REFERENCES

Aderem, A. and Underhill, D.M. 1999. Mechanisms of phagocytosis in macrophages. Annual Reviews Immunology 17:593-623.

Akifumi, M., Hideki, M., Yasuyoshi, T., Kiyotaka, H., and Norihiro, T. 1998. Ascorbate-dependent enhancement of nitric oxide formation in activated macrophages. Nitric Oxide: Biology and Chemistry 2:235-241.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. 2002. Molecular biology of the cell. 4 th edition. New York.

Alipour, M., Omri, A. Smith, M., and Suntres, Z.E. 2007. Prophylactic effect of liposomal N-acetylcysteine against LPS-induced liver injuries. Journal of Endotoxin Research 13:297-304.

Allen, T.M, Austin, G.A., Chonn, A., Lin, L. and Lee, K.C. 1991. Uptake of liposomes by cultured mouse bone marrow macrophage: influence of liposome composition and size. Biochimica et Biophysica Acta 1061:56-64.

Aramaki, Y., Matsuno, R., and Tsuchiya, S. 2001. Involvement of P38 MAP kinase in the inhibitory effects of phosphatidylserine liposomes on nitric oxide production from macrophages stimulated with LPS. Biochemical and Biophysical Research Communications 280:982-987.

Aramaki, Y., Nitta, F., Matsuno, R., Morimura, Y., and Tsuchiya, S. 1996. Inhibitory effects of negatively charged liposomes on nitric oxide production from macrophages stimulated by LPS. Biochemical and Biophysical Research Communications 220:1-6.

Aronis, A., Madar, Z., and Tirosh, O. 2005. Mechanism underlying oxidative stress-mediated lipotoxicity: Exposure of J774.2 macrophages to triacylglycerols facilitates mitochondrial reactive oxygen species production and cellular necrosis. Free Radical Biology & Medicine 38:1221-1230.

Ayache, N., Boumediene, K., Mathy-Hartert, M., Reginstert, J.Y., Henrotin, Y, and Pujol, J.P. 2002. Expression of TGF-βs and their receptors is differentially modulated by reactive oxygen species and nitric oxide in human articular chondrocytes. Journal of the OsteoArthritis Reseach Society International 10:344-352.

Bogdan, C. 2001. Nitric oxide and the immune response. Nature Immunology 2:907-916.

Brosnan, C.F., Bornstein, M.B., and Bloom, B.R. 1981. The effects of macrophage depletion on the clinical and pathologic expression of experimental allergic encephalomyelitis. Journal of Immunology 126:614-620.

Cadenas, S., and Cadenas, A.M. 2002. Fighting the stranger-antioxidant protection against endotoxin toxicity. Toxicology 180:45-63.

Chellat, F., Merhi, Y., Moreau, A., and Yahia, L. 2005. Therapeutic potential of nanoparticulate systems for macrophage targeting. Biomaterials 26:7260-7275.

Coleman, J.W. 2001. Nitric oxide in immunity and inflammation. International Immunopharmacology 1:1397-1406.

Dedon, P.C., and Tannenbaum, S.R. 2004. Reactive nitrogen species in the chemical biology of inflammation. Archives of Biochemistry and Biophysics 423:12-22.

Fan, J., Shek, P.N., Suntres, Z.E., Li, Y.H., Oreopoulos, G.D., and Roststein, O.D. 2000. Liposomal antioxidants provide prolonged protection against acute respiration distress syndrome. Surgery 128:332-338.

Foong, W.C., and Green, K.L. 1988. Retention and distribution of liposome-entrapped [3H]methotrexate injected into normal or arthritis rabbit joints. Journal of Pharmacy and Pharmacology 40:464-468.

Forman, H.J., and Torres, M. 2001. Redox signaling in macrophages. Molecular Aspects of Medicine 22:189-216.

Freshney, R.L. (2000). Culture of animal cells: a manual of basic technique. 4 th edition. USA: Wiley-Liss.

Garcia, X., and Stein, F. 2006. Nitric oxide. Pediatric Infectious Diseases 17:55-57.

Gerwin, N., Hops, C., and Lucke, A. 2006. Intraarticular drug delivery in osteoarthritis. Advanced Drug Delivery Reviews 58:226-242.

Goldsby, R.A., Kindt, T.J., Osborne, B.A., and Kuby, J. (2003). Immunology. 5 th edition. New York: W.H Freeman and Company.

Gulati, M., Grover, M., Singh, S., and Singh, M. 1998. Lipophilic drug delivatives in liposomes. International Journal of Pharmaceutics 165:129-168.

Hassoun, E.A., Bagchi, D., Bagchi, M., and Stohs, S.J. 1995. Effect of vitamin E succinate on smokeless tobacco induced production of nitric oxide by rat peritoneal macrophages and J774.1 macrophage cells in culture. Free Radical Biology & Medicine 18:577-583.

Hinz, B., Brune, K., and Pahl, A. 2000. Nitric oxide inhibits inducible nitric oxide synthase mRNA expression in RAW 264.7 macrophages. Biochemical and Biophysical Research Communications 271:353-357.

Hunt, C.A. 1981. Liposome disposition in vivo. V. Liposome stability in plasma and implications for drug carrier function. Biochimica et Biophysica Acta 719:450-463.

Iuvone, T., D'Acquisto, F., Carnuccio, R., and Di Rosa, M. 1996. Nitric oxide inhibits LPS-induced tumor necrosis factor synthesis in vitro and in vivo. Pharmacology Letters 59:207-211.

Janeway, C.A. 1992. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunology Today 13:11-16.

Jara, L.J., Medina, G., Vera-Lastra, O., and Amigo, M. 2006. Accelerated atherosclerosis, immune response and autoimmune rheumatic diseases. Autoimmunity Reviews 5:195-201.

Kiefer, R., Kieseier, B.C., Stoll, G., and Hartung, H. 2001. The role of macrophages in immune-mediated damage to the peripheral nervous system. Progress in Neurobiology 64:109-127.

Lee, K., Hong, K., and Papahadjopoulos, D. 1992. Recognition of liposomes by cells: in vitro binding and endocytosis mediated by specific lipid headgrooups and surface charge density. Biochimica et Biophysica Acta 1103:185-197.

Liebler, D., Stratton, S., and Kaysem, K. 1997. Antioxidant actions of β-carotene in liposomal and microsomal membrane: role of carotenoid-membrane incorporation and α-tocopherol. Archives of Biochemistry and Biophysics 338:244-250.

Liu, D. 1997. Biological factors involved in blood clearance of liposomes by liver. Advanced Drug Delivery Reviews 24:201-213.

Makino, K., Tabata, J., Yoshioka, T., Fukuda, M., Ikekita, M., Ohshima, H., and Terada, H. (2003). Effects of liposomal phophatidylserine on phagocytic uptake of liposomes by macrophage-like HL-60RG cells. Colloids and Surfaces B: Biointerfaces 29:277-284.

Matsuno, R., Aramaki, Y., and Tsuchiya, S. 2001. Involvement of TGF-β in inhibitory effects of negatively charged liposomes on nitric oxide production by

macrophages stimulated with LPS. Biochemical and Biophysical Research Communications 281:614-620.

Mendez, C., Garcia, I., and Maier, R.V. 1995. Antioxidants attenuate endotoxin-induced activation of alveolar macrophages. Surgery 118:412-420.

Minko, T., Stefanov, A. Pozharov, V. 2002. Selected contribution lung hypoxia: antioxidant and antiapoptotic effects of liposomal α-tocopherol. Journal of Applied Physiology 93:1550-1560.

Napolitano, D.R., Mineo, J.R., de Souza, M.A., de Paula, J.E., Espindola, L.S., Espindola, F.S. 2005. Down-modulation of nitric oxide production in murine macrophages treated with crude plant extracts from the *Brazitian Cerrado*. Journal of Ethnopharmacology 99:37-41.

New, R.R.C. (1997). Liposomes: A practical approach. New York: Oxford University Press.

Oussoren, C. and Storm, G. 2001. Liposome to target the lymphatics by subcutaneous administration. Advanced Drug Delivery Reviews 50:143-156.

Pahan, K., Sheikh, F.G., Mamboodiri, A.M.S., and Singh, I. 1998. N-acetylcysteine inhibits induction of NO production by endotoxin or cytokine stimulated rat peritoneal macrophages, C_6 glial cells and astrocytes. Free Radical Biology & Medicine 24:39-48.

Paulos, C.M., Turk, M.J., Breur, G.J., and Low, P.S. 2004. Folate receptor-mediated targeting of therapeutic and imaging agent to activated macrophages in rheumatoid arthritis. Advanced Drug Delivery Reviews 56:1205-1217.

Ratnam, D.V., Ankola, D.D., Bhardwaj, V., Sahana, D.K., and Ravi Kumar, M.N.V. 2006. Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. Journal of Controlled Release 113:189-207.

Roerdink, F.N., Regts, J., Handel, T., Sullivan, S.M., Baldeschwieler, J.D. and Scherphof, G.L. 1989. Effect of cholesterol on the uptake and intracellular degradation of liposomes by liver and spleen; a combined biochemical and γ-ray perturbed angular correlation study, Biochimica et Biophysica Acta 980:234-240.

Rooijen, N.V. and Sanders, A. 1998. The macrophage as target or obstacle in liposome-based targeting strategies. Internation Journal of Pharmaceutics 162:45-50.

Ruben, Z., Katelijne, E., Arnold, G. 1997. The protective role of thiols against nitric oxide-mediated cytotoxicity in murine macrophage J774 cells. European Journal of Pharmacology 321: 87-96.

Schroit, A.J., Madsen, J., and Nayar, R. 1986. Liposome-cell interactions: in vitro discrimation of uptake mechanism and in vivo targeting strategies to mononuclear phagocytes. Chemistry and Physics of Lipids 40: 373-393.

Sinha, J., Das, N., and Basu, M.K. 2001. Liposome antioxidants in combating ischemia-reperfusion injury in rat brain. Biomedicine & Pharmacotherapy 55:264-271.

Stevenson, M., Baillie, A.J., and Richards, R.M.E. 1984. Quantification of uptake of liposomal carboxy fluorescein by professional phagocytes in-vitro. A flow microfluorimetric study on the J774 murine macrophage cell line. Journal of Pharmaceutical and Pharmacology 36:824-830.

Stone, W., and Smith, M. 2004. Therapeutic uses of antioxidant liposomes. Molecular Biotechnology 27:217-230.

Stuart, L.M., and Ezekowitz, R.A.B. 2005. Phagocytosis: Elegant Complexity. Immunity 22:539-550.

Suntres, Z.E., and Shek, P.N. 1994. Incorporation of α-tocopherol in liposomes promotes the retension of liposome-encapsulated Glutathione in the rat lung. Journal of Pharmaceutical and Pharmacology 46:23-28.

Traber, M.G., and Packer, L. 1995. Vitamin E: beyond antioxidant function. American Journal of Clinical Nutrition 62:1501s-1509s.

Vandana, S., Ram, S., Ilavazhagan, M., Kumar, G.D. and Banerjee, P.K. 2006. Comparative cytoprotective activity of vitamin C, E and beta-carotene against chromium induced oxidative stress in murine macrophages. Biomedicine & Pharmacotherapy 60:71-76.

Victor, V.M., Rocha, M., and De la Fuente, M. 2003. Regulation of macrophage function by the antioxidant *N*-acetylcysteine in mouse-oxidative stress by endotoxin. International Immunopharmacology 3:97-106.

Victor, V.M., Rocha, M., and De la Fuente, M. 2004. Immune cells: free radicals and antioxidants in sepsis. International Immunopharmacology 4:327-347.

Wilson, K.D., Raney, S.G., Sekirov, L., Chikh, G., deJong, S.D., Cullis, P.R., Tam, Y.K. 2007. Effects of intravenous and subcutaneous administration on the

pharmacokinetics, biodistribution, cellular uptake and immunostimulatory activity of CpG ODN encapsulated in liposomal nanoparticles. International Immunopharmacology 7:1064-1075.

Xiong, Y.Q., Mukhopadhyay, K., Yeaman, M.R., Adler-Moore, J., and Bayer, A.S. 2005. Functional interrelationships between cell membrane and cell wall in antimicrobial peptide-mediated killing of *Staphylococus aureus*. Antimicrobial Agents and Chemotherapy 49:3114-3121.

Yao, T., Degli, E.S., Huang, L. 1994. Inhibition of carbon tetrachloride-induced liver injury by liposomes containing vitamin E. American Journal of Physiology 267:C476-484.

Appendices

Appendix A: The representation of nitrite and calcein calibration lines

Appendix B: Quantitative determination of α-tocopherol (TOC) in liposomes by UV-spectrophotometric method

Quantitative determination of *N*-acetylcysteine (NAC) in liposomes by HPLC method

A representation of calcein calibration line by spectrofluorometric method

A representation of phospholipids calibration line by the Bartlett's assay

Appendix C: Results of statistical analysis

Appendix D: Results of cell viability tests

Appendix A

The representation of nitrite and calcein calibration line

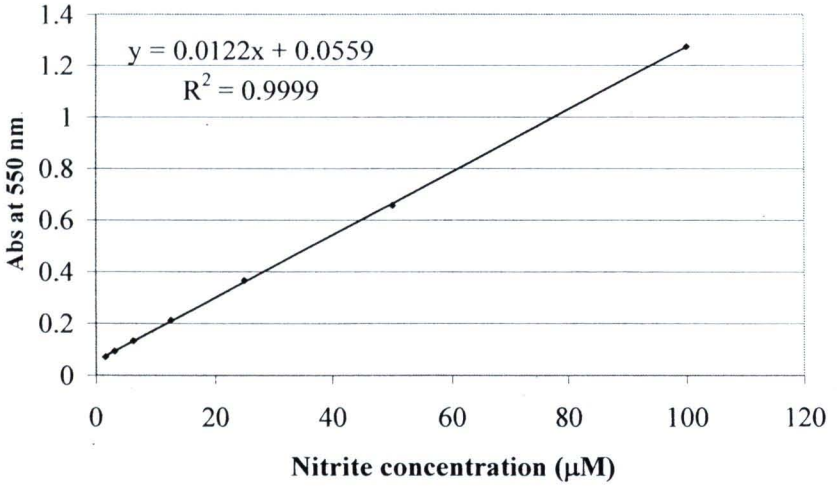


Figure A-1: A representation of nitrite calibration line by Griess reaction

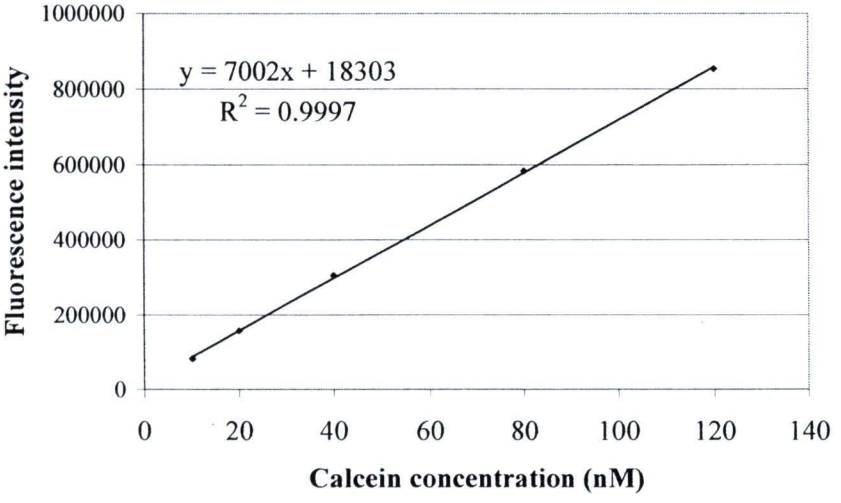


Figure A-2: A representation of calcein calibration line in presence of cell suspension were dissolved with 1% Triton®-X 100 by spectrofluorometric method

Appendix B

I Quantitative determination of α-tocopherol (TOC) in liposomes by UV-spectrophotometric method

1. Specificity

Since TOC gave significant signals at 292 nm, soybean phosphatidylcholine (PC) was spiked into the standard solutions of TOC at an amount equal to that determined from the Barlett's assay for each liposome preparation. Standard calibration lines were constructed from the absorbance and the nominal concentration of the standard solutions accordingly. The data are shown in Figures B-1 to B-3.

2. Linearity

Five standard solutions of TOC ranging from 20 to 100 μg/ml (0.046-0.232 mM) in chloroform:methanol (1:4) were prepared in the presence of 1 mg/ml PC. The absorbances at these solutions were plotted against the nominal concentration. Linear regression analysis of absorbance versus concentration was performed. The linearity was determined from the coefficient of determination. The result is shown in Table B-1 and Figure B-4.

3. Accuracy

TOC at 25, 50 and 75 μg/ml (0.058, 0.116 and 0.174 mM) and liposome suspension (1 mg/ml PC) were dissolved in chloroform:methanol (1:4). Three sets of each concentration were prepared. Each individual sample was analyzed by UV-spectrophotometric method at 292 nm, and analytical recovery of each sample was calculated in percent. The data are shown in Table B-2.

4. Precision

The precision was evaluated by analyzing three sets of three standard solutions (25, 50 and 75 μg/ml) of TOC. The mean, standard deviation (S.D.) and the coefficient of variation (%C.V.) of each standard solution were determined. The data are shown in Table B-3

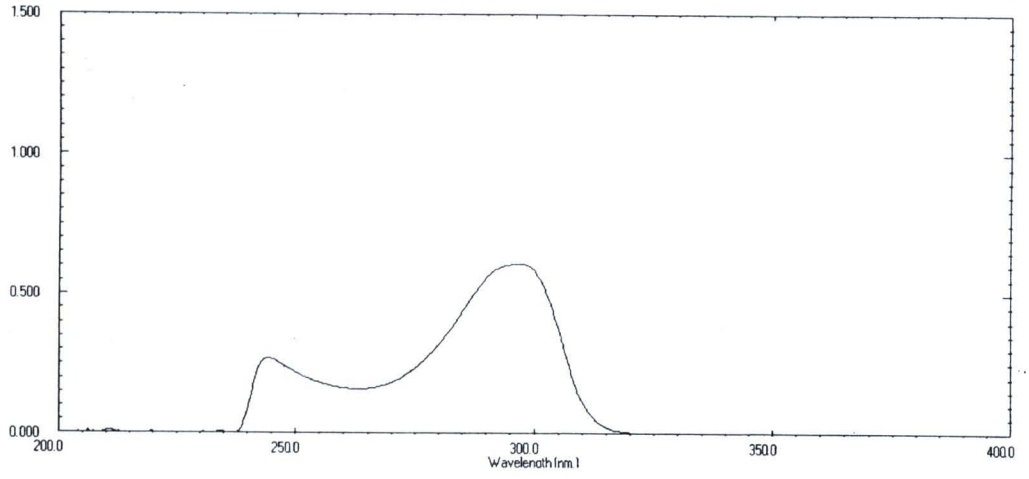


Figure B-1 The spectrum of TOC at 80 μg/ml in chloroform:methanol (1:4)

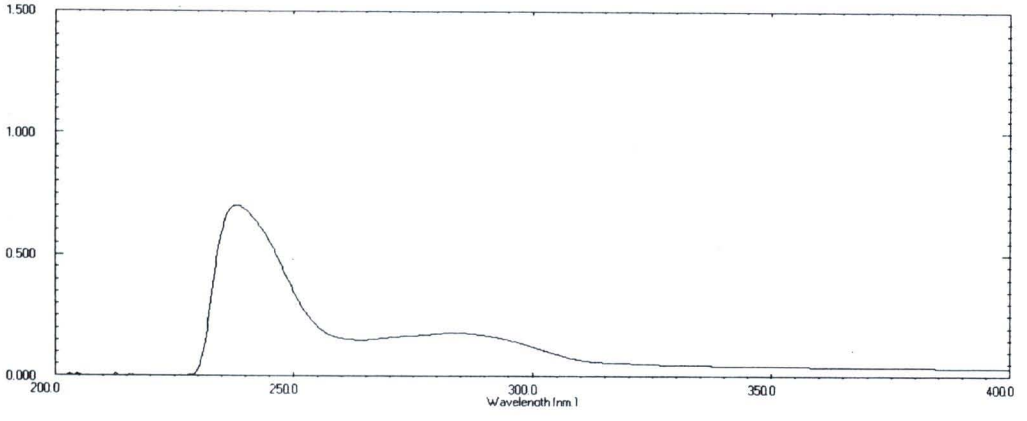


Figure B-2 The spectrum of blank liposomes 1 mg/ml in chloroform:methanol (1:4)

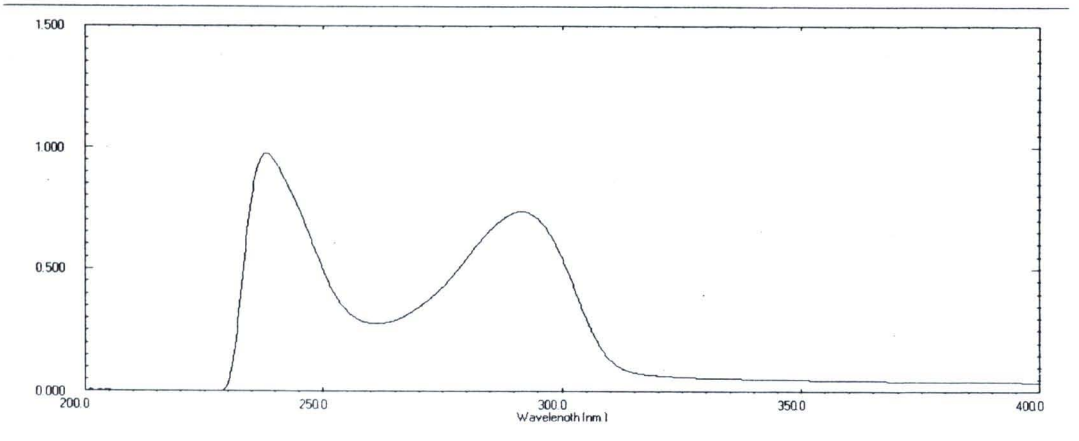


Figure B-3 The spectrum of TOC at 80 μg/ml and blank liposomes 1 mg/ml in chloroform:methanol (1:4)

Table B-1: Data for linearity of TOC standard calibration lines

Concentration (μg/ml)	Absorbance at 292 nm.			Mean	S.D.	%C.V.
	Set 1	Set 2	Set 3			
20	0.1689	0.1661	0.1682	0.1677	0.0015	0.869
40	0.3177	0.3146	0.3178	0.3167	0.0018	0.574
60	0.4716	0.4694	0.4689	0.4700	0.0014	0.306
80	0.6245	0.6239	0.6243	0.6242	0.0003	0.049
100	0.7733	0.7766	0.777	0.7756	0.0020	0.262
R^2	1	1	1	1	-	-

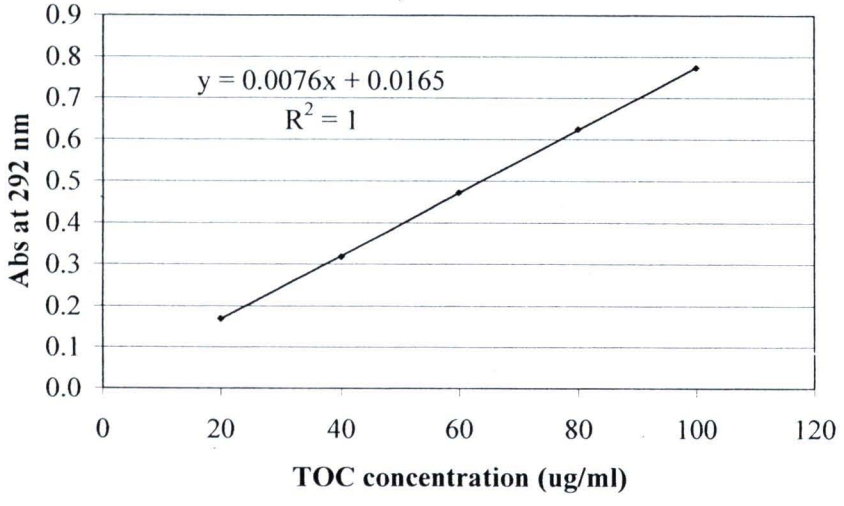


Figure B-4: A representation of TOC calibration line in the presence of 1 mg/ml PC by UV-spectrophotometric method

Table B-2: Analytical recovery of TOC by UV-spectrophotometric method

Nominal concentration of TOC (µg/ml)	Analytical recovery (%)									Mean ± S.D.
	Set 1			Set 2			Set 3			
25	98.00	98.79	98.89	101.14	101.14	99.43	101.53	101.79	101.47	100.24 ± 1.45
50	99.03	100.76	99.63	98.38	98.31	100.26	101.03	102.87	102.53	100.31 ± 1.66
75	99.74	100.79	101.28	99.38	101.71	100.29	100.67	100.7	101.74	100.70 ± 0.81

Table B-3: The precision of TOC analysis by UV-spectrophotometric method

Actual concentration of TOC (µg/ml)	Calculated concentration of TOC (µg/ml)									mean	S.D.	%C.V.
	Set 1			Set 2			Set 3					
25	24.5	24.7	24.72	25.29	25.29	24.86	25.38	25.45	25.37	25.06	0.363	1.45
50	49.51	50.38	49.82	49.19	49.16	50.13	50.51	51.43	51.26	50.15	0.827	1.65
75	74.8	75.59	75.96	74.53	76.28	75.22	75.50	75.53	76.30	75.52	0.610	0.81

II Quantitative determination of *N*-acetylcysteine (NAC) in liposomes by HPLC method

1. Specificity

To show that the assay method had specificity for NAC, all constituents of liposomes (PC, dicetylphosphate and cholesterol) were mixed with NAC solution and subjected to HPLC analysis. The chromatograms are shown in Figure B-5 to B-7.

2. Linearity

Six standard solutions of NAC ranging from 0.5 to 18 μg/ml (0.003 to 0.110 mM) in 1% Triton®-X 100 were prepared and subjected to HPLC assay. Linear regression analysis of absorbance versus concentration was performed. The linearity was determined from the coefficient of determination. The result is shown in Figure B-8.

3. Accuracy

NAC at 4.5, 9 and 13.5 μg/ml (0.028, 0.055 and 0.083 mM) and liposome suspension (1 mg/ml total lipid) were dissolved in 1% Triton®-X 100. Three sets of each concentration were prepared. Each individual sample was analyzed by HPLC method, and analytical recovery of each sample was calculated in percent. Data are shown in Table B-4.

4. Precision

The precision was evaluated by analyzing three sets of three standard solutions (4.5, 9 and 13.5 μg/ml) of NAC. The mean, standard deviation (S.D.) and the coefficient of variation (%C.V.) of each standard solution were determined. The data are shown in Table B-5.

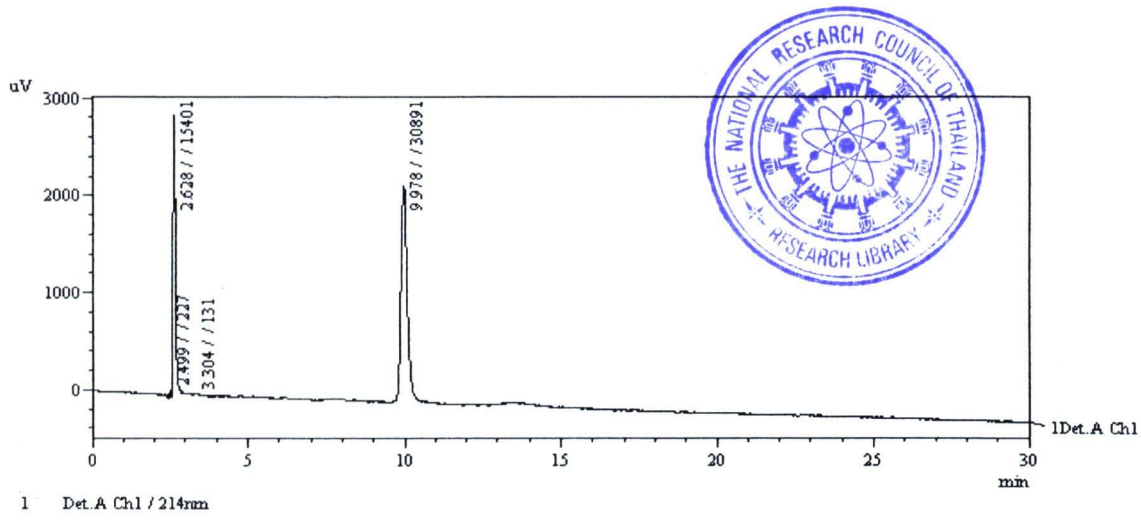


Figure B-5 HPLC chromatogram of NAC solution at 5 μg/ml

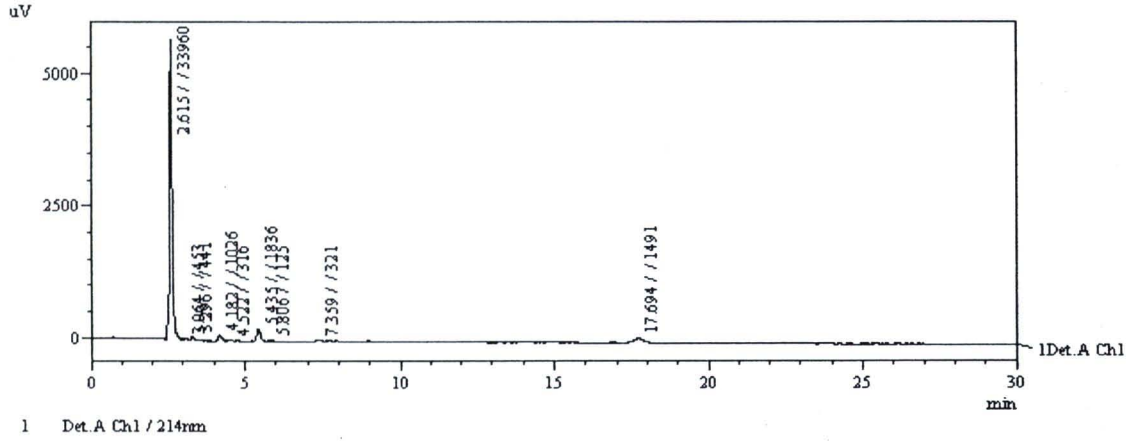


Figure B-6 HPLC chromatogram of blank liposomes 1 mg/ml in 1% Triton-X®100

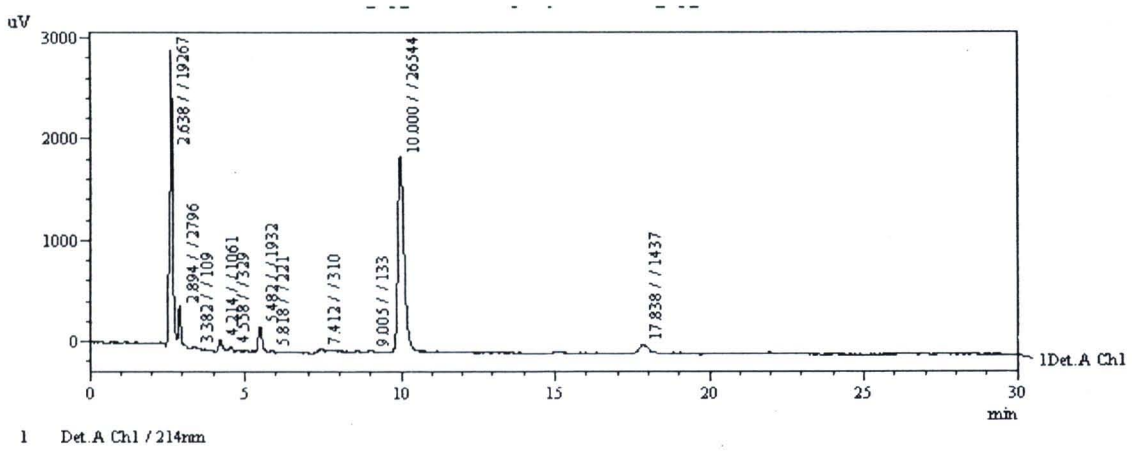


Figure B-7 HPLC chromatogram of NAC at 5 μg/ml and blank liposomes 1 mg/ml in 1% Triton-X®100

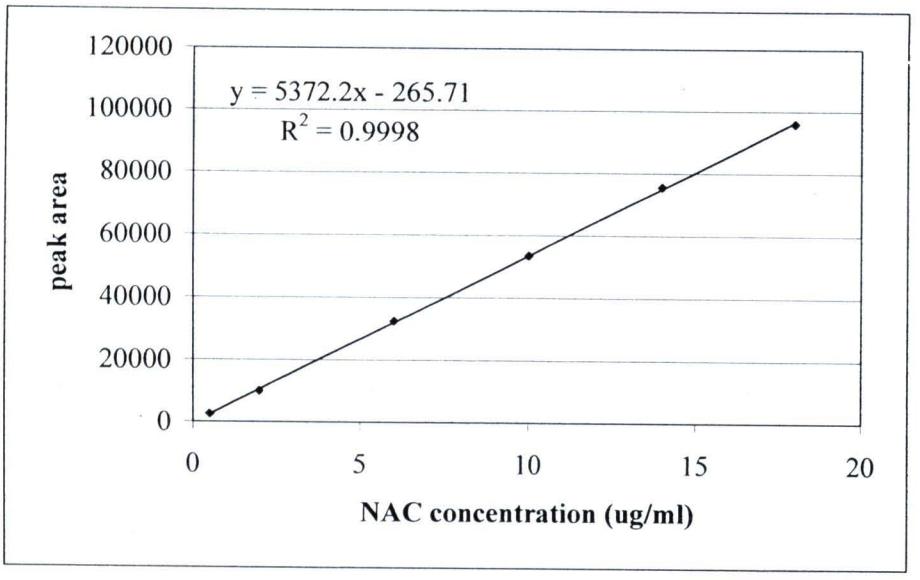


Figure B-8: A representation of NAC calibration line

Table B-4: Analytical recovery of NAC by HPLC method

Actual concentration of NAC (µg/ml)	% Analytical recovery			Mean ± S.D.
	1	2	3	
4.5	98.73	97.85	99.59	98.72 ± 0.867
9	102.23	103.61	100.16	102.0 ± 1.73
13.5	101.19	99.01	97.93	99.38 ± 1.658

Table B-5: The precision of NAC analysis by HPLC method

Concentration of NAC (µg/ml)	Calculated concentration of NAC (µg/ml)			mean	S.D.	%C.V.
	1	2	3			
4.5	4.44	4.40	4.48	4.44	0.039	0.879
9	9.20	9.32	9.01	9.18	0.156	1.698
13.5	13.66	13.37	13.22	13.42	0.224	1.669

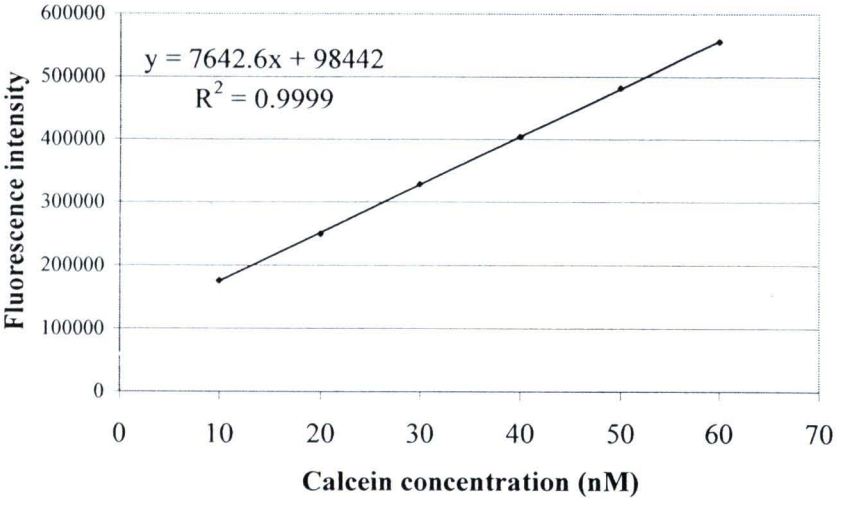


Figure B-9: A representation of calcein calibration line by spectrofluorometric method

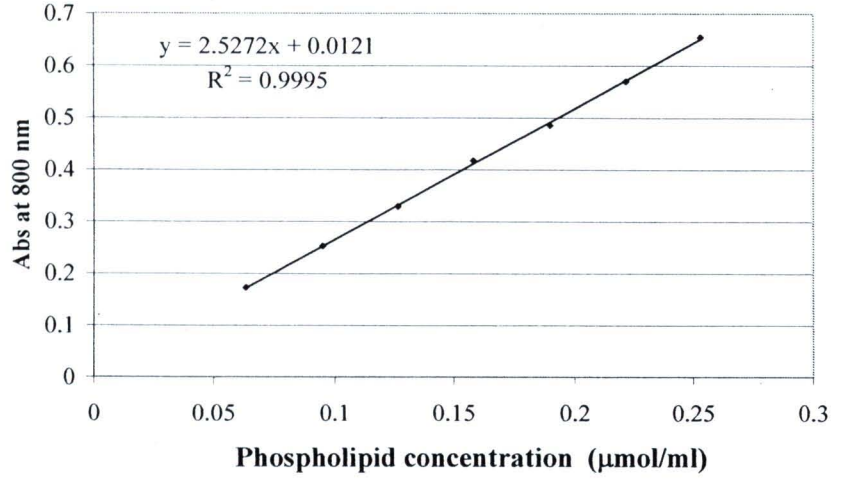


Figure B-10: A representation of phospholipid calibration line by Bartlett method

Appendix C

Appendix C-1 Statistical analysis of effect of TOC concentration on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition

Levene Statistic	df1	df2	P-value
6.813	3	32	.001

ANOVA

inhibition

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	52586.703	3	17528.901	314.874	.000
Within Groups	1781.426	32	55.670		
Total	54368.129	35			

Multiple Comparisons

Dependent Variable: inhibition

Dunnett T3

(I) TOC	(J) TOC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.25	6.83089	3.77468	.415	-5.1152	18.7770
	.50	-27.31646*	3.35482	.000	-37.7989	-16.8340
	1.00	-90.01509*	1.81048	.000	-95.4270	-84.6031
.25	.00	-6.83089	3.77468	.415	-18.7770	5.1152
	.50	-34.14735*	4.63295	.000	-47.9376	-20.3571
	1.00	-96.84597*	3.67249	.000	-108.6865	-85.0055
.50	.00	27.31646*	3.35482	.000	16.8340	37.7989
	.25	34.14735*	4.63295	.000	20.3571	47.9376
	1.00	-62.69862*	3.23941	.000	-73.0337	-52.3636
1.00	.00	90.01509*	1.81048	.000	84.6031	95.4270
	.25	96.84597*	3.67249	.000	85.0055	108.6865
	.50	62.69862*	3.23941	.000	52.3636	73.0337

*. The mean difference is significant at the .05 level.

Appendix C-2 Statistical analysis of effect of NAC concentration on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition2

Levene Statistic	df1	df2	P-value
5.355	4	40	.002

ANOVA

inhibition2

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	14404.327	4	3601.082	109.286	.000
Within Groups	1318.040	40	32.951		
Total	15722.367	44			

Multiple Comparisons

Dependent Variable: inhibition2

Dunnett T3

(I) nac1	(J) nac1	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
.0	.1	9.85542	2.81842	.057	-.2410	19.9519
	1.0	8.42745	2.68668	.095	-1.1773	18.0322
	10.0	-8.70125*	1.31090	.000	-13.1146	-4.2879
	20.0	-39.13124*	1.55312	.000	-44.4653	-33.7972
.1	.0	-9.85542	2.81842	.057	-19.9519	.2410
	1.0	-1.42797	3.80844	1.000	-13.6135	10.7576
	10.0	-18.55667*	3.00074	.001	-28.7916	-8.3217
	20.0	-48.98666*	3.11419	.000	-59.3925	-38.5808
1.0	.0	-8.42745	2.68668	.095	-18.0322	1.1773
	.1	1.42797	3.80844	1.000	-10.7576	13.6135
	10.0	-17.12870*	2.87735	.001	-26.8945	-7.3629
	20.0	-47.55869*	2.99548	.000	-57.5163	-37.6011
10.0	.0	8.70125*	1.31090	.000	4.2879	13.1146
	.1	18.55667*	3.00074	.001	8.3217	28.7916
	1.0	17.12870*	2.87735	.001	7.3629	26.8945
	20.0	-30.42999*	1.86363	.000	-36.4229	-24.4371
20.0	.0	39.13124*	1.55312	.000	33.7972	44.4653
	.1	48.98666*	3.11419	.000	38.5808	59.3925
	1.0	47.55869*	2.99548	.000	37.6011	57.5163
	10.0	30.42999*	1.86363	.000	24.4371	36.4229

*. The mean difference is significant at the .05 level.

Appendix C-3 Statistical analysis of effect of PC/CH liposome concentration on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition

Levene Statistic	df1	df2	P-value
1.332	3	32	.281

ANOVA

inhibition

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	13389.300	3	4463.100	257.866	.000
Within Groups	553.850	32	17.308		
Total	13943.150	35			

Multiple Comparisons

Dependent Variable: inhibition

Tukey HSD

(I) PCconc	(J) PCconc	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
.0	1.0	-24.55327*	1.96117	.000	-29.8668	-19.2398
	2.0	-40.03529*	1.96117	.000	-45.3488	-34.7218
	4.0	-51.47554*	1.96117	.000	-56.7891	-46.1620
1.0	.0	24.55327*	1.96117	.000	19.2398	29.8668
	2.0	-15.48202*	1.96117	.000	-20.7955	-10.1685
	4.0	-26.92227*	1.96117	.000	-32.2358	-21.6088
2.0	.0	40.03529*	1.96117	.000	34.7218	45.3488
	1.0	15.48202*	1.96117	.000	10.1685	20.7955
	4.0	-11.44026*	1.96117	.000	-16.7538	-6.1267
4.0	.0	51.47554*	1.96117	.000	46.1620	56.7891
	1.0	26.92227*	1.96117	.000	21.6088	32.2358
	2.0	11.44026*	1.96117	.000	6.1267	16.7538

*. The mean difference is significant at the .05 level.

Appendix C-4 Statistical analysis of effect of PC/CH/DCP liposome concentration on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition2

Levene Statistic	df1	df2	P-value
1.690	5	48	.155

ANOVA

inhibition2

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	11865.241	5	2373.048	337.622	.000
Within Groups	337.379	48	7.029		
Total	12202.620	53			

Multiple Comparisons

Dependent Variable: inhibition2

Tukey HSD

(I) DCPconc2	(J) DCPconc2	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	-23.31354*	1.24978	.000	-27.0227	-19.6043
	1.00	-28.03663*	1.24978	.000	-31.7458	-24.3274
	2.00	-34.15080*	1.24978	.000	-37.8600	-30.4416
	4.00	-43.58816*	1.24978	.000	-47.2974	-39.8790
	5.00	-43.32378*	1.24978	.000	-47.0330	-39.6146
.50	.00	23.31354*	1.24978	.000	19.6043	27.0227
	1.00	-4.72309*	1.24978	.005	-8.4323	-1.0139
	2.00	-10.83726*	1.24978	.000	-14.5465	-7.1281
	4.00	-20.27462*	1.24978	.000	-23.9838	-16.5654
	5.00	-20.01024*	1.24978	.000	-23.7194	-16.3010
1.00	.00	28.03663*	1.24978	.000	24.3274	31.7458
	.50	4.72309*	1.24978	.005	1.0139	8.4323
	2.00	-6.11416*	1.24978	.000	-9.8234	-2.4050
	4.00	-15.55153*	1.24978	.000	-19.2607	-11.8423
	5.00	-15.28715*	1.24978	.000	-18.9964	-11.5780
2.00	.00	34.15080*	1.24978	.000	30.4416	37.8600
	.50	10.83726*	1.24978	.000	7.1281	14.5465
	1.00	6.11416*	1.24978	.000	2.4050	9.8234
	4.00	-9.43737*	1.24978	.000	-13.1466	-5.7282
	5.00	-9.17299*	1.24978	.000	-12.8822	-5.4638
4.00	.00	43.58816*	1.24978	.000	39.8790	47.2974
	.50	20.27462*	1.24978	.000	16.5654	23.9838
	1.00	15.55153*	1.24978	.000	11.8423	19.2607
	2.00	9.43737*	1.24978	.000	5.7282	13.1466
	5.00	.26438	1.24978	1.000	-3.4448	3.9736
5.00	.00	43.32378*	1.24978	.000	39.6146	47.0330
	.50	20.01024*	1.24978	.000	16.3010	23.7194
	1.00	15.28715*	1.24978	.000	11.5780	18.9964
	2.00	9.17299*	1.24978	.000	5.4638	12.8822
	4.00	-.26438	1.24978	1.000	-3.9736	3.4448

*. The mean difference is significant at the .05 level.

Appendix C-5 Statistical analysis of effect of PC/CH/PG liposome concentration on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition

Levene Statistic	df1	df2	P-value
1.212	5	48	.318

ANOVA

inhibition

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	7131.400	5	1426.280	82.051	.000
Within Groups	834.381	48	17.383		
Total	7965.781	53			

Multiple Comparisons

Dependent Variable: inhibition

Tukey HSD

(I) PGconc1	(J) PGconc1	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	-13.50288*	1.96542	.000	-19.3360	-7.6697
	1.00	-16.48911*	1.96542	.000	-22.3223	-10.6560
	2.00	-23.35705*	1.96542	.000	-29.1902	-17.5239
	4.00	-32.20075*	1.96542	.000	-38.0339	-26.3676
	5.00	-33.33854*	1.96542	.000	-39.1717	-27.5054
.50	.00	13.50288*	1.96542	.000	7.6697	19.3360
	1.00	-2.98623	1.96542	.654	-8.8194	2.8469
	2.00	-9.85418*	1.96542	.000	-15.6873	-4.0210
	4.00	-18.69787*	1.96542	.000	-24.5310	-12.8647
	5.00	-19.83566*	1.96542	.000	-25.6688	-14.0025
1.00	.00	16.48911*	1.96542	.000	10.6560	22.3223
	.50	2.98623	1.96542	.654	-2.8469	8.8194
	2.00	-6.86794*	1.96542	.012	-12.7011	-1.0348
	4.00	-15.71164*	1.96542	.000	-21.5448	-9.8785
	5.00	-16.84943*	1.96542	.000	-22.6826	-11.0163
2.00	.00	23.35705*	1.96542	.000	17.5239	29.1902
	.50	9.85418*	1.96542	.000	4.0210	15.6873
	1.00	6.86794*	1.96542	.012	1.0348	12.7011
	4.00	-8.84370*	1.96542	.001	-14.6769	-3.0105
	5.00	-9.98149*	1.96542	.000	-15.8146	-4.1483
4.00	.00	32.20075*	1.96542	.000	26.3676	38.0339
	.50	18.69787*	1.96542	.000	12.8647	24.5310
	1.00	15.71164*	1.96542	.000	9.8785	21.5448
	2.00	8.84370*	1.96542	.001	3.0105	14.6769
	5.00	-1.13779	1.96542	.992	-6.9709	4.6954
5.00	.00	33.33854*	1.96542	.000	27.5054	39.1717
	.50	19.83566*	1.96542	.000	14.0025	25.6688
	1.00	16.84943*	1.96542	.000	11.0163	22.6826
	2.00	9.98149*	1.96542	.000	4.1483	15.8146
	4.00	1.13779	1.96542	.992	-4.6954	6.9709

*. The mean difference is significant at the .05 level.

Appendix C-6 Statistical analysis of inhibitory effect of TOC-loaded PC liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_load

Levene Statistic	df1	df2	P-value
.178	4	25	.948

ANOVA

inhibition_load

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	733.714	4	183.428	13.831	.000
Within Groups	331.544	25	13.262		
Total	1065.258	29			

Multiple Comparisons

Dependent Variable: inhibition_load

Tukey HSD

(I) PCTOC2	(J) PCTOC2	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-11.36823*	2.10252	.000	-17.5431	-5.1934
	3.00	-13.30514*	2.10252	.000	-19.4800	-7.1303
	4.00	-13.15425*	2.10252	.000	-19.3291	-6.9794
	5.00	-10.40565*	2.10252	.000	-16.5805	-4.2308
2.00	1.00	11.36823*	2.10252	.000	5.1934	17.5431
	3.00	-1.93692	2.10252	.886	-8.1117	4.2379
	4.00	-1.78602	2.10252	.912	-7.9609	4.3888
	5.00	.96258	2.10252	.990	-5.2122	7.1374
3.00	1.00	13.30514*	2.10252	.000	7.1303	19.4800
	2.00	1.93692	2.10252	.886	-4.2379	8.1117
	4.00	.15089	2.10252	1.000	-6.0239	6.3257
	5.00	2.89950	2.10252	.646	-3.2753	9.0743
4.00	1.00	13.15425*	2.10252	.000	6.9794	19.3291
	2.00	1.78602	2.10252	.912	-4.3888	7.9609
	3.00	-.15089	2.10252	1.000	-6.3257	6.0239
	5.00	2.74860	2.10252	.689	-3.4262	8.9234
5.00	1.00	10.40565*	2.10252	.000	4.2308	16.5805
	2.00	-.96258	2.10252	.990	-7.1374	5.2122
	3.00	-2.89950	2.10252	.646	-9.0743	3.2753
	4.00	-2.74860	2.10252	.689	-8.9234	3.4262

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Antioxidant-loaded liposomes at low antioxidant concentration

5.00 Antioxidant-loaded liposomes at high antioxidant concentration

Appendix C-7 Statistical analysis of inhibitory effect of TOC-loaded PC/DCP liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_load

Levene Statistic	df1	df2	P-value
15.012	4	25	.000

ANOVA

inhibition_load

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	12834.508	4	3208.627	49.203	.000
Within Groups	1630.315	25	65.213		
Total	14464.823	29			

Multiple Comparisons

Dependent Variable: inhibition_load

Dunnett T3

(I) DCPTOC	(J) DCPTOC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-6.81779*	1.52994	.011	-12.1473	-1.4883
	3.00	-56.92599*	3.67025	.000	-71.5535	-42.2985
	4.00	-28.73467*	4.46356	.006	-46.9470	-10.5224
	5.00	-37.45570*	4.75751	.002	-56.9876	-17.9238
2.00	1.00	6.81779*	1.52994	.011	1.4883	12.1473
	3.00	-50.10820*	3.62329	.000	-64.7952	-35.4212
	4.00	-21.91688*	4.42502	.023	-40.2010	-3.6328
	5.00	-30.63791*	4.72138	.007	-50.2428	-11.0330
3.00	1.00	56.92599*	3.67025	.000	42.2985	71.5535
	2.00	50.10820*	3.62329	.000	35.4212	64.7952
	4.00	28.19132*	5.54174	.005	8.7918	47.5909
	5.00	19.47029	5.78113	.063	-.9034	39.8440
4.00	1.00	28.73467*	4.46356	.006	10.5224	46.9470
	2.00	21.91688*	4.42502	.023	3.6328	40.2010
	3.00	-28.19132*	5.54174	.005	-47.5909	-8.7918
	5.00	-8.72104	6.31459	.816	-30.6382	13.1961
5.00	1.00	37.45570*	4.75751	.002	17.9238	56.9876
	2.00	30.63791*	4.72138	.007	11.0330	50.2428
	3.00	-19.47029	5.78113	.063	-39.8440	.9034
	4.00	8.72104	6.31459	.816	-13.1961	30.6382

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Antioxidant-loaded liposomes at low antioxidant concentration

5.00 Antioxidant-loaded liposomes at high antioxidant concentration

Appendix C-8 Statistical analysis of inhibitory effect of TOC-loaded PC/PG liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_load

Levene Statistic	df1	df2	P-value
2.886	4	40	.034

ANOVA

inhibition_load

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	10764.482	4	2691.120	69.807	.000
Within Groups	1542.036	40	38.551		
Total	12306.518	44			

Multiple Comparisons

Dependent Variable: inhibition_load

Dunnett T3

(I) PGTOC	(J) PGTOC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-12.10414*	2.93723	.016	-22.1570	-2.0513
	3.00	-38.07945*	3.01967	.000	-48.2458	-27.9132
	4.00	-30.70397*	3.64809	.000	-42.3909	-19.0171
	5.00	-39.34205*	3.53357	.000	-50.6942	-27.9899
2.00	1.00	12.10414*	2.93723	.016	2.0513	22.1570
	3.00	-25.97531*	1.73116	.000	-31.5325	-20.4181
	4.00	-18.59983*	2.68087	.000	-27.6779	-9.5218
	5.00	-27.23791*	2.52282	.000	-35.7158	-18.7600
3.00	1.00	38.07945*	3.01967	.000	27.9132	48.2458
	2.00	25.97531*	1.73116	.000	20.4181	31.5325
	4.00	7.37548	2.77095	.159	-1.8492	16.6002
	5.00	-1.26260	2.61834	1.000	-9.9133	7.3881
4.00	1.00	30.70397*	3.64809	.000	19.0171	42.3909
	2.00	18.59983*	2.68087	.000	9.5218	27.6779
	3.00	-7.37548	2.77095	.159	-16.6002	1.8492
	5.00	-8.63808	3.32353	.159	-19.2764	2.0003
5.00	1.00	39.34205*	3.53357	.000	27.9899	50.6942
	2.00	27.23791*	2.52282	.000	18.7600	35.7158
	3.00	1.26260	2.61834	1.000	-7.3881	9.9133
	4.00	8.63808	3.32353	.159	-2.0003	19.2764

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Antioxidant-loaded liposomes at low antioxidant concentration

5.00 Antioxidant-loaded liposomes at high antioxidant concentration

Appendix C-9 Statistical analysis of inhibitory effect of NAC-loaded PC/CH liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_load

Levene Statistic	df1	df2	P-value
3.310	4	40	.020

ANOVA

inhibition_load

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	5156.774	4	1289.194	98.045	.000
Within Groups	525.960	40	13.149		
Total	5682.735	44			

Multiple Comparisons

Dependent Variable: inhibition_load

Dunnett T3

(I) PCNAC	(J) PCNAC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-9.72026*	2.14355	.004	-16.6177	-2.8229
	3.00	-32.58344*	1.85829	.000	-38.5292	-26.6377
	4.00	-18.30860*	1.50632	.000	-23.3632	-13.2540
	5.00	-15.44597*	1.55697	.000	-20.5926	-10.2993
2.00	1.00	9.72026*	2.14355	.004	2.8229	16.6177
	3.00	-22.86319*	2.10331	.000	-29.6511	-16.0753
	4.00	-8.58835*	1.79990	.006	-14.7586	-2.4181
	5.00	-5.72571	1.84251	.079	-11.9519	.5005
3.00	1.00	32.58344*	1.85829	.000	26.6377	38.5292
	2.00	22.86319*	2.10331	.000	16.0753	29.6511
	4.00	14.27484*	1.44848	.000	9.4392	19.1105
	5.00	17.13748*	1.50109	.000	12.1998	22.0751
4.00	1.00	18.30860*	1.50632	.000	13.2540	23.3632
	2.00	8.58835*	1.79990	.006	2.4181	14.7586
	3.00	-14.27484*	1.44848	.000	-19.1105	-9.4392
	5.00	2.86264	1.03393	.117	-.4538	6.1791
5.00	1.00	15.44597*	1.55697	.000	10.2993	20.5926
	2.00	5.72571	1.84251	.079	-.5005	11.9519
	3.00	-17.13748*	1.50109	.000	-22.0751	-12.1998
	4.00	-2.86264	1.03393	.117	-6.1791	.4538

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Antioxidant-loaded liposomes at low antioxidant concentration

5.00 Antioxidant-loaded liposomes at high antioxidant concentration

Appendix C-10 Statistical analysis of inhibitory effect of co-incubation of TOC dispersion and blank PC liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_add

Levene Statistic	df1	df2	P-value
1.647	4	25	.194

ANOVA

inhibition_add

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	799.123	4	199.781	7.932	.000
Within Groups	629.632	25	25.185		
Total	1428.755	29			

Multiple Comparisons

Dependent Variable: inhibition_add

Tukey HSD

(I) PCTOC2	(J) PCTOC2	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-11.36823*	2.89743	.005	-19.8776	-2.8589
	3.00	-13.30514*	2.89743	.001	-21.8145	-4.7958
	4.00	-7.46565	2.89743	.106	-15.9750	1.0437
	5.00	-14.09868*	2.89743	.000	-22.6081	-5.5893
2.00	1.00	11.36823*	2.89743	.005	2.8589	19.8776
	3.00	-1.93692	2.89743	.961	-10.4463	6.5725
	4.00	3.90258	2.89743	.666	-4.6068	12.4120
	5.00	-2.73045	2.89743	.878	-11.2398	5.7789
3.00	1.00	13.30514*	2.89743	.001	4.7958	21.8145
	2.00	1.93692	2.89743	.961	-6.5725	10.4463
	4.00	5.83950	2.89743	.288	-2.6699	14.3489
	5.00	-.79353	2.89743	.999	-9.3029	7.7158
4.00	1.00	7.46565	2.89743	.106	-1.0437	15.9750
	2.00	-3.90258	2.89743	.666	-12.4120	4.6068
	3.00	-5.83950	2.89743	.288	-14.3489	2.6699
	5.00	-6.63303	2.89743	.182	-15.1424	1.8763
5.00	1.00	14.09868*	2.89743	.000	5.5893	22.6081
	2.00	2.73045	2.89743	.878	-5.7789	11.2398
	3.00	.79353	2.89743	.999	-7.7158	9.3029
	4.00	6.63303	2.89743	.182	-1.8763	15.1424

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Blank liposomes with additional antioxidant at low concentration

5.00 Blank liposomes with additional antioxidant at high concentration

Appendix C-11 Statistical analysis of inhibitory effect of co-incubation of TOC dispersion and blank PC/DCP liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_add

Levene Statistic	df1	df2	P-value
20.224	4	25	.000

ANOVA

inhibition_add

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	14189.754	4	3547.439	48.765	.000
Within Groups	1818.642	25	72.746		
Total	16008.396	29			

Multiple Comparisons

Dependent Variable: inhibition_add

Dunnett T3

(I) DCPTOC	(J) DCPTOC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-6.81779*	1.52994	.011	-12.1473	-1.4883
	3.00	-56.92599*	3.67025	.000	-71.5535	-42.2985
	4.00	-39.85549*	5.10757	.003	-60.9532	-18.7578
	5.00	-40.59039*	4.76944	.002	-60.1757	-21.0051
2.00	1.00	6.81779*	1.52994	.011	1.4883	12.1473
	3.00	-50.10820*	3.62329	.000	-64.7952	-35.4212
	4.00	-33.03771*	5.07393	.007	-54.2087	-11.8668
	5.00	-33.77261*	4.73339	.004	-53.4310	-14.1142
3.00	1.00	56.92599*	3.67025	.000	42.2985	71.5535
	2.00	50.10820*	3.62329	.000	35.4212	64.7952
	4.00	17.07050	6.07246	.151	-4.5246	38.6656
	5.00	16.33560	5.79094	.145	-4.0787	36.7499
4.00	1.00	39.85549*	5.10757	.003	18.7578	60.9532
	2.00	33.03771*	5.07393	.007	11.8668	54.2087
	3.00	-17.07050	6.07246	.151	-38.6656	4.5246
	5.00	-.73490	6.79349	1.000	-24.3174	22.8476
5.00	1.00	40.59039*	4.76944	.002	21.0051	60.1757
	2.00	33.77261*	4.73339	.004	14.1142	53.4310
	3.00	-16.33560	5.79094	.145	-36.7499	4.0787
	4.00	.73490	6.79349	1.000	-22.8476	24.3174

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Blank liposomes with additional antioxidant at low concentration

5.00 Blank liposomes with additional antioxidant at high concentration

Appendix C-12 Statistical analysis of inhibitory effect of co-incubation of TOC dispersion and blank PC/PG liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_add

Levene Statistic	df1	df2	P-value
7.894	4	40	.000

ANOVA

inhibition_add

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	7061.351	4	1765.338	89.186	.000
Within Groups	791.759	40	19.794		
Total	7853.110	44			

Multiple Comparisons

Dependent Variable: inhibition_add

Dunnett T3

(I) PGTOC	(J) PGTOC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-12.10414*	2.93723	.016	-22.1570	-2.0513
	3.00	-38.07945*	3.01967	.000	-48.2458	-27.9132
	4.00	-16.23077*	2.76929	.002	-26.1659	-6.2956
	5.00	-22.44726*	2.77582	.000	-32.3835	-12.5110
2.00	1.00	12.10414*	2.93723	.016	2.0513	22.1570
	3.00	-25.97531*	1.73116	.000	-31.5325	-20.4181
	4.00	-4.12663	1.24396	.055	-8.3160	.0627
	5.00	-10.34311*	1.25845	.000	-14.5559	-6.1303
3.00	1.00	38.07945*	3.01967	.000	27.9132	48.2458
	2.00	25.97531*	1.73116	.000	20.4181	31.5325
	4.00	21.84868*	1.42778	.000	16.9606	26.7367
	5.00	15.63219*	1.44042	.000	10.7285	20.5359
4.00	1.00	16.23077*	2.76929	.002	6.2956	26.1659
	2.00	4.12663	1.24396	.055	-.0627	8.3160
	3.00	-21.84868*	1.42778	.000	-26.7367	-16.9606
	5.00	-6.21649*	.79077	.000	-8.7470	-3.6860
5.00	1.00	22.44726*	2.77582	.000	12.5110	32.3835
	2.00	10.34311*	1.25845	.000	6.1303	14.5559
	3.00	-15.63219*	1.44042	.000	-20.5359	-10.7285
	4.00	6.21649*	.79077	.000	3.6860	8.7470

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Blank liposomes with additional antioxidant at low concentration

5.00 Blank liposomes with additional antioxidant at high concentration

Appendix C-13 Statistical analysis of inhibitory effect of co-incubation of NAC solution and blank PC/CH liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_add

Levene Statistic	df1	df2	P-value
2.267	4	40	.079

ANOVA

inhibition_add

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	6203.419	4	1550.855	111.468	.000
Within Groups	556.521	40	13.913		
Total	6759.940	44			

Multiple Comparisons

Dependent Variable: inhibition_add

Tukey HSD

(I) PCNAC	(J) PCNAC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-9.72026*	1.75835	.000	-14.7423	-4.6983
	3.00	-32.58344*	1.75835	.000	-37.6054	-27.5614
	4.00	-22.88795*	1.75835	.000	-27.9100	-17.8660
	5.00	-25.76721*	1.75835	.000	-30.7892	-20.7452
2.00	1.00	9.72026*	1.75835	.000	4.6983	14.7423
	3.00	-22.86319*	1.75835	.000	-27.8852	-17.8412
	4.00	-13.16770*	1.75835	.000	-18.1897	-8.1457
	5.00	-16.04696*	1.75835	.000	-21.0690	-11.0250
3.00	1.00	32.58344*	1.75835	.000	27.5614	37.6054
	2.00	22.86319*	1.75835	.000	17.8412	27.8852
	4.00	9.69549*	1.75835	.000	4.6735	14.7175
	5.00	6.81623*	1.75835	.003	1.7942	11.8382
4.00	1.00	22.88795*	1.75835	.000	17.8660	27.9100
	2.00	13.16770*	1.75835	.000	8.1457	18.1897
	3.00	-9.69549*	1.75835	.000	-14.7175	-4.6735
	5.00	-2.87926	1.75835	.483	-7.9013	2.1427
5.00	1.00	25.76721*	1.75835	.000	20.7452	30.7892
	2.00	16.04696*	1.75835	.000	11.0250	21.0690
	3.00	-6.81623*	1.75835	.003	-11.8382	-1.7942
	4.00	2.87926	1.75835	.483	-2.1427	7.9013

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Blank liposomes with additional antioxidant at low concentration

5.00 Blank liposomes with additional antioxidant at high concentration

Appendix C-14 Statistical analysis of inhibitory effect of co-incubation of NAC solution and blank PC/CH/DCP liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_add

Levene Statistic	df1	df2	P-value
1.184	4	25	.342

ANOVA

inhibition_add

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	2698.628	4	674.657	22.358	.000
Within Groups	754.395	25	30.176		
Total	3453.022	29			

Multiple Comparisons

Dependent Variable: inhibition_add

Tukey HSD

(I) DCPNAC	(J) DCPNAC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-24.506427*	3.171529	.000	-33.82080	-15.19205
	3.00	-26.859413*	3.171529	.000	-36.17379	-17.54504
	4.00	-14.071144*	3.171529	.001	-23.38552	-4.75677
	5.00	-16.951756*	3.171529	.000	-26.26613	-7.63738
2.00	1.00	24.506427*	3.171529	.000	15.19205	33.82080
	3.00	-2.352985	3.171529	.944	-11.66736	6.96139
	4.00	10.435284*	3.171529	.023	1.12091	19.74966
	5.00	7.554671	3.171529	.153	-1.75970	16.86904
3.00	1.00	26.859413*	3.171529	.000	17.54504	36.17379
	2.00	2.352985	3.171529	.944	-6.96139	11.66736
	4.00	12.788269*	3.171529	.004	3.47390	22.10264
	5.00	9.907657*	3.171529	.033	.59328	19.22203
4.00	1.00	14.071144*	3.171529	.001	4.75677	23.38552
	2.00	-10.435284*	3.171529	.023	-19.74966	-1.12091
	3.00	-12.788269*	3.171529	.004	-22.10264	-3.47390
	5.00	-2.880612	3.171529	.891	-12.19499	6.43376
5.00	1.00	16.951756*	3.171529	.000	7.63738	26.26613
	2.00	-7.554671	3.171529	.153	-16.86904	1.75970
	3.00	-9.907657*	3.171529	.033	-19.22203	-.59328
	4.00	2.880612	3.171529	.891	-6.43376	12.19499

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Blank liposomes with additional antioxidant at low concentration

5.00 Blank liposomes with additional antioxidant at high concentration

Appendix C-15 Statistical analysis of inhibitory effect of co-incubation of NAC solution and blank PC/CH/PG liposomes on NO production in LPS-stimulated J774A.1 cells

Test of Homogeneity of Variances

inhibition_add

Levene Statistic	df1	df2	P-value
18.584	4	25	.000

ANOVA

inhibition_add

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	12799.088	4	3199.772	38.770	.000
Within Groups	2063.311	25	82.532		
Total	14862.399	29			

Multiple Comparisons

Dependent Variable: inhibition_add

Dunnett T3

(I) PGNAC	(J) PGNAC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-1.565749	5.153574	1.000	-20.22016	17.08866
	3.00	-56.929810*	5.003387	.000	-75.38268	-38.47694
	4.00	-24.006971*	6.426616	.033	-46.31443	-1.69951
	5.00	-16.287042	5.786513	.142	-36.44490	3.87082
2.00	1.00	1.565749	5.153574	1.000	-17.08866	20.22016
	3.00	-55.364061*	3.632127	.000	-67.99455	-42.73358
	4.00	-22.441221*	5.427693	.026	-42.30890	-2.57354
	5.00	-14.721293	4.652117	.088	-31.21284	1.77025
3.00	1.00	56.929810*	5.003387	.000	38.47694	75.38268
	2.00	55.364061*	3.632127	.000	42.73358	67.99455
	4.00	32.922839*	5.285301	.003	13.20036	52.64531
	5.00	40.642768*	4.485170	.000	24.48617	56.79936
4.00	1.00	24.006971*	6.426616	.033	1.69951	46.31443
	2.00	22.441221*	5.427693	.026	2.57354	42.30890
	3.00	-32.922839*	5.285301	.003	-52.64531	-13.20036
	5.00	7.719929	6.031937	.866	-13.40484	28.84470
5.00	1.00	16.287042	5.786513	.142	-3.87082	36.44490
	2.00	14.721293	4.652117	.088	-1.77025	31.21284
	3.00	-40.642768*	4.485170	.000	-56.79936	-24.48617
	4.00	-7.719929	6.031937	.866	-28.84470	13.40484

*. The mean difference is significant at the .05 level.

1.00 Antioxidant solution at low concentration

2.00 Antioxidant solution at high concentration

3.00 Blank liposomes

4.00 Blank liposomes with additional antioxidant at low concentration

5.00 Blank liposomes with additional antioxidant at high concentration

Appendix C-16 Statistical analysis of effect of type of liposomes and contact time on intracellular uptake of calcein in J774A.1 cells

At 30 min

Test of Homogeneity of Variances

% uptake calcein at 30 min

Levene Statistic	df1	df2	P-value
2.571	2	6	.156

ANOVA

% uptake calcein at 30 min

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	0.0003423	2	0.0001712	3.385	.104
Within Groups	0.0003034	6	0.0000506		
Total	0.0006458	8			

Multiple Comparisons

Dependent Variable: % uptake calcein at 30 min

Tukey HSD

(I) condition	(J) condition	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	-.01474346	.00580626	.097	-.0325587	.0030717
	3.0	-.01022533	.00580626	.260	-.0280405	.0075899
2.0	1.0	.01474346	.00580626	.097	-.0030717	.0325587
	3.0	.00451813	.00580626	.729	-.0132971	.0223333
3.0	1.0	.01022533	.00580626	.260	-.0075899	.0280405
	2.0	-.00451813	.00580626	.729	-.0223333	.0132971

At 2 hours

Test of Homogeneity of Variances

% uptake calcein at 2 hr

Levene Statistic	df1	df2	P-value
1.046	2	6	.408

ANOVA

% uptake calcein at 2 hr

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	0.0006140	2	0.0003070	75.278	.000
Within Groups	0.0000245	6	0.0000041		
Total	0.0006385	8			

Multiple Comparisons

Dependent Variable: % uptake calcein at 2 hr

Tukey HSD

(I) condition	(J) condition	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	-.01792525*	.00164886	.000	-.0229844	-.0128661
	3.0	-.01708707*	.00164886	.000	-.0221462	-.0120279
2.0	1.0	.01792525*	.00164886	.000	.0128661	.0229844
	3.0	.00083818	.00164886	.870	-.0042210	.0058973
3.0	1.0	.01708707*	.00164886	.000	.0120279	.0221462
	2.0	-.00083818	.00164886	.870	-.0058973	.0042210

*. The mean difference is significant at the .05 level.

At 4 hours

Test of Homogeneity of Variances

% uptake calcein at 4 hr

Levene Statistic	df1	df2	P-value
5.517	2	6	.044

ANOVA

% uptake calcein at 4 hr

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	0.0003467	2	0.0001733	319.310	.000
Within Groups	0.0000033	6	0.0000005		
Total	0.0003500	8			

Multiple Comparisons

Dependent Variable: % uptake calcein at 4 hr

Dunnett T3

(I) condition	(J) condition	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	-.01169885*	.00071037	.006	-.0159238	-.0074739
	3.0	-.01425789*	.00023076	.000	-.0152084	-.0133074
2.0	1.0	.01169885*	.00071037	.006	.0074739	.0159238
	3.0	-.00255903	.00072657	.125	-.0065320	.0014139
3.0	1.0	.01425789*	.00023076	.000	.0133074	.0152084
	2.0	.00255903	.00072657	.125	-.0014139	.0065320

*. The mean difference is significant at the .05 level.

At 24 hours

Test of Homogeneity of Variances

% uptake calcein at 24 hr

Levene Statistic	df1	df2	P-value
6.851	2	6	.028

ANOVA

% uptake calcein at 24 hr

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	0.0104394	2	0.0052197	600.284	.000
Within Groups	0.0000522	6	0.0000087		
Total	0.0104916	8			

Multiple Comparisons

Dependent Variable: % uptake calcein at 24 hr

Dunnett T3

(I) condition	(J) condition	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.0	2.0	-.07983342*	.00098129	.000	-.0840241	-.0756428
	3.0	-.01894869*	.00282266	.039	-.0357440	-.0021534
2.0	1.0	.07983342*	.00098129	.000	.0756428	.0840241
	3.0	.06088473*	.00290867	.002	.0453675	.0764019
3.0	1.0	.01894869*	.00282266	.039	.0021534	.0357440
	2.0	-.06088473*	.00290867	.002	-.0764019	-.0453675

*. The mean difference is significant at the .05 level.

1.00 Calcein solution

2.00 Calcein-loaded PC/CH liposomes

3.00 Calcein-loaded PC/CH/DCP liposomes

Appendix C-17 Statistical analysis of effect of PC/CH/DCP liposomes on intracellular uptake of calcein solution in J774A.1 cells

Test of Homogeneity of Variances

% uptake calcein

Levene Statistic	df1	df2	P-value
.492	2	15	.621

ANOVA

% uptake calcein

Source of variation	Sum of Squares	df	Mean Square	F	P-value
Between Groups	0.0000033	2	0.0000017	3.828	.045
Within Groups	0.0000065	15	0.0000004		
Total	0.0000099	17			

Multiple Comparisons

Dependent Variable: % uptake calcein

Tukey HSD

(I) liposome	(J) liposome	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	.00035925	.00038104	.623	-.0006305	.0013490
	3.00	-.00067880	.00038104	.209	-.0016685	.0003109
2.00	1.00	-.00035925	.00038104	.623	-.0013490	.0006305
	3.00	-.00103806*	.00038104	.039	-.0020278	-.0000483
3.00	1.00	.00067880	.00038104	.209	-.0003109	.0016685
	2.00	.00103806*	.00038104	.039	.0000483	.0020278

*. The mean difference is significant at the .05 level.

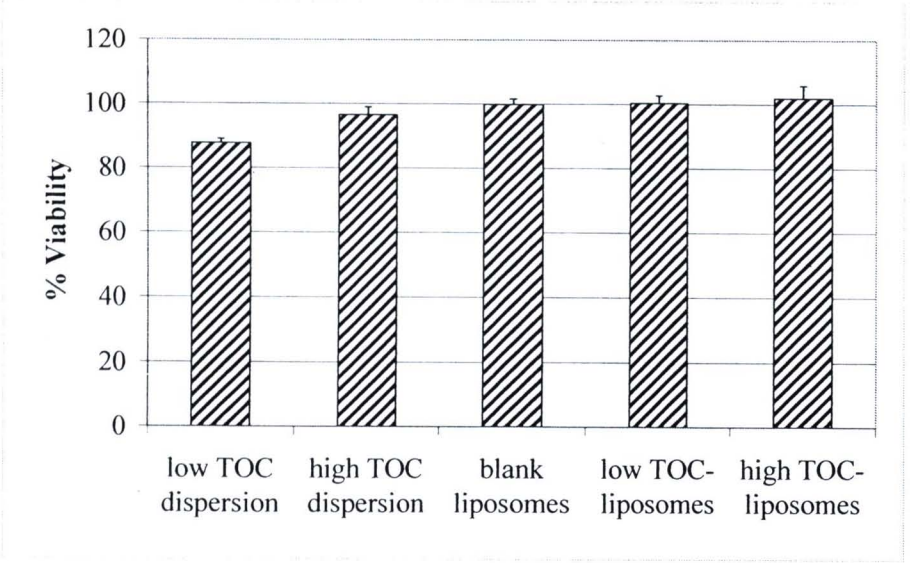
1.00 calcein solution

2.00 Blank PC/CH/DCP liposomes with additional calcein solution

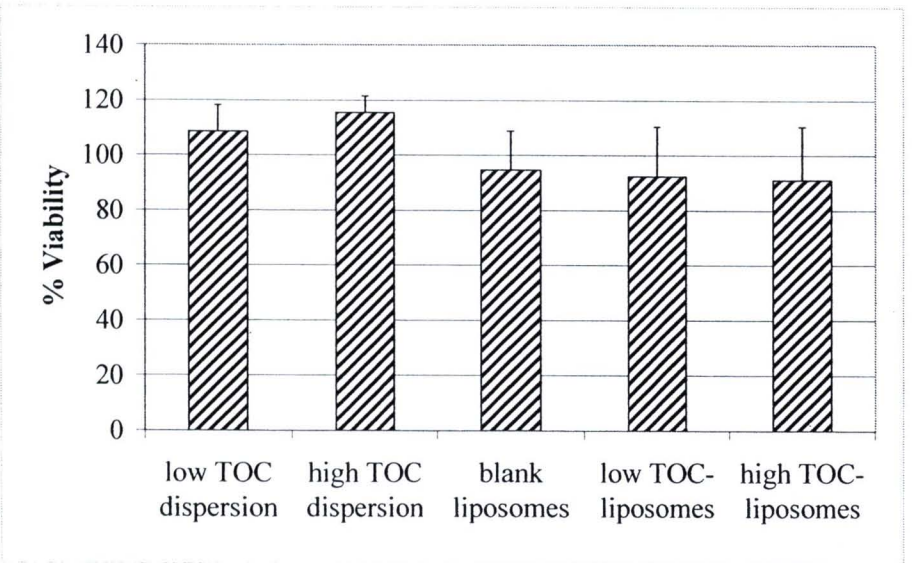
3.00 NAC-loaded PC/CH/DCP liposomes with additional calcein solution

Appendix D

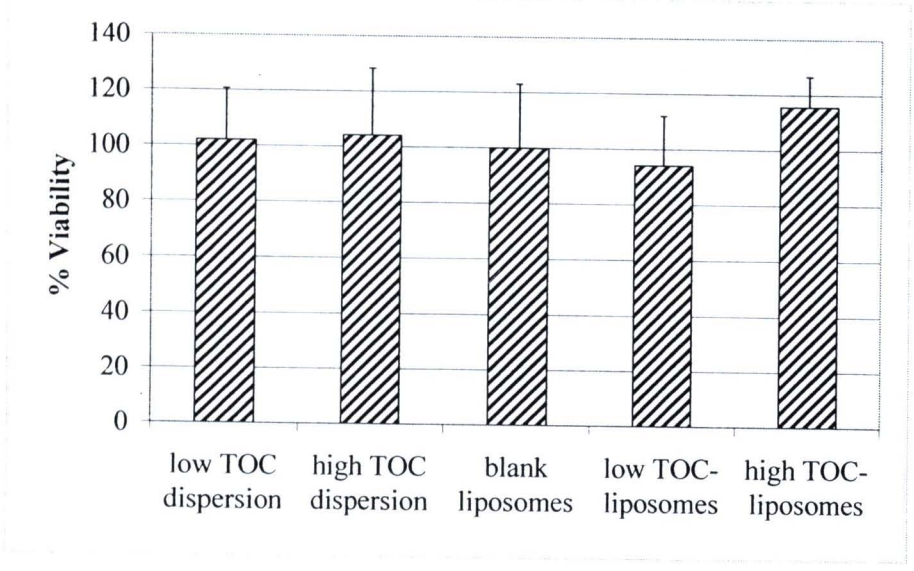
Results of cell viability tests



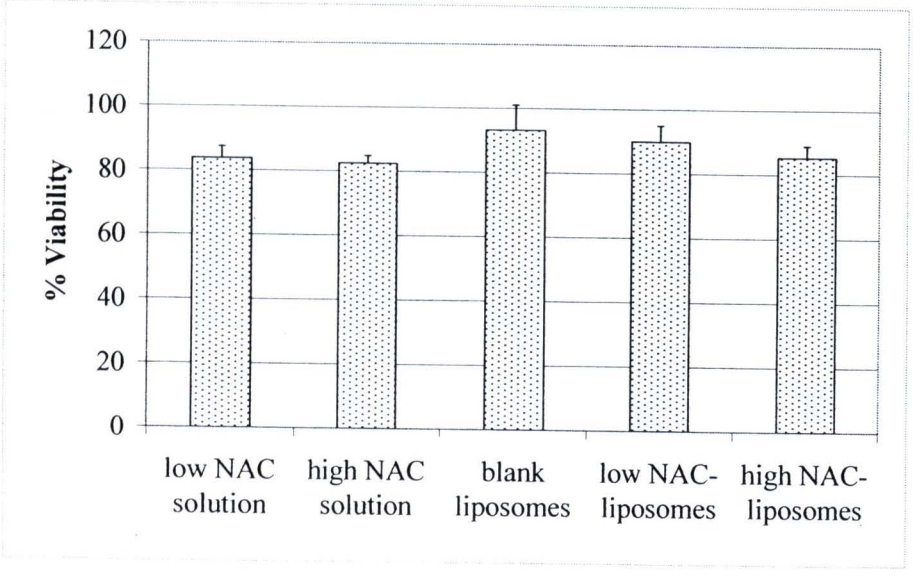
Appendix D-1: Effect of TOC-loaded PC liposomes on cell viability of J774A.1 cells by MTT assay. TOC was used at 0.25 mM (low TOC) and 0.5 mM (high TOC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=6).



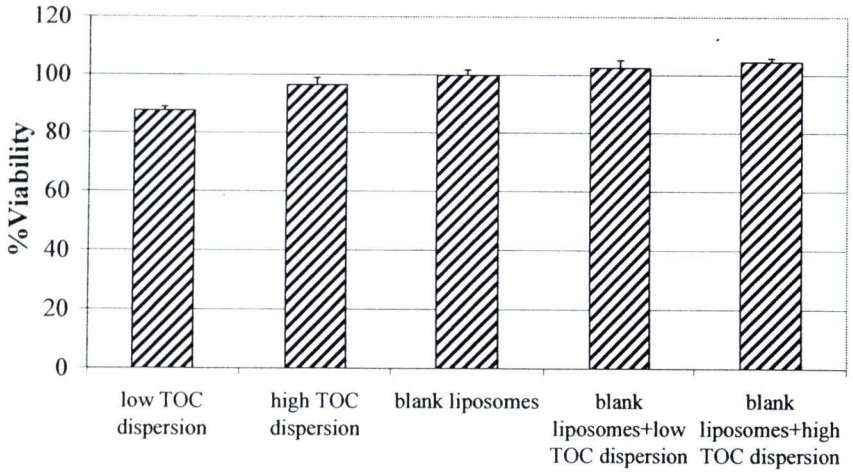
Appendix D-2: Effect of TOC-loaded PC/DCP liposomes on cell viability of J774A.1 cells by MTT assay. TOC was used at 0.25 mM (low TOC) and 0.5 mM (high TOC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=6).



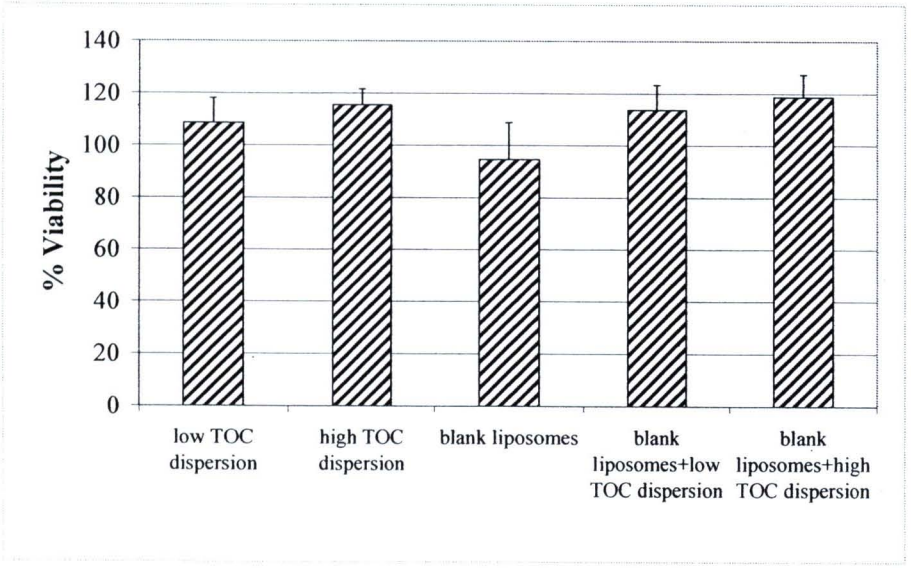
Appendix D-3: Effect of TOC-loaded PC/PG liposomes on cell viability of J774A.1 cells by MTT assay. TOC was used at 0.25 mM (low TOC) and 0.5 mM (high TOC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=9).



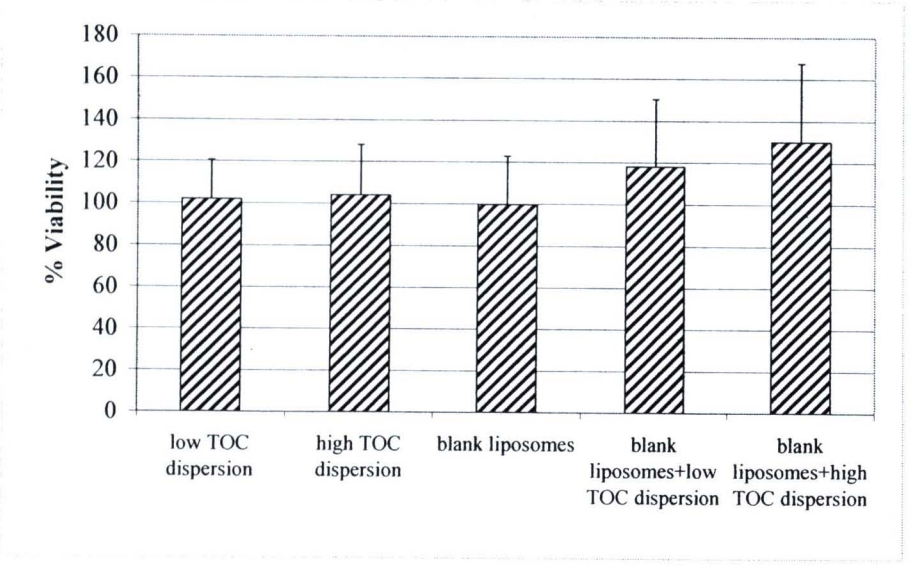
Appendix D-4: Effect of NAC-loaded PC/CH liposomes on cell viability of J774A.1 cells by MTT assay. NAC was used at 10 mM (low NAC) and 20 mM (high NAC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=9).



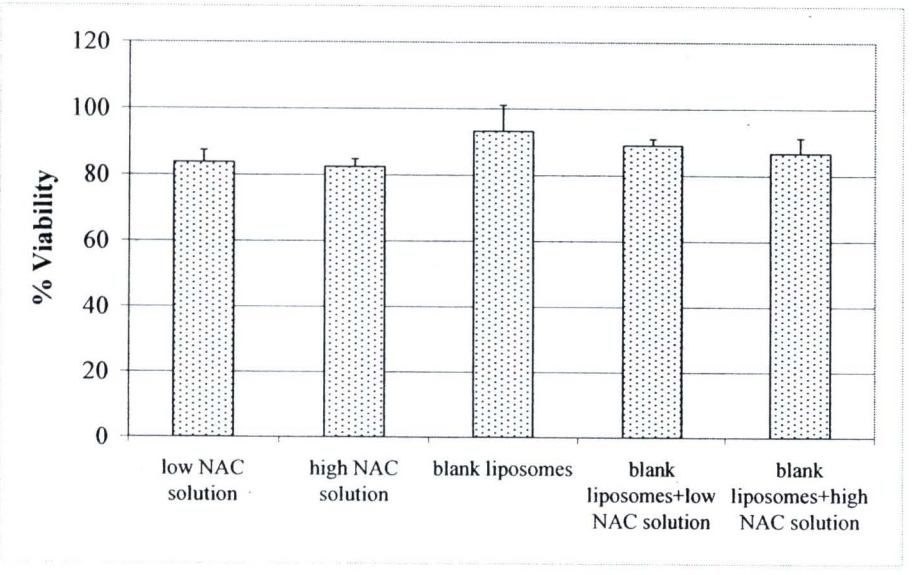
Appendix D-5: Effect of co-incubation of blank PC liposomes and TOC dispersion on cell viability of J774A.1 cells by MTT assay. TOC was used at 0.25 mM (low TOC) and 0.5 mM (high TOC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=6).



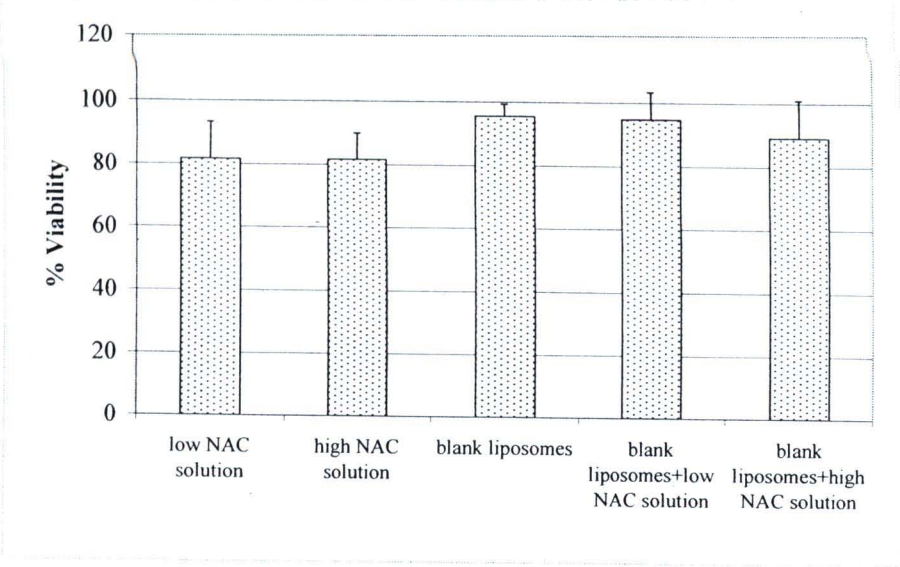
Appendix D-6: Effect of co-incubation of blank PC/DCP liposomes and TOC dispersion on cell viability of J774A.1 cells by MTT assay. TOC was used at 0.25 mM (low TOC) and 0.5 mM (high TOC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=6).



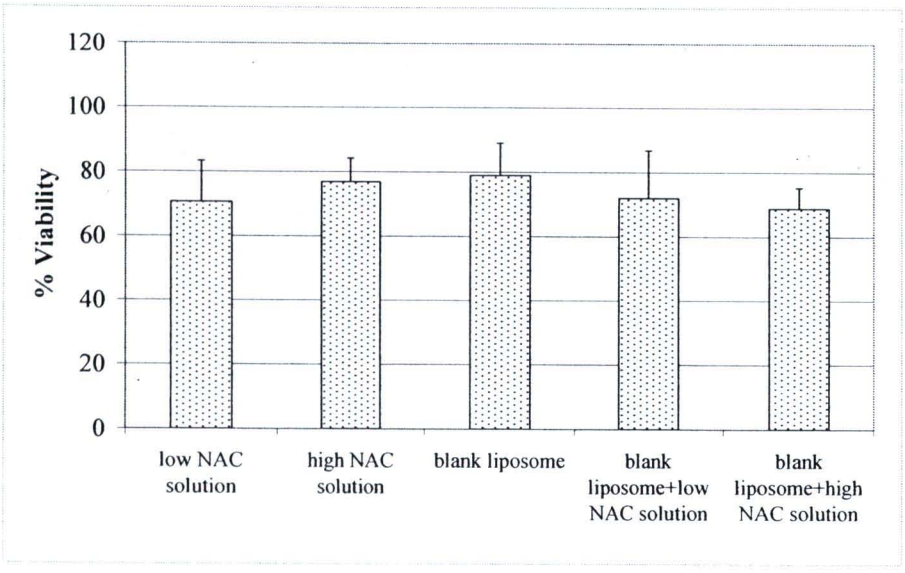
Appendix D-7: Effect of co-incubation of blank PC/PG liposomes and TOC dispersion on cell viability of J774A.1 cells by MTT assay. TOC was used at 0.25 mM (low TOC) and 0.5 mM (high TOC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=9).



Appendix D-8: Effect of co-incubation of blank PC/CH liposomes and NAC solution on cell viability of J774A.1 cells by MTT assay. NAC was used at 10 mM (low NAC) and 20 mM (high NAC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=9).



Appendix D-9: Effect of co-incubation of blank PC/CH/DCP liposomes and NAC solution on cell viability of J774A.1 cells by MTT assay. NAC was used at 10 mM (low NAC) and 20 mM (high NAC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=6).



Appendix D-10: Effect of co-incubation of blank PC/CH/PG liposomes and NAC solution on cell viability of J774A.1 cells by MTT assay. NAC was used at 10 mM (low NAC) and 20 mM (high NAC). Cell seeding density was $1x10^5$cells/ml. LPS concentration was 0.125 μg/ml. The displayed values are mean ± S.D. (n=6).

VITA

Miss Suphakanya Tantrabundit was born on January 11, 1980 in Khonkean, Thailand. She received the Bachelor's degree in Pharmacy from Chiangmai University in 2002. Since graduation, she has worked at the Mahasarakham University. She entered the Master of Pharmacy Program at Chulalongkorn University in 2004.