Effect of Template on Chiral Separation of Phenylalanine using Molecularly Imprinted Membrane in Aqueous Medium

[1,2] Noaman Ul-Haq* and [2] Joong Kon Park

[1]*School of Chemical and Materials Engineering, National University of Sciences and Technology, H-12, Islamabad, Pakistan*

[2]*Department of Chemical Engineering, Kyungpook National University, 1370 Sankyeok Dong, Buk-Gu,Daegu 702-01, Republic of Korea*

noaman@scme.nust.edu.pk*, parkjk@knu.ac.kr

Summary: Wet phase inversion method was used to prepare L-Phenylalanine (L-Phe) and D-Phenylalanine (D-Phe) imprinted poly [(acrylonitrile)-co-(acrylic acid)] membranes for chiral separation. Ultrafiltration experiments were conducted to evaluate the chiral separation ability of the prepared membrane towards racemate aqueous solution of Phenylalanine. The continuous permselectivity was observed by novel membrane. The chiral resolution ability of L-Phe imprinted membrane was much better than that of D-Phe. It was observed that both membranes simultaneously, selectively reject, selectively adsorbed and selectively permeate solute. The achieved adsorption selectivities of L-Phe imprinted membrane $[\alpha_{Ads}]_L$ and D-Phe imprinted membrane $[\alpha_{Ads}]_D$ were 2.6 and 2.40 respectively. Permselectivity of L-Phe imprinted membrane $[\alpha_{Perm}]_L$ was 2.56 while D-Phe imprinted membrane's permselectivity $[\alpha_{Perm}]_D$ was 2.03. The rejection selectivities of L-Phe and D-Phe imprinted membranes were $[\alpha_{Rej}]_L$=0.32 and $[\alpha_{Rej}]_D$ =0.28 respectively.

Keywords: Amino acid; Chiral resolution; Molecularly imprinted membrane; Molecular recognition; selectivity; Separation.

Introduction

In chemical and biological processes the selective separation and recognition of specific target molecule is an important issue [1]. The optical resolution of racemates has been essential in the perfume production, pharmaceutical industry, food preparation, and so forth due to the harmful effect of one of the enantiomer of racemate mixture. The resolution of racemates is the primary method to obtain pure enantiomers in industry [2-4].

The selective sorption abilities of molecularly imprinted polymers (MIPs) are remarkable by the virtue of their procedure of synthesis [5, 6]. Usually they are synthesized by very particular substrate that has the ability of selectively bound and separated. A heavily crosslinked rigid polymer is usually synthesized with template molecule. After completion of polymerization, a suitable solvent is used to remove template by washing the polymer. When polymer is exposed to a racemate solution, the polymer adsorbs one of the enantiomer with notable selectivity [7, 8]. The covalent and noncovalent are the major techniques for imprinting [9-12].

The prepared polymer can be cast in the form of membranes, known as Molecularly Imprinted Membranes (MIMs). MIMs are considered to be a promising material for selective separation with low energy consumption and can be scale up easily [13–18].

The appropriate polymer selection for the membrane is a major issue. Acrylic acid (AA) and acrylonitrile (AN) are most commonly available and have been considered as the most promising materials in a wet phase inversion method [19–25]. AA contains only one hydroxyl group that is sufficient to make hydrogen with amino acid of template i.e. phenylalanine. The AN in the polymer forms solid matrix for the membrane. While AA forms non-covalent interaction with template which helps in the fixation of template molecule in the membrane matrix [26].

Many researchers have used poly(AA-co-AN) imprinted membrane for the separation of target molecule (template). Trotta *et al.* imprinted poly(AA-co-AN) membrane with tetracycline hydrochloride [24] and naringin [25] for the separation of tetracycline hydrochloride from chloramphenicol; and to separate naringin from orange juice, respectively. Kobayashi *et al.,* [19–21] employed Theophylline (THO) as template molecule to imprint poly(AA-co-AN) membrane for the separation of THO and Caffeine (CAF). Cristallini *et al.,* [23] prepared Uric acid (UA) and THO imprinted poly(AA-co-AN) membranes for separation of UA and THO.

Several groups have devoted their efforts for the chiral separation of amino acids. But very few researchers have employed MIMs for the chiral separation of Phenylalanine. Takeda *et al.,* [27] used

*To whom all correspondence should be addressed.

Nylon 6, nylon 6,6, and terephthalic phenylene polyamide (TPPP) membranes were imprinted by L-Phe for chiral resolution of Phe in batch binding using ultrafiltration cell. Nylon 6, nylon 6,6, and TPPP, L-Phe imprinted membranes resolute 6.8, 4.2, and 1.7 partition coefficients of L – and D – forms respectively. Takeda *et al.,* might have got much better results if they would have considered the rejection of the solute by membranes. Jiang *et al.,* [28] imprinted chitosan (CS)/ γ-glycidoxypropyl-trimethoxysilane (GPTMS) hybrid membrane with L-Phe for chiral resolution of Phe by diffusion cells, improving significantly selectivity and achieved a separation factor of the order of 4.5 was achieved in 24 hrs.

First, the Kobayashi group [19-22, 26, 27] had introduced imprinting via phase separation starting with a solution containing the copolymer and the template; same approach has also been used, for instance, by the Drioli group [24, 25]. Later, Kobayashi introduced the method via polymerization of single functional monomer to imprint template and cast subsequent film using non-solvent induced phase separation method.

In this study we have prepared poly(AA-co-AN) MIM by wet phase inversion method, in which the in situ implantation of template (L-Phe or D-Phe) was done by non-covalent interactions for the optical resolution of Phenylalanine. Sorption and binding ability was studied along with the permeation selectivity of underivatized enantiomeric solution of Phe using Ultrafiltration experiments. Ultrafiltration technique showed significantly high selectivities in a short period of time. The selective rejection phenomenon of slote was also observed. The morphology of membrane was characterized by SEM. FT-IR spectroscopy was used to study the chemical structure of membrane.

Results and Discussions

Structure Analysis and Morphology of Membranes

The imprinting phenomenon is the virtue of hydrogen bonding [29]. It is appealing that one hydrogen bond is ample for the imprinting, selective recognition of template in aqueous medium. It is well known that weakening effect of water creates hurdle in the formation of noncovalent interactions. In current study the AA serves as functional monomer having single carboxylic group that is enough for the selective recognition of target molecule in water. The chemical functionality, shape and size of moieties and selective binding sites in the membrane structure were created after the removal of template.

The spectra of L-Phe imprinted poly(AA-co-AN) and D-Phe imprinted poly(AA-co-AN) membrane were analyzed by FT-IR. The interpretation of FT-IR spectra is summarized in Table-1. The OH dimmer and free OH stretching can be realized at 3466 cm^{-1} and 3242 cm^{-1} respectively, in L-Phe imprinted membrane, and in D-Phe imprinted membrane OH dimmer and free OH stretching appeared at 3461 cm^{-1} and 3243 cm^{-1}, respectively (Fig. 1). These free OH groups are might be due to the presence of COOH in imprinted poly(AA-co-AN) membranes. It is assumed that due to these free OH group form hydrogen bond with the template. SEM studies revealed that the average thickness of membrane was 25 μm and average thickness of dense top layer was 6 μm. The measured pore sizes of membrane were less than 25 nm.

Table-1: Assignment of FT-IR spectra L-Phe and D-Phe imprinted P(AA/AN) membranes.

Peak Assignment	Segment	L-Phe Imprinted Membrane	D-Phe Imprinted Membrane
OH Stretching Free COOH Group	AA	3466	3461
OH Stretching Dimerized COOH group	AA	3360	3360
OH Stretching Free COOH group	AA	3242	3243
CH Stretching	AA,AN	2939	2939
CN Stretching	AN	2244	2244
C=O Stretching	AA	1734	1734
NH Stretching	AN	1634	1634

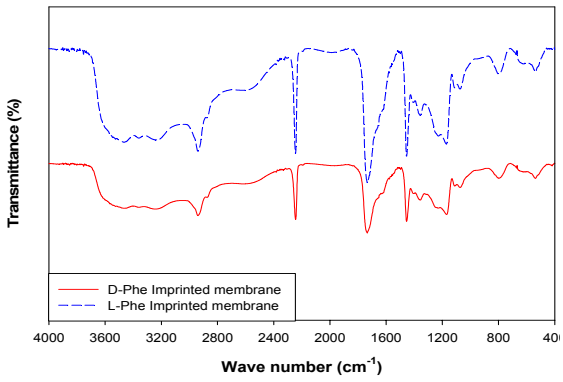


Fig. 1: FT-IR spectra of L-Phe & D-Phe imprinted P(AA/AN) membrane.

Effect on Swelling Separation Ability of Membrane

It is reported that the functional groups those are responsible for the recognition of template

may change their three-dimensional configuration due to swelling of polymer [30]. The swelling rate of L-Phe imprinted membrane was 73% and that of D-Phe imprinted membrane was 75%. It was observed that elasticity and swelling ability of membrane increased in the aqueous medium. According to the theory of "induced fit effect", Piletsky *et. al*. [31] concluded that solvation of the functional monomer binding ligands are the cause of swelling. Most of functional ligands (from the functional monomers) after the removal of template are probably produced inside the selective cavities. After selective rebinding, the volume of the polymer reduced nearly to the original volume. While Ulbricht observed that the increase in permeability is due to the swelling of membrane caused by the binding of template to the imprinting sites [32].

Template Effect on Selective Solute Rejection

During ultrafiltration process it was observed that solute not only adsorbed on membrane but also rejected by membrane. Fig. 2 shows that in L-Phe imprinted membranes, the rejection of D-Phe was higher than the rejection of L-Phe. In case of filtration from D-Phe membranes, the rejection of L-Phe was higher than that of D-Phe. The substrates (L-Phe or D-Phe used as template during the synthesis of membrane) after removal left imprinting cavities and channels (corresponding to the size and shape of L-Phe or D-Phe). The recognition of template took place by imprinted cavities and channels with in the membrane matrix worked as gate between pores [33]. When L-Phe imprinted membrane was used, these gates allowed L-Phe pass through it and rejected D-Phe. Similarly, when D-Phe imprinted membrane was used; L-Phe was rejected and D-Phe was allowed to pass through membrane. When L-Phe imprinted membranes were used; the rejection of D-Phe after 16 ml of filtration increased by 3.17-folds than rejection of L-Phe, and D-Phe imprinted membranes resulted rejection of L-Phe of the order of 3.53-times higher than the rejection of D-Phe. The rejection selectivity for L-Phe imprinted membrane was 0.32 and that of D-Phe imprinted membrane was 0.28. The nano pours and rough surface of membrane can also be the reason of rejection [34]. T. Gotoh *et al.,* have reported that when the amino acid concentration increases the rejection decreases [35]. NTR-7450 nanofiltration membrane was used for the separation of glutathione and its related amino acids (L-glutamate, L-cysteine, glycine, and L-glutamine). We used D-Phe and L-Phe imprinted membranes for chiral separation of Phe. We observed that the concentration of solute in retentate increased gradually with filtration time while rejection decreased. So we can assume that decrease in rejection with filtration time is due to the increase in concentration of solute in retentate. From above results we can also conclude that selective rejection is the combine effect of selective adsorption and selective permeation.

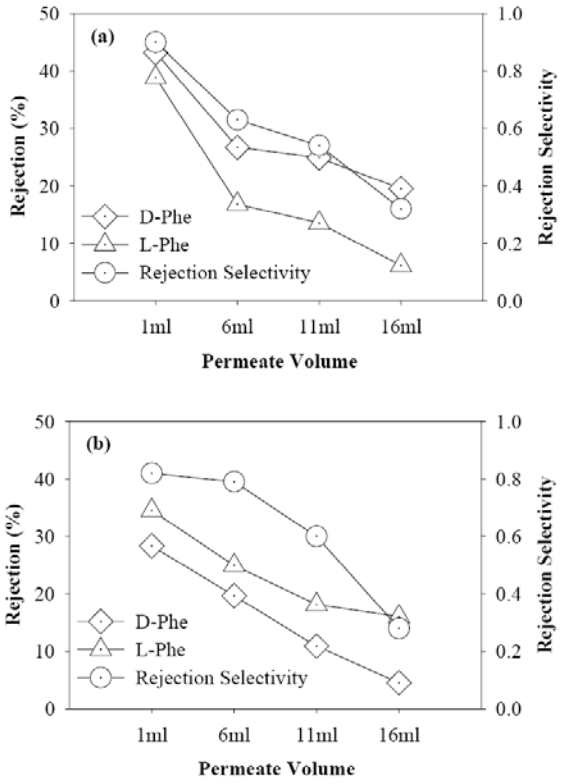


Fig. 2: Rejection profile of (a) L-Phe and (b) D-Phe imprinted AA/AN membranes after 16 ml filtration of 100-ppm racemate mixture of Phenylalanine.

Template Effect on Selective Solute Adsorption

The adsorbed amounts of D-Phe and L-Phe were 0.0647 mg/g of membrane and 0.1685 mg/g of membrane respectively, and adsorption selectivity $[\alpha_{Ads}]_L$ of 2.6 was achieved using L-Phe imprinted membranes. While D-Phe imprinted membrane showed adsorption selectivity $[\alpha_{Ads}]_D$ of 2.40 and adsorbed amount of D-Phe was 0.1674 mg/g of membrane while that of L-Phe was 0.0698 mg/g of membrane. It may be concluded that the selective performance of membrane is due to the "memory"

(the recognition imprinted sites) created in the membrane matrix after removal of template [28]. The high adsorption selectivity and strong binding affinity was observed in both L-Phe and D-Phe imprinted membranes (Table-2). Fig. 3 shows the chiral recognition ability of L-Phe and D-Phe imprinted membranes. There was preferential adsorption of L-Phe over D-Phe using L-Phe imprinted membrane and when D-Phe imprinted membrane was used the adsorbed amount of D-Phe was much more than the amount of L-Phe. The FT-IR spectra confirmed that the selective performance of L-Phe imprinted membrane is better than that of D-Phe imprinted membrane While the adsorption capacity of D-Phe was more than that of L-Phe it is might be due to swelling effect [31, 32] We can say that selective recognition of template by imprinted cavities in the membrane matrix directly effect on selective permeation and selective rejection.

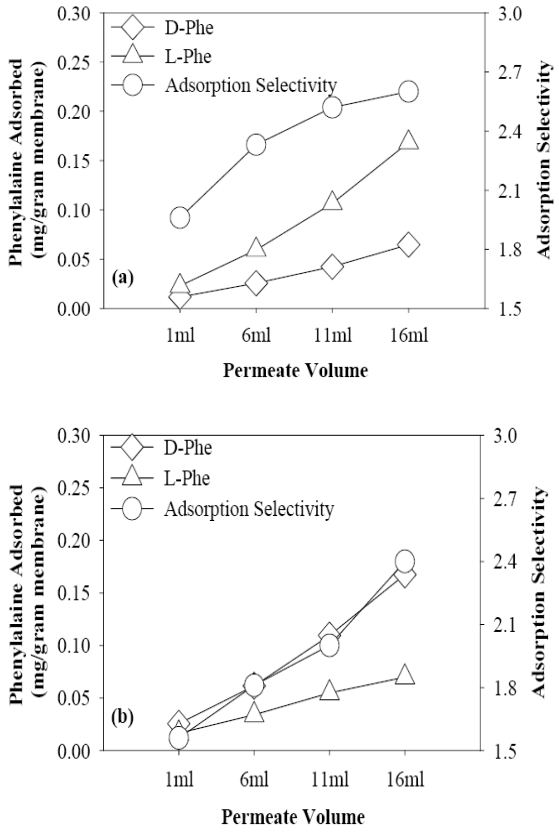


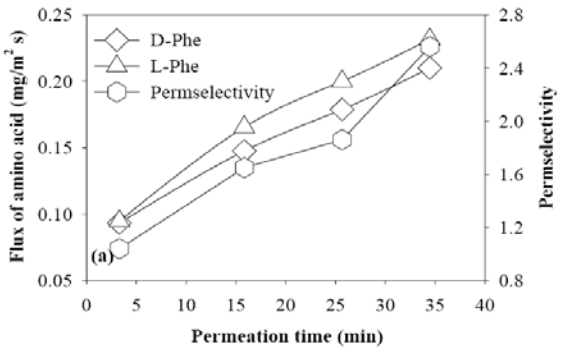
Fig. 3: Adsorption profile of (a) L-Phe and (b) D-Phe imprinted AA/AN membranes after 16 ml filtration of 100-ppm racemate mixture of Phenylalanine.

Template Effect on Selective Permeation

Fig. 4 shows typical permeation curves for concentration and flux versus time for separation of Phe isomers mixture obtained by permeation experiments using imprinted poly(AA-co-AN) membranes fixed in ultrafiltration kit by applying a pressure of 1 atm. The concentration of permeate increased gradually with time. The fluxes of the isomers in permeate also increased with time, increase in permeability is due to membrane swelling [31, 32]. Both L-Phe and D-Phe showed similar trend. The concentration and flux of the two isomers were different and chiral resolution of D, L-Phe was thus realized. The maximum separation factor (permselectivity) achieved in this study were about 2.56 and 2.03 for L-Phe imprinted poly(AA-co-AN) membranes and D-Phe imprinted poly(AA-co-AN) membranes, respectively (Table-2) and the permeability coefficient P was in 9 x 10^{-9} m^2/s. Fig. 4 illustrates that the permselectivity of L-Phe imprinted and D-Phe imprinted membrane increased with ultrafiltration. The permselectivity of L-Phe was found to be better than that of D-Phe imprinted membrane. It is be concluded that the template plays an important role on the performance of imprinting membrane used for chiral resolution, facilitated permeation through imprinted gates in the membrane and directly influence on selective rejection and selective adsorption.

Table-2: The selective separation profile of L-Phe and D-Phe imprinted membranes after 16 ml filtration of 100-ppm racemate mixture of Phenylalanine

Characterizations	L-Phe imprinted membrane	D-Phe imprinted membrane
S_{Ratio}	73%	75%
α_{Rej}	0.32	0.28
α_{Ads}	2.60	2.40
α_{Perm}	2.56	2.03
α_{Trans}	0.98	0.84



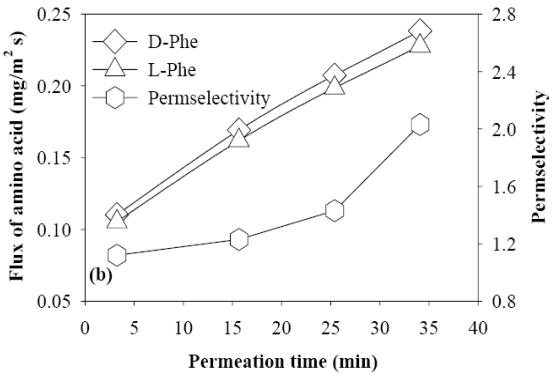


Fig. 4: Phe flux and permselectivity of (a) L-Phe and (b) D-Phe imprinted AA/AN membrane after 16 ml filtration of 100-ppm racemate mixture of Phenylalanine.

Template Effect on Solute Transport Mechanism

In MIMs transport of solute can be considered by (1) facilitated permeation and/or (2) retarded permeation [33]. The affinity binding is responsible for the facilitated permeation along with the preferential sorption of the template. In facilitated permeation transport of solute depend on the structure of membrane, concentration and distribution of MIP sites coupled with transport phenomenon [32]. The transmembrane pores having relatively small diameter might be responsible for the selective separation of enantiomers. Mostly liquid membranes are facilitated permeation membranes. The barrier structure of liquid membranes is non-porous. In retarded permeation other solute transports faster due to affinity binding, until a saturation of MIP sites with template is reached. MIP binding capacity helps to evaluate separation efficiency due to the saturation behavior. Those MIM can be solute adsorbers as selectivity is caused by specific adsorption [36]. Separation efficiency is determined by MIM binding capacity due to the saturation behavior. Based on the α_{Sep} data obtained by permeation of substrate and α_{Ads} data obtained by uptake values of membrane, transport selectivity α_{Trans} was calculated according to the solution-transport mechanism model using equation (8) and listed in Table-2. The transport selectivity of L-Phe imprinted membranes $[\alpha_{Trans}]_L$ was 0.98 and transport selectivity of L-Phe imprinted membranes $[\alpha_{Trans}]_D$ was 0.84. From these data we conclude that after 16 ml of permeation the permselectivity was higher than adsorption selectivity for both L-Phe and D-Phe imprinted membranes. From these three selectivity factors, it can be concluded that gate effect [33] plays an important role in selective adsorption of L-Phe and facilitated permeation of L-Phe. The D-Phe imprinted membrane showed similar behavior. D-Phe imprinted membrane rejected L-Phe and retarded transport of L-Phe while D-Phe was successfully recognized by membrane as adsorbed amount of D-Phe was much higher than that of L-Phe and permeation curves show facilitated permeation of L-Phe (template). Therefore, the separation mechanism of L-Phe and D-Phe imprinted poly(AA-co-AN) membranes for D,L-Phe isomer separation agreed well with the above mechanism (1). Thus we can conclude that the template recognition and increase in facilitated permeation are also functions of membrane swelling along with imprinted gates and cavities in the membrane matrix.

Experimental

Materials

The chemicals purchased from Sigma-Aldrich (USA) were 2,2-Azobiisobutyronitrile (AIBN), D-Phenylalanine (D-Phe), L-Phenylalanine (L-Phe), underivatized mixture of D,L-Phenylalanine (Phe) and Trifluoroacetic acid (TFA). Dimethyl sulfoxide (DMSO) was product of Kanto (Japan). Sulfate (CuSO4) and Acrylic acid (AA) were purchased from Junsei (Japan). Acrylonitrile (AN) was purchased from Yakuri (Japan). Scharlau (Spain) are the suppliers of the solvents i.e. acetonitrile and methanol used in HPLC. All reagents were of analytical grade and used without further purification.

Preparation of Molecularly Imprinted Membrane

To prepare molecularly imprinted poly(AA-co-AN) membranes by wet phase inversion method, imprinted polymer was prepared by radical polymerization. In 50 ml DMSO 7.19 ml AA, 0.5 g template (L-Phe or D-Phe) and 2 ml TFA were dissolved at 50 ^{O}C for 2 h in a polymerization reactor. To the above solution 37.72 g AN and a solution 50 ml DMSO and 0.22 g AIBN were added to above solution and nitrogen gas was purged for 5~10 minutes. The polymerization was done at 60 ^{O}C for 6 h under nitrogen atmosphere. The solution was stirred at uniform rotation speed of 200 rpm. 100 ml DMSO was added to the polymer and stirred for 20 h with a uniform rotation speed of 200 rpm at 25 ^{O}C. Then the polymer solution was placed in vacuum oven for 24 h, at 0.8 atm and 25 ^{O}C. With the help of

gardener knife polymer solution was cast on glass plate and coagulated in deionized water at 25 ^{O}C to get polymeric membrane. DMSO was removed from membrane by extensive washing. 5 % (V/V) acetic acid solution was used for the removal of template.

Characterization of poly(AA-co-AN) Membranes

FT-IR spectra of dried poly(AA-co-AN) samples (grounded with KBr pellets at room temperature) were recorded using a Mattson Galaxy 7020A FT-IR spectrophotometer (with a resolution of 0.025 cm^{-1} and wavelength range from 4000 cm^{-1} to 400 cm^{-1}) and a DTGS detector. The surface and cross-section morphology of poly(AA-co-AN) membranes were observed with Hitachi S-4300 Field Emission Scanning Electron Microscope (FE-SEM). Freeze dryer was used to dry samples of membrane, then samples were sputtered with gold and observed at 15 and 20 kV Energy Dispersive X-ray Spectrophotometer Image Processing System was used.

Separation Experiment

A 30 ml aqueous solution containing 100 mg Phe/l (50 mg for each enantiomer) with pH value of 2 was filtered through 5 sheets of membranes fixed in Millipore Ultrafiltration kit to determine the separation ability driven by a pressure of 1 atm. The amounts of L-Phe and D-Phe in samples were measured by HPLC consist of M 930 solvent delivery pump & M 720 UV Absorbance detector made of Young-Lin Instruments (Korea). The column TSKgel Enantio L2 made of Tosoh (Japan) with dimensions 4.6 mm id. X 250 mm was used. To check the reproducibility of results the experiments were repeated three times.

Rejection Selectivity of Membrane

The equation of rejection R used by other researchers [31] was modified using mass balance equation considering feed solution volume and concentration; permeate volume and concentration; volume and concentration of retentate; and amount of Phe adsorbed on membrane. The rejection R was calculated by following equations.

$$R_L = \frac{V_R\left[C_R - C_O\right]_L}{V_P\left[C_O\right]_L} \times 100 \qquad (1)$$

where R_L is rejection of L-Phe, subscript L represents L-Phe, V_R and V_P represents volume (ml) of retentate and permeate respectively; C_R and C_O are concentrations of Phe (mg/l) in retentate and in feed solution respectively. The rejection selectivity α_{Rej}, is defined as

$$\left[\alpha_{\mathrm{Re}\,j}\right]_L = \frac{R_L}{R_D} \qquad (2)$$

where $[\alpha_{Rej}]_L$ represents rejection selectivity when L-Phe imprinted membrane was used and R_D is rejection of D-Phe. If $[\alpha_{Rej}] < 1$, then it shows that the rejection of template was more than the counter enantiomer but, if $[\alpha_{Rej}] > 1$, this indicates that the rejection of counter enantiomer was more than template molecule.

Adsorption Selectivity of Membrane

The adsorption of L-Phe, Q_L (mg/g of membrane) on membrane was calculated by

$$Q_L = \frac{\left[M_O - \left(M_P + M_R\right)\right]_L}{W_D} \qquad (3)$$

where M_O, M_P and M_R are amounts of Phe (mg) in feed solution, in permeate and in retentate respectively; and W_D is dry weight of membrane. The adsorption selectivity α_{Ads} was calculated by using following equation [19-21].

$$\left[\alpha_{Ads}\right]_L = \frac{Q_L}{Q_D} \qquad (4)$$

where $[\alpha_{Ads}]_L$ represents adsorption selectivity when L-Phe imprinted membrane was used and Q_D is adsorption of D-Phe (mg/g of membrane). When $[\alpha_{Ads}] < 1$, then it shows that the adsorption of template was more than the counter enantiomer and $[\alpha_{Ads}] > 1$ show that adsorption of counter enantiomer was more than template enantiomer.

Solute Transportation Across Membrane

The L-Phe flux J_L (mg/m^{2}s) was calculated by the following equations: [37].

$$J_L = \frac{\lfloor M_P \rfloor_L}{AT} \quad (5)$$

where, A is the effective area (m^2) of membrane and T represents time (sec) required by solution to pass through membrane. The permeability coefficient P_L (m^2/s) of L-Phe is defined as:

$$P_L = \frac{J_L \partial}{\left[C_O - C_P\right]_L} \quad (6)$$

where □ is the membrane thickness (m) and C_P is concentration of Phe (mg/l) in permeate. The permselectivity $[\alpha_{Perm}]_L$ using L-Phe imprinted membrane was calculated by:

$$\left[\alpha_{Perm}\right]_L = \frac{P_L}{P_D} \quad (7)$$

where, P_D is permeability coefficient (m^2/s) of D-Phe. The $[\alpha_{Perm}] < 1$ illustrate that membrane showed facilitated permeation and $[\alpha_{Perm}] > 1$ shows that membrane retarted permeation of template.

The diffusion selectivity of the membranes was calculated by Jiang *et al.,* [28] method, for the chiral separation of amino acid. We calculated solute selectivity of the membrane using ultrafiltration technique considering solution transport mechanism by equ (8) after certain modifications.

$$\left[\alpha_{Trans}\right]_L = \frac{\left[\alpha_{Perm}\right]_L}{\left[\alpha_{Ads}\right]_L} \quad (8)$$

where $[\alpha_{Trans}]_L$ represents transport selectivity when L-Phe imprinted membrane was used. When $\alpha_{Trans} > 1$ then permselectivity is higher than adsorption selectivity and when $1 > \alpha_{Trans}$ then adsorption selectivity is higher than permselectivity.

Swelling Study of Imprinted Membranes

The Phe extracted membranes were soaked in distilled water for 72 h to ensure swelling equilibrium. Then the swollen membranes were taken out and water on the surface of membrane was blotted carefully with filter paper and weighed immediately. Then the membrane was dried under vacuum with a flat bottomed weighty object placed on the filter paper to avoid the shrinking. The following equation was used to determine swelling ratio (S_{Ratio}) of the membrane [38]:

$$S_{Ratio} = \frac{W_w - W_D}{W_D} \quad (9)$$

where, W_W is wet weight of membrane.

Conclusions

The L-Phe and D-Phe imprinted membranes prepared by AA and AN, successfully recognize template, facilitate permeation of template and reject other enantiomer. It was observed that one carboxylic molecule is sufficient for imprinting and recognition. The interacting imprinting sites in membrane matrix successfully bind template resulting in significantly improved chiral separation followed by ultrafiltration. Both L- and D-Phe imprinted membranes show similar trends. The results of L-Phe imprinted membranes were found to be remarkable. The L-Phe imprinted membrane was much better than D-Phe imprinted membrane, in terms of permselectivity, adsorption selectivity, rejection selectivity and transport selectivity.

References

1. O. Bruggemann, K. Haupt, L. Ye, E. Yilmaz and K. Mosbach*, Journal of Chromatography* A*,* **889,** 15 (2000).
2. W. S. Knowles, *Angewandte Chemie International Edition,* **41,** 1998 (2002).
3. R Noyori, *Angewandte Chemie International Edition,* **41,** 2008 (2002).
4. K. B. Sharpless, *Angewandte Chemie International Edition,* **41,** 2024 (2002).
5. Sellergren B (Ed.), In *Molecularly Imprinted Polymers*, *Man-Made Mimics of Antibodies and their Applications in Analytical Chemistry*, *Elsevier*, Amsterdam (2001).
6. T. Pap and G. Horvai, *Journal of Chromatography* B*,* **804,** 167 (2004).
7. A. Conesa and C. Palet, *Desalination,* **200,** 110 (2006).
8. M. Yoshikawa, J. Izumi, T. Kitao and S. Sakamoto, *Macromolecules,* **29,** 8197 (1996).

9. A. Kugimiya and T. Takeuchi, *Electroanalysis,* **11,** 1158 (1999).
10. D. A. Spivak and K. J Shea, *Macromolecules,* **31,** 2160 (1998).
11. B. R. Hart, D. J. Rush and K. J. Shea, *Journal of the American Chemical Society,* **122,** 460 (2000).
12. M. J. Whitcombe, M. E. Rodriguez, P. Villar and E. N Vulfson, *Journal of the American Chemical Society,* **117,** 7105 (1995).
13. H. H. Yang, S. Q Zhang, W. Yang, X. L Chen, Z. X Zhuang, J. G Xu, X. R. Wang, *Journal of the American Chemical Society,* **126,** 4054 (2004).
14. R. H. Schmidt, K. Mosbach and K. Haupt, *Advanced Materials,* **16,** 719 (2004).
15. D. J. Duffy, K. Das, S. L. Hsu, J. Penelle, V. M. Rotello and H. D. Stidham, *Journal of the American Chemical Society,* **124,** 8290 (2002).
16. N. Hilal and V. Kochkodan, *Journal of Membrane Science,* **213,** 97 (2003).
17. J. K. Mathew and J. Shea, *Journal of the American Chemical Society,* **118,** 8154 (1996).
18. M. Yoshikawa, T. Fujisawa, J. I. Izumi, T. Kitao and S. Sakamoto, *Analytica Chimica Acta,* **365,** 59 (1998).
19. H. Y. Wang, T. Kobayashi and N. Fujii, *Langmuir,* **12,** 4850 (1996).
20. H. Y. Wang, T. Kobayashi, T. Fukaya and N. Fujii, *Langmuir,* **13,** 5396 (1997).
21. T. Kobayashi, H. Y. Wang and N. Fujii, *Analytica Chimica Acta* **365,** 81 (1998).
22. K. Takeda and T. Kobayashi, *Journal of Membrane Science,* **275,** 61 (2006).
23. C. Cristallini, G. Ciardelli, N. Barbani and P. Giusti, *Macromolecular Bioscience,* **4,** 31 (2004).
24. F. Trotta, C. Baggiani, M. P. Luda, E. Drioli and T. Massari, *Journal of Membrane Science,* **254,** 13 (2005).
25. F. Trotta, E. Drioli, C. Baggiani and D. Lacopo, *Journal of Membrane Science,* **201,** 77 (2002).
26. T. Kobayashi, Y. Murawaki, P. S. Reddy, M. Abe and N. Fujii, *Analytica Chimica Acta,* **435,** 141 (2001).
27. K. Takeda, M. Abe and T. Kobayashi, *Journal of Applied Polymer Science,* **97,** 620 (2005).
28. Z. Jiang, Y. Yu and H. Wu, *Journal of Membrane Science,* **280,** 876 (2006).
29. T. Zhang, F. Liu, W. Chen, J. Wang and K. Li, *Analytica Chimica Acta,* **450,** 53 (2001).
30. T. A. Sergeyeva, S. A. Piletsky, A. A. Brovko, E. A. Slinchenko, L. M. Sergeeva and A. V. El'skaya, *Analytica Chimica Acta,* **392,** 105 (1999).
31. S. A. Piletsky, E. V. Piletskaya, T. L. Panasyuk, A. V. El'skaya, R. Levi, I. Karube and G. Wulff, *Macromolecules,* **311,** 2137 (1998).
32. M. Ulbricht, *Journal of Chromatography* B, **804,** 113 (2004).
33. S. A. Piletsky, T. L. Panasyuk, E. V. Piletskaya, L. A. Nicholls and M. Ulbricht, *Journal of Membrane Science,* **157,** 263 (1999).
34. M. D. Petala and A. I. Zouboulis, *Journal of Membrane Science,* **269,** 1 (2006).
35. T. Gotoh, H. Iguchi and K. Kikuchi, *Biochemical Engineering Journal,* **19,** 165 (2004).
36. D. K. Roper and E. N. Lightfoot, *Journal of Chromatography* A, **702,** 3 (1995).
37. R. J. Ansell, *Advanced Drug Delivery Reviews,* **57,** 1809 (2005).
38. F. Schneider, F. S. Piletsky, E. Piletska, A. Guerreiro and M. Ulbricht, *Journal of Applied Polymer Science,* **98,** 362 (2005).