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APPENDIX A

Calibrations

Table A1 Calibration curve of α -chymotrypsin at λ_{281} nm (fixed wavelength mode)

α -chymotrypsin (mg/ml)	Absorbance			Average	SD
0.000	0.0000	0.0000	0.0000	0.0000	0.0000
0.005	0.0117	0.0117	0.0117	0.0117	0.0000
0.010	0.0221	0.0221	0.0220	0.0221	0.0001
0.050	0.0929	0.0931	0.0935	0.0932	0.0003
0.100	0.1773	0.1762	0.1762	0.1766	0.0006
0.500	0.8579	0.8582	0.8585	0.8582	0.0003
1.000	1.6919	1.6953	1.6984	1.6952	0.0033

Table A2 Calibration curve of *p*-nitroaniline at λ_{365} nm (scanning wavelength mode)

<i>p</i> -nitroaniline (ppm)	Absorbance			Average	SD
0.00	0.0000	0.0000	0.0000	0.0000	0.0000
0.05	0.0000	0.0000	0.0000	0.0000	0.0000
0.50	0.0368	0.0395	0.0404	0.0389	0.0019
5.00	0.4332	0.4378	0.4420	0.4377	0.0044
15.00	1.8938	2.0700	2.2168	2.0602	0.1617

APPENDIX B

Forward and Backward Extractions

B1 Calculation percentage of forward extraction

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

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

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

Table B1 Concentration of α -chymotrypsin in aqueous phase of NaDEHP microemulsion system after forward extraction. (a): $[\text{protein}]_i = 0.4974$ mg/ml, (b): $[\text{protein}]_i = 0.4927$ mg/ml, (c): $[\text{protein}]_i = 0.5144$ mg/ml, (d): $[\text{protein}]_i = 0.4903$ mg/ml

Cosurfactant	NaCl (M)	Absorbance			Average	$[\text{protein}]_f$ (mg/ml)	% Forward
-	4.0	1.0694	1.0778	1.0811	1.0761	0.6329	0.00 (b)
0.1 M TBP	0.2	0.2447	0.2448	0.2450	0.2448	0.1440	71.05 (a)
15.0 mM CHAPS	4.0	0.7654	0.7656	0.7634	0.7648	0.4498	8.71 (b)
1.0 MM CHAPS	0.2	0.7661	0.7665	0.7668	0.7665	0.4508	9.38 (a)
5.0 mM CHAPS	0.2	0.6256	0.6264	0.6271	0.6264	0.3684	28.39 (c)
10.0 mM CHAPS	0.2	0.5785	0.5787	0.5788	0.5787	0.3403	31.58 (a)
15.0 mM CHAPS	0.2	0.8081	0.8100	0.8109	0.8097	0.4762	2.88 (d)

APPENDIX C

Activity Test

Calculation percentage of enzymatic activity

$$\% \text{ Activity} = \frac{\text{rate}_{\text{recovered}}}{\text{rate}_{\text{fresh}}} * 100$$

% Activity = Activity of recovered protein compared with fresh protein

rate recovered = rate of *p*-nitroaniline produced by recovered protein

rate fresh = rate of *p*-nitroaniline produced by fresh protein

Table C1 Activity of fresh α -chymotrypsin, [protein] = 49.74 mg/l

Reaction Time	Absorbance	[<i>p</i> -nitroaniline] (mg/l)	[<i>p</i> -nitroaniline]/[protein] (mg/l)/(mg/l)
0.0	0.0000	0.0000	0.0000
1.0	4.8249	50.3118	1.0114
2.0	5.7193	59.6382	1.1989
3.0	5.6522	58.9385	1.1848
4.0	5.6704	59.1283	1.1886
5.0	5.7037	59.4755	1.1956
6.0	5.6350	58.7591	1.1812

Table C2 Activity of recovered α -chymotrypsin from NaDEHP microemulsion system with 0.1 M TBP, [protein] = 255.80 mg/l

Reaction Time	Absorbance	[p-nitroaniline] (mg/l)	[p-nitroaniline]/[protein] (mg/l)/(mg/l)
0.0	0.0000	0.0000	0.0000
1.0	5.8526	61.0282	0.2386
2.0	7.0183	73.1835	0.2861
3.0	6.8309	71.2294	0.2785
4.0	7.0520	73.5349	0.2875
5.0	7.0509	73.5235	0.2874
6.0	7.0603	73.6215	0.2878
7.0	7.0507	73.5214	0.2874
8.0	7.0680	73.7018	0.2881

Table C3 Activity of recovered α -chymotrypsin from NaDEHP microemulsion system with 10.0 mM CHAPS, [protein] = 5.53 mg/l

Reaction Time	Absorbance	[p-nitroaniline] (mg/l)	[p-nitroaniline]/[protein] (mg/l)/(mg/l)
0.0	0.0000	0.0000	0.0000
1.0	-	-	-
2.0	0.3899	4.0652	0.7353
3.0	0.6748	7.0362	1.2727
4.0	2.9365	30.6204	5.5387

APPENDIX D

Water Content

Calculation of water to surfactant ratio (ω_0)

$$\omega_0 = \frac{[water]}{[surfactant]}$$

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

[surfactant] = Concentration of surfactant (M)

Table D1 Water content of microemulsion 0.1031 M NaDEHP/isooctane with 0.1 M TBP as a cosurfactant at pH 7.4, 25°C and various NaCl concentrations

NaCl (M)	Sample (mg)	Water content			
		(%)	(mg)	(M)	ω_0
0.2	7.4	6.23	0.46	2.56	24.84
	7.4	6.35	0.47	2.61	25.32
	7.5	6.40	0.48	2.67	25.87
	7.5	6.39	0.48	2.66	25.81
	7.6	6.37	0.48	2.69	26.08
Average		6.35			25.58
0.3	7.0	2.56	0.18	1.00	9.75
	7.5	2.60	0.20	1.08	10.51
	7.6	3.17	0.24	1.34	12.99
	7.4	2.61	0.19	1.07	10.40
	7.4	2.92	0.22	1.20	11.64
	7.0	2.76	0.19	1.07	10.40
Average		2.77			10.95
0.4	7.8	2.12	0.16	0.92	8.89
	7.6	1.89	0.14	0.80	7.76
	7.3	1.86	0.14	0.76	7.33
	7.3	1.94	0.14	0.79	7.65
	7.5	2.24	0.17	0.93	9.05
	7.4	1.86	0.14	0.77	7.43
Average		1.99			8.02

Table D2 Water content of microemulsion 0.1031 M NaDEHP/isooctane with CHAPS as a cosurfactant at pH 7.4, 25°C and various NaCl concentrations

CHAPS (mM)	NaCl (M)	Sample (mg)	Water content			
			(ppm)	(mg)	(M)	ω_0
0.0	4.0	29.1	23360	0.680	0.9441	9.1493
		29.2	24100	0.704	0.9774	9.4716
		29.1	23810	0.693	0.9623	9.3256
		29.3	23240	0.681	0.9457	9.1649
		29.5	22570	0.666	0.9247	8.9614
Average			23420			9.2146
15.0	4.0	15.4	61290	0.944	2.6219	25.4076
		15.3	64110	0.981	2.7247	26.4041
		15.1	55890	0.844	2.3443	22.7177
		15.2	63880	0.971	2.6972	26.1374
		15.2	67890	1.032	2.8665	27.7782
Average			62610			25.6890
1.0	0.2	14.4	208.3	0.003	0.0083	0.0807
		14.8	283.7	0.004	0.0117	0.1130
		14.5	262.0	0.004	0.0106	0.1023
		14.7	299.3	0.004	0.0122	0.1184
Average			263.3			0.1036
5.0	0.2	14.3	391.6	0.006	0.0156	0.1507
		14.4	284.7	0.004	0.0114	0.1104
		14.7	312.9	0.005	0.0128	0.1238
		14.8	351.3	0.005	0.0144	0.1400
Average			335.1			0.1312
10.0	0.2	14.7	340.1	0.005	0.0139	0.1346
		14.8	344.5	0.005	0.0142	0.1372
		14.3	328.6	0.005	0.0131	0.1265
		14.9	322.1	0.005	0.0133	0.1292
Average			333.8			0.1319
15.0	0.2	14.6	452.0	0.007	0.0183	0.1776
		14.9	416.1	0.006	0.0172	0.1669
		14.6	595.8	0.009	0.0242	0.2342
		14.3	524.4	0.007	0.0208	0.2019
Average			497.1			0.1951

Table D3 Water content of microemulsion 0.1031 M NaDEHP/isooctane at pH 7.4, 25°C after forward extraction

Cosurfactant	NaCl (M)	Sample (mg)	Water content			
			(ppm)	(mg)	(M)	ω_0
0.1 M TBP	0.2	15.0	129200	1.938	5.3833	52.0246
		15.5	124700	1.933	5.3690	51.8863
		15.5	125300	1.942	5.3949	52.1360
		15.6	124200	1.938	5.3820	52.0117
		15.4	125700	1.936	5.3772	51.9650
		15.3	125400	1.919	5.3295	51.5043
		15.7	123600	1.941	5.3903	52.0922
Average			125400			51.9457
15.0 mM CHAPS	4.0	14.4	493.0	0.007	0.0197	0.1913
		14.6	390.4	0.006	0.0158	0.1536
		14.6	383.0	0.006	0.0156	0.1509
		14.7	367.3	0.005	0.0150	0.1455
Average			408.6			0.1603
1.0 mM CHAPS	0.2	14.5	241.3	0.003	0.0097	0.0942
		14.7	285.7	0.004	0.0117	0.1131
		14.6	308.2	0.004	0.0125	0.1211
		14.7	326.5	0.005	0.0133	0.1292
Average			290.4			0.1144
5.0 mM CHAPS	0.2	14.3	307.6	0.004	0.0122	0.1184
		14.6	349.3	0.005	0.0142	0.1373
		14.4	430.5	0.006	0.0172	0.1669
		14.3	314.6	0.004	0.0125	0.1211
Average			350.5			0.1359
10.0 mM CHAPS	0.2	14.1	382.9	0.005	0.0150	0.1453
		14.5	448.2	0.006	0.0181	0.1749
		14.5	400.0	0.006	0.0161	0.1561
		14.3	412.5	0.006	0.0164	0.1588
Average			410.9			0.1588
15.0 mM CHAPS	0.2	14.4	347.2	0.005	0.0139	0.1346
		13.3	428.5	0.006	0.0158	0.1534
		14.5	296.5	0.004	0.0119	0.1157
		14.8	351.3	0.005	0.0144	0.1400
Average			355.9			0.1359

Table D4 Water content of microemulsion 0.1031 M NaDEHP/isooctane at pH 7.4, 25°C after backward extraction

Cosurfactant	NaCl (M)	Sample (mg)	Water content			
			(ppm)	(mg)	(M)	ω_0
0.1 M TBP	0.2 M	15.0	55660	0.083	0.2319	2.2412
		15.2	56710	0.086	0.2394	2.3140
		14.8	54590	0.081	0.2244	2.1689
		14.9	56710	0.084	0.2325	2.2467
		15.0	57000	0.086	0.2375	2.2952
		14.8	55400	0.082	0.2278	2.2010
		15.2	56050	0.085	0.2367	2.2870
		15.2	53810	0.082	0.2272	2.1956
		15.3	53520	0.082	0.2275	2.1982
		15.1	54030	0.082	0.2266	2.1901
Average			55290			2.2338
15.0 mM CHAPS	4.0	14.4	444.4	0.006	0.0178	0.1724
		14.6	404.1	0.006	0.0164	0.1590
		14.5	386.2	0.006	0.0156	0.1509
		14.5	413.7	0.006	0.0167	0.1616
Average			412.1			0.1610
1.0 mM CHAPS	0.2	14.3	237.7	0.003	0.0094	0.0915
		14.5	227.5	0.003	0.0092	0.0888
		14.5	289.6	0.004	0.0117	0.1130
		14.6	363.0	0.005	0.0147	0.1427
Average			279.5			0.1090
5.0 mM CHAPS	0.2	14.0	350.0	0.005	0.0136	0.1319
		14.5	310.3	0.004	0.0125	0.1211
		14.3	328.6	0.005	0.0131	0.1265
		14.2	316.9	0.004	0.0125	0.1211
Average			326.5			0.1252
10.0 mM CHAPS	0.2	14.4	361.1	0.005	0.0144	0.1400
		14.9	255.0	0.004	0.0106	0.1023
		14.3	265.7	0.004	0.0106	0.1023
		14.5	303.4	0.004	0.0122	0.1184
		14.9	342.2	0.005	0.0142	0.1373
Average			305.5			0.1200
15.0 mM CHAPS	0.2	14.3	328.6	0.005	0.0131	0.1265
		14.4	305.5	0.004	0.0122	0.1184
		14.3	265.7	0.004	0.0106	0.1023
		14.0	378.5	0.005	0.0147	0.1426
Average			319.6			0.1225

APPENDIX E
Dynamic Light Scattering

Table E1 Hydradynamic radius (Rh) of microemulsion 0.1 M NaDEHP/isooctane at pH 7.4, 25°C and various NaCl and cosurfactants

NaCl (M)	Cosurfactant Concentration	Size of microemulsion (nm)			%Inrange	Merit
		Zave (nm)	Poly	Mean (nm)		
0.2	0.1 M TBP	13.0	0.713	18.3	89.4	19.3
		14.8	0.606	19.8	93.1	16.8
		12.3	0.892	18.9	94.36	17.6
0.3	0.1 M TBP	10.8	0.120	11.5	96.1	25.2
		11.0	0.194	12.1	95.8	27.4
		9.0	0.193	10.9	99.4	28.2
0.4	0.1 M TBP	12.3	0.018	12.4	92.5	25.2
		12.2	0.099	12.8	95.9	27.5
		12.4	0.037	12.7	98.0	28.2
4.0	-	16.6	0.025	16.8	96.0	31.8
		17.2	0.188	18.9	98.6	32.9
4.0	15.0 mM CHAPS	22.2	0.567	29.2	98.4	38.5
		21.9	0.560	28.8	98.3	39.2
0.2	1.0 mM CHAPS	0.3	0.155	0.4	91.0	22.5
		0.4	0.121	0.5	98.7	14.9
0.2	5.0 mM CHAPS	0.6	0.133	0.7	94.0	10.2
		0.5	0.186	0.5	90.7	10.3
0.2	10.0 mM CHAPS	0.4	0.224	0.5	98.2	20.9
		0.3	0.175	0.4	88.8	16.1
0.2	15.0 mM CHAPS	0.4	0.112	0.4	84.4	17.8
		0.5	0.198	0.5	96.9	15.3

Table E2 Hydradynamic radius (Rh) of microemulsion 0.1 M HDEHP/isooctane after forward extraction

NaCl (M)	Cosurfactant Concentration	Size of microemulsion (nm)			%Inrange	Merit
		Zave (nm)	Poly	Mean (nm)		
0.2	0.2 M	48.0	1.000	77.7	96.5	44.4
	TBP	48.1	1.000	77.9	96.2	44.0
4.0	15.0 mM	1.2	0.287	1.4	87.5	10.1
	CHAPS	1.2	0.343	1.4	98.8	11.2
0.2	1.0 mM	0.3	0.181	0.3	84.8	17.4
	CHAPS	0.4	0.208	0.5	97.9	16.9
0.2	5.0 mM	1.3	0.325	1.5	89.5	10.7
	CHAPS	1.2	0.264	1.4	85.4	4.1
0.2	10.0 mM	1.1	0.301	1.3	56.5	4.8
	CHAPS	1.4	0.279	1.7	74.8	8.7
0.2	15.0 mM	1.3	0.303	1.5	94.3	10.5
	CHAPS	1.6	0.327	1.9	93.2	11.5

Table E3 Hydradynamic radius (Rh) of microemulsion 0.1 M HDEHP/isooctane after backward extraction

NaCl (M)	Cosurfactant Concentration	Size of microemulsion (nm)			%Inrange	Merit
		Zave (nm)	Poly	Mean (nm)		
0.2	0.2 M	2.4	0.335	2.8	84.1	10.9
	TBP	2.1	0.391	2.5	76.4	11.1
4.0	15.0 mM	0.9	0.192	1.0	96.9	20.5
	CHAPS	1.3	0.269	1.5	75.1	6.7
0.2	1.0 mM	1.2	0.330	1.5	89.4	10.8
	CHAPS	0.3	0.202	0.3	93.1	13.0
0.2	5.0 mM	1.2	0.466	1.5	96.3	11.6
	CHAPS	0.9	0.412	1.1	87.6	9.4
0.2	10.0 mM	1.1	0.243	1.2	77.4	13.9
	CHAPS	1.2	0.260	1.4	67.5	12.2
0.2	15.0 mM	1.1	0.212	1.2	95.2	26.9
	CHAPS	1.1	0.263	1.3	92.9	25.7

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