Kinetics and Modeling of (R,S)-1-Phenylethanol Acylation over Lipase

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ABSTRACT: The kinetics of the acylation of (R,S)-1-phenylethanol was investigated using lipase as a catalyst. The main parameters were temperature, reaction atmosphere, different acyl donors, and different amounts of acyl donor as well as the presence of some additives in the reaction mixture. The initial reaction rate increased with increasing temperature and with a decreasing amount of an acyl donor. The activated esters, such as isopropenyl- and vinyl acetate, exhibited very high acylation rates for R-1-phenylethanol, whereas low rates were obtained with ethyl acetate and 2-methoxyethyl acetate. The addition of water and acetophenone decreased the acylation rate. A kinetic model was developed based on a sequential step mechanism, in which enzyme was reacting in the first step with an acyl donor followed by the reaction of a modified enzyme complex with the reactant, R-1-phenylethanol. Comparison with experimental data obtained at different temperatures allowed simplification of this model, leading to a kinetic equation with just one apparent parameter. The influence of the amount of acyl donor, ethyl acetate, could be quantitatively described by taking into account the competitive inhibition of the ethanol produced. The rate constants and apparent activation energy for experiments performed under different temperatures and the amounts of acylation agent were determined. The apparent activation energy was 24.5 kJ/mol. © 2010 Wiley Periodicals, Inc. Int J Chem Kinet 42: 629–639, 2010

INTRODUCTION

Kinetic resolution of racemic compounds is an important practical method to produce enantiomerically pure alcohols, which are intermediates for synthesis of pharmaceuticals. The drawback with this method is the yield of the desired enantiomer, which is limited to 50% and separation of the enantiomers, typically with chromatographic technique, is needed. The products, optically active esters, can be easily transformed to alcohols, which are important intermediates for synthesis of pharmaceuticals.

Kinetic resolution of secondary alcohols over lipases has been investigated intensively [1]. Enzymes are active in apolar organic solvents, and the solvent selection is of crucial importance when working with lipases. It is known that enzyme conformation changes with changing the polarity of the solvent, and some residual water is needed for maintaining enzyme in its active conformation [1a].

Typically, activated esters, such as vinyl acetate or 4-chlorophenyl acetate, have been used as acyl donors in kinetic resolution of secondary alcohols [2,3]. This is because the reaction rate is higher when the acylation

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is irreversible. The kinetic resolution with, e.g., vinyl acetate is irreversible, because an unstable product, vinyl alcohol, is formed in stoichiometric amounts and quickly reacts further to form acetaldehyde via keto–enol tautomerization [2]. When a saturated acyl donor, such as ethyl acetate, is used, ethanol is formed as a by-product and the reaction is reversible. The equilibrium can be shifted toward the desired ester via, e.g., using an excess of acyl donor. The reaction rate is, however, quite slow in ethyl acetate [4], and ethanol decreases the reaction rate further by increasing the hydrophilicity of the reaction mixture [3].

The effect of temperature variations on the stereochemistry of enzymatic reactions has been addressed in the literature [5–9].

Dynamic kinetic resolution involves kinetic resolution together with the racemization of an undesired enantiomer yielding more than 50% of the desired product. An integrated catalytic reaction combining three different reactions, i.e., hydrogenation, kinetic resolution, and racemization in one step, is one-pot synthesis of chiral acetates via acetophenone hydrogenation to (*R*,*S*)-1-phenylethanol ((*R*,*S*)-1-PE) and the acylation of its *R*-enantiomer with lipase. In this reaction, homogeneous Ru-complex was used both as a hydrogenation catalyst for acetophenone and as a racemization catalyst for *S*-enantiomer [3]. Ethyl acetate is used as an acyl donor, since vinyl acetate will be hydrogenated immediately to ethyl acetate [3].

Kinetic modeling of lipase-catalyzed transesterification has been demonstrated in a few publications [2,10,11]. The modeling was based on a two-step sequence in the transesterification of 2-alkanol with vinyl acetate, in which an acyl-enzyme complex was formed in the first step followed by its reaction between the *R*- and *S*-1-phenylethanol in the second step [2]. The initial rates and the double reciprocal plots of inverse rate versus inverse of either acyl donor or reactant concentrations were taken as a basis for the earlier models.

To elucidate the reaction mechanism and to study the kinetics in one-pot synthesis of *R*-1-phenylethyl acetate, the kinetic resolution of (*R*,*S*)-1-PE (Fig. 1) was separately investigated in the current work under the analogous conditions as applied earlier in one-pot synthesis of *R*-1-phenylethyl acetate from acetophenone [12–14].

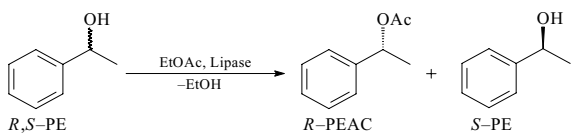

Figure 1 Reaction scheme for kinetic resolution of (*R*,*S*)-1-phenylethanol.

Note that lipase-catalyzed resolution for *R*,*S*-1-phenylethanol has been commercialized [15,16]. Precise information about the type of acylation agent is not available, and reactants such as succinic anhydride [15] and vinyl esters [16] were described. In the latter case, the reaction was considered [16] as irreversible since the reaction product vinyl alcohol rapidly tautomerizes into acetaldehyde. Despite commercial application of *R*,*S*-1-phenylethanol, no information about concentration profiles, values of kinetic constants, or reaction rates is readily available. The current contribution is aimed to fill this apparent void.

The main parameters in the current work were temperature, amount and type of acyl donor, reaction atmosphere, and the presence of water and other substrates, such as acetophenone. The kinetic model developed in this study was based not on transformed data, such as initial rates, but on primary data, i.e., concentration profiles. The kinetic parameters were determined for a series of experiments performed at different temperatures and concentrations of ethyl acetate.

EXPERIMENTAL

Kinetic resolution of (±)-1-phenylethanol (Acros Organics, Fairlawn, NJ; >98% GC) was performed in a glass reactor with the liquid phase volume of 50 mL. The initial concentration of the racemic substrate *R*,*S*-1-phenylethanol was 0.02 mol/L; thus the initial concentration of the respective enantiomers was 0.01 mol/L. Ethyl acetate (Sigma-Aldrich, Germany; >99.5% GC), vinyl acetate (Sigma-Aldrich; >99+%), 2-methoxy ethyl acetate (Sigma-Aldrich; 98%), and isopropenyl acetate (Sigma-Aldrich; >99%) were used as acyl donors. Toluene (J. T. Baker, The Netherlands; >99.5%, water content maximum 0.03%) or ethyl acetate (Sigma-Aldrich; >99.5%; hydranal-composite 2, water content <0.05%; Karl Fischer, Sigma-Aldrich) was used as a solvent; and a lipase (Novozym 435, *Candida antarctica* lipase immobilized on macroporous polyacrylate resin beads, bead size 0.3–0.9 mm, $S =$ 95.50 m^2, average pore diameter 17.9 μm, bulk density 430 kg/m^3, activity of 7000 PLU/g; Sigma-Aldrich) [17,18], was applied as an acylation catalyst. The stirring rate was 1000 rpm. The samples were taken after different reaction times and analyzed by a gas chromatograph equipped with a chiral column CP Chirasil Dex (250 μm × 0.250 μm × 25 m) and a flame ionization detector. The following temperature programme was used for analysis: 100°C (1 min)–0.30°C/min-130°C–15 °C/min–200°C (10 min). The temperature

of the injector and split ratio were 280°C and 100:1, respectively.

RESULTS AND DISCUSSION

Enantioselectivity

In all experiments conducted with (*R*,*S*)-1-phenylethanol, only (*R*)-1-phenylethanol was reactive independent of temperature, reaction atmosphere, or acyl donor type. Therefore within the precision of chiral GC analysis, it can be stated that (*S*)-1-phenylethanol was completely unreactive, leading to more than 99% enantioselectivity. As expected, no reaction was obtained starting from (*S*)-1-phenylethanol, in perfect agreement with the literature as it is generally known that the apparent rate constant in acylation of *S*-enantiomer is four to six orders of magnitude lower than that of *R*-enantiomer for conventional lipases [1b].

Effect of Reaction Temperature

The kinetic resolution of (*R*,*S*)-1-phenylethanol was performed under an argon atmosphere in a temperature range of 40–70°C in ethyl acetate as a solvent. The initial acylation rates of *R*-1-phenylethanol (no transformation of *S*-1-phenylethanol was observed) were calculated as

$$r_{\text{acylation}} = \frac{\dfrac{\Delta C_{\text{R-1-PE}}}{\Delta t}}{g_{\text{cat}}} \qquad (1)$$

$C_{\text{R-1-PE}}$, t, and g_{cat} are the concentration of *R*-1-phenylethanol, time, and catalyst amount, respectively. The initial acylation rates increased with increasing temperature (Table I, entries 1–4.). The initial acylation rate and the conversion after 1500 min increased, as expected, with increasing temperature. The apparent activation energy was determined by kinetic modeling (see the section Reaction Mechanism and Kinetics).

Effect of Reaction Atmosphere

The effect of hydrogen and argon was investigated to elucidate the effect of the reaction atmosphere on lipase activity in the acylation of *R*-1-phenylethanol. In one-pot synthesis of *R*-1-phenylethyl acetate, the reaction atmosphere is hydrogen and there is no knowledge of the effect of the reaction atmosphere on lipase. The results revealed the kinetic resolution of (*R*,*S*)-1-phenylethanol was faster in hydrogen than in argon (Fig. 2; Table I, entries 1, 2, and 4–6). The reason behind these results requires further investigation.

Effect of the Amount of Acyl Donor

The effect of the amount of acyl donor—ethyl acetate—was studied using one, two, or three equimolar amounts of ethyl acetate in the kinetic resolution of (*R*,*S*)-1-phenylethanol in toluene as a solvent (Table II). The initial rates and the conversions increased, as expected, with increasing amount of ethyl acetate, and the equilibrium was shifted toward the formation of the desired product. The initial reaction rate was, however, much slower with ethyl acetate in large excess (Table I, entry 4). The conversion of *R*-1-phenylethanol after 300 min in ethyl acetate was 23%, which was lower than with equimolar amounts or in a slight excess of acyl donor. This result can be explained by the fact that the enzyme conformation changes depending on liquid-phase composition, and usually lower activities were achieved in more polar solvents than in nonpolar solvents (see later).

Effect of Acyl Donor

The effect of the acyl donor was investigated at 70°C in toluene using three equimolar amounts of an acyl donor in the kinetic resolution of (*R*,*S*)-1-phenylethanol (Table III; Fig. 3). The following compounds were applied as acyl donors: ethyl acetate, vinyl acetate, 2-methoxy-ethyl acetate, and isopropenyl acetate. The initial acylation rates and conversions after 300 min are

Table I Kinetic Data from the Acylation of R-1-Phenylethanol over Lipase at Different Temperatures and under Hydrogen or Argon Atmosphere

Entry	Atmosphere	Temperature (°C)	Initial Acylation Rate (mmol/min/$g_{\text{cat.}}$)	Conversion after 1000 min (%)
1	Ar	40	0.002	31
2	Ar	55	0.0026	34
3	Ar	63	0.004	52
4	Ar	70	0.006	70
5	H_2	55	0.006	47
6	H_2	70	0.010	62

Ethyl acetate was used as a solvent and as an acyl donor.

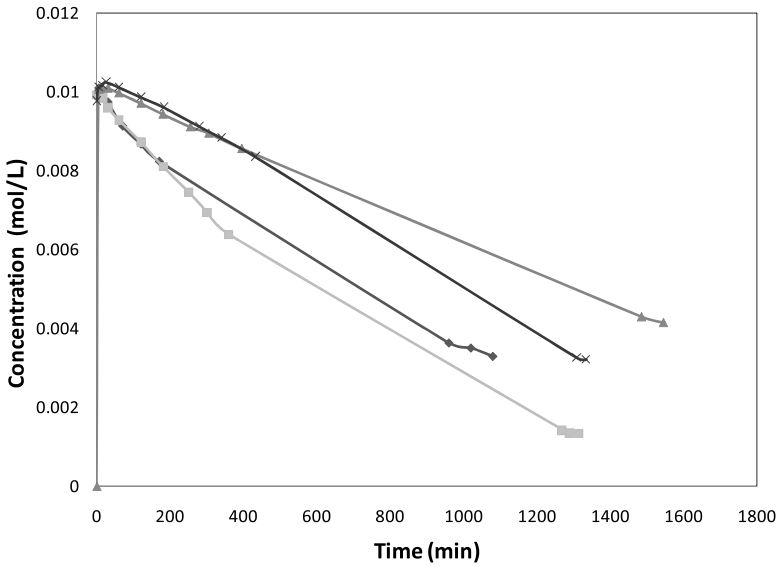

Figure 2 Concentration dependence of *R*-1-phenylethanol as a function of time in kinetic resolution of (*R*,*S*)-1-phenylethanol over lipase in ethyl acetate as an acyl donor. Symbols: experiments performed under (■) H_2 at 70°C, (♦) Ar at 70°C, (×) H_2 at 55°C, and (▲) Ar at 55°C.

Table II Kinetic Data from the Acylation of R-1-Phenylethanol over Lipase with Different Amounts of Acyl Donor, Ethyl Acetate, under Argon Atmosphere at 70°C

Entry	Solvent (Added Substrate)	Initial Acylation Rate (mmol/min/$g_{cat.}$)	Conversion after 300 min (%)
1	Toluene/3 equiv EtOAc	0.2	90
2	Toluene/2 equiv EtOAc	0.1	48
3	Toluene/1 equiv EtOAc	0.06	38

given in Table III. The following decreasing order of the initial rates and conversions was achieved for different acyl donors: isopropenyl acetate > vinyl acetate > ethyl acetate > 2-methoxy ethyl acetate. The acylation of *R*-1-phenylethanol exhibited the highest rate with isopropenyl acetate. Analogous results were achieved in the transesterification of 2-heptanol in chlorobenzene at 80°C, in which the conversion of the alkanol after 240 min was 35% and 18% with isopropenyl acetate and ethyl acetate, respectively [19]. The activated esters, i.e., those containing unsaturated groups or electronegative functional groups, are known to react faster than ethyl acetate as an acyl donor [3], and this was also the case in the current study, in which a 2.4 times higher initial acylation rate was achieved in toluene using 3 equiv of isopropenyl acetate (Table III, entry 2) compared to the case using 3 equiv of ethyl acetate in toluene (Table III, entry 4). The initial acylation rate in 2-methoxyethyl acetate was, as expected, lower than in ethyl acetate, since the methoxy group delocalizes the charge and thus 2-methoxy ethyl acetate releases an acyl group more slowly than ethyl acetate. An irreversible acylation occurred in the case of isopropenyl acetate and vinyl acetate [20,21] (Fig. 3). Unstable enols were formed and tautomerized rapidly to the corresponding ketone with unsaturated acyl donors. When vinyl acetate and isopropenyl acetate are used as acyl donors, either vinyl alcohol [2] or isopropenyl alcohol [4] is formed in the first step; thereafter these enols are converted via keto–enol tautomerization to corresponding ketones, i.e., acetaldehyde or acetone, respectively.

Table III Kinetic Parameters in the Acylation of R-1-Phenylethanol in Toluene over Lipase under Argon Atmosphere at 70°C Using Different Acyl Donors

Entry	Solvent (Added Substrate)	Initial Acylation Rate (mmol/min/$g_{cat.}$)	Conversion after 300 min (%)
1	Toluene/3 equiv vinyl acetate	0.32	97
2	Toluene/3 equiv Isopropenylacetate	0.48	100
3	Toluene/3 equiv 2-methoxyethyl acetate	0.08	70
4	Toluene/3 equiv ethyl acetate	0.2	90

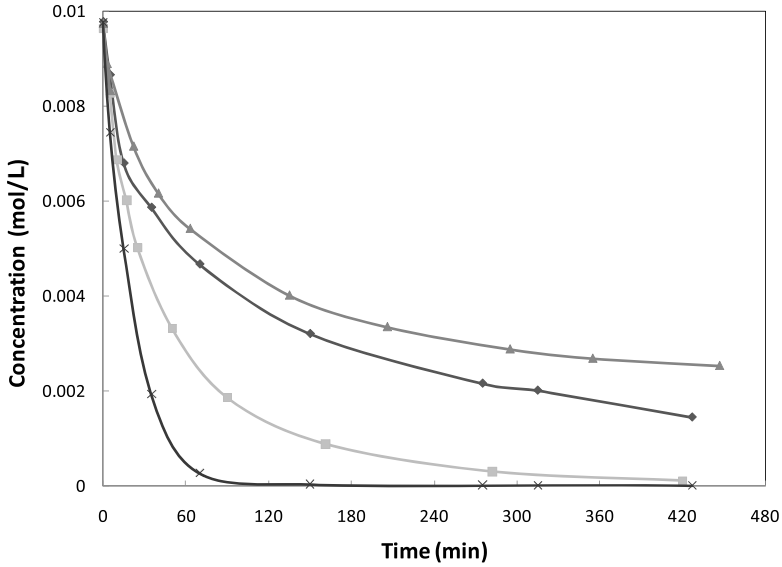

Figure 3 Concentration dependence of *R*-1-phenylethanol as a function of time in kinetic resolution of (*R*,*S*)-1-phenylethanol over lipase at 70°C under argon atmosphere. Symbols: (■) in toluene and 3 equiv of ethyl acetate, (▲) in toluene and 3 equiv of 2-methoxyethyl acetate, (♦) in toluene and 3 equiv of vinyl acetate, and (×) 3 equiv of isopropenyl acetate.

Effect of Additives

The acylation of (*R*,*S*)-1-phenylethanol in the presence of either water or acetophenone was investigated. The initial acylation rates with different reaction mixtures are given in Table IV. The following order of the decreasing initial rates was achieved: toluene (3 equiv ethyl acetate) > toluene (3 equiv ethyl acetate and acetotophenone) > toluene (3 equiv ethyl acetate and water). Typically, the effect of water on enzyme activity is given as a function of water activity (a_w) [22] and the reactants were preequilibrated to perform the investigations with known water amounts [23]. When a polar solvent would be used together with enzymes, the critical water content, i.e., with which an optimal enzyme activity can be achieved, is higher than in a nonpolar solvent due to the fact that water exhibits higher affinity to a polar solvent compared to a nonpolar one. Thus polar solvents can strip the essential water from the enzyme [24].

Another tool to investigate the hydrophobicity/hydrophilicity of organic solvents [25] and their effects on enzyme activities is the octanol/water partition coefficient; if its value, log *P*, is larger than four, biocatalysts are known to be active [26]. The influence of acetophenone is relevant, if one-pot synthesis of *R*-1-phenylethyl acetate would be studied. This order can be interpreted partially with solvent hydrophobicities, in which log *P*—partition coefficient in octanol/water—is one quantification tool. The log *P* values for different solvents are reported in Table V. When comparing the log *P* values with the initial

Table V Partition Coefficients for Different Solvents (Octanol/Water)

Solvent	Log *P*	Reference
Toluene	2.5	[1]
Acetophenone	1.14	[33]
Ethyl acetate	0.67	[34]
Ethanol	−0.31	[35]
Water	−1.15	[34]

Table IV Kinetic Results in the Acylation of R-1-Phenylethanol over Lipase Using Three Equivalents of Ethyl Acetate as an Acyl Donor and Adding Water and Acetophenone in the Reaction Mixture

Entry	Solvent (Added Substrate)	Initial Acylation Rate (mmol/min/$g_{cat.}$)	Conversion after 300 min (%)
1	Toluene/3 equiv EtOAc	0.2	90
2	Toluene/3 equiv EtOAc/AP	0.18	66
3	Toluene/3 equiv EtOAc/H_2O	0.0016	44

Reaction performed under argon atmosphere at 70 °C.
AP is acetophenone.

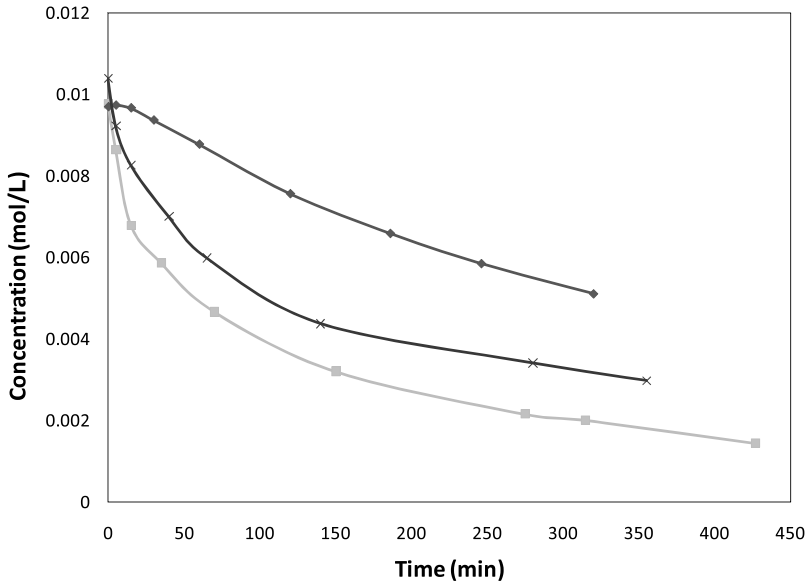

Figure 4 Concentration dependence of *R*-1-phenylethanol as a function of time in kinetic resolution of (*R*, *S*)-1-phenylethanol over lipase. Symbols: (■) in toluene and 3 equiv of ethyl acetate, (♦) in toluene and 3 equiv ethyl acetate, 0.5 g water added, and (×) in toluene and 3 equiv of ethyl acetate, 20 mg acetophenone added.

acylation rates, it turned out that the initial acylation rate decreased with increasing solvent polarity. One exception was found in this series, namely the log *P* value for acetophenone was higher than that of ethyl acetate and still an addition of acetophenone slightly decreased the initial acylation rate.

The conversions after prolonged reaction time followed the same order as the initial rates, and the kinetics is depicted in Fig. 4. The results thus showed that the presence of acetophenone slowed the rate of transesterification of *R*-1-phenylethanol.

REACTION MECHANISM AND KINETICS

The kinetics of the resolution of (*R*,*S*)-1-phenylethanol was investigated using lipase as a catalyst. Acyl-enzyme complexes are crucial intermediates in all lipase-catalyzed reactions [27]. From the mechanistic point of view, it has been suggested that the lipase first forms an acyl-enzyme complex with the acyl donor [28]. A sequential step mechanism was applied for enzymatic kinetic resolution of *R*-1-phenylethyl alcohol to *R*-phenylethyl acetate over immobilized lipase under Ar, using ethyl acetate as an acyl donor and solvent. The reaction sequence is depicted as follows:

$$\mathrm{E} + \mathrm{B} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{EQ}$$

$$\mathrm{EQ} + \mathrm{A} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} + \mathrm{I}$$

It is assumed that the acyl donor, ethyl acetate (B), binds first with the free enzyme (E) and forms a noncovalent enzyme-acyl complex, which releases a modified enzyme (EQ). The reactant, *R*-1-phenylethyl alcohol (A), combines with EQ, which subsequently relinquishes the *R*-phenylethyl acetate (P). Ethanol (I) was formed as a stoichiometric product.

The derivation of a two-step sequence with an irreversible second step is [29]

$$r = \frac{k_1 k_2 C_\mathrm{A} C_\mathrm{B}}{k_1 C_\mathrm{A} + k_2 C_\mathrm{B} + k_{-1}} \tag{2}$$

which after dividing the numerator and denominator by k_{-1} gives

$$r = \frac{(k_1/k_{-1}) k_2 C_\mathrm{A} C_\mathrm{B}}{k_1 C_\mathrm{A}/k_{-1} + k_2 C_\mathrm{B}/k_{-1} + 1} \tag{3}$$

The equation can be simplified for the low concentration domain of A; i.e., $k_1 C_\mathrm{A}/k_{-1} \ll k_2 C_\mathrm{B}/k_{-1} + 1$. The simplified model is written as follows:

$$r = \frac{k_2 K_1 C_\mathrm{A} C_\mathrm{B}}{\left(1 + \dfrac{k_2}{k_{-1}} C_\mathrm{B}\right)} \tag{4}$$

It should be noted that C_B was assumed to be constant in this series.

Acylation reactions were carried out at temperatures at 40, 55, and 70°C in an excess of acylation agent,

ethyl acetate; thus it can be assumed that $\frac{k_2}{k_{-1}} C_B \ll 1$, leading to

$$r = k' C_A \tag{5}$$

$$k' = \frac{k_1}{k_{-1}} k_2 \tag{6}$$

where r is the rate of reaction and C_A and k' are the concentration of R-1-phenylethyl alcohol and the appeared reaction rate constant, respectively.

The effect of the amount of acyl donor, ethyl acetate, was investigated in another series at 70°C. The experimental set was conducted using 0.5, 1, and 3 equiv molar amounts of ethyl acetate and modeled with the following equation:

$$r = k_2 K_1 C_A C_B = k'' C_A C_B \tag{7}$$

which is obtained from Eq. (3) when $1 \gg (k_1/k_{-1})C_A + (k_2/k_{-1})C_B$. It should be noted that since the rate constants of enzymatic reactions are significantly influenced by the reaction environment (solvent) the apparent rate constants $k'$and k'' are solvent dependent and thus are not equal to each other.

Since deactivation was observed in the acylation step and it is known from the literature [30] that lipase can be deactivated in the presence of ethanol, it was assumed that formed ethanol can act as a competitive inhibitor, leading to a conventional description of competitive inhibition

$$r = \frac{k'' C_A C_B}{(1 + K_I C_I)} \tag{8}$$

where K_I is the binding constant of ethanol and C_I is the concentration of the formed ethanol. In a special case of strong inhibition, $1 \ll K_I C_I$, Eq. (8) can be rewritten as

$$r = \frac{k'' C_A C_B}{K_I C_I} = k''' \frac{C_A C_B}{C_I} \tag{9}$$

where $k''' = k''/K_I$.

REACTOR AND PARAMETER ESTIMATION

The mass balances for a liquid-phase component (i) in a batch reactor were written as

$$\frac{dc_i}{dt} = r_i \tag{10}$$

The temperature dependence of the lumped kinetic constant k' is evaluated by the Arrhenius equation.

$$k'_i = k^0 \times e^{-\frac{E_{ai}}{R}\left(\frac{1}{T} - \frac{1}{T_{mean}}\right)} \tag{11}$$

The activation energy and the rate constants were determined by estimation of the parameters via minimization of the residual sum of the squares

$$Q = \sum_t \sum_i (c_{i,t,exp} - c_{i,t,\,mod\,el})^2 w_{i,t} \tag{12}$$

where $c_{i,t,exp}$, $c_{i,t,model}$, and w are the experimentally recorded concentrations, the concentrations predicted by the model, and the weight factors, respectively. The weight factor w was set to 1 for all experimental points. The software Modest was used for minimizing the objective function and the rate constants were estimated with the simplex method and then switched to Levenberg–Marquardt method [31]. The ordinary differential equations (ODE) describe the reactor model by the backward difference method. The equations for the reaction kinetic model are ODEs and were integrated starting from the initial conditions.

MODELING RESULTS

The fit of the model depicted by Eq. (6) to the experimental data performed under different reaction temperatures is presented in Fig. 5. The degree of explanation for this model was 99.21%. The estimated rate constant (k') and the activation energy (E_a) are listed in Table VI. The experimental data were very well described with the model according to Eq. (6) as can be seen from Fig. 5.

Equation (7) describes the effect of using different amounts of acyl donor, 0.5, 1, and 3 equiv molar amounts of ethyl acetate. The comparison of the estimated and measured data using different amounts of acyl donor demonstrated poor description (not shown). The reason for this is that lipase was deactivated in the presence of ethanol. An adequate fit of the model was

Table VI Estimated Parameters for Different Temperatures

Parameter	Value	Error (%)
k'	1.322×10^{-4} dm^3/(g min)	1.8
E_a	24.5 kJ/mol	5.0

The degree of explanation for the experiments was 99.21%.
$T_{mean} = 55$°C.

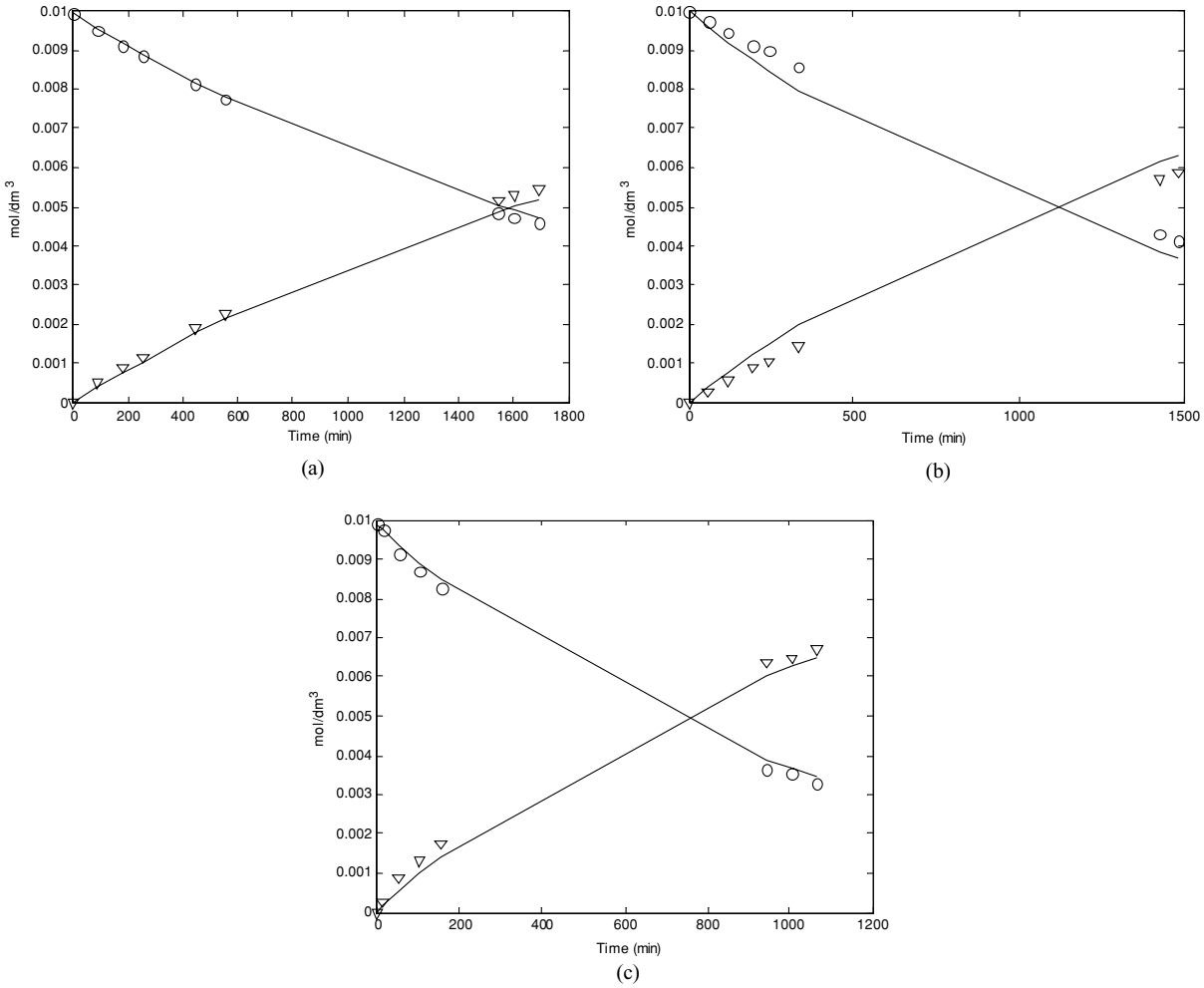

Figure 5 Comparison of the estimated and measured kinetic data. (a) 40°C, (b) 55°C, (c) 70°C; (○) *R*-1-phenylethyl alcohol; (∇) *R*-1-phenylethyl acetate. Mass of enzyme 62.5 mg.

achieved by introducing the competitive inhibition of ethanol into the model (Eq. (8)). It turned out, however, that parameters k'' and K_I were highly correlating; thus Eq. (9) is able to describe the experimental data reasonably well. The estimated kinetic parameter k''' for the experiments performed with 0.5, 1, and 3 equiv molar amounts of ethyl acetate is 1.642×10^{-3} dm^3/min g with a standard error of 7.5%. The residual sum of squares was 0.2717×10^{-4}; the degree of explanation was 90.25%, and the model fits the experimental data rather well as demonstrated in Fig. 6.

The correlation matrix of estimated kinetic parameters is presented in Table VII for experiments carried out under different temperatures. The correlation matrix describes the correlation among kinetic parameters. The diagonal elements of the correlation matrix are always equal to 1.000 since they show the correlation of a column with itself. Correlation on a scale with 1.000 indicates a perfect positive correlation; 0.000 means that there is no correlation at all, and −1.000 stands for a perfect negative correlation. The correlation matrix of experiments performed under different temperatures (Table VII) shows that there is a very low positive correlation between parameters E_a and k'.

The estimated kinetic constants were identified by parameter sensitivity analysis plots using the Markov chain Monte Carlo (MCMC) method (Fig. 7). The distribution of the parameters showed how well the parameters were identified [30]. The prediction distributions

Table VII Correlation Matrix of the Parameters for Different Temperatures

	k'	E_a
k'	1.000	
E_a	0.048	1.000

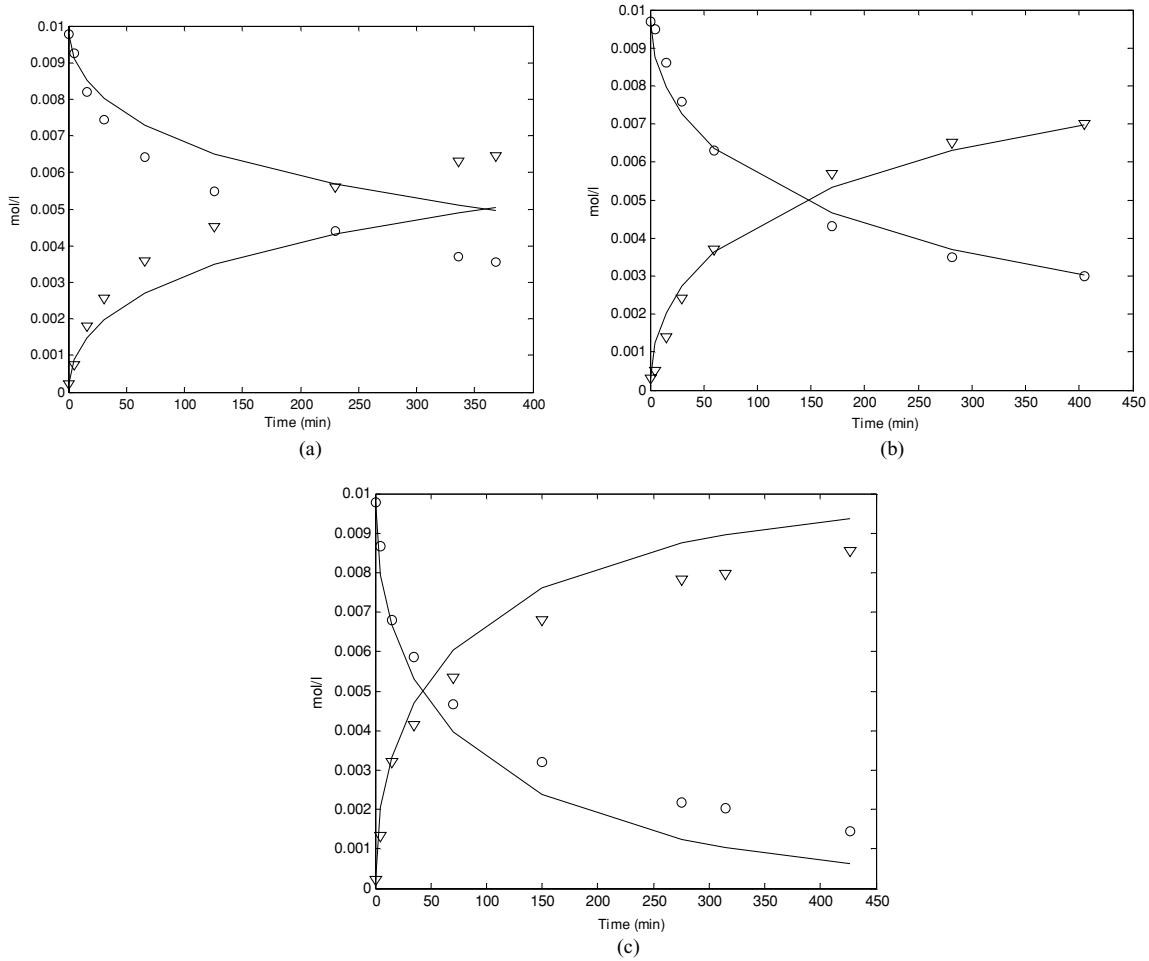

Figure 6 Comparison of the estimated and measured kinetic data using different amounts of acyl donor in one-pot synthesis of *R*-1-phenylethyl acetate. (a) 0.5 equiv of ethyl acetate at 70°C, (b) 1 equiv of ethyl acetate at 70°C, (c) 3 equiv of ethyl acetate at 70°C; (○) *R*-1-phenylethyl alcohol; (▽) *R*-1-phenylethyl acetate. Mass of enzyme 25 mg.

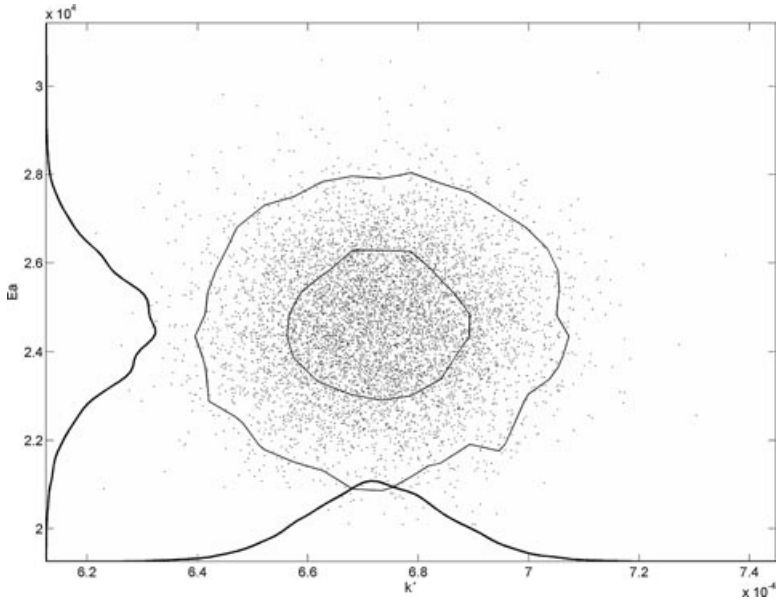

Figure 7 Parameter sensitivity analysis plots for experiments performed under different temperatures (2D and 1D marginal posterior distribution plots E_a and k' parameters).

revealed to which extent the parameter uncertainty is relevant with respect to the model predictions. The estimation of the model parameters was performed according to the Bayesian paradigm. All of the modeling parameters were treated as statistical distributions to obtain the optimum range of the estimated values. MCMC plots for experiments performed under different temperatures given in Fig. 7 reveal that the optimum values of kinetic parameters, E_a and k', were identified.

CONCLUSIONS

The kinetics of the acylation of (*R*,*S*)-1-phenylethanol was investigated using lipase as a catalyst.

The main parameters were temperature, reaction atmosphere, different acyl donors, and different amounts of acyl donor. Only one enantiomer was reactive, leading thus to more than 99% enantioselectivity. The initial reaction rate increased with increasing temperature, and the apparent activation energy was determined to be 24.5 kJ/mol. In hydrogen atmosphere, the acylation was faster than under argon.

When using different amounts of acyl donor, ethyl acetate, the initial reaction rates decreased, as expected, with increasing amount of acyl donor. At the same time, the conversion levels of (*R*,*S*)-1-phenylethanol increased.

The highest acylation rates were achieved with isopropenyl acetate followed by vinyl acetate and ethyl acetate, whereas very low rates were obtained with 2-methoxyethyl acetate. The conversion levels followed the same order.

Acetophenone and water as additives in the acylation of (*R*,*S*)-1-phenylethanol caused a decline in the reaction rates. The excess of hydrophilic water is known to retard the lipase activity, whereas the retarding effect of acetophenone on the acylation was not expected based on the octanol/water partition coefficient of acetophenone being higher than that for ethyl acetate. Acetophenone can, however, due to its structure interact with lipase, thus retarding the catalytic activity of lipase.

A kinetic model was developed based on a sequential step mechanism, in which enzyme was reacting in the first step with an acyl donor followed by the reaction of a modified enzyme complex with the reactant, *R*-1-phenylethanol, giving *R*-1-phenylethyl acetate, ethanol, and the free enzyme. Comparison of the model with the experimental data obtained at different temperatures allowed simplifications of this model, leading to a kinetic equation with just one apparent parameter. The influence of the amount of acyl donor, ethyl acetate, could be quantitatively described, taking into account competitive inhibition by the ethanol product. The kinetic model described the experimental data well.

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