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Macromolecular diffusion through polymer membranes

Shuji Sato and Sung Wan Kim

Department of Pharmaceutics, University of Utah, Salt Lake City, UT 84112 (U.S.A.)

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Summary

Macromolecular diffusion through various synthetic membranes including cellulose, hydrogels and biodegradable polymers were studied. The synthesized membranes were fabricated using proper solvents to produce porous membranes as well as dense membranes followed by characterization. Solutes with a wide range of molecular sizes were used to verify the proposed diffusion mechanism of 'pore' vs 'partition' type permeation. It was found that macromolecules not only permeate via a bulk water channel but through polymer matrix as well depending on the method of fabrication. The obtained data can provide useful information for the design of a macromolecules delivery system.

Introduction

Although macromolecule diffusion through polymer membranes is considered to be important in the development of new biomedical and pharmaceutical systems, few studies can be found in the literature.

Langer et al. (1976, 1978, 1980) and Davis (1972) developed monolithic devices for sustained release of macromolecules. Davis used poly(acrylamide) as a matrix for sustained release of insulin, but did not analyze diffusion coefficients. Langer et al. used poly(vinylalcohol), poly(hydroxyethyl methacrylate) and ethylene-vinyl acetate copolymer, and described the cumulative percent of protein released from the device.

Correspondence: S.W. Kim, Department of Pharmaceutics, University of Utah, Salt Lake City, UT 84112, U.S.A.

They demonstrated that macromolecular diffusion from monolithic devices results from diffusion through the channels introduced by proteins incorporated into the polymer matrix.

Peppas et al. (1983) investigated the diffusion of albumin and theophylline through amorphous cross-linked polymeric networks and studied the effects of degree of cross-linking, volume degree of swelling and initial thermodynamic state on the diffusion processes. They determined that the mesh size of the cross-linked network affects the rate of diffusion, decreasing mesh size slowed or even stopped diffusion, dependent on the size of solute. It was also found that the initial thermodynamic state of the polymer markedly affected drug release through polymeric materials.

Yasuda et al. (1968, 1969, 1971) developed the free volume theory for the diffusion of a solute through polymer membranes and extended the theory to the diffusional permeability of various molecular weight solutes. They showed that the natural logarithm of the relative diffusivity decreases proportionally to the reciprocal of membrane hydration and also to the square of the size of the solute. In free volume theory, the diffusion of the solute is interpreted in terms of an Eyring concept of diffusion (Eyring, 1936), that is, a solute diffuses by jumping from 'hole' to 'hole'. In this model, the diffusion coefficient can be written as:

$$D = \nu \exp(-F/kt) = \nu \exp(S/k) \exp(-E/kt) \tag{1}$$

where ν is the translational oscillation frequency of the diffusion species and F, S and E are the free energy, entropy and energy of activation for diffusion, respectively. The energy term describes the major part of the temperature dependence on diffusion phenomenon and the entropy term consists of two contributions: one is the conformational probability of formation of a hole sufficiently large for the passage of the diffusing molecule; that is, $\exp(-Br^2/V_f)$, and the other is the probability $\psi(r^2)$ of finding space in the swollen membrane for such a hole.

$$S/k = \ln W = -Br^2/V_f + \ln \psi(r^2) \tag{2}$$

where V_f is the total free volume in the membrane, r^2 is the effective cross-sectional area of the solute, B is a proportionality factor, Br^2 is a characteristic volume parameter describing the diffusion of permeant molecules, and $\psi(r^2)$ is the probability of finding a hole in the network of solvated polymer segments with a cross-section equal to or larger than r. From Eqns. 1 and 2, the diffusion coefficients of solutes through a polymer is given by:

$$D_s = \nu\psi(r^2) \exp(-Br^2/V_f) \exp(-E/kt) \tag{3}$$

Assuming the activation energy of diffusion is the same as in the membrane and $\psi = 1$, the diffusion coefficient of the solute in pure water is:

$$D_w = \nu \exp(-Br^2/V_{fw}) \exp(-E/kt) \tag{4}$$

where V_{fw} is the free volume of H_2O. It was assumed that the effective free volume available for the permeation of such solutes is essentially the free volume of water in the swollen polymer membrane, that is:

$$V_f = V_{fs} = HV_{fw} \tag{5}$$

where V_{fs} is the free volume of solute in H_2O and H is the degree of hydration. Dividing Eqn. 3 by Eqn. 4 and substituting for V_f with Eqn. 5 results in:

$$\ln \frac{D_s}{D_w} = \ln \psi(r^2) \left[-Br^2 \left(\frac{1}{V_{fw}} \right) \left(\frac{1-H}{H} \right) \right] \tag{6}$$

Eqn. 6 predicts that the diffusivity of the solute through polymer membranes increases with membrane hydration and decreases with the solute size.

Colton et al. (1971) studied the permeability of macromolecules through cellulosic membranes and basically found the same results as Yasuda et al. Klein et al. (1979) determined the permeability coefficients of proteins through hemodialysis membranes and compared those findings to the values calculated from the application of a hydrodynamic theory based on pore structure models. Sefton et al. (1980) measured the insulin permeability of hydrophilic polyacrylate membranes and found the membrane permeability to insulin to be proportional to the membrane hydration.

Kim et al. (1978, 1980), Wisniewski and Kim (1980) and Zentner et al. (1978, 1979) examined solute permeation through hydrogel membranes using poly(hydroxyethyl methacrylate) (p-HEMA) and its copolymers, with both hydrophilic and hydrophobic solutes. It was found that p-HEMA and its copolymers are permeable to both hydrophobic and hydrophilic solutes. For hydrophilic solutes, permeation probably occurs through the 'bulk-like' water regions of the hydrogel, also known as the 'pore' mechanism of diffusion. For the hydrophobic solutes, diffusion still occurs predominantly by 'pore' type mechanisms in hydrogels and to a lesser extent by 'partition' mechanisms. An important aspect is that the second mechanism of diffusion, that is, 'partition' mechanism was shown for solutes that are permeable through membranes devoid of bulk water. Thus, the permeation of protein through polymer membrane is probably via a 'partition' mechanism.

Polymer membranes used for the diffusion studies in this paper are as follows: cellulose acetate (dense and porous type), regenerated cellulose (dense and porous type), copolymer of lactic and glycolic acid (dense and porous type), poly(hydroxyethyl methacrylate) (p-HEMA) (dense and porous type), copolymer (hydroxyethyl methacrylate/methoxyethyl methacrylate) (p-HEMA/MEMA) (dense type) and copolymer (hydroxyethyl methacrylate/methoxyethoxyethyl methacrylate) (p-HEMA/MEEMA) (dense type). To examine the diffusion process, solutes with a wide range of molecular size were used including sodium acetate, glucose, maltose, insulin, cytochrome c and albumin. Based on the dependence of diffusivity on solute size and membrane hydration, the mechanisms of diffusion for these solutes are interpreted.

Materials and Methods

Preparation of polymer membranes

Dense type cellulose acetate membrane. Cellulose acetate powder (Aldrich Chemicals, acetyl content 39.8%) was dissolved in acetone to get a 10% (w/w) polymer solution, which was in turn filtered with a Buchner type funnel with fritted disc to remove dust and undissolved particles to avoid undetectable pinholes in the membrane. This filtered polymer solution was cast on a glass plate using a doctoring blade (Gardner Instruments, Bethesda, MD). After soaking in distilled water overnight, the membrane was removed from the glass plate.

Porous type cellulose acetate membrane. 20 parts (w/w) of double-distilled water was added to 80 parts (w/w) of the cellulose acetate–acetone solution mentioned above and the mixture was well stirred overnight. The mixture was cast on a glass plate using a doctoring blade and then immediately placed in a glass chamber filled with saturated acetone vapor for 30 min to control the evaporation rate of solvent from the membrane, and finally air dried. After soaking in distilled water overnight, the membrane was removed from a glass plate.

Dense and porous type regenerated cellulose membranes. These were prepared by soaking the dense or porous cellulose acetate membranes mentioned above in a mixture of 0.2 N aqueous sodium hydroxide solution and an equal volume of ethyl alcohol for 8 h at room temperature. This saponification process was monitored by infrared spectroscopy.

Dense type p-HEMA, poly-HEMA/MEMA and poly-HEMA/MEEMA membranes. Dense type membranes of three methacrylic hydrogels, p-HEMA, poly-HEMA/MEMA and poly-HEMA/MEEMA, were synthesized by free radical polymerization (Andrade, 1976). Poly-HEMA/MEMA (45/55% v/v) films were prepared from a mixture of hydroxyethyl methacrylate (Hydron Laboratories) and methoxyethyl methacrylate (Polysciences). Poly-HEMA/MEEMA (8/92% v/v films were prepared from a mixture of hydroxyethyl methacrylate (Hydron Laboratories) and methoxyethoxyethyl methacrylate (Polysciences).

Porous type p-HEMA membrane. These were prepared by the method described by Klomp et al. (1983). This procedure was developed and investigated in detail by Yasuda et al. (1966) and applied to a hybrid artificial pancreas by Klomp et al. (1983). The method is predicated on the coagulation phenomenon of polymer chains in a poor solvent while polymerization proceeds. A 24.88% (w/w) HEMA monomer and a 0.12% (w/w) ethylene glycol dimethacrylate (EDGMA) (Polysciences) in 55% (w/w) double distilled water were mixed at room temperature. The remaining water (20% w/w) was divided into two equal parts which were used to dissolve the two components of the redox initiators, that is, ammonium persulfate (0.25% w/w of monomers, Sigma Chemicals) and sodium metabisulfite (0.25% w/w of monomers, Sigma Chemicals). All solutions were cooled to 5°C prior to use, and then mixed together. The mixture was poured into a polymerization vessel which consisted of two glass plates. Polymerization was conducted at 5°C for 2 h and completed at room temperature for 24 h. The membranes were soaked in distilled water to remove unreacted monomer.

Biodegradable copolymer of lactic and glycolic acid. This was prepared by a ring-opening polymerization of a mixture of lactide and glycolide using tetraphenyltin as an initiator. The procedure was similar to the method described by Kulkarni et al. (1966), but slightly modified. This procedure consists of two steps: synthesis of lactide and glycolide, and conversion of lactide and glycolide to the copolymer.

Lactide and glycolide are the cyclic dimers of lactic acid and glycolic acid, respectively, and are synthesized by condensation reactions of the appropriate acids. A mixture of 500 g of 30% (w/w) aqueous solution of D,L-lactic acid (Sigma Chemicals) and 3 g of zinc oxide (Aldrich Chemicals) was placed in a 3-neck round-bottom flask and subjected to distillation for 6 h at 140°C, it was initially at atmospheric pressure and gradually the pressure was decreased to approximately 25 mm Hg. After the water had distilled off, a collecting flask was used to receive the lactide. Then, the pressure was decreased to 1 mm Hg and the temperature was gradually raised to 250°C and the lactide distilled under these conditions for 8 h. The crude product was recrystallized several times from ethyl acetate, and finally washed with ice-cold anhydrous ether. The final product was identified by the capillary melting point method. The lactide obtained was stored in a vacuum desiccator with phosphorus pentoxide. Glycolide was prepared from glycolic acid (Sigma Chemicals) by the same method.

For the synthesis of copolymers, the lactide and glycolide were placed in a polymerization tube dried at 120°C for 24 h, and then tetraphenyltin (Aldrich Chemicals) (0.03% w/w based on the total weight of lactide and glycolide) dissolved in a small amount of dry benzene, was added. The benzene was removed under vacuum. The polymerization tube was sealed under vacuum and placed in an oil bath at 180°C for 15 h. After polymerization, the polymerization tube was cut and the copolymer was taken out by dissolving it with solvent. Pure polylactic acid and the copolymer of 75% (w/w) lactic acid and 25% (w/w) glycolic acid were dissolved with chloroform and the copolymer of 50% (w/w) lactic acid and 50% (w/w) glycolic acid was dissolved with dimethyl sulfoxide (DMSO). The polymers were precipitated in double-distilled water and dried in vacuo at room temperature. The molecular weights of these polymers were measured by viscometry with a Ubbelohde type viscometer (Fisher Scientific).

Dense type membranes of pure poly-lactic acid and the copolymer of 75% (w/w) lactic acid and 25% (w/w) glycolic acid were cast from 5% (w/w) chloroform solution of a glass plate using a doctoring blade. The solvent was evaporated by air-drying.

Porous type membranes of pure poly-(lactic acid) and the copolymer of 75% (w/w) lactic acid and 25% (w/w) glycolic acid were cast from a chloroform–methyl alcohol solution of polymer (polymer–chloroform–methylalcohol 5 : 85 : 10 based on weight) on a glass plate using a doctoring blade. The solvent was evaporated for 30 min in a glass chamber which was filled with chloroform vapor and then air-dried.

Dense type membranes of the copolymer of 50% (w/w) lactic acid and 50% (w/w) glycolic acid were cast from a 10% (w/w) DMSO solution on a glass plate using a doctoring blade, and then dried at 90°C and 0.025 mm Hg in a vacuum oven for 12 h.

Porous type membranes of the copolymer of 50% (w/w) lactic acid and 50% (w/w) glycolic acid were prepared by the modified phase inversion technique. The 10% (w/w) DMSO solution was cast on a glass plate using a doctoring blade and then immediately coagulated by immersion into distilled water.

Characterization of polymer membranes

Infrared spectroscopy. The polymer samples were examined directly with a double beam infrared spectrophotometer (Beckman Nicrolab 620 MX Computing Infrared Spectrophotometer, Beckman Instruments). The samples were dried at room temperature and the resulting polymer films were transparent.

Melting point measurement. Melting point measurements were conducted with the capillary melting point method (Thomas Hoover Capillary Melting Point Apparatus).

Viscometry. Viscosities of the copolymers of lactic acid and glycolic acid were measured at $37.0 \pm 0.1°C$ to determine the molecular weights using the Ubbelohde type viscometer. Pure poly (lactic acid) and the copolymer of 75% (w/w) lactic acid and 25% (w/w) glycolic acid were dissolved in tetrahydrofuran and the copolymer of 50% (w/w) lactic acid and 50% (w/w) glycolic acid was dissolved in DMSO. Initially, a 1% (w/v) polymer solution was prepared, and viscosities were measured as the solution was diluted stepwise. Intrinsic viscosity was determined by extrapolation using the following equation:

$$[\eta] = \lim_{c \to \infty} \frac{[\eta_{sp}]}{c} \tag{7}$$

where $[\eta]$ is the intrinsic viscosity, η_{sp} is specific viscosity, C is the concentration of polymer solution in g/100 ml. The specific viscosity at each polymer concentration was calculated with the following equation:

$$\eta_{sp} = \eta_r - 1 = \frac{\eta}{\eta_0} - 1 \simeq \frac{t}{t_0} - 1 \tag{8}$$

where η_r is relative viscosity, η is the viscosity of the polymer solution, η_0 is the viscosity of the solvent, t is the falling time of polymer solution and t_0 is the falling time of solvent. Intrinsic viscosity $[\eta]$ can be related to molecular weight of polymer by the equation:

$$[\eta] = KM^{\alpha} \tag{9}$$

where M is the molecular weight of polymer and K and α are the constants which are dependent on the combination of polymer and solvent. The equation of Wise et al. (1978) was used:

$$[\eta] = 1.04 \times 10^{-4} M^{0.75} \tag{10}$$

For the copolymer of 50% (w/w) lactic acid and 50% (w/w) glycolic acid, only the intrinsic viscosity in DMSO was determined.

Scanning electron microscopy (SEM). The polymer samples were vacuum-dried overnight at room temperature. The upper surface, lower surface and the cross-section were examined with a scanning electron microscope. Since the hydrogel films, dense and porous type p-HEMA, dense type copoly-HEMA/MEMA and dense type copoly-HEMA/MEEMA, were polymerized in the mold, there was no difference between the upper and lower surfaces.

Measurements of partition coefficient and hydration

Partition coefficients, K_d, were determined by a solution depletion technique in which the solutions of various radiolabelled solutes were equilibrated with known volumes of polymers. Partition coefficients were calculated using the following equation:

$$K_d = \frac{V_s(C_0 - C_s)}{V_m C_s} \tag{11}$$

where, V_s is volume of solution, V_m is volume of polymer film, C_0 is the initial solute concentration in the solution and C_s is the solute concentration in the solution at the equilibrium. The initial solute concentrations were similar to those used in the diffusion experiments. The solulte concentrations in the solutions were measured until a constant value was obtained by liquid scintillation counting (Beckman LS 7500, Beckman Instruments), using scintillation fluid (Aquasol, Formula 950-A, New England Nuclear), or γ-ray counting (Beckman Biogamma II, Beckman Instruments). The thickness of the wet polymer membranes was measured by a direct measurement using a light wave micrometer (Lightwave Micrometer, Van Keuren), which was accurate to 2.5×10^{-5} cm.

Hydration of polymer membranes was calculated by the following equation:

$$H = \left(\frac{d_m}{d_w}\right) W_f \tag{12}$$

where H is hydration of polymer membrane, d_m is the wet membrane density, d_w is the density of water and W_f is the water content of wet polymer membrane. It was determined from the wet and dry weights of known volumes of polymer using the following equation:

$$W_f = \frac{(\text{wet weight}) - (\text{dry weight})}{(\text{wet weight})} \tag{13}$$

Diffusion experiments

A glass diffusion cell which consists of two compartments of equal volume (150 ml), was used in all diffusion experiments. The polymer membrane (area 14.9 cm^2) was clamped between the compartments. Each compartment was stirred at 1550 rpm

during the experiment to minimize the boundary layer effects. Initially, one compartment was filled with double-distilled water and the other compartment was filled with an aqueous solution containing the permeant solute in all cases except for insulin. For insulin, the diffusion was examined with phosphate-buffered saline (pH 8.0) with 2.0 mg/ml urea to avoid insulin aggregation during the diffusion experiment. The diffusion of sodium acetate, glucose and maltose was measured at an initial concentration of 1.0 mg/ml, and the diffusion of proteins (cytochrome c, insulin and albumin) was determined at an initial concentration of 0.01 mg/ml.

The diffusion process through polymer membranes was followed by determining the increase in solute concentration in the initially solute-free compartment by liquid scintillation counting (Beckman LS7500, Beckman Instruments) using scintillation fluid (Aquasol, Formula 950-A, New England Nuclear), γ-ray counting (Beckman Biogamma II, Beckman Instruments). The thickness of the wet membrane was measured using a lightwave micrometer (Van Keuren).

Analysis of diffusion data

All permeation coefficients were calculated from the following equation:

$$\ln\left(\frac{C_0V-(2V+K_dV_m)C_2}{C_0V-(2V+K_dV_m)C_1}\right)=-\frac{2UA}{V\ell}(t_{ss}-t_{oss}) \tag{14}$$

where C_0 is the initial concentration of solute in the donor phase, C_1 is the concentration in the acceptor phase at the time of onset of steady-state, C_2 is the concentration in the acceptor phase at any time during steady-state, V is the compartment volume, V_m is the volume of membrane, K_d is the partition coefficient, U is the permeation coefficient, A is the effective membrane area, ℓ is the membrane thickness, t_{ss} is any time during steady-state, and t_{oss} is the time of the onset of steady-state. Eqn. 14 was derived elsewhere (Zentner, 1979) from Fick's first law. A plot of:

$$\ln\left(\frac{C_0V-(2V+K_dV_m)C_2}{C_0V-(2V+K_dV_m)C_1}\right) \text{ versus } (t_{ss}-t_{oss}) \tag{15}$$

yields a straight line with a slope of $-2UA/V\ell$. The permeation coefficient U was calculated from known values of A, V and ℓ. The diffusion coefficient was obtained from the following equation:

$$D=\frac{U}{K_d} \tag{16}$$

This is an apparent diffusion coefficient which includes both contributions due to diffusion through the boundary and membrane layers. Analysis of the boundary layer was not included in this experiment as it is assumed that the boundary layer contribution in the experiments is similar in all systems of polymers and water-soluble solutes used in this study.

Results and Discussion

Characterization of polymer membranes

The regenerated cellulose membrane was prepared by saponification of the cellulose acetate membrane, and the saponification process was monitored by IR

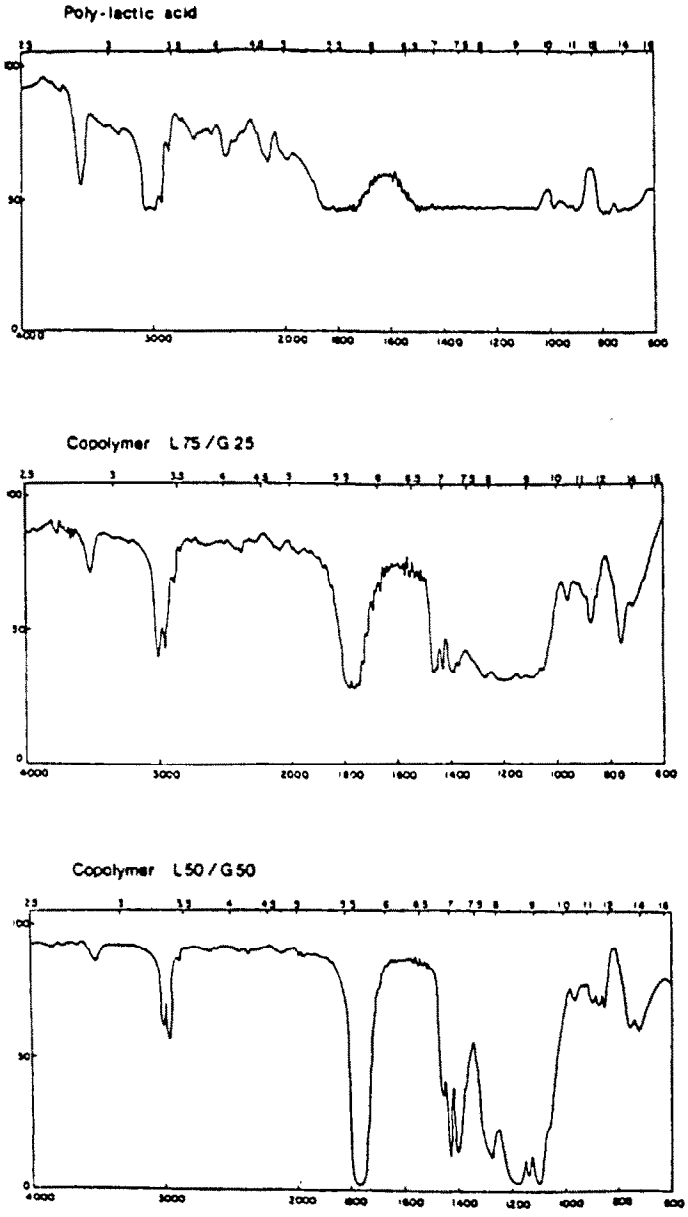

Fig. 1. Infrared spectra of copoly-lactic/glycolic acid.

TABLE 1

INTRINSIC VISCOSITIES OF COPOLYMERS OF LACTIC AND GLYCOLIC ACID

Ratio of lactic acid and glycolic acid	Intrinsic viscosity	Molecular [a] weight
Lactic acid 100% [b]	0.25	3.22×10^4
Lactic acid 75% [b] -glycolic acid 25%	0.59	1.01×10^5
Lactic acid 50% [c] -glycolic acid 50%	0.37	-

[a] $[\eta] = 1.04 \times 10^{-4} M^{0.75}$.
[b] In tetrahydrofuran at 37°C.
[c] In dimethylsulfoxide at 37°C.

spectroscopy. The ester band at 1740 cm^{-1} due to the carbonyl frequency of cellulose acetate disappeared and the hydroxyl group band at 3200–3400 cm^{-1} was strengthened by saponification of cellulose acetate. This was evidence which indicated that the saponification process from cellulose acetate to regenerated cellulose was complete.

Fig. 1 shows the IR spectra of the copolymers of lactic and glycolic acid. The upper spectrum is pure polylactic acid, the middle one is the copolymer of 75% (w/w) lactic acid and 25% (w/w) glycolic acid, and the lower one is the copolymer of 50% (w/w) lactic acid and 50% (w/w) glycolic acid. The ratio of the alkyl group band at ~ 2900 cm^{-1} to the carbonyl band ~ 1750 cm^{-1} increases with increasing lactic acid content of the copolymer. This is corroborated by an examination of the structures which shows lactic acid to have an additional methyl group.

The structures of poly(lactic acid) and poly(glycolic acid) are:

poly(lactic acid) $[-O-CH(CH_3)-CO-]_n$

poly(glycolic acid) $[-O-CH_2-CO-]_n$

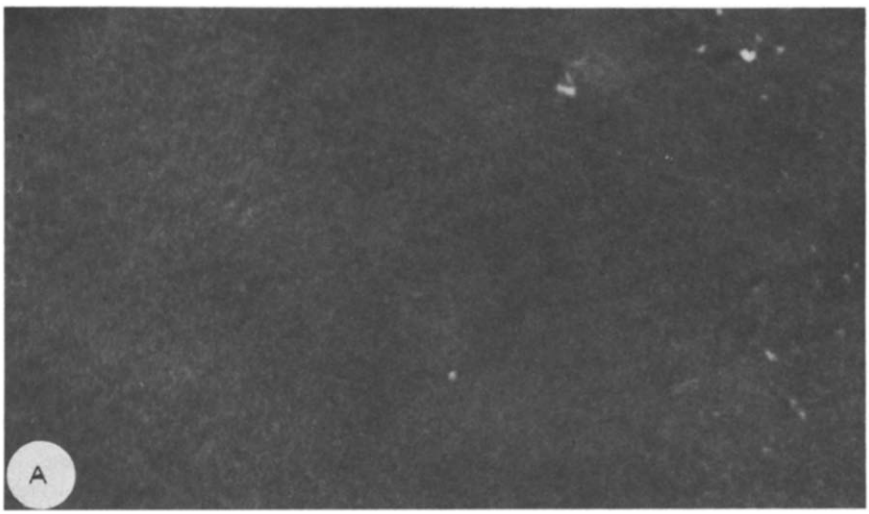

Fig. 2a.

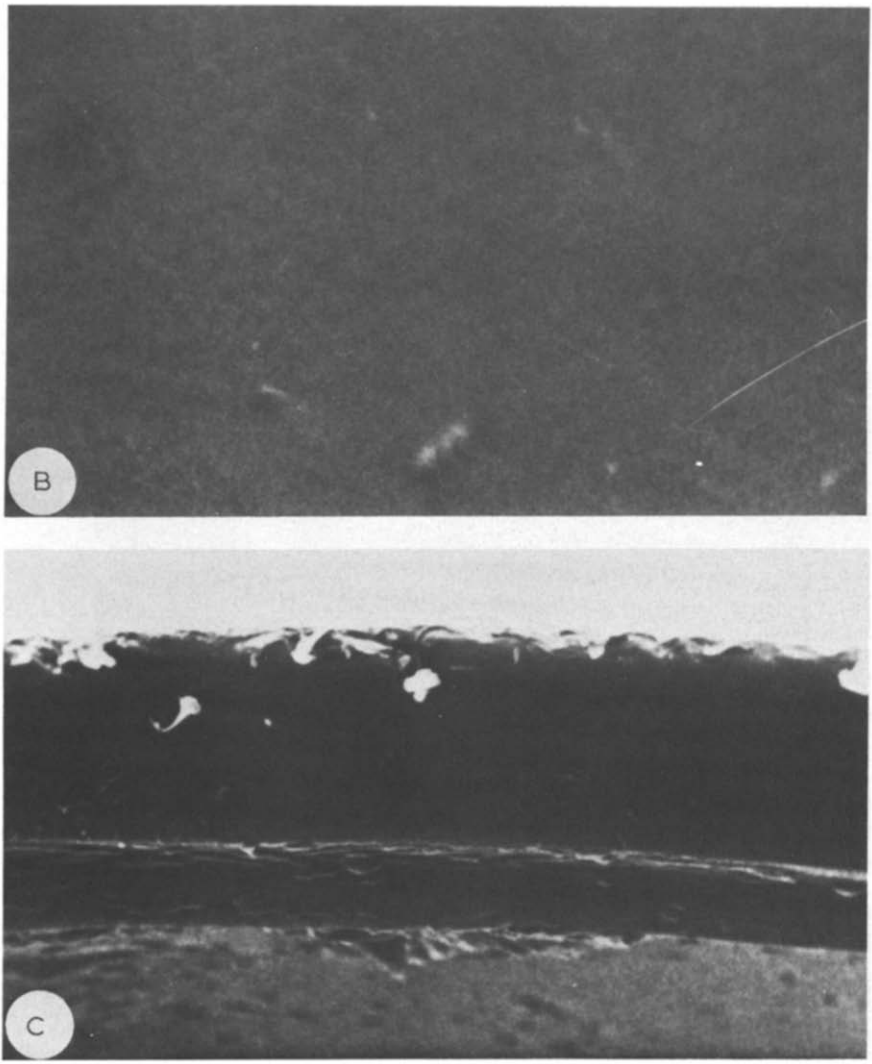

Fig. 2. Scanning electron micrographs of regenerated cellulose dense membrane. A: upper surface; B: lower surface; C: cross-section. 1800×.

Table 1 lists the intrinsic viscosities of the copolymers of lactic and glycolic acid measured with the Ubbelohde type viscometer. Molecular weights of pure polylactic acid and the copolymer of 75% (w/w) lactic acid and 25% (w/w) glycolic acid were estimated using Eqn. 10 from the intrinsic viscosities.

Figs. 2–7 include SEM photographs of regenerated cellulose acetate dense membrane, copolymer of lactic/glycolic acid (50/50) dense membrane, copolymer of lactic/glycolic (50/50) porous membrane, p-HEMA dense membrane, and p-HEMA porous membrane, respectively. The surfaces of all dense type membranes were flat

and no visible pores were seen in them, nor was any water channel present in the cross-sections of all dense type membranes. On the other hand, all porous type membranes exhibited many pores on the surfaces and water channels in the cross-sections, and the distributions of pores were asymmetrical between upper surfaces and lower surfaces in all cases except porous p-HEMA, where the distribution of pores was more symmetrical. The difference in the pore distribution can be attributed to the difference in methods of the membrane fabrication as mentioned previously.

Dependence of membrane diffusion coefficient on solute molecular size

The membrane diffusion coefficients (D) of several solutes (sodium acetate,

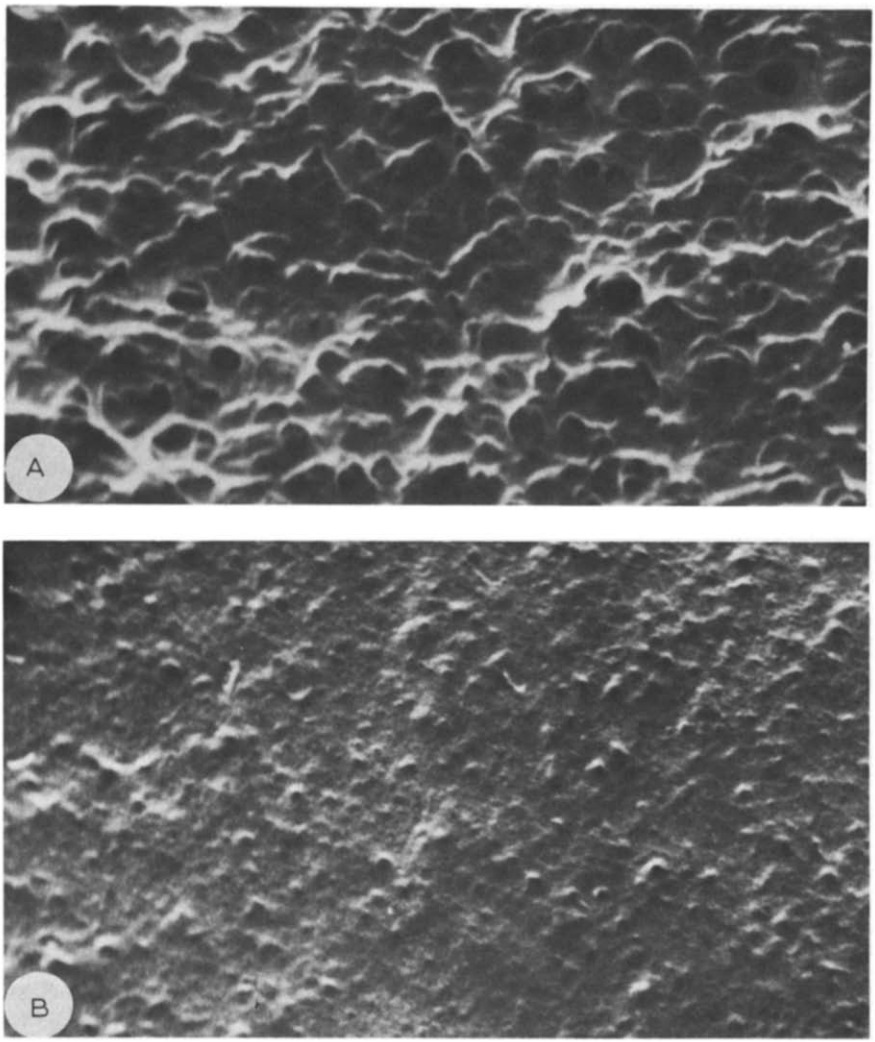

Fig. 3A and B.

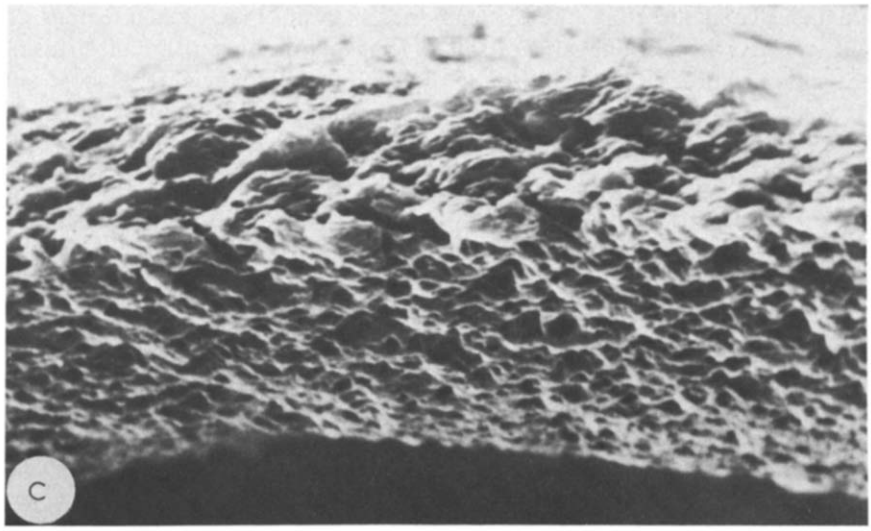

Fig. 3. Scanning electron micrographs of regenerated cellulose porous membrane. A: upper surface; B: lower surface; C: cross-section. 1950×.

glucose, maltose, insulin, cytochrome c and albumin) were determined using nine prepared membranes (dense cellulose acetate, porous cellulose acetate, dense regenerated cellulose, porous regenerated cellulose, porous copolymer of lactic/glycolic acid (50/50) dense p-HEMA, dense p-HEMA/MEMA, dense p-HEMA/MEEMA, and porous p-HEMA). The results are summarized in Tables 2–5 with the values of permeation coefficient (U) and partition coefficient (K_d). The natural logarithm of diffusion coefficients was plotted versus the cross-sectional area, r^2 (Å^2), in Fig. 8. The cross-sectional area of sodium acetate, glucose and maltose was estimated by atomic contributions according to Wilke (1946). The molecular volumes were calculated by summing up all atomic volumes, and then the molecular radii were calculated using the following equation assuming that the solutes were spherical.

$$r^2 = \left(\frac{3V}{4\pi N_0}\right)\frac{2}{3} \tag{17}$$

where r is the radius of solute, V is the molal volume of solute and N_0 is Avogadro's number. The cross-sectional areas of cytochrome c and albumin were found in Klein et al. (1979) and Colton et al. (1971). The cross-sectional area of insulin was also calculated by Eqn. 17, but its molecular volume was derived from the partial specific volume. Porous copolymer of lactic/glycolic acid (50/50), porous p-HEMA and porous regenerated cellulose showed the largest diffusivities. Dense and porous cellulose acetate showed the smallest diffusivities and dense regenerated cellulose, dense p-HEMA, p-HEMA/MEMA and p-HEMA/MEEMA had intermediate diffusivities. Biodegradable polymers other than porous 50/50 copolymer of lactic/glycolic acid, i.e. dense 50/50 copolymer, dense and porous 75/25 copolymer of lactic/glycolic acid, and dense and porous polylactic acid did not show any

detectable diffusivities therefore, these membranes were not investigated further.

The plot of the cross-sectional area of solute versus natural logarithm of diffusion coefficient showed linearity for small solute molecules (sodium acetate, glucose and maltose), which can be interpreted to mean a 'pore' mechanism of diffusion. Large molecules (insulin, cytochrome c and albumin) also diffused through the polymer membranes and these solutes showed deviations from linearity in the plot of the cross-sectional area of solute versus natural logarithm of diffusion coefficient, which indicates that a 'partition' mechanism dominates the diffusion of these large molecules through polymer membranes.

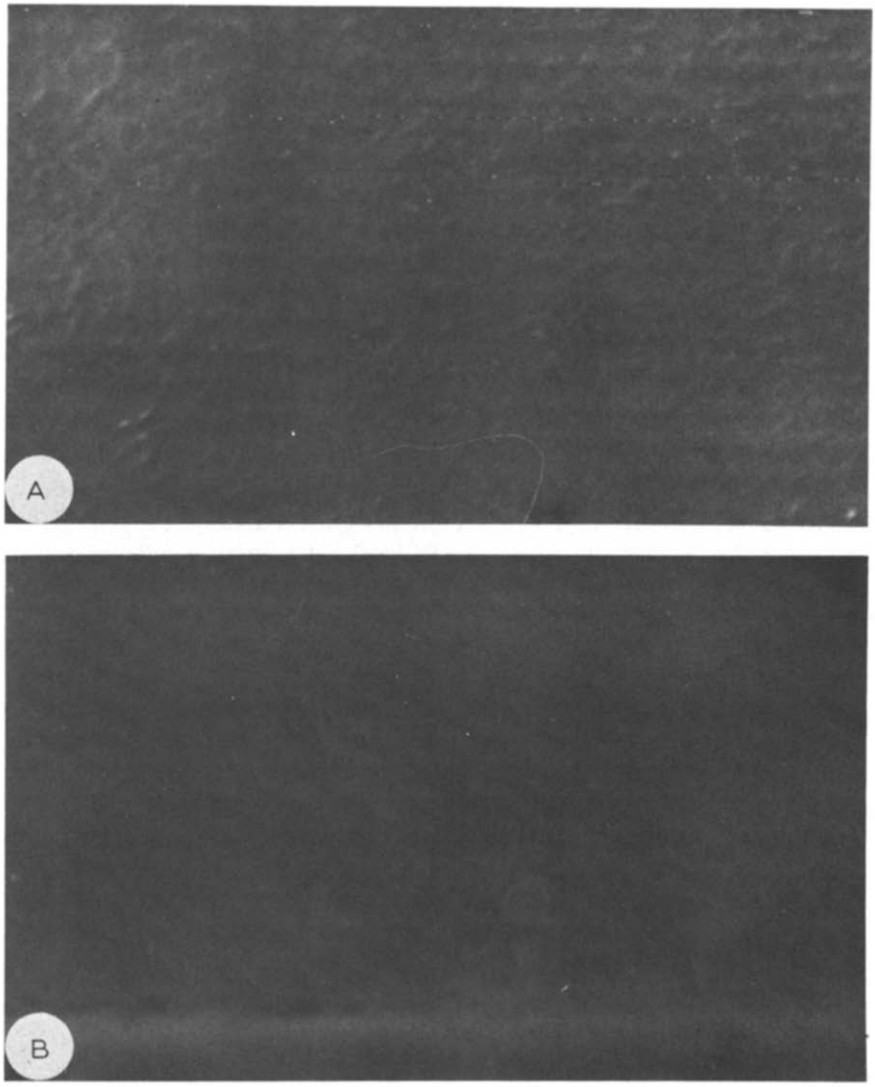

Fig. 4A and B.

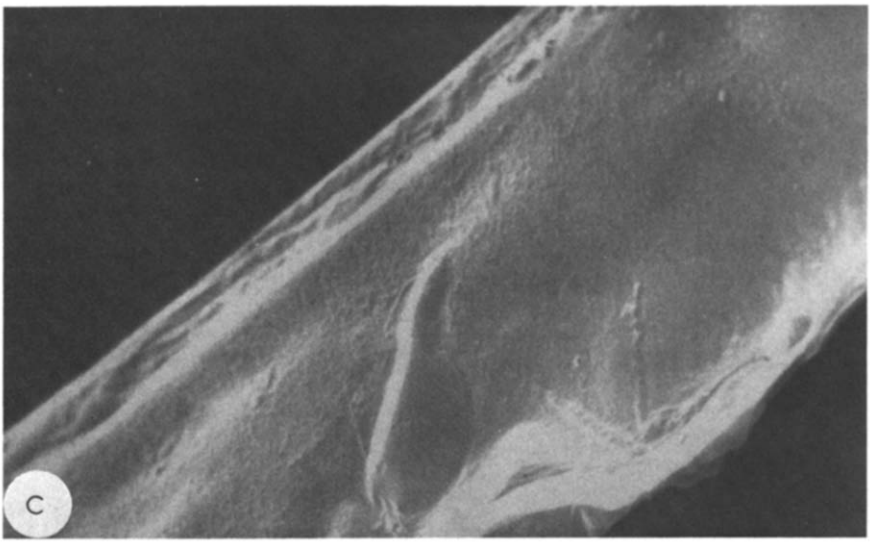

Fig. 4. Scanning electron micrographs of copoly-lactic/glycolic acid dense membrane. A: upper surface; B: lower surface; C: cross-section. 1500×.

The 'partition' mechanism was investigated by Kim et al. (1980) for the diffusion phenomena of hydrophobic solutes through hydrogels. When the content of cross-linker was not large, the permeation of hydrophobic solutes occurred predominantly by the 'pore' mechanism. Even when the membrane was cross-linked with a larger amount of cross-linker and the bulk-like water was not available for solute-diffusion, the hydrophobic solutes still diffused. This diffusion probably occurred by dissolution into the polymer matrix.

The fast diffusion of large solutes (insulin, cytochrome c and albumin) cannot be explained by the 'pore' mechanism alone, where the diffusivity of solute is supposed to decrease as the solute size increase, as analyzed by Yasuda et al. (1968, 1969, 1971). The 'partition' mechanism which was introduced to interpret the permeation of hydrophobic steroids through hydrogel membranes may explain the larger diffusivities of proteins which are greater than expected for the 'pore' mechanism.

Insulin showed relatively high diffusivity in urea–phosphate-buffered saline (PBS) (pH 8.0) compared to other proteins (cytochrome c and albumin) in water.

Effects of phosphate-buffered saline (PBS) and urea on diffusion coefficient of protein

As shown in Fig. 8, insulin showed relatively high diffusivity compared to cytochrome c and albumin. The diffusion coefficient of insulin was determined using phosphate-buffered saline (PBS) (pH 8.0) because insulin did not dissolve in water even when the pH was raised as high as 10 by adding a small amount of sodium hydroxide. It was necessary to add 2.0 mg/ml of urea to avoid insulin aggregation during the diffusion experiment. The diffusion coefficients of cytochrome c and albumin were measured in distilled water. It was assumed that PBS and/or urea

facilitated the diffusion of protein through a polymer membrane, this then explains the relatively high diffusivity of insulin.

The diffusion coefficients of cytochrome c and albumin through the porous cellulose acetate membrane were measured in PBS and aqueous urea solution (2.0 mg/ml) to determine whether the assumption mentioned above was valid. The results are presented in Table 6 and the plot of the natural logarithm of the diffusion coefficient versus the cross-sectional area of solute is shown in Fig. 9. It was shown that the diffusion coefficient of albumin could be increased to equal that of insulin in either PBS or urea solutions. The diffusion coefficient of cytochrome c could be

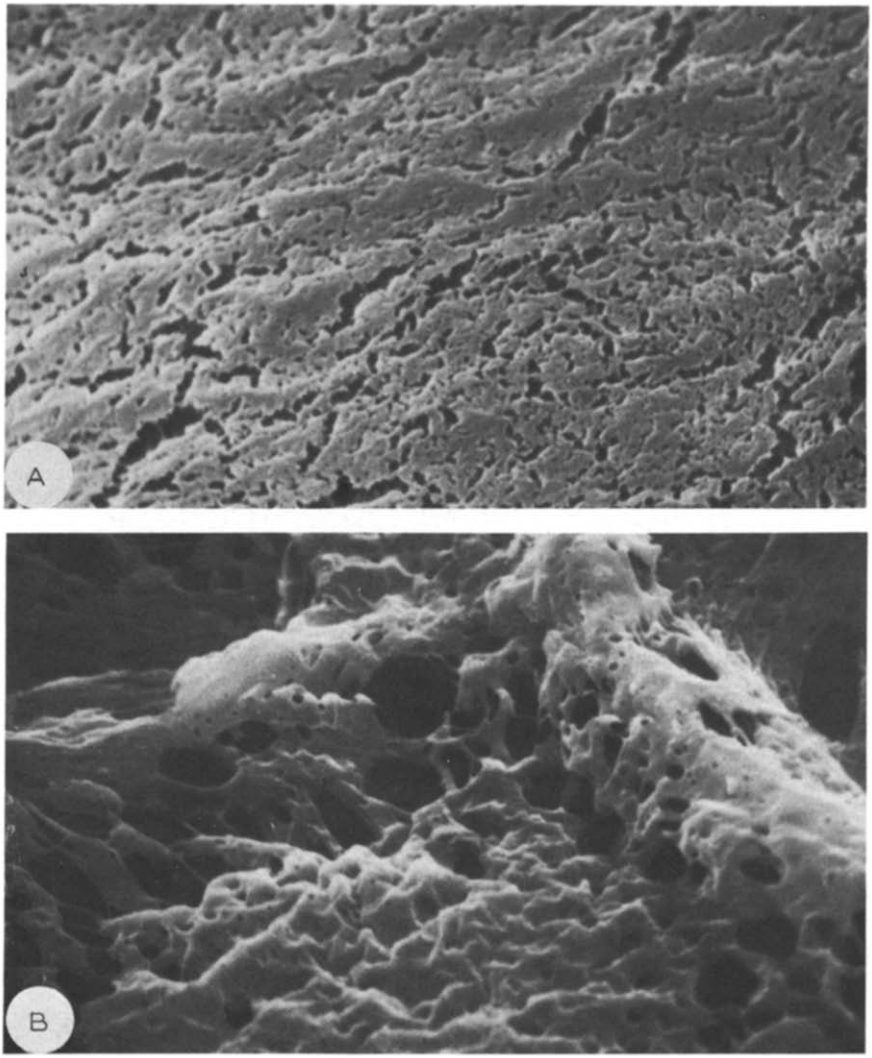

Fig. 5A and B.

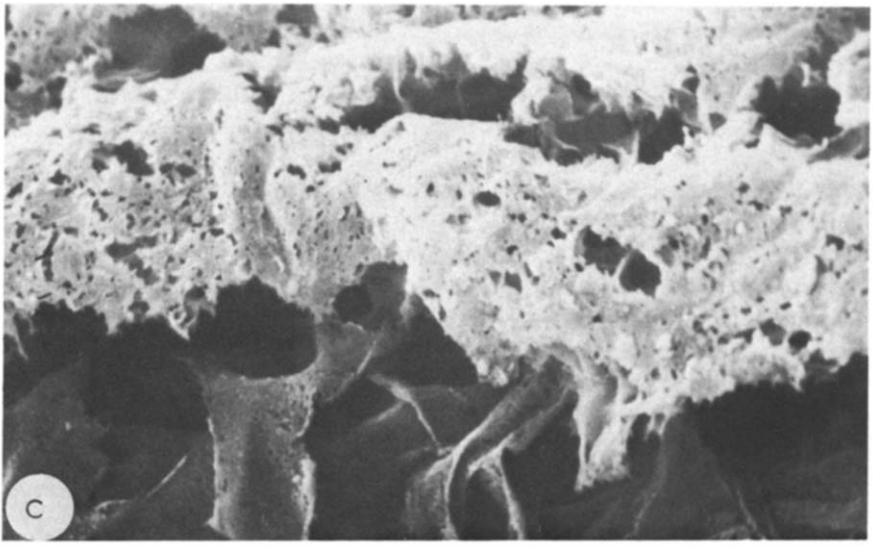

Fig. 5. Scanning electron micrographs of copoly-lactic/glycolic acid porous membrane. A: upper surface; B: lower surface; C: cross-section. 1450×.

increased to that of insulin only in PBS solution and not in urea solution. The effects of PBS and urea on protein diffusivity through polymer membrane were due to the reduction of partition coefficients of the protein in the polymer membrane. Since the diffusion coefficient is calculated by dividing the permeability by the partition coefficient, a reduction in the partition coefficient causes an increase in the diffusion coefficient even if the permeability remains unchanged.

The segregation of charge in cytochrome c has not been found in any other protein structure and probably occurs because cytochrome c interacts with two molecular complexes (the reductase and the oxidase) by electrostatic attraction. Thus, most of the interaction between cytochrome c and substrate can be attributed to the electrostatic force. Since an electrostatic interaction is weakened by increased ionic strength, it seems that the interaction between cytochrome c and cellulose acetate segments is weakened in PBS compared to pure water and the partition coefficient of cytochrome c in cellulose acetate membrane is decreased.

Albumin binds most anions (Scatchard et al., 1959; Scatchard and Yap, 1964). Meyer and Guttman (1968) reviewed comprehensively the binding of organic compounds to albumin and suggested the hydrophobic contribution to the binding force between albumin and an organic compound. Several studies have indicated a large electrostatic component in the binding between albumin and an ionic species and demonstrated a strong dependence of binding force on ionic strength (McMenamy et al., 1977; Pederson, 1972; Carr, 1953). Thus, albumin interacts with substrates via hydrophobic bonding and electrostatic interactions. Hydrophobic bonding is weakened by a water structure-breaking agent like urea. Therefore, both PBS and urea reduce the interaction between albumin and the cellulose acetate

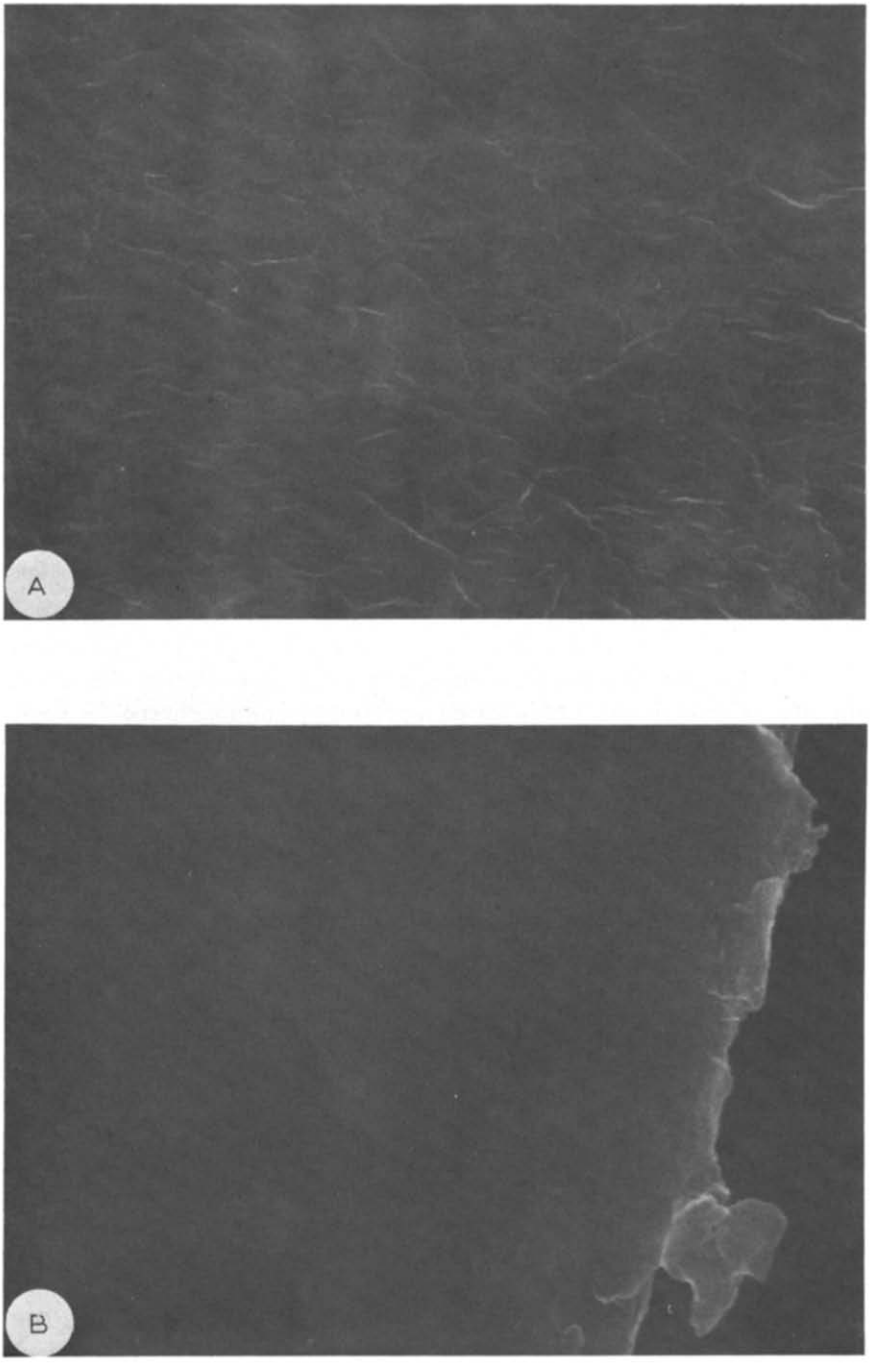

Fig. 6. Scanning electron micrographs of poly-HEMA dense membrane. A: surface; B: cross-section. 1500×.

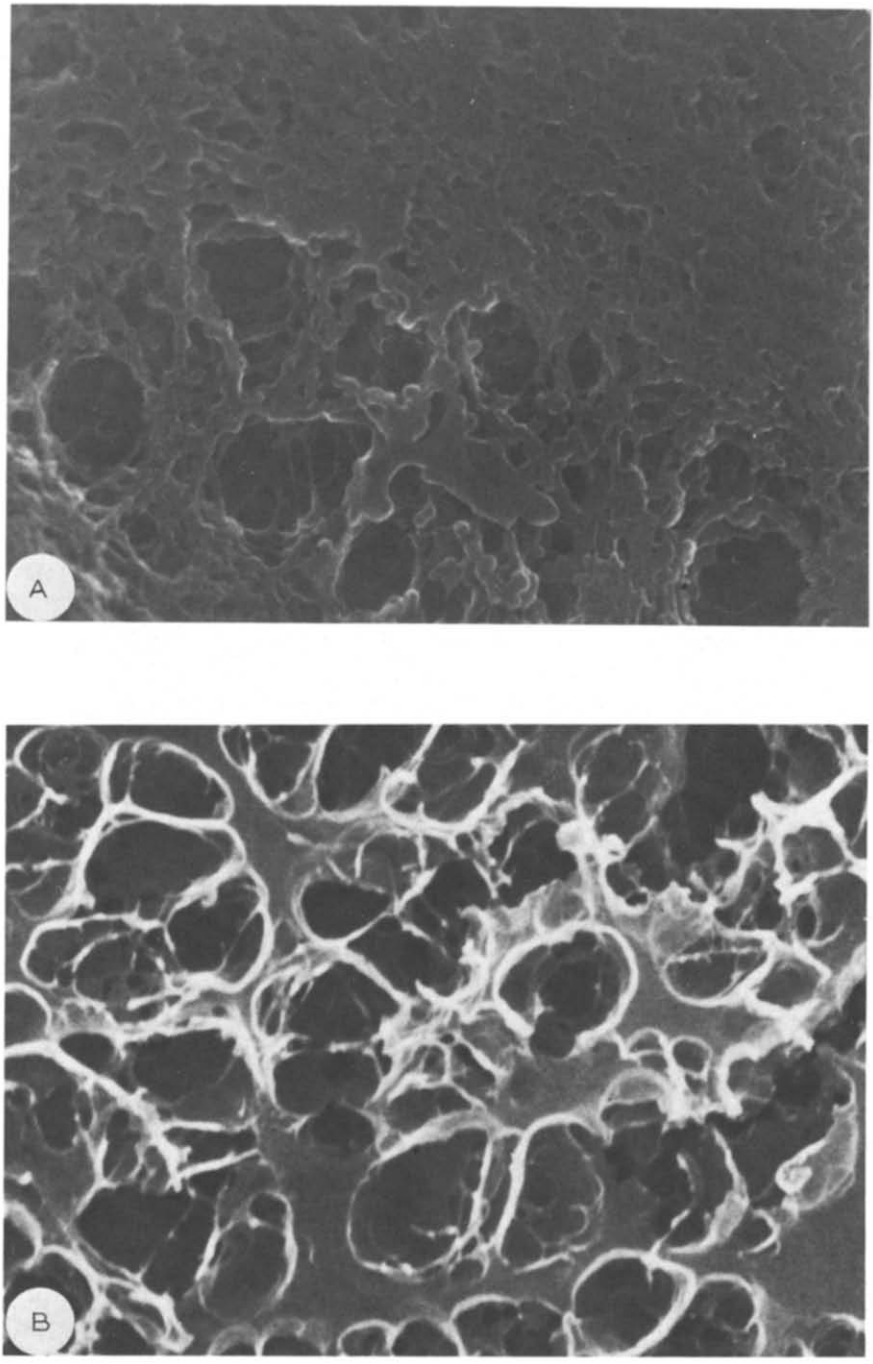

Fig. 7. Scanning electron micrographs of poly-HEMA porous membrane. A: surface; B: cross-section. 1500×.

TABLE 2

PERMEATION, PARTITION AND DIFFUSION COEFFICIENTS (cm^2/s) OF VARIOUS SOLUTES IN REGENERATED CELLULOSE

	Regenerated cellulose	
	Dense	Porous
Sodium acetate		
U	1.58×10^{-6}	1.77×10^{-6}
K_d	0.92	0.96
D	1.71×10^{-6}	1.84×10^{-6}
Glucose		
U	6.82×10^{-7}	1.06×10^{-6}
K_d	0.99	0.87
D	6.89×10^{-7}	1.22×10^{-6}
Maltose		
U	4.75×10^{-7}	5.21×10^{-7}
K_d	0.85	0.91
D	5.59×10^{-7}	5.93×10^{-7}
Insulin		
U	7.05×10^{-8}	4.93×10^{-8}
K_d	1.37	1.19
D	5.15×10^{-8}	4.14×10^{-8}
Cytochrome c		
U	2.05×10^{-7}	2.81×10^{-7}
K_d	124.5	111.3
D	1.64×10^{-9}	2.52×10^{-9}
Albumin		
U	1.78×10^{-9}	2.22×10^{-9}
K_d	1.79	1.37
D	9.94×10^{-10}	1.62×10^{-9}

U = permeation coefficient; K_d = partition coefficient; D = diffusion coefficient; (n = 3, S.D. < 5%).

segments and increase the diffusion coefficient of albumin through cellulose acetate membrane by decreasing the partition coefficient. The decrease of K_d values of the proteins due to PBS may also be explained in terms of the decreased solubilities of the proteins due to increased ionic strength of the PBS solution.

Relationship between diffusivities of insulin and glucose and hydration of polymer membrane

Table 7 shows the values of hydration (H) of the polymer membrane, and the diffusion coefficients (D) of glucose and insulin. The plot of $(\ln D)^{-1}$ of glucose and insulin versus X^{-1} $(= H/1 - H)$ of polymer membranes is shown in Fig. 10.

The plot of the reciprocal of the natural logarithm of the diffusion coefficients of glucose and insulin versus the swelling parameter X^{-1} $(= H/1 - H)$ of the polymer

TABLE 3

PERMEATION, PARTITION AND DIFFUSION COEFFICIENTS (cm^2/s) OF VARIOUS SOLUTES IN CELLULOSE ACETATE

	Cellulose acetate	
	Dense	Porous
Sodium acetate		
U	4.22×10^{-10}	1.72×10^{-7}
K_d	0.23	0.69
D	1.83×10^{-9}	2.49×10^{-7}
Glucose		
U	5.36×10^{-11}	7.41×10^{-9}
K_d	0.26	0.68
D	2.06×10^{-10}	1.09×10^{-8}
Maltose		
U	4.62×10^{-11}	2.28×10^{-10}
K_d	0.21	0.62
D	2.20×10^{-10}	3.67×10^{-10}
Insulin		
U	7.54×10^{-10}	5.92×10^{-9}
K_d	1.13	1.97
D	6.67×10^{-10}	3.01×10^{-9}
Cytochrome C		
U		1.55×10^{-8}
K_d		231.9
D		6.68×10^{-11}
Albumin		
U		1.20×10^{-9}
K_d		48.5
D		2.47×10^{-11}

U = permeation coefficient; K_d = partition coefficient; D = diffusion coefficient; (n = 3, S.D. < 5%).

membrane shows linearity at the low values of X^{-1} and then gradually plateaus when approaching the diffusion coefficient of the self-diffusion in water. Since X^{-1} is calculated using the following equation:

$$X^{-1} = \frac{H}{1-H} \tag{18}$$

X^{-1} has an extremely large value when the hydration (H) of the membrane approaches unity. The value of X^{-1} has a meaning only when X^{-1} is calculated for a low hydration value, therefore it is inaccurate to apply X^{-1} to a highly hydrated membrane.

The difference in $(\ln D)^{-1}$ values between insulin and glucose decreases as the

TABLE 4

PERMEATION, PARTITION AND DIFFUSION COEFFICIENTS (cm^2/s) OF VARIOUS SOLUTES IN COPOLYMER OF LACTIC AND GLYCOLIC ACID (50/50 % w/w)

	Copolymer (L50/G50)
Glucose	
U	5.17×10^{-6}
K_d	1.53
D	3.38×10^{-6}
Maltose	
U	1.07×10^{-6}
K_d	1.64
D	0.52×10^{-7}
Insulin	
U	5.47×10^{-7}
K_d	3.90
D	1.40×10^{-7}
Albumin	
U	2.44×10^{-7}
K_d	41.2
D	5.92×10^{-9}

U = permeation coefficient; K_d = partition coefficient; D = diffusion coefficient; (n = 3, S.D. < 5%).

X^{-1} value decreases and, finally, the $(\ln D)^{-1}$ value of glucose becomes higher than that of insulin, that is, insulin permeates faster than glucose when the hydration of the membrane is very low. This is partially because PBS and urea facilitate the diffusion of insulin, but it is also because insulin has the advantage of also diffusing

TABLE 5

PERMEATION, PARTITION AND DIFFUSION COEFFICIENTS (cm^2/s) OF VARIOUS SOLUTES IN METHACRYLIC HYDROGELS

	p-HEMA/MEMA	p-HEMA	p-HEMA/MEEMA	Porous p-HEMA
Glucose				
U	2.44×10^{-10}	4.18×10^{-8}	1.57×10^{-7}	5.81×10^{-7}
K_d	0.12	0.22	0.47	0.63
D	2.03×10^{-9}	1.90×10^{-7}	3.35×10^{-7}	9.22×10^{-7}
Insulin				
U	9.36×10^{-9}	5.84×10^{-8}	1.17×10^{-7}	2.35×10^{-7}
K_d	1.21	1.65	2.10	2.40
D	7.73×10^{-9}	3.54×10^{-8}	5.56×10^{-8}	9.79×10^{-8}

U = permeation coefficient; K_d = partition coefficient; D = diffusion coefficient; (n = 3, S.D. < 5%).

TABLE 6

EFFECTS OF PHOSPHATE-BUFFERED SALINE (PBS) AND UREA ON PERMEATION, PARTITION AND DIFFUSION COEFFICIENTS (cm^2/s) OF ALBUMIN AND CYTOCHROME C IN CELLULOSE ACETATE POROUS MEMBRANE

	In water	In PBS	In urea soln.
Cytochrome C			
U	1.55×10^{-8}	8.31×10^{-9}	1.32×10^{-8}
K_d	231.9	1.57	57.46
D	6.68×10^{-11}	5.29×10^{-9}	2.30×10^{-10}
Albumin			
U	1.20×10^{-9}	7.25×10^{-9}	3.01×10^{-9}
K_d	48.5	2.33	1.92
D	2.47×10^{-11}	3.11×10^{-9}	1.57×10^{-9}

U = permeation coefficient; K_d = partition coefficient; D = diffusion coefficient; (n = 3, s.d. < 5%)

via the 'partition' mechanism when the hydration of the membrane is very low.

As seen in Fig. 10, hydrogels (dense and porous p-HEMA, p-HEMA/MEMA and p-HEMA/MEEMA) have their own curves, indicating that hydrogels maintain a higher diffusivity for solute permeation than cellulosic membranes. The higher

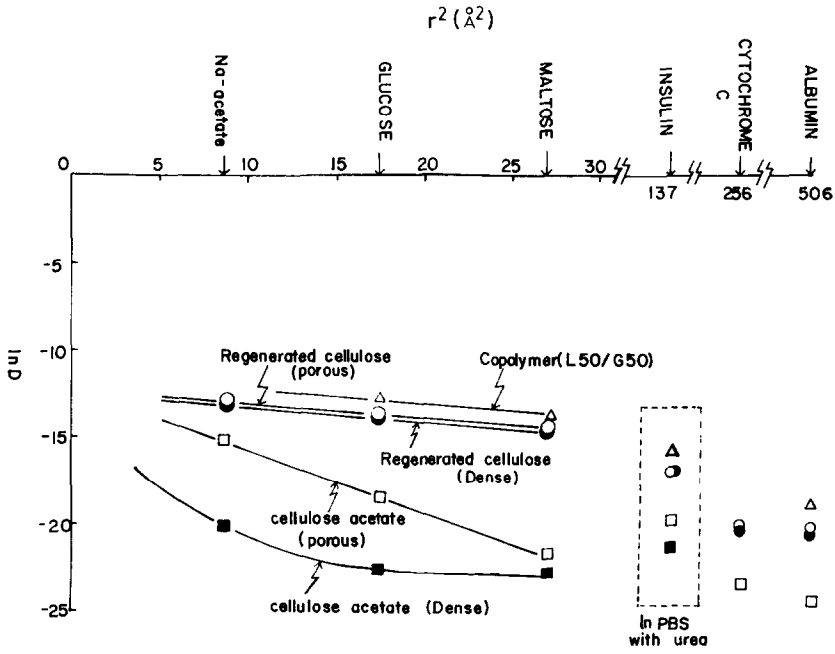

Fig. 8. Dependence of membrane diffusion coefficient on the solute molecular size.

TABLE 7

HYDRATION (H) OF POLYMER MEMBRANES, DIFFUSION COEFFICIENTS (cm^2/s) OF GLUCOSE AND INSULIN, AND VALUES OF $(\ln D)^{-1}$ AND X^{-1} (= H/1 − H)

Polymer membrane	Hydration (H)	Diffusion coefficient (D) (cm^2/s)	
		Glucose	Insulin
Cellulose acetate (dense)	0.20	2.06×10^{-10}	6.67×10^{-10}
Cellulose acetate (porous)	0.54	1.09×10^{-8}	3.01×10^{-9}
Regenerated cellulose (dense)	0.84	6.89×10^{-7}	5.15×10^{-8}
Regenerated cellulose (porous)	0.84	1.22×10^{-6}	4.14×10^{-8}
p-lactic/glycolic acid (L50/G50)	0.98	3.38×10^{-6}	1.40×10^{-7}
p-HEMA/MEMA	0.27	2.03×10^{-9}	7.73×10^{-9}
p-HEMA	0.49	1.90×10^{-7}	3.54×10^{-8}
p-HEMA/MEEMA	0.56	3.35×10^{-7}	5.56×10^{-8}
p-HEMA (porous)	0.74	9.22×10^{-7}	9.79×10^{-8}

D: n = 3, s.d. < 5%.

diffusivity of hydrogels can be explained in terms of the flexibility of the polymer chains which constitute the membrane.

Since cellulose consists of glucopyranose rings combined by β-1,4-linkages, there are only two kinds of movements of the main chain: one is the movement of the pyranose ring from 'chair form' to 'boat form', and the other is the rotation around

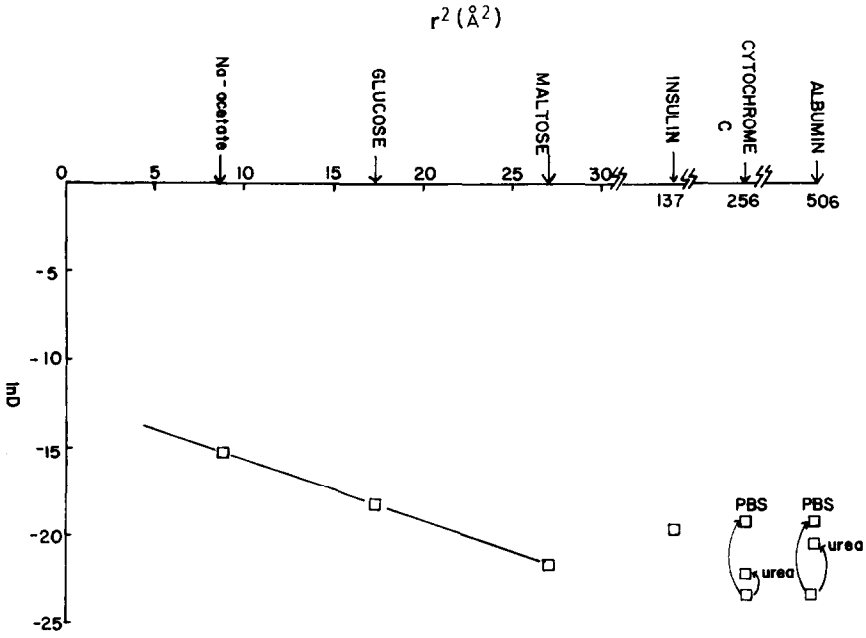

Fig. 9. Effects of phosphate-buffered saline (PBS) and urea on diffusion coefficients of albumin and cytochrome t in cellulose acetate porous membrane.

the β-1,4-glycoside linkage. In addition to the restriction of movement due to the intrinsic structure, the cellulose chains exist in a partially crystallized form made by interchain hydrogen bonding (Shiraishi et al., 1975). On the other hand, the main chain of a hydrogel consists of C–C single bonds, which allows the main chain of hydrogel to be very flexible. Thus, hydrogel chain segments appear to fluctuate and provide diffusing solutes with enough space for diffusion,

Diffusion coefficients of glucose and insulin through various hydrogel membranes, tabulated in Table 5, present an interesting feature. In p-HEMA/MEMA, the diffusion coefficients of glucose and insulin are much closer than other hydrogel systems. This indicates that diffusion in this hydrogel is more via the partition mechanism. However, in p-HEMA, there is an order of magnitude difference between the two diffusion coefficients which implies diffusion occurs mainly via the pore mechanism. This is largely dependent on solute size. Both glucose and insulin increase permeability as the H_2O content of the hydrogel increases, p-

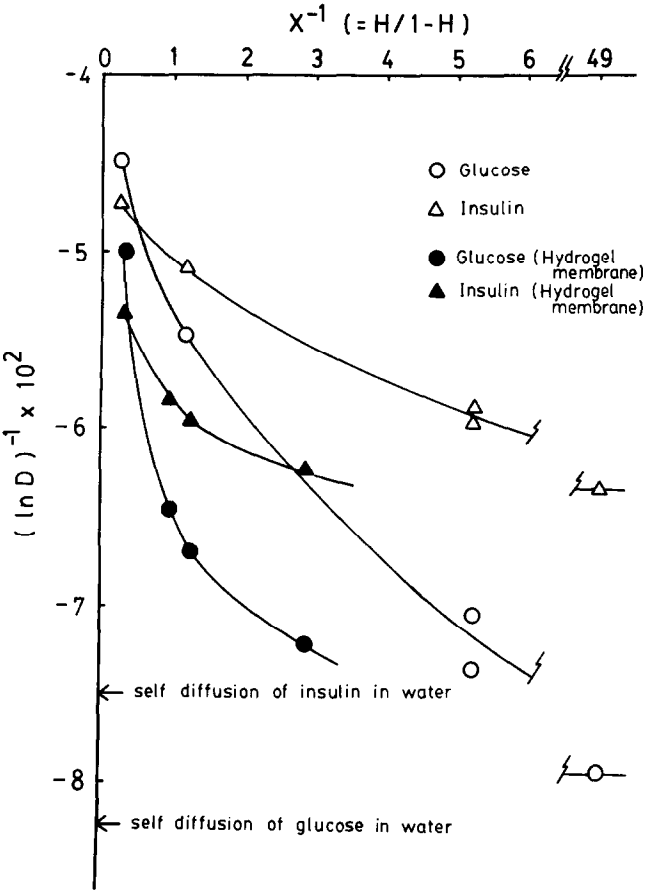

Fig. 10. Plot of $(\ln D)^{-1}$ of glucose and insulin versus X^{-1} (= H/1 – H) of polymer membranes.

HEMA/MEMA (~ 20% H_2O), p-HEMA (~ 40% H_2O), p-HEMA/MEEMA (~ 55% H_2O) and porous p-HEMA (high H_2O channel).

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References

Carr, C.W., Studies done on the binding of small ions in protein solutions with the use of membrane electrodes. II. The binding of calcium ions in solutions of bovine serum albumin. Arch. Biochem. Biophys., 43 (1953) 147–156.

Colton, C.K., Smith, D.A., Merrill, E.W. and Farrell, P.C., Permeability studies with cellulosic membranes. J. Biomed. Mat. Res., 5 (1971) 459–488.

Creque, H.M., Langer, R.S. and Folkman, J., One month of sustained release of insulin from a polymer implant. J. Diabetes, 29 (1980) 37–40.

Davis, B.K., Control of diabetes with polyacrylamide implants containing insulin. Experientia, 28 (1972) 348.

Eyring, H., Viscosity, plasticity and diffusion as examples of absolute reaction rates. J. Chem. Phys., 4 (1936) 283–291.

Flynn, G.L., Yalkolsky, S.H. and Roseman, T.J., Mass transport phenomena and models: theoretical concepts. J. Pharm. Sci., 63 (1974) 479–510.

Jhon, M.S. and Andrade, J.D., Water and hydrogels. J. Biomed. Mater. Res., 7 (1973) 509–521.

Kim, S.W., Cardinal, J.R., Wisniewski, S. and Zentner, G.M., Solute permeation through hydrogel films – hydrophobic vs hydrophilic solutes. In Rowland, S.P., (Ed.) ACS Symposium Series 127, American Chemical Society, Washington, DC, (1980), p. 347.

Klein, E., Holland, F.F. and Eberle, K., Comparison of experimental and calculated permeability and rejection coefficients for hemodialysis membranes. J. Membr. Sci., 5 (1979) 173–188.

Klomp, G.F., Hashiguchi, H., Ursell, P.C., Takeda, Y., Taguchi, T. and Dobelle, W.H., Macroporous hydrogel membranes for a hybrid artificial pancreas. II. Bicompatibility. J. Biomed. Mat. Res., 17 (1983) 865–871.

Korsmeyer, R.W. and Peppas, N.A., Macromolecular and modeling aspects of swelling controlled system. In Controlled Release Delivery Systems, M. Dekker, 1983, 77–90.

Kulkarni, R.K. Pani, K.C. Neuman, C. and Leonard, F., Polylactic acid for surgical implants. Arch. Surg., 93 (1966) 839–843.

Langer, R.S. and Folkman, J., Polymers for the sustained release of proteins and other macromolecules. Nature (Lond.) 263 (1976) 797–799.

Langer, R.S. and Folkman, J., Polymeric delivery system. In Kostelnik, R.J. (Ed.), Midland Macromolecular Monograph, Vol. 5, Gordon and Breach Science Publishers, New York, 1978, p. 175.

Langer, R.S., Rhine, W.D., Hsieh, D.S.T. and Bawa, R.S., In Bater, R. (Ed.), Controlled Release of Bioactive Materials. Academic Press, New York, 1980, p. 83.

LeBas, G., The Molecular Volume of Liquid Chemical Compounds, Longmans, Green and Co., London and New York, 1915.

McMenamy, R.H., Madeja, M.I. and Watson, F., Effects of salts and pH on the binding of thiocyanate with bovine plasma albumin. J. Biol. Chem., 243 (1969) 2328–2335.

McMenamy, R.H., Rosenoer, V.M., Oratz, M. and Rothschild, M.A., (Eds.), Albumin Structure, Function and Uses, Pergamon Press, New York, 1977, pp. 143–145.

Meyer, M.C. and Guttman, D.E., The binding of drugs by plasma proteins. J. Pharm. Sci., 57 (1968) 895–918.

Pederson, D.O., Binding of calcium of serum albumin. Scand. J. Clin. Lab. Invest., 29 (1972) 427–432.
Peters, T., Jr. and Red, R.G., In Peters, Jr., T., Sjoeholm, I. (Eds.), Albumin Structure, Biosynthesis, Function, Federation of European Biochemical Societies, 11th meeting, Vol. 50, Copenhagen, 1977, p. 11.
Refojo, M.F. and Yasuda, H., Hydrogels from 2-hydroxyethyl methacrylate and propylene glycol monoacrylate. J. Appl. Polymer Sci., 9 (1965) 2425–2435.
Ronel, S.H., D'Andrea, M.J., Hashiguchi, H., Klomp, G.F. and Dobelle, W.H., Macroporous hydrogel membranes for a hybrid art pancreas. I. Synthesis and chamber fabrication. J. Biomed. Mat. Res., 17 (1983) 855–864.
Roseman, T.J., Release of steroids from a silicone polymer. J. Pharm. Sci., 63 (1974) 479.
Scatchard, G., Scheinberg, I.H. and Armstrong, Jr., H., Physical chemistry of protein solutions. IV. the combination of human serum albumin with chloride ion. J. Am. Soc., 72 (1950) 535–540.
Scatchard, G., Wu, Y.V. and Shen, A.L., Physical chemistry of protein solutions. X. The binding of small anions by serum albumin. J. Am. Chem. Soc., 81, (1969) 6104–6109.
Scatchard, G. and Yap, W.T., Physical chemistry of protein solutions. XII. The effects of temperature and hydroxide ion on the binding of small anions to human serum albumin. J. Am. Chem. Soc., 86 (1964) 3434–3438.
Sefton, M.V. and Nishimura, E., Insulin permeability of hydrophilic polyacrylate membranes. J. Pharm. Sci., 69 (1980) 208–209.
Smith, M.H., In Sober, H.A. (Ed.), Handbook of Biochemistry, The Chemical Rubber Co., Cleveland, OH, 1968, p. C-3.
Wilke, C.R., Estimation of liquid diffusion coefficients. Chem. Eng. Prog., 45 (1949) 218–224.
Wise, D.L., Gregory, J.B., Newberne, P.M., Bartholow, L.C. and Stanbury, J.B., In Kostelnik, R.J. (Ed.), Polymeric Delivery Systems. Midland Macromolecular Monograph, Vol. 5, Gordon and Breach Science Pub., New York (1978) 121.
Wisniewski, S. and Kim, S.W., Permeation of water soluble solute through polyHEMA and polyHEMA crosslinked with EGDMA. J. Membr. Sci., 6 (1980) 299.
Yasuda, H., Lamaze, C.E. and Ikenberry, L.D., Permeability of solutes through hydrated polymer membranes. Makromolek. Chem., 118 (1968) 19–35.
Yasuda, H., Ikenberry, L.D. and Lamaze, C.E., Permeability of solutes through hydrated polymer membranes. Part II: permeability of water soluble organic solutes. Makromolek. Chem., 125 (1969) 108–118.
Yasuda, H., Peterlin, A., Colton, C.K., Smith, K.A. and Merrill, E.W., Permeability of solutes through hydrated polymer membranes. Part III: theoretical background for the selectivity of dialysis machines. Makromolek. Chem., 126, (1969) 177–186.
Yasuda, H. and Lamaze, C.E., Permselectivity of solutes in homogenous water swollen polymer membranes. J. Macromol. Sci.-Phys., B5(11) (1971) 111–134.
Yasuda, H., Gochin, M. and Stone, W., Jr., Hydrogels of poly(hydroxyethyl methacrylate) and hydroxyethyl methacrylate-glycerol, monomethacrylate copolymers. J. Polymer Sci., Part A-1, 4 (1966) 2913–2927.
Zentner, G.M., Cardinal, J.R. and Kim, S.W., Progestin permeation through polymer membranes: II. Diffusion studies of hydrogel membranes. J. Pharm. Sci., 67 (1978) 1352.
Zentner, G.M., Cardinal, J.R., Feijen, J. and Song, S., Progestin permeatimon through polymer membranes: IV. Mechanism of steroid permeation and functional group contributions to diffusion through hydrogel films. J. Pharm. Sci., 68 (1979) 970–978.
Zentner, G.M., Ph. D. dissertation, The University of Utah, Salt Lake City, UT, 1979.