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Chitosan gel beads immobilized Cu (II) for selective adsorption of amino acids

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Abstract

Chitosan (CS) gel beads were prepared by using phase inversion and precipitation technique. The gel beads could bind copper (II), by which Cu (II) ion-immobilized chitosan gel beads (CS-Cu^{2+} gel beads) were prepared, and the amount of the immobilized Cu (II) was about 35 mg/g when the CS gel beads were incubated in 150 ppm cupric sulfate solution. The CS-Cu^{2+} gel beads could selectively adsorb histidine (His) from the mixed solution containing His and tryptophan (Trp); and the selective coefficient which was defined as the adsorbed amount ratio of His to Trp was about 8.0 at the pH value of 7.4. The effect of the pH value on the amino acid adsorption was also studied. In order to investigate the relationship of the amino acid adsorption and protein adsorption, the adsorbed amounts for IgG and albumin were determined; and the results indicated that the CS-Cu^{2+} gel beads could adsorb a larger amount of IgG than albumin due to the larger amount of the exposed residual His. The study provided a sorbent and a method to selectively remove His and IgG.

Keywords: Chitosan; Gel beads; Cu (II); Amino acid; IgG

1. Introduction

Chitosan, poly(*b*-1-4)-2-amino-2-deoxy-D-glucopyranose, is produced by partially or fully alkaline *N*-deacetylation of chitin, which can be widely found in the exoskeleton of shellfish and crustaceans as the second most abundant natural biopolymers next to cellulose [1]. It is inexpensive, non-toxic, hydrophilic, biocompatible, and biodegradable [2,3]. In consideration of these excellent properties, chitosan and its derivatives have been found many applications in medicine, biotechnology, pharmacology and food technology [4]. It is well-known that chitosan is an outstanding sorbent of extremely high affinity for transition metal ions due to the abundant amino ($-NH_2$) and/or hydroxy (–OH) groups on chitosan chains served as conjunction sites [5,6]. Nevertheless, the amino group of chitosan is the principal group in binding metal ions, and it was widely accepted that a copper ion via four amino groups in a square-planar geometry immobilized on chitosan [3,7]. Its adsorption capacity of metal ions depends on the degree of deacetylation [4]. Chitosan has undoubtedly been one of the most popular adsorbents for the removal of metal ions from aqueous solution and is widely used in waste treatment applications [8].

Extensive researches were carried out to investigate the interactions between copper (II) and amino acids [9,10]. The amino group and carboxyl are the functional groups of amino acid, and they are the principal chelate groups to interact with metal ions. Ignoring the side chains of amino acids, the common structure was a five member chelate ring [9], in which the Cu^{2+} bound with the oxygen of hydroxyl and the nitrogen of amino group. However, it was not such simple, such as the effect of the imidazole of histidine and the sulfhydryl of cysteine. These side chains play important role in the complex of copper and amino acids. His has strong affinity for metal ions, especially for copper (II), which is attributed to its functional imidazole group.

Abbreviations: CS, chitosan; CS-Cu^{2+}, Cu (II) ion-immobilized chitosan; His, histidine; Trp, tryptophan; EDTA, Ethylenediamine tetraacetic acid disodium salt.

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Nowadays, immobilized metal ion affinity chromatography (IMAC) is widely used for the separation and purification of natural and recombinant proteins for its higher selectivity [11–13]. Proteins vary from each other mainly in the component of amino acids. The borderline metal ions, such as copper (II) could interact with both amino acids and proteins. Hari et al [14] have studied the selective adsorption of human IgG from a mixture of albumin, γ-globulin, fibrinogen, and IgG onto copper (II) ion-immobilized cellulose membrane. Our interest is focused on the inexpensive adsorbents for amino acid and protein adsorption, and that whether there is a relationship between the adsorption of amino acid and protein to the copper (II) ions; and thus to find a method to separate and purify amino acids and proteins.

In the present study, chitosan gel beads were prepared by a phase inversion method, and then copper (II) ions were immobilized on the gel beads in cupric sulfate solution. Considering the interactions among chitosan, copper (II) and amino acids, the capability of CS-Cu^{2+} gel beads for the selective adsorption of amino acids was investigated. The adsorption capacity of IgG was compared with albumin at the same conditions. The relationship between the amino acid adsorption and protein adsorption was discussed preliminarily.

2. Materials and methods

2.1. Materials

Chitosan powder, with a deacetylation degree of about 90% and viscosity below 100 cps, was purchased from Boao Biological Tech. Co., Ltd., Shanghai. Acetic acid (HAc), sodium hydroxide (NaOH), glutaraldehyde and cupric sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) were supplied by Kelong Chemical Reagent Factory, Chengdu. Ethylenediamine tetraacetic acid disodium salt (EDTA, $C_{10}H_{14}N_2O_8Na_2{\cdot}2H_2O$) was purchased from Kermel Chemical Reagent Co., Ltd. Shanghai. The amino acids (Tryptophan, Trp; histidine, His) were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai. All the other regents were of analytical grade, and were used without further purification.

2.2. Preparation of Cu (II) immobilized porous chitosan gel beads

Chitosan gel beads were prepared as in our previous work [16]. Required amount of chitosan was dissolved into 3% aqueous acetic acid solution, and the chitosan concentration was 4%. Then, the solution was dropped into a precipitation bath containing 1 mol/l aqueous sodium hydroxide solution, by using a 0.4 mm-diameter syringe needle. The injection speed was controlled at about 60 drops/min. The air gap from the needle to the surface of the NaOH solution was about 10 cm. After half an hour, the wet chitosan beads were collected and extensively rinsed with double distilled water to remove the residual NaOH and sodium acetate (CH_3COONa), and then stored in double distilled water until use.

Cu (II) solutions of were prepared by dissolving cupric sulfate pentahydrate into distilled water. Chitosan gel beads (about 1 g in dried weight) were dipped in 250 ml cupric sulfate solution (the concentration 150 ppm), agitated by a magnetic stirrer for about 10 h. The Cu (II) immobilized blue chitosan gel beads were collected and extensively rinsed with double distilled water to remove the free Cu (II) ions. Since chitosan was soluble in acidic conditions, the chitosan gel beads were cross-linked by glutaraldehyde as mentioned in our previous work [16].

2.3. Characterization of the gel beads

A scanning electron microscope (JSM-5900LV, JEOL) was used for the morphology observation of the gel cross-sections. The diameter and the porosity of the gel beads were calculated from the density of the chitosan and the weight change before and after drying. These had been mentioned in detail in our previous work [15,16].

2.4. The amount of Cu (II) immobilized to the chitosan gel beads

The concentration of Cu (II) in the solution after adsorption was determined by using an electro-conductivity analyzer. Then the amounts of Cu (II) immobilized on the beads, P (mg/g), were approximately calculated using the following equation:

$$P = V(C_o - C_e)/W_D \quad (1)$$

where C_o (mg/l) is the initial Cu (II) concentration; C_e (mg/l) is the final Cu (II) concentration after adsorption; V (l) is volume of the Cu (II) solution; and W_D (g) is the weight of the dried beads.

Ethylenediamine tetraacetic acid disodium salt (EDTA) was used to take off the cupric ions from the CS–Cu (II) gel beads. The CS–Cu (II) gel beads (about 15 mg in dried weight) were dipped in 5 ml of 1 mol/l EDTA aqueous solution, and shaking for 12 h at room temperature. Then, 2 ml of the solution was taken out and diluted with double distilled water to 25 ml. The concentration of the solution was determined using an atomic absorption spectrophotometer (Model AA220Fs, VARIAN, U.S.A.). Then the amounts of the immobilized cupric ions can be calculated.

2.5. Adsorption experiments

2.5.1. Single amino acid adsorption

The chitosan gel beads (about 15 mg in dried weight) were applied to 5 ml of the amino acid solution (initial concentration 200 mg/l) with a given pH adjusted with NaOH or HCl solution (1 mol/l) at 25 °C, and the concentration of the amino acid solutions at different time intervals was determined by a UV–VIS spectrophotometer (Model 752, shanghai spectrophotometer instrument Co., Ltd., china) at the wavelength of 280 nm for Trp and 570 nm for His (Ninhydin reactions), respectively. The adsorption capacities of the amino acids were calculated from the initial and final concentrations.

2.5.2. Two kinds of amino acids co-adsorption

The chitosan gel beads (15 mg in dried weight) were added to a 5 ml of the mixed amino acids solution containing Trp and