TRIS(HYDROXYMETHYL)AMINOMETHANE AFFINITY MEMBRANE FORADSORPTION OF LYSOZYME

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Abstract - Polyacrylonitrile (PAN) nanofiber membrane was prepared by electrospinning technique. The PAN membrane used in this work comprises a polyethylene terephthalate (PET) spunbond fabric as a supporting layer with upper and lower PAN nanofiber membrane. After 3 M NaOH and diluted HCl treatments, the weak cationic exchange membrane (i.e., P-COOH) was obtained. The P-COOH membrane was then functionalized with tri-s(hydroxymethyl) aminomethane as an affinity nanofiber membrane (i.e., P-Tris). In this study, lysozyme was chosen as a model protein. The physical properties of the nanofiber membranes were characterized in terms of fiber diameter, porosity and pore size, specific area of the surface, FTIR and SEM analysis. The adsorption experiments were carried out in a well-mixed system under the various operating conditions (e.g., modification pH, adsorption pH, and the molar ratio of reactants, P-COOH/Tris). The dynamic adsorption characteristics of the P-Tris membranes for lysozyme by membrane chromatography were assessed by measurements of the breakthrough curves. The influences of operating conditions (e.g., adsorption pH, lysozyme concentration, no. of sheet membrane, and flow rate) on the adsorption performance of membrane were investigated in an AKTA prime chromatographic system (GE Healthcare).

Index Terms - Polyacrylonitrilenanofiber,lysozyme,Tris, AKTA

I. INTRODUCTION

Tris(hydroxymethyl)aminomethane (Tris) is one of the important reagents and widely used in buffer solutions[1-4]. The specific interactions between Tris and protein have commonly been ignored when it is used as a buffer. However, in recent studies[1-4], it has been demonstrated that there is a specific interaction between lysozyme and Tris. This kind of interaction is considered as a weak specific one, which enables to elute lysozyme from Tris affinity column under a mild condition[1-4]. The PAN membrane is less suitable for use in adsorption of proteins due to its hydrophobicity as compared to the cellulose membrane with hydrophilic properties[5-13].

However, PAN membrane has some advantages, e.g., strong mechanical strength and chemical stability[5-13]. To improve the hydrophilicity of PAN membrane, introduce hydrophilic groups (e.g., Tris molecule) onto the PAN membrane surface may be an ideal method for combining both advantages of hydrophilic and hydrophobic membranes. Hence, PAN membrane was chosen for use in this work.

It has been demonstrated that Tris immobilized affinity chromatography is a highly efficient and low cost technique for purification of lysozyme, due to the presence of high stability and reproducibility. To overcome the problems observed in packed bed chromatography14-16, membrane chromatography may be one of the alternative techniques. Hence, amine Tris molecule with multi-hydroxyl groups was chosen as a weak affinity ligand in this work and was immobilized onto the membrane as an adsorber for adsorption of lysozyme.

II. EXPERIMENTAL

A. Materials

Polyacrylonitrile (PAN) yarn (Mw 120, 000, containing 93% acrylonitrile and 7% vinylacetate) was purchased from Fortune Industries Inc. (Tao-Yuan, Taiwan). Polyethyleneterephthalate (PET) spunbond fabric (basis weight 15 g/m^2, thickness 90 μm, fiber diameter 300-500 μm) was supplied from Freudenberg Far Eastern Spunweb Co., Ltd. (Tao-Yuan, Taiwan). Water soluble chitosan (molecular weight 50,000 g/mol) was obtained from Charming & Beauty Co., Ltd. The dried Spirulinaplatensis powder was obtained from Charming & Beauty Co. Ltd. (Taipei, Taiwan). All other chemicals used were of analytical grade. Electrospinning device was purchased from Falco Enterprise Co., Ltd. (New Taipei City, Taiwan) and electrospinning process was conducted at room temperature. The pH of the adsorption buffer was monitored with a pH meter (Mode SP-2200, Suntex Ins. Co., Taiwan). A UV-Vis spectrophotometer (Model Ultrospec 3100 pro, GE Healthcare Biosciences, Uppsala, Sweden) was used to measure the absorbance of lysozyme (Sigma) samples. AKTAprime system (GE Healthcare Biosciences, Uppsala, Sweden) was employed to membrane chromatography.

B. Preparation of PAN nanofiber membrane

PAN yarn (15 g) was completely dissolved in 100 mL dimethylacetamide (DMAc). A 10 mL prepared PAN solution was loaded into a syringe with 21-gauge stainless steel nozzle. The electrospinning process was performed at 25^{o}C and the operating parameters were determined based on the previous study by Chiu et al.[5-6]. The PAN nanofiber mat was collected on

nonwoven PET, which was wrapped around the grounded steel drum. The PAN-PET-PAN membranes (the number of PAN nanofiber layer was 2, designated as PAN) were subjected to heat pressing process at 100°C for 1 h and was further used in this study.

C. Preparation of polyacrylic acid membrane

The PAN membrane (diameter 25 mm) was placed in 5 mL 3 M NaOH at 85°C for 25 min. After alkaline treatment, the membrane was removed and successively washed with H_2O to remove the excess NaOH. The hydrolyzed membrane was then treated with 0.1 M HCl (designated as PAN-COOH), followed by washing and drying in an oven at 60°C before use. The surface density of carboxylic groups on the PAN-COOH membrane was determined by dyeing with toluidine blue O (TBO) as described by Ma et al. [7-8].

D. Preparation of modified Tris membrane

The carboxylated membrane was submerged into 5 mL 0.1M MES (4-Morpholineethanesulfonic acid hydrate) solution(pH 6.0),containing100μmol 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 100 μmol) and Tris (100 μmol). The mixture was shaken at 100 rpm and 25°C for 3 h. Finally, the Tris membrane was taken out, washed with H_2O to remove the free Tris and then dried in an oven at 60°C.

E. Membrane chromatography

In order to compare the adsorption performance of the modified Trisnanofiber membrane under different operating conditions, an experiment was carried out using one sheet of membrane (4.9 cm^2) at the fixed liquid velocity. The membrane adsorber was irrigated with buffer (20 mM sodium phosphate, pH 8.0) at a liquid velocity of 1.0 ml/min. After 5 ml of adsorption buffer had passed through the adsorber, 2.0 mg/ml lysozyme (12 ml) was loaded onto the membrane adsorber. After the measurement of breakthrough curve was completed.

The samples were collected and lysozyme samples were determined. Dynamic binding capacity is defined as the amount of protein adsorbed in per unit weight of membrane. It is commonly used to evaluate the adsorption efficiency of the membrane chromatography[15-17]. In this work, the dynamic binding capacity (DBC) at 10% breakthrough was calculated.

III. RESULTS AND DISCUSSION

A. **Membrane characteristics**

The SEM analysis (Fig. 1) revealed that the modification of PAN proceeded without significant changes in the morphology of the nanofiber membrane, including the average nanofiber diameter of 300-600 nm, average pore size of 0.46 μm, and porosity of approx. 80%.

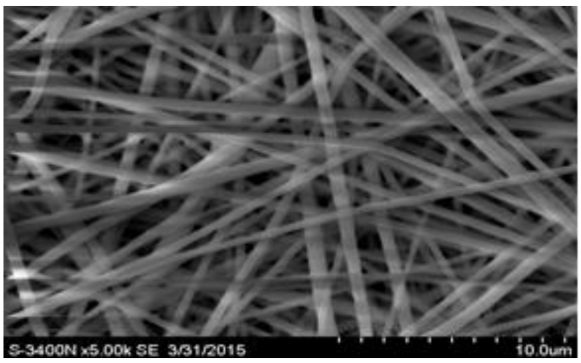


Fig. 1(a).SEM image of PAN (5kx).

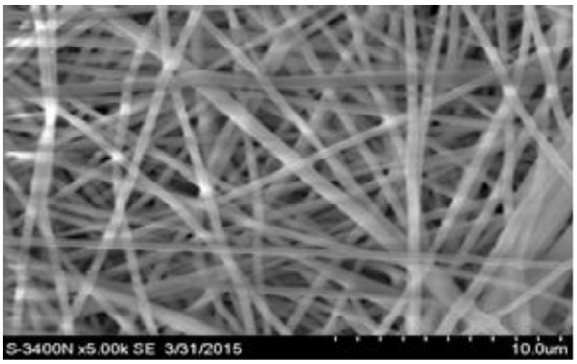


Fig. 1(b).SEM images of P-COOH (5kx).

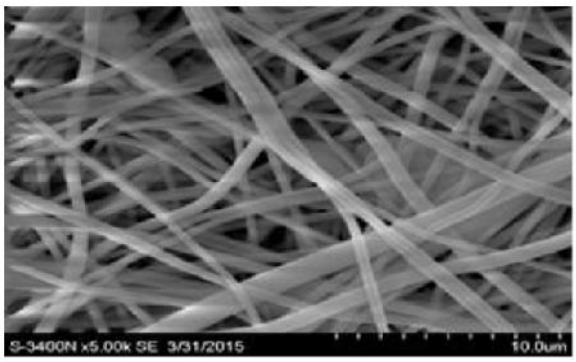


Fig. 1(c).P-Trisnanofiber membrane (5kx).

B. **Choice of adsorption pH**

The influence of solution pH on adsorption of lysozyme was investigated by Trisnanofiber membrane chromatography. As can be seen from Fig. 2, it was shown that the maximum adsorption capacity of Tris membrane for lysozyme occurred at pH 9.0. The results were obtained by frontal analysis and the order of binding capacity for lysozyme was pH 9 > pH 7 > pH 5. Hence, adsorption pH 9.0 was used for further experiments.

C. **Effect of feed concentration**

The effects of feed concentration on lysozyme adsorption are presented in Fig. 2(a). From the breakthrough curves, it was found that feed concentration of 0.5 mg/ml had the best adsorption performance. However, the dynamic binding capacity in this case was 524.7 mg/g-M, which was lower than either feed concentration of 1.0 mg/g-M (576.12 mg/g-M) or feed concentration of 2.0 mg/g-M (1068.2

mg/g-M). On the other hand, an increase in feed concentration resulted in the increase of dynamic binding capacity. The higher lysozyme concentration in the feed provided more lysozyme that flowed through the membrane. This is probably because the Tris affinity membrane had highly effective adsorption for lysozyme.

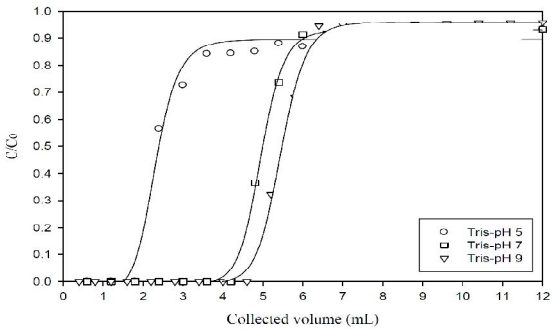


Fig. 2.Breakthrough curves forAdsorption of lysozyme at different operating pH

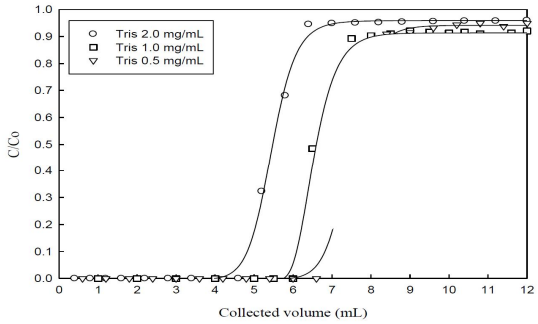


Fig. 2(a).Breakthrough curves forAdsorption of lysozyme at different concentration

D. Effect of flow rate

The breakthrough curves at three different flow rates were obtained in Fig. 3. It was found that the increase in flow rate did not cause a significant decrease. As the flow rate increased from 0.1 to 1.0 ml/min, the binding capacity of the Tris membrane increased from 1,043 to 1,300 mg/g-M. Clearly, increase of flow rate, shortens the contact time between lysozyme and the membrane. Hence, the high flow rate of 1.0 ml/min may be favorable to achieve a maximum production rate of lysozyme.

E. Effect of layer in membrane stack

The layer in membrane stack was changed to evaluate the DBC of Tris membrane. From the breakthrough curve in Fig. 3(a), the DBC was calculated to be 1043, 1228, and 699 mg/g-M with the layer of 1, 3, and 5, respectively. It seemed not expected that the DBC increased with increase in membrane layer. This may be due to increase the uncertainty of liquid flow distribution, especially for 5-layer membrane stack.

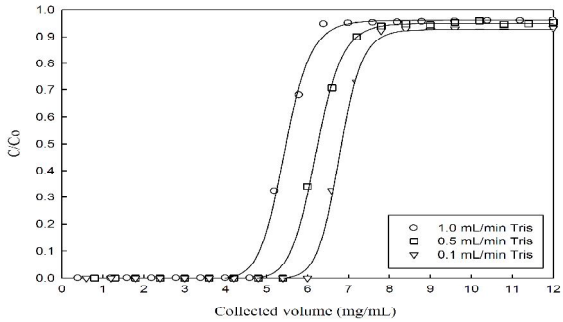


Fig. 3.Breakthrough curves for Adsorption of lysozyme at different flow rates

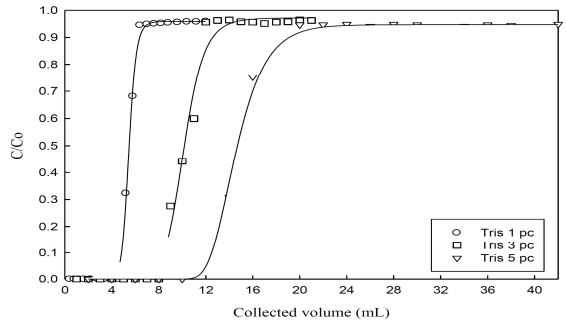


Fig. 3.(a)Breakthrough curves for adsorption of lysozyme at different layers of P-Tris membrane.

CONCLUSION

In this work, a highly efficient and low cost Tris affinity membrane has been developed for use in adsorption of lysozyme. The results showed that a high flow rate of 1.0 ml/min, high feed concentration of 2.0 ml/min, and 3-layer membrane were favorable to achieve a high dynamic binding capacity. Tris affinity membrane chromatography may have high potential on recovery of lysozyme from complex chicken egg white solution.

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