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# Optical resolution of racemic tryptophan through non-chiral membranes by ultrafiltration using chiral selector in solution

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Optical resolution of racemic mixture of tryptophan has been performed by ultrafiltration in solution containing bovine serum albumin (BSA) as a chiral selector, using non chiral polysulfone (hydrophobic) and polyamide (hydrophilic) membranes. The effectiveness of optical resolution was characterized in terms of enantiomeric enrichment (%ee) in permeate. Solute flux and enantiomeric enrichment of two different membranes were compared. Effects of solute and bovine albumin (chiral selector) concentration in feed solution, *p*H of the feed and operating pressure were studied in detail to optimize the enantiomeric enrichment process. It has been observed that enantioselectivity of ultrafiltration process is strongly *p*H-dependent and reaches a maxima at feed *p*H 9.0–9.2. The solute flux (racemic tryptophan dissolved in the liquid) as well as permeate flux (solution consisting racemic tryptophan, BSA and buffer) decreases but %*ee* increases with permeation time, which indicates that concentration polarization effects the enantioselectivity of the process adversely. Enantioselectivity increases with the BSA content in the feed solution. Optical resolution of tryptophan racemic mixture upto 33 %ee in single pass, at a permeate flux of 9 l/m$^2$-h was obtained.

**Keywords:** Enantioselectivity, Enantiomer enrichment, Ultrafiltration, Bovine serum albumin, Chiral selector
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Significant number of organic compounds such as amino acids, drugs, pesticides, insecticides etc. are optically active molecules and exist as a pairs of isomers. Their biological activities are largely influenced by stereo-chemical factors. For example, potency of certain drug molecules lies in one enantiomer, as *d*-isomer of amphetamine (a drug used as central nervous system stimulant) is several times more potent than *l*-isomer; *l*-isomers of codeine, morphine and heroin are potent analgesics whereas their *d*-isomers do not posses analgesic properties. Enantiomeric separation of racemic mixture is one of the major routes to obtain desired isomer. Enantioselective separation originated from the resolution of sodium ammonium tartarate in 1848 by Pasteur[1]. Conventional optical resolution methods such as fractional crystallization[2], microbiological methods[3], kinetic enzymatic resolution technology[4] and high performance liquid chromatography[5] are batch processes, and only a small amount of optically active compound can be treated in one batch operation. The membrane separation technique is simple and can be operated continuously. Membrane separation process has several other advantages *viz.*, modular, energy efficient etc., over conventional optical resolution methods mentioned above. Optical resolution by membranes was first investigated with liquid membranes containing chiral crown ethers[6-8]. But the stability and durability of liquid membranes are poor as compared to those of polymeric membranes. There have been pioneering reports on solid polymeric membranes for optical resolution[9-16]. Plasma-polymerized membranes of *d*-camphor and *l*-menthol[9,10], polymeric membranes having cyclodextrin moieties[11], poly (amino acid) membranes[12], polymer hollow fibre membranes[13] and molecular imprinted polymeric membranes containing DIDE derivatives[14,15] have been employed for optical resolution (DIDE is the polystyrene resin *DIDE-resin*, diimide-diepoxide). However, in majority of studies optical resolution were carried out in the dialysis mode. Recently ultrafiltration technique has been employed for optical resolution using chiral selective ligand in the solution[16-18]. Non chiral polysulfone membranes and modified polysulfone membranes have been applied for the enantiomer separation by ultrafiltration[16,17]. Poncet *et al.*[17] have used serum albumin as chiral selector for racemic tryptophan resolution using PES membrane. Romero and Zydney[18] have reported ultrafiltration of tryptophan

using bovine serum albumin and observed strong binding of l-tryptophan at low concentrations. Higuchi *et al*.[19] studied enantiomeric separation of amino acids in solution using ultrafiltration. However, the optical resolution by a membrane process is just in the beginning stage and has to be improved much more for its practical use. Here, the optical resolution of racemic tryptophan by ultrafiltration process in a solution system containing bovine serum albumin as chiral selector using hydrophobic polysulfone and hydrophilic polyamide membranes has been reported.

## Experimental Procedure

### Materials

*Bovine Serum Albumin* (BSA) molecular weight 69,000 Dalton (fatty acids free, 98% pure) was obtained from S D Fine Chemicals Ltd., Mumbai (India). Racemic tryptophan, *d*-tryptophan and *l*-tryptophan were procured from Sigma Chemical Company Ltd., (USA). Other chemicals and reagents used in the study were AR grade.

### Membrane fabrication and characterization

Ultrafiltration membranes were fabricated in the laboratory from polysulfone 'Udel 3500' (Union Carbide Inc., USA) and polyamide 'Nomex fiber grade' (Dupont de numerous). Polymers were dissolved in appropriate solvents in required concentration and membranes were fabricated by wet phase inversion method. Molecular weight cut off (MWCO) values of membranes were determined by Gel Permeation Chromatography (GPC) using 0.1% solution of dextran (as solute) and pore size analysis of the membranes were done on Capillary Flow Porometer (Porous Materials Inc., USA). Detailed characteristics of the membranes are given in Table 1.

### Ultrafiltration experiments

Ultrafiltration experiments were conducted in dead end mode on ultrafiltration kit model 402 (Amicon, USA), at different pressures (1–3 kg/cm$^2$). UF cell volume was 400 mL and it can accommodate flat circular shape membrane of diameter 7.8 cm having an effective membrane area of 47.75 cm$^2$. UF cell is connected with a solution reservoir to keep cell volume constant and is placed on magnetic stirrer whose rpm can be controlled.

Bovine serum albumin (0.1% *w/v*) dissolved in reverse osmosis water of conductivity 40-50 micro ohms (*p*H of the solution was adjusted by citric acid-sodium hydrogen phosphate and borate buffers) was passed through the membranes at 1, 2 and 3 kg/cm$^2$. It was observed that bovine serum albumin does not pass through the membrane. Concentration of BSA in permeate in absence of tryptophan was determined spectrophotometerically at its $\lambda_{max}$ (280 nm). The required amount of racemic tryptophan was added into bovine albumin solution and stirred at fixed rotation for 6 h before conducting ultrafiltration experiment. Concentrations of *d*-tryptophan and *l*-tryptophan in permeate were estimated on HPLC (Waters) fitted with Photodiode array detector at 278 nm using Crown pack column (CR+, Diacel Chemical Co.)

## Results and Discussion

For a membrane process, the membrane selectivity and flux are the two important parameters those exhibit membrane performance and productivity. In an enantiomeric separation process, the selectivity is the measure of optical purity of enantiomer. Optical purity is defined in terms of % enantiomeric enrichment or excess (%ee)

$$\%ee = \frac{\begin{array}{l}(\text{Conc. of major isomer inpermeate}) - \\ (\text{Conc. of minor isomer inpermeate})\end{array}}{\begin{array}{l}(\text{Conc. of major isomer inpermeate}) + \\ (\text{Conc. of minor isomer inpermeate})\end{array}} \times 100$$

Flux of membrane ($J_s$) explains the measure of productivity of the process, i.e., how fast is the membrane process. It is defined as amount of solute passes through per unit area of membrane per unit time at constant pressure

$$J_S = \frac{\text{Amount of solute}}{\text{Area of the memmbrane* time}}$$

Table 1— Membrane characteristics

| S. No. | Specification | | |
|---|---|---|---|
| 1 | Polymer name and grade | Polysulfone Udel 3500 | Polyamide Nomex fiber grade |
| 2 | Fabrication method | Wet phase inversion | Wet phase inversion |
| 3 | Molecular weight cut off (MWCO) | 20,000 Dalton | 20,000 Dalton |
| 4 | Bubble point diameter (micron) | 0.098 | 0.092 |
| 5 | Pure water permeability (PWP) at 3 kg/cm$^2$ pressure | 136 lm$^2$/h | 124 lm$^2$/h |

**Optical purity**

Optical purity of tryptophan has been determined in terms of % enantiomeric excess (%ee) in permeate. The effect of ultrafiltration time, *p*H, concentration of racemic tryptophan and bovine serum albumin (chiral selector) in feed solution on optical purity and solute flux were investigated in order to optimize the separation parameters.

From Fig. 1 it is evident that *d*-tryptophan is preferentially permeated through membrane and its concentration in permeate is higher than its concentration in feed solution. Both the membranes studied have MWCO values ~ 20 KD far less than the average molecular weight of BSA ~ 69 KD. Due to size exclusion properties of ultrafiltration membranes, theoretically BSA will not pass through these membranes. To confirm it, 0.01 % solution of BSA was passed through the membranes at 1, 2 and 3 kg/cm$^2$ feed pressures. BSA was not detected in permeate. BSA is known to posses several binding sites for hydrophobic amino acids such as tryptophan[20,21] and has high affinity for *l*-tryptophan. The binding constant of BSA with *l*-tryptophan is reported[21] to be $4.4 \times 10^4$ $M^{-1}$. When racemic mixture of tryptophan was added in the BSA solution, *l*-tryptophan molecules preferentially bind with BSA and *d*-tryptophan molecules remains in the BSA solution. Due to high affinity of BSA for *l*-tryptophan molecules, BSA-*l*-tryptophan adduct is formed in the solution. The molecular size of adduct is larger than the pores in the membrane, therefore the BSA-*l*-tryptophan adduct does not permeate through membrane hence, the concentration of free *l*-tryptophan in the feed solution decreases. BSA-*l*-tryptophan adduct is retained by the membrane and free (unbound) *d*-tryptophan along with remaining unbound *l*-tryptophan (because there is no 100% binding) permeate through the membrane. It results in an increase in the *d*-tryptophan concentration and decrease in *l*-tryptophan concentration in permeates and optical purity for *d*-tryptophan is observed in permeates. The enantiomeric separation of racemic tryptophan is resulted due to size exclusion properties of ultrafiltration membranes. With an increase in permeation time, there is an increase in the concentration of *d*-tryptophan in permeate (cumulative) and hence higher optical purity. However, % increase in *d*-tryptophan concentration (incremental) in permeate decreases gradually. Polyamide membrane shows slightly higher purity in comparison to polysulfone membrane due to its higher flux, hence higher concentration of *d*-tryptophan in permeate.

Enantioselectivity of the process is highly *p*H dependent and increases as *p*H of the feed solution increases (Fig. 2). Maximum enantiomeric excess is observed between *p*H 9.0 – 9.2, any further increase in *p*H resulted in the decrease in enantioselectivity. The adduct formation of BSA with tryptophan is reversible and depends on several factors *viz.*, *p*H of the feed, concentration of racemic tryptophan and BSA, reaction time, physicochemical factors etc. The polarity of BSA, being a low molecular weight protein, varies with the *p*H of the solution. At acidic *p*H ~ 2 - 3, it is positively charged, at *p*H 4.8 it is reported to be amphoteric or electrically neutral and at *p*H ≥ 6, it is negatively charged. Its negative charge increases as *p*H increases. At *p*H 6.8, its charge is –17.5 units; at *p*H 7.4 its charge is –22 units[22]. In strong negatively charge state, it has higher complexion with *l*-tryptophan. *l*-tryptophan has maximum complexion with BSA at *p*H~ 9 to 9.2[23,24] since electronegativity favour adduct formation. Therefore, optimum enantiomeric separation is observed at *p*H ~ 9. As the *p*H increases from 6 to 11, the amount of unprotonated BSA sites increases

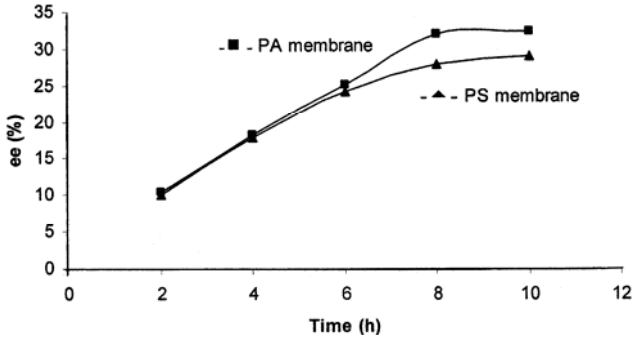


Fig. 1—Change in %ee of *d*-tryptophan with the permeation time; [BSA] = 0.07% (*w/v*), *p*H = 9.2 and racemic tryptophan = $3 \times 10^{-3}$ % (*w/v*).

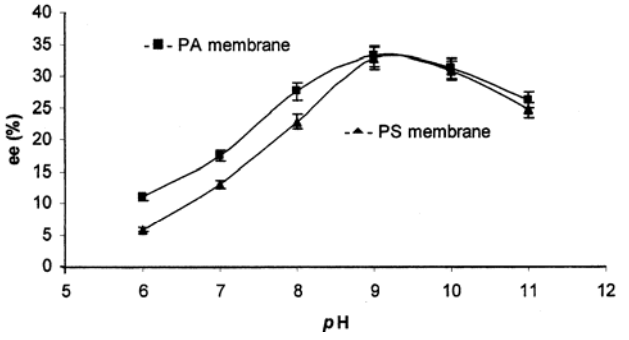


Fig. 2—Change in %ee of *d*-tryptophan with the *p*H of the feed solution; [BSA] = 0.07% (*w/v*), and racemic tryptophan = $3 \times 10^{-3}$ % (*w/v*).

leading to an increase of the apparent complexation. But at the same time, the amount of the zwitter-ion form of the amino acid decreases leading to a decrease of the apparent complexation. Below *p*H 9.4, the first effect is predominant whereas above *p*H 9.4 the second effect is predominant. Such opposite variations lead to a maximum of the apparent complexation around *p*H 9.4. Thus, the choice of the *p*H of the feed solution is critical while performing ultrafiltration experiments using the chiral recognition of tryptophan by bovine serum albumin.

Polyamide membrane being hydrophilic and negatively charged in comparison to polysulfone membrane favours complexion between negatively charged BSA and *l*-tryptophan, hence shows enhance enantiomeric enrichment in comparison to poly-sulfone membrane.

At constant BSA concentration in the feed solution (0.07% *w/v*), the concentration of racemic tryptophan in feed solution was varied from 1 x $10^{-3}$ to 6 x $10^{-3}$ % *w/v*. An increase in concentration of racemic tryptophan in feed shows higher optical purity initially at low concentration (Fig. 3). Increase in racemic concentration in feed provides more number of *l*-tryptophan molecules available for complexion with BSA, at the same time, concentration of *d*-tryptophan molecules increases in feed. As a result more *d*-tryptophan permeates through membrane. Further increase in racemic concentration reduces the optical purity due to the site saturation mechanism of BSA.

At constant *p*H (*p*H 9), BSA content in the feed solution was varied from 0.01 to 0.1%*w/v*. Initially enantioselectivity increases and reaches maximum (33%ee) at BSA concentration of 0.07 – 0.08% (*w/v*), thereafter enantioselectivity decreases (Fig. 4). This is due to the fact that with an increase in the concentration of BSA, it complexes with more number of *l*-tryptophan molecules, consequently number of free *l*-tryptophan molecules decreases and concentration of *d*-tryptophan increases in the feed and therefore, more *d*-tryptophan and fewer *l*-tryptophan molecules passes in permeate. As a result higher enantiomeric enrichment is observed. Further, the decrease in enantioselectivity at higher BSA concentration (> 0.08%) is perhaps due to the saturation effect. At higher BSA concentration protein-protein interaction seems predominant and protein-tryptophan interaction, which is essential for BSA-tryptophan complexion, is ineffective.

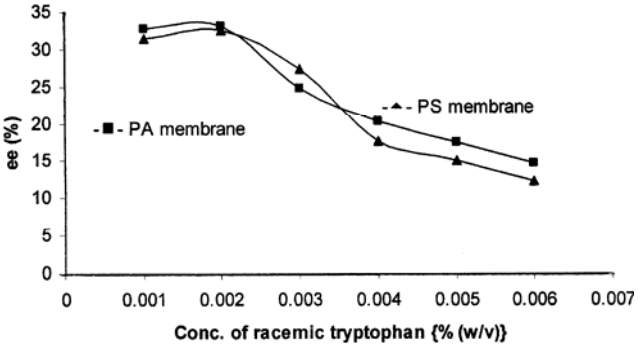


Fig. 3—Change in %ee of *d*-tryptophan as a function of racemic tryptophan concentration in the feed solution at a BSA concentration of 0.07% (*w/v*) and *p*H = 9.2.

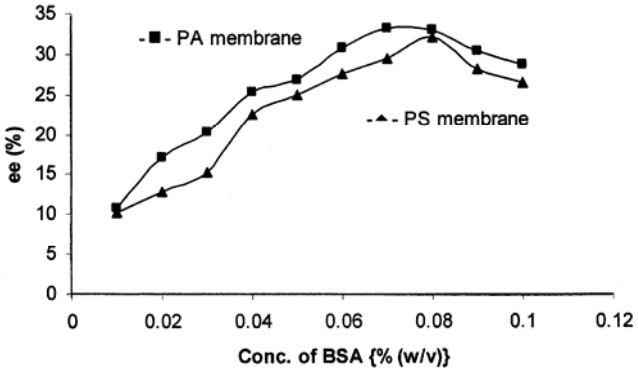


Fig. 4—Change in %ee of *d*-tryptophan with the change in concentration of bovine serum albumin (BSA); *p*H = 9.2 and racemic tryptophan = 3 x $10^{-3}$ % (*w/v*).

**Flux**

Permeability of membranes at different pressures and permeate time at constant pressure, for pure water, BSA solution and BSA-racemic tryptophan solution are given in Figs 5 and 6. Permeability of membranes increases as trans-membrane pressure increases. Permeability of BSA-racemic tryptophan solution is lowest. Permeability of BSA solution is lower than water but higher than BSA-racemic tryptophan solution. Polyamide membrane shows higher permeability for all in comparison to polysulfone membrane. It is because polyamide membrane is hydrophilic in nature and also slightly more negatively charged in comparison to polysulfone. Further, polysulfone membrane being hydrophobic would have higher protein adsorption on the surface that results low membrane permeability. Decrease in flux at higher pressure indicates compaction phenomenon. Polysulfone membrane has more compaction thereby lower permeability at higher pressures than polyamide membrane. Higher liquid permeability of polyamide membrane results in greater optical purity.

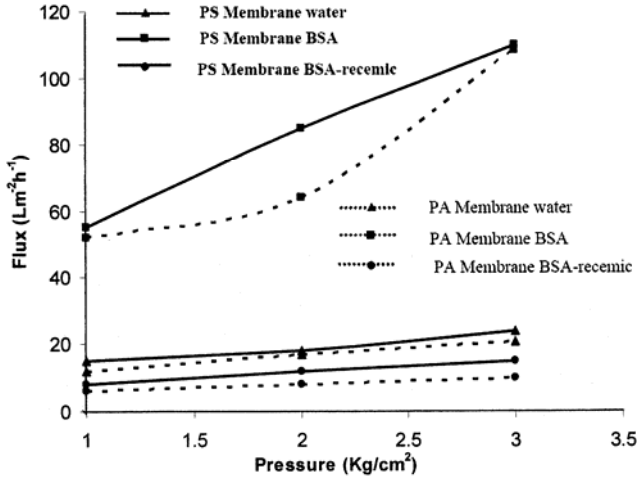


Fig. 5—Permeate flux of membranes for different feeds after 30 min

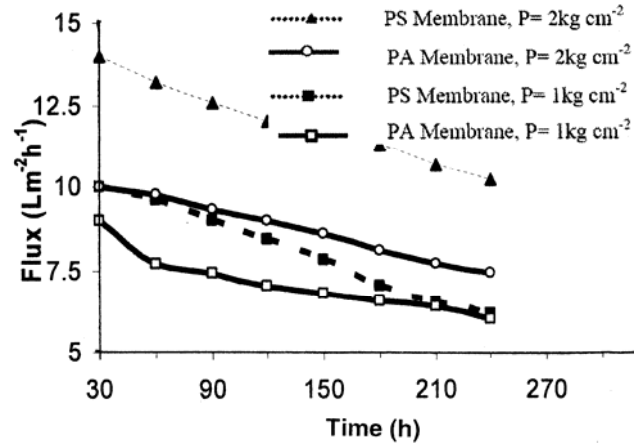


Fig. 6—Permeate flux of membranes with time at pressures 1 and 2 kg/cm$^2$

## Conclusion

The optical resolution of the racemic mixture is possible by ultrafiltration using BSA as chiral selector in solution due to size exclusion properties of ultrafiltration membranes. The enantioselectivity is strongly *p*H dependent and maximum selectivity is observed at *p*H 9.0 – 9.2. At constant *p*H and BSA concentration, any increase in concentration of racemic tryptophan in feed solution shows higher optical purity, moreover as concentration of racemic tryptophan increases optical purity deceases. Increase in BSA content in the feed solution increases enantioselectivity initially and reaches maxima (33%ee) at BSA concentration of 0.07 – 0.08% (*w/v*) Permeability of membranes increases as trans-membrane pressure increases from 1 to 3 kg/cm$^2$. Polyamide membrane shows higher permeability and enantioselectivity than polysulfone membrane.

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