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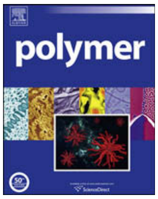

Protein adsorption and separation with chitosan-based amphoteric membranes

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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

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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:

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

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