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METHYL-β-CYCLODEXTRIN ASSISTED ENANTIOSELECTIVE ESTER HYDROLYSIS CATALYZED BY LIPASE IMMOBILIZED IN A POLYMER MEMBRANE

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ABSTRACT

Kinetic resolution of racemates by means of enzyme processes occurring in a membrane reactor is discussed. The reactor containing a lipase catalyst immobilized within the polyamide membrane was used to separate (*R,S*)-1-phenylethanol and (*R,S*)-1-phenyl-1-propanol that were produced by ester hydrolysis. The efficient separation was accomplished by the on-line extraction of the produced alcohols in an independent membrane extraction module. Methyl-β-cyclodextrin was used as an alcohol extractant to separate alcohols from both the unreacted esters and the produced acids. Thermal decomposition (at 60–70°C) of the formed complexes of methyl-β-cyclodextrin and alcohol is suggested as a convenient method for reextraction. Application of the hybrid reactor-extractor membrane system yielded higher reaction rates and greater enantiomer excess in relation to the unreacted R–ester.

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INTRODUCTION

Kinetic resolution of acid and/or alcohol racemates in enzyme-catalyzed processes is an alternative for producing optically pure compounds. However, potential limitations are often observed, such as low enzyme enantioselectivity and conversion (ranging up to 50%). Moreover, separation of products from remaining substrates may be laborious. Some interesting approaches to improve the enzymatic kinetic resolution have been reported, e.g., application of two enzymes of opposite enantioselectivities (1), tandem or sequential enzymatic reactions (2–4), and in situ racemization of a substrate. Other strategies leading to increased selectivity have been explored: imprinting of enzymes by a substrate analogue (5), cross-link crystallization (6), and preparation of special enzymes (7,8).

Because of the low solubility of organic substrates in water, most hydrolysis reactions must be performed in 2-phase systems. A convenient way to carry out such reactions is use of a membrane reactor with an enzyme catalyst immobilized within the membrane. A number of processes of that kind have been reported (9,10).

Recently, promising results were published that evidenced a positive influence of some compounds, such as crown ethers (11) and cyclodextrins (12,13) on enantioselectivities of subtilisin and bovine serum albumin. We have also reported (14) that β-cyclodextrin and its methylated derivative, methyl-β-cyclodextrin (met-β-CD) modify activity of free lipase derived from *Pseudomonas* during enzymatic ester hydrolysis. Later, we have found that met-β-CD participates in transferring 1-phenyl alcohols from *n*-heptane to water. Our aim was to study hydrolysis of two 1-phenylalcohol esters in a 2-phase enzyme membrane system and simultaneous separation of alcohol products. The alcohols were extracted on-line in a membrane extraction module through the use of met-β-CD.

Ability of cyclodextrins to form inclusion complexes with different substances is widely exploited, especially in the food and pharmaceutical industries (15). These compounds are useful in a number of applications (16–20) ranging from chiral separations in gaseous phases to high- performance liquid chromatography (HPLC) and production of drugs. While forming host-guest complexes with the substrates, cyclodextrins can change the formal solubility of a guest compound. Moreover, some cyclodextrins and their derivatives catalyze a number of reactions that usually proceed according to the Michaelis-Menten hypothesis and have been used even as simple enzyme models (21).

MATERIALS AND METHODS

Chemicals

Met-β-CD (randomly methylated, M.W. = 1415) as well as (±)-1-phenylethyl propionate and (±)-1-phenylethanol (Fluka Chenile AG, Buchs,

Switzerland) were used without additional purification. Racemic 1-phenylethyl acetate and 1-phenyl-1-propyl acetate were synthesized according to a standard procedure using acetic anhydride. *n*-Hexane, methyl *tert*-butyl ether (MTBE) and other solvents (Aldrich) were of HPLC grade. Polyamide hollow-fiber membranes (i.d. = 0.6 mm; o.d. = 1.2 mm; cutoff 50 kd) were obtained as a gift from Berghof, Germany. Lipase from *Pseudomonas*, E.C. 3.1.1.3, 2160 units[1] per mg solid, was purchased from Sigma.

Analytical

Concentrations of all the esters and alcohols were determined by HPLC with a Chiralcel OD—H column (Daicel Chemical Ind, Japan) and a mixture of *n*-hexane and MTBE (80:20 vol %) as an eluent. The optimal flow rate was 1.5 mL/min. Chromatographic data were obtained from a system consisting of a precision isocratic pump (Spectra Physics SP 8810), a Rheodyne injector with a 10-μL loop, and a refractive index detector (RI SE-61, Shodex, Japan). Then, quantitative data were handled with a PL Logical PC program. Experimental conditions were found under which separate peaks corresponding to all the enantiomers were observed. A sample chromatogram is shown in Fig. 1.

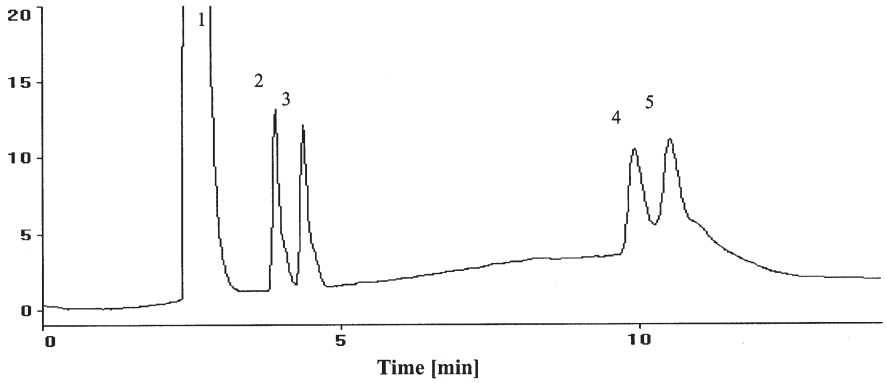

Figure 1. Chromatogram of a mixture of (*R,S*)-1-phenylethanol (peaks 4 and 5) and (*R,S*)-1-phenylethyl propionate (peaks 2 and 3, respectively) in *n*-heptane. For a mixture of (*R,S*)-1-phenyl-1-propanol and (*R,S*)-1-phenyl-1-propyl acetate, the retention times are equal to 8.1, 8.8, 4.2, and 4.7 minutes, respectively. Flow rate = 1.0 mL/min; eluent: *n*-hexane/MTBE, 80/20 (vol/vol).

[1]One unit of lipase is the amount of lipase that liberates 1 μmol of free fatty acid in 1 hour during the hydrolysis of olive oil at pH = 7.2 and T = 310 K.

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Reactor Operation

Results of some enzymatic ester hydrolyses performed in the enzyme membrane reactor have been previously published (4). The enzyme membranes were prepared by the chemical immobilization of lipase derived from *Pseudomonas* (via diamine and glutaraldehyde), within a polyamide hollow-fiber membrane. The total amount of lipase immobilized in 5 fibers placed in the membrane module was 1.4 mg, and the inner geometrical area of the membrane was equal to 17 cm^2 (5 fibers 18-cm long).

The hydrolyses were carried out at 303 K in a 2-phase system with the organic phase (ester solution in *n*-heptane) circulating in the shell and the aqueous buffer solution (pH = 8.0) circulating countercurrently on the lumen side of the membranes. Small samples of the organic phase were taken to determine the reagent concentrations by HPLC.

Extraction of Alcohols by Met-β-CD

Membrane extraction is an alternative to the classic solvent-extraction method (15,16), the extraction of alcohols with met-β-CD (instead of a normal 2-phase liquid extraction) was performed in the polyamide hollow-fiber membrane module. The extraction module consisted of 10 polyamide fibers (18-cm long). All the experiments were carried out at 298 K. A scheme for the module is shown in Fig. 2.

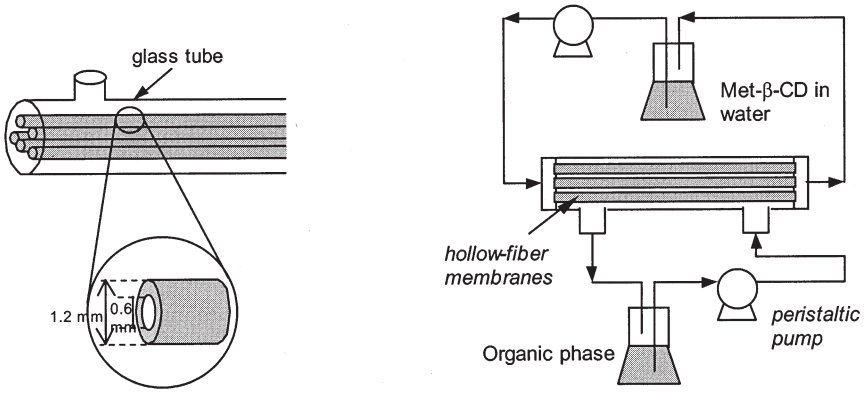

Figure 2. Scheme for the membrane extraction module.

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RESULTS AND DISCUSSION

Ester Hydrolysis with Native Lipase

The results of the enzyme membrane reactor process were compared with those of the experiments with a native enzyme. Hydrolyses of both (±)-1-phenylethyl propionate and (±)-1-phenylethyl acetate catalyzed by the native lipase, with and without the met-β-CD additive, were also performed. As seen in Table 1, the initial rates of the reaction in the presence of met-β-CD were higher than those of the reaction without this compound, although met-β-CD itself does not catalyze the discussed process. Also the measured concentrations of the alcohol products in the organic phase were much lower than the concentrations of these products calculated from both the conversion of the substrate and the coefficient of alcohol partition between *n*-heptane and water.

The relationship between the amounts of both 1-phenylethanol and 1-phenyl-1-propanol removed from the *n*-heptane solution and the amount of added cyclodextrin is shown in Fig. 3. Because the molar ratio of extracted alcohol to met-β-CD dissolved in water is close to 1, the methylated cyclodextrin may form an inclusion complex with 1–phenyl alcohols (1:1).

The evident deviation of the experimental points from a straight line at $n_{CD} > 3 \times 10^{-4}$ mol (Fig. 3) cannot be explained at present. However, the effect might result from the possible equilibrium of the alcohol association in the aqueous phase.

The reaction of complexation is given by the equation:

$$\text{alcohol}_{org} + \text{met-}\beta\text{-CD}_{aq} \overset{K_{eq}}{\rightleftharpoons} [\text{alcohol-met-}\beta\text{-CD}]_{aq} \quad (1)$$

At equilibrium, the complexation constant is defined as

$$K_{eq} = \frac{[\text{alcohol-(met-}\beta\text{-CD)}]_{aq}}{[\text{alcohol}]_{org}[\text{met-}\beta\text{-CD}]_{aq}} \quad (2)$$

Table 1. Initial Reaction Rates (v_0) and Enantiomeric Ratios (E) for Ester Hydrolyses Both With and Without Methyl-β-CD

	v_0 [mmol/dm^3·h]		E [–]	
Ester	1	2	3	4
(±)-1-phenylethyl acetate	0.26	0.41	22	18
(±)-1-phenyl-1-propyl acetate	0.75	1.20	49	58

Columns 1 and 3: without met-β-CD; columns 2 and 4: with 0.4 g met-β-CD.
Amount of native lipase = 0.1 mg (6×10^{-7} mol/dm^3); initial ester concentration = 0.05 mol/dm^3 in *n*-heptane; T = 303 K; pH = 8.0.

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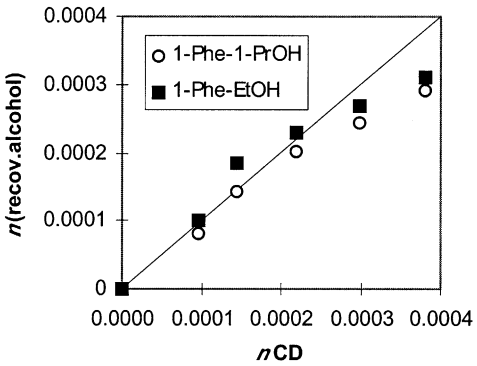

Figure 3. Amount of alcohol removed from the organic phase (moles) versus amount of met-β-CD in the aqueous phase (moles); $T = 303$ K.

where $[\text{alcohol-(met-β-CD)}]_{aq}$ is the concentration of the alcohol-(met-β-CD) complex in the aqueous phase; $[\text{alcohol}]_{org}$ is the concentration of alcohol in the organic phase; and $[\text{met-β-CD}]_{aq}$ is the concentration of free met-β-CD in water. The equilibrium value of $[\text{alcohol-(met-β-CD}]_{aq}$ was assumed to be equal to the concentration of alcohol in the aqueous phase ($[\text{alcohol}]_{aq}$) reduced by the amount of alcohol present in this phase due to partition of the alcohol between the phases. The latter amount was evaluated from the coefficient of partition. The calculated values of K_{eq} for complexation of both alcohols are given in Table 2.

Table 2. Coefficients of Partition (P) of 1-Phenyl Alcohols Between Water and *n*-Heptane and Equilibrium Constants (K_{eq}) for Complexation of 1-Phenyl Alcohols with Met-β-CD (293 K)

Alcohol	P	K_{eq}
1-phenylethanol	0.13	71 ± 1
1-phenyl-1-propanol	0.08	88 ± 2

$P = \dfrac{[alcohol]_{aq}}{[alcohol]_{org}}$ determined without met-β-CD).

Total concentration of met-β-CD in water was 0.250 mol/L, and initial alcohol concentrations in the organic phase were 0.205 and 0.184 mol/L for 1-phenyl-1-propanol and 1-phenylethanol, respectively. Total volume of each phase was 5 cm^3.

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The complexation constants of 1-phenyl alcohols (71 and 88) are distinctly different from those calculated for phenyl esters: 1-phenylethyl acetate (0.04) and 1-phenyl-1-propyl acetate (0.07). The differences in complexation constants explain the ability of met-β-CD to extract the mentioned alcohols from mixtures with esters in the organic phase.

The following arguments may explain the alcohol-extraction result: The internal surface of the CD cavity is hydrophobic, and a nonpolar part of the molecule (e.g., phenyl or naphthyl substituents of suitable size) can penetrate into the cyclodextrin ring. When such a guest is able to form additional interactions with the functional groups of the modified cyclodextrin, as with met-β-CD, inclusion complexes can be formed. This happens with an alcohol molecule that can form a hydrogen bond with the CH_3O–group (proton acceptor), whereas with esters such interactions are less probable. The ring and possible inclusion mechanisms are illustrated schematically in Fig. 4.

Membrane Extraction of 1-Phenylethanol by Methyl-β-cyclodextrin

Results of membrane extraction of 1-phenylethanol from a mixture with the phenyl ester in *n*-heptane to an aqueous solution of excess met-β-CD are presented in Fig. 5. The figure shows the alcohol recovery and its molar flux as functions of the flow rate of the aqueous stripping phase.

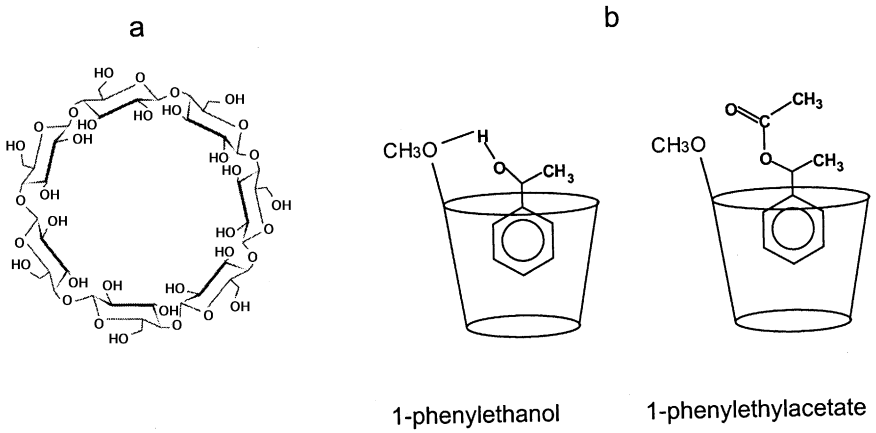

Figure 4. β-cyclodextrin structure (a) and a scheme for inclusions of alcohol and ester to met-β-CD (b).

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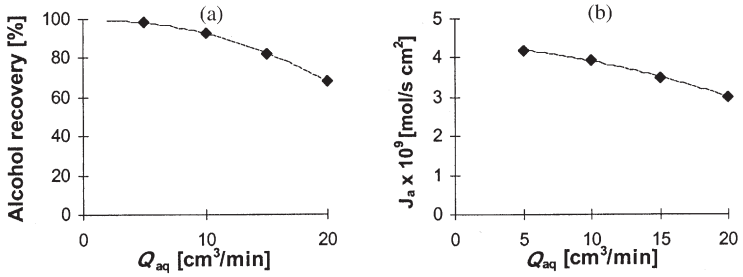

Figure 5. Effectiveness of 1-phenylethanol extraction from an equimolar mixture with its ester in *n*-heptane as percentage of (a) alcohol recovery and (b) flux of alcohol (J_a) versus flow rate of the aqueous phase (Qaq). Initial concentrations of alcohol and ester were 0.01 mol/L, and the stripping phase was constituted with 0.0015 mol met-β-CD in 50 mL of water (pH = 7.0).

The flux of alcohol was calculated through the following relation:

$$J_a = K_w \Delta C \tag{3}$$

where K_w is the overall coefficient of effective mass transfer and is defined by the equation

$$K_w = \frac{Q_{aq} C_{aq,o}}{S \Delta C} \tag{4}$$

Q_{aq} denotes the flow rate of the aqueous phase; $C_{aq,o}$ is the outlet alcohol concentration on the aqueous side; S is the membrane surface area; and ΔC is a mean concentration driving force. The ΔC is calculated as

$$\Delta C = \frac{\Delta C_i - \Delta C_o}{\ln\left(\frac{\Delta C_i}{\Delta C_o}\right)} \tag{5}$$

where ΔC_i and ΔC_o denote concentration differences between the organic and aqueous phases, at the inlet and outlet of the reactor, respectively.

Alcohol recovery clearly depends on the flow rate of the aqueous phase (Fig. 5). Both the flux of alcohol and the overall coefficient of effective mass transfer decrease with the flow rate. This decrease is caused by a reduced contact time with the aqueous phase. At sufficiently low flow of the stripping (aqueous) phase and at the proper concentration of met-β-CD, nearly 100% extraction can be accomplished.

Ester Hydrolysis Followed by the Extraction of Alcohol in the Hybrid Membrane System

To provide simultaneous hydrolysis of ester and removal of the produced alcohol from the organic phase, we proposed the sequenced hydrolysis of ester

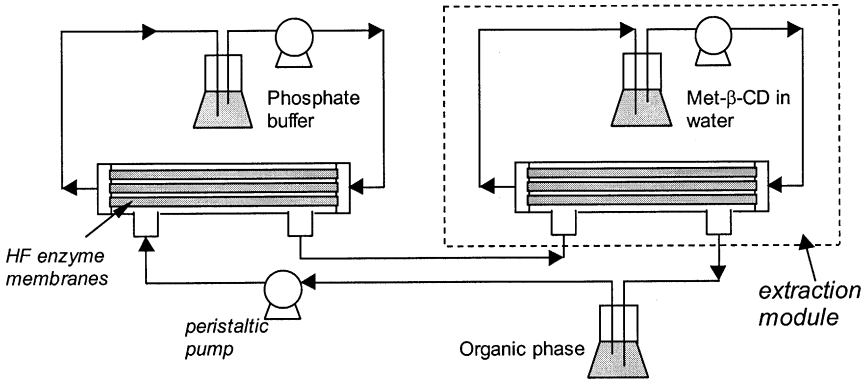

Figure 6. Ester hydrolysis in a hollow fiber (HF) enzyme membrane reactor with subsequent product extraction with met-β-CD.

and extraction of the produced alcohol in a separate module. A scheme for this hybrid reaction-extraction system is shown in Fig. 6, and the system parameters are given in Table 3. Performance of the system is presented in Fig. 7 as a plot of the amount of the removed alcohol with regard to the flow rate of the stripping phase.

Because the reaction rates of ester hydrolysis appeared to depend on the flow rate of the aqueous phase, the flow rate in further experiments was fixed at 7.5 cm^3/min with a constant flow rate of the organic phase equal to 10 cm^3/min. Concentrations of enantiomeric alcohols and esters over time in the system with and without the extraction module is presented in Fig. 8.

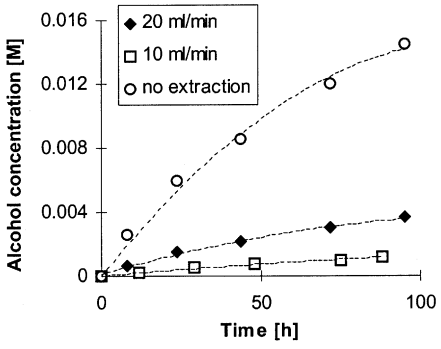

Figure 7. Concentration of alcohol (total, $R + S$) produced in the organic phase during hydrolysis of (±)-1-phenylethyl acetate versus time at various flow rates of the stripping aqueous phase in the extraction module.

Table 3. Conditions for Hydrolysis of Both (±)-1-Phenylethyl Acetate and (±)-1-Phenyl-1-Propyl Acetate in a Two-Phase Membrane Reactor

Membrane Reactor	
Enzyme membrane	Polyamide hollow-fiber membrane
Number/length of fibers	5; 18 cm
Membrane surface area (geometric)	17 cm^2 (inner side)
Internal membrane volume	0.61 cm^3
Source/amount of lipase	*Pseudomonas* 1.8 mg (0.1 mg/cm^2)
Phase composition in:	
Lumen	0.5 mol/L aqueous solution of phosphate buffer, pH = 8.0
Shell	0.05 mol/L solution of ester in *n*-heptane
Phase volumes; Flow rates:	
Aqueous phase	50 cm^3; 5–20 cm^3/min
Organic phase	50 cm^3; 5–25 cm^3/min
Temperature	303 K
Membrane Extractor	
Membranes	Polyamide hollow-fiber membranes
Number/length of fibers	10; 18 cm
Membrane surface area	34 cm^2 (inner side)
Stripping phase:	
Composition	0.02 mol/L aqueous solution of met-β-CD (3 g/50 cm^3), pH = 7.0
Phase volume; Flow rate	50 cm^3; 5–20 cm^3/min

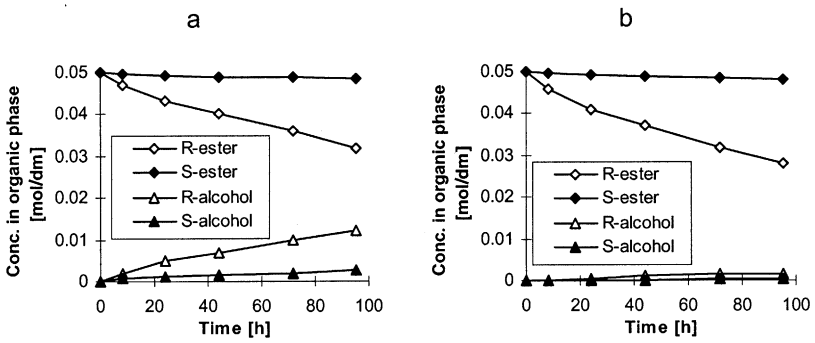

Figure 8. Concentrations of reagents in the organic phase during hydrolysis of (±)-1-phenylethyl acetate in the enzyme membrane reactor: (a) single ester hydrolysis and (b) hydrolysis in the hybrid reactor-extractor system with extraction by met-β-CD. Flow rate of the organic phase: 10 mL/min; flow rate of the aqueous phase containing met-β-CD: 7.5 mL/min.

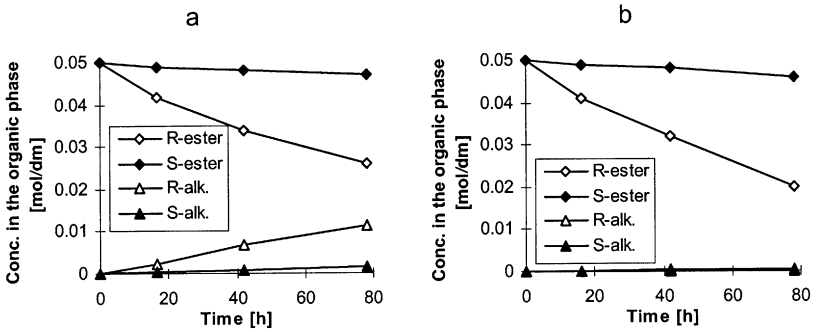

Figure 9. Concentrations of reagents in the organic phase during hydrolysis of (±)-1-phenyl-1-propyl acetate in the enzyme membrane reactor: (a) without extraction and (b) in the hybrid reactor-extractor system with extraction by met-β-CD.

Better results were obtained in the experiments under similar flow conditions (the flow rate of the aqueous phase equal to 7.5 mL/min) with racemic (±)-1-phenyl-1-propyl acetate. Removal of chiral 1-phenyl-1-propanol from the organic phase was almost complete. The results are given in Fig. 9.

An advantage of the hybrid system is evident when the initial reaction rates in the processes with and without the extraction are compared. The data for hydrolyses of both esters are summarized in Table 4. As the data show (Table 4), the reaction rates for the hydrolyses in the hybrid system (i.e., in the presence of the met-β-CD extractant) are higher than those in the single reactor as in the case of the batch process (Table 1). This result was found for both esters. It is to be mentioned that the enantiomeric ratios in the two processes remained unchanged.

Additional experiments indicated that the extracted alcohol can easily be re-extracted to *n*–heptane at elevated temperatures (60–70°C). Results of such a process, i.e., of decomplexation, are shown in Fig. 10.

Table 4. Initial Reaction Rates for Ester Hydrolyses in Both the Single Membrane Reactor and the Hybrid Reactor-Extractor System

	Initial Reaction Rates (mmol/dm^3·h)	
Ester	Single Reactor	Hybrid System
(±)-1-phenylethyl acetate	0.33	0.61
(±)-1-phenyl-1-propyl acetate	0.37	0.50

Amount of lipase in the membrane = 1.4 mg; initial ester concentration = 0.1 mol/dm^3; Q_{org} = 10 cm^3/min; Q_{aq} = 7.5 cm^3/min.

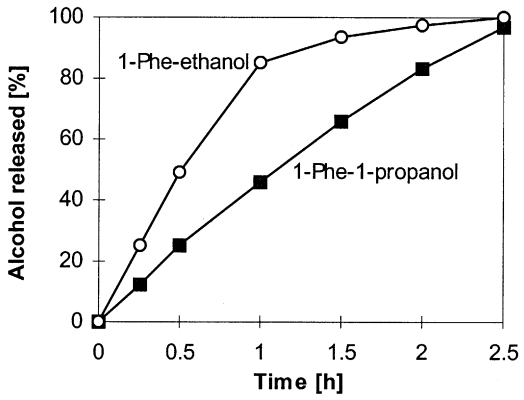

Figure 10. Releasing of alcohols from the aqueous phase to *n*-heptane at 60(C. Initial amounts of 1-phenylethanol and 1-phenyl-1-propanol in the aqueous phase were 5×10^{-5} and 4×10^{-5} mol, respectively; the volume of the *n*-heptane phase was 5 cm^3; and the surface area of the phase was 2 cm^2.

Due to a higher constant of complexation (K_{eq}) of 1-phenyl-1-propanol than that of 1-phenylethanol, the rate of transferring 1-phenyl-1-propanol to *n*-heptane is lower and equals 0.11 mol/h·m^2 (in relation to the phase surface area). In the case of 1-phenylethanol, the rate is 0.16 mol/h·m^2. The transfer of both alcohols to the organic phase was completed within 2.5 hours.

CONCLUSIONS

Met-β-CD forms water-soluble inclusion complexes with 1-phenyl-ethanol and 1-phenyl-1-propanol. Therefore, it can be used as an efficient extractant of these alcohols from mixtures with phenyl esters in *n*-heptane. The process completed in a polyamide hollow-fiber membrane module was a convenient method for the extraction.

Decomposition of the complexes occurs easily at elevated temperatures; thus, the process can be applied as a simple method for reextraction of the alcohols. On-line connecting of the extraction module to the organic phase circuit of the enzyme membrane reactor for the ester hydrolysis results in an enhanced rate of this reaction and in a higher extent of conversion. The enantiomeric purity of the produced alcohols remains unchanged.

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