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Separation of propranolol enantiomers through membranes based on chiral derivatized polysulfone

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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 unmodified 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 96 h of performance. Modelling of the propranolol transport rate is also performed.

Keywords: Chiral membranes; Enantioseparation; Polysulfone

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 energy 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.

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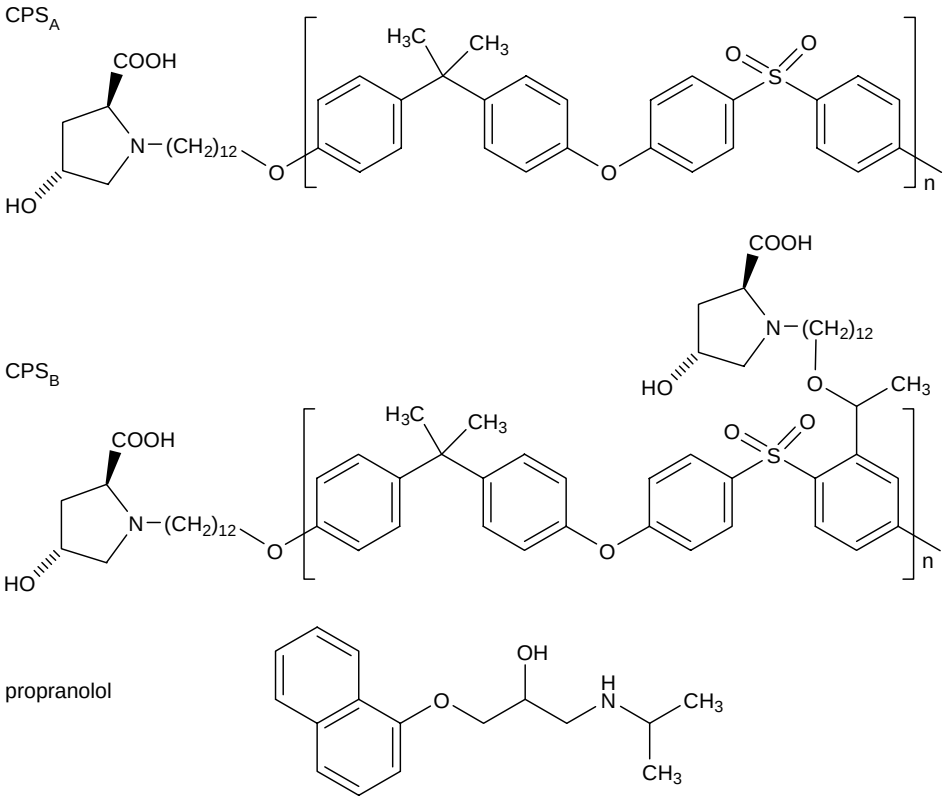

Fig. 1. Structures of CPS_A, CPS_B and the racemic propranolol.

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 (Fig. 1). The chiral polymeric membrane systems obtained from these materials have been characterized in terms of transport rate and enantioselectivity. The former has been modelled in order to estimate mass transfer coefficients. Scanning electron microscopy (SEM) has been used to study the superficial and internal morphology of the prepared polymeric membranes.

2. Experimental

2.1. Reagents

R-Propranolol hydrochloride, *S*-propranolol hydrochloride and racemic propranolol hydrochloride, all AR grade, were supplied by Sigma-Aldrich (Germany). Triethanolamine and hydroxypropyl-β-cyclodextrin (HP-β-CD) both AR 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. Polymer synthesis

Udel polysulfone (ULTRASON S 3010, BASF) (PS) was chemically derivatized to introduce the chiral carrier. Two synthetic pathways, A and B, were applied to obtain two different chiral polysulfones, namely CPS_A and CPS_B, respectively.

CPS_A was obtained following a three step synthesis: alkylation of unmodified polysulfone on the residual hydroxyl groups of PS with 12-bromo-1-dodecanol; substitution of the new resulting hydroxyl groups by chlorine; and final substitution of chlorine atoms by (4*R*)-hydroxy-L-proline.

CPS_B was synthesized using the same procedure but performing a previous introduction of additional hydroxyl groups by lithiation of certain aromatic rings, and subsequent reaction with acetaldehyde following the procedure described by Guiver and co-workers [18]. Thus, the introduction of more hydroxyl groups on the PS increases the number of chiral carrier binding sites. The preparation and characterization details of these materials will be described elsewhere [19].

2.3. Membranes

Both polymers, CPS_A and CPS_B, showed a limited solubility in *N*,*N*-dimethylformamide (DMF, AR grade, Sigma-Aldrich). Therefore, membrane-casting solutions were prepared by dissolving mixtures of unmodified polysulfone (PS) (BASF, Spain) (100, 95, 90, 85, 80 and 75%) and chiral polysulfone CPS_A (0, 5, 10, 15, 20 and 25%). In the case of CPS_B, it was not possible to obtain homogeneous casting solutions in DMF from CSP_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.

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 [20]. An ice-water bath was used to induce coagulation.

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

2.4. 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 [21]. In each experiment, the stirring rates of feed and stripping solutions were kept constant throughout the experiment at 1200 rpm [22]. 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 0.1 g/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].

2.5. 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 100 mM phosphoric acid, containing 17.4 mM hydroxypropyl-β-cyclodextrin (HP-β-CD), adjusted at pH 4.4 with triethanolamine [23,24]. The applied voltage was 23 kV and UV detection was set at 210 nm. Samples were injected using the hydrodynamic mode for 5 s 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.

2.6. Calculations

Both, the transport rate of racemic propranolol through the chiral polymeric membranes, and their enatioselectivity 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):

$$R = \frac{Cs_t}{Cf_0} 100 \tag{1}$$

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

$$\alpha = \frac{Ca_{t,S}/Ca_{t,R}}{Cf_{0,S}/Cf_{0,R}} \tag{2}$$

where $Ca_{t,S}$ and $Ca_{t,R}$ are the concentrations of *S*- and *R*-enantiomers of propranolol, at any time ('a' denotes the aqueous phase, feed or striping), while $Cf_{0,S}$ and $Cf_{0,R}$ 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 Cs_t for both enantiomers versus time, by using Scientist® (Micromath Scientific Software, USA). Differential equations used for modelling purposes are conveniently developed elsewhere [25].

3. Results and discussion

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.

3.1. 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 cross-section images of membranes prepared from CPS_A are shown in Fig. 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 [20]. 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.

3.2. 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 unmodified PS were also carried out for comparative reasons. In Fig. 3, the re-extraction percentage of propranolol (R) at 96 h 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 though 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

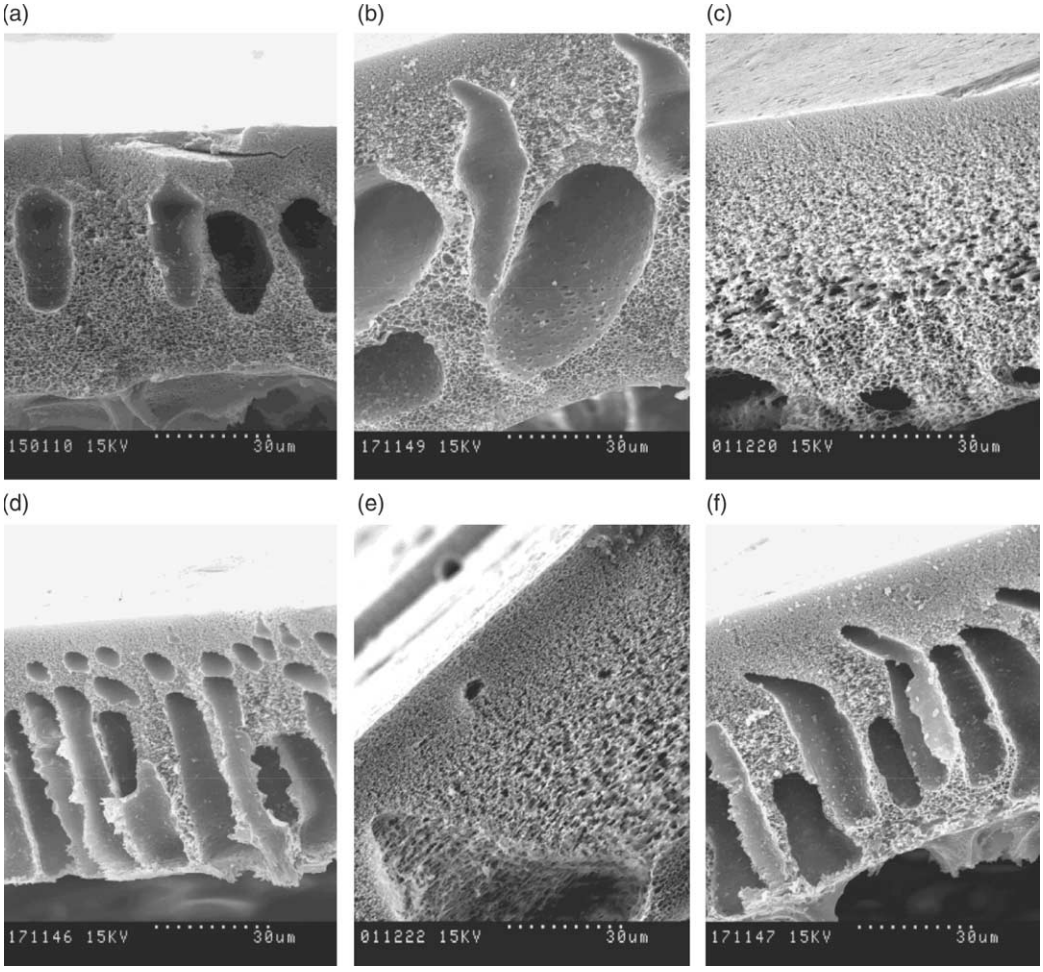

Fig. 2. Cross-section SEM images of chiral polysulfone membranes prepared from casting solutions containing (a) 0%, (b) 5%, (c) 10%, (d) 15%, (e) 20% and (f) 25% of CPS_A.

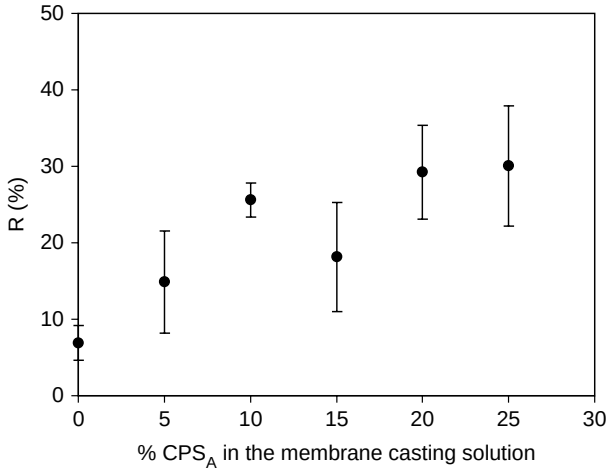

Fig. 3. Influence of the CPS_A content in the membrane on the re-extraction percentage of propranolol. Values correspond to 96 h of experiment. Error bars correspond to standard deviation.

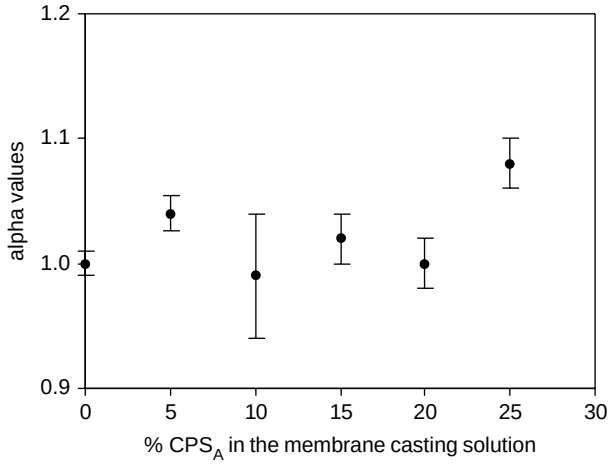

Fig. 4. Influence of the CPS_A content in the membrane on the enantioselectivity, expressed in terms of alpha values. Values correspond to 96 h of experiment. Error bars correspond to standard deviation.

'jumping' mechanism [20]. 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% (Fig. 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 (Chiral Activated Membranes) [26]. The latest mentioned PS membranes (CAM) contain the chiral carrier physically trapped but not bonded, in the polymer matrix, as a result of the carrier directly addition, in its free form, to the membrane casting solutions. 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% [26], which is in the same range of re-extraction 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.

3.3. Study of the enantioselectivity

The enantioselectivity of the studied membranes (at 96 h of experiment) in terms of alpha values versus the CPS_A content is shown in Fig. 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 Fig. 5. Here, the alpha values determined in

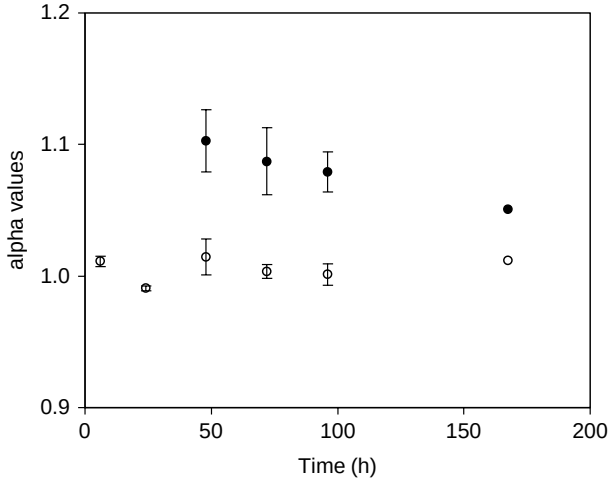

Fig. 5. Evolution of alpha values in the feed (empty symbols) and stripping (full symbols) aqueous solutions versus time, when using a membrane prepared from a casting solution containing 25% of CPS_A. Error bars correspond to standard deviation.

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 Fig. 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 [26]. 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 [20]. 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.

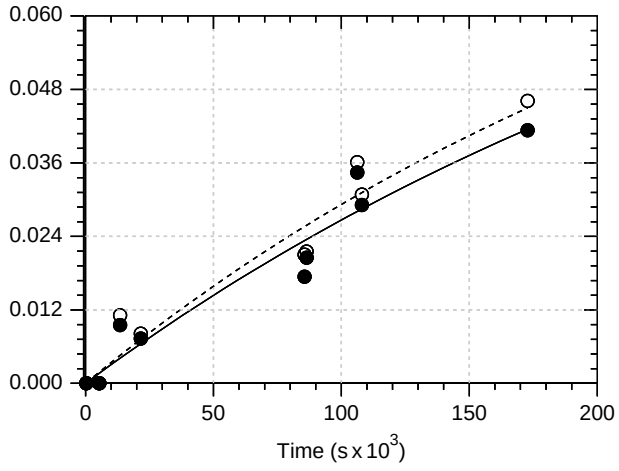

Fig. 6. *S*- and *R*-Propranolol concentrations (full and empty symbols, respectively) in the stripping solution (*y*-axis/mM) plotted versus time. Experimental values were fitted into a curve by using the Scientist software.

Table 1
Re-extraction mass transfer coefficients of *S*- and *R*-propranolol obtained for two different membrane systems, chiral polysulfone membranes (CPS_A) and CAM

	S-propranolol mass transfer coefficients (m/s)	*R*-propranolol mass transfer coefficients (m/s)
CPS_A	$(1.75\pm0.4)\times10^{-8}$	$(1.57\pm0.4)\times10^{-8}$
CAM	$(8.22\pm0.1)\times10^{-8}$	$(8.47\pm0.1)\times10^{-8}$

3.4. Transport modelling

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 modelled by using the Scientist software (Fig. 6), and the corresponding mass transfer coefficients of the membrane system were determined. The values obtained for each enantiomer in the re-extraction 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 [25]. 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_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_A was required to reach the highest enantioselectivity transport. This corresponds to an alpha value of 1.1 at 96 h of experiment. The enantioselective propranolol transport across the studied chiral polysulfone (CPS_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_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_A membranes.

In summary, CPS_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_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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