CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of *P. emblica* extract nanoliposomes

In this investigation, *P. emblica* extract nanoliposomes were prepared by modified ethanol injection method (New, 1987). The organic solvent, the sonication time, and the temperature for evaporation of organic solvent were adjusted to obtain suitable conditions of preparation of *P. emblica* extract nanoliposomes. The liposomal suspensions were opalescence. The stable membrane was produced by using SPC: Tween®80 at 5:1 by weight. The SPC was dissolved in ethanol to obtain clear solution and ethanol was evaporated out using rotary evaporation. The temperature of water bath was used at 40°C and the pressure was reduced to 100 mbar it was suitable in this nanoliposomes preparation. The concentration of *P. emblica* extract was 1% w/v dissolved in citrate-phosphate phosphate buffer pH 5.5 (Nimmannit, 2007) which is the stable pH of *P. emblica* extract. The appearance of *P. emblica* extract liposomes exhibited good feature with spherical vesicles. The concentration of *P. emblica* extract in preparation was further increased to 2% w/v but it did not perform a good appearance because it started to precipitate to the bottom of the flask. Thus, the appropriate concentration of *P. emblica* extract was 1% w/v. After that, sizing of liposomes was performed by Lipex Extruder using 100 nm polycarbonate membrane filtered to reduce the size as nanoliposomes.

2. Physical Characterization of *P. emblica* extract nanoliposomes

2.1 Determination of particle size and size distribution

The particle size and size distribution of *P. emblica* extract nanoliposomes 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 ($D_{4,3}$).

Table 5 Mean particle size and span of *P. emblica* extract nanoliposomes in formulation 1-6 (Mean ± SD, n=3)

Formulation	SPC:Tween®80	Mean particle size ($D_{4,3}$, nm)	Span
1	1:1 (1%)	867.33±70.06	2.314±0.09
2	1:1 (2%)	1221.00±25.06	2.789±0.02
3	3:1 (1%)	660.00±2.00	1.694±0.02
4	3:1 (2%)	1053.33±61.10	2.729±0.03
5	5:1 (1%)	566.67±43.50	1.751±0.09
6	5:1 (2%)	938.00±21.17	2.176±0.05

The mean particle size of *P. emblica* extract nanoliposomes was in range of 566-1,221 nm. The polydispersity of particles were expressed by the span. The span value of *P. emblica* extract nanoliposomes was being approximately in the range of 1.694-2.789. The high span value indicated a wide particle size distribution. The influences of *P. emblica* extract concentration on the particle size and particle size distribution (span) of *P. emblica* extract nanoliposomes are shown in Table 5.

2.2 Transmission electron microscope

The TEM images of the *P. emblica* extract nanoliposomes were illustrated in Figure 19 and 20, respectively. It was found that *P. emblica* extract nanoliposomes showed a spherical or ellipsoidal shape with 100-250 nm in diameter. There was some

aggregation and overlapping of nanoliposomes. In Figure 19, TEM micrograph shows the majority of nanoliposomes consisted of several bilayers.

Transmission electron microscope showed some deformed structures, apparently due to osmotic pressure change during the staining process. Figure 19 shows some lamellar patterns on liposomes. It might be multilamellar but it is usually difficult to report for multilamellar vesicle from transmission microscopy. It has been suggested that the apparent multilamellarity might be an artifact of negative staining and that multilamellations were seen when liposomes were partially overlapping thus do not reflect the actual internal structure. When negative staining was performed properly, a monolayer of liposomes embedded in negative stain could be spread across the grid. This would result in the formation of artefactual aggregates of liposomes. These aggregates could be indistinguishable from those formed prior to staining. However, if an isolated multilamellar structure was seen, it could highly suggestive that the sample could be multilamellar (New, 1987).

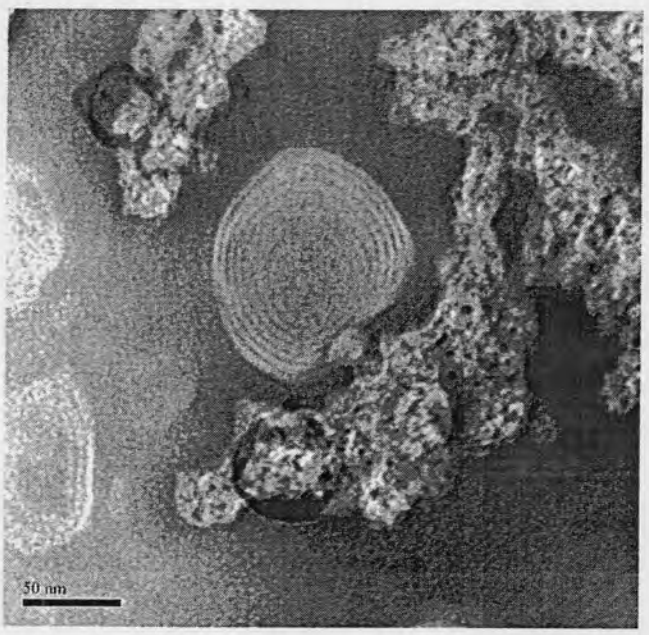


Figure 19 Transmission electron micrographs of *P. emblica* extract nanoliposomes, presence of multilamellar vesicles (x 450,000)

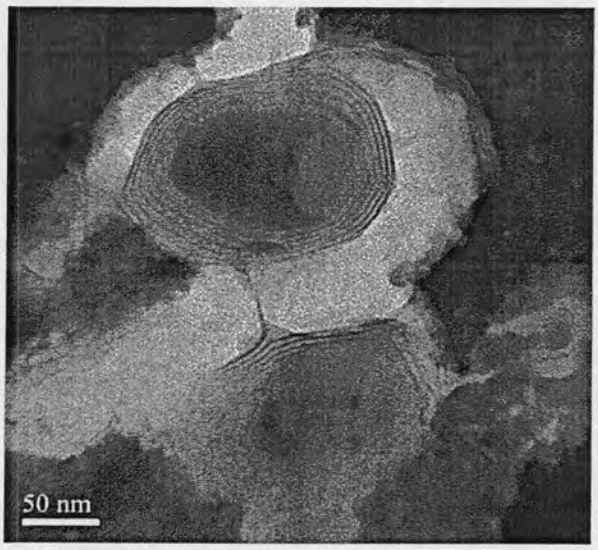


Figure 20 Transmission electron micrographs of *P. emblica* extract nanoliposomes, presence of multilamellar vesicles (x 180,000)

According to the TEM images of the *P. emblica* extract nanoliposomes show above, it was found that *P. emblica* extract nanoliposomes were multilamellar vesicle, which showed approximately 100-200 nm in diameter (Figure 19) and 150-250 nm in diameter (Figure 20), respectively. All of TEM micrographs confirmed the present of multilamellar vesicle of *P. emblica* extract nanoliposomes and provided morphological information. An aggregation of spherical shape of *P. emblica* extract nanoliposomes was obtained. The particle sizes were considerably smaller when estimated with TEM than when measured by Mastersizer. TEM images showed particle size between 100-250 nm, whereas Mastersizer indicated that the smallest population has average mean diameter at least 500-600 nm. This apparent discrepancy could be explained by the aggregation of *P. emblica* nanoliposomes in range of nanoparticles as shown in Figure 21. Thus, nanoliposomes prepared by modified ethanol injection method was not stable because there was aggregation after sizing by lipid extruder and Mastersizer can analyze the particle size greater than measuring by TEM.

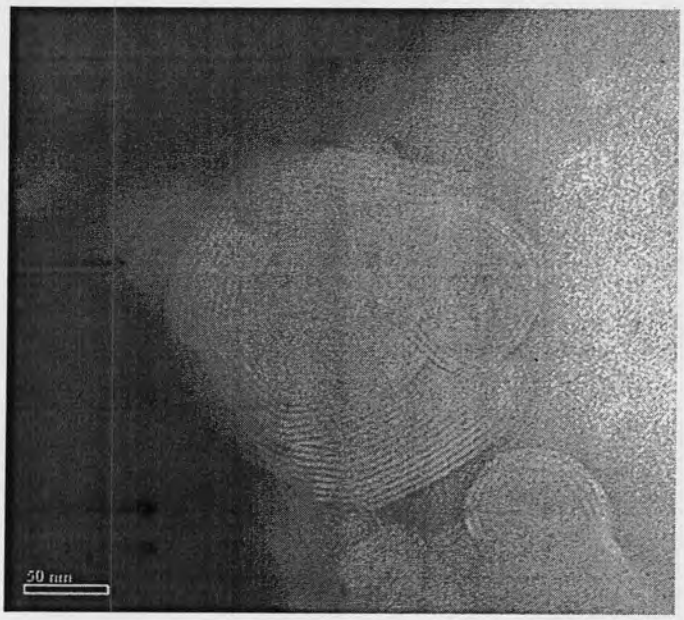


Figure 21 Transmission electron micrographs show aggregation of *P. emblica* nanoliposomes in range of nanoparticles

2.3 Determination of *P. emblica* extract nanoliposomes encapsulation efficiency

P. emblica extract nanoliposomes were ultracentrifuged at 65,000 rpm for 1.30 h. Then the upper part or supernatant with colorless and the precipitant with milky or yellow 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. The ultracentrifugation at 36,000 rpm of these liposomes was not completely effective to separate nanoliposomes from the suspensions. The encapsulation efficiency of *P. emblica* extract nanoliposomes was calculated from the amount of *P. emblica* extract in supernatant and precipitant.

The encapsulation efficiency was expressed as the mean percentage of encapsulated *P. emblica* extract in nanoliposomes as show in Table 6. Also, mean % recovery was shown in Table 6.

Table 6 % Encapsulation efficiency and % recovery of *P. emblica* extract nanoliposomes in formulation 1-6 (Mean ± SD, n=3)

P. emblica extract nanoliposomes formulation	% Encapsulation efficiency	% Recovery
1	22.70±1.21	72.49±2.41
2	20.48±0.26	68.22±0.78
3	36.17±1.04	76.55±1.94
4	29.40±0.48	66.71±0.50
5	50.56±0.98	76.33±1.61
6	32.73±0.33	67.26±0.19

P. emblica extract nanoliposomes in the formulation 5 got 50.56% that was the highest percent encapsulation with percent recovery of 76.33 (Table 6).

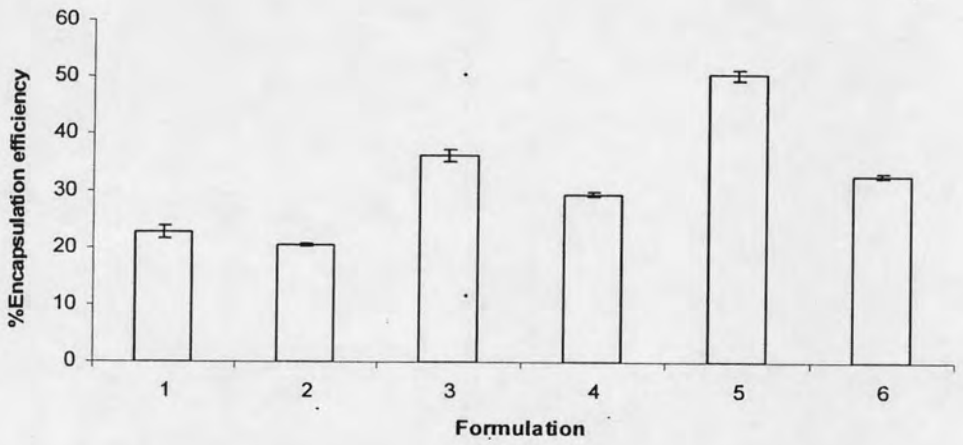


Figure 22 % Encapsulation efficiency of *P. emblica* extract nanoliposomes (Formulation 1-6)

Figure 22 shows % encapsulation efficiency of *P. emblica* extract nanoliposomes and concentration of *P. emblica* extract. Percent encapsulation efficiency of *P. emblica* extract nanoliposomes prepared with the concentration of 1% of *P. emblica* extract was higher than *P. emblica* extract nanoliposomes prepared with the concentration of 2% of *P. emblica* extract, suggesting that 1% was an optimal concentration for preparing *P. emblica* extract nanoliposomes.

3. Preparation of facial patch containing *P. emblica* extract nanoliposomes

3.1 Determination of the amount of plasticizers

Table 7 shows the ingredients in each formula containing different concentrations of glycerin which varying from 1-5% w/v. In this study, the 2.5% w/v polyvinyl alcohol was dispersed in distilled water and stirred continuously at room temperature until dissolved. The plasticizers were added to the mixture. The ultrasonic bath was used to remove air bubbles for 1 h. A film was cast by pouring the mixture into 9 cm diameter petridish and the mixture was dried in hot air oven to obtain the film.

The films were clear with colorless, smooth, and flexible film products. The thickness of the film was in the range 0.05±0.002 to 0.08±0.002 mm. The mechanical properties of all films were shown in Table 8.

Several of the mechanical properties parameters (tensile strength, % elongation, and Young's modulus) gave the difference in film characteristics. The parameter was shown the strength and elasticity of the film. A soft and weak polymer was characterized by a low tensile strength, low % elongation, and Young's modulus. A hard and brittle film was defined by a moderate tensile strength, low % elongation, and high Young's modulus. A soft and tough film was determined by a moderate tensile strength, high % elongation, and low Young's modulus. Finally, a hard and tough film was characterized by high tensile strength, % elongation, and Young's modulus (Peh and Wong, 1999). In addition, the facial patch should have a moderate tensile strength, high % elongation, and low Young's modulus.

Table 7 Formulations of facial patch containing different concentration of glycerin

Ingredients	Concentration of ingredients in facial patch formula				
	1	2	3	4	5
PVA	2.5	2.5	2.5	2.5	2.5
Glycerin	1	2	3	4	5
DI water qs. to	100	100	100	100	100

Table 8 Mechanical properties data of film formula 1-5 (mean ± SD, n = 5)

Formula	Thickness (mm.)	Tensile strength (MPa)	% Elongation	Young's modulus (MPa)	Work of failure (MJ)	Appearance of the patch
1	0.05±0.003	28.14±2.15	45.51±6.04	175.3±24.45	4.096±0.700	Soft, tough
2	0.05±0.002	35.24±7.65	16.05±2.394	390.1±20.11	1.016±0.4135	Hard, brittle
3	0.06±0.001	29.08±6.52	12.29±2.427	345.5±40.08	0.825±0.4027	Hard, brittle
4	0.08±0.002	16.23±1.52	46.82±9.94	195.80±17.02	4.795±1.132	Soft, tough
5	0.06±0.001	29.55±2.506	13.58±1.671	370.3±19.55	0.638±0.125	Hard, brittle

In the Table 8, the formula 1 which contained 2.5% PVA and 1% glycerin demonstrated the moderate tensile strength of 28.14±2.150 MPa, the breaking stress was moderate and high % elongation of 45.51±6.04, but low Young's modulus of 175.30±24.45 MPa resulted in the film was soft and tough.

3.2 Preparation of backing layer of facial patch containing *P. emblica* extract nanoliposomes

According to the film no.1 was selected, PVA 2.5% and glycerin 1% were used and were dispersed in distilled water and continuously stirred until dissolved. Films were cast by pouring the mixture into 9 cm diameter petridish and drying in hot air oven at 40°C for 24 h. Then, the clear, smooth, and flexible films were obtained.

3.3 Determination of the amount of aqueous solution for casting

The thickness of facial patch is important to give the good satisfactory application. The facial patch should have an appropriate thickness. The mixture solution in the formula 1 was chosen to cast in the amount of 10, 15, and 20 g and was cast into the 9 cm diameter petridish as film no. 6, 7, and 8. The thicknesses of the films were 0.04±0.005, 0.06±0.005, and 0.08±0.005 mm respectively. The mechanical properties of the film were shown in Table 9.

Table 9 Mechanical properties data of film no. 6, 7, and 8 (mean ± SD, n = 5)

Formula	Amount of mixture (g)	Thickness (mm.)	Tensile strength (MPa)	% Elongation	Young's modulus (MPa)	Work of failure (MJ)	Appearance of the patch
6	10	0.04±0.005	50.90±10.28	17.68±1.441	500.00±40.98	2.022±0.23	Hard, brittle
7	15	0.06±0.005	50.4±5.60	31.56±1.471	270.2±10.79	7.59±1.03	Soft, tough
8	20	0.08±0.005	39.91±5.54	32.12±2.25	247.7±10.46	5.51±2.15	Soft, tough

In Table 9, the film no. 8 that contained 2.5% w/v PVA and 1% w/v glycerin was selected due to the amount of mixture solution which performed the greatest mechanical properties value of moderate tensile strength, high % elongation, and low Young's modulus and appearance had a suitable thickness of the film.

3.4 Preparation of facial patch containing *P. emblica* extract nanoliposomes

The film formula no. 8 was selected as a backing layer. The mixture of 1% w/v of *P. emblica* extract nanoliposomes, 2.5% PVA and 1% w/v glycerin were cast onto the well dry backing layer and drying in hot air oven. The obtained facial patch containing *P. emblica* extract nanoliposomes (formula 9) was shown in Figure 23. The concentration of *P. emblica* extract was determined and calculated in term of gallic acid equivalents using HPLC.

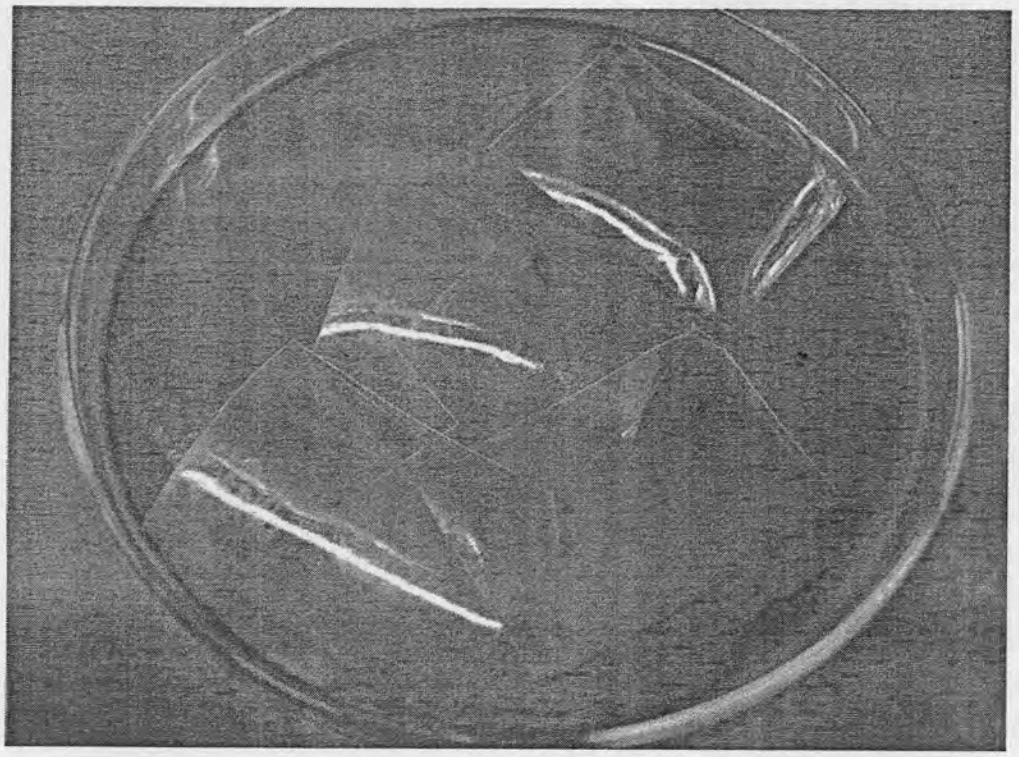


Figure 23 Facial patch containing *P. emblica* extract nanoliposomes formula no. 9

4. Physical evaluation of facial patch containing *P. emblica* extract nanoliposomes

The facial patch containing *P. emblica* extract nanoliposomes gave a transparent with colorless to opalescence film. The mechanical properties of facial patch containing *P. emblica* extract nanoliposomes were shown in Table 10.

Table 10 Mechanical properties data of facial patch containing *P. emblica* extract nanoliposomes formula no.9

Formula	Thickness (mm.)	Tensile strength (MPa)	% Elongation	Young's modulus (MPa)	Work of failure (MJ)	Appearance of the patch
9	0.08±0.005	40.3±4.32	37.32±5.31	246.1±12.21	2.13±2.3	Soft, tough

5. Determination of *P. emblica* extract

The *P. emblica* extract demonstrated a major peak of gallic acid 270 nm at the resolution time 7.906 min. The chromatogram of *P. emblica* extract was shown in Figure 24.

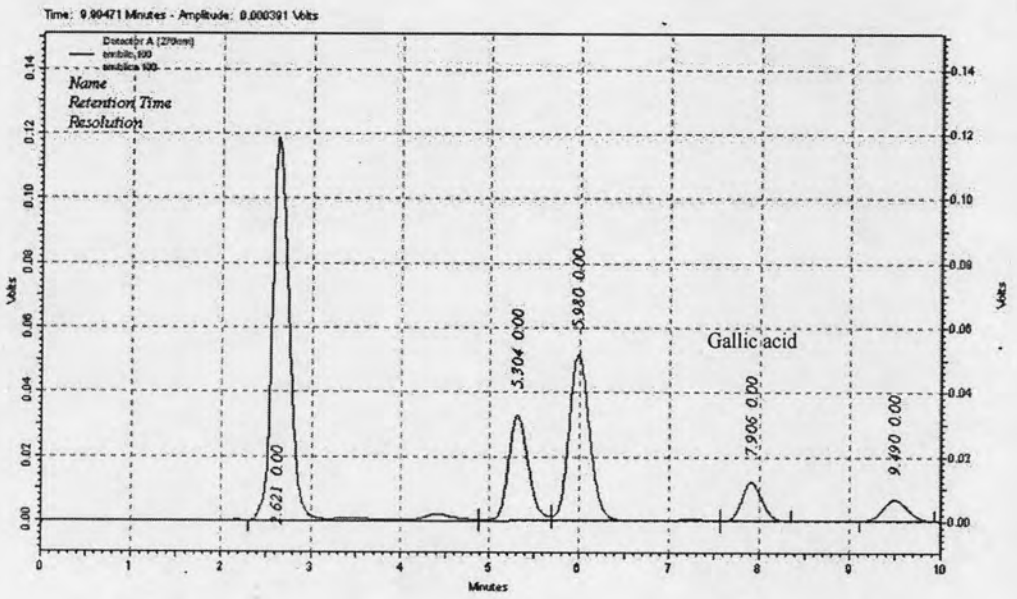


Figure 24 The HPLC chromatogram of *P. emblica* extract

5.1 Quantitative analysis of gallic acid using High Performance Liquid Chromatography (HPLC)

The calibration curve of standard gallic acid was shown in Figure 25. The data of validation of HPLC method of gallic acid was shown in Appendix II. The concentration of *P. emblica* extract 1% has the concentration of gallic acid 0.15%.

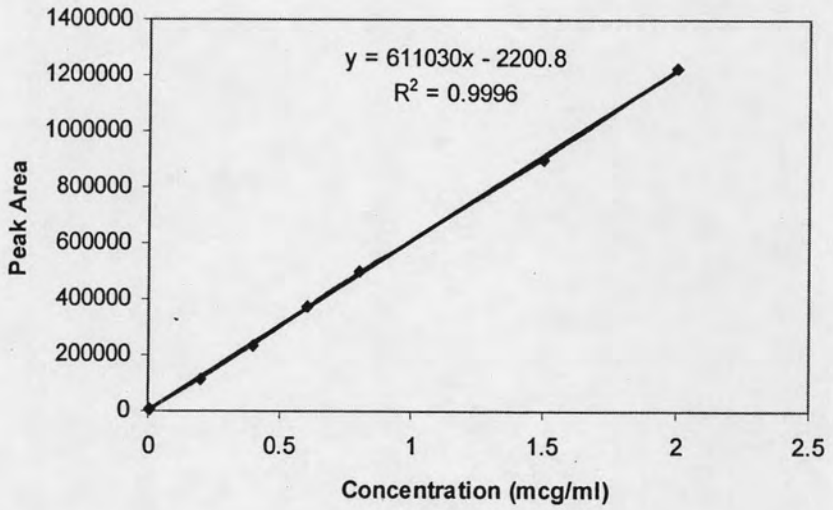


Figure 25 Calibration curve of the standard gallic acid .

6. *In vitro* diffusion study of *P. emblica* extract from nanoliposomes from the facial patch

The *in vitro* release of *P. emblica* extract nanoliposomes from the facial patch was studied using modified Franz's diffusion cell. The release profile of the facial patches containing *P. emblica* extract and the facial patches containing *P. emblica* extract nanoliposomes (n=3) were shown in Table 11 and Table 12, respectively. The concentration of *P. emblica* extract in the facial patches was 3 mg/cm^2. From the Table 11, the concentration of *P. emblica* extract was released more than 99% within 4 hours.

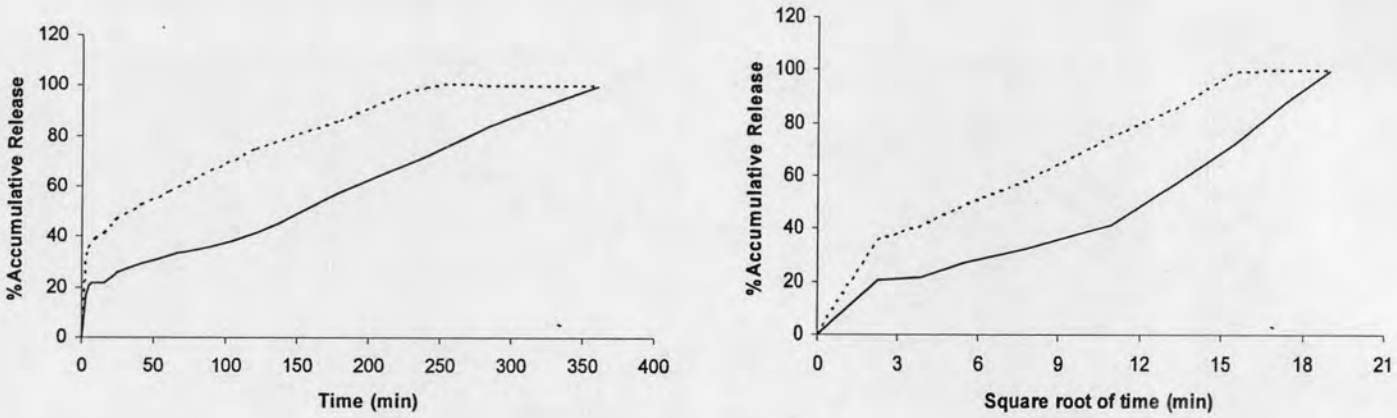
Table 11 The release profile of the facial patches containing *P. emblica* extract

Time (min)	Gallic acid (mcg/ml)	total gallic acid in franz cell	cumulative amount gallic acid in franz	%Accumulative Release	$(Time)^{1/2}$
5	0.1340	1.8760	2.8346	35.4321	2.2361
15	0.2239	3.1351	3.2691	40.8640	3.8730
30	0.2622	3.6708	3.8947	48.6836	5.4772
60	0.3116	4.3625	4.6247	57.8086	7.7460
120	0.4021	5.6298	5.9414	74.2675	10.9545
180	0.4627	6.4779	6.8800	86.0002	13.4164
240	0.5330	7.4621	7.9249	99.0607	15.4919
300	0.5319	7.4466	7.9796	99.7452	17.3205
360	0.5306	7.4280	7.9599	99.4986	18.9737

From Table 12, the concentration of *P. emblica* extract was released from nanoliposomes in facial patch more than 99% within 6 hours. The results show that facial patch containing *P. emblica* extract nanoliposomes could prolong the release of *P. emblica* extract comparing to the facial patch containing *P. emblica* extract as shown in Figure 26.

Table 12 The release profile of the facial patches containing *P. emblica* extract nanoliposomes

Time (min)	Gallic acid (mcg/ml)	total gallic acid in franz cell	cumulative amount gallic acid in franz	%Accumulative Release	$(Time)^{1/2}$
5	0.0770	1.0775	1.6168	20.3374	2.2361
15	0.1186	1.6597	1.7367	21.8451	3.8730
30	0.1453	2.0344	2.1530	27.0814	5.4772
60	0.1733	2.4256	2.5709	32.3387	7.7460
120	0.2226	3.1169	3.2902	41.3862	10.9545
180	0.3107	4.3505	4.5731	57.5233	13.4164
240	0.3882	5.4341	5.7449	72.2624	15.4919
300	0.4714	6.5997	6.9879	87.8978	17.3205
360	0.5335	7.4690	7.9404	99.8793	18.9737



(a) **(b)**

Figure 26 The release of *P. emblica* extract from facial patch

- - - Facial patch containing *P. emblica* extract

——Facial patch containing *P. emblica* extract nanoliposomes

(a) A plot of % Accumulative release against time

(b) A plot of % Accumulative release against $(time)^{½}$

Figure 26 (a) of facial patch containing *P. emblica* extract show exponential curve when plot % accumulative release versus time and also show linearity when plot % accumulative release versus square root of time. There are three stages involved in Figure 26 (b) which are burst stage, release stage, and maintained stage. The burst stage occurs at the very beginning of the graph while the release stage is considered when the graph shows an increased straight line and the maintained stage occurs when the graph shows the stable line at last. The results show that facial patch containing *P. emblica* extract operates under Higuchi condition. While, Figure 26 (a) of facial patch containing *P. emblica* extract nanoliposomes show linearly which it operates under zero order, indicating that the released rate of facial patch containing *P. emblica* extract nanoliposomes is independent of the concentrations.

7. *In vivo* skin moisturizing and elasticity efficacy test of facial patch containing *P. emblica* extract nanoliposomes

Moisturizing effect of facial patches containing *P. emblica* extract nanoliposomes

Skin hydration was evaluated by Skin Diagnostic SD 27. The forehead was used as tested area. The results of skin hydration was measured as moisture content at tested area after using facial patches containing *P. emblica* extract nanoliposomes of each group were shown in Table 13.

Table 13 The results of average skin hydration after using facial patches containing *P. emblica* extract nanoliposomes from week 0, 1, 2, 3, and 4 (n=3)

Group	Subject	Moisture content (Right forehead)					Moisture content (Left forehead)				
		Week 0	Week 1	Week 2	Week 3	Week 4	Week 0	Week 1	Week 2	Week 3	Week 4
Liposomes	1	32.67	45	44	48.33	50	30.67	34.67	26.67	32.67	46.33
	2	42	46	48.33	54.33	55.67	51.33	57.67	62	64	68.67
	3	12.33	22.33	28.33	31	36.33	3.33	27.67	28	30.33	30.67
	4	2.67	16.67	26	23.67	29.67	19.33	26.67	31.67	35	39.67
	5	47	30	30.33	29	32.67	4	24.67	28	34.33	31.67
	6	22.33	48.33	38.67	48.33	47	47	49	46.33	48.33	50.33
	7	48.33	41.67	46	46.67	46.33	22	39.33	33.67	36.67	41.67
Emblica	8	27	39.67	44	57	58	60.67	54	39.67	66.67	69.67
	9	26.33	35.67	40.67	29.67	46.67	28.67	38	42	48.67	60.33
	10	20.37	45.33	31.67	47	49.67	34.67	56	55.33	61	67.67
	11	27.67	19.33	17.67	35.33	37	10.33	13.33	29.33	37	42
	12	0	34	40.67	44.67	49	33	36	39.67	51.67	59.67
	13	49.33	8.67	15.67	24.67	33	2	18.67	29	39.67	45
	14	49.33	52.67	52.67	44.67	50	43	52.33	62.33	63	75
Liposomes + Emblica	15	18.33	32.67	37.67	40.33	37.33	18.67	35.67	39	47.67	50.67
	16	50.67	53.67	52.33	56	53	46.67	60	61.67	65.67	74.33
	17	2.67	16	24	29	28.33	7.67	30	34	36	50.67
	18	7	22	25.33	27.67	30	13	21.33	35.67	37.67	48.67
	19	12	12.33	15	20.33	22.33	14.33	16.67	29.67	32	38.67
	20	41.33	41.67	46.67	56.67	56.33	29	42.67	55.33	62.67	63.67
	21	19	24.67	22.67	32.33	31.67	10.67	23.67	35.67	44	51.33

From Table 13, the p-value < 0.05, therefore the moisture content was significantly increased due to an increased of skin hydration. This result could explain that the moisture was more up taken into the skin, thus the facial patches containing *P. emblica* extract nanoliposomes can keep the moisture content onto the skin.

The average values of moisture content of each group from week 0 to week 4 were shown in Table 14 and the pairwise comparison of each group was shown in Table 15. The results show that the group of Liposomes + Emblica was significantly different from the control group.

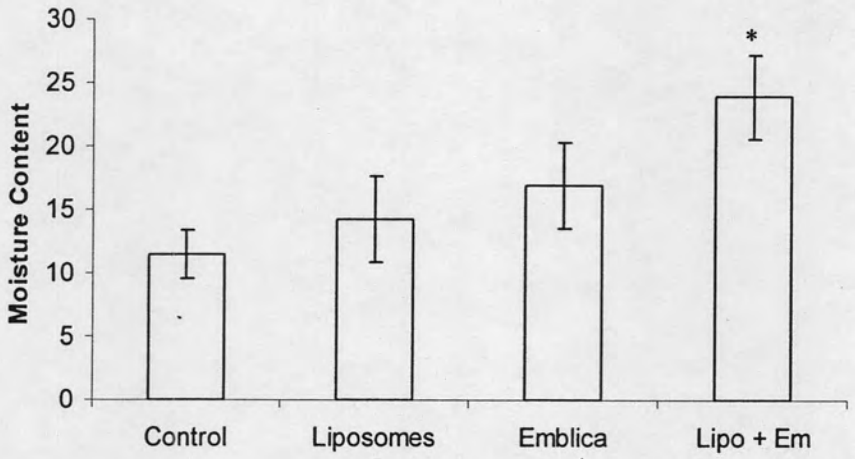
Table 14 Estimated marginal means of moisture content of each group

Group	Mean	Std. Error
Control	11.492	1.930
Liposomes	14.238	3.342
Emblica	16.905	3.342
Liposomes + Emblica	23.905	3.342

Table 15 Pairwise comparison of each group by SPSS program

Group (I)	Group (J)	Mean difference (I-J)	Std. Error	Sig.(a)
Control	Liposomes	-2.746	3.859	0.481
	Emblica	-5.413	3.859	0.169
	Liposomes + Emblica	-12.413 (*)	3.859	0.003

* = Significant difference ($p < 0.05$)



* = Significant difference ($p < 0.05$)

Figure 27 Average moisture content of each group after using facial patch containing different formula

Figure 27 show the results of average skin hydration after using facial patch containing different formulas; liposomes, emblica extract, and *P. emblica* extract nanoliposomes.

Table 16 and Figure 28 show the average value of skin hydration of each group after using the patches for 4 weeks. The results show that the group of liposomes + emblica gives the highest skin hydration comparing to the other groups.

Table 16 Average value of skin hydration of each group after using the patches for the last 4 weeks

Group	Week	Moisture content
Control	0	25.81
	1	32.78
	2	34.68
	3	39.84
	4	41.9
Liposomes	0	25.38
	1	37.1
	2	37.05
	3	40.19
	4	44.14
Emblica	0	30.33
	1	38.33
	2	42.48
	3	52.52
	4	59.9
Liposomes + Emblica	0	20
	1	32.86
	2	40.71
	3	46.52
	4	54 (*)

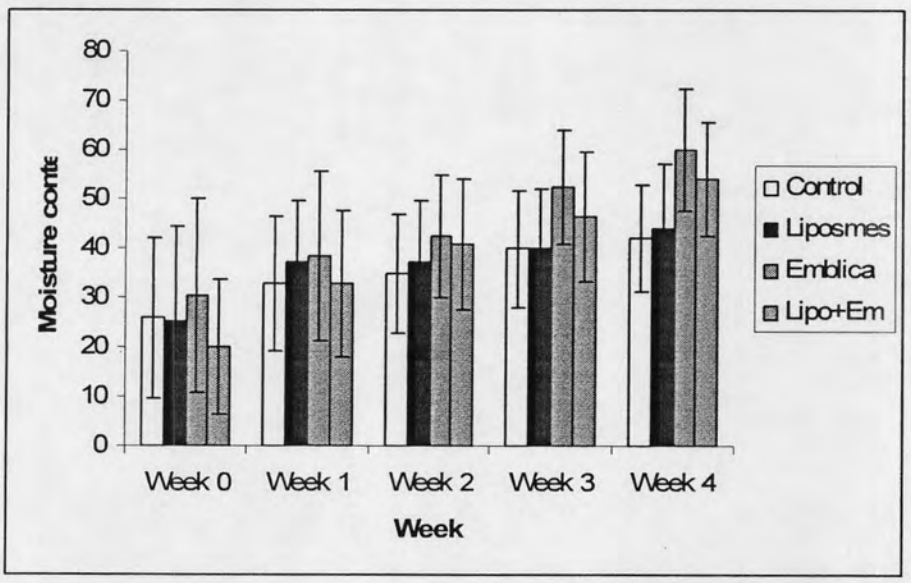


Figure 28 Graphs of average value of skin hydration of each group after using the facial patches for the last 4 weeks

The elasticity measurement using DermaLab® Elasticity probe

The elasticity (Young's modulus value) was measured by DermaLab® Elasticity probe, the differences of Young's modulus between week 0 and 4 were calculated. The macroscopic photograph was observed to evaluate an improvement of the area of tested. Three measurements were performed in each testing and were shown in Table 17. The Young's modulus was reduced as a result of using the facial patches containing *P. emblica* extract nanoliposomes which resulted in increase the elasticity of the skin significantly ($p < 0.05$).

Table 17 The Young's modulus of skin on forehead in 21 volunteers

Group	Subject	Young's modulus (Right forehead)					Young's modulus (Left forehead)				
		Week 0	Week 1	Week 2	Week 3	Week 4	Week 0	Week 1	Week 2	Week 3	Week 4
Liposomes	1	11.96	12.07	11.58	11.87	11.27	10.87	10.63	10.61	10.24	9.39
	2	10.26	9.58	8.80	9.89	9.55	10.80	8.50	8.50	8.48	9.33
	3	12.14	12.56	11.58	10.93	11.12	11.91	11.04	10.89	11.34	10.49
	4	11.51	11.70	10.84	10.71	11.43	11.09	10.39	11.00	10.91	9.80
	5	10.46	11.75	10.57	10.64	10.85	10.75	9.95	10.78	9.62	9.59
	6	9.18	7.90	7.06	7.98	8.63	10.00	8.06	8.91	9.19	7.37
	7	10.3	10.31	9.83	9.47	8.36	9.43	9.25	9.98	9.24	8.30
Emblica	8	10.80	9.87	9.32	8.71	9.14	10.40	9.58	8.55	7.76	7.73
	9	9.54	10.58	8.57	10.51	9.03	10.74	10.54	9.53	8.80	8.63
	10	11.78	11.60	11.31	10.32	10.02	12.34	11.89	10.44	10.61	9.52
	11	11.7	10.95	10.90	11.06	9.81	11.75	10.61	9.67	9.72	8.43
	12	11.34	8.77	10.44	9.96	9.47	9.95	8.24	9.76	9.91	8.80
	13	10.58	10.41	9.77	10.00	9.86	9.80	9.49	8.79	9.08	8.43
	14	10.51	9.36	10.60	9.31	9.90	10.68	9.36	8.11	8.09	8.28
Liposomes + Emblica	15	10.46	10.81	10.39	10.35	9.87	11.32	10.66	9.38	9.48	7.98
	16	11.45	11.21	10.68	11.02	11.10	11.18	10.73	10.01	8.73	8.29
	17	12.79	12.35	11.37	12.18	11.59	13.23	12.05	10.63	9.23	9.19
	18	9.59	9.83	10.07	9.39	9.25	10.55	10.43	9.65	8.35	7.75
	19	11.17	11.08	10.47	9.69	10.28	10.96	10.54	9.49	8.83	8.08
	20	10.74	10.30	9.86	9.43	9.23	11.41	10.04	9.32	9.37	8.06
	21	12.98	12.40	11.85	11.38	11.17	12.39	11.60	9.51	9.31	9.01

The Young's modulus value of the skin was reduced. Thus, the elasticity of the skin was increased resulted in elevating and firming of the skin at the forehead and the wrinkle was also reduced as well.

The average values of Young's modulus of each group from week 0 to week 4 were shown in Table 18 and the pairwise comparison of each group was shown in Table19. The results show that the group of Liposomes + Emblica was significantly different from the control group. Young's modulus of each group after using facial patch containing different formulas were shown in Figure 29

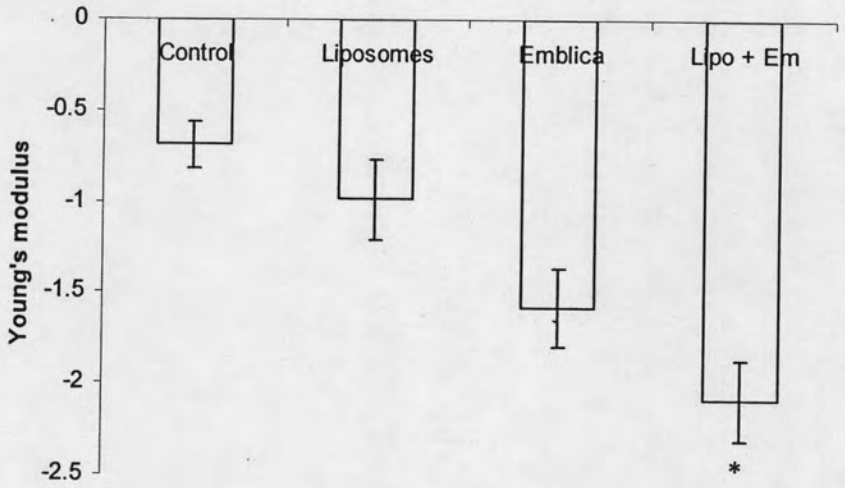
Table 18 Estimated marginal means of Young's modulus of each group

Group	Mean	Std. Error
Control	-0.691	0.125
Liposomes	-0.988	0.216
Emblica	-1.579	0.216
Liposomes + Emblica	-2.085	0.216

Table 19 Pairwise comparison of each group by SPSS program

Group (I)	Group (J)	Mean difference (I-J)	Std. Error	Sig.(a)
Control	Liposomes	0.297	0.250	0.241
	Emblica	0.889	0.250	0.001
	Liposomes + Emblica	1.394 (*)	0.250	0.000

* = Significant difference ($p < 0.05$)



* = Significant difference ($p < 0.05$)

Figure 29 Average Young's modulus of each group after using facial patch containing different formulas

From the macroscopic photograph of the forehead area in 21 volunteers compared between before and after 4 weeks application. Also, the facial patches containing *P. emblica* extract nanoliposomes could reduce the wrinkle line significantly from the observation. The results were conformed by the macroscopic photograph of the skin as shown in Figure 31.

Table 20 and Figure 30 show the average value of Young's modulus of each group after using the facial patches for 4 weeks. The results show that the Young's modulus was the most reduced in liposomes + emblica group.

Table 20 Average value of Young's modulus of each group after using the facial patches for the last 4 weeks

Group	Week	Young's modulus
Control	0	11.01
	1	10.74
	2	10.28
	3	10.23
	4	10.05
Liposomes	0	10.70
	1	9.69
	2	10.10
	3	9.86
	4	9.19
Emblica	0	10.81
	1	9.96
	2	9.27
	3	9.14
	4	8.56
Liposomes + Emblica	0	11.58
	1	10.88
	2	9.72
	3	9.05
	4	8.34 9 (*)

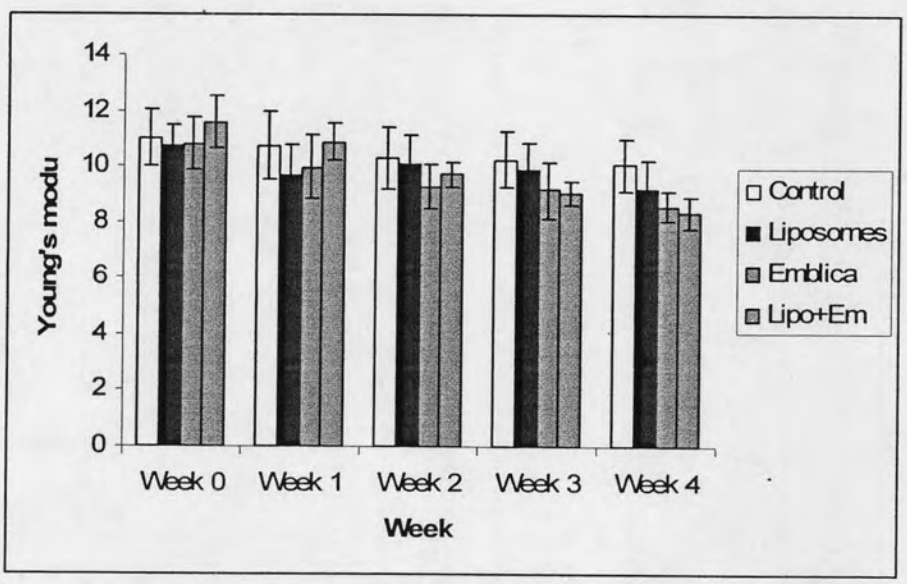
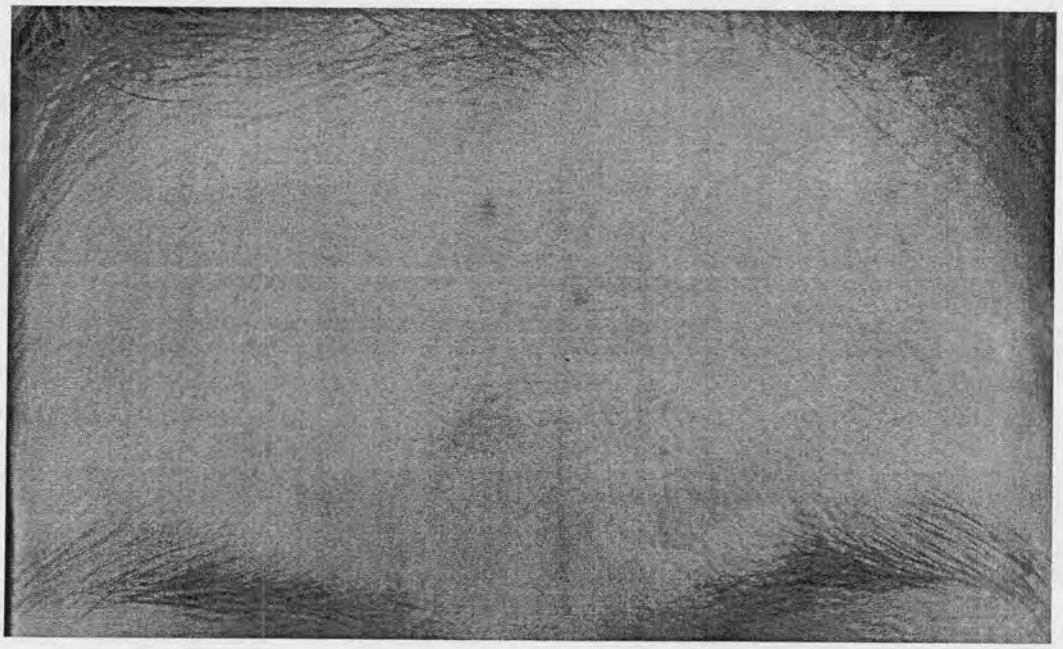
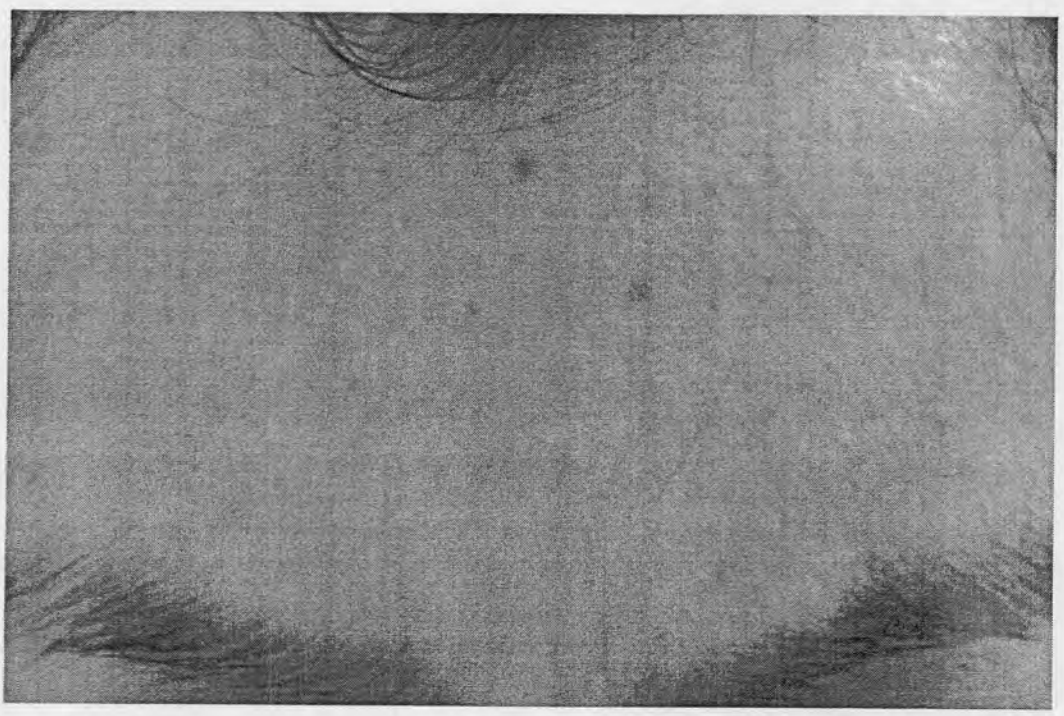


Figure 30 Graphs of average value of Young's modulus of each group after using the facial patches for the last 4 weeks



(a)



(b)

Figure 31 The macroscopic photograph of the wrinkle line: (a) before application and (b) after application