Initial Flux and Rejection Characteristics of Partially Permeable Ultrafiltration Membranes

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Synopsis

Solutions of bovine serum albumin (BSA) were ultrafiltered with and without stirring through membranes partially permeable to the solute, over a range of pH values. At the isoelectric point, flux was a minimum and rejection was a maximum. For all conditions, the flux for stirred ultrafiltration was greater than without stirring, as expected from conventional theory, and in contrast to recently reported "anomalous" behavior measured at the isoelectric point. Some evidence of unusual behavior at the isoelectric point was obtained when the flux of a freshly ultrafiltered solution of BSA was compared to that when the permeate and retentate were recombined, and when the retentate concentration was adjusted to the original concentration. For pH values other than the isoelectric point, the fluxes were similar for each set of experiments. At the isoelectric point, it was also found that flux was insensitive to changes in stirring speed. The unusual behavior at the isoelectric point is attributed to protein aggregation and precipitation causing loss of membrane permeability.

INTRODUCTION

In a recent note,[1] Swaminathan et al. reported some unusual flux behavior for stirred cell ultrafiltration (UF) of proteins through membranes with partial solute permeability. To summarize, they found: (1) higher fluxes were achieved without stirring than with stirring for partially permeable membranes; (2) the flux for stirred cell UF of a fresh 0.05% bovine serum albumin (BSA) solution was considerably higher than for stirred cell UF of the retentate adjusted to the original concentration, with the same but cleaned membrane.

These results were obtained at a pH value of 4.8, corresponding to the isoelectric point of the protein used. At this pH, the protein has no net charge, and it may form aggregates.[2] Indeed, Swaminathan et al.[1] hypothesized that their unusual results may have been caused by the effect of stirring on the size of the protein aggregates in the polarized layer, with smaller aggregates resulting in reduced permeability and lower flux. On this basis, UF without stirring would have larger aggregates and higher flux. Similarly, the fresh BSA solution would have larger aggregates than the rediluted retentate which would have been previously subject to stirring. These unusual phenomena invite further investigations, particularly to see if they are readily obtainable over a range of pH values, including those where aggregate formation is not prevalent.

As part of a study of protein fractionation, the present authors have been examining the initial-time and steady-state flux and rejection characteristics of partially permeable membranes with BSA solutions. Because pH affects the charge and shape of the proteins, it has been varied either side of the isoelectric point; both stirred and nonstirred UF have been examined. While our experi-

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mental conditions were close to those used by Swaminathan et al., our flux data show different trends, more in line with those of retentive membranes. The simultaneous rejection data provide insight into this behavior. Additional studies of stirred cell UF with rediluted retentate and with recombined retentate and permeate and studies with increased stirring show that operation at the isoelectric point is qualitatively different from that at other pH conditions.

EXPERIMENTAL

All experiments were done in a magnetically stirred cell with 15 cm^2 membrane area using Amicon Diaflo XM-100A membranes and an 0.05 wt % solution of BSA (Calbiochem A grade, 100% purity), buffered with 0.1*M* citric acid–sodium phosphate. Three pH levels were studied: pH 3.0 (BSA molecules positively charged), pH 4.8 (the isoelectric point), and pH 7.4 (BSA negatively charged). The applied pressure was 100 kPa for both stirred and nonstirred experiments, and the temperature was 25°C.

Prior to an experiment, the cell was dried before fitting a new or cleaned membrane. An initial batch volume of 100 ml of solution was then introduced and the appropriate pressure applied by compressed nitrogen so that ultrafiltration commenced immediately, without preequilibration of the membrane. The experiment was conducted as a batch concentration to about half the initial volume. Fluxes were measured by noting the time for collection of 5-ml quantities of permeate. Analysis for BSA content in the permeate was by UV spectrophotometry, and the rejection associated with each 5 ml of permeate was calculated by

$$\text{rejection} = 1 - (\text{BSA concentration in permeate sample})/(\text{BSA concentration in the cell according to mass balance})$$

Overall mass balances involving analysis of feed, final retentate, and accummulated permeate were found to close within ± 1%. At each pH, the flux and rejection histories were measured in batch concentration runs with stirring and a second run without stirring. Stirring speeds of 500 and 1000 rpm were used, with the majority being at 500 rpm. A new membrane from the same batch of membranes was used for each run, except for those at 1000 rpm, which used a cleaned membrane. Two additional batch concentration runs were performed at each pH with stirring using retentate diluted to the original concentration and using recombined retentate and permeate from the previous run. Before each of these additional runs, the membranes were cleaned by the procedure recommended by Amicon using dilute (0.1*M*) NaOH solution for a period of 15 min. This cleaning procedure typically returned the water flux to 90% of its initial value, but was not able to restore fully the flux, even when repeated several times. Table I compares the conditions used in this work with those used by Swaminathan et al.[1,3]

RESULTS AND DISCUSSION

The flux histories at the three pH levels for both stirred and nonstirred conditions are shown in Figure 1. For comparison pseudo steady-state flux values based on the stirred and nonstirred data of Swaminathan et al.[1] for Amicon

TABLE I
Comparison of Pertinent Experimental Conditions Used in this Work and by Swaminathan et al.[1,3]

	This work	Swaminathan
Membrane	XM100A, new and used	XM100A, used
Solution	BSA 0.05 wt % (in citric phosphate buffer)	BSA 0.05 wt % (in citric phosphate buffer)
pH	3.0, 4.8, 7.4	4.8
rpm	500, 1000	800
Cell diameter	4.4 cm	6.5 cm
ΔP	100 kPa	138 kPa
Mode of operation	batch concentration	diafiltration

XM-100A membranes are also included. Swaminathan's stirred result has been corrected for differences in stirring speed through the relationship

$$\text{flux} \propto (\text{rpm})^{0.42}$$

from Blatt et al.[4] It is evident from this work that for stirred UF with partially permeable membranes, the flux varies significantly with pH, having a minimum value at the isoelectric point (pH 4.8).

This behavior has been reported previously for retentive membranes with protein solutions[5,6] and is believed to be associated with protein aggregation at the isoelectric point. It is of interest to note that the corrected pseudo steady-state flux for the stirred UF of BSA at pH 4.8 reported by Swaminathan et al. is not very different from the value reported in this work. Corrections for differences in pressure drop and temperature could not be made due to the absence of data, but they would tend to narrow the gap between our flux value and that of Swaminathan et al.

Figure 1 also shows that our nonstirred fluxes vary with pH, although rather less distinctly than for stirred conditions. However, the most obvious feature of the results is that the nonstirred fluxes are significantly lower than the stirred

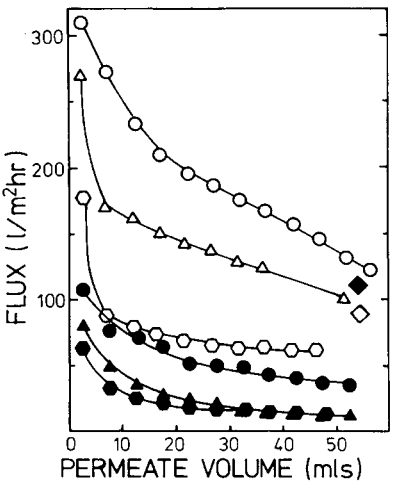

Fig. 1. Flux profiles for stirred (open symbols) and nonstirred (filled symbols) UF at pH 3.0, 4.8 and 7.4: (○, ●) pH 7.4; (△, ▲) pH 3.0; (○, ●) pH 4.8; (◇, ◆) pH 4.8, Swaminathan et al.[1]

fluxes, in contrast with the "anomalous" behavior reported by Swaminathan et al. This effect occurs at all pH values.

Conventional concentration polarization theory for partially permeable membranes relates flux J to concentrations by[7]

$$J = k \ln \frac{C_W - C_P}{C_F - C_P} \tag{1}$$

where k is the mass transfer coefficient and C_F, C_P, and C_W are the solute concentrations in the feed, permeate, and at the membrane–feed solution interface, respectively. Equation (1) indicates that a low mass transfer coefficient k resulting from the absence of stirring should lead to a lowered flux. Our results are in qualitative agreement with this.

In terms of rejection coefficient $[\delta = 1 - (C_P/C_F)]$, eq. (1) becomes

$$J \simeq k \ln \frac{C_W}{\delta C_F} \quad \text{for } \delta \to 1.0 \tag{2}$$

or

$$J \simeq k \ln \frac{C_W - C_F}{\delta C_F} \quad \text{for } \delta \to 0.0 \tag{3}$$

Accordingly, flux and rejection are interdependent, with the general expectation being that as rejection increases, flux decreases. This applies to pregel polarized operation and may become invalid following gel polarization which could give secondary membrane rejection. The time-dependent rejections for stirred and nonstirred UF are shown in Figures 2 and 3, respectively; the corresponding flux data are shown in Figure 1. For the stirred UF results, the pH 7.4 rejections are the lowest, and this is reflected in the higher fluxes at this condition. However, the pH 3.0 and 4.8 rejections are very similar although the fluxes are significantly different. The lower flux at pH 4.8 is not unexpected and can be explained by changes in solute properties, such as diffusivity, at the isoelectric point, giving a lower value of the mass transfer coefficient k. Another factor, discussed below, may be the greater tendency to lose membrane free area through protein precipitation and adsorption at the isoelectric point.

The similar (and higher) rejections at pH 4.8 and 3.0 may be caused by the effect of pH on the size and shape of the protein. At pH 4.8, the isoelectric point, BSA may aggregate[2] and this would tend to increase solute rejection. At pH

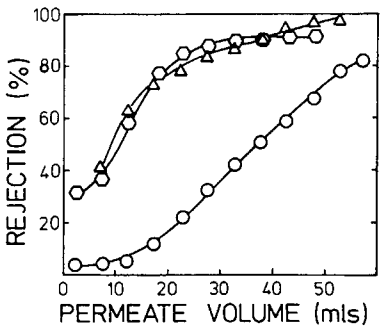

Fig. 2. Rejection profiles for stirred UF: (○) pH 7.4; (△) pH 3.0; (○) pH 4.8.

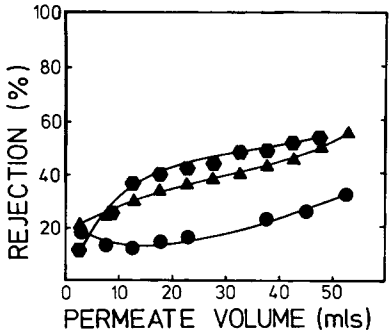

Fig. 3. Rejection profiles for nonstirred UF: (●) pH 7.4; (▲) pH 3.0; (●) pH 4.8.

3.0 the BSA molecule expands becoming longer and more asymmetric without change in molecular weight,[8] and this would also increase rejection. Similar reasoning can explain the effect of pH on rejection for non-stirred UF, as shown in Figure 3. The non-stirred UF rejections are generally lower than the stirred UF rejections, and this may be because without stirring C_W increases, due to polarisation, causing an increase in C_p.

Swaminathan et al. explained their 'anomalous' flux results by suggesting that changes occurred in the structure of the aggregates of protein molecules as a result of stirring. Under non-stirred conditions the large aggregates formed a 'porous' gel layer whilst stirring broke up these aggregates and formed a more compact gel layer of reduced permeability. However, it should be noted that if this aggregate break-up hypothesis is correct, it would also apply to retentive membranes, but as is generally accepted and acknowledged by Swaminathan et al. the effect of stirring is to increase flux with such membranes.

In support of their aggregate break-up hypothesis Swaminathan et al. showed that the flux with a fresh BSA solution at the isoelectric point was significantly higher than for the retentate adjusted to the original concentration. Figure 4 compares the flux data at pH 4.8 derived from Swaminathan et al.[1] with the flux histories obtained in this work at pH 3.0 and 4.8 for a fresh BSA solution, recombined retentate and permeate, and for rediluted retentate. Our results indicate a difference in behavior at pH 3.0 compared with that at the isoelectric point, pH 4.8. (Data at pH 7.4 are qualitatively similar to those at pH 3.0). At the lower pH there is little difference between experiments, whereas at the isoelectric point the flux profiles drop noticeably for subsequent experiments. However, the differences in flux are not as great as those reported by Swaminathan. Figure 5 shows that the rejection profiles mirror the flux profiles, as expected from eqs. (2) and (3). The most likely explanation for our results is that the water flux is not completely restored after cleaning, and that the effective free area of the membrane is slightly reduced and this results in lower flux values and higher rejections. Similar findings for flux with retentive membranes are reported elsewhere.[9]

Table I summarizes the differences between the experimental conditions used in this work and by Swaminathan et al. It is unlikely that variations in pressure drop could account for the different findings. With respect to mode of operation, in this work the retentate concentration would have increased slightly with time and for Swaminathan et al. it would have decreased with time. However, this

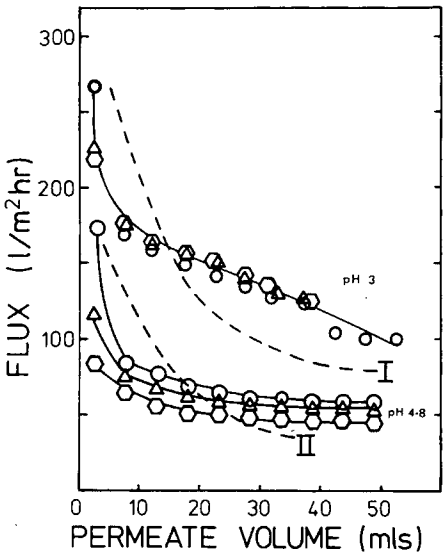

Fig. 4. Flux profiles for stirred UF of fresh solution, recombined retentate and permeate, and rediluted retentate at pH 3.0 and 4.8. Results from Swaminathan for fresh (I) and rediluted retentate (II) solutions: (○) fresh; (△, ○) rediluted or recombined; (I) Swaminathan (fresh, pH 4.8); (II) Swaminathan (rediluted, pH 4.8).

difference is again unlikely to account for the different trends in the results, as evidenced by the fact that the trends apply from the beginning of each experiment when conditions would have been very similar for the two modes.

The most significant differences between the two sets of experiments are the larger cell diameter and higher stirring speed used by Swaminathan which result in a higher average rotational *Re* compared with this work. In order to test the effect of changes in *Re* for the solution at pH 4.8, we doubled the stirring speed (*Re* α rpm); note that even at 1000 rpm, our *Re* was only 60% of that used by Swaminathan et al. Figures 6 and 7 show that there is no change in permeate flux or membrane rejection as the stirring speed increases from 500 to 1000 rpm. These experiments were done with the same used but cleaned membrane, the water flux being restored before each experiment. Figure 6 also shows that the flux profiles for a new membrane (from Fig. 1) are significantly higher than for

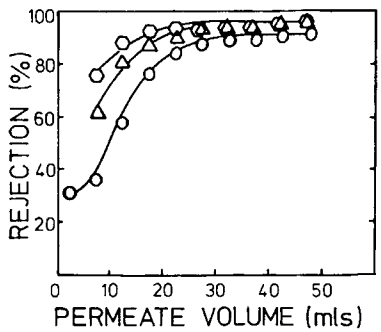

Fig. 5. Rejection profiles for stirred UF of fresh BSA solution, recombined retentate and permeate, and rediluted retentate at pH 4.8: (○) fresh; (○) recombined retentate and permeate; (△) rediluted retentate.

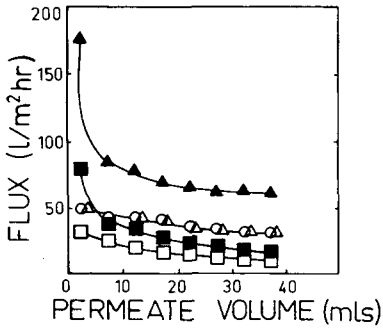

Fig. 6. Effect of stirring speed on flux profiles for UF at pH 4.8. Used, cleaned membrane: (○) 1000 rpm; (△) 500 rpm; (□) 0 rpm. New membrane (Fig. 1): (▲) 500 rpm; (■) 0 rpm.

a used membrane, showing the effect of loss of free area. However, for both new and used membranes, flux is enhanced for the stirred conditions.

It is interesting to note that although eq. (1) predicts a higher flux at 1000 rpm, there is no increase at the isoelectric pH. At pH 7.4, however, an increase in stirring speed leads to increased flux as shown in Table II for the steady state UF of a 0.1% BSA solution in 0.1*M* phosphate buffer. These observations, as well as those reported in Figure 4, do suggest that the UF of protein solutions at the isoelectric point is less predictable than at other pH values. This provides limited support for the "anomalous" flux data of Swaminathan et al.[1] Never-

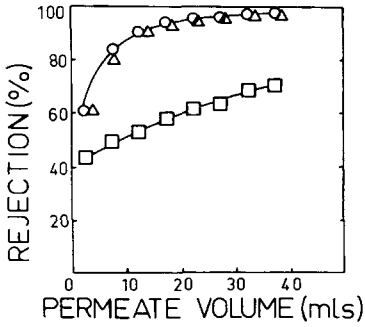

Fig. 7. Effect of stirring speed on rejection profiles for UF at pH 4.8: (○) 1000 rpm; (△) 500 rpm; (□) 0 rpm.

TABLE II
Effect of Stirring Speed and pH on Flux

pH	Flux, L/m² h				
	0 rpm	250 rpm	500 rpm	800 rpm	1000 rpm
4.8 Swaminathan	115[b]	—	—	92[b]	—
4.8 This work	15[a]	—	60[a]	—	—
	10[b]	—	32[b]	—	32[b]
7.4 0.1%BSA This work	27[b]	111[a]	134[b]	152[b]	—

[a] New membrane.
[b] Used membrane.

theless, none of our data at the isoelectric point show evidence of higher flux without stirring. The most probable explanation for our results at the isoelectric point and those of Swaminathan is that at this pH condition there is greater tendency for protein aggregates to precipitate and plug the membrane causing loss of free area. In Swaminathan's case, it would appear that the higher rotational *Re* may have caused rapid aggregate breakup and more plugging, so that from the initial stages of an experiment the free area with stirring was significantly less than without stirring.

In summary, our results suggest that the UF of protein solutions through partially permeable membranes is in qualitative agreement with conventional theory. An exception to this is operation at the isoelectric point where flux becomes insensitive to stirring. The "anomalous" behavior reported by Swaminathan et al. possibly results from their use of isoelectric conditions coupled with high shear.

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