REFERENCES

Adachi, M., Yamazaki, M., Harada, M., Shioi, A., and Katch, S. (1997). Bioaffinity separation of trypsin using trypsin inhibitor immobilized in reverse micelles composed of a nonionic surfactant. Biotechnology and Bioengineering, 53 (4), 406-408.

Andrade, S.M., Costa, S.M.B., and Pansu, R. (2000). The influence of water on the photophysical and photochemical properties of Piroxicam in AOT/isooctane/water reverse micelles. Photochemistry and Photobiology, 71(4), 405-412.

Bailey, J.E. and Ollis, D.F. (Eds.). (1986). Biochemical engineering fundamentals. 2nd ed. New York: McGrew-Hill book company.

Changez, M. and Varshney, M. (2000). Aerosol-OT microemulsions as transdermal carriers of tetracaine hydrochloride. Drug Development and Industrial Phamacy, 26(5), 507-512.

Clint, J.H. (Eds.).(1992). Surfactant aggregation. New York: Chapman and Hall.

Das, P.K., Srilakshmi, G.V., and Chaudhuri, A. (1999). Experimental probing of water and counterion concentrations inside a reversed micelle water-pool: an overlooked parameter in micellar enzymology. Langmuir, 15(2), 981-987.

Gasco, M.R. (1997). Microemulsions in pharmaceutical field: perspectives and applications. In C. Solans , R. Pons, H. Kunieda (Eds.), Surfactant science series volume 66. Industrial application of microemulsion. (pp. 97-122). New York: Marcel Dekker.

Hatton, A. (1997). Reversed micellar extraction of proteins. In C. Solans , R. Pons, H. Kunieda (Eds.), Surfactant science series volume 66. Industrial application of microemulsion. (pp. 55-90). New York: Marcel Dekker.

Hu, Z. and Gulari, E. (1996). Protein extreaction using the sodium bis (2-ethylhexyl) phosphate (NaDEHP) reverse micellar system. Biotechnology and Bioengineering, 50, 203-206.

Jarudilokkul, S.,Poppenborg, L.H., and Stuckey , D.C. (2000). Selective reverse micellar extraction of three proteins from filtered fermentation broth using response surface methodology. Separation Science and Technology, 35(4), 503-517.

Jarudilokkul, S., Poppenborg, L.H., and Stuckey , D.C. (1999). Backward extraction of reverse micellar encapsulated proteins using a counterionic surfactant. Biotechnology and Bioengineering, 62(5), 593-601.

Kelley, B.D., Wang, D.I.C., and Hatton, T.A. (1993). Affinity-based reversed micellar protein extraction: I. Principles and protein-ligand systems. Biotechnology and Bioengineering, 42, 1199-1208.

Marcozzi, G., Correa, N., Luisi, P.L., and Caselli, M. (1991). Protein extraction by reverse micelles: A study of the factors affecting the forward and backward transfer of α-chymotrypsin and its activity. Biotechnology and Bioengineering, 38, 1239-1246.

Pires, M.J., Aires-Barros, M.R.,and Cabral, J.M.S. (1996). Liquid-liquid extraction of proteins with reversed micelles. Biotechnology Progress, 12, 290-301.

Poppenborg, L.H., Brillis, A.A., and Stuckey, D.C. (2000). The kinetic separation of protein mixtures using reverse micelles. Separation Science and Tecchnology, 35(6),843-858.

Solans, C., Pons, R., and Kunida, H. (Eds.). (1997). Surfactant science series volume 66. Industrial application of microemulsion. New York: Marcel Dekker.

Vermathen, M., Louie E.A., Chodosh, A.B., Ried, S., and Simonis, U. (2000). Interaction of water-insoluble tetraphenylporphyruins with micelles probed by UV-Visible and NMR spectroscopy. Langmuir, 16(1), 210-221.

APPENDIX A

Water Content

Calculation of water content (ω_o)

$$\omega_o = \frac{[\text{Water}]_{oil}}{[\text{Surfactant}]_{oil}}$$

[Water] = Concentration of water in oil phase (M)

[Surfactant] = Concentration of surfactant in oil phase (M)

Table A1 Water content of microemulsion 0.1 M NaDEHP/isooctane with 0.1 M TBP as a cosurfactant and adjusting pH equal 7.5 at various salt concentrations. (Density of isooctane = 0.692 g/cm^3)

NaCl (M)	Sample (mg)	Water content			
		(%)	(mg)	(M)	ω_o
0.1	7.92	16.74	1.3	7.3470	73.470
	7.33	17.84	1.3	7.2351	72.351
	7.81	17.52	1.4	7.5920	75.920
			Ave ω_o		73.91±1.49
0.2	7.21	5.78	0.4	2.3108	23.1080
	6.42	6.20	0.4	2.2055	22.0551
	5.52	7.21	0.4	2.2028	22.0275
			Ave ω_o		22.40±0.50
0.5	7.51	3.65	0.3	1.5221	15.2208
	7.12	3.69	0.3	1.4596	14.5960
	6.82	3.76	0.3	1.4261	14.2614
			Ave ω_o		14.69±0.40
1.0	7.24	3.04	0.2	1.2232	12.2316
	7.33	2.96	0.2	1.2046	12.0456
	7.12	3.03	0.2	1.1977	11.9774
			Ave ω_o		12.08±0.11
1.5	7.08	2.58	0.2	1.0136	10.1362
	5.06	2.92	0.1	0.8211	8.2113
	6.41	2.63	0.2	0.9348	9.3479
			Ave ω_o		9.23±0.79

NaCl (M)	Sample (mg)	Water content			
		(%)	(mg)	(M)	ω_o
2.0	6.84	2.38	0.2	0.9052	9.0516
	6.55	2.36	0.2	0.8599	8.5987
	7.37	2.19	0.2	0.8963	8.9627
			Ave ω_o		8.87±0.19
3.0	7.76	1.82	0.1	0.7846	7.3459
	5.95	2.03	0.1	0.6720	7.8462
	6.97	1.90	0.1	0.7349	6.7202
			Ave ω_o		7.31±0.46
4.0	7.45	1.61	0.1	0.6655	6.6553
	5.77	1.82	0.1	0.5847	5.8469
	7.49	1.6	0.1	0.6658	6.6578
			Ave ω_o		6.39±0.38

Table A2 Water content of microemulsion 0.1 M NaDEHP/isooctane with 0.1 M 2-ethyl-1-hexanol as cosurfactant at various salt concentrations.

NaCl (M)	Sample (mg)	Water content			
		(%)	(mg)	(M)	ω_o
0.1	6.92	7.13	0.5	2.7220	27.2205
	7.31	6.73	0.5	2.7390	27.3898
	6.82	6.69	0.5	2.5240	25.2399
			Ave ω_o		26.62±0.98
0.2	0.0072	4.61	0.3	1.8342	18.3415
	0.0075	4.42	0.3	1.8323	18.3226
	0.0075	4.37	0.3	1.8099	18.0994
			Ave ω_o		18.25±0.11
0.5	0.0076	2.84	0.2	1.1952	11.9522
	0.0074	2.84	0.2	1.1600	11.6002
	0.0077	2.86	0.2	1.2174	12.1740
			Ave ω_o		11.91±0.24
1.0	7.31	2.68	0.2	0.9594	10.8106
	7.12	2.55	0.2	0.9984	9.9836
	7.42	2.53	0.2	1.0333	10.3326
			Ave ω_o		10.38±0.34
1.5	7.31	2.36	0.2	0.9594	9.5942
	5.23	2.58	0.1	0.7442	7.4418
	7.32	2.21	0.2	0.8918	8.9180
			Ave ω_o		8.65±0.90

NaCl (M)	Sample (mg)	Water content			
		(%)	(mg)	(M)	ω_o
2.0	7.23	2.07	0.1	0.8242	8.2415
	6.64	2.11	0.1	0.7675	7.6752
	7.62	1.96	0.1	0.8221	8.2211
			Ave ω_o		8.05±0.26
3.0	7.12	1.80	0.1	0.7118	7.1179
	7.43	1.72	0.1	0.7045	7.0451
	7.12	1.75	0.1	0.6897	6.8967
			Ave ω_o		7.02±09
4.0	6.52	1.42	0.1	0.5102	5.1019
	7.12	1.44	0.1	0.5632	5.6323
	7.13	1.49	0.1	0.5860	5.8602
			Ave ω_o		7.02±09

Table A3 Water content of microemulsion 0.1 M NaDEHP/isooctane with 0.1 M 1-heptanol as cosurfactant at various salt concentrations.

NaCl (M)	Sample (mg)	Water content			
		(%)	(mg)	(M)	ω_o
0.1	28.4	5.2	1.5	2.054	20.54
	28.6	5.68	1.6	2.2566	22.566
	28.5	5.45	1.6	2.1589	21.589
			Ave ω_o		21.56±0.83
0.2	28.9	4.415	1.3	1.7721	17.721
	28.8	4.399	1.3	1.7578	17.578
	31.4	4.003	1.3	1.7469	17.469
			Ave ω_o		17.59±0.10
0.5	28.0	3.34	0.9	1.299	12.99
	28.7	3.28	0.9	1.306	13.06
	28.7	3.31	0.9	1.3185	13.185
			Ave ω_o		13.08±0.08
1.0	28.4	2.67	0.8	1.0513	10.513
	28.5	2.66	0.8	1.0499	10.499
	28.8	2.70	0.8	1.0784	10.784
			Ave ω_o		10.60±0.13
1.5	28.7	2.37	0.7	0.9427	9.4272
	28.6	2.37	0.7	0.9396	9.3957
	27.8	2.43	0.7	0.9368	9.368
			Ave ω_o		9.40±0.02

NaCl (M)	Sample (mg)	Water content			
		(%)	(mg)	(M)	ω_o
2.0	29.3	2.225	0.7	0.9039	9.0391
	28.2	2.262	0.6	0.8863	8.8626
	27.5	2.349	0.6	0.8965	8.9654
			Ave ω_o		8.96±0.07

Table A4 Water content after backward extraction of reverse micelles when using TBP as a cosurfactant.

Sample (mg)	Water content			
	(%)	(mg)	(M)	ω_o
36.46	0.4712	0.1718	0.19089	1.90888
36.40	0.4615	0.168	0.18665	1.86651
36.35	0.478	0.1738	0.19306	1.93059
		Ave ω_o		1.90±0.02

APPENDIX B

Analysis

Table B1 Calibration curve of α-chymotrypsin at λ_{281}nm.

Protein (μg/ml)	Absorbance			Average
0	0	0	0	0
1	0.003	0.004	0.003	0.0033
5	0.007	0.006	0.007	0.0067
50	0.094	0.094	0.094	0.0940
100	0.184	0.183	0.183	0.1833
500	0.913	0.914	0.914	0.9137
1000	1.827	1.827	1.827	1.8270

Table B2 Calibration curve of *p*-nitroaniline at λ_{410} nm.

Conc.(ppm)	Absorbance			Average
0	0	0	0	0
0.01	0.003	0.004	0.003	0.0033
0.1	0.009	0.009	0.009	0.0090
1	0.06	0.06	0.06	0.0600
3	0.188	0.188	0.188	0.1880
5	0.316	0.316	0.317	0.3163
10	0.613	0.613	0.611	0.6123
15	0.9	0.9	0.901	0.9003

Table B3 Calibration curve of activity test of fresh protein at λ_{410} nm.

Protein (μg/ml)	Absorbance			Average
0	0	0	0	0
1	0.002	0.003	0.003	0.0027
5	0.007	0.007	0.007	0.0070
50	0.019	0.019	0.019	0.0190
100	0.039	0.04	0.041	0.0400
500	0.177	0.177	0.178	0.1773
1000	0.374	0.374	0.375	0.3743

APPENDIX C

Forward and Backward Extractions

C1 Calculation percentage of forward extraction

$$\% \text{ Forward extraction} = \frac{([\text{protein}]_i - [\text{protein}]_f) \times 100}{[\text{protein}]_i}$$

$[\text{protein}]_f$ = Protein concentration in aqueous phase after forward extraction (mg/ml)

$[\text{protein}]_i$ = Protein concentration in aqueous phase before forward extraction (mg/ml)

Table C1.1 Effect of pH in aqueous phase on percentage of α-chymotrypsin forward extraction. $[\text{protein}]_i$ = 0.5 mg/ml

pH	Absorbance			Average	$[\text{protein}]_f$ (mg/ml)	*Ave %E
7.5	0.05	0.05	0.05	0.05	0.027	94.58±0.05
8.3	0.083	0.083	0.083	0.083	0.045	91.04±0.19
9.5	0.129	0.129	0.129	0.129	0.070	85.88±1.45
10.5	0.221	0.221	0.222	0.2213	0.121	77.06±2.88
11.5	0.51	0.51	0.51	0.51	0.279	43.84±0.47

*Ave % E = average of three data sets

Table C1.2 Effect of salt concentration in aqueous phase on forward extraction.

NaCl (M)	Absorbance			Average	$[\text{protein}]_f$ (mg/ml)	*Ave %E
0.1	0.048	0.048	0.049	0.048	0.026	93.71±0.45
0.2	0.071	0.072	0.072	0.072	0.039	92.14±0.23
0.5	0.676	0.675	0.674	0.675	0.369	25.17±1.02
1.0	0.843	0.842	0.842	0.842	0.461	7.61±0.27
1.5	0.88	0.881	0.882	0.881	0.482	5.89±1.64

*Ave % E = average of three data sets

Table C1.3 Effect of protein concentration in aqueous phase on forward extraction.

protein (mg/ml)	Absorbance			Average	$[protein]_f$ (mg/ml)	Ave %E
0.1	0.02	0.022	0.02	0.021	0.011	88.696
0.5	0.052	0.052	0.051	0.052	0.029	94.298
1	0.194	0.193	0.195	0.194	0.108	89.250

Table C1.4 Effect of type of cosurfactant on forward extraction.

0.1 M 1-heptanol as a cosurfactant.

no	Absorbance			average	$[protein]_f$ (mg/ml)	% E
1	0.22	0.223	0.226	0.223	0.1221	75.57
2	0.233	0.234	0.233	0.2333	0.1278	74.44
3	0.295	0.296	0.295	0.2953	0.1618	67.64

0.1 M 2-ethyl-1hexanol as a cosurfactant.

no	Absorbance			average	$[protein]_f$ (mg/ml)	% E
1	0.295	0.299	0.301	0.2983	0.1631	67.38
2	0.117	0.12	0.119	0.1187	0.0647	87.05
3	0.204	0.207	0.209	0.2067	0.1129	77.42

C2 Calculation percentage of backward extraction

$$\% \text{ Backward extraction} = \frac{([protein]_i - [protein]_f) \times 100}{[protein]_i}$$

$(protein)_f$ = Protein concentration in aqueous phase after backward extraction(mg/ml)

$(protein)_i$ = Protein concentration in aqueous phase after forward extraction (mg/ml)

Table C2.1 Effect of salt concentration in aqueous phase on backward extraction.

NaCl (M)	Absorbance			Average	$[protein]_f$ (mg/ml)	%E
0.1	0.656	0.656	0.657	0.656	0.359	75.76±4.44
0.2	0.624	0.622	0.623	0.623	0.341	73.94±3.05
0.5	0.175	0.175	0.175	0.175	0.096	72.97±5.69
1.0	0.053	0.053	0.053	0.053	0.029	73.84±0.25
1.5	0.025	0.025	0.025	0.025	0.013	74.82±3.85

Table C2.2 Effect of protein concentration in aqueous phase on backward extraction.

protein (mg/ml)	Absorbance			Average	$[protein]_f$ (mg/ml)	%E
0.1	0.02	0.022	0.02	0.021	0.011	88.70
0.5	0.052	0.052	0.051	0.052	0.029	94.30
1	0.194	0.193	0.195	0.194	0.108	89.25

Table C2.3 Effect of type of cosurfactant on backward extraction.

0.1 M 1-heptanol as a cosurfactant

No.	Absorbance			Average	$[protein]_f$ (mg/ml)	%E
1	0.48	0.478	0.479	0.479	0.2626	69.49
2	0.489	0.49	0.491	0.49	0.2686	72.17
3	0.513	0.514	0.515	0.514	0.2818	83.33
				Ave % E		75.00±5.99

0.1 M 2-ethyl-1-hexanol

No.	A			Average	$[protein]_f$ (mg/ml)	%E
1	0.46	0.459	0.46	0.4597	0.2514	74.63
2	0.551	0.553	0.55	0.5513	0.3016	69.30
3	0.473	0.473	0.472	0.4727	0.2585	66.80
				Ave % E		70.24±3.27

C3 Calculation of activity test

$$\% \text{ Activity} = \frac{[p\text{-nitroaniline}]_f \times 100}{[p\text{-nitroaniline}]_i}$$

$[p\text{-nitroaniline}]_f$ = Concentration of *p*-nitroaniline from hydrolysis reaction of protein after backward extraction

$[p\text{-nitroaniline}]_i$ = Concentration of *p*-nitroaniline from hydrolysis reaction of fresh protein

Table C3.1 Effect of salt concentration in aqueous phase on activity test.

NaCl (M)	*p*-nitroaniline after activity test					*$[p\text{-nitroaniline}]_i$ (ppm)	%Activity
	Absorbance			Ave Abs	$[p\text{-nitroaniline}]_f$ (ppm)		
0.1	0.07	0.07	0.07	0.071	1.114	2.150	51.79±1.16
0.2	0.06	0.06	0.06	0.063	0.970	2.040	47.53±4.37
0.5	0.02	0.02	0.02	0.021	0.277	0.538	51.52±6.49
1.0	0.01	0.01	0.01	0.008	0.067	0.128	52.39±3.70
1.5	0.01	0.01	0.01	0.005	0.012	0.030	38.98±4.20

*$[p\text{-nitroaniline}]_i$ calculate from fresh protein and calibration curve of *p*-nitroaniline and activity test.

Table C3.2 Effect of protein concentration in aqueous phase on activity test.

Protein (mg/ml)	Absorbance			Ave Abs	[*p*-nitroaniline]$_f$ (ppm)	[*p*-nitroaniline]$_i$ (ppm)	%Activity
0.1	0.006	0.006	0.007	0.006	0.034	0.119	28.31
0.5	0.07	0.07	0.07	0.070	1.114	2.150	51.79
1	0.128	0.127	0.127	0.127	2.044	2.983	68.52

*[*p*-nitroaniline]$_i$ calculate from fresh protein and calibration curve of *p*-nitroaniline and activity test.

Table C3.3 Effect of type of cosurfactant on activity test.

0.1 M 1-heptanol.

No.	Absorbance			Ave Abs	[*p*-nitroaniline]$_f$ (ppm)	[*p*-nitroaniline]$_i$ (ppm)	%Activity
1	0.021	0.018	0.019	0.01933	0.24972	1.55935	16.01
2	0.019	0.019	0.02	0.01933	0.24972	1.59612	15.65
3	0.021	0.023	0.024	0.02267	0.30509	1.67701	18.19

0.1 M 2-ethyl-1-hexanol.

No.	A			Ave Abs	[*p*-nitroaniline]$_f$ (ppm)	[*p*-nitroaniline]$_i$ (ppm)	%Activity
1	0.006	0.006	0.006	0.006	0.0282	1.4883	1.89
2	0.005	0.005	0.005	0.005	0.0116	1.8008	0.65
3	0.009	0.009	0.009	0.009	0.0781	1.5373	5.08

APPENDIX D

Dynamic Light Scattering

Table D1 Hydrodynamic radius (Rh) of reverse micelles at various type of cosurfactant.

Cosurfactant	Size of reverse micelles (nm)									
	Before forward extraction					After forward extraction				
	Zave (nm)	Poly	Fit error	% In range	% Merit	Zave (nm)	Poly	Fit error	% In range	% Merit
TBP	17.7	0.463	0.0089	86.6	16	44.5	0.078	0.0008	97.7	21.9
	18.1	0.451	0.0014	80.9	16.6	44.7	0.051	0.00154	98.8	22
	16.8	0.386	0.0005	86.2	15.4	43.7	0.061	0.00037	95.2	22.1
1-heptanaol	6.4	0.117	0.0266	83.5	20.1	11.3	0.184	0.04631	74.8	23.4
	5.9	0.11	0.0321	89.5	19.8	9.7	0.159	0.05502	75.3	22.5
	5.8	0.107	0.029	85.5	20.0	9.7	0.156	0.04467	75.0	22.3
2-ethyl-1-hexanol	5.3	0.094	0.0213	97.8	20.2	11.4	0.176	0.01745	73.6	14.0
	5.7	0.092	0.0582	92.5	20.5	9.6	0.239	0.06368	88.9	14.7
	5.5	0.089	0.0207	84.2	20.5	9.5	0.192	0.05919	92.1	14.7

Table D2 Hydrodynamic radius (Rh) of reverse micelle at various protein concentration.

Protein concentration (mg/ml)	Before forward extraction					After forward extraction				
	Zave[1] (nm)	Poly[2]	Fit error[3]	% In range[4]	% Merit[5]	Zave (nm)	Poly	Fit error	% In rage	% Merit
0.1	17.7	0.463	0.0089	86.6	16	41.6	0.072	0.00036	96.9	32.6
	18.1	0.451	0.00144	80.9	16.6	41.2	0.092	0.0025	99.5	32.8
	16.8	0.386	0.0005	86.2	15.4	40.9	0.071	0.0031	96.0	33.2
0.5	17.7	0.463	0.0089	86.6	16.0	44.5	0.078	0.0008	97.7	21.9
	18.1	0.451	0.00144	80.9	16.6	44.7	0.051	0.00154	98.8	22.0
	16.8	0.386	0.0005	86.2	15.4	43.7	0.061	0.00037	95.2	22.1
1	17.7	0.463	0.0089	86.6	16.0	37.7	0.075	0.0023	99.8	36.2
	18.1	0.451	0.00144	80.9	16.6	38.0	0.043	0.00022	98.7	36.5
	16.8	0.386	0.0005	86.2	15.4	38.0	0.043	0.00023	98.5	36.0

[1] The Z average size result of current record that is the average diameter size of particle.

[2] The polydispersity calculated using the initial cumulants fit to the current size result. If the value is close to 1.0, particle size distribution is very wide.

[3] The value calculated for the correlation coefficient as corresponding exactly to the size distribution resulting from the fitting procedure. The smaller value, the better fitting.

[4] In range value calculated from the ratio of the far point. A higher value (85-100%) indicates that the correlation function has nearly decayed to 0 by the measured far point, and hence the sample time is set to a suitable value, and the experiment a well founded one. The value between 85-100, the average diameter size is the exact result.

[5] Merit value for the current record. The percentage of (correlation-baseline)/baseline, normally10-60%. The value between 10-60, the signal to noise ratio is good.

CURRICURUM VITAE

Name: Ms. Wassana Sooksomsin

Date of Birth: May 10, 1978

Nationality: Thai

University Education:

1996-1999 Bachelor Degree of Science in Industrial Chemistry, Faculty of Applied Science, King Mongkut's Institute of Technology North Bangkok, Bangkok, Thailand