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Kinetic resolution of chiral alcohols in bifunctional membrane exhibiting enzyme activity and enantioselective permeation

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Abstract

The paper deals with the kinetic resolution of chiral alcohols in a transesterification process performed in the reactor containing a bifunctional polymer membrane. The membrane was prepared using an asymmetric polyamide film. It exhibits enantioselective permeabilities caused by an embedded imprinted polymer as well as catalytic properties induced by the immobilised lipase. The transesterification of vinyl acetate with (±)-*trans*-2-phenyl-1-cyclohexanol, performed in the reactor with the bifunctional membrane, gives chiral products of high purity. In this case, the enantiomeric excess (e.e.) of the product exceeds 98% while it reaches 80% only when the reactor with a common enzyme membrane is used.

Keywords: Enantioseparation; Membrane reactor; Molecularly imprinted polymer; Kinetic resolution; Lipase

1. Introduction

The need for preparing pharmaceuticals and agrochemical products from pure enantiomers is obvious nowadays. From among many methods for selecting such enantiomerically pure substances, the processes performed in the membrane reactors are considered as especially useful [1–3]. One of these processes consists in the kinetic resolution of products obtained mostly in hydrolyses of esters, esterifications, and transesterifications that are performed in the reactors containing enzyme membranes. Suitable enzymes (lipases or esterases) can be immobilised in or on the membrane structure [1–3]. These enzymes are easily accessible, relatively cheap, and do not require addition of coenzymes, what simplifies the process engineering. The biocatalyst should react selectively with only one enantiomer of a racemic substrate. However, the catalytic activity and enantioselectivity observed in the processes with non-natural substrates are usually lower as compared to those in the reactions with the natural ones. In such cases, an efficient method for enhancing the process selectivity must be applied. One of the ways to solve this problem is the enzyme engineering. Others consist in a modification of the enzyme enantioselectivity by the addition of, e.g. chiral (usually macrocyclic) additives, like cyclodextrins or crown ethers, and simply in shifting the equilibrium of thermodynamically unfavourable reactions [4–6]. The dynamic kinetic resolution as well as tandem or sequential reactions catalysed by one or two enzymes are further examples of the last method. The enantiomeric excess of both the product and remaining substrate in these processes is higher than that in the single process, due to a higher concentration of the favourable substrate enantiomer. Some

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research concerning the last topic has been done also in our laboratory [7,8].

Beside the resolutions based on enantioselective catalytic reactions, simple filtration through special selective membranes should also be mentioned [9,10]. Membranes suitable for such a resolution must exhibit molecular recognition properties. They should be formed mostly of, or should contain, the polymer imprinted by a selected chiral-molecule template.

The molecular imprinting is a method for creating polymer matrices that display a selective molecular recognition behaviour [11]. Such materials enable the separation based on affinity, particularly when used as stationary phases in HPLC, in capillary electrophoresis, and as selective membranes [12,13]. Several other applications of the molecularly imprinted polymers (MIPs) have also been reported, e.g. shifting the reaction equilibrium by a product removal [14]. Application of the molecularly imprinted membranes may be more promising because of lower diffusion-related limitations in a thin membrane, when compared to those occurring in packed columns, and ease of enlargement of a module area. However, the enantioselectivity of dense polymer membranes obtained by embedding a chiral selector or by molecular imprinting is very low till now and usually does not exceed 3 [15,16]. This fact practically eliminates commercial application of these membranes.

In this paper, we report the results on the kinetic resolution of selected chiral alcohols by transesterification of vinyl acetate, performed in the reactor containing a special bifunctional membrane. Pores of the membrane are filled with an imprinted polymer exhibiting enantioselective transport properties, while the membrane skin layer contains the immobilised enzyme (lipase). Implementation of this molecularly imprinted polymer into the membrane has been accomplished by polymerisation of the proper monomer introduced to the membrane together with the chiral template molecules. Thus, the membrane exhibits both the enantioselective catalytic action in transesterification and enantioselective permeability.

The aim of this work was to obtain enantiomerically pure compounds by the kinetic resolution of (±)-*trans*-2-phenyl-1-cyclohexanol and (±)-menthol in transesterifications catalysed by lipase exhibiting moderate or low enantioselectivity towards these substrates. The selected alcohols are representatives of an important class of the chiral compounds of potentially broad applications. Vinyl acetate was used as an acyl donor as it favours shifting the reaction equilibrium to products [7,17].

The complete results are presented for the processes performed with *trans*-2-phenyl-1-cyclohexanol only. The reactions involving menthol seemed to be not purposeful to be presented as the obtained resolution was very low. The reaction with *trans*-2-phenyl-1-cyclohexanol is outlined in the scheme shown in Fig. 1.

2. Experimental

2.1. Materials

All the chiral alcohols (>99%): (1*R*,2*S*,5*R*)-(−)-menthol, (1*S*,2*R*,5*S*)-(+)-menthol, and their racemic mixture as well as (±)-*trans*-2-phenyl-1-cyclohexanol were purchased from Aldrich Chemical Co. (Gillingham-Dorset, UK) and were used without any further purification. Vinyl acetate (>99%) was obtained from Fluka Chemie AG (Buchs, Switzerland) and was distilled just before the experiments. Ethyleneg-

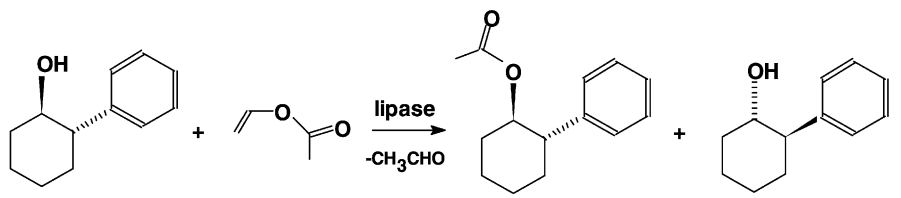

Fig. 1. Scheme for kinetic resolution of *trans*-2-phenyl-1-cyclohexanol catalysed by lipase from *Pseudomonas* sp.

Table 1
Chromatographic data for resolution of chiral alcohols and their esters

Compound	Retention time (min)	k'
Menthol (1*R*,2*S*,5*R*)	11.036	1.06
(1*S*,2*R*,5*S*)	11.490	
trans-2-Phenyl-1-cyclohexanol (1*S*,2*R*)	6.022	1.16
(1*R*,2*S*)	6.805	
trans-2-Phenyl-1-cyclohexyl acetate (1*S*,2*R*)	8.132	1.20
(1*R*,2*S*)	9.653	

lycoldimethacrylate (EGDMA)and (±)-camphorquinone were purchased from Aldrich.

Lipase from *Pseudomonas* sp. (activity of 30 units/mg solid) was obtained from Sigma Chemical Co. (St. Louis, USA). One unit of the lipase enables to yield 1.0 mmol/min of glycerol from triglyceride at pH 7.0 and 37 °C. *n*-Hexane and 2-propanol (HPLC grade) were purchased from Labscan Ltd., Dublin, Ireland.

2.2. Analytical methods

Concentrations of the particular enantiomers were estimated by means of the HPLC system consisted of a Shimadzu LC-10AD isocratic pump, Rheodyne 7725i injector, and Shimadzu UV SPD-10A detector (detection at 254 nm). The columns Chiralcel-OJ (250 mm × 4.6 mm) and Chiralcel-OD-H (both Daicel, Japan) were used for the reactions with menthol and *trans*-2-phenyl-1-cyclohexanol, respectively. Mobile phase elution (1 ml/min) was performed isocratically, using a filtered and degassed mixture of *n*-hexane and 2-propanol (95/5 (v/v)). The injected sample volume was 20 μl. Retention times were as shown in Table 1.

2.3. Preparation and characteristics of imprinted membranes

Flat sheet polyamide-6 membranes were obtained by wet phase inversion, according to the procedure described in the previous paper [13]. The membranes (surface area of 25 cm^2) were dried and then soaked with a polymerisation solution consisting of acrylic acid, EGDMA as a cross-linking agent, a selected template, and *n*-hexane as a porogen, followed by addition of (±)-camphorquinone as an initiator. The membranes were irradiated for 15 min with the UV-light by means of an Optilux-150 UV-lamp (Demetron Research Corporation, Danbury, USA) and left for 24–48 h to attain a complete polymerisation. The porogen was removed by evaporation. Finally, the membranes were washed with methanol (to remove the template) and then with water. Both solvents were forced to permeate through the membranes by applying nitrogen under increased pressure. Mass of the polymer in a membrane was equal to 15–25% of the total membrane weight. The prepared membranes were characterised by the hydrodynamic permeabilities and pore-size distributions estimated with use of a Coulter Porosimeter II (Coulter, UK).

The rest of the polymerisation solution was also exposed to the UV light and, after a complete polymerisation, the resulting polymer was washed and used for the evaluation of sorption (Table 2).

2.4. Preparation of a bifunctional membrane

According to the procedure described previously [7], lipase was covalently immobilised in the skin layer of the molecularly imprinted membrane placed in a membrane cell. Glutar dialdehyde and ethylene diamine were used as a spacer and binding agent, respectively. One milligram of the lipase from

Table 2
Composition of the polymerisation mixtures for MIP and CP

Component	Compound	CP mass (g)	MIP mass (g)
Template	(1*R*,2*S*,5*R*)-(−)-menthol (MIP-M) or	–	0.1
	(1*R*,2*S*)-(−)-*trans*-2-phenyl-1-cyclohexanol (MIP-Ph)	–	0.1
Monomer	Acrylic acid	0.4	0.4
Crosslinker	EGDM	1.0	1.0
Initiator	(±)-Camphorquinone	0.014	0.015

Pseudomonas sp. related to the 30 cm^2 surface of the polyamide membrane. The comparative polymer (CP) enzyme membrane was prepared in the same way from the non-imprinted PA-6 membrane. Before using it in transesterification, the membrane was conditioned in a 0.1 M phosphate buffer (pH 8.0).

2.5. Determination of permeability of enantiomers through membranes

A membrane with the effective surface area of 30 cm^2 was placed in a temperature-controlled (25 °C) diffusion cell (Fig. 2). The feed solution contained the known amount of a racemic compound in *n*-hexane while the pure solvent was the stripping phase. Both compartments (each of 160 cm^3) were rigorously stirred. Small samples (0.2 cm^3) were taken from each compartment and concentrations of the enantiomers were estimated by the chiral HPLC.

3. Results

3.1. Sorption of different enantiomers in the molecularly imprinted polymers

Before the sorption experiments, the MIPs were ground and carefully dried. Each sample (0.1 g) of the imprinted polymer and comparative one (synthesised without a template) was incubated in the *n*-hexane solution of a selected alcohol racemate (5 cm^3, 0.01 mol dm^{-3}). After a complete sorption was attained, the sample was centrifugated and the composition of the supernatant was analysed by the chiral HPLC. The amount of the compound sorbed, A_m, was calculated with the following equation:

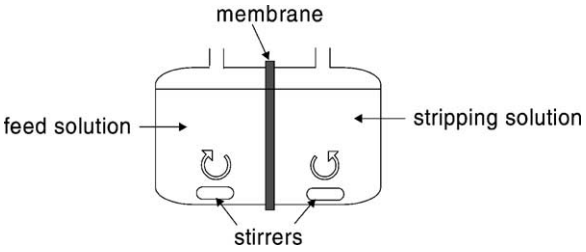

Fig. 2. Scheme for the diffusion cell designed for the determination of enantiomer permeabilities through the imprinted membranes.

$$A_m = \frac{(c_0 - c_e)V}{W}$$

where c_0 and c_e are the initial and equilibrium concentrations, respectively, and V and W, the volume of solution and weight of dry polymer used for the binding experiment, correspondingly.

Sorption selectivities of the polymers (α_s) were estimated as the ratios of the enantiomer sorption in the imprinted polymer to that in the comparative (non-imprinted) polymer. The results are summarized in Table 3.

According to the presented data, the imprinted polymers exhibit sorption selectivity towards a definite enantiomer, namely, the template analogue. Both the maximum sorption of this enantiomer and sorption selectivity for *trans*-2-phenyl-1-cyclohexanol are significantly higher than that for menthol. However, the sorption selectivity was lower than two in all cases. This indicates that the bigger the differences in the three-dimensional structure of the enantiomers, the better the polymer cavity reflects the shape of the template molecule and the higher the selectivity of the imprinted matrix. Even in the case of monofunctional compounds, such as the studied alcohols, one can

Table 3
Results of sorption of (±)-menthol and (±)-*trans*-2-phenyl-1-cyclohexanol in the MIP and CP polymers

Polymer	Compound sorbed	Maximum amount of sorbed compound[a] (μmol g^{-1})	Sorption selectivity, α_S[b]
CP	(±)-Menthol	11.3 (±0.5)	1.00
	(±)-*trans*-2-Phenyl-cyclohexanol	10.5 (±0.7)	1.01
MIP-M[c]	(±)-Menthol	23.1 (±1.2)	1.25
MIP-Ph[d]	(±)-*trans*-2-Phenyl-cyclohexanol	25.8 (±0.9)	1.97

[a] Total amount of the compound sorbed per 1 g of MIP (both enantiomers).
[b] Ratio of the sorption of the template enantiomer to that of the other one, respectively.
[c] Imprinted with (1*R*,2*S*,5*R*)-(−)-menthol.
[d] Imprinted with (1*R*,2*S*)-(−)-*trans*-2-phenyl-1-cyclohexanol.

Table 4
Characteristics of the imprinted and comparative (non-imprinted) polyamide membranes

Membrane	Mean pore diameter (nm)	Pore size range	Number of pores per cm^2	Hydrodynamic permeability (m/Pa s)
PA	123	80–164	3.3×10^5	9.3×10^{-8}
IMP-M[a]	101	74–138	2.8×10^5	4.1×10^{-8}
IMP-Ph-10[b]	83	53–111	2.7×10^5	3.7×10^{-8}
IMP-Ph-20[c]	94	70–121	2.4×10^5	4.3×10^{-8}

[a] Membrane imprinted with (1*R*,2*S*,5*R*)-(−)-menthol, containing 10% (w/w) of the template in the polymerization solution.
[b] Membrane imprinted with (1*R*,2*S*)-(−)-*trans*-2-phenyl-1-cyclohexanol, containing 10% (w/w) of the template in the polymerization solution.
[c] Membrane imprinted with (1*R*,2*S*)-(−)-*trans*-2-phenyl-1-cyclohexanol, containing 20% (w/w) of the template in the polymerization solution.

obtain certain selectivity, provided that there are relatively large rings or substituents in their molecules.

3.2. Morphology and transport characteristics of the membranes

Mean pore sizes, pore-size distributions, and hydrodynamic permeabilities of the imprinted membranes are summarized in Table 4 while the SEM image of an imprinted membrane is shown in Fig. 3.

As follows from Table 4, the imprinted membranes have smaller pores and lower hydrodynamic permeabilities in comparison to the non-imprinted PA membrane. This is certainly caused by a partial clogging of the pores by an imprinted polymer layer.

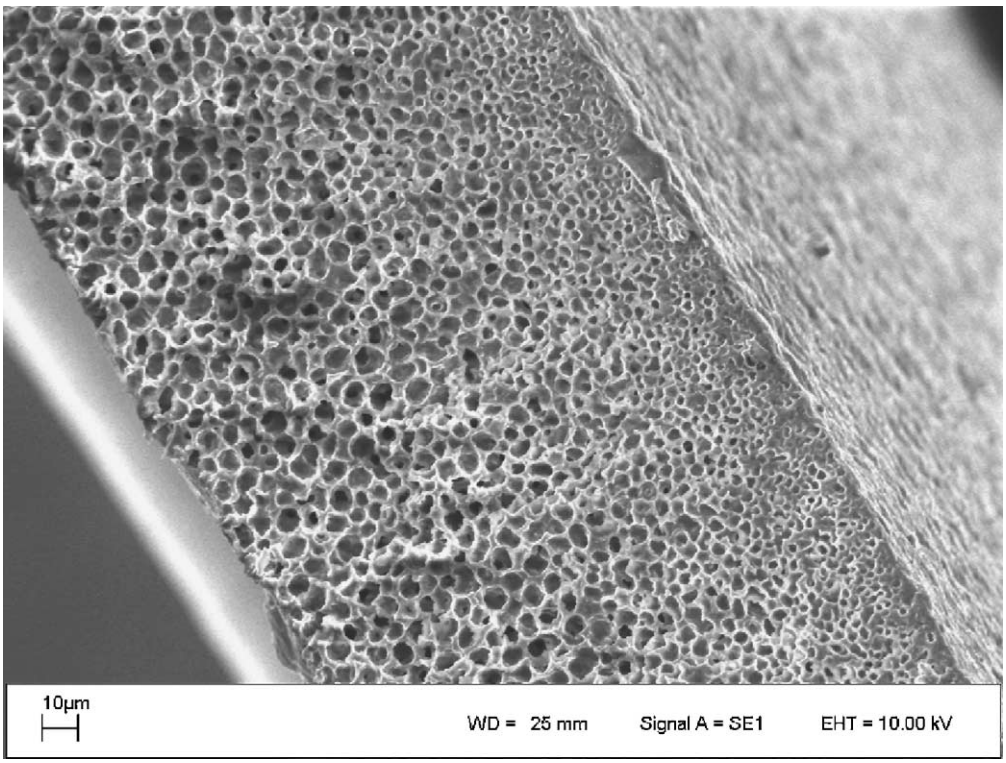

Fig. 3. SEM image of the imprinted polyamide membrane (LEO 1430VP, LEO Electron Microscopy Ltd., Cambridge, UK).

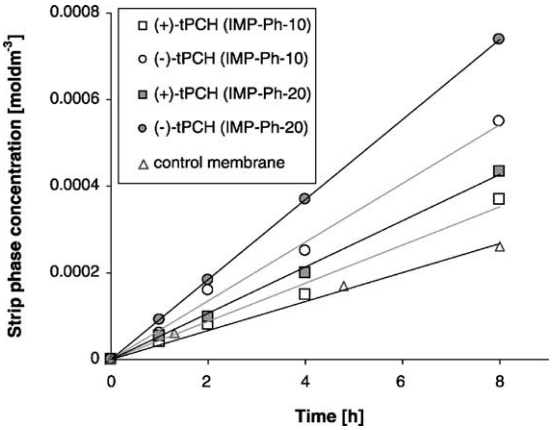

Fig. 4. Concentration changes of *trans*-2-phenyl-1-cyclohexanol enantiomers vs. time of permeation through the imprinted and comparative (non-imprinted) membranes. Squares relate to (1*R*,2*S*)-(−)-*trans*-2-phenyl-1-cyclohexanol, circles to (1*S*,2*R*)-(+)-*trans*-2-phenyl-1-cyclohexanol. Two different membranes were used (IMP-Ph-10 (empty symbols), and IMP-Ph-20 (filled symbols).

3.3. Permeability of enantiomers through the imprinted membranes

Selectivity of the imprinted membranes in transport of enantiomers has been estimated from the concentrations of particular enantiomers, changing during permeation of these compounds through the membrane in the "diffusion cell" (presented in Section 2.5). The results, shown in Fig. 4, have been used to calculate permeability coefficients (P) for the two enantiomers and to compare them to the results obtained for the non-imprinted PA membrane.

The values of P ($\mathrm{m\,s^{-1}}$) were calculated from the slope of the relationship between the solute flux, J ($\mathrm{mol\,m^{-2}\,s^{-1}}$), and the difference in concentration of the compound between the two compartments (Fig. 5), according to the equation:

$$J = \frac{Q}{At} = P\,\Delta C$$

where Q (mol) is the amount of permeated compound, ΔC ($\mathrm{mol\,m^{-3}}$) the difference in concentration of the compound between the two compartments, A ($\mathrm{m^2}$) an effective membrane area, and t (s) time.

Separation factors (SF) were calculated as the ratios of permeability coefficients of the enantiomers permeating through the membrane. The values of these coefficients are summarised in Table 5.

As seen, the enantiomer used as the imprinted template permeates in preference to the other one. This tendency agrees with the sorption preference presented earlier. However, the separation factors are lower than the sorption selectivities (Tables 3 and 5). Moreover, the separation is superior in the membrane with a higher concentration of the chiral sites.

The results confirm that the mechanism of the guest-molecule transport in the membrane is quite different from the behaviour of, e.g. a chiral chromatographic packing material. In the latter, the enantiomers

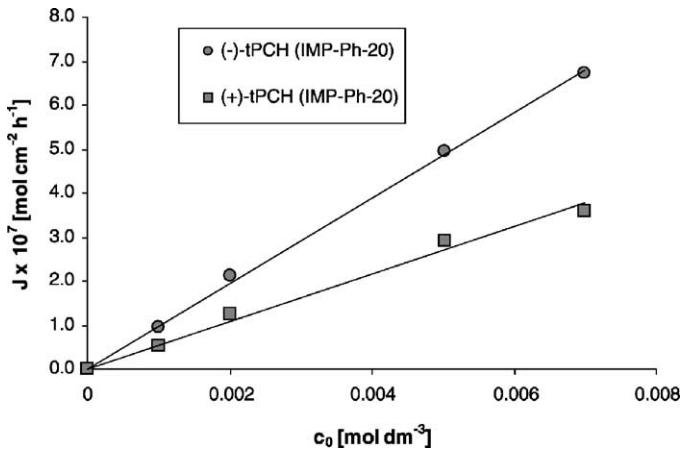

Fig. 5. Fluxes of the enantiomers of *trans*-2-phenyl-1-cyclohexanol permeated through the membrane vs. the initial concentration of the alcohol.

Table 5
Permeability coefficients and separation factors for *trans*-2-phenyl-1-cyclohexanol enantiomers (293 K)

Enantiomer	Permeability coefficient, P ($m\,s^{-1}$)		
	Control membrane	IMP-Ph-10	IMP-Ph-20
(1*R*,2*S*)-(−)-*trans*-2-phenyl-1-cyclohexanol	1.28×10^{-7}	1.93×10^{-7}	2.69×10^{-7}
(1*S*,2*R*)-(+)-*trans*-2-phenyl-1-cyclohexanol	1.28×10^{-7}	1.28×10^{-7}	1.50×10^{-7}
Separation factor[a]	1.0	1.5	1.7

[a] Separation factor (SF): P_-/P_+, where P_- and P_+ are permeability coefficients of the (−)- and (+)-enantiomers, respectively.

would permeate and be washed out in reverse order. The prepared membrane behaves as the phase with a fixed chiral selector rather than a typical affinity membrane [9,18]. Thus, the shape-selective open cavities recognize the template analogue enantiomer molecules so that they pass through the membrane in preference to the others. The template enantiomer is transported from one binding site inside the membrane to another, while the second enantiomer can either diffuse through the membrane, or diffuse back to the feed solution. Concentration of the template analogue in the membrane is then higher than of the second enantiomer, so it can diffuse to the stripping phase at higher rate. The explanation is valid if we consider the system without any external pressure added. The situation is quite different if the mixture of enantiomers permeates under pressure. Longer residence time of the template analogue would cause higher flux of the second enantiomer. Similar relationships were reported by, e.g. Dzgoev and Haupt [10] who prepared enantioselective membranes imprinted with CBZ-L-tyrosine. However, opposite results, typical for chromatographic affinity membranes, were also reported [15,16,19].

Relatively low permeability selectivities make application of such membranes for separation of enantiomers rather unsuitable because the enantiomer ratio after a single run is in the range of 1.5–1.7 only. However, the obtained enrichment of alcohol in the more reactive enantiomer that takes part in the enzymatic transesterification should result in a higher optical purity of the reaction product. We have proved validity of this idea in the separation of (±)-*trans*-2-phenyl-1-cyclohexanol performed in the system containing a bifunctional membrane.

3.4. Kinetic resolution of alcohols in the reactor with bifunctional membranes

According to the results of our previous experiments, lipase from *Pseudomonas* sp. catalyses preferentially 1*R*-enantiomer of chiral cyclohexanols [20]. The enantiomeric ratio (E) of transesterification of vinyl acetate with (±)-*trans*-2-phenyl-1-cyclohexanol in *n*-hexane was equal 20 in the system containing the native enzyme. It is a rather moderate enantioselectivity and the enantiomeric excess of the product (acetate) did not exceed 87%. This process, performed in the reactor with the same lipase immobilised in the polyamide-6 membrane, exhibited the same enantioselectivity. The yield of such a reactor, expressed in moles of the produced alcohol per unit time and gram of the enzyme, was evidently lower and equalled ca. 20% of that exhibited by the lipase suspension. However, the immobilised lipase worked much longer than the native enzyme did, retaining the catalytic activity for more than 180 h.

Transesterifications in the reactor with the bifunctional membrane were carried out using the same arrangement as that in the case of the common enzyme membrane. The *n*-hexane solution of the racemic mixture of alcohols was placed on the membrane side containing the imprinted polymer layer. This solution was circulated countercurrently to the circulation of the solution of vinyl acetate in *n*-hexane), placed on the enzyme layer side of the membrane. The scheme for this reactor is given in Fig. 6.

Concentrations of both alcohol and ester enantiomers in the feed and stripping solutions were determined in dependence on time. The results obtained using the bifunctional membrane are shown in Fig. 7a as a plot of the concentrations of the product and unreacted alcohol in the stripping phase versus time.

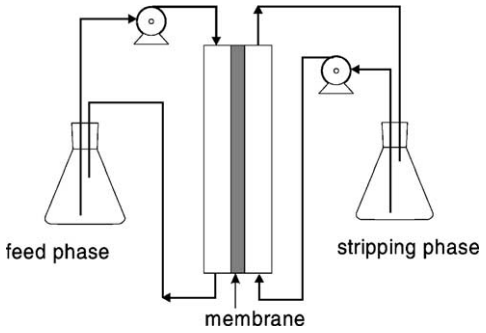

Fig. 6. Scheme for the membrane reactor utilising the kinetic resolution of (±)-*trans*-2-phenyl-1-cyclohexanol in transesterification of vinyl acetate.

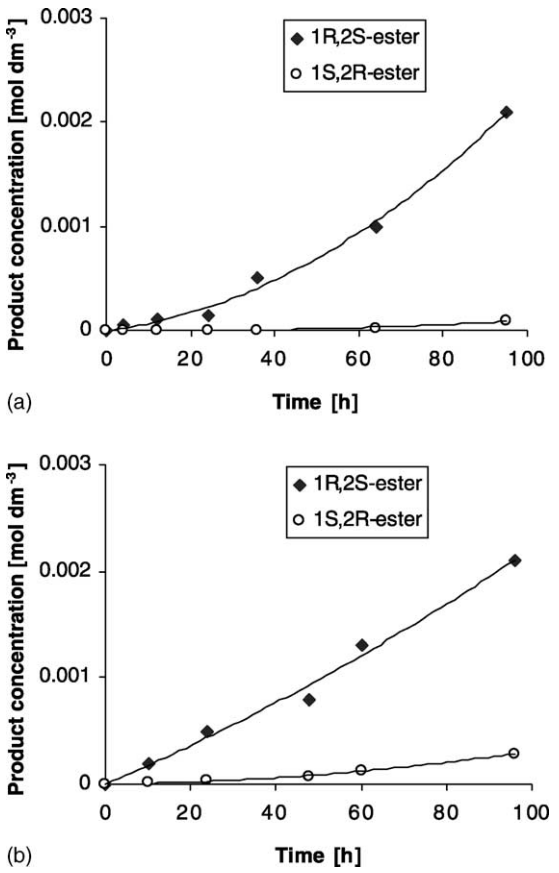

Fig. 7. Concentration of the products of vinyl acetate transesterification with *trans*-2-phenyl-1-cyclohexanol vs. the reaction time; (a) in the reactor with the bifunctional membrane, (b) in the reactor with the common enzyme membrane. Initial substrate concentration: 0.01 mol dm^{-3} in *n*-hexane.

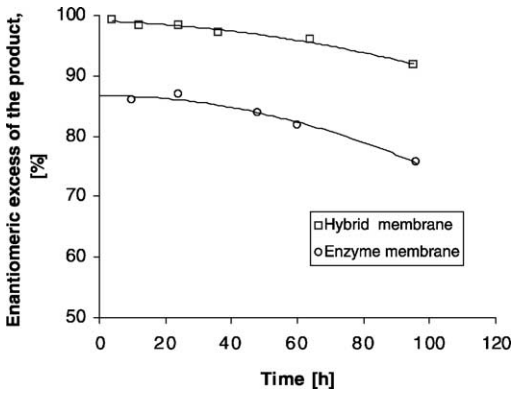

Fig. 8. The product enantiomeric excess in transesterification of vinyl acetate by (±)-*trans*-2-phenyl-1-cyclohexanol in the reactor with the hybrid enzyme membrane.

The data for the process performed with the common enzyme membrane are presented in Fig. 7b.

As seen, the process with use of the bifunctional membrane yields higher amounts of optically pure products than the other one does. In the former case, the concentration of the unwanted 1*S*,2*R*-ester becomes noticable after ca. 50 h performance while this happens after already ca. 15 h for the common enzyme membrane. The difference between the enantiomer concentrations results in distinctly different values of the enantiomeric excesses (Fig. 8).

The enantiomeric excess (e.e.) of the product formed with use of the bifunctional membrane in the stripping phase is clearly higher while the reactor productivity is lower (Table 6). This disadvantage is caused by the lower permeability of the membrane with the imprinted polymer.

The obtained results indicate that, within the imprinted polymer layer of the membrane, a sufficient separation of alcohol enantiomers occurs, which

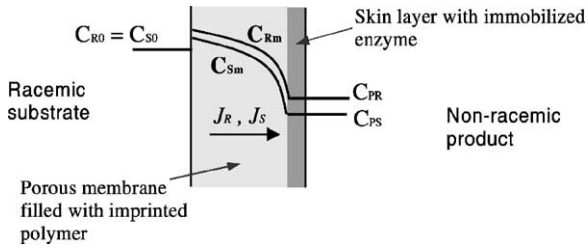

Fig. 9. Scheme for concentration profiles occurring during the transport through an enantioselective membrane.

Table 6
Initial reaction rates and enantiomeric excesses of products in transesterifications performed when using the common enzyme or the bifunctional membrane

Alcohol	Template	Productivity (mmol h^{-1} m^{-2})	e.e. of the product[a] (%)
(±)-*trans*-2-Phenyl-1-cyclohexanol	(1*R*,2*S*)-(−)-*trans*-2-phenyl-1-cyclohexanol	10	98.5
(±)-*trans*-2-Phenyl-1-cyclohexanol	None	16	88.2

[a] At the reaction time of 40 h and conversion of 30%.

results in higher permeability of one enantiomer over the other. Consequently, the substrate being in contact with the biocatalyst is not a racemate but is enriched in the more reactive enantiomer and, thus, the yield of the preferred enantiomer in the chemical conversion is much higher. The mechanism of the overall separation of the enantiomers within the bifunctional membrane is shown schematically in Fig. 9.

4. Conclusions

The enantioselective permeation membrane can be formed by the direct polymerisation of a proper monomer solution containing an appropriate enantiomer that is introduced as a template into the ultrafiltration polyamide-6 membrane. The permeation rate of this enantiomer is higher as compared to that of the other one and it depends on the concentration of the template. The selectivity factor reaches 1.97 in the separation of *trans*-2-phenyl-1-cyclohexanol, which is too low for this method to be utilised for the direct separation of that enantiomer. It is, however, sufficient to enhance the kinetic resolution in the enzyme process performed in the membrane that has additionally been modified by immobilising an enzyme. Such a modification causes an increase in the enantiomeric excess of the product of vinyl acetate transesterification with the above-mentioned alcohol from 88.2 to 98.5% at the 30% conversion. The bifunctional membrane with the layers of the imprinted polymer and enzyme can be used for the separation of chiral substrates by the kinetic resolution process performed in the membrane reactor.

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References

[1] D.R. Wu, S.M. Cramer, G. Belfort, Kinetic resolution of racemic glycidyl butyrate using a multiphase membrane enzyme reactor: experiments and model verification, Biotechnol. Bioeng. 41 (1993) 979–992.

[2] J.L. Lopez, S.L. Matson, A multiphase/extractive enzyme membrane reactor for production of diltiazem chiral intermediate, J. Membr. Sci. 125 (1997) 189–198.

[3] K. Sakaki, L. Giorno, E. Drioli, Lipase-catalyzed optical resolution of racemic naproxen in biphasic enzyme membrane reactors, J. Membr. Sci. 184 (2001) 27–38.

[4] K. Griebenov, Y.D. Laureano, A.M. Santos, I.M. Clemente, L. Rodriguez, M.W. Vidal, G. Barletta, Improved enzyme activity and enantioselectivity in organic solvents by methyl-β-cyclodextrin, J. Am. Chem. Soc. 121 (1999) 8157.

[5] A. Ghanem, V. Schurig, Peracetylated β-cyclodextrin as additive in enzymatic reactions: enhanced reaction rate and enantiomeric ratio in lipase-catalyzed transesterifications in organic solvents, Tetrahedron: Asymmetry 12 (2001) 2761–2766.

[6] D.-J. van Unen, J.F.J. Engbersen, D.N. Reinhoudt, Studies on the mechanism of crown-ether-induced activation of enzymes in non-aqueous media, J. Mol. Catal. B: Enzym. 11 (4-6) (2001) 877–882.

[7] J. Ceynowa, I. Koter, Selection of pure enantiomers of 1-phenyl alcohols by sequenced processes of ester hydrolysis and transesterification in enzyme membrane reactors, Sep. Sci. Technol. 34 (1999) 2663.

[8] J. Ceynowa, I. Koter, Methyl-β-cyclodextrin assisted enantioselective ester hydrolysis catalyzed by lipase immobilized in a polymer membrane, Sep. Sci. Technol. 36 (13) (2001) 2885–2898.

[9] G. Wulff, Molecular imprinting in cross-linked materials with the aid of molecular templates—a way towards artificial antibodies, Angew. Chem., Int. Ed. Engl. 34 (1995) 1812–1832.

[10] P.A.G. Cormack, K. Mosbach, Molecular imprinting: recent developments and the road ahead, React. Funct. Polym. 41 (1999) 115–124.

[11] T. Takeuchi, J. Haginaka, Separation and sensing based on molecular recognition using molecularly imprinted polymers, J. Chromatogr. B 728 (1999) 1–20.

[12] O. Brüggemann, Chemical reaction engineering using molecularly imprinted polymeric catalysts, Anal. Chim. Acta 435 (2001) 197–207.

[13] J. Ceynowa, I. Koter, Lipase-catalyzed kinetic resolution of (*R*,*S*)-1-phenylethyl propionate in an enzyme membrane reactor, Acta Biotechnol. 17 (1997) 253–261.
[14] M. Barboiu, C. Guizard, C. Luca, N. Hovnanian, J. Palmieri, L. Cot, Facilitated transport of organics of biological interest. Part I. A new alternative for the separation of amino acids by fixed-site crown-ether polysiloxane membranes, J. Membr. Sci. 172 (2000) 91–103.
[15] J. Randon, F. Garnier, J.L. Rocca, B. Maïsterrena, Optimization of the enantiomeric separation of tryptophan analogs by membrane processes, J. Membr. Sci. 175 (2000) 111–117.
[16] A. Dzgoev, K. Haupt, Molecularly imprinted polymer membranes for chiral separation, Chirality 11 (1999) 465–469.
[17] S.A. Piletsky, T.L. Panasyuk, E.V. Piletskaya, I.A. Nicholls, M. Ulbricht, Receptor and transport properties of imprinted polymer membranes—a review, J. Membr. Sci. 157 (1999) 263–278.
[18] E.M. van der Ent, K. Van't Riet, J.T.F. Keurentjes, A. Van der Padt, Design criteria for dense permeation-selective membranes for enantiomer separations, J. Membr. Sci. 185 (2001) 207–221.
[19] F. Trotta, E. Drioli, C. Baggiani, D. Lacopo, Molecular imprinted polymeric membrane for naringin recognition, J. Membr. Sci. 201 (2002) 77–84.
[20] J. Ceynowa, M. Rauchfleisz, Resolution of racemic *trans*-2-methyl-1-cyclohexanol by lipase-catalysed transesterification in a membrane reactor, J. Mol. Catal. B: Enzym. 15 (1–3) (2001) 71–80.