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A Quantitative Structure Activity Relationship Study on Permeation of Amino Acids in Enantioselective Membranes

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ABSTRACT

The permeation of seven isomers of amino acids (AA) namely Tryptophan (Trp), Phenylalanine (Phe), Tyrosine (Tyr), Phenylglycine (Phegly), Methionine (Meth), Threonine (Thr)and Serine (Ser) was studied using an enantioselective membrane prepared from polysulphone containing L-tryptophan-glutaraldehyde condensation product in the polymer matrix. The experiments were conducted in dialysis mode under optimized condition of the aqueous phase pH and salt concentration in a two compartment membrane cell. The membrane was found to exhibit selectivity towards all the D-isomers of amino acids studied in this work. In order to deduce implication on the design of suitable membrane system for specific application, a quantitative structure activity relationship analysis has been made using hydrophobicity as the molecular descriptor. In this analysis, the solute fluxes were analysed on the basis of a permeation model consisting of diffusing flow alone. Finally, the model parameters of the diffusive flux of both isomers were correlated with hydrophobicity taken as the molecular property.

Keywords : Amino acids, enantioselective membrane, glutaraldehyde, dialysis, diffusive flux, hydrophobicity

1.0 INTRODUCTION

Amino acids are produced in large amounts through chemical synthesis and/or by microbial fermentation of cane or beet molasses with generally selected bacterial strain [1]. These are obtained in aqueous solution and have to be separated from excess reagents or substrates and nutrients in case of fermentation, from impurities introduced through feed and from by products. For their recovery, separation and purification from the fermentation broth a series of separation steps like filtration, crystallization and ion exchange are used. Final purification and recovery are achieved by chromatography techniques and fractional crystallization. However, there is an increasing trend of

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using single enantiomer of amino acids and other compounds in food and pharmaceutical industries, instead of racemates which actually are composed of equimolar amounts of enantiomers. Conventionally, large scale production of optical isomers is based on chromatographic processes with chiral stationary phases.

Chromatography, being time consuming and cost effective, there is a global drive towards study on membrane transport processes with the ultimate aim of designing suitable membrane system for biotechnological applications. In general, two types of membranes have been studied for transport of amino acids, i.e. liquid membrane which mostly utilises the principle of reactive extraction with a chiral selector often providing facilitated transport and the other is the solid membranes made mostly of polymeric materials.

Macrocyclic ionophores, particularly chiral crown ethers have opened up the promising field of enantioselective amino acid transport in liquid membrane system. Several amino acids were resolved in a polymer (microporous polypropylene Celgard 2400) supported liquid membrane using chiral crown ether, 2,3,4,5-bis[1,2-(3-phenylnaphthol)]-1,6,9,12,15,18-hexeoxacycloeicosa 2,4-diene [2] in which uphill transport data are available for single isomer as well as racemic mixtures.

From the point of view of stability of liquid membrane, amino acid resolution by selective transport in solid membrane seems to be a better proposition. Selective permeation of L-isomers with respect to D-isomers of tryptophan and tyrosine was attained by membranes consisting of n-acylated (benzoyl acetanoyl lauroyl) chitosan carrying hydrophobic groups [3]. Chitin-Chitosan membranes also allowed separations of amino acids as well as polypeptides [4]. Poly (L-glutamates) having amphiphilic side chains was designed as membrane materials for optical resolution of racemic mixture of tryptophan [5].

Another case of enantioselective polymeric membrane [6] exhibiting flux hydrophobicity relationship is poly(m-phenylene isopthalimide) ultrafiltration membrane coated with poly(L-glutamates) with ampiphilic side chains of (n-nonyphenoxy) oligo(oxyethylene). Molecularly imprinted membrane bearing tetrapeptide derivative i.e. H-Asp(Oc Hex)- Iie-Asp(Oc Hex)-Glu(O Bzl)CH_2 was shown to exhibit excellent enantioselectivity for amino acids through a recognition behaviour specific to type of the membrane [7].

The β-cyclodextrin polymeric membrane based on poly (vinyl alcohol) for enantioselective permeation of DL-tryptophan was described by Ishihara *et al.* [8]. The maximum of enantiomeric excess for DL-tryptophan was 25.4% with permeation rate of D-tryptophan up to 1.48×10^{-5} gm m^{-2} h^{-1}.

The separation of racemic tryptophan (Trp) analogs had been performed by two ways: ultrafiltration (UF) in a solution system using bovine serum albumin (BSA) as a free chiralselector and dialysis using BSA grafted Nylon membrane [9]. The chiral selector was able to bind the L-Trp with a high stereo specificity. However, both D- and L- amino acid bind competitively on the same protonated protein (BSA) site. Enatioselective transportation of amino acids was performed under concentration gradient through BSA grafted Nylon membrane but enantioselective excess could not be retained after 2 hours.

It is apparent that most of the studies pertains to single isomer or zwitterions in a specific membrane system [10]. So we have made an attempt to study primarily with the objective of further elucidating the enantioselectivity of various amino acids in a single enantioselective membrane and deduce correlation with the chemical structure of amino acids. The present study on amino acid enantioselective membrane relies on a simple, less expensive membrane material based on glutaraldehyde/L-tryptophan condensate physically embedded membrane [11].

2.0 EXPERIMENTAL STUDY

2.1 Materials and Methods

Polysulfone (average molecular weight 30,000) was supplied by Aldrich Chemical Company, USA. DL-tryptophan, phenylalanine, tyrosine, phenylglycine, methionine, threonine and serine were special grade purchased from Sisco Research Laboratory Pvt. Ltd (SRL), India. Glutaraldehyde (25% aqueous solution) and N-methyl pyrrolidine (NMP) supplied by Kemphasol and SRL respectively were used as received without further purification.

The condensation product of L-amino acid/glutaraldehyde were prepared by the method reported by Masawaki *et al*. [11]. 10 ml of 25% aqueous solution of glutaraldehyde was added slowly with constant stirring to 1 weight% 200 ml aqueous solution of L-tryptophan. The reaction mixture was kept overnight for completion of product formation. The product was filtered off and washed several times with distilled water until complete removal of glutaraldehyde. Then, the product was allowed to dry in room temperature. Finally, it was kept in a desicator for 48 hours to remove the moisture. The final dried product was characterized by IR.

The membrane were cast from a solution consisted of polysulfone (PS), amino acid condensates, an organic additive (glycerol) in N-methyl-2-pyrrolidine (NMP) and a swelling gel $LiNO_3$. The weight ratio of the components were 1/.5/.2/5/.2 respectively. All the components were stirred at 45°C until a homogeneous solution was achieved. Film was cast on a glass plate with a Doctor's knife while the solution mixture was at that temperature by the common phase inversion process. The solvent was evaporated in an oven at 80°C for 2 hours. Gelation was done in ice cold water for 24 hours. The casted membrane was characterized for pore size and surface morphology using modified bubble point method [12] and scanning electron micrograph.

Pure water permeation was measured with the membrane using the same cell assembly mentioned in Section 2.2 at 200 Kpa.

2.2 Estimation of Permeation Parameters

2.2.1 Permeation Experiment

The permeation experiments were carried out in a Teflon cell as shown in Figure 1. The volume of the two compartments of the cell was 120 ml. One of the compartments was connected to a reservoir of 500 ml in volume. The effective area of the membrane was 7.09 cm^2.

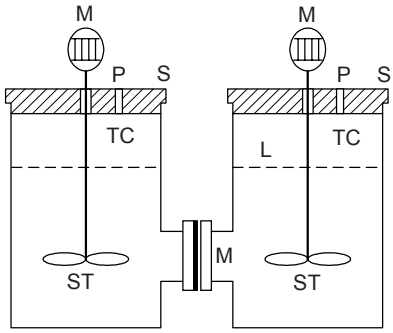

Figure 1 Schematic diagram of the membrane cell. TC: Teflon cell, M: Membrane, ST: Stirrer, L: Liquid level, M: Motor, P: Sample port, S: Stopper

Before mounting the membrane into the membrane holder of the cell, it was kept immersed in distilled water for about 24 hours. The operating temperature was 28°C. To carry out the dialysis experiment, the left compartment of the cell was filled with an aqueous solution of amino acid and 0.02 wt% of sodium azide was added onto the solution as a fungicide [13]. The second compartment was filled with distilled water. The solutions in both compartments were continously stirred using a magnetic stirrer and circulated with perislatic pumps. The concentration of the amino acid solution on the compartment was assumed to be constant at its initial value as only a very small amount of solute was transported to the other compartment.

Sample solutions were collected from the permeate side at a regular time interval and quantitative estimation of amino acid isomer in the receiving phase was performed by HPLC (WATERO 510) equipped with a UV detector (254 nm) by using a CHIRALPAK WH column (250 mm × 4.6 mm id) (Daicel Chemical Ind. Ltd.).

2.2.2 Permeation Model

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

$$J_i = -Ds_i \frac{\delta Cm_i}{\delta x} \quad (i = D, L) \tag{1}$$

The unsteady mass balance of the diffusing solute within the membrane is represented by:

$$\frac{\delta Cm_i}{\delta t} = Ds_i \frac{\delta^2 Cm_i}{\delta x^2} \quad (i = D, L) \tag{2}$$

The partition equilibrium between the solution and the membrane for the solute is also as follows:

$$Cm_i = Ks_i.C_i \quad (i = D, L) \tag{3}$$

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

When one side of the membrane is kept at a constant concentration C_{1i} and the membrane is initially at zero concentration of solute component, then the total amount of diffusing solute of component i is Q_{ti} at time t_i, which has passed through the membrane in time t becomes linearly dependent on the operating time t. Q_{ti} can be expressed as:

$$Q_{ti} = \frac{Ps_i C_{1i}}{\delta}\left(t - \frac{\delta^2}{6Ds_i}\right) \quad (i = D, L) \tag{4}$$

with

$$Ps_i = Ds_i \times Ks_i \tag{5}$$

Using Equations (4) and (5), the Q_{ti} vs t plot of experimental data Ps_i and Ds_i were evaluated from the slope and the intercept of the straight lines respectively and the Ks_i value can be calculated by Equation (5).

Now in the case of L-isomer, a chemical equilibria may be established by an exchange reaction between "adsorbed" or free solute molecule S_{LF} and "associated" or bound solute molecule on the L-Trp site in the condensate matrix.

$$S_{LF} \rightleftharpoons S_{LB} \tag{6}$$

The apparent self association constant, H of the exchange reaction between S_{LF} and S_{LB} is defined by equation using their respective concentrations, C_{mLF} and C_{mLB}.

$$H = C_{mLB} / C_{mLF} \tag{7}$$

By assuming that the self associated solute does not diffuse, the solute flux of L-isomer can be expressed as:

$$J_L = -D_{SL} \frac{\delta C_{mLF}}{\delta x} \tag{8}$$

The total concentration of L-isomer C_{mL} on the whole pore surface of the membrane is expressed as:

$$C_{mL} = C_{mLF} + C_{mLB} \tag{9}$$

Finally, the following flux equation may be derived from Equations (3), (7), (8) and (9):

$$J_L = \frac{P_{SL}}{(1+H)} \frac{\delta C_L}{\delta x} \tag{10}$$

where, P_{SL} is the solute permeability of L-Trp and other L-AA. The observed solute permeability is expressed as:

$$P_{SL(obs)} = \frac{P_{SL}}{(1+H)} \tag{11}$$

The D-isomer permeates without association. The observed flux of D-isomer $P_{SD(obs)}$ is considered to be nearly equal to P_{SL} because of identical physical properties. Then $P_{SL\ (obs)}$ may be represented by introducing $P_{SD(obs)}$ as:

$$P_{SL(obs)} = P_{SD(obs)} / (1+H) \tag{12}$$

3.0 RESULTS AND DISCUSSION

3.1 Characterisation of the Condensation Product

Typical IR spectra of pure L-tryptophan and L-tryptophan/glutaraldehyde condensation product are shown in Figures 2 and 3 respectively. In IR spectra of the condensation product the band at 3039.68 cm^{-1}, which is attributed to the $^{+}NH_3$ stretching vibrational mode [14] seems to disappear. A new band appears at 3404.53 cm^{-1} attributes to the $-^{+}$N-H stretching vibration [14] which implies that condensation occurred in between the amine group of the acid and carbonyl group of glutaraldehyde.

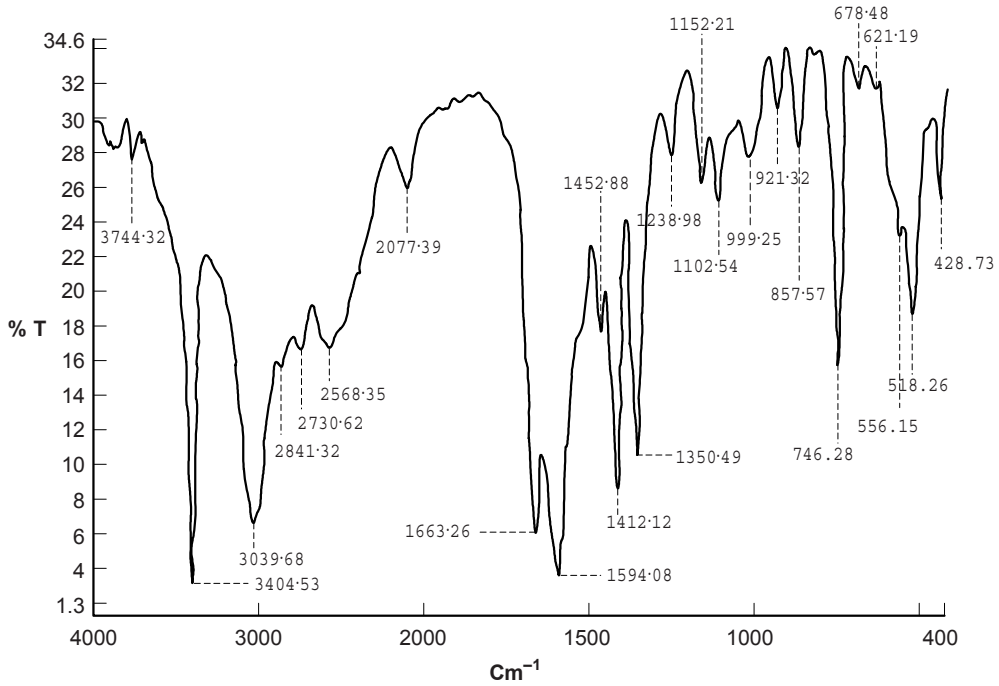

Figure 2 IR spectra of pure L-tryptophan

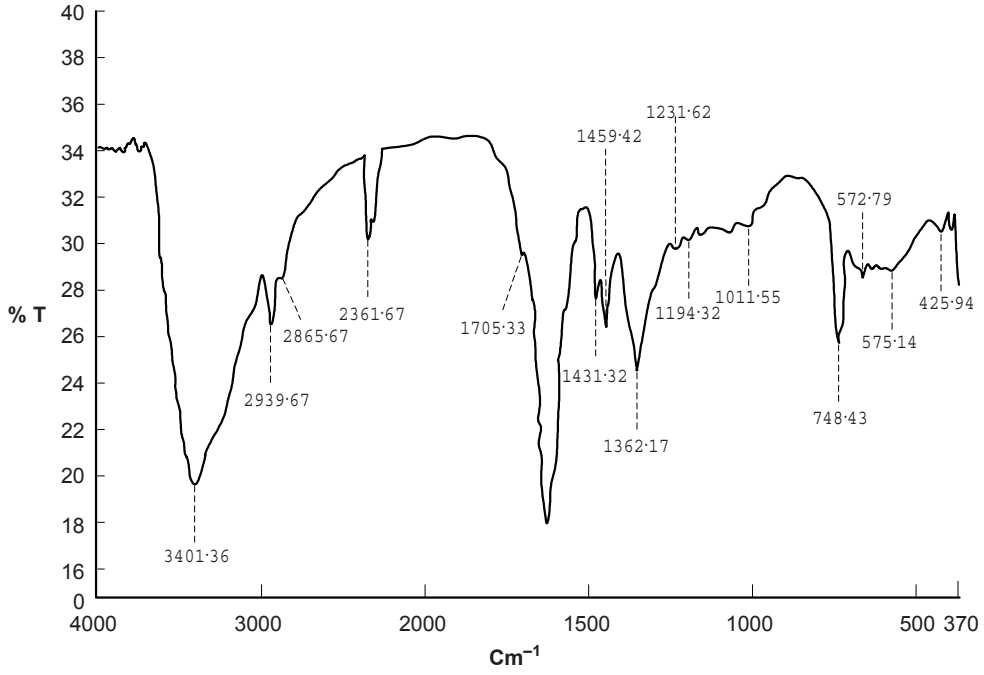

Figure 3 IR spectra of pure L-tryptophan/Glutaraldehyde condensation product

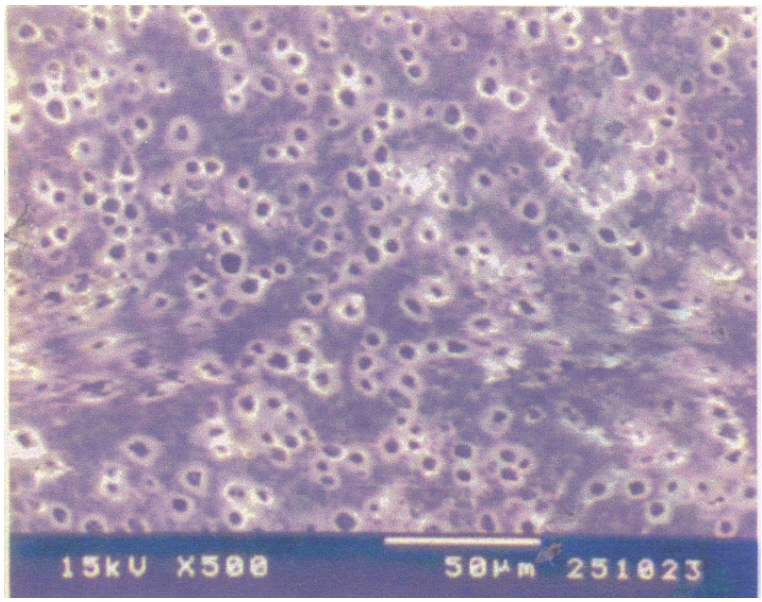

Figure 4 Photomicrograph of polysulfone membrane embedded with L-tryptophan [Additive: ethylene glycol monomethyl Methacrylate (center view)]

3.2 Characterisation of the Membrane

Membrane is characterized by membrane surface morphology, pore size and flux of pure water permeation. In this work, we report studies of the membrane surface using scanning electron micrography (SEM). The scanning electron micrograph shown in Figure 4 reveals that there is apparently no visible pore on the membrane surface. The physical properties of the membranes are shown in Table 1. The film measured approximately 140 mm and it is taken as the membrane thickness. The pure water permeability is 4.73×10^{-7} $lm^{-2}h^{-1}$ and the largest pore diameter determined by modified bubble point method [12], which is originally developed by Capanelli *et al*. [15]. In this method, an immiscible pair, consisting of a test liquid to be forced through the membrane, and a wetting liquid to fill the membrane pores, the test liquid will pass through the pores with radii larger than that given by Canton equation at the corresponding pressure

$$r = 2\delta \cos \theta / \Delta P \tag{13}$$

Table 1 Physical properties of the membrane prepared from various amino acid condensates

	Membrane thickness(mm)	Pore pure diameter (nm)	Water permeability (mM $Pa^{-1}s^{-1}$)
L-phenylalanine	150	5	7.32×10^{-7}
L-phenylglycine	156	6	7.64×10^{-7}
L-tyrosine	135	4	5.25×10^{-7}
L-tryptophan	140	3	4.73×10^{-7}

where ΔP is the pressure drop across the membrane, r is the pore radius, δ is the interfacial tension of liquid pair and θ is the contact angle at the membrane surface. Pore smaller than Cantars size will remain closed by the interfacial tension. The flux is governed by the Hagen-Poiseullis equation (however, based on certain assumption):

$$JiA = \sum_{I=1}^{I} \pi \eta_k r_1 \Delta P_1 / 8 I_n \tag{14}$$

where A is the area of the tested membrane, I is the equivalent length of the pore, η is the dynamic viscocity of the test liquid, r_k is the radius of the k^{th} pore ($r_k = r_{\min}$ when $k = 1$) and η_k is the numbers of pores having radius r_k. The pore size distribution was calculated from pressure and flux data. The immiscible liquid pair used was isobutanol, methanol and water (15:7:25 v/v with $\delta = 3.5 \times 10^{-4}$ Pa.m). In our work, the mean pore size is only determined from a plot of J vs ΔP (slope of the plot using Equation (14)) and it was found that the largest pore diameter determined by this method was 3 nm, it implies that the membrane is suitable for dialysis mode of experiment.

3.3 Transport Mechanism

Membrane is constructed of porous polysulfone (PS) matrix alternated with L-tryptophan condensate as depicted in Figure 5 which also depicts the transport mechanism. In the PS part, no difference in permeation of the D and L amino acid exists because of identical pore dimensions. In the L-AA condensate part, the L-isomer forms self-associates by hydrogen bonding [11] of the amino groups and carbonyl group of the membrane. It is postulated that the self-associates between L-L forms of the condensate and amino groups are more stable than those between L-D form [11], accordingly the D-isomer permeates through the membrane.

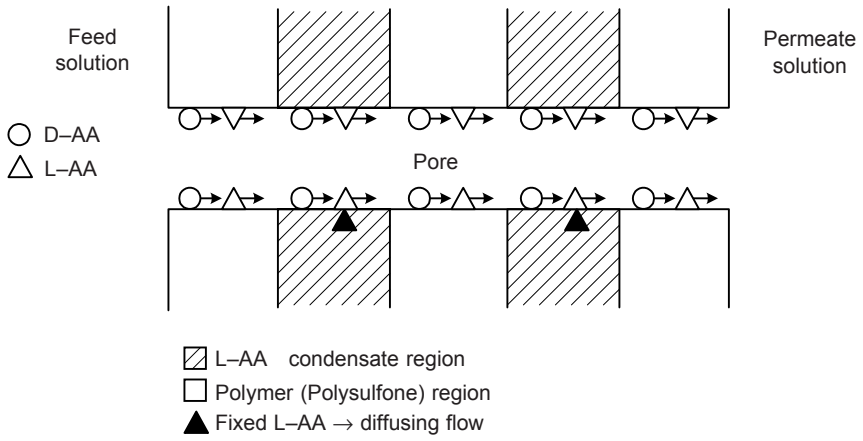

Figure 5 Permeation model of D- & L-amino acid through membrane pore under the mode of dialysis

3.4 Permeation Rate and Enantioselectivity

The concentration of amino acid isomer in the permeat solution was determined from typical HPLC chromatogram (Figures 6 and 7). From the measured concentration of permeates in dialysis

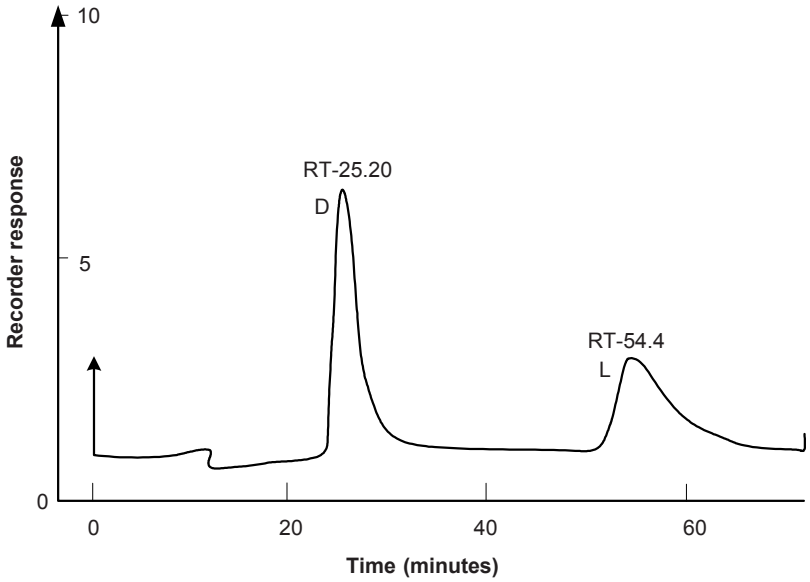

Figure 6 Typical HPLC chromatogram of phenylalanine (feed solution)

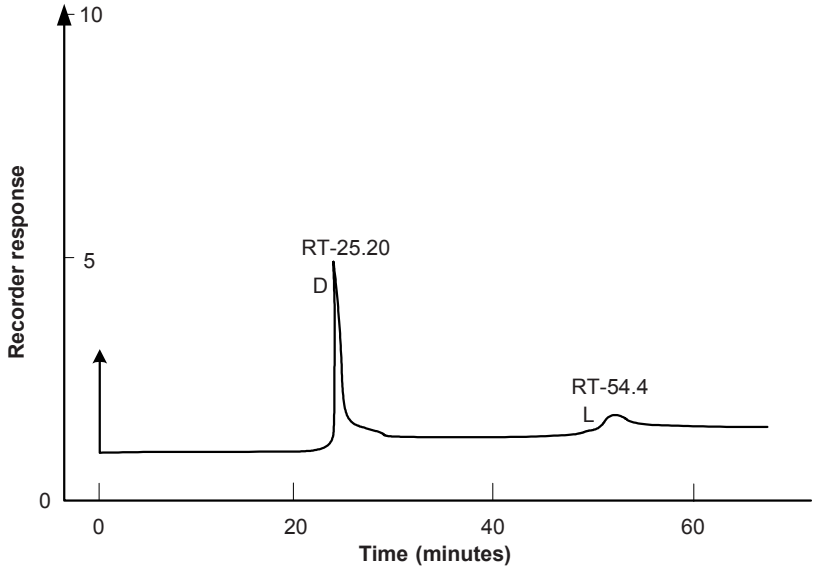

Figure 7 Typical HPLC chromatogram of phenylalanine (permeate solution)

experiments the total amount of diffusing solutes (Q) of D and L isomers of tryptophan, phenylalanine, tyrosine, phenylglycine, methionine, threonine and serine had been calculated and the results were presented graphically in Figures 8 and 9. The L-isomers were found to be less permeated apparently because of self association behaviour according to the postulated hypothesis [11]. A very low permeation of L-isomer of tryptophan can be explained because of higher specific interaction between the embedded amino acid and the same permeated amino acid. By regression analysis of the Q_{ti}

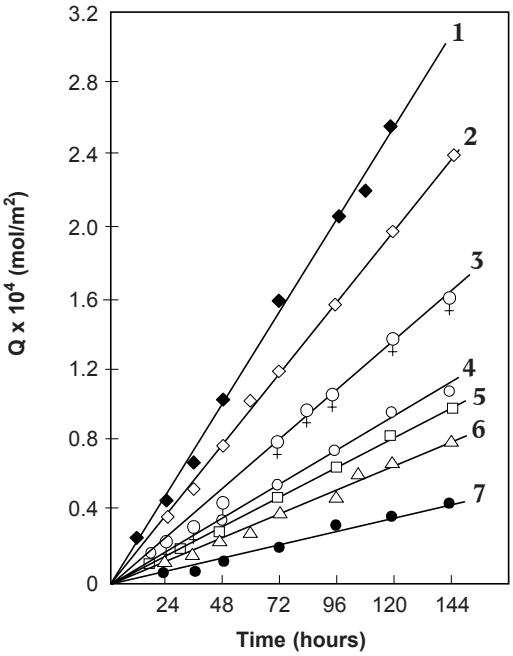

Figure 8 Plot of total amount of diffusing solute vs time of L-amino acids (L-tryptophan embedded membrane). 1: L-serine, 2: L-threonine, 3: L-methionine, 4:L-phenylglycine, 5: L-tyrosine, 6: L-phenylalanine, L-tryptophan

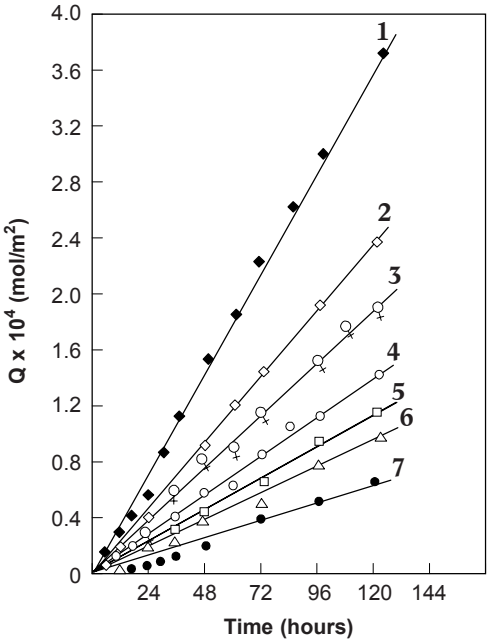

Figure 9 Plot of total amount of diffusing solute vs time of D-amino acids (L-tryptophan embedded membrane). 1: D-serine, 2: D-threonine, 3: D-methionine, 4:D-phenylglycine, 5: D-tyrosine, 6: D-phenylalanine, D-tryptophan

Table 2 Diffusion coefficient (D_{si}), partition coefficient (K_{si}) and permeation coefficient (P_{si}) of amino acids in L-tryptophan embedded membrane: C_{1i} = 10 mM, T = 28°C

Amino acids	D_{si} (m^2/s)	K_{si} (-)	P_{si} (m^2/s)
L-Ser	0.23×10^{-12}	4.87	1.12×10^{-12}
D-Ser	1.34×10^{-12}	1.6	2.20×10^{-12}
L-Thr	0.12×10^{-12}	7.8	0.94×10^{-12}
D-Thr	1.00×10^{-12}	1.9	1.90×10^{-12}
L-Meth	0.08×10^{-12}	9.0	0.72×10^{-12}
D-Meth	0.54×10^{-12}	2.8	1.50×10^{-12}
L-Pgly	0.07×10^{-12}	8.0	0.56×10^{-12}
D-Pgly	0.32×10^{-12}	3.7	1.20×10^{-12}
L-Tyr	0.06×10^{-12}	8.1	0.48×10^{-12}
D-Tyr	0.27×10^{-12}	4.0	1.08×10^{-12}
L-Phe	0.05×10^{-12}	8.4	0.42×10^{-12}
D-Phe	0.20×10^{-12}	5.0	1.00×10^{-12}
L-Trp	0.03×10^{-12}	5.3	0.16×10^{-12}
D-Trp	0.10×10^{-12}	1.2	0.52×10^{-12}

Table 3 Diffusion coefficient (D_{si}), partition coefficient (K_{si}) and permeation coefficient (P_{si}) of amino acids in L-tyrosine embedded membrane: C_{1i} = 10 mM, T = 28°C

Amino acids	D_{si} (m^2/s)	K_{si} (-)	P_{si} (m^2/s)
L-Ser	0.37×10^{-12}	3.68	1.36×10^{-12}
D-Ser	1.53×10^{-12}	1.66	2.54×10^{-12}
L-Thr	0.28×10^{-12}	3.74	1.05×10^{-12}
D-Thr	1.42×10^{-12}	1.69	2.40×10^{-12}
L-Meth	0.25×10^{-12}	3.8	0.94×10^{-12}
D-Meth	1.12×10^{-12}	1.7	1.90×10^{-12}
L-Pgly	0.19×10^{-12}	3.84	0.84×10^{-12}
D-Pgly	1.06×10^{-12}	1.73	1.84×10^{-12}
L-Tyr	0.14×10^{-12}	3.88	0.54×10^{-12}
D-Tyr	0.71×10^{-12}	1.8	1.27×10^{-12}
L-Phe	0.11×10^{-12}	4.05	0.45×10^{-12}
D-Phe	0.49×10^{-12}	1.96	0.96×10^{-12}
L-Trp	0.08×10^{-12}	4.5	0.33×10^{-12}
D-Trp	0.34×10^{-12}	2.2	0.74×10^{-12}

versus time curves, the intercept and slope of the lines were calculated, and hence diffusion coefficient (D_{si}), permeation coefficient (P_{si}) and partition coefficient (K_{si}) for all the amino acids had been calculated using Equations (4) and (5). The values are listed in Tables 2-5. The effect of solute concentration is shown in Figure 10 from which it is apparent that an increase in solute concentration increases the permeation rate in conformity with the theory of diffusion.

The enantioselectivity is defined as $\alpha = (C_{pD}/C_{fD})/(C_{pL}/C_{fL})$, where C_{pD} and C_{pL} are the concentrations of D and L isomers respectively in the permeate side and C_{fD} and C_{fL} are the

Table 4 Diffusion coefficient (D_{si}), partition coefficient (K_{si}) and permeation coefficient (P_{si}) of amino acids in L-phenylalanine embedded membrane: C_{1i} = 10 mM, T = 28°C

Amino acids	D_{si} (m^2/s)	K_{si} (-)	P_{si} (m^2/s)
L-Ser	0.76×10^{-12}	2.9	2.2×10^{-12}
D-Ser	2.03×10^{-12}	1.41	2.86×10^{-12}
L-Thr	0.56×10^{-12}	3.3	1.84×10^{-12}
D-Thr	1.94×10^{-12}	1.46	2.83×10^{-12}
L-Meth	0.49×10^{-12}	3.55	1.73×10^{-12}
D-Meth	1.27×10^{-12}	1.84	2.34×10^{-12}
L-Pgly	0.37×10^{-12}	4.2	1.54×10^{-12}
D-Pgly	1.93×10^{-12}	1.99	1.85×10^{-12}
L-Tyr	0.33×10^{-12}	4.47	1.48×10^{-12}
D-Tyr	0.58×10^{-12}	2.4	1.38×10^{-12}
L-Phe	0.21×10^{-12}	4.57	0.97×10^{-12}
D-Phe	0.41×10^{-12}	2.94	0.21×10^{-12}
L-Trp	0.13×10^{-12}	5.87	0.74×10^{-12}
D-Trp	0.28×10^{-12}	3.3	0.93×10^{-12}

Table 5 Diffusion coefficient (D_{si}), partition coefficient (K_{si}) and permeation coefficient (P_{si}) of amino acids in L-phenylglycine embedded membrane:C_{1i} = 10 mM, T = 28°C

Amino acids	D_{si} (m^2/s)	K_{si} (-)	P_{si} (m^2/s)
L-Ser	1.3×10^{-12}	2.2	2.86×10^{-12}
D-Ser	2.4×10^{-12}	1.78	4.3×10^{-12}
L-Thr	1.17×10^{-12}	2.4	2.81×10^{-12}
D-Thr	2.22×10^{-12}	1.8	4.0×10^{-12}
L-Meth	1.1×10^{-12}	2.49	2.74×10^{-12}
D-Meth	1.82×10^{-12}	1.96	3.57×10^{-12}
L-Pgly	0.7×10^{-12}	3.03	2.1×10^{-12}
D-Pgly	1.55×10^{-12}	2.2	3.4×10^{-12}
L-Tyr	0.36×10^{-12}	3.25	1.17×10^{-12}
D-Tyr	1.16×10^{-12}	2.36	2.73×10^{-12}
L-Phe	0.21×10^{-12}	4.04	0.84×10^{-12}
D-Phe	1.05×10^{-12}	2.33	2.45×10^{-12}
L-Trp	0.12×10^{-12}	4.12	0.5×10^{-12}
D-Trp	0.64×10^{-12}	2.86	1.82×10^{-12}

concentration of the D and L isomers respectively in the feed side. Since C_{fD} and C_{fL} are considered to remain constant, therefore, $\alpha = (C_{pD}/C_{pL})$. The values of enantioselectivity are listed in Table 6.

Table 6 Enantioselectivity (α) of various amino acids in the studied membranes

Amino acid	L-Trp embedded membrane	L-Tyr embedded membrane	L-Pgly embedded membrane	L-Phe embedded membrane
Tryptophan	1.89	1.33	1.93	1.49
Tyrosine	1.18	1.59	1.86	1.47
Phenylalanine	1.52	1.83	2.13	1.55
Phenylglycine	1.52	1.56	1.76	1.36
Methionine	1.49	1.30	1.69	1.27
Threonine	1.21	1.14	1.62	1.11
Serine	1.54	1.34	1.21	1.23

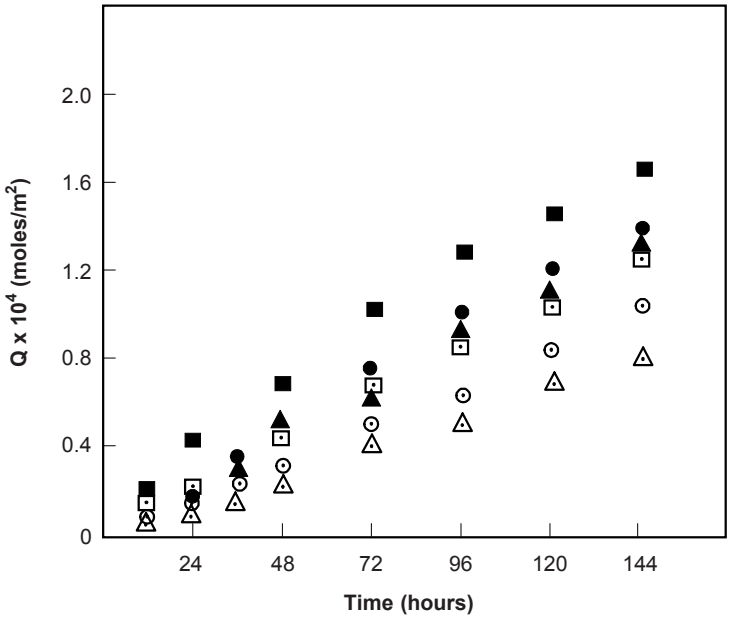

Figure 10 Plot of total amount of diffusing solute vs time of Phenylalanine isomers (L-tryptophan embedded membrane) at different solute concentration
Open symbol: L-isomer, closed symbol: D-isomer. ΔΔ 10 mM, OO 25 mM, □□50 mM

3.5 Effect of Molecular Structure

The effect of molecular structure on permeation behaviour has been analysed from a correlating approach using hydrophobicity as the molecular property and diffusion (D_{si}), partition (K_{si}) and permeation (P_{si}) co-efficients, from permeation experiment. From Figures 8 and 9 it appears that a membrane having L-tryptophan embedded in the matrix is capable of discriminating other amino acid isomers but permeation of other amino acids occurs at different rates rendering effective separation. Since solid membrane transport phenomena is described by diffusion, partition and permeation co-efficient, so it has been tried to correlate these coefficients with hydrophobicity. The

hydrophobicity value of all the amino acids except phenylglycine has been taken from literature [14]. The measurement of hydrophobicity of phenylglycine molecule was made by using a method suggested by Nozaki and Tanford [2].

This method relies on the measurement of solute solubility in ethanol that provides a semiquantitative estimation of interaction inside a native molecule for hydrophobic moiety relative to the same when exposed in water. Hydrophobicity was calculated using the simplified formula:

$$F = RT \ln[N_{iw}/N_i] \tag{15}$$

where F, R and T are the hydrophobicity scale, universal gas constant and absolute temperature respectively (in appropriate units). N_i and N_{iw} are the solubilities in terms of mole fraction in ethanol and water respectively. The effect of hydrophobicity on diffusion, partition and permeation co-efficients are shown in Figures 11 through 13 which rather clearly indicate strong influence of chemical structure and side chain hydrophobicity of the concerned molecule.

From the plot, the following correlation could be deduced:

$$P_{si} = -2.45 \times 10^{-15} H_p + 1.04 \times 10^{-11} \text{ (L-isomer)} \tag{16}$$

$$P_{si} = -4.2 \times 10^{-15} H_p + 2.06 \times 10^{-11} \text{ (D-isomer)} \tag{17}$$

$$\text{Log}D_{si} = -2.34 \times 10^{-4} H_p - 11.73 \text{ (L-isomer)} \tag{18}$$

$$\text{Log}D_{si} = -3.31 \times 10^{-4} H_p - 10.848 \text{ (D-isomer)} \tag{19}$$

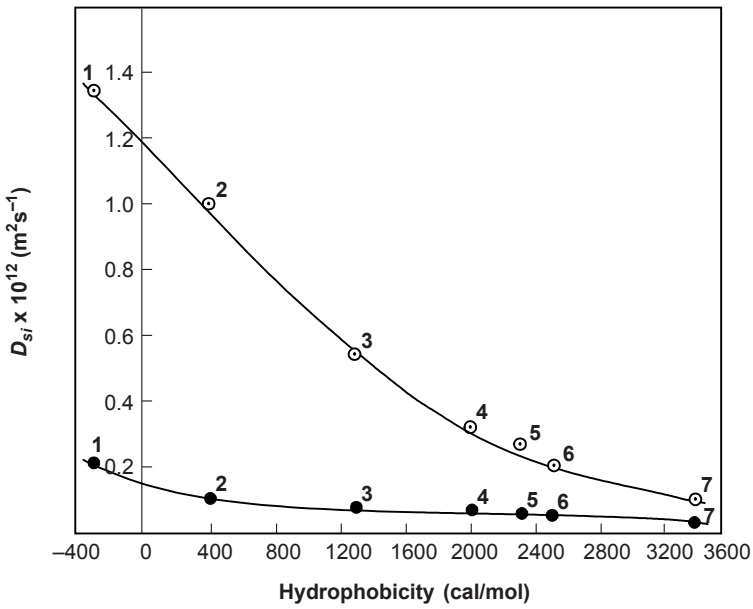

Figure 11 Hydrophobicity vs diffusion coefficient of amino acid isomers for L-tryptophan embedded membrane
Open symbol: D-isomer, closed symbol: L-isomer, 1: Serine, 2: Threonine, 3: Methionine, 4: Threonine, 5: Tyrosine, 6: Phenylalanine, 7: Tryptophan

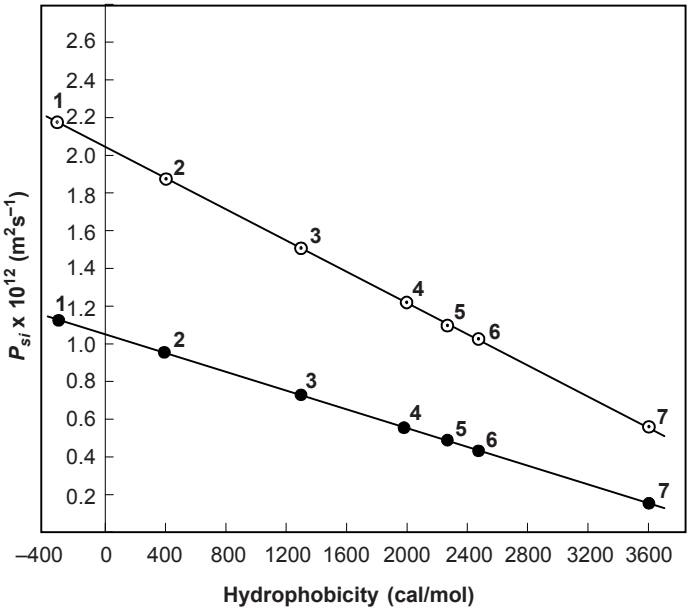

Figure 12 Hydrophobicity vs permeation coefficient of amino acid isomers for L-tryptophan embedded membrane
Open symbol: D-isomer, closed symbol: L-isomer, 1: Serine, 2: Threonine, 3: Methionine, 4: Threonine, 5: Tyrosine, 6: Phenylalanine, 7: Tryptophan

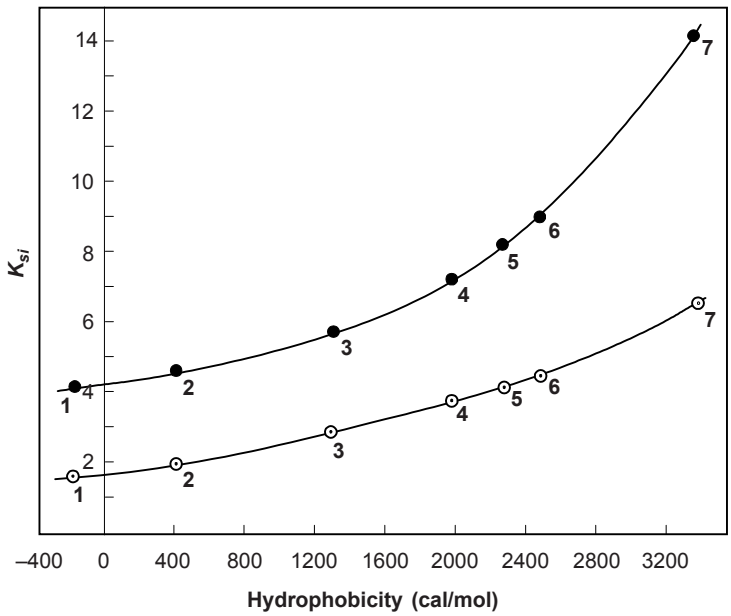

Figure 13 Hydrophobicity vs partition coefficient of amino acid isomers for L-tryptophan embedded membrane
Open symbol: D-isomer, closed symbol: L-isomer, 1: Serine, 2: Threonine, 3: Methionine, 4: Threonine, 5: Tyrosine, 6: Phenylalanine, 7: Tryptophan

$$\text{Log}K_{si} = 1.44 \times 10^{-4} H_p + 0.602 \text{ (L-isomer)} \quad (20)$$

$$\text{Log}K_{si} = 1.69 \times 10^{-4} H_p + 0.21 \text{ (D-isomer)} \quad (21)$$

with a standard deviation of ±10% for all the correlation.

4.0 CONCLUSION

Membrane prepared from condensation product of glutaraldehyde-L-tryptophan embedded in polysulfone matrix could discriminate all seven racemic amino acids studied in this work implying that it is possible to achieve effective separation of the D and L isomers with reasonably high enantioselectivity for D-isomers. The permeation parameters seem to correlate well with the solute molecular structure expressed in terms of hydrophobicity and permeation of amino acids in an enantioselective membrane is predictable and depends on their molecular structure. This findings of the present investigation indeed would help in deducing implication for design and development of membrane separation process for recemates mixture.

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NOMENCLATURE

C concentration of amino acid on the outer membrane surface (mM)
C_m concentration of amino acid in the membrane (mM)
C_1 concentration of amino acid in compartment 1 of the dialysis cell (mM)
D_s diffusion coefficient (m^2s^{-1})
H apparent self-association parameter (-)
H_p hydrophobicity (cal mol^{-1})
J solute flux (mol m^2 s^{-1})
K_s partition coefficient (-)
P_s solute permeability (m^2 s^{-1})
Q_t total amount of solute permeated (mol m^{-2})
T temperature (^oC)
T time (s)
d membrane thickness (m)

SUBSCRIPTS

B associated solute
D D-amino acid
F feed solution

i solute of the component I
P permeate solution
L L-amino acid

REFERENCES

[1] Thien, M. P., T. A. Hatton, and D. I. C. Wang. 1988. Separation and Concentration of Amino Acids Using Liquid Emulsion Membranes. *Biotech. Bioengg*. 32: 604-615.

[2] Nozaki, Y. and C. Tanford. 1971. The Solubility of Amino Acids and Two Glycine Peptides in Aqueous Ethanol and Dioxane Solution. *J. Biol. Chem*. 246: 2211.

[3] Sahoo, G. C., A. C. Ghosh, and N. N. Dutta. 1997. Recovery of Cephalexin from Dilute Solution in a Bulk Liquid Membrane. *Process Biochem*. 32(4): 265-272.

[4] Sahoo, G. C., N. N. Dass, and N. N. Dutta. 2000. Liquid Membrane Transport of Cephalosporin Antibiotics: Effect of Chemical Nature of Solute. *Chem. Eng. Commun*. 179: 89-99.

[5] Hano, T., T. Ohtake, M. Matsumoto, D. Kitayama, F. Hori, and F. Nakashio. 1991. Extraction Equilibria of Amino Acids with Quaternary Ammonium Salt. *J. Chem. Engg.* Jan. 24: 20-24.

[6] Itoh, H., M. P. Thien, T. Hatton, and D. I. C. Wang. 1990. A Liquid Emulsion Membrane Process for the Separation of Amino Acids. *Biotech. Bioengg.* 35: 853-860.

[7] Teramota, M., T. Yamashiro, A. Inoue, A. Yamamoto, H. Matsuyama, and Y. Miyake. 1991. Extraction of Amino Acids by Emulsion Liquid Membrane Containing Di(2-ethyl-hexyl)-phosphoric Acid as Carrier: Biotechnological Coupled, Coupled Facilitated Fransport Diffusion. *J. Membr. Sci.* 58: 11-32.

[8] Ishihara, I., N. Suzuki, and K. Matsui. 1987. *J. Chem. Soc. Japan*. 446.

[9] Scrimin, P., P. Tecilla, and U. Tonellato. 1995. Chiral Lipophilic Ligand 2. Cu(II)-Mediated Transport of α-Amino Acids Across a Bulk Liquid Membrane. *Tetrahedron*. 5: 1217-1230.

[10] Yamaguchi, T., K. Nishimura, T. Shinbo, and M. Sugiura. 1985. Enantiomer Resolution af Amino Acids by a Polymer Supported Liquid Membrane Containing a Chiral Crown Ether. *Chem. Lett.* 1549-1552.

[11] Masawaki, T., M. Sasai, and S. Tone. 1992. Optical Resolution of an Amino Acid by an Enantioselective Ultrafiltration Membrane. *J. Chem. Engg.* Jan, 25(1): 33-38.

[12] Maruyama, A., N. Adachi, T. Takatsuki, M. Torii, K. Sanui, and N. Ogata. 1990. Enantioselective Permeation of α-Amino Acids through Poly(amino acid) Derived Membrane. *Macromol*. 23: 2748-2752.

[13] Yoshikawa, M., J. I. Izumi, T. Kitao, S. Koya, and S. Sakamoto. 1996. Enantioselective Electrodialysis of N-a-Acetyltryptophans through Molecularly Imprinted Polymeric Membranes. *Chem. Lett*. 8: 611-612.

[14] Dobashi, A., N. Saito, Y. Motoyama, and T. Hara. 1986. Self Induced Nonequivalence in the Association of D- and L-Amino Acid Derivatives. *J. Am. Chem. Soc.* 108: 307-308.

[15] Capanelli, G., F. Vigo, and S. Munari. 1983. Ultrafiltration Membranes–Characterization Methods. *J. Membr. Sci.* 15: 289-313.