ENANTIOSELECTIVE SEPARATION OF PROPRANOLOL BY CHIRAL ACTIVATED MEMBRANES

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ABSTRACT

The employment of racemic drugs has become unattractive due to policy changes introduced by regulatory agencies. As a consequence, pharmaceutical industries have been forced to develop new technologies for preparing non-racemic drugs. Resolution can be performed by membrane technology due to its several advantages over traditional methods. And, in particular chiral activated membranes can be used for the enantioselective separation in continuous mode with a longer lifetime than corresponding liquid membrane systems.
In this work, chiral activated membranes have been tested for the enantioseparation of the racemic drug propranolol. Polysulfone (PS) based membranes have been prepared for this purpose, using N-hexadecyl-L-hydroxyproline (HHP) and L-di-n-dodecyltartrate (L-DDT) as chiral carriers, respectively. Kinetic experiments have been carried out using a membrane module with two rectangular channels separated by the membrane, which allows for a well-defined hydrodynamics of the feed and stripping phases. Propranolol transport across the membrane depends on its diffusion rate from the bulk solution to the membrane surface and also on the relative amount of carrier present in the membrane. Therefore, the influence of both the solute/carrier ratio and the flow rates of the feed and stripping phases on the rate of extraction and on the selectivity of the process was evaluated for both chemical systems. Modelling of kinetic experiments has been performed and mass transfer coefficients obtained for both systems were compared.

*Keywords: fixed carrier membranes, enantioseparation, racemic propranolol, chiral resolution, facilitated transport.*

**INTRODUCTION**

It is known that chirality strongly influences some chemical and biochemical reactions due to the different behaviour of the corresponding enantiomers. However, many chemical species with biological activity are prepared and used as racemic mixtures, despite the desired activity being often carried out by only one of the two enantiomers while the other undertakes no specific activity [1]. In certain cases, the later enantiomer may inhibit the desired effect of the first enantiomer or even have adverse side effects [2].
In the case of racemic propranolol, a β-blocking drug used for treating some cardiovascular anomalies, the S-isomer shows far more blocking activity than the R-isomer [3,4]. Thus, obtaining and identifying enantiopure compounds has become one of the most important demands, specially by pharmaceutical industries, which have been forced by regulatory agencies to develop methodologies for producing pure enantiomers. Most methods employed up to date, in the pharmaceutical industry, to obtain enantiopure compounds, such as stereoselective asymmetric synthesis, biotransformation, chiral separation processes based on the enzymatic kinetic resolution technique or diastereomeric crystallisation, have been shown to have several drawbacks. They require a considerable number of different steps and a high-energy consumption in order to produce a reasonable amount of one optically pure enantiomer [5-7]. Recently, other separation processes based on chiral stationary phases have increasingly gained attention at an industrial level, as both pure enantiomers can be obtained at the same time with far less difficulty [8]. In this context, the use of membrane technology for chiral separations offers several advantages over traditional methods, namely, low time cost, simplicity of operation and easy scale-up. Furthermore, when using chiral activated membranes only a small quantity of an expensive chiral selector is required [9].

Different enantioselective carriers have already been tested for the enantioseparation of racemic propranolol by using liquid membrane (LM) systems, such as N-n-alkyl-hydroxyprolines [10,11], or dialkyl tartrates [12]. In both cases, the carrier is present in the membrane and selectively forms a complex with one of the enantiomers, which is transported across the membrane by an ion pairing mechanism [6,10]. These transport systems are driven by a proton gradient between both feed and stripping aqueous phases.
However, liquid membranes systems have been shown to have problems of stability and short lifetime when tested under industrial separation conditions [13,14]. In an attempt to overcome these drawbacks, solid polymeric activated membrane systems have been proposed to resolve racemic mixtures [15-17].

In the present work, chiral activated membranes, based on a polysulphone polymeric support, containing N-hexadecyl-L-hydroxyproline (system A) or L-di-n-dodecyltartrate (system B) have been prepared and applied for the enantioselective transport of racemic propranolol.

Propranolol transport across the membrane is limited by its diffusion rate from the bulk solution to the membrane surface; therefore, the hydrodynamic conditions of the feed and stripping phases are important parameters.

On the other hand, for fixed site membranes the transport of solute is presumably achieved by diffusion in the membrane from a carrier site to a neighbour site. If the carriers are at a critical distance, the solute may be able to jump from site to site, thus increasing the transport rate significantly [18,19].

The influence of both, the solute/carrier ratio and the hydrodynamic conditions of the feed and stripping phases on the enantioselectivity and rate of solute transport were studied for the two systems (A and B). Mass transfer coefficients were calculated in order to be able to compare the two systems, operated under different experimental conditions.

THEORY

Transport Modelling
Transport mechanism schemes for the two systems propranolol/N-hexadecyl-L-hydroxyproline (system A) and propranolol/L-di-n-dodecyltartrate (system B) are shown in Figures 1 and 2, respectively. Both chemicals systems were previously studied in other membrane configurations and as a result the transport mechanisms involved in both cases were proposed [4,11].

In system A, the enantioselective interaction takes place in the feed side membrane surface, where the S-propranolol enantiomer preferentially forms an ionic pair with the carrier N-hexadecyl-L-hydroxyproline, and it is transported across the membrane. Propranolol transport is coupled by a proton antiport [11].

For system B, hydrophobic L-di-n-dodecyltartrate forms a non-polar complex with boric acid and, preferentially, with the corresponding enantiomer of propranolol. Thus, both propranolol and boric acid are extracted and form a tetrahedral complex involving hydrogen bonding with the tartrate [4].

A membrane module with two rectangular channels separated by the membrane was used for the kinetic experiments (details are given below). The well-defined hydrodynamic conditions established in both channels allow for a rigorous evaluation of the mass transfer coefficients.

Due to the small membrane area, the concentration change during a single pass is negligible. Therefore, in order to increase the change in concentration, extraction and stripping were carried out with recirculation of the feed and stripping phases through the module and back into the feed and stripping reservoirs, respectively. The solute concentration was measured along time.

The differential mass balances to each one of the enantiomers in the stripping phase are:
where $Q$ is the flowrate, $C$ is the solute concentration, $K$ is the overall mass transfer coefficient and $dA = w \, dz$ is the differential membrane area, where $w$ is the channel width and $dz$ the differential length; the subscripts SR and SS refer to the enantiomers $R$ and $S$ in the stripping phase, respectively, and the superscript * refers to the equilibrium concentration.

The transient mass balances concerning the stripping reservoir may be expressed as:

$$-V_S \left( \frac{dC_{SR}}{dt} \right) = Q_S \left( C_{SR}^{\text{in}} - C_{SR}^{\text{out}} \right)$$  (3)

$$-V_S \left( \frac{dC_{SS}}{dt} \right) = Q_S \left( C_{SS}^{\text{in}} - C_{SS}^{\text{out}} \right)$$  (4)

assuming that the solute concentration in the reservoir and at the module inlet is identical for both enantiomers, $C_{SR}^{\text{in}} = C_{SR}^{\text{in}}$ and $C_{SS}^{\text{in}} = C_{SS}^{\text{in}}$.

These four equations can be reduced to only two, taking into account that the driving forces, $(C_{SR}^* - C_{SR})$ and $(C_{SS}^* - C_{SS})$ can be considered constant along the membrane:

$$V_S \left( \frac{dC_{SR}}{dt} \right) = K_{SR} A \left( C_{SR}^* - C_{SR} \right)$$  (5)

$$V_S \left( \frac{dC_{SS}}{dt} \right) = K_{SS} A \left( C_{SS}^* - C_{SS} \right)$$  (6)

These equations may be integrated after substitution of the equilibrium concentrations, $C_{SR}^*$ and $C_{SS}^*$. Since there is a very small quantity of carrier within the membrane and both the feed and stripping phases have equal volumes, then the stripping equilibrium concentration equals the feed phase concentration for both enantiomers, $C_{SR}^* = C_{FR}$ and $C_{SS}^* = C_{FS}$, equations (5) and (6) become:

$$V_S \left( \frac{dC_{SR}}{dt} \right) = K_{SR} A \left( C_{FR} - C_{SR} \right)$$  (7)

$$V_S \left( \frac{dC_{SS}}{dt} \right) = K_{SS} A \left( C_{FS} - C_{SS} \right)$$  (8)
The overall mass transfer coefficients $K_{SR}$ and $K_{SS}$ for each enantiomer are calculated by least squares minimization of the residuals of the experimental data of $C_{SR}$ versus time and $C_{SS}$ versus time using Equations (7) and (8).

Enantioselectivity

The enantioselectivity of the membrane process is given in terms of enantiomeric excess. The enantiomeric excess is defined by the ratio of the difference between the concentration of both enantiomers in the feed or stripping phase to the total amount of both enantiomers present at any time, and was calculated according to [20]:

$$\text{Enantiomeric excess} = \left\{\frac{(C_{SS}-C_{SR})}{(C_{SS}+C_{SR})}\right\} \times 100 \quad (9)$$

EXPERIMENTAL

Reagents

$R$-propranolol hydrochloride, $S$-propranolol hydrochloride and racemic propranolol hydrochloride, all p.a. grade, were supplied by Sigma-Aldrich (Germany). N-hexadecyl-L-hydroxyproline (HHP), isopropyl myristate (IPM), triethanolamine and hydroxypropyl-$\beta$-cyclodextrin (HP-$\beta$-CD), all p.a. grade, were also purchased from Sigma-Aldrich (Germany). All other reagents used (such as acids and inorganic salts) were of analytical grade. MilliQ water was used for all aqueous solutions. Propranolol hydrochloride and tartaric acid were purchased from Aldrich Chemical Co. (USA), boric acid from Riedel–de Haën AG (Germany) and chloroform from Merck
L-di-n-dodecyltartrate (L-DDT) was prepared as described by Abe et al. [21].

Membrane Preparation

Polysulfone (PS) casting solution (15 % wt.) was prepared by dissolving PS (Basf) in a.r. grade N,N-dimethylformamide (DMF, Sigma-Aldrich). PS membranes were obtained by phase inversion technique of the polysulfone casting solution over a non-woven fabric, which assured re-enforced PS membranes [22]. Membranes were chiral activated by addition of one of the two different chiral carriers investigated, to the casting solution; N-hexadecyl-L-hydroxyproline (HHP), previously dissolved in isopropyl myristate (IPM), or L-di-n-dodecyltartrate (L-DDT).

Apparatus

Experiments have been carried out using a membrane module with two rectangular channels separated by the membrane. This configuration allows for a well-defined hydrodynamics of the phases. Aqueous fixed volume feed and stripping solutions were pumped to each membrane side in counter-current mode. All experiments were performed at 24 ± 1 °C. Figure 3 shows the experimental set-up.

Procedure

Two different chemical systems, N-hexadecyl-L-hydroxyproline (system A) and L-di-n-dodecyltartrate (system B), were characterized for the enantioselective resolution of
racemic propranolol, by studying the influence of both the solute to carrier ratio (S/C) and the flow rate of the feed and stripping phases on the transport rate and on the selectivity through the chiral activated membranes. With this purpose, two different solute/carrier ratios (50 and 100) were investigated for system A and for system B (12 and 120). Two different flow rates (10 and 100 ml/min) were also assayed for both systems.

For system A, the influence of the solute to carrier ratio (S/C) was studied by varying the N-hexadecyl-L-hydroxyproline (HHP) concentration in the membrane, while in the case of system B it was the propranolol concentration that was varied. The variation of S/C in system A was limited by the sensibility of the propranolol (solute) determination. Therefore, the carrier amount in the membrane should be varied to reach lower S/C ratios. On the contrary, the variation of S/C in system B was limited by the change of membrane properties with the carrier incorporation. Hence, in the latest case, solute concentration was varied.

In the case of system A, the feed phase contained 0.1g/L (0.34mM) of racemic propranolol and was adjusted at pH 8 with a borax buffer, and the stripping phase was buffered with disodium phosphate, at pH 7. For system B, the feed phase contained 1mM and 10 mM of racemic propranolol in a 0.1 M boric acid media at pH 5.2, adjusted with acetate buffer, while the stripping solution was maintained at pH 2 with a 1 M solution of formic acid.

A blank experiment using the polysulfone membrane without carrier was also performed for both systems.

Propranolol Determination
Two different detection techniques have been used to follow each chemical system (A and B). In the first case (A), a capillary electrophoresis system (P/ACE SYSTEM MDQ, Beckman, USA) was used to analyse the concentration of both enantiomers in the collected samples. Determination was performed using 50 µm internal diameter uncoated fused-silica capillaries of 60 cm (50 cm to the detector). Before each set of analyses, the capillary was rinsed with a 0.1 M NaOH solution, MilliQ water and finally with the separation buffer solution. The latter consisted of 100 mM phosphoric acid adjusted at pH 4.4 with triethanolamine, containing 17.4 mM hydroxypropyl-β-cyclodextrin (HP-β-CD) [23,24]. The applied voltage was 23kV and UV detection was carried out at 210nm. Samples were injected using the hydrodynamic mode for 5s, at 0.3 psi. The capillary was thermo stated at 20ºC. Between consecutive determinations, the capillary was rinsed with MilliQ water. At the end of the day, the capillary was washed with NaOH 0.1 M, MilliQ water and MeOH, which was used for removing organic material and for facilitating capillary drying.

For system B, the quantification of both propranolol enantiomers in the aqueous phase was performed by HPLC using a UV detector (Merck, Hitachi, Japan) at a wavelength of 254 nm. A Chiralcel OD-R (Daicel, Japan) column was used and the mobile phase was a 0.1 M aqueous solution of potassium hexafluorophosphate: acetonitrile (60:40).

Calculation Methods

The overall mass transfer coefficients were evaluated by fitting the experimental data, i.e., the experimental values of $C_s$ for both $R$ and $S$ enantiomers versus time, using
Scientist® (Micromath Scientific Software, U.S.A). The errors associated with the determined parameters were calculated for a confidence interval of 95%.

RESULTS AND DISCUSSION

Influence of the Solute to Carrier Ratio

With the purpose to investigate the influence of the solute to carrier ratio on the transport rate and on the enantioselectivity of racemic propranolol transport across the chiral activated membranes, the concentration of carrier within the membrane was adjusted to reach the solute/carrier ratios (S/C) of 50 and 100 for system A; the initial concentration of propranolol in the feed solution was also varied to obtain the solute/carrier ratios (S/C) of 12 and 120 for system B. The results obtained are shown in Figure 4 (system A) and Figure 5 (system B). It can be observed that the transport rate of propranolol across the membrane increases when decreasing the solute/carrier ratio in both membrane systems, i.e., when increasing the relative amount of carrier in the membrane. This behaviour is usually present in membrane transport systems based in facilitated transport mechanism. Here, the amount of active sites in the membrane (i.e.; the amount of carrier) is the limiting step of the process [22,25].

The blank experiments for both systems exhibited practically no transport, which was expected since the solute diffusion through the solid membrane without a carrier is extremely slow.
Influence of the Flow Rate

Two different flow rates of feed and stripping phases (100 and 10 ml/min.) were studied for both systems A and B, in order to determine its influence on the transport rate of propranolol across de chiral activated membranes. As can be seen in Figures 6 (a and b) and 7, for systems A and B respectively, lower flowrates cause a decrease of the transport rate of propranolol. This behaviour may be due to the corresponding decrease of the Reynolds number, when decreasing the flow rate. Therefore, the propranolol transport across the membrane is limited by its diffusion rate from the bulk solution to the membrane surface. Furthermore, in the case of system A, the differences on the transport rate between the two flow rates investigated are, as expected, more important at low solute to carrier ratio, due to the faster transport step within the membrane, for this case. Under these conditions, the maximum transport rate that may be attained, in the absence of boundary layer resistances, is much higher than for high S/C ratios.

Enantiomeric Selectivity

In this study, enantioselectivity was only found for system B. When working with system A, although the amount of carrier in the membrane relatively to the amount of solute in the feed solution was high, enantioselectivity was not observed due to the high transport rates obtained with the experimental conditions employed. Often, in these membrane systems, a compromise must be reached between both the external mass transfer conditions and the amount of carrier incorporated in the membrane. These two factors influence the transport rate of the solute through the membrane as well
as the process enantioselectivity. The solute transport rate increases when increasing the Reynolds number and also when increasing the carrier concentration in the membrane, whereas the enantioselectivity increases with the increase of carrier concentration just until an optimum. This can be related to an enhancement of transport rate at high carrier concentration, which makes the process less enantioselective [17].

For system B, and for the higher solute carrier ratio S/C=120 there was no enantioselectivity (Figure 8). However, an enantiomeric excess of 38% was obtained for the solute carrier ratio S/C=12 which decreased to 3% at the end of the experiment. This behaviour also observed by other authors [1], showing that, enantioselectivity is kinetically driven and no selectivity is noticeable once equilibrium is set in.

Transport Modelling

The overall mass transfer coefficients for the two enantiomers, $K_{SR}$ and $K_{SS}$, were obtained by least squares minimization of residuals of the experimental data of $C_{SR}$ versus time and $C_{SS}$ versus time using Equations (7) and (8).

Tables 1 and 2 show the values obtained varying the flowrate and varying the solute/carrier ratio for system A, and Tables 3 and 4 show the corresponding results for system B.

For system A, Table 1 shows that, for the lower flowrate (10ml/min) the values of the mass transfer coefficients are 30% lower than the values obtained for the higher flowrate (100ml/min).

For system B similar results are obtained. Table 3 shows that, for the lower flowrate the values of the mass transfer coefficients are 28% lower than the values obtained for the
higher flow rate. Thus, as mentioned, the propranolol transport across the membrane is limited by its diffusion rate from the bulk solution to the membrane surface at lower flow rates.

The solute to carrier ratio has a very high influence on the transport rate as expected, since the relative quantity of carrier is the limiting step of the process. For system A, the mass transfer coefficients obtained for the lower solute carrier ratio (S/C=50) are four times higher than the values obtained for the higher solute carrier ratio (S/C=100). It was expected that the transport of solute would be the double due to the double carrier concentration used. This high increase on the transport, also reported by other authors [19], is probably related with the decrease of the distance between the carrier sites allowing the solute to be transported by a more effective jumping mechanism.

For system B, the mass transfer coefficients obtained for the lower solute carrier ratio (S/C=12) are 2.5 times higher than the values obtained for the higher solute carrier ratio (S/C=120). In this case, the quantity of carrier was not changed but the quantity of solute decreased, so the transport rate is higher due to the higher availability of the carrier, corresponding to a relative increase of carrier in the system.

To sum up, it may be stated, that the increase of transport rate experienced by both systems when lowering the S/C ratio depends not only on the solute to carrier ratio itself, but also depends on the absolute number of carrier sites inside the membrane, which actually is the rate determining step of the process.

CONCLUSIONS
The transport of propranolol across the membrane is limited not only by its diffusion rate from the bulk solution to the membrane surface but also by the relative amount of carrier in the membrane.

The decrease of the flow rate of the feed and stripping phases causes a decrease on the transport rate of propranolol in both systems A and B.

The transport rate of propranolol across the membrane increases when decreasing the solute to carrier ratio in both membrane systems, due to the relative increase of carrier amount in the membrane.

In both cases (systems A and B), the variation of the solute to carrier ratio has much more influence on propranolol transport rate than the variation of the aqueous phases flow rates. Enantioselectivity is only clearly observed when working with system B and for the lowest S/C ratio (S/C = 12), corresponding to the higher relative carrier amount in the membrane. It is also observed a decrease of the enantiomeric excess with time for that experiment. It appears that, enantioselectivity is kinetically driven and no selectivity is noticeable once equilibrium is set in.

The chemical conditions of system A do not allow achieving such lower S/C ratios, thus enantioselectivity could not be observed.

ACKNOWLEDGEMENTS

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### Table 1 – Mass transfer coefficients for two different flowrates with system A

(S/C=100)

<table>
<thead>
<tr>
<th></th>
<th>(K_{SR} (m/s))</th>
<th>(K_{SS} (m/s))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Q=100\text{ml/min})</td>
<td>((2.92 \pm 0.50) \times 10^{-8})</td>
<td>((3.12 \pm 0.50) \times 10^{-8})</td>
</tr>
<tr>
<td>(Q=10\text{ml/min})</td>
<td>((2.03 \pm 0.60) \times 10^{-8})</td>
<td>((2.10 \pm 0.60) \times 10^{-8})</td>
</tr>
</tbody>
</table>
Table 2 – Mass transfer coefficients for two different solute to carrier ratios with system A

(Q=100ml/min)

<table>
<thead>
<tr>
<th>S/C</th>
<th>$K_{SR}$ (m/s)</th>
<th>$K_{SS}$ (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>$(1.26\pm0.10)\times10^{-7}$</td>
<td>$(1.22\pm0.10)\times10^{-7}$</td>
</tr>
<tr>
<td>100</td>
<td>$(2.92\pm0.50)\times10^{-8}$</td>
<td>$(3.12\pm0.50)\times10^{-8}$</td>
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</tbody>
</table>
Table 3 – Mass transfer coefficients for two different flowrates with system B

(S/C=12)

<table>
<thead>
<tr>
<th>Flowrate (ml/min)</th>
<th>$K_{SR}(m/s)$</th>
<th>$K_{SS}(m/s)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>$(5.40\pm0.30)*10^{-8}$</td>
<td>$(5.30\pm0.30)*10^{-8}$</td>
</tr>
<tr>
<td>10</td>
<td>$(4.49\pm0.50)*10^{-8}$</td>
<td>$(4.33\pm0.50)*10^{-8}$</td>
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</tbody>
</table>
Table 4 – Mass transfer coefficients for two different solute to carrier ratios with system B

(Q=100ml/min)

<table>
<thead>
<tr>
<th></th>
<th>$K_{SR}$ (m/s)</th>
<th>$K_{SS}$ (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/C=12</td>
<td>(5.40±0.30)*10^{-8}</td>
<td>(5.30±0.30)*10^{-8}</td>
</tr>
<tr>
<td>S/C=120</td>
<td>(2.13±0.50)*10^{-8}</td>
<td>(2.11±0.50)*10^{-8}</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Scheme of steps taking place in the membrane for propranolol/N-hexadecyl-L-hydroxyproline (HHP) system A. The carboxylic group of the carrier (RCOOH) has a pKa of 9.5.

Figure 2. Scheme of steps taking place in the membrane for propranolol/L-di-n-dodecyltartrate (L-DDT) system B.

Figure 3. Experimental set-up.

Figure 4. Influence of the solute to carrier ratio (S/C) on the propranolol transport across the chiral activated membrane for system A. Open and filled symbols correspond to the $S$ and $R$ enantiomers, respectively. Flow rates of feed and stripping phases were maintained at 100 ml/min.

Figure 5. Influence of the solute to carrier ratio (S/C) on the propranolol transport across the chiral activated membranes for system B. Open and filled symbols correspond to the $S$ and $R$ enantiomers, respectively. Flow rates of feed and stripping phases were maintained at 100 ml/min.

Figure 6. Influence of flow rates of feed and stripping phases on the propranolol transport across the chiral activated membranes for system A, with a solute to carrier ratio (S/C) of a) 50 and b) 100. Open and filled symbols correspond to $S$ and $R$ enantiomers, respectively.

Figure 7. Influence of flow rates of feed and stripping phases on the propranolol transport across the chiral activated membranes for system B, with a solute to carrier ratio (S/C) of 12. Open and filled symbols correspond to $S$ and $R$ enantiomers, respectively.

Figure 8. Enantiomeric excess for system B, with different solute to carrier ratios (S/C).
Figure 1

FEED PHASE  MEMBRANE PHASE  STRIPPING PHASE

R₂NH₂⁺  RCOOH  RCOO⁻R₂NH₂⁺

R₂NH + H⁺  →  R₂NH₂⁺ + H⁺

pKa = 9.4
Figure 2

FEED PHASE  MEMBRANE PHASE  STRIPPING PHASE

\[ \text{pKa} = 9.4 \]

\[ \begin{align*}
\text{R}_2\text{NH} & + \\
\text{H}^+ & \\
\text{R}_2\text{NH}_2^+ & + \\
\text{H}_3\text{BO}_3 & \\
\text{B(OH)}_2\text{-R}_2\text{NH}_2^+ & + \\
\text{H}^+ &
\end{align*} \]

\[ \begin{align*}
\text{R}_2\text{NH}_2 & + \\
\text{H}^+ & \\
\text{R}_2\text{NH}^+ & + \\
\text{H}_3\text{BO}_3 & \\
\text{B(OH)}_2\text{-R}_2\text{NH}_2^+ & + \\
\text{H}^+ &
\end{align*} \]
Figure 3

[Diagram showing a membrane system with pump and feed connections. The membrane area is 34.5 cm².]
Figure 4

- △ Blank
- ○ S/C = 50
- □ S/C = 100

Time (h) vs. Stripping conc. (mM)
Figure 5

The figure illustrates the relationship between time (h) and stripping concentration/feed initial concentration. Two sets of data points are shown, each representing different S/C ratios: S/C = 12 (circles) and S/C = 120 (squares). The concentration values range from 0.00 to 0.20. The graph shows a clear increase in concentration with time, with data points clustering higher on the graph for the S/C = 12 ratio compared to S/C = 120.
Figure 6
a)
b)

![Graph showing the relationship between Stripping conc. (mM) and Time (h) for two flow rates: 100 ml/min. and 10 ml/min.](image)
Figure 8

![Graph showing enantiomeric excess (%) vs. time (h) for S/C = 12 and S/C = 120.](image)
Chiraly derivatized polysulfones for enantioselective membranes: preparation and characterization

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Chiraly derivatized polysulfones for enantioselective membranes: preparation and characterization

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Abstract

Several amines derived from L-proline have been bonded as chiral selectors to Udel polysulfone through a long chain spacer. The residual functionality of the polymer has been considered in this derivatization, together with the further introduction of reactive hydroxyl groups on the starting polymeric material. However, the physico-chemical properties of the polymers obtained following the first of these two pathways resulted more appropriate for the preparation of membranes. The preparation and characterization of the obtained polymers is described.

1. Introduction

Chiral drugs, pesticides and flavours produce different biological effects depending on the enantiomer interacting with the biological system responsible for this response, which is often able to recognize them.\textsuperscript{1} As a consequence mainly the pharmaceutical industry faces the problem to produce single enantiomers separately with the aim to avoid inconveniences related to these differences in drugs and to meet the authorities' requirements to this respect.\textsuperscript{2}

Classically, resolution of enantiomers has been achieved by crystallization of their mixtures. Nevertheless, the scientific and economic relevance of chiral substances

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has favoured the developments in separation techniques in the last two decades.\(^3\) Despite the fact that in former days liquid chromatography was reputed to be a very expensive and inefficient purification technique for preparative purposes it is nowadays one of the first choices to carry out large scale chiral separations. The application to the resolution of enantiomers, where the resulting products have a high added value, and techniques such as Simulated Moving Bed (SMB),\(^4\) can partially balance the classically attributed high costs of liquid chromatography. However, the search for separation processes industrially applicable is still open to other techniques. In this context, membrane based separation techniques, well established process methods in the industrial treatment of fluids, have a high potential in enantioseparation. Several properties intrinsic to these techniques make them highly interesting. Thus, membrane separations can be performed in continuous mode. They are cost effective, and show a low energetic demand together with a relatively simple set-up. Nevertheless, the extent of their applicability to enantioseparations has yet to be demonstrated.\(^5\)

Different membrane configurations and chiral selectors (CS) have been already tested.\(^6\) In principle, polymeric solid membranes are considered to be more stable than liquid membranes and to show a more convenient lifetime than these later to assure commercial applicability. On the contrary, they have the disadvantage of the slow diffusion.\(^7\) Nevertheless, solid membranes constituted by chiral polymers have been tested in the enantioseparation of several racemates. Different approaches have been undertaken. Thus, the chiral polymer can be obtained by the polymerization of chiral monomers\(^8\) or by the molecular imprinting technique.\(^9\) Alternatively, the CS can be fixed on a pre-existing ultrafiltration membrane.\(^10\)

In the study here presented Udel polysulfone, a polymer commonly used in the manufacture of membranes, has been considered as the basic material on which the CS has been chemically bonded. Regarding the CSs, L-proline derivatives have been used as CS for enantiodiscrimination in techniques such as chiral gas chromatography,\(^11\) chiral countercurrent chromatography (CCC),\(^12\) capillary electrophoresis\(^13\) or as chiral ligands for enantioselective catalitic reactions.\(^14\) L-Proline derivatives have been extensively used as chiral moiety in HPLC chiral stationary phases, technique in which high enantioselectivity is sometimes attained for certain enantiomeric mixtures.\(^15\) However,
only \(N-n\)-alkyl-(4\(R\))-hydroxy-L-proline has been used as chiral carrier, either in supported liquid membrane (SLM) systems\(^{16,17}\) as in solid polymeric membrane systems\(^{18}\) in the separation of racemic propranolol.

In this study the preparation and characterization of modified polysulfones containing the CSs shown in Figure 1 is described. Firstly, the introduction of hydroxyl groups on the starting material, following the method described by Guiver and co-workers\(^{19}\) was considered. Moreover, the possibility to take advantage of the remaining active functionality on the polymer was also taken into account. The CSs are bonded through an alkylation reaction, thanks to their nucleophilic character, to a long chain spacer previously fixed on the polysulfone.

Figure 1

### 2. Experimental Section

#### General

Sodium hydride (60% dispersion in mineral oil), \(N\text{-}\text{tert}\)-butoxycarbonyl-L-proline, \(N\text{-}\text{tert}\)-butoxycarbonyl-(4\(R\))-hydroxy-L-proline, 12-bromo-1-dodecanol (99\%) and \(n\)-butyllithium (\(n\)-BuLi, 2.5M in hexane) were supplied by Aldrich. Polysulfone (PS, BASF, Spain) was dried for at least 3 hours at 135°C prior use. Absolute dimethylformamide (DMF, Fluka, 99.8\%), 2-propanol and hexane (p.a. grade, Panreac, Spain) were employed as delivered. Tetrahydrofuran (THF) was distilled over sodium just before use.

IR spectra were obtained using a FTIR Nexus Thermo Nicolet spectrophotometer. NMR spectra were recorded on a Varian Mercury 400 instrument. Chemical shifts are quoted in \(\delta\) values downfield from TMS, and J values are given in Hz. The signals of the solvent (CDCl\(_3\)) were used as a reference. 2D hydrogen-hydrogen (COSY), hydrogen-carbon for one bond (HSQC) and three bond spin correlations (HMBC) spectra were recorded for some of the prepared polymers. HR-MAS NMR experiments were performed on a Brucker Avance DMX500 instrument. The DS of modified polymers were determined using \(^1\text{H}\) NMR by comparative integration of selected signals. Glass transition temperatures (\(T_g\)) were obtained from differential
scanning calorimetry (DSC) performed on a DSC30 Mettler Toledo instrument. Samples were heated initially to at least 30ºC above $T_g$ at 10ºC/min under a flow of 50mL/min of nitrogen gas, quenched with liquid nitrogen, and reheated at 10ºC/min for the $T_g$ measurement. Gel permeation chromatography (GPC) measurements were made on THF polymer solutions using a µ-Styragel™ HR1 column (300 x 4.6 mm i.d.). A Waters HPLC system equipped with an index refraction detector and the corresponding software were used. MALDI-TOF mass spectra were recorded on a 4700 Proteonomics analyzer (Applied Biosystems) in reflectron mode at a wavelength of 337 nm (N₂ laser light). Elemental analyses were performed on a CE Instruments apparatus Mod. EA 1108 (Carlo Erba Instruments, Milan, Italy) using standard conditions by the Serveis Científico-Tècnics de la Universitat de Barcelona (Spain).

**Preparation of the chiral selectors b and c (Scheme 1)**

Following the procedure already described for N-tert-butoxycarbonyl-(4R)-hydroxy-L-proline,²⁰ to a solution of the appropriate N-protected amino acid (1.3 mmol) and EEDQ (1.7 mmol) in CH₂Cl₂ (20 mL), 3,5-dimethylaniline (1.7 mmol) was added. The solution was stirred at r.t. for 24 h. The organic solution was washed successively with 2N HCl and aq. 5% NaHCO₃. The obtained prolinamides were purified by chromatography on silica gel and properly characterized by IR, ¹H NMR and ¹³C NMR.²⁰ A solution of the obtained amide (0.8 mmol) in trifluoroacetic acid and CH₂Cl₂ (20 mL, 30:70) was stirred at r.t. for 30 min. The mixture was basified with NH₄OH and extracted with CH₂Cl₂. The solvent was evaporated and the residual solid collected. The obtained compounds were purified by the crystallization from ethanol-diethyl ether of the corresponding hydrochloride derivative. Amines b and c were obtained in global yields of 80% from the starting protected amino acid.

**N-(3,5-dimethylphenyl-L-prolinamide (b)** ¹H-NMR (400 MHz, CDCl₃) δ: 1.80-2.10 (m, 3H, C₃H₃ + C₄H₂); 2.33 (s, 6H, 2CH₃Ar); 2.67 (m, 1H, C₃Hb); 3.00 (bs, 1H, NH); 3.52 (m, 2H, C₅H₂); 4.96 (m, 1H, C₂H); 6.72 (s, 1H, C₂H); 7.22 (s, 2H, C₂H); 9.51 (bs, 1H, NH). ¹³C-NMR (100.6 MHz, CDCl₃) δ: 21.2 (2CH₃Ar); 25.3 and 26.3 (C₃H₂ and C₄H₂); 50.5 (C₅H₂); 60.5 (C₂H); 117.4 (C₂H₂); 125.6 (C₂H); 137.9 and 138.1 (C₁' and C₃'₅'); 172.5 (CONH).
N-(3,5-dimethylphenyl)-(4\text{R})-hydroxy-L-prolinamide (c) \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3})
\[ \delta: 2.25 \text{ (m, 1H, C}^3\text{H}_a); 2.30 \text{ (s, 6H, 2CH}_3\text{Ar); 2.60 \text{ (m, 1H, C}^3\text{H}_b); 3.00 \text{ (bs, 1H, NH); 3.15 \text{ (m, 1H, C}^5\text{H}_a); 3.39 \text{ (m, 1H, C}^5\text{H}_b); 4.19 \text{ (dd, 1H, C}^2\text{H); 5.45 \text{ (m, 1H, C}^4\text{H); 6.75 \text{ (s, 1H, C}^4\text{H); 7.24 \text{ (s, 2H, C}^{2,6}\text{H); 9.55 \text{ (bs, 1H, NH).}} } \text{\textsuperscript{13}C-NMR (100.6 MHz, CDCl\textsubscript{3})} \delta: 21.2 \text{ (2CH}_3\text{Ar); 39.6 \text{ (C}^3\text{H}_2); 55.0 \text{ (C}^5\text{H}_2); 60.0 \text{ (C}^2\text{H); 72.9 \text{ (C}^4\text{H); 117.2 \text{ (C}^{2,6}\text{H); 125.9 \text{ (C}^4\text{H); 137.1 \text{ (C}^1\text{); 138.6 \text{ (C}^{3,5}\text{); 173.5 \text{ (CONH).}} } \text{Anal. Calcd. for C}_{13}\text{H}_{18}\text{N}_2\text{O}_2: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.69; H, 7.93; N, 11.58.} \]

Preparation of the chiral selectors d and e (Scheme 1)

Selectors d and e were obtained following the procedure already described.\textsuperscript{20}

Preparation of methylcarbinol-polysulfone (PS1) (Scheme 2)

Following the procedure described by Guiver et al.\textsuperscript{18} PS (1g, 2.3 mmol of monomeric units) was mechanically stirred in anhydrous THF (20 mL) under argon atmosphere and cooled to approximately –78\textdegree C using a dry ice/acetone bath. n-BuLi (2.5 mL, 6.2 mmol) was injected drop-wise to the solution using a syringe pump. The mixture was stirred for 30 min. Acetaldehyde (0.6 mL, 10 mmol) was then added to the reaction mixture which was stirred for 2 additional hours at –78\textdegree C. Finally, the solution was gradually warmed up to room temperature for 2 hours. The polymer was precipitated into water/2-propanol (4:1) and washed successively with 2-propanol and water.

\[ \text{\textsuperscript{1}H-NMR (400 MHz, Pyr-d}_5) \delta: 1.45 \text{ (m, CH}_3\text{CH); 1.69 \text{ (s, PS CH}_3); 6.15 \text{ (m, CHOH); 6.8-7.5 \text{ (m, PS o-OAr and o-R); 8.0-8.5 \text{ (m, PS o-SO}_2).}} \]

Scheme 2

Alkylation of PS and PS1

PS or methylcarbinol-polysulfone (PS1) (500 mg) was mechanically stirred in dry DMF (5mL) under argon atmosphere until complete dissolution. Sodium hydride (500 mg, 2.1 mmol, 60 % dispersion in mineral oil) was added to the solution. 12-Bromo-
1-dodecanol (568 mg, 2.1 mmol) in dry DMF (10 mL) was injected drop-wise through a septum using a syringe. The mixture was stirred at room temperature for 24 h. The resulting solution was poured into water (40 mL) and a solid material was obtained by precipitation. The alkylated polysulfone thus obtained was successively washed with water (until complete removal of DMF) and hexane. Finally it was dried under vacuum conditions.

**12-Hydroxydodecanoyl-polysulfone PS2.** $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: (HR-MAS)

**12-Hydroxydodecanoyl-polysulfone PS4.** $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.26 (m, (CH$_2$)$_6$); 1.56 (m, C$_{11}$H$_2$); 1.68 (s, PS CH$_3$); 3.38 (m, CH$_2$O); 3.63 (m, CH$_2$OPS); 6.92 (d, PS Ar$^6$H); 7.01 (d, PS Ar$^{12}$H); 7.24 (d, PS Ar$^5$H); 7.84 (d, PS Ar$^{11}$H). $^{13}$C-NMR (100.6 MHz, CDCl$_3$) $\delta$: 26.4 (CH$_2$); 29.8 ((CH$_2$)$_6$); 31.1 (CH$_3$), 42.6 (Cq); 71.2 (CH$_2$O); 117.9 (Ar$^{12}$H); 120.0 (Ar$^6$H); 128.6 (Ar$^5$H); 129.9 (Ar$^{11}$H); 135.6 (Ar$^{10}$); 147.4 (Ar$^4$); 153.0 (Ar$^3$); 162.2 (Ar$^7$).

**Chlorination of the alkylated polysulfones PS2 and PS4**

The alkylated polysulfone, PS2 or PS4, (1 g) was dissolved in thionyl chloride (20 mL). The solution was vigorously stirred and heated to reflux for 2h. Afterwards, the excess of reagent was removed by distillation in vacuum and the rubbery solid recovered was washed with hexane until it became a powder.

**N-Alkylation of the proline-derived chiral selectors a-e**

Potassium carbonate (964 mg, 6.9 mmol) and the corresponding chiral selector a-e (4.6 mmol) were suspended in anhydrous DMF. Potassium iodide (154 mg, 0.9 mmol) and the chlorinated polysulfones (600 mg) were added to the stirred suspension. The mixture was heated to 70°C and maintained in these conditions for 72 h. The solvent was partially removed the under reduced pressure and a solid material precipitated on the addition of water. The resulting solid was washed with water and redissolved in CH$_2$Cl$_2$, the organic solution was washed with HCl 2N, dried over sodium sulphate and the solvent removed under reduced pressure. The obtained solid was dissolved in THF and reprecipitated in water and washed exhaustively with this solvent to obtain a powder.
12-Prolyldodecanoyl-polysulfone PS3. $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: (HR-MAS)

12-Prolyldodecanoyl-polysulfone PS5a. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.26 (m, (CH$_2$)$_6$); 1.56 (m, C$_{11}$H$_2$); 1.69 (s, PS CH$_3$, C$_3$H$_4$); 2.17 (C$_3$H$_6$); 2.73 (C$_5$H$_6$); 3.22 (C$_5$H$_6$); 3.39 (m, CH$_2$O); 3.63 (m, CH$_2$OPS); 4.11 (C$_2$H); 5.00 (C$_4$H); 6.90 (d, PS Ar$_6^6$H); 7.05 (d, PS Ar$_{12}^6$H); 7.22 (d, PS Ar$_5^3$H); 7.82 (d, PS Ar$_{11}^{11}$H).

12-Prolyldodecanoyl-polysulfone PS5b. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.26 (m, (CH$_2$)$_6$); 1.55 (m, C$_{11}$H$_2$); 1.69 (s, PS CH$_3$); 1.76 (m, C$_4$H$_2$); 2.04 (m, C$_3$H$_6$); 2.21 (m, C$_3$H$_6$); 2.33 (s, 2CH$_3$Ar); 3.00 (m, C$_5$H$_2$); 3.38 (m, CH$_2$OH); 3.62 (m, CH$_2$OPS); 3.84 (m, C$_2$H); 6.74 (s, C$_4$H); 6.93 (d, PS Ar$_6^6$H); 6.99 (d, PS Ar$_{12}^{12}$H); 7.23 (d, PS Ar$_5^5$H and C$_2^6$H); 7.84 (d, PS Ar$_{11}^{11}$H); 9.60 (bs, NH). $^{13}$C-NMR (100.6 MHz, CDCl$_3$) $\delta$: 21.5 (2CH$_3$Ar); 26.1 (CH$_2$ and C$_4$H$_2$); 29.8 ((CH$_2$)$_6$); 31.2 (PS CH$_3$); 39.3 (C$_3$H$_2$); 42.6 (Cq); 62.0 (C$_5$H$_2$); 68.7 (C$_2$H); 71.9 (CH$_2$O); 117.4 (C$_4^6$H); 117.9 (Ar$_{12}^{12}$H); 120.0 (Ar$_6^6$H); 126.0 (C$_4$H); 128.6 (Ar$_5^5$H); 129.8 (Ar$_{11}^{11}$H); 135.6 (Ar$_{10}^{10}$); 138.5 (C$_1$ and C$_3^5$); 147.4 (Ar$_4$); 153.1 (Ar$_1^1$); 162.2 (Ar$_7^7$); 172.0 (CON).

12-Prolyldodecanoyl-polysulfone PS5c. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.26 (m, (CH$_2$)$_6$); 1.55 (m, C$_{11}$H$_2$); 1.69 (s, PS CH$_3$); 2.01 (m, C$_3$H$_6$); 2.29 (m, C$_3$H$_6$); 2.30 (s, 2CH$_3$Ar); 2.86 (m, C$_5$H$_4$); 3.07 (m, C$_5$H$_6$); 3.39 (m, CH$_2$OH); 3.63 (m, CH$_2$OPS); 3.97 (m, C$_2$H); 4.40 (m, C$_4$H); 6.76 (s, C$_4$H); 6.96 (d, PS Ar$_6^6$H); 7.00 (d, PS Ar$_{12}^{12}$H); 7.24 (d, PS Ar$_5^5$H and C$_2^6$H); 7.84 (d, PS Ar$_{11}^{11}$H). $^{13}$C-NMR (100.6 MHz, CDCl$_3$) $\delta$: 21.5 (2CH$_3$Ar); 26.1 (CH$_2$); 29.7 ((CH$_2$)$_6$); 31.1 (PS CH$_3$); 39.3 (C$_3$H$_2$); 42.6 (Cq); 63.0 (C$_5$H$_2$); 68.7 (C$_2$H); 71.2 (CH$_2$O); 115.1 (C$_4^6$H); 117.9 (Ar$_{12}^{12}$H); 120.0 (Ar$_6^6$H); 127.9 (C$_4$H); 128.6 (Ar$_5^5$H); 129.8 (Ar$_{11}^{11}$H); 135.4 (Ar$_{10}^{10}$, C$_1$ and C$_3^5$); 147.4 (Ar$_4$); 153.0 (Ar$_1^1$); 162.2 (Ar$_7^7$).

12-Prolyldodecanoyl-polysulfone PS5d. $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 1.26 (m, (CH$_2$)$_6$); 1.56 (m, C$_{11}$H$_2$); 1.69 (s, PS CH$_3$); 1.80 (m, CH$_2$ chain); 2.10-2.30 (m and s, C$_3$H$_6$ and 4CH$_3$Ar); 2.60-2.80 (m, C$_5$H$_2$); 3.39 (m, CH$_2$OH); 3.63 (m, CH$_2$OPS); 3.98 (m, C$_2$H); 5.30 (m, C$_4$H); 6.72 and 6.76 (s and s, C$_4$H and C$_4^6$H); 6.90 (d, PS Ar$_6^6$H); 7.05 (d,
PS Ar$^{12}$H and C$^{2,6'}$H); 7.25 (d, PS Ar$^3$H and C$^{2,6'}$H); 7.84 (d, PS Ar$^{11}$H); 9.40 (bs, NHCO). $^{13}$C-NMR (100.6 MHz, CDCl$_3$) δ: 21.5 (2CH$_3$Ar); 26.1 (CH$_2$); 29.7 ((CH$_2$)$_n$); 31.1 (PS CH$_3$); 39.3 (C$_3$H$_2$); 42.6 (Cq); 63.0 (C$_5$H$_2$); 68.7 (C$_2$H); 71.2 (CH$_2$O); 115.1 (C$^{2,6'}$H); 117.9 (Ar$^{12}$H); 120.0 (Ar$^6$H); 127.9 (C$_4$H); 128.6 (Ar$^5$H); 129.8 (Ar$^{11}$H); 135.4 (Ar$^{16}$, C$^1$ and C$^{3,5'}$); 147.4 (Ar$^4$); 153.0 (Ar$^1$); 162.2 (Ar$^7$).

12-Prolyldodecanoyl-polysulfone PS5e. $^1$H-NMR (400 MHz, CDCl$_3$) δ: 1.26 (m, (CH$_2$)$_6$); 1.56 (m, C$_{11}$H$_2$); 1.69 (s, PS CH$_3$); 1.80 (m, CH$_2$ chain); 2.25 (m, C$_3$H$_a$); 2.30 (s, 2CH$_3$Ar); 2.36 (s, 2CH$_3$Ar); 2.60 (m, C$_3$H$_b$); 3.15 (m, C$_5$H$_a$); 3.39 (m, C$_5$H$_b$ and CH$_2$O); 3.63 (m, CH$_2$OPS); 4.19 (m, C$_2$H); 5.45 (m, C$_4$H); 6.75 (s, C$_4$H); 6.90 (d, PS Ar$^6$H); 7.05 (d, PS Ar$^{12}$H); 7.20 (s, C$_4$H); 7.24 (d, PS Ar$^5$H and C$^{2,6'}$H); 7.62 (s, C$^{2,6'}$H); 7.84 (d, PS Ar$^{11}$H); 9.55 (bs, NH). $^{13}$C-NMR (100.6 MHz, CDCl$_3$) δ: 20.9 (2CH$_3$Ar and 2CH$_3$Ar); 37.5 (C$_3$H$_2$); 53.9 (C$_5$H$_2$); 60.9 (C$_5$H); 78.2 (C$_4$H); 118.0 (C$^{2,6'}$H); 127.0 (C$_4$H); 127.5 (C$^{2,6'}$H); 130.0 (C$_{16}$); 135.5 (C$_4$H); 137.9 (C$_{16}$); 138.5 (C$_{3,5'}$ and C$_{3',5''}$); 166.5 (COO); 172.5 (CONH).

3. Results and discussion

3.1 Preparation and characterization of the chiral selectors b-e.

General methods were applied to prepare the L-proline and (4R)-hydroxy-L-proline derivatives. $N$-tert-Butoxycarbonyl derivatives of both amino acids were used as starting products. Their reaction with 3,5-dimethylaniline in the presence of 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as a coupling agent yielded the corresponding $N$-acyl-L-prolinamides (Scheme 1) in high yield. As usual in $N$-acylproline derivatives, the rotation of the $N$-acyl bond is restricted. This restriction resulted in the broadening of signals both in $^1$H and $^{13}$C-NMR spectra. The removal of the $N$-tert-butoxycarbonyl group with TFA produced the CSs b and c containing the free amino group in high yield. The CSs d and e were obtained as described.

3.2 Synthesis of the polymeric materials PS1, PS2 and PS3
Two different approaches were considered to bond the CSs to the raw PS. Firstly, taking into account the probable dependence of enantioselectivity on the CS density in the membrane, the previous inclusion of some additional functional groups on the PS was considered. In a parallel approach, the presence of the remaining phenolic hydroxyl groups on the polysulfone was taken into account.

The regioselective functionalization of polysulfone, as a method to introduce diverse groups on the polymer, has been extensively studied.21-23 The direct metallation with \( n \)-butyllithium is known as a well established process that takes place on the \( \text{ortho} \) position to the sulfone linkage. The subsequent reaction with electrophiles allows the introduction of diverse groups, being the degree of substitution dependent on the amount of metallating agent used. With the aim to increase the amount of hydroxyl groups on the polymer acetaldehyde was used as electrophile. The introduction of procedure to obtain derivatives such as \( \text{PS1} \) was largely studied and described by Guiver and co-workers.19 The material obtained following the conditions described in the experimental section (\( \text{PS1} \)) attained in our hands a derivatization degree of nearly two methylcarbinol groups per monomeric unit (calculated on the basis of the integration of selected \( ^1\text{H} \) NMR signals).

A long spacer was then introduced between the polymer matrix and the CS. This spacer may act in the membrane by allowing the CS to freely move within the membrane during the process, thus facilitating the transport phenomenon. Thus, the obtained \( \text{PS1} \) was further reacted with 12-bromo-1-dodecanol in the presence of a base, strong enough to promote the reaction of both kinds of hydroxyl groups, phenolic and alkyllic. However, the polysulfone derivative obtained, \( \text{PS2} \), resulted highly insoluble in the solvents commonly used in the preparation of membranes. Nevertheless, the inclusion of CS a on this material was undertaken. Thus, \( \text{PS2} \) was treated with an excess of thionyl chloride and the chlorinated polymer was reacted with CS a. The reaction took place in the presence of a base and potassium iodide to facilitate the substitution of chlorine atoms. \( \text{PS3} \) was successfully obtained by this procedure. However, the substitution degree in chiral entities was lower than expected, which indicates a low yield in the fixation of the CS. Moreover, the lack of solubility of this material, and the membrane making properties of the obtained polymer, were not adequate for the preparation of suitable
membranes. This insolubility also affected the characterization of the obtained products. Thus, the NMR spectra corresponding to PS2 and PS3 were registered in gel phase (HR-MAS).

3.3 Synthesis of the polymeric materials PS4 and PS5

In parallel, the residual functionality of PS was considered in order to bond the CS to the polymer. However, to assess the feasibility of this approach, the raw material was analyzed by mass spectrometry (MALDI-TOF). The results indicated that the average degree of polymerization of PS was of 8 monomer units. This result implies a maximum derivatization degree for the CS of one unit for each four monomer units of the polymer.

PS was alkylated with 12-bromo-1-dodecanol in the same conditions used formerly for PS1. The obtained material (PS4) showed a substitution degree in accordance with the previously determined polymerization degree. Analogously to the preparation of PS3, PS4 was treated with thionyl chloride and the obtained chlorinated polymer was used to bond CSs a-e. The coupling reaction yielded the chirally derivatized polysulfones PS5a-e in which the content of CS corresponded to the practically quantitative derivatization (Determined by integration of the $^1$H NMR signals).

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References


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Figure 1. L-Proline and 4(R)-hydroxy-L-proline-derived chiral selectors.
Figure 2. MS MALDI-TOF spectrum of the raw polysulfone used.
Figure 3. GPC profiles of (a) PS, PS1 and PS2; (b) PS, PS4, PS4-Cl and PS5a; (c) PS, PS5b-e.
Figure 4. DSC scans for PS, PS4, PS4-Cl and PS5b showing \( T_g \).

Scheme 1. (a) 3,5-Me2C6H3NH2, EEDQ, CH2Cl2, r.t. (b) TFA/CH2Cl2 (30:70), r.t. (c) 3,5-Me2C6H3NCO, pyridine, 115ºC. (d) 3,5-Me2C6H3COCl, 2N NaOH/CHCl3 (25:75), r.t.

Scheme 2. (a) 1) n-BuLi, THF, -78ºC; 2) CH3CHO. (b) 1) NaH, DMF; 2) Br(CH2)12OH, r.t. (c) 1) Cl2SO; 2) a-e, KI, K2CO3, DMF, 70ºC.
Figure 1
Scheme 1
Scheme 2

PS1

PS2

PS3

PS4

PS5a-e
CHARACTERIZATION OF MEMBRANES BASED IN CHIRAL DERIVATIZED POLYSULFONE. APPLICATION TO THE SEPARATION OF PROPRANOLOL ENANTIOMERS

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Abstract

The characterization of new synthesized chiral polymeric membranes, based in polysulfone polymer is here reported. Polysulfone was derivatized to chiral polysulfone, by bonding covalently the chiral carrier, N-dodecyl-4(R)-hydroxy-L-proline, to the polymer matrix. Two different chiral polysulfones, referred to as CPS_A and CPS_B, have been synthesized and used in the preparation of chiral polymeric membranes. However, as a consequence of the limited CPS_B solubility, only CPS_A resulted adequate to obtain useful membranes. Therefore, various chiral polysulfone membranes containing different amounts of CPS_A in raw polysulfone (PS) were prepared and properly characterized by Scanning Electron Microscopy (SEM) and by enantioselective transport experiments of racemic propranolol. Dialysis transport experiments allowed us to determine the influence of the carrier content in the membrane on the transport rate and on enantiomer separation. Membranes containing a CPS_A/PS ratio of 1:3 showed an alpha value of 1.1 at 96h of performance.

Modeling of the propranolol transport rate is also performed. Furthermore, chiral polysulfone membranes performance is compared with other membrane configurations, such as SLM or polymeric membranes. In comparison with other membrane types or configurations, those having lower transport rate show better enantioselectivities and vice versa.

**Keywords:** Chiral membranes, enantioseparation, propranolol, polysulfone, facilitated transport.
1. Introduction

Many compounds used in the agrochemical and the pharmaceutical industries are chiral and, as a consequence, the existence of enantiomers in these products has to be considered. The often encountered pharmacological differences between enantiomers justify the administration of chiral drugs as single enantiomers, and therefore, the need of methods to produce them [1]. Industrial scale production of single enantiomers may be achieved either by the enantioselective synthesis of the desired enantiomer or by resolution of a racemic mixture. Although both approaches have undergone outstanding developments in recent years, resolution is often considered the method of choice at the early stages of drug development [2]. Nowadays, liquid chromatography, applying the Simulated Moving Bed technology, is one of the most used methodologies in the industrial production of single enantiomers [3]. However, even if effective, the initial investment in instrumentation required in this technique often makes it prohibitive. In this context, membrane-based separation techniques are process methods with a high potential in enantioseparation due to their cost effectiveness, low energetic demand, set-up simplicity and the possibility to be used in continuous mode [4,5]. Nevertheless, the extent of their applicability in this field has yet to be demonstrated [6].

Different enantioselective carriers have already been tested in the enantioseparation of several racemates using diverse membrane configurations (see ref. [7] for a review). Among them, N-n-alkyl-4(R)-hydroxy-L-prolines have been used in supported liquid membrane (SLM) systems in the separation of racemic propranolol [8,9]. The selective transport results from an ion pairing mechanism. Thus, the chiral carrier selectively forms
a complex with one of the enantiomers, which is transported across the membrane [10]. These transport systems are driven by a proton gradient between feed and stripping aqueous phases.

However, supported liquid membrane systems have shown to have too low stability and short lifetime to assure good commercial applications [11]. In an attempt to obtain enantioselective membranes with improved stability and lifetime, chiral polymeric membranes, have been developed. Certain chiral solid polymeric membrane systems, obtained by the polymerization of chiral monomers [12,13] or by the molecular imprinting technique [14], have already been proposed to resolve enantiomeric mixtures [7]. Among them, the norbornadiene polymeric membrane described by Aoki and co-workers [13] was used in the enantioseparation of propranolol. In a different approach, the chiral carrier (an amino acid [15], bovine serum albumin (BSA) [16] or DNA [17], among others) has also been chemically immobilized on an ultrafiltration membrane.

In the present study, chiral polymeric membranes, based on a polysulfone polymeric support modified by covalently bonding N-dodecyl-4(R)-hydroxy-L-proline have been tested in the enantioselective transport of propranolol. Two different chiral polysulfone (CPS) polymers, CPS_A and CPS_B, were prepared (Figure 1). The chiral polymeric membrane systems obtained from these materials have been characterized in terms of transport rate and enantioselectivity. The former has been modeled in order to estimate mass transfer coefficients. Scanning Electron Microscopy (SEM) has been used to study the superficial and internal morphology of the prepared membranes.
2. Experimental

2.1 Reagents

*R*-propranolol hydrochloride, *S*-propranolol hydrochloride and racemic propranolol hydrochloride, all a. r. grade, were supplied by Sigma-Aldrich (Germany). Triethanolamine and hydroxypropyl-β-cyclodextrin (HP-β-CD) both a. r. grade, used for *SR*-propranolol quantification by capillary electrophoresis, were also purchased from Sigma-Aldrich (Germany). All other reagents used (such as acids and inorganic salts) were of analytical grade. MilliQ water was used for all aqueous solutions.

2.2 Membranes

Both polymers, CPS\textsubscript{A} and CPS\textsubscript{B}, showed a limited solubility in N,N-dimethylformamide (DMF, a.r. grade, Sigma-Aldrich), mainly in the case of CPS\textsubscript{B}. Therefore, membrane casting solutions were prepared by dissolving mixtures of raw polysulfone (PS) (BASF, Spain) (100, 95, 90, 85, 80 and 75 %) and chiral polysulfone CPS\textsubscript{A} (0, 5, 10, 15, 20 and 25 %) or CPS\textsubscript{B}, when possible, in DMF. The total amount of polysulfone (PS plus CPS) in the casting solutions was of 15 % wt. Membranes were obtained by phase inversion of the casting solutions over a non-woven fabric, which assured re-enforced membranes [18]. An ice-water bath was used to induce coagulation.

SEM images were obtained by using an scanning electronic microscope HITACHI S-570 (Hitachi LTD. Tokyo, Japan) in the UAB Microscopy Service.
2.3 Apparatus and Procedure

In the present study, two different membrane modules were employed, depending on the kind of experiment carried out. Thus, transport experiments were performed with a dialysis membrane cell consisting of two compartments (for the aqueous feed and stripping solutions) of 200 mL, connected through a circular window where the membrane was placed. The surface area of the membrane was of 12 cm$^2$. Maxon A-max (19 mm) stirring motors were employed to stir the solutions in both compartments [19]. In each experiment, the stirring rates of feed and stripping solutions were kept constant throughout the experiment at 1200 r.p.m [20]. The transport of each enantiomer was determined by monitoring their concentration in the feed and stripping solutions. For this purpose, samples of 0.5 mL were periodically withdrawn from both aqueous solutions over the whole experiment.

Kinetic experiments were carried out using a membrane module constituted by two stainless steel blocks with a rectangular channel (length 23 cm, width 1.5 cm and depth 0.3 cm) each, separated by the membrane. The membrane working area was of 34.5 cm$^2$. This configuration permits a well-defined hydrodynamics of the phases allowing us to model the transport in the system. A fixed volume of feed and stripping aqueous solutions were pumped at a 100 mL/min flow rate using a peristaltic pump (Masterflex model number 7521-35, Cole-Parmer Instruments Co., Illinois, USA) to each membrane side in counter-current mode.
All experiments were performed at 24 ± 1 °C and repeated an average of three times. In all experiments, the feed phase, containing 0.1g/L of racemic propranolol, was adjusted at pH 8 with borax buffer. The stripping phase was buffered with disodium phosphate at pH 7. All those chemical conditions (feed and stripping pH, and propranolol concentration) were previously optimized [9].

2.4 Enantioselective Propranolol Determination

A capillary electrophoresis (CE) system (P/ACE SYSTEM MDQ, Beckman, USA) was used to analyze the concentration of both enantiomers in the collected samples. Determination was performed in 50 μm i.d. uncoated fused-silica capillaries of 60 cm length (50 cm to the detector). The capillary was rinsed with 0.1 M NaOH solution, MilliQ water and finally with the separation buffer solution before each set of analyses. The latter consisted of 100mM phosphoric acid, containing 17.4 mM hydroxypropyl-β-cyclodextrin (HP-β-CD), adjusted at pH 4.4 with triethanolamine [21,22]. The applied voltage was 23kV and UV detection was set at 210nm. Samples were injected using the hydrodynamic mode for 5s at 0.3 psi. The capillary was thermostated at 20°C. The capillary was rinsed with MilliQ water between consecutive determinations. At the end of the day, the capillary was washed with NaOH 0.1 M, MilliQ water and MeOH, which was used to remove the possible remaining organic materials and water.

2.5 Calculations
Both, the transport rate of racemic propranolol through the chiral polymeric membranes, and their enantioselectivity were investigated. The transport rate is expressed in terms of Re-extraction percentage (R), which is calculated as the ratio of S- and R-propranolol concentration in the stripping phase at any time t (Cs_t) to the initial racemic propranolol concentration in the feed phase (Cf_0):

\[ R = \left( \frac{C_{s,t}}{C_{f,0}} \right) \times 100 \]  

(1)

The enantioselectivity of the process is given in terms of alpha values (\( \alpha \)). Alpha values were calculated using the following equation [17] for the feed and the stripping phase.

\[ \alpha = \left( \frac{C_{a,s}}{C_{a,r}} \right) / \left( \frac{C_{f,0,s}}{C_{f,0,r}} \right) \]

(2)

where \( C_{a,s} \) and \( C_{a,r} \) are the concentrations of S- and R-enantiomers of propranolol, at any time ("a" denotes the aqueous phase, feed or striping), while \( C_{f,0,s} \) and \( C_{f,0,r} \) correspond to the initial feed concentrations of each enantiomer. Therefore, alpha values over 1.0 will indicate an enantioselective transport of S-propranolol over R-propranolol.

Furthermore, the overall mass transfer coefficients were evaluated by fitting the experimental data, i.e., the experimental values of \( C_{s,t} \) for both enantiomers versus time, by using Scientist® (Micromath Scientific Software, U.S.A).

3. Results and discussion
3.1 Synthesis of CPS and membranes preparation

Udel polysulfone (PS) was chemically derivatized to introduce the chiral carrier following two synthetic pathways. Firstly, the possibility to take advantage of the residual functionality of the raw polymer was considered. CPS\textsubscript{A} (Figure 1) was the material resulting from this approach. Nevertheless, considering that the concentration of chiral carrier in the membrane may have an effect on enantioselectivity, the additional functionalization of the PS was also undertaken. The introduction of reactive groups onto the aromatic rings of the PS, following the procedure developed by Guiver and co-workers [23], originated the new polysulfone derivative CPS\textsubscript{B} (Figure 1). The preparation and characterization of these materials will be described elsewhere [24].

As a consequence of their different chemical structure, the physical properties of both materials differ significantly from each other and also from the original PS material. Thus, the new polysulfones showed a reduced solubility in DMF regarding that of PS. To minimize this problem, membrane casting solutions where prepared by mixing the new materials with PS at different ratios. However, it was not possible to obtain homogeneous casting solutions in DMF from CPS\textsubscript{B} and PS, even at 5% ratio of the former. Other organic solvents commonly used to prepare membrane casting solutions, such as chloroform or dimethylsulfoxide (DMSO), where also tested without success. In an attempt to test CPS\textsubscript{B}, a membrane was obtained from the DMF casting solution containing 5% of this material in PS. Unfortunately, the lack of homogeneity of this solution, prevented the preparation of a membrane suitable to be tested.
Various membranes were prepared from six different CPS$_A$/PS ratios up to a maximum of 1:3. They were morphologically characterized and tested in the separation of propranolol enantiomers.

3.2 Membrane characterization by SEM

Scanning Electron Microscopy (SEM) was used to characterize the chiral polysulfone membranes in order to investigate their surface and cross-section morphological structure. The membranes prepared from CPS$_A$/PS, have a homogeneous surface, whereas the membrane prepared from CPS$_B$/PS presents a top surface full of big holes. This was attributed to the strong effect of the structural chemical modification on the membrane forming properties in the new material. The presence of these holes prevented the use of the membrane.

The cross-section images of membranes prepared from CPS$_A$ are shown in Figure 2. A dense top surface and an asymmetric internal structure with the presence of macrovoids (of different size and distribution) are common features to all of them. These morphological particularities are typical characteristics of PS membranes obtained by phase inversion, when using DMF/water as solvent/non-solvent pair [18]. The presence of different ratios of CPS$_A$ in the casting solution causes slight variations in the structure and number of macrovoids. These minor morphological variations did not affect the membrane use. Thus, all membranes resulted appropriated for their application to the separation of enantiomers.

3.3 Permeation of propranolol across the prepared membranes
Dialysis experiments were performed on the five chiral polymeric membranes prepared from CPS$_A$ and PS. Blank experiments with a membrane obtained from raw PS were also carried out for comparative reasons. In Figure 3, the reextraction percentage of propranolol (R) at 96h is plotted versus the content of CPS$_A$ in the membrane. The fact that a certain transport is observed in the blank experiments indicates that a free, non-selective diffusion is occurring through the membranes. Nevertheless, a clear increase of R regarding that of the blank experiments was observed for the membranes containing the chiral polymer. Therefore, the incorporation of the chiral carrier in the membrane results in a facilitated transport of propranolol. A facilitated transport was also observed in the supported liquid membrane system containing the same carrier [9]. The diffusion of the ion-pair carrier-analyte can account for the facilitated transport in this latter system. However, considering that the chiral selector is here covalently bonded to the polymeric membrane, it can be stated that the facilitated transport through this solid system is attained by a “jumping” mechanism [18]. Moreover, it should be taken into account that the chemical conditions of the aqueous phases extend the "jumping" facilitated transport of propranolol to completeness while minimizing retroextraction [9].

The increase of R values results from increasing the content of CPS$_A$ in the membrane. From 0 to 10% of CPS$_A$, this increase seems to follow a linear progression while afterwards the values tend to reach a plateau around 25% (Figure 3). Thus, the maximum transport rate for this system is already reached when a 10% of CPS$_A$ is incorporated in the membrane.

The R values attained in this study are much lower than those obtained with the corresponding supported liquid membrane (SLM) system, which approach 90-100% [9],
as it is usual for these two types of membrane configuration. This is related to the fact that the diffusion coefficient of a solute is always orders of magnitude lower in a solid that in a liquid phase [7]. In comparison with other polymeric membranes, the R values here presented are much higher than the values attained with norbornadiene polymeric membranes reported by Aoki et al. [13], but comparable to R values achieved with other polysulfone N-hexadecyl-L-hydroxyproline based membranes referred to as CAM [25]. The latest PS membranes contain the chiral carrier physically trapped but not bonded, in the polymer matrix. So, in that case, using the same experimental set-up and chemical conditions, and for a carrier concentration equivalent to a 10-15 % of CPS_A, R values are around 20% [25], which is in the same range of reextraction percentage values corresponding to the membranes prepared in this study. The transport performance is not affected by the covalent bonding of the carrier to the polysulfone matrix.

3.4 Study of the enantioselectivity

The enantioselectivity of the studied membranes (at 96h of experiment) in terms of alpha values versus the CPS_A content is shown in Figure 4. The higher alpha value (1.1) was reached when a 25% of CPS_A was used. Compositions with lower content of CPS_A showed alpha values around 1.0. So, although the maximum propranolol transport rate was obtained for a membrane with 10% of CPS_A, the corresponding process is not enantioselective. As it usually occurs in these systems, for a given analyte concentration, a controlled increase of the chiral selector in the membrane is needed to attain enantioseparation. Moreover, a minimum content of chiral carrier is needed to accomplish for enantioselectivity. However, if carrier content becomes too high, the
enantioselectivity of the process tend to diminish, as a consequence of the increase in transport rate. This fact was also observed when studying this system either in SLM or CAM configurations, by using analogous experimental set-up and conditions [9].

The evolution of alpha with time, for a chiral polysulfone membrane prepared from a casting solution containing 25% of CPS$_A$, is plotted in Figure 5. Here, the alpha values determined in both feed and stripping aqueous solutions are plotted versus time. It is important to note that the stripping alpha values are always above 1.0, what means that an enantioresolution process is actually taking place, as expected. In Figure 5, it may be also observed that the stripping alpha values decrease along with time. This is a consequence of the free diffusion transport mechanism, whose influence increases relatively with time. Furthermore, alpha values over 1.0 in all cases, indicate that the $S$-enantiomer is the most rapidly transported. Taking into account the proposed transport mechanism, the faster transported propranolol enantiomer should be the one possessing higher affinity for the chiral groups pending from the membrane polymer. So, the $S$-enantiomer will jump faster between the chiral groups inside the membrane.

The findings reported above are in good agreement with previous studies concerning the same chiral system. When CAM configuration was employed, $S$-propranolol enantiomer was again the fastest transported [25]. On the contrary, when using SLM configuration the $R$-enantiomer was the first to reach the stripping solution [9]. In this later case, the higher diffusion coefficient of the free specie and its lower lipofilicity promotes its free diffusion. However, the enantiomer with a higher affinity for the chiral selector ($S$-propranolol) is longer retained in the liquid membrane phase.

Concerning alpha values, those determined with CPS$_A$ membranes are higher than those obtained for the SLM configuration [9]. Two main factors account for this result: on the
one hand, the lower participation of propranolol free diffusion, and relatively higher participation of facilitated transport across the CPS membranes, regarding those of SLM. On the other hand, the lower transport rate in CPS [18]. Concerning CAM configuration, the alpha values obtained here are comparable to those obtained for CAM. On the contrary, the alpha values here reported are lower than those obtained by Aoki and coworkers [13]. This may be related not only to the intrinsic nature of the chiral selector but also to transport rate, which is much higher in the former case.

3.5 Transport modeling

Modeling of the enantiomers transport across the membrane offers the possibility to compare mass transfer coefficients between different membrane systems. The transport rate of $S$- and $R$-propranolol through the chiral polysulfone membrane containing 25% in CPS$_A$ was modeled by using the Scientist software (Figure 6), and the corresponding mass transfer coefficients of the membrane system were determined. The values obtained for each enantiomer in the reextraction process are collected in Table 1. These mass transfer coefficients can be compared with those obtained from CAM system, whose values are also included in Table 1 [26]. In order to be compared, those values should be related to the actual molar concentration of chiral entities in each case, that is 1.01 and 0.34 for CPS$_A$ and CAM, respectively. As seen in Table 1, the transport rate of propranolol appears to be lower when the chiral carrier is covalently bonded to the PS. It is probably due to the reduced mobility of the carrier. Therefore, the facilitated transport takes place slower by the “jumping” mechanism than by facilitated diffusion (in CAM). However, the chiral polymeric (CPS$_A$) membranes show better enantioselectivity than the
previously studied CAM configuration, probably due to the slowness of the transport process.

4. Conclusions

The influence of the carrier concentration on transport rate and enantioselectivity for racemic propranolol has been determined. Whereas 10% content of CPS\textsubscript{A} in the membrane casting solution resulted enough to obtain membranes able to attain for a relatively high analyte transport rate, 25% content of CPS\textsubscript{A} was required to reach the highest enantioselectivity transport. This corresponds to an alpha value of 1.1 at 96h of experiment. The enantioselective propranolol transport across the studied chiral polysulfone (CPS\textsubscript{A}) membranes is proposed to occur by a facilitated transport mechanism that takes place by "jumping" the enantiomer between chiral entities. The S-enantiomer transport rate is higher than that of the R-propranolol.

Finally, the mass transfer coefficients of both enantiomers for the CPS\textsubscript{A} membrane system indicate that when the carrier is covalently bonded to the polymer matrix lower transport rates than those obtained for CAM configuration are encountered. Therefore, in the later case free diffusion is relatively higher than that of CPS\textsubscript{A} membranes.

In summary, CPS\textsubscript{A} membranes, synthesized here for the first time, show better enantioselective transport, and lower free diffusion for propranolol, in comparison with other configurations (SLM and CAM). These promising results may be further improved by working with a short time sequential CPS\textsubscript{A} membrane set-up system, or by improving the chiral selector. Thus, new CPS membranes can be a powerful tool as
enantioseparation technique that may offer a wide range of potential applications in the pharmacological field, among others.
Acknowledgements

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References


TABLES

Table 1. Reextraction mass transfer coefficients of $S$- and $R$-propranolol obtained for two different membrane systems, chiral polysulfone membranes ($\text{CPS}_\Lambda$) and CAM.

<table>
<thead>
<tr>
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<th>$S$-propranolol mass transfer coefficients (m/s)</th>
<th>$R$-propranolol mass transfer coefficients (m/s)</th>
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</thead>
<tbody>
<tr>
<td>CPS$_\Lambda$</td>
<td>$(1.75 \pm 0.4) \times 10^{-8}$</td>
<td>$(1.57 \pm 0.4) \times 10^{-8}$</td>
</tr>
<tr>
<td>CAM</td>
<td>$(8.22 \pm 0.1) \times 10^{-8}$</td>
<td>$(8.47 \pm 0.1) \times 10^{-8}$</td>
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</table>
FIGURE LEGENDS

Figure 1. Structures of CPS$_A$, CPS$_B$ and the racemic propranolol.

Figure 2. Cross-section SEM images of chiral polysulfone membranes prepared from casting solutions containing a) 0%, b) 5 %, c) 10%, d) 15%, e) 20% and f) 25% of CPS$_A$.

Figure 3. Influence of the CPS$_A$ content in the membrane on the Reextraction percentage of propranolol. Values correspond to 96h of experiment. Error bars correspond to standard deviation.

Figure 4. Influence of the CPS$_A$ content in the membrane on the enantioselectivity, expressed in terms of alpha values. Values correspond to 96h of experiment. Error bars correspond to standard deviation.

Figure 5. Evolution of alpha values in the feed (empty symbols) and stripping (full symbols) aqueous solutions versus time, when using a membrane prepared from a casting solution containing 25% of CPS$_A$. Error bars correspond to standard deviation.

Figure 6. $S$- and $R$-Propranolol concentrations (full and empty symbols, respectively) in the stripping solution plotted versus time. Experimental values were fitted into a curve by using the Scientist software.
Figure 1

CPS$_A$

CPS$_B$

propranolol
Figure 2

a) 

b) 
c) 

d) 
e) 
f)
Figure 3

![Graph showing the relationship between \% CPS\textsubscript{A} in the membrane casting solution and R (\%).]
Figure 4

% CPS$_A$ in the membrane casting solution

alpha values

0,9 1,0 1,1 1,2

0 5 10 15 20 25 30
Figure 5

![Graph showing alpha values over time](image-url)
Figure 6