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Article

Enantioselective Transport of *R*-Clenbuterol through a Bulk Liquid Membrane containing *O,O'*-Dibenzoyl-(2*S*, 3*S*)-tartaric acid

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Um método de membrana líquida (BLM) usando O, O'-dibenzoil-(2S, 3S)-ácido tartárico foi desenvolvido para o transporte enantioseletivo do clenbuterol racêmico. A influência do pH tampão e da relação entre as concentrações do veículo quiral e do clenbuterol racêmico foi estudada. Verificou-se que as escolhas de pH 7 em solução aquosa e a relação entre as concentrações de 1:4 foram apropriadas. O modelo de transporte cinético estabelecido envolveu duas reações consecutivas irreversíveis de primeira ordem e foram determinadas as constantes de reação aparente de pseudo-primeira ordem. Esse modelo foi adequado para a otimização dos sistemas BLM de transporte visando à produção em larga escala do enantiômero puro.

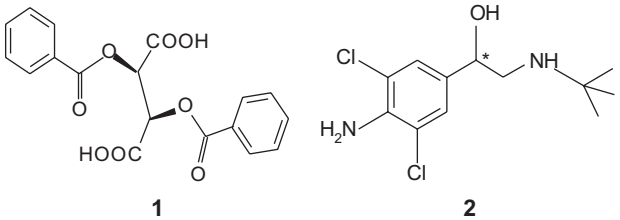
A method of bulk liquid membrane (BLM) using O, O'-dibenzoyl-(2S, 3S)-tartaric acid was developed for the enantioselective transport of racemic clenbuterol. The influence of buffer pH, concentration ratio of chiral carrier to clenbuterol were studied. It was an appropriate choice of such as pH 7 in aqueous solution and concentration ratio of 1:4. The model of kinetic transport was established by means of a kinetic model involving two consecutive irreversible first order reactions. The pseudo-first order apparent rate constants were determined. It is helpful for optimizing the transport of BLM systems and realizing the large-scale production of pure enantiomer.

Keywords: bulk liquid membrane, enantioselective transport, clenbuterol, *O*, *O'*-dibenzoyl-(2*S*, 3*S*)-tartaric acid, kinetics

Introduction

Liquid membranes are liquid phases, existing in either supported or unsupported form, serving as selective barriers between liquid or gas phases, and have shown great potential for use in chiral separations.[1] Bulk liquid membrane (BLM) is one of the types of liquid membranes.[2,3] In a BLM, a relatively thick layer of immiscible fluid is used to separate the feed and strip phases. There is no means of support for the membrane phase and it is kept apart from the external phases only by means of its immiscibility. A recent development in liquid membranes is the incorporation of selective carriers within the liquid membrane phase, chemically facilitating the transport of a specific compound across the membrane. With chiral carriers, it is possible to stereoselectively transport optical isomers.[4,5] The type of chiral carrier is evidently a very important parameter when designing an experiment.

O, *O'*-dibenzoyl-(2*S*, 3*S*)-tartaric acid ((+)-DBTA, (**1**), Figure 1) has been known as a chiral selector of enantiomers, such as ephedrine, chiral alcohols and *N*-methylamphetamine.[6] Moreover, it was also observed that (+)-DBTA can form complexes with some anilides.[7] Since the good complex forming abilities of (+)-DBTA arise from a number of facts. The carboxylic acid groups of (+)-DBTA can donate protons for hydrogen bonding, while it can also behave as a proton acceptor due to the eight oxygen atoms it contains. The benzoyl groups can take part in hydrophobic interactions while the other part of



1 **2**

Figure 1. Molecular structure: *O*, *O'*-dibenzoyl-(2*S*, 3*S*)-tartaric acid (**1**); clenbuterol (**2**).

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the molecule contains polar hydrophilic groups.[8] Clenbuterol (Cle, (**2**), Figure 1) served as a model in this study, belonging to the family of β-adronists, is a sympathomimetic drug with potent β_2-adrenoceptor stimulating properties and used for the treatment of pulmonary diseases. The (-)-*R*-enantiomer is described as responsible for the mimetic effect on β_2-receptors while the (+)-*S*-enantiomer reveals a blocking effect on the β_1-receptors.[9, 10]

However, no more (+)-DBTA and Cle have been tested while no discussion of the enantioselective transport kinetics through a BLM has been carried out. Based on previous results, this work presents more extensive results of the resolution method of BLM. First the influence of buffer pH, concentration ratio of chiral carrier to Cle were investigated. Second, the model of transport kinetics was analyzed in a more systematic way.

Theoretical

(+)-DBTA can form enantioselective complexes with Cle enantiomers according to the above mention, seemed promising enough to justify the next step of carrier-mediated transport in a single BLM to determine the feasibility of Cle enantiomer enrichment by a (+)-DBTA facilitated BLM.

From the partitioning experiment (concentration ratio of 1:1, 0.1 mol L^{-1}, pH 7, and *n*-octanol as solvent), the distribution coefficient (K_d) can be obtained from the equilibrium concentration ratio of Cle concentration of the organic phase to that of the aqueous phase. According to the study, $K_d = 0.026$. This value indicates that Cle prefers to remain in the feed phase, thus slowing the interface transfer, and thus, the overall mass transfer. However, the equilibrium constant (K_{eq}*) of complexation reaction on chiral carrier to Cle, is far bigger than the distribution coefficient, so the influence on mass transfer is small and can be neglected generally. *R*- and *S*-Cle form two diastereomeric complexes with (+)-DBTA through coulombic interactions, hydrogen bonding and van der Waals interactions, which different from chemical reaction. So we suppose that Cle enantiomer transport may obey the kinetic laws of two consecutive irreversible first order reactions.[2,3] The mechanism and the enantioselctive transport kinetics scheme of Cle enantiomers through a BLM are schematically described in equation (1):

$$\begin{aligned}&\left[Cle_{R(S)}\right]_f+\left[(+)-DBTA\right]_m \overset{k_1}{\Rightarrow}\\ &\qquad\left[Cle_{R(S)}-(+)-DBTA\right]_m \overset{k_2}{\Rightarrow}\\ &\qquad\qquad\left[Cle_{R(S)}\right]_s+\left[(+)-DBTA\right]_m\end{aligned} \tag{1}$$

k_1 and k_2 are the pseudo-first-order apparent rate constants of feed–membrane interfacial and membrane–strip interfacial transport of Cle enantiomer, respectively.

The average flux (*J*) of each enantiomer was calculated by:[11]

$$J=\frac{Vs\ \Delta Cs}{A\ t} \tag{2}$$

where *t* is the time over which the concentration difference, ΔCs, is measured in the strip, *Vs* the strip volume and *A* is the effective membrane area. This is the average flux from the start of the experiment till time *t*. The enantioselectivity was calculated in terms of the separation factor (α) and the percentage enantiomeric excess (*ee*, %):

$$\alpha=\frac{J_R}{J_S} \tag{3}$$

$$ee,\ \%=\frac{J_R-J_S}{J_R+J_S}\times 100 \tag{4}$$

Therefore, this kinetic behaviour can be described according to the following equations:[12]

$$\frac{d\mathrm{R_f}}{d\mathrm{t}}=-\mathrm{k_1R_f}\equiv J_f \tag{5}$$

$$\frac{d\mathrm{R_m}}{d\mathrm{t}}=\mathrm{k_1R_f}-\mathrm{k_2R_m} \tag{6}$$

$$\frac{d\mathrm{R_s}}{d\mathrm{t}}=\mathrm{k_2R_m}\equiv J_s \tag{7}$$

Integration of those differential equations gives:

$$R_f=\exp(-k_1t) \tag{8}$$

$$R_m=\frac{k_1}{k_2-k_1}[\exp(-k_1t)-\exp(-k_2t)] \tag{9}$$

$$R_s=1-\frac{1}{k_2-k_1}[k_2\exp(-k_1t)-k_1\exp(-k_2t)] \tag{10}$$

R_f, R_m and R_s represent the mole fractions of Cle enantiomer in the feed solution, membrane phase and strip solution, respectively. These equations show that the time dependence of R_f is monoexponential and the time dependence of both R_m and R_s is biexponential. R_m has a maximum, the time at which it occurs being obtained from $dR_m/dt = 0$.

$$t_{max} = \frac{\ln(k_1/k_2)}{k_1 - k_2} \quad (11)$$

the value of R_m at that time being:

$$R_{max} = (\frac{k_1}{k_2})^{-\frac{k_2}{k_1 - k_2}} \quad (12)$$

Experimental

Chemicals

Racemic Cle was obtained from Dazhong Pharmaceutic & Co. Inc. (Shanghai, China). (+)-DBTA was purchased from Lingxing & Co. Inc. (Zhejiang, China). HP-β-CD were obtained from Yiming Fine Chemical Factory (Taixing, China). *n*-Octanol was obtained from Yili & Co. Inc. (Beijing, China). All chemicals were of analytical reagent grade.

Analysis

Chiral capillary zone electrophoresis was performed using a P/ACE MDQ system equipped with a diode array detection system (Beckman Coulter, Fullerton, CA, USA). The system was computer-controlled, with an integrated P/ACE Station Software (32 Karat TM Version 7.0) package. The dimensions of the capillary were 60.2 cm × 50 μm i.d. with the detection window at the distance of 10 cm from the capillary outlet. The injection mode was hydrodynamic (5 s, 20 mbar pressure). Efficient chiral separation was achieved with 30 mmol L^{-1} HP-β-CD in Na_2HPO_4/ H_3PO_4 buffer pH 2.5, operated at 24 kV (20 ± 0.1 °C) and detection wavelength of 214 nm. The *R*-Cle was found to migrate first in the buffer system (Figure 2).[9,10]

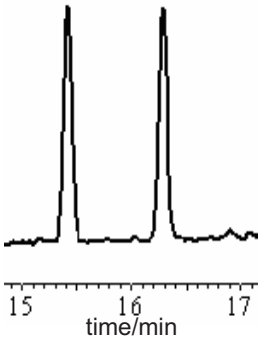

Figure 2. Resolution of Cle by capillary zone electrophoresis.

Procedures

The experimental studies were carried out applying the BLM technique, using a stirred transfer Lewis type cell with BLM layered over the feed and strip phases (Figure 3). The left aqueous feed solutions (40 mL) consisted of Cle of different concentration and 0.1 mol L^{-1} Na_2HPO_4/NaH_2PO_4 buffer. The strip solution (40 mL), which consisted of 0.1 mol L^{-1} Na_2HPO_4/NaH_2PO_4 buffer was located in the right part of the vessel. The organic membrane phase (60 mL), prepared by dissolving (+)-DBTA of different concentration in *n*-octanol, was added on top of the water phases. The contact area between the feed and membrane and the membrane and strip phases of the single BLM system were all 3.14 cm^2. The experiments were carried out at 25 °C by using a thermostated apparatus. pH measurements were made with a pH meter using a combined glass electrode. The feed solution, the membrane phase and the strip solution were stirred at 200 rpm.

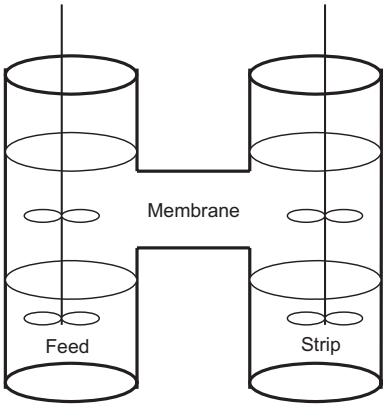

Figure 3. Transport cell of bulk liquid membrane.

Results and Discussion

Variation of separation factor with transport time

A typical graph of the variation of separation factor over transport time for the BLM is given in Figure 4. Separation factor decreases sharply during the initial induction period, then begin to decrease slowly and approximately equal to 1. This means that any enantioselectivity of (+)-DBTA for a specific Cle enantiomer in BLM system is based on the kinetically and not thermodynamically driven, namely, Gibbs free energy (ΔG) is positive value, which predicts that the complexation reaction is slow and is unlikely to occur unless the driving force, *i.e.* concentration gradient, remains strong enough. There is a bigger concentration gradient across the membrane between the feed and strip phases when the transport is in the initial induction period. Separation factor decreases with the decreasing concentration gradient. When the equilibrium is reached between the feed and the strip phases which would occur

once both the feed and strip phases have the same Cle concentration, then separation factor approximately equal to 1 and the transport will cease with it.

Influence of the concentration ratio of (+)-DBTA to Cle on flux and enantioselectivity

When comparing the flux and the enantioselectivity as a function of the concentration ratio of (+)-DBTA to Cle, the following observations were made (see Table 1). The enantiomer designated *R* form had the slightly higher flux in all cases investigated. This means that (+)-DBTA preferentially recognize *R*-Cle enantiomer relativing to *S*-Cle enantiomer. The flux and enantioselectivity decrease with transport time in the experiment. These are probably due to the loss of chiral carrier from the liquid membrane leading to reduced enantioselectivities and lower fluxes and enantioselectivities due to facilitated transport. The flux and enantioselectivity also decrease with the variation of the concentration ratio from 1:1 to 1:4. These were anticipated as less carrier was available with the variation of the concentration ratio, decreasing the transport rate of both enantiomers. The further varying concentration ratio did not significantly affect the enantioselectivity. This would imply the same amount of chiral carrier would perform the required upgrade of the racemic mixture regardless of initial concentration, thereby minimizing the amount of expensive of chiral carrier required for the process. However, the time achieved extraction equilibrium increases with a variation in concentration ratio. For obtaining a higher enantioselective extraction rate in all of the extraction processes, the concentration ratio of 1:4 was selected for the better experimental conditions.

Influence of pH in queous phase on flux and enantioselectivity

The influence of pH in the aqueous solutions is shown in Table 2. The flux for both enantiomers increased slightly with the fall of pH.[13] Because Cle belongs to the compounds of chiral amine, it means that, at lower pH, more Cle (pH 8-9, measured value by experiment) will be charged. Thus more charged Cle being in the aqueous phase may go against enantioselective comlexation reaction. The molecular size of charged Cle is smaller than that of complexation, it may be easier to through the BLM, the passive transpot, and therefore, flux increases.

Table 1. Effect of concentration ratio on flux ($\times 10^2$ mol cm^{-2} h^{-1}) and enantioselectivity

$C_{(+)\text{-DBTA}} : C_{Cle}$		time/h	2	4	6	8	12	24
1 : 1	J_R		1.381	1.095	0.861	0.719	0.574	0.378
	J_S		0.806	0.784	0.642	0.559	0.488	0.341
	α		1.714	1.397	1.340	1.284	1.177	1.108
	ee,%		26.29	16.55	14.57	12.52	8.10	5.15
1 : 2	J_R		1.293	1.042	0.822	0.684	0.540	0.357
	J_S		0.765	0.751	0.620	0.537	0.464	0.327
	α		1.690	1.387	1.327	1.273	1.163	1.092
	ee,%		25.66	16.23	14.01	12.04	7.57	4.39
1 : 4	J_R		1.247	1.001	0.788	0.647	0.510	0.334
	J_S		0.748	0.732	0.602	0.513	0.443	0.309
	α		1.668	1.369	1.310	1.261	1.152	1.080
	ee,%		25.01	15.52	13.38	11.55	7.03	3.89

Conditions: Buffer pH 7, 0.1 mol L^{-1} Cle.

Table 2. Effect of pH on flux ($\times 10^2$ mol cm^{-2} h^{-1}) and enantioselectivity

pH		time/h	2	4	6	8	12	24
5	J_R		1.396	1.115	0.879	0.732	0.589	0.386
	J_S		0.834	0.819	0.674	0.584	0.513	0.371
	α		1.671	1.361	1.304	1.253	1.146	1.090
	ee,%		25.20	15.31	13.20	11.25	6.41	1.98
7	J_R		1.381	1.095	0.861	0.719	0.574	0.378
	J_S		0.806	0.784	0.642	0.559	0.488	0.341
	α		1.714	1.397	1.340	1.284	1.177	1.108
	ee,%		26.29	16.55	14.57	12.52	8.10	5.15
9	J_R		1.285	1.025	0.799	0.669	0.535	0.361
	J_S		0.798	0.776	0.634	0.551	0.479	0.336
	α		1.611	1.321	1.261	1.215	1.116	1.073
	ee,%		23.38	13.83	11.51	9.67	5.52	3.59

Conditions: 0.1 mol L^{-1} Cle, the concentration ratio of 1 : 1 for (+)-DBTA to Cle.

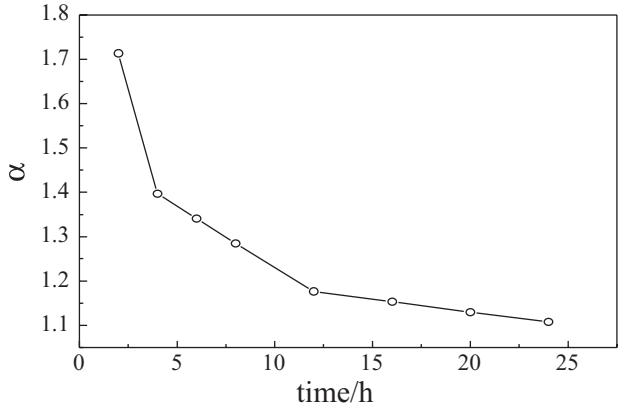

Figure 4. Variation of enantioselectivity with transport time for Cle enantiomers. Conditions: Buffer pH 7, 0.1 mol L^{-1} Cle, the concentration ratio of 1 : 1 for (+)-DBTA to Cle.

This also means that, with an increase in passive transport, the enantioselectivity should decrease for all the single BLM systems tested. So the enantioselectivities decrease with pH from 7 to 5. However, the enantioselectivities increase with pH from 9-7. Because *n*-octanol in membrane phase can dissolve a thimbleful of water, and the basic aqueous solution may infiltrate into membrane phase. As (+)-DBTA was acidic (pH 3-4, set value by Lingxing & Co. Inc.), the permeation may change the enantioselectivitive characteristics of chiral carrier. So it was an appropriate choice of such as pH 7 in aqueous solutions.

Analysis of enantioselective transport kinetics

In order to investigate the enantioselective transport kinetics of Cle through the BLM, the variations of R_f, R_m and R_s with time were investigated. The experimental results are shown in Figure 5. From Figure 5, R_f decreases mono-exponentially with time, and R_s follows a monotonically increasing while the time dependence of R_m presents a maximum. R_f and R_s approximately equal to 0.5 when the transport was carried out completely, which further verify that enantioselctive transport obeys generally the kinetic mechanism of two irreversible pseudo-first-order reactions. If $k_1 > k_2$ or $k_1 < k_2$, the experimental data can be fitted by the model of two consecutive irreversible pseudo-first-order reactions.

Since the enantioselective complexes which could not be stripped at once, formed by chiral carrier with two enantiomer, only could be stripped by means of diffusing through the boundary layer of membrane-feed solution interface, membrane phase, then reaching the membrane-strip phase. The diffusing process needed some transport time, so the enantioselective complexes were accumulated in organic membrane phase. Figure 4 also suggest that there is

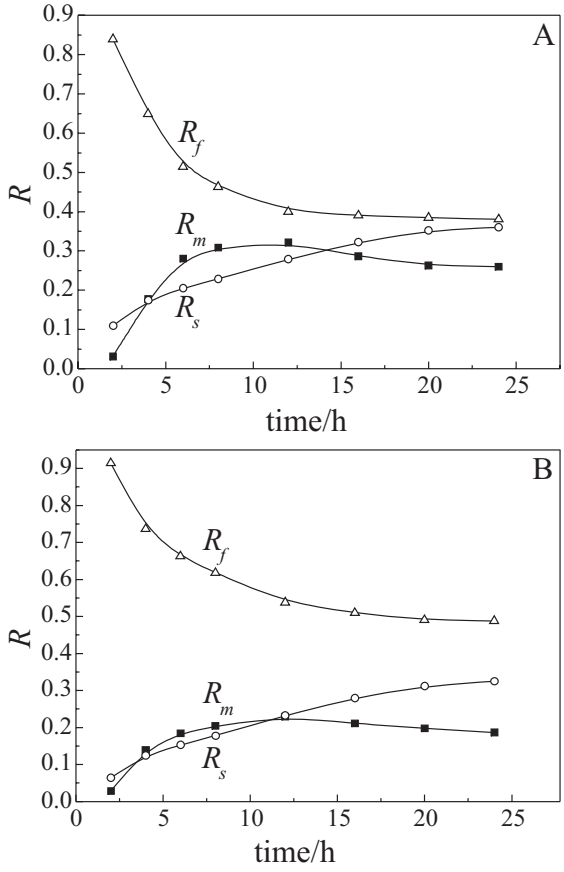

Figure 5. Variation of R_f, R_m and R_s of *R*-Cle over time (A); Variation of R_f, R_m and R_s of *S*-Cle over time (B). Conditions: Buffer pH 7, 0.1 mol L^{-1} Cle, the concentration ratio of 1:1 for (+)-DBTA to Cle

a higher *R*-Cle concentration in the beginning of the experiment, therefore determining $\alpha >> 1$ during some hours.

If the diffusion is the key step of rate controlled in this process, the mechanism and the enantioselctive transport kinetics scheme will result in large errors and not be described by equation (3), which does not include diffusing process. From the Table 3 we can see that the values of k_2 for *R*-Cle and *S*-Cle are bigger and adjacent, comparing to k_1 for *R*-Cle and *S*-Cle. The value of k_1 is higher for *R*-Cle than for *S*-Cle while the opposite is for k_2 value, showing that the enantioselective complxation reaction occurs in membrane-feed solution interface and exists of difference in enantioselectivity. These facts suggest that the interfacial reaction of membrane-feed solution interface is the key step of rate controlled and the transport can be described by equation (3).

Table 3. Kinetic parameters for enantioselective transport of Cle enantiomers

	$k_1/(\times 10^2\ h^{-1})$	$k_2/(\times 10^2\ h^{-1})$	$time_{max}$/h	R_{max}
R-Cle	6.701	9.909	11.921	0.334
S-Cle	4.337	10.655	13.583	0.227

Conclusions

R-Cle can be effectively transported through a BLM containing (+)-DBTA in *n*-Octanol. The influence of the molar concentration ratio of (+)-DBTA to Cle, and pH in aqueous phase on chiral transport performance of BLM were investigated. The enantiotransport in BLM was analysed by means of a kinetic model involving two consecutive irreversible first order reactions.

Acknowledgments

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References

1 Way, J. D.; Noble, R. D.; Flynn, T. M.; Sloan, E. D.; *J. Membr. Sci.* **1982**, *12*, 239.
2. Ma, M.; He, D. S.; Liao, S. H.; Zeng Y.; Xie, Q. J.; Yao, S. Z.; *Anal. Chim. Acta.* **2002**, *456*, 157.
3. Ma, M.; Chen, B.; Luo, X. B.; Tan, H.; He, D. S.; Xie, Q. J.; Yao, S. Z.; *J. Membr. Sci.* **2004**, *234*, 101.
4. Scrimin, P.; Tecilla, P.; Tonellato, U.; *Tetrahedron* **1995**, *51*, 217.
5. Morishima, I.; Iizuka, T.; *J. Am. Chem. Soc.* **1974**, *96*, 7367.
6. Tan, B.; Luo, G. S.; Wang, J. D.; *Tetrahedron Asymmetry* **2006**, *17*, 883.
7. Kassai, C.; Juvancz, Z.; Balint, J.; Fogassy, E.; Kozma, D.; *Tetrahedron Asymmetry* **2000**, *56*, 8355.
8. Tan, B.; Luo, G. S.; Xuan, Q.; Wang, J. D.; *Sep. Purif. Technol.* **2006**, *49*, 186.
9. Toussaint, B.; Hubert, Ph.; Tjaden, U. R.; van der Greef, J.; Crommen, J.; *J. Chromatogr. A.* **2000**, *871*, 173.
10. Gausepohl, C.; Blaschke, G.; *J. Chromatogr. B.* **1998**, *713*, 443.
11. Krieg, H. M.; Lotter, J.; Keizer, K.; Breytenbach, J. C.; *J. Membr. Sci.* **2000**, *167*, 33.
12. León, G.; Guzmán, M. A.; *Desalination* **2005**, *184*, 79.
13. Jiao, F. P.; Huang, K. L.; Ning, F. R.; Hu, W. G.; Yu, J. G.; *Sep. Sci. Technol.* **2006**, *41*, 1893.

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