

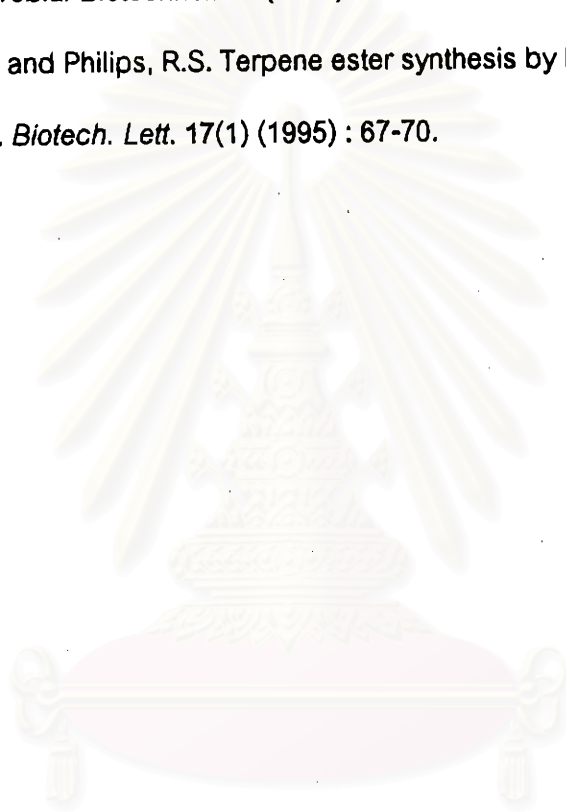
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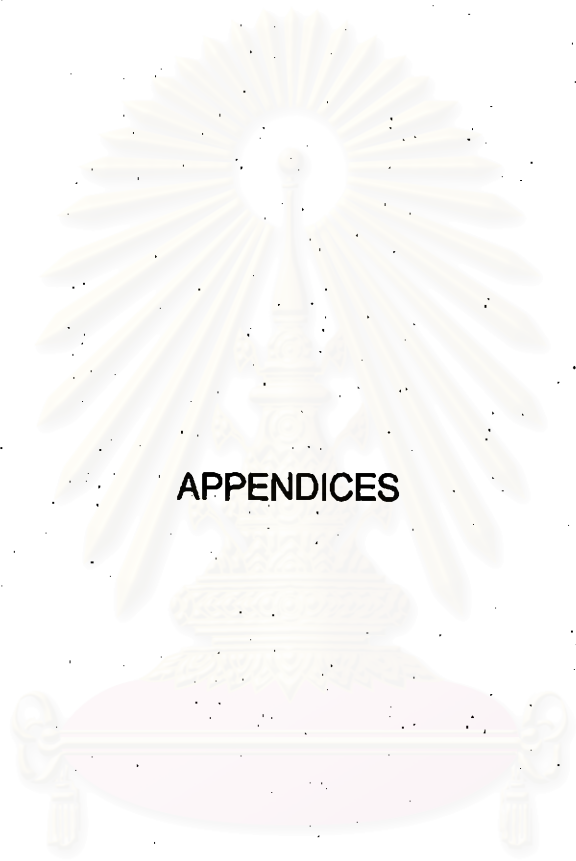
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APPENDICES

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Appendix A

A1 Experimental design

Two experimental designs were planned in this study. The first was 2^4 factorial design for an investigation of effects of key parameters on initial rate. The latter, central composite design (CCD), was an expansion of the first design to both extremes in order to construct response surface equation.

Four experimental parameters were of interest, X1: racemic menthol concentration (mM), X2: hexyl acetate (mM), X3: temperature ($^{\circ}\text{C}$), X4: stirring speed (rpm). In the first design, all factors were studied at two levels (+1 and -1) (show in table A1). Levels of these factors can be determined from their actual values as expressed in equations below. The number subtracted from the actual value is the actual value at level 0, while the divisor is the difference between actual values per level.

$$X1 = \frac{\text{concentration of racemic menthol} - 60}{20}$$

$$X2 = \frac{\text{concentration of hexyl acetate} - 260}{120}$$

$$X3 = \frac{\text{temperature} - 60}{15}$$

$$X4 = \frac{\text{stirring speed} - 155}{30}$$

Table A1 Experimental design planning. Codified and uncoded levels of the factors, X1: (\pm)menthol concentration (mM), X2: hexyl acetate concentration (mM), X3: temperature ($^{\circ}$ C) and X4: stirring speed (rpm) studied for the 2^4 experimental design

Type of Plan	Exp. no.	codified factors				Uncodified factors			
		X1	X2	X3	X4	X1	X2	X3	X4
2^4	1	-	-	-	-	40	140	45	125
	2	+	-	-	-	80	140	45	125
	3	-	+	-	-	40	380	45	125
	4	+	+	-	-	80	380	45	125
	5	-	-	+	-	40	140	75	125
	6	+	-	+	-	80	140	75	125
	7	-	+	+	-	40	380	75	125
	8	+	+	+	-	80	380	75	125
	9	-	-	-	+	40	140	45	185
	10	+	-	-	+	80	140	45	185
	11	-	+	-	+	40	380	45	185
	12	+	+	-	+	80	380	45	185
	13	-	-	+	+	40	140	75	185
	14	+	-	+	+	80	140	75	185
	15	-	+	+	+	40	380	75	185
	16	+	+	+	+	80	380	75	185

Table A2 Levels of experimental factors

Experiment factors	Levels				
	-2	-1	0	+1	+2
(±)menthol concentration (mM)	20	40	60	80	100
hexyl acetate concentration (mM)	20	140	260	380	500
temperature (°C)	30	45	60	75	90
stirring speed (rpm)	95	125	155	185	215

The effects of these experimental plans were determined by applying the Yates's algorithm (Box et al., 1978) to the uncodified experiments. Then, the factors are expanded to a central composite design (CCD), as shown in table A2 and A3, by introducing the extreme levels (Barker, 1985) : (±)menthol concentration at 20 and 100 mM, hexyl acetate concentration at 20 and 500 mM, temperature at 30 and 90 °C and stirring speed at 95 and 215 rpm, and the central point (Barker, 1985) : (±)menthol concentration at 60 mM, hexyl acetate concentration at 260 mM, temperature at 60 °C and stirring speed at 155 rpm.

Table A3 Experimental design planning, codified and uncoded levels for the central composite design (CCD)

Type of Plan	Exp. no.	Codified factors					Uncodified factors			
		X1	X2	X3	X4		X1	X2	X3	X4
CCD	17	-2	0	0	0		20	260	60	155
	18	+2	0	0	0		100	260	60	155
	19	0	-2	0	0		60	20	60	155
	20	0	+2	0	0		60	500	60	155
	21	0	0	-2	0		60	260	30	155
	22	0	0	+2	0		60	260	90	155
	23	0	0	0	-2		60	260	60	95
	24	0	0	0	+2		60	260	60	215
	25	0	0	0	0		60	260	60	155
	26	0	0	0	0		60	260	60	155
27	0	0	0	0		60	260	60	155	

The initial rates obtained from 2^4 factorial sets of the experiments are shown in table

A4.

Table A4 The initial rates of 2^4 factorial design experiments from table A1

Exp. No.	initial rate ($\mu\text{M/hr}$)
1	165
2	170
3	184
4	231
5	253
6	261
7	285
8	300
9	143
10	186
11	201
12	228
13	238
14	223
15	308
16	321

From these experimental data, the main and interaction effects can be determined using Yates's algorithm, and the results are shown in table A5.

Table A5 Yates's algorithm

exp. no.	design matrix variables				initial rate y ($\mu\text{mol/lit-hr}$)	algorithm						
	1	2	3	4		(1)	(2)	(3)	(4)	divisor	estimate	identification
1	-	-	-	-	165.00	335.00	750.00	1852.00	3700.00	16	231.2500	average
2	+	-	-	-	170.00	415.00	1102.00	1848.00	146.00	8	18.2500	1
3	-	+	-	-	184.00	514.00	758.00	78.00	422.00	8	52.7500	2
4	+	+	-	-	231.00	588.00	1090.00	68.00	64.00	8	8.0000	12
5	-	-	+	-	253.00	329.00	52.00	154.00	684.00	8	85.5000	3
6	+	-	+	-	261.00	429.00	26.00	268.00	-98.00	8	-12.2500	13
7	-	+	+	-	285.00	461.00	70.00	52.00	62.00	8	7.7500	23
8	+	+	+	-	303.00	629.00	-2.00	12.00	12.00	8	1.5000	123
9	-	-	-	+	143.00	5.00	80.00	352.00	-4.00	8	-0.5000	4
10	+	-	-	+	186.00	47.00	74.00	332.00	-10.00	8	-1.2500	14
11	-	+	-	+	201.00	8.00	100.00	-26.00	114.00	8	14.2500	24
12	+	+	-	+	228.00	18.00	168.00	-72.00	-40.00	8	-5.0000	124
13	-	-	+	+	238.00	43.00	42.00	-8.00	-20.00	8	-2.5000	34
14	+	-	+	+	223.00	27.00	10.00	68.00	-46.00	8	-5.7500	134
15	-	+	+	+	308.00	-15.00	-16.00	-32.00	74.00	8	9.2500	234
16	+	+	+	+	321.00	13.00	28.00	44.00	76.00	8	9.5000	1234

(1)

(2)

(3)

(4)

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In table A5 the design matrix gives the experimental conditions in standard order. Column y contains the corresponding initial rate on product yields for each run. These initial rates are now considered in successive pairs. The first eight entries in column (1) are obtained by adding the pairs together. Thus $165 + 170 = 335$, $184 + 231 = 415$, and so on. The second eight entries in column (1) are obtained by subtracting the top number from the bottom number of each pair. Thus $170 - 165 = 5$, $231 - 184 = 47$, and so on. In just the same way that column (1) is obtained from column y, column (2) is obtained from column (1). Finally, column (4) is obtained from column (3) in the same manner. To obtain the effects one has only to divide as before by the appropriate divisor, which is 16 for the first entry and 8 for the others. The first estimate is the grand average of all the observations. The remaining effects are identified by locating the plus sign occurs under each design matrix variable, so that the effect in the second row is the (\pm)menthol concentration effect. In the eighth row plus signs occurs in the 1, 2 and 3 column, so that the effects in that row is the 1x2x3 interaction.

From table A6, by considering the estimated value of main effects, it was found that changing of temperature will most affect the change of initial rates.

Estimated values of main and interaction effects demonstrated in table A6 were then run in order starting from the least to the highest values, and the probability of each values were calculated as shown in table A7. In order to indicate influences of each parameters on initial rate, these probability values were used to plot against estimated values (a normal plot) are shown in figure A1 (Box et al., 1978).

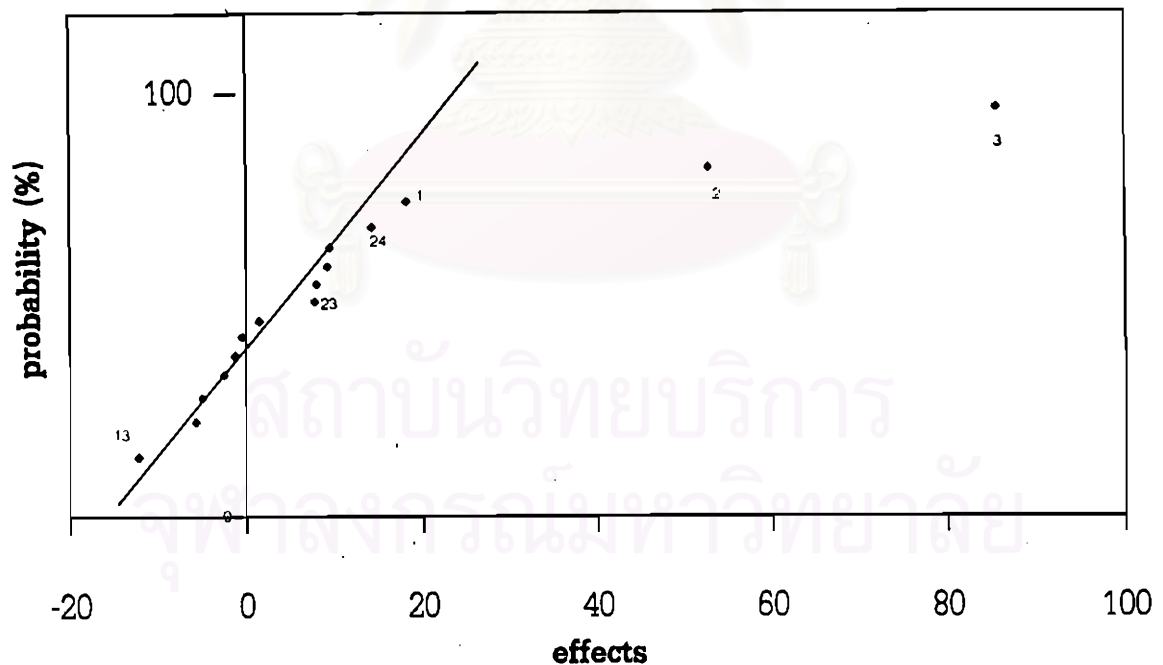
Table A6 Main and interaction effects of (±)menthol concentration, hexyl acetate concentration, temperature, and stirring speed on the estimated value

Effects	identity of effects	estimate
average		231.25
<u>Main effects</u>		
[(±)menthol] (1)	1	18.25
[hexyl acetate] (2)	2	52.75
temperature (3)	3	85.50
stirring speed (4)	4	-0.50
<u>Interaction effects</u>		
[(±)menthol] x [hexyl acetate]	12	8.00
[(±)menthol] x temperature	13	-12.25
[(±)menthol] x speed	14	-0.50
[hexyl acetate] x temperature	23	7.75
[hexyl acetate] x speed	24	14.25
temperature x speed	34	-2.50
	123	1.50
	124	-5.00
	134	-5.75
	234	9.25
	1234	9.50

Table A7 In order of effects and probability

order number (i)	1	2	3	4	5	6	7	8
effects	-12.25	-5.75	-5.00	-2.50	-1.25	-0.50	1.50	7.75
identity of effects	13	134	124	34	14	4	123	23
$P = 100(I-0.5)/15$	3.33	10.00	16.67	23.33	30.00	36.67	43.33	50.00

order number (i)	9	10	11	12	13	14	15
effects	8.00	9.25	9.50	14.25	18.25	52.75	85.50
identity of effects	12	234	1234	24	1	2	3
$P = 100(I-0.5)/15$	56.67	63.33	70.00	76.67	83.33	90.00	96.67

Figure A1 Plot of effects with probability from 2^4 factorial sets of the experiments

From figure A1, the result suggests that over the ranges of the parameters studied the main and interaction effects of 13, 23, 24, 1, 2, and 3 were the only effects distinguishable from noise. These parameters were then tested whether they really affect the rate of reaction (Box et al., 1978).

From effects in figure A1, a linear equation can be created to find a relationship between initial rate and effects as show in equation (A.1).

$$y = \text{average} + (-12.25/2)X_1X_3 + (7.75/2)X_2X_3 + (14.25/2)X_2X_4 \\ + (18.25/2)X_1 + (52.75/2)X_2 + (85.50/2)X_3 \quad (\text{A.1})$$

by y = response as initial rate ($\mu\text{mol/lit-hr}$)

X_1 = (\pm)menthol concentration

X_2 = hexyl acetate concentration

X_3 = temperature

X_4 = stirring speed

The test will be made by replacing variables X_1, X_2, X_3, X_4 with codified factors +1, -1 in table A1 of 2^4 factorial experiments.

For example experiment no. 1 of table A1, values of $X_1, X_2, X_3,$ and X_4 are -1. These values are then replaced in equation A.1.

Thus,

$$y = 231.25 + (-12.25/2)(-1)(-1) + (7.75/2)(-1)(-1) + (14.25/2)(-1)(-1) \\ + (18.25/2)(-1) + (52.75/2)(-1) + (85.50/2)(-1)$$

$$= 157.75 \mu\text{M/hr}$$

Results obtained by replacing values of 2^4 factorial experiments are shown in table A8. In order to testify the validity of equation A.1, the differences (the least to the highest values) between experimental values and calculated values are determined and plotted on a probability paper. Quite a good straight line was obtained as shown in figure A2 which supports the above speculation that parameters 13, 23, 24, 1, 2, and 3 were the only effects found in this study.



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Table A8 Comparison between initial rates from equation A.1 and experimental results

Order number	Initial rate from experiments	Initial rate from equation	Difference	Sort from minimum to maximum	Probability $P=100 \cdot (i-1/2)/15$ $i = \text{order number}$
1	165	157.75	7.25	-18.50	3.125
2	170	188.50	-18.50	-16.50	9.375
3	184	188.50	-4.50	-9.25	15.625
4	231	219.25	11.75	-5.50	21.875
5	253	248.00	5.00	-4.50	28.125
6	261	253.75	7.25	-1.75	34.375
7	285	294.25	-9.25	-0.50	40.625
8	303	300.00	3.00	-0.50	46.875
9	143	143.50	-0.50	3.00	53.125
10	186	174.25	11.75	4.25	59.375
11	201	202.75	-1.75	5.00	65.625
12	228	233.50	-5.50	6.75	71.875
13	238	233.75	4.25	7.25	78.125
14	223	239.50	-16.50	7.25	84.375
15	308	308.50	-0.50	11.75	90.625
16	321	314.25	6.75	11.75	96.875

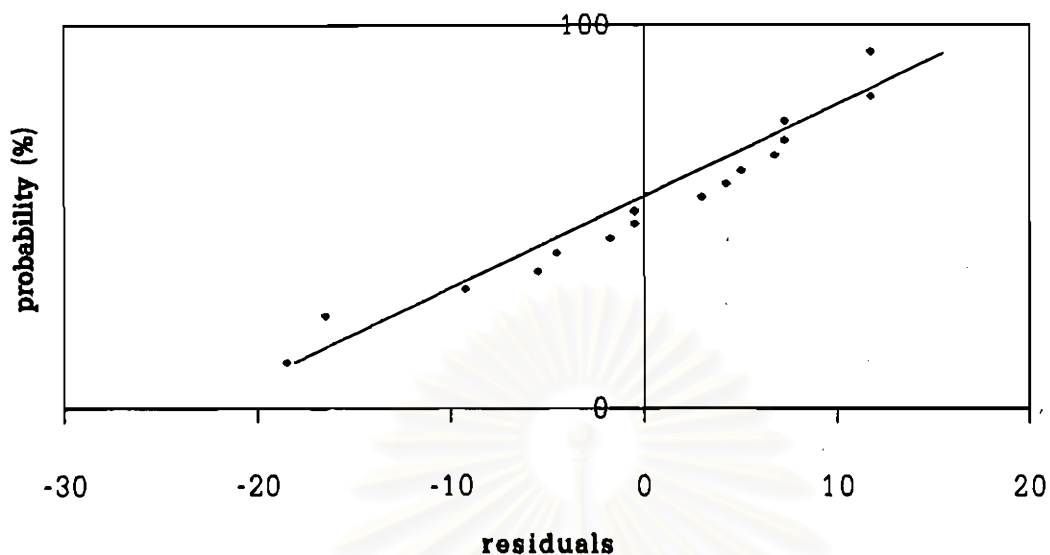


Figure A2 Plot of differences between the experiment of results and calculated results

(from equation A.1) on probability paper

From the experimental design planning at two levels, we could only construct the first order equation. So, the second order equation was then constructed for forecasting the reaction rate by experimenting key parameters at extreme levels (+2 and -2) (See table A3 and A9).

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Table A9 The initial rates of central composite design of factorial design experiments

Exp. No.	Initial rate ($\mu\text{M/hr}$)
17	152
18	256
19	96
20	318
21	98
22	100
23	285
24	260
25	250
26	298
27	309

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In order to achieve a more reliable initial values, the second order equation was then constructed using the principle of multiple regression which is as follows : (Myers, 1979)

$$y_i = \beta_0 + \sum_i \beta_i X_i + \sum_{i < j} \beta_{ij} X_i X_j + \sum_i \beta_{ii} X_i^2 + \epsilon_i \quad (A.2)$$

That is, if written in terms of the following vector of errors

$$y = \begin{bmatrix} y_1 \\ y_2 \\ \dots \\ y_n \end{bmatrix}, \quad X = \begin{bmatrix} 1 & X_{11} & X_{21} & \dots & X_{k1} \\ 1 & X_{12} & X_{22} & \dots & X_{k2} \\ \dots & \dots & \dots & \dots & \dots \\ 1 & X_{1n} & X_{2n} & \dots & X_{kn} \end{bmatrix}$$

and

$$\epsilon = \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \dots \\ \epsilon_n \end{bmatrix}$$

Then

$$y = X\beta + \epsilon \quad (A.3)$$

- Where
- y_i = response for the i th run
 - y = vector of response
 - β_i = regression coefficient for the i th run
 - β = vector of regression coefficient

- X_1, X_j = regression variables
 X = matrix of regression variables
 ε_i = random error for ith run
 ε = vector of random errors
 N = number of experiments

We wish to find the vector β that minimize errors using least square method, that is to determine vector β which results in a minimum value for

$$L = \sum_{i=1}^n \varepsilon_i^2 = \varepsilon' \varepsilon \quad (\text{A.4})$$

Then
$$L = (y - X\beta)'(y - X\beta) \quad (\text{A.5})$$

$$\frac{\partial L}{\partial \beta} = -2X'Y + 2X'X\beta = 0 \quad (\text{A.6})$$

Setting equation A.6 equals to zero and solving for β

Which equation A.6 can be simplified to

$$X'X \beta = X'y \quad (\text{A.7})$$

From equation A.5, set the matrix shown in table A10 and substitute the values into table A10 which results in table A11.

The values of vector β (determined by solving equation A.7) are shown in an equation which presents relationship as follows.

Table A10 Matrix from relation of four factors followed from Myers, 1979.

1	N	Σx_1	Σx_2	Σx_3	Σx_4	Σx_1^2	Σx_2^2	Σx_3^2	Σx_4^2	Σx_1x_2	Σx_1x_3	Σx_1x_4	Σx_2x_3	Σx_2x_4	Σx_3x_4	β_0	ΣY
2	Σx_1	Σx_1^2	$\Sigma x_1'x_1$	Σx_3x_1	Σx_4x_1	Σx_1^3	$\Sigma x_2^2x_1$	$\Sigma x_3^2x_1$	$\Sigma x_4^2x_1$	$\Sigma x_1^2x_2$	$\Sigma x_1^2x_3$	$\Sigma x_1^2x_4$	$\Sigma x_1x_2x_3$	$\Sigma x_1x_2x_4$	$\Sigma x_1x_3x_4$	β_1	Σx_1Y
3	Σx_2	Σx_2^2	Σx_2^2	Σx_3x_2	Σx_4x_2	$\Sigma x_1^2x_2$	Σx_2^3	$\Sigma x_3^2x_2$	$\Sigma x_4^2x_2$	$\Sigma x_1x_2^2$	$\Sigma x_1x_3x_2$	$\Sigma x_1x_4x_2$	$\Sigma x_2^2x_3$	$\Sigma x_2^2x_4$	$\Sigma x_3x_4x_2$	β_2	Σx_2Y
4	Σx_3	Σx_3^2	Σx_3^2	Σx_4x_3	$\Sigma x_1^2x_3$	$\Sigma x_2^2x_3$	Σx_3^3	$\Sigma x_4^2x_3$	$\Sigma x_1x_2x_3$	$\Sigma x_1x_3^2$	$\Sigma x_1x_4x_3$	$\Sigma x_2x_3^2$	$\Sigma x_2x_4x_3$	$\Sigma x_3^2x_4$		β_3	Σx_3Y
5	Σx_4	Σx_4x_1	Σx_4x_2	Σx_4x_3	Σx_4^2	$\Sigma x_1^2x_4$	$\Sigma x_2^2x_4$	$\Sigma x_3^2x_4$	Σx_4^3	$\Sigma x_1x_2x_4$	$\Sigma x_1x_3x_4$	$\Sigma x_1x_4^2$	$\Sigma x_2x_3x_4$	$\Sigma x_2x_4^2$	$\Sigma x_3x_4^2$	β_4	Σx_4Y
6	Σx_1^2	Σx_1^3	$\Sigma x_1^2x_2$	$\Sigma x_1^2x_3$	$\Sigma x_1^2x_4$	Σx_1^4	$\Sigma x_2^2x_1^2$	$\Sigma x_3^2x_1^2$	$\Sigma x_4^2x_1^2$	$\Sigma x_1^3x_2$	$\Sigma x_1^3x_3$	$\Sigma x_1^3x_4$	$\Sigma x_2x_3x_1^2$	$\Sigma x_2x_4x_1^2$	$\Sigma x_3x_4x_1^2$	β_{11}	$\Sigma x_{11}Y$
7	Σx_2^2	$\Sigma x_2^2x_1$	Σx_2^3	$\Sigma x_2^2x_3$	$\Sigma x_2^2x_4$	$\Sigma x_2^2x_1^2$	Σx_2^4	$\Sigma x_3^2x_2^2$	$\Sigma x_4^2x_2^2$	$\Sigma x_1x_2^3$	$\Sigma x_1x_3x_2^2$	$\Sigma x_1x_4x_2^2$	$\Sigma x_2^2x_3$	$\Sigma x_2^2x_4$	$\Sigma x_3x_4x_2^2$	β_{22}	$\Sigma x_{22}Y$
8	Σx_3^2	$\Sigma x_3^2x_1$	$\Sigma x_3^2x_2$	Σx_3^3	$\Sigma x_3^2x_4$	$\Sigma x_3^2x_1^2$	$\Sigma x_3^3x_2^2$	Σx_3^4	$\Sigma x_4^2x_3^2$	$\Sigma x_1x_2x_3^2$	$\Sigma x_1x_3^2$	$\Sigma x_1x_4x_3^2$	$\Sigma x_2x_3^2$	$\Sigma x_2x_4x_3^2$	$\Sigma x_3^2x_4$	β_{33}	$\Sigma x_{33}Y$
9	Σx_4^2	$\Sigma x_4^2x_1$	$\Sigma x_4^2x_2$	$\Sigma x_4^2x_3$	Σx_4^3	$\Sigma x_4^2x_1^2$	$\Sigma x_4^3x_2^2$	$\Sigma x_4^4x_3^2$	Σx_4^4	$\Sigma x_1x_2x_4^2$	$\Sigma x_1x_3x_4^2$	$\Sigma x_1x_4^2$	$\Sigma x_2x_3x_4^2$	$\Sigma x_2x_4^2$	$\Sigma x_3x_4^2$	β_{44}	$\Sigma x_{44}Y$
10	Σx_1x_2	$\Sigma x_1^2x_2$	$\Sigma x_1x_2^2$	$\Sigma x_1x_2x_3$	$\Sigma x_1x_2x_4$	$\Sigma x_1^3x_2$	$\Sigma x_1x_2^3$	$\Sigma x_1x_2x_3^2$	$\Sigma x_1x_2x_4^2$	$\Sigma x_1^2x_2^2$	$\Sigma x_1^2x_3x_2$	$\Sigma x_1^2x_4x_2$	$\Sigma x_2^2x_3x_1$	$\Sigma x_2^2x_4x_1$	$\Sigma x_1x_2x_3x_4$	β_{12}	$\Sigma x_{12}Y$
11	Σx_1x_3	$\Sigma x_1^2x_3$	$\Sigma x_1x_3x_2$	$\Sigma x_1x_3^2$	$\Sigma x_1x_3x_4$	$\Sigma x_1^3x_3$	$\Sigma x_1x_3x_2^2$	$\Sigma x_1x_3^3$	$\Sigma x_1x_3x_4^2$	$\Sigma x_1^2x_3x_2$	$\Sigma x_1^2x_3^2$	$\Sigma x_1^2x_4x_3$	$\Sigma x_2x_3^2x_1$	$\Sigma x_1x_2x_3x_4$	$\Sigma x_3^2x_4x_1$	β_{13}	$\Sigma x_{13}Y$
12	Σx_1x_4	$\Sigma x_1^2x_4$	$\Sigma x_1x_4x_2$	$\Sigma x_1x_4x_3$	$\Sigma x_1x_4^2$	$\Sigma x_1^3x_4$	$\Sigma x_1x_4x_2^2$	$\Sigma x_1x_4x_3^2$	$\Sigma x_1x_4^3$	$\Sigma x_1^2x_4x_2$	$\Sigma x_1^2x_4x_3$	$\Sigma x_1^2x_4^2$	$\Sigma x_1x_2x_3x_4$	$\Sigma x_2x_4^2x_1$	$\Sigma x_3x_4^2x_1$	β_{14}	$\Sigma x_{14}Y$
13	Σx_2x_3	$\Sigma x_1x_2x_3$	$\Sigma x_2^2x_3$	$\Sigma x_2x_3^2$	$\Sigma x_2x_3x_4$	$\Sigma x_2x_3x_1^2$	$\Sigma x_2^3x_3$	$\Sigma x_2x_3^3$	$\Sigma x_2x_3x_4^2$	$\Sigma x_2^2x_3x_1$	$\Sigma x_2x_3^2x_1$	$\Sigma x_1x_2x_3x_4$	$\Sigma x_2^2x_3^2$	$\Sigma x_2^2x_4x_3$	$\Sigma x_3^2x_4x_2$	β_{23}	$\Sigma x_{23}Y$
14	Σx_2x_4	$\Sigma x_1x_2x_4$	$\Sigma x_2^2x_4$	$\Sigma x_2x_4x_3$	$\Sigma x_2x_4^2$	$\Sigma x_2x_4x_1^2$	$\Sigma x_2^3x_4$	$\Sigma x_2x_4x_3^2$	$\Sigma x_2x_4^3$	$\Sigma x_2^2x_4x_1$	$\Sigma x_1x_2x_3x_4$	$\Sigma x_2x_4^2x_1$	$\Sigma x_2^2x_4x_3$	$\Sigma x_2^2x_4^2$	$\Sigma x_3x_4^2x_2$	β_{24}	$\Sigma x_{24}Y$
15	Σx_3x_4	$\Sigma x_1x_3x_4$	$\Sigma x_3x_4x_2$	$\Sigma x_3^2x_4$	$\Sigma x_3x_4^2$	$\Sigma x_3x_4x_1^2$	$\Sigma x_3x_4x_2^2$	$\Sigma x_3^3x_4$	$\Sigma x_3x_4^3$	$\Sigma x_1x_2x_3x_4$	$\Sigma x_3^2x_4x_1$	$\Sigma x_3x_4^2x_1$	$\Sigma x_3^2x_4x_2$	$\Sigma x_3x_4^2x_2$	$\Sigma x_3^2x_4^2$	β_{34}	$\Sigma x_{34}Y$

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Table A11 To replace a values from table A10

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			
1	27	0	0	0	0	24	24	24	24	0	0	0	0	0	0	X	β_0	6122
2	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0		β_1	354
3	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0		β_2	866
4	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0		β_3	688
5	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0		β_4	-54
6	24	0	0	0	0	48	16	16	16	0	0	0	0	0	0		β_{11}	5332
7	24	0	0	0	0	16	48	16	16	0	0	0	0	0	0		β_{22}	5356
8	24	0	0	0	0	16	16	48	16	0	0	0	0	0	0		β_{33}	4492
9	24	0	0	0	0	16	16	16	48	0	0	0	0	0	0		β_{44}	5880
10	0	0	0	0	0	0	0	0	0	24	0	0	0	0	0		β_{12}	64
11	0	0	0	0	0	0	0	0	0	0	24	0	0	0	0		β_{13}	-98
12	0	0	0	0	0	0	0	0	0	0	0	24	0	0	0		β_{14}	-10
13	0	0	0	0	0	0	0	0	0	0	0	0	24	0	0		β_{23}	62
14	0	0	0	0	0	0	0	0	0	0	0	0	0	24	0		β_{24}	114
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24		β_{34}	-20

$$\begin{aligned}
 y = & 285.67 + 14.75X_1 + 36.08X_2 + 28.67X_3 - 2.25X_4 \\
 & - 14.48X_{11} - 13.73X_{22} - 40.73X_{33} + 2.64X_{44} \\
 & + 4.00X_1X_2 - 6.12X_1X_3 - 0.62X_1X_4 \\
 & + 3.87X_2X_3 + 7.12X_2X_4 - 1.25X_3X_4
 \end{aligned} \tag{A.8}$$

The initial rate could be obtained by replacing variables X_1 , X_2 , X_3 , and X_4 with codified factors +1, -1 of 2^4 factorial experiments (show in table A1) including CCD value (shown in table A3), and the calculated results obtained are compared to the experimental values (see table A12).



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Table A12 Comparison between initial rates obtained experimentally and those calculated from the second order equation

Experimental number	Experimental values ($\mu\text{M/hr}$)	Calculation values ($\mu\text{M/hr}$)
1	165	149
2	170	184
3	184	191
4	231	242
5	253	213
6	261	224
7	285	271
8	303	298
9	143	134
10	186	167
11	201	204
12	228	253
13	238	193
14	223	201
15	308	280
16	321	301

Experimental number	Experimental values ($\mu\text{M/hr}$)	Calculation values ($\mu\text{M/hr}$)
CCD 1	152	198
2	256	257
3	96	158
4	318	303
5	98	66
6	100	180
7	285	300
8	260	291
9	250	285
10	298	285
11	309	285

The validity of equation A.6 is then tested using ANOVA analysis.

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Table A13 ANOVA analysis of second order equation for predicting initial rate values

Source	sum of square	DF	mean square	F-ratio
Regression	1488971	15	99265	2.39
lack of fit	23468	10	2347	
Error	1969	2	984	
Residual	25437	12	2120	
Total	1514408	27		

The critical F (degree of freedom 10, 2) value obtained from the statistical table at 95% confidence equals to 19.40, and the F-ratio found in table A13 is 2.39 which is less than the critical value. As a result, equation A.6 is reliable at 95% confidence in predicting an initial rate.

From second order equation (A.8), we can predict initial rate of the transesterification reaction in the range of experimental design. We will consider main and interaction effects of the reaction. The main effects found were menthol concentration, hexyl acetate concentration, and temperature, while interaction effects were between menthol concentration and temperature, hexyl acetate concentration and temperature, and hexyl acetate concentration and stirring speed. Since, all the main effect parameters were also included in interaction effect parameters, only interaction effect parameters will be discussed.

A2 To find the optimum point

From equation A.6 obtained using experimental design, the optimum conditions for the transesterification reaction of racemic menthol and hexyl acetate concentration by *Candida cylindracea* lipase can be determined using Myers's method (1979) as follows; So, we will find the suitable condition of all factors from this below equation. (Myers, 1979)

$$X = \frac{-B^{-1}b}{2}$$

Where

$$B = \begin{bmatrix} \beta_{11} & \beta_{12} & \dots & \beta_{1k} \\ \beta_{12} & \beta_{22} & \dots & \beta_{2k} \\ \dots & \dots & \dots & \beta_{3k} \\ \beta_{1k} & \beta_{2k} & \dots & \beta_{4k} \end{bmatrix} \text{ symmetry}$$

$$b = \begin{bmatrix} \beta_1 \\ \beta_2 \\ \dots \\ \beta_k \end{bmatrix}$$

and

$$X = \begin{bmatrix} X_1 \\ X_2 \\ \dots \\ X_k \end{bmatrix}$$

Where β_i = regression coefficient for the i th run

X_1 = menthol concentration

X_2 = hexyl acetate concentration

X_3 = temperature

X_4 = stirring speed

Since it was earlier found that

$$B = \begin{bmatrix} -14.48 & 4.00 & -6.12 & -0.62 \\ 4.00 & -13.73 & 3.87 & 7.12 \\ -6.12 & 3.87 & -40.73 & -1.25 \\ -0.62 & 7.12 & -1.25 & 2.64 \end{bmatrix}$$

$$B^{-1} = \begin{bmatrix} -0.0771 & -0.0111 & 0.0100 & 0.0164 \\ -0.0111 & -0.0332 & -0.0041 & 0.0649 \\ 0.0100 & -0.0041 & -0.0265 & 0.0009 \\ 0.0154 & 0.0349 & 0.0009 & 0.1539 \end{bmatrix}$$

$$b = \begin{bmatrix} 14.75 \\ 36.08 \\ 28.67 \\ -2.25 \end{bmatrix}$$

$$\text{So } X = \begin{bmatrix} 0.6433 \\ 0.8342 \\ 0.3803 \\ -1.4927 \end{bmatrix}$$

By changing regression variables to the actual values, optimum conditions for the transesterification studied are:

concentration of racemic menthol = 73 mM

concentration of hexyl acetate = 360 mM

temperature = 66 °C

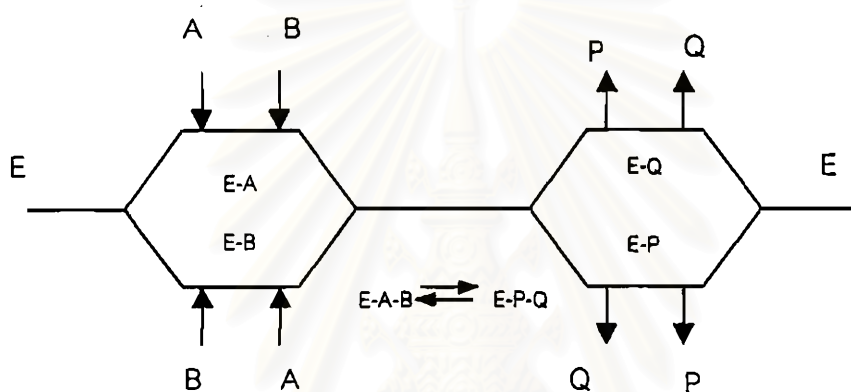
stirring speed = 110 rpm



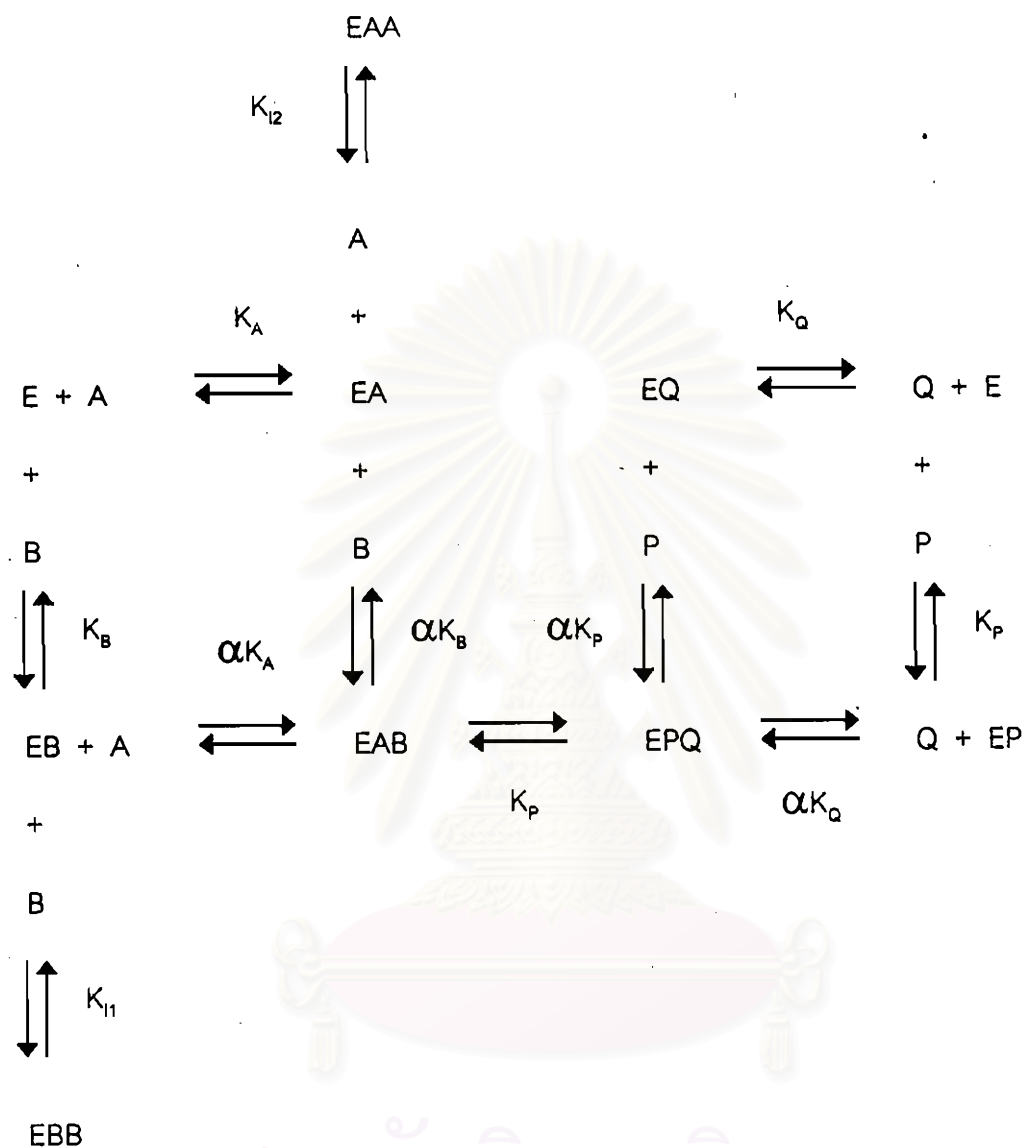
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Appendix B Random bi bi mechanism

Both substrate (A and B) randomly bind with enzyme to form enzyme-substrate complex (EAB). After which the product-complex (EPQ) is produced. In the same way as the substrate binding, both products are randomly released from the enzyme. The binding of substrates and releasing of products are in random fashion, and can be shown by the scheme as follows:



The schematic show in below.



Where

A = hexyl acetate

B = racemic menthol

P = hexanol

Q = (-)-menthyl acetate

E = enzyme lipase

K_{i1} = dissociation constant of reaction $EB + B \rightleftharpoons EBB$

K_{i2} = dissociation constant of reaction $EA + A \rightleftharpoons EAA$

K_A = dissociation constant of reaction $E + A \rightleftharpoons EA$

αK_A = dissociation constant of reaction $EB + A \rightleftharpoons EAB$

K_B = dissociation constant of reaction $E + B \rightleftharpoons EB$

αK_B = dissociation constant of reaction $EA + B \rightleftharpoons EAB$

EA = enzyme-A complex

EAA = enzyme-A2 molecules complex

EB = enzyme-B complex

EBB = enzyme-B2 molecules complex

Assumption :

1. Since all the binding and dissociation steps are very rapid compared to catalytic step, the rate limiting step will be indicated simply as The step $EAB \xrightleftharpoons{K_p} EPQ$,

therefore, $V = K_p[EAB]$

2. Rapid equilibrium approach is applied.

Since,

$$V = K_p[EAB]$$

and

$$\frac{V}{E_0} = \frac{K_p[EAB]}{[E] + [EA] + [EB] + [EAA] + [EBB] + [EAB]}$$

Where $[E_0] = [E] + [EA] + [EB] + [EAA] + [EBB] + [EAB]$

$[EA] = [E][A] / K_A$

$[EB] = [E][B] / K_B$

$[EAA] = [EA][A] / K_{i2} = [E][A][A] / K_{i2}K_A$

$$[EBB] = [EB][B] / K_{i1} = [E][B][B] / K_{i1}K_B$$

$$[EAB] = [E][A][B] / \alpha K_A K_B$$

So,

$$\frac{V}{K_p E_0} = \frac{[E][A][B]}{\alpha K_A K_B} \frac{1}{[E] + \frac{[E][A]}{K_A} + \frac{[E][B]}{K_B} + \frac{[E][A][A]}{K_{i2}K_A} + \frac{[E][B][B]}{K_{i1}K_B} + \frac{[E][A][B]}{\alpha K_A K_B}}$$

$$\frac{V}{V_{\max}} = \frac{[A][B]}{\alpha K_A K_B + \alpha K_B [A] + \alpha K_A [B] + [A][A] \frac{\alpha K_B}{K_{i2}} + [B][B] \frac{\alpha K_A}{K_{i1}} + [A][B]}$$

Therefore,

$$V = \frac{[A][B]V_{\max}}{\alpha K_A K_B + \alpha K_B [A] \left(1 + \frac{[A]}{K_{i2}}\right) + \alpha K_A [B] \left(1 + \frac{[B]}{K_{i1}}\right) + [A][B]}$$

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BIOGRAPHY

Anontapat Kalapaphongse was born on 10th June, 1972 in Bangkok, Thailand. He took the entrance examination to study in the faculty of Science, department of Chemical Technology, Chulalongkorn University. In 1995, he furthered his study in Master's degree program at the Department of Chemical Engineering, Chulalongkorn University. He actively participated in the Biochemical Engineering research group.



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