OPTICAL RESOLUTION OF AN AMINO ACID BY AN ENANTIOSELECTIVE ULTRAFILTRATION MEMBRANE

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An enantioselective membrane was prepared by introducing an amino acid condensate with glutaraldehyde into a polysulfone membrane matrix. The optical resolution of phenylalanine from aqueous racemate solutions was examined under the concentration and pressure gradients across the membrane respectively. The membrane was permeable with respect to D-phenylalanine in preference to L-phenylalanine, and the separation factor was 1.25 to 4.10.

The solute fluxes were analyzed on the basis of a permeation model consisting of both diffusing flow and viscous flow.

Using the solute fluxes of D- and L-amino acids, the separation factor could be successfully expressed by the solute permeability of amino acid and the volume flux.

Introduction

The optical resolution of the physiologically active L-amino acid from the racemate is essential in the production of foods, pharmaceuticals and other products and is usually accomplished by one of three methods: chromatography, crystallization and a membrane process. The membrane process is an energy-saving technique with no phase changes. Recently, Matson and Quinn[5] reported that L-amino acids from aqueous racemate solutions could be successfully separated using an impregnated-liquid membrane in combination with an enzyme-immobilized membrane. Yamaguchi *et al.*[8] applied the impregnated-liquid membrane with optically active crown ethers on a porous polymer membrane to optical resolution of amino acids. Some investigators have reported optical resolution of amino acids by a polymer membrane having cyclodextrin moieties[3] and by a poly(amino acid)-derived membrane.[4] However, these separation techniques are not suitable for the treatment of a large number of racemate solutions because the concentration gradient is the driving force and the solute flux is considerably small.

In the previous study,[6] one of the authors reported that the optical resolution of amino acids from aqueous solutions could be effectively done at elevated pressure, using an enzyme-immobilized composite membrane.

In the present work, an enantioselective polymer membrane is prepared by chemical synthesis and the optical resolution of an amino acid from aqueous racemate solutions is examined under concentration and pressure gradients across the membrane. The observed permeation flux and the separation factor are analyzed on the basis of a proposed permeation model.

1. Experimental

1.1 Materials

Polysulfone (P-1700, Union Carbide) was supplied by Daicel Chemical Industries, Ltd. D-, L- and DL-phenylalanine (Phe) were special grade, purchased from Wako Pure Chemical Industries, Ltd. Glutaraldehyde (25 wt% aqueous solution), *N*-methyl-2-

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pyrrolidone, and lithium nitrate were special grade and were used without further purification.

1.2 Preparation of the enantioselective membrane

Ten ml of glutaraldehyde, which was a 25 wt% aqueous solution, was added to an aqueous solution (1 wt%, 200 ml) of L-phenylalanine (L-Phe). The condensation reaction occurred, giving a deposit of brown precipitate. After 24 h the precipitate was filtered off, washed with distilled water, and dried in a desiccator at room temperature.

A membrane was cast from a solution of polysulfone (PS)/*N*-methyl-2-pyrrolidone (NMP)/$LiNO_3$/amino acid condensate having a weight ratio of 1/5/0.2/0.1. The solvent was evaporated in an oven at 80 °C for 120 min. The gelation was done in ice-cold water for more than 12 h. The casting conditions and the physical properties of the membrane are listed in **Table 1**. The membrane so prepared was recognized to possess the chiral recognition sites required for optical resolution of DL-Phe.

1.3 Optical resolution of DL-Phe by dialysis

A schematic diagram of the dialysis cell is shown in **Fig. 1**. Each compartment of the cell had a volume of 150 ml, and compartment 1 was connected to a reservoir of 300 ml in volume. The effective surface area of the membrane in the dialysis cell was 19.6 cm^2.

The membrane was kept in distilled water for 24 h before mounting it into the membrane holder of the dialysis cell, and was maintained in a thermostated water bath at 37 °C. Cell compartment 1 was filled with an aqueous solution of racemic phenylalanine (DL-Phe) with a concentration C_{1i} (i=D, L), of 25 $mol \cdot m^{-3}$. Cell compartment 2 was filled with distilled water. The solutions in both cell compartments were stirred well. During measurement, the concentration of aqueous amino acid solution in compartment 1 was considered to be maintained constant at its initial value owing to the transport of a very small amount of solute from compartment 1 to compartment 2.

1.4 Optical resolution of DL-Phe at elevated pressure

The permeation experiments were carried out using a batch-type cell[6] in which the solution was stirred well with a rotating impeller. The effective area of the membrane was 28.3 cm^2. The operating pressure was varied in the range of 0.05–2 MPa, and the temperature was 37°C. DL-Phe was used in aqueous solutions with concentrations of solute, C_{bi}, in the range of 1–4 $mol \cdot m^{-3}$.

The separation factor, α, of DL-amino acid is defined as follows:

$$\alpha = \frac{C_{pD}/C_{bD}}{C_{pL}/C_{bL}} \qquad (1)$$

Analysis of the concentration of D- or L-amino acid in permeate solution was performed by HPLC using an optical resolution column (Crownpak CR(+), Daicel Chemicals Industries, Ltd.).

Table 1. Casting conditions and physical properties of the membrane

solvent	NMP
swelling agent	$LiNO_3$
weight ratio of PS/NMP/$LiNO_3$/A*	1/5/0.2/0.1
solvent evaporation temperature [°C]	80
solvent evaporation period [min]	120
membrane thickness [μm]	158
volume water content [—]	0.63
pure water permeability [$m \cdot MPa^{-1} \cdot s^{-1}$]	6.92×10^{-7}

* L-Phenylalanine condensate

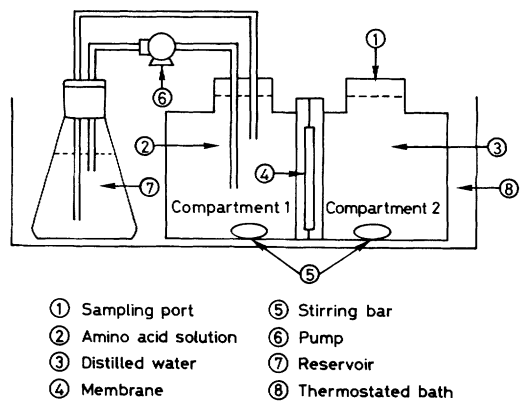

Fig. 1. Schematic diagram of dialysis cell

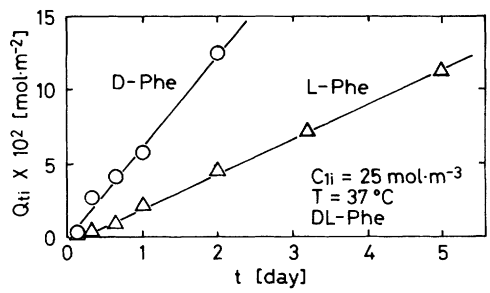

Fig. 2. Plots of Q_{ti} vs. time in dialysis

2. Results and Discussion

2.1 Determination of the diffusion and partition coefficients of D- and L-Phe by dialysis

The time course of the total amount of diffusing solute, Q_{ti}, which has passed through the membrane, is shown in **Fig. 2**. The total amount of diffusing solute increased linearly with time except for the initial period.

The solute flux across the membrane can be expressed as follows.

$$J_i = -Ds_i \frac{\partial Cm_i}{\partial x} \qquad (i=\mathrm{D, L}) \qquad (2)$$

The unsteady mass balance of the diffusing solute

within the membrane is represented by Eq. (3).

$$\frac{\partial Cm_i}{\partial t} = Ds_i \frac{\partial^2 Cm_i}{\partial x^2} \qquad (i = \mathrm{D, L}) \qquad (3)$$

The solution of Eq. (3) can be obtained by Laplace transform.[1] The partition equilibrium between the solution and the membrane for the solute is as shown in Eq. (4).

$$Cm_i = Ks_i \cdot C_i \qquad (i = \mathrm{D, L}) \qquad (4)$$

where C_i and Cm_i are the solute concentrations of component i outside and inside the membrane respectively.

If one face, $x=0$, of a membrane is kept at a constant concentration C_{1i} and the membrane is initially at zero concentration, then the total amount of diffusing solute of component i, Q_{ti}, which has passed through the membrane in time t becomes linearly dependent on process time t.[7] Q_{ti} can be expressed as Eq. (5).

$$Q_{ti} = \frac{Ps_i C_{1i}}{\delta}\left(t - \frac{\delta^2}{6Ds_i}\right) \qquad (i = \mathrm{D, L}) \qquad (5)$$

where the solute permeability, Ps_i, is the product of the diffusion coefficient, Ds_i, and the partition coefficient, Ks_i.

By matching Eq. (5) with the experimental data in Fig. 2, Ds_i and Ks_i were evaluated from the intercept and the slope of the straight lines, respectively. These values are listed in **Table 2**. The partition coefficient of D-Phe is slightly smaller than that of L-Phe. However, the diffusion coefficient of D-Phe is fairly larger than that of L-Phe.

2.2 Analysis of optical resolution mechanism by dialysis

The present membrane is constructed from two polymer matrices, *i.e.*, porous polysulfone alternated with L-phenylalanine condensate as depicted in **Fig. 3**. In the part of polysulfone portions there appears to be no permeability difference between D- and L-Phe in the membrane because of identical physical properties. In the L-Phe condensate portions the L-Phe tends to form self-associates by hydrogen-bonding of the amino group and the carbonyl group in the membrane. Dobashi *et al.*[2] reported that DL-*N*-acetylvaline-*tert*-butyl esters in solution associate themselves to form self-associates, and that the self-associates between L- and L-forms are more stable than those between L- and D-forms.

The following assumptions will be made:

1) In the polysulfone matrix, D- and L-Phe are adsorbed equally on the surface of the membrane pore.

In the amino acid condensate matrix, stereospecific affinities act between L-Phe in the aqueous solution and the L-Phe sites fixed within the membrane. The L-Phe solutes are stably associated with each other on the L-Phe sites of the surface of the membrane pore.

2) There is no interaction between D-Phe in the aqueous solution and the L-Phe sites fixed within the membrane. D-Phe is merely adsorbed on the surface of the membrane.

3) The partition equilibrium between the solute concentration, C_i, in the solution and that in the membrane, Cm_i, is according to Eq. (4).

4) As shown in Fig. 3, in the case of L-Phe a sort of chemical equilibrium may be established owing to an exchange reaction between "adsorbed" (or free) solute molecules, S_{LF}, and "associated" (or bound) solute molecules, S_{LB}, on the L-Phe sites in the amino acid condensate matrix.

$$S_{LF} \rightleftharpoons S_{LB} \qquad (6)$$

The apparent self-association constant, H, of the exchange reaction between S_{LF} and S_{LB} is defined by Eq. (7), using their respective concentrations, Cm_{LF} and Cm_{LB}.

$$H = Cm_{LB}/Cm_{LF} \qquad (7)$$

By assuming that the "associated" solute does not diffuse, the solute flux of L-Phe can be expressed as Eq. (8).

$$J_L = -Ds_L \frac{\partial Cm_{LF}}{\partial x} \qquad (8)$$

The total concentration of L-Phe, Cm_L, on the whole pore surface of the membrane is expressed as the summation of concentration of the adsorbed

Table 2. Diffusion and partition coefficients

	D-Phe	L-Phe
Ds_i [$m^2 \cdot s^{-1}$]	6.05×10^{-13}	2.09×10^{-13}
Ks_i [—]	3.49	4.06
Ps_i [$m^2 \cdot s^{-1}$]	2.11×10^{-12}	8.49×10^{-13}

$C_{1i} = 25\ mol \cdot m^{-3}$, $T = 37°C$

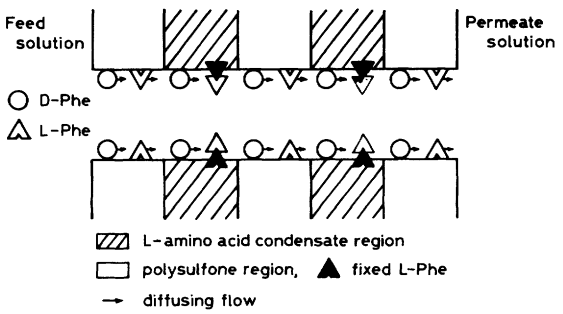

Fig. 3. Permeation model of D- and L-Phe through membrane pore under concentration gradient

L-Phe and that of the associated L-Phe.

$$Cm_L = Cm_{LF} + Cm_{LB} \tag{9}$$

From Eqs. (4), (7), (8), and (9), we have the following equation.

$$J_L = -\frac{Ps_L}{1+H}\frac{\partial C_L}{\partial x} \tag{10}$$

where Ps_L is the solute permeability of L-Phe.

Thus, the observed solute permeability of L-Phe, $Ps_L(\text{obs})$, is expressed by

$$Ps_L(\text{obs}) = \frac{Ps_L}{1+H} \tag{11}$$

On the other hand, D-Phe permeates through the amino acid condensate region in the polymer matrix with no interaction, because D-Phe does not associate with the L-Phe site fixed within the membrane. The observed solute permeability of D-Phe, $Ps_D(\text{obs})$, is considered to be nearly equal to Ps_L because the physical properties of D-Phe are the same as those of L-Phe in the polysulfone matrix. As a result, $Ps_L(\text{obs})$ is represented by introducing $Ps_D(\text{obs})$ as follows.

$$Ps_L(\text{obs}) = Ps_D(\text{obs})/(1+H) \tag{12}$$

Using the data in Table 2, H is evaluated to be 1.49 from Eq. (12). This value gives the degree of difference between the affinities of D- and L-Phe for the membrane.

2.3 Volume flux and solute flux of amino acid at elevated pressure

The effects of the operating pressure on the volume flux, J_v, and the solute flux, J_i (i=D, L), are shown in **Figs. 4** and **5** respectively.

As shown in Fig. 4, J_v was proportional to the operating pressure difference, ΔP, and the osmotic pressure nearly equals zero since the solution is dilute. From the observed data, J_v is correlated with ΔP as follows.

$$J_v = L_p \cdot \Delta P \tag{13}$$

where L_p is the pure water permeability and its value was $6.92 \times 10^{-7}\,\text{m}\cdot\text{MPa}^{-1}\cdot\text{s}^{-1}$.

As seen in Fig. 5, the behavior of J_D and of J_L against ΔP may be divided into two regions by the ranges of ΔP above and below a critical value of J_v respectively. The critical values were assigned as 1.2 MPa and 1.3 MPa for J_D and J_L respectively.

In the region of ΔP below the critical value, J_D and J_L gradually increase with increasing ΔP. In the region of ΔP above the critical value, J_D and J_L rise linearly with ΔP.

The porous membrane structure is assumed to have a distribution of membrane pore sizes. Most of the solutes permeate with the volume flux through the relatively large membrane pores at higher ΔP.

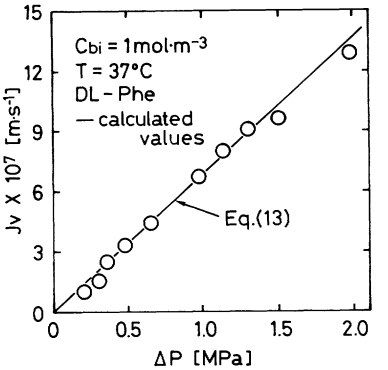

Fig. 4. Effect of operating pressure on volume flux

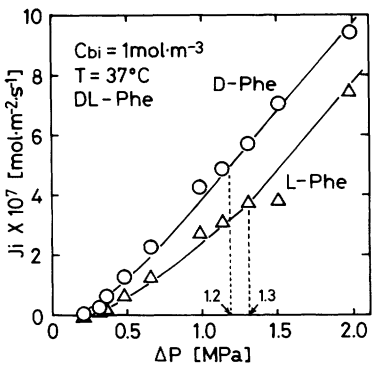

Fig. 5. Effect of operating pressure on solute flux

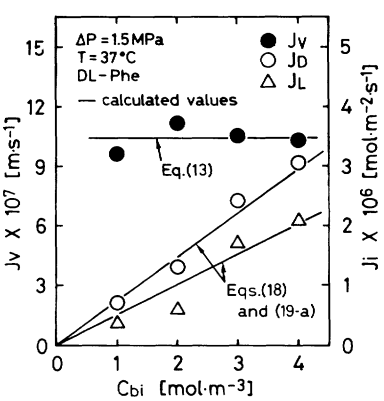

Fig. 6. Effects of solute concentration of feed solution on J_v and J_i

Figure 6 indicates the plots of J_v, J_D and J_L against the solute concentration, C_{bi}, in the feed solution at 1.5 MPa above the critical pressure. From Fig. 6, it is found that J_D and J_L are proportional to C_{bi} and that J_v is independent of C_{bi}.

To derive the relation between the solute flux and the volume flux at elevated pressure, we propose a transport model by considering the physical situation as depicted in **Fig. 7**. The model is postulated as follows:

1) The solute molecules adsorbed are mutually interactive at the surface of the membrane pore to form a multilayer.

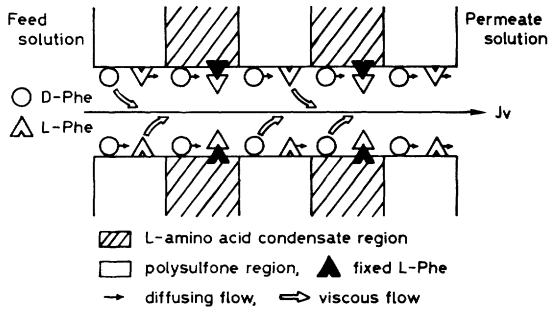

Fig. 7. Transport model of D- and L-Phe through membrane pore under pressure gradient

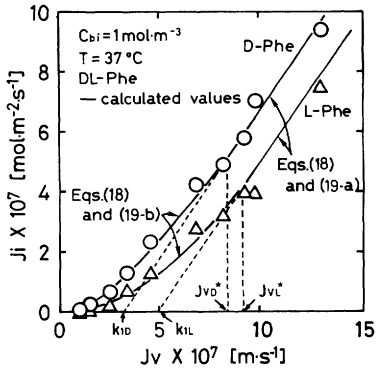

Fig. 8. Effect of volume flux on solute flux

2) At low volume flux, the solutes permeate reluctantly with the volume flux.
3) At high volume flux, the solutes in the feed solution permeate directly with the volume flux.

By converting ΔP to J_v by Eq. (13) using the data in Fig. 5, the relation between J_i and J_v is replotted in **Fig. 8** for D- and L-Phe respectively.

As seen in Fig. 8, the behavior of J_D and of J_L in the membrane against J_v can be divided into two regions, above and below the critical volume flux, J_{vi}^* (i=D, L), respectively. Here, J_{vD}^* and J_{vL}^* are evaluated corresponding to J_v at 1.2 MPa and 1.3 MPa, respectively.

From Figs. 6 and 8 it is ascertained that in the region of $J_v > J_{vi}^*$ an increase in J_i is proportional to an increase in J_v and that in C_{bi} respectively.

In the region of $J_v > J_{vi}^*$, considering the contributions of the diffusing flow and the viscous flow to J_i, the following relation between J_i and J_v is represented.

$$J_i = -Ps_i\frac{dC_i}{dx} + (J_v - k_{1i})C_{bi} \qquad (J_v > J_{vi}^*,\ i=\mathrm{D, L}) \tag{14}$$

where the first term of the right-hand side of Eq. (14) is concerned with the diffusing flow and the second term with the viscous flow.

In the region of J_v below J_{vi}^*, the solute, which weakly adsorbs on the surface of the membrane pore, reluctantly permeates with J_v. Modifying the second term of the right-hand side of Eq. (14) with respect to the change in J_v, we have the following equation.

$$J_i = -Ps_i\frac{dC_i}{dx} + \left(J_v - \frac{k_{2i}}{1+\dfrac{k_{3i}}{J_v}}\right)C_{bi} \qquad (0 < J_v < J_{vi}^*) \tag{15}$$

where the denominator of the second term of the right-hand side of Eq. (15), *i.e.*, $1+(k_{3i}/J_v)$, is a correction factor for the viscous flow.

The relation between J_i and J_v is derived as follows. In the present study, C_{bi}, C_{pi}, and J_v are constant at steady state and the following boundary conditions apply:

Table 3. Parameters evaluated from the experimental data

	D-Phe	L-Phe
k_{1i} [m·s^{-1}]	2.99×10^{-7}	4.94×10^{-7}
k_{2i} [m·s^{-1}]	4.02×10^{-7}	9.26×10^{-7}
k_{3i} [m·s^{-1}]	2.88×10^{-7}	7.94×10^{-7}
J_{vi}^* [m·s^{-1}]	8.40×10^{-7}	9.07×10^{-7}

$$\text{B. C.:}\quad \begin{aligned} C_i &= C_{bi} && \text{for}\quad x=0 \\ C_i &= C_{pi} && \text{for}\quad x=\delta \end{aligned} \tag{16}$$

Since the amino acid solution is dilute under the experimental conditions, the following relation holds.

$$J_i = C_{pi}\cdot J_v \tag{17}$$

Using Eq. (17), the integration of Eq. (15) under Eq. (16) across the membrane thickness yields Eq. (18).

$$J_i = \frac{Ps_i/\delta + (J_v - G_i)}{J_v + Ps_i/\delta}C_{bi}\cdot J_v \tag{18}$$

where

$$G_i = k_{1i} \qquad (J_v > J_{vi}^*) \tag{19-a}$$

$$G_i = k_{2i}/(1+k_{3i}/J_v) \qquad (0 < J_v < J_{vi}^*) \tag{19-b}$$

The parameters, k_{1i}, k_{2i}, k_{3i}, in Eq. (19) were determined by the least-squares method and the evaluated values are summarized in **Table 3**.

Using the parameters in Tables 2 and 3, the calculated values from Eqs. (18) and (19) were fitted well with the experimental values as shown in Fig. 8.

2.4 Separation factor in optical resolution at elevated pressure

Figure 9 shows the effect of the concentration of DL-Phe in the feed solution on the separation factor. The separation factor is independent of C_{bi} in the range of the experimental conditions. The effect of the volume flux on the separation factor is shown in **Fig. 10**.

Substituting Eq. (17) into Eq. (1), the separation factor can be rewritten as

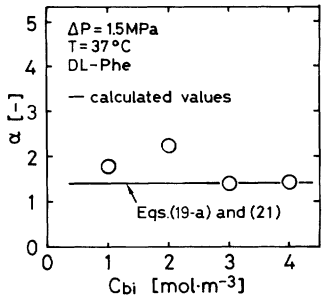

Fig. 9. Effect of solute concentration of feed solution on separation factor

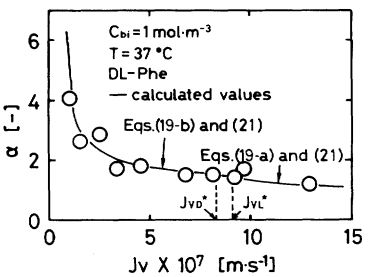

Fig. 10. Effect of volume flux on separation factor

$$\alpha = \frac{C_{bL}}{C_{bD}} \frac{J_D}{J_L} \tag{20}$$

Combining Eqs. (18) and (20) yields Eq. (21).

$$\alpha = 1 + \frac{G_L \cdot \delta(Ps_D + J_v \cdot \delta) - G_D \cdot \delta(Ps_L + J_v \cdot \delta)}{(Ps_D + J_v \cdot \delta)\{Ps_L + (1 - G_D)J_v \cdot \delta\}} \tag{21}$$

The calculated values from Eq. (21) together with Eqs. (19-a) and (19-b) are shown in Figs. 9 and 10 respectively. These were fitted well with the experimental values.

As the solute flux increases with increasing ΔP, the separation factor of DL-Phe approaches unity. When the operating pressure is lower, the optical resolution is favorably performed by the difference of affinities for the fixed L-Phe in the membrane between D- and L-amino acids.

Conclusion

An enantioselective membrane was prepared by introducing an amino acid condensate with glutaraldehyde into a polysulfone membrane matrix. The test membrane was permeable with respect to D-Phe in preference to L-Phe and was usable for optical resolution of DL-Phe.

The solute permeabilities of D- and L-Phe were measured by a dialysis experiment. The difference between them could be explained by introducing the self-association constant of L-Phe in the membrane. The volume flux and the solute fluxes increase with increasing operating pressure. The solute fluxes were analyzed by a permeation model consisting of both the diffusing flow and the viscous flow.

Based on the permeation model, the separation factor was successfully expressed in terms of the solute permeability of amino acid and the volume flux. In the range of the diffusion flow under low operating pressure the separation factor showed a high value and the membrane was applicable to the optical resolution of amino acids.

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Nomenclature

C	=	concentration of Phe on the outer membrane surface	[mol·m^{-3}]
Cm	=	concentration of Phe in the membrane	[mol·m^{-3}]
C_1	=	concentration of Phe in compartment I of the dialysis cell	[mol·m^{-3}]
Ds	=	diffusion coefficient	[m^2·s^{-1}]
H	=	apparent self-association constant	[—]
J	=	solute flux	[mol·m^{-2}·s^{-1}]
J_v	=	volume flux	[m·s^{-1}]
J_v^*	=	critical volume flux	[m·s^{-1}]
k_1	=	viscous flow parameter of Eq. (14)	[m·s^{-1}]
k_2	=	viscous flow parameter of Eq. (15)	[m·s^{-1}]
k_3	=	viscous flow parameter of Eq. (15)	[m·s^{-1}]
Ks	=	partition coefficient	[—]
L_p	=	pure water permeability	[m·MPa^{-1}·s^{-1}]
ΔP	=	pressure difference	[Pa]
Ps	=	$Ds \cdot Ks$, solute permeability	[m^2·s^{-1}]
Q_t	=	total amount of solute permeation	[mol·m^{-2}]
T	=	temperature	[°C]
t	=	time	[s]
x	=	coordinate in the membrane	[m]
α	=	separation factor	[—]
δ	=	membrane thickness	[m]

〈Subscripts〉

B	=	associated solute
b	=	feed solution
D	=	D-amino acid
F	=	adsorbed solute
i	=	solute of the i component
p	=	permeate solution
L	=	L-amino acid

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