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Appendix I

Encapsulation efficiency data of lactic acid from studying some factors affecting encapsulation of lactic acid in liposomes

HPLC Assay for lactic acid

Calibration curve data for Factor1

For sample No 1A, 3C, 4A, 4B, and 4C

Concentration (mcg/ml)	20.64	51.60	103.20	206.40	309.60	412.80	516.00
Peak area ratio	0.066	0.120	0.352	0.722	1.067	1.437	1.888

$$R^2 = 0.9981$$

$$y = 0.0037x - 0.0395$$

For sample No 1C

Concentration (mcg/ml)	20.80	52.00	104.00	208.00	312.00	416.00
Peak area ratio	0.062	0.193	0.405	0.788	1.183	1.646

$$R^2 = 0.9990$$

$$y = 0.0039x - 0.0185$$

For sample No 1B, and 2C

Concentration (mcg/ml)	20.08	50.20	100.40	200.80	301.20	502.00
Peak area ratio	0.119	0.177	0.362	0.729	1.118	1.831

$$R^2 = 0.9990$$

$$y = 0.0036x - 0.0141$$

For sample No 2B, 2B, 3A, and 2C

Concentration (mcg/ml)	51.40	102.80	205.60	308.40	411.20	514.00
Peak area ratio	0.121	0.390	0.818	1.265	1.698	2.110

$$R^2 = 0.9993$$

$$y = 0.0043x - 0.0685$$

**Calibration curve data
for Factor2**

For sample No 6A, 7A, 8A, and 9A

Concentration (mcg/ml)	20.16	50.40	100.80	201.60	302.40	403.20
Peak area ratio	0.056	0.166	0.340	0.708	1.052	1.472

$$R^2 = 0.9990$$

$$y = 0.0037x - 0.0245$$

For sample No 6B, 7B, and 8B

Concentration (mcg/ml)	20.56	51.40	102.80	205.60	308.40	411.20
Peak area ratio	0.079	0.195	0.372	0.754	1.135	1.620

$$R^2 = 0.9972$$

$$y = 0.0039x - 0.0185$$

For sample No 6C, 7C, 8C, 9B, and 9C

Concentration (mcg/ml)	20.16	50.40	100.80	201.60	302.40	403.20	504.00
Peak area ratio	0.079	0.195	0.372	0.754	1.135	1.620	1.422

$$R^2 = 0.9998$$

$$y = 0.0028x - 0.0077$$

**Calibration curve data
for Factor3: pH3**

Neutral liposomes

Concentration (mcg/ml)	20.40	51.00	102.00	204.00	306.00	510.00
Peak area ratio	0.061	0.206	0.415	0.839	1.256	2.118

$$R^2 = 0.9999$$

$$y = 0.0042x - 0.0151$$

Positive liposomes

Concentration (mcg/ml)	20.24	50.60	101.20	202.40	303.60	506.00
Peak area ratio	0.057	0.174	0.354	0.738	1.080	1.824

$$R^2 = 0.9999$$

$$y = 0.0036x - 0.0108$$

Negative liposomes

Concentration (mcg/ml)	20.24	50.60	101.20	202.40	303.60	506.00
Peak area ratio	0.074	0.179	0.382	0.756	1.117	1.886

$$R^2 = 0.9998$$

$$y = 0.0037x - 0.0029$$

**Calibration curve data
for Factor3: pH4**

Neutral liposomes

Concentration (mcg/ml)	20.40	51.00	102.00	204.00	306.00	510.00
Peak area ratio	0.053	0.172	0.349	0.702	1.084	1.791

$$R^2 = 0.9999$$

$$y = 0.0035x - 0.0142$$

Positive liposomes

Concentration (mcg/ml)	20.56	51.40	102.80	205.60	308.40	514.00
Peak area ratio	0.073	0.174	0.366	0.734	1.127	1.871

$$R^2 = 0.9999$$

$$y = 0.0037x - 0.0098$$

Negative liposomes

Concentration (mcg/ml)	20.48	51.20	102.40	204.80	307.20	512.00
Peak area ratio	0.067	0.189	0.405	0.730	1.063	1.841

$$R^2 = 0.9987$$

$$y = 0.0036x - 0.0066$$

**Calibration curve data
for Factor3: pH5**

Neutral liposomes

Concentration (mcg/ml)	20.48	51.20	102.40	204.80	307.20	510.00
Peak area ratio	0.068	0.195	0.389	0.799	1.244	1.945

$$R^2 = 0.9982$$

$$y = 0.0039x - 0.0032$$

Positive liposomes

Concentration (mcg/ml)	20.24	50.60	101.20	202.40	303.60	506.00
Peak area ratio	0.072	0.192	0.394	0.791	1.207	2.047

$$R^2 = 0.9999$$

$$y = 0.0037x - 0.0098$$

Negative liposomes

Concentration (mcg/ml)	20.32	50.80	101.60	203.20	304.80	508.00
Peak area ratio	0.075	0.182	0.393	0.763	1.156	1.940

$$R^2 = 0.9987$$

$$y = 0.0038x - 0.0058$$

**Calibration curve data
for Factor4:**

Concentration (mcg/ml)	20.08	50.20	100.40	200.80	301.20	502.00
Peak area ratio	0.061	0.219	0.521	1.093	1.625	2.835

$$R^2 = 0.9996$$

$$y = 0.0057x - 0.0641$$

Phospholipid assay**Phosphorus Content (Factor1)**

Sample No.	Lipid Conc. (mg/ml)	Absorbance at 800nm (A1)		Mean	Abs at 800 nm (blank) (Ao)	A1-Ao (nm)	Amount of EPC (mg/ml)
		n1	n2				
1A	10.00	0.578	0.606	0.592	0.289	0.303	8.92
1B	10.00	0.611	0.545	0.578	0.289	0.289	8.54
1C	10.00	0.622	0.586	0.604	0.289	0.315	9.24
2A	20.00	0.473	0.517	0.495	0.289	0.206	21.04
2B	20.00	0.448	0.488	0.468	0.289	0.179	18.63
2C	20.00	0.465	0.493	0.479	0.289	0.19	19.61
3A	30.00	0.598	0.636	0.617	0.289	0.328	31.95
3B	30.00	0.571	0.611	0.591	0.289	0.302	29.63
3C	30.00	0.633	0.607	0.62	0.289	0.331	32.22
4A	40.00	0.743	0.701	0.722	0.289	0.433	41.34
4B	40.00	0.704	0.734	0.719	0.289	0.43	41.07
4C	40.00	0.730	0.698	0.714	0.289	0.425	40.62

Note: 2A-4C dilution factor 3=>10

Standard Curve

Conc. (umol/ml)	0	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.289	0.431	0.534	0.626	0.704	0.792	0.887
Abs-AbsBK		0.142	0.245	0.337	0.415	0.503	0.599

$$R^2 = 0.9986$$

$$y = 2.8009x - 0.0298$$

Phosphorus Content (Factor2)

Sample No.	Lipid Conc. (mg/ml)	Absorbance at 800 nm (A1)		Mean	Abs at 800 nm (blank) (Ao)	A1-Ao (nm)	Amount of EPC (mg/ml)
		n1	n2				
6A	10.00	0.655	0.689	0.672	0.306	0.366	10.54
6B	10.00	0.612	0.622	0.617	0.306	0.311	9.11
6C	10.00	0.593	0.587	0.59	0.306	0.284	8.40
7A	20.00	0.617	0.633	0.625	0.306	0.319	9.31
7B	20.00	0.582	0.590	0.586	0.306	0.28	8.30
7C	20.00	0.598	0.622	0.61	0.306	0.304	8.92
8A	30.00	0.625	0.575	0.6	0.306	0.294	8.66
8B	30.00	0.586	0.590	0.588	0.306	0.282	8.35
8C	30.00	0.643	0.665	0.654	0.306	0.348	10.07
9A	40.00	0.598	0.574	0.586	0.306	0.28	8.30
9B	40.00	0.604	0.554	0.579	0.306	0.273	8.11
9C	40.00	0.630	0.616	0.623	0.306	0.317	9.26

Standard Curve

Conc. (umol/ml)	0	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.306	0.451	0.546	0.634	0.731	0.818	0.911
Abs-AbsBK		0.145	0.240	0.328	0.425	0.512	0.605

$$R^2 = 0.9988$$

$$y = 2.8688x - 0.0373$$

Standard Curve (Factor3)**pH3:sample10-12, 16-18**

Conc. (umol/ml)	0	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.301	0.476	0.577	0.674	0.770	0.871	0.973
Abs-AbsBK		0.175	0.276	0.373	0.469	0.570	0.672

$$R^2 = 0.9999$$

$$y = 3.0920x - 0.0227$$

pH3:sample13-15

Conc. (umol/ml)	0	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.321	0.454	0.546	0.638	0.730	0.816	0.911
Abs-AbsBK		0.133	0.225	0.317	0.409	0.495	0.590

$$R^2 = 0.9999$$

$$y = 2.8456x - 0.0483$$

Phosphorus Content (Factor3)pH3

Sample No.	Lipid Conc. (mg/ml)	Absorbance at 800nm (A1)		Mean	Abs at 800 nm (blank) (Ao)	A1-Ao (nm)	Amount of Lipid (mg/ml)
		n1	n2				
10A	10.00	0.659	0.642	0.651	0.301	0.350	9.04
10B	10.00	0.687	0.688	0.688	0.301	0.387	9.94
10C	10.00	0.651	0.662	0.657	0.301	0.356	9.19
11A	10.00	0.631	0.632	0.632	0.301	0.331	8.58
11B	10.00	0.601	0.613	0.607	0.301	0.306	7.97
11C	10.00	0.622	0.610	0.616	0.301	0.315	8.19
12A	10.00	0.662	0.659	0.661	0.301	0.36	9.28
12B	10.00	0.691	0.666	0.679	0.301	0.378	9.72
12C	10.00	0.671	0.686	0.679	0.301	0.378	9.72
13A	10.00	0.550	0.684	0.617	0.321	0.296	9.07
13B	10.00	0.546	0.603	0.5745	0.321	0.2535	7.95
13C	10.00	0.555	0.563	0.559	0.321	0.238	7.55
14A	10.00	0.541	0.569	0.555	0.321	0.234	7.44
14B	10.00	0.570	0.589	0.5795	0.321	0.259	8.10
14C	10.00	0.570	0.551	0.5605	0.321	0.24	7.60
15A	10.00	0.596	0.594	0.595	0.321	0.274	8.49
15B	10.00	0.584	0.598	0.591	0.321	0.270	8.39
15C	10.00	0.578	0.599	0.5885	0.321	0.268	8.34
16A	10.00	0.698	0.691	0.695	0.301	0.394	10.11
16B	10.00	0.693	0.713	0.703	0.301	0.402	10.30
16C	10.00	0.713	0.699	0.706	0.301	0.405	10.37
17A	10.00	0.692	0.696	0.694	0.301	0.393	10.08
17B	10.00	0.688	0.681	0.685	0.301	0.384	9.86
17C	10.00	0.696	0.689	0.693	0.301	0.392	10.06
18A	10.00	0.676	0.687	0.682	0.301	0.381	9.79
18B	10.00	0.678	0.651	0.665	0.301	0.364	9.38
18C	10.00	0.700	0.683	0.692	0.301	0.391	10.03

Phosphorus Content (Factor4)

Sample No.	Lipid Conc. (mg/ml)	Absorbance at 800nm (A1)		Mean	Abs at 800 nm (blank) (Ao)	A1-Ao (nm)	Amount of Lipid (mg/ml)
		n1	n2				
22AA	10.00	0.624	0.622	0.623	0.364	0.259	10.73
22BB	10.00	0.681	0.649	0.665	0.364	0.301	12.96
22CC	10.00	0.678	0.600	0.639	0.364	0.275	11.56
3AA	20.00	0.709	0.593	0.651	0.364	0.287	11.02
3BB	20.00	0.593	0.721	0.657	0.364	0.293	11.28
33BB	20.00	0.619	0.645	0.632	0.364	0.268	10.08

Standard Curve (Factor4)

Conc. (umol/ml)	0	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.364	0.476	0.577	0.674	0.770	0.871	0.973
Abs-AbsBK		0.259	0.357	0.446	0.568	0.660	0.750

$$R^2 = 0.9983$$

$$y = 3.1125x - 0.0585$$

$$y = 2.8009x - 0.0298$$

Effect of ionic strengths on entrapment of lactic acid in liposomes

a) pH3 : Neutral liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrap-ment	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
10A	10.13	3.01	0.1	neutral	0.6049	0.2585	7.38	1.35	8.73	86.15	15.43		9.04	1.24			
10B	10.13	3.01	0.1	neutral	0.5722	0.2833	6.99	1.47	8.46	83.51	17.36	16.04	9.94	1.23	1.21	0.05	4.16
10C	10.13	3.01	0.1	neutral	0.5723	0.2424	6.99	1.27	8.26	81.54	15.34		9.19	1.15			
11A	10.00	3.08	0.3	neutral	0.6463	0.1842	7.87	0.98	8.85	88.54	11.08		8.58	0.95			
11B	10.00	3.08	0.3	neutral	0.6940	0.2255	8.44	1.18	9.63	96.26	12.30	12.01	7.97	1.24	1.15	0.17	14.78
11C	10.00	3.08	0.3	neutral	0.7007	0.2356	8.52	1.23	9.76	97.55	12.65		8.19	1.25			
12A	10.00	2.93	0.5	neutral	0.6829	0.2392	8.31	1.25	9.56	95.61	13.09		9.28	1.12			
12B	10.00	2.93	0.5	neutral	0.6977	0.2770	8.49	1.44	9.92	99.23	14.48	14.09	9.72	1.23	1.21	0.07	6.18
12C	10.00	2.93	0.5	neutral	0.7040	0.2850	8.56	1.48	10.04	100.37	14.71		9.72	1.26			

Note: dilution factor of amount of lactic acid in free drug = 62/3000

: dilution factor of amount of lactic acid in free drug = 0.05

b) pH3 : positive liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment(mg/ml)	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
13A	10.06	2.91	0.10	positive	0.60	0.26	8.47	1.56	10.03	99.73	15.59	13.14	9.07	1.29			
13B	10.06	2.92	0.10	positive	0.63	0.21	8.90	1.24	10.14	100.81	12.26	12.05	7.97	1.17	1.21	0.07	5.84
13C	10.06	2.93	0.10	positive	0.64	0.19	9.01	1.18	10.18	101.23	11.57	12.05	7.55	1.17			
14A	10.09	2.92	0.30	positive	0.65	0.21	9.24	1.29	10.54	104.42	12.29	12.05	7.44	1.30			
14B	10.09	2.92	0.30	positive	0.65	0.21	9.11	1.28	10.39	103.00	12.31	12.05	8.10	1.18	1.22	0.07	5.69
14C	10.09	2.92	0.30	positive	0.65	0.20	9.18	1.20	10.38	102.84	11.56	12.05	7.60	1.18			
15A	10.17	2.93	0.50	positive	0.67	0.19	9.43	1.15	10.58	104.05	10.84	12.06	8.49	1.01			
15B	10.17	2.93	0.50	positive	0.70	0.23	9.93	1.37	11.30	111.12	12.12	12.06	8.39	1.22	1.19	0.17	14.06
15C	10.17	2.93	0.50	positive	0.70	0.25	9.82	1.49	11.31	111.24	13.21	12.06	8.34	1.34			

Note: dilution factor of amount of lactic acid in free drug = 62/3000

: dilution factor of amount of lactic acid in free drug = 0.05

c) pH3 : Negative liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment(mg/ml)	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
16A	10.18	2.92	0.1	negative	0.6039	0.2374	8.20	1.34	9.54	93.73	14.07		10.11	1.11			
16B	10.18	2.92	0.1	negative	0.5941	0.2297	8.07	1.30	9.37	92.01	13.87	14.47	10.30	1.05	1.11	0.06	5.00
16C	10.18	2.92	0.1	negative	0.5810	0.2560	7.89	1.45	9.34	91.72	15.49		10.37	1.16			
17A	10.30	2.92	0.3	negative	0.6266	0.2447	8.51	1.38	9.89	96.02	13.98		10.08	1.14			
17B	10.30	2.92	0.3	negative	0.6043	0.2205	8.21	1.25	9.45	91.78	13.20	13.98	9.87	1.05	1.13	0.07	5.81
17C	10.30	2.92	0.3	negative	0.6065	0.2525	8.24	1.43	9.66	93.80	14.77		10.06	1.18			
18A	10.00	2.93	0.5	negative	0.5884	0.2249	7.99	1.27	9.26	92.63	13.74		9.79	1.08			
18B	10.00	2.93	0.5	negative	0.604	0.2128	8.20	1.20	9.41	94.06	12.81	14.02	9.38	1.07	1.13	0.09	8.04
18C	10.00	2.93	0.5	negative	0.5952	0.2630	8.08	1.49	9.57	95.68	15.52		10.03	1.23			

Note: dilution factor of amount of lactic acid in free drug = 62/3000

: dilution factor of amount of lactic acid in free drug = 0.05

d) pH4 : Neutral liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
19A	10.10	4.09	0.1	neutral	0.6429	0.2766	9.39	1.72	11.10	109.94	15.46		9.91	1.44			
19A	10.10	4.09	0.1	neutral	0.6252	0.2411	9.13	1.51	10.64	105.36	14.17	14.50	9.44	1.33	1.36	0.07	5.19
19A	10.10	4.09	0.1	neutral	0.6381	0.2397	9.32	1.50	10.82	107.11	13.86		9.51	1.31			
20A	10.89	3.94	0.3	neutral	0.6658	0.2476	9.71	1.55	11.26	103.40	13.73		9.75	1.32			
20A	10.89	3.94	0.3	neutral	1.0289	0.2458	9.93	1.54	11.47	105.32	13.39	13.54	9.21	1.39	1.32	0.07	5.03
20A	10.89	3.94	0.3	neutral	0.9154	0.2197	8.85	1.38	10.23	93.98	13.49		9.16	1.26			
21A	10.98	3.90	0.5	neutral	0.9749	0.2671	9.42	1.66	11.08	100.92	14.99		8.97	1.54			
21B	10.98	3.90	0.5	neutral	0.8719	0.1931	8.44	1.22	9.66	88.01	12.67	13.50	9.02	1.13	1.34	0.21	15.37
21C	10.98	3.90	0.5	neutral	1.0397	0.2365	10.04	1.48	11.52	104.90	12.85		9.16	1.35			

f) pH4 : Negative liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment(mg/ml)	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
25A	10.03	3.91	0.1	negative	0.5904	0.1825	8.29	1.09	9.38	93.49	11.58		10.11	0.89			
25B	10.03	3.91	0.1	negative	0.5803	0.1873	8.15	1.11	9.26	92.37	12.01	12.08	10.30	0.90	0.90	0.00	0.35
25C	10.03	3.91	0.1	negative	0.5516	0.1887	7.75	1.12	8.87	88.47	12.63		10.37	0.90			
26A	10.00	3.93	0.3	negative	0.6228	0.1543	8.74	0.92	9.67	96.65	9.56		10.08	0.76			
26B	10.00	3.93	0.3	negative	0.6369	0.1631	8.94	0.97	9.91	99.12	9.83	9.79	9.87	0.82	0.80	0.04	4.41
26C	10.00	3.93	0.3	negative	0.6413	0.1672	9.00	1.00	10.00	99.96	9.98		10.06	0.83			
27A	10.58	3.91	0.5	negative	0.6151	0.1681	8.63	1.00	9.64	91.09	10.41		9.79	0.85			
27B	10.58	3.91	0.5	negative	0.6039	0.1924	8.48	1.14	9.62	90.94	11.87	12.29	9.38	1.01	0.99	0.12	12.45
27C	10.58	3.91	0.5	negative	0.5492	0.2231	7.72	1.32	9.04	85.43	14.59		10.03	1.09			

e) pH4 : positive liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment(mg/ml)	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
22A	10.38	3.97	0.10	positive	0.6360	0.2823	8.73	1.63	10.36	99.79	15.75		8.20	1.49			
22B	10.38	3.97	0.10	positive	0.6165	0.2402	8.46	1.40	9.86	94.99	14.16	15.22	7.99	1.31	1.40	0.09	6.46
22C	10.38	3.97	0.10	positive	0.6584	0.2925	9.03	1.69	10.72	103.26	15.75		9.07	1.39			
23A	10.22	3.90	0.30	positive	0.6636	0.2174	9.10	1.27	10.37	101.46	12.24		8.63	1.10			
23B	10.22	3.90	0.30	positive	0.7026	0.2491	9.63	1.45	11.07	108.35	13.06	12.32	8.65	1.25	1.16	0.08	7.07
23C	10.22	3.90	0.30	positive	0.5587	0.1717	7.68	1.01	8.70	85.09	11.66		6.78	1.12			
24A	10.13	3.90	0.50	positive	0.6650	0.2557	9.12	1.48	10.60	104.66	13.99		7.91	1.40			
24B	10.13	3.90	0.50	positive	0.6973	0.2381	9.56	1.38	10.94	108.00	12.66	13.59	8.17	1.27	1.32	0.07	5.39
24C	10.13	3.90	0.50	positive	0.6688	0.2600	9.17	1.51	10.68	105.40	14.11		8.73	1.29			

g) pH5 : Neutral liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
1A	10.24	4.90	0.1	neutral	0.6831	0.2328	8.72	1.20	9.91	96.81	12.08		9.91	1.01			
1B	10.24	4.90	0.1	neutral	0.67	0.2265	8.55	1.16	9.71	105.36	11.99	11.92	8.88	1.09	1.03	0.06	5.64
1C	10.24	4.90	0.1	neutral	0.6428	0.2117	8.20	1.09	9.29	107.11	11.71		9.23	0.98			
2A	10.11	4.99	0.3	neutral	0.6469	0.2156	8.25	1.11	9.36	103.40	11.83		9.68	0.95			
2B	10.11	4.99	0.3	neutral	0.5284	0.1517	6.73	0.77	7.51	105.32	10.31	11.34	8.91	0.72	0.88	0.13	15.08
2C	10.11	4.99	0.3	neutral	0.6152	0.2058	7.85	1.06	8.90	93.98	11.87		9.23	0.95			
3A	10.15	5.11	0.5	neutral	0.5802	0.2782	7.40	1.43	8.83	100.92	16.24		8.34	1.43			
3B	10.15	5.11	0.5	neutral	0.5993	0.3287	7.64	1.70	9.34	88.01	18.17	18.41	10.26	1.38	1.40	0.03	1.97
3C	10.15	5.11	0.5	neutral	0.5430	0.3522	6.92	1.82	8.74	104.90	20.82		10.85	1.40			

h) pH5 : positive liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment(mg/ml)	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
4A	10.01	4.90	0.10	positive	0.5795	0.1971	7.28	1.08	8.36	83.56	12.94		7.72	1.05			
4B	10.01	4.90	0.10	positive	0.5710	0.2522	7.18	1.36	8.54	85.29	15.93	15.59	8.72	1.17	1.19	0.15	12.41
4C	10.01	4.90	0.10	positive	0.6415	0.3301	8.04	1.75	9.79	97.81	17.90		9.78	1.34			
5A	10.00	4.91	0.30	positive	0.6527	0.2211	8.17	1.20	9.38	93.78	12.83		8.54	1.06			
5B	10.00	4.91	0.30	positive	0.6631	0.2075	8.30	1.13	9.44	94.36	12.03	13.80	8.22	1.03	1.08	0.06	5.92
5C	10.00	4.91	0.30	positive	0.6075	0.2825	7.62	1.51	9.14	91.36	16.56		9.82	1.15			
6A	10.13	5.02	0.50	positive	0.6552	0.2242	8.20	1.22	9.42	93.03	12.93		10.19	0.90			
6B	10.13	5.02	0.50	positive	0.6905	0.1947	8.64	1.07	9.71	95.81	11.03	14.08	7.70	1.04	1.03	0.13	12.96
6C	10.13	5.02	0.50	positive	0.5961	0.3147	7.48	1.68	9.16	90.42	18.29		10.78	1.16			

i) pH5 : Negative liposomes

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid		Total lactic acid(mg/ml)	% recovery	%Entrapment(mg/ml)	Mean	Amount of EPC (mg/ml)	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
7A	10.06	4.93	0.1	negative	0.5479	0.2343	7.29	1.28	8.57	85.19	14.99	14.50	9.67	1.11			
7B	10.06	4.93	0.1	negative	0.6365	0.2405	8.45	1.32	9.77	97.11	13.49	14.50	10.49	1.05	1.12	0.08	7.37
7C	10.06	4.93	0.1	negative	0.6401	0.2750	8.50	1.50	10.00	99.42	15.02		10.34	1.21			
8A	10.14	4.96	0.3	negative	0.6410	0.2217	8.51	1.22	9.73	95.93	12.51		9.94	1.02			
8B	10.14	4.96	0.3	negative	0.6164	0.2904	8.18	1.58	9.77	96.35	16.22	13.98	10.56	1.25	1.10	0.13	12.19
8C	10.14	4.96	0.3	negative	0.5868	0.2156	7.80	1.18	8.98	88.58	13.19		9.70	1.02			
9A	9.99	4.93	0.5	negative	0.6369	0.2232	8.46	1.23	9.68	96.92	12.66		9.32	1.10			
9B	9.99	4.93	0.5	negative	0.571	0.258	7.59	1.41	9.00	90.10	15.68	14.23	9.70	1.21	1.13	0.07	5.99
9C	9.99	4.93	0.5	negative	0.5514	0.2240	7.33	1.23	8.56	85.70	14.36		9.36	1.09			

Factoe4: Effect of cholesterol on entrapment of lactic acid in liposomes

- mol. ratio EPC:Chol=1:1(Sample No.3)

- mol. ratio EPC:Chol:SA =4.5:4.5:1(Sample No.22)

Sample No.	Conc. (mg/ml)	pH	ionic strength	charge	Peak Area Ratio		Amount of lactic acid(mg/ml)		Total lactic acid(mg/ml)	% recovery	%Entrapment	Mean	Amount of EPC (mg/m	mol. drug per mol. lipid	Mean	SD	%CV
					Free drugs	Pellets	Free drugs	Pellets									
22AA	10.06	3.99	0.1	neutral	0.9260	0.3185	8.69	1.39	10.07	100.12	13.77		4.83	1.08			
22BB	10.06	3.99	0.1	neutral	0.8276	0.2619	7.82	1.18	9.00	89.50	13.13	12.68	5.83	0.76	0.88	0.17	19.15
22CC	10.06	3.99	0.1	neutral	0.9637	0.2478	9.02	1.13	10.15	100.86	11.15		5.20	0.81			
3AA	10.16	5.16	0.5	positive	0.9429	0.2537	8.83	1.15	9.99	98.28	11.54		5.51	0.87			
3BB	10.16	5.16	0.5	positive	0.983	0.2607	9.19	1.18	10.36	102.00	11.36	10.81	5.64	0.87	0.84	0.05	5.56
3CC	10.16	5.16	0.5	positive	0.9709	0.1992	9.08	0.95	10.03	98.76	9.51		5.04	0.79			

Note: dilution factor of amount of lactic acid = 0.05

: dilution factor of amount of lactic acid = 0.05

Appendix II

Release data of lactic acid from liposomes at 4°C and 37°C

**Calibration curve data for
Release studies at 4°C**

Positive liposomes

Concentration (mcg/ml)	20.40	30.60	51.00	102.00	204.00	306.00
Peak area ratio	0.065	0.100	0.173	0.354	0.730	1.079

$$R^2 = 0.9998$$

$$y = 0.0036x - 0.0083$$

Neutral liposomes

Concentration (mcg/ml)	10.52	21.04	52.60	105.20	210.40	315.60
Peak area ratio	0.039	0.057	0.148	0.363	0.723	1.057

$$R^2 = 0.9986$$

$$y = 0.0034x - 0.0085$$

Positive liposomes

with Cholesterol

Concentration (mcg/ml)	10.28	20.56	30.84	51.40	102.80	205.60
Peak area ratio	0.065	0.154	0.230	0.372	0.774	1.608

$$R^2 = 0.9996$$

$$y = 0.0079x - 0.0188$$

Neutral liposomes

with Cholesterol ;for sample at 2, and 4 hrs.

Concentration (mcg/ml)	10.16	20.32	30.48	50.80	101.60	203.20
Peak area ratio	0.060	0.125	0.196	0.341	0.719	1.640

$$R^2 = 0.9967$$

$$y = 0.0082x - 0.0550$$

for sample at 8, 12, 18, and 24 hrs.

Concentration (mcg/ml)	10.32	20.64	30.96	51.60	103.20	206.40
Peak area ratio	0.073	0.124	0.204	0.329	0.724	1.438

$$R^2 = 0.9992$$

$$y = 0.0070x - 0.0161$$

**Calibration curve data for
Release studies at 37°C**

Positive liposomes

Concentration (mcg/ml)	20.56	30.84	51.40	102.80	205.60	308.40
Peak area ratio	0.068	0.110	0.183	0.370	0.751	1.104

$$R^2 = 0.9998$$

$$y = 0.0036x - 0.0019$$

Neutral liposomes

Concentration (mcg/ml)	20.24	30.36	50.60	101.20	202.40	303.60
Peak area ratio	0.048	0.088	0.161	0.392	0.777	1.184

$$R^2 = 0.9995$$

$$y = 0.0040x - 0.0319$$

Positive liposomes

with Cholesterol

Concentration (mcg/ml)	10.40	20.80	31.20	52.00	104.00	208.00
Peak area ratio	0.037	0.130	0.208	0.349	0.733	1.439

$$R^2 = 0.9994$$

$$y = 0.0070x - 0.0186$$

Neutral liposomes

with Cholesterol

Concentration (mcg/ml)	10.32	20.64	30.96	51.60	103.20	206.40
Peak area ratio	0.073	0.124	0.204	0.329	0.724	1.438

$$R^2 = 0.9992$$

$$y = 0.0070x - 0.0161$$

Release of entrapped lactic acid from positive liposomes at 4 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.1581	0.1298	0.139	0.55	0.46	0.47	11.33	9.57	9.73	10.21	0.97
4	0.2053	0.1983	0.158	0.79	0.77	0.61	16.46	15.90	12.71	15.02	2.02
8	0.3480	0.3268	0.323	1.38	1.31	1.23	28.63	27.18	25.49	27.10	1.58
12	0.6528	0.4579	0.406	1.60	1.60	1.72	33.06	33.19	35.59	33.95	1.42
18	0.6892	0.6358	0.615	1.92	1.73	1.62	39.69	35.88	33.58	36.38	3.08
24	0.6800	0.7086	0.656	2.11	1.83	1.84	43.64	37.95	38.14	39.91	3.23
total				4.83	4.83	4.83					

Release of entrapped lactic acid from neutral liposomes at 4 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.0337	0.0715	0.069	0.15	0.28	0.27	3.65	7.06	6.78	5.83	1.89
4	0.0835	0.0966	0.093	0.35	0.42	0.39	8.58	10.41	9.69	9.56	0.92
8	0.1409	0.1262	0.113	0.60	0.59	0.51	14.88	14.56	12.77	14.07	1.14
12	0.1548	0.1599	0.124	0.74	0.79	0.63	18.29	19.50	15.53	17.77	2.04
18	0.1722	0.1777	0.153	0.89	0.95	0.80	22.16	23.53	19.88	21.86	1.85
24	0.1736	0.1896	0.169	1.00	1.10	0.95	24.92	27.30	23.58	25.27	1.89
total				4.03	4.03	4.03					

Release of entrapped lactic acid from positive liposomes with cholesterol at 4 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.1447	0.2225	0.1462	0.25	0.36	0.24	12.04	17.59	11.79	13.81	3.28
4	0.3974	0.3476	0.3476	0.67	0.61	0.58	32.68	29.73	28.26	30.22	2.25
8	0.5843	0.4724	0.4726	1.05	0.88	0.85	51.65	43.39	41.77	45.60	5.30
12	0.5342	0.4411	0.4407	1.13	0.96	0.93	55.43	47.22	45.60	49.42	5.27
18	0.5023	0.441	0.4426	1.22	1.08	1.05	59.95	52.92	51.45	54.77	4.54
24	0.4900	0.4104	0.4487	1.33	1.15	1.17	65.55	56.41	57.64	59.87	4.96
total				2.04	2.04	2.04					

Release of entrapped lactic acid from neutral liposomes with cholesterol at 4 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.124	0.0862	0.1025	0.26	0.20	0.22	13.02	10.18	11.13	11.44	1.45
4	0.2074	0.1781	0.219	0.42	0.37	0.42	21.31	18.54	21.31	20.39	1.60
8	0.3497	0.2643	0.2485	0.73	0.56	0.54	36.59	28.26	27.20	30.69	5.14
12	0.3804	0.3594	0.3056	0.88	0.80	0.71	44.48	40.35	35.72	40.18	4.39
18	0.3904	0.3711	0.3001	1.01	0.93	0.79	51.03	46.70	39.90	45.88	5.61
24	0.3436	0.3694	0.3081	1.05	1.04	0.90	52.90	52.14	45.14	50.06	4.28
total				1.99	1.99	1.99					

Release of entrapped lactic acid from positive liposomes at 37 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.2790	0.2797	0.3046	0.92	0.92	0.98	24.38	24.22	25.83	24.81	0.89
4	0.4870	0.4881	0.4987	1.76	1.75	1.77	46.54	46.25	46.68	46.49	0.22
8	0.7780	0.7669	0.7909	2.99	2.93	2.98	78.97	77.41	78.65	78.35	0.82
12	0.7160	0.7489	0.6868	3.22	3.30	3.09	85.01	87.12	81.48	84.54	2.85
18	0.6556	0.6505	0.6625	3.42	3.40	3.39	90.29	89.68	89.53	89.83	0.40
24	0.5731	0.6697	0.5837	3.52	3.82	3.51	92.77	100.90	92.64	95.44	4.73
total				3.79	3.79	3.79					

Release of entrapped lactic acid from neutral liposomes at 37 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.142	0.1408	0.1909	0.51	0.52	0.64	10.38	10.48	12.92	11.26	1.44
4	0.2	0.208	0.2131	0.77	0.81	0.82	15.59	16.31	16.43	16.11	0.45
8	0.3736	0.3428	0.3851	1.40	1.34	1.43	28.29	26.92	28.89	28.03	1.01
12	0.4289	0.4438	0.4887	1.77	1.83	1.94	35.69	36.81	39.11	37.20	1.75
18	0.5727	0.594	0.644	2.43	2.52	2.65	48.91	50.75	53.35	51.00	2.23
24	0.5808	0.6107	0.6647	2.69	2.88	3.04	54.27	58.06	61.37	57.90	3.55
total				4.96	4.96	4.96					

Release of entrapped lactic acid from positive liposomes with cholesterol at 37 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.6125	0.61	0.641	1.07	1.05	1.08	44.83	44.23	45.53	44.86	0.6515
4	0.6418	0.8081	0.8653	1.30	1.57	1.64	54.47	65.73	68.92	63.04	7.5897
8	0.8147	0.8762	0.9408	1.78	1.92	2.02	74.67	80.43	84.71	79.94	5.0368
12	0.7951	0.8023	0.8342	1.98	2.05	2.12	83.28	85.97	88.87	86.04	2.7936
18	0.6902	0.7224	0.7757	2.04	2.15	2.26	85.59	90.21	95.09	90.30	4.7465
24	0.6184	0.6215	0.6000	2.12	2.19	2.20	89.04	91.98	92.48	91.17	1.86
total				2.38	2.38	2.38					

Release of entrapped lactic acid from neutral liposomes with cholesterol at 37 ° C

Time(hr.)	Peak Area Ratio (PAR)			Amount of lactic acid (mg)			% Released			Mean	SD
	n1	n2	n3	n1	n2	n3	n1	n2	n3		
0	0	0	0	0	0	0	0	0	0	0	0
2	0.1641	0.1831	0.1849	0.30	0.33	0.33	14.10	15.45	15.26	14.94	0.73
4	0.3103	0.2833	0.2984	0.60	0.56	0.57	27.94	25.86	26.55	26.78	1.06
8	0.3747	0.4683	0.4079	0.81	0.95	0.84	37.28	44.13	39.04	40.15	3.56
12	0.4035	0.4475	0.5159	0.97	1.06	1.14	44.68	48.94	52.82	48.81	4.07
18	0.555	0.509	0.5682	1.34	1.29	1.38	62.07	59.81	63.83	61.90	2.02
24	0.4758	0.5114	0.5295	1.37	1.45	1.48	63.41	66.93	68.64	66.33	2.66
total				2.16	2.16	2.16					

Appendix III

Stability data of lactic acid liposomes

**Calibration curve data for
Stability studies****Day0**

Concentration (mcg/ml)	20.40	51.00	81.60	102.00	204.00	306.00	510.00
Peak area ratio	0.068	0.110	0.183	0.370	0.751	1.104	1.9704

$$R^2 = 0.9993$$

$$y = 0.0039x - 0.0125$$

Day7

Concentration (mcg/ml)	30.48	50.80	101.60	203.20	304.80	508.00
Peak area ratio	0.102	0.174	0.390	0.733	1.114	1.890

$$R^2 = 0.9993$$

$$y = 0.0039x - 0.0125$$

Phosphorus content**Std Curve: Stability studies****Day0**

Conc. (umol/ml)	0	0.032	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.278	0.464	0.464	0.533	0.603	0.687	0.763	0.792
Abs-AbsBK		0.368	0.186	0.255	0.325	0.409	0.485	0.514

$$R^2 = 0.9980$$

$$y = 2.4232x - 0.0203$$

Day7:Formulation1-6

Conc. (umol/ml)	0	0.032	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.343	0.439	0.504	0.568	0.650	0.674	0.716	0.844
Abs-AbsBK		0.096	0.161	0.225	0.307	0.373	0.425	0.501

$$R^2 = 0.9982$$

$$y = 2.1105x - 0.0281$$

Day7:Formulation7-8

Conc. (umol/ml)	0	0.032	0.064	0.096	0.128	0.160	0.192	0.224
Abs at 800nm	0.301	0.372	0.450	0.532	0.589	0.674	0.748	0.815
Abs-AbsBK		0.071	0.149	0.231	0.288	0.373	0.447	0.514

$$R^2 = 0.9988$$

$$y = 2.3069x - 0.0009$$

Phosphorus Content (Stability studies)

Day0

Formula	Lipid Composition	Lipid Conc. (mg/ml)	Abs at 800 nm (A1)		Mean	Abs at 800 nm (Ao)	A1-Ao (nm)	Amount of Lipid (mg/ml)
			n1	n2				
1	EPC/SA (9:1)	10.00	0.580	0.558	0.569	0.278	0.291	9.31
	pH4	10.00	0.541	0.581	0.561	0.278	0.283	9.03
	No vit E	10.00	0.522	0.570	0.546	0.278	0.268	8.52
2	EPC/SA (9:1)	10.00	0.514	0.600	0.557	0.278	0.279	8.90
	pH4	10.00	0.535	0.539	0.537	0.278	0.259	8.21
	With vit E	10.00	0.540	0.552	0.546	0.278	0.268	8.52
3	EPC	10.00	0.605	0.601	0.603	0.278	0.325	9.43
	pH5	10.00	0.531	0.597	0.564	0.278	0.286	8.22
	No vit E	10.00	0.544	0.568	0.556	0.278	0.278	7.98
4	EPC	10.00	0.590	0.580	0.585	0.278	0.307	8.87
	pH5	10.00	0.637	0.629	0.633	0.278	0.355	10.36
	With vit E	10.00	0.599	0.585	0.592	0.278	0.314	9.09
5	EPC/Chol/SA (4.5:4.5:1), pH4	10.00	0.452	0.496	0.474	0.278	0.196	12.08
		10.00	0.423	0.427	0.425	0.278	0.147	8.71
	No vit E	10.00	0.413	0.479	0.446	0.278	0.168	10.16
6	EPC/Chol/SA (4.5:4.5:1), pH4	10.00	0.421	0.459	0.440	0.278	0.162	9.75
		10.00	0.435	0.399	0.417	0.278	0.139	8.16
	With vit E	10.00	0.425	0.447	0.436	0.278	0.158	9.47
7	EPC/Chol (1:1)	10.00	0.436	0.444	0.440	0.278	0.162	8.77
	pH5	10.00	0.440	0.424	0.432	0.278	0.154	8.28
	No vit E	10.00	0.425	0.489	0.457	0.278	0.179	9.82
8	EPC/Chol (1:1)	10.00	0.446	0.448	0.447	0.278	0.169	9.2
	pH5	10.00	0.469	0.439	0.454	0.278	0.176	9.64
	With vit E	10.00	0.453	0.467	0.460	0.278	0.182	10.01

Phosphorus Content (Stability studies)

Day7

Formula	Lipid Composition	Lipid Conc. (mg/ml)	Abs at 800 nm (A1)		Mean	Abs at 800 nm (Ao)	AI-Ao (nm)	Amount of Lipid (mg/ml)
			n1	n2				
			1A	EPC/SA (9:1)				
1B	pH4	10.00	0.641	0.671	0.656	0.343	0.313	11.27
1C	No vit E	10.00	0.621	0.681	0.651	0.343	0.308	11.05
2A	EPC/SA (9:1)	10.00	0.584	0.598	0.591	0.343	0.248	8.7
2B	pH4	10.00	0.600	0.652	0.626	0.343	0.283	10.06
2C	With vit E	10.00	0.671	0.631	0.651	0.343	0.308	11.05
3A	EPC	10.00	0.663	0.665	0.664	0.343	0.321	10.41
3B	pH5	10.00	0.640	0.644	0.642	0.343	0.299	9.63
3C	No vit E	10.00	0.670	0.682	0.676	0.343	0.333	10.84
4A	EPC	10.00	0.649	0.709	0.679	0.343	0.336	10.95
4B	pH5	10.00	0.654	0.646	0.650	0.343	0.307	9.92
4C	With vit E	10.00	0.975	0.385	0.680	0.343	0.337	10.99
5A	EPC/Chol/SA	10.00	0.513	0.499	0.506	0.343	0.163	10.64
5B	(4.5:4.5:1), pH4	10.00	0.482	0.512	0.497	0.343	0.153	9.91
5C	No vit E	10.00	0.506	0.500	0.503	0.343	0.16	10.44
6A	EPC/Chol/SA	10.00	0.485	0.515	0.500	0.343	0.157	10.18
6B	(4.5:4.5:1), pH4	10.00	0.492	0.536	0.514	0.343	0.171	11.26
6C	With vit E	10.00	0.523	0.507	0.515	0.343	0.172	11.33
7A	EPC/Chol (1:1)	10.00	0.463	0.533	0.498	0.301	0.197	12.93
7B	pH5	10.00	0.503	0.505	0.504	0.301	0.203	13.26
7C	No vit E	10.00	0.428	0.428	0.428	0.301	0.127	8.32
8A	EPC/Chol (1:1)	10.00	0.509	0.497	0.503	0.301	0.202	13.19
8B	pH5	10.00	0.498	0.508	0.503	0.301	0.202	13.19
8C	With vit E	10.00	0.431	0.435	0.433	0.301	0.132	8.64

Stability of liposomes(Day0)

Lipid composition	Lactic Conc. (mg/ml)	Peak area ratio		Amount of lactic acid		Total lactic acid (mg/ml)	%recovery	%Entrapment (mg/ml)	Mean	SD	Amount of Lipid (mg/ml)	mol.drug per mol. lipid	Mean	SD	%CV
		Free drugs	Pellets	Free drugs (mg/ml)	Pellets (mg/ml)										
EPC:SA/pH3.98 (9:1) (No Vit.E)	10.07	0.5670	0.2826	7.43	1.56	8.99	89.31	17.39			9.31	1.40			
	10.07	0.6163	0.2851	8.06	1.58	9.64	95.72	16.36	16.60	0.70	9.03	1.45	1.45	0.05	3.36
	10.07	0.6118	0.2763	8.00	1.53	9.53	94.68	16.05			8.52	1.50			
EPC:SA/pH4 (9:1) (With Vit.E)	9.86	0.6203	0.2727	8.11	1.51	9.62	97.61	15.70			8.90	1.41			
	9.86	0.6165	0.2660	8.06	1.48	9.54	96.75	15.47	15.67	0.19	8.21	1.50	1.47	0.05	3.25
	9.86	0.6225	0.2766	8.14	1.53	9.67	98.10	15.84			8.52	1.50			
EPC/pH5 (No Vit.E)	10.28	0.5803	0.2709	7.60	1.50	9.10	88.54	16.50			9.43	1.33			
	10.28	0.5510	0.2546	7.22	1.42	8.64	84.04	16.38	15.73	1.23	8.22	1.43	1.37	0.06	4.09
	10.28	0.5921	0.2318	7.75	1.29	9.05	87.99	14.31			7.98	1.35			
EPC/pH5.04 (With Vit.E)	10.60	0.5911	0.2310	7.74	1.29	9.03	85.18	14.29			8.87	1.21			
	10.60	0.5843	0.2702	7.65	1.50	9.15	86.31	16.37	15.52	1.09	10.36	1.20	1.24	0.06	4.56
	10.60	0.5761	0.2564	7.55	1.42	8.97	84.63	15.88			9.09	1.31			

Stability of liposomes(Day7)

Lipid composition	Lactic Conc. (mg/ml)	Peak area ratio		Amount of lactic acid			Total lactic acid (mg/ml)	%recovery	%Entrapment (mg/ml)	Mean	SD	Amount of Lipid (mg/ml)	mol.drug per mol. lipid	Mean	SD	%CV
		Free drugs	Pellets	Free drugs (mg/ml)	Pellets (mg/ml)											
EPC:SA:Chol/pH3.96 (4.5:1:4.5) (No Vit.E)	10.23	0.6848	0.1476	9.40	0.88	10.28	100.54	8.60	9.84	10.64	0.69	0.84	0.14	17.05		
	10.23	0.7106	0.1977	9.75	1.16	10.91	106.68	10.67	9.84	9.91	0.98	0.84	0.14	17.05		
	10.23	0.681	0.1802	9.35	1.07	10.42	101.81	10.24	9.84	10.44	0.85	0.84	0.14	17.05		
EPC:SA:Chol/pH3.97 (4.5:1:4.5) (With Vit.E)	10.26	0.677	0.1431	9.29	0.86	10.15	98.97	8.47	9.87	10.18	0.70	0.78	0.08	10.08		
	10.26	0.6826	0.1974	9.37	1.16	10.53	102.66	11.04	9.87	11.26	0.86	0.78	0.08	10.08		
	10.26	0.6827	0.1778	9.37	1.05	10.43	101.61	10.10	9.87	11.33	0.77	0.78	0.08	10.08		
EPC:Chol/pH5.05 (1:1) (No Vit.E)	10.12	0.5521	0.2483	7.61	1.45	9.05	89.47	15.98	14.21	12.93	0.93	1.02	0.21	20.72		
	10.12	0.6021	0.2384	8.28	1.39	9.67	95.60	14.39	14.21	13.26	0.87	1.02	0.21	20.72		
	10.12	0.6601	0.2159	9.07	1.27	10.33	102.10	12.26	14.21	8.32	1.27	1.02	0.21	20.72		
EPC:Chol/pH5.05 (1:1) (With Vit.E)	10.29	0.6595	0.2443	9.06	1.42	10.48	101.88	13.59	13.05	13.19	0.90	1.01	0.21	20.77		
	10.29	0.6278	0.2378	8.63	1.39	10.02	97.36	13.86	13.05	13.19	0.88	1.01	0.21	20.77		
	10.29	0.7144	0.2215	9.80	1.30	11.10	107.85	11.69	13.05	8.64	1.25	1.01	0.21	20.77		

Stability of liposomes(Day0)

Lipid composition	Lactic Conc. (mg/ml)	Peak area ratio		Amount of lactic acid		Total lactic acid (mg/ml)	%recovery	%Entrapment (mg/ml)	Mean	SD	Amount of Lipid (mg/ml)	mol.drug per mol. lipid	Mean	SD	%CV
		Free drugs	Pellets	Free drugs (mg/ml)	Pellets (mg/ml)										
EPC:SA:Chol/pH3.96 (4.5:1:4.5) (No Vit.E)	10.23	0.7316	0.2030	9.54	1.14	10.68	104.42	10.69			12.08	0.79			
	10.23	0.7383	0.2127	9.63	1.19	10.82	105.76	11.03	10.67	0.36	8.71	1.14	0.95	0.18	18.99
	10.23	0.7408	0.1968	9.66	1.11	10.77	105.25	10.30			10.16	0.91			
EPC:SA:Chol/pH3.97 (4.5:1:4.5) (With Vit.E)	10.26	0.6596	0.1883	8.62	1.06	9.68	94.35	10.99			9.75	0.91			
	10.26	0.7359	0.1642	9.59	0.94	10.53	102.64	8.89	10.24	1.17	8.16	0.95	0.96	0.06	5.76
	10.26	0.7321	0.2063	9.55	1.16	10.71	104.34	10.83			9.47	1.02			
EPC:Chol/pH5.05 (1:1) (No Vit.E)	10.12	0.6336	0.1981	8.28	1.12	9.40	92.88	11.87			8.77	1.06			
	10.12	0.6451	0.2027	8.43	1.14	9.57	94.58	11.91	11.80	0.16	8.28	1.15	1.06	0.09	8.82
	10.12	0.6601	0.2015	8.62	1.13	9.76	96.41	11.62			9.82	0.96			
EPC:Chol/pH5.05 (1:1) (With Vit.E)	10.29	0.6574	0.2090	8.59	1.17	9.76	94.87	12.02			9.20	1.06			
	10.29	0.6278	0.1970	8.21	1.11	9.32	90.57	11.91	11.97	0.06	9.64	0.96	0.99	0.07	6.71
	10.29	0.6334	0.2003	8.28	1.13	9.41	91.43	11.99			10.01	0.94			

Stability of liposomes(Day7)

Lipid composition	Lactic Conc. (mg/ml)	Peak area ratio		Amount of lactic acid		Total lactic acid (mg/ml)	%recovery	%Entrapment (mg/ml)	Mean	SD	Amount of Lipid (mg/ml)	mol.drug per mol. lipid	Mean	SD	%CV
		Free drugs	Pellets	Free drugs (mg/ml)	Pellets (mg/ml)										
EPC:SA/pH3.98 (9:1) (No Vit.E)	10.07	0.5595	0.2316	7.71	1.35	9.06	89.98	14.94			10.02	1.13			
	10.07	0.6319	0.2588	8.69	1.51	10.19	101.20	14.78	16.04	2.04	11.27	1.11	1.15	0.05	4.51
	10.07	0.5156	0.2762	7.11	1.60	8.72	86.56	18.39			11.05	1.21			
EPC:SA/pH4 (9:1) (With Vit.E)	9.86	0.5843	0.2183	8.04	1.28	9.32	94.54	13.73			8.70	1.22	1.16	0.05	4.67
	9.86	0.5648	0.2355	7.78	1.38	9.15	92.84	15.03	14.98	1.22	10.06	1.14			
	9.86	0.5614	0.2563	7.73	1.49	9.22	93.55	16.17			11.05	1.12			
EPC/pH5 (No Vit.E)	10.28	0.5657	0.2399	7.79	1.40	9.19	89.41	15.24			10.41	1.12	1.15	0.05	4.06
	10.28	0.5716	0.2238	7.87	1.31	9.18	89.31	14.27	15.41	1.23	9.63	1.13			
	10.28	0.5686	0.2705	7.83	1.57	9.40	91.45	16.71			10.84	1.21			
EPC/pH5.04 (With Vit.E)	10.60	0.5975	0.2691	8.22	1.56	9.78	92.30	15.98			10.95	1.19	1.13	0.05	4.34
	10.60	0.5846	0.2283	8.05	1.34	9.38	88.50	14.24	15.23	0.90	9.92	1.12			
	10.60	0.5729	0.2475	7.89	1.44	9.33	88.02	15.46			10.99	1.09			

Retention of lactic acid in different liposomes at 4°C

Lipid composition	Lactic acid entrapped/lipid (mol/mol)						%Remaining			Mean	SD	%CV
	Day0			Day7			n1	n2	n3			
	n1	n2	n3	n1	n2	n3						
EPC:SA/pH3.98 (9:1) (No Vit.E)	1.40	1.45	1.50	1.13	1.11	1.21	80.71	76.55	80.67	79.31	2.39	3.01
EPC:SA/pH4 (9:1) (With Vit.E)	1.41	1.50	1.50	1.22	1.14	1.12	86.52	76.00	74.67	79.06	6.50	8.22
EPC/pH5 (No Vit.E)	1.33	1.43	1.35	1.12	1.13	1.21	84.21	79.02	89.63	84.29	5.30	6.29
EPC/pH5.04 (With Vit.E)	1.21	1.20	1.31	1.19	1.12	1.09	98.35	93.33	83.21	91.63	7.71	8.42
EPC:SA:Chol/pH3.96 (4.5:1:4.5) (No Vit.E)	0.79	1.14	0.91	0.69	0.98	0.85	87.34	85.96	93.41	88.90	3.96	4.45
EPC:SA:Chol/pH3.97 (4.5:1:4.5) (With Vit.E)	0.91	0.95	1.02	0.70	0.86	0.77	76.92	90.53	75.49	80.98	8.30	10.25
EPC:Chol/pH5.05 (1:1) (No Vit.E)	1.06	1.15	0.96	0.93	0.87	1.27	87.74	75.65	132.29	98.56	29.83	30.27
EPC:Chol/pH5.02 (1:1) (With Vit.E)	1.06	0.96	0.94	0.90	0.88	1.25	84.91	91.67	132.98	103.18	26.02	25.22

VITA

Miss Orawan Niyompattamah was born on January 4, 1968 in Bangkok, Thailand. She received her Bachelor Degree of Science in Pharmacy from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in 1991. After graduation, she worked in the Department of Medical Sciences, Bangkok from 1991 to 1993. In 1993-1995, she worked at Bangkok General Hospital, Bangkok before entering the Master's Degree program in Pharmacy at Chulalongkorn University.