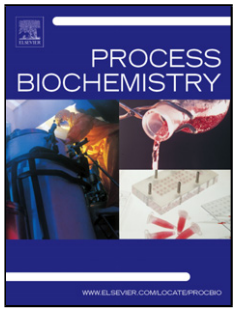

Title: Modeling and optimization of lipase-catalyzed hydrolysis for production of (*S*)-2-phenylbutyric acid enhanced by hydroxyethyl-β-cyclodextrin

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Modeling and optimization of lipase-catalyzed hydrolysis for production of (*S*)-2-phenylbutyric acid enhanced by hydroxyethyl-β-cyclodextrin

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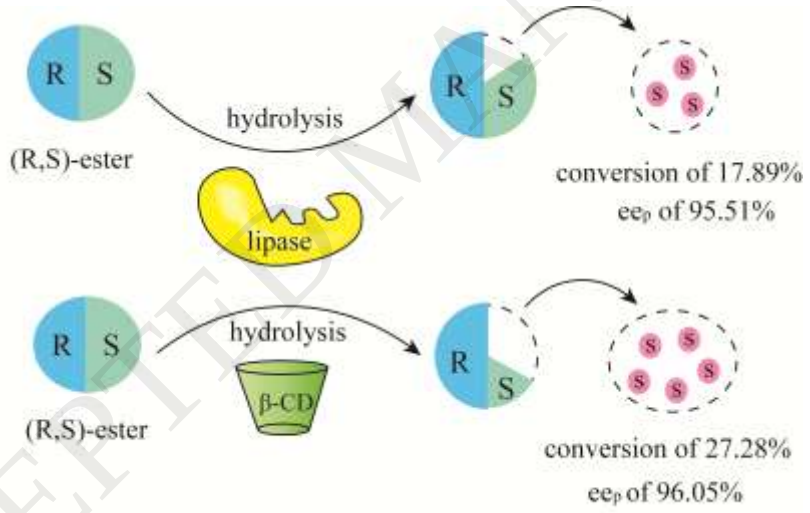
Graphical abstract



Research highlights

- Chiral resolution of 2-phenylbutyric acid by lipase-catalyzed with ee_p of 96.05%.
- Enhancement of substrate conversion by β-cyclodextrin derivatives is up to 1.5 times.
- The reaction system may be extended to a large number of aromatic acid enantiomers.

ABSTRACT

An efficient reactive system was established to produce (*S*)-2-phenylbutyric acid (2-PBA) through the enzymatic enantioselective hydrolysis of 2-phenylbutyrate ester (2-PBAE) in aqueous medium. Lipase CALA from *Canadian antarctica* and hexyl 2-phenylbutyrate (2-PBAHE) were identified upon screening as the best enzyme and substrate, respectively. Adding hydroxyethyl-β-cyclodextrin (HE-β-CD) to improve the solubility of the substrate resulted in a 1.5 times increase in substrate conversion while retaining a high enantioselectivity compared with that when HE-β-CD was not added. The effects of lipase concentration, substrate concentration and HE-β-CD concentration, temperature, pH, and reaction time on enantiomeric excess and conversion rate were investigated, and the optimal conditions were identified using response surface methodology (RSM). Under the optimal conditions, namely 50 mg/mL lipase CALA, 30 mmol/L substrate, 60 mmol/L HE-β-CD, pH of 6.5, temperature of 83 °C and reaction time of 18 hours, the enantiomeric excess and overall conversion rate were 96.05% and 27.28%, respectively. This work provides an efficient alternative method for improving the conversion of aromatic ester substrates by including β-cyclodextrin in an aqueous hydrolysis reaction system.

Keywords: Lipase; Enantioselective hydrolysis; 2-Phenylbutyric acid; Response surface methodology

1. Introduction

2-Phenylbutyric acid (2-PBA) (Fig. 1) has a wide range of applications, especially in the pharmaceutical industry. It is an important intermediate for the synthesis of a variety of aromatic acid drugs, which are commonly used to treat indigestion and some other diseases

and for their anti-thrombotic effect. For example, indobufen (2-(4-(1-oxoisoindolin-2-yl) phenyl) butanoic acid), synthesized from 2-phenylbutyric acid [1], is a new drug with reversible, selective multi-target, and anti-thrombotic properties [2,3]. Studies have shown that the anti-thrombotic activity of (*S*)-indobufen is more pronounced than that of (*R*)-indobufen, and a similar phenomenon has also been observed for most of the other aromatic acids [4]. Therefore, obtaining optically pure (*S*)-2-PBA is of great importance in the pharmaceutical industry. Currently, capillary electrophoresis [5], chromatography [6], and liquid-liquid extraction [7] are applied for the resolution of 2-PBA enantiomers. However, the low stability and sensitivity of capillary electrophoresis, high separation cost and low capacity of chromatographic techniques, and low selectivity of liquid-liquid extraction limit their applications. Hence, a new and efficient method for the resolution of 2-PBA enantiomers is urgently needed.

Over the past decades, enzymatic kinetic resolution has been widely used for enantiomeric resolution because of its excellent stereoselectivity, mild reaction conditions, and few side reactions [8,9], and most importantly, it is environmentally friendly [10]. Lipase, a type of hydrolases, is one of the most common classes of enzymes [11]. As reported in the literature, some free lipases or immobilized lipases, including lipase AYS, lipase AK, lipase PS, Novozym 40086, lipozyme TL IM, Novozym 435, and lipozyme RM IM, have been widely used for the chiral resolution of racemic esters, carboxylic acids, and alcohols [12-21]. Lipase CALA is a member of the α/β-hydrolase family. The excellent thermal stability, activity, and stereoselectivity of lipase CALA make it critically important for industry [22-25]. Enantioselective hydrolysis is a common method for the enzymatic kinetic resolution of

enantiomers [26,27]. However, the substrate conversion is generally low because of the poor solubility of the racemic substrate in the aqueous phase. Many methods have been utilized to address this problem, such as, modification of the lipase [28,29], immobilization of the lipase [30,31], and addition of a surfactant [32] or crown ether [33].

Cyclodextrin (CD) derivatives have attracted a large amount of attention in the fields of catalysis and separation [34,35], and they have a three-dimensional ring structure that forms a conical hollow cylinder with a hydrophobic interior and hydrophilic exterior. This distinct structure of CD derivatives can effectively increase the solubility of some hydrophobic drugs in water by forming inclusion complexes [14,36], which may be useful for enhancing the conversion of an ester substrate for enzyme-catalyzed hydrolysis. The results from several studies on enzymatic kinetic resolution in aqueous systems have supported this hypothesis [14,37,38].

The efficiency of lipase-catalyzed resolution depends on many process factors, and process optimization requires many experiments. Response surface methodology (RSM) is a powerful tool that has been used to evaluate the interactive effects among multiple process factors and describe the overall process [39]. In the present work, the lipase-catalyzed hydrolysis of 2-phenylbutyrate ester (2-PBAE) to prepare (*S*)-2-PBA with lipase CALA in aqueous medium was studied. β-CD derivatives were used as a green additive to improve the conversion of insoluble substrates in the aqueous hydrolysis reaction system. RSM was used to simulate and optimize the reaction process.

2. Experimental

2.1. Lipase and reagents

Commercially available free lipases, including lipase AYS from *Candida rugosa* (white powder, activity 30000 U/g), lipase AK from *Pseudomonas fluorescens* (light yellow powder, activity 20000 U/g), and lipase PS from *Burkholderia cepacia* (white powder, activity 30000 U/g), were purchased from Chengna Chemical Co., Ltd. (Nanjing, China). Lipase CALA from *Candida antarctica* (brown liquid, activity 6000 LU/g) was purchased from Gao Ruisen Technology Co., Ltd. (Beijing, China). Four immobilized lipases, including Novozym 435 from *Candida antarctica* immobilized on macroporous resin (activity 10000 U/g), lipozyme RM IM from *Rhizomucor miehei* immobilized on macroporous anion exchange resin (activity 250 IUN/g), Novozym 40086 from *Aspergillus oryzae* immobilized on acrylic resin beads (activity 275 IUN/g), and lipase TL IM from *Thermomyces lanuginosus* immobilized on silica (activity 250 IUN/g), were purchased from Novozymes Biopharma DK A/S (Denmark). All of the lipases were used without further treatment.

(*R*,*S*)-2-PBA containing equal proportions (50%) of the two enantiomers (purity > 98%) was obtained from Huacheng Chemical Industry Co., Ltd. (Shanghai, China). Tris (hydroxymethyl) methyl aminomethane was obtained from Shanpu Chemical Co., Ltd. (Shanghai, China). β-CD derivatives were purchased from Qianhui Fine Chemical & Co. Inc. (Shandong, China). Nine alcohols were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The solvents for chromatography were of high performance liquid chromatography (HPLC) grade and other reagents were of analytical grade, which were purchased from different suppliers.

2.2. Synthesis of 2-PBAE

(*R*,*S*)-2-PBAE was obtained by esterification reactions between (*R*,*S*)-2-PBA and different

alcohols, where (*R,S*)-2-PBA and an alcohol at a molar ratio of 1:1 were dissolved in 40 mL toluene and reacted at 110 °C with stirring for 12 hours in the presence of 0.05 g p-toluenesulfonic acid as a catalyst. After completion of the reaction, the resultant mixture was washed with saturated $NaHCO_3$ solution (3 × 40 mL) to remove the excess substrate and then washed with deionized water until neutral. The organic phase was separated, dried over anhydrous $MgSO_4$ for 12 hours, filtered and concentrated under reduced pressure to give the desired yellow liquid product. The yield and purity of 2-PBAE were higher than 90% and 95%, respectively. Because the esterification is a non-selective reaction, the proportion of each enantiomer before and after the reaction did not change.

2.3. Lipase-catalyzed hydrolysis of 2-PBAE

The lipase-catalyzed hydrolysis reaction was carried out as follows: a certain amount of substrate was first placed in a 25-mL reaction tube, and 1 mL 0.1 mol/L Tris/HCl buffer solution containing HE-β-CD was decanted into the tube. A thermostatic apparatus (IKA® RCT B S025) was employed to maintain a constant temperature for the reaction. After reaching the required temperature, lipase was added to start the reaction, and the reaction system was stirred with a magnetic stirrer at 600 rpm. The reaction was terminated with 1 mL of acetonitrile, and then, the reaction mixture was filtered. The reaction mechanism is shown in Fig. 2.

2.4. HPLC analysis

The concentrations of 2-PBA enantiomers were measured with a HPLC instrument (Waters 1525 binary pump system) equipped with UV/visible detection set at 225 nm (Waters 2489). The analysis was performed on an Inertsil ODS-3 column (250 mm × 4.6 mm, 5 μm). The mobile phase was consisted of methanol and 0.5% acetic acid aqueous solution containing

25 mmol/L HP-β-CD (36:64, v/v, pH = 4.0 adjusted with triethylamine). The flow rate was 0.8 mL/min, and the column temperature was set at 30 °C. The sample volume was 10 μL [7]. The retention times of (*S*)-2-PBA and (*R*)-2-PBA were 34.21 and 37.56 min, respectively. The purity of all synthesized esters was also confirmed by HPLC with a mobile phase consisting of acetonitrile and water (7:3, v/v).

The enantiomeric excess of the product (ee_p) and conversion rate (c) are defined as

$$ee_p = \frac{[(S)-acid]-[(R)-acid]}{[(S)-acid]+[(R)-acid]} \times 100\% \quad (1)$$

$$c = \frac{[(S)-acid]+[(R)-acid]}{[(S)-ester]_0+[(R)-ester]_0} \times 100\% \quad (2)$$

The enantioselectivity, E, is expressed as

$$E = \frac{\ln[1-(1+K)c(1+ee_p)]}{\ln[1-(1+K)c(1-ee_p)]} \quad (3)$$

where K is the reaction equilibrium constant and the subscript 0 is the initial concentration.

2.5. Experimental design and data analysis

Based on a single factor investigation, RSM was used to optimize the process of the lipase-catalyzed hydrolysis 2-PBAE. Compared with the common method, the RSM and central composite design can reflect the effects of process variables and the interactions among them more clearly, allowing the number of required experimental runs and time for process optimization to be reduced greatly.

The independent variables examined in this study were temperature, pH of the aqueous phase, HE-β-CD concentration, enzyme concentration, substrate concentration, and reaction time. The dependent variables were enantiomeric excess of the product and conversion of the ester substrate. The code and value range of the independent variables are shown in Table 1.

The experimental data were regression analyzed with the response surface to satisfy the following quadratic polynomials equations:

$$ee_p = \alpha_0 + \sum_{i=1}^{k} \alpha_i X_i + \sum_{i=1}^{k} \alpha_{ii} X_i^{\,2} + \sum_{i=1} \sum_{j=i+1}^{k} \alpha_{ij} X_i X_j \quad (4)$$

$$c = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^{\,2} + \sum_{i=1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad (5)$$

where ee_p and c are the predicted values of response, α_0 and β_0 are constants, α_i and β_i are linear coefficients, α_{ii} and β_{ii} are squared coefficients, α_{ij} and β_{ij} are cross-product coefficients, k is the number of factors, and X_i and X_j are the independent variables. The experimental data obtained were analyzed by analysis of variance (ANOVA) and fitted to a second-order polynomial equation using multiple regression analysis.

3. Results and discussion

3.1. Lipase-catalyzed hydrolysis reaction system

3.1.1. Screening of lipase

The activities of eight lipases were evaluated to screen the optimum lipase for the hydrolysis resolution of racemic hexyl 2-phenylbutyrate (2-PBAHE). As shown in Table 2, lipase AK and lipozyme TL IM had no catalytic activity for the hydrolysis of the substrate. Lipase PS and Novozym 40086 had more activity toward (*R*)-2-PBAHE. Lipase CALA exhibited the highest selectivity (ee_p up to 64.46%) among the lipases; however, the conversion rate of 5.45% was relatively low. In the case of Novozym 435, the highest conversion rate of 36.6% was obtained, but with nearly no selectivity toward 2-PBAHE. To obtain a high optical purity of the target product, lipase CALA was selected for further investigation.

3.1.2. Influence of substrate structure

The lipase-catalyzed hydrolysis reaction occurs by forming an intermediate complex between the active site of the lipase and the substrate, following a strict molecular match. Many studies have noted that substrate access to the active site of the lipase is one of the main factors controlling the specificity of catalysis and enantioselectivity [8]. 2-PBAEs with different carbon chain lengths were investigated under identical conditions. As shown in Table 3, the carbon chain length of 2-PBAE had a significant effect on ee_p and c in the lipase-catalyzed hydrolysis reaction. For substrates with a straight chain, both ee_p and c increased gradually, with the increase in carbon chain length ranging from methyl ester to hexyl ester. For branched chains, ee_p and c were obviously lower than those for a straight chain with the same number of carbon atoms. Notably, there were rapid decreases in ee_p and c for octyl ester. These results suggest that the substrate specificity may be depend on the binding between the length and shape of the substrate and the activity center of lipase. Short-chain substrates with lower specific interactions and long-chain substrates with larger steric hindrance result in decreased c [40]. Based on the above results, the 2-PBAHE was selected as the optimal substrate for further experiments.

3.1.3. Screening of CD derivatives

As shown in Table 3, a high ee_p was achieved with lipase CALA as the catalyst, but the c of (*S*)-2-PBAHE was relatively low for industrial-scale production. This may be due to the low solubility of 2-PBAHE in this aqueous reaction system. Thus, increasing the solubility may be an effective way to improve the conversion. CD derivatives have a special structure with an outer hydrophilic area and inner hydrophobic cavity. Many studies have suggested that they can form inclusion complexes with a variety of hydrophobic aromatic compounds

[14,34,36,38,]. It was found in our previous work that β-CD derivatives can significantly increase the solubility of hydrophobic enantiomers and recognize a series of enantiomers [7,41,42]. In this study, five β-CD derivatives were separately added to the aqueous reaction system (Fig. 3). As shown in Table 4, each β-CD derivative had a different effect on *c*, possibly because the solubility was enhanced through the formation of inclusion complexes between 2-PBAHE and β-CD derivatives. The stability of the inclusion complexes can be influenced by the chemical structure of β-CD derivatives. It was observed that adding SBE-β-CD resulted in nearly no improvement in *c*. This phenomenon can be explained by the dissociation of SBE-β-CD, which makes it highly polar and then hinders the inclusion of the hydrophobic 2-PBAHE. The *c* increased when HP-β-CD, CM-β-CD, HE-β-CD or Me-β-CD was used, and ee_p remained nearly unchanged. The reason for these results may be that more stable inclusion complexes are formed between the above β-CD derivatives and 2-PBAHE because of the better alignment of their molecular structure. Among them, HE-β-CD was selected as the most suitable additive because of its low price and easy access.

3.2. Regression analysis and model fitting

The effects of six independent variables on the dependent variables (ee_p and *c*) were investigated (Table S1). These experimental results were fitted with a second-order polynomial equation, and the values of the regression coefficients were calculated. The multiple quadratic regression models for ee_p and *c* can be expressed by the second-order polynomial equations (6) and (7):

$$ee_p(\%) = 92.85 + 2.92A + 8.62B - 0.96C - 3.77D - 0.14E - 3.32F + 8.75BF + 0.7CF + 0.084DF - 9.36A^2 - 7.76B^2 - 1.63C^2 - 2.94D^2 - 0.24E^2 - 0.13F^2 \quad (6)$$

$$c(\%) = 27.70 - 3.09\,A - 9.49\,B + 6.72\,C + 8.73\,D - 10.80\,E + 9.70\,F - 11.32\,BF + 4.80\,CF + 2.39\,DF - 7.66\,A^2 - 7.37\,B^2 - 2.74\,C^2 - 2.59\,D^2 + 1.64\,E^2 - 3.46\,F^2 \quad (7)$$

The performance of the models was checked to prevent misleading results. Pareto analysis of variance (ANOVA) was performed to test the accuracy of the models (Table S2). The smaller the *p*-value was, the more significant the corresponding coefficient. A *p*-value < 0.0500 indicated that the model terms were significant. As shown in Table S2, the *p*-values for all the corresponding coefficients were lower than 0.0001, indicating that the quadratic models (6) and (7) were significant and adequate to simulate the relationship between the responses and the independent variables. Moreover, the *p*-value can also be used to check the significance of each coefficient and indicate the pattern of interactions among variables. In this case, A, C, D, A^2, B^2, C^2, and D^2 were significant for model ee_p, and the order of significance was A > C > D > E > F > B. Similarly, A, C, D, E, A^2, B^2, and F^2 were significant for model *c*, and the order of significance was A > C > E > D > F > B.

From the analysis of variance results shown in Table S2, the R^2 values of ee_p and *c* were 0.9850 and 0.9952, respectively, indicating that the models were reliable. The "Pred R-Squared" values of 0.9681 and 0.9770 were in reasonable agreement with the "Adj R-Squared" values of 0.9769 and 0.9926, respectively. The coefficients of variation of the models were 0.45% and 4.69%, respectively, indicating that the models had good stability. Therefore, the ee_p and *c* of the lipase-catalyzed hydrolysis resolution 2-PBAHE for the preparation of (*S*)-2-PBA can be analyzed and predicted by these models.

3.3. Effects of multiple factors

3.3.1. Effects of temperature and pH value

Fig. 4a and Fig. 4b show the surface response plots for ee_p and *c* as functions of pH from

4.0 to 8.0 and temperature from 65 to 90 °C. As shown in Fig. 4, *c* and ee_p increased at first and then decreased as the temperature increased. As the temperature increased, the chance of collision between the enzyme and the substrate molecules increased, which may favor the formation of the enzyme-substrate complex, then promoting *c*. However, an excessive temperature may result in the decomposition of weak ions and hydrogen bonding, causing the denaturation of the protein structure [43]. The ee_p also decreased because the conformation of the active site of the enzyme was altered at high temperatures, affecting the enantioselectivity of the enzyme. Moreover, as shown in Fig. 4, an extreme pH will lead to a decrease in ee_p. The active center of the lipase is a "catalytic triad" composed of Ser, His and another amino acid residue. The imidazole moiety of the His directly participates in the reaction, which is the key step for the catalytic properties of lipase [44]. An extreme pH can disrupt the lipase tertiary structure and hydrogen bonding, resulting in the loss of enantioselectivity and catalytic activity [12,13]. In addition, the pH can also alter the existing state of the substrate and lipase, which affects the binding and catalytic ability of the enzyme towards the substrate. Fig. 4a shows that the highest ee_p was obtained at a pH of 6.5. To obtain an ee_p value greater than 96%, a temperature of 83 °C and pH of 6.5 were set as the optimal conditions based on the response surface graphs.

3.3.2. Effects of the concentrations of HE-β-CD and the substrate

As shown in Table 4, the addition of HE-β-CD improved the conversion. In order to further optimize the reaction system, the effects of the concentrations of HE-β-CD and the substrate and their mutual interaction on *c* and ee_p were studied. As shown in Fig. 5, under a fixed substrate concentration, ee_p first increased and then decreased as the HE-β-CD

concentration increased. A possible reason for this result is that HE-β-CD can form inclusion complexes with 2-PBAHE and has a stronger inclusion ability towards (*S*)-2-PBAHE than (*R*)-2-PBAHE. However, an excessive HE-β-CD concentration (＞60 mmol/L) may lead to more (*R*)-2-PBAHE being included, resulting in a drop in ee_p. Because the 2-PBAHE molecular is soluble in the reaction system when HE-β-CD was added, *c* increased rapidly with the increase in HE-β-CD concentration, and then, the trend became more subtle. There was a decrease in *c* as the substrate concentration increased with a fixed HE-β-CD concentration, possibly because the active sites of the enzyme are limited or a part of the substrate could not be catalyzed at the fixed time, which lead to a decrease in *c*. It was concluded that the appropriate concentration of HE-β-CD was 60 mmol/L to obtain a high ee_p (>95%) and that the concentration of the substrate should not be too high.

3.3.3. Effects of the concentrations of lipase CALA and the substrate

The effects of the concentrations of lipase and the substrate on ee_p and *c* were further studied. As shown in Fig. 6, the concentration of the substrate had few effects on the ee_p but a large effect on conversion, the same phenomenon observed in Fig. 5. The *c* increased continuously as the lipase concentration increased, while the ee_p showed a small change from 20 to 50 mg/mL and then decreased dramatically with a further increase to 90 mg/mL. This phenomenon was consistent with the literature [16]. This can be explained by the fact that the available active sites for substrate binding increase, and the reaction rates for the two enantiomers of the substrate both increase at a high lipase concentration. (*S*)-2-PBAHE reaches its reaction equilibrium faster, and the apparent rate decreases gradually. However, (*R*)-2-PBAHE is far from reaction equilibrium, and the reaction rate is enhanced by increasing

the concentration of lipase, which results in a decrease in ee_p. From the perspective of economic efficiency, a higher substrate concentration is preferred while obtaining a suitable conversion rate. To obtain a higher ee_p and satisfactory c, the proposed concentrations of the lipase and substrate were 50 mg/mL and 30 mmol/mL, respectively.

3.3.4. Effects of the concentration of HE-β-CD and reaction time

As shown in Fig. 7, ee_p and c were clearly influenced by the concentration of HE-β-CD and reaction time. The ee_p first increased to a maximum value and then decreased with the further increase in HE-β-CD concentration, showing a similar trend as that observed in Fig. 5. At any HE-β-CD concentration, ee_p decreased with time (Fig. 7a). As shown in Fig. 7b, the c increased slightly under a low HE-β-CD concentration, even though the reaction time continuously increased. However, under a relatively high concentration of HE-β-CD, c increased rapidly at first and then increased slowly after 18 hours. This phenomenon can be explained by the fact that a larger number of complexes between HE-β-CD and the substrate are formed in the aqueous phase at high HE-β-CD concentrations, which enhances the reaction as explained above. Therefore, the concentration of HE-β-CD was set at 60 mmol/L to achieve a maximum ee_p and relatively high c. Prolonging the reaction time can increase c, but ee_p will decrease after 18 hours of reaction time. Therefore, a reaction time of 18 hours was selected as the suitable reaction time.

3.3.5. Effects of the concentration of lipase CALA and reaction time

In a lipase-catalysis reaction, the reaction rate can be increased by increasing the concentrate of lipase, but the cost is also increased. Therefore, the concentration of lipase and reaction time should be optimized. As shown in Fig. 8a, ee_p decreased rapidly as the lipase

concentration and reaction time increased. In addition, as show in Fig. 8b that increasing the lipase concentration shortened the reaction time to achieve a high conversion. Combining the results in Figs. 8a and 8b, 50 mg/mL lipase and a reaction time of 18 hours were proposed for the hydrolysis of 2-PBAHE to prepare (*S*)-2-PBA, which conform with the results shown in Fig. 6 and Fig. 7.

3.4. Validation of the model

In order to further test the validity of the model predictions, additional independent experiments were carried out at the suggested optimum conditions: a 1 mL Tris/HCl buffer solution system containing 60 mmol/L HE-β-CD, 50 mg/mL lipase CALA, and 30 mmol/L substrate at 83 °C and pH of 6.5 and reacting for 18 hours. Three groups of parallel experiments were performed. Fig. 9 shows the chromatograms of the products of the lipase CALA-catalyzed hydrolysis of (*R*,*S*)-2-PBAHE. The values of ee_p and c were 96.05% and 27.28%, respectively. The predicted values of the model were 95.31% and 28.96%, respectively. The relative errors of ee_p and c between the experimental value and the predicted value were less than 1.00%, indicating that the model is reliable to predict the preparation of (*S*)-2-PBA by the lipase CALA-catalyzed hydrolysis of 2-PBAHE.

4. Conclusions

The lipase-catalyzed hydrolysis of 2-PBAE to prepare (*S*)-2-PBA was studied systematically. The effects of various factors on enantiomeric excess and conversion rate were investigated, such as the type of β-CD derivatives, substrate concentration, lipase concentration, HE-β-CD concentration, temperature, pH, and reaction time. Lipase CALA was selected as the most effective catalyst. The addition of HE-β-CD increased the conversion by 1.5 times. RSM

was used to obtain the optimal process parameters and understand the relationship between the resolution efficiency and process parameters. The reaction system had an optimal temperature and pH. The concentration of the substrate had an effect on *c* but not on ee_p. Under the optimal conditions: 50 mg/mL lipase CALA, 30 mmol/L substrate, 60 mmol/L HE-β-CD, pH of 6.5, temperature of 83 °C and reaction time of 18 hours, the experimental values of ee_p and *c* were 96.05% and 27.28%, respectively, which are in good agreement with the predicted values. This work provides a simple and feasible method for obtaining optically pure (*S*)-2-PBA and demonstrates that β-CD derivatives are useful for improving the conversion of an aromatic ester substrate for the hydrolysis process in an aqueous reaction system. The results provide theoretical guidance for process design and operation. However, before applying the resolution system to industrial-scale equipment, for example, a stirred tank reactor, additional factors, including scaling effects, should be considered.

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Figure legends

Fig. 1. The chemical structure of (*S*)-2-PBA.

Fig. 2. Reaction diagram of hydrolysis resolution of (*R,S*)-2-PBAE by lipase.

Fig.3. The mechanism of inclusion interaction between β-CD derivatives and 2-PBAHE.

Fig. 4. Effects of temperature and pH value on (a) ee_p and (b) *c*. Conditions: 60 mmol/L HE-β-CD, 30 mmol/L substrate, 50 mg/mL enzyme and reaction time of 18 hours.

Fig. 5. Effects of the concentrations of HE-β-CD and the substrate on (a) ee_p and (b) *c*. Conditions: 50 mg/mL enzyme, temperature of 83 °C, pH of 6.5 and reaction time of 18 hours.

Fig. 6. Effects of the concentrations of enzyme and the substrate on (a) ee_p and (b) *c*. Conditions: 60 mmol/L HE-β-CD, temperature of 83 °C, pH of 6.5 and reaction time of 18 hours.

Fig. 7. Effects of the concentrations of HE-β-CD and reaction time on (a) ee_p and (b) *c*. Conditions: 30 mmol/L substrate, 50 mg/mL enzyme, temperature of 83 °C and pH of 6.5.

Fig. 8. Effects of the concentrations of enzyme and reaction time on (a) ee_p and (b) *c*. Conditions: 60 mmol/L HE-β-CD, 30 mmol/L substrate, temperature of 83 °C and pH of 6.5.

Fig. 9. Chromatograms of (a) the sample of racemic (*R,S*)-2-PBA and (b) the hydrolysis products of (*R,S*)-2-PBAHE.

Fig. 1

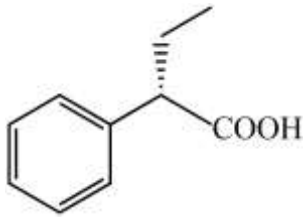
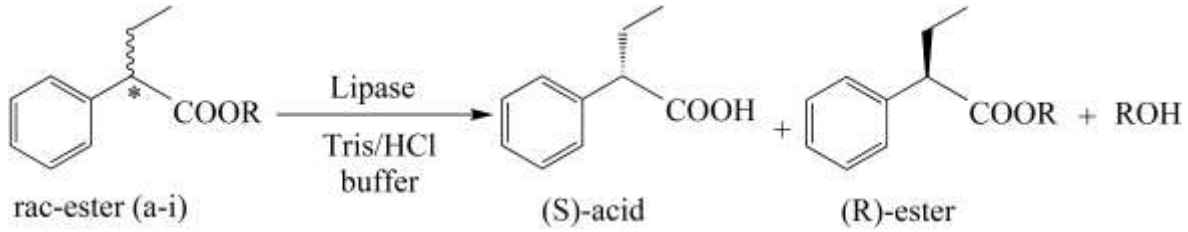


Fig. 2



rac-ester (a-i) (S)-acid (R)-ester

a: R = CH_3
b: R = C_2H_5
c: R = n-C_4H_9
d: R = iso-C_4H_9
e: R = n-C_5H_{11}
f: R = iso-C_5H_{11}
g: R = C_6H_{13}
h: R = C_7H_{15}
i: R = C_8H_{17}

Fig. 3

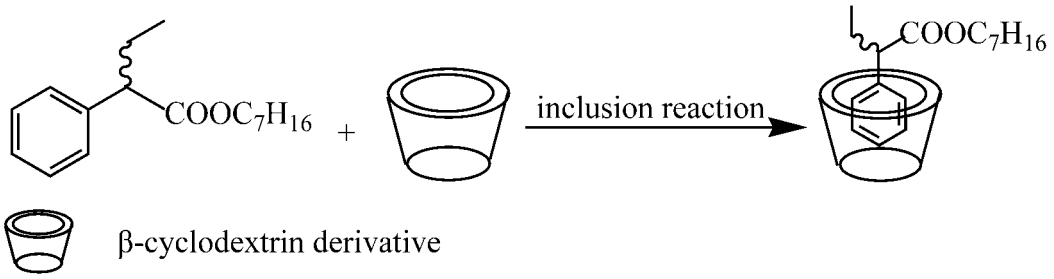


Fig. 4

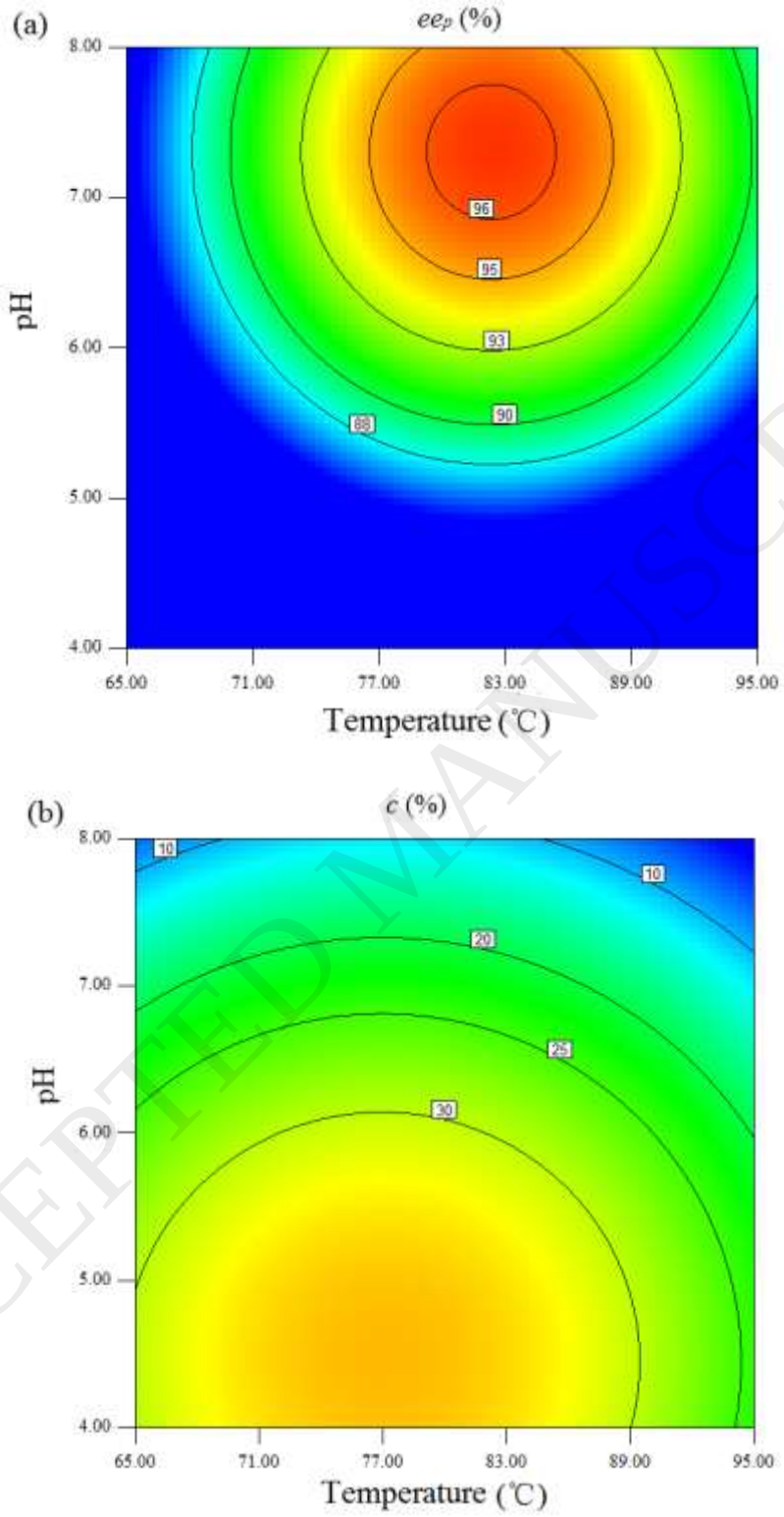


Fig. 5

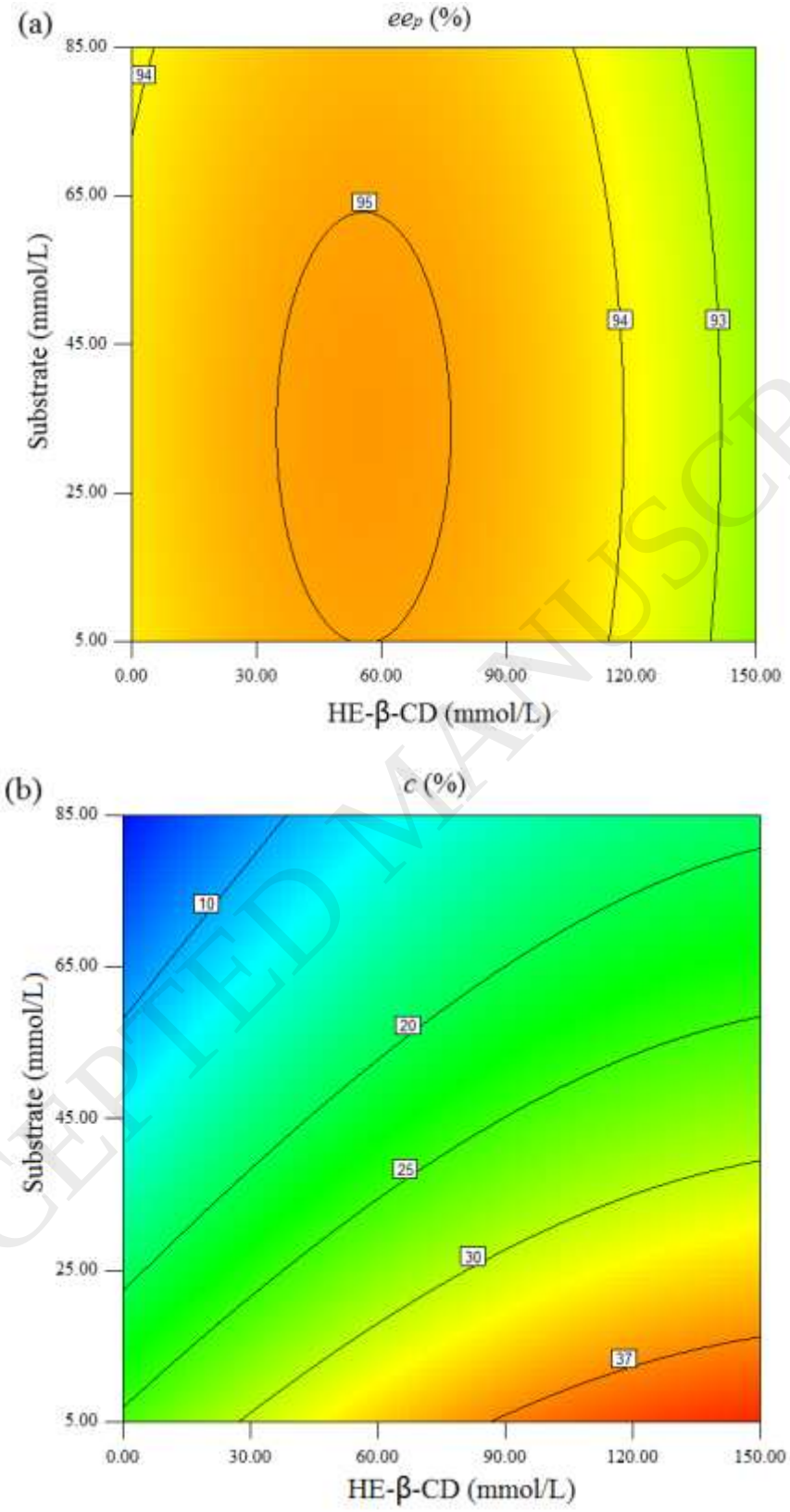


Fig. 6

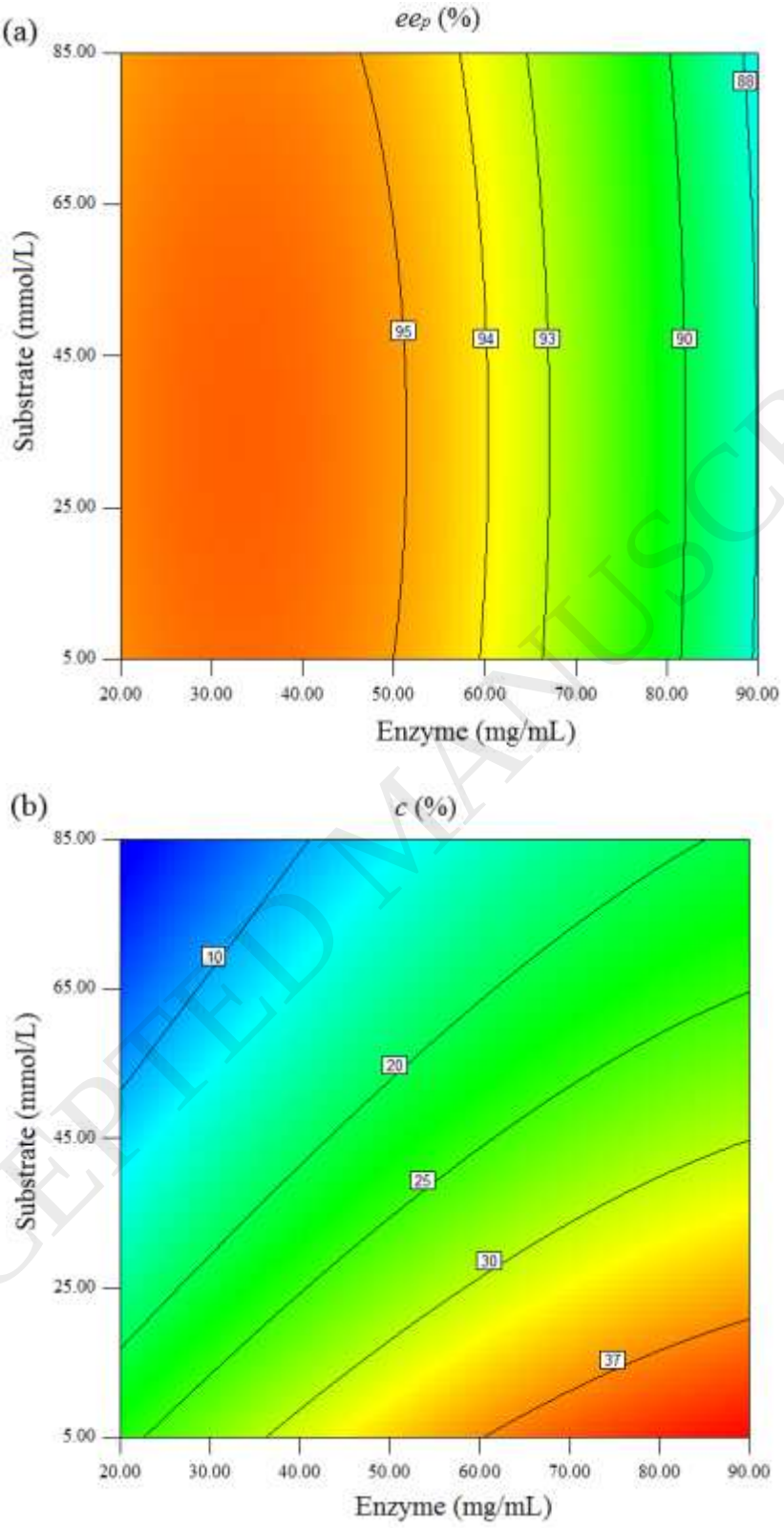


Fig. 7

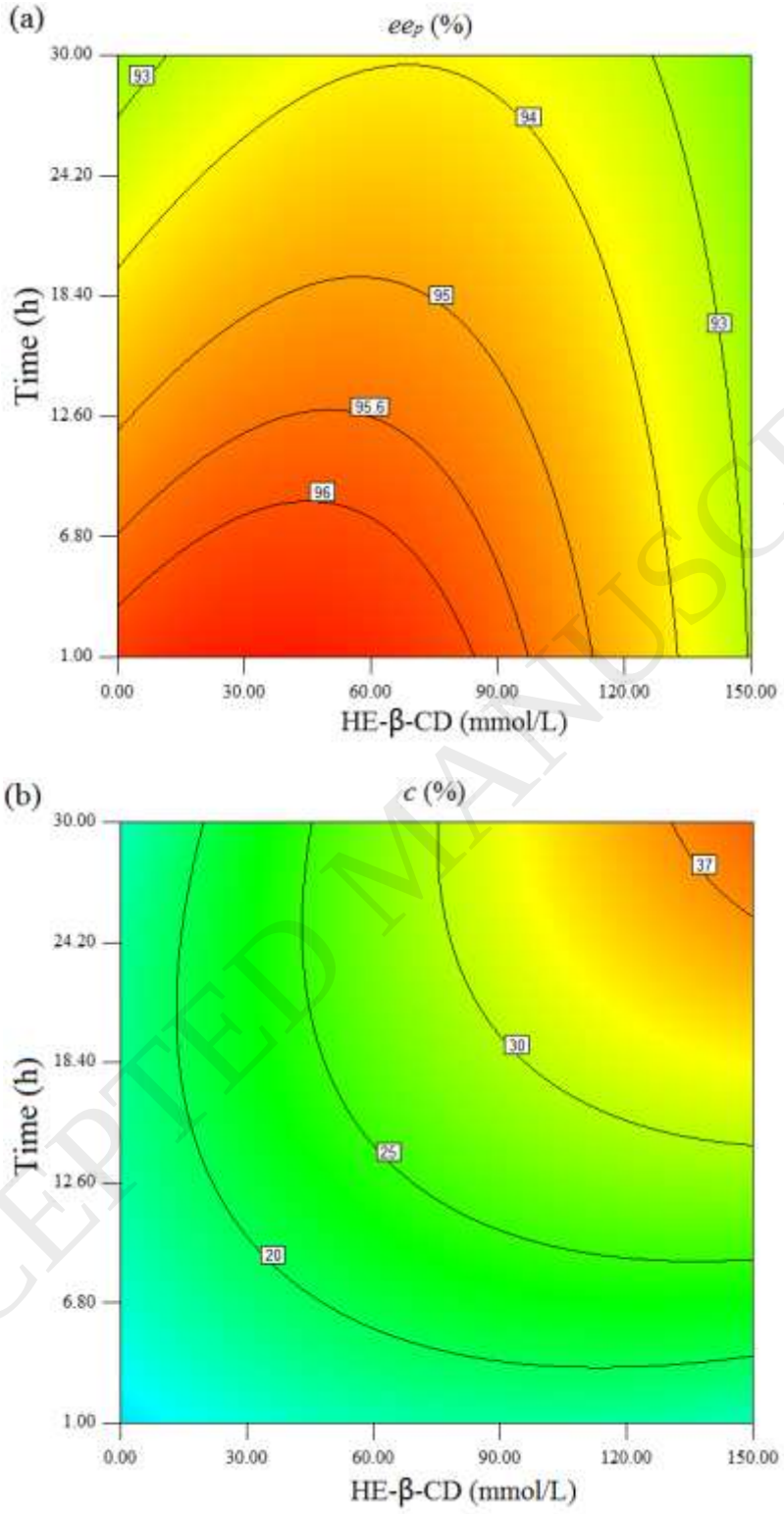


Fig. 8

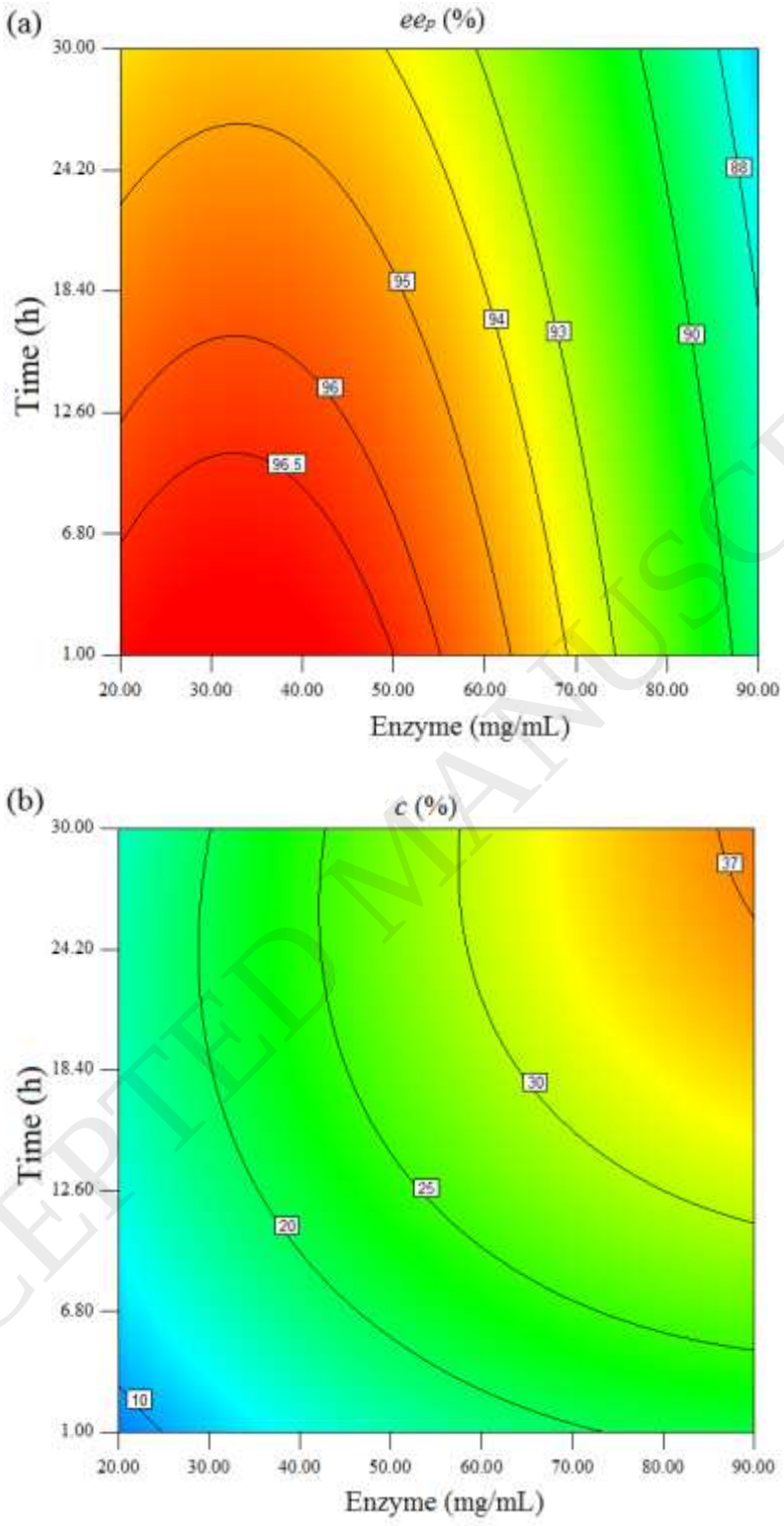


Fig. 9

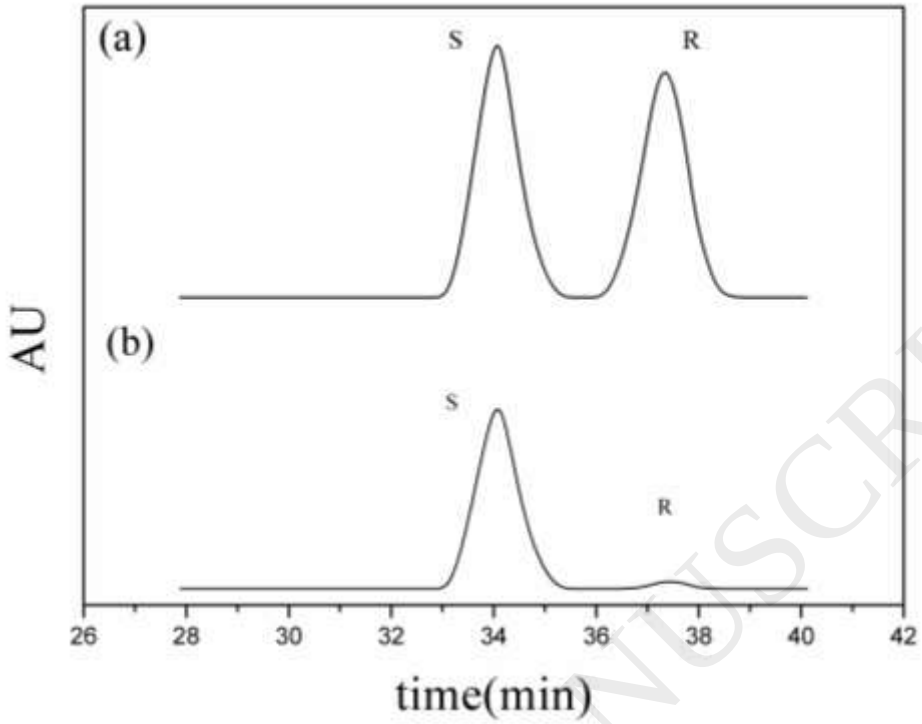


Table 1. Independent variables and their ranges.

Independent variable	Code	Units	Low	High
Temperature	A	°C	65	95
pH	B	1	4.0	8.0
HE-β-CD concentration	C	mmol/L	0	150
Enzyme concentration	D	mg/mL	20	90
Substrate concentration	E	mmol/L	5	85
Reaction time	F	h	1	30

Table 2. Effect of different lipases.

Enzyme	ee_p(%)	c(%)	E	Selectivity
Lipase AYS	21.36	0.98	1.55	S
Lipase CALA	64.46	5.45	4.80	S
Lipase AK	N	N	N	N
Lipase PS	36.76	0.47	2.17	R
Novozym 40086	19.30	14.95	1.53	R
Lipozyme TL IM	N	N	N	N
Novozym 435	0.32	36.66	1.01	S
Lipozyme RM IM	25.06	0.49	1.67	R

N: Not reacted.

Conditions: 20 mmol/L substrate, 10 mg/mL enzyme, temperature of 50 °C, pH of 6.5 and reaction time of 4 hours.

Table 3. Effects of different substrates.

Substrate	ee_p(%)	c(%)	E
a	91.52	9.36	24.79
b	94.68	11.52	41.31
c	94.84	12.28	43.54
d	93.14	9.35	30.95
e	95.44	12.55	49.03
f	94.96	10.82	43.33
g	96.74	15.44	71.75
h	96.20	14.31	60.44
i	91.54	7.81	24.44

Conditions: 15 mmol/L substrate, 30 mg/mL enzyme, temperature of 83 °C, pH of 6.5 and reaction time of 23 hours.

Table 4. Effects of different β-CD derivatives.

Cyclodextrin	ee_p(%)	c(%)	E
Non-cyclodextrin	95.72	14.07	53.32
Hydroxyethyl-β-cyclodextrin (HE-β-CD)	96.18	18.72	63.77
Hydroxypropyl-β-cyclodextrin(HP-β-CD)	95.20	17.90	49.82
Carboxymethyl-β-cyclodextrin (CM-β-CD)	95.06	16.16	47.24
Sulfobutyl-β-cyclodextrin (SBE-β-CD)	95.36	14.20	49.15
Methylated-β-cyclodextrin (Me-β-CD)	96.18	17.49	62.69

Conditions: 15 mmol/L substrate, 15 mmol/L β-CD derivatives, 30 mg/mL enzyme, temperature of 83 °C, pH of 6.5 and reaction time of 16 hours.

Table 5. Verification of the predicted models.

Model parameters	Model of ee_p	Model of c
R-Squared	0.9850	0.9952
Adj R-Squared	0.9769	0.9926
Pred R-Squared	0.9681	0.9770
Adeq Precision	47.91	75.11
Coefficient of variation (%)	0.45	4.69