Reflection Coefficients of Homopore Membranes: Effect of Molecular Size and Configuration

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ABSTRACT Osmotic water flow through membranes with uniform defined pores was measured for a variety of macromolecular solutes. Water flow increased linearly with applied hydrostatic pressure, allowing the effective osmotic pressure of the solutes to be estimated by extrapolation. Reflection coefficients for each solute-membrane combination were calculated and correlated with the ratio of solute size to pore size. For the same mean molecular size, proteins were found to have larger reflection coefficients than dextrans. Molecular rigidity may play a role in this difference in behavior.

INTRODUCTION

Practically speaking all real porous membranes are permeable to some solutes, and because of this fact, are not ideal in the sense that the theoretical thermodynamic osmotic pressure cannot be attained across the membrane for all solutes. To measure osmotic pressure by membrane methods, one usually chooses a membrane which has pores which are much smaller than the solute of interest. Due to limitations on availability membranes with small pores, this method is restricted to solutes with molecular weights of about 200 or larger.

In biological systems, there are many anatomical units which behave as porous membranes and osmotic barriers to some extent: the endothelial lining of blood capillaries, the glomerulus of the kidney, and perhaps the plasma membrane of cells as well.

The fact that these membranes are not ideal semipermeable barriers is well recognized in the biological literature, and is the basis for characterization of osmotic forces acting across biological membranes in terms of effective osmotic pressures. For example, the osmotic pressures of lipid-insoluble solutes are effective across cell membranes, whereas only osmotic pressures of macromolecular solutes are effective across capillary membranes (colloid osmotic pressure, or oncotic pressure).

The relationship between effective osmotic pressure and molecular size was put in more quantitative terms by Staverman (1) who defined the reflection coefficient as the ratio of real to ideal osmotic pressures for a given membrane-
solute combination. The reflection coefficient normally ranges over values from 0 to 1, instead of either 0 or 1 prescribed by the oncotic pressure concept.

Osmotic flow rates through many artificial (2) and natural membranes (3) have been measured using a wide variety of solutes as osmotic agents, and to this extent it has been verified that membrane pore size and molecular size are important parameters in determining the reflection coefficient. However, only very few experimental studies (4, 5) have been conducted with defined membranes to elucidate the relationship between solute size and configuration and pore size on the osmotic behavior of solutes.

The development of technology to prepare membranes with straight through pores (6) has made it possible to conduct experiments on the effect of pore size on osmosis. In this work we have measured osmotic flow rates across membranes with pore diameters in the range of 300-600 Å, using solutes with molecular diameters in the range of 50-200 Å in diameter.

**METHODS**

*Transport Apparatus*

The membrane was mounted between two cylindrical chambers fitted with disk-shaped magnetic stirrers, Fig. 1a. To minimize boundary layer effects, the membrane was
supported only on its periphery so that the circulation induced by the magnetic stirrers could reach both sides of the membrane equally well. One disadvantage of this configuration is that the membrane flexes when exposed to a hydrostatic pressure difference. This limits the hydrostatic pressure that can be imposed on the membrane before causing permanent distortion or continuous creep. In the apparatus used, a continuous distortion of the membrane would displace liquid from the downstream chamber and appear as an erroneously higher osmotic flow. However, no detectable creep of the membrane was observed over a 24-h period with a 50-cm H₂O pressure difference.

Liquid flow through the membrane was measured by weighing the effluent from the downstream chamber following a suggestion of Zelman et al. (7), Fig. (6). A fine polyethylene tube connected the transport cell to a weighing bottle situated on the pan of recording analytical balance. The tube passed through a small hole in the cap of the bottle and beneath the surface of liquid to prevent drop formation. Evaporation from the bottle was <0.0024 g/h, about 50 times less than the lowest liquid flow rates encountered in the osmotic experiments.

The hydrostatic pressure across the membrane was controlled by means of a reservoir with a large surface area which was attached to the upstream side of the apparatus. The liquid level in the weighing bottle corresponded to the centerline of the transport chamber, and the difference in liquid level across the membrane was measured with a cathetometer with a reproducibility of ±0.1 mm.

When the hydrostatic pressure was changed by moving the reservoir vessel, the measured flow rates reached new steady values within 10 min.

The stirrers in the chambers consisted of permanent magnet discs encapsulated in plastic (polystyrene) in a cylindrical form. Mass transfer characteristics at the membrane surface have been published by Colton and Smith (8) for this configuration. We measured the mass transfer rates at various stirrer speeds by a polarographic technique (9). The membrane was replaced with a platinum sheet, a solution of potassium ferricyanide placed in one of the chambers, and current-voltage curves obtained at different rotation speeds. The form of the correlation given by Colton and Smith (8) was confirmed with the constant \( \alpha = 0.058 \) for this particular apparatus

\[
\left( \frac{k}{\omega b} \right) \left( \frac{\eta}{D} \right)^{2/3} = \alpha \left( \frac{\omega b^3}{\eta} \right)^{-0.33},
\]

where \( k \) = mass transfer coefficient (cm/s), \( \omega \) = stirring rate (rad/s), \( b \) = membrane radius (cm), \( \eta \) = kinematic viscosity (poise), and \( D \) = diffusivity (cm²/s). Stirring rates of ~200 rpm resulted in a mass transfer coefficient of ~2 \( \times \) 10⁻⁴ cm/s. Typically the linear superficial velocity of liquid across the membrane was ~2 \( \times \) 10⁻⁵ cm/s, and because this is on the order of one-tenth of the mass transfer coefficient near the membrane surface, it can be assumed that the solute concentration at the membrane surface was the same as in the bulk of the solution.

Membrane Characterization

Membranes with nominal pore diameters of 300 and 500 Å were obtained in sheet form from Nuclepore Corp. (Pleasanton, Calif.). Nominal pore density was given as \( 6 \times 10^8 \) pores/cm². Pore densities were determined for samples of each sheet by quantitative scanning electron microscopy. The linear magnification of the SEM was calibrated with grids and the number of pores per unit area estimated from fields containing ~500 pores at \( \times \) 10,000 magnification. To obtain good contrast (Fig. 2) it was necessary to observe the sample at a 45° angle; however, this geometric distortion was compensated for by
observing grids under the same conditions. The nominal 500 Å membrane had a pore density of $5.3 \pm 0.6 \times 10^8$ pores/cm$^2$.

The nominal thickness of the plastic film, and therefore pore length was given by the manufacturer as $5.4 \times 10^{-4}$ cm. An experimental estimate of $5.83 \times 10^{-4}$ cm for the film thickness was obtained by weighing a known area of the sheet and calculating the thickness using a value of 1.19 g/cm$^3$ for the density of polycarbonate.

Pore diameter estimates were calculated, as in previous studies (9), from measured air flow and water flow rates through the membrane at known pressure gradients and the use of Knudsen and Poiseuille flow equations, respectively. Ultrafiltered distilled water was used in all experiments to minimize plugging of the membrane by particulates. Water was deaerated immediately before use to prevent bubble formation in the osmosis cell during an experiment.

Gamma-globulin was dissolved in a buffer of 0.15 M NaCl and 0.05 M sodium phosphate (dibasic) which was preadjusted to pH 7.0 by addition of 0.2 N HCl. This solution (usually 10% by weight) was carefully pressure-filtered through a series of Gelman membrane filters (1.2, 0.45, and 0.20 μm, Gelman Instrument Co., Ann Arbor, Mich.). The solution was refrigerated at ~10°C and carefully deaerated by slowly heating the solution to slightly above 27°C (temperature of the experiments). The γ-globulin

![Figure 2. Scanning electron micrograph of Nuclepore membrane. Nominal pore radius: 150 Å (×31,500).](image-url)
used was obtained from Calbiochem (San Diego, Calif.) and is 98% electrophoretically pure, bovine γ-globulin, fraction II, grade A.

Bovine albumin from Sigma Chemical Co., St. Louis, Mo. (Cohn fraction V, 96-99% pure) was dissolved in a pH 5.05 buffer at a concentration of 8% by weight. The buffer was made by adjusting to pH 5.05 a 0.15 M NaCl and 0.05 M sodium citrate solution using 2 N HCl. The albumin was filtered under pressure and refrigerated until further use.

Various molecular weight Dextrans were obtained from Pharmacia Fine Chemicals (Piscataway, N.J.) and dissolved in distilled water (no buffers used). The solutions were then filtered and stored in the refrigerator. The two preparations used were T-70 (mol wt\(_N\) = 42,500, intrinsic viscosity = 0.26) and T-500 (mol wt\(_N\) = 188,000, intrinsic viscosity = 0.54), these solutes were used at concentrations of 3.3 and 6.6% by weight, respectively.

**Osmotic Pressure of Solutions**

The thermodynamic osmotic pressure of solutions was determined using a Wescan membrane osmometer (originally manufactured by Melabs, Wescan Instruments, Inc., Santa Clara, Calif.), with a Schleicher and Schuell, Inc. (Keene, N.H.) type RC-51 membrane. For osmotic pressure measurements of protein solutions, the osmometer reference chamber was filled with the same buffer solutions as were used to prepare the protein solution. Also, the experiments were conducted at the isoionic pH of the proteins to minimize Donnan effects.

**RESULTS**

The measured osmotic pressures of the four solutes used in this study are given in Fig. 3. Inasmuch as osmotic pressure is known to be at least a quadratic function of concentration for macromolecules (10), the data is plotted in the conventional manner \( \Pi/C \) vs. \( C \). The intercept of these lines (infinite dilution) provide estimates of molecular weights (MW) by the equation

\[
(\Pi/C)_o = RT/MW.
\]  

Estimated molecular weights by this procedure, shown in Table I, correspond very closely to the literature values. The thermodynamic osmotic pressure of any solution used in later experiments is readily obtained from these graphs.

It was necessary to show that the Nuclepore membranes did not plug up over time and that they did not continue to stretch during the course of an experiment. Water flow through a membrane was monitored over several days to check these points, Fig. 4. All the flow data lie on a single line with applied hydrostatic pressure. If membrane plugging occurred, then the measured flow at a given hydrostatic head would have decreased with time. If the membrane slowly stretched with time then the curve would have had an upward curvature. Because all the flow data lie on a single line which goes through the origin, we have some confidence that neither plugging or membrane creep was important.

Pore sizes for each membrane were estimated from measurements of water and air flow rates through the membranes at known pressure differences. Poiseuille's formula was assumed for water flow:

\[
Q = \frac{\pi \Delta P \pi A R_p^4}{8\eta L};
\]
Figure 3. Osmotic pressure of test solutes as a function of concentration.

Table I

| Solute          | (I/C)_w | Measured MW | Literature MW |
|-----------------|---------|-------------|---------------|
| Dextran T-70    | 0.605   | 42,500      | 42,500*       |
| Dextran T-500   | 0.135   | 188,000     | 188,000*      |
| Bovine albumin  | 0.34    | 74,700      | 69,000±       |
| Gamma-globulin  | 0.106   | 240,000     | 156,000–200,000|

* Supplied by manufacturer, Pharmacia, Inc. T-70: M_w = 42,500, M_a = 70,000. T-500: M_w = 188,000, M_a = 476,000.
± Scatchard et al. (11).
$ Oncley et al. (12).

and Kundsen's equation is valid for gas flow in this range of pore sizes and pressures:

\[
Q = \frac{8\pi}{3} \frac{\Delta P}{P_L} \left( \frac{RT}{2\pi M} \right)^{1/2} nA R_p^3; \tag{4}
\]
where \( R_p \) = pore radius; \( P \) = upstream pressure; \( \Delta P \) = pressure difference across membrane; \( Q \) = volumetric flow rate; \( n \) = pore density, number per area; \( L \) = membrane thickness; \( A \) = membrane area; \( T \) = absolute temperature; \( M \) = molecular weight of gas.

These values were determined for each piece of Nuclepore membrane that was used in an osmotic experiment. Pore diameters calculated from the air flow measurements were considerably higher than that determined by water flow, but we have no explanation for this. Pore diameter estimates on membrane sections from a given sheet, shown in columns 2 and 5 of Table II, varied by \( \pm 5\% \), indicating both the uniformity of membranes and reproducibility in the experimental manipulations.

Solvent flow rates across microporous Nuclepore membranes were measured with various macromolecular solutes in the down-stream chamber to determine the effective osmotic pressure of the solutes for different non-ideal membranes.

Typically, for a given applied hydrostatic head, the flow of water or buffer across the membrane was greater when a macromolecular solute was placed in the downstream chamber. Also, as shown in Fig. 5, the flow rate through the membrane increases linearly with applied pressure. However, it was often found that the slope of the flow vs. pressure line decreased when a solute was present in the downstream chamber; e.g., lines a, b, and c are for the same membrane.

**DISCUSSION**

The linear equation proposed by Kedem and Katchalsky (2)

\[
J_{v,1 \rightarrow 2} = L_p (\Delta P - \sigma \Delta \Pi) = L_p \left[ (P_1 - P_2) - \sigma (\Pi_1 - \Pi_2) \right]
\]  

appears to be valid for this single solute system, Fig. 5. At the intercept of the pressure axis the flow is zero \( (J_v = 0) \) and then the reflection coefficient is found...
algebraically by the following expression since $P_2$ is zero, i.e., atmospheric pressure

$$\sigma = - \frac{P_1^o}{(\Pi_2 - \Pi_1)}$$

(6)

### Table II

**Characteristics of Individual Samples of Homopore Membranes**

| Nominal pore radius A | Pore radius from water flow A | Pore radius from air flow A |
|-----------------------|------------------------------|----------------------------|
| 250                   | 238                          | 290                        |
| 150                   | 138                          | 152                        |
|                       | 127                          | 147                        |
|                       | 124                          | 139                        |
|                       | 123                          | 136                        |
|                       | 120                          | 166                        |

Figure 5. Measured osmotic flow rate ($J_o$) vs. applied hydrostatic head ($\Delta P$) for microporous membranes. Dextran T-500, 6.6% by weight; $\gamma$-globulin, 10.0% by weight; membrane nominal pore diameter, 300 Å.

In each experiment the difference across the membrane in thermodynamic osmotic pressure ($\Pi_2 - \Pi_1$) was obtained from Fig. 3 using the bulk solute concentrations. The electrochemically measured mass transfer coefficients showed that boundary ("unstirred") layer corrections were negligible.

Values for reflection coefficients obtained in this manner for $\gamma$-globulin, albumin, Dextran T-70, and Dextran T-500 are shown in column 6 of Table III.
The slope of the flow vs. pressure lines in Fig. 5 give the numerical values of the filtration coefficient since the term $\sigma \Delta \Pi$ is a constant in each experiment.

We found that the filtration coefficient ($L_p$) obtained when a solute was placed in the downstream chamber usually was less than that measured for water alone, and the reduction in flow was much more for protein solutions than for dextran solutions. Two possible explanations for the decrease in bulk flow are plugging of pores and (or) reduction in pore diameter by adsorption of solutes to the pore wall. To check this, in some experiments after a run, the chambers were flushed with water and the filtration coefficients were redetermined. In the dextran runs, the filtration coefficient returned to its original value, but after exposure to albumin the filtran coefficient remained depressed at the same value as in the presence of albumin. This indicates that albumin was probably irreversibly adsorbed to the pore walls.

It should be noted that one reason for applying the excess hydrostatic pressure to the pure solvent chamber was to minimize chances of plugging pores in the membrane. The solvent could be ultrafiltered before use to remove particulates, whereas if the solute solution were passed through an ultrafilter, there would be a change in concentration of the ultrafiltrate due to sieving effects.

| Solute and concentration | Stokes-Einstein solute radius $R_s$ | Nominal pore diameter $A$ | Membrane pore radius by water flow $A'$ | Pore radius from $L_p$ $R_p$ | $R_p/R_s$ | Reflection coefficient $\sigma$ |
|--------------------------|------------------------------------|--------------------------|---------------------------------|----------------------------|--------|-----------------------------|
| Dextran T-70 4.0 %       | 45                                 | 300                      | 182                             | 182                        | 0.25   | 0.135                       |
| Dextran T-70 4.0 %       | 45                                 | 500                      | 225                             | 225                        | 0.19   | 0.17                        |
| Dextran T-70 3.7 %       | 45                                 | 500                      | 238                             | 238                        | 0.19   | 0.11                        |
| Dextran T-70 4.0 %       | 45                                 | 500                      | 222                             | 222                        | 0.20   | 0.16                        |
| Dextran T-500 3.3 %      | 96                                 | 500                      | 210                             | 198                        | 0.48   | 0.20                        |
| Dextran T-500 3.3 %      | 96                                 | 500                      | 214                             | 200                        | 0.48   | 0.20                        |
| Dextran T-500 3.3 %      | 96                                 | 500                      | 157                             | 150                        | 0.64   | 0.27                        |
| Dextran T-500 3.3 %      | 96                                 | 500                      | 150                             | 150*                       | 0.64   | 0.13                        |
| Dextran T-500 3.3 %      | 96                                 | 500                      | 128                             | 128*                       | 0.75   | 0.36                        |
| Dextran T-500 6.6 %      | 96                                 | 500                      | 157                             | 157                        | 0.70   | 0.26                        |
| Albumin 8.0 %            | 37                                 | 300                      | 157                             | 140                        | 0.26   | 0.19                        |
| Albumin 4.0 %            | 37                                 | 300                      | 157                             | 154                        | 0.24   | 0.24                        |
| Albumin 4.0 %            | 37                                 | 500                      | 210                             | 195                        | 0.19   | 0.33                        |
| Albumin 4.0 %            | 37                                 | 500                      | 204                             | 192                        | 0.19   | 0.39                        |
| Albumin 4.0 %            | 37                                 | 500                      | 221                             | 204                        | 0.18   | 0.09                        |
| Albumin 4.0 %            | 37                                 | 300                      | 163                             | 141                        | 0.26   | 0.54                        |
| $\gamma$-Globulin 10.0 % | 56                                 | 300                      | 184                             | 98                         | 0.57   | 0.82                        |
| $\gamma$-Globulin 10.0 % | 56                                 | 500                      | 197                             | 132                        | 0.43   | 0.37                        |
| $\gamma$-Globulin 10.0 % | 56                                 | 300                      | 135                             | 98                         | 0.57   | 0.54                        |
| $\gamma$-Globulin 10.0 % | 56                                 | 500                      | 202                             | 131                        | 0.43   | 0.76                        |

* Estimated
Also, the reproducibility of water flow through the membrane over several days, Fig. 2, argues against the possibility of pores becoming plugged. Therefore, we are inclined to think that reduction in flow in the presence of macromolecules was due to solute adsorption on the pore walls.

New estimates of pore diameters for each experiment were obtained from the lower measured values of \( L_p \) and the water flow equation, since \( L_p = Q/AAP \). These calculated pore diameters are listed in column 4 of Table III. Because of the fourth power dependency of flow on pore diameter, a large reduction in flow rate can be accounted for by a modest decrease in pore size.

To compare the effects of solute dimensions on reflection coefficients, it is necessary to have an estimate of the molecular size of each macromolecule used. In particular, the proteins are not spherical in shape, and therefore, there is a question as to which dimension is the most characteristic in this situation. Fish et al. (13) showed that the distribution coefficient of linear and oval shaped proteins in microporous resins correlated best with the Stokes-Einstein radius of the molecule rather than either the length or diameter. The Stokes-Einstein radius for each solute was obtained from published values of diffusion coefficients by the equation

\[
R_{se} = \frac{RT}{6\pi\eta ND}
\]

where \( N \) = Avogadro's number, and the results are shown in column 2 of Table III. The dextrans used in this study are not a single well-defined species. For dextran T-70 80% of the molecules have Stokes-Einstein radii between 38 and 60Å, and for dextran T-500 the corresponding limits are 60Å and 110Å (we thank the reviewers for pointing out the size distribution range). However, for the experiments reported here, a systematic error of < 4% is incurred by attributing the measured reflection coefficient to the diameter of a molecule with the number average molecular weight of the mixture.

![Figure 6](image-url)
Using the pore diameters estimated from $L_p$ (column 4) and the molecular diameters estimated from the Stokes-Einstein radius (column 2), the ratio of molecular size to pore size $R_e/R_p$, was calculated (column 5). Now the reflection coefficient can be plotted against relative molecular size (column 6 vs. column 5), and the composite of all runs is shown in Fig. 6. Although there is quite a bit of scatter in the data, there appears to be a definite clustering of the protein data and the dextran data. For a given ratio of molecular to pore size, the proteins have greater reflection coefficients. This may be due to the fact that proteins are more rigid structures than the dextrans and that within a pore the dextrans can distort to give an apparently lower molecular size. A somewhat similar distinction in behavior between dextrans and proteins in a biological membrane was noted by Renkin (14) with respect to glomerular filtration.

The implications here are that the molecular configuration and rigidity of solutes play an important role in the osmotic behavior of "leaky" membranes.

The line in Fig. 6 is the theoretical relationship between reflection coefficients and molecular size as presented by Curry (15) and Anderson and Malone (16); our data does not appear to provide definitive confirmation of the theory for either proteins or dextrans.

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