CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of nanoparticles and Coenzyme Q_{10}-loaded nanoparticles from microemulsion system

1.1 Preparation of nanoparticles from microemulsion system

A use of warm microemulsion as a precursor of nanoparticle production has been done and modified by different research groups (Cavalli *et al.*, 1999; Cui and Mumper, 2002 a and b; Gasco, 1997; Heydenreich *et al.*, 2003; Igartua *et al.*, 2002; Ugazio, Cavalli and Gasco, 2002). This method was based on the natural and spontaneous formation of a microemulsion and used a property of lipid being solid at room temperature. In this technique, microemulsion needs to be produced at a temperature above the melting point of the lipid. The lipid was melted and a mixture of water and surfactant at a few higher temperatures than lipid phase (~ 5 °C) were then added under mild stirring to the melted lipid. A transparent, thermodynamically stable system represents the microemulsion formation (Müller, Mäder and Gohla, 2000). The nanoparticles were then produced from direct cooling the warm oil in water (o/w) microemulsions to room temperature under mild stirring.The size of nanoparticle was affected by the composition of microemulsion system, particularly by the surfactants used. Oyewumi and Mumper (2002) suggested that the microemulsion having the amphiphatic matrix materials (oil phase) combined with surfactants having moderate HLB values (i.e., 14-17) promoted the formation of very small and stable nanoparticles.

In this study, an experiment was performed to verify the suitable microemulsion system which gave the small and stable nanoparticles, whereas a less amount of surfactants was used. The microemulsion was composed of oil (matrix material), the surfactant and water phases. Lawrence and Rees (2000) suggested that the surfactant with HLB in the range of 8-18 was preferred to the formation of o/w microemulsion. The non-ionic surfactants were widely used in the production of stable microemulsion and had the advantage over ionic surfactants of being less toxic and less sensitive to electrolytes and pH variation (Attwood, 2002). The oil phase used in this experiment was the non-ionic emulsifying wax or Brij® 72 (polyoxyl-2-stearyl ether; HLB = 4.9; melting point (MP) = 43°C) at the concentration of 2 mg/mL. The compositions of emulsifying wax were a blend of cetostearyl alcohol (HLB = 13-14; MP = 48 – 53 °C) and one of the surfactant; Tween® 20 (Polyoxyethylene-20-sorbitan monolaurate; HLB = 16.7), Tween® 60 (polyoxyethylene-20-sorbitan monostearate; HLB = 14.9), and cetomacrogol 1000 (polyoxyethylene glycol 1000; HLB = 15.8), at a weight ratio of 4:1. The non-ionic surfactant used to stabilize oil droplet was Brij® 78 (polyoxyl-20-stearyl ether; HLB = 15.3) or Tween® 80 (polyoxylethylene 20 sorbitan monooleate; HLB = 15) (Wade and Weller, 1994). All of ingredients used in this experiment were potentially biocompatible. The details of each ingredient used in this study are summarized in appendix A.

The microemulsion systems, which used as nanoparticles templates, were achieved by varying the oil phase (emulsifying wax or Brij® 72), surfactant phase (100 mM Brij® 78 stock solution or 10% Tween® 80 stock solution) and water phase. The size and size distribution of achieved nanoparticles were determined by the photon correlation spectroscopy (PCS) at 4 and 24 hours after the preparation. The data of size and size distribution of all formulation is described in Tables C1 and C2 in appendix C.

1.1.1 Nanoparticles containing Brij® 78 as a surfactant

The systems containing Brij® 78 as a surfactant and four types of core materials, namely, 4:1 cetostearyl alcohol (CT):Tween® 20, 4:1 CT:Tween® 60, 4:1 CT:Cetomacrogol 1000 and Brij® 72, were determined for particle size and size distribution (Table 4-1). It was noted that the amount of Brij® 78 used was the concentration around the phase boundary of microemulsion formation as described in Tables B1-B4 in appendix B.

It was found that all of the formulations containing emulsifying wax as a core material could form the nanoparticles having diameter of less than 110 nm after 4 hours of preparation. While the system consisted of Brij® 72 and Brij® 78 formed the particles larger than 100 nm (approximately 400-500 nm) with slightly increased in size after storage for 24 hours at room temperature. The results also showed that the concentration of the surfactant influenced the size and size distribution of nanoparticles. An increase in Brij® 78 concentration might contribute to the reduction of mean particle size and size distribution except in the system consisted of Brij® 72 and Brij® 78. For example, the mean sizes of nanoparticles after 4 hours, prepared using cetostearyl alcohol blended with cetomacrogol as an oil phase and Brij® 78 at concentrations of 7 mM, 9 mM and 11 mM, were 92.17 ± 0.83, 59.50 ± 0.95 and 51.60 ± 1.37 nm, respectively. However, nanoparticles prepared from emulsifying wax and Brij® 78 could not retain their small size after storage for 24 hours at room temperature. The tendency of the nanoparticles to increase in size during storage is possibly related to Ostwald ripening phenomena in order to reduce the overall free energy of the system (Koziara *et al.*, 2003; Lockman *et al.*, 2003).

Table 4-1 Average diameter (z-average) and polydispersity index (PI) of nanoparticles consisting of oil (wax) at 2 mg/mL and various concentrations of Brij® 78 after 4 and 24 hours (mean ± S.D., n=3).

Rx	Brij® 78 (mM)	Wax	4 hours		24 hours	
			Size (nm)	PI	Size (nm)	PI
A9	8	CT + Tween® 20	92.73 ± 1.63	0.32 ± 0.00	552.60* ± 273.79	1
A11	10		54.77 ± 1.51	0.32 ± 0.02	1595.83* ± 549.69	1
A13	12		55.00 ± 0.75	0.35 ± 0.04	1040.23* ± 511.63	1
A24	7	CT + Tween® 60	103.63 ± 2.40	0.24 ± 0.01	329.57* ± 39.68	1
A26	9		72.37 ± 1.25	0.35 ± 0.01	471.53* ± 253.64	0.95 ± 0.05
A28	11		64.50 ± 3.40	0.41 ± 0.03	617.23* ± 214.31	1
A40	7	CT + Cetoma -crogol	92.17 ± 0.83	0.17 ± 0.01	120.70* ± 1.35	0.31 ± 0.05
A42	9		59.50 ± 0.95	0.26 ± 0.04	143.37* ± 21.87	0.58 ± 0.05
A44	11		51.60 ± 1.37	0.26 ± 0.04	164.77* ± 12.33	0.67 ± 0.08
A53	4	Brij® 72	467.53 ± 21.06	0.38 ± 0.01	588.73* ± 9.02	0.49 ± 0.02
A55	6		548.60 ± 2.71	0.38 ± 0.02	604.17* ± 19.80	0.52 ± 0.04
A57	8		409.67 ± 7.12	0.21 ± 0.04	475.70* ± 12.80	0.36 ± 0.01

* Indicate the size of the nanoparticles after 24 hours of preparation was significantly higher than the size of nanoparticles after 4 hours of preparation ($P<0.05$).

1.1.2 Nanoparticles containing Tween® 80 as a surfactant

The systems consisting of Tween® 80 as a surfactant and four different oil phases (as described in 1.1.1) were studied. The amounts of surfactant that could form microemulsion around phase boundary (Tables B5-B8 in appendix B) were used

to prepared nanoparticles and determined for their particle size and size distribution as summarized in Table 4-2.

Table 4-2 Average diameter (z-average) and polydispersity index (PI) of nanoparticles consisting of oil (wax) at 2 mg/mL and various concentrations of Tween® 80 (T80) after 4 and 24 hours (mean ± S.D., n=3).

Rx	T 80 (mM)	Wax	4 hours		24 hours	
			Size (nm)	PI	Size (nm)	PI
B14	26	CT + Tween® 20	171.37 ± 6.80	0.45 ± 0.02	184.57 ± 14.70	0.41 ± 0.04
B16	28		230.83 ± 2.06	0.44 ± 0.045	230.10 ± 13.27	0.43 ± 0.02
B18	30		1004.60 ± 56.56	0.58 ± 0.02	1646.6* ± 66.62	0.96 ± 0.07
B32	25	CT + Tween® 60	102.00 ± 5.79	0.42 ± 0.04	101.90 ± 6.93	0.39 ± 0.03
B34	27		121.60 ± 12.42	0.44 ± 0.06	124.57 ± 10.16	0.41 ± 0.03
B36	29		200.17 ± 18.49	0.47 ± 0.05	199.20 ± 13.50	0.42 ± 0.03
B50	23	CT + Cetoma -crogol	73.93 ± 4.50	0.39 ± 0.03	83.67 ± 3.97	0.36 ± 0.02
B52	25		81.90 ± 6.63	0.41 ± 0.03	89.37 ± 7.05	0.38 ± 0.04
B54	27		72.30 ± 3.93	0.38 ± 0.02	85.20 ± 4.83	0.34 ± 0.02
B66	17	Brij® 72	73.20 ± 2.98	0.35 ± 0.02	92.10 ± 12.70	0.39 ± 0.02
B68	19		72.30 ± 6.16	0.41 ± 0.01	99.10* ± 6.71	0.41 ± 0.02
B70	21		126.40 ± 7.71	0.48 ± 0.02	146.40 ± 12.17	0.46 ± 0.02

* Indicate the size of the nanoparticles after 24 hours of preparation was significantly higher than the size of nanoparticles after 4 hours of preparation ($P<0.05$).

From the results, systems containing the blend of cetostearyl alcohol with Tween® 20 or Tween® 60 and Brij® 72 as core materials showed an increase in particle size upon increasing the concentration of Tween® 80 and the size of most of nanoparticles seemed to be slightly increased after 24 hours.

The systems which were composed of a combination of cetostearyl alcohol and cetomacrogol as an oil phase and Tween® 80 as surfactant phase could form the nanoparticles with the size below 100 nm (72-82 nm) and narrow size distribution (PI 0.3-0.4). The nanoparticles showed a slightly decrease in particle size upon increasing the concentration of Tween® 80 and seemed to be stable over a period of 24 hours with the mean sizes were in the range of 83-89 nm. Consequently, these systems were selected to study the effect of cooling method on the size of nanoparticles and were used to incorporate Coenzyme Q_{10}.

Compared the type of surfactants used, the nanoparticles prepared using Tween® 80 were generally more stable than those using Brij® 78 even though Tween® 80 (HLB=15.0) and Brij® 78 (HLB=15.3) has closely HLB value. Moreover, the concentrations of these two surfactants used to produce nanoparticle were either above the CMC (the CMC of Tween® 80 and Brij® 78 were 0.1 and 0.75 mM, respectively). Attwood (2002) suggested that the non-ionic surfactants were not have electrical repulsive force contributing to stability but formed an adsorbed layer at the particle surface resulting in a steric interaction. Moreover, these polymeric materials might increase the viscosity of the aqueous vehicles and thus slow the rate of sedimentation of the particles. Generally, steric forces are dependent upon length of polymer chains with longer the chains, greater the stabilization (Lawrence, 2004). The bulkies structure of Tween® 80 (C_{64} H_{124} O_{26}) and the appropriate geometric packing with lipid material core might be responsible to the greater stability of the system after storage. However, the amount of Tween® 80 used for stabilization of the particles in nanometric range was at least 20 mM while the amount of Brij® 78 used was about

two times lower (about 10 mM). However, when the concentration of Brij® 78 was increased to 20 mM, it could not retain the nanometric size range of obtained particles even after 4 hours of preparation. Again, when the concentration of Tween® 80 was decreased to 10 mM, it could not to form microemulsion template and consequently, nanoparticle were not achieved.

1.1.3 Effect of cooling method

According to the studies described above, nanoparticles were obtained by simple cooling the warm microemulsion (60°C) down to room temperature (25°C) within 10 minutes under mild stirring. To investigate the effect of cooling rate on nanoparticle sizes, another cooling method was studied by rapidly cooling the warm microemulsion (60°C) down to 5°C on an ice-bath under gentle stirring in 10 minutes. The systems containing 4:1 cetostearyl alcohol to cetomacrogol and Tween® 80 (Rx B50, B52 and B54) were studied. The nanoparticles dispersion was kept at room temperature (25°C) and the sizes were measured at 4, 24, 48 hours and 1 week after preparation. The results are summarized in Table 4-3 and Figure 4-1.

The results showed that cooling rate could affect the size of the cured nanoparticles. Al-Kassas, Gilligan and Po (1993) have studied the effect of the rate and method of cooling on the properties of drug matrices. They found that crystallization and the size of crystals were a function of the rate of cooling. Generally, when cooling was slow, larger crystals were formed.

Table 4-3 Average diameter (z-average) and polydispersity index (PI) of nanoparticles containing cetostearyl alcohol and cetomacrogol at 2 mg/mL and Tween® 80 (T80) prepared by 2 different cooling methods (mean ± S.D., n=3).

T80 (mM)	Time	Cooling method for cured nanoparticles			
		Simple cooling		Rapid cooling	
		Size (nm)	PI	Size (nm)	PI
23 (B50)	4 hrs	73.93 ± 4.50	0.39 ± 0.03	55.77* ± 3.18	0.48 ± 0.03
	24 hrs	83.67 ± 3.97	0.36 ± 0.02	74.60 ± 2.44	0.43 ± 0.01
	48 hrs	117.70 ± 5.73	0.40 ± 0.02	80.70* ± 6.34	0.42 ± 0.04
	1 wk	122.27 ± 3.20	0.40 ± 0.02	91.70* ± 5.96	0.39 ± 0.03
25 (B52)	4 hrs	81.90 ± 6.63	0.41 ± 0.03	52.90* ± 1.92	0.56 ± 0.01
	24 hrs	89.37 ± 7.05	0.38 ± 0.04	92.47 ± 0.76	0.46 ± 0.07
	48 hrs	106.13 ± 3.53	0.45 ± 0.01	97.23* ± 4.37	0.38 ± 0.02
	1 wk	114.27 ± 5.36	0.44 ± 0.04	107.73* ± 5.22	0.39 ± 0.02
27 (B54)	4 hrs	72.30 ± 3.93	0.38 ± 0.02	21.20* ± 1.97	0.42 ± 0.02
	24 hrs	85.20 ± 4.83	0.34 ± 0.02	47.10* ± 4.61	0.51 ± 0.01
	48 hrs	100.73 ± 9.75	0.42 ± 0.04	62.00* ± 7.18	0.50 ± 0.05
	1 wk	114.93 ± 2.75	0.39 ± 0.02	96.83* ± 7.00	0.46 ± 0.04

* Indicate the size of the nanoparticles prepared by rapid cooling method was significantly lower than the size of nanoparticles prepared by simple cooling method ($P<0.05$), when compare at the same formulation and time after preparation.

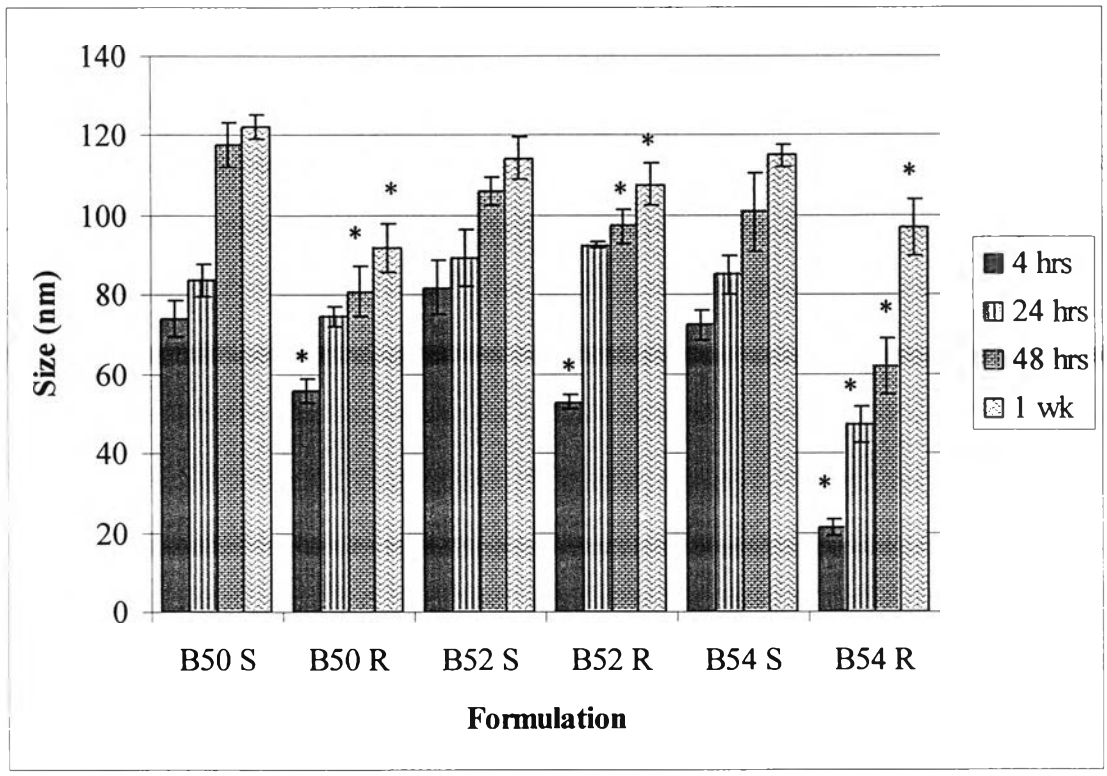


Figure 4-1 Average diameter of nanoparticles received from different cooling methods, simple cooling (S) and rapid cooling (R) (mean ± S.D., n=3).

* Indicate the size of the nanoparticles prepared by rapid cooling method was significantly lower than the size of nanoparticles prepared by simple cooling method ($P<0.05$), when compare at the same formulation and the same time after preparation.

The rapid cooling method resulted in smaller sizes of nanoparticles than those using simple cooling method. After 1-week storage, the overall sizes of nanoparticles from rapid cooling method were still less than 100 nm. So, the immediate cooling of warm microemulsion in an ice-bath under mild stirring for preparation of nanoparticles was used for further preparation of nanoparticles. However, Oyewumi and Mumper (2002) found that the rapid and simple methods provided the nanoparticles having the size below 100 nm.

In addition, the increasing tendency of the nanoparticles size were observed with the prolong time. The reason might be relate to Ostwald ripening events due to a deposition of the dissolved material on larger surfaces, resulting in the growth of particles. In details, Ostwald ripening depends on both the granulometry and the Laplace pressure being higher for lower size droplets. A species flux occurs from small to large droplets *via* the continuous phase and the average diameter consequently increases (Heurtault *et al.*, 2003; Koziara *et al.*, 2003).

1.2 Preparation of Coenzyme Q_{10}-loaded nanoparticles from microemulsion system

The nanoparticles from microemulison system selected for incorporation of Coenzyme Q_{10} were a mixture of 4:1 cetostearyl alcohol to cetomacrogol and Tween® 80 due to its small size (below 100 nm) and uniformity (PI 0.3-0.4) after storage. The preparation process of Coenzyme Q_{10}-loaded nanoparticle was the same as described previously for drug-free nanoparticles with a use of rapid cooling method. Since Coenzyme Q_{10} is hydrophobic molecules (Bunjes *et al.*, 2001), it was incorporated in nanoparticles by the addition to the oil phase of microemulsion template.

The effect of surfactant (Tween® 80), emulsifying wax and Coenzyme Q_{10} concentrations on the size of cured Coenzyme Q_{10}-loaded nanoparticles was studied. The data of their appearance are presented in Table B9 in appendix B. The data of size and size distribution of Coenzyme Q_{10}-loaded nanoparticles are described in Table C4 in appendix C.

1.2.1 Effect of non-ionic surfactant concentration

The nanoparticles were prepared using 2 mg/mL of emulsifying wax and various concentrations of Tween® 80 (20-60 mM). The concentrations of Coenzyme Q_{10} were 1 and 2 mg/mL.

With respect to particle size and size distribution analysis as illustrated in Table 4-4, the diameters of all formulations after 24-hour storage at room temperature were below 100 nm with a size distribution (PI) of 0.4-0.6.

Table 4-4 Average diameter (z-average) and polydispersity index (PI) of Coenzyme Q_{10}-loaded nanoparticles at 24 hours after preparation (mean ± S.D., n=3).

Tween® 80 (mM)	Rx	Coenzyme Q_{10} 1 mg/mL		Rx	Coenzyme Q_{10} 2 mg/mL	
		Mean size (nm)	PI		Mean size (nm)	PI
20	C11	70.90 ± 3.16	0.48 ± 0.02	C21	91.10 ± 2.52	0.47 ± 0.01
24	C12	63.17 ± 1.94	0.50 ± 0.01	C22	57.00* ± 6.48	0.55 ± 0.00
30	C13	31.63* ± 3.27	0.55 ± 0.01	C23	43.73* ± 5.30	0.53 ± 0.01
35	C14	49.50 ± 5.89	0.57 ± 0.00	C24	67.03 ± 8.85	0.51 ± 0.02
40	C15	42.10* ± 12.20	0.62 ± 0.01	C25	41.77* ± 10.85	0.60 ± 0.01
45	C16	39.60* ± 13.05	0.61 ± 0.02	C26	37.53* ± 12.57	0.61 ± 0.03
50	C17	31.47* ± 8.10	0.58 ± 0.06	C27	37.83* ± 13.74	0.60 ± 0.02
60	C18	28.17* ± 6.69	0.58 ± 0.04	C28	35.87* ± 6.64	0.52 ± 0.16

* Indicate the size of Coenzyme Q_{10} loaded-nanoparticles was significantly lower than the size of Coenzyme Q_{10} loaded-nanoparticles prepared by using 20 mM Tween® 80 ($P<0.05$) when compare in the same Coenzyme Q_{10} concentration (1 and 2 mg/mL).

Importantly, the results showed that the amounts of Tween® 80 had an influence on the size and size distribution of Coenzyme Q_{10} loaded-nanoparticles. The increase in the concentration of Tween® 80 contributed to the reduction of mean particle sizes. In some formulations, it was found that an increase in Coenzyme Q_{10} concentration from 1 to 2 mg/mL caused an increase in size of nanoparticles. Mehnert and Mäder (2001) explained that high concentration of the surfactant reduced the surface tension which was connected to tremendous increase in surface area and subsequently, very small particles were achieved.

1.2.2 Effect of emulsifying wax concentration

In this experiment the concentration of wax (the blend of 4:1 cetostearyl alcohol to cetomacrogol) used were increased from 2 to 4 and 6 mg/mL and the amount of Tween® 80 around the microemulsion phase boundary was used for an investigation. It was noted that the concentration of Tween® 80 which could form microemulsion were 48 and 72 mM for the systems containing wax 4 and 6 mg/mL, respectively. Moreover, it was observed that an initial weight ratio of emulsifying wax to Tween® 80 which could from microemulsion was a weight ratio of 1:15.72 wax to Tween® 80 (derived from 2 mg/mL (0.2%) wax and 24 mM (3.144%) Tween® 80, 4 mg/mL (0.4%) wax and 48 mM (6.288%) Tween® 80 and 6 mg/mL (0.6%) wax and 72 mM (9.432%) Tween® 80. The concentrations of Coenzyme Q_{10} were at 1 and 2 mg/mL. The results of size and size distribution are summarized in Table 4-5.

From the result, when the wax concentration increased, the nanoparticles were larger and the size distribution was less. For example, the mean diameter and PI of nanoparticles containing 2, 4 and 6 mg/mL wax at 60 mM Tween® 80 were 28.17 ± 6.69, 62.10 ± 5.76 and 122 ± 4.90 nm and 0.581 ± 0.039, 0.512 ± 0.032 and 0.300 ± 0.015 at 1 mg/mL of Coenzyme Q_{10}, respectively. This result was in agreement with the study of Oyewumi and Mumper (2002) who reported the

particle size increased in dose dependent manner with concentration of emulsifying wax. Again, an increase in the Tween® 80 concentration contributed to the reduction of particles size. The reason might be due to the increase concentration of core material led to increasing in obtained nanoparticles size.

Table 4-5 Average diameter (z-average) and polydispersity index (PI) of Coenzyme Q_{10}-loaded nanoparticles, consisting of 2, 4 or 6 mg/mL of wax at 24 hours after preparation (mean ± SD, n=3).

Tween®80 (mM)	Wax (mg/mL)	Rx	Coenzyme Q_{10} 1 mg/mL		Rx	Coenzyme Q_{10} 2 mg/mL	
			Mean size (nm)	PI		Mean size (nm)	PI
45	2	C16	39.60 ± 13.05	0.61 ±0.02	C26	37.53 ± 12.57	0.61 ± 0.03
60		C18	28.17 ± 6.69	0.58 ±0.04	C28	35.87 ± 6.64	0.52 ± 0.16
45	4	D13	100.93 ±10.33	0.38 ±0.03	D23	78.40 ± 6.88	0.46 ± 0.03
48		D14	90.27 ± 4.27	0.37 ±0.01	D24	86.70 ± 4.40	0.47 ± 0.01
50		D15	82.90 ± 6.84	0.39 ±0.05	D25	77.13 ± 7.24	0.46 ± 0.03
60		D17	62.10* ± 5.76	0.51 ±0.03	D27	62.73* ± 6.98	0.53 ± 0.04
60	6	E15	122.73* ± 6.39	0.30 ±0.02	E25	108.10* ±4.90	0.38 ± 0.02
72		E16	126.10 ± 2.69	0.48 ±0.01	E26	90.20 ± 5.30	0.48 ± 0.02
80		E17	90.30 ± 3.44	0.44 ±0.02	E27	89.70 ± 5.10	0.47 ± 0.01
90		E18	94.07 ± 9.11	0.51 ±0.02	E28	96.00 ± 7.30	0.48 ± 0.02

* Indicate the size of the Coenzyme Q_{10} loaded-nanoparticles was significantly higher than the size of Coenzyme Q_{10} loaded-nanoparticles prepared by using 2 mg/mL wax ($P<0.05$), when compare in the same Tween® 80 (60 mM) and Coenzyme Q_{10} concentration (1 and 2 mg/mL).

Moreover, there was a direct linear relationship between lowest concentrations of Tween® 80 and each amount of wax that could form microemulsion as shown in Figure 4-2.

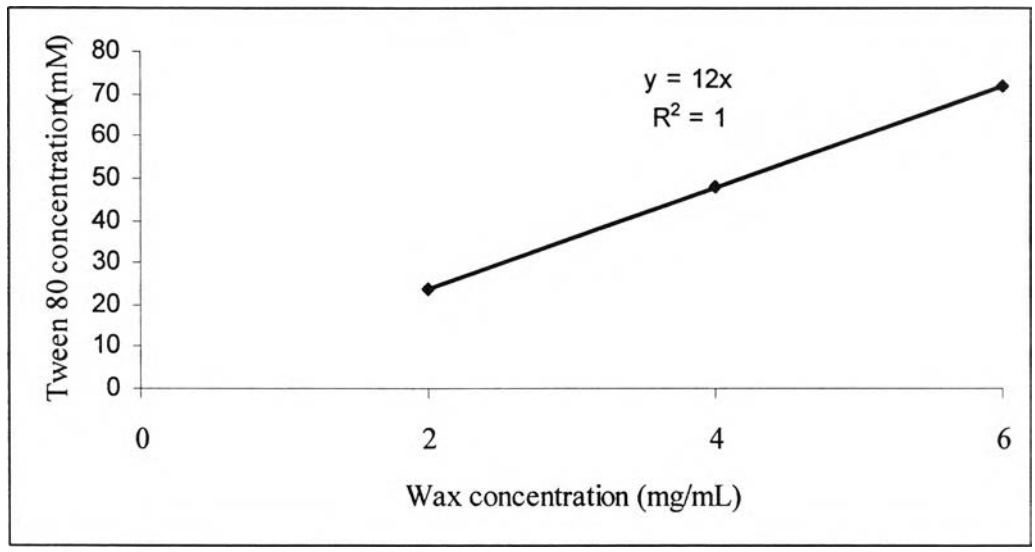


Figure 4-2 Correlation between concentrations of wax and lowest concentrations of Tween® 80 for microemulsion formation.

1.2.3 Effect of Coenzyme Q_{10} concentration

As mentioned before, lowest concentrations of Tween® 80 that could form microemulsion for each amount of wax, 24 mM (3.144%), 48mM (6.288%) and 72 mM (9.432%), for 2, 4 and 6 mg/mL wax, respectively, were used to incorporate the various amount of Coenzyme Q_{10} (1-4 mg/mL) in the nanoparticles. The systems without Coenzyme Q_{10} loading were also investigated for the size and size distribution. The results of size and size distribution are shown in Table 4-6 and Figure 4-3.

For the nanoparticles containing 1 mg/mL Coenzyme Q_{10} had the larger size compared to the drug-free nanoparticles. Upon increasing Coenzyme Q_{10} from 1 to 2 and 3 mg/mL, a slight decrease in nanoparticle sizes was seen. However, the particle size was increased again at the highest concentration of Coenzyme Q_{10} (4 mg/mL).

Table 4-6 Average diameter (z-average) and polydispersity index (PI) of Coenzyme Q_{10}-loaded nanoparticles with various amount of Coenzyme Q_{10} at 24 hours after preparation (mean ± S.D., n=3).

Rx	Wax (mg/mL)	Tween® 80 (mM)	Coenzyme Q_{10} (mg/mL)	Mean size (nm)	PI
F2	2	24	-	60.50 ± 4.00	0.40 ± 0.01
C12			1	63.17 ± 1.94	0.50 ± 0.01
C22			2	57.00 ± 6.48	0.55 ± 0.00
C32			3	45.57* ± 4.90	0.58 ± 0.01
C42			4	84.07* ± 4.68	0.67 ± 0.01
F4	4	48	-	92.00 ± 4.70	0.39 ± 0.02
D14			1	90.27 ± 4.27	0.37 ± 0.01
D24			2	86.77 ± 4.40	0.47 ± 0.01
D34			3	85.70 ± 5.57	0.49 ± 0.02
D44			4	86.33 ± 9.65	0.52 ± 0.02
F6	6	72	-	109.80 ± 7.80	0.45 ± 0.02
E16			1	126.10* ± 2.69	0.48 ± 0.01
E26			2	90.17* ± 5.32	0.48 ± 0.02
E36			3	88.77* ± 3.21	0.50 ± 0.02
E46			4	118.73 ± 6.82	0.46 ± 0.02

* Indicate the size of the Coenzyme Q_{10} loaded-nanoparticles was significantly different from the size of Coenzyme Q_{10} free nanoparticles ($P<0.05$), when compare in the same Tween® 80 (24, 48 and 72 mM) and wax concentration (2, 4 and 6 mg/mL).

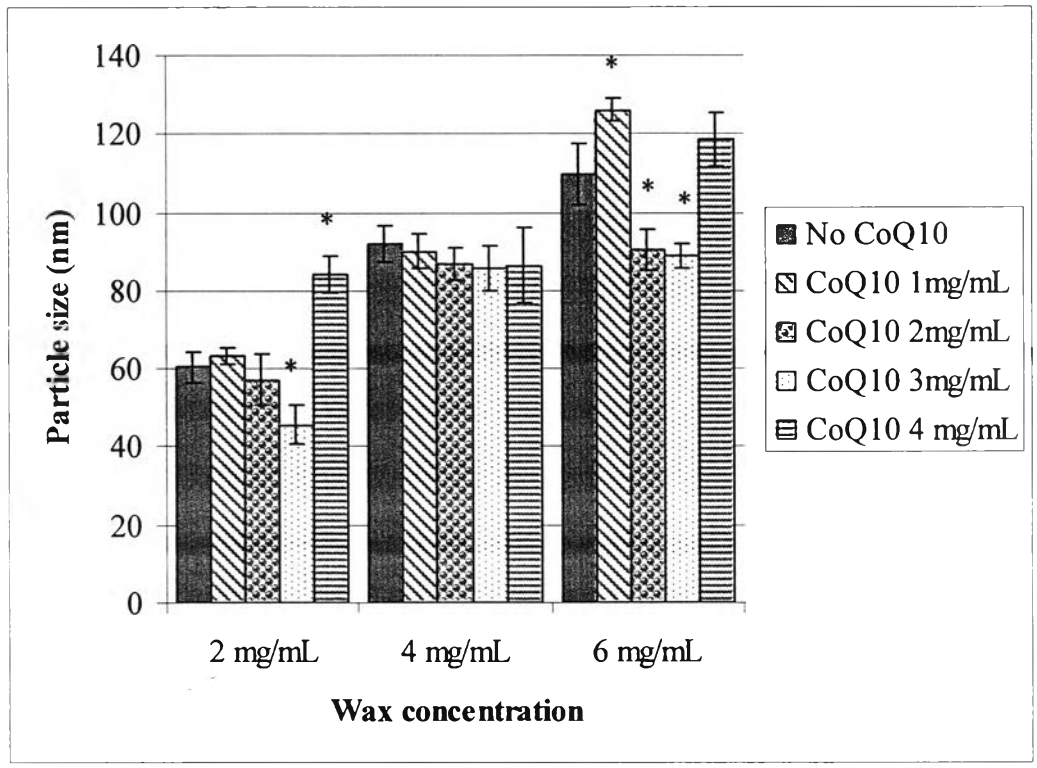


Figure 4-3 Mean particle size of Coenzyme Q_{10}-loaded nanoparticle using the various amount of Coenzyme Q_{10} and wax (mean ± S.D., n = 3).

* Indicate the size of the Coenzyme Q_{10} loaded-nanoparticles was significantly different from the size of Coenzyme Q_{10} free nanoparticles ($P<0.05$), when compare in the same Tween® 80 (24, 48 and 72 mM) and wax concentration (2, 4 and 6 mg/mL).

The small sizes of Coenzyme Q_{10}-loaded nanoparticles were seen at the system containing lower amount of wax. Importantly, the incorporation of Coenzyme Q_{10} did not interfere with the nanoparticles engineering process most likely due to the hydrophobicity and low melting point that were compatible with the nanoparticles matrix materials (Oyewumi and Mumper, 2002). From chemical structure of Coenzyme Q_{10}, it was assumed to mainly insert itself into the lipid core of nanoparticles while leaving its hydrophilic head on the surface (Bunjes *et al.*, 2001). Moreover, if it is completely incorporated into the core of nanoparticles, the size would be greater upon increasing the amount of drug concentration.

The effect of ingredients used in the nanoparticles will be evaluated for the entrapment efficiency in order to gain the suitable formulation which will be discussed later.

2. Determination of entrapment efficiency

From the HPLC analysis, the method was validated and the data analysis is presented in Appendix D. The resolution time of Coenzyme Q_{10} was about 5.5 min detected at 275 nm (USP 27, 2004). The amount of Coenzyme Q_{10} entrapped in the nanoparticles was calculated using the linear equation derived from the regression analysis of the calibration curve, as $y = 0.00006x + 0.1498$ ($R^2 = 0.9998$); where y represented Coenzyme Q_{10} concentrations and x was the peak area obtained from the HPLC analysis. The HPLC profile and the calibration curve of standard Coenzyme Q_{10} stock solution are illustrated in Figures 4-4 and 4-5, respectively.

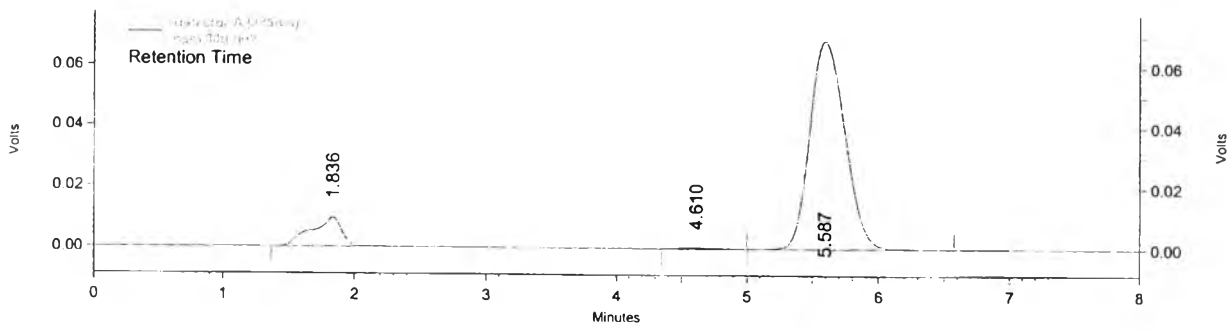


Figure 4-4 The HPLC profile of Coenzyme Q_{10}.

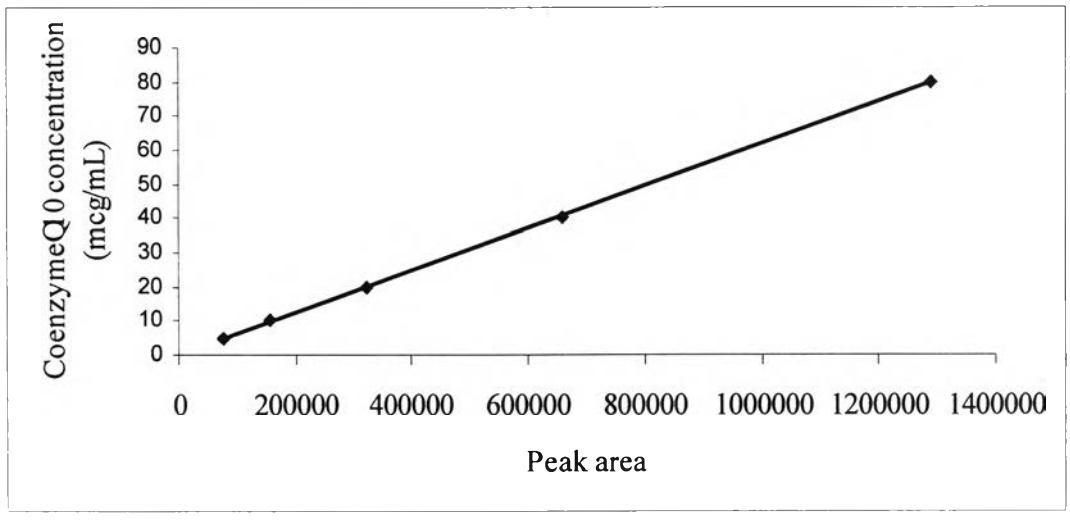


Figure 4-5 Calibration curve of the standard Coenzyme Q_{10}.

The Coenzyme Q_{10}-loaded nanoparticles prepared using 24 mM Tween® 80 and 2 mg/mL wax (Rx C12, C22, C32 and C42), 48 mM Tween® 80 and 4 mg/mL wax (Rx D14, D24, D34 and D44) and 72 mM Tween® 80 and 6 mg/mL wax (Rx E14, E24, E34 and E44) were subjected to evaluation of entrapment efficiency after loading various amount of Coenzyme Q_{10} (1-4 mg/mL). It should be mentioned that the centrifugal ultrafiltration technique used in the efficiency of incorporation was suitable as the high percent recovery was observed (> 85%).

From the results, the system containing less amount of wax (2 mg/mL) had less efficiency (< 50 %) to entrap the Coenzyme Q_{10} (Table 4-7 and Table D5 in appendix D). The reason might be due to a low amount of core matrix material and, thus, a limited matrix volume compared to those containing higher concentration of wax. However, in the same weight ratio of drug to matrix material at 0.5 (Rx C12, D24 and E36), it was found that the system containing 2 mg/mL wax still had lower entrapment efficiency than the system containing 4 and 6 mg/mL wax which might be due to the very small size of particles.

Table 4-7 Entrapment efficiency (%) and recovery (%) of Coenzyme Q_{10}-loaded nanoparticles (mean ± S.D., n =2).

Rx [a]	Drug/ material (wt ratio)	Amount of CoenzymeQ_{10} (mg)				% Entrapment	% Recovery
		Loaded [b]	Un-entrapped	Entrapped	Total [c]		
C12	0.5	0.94	0.59	0.23	0.82	28.33 ± 16.50	87.69 ± 2.36
C22	1	1.82	0.92	0.82	1.74	47.45 ± 6.17	95.64 ± 5.32
C32	1.5	2.80	1.69	1.03	2.72	37.79 ± 3.22	96.98 ± 1.15
C42	2	3.61	1.78	1.73	3.51	42.13 ± 17.70	97.09 ± 77.76
D14	0.25	1.15	0.20	1.02	1.21	83.81 ± 0.89	105.46 ± 19.81
D24	0.5	1.79	0.40	1.63	2.03	80.19 ± 1.07	113.77 ± 22.63
D34	0.75	2.81	0.56	2.08	2.64	78.74 ± 0.59	93.79 ± 1.07
D44	1	3.57	0.76	2.68	3.44	78.00 ± 1.85	96.24 ± 3.30
E16	0.17	1.03	0.20	0.75	0.95	78.95 ± 13.40	92.23 ± 0.00
E26	0.33	1.67	0.47	1.36	1.83	74.81 ± 5.76	109.58 ± 18.13
E36	0.5	2.73	0.67	1.98	2.64	74.56 ± 23.31	96.88 ± 4.41
E46	0.67	4.11	0.41	3.38	3.78	89.29 ± 1.19	91.97 ± 1.03

[a] Rx: the first number after the capital letter referred to the amount (mg/mL) of Coenzyme Q_{10} used.

[b] Loaded = the initial concentration of Coenzyme Q_{10} loaded.

[c] Total = sum of unentrapped and entrapped Coenzyme Q_{10}.

The Coenzyme Q_{10} entrapment of more than 74% was found in the nanoparticles containing 4 and 6 mg/mL of wax or having the weight ratio of drug to matrix material lower or equal to 1. Sufficient drug loading capacity is one of the prerequisites for the nanopaticles as carriers for topical drug delivery. In addition, size of nanoparticles is also critical. Dingler (1999) suggested that the small sizes of nanoparticles (<100 nm) will be in close contact with the stratum corneum that can increase the amount of encapsulated agents penetrating into the viable skin. These

ultrafine particles also showed an occlusive effect which might promote the penetration of active ingredients into the upper part of the epidermis.

From general trend of result derived from PCS measurement and incorporation studies, the formulation chosen for the further studies was the system compositing 4 mg/mL wax and 48 mM Tween®80 due to the higher amounts of Coenzyme Q_{10} (78-83%) were entrapped and smaller nanoparticles size (86-90 nm). Although, the systems with highest wax concentration (6 mg/mL) also provided high entrapment efficiency but the particles sizes were occasionally greater than 100 nm that might reduce the property in promotion of penetration.

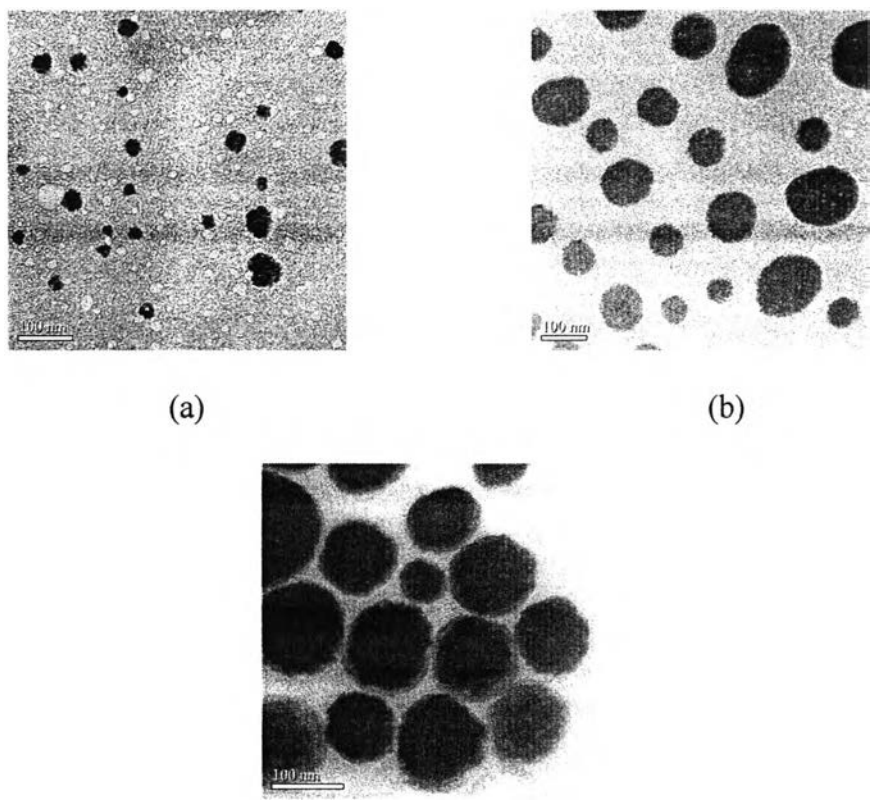
3. Characterization of Coenzyme Q_{10}-loaded nanoparticles by Transmission Electron Microscopy (TEM)

The Coenzyme Q_{10}-loaded nanoparticles prepared from microemulsion systems consisting of 4 mg/mL wax and 48 mM Tween® 80 and either 2 mg/ml (Rx D24) or 4 mg/mL (Rx D44) Coenzyme Q_{10} was determined for their size and morphology by Transmission Electron Microscopy (TEM).

The results from TEM analysis shown in Figure 4-6, illustrating that the sizes of nanoparticles were below 100 nm. The finding were in agreement with the light scattering (PCS) experiment in which the average particle sizes from the PCS results were 86.77 ± 4.40 nm and 86.33 ± 9.65 nm for systems containing 2 and 4 mg/mL Coenzyme Q_{10}, respectively. The Coenzyme Q_{10}-loaded nanoparticles were spherical and narrow in size distribution. However, there were some white spot appear in the TEM photograph that might be the residue of phosphotungstic acid (PTA) which used in staining process.

(a) (b)

Figure 4-6 Photograph under transmission electron microscope (TEM) of Coenzyme Q_{10}-loaded nanoparticles (a) Rx D24 and (b) Rx D44.



(a) (b)

(c)

Figure 4-7 Photograph under transmission electron microscope (TEM) of Coenzyme Q_{10}-loaded nanoparticles containing 2 mg/mL Coenzyme Q_{10} with the different concentrations of wax (a) 2 mg/mL, (b) 4 mg/mL and (c) 6 mg/mL.

In addition, upon increasing the concentration of wax at the constant amount of Coenzyme Q_{10} (2 mg/mL), the size of nanoparticles from TEM seemed to be slightly increased (Figure 4-7). The TEM results were also followed the same trend as the PCS measurement in which the sizes of Coenzyme Q_{10}-loaded nanoparticles were 57.00 ± 6.48, 86.77 ± 4.40 and 90.17 ± 5.32 nm for 2, 4 and 6 mg/mL wax, respectively.

4. Thermal analysis of Coenzyme Q_{10}-loaded nanoparticles by Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry (DSC) analysis was used to investigate the physical state of the obtained Coenzyme Q_{10}-loaded nanoparticle. It was noted that the sample was subjected to freeze-drying process before the experiment. DSC is a thermal analytical technique, which measures heat flow associated with transitions in materials as a function of temperature. DSC provides useful information about the physical and chemical changes that involve endothermic or exothermic process in heat capacity including melting and recrystallization behavior, the timing of polymorphic transitions and enthalpy (Cui, Hsu and Mumper, 2003; Heurtault *et al.*, 2003; Hou *et al.*, 2003; Müller, Mäder and Gohla, 2000).

The exothermic melting peak were present for Coenzyme Q_{10} alone, wax and a physical mixture of Coenzyme Q_{10} and wax as shown in Figures 4-8 and 4-9. The melting peak of Coenzyme Q_{10} was present at 51°C with slightly splitting peak that might due to any impurities in sample and the melting temperatures of wax were 39 and 52°C. Moreover, the DSC profile of a physical mixture of Coenzyme Q_{10} and wax showed the apparent exothermic shift peak at 47°C, which may be attributed to the fusion process of wax and Coenzyme Q_{10} as well as some interactions between wax and Coenzyme Q_{10} in the mixture. In contrast, there was no any melting peak

observed in the thermogram of Coenzyme Q_{10}-loaded nanoparticles. This finding indicated that the nanoparticles were structurally different from the physical mixture.

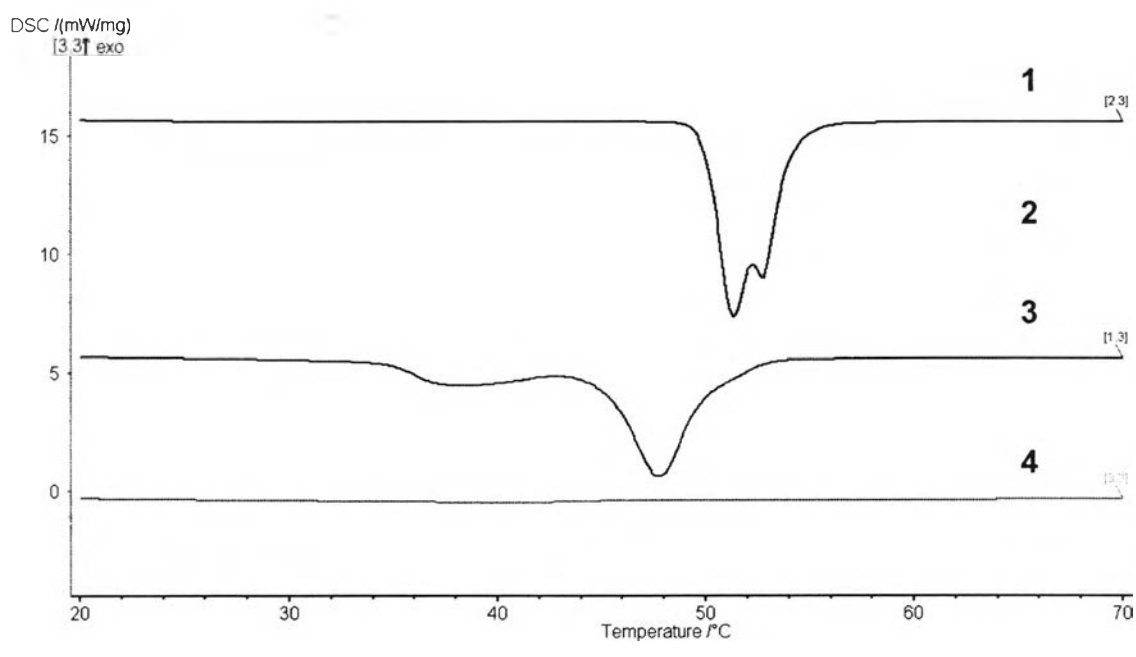


Figure 4-8 The DSC thermogram of (1) Coenzyme Q_{10}, (2) wax, (3) physical mixture of Coenzyme Q_{10} and wax at a weight ratio of 1:1, and (4) nanoparticles containing 4 mg/mL Coenzyme Q_{10}.

The melting peak of the materials to break-up of the crystal lattice observed in thermogram is normally referred to the crystallinity of the material. Whereas, in the amorphous state of the same material, molecules are not packed in a repeating long-range ordered fashion; hence no melting peak represented the breaking of crystal lattice. Consequently, the absence of melting peak in Coenzyme Q_{10}-loaded nanoparticles suggested that Coenzyme Q_{10} was dispersed in matrix material as an amorphous state and the drug-loaded nanoparticles not a simple physical mixture of their individual component (Buckton, 2002).

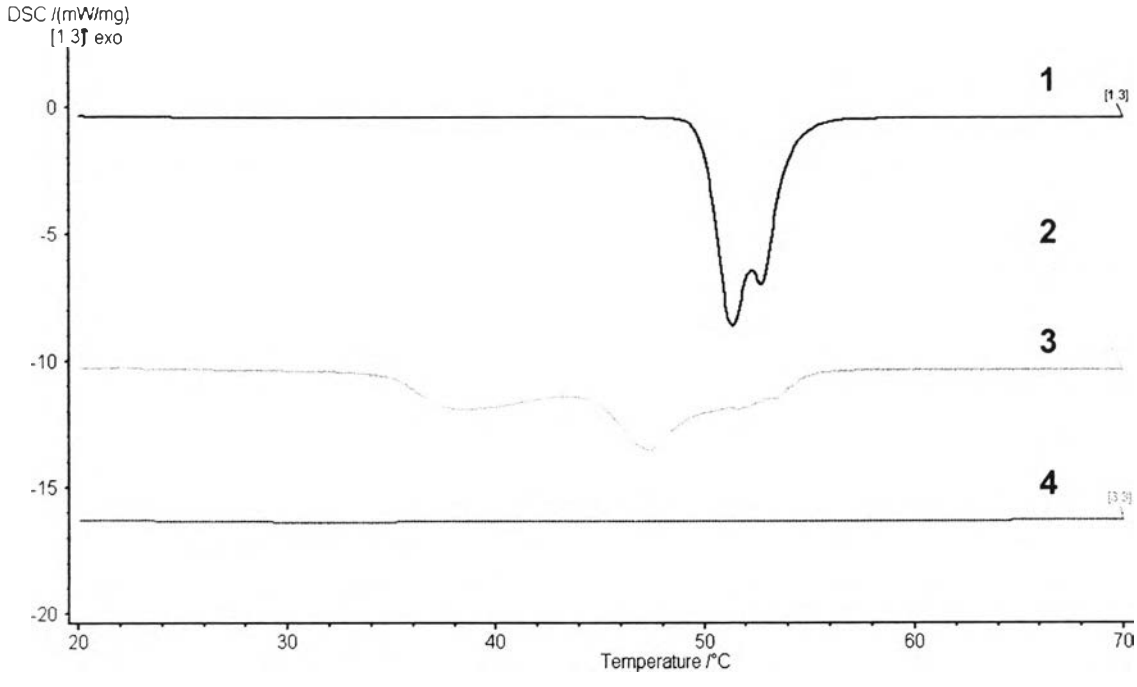


Figure 4-9 The DSC thermogram of (1) Coenzyme Q_{10}, (2) wax, (3) physical mixture of Coenzyme Q_{10} and wax at a weight ratio of 1:2, and (4) nanoparticles containing 2 mg/mL Coenzyme Q_{10}.

The effect on the crystalline habits of drugs may be related to the preparative method of the nanoparticles. In this experiment, the nanoparticles were prepared by rapid cooling of the warm o/w microemulsion in an ice-bath under mild stirring. In the microemulsion state, Coenzyme Q_{10} was partitioned partly in the internal oil phase and partly at the interphase between internal and continuous phase, depending on their lipophilicity. When nanoparticles were formed by a quick quenching of the microemulsion, the presence of the droplet structure of the microemulsion did not allow the Coenzyme-Q_{10} molecules to nucleate and form the crystal lattice, and consequently the Coenzyme Q_{10} molecules remain dispersed in the lipid matrix of the nanoparticles in an amorphous state. Moreover, the amorphous state is mostly detected after lyophilization that resulting in a higher energy state than crystalline form. This behavior might improve the solubility of Coenzyme Q_{10} in water (Cavalli

et al., 1997; Cavalli *et al.*, 1999). However, the similar result will be obtained when using simple cooling method in preparation process due to the same reason as in rapid cooling method.

5. *In vitro* release study

It needs to be verified that nanoparticles were able to release incorporated Coenzyme Q_{10} in order to achieve therapeutic effect of drug. The *in vitro* release of Coenzyme Q_{10} from nanoparticles containing 4 mg/mL wax, 48 mM Tween® 80 and 2 mg/mL (Rx D24) and 4 mg/mL (Rx D44) Coenzyme Q_{10}, was studied.

Lockman *et al.* (2003) suggested that drug release is dependent on the structure of the nanoparticles and type and length of polymer. From the result, the release profile of Coenzyme Q_{10} from lipid core material of nanoparticles exhibited a biphasic pattern characterized by a rapidly initial release in the first ten hours, followed by a slower release up to 120 hours (Figure 4-10, Table 4-8 and Tables D6-D7 in appendix D).

Table 4-8 Percentage of Coenzyme Q_{10} from nanoparticles, Rx D24 and D44 (mean ± S.D., n=3).

Time (hours)	Percentage Coenzyme Q_{10} released	
	Rx D24	Rx D44
2	34.74 ± 2.97	28.56 ± 1.35
6	59.70 ± 2.10	48.71 ± 1.97
10	71.92 ± 1.40	56.98 ± 3.40
20	82.88 ± 0.41	61.79 ± 3.12
60	90.75 ± 1.44	65.47 ± 3.13
100	95.75 ± 1.41	68.41 ± 3.04
120	97.18 ± 1.39	70.61 ± 2.91

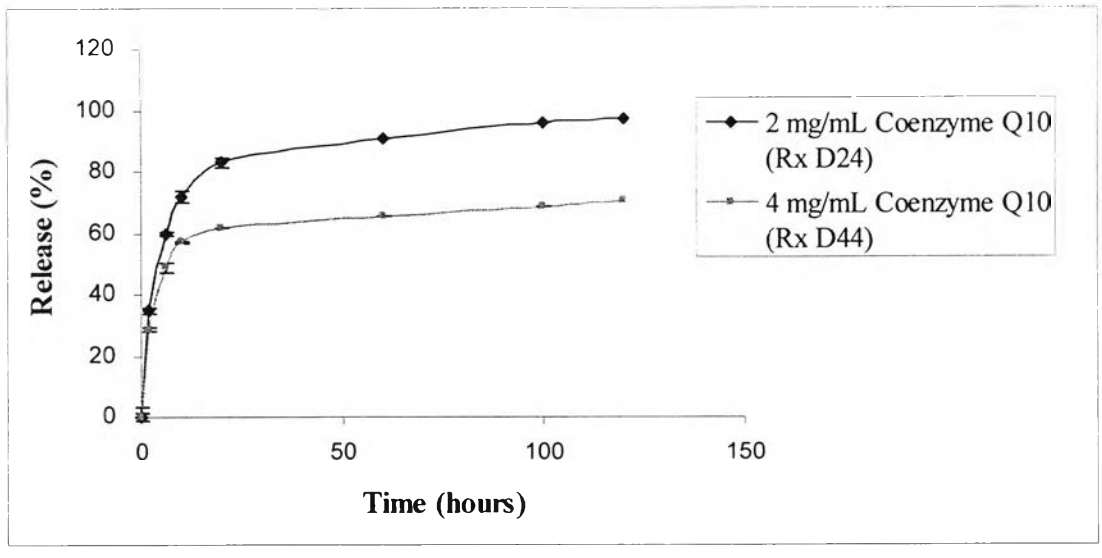


Figure 4-10 Release patterns of Coenzyme Q_{10} from nanoparticles, Rx D24 and D44 (mean ± S.D., n=3).

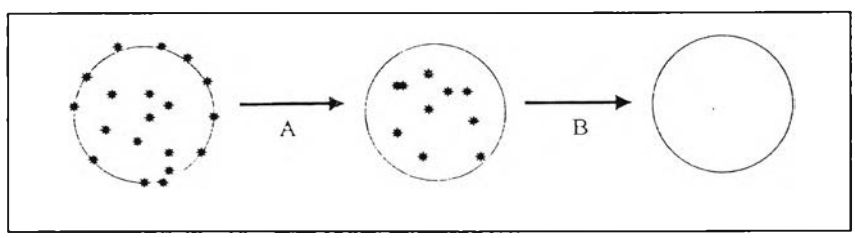


Figure 4-11 Mechanism of Coenzyme Q_{10} release from nanoparticle matrix. A. initial rapid desorbtion process (burst release) and B. slow controlled release process (Lockman *et al.*, 2002).

Figure 4-11 illustrated the mechanism of Coenzyme Q_{10} release from nanoparticle matrix. This model suggested that the initial rapid release (burst release) might be due to the release of Coenzyme Q_{10} located near the surface or loosely adsorbed on the surface of nanoparticles, thus the drug could be released immediately. Once the rapid component of release was complete, there was a slower, much more controlled release of Coenzyme Q_{10} owing to either nanoparticle degradation or diffusion of Coenzyme Q_{10} through nanoparticle matrix which indicated that the wax

matrix could retard the diffusion of Coenzyme Q_{10} which might mostly disperse in the wax matrix (Lockman *et al.*, 2002). For transdermal application, both burst release and sustained release are of interest. Burst release can be useful to improve the penetration of drug, while sustained release will supply the skin over a prolonged period of time with the drug (Mei *et al.*, 2003).

It was noted that, when concerning in cost effectiveness, Rx D24 will give the better outcome than Rx D44 due to with the similar entrapment efficiency (around 80%) Rx D24 lost the active drug in lower amount than Rx D44. Moreover, from the release profile, Rx D24 gave the higher percentage release than Rx D44 makes the more attractive in Rx D24.

6. Stability studies of Coenzyme Q_{10}-loaded nanoparticles

6.1 Stability of Coenzyme Q_{10}-loaded nanoparticles dispersion

The stability of Coenzyme Q_{10}-loaded nanoparticles dispersion at drug concentrations of 2 mg/mL (Rx D24) and 4 mg/mL (Rx D44) was observed after storage in light-protectant container at room temperature (25°C) and in a refrigerator (4°C) for 4 and 8 weeks. The samples were measured of the particles size by PCS and the amount of Coenzyme Q_{10} remained in the formulation by HPLC analysis. The results are summarized in Table 4-9, Table C5 in appendix C for PCS results and Table D8 in appendix D for HPLC results).

Table 4-9 The size of nanoparticles, Rx D24 and D44 (mean ± S.D., n = 3) and remaining amount of Coenzyme Q_{10} (mean ± S.D., n = 2).

Time (weeks)	Size (nm)				% remaining			
	4°C		25°C		4°C		25°C	
	Rx D24	Rx D44	Rx D24	Rx D44	Rx D24	Rx D44	Rx D24	Rx D44
0	86.77 ± 4.40	86.33 ± 9.65	86.77 ± 4.40	86.33 ± 9.65	100	100	100	100
4	98.63 ± 3.31	88.03 ± 7.06	ND	ND	99.42 ± 0.16	97.58 ± 0.62	88.83 ± 0.45	92.57 ± 0.04
8	97.10 ± 5.17	91.43 ± 7.98	ND	ND	97.91 ± 0.00	96.99 ± 0.08	82.20 ± 0.63	88.74 ± 0.27

ND = not determined; drug-loaded nanoparticles precipitated

The sizes Coenzyme Q_{10}-loaded nanoparticles after storage at 4 °C for 4 and 8 weeks were not significant different from the original sizes for Both Rx D24 and D44.

Coenzyme Q_{10}-loaded nanoparticles dispersion stored at 4°C were more stable than those stored at 25°C as no precipitation occurred. It was noticed that after 1 week of storage at room temperature, the Coenzyme Q_{10}-loaded nanoparticles began to precipitate out. Heurtault *et al.* (2003) found that the parameter including temperature had an effect on the stability of a nanoparticulate system. The induction of energy into the system such as input temperature led to the particle growth and subsequent destabilization of system. Moreover, the results might be due to a decrease in the high level of film rigidity of the surfactant (also named microviscosity). The microviscosity prevents fusion of the film layers after particle collision and is a temperature-dependent factor. When temperature increases, microviscosity then decreases leading to the destabilization of the system (Heurtault *et al.*, 2003; Freitas and Müller, 1998; Koziara *et al.*, 2003).

For the remaining amount of Coenzyme Q_{10} in the formulations, a decrease in amount of Coenzyme Q_{10} in dispersion might be due to chemical degradation of drug such as hydrolysis reaction. A loss of Coenzyme Q_{10} appeared to be less than 5% for the formulation kept at 4°C. At the higher temperature (25°C), the quantity of drug lost became greater than 10%, indicating any instability of Coenzyme Q_{10} against increasing temperature.

It could be recommended that nanoparticles dispersion containing Coenzyme Q_{10} should be stored at the lower temperature such as in the refrigerator in order to maintain the nanometric size and the level of drug remaining.

6.2 Stability of freeze-dried Coenzyme Q_{10}-loaded nanoparticles

Since it was previously found that the nanoparticles dispersion was physically unstable at room temperature together with a high loss of Coenzyme Q_{10} potency. Consequently, Coenzyme Q_{10}-loaded nanoparticles dispersions were subjected to freeze-drying process in order to increase the chemical and physical stability of Coenzyme Q_{10} over a period of time at room temperature. The transformation into dry product will prevent Ostwald ripening, avoid hydrolysis reaction (Lim and Kim, 2002; Mehnert and Mäder, 2001) and also offers principle possibilities for other studies such as thermal analysis by DSC and provides simple incorporation of Coenzyme Q_{10}-loaded nanoparticles into cream base for topical application.

From preliminary study, without any cryoprotectant added, Coenzyme Q_{10}-loaded nanoparticles dispersion were unable to form dry powder after subjected to freeze-drying process for 48 hours and the stick mass product were seen. Konan, Gurny and Alle'man (2002) suggested a use of cryoprotective excipients such as sugar to prevent particle aggregation during freeze-drying process. The cryoprotective effect

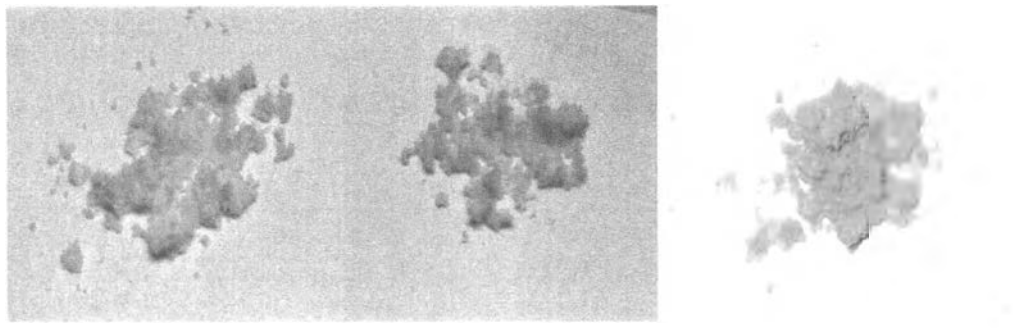
attribute to the ability of the additive (i.e., mannitol) to form a glassy amorphous matrix around the particles, thus preventing the particles from sticking together during the removal of water.

In this study, the cryoprotective agents namely, dextrose and mannitol solution at a concentration of 2, 4 and 8% w/w, were added to the particulate dispersion at a volume ratio of 1:1 cryoprotactant solution to Coenzyme Q_{10}-loaded nanoparticles dispersions, hence a final concentration of cryoprotectant solution in the formulation became to 1, 2 and 4% w/w, respectively. The appearance of freeze-dried powder of Coenzyme Q_{10}-loaded nanoparticles was observed (Table 4-10). The size of freeze-dried Coenzyme Q_{10}-loaded nanoparticles after stored at 25°C for 1 week was measured by reconstituted with warm water to obtain slightly turbid solution and the results are tabulated in Table 4-10 and Table C6 in appendix C)

Table 4-10 The appearance of freeze-dried Coenzyme Q_{10}- loaded nanoparticles and the size of reconstituted freeze-dried product after storage at 25°C for 1 week (mean ± S.D., n=3).

Rx	Appearance						Size after reconstitution (nm)		
	Dextrose (%w/w)			Mannitol (%w/w)			Mannitol (%w/w)		
	1	2	4	1	2	4	1	2	4
D24	S	S	S	Y,F	P,F	P,F	84.10 ± 1.06	85.97 ± 1.60	91.77 ± 1.66
D44	S	S	S	Y,F	P,F	P,F	80.03 ± 0.84	73.13 ± 0.90	83.77 ± 0.76

S = yellow, shrinkage and sticky mass; F = fluffy, shelf stable cake; Y = yellow color; P = Pale yellow



(a) (b) (c)

Figure 4-12 The appearance of freeze-dried Coenzyme Q_{10}-loaded nanoparticles containing (a) 2 mg/mL Coenzyme Q_{10} (yellow color), (b) 4 mg/mL Coenzyme Q_{10} (yellow color) and of (c) Coenzyme Q_{10} powder (orange color).

The freeze-dried product using dextrose as a cryoprotectant was undesirable as the product was a yellow shrinkage and sticky mass. On the other hand, the addition of mannitol provided a fluffy yellow freeze-dried product which was subsequent to further studies. All concentrations range of mannitol (1-4% w/w) used were sufficient to provide the size of reconstituted nanoparticles in nanometric range similar to the initial sizes of non-freeze dried product, 86.77 ± 4.40 nm and 86.33 ± 9.65 nm for system containing Coenzyme Q_{10} at 2 mg/mL (RxD24) and 4 mg/mL (RxD44). However, since the freeze-dried powders were very hygroscopic (as seen when stored unprotect), the product should be stored in cool, dry place.

The appearance of freeze-dried Coenzyme Q_{10}-loaded nanoparticles powder is shown in Figure 4-12. The concealing of Coenzyme Q_{10} color was occurred as seen from the pale yellow color of freeze-dried Coenzyme Q_{10}-loaded nanoparticles powder compared to the orange color of original Coenzyme Q_{10}. Dingler *et al.* (1999) offered that concealing of the color was important in the case of active ingredients which can lead to a less aesthetic appearance and unacceptance by the customer. Since Coenzyme Q_{10} was gradually decomposed and darkened when

exposed to light, the freeze-drying process might be of benefits to retard the unacceptable change of Coenzyme Q_{10}.

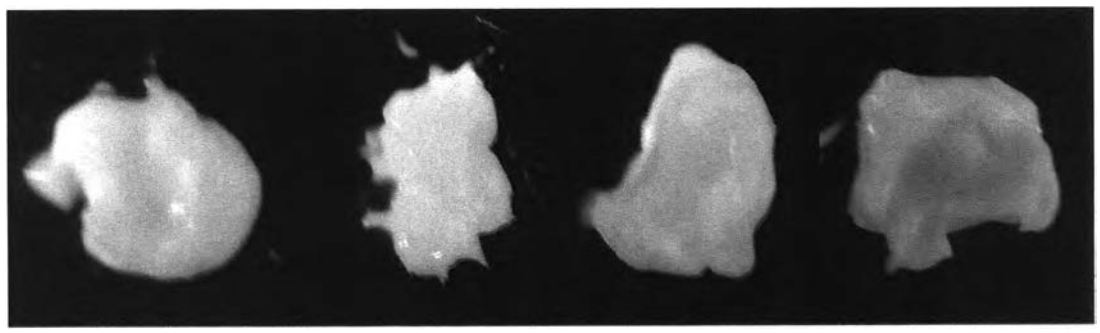
Moreover, the stability of freeze-dried Coenzyme Q_{10}-loaded nanoparticles using 2% w/w mannitol was studied after 4-week storage at 4 and 25°C. The stability result is expressed in Table 4-11 (see detail in Table D8 in appendix D). The results showed the high percentage remaining (>95%) of Coenzyme Q_{10} in the freeze-dried product compared to the non-freeze dried nanoparticles (<90%), especially when stored at 25°C.

Table 4-11 The stability of Coenzyme Q_{10} in freeze-dried nanoparticles, Rx D24 and D44, after storage for 4 week at 4°C and 25°C (mean ± S.D., n=2).

Rx	% Remaining of Coenzyme Q_{10} after 4-week storage	
	At 4°C	At 25°C
D24	99.79 ± 0.08	95.57 ± 0.12
D44	99.73 ± 0.37	98.31 ± 0.30

6.3 Stability of mixed-cream containing Coenzyme Q_{10}-loaded nanoparticles

The freeze-dried Coenzyme Q_{10}–loaded nanoparticles (Rx D24 and D44) were mixed with a commercially available cream base for investigation of the physical appearance using freeze-thaw experiment by storing the product at 4°C (in refrigerator) for 48 hours and then at 45°C (in hot air oven) for another 48 hours and for 6 cycles. For comparison, Coenzyme Q_{10} powder was subjected to combine with the commercial cream base. The results are presented in Figure 4-13 and Table 4-12.



(a) (b) (c) (d)

Figure 4-13 The appearance of (a) o/w cream base, mixed-cream containing freeze-dried nanoparticles load with Coenzyme Q_{10} (b) 2 mg/mL (Rx D24), (c) 4 mg/mL (Rx D44) and (d) mixed-cream containing Coenzyme Q_{10} powder.

Table 4-12 The stability of mixed cream containing the freeze-dried Coenzyme Q_{10}–loaded nanoparticles and Coenzyme Q_{10} crystal subjected to accelerated test.

Rx	Appearance	Stability at different freeze-thaw cycles					
		Cycle1	Cycle2	Cycle3	Cycle4	Cycle5	Cycle6
D24	Homogeneous, pale yellow cream	Stable	Stable	Stable	Stable	Stable	Stable
D44	Homogeneous, pale yellow cream	Stable	Stable	Stable	Stable	Stable	Stable
Co Q_{10} crystal	Non-homogeneous cream	ND	ND	ND	ND	ND	ND

ND = not determined; separation of drug crystal

From the results, the mixed cream containing freeze-dried Coenzyme Q_{10}–loaded nanoparticles appeared to possess the physical stability indicated by the lack of color change or separation during freeze-thaw test. The homogenous appearance of product was observed when the freeze-dried Coenzyme Q_{10}-loaded nanoparticles were used while the Coenzyme Q_{10} powder could not thoroughly mix to form a cream with homogeneous texture. The freeze-dried Coenzyme Q_{10}–loaded nanoparticle mixed cream also left no gritty residue after topical application which would be cosmetically attractive.

Furthermore, the amount of Coenzyme Q_{10} in the nanoparticles mixed-cream after being kept in light-resistant container for 4 weeks at room temperature (25°C) was determined by HPLC method.

Table 4-13 The stability of Coenzyme Q_{10} in freeze-dried Coenzyme Q_{10}-loaded nanoparticles mixed cream after storage for 4 weeks at 25°C (mean ± S.D., n = 2).

Rx	% Remaining of Coenzyme Q_{10}
D24	99.93 ± 0.01
D44	99.66 ± 0.21

The results showed that the Coenzyme Q_{10} still remained in the mixed-cream over 4 weeks at 25°C (Table 4-13 and Table D10 in appendix D). Hence, from the overall results, it could be possible to formulate the Coenzyme Q_{10}-loaded nanoparticles from microemulsion template which were stable and suitable to be employed as drug carrier for topical delivery of Coenzyme Q_{10}.