Protein fractionation in a vortex flow filter. II: Separation of simulated mixtures

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Abstract

This work investigates the fractionation of simulated mixtures of lysozyme/ovalbumin and lysozyme/myoglobin in a vortex flow ultrafilter. The operating parameters for the separations were identified from transmission studies conducted previously for each of these three proteins [3]. At low transmembrane pressure and high membrane rotation speed, the ultrafiltration characteristics of a dilute protein mixture were virtually identical to those of its individual components. The selectivity was controlled primarily by the extent of polarization of the smaller, preferentially transported species (lysozyme). Thus, high separation factors were obtained under operating conditions favoring lysozyme buildup at the membrane surface. It was further observed that better separation occurred at low fluxes (corresponding to low applied pressure) thereby indicating that the throughput and degree of resolution are mutually exclusive.

Keywords: Ultrafiltration; Vortex flow filter; Protein transmission; Concentration polarization; Protein fractionation; Separation factor

1. Introduction

The recovery of a specific protein from a fermentation broth broadly involves cell harvesting/cell debris removal, followed by concentration and fractionation of the macromolecular mixture. The first two steps commonly employ membrane based processes like microfiltration (MF) and ultrafiltration (UF). The technique offers the advantages of ease of operation, coupled with high throughputs while maintaining product purity under ambient conditions. In spite of these benefits, resolving proteins by UF has had few practical applications. This limitation is chiefly attributed to the constraints imposed by several factors, namely, non-uniform membrane pore size, concentration polarization, membrane fouling and solute-solute interactions. However, it is now well established that choosing hydrophilic membranes in combination with filtration modules offering improved mass transfer characteristics can restrict the deleterious effects of fouling and concentration polarization respectively. In addition, a judicious selection of the UF operating conditions can result in reasonably successful fractionations [1,2]. As such, selective filtration can be explored as a possible alternative to conventional processes like ammonium sulfate precipitation for the primary separation of protein mixtures.

In an earlier paper [3], we reported the UF characteristics of three different proteins viz. lysozyme,
ovalbumin and myoglobin in a vortex flow filter as a function of module hydrodynamics and solution environment. The study indicated that the protein transmission varied over a wide range and could be controlled by appropriate manipulation of the system parameters like transmembrane pressure (TMP), membrane rotation speed, protein concentration, ionic strength and the solution pH. This paper presents the separation of simulated lysozyme/ovalbumin and lysozyme/myoglobin mixtures under optimum experimental conditions identified from single protein studies. The aim was to obtain high separation factors and any moderate flux decline, though undesirable, was considered of secondary consequence. The strategy thus developed for simulated mixtures can be ultimately applied for practical separations such as the resolution of lysozyme and ovalbumin directly from crude egg white.

2. Previous studies

The fractionation of macromolecular mixtures by UF has generated considerable interest in the past decade. Chen and Zall [4] investigated the optimum conditions for the concentration/purification of D-like and B-like cathepsin proteases from clam visceras extract using a thin channel UF device equipped with a 30 kD molecular weight cutoff (MWCO) membrane. They observed that operation of the system at high recirculation velocity at pH 2.5 resulted in rapid concentration of the extract. However, at the expense of reduced flux, the separation of the two proteases appeared more feasible when operating at low applied pressure and recirculation rates. Slater et al. [5] studied the separation of bovine alkaline phosphatase (140 kD) from BSA (69 kD) using a regenerated cellulose membrane (MWCO 100 kD) in a laboratory scale stirrer unit operating between 0–500 rpm. The studies were done utilizing various buffers, operating pressures and stirrer speeds. The stirrer speed was found to be the most significant factor in determining flux decline and improving separation efficiency. The separation factor, however, was quite poor (< 2).

It is now well recognised that apart from molecular sieving, protein separation is influenced by the existence of solute–membrane and solute–solute interactions, in addition to protein adsorption and concentration polarization [6]. The interdisciplinary nature of this problem requires not only a successful exploitation of fluid mechanical and mass transfer processes but also of the surface and colloidal properties. The latter has focused attention upon various techniques of membrane modification, as well as use of novel membrane materials. Higuchi et al. [7] reported successful separations of BSA and γ-globulin in a stirred batch cell using surface modified polysulfone membranes (MWCO 200 kD) possessing –CH(CH₃)CH₂OH segments. In this instance, the smaller BSA was concentrated in the retentate and the larger γ-globulin in the permeate. They suggested that this separation was caused by the balance of hydrophilic and hydrophobic groups on the surface modified membrane.

Nakao et al. [8] proposed the use of charged UF membranes in preference to uncharged ones for the separation of macromolecules of similar dimensions. Apart from the pore size, a charged membrane possesses the additional variables of the sign and the density of charge. Such a membrane would expel similarly charged solutes and colloids so that it is comparatively less fouled by gel layer formation than the uncharged membrane. This has been verified in the UF of ovalbumin solutions using negatively charged sulfonated polysulfone membranes [9]. For their investigations on the separation of myoglobin–cytochrome c mixtures, Nakao et al. [8] prepared both negatively as well as positively charged polymers. The former was obtained by sulfonation of polysulfone while the latter was synthesized by chloromethylation of polysulfone followed by quaternization of the amino group. The membranes were cast from the solutions of the charged polymer, with the MWCO being determined by the choice of the casting conditions. These membranes were found to strongly reject like charged solutes while enabling electrically neutral molecules to pass through. Thus, it was possible to effectively separate myoglobin–cytochrome c mixtures by these membranes by setting the solution pH near the pI of one of the proteins. Positively charged UF membranes prepared from quaternized polyacrylonitrile grafted poly(N,N-dimethylaminoethyl methacrylate) have also been
tested for the permeation of BSA and γ-globulin [10]. In buffered saline solution, it was observed that below its pI, the albumin permeation was very low but increased markedly at higher pH when the protein became negatively charged.

Instead of using synthetic polymers, some workers have explored unconventional membrane materials for protein fractionation. Schnabel et al. [11] described the use of porous glass membranes of varying pore radii for the separation of a multicomponent mixture of lysozyme, BSA, IgG and ferritin. These membranes were characterized by a narrow pore-size distribution (standard deviation < 10%) ensuring sharp cutoff behaviour. A 7.3 nm pore size membrane allowed free passage of lysozyme while albumin, IgG and ferritin were retained in increasing degree in that order. Sakai et al. [12] reported the use of similar microporous glass membranes for low temperature plasma separation. Sara and Sleytr [13] developed UF membranes from surface or S-layers of *B. stearothermophilus* and *C. thermohydrosulphuricum* strains after crosslinking with glutaraldehyde. The S-layers are paracrystalline arrays of protein or glycoprotein subunits linked by non-covalent interactions which completely cover the cell surface. They are highly porous (porosity 20–50%) and possess 2–6 nm diameter channels. Because of their isoporous structure, these membranes displayed very steep rejection curves and thus acted as accurate molecular sieves. Fractionation of a multicomponent mixture of carbonic anhydrase, ovalbumin and BSA resulted in complete enrichment of ovalbumin and BSA in the retentate. Several commercial UF membranes (MWCO 20, 30, 50 and 100 kD) were unable to satisfactorily resolve this mixture; a fact which was attributed to their broad pore size distribution. In addition, Kuepcue et al. [14] demonstrated that the surface properties of the S-layer UF membrane could be altered by proper amidation of the free carboxyl groups on the surface and in the pores. Thus, it was possible to obtain a positively charged or neutral UF membrane apart from the inherently negative one.

An alternative strategy for obtaining effective separations involves manipulating the protein characteristics like size and surface charge. Pouliot et al. [15] convincingly demonstrated that the fractionation of casein hydrolysate is predominantly determined by the charge/hydrophilicity balance of the peptides. This can be accomplished by proper adjustment of the feed solution properties, notably the pH and ionic strength. Ohno et al. [16] reported separations of albumin (69 kD, pI 4.7) and immunoglobulins (155 kD, pI approximately 7.0) using 100 kD MWCO polyethersulfone membranes by maintaining the solution pH between 4 and 5 and the salt concentration below 0.2 M NaCl. The total protein concentration in this instance was kept less than 40 g/l. The protein selectivity decreased significantly both at high salt concentrations and at higher solution pH. In contrast, Higuchi et al. [7] were unable to obtain significant albumin–IgG separations at pH 5, using either native or surface modified polysulfone membranes of MWCO rating 200 kD. However, the surface modified membrane exhibited high selectivity for IgG (defined as the ratio of IgG in the permeate to that in the retentate divided by the same ratio for albumin) when the experiments were carried out at higher pH (7.2 and 9.0). This was due to nearly complete retention of the smaller albumin molecules in the presence of higher throughput of the larger IgG.

Nakao et al. [8] studied the effects of electrostatic interactions on solute sieving using very large pore size membranes which had essentially no steric selectivity for the different proteins. The protein sieving coefficients were strong functions of pH, with the smallest rejections obtained when the solution pH was equal to the protein isoelectric point. They demonstrated that these membranes could effect significant separation between myoglobin (17.5 kD, pI 6.8) and cytochrome c (12.4 kD, pI 9) based purely on electrostatic interactions.

Sudareva et al. [17] examined the influence of solution pH on the separation of mixtures of albumin/cytochrome c and ribonuclease/hemoglobin. In all cases, the separation attained with the binary mixtures was dramatically reduced compared to that predicted from single solute measurements. This was ascribed to the formation of complexes arising from electrostatic interactions between the oppositely charged proteins in the bulk solution. The selectivities were quite poor under all conditions and the maximum selectivity for ribonuclease was attained at the isoelectric point of the larger hemoglobin molecule. In contrast, Nakatsuka and Michaels [1] demonstrated that it was possible to obtain very good separation of myoglobin (pI 6.8) and BSA (pI 4.8)
using nonsorptive, regenerated cellulose membranes of MWCO rating 30 kD. The selectivity was relatively independent of solution ionic strength but decreased as the solution pH increased from 4.8 to 6.8. This decrease in selectivity was due to the increase in myoglobin retention at higher pH, which the authors attributed to the increase in myoglobin adsorption to the membrane pores at its isoelectric point.

Zhang and Spencer [18] performed a series of experiments on the separation of albumin and IgG using formed-in-place microfiltration membranes of titanium dioxide supported on a porous stainless steel backing. Data at pH 4.9 showed albumin selectivities of about 2–6 at very low salt concentrations, with the separation decreasing significantly with added salt. Data at neutral pH showed almost no separation under any conditions. However, very high selectivities were observed at pH 8 for dilute protein mixtures (< 1 g/l) at very low salt concentrations, with the filtrate being more concentrated in the larger immunoglobulins. This pH dependence was explained in terms of the alteration in the protein mobility within the membrane pores due to the effect of pH on the electrostatic interactions between the proteins and the membrane. The IgG selectivity at pH 8 was, in addition, a function of the membrane permeability and hence of the filtrate flux.

More recently, Saksena and Zydney [2] investigated the transport of BSA and IgG, both separately and in mixture, through 100 and 300 kD polyether-sulfone membranes in a stirred UF device at varying pH and ionic strength. They reported that the selectivity was a complex function of the filtrate flux due to the simultaneous convective and diffusive solute transport through the membrane and the bulk mass transfer limitations in the stirred cell. Under physiological conditions (pH 7 and 0.15 M NaCl), the low selectivity for this mixture (< 2) was attributed primarily to the result of protein adsorption. In contrast, selectivities as high as 50 were obtained at pH 4.8 and low salt concentration (0.0015 M NaCl) as a result of the electrostatic contributions to both bulk and membrane transport. They showed that the selectivity could actually be reversed, with the higher passage of the larger IgG molecules by using a 300 kD MWCO membrane at pH 7.4 and an ionic strength of 0.0015 M NaCl.

3. Experimental

3.1. Materials

The proteins, viz. chicken egg lysozyme (13.93 kD, pl 10.6), horse heart myoglobin (16.89 kD, pl 6.8) and chicken egg ovalbumin (43.5 kD, pl 4.6) were supplied by Sigma Chemical Co., St. Louis, MO, USA. All buffers and solutions were prepared in ultrapure water (resistivity 18.2 Mohm-cm) obtained from a Milli-Q unit (Millipore Corp., Bedford, MA, USA). The buffer reagents were analytical grade and were purchased locally from E. Merck.

3.2. Method

The UF was performed on a Benchmark™ Gx vortex flow filter using surface modified polyacrylonitrile membranes of MWCO rating 100 kD. Both the membranes and the filter unit were procured from Membrex Inc., NJ, USA. The details of the UF module as well as the experimental procedure are described elsewhere [3].

3.3. Data analysis

The protein samples were analyzed by a Pharmacia FPLC system (Pharmacia LKB Biotechnology, Uppsala, Sweden) with a LCC-500 Plus liquid chromatography controller and a REC 102 recorder. A Superdex™ 75 HR 10/30 gel filtration column with a total bed volume of 24 ml was used. The gel matrix is composed of highly crosslinked porous agarose beads which are covalently bonded to dextran and is recommended for proteins in the 3–70 kD molecular weight range [19]. 200 µl of the sample was automatically loaded onto the column by a MV-7 motor valve. The sample was eluted using a buffer flow rate of 0.8 ml/min which was accurately controlled by a high precision P-500 pump. The elution buffer (citric acid–phosphate buffer, pH 6.8, 0.15 M NaCl) was filtered through a 0.1 µm filter and degassed prior to use. The concentration of the eluted protein was detected online by a UV-MII monitor with a 280 nm filter.

The separation factor (SF) was defined as:

\[
SF = \left( \frac{\tau_{obs}}{\tau_{obs}} \right)_1 \left/ \left( \frac{\tau_{obs}}{\tau_{obs}} \right)_2 \right.
\]
where $\tau_{\text{obs}} (= C_p/C_b)$ is the observed transmission, $C_p$ and $C_b$ are the protein concentrations in the permeate and bulk feed, respectively, subscript 1 refers to the preferentially permeating component and subscript 2 to the rejected component.

4. Results and discussions

In our previous work on single proteins [3], we showed that under physiological conditions (pH 6.8, 0.15 M NaCl), the transmission of lysozyme (100–120%) differs perceptibly from that of ovalbumin (25–55%) and to a lesser extent, myoglobin (70–90%). Further, the transmission could be controlled by adjusting the UF operating conditions, the effect of which is summarized in Table 1. Based on this data, the separation of lysozyme–ovalbumin and lysozyme–myoglobin was investigated under different filtration conditions.

4.1. Lysozyme–ovalbumin separation

The selective filtration of lysozyme and ovalbumin mixtures was initially performed at different membrane rotation speeds (Fig. 1). This helped in gauging the extent to which concentration polarization influenced the separation characteristics. The feed solution composition of 0.1 g/l lysozyme and 2 g/l ovalbumin was chosen to simulate the approximately 1:19 ratio of these two proteins present naturally in egg white [20]. The volume flux [Fig. 1(a)] was reasonably linear at 3000 rpm but became constant above 40 kPa at 1000 rpm. Fouling was noticeably absent in both instances with less than 10% reduction in the membrane permeability. Apart from the reduced mass transfer at the lower rotation speed, the flux decline was probably further aggravated by the fact that mixing particles of different size increases the overall packing density and thus enhances the resistance to solvent permeation [21]. This effect would be particularly pronounced when the two solutes have opposite charge (as in this situation with lysozyme being positive and ovalbumin negative at pH 6.8) [22]. The corresponding transmission data is presented in Fig. 1(b). The difference in lysozyme and ovalbumin transmission was much larger at 3000 rpm, particularly at low TMP (< 30 kPa). Increased solute buildup of both the proteins at 1000 rpm narrowed this gap and thus had a deleterious effect upon the degree of separation. Consequently, higher separation factors (SF) defined as the ratio of lysozyme to ovalbumin transmission, i.e. $(\tau_{\text{obs}})_L/(\tau_{\text{obs}})_O)$ were attained under conditions of high rotation speed and low fluxes corresponding to low applied pressure [Fig. 1(c)].

A surprising aspect of these results is that the transmission characteristics of this mixture are almost identical to those of the corresponding single proteins [3], particularly at 3000 rpm. The lysozyme transmission, especially, continued to be 100% and above even in the presence of ovalbumin. This is contrary to the postulate that the polarization buildup of the rejected solute (ovalbumin) hinders the passage of a completely transmitting smaller molecule (lysozyme) [2,7,23–25]. However, Nakatsu and Michaels [1] reported an exception in the unstirred UF of BSA–myoglobin mixtures through a non-sorp-

<table>
<thead>
<tr>
<th>Protein</th>
<th>τ_{obs} range (%)</th>
<th>↑ Axial velocity (Re_{axial} 45–120)</th>
<th>↑ rpm</th>
<th>↑ Protein concentration (0–2.5 g/l)</th>
<th>↑ Ionic strength (0–1 M NaCl)</th>
<th>↑ pH</th>
</tr>
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<tr>
<td>Lysozyme</td>
<td>30–120</td>
<td>×</td>
<td>↑</td>
<td>↓</td>
<td>0–0.15 ↑</td>
<td>↑</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>0–70</td>
<td>×</td>
<td>↓</td>
<td>×</td>
<td>0.15–1 ↓ then ↓</td>
<td>↑</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>0–90</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>0–0.15 ↑</td>
<td>↑</td>
</tr>
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*a Re_{axial}: axial Reynolds number.

*b Ta: Taylor number; ↑, increases; ↓, decreases; ×, no change.
tive, regenerated cellulose membrane (MWCO 30 kD). In the experiments performed at low bulk concentrations (1 g/l) and low applied pressures (< 100 kPa), the protein rejections were approximately the same as for single solute measurements. They explained that the unobstructed passage of the smaller myoglobin molecules under these circumstances indicated that the BSA polarization layer, at least at low TMP, was freely permeable to myoglobin. However, in this investigation, the unexpectedly high passage of lysozyme in the permeate (up to 120%) is attributed to the presence of attractive protein–membrane interaction [26]. Therefore in the present instance, the smaller lysozyme is preferentially pulled towards the membrane surface and the polarization layer thus formed does not restrict the transport of the larger ovalbumin molecules.

The influence of lysozyme buildup was examined by varying the total protein concentration as illustrated in Fig. 2. Maintaining a constant solute ratio of 1:1, the total protein concentration was stepped up from 0.2 to 2 g/l. The volumetric flux decreased at higher concentrations and the pattern was in good agreement with that for lysozyme alone with increasing bulk concentration. Fouling could be ignored as the loss in membrane permeability was well below 7.5%. The lysozyme transmission [Fig. 2(b)] also remained above 100% and roughly concurred once more with the corresponding data obtained for the single protein [3]. The ovalbumin transmission was, however, adversely affected by increasing the protein concentration in the feed with values as low as 20% for the 1 g/l–1 g/l lysozyme–ovalbumin mixture. The ovalbumin transmission appeared to be controlled primarily by the lysozyme polarization layer which, in turn, was dependent upon the amount of lysozyme present in the solution. It was almost independent of varying ovalbumin concentration as exhibited by Fig. 3(b). The accompanying fluxes were also only marginally reduced by increasing the ovalbumin concentration from 0.1 to 2 g/l while keeping the lysozyme concentration fixed at 0.1 g/l [Fig. 3(a)]. Except at very low fluxes (< 30 × 10^{-6} m/s), the separation factors as shown in Fig. 3(c) were unaffected by a rise in the ovalbumin concentration. These results confirm that the lysozyme buildup on the membrane surface due to attractive protein–membrane interactions at pH 6.8 acted as a secondary barrier to the transport of ovalbumin across the membrane. Therefore, improved separation is possible by operating under conditions favoring selective polarization of lysozyme alone on the up-

![Graph](image-url)
stream side of the membrane as demonstrated in Fig. 2(c).

An alternative method of enhancing lysozyme polarization in the presence of low ovalbumin buildup is to appropriately manipulate the solution pH/ionic strength. It is evident from Table 1 that low salt concentrations result in lower transmission values of ovalbumin. Since lysozyme was attracted and ovalbumin was repelled at pH 6.8, reducing the amount of salt in the feed solution would heighten the pro-

Fig. 3. Effect of ovalbumin concentration on lysozyme (L)–ovalbumin (O) fractionation (500 ml/min crossflow, 3000 rpm, 0.15 M NaCl, pH 6.8). (a) Flux vs. TMP. (b) Transmission vs. flux. (c) Separation factor vs. flux.

Fig. 2. Effect of total protein concentration on lysozyme (L)–ovalbumin (O) fractionation (500 ml/min crossflow, 3000 rpm, 0.15 M NaCl, pH 6.8). (a) Flux vs. TMP. (b) Transmission vs. flux. (c) Separation factor vs. flux.
Flux (m/s) X 10^6

Flux vs. TMP

Transmission (%)

Transmission vs. flux

Separation factor (-)

Separation factor vs. flux

Fig. 4. Effect of ionic strength on lysozyme (L)–ovalbumin (O) (0.1 g/l–2 g/l) fractionation (500 mL/min crossflow, 3000 rpm, pH 6.8). (a) Flux vs. TMP. (b) Transmission vs. flux. (c) Separation factor vs. flux.

The results are presented in Fig. 4. Low ionic strength (0.05 M NaCl) was characterized by low flux, which improved with further addition of salt [Fig. 4(a)]. This trend was once more in agreement with the pattern displayed by lysozyme alone at varying NaCl concentrations [3]. However, fouling was totally absent at low ionic strengths in this instance. This was in complete contrast to the situation for pure lysozyme UF at 0.05 M NaCl which exhibited 52% reduction in the membrane permeability. This observation can be analyzed qualitatively as follows. The lysozyme–membrane attraction is particularly strong at low ionic strength. Consequently, lysozyme gets adsorbed on the membrane surface at 0.05 M NaCl, leading to low flux accompanied by high fouling. In the presence of ovalbumin, an additional associative interaction between the two species is also important. More so, because at pH 6.8 the molecules are oppositely charged and the low salt concentration will not provide effective ionic shielding [24]. As a result, the overall lysozyme–membrane interaction is weaker now than in the case of the pure lysozyme alone. This overcomes the fouling but the protein–membrane interaction is still sufficiently strong to cause enrichment in the permeate. This system thus provides an example where the solute–solute interaction between different components was actually beneficial to the separation process. It is in contrast to the generally accepted view that solute–solute interactions should be minimized during fractionation by operating at a pH where both the proteins are similarly charged [22]. Fig. 4(b) displays the corresponding transmission profiles. The lysozyme transmission was consistently well above 100% with values reaching 140% at 0.05 M NaCl while ovalbumin exhibited a steady increase in transmission upon salt addition. High separation factors (up to 30) were consequently achieved while operating at low ionic strength [Fig. 4(c)].

A common feature of the separation factor vs. flux profile in all the cases discussed above is the high degree of separation observed at low fluxes (corresponding to lowTMPs). This suggests that the throughput and the degree of resolution are mutually exclusive factors in protein fractionation by UF. As such, the purity of the product obtained may have to be compromised for achieving higher productivity to ensure that the process remains economically viable.
4.2. Lysozyme–myoglobin separation

From the results discussed above, it is apparent that enhanced polarization of lysozyme improves the rejection of the larger species (ovalbumin) thus leading to better resolution of the mixture. The same principle can be applied for the separation of a simulated mixture of lysozyme and myoglobin, the results of which are presented in Fig. 5. At low lysozyme concentration (0.2 g/l) the flux increased proportionately with increasing applied pressure. An increase in the amount of lysozyme in the solution results in increased curvature in the flux profile. The pattern was once more similar to the corresponding single protein data for varying lysozyme concentration [3]. Also, the membrane fouling was noticeably absent in both the instances. The accompanying transmission [Fig. 5(b)] continued to be between 100–115% for lysozyme and was only marginally affected by elevating the lysozyme concentration from 0.2 to 1 g/l. However, the myoglobin transmission which was in the 70–90% range in the presence of 0.2 g/l lysozyme, dramatically reduced to 40% for 1 g/l lysozyme. So, a better separation (SF of about 3) was obtained in the latter instance [Fig. 5(c)]. This experiment yet again demonstrates the advantage of operating under conditions that selectively polarize the smaller species. It was, thus, possible to resolve even close MW proteins like lysozyme (13.93 kD) and myoglobin (16.89 kD) in a single step using UF.

5. Conclusions

In this work, we have investigated the selective filtration of simulated lysozyme/ovalbumin and lysozyme/myoglobin mixtures in a vortex flow filter using 100 kDa "ultrahydrophilic" membranes. The salient features of these separations are summarized below.

- Under conditions of low TMP and high mass transfer, the UF characteristics of dilute mixtures of proteins are virtually identical to those of the corresponding single protein measurements. Thus, it is feasible to carry out successful separations for proteins which display a reasonable difference in transmission during UF of the single component.
- A high degree of separation is generally obtained at low fluxes (corresponding to low TMPs). Thus, the throughput and the degree of resolution are mutually exclusive factors in protein fractionation by UF.
It is possible to improve the separation of protein mixtures by operating under conditions favoring the polarization of the smaller solute on the membrane surface by appropriate adjustment of the solution conditions (viz. feed concentration, pH and ionic strength). This, in effect, increases the rejection of the larger species. Under these conditions, it is possible to resolve even proteins very close together in their molecular weights (as demonstrated for lysozyme and myoglobin mixtures in this work).

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**References**


