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Chitosan-based membrane chromatography for protein adsorption and separation

Yezhuo Liu, Zhicheng Feng, Zhengzhong Shao, Xin Chen *

State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Laboratory of Advanced Materials, Fudan University, 220 Handan Road, Shanghai 200433, People's Republic of China

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ABSTRACT

A chitosan-based membrane chromatography was set up by using natural chitosan/carboxymethylchitosan (CS/CMCS) blend membrane as the matrix. The dynamic adsorption property for protein (lysozyme as model protein) was detailed discussed with the change in pore size of the membrane, the flow rate and the initial concentration of the feed solution, and the layer of membrane in membrane stack. The best dynamic adsorption capacity of lysozyme on the CS/CMCS membrane chromatography was found to be 15.3 mg/mL under the optimal flow conditions. Moreover, the CS/CMCS membrane chromatography exhibited good repeatability and reusability with the desorption efficiency of ~90%. As an application, lysozyme and ovalbumin were successfully separated from their binary mixture through the CS/CMCS membrane chromatography. This implies that such a natural chitosan-based membrane chromatography may have great potential on the bioseparation field in the future.

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1. Introduction

With the development of biotechnology, the bioseparation techniques with adsorptive membranes become more and more important due to their efficiency in overcoming mass transfer limitation [1]. The advantage of membrane chromatography lies in the predominance of convective material transport [2] because the membrane adsorber acts as a short and wide chromatographic column [3]. Membrane chromatography is widely employed for purification and recovery of biomolecules such as polypeptides, proteins, nucleic acids, polynucleotides, and so on [4–6].

Ion exchange membrane, a widely used membrane adsorber, has attracted many interests because of its broad applicability, high resolution, and large adsorption capacity in large-scale protein purification processes [7]. It can separate the proteins with similar molecular weights but different charge conditions easily. For instance, Zeng and Ruckenstein [8] used macroporous anion-exchange chitosan membranes with controlled pore sizes to separate protein mixtures. They selected five proteins and the results showed that all these five proteins were recovered efficiently (91–98%). Lin et al. [9] employed both cellulose phosphate cation-exchange membrane and diethylaminoethyl cellulose anion-exchange membrane to separate lysozyme, bovine serum albumin, and γ-globulin. Avramescu et al. [10] also successfully separated bovine serum albumin and bovine hemoglobin through a novel ion-exchange membrane, which was prepared by the incorporation of various types of Lewatit ion-exchange resins into an ethylene–vinyl alcohol copolymer porous matrix.

The natural polymer-based ion-exchange membranes are obvious benefit for bioseparation because of their good biocompatibility. However, most of the ion-exchange membranes reported are from either synthetic polymers or synthetic/natural polymer blends/composites [11–14], and only few are from natural polymers, as mentioned above [9,15].

Chitosan (CS), derived from chitin that is the main structural component of the invertebrate exoskeleton and the fungal cell wall, is an abundant natural polymeric resource [16]. It is considered as an attractive material that can be potentially used in biomedical fields because of its favorable physicochemical and biological properties, such as good biocompatibility, non-toxicity, and antibacterial property [17,18]. In our previous research, we developed two natural polymer ion-exchange membranes, i.e., chitosan/carboxymethyl cellulose (CS/CMC) and chitosan/carboxymethyl chitosan (CS/CMCS) blend membrane [15,19–23]. We have performed the characterizations of those two materials by FTIR spectroscopy and X-ray diffraction, and also tested their mechanical properties as well as swelling ratios in different pH buffer solutions [22,23]. In the meantime, we have fully studied the adsorption properties of two model protein, lysozyme and ovalbumin on both blend membranes and successfully separated each protein from their binary mixture in static mode [15,19]. In this article, we reported our trials to use CS/CMCS membrane as a matrix to compose membrane chromatography and discussed the dynamic adsorption and separation properties of such a CS/CMCS membrane chromatography.

* Corresponding author. Tel.: +86 21 6564 2866; fax: +86 21 51630300.
E-mail address: chenx@fudan.edu.cn (X. Chen).

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2. Experimental

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). Sample of lysozyme and ovalbumin (Grade V, minimum purity of 98%) was obtained from Sigma-Aldrich. CS with 72% deacetylation degree was further deacetylated according to the literature [24]. The final deacetylation degree was 92% as determined by titration [25]. CMCS was prepared by the established procedure reported previously [26]. The substitution degree of carboxymethyl groups on CMCS was determined by ^{1}H NMR [26,27]. 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 [15].

2.2. Preparation of the macroporous CS/CMCS blend membranes

The macroporous CS/CMCS blend membranes were prepared following the method in our previous work [15,19]. In brief, 2 wt% CMCS aqueous solution was added dropwise into 2 wt% CS acetic acid solution under stirring, and then the porogen silica particles and crosslinking agent glutaraldehyde were added. The CMCS content in the final CS/CMCS blend membrane was set to 40 wt% and the crosslinking degree is 3%. In this paper, we used three different sizes of silica particle, thus obtained three different macroporous membranes. After 3 h of stirring, the solution was poured into a poly (ethylene terephthalate) box and allowed to dry. Then, the dried membranes were immersed into 5 wt% NaOH aqueous solution to dissolve the silica and generated the macroporous membranes. Finally, the membranes were further crosslinked under mild alkaline conditions using epichlorohydrin [28]. The pore size and the thickness of the membranes were determined from their SEM photographs taken with TS-5136MM scanning electron microscopy at 20 kV, using the software provided with the equipment. The average pore size was obtained by measuring at least 20 pores. The thickness of the membrane obtained here was used to calculate the volume of the membrane, and further calculate the dynamic adsorption capacity according to Eq. (1) (see below).

2.3. Dynamic adsorption of CS/CMCS membrane chromatography for lysozyme

We utilized the experimental setup similar to Chen and Juang's [29], but improved the membrane stack holder according to the literature of Ghosh and Wong [1] (Fig. 1). The CS/CMCS blend membranes were cut into 5 cm diameter slice and put into 0.01 mol/L borate buffer solution (pH = 9.2) for 4 h to reach the swelling equilibrium. Then three to five membranes were packed into the stack holder to form membrane stack. The diameter of effective membrane area in the stack holder was 4 cm. Lysozyme solutions with concentration from 0.27−0.75 mg/mL in 0.01 mol/L borate buffer solution were loaded through the stack holder with the flow rate of 2−4 mL/min controlled by a peristaltic pump (DDB-320, Shanghai Zhisun Instrument Co., Ltd.). After ~160 min of lysozyme loading, the membrane stack was washed with fresh 0.01 mol/L borate buffer solution to release the unbounded lysozyme, and then eluted with 0.01 mol/L borate buffer solution containing 1.5 mol/L NaCl to desorb lysozyme [3]. At last, the membrane stack was regenerated with 2 mol/L NaCl solution. The permeate in the adsorption-washing-elution process was collected with a fraction collector and the lysozyme concentration in permeate was measured with a Hitachi UV 2910 UV–vis spectrometer at 280 nm. We chose 0.01 mol/L borate buffer solution in this work because the CS/CMCS blend membrane showed maximum lysozyme adsorption capacity at this pH value (pH = 9.2) in our previous work in batch system [15].

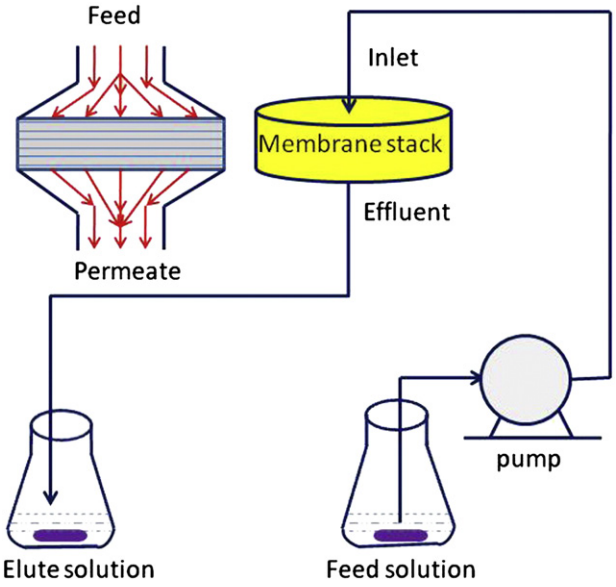

Fig. 1. Schematic diagram of experimental apparatus. The upleft insert shows the detailed schematic diagram of membrane stack holder.

The dynamic binding capacity at 10% breakthrough (defined as when the protein concentration in the permeate is equal to 10% of the feed concentration) of lysozyme was calculated according to the following equation [30]:

$$Q_{B10\%} = C_p\left(V_p - V_h\right)/V_b \quad (1)$$

where $Q_{B10\%}$ is the dynamic binding capacity (mg/mL) at 10% breakthrough, C_p is the concentration of lysozyme loaded (mg/mL), V_p is the volume of lysozyme solution loaded at 10% breakthrough (mL), V_h is the holdup volume of the apparatus (mL), and V_b is the volume of membranes (mL).

The desorption efficiency of lysozyme from the CS/CMCS membrane was calculated according to the following equation:

$$DS = q_e/(q_i - q_w) \quad (2)$$

where q_e is the lysozyme amount in elution, q_i is the initial lysozyme amount in feed, and q_w is the lysozyme amount that removes from the membrane during the washing process.

2.4. Separation of lysozyme–ovalbumin binary mixture through CS/CMCS membrane chromatography

Three layers of CS/CMCS blend membrane with average pore size of 55 μm were packed as membrane stack. 20 mL feed solution composed of 0.52 mg/mL of lysozyme and 0.52 mg/mL ovalbumin in 0.01 mol/L borate buffer solution was loaded through the membrane stack with the flow rate of 2 mL/min. Then, the membrane stack was washed with fresh 0.01 mol/L borate buffer solution, and eluted with 0.01 mol/L borate buffer solution containing 1.5 mol/L NaCl. HPLC (Waters 600E with BioSuite™ 250 SEC column) was used to examine the composition and concentration of the permeate from the whole separation process.

3. Results and discussion

3.1. Adsorption of lysozyme on the CS/CMCS membrane chromatography

Fig. 2 shows the typical adsorption–washing–elution curve of lysozyme on the CS/CMCS membrane chromatography. At first few

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