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Factors affecting selective rejection of proteins within a binary mixture during cross-flow ultrafiltration

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Abstract

The flux decline and rejection behavior in cross-flow ultrafiltration (UF) of BSA/lysozyme (Ly) mixtures, with and without ultrasound, were investigated in upward and downward modes. Polysulfone (PS) and polyvinylidine fluoride (PVDF) ultrafilters were selected. Experiments were conducted at different pH values (4.9–11), applied pressures (2–4 atm), added NaCl concentrations (0.01–1 g/L), and ultrasonic powers (180–250 W). It was shown that PS membrane yielded higher flux and lower Ly rejection than PVDF membrane. Low rejection of Ly was achieved at pH near isoelectric point of Ly (11.0), particularly under the conditions of lower applied pressures and ionic strengths. The flux and Ly rejection were strongly affected by the solution environment. BSA was almost retained in the retentate, but its existence had a significant effect on permeate flux. The flux was enhanced with ultrasound and the enhancement increased with increasing ultrasonic power at 25 kHz, likely due to the change of molecular sizes of the proteins under ultrasonic irradiation. With ultrasound, the level of flux enhancement in the upward mode was slightly better than that in the downward mode.

Keywords: Cross-flow ultrafiltration; Protein mixtures; Ultrasound; Separation; Flux enhancement

1. Introduction

Protein separation or purification is a crucial process in biotechnology due to its wide range of applications in biomedical and food industries. The techniques used for protein separation and purification such as chromatography, electrophoresis, and affinity operations have been recently established for producing small quantities of proteins in research laboratories. However, these techniques are rather difficult to scale-up, which limits production levels [1,2]. Besides, some methods like chromatography and electrophoresis require complex instrumentation support to run efficiently, and usually yield low throughput of the products at an extremely high process cost. Hence, the separation techniques that can yield high throughput of the products at a low cost are highly desired in biotechnological industries. Of these potential candidates, ultrafiltration (UF) has attracted a considerable amount of attention in recent years for the separation of proteins due to comparatively gentler towards the proteins than separation process on phase changes and more economical than gel chromatography [3–8].

The applications of UF are limited to systems where the solutes to be separated have more than 10-fold difference in molecular weight. In such cases, molecular size is the sole criteria for separation. However, it is possible to separate solutes having comparable molecular weights by adequately manipulating the parameters such as pH, ionic strength, and applied pressure [3]. van Eijndhoven et al. [4] have shown the possibility to improve the selectivity of albumin/hemoglobin by reducing salt concentration and adjusting the pH to near isoelectric point (p*I*) of hemoglobin. Feins and Sirkar [5] have separated two proteins with relatively close in molecular weight with internally staged UF. The fractionation of lysozyme (Ly)/ovalbumin and Ly/myoglobin mixtures by 100-kDa hydrophilic polyacrylonitrile membrane has been studied in a vortex flow ultrafilter [9]. Saksena and Zydney [3] have also studied the transport of IgG and BSAusing 100- and 300-kDa PS membranes in a stirred cell. The separation of Ly and BSA by Amicon PM 30 membrane and the effect of salt concentration and BSA-Ly interaction on the

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rate of Ly washout were also examined [10]. However, the main problem restricting practical applications is membrane fouling [9].

Flux decline during protein UF is mainly attributed to membrane fouling, resulting from the accumulation of proteins drawn toward filtering surface by convective flow of filtrate through the membrane. There are some strategies to increase the flux through pretreatment of the membranes such as plasma, grafting, irradiation with UV, and pre-adsorption of appropriate matter [11–13]. In general, these treatments are comparatively complicated and expensive to practical operations. A pulsed electrical field has been actually applied to clean the fouled membrane [14]. It was found that cleaning by electrical fields is moderately effective when the particles of the solute and the membrane have same sign of zeta potential.

Alternatively, ultrasound has been widely applied as a cleaning method due to the cavitation, phenomena or acoustic streaming or turbulence [15]. Ultrasound is the sonic wave at frequencies ranging from 16 kHz to 10^{19} Hz [16]. When the ultrasonic energy at high power is applied to a liquid, cavitation takes place, which means the formation, growth, and sudden collapse of bubbles in liquids. The acoustic streaming and shear forces imposed by cavitation bubbles reduce the fouling on membrane surface. This leads to an increase of permeate flux. There are many studies on the enhancement of flux through various membranes with ultrasound [17–24]. For example, Li et al. [17] have examined the influence of ultrasound on the diffusion of electrolytes through a cellophane membrane, and observed that the diffusion with ultrasound is faster than that without ultrasound. Band et al. [18] have studied the effect of specially modulated ultrasound signals on water desalination with an ion-exchange hollow fiber. The enhancement increased with increasing ultrasonic power for Na^+–H^+ exchange. Chai et al. [19] have applied ultrasound to clean polymeric UF and MF membranes fouled by peptone permeation, and observed that cleaning of the fouled membranes by ultrasound in association with water cleaning is effective. It was noticed that the effectiveness of ultrasound in membrane filtration depends on many factors including ultrasonic frequency and power, ultrasonic irradiation angle, and the position of ultrasonic vibration plate in the membrane module. Although many researchers have studied the effect of the parameters such as pH value, applied pressure, NaCl concentration, membrane orientation, and ultrasonic power on the performance of filtration processes, most of the previous studies focused on their influences on the volumetric flux particularly for the parameter of ultrasound. The effect of ultrasonic power on the separation capability of protein mixtures by cross-flow UF remains unclear.

In this work, Ly and BSA were chosen as model proteins and the membranes with a MWCO of 30 kD were selected. The effect of ultrasound on separation ability, flux, and protein structure during cross-flow UF of binary protein solutions was investigated in order to better understand the ultrasound contribution and thereby improve the performance of UF process. Factors affecting UF flux and solute rejection such as solution pH, ionic strength, applied pressure, ultrasonic power, and the position of ultrasonic vibration plate were examined. The enhancement of UF flux with ultrasound in continuous membrane processes was finally demonstrated.

2. Materials and methods

2.1. Reagents and membranes

Lysozyme (Ly, MW 14,300) and bovine serum albumin (BSA, MW 66,430) were offered from Sigma Co. The p*I* values for Ly and BSA are 11.0 and 4.9, respectively. The single protein solution was prepared by dissolving protein in 50 mM phosphate buffer, in which the pH was adjusted in the range 4.9–11.0. The solution was gently agitated for 1 h to ensure homogeneity at 25 °C. Prior to use, the buffer was filtered through a 0.45-μm Durapore membrane (Millipore, Bedford, MA). The binary protein solution was obtained by mixing single solutions with gentle agitation for 20 min, and the solutions were pre-filtered through a 0.45-μm Durapore membrane to remove any undissolved proteins and large particulates. The ionic strength of protein solutions was adjusted by the addition of NaCl. The particle sizes of protein molecules were measured instantly after ultrasonic irradiation for 3 min at 25 °C (Zeta Size Nano Series, NANO-2S).

Polysulfone (PS) and polyvinylidine fluoride (PVDF) flat membrane used were supplied from Osmonics Co. Both asymmetric membranes had a MWCO of 30,000 and a dimension of 15 cm × 7.5 cm. The average pore size of the dense skin layer on PS and PVDF membrane was measured to be 0.028 and 0.025 μm, respectively, by capillary flow porosmetry (Porous Materials CFP-1500 AEXL, USA). The zeta potentials of both membranes were measured by the Center for Membrane Technology, CYCU, Taiwan [25]. Prior to use, these membranes were soaked overnight in protein solutions to ensure the attainment of equilibrium between membrane and protein molecules. The contact angle of deionized water droplets on the membrane surface was measured at 25 °C and a relative humidity of ambient air of about 60% (CA-VP150).

2.2. Cross-flow UF experiments

Fig. 1 shows the experimental set-up, in which the retentate was in totally re-circulating mode. The UF cell, equipped with a 2-L feed reservoir, had a channel dimension of 2 mm high, 75 mm wide, and 150 mm long. The constant cross-flow velocity of 1.5 cm/s was chosen. The temperature was fixed at 25 °C controlled by cooling water. It was experimentally found that the permeate flux, without ultrasound, could reach steady state within about 30–35 min. Except in continuous runs, the flux was thus started to measure after 35-min operation and the permeate was collected (30 cm^3) to analyze the concentrations of proteins. Thus, the permeate flux (J_v) at each run was calculated in the time intervals t_1 and t_2 by

$$J_v = \frac{V_{t_2} - V_{t_1}}{A(t_2 - t_1)} \quad (1)$$

where A is the effective membrane area and V is the volume of the permeate.