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# Optical Resolution of α-Amino Acids by Reverse Osmosis using Enantioselective Polymer Membrane Containing Chiral Metal-Schiff Base Complex

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**The optical resolution of α-amino acids, arginine and alanine was performed by reverse osmosis at 517.10 kPa and 1034.21 kPa pressures using enantioselective composite nanofiltration membrane prepared by interfacial co-polymerizing, a mixture of Zinc metal Schiff's base complex and piperazine with trimesoyl chloride in-situ on the top of polysulfone ultrafiltration membrane. The chemical composition of the enantioselective layer was determined by ATR-FTIR and X-ray Fluorescence Spectroscopy and surface morphology was studied by scanning electron microscopy. The effect of process parameters such as the operating pressure, permeation time, and concentration of the feed on the performance of membrane was studied. The volumetric flux of aqueous solution of α-amino acids through membrane follows Hegen-Poiseuillie equation. The composite membrane permeates d-enantiomers of α-amino acids preferentially; 54% enantiomeric excess in for d-arginine was achieved. The enantioselective permeability of the membrane is found to be time dependent. The enantioselective property of the membrane has arisen due to a homo-Chiral environment created in the membrane by incorporating chiral ligand Schiff complex in thin film of poly (piperazine trimesamide) polymer on the top of the polysulfone membrane. The composite membrane also exhibits the separation of α-amino acids from their aqueous solutions.**

**Keywords** α-amino acids; composite membrane; enantioselective membrane; metal Schiff base complex; optical resolution; trimesoyl chloride

## INTRODUCTION

The separation of chiral compounds is of great interest because the demand for optically pure chiral compounds including drugs, agrochemicals, food additives, perfumes, specialty materials, is increasing constantly (1). Therefore, to develop a technically feasible and economically viable optical resolution method which could be operated continuously and commercialize easily is of great significance. The existing optical resolution methods such as preferential crystallization, kinetic resolution, capillary electrophoresis, enantioselective chromatography, etc. are batch processes hence they resolve a small amount in one operation. Moreover, few methods require resolution aids (Chiral reagents or chiral solvents) that may not be recoverable (2–4). In the last two decades membrane separation processes have emerged advantageous over conventional separation processes on account of being modular, economical, eco-friendly, simple in operation, and easy to scale up; therefore a membrane based optical resolution process looks highly attractive. The membrane based optical resolution was first investigated using liquid membranes containing chiral carriers such as chiral crown ethers, calixarene, amino acids derivatives, etc. (5–8). Though liquid membranes showed high enantio-selectivity, they were found to be very poor in stability (8). Solid polymer membranes have superior stability hence they are considered to be more suitable. Various kinds of polymeric membranes have been reported for optical resolution, including plasma-polymerized membranes of *d*-camphor and *l*-menthol (9), membranes having cyclo-dextrin moieties (10), membranes made from polymeric chiral crown ethers (11), poly (amino acid) membranes having amphiphilic side chains (12,13), membranes bearing an amino acid condensate (14), (+)-poly{1-[dimethyl(10-pinanyl)silyl]-1-propyne} membrane (15), polysulfone membranes with immobilized bovine serum albumin (16,17), a sericine membrane (18), a cellulose tris (3,5-dimethylphenyl) carbamate membrane (19), a poly{γ-[3-(pentamethyldisiloxyl)-propyl]-L-glutamate} membrane, (20) PMMA membrane containing (−)-oligo {methyl (10-pinanyl)siloxane} (21), β-cyclodextrin immobilized cellulose acetate dialysis membranes (22), cellulose acetate butyrate membrane (23), molecularly imprinted membranes (24), affinity ultrafiltration membranes, (25), etc.

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In general polymer membranes described for optical resolution have been fabricated from chiral polymers and their derivatives; most of these membranes are brittle, fragile, and possess inferior mechanical properties (26). Inferior mechanical properties restricted their uses in the dialysis mode of separation. The dialysis mode of separation results in a very low rate of permeation because the driving force for transport is solute concentration gradient only. Other types of polymer membranes are prepared from non-chiral polymers having enantiomers recognizing molecules viz.; amino acids, proteins, oligo-peptides, etc. grafted on to polymer backbone as groups or side chains. Such membranes possess superior mechanical properties; however, during permeation recognition sites get saturated quickly because of being fixed in the polymer matrix, thereby selectivity decreases sharply with time. The development of a suitable enantioselective membrane may realize the optical resolution by membrane process in the true sense.

Chiral ligands exchange chromatography using chiral stationary phases have been used for the separation of racemic mixtures of chiral drugs (27,28). We have studied the possibility of fabricating enantioselective membrane based on ligands exchange chromatography incorporating reactive metal complex in polymer membrane. The disalicylaldehyde-arginine Schiff base complex of zinc metal was incorporated by in-situ copolymerizing it with piperazine and trimesoyl chloride interfacially on the top of polysulfone ultrafiltration membrane. The optical resolution of α-Amino acids, arginine and alanine, has been performed using composite membrane based on Zn metal-Schiff base complex.



## EXPERIMENTAL

### Materials

Trimesoyl chloride (TMC), piperazine, racemic arginine and its enantiomers, racemic alanine and its enantiomers were obtained from Sigma–Aldrich (USA). Salicyladehyde (SD Fine Chemicals), zinc chloride (Analytical Grade) and all other chemicals were LR grade and used without any further purification, unless otherwise stated.

### Methods

*Synthesis of Metal Schiff Base Complexes*

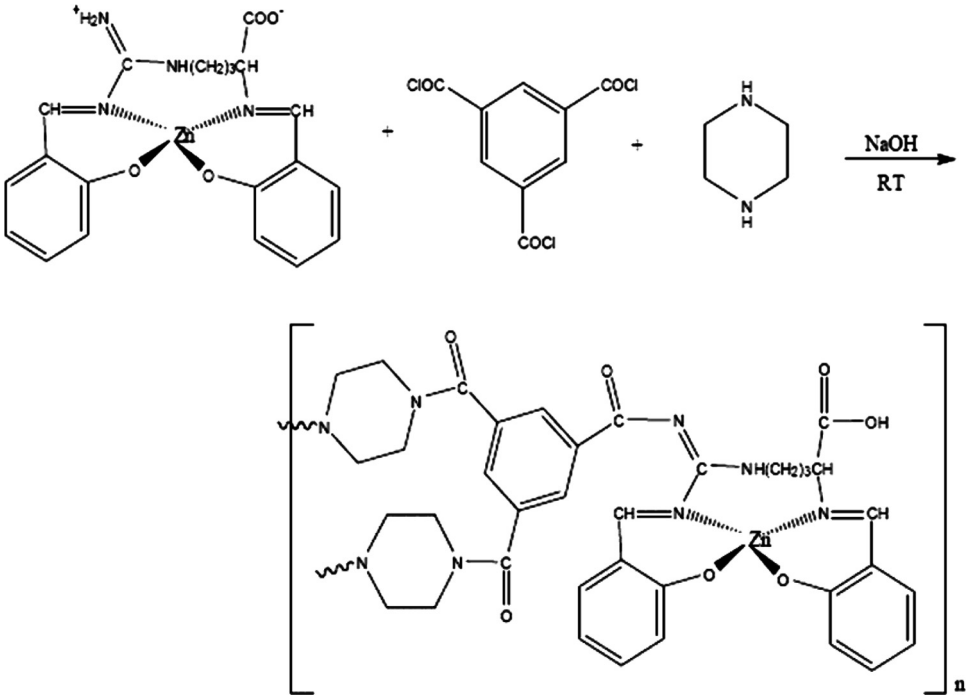
Schiff base metal complex was synthesized as per method reported by J. Mao et al. (29). 1 mM of l-arginine was dissolved in 50 mL warm methanol and an equimolar quantity of salicylaldehyde was added drop-wise to the solution. The mixture was refluxed at 70°C for 30–45 minutes under continuous stirring. A bright yellow solid salicylaldehyde-arginine Schiff base (SALA) was obtained after evaporating the solvent. 1 mM of SALA prepared in previous step and 1 mM of LiOH were dissolved in 40 mL warm methanol; the residue if any was filtered out, and 1.2 mM of salicylaldehyde was added dropwise into the filtrate. The mixture was refluxed for 1 hour, cooled, and filtered. A yellow solid precipitated of disalicylaldehyde-arginine Schiff base (D-SALA) was obtained. It was washed with cold isopropyl alcohol and dried in vacuum. The zinc metal Schiff's base complex was synthesised by adding 2 mM ethanolic solution of zinc chloride drop-wise into 1 mM ethanolic solution of the D-SALA, under continuous stirring within 30 minutes at ambient temperature; a precipitate of zinc metal Schiff's base complex was then isolated gradually. The precipitate was filtered, washed by ethanol, and dried in vacuum. Re-crystallization from anhydrous ethanol yields analytically pure complex as a fine powder.

### Preparation of Composite Membrane

Zinc metal Schiff base complex 1% (w/w) and piperazine 1% (w/w) were dissolved in 0.1 N aqueous solution of NaOH. Trimesoyl chloride 0.5% (w/w) was dissolved in n-hexane. Polysulfone ultrafiltration membrane having 15% polysulfone content prepared by phase inversion method as per details given elsewhere (30) was used as support membrane for preparing thin film composite membrane. The polysulfone membrane was washed thoroughly with ion free water many times before coating a thin enantioselective layer on it. To coat a thin enantioselective layer, the membrane was immersed in aqueous solution of zinc Schiff base complex and piperazine for 5 minutes. After 5 minutes the extra solution was drained off and the membrane was air dried for 10 min at ambient temperature. The membrane was then immersed in TMC solution for 2 minutes. After 2 minutes the extra TMC solution was drained off. The membrane was air dried for 2 hours, and was subjected to heat treatment at 80–90°C in a hot air oven for 10 minutes. The membrane was cooled to room temperature and preserved in de-ionized water. The schematic representation of interfacial reaction is given in Scheme 1.

### Optical Resolution of α-Amino Acids

Optical resolution of racemic α-amino acids, arginine and alanine, was performed in a closed loop and cross-flow mode on a reverse osmosis permeation module having four cells connected in series. Each cell has a circular shape membrane having an effective membrane area of 0.00195 $m^2$. Volumetric flux ($J_V$) of the membrane was recorded at various trans-membrane pressures between 344 kPa to 1034.21 kPa for 2 to 10 hours at constant temperature (25°C) using aqueous solutions of concentrations of 5.7 and 11.4 mM. The effect of trans-membrane pressure, permeation time, and concentration of feed solution on volumetric flux ($J_V$), solute flux ($J_S$), enantiomeric excess of isomer, and separation of the solute was studied.




SCH. 1. Schematic representation of interfacial polymerization reaction of metal Schiff-base complex and piperazine with trimesoyl chloride.



*Analysis of Permeate*

The concentrations of enantiomers in the feed and the permeate were determined by high pressure liquid chromatography (HPLC, Waters) equipped with PDA detector and Chiral Chrompak CR (+) column (Daicel Chemical Industries Ltd.). Mobile phase for HPLC analysis was perchloric acid at flow rate of 0.4 mL/min. HPLC chromatograms of arginine feed solution, permeate, and d and l-arginine standards are given in Fig. 5 and of alanine feed solution, permeate, and l-alanine standard are given in Fig. 6.

## Theory and Explanation

The performance of a membrane-based optical resolution process is explained in terms of membrane permeability for enantiomers, enantiomers selectivity, and the separation of the solute.

**Permeability**: Flux is a measure of permeability of membrane and is described in terms of volumetric flux ($J_V$) and solute flux ($J_S$).

**Volumetric Flux ($J_V$)**: It is the volume of liquid in liters permeated through per unit area of membrane per unit time. Volumetric flux ($J_V$) through a microporous membrane is explained by the Hegen-Poiseuillie equation as:

$$J_v = \frac{n\pi r^4 \Delta p}{8\mu l} \tag{1}$$

Here, n is the number of pores, r is the radius of the membrane pores, $\Delta p$ is the trans-membrane pressure, $\mu$ is the viscosity of the fluid, and l is the membrane thickness.

**Solute Flux ($J_S$)**: It is the amount of solute in grams or moles that passes through per unit area of membrane per unit time at constant pressure.

$$J_{(s)} = \frac{\text{Amount of solute permeated}}{\text{Area of membrane X time}} \tag{2}$$

**Solute Separation**: The separation of a solute by membrane is expressed as % separation by the following equation:

$$\%\,\text{Separation} = \frac{\text{Amount of solute in feed} - \text{Amount of solute in permeate}}{\text{Amount of solute in feed}} \times 100 \tag{3}$$

**Enantioselective Permeability**: The enantioselective permeability of a membrane is a measure of optical purity of enantiomers and is defined in terms of the % enantiomeric enrichment or excess (%ee) and separation factor ($\alpha$). The enantioselective permeability of the membrane and the separation factor was calculated using the area of peaks for enantiomers in HPLC chromatograms.

**Enantiomeric Excess (%ee)**: %ee is a measure of excess of one of the paired enantiomers in the permeate of the

membrane and is given by the following expression:-

$$\%ee = \frac{C^{dP} - C^{lP}}{C^{df} + C^{lf}} \times 100 \quad (4)$$

Here, $C^{dP}$ and $C^{lP}$ are concentrations of a major permeating isomer and minor permeating isomer in permeate respectively. $C^{df}$ and $C^{lf}$ are concentrations of d-enantiomer and l-enantiomer in feed.

**Separation factor (α)**: The separation factor (α) is a ratio of two enantiomers in permeate and feed solutions as defined by the following equation:

$$\alpha = \frac{C^{dP}/C^{lP}}{C^{df}/C^{lf}} \quad (5)$$

If the feed is a solution of racemic compound, as is the case here, the separation factor (α) may be defined as:

$$\alpha = C^{dP}/C^{lP} \quad (6)$$

## RESULTS AND DISCUSSION

### Characterization of Arginine Schiff's Base Metal Complex and Composite Membrane

*Arginine Schiff's Base Metal Complex*

Salicylaldehyde arginine Schiff base ligands (SALA & D-SALA) and metal complex were characterized by element analysis (CHN&S analyzer, Perkin–Elmer 2400), IR analysis (Perkin-Elmer Spectrum, GX), $^1$H NMR (Bruker Avance-II spectrophotometer, 200 MHz) and $^{13}$CNMR (Bruker Avance-II spectrophotometer, 500 MHz) using DMSO-$d_6$ (solvent), and TMS as an internal reference.

**Salicylaldehyde-arginine Schiff (SALA)**: Elemental analysis of the Salicylaldehyde-arginine Schiff base:

Molecular formula, $C_{13}H_{18}N_4O_3$ (278.14)

Calculated values (%): C 56.10, H 6.52, N 20.13.

Found (%): C 55.86, H 6.45, N 19.87.

IR spectra (Fig. 1a): the presence of C=N stretching at 1472 $cm^{-1}$, $CH_2$ absorption at 1456 $cm^{-1}$, $-NH_2$ (primary) at 3351 $cm^{-1}$ and −OH (phenolic) at 3163 $cm^{-1}$ confirm the formation of target compound.

$^1$H NMR (DMSO): δ 8.52 (s, 1H), 8.36 (s, 1H), 7.40–7.38 (m, 4H), 7.31–6.69 (m, 4H), 3.57 (t, 1H), 3.56 (s, 1H), 3.39 (t, 2H), 1.93–1.76(m, 2H), 1.34 (t, 2H).

$^{13}$CNMR (DMSO): δ 174.18 (C O), 163.92 (C N), 158.6 (C N), 157.33 (C), 132.8–115.66 (5C, phenyl), 63.70 (CH), 32.00–25.38 (3CH2).

**Disalicylaldehyde arginine Schiff base (D-SALA)**: Elemental analysis of DSALA:

Molecular Formula, $C_{20}H_{22}LiN_4O_4$ (389.35)

Calculated values (%): C 61.70, H 5.70, N 14.39

Found (%): C 60.98, H 5.47, N 14.67.

IR spectra (Fig. 1b): the presence of C=N stretching at 1472 $cm^{-1}$, $CH_2$ absorption at 1456 $cm^{-1}$, and −OH (phenolic) at 3163 $cm^{-1}$ and disappearance of $-NH_2$ (primary) peak at 3351 $cm^{-1}$ confirm the formation of D-SALA.

$^1$H NMR (DMSO): δ 10.25 (s, 1H), 8.99 (s, 1H), 8.36 (s, 1H), 7.78–7.77 (m, 4H), 6.89–6.51 (m, 8H), 3.99 (t, 1H), 3.11 (t, 2H), 1.91–1.76 (m, 2H), 1.50 (t, 2H).

$^{13}$C NMR (DMSO): δ 174.20 (C=O), 163.99 (C=N), 159.00 (C=N), 157.34 (2C), 148.69 (C N), 123.89–114.76 (10C, 2phenyl), 69.06 (CH), 31.10–25.39 (3CH2).

**Schiff's base complex of Zn**: Elemental analysis of complex:

Molecular Formula, $C_{20}H_{20}N_4O_4Zn$ (445.81)

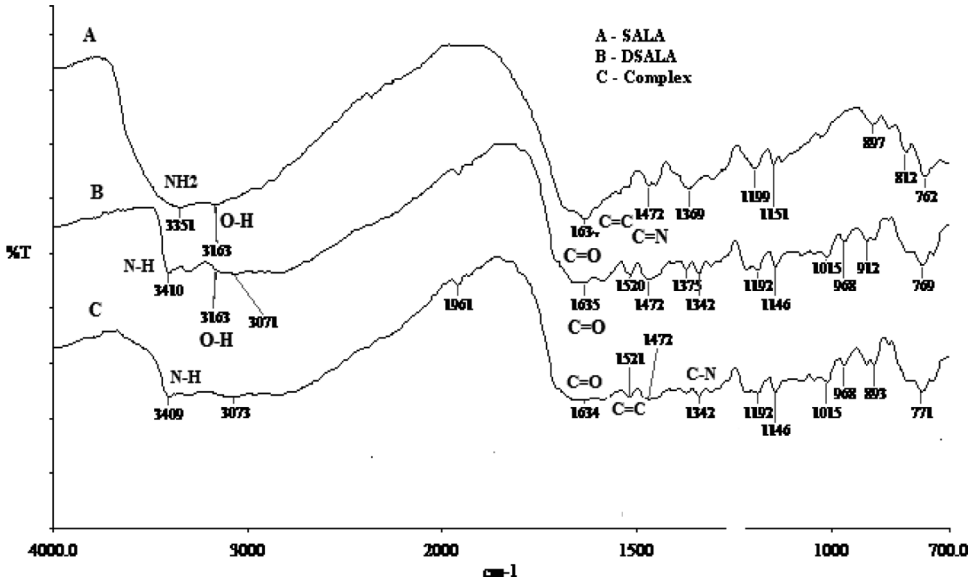



FIG. 1. FTIR spectra of ligands SALA (A), D-SALA (B) and Zn Metal complex (C).

Calculated values (%): C 53.88, H 4.52, N 12.87, and Zn 13.32
Found (%): C 53.52, H 4.61, N 12.41 and Zn (by ICP) 12.80.
IR spectra (Fig. 1c): the presence of C=N stretching at 1472 $cm^{-1}$, C−N=C at 1342 $cm^{-1}$, $CH_2$ absorption at 1456 $cm^{-1}$, C=O at 1652 $cm^{-1}$, C=C at 1521 $cm^{-1}$ etc. and disappearance of −OH (phenolic) at 3163 $cm^{-1}$ confirm the formation of metal complex.
$^1$H NMR (DMSO) of Zn metal complex: $\delta$ 8.53 (s, 1H), 8.37 (s, 1H), 7.40–7.37(m, 2H), 7.40–7.28 (m, 8H), 2.59 (t, 1H), 1.93 (t, 2H), 1.76–1.74 (m, 2H), 1.43 (t, 2H). The absence of OH peak at $\delta$ 3.12 confirms the formation of metal complex.

*Composite Membrane*

The composite membrane was characterized by the ATR-FTIR spectrophotometer for the chemical structure of its top layer and by Scanning Electron microscopy for morphology. ATR-FTIR spectra of polysulfone membrane before coating and after coating were recorded on a Perkin-Elmer spectrometer (Perkin-Elmer GX, ATR-FTIR) using a Germanium crystal at a nominal incident angle of 45° at a speed of 100 scans at a resolution of 2 $cm^{-1}$. ATR-FTIR spectra of membrane before and after coating with thin film of polypiperazineamide containing Zn metal complex is given as Fig. 2. The peaks at 1487–90 $cm^{-1}$, and 1584 $cm^{-1}$ are characteristics of polysulfone support. The appearance of absorption bands in 1472–1644 $cm^{-1}$ region can be attributed to the C=O, C=N stretching vibrations. The peak at 1667 $cm^{-1}$ in the spectra of coated membrane is indicative of amide formation. Characteristic absorption at 1731 $cm^{-1}$ (imide ring C=O), 1369 $cm^{-1}$ (C−N−C, imide in the plane), and 747 $cm^{-1}$ (C−N−C, out-of-plane bending, imide).The presence of Zn metal on the composite membrane was confirmed by X-ray Fluorescence Spectroscopy (WDX mode).

The surface morphology of the top layer and the cross section of the membrane were studied by scanning electron microscopy (Leo,). SEM photographs are given in Figs. 3 (surface) and 4 (transverse section). In the transverse section the selective is measured ∼4 μm thick and the microporous polysulfone layer of the composite membrane is measured ∼60 μm thick.

## Volumetric Flux ($J_v$)

The pure water permeability (PWP) of the membrane and the volumetric flux for 5.7 mM and 11.4 mM concentrated solutions of racemic alanine and arginine as a function of trans-membrane pressure (Δp) are depicted in Fig. 7. The volumetric flux of amino acids solutions is less than pure water flux of membrane at corresponding pressure. The volumetric flux increases with trans-membrane pressure as per Hegen-Poiseuillie equation (1); however, the increase rate is not systematic. Initially the increase rate is higher and declines gradually. Further more, the concentrated solution permeates slowly compared to less concentrated solution at identical pressure. The time variation in volumetric flux with time is shown in Fig. 8. It is seen that flux decreases as the permeation time proceeds. The decline in flux with time results mainly due to increase in osmotic pressure of the feed solution near the membrane surface at the upstream side and partially due to fouling of the membrane caused by solute adsorption or particulate blocking of the membrane pores (31). The volumetric flux follows a similar pattern for



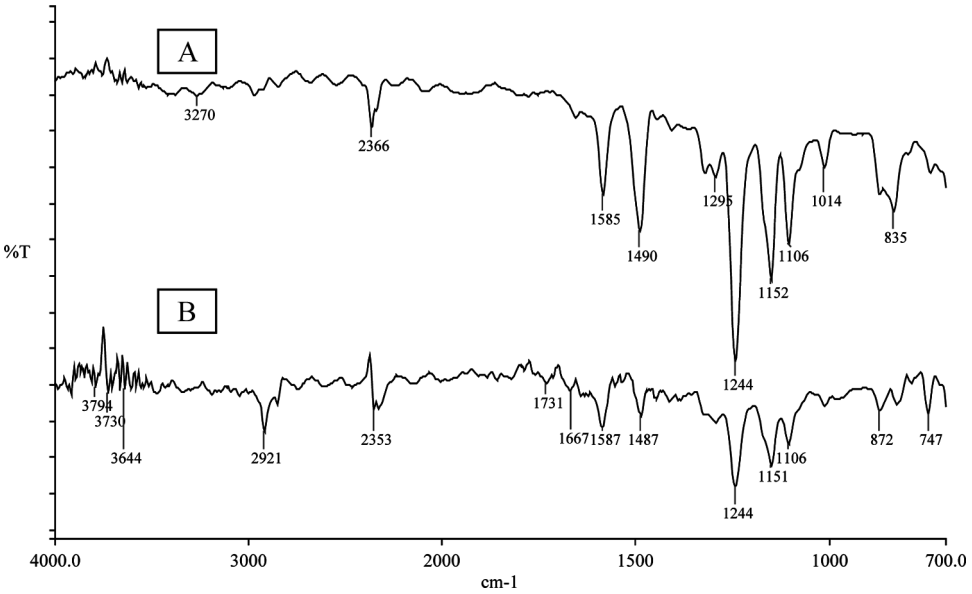



FIG. 2. ATR-FTIR spectra of polysulfone support membrane before coating (A) and after coating a thin enantioselective layer on it (B).

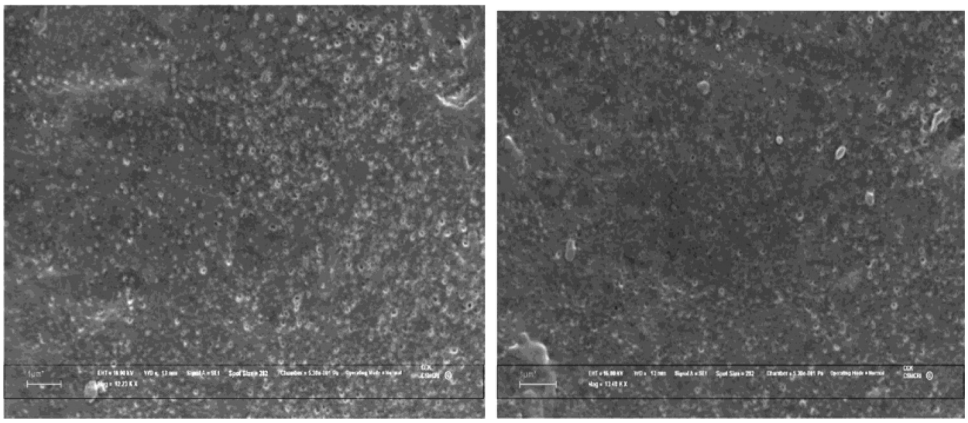


FIG. 3. SEM photographs of top surface of composite membrane.

solutions of arginine and alanine of similar concentration. The observations indicate that the volumetric flux is independent of molecular sizes of permeating solutes within close range molecular sizes.

### Solute Flux ($J_s$)

Solute flux of 5.7 mM and 11.4 mM concentrated solutions of racemic arginine and alanine at 517.1 kPa and 1034.21 kPa pressures, as a function of permeation time is shown in Fig. 9. The solute flux is high at high trans-membrane pressure and less at low trans-membrane pressure; for example the solute flux is 52.46 $mg/m^2 h$ for 11.4 mM concentrated feed solution of arginine at 1034.21 pKa whereas the solute flux is 34.86 $mg/m^2 h$ at 517.1 kPa. The solute flux decreases with time and the decrease in flux is more pronounced for more concentrated feed solutions (11.4 mM) compared to less concentrated solutions (5.7 mM). Similar observations were recorded for both types of feed (arginine and alanine) solutions. The reduction in solute flux is due to clogging of membrane pores by solute molecules and by interaction of the solute with the membrane surface. It is observed that arginine permeates more compared to alanine at any time of permeation at corresponding pressure. It is due to the fact that the membrane top layer is homo-chiral for arginine molecules hence it interacts preferentially with the membrane resulting in fast diffusion through the membrane. The preferential diffusion of arginine through the membrane might be the cause of a high permeation rate compared to alanine.

### Solute Separation

Rejections of arginine and alanine from 5.7 mM and 11.4 mM concentrated feed solutions at 517.1 kPa and 1034.21 kPa pressures for permeation time from 2 to 10 hours are shown in Fig. 10. Alanine is rejected more than arginine; for example, from 5.7 mM concentrated feed solution of arginine at 517.1 kPa trans-membrane pressure

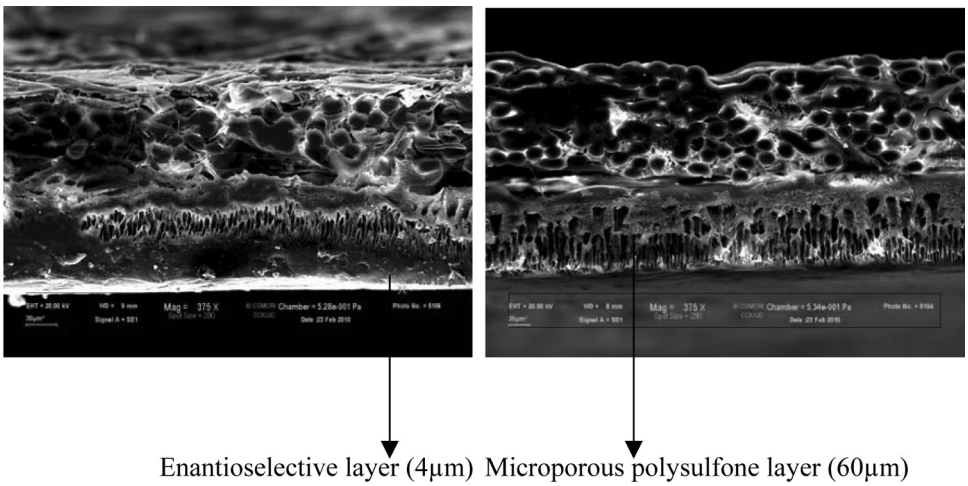
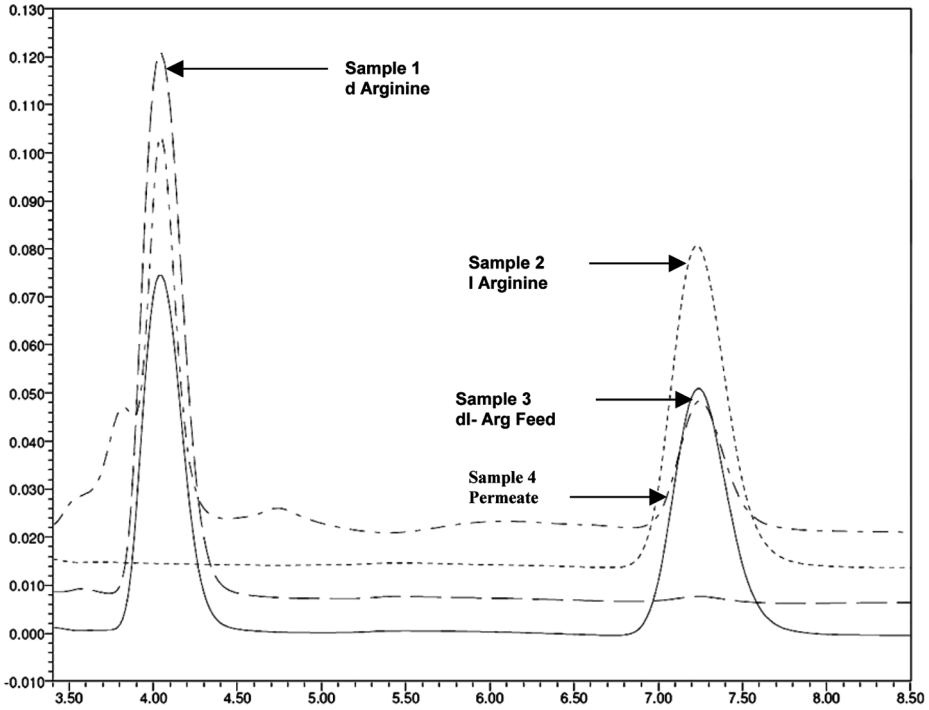



FIG. 4. SEM photographs of cross section of composite membrane.




**Peak report**

| Sample details | Name of Peak | Retention Time | Area Percentage |
|---|---|---|---|
| **Sample 1 (1000ppm d-arg.)** | d-Arginine | 4.04 | 100.00 |
| **Sample 2 (1000ppm l-arg.)** | l-Arginine | 7.23 | 100.00 |
| **Sample 3 Feed (1000ppm racemic arg)** | d-Arginine | 4.02 | 50.95 |
| | l-Arginine | 7.23 | 49.05 |
| **Sample 4 (Permeate)** | d-Arginine | 4.04 | 70.67 |
| | l-Arginine | 7.24 | 29.33 |

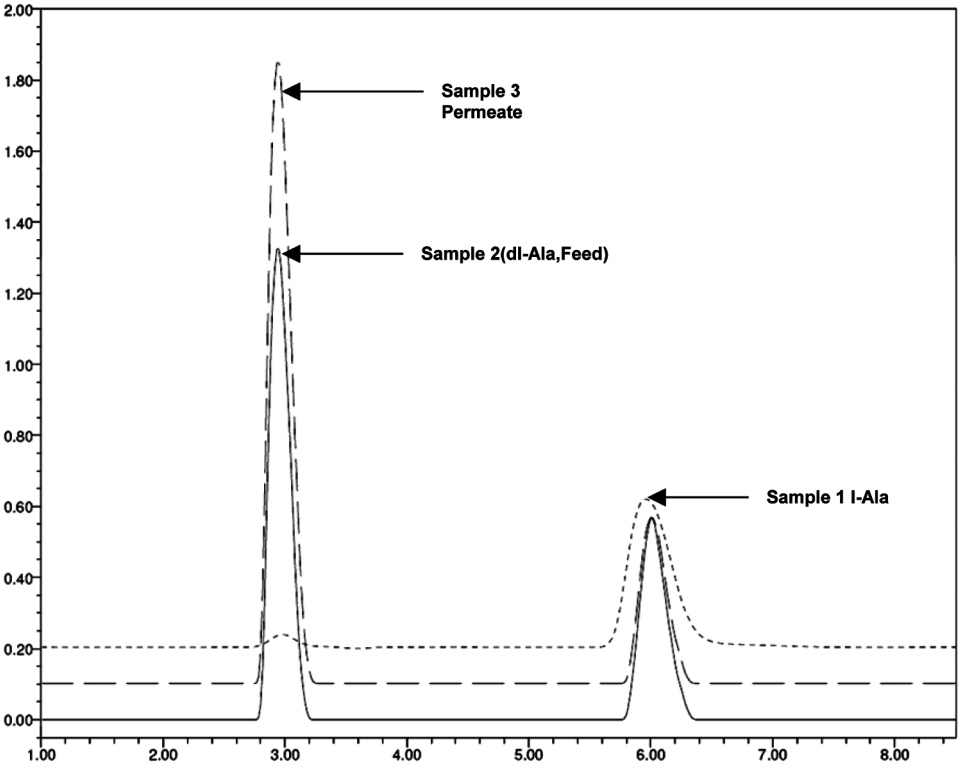
FIG. 5. HPLC Chromatograms of l-alanine (standard), racemic alanine (feed solution), and permeate.



arginine is rejected ~38 to 41% whereas alanine at similar concentration and pressure is rejected ~52–59%. The rejection of the solute by a nanofiltration and reverse osmosis membranes is well described by a solute-diffusion model and is chiefly governed by the interaction of the solute with the membrane material (32). Higher interaction between the membrane and the solute at the membrane surface facilitates the diffusion of the solute through the membrane. The top layer of the membrane is homo-chiral for arginine molecules hence arginine molecules interact preferentially resulting in fast diffusion through the membrane. The fast diffusion of arginine through the membrane is the cause of low rejection compared to the rejection of alanine because the membrane did not interact preferentially with alanine, hence is rejected more. Further, rejection is higher at low permeation pressure, for example the rejection of alanine from 5.7 mM concentrated feed solution at 517.1 kPa pressure is 58.8% whereas at 1034.21 kPa pressure rejection is 63.1% for the same feed solution, i.e., 7.3% increase in rejection by a two-fold increase in trans-membrane pressure. The rejection of amino acids is time dependent and decreases rapidly up to 6 hours, thereafter rejection is practically stable.

## Enantioselective Permeability

The variation in concentrations of d- and l-enantiomers of arginine and alanine in permeate is given in Figs. 11 and 12 respectively. It is seen that the concentration of d-enantiomer in the permeate is more compared to l-enantiomer which is indicative of preferentially permeation of d-enantiomer. This is indicative of the enantioselective permeability of the membrane to d-enantiomers of arginine and alanine. It is evident from observations that the enantio-selectivity of the membrane for d-arginine is




**Peak report**

| Sample details | Name of Peak | Retention Time | Area Percentage |
|---|---|---|---|
| **Sample 1 (1000ppm l-ala.)** | l-Alanine | 6.01 | 100.00 |
| **Sample 2 (1000ppm racemic ala., Feed)** | d-Alanine | 2.94 | 52.80 |
| | l-Alanine | 6.00 | 47.20 |
| **Sample 3 (Permeate)** | d-Alanine | 2.94 | 64.70 |
| | l-Alanine | 6.01 | 35.30 |

FIG. 6. HPLC Chromatograms of l-arginine (standard), d-arginine (standard), racemic arginine (feed solution), and permeate.

higher compared to d-alanine. The enantioselective permeability of membrane attributes enantiomeric excess (%ee) of d-isomers in permeates. The enantiomeric excess (%ee) of d-enantiomers in permeates as a function of permeation time for 5.7 mM and 11.4 mM concentrated solutions of racemic arginine and alanine at 517.1 kPa and 1034.21 kPa pressures is given in Fig. 13. It is observed that the enantioselective permeability increases with time as a result of higher enantiomeric excess which is achieved as time progresses. Further higher enantiomeric excess is achieved at low permeation pressure and higher feed concentration. The highest enantiomeric excess achieved is 54% for 11.4 mM concentrated solution of arginine at 517.1 kPa pressure after 10 hrs of permeation.

The characteristic ''Enantio-selectivity'' in membrane is essential to resolve optical isomers. It is well known that optical isomers can be distinguished in a chiral environment only. The composite membrane distinguished enantiomers and permeates d-enantiomers preferentially by the interaction between the chiral environment present in the membrane and the enantiomers. Metal Schiff-base complex of arginine incorporated in the top layer of the membrane responsible for chiral environment in the membrane as a result of which the composite membrane exhibit enantioselective property and capable of resolving racemic mixtures of arginine and alanine. According to the 3-point interaction rule of chiral recognition in ligand-exchange discrimination between the two enantiomers is achieved by reversible complexion of metal ions with a chiral complexing agent, typically α-amino acids (33). One of the two complexes which have three points on the plane is more stable than the other one (34). The selective layer of the composite membrane consists of a metal complex having chiral ligands (l-arginine) hence the enantiomeric

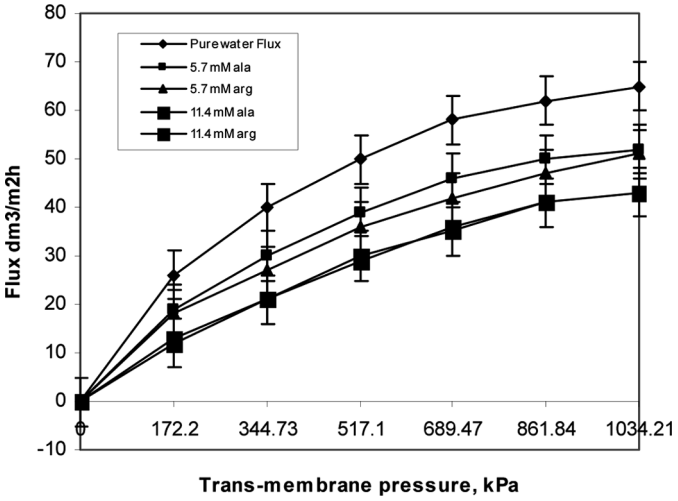



FIG. 7. Variation in volumetric flux ($J_V$) of membrane with trans-membrane pressure.

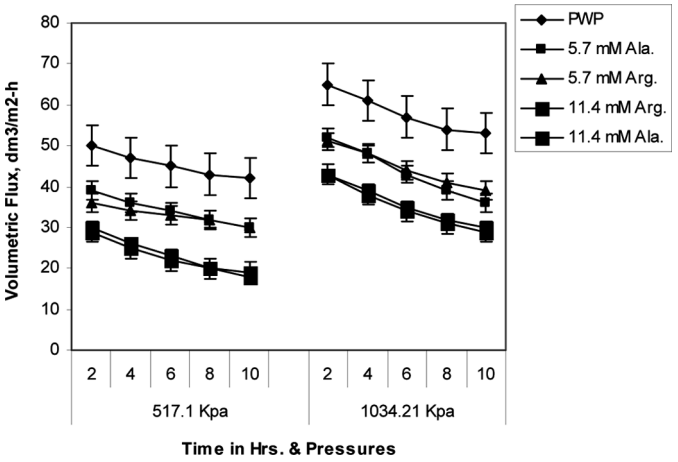



FIG. 8. Variation in volumetric flux of membrane with time.

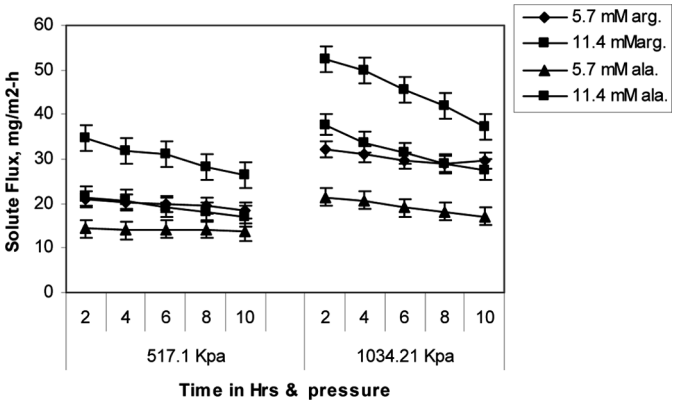



FIG. 9. Variation in solute flux ($J_s$) of arginine and alanine with time at 517.1 kPa and 1034.21 kPa pressures.

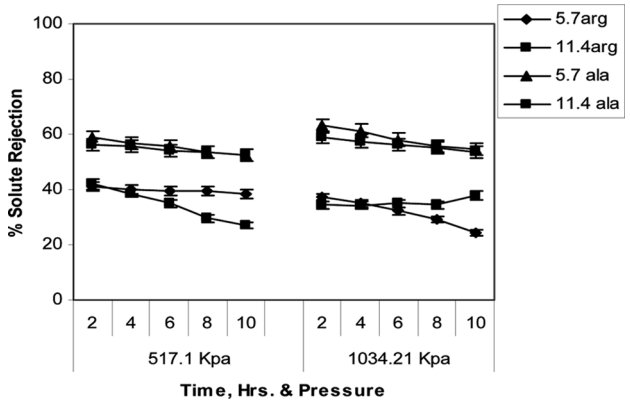



FIG. 10. Variation in solute rejection with time for 5.7 mM and 11.4 mM concentrated solutions of racemic arginine and alanine at 517.1 kPa and 1034.21 kPa pressures.

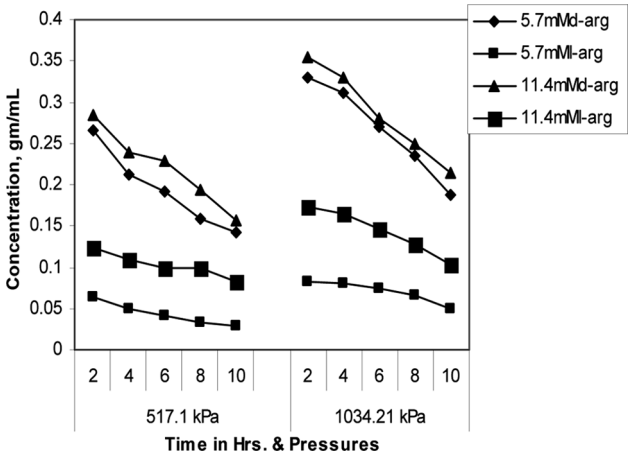



FIG. 11. Variation in concentrations of d- and l-enantiomers of arginine in permeate with time.

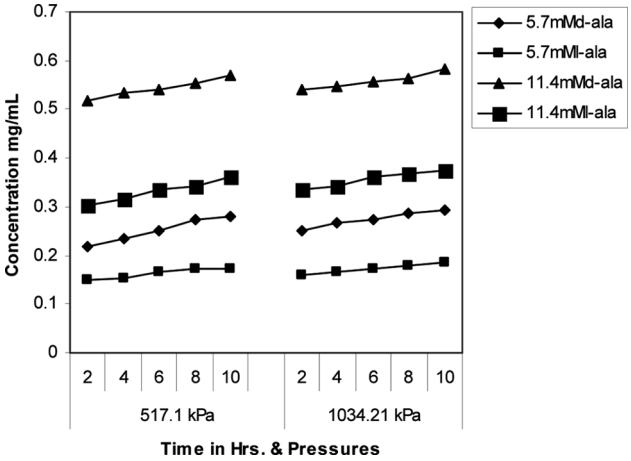



FIG. 12. Variation in concentrations of d- and l-enantiomers of alanine in permeate with time.

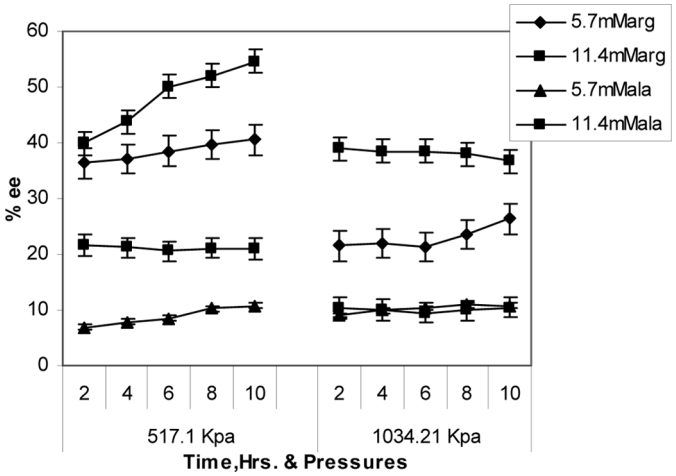



FIG. 13. Enantiomeric excess (%ee) of d-isomers with time for 5.7 mM and 11.4 mM solutions of racemic arginine and alanine at 517.1 kPa and 1034.21 kPa pressures.

separation is expected to be performed by forming a complex between metal ions present on the membrane and permeating enantiomers of α-amino acids. First, the disalicylaldehyde Schiff base ligand and bivalence metal ion ($Zn^{++}$) forms a reversible complex with permeating molecules by the hydroxyl and amino group. The complex formed by l-enantiomer is comparatively more stable, whereas the d-enantiomers form feebly, hence permeated preferentially.

Enantioselective property of the membrane is also described by a factor called separation factor (α), the ratio of two enantiomers in the permeate. The separation factor as a function of permeation time for 5.7 mM and 11.4 mM concentrated solutions of racemic arginine and alanine at 517.1 kPa and 1034.21 kPa pressures is given in Fig. 14. It is observed that the separation factor is high for arginine (α is 6.84) compared to alanine (α is 1.71). Higher separation factor is achieved at low permeation pressure compared to high permeation pressure (α is 6.84 at 517.1 kPa pressure against 3.83 at 1034.21 kPa). The separation factor remains more or less constant (during permeation time up to 10 hrs.) considering the range of practical errors. The separation factor is indicative of enantioselective property of membrane which is preferentially permeable to d-enantiomers, therefore the ration of d-isomer to l-isomer is higher in permeate.

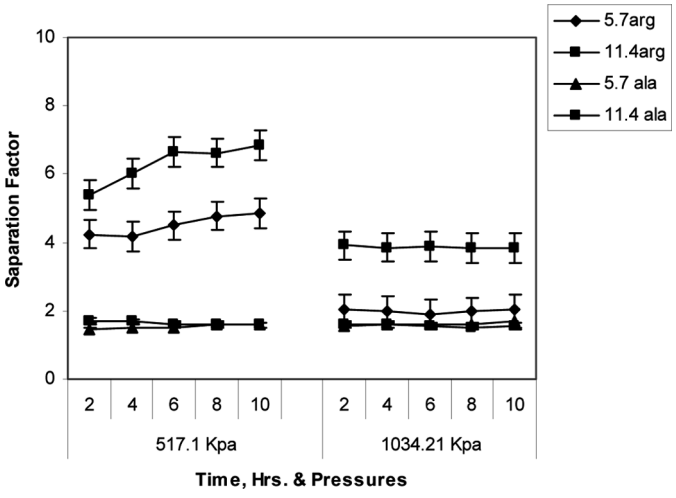



FIG. 14. Variation in separation factor with time for 5.7 mM and 11.4 mM solutions of racemic arginine and alanine at 517.1 kPa and 1034.21 kPa pressures.

## CONCLUSIONS

The study concludes that metal-Schiff base complexes can be incorporated in the membrane to develop enantioselective property. The composite membrane fabricated by in-situ interfacial copolymerization of the metal-Schiff base complex and piperazine with trimesoyl chloride on the top of polysulfone membrane has an enantioselective property which is attributed to the presence of chiral environment which is essential to achieve enantioselective property. The composite membrane permeates preferentially d-enantiomers of α-amino acids arginine and alanine. The membranes exhibit higher enantio-selectivity (54%) for arginine compare to alanine (21%). The enantio-selectivity of the membrane shows time dependency and increases with time. In addition to the enantioselective property the membranes also exhibit separation property and reject α-amino acids arginine and alanine from their aqueous solution. The membrane shows higher rejection for alanine compare to arginine. Though %ee achieved is less, however, nanofiltration being a pressure driven process the productivity is high and continuous. This kind of membrane might be useful for multistage process to achieve a high degree of enantioselectivity using permeate of the first stage which is 54% enantiomeric rich as feed for the second stage. The permeation of the second stage is expected to have higher %ee.

## ABBREVIATIONS

| | |
|---|---|
| $J_V$ | Volumetric flux |
| $J_s$ | Solute flux |
| %ee | Enantiomeric enrichment or excess |
| α | Separation factor |
| $C^{df}$ | Concentrations of minor permeating isomer in the feed |
| $C^{lf}$ | Concentrations of major permeating isomer in the feed |
| $C^{lP}$ | Concentrations of minor permeating isomer in permeate |
| $C^{dP}$ | Concentrations of major permeating isomer in permeate |

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