CHAPTER 3
RESULTS

3.1 Characteristics of liposome formulations

Eight liposome formulations were prepared and their characteristics in physical appearances, pH, charges/zeta potential and size were investigated.

3.1.1 The physical appearances of liposome formulations

The appearances of eight liposome formulations which were freshly prepared and kept at 4±1°C for one day were shown in Table 3.1 and Figures 3.1 to 3.2.

Table 3.1 : The appearances of liposome formulations

Formulations	Freshly prepared	Kept at 4±1°C for one day
1:1	translucent, white dispersion	translucent, white dispersion with the 1 mm height of sediment at the bottom
7:2	translucent, white dispersion	translucent, white dispersion with a thin layer sediment at the bottom
7:2:1(+)	translucent, white dispersion	translucent, white dispersion
7:2:1(-)	translucent, white dispersion	translucent, white dispersion
1:1AmB	translucent, yellow dispersion	translucent, yellow dispersion with the 0.5 mm height of sediment at the bottom
7:2AmB	translucent, yellow dispersion	translucent, yellow dispersion with the 2 mm height of sediment at the bottom
7:2:1(+)AmB	translucent, yellow dispersion	half translucent, yellow dispersion with half sediment at the bottom
7:2:1(-)AmB	translucent, yellow dispersion	translucent, yellow dispersion with a thin layer sediment at the bottom

Figure 3.1 : The physical appearance of liposome formulations from left to right 1:1, 7:2, 7:2:1(+) and 7:2:1(-) kept at 4±1°C for one day

Figure 3.2 : The physical appearance of liposome formulations from left to right 1:1AmB, 7:2AmB, 7:2:1(+)AmB and 7:2:1(-)AmB kept at 4±1°C for one day

3.1.2 pH measurement of liposome formulations

The pH's of eight liposome formulations are listed in Table 3.2.

Table 3.2 : The pH's of liposome formulations comparing to phosphate buffer (pH 7.4) solution

Formulations	pH measurement			Mean	SD	%CV
	1st	2nd	3rd			
phosphate buffer (pH 7.4)	7.48	7.48	7.50	7.487	0.012	0.154
1:1	7.51	7.52	7.52	7.517	0.006	0.077
7:2	7.51	7.52	7.52	7.517	0.006	0.077
7:2:1 (+)	7.53	7.54	7.54	7.537	0.006	0.077
7:2:1 (-)	7.49	7.48	7.48	7.483	0.006	0.077
1:1 AmB	7.54	7.55	7.53	7.540	0.010	0.133
7:2 AmB	7.56	7.55	7.52	7.543	0.021	0.276
7:2:1(+) AmB	7.49	7.54	7.54	7.523	0.029	0.384
7:2:1(-) AmB	7.50	7.48	7.48	7.487	0.012	0.154

3.1.3 Charges and zeta potential

The charges and zeta potential values of eight liposome formulations are shown in Table 3.3 and Figure 3.3.

Table 3.3 : Charges and zeta potential values of eight liposome formulations

Formula -tions	Zeta potential (millivolts) ($\bar{x} \pm SD$)			Mean	SD	%CV	Charge observed from direction of particle movement
	1	2	3				
1:1	42.28 ±19.37	31.78 ±15.43	44.73 ±19.62	39.60	6.88	17.37	Negative
7:2	31.75 ±18.37	35.78 ±21.25	41.40 ±20.50	36.31	4.85	13.35	Negative
7:2:1(+)	35.89 ±13.50	30.28 ±6.13	31.56 ±9.63	32.58	2.94	9.02	Positive
7:2:1(-)	68.99 ±22.62	69.92 ±16.50	67.72 ±22.25	68.88	1.10	1.60	Negative
1:1 AmB	56.77 ±25.50	57.27 ±25.62	57.21 ±28.12	57.08	0.27	0.48	Negative
7:2 AmB	59.21 ±24.37	58.21 ±20.25	60.65 ±25.74	59.36	1.23	2.07	Negative
7:2:1(+) AmB	48.70 ±11.31	47.31 ±10.25	46.31 ±6.56	47.44	1.20	2.53	Positive
7:2:1(-) AmB	56.91 ±24.00	64.78 ±25.62	66.96 ±26.25	62.88	5.29	8.41	Negative

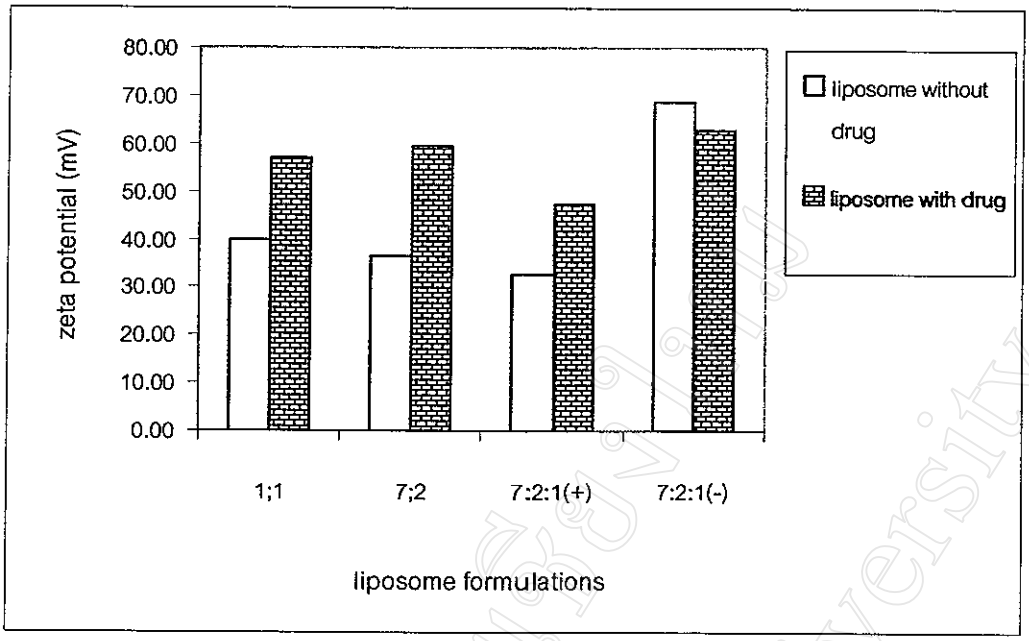


Figure 3.3 : Histograms of zeta potential of liposome formulations with and without the entrapped AmB

3.1.4 Size determination by SEM

Size determination of liposome formulations by SEM at magnification in the ranges of 10,000 to 30,000 was performed. Figures 3.4 to 3.7 showed the micrograph of liposome formulations without the entrapped drugs where Figures 3.8 to 3.11 were for those with the entrapped drug. Sizes of these liposome formulations were measured and calculated from 100 particles of the micrograph, except 8 particles for the 7:2:1(+)AmB liposome since the micrograph was not sharp enough. Table 3.12 summarized mode, mean, standard deviation and variation coefficient of the liposome size, where Table 3.13 showed the size frequency distribution. Figures 3.12 and 3.13 demonstrated size distribution of liposome formulations with and without the entrapped AmB respectively.

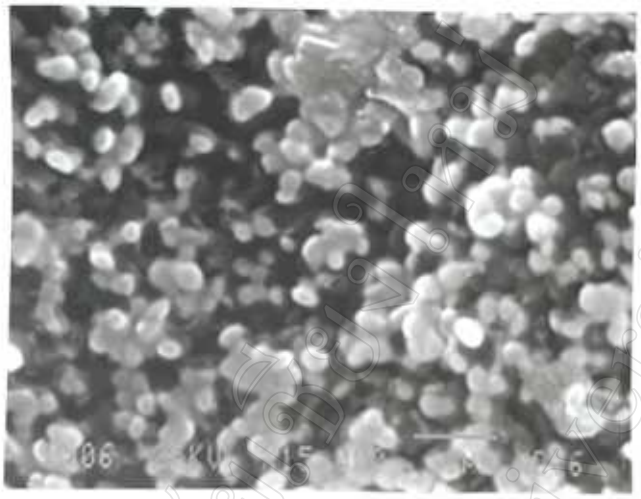


Figure 3.4 : Scanning electron micrograph of the 1:1 liposome formulation, 15000x

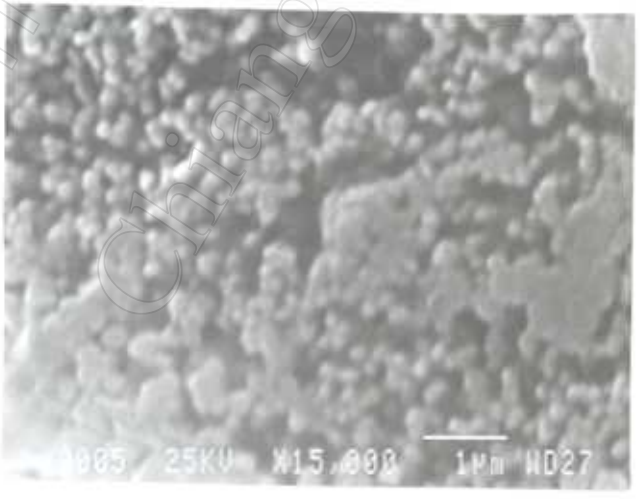


Figure 3.5 : Scanning electron micrograph of the 7:2 liposome formulation, 15000x

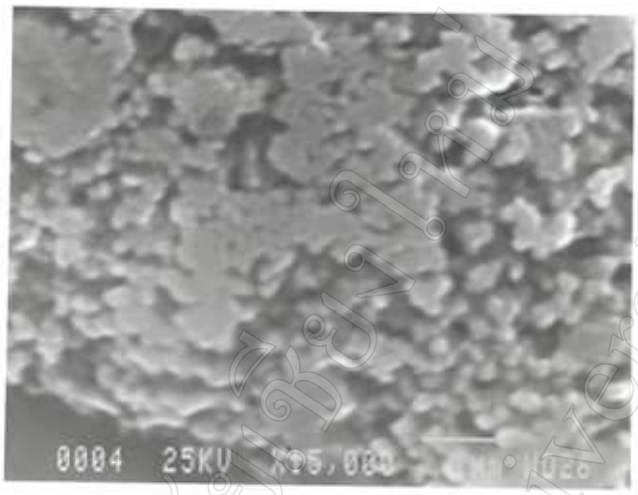


Figure 3.6 : Scanning electron micrograph of the 7:2:1(+) liposome formulation, 15000x

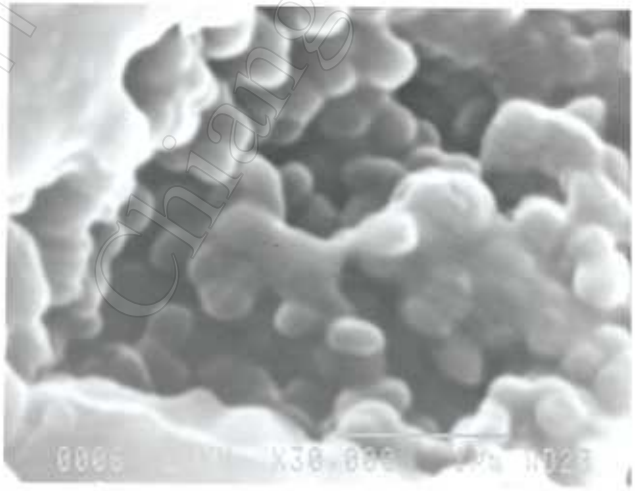


Figure 3.7 : Scanning electron micrograph of the 7:2:1(-) liposome formulation, 30000x

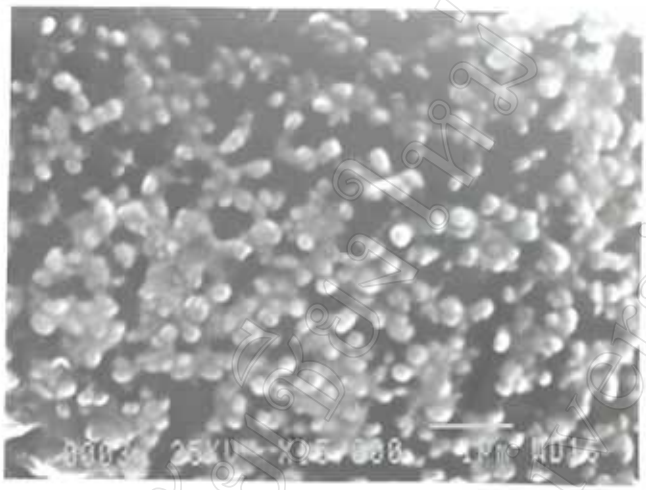


Figure 3.8 : Scanning electron micrograph of the 1:1AmB liposome formulation, 15000x

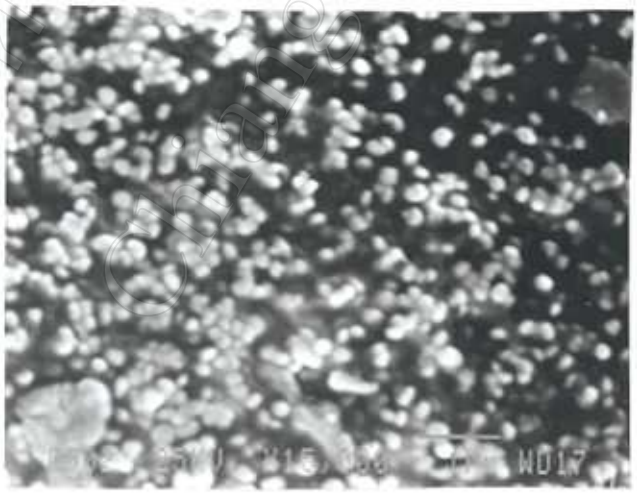


Figure 3.9 : Scanning electron micrograph of the 7:2AmB liposome formulation, 15000x

Figure 3.10 : Scanning electron micrograph of the 7:2:1(+)AmB liposome formulation, 12000x

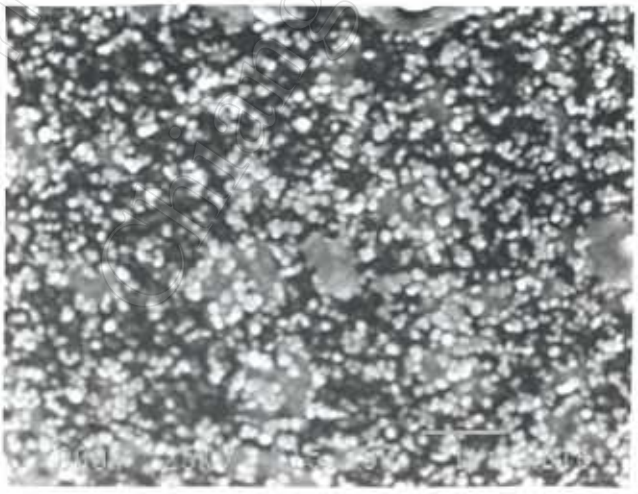


Figure 3.11 : Scanning electron micrograph of the 7:2:1(-)AmB liposome formulation, 15000x

Table 3.4 : Sizes (μm) of the 1:1 liposome formulation (100 particles)

No.	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Sizes (μm)	0.231	0.246	0.154	0.231	0.231	0.192	0.231	0.192	0.192	0.308
	0.231	0.215	0.231	0.308	0.231	0.215	0.308	0.154	0.154	0.231
	0.192	0.192	0.231	0.269	0.154	0.169	0.308	0.308	0.154	0.269
	0.192	0.138	0.154	0.215	0.231	0.292	0.308	0.308	0.215	0.269
	0.231	0.154	0.231	0.192	0.231	0.308	0.192	0.292	0.169	0.308
	0.192	0.231	0.231	0.231	0.231	0.308	0.269	0.231	0.231	0.215
	0.192	0.231	0.215	0.231	0.231	0.231	0.192	0.231	0.308	0.308
	0.246	0.231	0.154	0.192	0.308	0.154	0.215	0.192	0,269	0.231
	0.215	0.269	0.192	0.231	0.231	0.231	0.269	0.192	0.231	0.192
	0.246	0.169	0.231	0.215	0.246	0.246	0.215	0.231	0.269	0.269

Table 3.5 : Sizes (μm) of the 7:2 liposome formulation (100 particles)

No.	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Sizes (μm)	0.179	0.214	0.143	0.214	0.214	0.107	0.179	0.214	0.214	0.143
	0.214	0.214	0.179	0.179	0.250	0.107	0.143	0.214	0.143	0.143
	0.143	0.214	0.143	0.179	0.143	0.107	0.143	0.143	0.214	0.107
	0.107	0.250	0.179	0.143	0.179	0.143	0.179	0.214	0.179	0.143
	0.143	0.179	0.179	0.143	0.214	0.107	0.143	0.179	0.179	0.143
	0.143	0.214	0.107	0.143	0.250	0.107	0.179	0.214	0.179	0.143
	0.143	0.179	0.143	0.143	0.179	0.179	0.143	0.107	0.214	0.107
	0.143	0.107	0.214	0.143	0.143	0.107	0.214	0.143	0.214	0.107
	0.143	0.143	0.143	0.179	0.250	0.214	0.179	0.214	0.179	0.179
	0.143	0.143	0.143	0.107	0.250	0.143	0.184	0.214	0.143	0.143

Table 3.6 : Sizes (μm) of the 7:2:1(+) liposome formulation (100 particles)

No.	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Sizes (μm)	0.143	0.143	0.143	0.179	0.143	0.107	0.214	0.214	0.214	0.143
	0.214	0.143	0.107	0.214	0.107	0.107	0.214	0.214	0.214	0.214
	0.107	0.107	0.143	0.143	0.107	0.143	0.107	0.179	0.179	0.107
	0.143	0.143	0.143	0.214	0.179	0.214	0.214	0.179	0.107	0.143
	0.107	0.143	0.179	0.143	0.179	0.143	0.179	0.179	0.107	0.143
	0.143	0.179	0.143	0.143	0.143	0.214	0.143	0.214	0.107	0.143
	0.143	0.179	0.179	0.107	0.107	0.214	0.143	0.143	0.143	0.143
	0.143	0.143	0.214	0.107	0.179	0.214	0.143	0.214	0.107	0.214
	0.179	0.214	0.179	0.179	0.214	0.143	0.143	0.214	0.143	0.143
	0.143	0.143	0.143	0.214	0.143	0.143	0.143	0.143	0.143	0.143

Table 3.7 : Sizes (μm) of the 7:2:1(-) liposome formulation (100 particles)

No.	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Sizes (μm)	0.357	0.250	0.280	0.320	0.200	0.280	0.240	0.240	0.240	0.280
	0.250	0.250	0.280	0.280	0.160	0.160	0.280	0.240	0.320	0.320
	0.250	0.357	0.280	0.280	0.160	0.240	0.360	0.240	0.200	0.280
	0.321	0.321	0.280	0.240	0.160	0.200	0.200	0.200	0.200	0.320
	0.214	0.214	0.240	0.280	0.200	0.160	0.160	0.160	0.160	0.160
	0.286	0.321	0.320	0.200	0.280	0.160	0.200	0.200	0.320	0.200
	0.321	0.250	0.360	0.160	0.280	0.280	0.200	0.200	0.280	0.280
	0.214	0.250	0.320	0.160	0.200	0.160	0.240	0.200	0.320	0.280
	0.286	0.286	0.320	0.240	0.280	0.160	0.200	0.200	0.320	0.240
	0.250	0.214	0.360	0.200	0.240	0.160	0.280	0.240	0.240	0.160

Table 3.8 : Sizes (μm) of the 1:1AmB liposome formulation (100 particles)

No.	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Sizes (μm)	0.231	0.154	0.115	0.192	0.115	0.192	0.115	0.269	0.192	0.115
	0.231	0.154	0.077	0.115	0.192	0.154	0.192	0.154	0.192	0.115
	0.231	0.154	0.231	0.154	0.154	0.308	0.154	0.154	0.115	0.231
	0.231	0.231	0.231	0.154	0.115	0.192	0.154	0.154	0.115	0.231
	0.231	0.115	0.154	0.077	0.269	0.154	0.231	0.231	0.192	0.231
	0.231	0.154	0.192	0.192	0.192	0.154	0.192	0.154	0.215	0.154
	0.231	0.154	0.154	0.308	0.231	0.192	0.154	0.154	0.231	0.154
	0.308	0.077	0.269	0.269	0.154	0.154	0.154	0.115	0.115	0.192
	0.231	0.154	0.115	0.154	0.154	0.154	0.154	0.154	0.154	0.308
	0.154	0.154	0.154	0.115	0.154	0.154	0.231	0.154	0.154	0.154

Table 3.9 : Sizes (μm) of the 7:2AmB liposome formulation (100 particles)

No.	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Sizes (μm)	0.146	0.115	0.177	0.154	0.138	0.146	0.154	0.292	0.169	0.231
	0.115	0.115	0.231	0.154	0.138	0.154	0.115	0.162	0.169	0.154
	0.100	0.169	0.231	0.231	0.154	0.154	0.138	0.223	0.208	0.154
	0.077	0.100	0.154	0.192	0.138	0.231	0.138	0.231	0.115	0.115
	0.154	0.115	0.231	0.192	0.154	0.115	0.162	0.223	0.154	0.308
	0.192	0.208	0.077	0.115	0.154	0.115	0.169	0.154	0.146	0.308
	0.138	0.154	0.115	0.077	0.077	0.077	0.131	0.154	0.115	0.231
	0.146	0.177	0.192	0.077	0.115	0.092	0.223	0.231	0.169	0.231
	0.077	0.169	0.154	0.154	0.138	0.177	0.138	0.192	0.138	0.215
	0.177	0.115	0.154	0.154	0.169	0.231	0.169	0.223	0.146	0.215

Table 3.10 : Sizes (μm) of the 7:2:1(-)AmB liposome formulation (100 particles)

No.	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
Sizes (μm)	0.115	0.138	0.146	0.100	0.100	0.154	0.154	0.115	0.192	0.146
	0.115	0.077	0.077	0.115	0.077	0.154	0.177	0.115	0.115	0.115
	0.077	0.146	0.092	0.077	0.131	0.177	0.154	0.154	0.177	0.146
	0.138	0.146	0.115	0.077	0.077	0.154	0.146	0.115	0.138	0.154
	0.069	0.092	0.115	0.077	0.077	0.154	0.131	0.177	0.138	0.131
	0.062	0.131	0.100	0.131	0.077	0.154	0.115	0.115	0.146	0.115
	0.077	0.146	0.100	0.100	0.077	0.115	0.115	0.100	0.146	0.192
	0.069	0.115	0.054	0.100	0.077	0.115	0.146	0.154	0.154	0.115
	0.077	0.115	0.077	0.115	0.115	0.177	0.192	0.192	0.115	0.154
	0.077	0.077	0.100	0.100	0.115	0.100	0.154	0.192	0.154	0.177

Table 3.11 : Sizes (μm) of the 7:2:1(+)AmB liposome formulation (8 particles)

No.	1-8
Sizes (μm)	0.363, 0.455, 0.455, 0.363, 0.273, 0.363, 0.273, 0.273

Table 3.12 : Mode, mean, standard deviation and variation coefficient of particle sizes (μm) of liposome formulations

Formulations	Mode	Mean	SD
1:1	0.231	0.229	0.045
7:2	0.143	0.166	0.040
7:2:1(+)	0.143	0.159	0.040
7:2:1(-)	0.280	0.246	0.058
1:1AmB	0.154	0.178	0.052
7:2AmB	0.154	0.162	0.050
7:2:1(+)AmB	0.364	0.352	0.076
7:2:1(-)AmB	0.115	0.122	0.035

Table 3.13 : Sizes frequency distributions of 100 particles of each liposome formulation

Size ranges (μm)	No. of particles						
	1:1	7:2	7:2:1(+)	7:2:1(-)	1:1 AmB	7:2 AmB	7:2:1(-) AmB
0.0585-0.0985	0	0	0	0	3	8	23
0.0985-0.1385	1	14	17	0	14	26	42
0.1385-0.1785	12	37	44	16	41	39	30
0.1785-0.2185	26	44	39	22	15	9	5
0.2185-0.2585	37	5	0	21	19	15	0
0.2585-0.2985	11	0	0	22	4	1	0
0.2985-0.3385	13	0	0	14	4	2	0
0.3385-0.3785	0	0	0	5	0	0	0

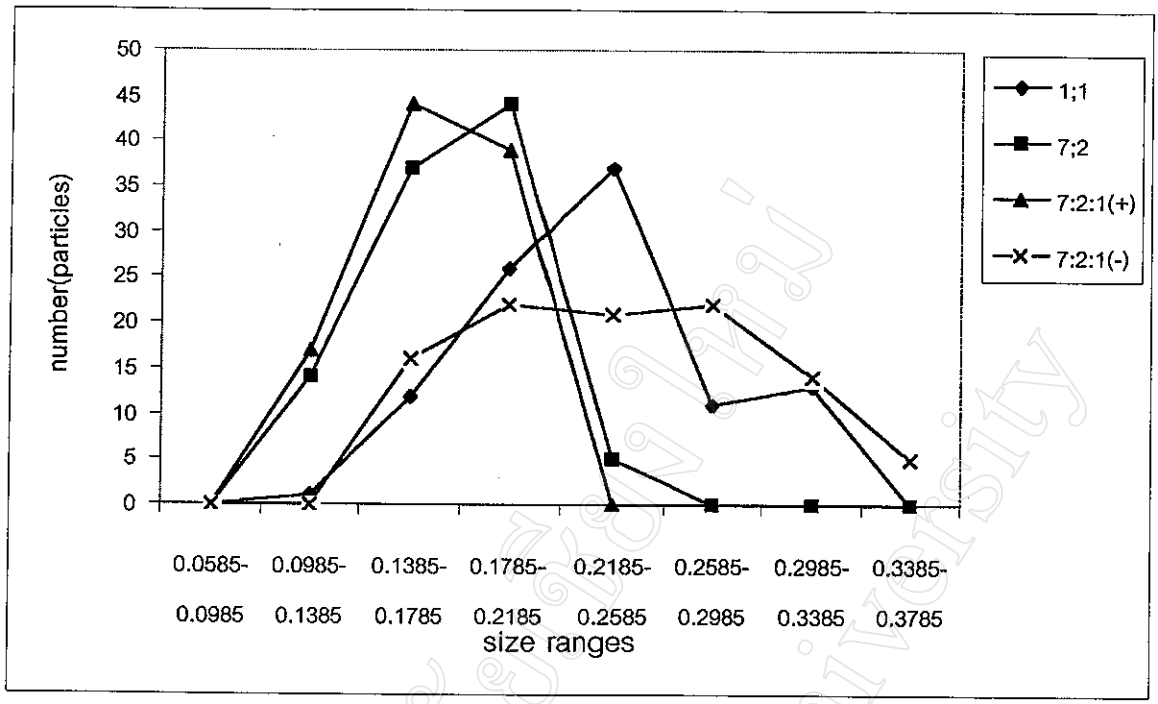


Figure 3.12 : Size distribution of liposome formulations without the entrapped amphotericin B

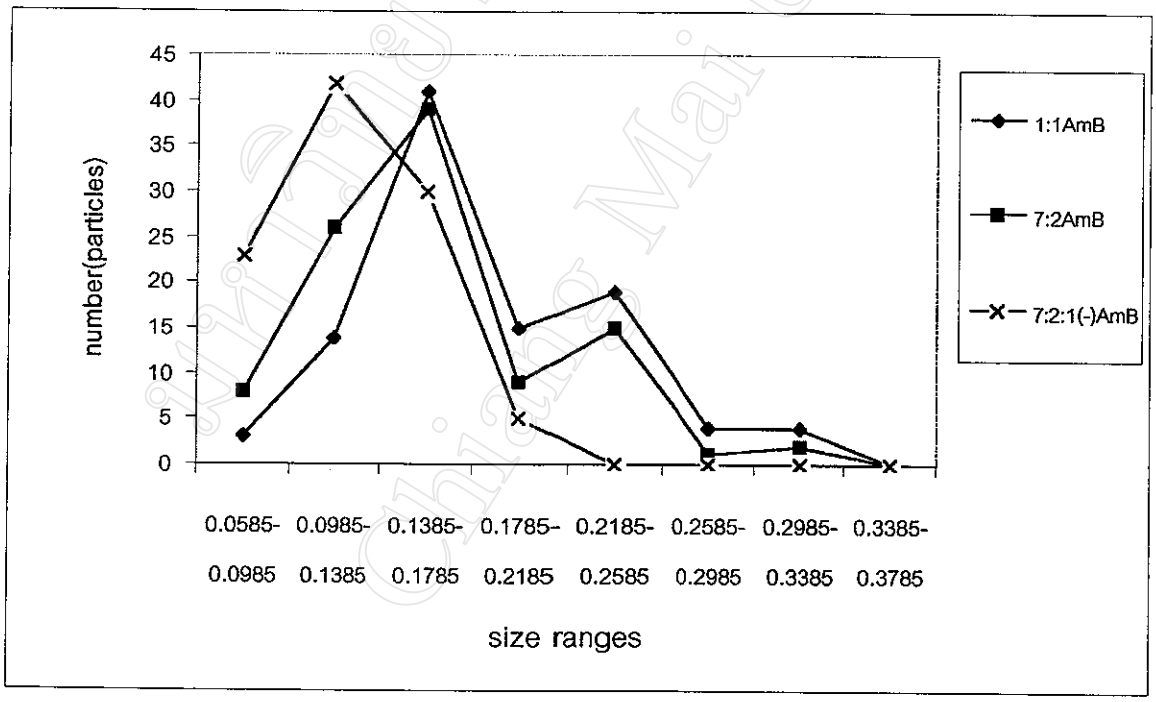


Figure 3.13 : Size distribution of liposome formulations with the entrapped amphotericin B

3.1.5 Lamellarity determined by TEM

The lamellarity of the 7:2 liposome formulation entrapped with AmB determined by TEM was showed in Figure 3.14.

Figure 3.14 : The lamellarity of the 7:2AmB liposome formulation

3.1.6 Investigation of the transition temperature and the enthalpy of transition by DSC

The scanned DSC curves of amphotericin B, Fungizone®, hydrogenated soya phosphatidylcholine, cholesterol, stearylamine and dicetyl phosphate powder and the eight lyophilized liposome formulations were shown in Figures 3.15 to 3.21 respectively. The transition temperature and enthalpy of transition obtained from these DSC curves were concluded in Table 3.14 to 3.16.

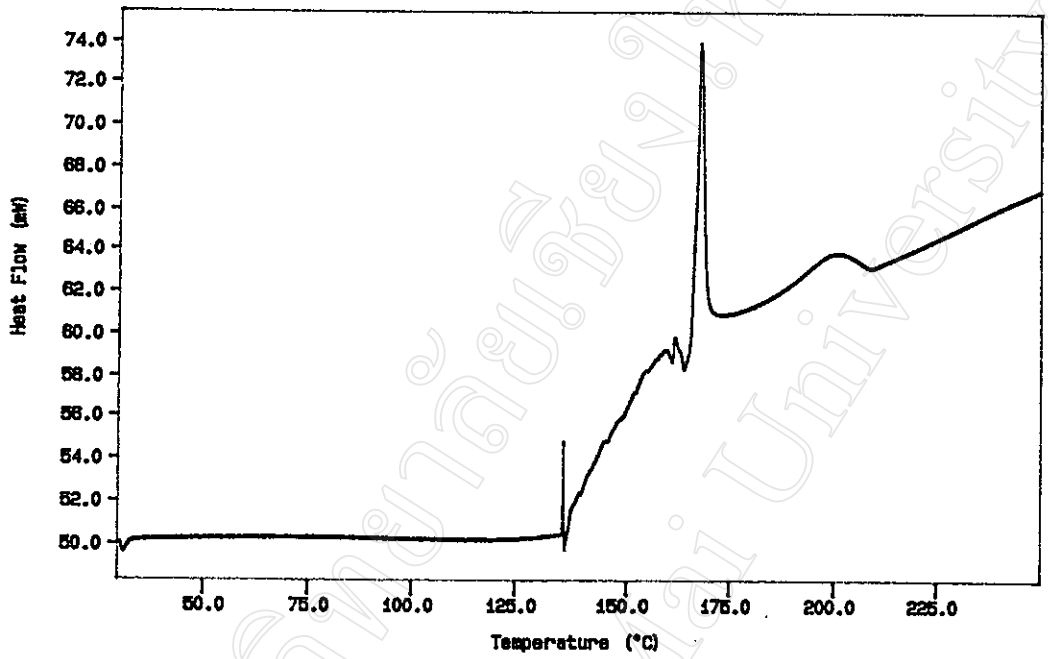


Figure 3.15 : The DSC curve of amphotericin B powder at rate 5°C/min

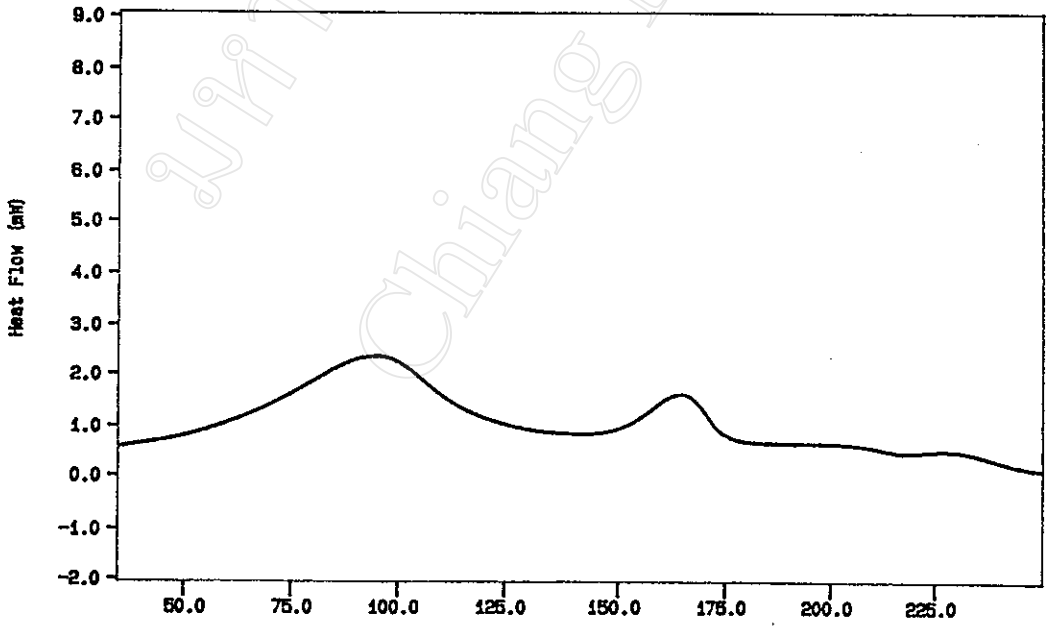


Figure 3.16 : The DSC curve of Fungizone®powder at rate 5°C/min

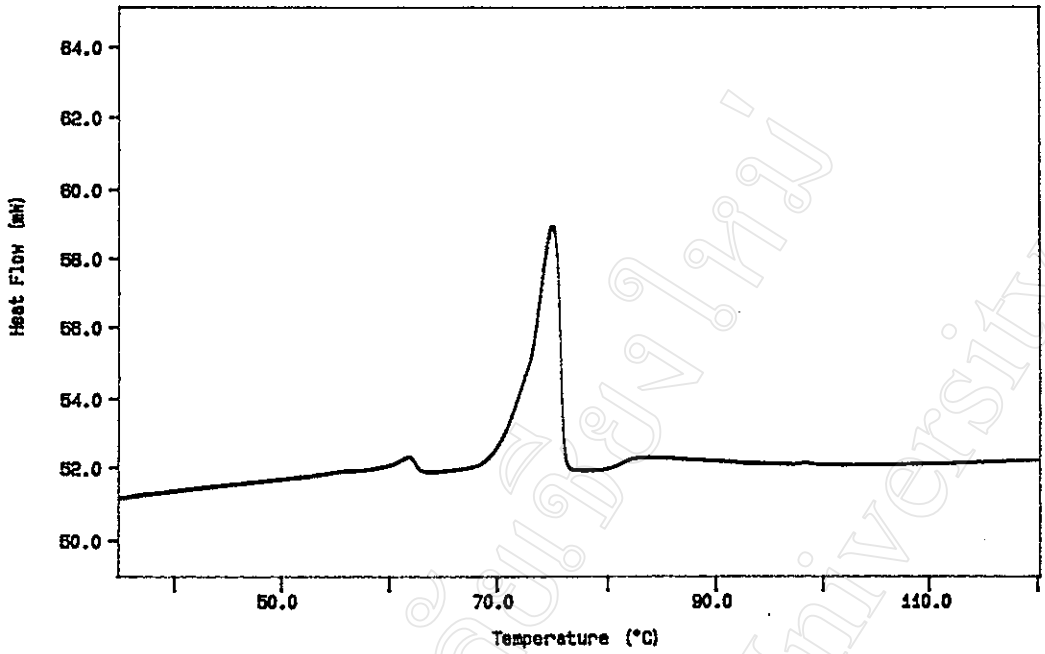


Figure 3.17 : The DSC curve of hydrogenated soya phosphatidylcholine (Emulmetik950®) powder at rate 5°C/min

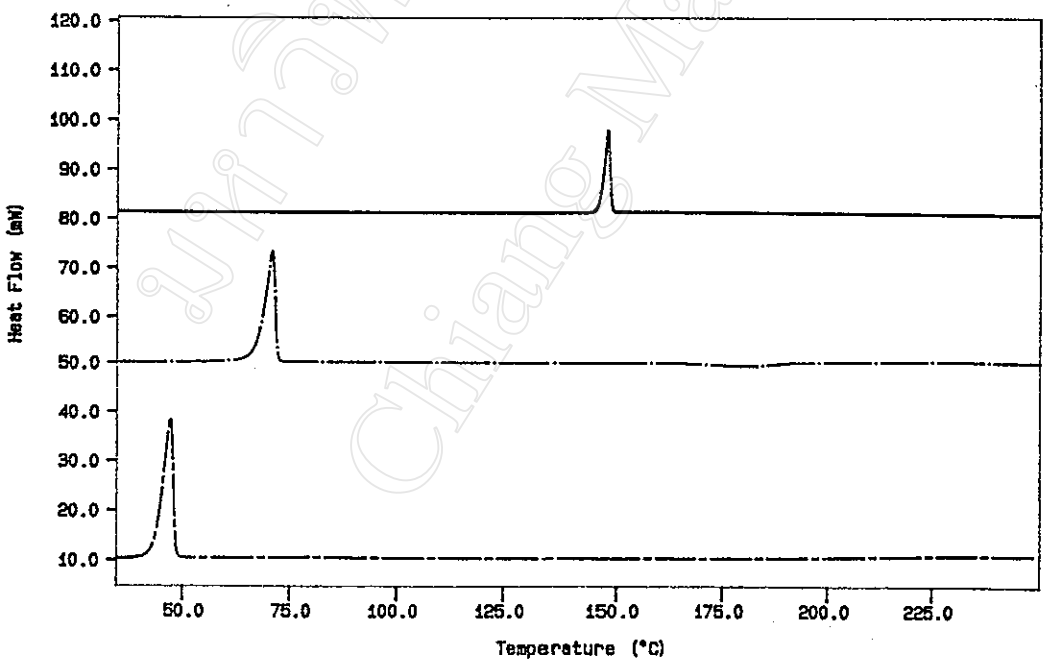


Figure 3.18 : The DSC curves of cholesterol (——) , dicetyl phosphate (– • –) and stearylamine (– – –) powder at rate 5°C/min

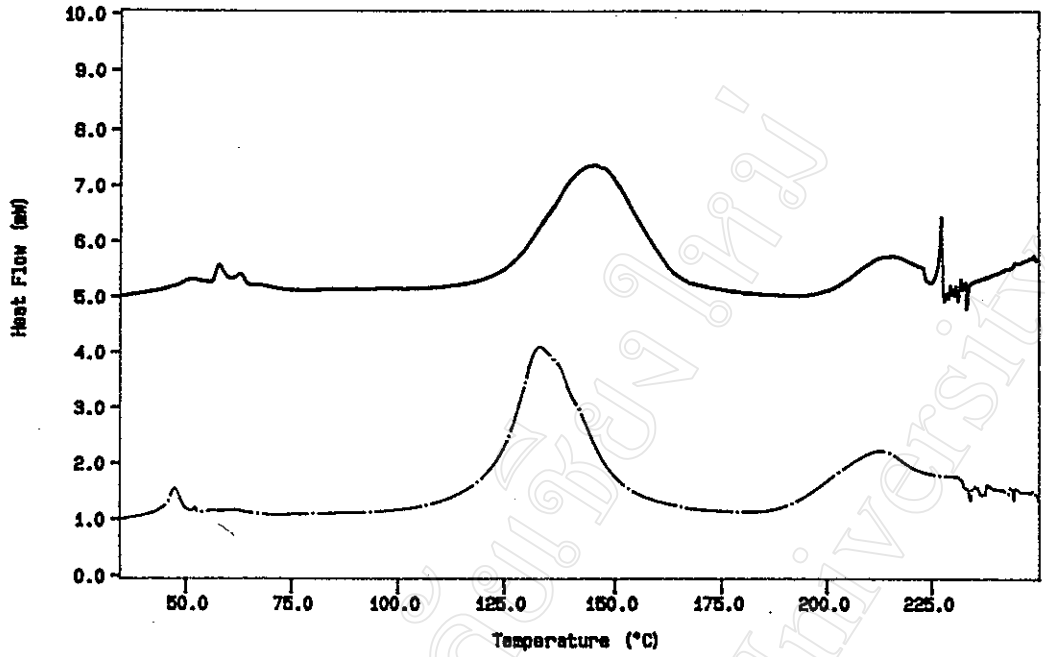


Figure 3.19 : The DSC curves of 1:1 (——) and 1:1AmB (– • –) lyophilized liposome formulations at rate 5°C/min

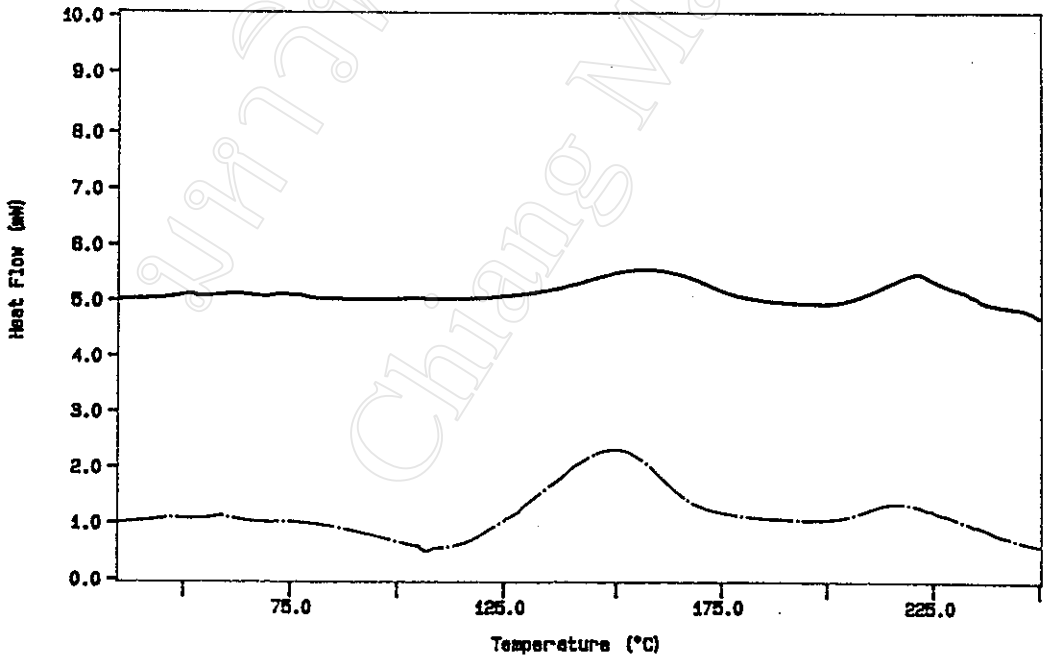


Figure 3.20 : The DSC curves of 7:2 (——) and 7:2AmB (– • –) lyophilized liposome formulations at rate 5°C/min

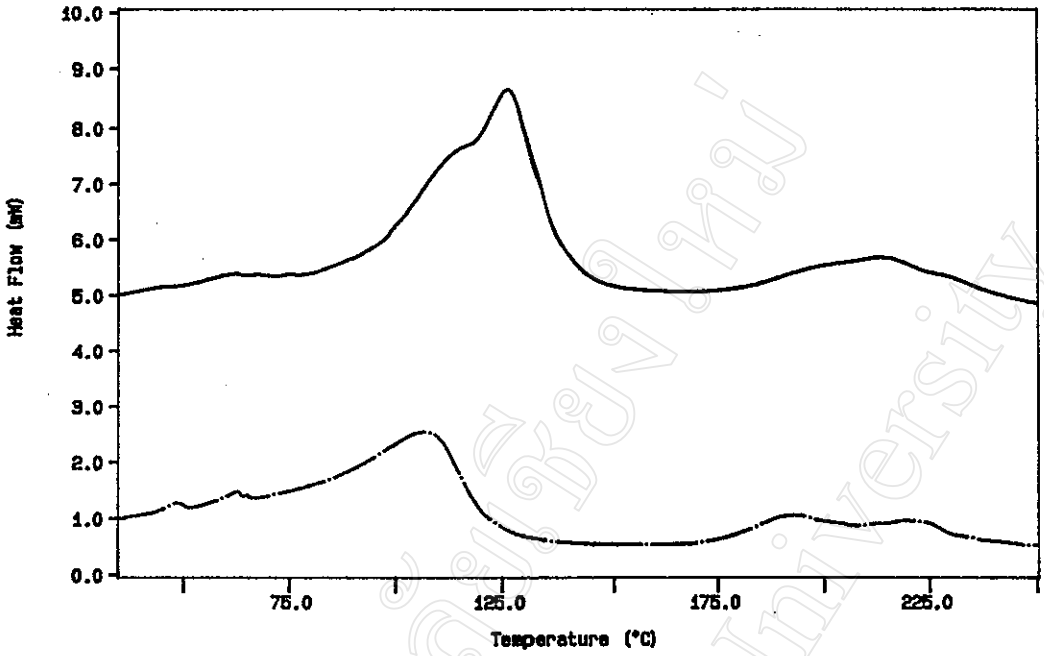


Figure 3.21 : The DSC curves of 7:2:1(+) (——) and 7:2:1(+)AmB (– • –) lyophilized liposome formulations at rate 5°C/min

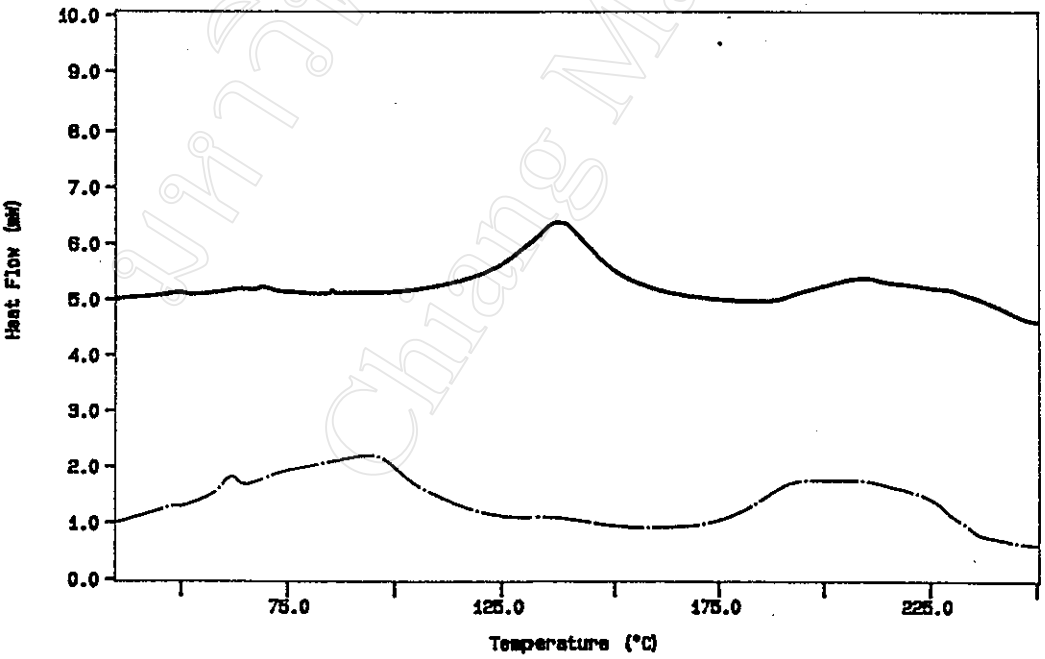


Figure 3.22 : The DSC curves of 7:2:1(-) (——) and 7:2:1(-)AmB (– • –) lyophilized liposome formulations at rate 5°C/min

Table 3.14 : The transition temperatures of FGZ, HSC, CHL, DCP, SA and the eight liposome formulations calculated from the DSC curves

Compounds		Transition temperature(°C)			
		1	2	Mean	SD
FGZ	1st Peak	91.859	94.340	93.100	1.754
	2nd Peak	164.563	164.758	164.661	0.138
HSC	1st Peak	61.789	61.855	61.822	0.047
	2nd Peak	76.377	75.388	75.883	0.699
CHL	-	147.514	148.510	148.012	0.704
DCP	-	68.012	70.062	69.037	1.450
SA	-	44.821	47.125	45.973	1.629
1:1	1st Peak	145.483	158.602	152.043	9.277
	2nd Peak	212.698	218.413	215.555	4.041
7:2	1st Peak	156.935	136.820	146.878	14.223
	2nd Peak	221.220	207.349	214.285	9.808
7:2:1(+)	1st Peak	135.482	126.529	131.006	6.331
	2nd Peak	217.595	213.235	215.415	3.083
7:2:1(-)	1st Peak	137.781	148.239	143.010	7.395
	2nd Peak	209.547	219.550	214.549	7.073
1:1 AmB	1st Peak	133.470	135.961	134.716	1.761
	2nd Peak	211.094	215.651	213.373	3.222
7:2 AmB	1st Peak	149.296	131.239	140.268	12.768
	2nd Peak	217.246	211.323	214.285	4.188
7:2:1(+) AmB	1st Peak	107.145	110.360	108.753	2.273
	2nd Peak	192.673	219.365	206.019	18.874
7:2:1(-) AmB	1st Peak	94.103	75.831	84.967	12.920
	2nd Peak	207.189	194.207	200.698	9.180

Table 3.15 : The enthalpy of transition (ΔH, J/g) of FGZ, HSC, CHL, DCP, SA and the eight liposome formulations calculated from the DSC curves

Compounds		Enthalpy of transition (J/g)			
		1	2	Mean	SD
FGZ	1st Peak	237.109	212.058	224.584	17.714
	2nd Peak	46.601	44.987	45.794	1.141
HSC	1st Peak	1.650	1.658	1.654	0.006
	2nd Peak	30.917	35.580	33.249	3.297
CHL	-	67.617	68.520	68.069	0.639
DCP	-	201.366	207.990	204.678	4.684
SA	-	278.601	276.808	277.705	1.268
1:1	1st Peak	178.094	112.064	145.079	46.690
	2nd Peak	25.308	23.120	24.214	1.547
7:2	1st Peak	92.919	146.340	119.630	37.774
	2nd Peak	53.236	78.494	65.865	17.860
7:2:1(+)	1st Peak	513.365	455.498	484.432	40.918
	2nd Peak	44.704	90.529	67.617	32.403
7:2:1(-)	1st Peak	169.450	186.180	177.815	11.830
	2nd Peak	74.732	51.237	62.985	16.613
1:1 AmB	1st Peak	178.727	166.297	172.512	8.789
	2nd Peak	33.747	33.026	33.387	0.510
7:2 AmB	1st Peak	275.595	223.141	249.368	37.091
	2nd Peak	51.755	34.729	43.242	12.039
7:2:1(+) AmB	1st Peak	261.207	338.684	299.946	54.785
	2nd Peak	78.766	97.64	88.203	13.346
7:2:1(-) AmB	1st Peak	189.316	178.480	183.898	7.662
	2nd Peak	149.012	155.825	152.419	4.818

Table 3.16 : Conclusion of the mean ($\bar{x} \pm SD$) of transition temperature (T_c) and enthalpy of transition (ΔH) of the compositions in liposome

Compounds	Transition temperature, T_c (°C)		Enthalpy of transition, ΔH (J/g)	
	1st Peak	2nd Peak	1st Peak	2nd Peak
FGZ	93.100 ± 1.754	164.661 ± 0.138	224.584±17.714	45.794±1.141
HSC	61.822 ± 0.047	75.883 ± 0.699	1.654±0.006	33.249±3.297
CHL	148.012 ± 0.704	-	68.069±0.639	-
DCP	69.037 ± 1.450	-	204.678±4.684	-
SA	45.973 ± 1.629	-	277.705±1.268	-

Table 3.17 : Conclusion of the mean ($\bar{x} \pm SD$) of transition temperature (T_c) of the liposome formulations

Compounds	Liposomes without drug		Liposomes with drug	
	1st Peak	2nd Peak	1st Peak	2nd Peak
1:1	152.043 ± 9.277	215.555 ± 4.041	134.716 ± 1.761	213.373 ± 3.222
7:2	146.878 ± 4.233	214.285 ± 9.808	140.268 ± 2.768	214.285 ± 4.188
7:2:1(+)	131.006 ± 6.331	215.415 ± 3.083	108.753 ± 2.273	206.019 ± 8.874
7:2:1(-)	143.01 ± 7.395	214.549 ± 7.073	84.967 ± 12.920	200.698 ± 9.180

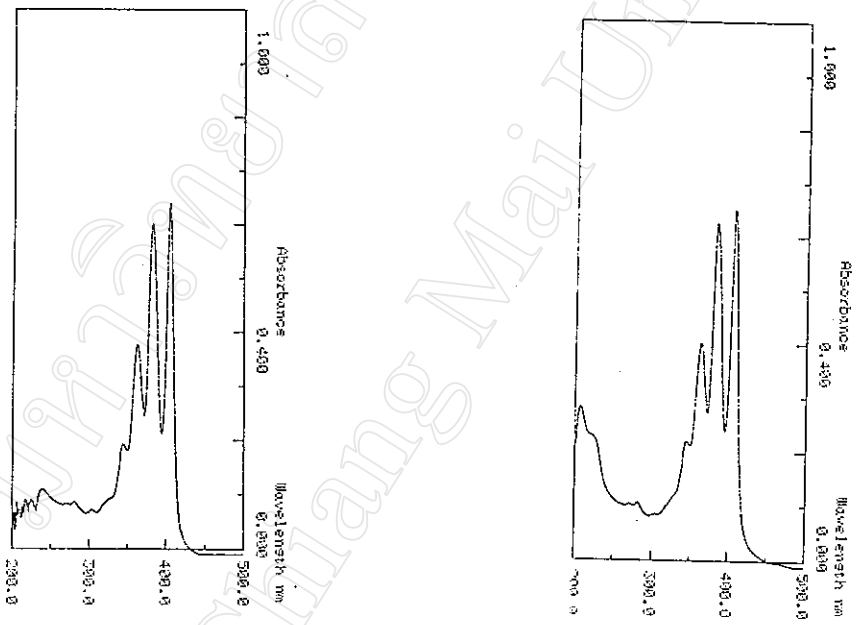
Table 3.18 : Conclusion of the mean($\bar{x} \pm SD$)of enthalpy of transition (ΔH) (J/g) of the liposome formulations

Compounds	Liposomes without drug		Liposomes with drug	
	1st Peak	2nd Peak	1st Peak	2nd Peak
1:1	145.079±46.690	24.214±1.547	172.512±8.789	33.387±0.510
7:2	119.63±37.774	65.865±17.860	249.368±37.091	43.242±12.039
7:2:1(+)	484.432±40.918	67.617±32.403	299.946±54.785	88.203±13.346
7:2:1(-)	177.815±11.830	62.985±16.613	183.898±7.662	152.419±4.818

3.2 Qualitative and quantitative analysis of amphotericin B, Fungizone® and the drug in liposome formulations by HPLC

3.2.1 UV absorption spectra

UV absorption spectra of amphotericin B in DMSO/methanol solution, Fungizone® in methanol and the liposome with the entrapped drug in methanol gave absorption peaks at four wavelengths at 406, 382, 363 and 345 nm while the liposomes without the entrapped drug in methanol did not show any peak at wavelength more than 300 nm. The absorption spectra of all of these samples are shown in Figures 3.23 and 3.24.



(A) (B)

Figure 3.23 : UV absorption spectra of amphotericin B in DMSO/methanol solution (A) and Fungizone® in methanol (B) (5 μg/ml)

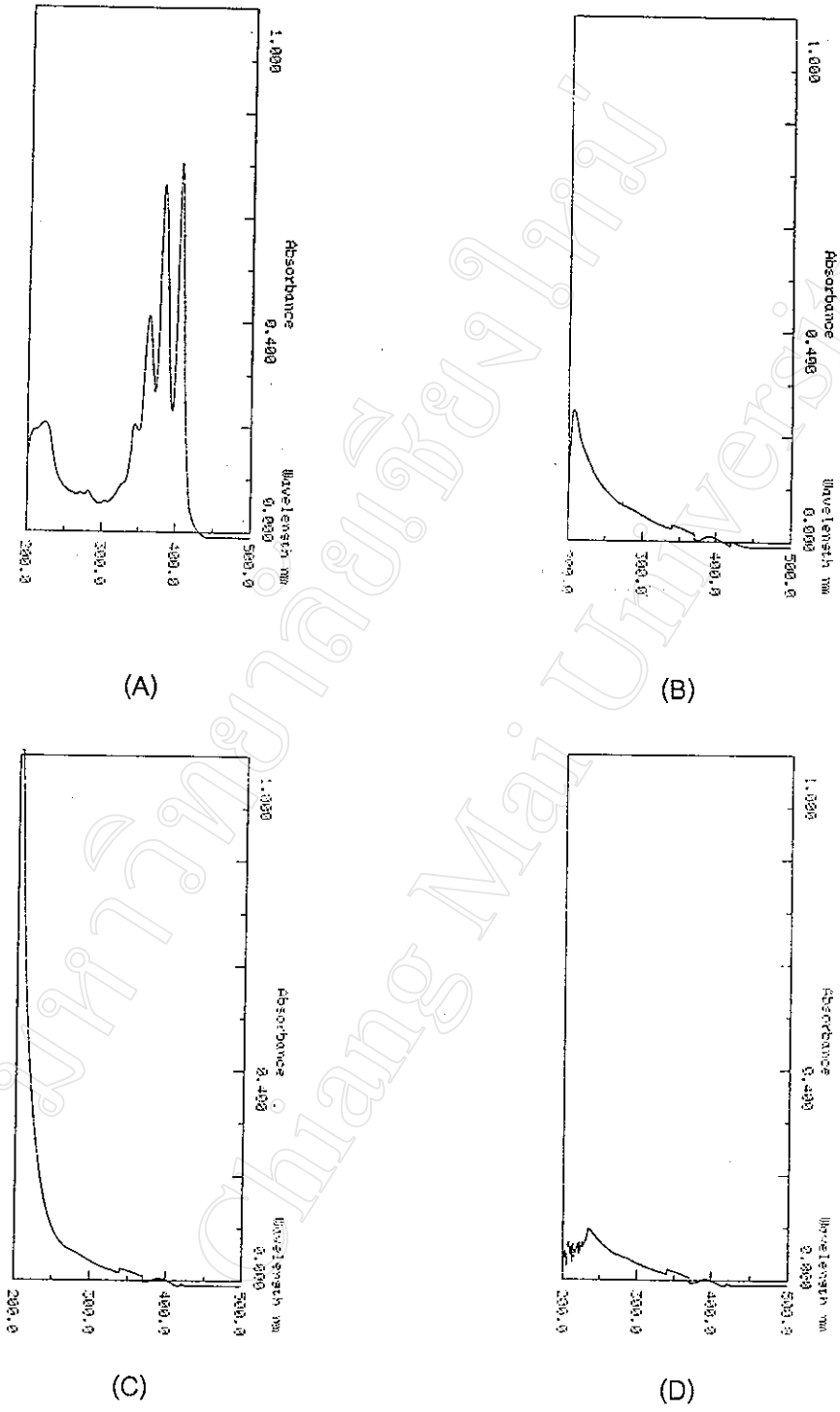


Figure 3.24 : UV absorption spectra of liposomes with the drug in methanol(A)(about 5µg/ml), liposomes without the drug in methanol (B) methanol (C) and DMSO/methanol solution (D)

3.2.2 Chromatogram of amphotericin B and Fungizone® by HPLC with UV detection at 382 nm

Chromatograms of methanol , Fungizone® in methanol and amphotericin B in methanol were shown in (A), (B) and (C) of Figure 3.25 respectively. In comparing the chromatograms, amphotericin B from Fluka BioChemika gave retention time at 4.678 mins where amphotericin B in the form of Fungizone® showed retention time at 4.693 mins.

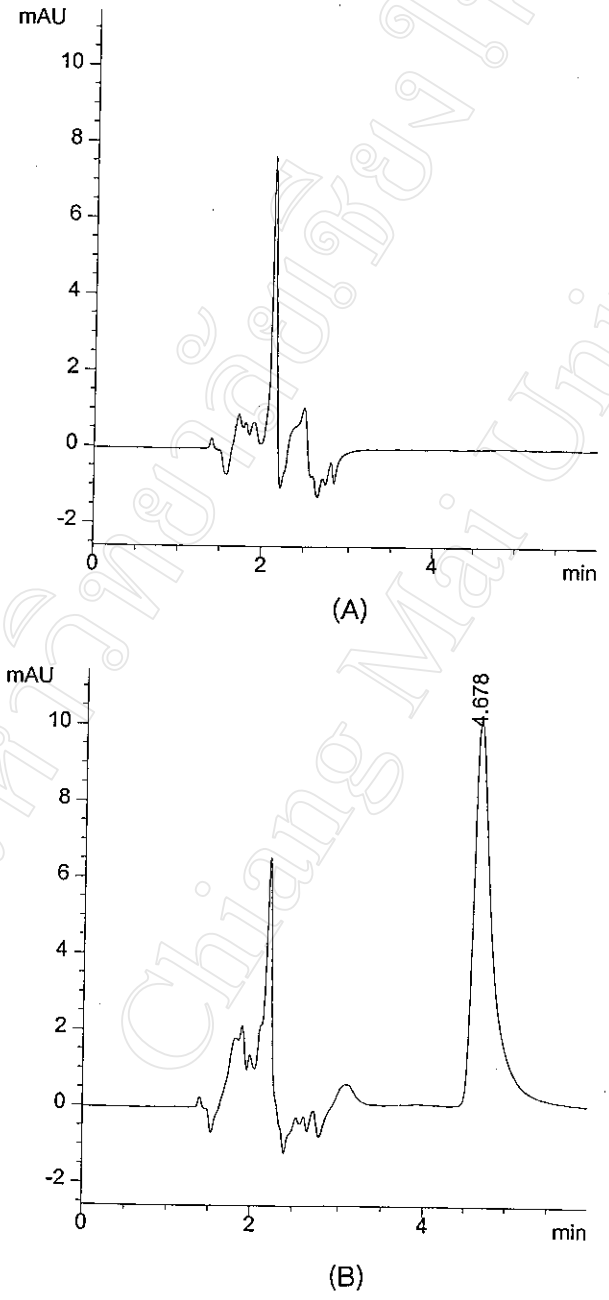
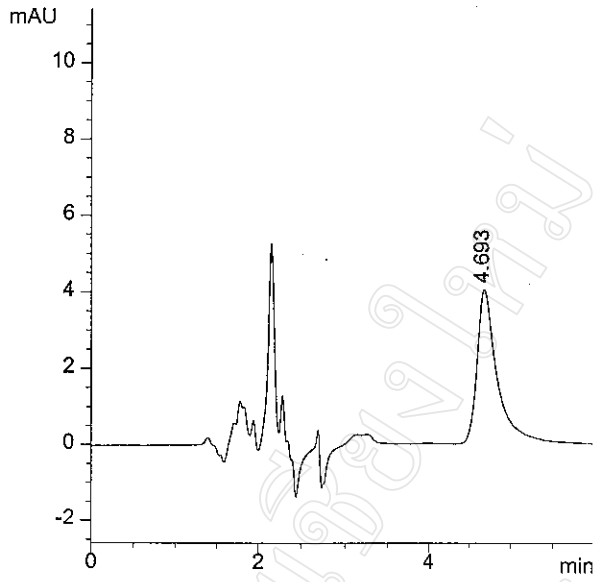


Figure 3.25 : Chromatograms of methanol (A), amphotericin B in methanol (B) and Fungizone® in methanol (C).



(C)

Figure 3.25 : Chromatograms of methanol (A), amphotericin B in methanol (B) and Fungizone® in methanol (C) (continue)

3.2.3 The amphotericin B standard curve preparation

Reference standard amphotericin B at concentrations of 0.02, 0.10, 0.50, 1.00 and 1.50 µg/ml were prepared in duplicate and injected into HPLC three times per sample. The peak areas of each concentration were shown in Table 3.19 and 3.20 and the standard curve was constructed as in Figure 3.26.

Table 3.19 : Peak areas in various concentrations of the reference standard amphotericin B

Conc.	1				2			
(µg/ml)	Inject 1	Inject 2	Inject 3	**Mean 1**	Inject 1	Inject 2	Inject 3	**Mean 2**
0.02	3.11	3.28	2.83	3.07	2.98	3.07	3.22	3.09
0.10	25.64	24.95	25.21	25.26	23.85	23.22	23.06	23.37
0.50	163.32	162.46	162.38	162.72	172.13	170.67	173.56	172.12
1.00	353.12	351.89	350.40	351.80	346.61	339.46	338.58	341.55
1.50	531.33	532.27	531.85	531.82	517.80	515.15	515.65	516.20

Table 3.20 : The peak areas from duplicate analysis of the standard amphotericin B at various concentrations

Conc.(μg/ml)	Mean 1	Mean 2	Mean	SD	%CV
0.02	3.07	3.09	3.08	0.01	0.37
0.10	25.26	23.37	24.32	1.34	5.49
0.50	162.72	172.12	167.42	6.65	3.97
1.00	351.80	341.55	346.68	7.25	2.09
1.50	531.82	516.20	524.01	11.04	2.11

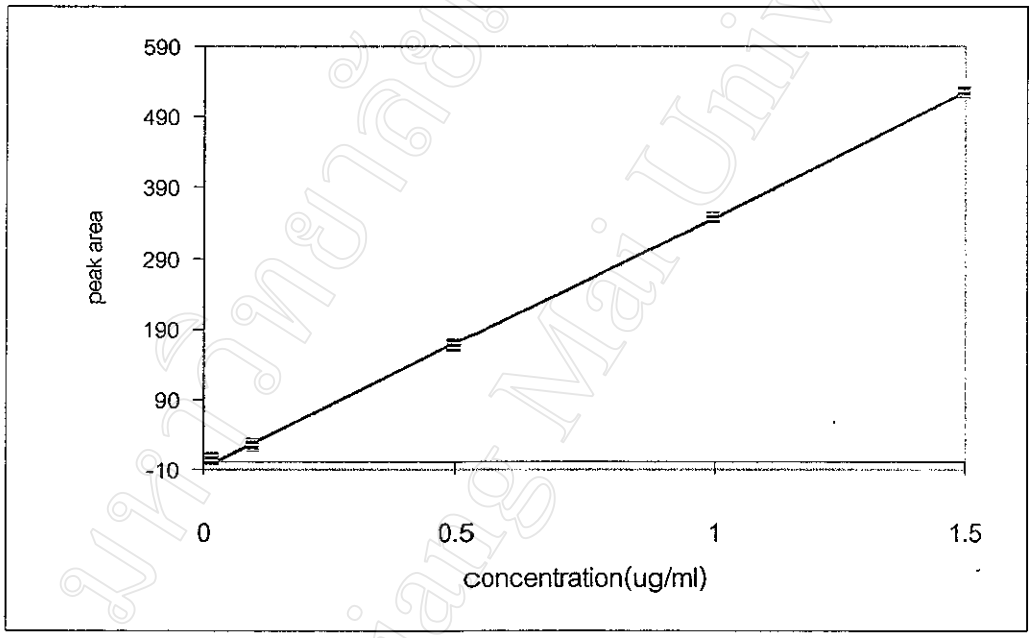


Figure 3.26 : The standard curve of standard amphotericin B with r^2 = 0.9999 and y = 354.34x - 8.01 from the linear regression analysis

3.2.4 Contents (mg) determination of amphotericin B in Fungizone® powder (1 mg)

From the labelled amount, one vial of Fungizone® was composed of amphotericin B 50 mg, sodium deoxycholate 41 mg and sodium phosphate 20.2 mg that made up total of 111.2 mg. This contained 45% of amphotericin B in the product. The contents of amphotericin B in the form of Fungizone® determined by HPLC were demonstrated in Table 3.21. The samples were performed in triplicate.

Table 3.21 : The contents of amphotericin B in 1 mg of Fungizone®powder determined by HPLC

Analysis	Peak area		
	1	2	3
Inject 1	68.26	85.91	69.82
Inject 2	70.84	84.43	70.69
Inject 3	70.83	78.91	67.14
Mean	69.98	83.08	69.22
SD	1.49	3.69	1.85
Conc. (μg/ml)	0.22	0.26	0.22
Content in 1 mg (mg)	0.40	0.46	0.40

The contents of amphotericin B in 1 mg of Fungizone® were 0.42 ± 0.037 which was 42 ± 3.7% of the total weight of Fungizone® product. This value was close to the label amount which was 45%.

3.3 Determination of the percentages of entrapment of amphotericin B in liposomes

The percentages of amphotericin B entrapped in liposomes were calculated from the amphotericin B contents assayed by HPLC from the entrapped pellets and the free drugs in the supernatant of 1:1AmB, 7:2AmB, 7:2:1(+)AmB and 7:2:1(-)AmB liposome formulations. The chromatogram of the entrapped amphotericin B gave the peak at 4.6 mins the same as the standard amphotericin B. However, the chromatogram of all samples of the unentrapped amphotericin B in the supernatant showed peak at about four mins (Figure 3.27). The chromatograms of blank liposome (no drug) of all formulations when assayed the dispersion for the total drug (A), the pellet (B) and the unentrapped drug from the supernatant (C), they showed no peaks at retention time more than 3 mins (Figure 3.28).

Peak areas from HPLC analysis of total amount of amphotericin B, the entrapped and unentrapped of 1:1, 7:2, 7:2:1(+) and 7:2:1(-) formulations were presented in Table 3.22 to 3.24, 3.25 to 3.27, 3.28 to 3.30 and 3.31 to 3.33 respectively. The average peak areas were calculated and were further used to calculate the amount of amphotericin B entrapped in liposomes as shown in Tables 3.34, 3.35, 3.36 and 3.37 for 1:1, 7:2, 7:2:1(+) and 7:2:1(-) amphotericin B liposomes respectively. Table 3.38 showed the average percentages of the entrapment. The loading of amphotericin B in liposomes was shown in Table 3.39.

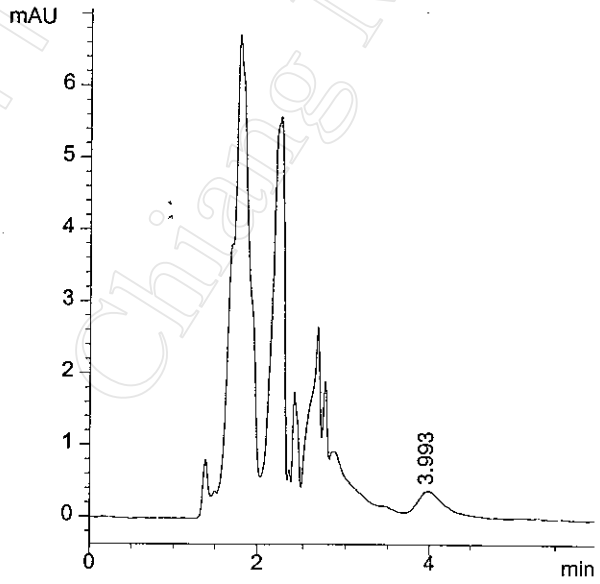
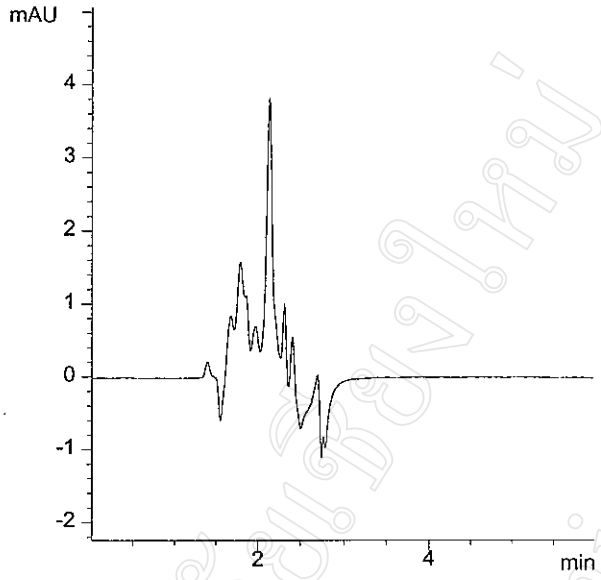
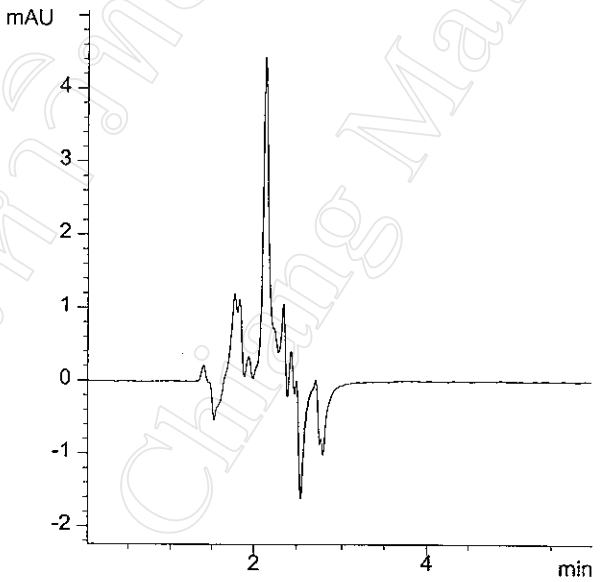


Figure 3.27 : The HPLC chromatogram of the unentrapped amphotericin B in the supernatant of the 1:1AmB liposome formulation

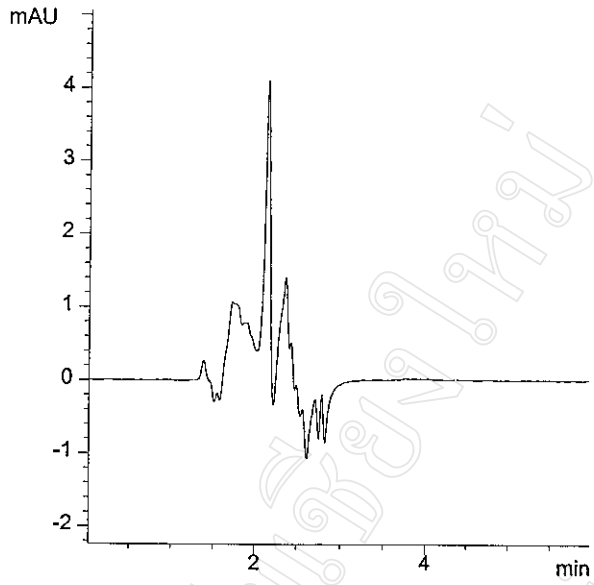


(A)



(B)

Figure 3.28 : The HPLC chromatograms of the liposome formulation without the entrapped drug when assayed for the total amount of drug (liposome dispersion) (A) , the entrapped drug (pellets) (B) and the free drug (supernatant) (C)



(C)

Figure 3.28 : The HPLC chromatograms of the liposome without the drug when assayed for the total amount of drug (liposome dispersion) (A) , the entrapped drug (pellets) (B) and the free drug (supernatant) (C) (continue)

Table 3.22 : Peak areas from HPLC analysis of the total amount of AmB in 1:1AmB liposomes

Total drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	53.51	53.19	53.80	53.50	58.33	58.11	58.33	58.25
2	56.11	55.83	55.97	55.97	59.29	59.52	58.33	59.05
3	58.63	58.68	58.73	58.68	60.95	61.82	61.95	61.57
Mean	-	-	-	**56.05**	-	-	-	**59.62**
SD	-	-	-	2.59	-	-	-	1.73
%CV	-	-	-	4.62	-	-	-	2.91

Table 3.23 : Peak areas from HPLC analysis of the entrapped AmB in 1:1AmB liposomes

Entrapped drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	108.05	108.73	108.43	108.40	104.26	104.79	105.01	104.69
2	113.42	113.29	113.18	113.30	108.23	107.02	106.60	107.28
3	112.52	112.22	111.67	112.14	105.82	104.64	105.68	105.38
Mean	-	-	-	**111.28**	-	-	-	**105.78**
SD	-	-	-	2.56	-	-	-	1.34
%CV	-	-	-	2.30	-	-	-	1.27

Table 3.24 : Peak areas from HPLC analysis of the unentrapped AmB in 1:1AmB liposomes

Free drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	42.47	42.27	42.19	42.31	368.20	365.37	363.95	365.84
2	38.57	38.48	38.25	38.43	353.88	353.43	351.49	352.94
3	38.70	38.15	38.67	38.51	359.88	358.08	357.00	358.32
Mean	-	-	-	**39.75**	-	-	-	**359.03**
SD	-	-	-	2.22	-	-	-	6.48
%CV	-	-	-	5.57	-	-	-	1.80

Table 3.25 : Peak areas from HPLC analysis of the total amount of AmB in 7:2AmB liposomes

Total drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	69.16	69.46	69.41	69.34	67.09	67.17	67.76	67.34
2	70.54	69.92	70.43	70.30	67.23	66.94	66.97	67.05
3	66.90	67.69	68.28	67.62	66.87	67.08	67.25	67.07
Mean	-	-	-	**69.09**	-	-	-	**67.15**
SD	-	-	-	1.35	-	-	-	0.16
%CV	-	-	-	1.96	-	-	-	0.24

Table 3.26 : Peak areas from HPLC analysis of the entrapped AmB in 7:2AmB liposomes

Entrapped drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	128.14	129.20	130.63	129.33	116.96	116.75	116.93	116.88
2	127.76	127.26	127.61	127.54	114.78	115.34	115.13	115.09
3	128.72	127.59	128.11	128.14	118.39	119.19	120.28	119.29
Mean	-	-	-	**128.34**	-	-	-	**117.08**
SD	-	-	-	0.91	-	-	-	2.11
%CV	-	-	-	0.71	-	-	-	1.80

Table 3.27 : Peak areas from HPLC analysis of the unentrapped AmB in 7:2AmB liposomes

Free drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	4.59	5.04	4.76	4.80	16.23	15.78	15.69	15.90
2	4.61	4.72	4.27	4.53	16.62	16.47	16.50	16.53
3	4.60	4.68	4.65	4.64	16.71	16.20	17.17	16.69
Mean	-	-	-	**4.66**	-	-	-	**16.37**
SD	-	-	-	0.13	-	-	-	0.42
%CV	-	-	-	2.86	-	-	-	2.55

Table 3.28 : Peak areas from HPLC analysis of the total amount of AmB in 7:2:1(+)AmB liposomes

Total drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	63.98	63.73	67.33	65.01	55.23	54.97	54.89	55.03
2	66.42	65.07	67.09	66.19	56.50	57.29	57.07	56.95
3	67.19	67.64	67.85	67.56	55.31	54.41	54.98	54.90
Mean	-	-	-	**66.25**	-	-	-	**55.63**
SD	-	-	-	1.27	-	-	-	1.15
%CV	-	-	-	1.92	-	-	-	2.06

Table 3.29 : Peak areas from HPLC analysis of the entrapped AmB in 7:2:1(+)AmB liposomes

Entrapped drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	131.67	132.03	132.25	131.98	106.35	106.58	106.41	106.45
2	122.46	126.74	126.11	125.10	100.00	100.10	100.06	100.05
3	128.14	128.56	128.59	128.43	106.78	106.89	106.72	106.80
Mean	-	-	-	**128.51**	-	-	-	**104.43**
SD	-	-	-	3.44	-	-	-	3.80
%CV	-	-	-	2.68	-	-	-	3.64

Table 3.30 : Peak areas from HPLC analysis of the unentrapped AmB in 7:2:1(+)AmB liposomes

Free drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	3.11	3.35	3.17	3.21	5.87	6.46	6.81	6.38
2	3.38	3.58	3.28	3.41	6.22	6.12	6.04	6.13
3	4.52	3.93	3.90	4.12	6.38	6.28	6.20	6.29
Mean	-	-	-	**3.58**	-	-	-	**6.26**
SD	-	-	-	0.48	-	-	-	0.13
%CV	-	-	-	13.28	-	-	-	2.02

Table 3.31 : Peak areas from HPLC analysis of the total amount of AmB in7:2:1(-)AmB liposomes

Total Drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	77.09	69.61	69.35	72.02	50.98	50.88	51.12	50.99
2	70.25	70.45	70.57	70.43	51.41	53.33	52.66	52.46
3	73.41	72.74	73.28	73.14	54.01	54.78	54.49	54.43
Mean	-	-	-	**71.86**	-	-	-	**52.63**
SD	-	-	-	1.37	-	-	-	1.72
%CV	-	-	-	1.90	-	-	-	3.28

Table 3.32 : Peak areas from HPLC analysis of the entrapped AmB in 7:2:1(-)AmB liposomes

Entrapped drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	135.37	135.55	136.73	135.88	90.92	90.51	90.58	90.67
2	133.81	133.23	132.64	133.23	92.98	93.44	92.82	93.08
3	132.84	131.70	131.31	131.95	97.21	97.07	97.49	97.26
Mean	-	-	-	**133.69**	-	-	-	**93.67**
SD	-	-	-	2.01	-	-	-	3.33
%CV	-	-	-	1.50	-	-	-	3.56

Table 3.33 : Peak areas from HPLC analysis of the unentrapped AmB in 7:2:1(-)AmB liposomes

Free drug	Peak areas of liposome lot 1				Peak areas of liposome lot 2			
	Inject 1	Inject 2	Inject 3	Mean 1	Inject 1	Inject 2	Inject 3	Mean 2
1	87.14	86.89	86.79	86.94	148.29	146.90	147.23	147.47
2	82.08	81.63	81.52	81.75	149.92	150.21	151.08	150.41
3	77.97	76.70	77.69	77.45	151.79	151.48	150.90	151.39
Mean	-	-	-	**82.05**	-	-	-	**149.76**
SD	-	-	-	4.75	-	-	-	2.04
%CV	-	-	-	5.79	-	-	-	1.36

Table 3.34 : Mean peak areas, concentrations, percentages of drug (total, entrapped and unentrapped AmB) of 1:1AmB liposome formulation

1:1AmB	Description	Total drug	Entrapped drug	Free drug
Liposome lot 1	Mean of peak area	56.05	111.28	39.75
	Concentration (μg/ml)	0.18	0.34	0.13
	Amount (μg)	180.79	168.33	1.35
	Percentages of drug (%)	100.00	93.13	0.75
Liposome lot 2	Mean of peak area	59.62	105.78	359.03
	Concentration (μg/ml)	0.19	0.32	1.04
	Amount (μg)	190.87	160.57	10.36
	Percentages of drug (%)	100.00	84.21	5.43

Note : Dilution factors for the total, entrapped and free drug were 1,000, 500 and 11.

Table 3.35 : Mean peak areas, concentrations, percentages of drug (total, entrapped and unentrapped AmB) of 7:2AmB liposome formulation

7:2AmB	Description	Total drug	Entrapped drug	Free drug
Liposome lot 1	Mean of peak area	69.09	128.34	4.66
	Concentration (μg/ml)	0.22	0.38	0.04
	Amount (μg)	217.58	192.40	0.36
	Percentages of drug (%)	100	88.43	0.17
Liposome lot 2	Mean of peak area	67.15	117.08	16.37
	Concentration (μg/ml)	0.21	0.35	0.07
	Amount (μg)	212.12	176.52	0.69
	Percentages of drug (%)	100	83.22	0.33

Note : Dilution factors for the total, entrapped and free drug were 1,000, 500 and 11.

Table 3.36 : Mean peak areas, concentrations, percentages of drug (total, entrapped and unentrapped AmB) of 7:2:1(+)AmB liposome formulation

7:2:1(+) AmB	Description	Total drug	Entrapped drug	Free drug
Liposome lot 1	Mean of peak area	66.25	128.51	3.58
	Concentration (μg/ml)	0.21	0.39	0.03
	Amount (μg)	209.58	192.63	0.33
	Percentages of drug (%)	100	91.91	0.16
Liposome lot 2	Mean of peak area	55.63	104.43	6.26
	Concentration (μg/ml)	0.18	0.32	0.04
	Amount (μg)	179.60	158.66	0.40
	Percentages of drug (%)	100	88.34	0.22

Note : Dilution factors for the total, entrapped and free drug were 1,000, 500 and 11.

Table 3.37 : Mean peak areas, concentrations, percentages of drug (total, entrapped and unentrapped AmB) of 7:2:1(-)AmB liposome formulation

7:2:1(-) AmB	Description	Total drug	Entrapped drug	Free drug
Liposome lot 1	Mean of peak area	71.86	133.69	82.05
	Concentration (μg/ml)	0.23	0.40	0.25
	Amount (μg)	225.41	199.95	2.54
	Percentages of drug (%)	100.00	88.71	1.13
Liposome lot 2	Mean of peak area	52.63	93.67	149.76
	Concentration (μg/ml)	0.17	0.29	0.45
	Amount (μg)	171.12	143.48	4.45
	Percentages of drug (%)	100.00	83.85	2.60

Note : Dilution factors for the total , entrapped and free drug were 1,000, 500 and 11.

Table 3.38 : The average percentages of the entrapment of AmB and the free AmB in liposome formulations

Formu-lations	Percentages(%)	Liposome lot 1	Liposome lot 2	Average percentages	SD	%CV
1:1AmB	Total drug	100.00	100.00	**100.00**	0.00	0.00
	Entrapped drug	93.13	84.12	**88.63**	6.37	7.18
	Free drug	0.75	5.43	**3.09**	3.31	107.21
7:2AmB	Total drug	100.00	100.00	**100.00**	0.00	0.00
	Entrapped drug	88.43	83.22	**85.82**	3.68	4.29
	Free drug	0.17	0.33	**0.25**	0.11	46.06
7:2:1(+) AmB	Total drug	100.00	100.00	**100.00**	0.00	0.00
	Entrapped drug	91.91	88.34	**90.13**	2.53	2.80
	Free drug	0.16	0.22	**0.19**	0.05	24.28
7:2:1(-) AmB	Total drug	100.00	100.00	**100.00**	0.00	0.00
	Entrapped drug	88.71	83.85	**86.28**	3.43	3.98
	Free drug	1.13	2.60	**1.86**	1.04	55.91

Table 3.39 : The amount of AmB per total lipid (μg/mg) in liposome formulations

Formulations	Total lipid (mg) in 1 ml of sample	Amounts (μg) of the entrapped AmB in 1 ml of sample		Loading of AmB in liposome (AmB (μg) /lipid (mg)) (μg/mg)
		Lot 1	Lot 2	
1:1AmB	5	168.33	160.57	32.89 ± 1.10
7:2AmB	5	192.40	176.52	36.89 ± 2.25
7:2:1(+)AmB	5	192.63	158.66	35.13 ± 4.80
7:2:1(-)AmB	5	199.95	143.48	34.34 ± 7.99

3.4 Stability study of amphotericin B entrapped in liposome formulations

3.4.1 Physical stability

The physical appearances of 1:1, 7:2, 7:2:1(+), 7:2:1(-) liposomes with and without the entrapped the drug , Fungizone® solution and Fungizone® powder freshly prepared and stored at 4±1°C, 30±1°C and 45±1°C for 90 days were investigated as shown in Table 3.40. Figure 3.29 to 3.31 showed the appearances of all of these samples when freshly prepared and after 90 days at 4±1°C, 30±1°C and 45±1°C respectively.

Table 3.40 : The physical changes of 1:1, 7:2, 7:2:1(+), 7:2:1(-) liposomes with and without entrapped drug, Fungizone® solution and Fungizone® powder right after the preparation and stored at 4±1°C, 30±1°C and 45±1°C

Formulations	Freshly prepared		90 days					
	Sedimentation	Supernatant	Sediment			Supernatant		
			4°C	30°C	45°C	4°C	30°C	45°C
1:1	Yes	+1	Yes	Yes	No	+2	+2	+3
1:1AmB	Yes	+1	Yes	Yes	No	+2	+2	+3
7:2	Yes	+1	Yes	Yes	Yes	+1	+1	+1
7:2AmB	Yes	+1	Yes	Yes	Yes (pale)	+2	+2	+3
7:2:1(+)	No	+1	No	Yes	No	+2	+2	+3
7:2:1(+)AmB	Yes	transparent	Yes	Yes	Yes (pale)	+1	+1	+2
7:2:1(-)	No	+1	No	No	No	+1	+1	+1
7:2:1(-)AmB	Yes	+1	Yes	Yes	No	+2	+2	+3
Fungizone® solution.	clear yellow colour		pale yellow at at 4°C, 30°C and 45°C and increase turbidity at 45°C.					
Fungizone® powder	lyophilized powder, fluffy		intense yellow, flat at 4°C, 30°C and 45°C.					

Note : +1 to +3 indicated the degree of turbidity

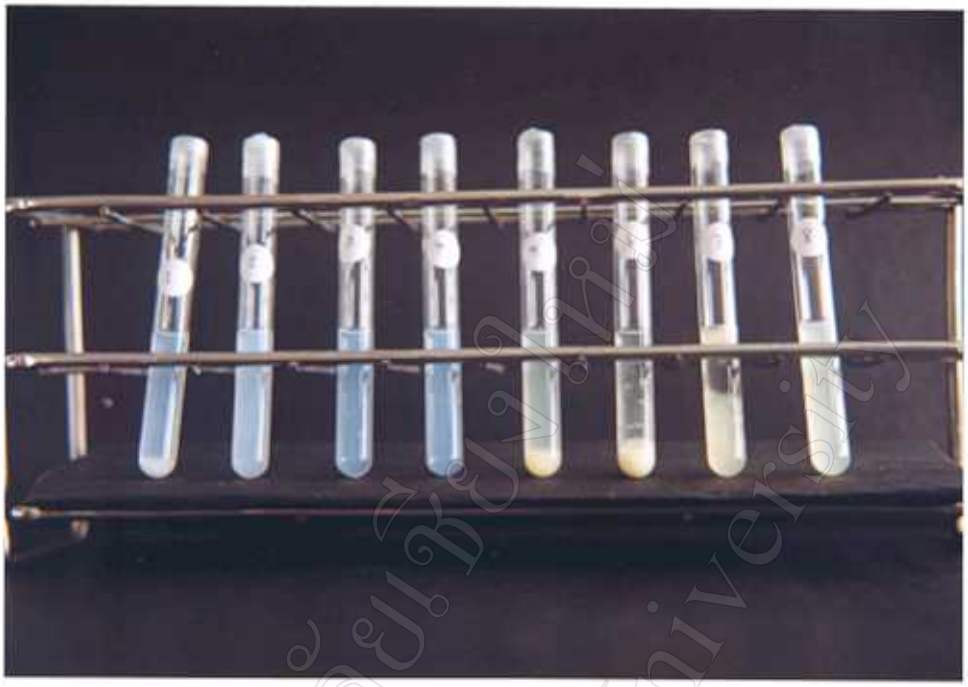
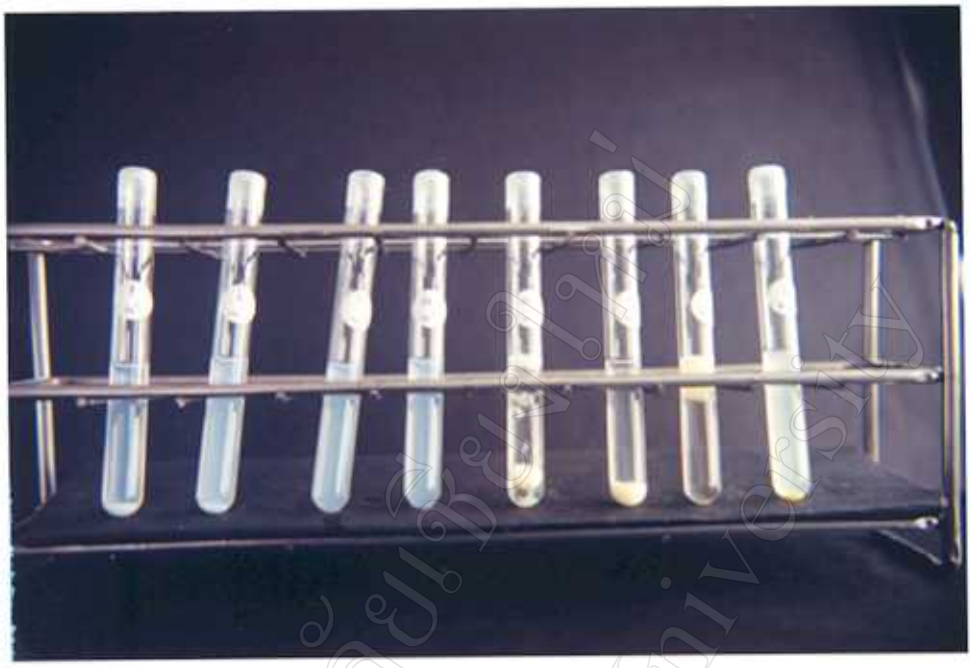
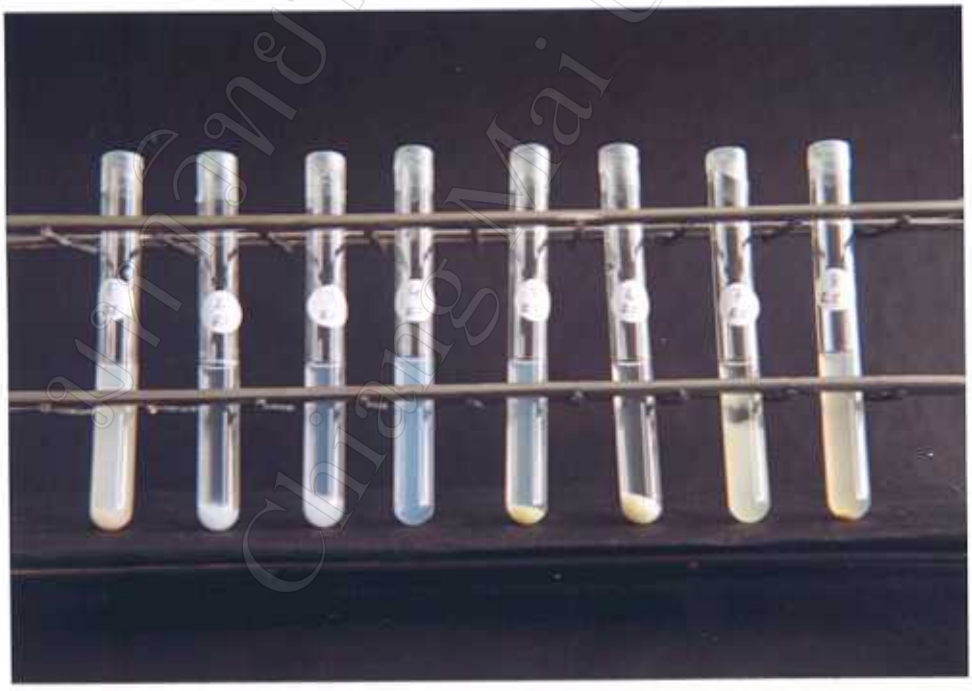


Figure 3.29 : The physical appearance of freshly prepared 1:1 (No.1), 7:2 (No.2), 7:2:1(+) (No.3), 7:2:1(-) (No.4), 1:1AmB (No.5), 7:2AmB (No.6), 7:2:1(+)AmB (No.7), 7:2:1(-)AmB (No.8)



(A)

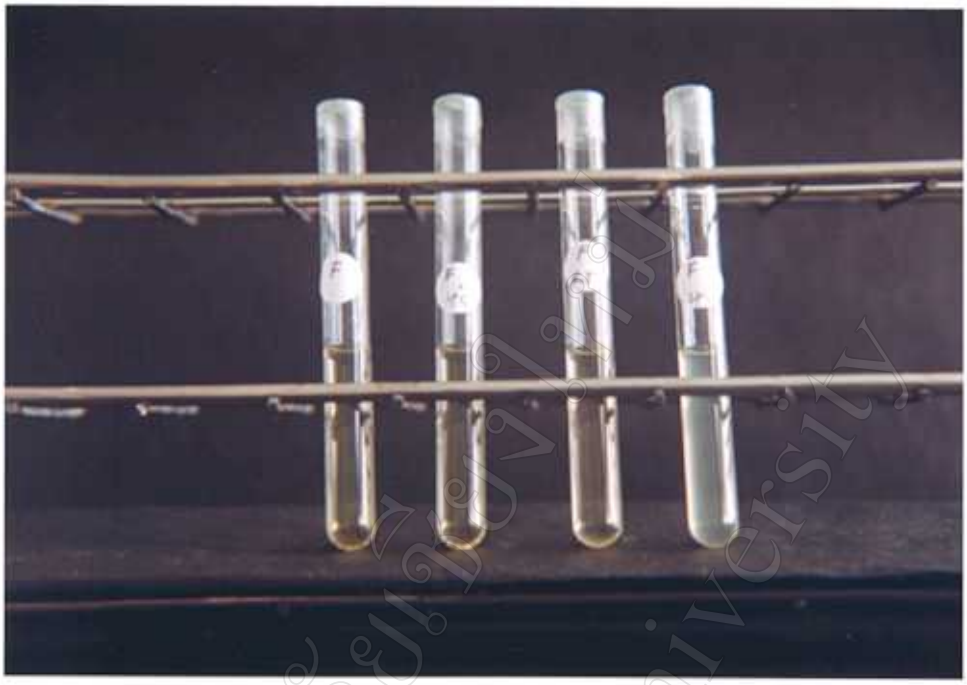


(B)

Figure 3.30 : The physical appearance of 1:1 (No.1), 7:2 (No.2), 7:2:1(+) (No.3), 7:2:1(-) (No.4), 1:1AmB (No.5), 7:2AmB (No.6), 7:2:1(+)AmB (No.7), 7:2:1(-)AmB (No.8) when stored at 4°C (A), 30°C (B) and 45°C (C) for 90 days

(C)

Figure 3.30 : The physical appearance of 1:1 (No.1), 7:2 (No.2), 7:2:1(+) (No.3), 7:2:1(-) (No.4), 1:1AmB (No.5), 7:2AmB (No.6), 7:2:1(+)AmB (No.7), 7:2:1(-)AmB (No.8) when stored at 4°C (A), 30°C (B) and 45°C (C) for 90 days (continue)



(A)

(B)

Figure 3.31 : Fungizone® solution (A) and Fungizone® powder (B) at initial and at 90 days when stored at 4°C, 30°C and 45°C (from left to right respectively)

3.4.2 Chemical stability

The remaining amounts of amphotericin B sampling at 0, 5, 20, 40 and 90 days of 1:1AmB, 7:2AmB, 7:2:1(+)AmB, 7:2:1(-)AmB liposome formulations, Fungizone® solution and Fungizone® powder were determined by HPLC and converted to percentages of amphotericin B remaining comparing to at initial as shown in Tables 3.41 to 3.46 respectively. Table 3.47 and Figure 3.32 summarized and compared the percentages of amphotericin B remaining at each temperature.

The kinetic chemical stability study of amphotericin B was interpreted and the shelf life as well as the degradation rate of amphotericin B in liposome formulations, Fungizone® solution and Fungizone® powder were calculated by substituting in the equations of zero order, first order and Higuchi model. These equations were demonstrated in Table 3.48. The r square values from these equations were determined. The Higuchi model appreciate to give the r square value higher than the other two equations. Thus, The Higuchi model seems to be the most acceptable approach for our study than the others. The slopes of each temperature from the regression with Higuchi model were substituted in the Arrhenius equation in order to predict the degradation rate of amphotericin B at the specific temperature. The degradation rate can then be converted to the periods when the contents of amphotericin B remaining 90% (shelf life, t_{90}). The degradation rates of each temperature and shelf lives were shown in Tables 3.49 to 3.56.

Table 3.41 : The remaining amounts of amphotericin B in the 1:1AmB liposome sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C

1:1AmB		4°C	30°C	45°C
0 day	Average peak areas (SD,%CV)	59.62 (1.73, 2.91)	59.62 (1.73, 2.91)	59.62 (1.73, 2.91)
	Concentration (µg/ml)	190.87	190.87	190.87
	% remaining	**100.00**	**100.00**	**100.00**
5 days	Average peak areas (SD,%CV)	59.83 (1.14, 1.90)	61.16 (1.33, 2.18)	45.03 (1.46, 3.25)
	Concentration (µg/ml)	191.45	195.21	149.68
	% remaining	**100.31**	**102.27**	**78.42**
20 days	Average peak areas (SD,%CV)	56.86 (1.49, 2.61)	58.66 (1.51, 2.57)	27.14 (0.33, 1.21)
	Concentration (µg/ml)	183.06	188.14	99.20
	% remaining	**95.91**	**98.57**	**51.97**
40 days	Average peak areas (SD,%CV)	56.57 (1.17, 2.07)	56.34 (1.23, 2.18)	19.01 (0.74, 3.87)
	Concentration (µg/ml)	182.24	181.60	76.25
	% remaining	**95.48**	**95.14**	**39.95**
90 days	Average peak areas (SD,%CV)	56.26 (1.21, 2.15)	56.56 (1.70, 3.00)	9.37 (0.27, 2.83)
	Concentration (µg/ml)	181.38	182.21	49.04
	% remaining	**95.03**	**95.46**	**25.70**

Table 3.42 : The remaining amounts of amphotericin B in the 7:2AmB liposome sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C

7:2AmB		4°C	30°C	45°C
0 day	Average peak areas (SD,%CV)	67.15 (0.16, 0.24)	67.15 (0.16, 0.24)	67.15 (0.16, 0.24)
	Concentration (μg/ml)	212.12	212.12	212.12
	% remaining	**100.00**	**100.00**	**100.00**
5 days	Average peak areas (SD,%CV)	66.32 (0.97, 1.47)	65.38 (3.73, 5.71)	59.93 (1.93, 3.22)
	Concentration (μg/ml)	209.76	207.10	191.74
	% remaining	**98.89**	**97.64**	**90.39**
20 days	Average peak areas (SD,%CV)	64.09 (0.55, 0.86)	64.21 (1.05, 1.63)	54.07 (0.73, 1.34)
	Concentration (μg/ml)	203.46	203.81	175.19
	% remaining	**95.92**	**96.08**	**82.59**
40 days	Average peak areas (SD,%CV)	59.96 (0.49, 0.81)	63.04 (0.59, 0.94)	47.06 (0.55, 1.17)
	Concentration (μg/ml)	191.81	200.52	155.42
	% remaining	**90.43**	**94.53**	**73.27**
90 days	Average peak areas (SD,%CV)	62.38 (2.63, 4.22)	61.25 (0.52, 0.85)	39.30 (0.92, 2.34)
	Concentration (μg/ml)	198.66	195.46	133.53
	% remaining	**93.66**	**92.15**	**62.95**

Table 3.43 : The remaining amounts of amphotericin B in the 7:2:1(+)AmB liposome sampling at 0, 5, 20, 40 and 90 days when 4°C, 30°C and 45°C

7:2:1(+)AmB		4°C	30°C	45°C
0 day	Average peak areas (SD,%CV)	55.63 (1.15, 2.06)	55.63 (1.15, 2.06)	55.63 (1.15, 2.06)
	Concentration (µg/ml)	179.60	179.60	179.60
	% remaining	**100.00**	**100.00**	**100.00**
5 days	Average peak areas (SD,%CV)	60.08 (1.18, 1.96)	58.06 (2.53, 4.36)	45.52 (1.13, 2.49)
	Concentration (µg/ml)	192.15	186.47	151.08
	% remaining	**107.00**	**103.82**	**84.12**
20 days	Average peak areas (SD,%CV)	56.81 (2.50, 4.39)	55.95 (0.90, 1.62)	36.41 (0.08, 0.23)
	Concentration (µg/ml)	182.93	180.51	125.37
	% remaining	**101.85**	**100.51**	**69.80**
40 days	Average peak areas (SD,%CV)	56.18 (1.78, 3.18)	56.14 (0.33, 0.59)	29.72 (0.82, 2.77)
	Concentration (µg/ml)	181.17	181.05	106.47
	% remaining	**100.87**	**100.81**	**59.28**
90 days	Average peak areas (SD,%CV)	55.14 (0.94, 1.71)	56.33 (0.93, 1.65)	17.59 (0.10, 0.54)
	Concentration (µg/ml)	178.21	181.58	72.26
	% remaining	**99.23**	**101.10**	**40.23**

Table 3.44 : The remaining amounts of amphotericin B in the 7:2:1(-)AmB liposome sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C

7:2:1(-)AmB		4°C	30°C	45°C
0 day	Average peak areas (SD,%CV)	52.63 (1.72, 3.28)	52.63 (1.72, 3.28)	52.63 (1.72, 3.28)
	Concentration (µg/ml)	171.13	171.13	171.13
	% remaining	**100.00**	**100.00**	**100.00**
5 days	Average peak areas (SD,%CV)	53.81 (1.41, 2.62)	54.70 (2.74, 5.07)	38.48 (0.78, 2.03)
	Concentration (µg/ml)	174.47	175.20	131.22
	% remaining	**101.96**	**102.38**	**76.68**
20 days	Average peak areas (SD,%CV)	52.07 (0.31, 0.60)	53.05 (0.58, 1.10)	30.32 (0.72, 2.38)
	Concentration (µg/ml)	169.57	172.32	108.18
	% remaining	**99.09**	**100.70**	**63.21**
40 days	Average peak areas (SD,%CV)	49.26 (0.55, 1.11)	49.48 (1.45, 2.93)	24.40 (0.54, 2.20)
	Concentration (µg/ml)	161.62	162.25	91.47
	% remaining	**94.45**	**94.82**	**53.45**
90 days	Average peak areas (SD,%CV)	49.62 (0.12, 0.24)	46.32 (0.67, 1.45)	17.98 (0.93, 5.16)
	Concentration (µg/ml)	162.65	153.32	73.36
	% remaining	**95.04**	**89.60**	**42.87**

Table 3.45 : The remaining amounts of amphotericin B in Fungizone® solution sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C

Fungizone® solution		4°C	30°C	45°C
0 day	Average peak areas (SD,%CV)	69.97 (1.49, 2.13)	69.97 (1.49, 2.13)	69.97 (1.49, 2.13)
	Concentration (µg/ml)	220.08	220.08	220.08
	% remaining	**100.00**	**100.00**	**100.00**
5 days	Average peak areas (SD,%CV)	64.45 (1.34, 2.08)	55.78 (4.34, 7.79)	49.88 (2.04, 4.09)
	Concentration (µg/ml)	204.49	180.03	163.37
	% remaining	**92.92**	**81.80**	**74.23**
20 days	Average peak areas (SD,%CV)	65.19 (0.65, 1.00)	47.60 (2.01, 4.23)	35.71 (0.97, 2.72)
	Concentration (µg/ml)	206.58	156.93	123.38
	% remaining	**93.86**	**71.30**	**56.06**
40 days	Average peak areas (SD,%CV)	60.98 (4.42, 7.25)	28.95 (1.57, 5.42)	37.48 (0.71, 1.89)
	Concentration (µg/ml)	194.70	104.31	128.37
	% remaining	**88.47**	**47.39**	**58.33**
90 days	Average peak areas (SD,%CV)	48.74 (4.61, 9.46)	18.61 (0.90, 4.85)	30.81 (0.59, 1.90)
	Concentration (µg/ml)	160.17	75.12	109.55
	% remaining	**72.78**	**34.13**	**49.78**

Table 3.46 : The remaining amounts of amphotericin B in Fungizone® powder sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C

Fungizone® powder		4°C	30°C	45°C
0 day	Average peak areas[a](SD,%CV)	69.97 (1.49, 2.13)	69.97 (1.49, 2.13)	69.97 (1.49, 2.13)
	Concentration (mg/mg)	0.4000	0.4000	0.4000
	% remaining	**100.00**	**100.00**	**100.00**
5 days	Average peak areas (SD,%CV)	54.89 (0.68, 1.24)	53.42 (1.63, 3.06)	53.45 (2.46, 4.60)
	Concentration (mg/mg)	0.3550	0.3467	0.3469
	% remaining	**88.75**	**86.68**	**86.73**
20 days	Average peak areas (SD,%CV)	63.50 (1.91, 3.00)	55.65 (0.92, 1.65)	54.99 (3.25, 5.91)
	Concentration (mg/mg)	0.4036	0.3593	0.3556
	% remaining	**100.90**	**89.83**	**88.90**
40 days	Average peak areas (SD,%CV)	58.93 (0.53, 0.90)	44.58 (2.40, 5.39)	49.24 (3.33, 6.77)
	Concentration (mg/mg)	0.3778	0.2968	0.3231
	% remaining	**94.46**	**74.21**	**80.78**
90 days	Average peak areas (SD,%CV)	46.53 (4.16,8.93)	43.55 (1.34,3.07)	40.05 (3.18,7.94)
	Concentration (mg/mg)	0.3079	0.2910	0.2712
	% remaining	**76.97**	**72.76**	**67.81**

Note : [a] : The values were from Fungizone® solution

Table 3.47 : Comparison of the percentages of the remaining amphotericin B in liposome formulations, Fungizone® solution and Fungizone® powder sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C

4°C

Day(s)	1:1AmB	7:2AmB	7:2:1(+) AmB	7:2:1(-) AmB	Fungizone® solution	Fungizone® powder
0	100.00	100.00	100.00	100.00	100.00	100.00
5	100.31	98.89	106.99	101.96	92.91	88.75
20	95.91	95.92	101.85	99.09	93.86	100.90
40	95.48	90.43	100.87	94.45	88.47	94.46
90	95.03	93.66	99.23	95.04	72.78	76.97

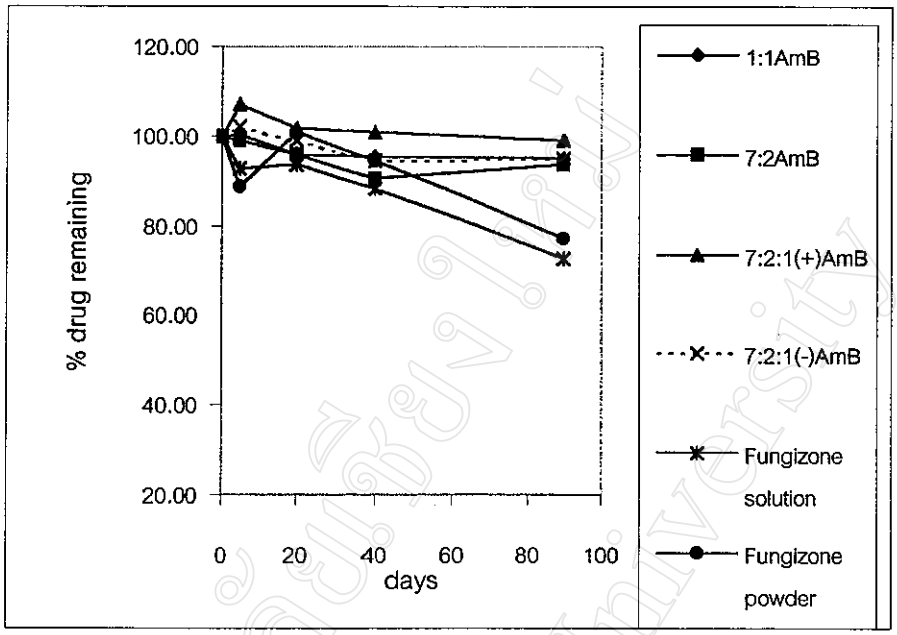
30°C

Day(s)	1:1AmB	7:2AmB	7:2:1(+) AmB	7:2:1(-) AmB	Fungizone® solution	Fungizone® powder
0	100.00	100.00	100.00	100.00	100.00	100.00
5	102.27	97.64	103.82	102.38	81.80	86.68
20	98.57	96.08	100.51	100.70	71.30	89.83
40	95.14	94.53	100.81	94.82	47.39	74.21
90	95.46	92.15	101.10	89.60	34.13	72.76

45°C

Day(s)	1:1AmB	7:2AmB	7:2:1(+) AmB	7:2:1(-) AmB	Fungizone® solution	Fungizone® powder
0	100.00	100.00	100.00	100.00	100.00	100.00
5	78.42	90.39	84.12	76.68	74.23	86.73
20	51.97	82.59	69.80	63.21	56.06	88.90
40	39.95	73.27	59.28	53.45	58.33	80.78
90	25.70	62.95	40.23	42.87	49.78	67.81

4°C



30°C

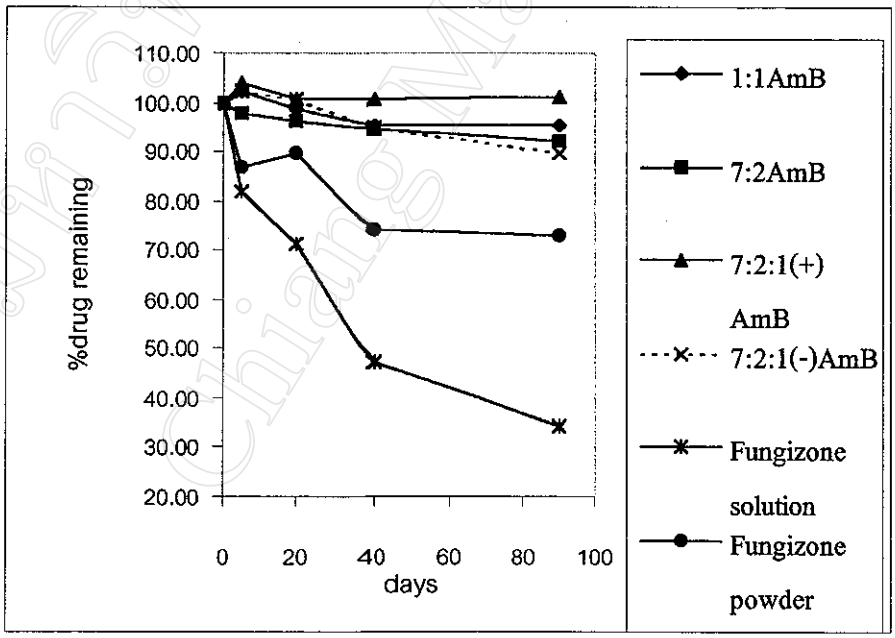


Figure 3.32 : The comparison of the percentages of the remaining amphotericin B in liposome formulations, Fungizone® solution and Fungizone® powder sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C

45°C

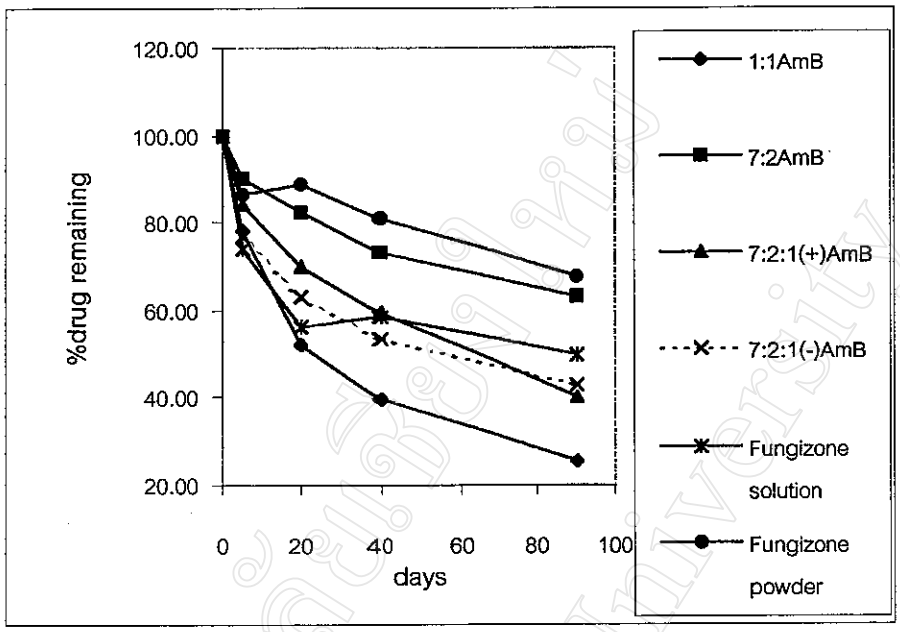


Figure 3.32 : The comparison of the percentages of the remaining amphotericin B in liposome formulations, Fungizone® solution and Fungizone® powder sampling at 0, 5, 20, 40 and 90 days when kept at 4°C, 30°C and 45°C (continue)

Table 3.48 : The equations used to calculate the degradation rate of AmB in various formulations

Types of equation	Equation	Term descriptions
Zero order	$C = C_0 + kt$	C_0 = initial concentration C = concentration at given t t = time k = rate constant
First order	$\ln C = \ln C_0 + kt$	
Higuchi model	$C = C_0 + kt^{0.5}$	
Arrhenius	$\ln k = \ln A - (Ea / RT)$	ln A = frequency factor Ea = activation energy R = gas constant T = absolute temperature
Shelf life (only for Higuchi model)	$(t_{90})^{0.5} = (C - C_0) / \text{antiln } k_s$ $t_{90} = ((t_{90})^{0.5})^2$	k_s = rate constant at the specific temperature

Table 3.49 : Calculation of the degradation rate (slope) and the shelf life of the 1:1AmB liposome formulation

1:1 AmB	Zero order			First order			Higuchi model		
	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square
4°C	0.056	99.07	0.6196	0.0006	4.60	0.6225	0.6264	100.17	0.7866
30°C	0.0667	100.36	0.6435	0.0007	4.61	0.6470	0.6828	101.36	0.6788
45°C	0.7208	81.55	0.7723	0.0141	4.41	0.9085	8.0095	95.28	0.9602
Degra-dation rate, k (% / day $^{0.5}$)	Linear regression of slope from Higuchi model ; ln k = 16.29 – 4738.45 (1/T) r^2= 0.6019 Predict at 30°C ; ln k_{303} = 16.29 – 4738.45 (1/(273+30)) = 0.65 antiln k_{303} 1.92 ; k = degradation rate = 1.92 (% / day $^{0.5}$)								
Shelf life (t_{90})	$(t_{90})^{0.5}$ = (90 – 100) / (-1.92) = 5.21 t_{90} = $(5.21)^2$ = 27.13 days								

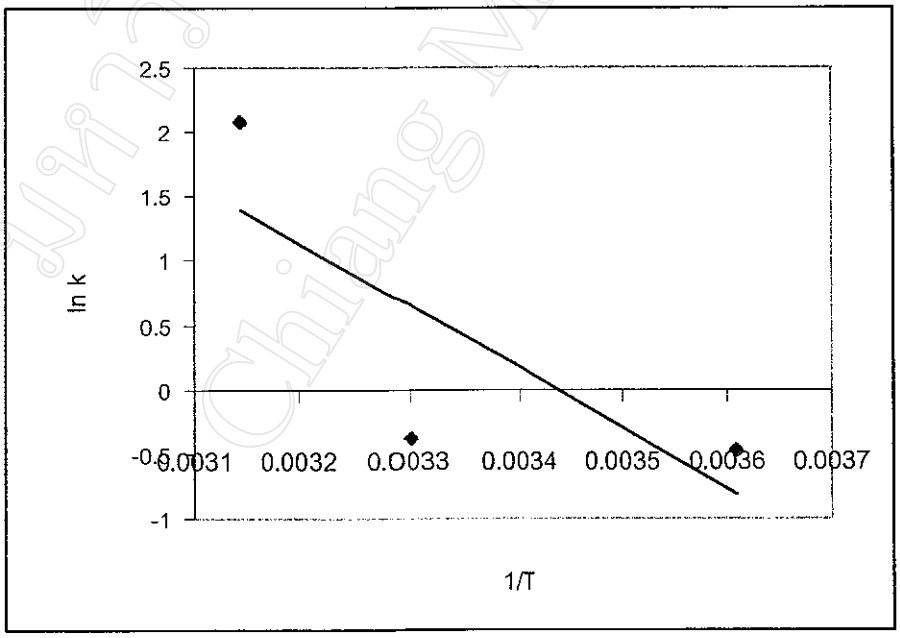


Figure 3.33 : The Arrhenius plot of the 1:1AmB liposome formulation

Table 3.50 : Calculation of the degradation rate (slope) and the shelf life of the 7:2AmB liposome formulation.

7:2 AmB	Zero order			First order			Higuchi model		
	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square
4°C	0.0727	98.03	0.4629	0.0008	4.59	0.4545	0.8656	99.68	0.6618
30°C	0.0767	98.46	0.8775	0.0008	4.59	0.8861	0.8136	99.75	0.9952
45°C	0.3732	93.41	0.8886	0.0048	4.54	0.9282	3.9373	99.57	0.9962
Degra-dation rate, k (% / day $^{0.5}$)	Linear regression of slope from Higuchi model ; ln k = 9.61 – 2764.87 (1/T) r^2= 0.5382 Predict at 30°C ; ln k_{303} = 9.61 – 2764.87 (1/(273+30)) = 0.49 antiln k_{303} 1.62 ; k = degradation rate = 1.62 (% / day $^{0.5}$)								
Shelf life (t_{90})	$(t_{90})^{0.5}$ = (90 – 100) / (-1.62) = 6.17 t_{90} = $(6.17)^2$ = 38.10 days								

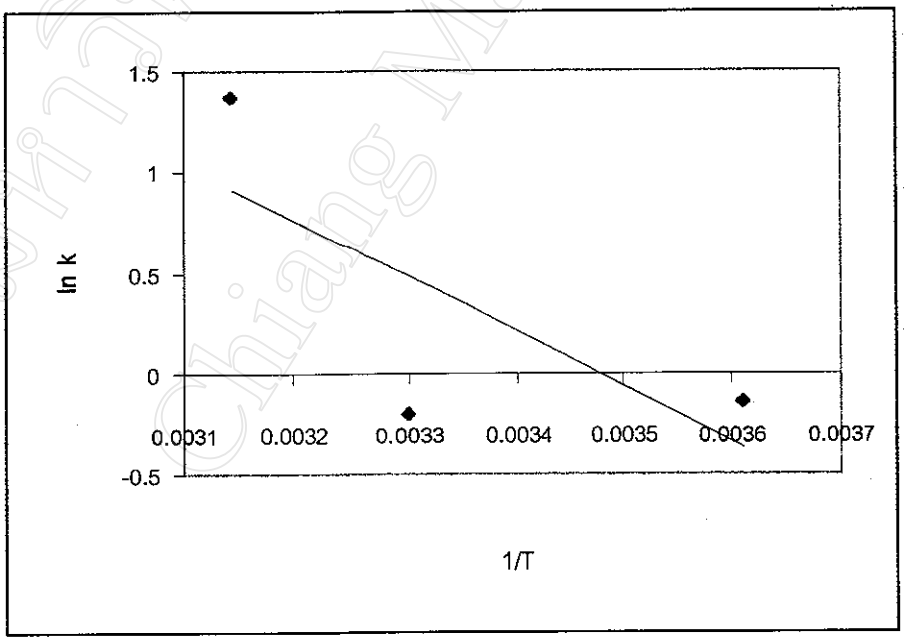


Figure 3.34 : The Arrhenius plot of the 7:2AmB liposome formulation

Table 3.51 : Calculation of the degradation rate (slope) and the shelf life of the 7:2:1(+)AmB liposome formulation

7:2:1 (+) AmB	Zero order			First order			Higuchi model		
	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square
4°C	0.0451	103.19	0.2869	0.0004	4.64	0.2909	0.3389	103.31	0.1636
30°C	0.0062	101.44	0.0226	0.0001	4.62	0.0215	0.0324	101.39	0.0063
45°C	0.5917	89.03	0.8877	0.0094	4.50	0.9607	6.2528	98.85	0.9983
Degra-dation rate, k (% / day $^{0.5}$)	Linear regression of slope from Higuchi model ; ln k = 13.52 – 4301.47 (1/T) r^2= 0.1494 Predict at 30°C ; ln k_{303} = 13.52 – 4301.47 (1/(273+30)) = -0.68 antiln k_{303} 0.51 ; k = degradation rate = 0.51 (% / day $^{0.5}$)								
Shelf life (t_{90})	$(t_{90})^{0.5}$ = (90 – 100) / (-0.51) = 19.60 t_{90} = $(19.60)^2$ = 384.5 days								

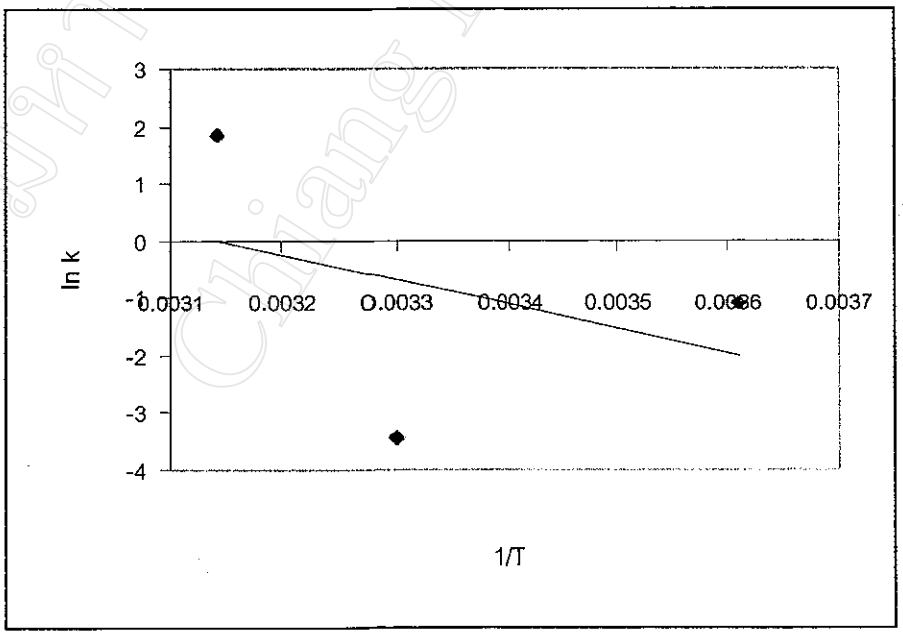


Figure 3.35 : The Arrhenius plot of the 7:2:1(+)AmB liposome formulation

Table 3.52 : Calculation of the degradation rate (slope) and the shelf life of the 7:2:1(-)AmB liposome formulation

7:2:1 (-)AmB	Zero order			First order			Higuchi model		
	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square
4°C	0.0721	100.34	0.6555	0.0007	4.61	0.6562	0.7323	101.40	0.6814
30°C	0.1372	101.75	0.9110	0.0014	4.62	0.9174	1.2447	103.10	0.7552
45°C	0.5222	83.44	0.7395	0.0082	4.42	0.8436	5.8867	93.76	0.9459
Degra-dation rate, k (% / day $^{0.5}$)	Linear regression of slope from Higuchi model ; ln k = 14.26 – 4086.11 (1/T) r^2= 0.7988 Predict at 30°C ; ln k_{303} = 14.26 – 4086.11 (1/(273+30)) = 0.77 antiln k_{303} 2.16 ; k = degradation rate = 2.16 (% / day $^{0.5}$)								
Shelf life (t_{90})	$(t_{90})^{0.5}$ = (90 – 100) / (-2.16) = 4.63 t_{90} = $(4.63)^2$ = 21.43 days								

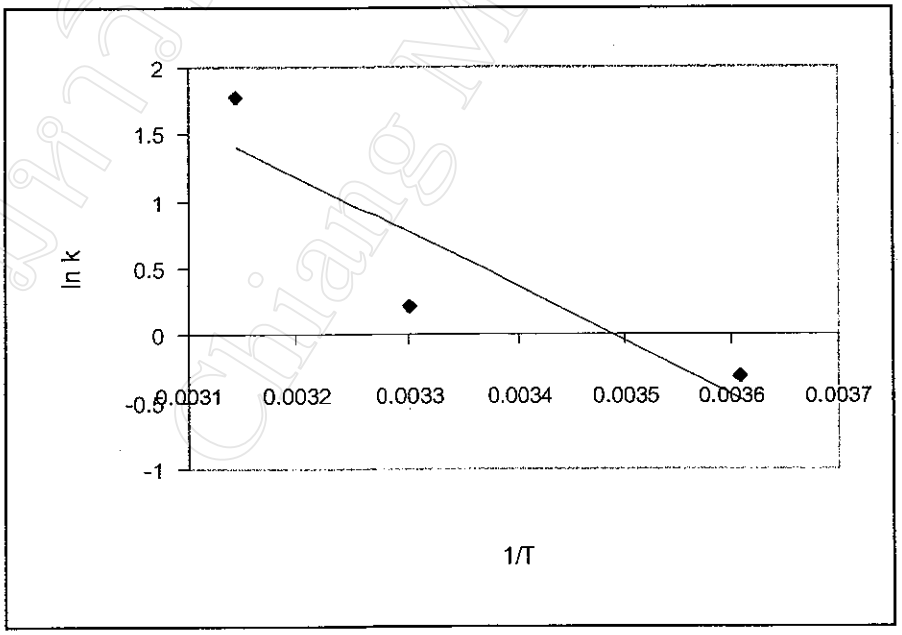


Figure 3.36 : The Arrhenius plot of the 7:2:1 (-)AmB liposome formulation

Table 3.53 : Calculation of the degradation rate (slope) and the shelf life of Fungizone® solution

Fungizone solution	Zero order			First order			Higuchi model		
	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square	Slope (-)	Inter-cept	R square
4°C	0.2741	98.10	0.9480	0.0032	4.59	0.9507	2.6201	101.41	0.8725
30°C	0.6712	87.73	0.8587	0.0115	4.49	0.9347	7.1270	99.03	0.9748
45°C	0.4107	80.41	0.5503	0.0060	4.37	0.6215	4.9702	90.07	0.8118
Degradation rate, k (% / day $^{0.5}$)	Linear regression of slope from Higuchi model ; ln k = 7.00 – 1638.38 (1/T) r^2= 0.5867 Predict at 30°C ; ln k_{303} = 7.00 – 1638.38 (1/(273+30)) = 1.59 antiln k_{303} 4.92 ; k = degradation rate = 4.92 (% / day $^{0.5}$)								
Shelf life (t_{90})	$(t_{90})^{0.5}$ = (90 – 100) / (-4.92) = 2.03 t_{90} = $(2.03)^2$ = 4.12 days								

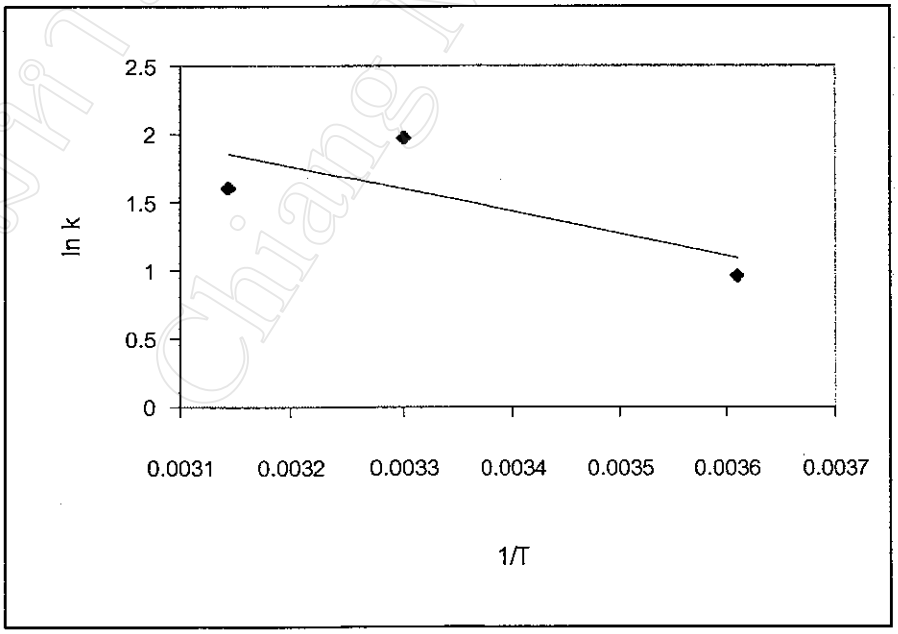


Figure 3.37 : The Arrhenius plot of Fungizone® solution

Table 3.54 : Calculation of the degradation rate (slope) and the shelf life of Fungizone® powder

Fungi -zone pow -der	Zero order			First order			Higuchi model		
	Slope (-)	Inter- cept	R square	Slope (-)	Inter- cept	R square	Slope (-)	Inter- cept	R square
4°C	0.2117	98.78	0.6184	0.0024	4.59	0.6404	1.8552	100.57	0.6915
30°C	0.2596	92.74	0.6935	0.0031	4.53	0.7164	2.8402	97.49	0.8359
45°C	0.3017	94.19	0.8703	0.0037	4.55	0.9028	3.0789	98.71	0.9128
Degra- dation rate, k (% / day $^{0.5}$)	Linear regression of slope from Higuchi model ; ln k = 4.71 – 1128.92 (1/T) r^2= 0.9658 Predict at 30°C ; ln k_{303} = 4.71 – 1128.92 (1/(273+30)) = 0.98 antiln k_{303} 2.68 ; k = degradation rate = 2.68 (% / day $^{0.5}$)								
Shelf life (t_{90})	$(t_{90})^{0.5}$ = (90 – 100) / (-2.68) = 3.73 t_{90} = $(3.73)^2$ = 13.91 days								

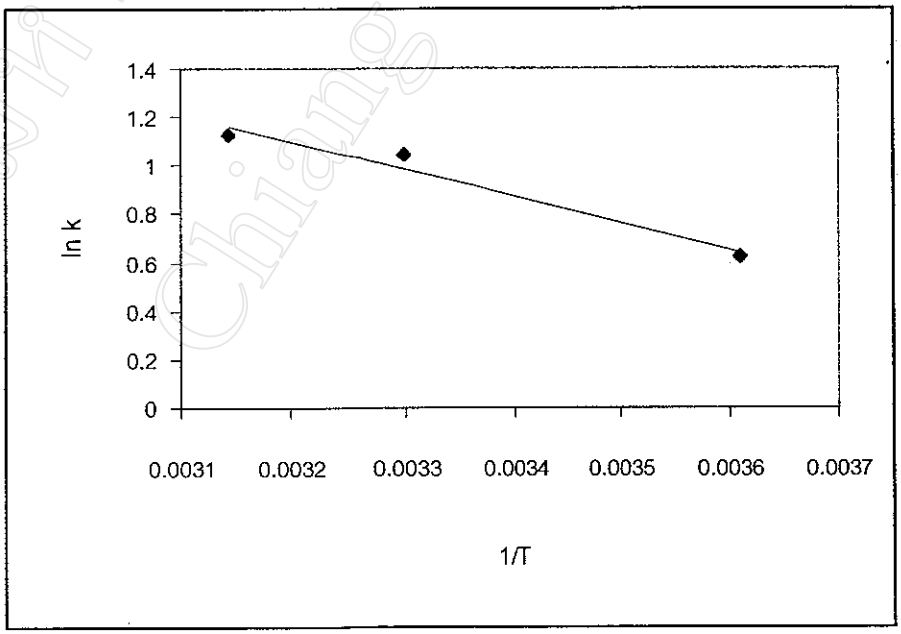


Figure 3.38 : The Arrhenius plot of Fungizone® powder

Table 3.55 : Conclusion of the degradation rates (k) of liposome formulations, Fungizone® solution and Fungizone® powder

Formulations	Degradation rates (k) of AmB stored at different temperatures (% / day $^{0.5}$)					
	From the experiment			Predicted from Arrhenius equation		
	4°C	30°C	45°C	4°C	30°C	45°C
1:1AmB	0.63	0.68	8.01	0.44	1.92	4.01
7:2AmB	0.87	0.81	3.94	0.69	1.62	2.50
7:2:1(+)AmB	0.34	0.03	6.25	0.13	0.51	0.99
7:2:1(-)AmB	0.73	1.25	5.89	0.61	2.16	4.10
Fungizone solution	2.62	7.13	4.97	2.96	4.92	6.35
Fungizone powder	1.86	2.84	3.08	1.88	2.68	3.19

Table 3.56 : Predicted shelf life of liposome formulations, Fungizone® solution and Fungizone® powder

Formulation	Predicted shelf life (days)		
	4°C	30°C	45°C
1:1AmB	511.73	27.13	6.21
7:2AmB	210.21	38.10	16.02
7:2:1(+)AmB	5,556.45	384.50	101.33
7:2:1(-)AmB	267.13	21.43	5.95
Fungizone solution	11.41	4.12	2.48
Fungizone powder	28.11	13.91	9.82

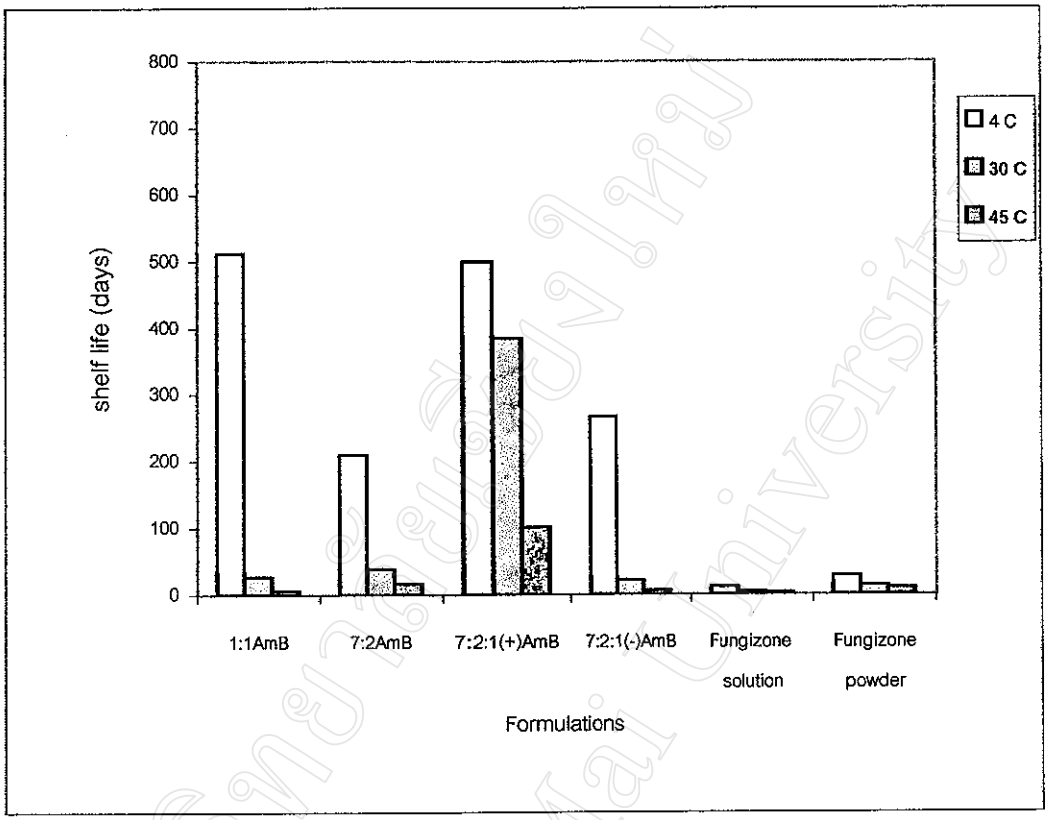


Figure 3.39 : The histograms of the predicted shelf life (days) at 4±1°C, 30±1°C and 45±1°C of liposome formulations, Fungizone®solution and Fungizone®powder

3.5 The transdermal absorption of amphotericin B liposome formulations through rat skin, by the vertical Franz diffusion cells

3.5.1 Validation of the experiment

Before the experiment, all related control samples were first validated by HPLC analysis. These samples were the 50:50 v/v of ethanol/water solution in receiver chamber, viable epidermis and dermis without amphotericin B, stratum corneum in stripped tape, unstripped tape in methanol, deionized water, phosphate buffer (pH 7.4) and 1:9 v/v of DMSO/methanol solution. The HPLC chromatogram of these samples were demonstrated in Figures 3.40 to 3.46. There was no peak between 4 to 4.6 mins where peaks of amphotericin B appeared. Thus, there were no interferences from these control samples on the assay of amphotericin B by HPLC.

The 1:1, 7:2, 7:2:1(+) and 7:2:1(-) liposomes also did not show the peak between 4 to 4.6 mins as well as those already presented in the result of the entrapment topic. The samples from transdermal absorption study were assayed for the peak areas from HPLC analysis that were converted to the amount (μg) and flux (ng/cm^2 per h) of amphotericin B of different formulations in different strata of skin as shown in Tables 3.57 to 3.59 and Figure 3.47.

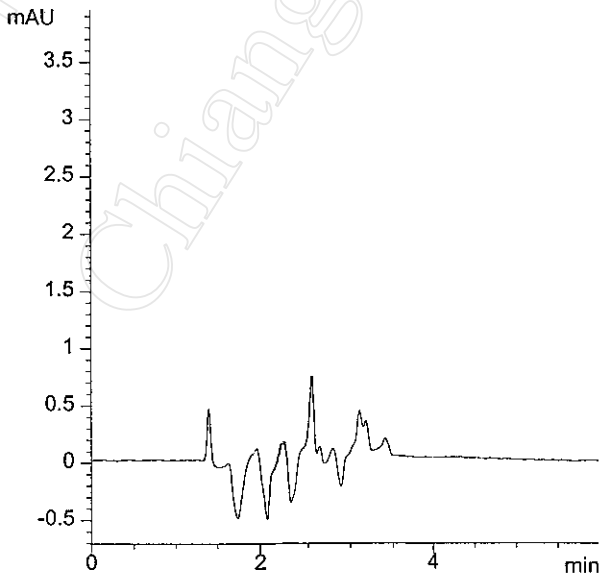


Figure 3.40 : The HPLC chromatogram of 50:50 v/v of ethanol/water solution.

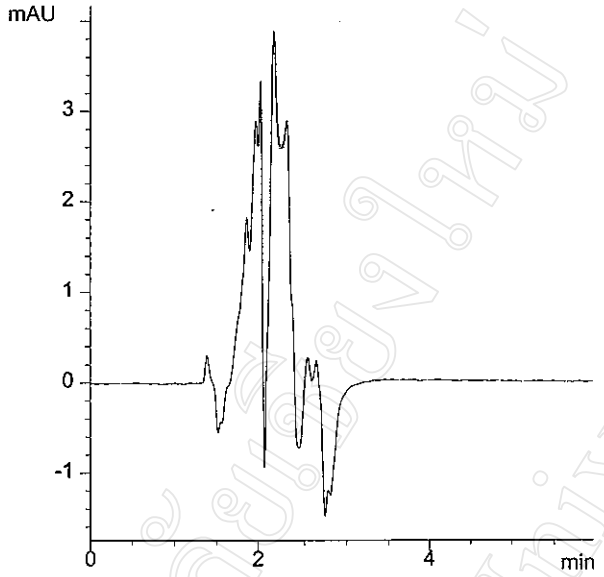


Figure 3.41 : The HPLC chromatogram of viable epidermis and dermis without amphotericin B

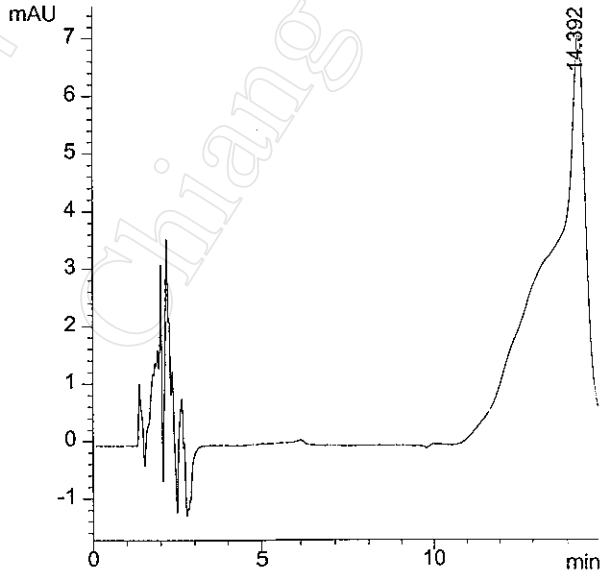


Figure 3.42 : The HPLC chromatogram of stratum corneum in the striped-tape

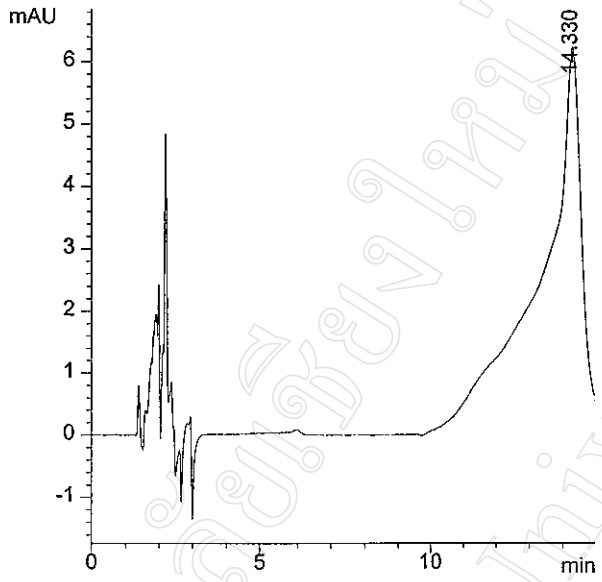


Figure 3.43 : The HPLC chromatogram of unstripped tapes extracted in methanol.

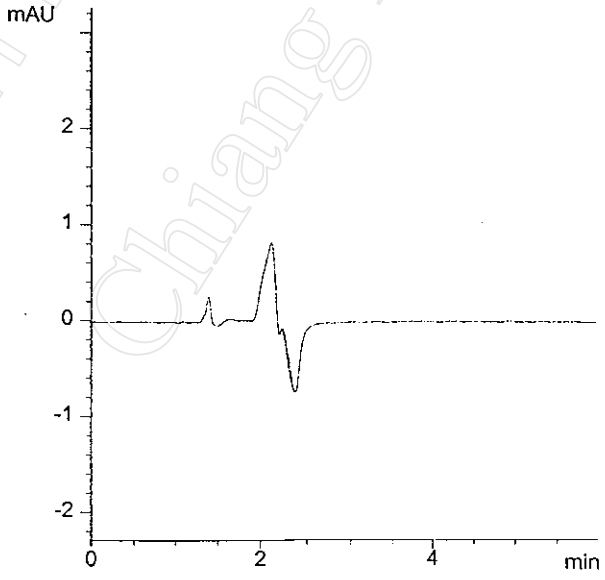


Figure 3.44 : The HPLC chromatogram of water

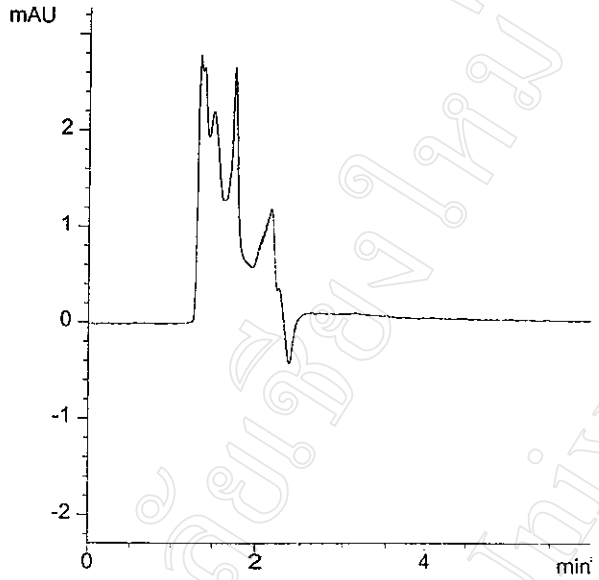


Figure 3.45 : The HPLC chromatogram of phosphate buffer pH 7.4

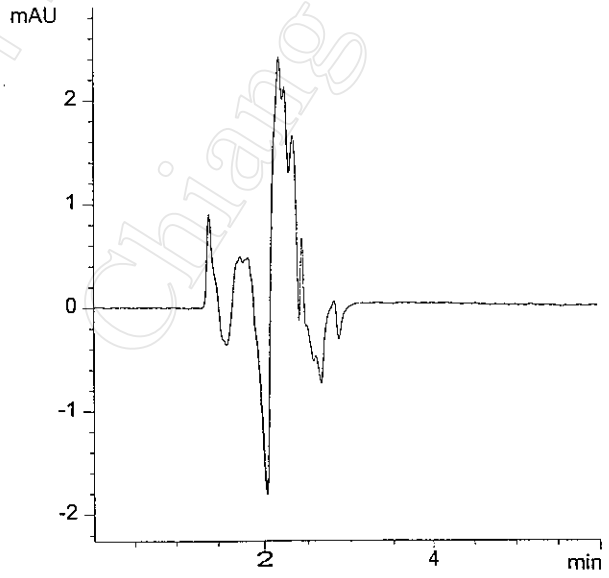


Figure 3.46 : The HPLC chromatogram of 1:9 v/v of DMSO/methanol solution

Table 3.57 : The peak areas from transdermal absorption study of amphotericin B in various formulations

Formulations		Peak areas of amphotericin B at initial (in 2 ml of the sample)	Peak areas of amphotericin B at 24 hours			
			Stratum corneum	Viable epidermis and dermis	Receiver medium	Donor
Fungizone® Solution	1	66.47 ± 0.16[a]	70.70 ±0.09[b]	65.87±0.52[b]	0	94.71±0.06[e]
	2	65.21 ± 0.39[a]	62.32±0.28[b]	71.41±0.19[b]	0	99.98±0.19[e]
AmB in DMSO / methanol solution	1	52.61 ± 0.09[a]	36.32±0.32[b]	13.96±1.32[b]	2.78±0.13[d]	84.63±0.29[e]
	2	49.69 ± 0.28[a]	35.90±0.12[b]	34.65±0.28[b]	2.55±0.15[d]	79.22±0.35[e]
AmB in Phosphate buffer pH 7.4	1	9.27 ± 0.08[a]	6.76±0.18[c]	0	0	55.83±3.62[f]
	2	9.37 ± 0.19[a]	20.15±0.32[c]	0	0	52.31±0.25[f]
1:1 AmB	1	64.32 ± 0.68[a]	19.24±0.94[c]	8.63±0.14[c]	0	85.98±0.74[e]
	2	67.29 ± 0.78[a]	22.30±0.41[c]	7.60±0.23[c]	0	89.34±0.57[e]
7:2 AmB	1	69.31 ± 1.30[a]	20.09±0.23[c]	4.60±0.28[c]	0	93.35±0.38[e]
	2	66.91 ± 0.82[a]	12.96±0.16[c]	5.94±0.26[c]	0	95.57±0.36[e]
7:2:1 (+)AmB	1	72.53 ± 0.35[a]	462.82±0.9[c]	71.51±1.55[c]	0	96.44±1.16[e]
	2	58.32 ± 0.37[a]	14.27±0.01[b]	43.29±0.38[c]	0	92.34±0.38[e]
7:2:1 (-)AmB	1	58.41 ± 0.43[a]	256.32±3.9[c]	118.61±0.2[c]	0	80.93±0.32[e]
	2	59.50 ± 0.51[a]	202.82±0.9[c]	140.12±1.4[c]	0	80.50±0.76[e]

Note : The dilution factor of a, b, c, d, e and f were 1000, 50, 5, 12, 1250, 25 respectively.

Table 3.58 : The amounts (μg) of amphotericin B in various formulations in different strata of the skin at 37±1°C for 24 hrs

Formulations		Amount of amphotericin B at initial (in 2 ml of the sample) (μg)	Amount of amphotericin B, (μg) at 24 hours.				
			Stratum corneum	Viable epidermi -s and dermis	Receiver medium	Donor	Sum of amount at 24 hours
Fungizone® solution	Mean	416.83	5.26	5.41	0	371.65	382.31
	SD	4.99	0.42	0.28	0	13.15	13.01
	%CV	1.20	7.96	5.11	0	3.54	3.40
AmB in DMSO / methanol solution	Mean	333.92	3.11	2.28	0.18	317.27	322.85
	SD	11.62	0.02	1.03	0.003	13.49	12.48
	%CV	3.48	0.67	45.29	1.53	4.25	3.87
AmB in Phosphate buffer pH 7.4	Mean	9.76	0.15	0	0	4.38	4.53
	SD	0.01	0.07	0	0	0.18	0.10
	%CV	0.09	44.13	0	0	4.00	2.30
1:1 AmB	Mean	416.68	0.20	0.11	0	337.49	337.81
	SD	11.87	0.02	0.01	0	8.38	8.39
	%CV	2.85	7.54	4.51	0	2.48	2.48
7:2 AmB	Mean	429.65	0.17	0.09	0	361.48	361.75
	SD	9.56	0.04	0.01	0	5.52	5.49
	%CV	2.23	20.55	7.14	0	1.53	1.52
7:2:1 (+) AmB	Mean	414.50	2.45	0.46	0	361.22	364.13
	SD	56.70	1.24	0.14	0	10.22	11.60
	%CV	13.68	50.56	30.51	0	2.83	3.19
7:2:1 (-) AmB	Mean	377.97	1.68	0.97	0	313.00	315.65
	SD	4.38	0.27	0.11	0	1.07	1.23
	%CV	1.16	15.92	11.07	0	0.34	0.39

Table 3.59 : The flux (ng/cm^{2}per h) of amphotericin B of various formulation in different strata of the rat skin at 37±1°C for 24 hrs

Formulations		Flux (ng/cm^{2}per h) at 24 hours		
		Stratum corneum	Viable epidermis and dermis	Receiver medium
Fungizone® solution	Mean	123.77	127.31	0
	SD	9.85	6.51	0
	%CV	7.96	5.11	0
AmB in DMSO / methanol solution	Mean	73.28	53.67	4.26
	SD	0.49	24.31	0.07
	%CV	0.67	45.29	1.53
AmB in Phosphate buffer pH 7.4	Mean	3.57	0	0
	SD	1.57	0	0
	%CV	44.13	0	0
1:1 AmB	Mean	4.78	2.67	0
	SD	0.36	0.12	0
	%CV	7.54	4.51	0
7:2 AmB	Mean	4.07	2.21	0
	SD	0.84	0.16	0
	%CV	20.55	7.14	0
7:2:1 (+) AmB	Mean	57.6	10.86	0
	SD	29.13	3.32	0
	%CV	50.56	30.51	0
7:2:1 (-) AmB	Mean	39.46	22.82	0
	SD	6.28	2.53	0
	%CV	15.92	11.07	0

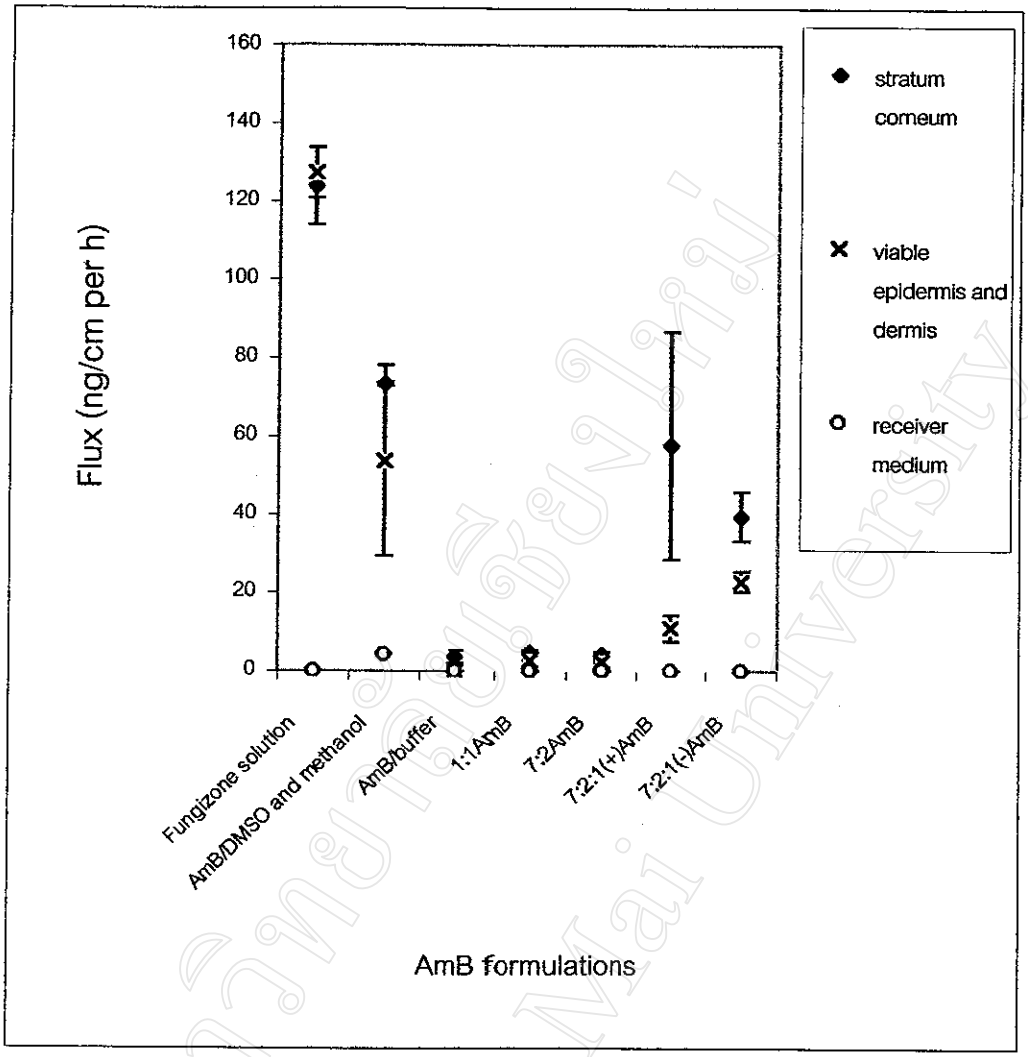


Figure 3.47 : The flux of amphotericin B in various formulations in different strata of the rat skin