Macroporous Chitosan/Carboxymethylcellulose Blend Membranes and Their Application for Lysozyme Adsorption

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ABSTRACT: The adsorption of lysozyme was investigated with novel macroporous chitosan (CS)/carboxymethylcellulose (CMC) blend membranes. The CS/CMG blend membranes were prepared by a simple solution-blending method with glutaraldehyde as a crosslinking agent for CS and with silica particles as porogens. The CS/CMC blend membranes were insoluble in aqueous media when the CMC concentration in the membranes did not exceed 30 mol %. The protein adsorption on these membranes from aqueous solutions containing different amounts of lysozyme at different pHs was investigated in batch systems. The results showed that the lysozyme adsorption capacity had a maximum at pH 9.2, and this indicated that the CS/CMC blend membranes could act as cation-exchange membranes. Moreover, the blend membranes showed the best adsorption properties for lysozyme when the CMC concentration was 20 mol %. In addition, the lysozyme adsorption capacity of the blend membranes increased with an increase in the initial lysozyme concentration and the adsorption temperature. The maximum adsorption capacity of the macroporous CS/CMC blend membranes was as high as 240 mg/g (170 mg/mL), and more than 95% of the adsorbed lysozyme was desorbed in a pH buffer at 11.8. The blend membranes also demonstrated good reusability after several adsorption–desorption cycles. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 1267–1274, 2005

Key words: biopolymers; chromatography; ion exchangers; polyelectrolytes; proteins

INTRODUCTION

Ion-exchange membranes are among the major types of membrane materials for protein separation.[1] Ion-exchange separations take advantage of electrostatic interactions between the surface charges of biomacromolecules (proteins, polypeptides, nucleic acids, or amino acids) and the charged groups on membranes.[2] According to the nature of ion-exchange separation (i.e., separation can be performed only through a change in the pH of the buffer), many ion-exchange membranes have been studied and reported.[1–6] Normally, various amino groups[7–10] are used in anion-exchange membranes, whereas sulfonic[10] and carboxyl groups[11] are used in cation-exchange membranes. In the literature, most ion-exchange membranes are made of synthetic polymers; only a few are from natural polymers.

Usually, natural polymers have several unique advantages over synthetic polymers, such as nontoxicity, good biological compatibility, and high hydrophilicity, which are important for biomacromolecular separation. However, the poor mechanical properties and poor solvent resistance of natural polymers prevent them from being widely applied in the field of separation. Among natural polymers, only chitosan (CS), cellulose, and alginate can be found in the literature as ion-exchange membrane materials. CS can be used as an anion-exchange membrane directly.[7] Cellulose derivatives, such as cellulose phosphate[12] and cellulose acetate,[13] and alginate[11,14] are used as cation-exchange membranes.

Both CS and cellulose are polysaccharides, so the structures of these two natural polymers are very similar. The only difference between CS and cellulose is that the amino group in CS is substituted with the hydroxyl group in cellulose. Carboxymethylcellulose (CMC) is one kind of cellulose derivative formed by the carboxymethylation of the hydroxyl group in cellulose. Although CMC can act as an ion-exchange material for carboxyl groups,[15,16] few reports can be found about its application for ion-exchange membranes because of its water-soluble nature. In this research, we modified CMC by forming a polyelectro-

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lyte complex with CS to prepare a novel macroporous CS/CMC blend membrane, and we successfully used this material as a cation-exchange membrane to absorb lysozyme.

EXPERIMENTAL

Materials

CS (molecular weight = 300,000, deacetylation degree = 90%) was purchased from Jinan Haidebei Marine Biological Product Co., Ltd. (Jinan, China). Silica particles were purchased from Wusi Chemical Reagent Co., Ltd. (Shanghai, China). Sodium carboxymethylcellulose, lysozyme, and all other chemical reagents (analytical-reagent-grade) were purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China), and were used without further purification.

Preparation and characterization of the macroporous CS/CMC blend membranes

Two grams of CS was dissolved in 100 mL of a 2 vol % aqueous acetic acid solution, and 2 g of sodium carboxymethylcellulose was dissolved in 100 mL of deionized water. Both CS and sodium carboxymethylcellulose solutions were filtered to remove trace precipitates before they were mixed. The sodium carboxymethylcellulose solution was added dropwise to the CS solution at 60°C under stirring. After the complete mixing of the CS and sodium carboxymethylcellulose solutions, silica particles and a glutaraldehyde solution (concentration = 1×10^{-5} mol/L) were added, and this was followed by vigorous stirring for uniform dispersion. The weight ratio of the silica particles to the polymer (the weights of both CS and sodium carboxymethylcellulose) was set to 10:1 according to our previous study,[17] and the amount of glutaraldehyde was calculated from the final crosslinking degree of CS, which was 1 mol %. After 3 h of stirring, the solution was poured into a poly(ethylene terephthalate) box and allowed to dry in a fume hood overnight at approximately 25°C and 50% relative humidity. When the membranes dried, they were immersed in a 5 wt % aqueous NaOH solution and kept there for 2 h at 80°C to dissolve the silica and generate the macroporous CS/CMC blend membranes. Finally, the macroporous membranes were washed with distilled water to remove the remaining NaOH, and they were stored in deionized water for further use.

The morphology of the macroporous CS/CMC blend membranes was observed with a Philips XL30 scanning electron microscope (Philips Analytical B.V., Almelo, the Netherlands) at 20 kV. Both the surface and cross section (prepared by the fracturing of the membrane under liquid nitrogen) were scanned after they were coated with a thin layer of gold.

Lysozyme adsorption on the CS/CMC blend membranes

The adsorption of lysozyme from the aqueous medium on the macroporous CS/CMC blend membranes ([CMC] = 0–30 wt %) was studied at various pHs in an acetate buffer (0.2*M*, pH 4.0 or 5.4), a phosphate buffer (0.2*M*, pH 7.0 and 8.0), a sodium borate buffer (0.1*M*, pH 9.2), or a Britton–Robinson buffer (a mixture of 0.04*M* phosphoric acid, acetic acid, and boric acid plus 0.2*M* sodium hydroxide, pH 10.0, 11.0, or 11.8). To compare the adsorption properties of the membranes, we set the volume of the adsorption medium with respect to the weight of the CS/CMC blend membranes to a constant of 290; this meant a large piece of membrane (ca. 5.0 cm × 1.5 cm) immersed in 20 mL of the lysozyme solution. The adsorption experiments were conducted in an oscillator with a water bath (model SHZ-B, Shanghai Yuejin Medical Instrument Co., Ltd., Shanghai, China). The time to adsorption equilibrium was found to be 5–6 h, and so for all the adsorption measurements reported here, a 12 h adsorption duration was employed. The initial lysozyme concentration was 0–0.9 mg/mL, and the adsorption temperature was varied from 15 to 30°C.

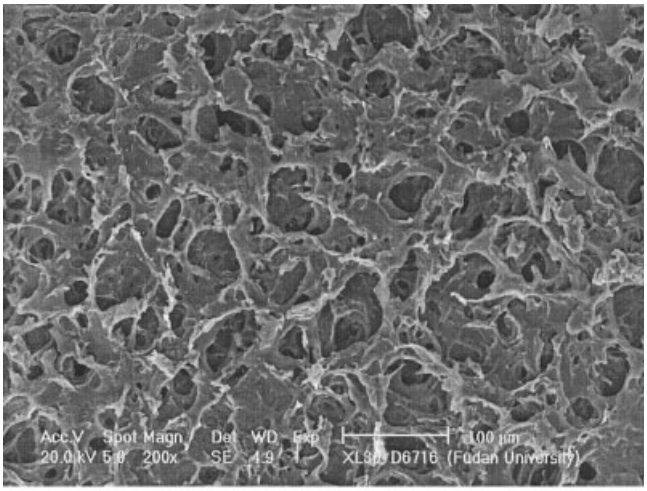
The amounts of the adsorbed lysozyme on the macroporous CS/CMC blend membranes were determined from the initial and final concentrations of the lysozyme within the adsorption medium. The lysozyme solution was measured at 280 nm with a Lambda 35 ultraviolet–visible spectrophotometer (Jobin Yvon S.A.S., Longjumeau, France). The adsorption capacity of the lysozyme was calculated with the following expression:

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

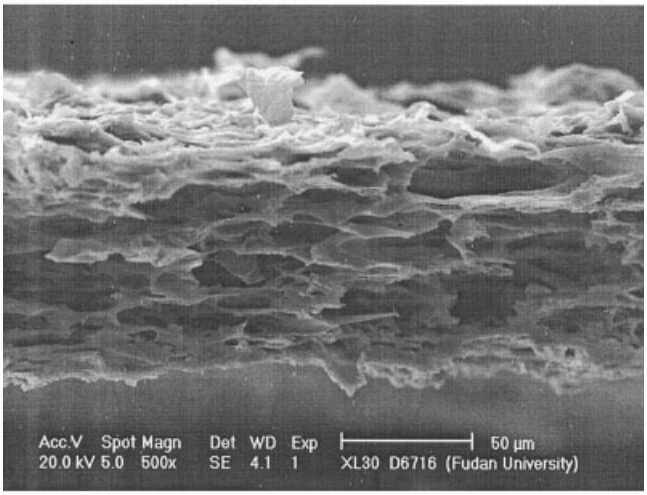
where C_0 and C_1 are the initial and final concentrations of the lysozyme solution, respectively; V is the volume of the lysozyme solution; and W is the weight of the dry CS/CMC blend membrane. All the adsorption data were averages of at least three experiments.

Stability of the CS/CMC blend membranes after repeated use

To determine the reusability of the CS/CMC blend membrane, we repeated the adsorption–desorption cycle three times with the same blend membrane. The desorption experiments were performed in a buffer solution at pH 11.8. The lysozyme-adsorbed (at pH 9.2) blend membrane was placed in the desorption medium for 12 h with oscillation at 25°C. The final



(a)



(b)

Figure 1 SEM photographs of a macroporous CS/CMC blend membrane: (a) the surface and (b) the cross section ([CMC] = 20 mol %).

lysozyme concentration within the desorption medium was determined as previously described.

RESULTS AND DISCUSSION

Characteristics of the CS/CMC blend membranes

CMC is a cellulose derivative that contains a carboxyl group on each polymer unit. This characteristic provides it with a cation-exchange capacity because —COOH becomes —COO^- when the pH value is higher than 7. However, CMC is soluble in both neutral and base media, so it cannot be used as a cation-exchange membrane in its native state. To make it insoluble, CS is chosen to form a polyelectrolyte complex with CMC, as described in the literature.[18,19] In this study, we also used glutaraldehyde to crosslink the CS component in the blend membranes to form a semi-interpenetrating network[20] for further enhancement of the membrane stability. The CS/CMC blend membranes that we prepared were very stable and maintained their strength in aqueous media. On the other hand, silica particles were used as porogens because several reports have shown that this method can produce porous membranes with high porosity and large pore size.[7,17] Figure 1 shows the scanning electron microscopy (SEM) photographs of the surface and cross section morphology of the macroporous CS/CMC blend membranes. The pictures clearly indicate that the pores were well distributed and continuous within the membranes. This would meet the requirements of protein separation in future investigations because high permeability could be achieved.[7] Some membrane characteristics are summarized in Table I.

Effect of the adsorption time

The adsorption equilibrium time of the lysozyme molecules on the CS/CMC blend membranes were investigated, and the results are presented in Figure 2. The adsorption capacity gradually increased with the adsorption time until adsorption equilibrium was reached at about 5 h. To ensure that the adsorption data were all from an equilibrium state, we measured the lysozyme adsorption capacities under different conditions after 12 h of adsorption.

Effect of the pH on the lysozyme adsorption

Electrostatic interactions are important for protein adsorption on ion-exchange membranes. Figure 3 shows the influence of the pH on the lysozyme adsorption on the CS/CMC blend membranes. The maximum lysozyme adsorption was obtained at pH 9.2. Significantly lower lysozyme adsorption was observed when the pH was lower than 7 or higher than 11. These results indicated that the pH of the medium had an important effect on the adsorption capacity of the lysozyme and that there was a preferential interaction between the lysozyme and the CS/CMC blend membranes around pH 9.2.

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 isoelectric point (pI) of the lysozyme is 11, the ly-

TABLE I
Characteristics of the Macroporous CS/CMC Blend Membranes

Thickness	100 μm
Average pore size	30 μm
Porosity	47%
Density	0.708 g/mL

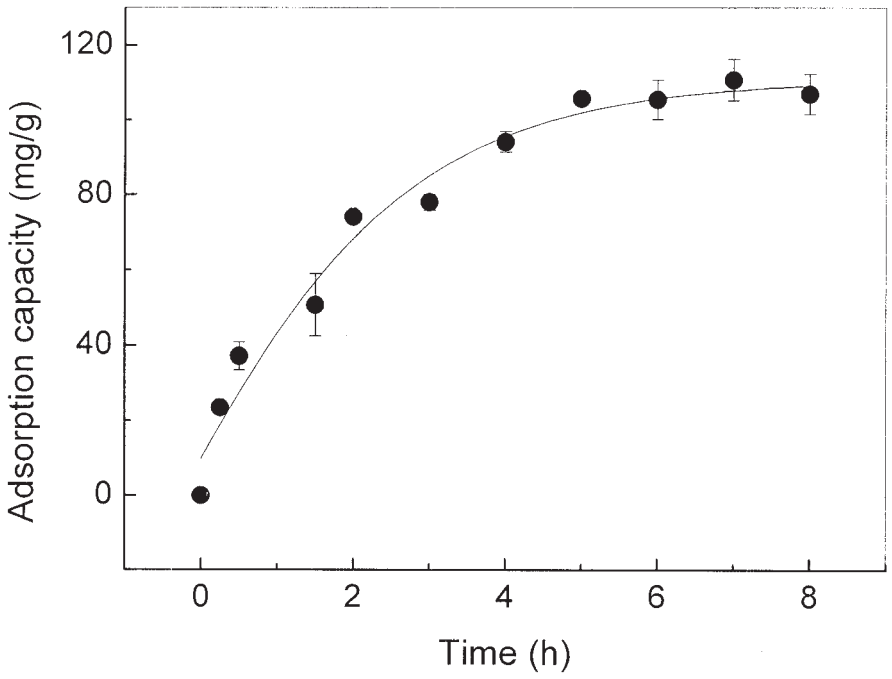


Figure 2 Adsorption time of the lysozyme for the CS/CMC blend membranes ([CMC] = 20 mol %, initial lysozyme concentration = 0.5 mg/mL, pH = 8.0, temperature = 30°C).

sozyme is cationic at a pH lower than 11, but it is anionic when the pH is greater than 11.[21,22] On the other hand, the CS/CMC membranes were positively charged in an acidic medium and negatively charged in a basic medium because they had a polyelectrolyte complex. Therefore, we examined the pH influence of the lysozyme adsorption on the CS/CMC blend membranes in three different pH ranges. First, when the pH was lower than 7, the CS/CMC blend membranes were positively charged because of the —NH_3^+ groups

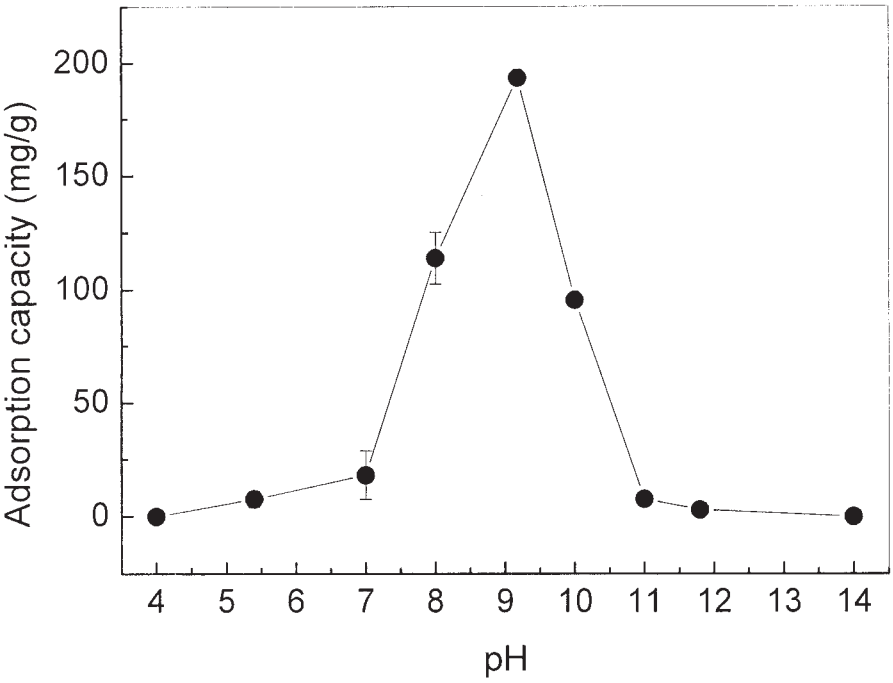


Figure 3 Influence of the pH on the lysozyme adsorption on the CS/CMC blend membranes ([CMC] = 20 mol %, initial lysozyme concentration = 0.5 mg/mL, temperature = 30°C).

TABLE II
Effect of the CMC Content on the Adsorption of the Lysozyme[a]

CMC (mol %)	Lysozyme adsorption capacity (mg/g)
0	11.7 ± 1.4
10	60.9 ± 1.2
20	91.0 ± 5.5
30	66.4 ± 1.7

[a] Initial lysozyme concentration = 0.5 mg/mL, pH = 9.2, temperature = 15°C).

in the CS macromolecular chains. In the meantime, the lysozyme was also positively charged for pH values lower than its pI. As a result, little lysozyme was adsorbed onto the CS/CMC blend membranes because of the electrostatic repulsion between the lysozyme and the membranes. Second, when the pH was higher than 7 but lower than 11, the CS/CMC blend membranes were negatively charged because of the —COO^- groups in the CMC macromolecular chains, and the lysozyme was still positively charged (pH < pI). Therefore, the lysozyme could be adsorbed onto the CS/CMC blend membranes because the lysozyme and the membranes had opposite charges. Figure 3 clearly shows that the lysozyme adsorption increased significantly in this region and reached a maximum around pH 9.2. Lastly, when the pH value was greater than 11, both the CS/CMC membranes and lysozyme were negatively charged, and there was almost no lysozyme adsorption on the membranes, like that at pH < 7.0.

Effect of the CMC content in the membranes

As previously discussed, CMC was the effective component in the CS/CMC blend membranes that adsorbed lysozyme between pH 7 and 11. Ideally, the more CMC was in the blend membranes, the more lysozyme was adsorbed. However, the results indicated that the lysozyme adsorption showed a maximum when the CMC concentration was 20 mol % (Table II). The lysozyme adsorption capacity of the membranes containing 30 mol % CMC was lower than that of the membranes containing 20 mol %. This phenomenon may have been due to the high carboxymethyl group density on the surface of the membranes, which caused large steric hindrance when the CMC content increased. As a result, the lysozyme molecules were hindered from being close to the membranes, and the adsorption capacity decreased. Similar results can be found in the literature.[23] Moreover, the stability and mechanical properties of the CS/CMC blend membranes became worse when the CMC content exceeded 30 mol %.

The pure macroporous CS membrane also adsorbed some lysozyme molecules, but much less than the CS/CMC blend membrane did. Complete deacetylation from chitin is very difficult, so there are always some acetyl groups remaining in CS (the deacetylation degree of the CS sample that we used was 90%). Therefore, these *N*-acetyl-D-glucosamine units could also bind some lysozyme molecules, as reported in the literature.[24]

Effect of the initial lysozyme concentration

The lysozyme adsorption isotherm of the CS/CMC blend membranes is presented in Figure 4. An increase in the lysozyme concentration in the adsorption medium led to an increase in the amount of adsorbed lysozyme on the membranes. The relationship between the initial lysozyme concentration and the lysozyme adsorption capacity on the membranes was linear when the initial lysozyme concentration was no more than 0.6 mg/mL. The slope of the line (~360 mL/g) was quite close to the ratio of the adsorption medium volume to the weight of the dry membrane (~390 mL/g) that we set for the adsorption experiment. This meant that almost all the lysozyme in the adsorption medium was adsorbed onto the membranes when the initial lysozyme concentration was 0–0.6 mg/mL. However, when the initial concentration continuously increased to 0.8 mg/mL, although the lysozyme adsorption capacity still increased, it was on the low side of the line. The lysozyme adsorption began to level off with a further increase in the initial lysozyme concentration, and this could be explained by the saturation of the ion-exchange group (—COO^-) in the membrane with the adsorbed lysozyme molecules, as a result of which the maximum adsorption capacity (240.0 mg/g) was achieved.[21,22]

Effect of the adsorption temperature

Figure 5 shows the relationship between the adsorption temperature and the lysozyme adsorption capacity on the CS/CMC blend membranes. From 15 to 30°C, the adsorption capacity increased almost 2.4 times. This indicated that the increase in the temperature was favorable to lysozyme adsorption on the CS/CMC blend membranes. Moreover, because the natural logarithm of the adsorption capacity (ln Q_a) was linearly related to the reciprocal of the absolute temperature (T^{-1}), we could calculate the apparent adsorption activation energy (E_a) of the lysozyme adsorption on the CS/CMC blend membranes with an Arrhenius equation ($Q_a = Q_0 \exp(-E_a/RT)$, where T is the temperature, Q_a is the adsorption capacity, and Q_0 is a constant, R is the universal gas constant). It was 38.69 kJ/mol.

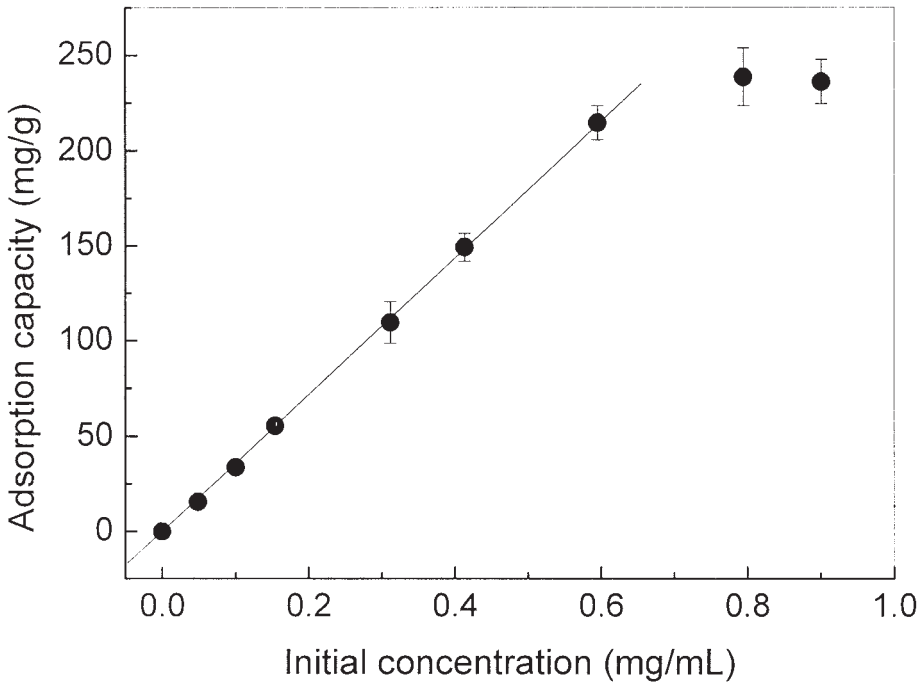


Figure 4 Influence of the initial concentration on the lysozyme adsorption on the CS/CMC blend membranes ([CMC] = 20 mol %, pH = 9.2, temperature = 30°C).

Desorption and reusability of the CS/CMC blend membranes

The desorption of the adsorbed lysozyme from the CS/CMC blend membranes was performed in a pH 11.8 buffer. At this pH, almost no lysozyme was absorbed onto the membrane, as mentioned previously, and the membrane was very stable because the main component of the membrane, CS, did not swell in the basic medium. The CS/CMC blend membranes were first placed in an adsorption medium (pH 9.2) that

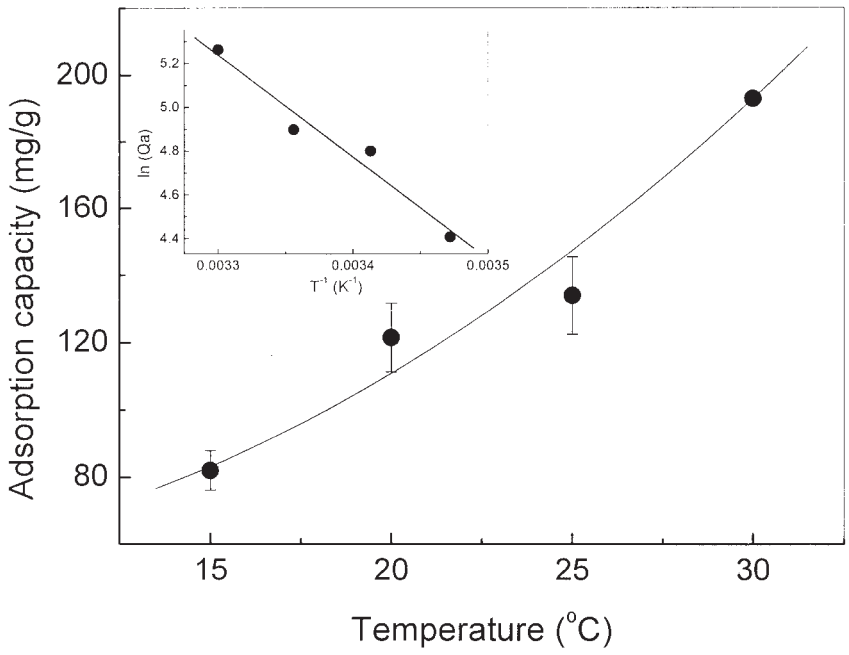


Figure 5 Influence of the adsorption temperature on the lysozyme adsorption on the CS/CMC blend membranes ([CMC] = 20 mol %, initial lysozyme concentration = 0.5 mg/mL, pH = 9.2).

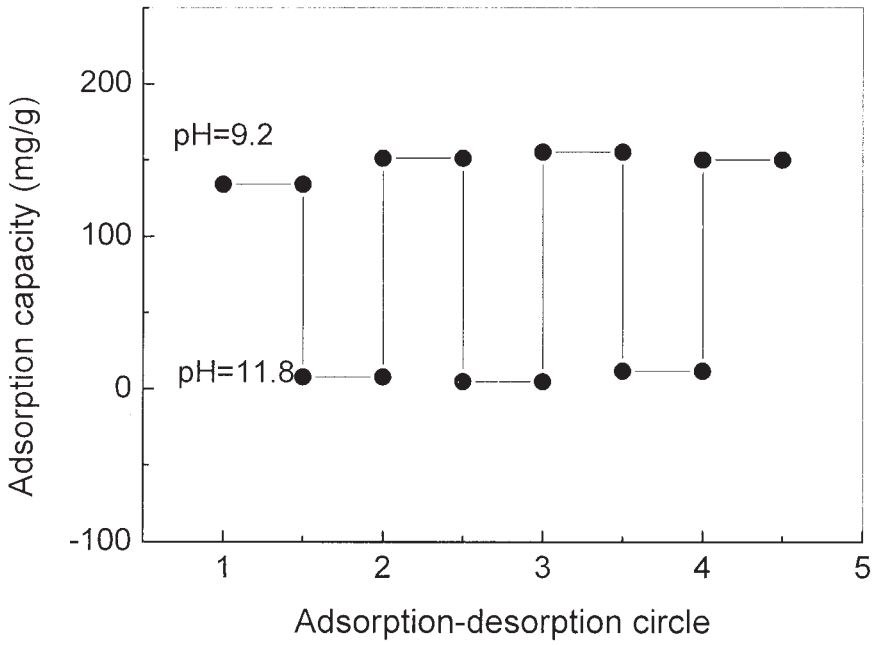


Figure 6 Adsorption–desorption circles of the lysozyme on the CS/CMC blend membranes ([CMC] = 20 mol %, initial lysozyme concentration = 0.5 mg/mL, temperature = 25°C).

contained 0.5 mg/mL lysozyme. After the lysozyme was adsorbed onto the membranes, the lysozyme-loaded membranes were transferred to a desorption medium at pH 11.8, and this allowed the lysozyme to release. Both adsorption and desorption procedures were run for 12 h. The adsorption–desorption cycle was performed three times, and the results are presented in Figure 6. More than 95% of the lysozyme was released in the desorption medium, and the adsorption–desorption curves were almost identical in all three rounds. A relatively high lysozyme adsorption capacity in the second and third rounds may have been due to the swelling of the CS/CMC blend membranes, which exposed more —COO^- groups to the lysozyme. Furthermore, after the third round of the adsorption–desorption circle, the membranes were placed in the adsorption medium (0.5 mg/mL lysozyme, pH 9.2) again, and they adsorbed the same amount of lysozyme as before. Then, the lysozyme-loaded membranes were placed in a neat pH 9.2 buffer (not containing lysozyme), and the results showed that the desorption ratio was less than 3%. This indicated that the desorption of the lysozyme from the CS/CMC blend membranes was not ad-

TABLE III
Comparison of the Maximum Adsorption Capacities of the Lysozyme onto Various Adsorbents

Material	Maximum adsorption capacity	Reference
CS/CMC blend membrane	170.0 mg/mL (or 1645 $\mu g/cm^2$)	This work
Procion Green H-E4BD immobilized porous Poly(hydroxyethylmethacrylate) ion-exchange membrane	13.33 mg/mL (or 392 $\mu g/cm^2$)	21
Procion Green H-4G immobilized Poly(2-hydroxyethylmethacrylate)/chitosan interpenetrating network membrane	20.28 mg/mL (or 365 $\mu g/cm^2$)	22
Macroporous chitin affinity membrane	50 mg/mL	22
Cibacron Blue F3GA immobilized poly(2-hydroxyethylmethacrylate) membrane	135 $\mu g/cm^2$	22
Acrylate-grafted slica membranes	12.2–12.6 $\mu g/cm^2$	22
Cibacron Blue F3GA immobilized porous silica	30 $\mu g/cm^2$	22
Quartenery aminopropyl dimethlsilyl methylated silica membranes	4–25 $\mu g/cm^2$	22
Triazine dye immobilized microfiltration membrane	78–122 $\mu g/cm^2$	22
Sulfone-modified poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) microporous polymeric membrane	260 $\mu g/cm^2$	22
Procion Yellow HE-3G attached poly(vinyl alcohol) modified poly(styrene divinylbenzene)	0.02 $\mu g/cm^2$ 0.01 $\mu g/cm^2$	22 22

sorption equilibrium but was mainly a cation-exchange effect.

CONCLUSIONS

CS/CMC blend membranes were prepared by a simple solution-blending method followed by a crosslinking procedure to form a semi-interpenetrating network to prevent CMC from dissolving in an aqueous medium. When the pH value of the adsorption medium was between 7 and 11, they could be used as cation-exchange membranes for the adsorption of lysozyme. The maximum lysozyme adsorption capacity of these membranes was 240.0 mg/g (or 170.0 mg/mL based on the membrane density or 1645 $\mu g/cm^2$ based on the plain surface area[21,22]), which was considerably higher than that of some other materials published before (Table III). The adsorbed lysozyme could be desorbed more than 95% through a change in the pH of the aqueous medium. The repeated adsorption–desorption processes revealed that these novel macroporous CS/CMC blend membranes had good properties for the adsorption of a model adsorbate lysozyme and could have wide applications in processing large volumes of biological fluids containing target proteins.

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