Sorption and permeation of poly(n-hexyl L-glutamate)—organic solvent systems

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To elucidate the permeation mechanism in polypeptide membranes quantitatively, sorption and permeation of organic solvents on and through poly(n-hexyl L-glutamate) (PHeLG) membrane are studied. Sorption isotherms of solvents are monotonous curves characteristic of random mixing of solvent with the side chains of PHeLG. Values obtained for the permeability and diffusion coefficients are explained systematically by the solvent size and affinity to PHeLG. The free volume fraction of PHeLG is larger than that of usual amorphous polymers. Using a membrane prepared by the application of electric field to concentrated PHeLG solution, the influence of orientation of α-helix to the permeation behaviour is examined. While the sorption is affected little by orientation, the permeability and diffusion coefficients decrease systematically with increase of orientation. It is also confirmed that the critical solvent concentration for the orientation in PHeLG—benzene system is about 40 wt %.

INTRODUCTION

It has been elucidated that the permeability of various small molecular substances through synthetic polypeptide membrane is high due to the permeation of small molecules through the side chain region between helical polypeptide molecules[1–4]. It is quite interesting to study the relationship among the sorption, diffusional permeation, solvent nature and side chain structure of the polymer more quantitatively.

Previously, sorption of benzene has been studied in various poly(n-alkyl L-glutamates) whose side chains have different lengths. The sorption isotherms of benzene by poly(n-butyl L-glutamate) and poly(n-octyl L-glutamate) were monotonous curves concave upwards, which is characteristic of the dissolution (random mixing) of solvent in polymer brought about by the flexibility of the side chain. The isotherms have been analysed by Flory's random mixing model of side chains and solvent[5,6], modified by elastic free energy considerations. Such a system which shows the simple dissolution mode may be convenient for analysing the relation between diffusion and polymer structure.

In this study, poly(n-hexyl L-glutamate) (PHeLG) was selected as the membrane material. The PHeLG may show the same sorption mode as those of the above described polymers, since the side chain length was just intermediate of those of the above polymers. Also, the PHeLG membrane has enough strength for the permeation experiment. The solvents selected were benzene, ethylene dichloride, dioxane and cyclohexane, which were different in molecular size and in affinity to the polymer. The sorption and diffusional permeation results were analysed in terms of the molecular parameters of the solvents and the molecular characteristics of the side chain region of PHeLG.

EXPERIMENTAL

Materials

PHeLG was prepared by ester exchange[3] with poly(γ-methyl L-glutamate) supplied by Kyowa Hakko K. K. The exchange was over 98% which was confirmed by high resolution n.m.r. The molecular weight of PHeLG obtained was about 1.6×10^5 by viscosity measurement of dichloroacetic acid solution. The PHeLG membrane was prepared by dry method from 5% ethylene dichloride solution, and was annealed at 70°C under vacuum for one day. The method for producing the orientation of the membranes is described later. The thickness of the membrane is about 2.0×10^{-2} cm for the sorption experiments, and 2.5 to 4.0×10^{-2} cm for permeation experiments.

Benzene, ethylene dichloride, dioxane and cyclohexane used as penetrants were extra pure grade.

Sorption and permeation experiments

Sorption experiments were made by a gravimetric sorption apparatus[8] using a quartz spring balance. Sorption isotherms were expressed as the relation between amount sorbed of solvent g/(g of polymer) and relative vapour pressure of solvent. The isotherms were observed at 25°C.

For permeation experiments, Rouse's apparatus[9] was used. On the feed side or high pressure side of the membrane, solvent vapour was supplied at controlled pressure, by the temperature regulation of liquid solvent in a trap connected to the cell. Pressure of the permeant side or low pressure side cell was maintained to be essentially zero by using the liquid nitrogen trap for the permeant vapour. From the slope of the steady state straight line of permeation curve (amount

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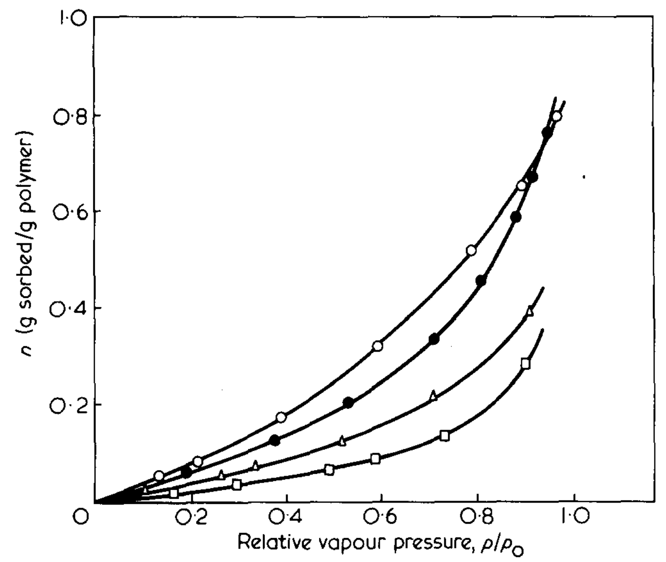

Figure 1 Sorption isotherms (amount sorbed n vs. relative vapour pressure (p/p_0) of PHeLG – organic solvent systems: ○, ethylene dichloride; ●, benzene; △, dioxane; □, cyclohexane

permeated *vs.* time) the permeability coefficient $\bar{P}$ was calculated as usual, and the diffusion coefficient $\bar{D}$ was obtained by $\bar{P}/S$, where the solubility coefficient S is the rate of concentration in membrane to pressure in the sorption isotherm. The bar in $\bar{P}$ and $\bar{D}$ denotes concentration averages.

Orientation under electric field

To obtain orientation, an electric field was applied to PHeLG membrane plasticized by benzene vapour. A PHeLG membrane of thickness 2.4×10^{-2} cm was fixed to the pole plates of the orientation cell, which was then evacuated by the rotary pump, and saturated benzene vapour at a certain temperature was introduced to the cell by the same method as that described in the permeation apparatus. After sufficient time in which the benzene content in the membrane reached an equilibrium, an electric field of 500 Volt/cm (direct current) was applied during 24 h.

Estimation of the orientation was obtained using an X-ray diffraction apparatus (JDX-5P, Nihon Denshi K. K., Japan) and was expressed by the ratio of equatorial to meridian intensity I_e/I_m of the (100) diffraction of the PHeLG using X-ray diffractometer.

RESULTS AND DISCUSSION

Sorption isotherms of PHeLG – solvent systems

The sorption isotherms of various organic solvent – PHeLG systems are shown in *Figure 1*. All of the isotherms increase monotonously with the relative vapour pressure and are concave upwards. This behaviour is similar to that of poly(n-butyl L-glutamate) and poly(n-octyl L-glutamate) described above[7] and reflects the random mixing of solvent and side chains of the polymer. The second order transition temperature of PHeLG is about −10°C[10] and so molecular movement of the side chains is sufficient at the experimental temperature.

From *Figure 1*, the affinity of solvent to PHeLG is considered to be the order of benzene ≃ ethylene dichloride > dioxane > cyclohexane. As was elucidated in previous study[7], the isotherms of the similar system can be expressed by Flory's random mixing theory of side chain and solvent modified by an elastic free energy contribution in the high relative pressure range. Thus, the Flory's theory was applied to the low pressure region of the present isotherms, and the interaction parameter χ_1 was obtained. The values obtained for the present systems are as follows; benzene: −0.24, ethylene dichloride: −0.12, dioxane: 0.47, and cyclohexane: 0.56. These values show the affinity with the polymer quantitatively.

Permeability and diffusion coefficients of PHeLG – solvent systems

The steady state permeability coefficient $\bar{P}$ and diffusion coefficient $\bar{D}$ are shown in *Figure 2*, and *Figure 3*, respectively. For benzene, only the data for relative vapour pressure less than 0.8 are described. Both $\bar{P}$ and $\bar{D}$ increase with relative vapour pressure almost exponentially. Ethylene dichloride, which has strong affinity with PHeLG and the smallest size, shows the highest $\bar{P}$ and $\bar{D}$. Inversely, cyclohexane with the lowest affinity and the largest molecular size shows the smallest $\bar{P}$ and $\bar{D}$. $\bar{D}$'s of benzene and dioxane are the same order of the magnitude reflecting almost the same molecular size (*Table 1*). However, the $\bar{P}$ of benzene is larger than that of dioxane reflecting the stronger affinity with PHeLG.

To study the molecular interrelationship between solvent and polymer in the diffusional permeation, diffusion coefficients corrected for the effect of swelling of the membranes should be estimated. In the present case, since the polymer sorbs solvent as high as 100% in the extreme case, the effect of swelling must not be neglected.

First, the experimental $\bar{D}$ may be regarded as the concentration average of the polymer fixed diffusion coefficient $\bar{D}^p$, and the relation between D^p and volume fixed mutual

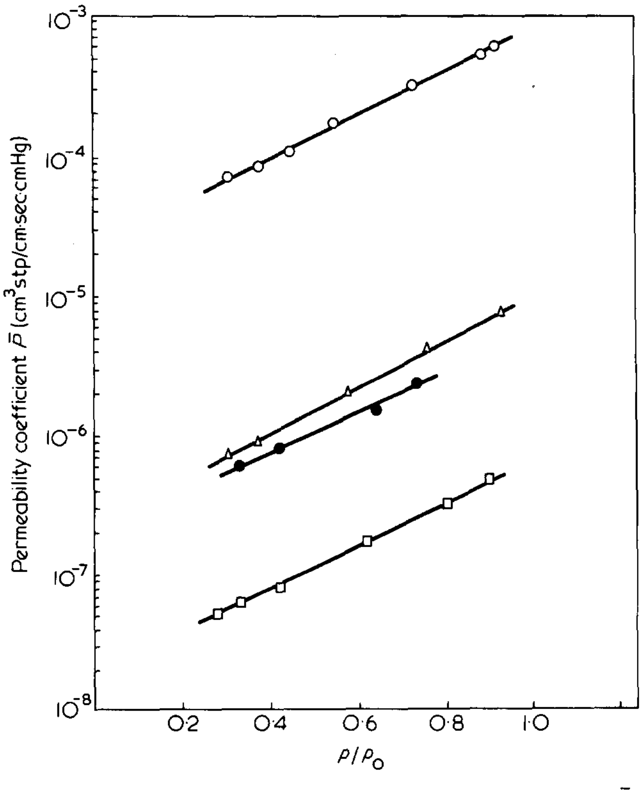

Figure 2 Relation between steady state permeability coefficient $\bar{P}$ at 25°C and applied relative vapour pressure of solvent p/p_0. For legend see *Figure 1*

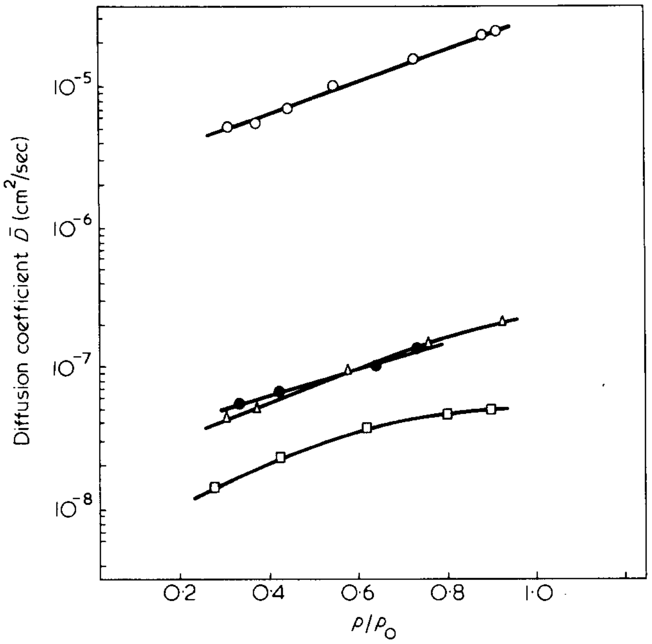

Figure 3 Relation between steady state diffusion coefficient $\bar{D}$ at 25°C and applied relative vapour pressure of solvent p/p_0. For legend see *Figure 1*

Table 1 Characteristic constants for diffusion of solvent in PHeLG membrane

Solvent	Ethylene dichloride	Benzene	Dioxane	Cyclohexane
Molar volume V(cm³/mol)	78.7	88.9	85.1	108.2
Interaction parameter, χ_1	−0.12	−0.24	0.47	0.52
$\gamma/f(0)$	6.2	5.7	3.8	3.3
D_0(cm²/sec)	2.7 x 10⁻⁶	2.2 x 10⁻⁸	3.0 x 10⁻⁸	6.0 x 10⁻⁹
$[f(0)/B_d]\ V$	2.0	2.1	2.5	2.8

diffusion coefficient D^v is expressed as follows[11]:

$$D^p = D^v \cdot (v_p)^2 \qquad (1)$$

where, v_p is the volume fraction of polymer in the system. Then, the average of D^p is written as follows:

$$\bar{D}^p = \frac{1}{C_s}\int_0^{C_s} D^p dC_s = \frac{1}{C_s}\int_0^{C_s} D^v (v_p)^2 dC_s$$

$$= \frac{1}{C_s}\int_0^{C_s} D^v (1 - v_s)^2 dC_s \qquad (2)$$

where, v_s is the volume fraction of solvent in the system, C_s is the mass of solvent per unit mass of polymer (g/g of polymer). Thus, the concentration dependent D^v is calculated by the numerical differentiation of $\bar{D}^p \cdot C_s$ *vs.* C_s relation (C_s is the surface concentration in this case) and dividing the result by $(1 - v_s)^2$. To obtain the intrinsic diffusion coefficient $\mathscr{D}^s$ which is directly related to a molecular motion in the system, next relation is used.

$$\mathscr{D}^s = D^v(1 - v_s) \qquad (3)$$

Calculated $\mathscr{D}^s$ by this method are summarized in *Figure 4* for all solvent systems. The magnitude of the $\mathscr{D}^s$ for the different solvents is ethylene dichloride > benzene ≃ dioxane > cyclohexane, which reflects again the molecular size of solvent. Furthermore, it is noticed that the concentration (v_s) dependence of $\mathscr{D}^s$ for ethylene dichloride and benzene, which are good solvents for PHeLG, are nearly expressed by straight lines in the semi-logarithmic plot. On the other hand, for dioxane and cyclohexane, whose affinity for PHeLG is relatively poor, ln $\mathscr{D}^s$ becomes saturated in the high v_s region, due to the poor plasticizing effect of the side chain region of the PHeLG.

Concentration dependence of diffusion coefficients of PHeLG – solvent systems

The diffusion coefficient which is quantitatively related to the mobility of the solvent molecule is the thermodynamic diffusion coefficient D_T[12], which is calculated by the following equation,

$$D_T(v_s) = \mathscr{D}^s(d\ln v_s/d\ln a_s) \qquad (4)$$

where, a_s is the activity of the solvent and approximated by its relative pressure. The term in parentheses of the right hand side of equation (4) is the thermodynamic correction factor, calculated using *Figure 1.*

According to the free volume theory for diffusion[13], the D_T (v_s) at a certain temperature is related to the free volume fraction $f(v_s)$ of the system of concentration v_s and to the relative critical size for the diffusant molecule B_d.

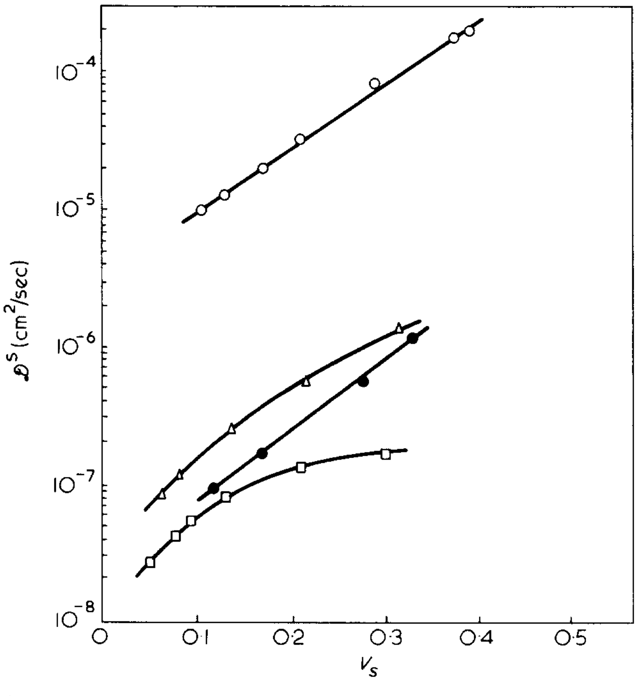

Figure 4 Concentration dependence of intrinsic diffusion coefficient $\mathscr{D}^s$. v_s is the volume fraction of solvent in the system. For legend see *Figure 1*

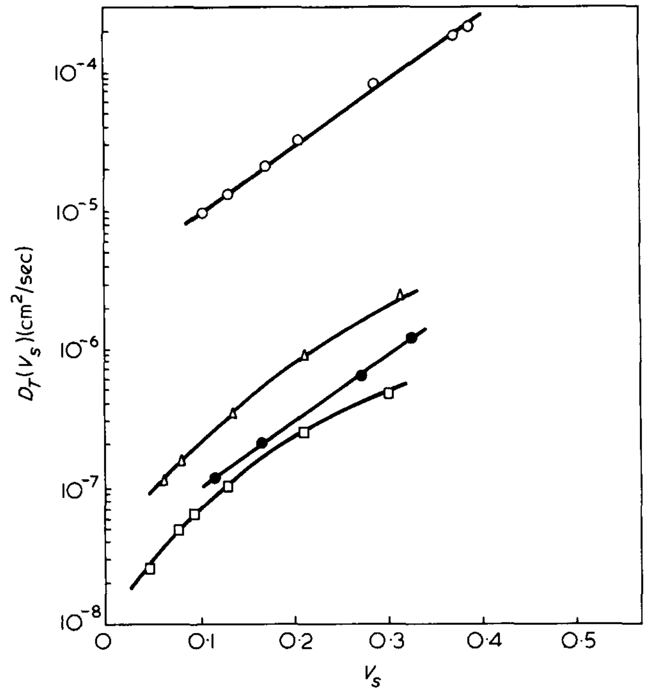

Figure 5 Relation between thermodynamic diffusion coefficient $D_T(v_s)$ and volume fraction of solvent v_s. For legend see *Figure 1*

$$D_T(v_s) = ART\exp[-B_d/f(v_s)] \qquad (5)$$

The free volume fraction $f(v_s)$ is expressed as follows assuming the additivity rule:

$$f(v_s) = f(0) + \beta v_s = f(v_{s,r}) + \beta(v_s - v_{s,r}) \qquad (6)$$

$$\beta = \gamma - f(0) \qquad (7)$$

where $f(0)$ and γ are the free volume fraction of pure polymer and pure solvent, respectively, and $v_{s,r}$ is a reference concentration. If we denote $D_T(v_{s,r})$ as the reference diffusion coefficient at $v_s = v_{s,r}$, substituting equation (6) to equation (5) and rearranging yields Equation (8).

$$\left[\ln\left\{\frac{D_T(v_s)}{D_T(v_{s,r})}\right\}\right]^{-1} = \frac{1}{B_d}\left[f(v_{s,r}) + \frac{[f(v_{s,r})]^2}{\beta}\cdot\frac{1}{(v_s - v_{s,r})}\right] \qquad (8)$$

which predicts a linear relationship between left hand term and $1/(v_s - v_{s,r})$.

The thermodynamic diffusion coefficient obtained by equation (4) and *Figure 1* is shown in *Figure 5*. As the reference concentration $v_{s,r}$, the smallest one in the series is selected. Application of Equation (8) to the concentration dependence of D_T is shown in *Figure 6*. The expected linear relationships between $[\ln\{D_T(v_s)/D_T(v_{s,r})\}]^{-1}$ and $1/(v_s - v_{s,r})$ are obtained for all solvents. Dividing the slope by the intercept, $\beta/f(v_{s,r})$ is obtained, and then using equation (6) and equation (7), $\beta/f(0)$ is obtained, from which the ratio of free volume fraction of the solvent to that of the polymer $\gamma/f(0)$ is calculated by

$$\gamma/f(0) = 1 + \beta/f(0) \qquad (9)$$

The values thus obtained are summarized in *Table 1* together with the molar volume V, interaction parameter χ_1, extrapolated diffusion coefficient D_0 and $f(0)/B_d \times V$. Since the V/B_d may be almost constant for all penetrants, $[f(0)/B_d] \times V$ corresponds to the free volume fraction of pure polymer $f(0)$. The fact that the $[f(0)/B_d] \times V$ is almost constant confirms again the adequacy of the application of equation (8).

The value of $\gamma/f(0)$ is 3.3 ~ 6.2, which is smaller than that of the usual amorphous polymers, where the free volume fraction of polymer is one order smaller than that of the solvent[13]. The present result indicates that molecular movement in the side chain region of PHeLG is similar to that of a small molecule, and this is the reason why the diffusional permeability through polypeptide membrane is so high. Furthermore, compared with the usual amorphous polymer–solvent systems which show a marked concentration dependence[13], the present systems show a relatively small concentration dependence. This is also due to the large free volume fraction of pure PHeLG which reduces the effect of added solvent.

The absolute values of $f(0)$, γ and B_d, should be treated with caution because the membrane is not uniform on the whole, as it consists of helix and side chain regions. However, the tendency among the solvents is sufficiently meaningful since the basic concept of equation (5) is adequate to any system.

Though the D_0 value should correspond with the molecular volume of the solvent, the D_0 found for ethylene dichloride is much larger than for the other solvents. This may be due to the rather linear shape of the molecule, and in fact the length of short axis is less than half of that of cyclohexane.

Diffusional permeability of oriented PHeLG membrane

In PHeLG–benzene system, a drastic decrease of permeability of diffusion coefficient is observed in the relative vapour pressure range larger than 0.8, as is shown in *Figure 7*. The fact that the membrane that had experienced the

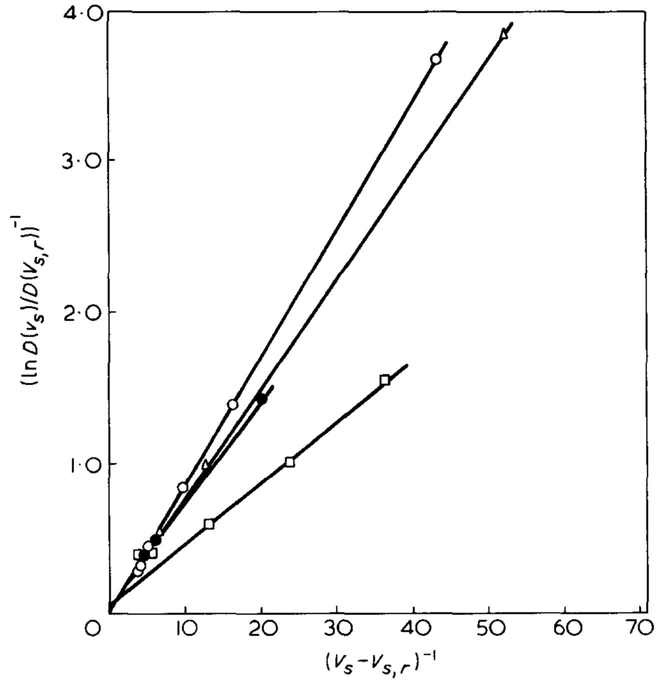

Figure 6 Application of equation (8) to the concentration dependence of D_T. For legend see *Figure 1*

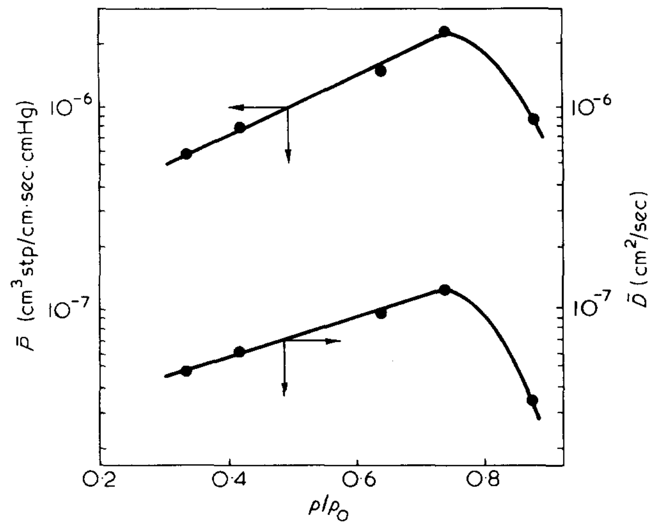

Figure 7 Relation between permeability coefficient $\bar{P}$ or diffusion coefficient $\bar{D}$ and relative vapour pressure for PHeLG – benzene system over the whole relative vapour pressure range

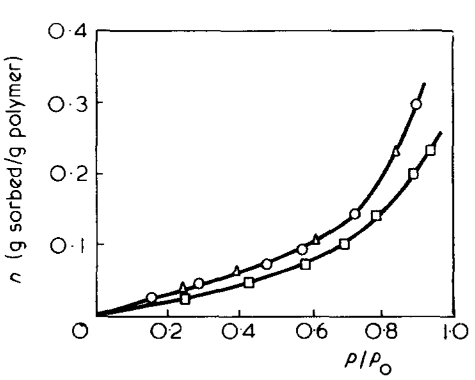

Figure 8 Sorption isotherms of oriented PHeLG – cyclohexane systems at 25°C: ○, I_e/I_m (orientation factor) = 1.0; △, I_e/I_m = 1.9; □, I_e/I_m = 4.0

permeation experiment at a high vapour pressure shows from X-ray photography a strong uni-directional orientation parallel to the membrane surface, suggests that the decrease of permeability is due to the orientation of the helix structure during the sorption and desorption cycles of the experiment.

The effect of orientation of PHeLG membrane on the permeability of solvent was examined more systematically. Cyclohexane was selected as the penetrant, because it has no influence on the membrane structure after the permeation experiment as shown by X-ray diffraction and its structure is analogous to benzene.

Oriented PHeLG was prepared by the method described in the experiment section. The membranes of orientation factor 1.7 and 1.9 were used for sorption and permeation experiments and that of 4.0 was also used for the sorption experiment. A large size membrane of such high degree of orientation for permeation experiment is difficult to prepare at present.

Sorption isotherms, permeability coefficients and diffusion coefficients are shown in *Figure 8* and *Figure 9*. Sorption behaviour is not influenced by orientation up to an orientation factor 1.9, but it is lowered to some extent at a high degree of orientation 4.0. On the other hand the permeability decreases markedly with the increase of orientation over the whole range of orientation. Thus the diffusion coefficient also shows the same tendency as that of permeability. Though the fact that the orientation affects the molecular motion rather than the sorption behaviour is quite interesting, more detailed explanation relating to the polymer morphology is not clear at present.

Critical condition for the orientation of PHeLG – benzene system

Orientation of α-helix during the permeation experiment is driven by the mechanical field, but in that case quantitative uniform regulation of orientation is difficult. Here, instead of a mechanical field, we used an electric field to induce the uniform orientation of the α-helix in concentrated solution[14].

Figure 10 shows the relation between orientation factor I_e/I_m and the concentration of solvent in PHeLG—benzene mixture. The concentration is controlled by the vapour pressure equilibriated with the PHeLG membrane as is described in the experimental section. Up to 35 wt% of benzene no

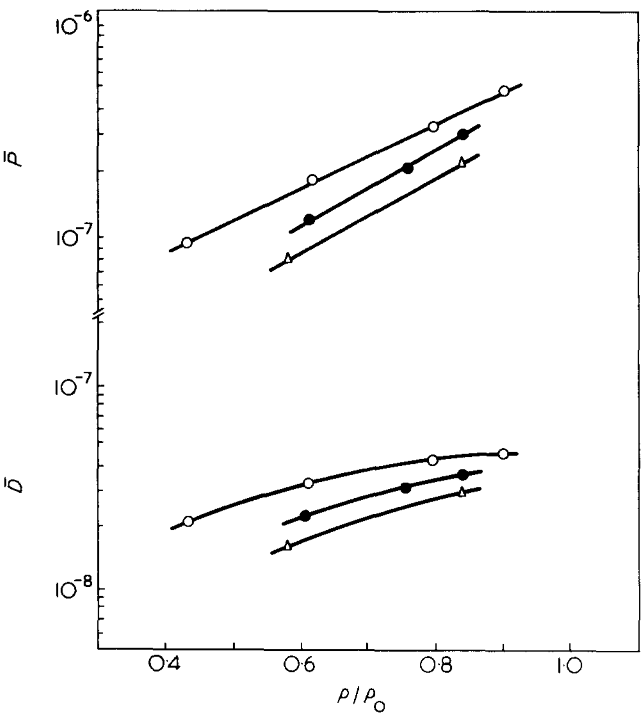

Figure 9 Relations between steady state permeability coefficient $\bar{P}$ and diffusion coefficient $\bar{D}$ and applied relative vapour pressure p/p_0 through oriented PHeLG membranes at 25°C: ○, I_e/I_m = 1.0; ●, I_e/I_m = 1.7; △, I_e/I_m = 1.9

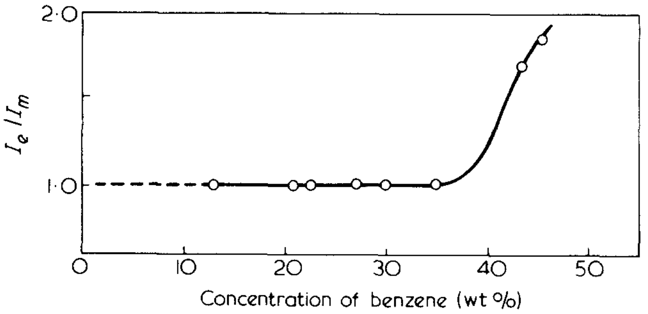

Figure 10 Relation between orientation factor I_e/I_m and concentration and PHeLG – benzene mixture oriented under electric field

orientation is observed. At over 40 wt%, orientation in the direction of electric field is observed. Thus, a benzene concentration of about 40 wt% is considered to be the critical condition for the orientation.

Referring to *Figure 1*, the concentration of about 40 wt% corresponds to the relative vapour pressure of about 0.8, and the pressure just accords with the onset of the decrease of permeability. Thus the unusual behaviour of *Figure 7* is explained adequately by the orientation of the α-helix in the membrane.

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