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APPENDICES

Appendix A Water Content and Size of Microemulsion

Table A-1 Water/surfactant molar ratio and size of reverse micelle when varying salt concentration; 100 mM NaDEHP/isooctane, 50 mM cosurfactant, no substrate

[NaCl], M	W_0	Rh, nm
0.0	26.9	41.3
0.5	15.8	19.5
1.0	11.3	12.0

Table A-2 Water/surfactant molar ratio and size of reverse micelle when varying substrate concentration; 100 mM NaDEHP/isooctane, 50 mM cosurfactant, 0.5 M NaCl

Substrate	W_0	Rh, nm
No substrate	15.8	19.5
25 mM caprylic acid/75 mM hexanol	10.7	-
50 mM oleic acid/75 mM hexanol	7.7	5.2
75 mM oleic acid/100 mM hexanol	7.2	4.6

Appendix B Calibration Curve

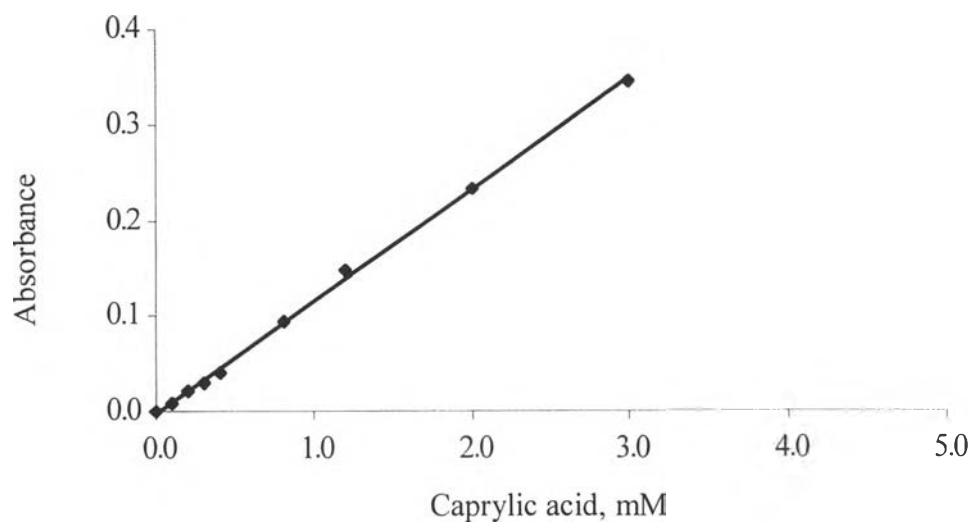


Figure B-1 Calibration curve of caprylic acid.

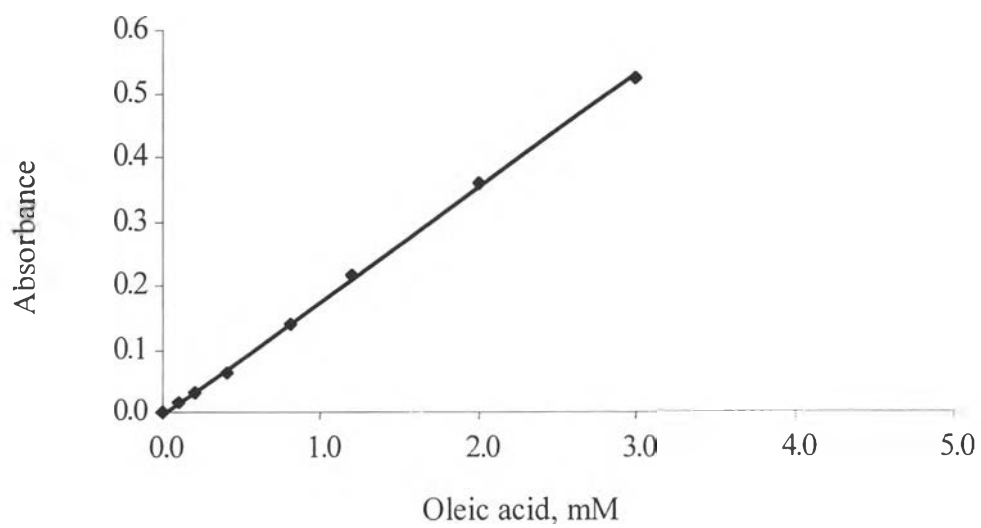


Figure B-2 Calibration curve of oleic acid.

Appendix C Determination of Lipase

Condition	Before encapsulation		Lipase remaining	Encapsulated lipase	
	Lipase,			Lipase,	%
	Absorbance	mg/l	Absorbance	mg/l	Encapsulation
50 mM caprylic acid/75 mM hexanol /0.2 M NaCl	0.639	0.042	0.259	0.025	59.471
50 mM oleic acid/75 mM hexanol/1.0 M NaCl	0.534	0.035	0.177	0.023	66.887
50 mM oleic acid/75 mM hexanol/0.5 M NaCl	0.534	0.035	0.265	0.018	50.332

Appendix D Esterification Reaction

Activity of lipase and reaction conversion(at 30 min) of esterification reaction in 100 mM NaDEHP/50 mM hexanol as a co surfactant/isooctane reverse micellar system (35°C and pH 7.4).

D1 Effect of W_0

Table D1-1 50 mM oleic acid with 75 mM hexanol

[NaCl]	W_0	Activity mM/min*mg protein	%Conversion
0.2	10	29.41	68.94
0.5	8.9	33.61	54.49
0.8	7.9	52.29	61.31
1	7.5	34.60	38.56

Table D1-2 50 mM caprylic acid with 75 mM hexanol

[NaCl]	W_0	Activity mM/min*mg protein	%Conversion
0.2	8.9	40.04	87.63
0.5	8.3	56.49	81.45
0.8	7.5	22.98	74.63
1	6.5	22.78	77.09

D2 Effect of type of substrate**Table D2-1** 50 mM fatty acid with 75 mM hexanol/0.5 NaCl

[fatty acid] (mM)	W_0	Activity mM/min*mg protein	%Conversion
caprylic acid	7.4	48.63	42.84
oleic acid	7.3	98.78	65.54

Table D2-2 25 mM fatty acid with 75 mM hexanol/0.5 NaCl

[fatty acid] (mM)	W_0	Activity mM/min*mg protein	%Conversion
caprylic acid	8.4	16.01	42.07
oleic acid	8.2	61.06	46.48

D3 Effect of type of fatty acid concentration**Table D3-1** Caprylic acid with 75 mM hexanol/0.5 NaCl

[Caprylic acid], mM	W_0	Activity mM/min*mg protein	%Conversion
0	-	0.00	0
20	9.15	16.13	83.90
35	7.67	99.07	67.94
50	7.4	48.63	42.84
65	6.73	27.57	35.98

Table D3-2 Oleic acid with 75 mM hexanol/0.5 NaCl

[oleic acid], mM	W_0	Activity mM/min*mg protein	%Conversion
0	-	0.00	0
15	9.37	23.58	44.57
30	8.4	61.06	46.48
45	7.9	129.47	36.9
60	6.9	109.41	27.32

D4 Effect of type of hexanol concentration**Table D4** 40 mM oleic acid with hexanol /0.5 NaCl

[hexanol], mM	W_0	Activity mM/min*mg protein	%Conversion
0	0	0.00	0
20	7.8	14.93	39.48
35	7.53	23.96	45.66
75	7.6	51.00	54.61
120	7.29	57.30	34.66

Appendix E FT-IR Spectra

E1 Spectra of substrates

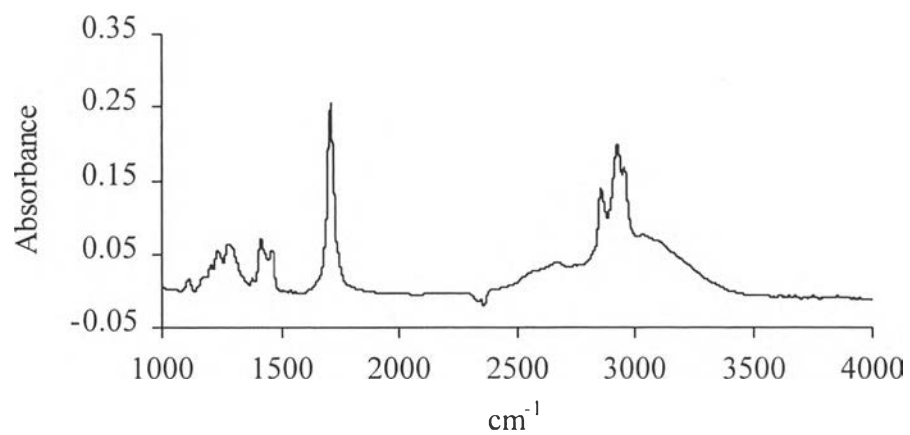


Figure E1-1 Spectrum of caprylic acid.

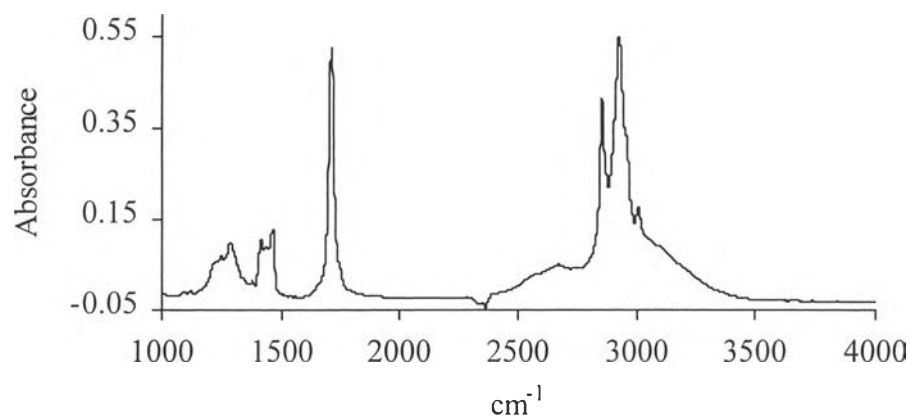


Figure E1-2 Spectrum of oleic acid.

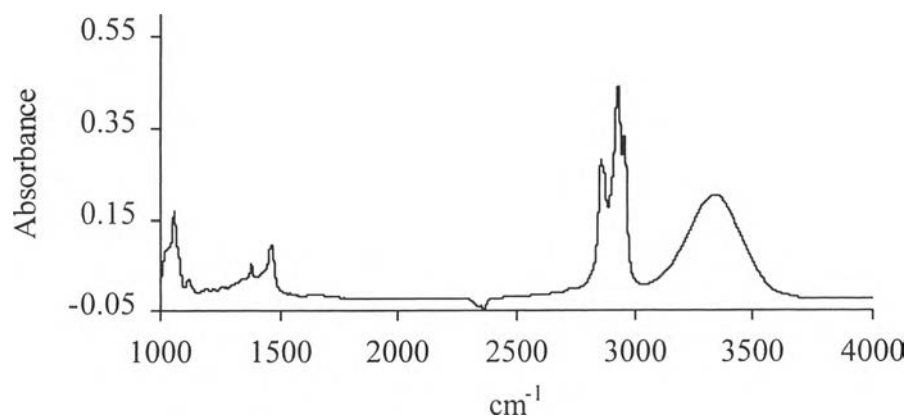


Figure E1-3 Spectrum of hexanol.

E 2 Spectra of Other Components in Microemulsions

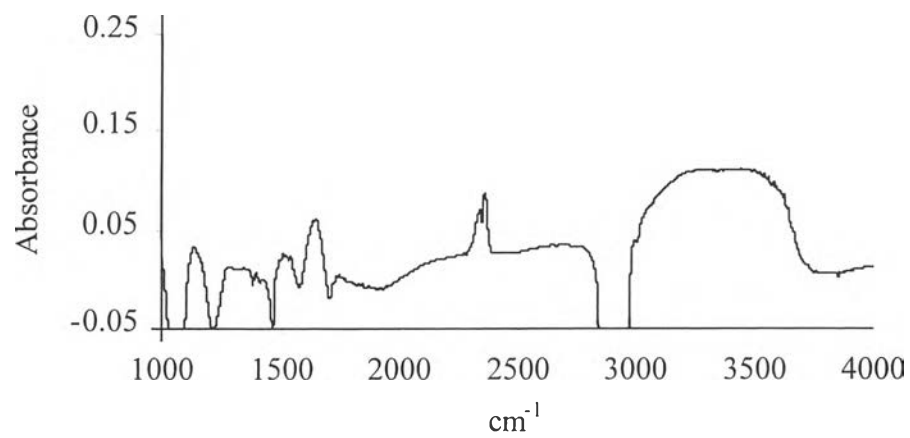


Figure E2-1 Spectrum of Rice bran lipase.

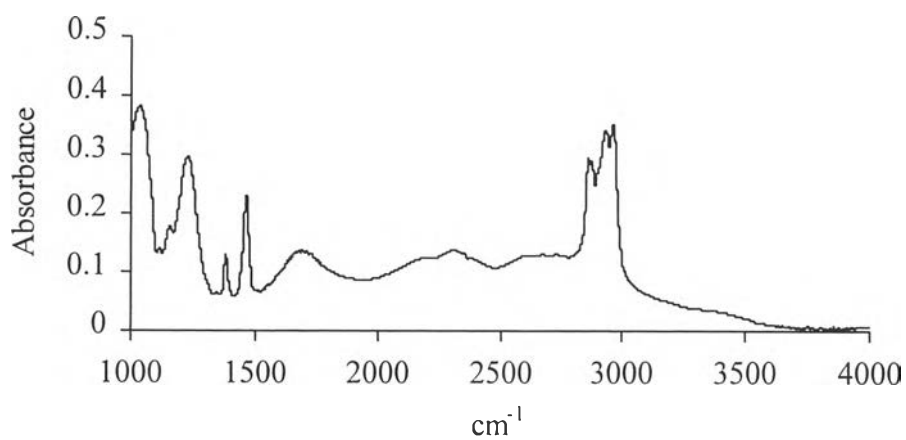


Figure E2-2 Spectrum of sodium bis(2-ethylhexyl)hydrogen phosphate (NaDEHP).

E 3 Spectra of Ester Sample

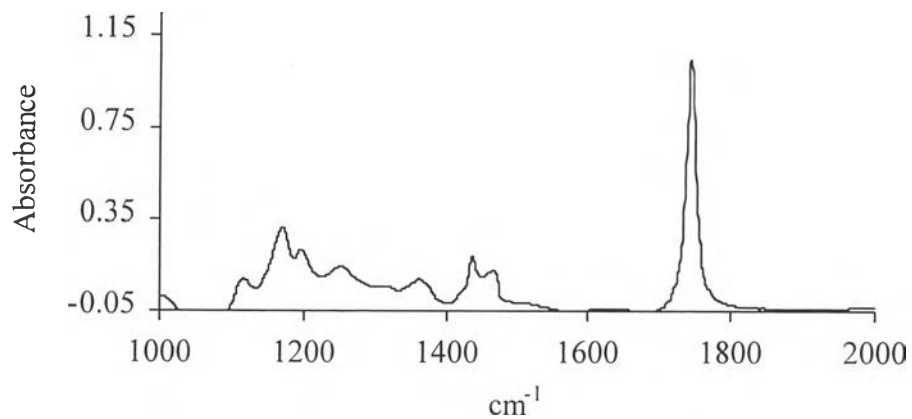


Figure E3-1 Spectrum of methyl ester.

Appendix F Example of Calculation

F1 Example of water to surfactant ratio, W_0 determination

Condition: 100 mM NaDEHP/50 mM hexanol/0.5M NaCl

substrate: 50 mM caprylic acid, 75 mM hexanol

average water content (W) = 2.522 % (W/W)

average weight of total sample (Wt) = 0.2845 mg

volume of sample (V) = 0.04 ml

molar concentration of NaDEHP in organic phase [NaDEHP] = 100 mM

$$W_0 = \frac{(W/100)(Wt) * 1000}{(Mw)(V) * [NaDEHP]}$$

$$W_0 = \frac{(2.522/100)(28.45)(1000)}{(18)(0.04)(100)}$$

$$W_0 = 10$$

F2 Example of enzyme determination

Absorbance of RBL at 276 nm determined by UV-VIS (A_{276}) = 0.6385

molar extinction coefficient of RBL at 276 nm (E) = 15.25

$$\text{Amount of enzyme} = \frac{A_{276}}{E} \quad \text{mg/l}$$

$$\text{Amount of enzyme} = \frac{0.6385}{15.25} = 0.042 \text{ mg/l}$$

F3 Example of activity determination

Condition: 100 mM NaDEHP/50 mM hexanol/0.5 M NaCl

substrate: 50 mM caprylic acid, 75 mM hexanol

initial rate from curve fitting = 8.9643 mM/min

volumn of aquese phase (V) = 7.5 ml

amount of lipase determine by UV-VIS = 0.025 mg/ml

$$= 0.025 * 7.5 = 0.19 \text{ mg}$$

$$\text{Activity} = \frac{\text{initial rate, mM/min}}{\text{amount of lipase, mg}}$$

$$= \frac{8.9643}{0.19} = 47.181 \text{ mM/min} * \text{mg of protein}$$

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