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Resolution of (*R*,*S*)-ibuprofen catalyzed by immobilized Novozym40086 in organic phase

Xin Yuan | Lujun Wang | Guangyong Liu | Guilin Dai | Kewen Tang

Department of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang, China

Correspondence
Kewen Tang, Department of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang 414006, Hunan, China.
Email: tangkewen@sina.com

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Abstract

The enantioselective esterification of ibuprofen catalyzed by Novozym40086 was successfully conducted in organic solvent. Removing-water reagent was added into the reaction mixture to remove water produced in the esterification. The effects of temperature, *n*-hexanol concentration, ibuprofen concentration, and loading of enzymes were investigated. Under the condition of equilibrium, the thermodynamic equilibrium constant (K) of 7.697 and enantioselectivity (E) of 8.512 were obtained. The esterification reaction achieved its equilibrium in approximately 30 hours with conversion of 56% and ee_S of 93.78%. The predicted values of X and ee_S were 67.90% and 95.60%, respectively. The experimental value is approximately equal to the theoretical value, which indicates the feasibility of ideal models.

KEYWORDS

enantioselectivity, enzymatic kinetic resolution, esterification, ibuprofen, immobilized Novozym40086

1 | INTRODUCTION

The living system is a chiral environment. From chemical molecular level, chirality is an inherent characteristic in living system. The chiral environment has a significant effect on biologically active substances.[1-3] A pair of enantiomers possess identical physical and chemical properties including chemical activity, solubility, density, melting, and boiling point, but they often exhibit marked differences in biological activities.[2] In many cases, one enantiomer shows desired physiological effect, while the other shows no effect or is even harmful to human health.[4] Additionally, taking in optically pure drugs can reduce the dose, reduce the metabolic burden, and reduce the side effects caused by the undesired enantiomer.[2,3,5] Nowadays, the market of single enantiomer is increasing and how to obtain single-enantiomer has become a hot research field. A variety of chiral technologies have been developed to produce optically pure drugs to meet the growing market demand.[6]

The methods of obtaining single-enantiomer drugs mainly contain asymmetric synthesis and racemic resolution. Asymmetric synthesis includes fermentation, chiral source synthesis, and asymmetric catalytic synthesis.[7] Although new impressive progress has been obtained in asymmetric synthesis, the most common way in industry to obtain optically pure compounds is still via chiral resolution of racemic mixtures. A series of resolution methods have been developed, such as membrane separation,[8-10] chromatography,[11,12] enantioselective liquid-liquid extraction (ELLE),[13-15] and enzymatic kinetic resolution.[16-28] Membrane separation is still one of the most widely applied technologies in manufacture pharmaceutical. But it has some disadvantages, for example, low transport rates, fouling of membrane, and instability over long periods of time.[4] Chromatography, a key technology for chiral separation, plays a crucial role in all fields, such as pharmaceuticals, fine chemicals, food, and beverage

Xin Yuan and Lujun Wang contributed equally to this work.

industries. Chromatography also possesses some advantages, such as rapid separation, wide application scope, convenient operation, high precision, and efficiency.[29] However, large quantities of solvents are required and much money is invested in expensive stationary phase and high-voltage equipment.[1,30] ELLE is regarded as a promising technology because it is easy to realize continuous production, easy industrial amplification, simple operation, low production cost, and wide application range.[7,30,31] The key problem for large-scale industrial applications of chiral extraction is lacking chiral extractant with high selectivity and versatility. These drawbacks limit the practical application of ELLE.[29]

Enzymatic kinetic resolution is an appealing alternative for chiral separation.[32] It can be applied in transesterification,[16-19] hydrolysis,[20-22] esterification,[23-28] etc. Among enzymes used in kinetic resolution, lipases (triacyglycerol acylhydrolases) are the most popular,[33,34] which shows the combination of wide substrate specificity and high regioselectivity and enantioselectivity. As lipases act as catalysts, the catalytic mechanisms are generally recognized as the "interfacial activation" mechanism.[35-37] Two different structural forms of lipases are assumed as follows: the closed form and the open form. The closed form is considered as an inactive form, in which the active sites are secluded from the reaction medium by a helical oligopeptide chain called "lid." However, the lid is displaced and the active sites are exposed to reaction medium in the open form. For example, when a hydrophobic substrate is catalyzed, such as an aromatic substance, only the open form interacts with substrate.[36,37] Enzyme catalytic reaction has been widely used due to its high stereoselectivity, broad substrate specificity, enantioselectivity, and reactivity. At the same time, the enzymatic kinetic resolution has a high thermostability and conformational stability in hydrophilic and hydrophobic environments.[32]

2-Arylpropionic acids (profens) are known as major nonsteroidal anti-inflammatory drugs, which are used in the treatment of cephalgia, muscular strain, headache, and rheumatoid arthritis.[38-40] This drug class has a chiral carbon atom within the propionic acid moiety. Ibuprofen is one of the most frequently used drugs.[41] The pharmacological activity of ibuprofen is mainly shown by the (*S*)-enantiomer, which is 160-fold more active than the corresponding (*R*)-enantiomer.[42] Therefore, obtaining the optically pure (*S*)-enantiomer is of great significance.

Since lipases can be easily recovered from the reaction medium, it can be immobilized on different carriers. Immobilization of enzymes can change their secondary structure modifying their activities. In this work, four immobilized lipases were investigated as catalysts, including Novozym 435, Novozym 40086, lipozyme RM IM, and TM IM. Novozyme 435 is CAL-B immobilized onto a macroporous acrylic polymer resin. Novozym 40086, as a new commercial immobilized lipase from *Rhizomucor miehei* immobilized on acrylic resin beads, was a novel and efficient biocatalyst for the esterification. Lipozyme TL IM is a 1,3 specific lipase originating from *Thermomyces lanuginosus* and immobilized on a noncompressible silica gel carrier. Lipozyme RM IM is a 1,3 specific lipase originating from *Rhizomucor miehei* and immobilized on a resin carrier.

In this study, the enantioselective esterification of ibuprofen catalyzed by different immobilized lipases was successfully conducted in organic solvent. Novozym 40086 was selected as the catalyst with the highest enantioselectivity obtained. Two idealized models were established, and matlab was used to simulate the effect of substrate concentrations on ee. There is a good agreement between experimental data and predicted values.

2 | EXPERIMENTAL

2.1 | Enzymes and chemicals

Novozym 435 from *Candida antarctica* and lipozyme TL IM from *Thermomyces lanuginosus* were obtained from Novo Nordisk company (Beijing, China). Lipozyme RM IM and from *Rhizomucor miehei* and Novozym40086 from *Aspergillus oryzae* were obtained from Novo Nordisk company (Beijing, China). Other property of the enzymes was listed in Table 1. (*R*,*S*)-ibuprofen was obtained from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Solvents for chromatography were of high-performance liquid chromatography (HPLC) grade. All other reagents used in this work were of analytical grade and obtained from different commercial suppliers.

2.2 | Analytical methods

The concentrations of (*R*)-ibuprofen and (*S*)-ibuprofen were analyzed by HPLC (waters e2695, Waters Corporation, USA). An ultraviolet (UV) detection wavelength was set at 230 nm. A chiral RJ-OH column (150 mm × 4.6 mm i.d., 5 μm) was employed, and the column temperature was maintained at 30°C. The mobile phase was made up of methanol and 0.6% (V/V) acetic acid aqueous solution (pH = 3.00, adjusted with triethylamine). The volume ratio was 20:80. The flow rate of the mobile phase was set at 1.0 mL min^{-1}. The injection volume was 10 μL. The retention time of (*R*)-ibuprofen was less than that of (*S*)-ibuprofen. This analytical method was adjusted according to the reference.[43] The product of ibuprofen *n*-hexyl ester was analyzed by HPLC

TABLE 1 The property of lipases used in this paper

Lipases	Source	Status	Optimum *T* and pH	Activity
Novozym 435	*Candida antarctica*	Immobilized	T = 30–60°C pH = 5–9	1000[a]
Novozym40086	*Aspergillus oryzae*	Immobilized	T = 30–50°C pH = 7–10	275[b]
Lipozyme RM	*Rhizomucor miehei*	Immobilized	T = 50°C pH = 7	250[b]
Lipozyme TL	*Thermomyces lanuginosus*	Immobilized	T = 50–75°C pH = 6–8	250[b]

[a]plu/g = Propyl Laurate Unit.

[b]IUN/g = Interesterification Unit.

(waters e2695, Waters Corporation, USA). A Diamonsil C_{18} column (250 mm × 4.6 mm i.d., 5 μm) was employed, and the column temperature was maintained at 30°C. An UV detection wavelength was set at 225 nm. The mobile phase was made up of acetonitrile and 0.5% (V/V) phosphoric acid aqueous solution. The volume ratio was 90:10. The flow rate of the mobile phase was set at 1.0 mL min^{-1}. The injection volume was 10 μL. The retention time of ibuprofen was less than that of ibuprofen *n*-hexyl ester.

2.3 | Enzyme-catalyzed esterification of ibuprofen

The enzyme-catalyzed esterification reaction was carried out in a 25-mL reaction flask. A certain concentration of (*R*,*S*)-ibuprofen and concentration of *n*-hexanol were added to 2-mL methyl tert-butyl ether (MTBE), and then lipase was added to initialize the reaction. The reaction was maintained under an agitate speed of 400 rpm. The schematic diagram is shown in Figure 1. When the reaction was completed, the reaction flask was cooled in an ice-water bath for 10 s, and the immobilized enzyme was separated by filtration. Adding 0.5-mL filtrate to a 10-mL centrifuge tube and diluting the sample with MTBE, the enantiomeric excess of substrate was determined by HPLC. The sample was diluted 10-fold with acetonitrile, and the products were analyzed by HPLC. All experiments were repeated three times under identical conditions, and the precision level of the replicated values was within ±3%.

3 | METHODOLOGY

In this reversible reaction, the reverse reaction has a significant impact on reaction equilibrium. The process is very complex, in which influences of various factors are hard to be investigated. In order to simplify the process, this study assumes an ideal reaction system.

In ideal reaction system, the reaction is completely unaffected by its reverse reaction when it does not reach equilibrium. Therefore, the influence of reverse reaction ban be ignored. When the reaction reaches equilibrium, the reaction rate is zero and the ee reaches its maximum value. For this ideal process, the modeling process is as follows.

3.1 | Determination of enantiomeric excess (ee) and conversion (c)

The enantiomeric excess (ee_s) indicates the purity of a single enantiomer in reaction substrate (Equation 1)

$$ee_S = \frac{[S]-[R]}{[S]+[R]}. \quad (1)$$

Conversion (X) of (*R*,*S*)-ibuprofen was calculated as follows:

$$X = \frac{[S]+[R]}{[S]_0+[R]_0}, \quad (2)$$

where $[S]$ and $[R]$ are the substrate concentrations of (*S*)-ibuprofen and (*R*)-ibuprofen after the reaction,

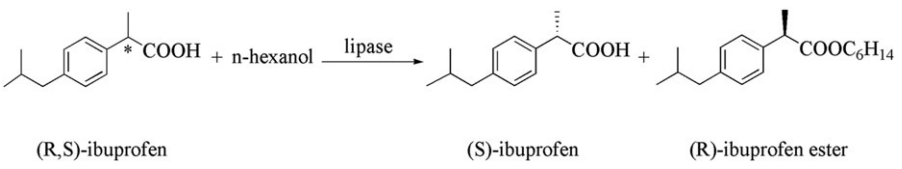

FIGURE 1 Biocatalysed esterification of (*R*,*S*)-ibuprofen with *n*-hexanol

respectively; $[S]_0$ and $[R]_0$ are the initial concentration of (*S*)-ibuprofen and (*R*)-ibuprofen, respectively.

3.2 | Establishment of relationship between $ee_{s,max}$ and equilibrium conversion of fast-reaction

Assuming that the initial concentrations of (*R*) and (*S*)-enantiomers are equal, the reaction of (*S*)-enantiomer is faster than that of (*R*)-enantiomer and ee_s can be defined by the following equation:

$$ee_S = \frac{X_S - X_R}{2 - X_S - X_R}. \tag{3}$$

Since it is unaffected by the reverse-reaction, the enantioselectivity (E) of the reaction is a constant that is only related to the reaction system and can be calculate by the following equation

$$E = \frac{\ln[(1-X)(1-ee_S)]}{\ln[(1-X)(1+ee_S)]} \tag{4}$$

due to

$$\begin{cases} X_S + X_R = 2X \\ X_S - X_R = ee_S(2 - X_S - X_R) = 2ee_S(1-X) \end{cases}. \tag{5}$$

Equation 5 can be transformed into the following equation

$$\begin{cases} 1 - X_S = (1-X)(1-ee_S) \\ 1 - X_R = (1-X)(1+ee_S) \end{cases}. \tag{6}$$

The relationship between X_R and X_S was obtained according to Equations 4 and 6.

$$1 - X_S = (1-X_R)^E. \tag{7}$$

Combining Equations 3 and 7, Equation 8 can be reduced

$$ee_S = \frac{2}{(1-X_S)^{\frac{E-1}{E}} + 1} - 1. \tag{8}$$

For an ideal system, ee_s will reach the maximum when X_S is equal to X_e.

$$ee_{S,\max} = \frac{2}{(1-X_e)^{\frac{E-1}{E}} + 1} - 1 \tag{9}$$

From the Equation 10, it can be seen that the maximum value of ee_s can be obtained, which is only related to the equilibrium conversion (X_e). The $ee_{s,max}$ can be achieved by increasing the forward-reaction rate or decreasing the inverse-reaction rate. Therefore, adding excess concentration of alcohol or removing the water can boost the process of esterification reaction to obtain higher X_e and $ee_{s,max}$. The results can be proofed by follows description:

The equilibrium conversion ratio (X_e) of the enantiomers is related to the concentration of each reaction component and the thermodynamic equilibrium constant (K) in the reaction system. For esterification reaction, the following equation describes the reaction.

$$R_1COOH + R_2OH \rightleftharpoons H_2O + R_1COOR_2$$

A represents R_1COOH, B represents R_2OH, P represents H_2O, Q represents R_1COOR_2, and its thermodynamic equilibrium constant (K) is given by following equation.

$$K = \frac{C_P \cdot C_Q}{C_A \cdot C_B}. \tag{10}$$

The concentration of each component is an equilibrium concentration. C_A represents concentration of (*R*, *S*)-ibuprofen, C_B represents concentration of alcohol, C_P represents concentration of water, and C_Q represents concentration of ester. The equilibrium conversion rate (X_e) of (*R*,*S*)-ibuprofen is defined by following equation.

$$X_e = \frac{C_Q}{C_A + C_Q}. \tag{11}$$

Combining Equations 10 and 11, X_e can be deduced

$$X_e = \frac{KC_B}{C_P + KC_B}. \tag{12}$$

3.3 | Ideal model of increase the alcohol concentration

Combining eqs. (9) and (12), $ee_{s,max}$ can be deduced.

$$ee_{S,\max} = \frac{2}{\left(\frac{C_P}{C_P + KC_B}\right)^{\frac{E-1}{E}} + 1} - 1 \tag{13}$$

According to the mass balance, the concentrations of components can be expressed as

$$\begin{cases} C_P = C_{A,0} \cdot X_{opt} \\ C_B = C_{B,0} - C_{A,0} \cdot X_{opt} \end{cases}, \tag{14}$$

where $C_{A,0}$ and $C_{B,0}$ are the initial concentrations of the enantiomeric acid and alcohol, respectively; X_{opt} is the

overall conversion rate of the enantiomers when the system ee_s reaches the maximum ($ee_{s,max}$). Combining Equations 13 and 14, the relationship between $ee_{s,max}$ and X_{opt} can be calculated as follows.

$$ee_{S,\max} = \frac{2}{\left(\frac{X_{opt}}{X_{opt}+K\left(C_B/C_A-X_{opt}\right)}\right)^{\frac{E-1}{E}}+1} - 1. \quad (15)$$

3.4 | Ideal model of water concentration

The concentration of water can be reduced by adding a water removal agent in the reaction system. The removal of water is performed by the hydration between water removal agent and water. The amount of agent is excess, which can ensure the water concentration (C_P) to be constant.[44] Therefore, Equation 13 can be deduced as follows.

$$ee_{S,\max} = \frac{2}{\left(\frac{C_P}{C_P+K\left(C_B\text{-}C_AX_{opt}\right)}\right)^{\frac{E-1}{E}}+1} - 1. \quad (16)$$

4 | RESULTS AND DISCUSSION

4.1 | Screening of lipases

Different lipases may show a large difference in the catalytic efficiency of enantioselective esterification of (*R*,*S*)-ibuprofen. Therefore, it is needed to obtain a lipase with good catalytic performance. The influences of four commercially available lipases on enantioselective esterification of (*R*,*S*)-ibuprofen with *n*-hexanol were investigated, in which MTBE acted as organic solvent. Table 2 displays the results of ee_s, *X*, and ee_p after 24 hours. Three lipases (lipase 435, 40086, and RM) show excellent catalytic performance, while lipase TM shows no catalytic performance. Compared with other lipases, Novozym40086 shows the highest catalytic performance and selectivity. Therefore, Novozym40086 was selected as the catalyst.

4.2 | Effect of temperature

Temperature has an improtant impact on both enzyme activity and enantioselectivity. The influence of temperature on conversion and enantiomeric excess of esterification of (*R*,*S*)-ibuprofen was tested with ranging from 40 to 80°C. As shown in Figure 2, the conversion of (*R*,*S*)-ibuprofen and ee increase with the increase of temperature (≤70°C), and then they decrease with further increase of temperature (≥70°C). The maximum of conversion and ee_S are reached at 70°C. *E* has no obvious change in this range. Based on above results, 70°C is selected as the optimum reaction temperature.

4.3 | Effect of (*R*,*S*)-ibuprofen concentration

Concentration of (*R*,*S*)-ibuprofen has a significant influence on conversion, ee_S and *E*. Figure 3 shows the influence of different initial (*R*,*S*)-ibuprofen concentration on catalytic performance. As depicted in Figure 3, conversion and ee are decreased with increasing (*R*,*S*)-ibuprofen concentration from 100 to 1200 mmol L^{-1}. *E* is nearly unchanged with a relative high value in the tested concentration of (*R*,*S*)-ibuprofen. The results indicate that the lower (*R*,*S*)-ibuprofen concentration is, the higher conversion and ee_S are. However, the productivity should also be considered, which will be low with low (*R*,*S*)-ibuprofen concentration. Based on these considerations, the initial (*R*,*S*)-ibuprofen concentration was selected at 600 mmol/L.

4.4 | Effect of *n*-hexanol concentration

The estrification reaction catalyzed by enzyme is reversible. To prompt the formation of product, excessive concentration of *n*-hexanol is added. However, alcohols act as a nucleophile and excessive *n*-hexanol concentration will affect the enzyme activity and the enantioseletivity. As shown in Figure 4, the conversion of (*R*,*S*)-ibuprofen and ee_s are decreased with increasing *n*-hexanol concentration from 450 to 2000 mmol/L. *E* has no obvious

TABLE 2 Results of esterification of (*R*,*S*)-ibuprofen catalyzed by different lipases

Lipases	Source	ee_s, %	X, %	ee_p, %
Novozym 435	*Candida antarctica*	5.42	33.35	25.71
Novozym 40086	*Rhizomucor miehei*	71.03	25.60	87.40
Lipozyme RM	*Rhizomucor miehei*	18.41	12.21	39.51
Lipozyme TL	*Thermomyces lanuginosus*	0	0	0

Note. Conditions: C_A = 100 mmol/L, C_B = 100 mmol/L, and T = 70°C.

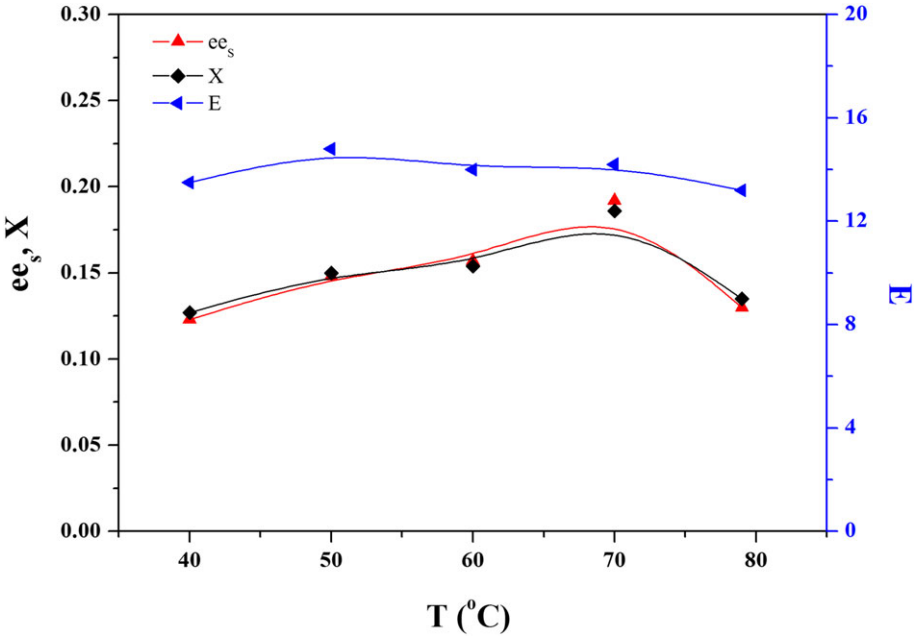

FIGURE 2 Effects of reaction temperature on *X*, ee_S, and *E*. Conditions: 100 mmol/L C_A, 100 mmol/L C_B, 100 mg/mL Novozym40086, and reaction time 1 hour

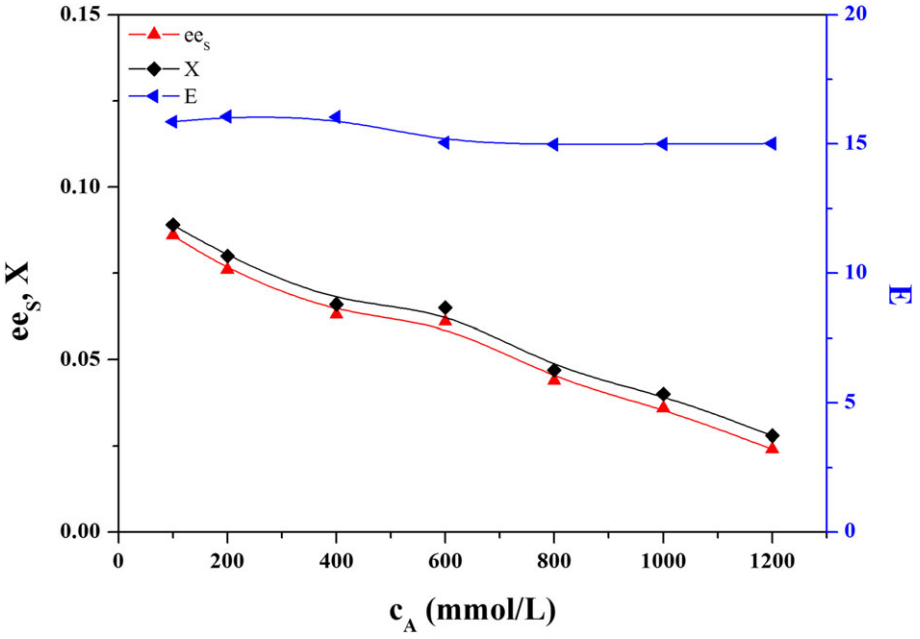

FIGURE 3 Effects of (*R*,*S*)-ibuprofen concentration on *X*, ee_S, and *E*. Conditions: 600 mmol/L C_B, 100 mg/mL Novozym40086, temperature 70°C, and reaction time 1 hour

change with *n*-hexanol concentration ranging from 450 to 800 mmol/L and then decreases with further increase of *n*-hexanol concentration (≥800 mmol/L). The results indicate that high *n*-hexanol concentration probably inhibits the enzyme activity and the enantioseletivity. Furthermore, high *n*-hexanol concentration may increase the occurrence of nonenantioselectivity reaction.[45] Therefore, the *n*-hexanol concentration chose 800 mmol/L to obtain higher X.

4.5 | Effect of enzyme amount

Lipase as a catalyst, increasing the amount of lipase can accelerate the reaction. In order to obtain optimal enzyme amount, the effect of enzyme amount on enantioselective esterification of (*R*,*S*)-ibuprofen was investigated. As shown in Figure 5, the conversion and ee_s are increased linearly with increasing the amount of immobilized enzyme. The *E* is decreased slowly with

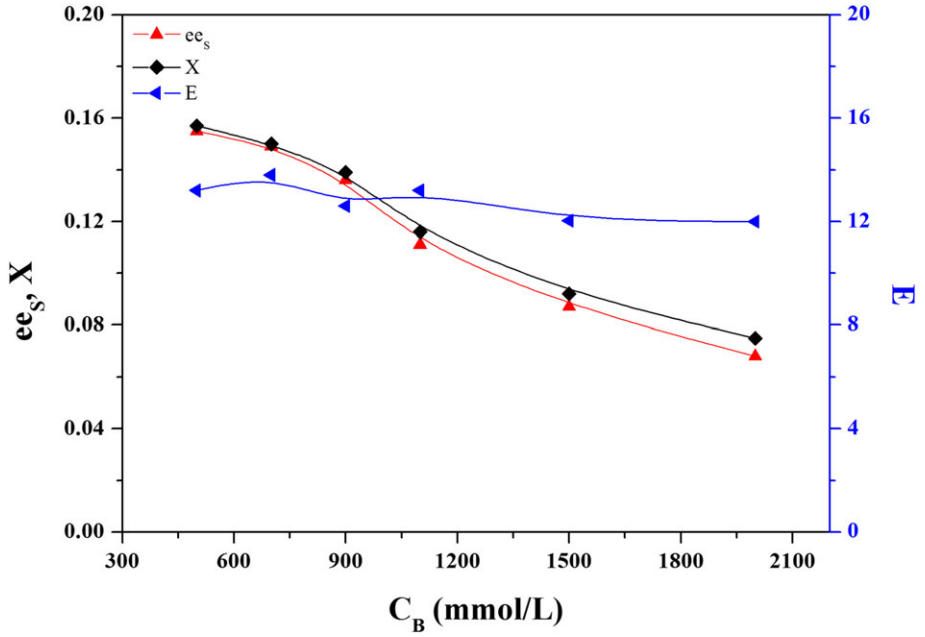

FIGURE 4 Effects of *n*-hexanol concentration on *X*, ee_S, and *E*. Conditions: 600 mmol/L C_A, 100 mg/mL Novozym40086, temperature 70°C, and reaction time 1 hour

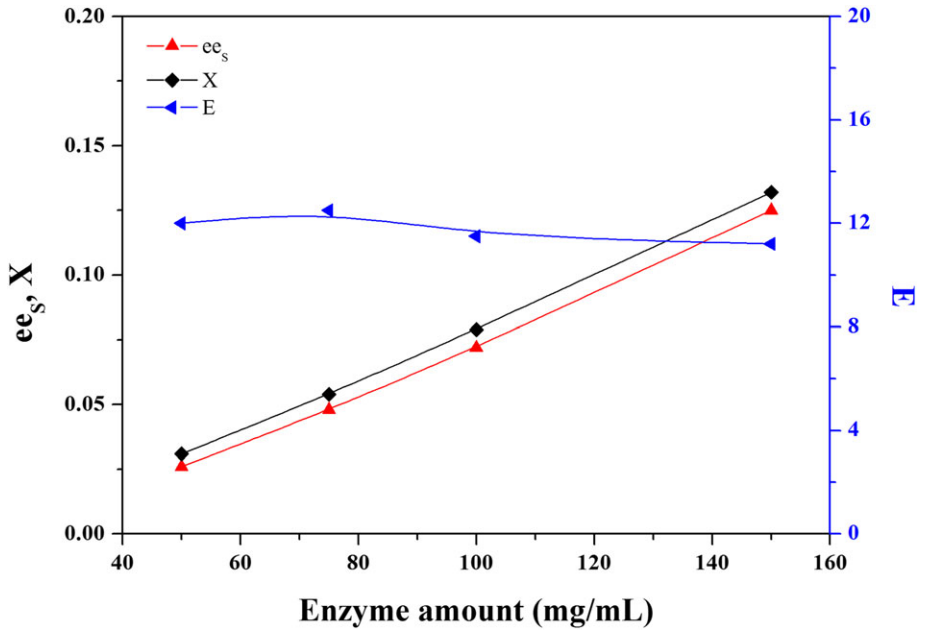

FIGURE 5 Effects of enzyme amount on *X*, ee_S, and *E*. Conditions: 600 mmol/L C_A, 2000 mmol/L C_B, 100 mg/mL Novozym40086, temperature 70°C, and reaction time 3 hours

the increase of immobilized enzyme. This phenomenon indicates that the increase of the amount of enzyme shows no inhibitation of lipase catalytic activity. Using immobilized enzyme can avoid the tendency of lipases to give aggregates.[46] Therefore, the amount of enzyme can be selected based on actual requirements (such as controlling the reaction rate and controlling the production cost).

4.6 | Determination of thermodynamic equilibrium constant (K) and enantioselectivity (E)

Under specified solvent and temperature, *K* is constant and *E* can be considered as a constant before the fast-reacting species attaining equilibrium. According to Equations 5 and 16, *K* and *E* are important parameters

for determining $ee_{s,max}$, so the determination of them is needed before the calculation of $ee_{s,max}$. When fast-reaction reached the thermodynamic equilibrium, the ee_s was up to the maximum ($ee_{s,max}$). ee_s is a function of conversion, and conversion is a function of time. Therefore, the Novozym40086 catalysis esterification of (*R*,*S*)-ibuprofen with different reaction time was investigated. When the ee_s reaches the maximum, the conversion of the fast-reaction was up to the highest degree and then was constant. At this time, *K* and *E* were calculated by the Equations 5 and 11, respectively. As shown in Figure 6, it is also found that there is a rapid increase of X_S, X_R, and ee at first, and then a slow increase of them happens after 6 hours. Therefore, the reaction can be

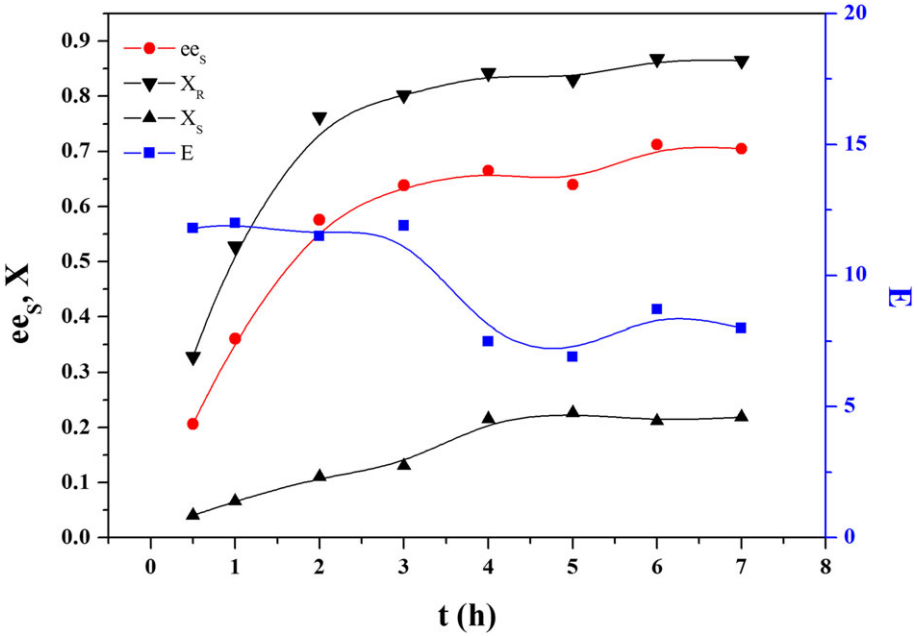

FIGURE 6 Effects of reaction time on X_R, X_S, ee_S, and *E*. Conditions: 100 mmol/L C_A, 100 mmol/L C_B, 100 mg/mL Novozym40086, and temperature 70°C

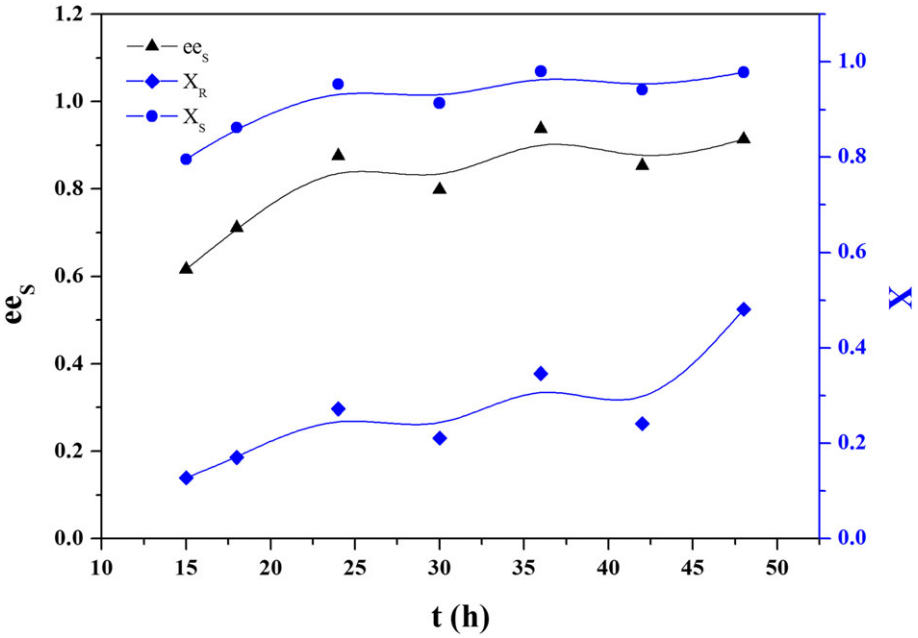

FIGURE 7 Effects of reaction time on conversion X_R, X_S, and ee_S. Conditions: 600 mmol/L C_A, 800 mmol/L C_B, 100 mg/mL Novozym40086, and temperature 70°C

regarded as reaching equilibrium at 6 h. K and E were calculated by Equations 5 and 11, with values of 7.697 and 8.512, respectively.

4.7 | Calculation and experimental verification

The factors affecting X_R, X_S, and ee_S were optimized, such as temperature, Novozym40086 amount, and substrate concentrations. Under these conditions, the influences of reaction time on X_R, X_S, and ee were investigated. From Figure 7, it can be found that X_R, X_S, and ee increase with reaction time. The X_S and ee reach the maximum at 36 hours. The ee_s is up to 93.78%, which is close to the expected ee_S value 95.6% estimated by Equations 5 and (16). The relevant parameters of the enzyme catalytic reaction have been depicted in Table 3.

TABLE 3 The relevant parameters of the enzyme catalytic reaction

Parameter	Value
Expected value of $ee_{s,max}$	95.6%
K	7.697
E	8.512
C_P/mmol/L	40
C_A/mmol/L	600
C_B/mmol/L	800

4.8 | Model optimization and validation

Combined with Equations 3, 11, and 16, matlab was used to simulate the effect of substrate concentrations on ee_S. As shown in Figure 8, ee_S increases rapidly and then shows no change when C_B is more than 800 mmol/L. The ee decreases with the increase of C_A, and it can keep a relative high value (≥95%). Taking the economy of *n*-hexanol and the productivity of (*R*,*S*)-profen into consideration, 800 mmol/L C_B and 600 mmol L^{-1} C_A are selected to achieve high X and ee.

4.9 | Application

The optimal reaction conditions were obtained according to the above results, including 800 mmol/L *n*-hexanol, 600 mmol/L (*R*,*S*)-ibuprofen, 100 mg/mL Novozym40086, 70°C, agitation speed of 400 rpm, and 30 hours for reaction time. Under the optimal conditions, the experimental values of X and ee_S are 59.90% and 93.78%, respectively, and the predicted values of X and ee_S are 67.90% and 95.60%, respectively, which indicate that the experimental values are well in agreement with the model prediction. The enantiomeric excess of the substrate was analyzed by HPLC (Figure 9). Figure 9A,B

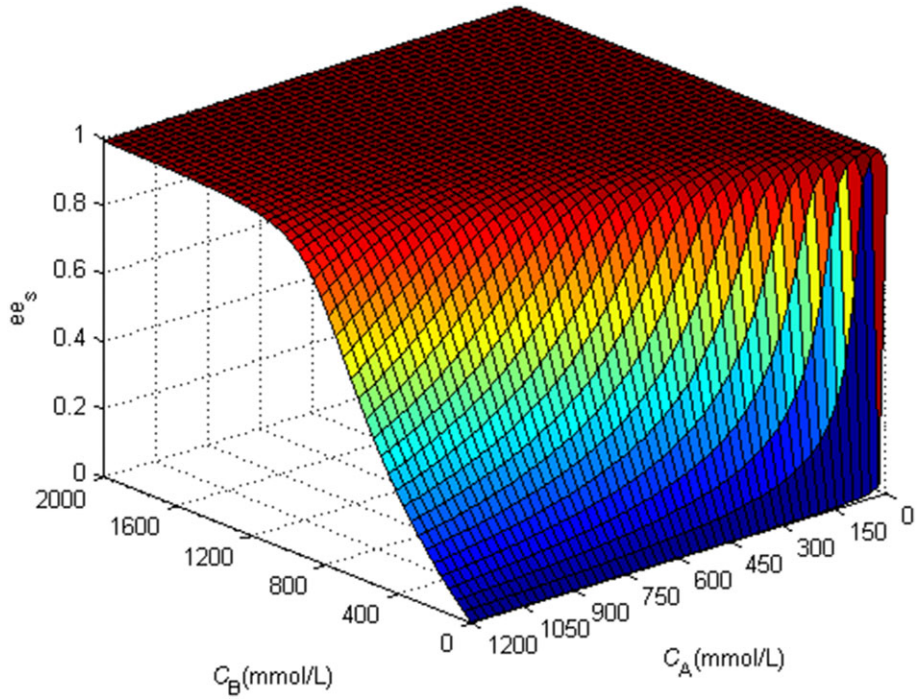

FIGURE 8 Dependence of predicted ee_s on C_A and C_B. Conditions: 100 mg/mL Novozym40086 and temperature 70°C

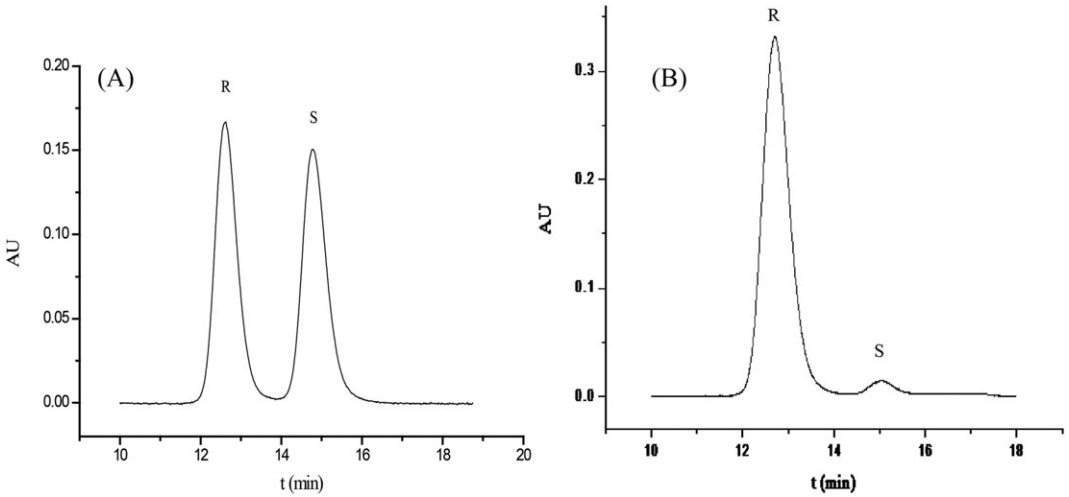

FIGURE 9 High-performance liquid chromatography chromatograms of the ibuprofen enantiomers. A, Racemate of (*R*,*S*)-ibuprofen. B, The remaining (*R*,*S*)-ibuprofen after esterification

shows the chromatograms of racemic (*R*,*S*)-ibuprofen and the remaining substrate after esterification by lipase.

5 | CONCLUSION

The effects of reaction temperature, initial concentration of ibuprofen, initial concentration of *n*-hexanol, and amount of enzyme on esterified ibuprofen by Novozym40086 were investigated. It was found that the temperature, Novozym40086 amount, substrate concentration, and reaction time have significant impacts on the conversion of substrate. At the same time, the substrate concentration and reaction time also have significant impacts on the optical purity of product. The other factors have no significant effect on the optical purity of product. The removing-water reagent added to the reaction system can enhance the substrate conversion. Under equilibrium, the thermodynamic equilibrium constant ($K = 7.697$) and enantioselectivity ($E = 8.512$) were obtained. The esterification reaction achieved its equilibrium in approximately 36 hours with a conversion of 56% and ee_s of 93.78%. Under equilibrium conditions, the theoretical value of ee_s was up to 95.60%. The experimental value is approximately equal to the theoretical value, so we assume that the two ideal models are feasible.

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ORCID

Kewen Tang https://orcid.org/0000-0002-1194-2664

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