Preparation and Characterization of Chitosan/Cu(II) Affinity Membrane for Urea Adsorption

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ABSTRACT: We used silica particles as a porogen to prepare macroporous chitosan membranes and subsequently prepared macroporous chitosan/Cu(II) affinity membranes for urea adsorption. The morphology, porosity, Cu(II) adsorption capacity, and swelling ratio of the macroporous membrane were measured. SEM photographs show the pores in the membrane dispersed uniformly, a feature that didn't change much after the adsorption of Cu(II). The porosity of the membrane had a maximum value when the silica/chitosan ratio was about 12. The Cu(II) adsorption capacity in the membrane leveled off when the initial concentration of $CuSO_4$ solution exceeded 5×10^{-2} mol/L. The macroporous chitosan/Cu(II) affinity membrane was successfully used for urea adsorption. The maximum urea adsorption capacity was 78.8 mg/g membrane, which indicates that the membrane has a great potential for hemodialysis for urea removal. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 1108–1112, 2003

Key words: biopolymers; metal-polymer complex; macroporous polymer; adsorption; morphology

INTRODUCTION

Uremia is a fatal human disease caused by the failure of the kidneys to clean toxins out of the body. Although kidney transplant is the first choice to cure uremia, hemodialysis and hemoperfusion are still common methods of treating this disease because of the lack of kidney donors and the expense of transplant. Among the toxins that need to be removed in blood purification, urea is a major component. Several materials have been reported to be urea absorbents, including activated carbon,[1] polyethylenepolyamine/Cu(II) complex,[2] tolylene diisocyanate (TDI) crosslinked β-cyclodextrin,[3] and others. However, because of the low adsorption capacity and poor biocompatibility of these materials they still cannot be used practically.

Chitosan [poly(2-amino-2-deoxy-D-glucose)] is the deacetylated form of chitin, which is the second most abundant biopolymer in the world after cellulose. Both chitin and chitosan are widely used in many fields, such as the food industry, the textile industry, papermaking, agriculture, and cosmetics.[4] Moreover, in the past few decades, chitosan has been used in biomedical and biotechnical research as a membrane material (e.g., for the separation of biomacromolecules) due to its hydrophilicity, nontoxicity, biological compatibility, biodegradability and easy membrane-forming nature.[5] Compared to particle absorbents, a membrane is more suitable for hemodialysis and hemoperfusion because of its unique advantages in the absorption and separation processes, such as its large surface, short diffusion path, and high productivity.[6] Based on the reasons mentioned above, our article focuses on the development of affinity membranes using natural polymers (mainly chitosan) for the absorption of urea. In our previous work, we successfully prepared a chitosan-silk fibroin/Cu(II) complex membrane,[2] but the urea adsorption capacity was still not satisfactory. In this article, we report the preparation of a macroporous chitosan/Cu(II) affinity membrane and its application to urea adsorption.

EXPERIMENTAL

Materials

Chitosan (deacetylation degree of about 65%) was obtained from Dalian Chitin Co., Ltd. (Liaoning, China). Silica particle was purchased from Shanghai Wusi Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents were analytical reagent grade and used without further purification.

Chitosan was further deacetylated in 50 wt % NaOH solution at a ratio of 0.1 kg/L in a stainless steel kettle at 373 K for 5 h under N_2 atmosphere. The resulting chitosan was washed to neutral and dried for further use. The degree of N-deacetylation and the molecular weight of the chitosan were determined by titration and viscometry methods, respectively, as described in our previous work.[4] The final deacetylation

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degree was 95% and the molecular weight was about 300 kDa.

Preparation and characterization of macroporous chitosan membranes

The macroporous chitosan membranes were prepared according to the method described by Zeng and Ruckenstein.[7] Two grams of chitosan were dissolved in 100 mL of 2 vol % aqueous acetic acid. After filtration of the chitosan solution, the silica particles were added, followed by vigorous stirring for 3 h at room temperature in order to disperse them uniformly. Then the solution was poured into a PET box and allowed to dry in a fume hood overnight at approximately 25°C and 50% relative humidity. After the membranes dried, they were immersed in a 5 wt % aqueous NaOH solution and kept for 2 h at 80°C to dissolve the silica and to generate macroporous chitosan membranes. Finally, the macroporous membranes were washed with distilled water to remove the remaining NaOH, dried and stored in a desiccator for further use.

In order to calculate the porosity of the macroporous chitosan membrane, a corresponding dense chitosan membrane was also prepared. After filtration, 2 wt % chitosan aqueous acetic solution was poured into a PET box and allowed to dry under the same conditions used for the preparation of the macroporous membrane.

The morphology of the macroporous chitosan membrane was observed by a Philip XL30 scanning electron microscope (SEM) at 20 kV. Both the surface and a cross section (prepared by fracturing the membrane under liquid nitrogen) were scanned after coating with a thin layer of gold.

The porosity of the macroporous chitosan membrane was calculated as follows:

$$\text{Porosity (\%)} = (1 - \rho_1/\rho_2) \times 100\% \quad (1)$$

where ρ_1 is the density of the macroporous chitosan membrane, and ρ_2 is the density of the corresponding dense chitosan membrane.

Preparation and characterization of macroporous chitosan/Cu(II) affinity membranes

The macroporous chitosan membranes were cut into small pieces (1.6 cm × 5.0 cm) and kept in $CuSO_4$ solutions with different initial concentrations and stirred for 12 h at room temperature. After rinsing with distilled water and drying, macroporous chitosan/Cu(II) affinity membranes were obtained.

The morphology of the affinity membrane was observed by SEM using the same method described above.

The Cu(II) adsorption capacity and the swelling ratio to water of the membranes were obtained as follows:

$$\text{Adsorption Capacity} = (W_1 - W_0)/W_0 \times 100\% \quad (2)$$

$$\text{Swelling Ratio} = (W_2 - W_1)/W_1 \times 100\% \quad (3)$$

where W_0 is the weight of the original macroporous chitosan membrane, W_1 is the weight of the membrane after adsorption of Cu(II) (i.e., dry chitosan/Cu(II) affinity membrane) and W_2 is the weight of the chitosan/Cu(II) affinity membrane after being swollen in water.

Adsorption of urea onto macroporous chitosan/Cu(II) affinity membranes

The macroporous chitosan/Cu(II) affinity membranes were added to the urea solution at a concentration of 1300 mg/L in phosphate buffer (pH = 7.0) and stirred for 12 h at room temperature. The ratio of the weight of the membrane to the volume of urea solution was fixed (~ 1.2 mg/mL). The adsorption capacity of urea was calculated with the following equation:

$$\text{Adsorption Capacity} = (C_0 - C_1) \times V/W \quad (4)$$

where C_0 and C_1 are the initial and final concentrations of urea solution respectively (determined according to the method described in the literature[3]), V is the volume of urea solution, and W is the weight of the dry chitosan/Cu(II) affinity membrane, respectively.

RESULTS AND DISCUSSION

Macroporous chitosan membranes

Generally, the porogens employed to generate porous membranes via the phase-inversion method are liquid compounds, such as acetone, dimethyl formamide, dimethyl sulfoxide, benzene, etc.[7] For instance, there are reports about preparing chitosan microporous membranes using water[8] or poly(ethylene glycol)[9] as the porogen. Unlike in microfiltration or ultrafiltration, the pore size of the membrane is not the key point in affinity separation; rather it is the affinity of the membrane for the substance to be separated. Therefore, a large pore size in affinity membranes can increase the fluid rate and enhance the productivity.

Silica particle has been reported as an ideal porogen to generate macroporous chitosan membranes with controlled porosity and good mechanical properties.[7] Figure 1 shows the SEM photographs of a macroporous chitosan membrane prepared using silica particle as the porogen. The pictures indicate that the pores are well distributed and continuous within the membranes,

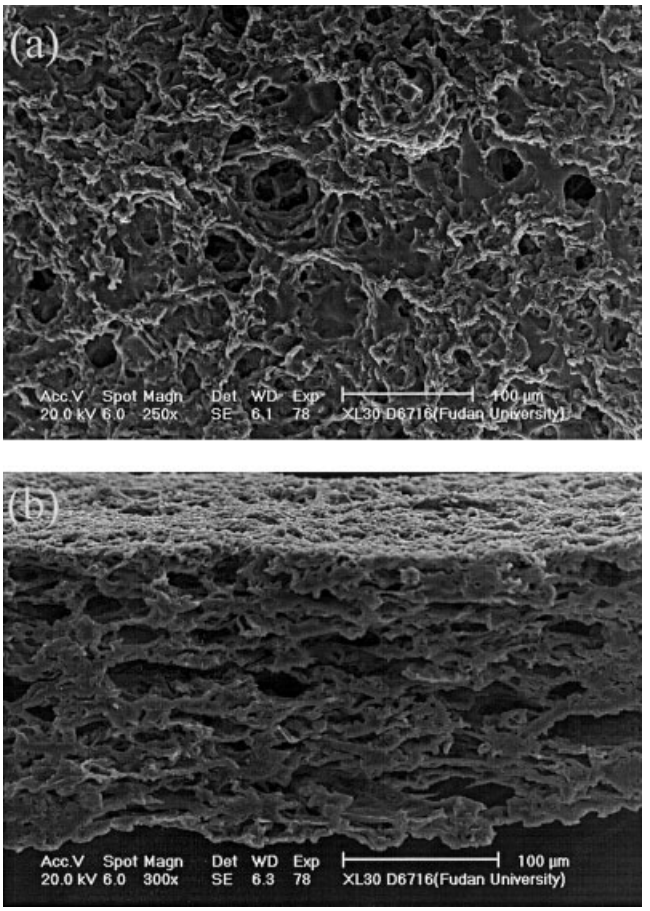

Figure 1 SEM photographs of macroporous chitosan membrane: (a) surface, (b) cross section (silica/chitosan = 10/1).

which meets the requirements for affinity separation. From the SEM pictures, we calculated the average pore size of the macroporous membrane to be 25–35 μm using an approach similar to one described in the literature.[10] We also compared the morphology of the membranes prepared under different conditions (room temperature for about 2 days, and room temperature in a fume hood overnight and an oven at 80°C for 8 h) in order to investigate the influence of dry time on morphology. The results indicate that the pore distribution of the membrane does not change much, which is similar to the findings in the literature.[7]

The more porosity in the membrane, the more surface for affinity adsorption, so we tried to vary the ratio of silica particle to chitosan in membrane preparation to obtain a membrane with maximum porosity. As shown in Figure 2, at the beginning an increase in the silica/chitosan ratio increased the porosity of the membrane. The porosity reached a maximum when the silica/chitosan ratio was around 12. To our surprise, the porosity decreased when the silica/chitosan ratio exceeded 12. After studying SEM pictures (Fig. 3), we concluded that, with the increase of the silica/chitosan ratio, the walls of the pores became increasingly thin. Because the walls eventually fail to

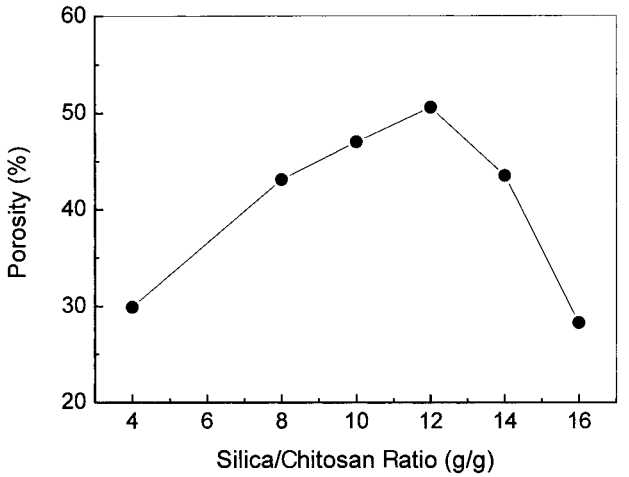

Figure 2 Influence of silica/chitosan ratio on the porosity of macroporous chitosan membranes.

hold the weight of the matrix, they collapse, resulting in low porosity. Taking into account the easiness of preparation and the relative high porosity, we chose a silica/chitosan ratio of 10 for macroporous chitosan membrane preparation for further use.

Macroporous chitosan/Cu(II) affinity membranes

Chitosan is a well-known adsorbent for metal ions, such as Cu(II), Ni(II), Cr(II), Zn(II) and Hg(II), because the amine group (—NH_2) and the hydroxyl group (—OH) can serve as coordinate sites for metals.[11–14] There are many articles in the literature on chitosan/Cu(II) complexes,[15–17] but few of them are about chitosan/Cu(II) complex membranes. In our previous work, we reported the preparation of chitosan/Cu(II) complex membranes[2,18] as well as the influence of membrane structure on Cu(II) adsorption capacity;[19] however, these articles were base on dense membranes. In this study, we use a macroporous chitosan

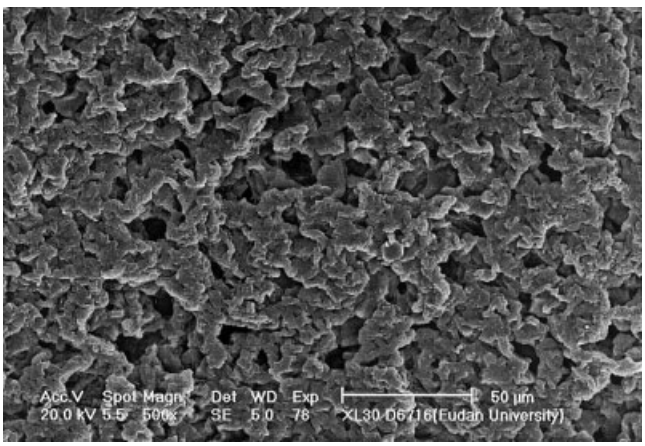

Figure 3 SEM photograph of macroporous chitosan membrane (surface, silica/chitosan = 16/1).

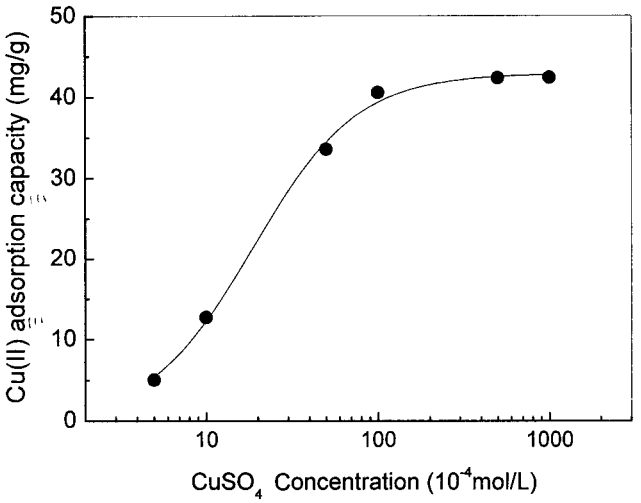

Figure 4 Influence of initial $CuSO_4$ concentration on Cu(II) adsorption capacity of macroporous chitosan membrane.

membrane as the matrix to prepare a novel macroporous chitosan/Cu(II) affinity (complex) membrane.

Figure 4 represents the effect of loading the concentration of $CuSO_4$ on the Cu(II) adsorption capacity of the macroporous chitosan membrane. With the increase of loading concentration, the amount of Cu(II) adsorbed on to the membrane increased. When the loading concentration was higher than 5×10^{-2} mol/L, the adsorption capacity of the Cu(II) leveled off, which meant the adsorption equilibrium had been reached. The morphology of the macroporous chitosan membrane did not change much after the adsorption of Cu(II) (Fig. 5).

As our chitosan/Cu(II) affinity membrane is supposed to be used in hemodialysis to remove urea, we need to consider its hydrophilicity. We know that hydrophilicity is favored in biotechnology and medical utilization; however, if the hydrophilicity is too high, excess swelling of the membrane will cause many problems, such as the leakage of the membrane into the device during the process. Chitosan is a high hydrophilic material because it has plenty of amine groups and hydroxyl groups in its structure. Its swelling ratio to water was beyond 400%, as shown in Figure 6. After the adsorption of Cu(II), the swelling ratio declined to 100–200%, which would be more suitable for its application. The reason for the decrease of the swelling ratio was probably Cu(II) acting as a crosslink agent to crosslink chitosan.[20] Thus we assumed that when the chitosan membrane was immersed in $CuSO_4$ solution, the hydrophilic groups in chitosan (mainly $—NH_2$ groups) were coordinated with Cu(II) ions to form a "crosslinked" structure. The more Cu(II)ions coordinated the hydrophilic groups in chitosan, the higher the crosslinking degree of the membrane, and the lower swelling ratio.

In order to support our assumption, we need to discuss the Cu(II)-chitosan complexation mechanism,

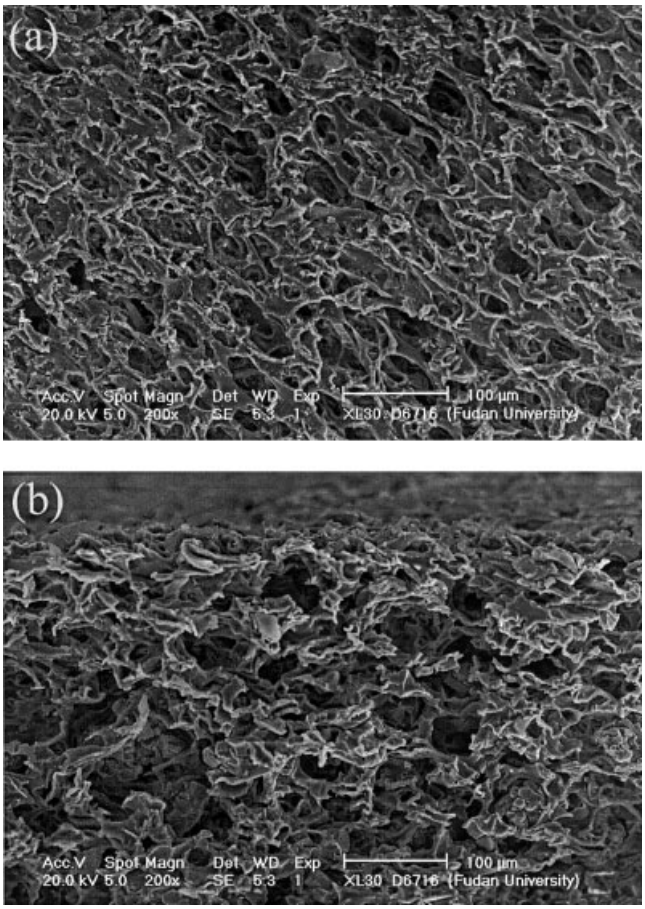

Figure 5 SEM photographs of macroporous chitosan/Cu(II) affinity membrane: (a) surface, (b) cross section (silica/chitosan = 10/1, initial $CuSO_4$ concentration = 10^{-2} mol/L).

although the main purpose of this article is not fundamental study. There have been many studies on the mechanism of the complexation, and different models

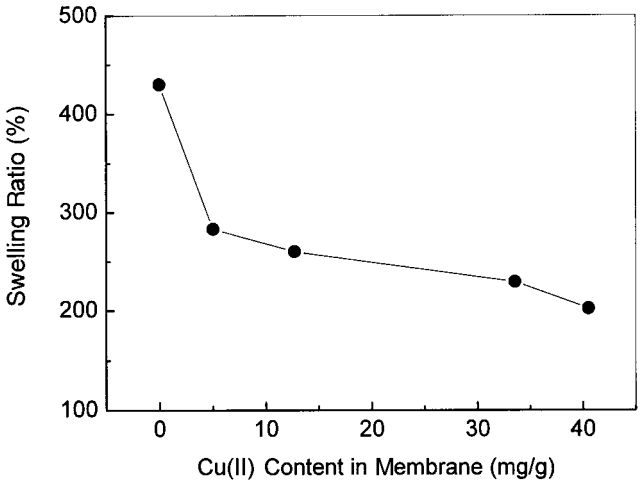

Figure 6 Influence of Cu(II) content in membrane on swelling ratio of macroporous chitosan/Cu(II) affinity membrane.

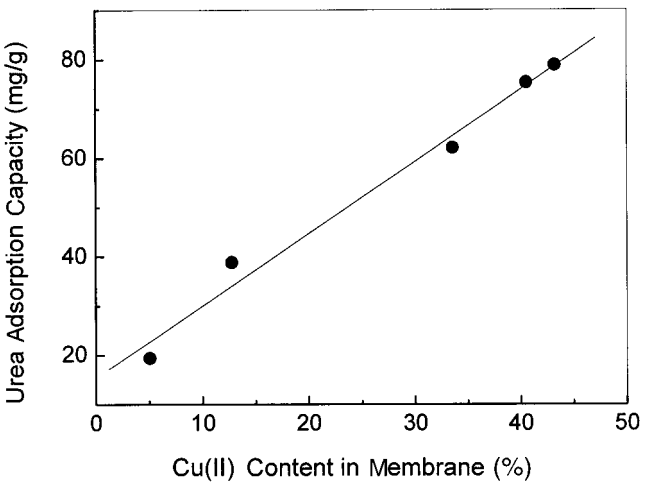

Figure 7 Influence of Cu(II) content in membrane on urea adsorption capacity of macroporous chitosan/Cu(II) affinity membrane.

have been proposed; however, they seem to disagree with each other (for detail, see ref. 21 and the references therein). The newest model proposed by Rhazi et al. suggests that Cu(II) coordinates with two —NH_2 groups in chitosan to form a $\{[Cu(—NH_2)_2]^{2+}, 2OH^-\}$ complex under neutral conditions.[21] As we know, chitosan is a semi-rigid polymer, so two —NH_2 groups coordinated with Cu(II) ions were unlikely to be in the same chitosan chain because of steric hindrance; therefore Cu(II) could act as a crosslinking agent to crosslink two chitosan chains.

Adsorption of urea onto macroporous chitosan/Cu(II) affinity membrane

One of the applications of the chitosan/Cu(II) complex is initial polymerization,[18,22] while another potential use is absorbtion of urea.[2] It is generally accepted that urea can coordinate with Cu(II) with its oxygen atom,[23] so it is practical to use the chitosan/Cu(II) complex membrane as an affinity membrane to absorb urea. According to the model discussed above,[21] only two coordinate sites on Cu(II) are occupied by —NH_2 groups in chitosan, so there are two other coordinate sites that can be used to coordinate urea. It is impossible for Cu(II) to link all four of its coordinate sites with —NH_2 groups in chitosan because of steric hindrance.

Figure 7 demonstrates urea adsorption onto the macroporous chitosan/Cu(II) affinity membrane. As presented in the figure, urea adsorption capacity increases linearly with the increase of Cu(II) content in the membrane. This means that the urea adsorption by the macroporous chitosan/Cu(II) affinity membrane simply depends on the Cu(II) content in the membrane. As noted in our previous report,[2] the urea adsorption capacity of dense chitosan/Cu(II) complex membrane is $\sim$ 14 mg/g membrane, while blending with 20 % silk fibroin, increases the capacity to $\sim$ 20 mg/g membrane. Here, because of the large surface area of the macroporous chitosan membrane that absorbs more Cu(II), the urea adsorption capacity increases significantly. The maximum urea adsorption capacity of this macroporous chitosan/Cu(II) affinity membrane is 78.8 mg/g membrane, which is higher than the capacities of other kinds of urea absorbents, such as activated carbon (9.0 mg/g),[1] polyethylenepolyamine/Cu(II) complex (75.2 mg/g)[2] and TDI crosslinked β-cyclodextrin (50.6 mg/g).[3] Considering its high urea adsorption capacity and advantages of membrane material, the macroporous chitosan/Cu(II) affinity membrane has strong implications for use in urea adsorption in the treatment of uremia by hemodialysis.

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