CHAPTER 3
RESULTS

3.1 Characteristics of liposome formulations

Nine liposome formulations were prepared and their characteristics in physical appearances, pH and sizes were investigated.

3.1.1 The physical appearances of liposome formulations

Nine liposome formulations were prepared and their physical appearances after 24 hrs were shown in Table 3.1. Liposome formulations number 1 to 3 showed sedimentation after 24 hrs. Formulations number 4 to 9 gave favorable physical appearances and were selected for further studies.

Table 3.1 : Physical appearances of nine liposome formulations prepared after 24 hrs

No.	Formulation	Physical Appearance
1	7:2	Translucent, white dispersion with sedimentation
2	7:2 (5%TA)	Translucent, white dispersion with sedimentation
3	7:2 (10%TA)	Translucent, white dispersion with sedimentation
4	7:2:1 (+)	Translucent, white dispersion with no sedimentation
5	7:2:1 (5%TA,+)	Translucent, white dispersion with no sedimentation
6	7:2:1 (10%TA,+)	Translucent, white dispersion with no sedimentation
7	7:2:1 (-)	Translucent, white dispersion with no sedimentation
8	7:2:1 (5%TA,-)	Translucent, white dispersion with no sedimentation
9	7:2:1 (10%TA,-)	Translucent, white dispersion with no sedimentation

Figure 3.1 : Physical appearances of the freshly prepared liposome formulations from left to right : 7:2:1 (+), 7:2:1, (5%TA,+), 7:2:1, (10%TA,+), 7:2:1 (-), 7:2:1, (5%TA,-) and 7:2:1, (10%TA,-) respectively.

3.1.2 pH measurement of liposome formulations

The pH values of six liposome formulations measured by a pH meter were listed in Table 3.2. When TA dissolved in DI water and entrapped in both positively and negatively charged liposome formulations, the pH values were increased except the blank negative liposome.

Table 3.2 : The pH values of six liposome formulations comparing to DI water, 5%TA solution and 10%TA solution in DI water

Formulations	pH values			Mean	SD	%CV
	1	2	3			
DI water	6.24	6.30	6.35	6.2967	0.0551	0.8747
5%TA in DI water	7.55	7.49	7.50	7.5133	0.0321	0.4278
10%TA in DI water	7.58	7.58	7.56	7.5733	0.0115	0.1525
7:2:1 (+)	7.64	7.53	7.60	7.5900	0.0557	0.7336
7:2:1 (5%TA,+)	7.93	7.92	7.90	7.9167	0.0153	0.1930
7:2:1 (10%TA,+)	7.89	7.84	7.84	7.8567	0.0289	0.3674
7:2:1 (-)	3.55	3.27	3.49	3.4367	0.1474	4.2897
7:2:1 (5%TA,-)	6.95	6.79	6.95	6.8967	0.0924	1.3394
7:2:1 (10%TA,-)	7.13	7.12	7.12	7.1233	0.0058	0.0811

3.1.3 Particle size and particle size distribution of liposomes

The mean particle size of six liposome formulations after 10-days preparation by a Light Scattering Particle Analyzer was shown in Table 3.2. Blank positively and negatively charged liposome and the positively charged liposome with the entrapped 5% and 10% TA had larger particle size of about 10 times than the negatively charged liposome with the entrapped drug. The size distribution of liposome formulations were shown in Figures 3.2 to 3.13.

Table 3.3 : Mean particle size (μm) of six liposome formulations

Formulations	Particle size (μm)		Mean
	Lot.1	Lot.2	
7:2:1 (+)	18.27	17.52	17.89
7:2:1 (5%TA,+)	17.17	17.83	17.50
7:2:1 (10%TA,+)	35.53	36.18	35.85
7:2:1 (-)	24.81	24.75	24.78
7:2:1 (5%TA,-)	2.75	2.78	2.76
7:2:1 (10%TA,-)	2.07	2.03	2.05

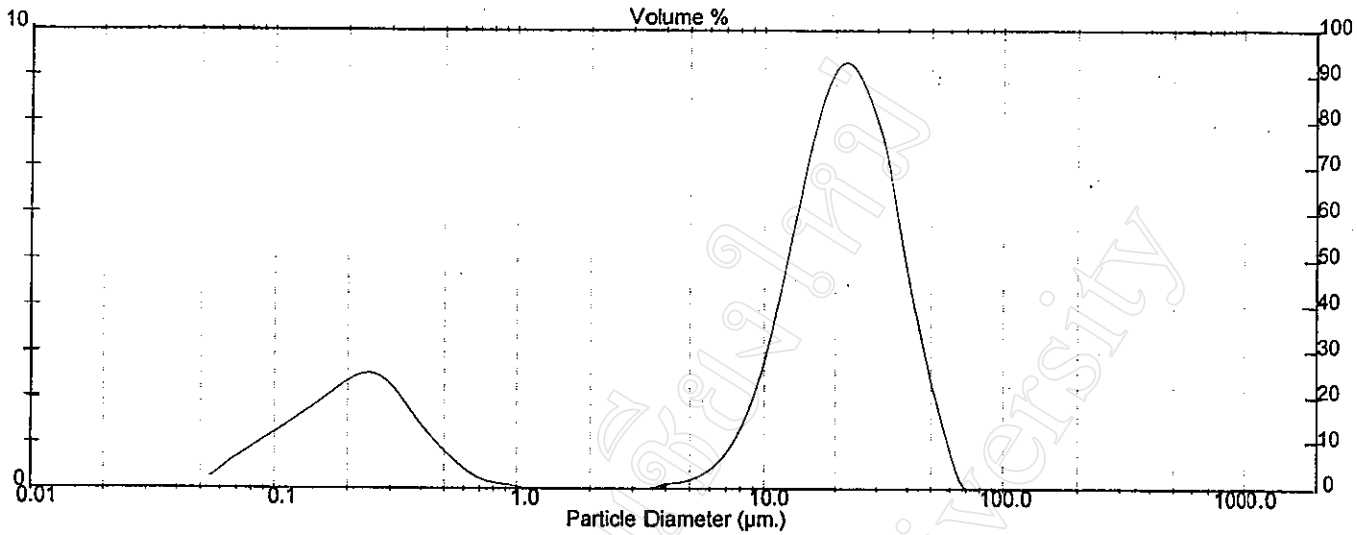

Figure 3.2 : Particle size (µm) distribution of 7:2:1 (+) Lot.1

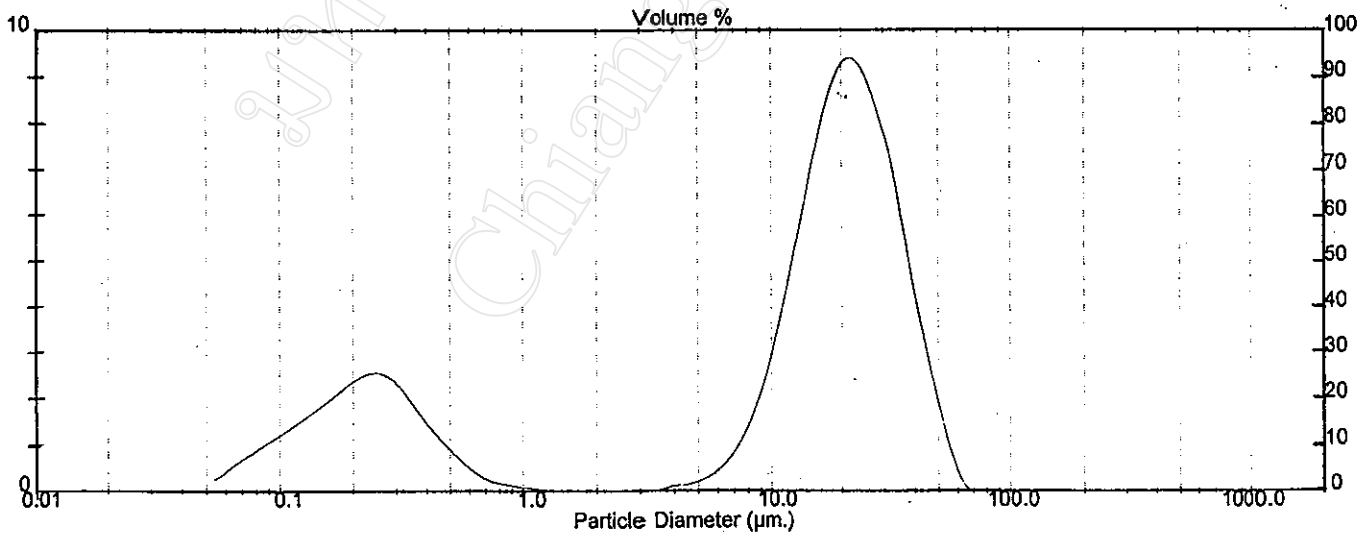

Figure 3.3 : Particle size (µm) distribution of 7:2:1 (+) Lot.2

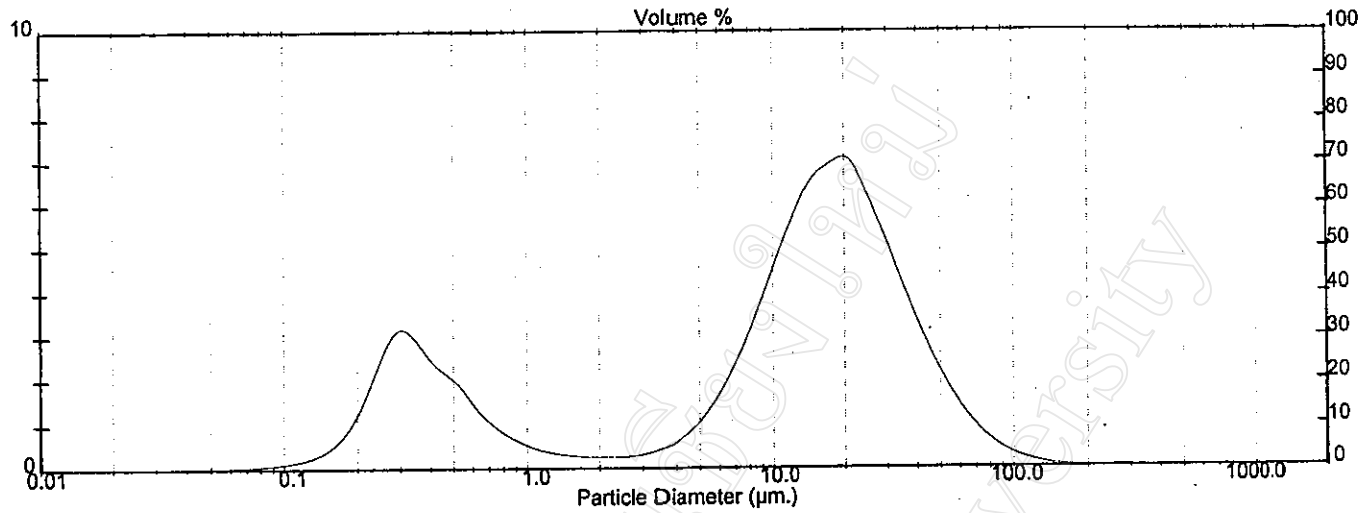

Figure 3.4 : Particle size (µm) distribution of 7:2:1 (5%TA,+) Lot.1

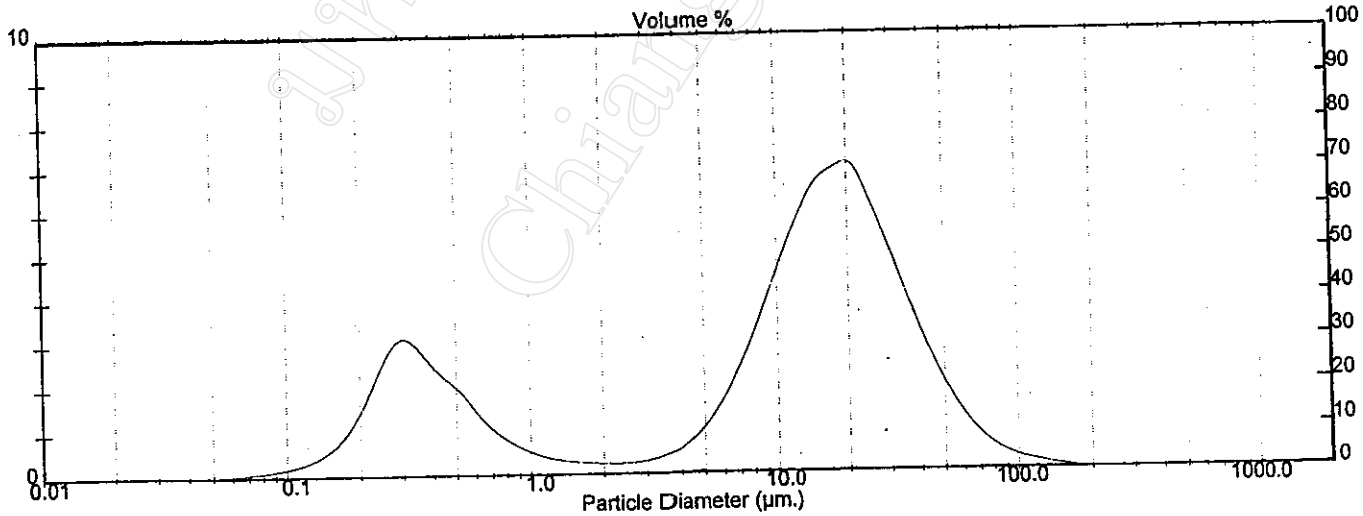

Figure 3.5 : Particle size (µm) distribution of 7:2:1 (5%TA,+) Lot.2

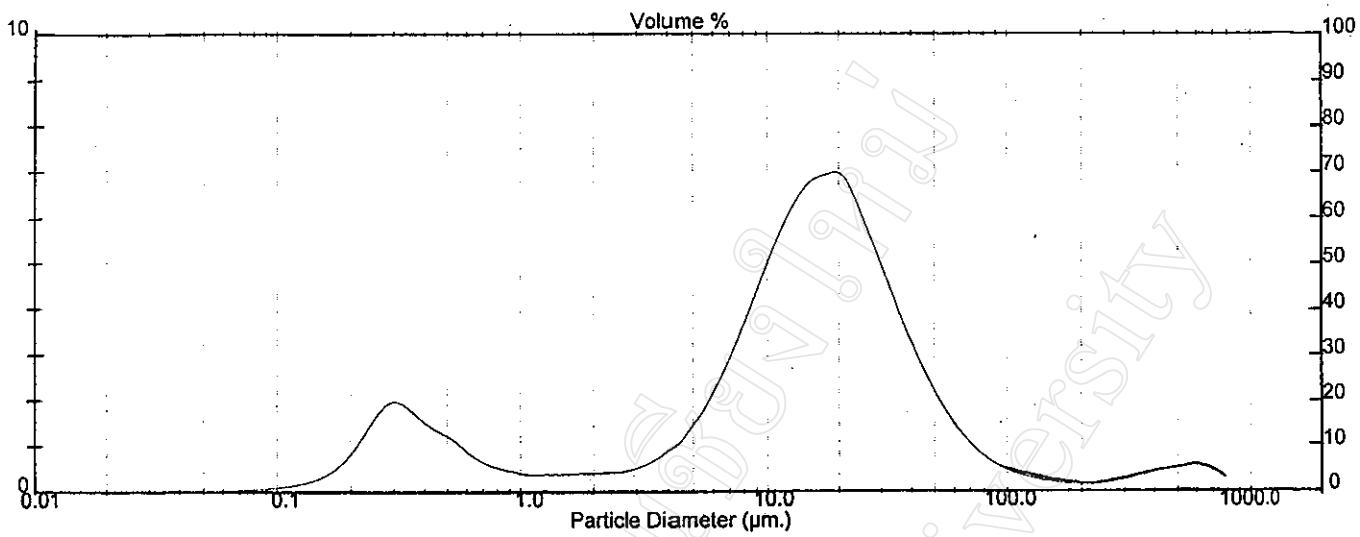

Figure 3.6 : Particle size (µm) distribution of 7:2:1 (10%TA,+) Lot.1

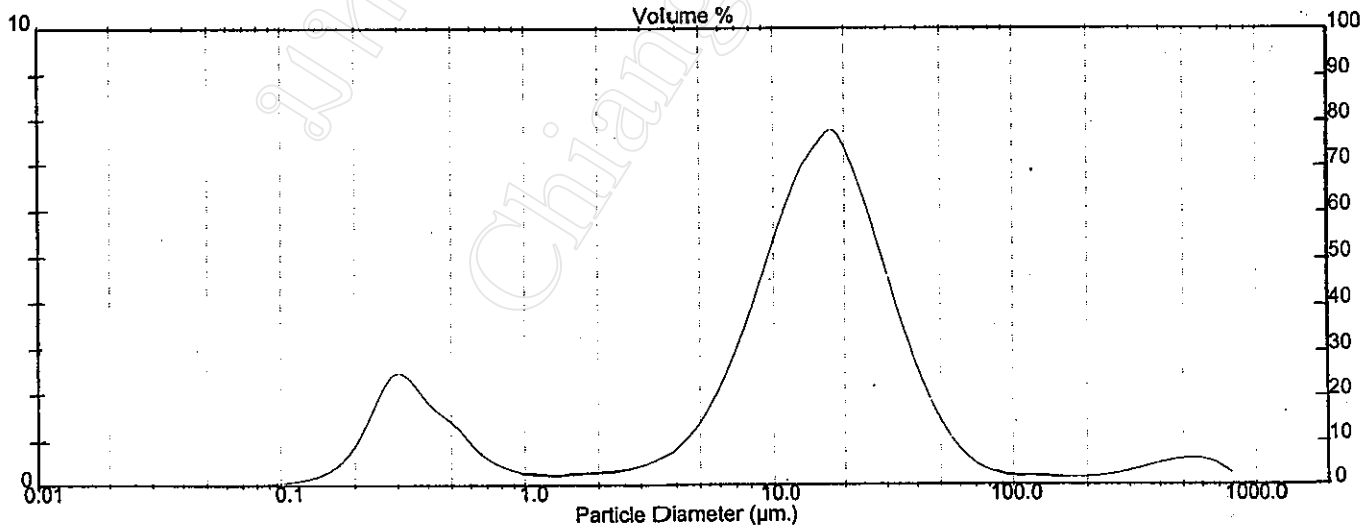

Figure 3.7 : Particle size (µm) distribution of 7:2:1 (10%TA,+) Lot.2

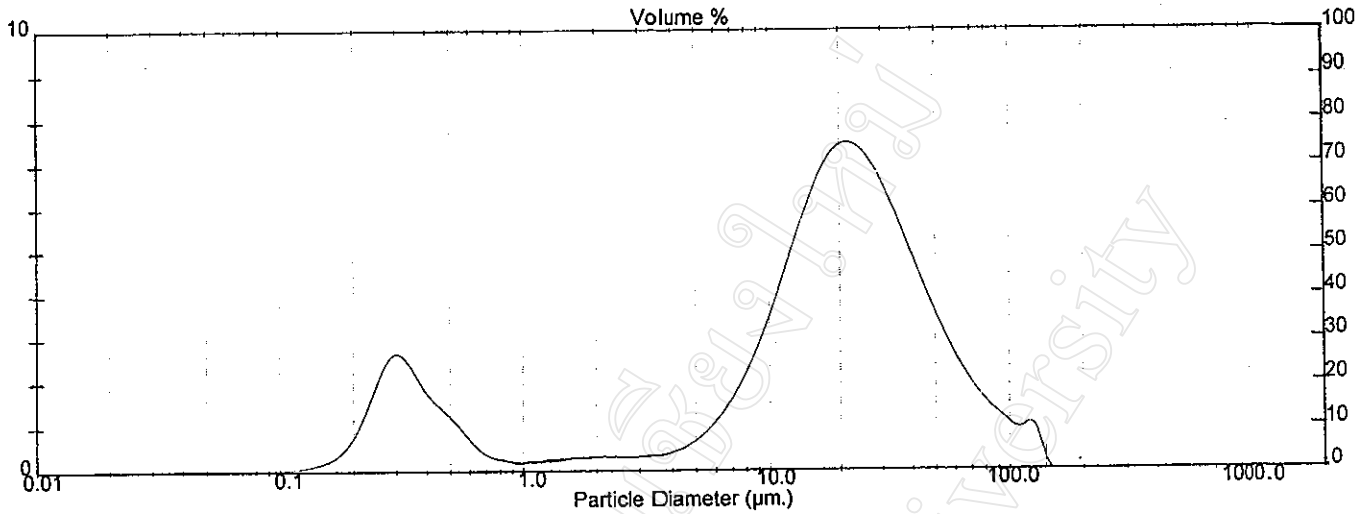

Figure 3.8 : Particle size (µm) distribution of 7:2:1 (-) Lot.1

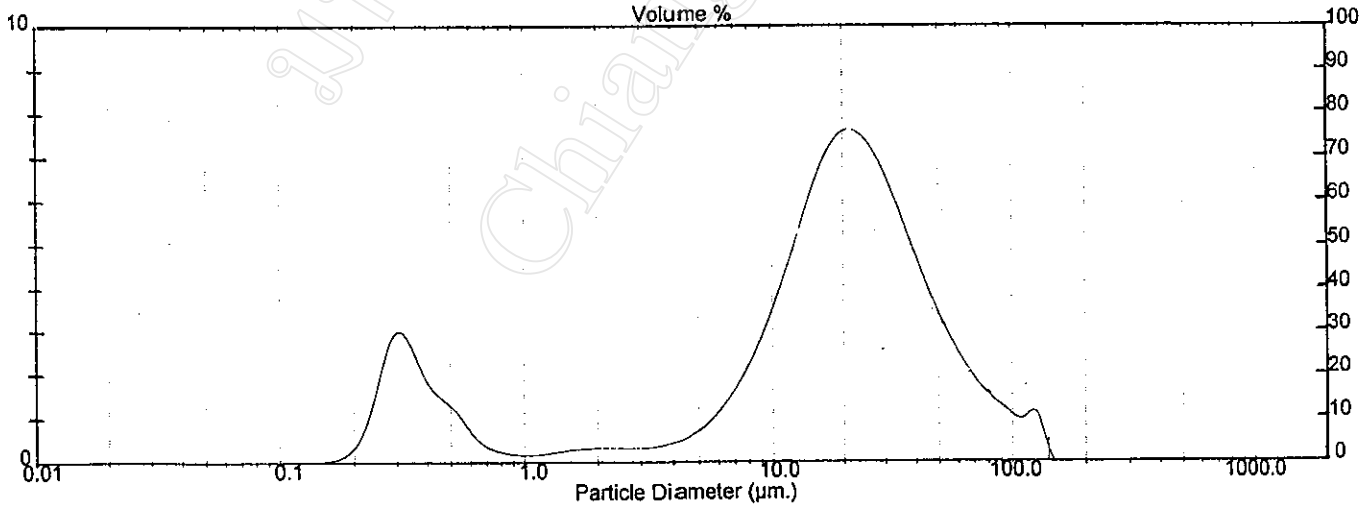

Figure 3.9 : Particle size (µm) distribution of 7:2:1 (-) Lot.2

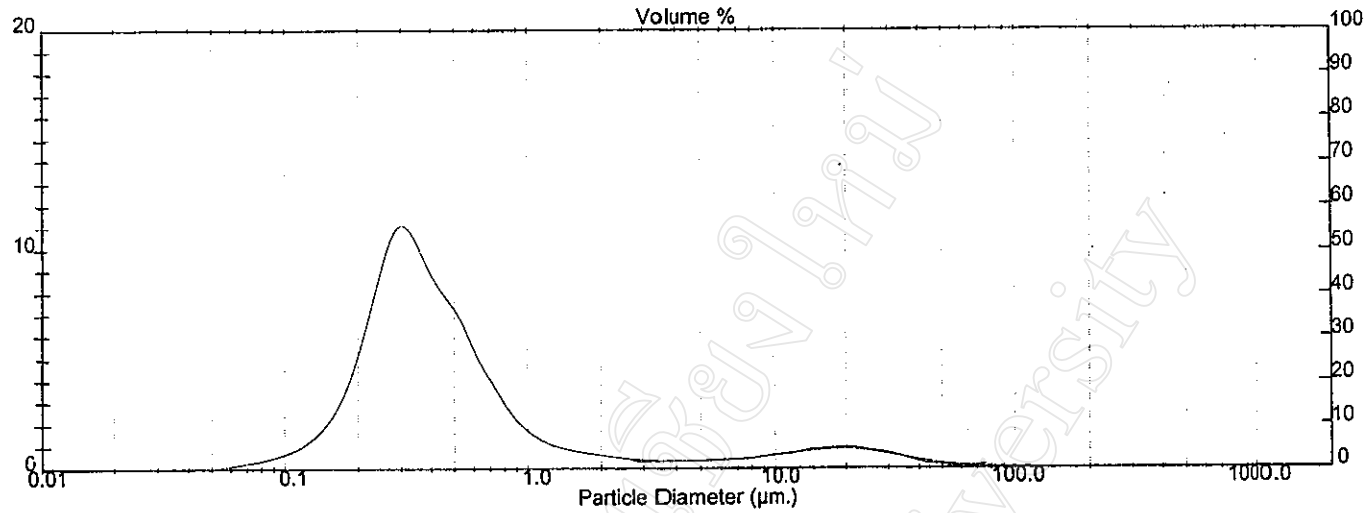

Figure 3.10 : Particle size (µm) distribution of 7:2:1 (5%TA, -) Lot.1

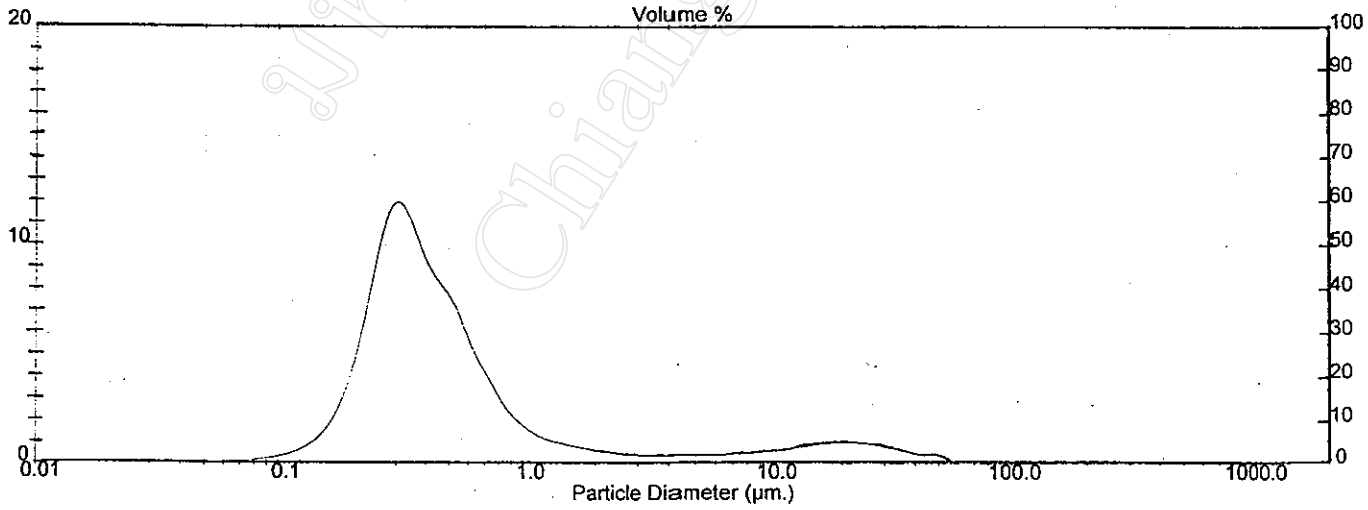

Figure 3.11 : Particle size (µm) distribution of 7:2:1 (5%TA, -) Lot.2

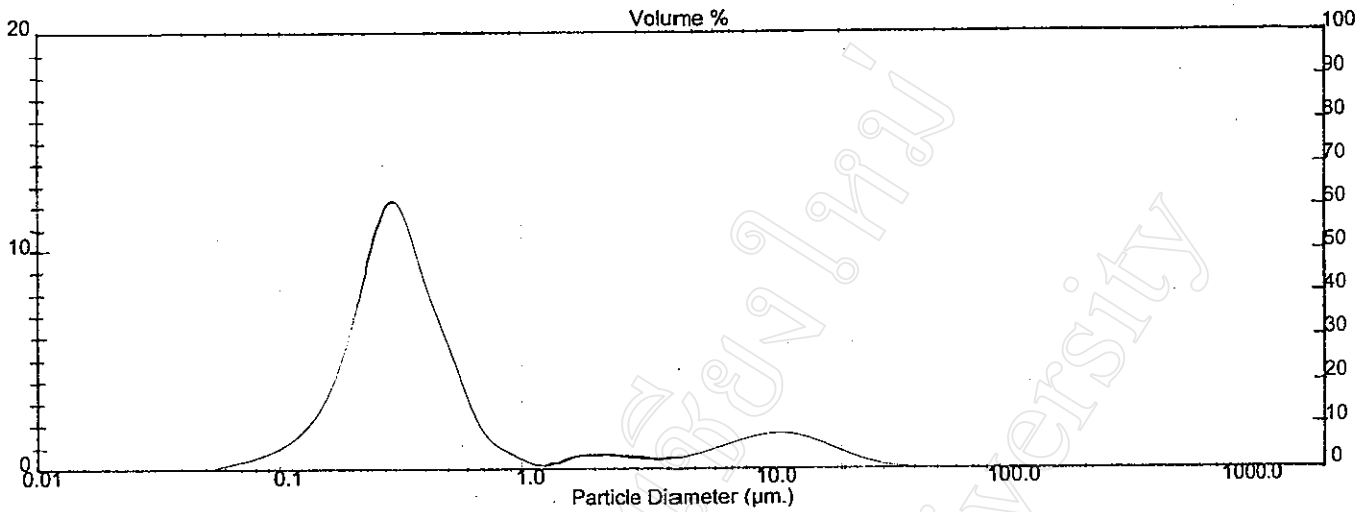

Figure 3.12 : Particle size (µm) distribution of 7:2:1 (10%TA, -) Lot.1

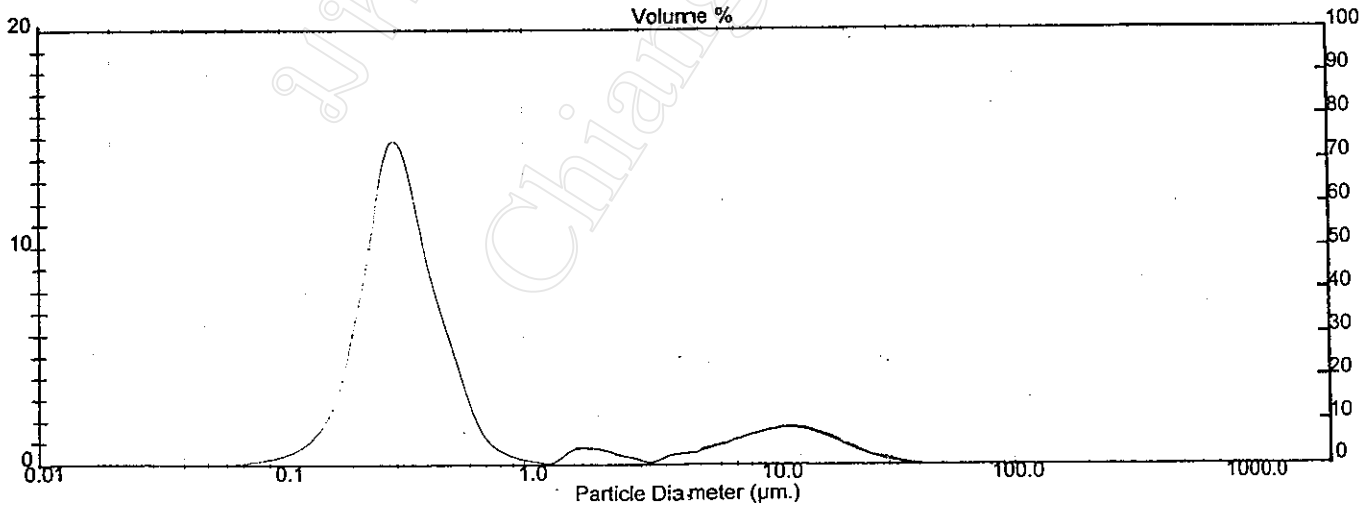

Figure 3.13 : Particle size (µm) distribution of 7:2:1 (10%TA, -) Lot.2

3.1.4 Morphology of liposome formulations determined by a transmission electron microscopy (TEM)

The morphology and lamellarity of multilamellar liposome formulations was determined by a transmission electron microscopy. The micrographs of 7:2:1 (+),7:2:1 (5%TA,+) and 7:2:1 (5%TA,-) were shown in Figure 3.14 to 3.16 respectively. About 8 to 15 bilayers were seen.

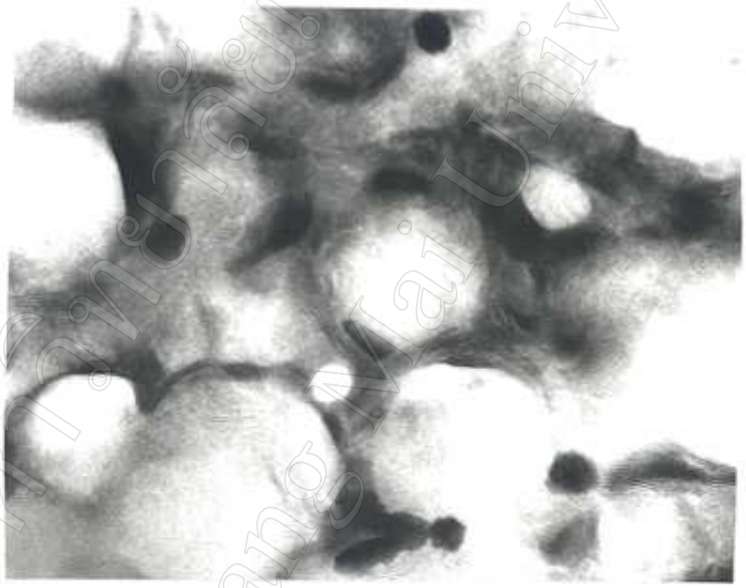

Figure 3.14 : The morphology and lamellarity of the blank 7:2:1 (+) liposome formulation, 6000x

Figure 3.15 : The morphology and lamellarity of 7:2:1 (5%TA,+) liposome formulation, 5000x

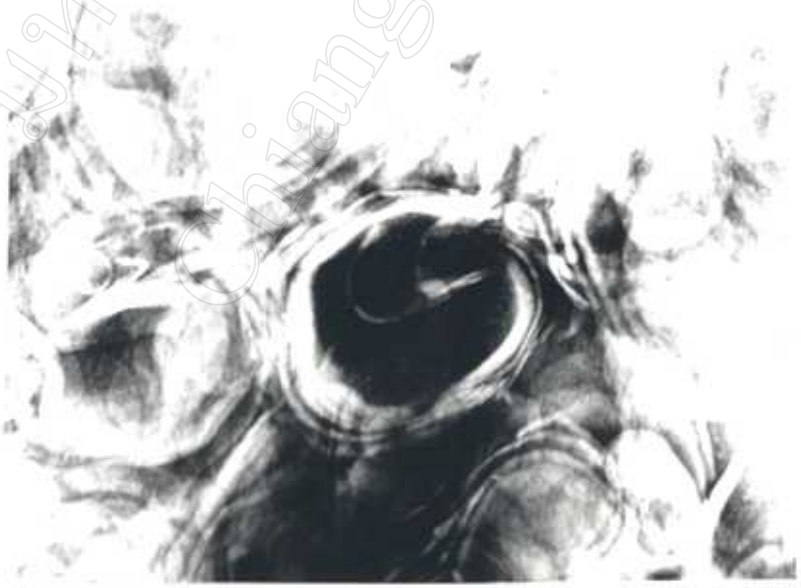

Figure 3.16 : The morphology and lamellarity of 7:2:1 (5%TA,-) liposome formulation, 6000x

3.1.5 Thermal analysis of liposome formulations

3.1.5.1 Glass transition temperature (T_g)

Glass Transition Temperatures of Emulmetik 950®, cholesterol, stearylamine, dicetyl phosphate, tranexamic acid, 7:2:1(10%TA, +) and 7:2:1(10%TA, -) liposome formulations by a thermogravimetric analyzer with the scanning rate of 20 ^{0}C / min from 40 ^{0}C to 500 ^{0}C were shown in Table 3.4 and Figures 3.17 to 3.24. The results indicated that Emulmetik 950®, cholesterol, dicetyl phosphate, tranexamic acid, 7:2:1 (10%TA, +) and 7:2:1(10%TA, -) liposome formulations decomposed at temperature approximate by higher than 200 ^{0}C except stearylamine which decomposed at lower than 200 ^{0}C.

Table 3.4 : The glass transition temperature (^{0}C) of Emulmetik 950®, cholesterol, stearylamine, dicetyl phosphate, tranexamic acid 7:2:1(10%TA, +) and 7:2:1 (10%TA, -) liposome formulations.

Formulation	The glass transition temperature (^{0}C)
Emulmetik 950®	257.77
Cholesterol	246.66
Stearylamine	162.22
Dicetyl Phosphate	218.47
Tranexamic Acid	223.63
7:2:1(10%TA,+)	222.22
7:2:1(10%TA,-)	229.63

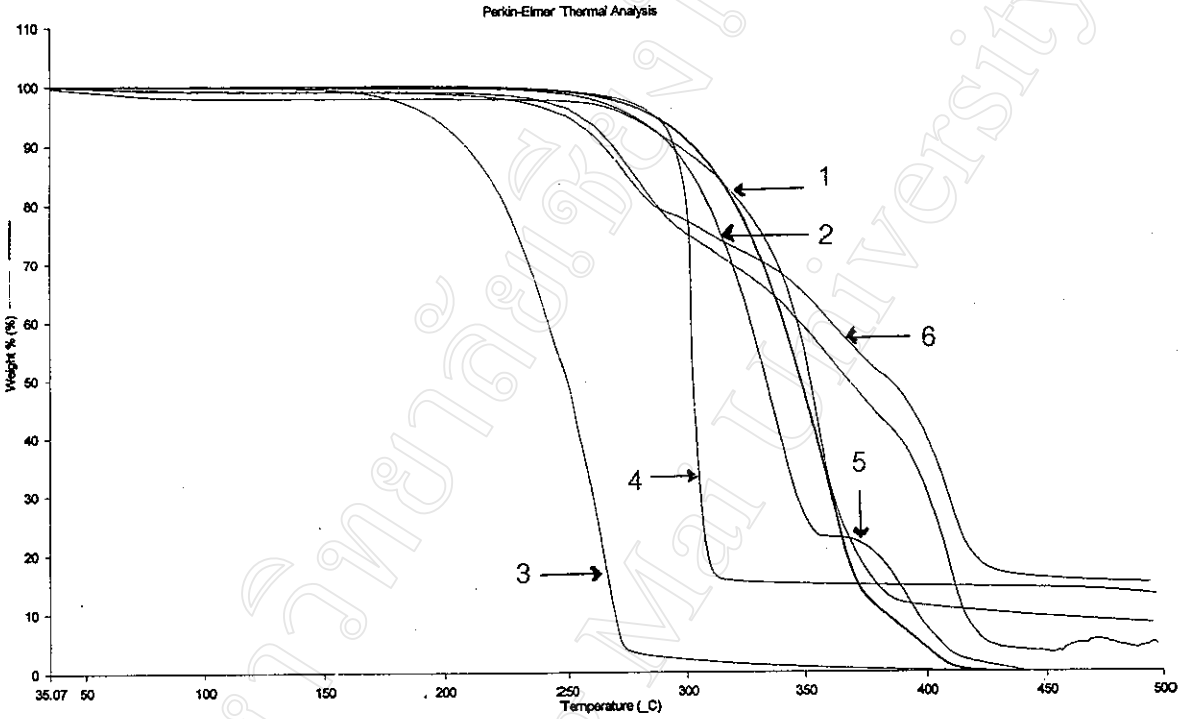

Figure 3.17 : The glass transition temperatures (^{0}C) of Emulmetik 950® (No.1), cholesterol (No.2), stearylamine (No.3), dicetyl phosphate (No.4), tranexamic acid, 7:2:1 (10%TA, +) (No.5), and 7:2:1(10%TA, -) (No.6) liposome formulations

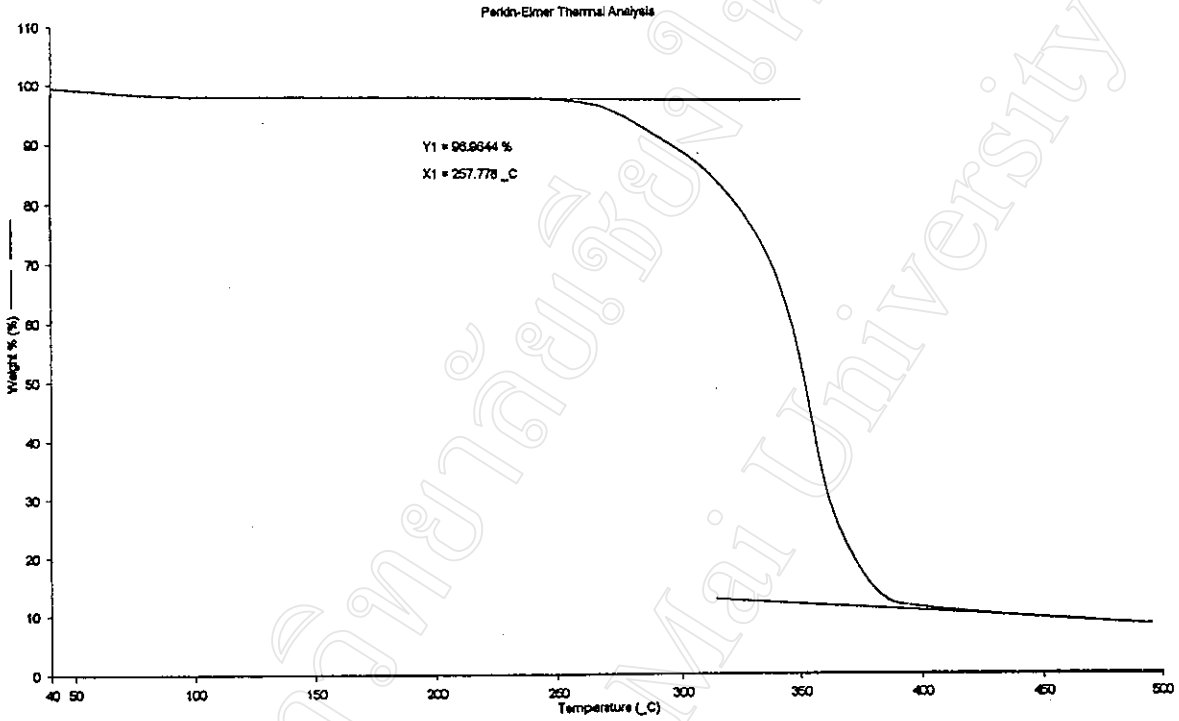

Figure 3.18 : The glass transition temperatures of Emulmetik 950® was 257.77 ^{0}C

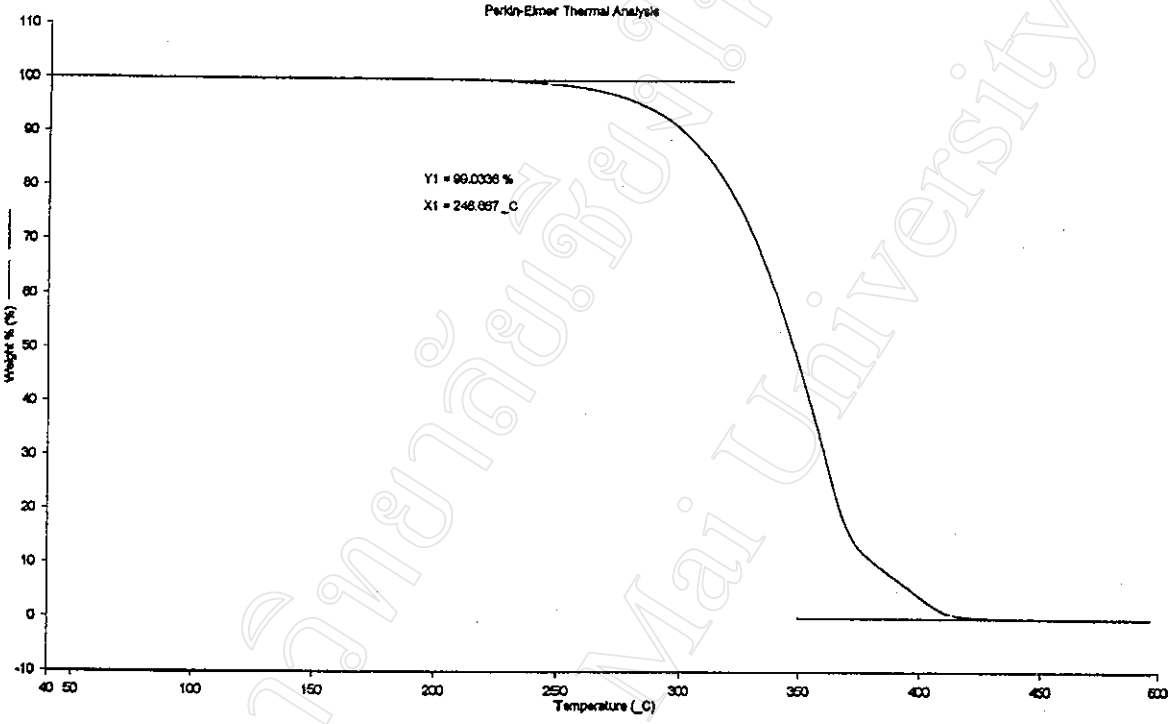

Figure 3.19 : The glass transition temperatures of cholesterol was 246.66 ^{0}C

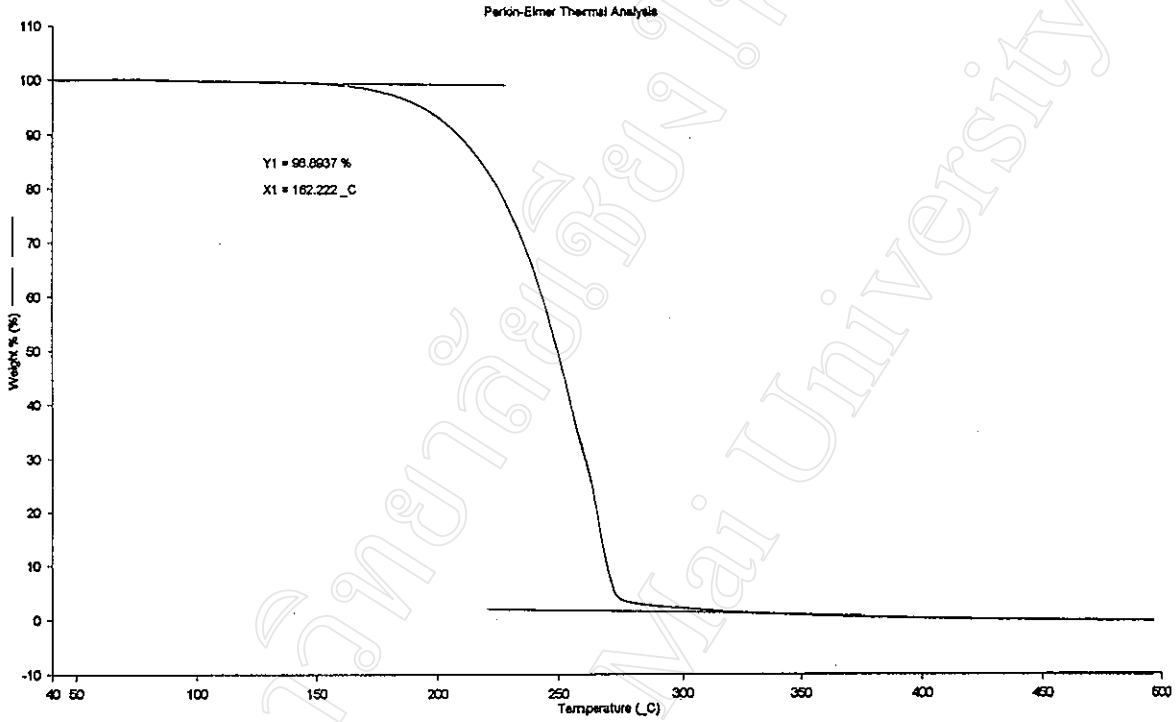

Figure 3.20 : The glass transition temperatures of stearylamine was 162.22 ^{0}C

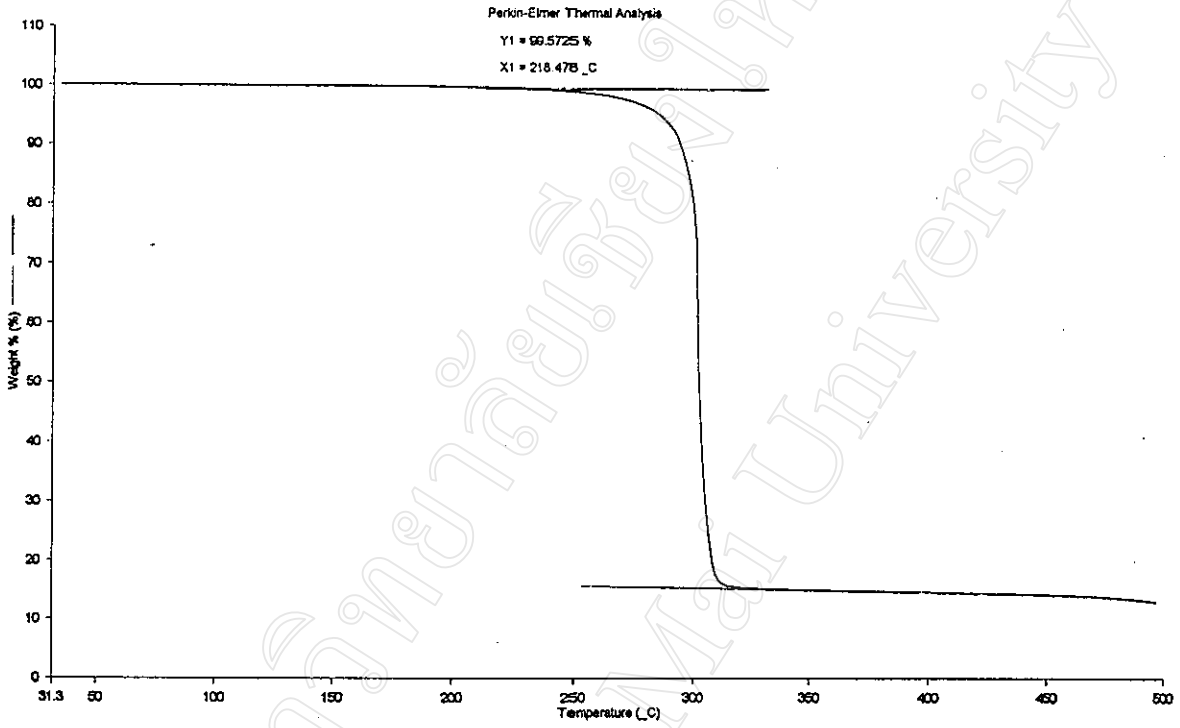

Figure 3.21 : The glass transition temperatures of dicetyl phosphate was 218.47 ^{0}C

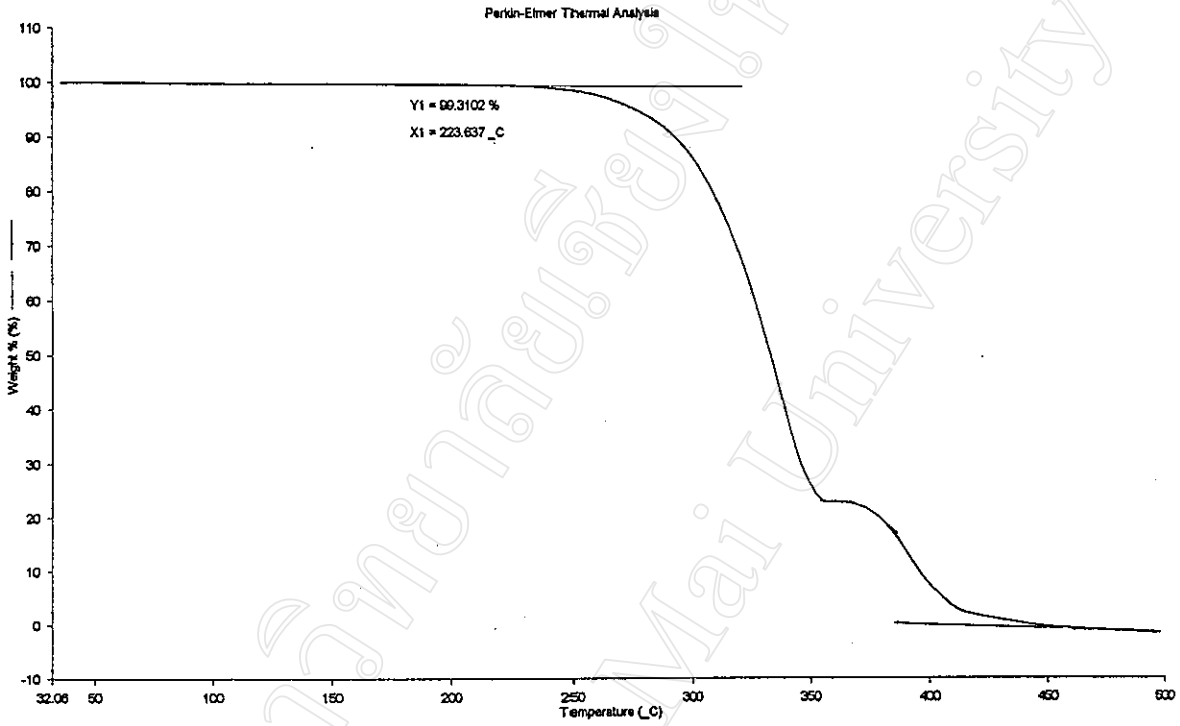

Figure 3.22 : The glass transition temperatures of tranexamic acid 223.63 ^{0}C

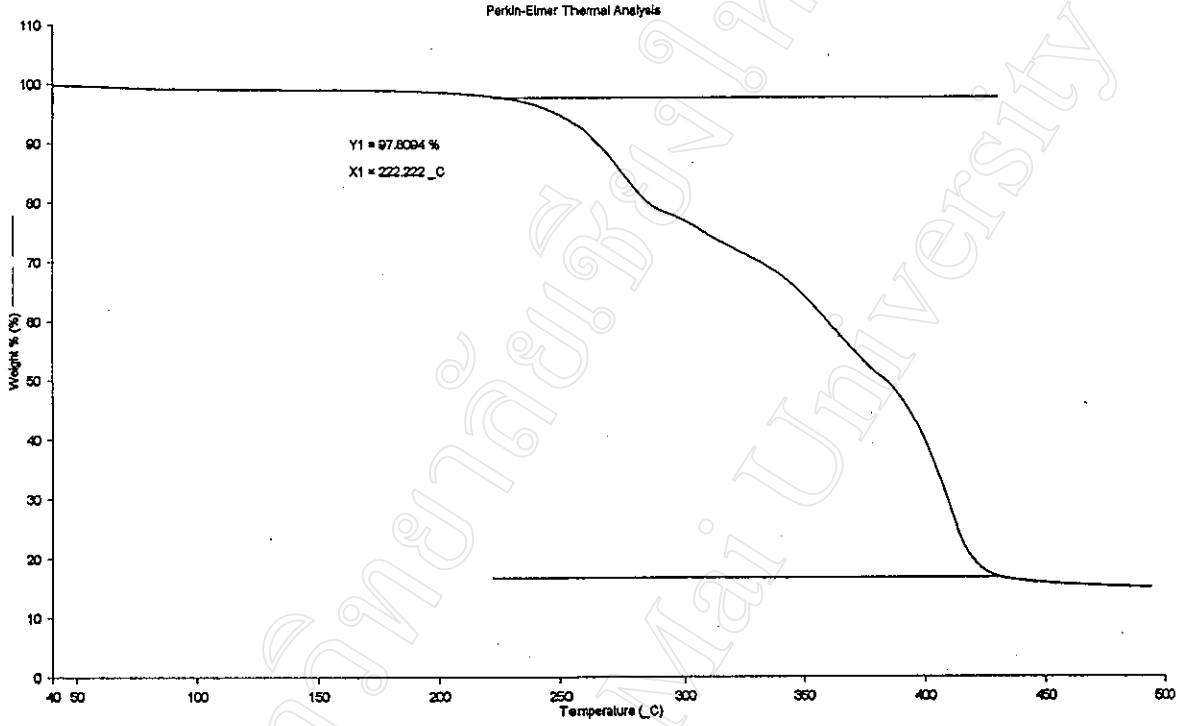

Figure 3.23 : The glass transition temperatures of 7:2:1 (10%TA, +) liposome formulations was 222.22 ^{0}C

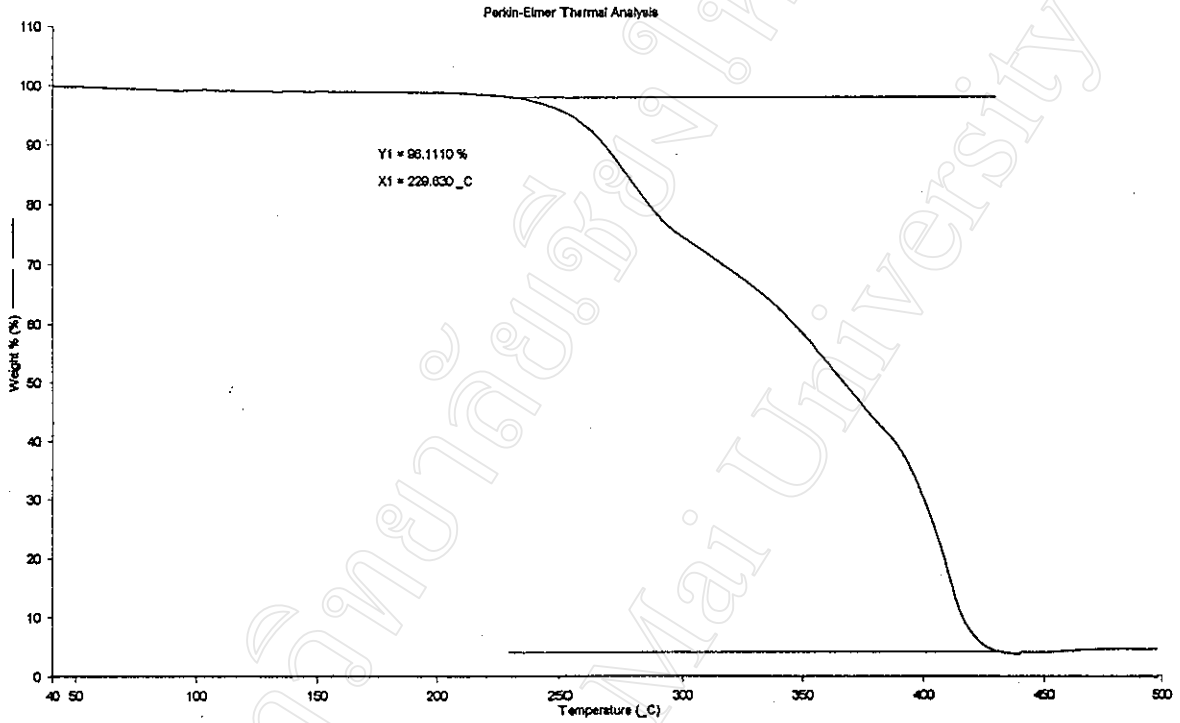

Figure 3.24 : The glass transition temperatures of 7:2:1 (10%TA, -) liposome formulations was 229.63 ^{0}C

3.1.5.2 Transition temperature (T_c) and enthalpy of transition (ΔH)

Transition temperatures and enthalpy of transition of Emulmetik 950®, cholesterol, stearylamine, dicetyl phosphate, tranexamic acid 7:2:1(+), 7:2:1(5%TA,+), -), 7:2:1(10%TA, +) 7:2:1(-), 7:2:1(5%TA, -) and 7:2:1(10%TA, -) liposome formulations by DSC with the scanning rate of 5 ^{0}C / min from 20 to 200 ^{0}C were shown in Tables 3.5 to 3.6 and Figures 3.25 to 3.29. The transition temperatures of the positively and negatively charged liposome formulations with and without the entrapped drug were not different. The enthalpy of transition of the positively and negatively charged liposome formulations without the entrapped drug was higher than that of the positively and negatively charged liposome formulations with the drug. The tranexamic acid exhibited no transition temperature and the enthalpy of transition when scanned in the range of 20 to 200 ^{0}C.

Table 3.5 : The transition temperatures (^{0}C) of Emulmetik 950®, cholesterol, stearylamine, dicetyl phosphate, tranexamic acid 7:2:1(+),7:2:1(5%TA,+), 7:2:1 (10%TA,+),7:2:17:2:1(-), (5%TA, -),7:2:1(10%TA, -) liposome formulations

Formulation	Transition temperature (^{0}C)		
	1	2	Mean
Emulmetik 950®	61.50	61.00	61.75
Cholesterol	149.50	149.83	149.66
Stearylamine	57.50	56.66	57.08
Dicetyl Phosphate	79.50	79.16	79.33
7:2:1(+)	78.00	77.50	77.75
7:2:1(5%TA,+)	76.50	76.00	76.25
7:2:1(10%TA,+)	76.33	77.83	77.08
7:2:1(-)	69.66	70.33	70.00
7:2:1(5%TA,-)	71.00	71.66	71.33
7:2:1(10%TA,-)	70.50	71.16	70.83

Note : There was no peak of tranexamic acid in the thermogram

Table 3.6 : The enthalpy of transition (ΔHJ/g) of Emulmetik 950®, cholesterol, stearylamine, dicetyl phosphate, tranexamic acid 7:2:1(+),7:2:1(5%TA,+), 7:2:1(10%TA, +) 7:2:17:2:1(-), (5%TA, -),7:2:1(10%TA, -) liposome formulations

Formulation	Enthalpy of transition (J/g)		
	1	2	Mean
Emulmetik 950®	31.56	45.46	38.51
Cholesterol	65.94	67.34	66.64
Stearylamine	256.30	331.19	293.74
Dicetyl Phosphate	185.71	188.25	186.98
7:2:1(+)	55.19	44.89	50.04
7:2:1(5%TA,+)	11.60	12.05	11.82
7:2:1(10%TA,+)	6.58	10.05	8.31
7:2:1(-)	52.87	37.85	45.36
7:2:1(5%TA,-)	12.70	16.66	14.60
7:2:1(10%TA,-)	4.06	8.60	6.33

3.2 Quantitative analysis of tranexamic acid (TA)

3.2.1 Standard curve of TA

The reference standard TA at concentrations of 4.00, 8.00, 12.00, 16.00, and 20.00 µg/ml in DI water were prepared in tripicate. The absorbance of each sample after derivertized with 2,4,6 trinitrobenzosulfonic acid was measured at λ 415 by a spectrophotometer in duplicate of 3 lots. The absorbances were shown in Tables 3.7 to 3.9 and the standard curve was constructed and presented in Figure 3.30. The data from the standard curve of TA when fitted with regression analysis gave r^2 close to 1 (0.9937). This confirmed that the quantitative analysis of TA used in this study gave high accuracy with precision.

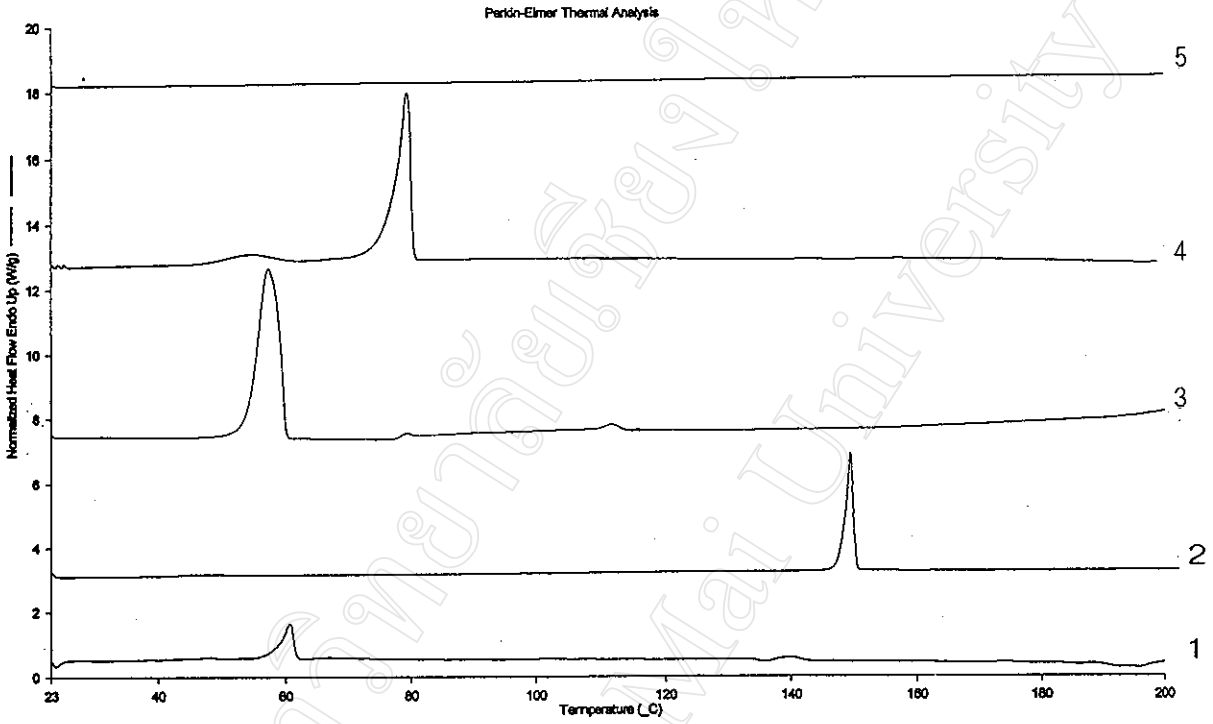

Figure 3.25 : DSC Thermograms of Emulmetik 950® (No.1), cholesterol (No.2), stearylamine (No.3), dicetyl phosphate (No.4), and tranexamic acid (No.5).

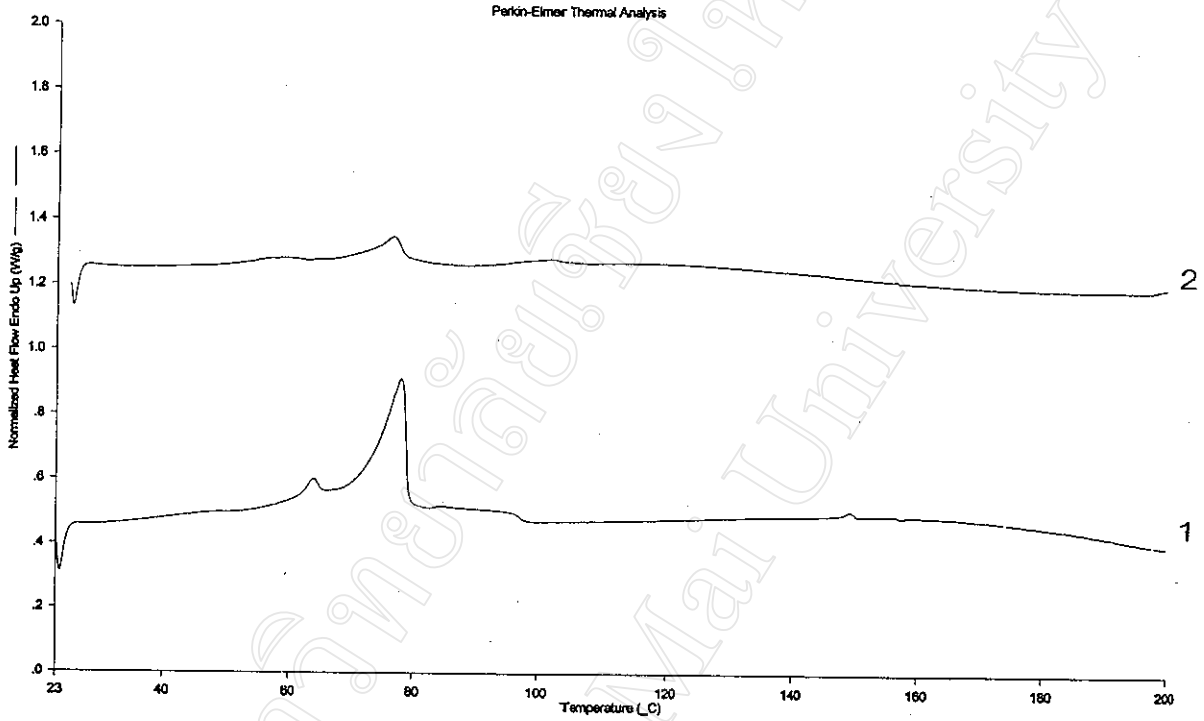

Figure 3.26 : DSC Thermograms of (7:2:1,+) (No.1), and 7:2:1 (5%TA,+) (No.2)

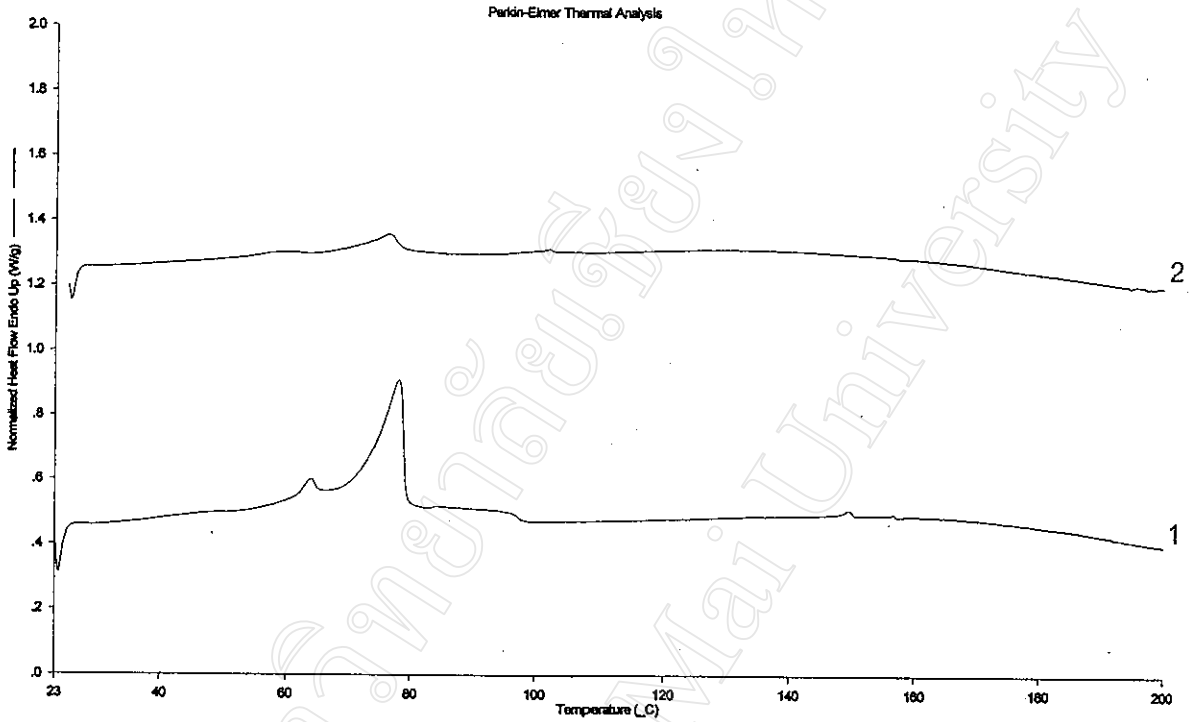

Figure 3.27 : DSC Thermograms of (7:2:1,+) (No.1), and 7:2:1 (10%TA,+) (No.2)

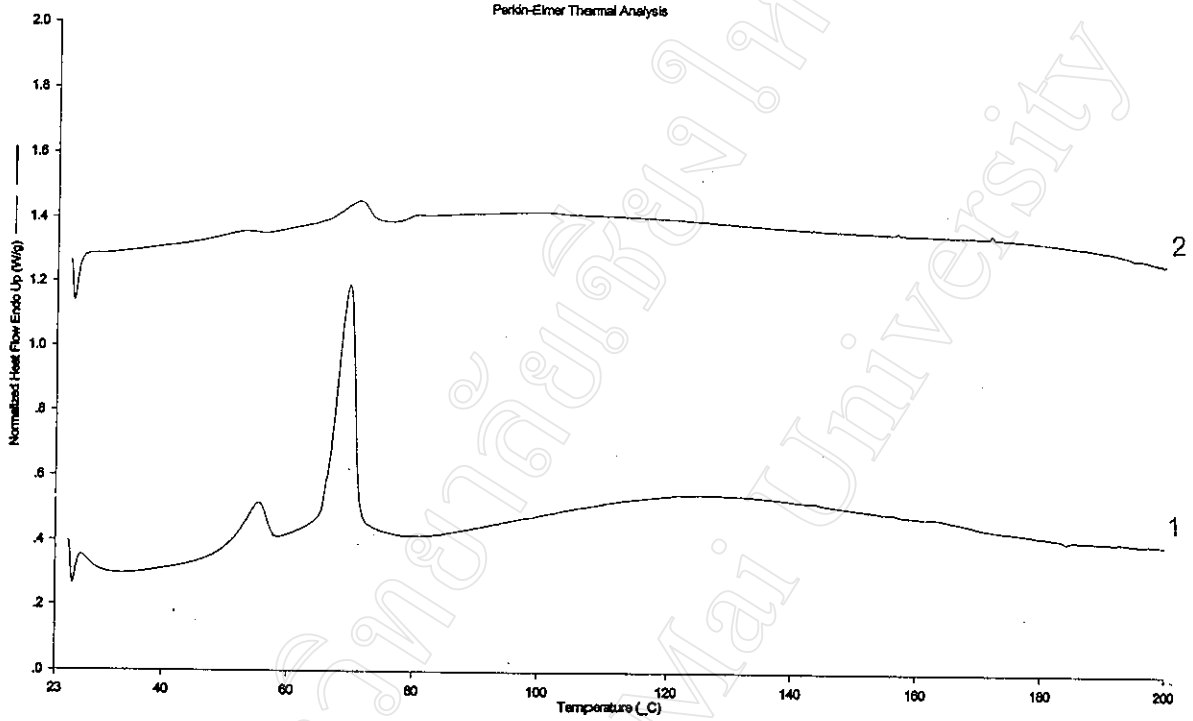

Figure 3.28 : DSC Thermograms of (7:2:1,-) (No.1), and 7:2:1 (5%TA,-) (No.2)

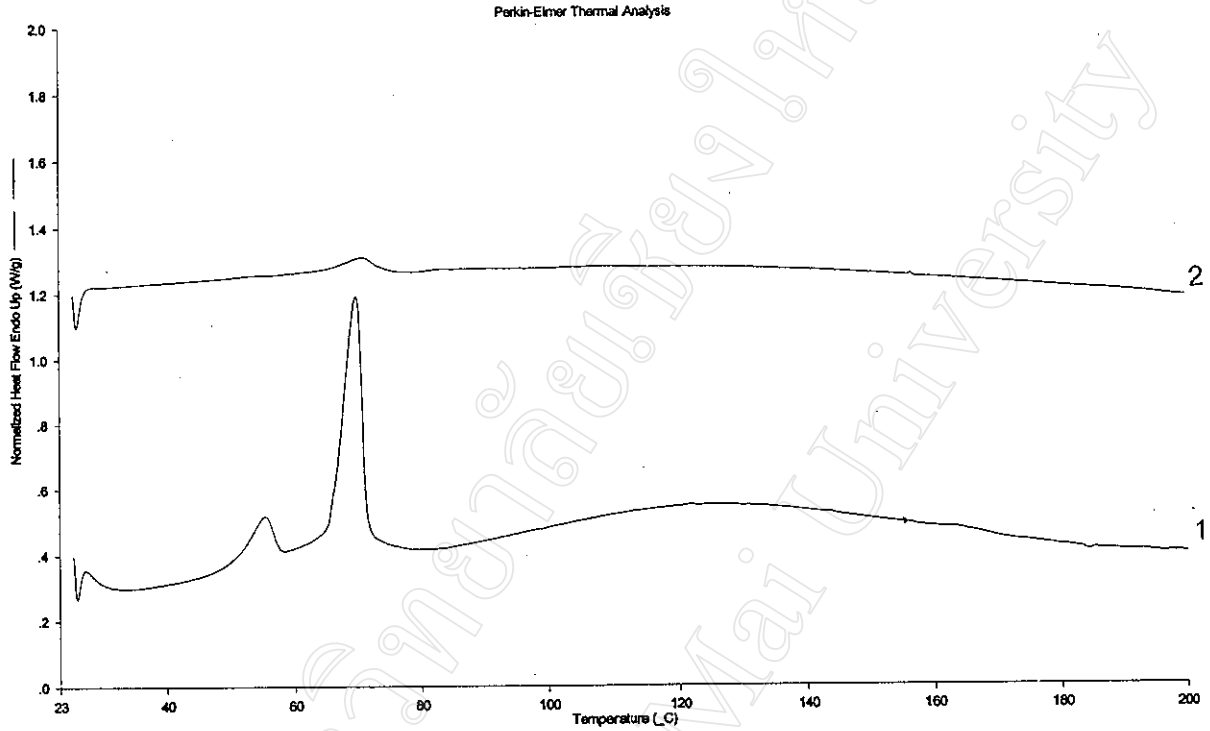

Figure 3.29 : DSC Thermograms of (7:2:1,-) (No.1), and 7:2:1 (10%TA,-) (No.2)

Table 3.7 : Absorbance (λ = 415 nm) of various concentrations of the reference standard TA in DI water (Lot.1)

Conc. (µg/ml)	Absorbance Lot. 1		
	1	2	Mean
0	0	0	0
4	0.190	0.190	0.190
8	0.400	0.400	0.400
12	0.628	0.630	0.629
16	0.794	0.794	0.794
20	0.930	0.928	0.929

Table 3.8 : Absorbance (λ = 415 nm) of various concentrations of the reference standard TA in DI water (Lot.2)

Conc. (µg/ml)	Absorbance Lot. 2		
	1	2	Mean
0	0	0	0
4	0.188	0.188	0.188
8	0.400	0.400	0.400
12	0.617	0.617	0.617
16	0.797	0.801	0.799
20	0.927	0.927	0.927

Table 3.9 : Absorbance (λ = 415 nm) of various concentrations of the reference standard TA in DI water (Lot.3)

Conc. (µg/ml)	Absorbance Lot. 3		
	1	2	Mean
0	0	0	0
4	0.192	0.192	0.192
8	0.399	0.399	0.399
12	0.631	0.631	0.631
16	0.804	0.804	0.804
20	0.927	0.927	0.927

Table 3.10 : Absorbance (λ = 415 nm) from tripicate analysis of the reference standard TA solution

Conc.(µg/ml)	Mean Lot.1	Mean Lot.2	Mean Lot.3	Mean	SD	%CV
0	0	0	0	0	0	0
4	0.190	0.188	0.192	0.1900	0.0020	1.0526
8	0.400	0.400	0.399	0.3997	0.0006	0.1445
12	0.629	0.617	0.631	0.6257	0.0076	1.2102
16	0.794	0.799	0.804	0.7990	0.0050	0.6258
20	0.929	0.927	0.927	0.9277	0.0012	0.1245

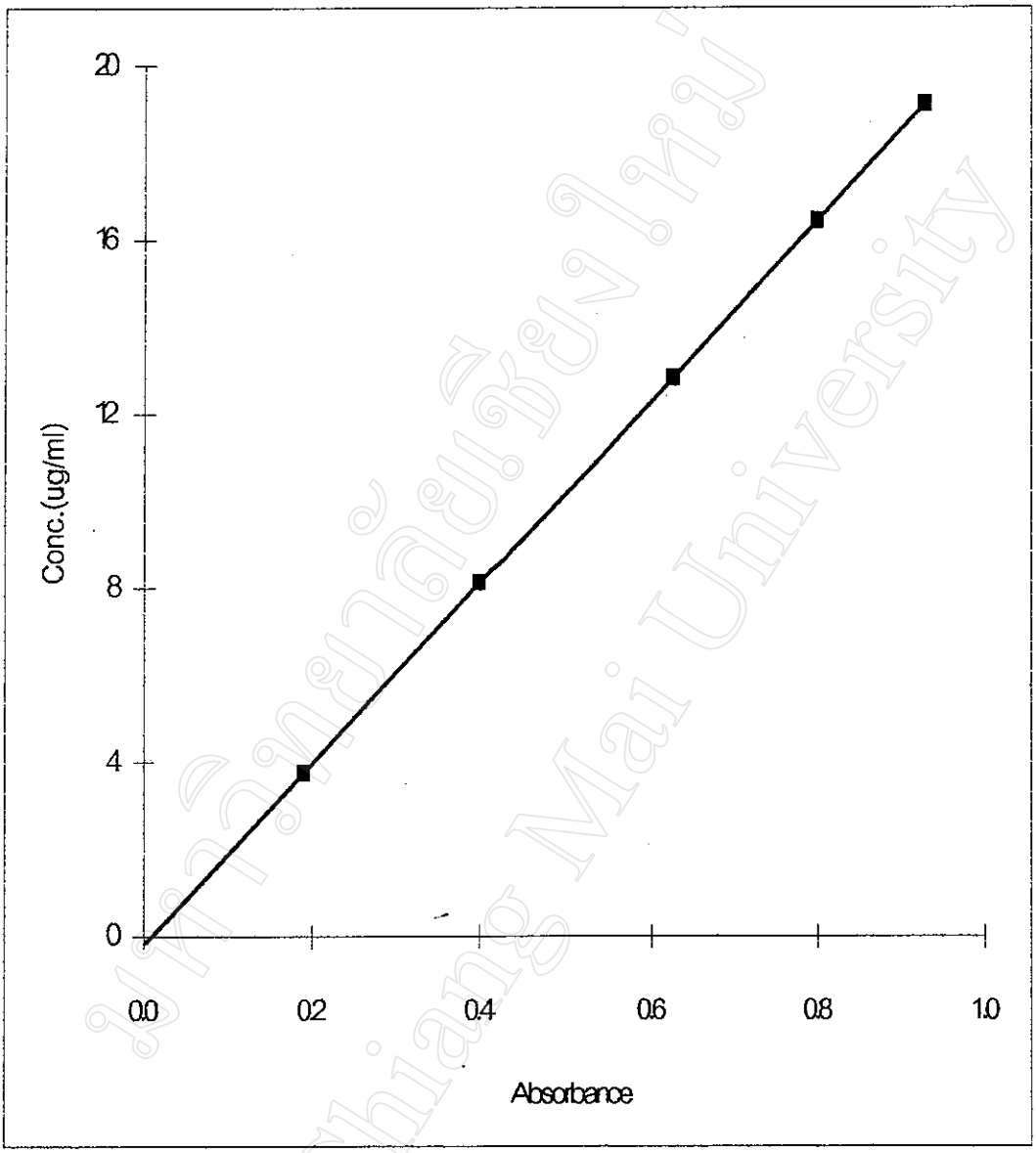

Figure 3.30 : The standard curve of standard TA in DI water (r^2 = 0.9937 and y = 20.7903 X - 0.1942)

3.2.2 Validation of the assay of tranexamic acid (TA) by a spectrophotometric method

In this study, spectrophotometric method was developed for the determination of TA by measuring the absorbance of the derivative of TA formed with 2,4,6 trinitrobenzosulfonic acid. The percentage recovery of TA in solution when assayed by a spectrophotometric method was compared with the potentiometric titration method using t test and F test. At the 95% confidence level there was no significant difference between these two methods. (Table 3.11). The percentage of recovery of TA in liposome formulations which assayed by spectrophotometric methods was showed in Table 3.12.

Table 3.11 : Comparison of the percentage recovery of TA determination by spectrophotometric and potentiometric titration methods

Method	2.5% TA solution (% Recovery)			Mean ± S.D.	%CV
	Lot.1	Lot.2	Lot.3		
Spectrophotometric	103.77	102.21	103.2	103.08 ± 0.79	0.77
Potentiometric titration	104.40	106.80	104.40	105.20 ± 1.38	1.31

Table 3.12 : Percentage recovery of TA in liposome formulations determined by a spectrophotometric method

Formulation	Liposome Lot.1		Liposome Lot.2		Mean ± S.D.
	1	2	1	2	
7:2:1 (5%TA,+)	94.09	94.46	93.12	93.42	93.77 ± 0.61
7:2:1 (10%TA,+)	103.79	103.40	104.74	104.52	104.66 ± 0.64
7:2:1 (5%TA,-)	91.44	91.44	95.38	94.86	93.28 ± 2.14
7:2:1 (10%TA,-)	103.45	104.01	103.69	103.54	103.67 ± 0.24

3.3 Determination of the percentages of tranexamic acid (TA) entrapped in liposome formulations

The amount of TA (mg) in supernatant and pellets and the percentages of TA entrapped in liposomes were presented in Tables 3.13 to 3.15 respectively. liposome formulations The percentages of loading of TA in lposome formulations were also shown in Table 3.16

Table 3.13 : Amount of TA (mg) in supernatant of the 1 g liposome formulations

Formulation	Supernatant				
	Liposome Lot.1		Liposome Lot.2		Mean ± S.D.
	1	2	1	2	
7:2:1 (5%TA,+)	39.87	40.05	40.05	40.05	40.01 ± 0.09
7:2:1 (10%TA,+)	87.86	87.51	89.30	89.12	88.45 ± 0.90
7:2:1 (5%TA,-)	38.34	38.34	40.51	40.33	39.38 ± 1.20
7:2:1 (10%TA,-)	89.57	89.94	90.31	90.13	89.99 ± 0.32

Table 3.14 : Amount of TA (mg) in pellet of the 1 g liposome formulations

Formulation	Pellet				
	Liposome Lot.1		Liposome Lot.2		Mean ± S.D.
	1	2	1	2	
7:2:1 (5%TA,+)	7.18	7.18	6.51	6.66	6.88 ± 0.35
7:2:1 (10%TA,+)	15.93	15.89	15.44	15.44	15.67 ± 0.27
7:2:1 (5%TA,-)	7.38	7.38	7.18	7.10	7.26 ± 0.14
7:2:1 (10%TA,-)	13.88	14.06	13.38	13.42	13.68 ± 0.34

Table 3.15 : Percentages of TA entrapped in various liposome from liposome formulations

Formulation	%TA entrapped in liposomes				Mean ± S.D.
	Liposome Lot.1		Liposome Lot.2		
	1	2	1	2	
7:2:1 (5%TA,+)	15.25	15.19	13.98	14.25	14.67 ± 0.65
7:2:1 (10%TA,+)	15.35	15.37	14.74	14.77	15.05 ± 0.35
7:2:1 (5%TA,-)	16.14	16.14	15.05	14.97	15.58 ± 0.66
7:2:1 (10%TA,-)	13.41	13.52	12.90	12.96	13.20 ± 0.31

Table 3.16 : Percentages of loading of TA in various liposome formulations

Formulation	Total lipid (mg) in1g of liposome	mg of TA per g of liposomes				% loading of liposome (mg /100 mg lipid)				mean % loading of TA in liposome (mg / 100mg lipid)
		Lot.1		Lot.2		Lot.1		Lot.2		
7:2:1 (5%TA,+)	25	7.18	7.18	6.51	6.66	28.70	28.70	26.04	26.63	27.52 ± 1.39
7:2:1 (10%TA,+)	25	15.93	15.89	15.44	15.44	63.17	63.56	61.76	61.73	62.70 ± 1.08
7:2:1 (5%TA,-)	25	7.38	7.38	7.18	7.10	29.53	29.53	28.70	28.41	29.04 ± 0.57
7:2:1 (10%TA,-)	25	13.88	14.06	13.38	13.42	55.51	56.25	53.51	53.66	54.73 ± 1.36

3.4 Stability study of tranexamic acid (TA) entrapped in liposome formulations

3.4.1 Physical stability

3.4.1.1 Physical appearance

The physical stability of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1 (5%TA, -) and 7:2:1(10%TA, -) liposome formulations when freshly prepared and kept at 4 ± 1 , 30 ± 1 and 45 ± 1 ^{0}C was observed at 30, 60 and 90 days. The physical appearance of liposome formulations was investigated and shown in Tables 3.17 to 3.20 and Figures 3.31 to 3.33. The negatively charged lipsome without the entrapped drug [7:2:1(-)] was sedimented at 45 ± 1 ^{0}C after 30 days and at 30 ± 1 ^{0}C after 60 days. After 90 days, the positively charged lipsome with and without the entrapped drug gave more turbidity more than the negatively charged lipsome with and without the drug.

Table 3.17 : The physical appearance of the freshly prepared 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) liposome formulations

Formulation	Physical appearance of the freshly prepared liposome formulations		
	Sedimentation	Flocculation	Turbidity
7:2:1 (+)	No	No	+1
7:2:1(5%TA,+)	No	No	+1
7:2:1(10%TA,+)	No	No	+1
7:2:1 (-)	No	No	+1
7:2:1(5%TA,-)	No	No	+1
7:2:1(10%TA,-)	No	No	+1

Note : +1 to +7 indicated the degree of increasing turbidity

Table 3.18 : The physical appearance of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) liposome formulations when kept at 4 ± 1 , 30 ± 1 and 45 ± 1 °C for 30 days

Formulation	Physical appearance of liposome formulations for 30 days								
	Sedimentation			Flocculaton			Turbidity		
	4 °C	30 °C	45 °C	4 °C	30 °C	45 °C	4 °C	30 °C	45 °C
7:2:1(+)	No	No	No	No	No	No	+1	+2	+2
7:2:1(5%TA,+)	No	No	No	No	No	No	+1	+2	+2
7:2:1(10%TA,+)	No	No	No	No	No	No	+1	+2	+2
7:2:1(-)	No	No	Yes	Yes	Yes	Yes	+1	+1	+1
7:2:1(5%TA,-)	No	No	No	No	No	No	+1	+1	+1
7:2:1(10%TA,-)	No	No	No	No	No	No	+1	+1	+1

Note : +1 to +7 indicated the degree of increasing turbidity

Table 3.19 : The physical appearance of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) liposome formulations when kept at 4 ± 1 , 30 ± 1 and 45 ± 1 °C for 60 days

Formulation	Physical appearance of liposome formulations for 60 days								
	Sedimentation			Flocculation			Turbidity		
	4 °C	30 °C	45 °C	4 °C	30 °C	45 °C	4 °C	30 °C	45 °C
7:2:1(+)	No	No	No	No	No	No	+1	+3	+6
7:2:1(5%TA,+)	No	No	No	No	No	No	+1	+3	+4
7:2:1(10%TA,+)	No	No	No	No	No	No	+1	+3	+3
7:2:1(-)	No	Yes	Yes	Yes	Yes	Yes	+3	+3	+3
7:2:1(5%TA,-)	No	No	No	No	No	No	+1	+1	+1
7:2:1(10%TA,-)	No	No	No	No	No	No	+1	+1	+1

Note : +1 to +7 indicated the degree of increasing turbidity

Table 3.20 : The physical appearance of 7:2:1(+), 7:2:1(5%TA,+), 7:2:1(10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) liposome formulations when kept at 4 ± 1 , 30 ± 1 and 45 ± 1 ^{0}C for 90 days

Formulation	Physical appearance of liposome formulations for 90 days								
	Sedimentation			Flocculation			Turbidity		
	4 ^{0}C	30 ^{0}C	45 ^{0}C	No	No	No	4 ^{0}C	30 ^{0}C	45 ^{0}C
7:2:1(+)	No	No	No	Yes	Yes	Yes	+1	+3	+7
7:2:1(5%TA,+)	No	No	No	No	No	No	+1	+5	+4
7:2:1(10%TA,+)	No	No	No	No	No	No	+1	+5	+4
7:2:1(-)	Yes	Yes	Yes	Yes	Yes	Yes	+3	+5	+5
7:2:1(5%TA,-)	No	No	No	No	No	No	+1	+1	+3
7:2:1(10%TA,-)	No	No	No	No	No	No	+1	+1	+1

Note : +1 to +7 indicated the degree of increasing turbidity

3.4.1.2 The amount of the remaining Tranexamic acid (TA) entrapped in liposomes of various liposome formulations

The amount of TA remaining in liposome formulations of 7:2:1(5%TA,+) , 7:2:1 (10%TA,+) , 7:2:1(5%TA,-) and 7:2:1(10%TA,-) when kept at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days were demonstrated in Tables 3.22 to 3.25 and Figures 3.34 to 3.36.

The kinetic stability study of TA remaining in liposome formulations was investigated. The shelf life as well as the leakage rate of TA from liposome formulations were calculated by substituting in the equation of zero order, first order, and Higuchi model. These equations were demonstrated in Appendix. The r square values from these equations were determined. From Table 3.29, the first order kinetics of all liposome formulations appeared to give the r square value higher than the zero order and Higuchi model. Thus, the first order kinetics was used to predict the degradation rate of TA in liposome formulations at each temperature. The leakage rate can then be converted to the periods when the contents of TA

Figure 3.31 : The physical appearance of the prepared liposome formulations when stored at 4 ± 1 ^{0}C for 90 days from left to right, 7:2:1(+), 7:2:1(5%TA,+), 7:2:1 (10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) respectively

Figure 3.32 : The physical appearance of the prepared liposome formulations when stored at 30 ± 1 °C for 90 days from left to right, 7:2:1(+), 7:2:1(5%TA,+), 7:2:1 (10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) respectively

Figure 3.33 : The physical appearance of the prepared liposome formulations when stored at 45 ± 1 °C for 90 days from left to right 7:2:1(+), 7:2:1(5%TA,+), 7:2:1 (10%TA,+), 7:2:1(-), 7:2:1(5%TA, -), and 7:2:1(10%TA, -) respectively

remaining 90% (shelf life, t_{90}). The leakage rates and the shelf life of each formulation at each temperature were shown in Table 3.21 and Figure 3.25. The leakage rate in the positively charged liposome was more than the negatively charged liposome and the leakage rate was increased with increasing temperature.

From Table 3.28 : The % remaining of TA in liposomes was calculated by fitting the data to zero order, first order and the Higuchi model. The correlation coefficient from the first order showed higher relation than the zero order and Higuchi model. Thus, the leakage rate from the first order was used to predict the shelf life of liposome formulations showed in Table 3.29. The histogram of the predicted shelf life from Table 3.29 was presented in Figure 3.37.

3.4.2 Chemical stability

The amount of TA in various liposome formulations showed the highest stability of about more than 90% of the drug remaining at 4, 30 and 45 °C for 90 days.

The kinetic chemical stability study of TAwas investigated. The shelf life as well as the degradation rate of TA in liposome formulations were calculated by substituting in the equation of zero order, first order, and Higuchi model. These equations were demonstrated in Appendix. The r square values from these equations were determined. From Table 3.37, the kinetics chemical of zero order, first order, and Higuchi model of all liposome formulations appeared to give the r square value no significant different. But, the degradation rate of the drug was increased with increasing temperature.

Table 3.21 : The percentage remaining amounts of TA entrapped in liposome of the 7:2:1 (5%TA,+) liposome formulation when kept at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

7:2:1(5%TA,+)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	40.01 ± 0.07	40.01 ± 0.07	40.01 ± 0.07
	Total TA in pellet of liposome 1 g (mg / g)	6.88 ± 0.42	6.88 ± 0.42	6.88 ± 0.42
	% remaining	100 ± 0.00	100 ± 0.00	100 ± 0.00
14 days	Total TA in supernatant of liposome 1 g (mg / g)	38.11 ± 0.19	37.71 ± 0.61	37.90± 1.15
	Total TA in pellet of liposome 1 g (mg / g)	5.95 ± 0.05	6.12± 0.03	5.88±0.01
	% remaining	92.19 ± 4.58	95.45 ± 4.50	91.61± 7.39
30 days	Total TA in supernatant of liposome 1 g (mg / g)	37.33± 0.19	37.33 ± 0.20	38.62±0.19
	Total TA in pellet of liposome 1 g (mg / g)	5.78 ± 0.20	5.56±0.29	5.70±0.51
	% remaining	91.62 ± 8.00	88.72±1.17	87.66±2.10
60 days	Total TA in supernatant of liposome 1 g (mg / g)	37.99 ±0.24	37.19±3.88	35.92±4.30
	Total TA in pellet of liposome 1 g (mg / g)	6.26±0.42	5.48±0.67	4.36±0.32
	% remaining	83.20±5.83	74.95±3.45	68.56±9.04
90 days	Total TA in supernatant of liposome 1 g (mg / g)	39.71±1.53	38.17±0.71	36.89±0.19
	Total TA in pellet of liposome 1 g (mg / g)	4.72±0.34	3.88±0.37	3.62±0.24
	% remaining	75.90±6.17	67.65±0.41	61.72±9.63

Table 3.22 : The percentage remaining amounts of TA entrapped in liposome of the 7:2:1 (10%TA,+) liposome formulation when kept at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

7:2:1(10%TA,+)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	88.99±0.32	88.99±0.32	88.99±0.32
	Total TA in pellet of liposome 1 g (mg / g)	6.88±0.42	6.88±0.42	6.88±0.42
	% remaining	100.00	100.00	100.00
14 days	Total TA in supernatant of liposome 1 g (mg / g)	89.00±0.19	91.08±0.46	87.15±0.89
	Total TA in pellet of liposome 1 g (mg / g)	15.56±0.05	14.55±0.03	15.79±0.12
	% remaining	99.42±2.01	96.50±1.31	97.43±2.41
30 days	Total TA in supernatant of liposome 1 g (mg / g)	87.03±0.19	90.67±0.20	87.03±0.19
	Total TA in pellet of liposome 1 g (mg / g)	14.86±0.35	14.76±1.05	14.14±0.83
	% remaining	98.73±0.35	93.41±3.56	88.90±2.31
60 days	Total TA in supernatant of liposome 1 g (mg / g)	88.94±1.69	80.46±4.06	77.26±2.15
	Total TA in pellet of liposome 1 g (mg / g)	13.29±3.09	10.73±0.05	11.05±0.53
	% remaining	82.21±5.58	78.58±5.28	73.70±0.08
90 days	Total TA in supernatant of liposome 1 g (mg / g)	87.29±0.65	83.95±0.71	84.55±1.27
	Total TA in pellet of liposome 1 g (mg / g)	12.08±0.03	10.15±0.21	9.75±0.35
	% remaining	78.26±1.26	72.02±3.32	69.06±1.63

Table 3.23 : The percentage remaining amounts of TA entrapped in liposome of the 7:2:1 (5%TA,-) liposome formulation when kept at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

7:2:1(5%TA,-)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	39.38±1.47	39.38±1.47	39.38±1.47
	Total TA in pellet of liposome 1 g (mg / g)	7.26±0.17	7.26±0.17	7.26±0.17
	% remaining	100.00	100.00	100.00
14 days	Total TA in supernatant of liposome 1 g (mg / g)	40.16±1.18	40.48±0.91	42.09±1.25
	Total TA in pellet of liposome 1 g (mg / g)	7.42±0.11	7.16±0.09	6.81±0.21
	% remaining	100.22±1.45	94.92±1.16	98.14±0.41
30 days	Total TA in supernatant of liposome 1 g (mg / g)	41.31±1.18	40.73±0.07	41.31±1.18
	Total TA in pellet of liposome 1 g (mg / g)	5.65±0.55	5.82±0.60	5.33±0.10
	% remaining	87.83±5.24	84.48±2.15	86.97±0.22
60 days	Total TA in supernatant of liposome 1 g (mg / g)	40.05±0.33	38.41±0.20	36.24±0.31
	Total TA in pellet of liposome 1 g (mg / g)	5.13±1.00	4.94±0.48	5.09±0.74
	% remaining	79.75±7.14	77.80±2.65	75.03±7.29
90 days	Total TA in supernatant of liposome 1 g (mg / g)	39.78±0.39	40.36±0.20	40.10±0.64
	Total TA in pellet of liposome 1 g (mg / g)	5.92±0.12	4.25±0.30	4.92±0.37
	% remaining	83.20±2.12	76.33±7.32	73.12±1.17

Table 3.24 : The percentage remaining amounts of TA entrapped in liposome of the 7:2:1 (10%TA,-) liposome formulation when kept at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

7:2:1(10%TA,-)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	89.99±0.33	89.99±0.33	89.99±0.33
	Total TA in pellet of liposome 1 g (mg / g)	13.68±0.40	13.68±0.40	13.68±0.40
	% remaining	100.00	100.00	100.00
14 days	Total TA in supernatant of liposome 1 g (mg / g)	87.66±1.22	93.48±0.13	90.51±0.90
	Total TA in pellet of liposome 1 g (mg / g)	14.37±0.10	14.08±0.03	14.08±0.33
	% remaining	103.02±1.11	99.21±3.24	102.05±0.22
30 days	Total TA in supernatant of liposome 1 g (mg / g)	91.51±1.18	91.51±0.07	90.06±1.18
	Total TA in pellet of liposome 1 g (mg / g)	12.80±0.35	12.97±1.33	12.85±0.10
	% remaining	96.52±0.75	97.65±6.05	93.80±0.83
60 days	Total TA in supernatant of liposome 1 g (mg / g)	81.10±1.12	97.31±0.20	83.97±0.14
	Total TA in pellet of liposome 1 g (mg / g)	11.47±0.27	11.65±0.38	10.51±0.25
	% remaining	93.20±1.82	87.40±0.12	84.29±4.51
90 days	Total TA in supernatant of liposome 1 g (mg / g)	81.64±1.15	90.23±0.20	83.97±2.33
	Total TA in pellet of liposome 1 g (mg / g)	11.42±0.12	11.90±0.69	10.66 ±0.05
	% remaining	93.09±2.77	88.29±5.57	85.43±5.39

Table 3.25 : Comparison of the percentages of the remaining TA entrapped in liposome of various liposome formulations when kept at 4 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	92.19	99.42	100.22	103.02
30	91.62	98.73	87.83	96.52
60	83.20	82.21	79.75	93.92
90	75.90	78.26	83.20	93.09

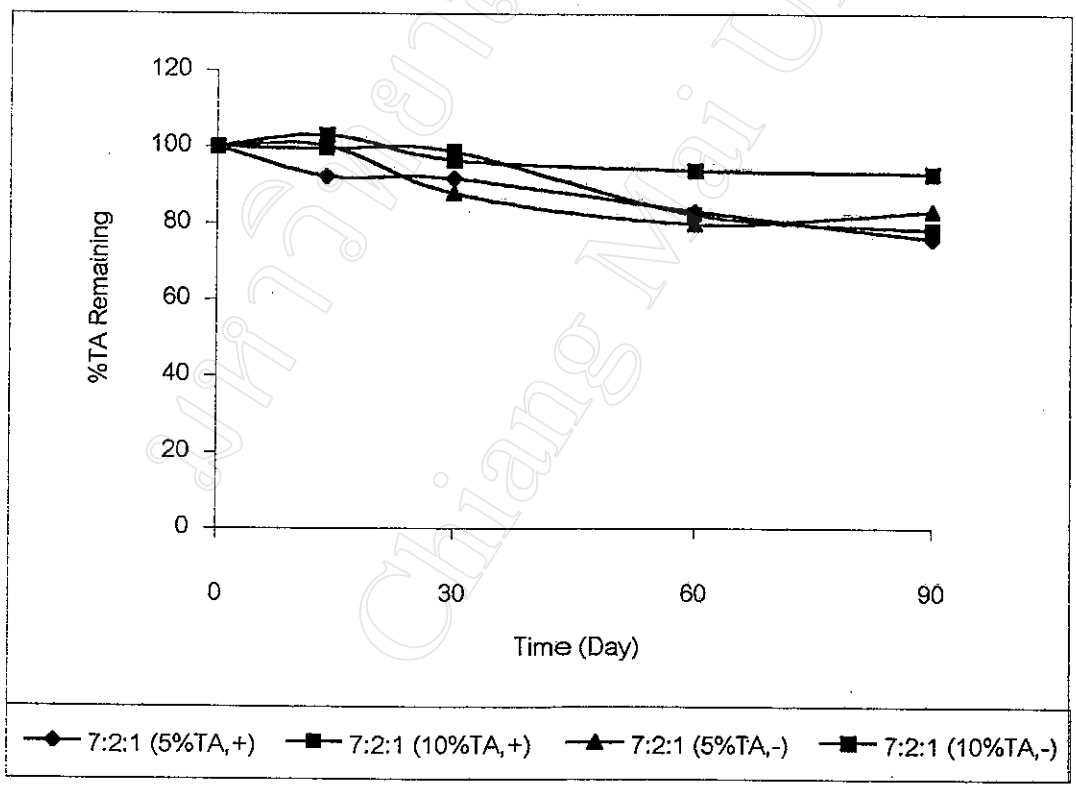

Figure 3.34 : The comparison of the percentages of the remaining total TA entrapped in liposome of various liposome formulations when kept at 4 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Table 3.26 : Comparison of the percentages of the remaining TA entrapped in liposome of various liposome formulations when kept at 30 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	95.45	96.50	94.92	99.21
30	88.72	93.41	84.48	97.65
60	74.95	78.58	77.80	87.40
90	67.65	72.02	76.33	88.29

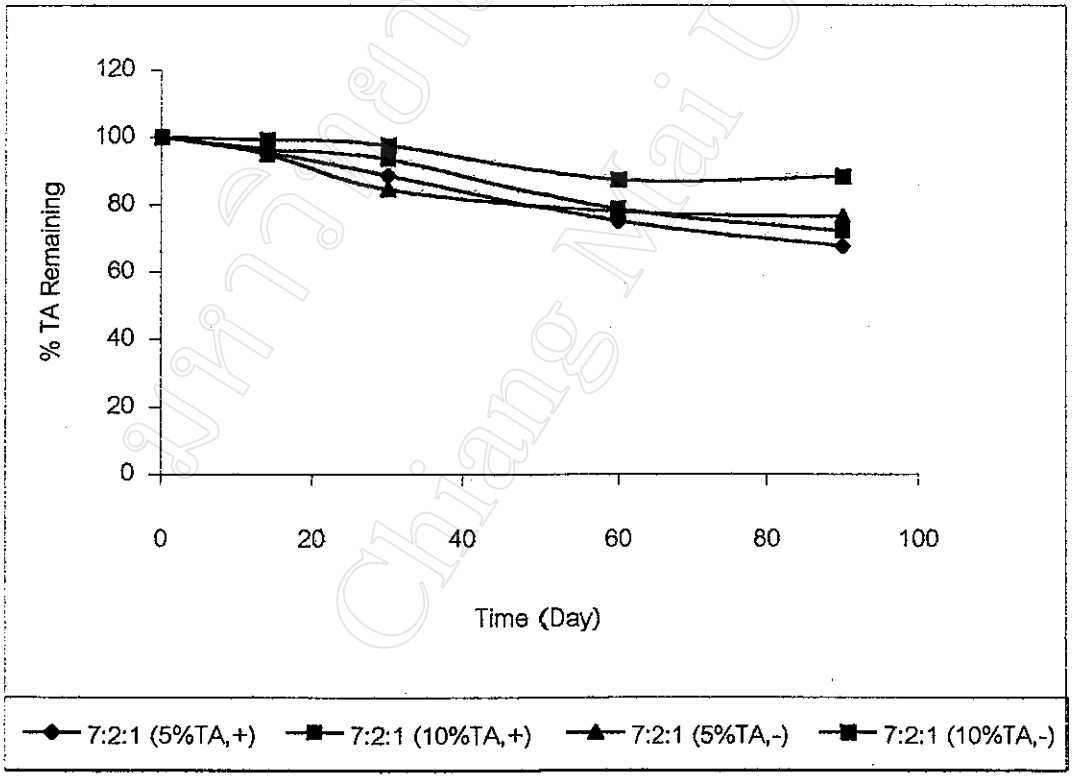

Figure 3.35 : The comparison of the percentages of the remaining total TA entrapped in liposome of various liposome formulations when kept at 30 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Table 3.27 : Comparison of the percentages of the remaining TA entrapped in liposome of various liposome formulations when kept at 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	91.61	97.43	98.14	102.05
30	87.66	88.90	86.97	93.80
60	68.56	73.70	75.03	84.29
90	61.72	69.06	73.12	85.43

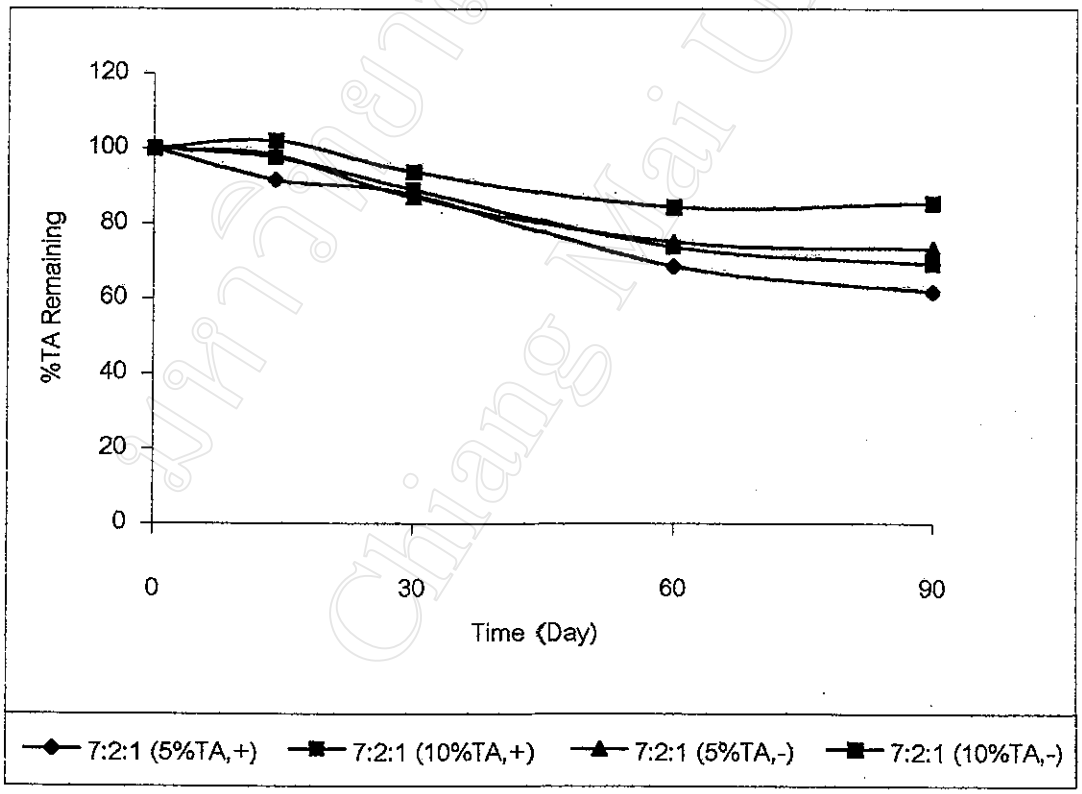

Figure 3.36 : The comparison of the percentages of the remaining total TA entrapped in liposome of various liposome formulations when kept at 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Table 3.28 : Determination of chemical kinetic of TA remaining entrapped in liposomes of liposome formulations fitted in zero order, first order and Higuchi model

Formulaton	Temperatur	Zero order			First order			Higuchi model		
	e	Slope (-)	Intercept	R square	Slope (-)	Intercept	R square	Slope (-)	Intercept	R square
7:2:1	4 °C	0.2510	98.3291	0.9697	0.0013	1.9941	0.9755	2.4378	101.4892	0.9392
(5%TA,+)	30 °C	0.3749	99.9001	0.9940	0.0020	2.0030	0.9919	3.5561	104.1730	0.9131
	45 °C	0.4383	98.9196	0.9734	0.0024	2.0002	0.9787	4.1999	104.1362	0.9176
7:2:1	4 °C	0.2780	102.5100	0.9076	0.0013	2.0129	0.9210	2.4823	104.8606	0.7433
(10%TA,+)	30 °C	0.3306	100.9294	0.9767	0.0017	2.0068	0.9764	3.0660	104.3276	0.8628
	45 °C	0.3758	100.4009	0.9626	0.0020	2.0048	0.9680	3.5596	104.6558	0.8869
7:2:1	4 °C	0.2254	98.9472	0.7433	0.0010	1.9960	0.8015	2.2717	102.2217	0.7751
(5%TA,-)	30 °C	0.2695	97.1629	0.8774	0.0014	1.9879	0.8920	2.7528	101.2740	0.9403
	45 °C	0.3311	99.5011	0.9294	0.0017	1.9994	0.9288	3.2113	103.6466	0.8880
7:2:1	4 °C	0.1000	101.1906	0.7554	0.007	2.0148	0.7897	0.9109	102.1306	0.6436
(10%TA,-)	30 °C	0.1568	100.5957	0.8595	0.0010	2.0030	0.8522	1.4662	102.2694	0.7688
	45 °C	0.2040	101.0327	0.8292	0.0013	2.0048	0.8322	1.9242	103.2969	0.7571

Note : see the equations of zero order, first order and Higuchi model in Appendix, (-) is minus, slope is leakage rate

Table 3.29 : The predicted shelf life of the TA in liposome of various liposome formulations

Formulation	Shelf life (days)		
	4 ^{0}C	30 ^{0}C	45 ^{0}C
7:2:1 (5%TA, +)	35.07	22.80	19.00
7:2:1 (10%TA, +)	35.07	26.82	22.80
7:2:1 (5%TA, -)	45.59	32.57	26.82
7:2:1 (10%TA, -)	65.13	45.59	35.07

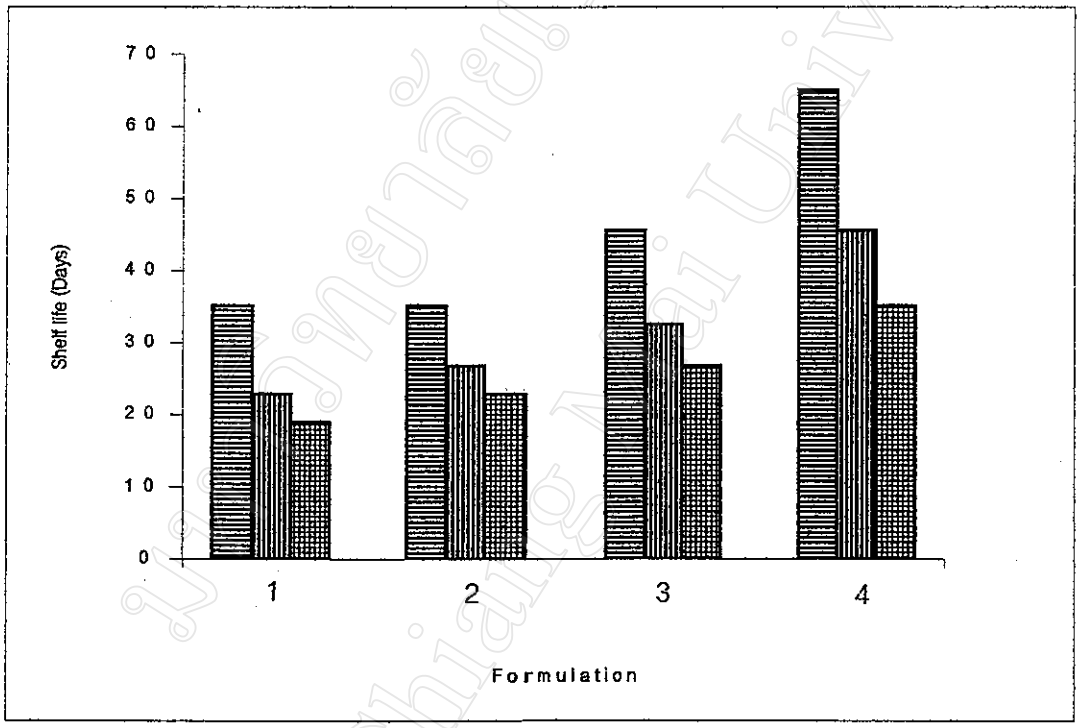

Figure 3.37 : The histograms of the predicted shelf life (days) at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C of liposome formulations.

▤ = 4 ± 1 ^{0}C ▥ = 30 ± 1 ^{0}C ▦ = 45 ± 1 ^{0}C

1 = 7:2:1 (5%TA,+) 2 = 7:2:1 (10%TA,+) 3 = 7:2:1 (5%TA,+) 4 = 7:2:(10%TA,+)

Table 3.30 : The total amounts of TA and the percentage of TA left comparing to at initial time in the 7:2:1(5%TA,+) liposome formulation (1 g) when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days

7:2:1(5%TA,+)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	40.01	40.01	40.01
	Total TA in pellet of liposome 1 g (mg / g)	6.88	6.88	6.88
	Total (mg)	46.89(100.00%)	46.89(100.00%)	46.89(100.00%)
14 days	Total TA in supernatant of liposome 1 g (mg / g)	38.11	37.71	37.90
	Total TA in pellet of liposome 1 g (mg / g)	5.95	6.12	5.88
	Total (mg)	44.06 (93.96%)	43.83 (93.47%)	43.73 (93.37%)
30 days	Total TA in supernatant of liposome 1 g (mg / g)	37.33	37.33	38.62
	Total TA in pellet of liposome 1 g (mg / g)	5.78	5.56	5.70
	Total (mg)	43.11 (91.94%)	42.89 (91.47%)	44.32 (94.52%)
60 days	Total TA in supernatant of liposome 1 g (mg / g)	37.99	37.19	35.92
	Total TA in pellet of liposome 1 g (mg / g)	6.26	5.48	4.36
	Total (mg)	44.25 (94.37%)	42.67 (91.00%)	40.28 (85.90%)
90 days	Total TA in supernatant of liposome 1 g (mg / g)	39.71	38.17	36.89
	Total TA in pellet of liposome 1 g (mg / g)	4.72	3.88	3.62
	Total (mg)	44.43 (94.75%)	42.05 (89.68%)	40.51 (86.39%)

Table 3.31 : The total amounts of TA and the percentage of TA left comparing to at initial time in the 7:2:1(10%TA,+) liposome formulation (1 g) when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days

7:2:1(10%TA,+)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	88.99	88.99	88.99
	Total TA in pellet of liposome 1 g (mg / g)	6.88	6.88	6.88
	Total (mg)	104.66 (100.00%)	104.66 (100.00%)	104.66 (100.00%)
14 days	Total TA in supernatant of liposome 1 g (mg / g)	89.00	91.08	87.15
	Total TA in pellet of liposome 1 g (mg / g)	15.56	14.55	15.79
	Total (mg)	104.56 (99.90%)	105.63 (100.93%)	102.94 (98.36%)
30 days	Total TA in supernatant of liposome 1 g (mg / g)	87.03	90.67	87.03
	Total TA in pellet of liposome 1 g (mg / g)	14.86	14.76	14.14
	Total (mg)	101.63 (97.10%)	105.43 (100.73%)	101.17 (96.67%)
60 days	Total TA in supernatant of liposome 1 g (mg / g)	88.94	80.46	77.26
	Total TA in pellet of liposome 1 g (mg / g)	13.92	10.73	11.05
	Total (mg)	102.86 (98.28%)	91.19 (87.13%)	88.31 (84.38%)
90 days	Total TA in supernatant of liposome 1 g (mg / g)	87.29	83.95	84.55
	Total TA in pellet of liposome 1 g (mg / g)	12.08	10.15	9.75
	Total (mg)	99.37 (94.95%)	94.10 (88.91%)	94.30 (90.10%)

Table 3.32 : The total amounts of TA and the percentage of TA left comparing to at initial time in the 7:2:1(5%TA,-) liposome formulation (1 g) when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days

7:2:1(5%TA,-)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	39.38	39.38	39.38
	Total TA in pellet of liposome 1 g (mg / g)	7.26	7.26	7.26
	Total (mg)	46.64(100.00%)	46.64(100.00%)	46.64(100.00%)
14 days	Total TA in supernatant of liposome 1 g (mg / g)	40.16	40.48	42.09
	Total TA in pellet of liposome 1 g (mg / g)	7.42	7.16	6.81
	Total (mg)	47.58 (102.02%)	47.64 (102.14%)	48.90 (104.85%)
30 days	Total TA in supernatant of liposome 1 g (mg / g)	41.31	40.73	41.31
	Total TA in pellet of liposome 1 g (mg / g)	5.65	5.82	5.33
	Total (mg)	46.96 100.69%)	46.55 (99.81%)	46.64 (100.00%)
60 days	Total TA in supernatant of liposome 1 g (mg / g)	40.05	38.41	36.24
	Total TA in pellet of liposome 1 g (mg / g)	5.13	4.94	5.09
	Total (mg)	45.18 (96.87%)	43.35 (92.95%)	41.33 (88.61%)
90 days	Total TA in supernatant of liposome 1 g (mg / g)	39.78	40.36	40.10
	Total TA in pellet of liposome 1 g (mg / g)	5.92	4.25	4.92
	Total (mg)	45.70 (97.98%)	44.61 (95.65%)	45.02 (96.53%)

Table 3.33 : The total amounts of TA and the percentage of TA left comparing to at initial time in the 7:2:1(10%TA,-) liposome formulation (1 g) when at 4 ± 1, 30 ± 1 and 45 ± 1 ^{0}C for 0, 14, 30, 60, and 90 days

7:2:1(10%TA,-)		4 ± 1 ^{0}C	30 ± 1 ^{0}C	45 ± 1 ^{0}C
0 day	Total TA in supernatant of liposome 1 g (mg / g)	89.99	89.99	89.99
	Total TA in pellet of liposome 1 g (mg / g)	13.68	13.68	13.68
	Total (mg)	103.67(100.00%)	103.67(100.00%)	103.67(100.00%)
14 days	Total TA in supernatant of liposome 1 g (mg / g)	87.66	93.48	90.51
	Total TA in pellet of liposome 1 g (mg / g)	14.37	14.08	14.08
	Total (mg)	102.03 (98.42%)	107.56(103.56%)	104.56(100.89%)
30 days	Total TA in supernatant of liposome 1 g (mg / g)	91.51	91.51	90.06
	Total TA in pellet of liposome 1 g (mg / g)	12.80	12.97	12.85
	Total (mg)	103.56 (99.92%)	104.48(100.78%)	102.91 (99.27%)
60 days	Total TA in supernatant of liposome 1 g (mg / g)	81.10	97.31	83.97
	Total TA in pellet of liposome 1 g (mg / g)	11.47	11.65	10.51
	Total (mg)	92.57 (89.29%)	108.96(105.10%)	94.48 (91.14%)
90 days	Total TA in supernatant of liposome 1 g (mg / g)	81.64	90.23	83.97
	Total TA in pellet of liposome 1 g (mg / g)	11.42	11.90	10.66
	Total (mg)	93.06 (89.77%)	102.13 (98.51%)	94.63 (91.28%)

Table 3.34 : Comparison of the percentages of total TA in liposome formulations when kept at 4 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	93.96	99.90	102.02	98.42
30	91.94	97.10	100.69	99.92
60	94.37	98.28	96.87	89.29
90	94.75	94.95	97.98	89.77

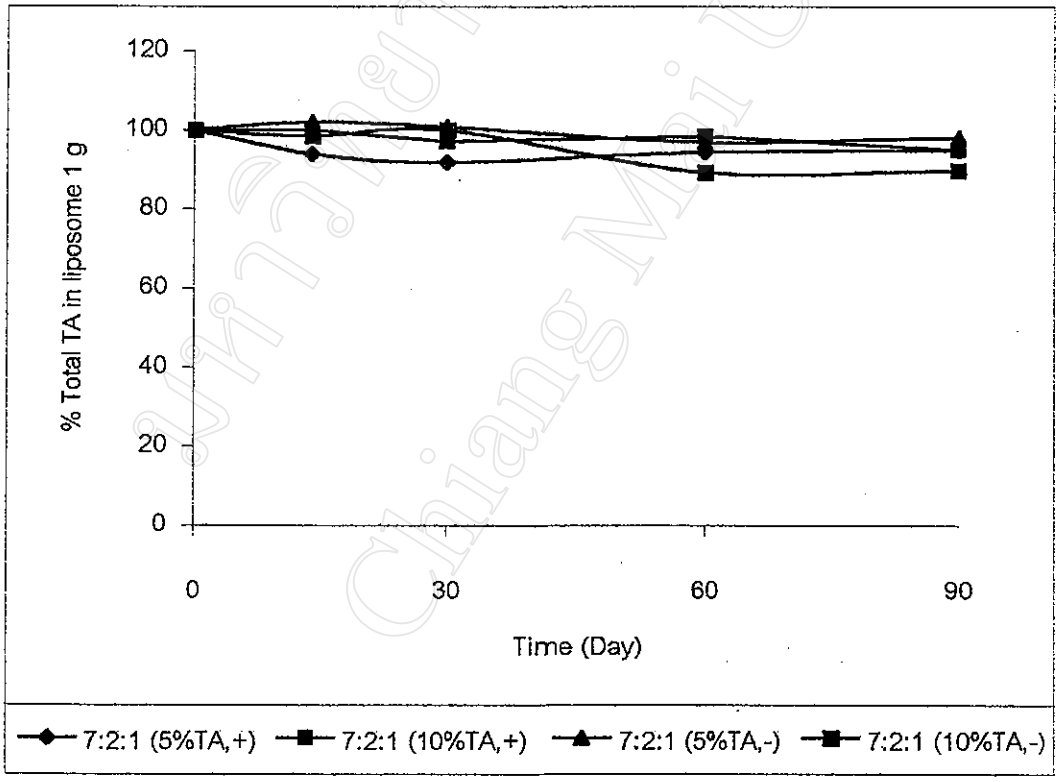

Figure 3.38 : The comparison of the percentages of total TA in liposome formulation when kept at 4 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Table 3.35 : Comparison of the percentages of total TA in liposome formulations when kept at 30 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	93.47	100.93	102.14	100.89
30	91.47	100.74	99.81	99.27
60	91.00	87.13	92.95	91.14
90	89.68	89.91	95.65	91.28

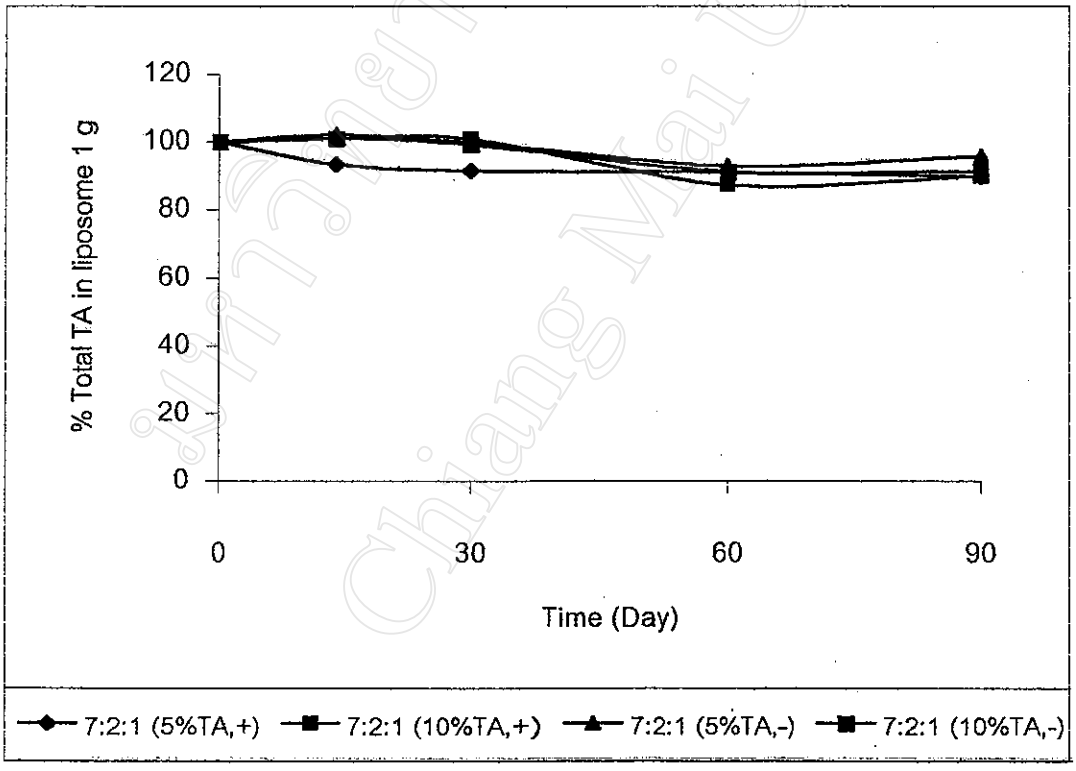

Figure 3.39 : The comparison of the percentages of total TA in liposome formulation when kept at 30 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Table 3.36 : Comparison of the percentages of total TA in liposome formulations when kept for 45 ± 1 ^{0}C at time 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	93.37	98.36	104.85	100.89
30	94.52	96.67	100.00	99.27
60	85.90	84.38	88.61	91.14
90	86.39	90.10	96.53	91.28

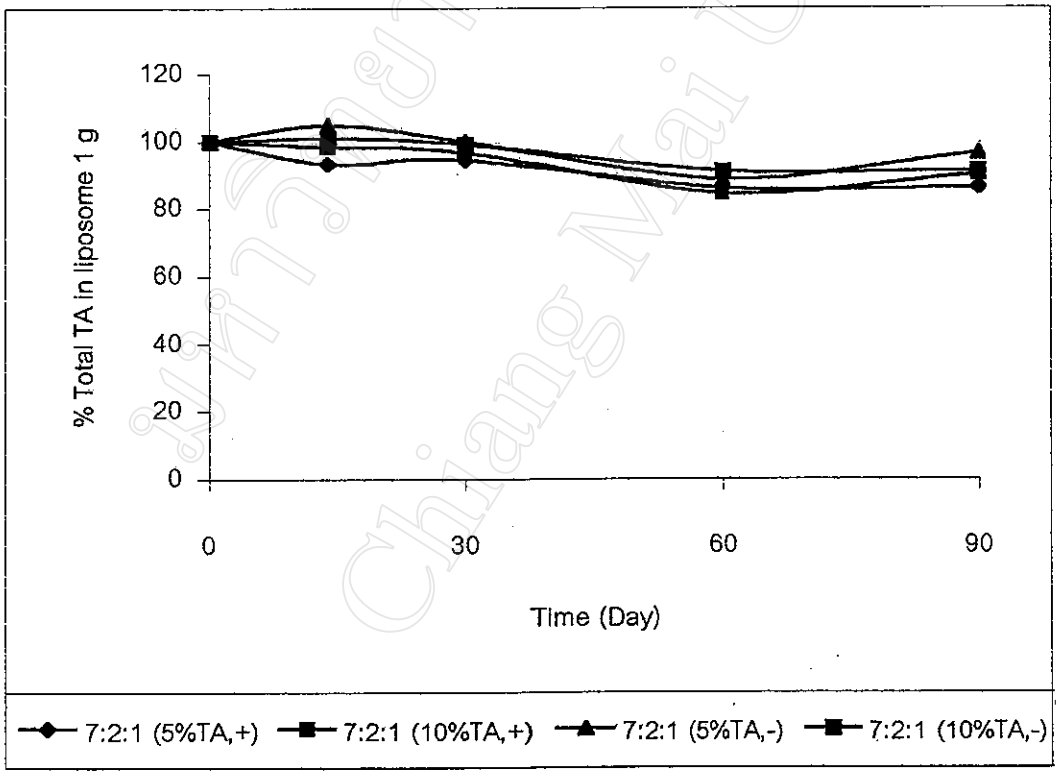

Figure 3.40 : The comparison of the percentages of total TA in liposome formulation when kept at 45 ± 1 ^{0}C for 0, 14, 30, 60 and 90 days

Table 3.37 : Determination of chemical kinetic of the percentages of total TA in liposome of liposome formulations fitted in zero order, first order and Higuchi model

Formulaton	Temperatur e	Zero order			First order			Higuchi model		
		Slope (-)	Intercept	R square	Slope (-)	Intercept	R square	Slope (-)	Intercept	R square
7:2:1 (5%TA,+)	4 °C	0.0317	96.2370	0.1481	0.0001	1.9830	0.1424	0.5177	97.7440	0.4040
	30 °C	0.0915	96.6748	0.6634	0.0004	1.9851	0.6752	1.0517	98.6898	0.9000
	45 °C	0.1486	97.8028	0.8260	0.0007	1.9904	0.8311	1.5251	101.1072	0.8933
7:2:1 (10%TA,+)	4 °C	0.0506	100.0124	0.7606	0.0002	2.0001	0.7606	0.4771	100.5708	0.6923
	30 °C	0.1554	101.7334	0.7144	0.0007	2.0079	0.709	1.3914	103.1055	0.5879
	45 °C	0.1457	99.5579	0.6591	0.0006	1.9981	0.6403	1.4420	101.5333	0.6625
7:2:1 (5%TA,-)	4 °C	0.0428	101.1759	0.5619	0.0001	2.0050	0.5626	0.3544	101.3876	0.3942
	30 °C	0.0803	101.2293	0.6149	0.0003	2.0053	0.6106	0.7197	101.9188	0.5061
	45 °C	0.1023	101.9703	0.3801	0.0004	2.0082	0.3708	0.9256	102.8965	0.3192
7:2:1 (10%TA,-)	4 °C	0.1349	100.7154	0.8013	0.0006	2.0034	0.8013	1.2350	102.0159	0.6895
	30 °C	0.1233	101.3023	0.8424	0.0005	2.0058	0.8419	1.1069	102.3741	0.6967
	45 °C	0.12333	101.3023	0.8424	0.0005	2.0058	0.8419	1.1069	101.3741	0.6967

Note : see the equations of zero order, first order and Higuchi model in Appendix, (-) is minus, slope is degradation rate

Table 3.38 : Comparison of the percentages of total TA in pellet of liposome formulations when kept at 4 ± 1 °C at time 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	86.48	99.30	102.20	105.04
30	84.01	93.17	77.82	88.30
60	90.99	88.83	70.66	83.85
90	68.60	77.09	81.54	83.48

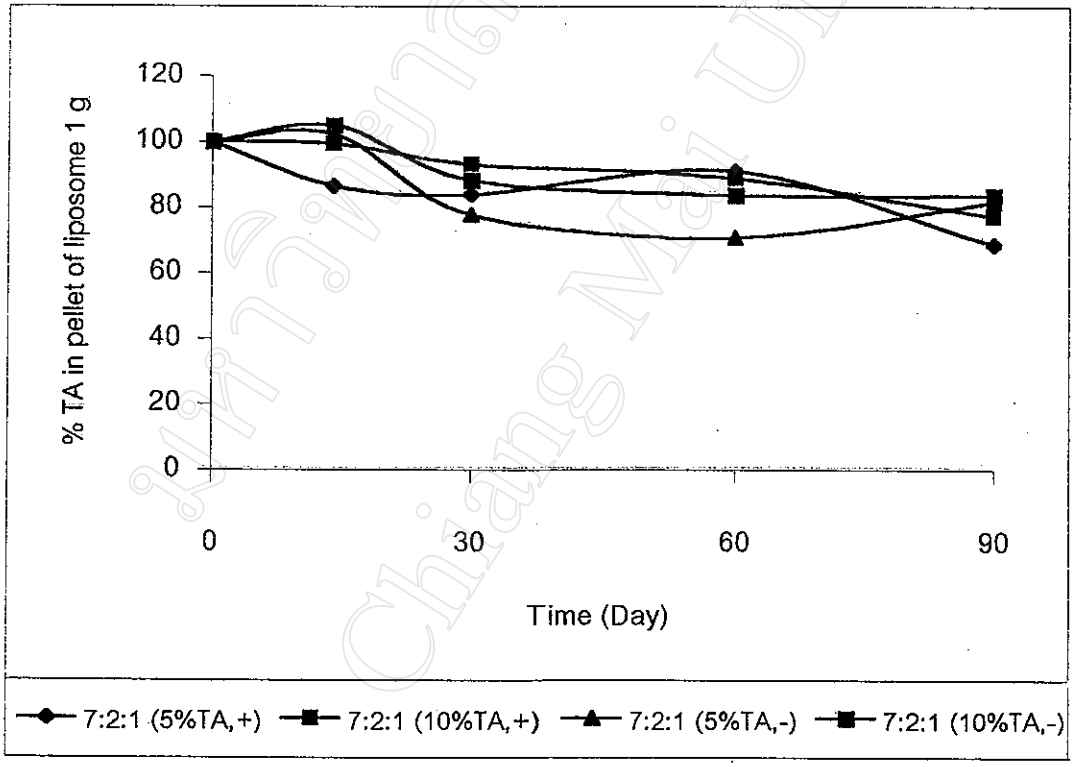

Figure 3.41 : The comparison of the percentages of total TA in pellet of liposome formulation when kept at 4 ± 1 °C for 0, 14, 30, 60 and 90 days

Table 3.39 : Comparison of the percentages of total TA in pellet of liposome formulations when kept at 30 ± 1 °C for time 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	88.95	92.85	98.62	102.92
30	80.81	94.19	80.17	94.81
60	79.65	68.47	68.04	85.16
90	56.40	64.77	58.54	86.99

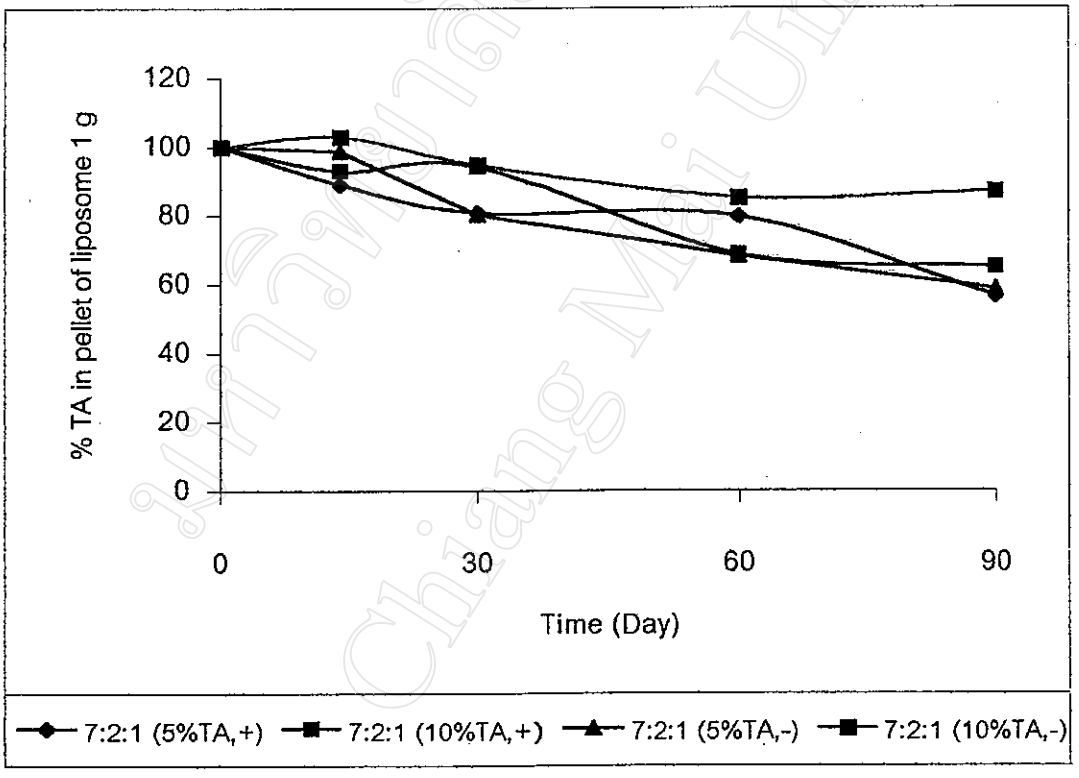

Figure 3.42 : The comparison of the percentages of total TA in pellet of liposome formulation when kept at 30 ± 1 °C for 0, 14, 30, 60 and 90 days

Table 3.40 : Comparison of the percentages of total TA in pellet of liposome formulations when kept at 45 ± 1 °C for time 0, 14, 30, 60 and 90 days

Day (s)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0	100.00	100.00	100.00	100.00
14	85.47	100.77	93.80	102.92
30	82.85	90.24	73.42	93.93
60	63.37	70.52	70.11	76.83
90	52.62	62.22	67.77	77.92

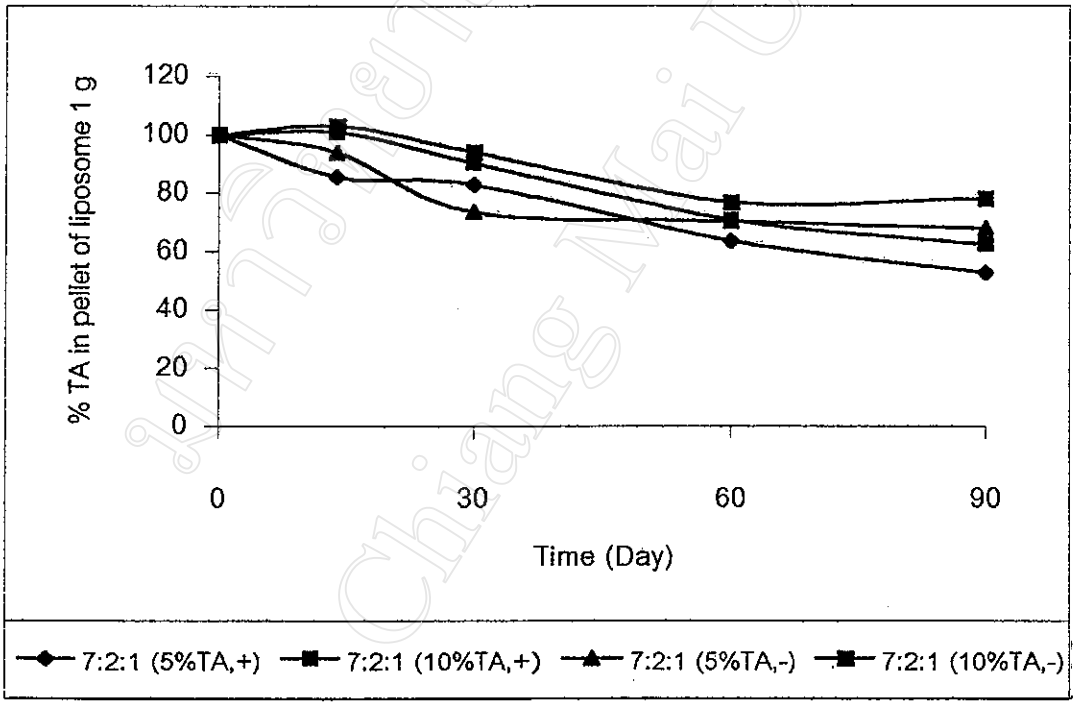

Figure 3.43 : The comparison of the percentages of total TA in pellet of liposome formulation when kept at 45 ± 1 °C for 0, 14, 30, 60 and 90 days

Table 3.41 : Determination of chemical kinetic of the percentages of total TA in pellet in liposomes of liposome formulations fitted in zero order, first order and Higuchi model

Formulaton	Temperatur e	Zero order			First order			Higuchi model		
		Slope (-)	Intercept	R square	Slope (-)	Intercept	R square	Slope (-)	Intercept	R square
7:2:1 (5%TA,+)	4 °C	0.2510	95.7563	0.6290	0.0013	1.9830	0.6410	2.5129	99.3145	0.6473
	30 °C	0.4215	97.5164	0.9078	0.0024	0.9957	0.8929	4.0564	102.6287	0.8635
	45 °C	0.5107	96.6799	0.9714	0.0030	0.9929	0.9850	4.9822	103.2281	0.9492
7:2:1 (10%TA,+)	4 °C	0.2528	101.4894	0.9612	0.0012	2.0084	0.9509	2.2883	103.7877	0.8084
	30 °C	0.4258	100.5793	0.9107	0.0023	2.0069	0.9132	3.9803	105.1198	0.8171
	45 °C	0.4730	103.1052	0.9509	0.0025	2.0192	0.9646	4.3258	104.6426	0.8236
7:2:1 (5%TA,-)	4 °C	0.2707	96.9491	0.4954	0.0013	1.9838	0.4764	2.9049	101.8168	0.5857
	30 °C	0.4918	100.1586	0.9480	0.0027	2.0061	0.9691	4.6973	105.9329	0.8880
	45 °C	0.3598	94.9836	0.7790	0.001	1.9764	0.7976	3.7724	100.9840	0.8792
7:2:1 (10%TA,-)	4 °C	0.2297	101.0463	0.7184	0.0010	2.0043	0.7671	2.2003	103.7782	0.6775
	30 °C	0.1912	101.3951	0.7911	0.0008	2.0063	0.7933	1.7851	103.4228	0.7081
	45 °C	0.3114	102.4023	0.8479	0.0015	2.0117	0.8497	2.8701	105.5089	0.7414

Note : see the equations of zero order, first order and Higuchi model in Appendix, (-) is minus, slope is degradation rate

3.5 The release study of tranexamic acid (TA) from solution and liposome of various liposome formulations and solution

The percentage of the cumulative amount of TA released from solution and liposome of various formulations at 0 to 24 hrs were shown in Tables 3.42 to 3.50 and Figures 3.44 to 3.46. The percentage of the cumulative amount of TA released from positively and negatively charged liposome with the same drug concentration were not significantly different. But, the higher entrapped drug concentration appeared to give higher rate of release. Liposome formulations gave the slower rate release of TA about 3 times than that from the solution (Table 3.52).

From Table 3.51 : The percentage of the cumulative amount of TA released from solution and liposome of various formulations at 0 to 24 hrs were calculated by fitting the data to zero order, first order and the Higuchi model. The correlation coefficient from the Higuchi model showed higher relation than the zero order and first order. The releasing rate of TA fitted with Higuchi model and the total TA released at 24 hrs from liposome and solution in Table 3.52.

Table 3.42 : Percentages cumulative TA released from 7:2:1 (5%TA,+) liposome formulation at 0 to 24 hrs

Time (hr)	7:2:1 (5%TA,+) liposome			Mean	SD
	Lot.1	Lot.2	Lot.3		
0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.49	0.87	1.12	0.83	0.32
0.50	1.23	1.64	1.70	1.52	0.25
1.00	1.28	1.40	1.77	1.48	0.25
2.00	5.78	5.85	6.11	5.92	0.17
4.00	9.99	9.78	10.09	9.96	0.16
6.00	12.97	12.11	13.17	12.75	0.56
8.00	15.46	14.18	16.49	15.38	1.16
24.00	28.04	26.61	29.50	28.05	1.45

Table 3.43 : Percentages cumulative TA released from 7:2:1 (10%TA,+) liposome formulation at 0 to 24 hrs

Time (hr)	7:2:1 (10%TA,+) liposome			Mean	SD
	Lot.1	Lot.2	Lot.3		
0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.45	1.42	1.75	1.21	0.68
0.50	1.95	2.64	2.42	2.34	0.35
1.00	2.03	2.75	2.52	2.43	0.37
2.00	6.29	9.03	9.70	8.34	1.81
4.00	14.47	15.15	15.93	15.18	0.73
6.00	18.64	18.96	19.41	19.00	0.39
8.00	22.33	21.95	23.82	22.70	0.99
24.00	36.25	34.94	37.42	36.20	1.24

Table 3.44 : Percentages cumulative TA released from 7:2:1 (5%TA,-) liposome formulation at 0 to 24 hrs

Time (hr)	7:2:1 (5%TA,-) liposome			Mean	SD
	Lot.1	Lot.2	Lot.3		
0.00	0.00	0.00	0.00	0.00	0.00
0.25	1.05	0.06	0.22	0.44	0.53
0.50	2.24	0.77	0.72	1.25	0.86
1.00	2.34	0.80	0.75	1.30	0.90
2.00	7.20	4.34	4.70	5.42	1.56
4.00	10.57	8.21	8.25	9.01	1.35
6.00	13.57	10.96	11.38	11.97	1.40
8.00	15.63	13.04	14.86	14.51	1.33
24.00	30.69	27.34	29.62	29.22	1.71

Table 3.45 : Percentages cumulative TA released from 7:2:1 (10%TA,-) liposome formulation at 0 to 24 hrs

Time (hr)	7:2:1 (10%TA,-) liposome			Mean	SD
	Lot.1	Lot.2	Lot.3		
0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.49	0.93	1.56	0.99	0.54
0.50	1.47	2.33	2.24	2.01	0.47
1.00	1.53	2.42	2.33	2.09	0.49
2.00	10.25	10.26	10.57	10.36	0.18
4.00	16.31	16.32	16.98	16.54	0.39
6.00	21.01	19.80	20.75	20.52	0.64
8.00	23.66	22.77	24.36	23.60	0.80
24.00	37.93	36.89	38.98	37.93	1.04

Table 3.46 : Mean percentages cumulative TA released from liposome formulation at 0 to 24 hrs

Time (hr)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)
0.00	0.00	0.00	0.00	0.00
0.25	0.83	1.21	0.44	0.99
0.50	1.52	2.34	1.25	2.01
1.00	1.48	2.43	1.30	2.09
2.00	5.92	8.34	5.42	10.36
4.00	9.96	15.18	9.01	16.54
6.00	12.75	19.00	11.97	20.52
8.00	15.38	22.70	14.51	23.60
24.00	28.05	36.20	29.22	37.93

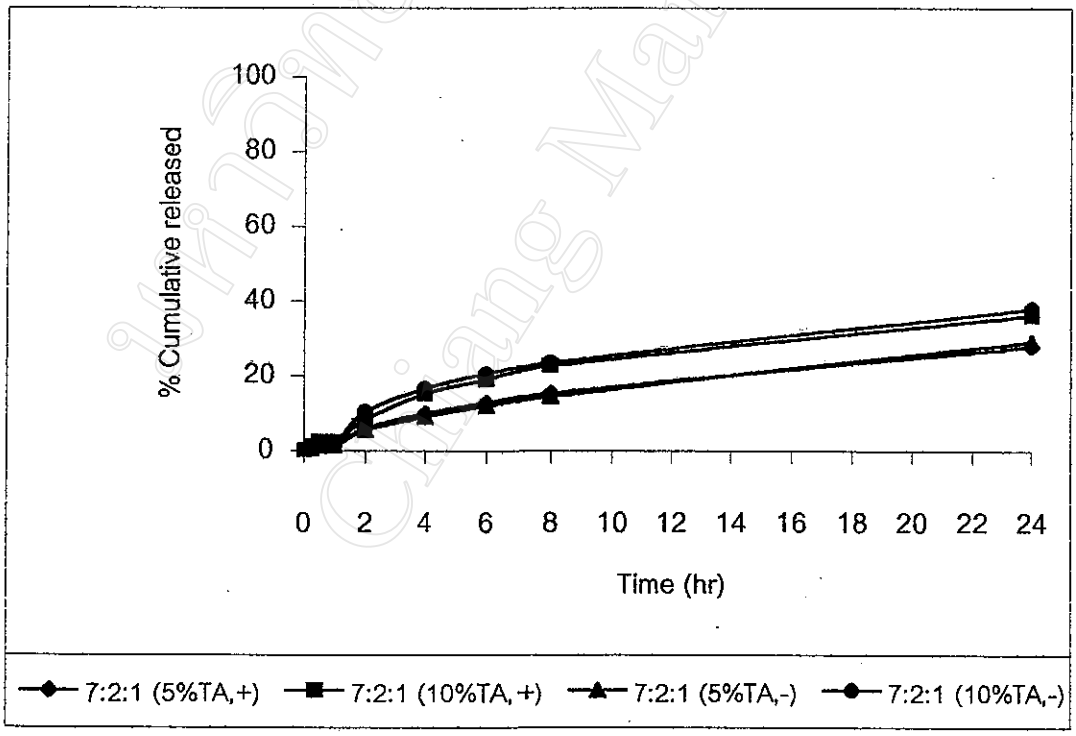

Figure 3.44 : Percentages cumulative TA released from the liposome formulations at 0 to 24 hrs

Table 3.47 : Percentages cumulative TA released from 5 % solution at 0 to 24

Time (hr)	5% TA solution (mg)			Mean	SD
	Lot.1	Lot.2	Lot.3		
0.00	0.00	0.00	0.00	0.00	0.00
0.25	3.40	2.82	2.79	3.00	0.35
0.50	5.19	4.90	5.43	5.18	0.27
1.00	14.77	11.07	10.69	12.18	2.25
2.00	30.54	24.83	30.82	28.73	3.38
4.00	43.01	36.07	42.26	40.45	3.81
6.00	61.71	51.21	51.38	54.76	6.02
8.00	63.09	51.83	62.69	59.20	6.39
24.00	76.08	83.20	85.79	81.69	5.03

Table 3.48 : Percentages cumulative TA released from 10% solution at 0 to 24 hrs

Time (hr)	10% TA solution (mg)			Mean	SD
	Lot.1	Lot.2	Lot.3		
0.00	0.00	0.00	0.00	0.00	0.00
0.25	2.98	4.16	4.30	3.82	0.73
0.50	5.34	6.10	5.67	5.71	0.38
1.00	11.40	14.54	14.01	13.31	1.68
2.00	19.85	25.18	20.36	21.80	2.94
4.00	36.85	42.57	42.10	40.51	3.17
6.00	46.78	59.64	56.41	54.28	6.69
8.00	56.09	58.87	58.50	57.82	1.51
24.00	81.81	91.18	88.98	87.32	4.90

Table 3.49 : Mean percentages cumulative traneamic acid released from 5Percentages and % solution at 0 to 24 hrs

Time (hr)	5% TA solution	10% TA solution
0.00	0.00	0.00
0.25	3.00	3.82
0.50	5.18	5.71
1.00	12.18	13.31
2.00	28.73	21.80
4.00	40.45	40.51
6.00	54.76	54.28
8.00	59.20	57.82
24.00	81.69	87.32

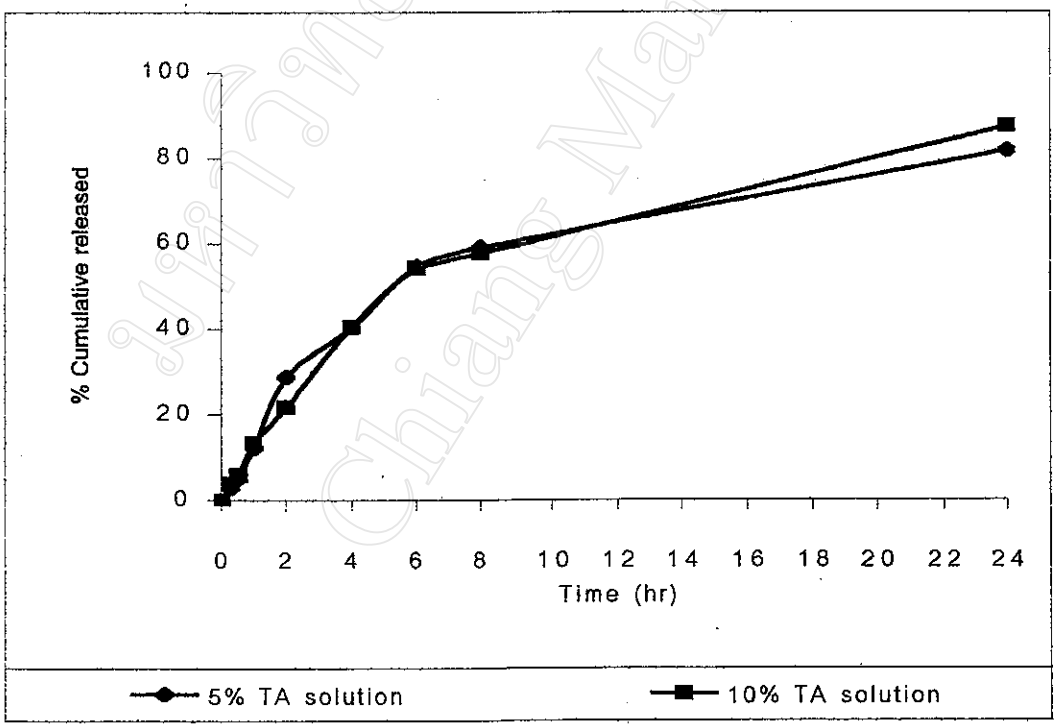

Figure 3.45 : Mean percentages cumulative traneamic acid released from 5% and 10% solution

Table 3.50 : Mean Percentages cumulative TA released from Liposome formulations and solution at 0 to 24 hrs

Time (hr)	7:2:1 (5%TA,+)	7:2:1 (10%TA,+)	7:2:1 (5%TA,-)	7:2:1 (10%TA,-)	5% TA solution	10%TA solution
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.83	1.21	0.44	0.99	3.00	3.82
0.50	1.52	2.34	1.25	2.01	5.18	5.71
1.00	1.48	2.43	1.30	2.09	12.18	13.31
2.00	5.92	8.34	5.42	10.36	28.73	21.80
4.00	9.96	15.18	9.01	16.54	40.45	40.51
6.00	12.75	19.00	11.97	20.52	54.76	54.28
8.00	15.38	22.70	14.51	23.60	59.20	57.82
24.00	28.05	36.20	29.22	37.93	81.69	87.32

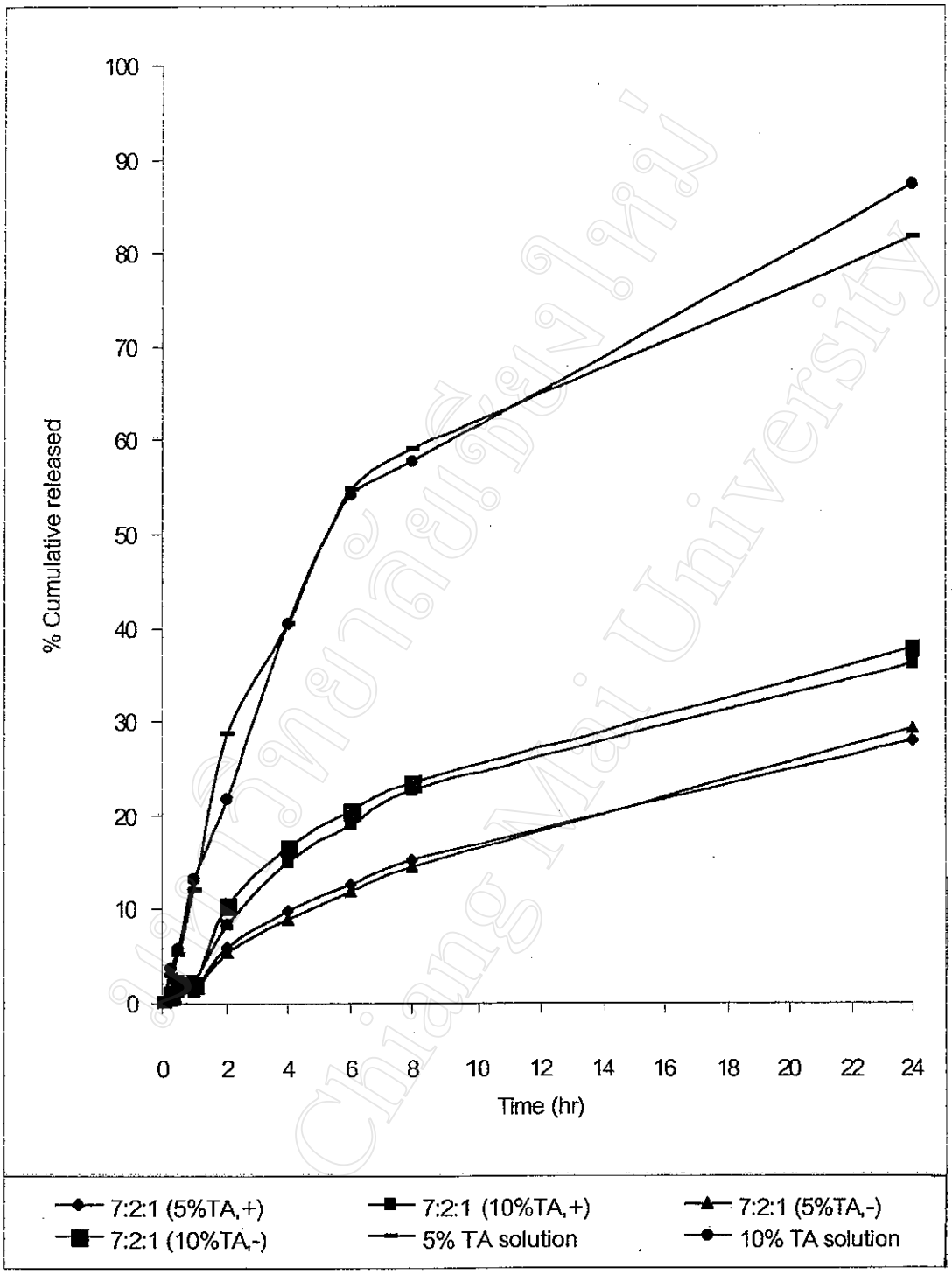

Figure 3.46 : Percentages cumulative TA released from liposome formulations and solution at 0 to 24 hrs

Table 3.51 : Determination of kinetic of the cumulative percentages of TA release from liposome formulations and solution fitted in zero order, first order and Higuchi model

Formulaton	Zero order			First order			Higuchi model		
	Slope (-)	Intercept	R square	Slope (-)	Intercept	R square	Slope (-)	Intercept (-)	R square
7:2:1 (5%TA,+)	1.1643	2.5121	0.9192	0.0541	0.4128	0.5778	6.1222	2.3060	0.9832
7:2:1 (10%TA,+)	1.5058	4.2786	0.8650	0.0511	0.5987	0.5486	8.1363	2.3357	0.9770
7:2:1 (5%TA,-)	1.2187	1.9278	0.9487	0.0603	0.3106	0.5630	6.2908	2.9097	0.9889
7:2:1 (10%TA,-)	1.5766	4.6565	0.8483	0.0537	0.5824	0.5084	8.5786	2.3738	0.9716
5% TA in DI water	3.3303	14.7584	0.7596	0.0455	1.0852	0.4615	18.8992	1.4574	0.9465
10% TA in DI water	3.5721	13.4591	0.9036	0.0454	0.0956	0.5220	19.7765	3.0660	0.9690

Note : see the equations of zero order, first order and Higuchi model in Appendix, (-) is minus, slope is release rate

Table 3.52 : The releasing rate of the percentage cumulative amount of TA released fitted with Higuchi model and total TA released at 24 hrs from the liposome formulations and solution

Formulations	Rate of release ($\%/hr^{1/2}$) ± S.D.	Total release at 24 hrs (%) ± S.D.
7:2:1 (5%TA,+)	6.12 ± 0.06	28.05 ± 1.45
7:2:1 (10%TA,+)	8.13 ± 0.05	36.20 ± 1.24
7:2:1 (5%TA,-)	6.29 ± 0.07	29.22 ± 1.71
7:2:1 (10%TA,-)	8.57 ± 0.04	37.93 ± 1.04
5% TA solution	18.89 ± 0.21	81.69 ± 5.03
10% TA solution	19.77 ± 0.20	87.32 ± 4.90