Anomalous Flux Behavior in Initial Time Stirred Protein Ultrafiltration Through Partially Permeable Membranes

The formation of a concentrated layer of retained macrosolutes on the membrane surface exposed to high pressure is a major problem in ultrafiltration, resulting in considerable solvent flux reduction. In protein ultrafiltration, there are often additional difficulties due to the formation of a gel layer on the membrane surface at high concentrations, leading to a condition known as gel polarization. The gel polarization model of Michaels[1] postulates that, for a fixed bulk concentration of a given macrosolute, the solvent flux is solely determined by the mass transfer conditions for the macrosolute, and variables that improve feed side mixing conditions increase the solvent flux and reduce the effect of polarization. Magnetically driven stirrers are therefore used in stirred cells to promote mixing in batch laboratory ultrafiltration (UF), and it has been shown[2] that stirring enhances the steady-state solvent flux in batch UF of protein solutions with totally retentive membranes. Almost all such investigations deal only with the steady-state performance. It has however been recently demonstrated[3] that the measurement of the ultrafiltration rate and the retention characteristics during the initial transient period before the pseudosteady state has been achieved, with the gel layer (or polarized layer) dynamically forming, is crucial to the understanding of the effects of gel polarization in protein ultrafiltration. The purpose of this note is to report the effect of stirring on the initial time ultrafiltration rate of protein solutions through partially permeable membranes. Furthermore, an attempt has been made to explain the observed unusual filtration behavior wherein the UF flux decreased with stirring for partially permeable membranes unlike that with almost completely retentive membranes.

EXPERIMENTAL

Bovine serum albumin (BSA) and obalubumin were used separately for the preparation of 0.05% protein solutions at their respective isoelectric pH values (4.8 for BSA and 4.6 for ovalubumin) from McIlvain's citric acid–phosphate buffer solutions. The solutions were ultrafiltered through synthetic anisotropic substituted olefin membranes Diaflo XM100A and XM300 (Amicon Corporation, Lexington, Mass.) with and without stirring at 1.38×10^5 Pa applied pressure (20 psig) for 15 min. The pure-water permeabilities and solute retention characteristics of these membranes are presented in Table I. The properties of the solutes, the details of the buffer solution preparation, the experimental setup, and the experimental procedure for initial time ultrafiltration measurements have been reported elsewhere.[3,4] The UF membranes of this study exhibit a pore size distribution.[5] All studies with stirring were conducted at a constant stirring rate of 800 rpm, and the stirrer blades were kept immersed to the same level in the liquid in each case in order to ensure identical stirring conditions.[3,4] Vortex formation was prevented by having several baffles on the wall of the cell.

TABLE I
Characteristics of Ultrafiltration Membranes[a]

Membrane	Molecular weight cut-off	Deionized water flow,[b] ml/cm²/min	Percentage solute retention[c] Ovalbumin (45,000)[d]	Hemoglobin (64,000)[d]	Bovine serum albumin (67,000)[d]
XM100A	100,000	0.4–1.4	—	45	45
XM300	300,000	0.75–2.0	—	10	10

[a] From Amicon Catalog, Publications No. 403 (1970) and 426 B (1972), Amicon Corporation, Lexington, Massachusetts.

[b] Average value at 10 psig pressure after 5 min.

[c] Average value after 10–30 min of continuous ultrafiltration in stirred cells at 10 psig pressure.

[d] Solute molecular weight.

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RESULTS AND DISCUSSION

The results of the initial time UF studies are presented in Figures 1 and 2 as the volume of ultrafiltrate collected against the time of filtration. The steady filtration rates achieved after the initial transient period have also been indicated in these figures. One notices that, for each of the membranes XM100A and XM300 (Figs. 1 and 2, respectively), the rate of ultrafiltration with stirring is substantially lower than that obtained under stagnant conditions during the initial transient period as well as afterward (i.e., pseudosteady-state period). This behavior is contrary to the usual one.[2] In fact, identical initial time UF studies with a PM-30 membrane which is almost completely retentive of both the solutes BSA and ovalbumin have shown[3,4] that stirring results in an increased ultrafiltration rate from the very beginning of ultrafiltration. That this unusual result with partially permeable membranes was not due to any accidental leakage in the membrane or in the cell was confirmed by the restoration of the original pure-water filtration rate after the experiments as well as by the reproducibility of the observed behavior. This unexpected behavior could not be explained in terms of fluid mechanical considerations, as these conditions were the same in all the experiments.

It is known that the steady-state UF performance of protein solutions is controlled by the polarized layer of membrane-retained macrosolutes. It is also known that proteins tend to agglomerate at their isoelectric pH. The polarized layer on the membrane is then likely to be composed of agglomerates of protein molecules. The solvent permeability of this layer is a strong function of the layer

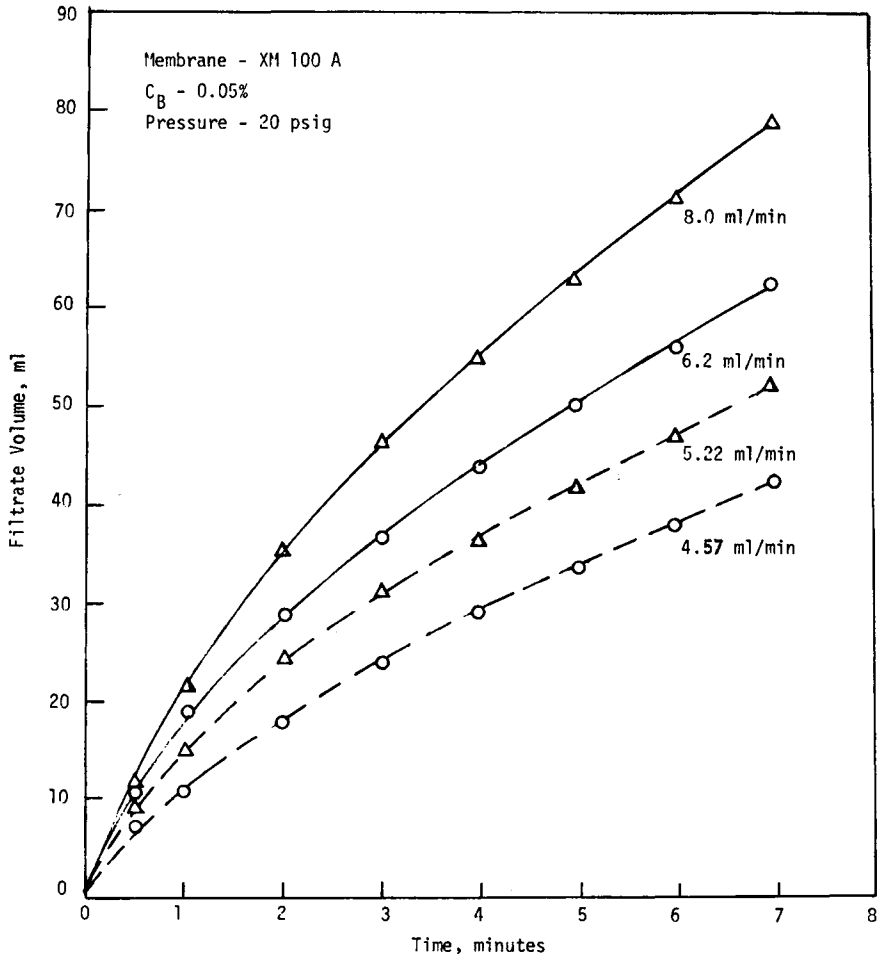

Fig. 1. Effect of stirring—XM 100 A membrane: (—) BSA; (---) ovalbumin; (○) stirred; (△) unstirred.

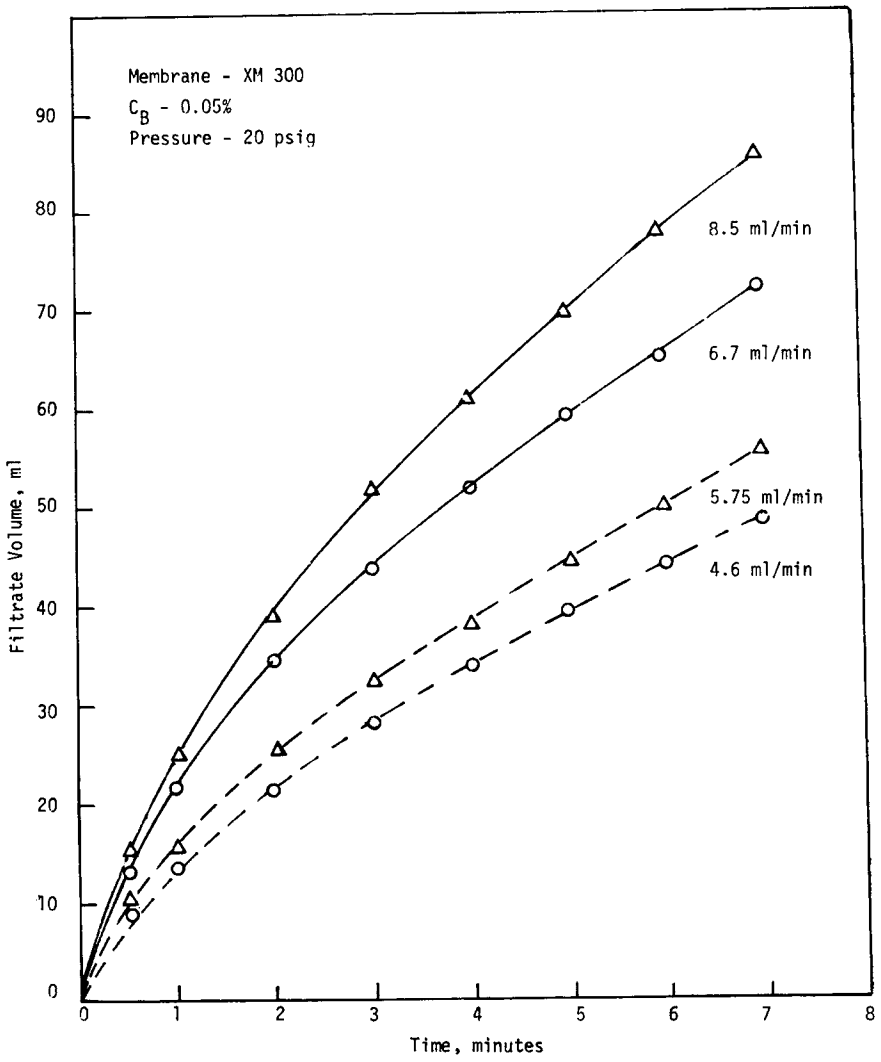

Fig. 2. Effect of stirring—XM 300 membrane: (—) BSA; (- - -) ovalbumin; (O) stirred; (Δ) unstirred.

porosity and the size of the particles that make up the layer. Other conditions remaining constant, the solvent permeability of a BSA layer, for example, is more than that of an ovalbumin layer.[3,4] The unusual filtration performance of these open membranes XM100A and XM300 could then presumably be due to some changes in the structure of the agglomerates of protein molecules in the polarized layer as a result of stirring and its consequences. To test the validity of this hypothesis, two further experiments were carried out. In the first experiment, a 0.05% BSA solution was ultrafiltered through a XM100A membrane at 20 psig under the usual stirred condition. The retentate from this experiment was then adjusted to 0.05% and refiltered at the same pressure in a second experiment through the same but cleaned membrane under identical stirring conditions. The results, presented in Figure 3, show that the ultrafiltration rate, in the second experiment with the retentate, was much lower than that in the first one with the fresh solution. Since the experimental conditions were the same in both the experiments, the large reduction in the filtration rate in the second experiment was probably due to a more pronounced change in the agglomerates of solutes molecules as a result of stirring in the first experiment resulting in smaller size aggregates and therefore a gel layer of lesser permeability. It should be pointed out here that the particular XM100A membrane used for these two experiments were taken from a batch different from the one used for the earlier

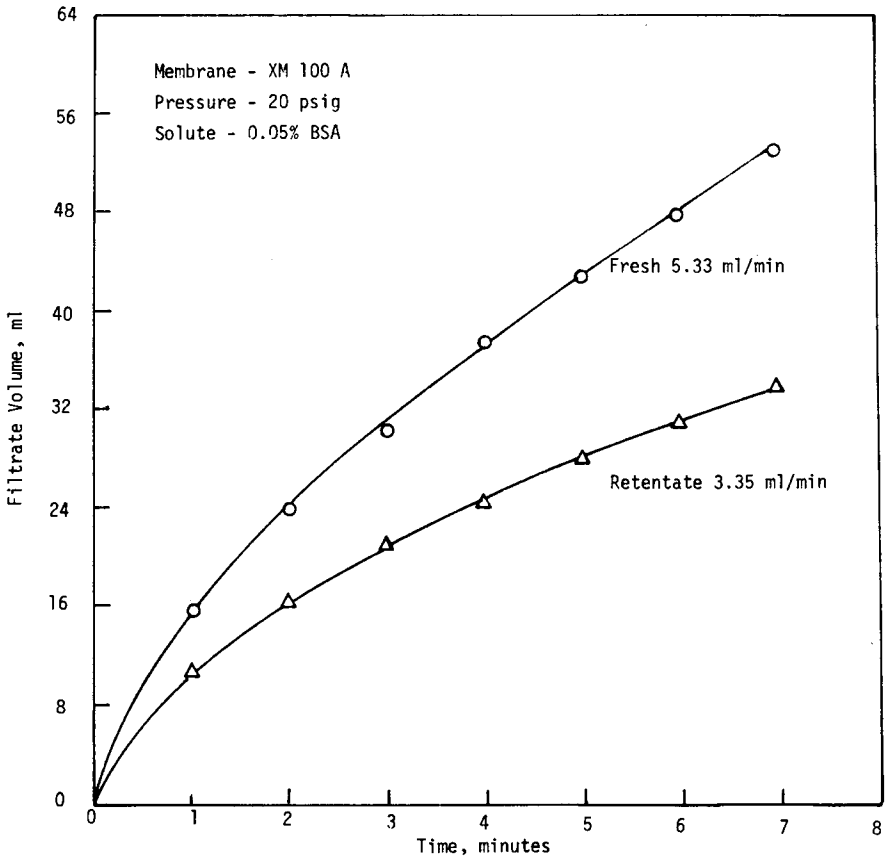

Fig. 3. Comparison of stirred ultrafiltration performance through a XM 100 A membrane of a fresh BSA solution with a solution of BSA obtained from the retentate of the fresh BSA solution.

experiments reported in Figure 1. This is the reason for the observed difference in the values of the steady state filtration rates between Figures 1 and 3 for the solute BSA since some variation exists in membrane flux performance from batch to batch.

One can also offer an alternative explanation of the above-mentioned anomalous flux behavior with the hypothesis of shear degradation of the protein molecules due to stirring resulting in a change in the structure and size of the protein molecules. However, at the low speed (800 rpm) used in the present study, shear degradation of the protein molecules is highly unlikely although several investigators have experimentally verified the shear degradation of synthetic and biological macromolecules due to high-speed stirring[6,7] (30,000, 4000 rpm), high shear in pressure capillaries,[8,9] and freezing and thawing.[10,11] Nevertheless, Van Oss et al.[12] have reported that stirred UF with a total retention membrane neither impairs the biological activity of the human serum albumins nor alters their molecular weight distributions.

It is worthwhile to point out here that this kind of anomalous solvent transport behavior with partially permeable membranes has not been reported earlier in the UF literature. Additional experimentation will be required to gain further insight into and to explain this anomalous flux behavior. A possible set of experiments would be:

(a) Measurement of the average molecular weight of the solutes in bulk solution before and after the stirring by intrinsic viscosity, light scattering, or ultracentrifugation; such measurements are to be simultaneously carried out on the ultrafiltrate as well as on a solution of the deposit formed on each membrane since partially permeable membranes are being used.

(b) Retention studies with noninteracting tracer molecules introduced into the bulk solution after the steady state has been attained to check the pore sizes of the deposited protein layer (which will be dependent on the molecular sizes of the protein aggregates) with and without stirring.

(c) Retention behavior of the protein solution with and without stirring since any change in the sizes of the protein aggregates will cause a different protein retention through the wide pore size distribution membranes; this experiment will reinforce any conclusion from (b) if the agglomeration effects are important.

(d) Electron-microscopic studies of the structure of the gel layer with and without stirring.

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