Optimization of Immobilization Conditions of *Candida antarctica* Lipase Based on Response Surface Methodology

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The conditions, including mass ratio of PEG4000 to lipase, pH, and mass ratio of diatomites to lipase, for immobilization of *Candida antarctica* lipase with PEG non-covalent modification were optimized by means of the response surface methodology (RSM). The immobilized lipase specific activity in the reaction of transesterification was selected as the response value. A mathematical model was developed to investigate the influences of various immobilization parameters and to predict the optimum immobilization conditions for lipase. The maximal specific activity was predicted to occur when PEG4000: lipase (*w*/*w*) was 7.61, diatomites: lipase (*w*/*w*), 9.92 and pH, 7.52, respectively. A repeat immobilization experiment of lipase was carried out under the optimized conditions for the verification of optimization. The maximal specific activity obtained experimentally was 56.11 U mg^{-1} pr., which was significantly higher than that obtained under unoptimized conditions.

Key words:
Response surface methodology, *Candida antarctica* lipase, immobilization, optimization, PEG

Introduction

Lipases (EC 3.1.1.3) are enzymes that may catalyze many different reactions such as hydrolysis, esterification and interesterification, which play an important role in industrial production. The industrial applications fields of lipases comprise organic synthesis, pharmaceuticals, food additives, detergents, and bio-surfactants.[1] In recent years, lipases have also been applied for biodiesel production,[2,3,4] which has attracted much attention. Lipases are produced by many microorganisms and higher eukaryotes like plants and animals. Compared to lipases from plants and animals, microbial lipases have obvious advantages including easy extraction and potential for an unlimited supply, which made them suitable for industrial applications. Moreover, microbial lipases are preferred enzymes in production of chiral compounds because of their chemo, regio and stereospecificity.[5] Lipases from *Candida antarctica* have many excellent characteristics including higher thermostability[6] in lower pH and high stereoselectivity in hydrolysis reaction.[7] However, at present, the high cost of lipase production remains the obstacle to its large-scale industrial applications as catalysts.[8]

Enzymes immobilization is considered an effective technology to obtain reproducible enzymes with high stability, and consequently sometimes the cost of enzymes as catalysts is reduced to some extent. However, only few immobilized enzymes are used in industry in large-scale. That is because of the high cost of carriers and reagents, low degree of immobilization and poor stability of immobilized enzymes limiting further industrial applications of lipase. Lipase has been successfully immobilized on many different carriers like gelatin,[9] macroporous absorbent resin[10] and nylon membrane.[11] Therein, Novozyme 435 (Novozymes) is a commercial lipase immobilized on the macroporous absorbent resin. Its immobilization cost, however, is relatively higher because macroporous absorbent resin is expensive. Therefore, it is useful to develop cost-effective systems with easy and feasible immobilized methods, good-performance carriers for large-scale industrial applications of immobilized lipase. In our study, we developed a new method to immobilize lipase from *C. antarctica* in which diatomites was used as absorbent carrier and PEG was used as a non-covalent reagent. To obtain the immobilized lipase with PEG non-covalent modification which has high specific activity of transesterification, the immobilization conditions were optimized using response surface methodology (RSM).[12] RSM is one of the popularly used optimization procedures, mainly developed based on full factorial central composite design (CCD). RSM is a collection of mathematical and statistical techniques that are useful for modeling and analysis in applications where a response of interest is influ-

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enced by several variables and the objective is to optimize this response. The first step in RSM is finding a suitable approximation for the true relationship between the response and independent variables. Usually a low-order polynomial in some region of the independent variables is employed for modeling. If the response is well modeled by a linear function of the independent variables, then the approximating function is the first-order. If there is curvature in the system, then a polynomial of higher degree must be used, such as the second-order model. RSM helps identify the effective factors, study interactions, select optimum conditions and quantify the relationships between one or more measured responses and the vital input factors in a limited number of experiments.

Materials and methods

Lipase and immobilization

Lipase that was produced from *Candida antarctica* DMS-3855 by our laboratory, was with transesterification activity of 317.6 U g^{-1}, specific activity in transesterification reaction of 5543.46 U g^{-1} protein (U g^{-1} pr.). CD03 type diatomites were employed in our immobilization experiments. Polyethylglycol (PEG4000) was purchased from Guangzhou Donghai Chemical Co., Ltd. All other chemicals used were of analytical grade.

C. antarctica DMS-3855 fermentation solution[13] was centrifuged at 4 °C and 5000 rpm for 10 min. An overview of our purification procedure on the obtained supernatant is given here with details available elsewhere.[14,15] Solid ammonium sulphate was added to form 40 % saturation after the extract was filtered to obtain the supernatant, and then 0.3 fold acetone of the original volume of supernatant was added. Acetone was added at 0 °C in all of our tests. The mixture was centrifuged for 10 min at 5000 rpm. Then solid ammonium sulphate was added to the supernatant to form 75 % saturation of original supernatant. The mixture was centrifuged for 10 min at 5000 rpm. The aqueous solution of the resulted precipitate was fractionally precipitated using acetone. The resulted lipase precipitate was dissolved and concentrated by super-filtration to remove ammonium sulphate. The lipase solution (phosphate buffer) was dried by vacuum refrigerated drier. The final solid powder was the purified lipase powder.

The immobilization procedure is given here. Diatomites were wetted with ethanol prior to use. After ethanol removal by filtration and washing with distilled water, the carrier was washed with phosphate buffer, pH 7.8. In this work, lipase was immobilized by "lyophilization in vacuum method".[16] The process was as follows. 100 mg lyophilizated lipase powders and the proper PEG powders were added into a phosphate buffer solution with proper pH value (Table 1, Table 4 and Table 5) at room temperature. Quantitative pretreatment carrier (pre-treated in phosphate buffer solution for 2 h) was added into the above mixture and the new mixture was placed in an oscillator at 30 °C and 150 rpm for 1 h. Finally, the immobilized lipase with PEG non-covalent modification was obtained after filtration at vacuum, washing with phosphate buffer solution and drying by vacuum. Aliquots were withdrawn from this mixture to determine enzyme concentration by UV adsorption measurements as a function of time.

Table 1 – *The experimental factors and levels for steepest ascent test*

Levels	Factors, real values		
	Z_1	Z_2	Z_3
−1	3	5	6.5
0	5	7	7.0
1	7	9	7.5

Enzyme transesterification activity assay

The immobilized lipase specific activity in the reaction of transesterification was assayed in the transesterification reaction between 1,2,3-oleic acid glyceride and methanol. In a typical experiment, 2 g of 1,2,3-oleic acid glyceride and 0.2074 g of methanol were mixed in a flask of 25 mL with molar ratio of 1 : 1, and the enzymatic transesterification started by adding 0.22 g of immobilized lipase to the substrate solution after preheating for 10 min at 40 °C. All reactions were carried out at a constant temperature of 40 °C with orbital shaking at 200 rpm. At scheduled times, aliquots were withdrawn from the reaction mixtures and filtrated with a 0.45 μm film, and heptadecanoic acid methyl ester was added into the filtrate as internal standard. The conversion was determined by GC [Make: Shimadzu, Column: SE54 capillary column (60 m × 0.25 mm × 0.5 μm), Detector: FID, Temperature: oven temperature from 55 °C (initial time 5 min) to 180 °C with a heating rate of 10 °C min^{-1}, Carrier gas: N_2]. Control experiments showed that the nonenzymic reaction was negligible in all conditions used. One activity unit (U) of lipase was defined as the amount of enzyme required to catalyze substrate to produce 1 μmol of oleic acid methyl ester per min at 40 °C and pH 8.0.

Experimental design and evaluation

"Steepest ascent method" experiment design

In regression design, primary estimates of experimental parameters may be far from their optimal values. To quickly approach the optimum value, the "steepest ascent method"[18] was used by designing some experiments in the grads direction of experiment variables. In our study, the different factors such as mass ratio of diatomites to lipase and pH value were chosen as the effect variables and expressed as Z_1, Z_2 and Z_3, respectively. The immobilized lipase specific activity in the reaction of transesterification (U mg^{-1} pr., Y) was chosen as the response value. The code factors of the above three independent variables were expressed as X_1, X_2 and X_3, respectively. According to our previous experimental results, a particular area had been chosen for optimization, namely mass ratio of PEG4000 to lipase (3–7), mass ratio of diatomites to lipase (5–9) and pH (6.5–7.5). The code level of each variable was designated as –1, 0 and + 1, respectively (Table 1). Eq. (1) was applied to calculate the coded level:

$$X_j = (Z_j - Z_{0j})/\Delta_j \quad (1)$$

Where $Z_{0j} = (Z_{1j} + Z_{2j})/2$; $\Delta_j = (Z_{2j} - Z_{1j})/2$; Z_{0j} are the zero level of Z_j; Δ_j is the semidiameter of Z_j

Eq. (2) was used to calculate Z_j by ascent step length (d b_j Δ_j).

$$Z_j = Z_{0j} + k\ d\ b_j\ \Delta_j \quad (2)$$

Where k = 1, 2,…,m; b_j are the linear regression coefficient of X_j; d is the coefficient of step length variety.

Design of RSM and evaluation

To search for the optimum immobilization conditions for the best specific activity, experiments of 3 factors and 3 levels were performed according to a central composite design of Box-Benhnken[19] experimental plan (Table 2). The three factors are mass ratio of PEG4000 to lipase, mass ratio of diatomites to lipase, and pH value, respectively. The Statistical Analysis System (STATGRAPHICS Centurion XV version 15.2.05) was used for experimental design of RSM, regression analysis of the data and estimated the determination coefficient (R^2) of the model. The surface plot and contour plot were also obtained.

The immobilized lipase specific activity in the reaction of transesterification was analyzed by multiple regression through the least squares method to fit the following eq. (3):

$$Y = A_0 + \Sigma A_i\ X_i + \Sigma A_{ii}\ X^2_{ii} + \Sigma\Sigma A_{ij}\ X_i\ X_j \quad (3)$$

Table 2 – *Box-Behnken Central Composite Design and experimental results for RSM*

RUNS	Independent variables, coded levels			Response
	X_1	X_2	X_3	Y/U mg^{-1} pr.
1	−1	−1	0	45.58
2	−1	1	0	44.21
3	1	−1	0	47.93
4	1	1	0	46.82
5	0	−1	−1	49.59
6	0	−1	1	47.88
7	0	1	−1	45.67
8	0	1	1	48.51
9	−1	0	−1	46.07
10	1	0	−1	42.69
11	−1	0	1	43.21
12	1	0	1	49.94
13	0	0	0	54.71
14	0	0	0	56.04
15	0	0	0	55.75

$X_1 = (Z_1 - 7.5)/1.0$; $X_2 = (Z_2 - 10.0)/1.0$; $X_3 = (Z_3 - 7.5)/0.2$

Where Y is the measured response variable, A_0 is the constant and A_i, A_{ii}, A_{ij} are the regression coefficients of the model obtained by multiple regression (which represent the linear, quadratic and cross-product effects of the factors on the response, respectively), and X_i, X_j represent the independent variables. The quality of fit of the polynomial model equation was expressed using coefficient of determination R^2.

Statistical analysis

Data from the Box-Behnken Central Composite Design for the immobilized lipase specific activity in the reaction of transesterification were subjected to a quadratic multiple regression analysis using least-squares regression to obtain the parameter estimated for the mathematical model. The regression analysis and analysis of variance (ANOVA) were carried out using the Multiple Regression procedure (The User's Guide to STATGRAPHICS® Centurion XV version 15.2.05; StatPoint, Inc., 2005) contained in the STATGRAPHICS statistical package to fit quadratic polynomial equations for all response variables. The response surface was generated by fitting the quadratic polynomial equation obtained from Multiple Regression analysis, holding one parameter at a constant value and changing the other two variables.

Results and discussion

Steepest ascent method

The linear regression model that fit the following eq. (4) was built by regression analysis:

$$Y = 40.4125 + 0.8125 \cdot X_1 + \\ + 1.0375 \cdot X_2 + 0.7625 \cdot X_3 \quad (4)$$

Where the determination coefficient (R^2) was 0.9496, it indicates that 94.96 % of the experimental data can be explained by the model. As may be seen from eq. (4), the linear coefficient of the three variables are both positive, which show that the activity of immobilized lipase can be enhanced by increasing the mass ratio of PEG4000 to lipase, the mass ratio of diatomites to lipase and pH value.

According to ascent step length 0.5 from the mass ratio of PEG4000 to lipase, the coefficient of step length variety (d) can be calculated by the following equation: d = 0.5/(b1 Δ1). So, d = 0.308. The step length of each factor is shown in Table 3. The "steepest ascent" experiments with 8 runs on the grads direction of each factor were performed. The experiment design and results of steepest ascent are shown in Table 4.

As can be seen from Table 4, the immobilized lipase specific activity in the reaction of transesterification was the highest, 52.95 U mg^{-1} pr. in the 5th experiment. Therefore, the value of each factor in the fifth run was chosen as the central point of RSM experiments.

Table 3 – *The linear regression model experiment and steepest ascent parameters*

RUNS	Independent variables, coded levels			Response
	X_1	X_2	X_3	Y/U mg^{-1} pr.
1	1	1	1	43.71
2	1	1	−1	41.15
3	1	−1	1	40.57
4	1	−1	−1	39.61
5	−1	1	1	41.22
6	−1	1	−1	39.80
7	−1	−1	1	39.37
8	−1	−1	−1	38.14
b_j	0.813	1.024	0.771	
$b_j \Delta_j$	1.628	2.048	0.385	
$d\ b_j \Delta_j$	0.501	0.631	0.118	
adjusted	0.5	0.6	0.1	

Table 4 – *Design and results of steepest ascent experiment*

RUNS	Z_j	Z_1	Z_2	Z_3	Response
					Y/U mg^{-1} pr.
1	$Z_{0j} + d\ b_j \Delta_j$	5.5	7.6	7.1	37.53
2	$Z_{0j} + 2d\ b_j \Delta_j$	6.0	8.2	7.2	39.82
3	$Z_{0j} + 3d\ b_j \Delta_j$	6.5	8.8	7.3	43.46
4	$Z_{0j} + 4d\ b_j \Delta_j$	7.0	9.4	7.4	47.18
5	$Z_{0j} + 5d\ b_j \Delta_j$	7.5	10.0	7.5	52.95
6	$Z_{0j} + 6d\ b_j \Delta_j$	8.0	10.6	7.6	48.67
7	$Z_{0j} + 7d\ b_j \Delta_j$	8.5	11.2	7.7	44.88
8	$Z_{0j} + 8d\ b_j \Delta_j$	9.0	11.8	7.8	42.64

RSM experiments with Central Composite Design

Based on the results (Table 4) of the steepest ascent experiment, RSM experiments were carried out in which 7.5 for mass ratio of PEG4000 to lipase, 10.0 for mass ratio of diatomites to lipase and 7.5 for pH value respectively were chosen as the central point. Factors and levels value of response surface analysis are shown in Table 5. Box-Behnken Central Composite Design and experimental results for RSM are shown in Table 2.

Table 5 – *Factors and levels value of response surface analysis*

Factors	Code	Levels		
		−1	0	1
PEG4000/lipase (*w*/*w*)	X_1	6.5	7.5	8.5
diatomites/lipase (*w*/*w*)	X_2	9.0	10.0	11.0
pH	X_3	7.3	7.5	7.7

Statistical analysis of the fitted quadratic regression model was tested by the Fisher's *F*-test for analysis of variance, and the results are shown in Table 6. The coefficient of determination (R^2) was 98.92 %. As can be seen, the determination coefficient (R^2) indicates that the sample variation of

Table 6 – *Analysis of variance (ANOVA) for the fitted quadratic polynomial model*

Source	Sum of squares	Df	Mean square	*F*-ratio	*p*-value
Model	254.568	8	31.821	68.63	0.0000
Residual	2.78207	6	0.463679		
Total	257.35	14			

R^2 = 0.98919
adjusted R^2 = 0.974776

98.92 % for specific activity of transesterification is attributed to the independent variables, and the model cannot explain only 1.08 % of the total variation. The present R^2 value reflects a very good fit between the observed and predicted responses, and it was considered reasonable to use the regression model to analyze trends in the responses. The adjusted R^2 statistic is 97.48 %. The F-ratio of quadratic regression model was 68.83 with a low probability value [$p < 0.0001$] which means the model is well accurate for predicting the specific activity.

Statistical analysis of effect estimates for response surface quadratic model was determined by t-value and p-value, which is shown in Table 7. The results presented in Table 7 demonstrate that the regression coefficients of all the quadratic terms were significant at the 1 % level as was one cross-product (X_1, X_3). In addition, regression coefficients of all the linear terms and the quadratic coefficient of cross-product (X_2, X_3) were significant at the 5 % level.

Table 7 – *Significance test of regression coefficient*

Parameter	Estimate	Standard error	t-statistic	p-value
Constant	55.5	0.393141	141.171	<0.0001**
X_1	1.05	0.240749	4.36659	0.0047*
X_2	−0.71	0.240749	−2.94394	0.0258*
X_3	0.69	0.240749	2.86606	0.0286*
$X_1 \cdot X_1$	−5.91	0.354372	−16.6844	<0.0001**
$X_1 \cdot X_3$	2.53	0.34047	7.42356	0.0003**
$X_2 \cdot X_2$	−3.48	0.354372	−9.81312	0.0001**
$X_2 \cdot X_3$	1.14	0.34047	3.34097	0.0156*
$X_3 \cdot X_3$	−4.11	0.354372	−11.598	<0.0001**

*significant, **high significant

Smaller p values indicate a higher level of significance for the corresponding coefficient.[20] Accordingly, an order of the effect of variables on the specific activity of immobilized lipase is the mass ratio of PEG4000 to lipase, the mass ratio of diatomites to lipase and pH value.

The quadratic regression eq. (5) obtained by the stepwise regression method is as follows:

$$Y = 55.5 + 1.05125 \cdot X_1 - 0.70875 \cdot X_2 + 0.69 \cdot X_3 - 5.9125 \cdot X_1 \cdot X_1 + 2.5275 \cdot X_1 \cdot X_3 - 3.4775 \cdot X_2 \cdot X_2 + 1.1375 \cdot X_2 \cdot X_3 - 4.11 \cdot X_3 \cdot X_3 \quad (5)$$

The model (eq. (5)) indicated that the mass ratio of PEG4000 to lipase (X_1) had a significant effect ($p < 0.001$) on Y as it had the largest coefficient followed by pH (X_3) and mass ratio of diatomites to lipase (X_2). The positive coefficients of X_1, X_3 and the interaction terms (X_1X_3 and X_2X_3) indicated a direct effect on the specific activity. In contrast, the quadratic terms (X_1^2, X_2^2 and X_3^2) had an inverse effect on lipase activity.

Analysis of optimum conditions

For detecting the effect of cross product on the specific activity, the quadratic regression equation with regard to three variables was obtained by fixing the other variables. According to the regression eq. (5), the response surface plots and contour plots were generated using STATGRAPHICS software and are depicted in Figs. 1–3.

These three-dimensional plots and their respective contour plots provide a visual interpretation of the interaction between two variables and facilitate the location of optimum experimental conditions. The relationship between the experimental factors and response can be understood by examining the series of contour plots and response surface plot generated by holding pH (Fig. 1), mass ratio of diatomites to lipase (Fig. 2) and mass ratio of PEG4000 to lipase (Fig. 3) at a constant level.

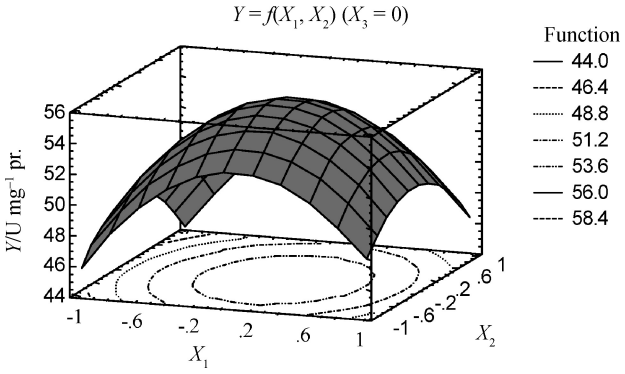

Fig. 1 – *Response surface plot and contour plot for the immobilized lipase specific activity (Y) as a function of PEG4000 (X_1) and diatomites (X_2)*

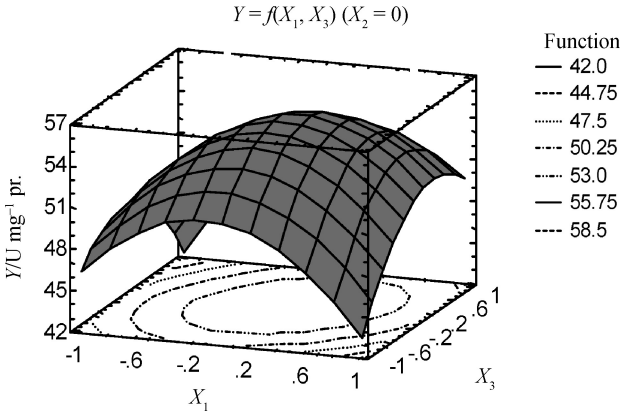

Fig. 2 – *Response surface for the immobilized lipase specific activity (Y) as a function of PEG4000 (X_1) and pH value (X_3)*

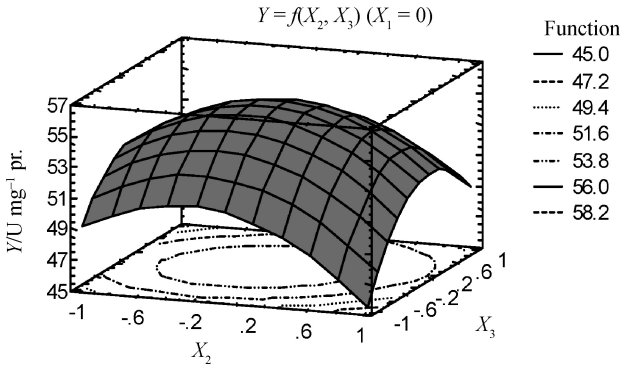

Fig. 3 – *Response surface for the immobilized lipase specific activity (Y) as a function of diatomites (X_2) and pH value (X_3)*

As seen in Table 1, the mass ratio of PEG4000 to lipase, mass ratio of diatomites to lipase and pH value were 5, 7, 7, respectively. Under above unoptimized conditions, the specific activity was only 44.5 U mg^{-1} pr. (Table 8). The canonical analysis revealed a minimum region for the model. The presented stationary point had the following critical values: mass ratio of PEG4000 to lipase = 7.61, mass ratio of diatomites to lipase = 9.92, and the pH 6.74. Under these conditions the predicted concentration of the specific activity was 55.63 U mg^{-1} pr., approximately 26.1 % higher than that of unoptimized conditions. The specific activity derivate with the activity of the free lipase was 5.5 U mg^{-1} pr., and the optimized maximal specific activity was 11.1 times of free lipase activity.

Table 8 – *Validation to quadratic regression model and the contrast of experimental results under optimized and unoptimized conditions*

	1	2	3	Mean
Actual/U mg^{-1} pr.	56.13	55.98	56.23	56.11
Predicted/U mg^{-1} pr.				55.63
Unoptimized/U mg^{-1} pr.				44.5

Fig. 1 shows the effect of mass ratio of PEG4000 to lipase (X_1) and mass ratio of diatomites to lipase (X_2) on the specific activity by fixing that pH value is 7.5 ($X_3 = 0$). This surface indicates the conditions that maximal specific activity are X_1 of 0.1117 and X_2 of –0.0845. According to eq. (1), the non-coded variables values for X_1, X_2 were obtained as follows: mass ratio of PEG4000 to lipase is 7.61; mass ratio of diatomites to lipase is 9.92. Through this analysis, it is evident that the specific activity increased as the mass ratio of both PEG4000 to lipase or diatomites to lipase increased, reached the maximal value, and then gradually decreased. The optimal mass ratios of both PEG4000 to lipase and diatomites to lipase were 7.61 and 9.92, respectively. Excessive PEG4000, used as non-covalent modification reagents, and diatomites, used as modification carriers, may inactivate lipase. On the other hand, an excess of PEG4000 and diatomites may cause a diffusion limit and hinder mass transfer and as a consequence, reduce the lipase activity.

According to Francesco *et al.,*[21] the enzyme formulation, lipase + PEG, appears to be of much interest as biocatalysts in organic synthesis, because they have shown much higher transesterification activity. Hence, PEG could act as lyoprotectants, preventing the formation of intermolecular interactions during the lyophilization process that might be responsible for protein denaturation. Yoshihiko *et al.*[22] also found that the amphiphilic nature of polymers was important to prepare the polymer–enzyme complex in an aqueous buffer solution, and subsequently used in an organic media. The polymer-enzyme complex was a good soluble biocatalyst for nonaqueous enzymology, and the solubilization of modified enzymes in organic solvents was crucial for their activation. Their optimal molar ratio of the PEG4000 to chymotrypsin was ca. 8.

In agreement with our findings, Gross *et al.*[23] demonstrated that an intermediate enzyme loading can be found where no further improvement in activity is detected when more support material is provided per unit mass of enzyme. Their data suggest that this optimum enzyme/support ratio is highly dependent on the surface chemistry and morphology of the adsorbent. This research group has also reported that *Candida antarctica* Lipase B (CALB) molecules spread upon adsorption on their supports as has been suggested elsewhere for "soft" proteins.[24]

Fig. 2 shows the effect of mass ratio of PEG4000 to lipase (X_1) and pH value (X_3) on the specific activity by fixing the mass ratio of diatomites to lipase to 10.0 ($X_2 = 0$).

Fig. 3 shows the effect of the mass ratio of diatomites to lipase (X_2) and pH value (X_3) on the specific activity by fixing the mass ratio of PEG4000 to lipase to 7.5 ($X_1 = 0$).

From Fig. 2 and Fig. 3, we can see that the pH value has certain effects on the activity of immobilized lipase. Therefore, the ionization state of enzyme was decided by pH value of buffer in which the immobilized enzyme was prepared. When X_3 is 0.1066 (pH value is 7.52), the specific activity was maximal. This result strongly suggested that the polymer-enzyme complex demonstrated the "pH memory" effect and the pH adjustment at its preparation stage was crucial for its activation in organic

media. Enzymes demonstrate a "pH memory" effect, i.e. the ionization state of an enzyme in organic media remains the same as in the last aqueous solution from which the enzyme is recovered.[25]

Validation of the models

Three repeated experiments were performed at the predicted optimal parameters to test the suitability of the model (eq. (5)). The results showed (Table 8) that the predicted values 55.63 U mg^{-1} pr. were greatly approached to the observed values 56.11 U mg^{-1} pr. This indicated that the model could be considered quite reliable for predicting the effect of each factor on the specific activity.

Conclusions

In this study, the response surface methodology (RSM) model was employed to study the combined effects of immobilization conditions on the immobilized lipase specific activity in the reaction of transesterification with PEG non-covalent modification. The immobilization conditions were optimized based on RSM and they were found optimum at a mass ratio of PEG to lipase of 7.61, mass ratio of diatomites to lipase of 9.92, and the pH value of 7.52 with an increase of the specific activity by 26.1 %. Validation experiments were also carried out to verify the availability and the accuracy of the model, and the result showed that the predicted value (55.63 U mg^{-1} pr.) was in very good agreement with the experimental value (56.11 U mg^{-1} pr.). Results of this study might serve as a guideline for the optimization of immobilization conditions of immobilized lipases with PEG non-covalent modification.

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