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Ultrafiltration of Albumin-Ethanol Solutions on Mineral Membranes

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ABSTRACT

This paper investigates the ultrafiltration of albumin-ethanol solutions on ZrO_2 mineral membranes for the preparation of human albumin from plasma. The classical process consists of a preconcentration phase of a 20% ethanol-albumin 7.5 g/L solution to raise albumin concentration to 80 g/L, then a diafiltration to reduce ethanol concentration to less than 0.3 g/L, and a final concentration to adjust albumin concentration to its final value of 210 g/L. The potential advantages of mineral membranes relative to the polysulfone membranes presently used are a longer membrane life and higher permeate fluxes in the presence of ethanol. In addition, they lend themselves to the use of back flushing or pulsatile flows for reducing membrane fouling. Using 2.7 mm i.d. Carbosep membranes with a 10 kd cut-off and velocities of 7 m/s, permeate fluxes of 40 L/h·m^2 at 4°C were obtained with 50 g/L albumin, 20% ethanol solutions representative of the preconcentration phase, while 45 to 50 L/h·m^2 were obtained at albumin concentrations of 100 g/L without ethanol at 8°C, representative of the final concentration phase. These fluxes compare favorably with fluxes obtained previously in our laboratory with polysulfone membranes which were respectively of 22 and 40 L/h·m^2 for the same solutions. This study confirms the expectation of a smaller reduction in the presence of ethanol of the permeate flux for the mineral membranes while albumin concentration in the permeate remained generally under 0.4 g/L irrespective of retentate concentration. The superposition of pressure and flow pulsations on the filter inlet by a piston-in-cylinder system decreases concentration polarization and increases permeate flux by 50 to 60% as compared with steady flows under the same conditions.

Key Words. Albumin ultrafiltration; Ethanol diafiltration; Zirconium oxide membrane

INTRODUCTION

Human albumin is generally prepared in plasma fractionation centers by the method of Cohn et al. (1) which consists in precipitation of immunoglobulins by addition to the plasma of 40% in volume of ethanol. The supernatant to collected and diluted with osmosed water to reduce ethanol concentration to 20%. The solution is then ultrafiltered and diafiltered until albumin is concentrated to 210 g/L while reducing ethanol concentration to about 0.1 g/L. The classical process (2, 3) consists of three steps:

- A preconcentration phase to raise albumin concentration to about 80 g/L. Since ethanol is completely transmitted by the membrane, its concentration remains constant during this phase. The fluid temperature is kept at 0°C in this phase to avoid albumin degeneration by ethanol.
- A diafiltration with osmosed water at constant volume. It is during this phase that ethanol concentration reaches its final level due to dilution. Since albumin is completely rejected by the membrane, its concentration remains constant and the temperature can be increased up to 8°C during the process since ethanol is progressively eliminated.
- A final concentration phase without water addition to adjust albumin concentration to its final value. This phase is carried out at 10°C.

This process is generally carried out with polysulfone cassettes of 10 kd cut-off which have a very high albumin retention and a low internal volume. Both properties are important for minimizing albumin losses. However, the drawbacks of such membranes is their limited lifetime of about 1 year and their relatively low permeate flux when ethanol concentration exceeds 10%. They are also damaged by ethanol concentrations above 25 to 30%. Thus, this paper investigates the possibility of replacing organic membranes by mineral ones. The potential advantages of mineral membranes are the following:

- A longer membrane lifetime: 5-year duration has been reported in a variety of applications (4).
- A better tolerance to high ethanol concentrations, thereby allowing the reduction or even the elimination of the initial dilution.
- Permitting to use backflushing or pulsatile flows to decrease membrane fouling and improve performance.

Their drawbacks relative to cassette-type membranes are:

- Availability presently restricted to tubular modules which require higher feed flow and have high internal volumes.

- Higher risk of albumin adsorption by the membrane due to their thick support.
- Higher initial cost, both in membranes and in pumps.

This paper describes a study of ultrafiltration of albumin ethanol solutions with ZrO_2 membranes on a carbon support (Carbosep). In order to reduce the feed flow and the internal volume, recently available 2.7 mm i.d. membranes were used rather than the traditional 6 mm i.d. ones. Two types of tests were carried out: first, tests with steady flow both at constant and variable protein concentrations with various transmembrane pressures and fluid velocities; second, the effect of superposing pressure and flow pulsations on the feed flow at various frequencies were investigated according to a technique previously used in our laboratory in the ultrafiltration of milk (5) and the microfiltration of wine and apple juice (6, 7).

MATERIAL AND METHODS

The laboratory-scale experimental set-up consisted of a feed reservoir of 5 L capacity placed in a thermostated bath and a volumetric gear pump used for circulating the solution at different flow rates. The flow circuit used for both steady and pulsatile flow experiments is shown in Fig. 1. A pulsation generator was introduced between the pump and the membrane unit (module) when the experiments were performed with pulsating flow. The pulsating mechanism consists of a piston-in-cylinder with an adjustable stroke length and frequency of pulsations, driven by compressed air at 6 bar. The motion of the piston superimposes flow and pressure pulsations at the inlet of the module. A one-way valve was installed between the pump and pulsating system in order to prevent backflow to the reservoir during the piston forward stroke. The details of the pulsating mechanism are given in Reference 8.

Pressures at the inlet and outlet of the module were measured with pressure transducers (Validyne Engineering Corporation, USA) and the instantaneous feed flow was measured by an electromagnetic flowmeter (Gould Electronics, USA) in pulsating flow experiments. The time-averaged permeate flow rate was measured by an electronic scale (Sartorius, Germany). The instantaneous signals from all devices were fed into a PC microcomputer through an analog digital interface. Figure 2 shows the instantaneous variations of transmembrane pressure (P_{TM}) and feed flow (Q_b) with time for a frequency of 1 Hz, a pulsed volume of 8 mL, and a solution of 50 g/L albumin in 20% ethanol for pulsating flow experiment. By adjusting the timing of air compression by an air flow control valve and a pressure control value, the frequency of pulsations was changed

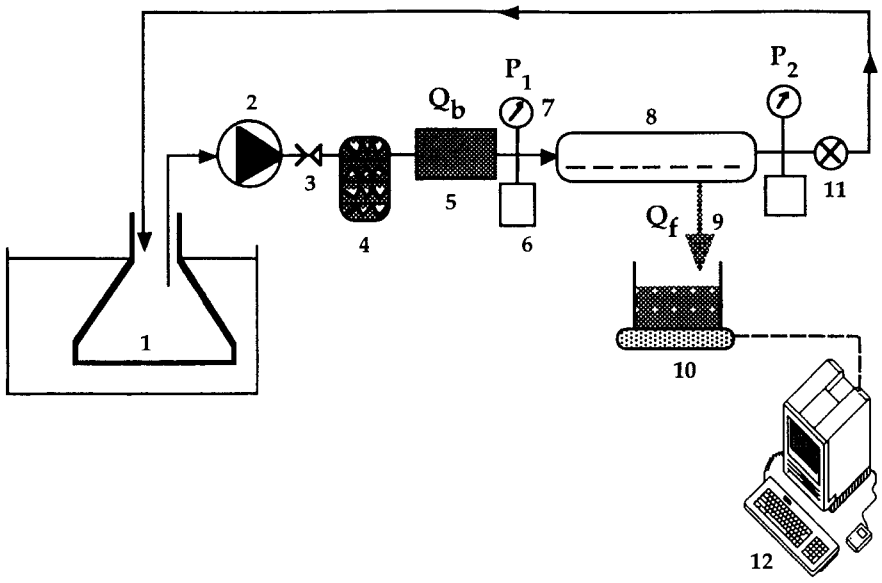


FIG. 1 Schematic diagram of filtration unit: (1) feed reservoir, (2) feed pump, (3) nonreturn valve, (4) pulsation generator, (5) electromagnetic flow meter, (6) pressure transducer, (7) pressure manometer, (8) filtration module, (9) permeate, (10) electronic scale, (11) control valve, (12) microcomputer.

between 0.5 and 2 Hz, and by limiting the piston stroke length using a microvalve the pulsed volume was varied between 6 and 12 mL. The retentate and permeate were returned to the feed reservoir for experiments on albumin filtration, and only the retentate was returned to the feed reservoir when experiments on albumin concentrations were conducted.

Membranes

Mineral membranes Carbosep M5 (TechSep, Miribel, France) of 10 kd cut-off were used. This membrane has a very thin layer of zirconium oxide on a porous support of carbon. Experimental units of 2.7 mm i.d. were used. A module consisting of three tubes of 36 cm length each provided a membrane surface area of about 0.0092 m^2.

Test Solutions

Bovine albumin, purity 96–99% (Sigma), and ethanol, purity 95% (Carlo Erba), were used for preparing different solutions in demineralized water at different concentrations of albumin and ethanol. To simulate the various

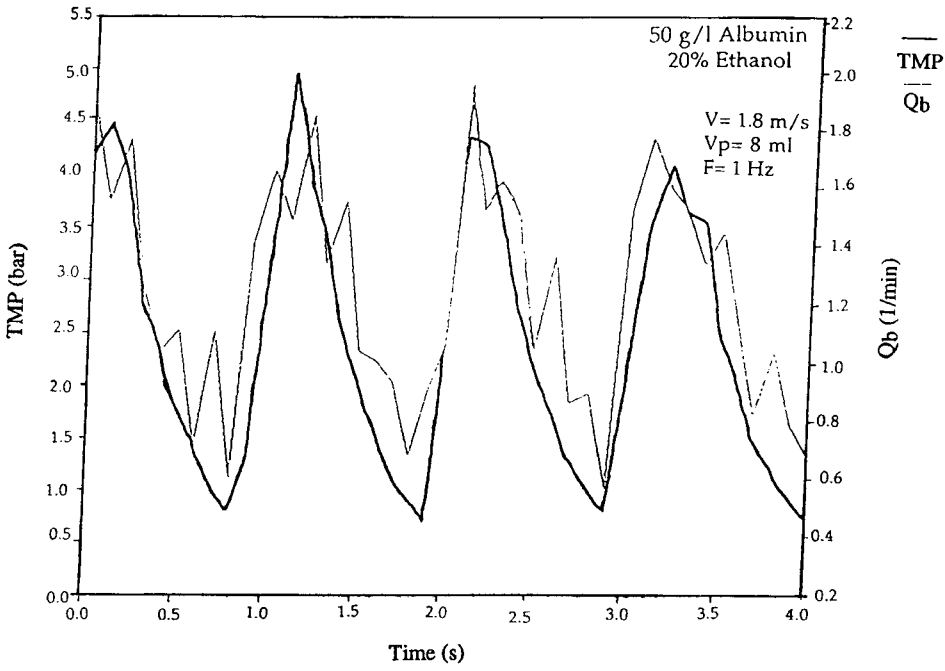


FIG. 2 Instantaneous variations of transmembrane pressure and feed flow at a frequency of 1 Hz, pulsed volume of 8 mL, and for a solution of 50 g/L albumin in 20% ethanol.

phases of the process, three different solutions were prepared at 4°C:

1. Albumin: 50 g/L; ethanol: 20%
2. Albumin: 50 g/L; ethanol: 30%
3. Albumin: 120 g/L without ethanol

In order to obtain good mixing, albumin was first dissolved in water without any stirring for a long time and then the required quantity of ethanol was added.

The solution was adjusted at a pH of 8.4 by adding NaOH, which also permitted a well-mixed solution to be obtained. The density and viscosity were also measured (Table 1). The variation in viscosity of the solution at different concentrations of albumin and at 0 and 20% ethanol is shown in Fig. 3. It was found that the viscosity increased with an increase in ethanol concentration; for example, at 50 g/L albumin concentration, the viscosity increased from 2.6×10^{-3} Pa·s at 0% to 3.6×10^{-3} Pa·s at 20% ethanol. This increase in viscosity explains in part the decrease in permeate flux in the presence of ethanol.

TABLE 1
Measurement of Density and Viscosity of Different Solutions

Solution	50 g/L albumin, 20% ethanol	50 g/L albumin, 30% ethanol	120 g/L albumin, 0% ethanol
Density (kg/m^3)	995	985	1010
Viscosity (Pa·s), 8°C	3.60×10^{-3}	4.16×10^{-3}	3.10×10^{-3}

Albumin Concentration Measurement

Two types of methods were used for measuring the concentration of albumin:

a) In the retentate (concentration higher than 1 g/L)
b) In the permeate (concentration less than 1 g/L)

For concentrations higher than 1 g/L, eight samples with different albumin concentrations, between 0 and 60 g/L, were prepared in demineralized water. Green Bromocresol solution (5 mL) was mixed in 5 mL of a sample of known albumin concentration. When mixing was complete, the optical density (O.D.) of the sample was measured at 630 nm using a UV spectrophotometer against a water sample. Figure 4(a) represents the calibration curve of optical density variation with albumin concentration. When the

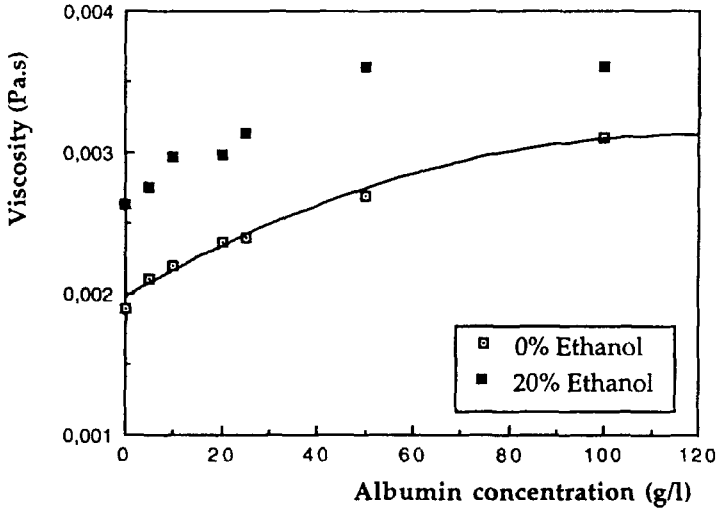
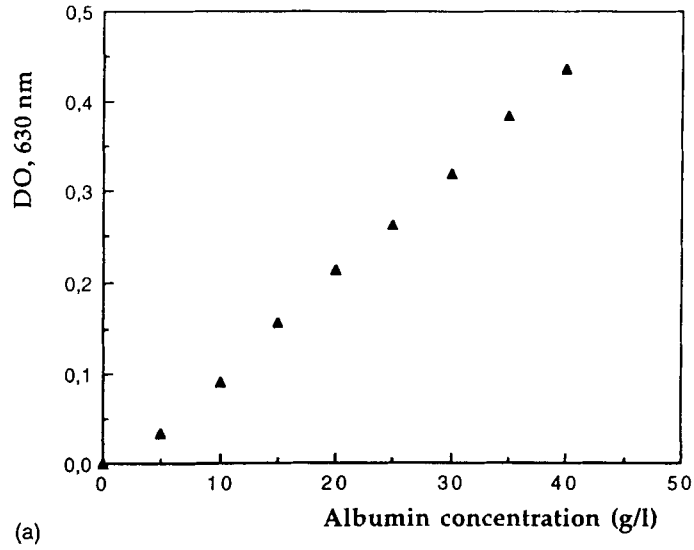
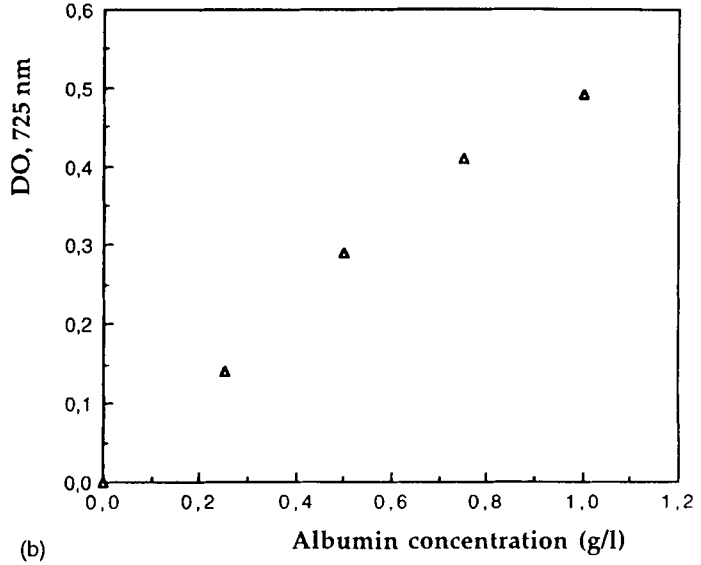


FIG. 3 Variation of solution viscosity at different concentrations of albumin and ethanol.



(a)



(b)

FIG. 4 Calibration graph showing variation of optical density with albumin concentrations at (a) 630 nm and (b) 725 nm.

albumin concentration was higher than 60 g/L, the samples were diluted before measurement.

When the albumin concentration was less than 1 g/L, the Lowry technique with Folin reagent was used. Four samples were prepared with known albumin concentrations between 0 and 1 g/L. We added 2.2 mL Biuret solution to a 5-mL sample, stirred it very well for 10 minutes at room temperature, and then 0.1 mL of Folin reagent was added. The optical density was measured at 725 nm. The color was stable after 30 minutes. The corresponding calibration curve is also shown on Fig. 4(b).

EXPERIMENTS

A fresh batch of solution was used for each run. After each experiment the membranes were cleaned with a base–acid wash (rinsing with hot water at 50–80°C for 30 minutes, rinsing with 0.5% NaOH at 40°C for 10 minutes, washing with hot water at 45°C for 10 minutes, and again rinsing with normal demineralized water for 10 minutes). The filtration rate of demineralized water at 20°C was measured after each washing to check the hydraulic permeability.

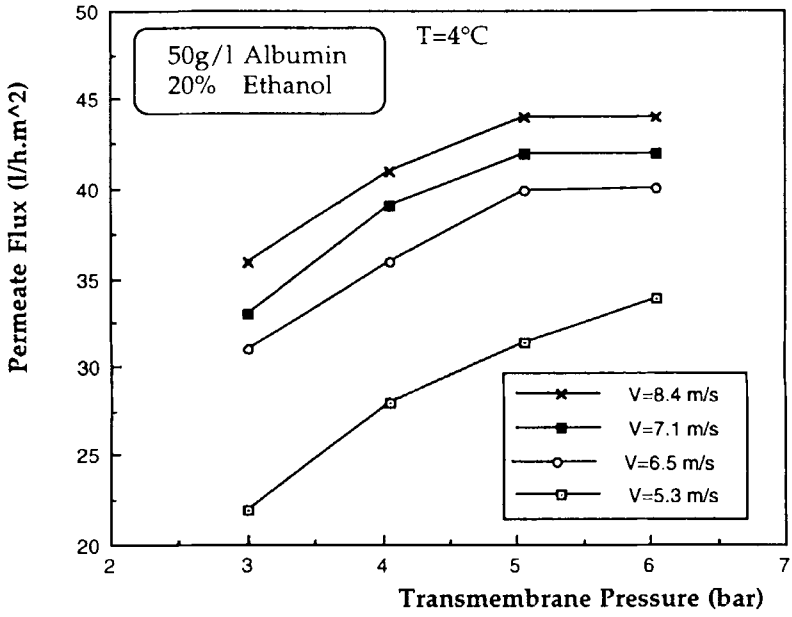


FIG. 5 Variation of permeate flux with transmembrane pressure at different velocities and for a solution of 50 g/L albumin in 20% ethanol.

After cleaning, whenever the initial water permeability was not obtained either due to membrane fouling or for other reasons, the membranes were again cleaned before the next experiment. The flow velocity was varied between 1 and 9 m/s and the transmembrane pressure between 1.5 and 6 bar during the different tests.

Steady Flow Results

Tests at Constant Albumin Concentration

In these tests both the permeate and retentate were recirculated to the tank. Tests were carried out at velocities ranging from 5.3 to 8.4 m/s for two different solutions: a solution of 50 g/L albumin with 20% ethanol tested at 4.5°C was representative of the preconcentration phase and a solution of 120 g/L albumin without ethanol, tested at 8°C, was representative of the final concentration phase. The variation of permeate flux with transmembrane pressure at various velocities is presented in Figs. 5 and 6 for the two solutions. Darcy's resistance of the clean membrane was $0.53 \times 10^{12}\ m^{-1}$. It can be seen that the permeate increases both with transmembrane pressure up to 5 or 6 bars and with increasing velocity.

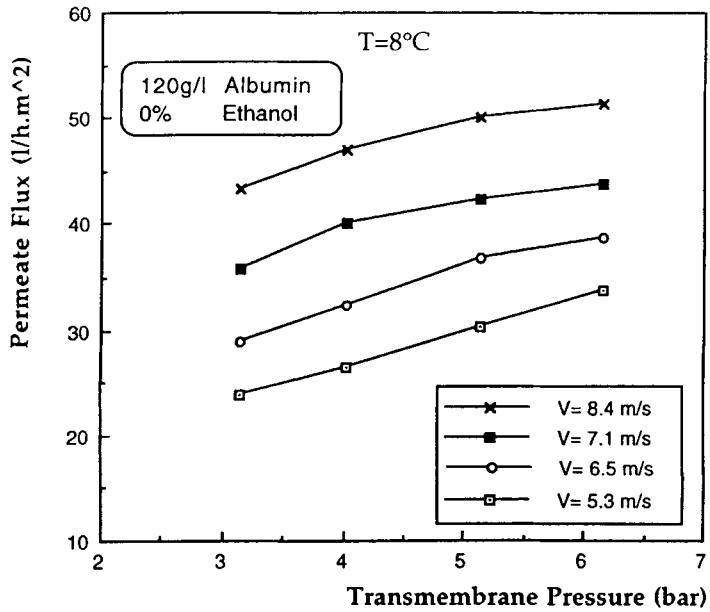


FIG. 6 Same as Fig. 5 but for a solution of 120 g/L albumin without ethanol.

The presence of ethanol lowers the permeate flux even though the albumin concentration is much less than without ethanol.

To further investigate the effect of ethanol on the permeate flux, we conducted a series of tests with a solution of 50 g/L albumin at 30% ethanol for velocities ranging from 1.5 to 8.4 m/s (see Fig. 7). The increase in ethanol causes a general reduction in permeate flux, as shown in Table 2.

The variation of pressure drop with velocity in the retentate across the filtration module is shown in Fig. 8 for the various solutions tested. It varies as $V^{1.43}$, which indicates that the flow is either not fully turbulent or not completely developed, even though the length-to-diameter ratio of the tubes was 133.

Concentration Tests

In these tests the permeate was not returned to the tank. Since the dead volume was 0.3 L and the initial volume 2.5 L, the maximum concentration factor which could be reached in a single experiment was about 8. In order to simulate the preconcentration phase in which the initial albumin concentration is very low (7.5 g/L), the experiment was carried out in two stages. In the first stage, albumin was concentrated from 7.5 to 40 g/L. Then the retentate was mixed with a fresh solution at 40 g/L and 20% ethanol, and the solution was concentrated to 180 g/L. The total duration of the experiment was 14 hours and 30 minutes. The result is shown on

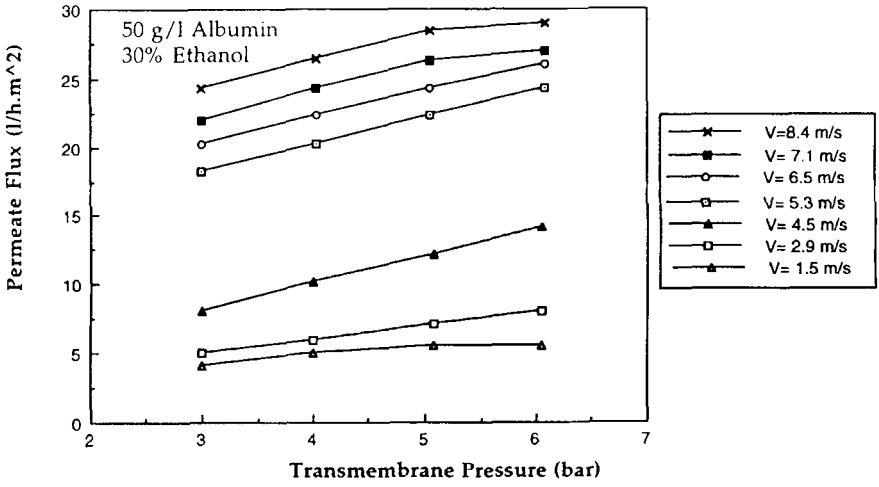


FIG. 7 Same as Fig. 5 but for a solution of 50 g/L albumin in 30% ethanol.

TABLE 2
Effect of Ethanol Concentration on Permeate Flux. Viscosity Increases by 15% When Ethanol Concentration Increases from 20 to 30%

	Permeate flux, Jf (L/h·m^2)							
	TMP = 4 bar; V =				TMP = 5 bar, V =			
Solution	5.3 m/s	6.5 m/s	7.1 m/s	8.4 m/s	5.3 m/s	6.5 m/s	7.1 m/s	8.4 m/s
50 g/L albumin, 20% ethanol	27.9	35.9	38.9	40.8	31.15	39.99	41.9	44.2
50 g/L albumin, 30% ethanol	20.2	22.4	24.3	26.38	22.4	24.4	26.38	28.5
% Decrease in Jf	27	37	37	35	28	39	37	36
120 g/L albumin, 0% ethanol	27.59	37.6	37.53	35.43	28.08	38.98	37.04	35.52

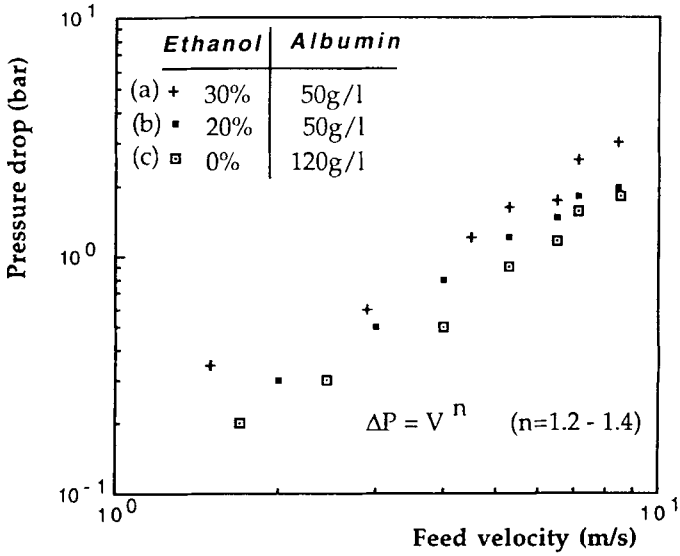


FIG. 8 Variation of pressure drop with feed velocity for three solutions: (a) 50 g/L albumin in 30% ethanol, (b) 50 g/L albumin in 20% ethanol, and (c) 120 g/L albumin without ethanol.

Fig. 9 in semilog coordinates together with the simulation of the final concentration phase without ethanol which was concentrated from 70 to 220 g/L.

It can be seen that the permeate flux does not obey the well-known logarithmic thin film law of Blatt et al. (9) in either case. The decay of permeate flux with increasing concentration is less than expected, especially when ethanol is present. For the final concentration without ethanol, the permeate flux drops linearly with ln C_p until the albumin concentration C_p reaches 150 g/L, giving a gel concentration, when extrapolated to $J = 0$, of 700 g/L. However, the flux appears to remain constant above 150 g/L. This maybe be explained by the rise in fluid temperature caused by the pumping of a small volume of fluid.

Albumin Losses in Permeate

Albumin concentrations in the permeate are plotted in Fig. 10 as a function of time for the experiments described earlier with various test solutions. Most concentrations measured are below 0.4 g/L, and there is no

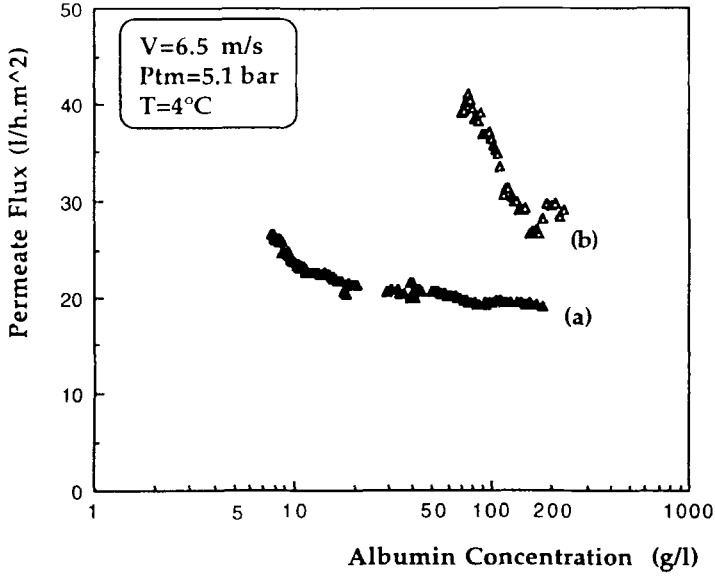


FIG. 9 Effect of albumin solution concentration on permeate flux at a velocity of 6.5 m/s and a transmembrane pressure of 5.1 bar for (a) a solution of 7.5 g/L albumin in 20% ethanol, first phase concentration from 7.5 to 40 g/L, second phase concentration from 40 to 180 g/L; (b) final phase concentration from 70 to 220 g/L without ethanol.

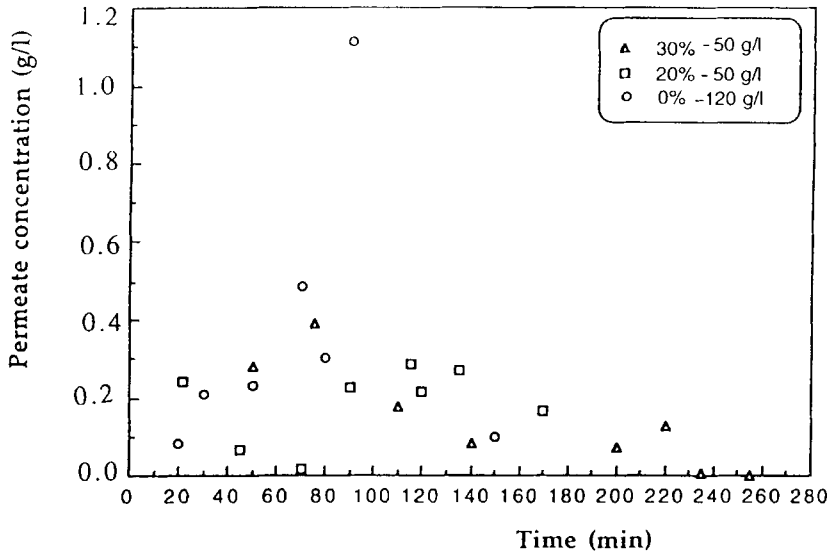


FIG. 10 Variation of albumin concentration in permeate with time for various test solutions: 50 g/L albumin in 20% ethanol, 50 g/L albumin in 30% ethanol, and 120 g/L albumin without ethanol.

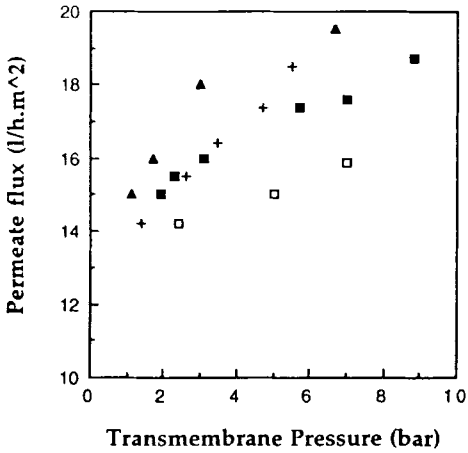
systematic variation with time or with albumin concentration in the retentate.

RESULTS WITH PULSATILE FLOWS

In this case the transmembrane pressure and the feed flow varied with time according to Fig. 2. The transmembrane pressure and velocity used in the following figures are therefore mean time values which were calculated by a microcomputer. Mean velocities were less than in the case of steady flow since the goal was to reduce pumping energy as well as to increase flux.

Effect of Frequency *F* and Pulsed Volume V_p

Tests were run with the 50 g/L–20% ethanol solution at a mean velocity of 1.8 m/s and frequencies of 1, 1.25, and 1.66 Hz for two-stroke volumes of the piston, 8 and 12 mL (Fig. 11). The permeate flux at the same transmembrane pressure increased with increasing frequency, but little improvement was obtained when the pulsed volume was raised from 8 to 12 mL. This is consistent with our earlier observation (10) that the optimal



T=4°C, 50 g/l Albumin - 20% Ethanol

	V (m/s)	Vp (ml)
□ F=1 Hz ■ F=1.25 Hz ▲ F=1.66 Hz	1.8	8
+ F=1.25 Hz	1.8	12

FIG. 11 Effect of pulsation frequency and pulsed volume on permeate flux variation with transmembrane pressure for a solution of 50 g/L albumin in 20% ethanol.

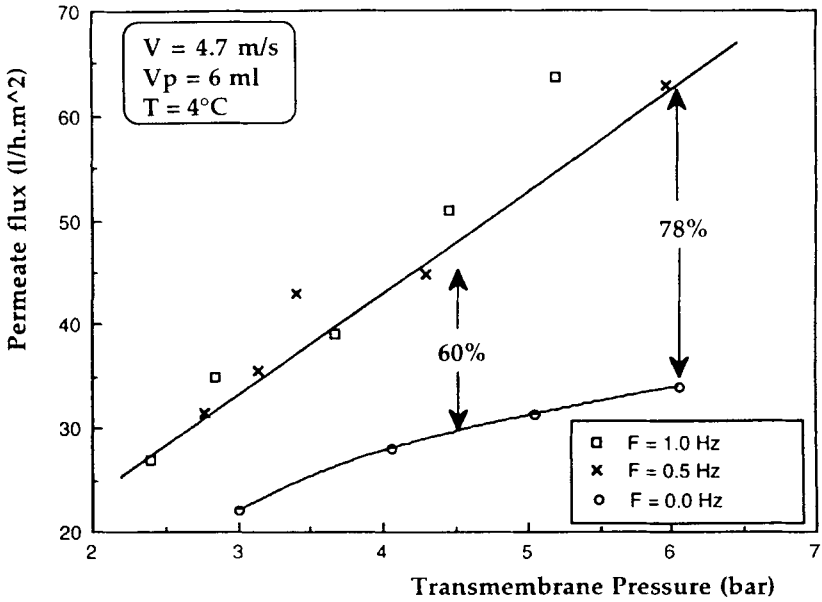


FIG. 12 Comparison of permeate flux variation with transmembrane pressure between steady flow and pulsating flow at different frequencies and for a solution of 50 g/L albumin in 20% ethanol.

pulsed volume is equal to the membrane internal volume, which is 6 mL in this case.

A comparison between steady flow and pulsatile flow filtration for the same mean time velocity of 4.7 m/s is shown in Fig. 12 for the 50 g/L–20% ethanol solution. The increase in permeate flux due to pulsations grows with transmembrane pressure and varies from 60% at 4.5 bar to 78% at 6 bars.

Similar flux increments with pulsations obtained with a 120-g albumin solution without ethanol are shown in Fig. 13 for a mean velocity of 4 m/s and frequencies ranging from 0.5 to 2 Hz for two values of pulsed volume (6 and 8 mL). An increase up to 75% can be obtained at a pressure of 4.5 to 5 bar with a 8-mL pulsed volume at a frequency of 1.5 Hz.

Effect of Pulsations on Albumin Retention

The albumin concentrations measured in the permeate at various frequencies are displayed in Fig. 14 for a 50 g/L–20% ethanol solution and in Fig. 15 for a 120 g/L albumin solution without ethanol. The albumin concentration rarely exceeds 0.8 g/L in the permeate, but seems to increase slightly with frequency. The permeate concentration does not seem to increase with retentate concentration and remained under 0.5 g/L with

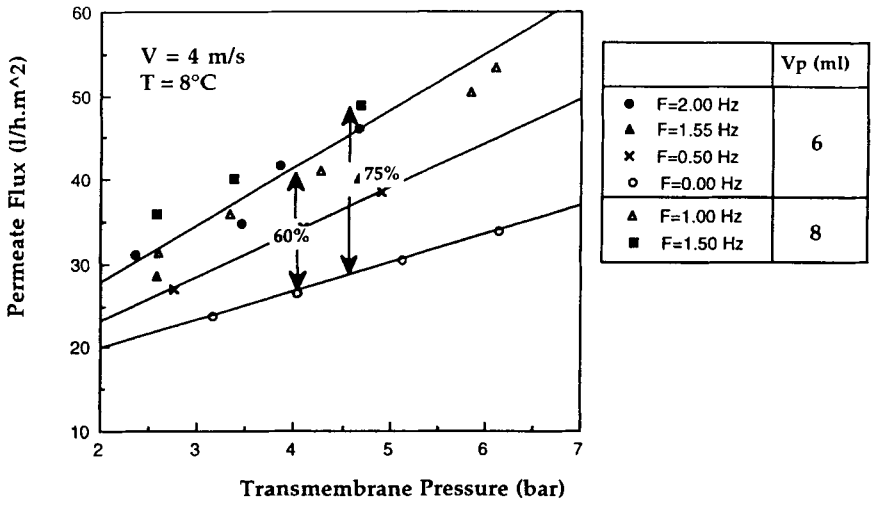


FIG. 13 Comparison of permeate flux variation with transmembrane pressure between steady flow and pulsating flow at different pulsed frequencies and for a solution of 120 g/L albumin without ethanol.

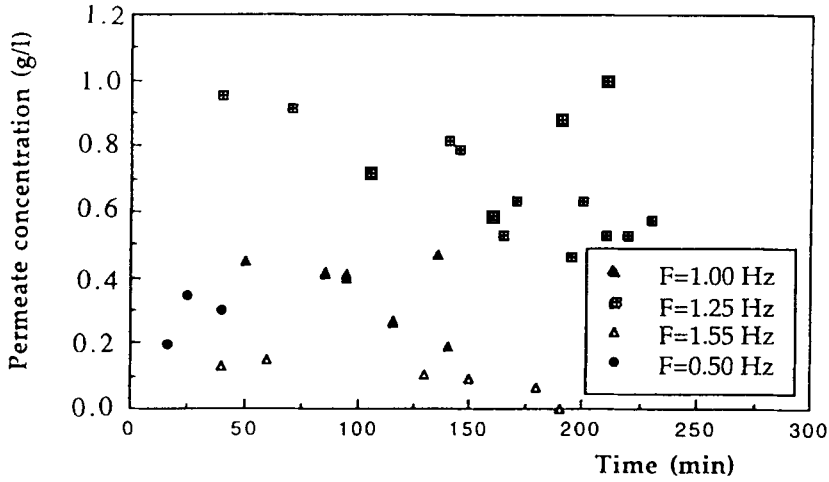


FIG. 14 Variation of albumin concentration in permeate with time for a solution of 50 g/L albumin in 20% ethanol using pulsating flow.

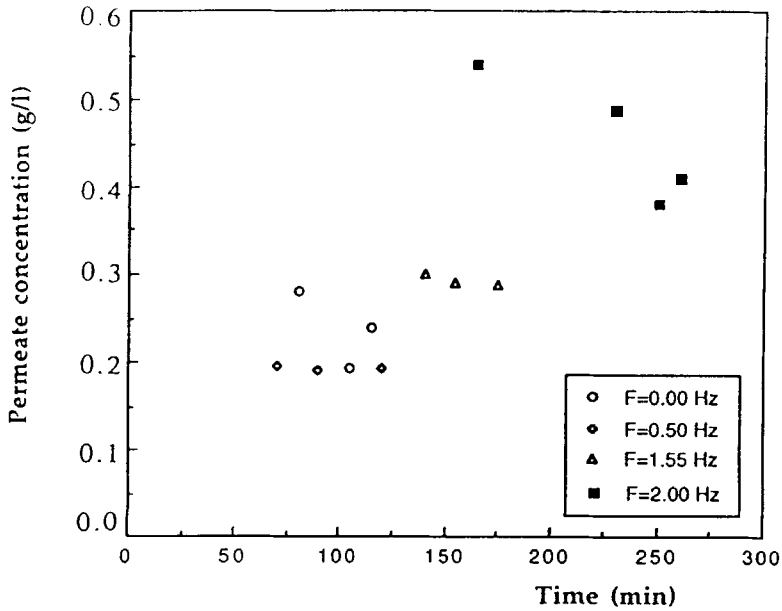


FIG. 15 Variation of albumin concentration in permeate with time for a solution of 120 g/L albumin without ethanol using pulsating flow.

the 120 g/L solution which corresponds to an albumin sieving coefficient of 0.995.

DISCUSSION

Our results show that even with mineral membranes, permeate flux drops significantly in the presence of ethanol. The reduction, however, does not seem as severe as with organic membranes.

This reduction is a little difficult to infer from the present data since albumin concentration was lowered when ethanol was added. However, a comparison can still be made since we earlier collected more extensive data on organic membranes (11).

For instance, with a 10-kd polysulfone membrane at 100 g/L without ethanol, a typical permeate flux at 4 bars was 70 L/h·m^2. In the same conditions, the flux dropped to 22 L/h·m^2 with a 50 g/L albumin–20% ethanol solution, which represents a 69% reduction. With mineral membranes at a velocity of 8.4 m/s and a pressure of 6 bars, the corresponding figures were 50 and 43 L/h·m^2 respectively, i.e., a 15% reduction.

At a velocity of 6.5 m/s, there is no reduction at all since the fluxes are equal to 40 L/h·m^2 in both cases. Of course, the actual reduction due to ethanol at the same albumin concentration is higher than these figures indicate. In the case of mineral membranes, the reduction cannot be due to a change in membrane characteristics (such as shrinking) but can be attributed to both an increase in permeate viscosity and to a change in permeability of the protein layer on the membrane due to ethanol. This argument is supported by the fact that the permeate follows different laws of variation with the wall shear stress (as shown in Fig. 16), depending on ethanol concentration.

Organic versus Mineral Membranes

Concerning the comparison of performances of organic and mineral membranes, it is clear from the preceding section that mineral membranes are capable of yielding higher permeate fluxes in the presence of ethanol than are organic membranes. However, one must bear in mind that the high velocities necessary with the presently available mineral membranes will require much higher feed flows per unit membrane area than cassette-type organic membranes. For instance, the feed flow necessary for a 0.435-m^2 cassette polysulfone membrane is 100 L/h or 215 L/h·m^2. For a standard configuration of two 120 cm long mineral membranes, the corresponding feed flow for a velocity of 1 m/s is 1010 L/h·m^2. Therefore, large

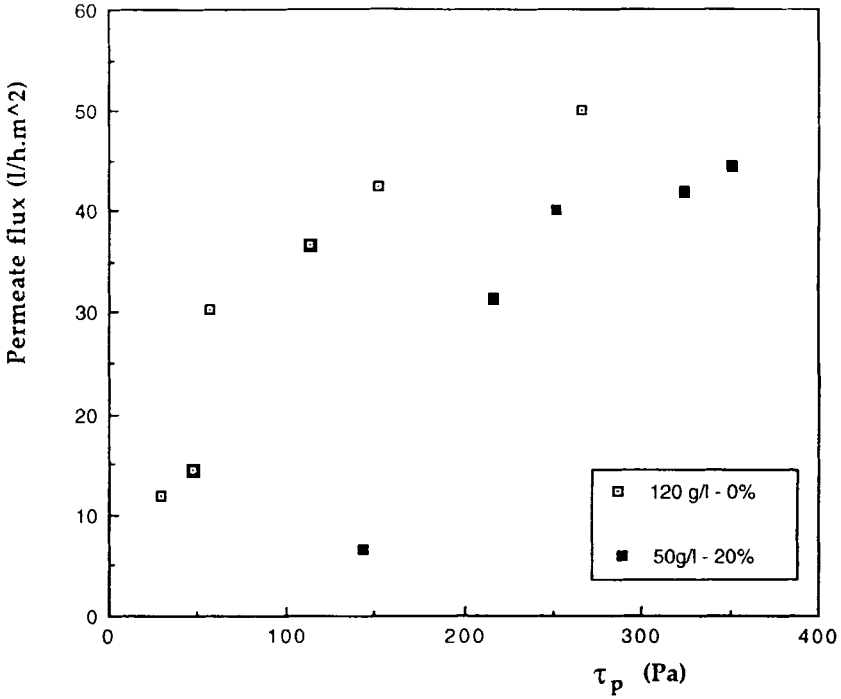


FIG. 16 Variation of permeate flux with wall shear stress for solutions of 50 g/L albumin in 20% ethanol and 120 g/L albumin without ethanol.

velocities, such as those tested in this study, may not prove economically feasible in terms of investment cost and electrical energy for the pumps.

Benefits of Pulsatile Flow

One of the main benefits of superimposing pulsations during filtration is that higher permeate fluxes are obtained at lower velocities than during steady flow. For instance, with pulsations at 0.5 Hz, Fig. 12 showed that for the 50 g/L–20% ethanol solution, it reached 60 L/h·m^2 at 6 bars, while it was only 44 L/h·m^2 for steady flow at 8.4 m/s. A reduction of velocity of 44% from 8.4 to 4.7 will lead to a power reduction for the steady flow pump of 76% since the hydraulic power is proportional to $V^{2.43}$ in our case (see Fig. 8). Even taking into account the additional power necessary for the pulsation generator, the specific energy per cubic meter of permeate should be greatly reduced by pulsations.

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