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Protein Ultrafiltration: an Experimental Study

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Synopsis

An experimental study was carried out on the ultrafiltration (UF) of protein-containing solutions under different conditions, as compared with a solution of a linear synthetic polymer. Three different fluidynamic regimes were investigated, namely, unstirred batch system, stirred batch system, and recirculating system. The results obtained substantially agree with the predictions of the gel polarization model. A significant effect of the electrolytes on the UF flux has been observed, which can be attributed to solute–solute interaction. The influence of different UF membranes has also been investigated.

INTRODUCTION

During the last decade, the concentration, purification, and separation of macromolecules by filtration through semipermeable membranes has been put into practice so that today membrane separation is considered as a unit operation. One of the fields in which this process is already at an industrial stage is the treatment of protein-containing solutions.[1]

The process, generally termed ultrafiltration (UF), has the following advantages with respect to other separation processes: (a) it is athermal; (b) it involves no change of phase; (c) it is relatively nondestructive to the easily denaturated proteins; (d) it requires low hydrostatic pressure; and (e) it can be performed at relatively low temperature, thus limiting any thermal denaturation of the protein molecule.

One of the problems of the UF of protein solutions is a marked decline of the permeate flux with time. This usually is attributed to the deposition of a concentrated layer of macrosolute molecules at the membrane/solution interface, the layer acting as an additional barrier to the flow of solvent and microsolutes. The prediction, by an analytical model, of the stationary UF rate and of the time necessary to approach this value is of particular interest.

In the present work, which is part of a research program on UF process modeling, results are reported of an investigation on the flux decay as a function of time and of the rejection behavior of three different kinds of cellulose acetate membranes toward macrosolute and microsolutes. (A preliminary account of this subject has been given at the First World Filtration Congress, Paris, May 14–17, 1974.)

The experiments were carried out at various applied pressures in three UF processing modes characterized by different fluidynamic conditions: namely, an unstirred batch system, a stirred batch system, and fluid recirculation on the active side of the membrane.

GEL POLARIZATION MODEL

Concentration polarization phenomena, which strongly affect all membrane processes, are particularly important in the UF of macromolecular solutions. In fact, when this kind of solution is processed, the concentration polarization modulus c_s^m/c_s', where c_s^m is the solute concentration at the membrane surface and c_s' the feed brine concentration, can reach very high values under typical operating conditions. In many cases, the modulus becomes so large that the concentration of macrosolutes at the membrane surface reaches the gelation point. It is, therefore, the permeability and selectivity properties of this gel layer that can determine the membrane performance in many UF processes.

The gel polarization model is now commonly used in treating the flux decay and rejection variation in this sort of separation process. The gel formation process can be considered to occur in all the three fluidynamic regimes under investigation here, even in the recirculating system in turbulent flow.[2]

In an unstirred batch system, we have attempted a correlation between the UF rate and the time using the Liu and Williams approach.[3] As shown in a previous paper on the UF of macromolecular solutions,[4] the UF rate versus time qualitatively fits the theoretical log–log curve predicted by Liu and Williams. The shift in the absolute value observed in that earlier work was attributed to decrease with time of the diffusion coefficient of the solute.

In a stirred batch system, one can use the following correlations proposed by Colton[5]:

Laminar boundary layer over the membrane surface:

$$\frac{k_s r}{D_s} = 0.285 \left(\frac{\omega r^2}{\nu}\right)^{0.55} \left(\frac{\nu}{D_s}\right)^{0.35} \text{ when } 8{,}000 < \frac{\omega r^2}{\nu} < 32{,}000$$

Turbulent boundary layer over the membrane surface:

$$\frac{k_s r}{D_s} = 0.0443 \left(\frac{\omega r^2}{\nu}\right)^{0.75} \left(\frac{\nu}{D_s}\right)^{0.33} \text{ when } 32{,}000 < \frac{\omega r^2}{\nu} < 82{,}000$$

where k_s = mass transfer coefficient, cm/sec; r = cell radius, cm; ω = stirrer speed, radians/sec; ν = kinematic viscosity, cm²/sec; and D_s = solute diffusivity, cm²/sec.

In a flow recirculating system, if one assumes that the limiting resistence to flow is in the dynamically formed secondary membrane or gel layer, it is possible to calculate the transport rate of water through the membrane (flux) on the basis of the mass transfer of membrane-retained species from the membrane surface back into the bulk stream. This is so because the dynamic gel layer is assumed to have a fixed gel concentration (c_g), but it is free to vary in thickness or porosity. Thus, at steady state, the flux of permeate, J, is a dependent variable which is constant for a given c_b and k_s, and is given by eq. (1):

$$J = k_s \ln c_g/c_b, \tag{1}$$

where c_b = bulk concentration, g/l.; c_g = gel concentration, g/l.; and $k_s = D_s/\delta$, cm/sec, D_s being the solute diffusivity coefficient, cm²/sec, and δ the thickness of the boundary layer over which the concentration varies, cm.

Before discussing the application of this model to specific flow regimes, a number of fairly subtle implications resulting from it should be noted.

The flux through the membrane is invariant with transmembrane pressure drop or permeability and is dependent only on the solute characteristics D_s and c_g and the boundary layer thickness δ. Thus, fluid management techniques must be directed toward decreasing the boundary layer thickness or, to put it another way, toward increasing the mass transfer coefficient k_s. The mass transfer coefficient is evaluated making use of the well-known mass transfer–heat transfer analogies. For example, the Graetz or Leveque solutions[6] for convective heat transfer in laminar flow channels, properly adapted for mass transfer case, can be used. The solution is based on the hypothesis of a laminar parabolic velocity profile completely established at the channel entrance and the concentration profile under development down the full length of the channel. The Leveque solution assumes the form

$$k_s = B\left[\dot{\gamma}_w \frac{D_s^2}{L}\right]^{1/3} \tag{2}$$

where B is a constant dependent on the wall boundary condition, $\dot{\gamma}_w$ is the fluid shear rate at the membrane surface, and L is the length of the flow channel over the membrane; $\dot{\gamma}_w$ is defined as

$$\dot{\gamma}_w = \left.\frac{\partial v}{\partial x}\right|_{x=0}$$

where v is the velocity inside the boundary layer varying from 0 at $x = 0$ (membrane surface) to v_b (bulk velocity) at $x = \delta$. For practical purposes, $\dot{\gamma}_w = 8v_b/d$ for circular tubes (d = diameter of tube), $\dot{\gamma}_w = 6v_b/h$ for flat rectangular channels (h = channel height).

Combining eqs. (1) and (2), one obtains the following relationship:

$$J = B \ln c_g/c_b \left[\dot{\gamma}_w \frac{D^2_s}{L}\right]^{1/3}. \tag{3}$$

EXPERIMENTAL

Materials

For the retention and decay studies, the following aqueous solutions were used: 0.1% bovine serum albumin (BSA), 0.02% cytochrome *c* (cyt.-*c*) and 0.1% sodium polymethacrylate (NaPMA), a linear synthetic polymer. The concentration of the protein solution was measured spectrophotometrically, while for the NaPMA solution the concentration was measured viscometrically.

Membrane types HF-35 (no NaCl rejection, flux 225–300 gal/day-ft²) KPOO (no NaCl rejection), and KP90 (90% rejection and flux of 22–33 gal/day-ft²), all manufactured by the Eastman Chemical Co., were used. The membrane properties reported above refer to the following standard test conditions: 600 psi, 78°F, and 5,000 ppm (0.5%) NaCl solution. Flux and rejection were

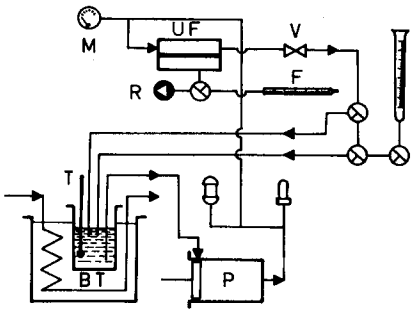

Fig. 1. Schematic diagram of recirculating ultrafiltration apparatus: VP, volumetric pump; UF; ultrafiltration cell; TR, thermostated reservoir; M, manometer.

measured during a 30-min test in a 2-in. cell with 800 ml/min brine feed. The membanes were stored in water containing 0.01% thimerosal as a bactericide.

Equipment

Unstirred Batch System. All experiments were carried out in an apparatus substantially similar to the one previously described,[4,7] lined with nylon tubes, while the connections were grade 316 stainless steel. The system was pressurized by an air tank. The cell containing the membrane and the solution reservoir was placed in a thermostated bath. The pressure was read at an open-air low-pressure Hg manometer with an accuracy of ±1 mm Hg. The applied pressure varied in the range of 1–5 atm with the 0.1% BSA solution. Unless otherwise stated, the temperature was 16°C.

Stirred Batch System. The above-mentioned assembly was used except for the cell; this was a standard stainless steel UF cell magnetically stirred at a constant stirring rate of 1800 rpm. The membrane was supported by a sintered stainless steel porous plate. The influence of the applied pressure in the range of 0.5–3.0 atm was investigated with a 0.1% BSA solution.

Recirculating System. This is illustrated in Figure 1. The influence of the axial flow rate on the UF rate was investigated at a constant applied pressure of 10 atm and an initial BSA concentration of 0.1%. In particular, the axial flow rate was increased in the range of 170–270 ml/min, corresponding to a laminar regime in the cell with a Reynolds number of 80–120. However, the existence of a fully developed laminar regime is uncertain in the cell, owing to the existence of a significant entrance effect. The length of the disturbance zone, as pointed out by Newman,[8] is given by the relationship

$$x < 0.005\ Re\ Sc\ D_s$$

where Sc is the Schmidt number.

RESULTS AND DISCUSSION

Figure 2 shows the effluent fluxes, J, plotted as a function of time in the case of an unstirred batch system. The observed flux decay is a function of the applied pressure as long as the asymptotic region is not reached. Particularly in the transient phase, high applied pressures lead to high effluent rates, as expected. On the contrary, the values of the asymptotic UF rate seems to be pressure inde-

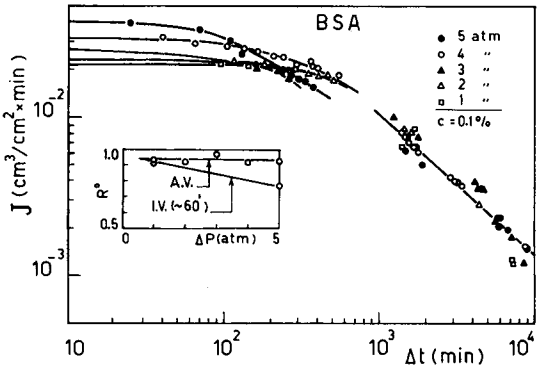

Fig. 2. Ultrafiltration flux decay with time of 0.1% (w/v) BSA solution at various applied pressures in an unstirred batch system (T = 16°C). *Inset:* rejection coefficient as a function of the applied pressure (A.V., asymptotic values; I.V., initial values measured after 60 min).

pendent. This result is in agreement with the previously discussed gel polarization model. In the inset of Figure 2, the observed rejection coefficient is plotted as a function of the applied pressure measured after 60 min in the transient region (see curve I.V.) and also in the asymptotic region (see curve A.V.). At all the applied pressures, the rejection coefficients appear to increase with time, at least during the time interval explored. However, a different behavior is shown by the solute rejection, R^0, as a function of the applied pressure. The rejection coefficient decreases with pressure when measured in the initial transient phase (see curve I.V.), while it appears to be constant when measured in the asymptotic region (see curve A.V.).

A set of experiments on the same BSA solution was conducted at different temperature, all the other significant parameters being held constant. The results indicate that the UF rate does not change significantly over a temperature interval of 9° to about 16°C.

The experimental results obtained in a stirred batch system are reported in Figure 3. An effluent rate decay as a function of time is absent, in contrast with the decay observed in the unstirred batch system at the same applied pressure. The gel polarization model again appears to be valid in this system. In the inset of Figure 3, the asymptotic UF rate is plotted against the applied pressure. It appears that at least for applied pressures higher than 2 atm, the flux J changes very slowly with pressure.

Figure 4 shows in a log–log fashion the UF fluxes (cm³/cm² min), as measured in a recirculating system, against the wall shear rate per unit channel length for BSA and NaPMA solutions. The experimental data fit a curve characterized by a slope of 0.43, which is very close to the one (0.42) reported by Porter[9] for similar experiments. In the inset of Figure 4, the observed rejection coefficient R^0 is plotted against the axial flow rate. From the data it appears that, for this kind of natural macromolecule, the rejection decreases on increasing the axial velocity. This behavior is different from that observed with the NaPMA solution. This synthetic polymer can be assumed to be linear, whereas BSA is a globular protein.

The experimental results obtained with this solution seem to indicate that a power law similar to eq. (3) fits the experimental data well. The experimental

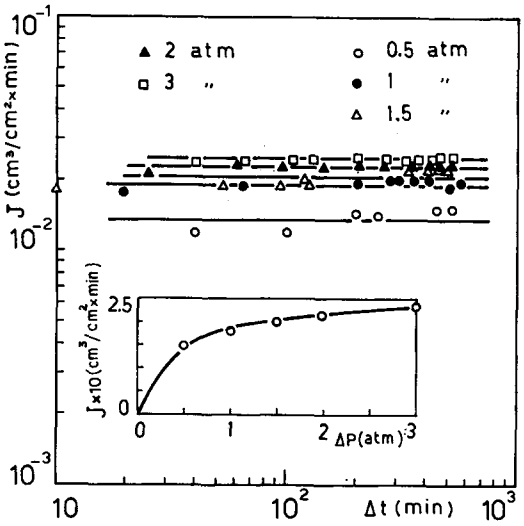

Fig. 3. Ultrafiltration flux decay with time of 0.1% (w/v) BSA solution at various applied pressures in a stirred batch system (T = 16°C). *Inset:* asymptotic flux as a function of applied pressure.

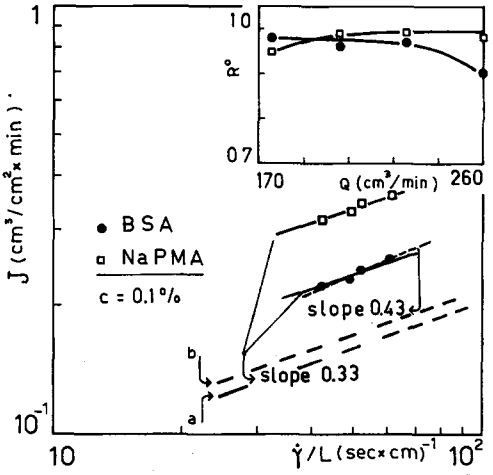

Fig. 4. Ultrafiltration flux dependence on wall shear rate per unit channel length for BSA and NaPMA solutions in a recirculating system (P = 10 atm; T = 16°C). Curves a and b are calculated on the basis of different diffusion coefficients (see text). *Inset:* rejection coefficient as a function of the axial flow rate Q.

results are compared with the theoretical ones, calculated on the basis of eq. (3), in figure 4. The curves were obtained using two different values of the diffusion coefficient D_s, one corresponding to the NaPMA diffusion coefficient measured at a concentration c_0 = 0.1% (see curve b), i.e., $D_s = 3 \times 10^{-6}$ cm²/sec, and the other measured at the hypothetical gel concentration c_g = 7.5%, i.e., $D_s = 2.7 \times 10^{-6}$ cm²/sec (see curve a). The rejection coefficient behavior for this linear polymer changes when the axial velocity is increased, as shown in the inset. In fact, R^0 increases with increasing flow rate.

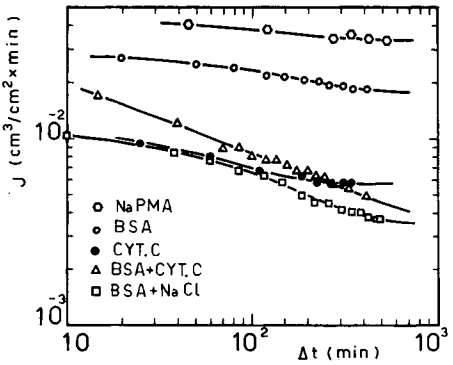

Fig. 5. Ultrafiltration flux decay with time for various macromolecular solutions in a recirculating system (P = 10 atm; T = 16°C; Q = 210 cm³/min).

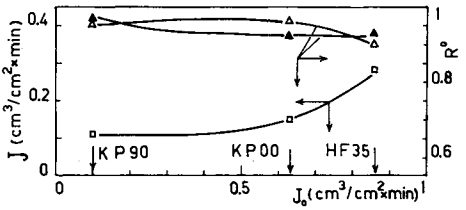

Fig. 6. Influence of membrane permeability on the ultrafiltration flux of a BSA–cyt.-c mixture (□), and on the rejection coefficient of BSA (▲) and cyt.-c (△) in a recirculating system.

In Figure 5 are compared some of the experimental data obtained in a recirculating system with different protein solutions and with a NaPMA solution under the same experimental conditions (T = 16°C; Q = 210 cm³/cm; c_0 = 0.1% for BSA, 0.02% for cyt.-c, and 0.5% for NaCl; P = 10 atm, membrane HF-35). A strong influence on the BSA UF flux can be observed when cyt.-c or NaCl was added to the BSA solution. For a better understanding of this effect, additional experiments were carried out by adding different quantities of NaCl to a fixed amount of BSA. The BSA UF rate decreases with increasing NaCl concentration, reaches a minimum, and then increases again up to value very close to the one measured in the absence of salt. This effect may be attributed to electrostatic solute–solute interaction resulting in a contraction of the BSA structure (shielding effect) and a subsequent flux reduction. The fact that cyt.-c behaves as NaCl in influencing the BSA UF rate is very likely due to the marked cationic character of the cyt.-c. A similar explanation has been recently suggested by Hopfenberg et al.[10] who observed the same effect working with solutions of charged and uncharged starch in the presence of different electrolytes.

In regard to the effect on flux on varying the initial UF rate for different kinds of membrane, it should be observed that the flux is not constant, independent of the membrane type, in contrast to what has been observed with a single protein component.[11] As appears from Figure 6, the rejection coefficient is fairly constant while the UF rate is markedly influenced by J_0. This might be explained by a different property of the gel layer when protein mixtures are ultrafiltered.

By comparing the results obtained with proteins with those obtained in the UF of linear polymers (see Figs. 4 and 5), it can be observed that at the same value

of c_0 and P, linear polymers show a much less marked flux decay and a higher absolute flux value. From the difference in molecular weight (mol. wt of BSA is three times higher than that of NaPMA) and in diffusion coefficient, such a result was to be expected for the flux but not for rejection coefficient values. This phenomenon might be connected to the fact that the CA membranes used have to be considered as weakly negatively charged, as recently suggested by Pusch,[12] who showed the presence of a low charge density (about 3.5 meq/g of dry membrane) in this type of membrane. Therefore, an electrostatic interaction between the NaPMA and the membrane cannot be excluded.

Flux measurements agree, at least qualitatively, with the results reported in the literature. As observed by other authors,[9,13] the relationship between flux and shear rate per unit channel length ($\dot{\gamma}_w/L$), also linear on a log–log plot, is different from that predicted theoretically, see eq. (3), being 0.43 instead of 0.33. However, the last value has been obtained in experiments with linear synthetic macromolecules (NaPMA).

From the analysis of the experimental data reported above, it appears that a gel polarization model is substantially suitable for describing, at least qualitatively, the UF rate decay and rejection behavior of globular proteins such as BSA and cyt.-*c*.

In fact, in a gel polarization mode, an increase in transmembrane pressure drop, which provides an increased driving force for UF but does not aid back-transport, would simply result in the buildup of a thicker or denser cake of retained species, and the steady state UF rate would be reduced to its initial value (see Figs. 2 and 3).

This pressure independence has indeed been shown for many systems, particularly at high pressure values. However, deviations occur in some systems at low pressure (see Fig. 3). It is assumed that in this region the concentration polarization modulus c_s^m/c'_s is not sufficiently large to create the formation of the gel layer at the membrane–solution interface. Thus, the final resulting UF flux is determined by a balance of forward-convective transport of solute and back-diffusive transport. As the pressure is increased, more solute is brought to the membrane surface and c_s^m presumably increases, thereby increasing the back-transport rate of solute into the flowing stream.

The rejection coefficient measured in a recirculating system appears to slightly increase with increasing axial flow rate, as expected. Rejection appears to be pressure independent in the range of 0.5–3.0 atm in the stirred system. This same result was obtained by working with NaPMA. However, other authors[14] reported that the observed rejection coefficient generally decreases with increasing applied pressure. This behavior was ascribed to a compaction of the gel, resulting in a higher concentration of the macromolecules at the membrane surface. A similar effect was observed in the present investigation only when the rejection coefficient were measured in the transient far from the asymptotic region (see inset in Fig. 2). Further, the rejection coefficient was observed to increase with time. This probably means that the gel layer controls the process, not only as far as flux is concerned, but also in regard to rejection. Therefore, it appears that a rigorous analytical model should take into account the possible variation of rejection coefficient with the other system parameters.

In conclusion, the results of the present investigation show that the UF rate and rejection coefficient of protein solutions are influenced by different condi-

tions, particularly the presence of electrolytes or other proteins. Furthermore, gel formation seems to be peculiar to protein UF, so that it could be of interest to investigate the activity of proteins in the gel form. Work along this line is in progress with catalytically active proteins.

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