Korean J. Chem. Eng., **31**(12), 2109-2123 (2014)
DOI: 10.1007/s11814-014-0284-z
pISSN: 0256-1115
eISSN: 1975-7220

REVIEW PAPER

Methods for separation of organic and pharmaceutical compounds by different polymer materials

Pravin Ganeshrao Ingole*,† and Neha Pravin Ingole**

*Korea Institute of Energy Research, 71-2, Jang-dong, Yuseong-gu, Daejeon 305-343, Korea
**Department of Biology, Chungnam National University, Daejeon 305-764, Korea
(*Received 27 May 2014 • accepted 23 September 2014*)

Abstract−The discrimination of enantiomers is a challenging task in separation technology, and using a membrane is most promising for separating enantiomers from racemic mixture. The optical resolution of chiral compounds is of interest to researchers working in a variety of fields from analytical, organic and medicinal chemistry, to pharmaceutics and materials, to process engineering for fabricating pharmaceuticals, agrochemicals, fragrances and foods, and so on. There is considerable demand for separation techniques appropriate for the large-scale resolution of chiral molecules. The separation of chiral compounds using chiral or achiral/non-chiral polymeric membranes with or without chiral selector represents a promising system for future commercial applications. This review focuses on an active field of chiral separation, membrane-based enantioseparation technique, which has potential for large-scale production of single-enantiomers. Enantiomeric separation by membrane processes has been studied using various configurations of liquid and solid polymer membranes. Selectivity and permeability of liquid-membranes is reasonably good because the rate of diffusion of solute molecules is high in liquids but has inferior durability and stability. Solid polymer membranes have inferior permeability because diffusion of solute through solids is slow but quite stable and durable; however, commercial application of membrane technology for optical resolution is yet to be realized. Several chiral separation membranes were prepared from chiral polymers where enantioselectivity was generated from chiral carbons in the main chain. However, it is rather tricky to generate excellent chiral separation membranes from chiral polymers alone, because racemic penetrants mainly encounter the flexible side chains of the membrane polymers.

Keywords: Chiral Separation, Optical Resolution, Enantioselective Polymeric Membranes, Pharmaceutical

INTRODUCTION

Many pharmaceutical, drug and flavor compounds are racemic mixtures. However, it is well known that only one of the two enantiomers performs the required biological action or what we called practical action. The surplus one, which means an impurity, may cause unwanted side effects. Therefore, chiral resolution becomes a very important separation process, particularly in the field of medicine and agriculture chemicals [1,2]. Enantiomers of a molecule have identical physical properties such as melting point and vapor pressure with one exception: they scatter polarized light differently [3]. For example, if linearly polarized light passes through a solution of chiral molecules (all of the same enantiomer), the plane of polarization will rotate. Most importantly, the two enantiomers of a molecule will rotate the plane of polarization in opposite directions. This phenomenon is called optical rotation and the compounds possessing this property are called as optically active. Enantiomers are most commonly formed when a carbon atom contains four different groups or atoms.

Chirality plays an important role in human life. The best example of chiral influence is given by nature itself. Most recognition systems in nature (e.g., enzymes, receptors) [4-11] distinguish pairs of enantiomers. The majority of biologically active molecules, including naturally occurring amino acids and sugars, are chiral [12,13]. Enantiomers of a drug have similar physicochemical properties but differ in their biological properties [14,15]. The distribution, metabolism and excretion in the body usually favor one enantiomer over the other because enantiomers stereo-selectively react in biological systems. Enantioselective HPLC-DAD method is used for the determination of etodolac enantiomers in tablets, human plasma and application to comparative pharmacokinetic study of both enantiomers after a single oral dose to twelve healthy volunteers [16]. Furthermore, biological transformation of drugs can be stereoselective, so the enantiomeric composition of chiral compounds may be changed. Additionally, due to different pharmacological activity, chiral drugs can differ in toxicity [17]. Thalidomide is an excellent example, due to the administration of the racemic thalidomide to pregnant women during 1960's thousands of babies were born with physical deformities. (+)-enantiomer of thalidomide is harmless (has tranquilizing properties) but (−)-enantiomer is teratogenic and leads to malformations of embryos if administered to pregnant woman. Unfortunately, many chiral drugs are still produced as racemate because either their chiral separation is difficult, or the cost of their stereoselective synthesis is too high, or simply at the time of the discovery of the drug, only racemic mixture was considered in the animal; and the clinical pharmacology, toxicology and teratology studies and knowledge of pharmacodynamic, pharmacokinetic or toxicological properties of individual enantiomers is still limited [18]. The facile synthesis of nanophase separated amphiphilic polymer conetworks allows the preparation of chiral membranes with precise mesh

†To whom correspondence should be addressed.
E-mail: ingolepravin@gmail.com

size and morphology. These chiral resolution model membranes can be used in virtually every solvent. Further, the solvent controls the permeation path of the substrate through the membrane [19].

A broad and serious examination of the role of chirality in new-drug development only began in 1992 after the announcement of a new policy by United States Foods and Drugs Administration (USFDA) for chiral drugs, wherein the testing of optically pure enantiomers of a chiral drug was made necessary for their efficacy [20]. Regulatory agencies throughout the world are currently reviewing the importance of chirality with regard to pharmaceutical and agrochemical products [21-26]. New guidelines from such agencies have been key drivers for the focus on single enantiomer products in these industries. The need for administration of the optically pure active drug "eutomer" has been realized; therefore, the USFDA has indicated that each enantiomer of a chiral drug should be tested independently for its therapeutics action and efficacy [27]. Recently, dynamic high performance liquid chromatography on chiral stationary phases has been used at low temperature separation of the interconverting enantiomers of diazepam, flunitrazepam, prazepam and tetrazepam [28]. These scientific and regulatory developments have created the necessity for new development in various technologies for isolating, preparing and utilizing optically pure compounds in commercial applications.

In this review article, we wish to present the theoretical as well as practical background regarding enantiomeric separation. The modifications of polymer membranes through different techniques are discussed. Simply, it aims to give some significant information showing the progress realized in the knowledge of the phenomena, which shows the performance of chiral selective membranes. This review study will help in designing the polymer back-bone for membrane and membrane fabrication technique.

OPTICAL RESOLUTION

Isolation of optically pure enantiomers is important in the pharmaceutical, agrochemical, fragrance, and food additive industries, since chirality plays a crucially important role in biological processes. Earliest optical resolution was performed by Pasteur in 1848, when he observed that sodium ammonium salts of tartaric acid crystallize into right handed and left handed crystals, and then he used a microscope and tweezers to separate the crystals from each other. After the separation of enantiomers of sodium ammonium salts of tartaric acid physically by Pasteur, researchers devised a number of methods for optical resolution, as it has been perceived as an important method of preparation of optically pure isomers of a compound. The various methods were developed for optical resolution and still new methods are being investigated [29,30]. In recent years extensive development has taken place in the enantiomeric separation and analysis of chiral compounds due to increased demand of chiral pure compounds in drug development [31]. The most popular separation techniques include diastereomeric crystallization, [32, 33] biocatalysis, chromatographic techniques (thin layer chromatography, gas chromatography, super- and sub-critical fluid chromatography, nano-LC, high-performance liquid chromatography), affinity electrokinetic chromatography, electromigration techniques, capillary electrophoresis (CE) techniques such as capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC),

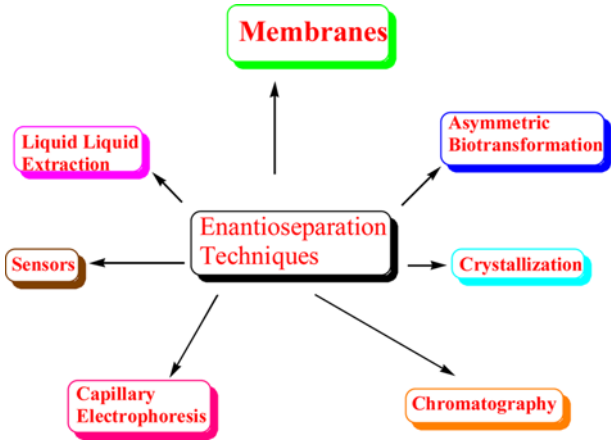

Fig. 1. Enantiomer separations techniques.

microemulsion electrokinetic chromatography (MEEK), moving bed chromatography [34-36]. The presence of homo-chiral environment "prerequisites" is must for the separation of enantiomers; therefore, all methods of chiral separation make use of chiral environment to distinguish paired enantiomers of a compound. The commonly used optical resolution methods include preferential crystallization; microbiological methods, kinetic resolution, capillary electrophoresis and enantioselective chromatography [37-49] are shown in Fig. 1.

Optical resolution of enantiomer is in demand in pharmaceuticals, food products and as ligands in chiral synthesis [50]. The conventional optical resolution methods are batch processes which can resolve only small amount of racemate in single operation; moreover it requires chiral reagents or solvents for resolution which cannot be recovered in some cases and in some case recovery is not economically viable. Hence optical resolution by traditional methods is expensive. Developing a versatile chiral separation method is an emerging field of research in separation science. Membrane separation processes are technically suitable for commercial applications such as continuous operation mode, easy adaptation to different production-relevant process configurations, convenient up-scaling and, in most cases, ambient temperature processing. Therefore, membrane based separation processes are highly applicable for enantiomeric separation at large scale.

MEMBRANE SEPARATION TECHNOLOGY

A membrane is a thin film considered to be barrier which selectively passes some components from one side to another side. Semi permeable membrane has been known since 1748, when Abbe Nollet observed that water diffuses from dilute solution to more concentrated solution when separated by parchment paper (semipermeable membrane) [51]. In 1855, Adolf Fick developed the first synthetic membrane from nitrocellulose and postulated Fick's Law of diffusive flux [52]. In a real sense membrane technology had its beginning in the 1960's with the development of asymmetric membrane from cellulose acetate at University College of Los Angles by Leob and Sourirajan. The development of thin film composite membrane during the 1970's by Cadotte et al. at Film Tech Co. USA opened wide vistas for membrane separations processes. Initial appli-

cations of membrane technology were limited to water desalination. During last three decades membrane-based separation processes have realized wide applications in chemical process industries and replaced conventional separation processes [53].

Solid polymer membranes can be broadly classified into the following three types: symmetric, asymmetric and composite.

1. Symmetric Membranes

Symmetric membranes are also known as homogeneous membranes. Such membranes may be dense and microporous; however, their morphological structure is homogeneous throughout the thickness of the membrane. Separation is achieved under the influence of a pressure difference across the membrane or concentration gradient or electrical potential gradient. Using these membranes, chemical species of similar size and diffusivity can be separated efficiently when their concentrations differ significantly. Symmetrically porous membranes have cylindrical, sponge, web or slit-like structure. Common methods of their preparation are irradiation, stretching of a melt-processed semi-crystalline polymer film, vapor-induced phase separation and temperature-induced phase separation. A schematic presentation of symmetric membranes of different morphology is given in Fig. 2. Dense symmetric membranes with thicknesses greater than 10 μm can be made by solution casting and subsequent solvent evaporation or by melt extrusion. As these membranes are relatively thick, they are highly useful in gas separations. Dense symmetric membranes are frequently used in electrodialysis applications.

2. Asymmetric Membranes

Asymmetric membranes are also known as heterogeneous membranes. An asymmetric membrane consists of a very thin (0.1-1.0 micron) and dense skin layer on a highly porous (100-200 microns) thick substructure (bottom layer). The thin skin acts as the selective layer. The pores in the bottom layer of the membrane are relatively larger than those of the skin layer. Material passing through fine pores of the skin layer can readily be transported through the open-sponge-like structure of the bottom layer. Its separation characteristics are determined by the nature of membrane material or pores sizes; and the mass transport rate is determined mainly by the skin thickness. Porous sub-layer acts as a support for the thin, fragile skin and has little effect on the separation characteristics (Fig. 3). Asymmetric microporous membranes are mostly described by their nominal molecular weight cut-off (MWCO); the membranes have more than 90% rejection for the smallest molecular weight species.

Asymmetric membranes can be further classified based on the pore sizes into the following groups:

2-1. Microfiltration (MF) Membranes

MF membranes contain pores of approximately 0.03 to 10 microns. The MWCO of these membranes is greater than 100,000 Daltons. These membranes are capable of removing particulate materials like sand, silt, clays, Giardia lamblia and Cryptosporidium cysts, algae and some bacterial species.

2-2. Ultrafiltration (UF) Membranes

UF membranes consist of pores of approximately 0.002 to 0.1 microns. Their MWCO value is approximately 10,000 to 100,000 Daltons. These membranes are most commonly used to separate sugars, bio-molecules such as proteins, enzymes, polymers and colloidal particles.

2-3. Nanofiltration (NF) Membranes

NF membrane contains pores in the range of 0.0001 micron and smaller. These membranes can remove the smaller solute molecules, including divalent and multivalent ions. The MWCO for nanofiltration membranes is approximately 200 to 1000 Daltons.

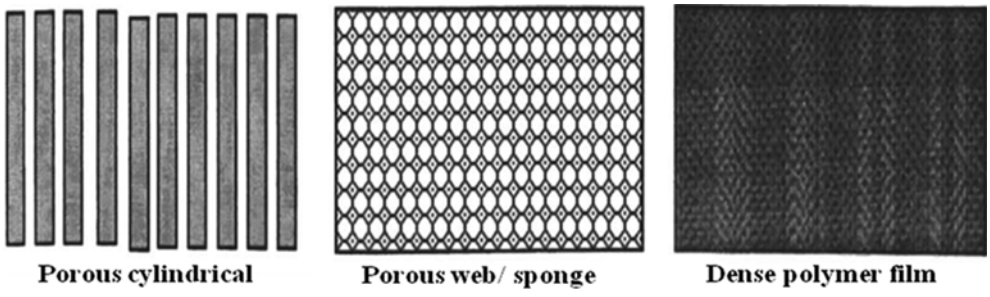

Fig. 2. Schematic presentation of symmetric membranes.

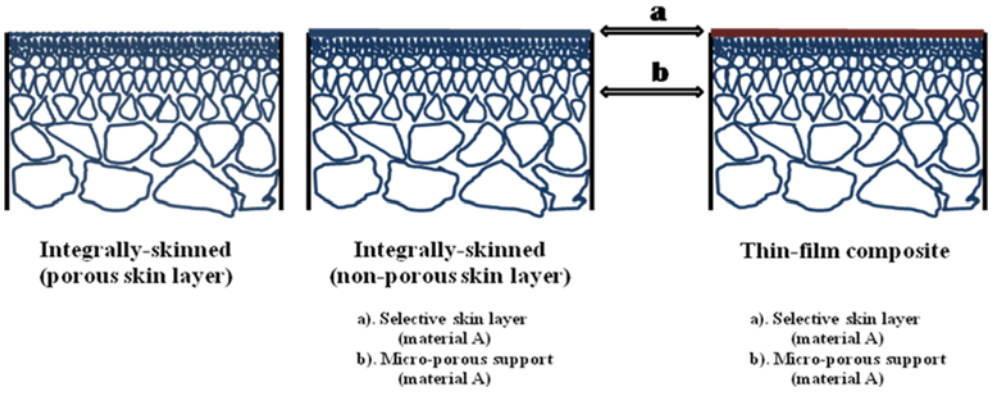

Fig. 3. Schematic presentation of asymmetric membranes.

2-4. Reverse Osmosis (RO) Membranes

RO membranes have a dense barrier layer in the polymer matrix where most separation occurs. The pores in a reverse osmosis membrane are very fine and considered to be of approximately 0.0005 micron. The membrane is designed to allow only water to pass through this dense layer while preventing the passage of solutes (such as salt ions).

3. Composite Membranes

Thin film composite (TFC) membranes are specific membranes made up of more than one material and also consist of more than one layer (at least two layers), a porous nonselective support layer and an ultra-thin selective barrier layer on top of micro-porous support. The layers of composite membranes are normally made up of different materials and are prepared in different operations. The thin film composite membrane was first developed by Cadotte et al. at Film Tech Co. USA during the 1970's via interfacial polymerization of two reactive monomers dissolved in two immiscible solutions on the top of polysulfone support (UF membrane) [54,55].

OPTICAL RESOLUTION BY MEMBRANES

Membrane separation processes are technically suitable for large-scale applications as they have the necessary features for commercial applications: continuous operation mode, easy adaptation to different production-relevant process configurations, convenient up-scaling and, in most cases, and ambient temperature processing [56]. Therefore, membrane processes are highly relevant for preparative scale enantiomeric separation [57-65]. Optical resolution by membranes is a relatively new application of membrane technology that had its beginning with the pioneering work of Cram et al. [66] of the University of California at Los Angeles. Lehn et al. [67,68] in 1973 reported the transport of amino acids and dipeptides through bulk liquid membrane separating two aqueous phases using charged lipophilic carriers and predicted that chirospecific transport allowing separation of racemic mixtures may be envisaged using either an optically active membrane phase or a chiral carrier. At the same time Cram et al. [69] demonstrated chiral recognition properties of optically pure crown ethers. These two observations laid the foundation of enantioselective extraction in bulk organic liquids. Cram demonstrated efficient enantiomeric separation using optically pure 22 membered rings system containing six roughly coplanar ether oxygen's regularly spaced by attachment to one another through four ethylene units (chiral host) and racemic mixtures of chloride and bromide salts of amino acids (guests) with the help of 'W' shaped assembly named as catalytic resolution machine [70-74]. Pirkle et al. developed a prototype membrane device using silicone rubber tubing (0.063 OD and 0.030 ID) wrapped around two spools; each spools was immersed in separate temperature controlled baths containing 4 : 1 water - methanol solution [75-77]. Dodecane was pumped through silicon tubing slowly at the rate of 1 mL/min. Racemic N-(3,5-dinitrobenzoyl) amino ester or amide was dissolved in an upstream source kettle. N-(3,5-dinitrobenzoyl) amino ester or amide diffused slowly through the walls of tubing and entered in dodecane solution that swept downstream where it diffused to methanol-water solution in the receiving kettle [78]. Transport rate of racemic N-(3,5-dinitrobenzoyl) amino ester in dodecane was very low. They observed different rate of transport for pair enantiomers of various N-(3,5-dinitrobenzoyl) amino acids derivatives in dodecane in presence of (S)-N-(1-naphthyl) leucine octadecyl ester. For example S-enantiomers of N-(3,5-dinitrobenzoyl) leucine n-butyl ester enters nine-times faster than its R-enantiomers. Successful demonstration of highly efficient enantioseparation of amino acid salts by Cram et al. [73] on catalytic resolution machine and enantioselective extraction of amino acids derivatives on prototype membrane unit (as described above) using transport agent ((S)-N-(1-naphthyl) leucine octadecyl ester) by Pirkle et al. [75] gave research momentum in this area.

Chiral separation membranes preferentially allow a specific enantiomer to adsorb or diffuse into the membrane. This specificity is generated by chiral recognition sites in the membranes such as chiral side chains, chiral backbones, or immobilized chiral selectors in polymeric chiral separation membranes. These enantioselective membranes act as selective barriers in the resolution process, and preferentially transport one enantiomer due to the stereospecific interaction between the enantiomer and chiral recognition sites. Adsorption-type enantioselective membranes and membrane-assisted resolution systems with non-enantioselective solid membranes have attracted much attention recently.

Two types of enantioselective membranes have been reported: liquid membranes and solid polymer membranes.

1. Optical Resolution through Liquid Membranes

A typical liquid membrane consists of an organic liquid sandwiched between two aqueous solutions. The analyte is dissolved in one of the aqueous solutions (feed solution). Molecules of the analyte diffuse through organic liquid to another aqueous solution (receiving phase) depending upon the diffusivity of analyte molecules (passive transport). A chiral carrier dissolved in organic liquid forms a complex with one enantiomer at the interface of feed and organic solution. The complex is then transported through the organic liquid, enantiomer is released at the interface of organic liquid and receiving phase, and carrier molecule gets itself free by releasing enantiomer in the receiving phase. Such separation is known as carrier facilitated transport. Carrier-facilitated transport of chiral compounds has been studied extensively using different types of chiral carriers [79-81]. The liquid membranes containing various kinds of chiral selector and enantiomers recognizing carriers such as chiral crown ethers [82], cyclodextrins [83-87], polysaccharides [88], biomacromolecules, [89] and calixarenes [90], were studied. At the interface, one enantiomer forms a complex with the chiral carrier preferentially depending upon the nature of carrier. A hollow fiber supported liquid membrane has been used for the enantioseparation of (R,S)-amlodipine and levocetirizine as well as the effects of thermodynamics on mass transfer prediction have been investigated [91,92]. Although studies on liquid membranes indicated very high enantioselectivity for certain isomers, the liquid membranes showed poor stability due to loss of solvent and carrier with time. Thus membranes lost enantioselectivity with time which restricted their applications at commercial scale. Solid polymer membranes being superior in stability and durability hence are considered more suitable for enantioseparation.

2. Optical Resolution through Solid Polymer Membranes

Based on solid polymer membranes, two different approaches have been documented for optical resolution of racemic mixtures: optical resolution through non-chiral membranes (better known as

membrane-assisted optical resolution); and optical resolution through chiral membranes.

MEMBRANE-ASSISTED OPTICAL RESOLUTION

Non-enantioselective membranes combined with other chiral recognition techniques, referred to as membrane-assisted resolution techniques, are an alternative method for optical resolution. In this technique, non-enantioselective membranes serve as barriers or filtration media to selectively separate optically pure enantiomers from a solution containing a racemic mixture and stereoselective binding agents. Optical resolution is performed using non-enantiomer selective membranes, and separation is achieved using enantiomer recognizing carrier or selector is added in the permeating solution. Higuchi et al. [93] investigated the optical resolution of racemic phenylalanine and leucine in a solution system containing bovine serum albumin (BSA). It was found that D-phenylalanine preferentially existed in the permeant at pH 7.0 due to the binding of BSA to L-phenylalanine, and that the concentration ratio of D-isomer to L-isomer in the permeant increased with a decrease in the feed concentration of the racemate in the solution system. This observation was explained by a site saturation mechanism. A minimum point was observed in the plot of the separation factor vs. feed concentration of leucine for the optical resolution of leucine in the solution system. It is suggested that BSA in the solution has two binding sites to leucine (i.e., binding sites to D-leucine and L-leucine).

1. Optical Resolution through Chiral Membranes

Enantioselective membranes are able to resolve optical isomers due to the presence of chiral recognition sites such as chiral side chains, chiral backbones, and chiral selectors. The enantioselective membranes act as selective barriers in the resolution process, and they selectively transport one enantiomer due to the stereo-specific interaction between the enantiomer and chiral recognition sites, thereby producing a permeant solution enriched with one enantiomer. Optical resolution through a polymer membrane was first demonstrated by Muruyama et al. [42] using a membrane of poly-L-glutamates with amphiphilic n-nonylphenoxy-oligoethyleneglycol side chains. The selectivity of membrane for isomers of tryptophan and tyrosin was demonstrated. Considerable efforts have been made in recent years to develop chiral selective membranes and membrane-based process for optical resolution separation [79,94]. In this approach polymer membranes having inbuilt capability to separate enantiomers were prepared and experimented. Enantioselective solid membranes can be categorized into two kinds: inherent chiral membranes and membranes functionalized with immobilized chiral selectors.

The membranes were prepared from various polymers using different approaches. Inherent chiral membranes are most often fabricated by casting membrane-forming solutions of chiral polymers. The chiral polymers include those with chiral backbones and/or chiral side chains (e.g., polyamino acids, polysaccharides, or polypeptides). Second types of membranes are made of achiral polymers containing chiral selectors are incorporated through different methods including immobilization on the surface, in the pores, or in bulk configuration on base membranes by impregnation, covalent grafting, or molecular imprinting. Many researchers have performed comprehensive investigations on various chiral polymers as membranes for optical resolution [95-103]. Several different macromolecular architectures have been studied in detail:

- Polymers with bulky chiral pendant groups; e.g. pinanyl, on a poly(prop-1-in) backbone
- Blends of chiral polymers with achiral polymers
- Graft copolymers with chiral macromolecular side chains on an achiral backbone
- Polymers with a chiral main chain; e.g. poly(amino acids)

1. Membranes having enantiomers recognizing moiety (chiral selector) immobilized or grafted
2. Membranes based on chiral stationary phase polymers
3. Inherent chiral membranes or membranes made of chiral polymers
4. Molecularly imprinted polymer membranes

1-1. Enantioselective Membranes Having Immobilized or Grafted Chiral Selector/Membrane Reactor

Long et al. reported the production of (S)-ibuprofen acid using lipase-catalyzed hydrolysis reaction in a hollow fiber membrane reactor, as an efficient and cleaner route to obtain single-enantiomer compound [104]. The performance of an enzymatic membrane reactor (EMR) was evaluated by varying operating temperature, organic phase flow rate, aqueous phase flow rate and enzyme loadings. Solid polymer membranes have been prepared by covalently grafting of the selector polypeptide onto various types of membranes for example poly(ethylene terephthalate) particle-track membranes [105,106] by glow discharge. Yang and Hage reported that HSA (human serum albumin) binds with D-tryptophan and L-tryptophan also with (*R*)- and (*S*)-ibuprofen [107]. Based on their observation HAS-based chiral stationary phases were developed for the enantioseparation of tryptophan, ibuprofen, warfarin etc. HSA was immobilized on membranes for enantioseparation of ibuprofen [108]. The protein-immobilized enantioselective ceramic membranes were prepared by chemically immobilizing bovine serum albumin by glutaraldehyde linkage on the surface of a porous ceramic membrane. The membrane was found suitable for the selective separation of tryptophan enantiomers [109].

Bovine serum albumin (BSA) as a chiral ligand was captured uniformly throughout a porous hollow-fiber membrane at a level of 160 mg/g by the polymer chains grafted onto the membrane. Subsequently, BSA was cross-linked with a 0.025% (w/w) of glutaraldehyde in a Tris-HC1 buffer (pH 8) for 4 h. BSA immobilized membranes were used for chiral separation of tryptophan. DL tryptophan in Tris-HC1 buffer (pH 8.0) as a mobile phase was permeated through the cross-linked-BSA-immobilized porous hollow fiber resulted in a separation factor of 12. Thus BSA immobilized hollow fiber is applicable for the chiral separation of enantiomers in a mobile phase in a wide range of pH and organic modifiers [110]. Chiral selectors usually used in liquid membranes, such as N-dodecyl-4(R)-hydroxy-L-proline, were also covalently bound on polysulfone matrix to fabricate enantioselective membranes for separation of propranolol, and a value of 1.1 toward S-Prp at 96 h has been reported [111]. DNA has been discovered to have several novel functions aside from carrying genetic information, such as electron transfer and DNA enzymatic activity. DNA can also intercalate some enantiomers with a binding constant that depends on the stereo-enantiomer. DNA has a binding site for enantiomers and its binding constant depends on the stereo-enantiomer. Higuchi et al. established that it can be used

as a bio-macromolecular chiral selector in chiral separation technology [112]. Calf thymus DNA (ct DNA) was used for the chiral separation of racemic tyrosine and racemic tryptophan. Their D-enantiomers preferentially existed in the permeant solution while L-enantiomers preferentially existed in the feed solution [113]. Surface modified polysulfone membranes by BSA have been used for the separation of mixed proteins and optical resolution of tryptophan [114-116]. These membranes have very good durability; however, during permeation recognition sites get saturated quickly (being fixed) so selectivity of such membranes decreases abruptly with time. Chiral separation by DNA immobilized membranes is based on the interaction between DNA and a specific stereo-enantiomer; thus immobilized DNA membranes were categorized as channel-type membranes.

The optical resolution of racemic tryptophan, phenylalanine and tyrosine was carried out by ultrafiltration using the plasma-polymerized membrane prepared by polymerizing *l*-menthol onto a cellulose acetate membrane having of 0.2 μm membrane pore diameter exposed to plasma irradiation for 60 min. The optical resolutions of amino acids by ultrafiltration membranes fixed with plasma polymerized l-menthol have been reported [117]. *l*-Menthol was fixed on cellulose acetate membranes having pore diameters of 0.2, 0.45 and 0.8 μm by the plasma polymerization method plasma irradiation. The fractional fixation of *l*-menthol increased with increasing time of plasma irradiation, resulting in a decreasing pure water permeability of the membrane. The membrane was permeable with respect to D-isomers of all three racemic amino acids in preference to L-isomers. A higher separation factor was obtained at lower volume flux. The maximum value of the separation factor obtained for DL-Trp was 8 at $J_v=1.5\times10^{8}$ m·s^{-1}.

Tone et al. performed optical resolutions of racemic tryptophan (DL-Trp) solutions by dialysis and ultrafiltration using the plasma polymerized membranes. Four kinds of terpenes, (*l*-menthol, S-(−)-*β*-citronellol, [(1S)-endo]-borneol, and R-limonene) were fixed on a cellulose acetate membrane by plasma polymerization method at various periods of plasma irradiation [118]. The fractional fixations of terpenes increased as the time of plasma irradiation increased. All the membranes were permeable with respect to the D-isomer tryptophan in preference to the L-isomer. The maximum value of separation factor obtained for DL-Trp was 9.5 at volume flux $J_v=4.7\times10^{8}$ m s^{-1}. The solute permeabilities and apparent viscous parameters were evaluated from the dialysis and ultrafiltration data, respectively. A linear relation between the solute permeability and the ratio of apparent viscous parameters was obtained. It is indicated that the strength of chemical interaction between the amino acid solute and the terpene fixed in the membrane has a high influence on the solute flow inside the membrane pore. Polypeptide-modified poly (vinylidene fluoride) (PVDF) membranes were prepared for the separation of chiral molecules in ultrafiltration. Poly(*γ*-benzyl-L-glutamates) (PBLG) were vapor-deposited on the PVDF membranes; and the PBLG on PVDF membranes were modified through debenzylation or an ester exchange reaction to produce poly(L-glutamic acid) (PLGA) and polyglutamates with triethylene glycol monomethyl ether side chains (PLTEG). The enantioselectivities for chiral *α*-amino acids (tryptophan, phenylalanine and tyrosine) and chiral drugs (propranolol, atenolol, and ibuprofen) were measured by concentration-driven experiments, and were found in the range from 1.04 to 1.47 [40]. The selectivity increased with the increases in the helical content of PLGA immobilized on PVDF membranes. The enantioselectivity was higher for chemically grafted polypeptide-modified PVDF membranes compared to polypeptide physisorbed PVDF membranes, attributed to the higher molecular weight and density of the polypeptide chains, which enhance the interaction between the chiral compounds and the surface-bound polypeptides [40]. Gumi et al. prepared chiral separation membranes from polysulfone grafted with *N*-dodecyl-4(*R*)-hydroxy-L-proline as a chiral selector. The membranes prepared from the chiral derivatized polysulfone showed a separation factor of 1.1 in the dialysis permeation of racemic propranol, where S-propranol preferentially permeated through the membranes [111]. Aoki et al. prepared chiral separation membranes from poly[*p*-(oligopinanylsiloxanyl) phenylacetylenes with a chiral helical main chain. Membranes with a high chiral helicity in the main chain showed good enantioselectivity for racemic phenylalanine, tryptophan, valine, and 2-phenethyl alcohol based on diffusion selectivity, while there was almost no sorption selectivity [119]. Chiral separation of racemic amino acids through polymeric membranes with saccharide side chains has also been reported. The chiral separation by immobilizing large molecules as chiral selectors on the membrane performed by three mechanisms: affinity membranes, selective sorption membranes, and selective diffusion membranes. The chiral separation mechanism of affinity membranes is based on the selective adsorption of specific isomers compared to the other isomers.

1-2. Membranes Based on Chiral Stationary Phase Polymers

The polymer membranes have been prepared from chiral compounds such as cellulose, polysaccharides (sodium alginate) and chitosan because they contain a large number of chiral active carbon on the backbone of the ring structure. Chiral carriers or recognizing compounds were immobilized on the achiral polymers to prepare optical resolution membranes: for example, DNA can bind both L- and D-amino acids; it has a higher anity toward the former (e.g., L- vs D-Phe) [120,121]. In DNA-immobilized cellulose and chitosan membranes, the pore size influences the preferential permeation of the isomer under a pressure gradient. For example, due to an interaction between L-Phe and DNA immobilized on the surface of the cellulose membranes, D-Phe preferentially entered the pores of the membranes when the pore size was <2 nm (molecular weight cut-off [MWCO]<5000) and then permeated through the membranes. In contrast, when the pore size was >2 nm (MWCO >5000), L-Phe preferentially permeated through the membranes due to immobilization of DNA both on the surfaces and inside the pores. Interestingly, the opposite eect was observed when the membranes were used to separate D- and L-Try due to the different affinities of the two amino acids toward DNA. Higuchi et al. [122] achieved a high degree of enantioseparation using multiple steps of ultrafiltration through DNA-immobilized membranes. For example, they showed that only four stages are needed to obtain 99% purity and a relatively high α value of 4. Therefore, it is expected that multistage separation will be useful for large-scale applications.

Kim et al. [123] prepared a membrane by cross-linking polysaccharides with glutaraldehyde for enantioseparation of *α*-amino acids (e.g., tyrosine (Tyr)) by a pressure gradient. In this system, a lower degree of crosslinking, higher concentration of a feed solution, higher operating pressure, and smaller solute size leads to a lower enanti-

omeric excess value. Herewith, maximum %ee value of over 98% was obtained for D-Try using a chitosan membrane. A diffusion-enantioselective polysulfone membrane also separated a mixture of R/S-Prp with α value of 1.7 [124]. The membrane was prepared by sol-gel phase inversion using a casting solution containing the chiral selector N-hexadecyl-L-hydroxyproline (1.2 wt%). The α value decreased after 48 h and became higher when the transport rates were low. Membranes made from polysaccharides such as sodium alginate (SA) and chitosan (CS) were used for the optical resolution of α-amino acids. The enantioseparation of racemic kynurenine has been accomplished using BSA-grafted nylon hollow fiber membranes with pore sizes between 0.1 and 1.2 mm and a diameter of 2.5 cm [125]. During concentration-driven experiments, the maximal ee value for L-kynurenine was close to 30% but dropped to 0 after 2 h due to the non-selective diffusion of both the enantiomers. For the diffusion-enantioselective membranes, however, it may be possible to prevent such diffusion by reducing the pore size of the membranes and creating multilayered BSA-grafted membranes. Antibody-immobilized membranes have been developed by Lee and co-workers [126] for the enantioseparation of a racemic drug, 4-[3-(4-uorophenyl)-2-hydroxy-1-[1,2,4]-triazol-1-yl-propyl]-benzonitrile. The immobilized antibody facilitated the transport of selectively bound *RS*-enantiomer relative to the *SR*-antipode.

1-3. Chiral Polymer Membranes

Solid membranes were prepared using various chiral polymers. Optical resolution membranes incorporating norbornadiene and disubstituted acetylene polymers have been reported. Membranes made from polymer having side chiral groups such as (+)-poly{l-[dimethyl(10-pinanyl)silyl]-l-propyne} ((+)-PDPSP) demonstrated selective permeation of a racemic mixture of Tryptophan, 1,3-butanediol and several other compounds with %ee value between 12% and 48%. (+)-poly{2-[dimethyl-(10-pinanyl)silyl]norbornadiene} ((+)-PDPSN) membranes were successfully used to optically separate racemic Prp, a hydrophobic compound that could not be separated by (+)-PDPSP membranes. These (+)-PDPSN membranes can separate R-Prp from a racemic mixture with a %ee value of 45%, which was sustained for as long as 2,000 h. Furthermore, the (+)-PDPSN membranes were more flexible than the (+)-PDPSP membranes, as they have more flexible main chains. This led to a higher permeability value for Try than that attained with the (+)-PDPSP membranes. Moreover, these tough (+)-PDPSN membranes are expected to allow the use of a pressure difference as the driving force for separation Diphenylacetylenes with chiral pinanyl groups are easily employed for forming chiral polymeric membranes because of their excellent solubility and film-forming properties as well as their helical conformations. Poly{(−)-1-4-[dimethyl-(10-pinanyl)silyl]phenyl-1-propyne} membranes showed a higher enantioselectivity (α=3.16) toward R-Try than membranes made from two enantioselective disubstituted acetylene polymeric membranes, namely, poly{(−)-1-4-[dimethyl(10-pinanyl)silyl]-phenyl-2-phenylacetylene} and poly-{(−)-1-3-[dimethyl(10-pinanyl)silyl]-phenyl-2-phenylacetylene}, which have thermally stable helical conformations. This was probably due to the greater stiffness of poly{(−)-1-4-[di-methyl(10-pinanyl)silyl]-phenyl-2-phenylacetylene} and poly{(−)-1-3-[dimethyl(10-pinanyl)silyl]-phenyl-2-phenylacetylene} so that they contained more and larger molecular-scale voids than poly{(−)-1-4-[dimethyl(10-pinanyl)silyl]phenyl-1-propyne} [127]. Teraguchi and Masuda proposed a strategy for preparing poly(diphenylacetylene) by desilylation of poly{(−)-1-4-[di-methyl(10-pinanyl)silyl]-phenyl-2-phenylacetylene} [128]. The resulting poly(diphenylacetylene) maintained the helical structure of poly{(−)-1-4-[dimethyl(10-pinanyl)silyl]-phenyl-2-phenylacetylene} and is insoluble in organic solvents. Therefore, it is expected that poly(diphenylacetylene) can be used to fabricate chiral membranes by the solution casting method. The concept of membrane-assisted enantiomer enrichment of the aroma compound linalool was investigated in membrane pervaporation through a combination of complexation to cyclodextrin and selectively permeating through using PDMS membrane at a feed temperature of 45 °C. The highest enantiomeric excess obtained was 14% [129].

The chiral separation ability of the molecularly imprinted membranes from resulting novel polymeric materials has been investigated [130]. The solid membranes are composed of polymers with a glutamate backbone; α-helical conformation is important in the separation of chiral compounds and is required for the enantiomeric binding of Trp with PLGA. The enantioselectivity of Trp increased from 1.04 to 1.28 at a pH of 3.3. PLGA was converted from a random coil to an α-helix as the concentration of ethanol increased and as pH decreased. Novel polyamides with asymmetric carbon in their main chains were obtained from aromatic diamines, 4,4′-diaminodiphenylmethane (DADPM) or 1,3-phenylenediamine (1,3-PDA), and *N*-α-protected L-glutamic acid, *N*-α-acetyl-L-glutamic acid (Ac-L-Glu-OH). These polyamides showed optical rotation [131].

1-4. Molecularly Imprinting Polymer Membranes (MIPM)

In recent years the applications of molecular imprinting technique have proven quite useful in preparing high performance enantioseparation membranes [132]. Molecularly imprinted polymeric membranes have gained impetus since 1990. MIPM has enantiospecific recognition sites in polymer matrix introduced at the time of preparation by molecular imprinting technique. MIPM are highly cross-linked membranes in which chiral template molecule is fixed at the time of polymer preparation or membrane fabrication. Template is later leached out from the membrane that leaves impressions in the membranes. These impressions act as recognition sites for the same template preferentially. The technique is complicated and yet to be understood fully. Novel polymeric materials with chiral environment were obtained by the reaction of lithiated polysulfone with the chiral terpenoid myrtenal. The molecularly imprinted polymer membranes were obtained from the resulting novel polymeric materials, and their chiral separation ability was investigated [133]. Molecularly imprinted polymeric membranes with tripeptide residue H-Glu(OBzl)-Glu(OBzl)-Glu(OBzl)-CH_2- (EEE) were prepared during the membrane preparation process in the presence of a print molecule. The Boc-L-Trp imprinted polymeric membranes thus obtained showed adsorption selectivity toward the print molecule analogue, Ac-L-Trp. From the adsorption isotherms of Ac-Trp's, the chiral recognition site, which was formed by the print molecule in the membrane preparation process, exclusively recognizes Ac-L-Trp, and the opposite isomer, Ac-D-Trp, is rejected [134].

MECHANISM OF SOLUTE TRANSPORT THROUGH MEMBRANES

Generally, transport through a membrane occurs due to the dif-

ference in their chemical potential or electrical potential between both sides of the membrane. The mechanism of transport strongly depends on membrane morphology. Solid polymer membranes have two typical morphologies: porous membranes and dense membranes.

1. Transport through Porous Membranes

Transport through porous membranes is governed by membrane porosity and interaction with the internal membrane surface. The transport of solute in the porous membranes in its first place is governed by membrane morphology. The morphology includes the surface and volume porosity, pore size distribution, and tortuosity is a factor used to correct for the deviation of pore shape from perfect cylinders. Permeability of the membrane is termed as flux; the amount of solute that passes through per unit area of the membrane. However, since transport is not an intrinsic membrane material property, permeability in porous membranes is not normalized for the membrane thickness. Pore sizes range from micrometers down to below 1 nm. Porosities range from more than 80% for micrometer-sized pores to less than 2% for nanometer-sized pores. Selectivity of porous membranes is defined by the term rejection or retention R. Retention is the ratio of concentration of component in permeant and feed.

$$R=\text{Conc. in permeant/Conc. in feed} \quad (1)$$

The retention or rejection of a solute depends on the ratio of molecular size to pore size. Thus the performance of dense membranes is strictly material dependent, while the performance of porous membranes is morphology and material dependent.

The mechanism of separation of enantiomers by membrane is entirely different from conventional membrane separation. As the enantiomers are identical in all respects except in optical rotation and can only be differentiated in a chiral environment, therefore it is essential to separate a racemic mixture by a membrane introduction of chiral environment in the membrane or in membrane process.

2. Transport through Dense Membranes

Dense membranes are permeable for small molecules as ions and gas molecules. Transport through dense membranes is described by the solution-diffusion model proposed by Wijmans and Baker, wherein the transport of molecules in dialysis, gas permeation, and pervaporation and ions in reverse osmosis have been explained satisfactorily. According to this model the permeability P of any substance is a product of its diffusion coefficient, D ($cm^2\ s^{-1}$) and solubility coefficient S ($cm^3\ cm^{-3}\ atm^{-1}$) of the component.

$$P=D\times S \quad (2)$$

Since the diffusion coefficient, and solubility coefficient of a substance depend on its interactions with the membrane material, transport of a substance through membrane depends solely on the nature of membrane material. The permeability of a dense membrane equals to flow, normalized for the membrane surface area, the difference in partial pressure and the membrane thickness. The value of the permeability is an intrinsic property of the membrane material and gives an indication of the membrane transport capacity. The other important characteristic of dense membrane is selectivity (α) for a component, which is defined as the ratio of the pure permeabilities of two components of mixture and gives an indication of the separation efficiency of the membrane. The combination of permeability and selectivity indicates the general performance of the membrane material.

MECHANISM OF ENANTIOSEPARATION BY SOLID MEMBRANES

The preparation of single enantiomer medicine has been one of the most important fields in the development of research. Through solid membrane, separation has become the most important method of enantioseparation. Enantioselective membranes perform enantioseparation due to the presence of chiral environment. Therefore, enantioselective membranes act as a selective barrier and selectively transport one enantiomer due to the stereospecific interaction between the enantiomer and chiral recognition sites. The different binding affinities of two enantiomers may be the result of different hydrogen bonding, hydrophobic, Coulomb, van der Waals interactions and steric effects with the chiral sites. Two mechanisms have been perceived in enantiomeric separations: facilitated and retarded transport. The separation of enantiomers by liquid membranes is usually based on facilitated transport, whereas separation by solid membranes is based on both mechanisms, facilitated and retarded transport [135].

1. Facilitated Transport

In facilitated transport processes [136], one enantiomer preferentially adsorbs to the chiral recognition sites in the enantioselective membranes near the feed phase due to a higher binding affinity. From there, it continuously adsorbs and desorbs from one chiral site to the next, and at last is transported toward the stripping phase. The other enantiomer, which has no or less specific binding affinity for the chiral environment, passes through the membrane by diffusion. In other words, this transport mechanism is based on the differential diffusion rates of two enantiomers. The facilitated transport is concentration driven. Generally, most chiral liquid and solid membranes composed of a chiral polymer or coated with an enantioselective polymeric layer utilize this class of transport. This type of transport is also utilized by chiral selector-immobilized membranes with relatively low binding affinities for two enantiomers. Chiral resolution solid membranes function based on facilitated transport mechanism may be adsorption-enantioselective membranes or diffusion-enantioselective membranes.

2. Retarded Transport

The membrane follows the retarded transport mechanism where the driving force is a pressure gradient. In contrast to the facilitated transport mechanism, retarded transport retains the adsorbed enantiomer in the membrane phase, while permitting the other enantiomer to pass through the membrane more easily due to its lower affinity for the chiral recognition site. Membranes that function based on the retarded transport mechanism are called adsorption-enantioselective membranes and they usually incorporate chiral selectors [137]. In an adsorption-enantioselective membrane, the binding affinity between chiral recognition sites and enantiomers is stronger than that of a diffusion-enantioselective membrane, and this interaction force always exists between one enantiomer and one chiral site. Separation efficiency of these membranes is mainly determined by the binding capacity. The adsorption-enantioselective membranes are expected to simultaneously possess relatively high flux and high enantioselectivity, and thus have more potential than diffusion-enantioselective membranes to carry out industrial-scale productions of optically pure compounds.

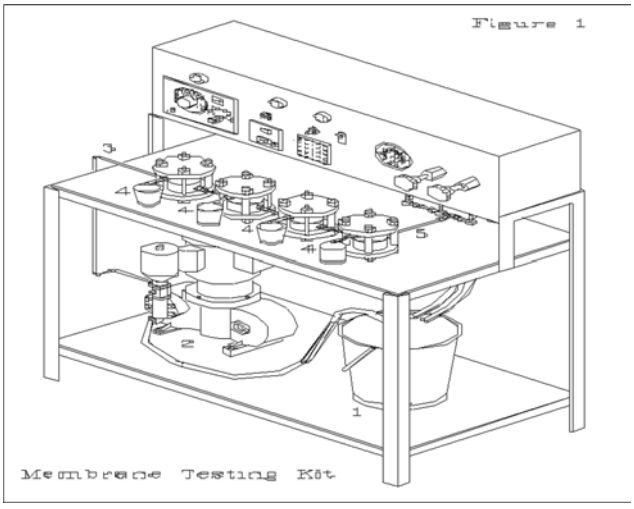

Fig. 4. Membrane permeation testing kit.

PERFORMANCE PARAMETERS OF MEMBRANE PERMEATION

The performance of the membranes can be determined by permeating aqueous solutions of racemic mixtures of chiral compounds preferably amino acids through membranes under pressure on reverse osmosis testing modules. The reverse osmosis testing module consists of four test cells arranged in series such that out of the previous cell is the feed for next cell, and finally the output of the last cell goes to the feed reservoir. Such an arrangement is specified as closed loop permeation. Here the concentration of the solute in permeant is the concentration of solute at that particular time and the feed concentration remains constant throughout the experiment. Each test cell has a circular membrane of effective area 0.00195 m^2. The diagram of the testing module is given in Fig. 4.

The performance of a membrane is expressed by two most important parameters: permeability and selectivity.

1. Permeability

The permeability is expressed as permeation flux. According to the preferential sorption model, the flux through a porous membrane is given by expression (Eq. (3)):

$$Jv=A^{\wedge}\{\Delta P-[\pi(X\,F)-\pi(X\,P)]\} \quad (3)$$

where A^ is the pure water permeability constant of the membrane and π (X) represents the osmotic pressure of the feed or permeant side with solute mole fraction X.

Flux is enhanced by employing a driving force (e.g., a pressure-driven process or electrodialysis), an ultrathin film, or a membrane with a high porosity [88,126,138,139].

The solute permeability, the amount of solute that passes through per unit area of membrane per unit time is known as the solute permeability and is expressed as solute flux (J_S). The solute flux is calculated using solute concentration in permeant and liquid permeability of membrane according to Eq. (4):

$$J_S=Q/At \quad (4)$$

Here, Q is amount of solute permeated in grams or moles, A is area of membrane in square meters and t is permeation time in hours.

2. Selectivity

The membrane selectivity is given by its ability to separate a particular solute from the solution and is expressed in terms of rejection percentage (% R) in accordance with Eq. (5):

$$\%\,R=(1-C_p/C_f)\times 100 \quad (5)$$

Here, C_p and C_f are concentrations of solute in permeant and feed, respectively.

3. Enantioselectivity

The performance of an enantioselective membrane is expressed by enantioselectivity which means preferential transport of one of the paired isomers. It is defined as the excess of one enantiomer over its analogous in permeant achieved through membrane permeation and is reported as percentage enantiomeric enrichment or excess (%ee). It is expressed as percentage enantiomeric excess (%ee) and as separation factor (α). The enantiomeric excess is a measure of excess of one of the paired enantiomers in the sample. The enantiomeric excess was determined by measuring the concentrations of enantiomers in permeant using expression (Eq. (6)):

$$\%ee=\frac{C^{Dp}-C^{Lp}}{C^{Dp}+C^{Lp}}\times 100 \quad (6)$$

Here, C^{Dp} and C^{Lp} are concentrations of D- and L-enantiomers in permeant.

4. Separation Factor (α)

The separation factor (α), which is another important parameter that describes the enantiomeric separation, is the ratio of enantiomers in permeant solution to feed solution as given by the Eq. (7):

$$\alpha=\frac{C^{Dp}/C^{Lp}}{C^{Df}/C^{Lf}} \quad (7)$$

If the feed is the solution of racemic compound, as is the case here, the separation factor (α) defined as (Eq. (8)):

$$\alpha=C^{D}_{P}/C^{L}_{P} \quad (8)$$

PREPARATION OF ASYMMETRIC MICRO-POROUS MEMBRANE FROM POLYSULFONE

The microporous polysulfone membrane was prepared by phase inversion technique similar method as discussed elsewhere [140,141]. A polymer solution is spread in the form of thin film of uniform

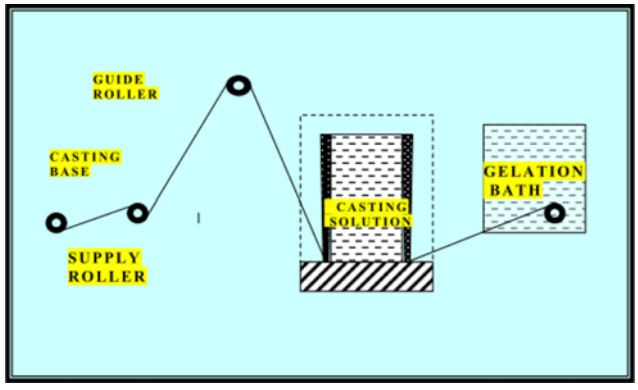

Fig. 5. Asymmetric membrane preparation machine.

thickness on a support and is precipitated by using a motorized casting machine as shown in Fig. 5. The precipitation (phase inversion) can be induced in several ways, including thermal gelation, solvent evaporation, or non-solvent immersion precipitation [142]. The phase inversion by non-solvent immersion precipitation is the most important method for asymmetric membrane preparation and was first employed by Loeb and Sourirajan to prepare asymmetric cellulose acetate membranes [143]. In this technique a polymer solution is cast into a film on a support and then immersed rapidly into a non-solvent (water, for example), which precipitates the polymer into a very thin dense polymer rich skin layer of the membrane and beneath the skin layer a more porous polymer sub-layer. The preparation of asymmetric microporous membrane according to phase inversion process consists of three main steps:

1) Dissolution of the polymer in a suitable solvent, usually, N,N-dimethylformamide, dimethylacetamide or N-methyl-2-pyrrolidone,

2) Casting the resulting solution as a thin film on the surface of a proper support, usually a nonwoven fabric, and

3) Finally immersing the cast film in polymer non-solvent (usually water).

The polymer concentration in solution controls the viscosity of solution. Generally, 15-18% (W/W) polymer concentration in a suitable solvent is considered to be ideal for a suitable asymmetric membrane preparation [144]. Higher viscous solution gives a dense membrane that results in a low porosity membrane. The thickness of polymer solution on the support at the point of spreading solution determines the thickness of the membrane; thicker membranes exhibit less water flux due to thick dense layer and decreased porosity. The evaporation time (time allowed from casting roller to point of immersion of film in the coagulation bath) is directly related to the thickness of top dense layer of the membrane. Longer evaporation gives a membrane of thicker and dense top layer, thereby having low permeability. The temperature and composition of the coagulating bath also affect the membrane morphology and in turn the properties of membrane. Lower temperature of coagulation solution induces fast precipitation, resulting low porosity. Addition of surfactant in coagulation solution improves the wettability and gives rise to a uniformly thick membrane.

PREPARATION OF THIN FILM COMPOSITE MEMBRANE

The most important thin-film composite membranes are made by coating an ultrathin film of a desired polymer on micro-porous membrane, preferably an asymmetric ultrafiltration membrane called a support membrane. The diagrammatic representation of thin film composite membrane showing different layers of composite membrane is given in Fig. 6. Formation mechanism of thin-film layers on polysulfone support is shown in Fig. 7. The ultrathin layer on asymmetric membrane can be prepared by different ways, such as dipping membrane in polymer solution, spraying polymer solution on the membrane, or generating polymer film in-situ on membrane by interfacial polymerization; however interfacial polymerization is most preferred because it offers a uniform and defect-free membrane. The interfacial polymerization technique of thin film composite membrane preparation was developed by Cadotte et al. [145]. This technique involves coating of micro-porous asymmetric membrane of desired porosity with a monomer wherein monomer on the membrane is then reacted with another monomer, cross-linking agent. This results in formation of a dense and cross-linked polymer layer on the asymmetric membrane because a cross-linking reaction occurs mostly at the surface of asymmetric membrane. The selective layer of the composite membranes is much thinner than that of asymmetric membranes; therefore, composite membranes exhibit much higher selectivity and permeability. The ultrathin layer of a composite membrane may be of polyamide, polyurea, polyether urea, polyurethane or polyester polymer. Most widely used thin-film composite membranes consist of cross-linked aromatic polyamide polymer layer [144]. A systematic representation of interfacial polymerization reaction between 1, 3, benzenediamine and 1,

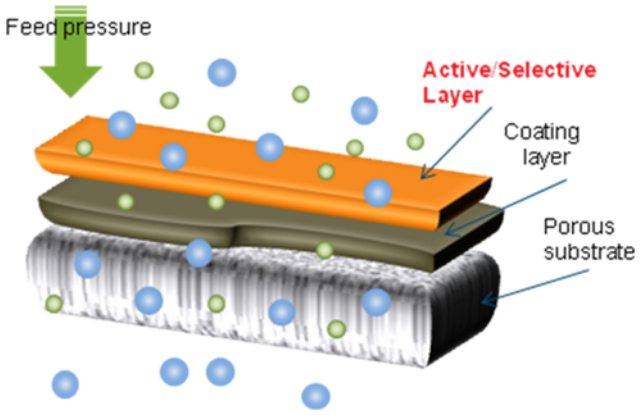

Fig. 6. Thin film composite membrane.

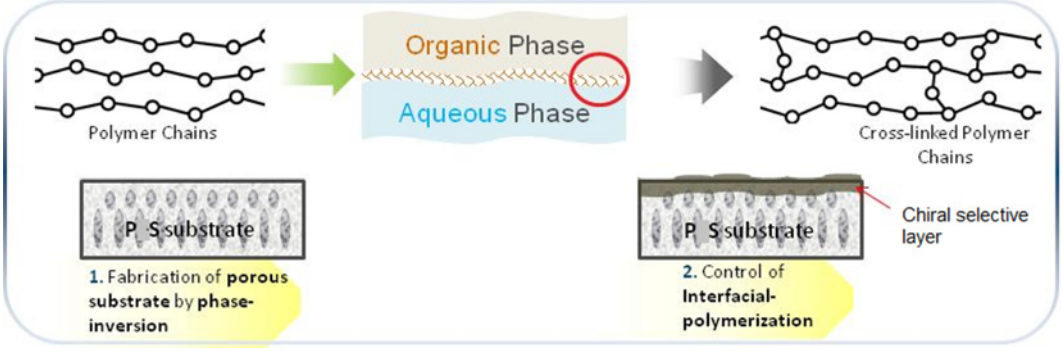

Fig. 7. Mechanism formation of TFC membrane.

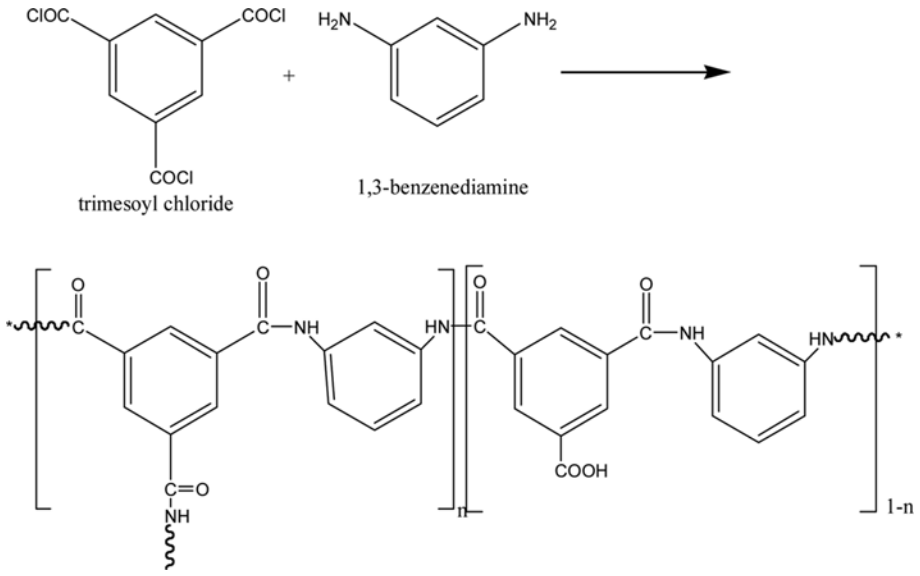

Fig. 8. A systematic representation of interfacial polymerization reaction.

3, 5, benzene tricarbonyl trichloride is shown in Fig. 8. The formation of thin film on top of a micro-porous membrane consists of the following steps:

1. Immersion of water-wet porous polysulfone membrane in an aqueous amine solution.
2. Proper drying of the amine wetted polysulfone membrane.
3. Contacting of the optimally dried polysulfone support with acid chloride.
4. Curing of the nascent polyamide composite membrane.

A REMARKABLE DISCOVERY HAS BEEN MADE RECENTLY

1. Immobilization of Chiral Selector BSA on Polysulfone Membrane

BSA was immobilized on polysulfone membrane fabricated by ultrafiltration permeation technique using BSA solution (2 mg/ml) in phosphate buffer (at pH 7) as feed permeating through membrane for 4 h at 344.7 kPa pressure. The membrane was removed from filtration unit and washed with deionized water to remove excess amount of BSA from the membrane surface. BSA adsorbed on the membrane surface and in the pores was cross-linked by passing glutaraldehyde (5% solution) through the membranes for 4 h at 344.7 kPa pressure, and finally membranes were cured at 600C for 10 min. The amount of BSA immobilized on the membrane was estimated by determining the concentration of BSA in feed and permeant solutions by UV-Vis spectrophotometer at 280 nm. The volumetric ux (Jv), the solute ux (Js), the separation factor (α), and the enantiomeric excess (%ee) of two types of membranes at different trans-membrane pressures and permeation times were determined. BSA semi-IPN membrane exhibits higher volumetric as well as solute uxes compared to BSA-immobilized membrane. A separation factor (α) to the order of 1.89 was achieved with BSA-immobilized membrane after 8 h of ultraltration, and in the same duration BSA-IPN membrane exhibited a separation factor (α) to the order of 1.62. BSA-immobilized membrane exhibits higher enantiomeric excess (30.8%) compared to BSA semi-IPN membrane (23.8%) after 8 h [146]. BSA molecules available on membrane as immobilized or as semi-IPN undergo complexion with tryptophan enantiomers differently. BSA-immobilized membrane had better separation and enantiomeric purity.

2. Preparation of Chiral-selective Composite Membranes

Chiral-selective composite membranes were prepared by forming a chiral-selective barrier layer on the top of polysulfone asymmetric membrane. The chiral-selective layer of composite membrane was prepared by interfacial polymerization of highly reactive chiral carbon containing monomers in situ on the top surface of polysulfone membrane. To prepare a chiral-selective barrier layer on the top surface of polysulfone membrane, in-situ interfacial polymerization reaction was performed using a motor-driven prototype coating machine. The schematic representation of preparation process of barrier layer of composite membrane by interfacial polymerization is given in Fig. 7.

3. Preparation of Chiral-selective Composite Membrane Having Zn Metal Schiff Base Complex of Chiral Ligands

The composite membrane containing a Schiff base complex of zinc ions having chiral ligands was prepared by reacting zinc salt with the Schiff base complex and piperazine with trimesoyl chloride interfacially on the top of polysulfone asymmetric membrane. The reaction scheme of interfacial polymerization of Schiff base complex and piperazine with trimesoyl chloride is represented in Fig. 9. The optical resolution of α-amino acids, arginine and alanine was performed by reverse osmosis at 517.10 kPa and 1,034.21 kPa pressures. 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 composite membrane permeates D-enantiomers of α-amino acids preferentially; 54% enantiomeric excess (α=6.84) in for D-arginine was achieved [56]. The enantioselective permeability of the membrane is found to be time

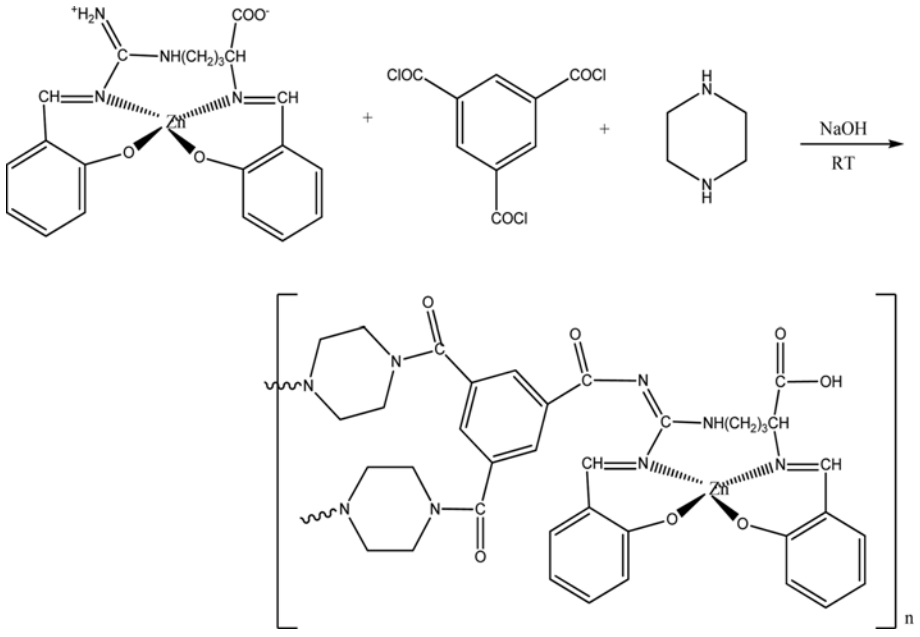

Fig. 9. Reaction scheme of interfacial polymerization of metal Schiff-base complex and piperazine with trimesoyl chloride [153].

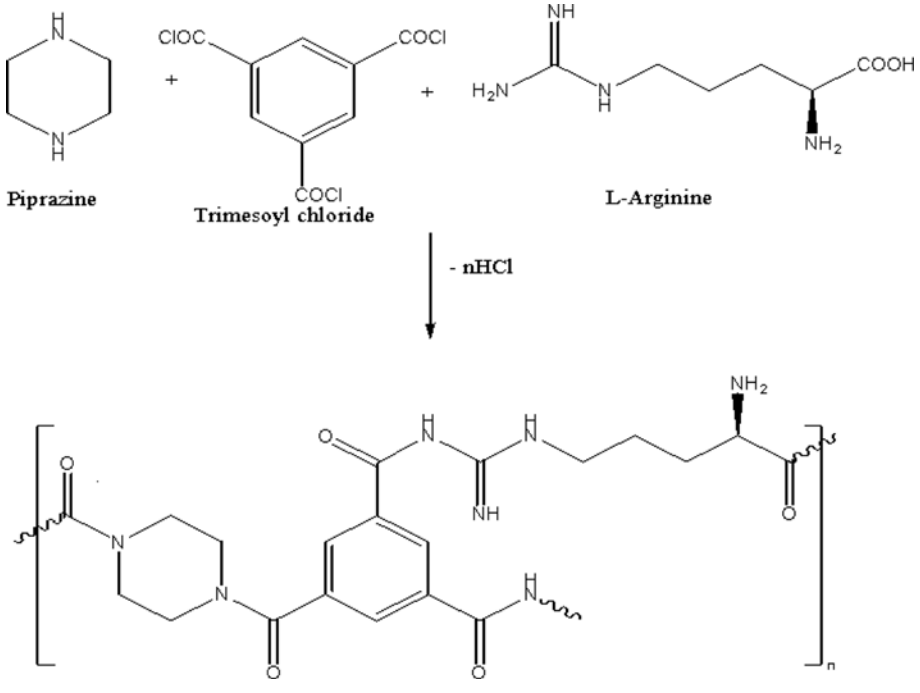

Fig. 10. Reaction scheme of interfacial polymerization of piperazine and L-arginine with trimesoyl chloride [152].

dependent. The enantioselective property of the membrane has arisen due to a homo-chiral environment created in the membrane by incorporating a chiral ligand Schiff complex in thin film of poly (piperazine trimesamide) polymer on the top of the polysulfone membrane.

4. Preparation of Chiral Selective Composite Membrane from Dibasic *α*-amino Acids, Piperazine with Trimesoyl Chloride

The polysulfone membrane was used for preparation of composite membrane. The composite membranes capable of recognizing enantiomers of *α*-amino acid derivatives were prepared by interfacial polymerization of L-enantiomer of amino acids and piperazine with trimesoyl chloride in-situ on the surface of polysulfone membrane. The schematic representation of interfacial polymerization reaction of piperazine and L-arginine with trimesoyl is given in Fig. 10.

The enantioselective performance of membranes was examined and correlated to the composition of selective layer through optical resolution of racemic arginine in reverse osmosis mode. The membranes having chiral environment performed enantiomeric separation by permeating D-arginine preferentially over 89% enantiomeric excess. The membranes without chiral environment, though, exhibited separation of arginine but did not perform enantiomeric sepa-

ration. The trans-membrane pressure and concentration of feed solution have noticeable effects on enantioseparation. Higher enantiomeric excess resulted from dilute feed and at low trans-membrane pressure [147-151]. The membrane prepared from L-arginine (2%) and trimesoyl chloride (1%) solutions exhibited highest enantiomeric excess (89%) [152]. The enantioselectivity of membranes was independent of time. The separation factor (α) for D-arginine achieved as high as ~17. There are a large number of recent research articles has been published in different journals regarding enantiomer separations. For comparison with different chiral separation techniques, the membrane separation technique is very convenient and easy to scale up.

CONCLUSIONS

The present study will contribute greatly towards the field of chiral separation (especially in pharmaceuticals industries) for the separation of two enantiomers from their racemic mixtures. The increasing need for single enantiomers of all chiral drugs and intermediates in chemical and pharmaceutical industry has created a significant demand for efficient processes to resolve racemic mixtures on industrial scale. However, commercial applications of membrane technology for optical resolution have not been realized. Despite extensive research on development of membranes with fixed chiral selector, the possibility of chiral separation by such membranes is still controversial, especially in case of porous membranes. Studies on possible mechanisms of chiral separation by the membranes should be continued. Development of membranes with chiral recognizing properties and different structures is important for both mechanistic studies and application of chiral separation. Finally, advanced polymeric materials are playing an important role in the development of chiral separation membranes for pharmaceutical applications.

REFERENCES

1. P. G. Ingole, N. R. Thakare, K. H. Kim, H. C. Bajaj, K. Singh and H. K. Lee, *New J. Chem.*, **37**, 4018 (2013).
2. N. M. Maier, P. Franco and W. Linder, *J. Chromatogr. A*, **906**, 3 (2001).
3. J. M. Daniels, E. R. Nestmann and A. Kerr, *J. Drug Inf.*, **31**, 639 (1997).
4. A. Ghanem and H. Y. Aboul-Enein, *Tetrahedron: Asym.*, **15**, 3331 (2004).
5. A. Ghanem and H. Y. Aboul-Enein, *Chirality*, **17**, 1 (2005).
6. J. M. Keith, J. F. Larrow and E. N. Jacobsen, *Adv. Synth. Catal.*, **343**, 5 (2001).
7. H. E. Schoemaker, D. Mink and M. G. Wubbolts, *Science*, **299**, 1694 (2003).
8. U. T. Bornscheuer, *Angew. Chem. Int. Ed.*, **42**, 3336 (2003).
9. H. Pellissier, *Tetrahedron*, **59**, 8291 (2003).
10. U. T. Strauss, U. Felfer and K. Faber, *Tetrahedron: Asym.*, **10**, 107 (1999).
11. U. T. Strauss and K. Faber, *Tetrahedron: Asym.*, **10**, 4079 (1999).
12. G. L. J. A. Rikken and E. Raupach, *Nature*, **405**, 932 (2000).
13. G. M. Maggiora, B. Mao and K. C. Chou, *New Developments in Molecular Chirality, Ed. P. G. Mezey, Kluwer Academic Publishers, Dordrecht.*, **5**, 93 (1991).
14. W. O. Foye, T. L. Lemke and D. A. Williams, *Foye's principles of medicinal chemistry*, Lippincott Williams & Wilkins (2007).
15. I. Aranaz, M. Mengíbar, R. Harris, I. Paños, B. Miralles, N. Acosta, G. Galed and Á. Heras, *Current Chem. Biolo.*, **3**, 203 (2009).
16. I. I. Hewala, M. S. Moneeb, H. A. Elmongy and A.-A. M. Wahbi, *Talanta*, **130**, 506 (2014).
17. H. Caner, E. Groner and L. Levy, *Drug Discovery Today*, **9**, 105 (2004).
18. Anon, *FDA's Policy statement for the development of new stereoisomeric drugs, Chirality*, **4**, 338 (1992).
19. J. Tobis, L. Bocha, Y. Thomanna and J. C. Tiller, *J. Membr. Sci.*, **372**, 219 (2011).
20. Food & Drug Administration, Policy Statement for the Development of New Stereoisomeric Drugs, 57 Fed. Reg., **22**, 249 (1992).
21. N. M. Maier, P. Franco and W. Lindner, J. *Chromatogr. A*, **906**, 3 (2001).
22. C. A. M. Afonso and J. G. Crespo, *Angew. Chem., Int. Ed.*, **43**, 5293 (2004).
23. K. B. Jirage and C. R. Martin, *Trends Biotechnol.*, **17**, 197 (1999).
24. P. M. Masters, *Forensic Sci. Int.*, **32**, 179 (1986).
25. J. R. Cronin and S. Pizzarello, *Science*, **275**, 951 (1997).
26. M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Kesseler, R. Sturmer and T. Zelinski, *Angew. Chem., Int. Ed.*, **43**, 788 (2004).
27. A. R. Fassihi, *Int. J. Pharm.*, **92**, 1 (1993).
28. R. Sabia, A. Ciogli, M. Pierini, F. Gasparrini and C. Villani, *J. Chromatogr. A* (2014), DOI:10.1016/j.chroma.2014.07.097.
29. W. R. Brode, *J. Opt. Soc. Am.*, **41**, 987 (1951).
30. T. J. Ward and T. M. Oswald, *Encyclopaedia of Analytical Chemistry* (2006).
31. C. M. Kraml, D. Zhou, N. Byrne and O. McConnell, *J. Chromatogr. A*, **1100**, 108 (2005).
32. H. Nohira, K. Watanabe and M. Kurokawa, *Chem. Lett.*, **5**, 299 (1976).
33. J. Jacques and A. Collet, *Wiley Interscience*, New York (1981).
34. B. S. Sekhon, *Int. J. Chem. Technol. Res.*, **2**, 1584 (2010).
35. A. S. Bommarius and B. R. Riebel-Bommarius, *Biocatalysis: Fundamentals and Applications*, Wiley (2007).
36. E. Lee, M. B. Park, J. M. Kim, W. S. Kim and I. H. Kim, *Korean J. Chem. Eng.*, **27**, 231 (2010).
37. R. D. Noble, *J. Membr. Sci.*, **75**, 121 (1992).
38. M. Ulbricht, *Polymer*, **7**, 2217 (2006).
39. M. Nakamura, S. Kiyohara, K. Saito, K. Sugita and T. Sugo, *Anal. Chem.*, **71**, 1323 (1999).
40. N. H. Lee and C. W. Frank, *Polymer*, **43**, 6255 (2002).
41. M. Newcomb, R. C. Helgeson and D. J. Cram, *J. Am. Chem. Soc.*, **96**, 7367 (1974).
42. A. Maruyama, N. Adachi, T. Takatsuki, M. Torii, K. Sanui and N. Ogata, *Macromolecules*, **23**, 2748 (1990).
43. T. Aoki, K. Shinohara, T. Kaneko and E. Oikawa, *Macromolecules*, **29**, 4192 (1996).
44. T. Aoki, Y. Kobayashi, T. Kaneko, E. Oikawa, Y. Yamamura, Y. Fujita, M. Teraguchi, R. Nomura and T. Masuda, *Macromolecules*, **32**, 79 (1999).
45. T. Aoki, *Prog. Polym. Sci.*, **24**, 951 (1999).
46. T. Aoki, M. Ohshima, K. Shinohara, T. Kaneko and E. Oikawa, *Polymer*, **38**, 235 (1997).
47. M. Teraguchi and T. Masuda, *Macromolecules*, **35**, 1149 (2002).

48. M. Teraguchi, J. Suzuki, T. Kaneko, T. Aoki and T. Masuda, *Macromolecules*, **36**, 9694 (2003).
49. M. Teraguchi, K. Mottate, S. Y. Kim, T. Aoki, T. Kaneko, S. Hadano and T. Masuda, *Macromolecules*, **38**, 6367 (2005).
50. Y. Masakazu and H. Akon, *Enantioselective Membranes*, DOI: 10.1002/9781118522318.emst131 John Wiley & Sons, Inc. All (2013).
51. H. K. Lonsdale, *J. Membr. Sci.*, **10**, 81 (1982).
52. S. V. Madihally, *Principles of Biomedical Engineering, Artech House*, Boston London (2010).
53. J. P. G. Villaluenga and A. Tabe-Mohammadi, *J. Membr. Sci.*, **169**, 159 (2000).
54. J. E. Cadotte, K. E. Cobian, R. H. Forester and R. J. Petersen, NTIS Report No. PB-253193 (1976).
55. S. A. Altinkaya, *Desalination*, **199**, 459 (2006).
56. K. Singh, P. G. Ingole, H. C. Bajaj, A. Bhattacharya and H. R. Brahmbhatt, *Sep. Sci. Technol.*, **45**, 1374 (2010).
57. E. Vedejs and M. Jure, *Angew. Chem., Int. Ed.*, **44**, 3974 (2005).
58. G. R. Cook, *Curr. Org. Chem.*, **4**, 869 (2000).
59. J. M. Keith, J. F. Larrow and E. N. Jacobsen, *Adv. Synth. Catal.*, **343**, 5 (2001).
60. M. Tokunaga, J. F. Larrow, F. Kakiuchi and E. N. Jacobsen, *Science*, **277**, 936 (1997).
61. S. Ramdeehul, P. Dierkes, R. Aguado, P. C. J. Kamer, P. W. N. M. Van Leeuwen and J. A. Osborn, *Angew. Chem., Int. Ed.*, **37**, 3118 (1998).
62. H. Pellissier, *Tetrahedron*, **59**, 8291 (2003).
63. V. Schurig, *J. Chromatogr. A*, **906**, 275 (2001).
64. S. Fanali, P. Catarcini, G. Blaschke and B. Chankvetadze, *Electrophoresis*, **22**, 3131 (2001).
65. Y. Nagata, T. Iida and M. Sakai, *J. Mol. Catal. B: Enzym.*, **12**, 105 (2001).
66. S. S. Peacock, D. M. Walba, F. C. A. Gaeta, R. C. Helgeson and D. J. Cram, *J. Am. Chem. Soc.*, **102**, 2043 (1980).
67. B. Dietrich, J. M. Lehn and J. P. Sauvage, *Tetrahedron*, **29**, 1647 (1973).
68. B. Dietrich and J. M. Lehn, *Tetrahedron Lett.*, **l5**, 1225 (1973).
69. E. P. Kyba, J. M. Timko, L. J. Kaplan, F. de Jong, G. W. Gokel and D. J. Cram, *J. Am. Chem. Soc.*, **100**, 4555 (1978).
70. D. J. Cram and J. H. Cram, *ACC. Chem. Res.*, **11**, 8 (1978).
71. L. R. Sousa, D. H. Hoffman, L. Kaplan and D. J. Cram, *J. Am. Chem. Soc.*, **96**, 7100 (1974).
72. L. R. Sousa, G. D. Y. Sogah, D. H. Hoffmann and D. J. Cram, *J. Am. Chem. Soc.*, **100**, 4569 (1978).
73. G. D. Y. Sogah and D. J. Cram, *J. Am. Chem. Soc.*, **101**, 3035 (1979).
74. G. D. Y. Sough and D. J. Cram, *J. Am. Chem. Soc.*, **97**, 1259 (1975).
75. W. H. Pirkle, US Patent, 5,080,795 (1992).
76. W. H. Pirkle, WO 9,117,816 (1992).
77. W. H. Pirkle and W. E. Bowen, *Tetrahedron: Asymmetry*, **5**, 773 (1994).
78. W. H. Pirkle and E. M. Doherty, *J. Am. Chem. Soc.*, **111**, 4113 (1989).
79. C. Thoelen, M. De Bruyn, E. Theunissen, Y. Kondo, I. F. J. Vankelecom, P. Grobet, M. Yoshikawa and P. A. Jacobs, *J. Membr. Sci.*, **186**, 153 (2001).
80. T. Aoki, S. Tomizawa and E. Oikawa, *J. Membr. Sci.*, **99**, 117 (1995).
81. E. Yashima, J. Noguchi and Y. Okamoto, *J. Appl. Polym. Sci.*, **54**, 1087 (1994).
82. T. Kakuchi, T. Takaoka and K. Yokota, *Polym. J.*, **22**, 199 (1990).
83. Y. Xiao, H. M. Lim, T. S. Chung and R. Rajagopalan, *Langmuir*, **23**, 12990 (2007).
84. Z. Zhou, Y. Xiao, T. A. Hatton and T. S. Chung, *J. Membr. Sci.*, **339**, 21 (2009).
85. Y. Zhang, H. Yu, Y. Wu, W. Zhao, M. Yang, H. Jing and A. Chen, *Ana. Biochem.*, **462**, 13 (2014).
86. K. He, F. Qiu, J. Qin, J. Yan and D. Yang, *Korean J. Chem. Eng.*, **30**, 2078 (2013).
87. X. Liu and L. Meng, *Korean J. Chem. Eng.*, **30**, 918 (2013).
88. J. H. Kim, J. H. Kim, J. Jegal and K. H. Lee, *J. Membr. Sci.*, **213**, 273 (2003).
89. W. Li, Y. Li, Y. Fu and J. Zhang, *Korean J. Chem. Eng.*, **30**, 1448 (2013).
90. L. Chen, X. He, B. Zhao and Y. Liu, *Anal. Chim. Acta*, **417**, 51 (2000).
91. N. Sunsandee, N. Leepipatpiboon, P. Ramakul, T. Wongsawa and U. Pancharoen, *Sep. Purif. Technol.*, **102**, 50 (2013).
92. N. Sunsandee, N. Leepipatpiboon and P. Ramakul, *Korean J. Chem. Eng.*, **30**, 1312 (2013).
93. A. Higuchi, M. Hara, T. Horiuchi and T. Nakagawa, *J. Membr. Sci.*, **93**, 157 (1994).
94. K. Shinohara, T. Aoki and E. Oikawa, *Polymer*, **36**, 2403 (1995).
95. K. Sakaki, S. Hara and N. Itoh, *Desalination*, **149**, 247 (2002).
96. Y. J. Wang, Y. Hu, J. Xu, G. S. Luo and Y. Y. Dai, *J. Membr. Sci.*, **293**, 133 (2007).
97. A. Bodalo, J. L. Gomez, E. Gomez, M. F. Maximo and M. C. Montiel, *Enzyme Microb. Technol.*, **35**, 261 (2004).
98. A. Liese, U. Kragl, H. Kierkels and B. Schulze, *Enzyme Microb. Technol.*, **30**, 673 (2002).
99. W. S. Long, S. Bhatia and A. Kamaruddin, *J. Membr. Sci.*, **219**, 69 (2003).
100. E. Miyako, T. Maruyama, N. Kamiya and M. Goto, *Chem. Eur. J.*, **11**, 1163 (2005).
101. L. M. Robeson, B. D. Freeman, D. R. Paul and B. W. Rowe, *J. Membr. Sci.*, **341**, 178 (2009).
102. L. M. Robeson, *J. Membr. Sci.*, **320**, 390 (2008).
103. B. D. Freeman, *Macromolecules*, **32**, 375 (1999).
104. W. S. Long, A. Kamaruddin and S. Bhatia, *J. Membr. Sci.*, **247**, 185 (2005).
105. A. Papra, H. G. Hicke and D. Paul, *J. Appl. Polym. Sci.*, **74**, 1669 (1999).
106. Y. Ito, Y. Ochiai, Y. S. Park and Y. Imanishi, *J. Am. Chem. Soc.*, **119**, 1619 (1997).
107. J. Yang and D. S. Hage, *J. Chromatogr.*, **645**, 241 (1993).
108. T. Kimura, K. Nakanishi, T. Nakagawa, A. Shibukawa and K. Matsuzaki, *Pharm. Res.*, **22**, 667 (2005).
109. C. L. Su, R. J. Dai, B. Tong and Y. L. Deng, *Chinese Chem. Lett.*, **17**, 649 (2006).
110. W. R. Bowen and R. R. Nigmatullin, *Sep. Sci. Technol.*, **37**, 3227 (2002).
111. T. Gumi, C. Minguillon and C. Palet, *Polymer*, **46**, 12306 (2005).
112. A. Higuchi, H. Yomogita, B. O. Yoon, T. Kojima, M. Hara, S. Maniwa and M. Saitoh, *J. Membr. Sci.*, **205**, 203 (2002).
113. H. A. Wagenknecht, E. D. A. Stemp and J. K. Barton, *J. Am. Chem. Soc.*, **122**, 1 (2000).
114. A. Higuchi, Y. Ishida and T. Nakagawa, *Desalination*, **90**, 127 (1993).

115. F. Garnier, J. Randon and J. L. Rocca, *Sep. Purif. Technol.*, **16**, 243 (1999).
116. S. Edmondson, V. L. Osborne and W. T. S. Huck, *Chem. Soc. Rev.*, **1**, 14 (2004).
117. S. Tone, T. Masawaki and T. Hamada, *J. Membr. Sci.*, **103**, 57 (1995).
118. S. Tone, T. Masawaki and K. Eguchi, *J. Membr. Sci.*, **118**, 31 (1996).
119. T. Aoki, T. Fukuda, K. I. Shinohara, T. Kaneko, M. Teraguchi and M. Yagi, *J. Polym. Sci., Polym. Chem.*, **42**, 4502 (2004).
120. A. Higuchi, A. Hayashi, N. Kanda, K. Sanui and H. Kitamura, *J. Mol. Str.*, **739**, 145 (2005).
121. Y. Matsuoka, N. Kanda, Y. M. Lee and A. Higuchi, *J. Membr. Sci.*, **280**, 116 (2006).
122. A. Higuchi, Y. Higuchi, K. Furuta, B. O. Yoon, M. Hara, S. Maniwa, M. Saitoh and K. Sanui, *J. Membr. Sci.*, **221**, 207 (2003).
123. I.-C. Kim, J. Jegal and K.-H. Lee, *J. Polym. Sci., Part B: Polymer Physics*, **40**, 2151 (2002).
124. T. Gumi, M. Valiente and C. Palet, *J. Membr. Sci.*, **256**, 150 (2005).
125. J. Randon, F. Garnier, J. L. Rocca and B. Maisterrena, *J. Membr. Sci.*, **175**, 111 (2000).
126. S. B. Lee, D. T. Mitchell, L. Tron, T. K. Nevanen, H. Soderlund and C. R. Martin, *Science*, **296**, 2198 (2002).
127. T. Aoki, K. Shinohara and E. Oikawa, *Makromol. Chem. Rapid Commun.*, **13**, 565 (1992).
128. M. Teraguchi and T. Masuda, *Macromolecules*, **35**, 1149 (2002).
129. J. Paris, C. Molina-Jouve1, D. Nuel, P. Moulin and F. Charbit, *J. Membr. Sci.*, **237**, 9 (2004).
130. M. Yoshikawa, K. Murakoshi, T. Kogita, K. Hanaoka, M. D. Guiver and G. P. Robertson, *Europ. Polym. J.*, **42**, 2532 (2006).
131. M. Nakagawa, Y. Ikeuchi and M. Yoshikawa, *Polymer*, **49**, 4612 (2008).
132. M. Yoshikawa, Y. Kondo and Y. Morita, *Bioseparation*, **10**, 323 (2001).
133. P. G. Ingole, K. Singh and H. C. Bajaj, *Arab. J. Chem.* (2011), DOI: 10.1016/j.arabjc.2011.10.011.
134. M. Yoshikawa, T. Ooi and J.-I. Izumi, *Eur. Polym. J.*, **37**, 335 (2001).
135. J. T. F. Keurentjes, L. J. W. M. Nabuurs and E. A. Vegter, *J. Membr. Sci.*, **113**, 351 (1996).
136. M. Ulbricht, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **804**, 113 (2004).
137. E. M. Van der Ent, K. Van't Riet, J. T. F. Keurentjes and A. Van der Padt, *J. Membr. Sci.*, **185**, 207 (2001).
138. B. B. Lakshmi and C. R. Martin, *Nature*, **388**, 758 (1997).
139. K. B. Jirage and C. R. Martin, *Trends Biotechnol.*, **17**, 197 (1999).
140. P. G. Ingole, W. Choi, K. H. Kim, H. D. Jo, W. K. Choi, J. S. Park and H. K. Lee, *Desalination*, **345**, 136 (2014).
141. P. G. Ingole, W. Choi, K. H. Kim, C. H. Park, W. K. Choi and H. K. Lee, *Chem. Eng. J.*, **243**, 137 (2014).
142. A. Prakash Rao, S. V. Joshi, J. J. Trivedi, C. V. Devmurari and V. J. Shah, *J. Membr. Sci.*, **211**, 13 (2003).
143. S. Leob and S. Sourirajan, *Reverse Osmosis*, National Research Council of Canada, Ottawa, Canada (1985).
144. S. H. Yun, P. G. Ingole, K. H. Kim, W. K. Choi, J. H. Kim and H. K. Lee, *Chem. Eng. J.*, **258**, 348 (2014).
145. J. E. Cadotte, R. J. Petersen, R. E. Larson and E. E. Erickson, *Desalination*, **32**, 25 (1980).
146. K. Singh, H. C. Bajaj, P. Ingole and A. Bhattacharya, *Sep. Sci. Technol.*, **45**, 346 (2010).
147. K. Singh, H. C. Bajaj and P. G. Ingole, *Patent Number(s):* WO2010109490-A1; IN200900629-I1 (2010).
148. P. G. Ingole, K. Singh and H. C. Bajaj, *Sep. Sci. Technol.*, **46**, 1898 (2011).
149. K. Singh, P. G. Ingole, J. Chaudhary, H. Bhrambhatt, A. Bhattacharya and H. C. Bajaj, *J. Membr. Sci.*, **378**, 531 (2011).
150. P. G. Ingole, K. Singh and H. C. Bajaj, *Ind. J. Chem. Technol.*, **18**, 197 (2011).
151. P. G. Ingole, K. Singh and H. C. Bajaj, *J. Trends Chem.*, **1**, 1 (2010).
152. K. Singh, P. G Ingole, H. Bhrambhatt, A. Bhattacharya and H. C. Bajaj, *Sep. Purif. Technol.*, **78**, 138 (2011).
153. P. G. Ingole, K. Singh and H. C. Bajaj, *Desalination*, **281**, 413 (2011).
154. P. G. Ingole, H. C. Bajaj and K. Singh, *Desalination*, **305**, 54 (2012).
155. P. G. Ingole, H. C. Bajaj and K. Singh, *Desalination*, **343**, 75 (2014).
156. P. G. Ingole, H. C. Bajaj and K. Singh, *Procedia Engineering*, **44**, 358 (2012).
157. P. G. Ingole, H. C. Bajaj and K. Singh, *RSC Advances*, **3**, 3667 (2013).
158. K. Singh, P. G. Ingole, H. C. Bajaj and H. Gupta, *Desalination*, **298**, 13 (2012).
159. P. G. Ingole, K. Singh, H. C. Bajaj, D. N. Srivastava and B. Rebary, *Sep. Sci. Technol.*, **48**, 1777 (2013).
160. P. G. Ingole, K. Singh and H. C. Bajaj, *Adv. Mater. Res.*, **2**, 1 (2013).
161. K. Singh, H. C. Bajaj and P. G. Ingole, *Patent Number(s):* WO2013118148-A1; PCT/IN2013/000081 (2010).