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Enantiomeric separation of antidepressant drug fluoxetine based on chiral membranes

Sema Salgın*, Uğur Salgın, Nagihan Tuzlalı

Cumhuriyet University, Faculty of Engineering Department of Chemical Engineering 58140, Sivas, Turkey, Tel. +90 (346) 2191010/2804, Fax +90 (346) 2191179, email: ssalgin@cumhuriyet.edu.tr (S. Salgın), usalgin@cumhuriyet.edu.tr (U. Salgın), nagehansoyer@hotmail.com (N. Tuzlalı)

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ABSTRACT

In this study, the enantiomeric separation of antidepressant drug fluoxetine (FLX) was carried out using chiral selector-immobilized polyethersulfone (PES) membranes. For this purpose, the chiral selectors BSA, *Candida rugosa* lipase (CRL) and α-cyclodextrine (α-CD) were adsorbed on PES membranes by the static adsorption at different pH values. Chemical and electrokinetic characterizations of modified membranes were done by Fourier transforms infrared (FTIR) spectroscopy and by an electrokinetic analyzer (EKA), respectively. FTIR and EKA analysis were particularly employed in order to evaluate the immobilization efficiency. Racemic FLX was ultrafiltrated with the chiral selective membranes using the stirred ultrafiltration cell. The effectiveness of chiral separation was characterized in terms of the values of enantiomeric excess (ee%) in permeate. During the ultrafiltration, the samples taken from permeate were analyzed with liquid chromatography system using Chiralpak AD-H column for the determination of R-FLX and S-FLX enantiomers. The ee_s values of BSA and CRL in the permeate were approximately equal.

Keywords: Chiral membrane; Chiral selector; Enantiomeric separation; Fluoxetine

1. Introduction

Enantiomeric forms of a drug can have dramatically different in potency, toxicity, and behavior in biological systems. One of the enantiomers shows the required biological action while the other enantiomer performs undesired activities, creates side effects or presents toxicity. Even when side effects are not serious, the inactive enantiomer must be metabolized and thus represents an unnecessary burden for the organism [1].

Chiral separation of enantiomeric drugs has attracted great attention because the Food and Drug Administration is expressing a strong preference that all medicinal drugs be sold in enantiopure form. In view of this, enantiomeric separation of drugs has gained vital importance for the pharmaceutical companies. The traditional chiral separation methods are diastereomeric or preferential crystallization, chemically or enzymatic kinetic resolution and column chromatography [2-4]. The increasing demand of optically pure compound in respect of high purities and amounts in the pharmaceutical, food and chemical industries necessitates the development of new separation and production techniques.

The membrane-based chiral separation offers several advantages such as high throughput, energy saving, continuous process, easily scale up and more economical than many other separation processes [5–7]. The chiral separation with membranes can be divided into affinity membranes system using chiral selectors or ligands in feed solution (non-chiral membrane), supported liquid membranes including enantiomer-recognizing carriers and solid membranes (chiral membrane). In the latter membrane systems, chiral separation was carried out by chiral polymers, molecularly imprinted membranes and immobilized chiral

*Corresponding author.

selector membranes [5–11]. Chiral separation of racemic drugs through polymeric membranes with an immobilized chiral selector could be very promising for commercial systems in the future.

Chiral selectors show different interactions with a pair of enantiomers. The mechanism of enantio-recognition is described by a three point rule. According to this rule, the enantiomers should have at least three different interactions with chiral selector and at least one of these interactions is stereochemically dependent, then the enantiomers can in principle be resolved. The free energy difference of each interaction should be different for a successful chiral separation [12]. A chiral selector should be stereoselective and form a transient diasteromeric complex with each enantiomer and it should be soluble and chemically stable in the used electrolyte. It should also not interfere with the detection and should exhibit fast complexation kinetics [13]. In membrane-based chiral separation processes, the most commonly used chiral electors are cyclodextrins and their derivatives [7,14,15], BSA [16–18] and DNA [19–22]. In these studies, chiral separation of amino acids was investigated.

FLX, trademarked as Prozac®, is one of the widely used antidepressants of the selective serotonin reuptake inhibitors for treatment of depressive disorders [23,24]. It works by modulating serotonin concentration in presynaptic neurons [25]. However, FLX produces undesired side effects including anxiety, sleep disturbances, sexual dysfunction and gastrointestinal disturbances [26]. FLX is currently marketed as a racemic mixture of two enantiomers [27], but the enantiomers of FLX do not have similar activity [28]. The attempt to develop a single enantiomer formulation of FLX for the treatment of depression has been unsuccessful. There is some study related to analyze and quantify of this racemic drug in the literature and the enantiomeric separation of FLX in these studies were achieved by chromatography and electrophoresis techniques [23–25,27,28].

In this study, for the first time, the enantiomeric separation of the racemic antidepressant drug FLX with chiral selective PES membrane was achieved. In the experiments of the chiral membranes preparation, the effects of chiral selector type and pH on binding of chiral selector on membranes were investigated for the static adsorption method. The efficacy of adsorption at different experimental conditions was determined by illustrating the changes occurred on the membrane surface after immobilization using FTIR-ATR spectrometer. Zeta potentials of membranes and chiral selectors were determined by electrokinetic analyzer (EKA) and Zetasizer equipments, respectively. Racemic FLX was ultrafiltrated with the chiral selective membranes using the stirred ultrafiltration cell. The effectiveness of chiral membrane was characterized in terms of the values of enantiomeric excess (ee%) in permeate.

2. Materials and methods

2.1. Materials

In the experiments R-FLX hydrochloride (Sigma F1678), S-FLX hydrochloride (Sigma F1553), Na_2HPO_4 (Merck 106586), $NaH_2PO_4{\cdot}2H_2O$ (Merck 106345), BSA (Sigma A 9647), CRL (Sigma L1754), α-CD (Sigma C4642), acetonitrile (Merck 100030), methanol (Sigma-Aldrich 34885) were used. All aqueous solutions were prepared from deionized water (Milli Q system, Millipore, Gradient model) with a resistivity of 18.2 MΩ cm. PES ultrafiltration membranes (14659-47D, Sartorius AG-Germany) with nominal cut-off values of 30 kDa and diameter of 47 mm were used in the experiments. Before all experiments, the membranes were rinsed with Milli-Q deionized water to remove preservatives.

2.2. Chiral membrane preparation

Static adsorption experiments of chiral selectors (BSA, CRL and α-CD) on PES membranes were carried out in an orbital shaker (Lab-line, MAXQ 4000) operated at 200 rpm and 20 ± 3°C in 0.1 M $NaH_2PO_4{\cdot}2H_2O$-Na_2HPO_4 buffer solution at pH = 4.8, 5.8, 6.6 and 7.2. Membranes were brought into contact with chiral selector solutions until equilibrium time of 4 h. Adsorption experiments were performed for the chiral selector concentration 1 g/L. After adsorption, membranes were rinsed with buffer solution to remove unbounded chiral selector.

2.3. FTIR-ATR analysis

A FTIR-ATR spectrometer (Spectrum 100, Perkin Elmer) was used to characterize virgin and the chiral selector linked membranes. The ATR accessory of the FTIR contained a ZnSe crystal. The spectra of membranes were recorded at a resolution of 4 cm^{-1}. The original and modified membranes were rinsed with buffer and completely dried at room temperature before analyses.

2.4. Zeta potentials measurements

The zeta potential of PES membranes was analyzed by an Electrokinetic Analyzer (EKA SurPASS-type A, Anton Paar GmbH, Graz, Austria). The apparent zeta potential of membranes was calculated through the streaming potential data using the Helmholtz-Smoluchowksi equation. The details of the system and procedure have been described by Salgın et al. [29].

The zeta potential of chiral selectors was performed using Zetasizer NanoZS instrument (Malvern Instruments Ltd., United Kingdom). The zeta potential was calculated through the electrophoretic mobility using the Henry equation [30].

2.5. Separation of racemic FLX with chiral selective membrane

Separation performance of chiral selective membrane was examined using a stirred ultrafiltration (UF) cell (8050, Amicon) at room temperature. For UF experiments, the racemic mixtures of FLX were prepared by mixing equal amounts of R-FLX and S-FLX. A racemic mixture of 0.2 g/L was prepared in deionized water. The membrane with effective membrane area of 0.00134 m^2 was fixed tightly in a stirred ultrafiltration cell which possessed 50 mL volume, and 20 mL of 0.2 g/L of racemic FLX aqueous solutions was used as feed solutions. The experiments were carried out until the value of ee% reached a constant value. 0.3 MPa of constant pressure was applied through using nitrogen

gas. The permeation separation proceeded at room temperature. The samples were taken in permeate and were analyzed by HPLC system (LC 10, Shimadzu) using ChiralPAK AD-H (Daicel) column (λ = 254 nm, 0.25 mL/min, 90% acetonitrile –10% methanol (v/v) mobile phase) to calculate the enantioselectivity. The enantioselectivity of transport through membranes was expressed as the enantiomeric excess of the R-FLX (ee_R) and S-FLX (ee_S) respectively. Eqs. (1) and (2) were used to calculate the *ee*% value that is defined as the ratio of the concentration difference over the total concentration of both enantiomers in the permeate [3].

$$ee_R = \frac{C_R - C_S}{C_R + C_S} \times 100 \quad (1)$$

$$ee_S = \frac{C_S - C_R}{C_R + C_S} \times 100 \quad (2)$$

where C_R and C_S denote concentration of R- and S-enantiomers of FLX in the permeate, respectively.

3. Results and discussion

3.1. Effect of pH on the preparation of chiral selective membranes

Static adsorption of the chiral selectors BSA, CRL and CD on PES membranes was investigated at 200 rpm and 20 ± 3°C in 0.1 M $NaH_2PO_4{\cdot}2H_2O$-Na_2HPO_4 buffer solution at pH = 4.8, 5.8, 6.6 and 7.2. FTIR spectra of original PES membrane and chiral selector adsorbed membrane are presented in Fig. 1. FTIR spectra were used to determine the most suitable pH for the preparation of chiral selective membrane. One characteristic peak was inspected as the evidence of the adsorption of protein based chiral selectors as BSA and CRL on the membrane surface, which are observed at 1660–1663 cm^{-1}, the amide I band (Fig. 1a,b). The amide I band originates predominantly from C=O streching vibration of the peptide groups [31,32]. The spectra of BSA and CRL adsorbed membranes at all pH values revealed the presence of the amide I bands. The intensity of amide I band is proportional to adsorption degree. The highest intensity of amide I band for BSA and CRL was observed on the chiral membrane at pH = 4.8 where the amount of adsorption was also the highest. In the case of α-CD adsorption (Fig. 1c), it is observed that the textural properties of PES membrane are slightly affected by adsorption of α-CD. The charasteristic peak of α-CD at 2935 cm^{-1} corresponds to *stretching* vibration of C-H [33]. As shown in Fig. 1c, it is conceivable that the slightly increased of the 2935 cm^{-1} band was corresponded to the adsorption of α-CD. The highest intensity of C-H vibration was observed on the chiral membrane at pH = 4.8. In addition, it should be noted that the vibration mode of phosphate anion originated from $NaH_2PO_4{\cdot}2H_2O$-Na_2HPO_4 buffer at ~949 cm^{-1} is observed for all chiral selector adsorbed membranes [29].

Zeta potentials of the original PES membranes and chiral selectors have a significant effect on the degree of adsorption caused by the membrane-chiral selector interfacial interactions. In Figs. 2 and 3, there are plotted zeta potential changes of chiral selector and original PES membrane in 0.1 M $NaH_2PO_4{\cdot}2H_2O$-Na_2HPO_4 solution as a function of pH, respectively. All of the chiral selectors had negative zeta potential values under the studied conditions. Chiral selectors did not give any isoelectric point (IEP), that is the pH value at which the zeta potential is zero. With decreasing pH, the absolute zeta potentials of chiral selectors decreased.

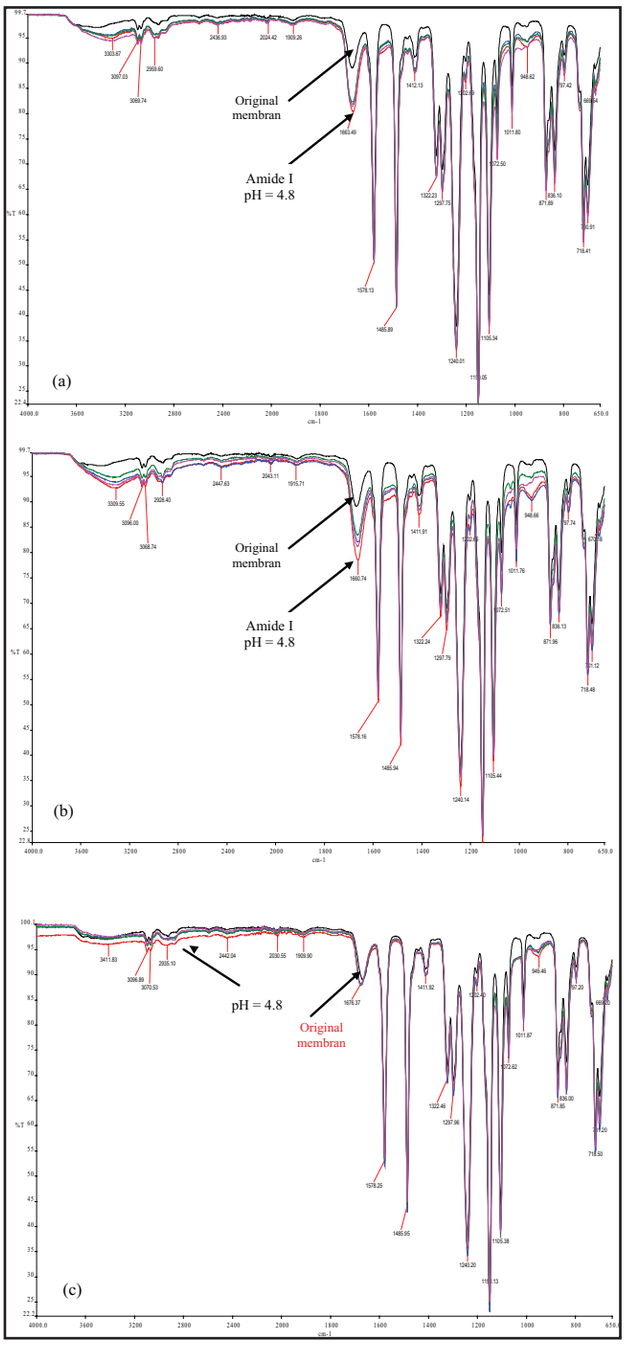

Fig. 1. FTIR spectra of original and chiral selector adsorbed PES membranes in 0.1 M $NaH_2PO_4{\cdot}2H_2O$-Na_2HPO_4 solution at pH 4.8, 5.8, 6.6 ve 7.2 a) BSA adsorbed membrane, b) CRL adsorbed membrane and c) α-CD adsorbed membrane.

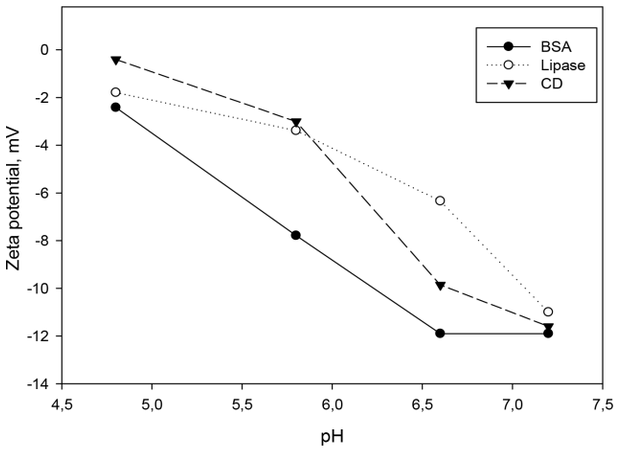

Fig. 2. The zeta potential of chiral selectors as a function of pH.

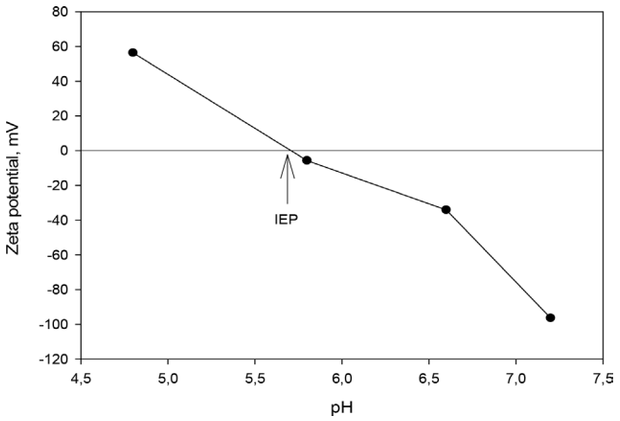

Fig. 3. The zeta potential of the original PES membrane as a function of pH.

At low pH, the original PES membrane gave positive zeta potential which decreased when pH was raised. IEP of PES membrane was pH 5.67. PES membrane had negative zeta potential values above IEP due to anion adsorption to hydrophobic PES surfaces [29,34].

It was observed that zeta potentials of BSA and CRL adsorbed PES membranes changed at pH = 4.8 where the amount of adsorption was also the highest. As a result of EKA analysis, the zeta potentials were determined to be 173 mV and 134.5 mV after the adsorption of BSA and CRL on membranes, respectively. This increase in zeta potentials of chiral membranes when compared to the original PES membrane (56.34 mV) indicates that a new group bonded to the membrane surface. Below the IEP of PES membrane, i.e., at pH 5.67, PES membrane had a positive charge; contrariwise, BSA and CRL had a negative charge. Therefore, below the IEP, attractive forces were dominant over repulsion forces and BSA and CRL showed a tendency to be adsorbed. The zeta potential of α-CD adsorbed membrane was determined to be 54.2 mV. The results of FTIR and zeta potential analysis of α-CD adsorbed membrane were not significantly changed when compared to the original PES membranes. The PES membrane used in this experiment had a 30 000 Da MWCO comparable to the MWof α-CDs (MW = 972.84 Da). It can be considered that the majority of α-CD was adsorbed inside the membrane pore walls and that only trace amounts adsorbed on membrane surfaces.

3.2. Separation performance of chiral selective membrane

The optimum pH was determined to be pH = 4.8 for the adsorption of chiral selectors on PES membrane. Separation performance of the prepared chiral selective membrane was examined using a stirred UF cell at room temperature. Initial racemic FLX concentration in feed chamber was 0.2 g/L. Enantio-separation was observed for all membranes adsorbed with BSA, CRL and α-CD chiral selectors. The all chiral selectors exhibited a stronger interaction with R-FLX than with S-FLX. It is conceivable that the more weakly bound S-enantiomer of FLX was preferentially permeated through the membrane. The enantiomeric excess of S-FLX were 10.86, 10.32 and 7.80% for BSA, CRL and α-CD, respectively.

4. Conclusion

In this study, the chiral selective membranes were prepared by the static adsorption of BSA, CRL and CD chiral selectors on PES membranes. The enantio-separation of racemic antidepressant drug FLX was achieved with the prepared chiral selective membranes. The chiral selectors BSA and CRL had similar values of ee_s. The multi-stage ultrafiltration process can be suggested to obtain higher ee %.

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