JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Optimization of Lipase-Catalyzed Regioselective Acylation of Pyridoxine (Vitamin B_6)

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Response surface methodology was successfully applied to optimize lipase-catalyzed regioselective esterification of pyridoxine (PN). Effects of various reaction conditions, including reaction temperature, time, enzyme loading, substrate molar ratio, and water activity, were investigated. A central composite design was employed to search for the optimal conversion of PN. A quadratic polynomial regression model was used for analysis of the experimental data at a 95% level ($p < 0.05$). The analysis confirmed that the water activity was the most significant factor affecting the conversion of PN. It was also suggested that the conversion was strongly affected by independent variables of temperature, time, substrate molar ratio, and water activity as well as interaction terms of temperature and enzyme loading/substrate molar ratio/water activity, time and enzyme loading/substrate molar ratio, substrate molar ratio, and water activity. The coefficient of determination of the model was found to be 0.963. Three sets of optimum reaction conditions were established, and the verified experimental trials were performed for validating the optimum points. A scale-up experiment was also done under the first set of optimal conditions.

KEYWORDS: Response surface methodology; pyridoxine; regioselective esterification; conversion; response value; optimization

INTRODUCTION

Pyridoxine (PN) is one of the three members (PN, pyridoxal, and pyridoxamine) of the vitamin B_6 group (*1*), and it is the most important form of commercial vitamin B_6. As depicted elsewhere, PN and its ester derivatives have broad applications in the food industry (*2*, *3*), cosmetics (*4*), and medical supplies (*5*). Unfortunately, the PN molecule has three hydroxyl groups and leads to a high solubility in water, which brings about a great deal of mass loss when it is used. To increase the lipid solubility of PN, which would be expected to increase their skin and cellular absorption, esterification methodology provides an excellent route to obtain a more apolar derivative (The esterification of PN has cut down the amount of hydroxyl groups and led to a decreasing solubility in water, which brings about the decrease of the water-soluble mass loss of PN when it is used.) (*6*). Also, there is a growing demand for regioselective esterification of PN that could provide precursors to synthesize other vitamin B_6 derivatives (*6*−*10*). Recently, 5-AcPN (5-*O*-acetylpyridoxine) has been obtained through lipase-catalyzed esterification of PN in our laboratory (*1*). As demonstrated in previous work (*1*), the conversion of PN was effected by reaction conditions such as temperature, time, enzyme loading, substrate molar ratio, and water activity.

A statistical optimization method overcomes the limitations of classic empirical methods and proves to be a powerful tool for the optimization of the target value (*11*, *12*). Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for designing experiments, building models, and analyzing the effects of several independent variables (factors) (*11*, *13*). The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple factors and their interactions. Also, study of the individual and interactive effects of these factors will be helpful in efforts to find the target value. Hence, RSM provides an effective tool for investigating the aspects affecting desired response if there are many factors and interactions in the experiment. To determine a suitable polynomial equation for describing the response surface, RSM can be employed to optimize the process.

The previous work (*1*) showed that the results were of general interest for developing industrial processes for the preparation of 5-AcPN. The present work focuses on optimization of the conversion of PN and also a decrease of the consumption of Novozym 435 and vinyl acetate. Central composite experimental design (CCD) (*14*), which is a 2^k factorial design with star points and center points, was used to fit an empirical, full second-order polynomial model. Five factors, including reaction temperature, reaction time, enzyme loading, substrate molar ratio, and water activity, were selected as independent variables while the conversion of PN was the dependent variable. During searching for optimal conditions for the response value by RSM, a small number of trials were performed to design an experiment

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10.1021/jf063613p CCC: $37.00
Published on Web 05/08/2007

and to evaluate the interactions of these factors. The interactions between factors influencing the esterification of PN were established, and the model describing the effect of the factors was also assessed.

MATERIALS AND METHODS

Materials. PN was purchased from Sigma Chemical Co. (St. Louis, MO). Novozym 435 (immobilized on acrylic resin, 7400 PLU/g) was from Novo Industrie (Bagsvaerd, Denmark). Acetonitrile was of chromatographic grade and was obtained from Merck & Co. (Darmstadt, Germany). 5-AcPN (>99%) and 4,5-*di*-AcPN (>99%) standards were purchased from Sigma Chemical Co., while all other reagents were purchased from local sources.

Enzymatic Reactions. All enzymatic reactions were carried out in a temperature-controlled incubator shaker at 210 r/min (3.5 s^{-1}). The procedure for the enzymatic esterification of PN in acetonitrile was described as a representative (*1*). Reactions were performed by adding Novozym 435 lipase (35 mg) and acyl donor (0.18 mmol) to a solution of the PN (10.2 mg, 0.06 mmol) in acetonitrile (3 mL) and shaking the reaction mixtures at 35 °C during the times indicated in the text. Samples of the biotransformation were withdrawn at different times, then diluted with deionized water, and analyzed by high-performance liquid chromatography (HPLC).

HPLC Analysis. PN transformational resultants were analyzed using an HPLC [Agilent (Waldbronn, Germany) 1100 (quaternary pump, degasser)] equipped with an UV detector. HPLC analysis was conducted by employing a C-18 column [Zorbax 300SB-C18 4.6 mm i.d. × 150 mm (5 μm), Agilent Technologies, Palo Alto, CA] with detection at 292 nm. The mobile phases were solution A (water containing 0.1% acetic acid) and solution B (acetonitrile containing 1% acetic acid) in all cases at a 1 mL min^{-1} flow of A/B gradient (0% B at 0 min, linear gradient to 100% at 7 min, maintained for 1 min, and finally to 0% at 9 min). The percentages of several resultants were computed from the respective peak areas.

The conversion of the reaction was quantified in terms of the mole percentage transesterification, that is, the ratio of PN consumed to the total amount of PN before reaction. In addition, the regioselectivity (Rs) is defined as:

$$\mathrm{Rs} = \frac{M_1}{M_2} \times 100\%$$

where M_1 is the mole percentage of 5-AcPN formed and M_2 is the mole percentage of PN consumed.

Setting Initial a_w in Closed System. Because of the distribution of water in the organic reaction system (e.g., bound to the enzyme or dissolved in the organic solvent), the thermodynamic water activity (a_w) is a better parameter than the water content to determine the amount of water associated with the enzyme and thereby to truly correlate with the enzyme catalytic activity (*15*, *16*). Among the available methods for controlling a_w, the simple and convenient way is preequilibration of the reaction components in the presence of saturated salt solutions, and the method is particularly suitable for the reaction, which doesn't generate water.

The a_w values of the acetonitrile, enzyme, and substrate were adjusted before starting the reaction by the following method: acetonitrile, PN, and Novozym 435 lipase were incubated in a chamber containing a desired saturated salt solution (*17*), and the system was allowed to reach equilibrium for 4 days at 20 °C for a desired a_w. Molecular sieves were used to generate the nearly anhydrous condition ($a_w = 0.05$). The following salts were used in this work: LiCl, KAc, $MgCl_2$, $CuCl_2$, and NaCl. The water activity (a_w) of the preequilibrated system was measured with an Aqualab Water Activity Meter (Decagon Devices, Washington, DC) at the set temperature.

The reactions were initiated by mixing the preequilibrated phases and Novozym 435 lipase in closed 10 mL centrifuge tube. The resultant mixture was assayed by HPLC in regular time.

Experimental Design. RSM was employed to analyze the operating conditions of PN acylation to obtain a high percent conversion. The experimental design was carried out by five chosen independent process variables with five levels (**Table 1**). The studied factors were as follows: water activity (a_w), reaction temperature [T (°C)], reaction time [t (h)], substrate molar ratio (acyl:PN), and enzyme loading (mg mL^{-1}). A CCD was employed to design the experiments. According to statistic theory, a CCD design of five factors consisted of 32 experiments, including 15 factorial points (cubic point) and 11 axial points (star point) as well as six replicates at the center point. Six replications (treatments 26−32) at the center of the design were used to estimate the pure error. For each factor, the experimental range and the central point were based on the results of preliminary trials (*1*).

Table 1. Coded Levels for Independent Factors Used in the Experimental Design

		coded levels				
factors	symbol	−2	−1	0	1	2
reaction temperature (°C)	x_1	25	30	35	40	45
reaction time (h)	x_2	1	1.5	2	2.5	3
enzyme loading (mg/mL)	x_3	8.3	10	11.7	13.3	15
molar ratio (acyl:PN)	x_4	1	2	3	4	5
water activity (a_w)	x_5	0.05	0.10	0.15	0.20	0.25

The software of Design-Expert 6.0 (Stat-Ease, United States) was used for designing and regressing the experimental data. The coded values of these factors were obtained according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \tag{1}$$

where x_i is the coded value of the factor, X_i is the real value of the factor, X_0 is the real value of the factor at the center point, and ΔX_i is the step change value of the factor.

The independent variables (factors) and their levels, real values as well as coded values, are presented in **Table 1**. The conversion of PN (Y) was the response of the experimental design. Because of the variation of Rs (regioselectivity) being so tiny with the change of reaction condition in the experimental range (*1*), Rs would not be taken as the response.

The model equation was used to predict the optimum value and subsequently to elucidate the interaction between the factors. The quadratic equation model for predicting the optimal point was expressed according to eq 2 (*19*):

$$Y = \beta_0 + \sum_{i=1}^{5} \beta_i x_i + \sum_{i=1}^{5} \beta_{ii} x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta_{ij} x_i x_j \tag{2}$$

where β_0, β_i, β_{ii}, and β_{ij} are regression coefficients (β_0 is a constant term, β_i is a linear effect term, β_{ii} is a squared effect term, and β_{ij} is an interaction effect term) and Y is the predicted response value.

RESULTS AND DISCUSSION

Effect of Reaction Time. Figure 1 shows the time course for the regioselective esterification of PN by Novozym 435 at 35 °C. The percent molar conversion of PN increased to 70% at 2 h; therefore, the range of reaction time from 1 to 3 h was chosen in this study. The selection of reaction time range needs to be extremely precise in the study of CCD, otherwise, the optimal condition of synthesis cannot be found inside the experimental region through the analyses of statistics and contour plots. Also, as shown in **Figure 1**, the Rs exceeded 90% and kept stable in the total time range.

RSM Experiments and Fitting the Models. The results at each point based on experimental design are shown in **Table 2**. The coded values of each factor in brackets correspond to the real value of the factor levels. For each factor, a conventional level was set to zero as a coded level. The quadratic model (eq 2) was chosen to fit the experimental data in this work. In

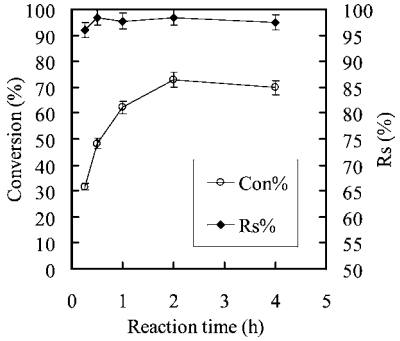

Figure 1. Time course of the regioselective esterification of PN in acetonitrile. Conditions: T, 35 °C; a_w, 0.16; acyl (vinyl acetate)/PN, 3:1; and enzyme, 5 mg/mL.

Table 2. Experimental Design and Results of the CCD Design

	variable level					
trial	T (°C)	t (h)	enzyme (mg/mL)	acyl:PN	a_w	response Y (%)
1	30 (–1)	1.5 (–1)	10.0 (–1)	2.0 (–1)	0.20 (1)	51.75
2	40 (1)	1.5 (–1)	10.0 (–1)	2.0 (–1)	0.10 (–1)	98.74
3	30 (–1)	2.5 (1)	10.0 (–1)	2.0 (–1)	0.10 (–1)	97.62
4	40 (1)	2.5 (1)	10.0 (–1)	2.0 (–1)	0.20 (1)	49.47
5	30 (–1)	1.5 (–1)	13.3 (1)	2.0 (–1)	0.10 (–1)	97.29
6	40 (1)	1.5 (–1)	13.3 (1)	2.0 (–1)	0.20 (1)	56.82
7	30 (–1)	2.5 (1)	13.3 (1)	2.0 (–1)	0.20 (1)	52.48
8	40 (1)	2.5 (1)	13.3 (1)	2.0 (–1)	0.10 (–1)	98.97
9	30 (–1)	1.5 (–1)	10.0 (–1)	4.0 (1)	0.10 (–1)	97.13
10	40 (1)	1.5 (–1)	10.0 (–1)	4.0 (1)	0.20 (1)	71.28
11	30 (–1)	2.5 (1)	10.0 (–1)	4.0 (1)	0.20 (1)	67.36
12	40 (1)	2.5 (1)	10.0 (–1)	4.0 (1)	0.10 (–1)	99.45
13	30 (–1)	1.5 (–1)	13.3 (1)	4.0 (1)	0.20 (1)	59.37
14	40 (1)	1.5 (–1)	13.3 (1)	4.0 (1)	0.10 (–1)	98.98
15	30 (–1)	2.5 (1)	13.3 (1)	4.0 (1)	0.10 (–1)	98.96
16	40 (1)	2.5 (1)	13.3 (1)	4.0 (1)	0.20 (1)	95.63
17	25 (–2)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	95.08
18	45 (2)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	99.01
19	35 (0)	1.0 (–2)	11.7 (0)	3.0 (0)	0.15 (0)	88.94
20	35 (0)	3.0 (2)	11.7 (0)	3.0 (0)	0.15 (0)	99.30
21	35 (0)	2.0 (0)	8.3 (–2)	3.0 (0)	0.15 (0)	97.12
22	35 (0)	2.0 (0)	15.0 (2)	3.0 (0)	0.15 (0)	99.15
23	35 (0)	2.0 (0)	11.7 (0)	1.0 (–2)	0.15 (0)	75.98
24	35 (0)	2.0 (0)	11.7 (0)	5.0 (2)	0.15 (0)	98.37
25	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.05 (–2)	98.04
26	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.25 (2)	17.15
27	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	98.51
28	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	92.41
29	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	96.87
30	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	98.83
31	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	94.49
32	35 (0)	2.0 (0)	11.7 (0)	3.0 (0)	0.15 (0)	99.25

addition, the α-level at which every term in the selected model should be significant was set as 0.05.

The effects of factors as well as their interactions could be discussed from the Pareto chart illustrated by **Figure 2**. The length of each bar was proportional to the absolute value of its associated regression coefficient or estimated effect. The order in which the bars were displayed corresponded to the order of the size of the effect. The chart included a vertical line that corresponded to the 95% limit, indicating statistical significance. A factor was, therefore, significant if its corresponding bar crossed this vertical line. As indicated in **Figure 2**, several different conclusions could be obtained as follows: (i) Independent variable enzyme loading (x_3), a quadratic terms of x_1^2, x_2^2, and x_3^2 and the interaction terms of x_1x_2, x_2x_5, x_3x_4, and x_3x_5, did not produce a significant effect on the conversion of PN within the designed intervals. (ii) The conversion of PN

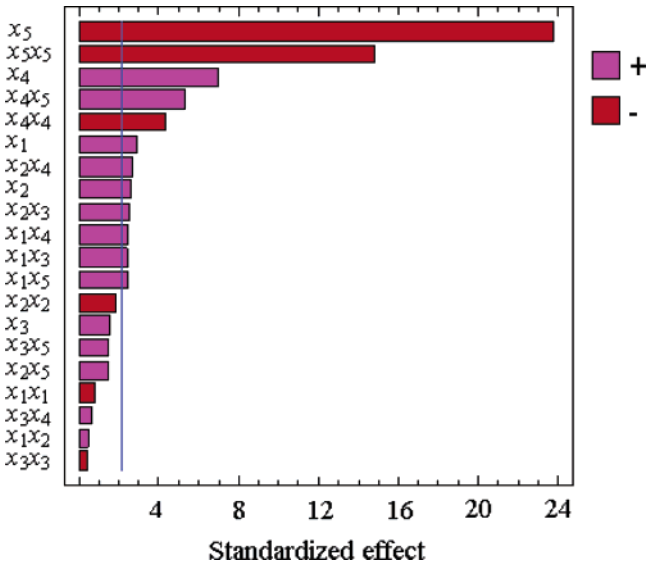

Figure 2. Pareto chart of standardized effects. Positive effects are in pink, and negative effects are in red. The perpendicular line indicates the confidence level of 95%, and factors with standardized effect values to the right of this line are statistically significant.

was greatly affected by x_1, x_2, x_4, and x_5. Likewise, significant interactions were found between temperature and enzyme loading (x_1x_3) ($p < 0.05$), temperature and substrate molar ratio (x_1x_4), temperature and water activity (x_1x_5), time and enzyme loading (x_2x_3), time and substrate molar ratio (x_2x_4), and substrate molar ratio and water activity (x_4x_5). At the same time, the analysis also confirmed that water activity (x_5) is the most significant factor affecting the conversion of PN (*3*). The regression coefficient of water activity was negative, which suggested that a low level of water activity of the reaction system would benefit the conversion of PN. On the other hand, the effects of the terms would be contrary if the coefficients were positive. According to the statistical method, the data were fitted to the response surface model to effectively evaluate the true relationship between the conversion of PN and these factors. A quadratic regression model was obtained by using coded values from the estimation of data:

$$\text{conversion\%} = 95.50 + 2.30x_1 + 2.05x_2 + 5.41x_4 - 18.53x_5 - 2.89x_4^2 - 10.29x_5^2 + 2.33x_1x_3 + 2.35x_1x_4 + 2.32x_1x_5 + 2.41x_2x_3 + 2.54x_2x_4 + 5.08x_4x_5$$

where x_i is the coded value of each factor.

Analysis of Variance (ANOVA) and Adequacy Test of the Model. For the model fitted, software generated model coefficients, F values and p values (prob > F, which indicates the insignificant probabilities); hence, one could justify the significance of each experimental variable. The corresponding variable would be more significant if the absolute F value became larger and the p value became smaller (*18*). As can be seen from **Table 3**, the regression quadratic model was highly significant ($p < 0.0001$) and the lack of fit was insignificant ($p > 0.05$), which indicated that the model was adequate to explain most of the variability for the conversion of PN. Nevertheless, a fitted response surface might give poor or misleading results if the model does not exhibit an adequate fit (*19*), which made the checking of model adequacy essential (**Table 4**). The low value of CV gave better reproducibility for the experimental point (*20*), and the CV for the conversion of PN proved to be within the acceptable range as given in **Table 4**. The large value of

Table 3. Analysis of Variance (ANOVA) for the Quadratic Model for the Conversion of PN

source	sum of square	DF	mean square	*F* value	prob > *F*
model	13385.20	13	1029.63	63.15	<0.0001
residual	293.49	18	16.31		
lack of fit	255.87	13	19.68	2.62	0.1479
pure error	37.63	5	7.53		
total	13678.69	31			

Table 4. Statistical Analysis for the Conversion of PN

standard deviation	4.04	*R*-squared	0.979
mean	85.62	adjusted *R*-squared	0.963
CV	4.72	predicted *R*-squared	0.896
PRESS	1420.03	Adeq precision	32.75

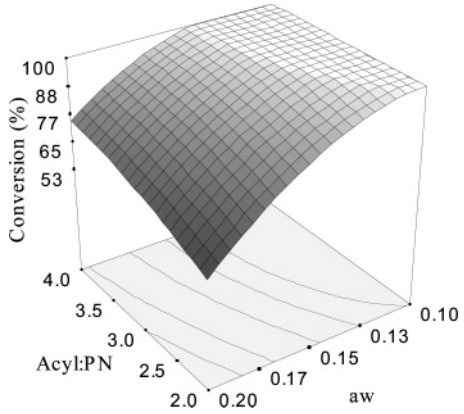

Figure 3. Response surface plots for the effects of substrate molar ratio and water activity. Conditions: *T*, 35 °C; *t*, 2 h; and enzyme, 11.7 mg/mL.

"adj *R*-squared" (0.963) indicated that the model could explain 96.3% of the total variation in the response. "Adeq precision" measured the signal-to-noise ratio, where a value greater than 4 was desirable. Consequently, this model could be used to navigate the design space.

Analysis of Response Surfaces. The three-dimensional surfaces plots were drawn to illustrate the main first-order effects and interactive effects of the independent variables (factors) on the dependent variable (response). **Figure 3** showed the effect of water activity and substrate molar ratio on the reaction. From the analysis of the response surface plots, water activity performed a significant influence on the response surface. As demonstrated elsewhere (*15*, *21*, *22*), the amount of water presented in the reaction systems was one of the most important factors, not only because of its influence on the lipase activity but also because of its influence on the hydrolysis of PN ester. Especially, acetonitrile possessed strong polarity and was capable of competing with lipase for its essential water effectively. In **Figure 3**, the high water activity of the organic solvent had a negative effect on the response value. That is to say, low water content of the reaction system could improve the esterification of PN. In contrast, the effect of substrate molar ratio was contrary, namely, the conversion of PN would be positively correlated to substrate molar ratio. As observed in **Figure 3**, a strong interaction between water activity and substrate molar ratio, which was reflected by the corresponding *p* value (<0.0001), was deduced from the elliptical nature of the contour curve. Not surprisingly, a combination of lower water activity and higher amount of vinyl acetate led to high conversion.

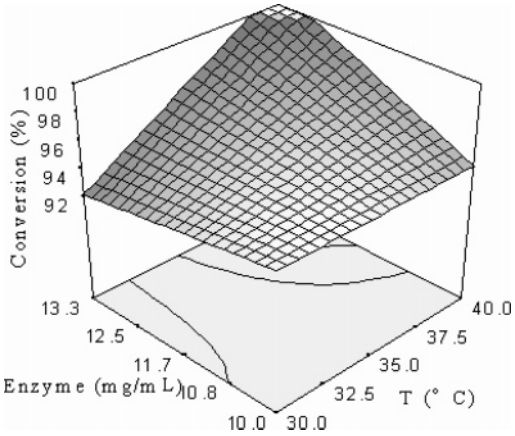

Figure 4. Response surface plots for the effects of reaction temperature and enzyme loading. Conditions: *t*, 2 h; acyl/PN, 3; and a_w, 0.15.

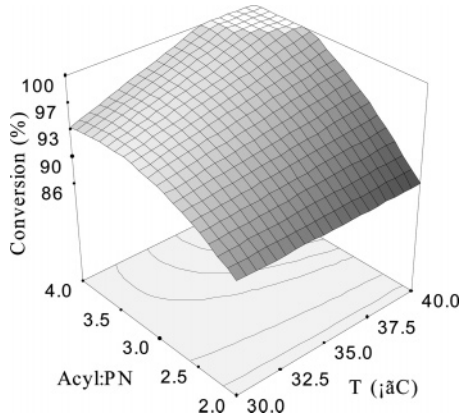

Figure 5. Response surface plots for the effects of reaction temperature and substrate molar ratio. Conditions: *t*, 2 h; enzyme, 11.7 mg/mL; and a_w, 0.15.

Figure 4 depicted response surface of the effects of the two factors, namely, reaction temperature and enzyme loading. It was evident that the interaction between reaction temperature and enzyme loading was significant. As presented in **Figure 4**, the conversion of PN grew fairly as the amount of lipase increased. It must be pointed out that at low temperature (<32 °C), slight conversion decline with the increase of enzyme loading has been observed, which could be attributed to the fact that there was great resistance of mass transfer with high enzyme loading when the temperature was low. Therefore, lipase should not be loaded too much to prevent diffused resistance.

The effect of substrate molar ratio could be in two ways. On one hand, the increase of vinyl acetate amount would raise the reaction rate of transesterification and repress hydrolysis. On the other hand, it could also increase the theoretical reaction equilibrium value (*1*). **Figure 5** denoted the effects of reaction temperature and substrate molar ratio on the response. The substrate molar ratio played an important role in the process of reaction as was evident from its first-order effect. In **Figure 5**, the degree of conversion was remarkably low at low acyl:PN (vinyl acetate:PN). Nevertheless, the temperature exerted a little influence on the response surface. As mentioned above, a high amount of vinyl acetate was required for the reaction to obtain a high degree of conversion, which was believed to be related to the increasing reaction equilibrium value. These results obtained by the regression model were in agreement with the experimental results of previous work (*1*).

As presented in **Figure 6**, similar to **Figure 5**, an increasing substrate molar ratio could raise the conversion of PN strongly,

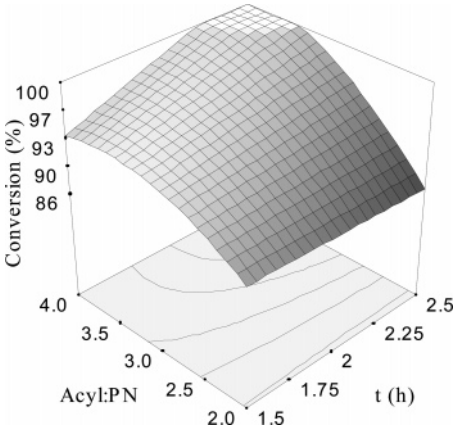

Figure 6. Response surface plots for the effects of reaction time and substrate molar ratio. Conditions: *T*, 35 °C; enzyme, 11.7 mg/mL; and a_w, 0.15.

Table 5. Optimum Conditions Found by the Model for the Conversion of PN

run	1	2	3	4
reaction temp (°C)	37.4	32.9	34.5	30.0
reaction time (h)	1.5	2.2	3.5	1.5
enzyme loading (mg/mL)	7.0	10.4	11.0	13.3
molar ratio (acyl:PN)	1.8	2.4	3.0	2.0
water activity (a_w)	0.10	0.12	0.15	0.20
	conversion of PN			
predicted value (%)	102.4	100.9	98.5	48.0
experimental value (%)[a]	98.7 ± 1.1[b]	99.2 ± 0.6[b]	97.4 ± 1.5[b]	45.1 ± 1.4[b]

[a] Mean ± standard deviation of triplicate determinations from different experiments. [b] Regioselectivities (Rs) obtained under these reaction conditions are 95.8, 94.2, and 94.5%, respectively.

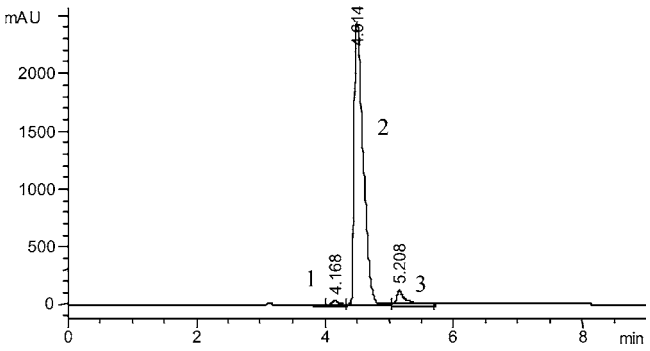

Figure 7. HPLC chromatography spectrum of the reaction productions. Peak 1 is PN, peak 2 is 5-AcPN, and peak 3 is 4,5-*di*-AcPN.

which reflected a general effect of substrate concentration on the reaction rate and thermodynamic equilibrium. Especially, when the value of acyl:PN was about 3 or higher, there was a fast reaction rate and the conversion of PN could exceed 95% at 2 h. On the other hand, reaction time had an observable effect on the response value at a high substrate molar ratio. However, the degree of conversion did not increase any longer after 1.5 h when the substrate molar ratio was smaller than 2.5. These observations suggested that, to some extent, sufficient vinyl acetate could shift the equilibrium toward the ester formation.

As we all know, it is of general interest for developing industrial processes for the preparation of 5-AcPN useful for food additives and cosmetic formulations and the synthesis of other PN derivatives. To reduce the cost of producing 5-AcPN, it is essential to control the consumption of lipase and vinyl acetate. On the basis of the discussion above, it was possible to obtain a high degree of conversion under low enzyme loading and a low substrate molar ratio. Three sets of predicted reaction conditions were given by the design to obtain the optimum conversion and regioselectivity (**Table 5**).

Verification of Results. To validate the predicted results, experiments using the improved formula were performed, and the observed values were shown in **Table 5**. On the basis of the solution given by the design, four runs of experiments were established at the fixed conditions. **Figure 7** showed the HPLC spectrum of one of these experiments, and peaks 1, 2, and 3 represented PN, 5-AcPN, and 4,5-*di*-AcPN (4,5-*di*-*O*-acetylpyridoxine), respectively. The experimental values were found to be reasonably close to the predicted ones, which confirmed the validity and adequacy of the predicted models. Moreover, the verification experiments also proved that the predicted values of conversion could be satisfactorily achieved within a 95% confidence interval of experimental values.

We then performed a scale-up experiment in a 100 mL reactor under the first set of optimal conditions. We used 680.0 mg of PN, 619.8 mg of vinyl acetate, and 700 mg of Novozym 435 in the reaction. As a result, 769.3 mg of 5-AcPN was prepared with PN conversion of 98.3% and regioselectivity of 94.7%. The byproduct was 51.6 mg of 4,5-*di*-AcPN obtained. The value of PN conversion was also close to the predicted value in the scale-up experiment.

Conclusions. Novozym 435 lipase was used as a biocatalyst to produce 5-AcPN. RSM was successfully applied to determine the optimum operational conditions for maximum conversion of PN. The five independent variables involved in the optimization were reaction temperature (x_1), reaction time (x_2), enzyme loading (x_3), substrate molar ratio (x_4), and water activity (x_5). The results showed a significantly good fit to this model. The *F* test and *p* value indicated that the largest effect in the response surface was the water activity (x_5) and substrate molar ratio (x_4). This was followed by the quadratic effect of x_5^2 and the interaction effect of x_1x_4, x_2x_3, x_2x_4, and x_4x_5. From the RSM results, four sets of operation conditions were observed. Furthermore, under optimized conditions, the experimental values agreed well with the values predicted. A scale-up experiment was also done under the first set of the optimal conditions. The experimental conditions allowed a fast, quantitative, and maximum production of 5-AcPN from PN.

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Received for review December 13, 2006. Revised manuscript received March 22, 2007. Accepted April 2, 2007. This work was supported by the Program of Introducing Talents of Discipline to Universities (No. B06006) and the Program for Changjiang Scholars and Innovative Research Team in University from the Ministry of Education of China.

JF063613P