Enantiomeric separation of \(\alpha\)-amino acids by imprinted terpolymer membrane

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Abstract
In this work, molecularly imprinted polymer membrane (\(\alpha\)-arginine (Arg) imprinted terpolymer P\((\text{AN-co-AA-co-AAm})\) membrane) was prepared by the wet phase inversion method. Acrylamide (AAm) and acrylic acid (AA) were used as the functional monomers and acrylonitrile (AN) was used as a cross linker. The removal of template molecules from the membrane matrix increased the number of free –COOH groups and reduced dimerized –COOH groups, which is an indirect evidence of the formation of recognition sites. Optical resolution was performed in ultrafiltration cell using aqueous solutions of racemic mixtures of \(\alpha\)-amino acids (arginine and asparagine). The imprinted membrane permeated \(\delta\)-enantiomers preferentially achieving 93\% and 72\% enantiomeric excess for \(\delta\)-arginine and \(\delta\)-asparagine, respectively.

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1. Introduction
The molecular imprinting technique is used to create specific recognition sites in polymers matrix (Vlatakis et al., 1993; Haupt, 2002). It is well known that molecularly imprinted polymers (MIPs) possess high selectivity and sensitivity for template molecules. Molecularly imprinted polymer membranes (MIPM) are widely used for the separation of compounds for which they have got recognition sites. In recent years, a variety of approaches have been applied to develop polymer membranes; for ex. in-situ polymerisation (Singh et al., 2011a,b) deposition on support membranes, phase inversion precipitation, spin coating, or grafting to a polymer or on the surface of membrane (Geismann et al., 2007). The method for preparing a MIP film coating on an electrode was demonstrated by modifying a quartz crystal microbalance (QCM) electrode with a vinyl-terminated self assembly monolayer...
and photo or electro polymerising the film in situ at the electrode (Cao et al., 2001; Malitesta et al., 1999; Panasyuk et al., 1999; Deore and Nagaoka, 2000; Peng et al., 2000). Synthesis of imprinted films of titanium dioxide by sequential chemisorption and activation of gold coated quartz crystal microbalance electrode has also been demonstrated (Lahav et al., 2001a,b; Lee et al., 1998). Furthermore, imprinted films have been synthesised by polycondensation of urethanes (Dickert et al., 1999, 1998, 2000), for the separation of chiral compounds polymer membranes have been synthesised by using interfacial polymerization methods (Singh et al., 2009, 2010a,b; Ingole et al., 2011a,b).

Composite membranes were prepared by the deposition of MIP layer on to the surface of polyvinylidene fluoride (PVDF) microfiltration membrane pre-coated with a photo initiator benzoin ethyl ether using 2-(dimethylamino) ethyl methacrylate as a functional monomer and trimethylopropane trimethacrylate as a cross-linker (Kochkodan et al., 2001, 2002; Hilal and Kochkodan, 2003). Phase inversion precipitation is another technique that can be used to synthesise imprinted membranes, which involves spreading a liquid phase of the cast solution containing the imprinting mixture on a glass plate and coagulating the imprinted polymer membrane in a non solvent or poor solvent for polymer (Wang et al., 1996, 1997a; Matthew and Shea, 1996). Although it involves soluble polymers, it cannot be used with conventional imprinting approaches in highly cross-linked polymers. Alternatively imprinted membrane may be prepared by solubilising pre-polymer that establishes interactions between the template and polymer during the period of adopting final conformation (Yoshikawa et al., 1996, 1999).

The grafting of imprinted polymer on support having immobilized initiator such as benzophenone on it subsequently polymerizes the imprinted polymer at the point of its attachment on the support (Piletsky et al., 2000; Sulitzyk et al., 2002; Wang et al., 1997b; Quaglia et al., 2001; Titirici and Hall, 2002). However, gelation and polymerisation in solution can occur and such side reactions are difficult to avoid. The problem has been solved by using initiators where one of the radicals formed by their decomposition is unable to initiate polymerisation but is capable of recombining and therefore terminating the growing polymers (Ruckert et al., 2002; Sellergren et al., 2002).

The present communication reports the enantiomeric separation of \( \alpha \)-amino acids by imprinted terpolymer membrane. The terpolymer membrane was characterized with Fourier Transform-Infrared spectra (FT-IR) using KBr pellet (Perkin-Elmer, GX). Energy-dispersive X-ray spectroscopy (EDX) analysis was performed on EDX analyzer (Leo, 1430UP, Oxford instruments) to estimate the amide content of the membrane after amide formation. The surface morphology of dried, fractured (for transverse section) and gold sputtered membrane samples was studied using scanning electron microscope (Leo, 1430UP, Oxford instruments) at 5 kV voltage in back scattering mode of electron detection.

2.2.2. Characterization of imprinted terpolymer membrane

The terpolymer membrane was characterized with Fourier Transform-Infrared spectra (FT-IR) using KBr pellet (Perkin-Elmer, GX). Energy-dispersive X-ray spectroscopy (EDX) analysis was performed on EDX analyzer (Leo, 1430UP, Oxford instruments) to estimate the amide content of the membrane after amide formation. The surface morphology of dried, fractured (for transverse section) and gold sputtered membrane samples was studied using scanning electron microscope (Leo, 1430UP, Oxford instruments) at 5 kV voltage in back scattering mode of electron detection.

2.2.3. Enantioseparation of racemic mixture of arginine and asparagine

The pressure driven permeation experiments were performed in an ultrafiltration cell (Amicon Inc. USA) having an effective membrane area of \( 1.994 \times 10^{-3} \) m\(^2\). Volumetric flux (Jv) was recorded at constant temperature (25°C) using 5.7 mM concentrated aqueous solutions of racemic arginine and asparagine as the feeds.

2.2.4. Analysis of permeates

The concentration of \( \alpha \)-amino acid in permeate was determined by UV–Vis spectrophotometer (Shimadzu UV-2550) at \( \lambda \)-max 284 nm. The concentrations of enantiomers in the permeate were determined using high pressure liquid chromatography (Jasco) equipped with PDA detector. The chromatograms were recorded at 200 nm using Chiral Crownpak CR (+) column (4.6 mm (i.d.) x 150 mm (l)), Daicel Chemical Industries Ltd., Japan) and Perchloric acid (pH 1.5) as the mobile phase at a flow rate of 0.6 ml/min at 25°C.

2.3. Explanation

The performance of membrane-based optical resolution process is explained in terms of membrane permeability and separation capability of membrane.

2.3.1. Permeability

The membrane permeability is a measure of the productivity of membrane process and is expressed in terms of solute flux (Js).
2.3.2. Solute flux ($J_s$)

The amount of solute in grams or moles passes through per unit area of membrane per unit time at constant pressure is termed as solute flux. It is calculated by measuring the volumetric flux and the concentration of solute per unit volume of permeate.

$$J_s = \frac{Q}{At}$$ (i)

where, $Q$ is the amount of solute in grams or moles, $A$ is area of membrane in square meters, and $t$ is time in h.

2.3.3. Separation

The separation of a particular type of solute from feed stream is the basic characteristic of membranes. Therefore, solute separation is considered a measure of effectiveness of membrane process and is expressed by the following equation as percentage of solute separated from solution:

$$\%R = (1 - \frac{C_p}{C_f}) \times 100$$ (ii)

where $C_p$ and $C_f$ are concentrations of solute in permeate and feed, respectively.

2.4. Enantiomeric selectivity

The enantioselectivity is a measure of optical purity and is defined in terms of the percentage enantiomeric enrichment or excess (\% ee) and separation factor ($\alpha$). The enantioselectivity of the membrane is calculated using following equation:

$$ee(\%) = 100 \times \frac{(D - L)}{(D + L)}$$ (iii)

The separation factor ($\alpha$) is the ratio of two enantiomers in permeate and feed solution as estimated by the following equation:

$$\alpha = \frac{C_{Dp}}{C_{Lp}} = \frac{C_{Df}}{C_{Lf}}$$ (iv)

If the feed solution is of the racemic compound, then Eq. (iv) may be reduced to following equation:

$$\alpha = \frac{C_{Dp}}{C_{Lp}}$$ (v)

2.5. Leaching of imprinted molecule

The template molecule, D-Arg was removed from terpolymer membrane by washing it with 5% acetic acid at 25°C in a shaking incubator (Julabo, SW23) at 150 rpm for 2 h and then rinsed with distilled water. This membrane was washed with distilled water and wash water was analyzed by UV spectrophotometer to observe the presence of template molecule (D-Arg). The membrane was continuously washed till it was free from template (see Fig. 1). The MIP membrane was kept in distilled water until its next use.

3. Results and discussion

3.1. Characterization of the membranes

3.1.1. ATR-FTIR spectroscopy

The FT-IR spectra of the membrane showed characteristics spectral band of free acid and amide group (Fig. 2). The appearance of absorption bands of O-H stretching at 3500 cm$^{-1}$, free COOH at 3450 cm$^{-1}$, dimerized COOH at 3220 cm$^{-1}$, C=O stretching at 1720 cm$^{-1}$, C=O stretching (amide) 1678 cm$^{-1}$ and peak in the region of 1460 cm$^{-1}$ may be attributed to the C=N group. Such absorbance was observed in the terpolymer membrane (Park and Kim, 2004).

3.1.2. Scanning electron microscopy (SEM)

The electron micrographs of membrane given in Fig. 3 indicate rough surface and porosity. Surface view of terpolymer membrane (Fig. 3 (A)) indicates that the surface is rough, has ridges and valley type impressions all over. In some places depression is less and in some places more. The surface view also indicates the presence of nano size pores as an indicative of porosity in
the membrane. The transverse section of membrane (Fig. 3 (B)) shows various size macro voids which were formed on leaching template molecules from the membrane.

3.2. Permeability

The volumetric flux from 5.7 mM concentrated aqueous solutions of racemic arginine and asparagine at 15 psi and 30 psi against permeation has been plotted as (Fig. 4). It is observed that volumetric flux from both solutions decreased with time. The volumetric flux from arginine solution at 15 psi and 30 psi is 10.13 L m⁻² h⁻¹ and 12.36 L m⁻² h⁻¹ and from asparagine solution is 11.87 L m⁻² h⁻¹ and 13.99 L m⁻² h⁻¹ at corresponding pressures. This indicates higher flux of asparagine solution compared to arginine solution because of marginally higher molecular size of size of arginine compared to asparagine molecule. The viscous flow of liquid through fine size pores of the membrane may face resistance due to tortuosity of pores and size of the molecule flowing. The concentration of feed solutions 5.7 mM was taken for sake of optimizing flux at moderate concentration. The volumetric flux is taken as proportional to trans-membrane pressure however as the pressure across the membrane is increased when the membrane pores deform and pore deformation is considered to be directly proportional to applied pressure. Initially deformation rate was higher which declined gradually. The thickness of membrane was observed to vary over 100 μm range.

3.3. Solute flux (Js)

Solute flux of racemic arginine and asparagine from 5.7 mM concentrated solutions at 15 and 30 psi as a function of permeation time depicted in Fig. 5 shows that the solute flux decreases with permeation time due to the concentration polarization and plugging of pores of the membrane with time. Asparagine flux was 7.38 g m⁻² h⁻¹ after 2 h at 15 psi pressure and arginine flux was 5.38 g m⁻² h⁻¹ at the corresponding time and pressure; similarly at 30 psi after 2 h of permeation, asparagine flux was 20.13 g m⁻² h⁻¹ and arginine flux was 12.76 g m⁻² h⁻¹. Thus, flux of asparagine is more compared to arginine at the corresponding pressure and time that indicates asparagine permeated faster than arginine through the membrane.

3.4. Selectivity

The percentage rejection of arginine and asparagine from 5.7 mM feed solutions with respect to permeation time is depicted in Fig. 6. It is observed that rejection increased with permeation time and overall rejection was in the range of 45–65%. The rejection of arginine was marginally higher than that of asparagine. The rejection of solute by a membrane occurs as a result of solute-membrane interactions such as adsorption, electrostatic, hydrophobic/hydrophilic etc. (Vito and Punzi, 1999).
Higher interactions favor rejection of solute. The rejection of solute by membrane could be described by solute-diffusion model. The rejection of $\alpha$-amino acids decreases marginally with time.

3.5. Enantiomeric selectivity

Molecularly imprinted membranes (MIMs) were fabricated on a glass plate by incorporating optically pure print or template molecules into the membranes and then extracting the template molecules to form voids that recognize the template molecule and the family or analog of the print molecules. Use of membrane for the optical resolution of $\alpha$-amino acids by permeating their aqueous solutions through the membrane, the print molecules and their analogs are selectively adsorbed to the print sites and the other enantiomers are excluded. MIMs are adsorption-enantioselective membranes as the membrane distinguishes enantiomers and performs functional separation due to stereoselective interactions between enantiomers and chiral recognition sites. The resulting membranes showed adsorption selectivity toward the print molecule and analogs. The variation in rate of penetration and adsorption of enantiomers on to the membrane surface becomes the basis of separation of enantiomers. The percentage enantiomeric excess (%ee) of $\alpha$-arginine and $\alpha$-asparagine from 5.7 mM solution of racemic arginine and asparagine was observed (Fig. 7). The maximum enantiomeric excess (93%) was achieved from terpolymer membrane. % ee increases with permeation time due to increased interactions between recognition sites and enantiomers. The concentration profile of D and L enantiomers of arginine and asparagine in permeate were practically stable throughout observation period (10 h) suggesting the existence of temporary and reversible interactions between membrane and enantiomers.

3.6. Separation factor ($\alpha$)

Enantioselective property of membranes may also be described as separation factor ($\alpha$) a ratio of enantiomers in permeate solution to feed solution as given by the following equation for racemic feed:

$$\alpha = \frac{C_{Dp}}{C_{Lp}}$$  \(\text{(vi)}\)

The separation factor for D-arginine and D-asparagine from 5.7 mM feed solutions is given in Fig. 8 elucidating that separation factor varies over the range of 1.8–5.6. The maximum separation factor achieved is, $\alpha = \sim 5.56$ with this terpolymer membrane.

4. Conclusions

Molecularly imprinted polymeric membranes showing optical resolution can be prepared using two functional monomers of AAm and AA to give the terpolymer P(AN-AA-AAm) matrix with many recognition sites and amide groups created by coupling reaction during polymerization by applying an alternative molecular imprinting technique. The membrane imprinted by D-isomer recognizes D-isomer in preference to the corresponding L-isomer, and vice versa. Ultrafiltration technique used for the separation of the racemic amino acid showed that permeselectivity directly reflects its adsorption selectivity. Higher enantioselectivity (93%) was observed for arginine as compared to asparagine (72%). The enantioselectivity of membrane shows time dependency and increases with time.

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