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APPENDICES

Appendix A Microemulsion studies.

Table A-1 Water content of 100mMNaDEHP reverse micellar system with no cosurfactant

NaCl (M)	% water	Wo
0	0.02	1.52
0.5	0.02	1.57
1.0	0.01	1.06
1.5	0.02	1.27
2.0	0.05	2.97
2.5	0.03	1.77
3.0	0.04	2.36
3.5	0.05	3.32
4.0	0.17	9.62
4.5	1.11	6.25
5.0	0.30	17.02

Table A-2 Water content of 100mM NaDEHP/ 50mM Hexanol isooctane reverse micellar system

NaCl (M)	% water	Wo
0.1	0.54	0.20
0.5	17.38	6.96
1.0	25.70	10.49
2.0	15.07	6.09
3.0	10.50	4.34

Table A-3 Water content of 100mM NaDEHP/ 100mM Hexanol isooctane reverse micellar system

NaCl (M)	% water	Wo
0.1	0.60	2.39
0.5	17.22	7.14
1.0	31.55	12.96
2.0	14.23	5.74
3.0	12.97	5.12

Table A-4 Water content of 100mM NaDEHP/ 150mM Hexanol isooctane reverse micellar system

NaCl (M)	% water	Wo
0.1	0.98	1.98
0.5	6.32	11.87
1.0	8.28	16.83
2.0	5.43	11.45
3.0	4.99	10.29

Appendix B Esterification reaction.

Table B-1 Water-to-surfactant molar ratio of 100mM NaDEHP/ 100mM hexanol/ 100 mM caprylic acid in isooctane/ 1.0M NaCl/ 0.02-0.08 mg/ml CALB reverse micellar system

Enzyme Conc. (mg/ml)	CALB		TLL	
	Wo		Wo	
	Before	After	Before	After
0.02	9.86	10.48	8.76	8.83
0.04	8.36	9.87	8.50	8.87
0.08	8.59	9.77	9.16	9.35

Table B-2 Rate of esterification of 100mM NaDEHP/ 100mM hexanol/100 mM caprylic acid in isooctane/ 1.0M NaCl/ 0.02-0.08 mg/ml CALB reverse micellar system

Time (min)	Substrate Concentration (mM)		
	Lipase Conc.		
	0.02 mg/ml	0.04mg/ml	0.08 mg/ml
0	95.15	96.07	96.55
5	91.07	90.68	91.02
10	84.35	84.62	85.09
20	76.92	83.40	80.17
50	68.16	73.70	65.89
100	65.58	62.05	60.16
150	56.67	56.41	56.46
200	48.93	46.43	47.41

Table B-3 Rate of esterification of 100mM NaDEHP/ 100mM hexanol/ 100 mM caprylic acid in isooctane/ 1.0M NaCl/ 0.02-0.08 mg/ml TLL reverse micellar system

Time (min)	Substrate Concentration (mM)		
	Lipase conc.		
	0.02 mg/ml	0.04mg/ml	0.08 mg/ml
0	95.89	95.73	95.81
5	93.71	93.90	93.84
10	89.09	88.63	88.68
20	80.11	80.01	80.03
50	73.69	73.81	73.77
100	70.20	70.17	70.36
150	69.47	69.30	69.44
200	68.73	68.58	68.44

Table B-4 Water-to-surfactant molar ratio of 25-150mM NaDEHP/100mM hexanol/ 100mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml lipases reverse micellar system

NaDEHP (mM)	CALB		TLL	
	Wo		Wo	
	Before rxn.	After rxn.	Before rxn.	After rxn.
25	1.23	1.93	1.11	1.18
50	2.63	2.77	3.09	3.22
75	4.04	4.87	4.81	5.79
100	6.67	8.24	8.44	10.62
125	7.86	9.15	9.50	11.13
150	9.28	10.15	13.67	14.86

Table B-5 Water-to-surfactant molar ratio of 25-150mM NaDEHP/ 100mM hexanol/ 100mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml lipases reverse micellar system

NaDEHP (mM)	CALB		TLL	
	Wo		Wo	
	Before rxn.	After rxn.	Before rxn.	After rxn.
25	0.84	0.97	0.78	0.91
50	2.86	3.39	2.54	3.01
75	5.16	6.21	5.11	6.09
100	7.08	8.60	7.00	8.45
125	9.31	11.39	9.12	9.97
150	10.61	11.91	11.91	12.71

Table B-6 Rate of esterification of 25-150mM NaDEHP/ 100mM hexanol/ 100mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB reverse micellar system

Time (min)	Oleic acid conc. (mM)					
	NaDEHP conc. (mM)					
	25	50	75	100	125	150
0	97.12	98.20	98.02	97.85	96.94	97.35
5	86.49	81.37	78.82	77.21	84.89	85.88
10	85.71	71.08	60.68	60.74	78.72	79.64
20	84.15	67.45	50.58	39.96	65.52	60.00
50	80.75	61.71	45.28	30.79	50.71	50.96
100	76.80	60.48	43.19	29.39	47.27	47.52
150	75.54	58.43	43.83	28.71	46.15	47.83
200	74.39	58.32	43.32	28.48	45.10	48.15

Table B-7 Rate of esterification of 25-150mM NaDEHP/ 100mM hexanol/ 100mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL reverse micellar system

Time (min)	Oleic acid conc. (mM)					
	NaDEHP conc. (mM)					
	25	50	75	100	125	150
0	98.10	98.75	96.19	96.77	97.41	96.55
5	88.41	82.49	75.37	60.48	73.52	73.78
10	84.32	79.54	65.57	54.94	67.38	56.42
20	82.13	63.11	49.09	39.34	58.61	56.70
50	82.65	62.62	47.07	35.64	45.68	55.23
100	76.86	59.19	42.68	35.68	43.19	55.63
150	76.83	59.18	42.49	32.66	41.52	54.55
200	76.33	58.19	42.87	32.36	40.77	54.20

Table B-8 Rate of esterification of 25-150mM NaDEHP/ 100mM hexanol/ 100mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB

Time (min)	Oleic acid conc. (mM)					
	NaDEHP conc. (mM)					
	25	50	75	100	125	150
0	98.70	98.21	98.02	98.37	97.82	97.63
5	88.35	81.23	80.46	77.12	79.94	80.81
10	87.64	72.52	70.75	68.48	68.80	75.09
20	85.38	70.74	61.64	57.91	54.69	70.47
50	82.66	65.26	54.36	50.39	47.25	65.37
100	80.16	63.53	53.41	47.12	42.32	60.68
150	78.77	60.56	54.58	46.89	42.07	58.79
200	75.08	59.31	53.39	46.72	41.48	58.40

Table B-9 Rate of esterification of 25-150mM NaDEHP/ 100mM hexanol/ 100mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL reverse micellar system

Time (min)	Oleic acid conc. (mM)					
	NaDEHP conc. (mM)					
	25	50	75	100	125	150
0	99.01	98.63	98.50	98.81	98.53	98.33
5	96.21	94.32	90.99	90.53	85.40	93.61
10	90.73	90.10	84.57	79.73	87.52	90.07
20	86.74	86.06	69.85	65.58	72.08	80.66
50	85.17	75.45	61.32	50.67	62.09	70.20
100	82.65	71.30	53.99	38.79	58.77	61.38
150	82.34	65.48	53.12	35.25	55.54	62.08
200	81.88	65.08	52.20	34.98	53.28	56.68

Table B-10 Rate of esterification of 100mM NaDEHP/ 100mM hexanol/ 25-150mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB reverse micellar system

Time (min)	Oleic acid conc. (mM)					
	25	50	75	100	125	150
0	24.13	49.34	74.29	98.70	124.27	147.17
5	13.24	32.19	59.30	83.95	111.17	141.17
10	7.42	17.71	38.39	70.25	100.32	127.90
20	3.82	15.83	20.09	61.00	84.76	118.21
50	1.08	4.42	19.24	60.23	71.23	106.82
100	0.93	1.14	19.26	48.81	69.06	96.56
150	1.01	0.98	18.76	46.73	69.86	90.57
200	1.03	1.16	18.47	40.33	69.31	94.86

Table B-11 Rate of esterification of 100mM NaDEHP/ 100mM hexanol/ 25-150mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL in reverse micellar system

Time	Oleic acid conc. (mM)					
(min)	25	50	75	100	125	150
0	24.70	49.22	73.92	99.77	124.48	149.17
5	17.87	41.04	65.67	86.86	115.93	142.45
10	11.89	33.98	48.67	77.90	108.18	139.25
20	5.67	18.02	35.70	66.89	92.10	120.64
50	2.42	9.42	25.99	59.90	76.08	108.62
100	1.66	2.57	19.34	57.53	72.09	102.31
150	1.02	1.29	19.42	52.18	70.63	96.41
200	1.18	1.12	19.15	49.30	72.08	90.12

Table B-12 Rate of esterification of 100mM NaDEHP/ 100mM hexanol/ 25-150mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB in reverse micellar system

Time	Oleic acid conc. (mM)					
(min)	25	50	75	100	125	150
0	24.16	48.04	74.39	98.56	124.06	148.45
5	16.03	40.98	64.61	86.00	115.64	144.91
10	10.83	30.69	44.27	70.00	107.21	134.29
20	7.16	18.61	30.80	61.91	90.89	119.67
50	5.00	6.09	21.62	57.06	73.99	117.29
100	1.58	1.40	19.21	46.03	72.91	112.08
150	1.20	1.58	17.17	44.76	72.91	113.50
200	1.52	2.08	17.97	43.94	71.43	111.09

Table B-13 Rate of esterification of 100mM NaDEHP/ 100mM hexanol/ 25-150mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL reverse micellar system

Time	Oleic acid conc. (mM)					
(min)	25	50	75	100	125	150
0	24.26	49.58	73.95	98.20	124.24	148.89
5	19.36	41.92	67.61	90.76	117.73	144.21
10	15.09	36.33	52.79	79.74	110.87	140.18
20	12.17	21.02	39.91	69.74	97.28	128.32
50	7.59	12.17	35.43	60.55	85.86	121.83
100	4.56	5.25	21.56	60.05	77.57	116.84
150	2.36	2.42	19.73	59.45	75.59	112.63
200	1.46	2.47	18.99	59.54	74.87	109.95

Table B-14 Rate of esterification, conversion and specific activity of 25-150mM NaDEHP/ 100mM hexanol/ 100mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB reverse micellar system

NaDEHP (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	1.23	1.93	1.39	2.70	16.85	30.43
50	2.63	2.77	5.52	3.73	37.16	41.95
75	4.04	4.87	20.49	4.24	43.80	47.70
100	6.67	8.24	23.49	4.56	68.53	51.32
125	7.86	9.15	16.45	3.02	47.69	34.02
150	9.28	10.15	9.38	2.82	47.66	31.80

Table B-15 Rate of esterification, conversion and specific activity of 25-150mM NaDEHP/ 100mM hexanol/ 100mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL reverse micellar system

NaDEHP (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	1.11	1.18	6.72	2.32	15.75	13.25
50	3.09	3.22	4.33	3.50	36.59	20.02
75	4.81	5.79	19.55	4.93	51.06	28.15
100	8.44	10.62	21.56	7.90	63.17	45.17
125	9.50	11.13	17.09	5.30	53.10	30.27
150	13.67	14.86	8.74	5.24	42.80	29.97

Table B-16 Rate of esterification, conversion and specific activity of 25-150mM NaDEHP/ 100mM hexanol/ 100mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB reverse micellar system

NaDEHP (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	0.84	0.97	16.25	2.33	16.25	26.24
50	2.86	3.39	33.54	3.75	33.54	42.27
75	5.16	6.21	44.54	3.91	44.54	44.01
100	7.08	8.60	48.77	4.58	48.77	51.52
125	9.31	11.39	51.70	4.01	51.70	45.18
150	10.61	11.91	33.04	3.84	33.04	43.22

Table B-17 Rate of esterification, conversion and specific activity of 25-150mM NaDEHP/ 100mM hexanol/ 100mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL reverse micellar system

NaDEHP (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	0.78	0.91	17.41	0.76	13.98	8.53
50	2.54	3.01	18.29	1.14	23.50	12.79
75	5.11	6.09	19.13	1.80	37.75	20.29
100	7.00	8.45	20.70	1.89	48.72	21.32
125	9.12	9.94	9.34	2.92	36.99	32.09
150	11.91	12.71	6.69	1.28	29.61	14.40

Table B-18 Rate of esterification, conversion and specific activity of 100mM NaDEHP/ 100mM hexanol/ 25-150mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB reverse micellar system

Oleic Acid (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	10.31	10.79	4.65	2.35	95.51	26.49
50	9.17	9.95	8.58	3.56	91.04	40.12
75	8.43	9.37	11.15	3.14	74.11	35.37
100	7.01	8.18	16.59	3.21	38.98	36.14
125	6.41	7.14	11.39	2.77	42.68	31.15
150	5.86	6.38	8.80	1.77	27.42	19.86

Table B-19 Rate of esterification, conversion and specific activity of 100mM NaDEHP/ 100mM hexanol/ 25-150mM oleic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL reverse micellar system

Oleic Acid (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	10.22	10.57	3.25	1.43	90.20	16.05
50	9.47	10.14	5.96	1.79	80.86	20.17
75	8.04	8.84	9.54	1.87	64.83	21.01
100	7.21	8.18	12.40	2.63	39.97	29.59
125	6.61	7.12	7.50	1.81	38.88	20.42
150	6.25	6.68	4.97	1.77	27.42	19.86

Table B-20 Rate of esterification, conversion and specific activity of 100mM NaDEHP/ 100mM hexanol/ 25-150mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml CALB reverse micellar system

Caprylic Acid (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	12.24	11.61	3.50	1.79	79.32	20.21
50	10.14	10.74	7.05	1.80	87.32	20.32
75	9.65	10.52	9.91	2.08	70.98	23.39
100	8.06	9.06	13.44	2.80	42.11	31.52
125	7.16	7.69	9.16	1.87	40.36	21.08
150	6.44	6.76	6.84	1.02	20.39	11.47

Table B-21 Rate of esterification, conversion and specific activity of 100mM NaDEHP/ 100mM hexanol/ 25-150mM caprylic acid in isooctane/ 1.0M NaCl/ 0.04mg/ml TLL reverse micellar system

Caprylic Acid (mM)	Wo			Initial Rate (mM min^{-1})	% Conversion	Specific Activity (mM min^{-1} mg $lipase^{-1}$)
	Before rxn.	After rxn.	% Increase			
25	11.43	11.75	2.85	1.13	68.71	12.71
50	40.08	10.51	4.24	1.62	75.45	18.20
75	9.75	10.52	7.87	1.48	52.09	16.64
100	4.85	9.43	11.08	1.85	38.38	20.80
125	7.15	7.61	6.40	1.45	30.89	16.37
150	6.50	6.70	3.15	1.16	18.17	13.04

Appendix C Calibration curve.

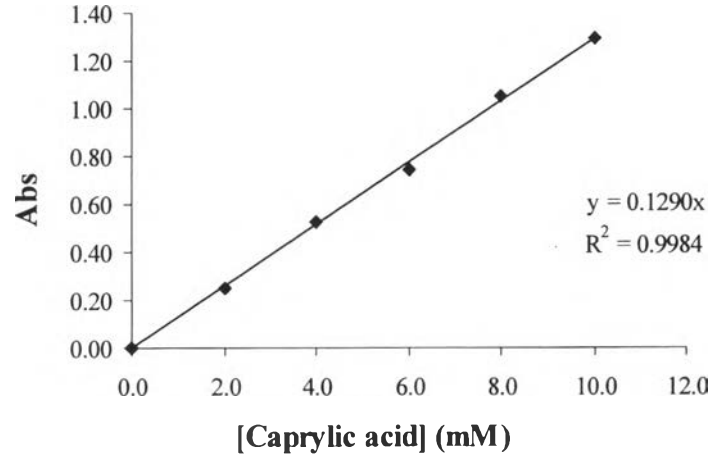

Figure C-1 Calibration curve of caprylic acid.

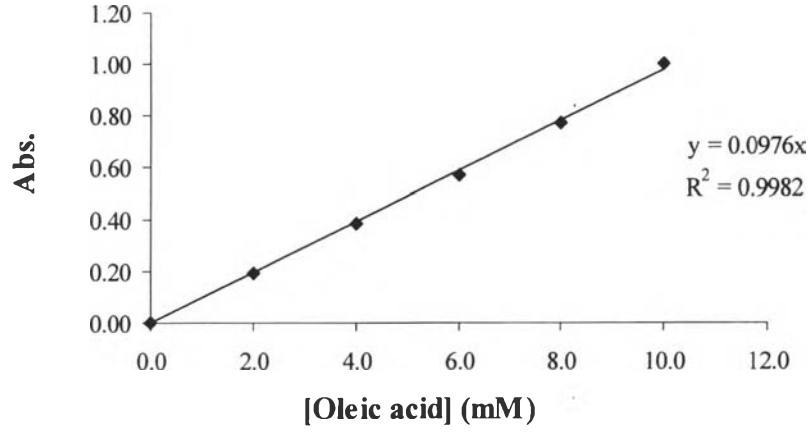

Figure C-2 Calibration curve of oleic acid.

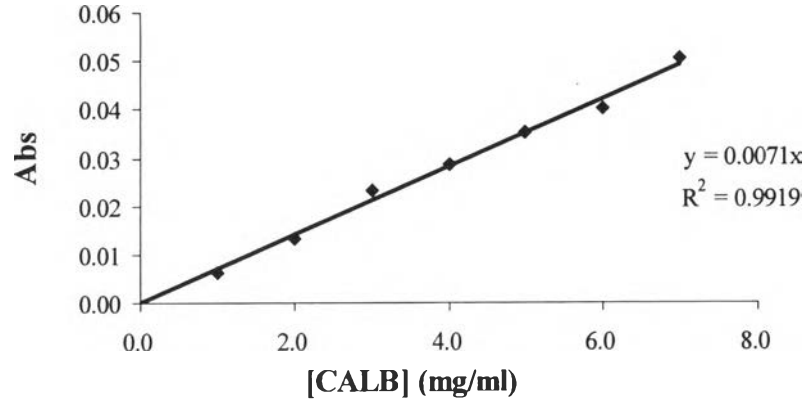

Figure C-3 Calibration curve of CALB.

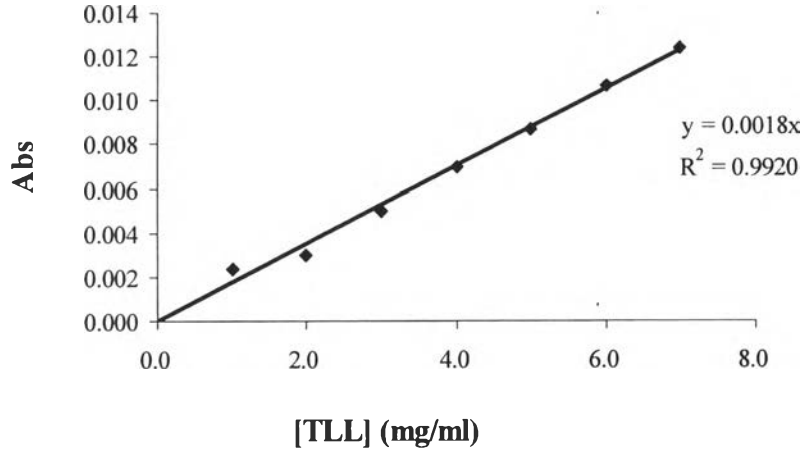

Figure C-4 Calibration curve of TLL.

Appendix D FTIR spectra.

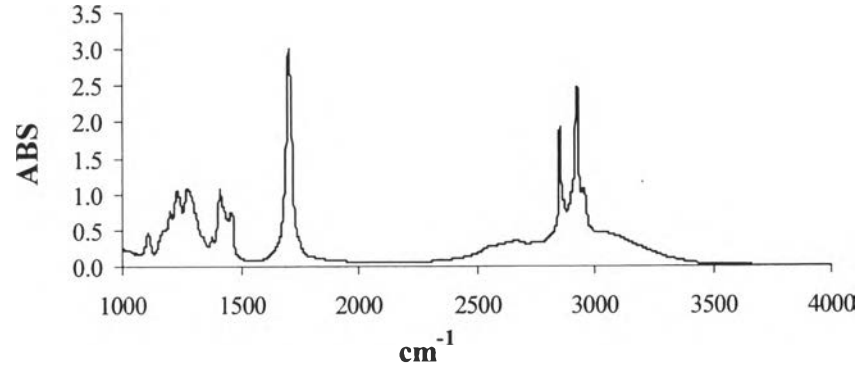

Figure D-1 Srectrum of oleic acid.

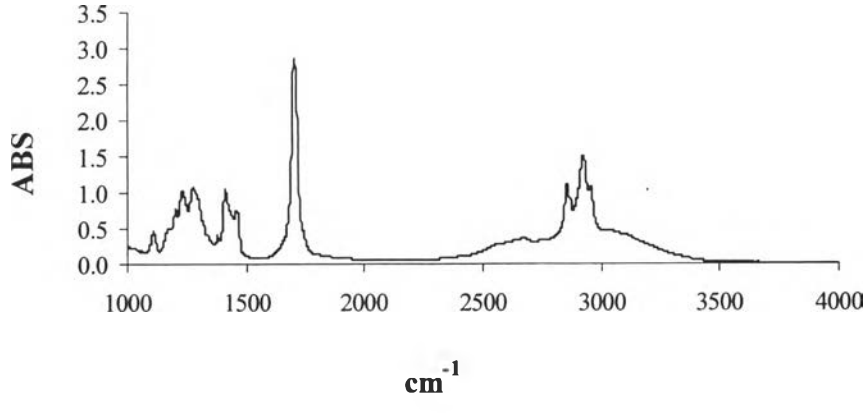

Figure D-2 Srectrum of caprylic acid.

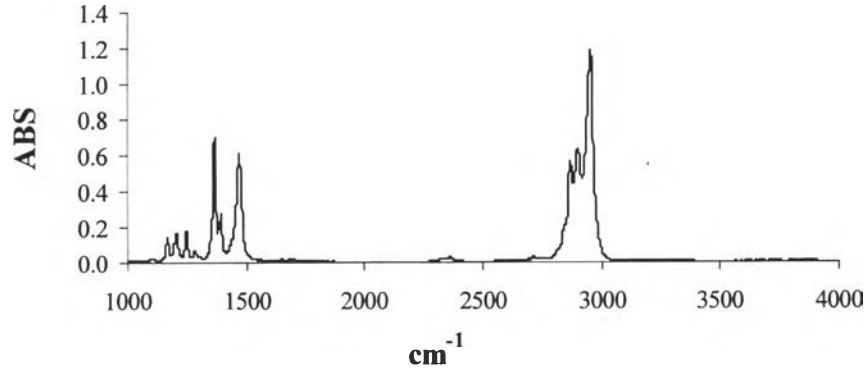

Figure D-3 Srectrum of hexanol.

Appendix E Examples of calculation.

F1 Example of water to surfactant ratio, W_0 determination

Condition: 25mM NaDEHP

100mM Hexanol

Average weight of sample injected = 0.1938 mg

Water content = 0.7972%

Sample volume = 0.05 ml

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

$$= \frac{(0.7972/100)(19.38)(1000)}{(18)(0.05)(50)}$$

$$= 3.43$$

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