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ORIGINAL ARTICLE

Enantiomeric separation of α-amino acids by imprinted terpolymer membrane

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Abstract In this work, molecularly imprinted polymer membrane (D-arginine (Arg) imprinted terpolymer P(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 α-amino acids (arginine and asparagine). The imprinted membrane permeated D-enantiomers preferentially achieving 93% and 72% enantiomeric excess for D-arginine and D-asparagine, respectively.

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

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Abbreviations: J_V, volumetric flux (L m^{-2} h^{-1}); J_S, solute flux (g m^{-2} h^{-1}); *R*, rejection of solute (%); %ee, percentage enantiomeric excess; α, separation factor; C_p, concentrations of solute in permeate (g/L); C_f, concentrations of solute in feed (g/L); C^{Dp}, concentrations of D-enantiomers in permeate (mg/L); C^{Lp}, concentrations of L-enantiomers in permeate (mg/L); C^{Df}, concentrations of D-enantiomer in the feed (g/mL); C^{Lf}, concentrations of L-enantiomer in the feed (g/mL).

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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 polyvinylidine 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; Mathew 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 prepolymer 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; Sulitzky 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 α-amino acids in pressure driven ultrafiltration technique using MIP membrane prepared by the wet phase inversion method. The MIP membrane exhibited satisfactory enantiomer separation with reasonably good productivity.

2. Experimental

2.1. Materials

Acrylonitrile (AN), acrylic acid (AA) and acrylamide (AAm) were procured from SD fine chemicals Ltd. (India). Racemic arginine (Arg.), Racemic asparagine (Asp.), D-arginine (D-Arg), L-arginine (L-Arg), D-asparagine (D-Asp) and L-asparagine (L-Asp) were purchased from Sigma-Aldrich USA. Dimethyl sulfoxide (DMSO) and other solvents were of analytical grade. All reagents were used as received without further purification.

2.2. Methods

2.2.1. Preparation of terpolymer membrane

A terpolymer membrane was prepared by the wet phase inversion method by mixing 0.2 g of D-Arginine dissolved in a solution containing 3 g AA, 3 g AAm and 20 g DMSO. Then 12.2 g of AN was added to the above mixture. 0.09 g of azoisobisbutyronitrile (AIBN) initiator dissolved in 20 g of DMSO was added to the reaction vessel and reaction mass was heated at 65 °C for 6 h under nitrogen atmosphere. The resultant polymer solution was cast on a glass plate as a film maintaining thickness of 100 μm which was then immersed in distilled water at 25 °C to coagulate the polymer film. The template molecule, D-Arg was removed from the membrane by leaching to create molecule recognition sites. After leaching terpolymer membrane was stored in distilled water.

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 1.994×10^{-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 α-amino acid in permeate was determined by UV–Vis spectrophotometer (Shimadzu UV-2550) at λ-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.) × 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).