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Chromatography separation of proteins by macroporous chitosan and chitin affinity membranes

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1. INTRODUCTION

Affinity membranes have emerged principally to overcome the problems of limited specificity experienced with membranes that operate purely on a sieving mechanism and as an alternative to the traditional affinity resins. It is a logical expectation that affinity membranes might combine the outstanding selectivity of affinity resins with the high productivity associated with filtration membranes. Comparing to the affinity column chromatography, the affinity membrane chromatography offers solute (ligate) into close proximity to bound ligand through convective transport. The resulting reduction in mass transfer resistance enables a number of advantages, such as higher flow rates, faster binding, lower pressure drops and higher productivity [1].

Materials for preparation of affinity membranes should be hydrophilic, should have a large number of reactive groups available for ligand coupling, chemical stability and good mechanical properties, and should allow the easy formation of a microporous or a macroporous structure. In view of these criteria, chitosan and chitin are expected to be most suitable materials. The chitosan and chitin contain a large number of reactive hydroxyl (OH) and amine (NH_2) groups, which can easily couple ligands. Also they have an excellent film-forming ability and good mechanical properties. Recently Zeng and Ruckenstein [2] prepared macroporous chitosan and chitin membranes with controlled porosity using a novel method. The method consisted of casting a suspension of silica particles of selected size in an acidic chitosan solution, removing the solvent by evaporation, and dissolving the silica particles by immersing the membranes into alkaline solutions. Using this novel method, they successfully prepared the macroporous chitosan membranes and also prepared the macroporous chitin membranes by acetylating the chitosan membranes with 5 vol% acetic anhydride in methanol.

We prepared the macroporous chitosan and chitin membranes by using silica particles as porogen, and then obtained the macroporous affinity chitosan membrane followed by coupling of the Cibacron Blue 3GA reactive dye to the chitosan membrane and the macroporous chitin membrane by acetylating the chitosan membrane. Using these affinity membranes, we performed the experiments of chromatography separation of BSA and lysozyme proteins.

2. EXPERIMENTAL

The macroporous chitosan and chitin membranes were prepared using the methods suggested by Zeng and Ruckenstein [2]. The method consisted in the dissolution of 0.5 g of chitosan (average molecular weight 750,000, from Fluka Chemi AG, Switzerland) in a 50 ml of 1 vol% acetic acid solution. To this solution, silica particles of selected size (average diameter; 3 ㎛, 5 ㎛ and 10 ㎛, from Sambo Chemical Co., Korea) were added (weight ratio of chitosan to silica 1 : 0.25, 1 : 0.5, 1 : 1, 1 : 1.5 and 1 : 2) and dispersed uniformly in the chitosan solution by vigorously stirring.

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The film was obtained by casting the chitosan solution containing silica particles onto a glass plate using a film applicator (Type YBA-3, from Yoshimitsu Co., Japan) and drying for 24 hr in a hood to evaporate the liquid. We obtained the macroporous chitosan membranes with 360 ㎛ thickness by immersing the film detached from glass plate in 5 wt% NaOH solution and kept for 2 hr at 80℃ in order to dissolve the silica particles. The macroporous chitin membranes were obtained by acetylating the chitosan membranes with acetic anhydride. To determine the optimal conditions for the preparation of macroporous chitosan and chitin membranes, we measured pure water flux, tensile strength, porosity, specific surface area and SEM views of pore structure of the membranes.

Cibacron Blue 3GA (CB3GA, from Sigma Co., USA) reactive dye which is an affinity ligand for serum albumin proteins was immobilized to the macroporous chitosan membrane using the modified Atkinson method described in our previous works [3]. The dye content in an affinity chitosan membrane was determined by hydrolyzing the membrane in 12 N HCl solutions at 80℃ for 15 min [4]. The chitosan membrane coupled with CB3GA was used as an affinity membrane for the separation of BSA protein. The chitin membrane without further chemical modification was used as an affinity membrane for the separation of lysozyme. The equilibrium binding capacities of BSA and lysozyme onto the affinity chitosan and chitin membranes respectively were determined by performing the batch adsorption experiments.

The characteristics of protein separation were investigated through the experiments of elution membrane chromatography of BSA and lysozyme using the membrane module containing 10 sandwiched affinity chitosan and chitin membranes. The schematic diagrams of the chromatography experimental system are shown in Fig. 1. The protein (BSA or lysozyme) solutions having different concentrations of 0.1~1.5 mg protein/ml were loaded into the membrane module until saturation was achieved at different flow rates of 1.5~10 ml/min. These were followed by washing with buffer solutions (0.05 M Tris buffer/0.05 M NaCl for chitosan membrane, 0.1 M phosphate buffer/1 M NaCl for chitin membrane, pH 8) at a flow rate of 5 ml/min, and then finally eluted with 0.5 N NaSCN in Tris buffer for BSA or 0.1 M acetic acid solution for lysozyme at different flow rates of 2~10 ml/min. The dynamic binding capacities of BSA and lysozyme onto the affinity chitosan and chitin membranes respectively were determined from the chromatograms with peaks of loading/washing/elution.

3. RESULTS AND DISCUSSION

Fig. 2 represents SEM photographs for the surface of macroporous chitosan membranes prepared with different added amount of silica particle in the chitosan solution. Fig. 2 shows that as increasing the added amount of silica particle having size of 5 ㎛, the porosity is increased and the pores are distributed more uniformly. The optimal conditions for the preparation of macroporous chitosan and chitin membranes are determined by measuring pure water flux, tensile strength, porosity, specific surface area and SEM views of pore structure of the membranes. The optimal preparation conditions are as follows; the weight ratio of chitosan to silica (C : S) is 1 : 1.5, the silica particle size is 5 ㎛. Characteristics of macroporous affinity chitosan and chitin membranes obtained at the optimal preparation conditions are shown in Table 1.

Results of elution chromatograms for the BSA separation by using affinity chitosan membranes show that the eluted BSA yields are decreased from 90 % to 20 % with increasing the flow rate, loading amount and solution concentration of BSA at the loading step but are increased from 60 % to 70 % with increasing the flow rate at the elution step.

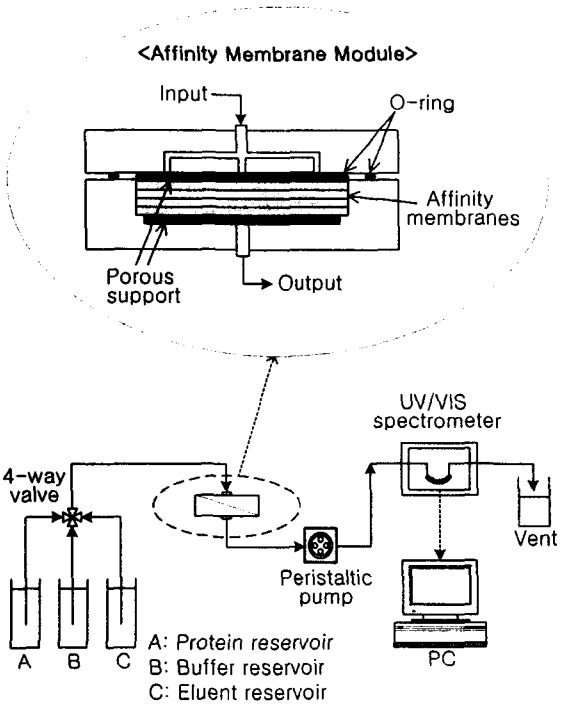
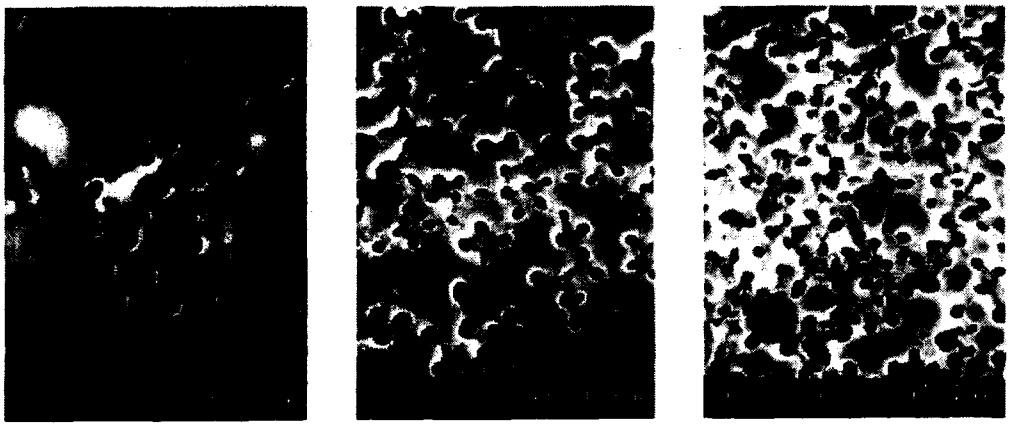
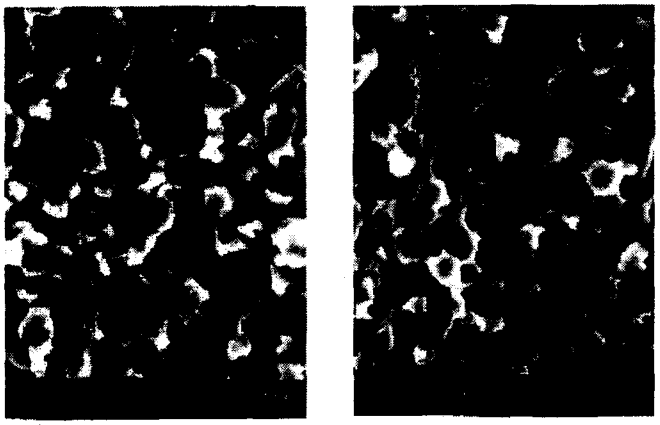


Fig. 1. Experimental system setup for the elution membrane chromatography.



(a) C : S = 1 : 0.25 (b) C : S = 1 : 0.5 (c) C : S = 1 : 1



(d) C : S = 1 : 1.5 (e) C : S = 1 : 2

Fig. 2. SEM photographs of the macroporous chitosan membranes with silica content.

Table 1. Characteristics of macroporous affinity chitosan and chitin membranes.

	Affinity chitosan membrane	Affinity chitin membrane
In 5 vol% acetic acid solution (pH 2.5)	Insoluble (after crosslinked)	Insoluble
Thickness	360 ㎛	360 ㎛
Pore volume[a]	0.002 cc/g	0.00185 cc/g
Specific surface area[a]	2.66 m^2/g	2.01 m^2/g
Ligand(CB3GA)	40.2 μmol/ml memb.	Non
Equilibrium binding capacity of protein	1.65 mg BSA/ml memb.	1.94 mg lysozyme/ml memb.

Also we find similar results for the lysozyme separation by using affinity chitin membranes. These results suggested that the macroporous chitosan and chitin affinity membranes can be efficient for proteins purification.

4. CONCLUSIONS

We prepared the macroporous chitosan and chitin membranes by using silica particles as porogen and then obtained the macroporous affinity chitosan membrane followed by coupling of the Cibacron Blue 3GA reactive dye to the chitosan membrane and the macroporous affinity chitin membrane by acetylating the chitosan membrane. Using these affinity membranes, we performed the experiments of chromatography separation of BSA and lysozyme proteins. The optimal conditions for preparation of macroporous chitosan and chitin membranes were determined as follows by measuring pure water flux, tensile strength of the membranes and SEM views of membrane pore structure; the weight ratio of chitosan to silica was 1 : 1.5, the silica particle size was 5 ㎛. Results of chromatography separation of BSA with the macroporous affinity chitosan membrane showed that the eluted BSA yields were decreased from 90 % to 20 % with increasing the flow rate, loading amount and solution concentration of BSA at the loading step but were increased from 60 % to 70 % with increasing the flow rate at the elution step. Also we found similar results for the chromatography separation of lysozyme with the macroporous affinity chitin membrane. These results suggested that the macroporous chitosan and chitin affinity membranes can be efficient for proteins purification.

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