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# Enhancement of enantioselectivity and reaction rate on the synthesis of (*S*)-ketoprofen hydroxyalkyl ester in organic solvents via isopropanol-dried immobilized lipase

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**Abstract: Lipase-catalyzed enantioselective esterification between (*R*,*S*)-ketoprofen and alkanediol in organic solvents was developed to produce (*S*)-ketoprofen hydroxyalkyl esters. The acyl acceptor of 1,6-hexanediol for the resolution of (*R*,*S*)-ketoprofen yielded only the enantioselectivity (the enantiomeric ratio of initial rate for (*S*)-ketoprofen to that of (*R*)-ketoprofen) $V_S/V_R = 8$, when crude Lipase MY originating from *Candida rugosa* was used. However, isopropanol-dried immobilized lipases (IPA-dried IM-lipase) effectively enhanced the enantioselectivity to greater than 20 in the esterification of (*R*,*S*)-ketoprofen when 1,4-butanediol, 1,5-pentanediol or 1,6-hexanediol was employed. IPA-dried IM-lipase and isooctane were selected to use for optimally immobilized lipase and reaction medium, respectively. The IPA-dried IM-lipase exhibited the highest enantioselectivity, $E = 26.7$, to the (*S*)-enantiomer with 1,5-pentanediol and the best enzyme activity to the (*S*)-enantiomer with 1,4-butanediol. The finding indicates that the carbon chain length of the alkanediol strongly affected the enzyme activity and enantioselectivity of lipase-catalyzed esterification. A maximum enantioselectivity of 37 at 27 °C was generated by IPA-dried IM-lipase for the enantioselective esterification of racemic ketoprofen with 1,4-butanediol. IPA-dried IM-lipase can effectively increase the enantioselectivity of lipase.**


**Keywords:** enantioselectivity; esterification; immobilized lipase; ketoprofen; prodrug

## INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a diverse group of drugs which exhibit pharmacological activity primarily toward the (*S*)-enantiomer; this group of drugs is used extensively for treating rheumatic diseases and related painful conditions.[1] 2-(4-Isobutylphenyl)propionic acid (ibuprofen), 2-(6-methoxy-2-naphthyl)propionic acid (naproxen) and 2-(3-benzoylphenyl)propionic acid (ketoprofen) are the best known. Ketoprofen and naproxen are administered through the oral or suppository routes for the treatment of pain and inflammation. When such drugs are given orally, gastrointestinal (GI) side effects, such as ulceration and hemorrhage, are the most frequently observed adverse reactions.[2] A series of acyloxyalkyl esters of ketoprofen and naproxen has been chemically synthesized and studied.[3,4] Hence, the topical administration of NSAIDs, like ketoprofen and naproxen, has been studied in order to minimize GI side-effects and other possible systemic side-effects, because the plasma peak level is high after oral administration.[5–7] The delivery route can increase the local drug concentration in the treatment of local inflammatory and pain processes.[8] The hydroxylalkyl esters of ketoprofen may have been the intermediates of acyloxyalkyl prodrugs (Scheme 1). The prodrugs of ketoprofen with the highest aqueous solubility were the most effective in delivering ketoprofen through the skin.[3]

Enzymatic resolution of racemic compounds continues to be a valuable means of obtaining optically-active pharmaceutical, agricultural and special chemicals.[9,10] The trend towards using an enzyme is especially strong in synthesizing new pharmaceuticals since almost all major pharmaceutical companies have elected to synthesize optically-pure chiral drugs.[11] *Candida rugosa* lipase is one of the most versatile and widely used enzymes in resolving chiral esters, acids, and alcohols in both aqueous and organic media. The lipases are used for the enantioselective esterification of many racemic compounds, enabling the kinetic resolution of pure enantiomers which serve as precursors for fine chemicals including pharmaceuticals and agrochemicals.[12] Changes in the $\alpha$-substituted aromatic groups at the 2-position

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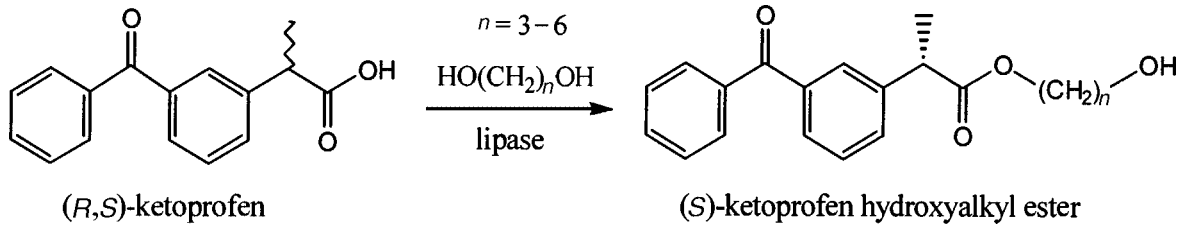


**Scheme 1.**

of propionic acids did not significantly affect the enantiospecific preference of *C rugosa* lipase. Generally, this lipase exhibited a high enantioselectivity of over around 40 toward ibuprofen, suprofen and naproxen, whereas it exhibited a low enantioselectivity of about 10 towards ketoprofen and flurbiprofen.[13–17]

The immobilization of lipase secured the reusability and minimized the cost of isolating the product. *C rugosa* lipases immobilized on polypropylene powders by adsorption frequently show good activity in organic media.[18,19] In the authors' previous study, a lipase-catalyzed enantioselective esterification process in organic solvents was developed for the synthesis of (*S*)-naproxen hydroxyalkyl ester,[20] the enantioselectivity of immobilized lipase associated with the isopropanol dehydration process can be effectively enhanced for the synthesis of (*S*)-naproxen morpholinoalkyl ester prodrugs in organic media.[21] In this work, the methodology is extended to produce the desired intermediates between racemic ketoprofen and the alcohol that contains a hydroxyalkyl group, by lipase-catalyzed esterification in organic solvents. Accordingly, the effect of the change in the straight alkyl chain length of the alkanediol on the enantioselectivity and the activity of the different means of preparation of immobilized enzyme are investigated.

## MATERIALS AND METHODS

### Materials

Racemic (*R*,*S*)-ketoprofen was purchased from Sigma. *C rugosa* lipase (triacylglycerol ester hydrolases, EC 3.1.1.3) from *C rugosa* (type VII, 835 μmol $h^{-1}$ $mg^{-1}$ for the hydrolysis of olive oil at pH 7.2 and 37 °C) was purchased from Sigma. Lipase MY (triacylglycerol ester hydrolases, EC 3.1.1.3) from *C rugosa* (30 U $mg^{-1}$ solid) was provided by Meito Sangyo. Lipase PS from *Burkholderia cepacia*, Lipase AK from *Pseudomonas fluorescens* and Lipase A from *Aspergillus niger* were obtained from Amano. Isooctane, cyclohexane, hexane and isopropanol of HPLC grade were dried over a molecular sieve and used without further purification. Other chemicals of analytical grade were commercially available.

### Analytical procedures

HPLC was used to monitor the esterification of racemic ketoprofen with various alcohols. *n*-Hexane/2-propanol/trifluoroacetic acid (90:10:0.05) by volume was used at 1 $cm^3$ $min^{-1}$ with detection at 270 nm and at a column temperature of 13 °C. Chiral columns (Chiralcel OD-H and Chiralcel OJ, Daicel Chemical, Japan) capable of separating the internal standard of phenylethanol, (*R*)- and (*S*)-ketoprofen, and their hydroxyalkyl prodrugs with the retention times listed in Table 1, were employed under the same conditions of mobile phase.

**Table 1.** Retention times (RT) of 2-phenylethanol, (*R*)-ketoprofen, (*S*)-ketoprofen, and their ester prodrugs

| Compound | RT (min) |
|---|---|
| 2-Phenylethanol[a] | 6.46 |
| 2-Phenylethanol[b] | 7.21 |
| (*R*,*S*)-Ketoprofen[a] | 7.38 |
| (*R*)-Ketoprofen[b] | 17.23 |
| (*S*)-Ketoprofen[b] | 25.78 |
| (*R*)-Ketoprofen 1,3-propanediol ester[a] | 19.95 |
| (*S*)-Ketoprofen 1,3-propanediol ester[a] | 18.57 |
| (*R*)-Ketoprofen 1,4-butanediol ester[a] | 19.31 |
| (*S*)-Ketoprofen 1,4-butanediol ester[a] | 17.55 |
| (*R*)-Ketoprofen 1,5-pentanediol ester[b] | 33.61 |
| (*S*)-Ketoprofen 1,5-pentanediol ester[b] | 31.57 |
| (*R*)-Ketoprofen 1,6-hexanediol ester[b] | 33.27 |
| (*S*)-Ketoprofen 1,6-hexanediol ester[b] | 30.45 |

[a]: Dacicel OD-H.
[b]: Dacicel OJ column.

### Lipase immobilization with different dry methods

The immobilized lipase (IM-lipase) was prepared by adsorbing *C rugosa* lipase on the adsorbent Accurel MP 1000, which had a particle size distribution between 355 and 425 mm. Briefly, dissolving 1200 mg of the crude lipase in 30 $cm^3$ phosphate buffer, pH 7, centrifuging at 4000 rpm for 15 min and removing the precipitate produced 25 $cm^3$ of clear enzyme solution. The solution was brought into contact with 1000 mg of the support that had been pre-wetted with 99.5% ethanol and pre-washed with 50% ethanol–water solution and pure deionized water, in succession. The lipase-support system was shaken at 4 °C for 6 h. Then, the support particles were separated from the solution by filtration. There are two different methods for drying the immobilized lipase. The first method is to lyophilize IM-lipase for 12 h and store at 4 °C. The second method is use to isopropanol, 20 $cm^3$ $g^{-1}$, to rinse and decant six times, and then to rinse IPA-dried IM-lipase with reaction solvent and decant six times.[21] The equivalent enzyme adsorbed on the support was determined after assaying the residual absorption intensity of the filtrate by employing the

Bio-Rad Protein Assay method with bovine serum albumin as the standard.

### Lipase, solvent and alcohol screening in esterification

Unless specified, 75 mg crude lipase was added to 15 $cm^3$ solvent containing 0.2 mM racemic ketoprofen and 5 mM alkanediol at 37 °C. 1,4-Butanediol was adsorbed as described by Castillo *et al*: 0.81 g of 1,4-butanediol and 1 g silica gel were carefully mixed until a homogeneous powder was obtained.[22] The resultant mixture was stirred with a magnetic stirrer, and samples were removed for HPLC analysis at different time intervals.

## RESULTS AND DISCUSSION

### Lipase screening

Table 2 presents the effect of lipases from different sources on the enantioselective esterification between racemic ketoprofen and 1,4-butanediol in isooctane. In lipase screening, lipases from *C rugosa* (Lipase MY and *C rugosa* lipase) and *P fluorescens* (Lipase AK) preferentially catalyzed the (*S*)-enantiomer. However, lipases from *B cepacia* (Lipase PS) and *As niger* (Lipase A) exhibited (*R*)-stereochemical preference. The desired product is (*S*)-ketoprofen hydroxyalkyl ester so the enzyme activity and enantioselectivity of Lipase MY all slightly exceeded that of *C rugosa* lipase.

**Table 2.** Screening lipase on enzyme chirality, $X_t$, $V_S/V_R$ and $ee_p$ from different sources in isooctane at 24h[c]

| Lipase source | $X_t$ (%)[a] | $V_S$ (mM $h^{-1}$ $mg^{-1}$)[b] | $ee_p$ (%)[c] | $V_S/V_R$[d] | Chirality |
|---|---|---|---|---|---|
| *C rugosa* lipase | 46.15 | $5.00 \times 10^{-5}$ | 70.3 | 5.0 | *S* |
| Lipase MY | 49.32 | $5.02 \times 10^{-5}$ | 73.2 | 5.6 | *S* |
| Lipase A | 9.13 | $6.00 \times 10^{-7}$ | 87.3 | 7.9* | *R* |
| Lipase AK | 3.54 | $1.30 \times 10^{-6}$ | 16.4 | 1.3 | *S* |
| Lipase PS | 1.02 | $1.00 \times 10^{-7}$ | 66.9 | 5.7* | *R* |

Reaction conditions: (*R*,*S*)-ketoprofen = 0.2 mM, 1,4-butanediol = 5 mM, Lipase MY = 5 mg $cm^{-3}$ and 37 °C.
[a] $X_t$: the conversion of (*R*,*S*)-ketoprofen at reaction time 24 h, $X_t = X_R + X_S$, where $X_R$ and $X_S$ are the conversion of (*R*)-naproxen morpholinoalkyl esters and (*S*)-naproxen morpholinoalkyl esters, respectively.
[b] $V_S$: the initial rate of (*S*)-ketoprofen.
[c] $ee_p$: the product enantiomeric excess, $ee_p = (X_S - X_R)/(X_S + X_R)$.
[d] $V_S/V_R$: the enantiomeric ratio of initial rate for (*S*)-ketoprofen to that of (*R*)-ketoprofen.

Lipase MY exhibited the highest enzyme activity, but the enantioselectivity ($V_S/V_R$, the enantiomeric ratio of initial rate for (*S*)-ketoprofen to that of (*R*)-ketoprofen) reached only 5.6. Lipase MY was selected as the best enzyme in the following experiments, after further investigating both the enzyme enantioselectivity for the (*S*)-enantiomer and the enzyme activity.

### Alcohol screening in esterification for comparison of isooctane and cyclohexane as reaction media

Several investigations have shown that isooctane and cyclohexane used as reaction media exhibited good enantioselectivity in lipase-catalyzed enantioselective esterification. However, the lipase can sustain higher enzyme activity in hydrophobic isooctane ($\log P = 4.5$) than in the less hydrophobic cyclohexane ($\log P = 3.2$) where log $P$ is defined as the logarithm of the partition coefficient of a given component in the octanol–water two-phase system).[20,21] Table 3 reveals that the alkanediol straight carbon chain length markedly affects the rate of enantioselective esterification when Lipase MY is used as a biocatalyst in isooctane and cyclohexane, respectively. As described above, the initial rate of the esterification of the (*S*)-enantiomer in isooctane still exceeds that in cyclohexane when different acyl acceptors (alcohol) are used. The lipase-catalyzed esterification rate of racemic ketoprofen varied with the different straight carbon chain length of acyl acceptors and the initial rate of the esterification of (*S*)-ketoprofen was maximum when 1,4-butanediol and 1,3-propanediol were employed in isooctane and cyclohexane, respectively. Similar results have already been exploited when *C rugosa* lipase was used as the biocatalyst in the enantioselective esterification of (*R*,*S*)-naproxen.[20] The results suggest that the mechanism of lipase-catalyzed esterification is as follows; an acyl donor (acid) attacks lipase to form an acyl–enzyme intermediate, and then an acyl acceptor (alcohol) reacts with the acyl enzyme intermediate to produce an ester. Both the acylation and the deacylation of the enzyme may contribute to the enantioselectivity of the lipase. If the results obtained herein are explained according to the above mechanism, then the reaction rate and enantioselectivity must be determined at the deacylation rate of the acyl–enzyme intermediate by the alcohol, to form

**Table 3.** Effect of alcohol on $V_S$, $X_t$, $ee_p$, and $V_S/V_R$ in esterification at 24h

| | Isooctane | | | | Cyclohexane | | | |
|---|---|---|---|---|---|---|---|---|
| Alcohol | $V_S$[a] | $X_t$ (%) | $ee_p$(%) | $V_S/V_R$ | $V_S$[a] | $X_t$ (%) | $ee_p$(%) | $V_S/V_R$ |
| 1,3-Propanediol | 4.10 | 36.15 | 53.0 | 5.2 | 2.30 | 27.83 | 48.8 | 6.1 |
| 1,4-Butanediol | 5.10 | 65.47 | 33.5 | 4.2 | 1.62 | 30.00 | 36.6 | 2.8 |
| 1,5-Pentanediol | 2.97 | 39.80 | 57.4 | 5.0 | 0.80 | 12.36 | 25.1 | 2.7 |
| 1,6-Hexanediol | 2.38 | 28.45 | 72.0 | 8.1 | 0.72 | 8.37 | 65.3 | 5.7 |

Reaction conditions: (*R*,*S*)-ketoprofen = 0.2 mM, alcohol = 1 mM, Lipase MY = 5 mg $cm^{-3}$ and 37 °C.
[a] ($\times 10^{-5}$ mM $h^{-1}$ $mg^{-1}$).

the second substrate.[23,24] When the straight carbon chain length of alkanediol exceeds 4, the reaction rate of esterification decreases as the carbon chain length of alkanediol increases. The chain length of the acyl acceptor was found to significantly affect the reaction rate of esterification, perhaps because a longer carbon chain length of alkanediol caused more steric hindrance and an adequate carbon chain length of alcohol was from three to four, matching the hydrophobic acyl-binding tunnel of the active site of lipase. The long carbon chain of alkanediol acted as a competitive substrate inhibitor of lipases in acyl transfer.[25] However, the crude Lipase MY exhibits poor enantioselectivity, with an enantioselectivity of under 10, for all alkanediols in Table 3. Similar results have been reported in relation to the enantioselective esterification of racemic ketoprofen with various alkanediols in organic solvents; these results have indicated that the enantioselectivity of lipases is low.[16,26,27]

## Effect of type of preparation of immobilized lipase (IM-lipase) on enantioselectivity and activity

The poor enantioselectivity of crude Lipase MY for the synthesis of (*S*)-naproxen hydroxyalkyl ester was improved in a previous work in which immobilized lipase was dehydrated by treatment with isopropanol.[20] Figure 1 compares the different types of preparations of immobilization lipase in terms of the specific initial rate of (*S*)-ketoprofen ($V_S$) (Fig 1(A)) and the enantioselectivity ($V_S/V_R$) (Fig 1(B)) associated with the different carbon chain lengths of alkanediol in isooctane. Comparison of crude Lipase MY and IPA-dried IM-lipase, both of the specific initial rate of (*S*)-ketoprofen ($V_S$) and the enantioselectivity ($V_S/V_R$) were increased five-fold using IPA-dried IM-lipase when 1,4-butanediol was used as the acyl acceptor for the enantioselective esterification of racemic ketoprofen. Immobilized lipase was dried by lyophilization, reducing the initial rate ($V_S$) of (*S*)-ketoprofen and the enantioselectivity ($V_S/V_R$) of crude Lipase MY. On the contrary, IPA-dried IM-lipase largely increased the enantioselectivity and enzyme activity. The enantioselectivity of IPA-dried IM-lipase exceeded 20 when 1,4-butanediol, 1,5-pentanediol and 1,6-hexanediol were used as acyl acceptors. Improving the enantioselectivity by IPA-dried IM-lipase has satisfied one of the practical criteria for dynamic kinetic resolution—that the enantioselectivity must exceed ~20.[28] A comparison of lyophilized IM-lipase and IPA-dried IM-lipase indicated that the methods of water removal differed greatly. The first is lyophilization, and the second is the use of isopropanol to strip water, followed by the replacement of the isopropanol with hydrophobic isooctane.

The lyophilization of immobilized lipase led to an undesirable change in the conformation of the active site. The IPA-dried IM-lipase did not easily change the conformation of the active site because its last step replaced isopropanol with hydrophobic solvent, providing the more strict surroundings of the immobilized lipase. Therefore, IPA-drying can be reasonably concluded to increase the enzyme activity and improve its enantioselectivity because isopropanol dehydration leaves the enzyme conformation close to its active form; the following replacement of hydrophobic solvent sustains the active conformation of immobilized lipase.

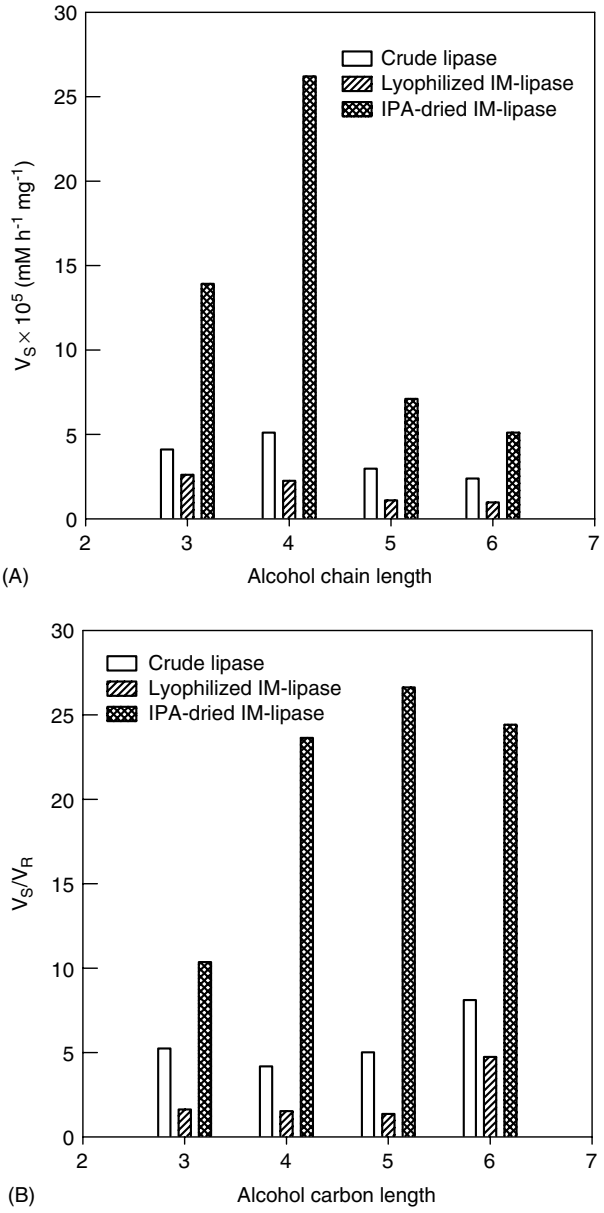


**Figure 1.** Effect of type of preparation of lipase on (A) the initial rate of the hydroxyalkyl ester prodrugs of (*S*)-ketoprofen and (B) the enantioselectivity for the esterification of racemic ketoprofen with hydroxyalkyl alcohol in isooctane. Conditions: (*R*, *S*)-ketoprofen = 0.2 mM, 37 °C, equivalent to 5 mg cm$^{-3}$ of free lipase MY, hydroxyalkyl alcohol = 1 mM.

## Effect of the use of silica on 1,4-butanediol in the lipase-catalyzed enantioselective esterification of (*R*,*S*)-ketoprofen for different preparations of lipase

Figure 2 plots the variation in the specific initial rate of (*S*)-ketoprofen ($V_S$) (Fig 2(A)) and the

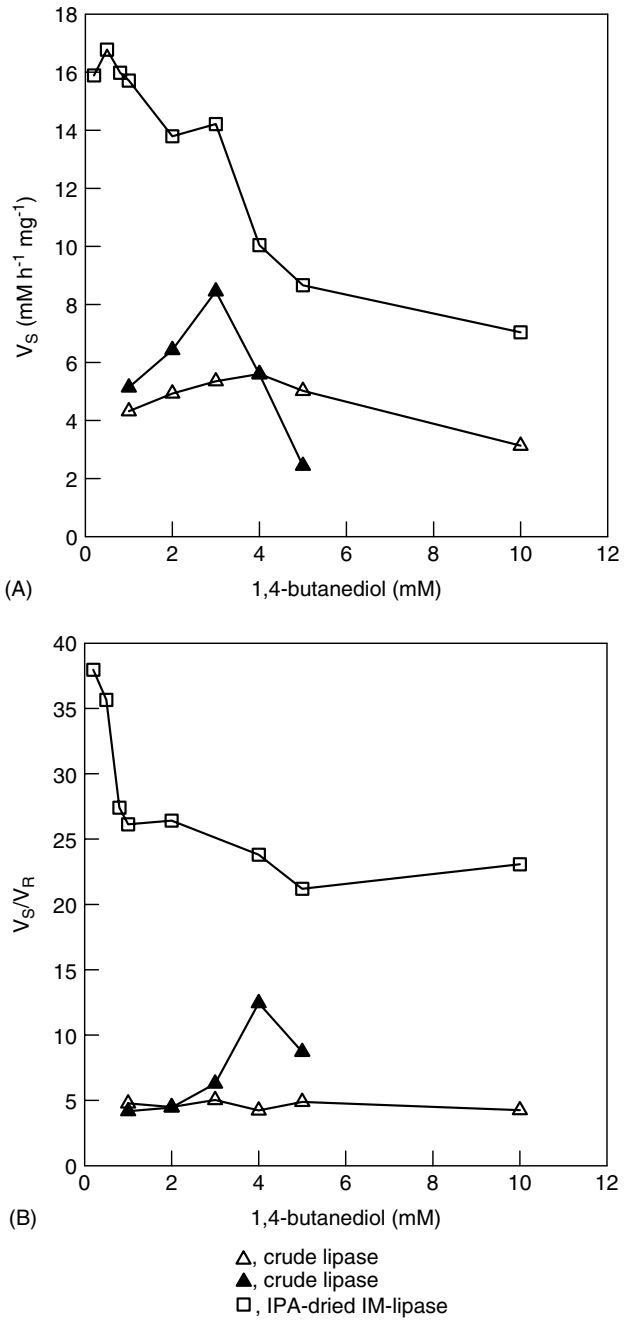


**Figure 2.** Effect of alcohol concentration on (A) the initial rate $V_S$ and (B) the enantioselectivity ($V_S/V_R$) of the hydroxybutyl ester prodrugs of racemic ketoprofen in isooctane for different types of preparation of lipase. For free 1,4-butanediol (filled), silica gel-adsorbed 1,4-butanediol (empty); Conditions: (*R*, *S*)-ketoprofen = 0.2 mM, 37 °C, equivalent to 5 mg cm$^{-3}$ of crude Lipase MY.

enantioselectivity ($V_S/V_R$) (Fig 2(B)) to compare various ways of preparing lipase to catalyze the esterification between (*R*,*S*)-ketoprofen and 1,4-butanediol in isooctane, respectively. 1,4-Butanediol is a polar alcohol, and is associated with two problems related to the lipase-catalyzed synthesis of (*S*)-ketoprofen hydroxybutyl ester in organic media. The first is the limited solubility of the polar substrate in hydrophobic solvent, in which the saturated concentration of 1,4-butanediol in isooctane is only about 5 mM at 37 °C The second is the aggregation of crude lipase caused by the high concentration of 1,4-butanediol in hydrophobic isooctane. Figure 2(A) reveals that the 1,4-butanediol continued to act as an enzyme inhibitor and the maximum concentration of free 1,4-butanediol was about 3 mM. The sharp fall in the specific initial rate ($V_S$) partially resulted from the aggregation of crude Lipase MY when the concentration of 1,4-butanediol approached saturation. 1,4-Butanediol adsorbed onto silica gel at 5 mM was used to elucidate the improvement in the inhibition of the enzyme by alkanediol and the overcoming of the limitation on the solubility of polar 1,4-butanediol. Meanwhile, IPA-dried IM-lipase was used further to solve the problem of the aggregation of crude lipase. The specific initial rate ($V_S$) of IPA-dried IM-lipase with silica gel-adsorbed 1,4-butanediol clearly exceeded that of crude Lipase MY at an equivalent enzyme concentration, and the maximum was shifted to around 0.5 mM.

Figure 2(B) shows that stable enantioselectivity was exhibited at concentrations of 1,4-butanediol adsorbed onto silica gel from 1 to 10 mM. The silica gel in esterification acts as a 'reservoir' for 1,4-butanediol, preventing the aggregation of crude Lipase MY, and thereby providing a stable reaction environment by the constant release of 1,4-butanediol to the reaction media during the reaction. When silica gel-adsorbed 1,4-butanediol was combined with IPA-dried IM-lipase, the enantioselectivity of IPA-dried IM-lipase exceeded 20, even when the concentration of 1,4-butanediol exceeded the saturated concentration of 5 mM in isooctane. Such combination can eliminate the limit on the solubility of 1,4-butanediol in isooctane and the aggregation of crude lipase powder in polar alkanediol at a high concentration.

Figure 3 compares free 1,4-butanediol with 1,4-butanediol adsorbed onto silica gel for the time course of the conversion of (*R*,*S*)-ketoprofen at reaction time 24 h ($X_t$) and the product enantiomeric excess ($ee_p$). The equilibrium of conversion decreased largely as the concentration of free 1,4-butanediol increased. The equilibrium of conversion exceeded 0.6 because the enantioselectivity of crude Lipase MY was poor. The increase in the concentration of free 1,4-butanediol greatly facilitates the aggregation of crude Lipase MY powder, severely reducing the reactive areas of crude lipase, causing the substrate (or the product) to become resistant to diffusion and generating a local highly polar environment around the lipase. The local polar environment was unfavorable to the progress of esterification, resulting in a decline in the equilibrium of conversion. Using the silica gel-adsorbed 1,4-butanediol, clearly improved the $X_t$ and $ee_p$. The shortcoming of the aggregation of crude lipase resulting from the high concentration of polar alkanediol was eliminated by the 1,4-butanediol adsorbed onto silica gel in the reservoir.

Figure 4 shows the progress of the lipase-catalyzed enantioselective reaction to compare the IPA-dried

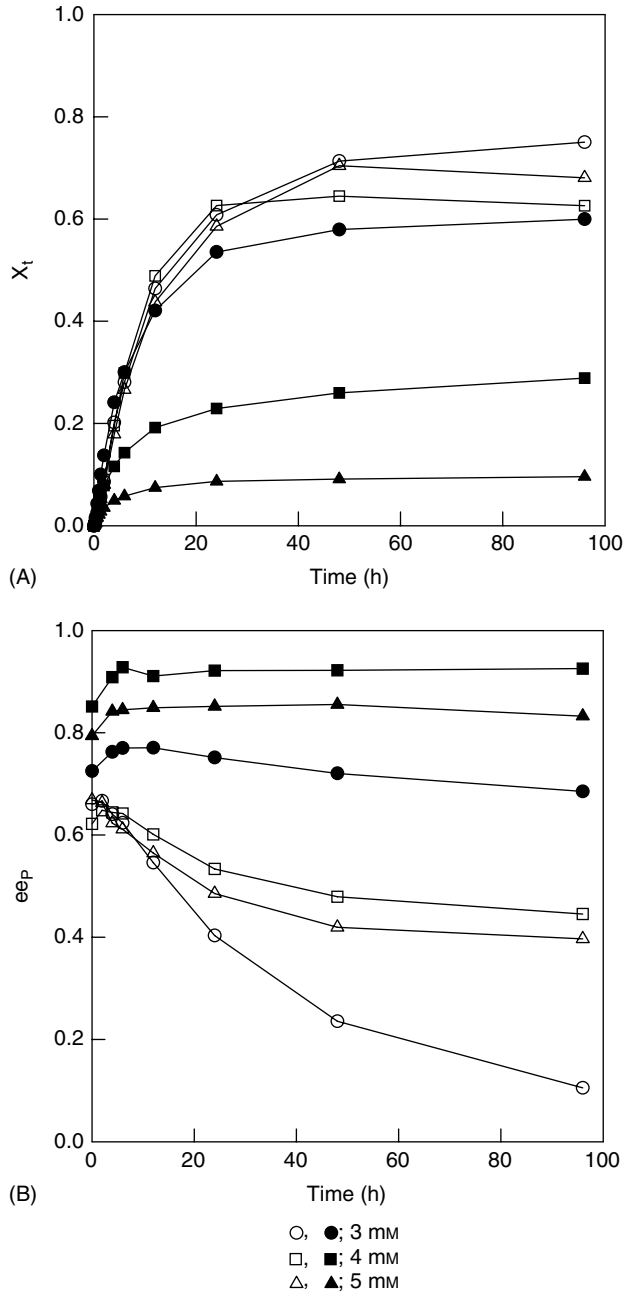


**Figure 3.** Effect of silica gel on (A) $X_t$ and (B) $ee_p$ of esterification of racemic ketoprofen with different concentrations of 1,4-butanediol (3 mM, 4 mM, 5 mM), catalyzed by crude Lipase MY. Filled symbols, free 1,4-butanediol, open symbols, silica gel-adsorbed 1,4-butanediol.

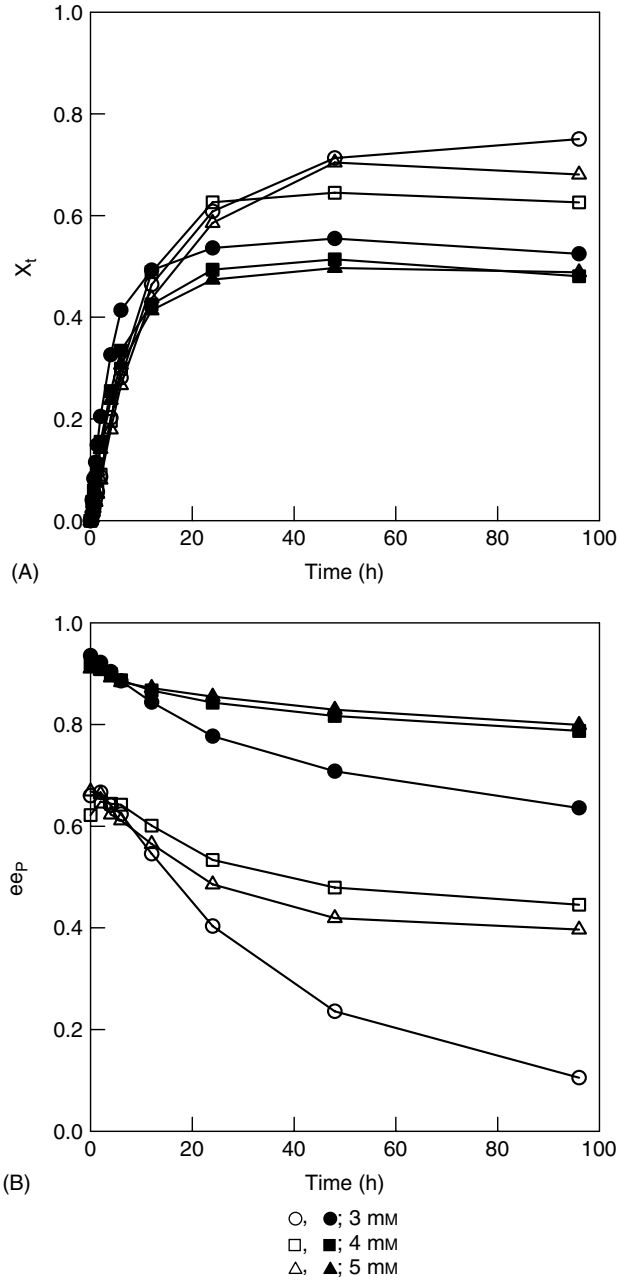


**Figure 4.** Effect of type of preparation of Lipase MY on (A) $X_t$ and (B) $ee_p$ of esterification of racemic ketoprofen with different concentrations of 1,4-butanediol (3 mM, 4 mM, 5 mM) adsorbed onto silica gel. Filled symbols, IPA-dried IM-lipase; open symbols, crude Lipase MY.

IM-lipase and crude Lipase MY, when the 1,4-butanediol adsorbed onto silica gel was used as the acyl acceptor. The graph reveals that the final conversion of IPA-dried IM-lipase is about 50% because of the improvement in the enantioselectivity of the IPA-dried IM-lipase. In contrast, in another reaction with crude Lipase MY, the poor enantioselectivity was responsible for a final conversion of over 60% and the low enantiomeric excess of the product. In the case of IPA-dried IM-lipase-catalyzed enantioselective esterification using 1,4-butanediol adsorbed onto silica gel, the enantioselectivity of the biocatalyst was improved, overcoming the limitation on the solubility of the polar alcohol and preventing the aggregation of crude lipase.

## Effect of temperature on the enantioselectivity and the initial rates of the esterification of racemic ketoprofen with 1,4-butanediol

Figure 5 shows that the initial rate $V_S$ of the enzymatic esterification of racemic ketoprofen with 1,4-butanediol in isooctane was maximum at 42 °C. The initial rates, $V_S$ and $V_R$, increased with temperature, but the rate of increase of the initial rate $V_R$ exceeded that of $V_S$, reducing the enantioselectivity in the reaction conditions. An asymmetric curve of

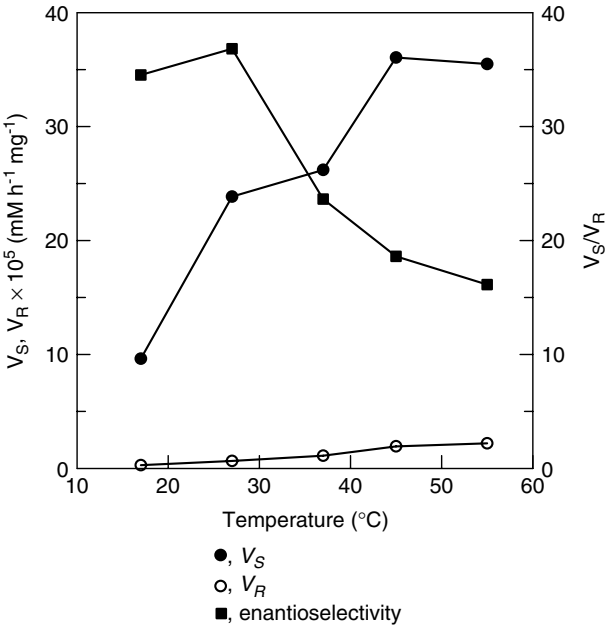


**Figure 5.** Effect of temperature of IPA-dry IM-lipase in isooctane on the initial rates $V_S$ and $V_R$ and the enantioselectivity. Conditions: (*R*,*S*)-ketoprofen = 0.2 mM, 1,4-butanediol = 1 mM, 37 °C, equivalent to 5 mg $cm^{-3}$ of crude *C rugosa* lipase.

enantioselectivity with a maximum value of 37 at the a reaction temperature of 27 °C was obtained.

## CONCLUSIONS

A lipase-catalyzed enantioselective esterification process in organic solvents was used to synthesize (*S*)-ketoprofen hydroxyalkyl ester. The Lipase MY that originates from *C rugosa* exhibited poor enantioselectivity with an (*S*)-stereochemical preference to obtain the desired (*S*)-ketoprofen esters. The effect of the carbon chain length of the alkanediol on the enzyme activity and the enantioselectivity of lipase-catalyzed esterification were investigated. IPA-dried IM-lipase and silica gel-adsorbed alkanediol were combined to improve the enantioselectivity, enhance the enzyme activity and overcome the low solubility of alkanediol in isooctane. IPA-dried IM-lipase has potential to be applied to dynamic kinetic resolution for racemic ketoprofen.

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## REFERENCES

1 Todd PA and Clissold SP, Naproxen: a reappraisal of its pharmacology, and therapeutic use in rheumatic diseases and pain states. *Drugs* **40**:91–137 (1990).
2 Shanbhag VR, Crider AM, Gokhale R, Harpalani A and Dick RM, Ester and amide prodrugs of ibuprofen and naproxen: synthesis, anti-inflammatory activity, and gastrointestinal toxicity. *J Pharm Sci* **81**:149–154 (1992).
3 Rautio J, Taipale H, Gynther J, Vepsalainen J, Nevalainen T and Jarvinen T, In vitro evaluation of acyloxyalkyl esters as dermal prodrugs of ketoprofen and naproxen. *J Pharm Sci* **87**:1622–1628 (1998).
4 Rautio J, Nevalainen T, Taipale H, Vepsalainen J, Gynther J, Laine K and Jarvinen T, Synthesis and in vitro evaluation of novel morpholinyl- and methylpiperazinylacyloxyalkyl prodrugs of 2-(6-methoxy-2-naphthyl)propionic acid (naproxen) for topical drug delivery. *J Med Chem* **43**:1489–1494 (2000).
5 Baixauli F, Inglés F, Alcantara P, Navarreté R, Puchol E and Vidal F, Percutaneous treatment of acute soft tissue lesions with naproxen gel and ketoprofen gel. *J Int Med Res* **18**:372–378 (1990).
6 Flouvat B, Roux A and Delhotal-Landes B, Pharmacokinetics of ketprofen in man after repeated percutaneous administration. *Arzneim-Forsch/Drug Res* **39**:812–815 (1989).
7 van den Ouweland FA, Eenhoorn PC, Tan Y and Gribnau FWJ, Transcutaneous absorption of naproxen gel. *Eur J Clin Pharmacol* **36**:209–211 (1989).
8 Suh H, Jun HW, Dzimianski MT and Lu GW, Pharmacokinetic and local tissue disposition studies of naproxen following topical and systemic administration in dogs and rats. *Biopharm Drug Dispos* **18**:623–633 (1997).
9 Klibanov AM, Asymmetric transformations catalyzed by enzymes in organic solvents. *Acc Chem Res* **23**:114–120 (1990).
10 Schoffers E, Golebiowski A and Johnson CR, Enantioselective synthesis through enzymatic asymmetrization. *Tetrahedron* **52**:3769–3826 (1996).
11 Schmid A, Dordick JS, Hauer B, Kiener A, Wubbolts M and Witholt B, Industrial biocatalysis today and tomorrow. *Nature* **409**:258–268 (2001).
12 Krishna SH and Karanth NG, Lipase and lipase-catalyzed esterification reactions in nonaqueous media. *Catalysis Reviews* **44**:499–591 (2002).
13 Chang CS and Tsai SW, A facile enzymatic process for the preparation of (*S*)-naproxen ester prodrug in organic solvents. *Enzyme Microb Technol* **20**:635–639 (1997).
14 Antona ND, Lombardi P, Nicolosi G and Salvo G, Large scale preparation of enantiopure *S*-ketoprofen by biocatalysed kinetic resolution. *Process Biochem* **38**:373–377 (2002).
15 Crescenzo GD, Ducret A, Trani M and Lortie R, Enantioselective esterification of racemic ketoprofen in non-aqueous solvent under reduced pressure. *J Mol Catal B: Enzym* **11**:939–947 (2001).
16 Park HJ, Choi WJ, Huh EC, Lee EY and Choi CY, Production of optically active ketoprofen by direct enzymatic esterification. *J Biosci Bioeng* **87**:545–547 (1999).
17 Morrone R, Nicolosi G, Patti A and Piattelli M, Resolution of racemic flurbiprofen by lipase-mediated esterification in organic solvent. *Tetrahedron: Asymmetry* **6**:1773–1778 (1995).
18 Al-Duri B and Yong YP, Characterisation of the equilibrium behaviour of lipase PS (from *Pseudomonas*) and lipolase 100L (from *Humicola*) onto Accurel EP100. *J Mol Catal B: Enzym* **3**:177–188 (1997).
19 Lin HY and Tsai SW, Dynamic kinetic resolution of (*R*,*S*)-naproxen 2,2,2-trifluoroethyl ester via lipase-catalyzed hydrolysis in micro-aqueous isooctane. *J Mol Catal B: Enzym* **24–25**:111–120 (2003).
20 Chang CS and Hsu CS, Lipase-catalyzed enantioselective esterification of (*S*)-naproxen hydroxyalkyl ester in organic media. *Biotechnol Lett* **25**:413–416 (2003).
21 Chang CS, Su CC, Zhuang JR and Tsai SW, Enhancement of enantioselectivity on the synthesis of (*S*)-naproxen morpholinoalkyl ester prodrugs in organic solvents using isopropanol-dried immobilized lipase. *J Mol Catal B: Enzym* **30**:151–157 (2004).
22 Castillo E, Dossat V and Marty A, The role of silica gel in lipase-catalyzed esterification reactions of high-polar substrates. *J AOCS* **74**:77–85 (1997).

23 Pan SH, Kawamoto T, Fukui T, Sonomoto K and Tanaka A, Stereoselective esterification of halogen-containing carboxylic acids by lipase in organic solvent: effects of alcohol chain length. *Appl Microbiol Biotechnol* **34**:47–51 (1990).
24 Holmquist M, Hæffner F, Norin T and Hult K, A structural basis for enantioselective inhibition of *Candida rugosa* lipase by long-chain aliphatic alcohols. *Protein Sci* **5**:83–88 (1996).
25 Holmquist M, Insights into the molecular basis for fatty acyl specificities of lipases from *Geotrichum candidum* and *Candida rugosa*. *Chem Phys Lipids* **93**:57–65 (1998).
26 Chamorro S, Alcántara AR, Casa RM and Sinisterra JV, Small water amounts increase the catalytic behaviour of polar organic solvents pre-treated *Candida rugosa* lipase. *J Mol Catal B: Enzym* **11**:939–947 (2001).
27 Crescenzo GD, Ducret A, Trani M and Lortie R, Enantioselective esterification of racemic ketoprofen in non-aqueous solvent under reduced pressure. *J Mol Catal B: Enzym* **9**:49–56 (2000).
28 Strauss UT, Felfer U and Faber K, Biocatalytic transformation of racemates into chiral building blocks in 100% chemical yield and 100% enantiomeric excess. *Tetrahedron: Asymmetry* **10**:107–117 (1999).