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APPENDIX A

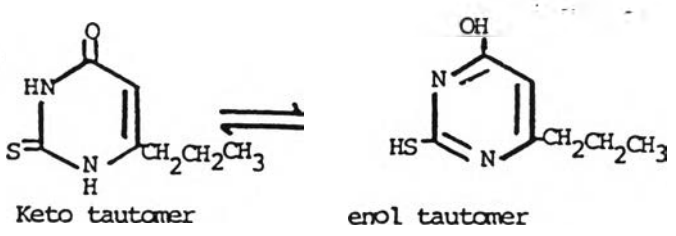
Molecular structure and physical properties of propylthiouracil (PTU)

(Aboul-Enein, 1977)

1. Molecular structure

1.1 Empirical : $C_7H_{10}N_2OS$

1.2 Structural :



Keto tautomer enol tautomer

1.3 Molecular weight : 170.23

2. Physical properties

2.1 Melting range : 219-221 °C

2.2 Solubility :

PTU is sparingly soluble in water (1:900 at 20 °C); soluble in 100 parts boiling water, in 60 parts of ethanol; in 60 parts of acetone. Practically insoluble in ether, chloroform, benzene. Freely soluble in aqueous solutions of ammonia and alkali hydroxide. A saturated aqueous solution is neutral or slightly acidic to litmus.

2.3 Ultraviolet spectrum :

PTU in neutral methanol absorbs ultraviolet radiation at 275 nm (a_m 15800) and at 214 nm (a_m 15600). In alkaline medium, it shows 3 maxima at 315.5 nm (a_m 10900), 260 nm (a_m 10700) and at 207.5 nm (a_m 15400).

2.4 Stability :

PTU is a relatively stable compound at room temperature. It is recommended that it should be kept in a well-closed containers protected from light.

APPENDIX B

BALB/c 3T3 fibroblast cell lines

(American Type Culture Collection, 1999)

Organism: *Mus musculus* (mouse)

Designation: BALB/3T3 clone A31

Depositors: S. Aaronson

Strain: BALB/c

Tissue: embryo; fibroblast

Tumorigenic: no

Morphology: fibroblast

Comments: Never allow cultures to become completely confluent before subculture. The serum used is calf serum, not fetal calf serum. The depositor recommends calf serum because fetal calf serum causes transformation and loss of contact inhibition. The serum initially employed and found satisfactory was from the Colorado Serum Co. Tested and found negative for ectromelia virus (mousepox).

Age stage: embryo; 14 to 17 day gestation

Reverse transcriptase: negative

Growth properties: adherent

Virus susceptibility: herpes simplex; vesicular stomatitis (Indiana)

Subculturing: Remove medium, add fresh 0.25% trypsin for 2 to 3 minutes, remove trypsin and allow the culture to sit for 10 to 15 minutes at 37 °C. Add fresh medium, aspirate and dispense into new flasks. For 60mm plates, use an inoculum of $3X10^5$ cells per plate and subculture every 3 days.

Split ratio: A subcultivation ratio of 1:3 is recommended

Fluid renewal: Twice per week

Propagation: ATCC medium: Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose and 1.0 mM sodium pyruvate, 90%; bovine calf serum, 10%

Passage submitted to the ATCC: 50

Virus resist: poliovirus 1

BioSafety level: 1

APPENDIX C

Solubility data of propylthiouracil (PTU)

The saturation solubility of PTU at 4-8 °C

a) Citrate buffer pH 5.5

Time (day)	Absorbance			PTU concentration (mg/ml)			Mean	SD
	1	2	3	1	2	3		
3	0.112	0.116	0.118	0.78	0.80	0.81	0.80	0.02
6	0.126	0.126	0.127	0.82	0.82	0.82	0.82	0.00
9	0.122	0.125	0.124	0.84	0.86	0.85	0.85	0.01
12	0.134	0.135	0.138	0.79	0.80	0.81	0.80	0.01

b) HEPES buffer pH 7.4

Time (day)	Absorbance			PTU concentration (mg/ml)			Mean	SD
	1	2	3	1	2	3		
3	0.166	0.163	0.166	1.09	1.07	1.09	1.08	0.01
6	0.165	0.169	0.165	1.04	1.06	1.04	1.05	0.01
9	0.192	0.173	0.200	1.25	1.14	1.29	1.22	0.08
12	0.193	0.195	0.191	1.12	1.13	1.11	1.12	0.01

c) Borate buffer pH 9.0

Time (day)	Absorbance			PTU concentration (mg/ml)			Mean	SD
	1	2	3	1	2	3		
3	0.286	0.324	0.339	1.78	1.99	2.08	1.95	0.15
6	0.314	0.302	0.316	1.90	1.83	1.91	1.88	0.04
9	0.293	0.277	0.304	1.83	1.74	1.90	1.82	0.08
12	0.334	0.320	0.323	1.91	1.83	1.85	1.86	0.04

APPENDIX D

Encapsulation efficiency data of PTU in liposomes

(Effects of preparation method, lipid concentration, and equilibrating time)

Effects of preparation method, lipid concentration, and equilibrating time on entrapment of PTU in liposomes

a) Immediately after preparation (PC 10 mg/ml)

Preparation method	n	PTU in system (mg)	Absorbance		PTU amount (mg)		Total PTU (mg)	%Recovery	%Entrapment	PC amount (mg)	mmol PTU/mol PC
			free drug	pellet	free drug	pellet					
Organic PTU method	1	1.1	0.892	0.133	0.90	0.13	1.03	93.64	12.98	8.91	66.39
	2	1.1	0.679	0.137	0.76	0.15	0.91	82.73	16.79	8.24	81.62
	3	1.1	0.748	0.159	0.83	0.18	1.01	91.82	17.53	8.88	87.90
	Mean						0.98	89.40	15.77	8.68	78.64
	SD						0.06	5.84	2.44	0.38	11.06
Organic-Aqueous PTU method	1	1.5	0.990	0.111	1.27	0.14	1.41	94.00	10.08	8.56	73.34
	2	1.5	0.935	0.116	1.20	0.15	1.35	90.00	11.04	8.98	73.13
	3	1.5	0.899	0.114	1.15	0.15	1.30	86.67	11.25	9.77	66.00
	Mean						1.35	90.22	10.79	9.10	70.82
	SD						0.06	3.67	0.62	0.61	4.18
Aqueous PTU method	1	1.1	0.744	0.110	0.96	0.14	1.10	100.00	12.88	9.56	65.11
	2	1.1	0.702	0.108	0.90	0.14	1.04	94.54	13.33	9.16	66.71
	3	1.1	0.803	0.087	1.03	0.11	1.14	103.64	9.78	9.64	51.04
	Mean						1.09	99.39	12.00	9.45	60.95
	SD						0.05	4.58	1.93	0.26	8.62

b) Immediately after preparation (PC 100 mg/ml)

Preparation method	n	PTU in system (mg)	Absorbance		PTU amount (mg)		Total PTU (mg)	%Recovery	%Entrapment	PC amount (mg)	mmol PTU/mol PC
			free drug	pellet	free drug	pellet					
Organic PTU method	1	1.1	0.321	0.310	0.32	0.78	1.11	100.91	70.71	109.29	31.54
	2	1.1	0.244	0.325	0.25	0.82	1.07	97.27	76.90	91.35	39.56
	3	1.1	0.277	0.333	0.31	0.93	1.24	112.73	75.03	99.67	40.99
	Mean						1.14	103.64	74.21	100.10	37.36
	SD						0.09	8.08	3.17	8.98	5.09
Organic-Aqueous PTU method	1	2.5	0.591	0.689	0.60	1.74	2.34	93.60	74.45	100.64	76.13
	2	2.5	0.536	0.726	0.54	1.83	2.37	94.80	77.20	92.31	87.46
	3	2.5	0.528	0.735	0.59	1.86	2.44	97.60	75.92	87.83	93.06
	Mean						2.38	95.33	75.86	93.59	85.55
	SD						0.05	2.05	1.38	6.50	8.62
Aqueous PTU method	1	1.1	0.186	0.251	0.24	0.80	1.04	94.54	77.14	89.05	39.87
	2	1.1	0.165	0.256	0.21	0.82	1.04	94.54	79.50	89.48	40.47
	3	1.1	0.177	0.271	0.23	0.87	1.10	100.00	79.29	91.05	42.11
	Mean						1.06	96.36	78.64	89.86	40.82
	SD						0.03	3.15	1.31	1.05	1.16

c) After one week of storage (PC 10 mg/ml)

Preparation method	n	PTU in system (mg)	Absorbance		PTU amount (mg)		Total PTU (mg)	%Recovery	%Entrapment	PC amount (mg)	mmol PTU/mol PC
			free drug	pellet	free drug	pellet					
Organic PTU method	1	1.1	0.878	0.126	0.96	0.14	1.10	100.00	12.55	10.35	58.65
	2	1.1	0.696	0.121	0.85	0.15	1.00	90.91	14.81	9.17	71.00
	3	1.1	0.663	0.118	0.81	0.14	0.95	86.36	15.11	8.75	72.54
	Mean						1.02	92.42	14.16	9.42	67.40
	SD						0.08	6.94	1.40	0.83	7.61
Organic-Aqueous PTU method	1	1.5	0.233	0.169	1.35	0.20	1.55	103.33	12.67	10.41	82.97
	2	1.5	0.236	0.172	1.29	0.19	1.47	98.00	12.72	9.74	84.90
	3	1.5	0.210	0.180	1.22	0.21	1.43	95.33	14.63	9.46	97.25
	Mean						1.48	98.89	13.34	9.87	88.37
	SD						0.06	4.07	1.12	0.49	7.75
Aqueous PTU method	1	1.1	0.831	0.132	0.96	0.15	1.12	101.82	13.71	8.33	81.04
	2	1.1	0.840	0.145	0.92	0.16	1.07	97.27	14.72	8.79	79.32
	3	1.1	0.876	0.151	1.02	0.18	1.19	108.18	14.70	9.52	81.06
	Mean						1.13	102.42	14.38	8.88	80.47
	SD						0.06	5.48	0.58	0.60	1.00

d) After one week of storage (PC 100 mg/ml)

Preparation method	n	PTU in system (mg)	Absorbance		PTU amount (mg)		Total PTU (mg)	%Recovery	%Entrapment	PC amount (mg)	mmol PTU/mol PC
			free drug	pellet	free drug	pellet					
Organic PTU method	1	1.1	0.205	0.303	0.22	0.83	1.05	95.45	78.70	100.64	36.27
	2	1.1	0.199	0.303	0.22	0.83	1.05	95.45	79.19	99.04	36.86
	3	1.1	0.215	0.311	0.26	0.95	1.21	110.00	78.34	90.07	46.43
	Mean						1.10	100.30	78.74	96.58	39.85
	SD						0.09	8.40	0.43	5.70	5.70
Organic-Aqueous PTU method	1	2.5	0.491	0.687	0.54	1.88	2.42	96.80	77.77	102.56	80.70
	2	2.5	0.444	0.697	0.48	1.91	2.39	95.60	79.69	89.11	94.23
	3	2.5	0.300	0.629	0.37	1.92	2.29	91.60	83.98	91.35	92.60
	Mean						2.37	94.67	80.48	94.34	89.17
	SD						0.07	2.72	3.18	7.21	7.39
Aqueous PTU method	1	1.1	0.223	0.297	0.26	0.86	1.12	101.82	76.90	89.40	42.46
	2	1.1	0.214	0.323	0.23	0.88	1.11	100.91	79.05	88.78	43.71
	3	1.1	0.217	0.338	0.25	0.98	1.23	111.82	79.57	87.86	49.16
	Mean						1.15	104.85	78.51	88.68	45.11
	SD						0.07	6.05	1.42	0.77	3.56

Phosphorus content

a) Immediately after preparation (PC 10 mg/ml)

Preparation method	Abs.			Abs. - Blank Abs.			PC amount (mg/ml)		
	1	2	3	1	2	3	1	2	3
Organic PTU method	0.502	0.481	0.501	0.322	0.301	0.321	8.91	8.24	8.88
Organic-Aqueous PTU method	0.872	0.901	0.957	0.504	0.533	0.589	8.56	8.98	9.77
Aqueous PTU method	0.942	0.914	0.948	0.574	0.546	0.580	9.56	9.16	9.64

b) Immediately after preparation (PC 100 mg/ml)

Preparation method	Abs.			Abs. - Blank Abs.			PC amount (mg/ml)		
	1	2	3	1	2	3	1	2	3
Organic PTU method	0.565	0.509	0.535	0.385	0.329	0.355	109.29	91.35	99.68
Organic-Aqueous PTU method	0.538	0.512	0.498	0.358	0.332	0.318	100.64	92.31	87.83
Aqueous PTU method	0.896	0.899	0.910	0.528	0.531	0.542	89.05	89.48	91.05

Calibration curve

: for PC 10 mg/ml organic-aqueous PTU method and aqueous PTU method

: and for 100 mg/ml aqueous PTU method

Concentration (umol/ml)	0.064	0.096	0.128	0.224
Abs.	0.601	0.779	0.952	1.447
Abs. - Blank Abs.	0.233	0.411	0.584	1.079

$y = 5.2679x-0.0975$; $R^2 = 0.9997$

Calibration curve

: for PC 10 mg/ml organic PTU method

: and for 100 mg/ml organic PTU method and organic-aqueous PTU method

Concentration (umol/ml)	0.064	0.096	0.128	0.160	0.192	0.224	0.256
Abs.	0.389	0.436	0.520	0.587	0.686	0.742	0.829
Abs. - Blank Abs.	0.209	0.256	0.340	0.407	0.506	0.562	0.649

$y = 2.3415x-0.0438$; $R^2 = 0.9951$

c) After one week of storage (PC 10 mg/ml)

Preparation method	Abs.			Abs. - Blank Abs.			PC amount (mg/ml)		
	1	2	3	1	2	3	1	2	3
Organic PTU method	0.547	0.510	0.497	0.367	0.330	0.317	10.35	9.17	8.75
Organic-Aqueous PTU method	0.602	0.580	0.571	0.339	0.317	0.308	10.41	9.74	9.46
Aqueous PTU method	0.534	0.549	0.573	0.271	0.286	0.310	8.33	8.79	9.52

d) After one week of storage (PC 100 mg/ml)

Preparation method	Abs.			Abs. - Blank Abs.			PC amount (mg/ml)		
	1	2	3	1	2	3	1	2	3
Organic PTU method	0.538	0.533	0.505	0.358	0.353	0.325	100.64	99.04	90.07
Organic-Aqueous PTU method	0.544	0.502	0.509	0.364	0.322	0.329	102.56	89.11	91.35
Aqueous PTU method	0.554	0.552	0.549	0.291	0.289	0.286	89.40	88.78	87.86

Calibration curve

: for PC 10 mg/ml organic-aqueous PTU method and aqueous PTU method

: and for 100 mg/mi aqueous PTU method

Concentration (umol/ml)	0.064	0.096	0.160	0.192	0.224	0.256
Abs.	0.406	0.512	0.657	0.731	0.814	0.882
Abs. - Blank Abs.	0.142	0.249	0.394	0.468	0.551	0.619

$y = 2.4447x-0.0004$; $R^2 = 0.9971$

Calibration curve

: for PC 10 mg/ml organic PTU method

: and for 100 mg/ml organic PTU method and organic-aqueous PTU method

Concentration (umol/ml)	0.064	0.096	0.128	0.160	0.192	0.224	0.256
Abs.	0.389	0.436	0.520	0.587	0.686	0.742	0.829
Abs. - Blank Abs.	0.209	0.256	0.340	0.407	0.506	0.562	0.649

$y = 2.3415x-0.0438$; $R^2 = 0.9951$

APPENDIX E

Encapsulation efficiency data of PTU in liposomes

(Effects of liposomal surface charge, pH, and cholesterol content)

Effect of liposomal composition on entrapment of PTU in liposomes

a) Neutral liposomes without cholesterol

pH	PTU in system (mg)	Absorbance	PTU amount (mg)	PC (mmol)	Chol (mmol)	Charge (mmol)	total lipid (mmol)	mmol PTU/mol lipid
5.5	2.2	0.242	1.40	0.12	0.00	0.00	0.12	71.40
5.5	2.2	0.260	1.43	0.10	0.00	0.00	0.10	81.98
5.5	2.2	0.246	1.58	0.12	0.00	0.00	0.12	77.84
Mean								77.07
SD								5.33
7.4	2.5	0.689	1.74	0.13	0.00	0.00	0.13	76.13
7.4	2.5	0.726	1.83	0.12	0.00	0.00	0.12	87.46
7.4	2.5	0.735	1.86	0.12	0.00	0.00	0.12	93.06
Mean								85.55
SD								8.62
9	3.2	0.114	1.32	0.11	0.00	0.00	0.11	68.43
9	3.2	0.287	1.38	0.11	0.00	0.00	0.11	73.17
9	3.2	0.298	1.68	0.12	0.00	0.00	0.12	83.55
Mean								75.05
SD								7.73

b) Neutral liposomes with cholesterol

pH	PTU in system (mg)	Absorbance	PTU amount (mg)	PC (mmol)	Chol (mmol)	Charge (mmol)	total lipid (mmol)	mmol PTU/mol lipid
5.5	1.4	0.154	0.80	0.10	0.08	0.00	0.18	26.39
5.5	1.4	0.123	0.81	0.08	0.07	0.00	0.15	30.84
5.5	1.4	0.131	0.86	0.10	0.08	0.00	0.18	28.31
Mean								28.51
SD								2.23
7.4	1.9	0.145	0.79	0.08	0.07	0.00	0.15	31.62
7.4	1.9	0.153	0.84	0.09	0.07	0.00	0.16	30.99
7.4	1.9	0.106	0.70	0.10	0.08	0.00	0.18	23.20
Mean								28.60
SD								4.69
9	3.2	0.211	1.14	0.08	0.06	0.00	0.14	48.14
9	3.2	0.220	1.19	0.07	0.06	0.00	0.13	51.51
9	3.2	0.190	1.23	0.09	0.08	0.00	0.17	43.24
Mean								47.63
SD								4.16

c) Positive liposomes without cholesterol

pH	PTU in system (mg)	Absorbance	PTU amount (mg)	PC (mmol)	Chol (mmol)	Charge (mmol)	total lipid (mmol)	mmol PTU/mol lipid
5.5	2.2	0.226	1.31	0.12	0.00	0.01	0.13	58.99
5.5	2.2	0.212	1.14	0.10	0.00	0.01	0.11	62.19
5.5	2.2	0.188	1.22	0.11	0.00	0.01	0.12	58.03
Mean								59.74
SD								2.18
7.4	2.5	0.319	1.57	0.11	0.00	0.01	0.12	77.54
7.4	2.5	0.105	0.70	0.10	0.00	0.01	0.11	37.68
7.4	2.5	0.206	1.33	0.11	0.00	0.01	0.12	66.00
Mean								60.41
SD								20.51
9	3.2	0.182	2.10	0.12	0.00	0.01	0.13	92.31
9	3.2	0.436	2.52	0.13	0.00	0.01	0.14	105.87
9	3.2	0.368	2.34	0.14	0.00	0.01	0.15	93.06
Mean								97.08
SD								7.62

d) Positive liposomes with cholesterol

pH	PTU in system (mg)	Absorbance	PTU amount (mg)	PC (mmol)	Chol (mmol)	Charge (mmol)	total lipid (mmol)	mmol PTU/mol lipid
5.5	1.8	0.200	1.07	0.09	0.08	0.01	0.18	34.29
5.5	1.8	0.133	0.87	0.08	0.07	0.01	0.16	30.87
5.5	1.8	0.145	0.95	0.08	0.08	0.01	0.17	32.12
Mean								32.43
SD								1.73
7.4	2.5	0.239	1.08	0.07	0.06	0.01	0.14	43.15
7.4	2.5	0.250	1.40	0.09	0.08	0.01	0.18	46.38
7.4	2.5	0.237	1.52	0.09	0.08	0.01	0.18	49.04
Mean								46.19
SD								2.95
9	3.2	0.371	1.89	0.09	0.07	0.01	0.17	63.77
9	3.2	0.349	1.98	0.10	0.08	0.01	0.19	59.32
9	3.2	0.271	1.74	0.08	0.07	0.01	0.16	62.20
Mean								61.76
SD								2.25

e) Negative liposomes without cholesterol

pH	PTU in system (mg)	Absorbance	PTU amount (mg)	PC (mmol)	Chol (mmol)	Charge (mmol)	total lipid (mmol)	mmol PTU/mol lipid
5.5	2.2	0.247	1.43	0.11	0.00	0.01	0.12	68.85
5.5	2.2	0.201	1.08	0.12	0.00	0.01	0.13	50.46
5.5	2.2	0.233	1.50	0.11	0.00	0.01	0.12	74.68
Mean								64.66
SD								12.64
7.4	2.5	0.328	1.63	0.08	0.00	0.01	0.09	109.82
7.4	2.5	0.389	2.21	0.12	0.00	0.01	0.13	102.70
7.4	2.5	0.268	1.72	0.11	0.00	0.01	0.12	82.66
Mean								98.40
SD								14.08
9	3.2	0.296	1.43	0.11	0.00	0.01	0.12	71.64
9	3.2	0.234	1.50	0.11	0.00	0.01	0.12	73.65
9	3.2	0.214	1.38	0.11	0.00	0.01	0.12	67.94
Mean								71.07
SD								2.90

f) Negative liposomes with cholesterol

pH	PTU in system (mg)	Absorbance	PTU amount (mg)	PC (mmol)	Chol (mmol)	Charge (mmol)	total lipid (mmol)	mmol PTU/mol lipid
5.5	2.2	0.151	0.54	0.07	0.06	0.01	0.14	22.00
5.5	2.2	0.106	0.71	0.07	0.06	0.01	0.14	29.22
5.5	2.2	0.122	0.80	0.08	0.07	0.01	0.16	29.60
Mean								26.94
SD								4.28
7.4	1.9	0.225	1.00	0.07	0.07	0.01	0.15	37.92
7.4	1.9	0.143	0.78	0.07	0.06	0.01	0.14	31.68
7.4	1.9	0.072	0.49	0.05	0.04	0.01	0.10	30.02
Mean								33.21
SD								4.16
9	2.6	0.188	1.05	0.08	0.07	0.01	0.06	39.22
9	2.6	0.162	1.05	0.08	0.07	0.01	0.16	38.75
9	2.6	0.206	1.33	0.08	0.07	0.01	0.16	48.75
Mean								67.94
SD								5.64

Phosphorus content

a) Without cholesterol

Charge	pH	Abs.			Abs. - Blank Abs.			PC amount (mmol/ml)		
		1	2	3	1	2	3	1	2	3
Neutral	5.5	0.696*	0.658*	0.951	0.356	0.318	0.648	0.12	0.10	0.12
	7.4	0.538	0.512	0.498	0.358	0.332	0.318	0.13	0.12	0.12
	9	0.691*	0.684*	0.946	0.350	0.343	0.643	0.11	0.11	0.12
Positive	5.5	0.710*	0.650*	0.612	0.370	0.310	0.310	0.12	0.10	0.11
	7.4	0.681*	0.576	0.600	0.340	0.273	0.297	0.11	0.10	0.11
	9	0.721*	0.654	1.043	0.380	0.350	0.740	0.12	0.13	0.14
Negative	5.5	0.716*	0.728*	0.624	0.376	0.388	0.322	0.11	0.12	0.11
	7.4	0.614*	0.648	0.636	0.273	0.344	0.332	0.08	0.12	0.11
	9	0.704*	0.630	0.628	0.364	0.327	0.325	0.11	0.11	0.11

b) With cholesterol

Charge	pH	Abs.			Abs. - Blank Abs.			PC amount (mmol/ml)		
		1	2	3	1	2	3	1	2	3
Neutral	5.5	0.645*	0.533	0.833	0.305	0.230	0.530	0.10	0.08	0.10
	7.4	0.523	0.540	0.832	0.220	0.238	0.529	0.08	0.09	0.10
	9	0.580*	0.575*	0.799	0.240	0.234	0.496	0.08	0.07	0.09
Positive	5.5	0.629*	0.530	0.776	0.288	0.228	0.473	0.09	0.08	0.08
	7.4	0.574*	0.546	0.801	0.234	0.244	0.498	0.07	0.09	0.09
	9	0.614*	0.571	0.752	0.274	0.268	0.448	0.09	0.10	0.08
Negative	5.5	0.594*	0.518	0.780	0.253	0.214	0.478	0.07	0.07	0.08
	7.4	0.608*	0.522	0.448	0.268	0.219	0.146	0.07	0.07	0.05
	9	0.540	0.544	0.785	0.237	0.241	0.482	0.08	0.08	0.08

Calibration curve : for neutral liposomes without cholesterol, pH 7.4

Concentration (umol/ml)	0.064	0.096	0.128	0.160	0.192	0.224	0.256
Abs.	0.389	0.436	0.520	0.587	0.686	0.742	0.829
Abs. - Blank Abs.	0.209	0.256	0.340	0.407	0.506	0.562	0.649

$y = 2.3415x-0.0438$; $R^2 = 0.9951$

Calibration curve : for unmarked data

Concentration (umol/ml)	0.064	0.096	0.128	0.160	0.192	0.224	0.256
Abs.	0.486	0.566	0.649	0.728	0.816	0.922	1.003
Abs. - Blank Abs.	0.183	0.263	0.346	0.425	0.513	0.619	0.700

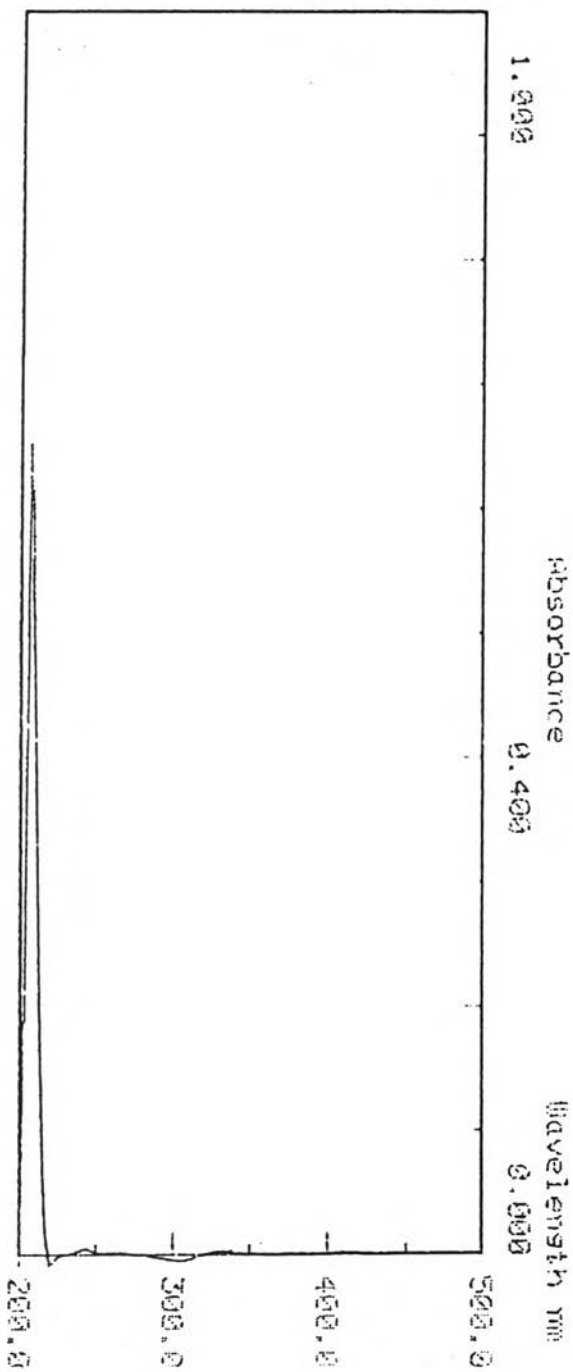
$y = 2.7109x-0.0017$; $R^2 = 0.9982$

Calibration curve : for marked data (*)

Concentration (umol/ml)	0.064	0.096	0.128	0.160	0.192	0.224	0.256
Abs.	0.561	0.637	0.718	0.842	0.909	1.006	1.141
Abs. - Blank Abs.	0.221	0.297	0.377	0.502	0.568	0.665	0.801

$y = 2.9777x-0.0134$; $R^2 = 0.9927$

The UV spectra of non-drug containing liposomal pellet analysed by the method used in quantitative analysis of PTU in liposomal pellet.



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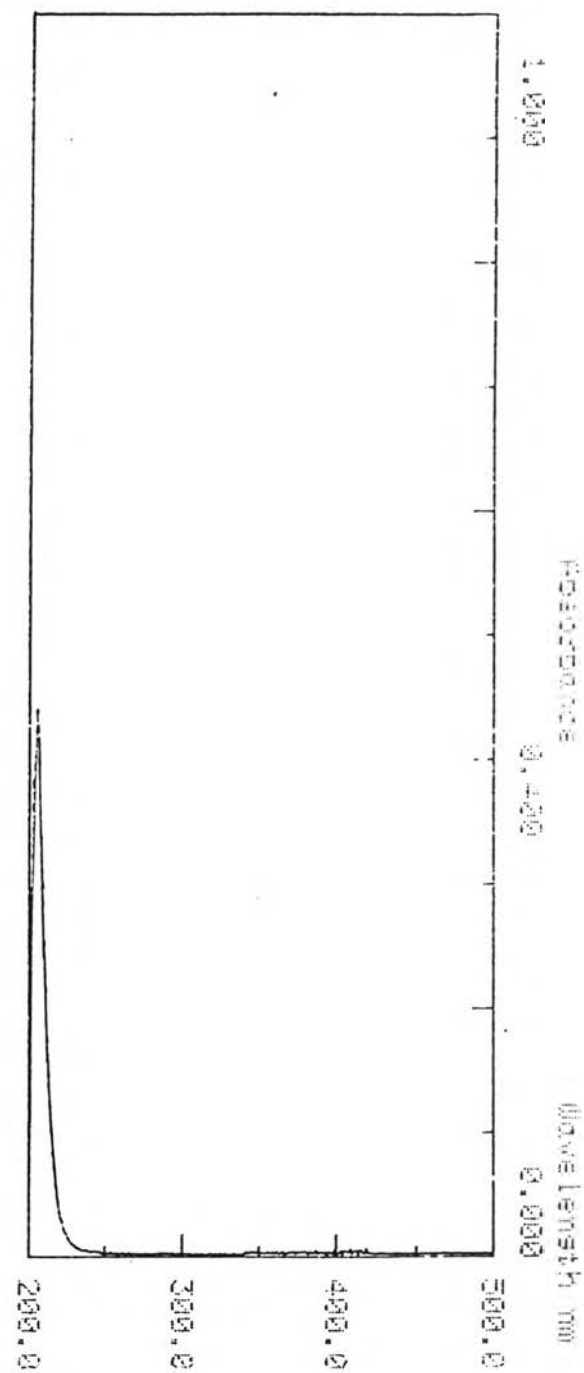
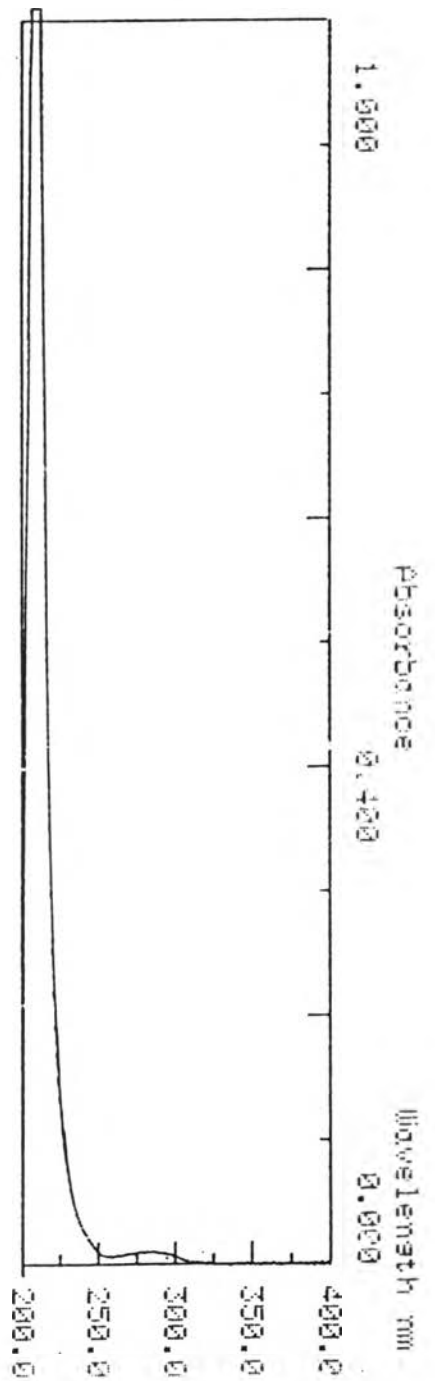


PC/CH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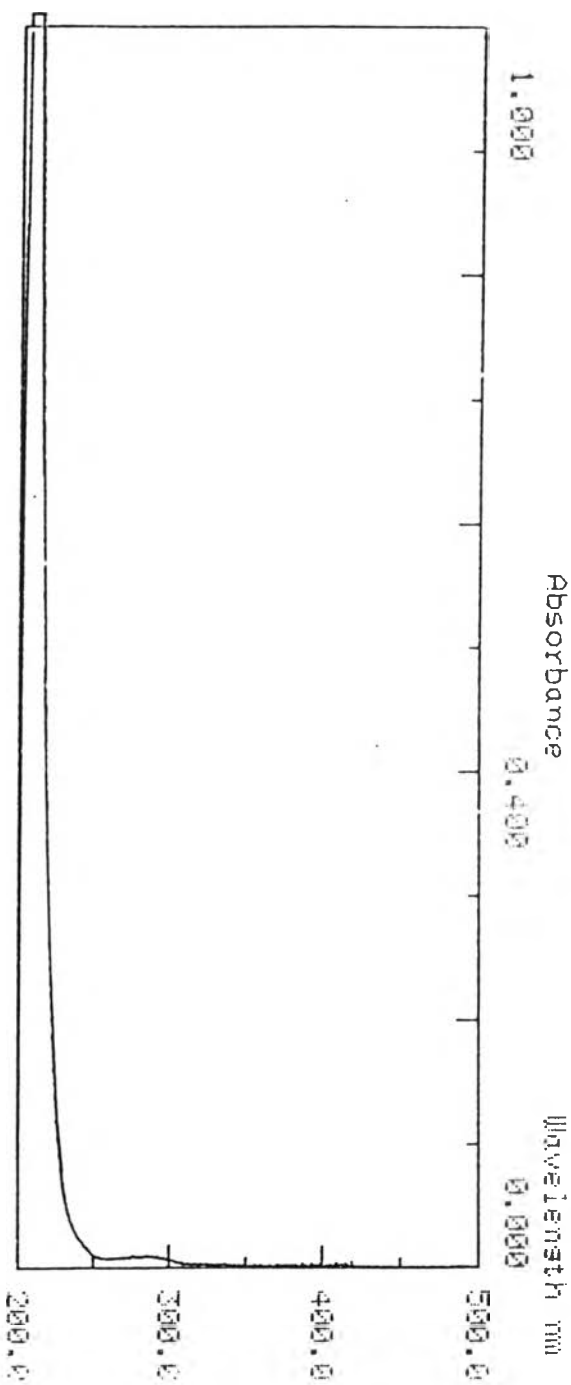
Absorbance
Wavelength nm

PC/SA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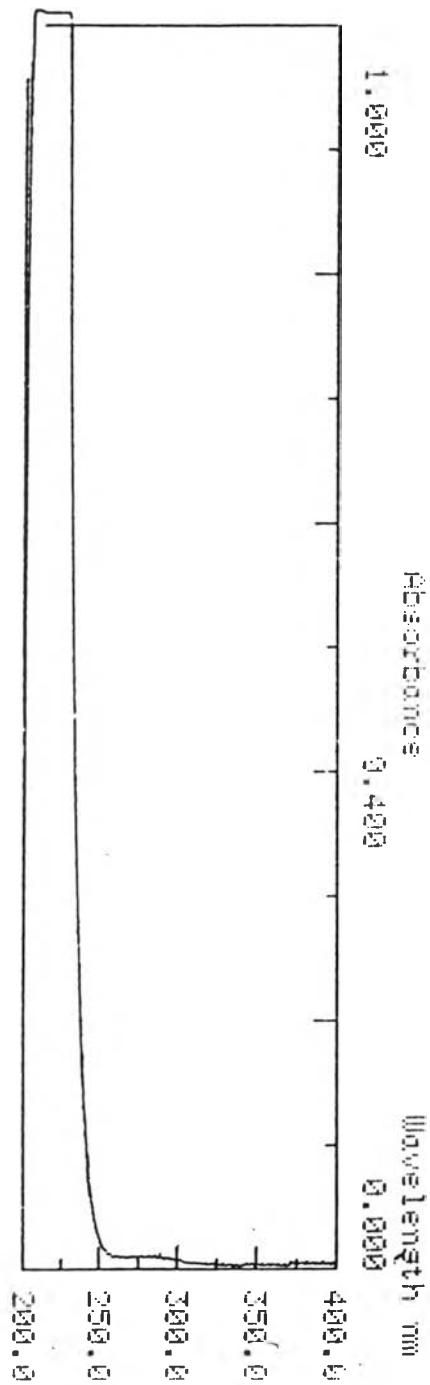
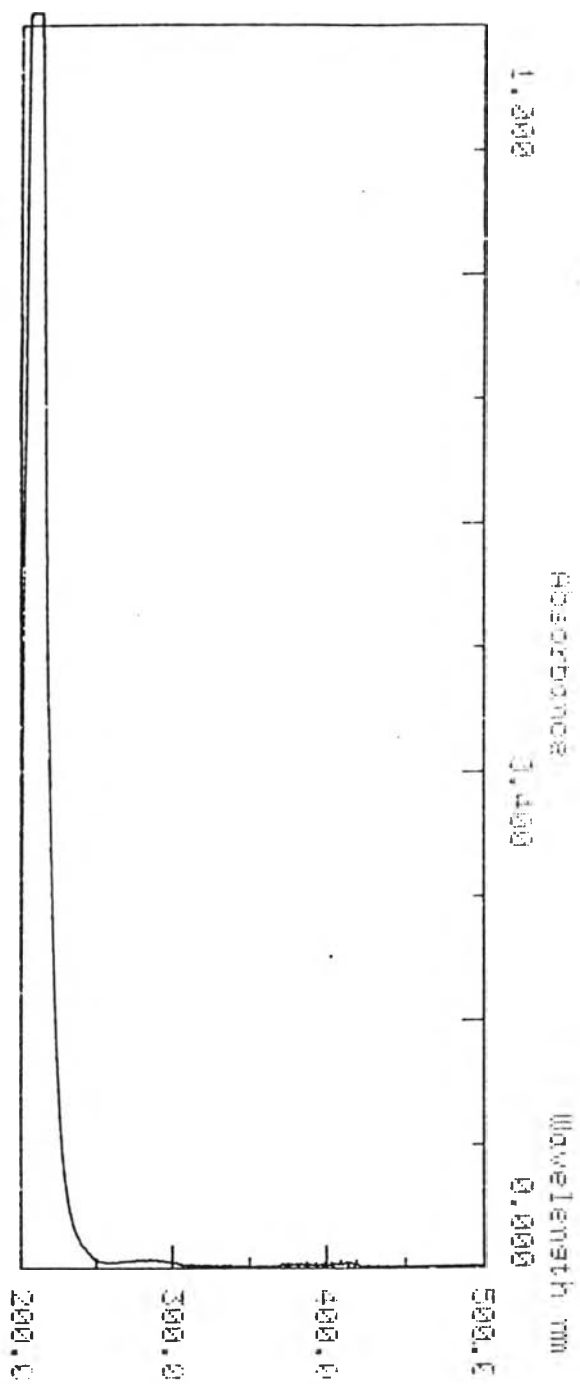
PC/CH/SA



PC/DCP



PC/CH/DCP

APPENDIX F

Release data of PTU

Drug release studies

a) Release of PTU from saturated solution

time (hr)	Absorbance					PTU amount (ug)					% Released					Mean	SD
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5		
0.5	0.249	0.279	0.274	0.259	0.302	580.26	619.35	614.93	578.87	656.14	32.24	34.41	34.16	32.16	36.45	33.88	1.78
1	0.310	0.338	0.298	0.319	0.380	985.54	1003.08	957.67	986.59	1099.46	54.75	55.73	53.20	54.81	61.08	55.91	3.03
2	0.409	0.358	0.343	0.441	0.536	1544.78	1353.26	1373.34	1597.60	1784.29	85.82	75.18	76.30	88.76	99.13	85.04	9.83
4	0.292	0.389	0.317	0.326	0.340	1701.58	1746.21	1676.23	1804.18	1841.07	94.53	97.01	93.12	100.23	102.28	97.44	3.82
6	0.161	0.265	0.190	0.196	0.199	1701.41	1820.77	1723.20	1855.00	1839.95	94.52	101.15	95.73	103.06	102.22	99.34	3.92
8	0.850	0.155	0.240	0.231	0.219	1695.73	1813.91	1766.61	1881.69	1825.48	94.21	100.77	98.14	104.54	101.42	99.82	3.87
12	0.428	0.915	0.636	0.577	0.683	1686.53	1815.05	1767.12	1874.74	1835.76	93.70	100.84	98.17	104.15	101.99	99.77	4.02
16	0.223	0.488	0.370	0.342	0.408	1683.55	1802.57	1774.23	1882.79	1837.52	93.53	100.14	98.57	104.60	102.08	99.79	4.16
20	0.048	0.344	0.221	0.234	0.267	1665.88	1814.56	1779.57	1894.53	1843.60	92.55	100.81	98.87	105.25	102.42	99.98	4.77
receptor volume (ml)	15.505	14.757	14.921	14.866	14.435												

b) Release of PTU from liposomes composed of PC, pH 7.4 (PTU content = 2.55 mg)

Time	Absorbance			PTU amount (ug)			%Released			Mean	SD
(hr)	1	2	3	1	2	3	1	2	3		
0.5	0.841	0.287	0.239	149.47	237.95	202.00	5.86	9.33	7.92	7.70	1.75
1	0.985	0.210	0.313	224.23	249.73	334.86	8.79	9.79	13.13	10.57	2.27
2	0.266	0.336	0.395	331.55	417.76	496.85	13.00	16.38	19.48	16.29	3.24
4	0.365	0.505	0.512	494.81	660.88	714.90	19.40	25.92	28.04	24.45	4.50
6	0.389	0.512	0.528	619.27	811.06	875.54	24.29	31.81	34.33	30.14	5.23
8	0.267	0.492	0.492	723.75	939.50	993.56	28.38	36.84	38.96	34.73	5.60
12	0.351	0.548	0.575	873.71	1129.04	1209.29	34.26	44.28	47.42	41.99	6.87
16	0.439	0.598	0.542	1052.09	1329.83	1343.92	41.26	52.15	52.70	48.70	6.45
20	0.421	0.536	0.506	1160.29	1446.62	1466.08	45.50	56.73	57.49	53.24	6.71
24	0.399	0.452	0.455	1259.55	1525.72	1563.95	49.39	59.83	61.33	56.85	6.50
48	0.667	0.726	0.607	1615.37	1895.66	1836.74	63.35	74.34	72.03	69.91	5.80
receptor volume (ml)	15.366	14.935	15.421								

c) Release of PTU from liposomes composed of PC:DCP, pH 7.4 (PTU content = 3.135 mg)

Time	Absorbance			PTU amount (ug)			%Released			Mean	SD
(hr)	1	2	3	1	2	3	1	2	3		
0.5	0.231	0.234	0.201	213.14	205.95	177.36	6.80	6.57	5.66	6.34	0.60
1	0.253	0.262	0.256	301.04	299.30	286.21	9.60	9.55	9.13	9.43	0.26
2	0.327	0.319	0.350	445.98	427.92	448.11	14.23	13.65	14.29	14.06	0.35
4	0.437	0.450	0.430	648.19	642.12	625.39	20.68	20.48	19.95	20.37	0.38
6	0.453	0.423	0.485	792.63	1130.55	803.54	25.28	36.06	25.63	28.99	6.13
8	0.493	0.556	0.566	911.65	1525.86	965.23	29.08	48.67	30.79	36.18	10.85
12	0.711	0.735	0.700	1210.38	1385.78	1191.11	38.61	44.20	37.99	40.27	3.42
16	0.395	0.819	0.684	1403.38	1594.83	1301.29	44.76	50.87	41.51	45.72	4.75
20	0.354	0.409	0.402	1463.40	1730.48	1525.26	46.68	55.20	48.65	50.18	4.46
24	0.360	0.422	0.402	1599.61	1899.05	1667.27	51.02	60.58	53.18	54.93	5.01
48	0.545	0.634	0.584	2084.83	2439.90	2149.06	66.50	77.83	68.55	70.96	6.04
receptor volume (ml)	16.005	15.257	15.421								

d) Release of PTU from liposomes composed of PC:CH:DCP, pH 7.4 (PTU content = 2.04 mg)

Time	Absorbance			PTU amount (ug)			%Released			Mean	SD
(hr)	1	2	3	1	2	3	1	2	3		
0.5	0.200	0.209	0.170	175.80	179.00	148.42	8.62	8.77	7.28	8.22	0.82
1	0.259	0.293	0.202	287.88	314.87	226.42	14.11	15.43	11.10	13.55	2.22
2	0.339	0.382	0.342	437.35	480.68	414.91	21.44	23.56	20.34	21.78	1.64
4	0.460	0.477	0.454	649.17	678.86	619.64	31.82	33.28	30.37	31.82	1.45
6	0.458	0.198	0.453	783.21	736.72	752.79	38.39	36.11	36.90	37.14	1.16
8	0.503	0.254	0.541	906.24	905.90	915.20	44.42	44.41	44.86	44.56	0.26
12	0.633	0.511	0.614	1116.50	1006.81	1079.59	54.73	49.35	52.92	52.34	2.74
16	0.357	0.265	0.625	1294.57	1104.84	1199.36	63.46	54.16	58.79	58.80	4.65
20	0.312	0.254	0.323	1336.52	1177.20	1320.20	65.52	57.71	64.72	62.65	4.30
24	0.314	0.214	0.286	1449.56	1193.14	1364.44	71.06	58.49	66.88	65.48	6.40
48	0.316	0.216	0.253	1563.33	1270.48	1402.71	76.63	62.28	68.76	69.22	7.19
receptor volume (ml)	15.366	14.935	15.421								

e) Release of PTU from liposomes composed of PC:SA, pH 9.0 (PTU content = 3.7 mg)

Time	Absorbance			PTU amount (ug)			%Released			Mean	SD
(hr)	1	2	3	1	2	3	1	2	3		
0.5	0.235	0.286	0.221	282.51	328.26	255.85	7.64	8.87	6.91	7.81	0.99
1	0.272	0.344	0.274	415.63	502.87	400.72	11.23	13.59	10.83	11.88	1.49
2	0.379	0.462	0.378	647.64	768.80	625.25	17.50	20.78	16.90	18.39	2.09
4	0.458	0.602	0.421	886.23	1104.86	817.91	23.95	29.86	22.11	25.31	4.05
6	0.547	0.614	0.460	1166.87	1346.01	1022.18	31.54	36.38	27.63	31.85	4.38
8	0.513	0.611	0.470	1332.09	1574.36	1207.35	36.00	42.55	32.63	37.06	5.04
12	0.602	0.669	0.528	1633.56	1872.08	1452.38	44.15	50.60	39.25	44.67	5.69
16	0.577	0.704	0.550	1830.52	2165.18	1677.33	49.47	58.52	45.33	51.11	6.74
20	0.552	0.698	0.539	2018.01	2424.16	1872.05	54.54	65.52	50.60	56.88	7.73
24	0.514	0.598	0.544	2180.27	2572.21	2081.30	58.93	69.52	56.25	61.57	7.02
48	0.731	0.695	0.674	2637.32	2910.08	2438.48	71.28	78.65	65.90	71.94	6.40
receptor volume (ml)	16.005	15.257	15.421								

Calibration data for PTU saturated solution and PTU liposomes composed of PC:SA, pH 9.0

Concentration (ug/ml)	1.4	2.8	4.2	5.6	7.0	8.4	9.8
Absorbance at 261 nm	0.095	0.189	0.279	0.371	0.459	0.553	0.651

$y = 0.066x+0.002$; $R^2 = 0.9999$

Calibration data for PTU liposomes composed of PC, pH 7.4

Concentration (ug/ml)	1.4	2.8	4.2	5.6	7.0	8.4	9.8
Absorbance at 275 nm	0.129	0.254	0.376	0.497	0.618	0.727	0.840

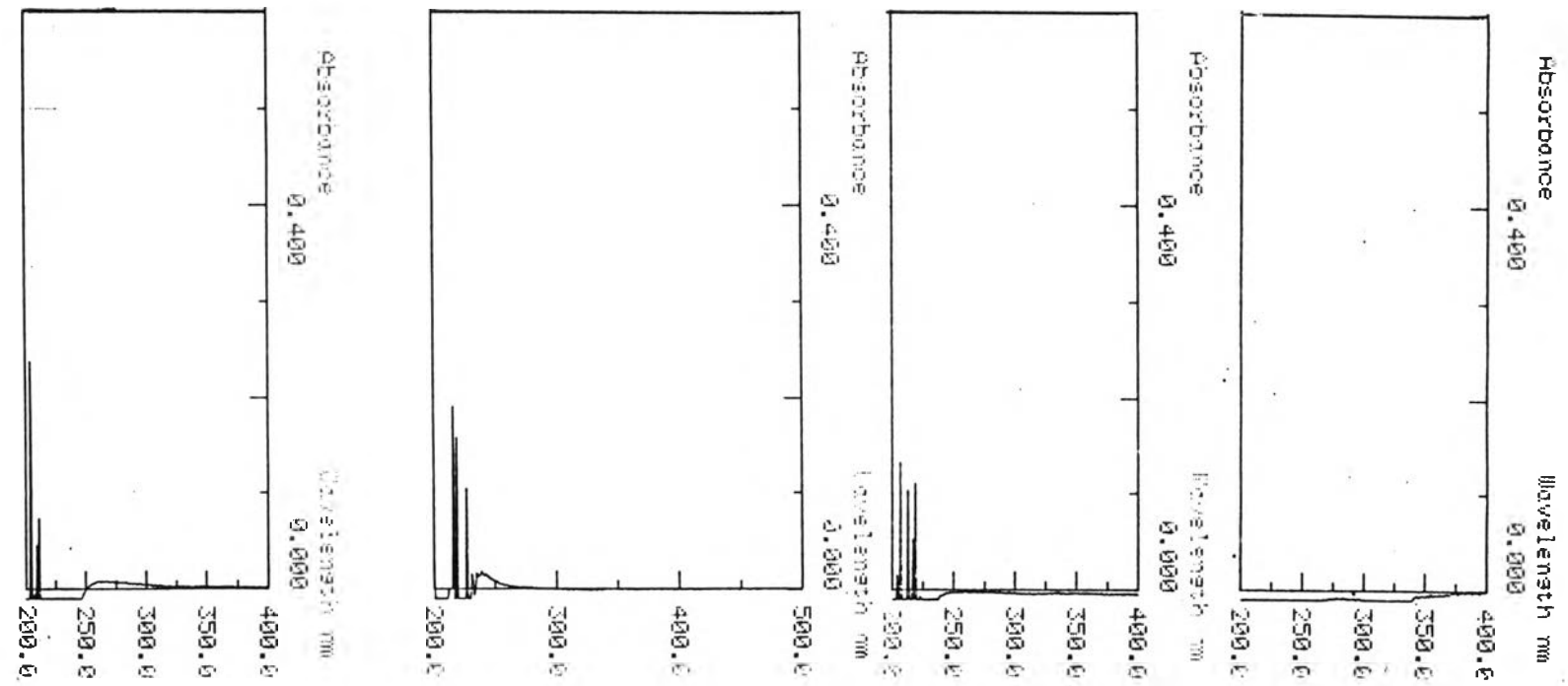
$y = 0.0847x+0.0171$; $R^2 = 0.9995$

Calibration data for PTU liposomes composed of PC:DCP and PC:CH:DCP, pH 7.4

Concentration (ug/ml)	1.4	2.8	4.2	5.6	7.0	8.4	9.8
Absorbance at 275 nm	0.117	0.241	0.363	0.484	0.596	0.704	0.810

$y = 0.0826x+0.011$; $R^2 = 0.999$

The UV spectra of the receptor fluid taken from non-drug containing liposomal systems.



(a) PC **(b) PC/DCP** **(c) PC/CH/DCP** **(d) PC/SA**

APPENDIX G

Water evaporation data

Water evaporation studies

a) Water evaporation from saturated solution of PTU

time(min)	weight (mg)			weight loss (mg)			cumulative weight loss (mg)			% cumulative weight loss			mean	SD
	1	2	3	1	2	3	1	2	3	1	2	3		
0	50.89	47.84	50.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	45.60	43.22	46.14	5.29	4.62	4.47	5.29	4.62	4.47	13.99	13.25	11.97	13.07	1.02
10	40.74	38.35	40.03	4.86	4.87	6.11	10.15	9.49	10.58	26.85	27.22	28.33	27.47	0.77
15	36.94	34.79	35.86	3.80	3.56	4.17	13.95	13.05	14.75	36.90	37.44	39.49	37.94	1.37
20	33.23	30.96	32.51	3.71	3.83	3.35	17.66	16.88	18.10	46.72	48.42	48.46	47.87	0.99
25	29.58	27.65	28.68	3.65	3.31	3.83	21.31	20.19	21.93	56.38	57.92	58.71	57.67	1.18
30	25.97	24.32	25.71	3.61	3.33	2.97	24.92	23.52	24.90	65.93	67.47	66.67	66.69	0.77
35	22.75	21.06	22.62	3.22	3.26	3.09	28.14	26.78	27.99	74.44	76.82	74.94	75.40	1.25
40	19.91	18.30	20.07	2.84	2.76	2.55	30.98	29.54	30.54	81.96	84.74	81.77	82.82	1.66
45	17.42	16.11	17.40	2.49	2.19	2.67	33.47	31.73	33.21	88.54	91.02	88.92	89.49	1.34
50	15.40	14.52	15.79	2.02	1.59	1.61	35.49	33.32	34.82	93.89	95.58	93.23	94.23	1.21
55	14.45	13.83	14.64	0.95	0.69	1.15	36.44	34.01	35.97	96.40	97.56	96.30	96.75	0.70
60	14.11	13.78	14.25	0.34	0.05	0.39	36.78	34.06	36.36	97.30	97.70	97.35	97.45	0.22
65	14.09	13.77	14.23	0.02	0.01	0.02	36.80	34.07	36.38	97.35	97.73	97.40	97.49	0.21
70	14.09	13.77	14.23	0.00	0.00	0.00	36.80	34.07	36.38	97.35	97.73	97.40	97.49	0.21
75	14.09	13.77	14.23	0.00	0.00	0.00	36.80	34.07	36.38	97.35	97.73	97.40	97.49	0.21
80	14.09	13.77	14.23	0.00	0.00	0.00	36.80	34.07	36.38	97.35	97.73	97.40	97.49	0.21
85	14.09	13.77	14.23	0.00	0.00	0.00	36.80	34.07	36.38	97.35	97.73	97.40	97.49	0.21
90	14.09	13.77	14.23	0.00	0.00	0.00	36.80	34.07	36.38	97.35	97.73	97.40	97.49	0.21

b) Water evaporation from PTU liposomes composed of PC, pH 7.4

time(min)	weight (mg)			weight loss (mg)			cumulative weight loss (mg)			% cumulative weight loss			mean	SD
	1	2	3	1	2	3	1	2	3	1	2	3		
0	53.65	60.27	50.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	50.08	56.80	47.60	3.57	3.47	2.80	3.57	3.47	2.80	8.84	7.44	7.53	7.94	0.78
10	46.84	54.44	45.41	3.24	2.36	2.19	6.81	5.83	4.99	16.87	12.50	13.43	14.27	2.30
15	44.53	52.18	43.60	2.31	2.26	1.81	9.12	8.09	6.80	22.60	17.35	18.30	19.42	2.80
20	42.78	50.14	41.94	1.75	2.04	1.66	10.87	10.13	8.46	22.93	21.72	22.77	22.47	0.66
25	41.15	48.42	40.52	1.63	1.72	1.42	12.50	11.85	9.88	30.97	25.41	26.59	27.66	2.93
30	39.50	46.83	39.19	1.65	1.59	1.33	14.15	13.44	11.21	35.06	28.82	30.17	31.35	3.28
35	37.82	45.45	37.93	1.68	1.38	1.26	15.83	14.82	12.47	39.22	31.78	33.56	34.85	3.88
40	36.25	44.12	36.60	1.57	1.33	1.33	17.40	16.15	13.80	43.11	34.63	37.14	38.29	4.36
45	34.88	42.89	35.75	1.37	1.23	0.85	18.77	17.38	14.65	46.51	37.27	39.42	41.07	4.84
50	33.77	41.65	34.73	1.11	1.24	1.02	19.88	18.62	15.67	49.26	39.93	42.17	43.79	4.87
55	32.64	40.60	33.65	1.13	1.05	1.08	21.01	19.67	16.75	52.06	42.18	45.08	46.44	5.08
60	31.60	39.58	32.81	1.04	1.02	0.84	22.05	20.69	17.59	54.63	44.37	47.34	48.78	5.28
65	30.67	38.59	31.95	0.93	0.99	0.86	22.98	21.68	18.45	56.94	46.49	49.65	51.03	5.36
70	29.87	37.65	31.07	0.80	0.94	0.88	23.78	22.62	19.33	58.92	48.51	52.02	53.15	5.30
75	29.17	36.78	30.20	0.70	0.87	0.87	24.48	23.49	20.20	60.65	50.38	54.36	55.13	5.18
80	28.45	35.94	29.21	0.72	0.84	0.99	25.20	24.33	21.19	62.44	52.18	57.02	57.21	5.13
85	27.81	35.02	28.17	0.64	0.92	1.04	25.84	25.25	22.23	64.02	54.15	59.82	59.33	4.95
90	27.23	34.00	27.48	0.58	1.02	0.69	26.42	26.27	22.92	65.46	56.34	61.68	61.16	4.58

c) Water evaporation from PTU liposomes composed of PC:DCP, pH 7.4

time(min)	weight (mg)			weight loss (mg)			cumulative weight loss (mg)			% cumulative weight loss			mean	SD
	1	2	3	1	2	3	1	2	3	1	2	3		
0	55.80	53.89	53.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	53.09	51.44	50.01	2.71	2.45	3.81	2.71	2.45	3.81	6.32	6.04	9.40	7.25	1.86
10	51.59	49.96	47.50	1.50	1.48	2.51	4.21	3.93	6.32	9.81	9.68	15.60	11.70	3.38
15	50.43	48.88	46.67	1.16	1.08	0.83	5.37	5.01	7.15	12.51	12.35	17.65	14.17	3.01
20	49.52	47.98	44.46	0.91	0.90	2.21	6.28	5.91	9.36	14.64	14.56	23.10	17.43	4.91
25	48.80	47.26	42.81	0.72	0.72	1.65	7.00	6.63	11.01	16.31	16.34	17.18	16.61	0.49
30	47.68	46.60	41.77	1.12	0.66	1.04	8.12	7.29	12.05	18.92	17.96	29.75	22.21	6.55
35	46.88	45.97	41.13	0.80	0.63	0.64	8.92	7.92	12.69	20.79	19.52	31.32	23.88	6.48
40	46.23	45.40	40.44	0.65	0.57	0.69	9.57	8.49	13.38	22.30	20.92	33.03	25.42	6.63
45	45.62	44.88	39.85	0.61	0.52	0.59	10.18	9.01	13.97	23.72	22.20	34.48	26.80	6.69
50	45.08	44.36	39.22	0.54	0.52	0.63	10.72	9.53	14.60	24.98	23.48	36.04	28.17	6.86
55	44.55	43.87	38.56	0.53	0.49	0.66	11.25	10.02	15.26	26.22	24.69	37.67	29.53	7.09
60	44.04	43.42	38.03	0.51	0.45	0.53	11.76	10.47	15.79	27.41	25.80	38.98	30.73	7.19
65	43.57	42.97	37.54	0.47	0.45	0.49	12.23	10.92	16.28	28.50	26.91	40.19	31.87	7.25
70	43.11	42.53	36.68	0.46	0.44	0.86	12.69	11.36	17.14	29.57	27.99	42.31	33.29	7.85
75	42.66	42.10	36.12	0.45	0.43	0.56	13.14	11.79	17.70	30.62	29.05	43.69	34.45	8.04
80	42.21	41.68	35.61	0.45	0.42	0.51	13.59	12.21	18.21	31.67	30.09	44.95	35.57	8.16
85	41.80	41.27	35.10	0.41	0.41	0.51	14.00	12.62	18.72	32.63	31.10	46.21	36.65	8.32
90	41.41	41.24	34.58	0.39	0.03	0.52	14.39	12.65	19.24	33.54	31.17	47.49	37.40	8.82

d) Water evaporation from PTU liposomes composed of PC:CH:DCP, pH 7.4

time(min)	weight (mg)			weight loss (mg)			cumulative weight loss (mg)			% cumulative weight loss			mean	SD
	1	2	3	1	2	3	1	2	3	1	2	3		
0	45.10	51.98	58.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	42.68	49.38	55.32	2.42	2.60	2.69	2.42	2.60	2.69	7.51	6.65	5.97	6.71	0.77
10	41.16	47.81	53.58	1.52	1.57	1.74	3.94	4.17	4.43	12.22	10.66	9.84	10.91	1.21
15	39.77	46.51	52.31	1.39	1.30	1.27	5.33	5.47	5.70	16.54	13.99	12.66	14.39	1.97
20	38.49	45.31	51.03	1.28	1.20	1.28	6.61	6.67	6.98	20.51	17.05	15.50	17.69	2.56
25	37.34	44.20	49.91	1.15	1.11	1.12	7.76	7.78	8.10	24.08	19.89	17.99	20.65	3.11
30	36.17	43.23	48.89	1.17	0.97	1.02	8.93	8.75	9.12	27.71	22.37	20.25	23.44	3.84
35	35.02	42.26	47.95	1.15	0.97	0.94	10.08	9.72	10.06	31.28	24.85	22.34	26.16	4.61
40	34.00	41.40	47.18	1.02	0.86	0.77	11.10	10.58	10.83	34.44	27.05	24.05	28.51	5.35
45	32.95	40.55	46.39	1.05	0.85	0.79	12.15	11.43	11.62	37.70	29.23	25.81	30.91	6.12
50	32.11	39.80	45.66	0.84	0.75	0.73	12.99	12.18	12.35	40.30	31.14	27.43	32.96	6.63
55	31.29	39.06	45.01	0.82	0.74	0.65	13.81	12.92	13.00	42.85	33.04	28.87	34.92	7.18
60	30.52	38.36	44.38	0.77	0.70	0.63	14.58	13.62	13.63	45.24	34.82	30.27	36.78	7.67
65	29.78	37.72	43.80	0.74	0.64	0.58	15.32	14.26	14.21	47.53	36.46	31.56	38.52	8.18
70	29.14	37.10	43.24	0.64	0.62	0.56	15.96	14.88	14.77	49.52	38.05	32.80	40.12	8.55
75	28.54	36.51	42.73	0.60	0.59	0.51	16.56	15.47	15.28	51.38	39.56	33.93	41.62	8.91
80	28.06	35.95	42.24	0.48	0.56	0.49	17.04	16.03	15.77	52.87	40.99	35.02	42.96	9.09
85	27.56	35.42	41.79	0.50	0.53	0.45	17.54	16.56	16.22	54.42	42.34	36.02	44.26	9.35
90	27.10	34.90	41.33	0.46	0.52	0.46	18.00	17.08	16.68	55.85	43.67	37.04	45.52	9.54

e) Water evaporation from PTU liposomes composed of PC:SA, pH 9.0

time(min)	weight (mg)			weight loss (mg)			cumulative weight loss (mg)			% cumulative weight loss			mean	SD
	1	2	3	1	2	3	1	2	3	1	2	3		
0	57.30	63.52	49.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	53.86	60.42	46.62	3.44	3.10	3.04	3.44	3.10	3.04	7.75	6.17	8.27	7.40	1.09
10	51.93	58.41	44.70	1.93	2.01	1.92	5.37	5.11	4.96	12.09	10.18	13.49	11.92	1.66
15	50.11	56.59	43.20	1.82	1.82	1.50	7.19	6.93	6.46	16.19	13.80	17.56	15.85	1.90
20	48.64	54.88	42.08	1.47	1.71	1.12	8.66	8.64	7.58	19.50	17.21	20.61	19.11	1.73
25	47.27	53.34	40.84	1.37	1.54	1.24	10.03	10.18	8.82	22.59	20.27	23.98	22.28	1.87
30	46.05	52.21	39.76	1.22	1.13	1.08	11.25	11.31	9.90	25.33	22.53	26.92	24.92	2.22
35	44.92	51.19	38.72	1.13	1.02	1.04	12.38	12.33	10.94	27.88	24.56	29.74	27.39	2.63
40	43.85	50.24	37.71	1.07	0.95	1.01	13.45	13.28	11.95	30.29	26.45	32.49	29.74	3.06
45	42.83	49.35	36.51	1.02	0.89	1.20	14.47	14.17	13.15	32.58	28.22	35.75	32.19	3.78
50	41.78	48.55	35.76	1.05	0.80	0.75	15.52	14.97	13.90	34.95	29.81	37.79	34.18	4.04
55	40.80	47.77	34.43	0.98	0.78	1.33	16.50	15.75	15.23	37.15	31.37	41.41	36.64	5.04
60	39.91	47.05	33.36	0.89	0.72	1.07	17.39	16.47	16.30	39.16	32.80	44.32	38.76	5.77
65	38.94	46.29	32.19	0.97	0.76	1.17	18.36	17.23	17.47	41.34	34.32	47.50	41.05	6.60
70	38.00	45.54	31.10	0.94	0.75	1.09	19.30	17.98	18.56	43.46	35.81	50.46	43.24	7.33
75	37.19	44.90	30.04	0.81	0.64	1.06	20.11	18.62	19.62	45.28	37.08	53.34	45.24	8.13
80	36.25	44.17	29.04	0.94	0.73	1.00	21.05	19.35	20.62	47.40	38.54	56.06	47.33	8.76
85	35.39	43.45	28.11	0.86	0.72	0.93	21.91	20.07	21.55	49.34	39.97	58.59	49.30	9.31
90	34.53	42.75	27.27	0.86	0.70	0.84	22.77	20.77	22.39	51.27	41.37	60.88	51.17	9.75

APPENDIX H

Biological activities of PTU liposomes

Biological activities

a) Effect of incubation time

Incubation time (day)	PTU conc. (ug/ml)	Viable cells			Viable cells : Control cells			Mean	SD
		1	2	3	1	2	3		
1	0 (control)	892800	834667	962667	100	100	100	100	0
	1.1	406400	760000	842667	45.52	91.05	87.53	74.70	25.33
	11	366400	658667	770667	41.04	78.91	80.06	66.67	22.20
	110	216000	541333	602667	24.19	64.86	62.60	50.55	22.85
	1100	86400	212000	160000	9.68	25.40	16.62	17.23	7.88
3	0 (control)	82400	277200	836000	100	100	100	100	0
	1.1	48800	246400	771000	59.22	88.89	92.22	80.11	18.17
	11	36800	229600	396667	44.66	82.83	47.45	58.31	21.28
	110	20800	114800	338667	25.24	41.41	40.51	35.72	9.09
	1100	3200	22400	128800	3.88	8.08	15.41	9.12	5.83

b) Antiproliferative effect of caffeine and PTU

Tested solution	Viable cells			Viable cells : Control cells			Mean	SD
	1	2	3	1	2	3		
DMEM+ (control)	354000	645333	809333	100.00	100.00	100.00	100.00	0.00
PTU	66000	188000	190667	18.64	29.13	23.56	23.78	5.25
Caffeine	172000	272000	305333	48.59	42.15	37.73	42.82	5.46

c) Antiproliferative effect of PTU solution in the presence and absence of CS in DMEM+

DMEM+	PTU conc. (ug/ml)	Viable cells			Viable cells : Control cells			Mean	SD
		1	2	3	1	2	3		
without CS	control	2200000	1514667	1341333	100	100	100	100	0
	1.1	1744000	1138667	1146667	79.27	75.18	85.49	79.98	5.19
	11	1428000	1053333	1080000	64.91	69.54	80.52	71.66	8.02
	110	1172000	736000	794667	53.27	48.59	59.24	53.70	5.34
	1100	677333	666667	666667	30.79	44.01	49.70	41.50	9.70
with CS	control	892800	834667	962667	100	100	100	100	0
	1.1	406400	760000	842667	45.52	91.05	87.53	74.70	25.33
	11	366400	658667	770667	41.04	78.91	80.06	66.67	22.20
	110	216000	541333	602667	24.19	64.86	62.60	50.55	22.85
	1100	86400	212000	160000	9.68	25.40	16.62	17.23	7.88

d) Antiproliferative effect of PTU liposomes (1 day incubation; PTU amount = 0.22 mg/well)

Formulations		Viable cells			Viable cells : Control cells			Mean	SD
		1	2	3	1	2	3		
solution (control)	without PTU	938667	851333	1048000	100.00	100.00	100.00	100.00	0.00
PC:DCP		837333	1096000	821333	89.20	116.76	87.50	97.82	16.42
PC:CH:DCP		452000	410667	620000	53.09	48.24	72.83	58.05	13.02
PC:CH		1213333	957333	1042667	115.78	91.35	99.49	102.21	12.44
solution	with PTU	1172000	736000	794667	53.27	48.59	59.24	53.70	5.34
PC:DCP		741333	864000	693333	78.98	92.05	73.86	81.63	9.38
PC:CH:DCP		546667	408000	424000	64.21	47.92	49.80	53.98	8.91
PC:CH		826667	736000	752000	78.88	70.23	71.76	73.62	4.62

e) Antiproliferative effect of PTU liposomes (3 days incubation; PTU amount = 0.22 mg/well)

Formulations		Viable cells			Viable cells : Control cells			Mean	SD
		1	2	3	1	2	3		
solution (control)	without PTU	369333	418667	374667	95.30	108.03	96.67	100.00	6.99
PC:DCP		361333	316000	378667	93.23	81.54	97.71	90.83	8.35
solution	with PTU	269333	133333	224000	69.50	34.40	57.80	53.90	17.87
PC:DCP		248000	209333	164000	63.99	54.01	42.32	53.44	10.85

APPENDIX I

Stability data of PTU liposomes

Stability data of PTU liposomes

a) Immediately after preparation

Lipid composition	n	PTU in system (mg)	Absorbance		PTU amount (mg)		Total PTU (mg)	%Recovery	Total lipid (mmol)	mmol PTU/mol PC
			free drug	pellet	free drug	pellet				
PC/ pH 7.4	1	9.1	0.240	0.309	6.90	1.78	8.68	95.43	0.12	86.34
	2	9.1	0.242	0.344	6.96	1.98	8.94	98.26	0.14	84.04
	3	9.1	0.269	0.251	7.28	1.36	8.64	94.94	0.08	101.60
	Mean						8.76	96.21	0.11	90.66
	SD						0.16	1.79	0.03	9.54
PC:DCP/ pH 7.4	1	9.1	0.263	0.217	7.57	1.25	8.82	96.88	0.06	117.91
	2	9.1	0.234	0.289	6.73	1.66	8.39	92.21	0.09	109.14
	3	9.1	0.255	0.340	6.90	1.84	8.74	96.05	0.10	112.48
	Mean						8.65	95.05	0.08	113.18
	SD						0.23	2.49	0.02	4.43
PC:CH:DCP/ pH 7.4	1	8.5	0.239	0.198	6.88	1.14	8.02	94.30	0.13	51.40
	2	8.5	0.217	0.350	6.24	2.01	8.25	97.09	0.18	66.22
	3	8.5	0.267	0.171	7.23	0.92	8.15	95.83	0.13	41.46
	Mean						8.14	95.74	0.15	53.03
	SD						0.12	1.40	0.03	12.46
PC:SA/ pH 9.0	1	14	0.342	0.436	9.84	2.51	12.35	88.20	0.16	94.97
	2	14	0.367	0.459	10.56	2.64	13.20	94.27	0.13	122.67
	3	14	0.404	0.424	10.93	2.29	13.22	94.45	0.12	115.38
	Mean						12.92	92.31	0.14	111.01
	SD						0.50	3.56	0.02	14.36

b) Week 4

Lipid composition	n	PTU in system (mg)	Absorbance	PTU amount (mg)	Total lipid (mmol)	mmol PTU/mol PC
PC/ pH 7.4	1	2.5	0.337	1.94	0.14	82.49
	2	2.5	0.234	1.34	0.10	78.93
	3	2.5	0.305	1.65	0.11	88.46
	Mean				0.12	83.29
	SD				0.02	4.82
PC:DCP/ pH 7.4	1	2.5	0.333	1.92	0.10	112.10
	2	2.5	0.324	1.86	0.10	109.71
	3	2.5	0.374	2.02	0.11	107.89
	Mean				0.10	109.90
	SD				0.01	2.11
PC:CH:DCP/ pH 7.4	1	1.9	0.220	1.27	0.11	67.62
	2	1.9	0.247	1.42	0.14	61.60
	3	1.9	0.203	1.10	0.14	45.03
	Mean				0.13	58.08
	SD				0.02	11.70
PC:SA/ pH 9.0	1	3.2	0.465	2.68	0.14	112.82
	2	3.2	0.378	2.17	0.12	102.36
	3	3.2	0.414	2.24	0.12	109.19
	Mean				0.13	108.12
	SD				0.01	5.31

c) Week 8

Lipid composition	n	PTU in system (mg)	Absorbance		PTU amount (mg)		Total PTU (mg)	%Recovery	Total lipid (mmol)	mmol PTU/mol PC
			free drug	pellet	free drug	pellet				
PC/ pH 7.4	1	9.1	0.280	0.306	7.78	1.69	9.47	104.04	0.12	78.93
	2	9.1	0.267	0.252	7.42	1.38	8.80	96.67	0.10	78.80
	3	9.1	0.281	0.261	7.81	1.43	9.24	101.49	0.09	96.01
	Mean						9.17	100.73	0.10	84.58
	SD						0.34	3.75	0.02	9.90
PC:DCP/ pH 7.4	1	9.1	0.272	0.306	7.56	1.69	9.25	101.60	0.10	96.68
	2	9.1	0.280	0.281	7.78	1.54	9.32	102.39	0.09	102.94
	3	9.1	0.296	0.272	8.22	1.49	9.71	106.73	0.09	97.78
	Mean						9.43	103.57	0.09	99.13
	SD						0.25	2.76	0.01	3.34
PC:CH:DCP/ pH 7.4	1	8.5	0.263	0.220	7.31	1.20	8.51	100.07	0.11	62.41
	2	8.5	0.281	0.198	7.81	1.08	8.89	104.54	0.12	50.87
	3	8.5	0.276	0.247	7.67	1.35	9.02	106.08	0.13	59.88
	Mean						8.80	103.56	0.12	57.72
	SD						0.27	3.12	0.01	6.07
PC:SA/ pH 9.0	1	14	0.409	0.392	11.36	2.17	13.53	96.65	0.15	85.45
	2	14	0.415	0.375	11.53	2.08	13.61	97.20	0.13	95.73
	3	14	0.406	0.360	11.28	1.99	13.27	94.77	0.10	117.15
	Mean						13.47	96.21	0.13	99.44
	SD						0.18	1.27	0.03	16.17

Phosphorus content

a) Immediately after preparation

Lipid composition	Abs.			Abs. - Blank Abs.			P content (mmol)			PC (mmol)			CH (mmol)			Charge (mmol)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
PC/ pH 7.4	0.635	0.685	0.513	0.349	0.399	0.227	0.121	0.138	0.079	0.121	0.138	0.079	0.000	0.000	0.000	0.000	0.000	0.000
PC:DCP/ pH 7.4	0.466	0.544	0.563	0.180	0.258	0.277	0.062	0.089	0.096	0.058	0.083	0.090	0.000	0.000	0.000	0.004	0.006	0.006
PC:CH:DCP/ pH 7.4	0.494	0.571	0.495	0.208	0.285	0.209	0.072	0.099	0.072	0.065	0.089	0.066	0.058	0.080	0.586	0.007	0.009	0.007
PC:SA/ pH 9.0	0.698	0.622	0.596	0.412	0.336	0.310	0.143	0.116	0.108	0.143	0.116	0.108	0.000	0.000	0.000	0.012	0.010	0.009

Calibration curve

Concentration (umol/ml)	0.064	0.096	0.128	0.160	0.192	0.224	0.256
Abs.	0.454	0.578	0.663	0.743	0.847	0.924	1.021
Abs. - Blank Abs.	0.168	0.292	0.377	0.457	0.561	0.638	0.735

$y = 2.8761x+0.0006$; $R^2 = 0.997$

b) Week 4

Lipid composition	Abs.			Abs. - Blank Abs.			P content (mmol)			PC (mmol)			CH (mmol)			Charge (mmol)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
PC/ pH 7.4	0.611	0.515	0.539	0.339	0.243	0.267	0.138	0.100	0.110	0.138	0.100	0.110	0.000	0.000	0.000	0.000	0.000	0.000
PC:DCP/ pH 7.4	0.515	0.514	0.540	0.243	0.242	0.268	0.100	0.100	0.110	0.094	0.093	0.103	0.000	0.000	0.000	0.007	0.007	0.007
PC:CH:DCP/ pH 7.4	0.414	0.450	0.461	0.142	0.178	0.189	0.061	0.075	0.079	0.055	0.068	0.072	0.049	0.061	0.064	0.006	0.007	0.008
PC:SA/ pH 9.0	0.586	0.552	0.542	0.314	0.280	0.270	0.128	0.115	0.111	0.128	0.115	0.111	0.000	0.000	0.000	0.011	0.010	0.010

Calibration curve

Concentration (umol/ml)	0.064	0.096	0.128	0.160	0.192	0.224
Abs.	0.430	0.499	0.585	0.660	0.747	0.837
Abs. - Blank Abs.	0.158	0.227	0.313	0.388	0.475	0.565

$y = 2.5478x-0.0128$; $R^2 = 0.9984$

c) Week 8

Lipid composition	Abs.			Abs. - Blank Abs.			P content (mmol)			PC (mmol)			CH (mmol)			Charge (mmol)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
PC/ pH 7.4	0.585	0.522	0.479	0.336	0.273	0.230	0.125	0.103	0.088	0.125	0.103	0.088	0.000	0.000	0.000	0.000	0.000	0.000
PC:DCP/ pH 7.4	0.520	0.480	0.485	0.272	0.232	0.236	0.102	0.088	0.090	0.096	0.082	0.084	0.000	0.000	0.000	0.007	0.006	0.006
PC:CH:DCP/ pH 7.4	0.408	0.426	0.439	0.160	0.177	0.190	0.062	0.069	0.073	0.057	0.062	0.066	0.051	0.056	0.059	0.006	0.007	0.007
PC:SA/ pH 9.0	0.618	0.562	0.491	0.370	0.313	0.242	0.137	0.117	0.092	0.137	0.117	0.092	0.000	0.000	0.000	0.012	0.010	0.008

Calibration curve

Concentration (umol/ml)	0.064	0.096	0.128	0.192	0.224	0.256
Abs.	0.415	0.503	0.592	0.767	0.853	0.959
Abs. - Blank Abs.	0.166	0.254	0.343	0.519	0.604	0.711

$y = 2.8002x-0.0154$; $R^2 = 0.9993$

VITA

Miss Rattana Rattanatraiphop was born on November 4, 1973 in Bangkok, Thailand. She received the Bachelor of Pharmacy (1st class honors) from the Faculty of Pharmacy, Chiangmai University, Chiangmai in 1996. After graduation, she had worked at the Research and Development Institute, Government Pharmaceutical Organization for two years. She entered the Master's Degree program in Pharmacy at Chulalongkorn University in 1998.