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Protein adsorption and separation with chitosan-based amphoteric membranes

Zhicheng Feng [a], Zhengzhong Shao [a], Jinrong Yao [a], Yufang Huang [b], Xin Chen [a,*]

[a] The Key Laboratory of Molecular Engineering of Polymers of MOE, Department of Macromolecular Science, Laboratory of Advanced Materials, Fudan University, 220 Handan Road, Shanghai 200433, People's Republic of China
[b] Department of Material Science, National Microanalysis Center, Fudan University, Shanghai 200433, People's Republic of China

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ABSTRACT

A macroporous amphoteric membrane was successfully prepared by solution blending of a natural polymer chitosan (CS) and its derivative carboxymethylchitosan (CMCS). The adsorption of two model proteins (ovalbumin and lysozyme) with very different p*I* values on this CS/CMCS blend membrane was investigated in batch systems. The results showed that both proteins could be effectively adsorbed on the membrane, but the adsorption capacities were influenced by the pH, the initial protein concentration and the CMCS content in the membrane. Because of the amphoteric nature of the protein and the CS/CMCS membrane, the pH for the maximum adsorption of ovalbumin and lysozyme was different, which is the basis for the separation of these proteins from binary mixtures. As the CS/CMCS blend membrane also showed good desorption properties for those two proteins, both ovalbumin and lysozyme were successfully separated from binary mixtures by adjusting only the pH of the feed and the desorption solutions.

1. Introduction

As biotechnology develops, bioseparation techniques with adsorptive membranes have become more important due to their efficiency in overcoming mass transfer limitations [1]. Some of the major problems associated with packed bed chromatography can be solved by using macroporous membranes as chromatography media because such membranes have short and wide chromatography columns [2–4]. Furthermore, the convective flow of the solution through the pores tremendously reduces the mass transfer resistance, and binding kinetics dominates the adsorption process [4]. In recent literatures, many different adsorptive membranes have been investigated for their effectiveness in applications based on membrane shape (flat sheet, hollow fiber, spiral wound, rod, etc.) as well as interaction modes (affinity, ion-exchange, hydrophobic interaction, reversed phase, etc.) [3–17].

Since the separation of biomacromolecules based on the electrostatic interactions between the surface charges of biomacromolecules and the charged groups on the membranes is relatively simple and efficient, many ion-exchange membranes were prepared [18–20], however most of them are either anion-exchange membranes (positively charged) or cation-exchange membranes (negatively charged), and only a few of them are based on natural polymers. In other words, natural amphoteric membranes for the separation of biomacromolecules have not yet been widely studied.

The increase of public awareness of the health and environmental issues accompanied by ever-stricter environmental regulations of the waste discharge turned attention of researchers to the natural polymers from renewable resources [21–23]. Polysaccharides, such as cellulose, starch, chitin and lignin, represent a vast family of natural polymers. Many of the commercially available polysaccharides are neutral or acidic, but chitin and its primary derivative chitosan (CS) are special in that they are basic. CS has both amino and hydroxyl reactive groups with polycation characteristics and good biocompatibility. Both types of functional groups offer several possibilities for derivatization and immobilization of biologically active species [24–27]. In our previous study, we successfully prepared a chitosan/carboxymethylcellulose (CS/CMC) amphoteric macroporous membrane that had good adsorption properties for the lysozyme and ovalbumin, two model proteins of different p*I*s [28]. The membrane could be used for the separation of either lysozyme or ovalbumin from their binary mixtures.

In attempt to improve the compatibility of membrane components, we now used carboxymethylchitosan (CMCS) instead of CMC. CMCS probably shares a number of structural features with the CS from which it is derived. This should facilitate preparation of homogenous mixtures. In addition, both CS and CMCS possess amino groups and may be cross-linked by glutaraldehyde into

* Corresponding author. Tel.: +86 21 6564 2866; fax: +86 21 6564 0293.
E-mail address: chenx@fudan.edu.cn (X. Chen).

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water insoluble interpenetrating networks (IPNs). Owing to these features, a CS/CMCS blend membrane should be superior to the CS/CMC blend membrane for protein adsorption and separation.

2. Experimental section

2.1. Materials

CS flake (deacetylation degree = 72%, molecular weight = 850,000) and CS powder (deacetylation degree = 99%, molecular weight = 40,000) were purchased from Jinan Haidebei Marine Biological Product Co., Ltd. (Jinan, China). Silica particles were purchased from Wusi Chemical Reagent Co., Ltd. (Shanghai, China). Ovalbumin (Grade V, min 98%) was purchased from Sigma. Lysozyme and all other chemical reagents (analytical grade) were purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China), and were used without further purification.

CS with 72% deacetylation degree was further deacetylated in 50 wt% NaOH solution at a ratio of 50 g/L in a stainless-steel kettle at 333 K for 24 h [29]. The resulting CS was washed to neutral and dried for further use. The final deacetylation degree was 92% as determined by titration [30].

2.2. Preparation of CMCS

CMCS was prepared by the established procedure reported in the literature [31]. 10 g CS powder with 99% deacetylation degree, 13.5 g sodium hydroxide, 80 mL isopropanol and 20 mL deionized water were added into a 500 mL flask. The flask was placed into a 50 °C water bath and the CS was left to swell and alkalized for 1 h. Then the mixture solution of 15 g monochloroacetic acid and 20 mL isopropanol was added dropwise into the reaction mixture and reacted for 4 h at the same temperature. The reaction was stopped by adding 200 mL 70 vol% ethyl alcohol into the reaction mixture. The solid in the flask was filtered and rinsed in 90 vol% ethyl alcohol to desalinate and dewater, and vacuum dried at room temperature to yield the product, the sodium salt of CMCS (NaCMCS). The substitution degree of carboxymethyl groups on CMCS was determined using the ^{1}H NMR method according to literature [31,32]. The total carboxymethyl substitution degree was 1.18, where the O-substitution degree was 1.00 and the N-substitution degree was 0.18, indicating most of the amino groups on the original CS molecular chains were preserved.

2.3. Preparation of the macroporous CS/CMCS blend membranes

The macroporous CS/CMCS blend membranes were prepared by the method similar to the CS/CMC membrane, as described in our previous work [28,33]. 2 wt% NaCMCS aqueous solution was added dropwise into 2 wt% CS acetic acid solution under stirring. The final CMCS content in the CS/CMCS mixture was set from 0 to 40 wt%. After complete mixing of the CS and NaCMCS solution, porogen silica particles (200–300 mesh, the weight ratio of silica to CS/CMCS blend was set to 10:1) and cross-linking agent glutaraldehyde solution (1×10^{-2} mol/L) were added. After 3 h of stirring, the solution was poured into a poly(ethylene terephthalate) box and allowed to dry. When the membranes dried, they were immersed in 5 wt% NaOH aqueous solution to dissolve the silica and generate the macroporous CS/CMCS blend membranes. As CMCS is water-soluble and CS is also soluble in weak acidic solution, the CS/CMCS membrane needs to be cross-linked to prevent it from dissolving in aqueous media. Therefore, besides forming Schiff bases with glutaraldehyde to cross-link [34], the CS/CMCS blend membranes were further cross-linked under mild alkaline conditions using epichlorohydrin [35]. After the cross-linking procedure, the membranes were washed extensively with deionized water and kept in deionized water for further use. The basic characterization of macroporous CS/CMCS blend membranes have already been reported in our previous papers [33,36], which shows the average pore size was 20–30 μm and the porosity was about 47%.

2.4. Protein adsorption on the CS/CMCS blend membranes

Lysozyme and ovalbumin were employed for adsorption studies. The membranes were cut into small pieces and put in test tubes containing protein solution, while keeping a constant factor of 400 in the ratio of volume of solution (mL) with respect to the weight (g) of the CS/CMCS blend membrane (ca. 0.05 g membrane in 20 mL solution). The adsorption of protein from the aqueous medium on the CS/CMCS blend membranes (CMCS content = 0–40 wt%) was studied at various pHs in different buffer solutions. Britton–Robinson buffer solution (a mixture of 0.04 mol/L phosphoric acid, acetic acid, and boric acid plus 0.2 mol/L sodium hydroxide) was used to study the pH effect on the protein adsorption. For other experiments, more simple 0.01 mol/L borax buffer solution (pH = 9.2) and 0.2 mol/L acetic acid–sodium acetate buffer solution (pH = 5.2) were used. The adsorption experiments were conducted in an oscillator with a water bath (model SHZ-B, Shanghai Yuejin Medical Instrument Co., Ltd., Shanghai, China) at 30 °C. The time to adsorption equilibrium was found to be 7–8 h, so for all the adsorption measurements reported here, 12 h adsorption duration was employed.

The amount of the protein adsorbed on the CS/CMCS blend membranes was determined by the difference between the initial and the final concentrations of the protein within the adsorption medium. The concentration of protein 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 protein was calculated with the following expression:

$$\text{Adsorption capacity} = (C_0 - C_1) \times V/W \quad (1)$$

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

2.5. Protein separation from their binary mixtures by the CS/CMCS blend membranes

A binary mixture of ovalbumin and lysozyme (both protein concentrations were 0.5 mg/mL) was put in test tubes with the CS/CMCS blend membranes at pH 5.2 or 9.2. After 12 h of adsorption, the CS/CMCS blend membranes were transferred to a desorption solution at pH 9.2 or 5.2, respectively, to desorb the absorbed protein. The ratio of the volume (mL) of either protein binary mixture solutions or desorption solutions to the weight of the CS/CMCS blend membrane (g) was kept to a constant of 100 (ca. 0.2 g membrane in 20 mL solution). Both the solutions after the adsorption and desorption procedures were collected to determine the purity of protein by SDS-PAGE in order to evaluate the separation efficiency [28].

3. Results and discussion

3.1. Protein adsorption on the CS/CMCS blend membranes

Both CS and CMCS are polysaccharides that have a number of different functional groups, such as amino, hydroxyl and carboxyl groups. Similar to CS and CMC, CS and CMCS can form

intermacromolecular complexes by the strong interactions between those functional groups, such as electrostatic interaction and hydrogen bonding, which have already been reported in the literature [37]. In our previous paper, we also proved the strong intermolecular interaction between CS and CMCS and the homogenous structure in CS/CMCS blend system with FTIR spectroscopy, X-ray diffractometry and SEM observation [36,38].

Ovalbumin (p$I = 4.6$) and lysozyme (p$I = 11.0$) were selected as model proteins to investigate their adsorption on the CS/CMCS blend membranes because there is quite a large difference of pI between them. The adsorption capacities of ovalbumin and lysozyme on the CS/CMCS blend membranes as a function of adsorption–time were first investigated, and the typical curves are presented in Fig. 1. The adsorption of both lysozyme and ovalbumin on the membrane shows the same trends, i.e., the adsorption capacity gradually increases while the adsorption rate gradually decreases with the adsorption–time, until the adsorption equilibrium is reached. The time to reach the adsorption equilibrium is about 8 h for lysozyme and 7 h for ovalbumin. Therefore, all the adsorption data in this article thereafter were obtained after 12 h adsorption to ensure that they were in an equilibrium state.

In order to analyze the adsorption kinetics of the proteins, first-order and the second-order kinetics models were applied to the experimental data. The first-order rate expression of Lagergren is as follows:

$$\frac{dq}{dt} = k_{1,ad}\left(q_{eq} - q\right) \tag{2}$$

where q (mg/g) is the amount of adsorbed protein on the membrane at time t and $k_{1,ad}$ (min^{-1}) is the rate constant of first-order biosorption. The integrated form of Eq. (2) is:

$$\log\left(q_{eq} - q\right) = \log\ q_{eq} - \frac{k_{1,ad}}{2.303}\,t \tag{3}$$

A straight line of $\log(q_{eq} - q)$ versus t would be needed to suggest the applicability of this kinetic model. However, to fit experimental data with Eq. (3), the equilibrium adsorption capacity q_{eq} must be known, and in most cases the first-order equation of Lagergren does not fit well for the whole range of contact time [39,40]. Therefore, a second-order kinetic model is proposed as follows:

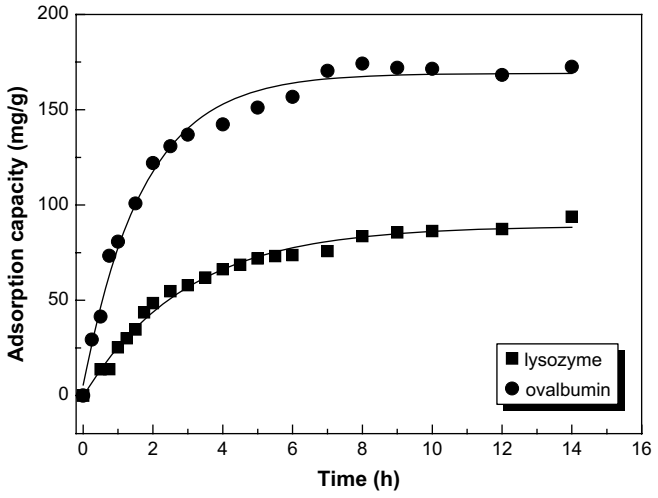

Fig. 1. Typical adsorption kinetics curve of ovalbumin and lysozyme on the CS/CMCS blend membranes (CMCS content: 40 wt%, initial protein concentration: 0.5 mg/mL, pH: ovalbumin, 5.2; lysozyme, 9.2).

$$\frac{dq}{dt} = k_{2,ad}\left(q_{eq} - q\right)^2 \tag{4}$$

where $k_{2,ad}$ (g/mg/min) is the rate constant of second-order biosorption. The integrated linear form of Eq. (4) is:

$$\frac{t}{q} = \frac{1}{k_{2,ad}q_{eq}^2} + \frac{1}{q_{eq}}\,t \tag{5}$$

If the second-order kinetics is applicable, a plot of t/q against t in Eq. (5) should give a linear relationship and there is no need to know any parameters beforehand. In addition, it describes the behavior over the whole range of adsorption and agrees with an adsorption mechanism being the rate controlling step [40].

A comparison of experimental adsorption capacity q_{exp}, the theoretical values calculated from the first-order kinetic model $q_{eq,1}$ and second-order kinetic model $q_{eq,2}$ of ovalbumin and lysozyme adsorbed on the CS/CMCS blend membranes are presented in Table 1. It shows that $q_{eq,2}$ of two proteins are both closer to q_{exp}, which indicates that the second-order kinetics model describes the data better. In addition, we find the correlation coefficients for the second-order kinetic model are higher than those of the first-order kinetic model when we fit our experimental data for both proteins, which also indicates the second-order kinetic model is more suitable for our protein adsorption process.

3.2. Effect of pH on the protein adsorption property of the CS/CMCS blend membranes

Like the protein adsorption on the CS/CMC blend membrane we reported previously [28], pH is also the main factor to influence the protein adsorption property of the CS/CMCS blend membrane. We know that both the protein and the CS/CMCS blend membrane are amphoteric, so the charges on their surface vary according to the environmental pH, and as a result the interaction between the protein and the membrane is different at different pH. Fig. 2 shows the influence of pH on the protein adsorption on the CS/CMCS blend membranes. Both ovalbumin and lysozyme have their maximum adsorption capacity at a specific pH, i.e., at pH = 5.2 for ovalbumin and around pH = 8.0 for lysozyme.

Lysozyme is a relatively small rigid protein [41], and we expect a hard protein to be more affected by charges at the surfaces [42]. This is indeed the case in the current research. The adsorption of lysozyme onto the CS/CMCS blend membrane is mainly ascribed to the amphoteric character of the protein and the membranes. It is found that the lysozyme has a large adsorption capacity when pH is in the range 8.0–9.2, which is similar to the lysozyme adsorption on CS/CMC blend membranes [28]. In this pH range, the lysozyme is positively charged and the CS/CMCS blend membrane is negatively charged, and the electrostatic interaction between them is very strong for the effective and significant adsorption. As the lysozyme adsorption at pH = 9.2 remains high, we choose this pH for other discussions in this article because we can use a simple borax buffer

Table 1
Kinetics model parameters for the adsorption of ovalbumin and lysozyme onto CS/CMCS blend membranes.[a]

Protein	q_{exp} (mg/g)	First-order kinetic model			Second-order kinetic model		
		$q_{eq,1}$ (mg/g)	$k_{1,ad} \times 10^3$ (min^{-1})	R^2	$q_{eq,2}$ (mg/g)	$k_{2,ad} \times 10^5$ (g/mg/min)	R^2
Lysozyme	94	78	3.87	0.972	104	6.16	0.992
Ovalbumin	172	140	6.29	0.956	192	6.67	0.996

[a] CMCS content: 40 wt%, initial protein concentration: 0.5 mg/mL, pH: ovalbumin, 5.2; lysozyme, 9.2.

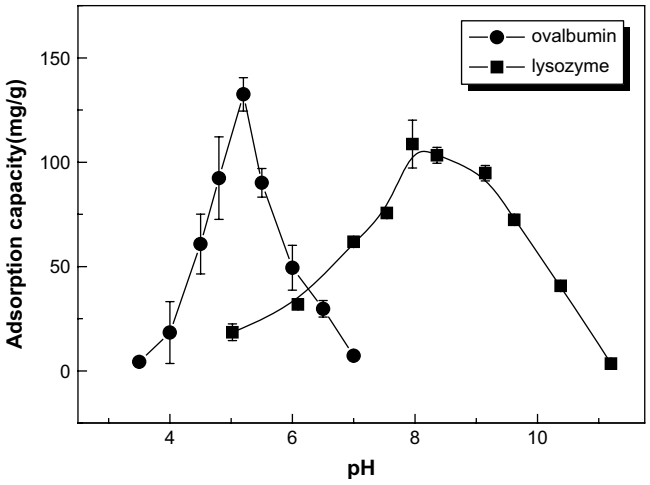

Fig. 2. Influence of pH on ovalbumin and lysozyme adsorption on the CS/CMCS blend membranes (CMCS content: 40 wt%, initial protein concentration: 0.5 mg/mL).

solution (pH = 9.2) instead of the complicated Britton–Robinson buffer solution. However, the lysozyme adsorption in the same pH may change a little because of the use of different pH buffer solutions.

For ovalbumin, the electrostatic interaction between the protein and the membrane is also a driving force for its adsorption because the ovalbumin is negatively charged and the membrane is positively charged at pH = 5.2, where it has the maximum adsorption capacity. However, electrostatic interaction seems not to be the only reason for the adsorption because there is still considerable adsorption capacity at pH = 4.6, the p*I* of the ovalbumin. Ovalbumin is a major globular protein component in egg white, which has 8.6% Glu and 9.9% Ser residues [43]. As we know, one common feature of globular proteins is that the hydrophilic residues are located on the external surface and most of the hydrophobic amino acids are buried in the protein core [44]. Thus, the carboxyl groups of Glu and hydroxyl groups of Ser on the surface of ovalbumin can form hydrogen bonds with the amino groups and/or hydroxyl groups on the CS/CMCS blend membrane, which gives an extra contribution to the adsorption capacity. Nevertheless, when pH < 4.6, there is electrostatic repulsion between the protein and the membrane [45], which is obviously not favorable for the hydrogen bonding. On the other hand, when pH > 4.6, the carboxyl groups in ovalbumin ionized to $-COO^-$, and the amino groups and hydroxyl groups on the membrane tend to form hydrogen bonds between themselves (because the amino groups are less protonized), this also reduce the hydrogen bonding between the ovalbumin and the CS/CMCS blend membrane. Therefore, the ovalbumin shows a maximum adsorption capacity on the CS/CMCS blend membrane at pH = 5.2, thanks to both electrostatic and hydrogen bond interactions. With the same consideration as lysozyme adsorption, we used 0.2 mol/L acetic acid–sodium acetate buffer solution (pH = 5.2) instead of the complicated Britton–Robinson buffer solution for other studies in this article. Similar to lysozyme mentioned above, we also found the ovalbumin adsorption capacity showing some difference in those two different buffer solutions.

3.3. Effect of CMCS content on the protein adsorption property of the CS/CMCS blend membranes

CMCS is the polyanion resource for the CS/CMCS blend membrane, but because of its water-soluble nature, its maximum content cannot exceed 40 wt%, otherwise the membrane is not stable in the buffer solution for the practical use, as CMCS will partially dissolve from the blend membrane. However, the structure of CS/CMCS blend membrane seems more stable than CS/CMC blend membrane because CMC started to dissolve from CS/CMC blend membrane into buffer solution when its content was higher than 20 wt% [28]. Fig. 3 shows the adsorption trend for ovalbumin and lysozyme on the CS/CMCS blend membranes with the increase in CMCS content. Interestingly, both proteins show the same tendency; i.e., the more the CMCS content, the higher the adsorption capacity. Both ovalbumin and lysozyme have the highest adsorption capacity when CMCS content in the CS/CMCS blend membrane is 40 wt%.

It is easy to understand that the lysozyme adsorption capacity increases with the increase in CMCS content because there are more carboxyl groups that can interact with lysozyme. However, things are different for ovalbumin. The absolute numbers of amino groups in the CS/CMCS blend membranes decreases with the increase in CMCS content, so it seems abnormal for the increase of the ovalbumin adsorption capacity with CMCS content. The same phenomenon was found in the CS/CMC blend membrane [28] and in other cases [46,47], which suggests an increase in the free amino groups. At pH = 5.2, the amino groups in CS are less protonized, so there is still strong hydrogen bonding between the amino groups and the hydroxyl groups within CS. Therefore, the free amino groups that can interact with ovalbumin are limited. Blending of CMCS into CS destroys the original hydrogen bonds within CS macromolecules and frees those amino groups. In addition, CMCS also has free amino groups, so the total free amino groups increase

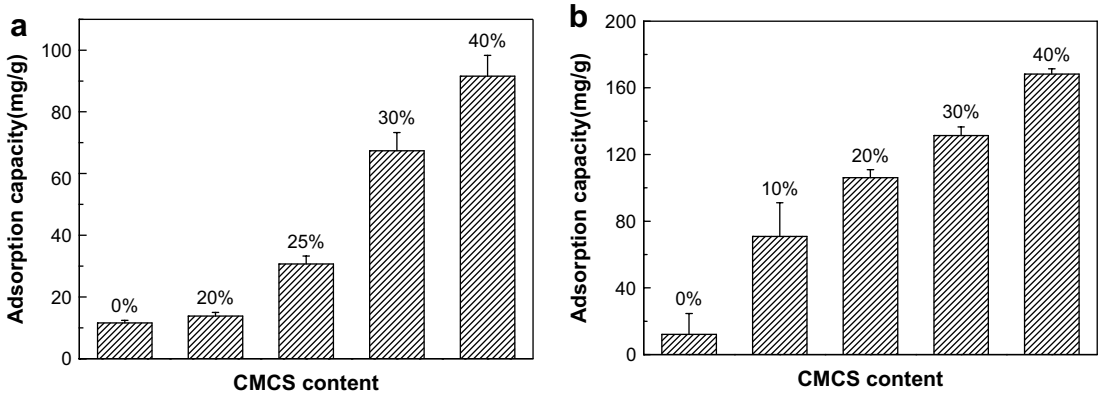

Fig. 3. Influence of CMCS content on lysozyme (a) and ovalbumin (b) adsorption on the CS/CMCS blend membranes (initial protein concentration: 0.5 mg/mL, pH: ovalbumin, 5.2; lysozyme, 9.2).

with the increase in CMCS content. Those free amino groups are the binding sites for ovalbumin, so the ovalbumin adsorption capacity also increases with the increase in CMCS contents. Moreover, we found the CS/CMCS blend membrane exhibited the higher ovalbumin adsorption capacity than CS/CMC blend membrane [28] because of its large amino group content.

3.4. Effect of protein initial concentration on the protein adsorption property of the CS/CMCS blend membranes

The ovalbumin and lysozyme adsorption isotherms of the CS/CMCS blend membranes are presented in Fig. 4. For lysozyme, the adsorption capacity first increases with the initial lysozyme concentration, then levels off when the initial lysozyme concentration is higher than 0.5 mg/mL until it is saturated at about 1.0 mg/mL. For ovalbumin, we are surprised to find that the adsorption capacity increases linearly until the ovalbumin solution is saturated at about 2.0 mg/mL. The difference of the adsorption behavior of two proteins may be attributed to their different adsorption mechanisms. As discussed before, the adsorption of lysozyme on the CS/CMCS blend membrane depends on the electrostatic interactions between the protein and the membrane, therefore when the negatively charged binding sites on the membrane are all occupied, no more lysozyme can be adsorbed. The ovalbumin adsorption on the CS/CMCS blend membrane not only depends on the electrostatic interaction, but also depends even more on the hydrogen bonding between the protein and the membrane. Thus, it is likely that, besides the electrostatic interaction, a large amount of ovalbumin is adsorbed on the CS/CMCS blend membrane through hydrogen bonding with numerous amino and hydroxyl groups in the membrane.

Two theoretical isotherm models (Langmuir and Freundlich) were used to analyze the experimental data. The Langmuir model is described by the following equation:

$$\frac{dq}{dt} = k_1 C(q_m - q) - k_2 q \tag{6}$$

where C is the concentration of protein in solution, q is the amount of protein adsorbed on the membranes and q_m is the maximum adsorption capacity of the membranes. At equilibrium, Eq. (6) leads to:

$$q_{eq} = \frac{q_m C_{eq}}{K_d + C_{eq}} \tag{7}$$

where K_d is a constant related to bonding energy of the adsorption.

The Freundlich model relates the adsorbed concentration as a power function in solute concentration. This empirical equation takes the form:

$$q_{eq} = K_F (C_{eq})^{1/n} \tag{8}$$

where K_F is adsorption capacity at unit concentration and $1/n$ is adsorption intensity. $1/n$ values indicate the type of isotherm to be irreversible ($1/n = 0$), favorable ($0 < 1/n < 1$), or unfavorable ($1/n > 1$) [48].

The adsorption constants and the correlation coefficients of the above two models evaluated from the isotherms are presented in Table 2. The results indicate that Freundlich model is more suitable for describing the biosorption equilibrium of lysozyme than Langmuir model in the concentration ranges we studied. However, both two models could, but not describe very well for ovalbumin adsorption. This accords with the adsorption mechanism discussed above, i.e., the adsorption of lysozyme is a simple heterogeneous adsorption, but the adsorption of ovalbumin is a combination of monolayer and heterogeneous adsorption.

The Freundlich constants n shown in Table 2 indicate that both proteins are adsorbed favorably by the CS/CMCS blend membrane because both of them are larger than 1.0. The q_m for lysozyme is close to the experimental equilibrium value (120 mg/g) which indicates that adsorption equilibrium is almost reached in our experiment. However, the q_m for ovalbumin is much larger than the highest adsorption capacity we can get (630 mg/g), confirming the adsorption equilibrium of ovalbumin is still not reached when the initial ovalbumin solution is saturated. Moreover, the large K_F values indicate easy adsorption for both proteins. The higher K_F value of ovalbumin accords to the result that CS/CMCS membranes have higher adsorption capacity for ovalbumin than lysozyme. Finally, a smaller value of K_d for lysozyme than ovalbumin implies the stronger bonding of lysozyme to the CS/CMCS blend membranes than ovalbumin, which provides further evidence for an alternative interaction (hydrogen bonding, which is weaker than electrostatic interaction) between the ovalbumin and the membrane other than the electrostatic interaction.

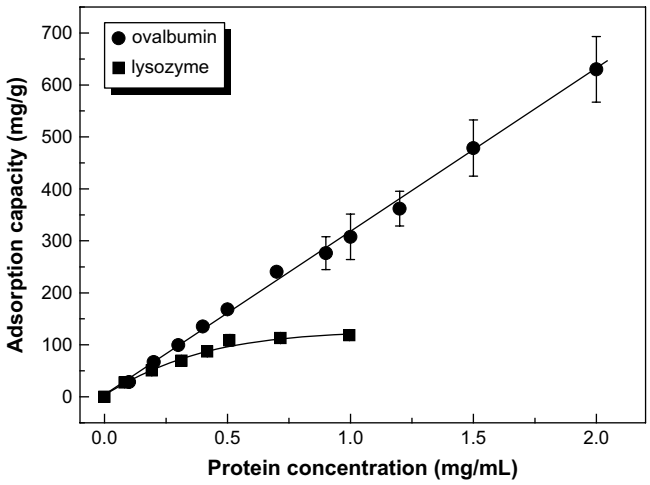

Fig. 4. Influence of the initial protein concentration on ovalbumin and lysozyme adsorption on the CS/CMCS blend membranes (CMCS content: 40 wt%, pH: ovalbumin, 5.2; lysozyme, 9.2).

3.5. Separation of lysozyme and ovalbumin from their binary mixture by the CS/CMCS blend membrane

The final goal for the design of such an amphoteric CS/CMCS blend membrane is to separate proteins simply by changing environmental pH. Before studying its protein separation capability, the desorption properties of the CS/CMCS blend membrane needs to be investigated. Similar to the CS/CMC blend membrane [28], more than 90% of the protein (both ovalbumin and lysozyme) can be desorbed from the membrane (ovalbumin desorbs at pH = 9.2, while lysozyme desorbs at pH = 5.2). In addition, the stability and

Table 2
Langmuir and Freundlich constants and correlation coefficients for the adsorption of ovalbumin and lysozyme on the CS/CMCS blend membranes.[a]

Protein	Langmuir constants			Freundlich constants		
	q_m (mg/g)	K_d (M)	R^2	n	K_F	R^2
Lysozyme	98	0.035	0.9442	2.59	148	0.9911
Ovalbumin	1021	0.452	0.9791	1.32	1004	0.9665

[a] CMCS content: 40 wt%, pH: ovalbumin, 5.2; lysozyme, 9.2.

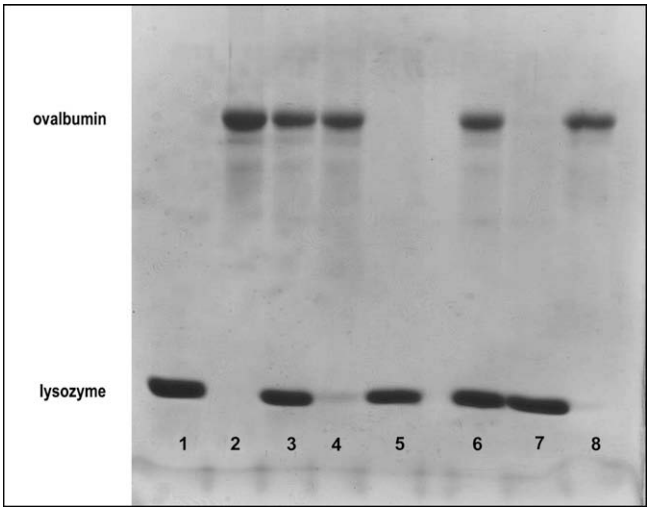

Fig. 5. SDS-PAGE assay of the separation of ovalbumin and lysozyme from their binary mixture with the CS/CMCS blend membrane (CMCS content: 40 wt%).

repeatability of the CS/CMCS blend membrane were found to be good after three adsorption–desorption cycles, which indicates the good reusability of the membrane.

From the adsorption and desorption results shown above, we may expect the separation of ovalbumin–lysozyme mixture by changing the environmental pH of the CS/CMCS blend membrane. A membrane with 40% CMCS content was utilized for the separation of ovalbumin–lysozyme mixture. The binary protein mixture contained 0.5 mg/mL lysozyme and 0.5 mg/mL ovalbumin, and separation results were qualitatively determined by SDS-PAGE (Fig. 5). Column 1 and column 2 represent the pure samples of lysozyme and ovalbumin. We first adjusted the protein mixture solution to pH 9.2 (column 3) and then put the CS/CMCS blend membrane to the mixture. From SDS-PAGE we found only ovalbumin remained in the solution after 12 h (column 4). Afterwards, the membrane was transferred to a buffer solution whose pH was 5.2 to desorb, and we found the desorbed solution contained only lysozyme (column 5) that obviously adsorbed from the protein mixture solution. On the contrary, if we adjust the protein mixture solution to pH 5.2 (column 6), after 12 h adsorption only lysozyme remained in the solution (column 7). The adsorbed ovalbumin could also be desorbed from the membrane in the buffer solution, whose pH was 9.2 (column 8). It needs to be noted that ovalbumin could be totally adsorbed by CS/CMCS blend membrane at pH 5.2, but some of the ovalbumin still remained in the buffer solution when using CS/CMC blend membrane in our previous study [28]. Thus it means both ovalbumin and lysozyme can be selectively separated from their binary mixture if we choose suitable pH values for the feed and the desorption solution.

4. Conclusions

An amphoteric membrane matrix was prepared by simple solution blending of natural polyelectrolyte CS and its derivative CMCS, followed by a cross-linking procedure. The capacity of the use of this macroporous natural amphoteric CS/CMCS blend membrane for protein adsorption and separation was extensively investigated. The effect of pH, initial protein concentration and CMCS content on the adsorption property of two model proteins, ovalbumin and lysozyme, on the CS/CMCS blend membrane was examined. The results showed that for both proteins, their adsorption capacity increased with the increase in CMCS content. Due to the amphoteric nature of the membrane, the environmental pH was found to be an important factor in the adsorption equilibrium of ovalbumin and lysozyme. The adsorption capacity varied with the change of environmental pH, and the maximum adsorption of ovalbumin and lysozyme was found at pH 5.2 and 8.0, respectively. Both the adsorption kinetics and the adsorption isotherm were fitted by different theoretical models. Though it was found that the adsorption mechanisms of ovalbumin and lysozyme on the CS/CMCS blend membrane may be different, they could still be separated effectively from their binary mixture by changing only the pH of the feed and the desorption solutions in batch systems. Moreover, the CS/CMCS blend membrane showed higher ovalbumin adsorption capacity and better protein separation property than the CS/CMC blend membrane we studied previously [28]. The continuous and dynamic separation test of the protein mixture and the quantitative analysis of the separation efficiency are still in process and we believe such a natural amphoteric membrane may have great potential on the membrane chromatography separation of biomacromolecules because all the materials are from nature.

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