Response Surface Methodological Approach for Optimization of Enzymatic Synthesis of Sorbitan Methacrylate

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Abstract

Sorbitan methacrylate was synthesized from sorbitan dehydrated from D-sorbitol using an immobilized lipase. To optimize the enzymatic synthesis of sorbitan methacrylate, response surface methodology was applied to determine the effects of five-level-four-factors and their reciprocal interactions on sorbitan methacrylate biosynthesis. A total of 30 individual experiments were performed, which were designed to study reaction temperature, reaction time, enzyme amount and substrate molar ratio. A statistical model predicted that the highest conversion yield of sorbitan methacrylate was 100%, at the following optimized reaction conditions: a reaction temperature of 43.06 ℃, a reaction time of 164.25 mins., an enzyme amount of 7.47%, and a substrate molar ratio of 3.98:1. Using these optimal factor values under experimental conditions in four independent replicates, the average conversion yield reached 98.7%±1.2% and was well within the value predicted by the model.

Introduction

In this study, we opted to use alcoholysis as the applied esterification process. Alcoholysis is the esterification of an ester (e.g. vinyl methacrylate) with the hydroxyl group in the acyl acceptor (e.g. 1,4-sorbitan), and this process generates another ester and an alcohol as by-products. In the enzymatic process used to

synthesize sorbitan methacrylate from 1,4-sorbitan, several factors, including the reaction solvent, reaction temperature, type and concentration of acyl donor, enzyme content, and initial substrate concentration, can affect both the conversion yield and the glycosylation rate. In cases in which vinyl methacrylate is employed as an acyl donor, during the glycosylation periods, vinyl alcohol is produced. Simultaneously, tautomeric isomerization to acetaldehyde occurs. Therefore, we can conclude that this process facilitates the irreversibility of glycosylation, resulting in higher conversion yields. In our study, we conducted the enzymatic synthesis of sorbitan methacrylate using Novozym 435 (derived from *Candida antarctica*), which is a well-known non-specific lipase. Novozym 435 facilitates reactions between a wide range of alcohols and vinyl esters, and is a remarkably heat-tolerant enzyme. Response Surface Methodology (RSM) uses multiple regression and correlation analyses as tools to test the effect of two or more independent factors on the dependent ones. Central composite rotatable design (CCRD) is a response surface methodology employed in the optimization process of biotechnological processes. In this study, we performed alcoholysis for the esterification of vinyl methacrylate (acyl donor) with the hydroxyl group in 1,4-sorbitan (acyl acceptor) using response surface methodology. This process was designed to generate sorbitan methacrylate, with vinyl alcohol as a by-product. Esterification for the enzymatic synthesis of 1,4-sorbitan ester using immobilized lipase (Novozym 435), was performed using vinyl methacrylate as acyl donors, and *t*-butanol as the organic solvent.

Materials and methods

Novozym 435 (Lipase B from *Candida antarctica*, EC 3.1.1.3, a non-specific lipase immobilized on a macroporous acrylic resin, 1-2% water content, 10,000 PLU (Propyl Laurate Units)/g) was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). All other chemicals were of analytical grade, and the solvent used was dried with molecular sieves for one day prior to use.

Enzymatic esterification

We applied esterification via alcoholysis. Esterification for the synthesis of 1,4-sorbitan esters with immobilized lipase (Novozym 435) was conducted using the apparatus.

Reaction temperature was controlled with a water bath, which had been equipped with a PID temperature controller. Mixing was conducted using a magnetic stirrer, spinning at approximately 200 rpm. The condenser prevented reactant (*t*-butanol) evaporation. Initial concentration of 1,4-sorbitan applied for RSM was 50 g/L.

Experimental design & statistical analysis

A five-level-four-factor CCRD was adopted in this study, requiring 30 experiments, which included 16 factorial points, 8 axial points, and 6 central points to provide information about the interior of the experiment region, allowing evaluation for curvature. The variables, and their levels, selected for the study of sorbitan methacrylate synthesis, were: reaction temperature (25-65℃), reaction time (30-240 mins.), enzyme amount (1-7 wt %) and substrate molar ratio (1:1-5:1; vinyl methacrylate:sorbitan). Table 1 shows the coded and uncoded independent factors (X_i), levels and experimental design. The experimental data (Table 1) were analyzed by means of RSM to fit the following second-order polynomial equation with Design-Expert 6 software (Stat-Ease, Inc., USA). Second -order coefficients were generated by regression with stepwise elimination. Response was first fitted to the factors by multiple regression. The quality of fit of the model was evaluated by the coefficients of determination (R^2) and the analysis of variances (ANOVA). The insignificant coefficients were eliminated after examining the coefficients and the model was finally refined. The quadratic response surface model was fitted to the following equation:

$$Y=\beta_{k0}+\sum_{i=1}^{4}\beta_{ki}x_i+\sum_{i=1}^{4}\beta_{kii}x_i^2+\sum_{i=1}^{3}\sum_{j=i+1}^{4}\beta_{kij}x_i x_j \qquad (1)$$

Where Y is the response factor (conversion yield), x_i the ith independent factor, β_0 the intercept, β_i the first-order model coefficients, β_{ii} the quadratic coefficients for the factor i and β_{ij} is the linear model coefficient for the interaction between factors i and j.

Quantitative analysis

Enzymatic reactions were monitored by analysis of the conversion yield of 1,4-sorbitan, using vinyl methacrylate. The reactant and product measurements

were taken by HPLC with a ZORBAX Carbohydrate column (5μm, 120Å, 250 mm×4.6 mm, Agilent), maintained at a constant 35 °C. A mixture of acetonitrile:water (75:25, v/v) was used as a mobile phase, at a 1.0 mL/min flow rate. Detection was carried out with an RI detector (Shimazu RID-10A, Japan).

Results and Discussion

This study was performed in order to optimize the chemo-enzymatic synthesis of sorbitan methacrylate using response surface methodology. The study's principal objective was to determine the optimum conditions for enzymatic esterification for sorbitan methacrylate synthesis using immobilized lipase (Novozym 435) in *t*-butanol from 1,4-sorbitan, using response surface methodology. Response Surface Methodology (RSM) uses multiple regression and correlation analysis as tools to test the effect of two or more independent factors on the dependent factors. To obtain a proper model for the optimization of sorbitan methacrylate synthesis, the central composite rotatable design, which is generally the best design for response surface optimization, was selected with five-level four-factors: i.e., reaction temperature, reaction time, enzyme amount, and substrate molar ratio. Finally, the best fitting model was determined by regression and stepwise elimination. It was represented that four linear coefficients (X_1, X_2, X_3, X_4), three quadratic coefficients (X_1^2, X_2^2, X_3^2), and two cross-product coefficients (X_1X_4, X_3X_4) were significant (Table 2). The final estimative response model equation, eliminating the insignificant variables to estimate the enzymatic synthesis of sorbitan methacrylate, was as follows.

$$Y = -91.679 + 3.791\ X_1 + 0.474\ X_2 + 12.571\ X_3 - 16.287\ X_4 - 0.0627\ X_1^2 - 0.001\ X_2^2 - 0.759\ X_3^2 + 0.344\ X_1X_4 + 1.716\ X_3X_4 \quad (2)$$

where Y is the response factor, conversion yield (%). X_1, X_2, X_3, and X_4 are the values of the independent factors, reaction temperature (°C), reaction time (min), enzyme amount (wt %) and substrate molar ratio (-), respectively. The model coefficients and probability values are provided in Table 2. All *p*-values of coefficients were less than 0.1, and the coefficient of determination (R^2) was 0.94, which indicated that the model was suitable to adequately represent the real relationship among the factors selected. The obtained and estimated values were

also sufficiently correlated (Fig. 1). According to the analysis of factors, there was no lack of fit. This indicates that the model represents the actual relationships of reaction parameters well within the ranges selected. The optimal conditions for enzymatic synthesis of sorbitan methacrylate estimated by the model equation, were: X_1=43.06℃, X_2=164.25 mins., X_3=7.47%, and X_4=3.98:1 (Fig. 2). The theoretical conversion yield predicted under the above conditions was Y=100.2%. To confirm the prediction by the model, the optimal conditions were applied to four independent replicates for sorbitan methacrylate biosynthesis. The average conversion yield reached 98.7%±1.2% and was well within the estimated value of the model equation. Thus, demonstrating that response surface methodology with appropriate experimental design, can be effectively applied to optimize the process of factors in a biochemical reaction. This study focused on the application of response surface methodology to optimize the conditions for sorbitan methacrylate biosynthesis using immobilized lipase. It may provide useful information in the development of economic and efficient processes using immobilized enzyme systems.

Acknowledgements

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References

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Table 1. Factors and their levels for central composite design

Variable	Symbol	Coded factor levels				
		-2	-1	0	1	2
Reaction temperature (℃)	X_1	25	35	45	55	65
Reaction time (min)	X_2	30	82.5	135	187.5	240
Enzyme amount (wt %)	X_3	1	3	5	7	9
Substrate molar ratio (acyl donor/acyl acceptor)	X_4	1	2	3	4	5

Table 2. Regression coefficients and significance of response surface reduced quadratic model after a stepwise elimination.

Factor	Coefficient Estimate	Standard Error	*t*-value	Prob > |*t*|
Intercept	-91.679	2.552	-	-
X_1	3.791	1.510	-3.473	0.0019
X_2	0.474	1.510	2.901	0.0073
X_3	12.571	1.510	6.104	<0.0001
X_4	-16.288	1.510	3.006	0.0058
X_1^2	-0.062	1.398	-3.277	0.0032
X_2^2	-0.001	1.398	-1.717	0.0993
X_3^2	-0.759	1.398	-1.963	0.0624
X_1X_4	0.344	1.849	1.763	0.0925
X_3X_4	1.716	1.849	1.856	0.0783

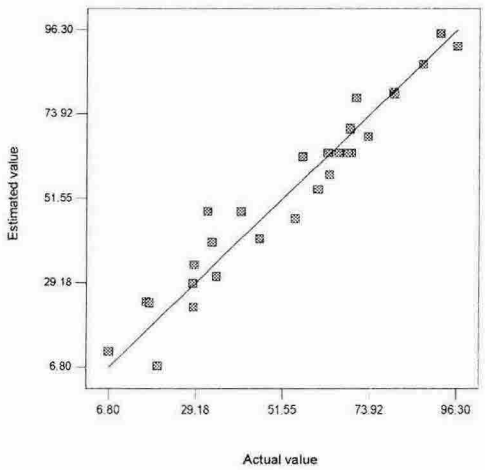

Fig. 1. Relationship between the obtained and estimated conversion yield of sorbitan methacrylate catalyzed by Novozym 435.

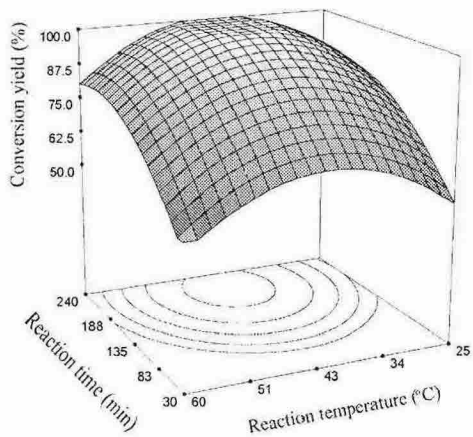

Fig. 2. Response surface plot representing the effect of reaction temperature and reaction time of the optimal enzymatic synthesis condition at the stationary point (enzyme amount 7.47%, and substrate molar ratio 3.98).