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Concentration of Bovine Serum Albumin Aqueous Solutions by Membrane Distillation

J. M. ORTIZ DE ZÁRATE, C. RINCÓN, and J. I. MENGUAL*
DEPARTAMENTO DE FÍSICA APLICADA I (TERMOLOGÍA)
FACULTAD DE FÍSICA
UNIVERSIDAD COMPLUTENSE
E-28040 MADRID, SPAIN

ABSTRACT

The use of membrane distillation in the concentration of protein solutions is proposed. Semidilute BSA solutions (concentrations ranging between 0.40 and 1.00%), at pH 7.4 were employed. Transmembrane volume fluxes and evolution of the BSA concentration were measured at different conditions. Temperatures ranged between 20 and 38°C. The filtration cell was operated at laminar regime with tangential velocities of 5.9 and 8.9 $cm \cdot s^{-1}$. Special attention was devoted to analysis of the temperature polarization and fouling effects. The results obtained show that fouling effects are practically absent in membrane distillation operations with BSA solutions, at least for concentrations up to 1.00% w/w and in the range of crossflow velocities employed. This may be due to the fact that the transmembrane fluxes are below the value of the critical flux for fouling.

Key Words. Membrane distillation; Fouling; Protein; Hydrophobic membranes

INTRODUCTION

Membrane distillation (MD) is a relatively new membrane process which has been mainly employed to concentrate salts and other low molecular weight products from aqueous solutions (1, 2). It is well known that

* To whom correspondence should be addressed.

MD is able to concentrate any nonvolatile solute. Consequently, it might be used in the concentration of proteins and other high molecular weight solutes. In this paper this possibility is discussed for a protein in the semidilute regime. MD experiments have been carried out with bovine serum albumin (BSA) aqueous solutions in different experimental conditions. The influence in the measured fluxes of some relevant parameters, such as temperature, solution concentration, etc., has been analyzed. As far as we know, this is the first attempt to study experimentally the concentration of protein aqueous solutions by MD.

The concentration of proteins and other biological solutes from aqueous solutions has been successfully developed by means of standard membrane techniques such as ultrafiltration (UF) and reverse osmosis (RO). UF concentrates proteins by removing water as well as any salts or other low molecular weight components of the feed solutions. In contrast, MD only removes the volatile components in the mixture, whereas salts and other nonvolatile components are retained, independent of their molecular weight. This kind of protein concentration is needed in some applications, such as the production of concentrated milk, where lactose has to be retained in addition to the proteins (3). This type of protein concentration is currently done by using RO. Therefore, concerning protein concentration, MD should be compared with RO.

As is well-known, the largest resistance to flux in the concentration of proteins by RO or by UF comes from membrane fouling (3–6). This fouling is caused by an irreversible attachment of a protein layer at the membrane surface. Typically, membrane fouling causes a decline in flux that can be observed a few hours after the beginning of an experiment. Several studies in the UF literature have demonstrated that fouling can be dramatically reduced by operating a system at a low value of the filtrate flux. Actually, Bacchin et al. (7) proposed that there is a critical flux below which the fouling effects in UF or RO processes are completely absent. In Ref. 7 a theoretical model explaining this fact was developed, and an equation that permits calculation of the critical flux presented. The model is based on hydrodynamic conditions (crossflow) and solution properties, so it is valid for both RO and UF. Since our experiments were carried out in a cell with crossflow geometry, the model should also be valid for our MD results. The transmembrane fluxes reported in the present paper are below the critical flux for fouling calculated according to the method of Bacchin et al. This value is 30 $L \cdot m^{-2} \cdot h^{-1}$, in the ranges of BSA concentrations and tangential velocities employed. Therefore, fouling is not expected to be present in our experiments. The only important resistance mechanism opposed to the flux in our system is due to the existence of temperature

polarization (TP). The temperature polarization coefficient, which is the parameter characterizing such a resistance, may be obtained by using the same models developed for the MD of pure liquids or low molecular weight solutions.

The second goal of the present paper is to verify experimentally the absence of fouling effects. In order to check them, a study of flux decline was carried out. In addition, some experiments were performed using pure water and NaCl aqueous solutions. Comparison of the fluxes obtained with these two systems and with protein solutions permits information about the abcence of fouling in the protein cases to be obtained.

THEORY

The system to be studied consists of a microporous hydrophobic membrane that separates two well-stirred aqueous solutions maintained at different temperatures. The temperature difference creates a water vapor pressure difference, which gives rise to a water flux in the vapor phase through the membrane pores from the warm to the cold side. This phenomenon is usually described by means of a linear relationship between the transmembrane flux, J, and the driving force, which is the water vapor pressure difference (8):

$$J = \frac{C}{\delta} \Delta P \tag{1}$$

where ΔP is the vapor pressure difference and δ is the membrane thickness. C is a phenomenological coefficient which measures the ability of the membrane to give MD fluxes. Coefficient C depends on different parameters (mean temperature, pressure, etc.) and on membrane characteristics (porosity, tortuosity, etc.). This coefficient may be related to the physical nature of the transport process, which is expected to be mainly a Knudsen-type diffusion (9).

As discussed in the Introduction, the only important resistance to the flux in our system is due to the phenomenon named temperature polarization (TP) (10). TP is due to the presence of liquid boundary layers adjoining the membrane at both sides. As an effect, the temperature difference on the two membrane surfaces (ΔT) is not the same as the one corresponding to the well-stirred bulk phases (ΔT_B). In other words, a part of the externally imposed temperature difference is dissipated through the boundary layers. This effect is quantified by the so-called temperature polarization coefficient (TPC), which is defined as the quotient TPC = $\Delta T/\Delta T_B$. As

a matter of fact, the temperature cannot be measured on the membrane surfaces and, consequently, the TPCs cannot be directly calculated but may only be obtained by using some model for TP. In the same way, the presence of the boundary layers gives rise to the phenomenon of concentration polarization (CP). The quantitative effect of CP was shown to be negligible as compared with that of TP for systems similar to the ones considered in the present paper (9, 11).

TP is controlled by U, the overall heat transfer coefficient for the boundary layers. As coefficient U increases, the difference between the bulk temperature and the temperature at the liquid–membrane surface is reduced. It is known that U depends strongly on the tangential velocity of the fluid in contact with the membrane. In order to evaluate U in MD experiments, a method due to Schofield et al. (8) is usually employed. Basically, it consists of measuring the transmembrane flux for a given tangential velocity for different values of the mean temperature. Then, the following equation is used:

$$\frac{\Delta T_B}{J\Delta H} = \frac{U\delta + 2\lambda}{\Delta HCU}\frac{1}{(dP/dT)} + \frac{2}{U} \tag{2}$$

where λ is the effective thermal conductivity of the membrane and ΔH is the enthalpy of vaporization of water. Equation (2) says that the bulk temperature difference (ΔT_B) divided by the product ($J\Delta H$) depends linearly on the inverse of the temperature derivative of the vapor pressure. That means that coefficient U may be obtained from the intercept of the corresponding straight line, which is $2/U$. It is worth noting that this U value is valid for the tangential velocity under which the set of measurements has been made [it is assumed that this coefficient is equal on both sides of the membrane, and that it is virtually independent on the mean temperature (12)]. On the other hand, the true vapor permeability coefficient (C) may be obtained from the U value and the slope of the straight lines in the Schofield plots, ($\Delta T_B/J\Delta H$) versus $1/(dP/dT)$.

The TPC value for each one of the experiments may be obtained from the Schofield model for TP by using the equation

$$\mathrm{TPC} = \frac{1 - \dfrac{2\Delta H}{U_{\mathrm{Calc}}}\dfrac{J}{\Delta T_B}}{1 + \dfrac{2\lambda}{\delta U_{\mathrm{Calc}}}} \tag{3}$$

where U_{Calc} is the U value calculated from the intercept of the Schofield straight lines, as explained above.

EXPERIMENTAL

Materials

Two PTFE commercial membranes, supplied by Gelman, were studied. They are grossly porous hydrophobic partitions with irregular cavities going through the membrane thickness. Their main characteristics, as specified by the manufacturer, are as follows:

TF-200: Nominal pore radius = 0.2 μm; thickness = 178 μm; porosity = 80%; liquid entry pressure of water ≈ 2.78 atm

TF-450: Nominal pore radius = 0.45 μm; thickness = 178 μm; porosity = 80%; liquid entry pressure of water ≈ 1.36 atm

The materials used were double-distilled water, NaCl p.a. grade from Probus, and BSA with purity >96% supplied by Fluka. BSA solutions were prepared at concentrations ranging from 0.40 to 0.60%. The pH values of the solutions were kept constant at 7.4 by using a buffer HNa_2PO_4:H_2NaPO_4, while NaN_3 at 0.02% was added as a bactericidal agent.

Apparatus

The experimental setup was a modified Minitan-S cell supplied by Millipore. This cell is designed for tangential flow processes. In our experiments the membrane is sandwiched between two equal acrylic manifolds. Silicone separators are placed between the membrane and the manifolds, providing gasket seals between the plates and creating a linear-flow sweeping flowpath for the liquids. The solutions are circulated tangentially to the membrane surfaces through nine rectangular channels (the cross area is 7×0.75 mm^2). The effective membrane area exposed to the flux is 30 cm^2 as quoted by the manufacturer. The circulation velocity was the same at both sides of the membrane. In these conditions the Reynolds number may be considered to be the same at both sides of the membrane, and no transmembrane pressure difference is expected to exist. This fact was checked at the end of the experiments by placing two pressure gauges at the cell inlets and pumping water through the circuit. The pressure difference measured was always lower than 0.1 atm. This pressure difference is lower than the liquid entry pressures for water in our membranes, which are 2.78 and 1.36 atm for membranes TF-200 and TF-450, respectively.

The experimental setup was completed with: Two graduated glass containers furnished with thermostating jackets; two low-pressure centrifugal pumps which were magnetically driven in order to avoid contamination of the circulating solutions; two floating ball flux meters; two Pt100 probes

placed in the cell inlets; two plug valves for controlling the tangential velocity at the chambers, and, finally, two circulating thermostats. The Pt100 probes were used for temperature measurement and control. A schematic of the complete setup is shown as Fig. 1. The described setup permits a different solution to be circulated at each side of the membrane. Both the velocity and the temperature of the solutions may be controlled. Temperature fluctuations at the cell inlet were lower than ± 0.5 K.

Experimental Procedures

The transmembrane volume fluxes were calculated from the measured volume variations of the containers. The concentration and pH of the solutions were assessed by sample extraction from the containers. The concentration of NaCl solutions was measured by means of a Möhr titration with ± 0.005 M uncertainty. The concentration of BSA solutions was obtained with the Bio-Rad method, with an uncertainty of $\pm 0.05\%$. A Shimadzu 160-A spectrophotometer set at 750 nm was used in this procedure. The pH values were assessed with a Sentron 1001 system (accuracy = 0.1).

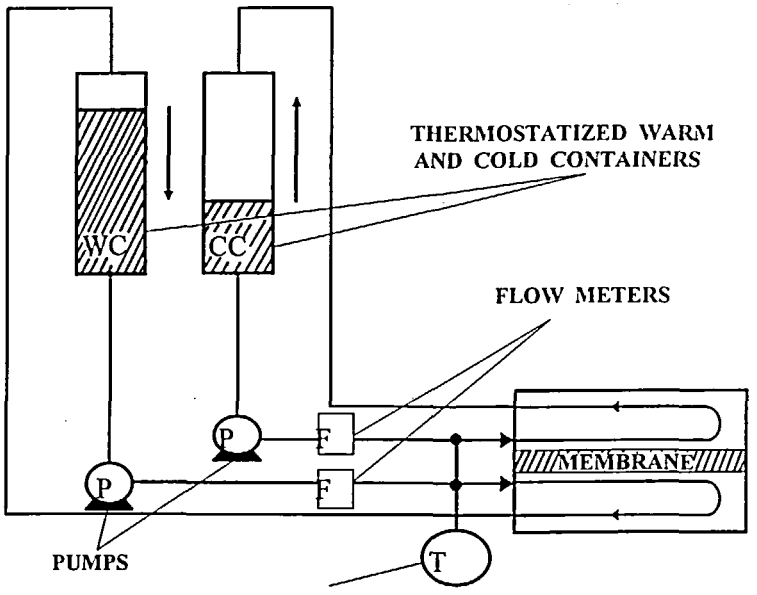

FIG. 1 Schematic of the experimental setup.

RESULTS

The experiments were carried out in four sets.

1. In the first set the TF-200 membrane was used, and 200 cm^3 of a NaCl solution and 100 cm^3 of pure water were initially placed in the warm and cold sides, respectively. Volume variations and concentration changes at the containers were measured for various warm and cold temperatures and for two tangential velocities.
2. In the second set the TF-200 membrane was used, and 200 cm^3 of a BSA solution and 100 cm^3 of pH 7.4 buffer were initially placed in the warm and cold sides, respectively. Volume variations and concentration changes in the containers were measured for various temperatures. Two tangential velocities and two initial protein concentrations were employed.
3. In the third set, carried out with the TF-450 membrane, 200 and 100 cm^3 of pure water were initially placed in the warm and cold sides, respectively. Volume variation of the containers was measured for various temperatures and tangential velocities.
4. In the fourth set, carried out with the TF-450 membrane, 200 cm^3 of a BSA solution and 100 cm^3 of pH 7.4 buffer were initially placed in the warm and cold sides, respectively. Volume variations and concentration changes of the containers were measured for various temperatures and initial protein concentrations and for two tangential velocities.

The temperature of each phase and the tangential velocity inside the MD cell were maintained at constant values in the experiments. Two different values for the tangential velocity, 5.9 and 8.9 $cm \cdot s^{-1}$, were employed. For these values the hydrodynamic conditions in the cell correspond to a laminar regime. NaCl experiments were carried out with an initial concentration of 0.06 M, whereas BSA experiments were carried out at 0.40 and 0.60% w/w initial concentrations. Volumes and concentrations at the containers were measured at regular time intervals. The experiments typically lasted between 7 to 8 hours. For the BSA solutions, pH was measured at the beginning and at the end of each run, and no significative change was detected. Protein concentration in the cold chamber at the end of the run was measured in several cases, and no significant trace was observed.

The experimental pairs of volume–time data are shown in Fig. 2 for a representative case of each one of the membranes. The triangles refer to membrane TF-200 and the diamonds to membrane TF-450. The open symbols indicate a warm container and the closed symbols indicate a

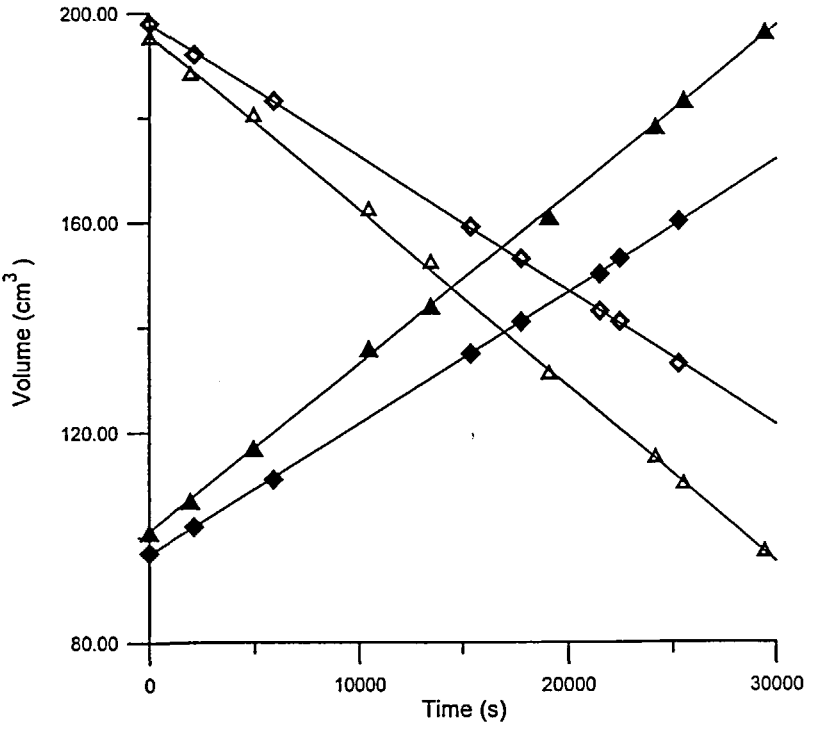

FIG. 2 Evolution of volume in the containers as a function of time for two BSA experiments. (△, ▲) Membrane TF-200 with T_w = 33.8°C, T_c = 22.9°C, c_0 = 0.61% w/w, and v = 8.9 cm·s^{-1}. (◇, ◆) Membrane TF-450 with T_w = 37.9°C, T_w = 27.4°C, c_0 = 0.60% w/w, and v = 5.9 cm·s^{-1}. The open symbols correspond to the volume of the warm container and the closed symbols to the volume of the cold container.

cold container. The BSA initial concentration was 0.60%, the tangential velocity was 5.9 cm·s^{-1}, and the temperatures were T_w = 33.8°C and T_c = 22.9°C for membrane TF-200, and T_w = 37.9°C and T_c = 27.4°C for membrane TF-450. In all cases the volume–time data may be adequately fitted to straight lines, which may be interpreted as indicating that the transmembrane flux does not decline over time in the considered conditions. This trend was observed for all the experimental conditions studied in this paper. In addition, the slope of the straight lines yields the corresponding value of the MD flux.

Tables 1 and 2 show the results for the studied systems in all the experimental conditions. Table 1 refers to both membranes and shows the values

TABLE 1
Temperatures, Tangential Velocity (v), Fluxes (J), Initial Concentration (c_0), and Concentration Reached after 7 Hours of Run (c_7) for the Experiments Carried Out with BSA Solutions

Membrane	T_{warm} (°C)	T_{cold} (°C)	v ($cm \cdot s^{-1}$)	$J \times 10^3$ $kg \cdot m^{-2} \cdot s^{-1}$	c_0 (% w/w)	c_7 (% w/w)
TF-450	39.8	31.6	5.9	0.702	0.39	0.51
	37.9	27.4	5.9	0.841	0.42	0.54
	36.4	25.3	5.9	0.861	0.42	0.55
	35.6	21.5	5.9	0.967	0.63	0.89
	36.0	23.2	5.9	0.926	0.60	0.90
	39.6	31.5	8.9	0.813	0.43	0.55
	37.8	29.5	8.9	0.807	0.45	0.52
	36.2	27.2	8.9	0.791	0.43	0.53
	34.9	25.2	8.9	0.842	0.42	0.53
	33.2	23.2	8.9	0.794	0.40	0.57
TF-200	36.9	26.7	5.9	1.022	0.43	0.79
	36.0	26.4	5.9	1.018	0.64	0.86
	33.9	22.9	5.9	1.008	0.62	0.85
	32.9	22.9	5.9	0.918	0.64	0.83
	31.4	21.0	5.9	0.830	0.45	0.72
	30.5	20.7	5.9	0.824	0.38	0.74
	36.9	29.9	8.9	0.784	0.40	0.54
	34.2	25.9	8.9	0.949	0.64	0.79
	33.8	22.9	8.9	1.089	0.61	0.96
	32.5	22.8	8.9	0.997	0.64	0.81
	31.0	20.8	8.9	0.891	0.42	0.60

of the flux (J), the initial concentration (c_0), and the concentration obtained after 7 hours of experiment (c_7) for the different temperatures and tangential velocities considered. Table 2 displays the transmembrane fluxes for the systems membrane TF-450/water and membrane TF-200/NaCl solution at the different temperatures and tangential velocities considered. A visual inspection of the last two columns of Table 1 shows that a measurable increase in the concentration of the solutions is reached. As a matter of fact, the BSA concentration increases between 15 and 50% in the case of membrane TF-450 and between 23 and 95% in the case of membrane TF-200. On the other hand, the order of the magnitude of the transmembrane fluxes presented in Tables 1 and 2 (around 10^{-3} $kg \cdot m^{-2} \cdot s^{-1}$) is similar to those appearing in the literature on MD (10, 13, 14) in spite of the lower temperature differences used in the present paper.

TABLE 2
Temperatures, Tangential Velocity (v), and Fluxes (J) for the Systems Membrane TF-450/Water and Membrane TF-200/NaCl Solution

System	T_{warm} (°C)	T_{cold} (°C)	Tangential velocity ($cm \cdot s^{-1}$)	$J \times 10^3$ $kg \cdot m^{-2} \cdot s^{-1}$
TF-450/water	56.7	50.7	5.9	0.821
	49.5	40.3	5.9	0.959
	49.2	40.4	5.9	0.968
	40.4	30.6	5.9	0.798
	38.9	27.7	5.9	0.808
	35.9	25.5	5.9	0.829
	35.1	23.3	5.9	0.759
	32.7	21.3	5.9	0.799
	49.1	40.1	8.9	1.199
	40.0	30.5	8.9	0.930
	38.6	28.8	8.9	0.925
	36.9	26.3	8.9	0.967
	35.4	24.2	8.9	0.957
	34.6	22.3	8.9	0.940
TF-200/NaCl solution	43.5	35.6	5.9	1.040
	43.5	27.0	5.9	1.944
	39.4	30.6	5.9	1.015
	39.3	30.4	5.9	1.012
	39.2	30.5	5.9	0.973
	38.9	27.1	5.9	1.318
	38.9	27.0	5.9	1.394
	38.3	27.0	5.9	1.280
	34.3	27.0	5.9	0.691
	33.6	27.1	5.9	0.697
	31.1	20.7	5.9	0.858
	43.2	26.8	8.9	2.063
	38.8	30.5	8.9	1.132
	38.0	26.9	8.9	1.443
	37.9	26.8	8.9	1.378
	33.9	26.7	8.9	0.755
	33.6	26.8	8.9	0.735
	30.6	20.6	8.9	0.927

The analysis of the TP effects was performed by the Schofield et al. method as described in the Theory Section. Schofield plots for the two values of the Reynolds number employed are shown in Fig. 3 for membrane TF-200 and in Fig. 4 for membrane TF-450. The data corresponding to NaCl in Fig. 3 and to pure water in Fig. 4 have been fitted to straight

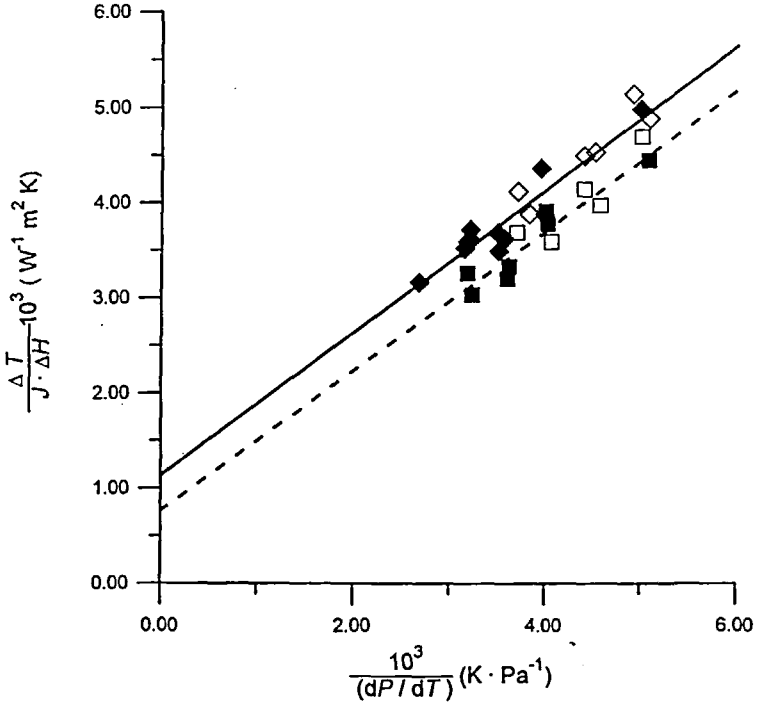

FIG. 3 Schofield plots for the TF-200 membrane. (◇) BSA solutions with tangential velocity, $v = 5.9$ cm·s^{-1}. (◆) NaCl solutions at $v = 5.9$ cm·s^{-1}; the solid line is the linear fitting of these points. (□) BSA solutions at $v = 8.9$ cm·s^{-1}. (■) NaCl solutions at $v = 8.9$ cm·s^{-1}; the dashed line is the fitting of these points. Both the BSA and the NaCl series include measurements made at different initial concentrations.

lines. An examination of both figures leads to three conclusions: 1) the experimental points may be adequately fitted by the linear relationship proposed by Schofield et al.; 2) there are not any difference between the fluxes measured for the BSA solutions, the pure water, and the NaCl solutions; and 3) the transmembrane flux is independent of the initial value of the BSA concentration. These results indicate, as expected, that no fouling was present in our protein concentration experiments.

On the other hand, the overall heat transfer coefficient may be evaluated from the intercept of the straight lines in Fig. 3 and in Fig. 4. The obtained U_{Calc} values ranged between 1.5 and 2.5 kW·m^{-2}·K^{-1}. By using Eq. (3), the corresponding TPC may be estimated for this calculation a typical

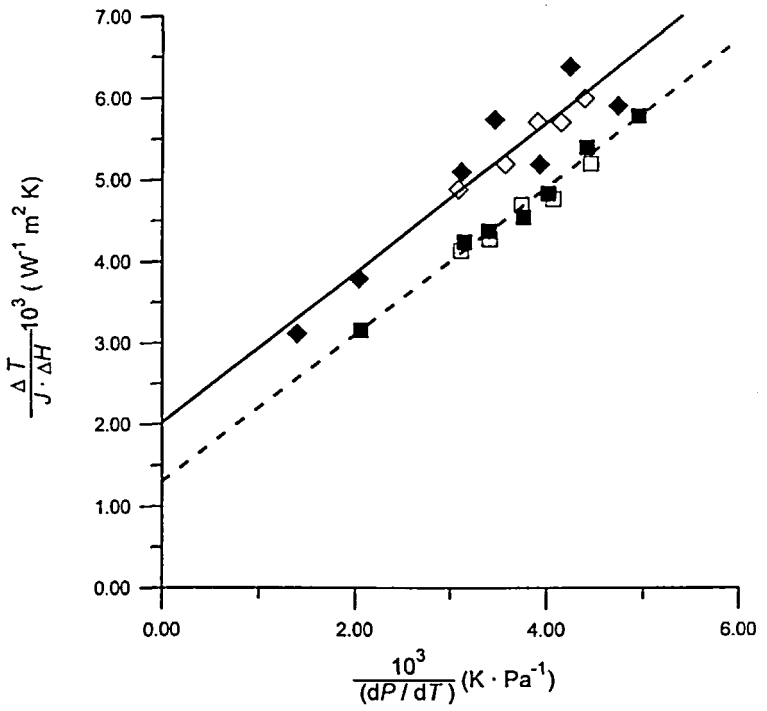

FIG. 4 Schofield plots for the TF-450 membrane. (◇) BSA solutions with tangential velocity, v = 5.9 cm·s^{-1}. (◆) Pure water at v = 5.9 cm·s^{-1}; the solid line is the linear fitting of these points. (□) BSA solutions at v = 8.9 cm·s^{-1}. (■) Pure water at v = 8.9 cm·s^{-1}; the dashed line is the fitting of these points. The two BSA series include measurements made at different initial concentrations.

value of $\lambda/\delta \approx 0.5$ kW·m^{-2}·K^{-1} was used (8)]. The obtained values vary between 0.6 and 0.7. These low values for the TPC mean that the TP effects are relatively high, since around 40–30% of the externally applied temperature difference between the two bulk phases is lost in the boundary layers. The present values for U_{Calc} agree with those in the literature for similar hydrodynamic conditions: Schofield et al. reported a U value of ~2.5 kW·m^{-2}·K^{-1} (8), Tomaszevska et al. of ~0.2–1.5 kW·m^{-2}·K^{-1} (15), and Fujii et al. of ~0.7 kW·m^{-2}·K^{-1} (16). In relation to the fouling effects in MD, the available literature is rather scarce. Calabrò et al. reported a flux decline in the case of orange juice concentration by MD (9), but this effect was explained as due to a progressive growing of the feed concentration.

CONCLUSIONS

It is possible to concentrate protein solutions at atmospheric pressure and low temperatures by using membrane distillation techniques.

Membrane fouling is absent in MD of protein solutions. This result agrees with predictions concerning the existence of a critical flux below which fouling is absent. This prediction was proposed in Ref. 7 for the study of membrane separation processes of proteins.

The only important limiting factor in MD of protein solutions is temperature polarization. To evaluate it, the techniques and models developed for MD of pure water or low molecular weight solutions may be employed.

MD of protein solutions could be an alternative to reverse osmosis. Althought RO is a more efficient process regarding the magnitude of the fluxes, the use of MD might be interesting under some circunstances; for instance, when it is possible to employ some source of wasteheat.

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