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Synthesis of Anhydrovinblastine by Response Surface Methodology

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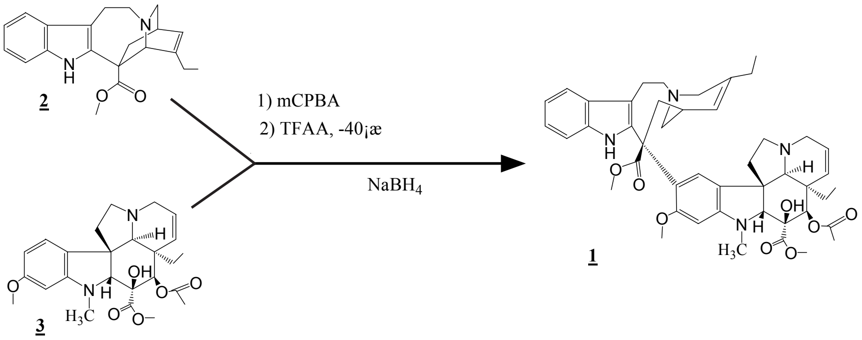
Anhydrovinblastine is the crucial intermediate of synthesizes vinorelbine and vinflunine, because of its complicated and difficult synthesizing processes, the yields of both preparation and synthesis are not high enough. Aiming at optimization of this process, experiment factors and levels were firstly selected by previously tests. According to the Box-Benhnken design principles, the method of response surface method with 3 factors and 3 levels was adopted. The factors influencing the technological parameters were determined by means of regression analysis. Response surface were finally graphed with the yield as the response value. The synthetic conditions are optimized are determined as follows: The vinblastine is dissolved in DMF and the concentration is 15 mmol/L; the reaction reagent is $SOCl_2$ and the stoichiometric proportions between reagent with vinblastine could be 50:1; the reaction temperature is 5 ºC and the duration is 12 h. The yield of anhydrovinblastine reaches 65.80 %.

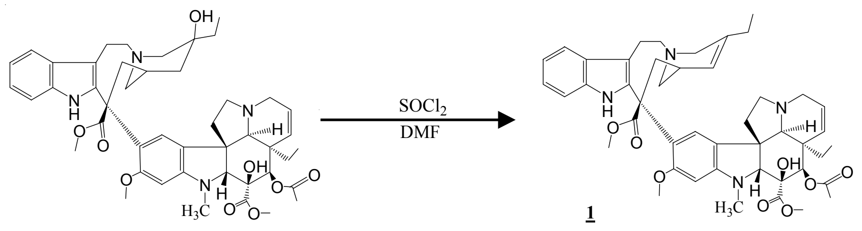
Key Words: Anhydrovinblastine, Process optimization, Synthesize, Response surface methodology.

INTRODUCTION

Vinorelbine[1,2] and vinflunine[3] are the semi-synthetic catharanthus alkaloids, which have high anticancer activity and low toxicity[4,5]. Among the preparation process of them, 3',4'-anhydrovinblastine (AVLB) **1** was a crucial intermediate, which was achieved through a biomimetic coupling reaction of catharanthine **2** and vindoline **3**[6,7] (**Scheme-I**). But this method needs two high purity monomers of **2** and **3**, the reaction needs -40 ºC low temperature condition and hard to control[8,9]. So, find a more convenient method to synthesize **1** is considerable. Teljse[10] obtained **1** from vinblastine by treatment with $SOCl_2$ or $POCl_3$ at room temperature but the yield just reach to 55 % (**Scheme-II**). The aim of this paper is to optimize this method by response surface methodology (RSM).



Scheme-I



Scheme-II

Response surface methodology (RSM) has been reported to be an effective tool for optimizing a process[11]. It is defined as the statistical tool that uses quantitative data from appropriate experimental design to determine and simultaneously solve multivariate equations[12]. The graphical representation of these equations are called as response surfaces, could be used to describe the individual and cumulative effect of the test variables on the response and to determine the mutual interaction between the test variables and their subsequent effect on the response[13,14].

In present research, response surface methodology (RSM) was applied to determine the optimum conditions for synthesize anhydrovinblastine from vinblastine sulfate.

EXPERIMENTAL

The raw material vinblastine sulfate afford by Haining Ltd., Hainan, China (HPLC > 65 %). The standard sample of AVLB (99 %, pure) afford by Sigma (USA). All chemicals used were of analytical grade. All glassware was over-dried at 120 ºC. The detect equipment is Waters Millipore HPLC.

Preparation of 3′,4′-anhydrovinblastine (AVLB): $SOCl_2$ are introduced in glass reactor, equipped with magnetic stirrer, then put in a bath at 0 ºC, DMF are added with argon bubbling. The mixture is agitated for 2 h at reaction temperature with argon bubbling, then DMF contain vinblastine sulfate are added and agitated for 6-12 h at this bath. Monitor the reaction with TLC and ending it when the row material used up. Then, cold water and ammonia are added to adjust pH 10.0. The mixture extracted with ether, the extract is dried on anhydrous sodium sulphate and concentrated at reduce pressure. The evaporation residue is dissolved in ethanol at 40 ºC and let it crystallize at 4 ºC, it is then filtered and dried.

Qualitative analysis of reaction products: The standard sample of AVLB (99 %, pure) dissolved in methanol and diluted to 5 μg/mL, 10 μg/mL, 15 μg/mL, 20 μg/mL, 25 μg/mL, injected 10 μL in the HPLC (Waters Millipore) separately which is equipped with 4.6 × 250 mm Dikma Diamonsil^{TM} C18 reverse phase column. The mobile phase was a mixture of methanol:acetonitrile: H_2O (25/35/40, v/v/v, pH 6). Flow rate was 1 mL/min and detection wavelength 267 nm.

Establish standard curve between sample concentration (X) and peak area (Y): Y = 35237X + 7471.5, r = 0.9989, RSD = 0.35 %. The reaction products dissolved in methanol with suitable concentration and calculate the purity of synthesized AVLB.

Box-Benhnken design and experimental results: Response surface methodology (RSM) was used for modelling the synthetic reaction and to optimize reaction conditions. The experimental design chosen for this study was that of Box-Benhnken design, a fractional factorial design for three variables. This design was preferred because relatively a few experimental combinations of the variables are adequate to estimate the complex response functions. Three levels, such as low, medium and high, denoted as -1, 0, +1, respectively in coded level of variables, were employed to fit a full quadratic response surface model and later approximated to obtain the optimal response.

The design variables selected in this study with actual and coded levels along with response variables are shown in Table-1. The experimental conditions were selected for each variable based on prior studies. Experiments were carried out according to the design points with independent variable such as reaction temperature (X_1), the substrate (vinblastine sulfate) concentration (X_2) and the stoichiometric proportions between dehydrating agent, $SOCl_2$ and the substrate (X_3). Where X_1 = (reaction temperature-12) /8, X_2 = (substrate concentration-15)/10 and X_3 = (the amount of $SOCl_2$-60)/20.

The experimental results (Y, % yield of AVLB) are also shown in Table-1.

Statistical analysis: The experimental data (Table-1) were analyzed by means of Design-Expert 7 software (Stat-Ease Inc., USA). The data were analyzed by the response surface regression (RSREG) procedure to fit the following second-order polynomial equation:

TABLE-1
RESULTS OF BOX-BENHNKEN EXPERIMENTAL DESIGN

Exp. No.	Coded level			Actual level			Y (%)
	X_1 (°C)	X_2 (mol/L)	X_3 (mol/mol)	X_1 (°C)	X_2 (mol/L)	X_3 (mol/mol)	
1	-1	-1	0	4	5	60	34.68
2	-1	1	0	4	25	60	61.53
3	1	-1	0	20	5	60	23.97
4	1	1	0	20	25	60	40.86
5	0	-1	-1	12	5	40	28.45
6	0	-1	1	12	5	80	30.78
7	0	1	-1	12	25	40	48.23
8	0	1	1	12	25	80	53.19
9	-1	0	-1	4	15	40	52.88
10	1	0	-1	20	15	40	35.76
11	-1	0	1	4	15	80	65.44
12	1	0	1	20	15	80	45.82
13	0	0	0	12	15	60	60.78
14	0	0	0	12	15	60	61.03
15	0	0	0	12	15	60	60.94

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} b_{ij} X_i X_j \qquad (1)$$

where Y is the response (% yield of AVLB); b_0, b_i, b_{ii} and b_{ij} are constant coefficients and X_i the uncoded independent variable. The quality of fit of the model was evaluated by the coefficients of determination (R^2) and the analysis of variances (Anova).

RESULTS AND DISCUSSION

Model fitting and Anova: This study was performed in order to determine the optimum conditions for synthesize AVLB from vinblastine sulfate by RSM. To obtain a proper model for the optimization of AVLB synthesis, the Box-Benhnken design, which is general the best design for response surface optimization, was selected with three-level-three-factor: *i.e.*, reaction temperature (X_1), the substrate (vinblastine sulfate) concentration (X_2) and the stoichiometric proportions between dehydrating agent, $SOCl_2$ and the substrate (X_3). Table-1 lists the experimental parameter settings and results based on the experimental design. All of the 15 designed experiments were performed and the results were multi-regression analyzed. Coefficients were evaluated by regression analysis and tested for their significance. Finally, the best-fitting model was determined by regression.

The experimental data (Table-1) were analyzed by means of Design-Expert 7 and the final estimative response model equation, was as follows:

$$Y = 60.92-8.52X_1 + 10.74X_2 + 3.74X_3 - 2.49X_1X_2 - 0.62X_1X_3 + 0.66X_2X_3 - 5.42X_1^2 - 15.23X_2^2 - 5.52X_3^2 \quad (2)$$

The Anova for response surface quadratic model is shown in Table-2. The model F-value of 44.30 implies the model is significant. There is only a 0.03% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case X_1, X_2, X_3, X_1^2, X_2^2, X_3^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The coefficient of determination (R^2) was 0.9875, which indicated that the model was suitable to adequately represent the real relationship among the factors selected.

TABLE-2
ANOVA FOR RESPONSE SURFACE QUADRATIC MODEL

Source	Sum of squares	d.f.	Mean square	F-value	Prob > F*
Model	2628.0700	9	528.730	44.85	0.0003
X_1	580.0400	1	580.040	87.09	0.0002
X_2	923.0000	1	923.000	138.59	<0.0001
X_3	111.8300	1	111.830	16.79	0.0094
X_1X_2	24.8000	1	24.800	3.72	0.1115
X_1X_3	1.5600	1	1.560	0.23	0.6486
X_2X_3	1.7300	1	1.730	0.26	0.6320
X_1^2	108.5500	1	108.550	16.30	0.0100
X_2^2	856.9600	1	856.960	128.67	<0.0001
X_3^2	112.4900	1	112.490	16.89	0.0093
Residual	33.3000	5	11.940	–	–
Lack of Fit	33.2700	3	19.880	691.63	0.0014
Pure Error	0.0320	2	0.016	–	–
Cor Total	2661.3700	14	–	–	–
R^2**	0.9875	–	–	–	–

*Prob > F = level of significance

**R^2 is the coefficient of determination.

Effect of parameters: Fig. 1 shows the response surface plots as function of reaction temperature, substrate concentration and interaction on AVLB synthesis at the stoichiometric proportions between $SOCl_2$ and the substrate is 60:1. The % yields increase with decrease in reaction temperature and is a clear indication that room temperature[10] is not the proper reaction condition. The % yields increased on going from 5 mmol/L to about 15 mmol/L and thereafter decreased further up to 25 mmol/L. The effect of reaction temperature and the amount of $SOCl_2$ on the % yields at

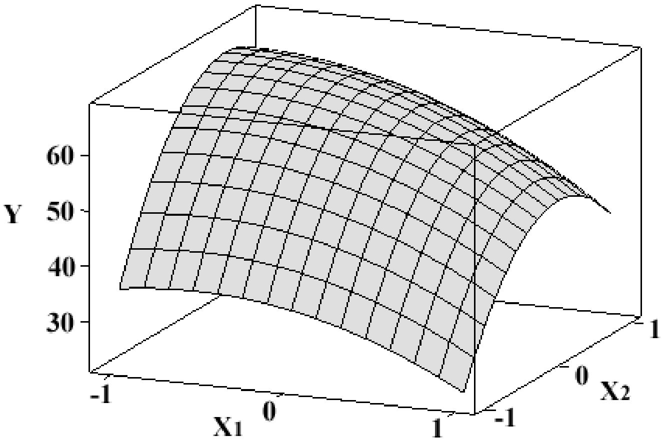


Fig. 1. Response surface plots as function of reaction temperature, substrate concentration and interaction on % yield

substrate concentration (15 mmol/L) is shown in Fig. 2. Too lower or higher amount of $SOCl_2$ are not the proper conditions. Fig. 3 shows the response surface plots of the amount of $SOCl_2$, substrate concentration and interaction on the % yield. The optimal reaction conditions will be shown below which can figure out from eqn. 2.

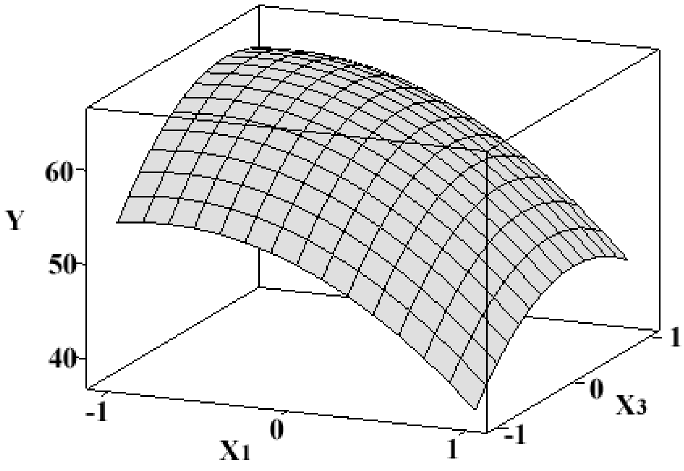


Fig. 2. Response surface plots as function of reaction temperature, the amount of $SOCl_2$ and interaction on % yield

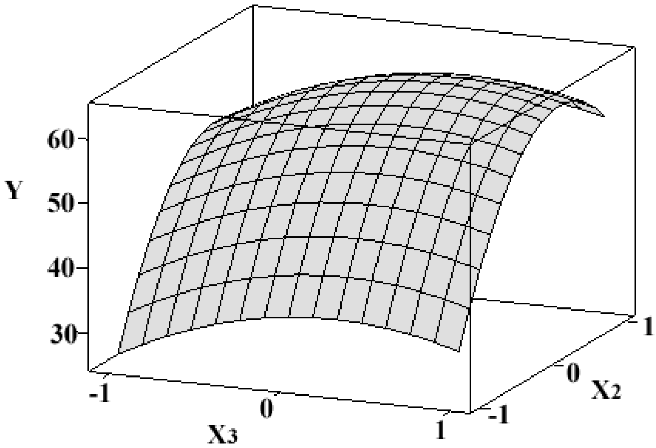


Fig. 3. Response surface plots as function of the amount of $SOCl_2$, substrate concentration and interaction on % yield

Optimization of reaction: Calculate the partial derivatives to X_1, X_2 and X_3 from eqn. 2 can obtain the following three equations:

$$10.84X_1 + 2.49X_2 + 0.62X_3 + 8.52 = 0 \quad (3)$$

$$2.49X_1 + 30.46X_2 - 0.66X_3 - 10.74 = 0 \quad (4)$$

$$0.62X_1 - 0.66X_2 + 11.04X_3 - 3.74 = 0 \quad (5)$$

The solution of equation is: $X_1 = -0.910$, $X_2 = 0.436$, $X_3 = 0.416$. The corresponding reaction conditions: reaction condition 5 ºC, substrate concentration 19 mmol/L and the stoichiometric proportions between $SOCl_2$ and the substrate is 68:1. Table-3 shows the predicted % yield and actual % yield at this optimal reaction conditions.

TABLE-3
EXPERIMENTAL AND PREDICTED RESULTS AT OPTIMAL REACTION CONDITIONS

	Optimal conditions	Yield (%)
Predicted results	$X_1 = -0.910$, $X_2 = 0.436$, $X_3 = 0.416$	67.90
Experimental results	X_1 (5 ºC), X_2 (19 mmol/L), X_3 (68:1, mol/mol)	65.80 ± 0.35

Table 3 clear indicate that the experimental results (65.80 ± 0.35) are close to the predicted results (67.90) and the RSD (3 %) indicate that the obtained model is credible.

Conclusion

Comparison of predicted and experimental values revealed good matching between them, implying that empirical models derived from RSM can be used to adequately describe the relationship between the factors and response in AVLB synthesis. This model can then be used to predict AVLB yield under any given conditions within the experimental range. We have demonstrated that optimum synthesis of AVLB can be successfully predicted by RSM. The predicted reaction conditions: reaction condition 5 ºC, substrate concentration 19 mmol/L and the stoichiometric proportions between $SOCl_2$ and the substrate is 68:1. The actual yield under these conditions reach to 65.80 % which is higher than previously reported (55 %)[10].

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