CHAPTER IV

RESULTS AND DISCUSSION

4.1 Morphological characteristics of *Momordica cochinchinensis* (Lour.) Spreng

The morphological characteristics of *Momordica cochinchinensis* (Lour.) Spreng fruit (Figure 14): the spines were smooth and dense. The oblong types were 8-12 cm in length and round types are 7-10 cm in length. The fruit weighs between 400 g and 600g. The mesocarp was about 1.25 cm thick, spongy and orange. The core was divided into cartilaginous chambers containing bright red fleshy seed pods. Each fruit had on average between 20 to 25 rounded, compressed and sculptured seeds. The aril and kernels contained oil. The average weight of the aril was about 10% of the total fruit weight. An average fruit weighing 1 kg yields approximately 100 g of fruit pulp and 120 g of seeds. The aril of a ripe fruit was bright red in color and has a palatable bland to nutty taste.

Figure 10 The characterization of *Momordica cochinchinensis* (Lour.) Spreng fruit

4.2 The aril oil of *Momordica cochinchinensis* (Lour.) Spreng

The wet aril should be dried in a hot air oven under 60°C because carotenoids was oxidized and degraded in high temperature condition. Dried aril (Figure 11) was heated slightly before pressing for oil because heating improves yield of oil, and separation of carotenoids from the plant protein. The aril oil was bright red (Figure 12).

The aril oil yield was 15% by weight of wet aril (Table 10).

Figure 11 The dried aril with seeds of *Momordica cochinchinensis* (Lour.) Spreng

Figure 12 The aril oil of *Momordica cochinchinensis* (Lour.) Spreng

Table 10 The percent yield of aril oil of *Momordica cochinchinensis* (Lour.) Spreng

Wet aril (g)	Dried aril (g)	Oil (g)	%Yield (g/100 g of wet aril)
100.0	75.0	15.0	15.0

4.3 Determination of antioxidant activities of aril oil of *Momordica cochinchinensis* (Lour.) Spreng

In the present study, antioxidant activity was measured by two different methods, namely 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

and ferric reducing antioxidant power (FRAP) assay. Both these assays have been frequently used to assess antioxidant activity. FRAP reflects total antioxidant power involving the single electron transfer reaction, whereas DPPH is based on free radical scavenging activity (71).

4.3.1 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of aril oil in butanol solution had shown in Figure 13, exhibited with IC_{50} of 6.31 ± 0.14 mg/ml. And the antioxidant activity of (±)-α-tocopherol in butanol solution had shown in Figure 14, with IC_{50} of 15.48 ± 0.08 µg/mL. In this analysis, the results shown that the antioxidant activity of aril oil was about 407 fold lower compared to (±)-α-tocopherol.

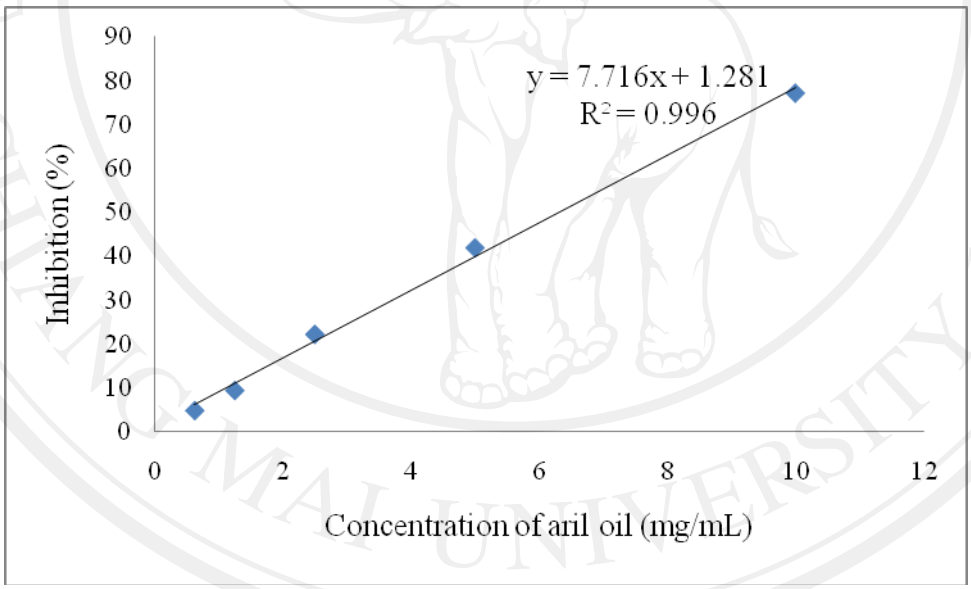


Figure 13 Linear regression plot of antioxidant activity against concentration of aril oil by DPPH assay

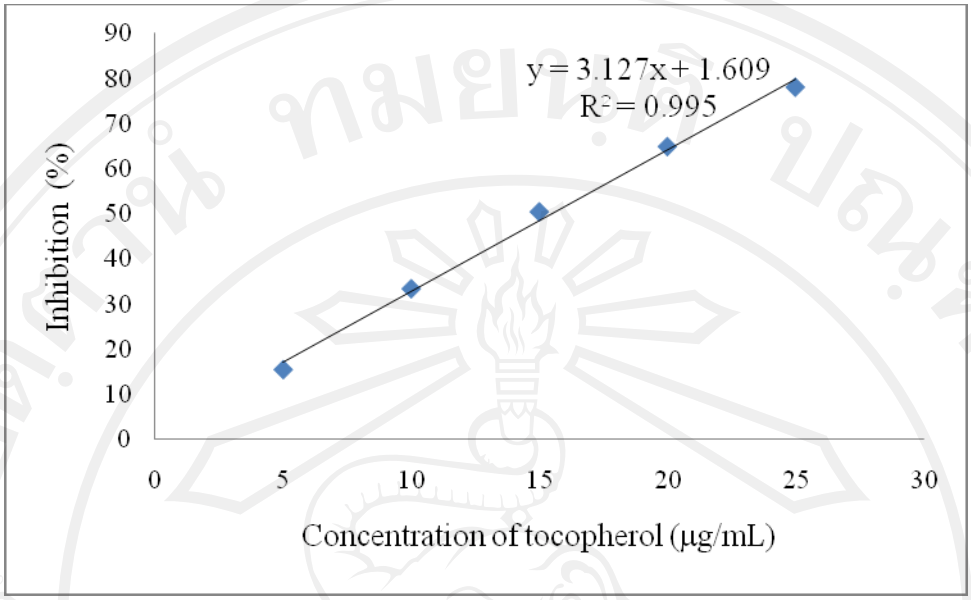


Figure 14 Linear regression plot of antioxidant activity against concentration of (±)-α-tocopherol standard by DPPH assay

4.3.2 Ferric reducing antioxidant power (FRAP) assay

The aril oil of *M. cochinchinnensis* exhibited about 106 folds lower antioxidant power with EC_1 values of 1.89±0.31 mM when compared to (±)-α-tocopherol having EC_1 values of 200.81±0.26 mM/mg (Figure 15). EC_1 value was calculated from absorbance/mg of sample prepared with standard curve of $FeSO_4.7H_2O$. The values are means ± standard deviation (n = 3).

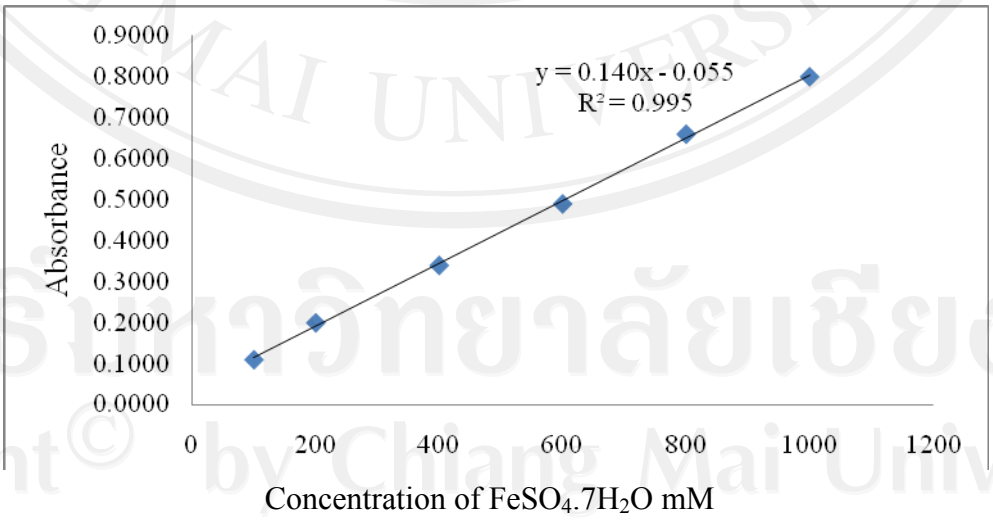


Figure 15 Linear regression plot absorbance against concentration of $FeSO_4.7H_2O$ standard by FRAP assay.

4.4 Determination of total carotenoids of aril oil of *Momordica cochinchinensis* (Lour.) Spreng

The total carotenoids concentration of aril oil was exhibited about 2,374.00 ± 5.525 ppm in Table 11.

Table 11 The absorbance and total carotenoids concentration of aril oil of *Momordica cochinchinnensis* (Lour.) Spreng

Sample	Absorbance (at 446 nm)	Total carotenoids (ppm)
1	0.742	2,368.216
2	0.745	2,377.792
3	0.745	2,377.792
Mean ± SD		**2374.00 ± 5.52**

4.5 Reversed phase - High Performance Liquid Chromatography (RP-HPLC) analysis

4.5.1 Calibration curve of β-carotene standard

Chromatogram of calibration curve of β-carotene standard between peak area of β-carotene (Y) and β-carotene concentration (X) were presented in figure 16 and 17, respectively. β-carotene standard could be separated absolutely by RP-HPLC with retention time of 13.0 min

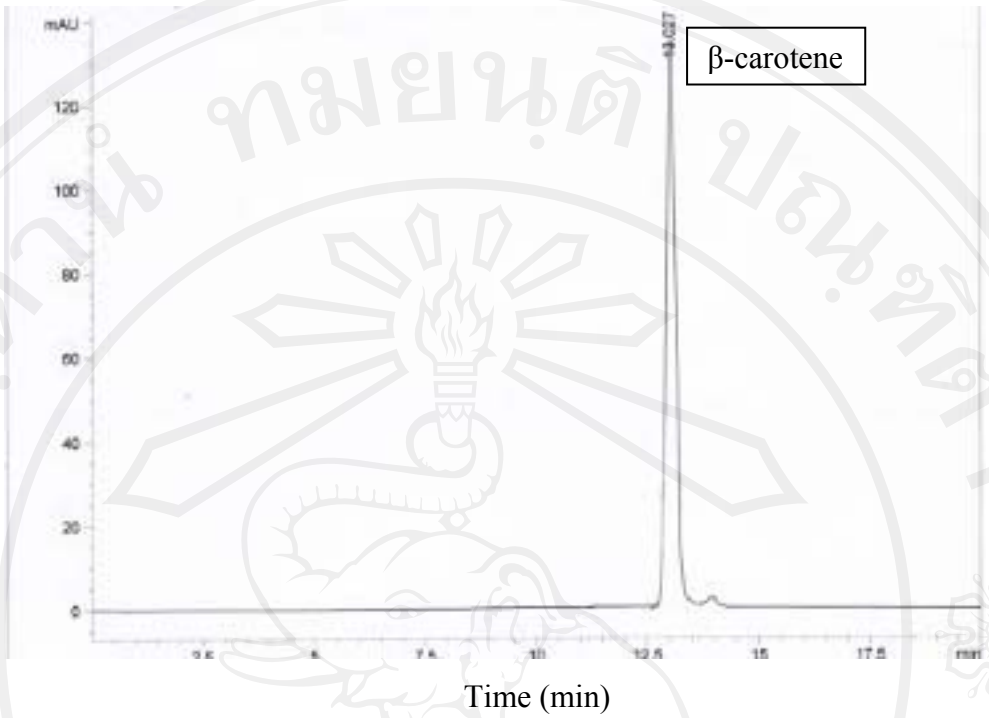


Figure 16 Chromatogram of β-carotene standard using RP-HPLC system

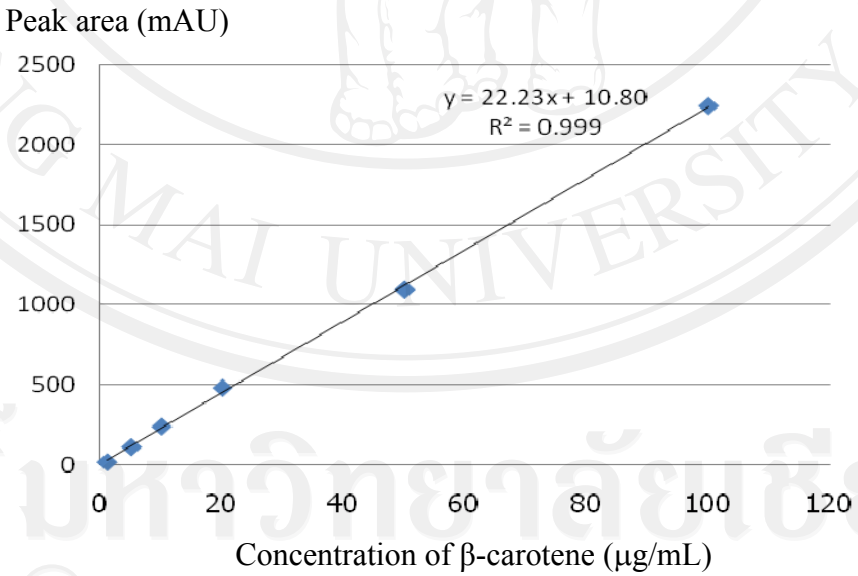


Figure 17 Standard curve of β-carotene standard

4.5.2 Analytical validation of HPLC equipment

Precision

The precision of the analytical method was presented in Table 12. The repeatability and the reproducibility coefficient of variation (%CV) of peak areas at each concentration of β-carotene standard varied from 20 to 100 µg/mL ranged from 0.49-1.90% and 1.12–1.91%, respectively.

Accuracy

The accuracy of the analytical method was presented in Table 13. The repeatability and the reproducibility coefficient of variation (% inaccuracy) of peak areas at each concentration of β-carotene standard varied from 20 to 100 µg/ml ranged from 1.17–4.51% and 1.29–3.97%, respectively.

Both of the percentage of coefficient of variation and the percentage of inaccuracy were in acceptable limit (<15%). These results indicated that the established HPLC method was suitable for the determination of β-carotene.

Table 12 The precision of HPLC method for standard peak of β-carotene

Target concentration (µg/mL)	Repeatability (n = 5)		Reproducibility (n = 5)	
	Mean ± SD of measured concentration (µg/mL)	% CV	Mean ± SD of measured concentration (µg/mL)	% CV
10	9.71±0.184	1.90	9.87±0.188	1.91
20	19.88±0.322	1.62	19.72±0.220	1.12
50	49.54±0.242	0.49	49.60±.0638	1.28

Table 13 The accuracy of HPLC method for standard peak of β-carotene

Target concentration (µg/mL)	Repeatability (n = 5)		Reproducibility (n = 5)	
	Mean ± SD of measured concentration (µg/mL)	% inaccuracy	Mean ± SD of measured concentration (µg/mL)	% inaccuracy
10	9.71±0.184	2.86	9.87±0.189	1.29
20	19.88±0.322	1.17	19.72±0.220	2.85
50	49.54±0.243	4.51	49.60±0.638	3.97

4.5.3 Determination of β-carotene of aril oil of *Momordica cochinchinensis* (Lour.) Spreng

The aril oil solution (20 mg/mL) was injected into the HPLC column. The peak area of the aril oil solution at the same retention time (13.00 min) as β-carotene standard (Figure 18) was calculated for β-carotene of concentration using the calibration curve equation in Table 14 as follows equations (6) and (7):

$$Y = 22.23X + 10.80 \quad (6)$$

$$R^2 = 0.999 \quad (7)$$

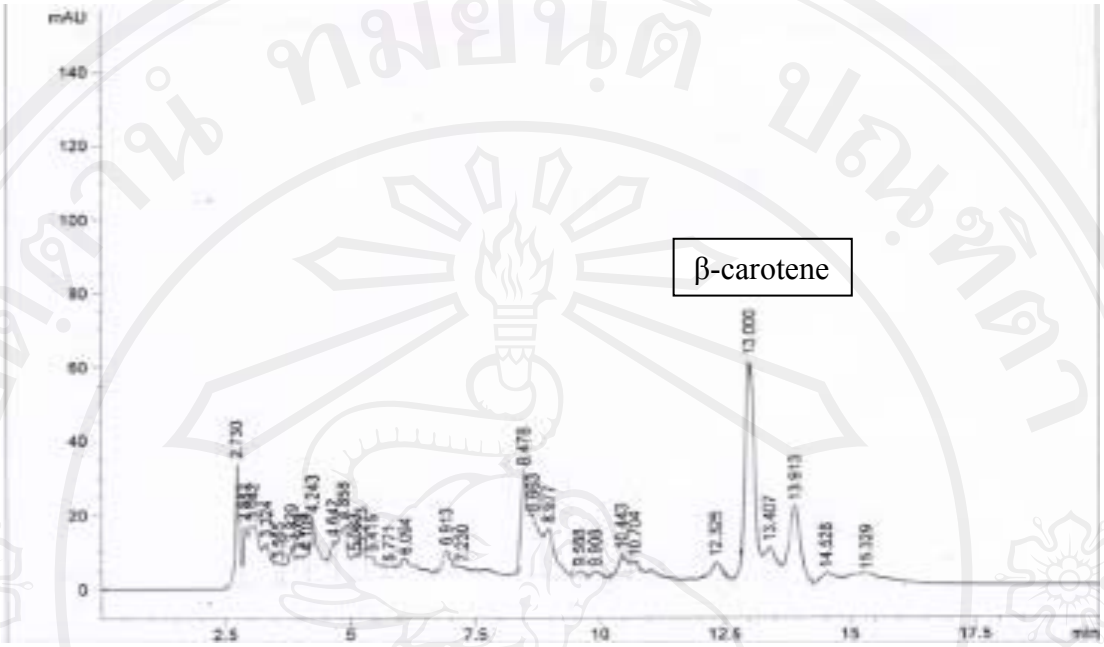
Where:

Y is the peak area

X is the concentration of sample

R^2 is the linear regression

Peak area (mAU)



Time (min)

Figure 18 Chromatogram of aril oil of *Momordica cochinchinensis* (Lour.) Spreng

Table 14 The concentration of β-carotene of aril oil of *Momordica cochinchinesis* (Lour.) Spreng

Calculation of β-carotene	
The average of peak area of the aril oil solution (20 mg/ml)	189.33±0.78
Concentration of β-carotene of the aril oil solution	8.03 µg/mL
Concentration of β-carotene in the aril oil	401.55 µg/g

4.6 Preparation of nanostructured lipid carriers (NLC)

4.6.1 Optimal condition for NLC preparation

Homogenization was one of the most frequently used techniques for preparing lipid nanoparticles. NLC were produced by hot high pressure homogenizer (HPH) following the compositions of formulation ingredients in Table 8.

Homogenization pressure could influenced the properties of emulsion as the shear forces and turbulence, both of which were pressure dependent, produced during homogenization could affected the Mean particle size, polydispersity index (PI) and zeta potential (ZP) values. As shown Table 15, increasing the homogenization

pressure resulted in decreases in the particles size and particle size distribution. However, the effect of pressure on zeta potential values did not show a consistent trend.

The effect of homogenization cycle on the properties of NLC was presented in Table 16. As expected, increasing the homogenization cycle resulted in decreasing in the particles sizes and particle size distribution. However, after eight homogenization cycle, a minor improvement on the particles size and particle size distribution was observed with fifth cycles of homogenization. Furthermore, the effect of pressure on zeta potential values did not show a consistent trend.

Table 15 Mean particle size, polydispersity index (PI) and zeta potential (ZP) values of the NLC formulation prepared at different homogenization pressure and homogenization cycle 5 cycles (n = 3)

Pressure (bar)	Mean particle size (nm)	PI	ZP (mV)
600	594 ± 4.6	0.48 ± 0.019	-27.3 ± 1.28
800	333 ± 4.5	0.54 ± 0.062	-31.4 ± 0.33
1000	142 ± 1.4	0.29 ± 0.017	-31.8 ± 0.46

Table 16 Mean particle size, polydispersity index (PI) and zeta potential (ZP) values of the NLC formulation prepared at different homogenization cycle and homogenization pressure 1000 bar (n = 3)

Cycle	Mean particle size (nm)	PI	ZP (mV)
3	557 ± 12.4	0.59 ± 0.076	-30.0 ± 0.152
5	142 ± 1.4	0.29 ± 0.017	-31.8 ± 0.460
8	137 ± 2.5	0.25 ± 0.013	-30.3 ± 0.755

4.6.2 Preparation aril oil of *Momordica cochinchinesis* (Lour.) Spreng loaded NLC

The three different concentrations of aril oil loaded NLC were developed by hot high pressure homogenizer (HPH) following the compositions of formulation ingredients in Table 17. Comparing formulations A1, A2 and A3 (1%, 3% and 5% aril oil, respectively), it was observed that the higher aril oil loading in NLC, the higher the particles size was obtained. However, the effect on particle size distribution and zeta potential values did not show a consistent trend.

Table 17 Mean particle size, polydispersity index (PI) and zeta potential (ZP) values of the three different concentrations of aril oil loaded NLC formulation prepared at homogenization pressure 1000 bar and homogenization cycle 5 times (n = 3)

Formulation	Mean particle size (nm)	PI	ZP (mV)
A1	130 ± 0.7	0.24 ± 0.047	-39.1 ± 0.34
A3	140 ± 1.7	0.23 ± 0.029	-37.8 ± 1.39
A5	146 ± 0.2	0.23 ± 0.037	-38.2 ± 1.44

The tone of the color increased with the increasing concentration of aril oil in formulation was shown in Figure 19. Because of the deep orange color of the NLC formulations contained 3 and 5% aril oil, they also formed yellowish orange when applied to the skin. Thus the NLC formulations contained 1% aril oil was selected to put in the selected cream base.

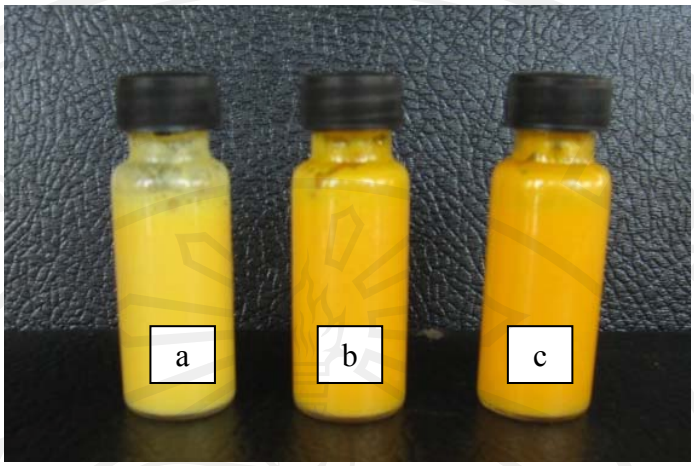


Figure 19 The NLC formulations contained three concentration of aril oil (a = 1% of aril oil; b = 3% of aril oil; c = 5% of aril oil)

4.6.3 Characterization of aril oil of *Momordica cochinchinensis* (Lour.) Spreng loaded NLC

After measurement particle size, polydispersion index and zeta potentail values by PCS, the morphology of NLC blank and aril oil loaded NLC was determined using a transmission electron microscopy (TEM) as described in section 3.6.3. The characteristics were round in shape and homogeneous shading. The morphology of NLC blank and aril oil loaded NLC were shown in figure 20 and 21, respectively. The particle size ranging approximately from 150 to 200 nm that correlated in the mean particle size and polydispersion index were shown in section 4.5.1 and 4.5.2.

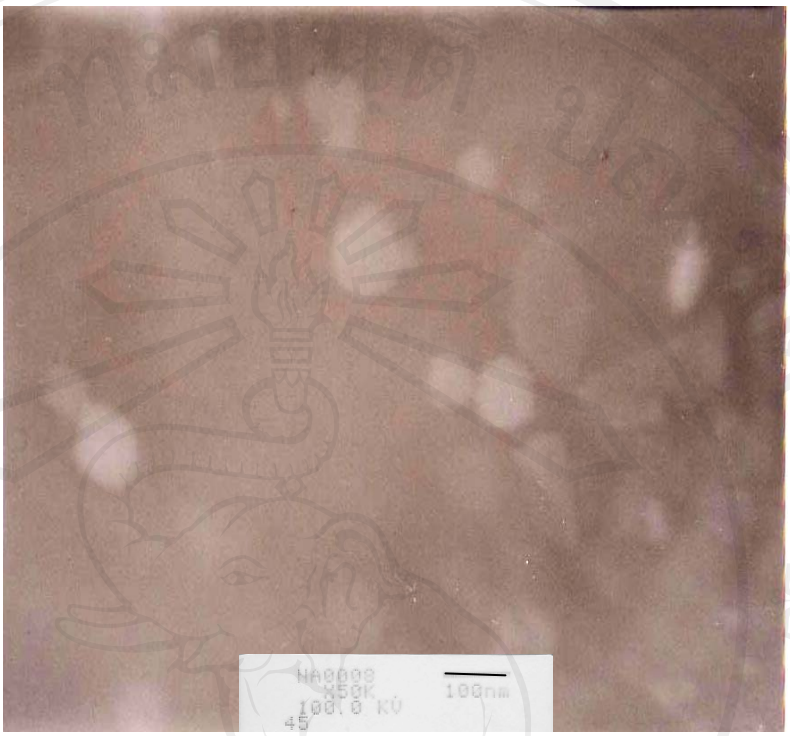


Figure 20 TEM photography of blank NLC

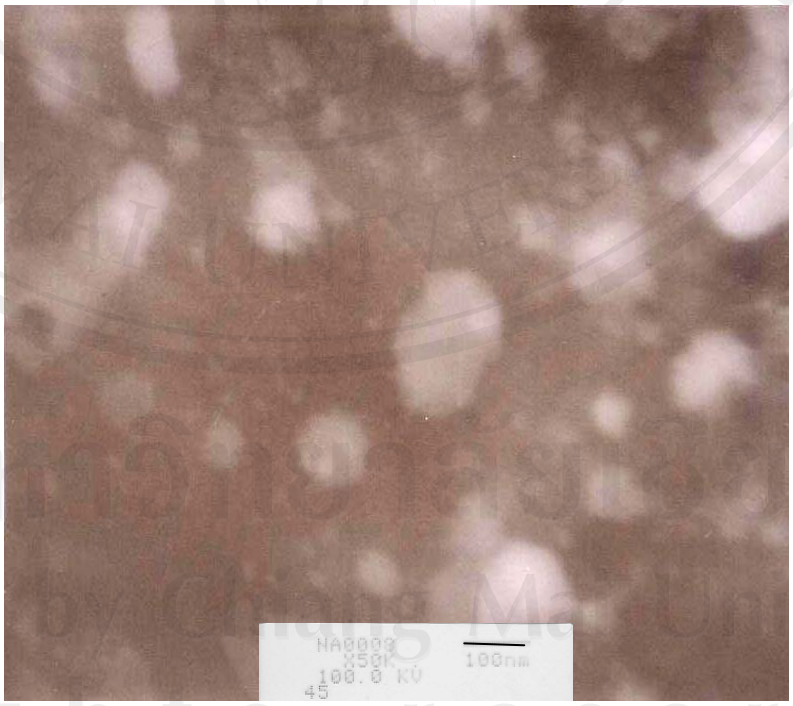


Figure 21 TEM photography of aril oil-loaded NLC

4.6.2 Stability test of the NLC and aril oil-loaded NLC formulation

After analysis of the obtained particle size and polydispersity index results of the NLC, 1% aril oil-loaded NLC (1% A-NLC), 3% aril oil-loaded NLC (3% A-NLC) and 5% aril oil-loaded NLC (5% A-NLC) formulations stored at 4°C and 25°C for 90 days (Table 18 - 21); the particles were less than 150 nm and polydispersity index were not above 0.3. And after 90 days at 45°C; the particles were more than 150 nm and polydispersity index were less than 0.3. For all test formulations did not result in particle aggregation.

The measurement of the zeta potential allows predictions about stability of colloidal dispersion. Usually, particle aggregation is less likely to occur for charged particles with high zeta potential (> |30| mV) due to electric repulsion. In general, lipid nanoparticles are negatively charged on the surface. All formulations reveal that during storage time, the zeta potential value of the surface of lipid nanoparticles slightly decreased. However, they were still below -30 mV (Table 18-21).

Table 18 Mean particle size, polydispersity index (PI) and zeta potential (ZP) values of NLC formulation before and after 90 days of storage at 4, 25 and 45°C (n = 3)

Formulation	Mean particle size (nm)	PI	ZP (mV)
NLC day 0 at 25°C	138 ± 1.3	0.25 ± 0.019	-32.1 ± 0.06
NLC day 90 at 4°C	140 ± 1.7	0.26 ± 0.074	-31.7 ± 0.61
NLC day 90 at 25°C	142 ± 0.2	0.26 ± 0.007	-31.4 ± 0.67
NLC day 90 at 45°C	179 ± 1.2	0.30 ± 0.042	-32.2 ± 0.27

Table 19 Mean particle size, polydispersity index (PI) and zeta potential (ZP) values of 1% aril oil-loaded NLC formulation (1% A-NLC) before and after 90 days of storage at 4, 25 and 45°C (n = 3)

Formulation	Mean particle size (nm)	PI	ZP (mV)
1% A-NLC day 0 at 25°C	130 ± 0.6	0.24 ± 0.047	-39.2 ± 0.35
1% A-NLC day 90 at 4°C	132 ± 0.4	0.25 ± 0.051	-38.3 ± 0.47
1% A-NLC day 90 at 25°C	137 ± 1.4	0.26 ± 0.043	-36.3± 0.88
1% A-NLC day 90 at 45°C	161 ± 2.7	0.28 ± 0.019	-33.9± 0.54

Table 20 Mean particle size, polydispersity index (PI) and zeta potential (ZP) values of 3% aril oil-loaded NLC formulation (3% A-NLC) before and after 90 days of storage at 4, 25 and 45°C (n = 3)

Formulation	Mean particle size (nm)	PI	ZP (mV)
3% A-NLC day 0 at 25°C	140 ± 1.7	0.21 ± 0.029	-37.2 ± 0.15
3% A-NLC day 90 at 4°C	142 ± 1.8	0.23 ± 0.020	-34.6 ± 1.39
3% A-NLC day 90 at 25°C	145 ± 0.9	0.23 ± 0.036	-34.4 ± 1.76
3% A-NLC day 90 at 45°C	169 ± 2.1	0.27 ± 0.024	-35.1± 0.35

Table 21 Mean particle size, polydispersity index (PI) and zeta potential (ZP) values of 5 % aril oil-loaded NLC formulation (5% A-NLC) before and after 90 days of storage at 4, 25 and 45°C (n = 3)

Formulation	Mean particle size (nm)	PI	ZP (mV)
5% A-NLC day 0 at 25°C	146 ± 0.2	0.23 ± 0.037	-38.3± 1.44
5% A-NLC day 90 at 4°C	148 ± 0.2	0.24 ± 0.075	-35.2± 1.25
5% A-NLC day 90 at 25°C	141 ± 0.9	0.26 ± 0.029	-34.6 ± 1.51
5% A-NLC day 90 at 45°C	174 ± 0.6	0.29 ± 0.009	-34.4± 0.35

4.7 Formulation cream containing aril oil and cream containing aril oil-loaded NLC

4.7.1 Preparation of cream base and cream containing aril oil

At freshly prepared, the cream base and cream containing aril oil had good appearance with white color and slightly yellowish orange color, respectively. The texture was homogeneous leaving no gritty residue after application. Cream containing aril oil gave a specific odor of its own.

After stability testing; some physical property changed. The pH and viscosity after 8 cycles of the heating-cooling cycling and 90 days of storage at 4, 25 and 45°C had a small change. The results were shown in Table 22 and 23. In physical properties, 90 days of storage at 4 and 25°C all properties were not different from freshly prepared. At 45°C the color of aril oil cream slightly paled but the other properties was not different from freshly prepared. All formulation had no phase separation during study period. The resulted physical properties showed that the cream base had good stability.

Table 22 Physical properties of the cream base before and after heating-cooling cycle and after 90 days of storage at 4, 25 and 45°C

Cream base	pH	Viscosity (Pas)
Day 0 at 25°C	6.30	7.67
Heating-cooling cycle	6.19	7.53
Day 90 at 4°C	6.28	7.51
Day 90 at 25°C	6.27	7.50
Day 90 at 45°C	6.21	7.41

Table 23 Physical properties of the cream containing aril oil before and after heating-cooling cycle and after 90 days of storage at 4, 25 and 45°C

Cream containing aril oil	pH	Viscosity (Pas)
Day 0 at 25°C	6.21	8.56
Heating-cooling cycle	6.18	8.41
Day 90 at 4°C	6.19	8.48
Day 90 at 25°C	6.17	8.43
Day 90 at 45°C	6.13	8.35

4.7.2 Preparation of cream containing NLC and cream containing aril oil-loaded NLC

The prepared cream containing NLC and cream containing aril oil-loaded NLC had good appearance with white color and slightly yellowish orange color, respectively (Figure 22). The texture of containing NLC and cream containing aril oil-loaded NLC were homogenous leaving no gritty residue after application. Cream containing aril oil-loaded NLC gave a specific odor of its own.

After accelerating stability test of the heating-cooling cycling and 90 days of storage at 4, 25 and 45°C, all of the prepared cream containing NLC and cream containing aril oil-loaded NLC exhibited good physical stability as shown by no phase separation. In physical properties, 90 days of storage at 4 and 25°C all properties were not different from freshly prepared. At 45°C the color of cream containing aril oil slightly paled but the other properties was not different from freshly prepared. The pH and viscosity of these formulations had a small change (Table 24 and 25). The resulted physical properties showed that all formulations had good stability.

Table 24 Physical properties of the cream containing NLC before and after heating-cooling cycle and after 90 days of storage at 4, 25 and 45°C

Cream containing NLC	pH	Viscosity (Pas)
Day 0 at 25°C	6.27	4.53
Heating-cooling cycle	6.14	4.27
Day 90 at 4°C	6.23	4.32
Day 90 at 25°C	6.22	4.18
Day 90 at 45°C	6.09	4.09

Table 25 Physical properties of the cream containing aril oil-loaded NLC before and after heating-cooling cycle and after 90 days of storage at 4, 25 and 45°C

Cream containing aril oil loaded NLC	pH	Viscosity (Pas)
Day 0 at 25°C	6.15	4.81
Heating-cooling cycle	6.08	4.42
Day 90 at 4°C	6.16	4.73
Day 90 at 25°C	6.14	4.66
Day 90 at 45°C	6.07	4.36

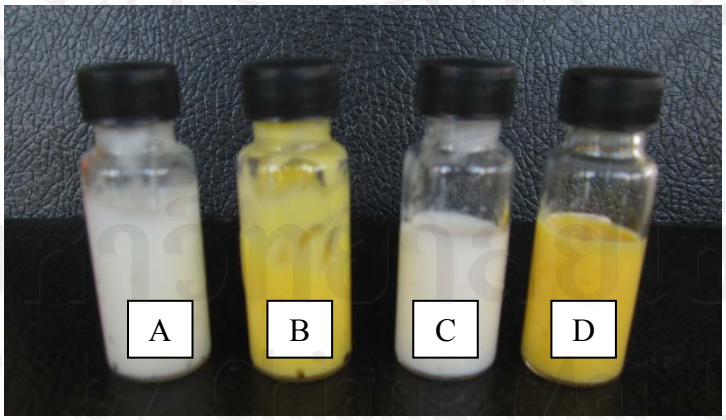


Figure 22 The physical appearance of four formulations (A = cream base; B = cream containing aril oil; C = cream containing NLC; D = cream containing aril oil-loaded NLC)

4.8 Chemical stability studies of β-carotene from cream containing aril oil and cream containing aril oil-loaded NLC for RP-HPLC analysis

The determination of β-carotene content was studies by accurately weighted 20 g of cream containing aril oil and cream containing aril oil-loaded NLC and prepared as followed in section 3.7.3. The solution was injected into RP-HPLC and measured.

Stability testing

The effect of temperature and light on β-carotene degradation reaction is generally recognized. The main reason of β-carotene in aril oil of *Momordica cochinchinnensis* (Lour.) Spreng was instable to the oxidation reaction. This results revealed that the temperature and light had a strong effect on the stability of β-carotene of cream containing aril oil and cream containing aril oil-loaded NLC (compared between 4, 25, 45°C and light) (Table 26 and 27). In all storages, compared between cream containing aril oil and cream containing aril oil-loaded NLC, the percentage of β-carotene of cream containing aril oil was lower than cream containing aril oil-loaded NLC.

Table 26 Percent remaining data of β-carotene. Samples were cream containing aril oil at 4, 25, 45°C and light after 90 days of storage

Cream containing aril oil		
Condition	**β-carotene concentration (μg/mL)**	**% Remaining**
Day 0 at 25°C	3.40±0.060	100.00
Day 90 at 4°C	2.52±0.059	73.98
Day 90 at 25°C	2.03±0.038	59.84
Day 90 at 45°C	0.94±0.046	27.79
Light	1.44±0.022	42.14

Table 27 Percent remaining data of β-carotene. Samples were cream containing aril oil loaded NLC at 4, 25, 45°C and light after 90 days of storage

Cream containing aril oil loaded NLC		
Condition	**β-carotene concentration (μg/mL)**	**% Remaining**
Day 0 at 25°C	3.35±0.069	100.00
Day 90, 4°C	2.68±0.026	79.87
Day 90, 25°C	2.45±0.059	73.38
Day 90, 45°C	1.26±0.044	37.83
Light	1.97±0.034	59.52

4.9 Primary skin irritation test

Skin irritation is one of the most common adverse effects in human depended on many factors, including the concentration, duration and frequency of exposure skin site, rate of penetration and intrinsic toxic potential of the substance. Skin irritation tests determine the level of damage caused to skin such as itching, swelling, and inflammation (13).

In human skin irritation study twenty volunteers were test with the five substances; cream base, cream containing aril oil cream, cream containing NLC, cream containing aril oil-loaded NLC and 1% sodium lauryl sulphate (SLS) as positive control. After 72 hours, the test sites were evaluated by visual scoring then calculate the primary irritation index (PII) and evaluated the type of skin irritation that shown in Table 28.

Table 28 The value of primary irritation index (PII) and type of skin irritation in volunteers

Sample	PII values	Type of skin irritation
Blank (no sample)	0.0	Non-irritation
Cream base	0.0	Non-irritation
Cream containing aril oil	0.0	Non-irritation
Cream containing NLC	0.0	Non-irritation
Cream containing aril oil loaded NLC	0.0	Non-irritation
1% SLS	0.5	Slightly irritation

The calculated PIIs were 0.0 at 72 hours for primary skin irritation test off five samples. This result indicated that there was no irritation in normal volunteers for cream base, aril oil cream, NLC in cream and aril oil loaded NLC in cream but 1% SLS had slightly irritation with PII = 0.5

4.10 Wrinkle reducing capacity tests of aril oil cream and aril oil loaded NLC in cream

The wrinkle reducing property of cream base, cream base, cream containing aril oil, cream containing NLC and cream containing aril oil-loaded NLC were evaluated by using Skin Visiometer® and analyzed in four parameters (Roughness-R*a*, Rz, volume, surface). Paired samples test were used to examine change in values, before and after of each treatment (untreated, treated, placebo). All of 20 subjects completed this clinical trial. The results which shown in Table 29 was mean value of 20 volunteers. After 8 weeks of treatment, application of cream containing aril oil cream area and cream containing aril oil-loaded NLC area exhibited significantly reducing of wrinkle in R*a*, Rz, volume and surface parameter (cream containing aril oil area: -14.83, -10.18, -12.30, and -19.34%, cream containing aril oil-loaded NLC area: -19.95, -13.41, -17.60, and -25.70%, respectively) (P<0.01).

The placebo area; cream base and cream containing NLC presented significantly reducing of the wrinkle in R*a*, Rz, volume and surface parameter (cream

base area; -5.3, -6.52, -8.36 and -14.69%, cream containing NLC area; -12.57, -10.33,-14.60 and -21.46%) (P<0.01). This result may mention that the wrinkle reducing property of cream base produced from the ingredient such as vitamin E acetate and cream containing NLC produced from the ingredient such as jojoba oil and α-tocopherol. In addition, the untreated area exhibited significantly increasing of wrinkle in R*a*, volume and surface parameter (16.59, 7.04 and 9.41%) (P<0.01) except Rz parameter, it exhibited non-significant change. This result indicates that aril oil cream and aril oil-loaded NLC in cream had the capability to reduce skin wrinkle.

The cream base area, cream containing NLC area, cream containing aril oil area and cream containing aril oil-loaded NLC area were analyzed by pared t-test (P<0.01) to determined the difference. As assessed to % efficiency value, application of aril oil cream area and aril oil loaded NLC in cream area exhibited significantly different evolution with untreated area for all parameters (Figure 23-26). The cream containing aril oil area and cream containing aril oil-loaded NLC area exhibited significantly different evolution with cream base area and cream containing NLC area for all parameter, respectively. Between cream base area, cream containing NLC area and untreated area, they shown significantly different in R*a*, volume and surface parameter except Rz parameter it exhibited non-significantly change.

Table 29 The wrinkle reducing property on the R*a*, Rz, volume and surface parameters of five treatment sites

Area	Wrinkle reducing parameter (after - before)			
	Roughness		Volume	Surface
	R*a*	**Rz**		
cream containing NLC	-7.36 ± 1.12	- 1.52 ± 0.35	-8.06 ± 4.83	-1.51 ± 0.68
cream containing aril oil-loaded NLC	-12.52 ± 1.08	-2.01± 0.48	-9.90 ± 5.33	-1.88 ± 0.64
Untreated area	9.45 ± 1.19	1.18 ± 1.63	3..85 ± 5.46	0.6 ± 0.11
cream base	-2.99 ± 0.54	-0.96 ± 0.29	-4.56 ± 5.59	-0.98 ± 0.72
cream containing aril oil	-8.95 ± 1.01	-1.47 ± 0.32	-6.81 ± 4.76	-1.36 ± 0.70

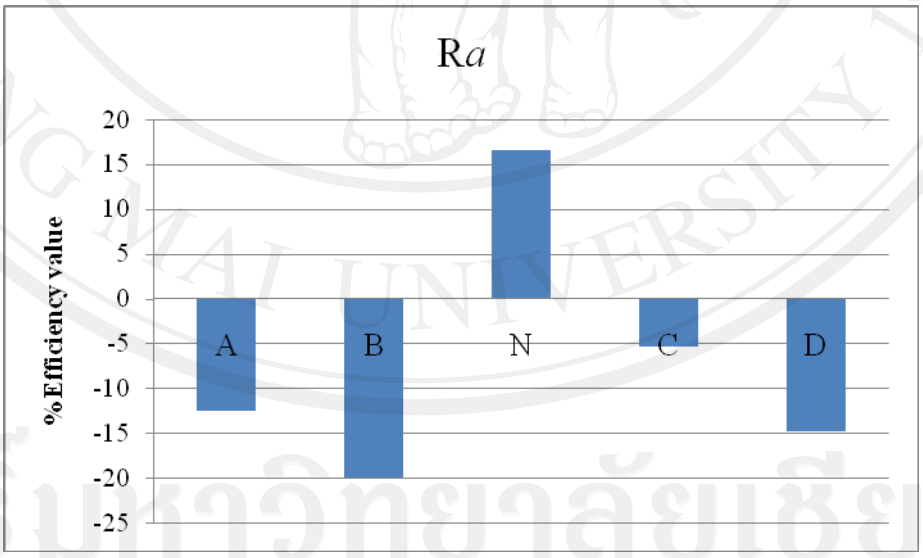


Figure 23 % Efficiency value of R*a* parameter of five test sites (A = cream containing NLC area; B = cream containing aril oil-loaded NLC area; N = untreated area; C = cream base area; D = cream containing aril oil area)

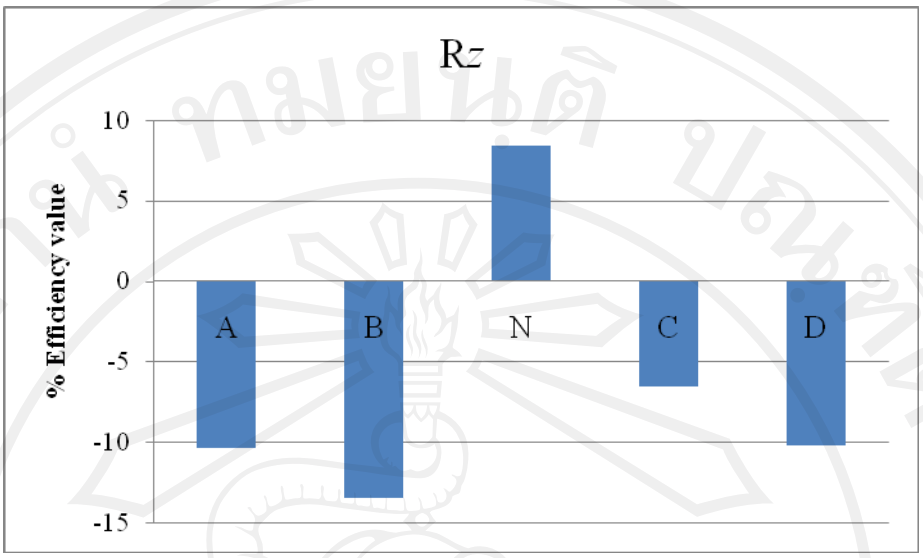


Figure 24 % Efficiency value of Rz parameter of five test sites (A = cream containing NLC area; B = cream containing aril oil-loaded NLC area; N = untreated area; C = cream base area; D = cream containing aril oil area)

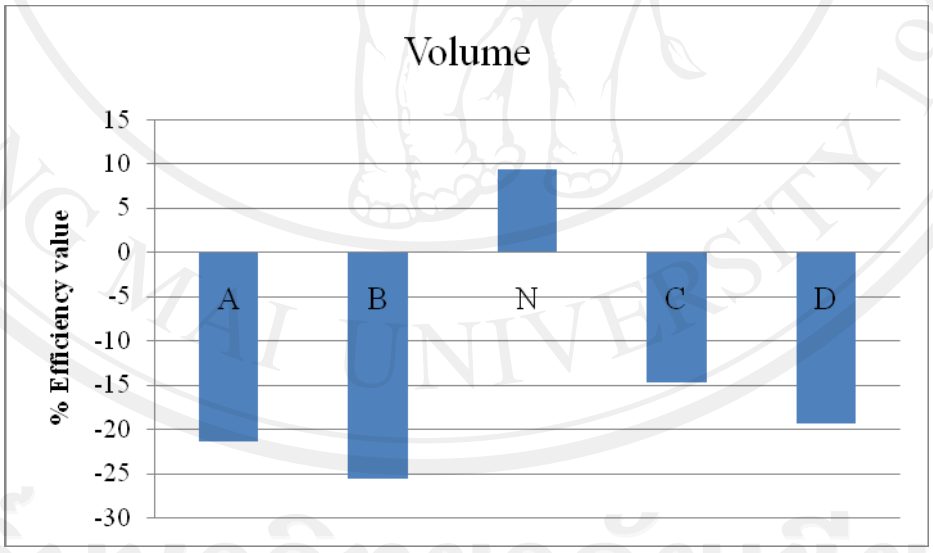


Figure 25 % Efficiency value of volume parameter of five test sites (A = cream containing NLC area; B = cream containing aril oil-loaded NLC area; N = untreated area; C = cream base area; D = cream containing aril oil area)

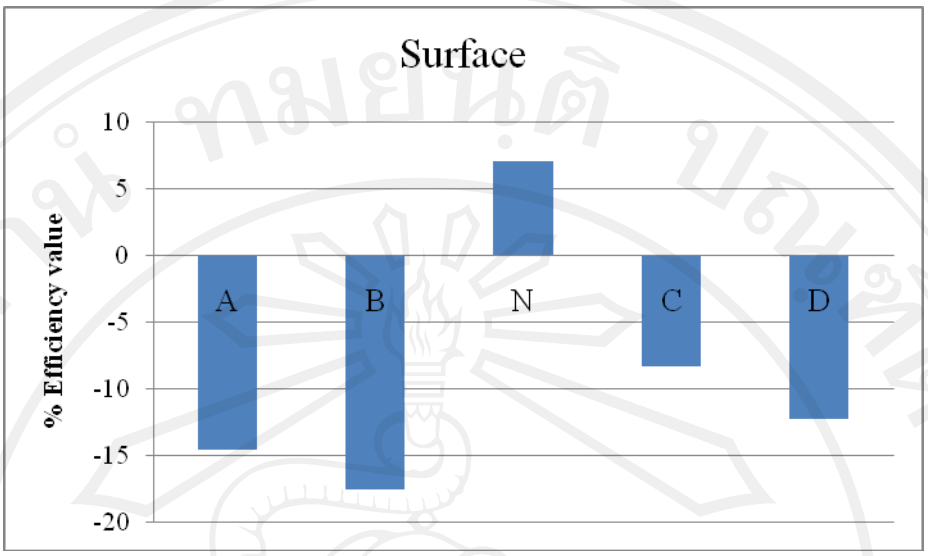


Figure 26 % Efficiency value of surface parameter of five test sites (A = cream containing NLC area; B = cream containing aril oil-loaded NLC area; N = untreated area; C = cream base area; D = cream containing aril oil area)