capacity was obtained with Cu–CTS–SiO_2 prepared by 2% chitosan with 10% PEG 20000 in coating

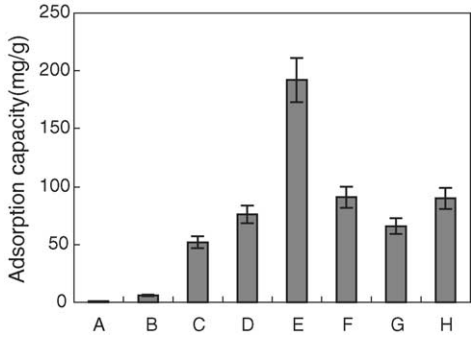

Fig. 2. Comparison of adsorption capacity between Cu–CTS–SiO_2 prepared in different condition. (A) Non-porous silica gel; (B) CTS–SiO_2 (no Cu^{2+}), (C) 2% CTS–10% PEG 10000; (D) 2% CTS–5% PEG 20000; (E) 2% CTS–10% PEG 20000; (F) 2% CTS–25% PEG 20000; (G) 5% CTS–10% PEG 20000; (H) 5% CTS–25% PEG 20000.

solution. It was obviously that a thicker layer of chitosan could be formed on silica gel with the higher content of chitosan in coating solution. Nevertheless, a thick chitosan layer and higher metal loading (Table 1) did not show any advantage on the protein adsorption capacity. The result inferred that the amount of immobilized metal ion was not the only factor affecting the protein binding capacity, the surface pore structure might also affected the protein binding. Fig. 2 showed that the molecular size of PEG and its content in coating solution could significantly affect the adsorption capacity of the adsorbent. For instance, BAS adsorption capacity on PEG 20000 imprinted Cu–CTS–SiO_2 (Fig. 2E) was almost four times larger than that with PEG 10000 (Fig. 2C) when the other conditions were the same. The phenomenon was probably due to the pore size formed by PEG 20000 was more accessible for BSA compared with PEG 10000, and might significantly increased the chance of interaction of BSA with immobilized Cu^{2+}. The results indicated that the pore size control in preparation of IMAC adsorbent might be very important regarding its adsorption capacity and selectivity.

Fig. 2A and B also showed that the non-porous silica gel support and the CTS–SiO_2 almost did not adsorb significant amount of BSA. The percentage of non-specific interaction on CTS–SiO_2 support only account for about 3.1% in the highest protein adsorption capacity on Cu–CTS–SiO_2. This value was much lower than the result of porous silica gel based matrix [24], in which the ratio was about 8.8% calculated by the reported value. The results not only proved that the adsorption of BSA on the Cu–CTS–SiO_2 was through a specific metal chelating force, but also proved that the non-porous silica gel had minimal non-specific interaction with protein. This might be ascribed to low surface area of the non-porous silica gel and shortage of the surface pores for protein accessing. The situation was very different with the result when porous silica was used as the support for chitosan layer [24]. Usually, porous silica gel could adsorb protein [23] and a complete hydrophilic polymer coating could decrease the non-specific interaction. But if the surface coverage was not complete such as in the situation of low ratio of crosslinking, the residual non-specific interaction might still exist. On the other hand, when the high ratio of crosslinking was performed, suitable pore structure might be lost. By introducing the method of molecular imprinting, the macroporous structure of the coated chitosan film could be obtained even if the chitosan was crosslinked in high ratio.

Fig. 3 presented the surface morphologies of CTS–SiO_2 prepared in different condition with variation of chitosan concentration and imprinted molecular PEG 10000 or PEG 20000. The surface morphology of silica gel was also presented (Fig. 3A). It could be found that the surface of the nonporous silica gel was smooth. After the coating, PEG removing and cross-linking steps in different condition, the surface morphologies were totally changed with a rugged and porous surface formed. The pore structure of CTS–SiO_2 coated in different condition was totally different (Fig. 3B–D). A dense and macroporous surface was formed

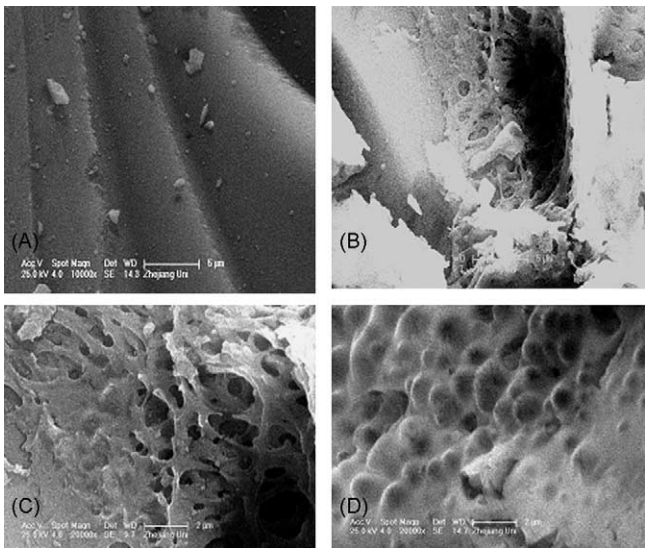

Fig. 3. SEM of the surface of the Cu–CTS–SiO_2 prepared in different coating solution. (A) Silica gel; (B) coated with 5% chitosan and 25% PEG 20000; (C) coated with 2% chitosan and 10% PEG 20000; (D) coated with 2% chitosan and 10% PEG 10000.

on CTS–SiO_2 coated with 2% chitosan and 10% PEG 20000 (Fig. 3C), which might contribute to the largest adsorption capacity. The results also inferred that in IMAC processes, the porosity and pore size of the matrix could affect the accessibility of binding sites for protein adsorption. So, the selective adsorption of metal binding protein by IMAC adsorbent could be achieved based on surface pore control as well as exploiting the differences in metal binding affinity. This would be an attractive aspect in protein separation by IMAC.

3.4. Adsorption kinetics

Fig. 4 was the kinetics curve of BSA adsorption to Cu–CTS–SiO_2. The curve indicated that the adsorption was fast in initial step. After 60 min, the rate of adsorption was decreased. The maximum adsorption capacity for BSA was reached after 100 min. In initial fast adsorption step, BSA might enter some easy accessible pore sites and bind with

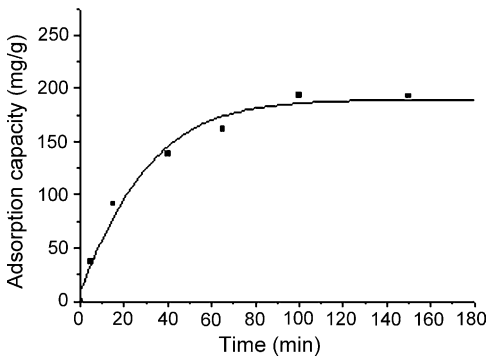

Fig. 4. Kinetic curve of BSA adsorption on Cu–CTS–SiO_2.

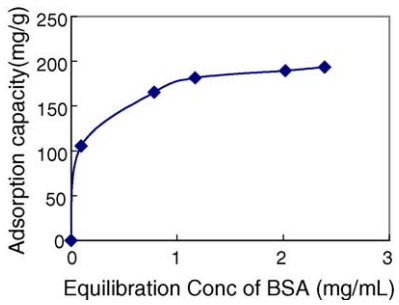

Fig. 5. Adsorption isotherm of BSA at pH 6, $T = 25\,^{\circ}C$.

the copper ions, while in the slow adsorption step, some protein might diffuse into deeper and smaller pore.

3.5. Adsorption isotherm

Adsorption isotherm of BSA on Cu–CTS–SiO_2 was similar to the Langmuir sorption isotherm. Data shown in Fig. 5 were fitted to the linear form of the Langmuir equation:

$$\frac{[C_e]}{[Q]} = \frac{1}{k[Q]_{max}} + \frac{[C_e]}{[Q]_{max}}$$

where $[C_e]$ and $[Q]$ represent equilibrium concentration of protein and the amount of adsorbed protein per unit weight of immobilized chitosan, respectively; $[Q]_{max}$ is the maximum amount of protein adsorbed on per gram weight of chitosan; and k the adsorption–desorption equilibrium constant related to the binding energy. The data were well fitted to the linear form of Langmuir equation expressed as following:

$$\frac{[C_e]}{[Q]} = 0.0006 + 0.005[C_e], \quad r^2 = 0.9988$$

The calculated coefficient $k = 8.3$ and $[Q]_{max} = 200$ mg g^{-1} indicated the adsorbent had a considerable sorption capacity and could be used as a suitable adsorbent for BSA from aqueous solution.

In order to further verify that the new IMAC adsorbent had feasibility of pervasive application in metal binding protein adsorption and separation, the adsorption isotherm of trypsin that also had metal binding ability was performed. As illustrated in Fig. 6, the Cu–CTS–SiO_2 IMAC adsorbent

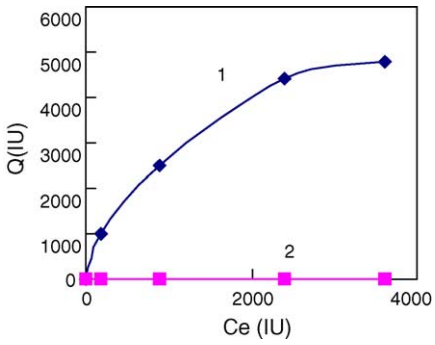

Fig. 6. Adsorption isotherm of trypsin on the Cu–CTS–SiO_2 (curve 1) and CTS–SiO_2 (curve 2).

could effectively bind with trypsin. The maximal adsorption capacity of the adsorbent for trypsin could reach 5000 IU per gram of the adsorbent. Meanwhile the Cu^{2+} free adsorbent (CTS–SiO_2) only adsorbed 20 IU of trypsin, which indicated that the support had low non-specific interaction with different kinds of protein.

3.6. Binding and elution of BSA from IMAC column packed by Cu–CTS–SiO_2

In IMAC column experiments, the sample solution was a crude BSA crystal sample dissolved in PBS. Fig. 7a shows that a relative small and wide peak was found in sample loading and washing step. The peak might be caused by unbound impurity protein or unbound BSA. A large peak was found after the column was eluted with 0.1 M imidazole solution. The content of protein concentration in collected peak fraction was determined by Bradford method. The recovery rate of BSA could reach 85%.

SDS–PAGE (Fig. 7b) proved that the main component in sample solution was BSA with some low molecular mass impurity polypeptide. After one step of IMAC purification, the collected BSA was purified.

3.7. Stability of Cu–CTS–SiO_2

The column filled with regenerated adsorbent can be reused. Fig. 8 summarized the results of regeneration and BSA adsorption capacity change of Cu–CTS–SiO_2. The adsorption capacity decreased obviously in initial four cycles, after that, the adsorption capacity tended to be stable. The initial reduction in adsorption might result from the losing of chitosan that was not sufficiently cross-linked. This part of chitosan could be a little solved in acid washing step during regeneration, while the remaining high dense cross-linked part could stabilize in acidic washing step and lead to a high stability of the adsorbent in subsequent regeneration cycles. On the other hand, after continuously washing the beads with 0.1 M NaOH solution at a flow rate of 1 mL min^{-1} for 24 h,

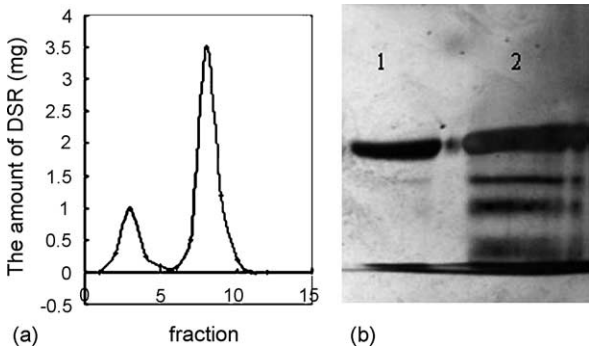

Fig. 7. (a) Elution curve of Cu–CTS–SiO_2 column. Fractions 1 and 2 are sample loading 5 mL per fraction; fractions 3–6 were eluted by binding buffer 5 mL per fraction; fractions 7–11 were eluted by imidazole 5 mL per fraction. (b) SDS–PAGE photograph of BSA sample of: the purified BSA (lane 1) and the loading sample (lane 2).

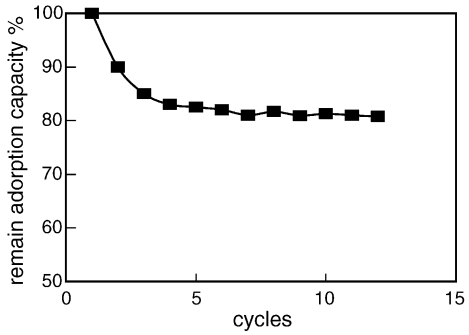

Fig. 8. The remain adsorption capacity for BSA during the regeneration cycles of the Cu–CTS–SiO_2 adsorbent.

only 5% weight loss was observed and the 92% protein binding capacity was reserved. This indicates that the bead was also stable after long-term contacting with alkaline solution. Overall, the absorbent was more stable in alkaline condition than the previous report [24], in which only about 75% BSA adsorption capacity was remained after long-term soaking in 0.1 M NaOH solution.

4. Conclusions

The experiment show a promising results that the new IMAC adsorbent with surface macroporous structure could be prepared by coating mixed solution of chitosan and PEG on non-porous silica gel and subsequently followed the step of PEG removal, chitosan deposition, cross-linking and copper ion chelating. Various porosity on the bead surface was formed by controlling the content of chitosan and PEG with different molecular mass in coating solution. The results proved that these kinds of ligand-free matrix had the advantage of low non-specific interaction, easy to prepare, high adsorption capacity for metal binding protein and high stability. More important, potential selective adsorption of metal binding protein could be achieved by designing the surface pore size using molecular imprinting method, as well as by utilizing the difference in metal chelating ability.

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