Retention of Proteins in Cross-Flow UF through Asymmetric Inorganic Membranes

P. Pradános, J. I. Arribas, and A. Hernández
Departamento de Fisica Aplicada II, Facultad de Ciencias, Universidad de Valladolid, 47071 Valladolid, Spain

The flow and retention of 0.1% w/w aqueous solutions of pepsin, bovine serum albumin (BSA), lipase, γ-globulin, and invertase with molecular weights of 36,000, 67,000, 80,000, 150,000, and 270,000 dalton (g/mol) are studied when they are tangentially filtered through an inorganic microporous membrane with a nominal pore size of 0.02×10^{-6} m made by Anopore, with transmembrane pressure differences up to 100 kPa. The data were analyzed within the frame of the film layer theory for the concentration polarization phenomenon. This allows the mass-transfer coefficient to be obtained for the cell used as a function of the feed circulation speed and the molecular weight of the solute. Apparent and true retention curves obtained lead to a size exclusion radius smaller than the nominal one but very similar to that obtained from scanning electronic photographs.

Introduction

Ultrafiltration is suited to both continuous and batch operations and offers several advantages over more traditional separation methods. For example, ultrafiltration is specially suitable to handle with heat labile substances due to no heat added. In addition, the products are not subjected to chemical denaturation when ultrafiltered, while a certain change in nature can appear with alternative processes like solvent extraction.

Due to all these factors, ultrafiltration is being used increasingly as a concentration and separation process in a variety of industries. For example, in biotechnological applications dealing with separation of proteins, ultrafiltration is used due to its gentle nature which makes it specially suitable for handling of sensible macromolecules (Forman et al., 1990; Bozzano and Glatz, 1991).

There are some disadvantages to ultrafiltration as well. Actually, once a membrane has been obtained that retains a macromolecular species, it should be convenient to secure high fluxes in order to increase the efficiency of the ultrafiltration step. Nevertheless, it is known that for high pressures the permeability decreases until a more or less flat plateau is reached for the flux. Hence, the volume flow cannot surpass a certain upper limit (Grieves et al., 1973).

The presence of this maximal flux has been attributed to the phenomenon of concentration-polarization, that is, the buildup of rejected solute in the boundary layer near the membrane surface. The mechanism by which flux reduction occurs has been variously thought to be, for example, a reduction in driving force resulting from the increased osmotic pressure at the membrane surface, the formation of a gel which offers a hydraulic resistance in addition to that of the membrane, or a fouling process (Vilker et al., 1981).

The fouling caused by physical or chemical adsorption can be avoided or limited if the solute-surface interactions are minimized by decreasing the membrane-protein affinity. This can be done, for example, by controlling the pH level, as shown by Nakao et al. (1988), with a subsequent possibility of a denaturation of the protein for extreme pH.

Also, low fouling levels can be obtained by using a filtration device provided with a feed chamber supplying a tangential flow over the membrane. Then, a high enough speed through the recirculation loop of the retentate should minimize the mean time of contact of the protein on the solid matrix. This would also clean the membrane surface by sweeping the protein molecules that could be otherwise entrapped on the pore entrances, causing pore clogging.

On the other hand, inorganic membranes seem to be especially interesting due to their high permeabilities and resistances to various organic and inorganic chemicals and high temperatures, with the subsequent possibility of sterilizing and reusing them. A particular type of inorganic membrane produced by anodic oxidation of aluminum seems to be especially interesting

Correspondence concerning this article should be addressed to A. Hernandez.

and easy to model. This anodic oxidation in a certain acid electrolyte results in a structure comprising a uniform columnar array of hexagonally close packed alumina cells each containing a cylindrical pore. According to O'Sullivan and Wood (1970), the cell and pore sizes are controlled by the anodizing voltage, whereas the film thickness is determined by the current density and the anodizing time. The porous films are detached from the aluminum substrate by a controlled reduction of the anodizing voltage, which results in a skin layer formed by smaller pores, as described by Fourneaux et al. (1989) and Rigby et al. (1990). The resulting membranes are highly porous and exhibit a capillary pore structure with very uniform pore sizes.

Usually, the manufacturers of ultrafilters specify a nominal molecular weight cutoff for their products. However, in practice, there is not a sharply defined molecular weight below which solutes pass the membrane and above which they are retained. Hence, if a process has to be designed including an ultrafiltration step, the corresponding retention characteristics should be studied in detail. In fact, the molecular weight cutoff depends on both the pressure difference acting through the membrane and the velocity of recirculation used in the retention loop.

Several proteins with various molecular weights are used in this work to characterize the retention properties of a commercially available alumina membrane manufactured by Anopore used in a tangentially driven device. Relatively high recirculation speeds are used with a constant pH. This neutral pH is used in order to reproduce the most usual conditions in the natural media and bioreactors. Moreover, the Anopore membranes seem to be almost discharged at neutral pH, which should reduce adsorption. On the other hand, if the proteins selected have a fairly strong negative charge at neutral pH, this should prevent the formation of protein aggregates.

For these membranes and solutes, the flux is measured vs. the applied pressure for several recirculation speeds. The apparent retention is measured to obtain the mass-transfer coefficient as a function of speed for several protein aqueous solutions. This allows the diffusivities of bovine serum albumin, lipase, and γ-globulin to be obtained, which fit well to a correlation of the Stokes-Einstein type.

Finally, the true retention is studied and the maximal retention curves are obtained and analyzed in terms of both the molecular weights and the gyration radii, leading to a sieve radius of 0.008 μm. This value is smaller than both the nominal one and the surface mean pore radius (0.012 μm). Nevertheless, this experimental sieve radius agrees with the minimum radius of the pores in the active layer, as seen by scanning electron microscopy (SEM images).

Experimental

Chemicals

An Anopore (Anodisc) filter supplied by Anotec Separations has been used. Here, it will be called A002 on account of its nominal pore diameter of 0.02 μm. This membrane consists of a system of large pores opened to a face and interconnected with a system of narrower and shorter pores that form the active layer. As shown by Fourneaux et al. (1989) and Thomas et al. (1991) by using SEM images, the asymmetric layer of fine pores is approximately 0.5 μm thick with a pore diameter of 0.025 μm while the average diameter of the larger pores is 0.15 μm. The total thickness of the membrane is 60 μm.

In order to avoid any irreversible change during operation, each membrane sample has been pressurized until 100 kPa for 45 min before being used (higher pressures would damage the membrane, while no permeability change could be detected for longer pressurizing periods). Aqueous solutions of several proteins (Pepsin, Bovine Serum Albumin, Lipase, γ-globulin, and Invertase) with molecular weights of 36,000, 67,000, 80,000, 150,000 and 270,000 daltons have been used. All the proteins were obtained from FLUKA AG: Pepsin (EC 3.4.23.1) crystalline powder from hog stomach, BSA of a purity >98%, lipase (EC 3.1.1.3) fine powder from rhizopus arrhizus, γ-globulin from bovine blood of a purity >98%, and invertase (EC 3.2.1.26) powder from baker's yeast containing up to 40% glucose.

The aqueous solution used is relatively dilute, 0.1% w/w, in order to minimize the solute-solute interactions. It has been prepared with distilled, degasified, and deionized water (resistivity higher than 18 MΩ•cm), and all solutions have been previously prefiltered with another Anopore filter A02 (whose nominal pore diameter is 0.2 μm) in order to eliminate large residual particles. All concentrations have been measured by using the assay of Lowry et al. (1951) with a spectrophotometer set at 750 nm.

A pH buffer (HNa_2PO_4:H_2NaPO_4 at 8.1×10^{-3} N and 1.9×10^{-3} N) of 7.4 has been used along with a bactericidal agent (NaN_3) at 0.02% w/w. According to Kirkwood (1967) and Malamud and Drysdale (1978), the isoelectric points are between 2.2 to 2.8 for pepsin, from 4.7 to 4.9 for BSA, 4.0 for lipase, from 6.1 to 6.47 for γ-globulin, and 3.4 for Invertase; therefore, all of the proteins are negatively charged at neutral pH. This and the presence of salts added as buffer and bactericide, which increase the ionic strength, prevent the formation of aggregates.

Experimental setup

All the experiments are performed in isothermal conditions at 298 K, by using a flat membrane tangential ultrafilter device that has been described elsewhere by Prádanos et al. (1992). The membrane cell is made of methacrylate and it is provided with four prismatic channels of $1.0\times5.25\times28.0$ mm on the membrane, whose hydraulic diameter is $d_h=1.68\times10^{-3}$ m, giving an effective membrane area of 5.08×10^{-4} m^2 and a total cross section of the retentate loop of 5.25×10^{-6} m^2.

The solutions are tangentially driven over the membrane and recirculated with speeds 0.048, 0.079, 0.159, 0.238, 0.317, 0.397, 0.476, 0.794, 1.190, 2.143, 3.175, 4.762 and 7.936 m/s. These speeds are defined as the volume flow through the hydraulic channel per unit time and unit cross-sectional area, hence, they are speeds averaged for a channel section. The applied transmembrane pressures are always below 100 kPa.

The solution is extracted from a thermostatted reservoir by means of a regulated impulsion pump. Two pressure transducers are placed before and after the membrane holder in the retentate loop. They have a range of 0–1,000 kPa over the atmospheric pressure, with a maximal error of ±0.25% full scale.

Given that the pressure loss along the hydraulic channel of the permeate loop is small and may be assumed as almost linear, the transmembrane pressure can be taken as the average

of the values taken up and down the membrane cell. In order to measure the retentate flow, two electromagnetic flowmeters are used, whose ranges are $1\times10^{-6}-1\times10^{-5}$ m^3/s and $1.67\times10^{-5}-1.67\times10^{-4}$ m^3/s, both with errors lower than a $\pm0.25\%$ full scale. The speed and pressure in the retentate loop are independently controlled by means of the pump regulation and an appropriate needle valve. The volume flow of permeate through the membrane is measured by timing and weighting with a high precision balance with errors lower than $\pm1\times10^{-7}$ kg.

Theory

Apparent and true retention

Concerning the output or permeate concentration, c_p, it is convenient to give it in terms of the feed concentration, c_0, through the so called observed or apparent retention coefficient:

$$R_o = 1 - \frac{c_p}{c_0} \tag{1}$$

This retention coefficient is a function of pressure, recirculation speed, and molecular weight, as pointed by Nakao and Kimura (1981).

Actually, there is a concentration c_m ($>c_0$) in contact with the membrane due to an accumulation phenomenon resulting from the balance of convection through the membrane and back diffusion which is called concentration polarization.

Then a true retention coefficient can be defined as:

$$R = 1 - \frac{c_p}{c_m} \tag{2}$$

which relates the actual concentrations on both faces of the membrane.

The concentration polarization phenomenon can be studied following the so called film-layer model, as formulated by Nakao et al. (1979, 1986), which assumes a zone where the concentration decreases from c_m on the membrane to c_0 at a distance δ inside the retentate phase. This hypothesis leads to a permeate volume flow per unit of exposed area of the membrane, J_v, given by:

$$J_v = K_m \ln \frac{c_m - c_p}{c_0 - c_p} \tag{3}$$

according to Jonsson and Boessen (1977), where $K_m = D/\delta$ is the so called mass-transfer coefficient and D is the diffusion coefficient. This coefficient could be evaluated by imposing a zero concentration on the permeate side of Eq. 3 and rearranging to:

$$J_v = K_m(\ln c_m - \ln c_0) \tag{4}$$

then, a semilog plot of J_v as a function of c_0 for c_m constant should lead to a straight whose slope would be K_m with a zero ordinate given by $K_m\ln(c_m)$. Nevertheless, this would need to work with very high feed concentrations near the gelation ($c_m \approx c_g$), with high viscosities and very important solute-solute interactions and formation of aggregates. This suggests looking for another low concentrations method, as described below.

Mass-transfer coefficient

By taking into account Eq. 2, Eq. 3 can be modified to:

$$\ln \frac{1-R_0}{R_0} = \ln \frac{1-R}{R} + \frac{J_v}{K_m} \tag{5}$$

According to the traditional film-layer theory, when there is almost gelation on the retentate face of the membrane, c_m increases very slowly and the volume flow is approximately independent of Δp. Then the transport through the membrane can be assumed to be mainly convective, leading to a virtually constant ratio c_p/c_m and a maximum true retention coefficient, R_{max}. Thus the first term of the righthand of Eq. 5 can be taken as constant and a plot of $\ln[(1-R_o)/R_o]$ against J_v would be a straight with $1/K_m$ as slope, and R_{max} can be obtained from the ordinate intercept.

On the other hand, the mass-transfer coefficient can be calculated on the basis of heat-transfer analogies by some kind of combination of dimensionless numbers, as shown by Wiley et al. (1985) and Gekas and Hallström (1987):

$$Sh = A\,(Re)^{\alpha}(Sc)^{\beta} \tag{6}$$

where A, α and β are constants and the Sherwood, Reynolds, and Schmidt numbers are:

$$Sh = \frac{K_m d_h}{D}$$

$$Re = \frac{v\rho d_h}{\eta}$$

$$Sc = \frac{\eta}{\rho D} \tag{7}$$

with ρ being the density of the retentate solution and η its viscosity. Then, by taking into account Eqs. 6 and 7:

$$K_m = \Phi v^{\alpha} \tag{8}$$

where Φ depends on the solute and the channel dimensions.

Then, Eq. 5 can be written as:

$$\ln \frac{1-R_0}{R_0} = \ln \frac{1-R}{R} + \frac{J_v}{\Phi v^{\alpha}} \tag{9}$$

Thus, the slope of a representation of $\ln[(1-R_o)/R_o]$ against J_v/v^{α} would be $1/\Phi$ and the ordinate intercept would give R_{max}, like in a direct representation of Eq. 5.

Several values have been proposed for the coefficients A, α, and β. According to Cheryan (1986) and Van den Berg et al. (1989), these exponents depend on the flow region and the lengths of the channel entrance. Among the plethora of available correlations it seems that for a laminar flow and $L_v < L < L_c$ (L_c being the length of development of the concentration limit

Table 1. Reynolds Numbers and Speeds on the Membrane Surface

Regime	Laminar										Turbulent	
v(m/s)	0.048	0.079	0.159	0.238	0.317	0.397	0.476	0.794	1.190	2.143	3.175	7.936
Re	90	148	298	446	594	744	892	1488	2230	4017	5951	14874

layer, L_v the length of the boundary momentum layer, and L the channel length) the Graetz-Leveque coefficients can be used for a nontubular membrane (Mulder, 1991). Thus, the following values: $A=1.86$, $\alpha=0.33$, and $\beta=0.33$ will be used here in the laminar regime. The Reynolds numbers corresponding to the speeds used here are shown in Table 1.

In such conditions, the coefficient of Eq. 8 is:

$$\Phi = 1.86\left(\frac{D^2}{d_h L}\right)^{1/3} \tag{10}$$

showing that Φ depends on the solute exclusively through its diffusivity.

In fact, at least for the lower speeds, we are in conditions of laminar flow, as shown in Table 1. On the other hand, the values of L_v and L_c can be evaluated for tubular channels by using:

$$L_v = Bd_hRe$$

$$L_c = 0.1\gamma_w \frac{d_h^3}{D} \tag{11}$$

according to Cheryan (1986) and Van den Berg et al. (1989). In Eq. 11, B is 0.029 but can arrive in special cases until 0.05 and γ_w is the shear rate at the membrane surface:

$$\gamma_w = \frac{8v}{d_h} \tag{12}$$

By using these equations $L<L_c$ whereas $L_v<L$ is not totally assured for high Reynolds numbers. In any case, we will use here Eqs. 9 and 10 with $\alpha=0.33$.

Results and Discussion

Volume flow and pressures

The volume flow per unit of exposed area of the membrane, J_v, has been measured as a function of the pressure difference applied through the membrane. First of all, it has been done for pure water (with the pH buffer and the bactericidal agent) giving a hydrodynamic permeability of 1.2047×10^{-8} m/Pa·s.

Actually, the volume flow decreases with time until a stationary state is reached due to protein adsorption, according to Suki et al. (1984). This has also been shown by Prádanos et al. (1992) for this membrane and aqueous solutions of invertase. These stationary values of J_v for the 0.1% w/w solutions of bovine serum albumin, lipase, γ-globulin, and several speeds of recirculation of the feed are shown vs. Δp in Figure 1. Almost always, J_v increases faster with pressure in the small pressures range and slower for high pressures. This is usually thought to be a consequence of the concentration polarization which is specially relevant for high pressures. On the other hand, the pepsin solution passes freely through the membrane while invertase is totally retained, for all the recirculation speeds used. In fact, while invertase gives a total retention, the glucose contained in the commercial solution of invertase is not retained. This fact was previously used to eliminate these impurities, as tested by a Fehling essay. Then, only one speed (0.476 m/s) has been used to measure flux (J_v) vs. the applied pressure (Δp) for both these proteins. In Figure 2, the flux is shown for this speed and all the proteins. Hydraulic permeability decreases with molecular weight, except for invertase, which can be explained in terms of the permeation mechanism acting when the solute molecules are totally retained.

As shown in Figure 3, it is possible to have a qualitative change in the permeation process that should lead to higher fluxes for large retained molecules than for smaller molecules, as obtained for invertase (permeation type A) as compared

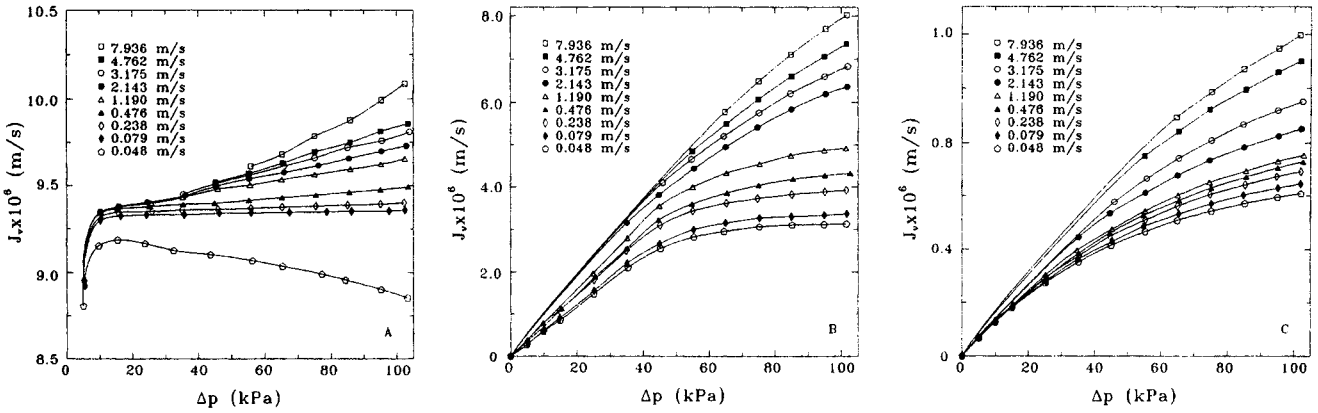

Figure 1. Volume flow vs. Δp for several recirculation speeds.

(A) Bovine serum albumin; (B) lipase; (C) γ-globulin.

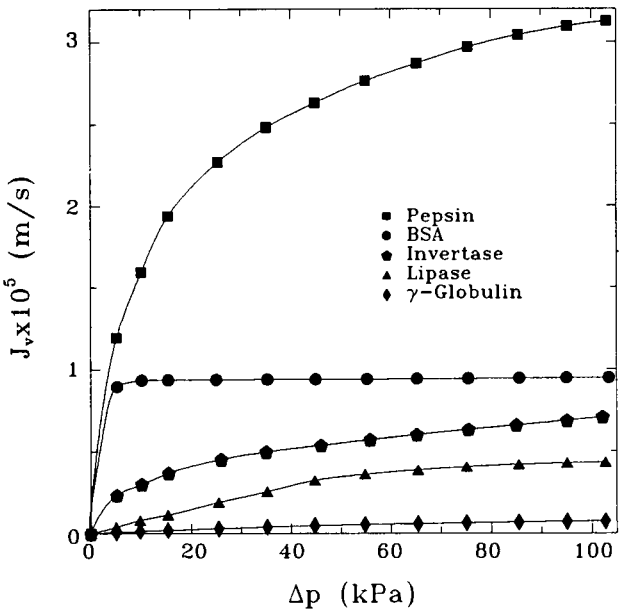

Figure 2. Volume flow vs. Δp for $v = 0.476$ m/s for all solutes.

with γ-globulin (type B) and lipase, BSA, and pepsin (type C). This could also explain the special behavior of J_v against pressure and speed for the second smallest protein, BSA, shown in Figure 1, especially for low recirculation velocities ($v = 0.048$ m/s for example) even with a decrease of J_v with pressure. This could be due to the fouling of the inner surfaces of the pores that should be less efficiently avoided by the circulation of the feed on the membrane if a permeation mechanism of type C is assumed.

Apparent retentions and mass-transfer coefficients

The apparent retention coefficient, R_o, can be calculated by Eq. 1 once the permeate concentration is measured for each applied pressure drop and recirculation speed. Then, the obtained R_o is plotted in Figure 4 as a function of Δp for several speeds in the laminar and turbulent ($v = 7.936$ m/s) ranges.

In laminar regime, it can be seen that for small retentions R_o increases with Δp for any recirculation speed (see Figure 4A). For higher retentions and also in laminar conditions, R_o decreases with pressure, sometimes after an initial increase (see Figures 4B and 4C).

These steps of increasing retention for increasing pressures can be a consequence of the entrance of more protein molecules inside the pores, until they are finally pulled through the pores when higher pressures are established. This phenomenon should be more relevant for small molecules (see Figure 3C) and molecules almost fitting inside the pores (see Figure 3B), that is for BSA and γ-globulin (Figure 4). Hence, with high molecular weight proteins, a constant R_o (a constant c_p) should be reached for higher pressures than with smaller proteins, as in fact is observed in Figure 4.

As foreseeable, for a given pressure, the observed retention increases with speed until for Reynolds numbers in the turbulent range it increases substantially due to the subsequent decrease in the thickness of the concentration-polarization layer. In the turbulent regime, R_o is almost constant for any pressure, possibly because the turbulent stirring prevails over the pressure effects.

In order to evaluate the mass-transfer coefficient as a function of v, we can plot the Eq. 5 for diverse constant recirculation speeds and variable Δp, as shown for BSA, Lipase and γ-globulin in Figures 5–7. The values of R_{max} shown in the figures have 95% confidence levels (t-tested).

Alternatively, we can measure R_o for several constant pressure differences and variable recirculation speeds within the laminar regime. Of course, the pressures should be high enough to be in the zone of J_v almost independent of Δp. Then, we can represent Eq. 9 with $\alpha = 0.33$ to obtain Φ directly, which does not depend either on v or on Δp, as shown in Figures 8–10. The values of Φ and R_{max} shown in the figures have 95% confidence levels (t-tested). The so obtained mass-transfer coefficients are presented in Figure 11 as a function of v. It can be seen that they always increase with v and are smaller for the higher molecular weights, as expected.

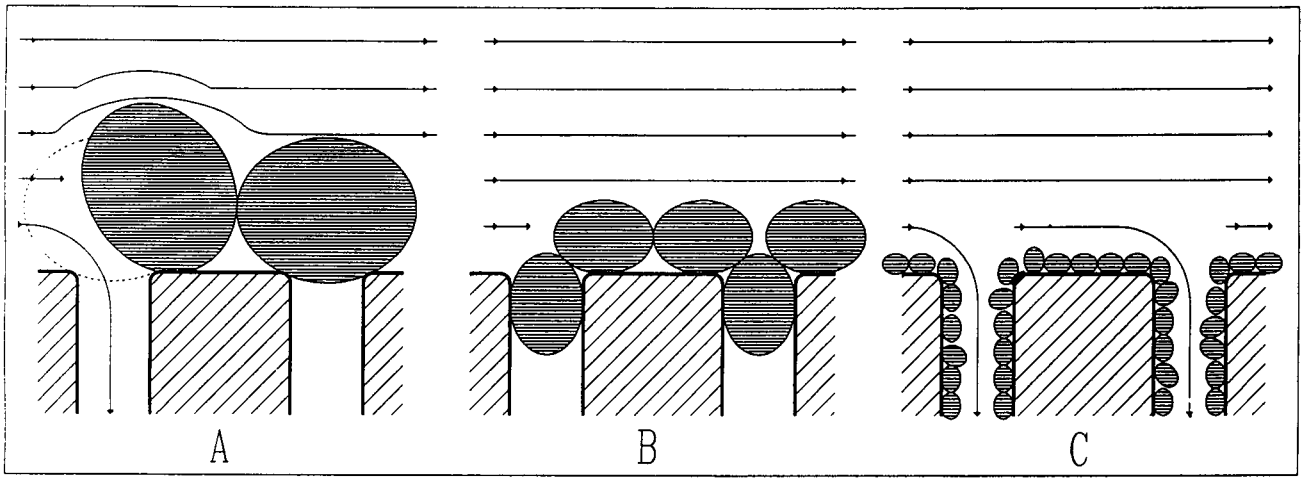

Figure 3. Possible mechanisms for permeation for (A) totally retained solutes; (B) partially retained; (C) freely passing solutes.

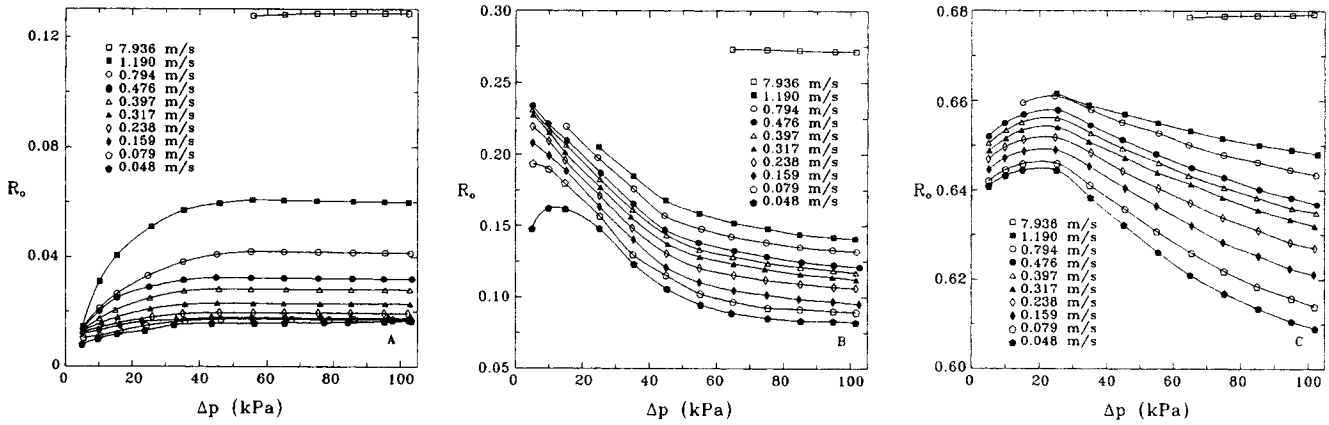

Figure 4. Apparent retention coefficient against Δp for several recirculation speeds and (A) BSA; (B) lipase; (C) γ-globulin.

Diffusivities and standard retention curves

The diffusivity can be evaluated from the values of K_m obtained by both methods and Eqs. 8 and 10. Then, the values for density and viscosity are taken as equal to the pure-water ones at 298.0 K: $\rho = 997.07$ kg/m^3 and $\eta = 0.8937 \times 10^{-3}$ kg/m·s leading to the values of D shown in Table 2. The values of D shown in the table have 95% confidence levels (t-tested).

In any case, D decreases with the molecular weight as is usual. On the other hand, it can be seen that the diffusivities obtained by the two methods are in reasonable agreement for lipase and γ-globulin, while they are less similar for BSA. This could be probably due to the errors, present in both methods, that become highly significant for the small retentions associated with bovine serum albumin. On the other hand, the errors linked to the use of not highly enough pressures are less significant for the constant pressure method.

Data on protein diffusivities are scarce in the literature but the infinite dilution diffusion coefficient for pure γ-globulin (Hess and Deutsch, 1948) seems to be 4.1×10^{-11} m^2/s while it should be 6.1×10^{-11} m^2/s for pure BSA (Brandrup and Immergut 1989). Nevertheless, these values cannot be used here, given that the ionic strength, which is fairly high due to the bactericidal and buffer solutions ($I = 0.021$ N), should affect the solute-solute and solute-solvent interactions.

It is known that the infinite dilution diffusivities depend on the molar volume of the solute. For solutes with roughly spherical molecules whose radius is large compared to that of the solvent, the Stokes-Einstein equation could be used:

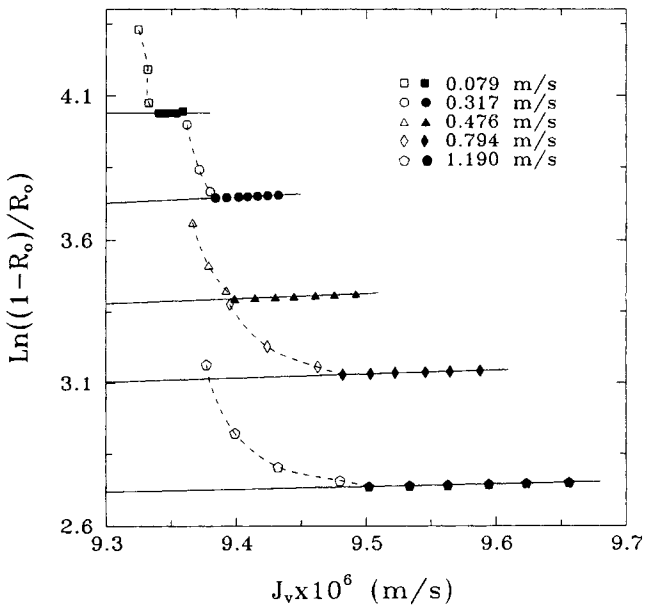

Figure 5. Representation of Eq. 5 for several recirculation speeds and BSA.

Only solid lines and ■ ● ▲ ◆ ● correspond to fluxes almost independent of Δp. The corresponding value of R_{max} is 0.14 ± 0.07.

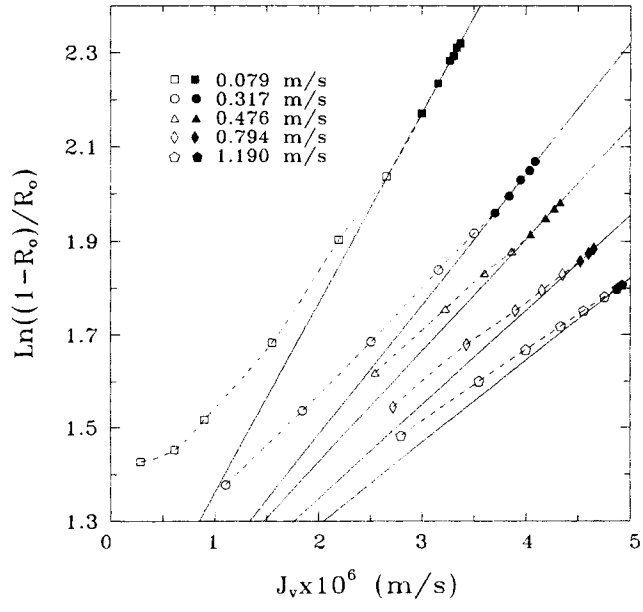

Figure 6. Representation of Eq. 5 for several recirculation speeds and lipase.

Only solid lines and ■ ● ▲ ◆ ● correspond to fluxes almost independent of Δp. The corresponding value of R_{max} is 0.281 ± 0.009.

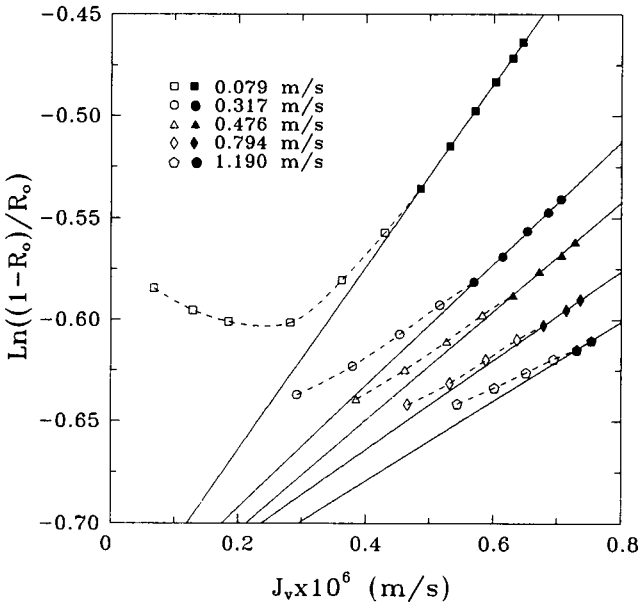

Figure 7. Representation of Eq. 5 for several recirculation speeds and γ-globulin.

Only solid lines and ■ ● ▲ ◆ ● correspond to fluxes almost independent of Δp. The corresponding value of R_{max} is 0.678 ± 0.004.

$$D = \frac{R_g T}{6\pi\eta r} \tag{13}$$

where R_g is the gas constant, T is the temperature, and r is the equivalent or gyration radius of the molecule.

If we introduce the molecular weight, M_w, and take into account that, in fact, the molecules can be taken as ellipsoids, we have:

$$D = \frac{\xi T}{\eta M_w^{1/3}} \tag{14}$$

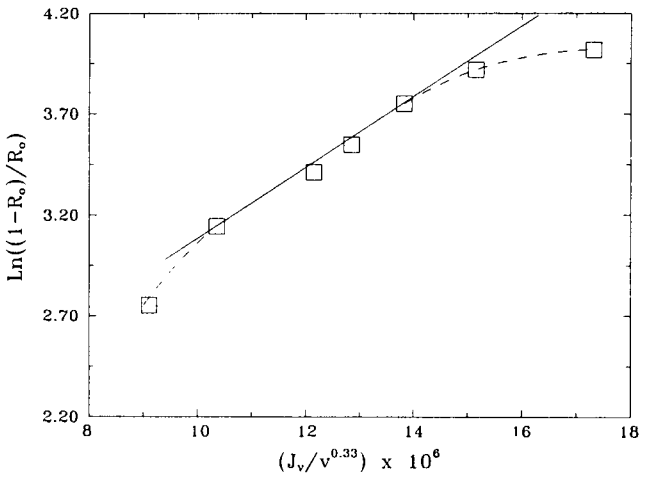

Figure 8. Representation of Eq. 9 for $\Delta p = 100$ kPa and BSA.

Only solid line corresponds to laminar regime (*Re* from 298 to 744). If we use all range of pressures with J_v independent of Δp, then $\Phi = (5.764 \pm 0.009) \times 10^{-6}$ (m/s)$^{2/3}$ and $R_{max} = 0.2088 \pm 0.0005$.

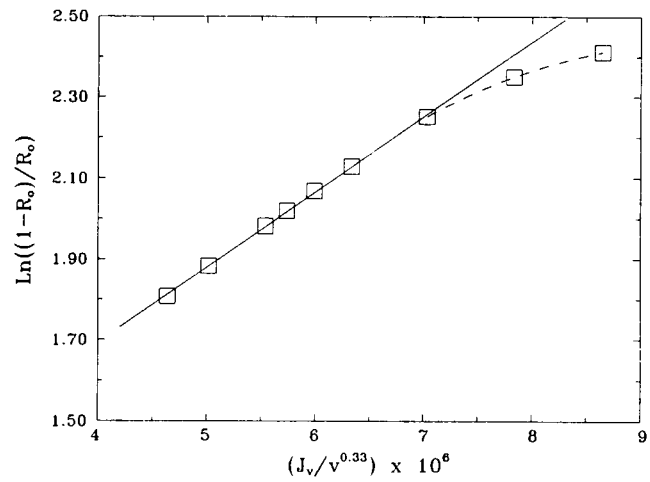

Figure 9. Representation of Eq. 9 for $\Delta p = 100$ kPa and Lipase.

Only solid line corresponds to laminar regime (*Re* from 298 to 2,230). If we use all range of pressures with J_v independent of Δp, then $\Phi = (5.47 \pm 0.08) \times 10^{-6}$ (m/s)$^{2/3}$ and $R_{max} = 0.275 \pm 0.003$.

ξ being a proportionality constant. By taking into account that the partial specific volume of proteins has been demonstrated to be between 0.69 and 0.78 cm^3/g, showing a Gaussian distribution with a mean value 0.73 cm^3/g and using Eq. 13, we should have $\xi = 11.06 \times 10^{-15}$. In fact, Young et al. (1980) have shown that for proteins at infinite dilution and without any ions in the solution, $\xi = 8.34 \times 10^{-15}$. Nevertheless, if one such equation is tried for our data (D_p in Table 2) we obtain a very good accordance with $\xi = 4.52 \times 10^{-15}$, as is shown in Figure 12.

Once the mass-transfer coefficients have been obtained by both methods, the concentration in contact with the membrane, c_m, and the true retention coefficient, R, can be calculated by Eqs. 2 and 3. It is always seen that R increases with

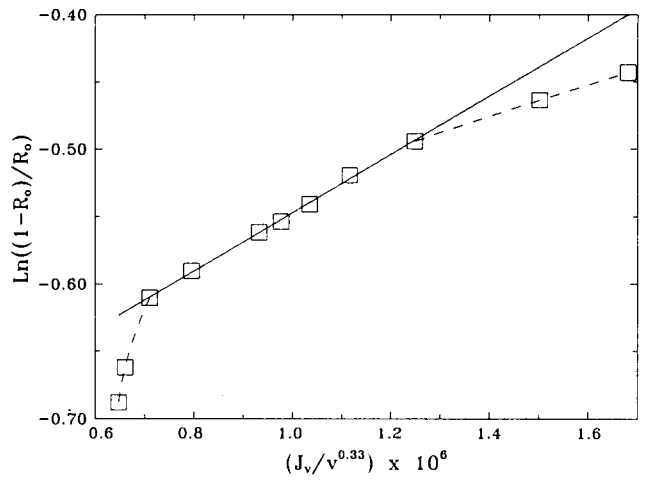

Figure 10. Representation of Eq. 9 for $\Delta p = 100$ kPa and γ-globulin.

Only solid line corresponds to laminar regime (*Re* from 298 to 2,230). If we use all range of pressures with J_v independent of Δp, then $\Phi = (4.62 \pm 0.04) \times 10^{-6}$ (m/s)$^{2/3}$ and $R_{max} = 0.6819 \pm 0.0005$.

Table 2. Diffusivities of BSA, Lipase and γ-Globulin

	D_v (10^{-11} m^2/s)	D_p (10^{-11} m^2/s)	ΔD (%)
BSA	5.42±1.75	3.74±0.12	31.0
Lipase	3.31±0.19	3.46±0.09	−4.5
γ-Globulin	2.90±0.20	2.68±0.04	7.6

Note: D_v is obtained for v=const., D_p corresponds to a constant applied pressure while $\Delta D = (D_v - D_p)/D_v$.

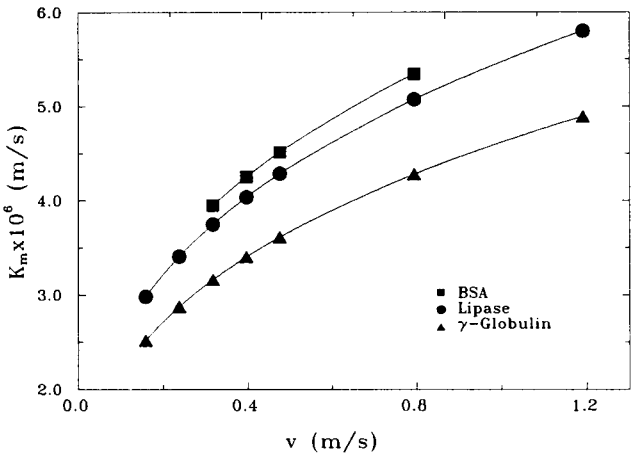

Figure 11. Mass-transfer coefficient as function of v in the laminar regime for proteins BSA, lipase, and γ-globulin.

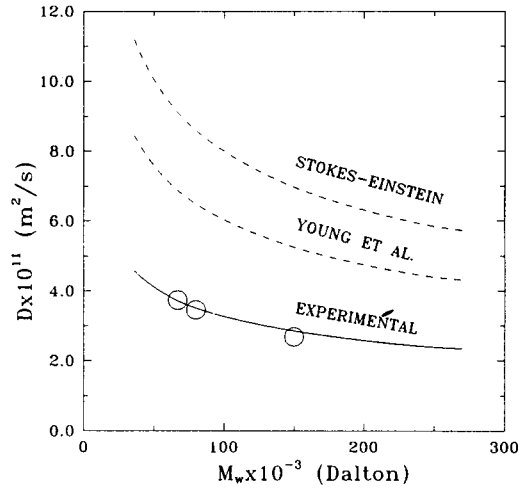

Figure 12. Diffusivities as function of molecular weight according to Eq. 13 with $\xi = 11.06 \times 10^{-15}$ (Stokes-Einstein), 8.34×10^{-15} (Young et al.) and 4.52×10^{-15} (experimental).

Δp to a more or less flat plateau corresponding to the region with volume flows independent of pressure.

The apparent and true retention coefficients are closer to each other for small Δp, while they separate for increasing pressures. Of course, the range of small pressures giving similar R and R_o are wider for high recirculation speeds. Both the coefficients are shown for γ-globulin against Δp for a low speed in Figure 13A, whereas they are plotted as a function of speed for a high Δp in Figure 13B.

The maximum retention corresponds to the above mentioned plateau and their values are given in Figures 5-10. It is worth noting that the results on R_{max} obtained by both methods are equal within the error range.

The gyration radii of the five proteins can be obtained from the general diagram of Sarbolouki (1982). Then the maximum retention coefficient is represented as a function of the logarithm of the molecular weight and the gyration radius in Figure 14. It is seen that a 100% retention is obtained for an equivalent molecular radius of 0.008 μm; this value is smaller than the

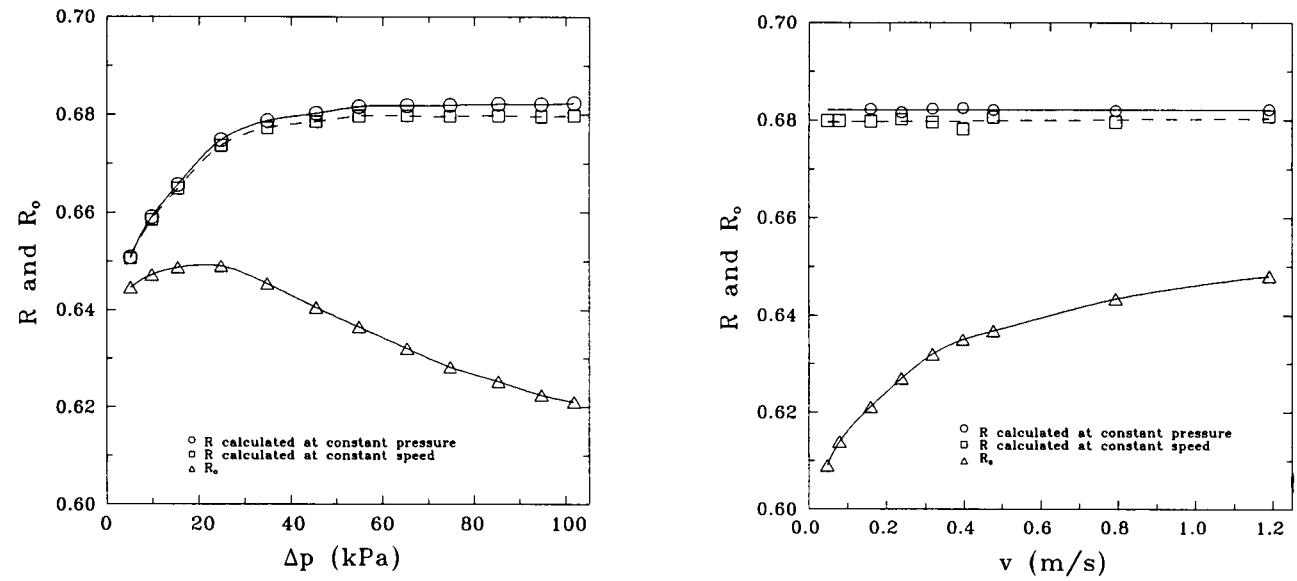

Figure 13. Observed and true retention coefficient for γ-globulin (A) as a function of Δp for v=0.159 m/s and (B) as a function of v for Δp=100 kPa.

Dashed lines conform to constant speed method to obtain K_m while solid ones correspond to constant pressure method.

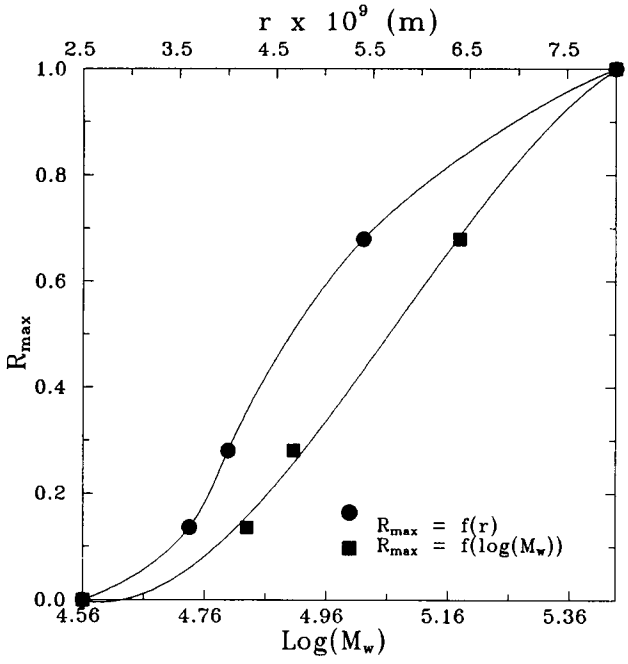

Figure 14. Maximal retention as function of gyration radii and logarithm of M_w.

surface mean pore radius (0.012 μm). In fact, transversal SEM images of these membranes show that the pores in the active layer are narrower inside them leading to a value of 0.0082 μm. On the other hand, some small adsorption should be present according to Kim et al. (1992) that could explain part of the small discrepancies between SEM and operation values of pore size.

Conclusions

We have characterized a microporous asymmetric membrane in cross-flow ultrafiltration of proteins, by measuring the apparent or observed coefficient of retention and correcting it to obtain the real one. This has been done in accordance with the film layer model for the concentration-polarization effect.

The mass-transfer coefficient of the proteins is calculated by two alternative methods. Between them, the constant pressure method seems to give better results, probably due to the more easy fixing of high pressure conditions that give J_v values that are really pressure-independent.

The Graetz-Leveque correlation has been used through Eq. 10. It is worth noting that a value of $\alpha = 1/3$ is predicted by many different correlations and has been proven to conform better to the data. On the other hand, this α and the equation for ϕ give diffusion coefficients closer to those available in the literature with a dependence on the molecular weight which is very similar to that predicted by Young et al. (1980).

Actually, the diffusivities are inversely proportional to the cubic root of the molecular weight, according to the predictions of the Stokes-Einstein equation but with a proportionality constant lower than calculated. This deviation can be caused by the presence of the bactericidal agent and the pH buffer, along with the relatively high concentrations that are reached on the membrane due to concentration-polarization.

It is seen that a 100% retention is obtained for an equivalent molecular radius of 0.008 μm. In fact this value is below the nominal pores size and is also smaller than mean pore radius obtained from SEM, which is 0.012 μm. Nevertheless this sieve pore size seems to agree with the narrower radius of the pores in the skin layer as seen in transversal electron photographs. This agreement allows to disregard significant changes of the effective operational pore radius due, for example, to protein adsorption.

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Notation

A = coefficient of the Chilton-Colburn correlation
B = coefficient to calculate the length of the concentration boundary layer
c_g = gelation concentration, mol/m^3
c_m = membrane concentration in contact with the high-pressure interface, mol/m^3
c_o = feed concentration, mol/m^3
c_p = permeate concentration, mol/m^3
d_h = diameter of the hydraulic channel, m
D = diffusion coefficient, m^2/s
D_p = diffusion coefficient obtained from constant pressure measurements, m^2/s
D_v = diffusion coefficient obtained from constant speed measurements, m^2/s
I = ionic strength, N
J_v = volume flown per unit of area and time through the membrane, m/s
K_m = mass-transfer coefficient, m/s
L = length of the hydraulic channel, m
L_c = length of formation of the boundary layer of concentration, m
L_v = length of formation of the boundary layer of momentum, m
M_w = molecular weight of the proteins, Dalton
r = gyration radius, m
R = true retention coefficient
R_g = gas constant, J/mol·K
Re = Reynolds number
R_{max} = maximal true retention coefficient
R_o = observed or apparent retention coefficient
Sc = Schmidt number
Sh = Sherwood number
T = temperature, K
v = recirculation speed of the feed solution, m/s

Greek letters

α = exponent of the Reynolds number in the Chilton-Colburn correlation
β = exponent of the Schmidt number in the Chilton-Colburn correlation
γ_w = shear rate at the membrane wall, s^{-1}
δ = thickness of the concentration polarization film layer, m
Δp = pressure drop through the membrane, Pa
η = solution viscosity, kg/m·s
ξ = proportionality constant in Eq. 13
ρ = solution density, kg/m^3
Φ = mass-transfer coefficient, m/s$^{1-\alpha}$

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