

Permeability of Poly-L-methionine Membrane and Its Oxidized Membrane to Water Vapor

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Synopsis

The permeability of poly-L-methionine (PLM) membrane and its oxidized form to water vapor was studied. Permeability coefficients of the PLM membrane were large, of the order of 10^{-7} cm³ (S.T.P.)-cm/cm²-sec-cm Hg. The sorption and permeation behavior of the PLM membrane was hydrophobic. The oxidized membrane was prepared by treating one or both sides of the PLM membrane with an aqueous solution of hydrogen peroxide. The membrane oxidized from one side is probably not layered but has a gradient of composition from one surface to the other. The amounts of water sorbed by the modified membrane increased with increase in oxidation time. The permeability coefficients of water vapor through the modified membrane were of the order of 10^{-6} cm³ (S.T.P.)-cm/cm²-sec-cm Hg.

INTRODUCTION

Few papers¹⁻⁴ on the transport properties of small molecules through synthetic polypeptides of rod-like, α -helical structure have been published. The studies on poly(*n*-alkyl-L-glutamate)¹⁻³ indicate that the diffusion of small molecules in polypeptide occurs through the side chain region between helices. Poly-L-methionine has a hydrophobic side chain, and it is known that its α -helical structure is stable.⁵

The purpose of the present investigation is to study the transport of water vapor through poly-L-methionine in order to confirm the above permeation mechanism and to elucidate the effect of the introduction of a hydrophilic group in the polymer side chain on the permeation of water vapor.

EXPERIMENTAL

Materials

Poly-L-methionine (PLM) was prepared by the polymerization of N-carboxyamino acid anhydride in methylene dichloride using triethylamine as an initiator at room temperature. The number-average degree of polymerization was 160, as determined by the titration of the amino endgroup with *N*/50 HClO₄ in acetic acid. PLM membranes were prepared by casting the diluted polymerization solutions onto glass plates. Membranes were washed with methanol to remove the unreacted L-methionine and low molecular weight PLM. The density of the membrane obtained by a flotation method using an aqueous calcium chloride solution at 20°C was 1.28 g/cm³, and the thickness was 0.049–0.051 mm.

Oxidation, introducing hydrophilic groups—sulfoxide and/or sulfone—to the PLM, was carried out as follows: PLM membranes were immersed in 15%

aqueous hydrogen peroxide at 20°C. As the reaction proceeded, the membranes gradually swelled and dissolved with time. Figure 1 shows the effect of membrane thickness on the dissolution time of the PLM membranes. One side of the PLM membranes was treated in contact with the solution, and the other side under vacuum.

Figure 2 shows the infrared spectra of the PLM membrane and a membrane treated with the hydrogen peroxide solution for 10 min. Absorption peaks appeared at 1650 cm^{-1} (amide I), 1545 cm^{-1} (amide II), and 615 cm^{-1} (amide V). Therefore, both membranes prepared were confirmed to have α -helical structures.⁶ For the treated membrane, an absorption peak also appeared in 1040 cm^{-1} ; we assign this to $>\text{S}=\text{O}$ stretching vibration. The intensity of this peak increased with increase in reaction time. Accordingly, we believe that part of the methionine residue is oxidized and that methionine sulfoxide residue is formed.

The polymers oxidized for various times, listed in Table I, were analyzed by NMR by use of a Varian EM360 spectrometer. From the spectra the extent of

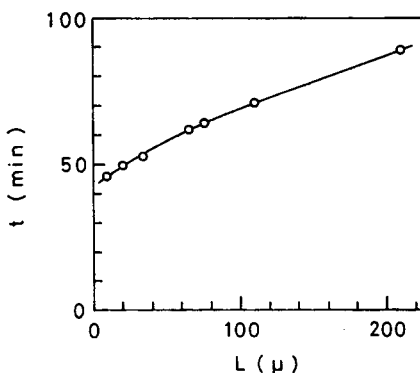


Fig. 1. Dissolution time t of poly-L-methionine membranes vs membrane thickness L .

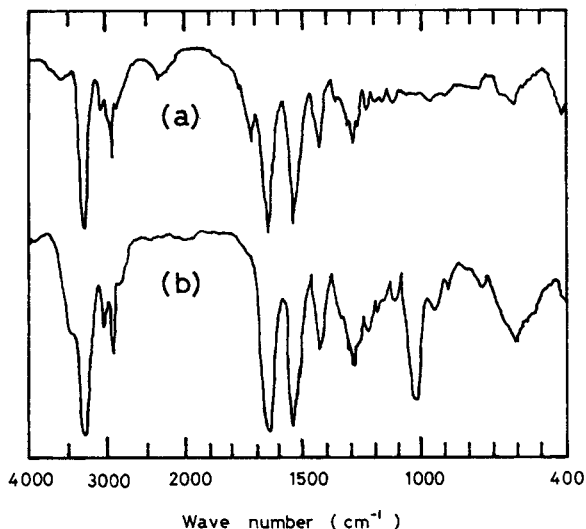


Fig. 2. Infrared spectra of poly-L-methionine membrane (a) and membrane treated with hydrogen peroxide solution for 10 min (b).

TABLE I
Relationship Between Reaction Time and Composition of Treated Membranes

Membrane	Original thickness, (mm)	Reaction time	Methionine sulfoxide composition, mole fraction
PLM=S ₁	0.051	35 min	0.31
PLM=S ₂	0.051	50 min	0.54
PLM=S ₃ ^a	0.051	3 day	

^a Powder.

methionine oxidation and the composition ratio can be calculated. Figure 3 gives the NMR spectra of the polymers in trifluoroacetic acid. As the oxidation proceeded, the intensity of the =S=CH₃ peak at 2.2 ppm decreased and was replaced at 3.0 ppm by the resonance attributed to the =SO=CH₃ group for PLM=S₁ and PLM=S₂. If sufficiently oxidized, PLM membranes dissolved in the aqueous hydrogen peroxide solution. When the solution was evaporated to dryness, the powder (PLM=S₃) was obtained. For the PLM=S₃ a resonance peak appeared at 3.2 ppm; we attribute this to the =SO₂=CH₃ group. Methionine sulfoxide residue probably formed in the first stage of the oxidation, and this was oxidized to the methionine sulfone in the highly oxidized membrane, causing membranes to dissolve in aqueous solution.

Composition ratios of the methionine sulfoxide for the PLM=S₁ and PLM=S₂ were estimated by comparing ratios of areas of the =S=CH₃ peak at 2.2 ppm and the =SO=CH₃ peak at 3.0 ppm. These data are summarized in Table I.

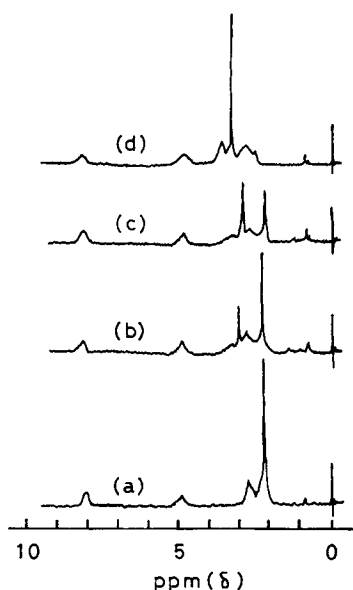


Fig. 3. Change in NMR spectra of poly-L-methionine upon oxidation: (a) PLM; (b) PLM=S₁; (c) PLM=S₂; (d) PLM=S₃.

Permeation and Sorption Experiments

Permeation measurements were made using Rouse's apparatus.⁷ Accordingly, the pressure of the lower-pressure side of the membrane is always near zero. The amount of water transferred through the membrane in time t , $Q(t)$, expressed as volume at standard conditions per unit membrane area [$\text{cm}^3(\text{S.T.P.})/\text{cm}^2$] is plotted versus time t , as shown in Figure 4 for the PLM-water system. The linear portions of the permeation curves correspond to steady-state permeation, from whose slopes the permeability coefficients, \bar{P} [$\text{cm}^3(\text{S.T.P.})\text{-cm}/\text{cm}^2\text{-sec}\text{-cm Hg}$], are determined. If solubility behavior is described by Henry's law dividing \bar{P} by the solubility coefficient S [$\text{cm}^3(\text{S.T.P.})/\text{cm}^3\text{-cm Hg}$] of penetrant to the polymer, the steady-state diffusion coefficient \bar{D} (cm^2/sec) is obtained; S is determined by the sorption isotherms described below.

The pressure of the higher-pressure side of the membrane is set up by controlling the temperature of the water-filled flask, which is connected to the higher-pressure side of the membrane in the vacuum system, and so the pressure corresponds to the saturated vapor pressure of the water at that temperature.

Sorption measurements for water vapor were carried out by the gravimetric method using the usual quartz spiral balance. Unless stated otherwise both the permeation and sorption measurements were made at 20°C.

RESULTS AND DISCUSSION

Permeability of Poly-L-methionine Membrane

The sorption isotherms of water vapor on the PLM at temperatures from 10° to 40°C are shown in Figure 5. The curves are independent of the temperature except at low relative pressures. This means that heats of sorption are small. PLM sorbs less water than does poly(γ -methyl-L-glutamate).¹ The PLM membranes have α -helical structure as judged by infrared absorption spectra. The sorption of low molecules onto polypeptides of rod-like, α -helical structure is reported to be controlled by polymer side chain-solvent mixing.^{8,9} Accordingly, the small sorption of water by PLM is attributed to the hydrophobicity of the side chain of the PLM. Averaged solubility coefficients S were calculated by dividing the sorption amount [$\text{cm}^3(\text{S.T.P.})/\text{cm}^3$] by vapor pressure. The deviation of solubility coefficients from Henry's law was small.

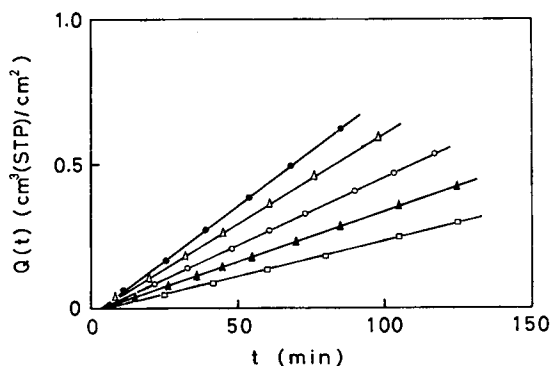


Fig. 4. Permeation curves of poly-L-methionine-water system (20°C). Membrane thickness is 0.049 mm. Applied pressure (cm Hg): (\square) 0.502; (\blacktriangle) 0.656; (\circ) 0.869; (\triangle) 1.103; (\bullet) 1.325.

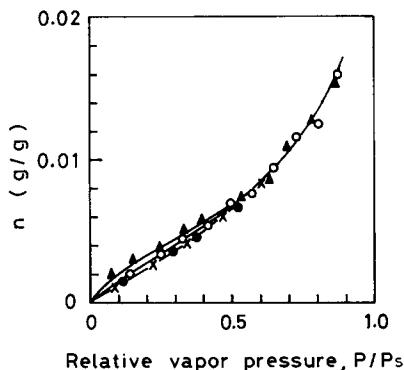


Fig. 5. Sorption isotherms of water on poly-L-methionine: (▲) 10°C; (○) 20°C; (×) 30°C; (●) 40°C.

The average permeability coefficients \bar{P} and average diffusion coefficients \bar{D} of the PLM membrane at temperatures from 10° to 40°C are plotted against the relative vapor pressure of water in Figure 6. \bar{P} increases slightly with relative vapor pressure and is independent of temperature. The latter indicates that the apparent activation energy of permeation is about zero. The \bar{P} of the PLM as a whole is about half that of poly(γ -methyl-L-glutamate).¹ The \bar{D} of the PLM is very large compared with that of vinyl polymers and about the same as that of poly(γ -methyl-L-glutamate).

The average diffusion coefficients \bar{D} are independent of the relative vapor pressure up to 0.9 relative vapor pressure and decrease with relative vapor

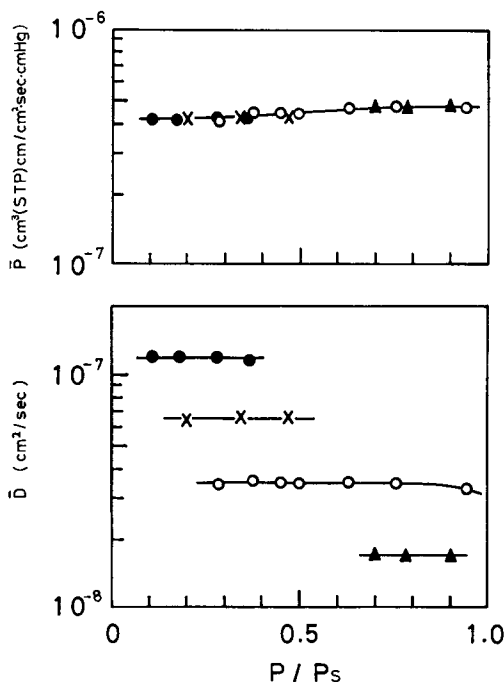


Fig. 6. Permeability coefficients \bar{P} and diffusion coefficients \bar{D} of poly-L-methionine membrane vs relative vapor pressure of water, P/P_s : (▲) 10°C; (○) 20°C; (×) 30°C; (●) 40°C.

pressures in the higher-pressure regions. From Figure 6, concentration-dependent diffusion coefficients $D(C)$ are estimated as a function of concentration C of penetrant in the polymer using the equation

$$\bar{D}(C_1) = \frac{1}{C_1} \int_0^{C_1} D(C) dC \quad (1)$$

As \bar{D} of the PLM is independent of the relative vapor pressure except in the higher-pressure region, \bar{D} equals $D(C)$. Diffusion coefficients of water vapor in poly(vinyl acetate) and hydrophobic polyolefin independent of the concentration of water have been reported¹⁰; this behavior seems to be characteristic of water permeation in hydrophobic polymers.

The decrease in \bar{D} at high relative vapor pressures is considered to be due to clustering of the water in the PLM. An approach to determining the degree of clustering was developed by Zimm and Lundberg.^{11,12} A clustering function G_{11}/V_1 is defined as

$$\frac{G_{11}}{V_1} = -(1 - v_1) \left[\frac{\partial \left(\frac{a_1}{v_1} \right)}{\partial a_1} \right]_{P,T} - 1 \quad (2)$$

where v_1 , V_1 , and a_1 are the volume fraction, partial molecular volume, and activity of water, respectively. According to this treatment, when $G_{11}/V_1 > -1$, clustering occurs; whereas when $G_{11}/V_1 < -1$, sorption occurs on sites with little tendency for clustering. The mean size of the clusters is $1 + v_1 G_{11}/V_1$. This treatment is applied to the sorption isotherm of water on the PLM at 20°C, and the results are shown in Figure 7. Takizawa¹ suggested that the cluster size ($1 + v_1 G_{11}/V_1$) in poly(γ -methyl-D-glutamate) agreed with the number of water molecules associated in the polymer, obtained by the application of polycondensation model of water in the polymer to the water sorption on the polymer,

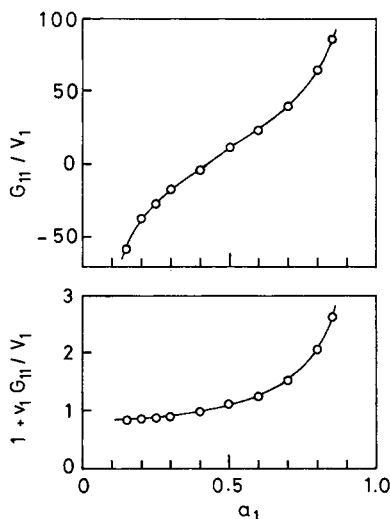


Fig. 7. Clustering function G_{11}/V_1 and mean size of the clusters, $1 + v_1 G_{11}/V_1$, vs activity of water a_1 (20°C).

and that a cluster of four or five water molecules could diffuse in the polymer. Figure 7 shows that clustering is evident in the PLM and that a cluster of three or four water molecules can diffuse in the PLM.

The $D(0)$ obtained by the extrapolation of the diffusion coefficient \bar{D} to zero penetrant concentration is plotted against the inverse temperature in Figure 8. Since a single straight line could easily be drawn through all the points in the range of the temperatures studied, the activation energy of diffusion, E_d , was calculated using the following equation:

$$D(0) = D_0 \exp(-E_d/RT) \quad (3)$$

The E_d obtained is about 12 kcal/mole and is about the same as that of poly(γ -methyl-L-glutamate),³ 11 kcal/mole, and smaller than that of the usual polymers.

These results suggest that the characteristic behavior of sorption and permeation of water vapor of the PLM membrane is consistent with the α -helical structure and hydrophobic side chain of PLM. Further, these results suggest that the diffusion of water molecules in the PLM may occur in the side chain region between helices.

Structure and Permeability of Poly-L-methionine Membrane Oxidized on One Side

One side of a PLM membrane 0.051 mm thick was oxidized with aqueous hydrogen peroxide solution for varying time intervals. The water vapor permeation was carried out with the oxidized side of the modified membrane facing water vapor in the high-pressure side of the permeation cell. The water permeation increased with oxidation time (Fig. 9).

The amount of water sorbed increased with increase in oxidation time (Fig. 10). Since membranes treated with the hydrogen peroxide solution have α -helical structures according to infrared absorption spectra, the increase in the amount of water sorbed is due to the change from the methionine residue to the methionine sulfoxide residue. The increase in permeability of water in Figure 9 is primarily due to the enhanced solubility of water in the membrane.

The permeation behavior of water vapor for the membrane whose one side was

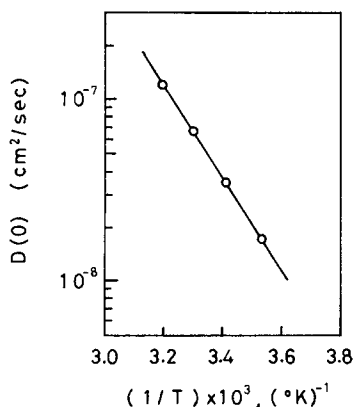


Fig. 8. Temperature dependence of diffusion coefficient $D(0)$.

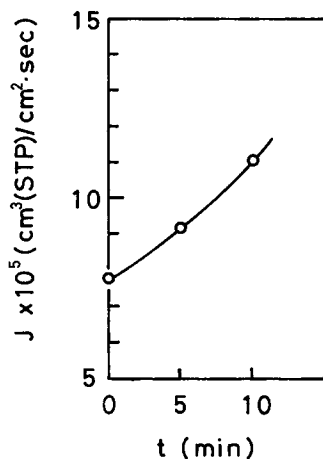


Fig. 9. Water flux J in modified poly-L-methionine membrane vs reaction time t . Applied relative vapor pressure is 0.5 (20°C).

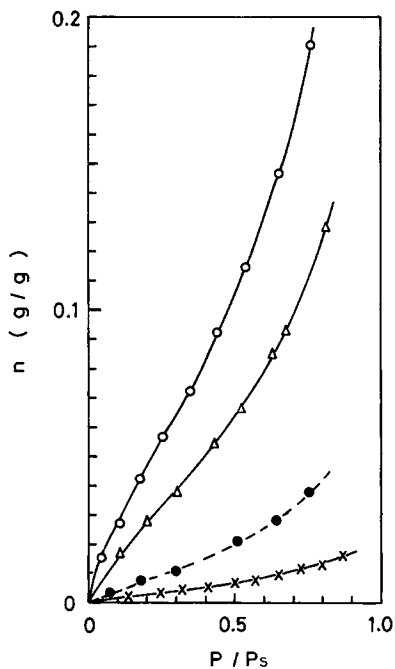


Fig. 10. Sorption isotherms of water (20°C): (x) PLM; (Δ) PLM=S₁; (O) PLM=S₂; (●) poly-L-methionine membrane treated on one side with hydrogen peroxide solution for 10 min; (---) calculated from eq. (6).

oxidized with the hydrogen peroxide solution for 10 min was examined with (a) the treated side of the membrane facing the high-pressure side and (b) the untreated side facing the high-pressure side. Figure 11 shows the relationship between the relative vapor pressure and the flux of water through the modified membrane. It is clear that when the treated side of the membrane faces the high-pressure side there is a higher flux of water than when the untreated side of the membrane faces the high-pressure side. The flux of water in the mem-

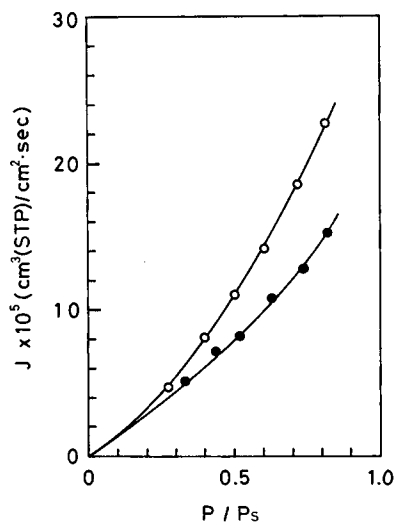


Fig. 11. Water flux vs relative vapor pressure (20°C) of poly-L-methionine membrane treated on one side with hydrogen peroxide solution for 10 min: (O) from treated side; (●) from untreated side.

brane whose untreated side faced the high-pressure side was about the same as that of the unoxidized membranes. Assumedly, the membrane has a gradient of chemical composition from one surface to the other.

It is of interest to know the distribution of the methionine sulfoxide residue in the membrane. One of us¹³ reported that when ethylene-vinyl acetate copolymer membranes were immersed in a sodium methoxide solution, partially hydrolyzed membranes consisting of three layers [(ethylene-vinyl alcohol copolymer)/(ethylene-vinyl acetate copolymer)/(ethylene-vinyl alcohol copolymer)] were prepared by adjusting the hydrolysis conditions.

Sternberg and Rogers¹⁴ reported that polyethylene membranes with nearly linear gradients of grafted poly(vinyl acetate) from one surface to the other were prepared and that the transport of methanol through the modified membranes proceeded at different rates, depending on the direction of flow relative to the gradient of grafted poly(vinyl acetate). Further, they derived a mathematical model which gave a quantitative description of the directional transport process. The relationship can be used to predict the transport behavior for single penetrants through asymmetric membranes from a knowledge of the properties of the individual components.

For the modified membrane whose diffusion-kinetic properties essentially have not been altered while its solution-thermodynamic properties have been greatly affected, the following equation is derived.¹⁴ Fick's generalized law of diffusion is represented by

$$J = \frac{d}{dx} (DC) \quad (4)$$

where J is the flux of the penetrant, D is a diffusion coefficient, and C is the concentration of the penetrant. The relation between the concentration of the penetrant and its vapor pressure is given by

$$C = Sp \quad (5)$$

where p is the vapor pressure of the penetrant and S is a solubility coefficient. For the modified membrane the diffusion coefficient is taken as that of the original membrane. The slight differences in diffusion coefficients due to slightly different morphologic regions across the membrane are too small to affect the overall behavior and are neglected. The solubility coefficient of the modified membrane is expressed by assuming the sum of the volume fraction contributions of the two components:

$$S = V_1S_1 + V_2S_2 \quad (6)$$

where S_1 is the solubility coefficient of the unmodified membrane, S_2 is the solubility coefficient of the completely modified membrane, and V_1 and V_2 are the volume fractions of component 1 and 2 in the membrane. The solubility coefficients of the pure components are a function of penetrant vapor pressure or concentration only. However, since the volume fractions at any point across the membrane thickness are explicit functions of the spatial coordinate, the solubility coefficient of the modified membrane is explicitly vapor pressure dependent and position dependent:

$$S = S(x,p) \quad (7)$$

Since we deal with time-independent rates (i.e., after the flux has reached steady state), the vapor pressure gradient in this case is only position dependent:

$$p = p(x) \quad (8)$$

Combining (4) and (5), while $D = \text{constant}$, gives

$$J = -D \left[S \left(\frac{dp}{dx} \right) + p \left(\frac{dS}{dx} \right) \right] \quad (9)$$

But from (7) and (8),

$$\frac{dS}{dx} = \left(\frac{\partial S}{\partial x} \right) + \left(\frac{\partial S}{\partial p} \right) \cdot \left(\frac{dp}{dx} \right) \quad (10)$$

Finally, combining (9) and (10) gives

$$J = -D \left\{ \left[S + p \left(\frac{\partial S}{\partial p} \right) \right] \left(\frac{dp}{dx} \right) + p \left(\frac{\partial S}{\partial x} \right) \right\} \quad (11)$$

The rearranged differential equation, converted into a finite difference equation, to be used in an iterative computation is

$$\frac{\Delta p}{\Delta x} = \left[-\left(\frac{J}{D} \right) - p \left(\frac{\delta S}{\delta x} \right) \right] / \left[S + p \left(\frac{\delta S}{\delta p} \right) \right] \quad (12)$$

By using eq. (12), we tried to estimate the composition of the methionine sulfoxide residue at any point across the modified membrane, to give the results in Figure 11. The diffusion coefficient of the modified membrane, which has a methionine sulfoxide residue of only 7 mole-%, may be taken as that of the PLM membrane. As can be seen from Figure 14, this assumption seems reasonable because the diffusion coefficient of the PLM= S_1 membrane, though dependent on the relative vapor pressure, approximately equals that of the PLM membrane. Accordingly, J and D in eq. (12) are shown in Figures 11 and 6, respectively. The solubility coefficient S_1 of the pure PLM membrane as a function of water vapor

pressure can also be obtained from Figure 5. However, the solubility coefficients S_2 of the pure poly(L-methionine sulfoxide) membrane cannot be obtained because such a pure membrane can not be prepared.

Therefore, we obtained S_2 the following way. Since the sorption isotherms for the membranes with different composition of methionine sulfoxide are shown in Figure 10, the amount sorbed is plotted against the methionine sulfoxide concentration at constant relative vapor pressure. The linear relationships can be seen in Figure 12. An imaginary sorption isotherm of water on poly(L-methionine sulfoxide) is drawn by extrapolation of this linear relation to $\phi_2 = 1$. The solubility coefficient S_2 of the pure poly(L-methionine sulfoxide) membrane is obtained from the imaginary sorption isotherm. The relationship of eq. (6) is confirmed using S_1 and S_2 for the modified membrane ($V_2 = 0.07$) and is shown in Figure 10 to represent the experimental data very well.

Since all factors in eq. (12), with the exception of $\Delta p/\Delta x$, are known, the concentration distribution of methionine sulfoxide residue in the modified membrane is obtained from eq. (12) by trial-and-error method using a computer and is shown in Figure 13. The initial condition on calculation is $p = C = 0$ at the low-pressure side of the membrane, $x = l$. The relation

$$p_i = p_{i-1} + \Delta p$$

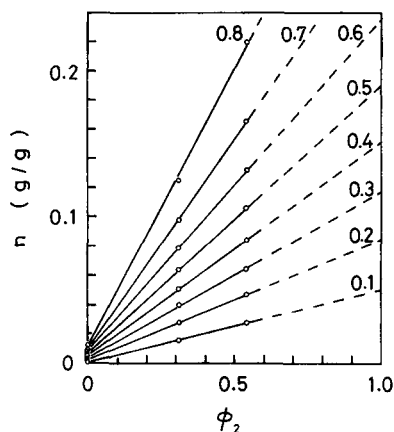


Fig. 12. Amount of water sorbed by modified poly-L-methionine membrane (n) vs mole fraction of methionine sulfoxide residue (ϕ_2). Figures on curves indicate relative vapor pressure.

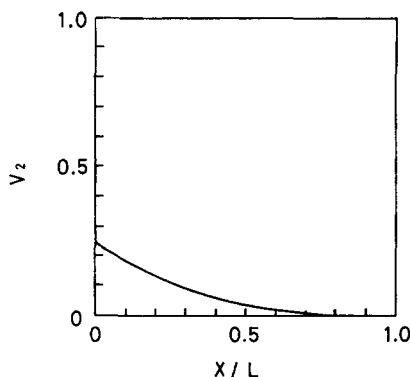


Fig. 13. Volume fraction of methionine sulfoxide residue vs relative thickness.

is used in conjunction with eq. (12). Figure 13 suggests that the modified membrane is not a two-layered membrane consisting of a layer of methionine sulfoxide residue and a layer of methionine residue but a membrane with a composition gradient of methionine sulfoxide residue from one surface to the other.

Accordingly, it is concluded that the membrane prepared by the immersion of the PLM membrane in the hydrogen peroxide solution (PLM=S₁ membrane) probably is a homogeneous membrane of methionine-methionine sulfoxide copolymer.

Permeability of PLM=S₁ Membrane

The permeability coefficient \bar{P} and diffusion coefficient \bar{D} of water vapor through the PLM=S₁ membrane are shown in Figure 14. \bar{P} is greatly dependent on the relative vapor pressure. The \bar{P} of the PLM=S₁ membrane is very large and about 20 times that of cellophane, which is the typical hydrophilic membrane.¹⁵ The diffusion coefficient \bar{D} of polymers with polar groups, such as cellophane¹⁵ and poly(glutamic acid),¹⁶ becomes very small in low relative vapor pressure because of the strong interaction between their polar groups. However, \bar{D} of the PLM=S₁ membrane is slightly lower than that of the PLM membrane at low relative vapor pressures, inspite of the addition of oxygen atoms to the side chain which must affect diffusion; this is due to the weak interaction between the side chains of the PLM=S₁. The \bar{D} of the PLM=S₁ membrane increases greatly with increase in relative vapor pressure; this indicates that the water in the PLM=S₁ membrane acts as a plasticizer. We conclude that the large value of \bar{P} in the PLM=S₁-water system results from the product of the large value

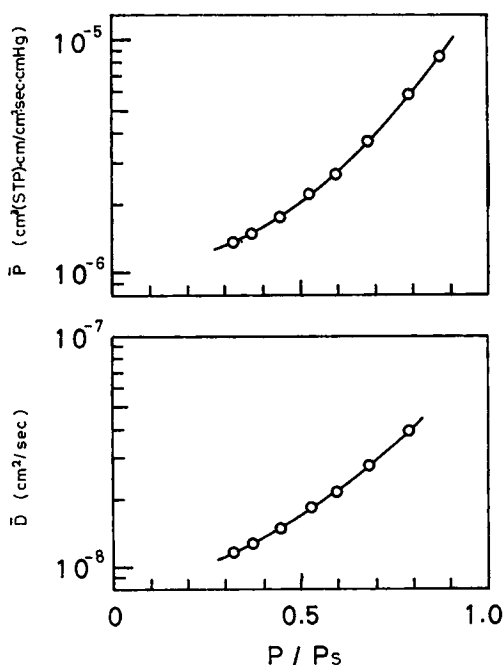


Fig. 14. Permeability coefficient \bar{P} and diffusion coefficient \bar{D} of PLM=S₁ membrane vs relative vapor pressure (20°C).

of \bar{D} characteristic of an α -helical structure and the large value of the solubility caused by the hydrophilicity of the sulfoxide group.

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