CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of *P. emblica* extract spray dried powder

Fifty kilograms of fresh fruit of *P. emblica* were washed, dried and commuted by a grinder. Then, juice was spray dried under the conditions of flow rate, inlet temperature and outlet temperature that yielded the bright yellow powder of *P. emblica* extract [Appendix II]. The amount of spray dried powder was 642 g. Percent yield of extract spray dried powder was 1.28%. The pH and moisture content of 1%w/v of extract spray dried powder was 2.58 and was 9.83%, respectively.

2. Quantitative analysis of total phenolic compounds in *P. emblica* extract by UV-VIS spectrophotometry

2.1 Folin-Ciocalteu reaction

Gallic acid and *P. emblica* extract were dissolved in water and reacted with Folin-Ciocalteu's reagent and sodium carbonate solution to get blue color. The solutions were scanned to get the maximum wavelength. The results show that the maximum absorbance was 747 nm. Then the experiment used this absorbance to measure the total phenolic compounds.

2.2 The calibration curve of gallic acid using UV-VIS spectrophotometry

The standard stock solution of gallic acid was prepared to make the concentration of 5, 10, 20, 40, 60 and 80 μg/mL. Distilled water was used as a blank.

One mL of each solution was reacted with 5 mL Folin-Ciocalteu's reagent and 4 mL sodium carbonate solution. The solution was incubated at room temperature, protected from light, for 2 h. The resultant solution was determined by UV-VIS spectrophotometer at 747 nm. The calibration curve was plotted between concentration of gallic acid and absorbance as shown in Figure 8; the data were shown in Appendix III-1.

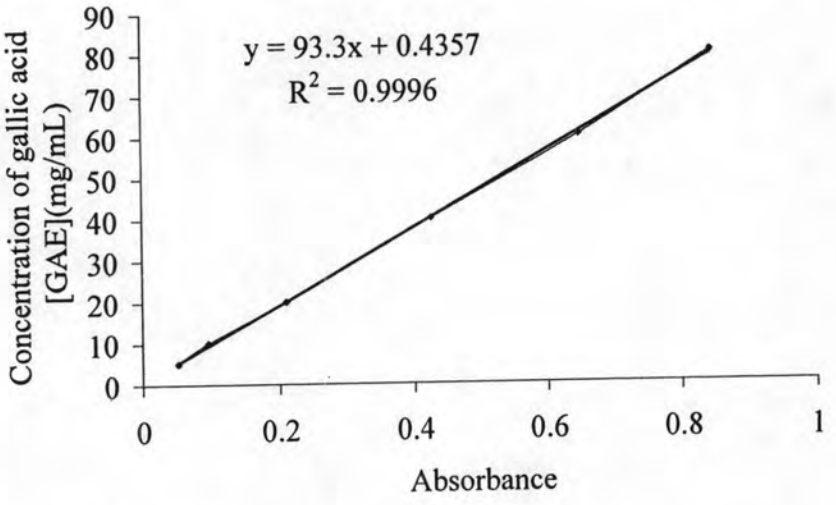

Figure 8 Calibration curve of gallic acid

2.3 The calibration curve of *P. emblica* extract in buffer solution pH 5.5

Five concentrations of *P. emblica* extract in buffer solution pH 5.5 were prepared and the absorbances were determined at 747 nm by UV-VIS spectrophotometer. The total phenolic compounds were calculated using absorbance [Appendix III-2] and GAE were calculated using calibration curve of gallic acid [Appendix III-3]. The calibration curve was plotted between gallic acid equivalent (GAE) and concentration of *P. emblica* extract as shown in Figure 9.

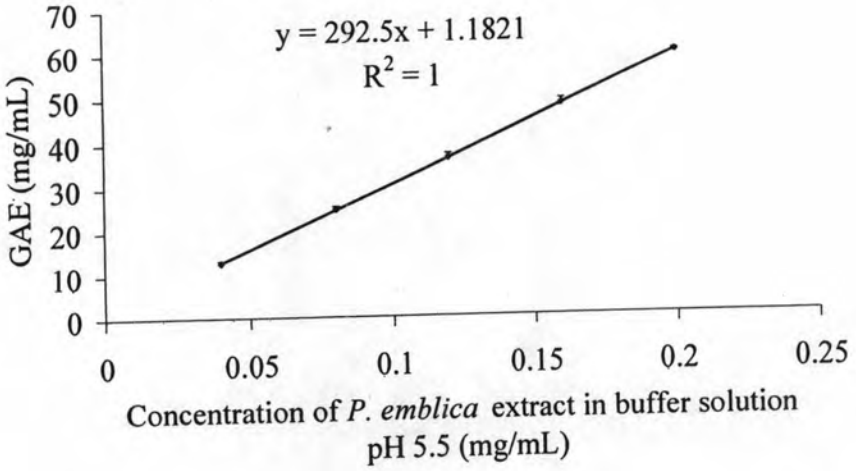

Figure 9 Calibration curve of *P. emblica* extract in buffer solution pH 5.5

2.4 The calibration curve of *P. emblica* extract in buffer solution pH 7.4

Five concentrations of *P. emblica* extract in buffer solution pH 7.4 were prepared and the absorbances were determined at 747 nm by UV-VIS spectrophotometer [Appendix III-4]. The total phenolic compounds were calculated [Appendix III-5] and the calibration curve was plotted between gallic acid equivalent (GAE) and concentration of *P. emblica* extract as shown in Figure 10.

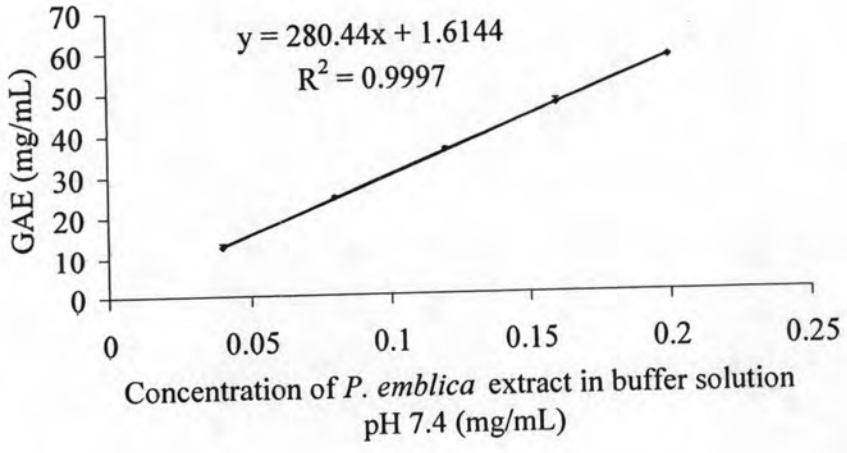

Figure 10 Calibration curve of *P. emblica* extract in buffer solution pH 7.4

The calibration curve of *P. emblica* extract in buffer solution pH 5.5 (Figure 9) and pH 7.4 (Figure 10) were used to calculate the amount of total phenolic compounds. Total phenolic compounds represented as GAE were 320 and 308 mg/g of *P. emblica* extract in buffer solution pH 5.5 and pH 7.4, respectively.

3. Preparation of *P. emblica* extract in liposomes

In this investigation phosphatidylcholine (PC) from egg yolk was selected to prepared liposomes because the transition temperature (T_c) was low (-15°C to -6°C), in which the lipid membrane can pass from tightly ordered gel to liquid crystal phase easily. Cholesterol was put together to strengthen the lipid membrane for increasing the stability of liposomes by insertion its hydroxyl group toward aqueous surface phosphate group and aliphatic chain aligned parallel to the acyl chain of PC. The stable membrane was produced by using PC: Cholesterol at 2:1 molar ratios (Brisaert, 2001). The PC (540 mg) and cholesterol (168 mg) were dissolved in ethanol to obtain clear solution and ethanol was evaporated out using rotary evaporation. The temperature, time and rotation speed were adjusted to get a good film forming. The water bath temperature at 40°C was suitable in this liposome preparation. For good film forming, the pressure was reduced to 100 mbar. The concentration of *P. emblica* extract was 1 mg/mL dissolved in phosphate-citrate buffer solution pH 5.5 (Nimmannit, 2550) which is the stable pH of *P. emblica* extract. Cholesterol ester was added to help vesicle forming and to increase stability of liposomes. The appearance of *P. emblica* extract in liposomes exhibited good feature with spherical vesicles. The concentration of *P. emblica* extract in preparation was further increased to 2, 3, 4 and 5 mg/mL. In addition, the preparations using PBS pH 7.4 and *P. emblica* extracts at the concentrations of 1, 2, 3, 4 and 5 mg/mL also performed a good appearance.

4. Determination of *P. emblica* extract encapsulation efficiency

P. emblica extract in liposomes pH 5.5 and 7.4 were ultracentrifuged at 65,000 rpm for 1h. Then the upper part or supernatant and the precipitant were obtained. Interestingly, when the liposomal suspension was ultracentrifuged at 36,000 rpm for 1 h the supernatant was colorless as same as ultracentrifuge at 65,000 rpm for 1 h but percent recovery was very low. Then the ultracentrifuge of 65,000 rpm could increase percent recovery but the ultracentrifugation of these liposomes was not completely effective to precipitant was turbid and before it was separated before measured the absorbance that the data show the percent recovery between 44.22-89.76%. The encapsulation efficiency of *P. emblica* extract in liposomes pH 5.5 and 7.4 were calculated from the amount of *P. emblica* extract in supernatant and precipitant. The experiment was set by varying the amount of *P. emblica* extract in liposomes as 1, 2, 3, 4 and 5 mg/mL at pH 5.5 and 7.4.

The supernatant was clear and fully to react with Folin-Ciocalteu's reagent and sodium carbonate solution to get blue color for determining the total phenolic compounds [Appendix IV-1 and IV-5]. The data was calculated to GAE by calibration curve of gallic acid [Appendix IV-2 and IV-6]. The precipitant was broken down by methanol and sonicated when the precipitant was reacted with Folin-Ciocalteu's reagent and sodium carbonate solution the solution was turbid so before determining total phenolic compounds by UV-VIS spectrophotometer the solution was centrifuge 3800 rpm for 5 min to separate the precipitated membrane from the solution the data were shown in Appendix VIII-3 and VIII-7. The absorbances were calculated to GAE by calibration curve of gallic acid to obtain the data of GAE [Appendix IV-4 and IV-8]

The encapsulation efficiency was expressed as the mean percentage of encapsulated *P. emblica* extract in liposomes pH 5.5 and 7.4 as show in Table 2 and 3 [Appendix IV-9 and IV-11]. Mean % recovery was shown in Table 3 [Appendix IV-10 and IV-12].

Table 2 Encapsulation efficiency of *P. emblica* in liposomes pH 5.5

P. emblica extract in liposoms	% Encapsulation efficiency	% Recovery
1 mg/mL pH 5.5	52.83	77.75
2 mg/mL pH 5.5	40.84	72.33
3 mg/mL pH 5.5	45.47	77.65
4 mg/mL pH 5.5	39.08	77.58
5mg/mL pH 5.5	43.70	66.38

Table 3 Encapsulation efficiency of *P. emblica* in liposomes pH 7.4

P. emblica extract in liposoms	% Encaosulation efficiency	% Recovery
1 mg/mL pH 7.4	35.41	89.74
2 mg/mL pH 7.4	36.43	87.51
3 mg/mL pH 7.4	31.67	81.27
4 mg/mL pH 7.4	33.38	89.76
5mg/mL pH 7.4	22.08	44.22

P. emblica extract in liposomes pH 5.5 at the concentration of 1 mg/ml got 52.83% that was the highest percent encapsulation in this buffer solution with percent recovery of 77.75 (Table 2) and *P. emblica* in liposomes pH 7.4 at the concentration of 2 mg/ml got 36.43% that was the highest percent encapsulation in this buffer solution pH 7.4 with percent recovery of 89.74 (Table3).

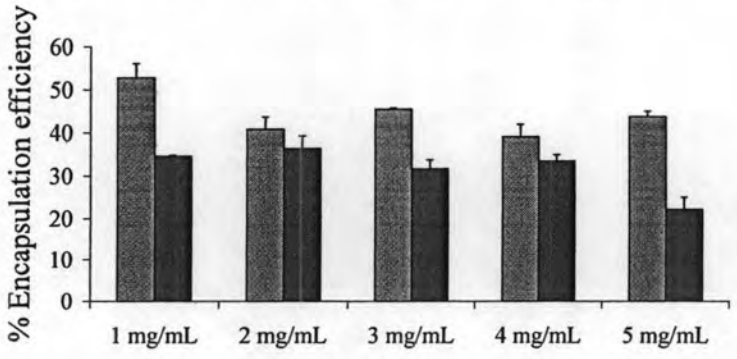

Figure 11 % Encapsulation efficiency of *P. emblica* extract in liposomes pH 5.5 ■ Liposome pH 5.5 and ■ Liposome pH 7.4

Figure 11 shwed % encapsulation efficiency of *P. emblica* extract in liposomes pH 5.5 and 7.4 and concentration. Percent encapsulation efficiency of *P. emblica* extract in liposomes pH 5.5 was higher than percent encapsulation efficiency of *P. emblica* extract pH 7.4 suggesting that pH 5.5 buffer solution was an optimal buffer solution for preparing *P. emblica* extract in liposomes.

5. Physical characterization of *P. emblica* extract in liposomes

5.1 Particle size and size distribution determination

The particle size and size distribution of *P. emblica* extract in liposomes were measured by Mastersizer, which was the most convenient method for particle size analysis. The particle size was described by the volume-weighted mean diameter. Before the particle size determination, optimal reflective index was needed to identify using fit curve method. For *P. emblica* extract in liposomes, the suitable reflective index was 1.52.

Curve of particle size distribution and mean particle size of freshly prepared *P. emblica* extract in liposomes pH 5.5 and 7.4 showed the symmetry particle size distribution as shown in Figure 12 and Figure 13, respectively.

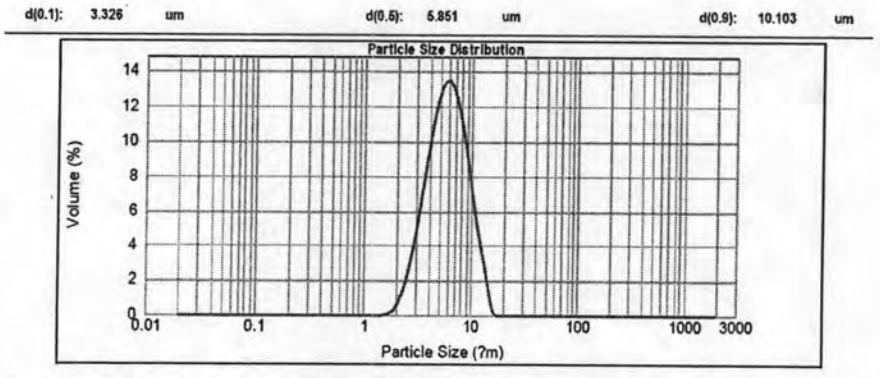

Figure 12 Particle size distribution of *P. emblica* extract in liposomes pH 5.5 Freshly prepared

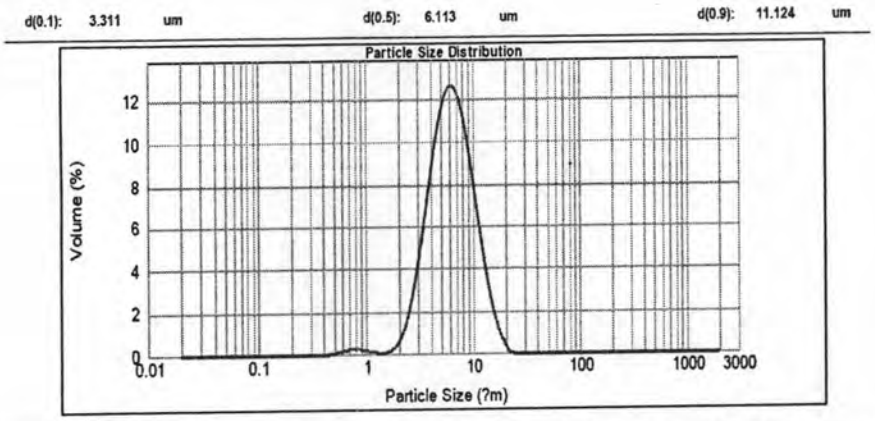

Figure 13 Particle size distribution of *P. emblica* extract in liposomes pH 7.4 freshly prepared

The mean particle size of *P. emblica* extract in liposomes pH 5.5 and 7.4 were in the range of 5.790 $\pm$ 0.756 μm and 6.188 $\pm$ 0.714 μm, respectively. The mean particle size of *P. emblica* extract in liposomes pH 5.5 and *P. emblica* extract in liposomes pH 7.4 were insignificantly different ($p<0.05$). The polydispersity of particles size were expressed by the span as shown in Table 2.

The span value of *P. emblica* extract in liposomes in buffer pH 5.5 and in buffer pH 7.4 were approximately in the range of 1.152 $\pm$ 0.114 and 1.283 $\pm$ 0.208, respectively.

Table 4 Mean particle size and span of *P. emblica* extract in liposomes in buffer pH 5.5 and buffer pH 7.4

Replication	pH 5.5		p H 7.4	
	Mean Particle size(μm)	**Span**	**Mean Particle size(μm)**	**Span**
1	5.189	1.184	5.651	1.292
2	6.26	1.138	6.385	1.233
3	6.546	1.137	6.832	1.209
4	5.975	1.185	6.276	1.263
5	5.132	1.186	5.578	1.371
6	5.851	1.158	6.113	1.278
7	6.308	1.038	6.610	1.202
8	5.764	1.144	6.100	1.293
9	5.084	1.200	5.521	1.410
Mean	5.790 ± 0.756	1.152±0.114	6.188 ± 0.714	1.283±0.208

5.2 Transmission electron microscope (TEM)

The highest % encapsulation of *P. emblica* extract in liposomes in buffer solution pH5.5 and 7.4 were selected to study the morphology by TEM. The negative staining of liposomes was performed properly, multilamellar of liposomes embedded in negative stain was spread across the grid. TEM can estimate some of sample in liposome.

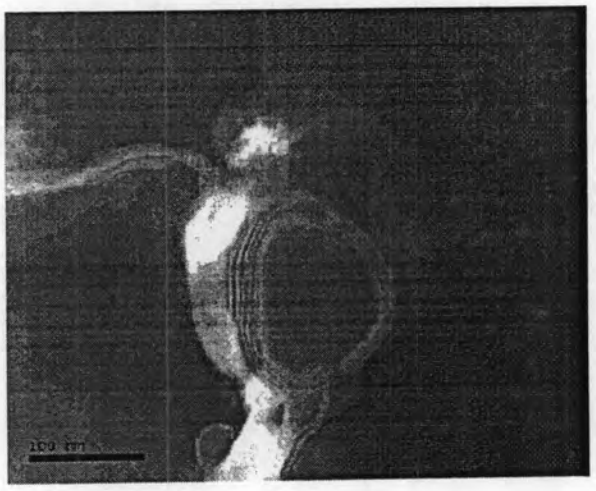

Figure 14 Transmission electron micrographs of *P. emblica* in liposomes pH 5.5 that present of multilamellar vesicle.

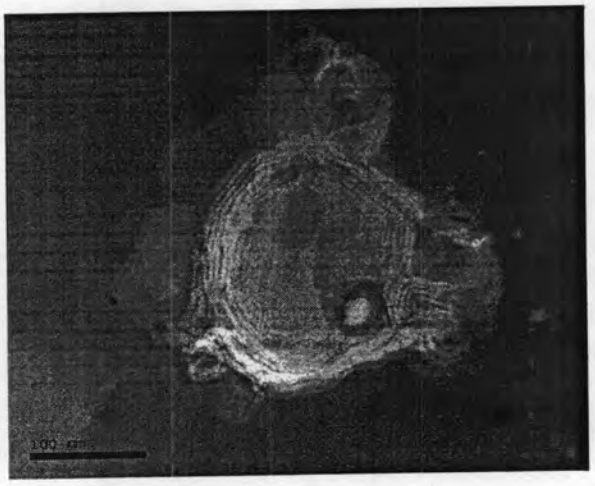

Figure 15 Transmission electron micrographs of *P. emblica* in liposomes pH 7.4 that present of multilamellar vesicle.

The TEM image of the *P. emblica* extract in liposomes pH 5.5 and 7.4 were illustrated in Figure 14 and 15 respectively. It was found that *P. emblica* extract in liposomes pH 5.5 and 7.4 are multilamellar vesicle, which showed approximately 200-300 nm in diameter (Figure 14) and 300-400 nm in diameter (Figure 15), respectively. All of TEM micrographs confirm the present of multilamellar vesicle of *P. emblica* extract in liposomes both pH 5.5 and 7.4. The particle size were

considerably smaller when estimated with TEM than when measured by Mastersizer. TEM images show particle size between 200-400 nm, whereas Mastersizer indicated that the smallest population has average mean diameter at least 5-6 μm. This apparent discrepancy can be explained by the aggregation of *P. emblica* in liposomes in range of nanoparticles. Then in Mastersizer could analyze the particle size greater than in TEM.

6. Stability of *P. emblica* extract in buffer solution

The stability of P. emblica extract in buffer solution pH 5.5 and 7.4 were investigated for the total phenolic compound using UV-VIS spectrophotometer and represented as GAE, GAE was calculated from the standard curve of gallic acid (Figure 8) using the following equation ; $y = 93 x + 0.04357$ (y = GAE and x = absorbance). The concentration of *P. emblica* extract were varied at 1, 2, 3, 4 and 5 mg/mL.

% Remaining of P. emblica extract in liposomes pH 5.5 and 7.4 at 12 weeks of storage were calculated from the following equation ;

$$\% \text{ Remaining} = \frac{\text{The amount of } P. \textit{ emblica} \text{ extract in buffer solution at 12 weeks} \times 100}{\text{The amount of } P. \textit{ emblica} \text{ extract in suspensions at time 0}}$$

The stability of *P. emblica* extract in buffer solution pH 5.5 was investigated upon storage in refrigerator ($4 \pm 1^{\circ}C$) and room temperature ($30 \pm 1^{\circ}C$) for 0, 1, 2, 4, 8 and 12 weeks. GAE data obtained from buffer solutions at $4 \pm 1^{\circ}C$ and $30 \pm 1^{\circ}C$, as shown in Table 5 and 6, respectively. The GAE values were plotted against time (Figure 16 and Figure 18). % remaining for all formulations observed at 12 weeks showed in Figure 17 and Figure 19.

Table 5 The amount of total phenolic compounds remaining calculated as GAE of *P. emblica* extract in buffer solution pH 5.5 in refrigerator ($4 \pm 1^{\circ}C$) at 0, 1, 2, 4, 8 and 12 weeks

Concentration (mg/ml)	GAE (μg/ml)					
	Time 0 week	Time1 week	Time 2 weeks	Time 4 weeks	Time 8 weeks	Time12 weeks
1	12.75	12.84	12.72	12.66	7.34	5.26
2	24.69	24.76	24.51	24.10	14.84	11.69
3	36.36	36.23	36.45	35.73	23.23	18.78
4	48.02	48.27	48.55	47.86	34.61	23.95
5	59.59	58.22	55.36	52.81	40.12	33.74

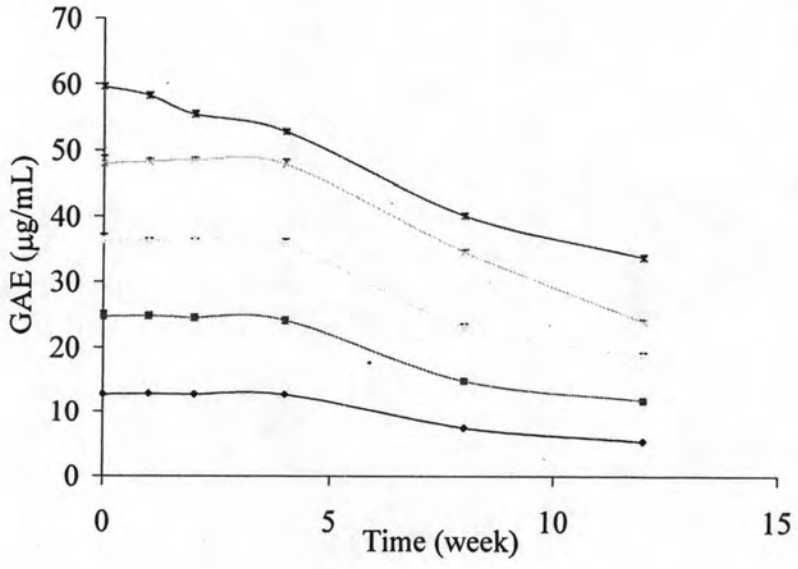

Figure 16 The GAE of *P. emblica* extract in buffer solution pH 5.5 stored in refrigerator ($4 \pm 1^{\circ}C$) at 0, 1, 2, 4, 8 and12 weeks (◆ = 1mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

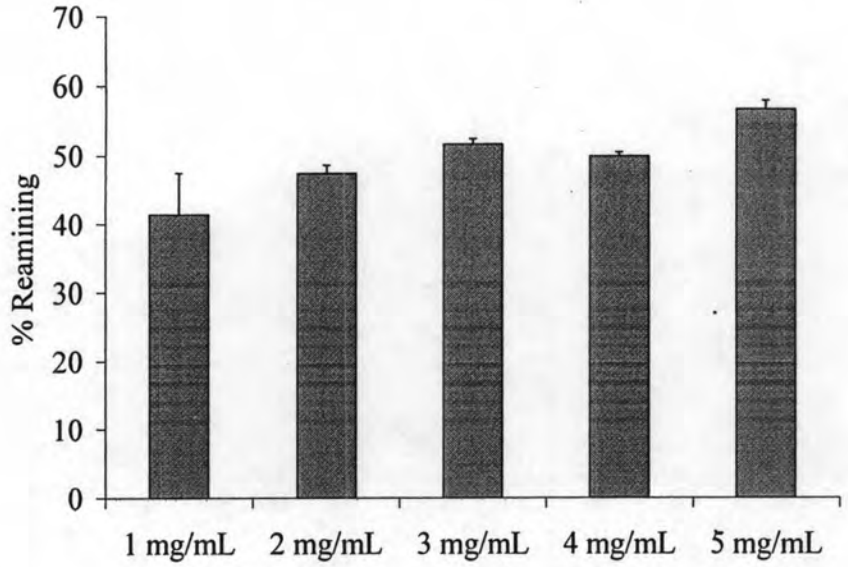

Figure 17 % Remaining of *P. emblica* extract in buffer solution pH 5.5 stored in refrigerator (4 ± 1 °C) at 12 weeks of storage

Table 6 The amount of total phenolic compounds remaining calculated as GAE of *P. emblica* extract in buffer solution pH 5.5 in room temperature (30 ± 1 °C) at 0, 1, 2, 4, 8 and 12

Concentration (mg/ml)	GAE (μg/ml)					
	Time 0 week	Time 1 week	Time 2 weeks	Time 4 weeks	Time 8 weeks	Time 12 weeks
1	12.75	12.75	12.50	12.60	8.80	5.38
2	24.69	24.82	24.91	24.88	19.62	12.35
3	36.36	34.33	36.23	36.26	22.36	18.72
4	48.02	47.99	47.80	48.21	32.41	28.89
5	59.59	58.72	57.01	55.92	49.11	40.87

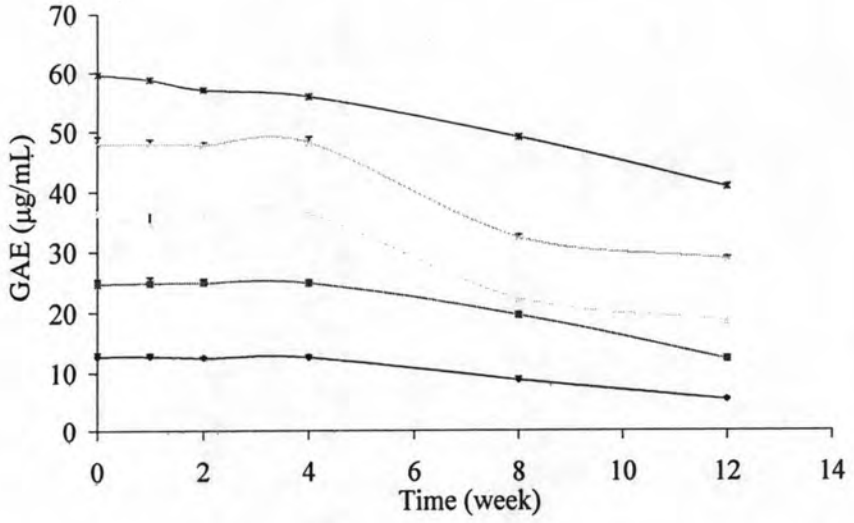

Figure 18 The GAE of *P. emblica* extract in buffer solution pH 5.5 stored in room temperature (30 ± 1 °C) at 0, 1, 2, 4, 8 and12 weeks (◆ = 1 mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

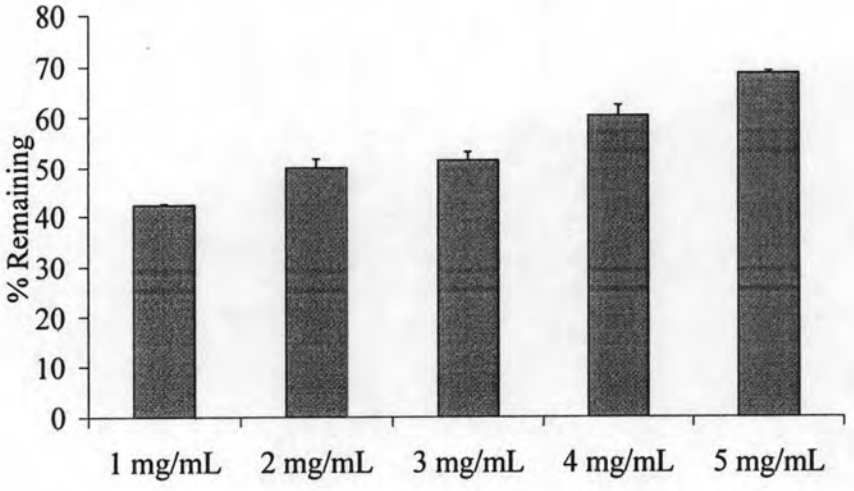

Figure 19 % Remaining of *P. emblica* extract in buffer solution pH 5.5 stored in room temperature (30 ± 1 °C) at 12 weeks of storage

The stability of *P. emblica* extract in buffer solution pH 7.4 was investigated upon storage in refrigerator (4 ± 1°C) and room temperature (30 ± 1°C) for 0, 1, 2, 4, 8 and 12 weeks. GAE data obtained from buffer solutions at 4 ± 1°C and 30 ± 1°C, as

shown in Table 7 and 8, respectively. The GAE values were plotted against time (Figure 20 and Figure 22). % remaining for all formulations observed at 12 weeks showed in Figure 21 and Figure 23.

Table 7 The amount of total phenolic compounds remaining calculated as GAE of *P. emblica* extract in buffer solution pH 7.4 in refrigerator (4 ± 1 °C) at 0, 1, 2, 4, 8 and 12 weeks

Concentration (mg/mL)	GAE (µg/mL)					
	Time 0 week	Time 1 week	Time 2 weeks	Time 4 weeks	Time 8 weeks	Time 12 weeks
1	12.47	13.12	12.66	12.63	10.14	6.47
2	24.32	23.89	23.64	23.89	11.10	9.33
3	35.61	34.96	34.89	35.14	19.66	18.57
4	46.43	47.12	46.28	46.06	27.62	24.85
5	57.50	56.32	54.92	53.03	41.11	33.56

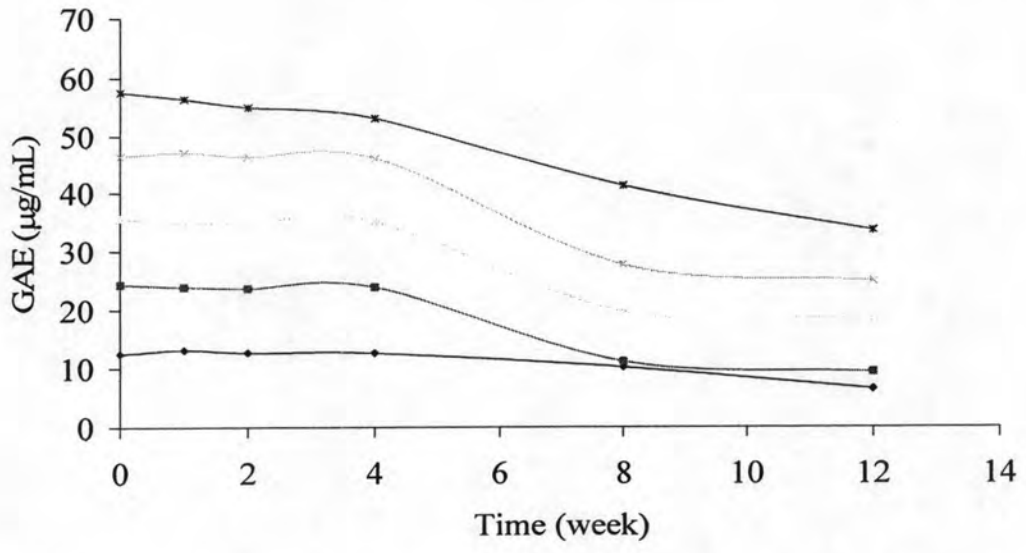

Figure 20 The GAE of *P. emblica* extract in buffer solution pH 7.4 stored in refrigerator (4 ± 1 °C) at 0, 1, 2, 4, 8 and12 weeks (◆ = 1mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

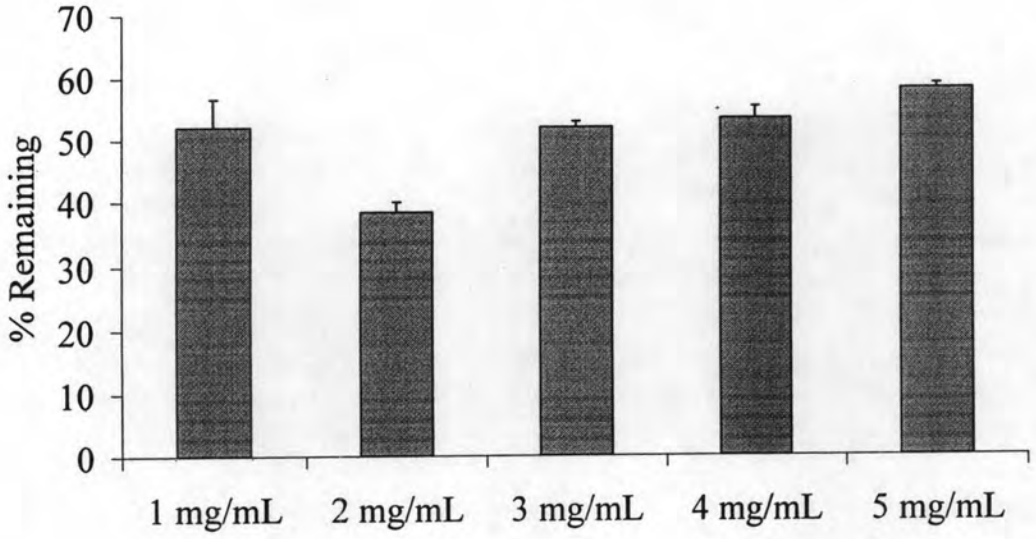

Figure 21 % Remaining of *P. emblica* extract in buffer solution pH 7.4 stored in refrigerator (4 ± 1 °C) at 12 weeks of storage

Table 8 The amount of total phenolic compounds remaining calculated as GAE of *P. emblica* extract in buffer solution pH 7.4 in room temperature (30 ± 1 °C) at 0, 1, 2, 4, 8 and 12 weeks

Concentration (mg/ml)	GAE (µg/ml)					
	Time 0 week	**Time 1 week**	**Time 2 weeks**	**Time 4 weeks**	**Time 8 weeks**	**Time 12 weeks**
1	12.4714	13.1867	12.7824	12.658	7.2155	4.1677
2	24.3205	23.9162	24.0095	23.4186	14.8039	7.371
3	35.6098	35.1433	34.8945	34.8945	23.2942	12.8757
4	46.4326	46.2771	46.1527	45.5307	27.8659	22.3612
5	57.5042	56.5712	56.0425	54.3942	40.0571	32.5309

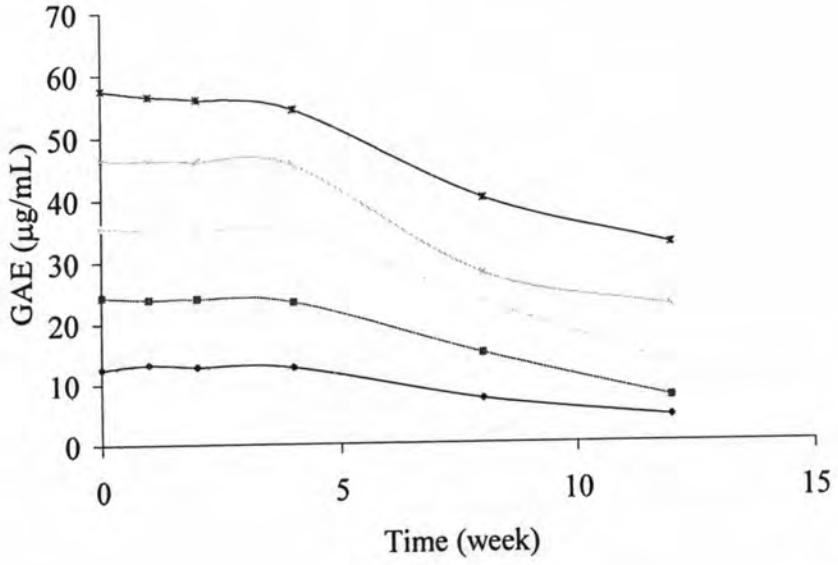

Figure 22 The GAE of *P. emblica* extract in buffer solution pH 5.5 stored in room temperature (30 ± 1 °C) at 0, 1, 2, 4, 8 and12 weeks (◆ = 1mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

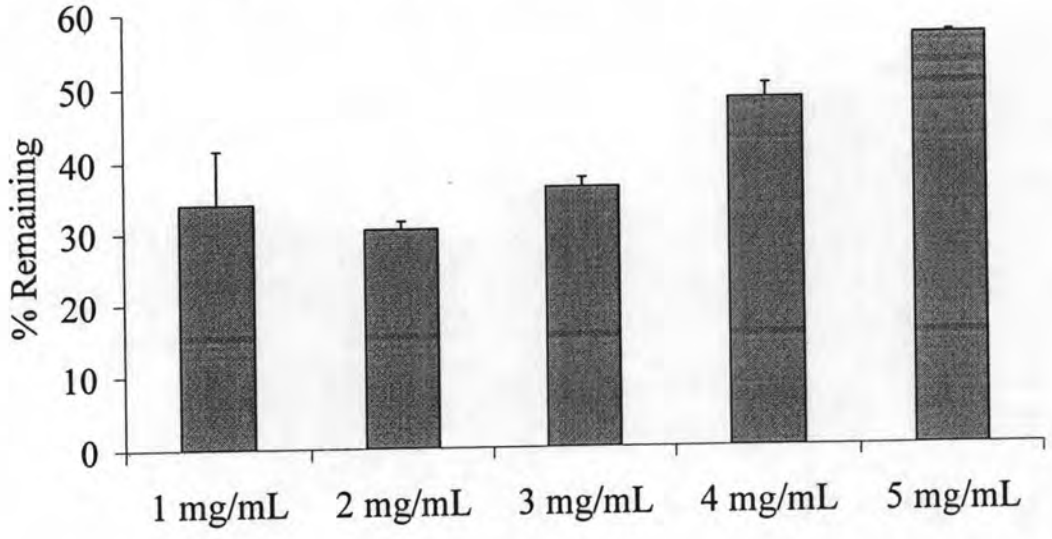

Figure 23 % Remaining of *P. emblica* extract in buffer solution pH 7.4 stored in room temperature (30 ± 1 °C) at 12 weeks of storage

In this study total phenolic compounds calculated as GAE in *P. emblica* extract in buffer solution pH 5.5 and 7.4 remain constant at each concentration by

determining before 4 weeks after that they were significantly dropped in the concentration of 1- 4 mg/ml except the concentration of 5%w/v that show significantly different after storage 1 week. One way ANOVA statistic analysis was used to determine the stability by using time (week) was the factor and GAE was the dependent value.

7. Stability of *P. emblica* extract in liposomes

Chemical stability

The stability of *P. emblica* extract in liposome pH 5.5 and 7.4 were investigated for the total phenolic compound using UV-VIS spectrophotometer and represented as GAE, GAE was calculated from the standard curve of gallic acid (Figure 8) using the following equation ; $y = 93 x + 0.04357$ (y = GAE and x = absorbance). The concentrations of *P. emblica* extract were varied at 1, 2, 3, 4 and 5 mg/mL.

% Remaining of *P. emblica* extract in liposomes pH 5.5 and 7.4 at 12 weeks of storage were calculated from the following equation ;

$$\% \text{ Remaining} = \frac{\text{The amount of } P. \textit{ emblica} \text{ extract in liposomal suspensions at various time} \times 100}{\text{The amount of } P. \textit{ emblica} \text{ extract in liposomal suspensions at time 0}}$$

The stability of *P. emblica* extract in liposomes pH 5.5 was investigated upon storage in the refrigerator ($4 \pm 1^{\circ}C$) and room temperature ($30 \pm 1^{\circ}C$) for 0, 1, 2, 4, 8 and 12 weeks. GAE data obtained from liposomal suspension at $4 \pm 1^{\circ}C$ and $30 \pm 1^{\circ}C$, as shown in Table 8 and Table 9, respectively. The GAE values were plotted against time (Figure 24 and Figure 26. % remaining for all formulations observed at 12 weeks showed in Figure 25 and Figure 27.

Table 9 The amount of total phenolic compounds remaining calculated as GAE of *P. emblica* extract in liposomes pH 5.5 stored in refrigerator(4 ± 1 °C) at 0, 1, 2, 4, 8 and 12 weeks

Concentration (mg/ml)	GAE (µg/ml)					
	Time 0 week	Time 1 week	Time 2 weeks	Time 4 weeks	Time 8 weeks	Time 12 weeks
1	11.73	11.88	11.76	11.51	11.41	8.18
2	23.39	23.67	23.70	23.67	23.20	17.76
3	34.21	34.09	33.96	33.90	33.84	28.95
4	45.59	45.56	45.19	45.16	44.75	38.10
5	48.86	49.01	48.67	48.45	48.24	40.31

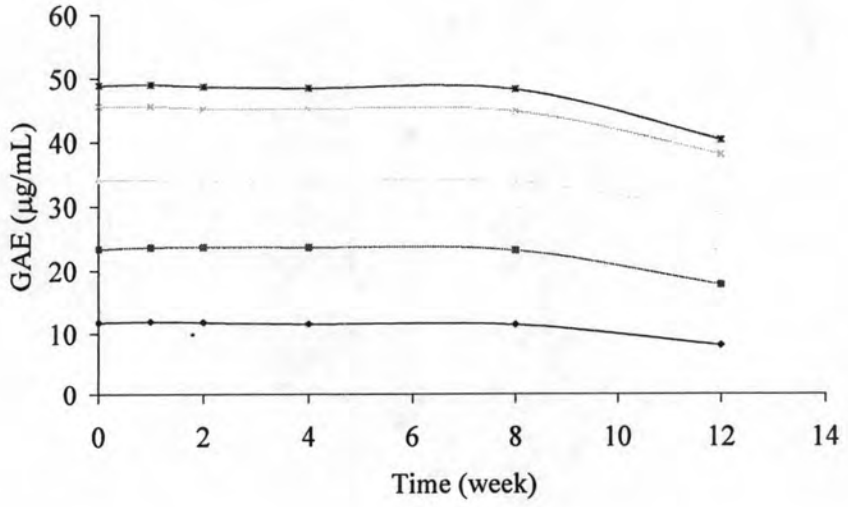

Figure 24 The GAE of *P. emblica* extract in liposomes pH 5.5 stored in refrigerator (4 ± 1 °C) at 0, 1, 2, 4, 8 and12 weeks(◆ = 1mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

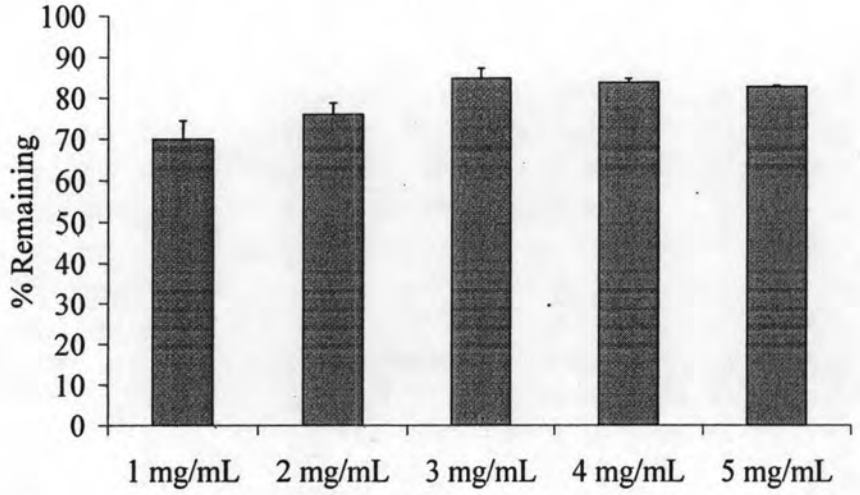

Figure 25 % Remaining of *P. emblica* extract in liposomes pH 5.5 stored in refrigerator (4 ± 1 °C) at 12 weeks of storage

Table 10 The amount of total phenolic compounds remaining calculated as GAE of *P. emblica* extract in liposomes pH 5.5 stored in room temperature (30 ± 1 °C) at 0, 1, 2, 4, 8 and 12 weeks

Concentration (mg/ml)	GAE (µg/ml)					
	Time 0 week	Time 1 week	Time 2 weeks	Time 4 weeks	Time 8 weeks	Time 12 weeks
1	11.73	11.91	11.76	11.29	11.45	7.62
2	23.39	23.39	23.79	23.45	23.23	16.17
3	34.21	33.99	34.30	33.99	33.68	28.15
4	45.59	45.56	45.44	45.31	45.25	37.88
5	48.86	48.77	47.61	46.56	44.72	38.53

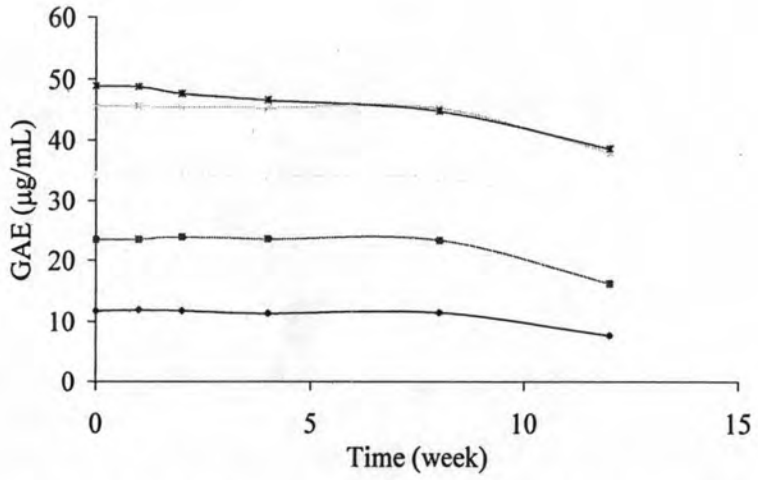

Figure 26 The GAE of *P. emblica* extract in liposomes pH 5.5 stored in room temperature (30 ± 1 °C) at 0, 1, 2, 4, 8 and12 weeks(◆ = 1mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

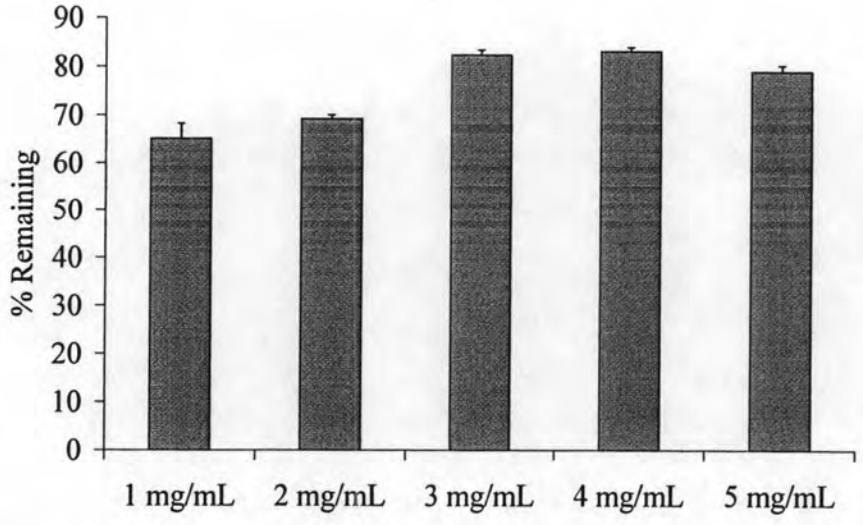

Figure 27 % Remaining of *P. emblica* extract in liposomes pH 5.5 stored in refrigerator (4 ± 1 °C) at 12 weeks of storage

The results show that 3 mg/mL *P. emblica* extract in liposomes pH 5.5 stored in refrigerator has the highest percent remaining but when analyze the data with one way ANOVA the results show that % remaining of *P. emblica* extract in buffer

solution pH 5.5 stored in refrigerator and room temperature in the concentration of 3, 4 and 5 mg/mL were in significantly different

The stability of *P. emblica* extract in liposomes pH 7.4 was investigated upon storage in the refrigerator (4 ± 1°C) and room temperature (30 ± 1°C) for 0, 1, 2, 4, 8 and 12 weeks. GAE data obtained from liposomal suspension at 4 ± 1°C and 30 ± 1°C, as shown in Table 10 and Table 11, respectively. The GAE values were plotted against time (Figure 28 and Figure 30. % remaining for all formulations observed at 12 weeks showed in Figure 29 and Figure 31.

Table 11 The amount of total phenolic compounds remaining calculated as GAE of *P. emblica* extract in liposomes pH 7.4 stored in refrigerator (4 ± 1 °C) at 0, 1, 2, 4, 8 and 12 weeks

Concentration (mg/ml)	GAE (µg/ml)					
	Time 0 week	**Time 1 week**	**Time 2 weeks**	**Time 4 weeks**	**Time 8 weeks**	**Time 12 weeks**
1	11.63	11.85	11.76	12.10	11.63	9.24
2	21.24	21.18	21.24	21.12	20.81	16.95
3	32.62	32.44	32.16	32.00	32.00	27.15
4	42.61	42.55	42.70	42.64	42.30	37.32
5	45.97	46.06	45.53	45.72	45.34	36.45

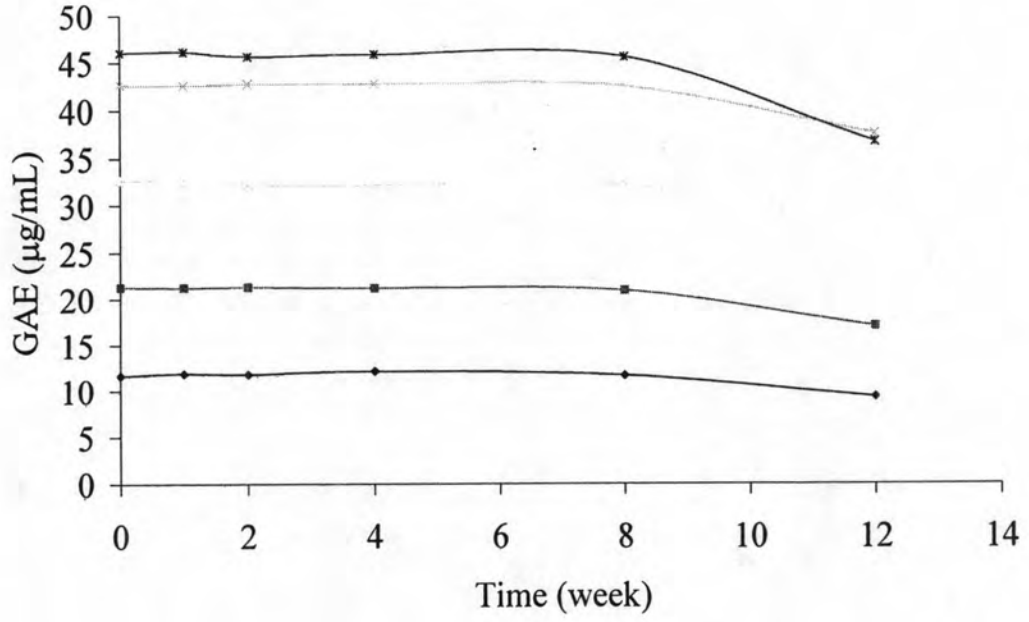

Figure 28 The GAE of *P. emblica* extract in liposomes pH 7.4 stored in refrigerator (4 ± 1 °C) at 0, 1, 2, 4, 8 and12 weeks(◆ = 1mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

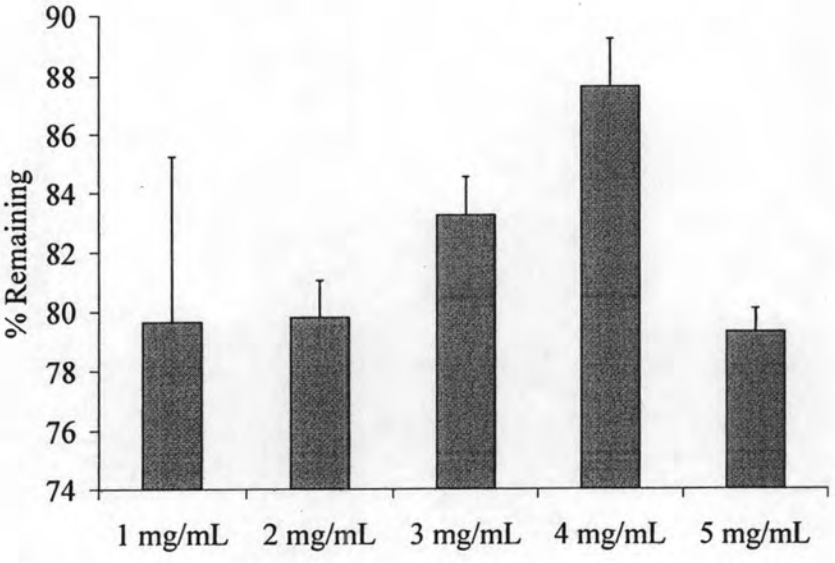

Figure 29 % Remaining of *P. emblica* extract in liposomes pH 5.5 stored in refrigerator (4 ± 1 °C) at 12 weeks of storage

Table 12 GAE of *P. emblica* extract in liposomes pH 7.4 stored in room temperature (30 ± 1 °C) at 0, 1, 2, 4, 8 and 12 weeks

Concentration (mg/ml)	GAE (µg/ml)					
	Time 0 week	Time 1 week	Time 2 weeks	Time 4 weeks	Time 8 weeks	Time 12 weeks
1	11.63	11.57	11.51	10.98	11.01	6.41
2	21.24	21.18	21.06	21.15	20.84	16.55
3	32.62	32.75	32.28	31.85	31.63	24.41
4	42.61	42.48	42.48	42.48	41.89	33.40
5	45.97	46.06	46.03	45.94	45.47	31.78

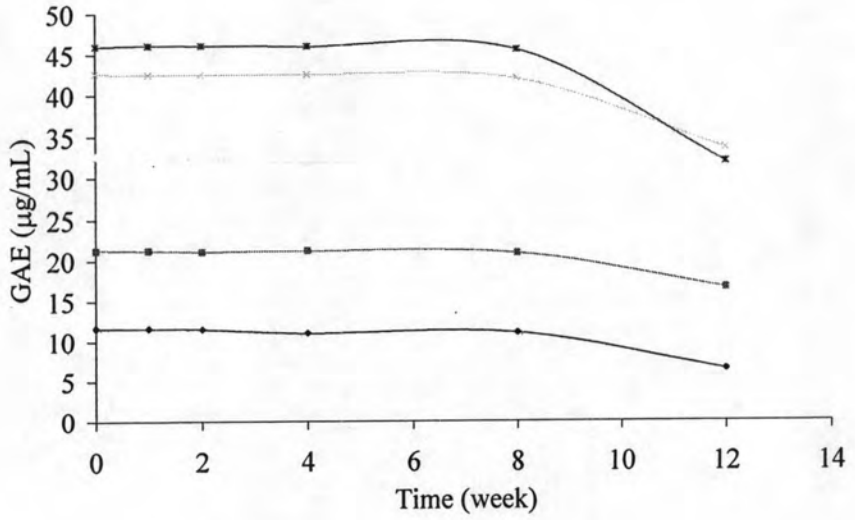

Figure 30 The GAE of *P. emblica* extract in liposomes pH 7.4 stored in refrigerator (30 ± 1 °C) at 0, 1, 2, 4, 8 and12 weeks(◆ = 1mg/mL *P. emblica* extract, ■ = 2 mg/mL *P. emblica* extract, ▲ = 3 mg/mL *P. emblica* extract, × = 4 mg/mL *P. emblica* extract and ∗ = 5 mg/mL *P. emblica* extract)

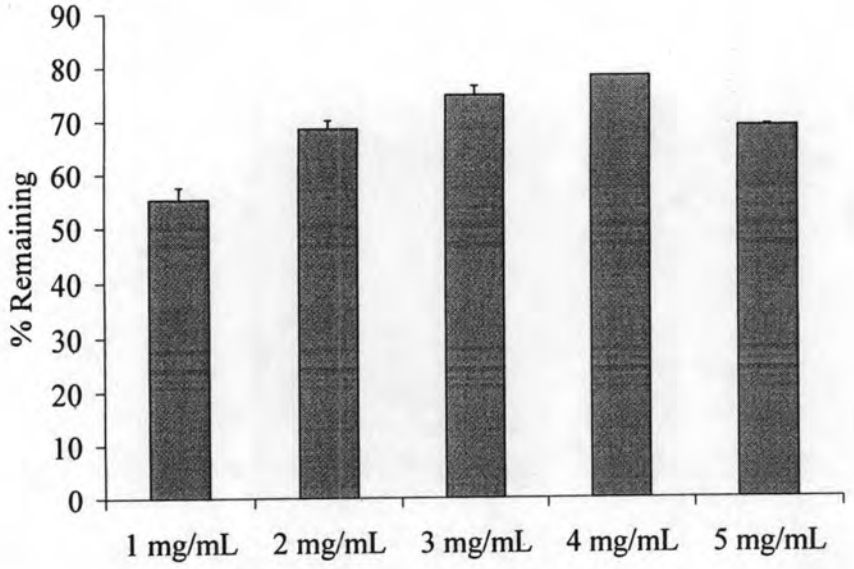

Figure 31 % Remaining of *P. emblica* extract in liposomes pH 7.4 stored in refrigerator (30 ± 1 °C) at 12 weeks of storage

The results show that 4 mg/mL *P. emblica* extract in liposomes stored in refrigerator has the highest percent remaining in buffer pH 7.4 at 12 weeks of storage (87.6%). The statistical analysis shows that % remaining of 3 mg/mL and 4 mg/mL *P. emblica* extract in liposomes pH 7.4 was not significantly different.

From the different buffer solution for preparing *P. emblica* extract in liposomes, the highest percent remaining in each preparation and storage were compareded

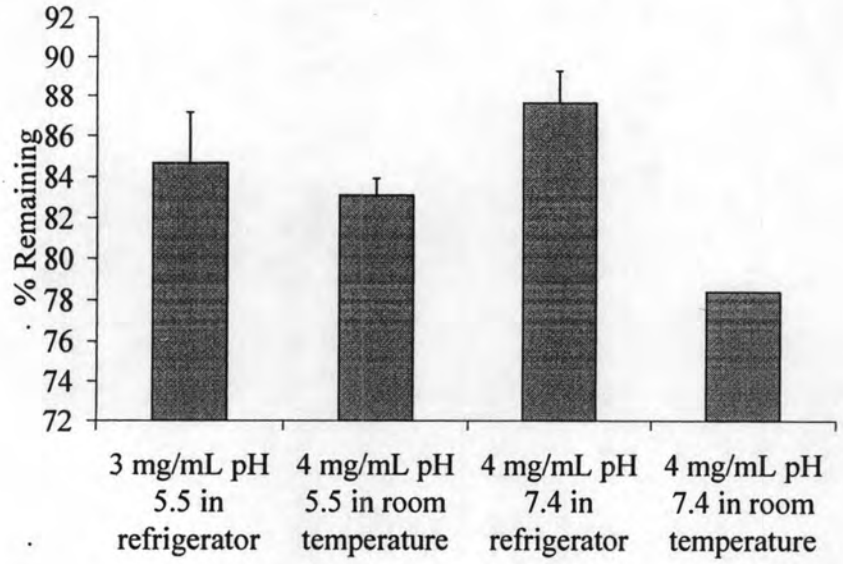

Figure 32 % Remaining of *P. emblica* extract in liposomes of the highest percent remaining in each condition

The results show that % remaining at 12 weeks of storage of *P. emblica* extract 3 mg/mL in liposomes pH 5.5 stored in the refrigerator and 4 mg/mL *P. emblica* extract in liposomes pH 7.4 stored in refrigerator were not significantly different ($p<0.05$)

% encapsulation efficiency is important for preparing liposomes. Consequently, the highest % encapsulation efficiency of each buffer solution stored in different condition was compared to show the highest % encapsulation efficiency of *P. emblica* extract in liposomes (Figure 33).

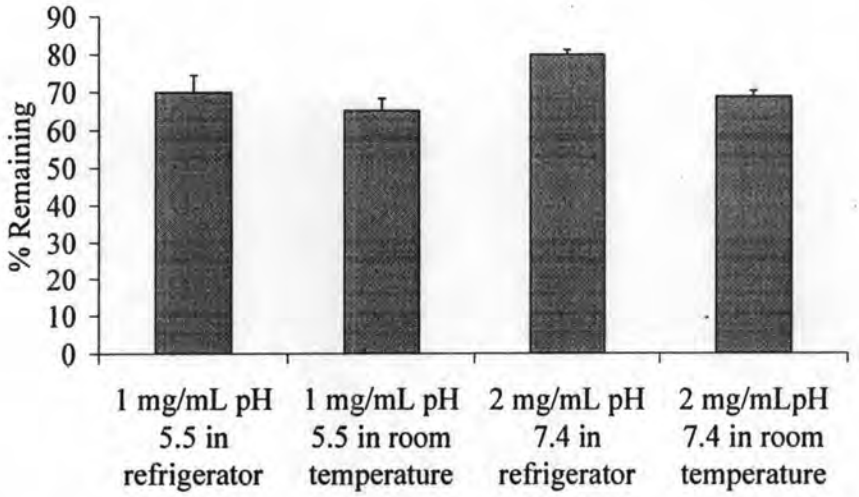

Figure 33 % Remaining of the highest % encapsulation at 12 weeks of storage

The graph show that 2 mg/mL *P. emblica* extract in liposomes has the highest percent remaining but when analyze the data with one way ANOVA the results show that % remaining of 2mg/mL *P. emblica* extract in liposomes pH 7.4 stored in refrigerator was insignificantly different from 1 mg/mL *P. emblica* in liposomes pH 5.5 stored in refrigerator.

To choose the best stability it can study by physical stability to confirm the stability of *P. emblica* extract in liposomes

In this study the chemical stability of each method of preparations was significantly decrease after 8 weeks of storage. Moreover the stability of *P. emblica* extract in liposomes were more stable than in buffer solution

Physical stability

7.2.1 Particle size and size distribution

The physical stability of liposomes during storage in refrigerator and in room temperature were studied in term of particle size and morphology. Particle size was measured at 0 week and after 12 weeks of storage. The data were shown in Table 13.

Table 13 show that the mean particle size of *P. emblica* extract in liposomes pH 5.5 freshly prepared (Figure 24) and storage in refrigerator (Figure 25) was changed from 5.790±0.776 to 5.553 ± 0.647 that was not significantly different ($p<0.05$) The span of freshly prepared and after storage 12 weeks in refrigerator (4 ± 1 °C) changed from 1.152±0.114 to 1.919 ± 0.362.

The mean particle size of *P. emblica* extract in liposomes pH 5.5 freshly prepared and storage in room temperature (30 ± 1 °C) as shown in Figure 26 was changed from 5.790±0.776 to 6.161 ± 0.504 and the span value changed from 1.152±0.114 to 7.621± 2.149 that was significantly difference ($p<0.05$) from freshly prepared. The changing curve of particle size shows markedly 2 peaks of aggregation in Figure 35 and 36 which different from freshly prepared.

P. emblica in liposomes pH 5.5 stored in refrigerator was more stable than stored in room temperature because the particle size distribution was not significantly different from freshly prepared.

The mean particle size of *P. emblica* extract in liposomes pH 7.4 freshly prepare was shown in Figure 37, storage in refrigerator was shown in Figure 38 and room temperature was shown in Figure 39 were changed from 6.188 ± 0.714 to 9.984± 1.047 and 9.164 ± 0.600 that was significantly different ($p<0.05$) from freshly prepare. The span value after storage 12 weeks in refrigerator (4 ± 1 °C) and in room temperature were changed from 1.283± 0.208 to 9.735± 1.892 and 10.174± 1.614, respectively that was significantly different from freshly prepare. The curve of particle size changing showed markedly 2 peaks of aggregation in Figure 38 and 39 which different from freshly prepared.

The suitable preparation of *P. emblica* extract in liposomes from this study was prepared by using 1mg/ml of *P. emblica* extract in liposomes pH 5.5 stored in refrigerator because it was not significantly different ($p<0.05$) of mean particle size from freshly prepared and span changed less than other preparations.

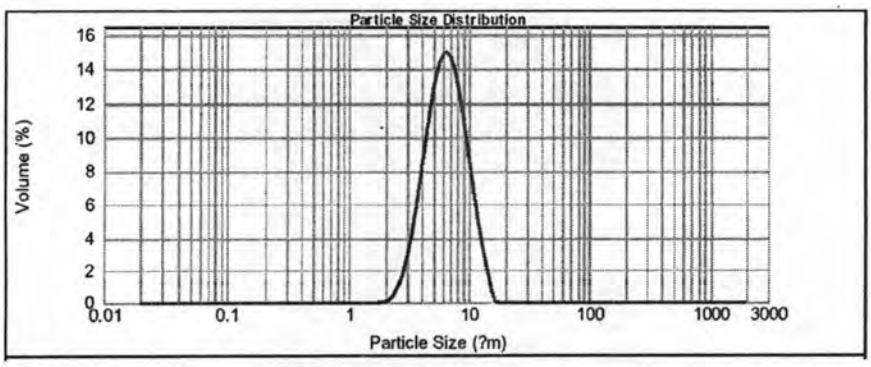

Figure 34 Particle size distribution of *P. emblica* in liposomes pH 5.5 at time 0

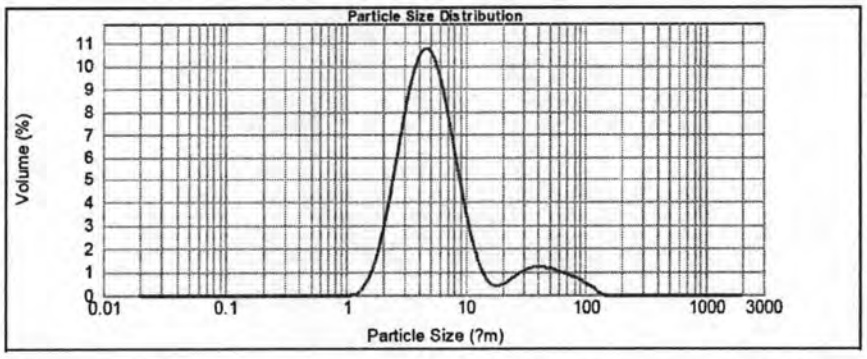

Figure 35 Particle size distribution of *P. emblica* extract in liposomes pH 5.5 in refrigerator after 12 weeks

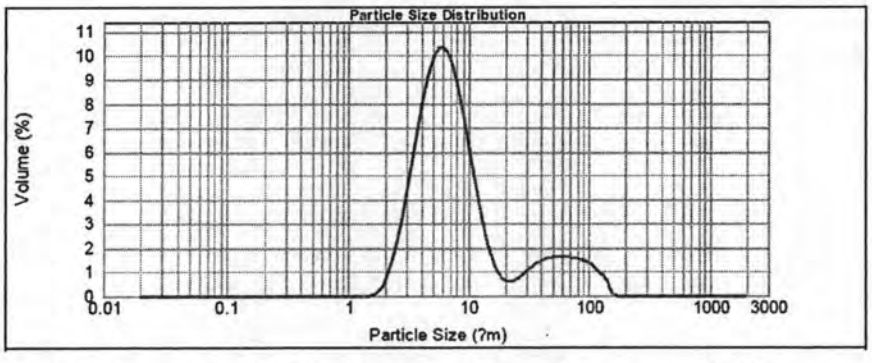

Figure 36 Particle size distribution of *P. emblica* extract in liposomes pH 5.5 in room temperature after 12 weeks

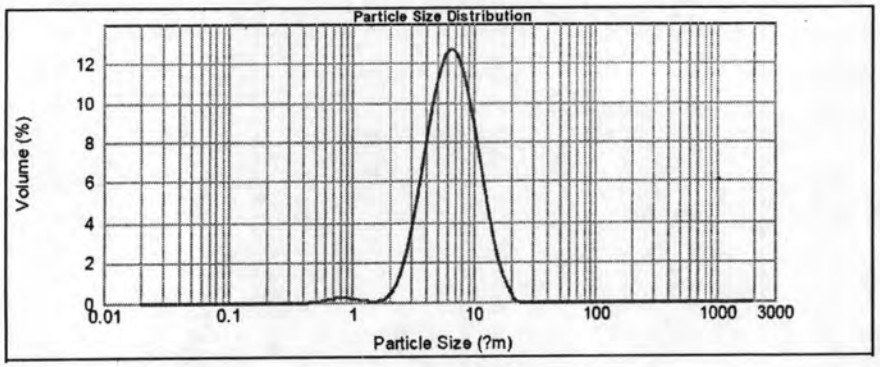

Figure 37 Particle size distribution of *P. emblica* in liposomes pH 7.4 at time 0

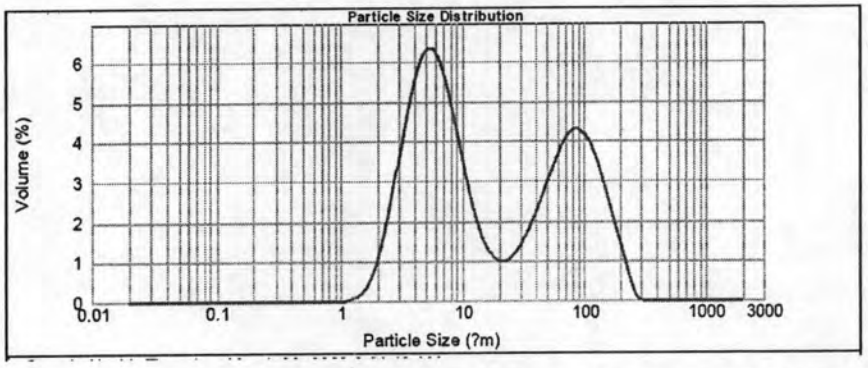

Figure 38 Particle size distribution of *P. emblica* extract in liposomes pH 7.4 in refrigerator after 12 weeks

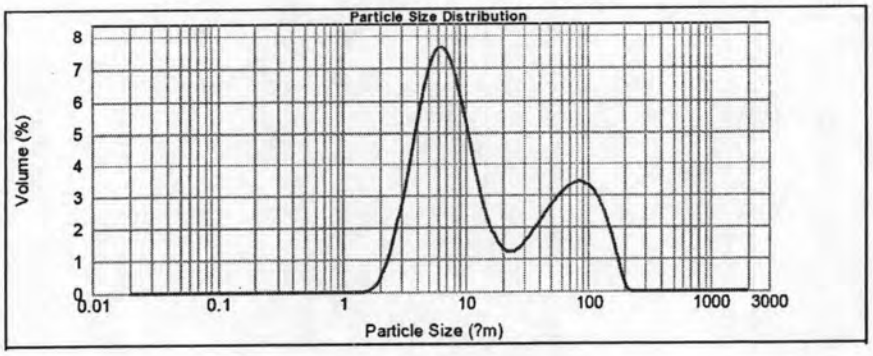

Figure 39 Particle size distribution of *P. emblica* extract in liposomes pH 7.4 in room temperature after 12 weeks

7.2.2 Transmission electron microscope (TEM)

TEM has been used extensively to probe lamellar liquid crystal morphology (Klenman et al., 1997) and provides valuable insight into bilayer organization and defects. The stability of liposomes using TEM was used to monitor changes in the size of liposomes and membrane phase.

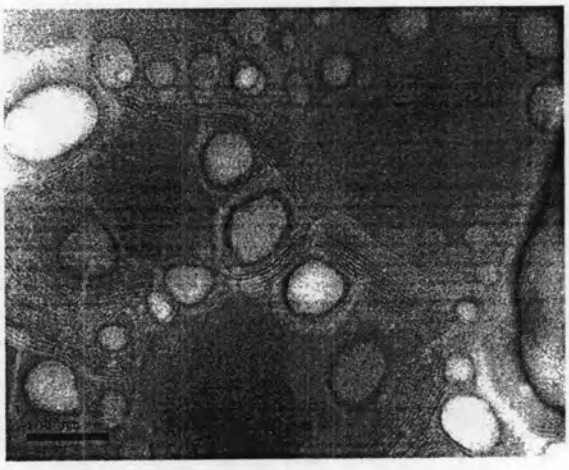

Figure 40 Morphology of *P. emblica* extract in liposomes pH 5.5 using TEM at 12 weeks x 150000 stored in refrigerator

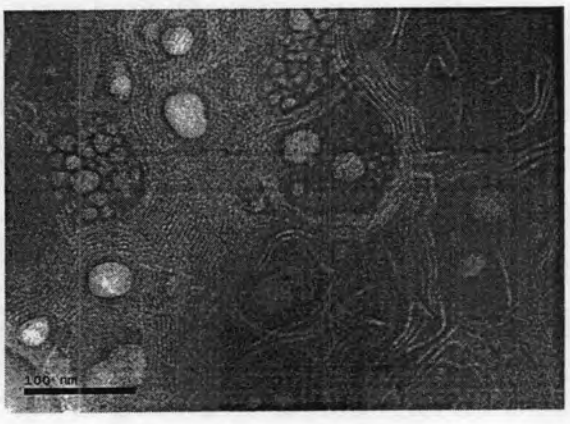

Figure 41 Morphology of *P. emblica* extract in liposomes pH 5.5 using TEM at 12 weeks x 200000 stored in room temperature

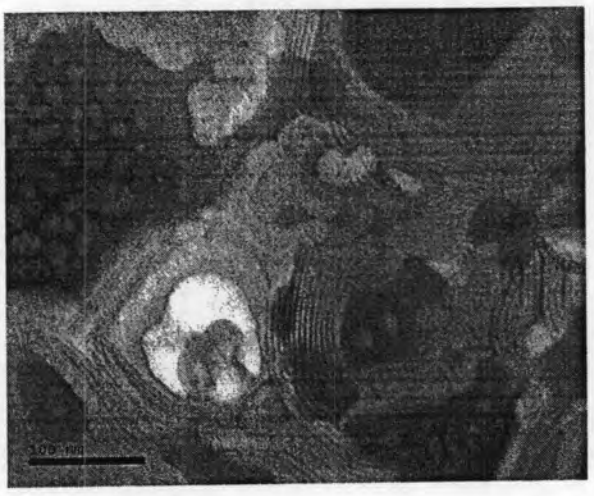

Figure 42 Morphology of *P. emblica* extract in liposomes pH 7.4 using TEM at 12 weeks x 200000 stored in refrigerator

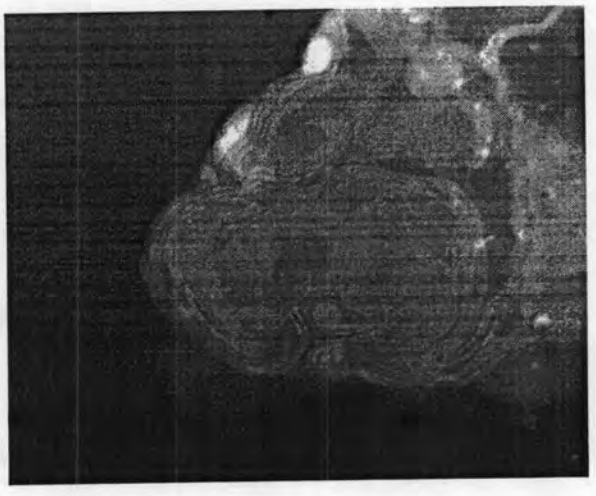

Figure 43 Morphology of *P. emblica* extract in liposomes pH 5.5 using TEM at 12 weeks using TEM x 200000 stored in room temperature

The shape and surface morphology of *P. emblica* extract in liposomes were investigated. Figure 40 showed multilamellar of single particle of *P. emblica* extract in liposomes pH 5.5 at 12 weeks in the refrigerator. It could suggest that this preparation was more stable than other preparation because liposomes still in the single vesicle there were small amount of liposomes that form aggregation. The result was used for confirming the result of particle size distribution.

TEM of *P. emblica* extract in buffer pH 5.5 stored in room temperature as shown in Figure 31 was larger size than storage in refrigerator. There were many liposomes formed aggregation. TEM still show the smaller size less than study by Mastersizer. Figure 32 shows TEM of *P. emblica* extract in buffer pH 7.4 stored in refrigerator, their particles show bigger size than prepared in buffer pH 5.5. Figure 33 shows the particle size of *P. emblica* extract stored in room temperature that formed aggregation of small particles. The result was as same as the result of Mastersizer that the particle size of 12 weeks storage were bigger than freshly prepared and the aggregation of many particles of liposomes which could confirm 2 peak of particle size distribution when measure with Mastersizer which storage at room temperature.

The morphology of *P. emblica* extract in liposomes showed bilayer organization insight liposome and also showed aggregations of small vesicles resulted in changing the size of liposomes and membrane phase.

8. Comparative stability of *P. emblica* extract in buffer solution and in liposomes

Percent remaining of total phenolic compounds calculated as gallic acid equivalent (GAE) in *P. emblica* extract was compared the % remaining of *P. emblica* extract in buffer solution pH 5.5, pH 7.4 and in liposomes pH 5.5 and 7.4 stored in a refrigerator (4 ± 1 °C) and room temperature (30 ± 1°C) at 12 weeks of storage. The results showed in Figure , Figure , Figure and Figure .

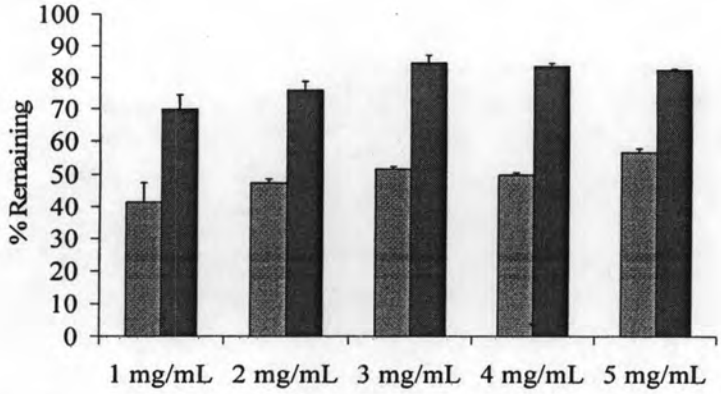

Figure 44 % Remaining of *P. emblica* extract in buffer solution and liposomes pH 5.5 stored in refrigerator (4 ± 1 °C) at 12 week of storage (*P. emblica* extract in buffer solution pH 5.5 and P. emblica extract in liposome pH 5.5)

The results show that *P. emblica* extract in liposomes pH 5.5 stored in refrigerator was significantly more stable than *P. emblica* extract in buffer solution pH 5.5 stored in refrigerator

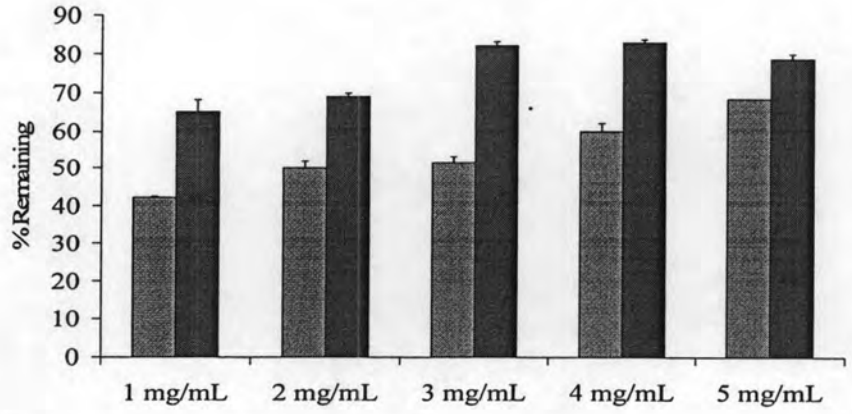

Figure 45 % Remaining of *p. emblica* extract in buffer solution and in liposomes pH 5.5 stored in room temperature (30 ± 1 °C) (*P. emblica* extract in buffer solution and *P. emblica* extract in liposome)

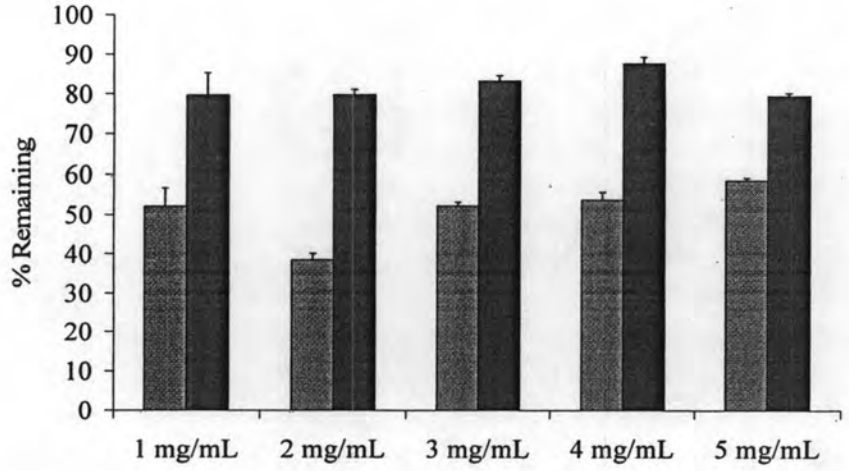

Figure 46 % Remaining of *P. emblica* extract in buffer solution and in liposomes pH 7.4 stored in refrigerator (4 ± 1 °C) at 12 week of storage (■ *P. emblica* extract in buffer solution pH 5.5 and ■ *P. emblica* extract in liposome pH 5.5)

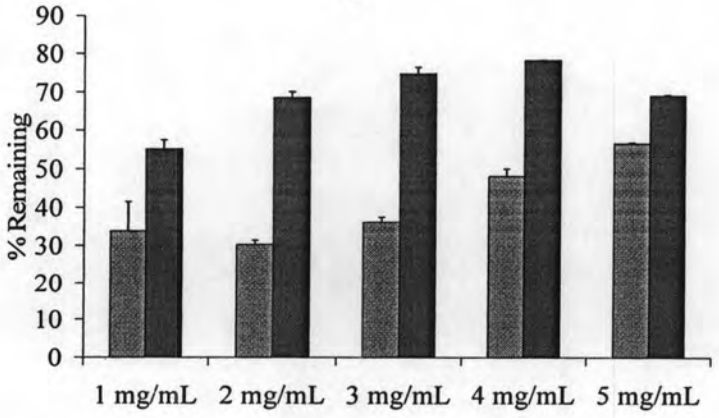

Figure 47 % Remaining of *P. emblica* extract in buffer solution and in liposomes pH 7.4 stored in room temperature (30 ± 1 °C) at 12 week of storage (■ *P. emblica* extract in buffer solution and ■ *P. emblica* extract in liposome)

The results showed that *P. emblica* extract in liposomes pH 5.5 and 7.4, stored in refrigerator (4 ± 1 °C) and room temperature (30 ± 1 °C) were significantly more stable than in buffer solution ($p<0.05$).