

APPENDIX





APPENDIX I

LIPOSOMES PREPARATION

Table 9 Mean peak area of tartaric acid standard with various concentration determined by HPLC to define calibration curve.

Tartaric acid standard concentration (mg/ml)	Peak area						
	sample 1		sample 2		sample 3		mean
	1	2	1	2	1	2	
0.5	1095540	1093109	1068811	1075041	1078979	1068050	1079922
1	2085199	2012595	2120849	2115296	2179175	2159170	2112047
2	3292050	3340076	3365220	3397418	3402065	3371242	3361345
5	8708564	8705045	8644095	8726190	8742037	8704235	8705028
10	17026640	17100222	16819709	16910712	17104010	17079939	17006872

Table 10 Mean peak area of 10 mg/ml of tamarind fruit pulp's extract determined by HPLC.

Sample	peak area		mean peak area	Tartaric acid content (mg/ml)	tartaric acid content (%)	Mean	SD
	1	2					
1	4316436	4385897	4351167	2.46	24.63	24.28	1.68
2	4569882	4509118	4539500	2.58	25.76		
3	3972006	4006993	3989500	2.25	22.46		

Table 11 Mean peak area of tartaric acid in supernatant of liposomes suspension after centrifugation. The value was calculated to entrapment efficiency of liposome.

Batch	Peak area			mean	Tartaric acid content (mg/ml)		Encapsulation efficiency (%)	mean	SD
	1	2	3		supernatant	added			
1	2105724	2106192	2102584	2104833	1.12	2	44.24		
2	2226789	2229975	2229736	2228833	1.19	2	40.52	41.39	2.53
3	2269749	2265153	2261597	2265499	1.21	2	39.42		
4	2699036	2706415	2701049	2702166	1.47	2	26.32		
5	2420305	2412080	2411116	2414500	1.30	2	34.95	32.63	5.53
6	2360189	2357832	2358479	2358833	1.27	2	36.62		
7	2439935	2447249	2441315	2442833	1.32	2	34.1		
8	2601085	2594469	2596946	2597500	1.41	2	29.46	30.24	3.54
9	2677272	2672053	2674175	2674500	1.46	2	27.15		
10-12	n.d.								
13	1947346	1950490	1945663	1947833	1.02	2	48.95		
14	1744119	1742026	1740357	1742167	0.90	2	55.12	51.13	3.46
15	1935401	1931072	1940028	1935500	1.01	2	49.32		

Table 11 (cont.)

Batch	Peak area			mean	Tartaric acid content (mg/ml)		Encapsulation efficiency (%)	mean	SD
	1	2	3		supernatant	added			
16	2502347	2509765	2514386	2508832	1.36	2	32.12	32.16	3.77
17	2381497	2381119	2380885	2381167	1.28	2	35.95		
18	2634522	2623057	2638922	2632167	1.43	2	28.42		
19	2767045	2758632	2760825	2762167	1.51	2	24.52	26.42	2.55
20	2731211	2735452	2729837	2732166	1.49	2	25.42		
21	2603221	2601905	2601375	2602167	1.41	2	29.32		
22	2365694	2360475	2362329	2362832	1.27	2	36.5	32.60	3.80
23	2495754	2504025	2498721	2499500	1.35	2	32.4		
24	2614134	2620645	2613722	2616167	1.42	2	28.9		
25	1302643	1297662	1298195	1299500	0.63	2	68.4	68.30	2.95
26	1210089	1204742	1203669	1206166	0.58	2	71.2		
27	1397965	1403974	1406561	1402833	0.69	2	65.3		

Table 12 Mean peak area of tartaric acid in supernatant of chitosan coated liposomes suspension after centrifugation. The value was calculated to encapsulation efficiency of chitosan/liposomes.

Batch	peak area 1			Tartaric acid content in supernatant (mg/ml)			Encapsulation efficiency (%)			Mean	SD
	1	2	3	1	2	3	1	2	3		
28	1846500	1745500	1799167	0.96	0.90	0.93	51.99	55.02	53.41	53.47	1.52
29	1939167	1961833	1735500	1.02	1.03	0.89	49.21	48.53	55.32	51.02	3.74
30	1994167	1942500	1891500	1.05	1.02	0.99	47.56	49.11	50.64	49.10	1.54
31	1901500	1749833	1605833	0.99	0.90	0.82	50.34	54.89	59.21	54.81	4.44
32	1565167	1705167	1849833	0.79	0.88	0.96	60.43	56.23	51.89	56.18	4.27
33	1991833	2072500	1816500	1.05	1.10	0.94	47.63	45.21	52.89	48.58	3.93
34	1750500	2013167	1835833	0.90	1.06	0.95	54.87	46.99	52.31	51.39	4.02
35	1675833	1435167	1621167	0.86	0.71	0.83	57.11	64.33	58.75	60.06	3.78
36	1732167	2001500	2027500	0.89	1.05	1.07	55.42	47.34	46.56	49.77	4.91
37	1551167	1554833	1793500	0.78	0.79	0.93	60.85	60.74	53.58	58.39	4.17
38	1813167	2131167	2005167	0.94	1.13	1.06	52.99	43.45	47.23	47.89	4.80
39	2227500	2342167	2091833	1.19	1.26	1.11	40.56	37.12	44.63	40.77	3.76

Table 12 (cont.)

Batch	peak area ¹			Tartaric acid content in supernatant (mg/ml)			Encapsulation efficiency (%)			Mean	SD
	1	2	3	1	2	3	1	2	3		
40	1368833	1531167	1301500	0.67	0.77	0.63	66.32	61.45	68.34	65.37	3.54
41	1378833	1293833	1451167	0.68	0.63	0.72	66.02	68.57	63.85	66.15	2.36
42	1171833	1416500	1299167	0.56	0.70	0.63	72.23	64.89	68.41	68.51	3.67
43	1201833	1321167	1368167	0.57	0.65	0.67	71.33	67.75	66.34	68.47	2.57
44	1235167	1500500	1416500	0.59	0.75	0.70	70.33	62.37	64.89	65.86	4.07
45	1471833	1729833	1569167	0.74	0.89	0.79	63.23	55.49	60.31	59.68	3.91
46	2157500	2217833	2221500	1.15	1.18	1.19	42.66	40.85	40.74	41.42	1.08
47	1702167	1984500	1971167	0.87	1.04	1.04	56.32	47.85	48.25	50.81	4.78
48	1494500	1732167	1647833	0.75	0.89	0.84	62.55	55.42	57.95	58.64	3.61
49	1684500	2013167	2221167	0.86	1.06	1.19	56.85	46.99	40.75	48.20	8.12
50	1442500	1649833	1708833	0.72	0.84	0.88	64.11	57.89	56.12	59.37	4.20
51	1487833	1701833	1665833	0.75	0.87	0.85	62.75	56.33	57.41	58.83	3.44

Table 12 (cont.)

Batch	peak area ¹			Tartaric acid content in supernatant (mg/ml)			Encapsulation efficiency (%)			Mean	SD
	1	2	3	1	2	3	1	2	3		
52	1217833	1245833	1289500	0.58	0.60	0.63	70.85	70.01	68.70	69.85	1.08
53	1183167	1367833	1356167	0.56	0.67	0.67	71.89	66.35	66.70	68.31	3.10
54	1551167	1264167	1381167	0.78	0.61	0.68	60.85	69.46	65.95	65.42	4.33

Table 13 Absorbance of amount of chitosan reacted with 50 ug/ml congo red determined by UV spectrophotometer at 575 nm.

level	chitosan concentration (ug/ml)	absorbance			mean	SD
		1	2	3		
1	10	0.18	0.16	0.15	0.16	0.02
2	20	0.32	0.38	0.35	0.35	0.03
3	30	0.52	0.52	0.5	0.51	0.01
4	40	0.66	0.68	0.66	0.67	0.01
5	50	0.84	0.82	0.85	0.84	0.02

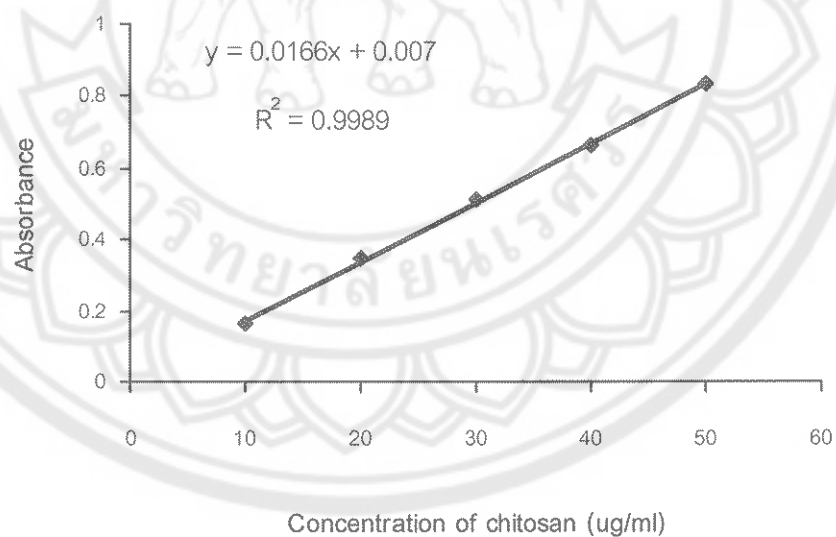


Figure 42 The calibration curve of amount of chitosan reacted with 50 ug/ml congo red determined by UV spectrophotometer at 575 nm

Table 14 Mean amount of chitosan reacted with liposome calculated from chitosan concentration in supernatant of chitosan coated liposomes suspension after centrifugation. The value was calculated from the calibration curve in figure 40

Batch	Absorbance			Chitosan concentration in supernatant (ug/ml)			Chitosan added (%)	Amount of chitosan added	% Chitosan reacted			Mean	SD
	1	2	3	1	2	3			1	2	3		
28	0.73	0.80	0.81	43.76	47.59	48.36	0.10	1 ml	56.24	52.41	51.64	53.43	2.46
29	0.80	0.81	0.77	47.77	48.35	46.26	0.10		52.23	51.65	53.74	52.54	1.08
30	0.75	0.80	0.81	45.05	47.59	48.36	0.10		54.95	52.41	51.64	53.00	1.73
31	0.84	1.12	1.02	50.25	67.30	61.25	0.50		89.95	86.54	87.75	88.08	1.73
32	0.82	0.69	0.62	48.70	41.25	36.80	0.50		90.26	91.75	92.64	91.55	1.20
33	0.19	0.41	0.64	11.20	24.40	38.25	0.50		97.76	95.12	92.35	95.08	2.71
34	0.30	0.58	0.78	17.70	34.60	46.40	1.00		98.23	96.54	95.36	96.71	1.44
35	0.27	0.27	1.13	15.90	15.90	67.50	1.00		98.41	98.41	93.25	96.69	2.98
36	0.40	1.24	0.54	23.80	74.30	32.40	1.00		97.62	92.57	96.76	95.65	2.70

Table 14 (cont.)

Batch	Absorbance			Chitosan concentration in supernatant (ug/ml)			Chitosan added (%)	Amount of chitosan added	% Chitosan reacted			Mean	SD
	1	2	3	1	2	3			1	2	3		
37	0.50	0.50	0.51	29.77	29.46	30.44	0.10	2 ml	70.23	70.54	69.56	70.11	0.50
38	0.48	0.50	0.53	28.35	29.57	31.58	0.10		71.65	70.43	68.42	70.17	1.63
39	0.62	0.61	0.60	36.79	36.16	35.48	0.10		63.21	63.84	64.52	63.86	0.66
40	0.30	0.30	0.14	17.40	17.40	7.95	0.50		96.52	97.75	98.41	97.56	0.96
41	0.39	0.39	0.25	23.20	23.20	14.90	0.50		95.36	95.34	97.02	95.91	0.96
42	0.28	0.28	0.28	16.70	16.70	16.20	0.50		96.66	93.25	96.76	95.56	2.00
43	0.30	0.25	0.35	17.70	14.60	20.50	1.00		98.23	98.54	97.95	98.24	0.30
44	0.40	0.22	0.62	23.60	12.90	36.80	1.00		97.64	98.71	96.32	97.56	1.20
45	0.30	0.32	0.61	17.50	18.80	36.50	1.00		98.25	98.12	96.35	97.57	1.06

Table 14 (cont.)

Batch	Absorbance			Chitosan concentration in supernatant (ug/ml)			Chitosan added (%)	Amount of chitosan added	% Chitosan reacted			Mean	SD
	1	2	3	1	2	3			1	2	3		
46	0.41	0.41	0.50	24.36	24.36	29.55	0.10		75.64	71.56	70.45	72.55	2.73
47	0.51	0.51	0.45	30.02	30.02	26.54	0.10		69.98	71.25	73.46	71.56	1.76
48	0.47	0.47	0.47	27.77	27.77	27.84	0.10		72.23	72.45	72.16	72.28	0.15
49	1.13	0.24	0.16	67.50	13.85	9.40	0.50		98.65	97.23	98.12	98.00	0.72
50	0.29	0.24	0.30	17.30	13.78	17.95	0.50	3 ml	96.54	97.25	96.41	96.73	0.45
51	0.38	0.38	0.24	22.20	22.20	13.75	0.50		95.56	95.56	97.25	96.12	0.98
52	0.30	0.42	0.30	17.50	24.60	17.50	1.00		98.25	97.54	98.25	98.01	0.41
53	0.26	0.30	0.42	15.40	17.50	24.60	1.00		98.46	98.25	97.54	98.08	0.48
54	0.34	0.26	0.21	19.80	15.40	12.50	1.00		98.02	98.46	98.75	98.41	0.37

Table 15 Cumulative amount of tartaric acid in liposomes formulation with initial tartaric acid content 1.37 mg/ml released from dialysis membrane detected by HPLC at 215 nm.

Time (hr)	Peak area			Tartaric acid content (mg/ml)			%Tartaric acid release			Mean	SD
	1	2	3	1	2	3	1	2	3		
0.5	602465	588122	592675	0.2138	0.2052	0.2079	15.65	15.02	15.22	15.30	0.32
1	798486	786875	755002	0.3314	0.3244	0.3053	24.26	23.75	22.35	23.45	0.99
2	1116764	1113804	1111755	0.5224	0.5206	0.5194	38.24	38.11	38.02	38.12	0.11
3	1428895	1416601	1399071	0.7096	0.7023	0.6917	51.95	51.41	50.64	51.33	0.66
4	1640397	1625599	1628786	0.8365	0.8277	0.8296	61.24	60.59	60.73	60.85	0.34
5	1684565	1668400	1672271	0.8630	0.8533	0.8557	63.18	62.47	62.64	62.76	0.37
6	1751954	1728732	1734196	0.9035	0.8895	0.8928	66.14	65.12	65.36	65.54	0.53
12	1864421	1861234	1859413	0.9710	0.9690	0.9679	71.08	70.94	70.86	70.96	0.11

Table 16 Cumulative amount of tartaric acid in 0.1% chitosan coated liposomes formulation with initial tartaric acid content 1.37 mg/ml release from dialysis membrane detected by HPLC at 215 nm.

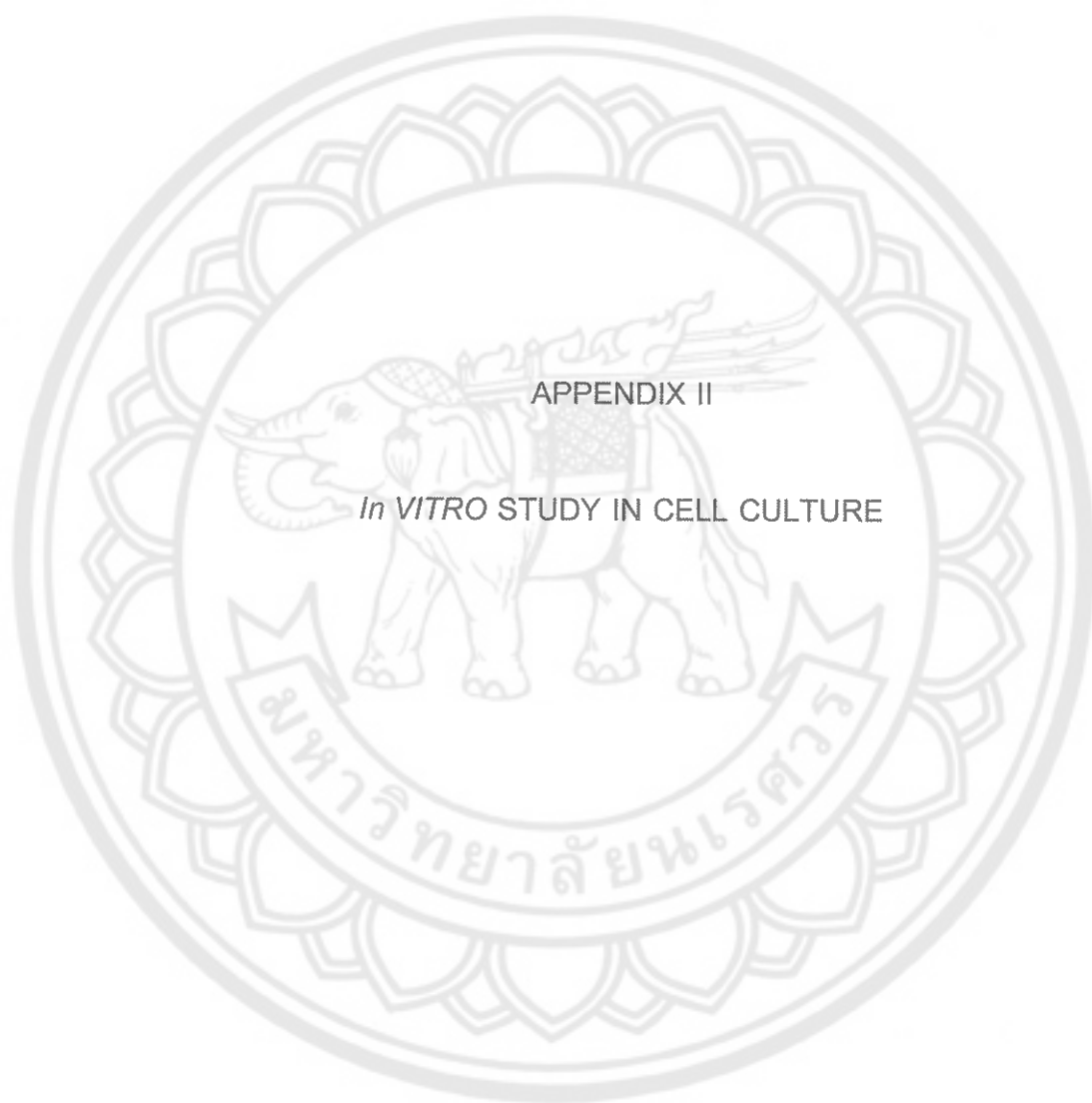
Time (hr)	Peak area			Tartaric acid content (mg/ml)			%Tartaric acid release			Mean	SD
	1	2	3	1	2	3	1	2	3		
0.5	478644	487543	477087	0.1395	0.1448	0.1386	10.45	10.85	10.38	10.56	0.25
1	581201	575195	572080	0.2010	0.1974	0.1955	15.06	14.79	14.65	14.83	0.21
2	824802	851943	840375	0.3472	0.3635	0.3565	26.01	27.23	26.71	26.65	0.61
3	974745	960730	967404	0.4371	0.4287	0.4327	32.75	32.12	32.42	32.43	0.32
4	1258168	1239703	1251494	0.6072	0.5961	0.6032	45.49	44.66	45.19	45.11	0.42
5	1372293	1364729	1356498	0.6757	0.6711	0.6662	50.62	50.28	49.91	50.27	0.36
6	1512447	1508220	1507330	0.7598	0.7572	0.7567	56.92	56.73	56.69	56.78	0.12
12	1630799	1613224	1608997	0.8308	0.8202	0.8177	62.24	61.45	61.26	61.65	0.52

Table 17 Cumulative amount of tartaric acid in 0.5% chitosan coated liposomes formulation with initial tartaric acid content 1.37 mg/ml release from dialysis membrane detected by HPLC at 215 nm.

Time (hr)	Peak area			Tartaric acid content (mg/ml)			%Tartaric acid release			Mean	SD
	1	2	3	1	2	3	1	2	3		
0.5	460402	450391	450391	0.1285	0.1225	0.1225	9.63	10.15	9.18	9.65	0.49
1	556508	549611	550946	0.1862	0.1821	0.1829	13.95	13.64	13.70	13.76	0.16
2	682424	675305	671078	0.2618	0.2575	0.2549	19.61	19.29	19.10	19.33	0.26
3	827695	807005	811899	0.3489	0.3365	0.3394	26.14	25.21	25.43	25.59	0.49
4	1117124	1107558	1099549	0.5226	0.5168	0.5120	39.15	38.72	38.36	38.74	0.40
5	1266176	1249491	1247712	0.6120	0.6020	0.6009	45.85	45.10	45.02	45.32	0.46
6	1335586	1326910	1329579	0.6537	0.6484	0.6500	48.97	48.58	48.70	48.75	0.20
12	1426575	1406330	1406108	0.7082	0.6961	0.6960	53.06	52.15	52.14	52.45	0.53

Table 18 Cumulative amount of tartaric acid in 0.5% chitosan coated liposomes formulation with initial tartaric acid content 1.37 mg/ml release from dialysis membrane detected by HPLC at 215 nm.

Time (hr)	Peak area			Tartaric acid content (mg/ml)			%Tartaric acid release			Mean	SD
	1	2	3	1	2	3	1	2	3		
0.5	473528	471080	469968	0.1364	0.1349	0.1343	10.22	10.11	10.06	10.13	0.08
1	512014	504228	516019	0.1595	0.1548	0.1619	11.95	11.60	12.13	11.89	0.27
2	594772	589433	576752	0.2092	0.2060	0.1984	15.67	15.43	14.86	15.32	0.42
3	720466	709879	699776	0.2846	0.2782	0.2722	21.32	20.64	20.39	20.78	0.48
4	968071	955613	948049	0.4331	0.4257	0.4211	32.45	31.89	31.55	31.96	0.45
5	1135588	1118903	1116901	0.5337	0.5236	0.5224	39.98	39.23	39.14	39.45	0.46
6	1205443	1193207	1187423	0.5756	0.5682	0.5648	43.12	42.57	42.31	42.67	0.41
12	1266621	1249936	1247489	0.6123	0.6023	0.6008	45.87	45.12	45.01	45.33	0.47



APPENDIX II

In VITRO STUDY IN CELL CULTURE

มหาวิทยาลัยพระนคร

Table 19 Absorbance of XTT assay of tamarind crude extract at different tartaric acid concentration influenced on keratinocyte proliferation at 490 nm.

Tartaric acid concentration (ug/ml)	absorbance					%proliferation
	1	2	3	mean	SD	
control	0.941	0.836	0.918	0.8983	0.06	100
50	0.977	0.979	0.936	0.9640	0.02	107.35
100	0.759	1.223	0.849	0.9437	0.25	105.12
200	0.732	0.692	0.793	0.7390	0.05	82.29
300	0.685	0.634	0.934	0.7510	0.16	83.63
400	0.696	0.843	0.682	0.7403	0.09	82.41
500	0.731	0.87	0.661	0.7540	0.11	83.96
1000	0.447	0.564	0.472	0.4943	0.06	54.56

Table 20 Absorbance of XTT assay of various formulation influenced on keratinocyte proliferation at 490 nm.

sample	absorbance					%proliferation
	1	2	3	mean	SD	
control	0.95	0.99	0.99	0.98	0.02	100
250 ug tartaric acid in liposomes	1.69	1.49	1.5	1.56	0.11	159
500 ug tartaric acid in liposomes	1.67	1.64	1.61	1.64	0.03	167
1000 ug tartaric acid in liposomes	2.17	1.88	1.89	1.98	0.16	202
500 ug tartaric acid in chitosan coated liposomes	1.48	1.49	1.52	1.50	0.02	153
1000 ug tartaric acid in chitosan coated liposomes	1.53	1.5	1.6	1.54	0.05	157
10%fetal bovine serum	1.002	0.831	0.833	0.89	0.10	138
Unloaded liposomes	0.856	0.671	0.911	0.81	0.13	126
Unloaded chitosan coated liposomes	0.663	0.841	0.733	0.75	0.09	115

Table 21 Cell number of MML-1 human melanocyte per time (day) determined by trypan blue method.

Day	cell number ($\times 10^5$)			mean	SD
	1	2	3		
0	1	1	1	1	0
1	5.1	4.8	4.6	4.83	0.25
2	6.7	6.5	6.9	6.70	0.20
3	14.5	13.9	15.1	14.50	0.60
4	13.5	14.1	13.2	13.60	0.46
5	13.6	13.2	13.8	13.53	0.31
6	12.9	13.4	12.5	12.93	0.45
7	12.1	12.6	11.5	12.07	0.55

Table 22 Absorbance determination of melanin content of MML-1 human melanocyte after treated with various formulations at 490 nm.

sample	absorbance at 490 nm					%melanin content
	1	2	3	mean	SD	
Control	0.068	0.063	0.062	0.064	0.003	100
500 ug tartaric acid	0.064	0.082	0.071	0.072	0.009	112.44
1000 ug tartaric acid	0.064	0.075	0.053	0.064	0.011	99.48
250 ug tartaric acid	0.079	0.086	0.064	0.076	0.011	118.65
500 ug tartaric acid in liposomes	0.082	0.061	0.076	0.073	0.011	113.47
1000 ug tartaric acid in liposomes	0.07	0.064	0.068	0.067	0.003	104.66
500 ug tartaric acid in chitosan/liposomes	0.065	0.068	0.056	0.063	0.006	97.93
1000 ug tartaric acid in chitosan/liposomes	0.062	0.074	0.059	0.065	0.008	101.04
Kojic acid 50 ug/ml	0.068	0.074	0.059	0.067	0.008	104.15
Kojic acid 500ug/ml	0.056	0.084	0.065	0.068	0.014	106.22