CHAPTER IV

RESULTS

1. Microemulsion formulation and phase diagram

The microemulsion systems which composed of tween 20 or tween 80 as a surfactant, glycerin, propylene glycol, or polyethylene glycol 400 as a cosurfactant, soybean oil, and water for injection in various ratio of surfactant and cosurfactant were investigated. The formability of each system is shown in Table 6. It could be seen that tween 20 as surfactant in the investigated formulations could not form microemulsion at all whereas some systems with tween 80 as surfactant were able to form microemulsion. Moreover, only systems with glycerin and polyethylene glycol 400 as cosurfactant were found to form microemulsion in the ratio of 1:1.5, 1:1, 1:0.7, 1:0.5 for tween 80:glycerin and 1:0.7, 1:0.5 for tween 80:polyethylene glycol 400 whereas systems with propylene glycol as cosurfactant could not form microemulsion at any ratio of surfactant:cosurfactant. The pseudo-ternary phase diagrams of microemulsion systems are presented in the Figures 8-9. The shaded area of the phase diagram represented the area where microemulsion existed as fluid, clear, transparent, and non-birefringent.

Comparison between microemulsion areas for the tween 80:glycerin systems of different ratio of surfactant:cosurfactant as shown in Figure 8 revealed that the largest microemulsion area was obtained from tween 80:glycerin system at the ratio of 1:0.5. This area decreased when the ratio of tween 80:glycerin decreased from 1:0.7 to 1:1 and 1:1.5. Furthermore, the maximum amount of the solubilized oil that obtained from tween 80:glycerin system at the ratio of 1:0.5 was 21% w/w. This amount was decreased to 18% w/w, 17% w/w, and 16% w/w when the ratio of tween 80:glycerin decreased from 1:0.7 to 1:1 and 1:1.5, respectively. For the tween 80:polyethylene glycol 400 systems as shown in Figure 9, the results were similarly to the tween 80:glycerin systems that the largest microemulsion area was obtained from tween80:polyethylene glycol 400 at the ratio of 1:0.5 and the area decreased when the

ratio of tween 80:polyethylene glycol 400 decreased to 1:0.7. The maximum of the solubilized oil was 12% w/w, which obtained from tween 80:polyethylene glycol 400 at the ratio of 1:0.5. The amount of oil was decreased to 11%w/w when the ratio of tween 80:polyethylene glycol 400 decreased to 1:0.7. From this study, the results indicated that when the surfactant:cosurfactant ratio increased the microemulsion area was increased. Glycerin as cosurfactant could form microemulsion better than polyethylene glycol 400. Furthermore, the amount of oil was solubilized in tween 80:glycerin system more than tween 80:polyethylene glycol 400 systems.

Microemulsion containing 8%, 6%, and 4% w/w of soybean oil were selected in this study due to low amount of surfactants. For designation of formulations, tween 80, glycerin, polyethylene glycol 400, soybean oil, water for injection and diazepam were T, G, P, O, W, and D, respectively. The number after surfactant and cosurfactant represented the ratio of surfactant or cosurfactant while the number after oil designated the amount of oil (%w/w) and the number after the drug represented the amount of diazepam. For example, the system of tween 80:glycerin at ratio 1:1.5, which had soybean oil 8%, designated T1 G1.5 O8, whereas this formulation containing diazepam 5 and 10 mg/ml designated T1 G1.5 O8 D5 and T1 G1.5 O8 D10, respectively. The formulations are listed in Table 7. They were in pseudo-ternary phase diagram of the system of soybean oil, water for injection, tween 80, and glycerin for tween 80:glycerin weight ratio of 1:1.5 and 1:1 (Figure 8 (A) and (B)) and the system of soybean oil, water for injection, tween 80, and polyethylene glycol 400 for tween 80:polyethylene glycol 400 weight ratio of 1:0.7 and 1:0.5 (Figure 9 (A) and (B)).

2. Preparation and physicochemical characterization of microemulsion

2.1 Determination of microemulsion type and non-birefringent property

In this study, the dilution test and the dye solubility test were employed. Table 8 summarizes the results of the tests for microemulsion type and non-birefringent property of microemulsion with and without diazepam.

Table 6 The formability of microemulsion systems.

Oil	Surfactant	Cosurfactant	Water	Ratio of surfactant : cosurfactant	Formability*
O	T20	G	W	1:1.5	-
O	T20	G	W	1:1	-
O	T20	G	W	1:0.7	-
O	T20	G	W	1:0.5	-
O	T20	PG	W	1:1.5	-
O	T20	PG	W	1:1	-
O	T20	PG	W	1:0.7	-
O	T20	PG	W	1:0.5	-
O	T20	P	W	1:1.5	-
O	T20	P	W	1:1	-
O	T20	P	W	1:0.7	-
O	T20	P	W	1:0.5	-
O	T	G	W	1:1.5	+
O	T	G	W	1:1	+
O	T	G	W	1:0.7	+
O	T	G	W	1:0.5	+
O	T	PG	W	1:1.5	-
O	T	PG	W	1:1	-
O	T	PG	W	1:0.7	-
O	T	PG	W	1:0.5	-
O	T	P	W	1:1.5	-
O	T	P	W	1:1	-
O	T	P	W	1:0.7	+
O	T	P	W	1:0.5	+

O = soybean oil
G = glycerin
T20 = tween 20
PG = propylene glycol
T = tween 80
P = polyethylene glycol 400
W = water for injection
\+ = The system could form microemulsion.
\- = The system could not form microemulsion.
* = The results from weight ratio of surfactants:oil = 9:1 to 1:9 and the characteristic of microemulsions was fluid, clear and transparent which confirmed by non-birefringent property and TEM.

Table 7 Composition of diazepam o/w microemulsions

System	Amount (%w/w)					Amount of diazepam (g)	
	O	T	G	P	W	Conc. 5 mg/ml	Conc. 10 mg/ml
T:G = 1:1.5	8.00	29.22	43.84	-	18.94	4.4389	8.8778
	6.00	27.60	41.40	-	25.00	4.4512	8.9023
	4.00	30.83	46.24	-	18.93	4.4338	8.8676
T:G = 1:1	8.00	36.53	36.53	-	18.94	4.4707	8.9413
	6.00	34.50	34.50	-	25.00	4.4819	8.9638
	4.00	38.53	38.53	-	18.94	4.4711	8.9421
T:P = 1:0.7	8.00	41.74	-	29.23	21.03	4.6335	9.2670
	6.00	35.69	-	24.98	33.33	4.6590	9.3179
	4.00	36.85	-	25.82	33.33	4.6464	9.2928
T:P = 1:0.5	8.00	47.31	-	23.66	21.03	4.6679	9.3358
	6.00	40.44	-	20.23	33.33	4.6895	9.3791
	4.00	41.78	-	20.89	33.33	4.6781	9.3563

From the dilution test on microemulsions without diazepam, the microemulsions became slightly turbid when water was added and mixed for a few minutes. After one hour standing at room temperature all preparations were still slightly turbid. Phase separation was not shown. In contrast all the preparations were turbid after adding soybean oil. After standing at room temperature for one hour phase separation occurred. The upper phase was slightly yellowish turbid solution whereas the lower phase was more turbid than the upper phase. For the microemulsions containing diazepam at concentration of 5 mg/ml and 10 mg/ml, the results from dilution test were similar to microemulsions without diazepam. When the system was added with water after shaking for a few minutes the preparation became slightly turbid and phase separation did not occur. When the system was added with soybean oil, the preparations were turbid and phase separation occurred. Thus, the results indicated that the external phase of all microemulsion preparations was hydrophilic phase.

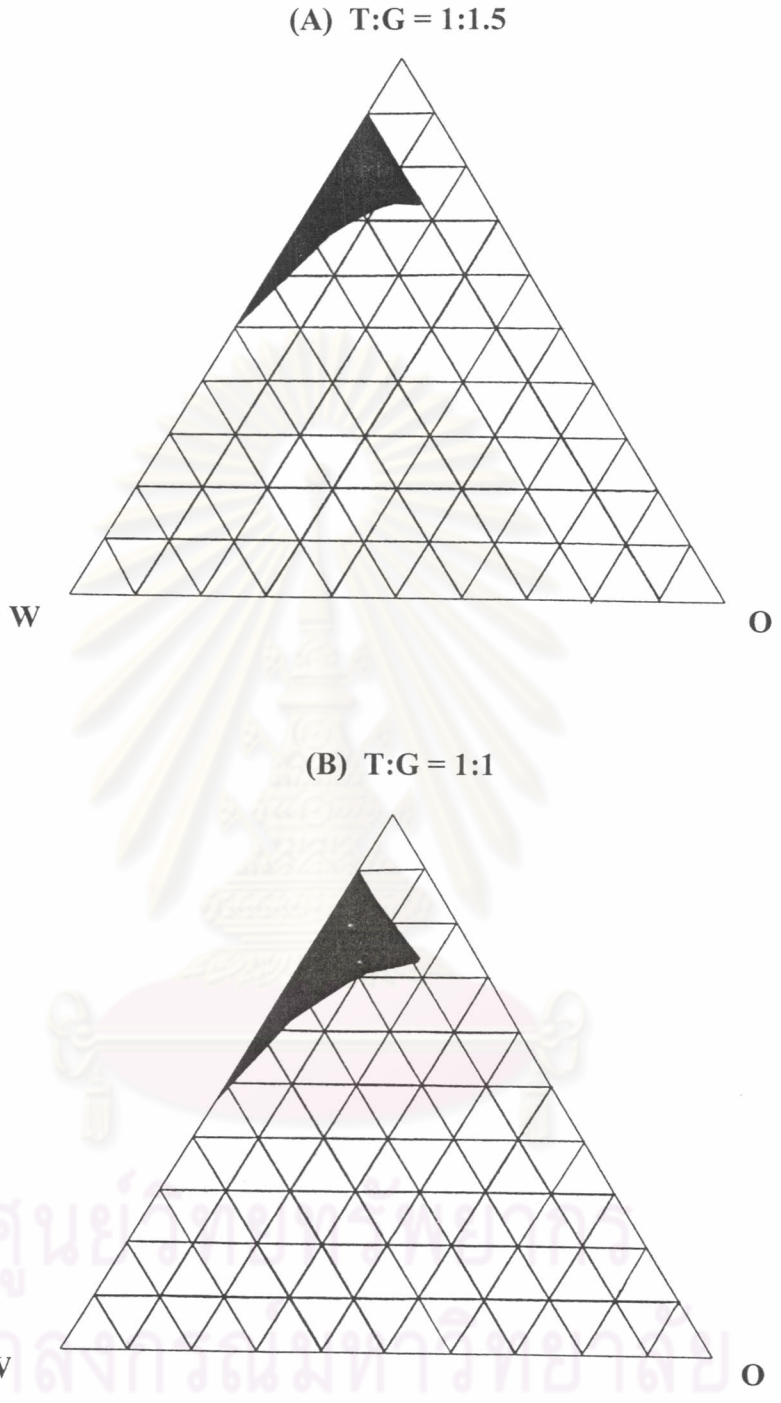

Figure 8 Pseudo-ternary phase diagram of the system of soybean oil (O), water for injection (W), tween 80 (T), and glycerin (G) at tween 80:glycerin weight ratio 1:1.5 (A), 1:1 (B), 1:0.7 (C), and 1:0.5 (D). The shaded area represents the microemulsion.

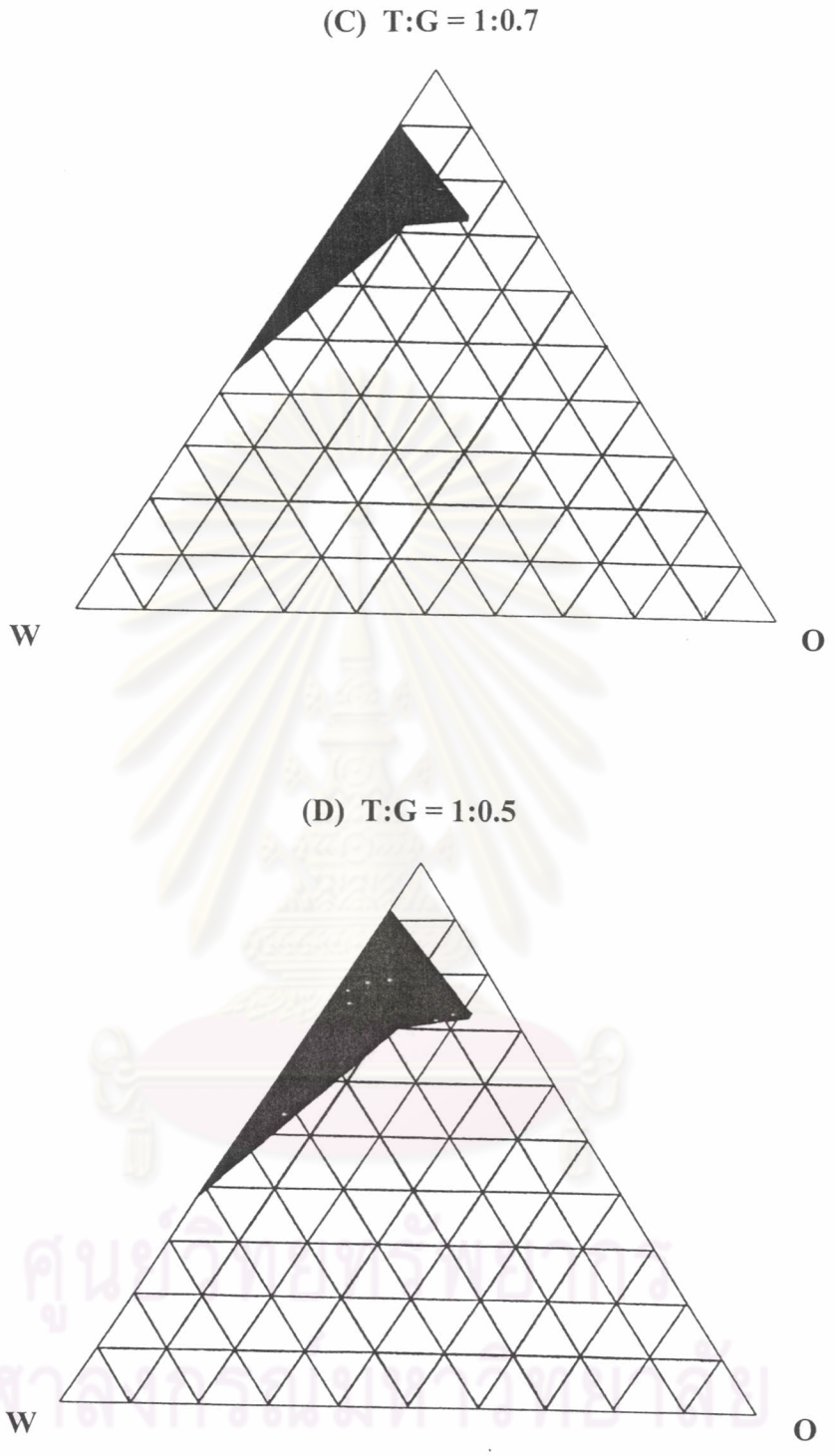

Figure 8 (Cont.) Pseudo-ternary phase diagram of the system of soybean oil (O), water for injection (W), tween 80 (T), and glycerin (G) at tween 80:glycerin weight ratio 1:1.5 (A), 1:1 (B), 1:0.7 (C), and 1:0.5 (D). The shaded area represents the microemulsion.

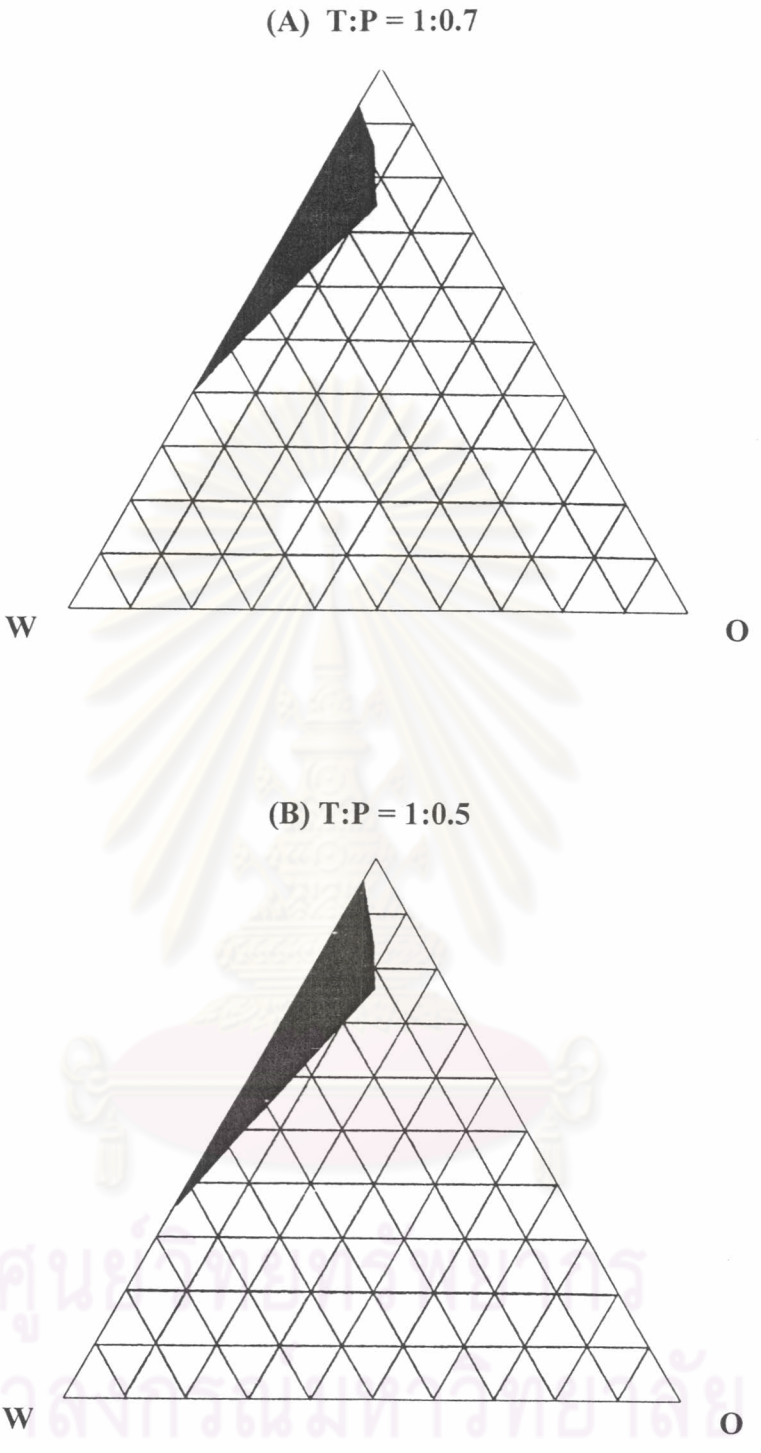

Figure 9 Pseudo – ternary phase diagram of the system of soybean oil (O), water for injection (W), tween 80 (T), and polyethylene glycol 400 (P) for tween 80 : polyethylene glycol 400 weight ratio 1:0.7 (A) and 1:0.5 (B). The shaded area represents the microemulsion.

Table 8 The microemulsion type and non-birefringent property of microemulsion with and without diazepam.

Formulation	Dilution Test		Dye solubility test		non-birefringent
	water dilution	oil dilution	water soluble dye[a]	oil soluble dye[b]	
T1 G1.5 O8	*	**	m	im	+
T1 G1.5 O8 D5	*	**	m	im	+
T1 G1.5 O8 D10	*	**	m	im	+
T1 G1.5 O6	*	**	m	im	+
T1 G1.5 O6 D5	*	**	m	im	+
T1 G1.5 O6 D10	*	**	m	im	+
T1 G1.5 O4	*	**	m	im	+
T1 G1.5 O4 D5	*	**	m	im	+
T1 G1.5 O4 D10	*	**	m	im	+
T1 G1 O8	*	**	m	im	+
T1 G1 O8 D5	*	**	m	im	+
T1 G1 O8 D10	*	**	m	im	+
T1 G1 O6	*	**	m	im	+
T1 G1 O6 D5	*	**	m	im	+
T1 G1 O6 D10	*	**	m	im	+
T1 G1 O4	*	**	m	im	+
T1 G1 O4 D5	*	**	m	im	+
T1 G1 O4 D10	*	**	m	im	+
T1 P0.7 O8	*	**	m	im	+
T1 P0.7 O8 D5	*	**	m	im	+
T1 P0.7 O8 D10	*	**	m	im	+
T1 P0.7 O6	*	**	m	im	+
T1 P0.7 O6 D5	*	**	m	im	+
T1 P0.7 O6 D10	*	**	m	im	+
T1 P0.7 O4	*	**	m	im	+
T1 P0.7 O4 D5	*	**	m	im	+
T1 P0.7 O4 D10	*	**	m	im	+
T1 P0.5 O8	*	**	m	im	+
T1 P0.5 O8 D5	*	**	m	im	+
T1 P0.5 O8 D10	*	**	m	im	+
T1 P0.5 O6	*	**	m	im	+
T1 P0.5 O6 D5	*	**	m	im	+
T1 P0.5 O6 D10	*	**	m	im	+
T1 P0.5 O4	*	**	m	im	+
T1 P0.5 O4 D5	*	**	m	im	+
T1 P0.5 O4 D10	*	**	m	im	+

[a] = Tartrazine

[b] = D&C red no.17

* = no separation

** = separation

+ = non-birefringent

m = miscible

im = immiscible

From the dye solubility test on drug free microemulsions, when tartrazine was added it was easily miscible with the microemulsions. The system exhibited clear, homogeneous yellow solution. In contrast when the oil soluble dye, D&C red No.17, was added the dye was not miscible with the microemulsion and the system became turbid. For the microemulsions containing diazepam at concentration 5 mg/ml and 10 mg/ml, the results from dye solubility test were not different from microemulsions without diazepam. Therefore, the results from dilution test and dye solubility test indicated that all preparations were o/w microemulsion. Furthermore, the preparations with and without drug and the boundary of microemulsion area were appeared dark when viewed under cross-polarizer indicating of non-birefringent property. Thus, it would be classified as a microemulsion.

2.2 Physical appearance

The physical appearances of the drug free and drug loaded microemulsions both before and after sterilization by autoclaving are listed in Table 9. The microemulsion without diazepam were clear, transparent, and one-phase yellow solution before autoclaving. After steam sterilization, the preparations containing polyethylene glycol 400 as cosurfactant showed good appearance both the surfactant:cosurfactant ratio of 1:0.7 and 1:0.5. They were clear, transparent, and one-phase yellow solution, whereas the preparations containing glycerin as cosurfactant showed phase separation both the surfactant:cosurfactant ratio of 1:1.5 and 1:1. The upper phase was clear, yellow solution and the lower phase was clear colorless solution. Although phase separation occurred during the sterilization process in the tween 80:glycerin systems, the preparations were recovered to one-phase microemulsions by gently shaking for a few seconds.

For microemulsions containing diazepam at the concentrations of 5 mg/ml and 10 mg/ml, before autoclaving the appearance of microemulsions was clear, transparent, and one-phase yellow solution although the drug was incorporated. Phase separation did not occur. Furthermore, the drug precipitation was not shown. After autoclaving, the results were similar to the microemulsion without diazepam that the preparations containing polyethylene glycol 400 as cosurfactant showed clear, transparent, and one-phase yellow solution. Both microemulsions containing two

levels of drug did not show any drug precipitation. For the preparations containing glycerin as cosurfactant, phase separation occurred in microemulsions containing diazepam of both concentrations. The upper phase was clear, yellow solution and the lower phase was clear, colorless solution. There was no drug precipitation in the microemulsions. Although phase separation occurred during the sterilization process in the tween 80:glycerin systems, the preparations recovered to one-phase microemulsions by gently shaking for a few seconds.

2.3 Refractive index

The refractive indices of microemulsion preparations are shown in Table 10 and Appendix C. For drug-free microemulsion, the refractive indices of microemulsion in tween 80:glycerin system at the ratio of 1:1.5 were 1.4478, 1.4389, and 1.4473 in Formulations T1 G1.5 O8, T1 G1.5 O6, and T1 G1.5 O4, respectively. The Formulation T1 G1.5 O6 was the lowest. When diazepam was incorporated at concentration 5 mg/ml and 10 mg/ml, the refractive indices of all formulations were slightly changed. There were in the range of 1.4396 to 1.4490. Low refractive indices were correspondingly obtained from Formulation T1 G1.5 O6 D5 and T1 G1.5 O6 D10. In consideration of microemulsion compositions, these formulations with low refractive indices had more amount of water in formulation than other microemulsions in the series. When decreasing the ratio of tween 80:glycerin to 1:1, the refractive indices of microemulsions showed similar results. Formulations T1 G1 O6, T1 G1 O6 D5, and T1 G1 O6 D10 had the low refractive indices and contained more water than other formulations.

In tween 80:polyethylene glycol 400 system at the ratio of 1:0.7, the refractive indices of microemulsion without drug were in the range 1.4394 to 1.4493. When the drug was loaded in microemulsions, the refractive indices were slightly different. Formulations T1 P0.7 O6, T1 P0.7 O6 D5, T1 P0.7 O6 D10, T1 P0.7 O4, T1 P0.7 O4 D5 and T1 P0.7 O4 D10 which had more amount of water in formulations had low refractive indices of about 1.43. Similar results to tween 80:polyethylene glycol 400 system at the ratio of 1:0.5, the refractive indices of drug-free microemulsion were

Table 9 The physical appearances of the microemulsions with and without diazepam both before and after sterilization by autoclaving.

Formulation	Macroscopic observation	
	Before autoclaving	after autoclaving
T1 G1.5 O8	*	**
T1 G1.5 O8 D5	*	**
T1 G1.5 O8 D10	*	**
T1 G1.5 O6	*	**
T1 G1.5 O6 D5	*	**
T1 G1.5 O6 D10	*	**
T1 G1.5 O4	*	**
T1 G1.5 O4 D5	*	**
T1 G1.5 O4 D10	*	**
T1 G1 O8	*	**
T1 G1 O8 D5	*	**
T1 G1 O8 D10	*	**
T1 G1 O6	*	**
T1 G1 O6 D5	*	**
T1 G1 O6 D10	*	**
T1 G1 O4	*	**
T1 G1 O4 D5	*	**
T1 G1 O4 D10	*	**
T1 P0.7 O8	*	*
T1 P0.7 O8 D5	*	*
T1 P0.7 O8 D10	*	*
T1 P0.7 O6	*	*
T1 P0.7 O6 D5	*	*
T1 P0.7 O6 D10	*	*
T1 P0.7 O4	*	*
T1 P0.7 O4 D5	*	*
T1 P0.7 O4 D10	*	*
T1 P0.5 O8	*	*
T1 P0.5 O8 D5	*	*
T1 P0.5 O8 D10	*	*
T1 P0.5 O6	*	*
T1 P0.5 O6 D5	*	*
T1 P0.5 O6 D10	*	*
T1 P0.5 O4	*	*
T1 P0.5 O4 D5	*	*
T1 P0.5 O4 D10	*	*

* = clear, transparent, one-phase yellow solution

** = two-phase separation (upper phase-yellow solution, lower phase-clear, colorless solution) but recovered to one-phase by gently shaking

Table 10 The pH, viscosity, and refractive index of the investigated microemulsions.

Formulation	pH		Viscosity* (cps) (mean±SD)	Refractive Index* (mean±SD)
	before autoclaving	after autoclaving		
T1 G1.5 O8	7.06	6.81	813.29 ± 2.86	1.4478 ± 0.0008
T1 G1.5 O8 D5	7.45	7.12	979.07 ± 4.74	1.4490 ± 0.0002
T1 G1.5 O8 D10	7.14	7.11	1062.03 ± 6.30	1.4483 ± 0.0006
T1 G1.5 O6	7.08	7.09	365.04 ± 5.84	1.4389 ± 0.0008
T1 G1.5 O6 D5	6.96	6.81	431.83 ± 1.34	1.4396 ± 0.0006
T1 G1.5 O6 D10	7.18	7.03	522.18 ± 3.83	1.4397 ± 0.0002
T1 G1.5 O4	6.95	6.84	725.27 ± 3.32	1.4473 ± 0.0003
T1 G1.5 O4 D5	7.12	7.05	854.31 ± 5.31	1.4480 ± 0.0002
T1 G1.5 O4 D10	7.25	7.22	940.33 ± 4.04	1.4468 ± 0.0002
T1 G1 O8	7.19	7.01	1085.44 ± 4.19	1.4494 ± 0.0008
T1 G1 O8 D5	7.39	7.18	1138.32 ± 3.68	1.4496 ± 0.0002
T1 G1 O8 D10	7.21	7.06	1234.68 ± 5.56	1.4491 ± 0.0003
T1 G1 O6	6.97	6.81	948.68 ± 4.42	1.4385 ± 0.0004
T1 G1 O6 D5	7.19	6.92	1008.88 ± 3.66	1.4395 ± 0.0006
T1 G1 O6 D10	7.25	7.01	1128.43 ± 6.50	1.4398 ± 0.0008
T1 G1 O4	7.07	6.77	1052.66 ± 2.63	1.4491 ± 0.0003
T1 G1 O4 D5	7.02	6.85	1145.26 ± 6.79	1.4487 ± 0.0002
T1 G1 O4 D10	7.26	6.76	1261.92 ± 4.53	1.4490 ± 0.0003
T1 P0.7 O8	6.7	6.23	705.55 ± 6.37	1.4493 ± 0.0004
T1 P0.7 O8 D5	6.85	6.64	770.98 ± 6.43	1.4479 ± 0.0004
T1 P0.7 O8 D10	7.12	6.78	848.85 ± 3.68	1.4486 ± 0.0005
T1 P0.7 O6	6.58	6.23	460.01 ± 4.02	1.4394 ± 0.0008
T1 P0.7 O6 D5	7.04	6.81	535.13 ± 2.98	1.4397 ± 0.0009
T1 P0.7 O6 D10	6.83	6.57	652.90 ± 3.33	1.4384 ± 0.0004
T1 P0.7 O4	6.91	6.66	452.59 ± 1.43	1.4394 ± 0.0006
T1 P0.7 O4 D5	7.18	6.75	514.31 ± 4.81	1.4384 ± 0.0007
T1 P0.7 O4 D10	7.24	6.88	652.65 ± 4.06	1.4396 ± 0.0009
T1 P0.5 O8	6.71	6.22	963.99 ± 5.55	1.4479 ± 0.0002
T1 P0.5 O8 D5	6.93	6.89	1043.35 ± 5.71	1.4490 ± 0.0003
T1 P0.5 O8 D10	7.11	6.74	1142.06 ± 3.32	1.4489 ± 0.0003
T1 P0.5 O6	6.86	6.32	774.28 ± 2.64	1.4377 ± 0.0010
T1 P0.5 O6 D5	6.94	6.53	834.69 ± 4.06	1.4377 ± 0.0007
T1 P0.5 O6 D10	7.02	6.84	915.69 ± 4.90	1.4380 ± 0.0003
T1 P0.5 O4	6.55	6.07	723.75 ± 4.85	1.4381 ± 0.0005
T1 P0.5 O4 D5	6.87	6.52	877.59 ± 3.40	1.4378 ± 0.0003
T1 P0.5 O4 D10	7.17	7.08	919.05 ± 6.25	1.4382 ± 0.0005

* = average from three determinations

in the range 1.4381 to 1.4479. When diazepam was incorporated at concentration of 5 mg/ml and 10 mg/ml, the refractive indices were slightly changed. The low refractive indices were obtained from Formulations T1 P0.5 O6, T1 P0.5 O6 D5, T1 P0.5 O6 D10, T1 P0.5 O4, T1 P0.5 O4 D5, and T1 P0.5 O4 D10, which had more amount of water than other formulations. From the above investigation, the refractive indices of microemulsions were decreased when increasing the amount of water in formulation. When diazepam was incorporated the refractive index of microemulsion was slightly changed.

2.4 Viscosity

The viscosities of the microemulsions with and without drug are shown in Table 10 and in Appendix C. For the preparations without diazepam, the viscosities of microemulsions of tween 80:glycerin system at the ratio of 1:1.5 were 813.29, 365.04, and 725.27 cps when decreasing the percentage of oil from 8 to 6 and 4% w/w, respectively. At the same percentages of oil but decreasing the ratio of surfactant:cosurfactant to 1:1, the viscosities of microemulsions were 1085.44, 948.68, and 1052.66 cps, respectively. Comparison in surfactant:cosurfactant ratio revealed that the viscosity of microemulsions was not increased when increasing the amount of oil phase as shown in Formulation T1 G1.5 O6 and T1 G1 O6. At the equal amount of oil in tween 80:glycerin systems, the viscosity of system at the ratio of 1:1 was higher than that of the ratio of 1:5 as shown in Figure 10.

The viscosity of drug free microemulsions in tween 80:polyethylene glycol 400 systems at the ratio of 1:0.7 revealed that Formulation T1 P0.7 O8 has the highest viscosity, which was 705.55. The viscosity decreased to 460.01 and 452.59 cps when decreasing the percentage of oil to 6 and 4% w/w, respectively. For microemulsions at the ratio of surfactant:cosurfactant 1:0.5, the viscosities of Formulations T1 P0.5 O8, T1 P0.5 O6, and T1 P0.5 O4 were 963.99, 774.28, and 723.75 cps, respectively. These results showed that within the same surfactant:cosurfactant ratio, the viscosity of microemulsions was increased when the amount of oil phase increased as shown in Figure 10. At the equal amount of oil in tween 80:polyethylene glycol 400 systems, the viscosity of system at the tween 80:polyethylene glycol 400 ratio of 1:0.5 was higher than that at the ratio of 1:0.7.

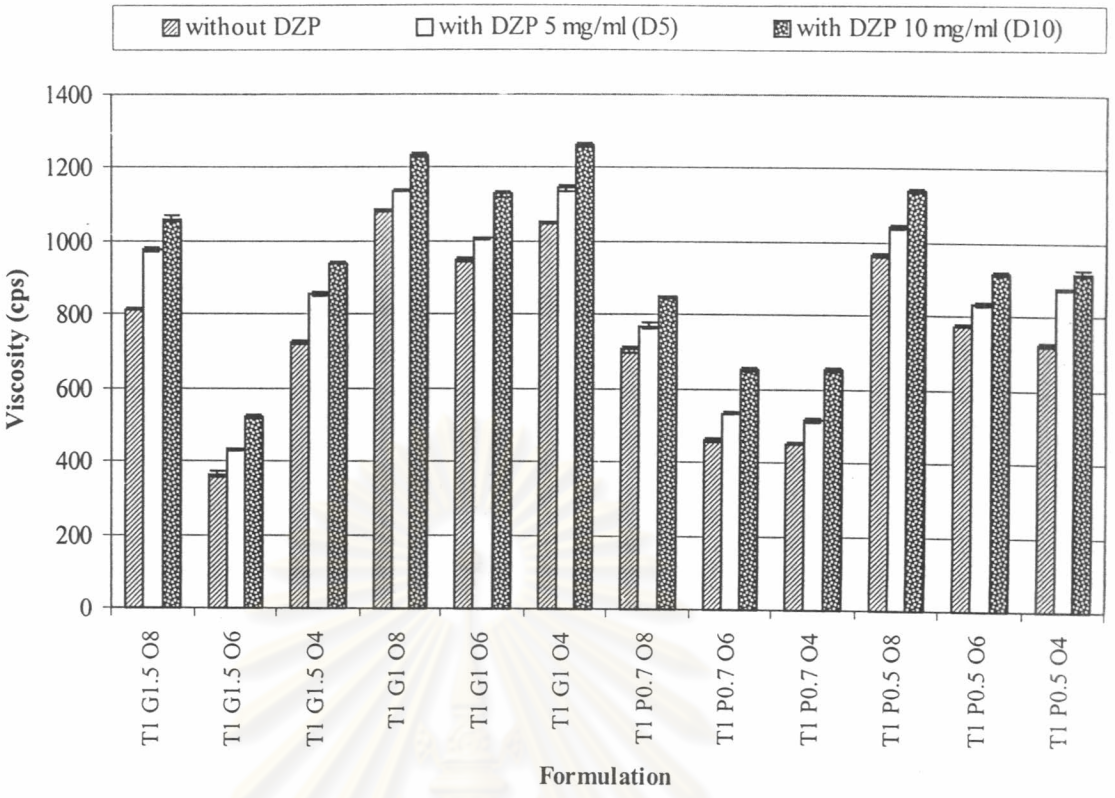

Figure 10 The viscosity of microemulsions with and without diazepam.

In tween 80:glycerin systems, the viscosity of drug loaded microemulsions at the ratio of surfactant:cosurfactant 1:1.5 increased when the drug was incorporated as shown in Figure 10. The viscosities of Formulations T1 G1.5 O8 D5, T1 G1.5 O6 D5, and T1 G1.5 O4 D5, which containing diazepam at the concentration of 5 mg/ml were higher than the formulation without diazepam. Furthermore, the viscosities of Formulations T1 G1.5 O8 D10, T1 G1.5 O6 D10, and T1 G1.5 O4 D10 which containing diazepam at concentration 10 mg/ml were higher than the formulation with lower amount of drug at the equal amount of oil. Similar results were obtained in microemulsion system at the ratio of surfactant:cosurfactant 1:1. Increasing the concentration of drug from 5 mg/ml to 10 mg/ml, the viscosity of microemulsions was increased.

The viscosities of microemulsions in tween 80:polyethylene glycol 400 system at the ratio of 1:0.7 increased when the drug was incorporated as seen in Figure 10. The viscosities of drug-loaded microemulsions at concentration 5 mg/ml in

Formulations T1 P0.7 O8 D5, T1 P0.7 O6 D5, and T1 P0.7 O4 D5 were higher than the corresponding formulations without diazepam. Furthermore, the viscosities of Formulations T1 P0.7 O8 D10, T1 P0.7 O6 D10, and T1 P0.7 O4 D10 which containing higher drug concentration were higher than the formulation of the lower drug concentration. The viscosities of tween 80:polyethylene glycol 400 system at ratio of 1:0.5 in Formulations T1 P0.5 O8 D5, T1 P0.5 O6 D5, and T1 P0.5 O4 D5 were 1043.35, 834.69, and 877.59 cps, respectively. And the viscosities of Formulations T1 P0.5 O8 D10, T1 P0.5 O6 D10, and T1 P0.5 O4 D10 were 1142.06, 915.69, and 919.05 cps, respectively. These results were similar to those from the microemulsions at the ratio of surfactant:cosurfactant 1:0.7 that the viscosities of microemulsion increased when incorporating and increasing the content of the drug in preparations.

The viscosities of microemulsions without drug in tween 80:glycerin systems were mostly more than tween 80:polyethylene glycol 400 systems except Formulation T1 G1.5 O6 that had the lowest viscosity. Similarly for microemulsions containing drug, the viscosities of tween 80:glycerin systems were mostly more than tween 80:polyethylene glycol 400 systems except Formulations T1 G1.5 O6 D5 and T1 G1.5 O6 D10 which had viscosity lower than other formulations.

From these results, it could be concluded that the viscosity of microemulsions was increased when the diazepam was incorporated. In addition, when the amount of diazepam increased from 5 mg/ml to 10 mg/ml, the viscosities were markedly increased in all preparations. Furthermore, the viscosities of microemulsions with and without drug in tween 80:glycerin systems were mostly more than tween 80:polyethylene glycol 400 systems.

2.5 pH

The pH's of microemulsions are shown in Table 10 and Figure 11. Before autoclaving, the pH's of microemulsions in tween 80:glycerin systems without drug were varied in the range 6.95 to 7.19. After autoclaving, the pH were decreased in all preparations to the range 6.77 to 7.09. Formulation T1 G1.5 O6 had the highest pH while the Formulation T1 G1 O4 had the lowest pH. In tween 80:polyethylene glycol

400 systems, the pH's of microemulsions before autoclaving were varied in the range 6.55 to 6.91. After steam sterilization, the pH were decreased in all preparations to the range 6.07 to 6.41. Formulation T1 P0.7 O4 had the highest pH while the Formulation T1 P0.5 O4 had the lowest pH.

In tween 80:glycerin systems, the pH's of drug-loaded microemulsions at the concentration 5 mg/ml before autoclaving were mostly higher than those without drug, except Formulations T1 G1.5 O6 D5 and T1 G1 O4 D5 which pH were decreasing. For microemulsions containing higher concentration of diazepam, the pH were increased in all preparations in the range 7.14 to 7.26 when compared to those without drug. After autoclaving, the pH's of microemulsions containing two levels of drug concentrations were decreased in all preparations. Similar results were obtained from tween 80:polyethylene glycol 400 systems, incorporating diazepam into the systems increased the pH. After autoclaving, the pH's of all preparations were decreased.

The pH was statistically significant decreased ($p<0.05$, tested by two tailed paired-sample T test) after autoclaving as shown in Figure 11. And the preparations containing diazepam showed higher pH than the drug-free microemulsions. Furthermore, the pH of tween 80:glycerin systems was mostly higher than the tween 80:polyethylene glycol 400 systems.

2.6 Particle size and shape

Validation of computerized program

Validation of computerized program was performed on three groups of particles. Each group composed of 50 particles. The results are listed in Table 11 which shows no statistical significant difference between the measurement by computerized program and precalibrated vernier on the particle diameter tested by 2 tailed paired-sample T test ($p>0.05$). The statistical evaluation is shown in Appendix F. Consequently, accurately particle diameter could be obtained from this program.

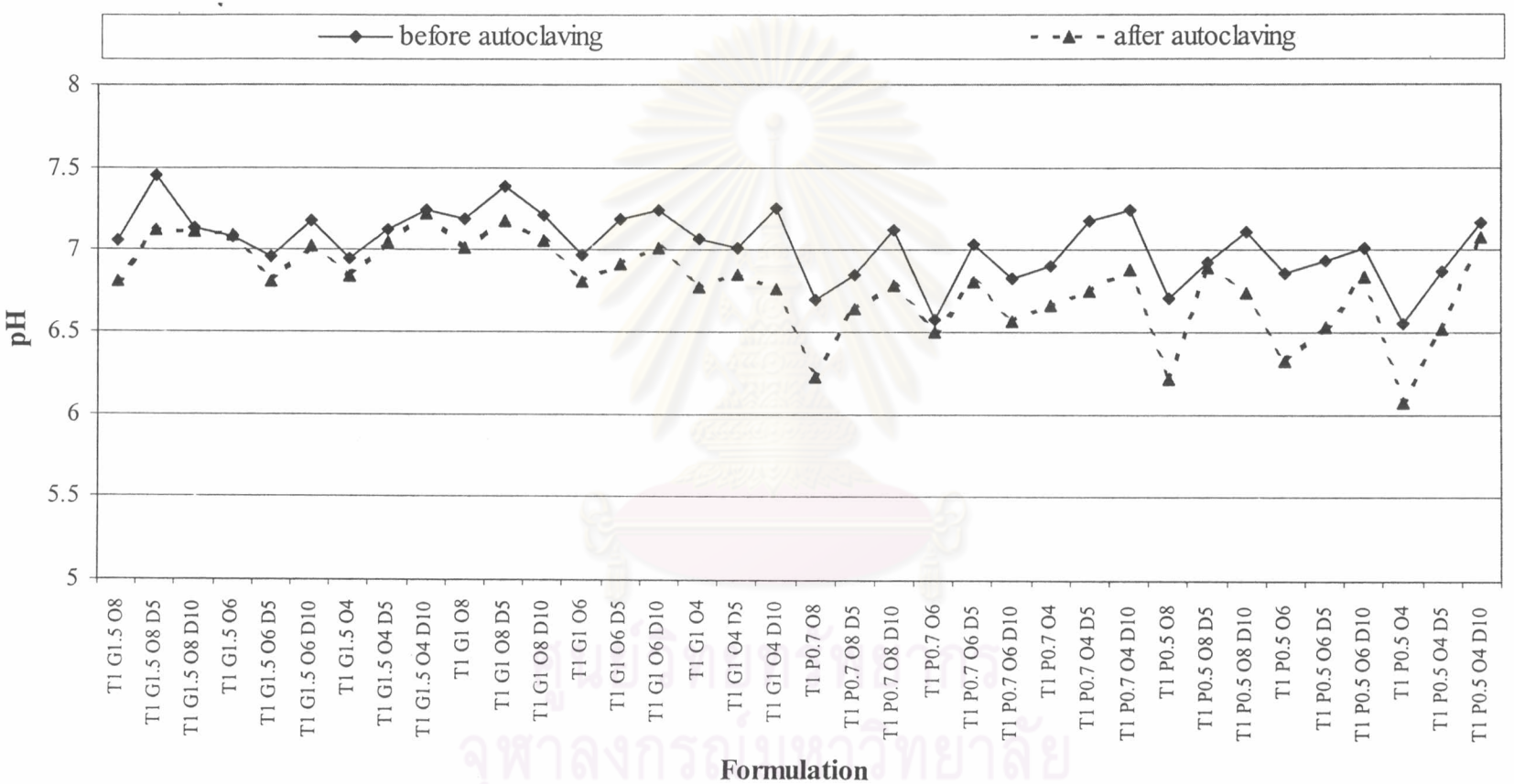

Figure 11 Comparison of the pH of microemulsions both before and after autoclaving.

The particle sizes of the microemulsions determined by TEM are listed in Table 12. Figures 12 to 75 show the TEM photomicrographs and particle size distribution of each formulation both before and after autoclaving. The mean particle diameter of each preparation was compared in Figures 76 and 77.

Tween 80:glycerin system at the ratio of 1:1.5

The TEM photomicrographs of microemulsions without diazepam and particle size distribution of formulations in tween 80:glycerin system at the ratio of 1:1.5 before autoclaving are shown in Figures 12, 13, 16, 17, 20, and 21, respectively. The shape of particles in Formulations T1 G1.5 O8, T1 G1.5 O6 were spherical particles whereas particle shape of Formulation T1 G1.5 O4 was irregular shape. Furthermore, wide particle size distribution was obtained in Formulations T1 G1.5 O8 and T1 G1.5 O4 while particle size distribution of Formulation T1 G1.5 O6 was narrow than that of Formulations T1 G1.5 O8 and T1 G1.5 O4. The largest particle size was obtained from Formulation T1 G1.5 O8. After autoclaving, the mean particle diameter was increased in all formulations as shown in Figure 76. The TEM photomicrographs of these formulations are shown in Figures 14, 18, and 22 and particle size distribution is shown in Figures 15, 19, and 23. All formulations produced spherical particles with wide particle size distribution.

When diazepam was added, the mean particle diameter of Formulations T1 G1.5 O4 D5 and T1 G1.5 O4 D10 was 84.65 and 83.31 nm after autoclaving. Similar formulations but without diazepam showed smaller particle sizes as shown in Figure 76. But statistically evaluation showed no statistical significant difference in mean particle diameter of these formulations ($p>0.05$, tested by ANOVA test). The TEM photomicrographs and particle size distribution of Formulations T1 G1.5 O4 D5 and T1 G1.5 O4 D10 are illustrated in Figures 24 to 27. They exhibited spherical particles in both formulations. Wide particle size distribution was obtained in Formulation T1 G1.5 O4 D5 whereas Formulation T1 G1.5 O4 D10 had narrow size distribution.

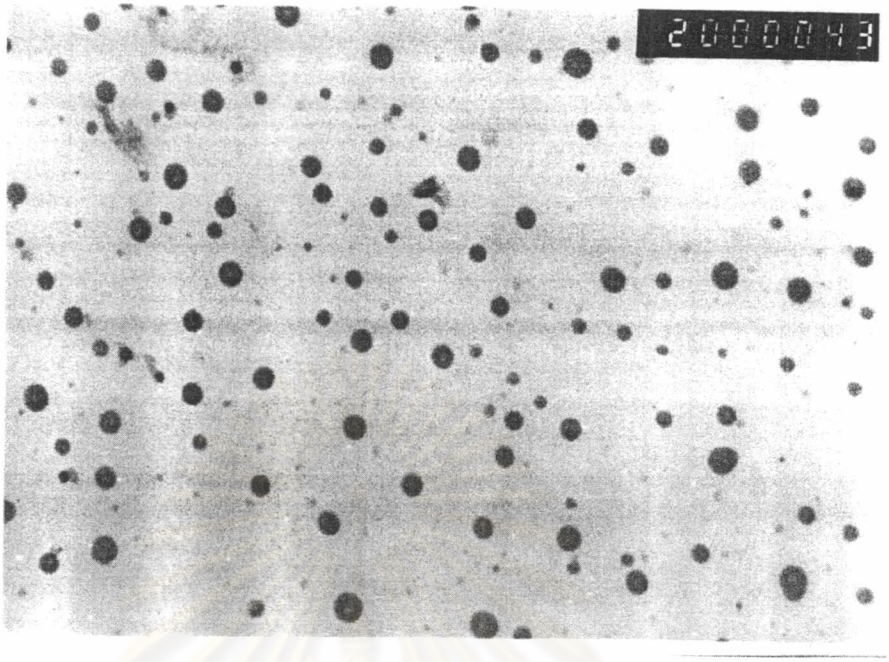

Figure 12 The TEM photomicrograph of the Formulation T1 G1.5 O8 before autoclaving (1 mm from picture equivalent to 33.33 nm).

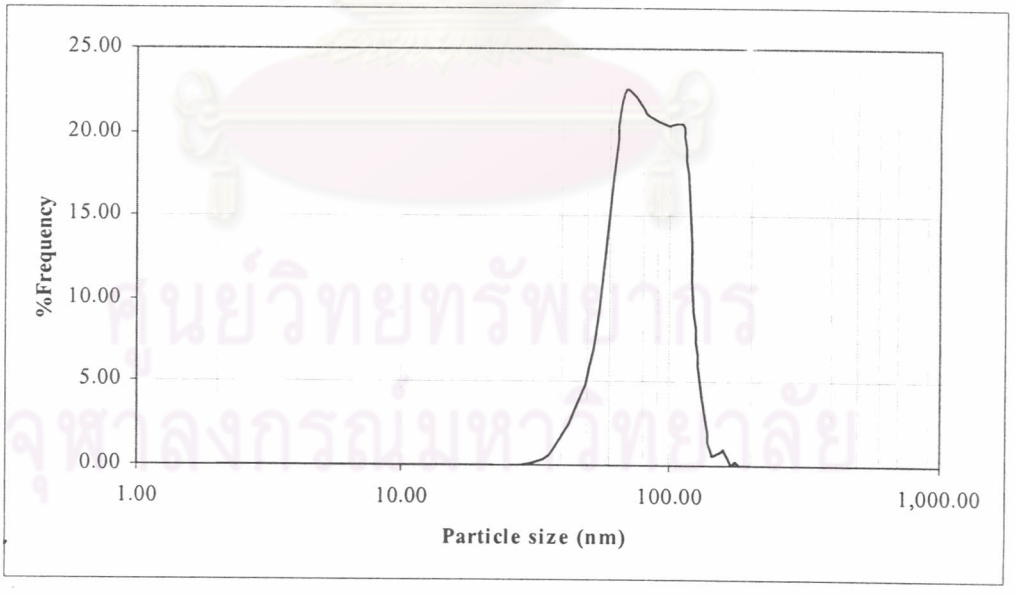

Figure 13 Particle size distribution of Formulation T1 G1.5 O8 before autoclaving.

Table 11 Comparison of the particle diameter obtained by a computerized program and precalibrated equipment.

Particle size (mm*)		Particle size (mm*)		Particle size (mm*)		Particle size (mm*)	
a	b	a	b	a	b	a	b
2.97	2.99	2.45	2.41	2.96	2.99	2.54	2.52
3.38	3.41	2.87	2.80	3.98	4.01	2.77	2.74
2.41	2.45	3.85	3.90	2.45	2.44	3.48	3.50
2.95	2.99	3.01	2.95	2.97	2.99	4.01	3.96
3.48	3.50	3.49	3.50	3.11	3.07	3.64	3.60
4.01	3.97	2.78	2.71	3.25	3.33	3.13	3.10
3.18	3.04	1.97	2.00	3.33	3.48	2.41	2.47
2.96	3.01	2.47	2.50	2.75	2.66	4.44	4.51
2.96	2.96	3.55	3.51	2.55	2.50	1.96	1.99
2.97	2.99	3.97	4.00	1.98	2.00	4.12	4.10
4.12	4.11	2.99	2.97	2.44	2.48	2.99	3.00
2.99	3.01	3.94	4.01	2.39	2.44	2.84	2.81
3.98	4.02	3.01	3.06	4.12	4.10	2.34	2.33
2.55	2.53	2.97	2.95	4.02	3.99	3.46	3.44
2.97	3.02	2.97	2.98	3.59	3.52	2.18	2.12
3.07	3.02	2.38	2.33	2.96	2.94	3.18	3.24
2.67	2.67	3.98	3.99	2.97	3.01	2.96	3.01
3.58	3.54	3.07	3.02	4.12	4.08	1.97	1.98
3.50	3.55	4.07	4.04	3.25	3.34	2.47	2.50
2.95	2.99	3.55	3.50	3.02	3.00	4.41	4.47
3.91	3.87	3.10	3.05	2.05	2.07	2.11	2.10
2.08	2.03	3.01	2.95	2.84	2.75	2.57	2.52
2.28	2.24	1.98	2.00	2.06	1.99	2.67	2.66
3.90	3.88	3.61	3.50	2.05	2.06	3.03	3.00
2.67	2.61	3.03	3.00	1.97	2.02	2.56	2.53
3.12	3.10	2.76	2.66	2.47	2.51	3.01	3.00
3.56	3.50	2.23	2.18	2.97	2.99	1.98	2.01
4.08	4.04	2.69	2.70	3.07	3.10	1.97	1.99
2.17	2.15	3.14	3.20	2.48	2.50	2.28	2.25
3.48	3.50	3.59	3.54	3.98	4.03	2.22	2.25
3.33	3.27	4.11	4.08	3.57	3.52	2.05	2.01
2.26	2.17	1.97	2.02	3.45	3.41	2.95	3.00
2.95	2.99	2.23	2.17	2.11	2.10	3.18	3.10
3.10	3.06	3.25	3.22	3.33	3.25	3.25	3.21
3.06	3.04	4.08	4.01	4.44	4.43	3.59	3.53
3.57	3.52	2.45	2.50	4.17	4.12	2.44	2.42
3.48	3.50	2.87	2.96	3.86	3.79	-	-
4.09	4.03	3.11	3.11	3.10	3.14	-	-

a = The results obtained from computerized program.

b = The results obtained from precalibrated equipment (digital vernier).

* = 1 mm that measured from TEM photomicrographs equivalent to 33.33 nm.

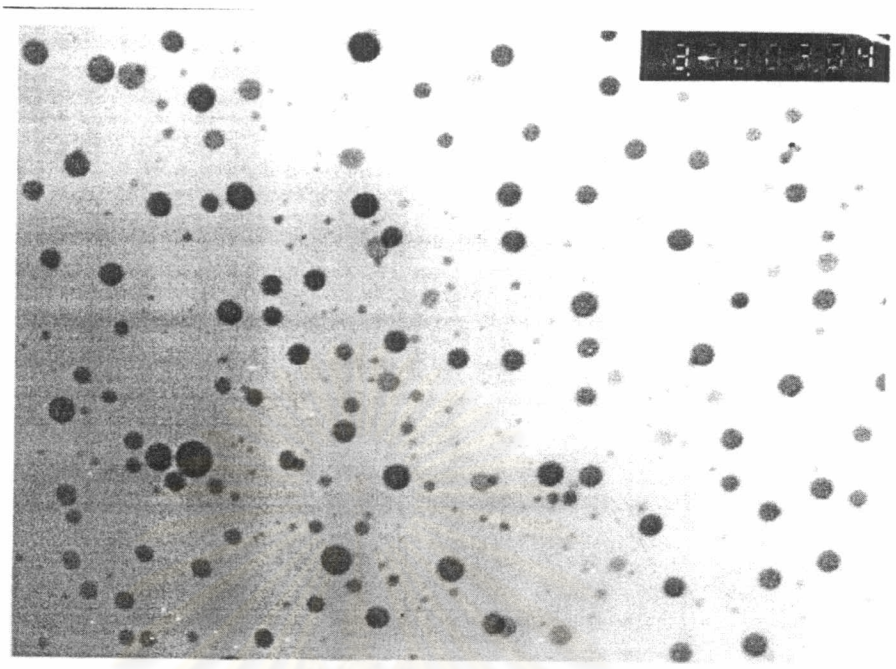

Figure 14 The TEM photomicrograph of the Formulation T1 G1.5 O8 after autoclaving (1 mm from picture equivalent to 33.33 nm).

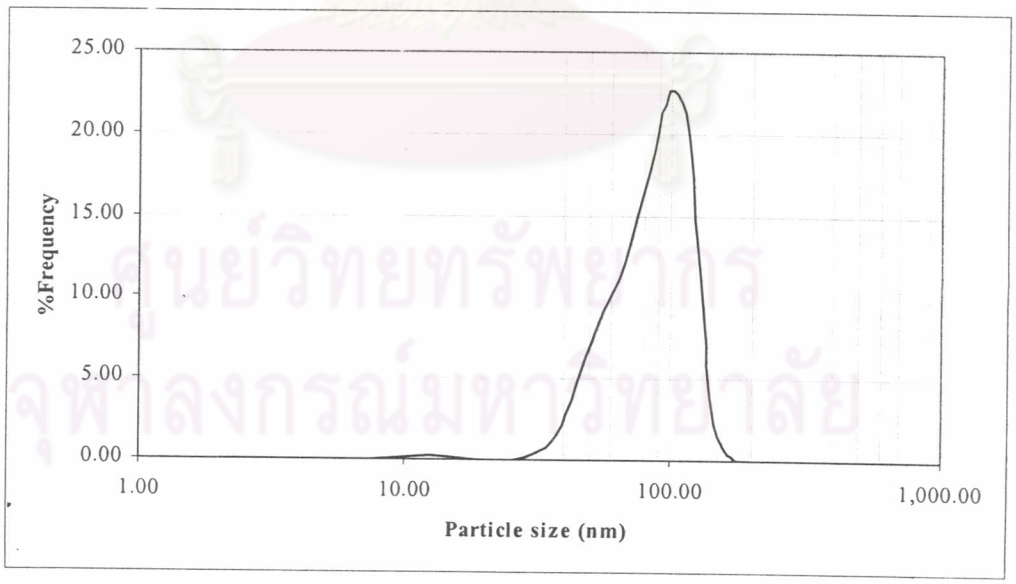

Figure 15 Particle size distribution of Formulation T1 G1.5 O8 after autoclaving.

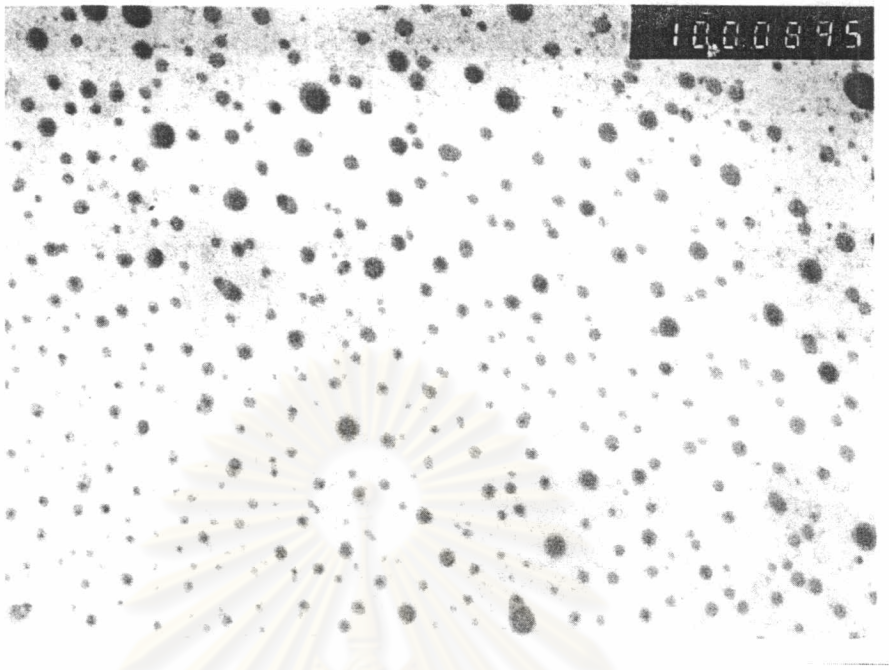

Figure 16 The TEM photomicrograph of the Formulation T1 G1.5 O6 before autoclaving (1 mm from picture equivalent to 58.82 nm).

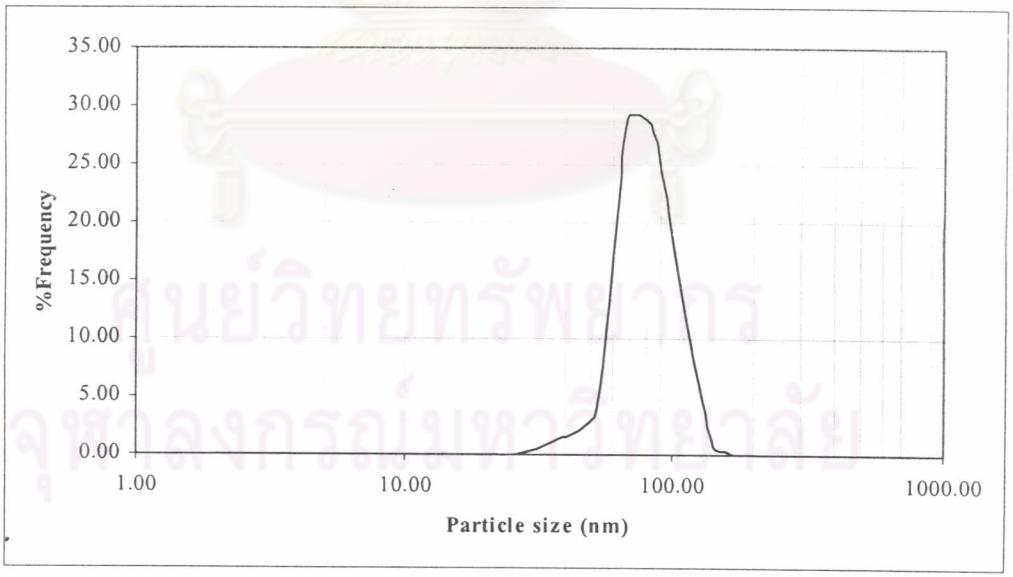

Figure 17 Particle size distribution of Formulation T1 G1.5 O6 before autoclaving.

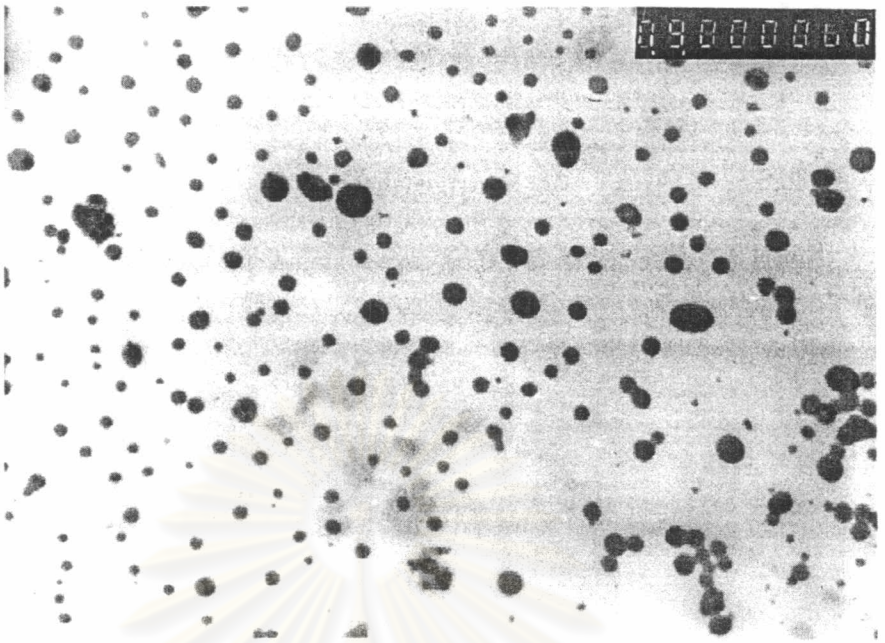

Figure 18 The TEM photomicrograph of the Formulation T1 G1.5 O6 after autoclaving (1 mm from picture equivalent to 33.33 nm).

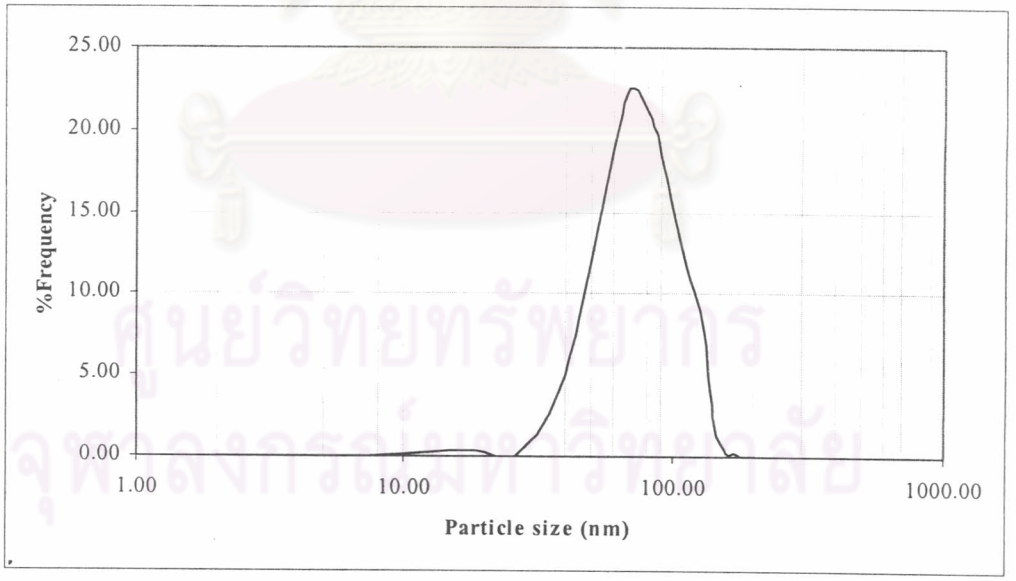

Figure 19 Particle size distribution of Formulation T1 G1.5 O6 after autoclaving.

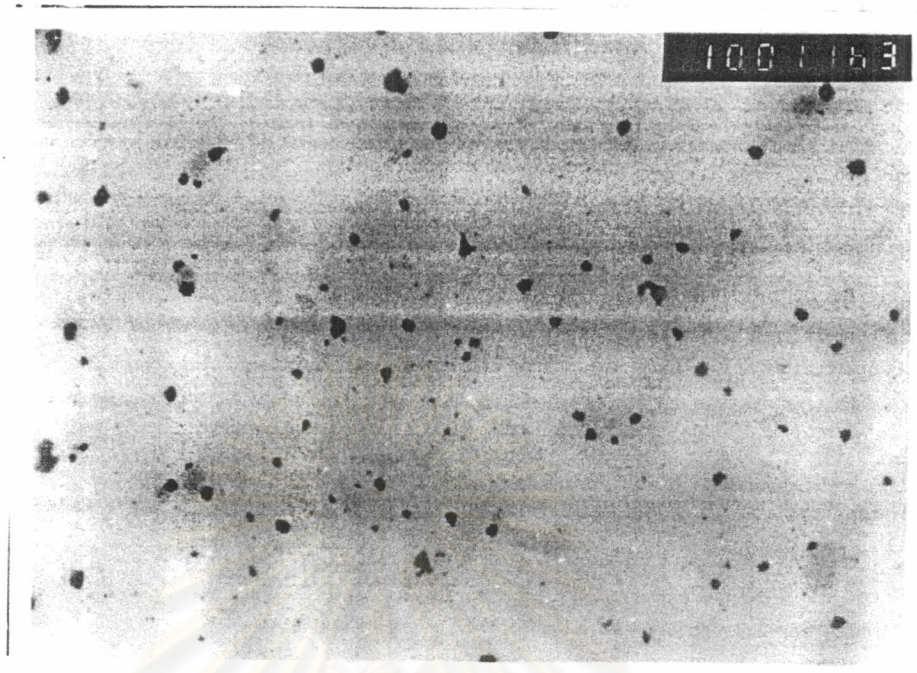

Figure 20 The TEM photomicrograph of the Formulation T1 G1.5 O4 before autoclaving (1 mm from picture equivalent to 58.82 nm).

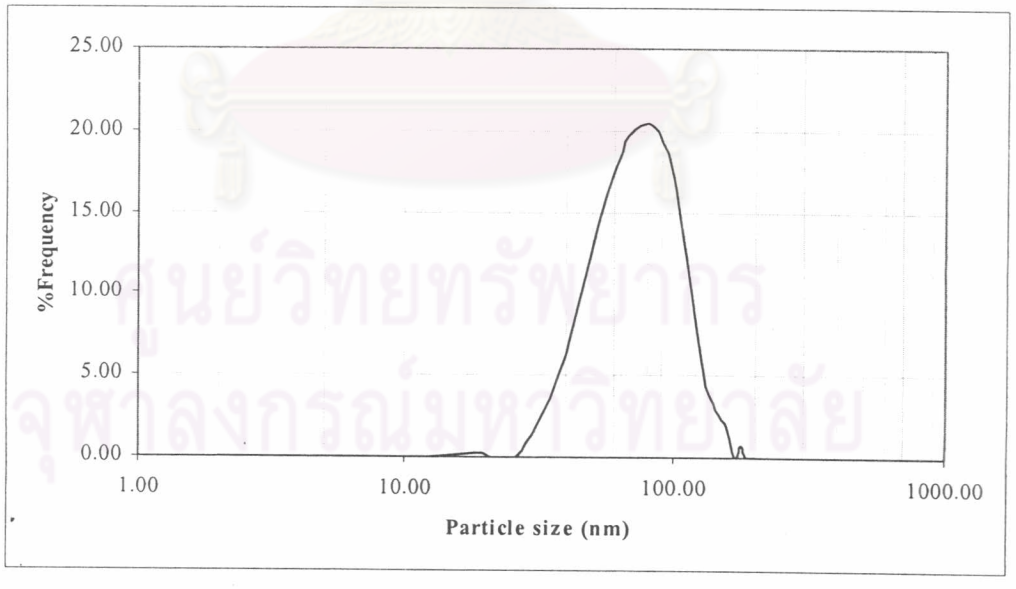

Figure 21 Particle size distribution of Formulation T1 G1.5 O4 before autoclaving.

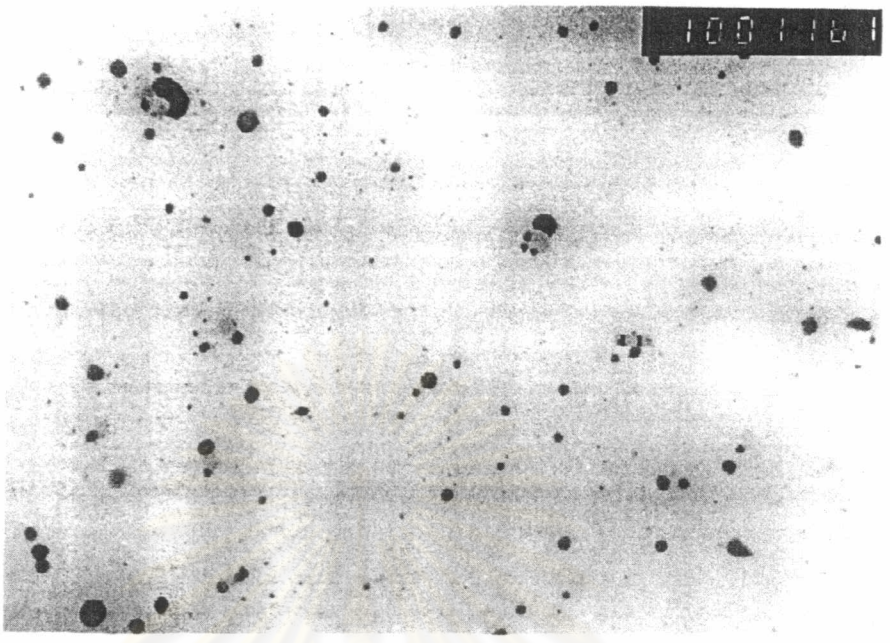

Figure 22 The TEM photomicrograph of the Formulation T1 G1.5 O4 after autoclaving (1 mm from picture equivalent to 58.82 nm).

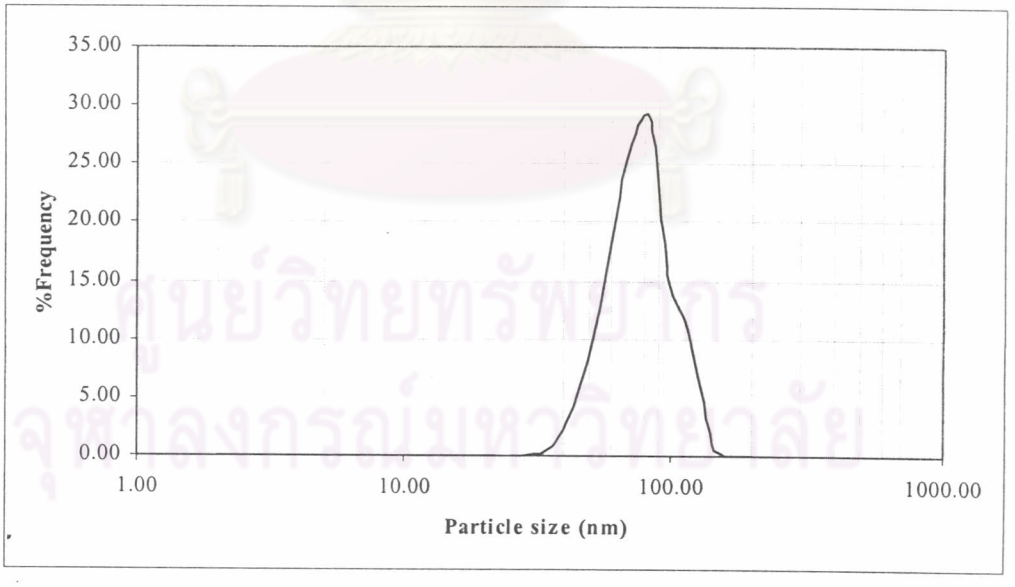

Figure 23 Particle size distribution of Formulation T1 G1.5 O4 after autoclaving.

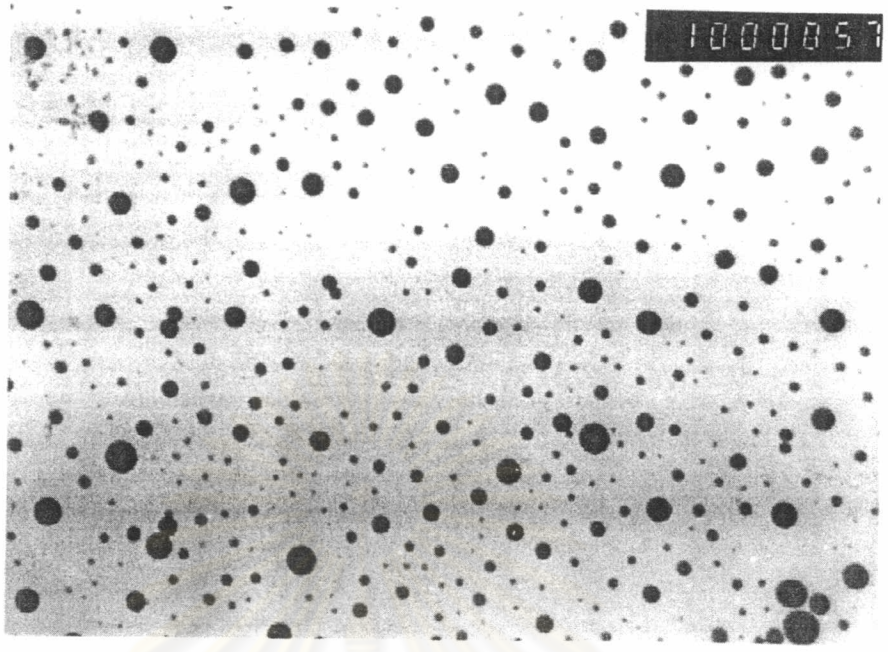

Figure 24 The TEM photomicrograph of the Formulation T1 G1.5 O4 D5 after autoclaving (1 mm from picture equivalent to 33.33 nm).

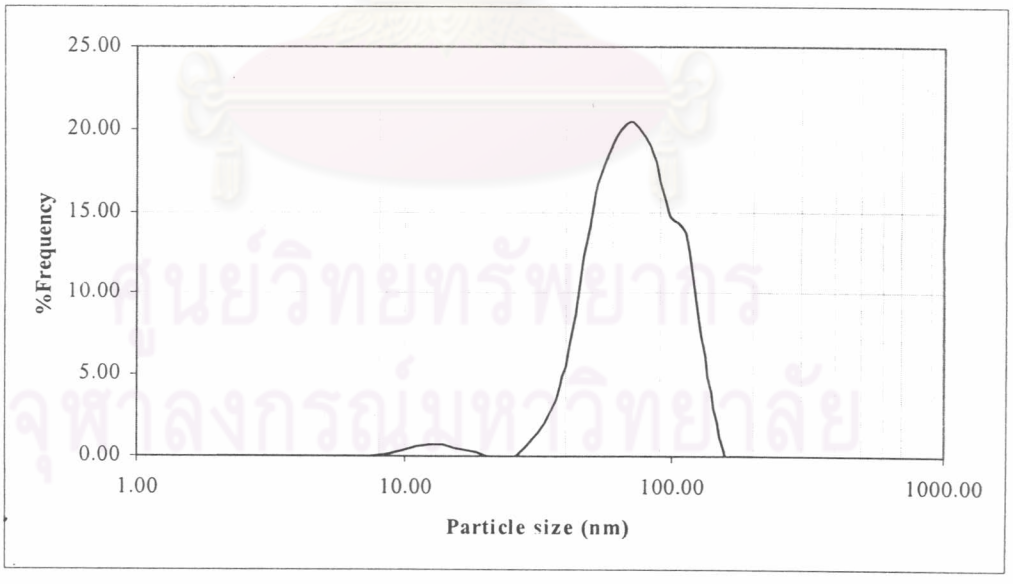

Figure 25 Particle size distribution of Formulation T1 G1.5 O4 D5 after autoclaving.

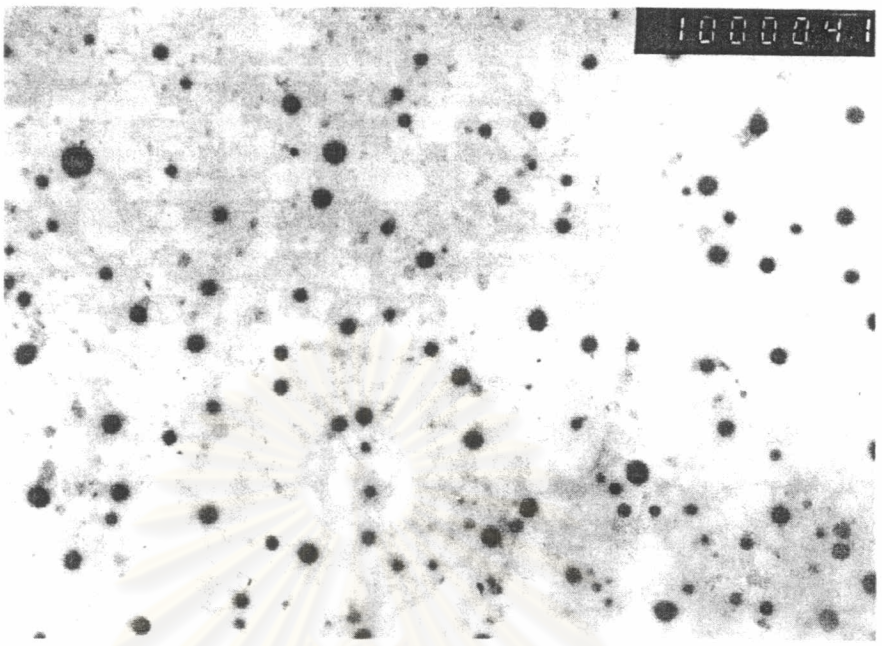

Figure 26 The TEM photomicrograph of the Formulation T1 G1.5 O4 D10 after autoclaving (1 mm from picture equivalent to 33.33 nm).

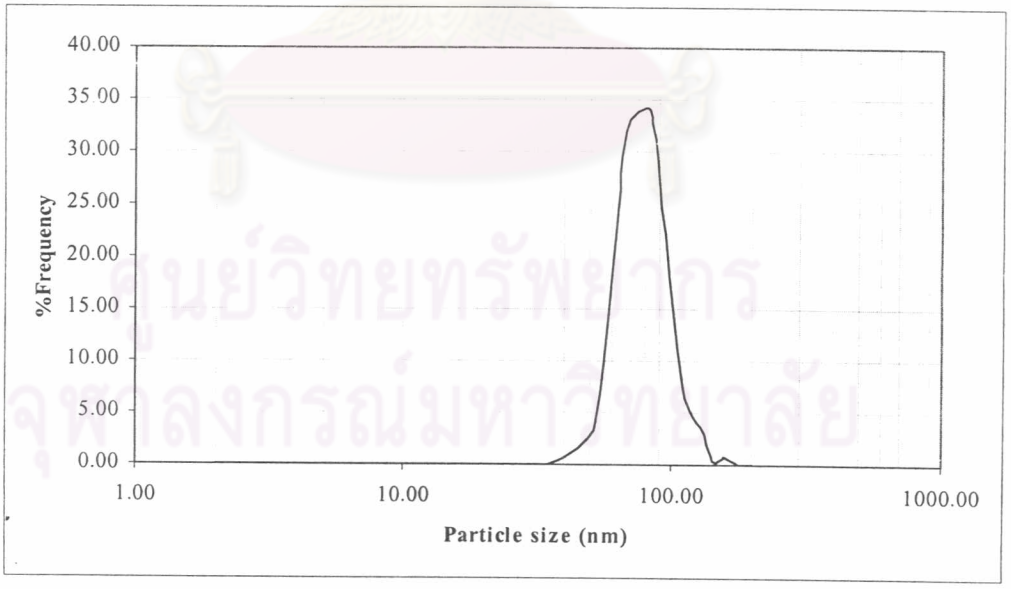

Figure 27 Particle size distribution of Formulation T1 G1.5 O4 D10 after autoclaving.

Tween 80:glycerin system at the ratio of 1:1

Figures 28, 32, and 36 illustrated the TEM photomicrographs of drug-free microemulsions before autoclaving that the shape of particles of all formulations was spherical. The particle size distribution of these formulations was wide as shown in Figures 29, 33, and 37. The mean particle diameter was 67.88, 68.09, and 62.51 nm in Formulation T1 G1 O8, T1 G1 O6, and T1 G1 O4, respectively. After autoclaving, their mean particle diameters were 76.69, 72.54, and 67.16 nm, respectively. It was found that the mean particle diameter of all formulations was increased after autoclaving as shown in Figure 76. The TEM photomicrographs revealed spherical particles in all formulations as seen in Figures 30, 34, and 38. The particle size of all formulations showed wide distribution as seen in Figures 31, 35, and 39.

In drug-loaded microemulsions, the TEM photomicrographs of Formulation T1 G1 O4 D5 and T1 G1 O4 D10 after autoclaving are shown in Figure 40 to 43. They were spherical particles with wide size distribution. The mean particle diameter of Formulations T1 G1 O4 D5 and T1 G1 O4 D10 was 65.49 and 68.74 nm. The mean particle diameter of Formulation T1 G1 O4 D5 was smaller than Formulation T1 G1 O4 while Formulation T1 G1 O4 D10 was slightly increasing as shown in Figure 76. However, results showed no statistical significant difference in mean particle diameter ($p>0.05$, tested by ANOVA test).

Tween 80:polyethylene glycol 400 system at the ratio of 1:0.7

In microemulsion without drug, the shape of particles as shown in TEM photomicrographs Formulations T1 P0.7 O8, T1 P0.7 O6, and T1 P0.7 O4 before autoclaving were spherical particles with wide size distribution as seen in Figures 44, 45, 48, 49, 50, and 53. The largest particle size was obtained from Formulation T1 P0.7 O8. After steam sterilization, the results revealed that particles size were increased in all formulations as illustrated in Figure 77. The TEM photomicrographs of these formulations are shown in Figures 46, 50, and 54. The shape of particle in all formulations was spherical and particle size distribution was wide as seen in Figures 47, 51, and 55.

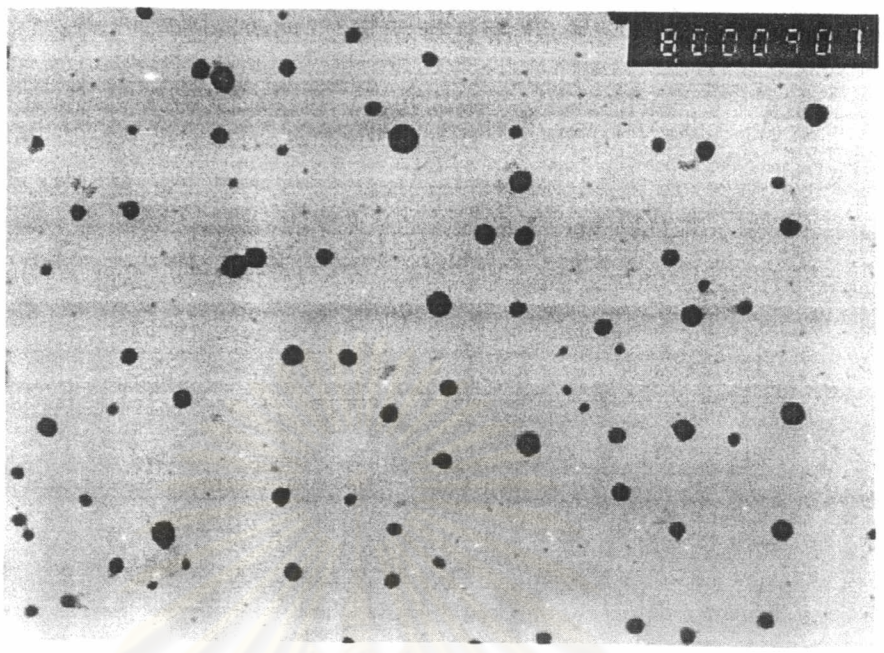

Figure 28 The TEM photomicrograph of the Formulation T1 G1 O8 before autoclaving (1 mm from picture equivalent to 33.33 nm).

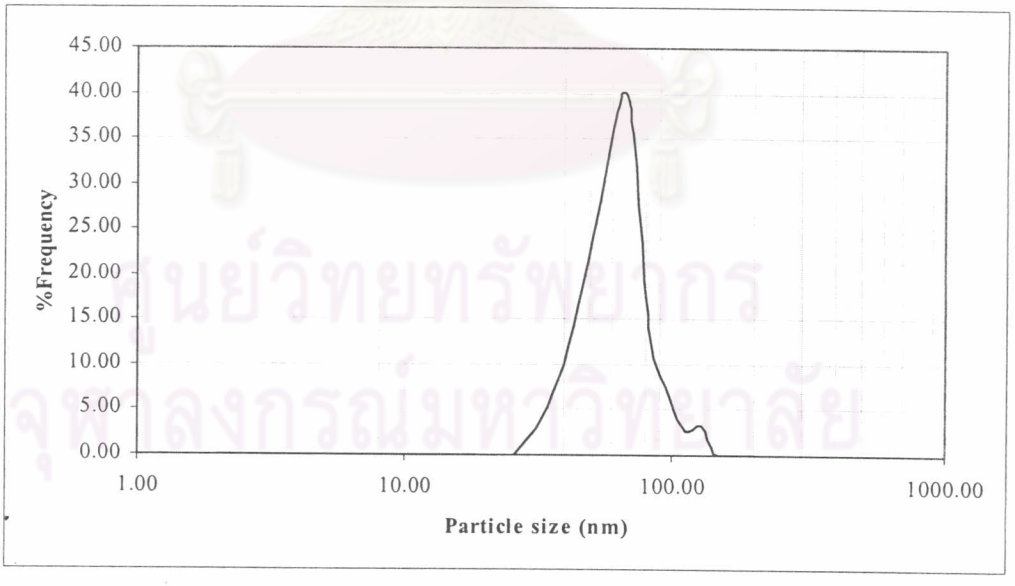

Figure 29 Particle size distribution of Formulation T1 G1 O8 before autoclaving.

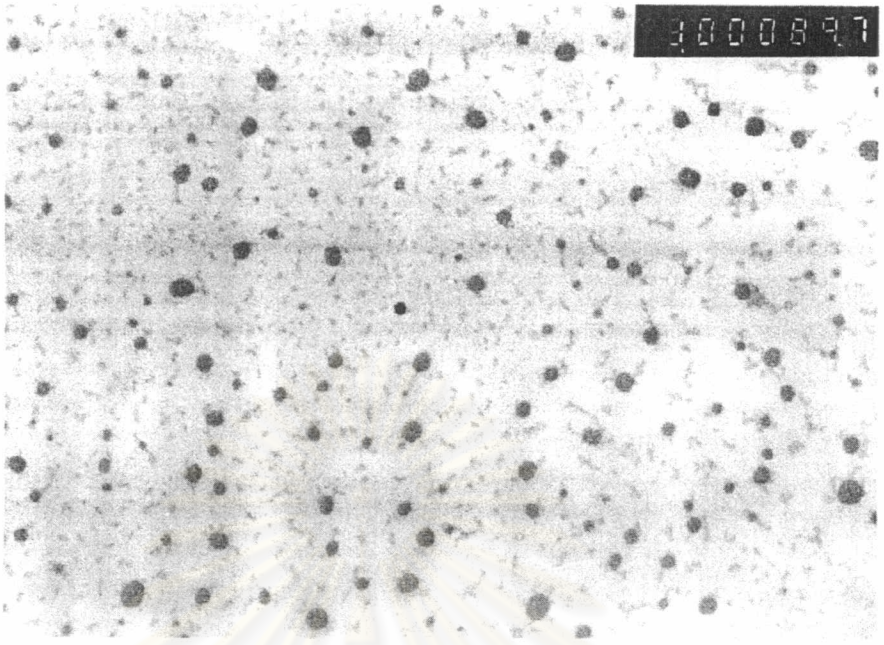

Figure 30 The TEM photomicrograph of the Formulation T1 G1 O8 after autoclaving (1 mm from picture equivalent to 58.82 nm).

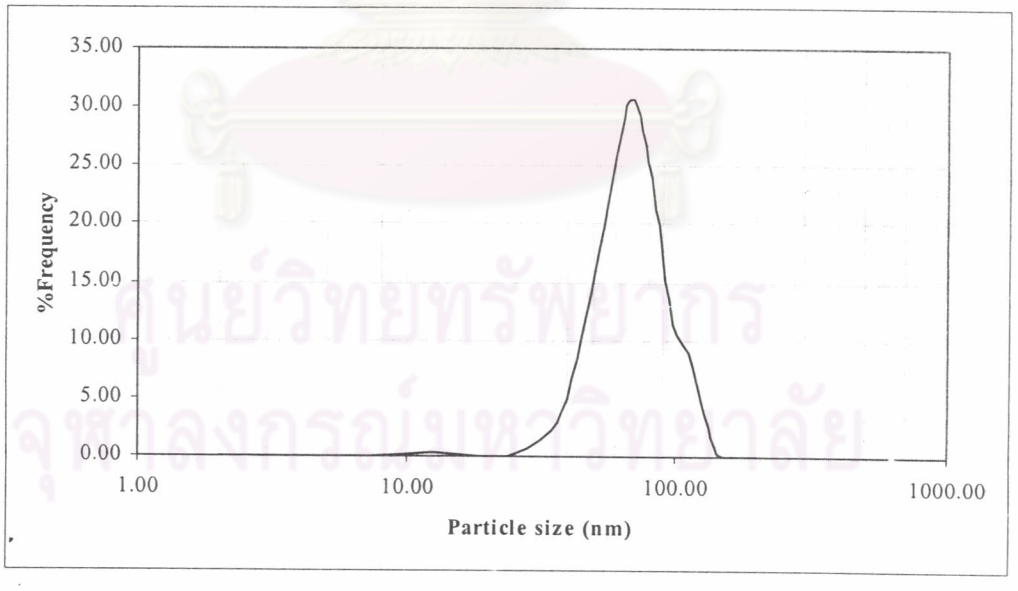

Figure 31 Particle size distribution of Formulation T1 G1 O8 after autoclaving.

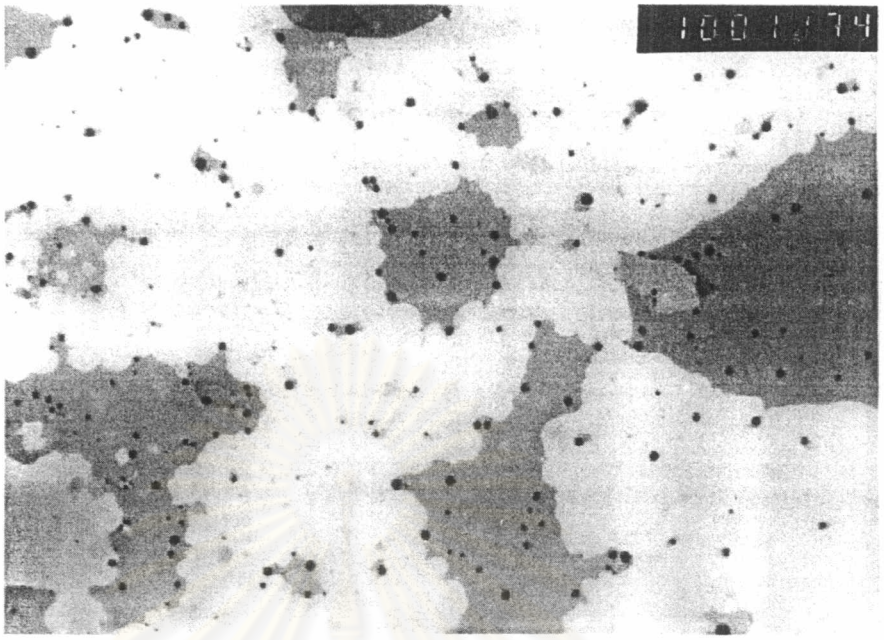

Figure 32 The TEM photomicrograph of the Formulation T1 G1 O6 before autoclaving (1 mm from picture equivalent to 58.82 nm).

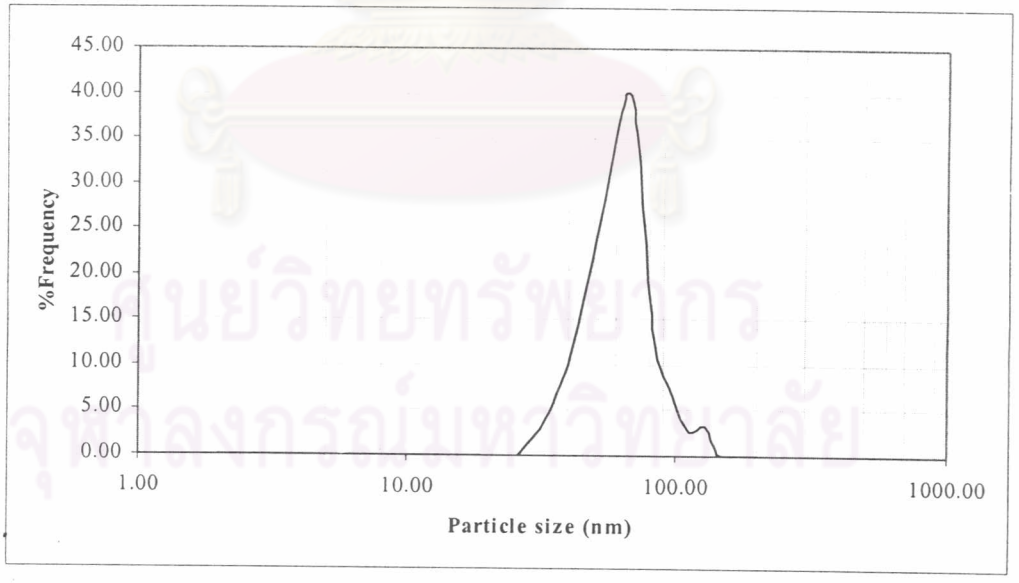

Figure 33 Particle size distribution of Formulation T1 G1 O6 before autoclaving.

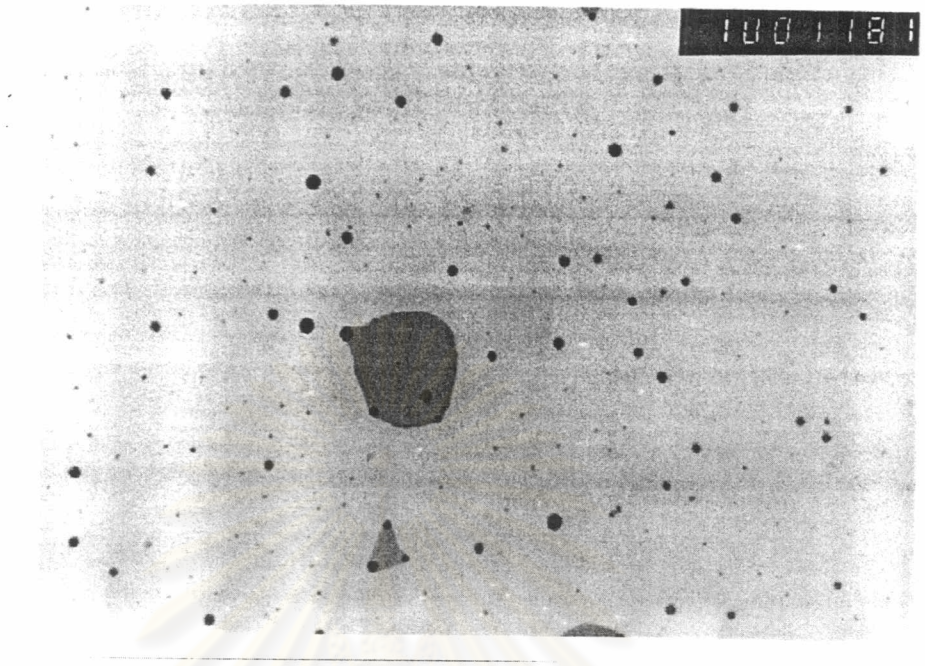

Figure 34 The TEM photomicrograph of the Formulation T1 G1 O6 after autoclaving (1 mm from picture equivalent to 58.82 nm).

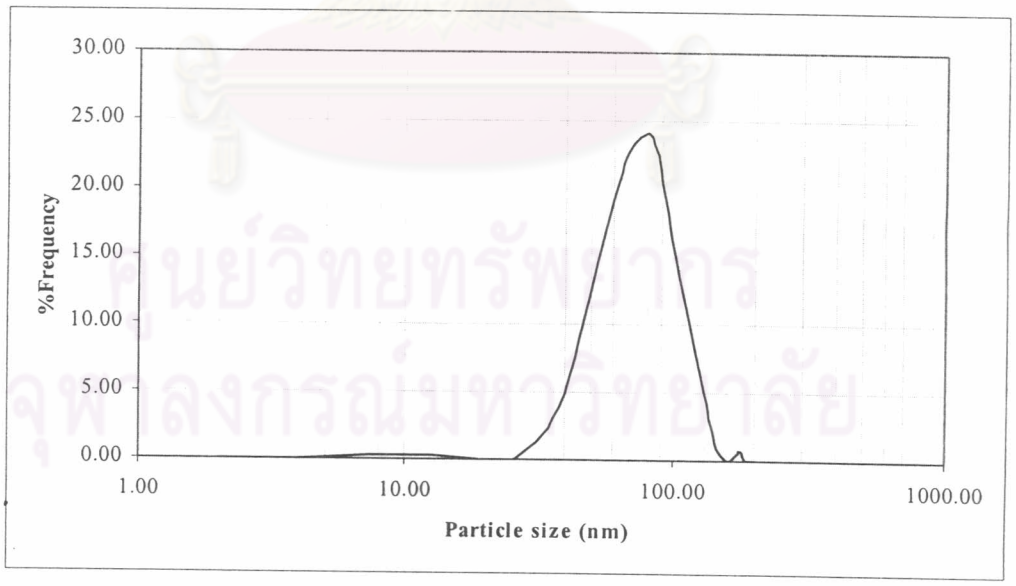

Figure 35 Particle size distribution of Formulation T1 G1 O6 after autoclaving.

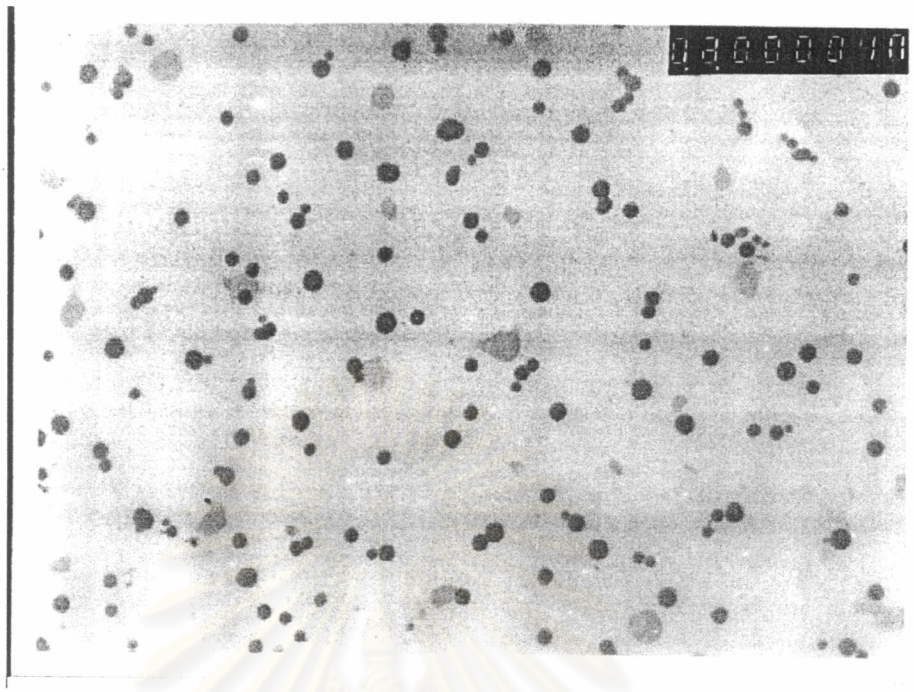

Figure 36 The TEM photomicrograph of the Formulation T1 G1 O4 before autoclaving (1 mm from picture equivalent to 33.33 nm).

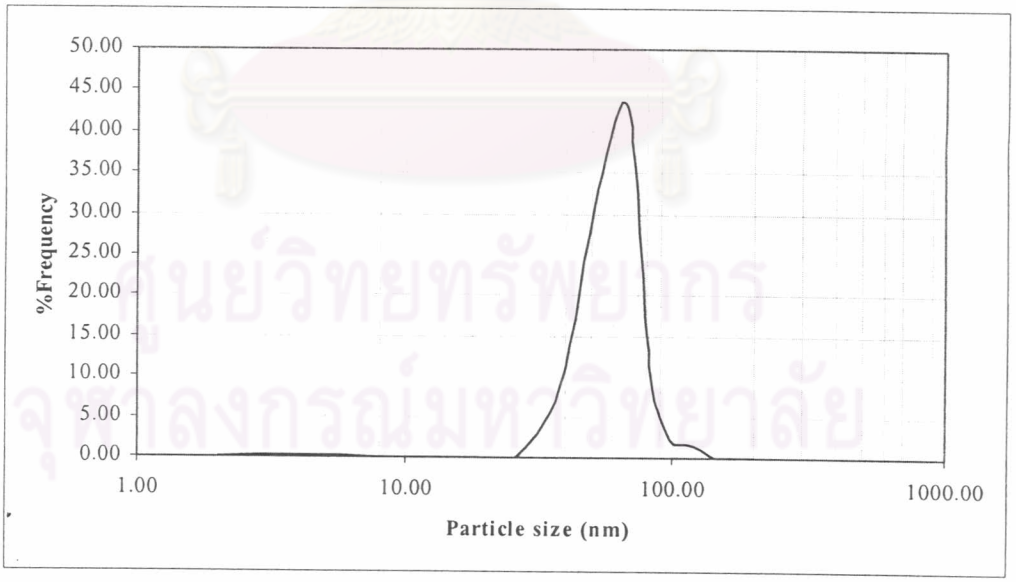

Figure 37 Particle size distribution of Formulation T1 G1 O4 before autoclaving.

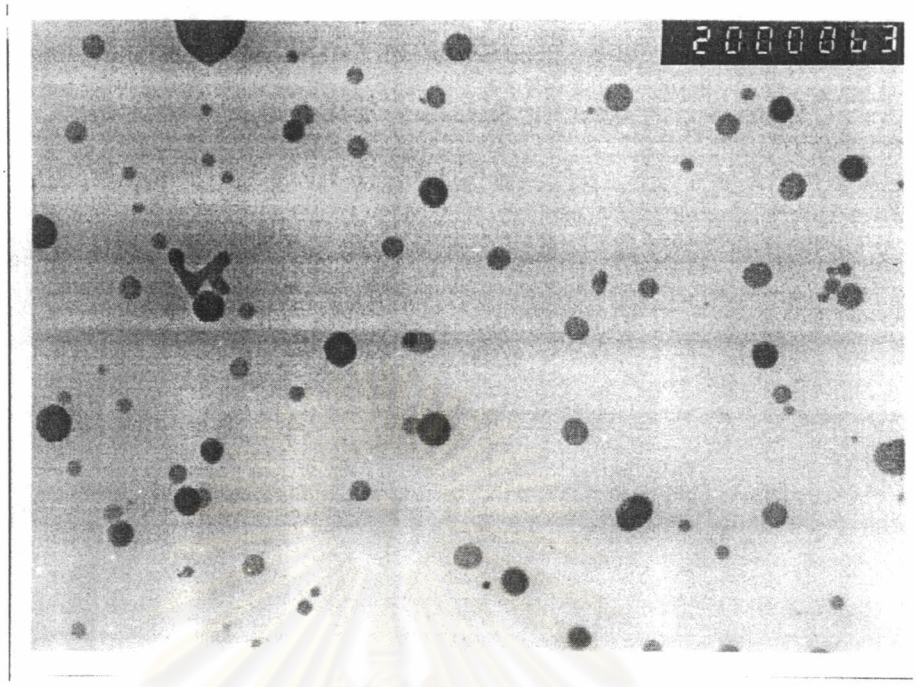

Figure 38 The TEM photomicrograph of the Formulation T1 G1 O4 after autoclaving (1 mm from picture equivalent to 33.33 nm).

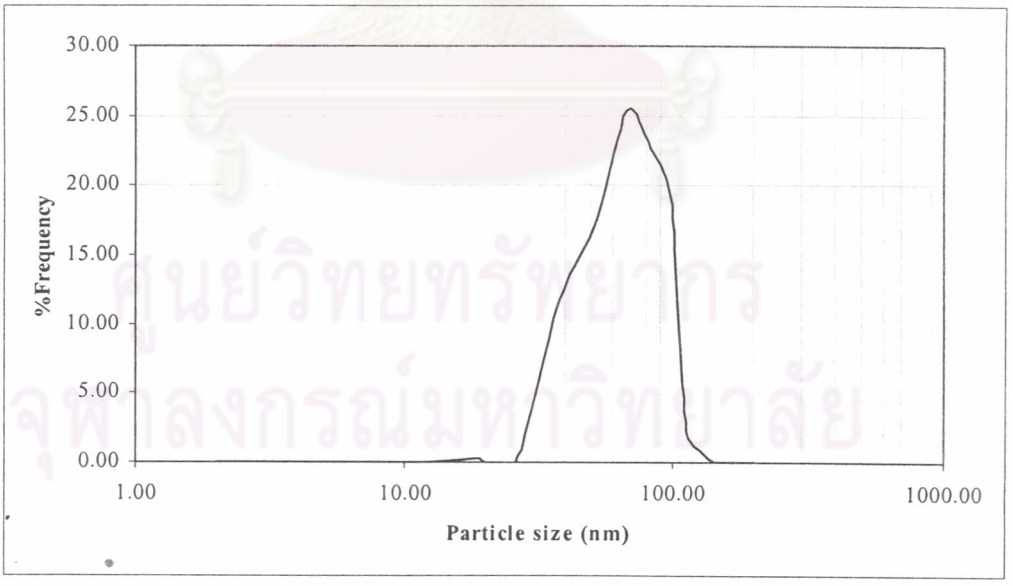

Figure 39 Particle size distribution of Formulation T1 G1 O4 after autoclaving.

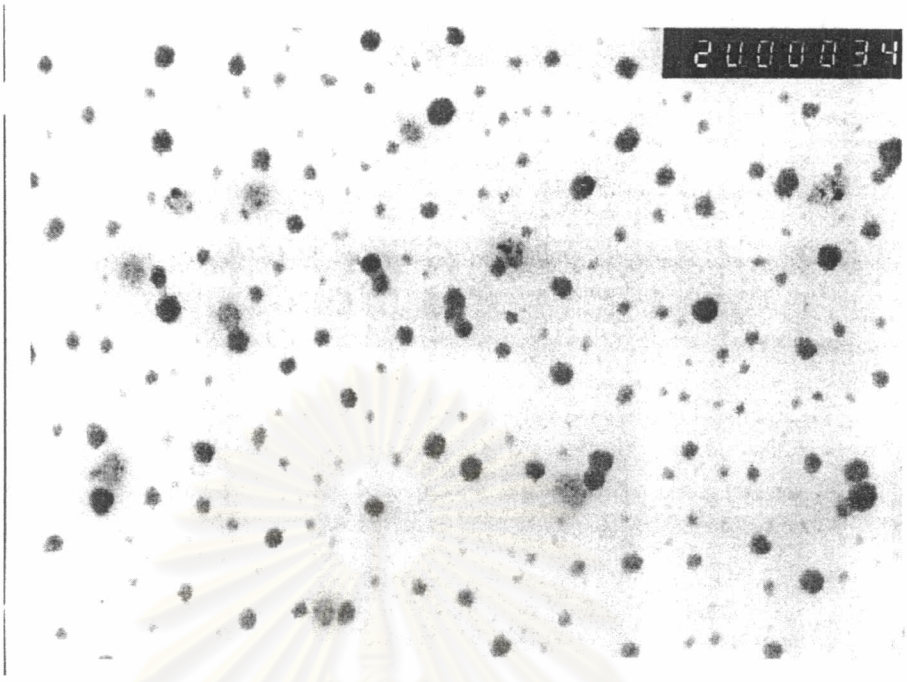

Figure 40 The TEM photomicrograph of the Formulation T1 G1 O4 D5 after autoclaving (1 mm from picture equivalent to 33.33 nm).

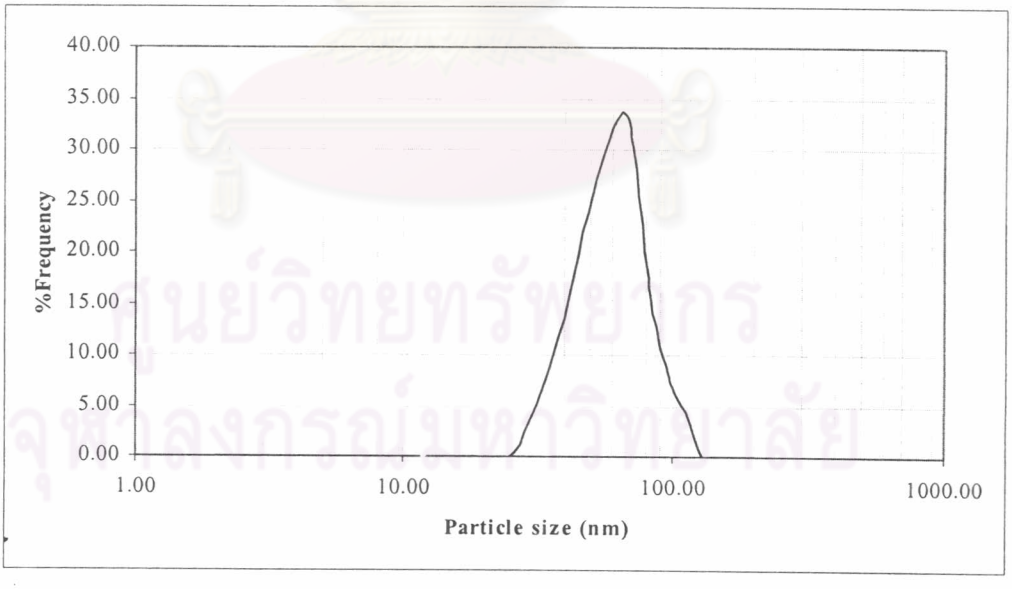

Figure 41 Particle size distribution of Formulation T1 G1 O4 D5 after autoclaving.

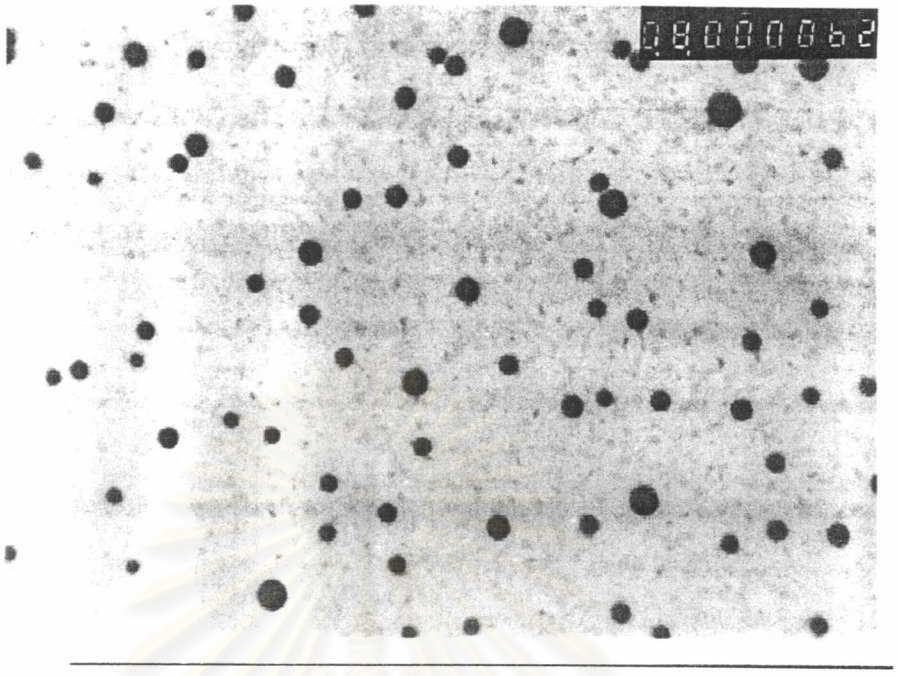

Figure 42 The TEM photomicrograph of the Formulation T1 G1 O4 D10 after autoclaving (1 mm from picture equivalent to 33.33 nm).

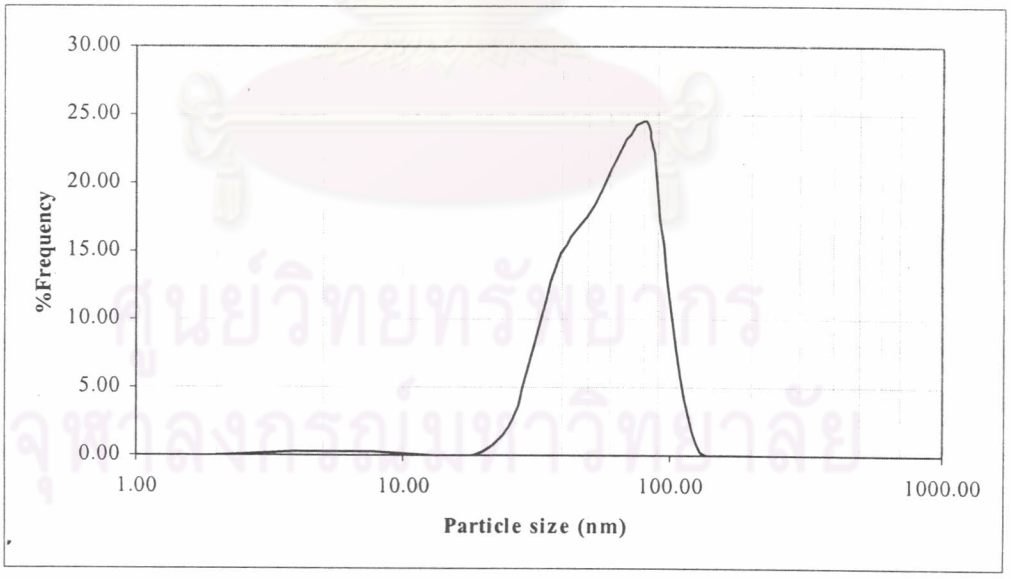

Figure 43 Particle size distribution of Formulation T1 G1 O4 D10 after autoclaving.

When diazepam was added, the mean particle diameter of Formulations T1 P0.7 O4 D5 and T1 P0.7 O4 D10 after autoclaving was smaller than drug-free microemulsion at the equal amount of oil as shown in Figure 77. The statistically evaluation showed statistical significant difference in mean particle diameter ($p<0.05$ tested by ANOVA test). Figures 56 to 59 exhibit the TEM photomicrographs and particle size distribution of each formulation. There were spherical particles and particle size distribution was wide.

Tween 80:polyethylene glycol 400 system at the ratio of 1:0.5

Before autoclaving, the TEM photomicrographs of microemulsions without drug were shown in Figures 60, 64, and 68 that the shape of Formulation T1 P0.5 O8 was spherical while Formulations T1 P0.5 O6 and T1 P1 O4 were of both irregular and spherical shape. Formulations T1 P0.5 O8 and T1 P0.5 O6 have wide particle size distribution whereas Formulation T1 P0.5 O4 had narrow particle size distribution as illustrated in Figures 61, 65, and 69. The particle size of Formulation T1 P0.5 O6 showed the largest particle size. After autoclaving, the results showed increasing of the mean particle diameter in all formulations as seen in Figure 77. The spherical particle was obtained in all formulations as illustrated in Figure 62, 66, and 70. Particle size distribution was wide in Formulation T1 P0.5 O8 while narrow particle size distribution was obtained from Formulations T1 P0.5 O6 and T1 P0.5 O4 as seen in Figures 63, 67, and 71.

The mean particle diameter after autoclaving of Formulations T1 P0.5 O4 D5 and T1 P0.5 O4 D10, which containing diazepam at concentration 5 mg/ml and 10 mg/ml, was 46.18 and 53.64 nm. The mean particle diameter of drug-loaded microemulsions was smaller than drug-free microemulsions as revealed in Figure 77. There were statistical significant differences in their mean particle diameter ($p<0.05$, tested by ANOVA test). The TEM photomicrographs and particle size distribution of the formulations illustrated in Figures 72 and 74. The shape of particles in Formulation T1 G1 O4 D5 was spherical particle while the shape of particles in Formulation T1 G1 O4 D10 was irregular and particle size distribution was narrow in both formulations as seen in Figures 72 to 75.

From the above investigation, the results did not clearly show the effect of the amount of oil on the particle size. In comparison of the mean particle diameter between before and after autoclaving exhibited that there was statistically different in the mean particle diameter tested by 2 tailed paired-sample T test ($p<0.05$) and statistical evaluation was showed in Appendix F. After autoclaving, the mean particle diameter of all preparations was increased. When diazepam was incorporated, it was found that the mean particle diameter after autoclaving in tween 80:glycerin systems showed no statistical significant difference ($p>0.05$) whereas in tween 80:polyethylene glycol 400 systems showed statistical significant difference ($p<0.05$) tested by ANOVA.

Comparison between ratio of surfactant:cosurfactant

For tween 80:glycerin systems both before and after autoclaving at the equal amount of oil, the mean particle diameter of microemulsions without diazepam at the ratio of surfactant:cosurfactant of 1:1 were smaller than those of 1:1.5 as seen Figure 76. In tween 80:polyethylene glycol 400 systems, the mean particle diameter of drug-free microemulsions at the ratio of surfactant:cosurfactant 1:0.5 were smaller than those of 1:0.7 as shown in Figure 77. From this study, the result indicated that the diameter of particle decreased when the surfactant:cosurfactant weight ratio was increased.

Comparison between surfactant:cosurfactant system

Comparison between tween 80:glycerin systems and tween 80:polyethylene glycol 400 systems both before and after autoclaving showed that microemulsions containing polyethylene glycol 400 as cosurfactant had particle size mostly smaller than microemulsions containing glycerin as cosurfactant. From this investigation, the result revealed that microemulsions using polyethylene glycol 400 as cosurfactant produced smaller particle than those using glycerin as cosurfactant.

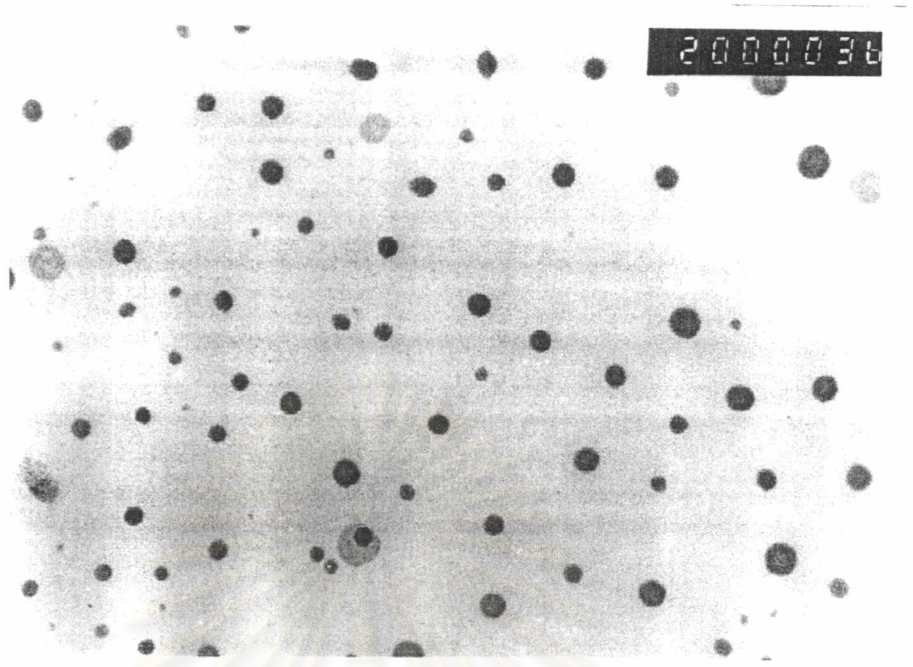

Figure 44 The TEM photomicrograph of the Formulation T1 P0.7 O8 before autoclaving (1 mm from picture equivalent to 33.33 nm).

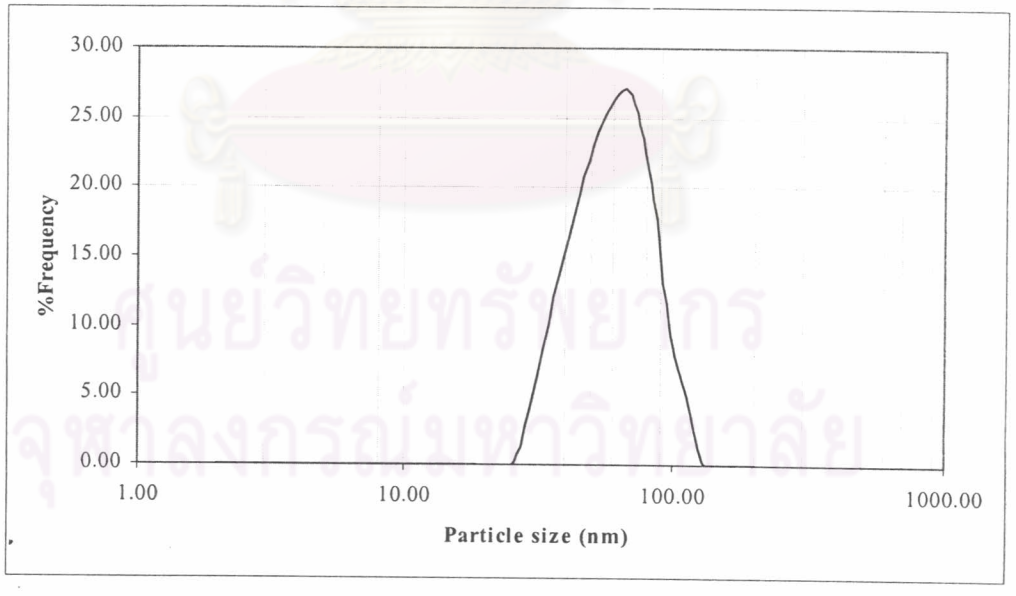

Figure 45 Particle size distribution of Formulation T1 P0.7 O8 before autoclaving.

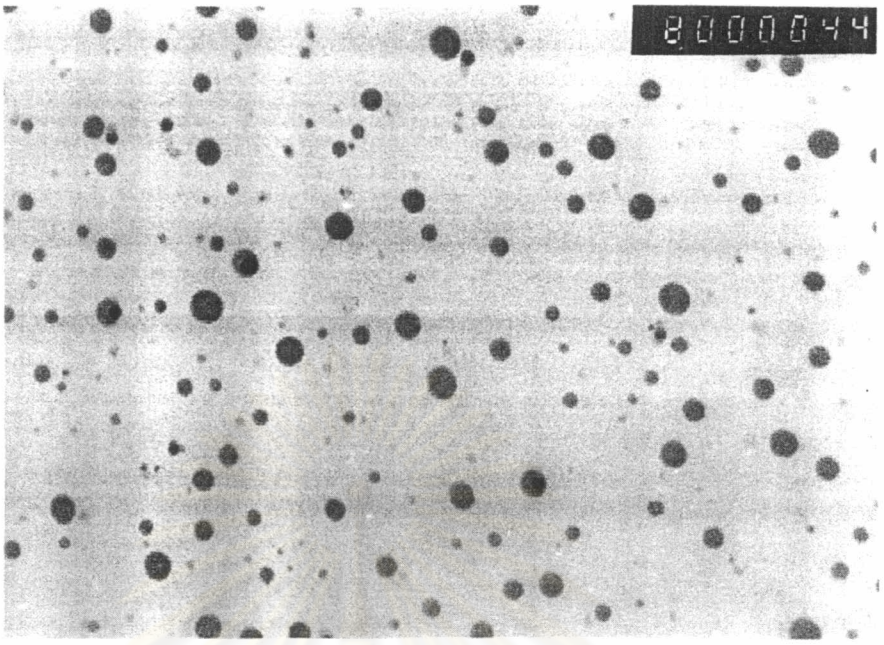

Figure 46 The TEM photomicrograph of the Formulation T1 P0.7 O8 after autoclaving (1 mm from picture equivalent to 33.33 nm).

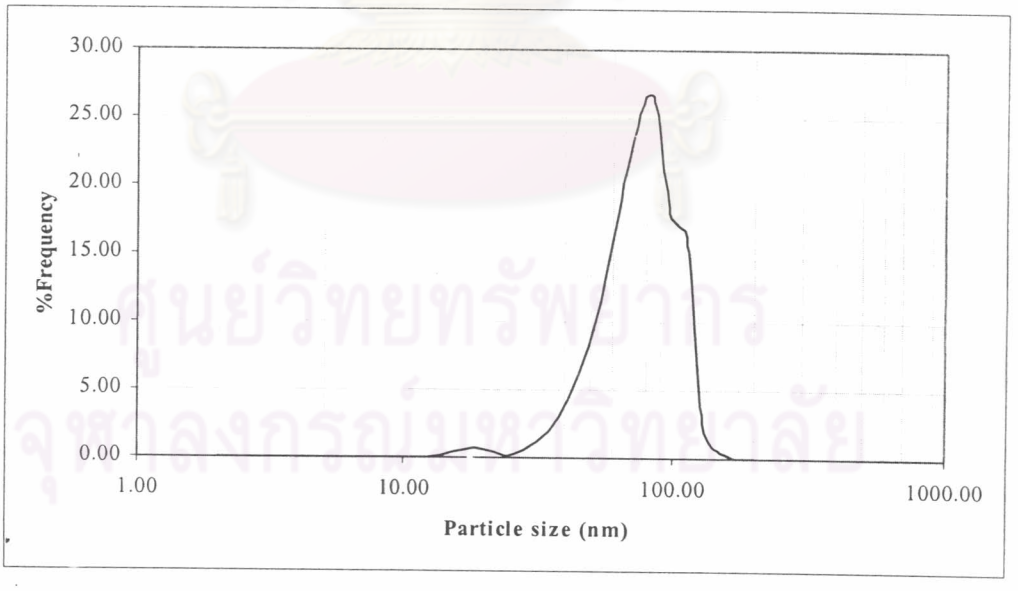

Figure 47 Particle size distribution of Formulation T1 P0.7 O8 after autoclaving.

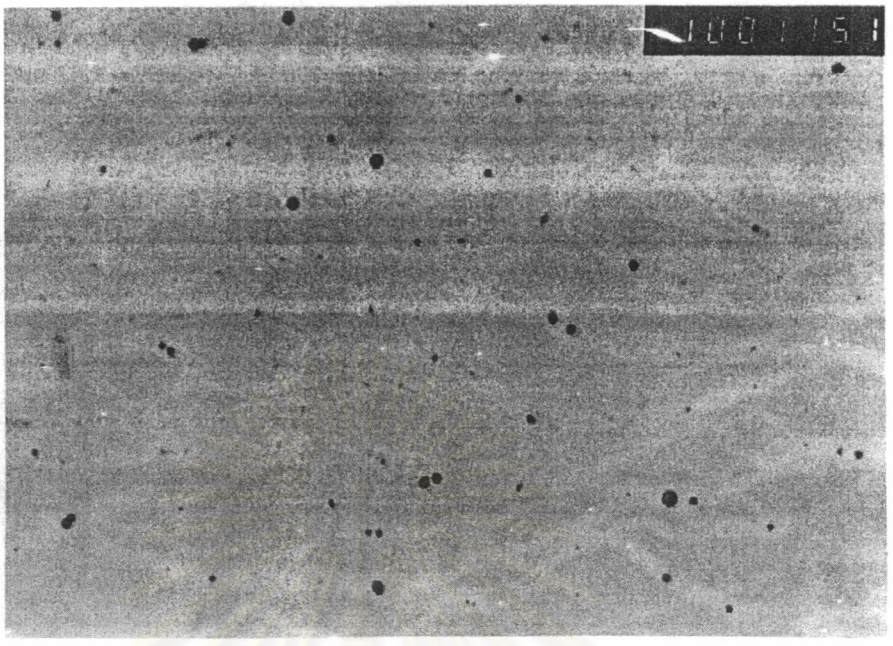

Figure 48 The TEM photomicrograph of the Formulation T1 P0.7 O6 before autoclaving (1 mm from picture equivalent to 58.82 nm).

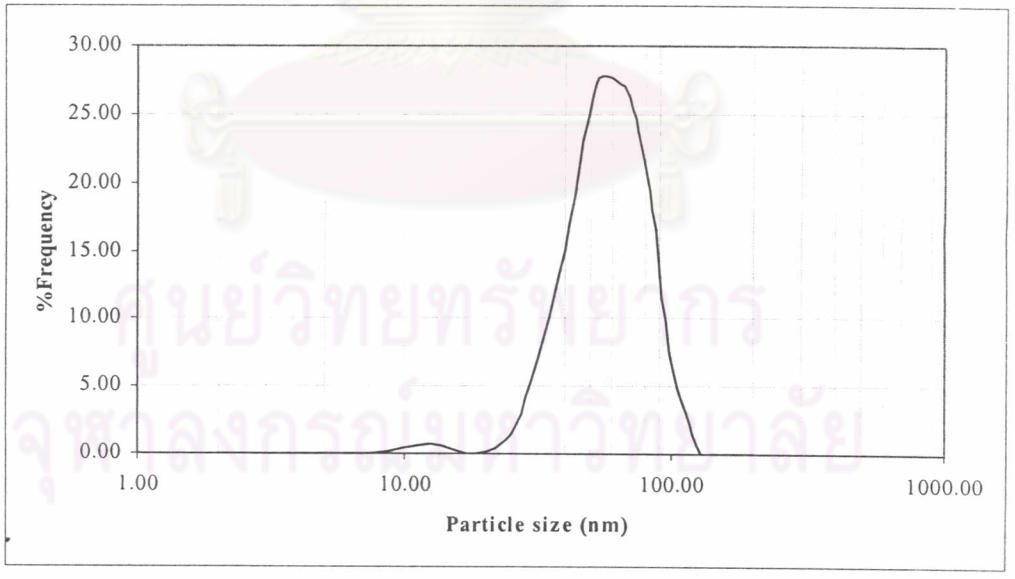

Figure 49 Particle size distribution of Formulation T1 P0.7 O6 before autoclaving.

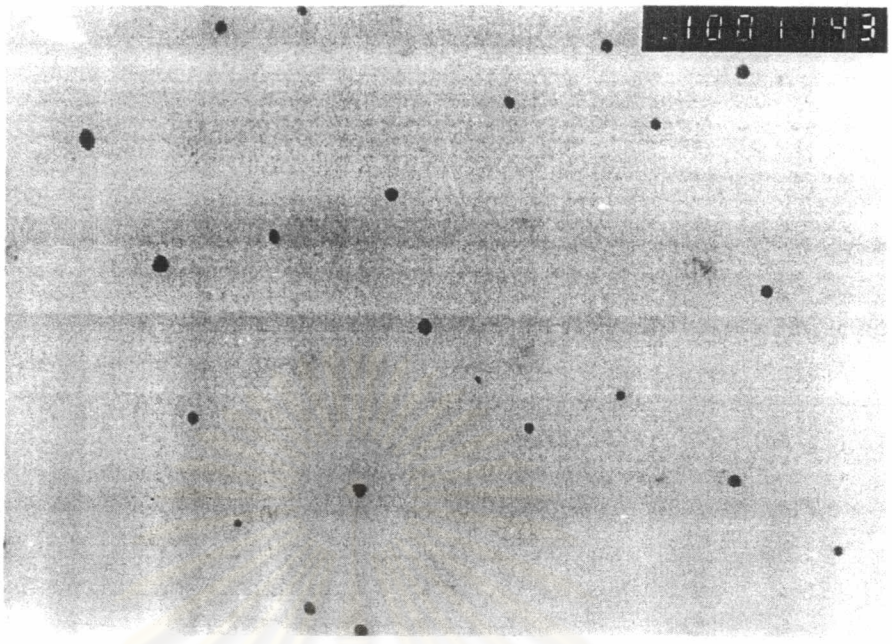

Figure 50 The TEM photomicrograph of the Formulation T1 P0.7 O6 after autoclaving (1 mm from picture equivalent to 58.82 nm).

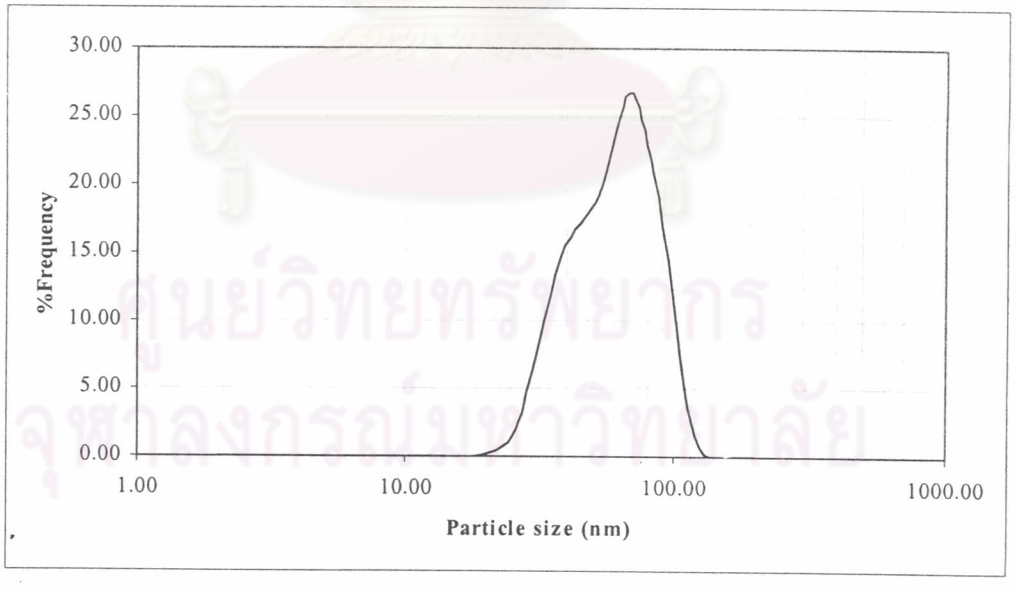

Figure 51 Particle size distribution of Formulation T1 P0.7 O6 after autoclaving.

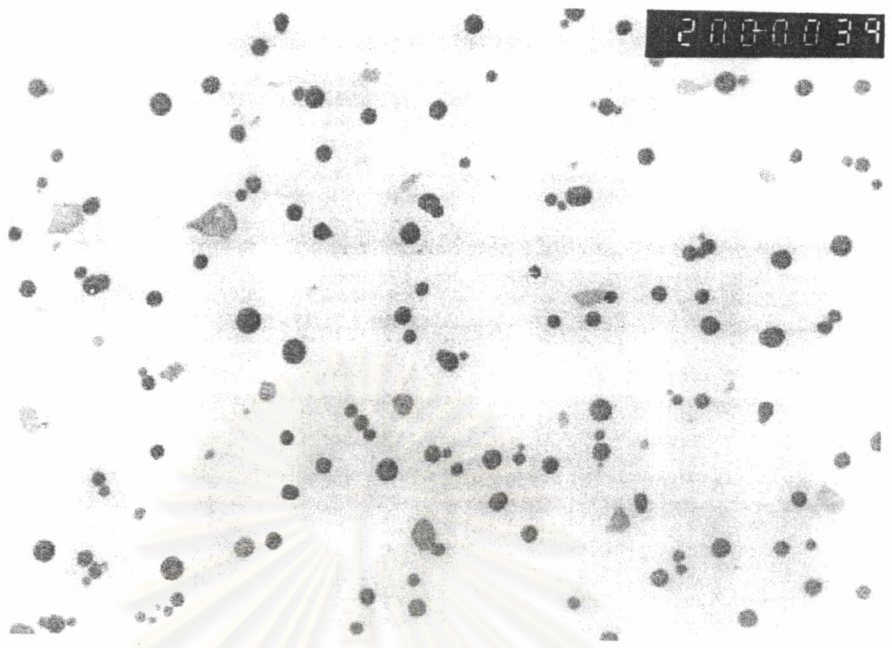

Figure 52 The TEM photomicrograph of the Formulation T1 P0.7 O4 before autoclaving (1 mm from picture equivalent to 33.33 nm).

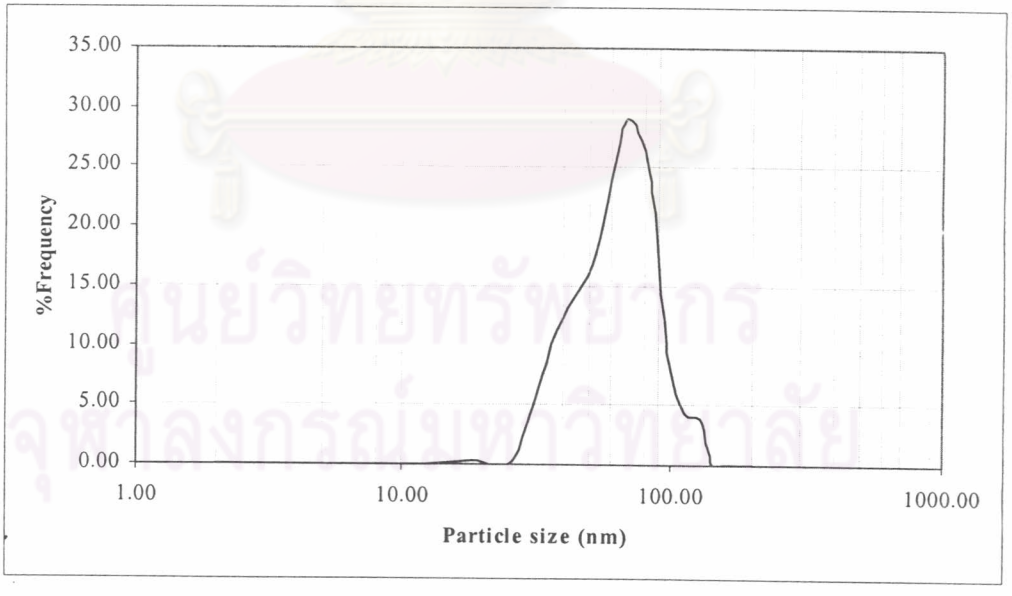

Figure 53 Particle size distribution of Formulation T1 P0.7 O4 before autoclaving.

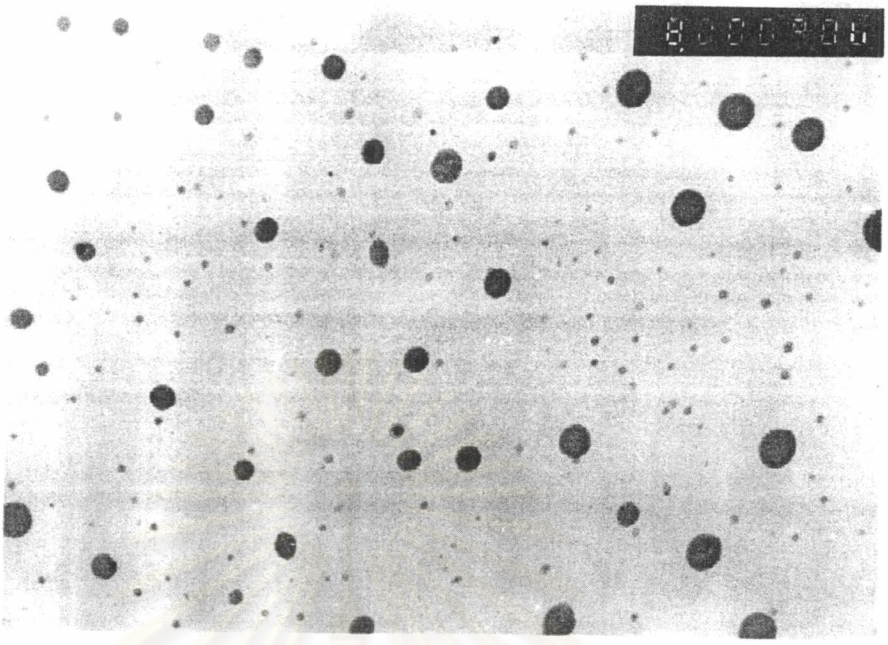

Figure 54 The TEM photomicrograph of the Formulation T1 P0.7 O4 after autoclaving (1 mm from picture equivalent to 33.33 nm).

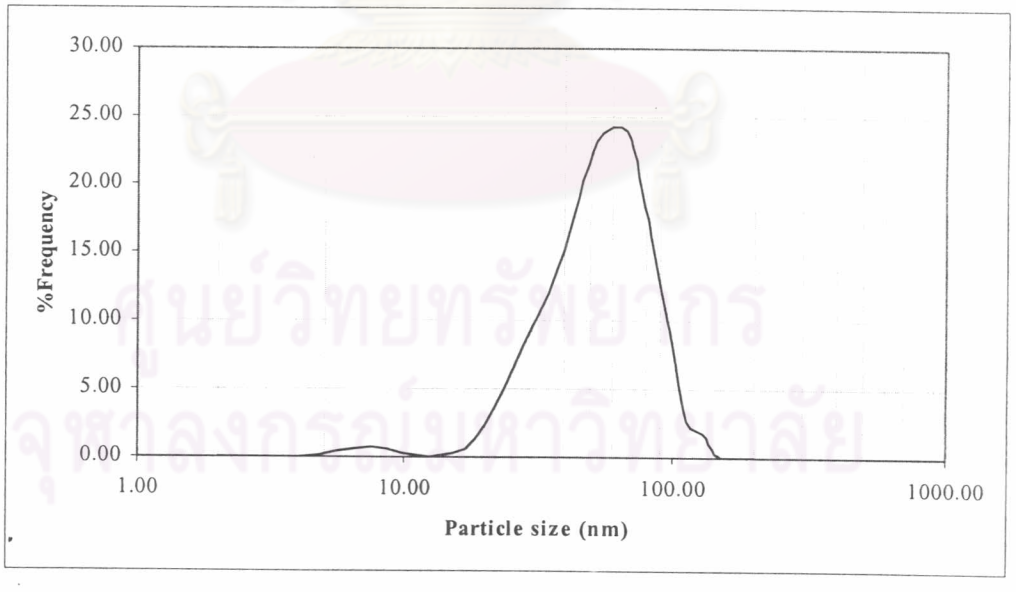

Figure 55 Particle size distribution of Formulation T1 P0.7 O4 after autoclaving.

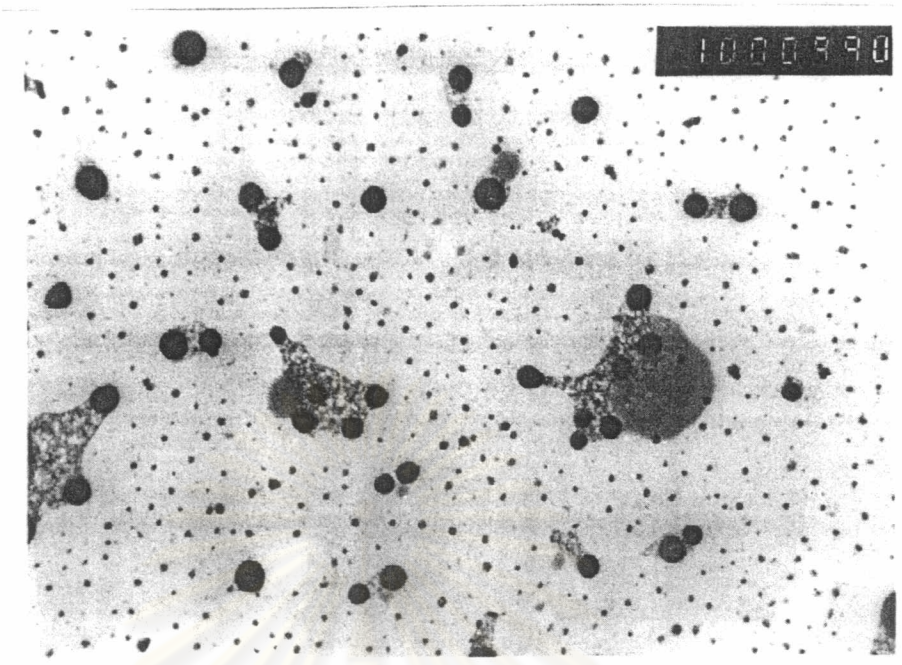

Figure 56 The TEM photomicrograph of the Formulation T1 P0.7 O4 D5 after autoclaving (1 mm from picture equivalent to 33.33 nm).

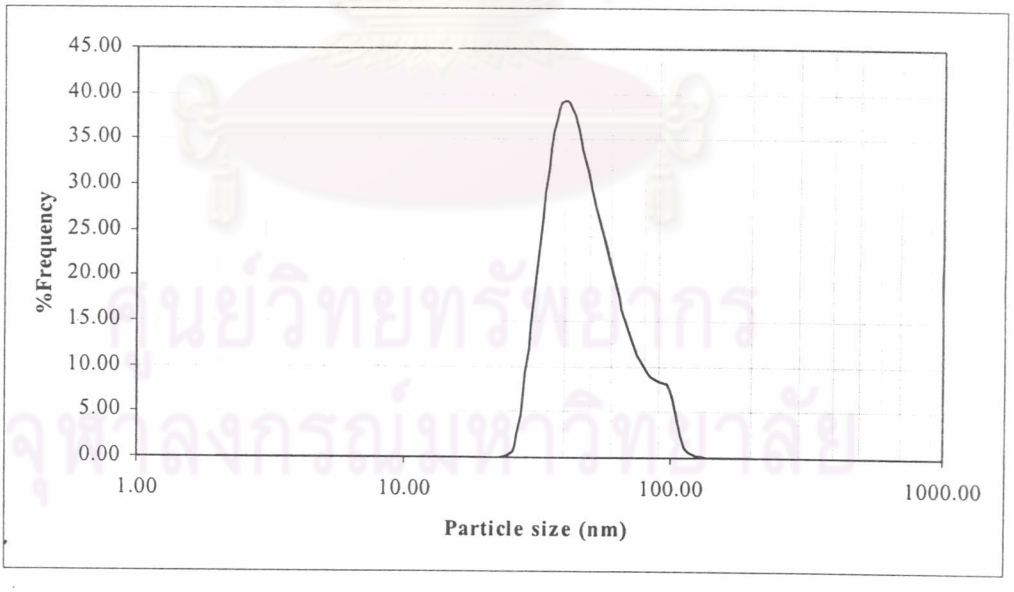

Figure 57 Particle size distribution of Formulation T1 P0.7 O4 D5 after autoclaving.

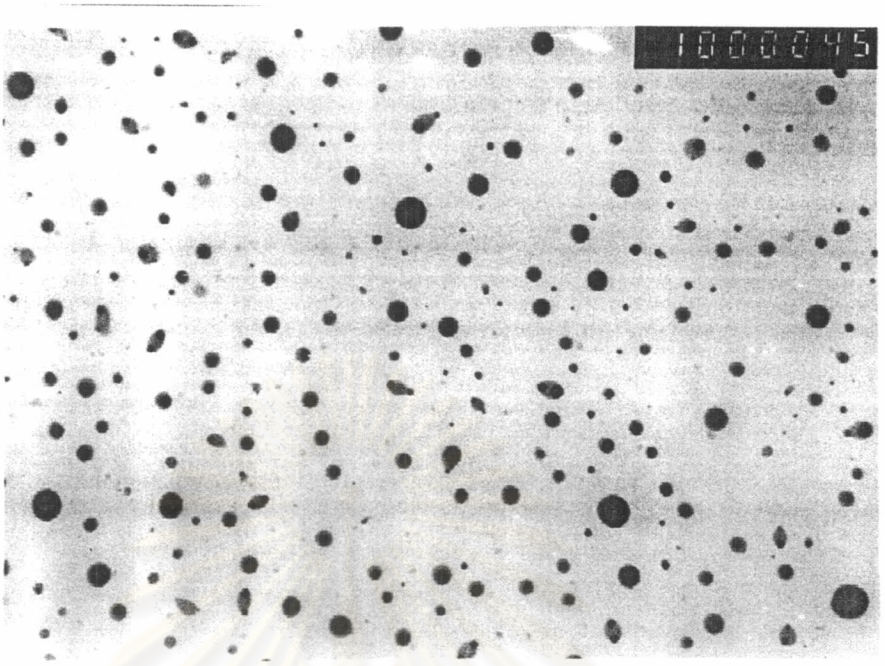

Figure 58 The TEM photomicrograph of the Formulation T1 P0.7 O4 D10 after autoclaving (1 mm from picture equivalent to 33.33 nm).

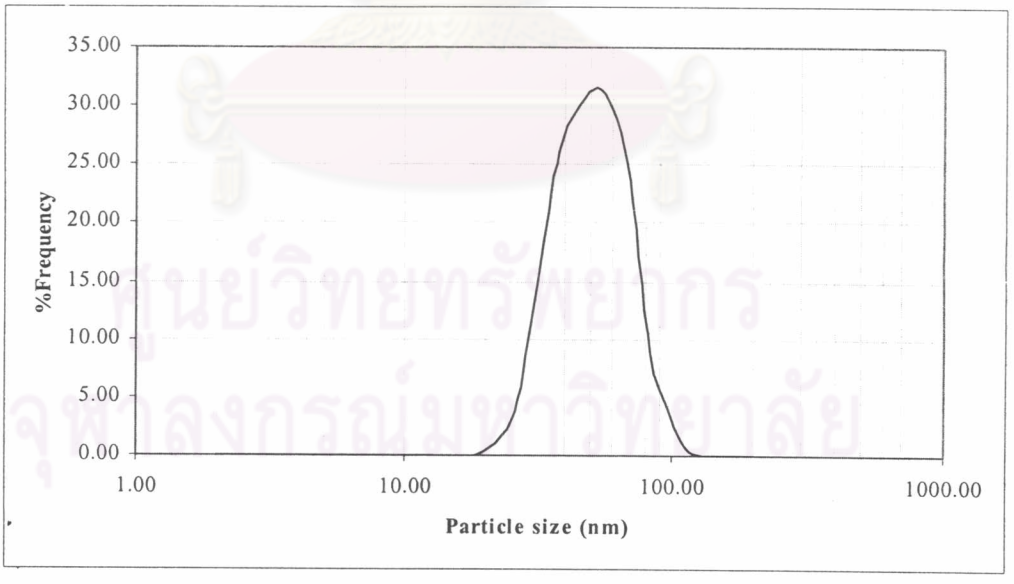

Figure 59 Particle size distribution of Formulation T1 P0.7 O4 D10 after autoclaving.

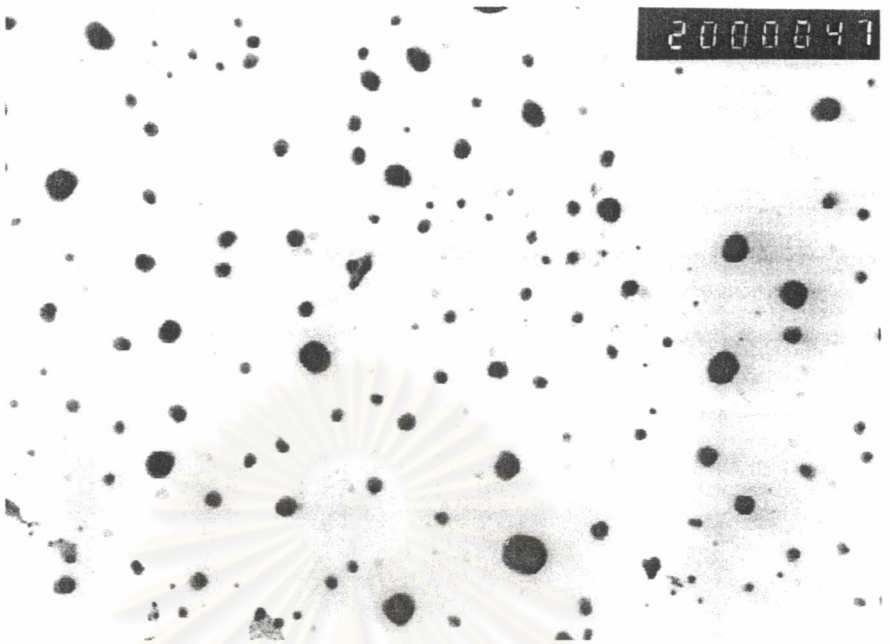

Figure 60 The TEM photomicrograph of the Formulation T1 P0.5 O8 before autoclaving (1 mm from picture equivalent to 33.33 nm).

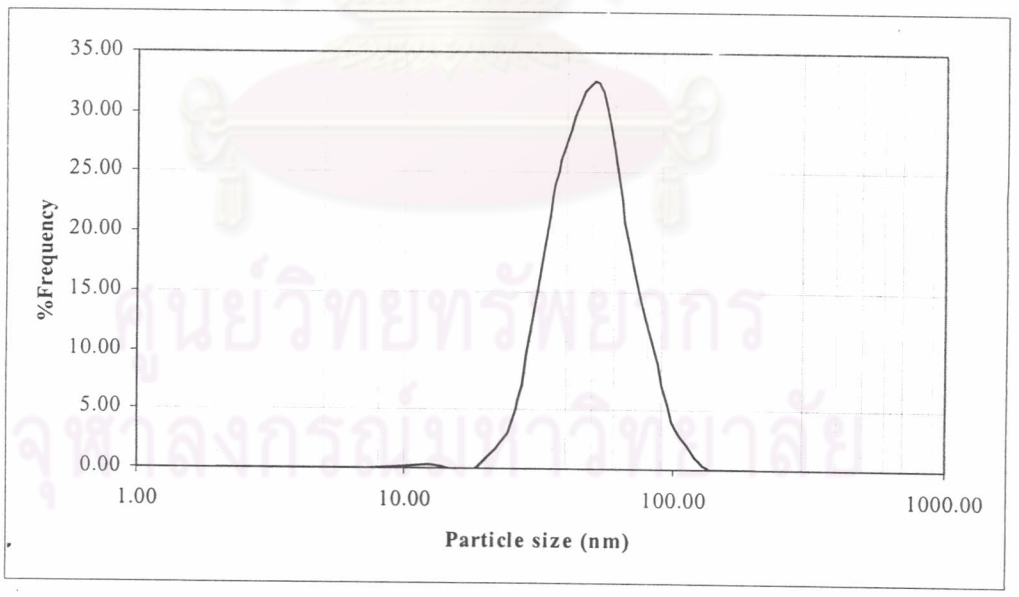

Figure 61 Particle size distribution of Formulation T1 P0.5 O8 before autoclaving.

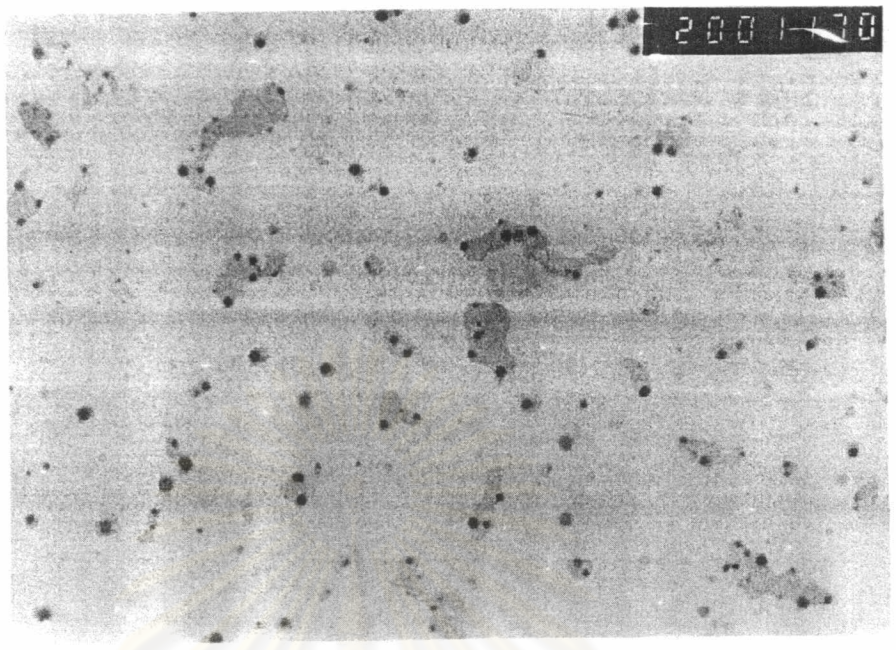

Figure 62 The TEM photomicrograph of the Formulation T1 P0.5 O8 after autoclaving (1 mm from picture equivalent to 58.82 nm).

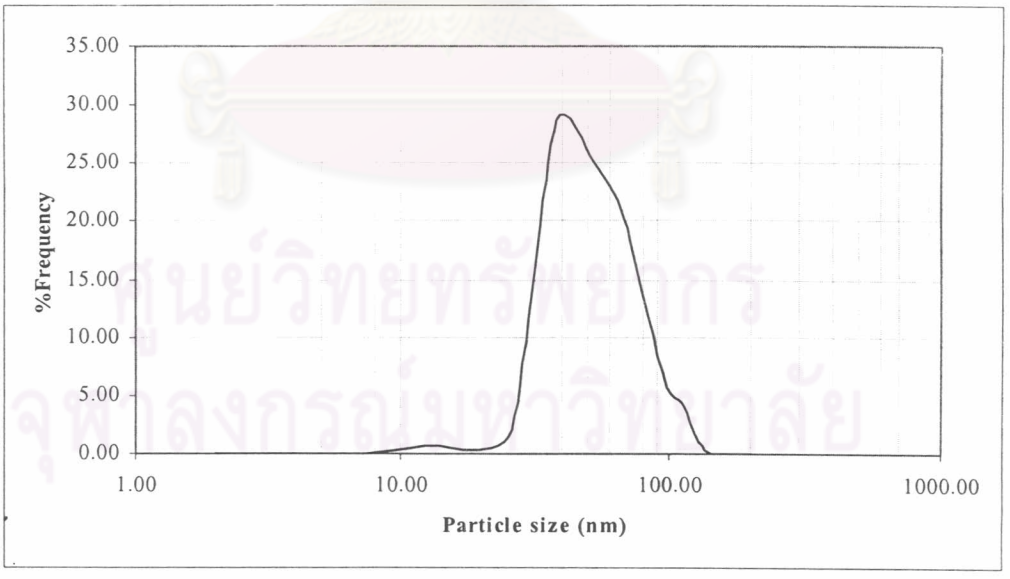

Figure 63 Particle size distribution of Formulation T1 P0.5 O8 after autoclaving.

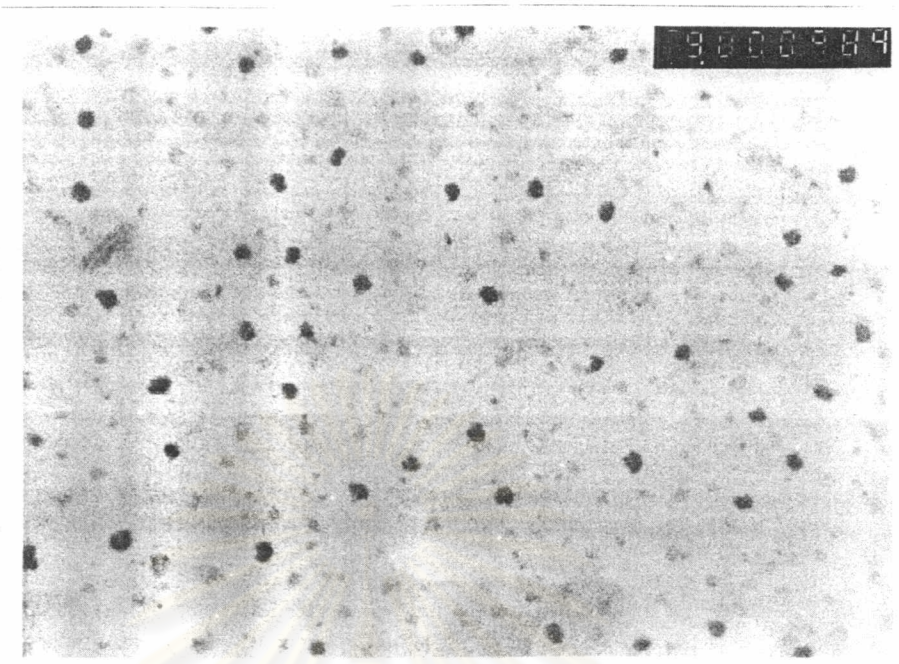

Figure 64 The TEM photomicrograph of the Formulation T1 P0.5 O6 before autoclaving (1 mm from picture equivalent to 33.33 nm).

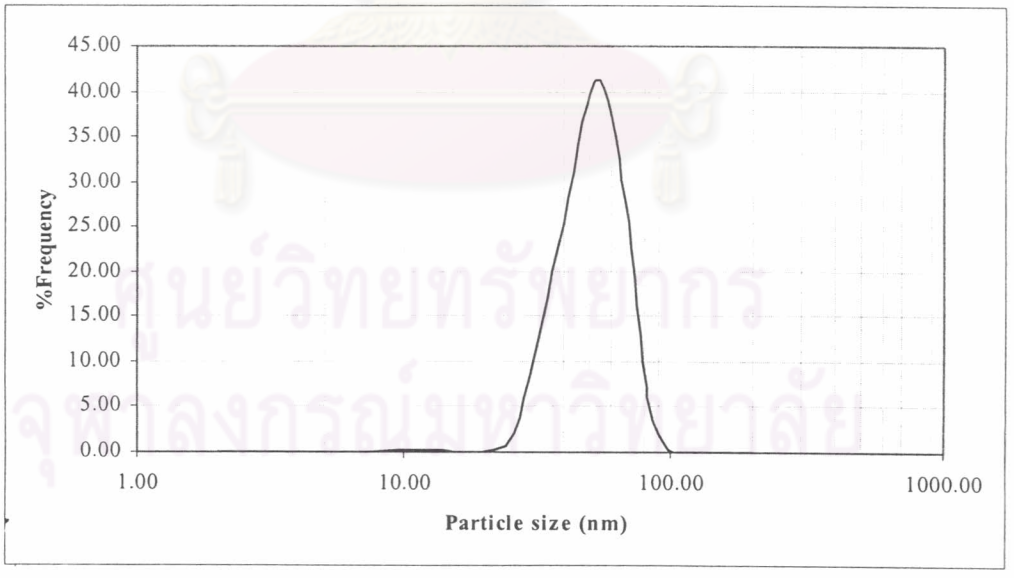

Figure 65 Particle size distribution of Formulation T1 P0.5 O6 before autoclaving

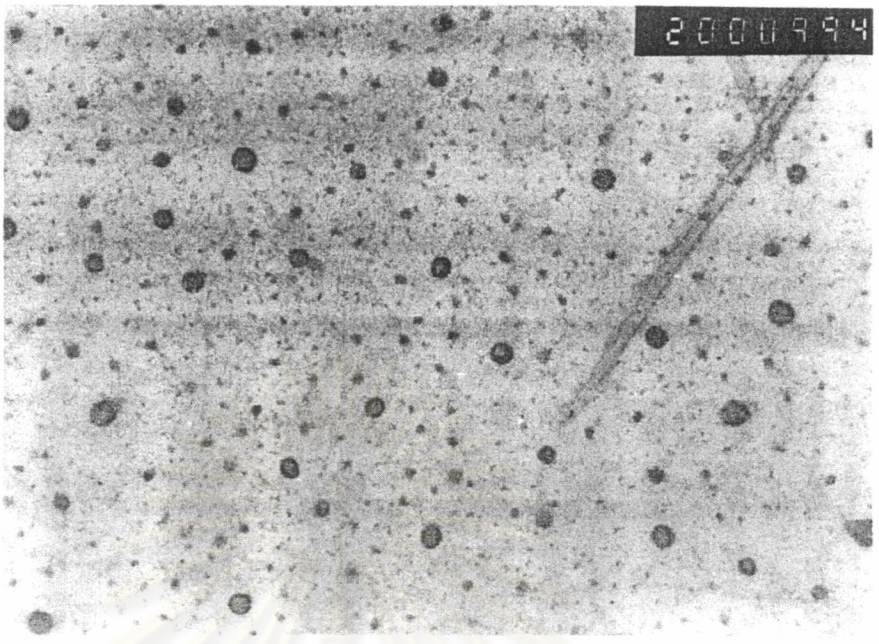

Figure 66 The TEM photomicrograph of the Formulation T1 P0.5 O6 after autoclaving (1 mm from picture equivalent to 33.33 nm).

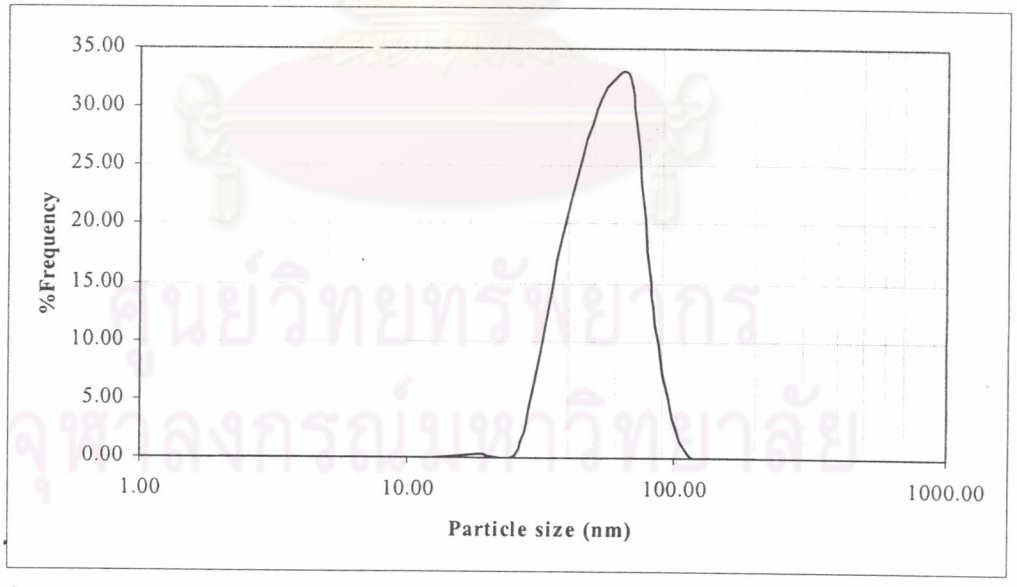

Figure 67 Particle size distribution of Formulation T1 P0.5 O6 after autoclaving.

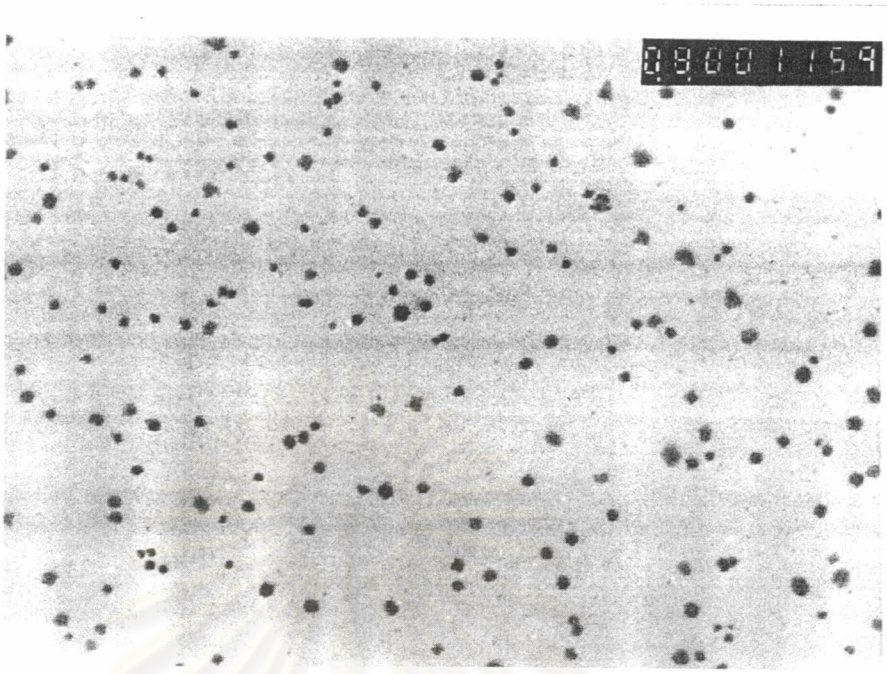

Figure 68 The TEM photomicrograph of the Formulation T1 P0.5 O4 before autoclaving (1 mm from picture equivalent to 33.33 nm).

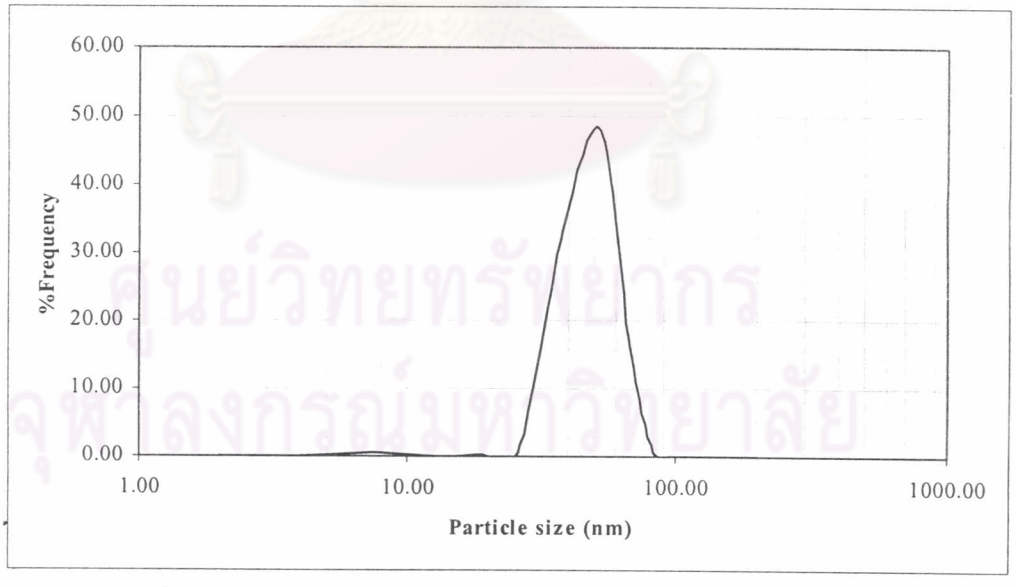

Figure 69 Particle size distribution of Formulation T1 P0.5 O4 before autoclaving.

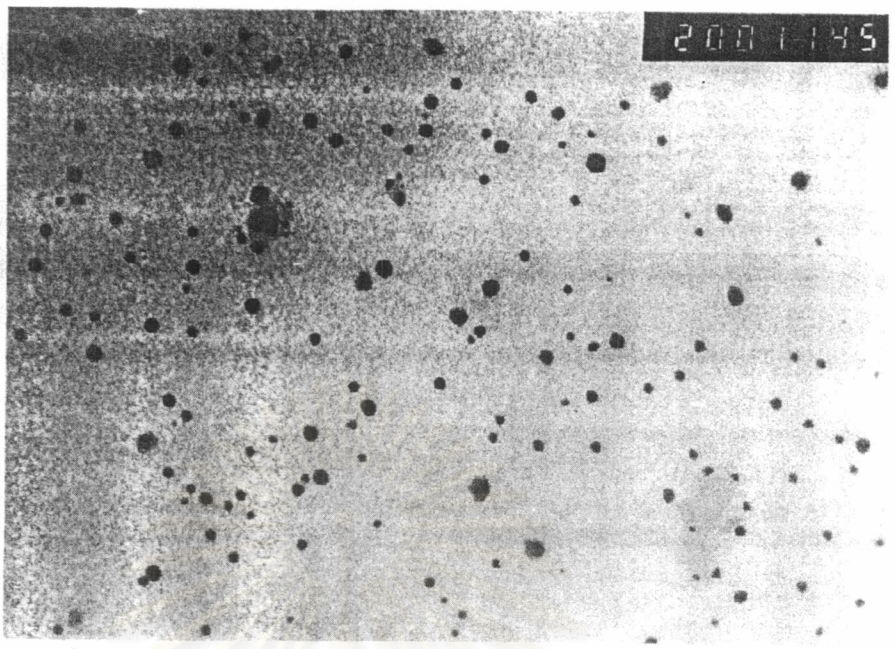

Figure 70 The TEM photomicrograph of the Formulation T1 P0.5 O4 after autoclaving (1 mm from picture equivalent to 33.33 nm).

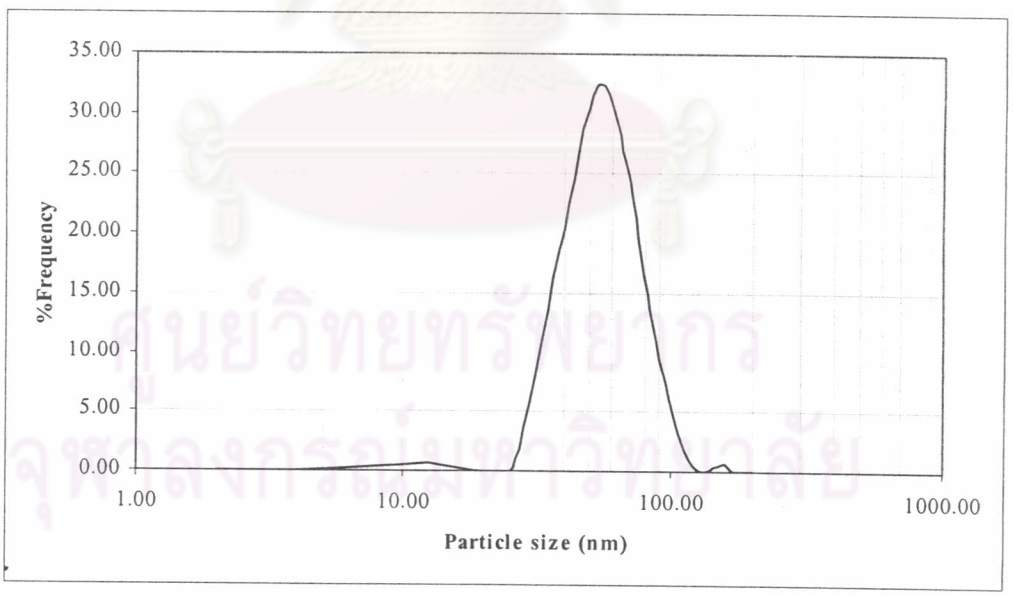

Figure 71 Particle size distribution of Formulation T1 P0.5 O4 after autoclaving.

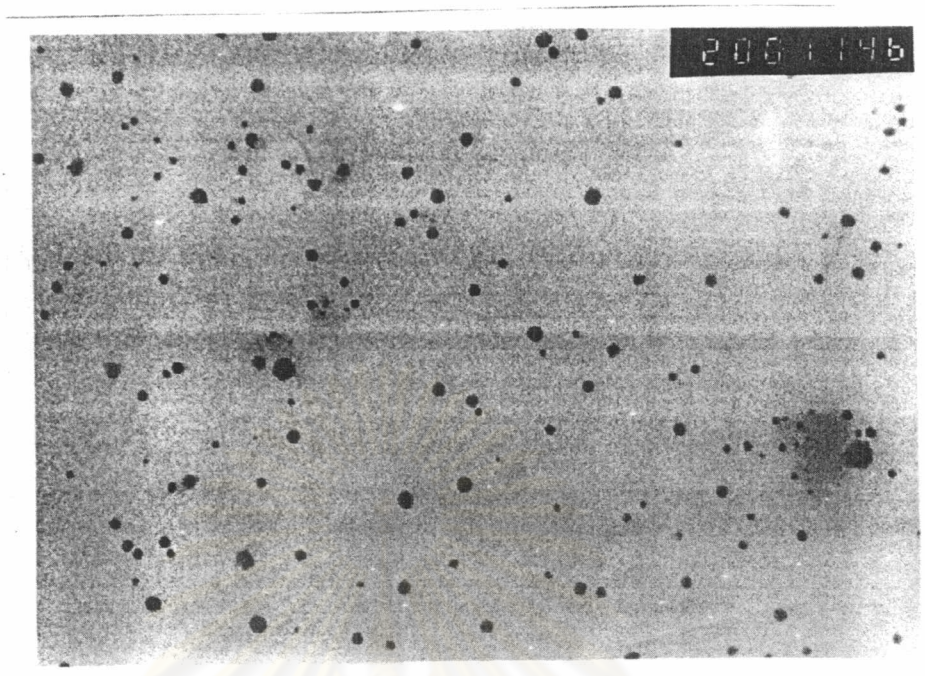

Figure 72 The TEM photomicrograph of the Formulation T1 P0.5 O4 D5 after autoclaving (1 mm from picture equivalent to 33.33 nm).

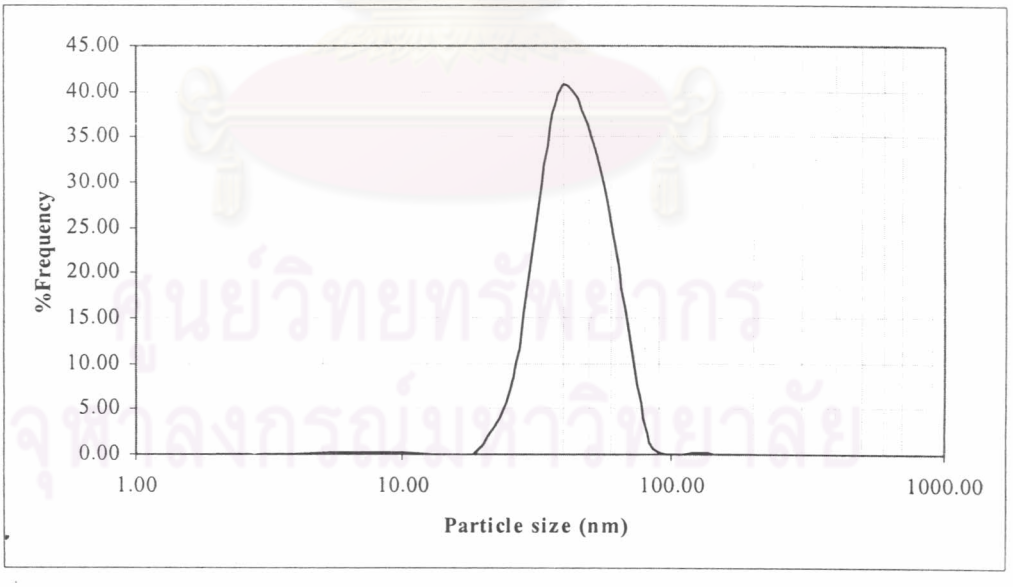

Figure 73 Particle size distribution of Formulation T1 P0.5 O4 D5 after autoclaving.

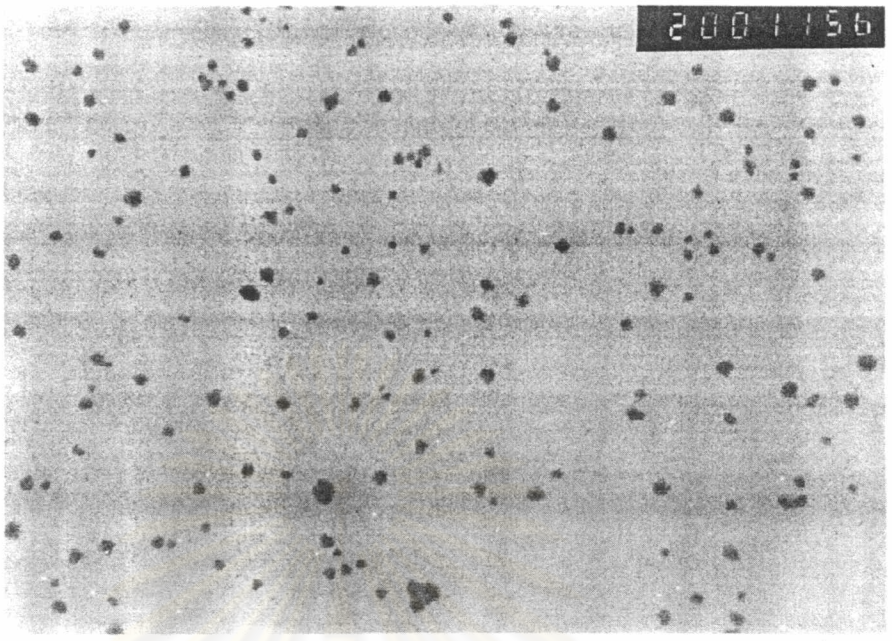

Figure 74 The TEM photomicrograph of the Formulation T1 P0.5 O4 D10 after autoclaving (1 mm from picture equivalent to 33.33 nm).

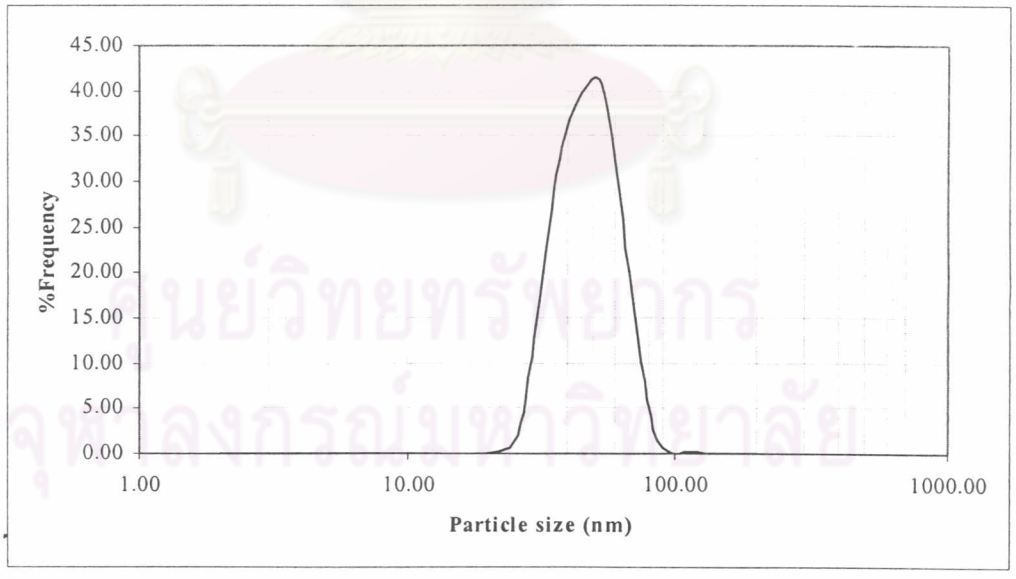

Figure 75 Particle size distribution of Formulation T1 P0.5 O4 D10 after autoclaving.

Table 12 Mean particle sizes of the microemulsions before and after autoclaving.

Formulation	Mean particle diameter [a] (nm)			
	before autoclaving	SD	after autoclaving	SD
T1 G1.5 O8	90.03	23.01	96.86*	25.87
T1 G1.5 O6	85.78	19.79	88.25*	26.32
T1 G1.5 O4	85.81	24.07	88.31*	21.18
T1 G1.5 O4 D5	-	-	84.65	29.15
T1 G1.5 O4 D10	-	-	83.31	17.56
T1 G1 O8	67.88	20.71	76.69*	22.44
T1 G1 O6	68.09	25.05	72.54*	23.88
T1 G1 O4	62.51	17.09	67.16*	25.38
T1 G1 O4 D5	-	-	65.49	18.68
T1 G1 O4 D10	-	-	68.74	23.07
T1 P0.7 O8	67.53	20.94	77.83*	22.83
T1 P0.7 O6	64.44	20.26	67.42*	21.72
T1 P0.7 O4	63.63	19.36	67.14*	27.51
T1 P0.7 O4 D5	-	-	56.26	21.09
T1 P0.7 O4 D10	-	-	59.52	17.91
T1 P0.5 O8	55.47	19.94	59.43*	23.19
T1 P0.5 O6	57.99	21.22	59.52*	15.82
T1 P0.5 O4	48.98	10.46	58.13*	17.34
T1 P0.5 O4 D5	-	-	46.18	13.92
T1 P0.5 O4 D10	-	-	53.64	12.98

[a] = mean particle diameter of microemulsions was calculated from 300 particles.

* = statistical significant difference ($p<0.05$) of mean particle diameter between before and after autoclaving.

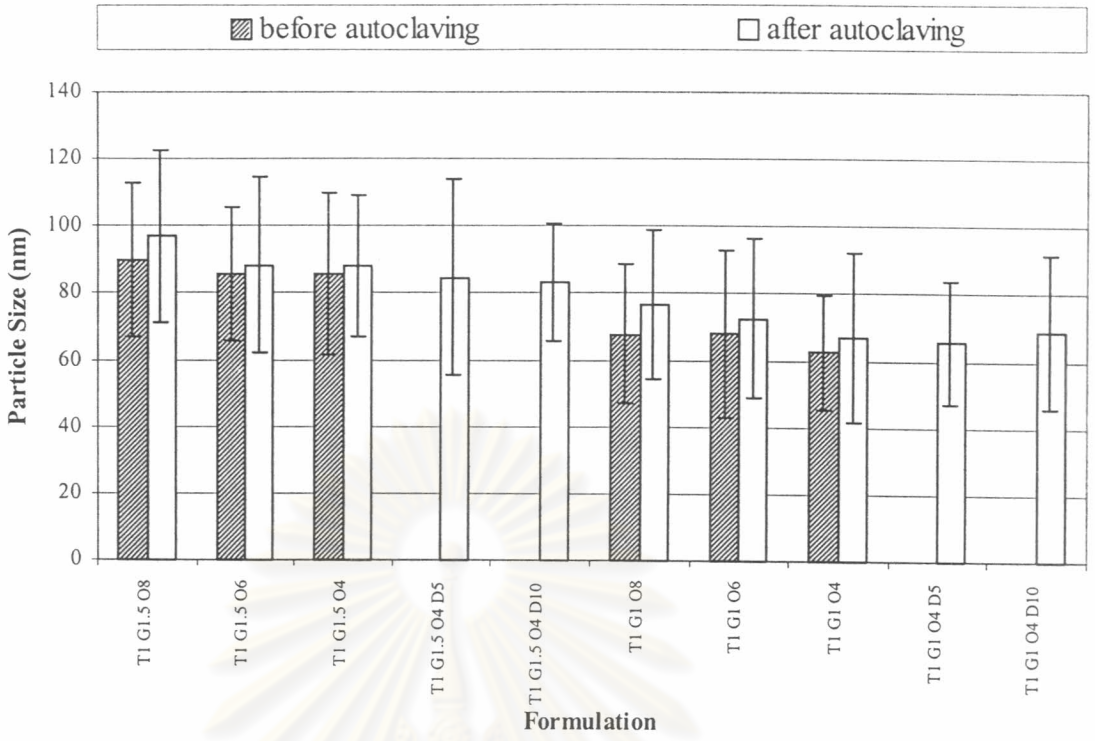

Figure 76 Comparison of the mean particle size of microemulsion in tween 80:glycerin systems between before and after autoclaving.

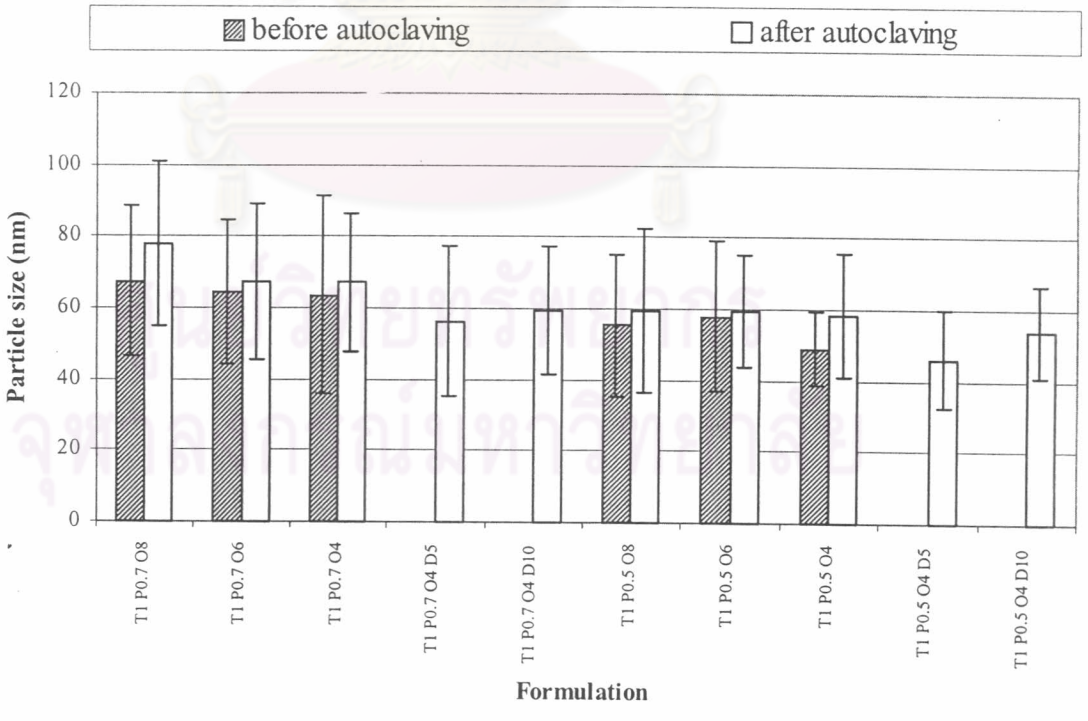

Figure 77 Comparison of the mean particle size of microemulsion in tween 80:polyethylene glycol 400 systems between before and after autoclaving.

2.7 *In vitro* drug diffusion

The diffusion profiles and the percentage of drug diffusion at different time interval of commercial diazepam injection and diazepam microemulsions illustrated in Figures 78-87. And raw data are described in Appendix E.

The commercial product, diazepam injection (10 mg/ 2 ml)

The commercial product, diazepam injection (10 mg/ 2 ml) was used to compare in the drug diffusion study. As shown in Figure 78 and 79, the amount of drug diffusion from commercial product at first 4 hours was rapid. The amount of drug diffusion at first 4 hours was 19.75% or 0.987 mg. After the fourth hour, the amount of drug diffusion decreased. The amount of drug diffusion in 48 hours was 56.24% or 2.81 mg.

Tween 80:glycerin system at the ratio of 1:1.5

The diffusion profiles of diazepam microemulsion of tween 80:glycerin system at the ratio of 1:1.5 are shown in Figures 80 and 81. The results showed that the drug diffusion from all formulations was sustained. The drug diffused to receptor compartment less than 20% in 48 hours. Among formulations containing diazepam at the concentration 5 mg/ml as shown in Figure 80, Formulation T1 G1.5 O4 D5 has more amount of drug diffusion than Formulations T1 G1.5 O6 D5 and T1 G1.5 O8 D5. The amount of drug diffusion in 48 hours was 0.970, 0.776, and 0.668 mg, respectively. Among formulations containing diazepam at the concentration 10 mg/ml, the amount of drug diffusion in Formulation T1 G1.5 O4 D10 was higher than Formulations T1 G1.5 O6 D10 and T1 G1.5 O8 D10 which the amount of drug diffusion in 48 hours were 1.721, 1.521, and 1.353 mg, respectively. From these results, the amount of drug diffusion increased when the amount of oil in formulation decreased. Furthermore, comparison between microemulsions at the equal amount of oil showed that the amount of drug diffusion increased when the concentration of drug in formulation was increased.

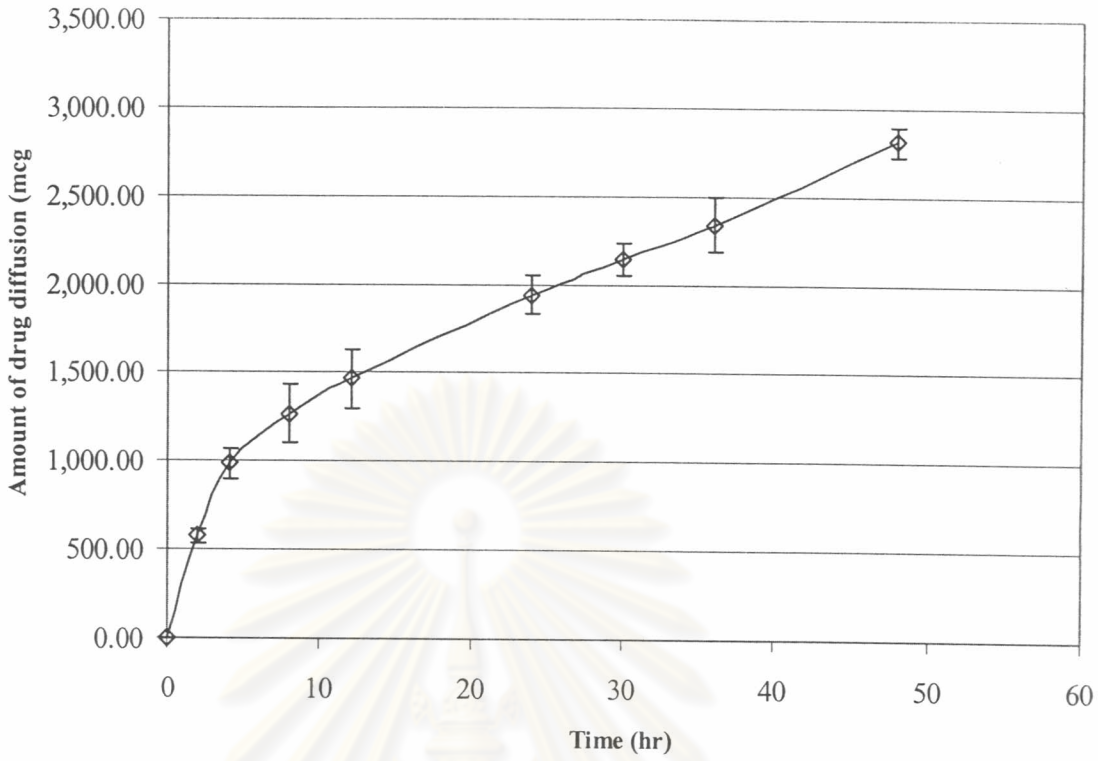

Figure 78 The diffusion profile of commercial diazepam injection.

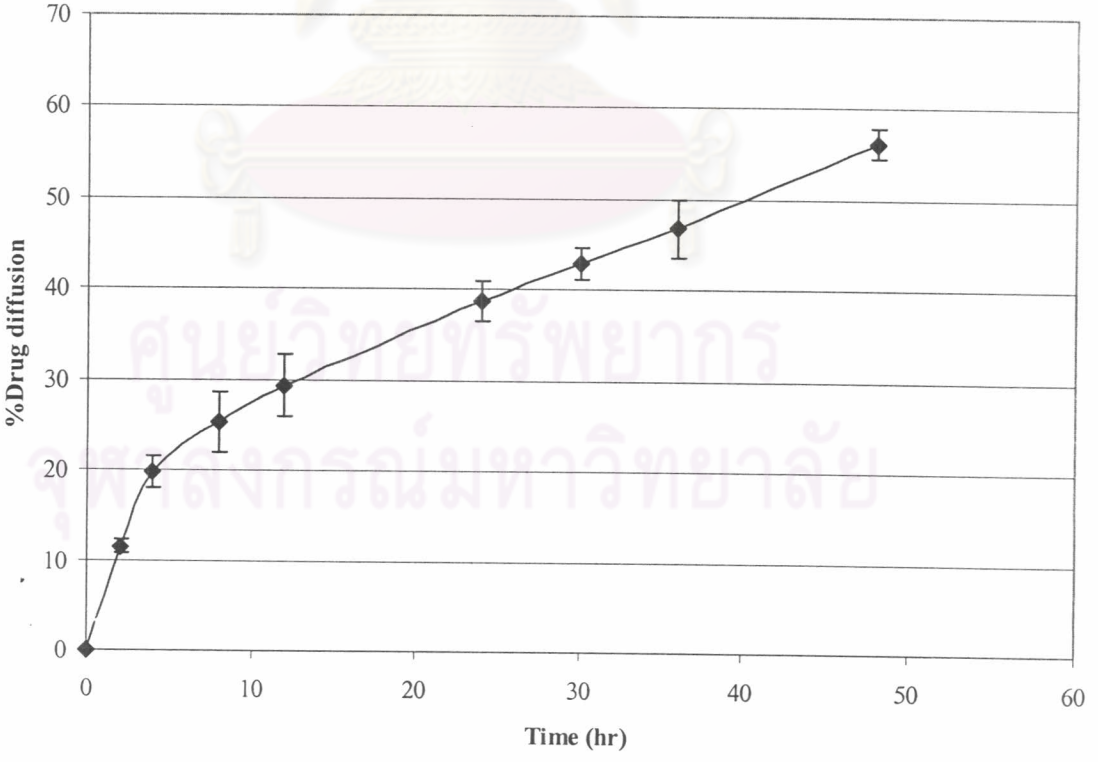

Figure 79 The percentage of drug diffusion from commercial diazepam injection at different time interval.

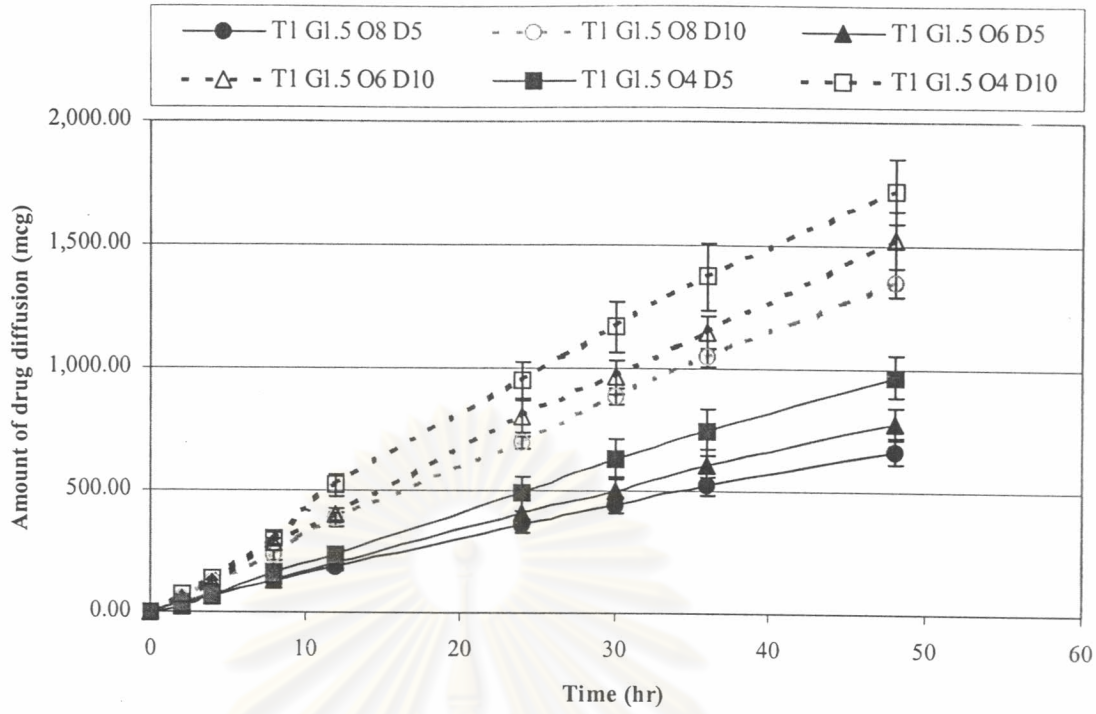

Figure 80 The diffusion profiles of diazepam microemulsions containing 1:1.5 of tween80:glycerin.

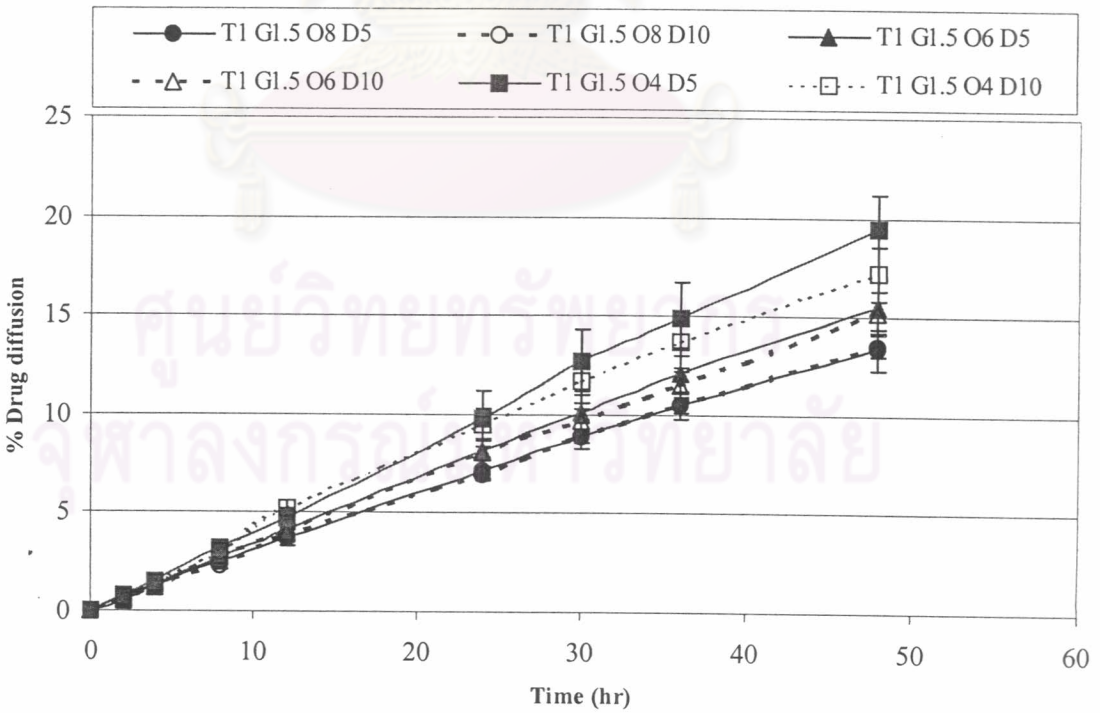

Figure 81 The percentage of drug diffusion from diazepam microemulsions containing 1:1.5 of tween80:glycerin at different time interval.

Comparison the diffusion profiles between Formulations T1 G1.5 O8 D5 and T1 G1.5 O8 D10 in Figure 81 showed that the diffusion profiles were no statistically significant difference in the diffusion pattern ($p>0.05$, tested by 2 tailed paired-sample T test). Similar results were obtained in Formulations T1 G1.5 O6 D5 and T1 G1.5 O6 D10, and Formulations T1 G1.5 O4 D5 and T1 G1.5 O4 D10 that the diffusion profiles were no statistically significant difference in the diffusion pattern ($p>0.05$). The results of statistical evaluation are shown in Appendix F. These results indicated the formulations which containing diazepam at concentration of 5 mg/ml and 10 mg/ml at equal amount of oil showed no statistically significant difference in the diffusion pattern ($p>0.05$).

The highest correlation coefficient of Formulations T1 G1.5 O8 D5 and T1 G1.5 O4 D5 was obtained from Power expression model as shown in Table 13. In Formulations T1 G1.5 O8 D10, T1 G1.5 O6 D5, and T1 G1.5 O6 D10, the highest correlation coefficient was obtained from cube root model. While the highest correlation coefficient of Formulation T1 G1.5 O4 D10 was obtained from Weibull model. From these results, it could be concluded that the drug diffusion kinetic of diazepam microemulsions in tween 80:glycerine system at the ratio of 1:1.5 was mostly fitted with cube root model.

Tween 80:glycerin system at the ratio of 1:1

The diffusion profiles in Figure 82 showed that Formulation T1 G1 O4 D5 had the highest amount of drug diffusion followed by Formulations T1 G1 O6 D5 to T1 G1 O8 D5, respectively. At higher drug concentration of 10 mg/ml, the amount of drug diffusion in Formulation T1 G1 O4 D10 was higher than Formulation T1 G1 O6 D10 and T1 G1 O8 D10, respectively. The results indicated that the amount of drug diffusion increased when the amount of oil in formulation decreased. Furthermore, at the equal amount of oil, microemulsions containing higher drug concentration had more amount of drug diffusion than microemulsions containing lower drug concentration.

The percentage of drug diffusion from diazepam microemulsions of this system was illustrated in Figure 83. The diffusion profiles between formulation containing diazepam at concentration 5mg/ml and 10 mg/ml at the equal amount of oil seemed to be similar. There was no statistically significant difference in their diffusion pattern ($p>0.05$, tested by 2 tailed paired-sample T test). These results were similar to microemulsions in tween 80:glycerin system at the ratio of 1:1.5. The results of statistical evaluation are shown in Appendix F.

Formulations T1 G1 O8 D5, T1 G1 O8 D10, and T1 G1 O6 D5 had the highest correlation coefficient when treated with Weibull model as shown in Table 13. While Formulation T1 G1 O6 D10, T1 G1 O4 D5, and T1 G1 O4 D10 had the highest correlation coefficient when treated with cube root model. From these results indicated that the drug diffusion kinetic of microemulsions in T80:Gly system at the ratio of 1:1 was fitted to cube root model and Weibull model.

Tween 80:polyethylene glycol 400 system at the ratio of 1:0.7

Figures 84 and 85 illustrate the diffusion profiles of diazepam microemulsion in this system. The amount of drug diffusion of all formulations was less than 20% in 48 hours. The diffusion profiles of microemulsions containing lower drug concentration as shown in Figure 84 revealed that the amount of drug diffusion decreased from Formulation T1 P0.7 O4 D5 to Formulation T1 P0.7 O6 D5 and T1 P0.7 O8 D5, respectively. The amount of drug diffusion in 48 hours of these formulations was 0.935, 0.750, and 0.697 mg. For microemulsions containing higher drug concentration, the results were similar to the lower drug concentration that the higher amount of drug diffusion was obtained from Formulation T1 P0.7 O4 D10 and decreasing in Formulation T1 P0.7 O6 D10 to T1 P0.7 O8 D10. The amount of drug diffusion in 48 hours of these formulations was 1.893, 1.532, and 1.333 mg, respectively. The results indicated that the amount of drug diffusion increased when decreasing the amount of oil in formulation. Furthermore, microemulsions which containing higher drug concentration had higher amount of drug diffusion than lower drug concentration at the equal amount of oil.

Comparison the diffusion profiles between drug loaded microemulsions both concentrations of drug at the equal amount of oil in Figure 85, indicated that there were no statistically significant difference in the diffusion pattern ($p>0.05$, tested by 2 tailed paired-sample T test). The results of statistical evaluation are shown in Appendix F. The highest correlation coefficient was obtained from Weibull model as exhibited in Table 13. From the results, it could be concluded that the drug diffusion kinetic of microemulsions in tween 80:polyethylene glycol 400 system at the ratio of 1:0.7 followed Weibull model.

Tween 80:polyethylene glycol 400 system at the ratio of 1:0.5

Figures 86 and 87 exhibit the diffusion profiles of microemulsions in tween 80:polyethylene glycol 400 at the ratio of 1:0.5. Figure 86 revealed that the diffusion profiles of Formulation T1 P0.5 O4 D5 had the highest amount of drug diffusion followed by Formulation T1 P0.5 O6 D5 to T1 P0.5 O8 D5, respectively. Similar results were obtained in microemulsions of higher drug concentration. The amount of drug diffusion in Formulation T1 P0.5 O4 D10 was higher than Formulation T1 P0.5 O6 D10 and T1 P0.5 O8 D10, respectively. From the above investigation, the results indicated that the amount of drug diffusion increased when the amount of oil in formulation decreased. Comparison between same formulations that containing higher and lower drug concentration, showed the amount of drug diffusion increased when the concentration of diazepam in formulation increased.

Comparison the diffusion profiles of microemulsions at the equal amount of oil in Figure 87, revealed that the formulations containing diazepam at concentration of 5 mg/ml and 10 mg/ml showed no statistically significant difference in the diffusion pattern ($p>0.05$ by 2 tailed paired-sample T test). The highest correlation coefficient of Formulations T1 P0.5 O8 D5, T1 P0.5 O6 D5, and T1 P0.5 O6 D10 was obtained from Weibull model as shown in Table 13. Whereas the highest correlation coefficient of Formulations T1 P0.5 O8 D10, T1 P0.5 O4 D5, and T1 P0.5 O4 D10 was obtained from cube root model. These results indicated that the drug diffusion kinetic of microemulsions in this system followed cube root model and Weibull model.

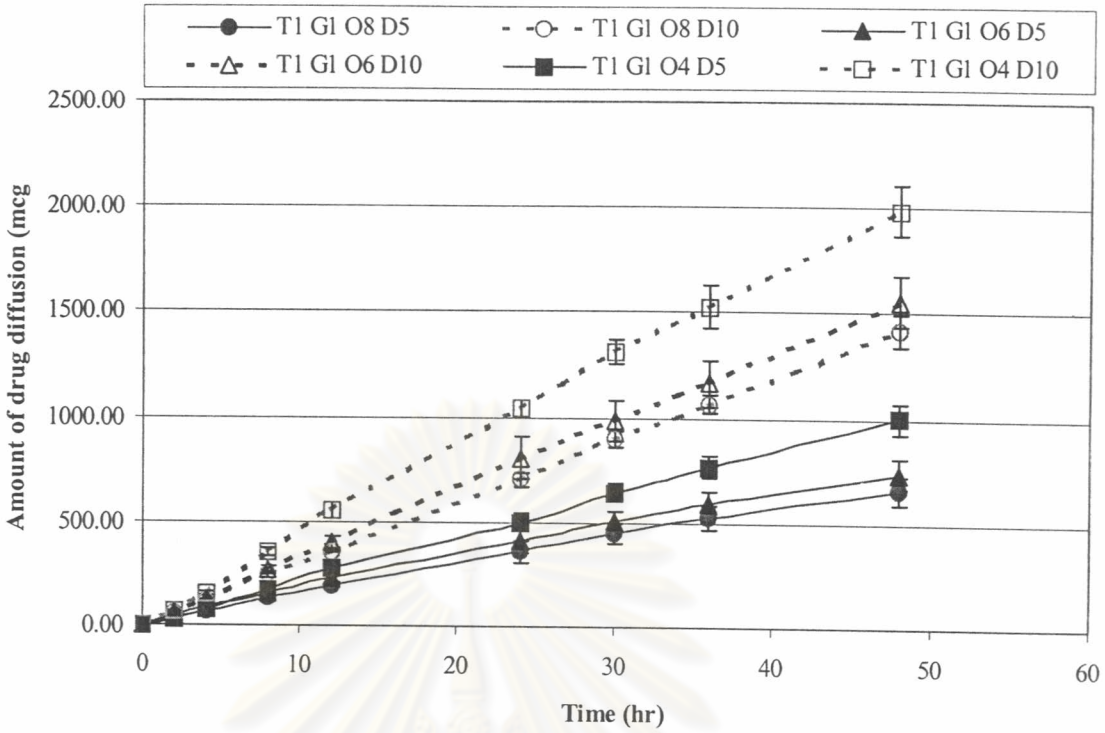

Figure 82 The diffusion profiles of diazepam microemulsion containing 1:1 of tween 80:glycerin.

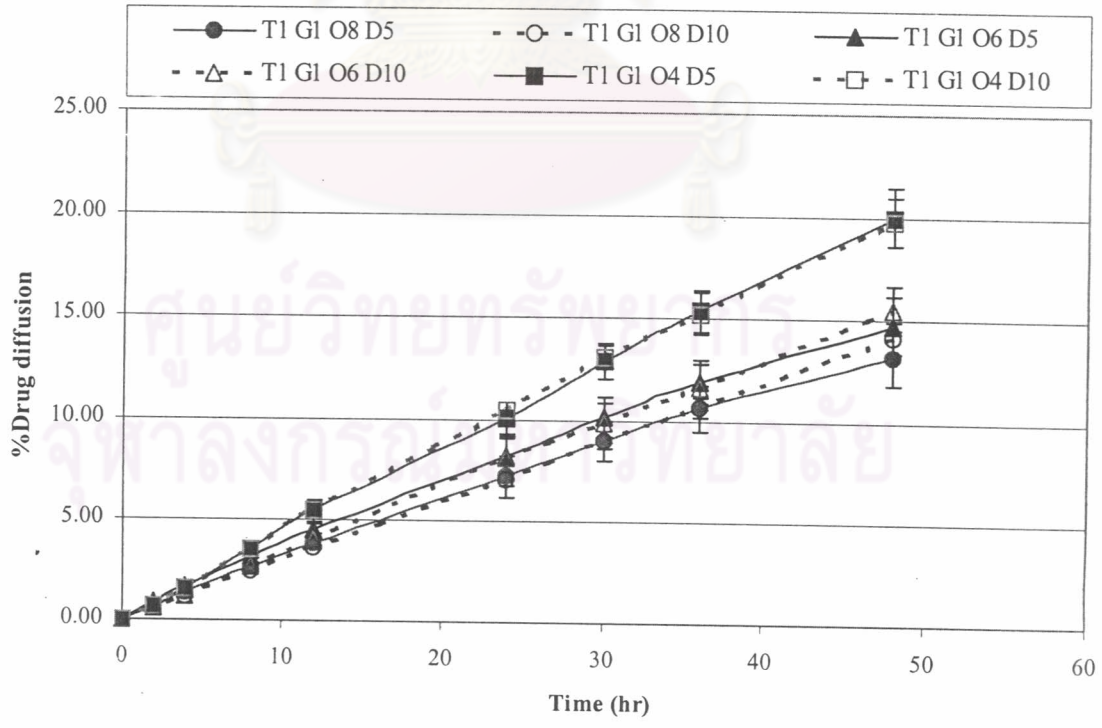

Figure 83 The percentage of drug diffusion from diazepam microemulsions containing 1:1 of tween80:glycerin at different time interval.

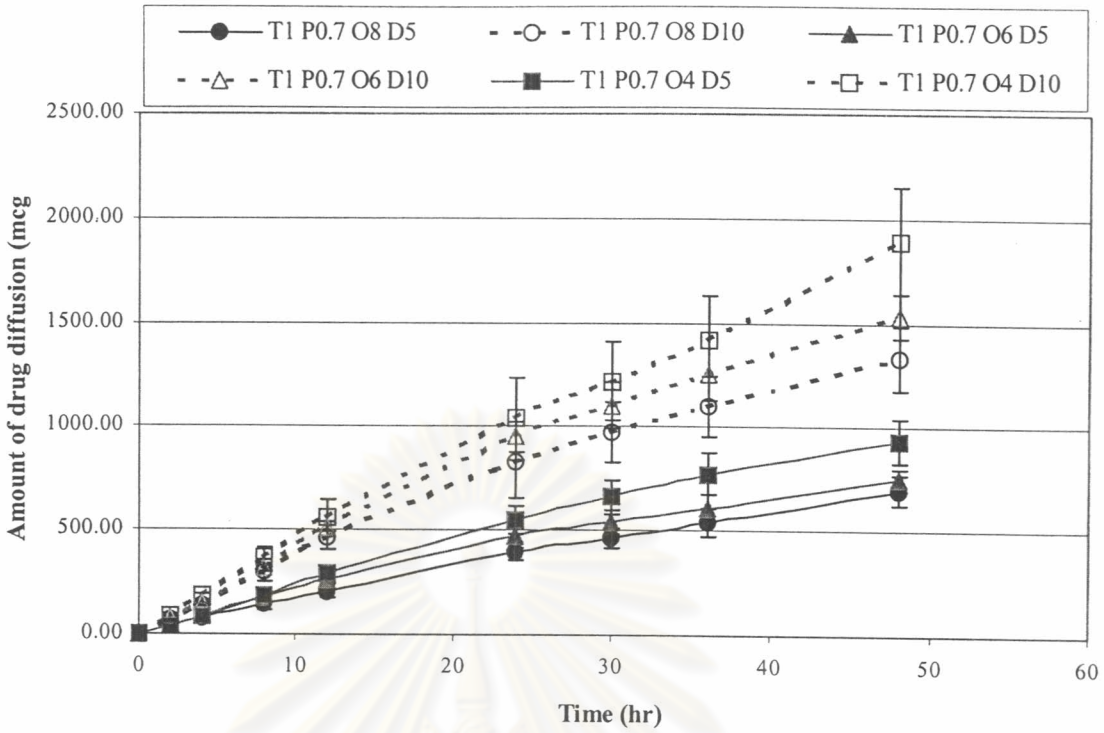

Figure 84 The diffusion profiles of diazepam microemulsions containing 1:0.7 of tween 80:polyethylene glycol 400.

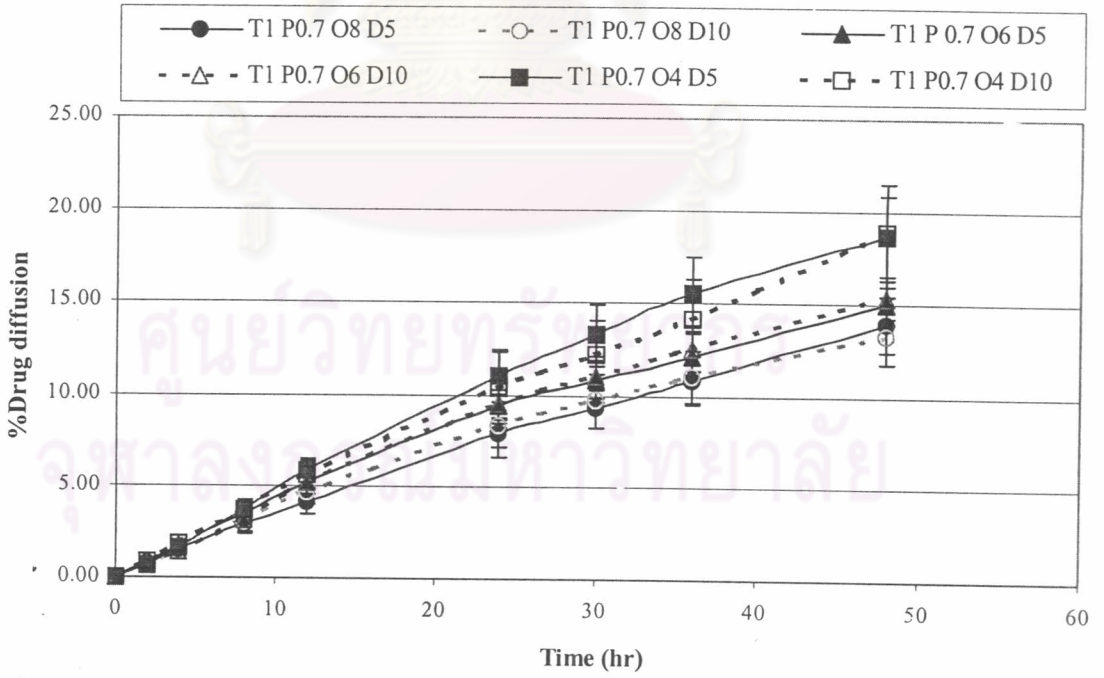

Figure 85 The percentage of drug diffusion from diazepam microemulsions containing 1:0.7 of tween 80:polyethylene glycol 400 at different time interval.

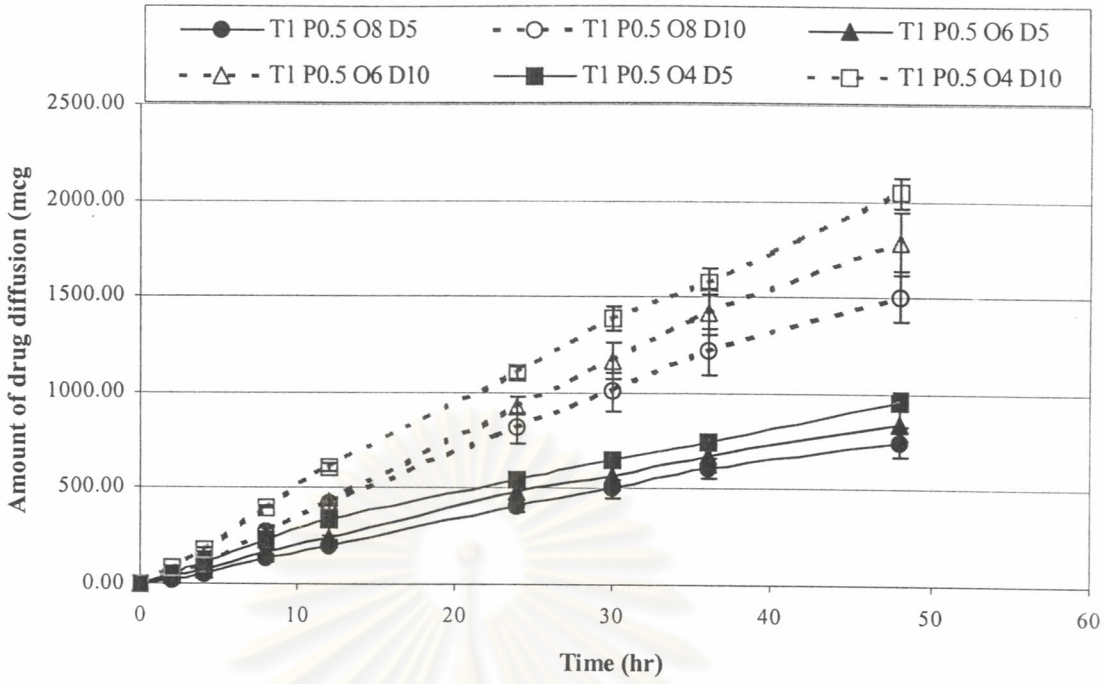

Figure 86 The diffusion profiles of diazepam microemulsions containing 1:0.5 of tween 80:polyethylene glycol 400.

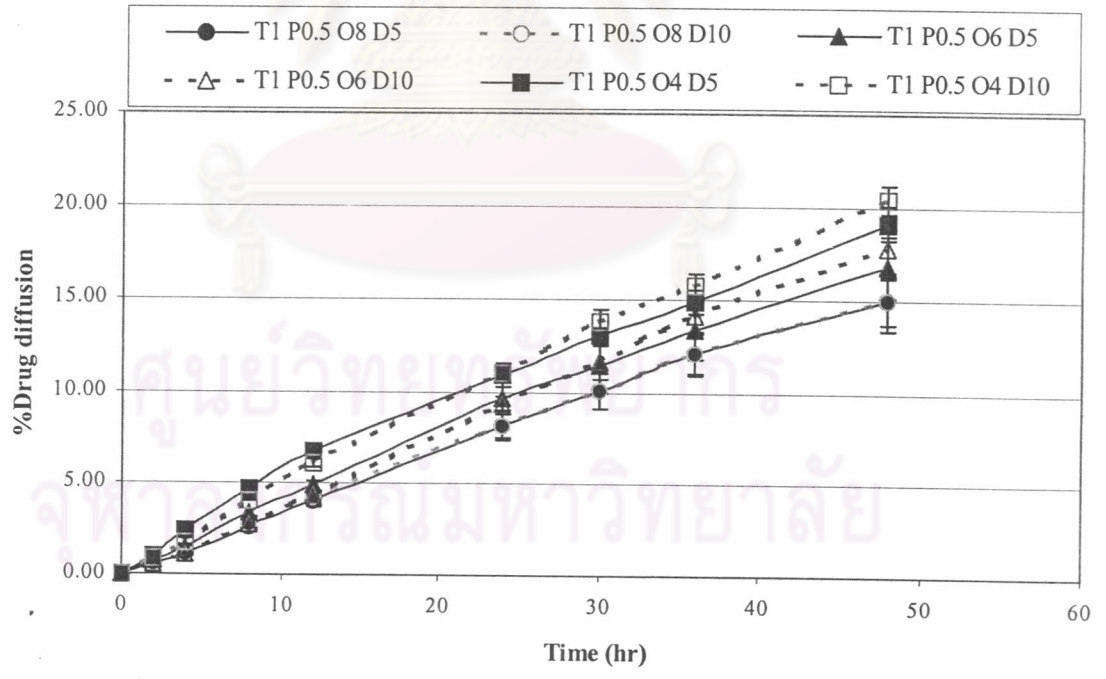

Figure 87 The percentage of drug diffusion from diazepam microemulsions containing 1:0.5 of tween 80:polyethylene glycol 400 at different time interval.

Table 13 The coefficient of determination of microemulsions in various drug diffusion kinetics calculated from total drug diffusion data.

Formulation	Zero order model	Higuchi model	Cube root model	Power expression model	Weibull model
T1 G1.5 O8 D5	0.9948	0.9455	0.9992	0.9994	0.9991
T1 G1.5 O8 D10	0.9988	0.9404	0.9994	0.9991	0.9993
T1 G1.5 O6 D5	0.9988	0.9368	0.9995	0.9937	0.9940
T1 G1.5 O6 D10	0.9991	0.9388	0.9995	0.9980	0.9986
T1 G1.5 O4 D5	0.9993	0.9301	0.9994	0.9997	0.9996
T1 G1.5 O4 D10	0.9954	0.9470	0.9972	0.9968	0.9977
T1 G1 O8 D5	0.9965	0.9495	0.9977	0.9996	0.9998
T1 G1 O8 D10	0.9994	0.9313	0.9996	0.9997	0.9998
T1 G1 O6 D5	0.9951	0.9552	0.9968	0.9997	0.9998
T1 G1 O6 D10	0.9996	0.9346	0.9998	0.9990	0.9995
T1 G1 O4 D5	0.9991	0.9352	0.9994	0.9949	0.9963
T1 G1 O4 D10	0.9983	0.9409	0.9994	0.9954	0.9968
T1 P0.7 O8 D5	0.9962	0.9530	0.9976	0.9988	0.9993
T1 P0.7 O8 D10	0.9838	0.9653	0.9866	0.9930	0.9942
T1 P0.7 O6 D5	0.9851	0.9665	0.9881	0.9955	0.9966
T1 P0.7 O6 D10	0.9858	0.9604	0.9887	0.9945	0.9956
T1 P0.7 O4 D5	0.9900	0.9541	0.9929	0.9926	0.9944
T1 P0.7 O4 D10	0.9970	0.9483	0.9982	0.9980	0.9987
T1 P0.5 O8 D5	0.9966	0.9401	0.9977	0.9971	0.9980
T1 P0.5 O8 D10	0.9967	0.9411	0.9979	0.9965	0.9974
T1 P0.5 O6 D5	0.9952	0.9497	0.9970	0.9974	0.9981
T1 P0.5 O6 D10	0.9978	0.9278	0.9983	0.9981	0.9987
T1 P0.5 O4 D5	0.9895	0.9663	0.9927	0.9881	0.9903
T1 P0.5 O4 D10	0.9964	0.9488	0.9982	0.9958	0.9972

The drug diffusion kinetic of microemulsions in tween 80:glycerin systems and tween 80:polyethylene glycol 400 systems followed cube root model and Weibull model. The diffusion rate of each formulation from cube root model showed in Table 14. The results revealed that formulations containing oil 8 % w/w had slow diffusion rate. The diffusion rate increased when the oil concentration decreased to 6% w/w and 4% w/w, respectively.

Table 14 The diffusion rate of microemulsions from cube root model.

Formulation	Diffusion rate ($mg^{1/3}hr^{-1}$)	Formulation	Diffusion rate ($mg^{1/3}hr^{-1}$)
T1 G1.5 O8 D5	$4.53*10^{-3}$	T1 P0.7 O8 D5	$4.72*10^{-3}$
T1 G1.5 O8 D10	$4.59*10^{-3}$	T1 P0.7 O8 D10	$4.65*10^{-3}$
T1 G1.5 O6 D5	$5.37*10^{-3}$	T1 P0.7 O6 D5	$5.20*10^{-3}$
T1 G1.5 O6 D10	$5.17*10^{-3}$	T1 P0.7 O6 D10	$5.40*10^{-3}$
T1 G1.5 O4 D5	$6.78*10^{-3}$	T1 P0.7 O4 D5	$6.74*10^{-3}$
T1 G1.5 O4 D10	$6.05*10^{-3}$	T1 P0.7 O4 D10	$6.46*10^{-3}$
T1 G1 O8 D5	$4.52*10^{-3}$	T1 P0.5 O8 D5	$5.27*10^{-3}$
T1 G1 O8 D10	$4.79*10^{-3}$	T1 P0.5 O8 D10	$5.28*10^{-3}$
T1 G1 O6 D5	$5.07*10^{-3}$	T1 P0.5 O6 D5	$5.89*10^{-3}$
T1 G1 O6 D10	$5.31*10^{-3}$	T1 P0.5 O6 D10	$6.34*10^{-3}$
T1 G1 O4 D5	$6.99*10^{-3}$	T1 P0.5 O4 D5	$6.83*10^{-3}$
T1 G1 O4 D10	$6.95*10^{-3}$	T1 P0.5 O4 D10	$7.16*10^{-3}$

2.8 Stability testing

The physical appearance, pH and refractive index of microemulsion preparations after stability testing are shown in Table 15. Figures 88 to 103 exhibit the TEM photomicrographs and particle size distribution of microemulsion after stability testing. The mean particle size and the percentage of drug in microemulsion preparations before and after stability testing are shown in Figures 104 to 106. All preparations were observed under accelerated conditions at 4°C for 48 hours and 45° C for 48 hours for 6 cycles.

Tween 80:glycerin system at the ratio of 1:1.5

The physical appearance of all formulations in this system was separated into two phases after stability testing under accelerated conditions. The upper phase was

yellow solution while the lower phase was clear, colorless solution. During stability testing by storing at 4°C, all formulations were turbid. When storing at 45°C, phase separation could be seen during the first cycle. Although phase separation occurred in all formulations, they were able to recover to one-phase microemulsion by gently shaking. In addition, the pH and refractive index of systems after stability testing was slightly varied when compared before stability testing as shown in Table 15. The TEM photomicrographs of Formulations T1 G1.5 O4 D5 and T1 G1.5 O4 D10 in Figures 88 and 90 exhibit that the particles are spherical. Particle size distribution of these formulations as shown in Figures 89 and 91 was wide. The particle size of Formulation T1 G1.5 O4 D5 after stability testing was larger than before stability testing while the particle size of Formulation T1 G1.5 O4 D10 was slightly changed as shown in Figure 104.

The chemical stability of preparations was studied by examining the content of diazepam after storage at accelerated condition. The results from the study are shown in Figure 105. The content of diazepam in Formulation T1 G1.5 O8 D10 was decreased to 95.12%. This formulation showed the highest decrease in this system. The content of drug in Formulations T1 G1.5 O8 D5, T1 G1.5 O6 D10, and T1 G1.5 O4 D5 was also decreased but they were remained more than 98%. While content of diazepam in Formulation T1 G1.5 O6 D5 and T1 G1.5 O4 D10 increased. However, all formulations have the content of diazepam in the range 90% - 110% of labeled amount within the acceptance in the monograph of diazepam injection (USP24/NF19, 2000).

Tween 80:glycerin system at the ratio of 1:1

After stability testing under accelerated conditions, phase separation was occurred in all formulations in this system as illustrated in Table 15. Similar results as tween 80:glycerin system at the ratio of 1:1.5, the upper phase was yellow solution while the lower phase was clear, colorless solution. Furthermore, all formulations were turbid when storing at 4°C. While storing at 45°C, phase separation could be seen during the first cycle. However, the preparations were able to recover the one-phase microemulsion by gently shaking. Refractive index and pH of systems after

stability testing were slightly changed as shown in Table 15. Figures 92 to 95 exhibit the TEM photomicrographs and particle size distribution of Formulations T1 G1 O4 D5 and T1 G1 O4 D10. Spherical particles were obtained from both formulations. Formulation T1 G1 O4 D5 had wide particle size distribution while Formulation T1 G1 O4 D10 had narrow particle size distribution. Furthermore, the particle size of these formulations was increased after stability testing as revealed in Figure 104.

Figure 105 shows the content of diazepam after storage at accelerated condition. The content of drug in Formulations T1 G1 O8 D5, T1 G1 O6 D5, T1 G1 O6 D10, and T1 G1 O4 D10 was decreased when compared to the content before stability testing. But they remained more than 96% except the content of Formulation T1 G1 O4 D10 which was decreased to 94.38%. While content of drug in Formulations T1 G1 O8 D10 and T1 G1 O4 D5 increased. However, the content of drug in microemulsions was in the range 90% - 110% of labeled amount that accepted in pharmacopoeia (USP24/NF19, 2000).

Tween 80:polyethylene glycol 400 system at the ratio of 1:0.7

All formulations in this system remained clear, transparent, and one-phase yellow solution at any time of observation. Refractive index and pH of all formulations was slightly changed after stability testing. The TEM photomicrographs of Formulations T1 P0.7 O4 D5 and T1 P0.7 O4 D10 in Figures 96 and 98 illustrated spherical particle. And narrow particle size distribution was obtained in both formulations as exhibited in Figures 97 and 99. The particle size was increased after stability testing as revealed in Figure 104.

The chemical stability of preparations was shown in Figure 106. Comparison between before and after stability testing, the content of diazepam in Formulations T1 P0.7 O8 D5, T1 P0.7 O6 D5, and T1 P0.7 O4 D5 was slightly decreased. They were in the range 99.43 to 101.39%. While the content of drug in Formulations T1 P0.7 O8 D10, T1 P0.7 O6 D10, and T1 P0.7 O4 D10 was increased but they were in the range 99.59 to 102.54%. These results indicated the content of diazepam in all formulations was in the range that pharmacopoeia acceptable.

Tween 80:polyethylene glycol 400 system at the ratio of 1:0.5

Similar to tween 80:polyethylene glycol 400 system at the ratio of 1:0.7, clear, transparent, and one-phase yellow solution was obtained in all formulations at any time of observation. In addition, refractive index and pH of all formulations was slightly changed. The shape of particle of Formulations T1 P0.5 O4 D5 and T1 P0.5 O4 D10 was spherical as illustrated in Figures 100 and 102. Particle size distribution of Formulations T1 P0.5 O4 D5 was narrow while Formulation T1 P0.5 O4 D10 had wide particle size distribution as revealed in Figures 101 and 103. The shape of particle of Formulations T1 P0.5 O4 D5 and T1 P0.5 O4 D10 was spherical as shown in Figures 100 and 102. Particle size distribution of both formulations was wide as revealed in Figures 101 and 103. And the mean particle diameter of both formulations was increased after stability testing.

The content of diazepam after stability testing was shown in Figure 106. The content of diazepam in Formulation T1 P0.5 O8 D5 was 95.26%. It was decreased but this content was close to the content before stability testing. The content of drug in Formulation T1 P0.5 O6 D5 was also decreased but it was close to 100%. While the content in Formulations T1 P0.0.5 O8 D10, T1 P0.5 O6 D10, T1 P0.5 O4 D5, and T1 P0.5 O4 D10 were increased but they were in the range 101.43 to 103.48%. Therefore, all formulations had the content of drug in the range 90% - 110% labeled amount.

From these studies, it could be concluded that after stability testing microemulsions showed good appearance both tween 80:glycerin systems and tween 80:polyethylene glycol 400 systems. Although phase separation was occurred in tween 80:glycerin systems but it recovered to one-phase by gently shaking. The pH and refractive index of formulations between before and after stability testing was no statistical significant difference ($p>0.05$, tested by 2-tailed paired-sample T test). However, particle size of microemulsions showed statistically significant increased after stability testing ($p>0.05$, tested by 2-tailed paired-sample T test). Furthermore, the content of diazepam remained in the range 90% - 110% that required in pharmacopoeia.

Table 15 The physical appearances, pH, and refractive index of microemulsion preparations after stability testing.

Formulation	Macroscopic observation	pH	Refractive index (mean ± SD)
T1 G1.5 O8 D5	**	7.07	1.4491 ± 0.0001
T1 G1.5 O8 D10	**	7.14	1.4481 ± 0.0005
T1 G1.5 O6 D5	**	6.78	1.4394 ± 0.0004
T1 G1.5 O6 D10	**	7.10	1.4394 ± 0.0003
T1 G1.5 O4 D5	**	7.00	1.4481 ± 0.0003
T1 G1.5 O4 D10	**	7.17	1.4472 ± 0.0003
T1 G1 O8 D5	**	7.11	1.4494 ± 0.0003
T1 G1 O8 D10	**	7.10	1.4492 ± 0.0002
T1 G1 O6 D5	**	6.81	1.4395 ± 0.0002
T1 G1 O6 D10	**	7.02	1.4397 ± 0.0003
T1 G1 O4 D5	**	6.88	1.4488 ± 0.0002
T1 G1 O4 D10	**	6.68	1.4490 ± 0.0002
T1 P0.7 O8 D5	*	6.58	1.4481 ± 0.0004
T1 P0.7 O8 D10	*	6.76	1.4486 ± 0.0003
T1 P0.7 O6 D5	*	6.77	1.4395 ± 0.0004
T1 P0.7 O6 D10	*	6.64	1.4386 ± 0.0003
T1 P0.7 O4 D5	*	6.86	1.4385 ± 0.0003
T1 P0.7 O4 D10	*	6.94	1.4395 ± 0.0005
T1 P0.5 O8 D5	*	6.90	1.4490 ± 0.0004
T1 P0.5 O8 D10	*	6.52	1.4490 ± 0.0002
T1 P0.5 O6 D5	*	6.68	1.4380 ± 0.0002
T1 P0.5 O6 D10	*	6.76	1.4380 ± 0.0003
T1 P0.5 O4 D5	*	6.67	1.4379 ± 0.0002
T1 P0.5 O4 D10	*	6.99	1.4382 ± 0.0002

* = clear, transparent, one phase yellow solution

** = two phase separation (upper phase-yellow solution, lower phase-clear, colorless solution) but recovered to one-phase by gently shaking.

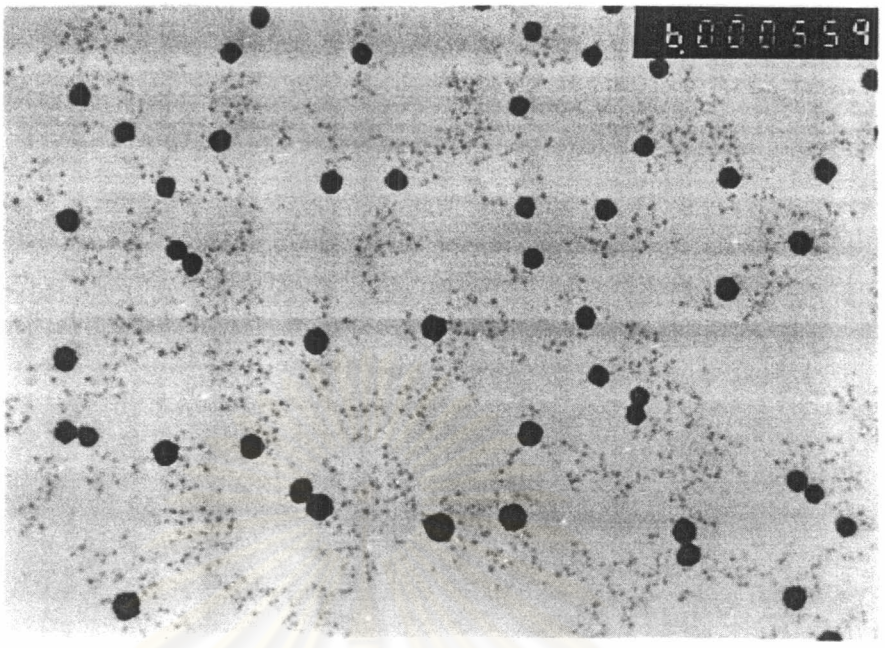

Figure 88 The TEM photomicrograph of the Formulation T1 G1.5 O4 D5 after stability testing (1 mm from picture equivalent to 33.33 nm).

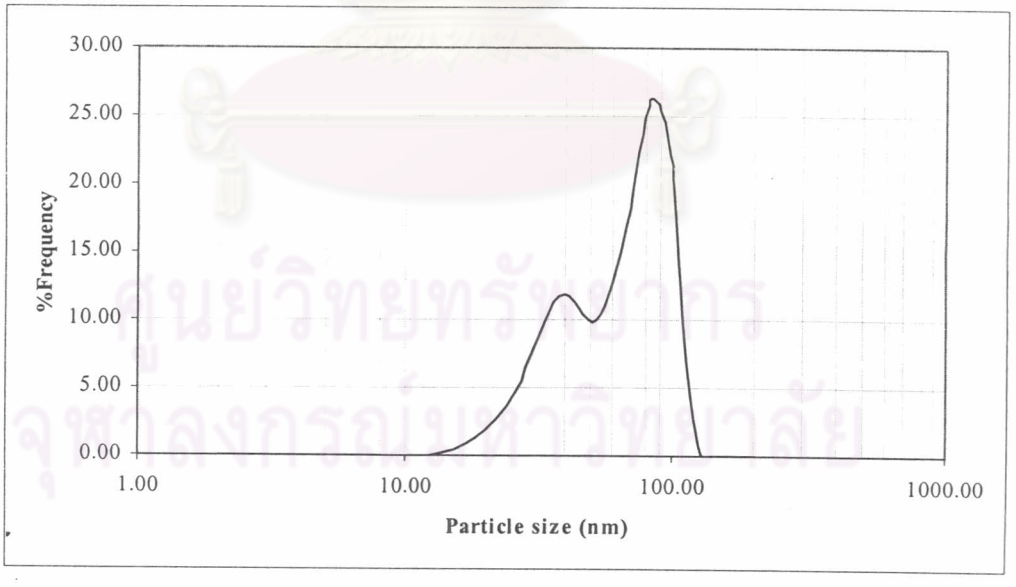

Figure 89 Particle size distribution of Formulation T1 G1.5 O4 D5 after stability testing.

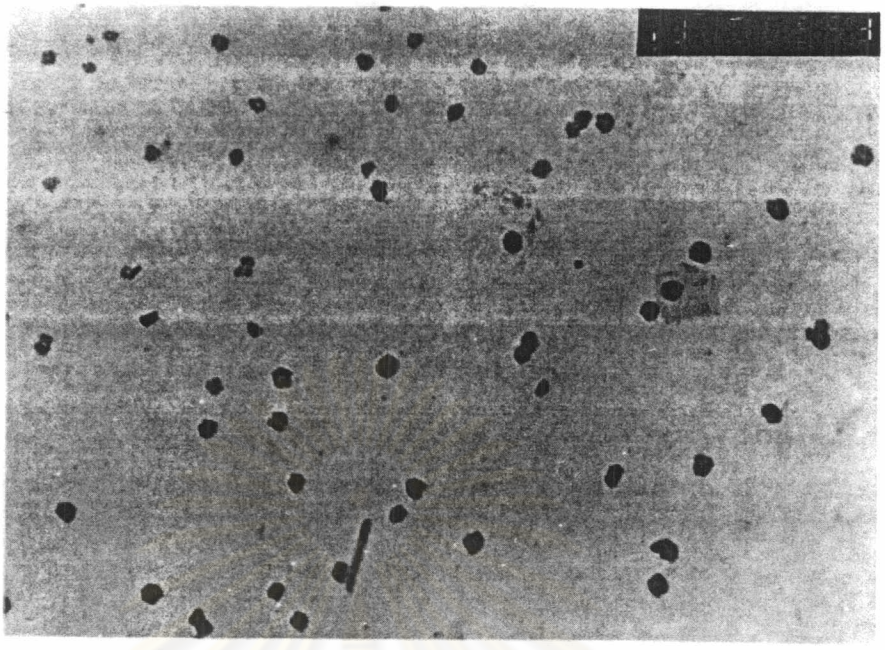

Figure 90 The TEM photomicrograph of the Formulation T1 G1.5 O4 D10 after stability testing (1 mm from picture equivalent to 58.82 nm).

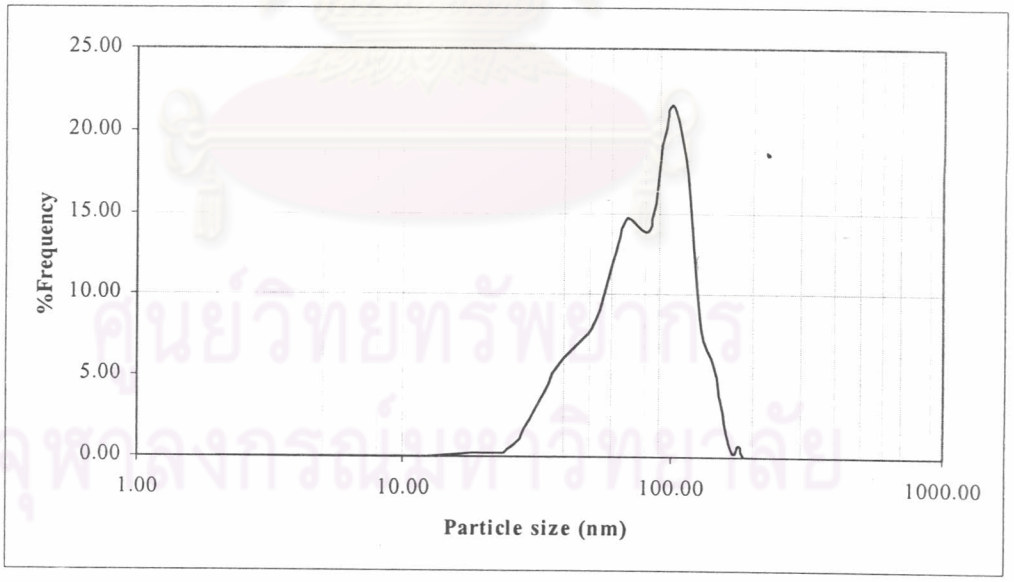

Figure 91 Particle size distribution of Formulation T1 G1.5 O4 D10 after stability testing.

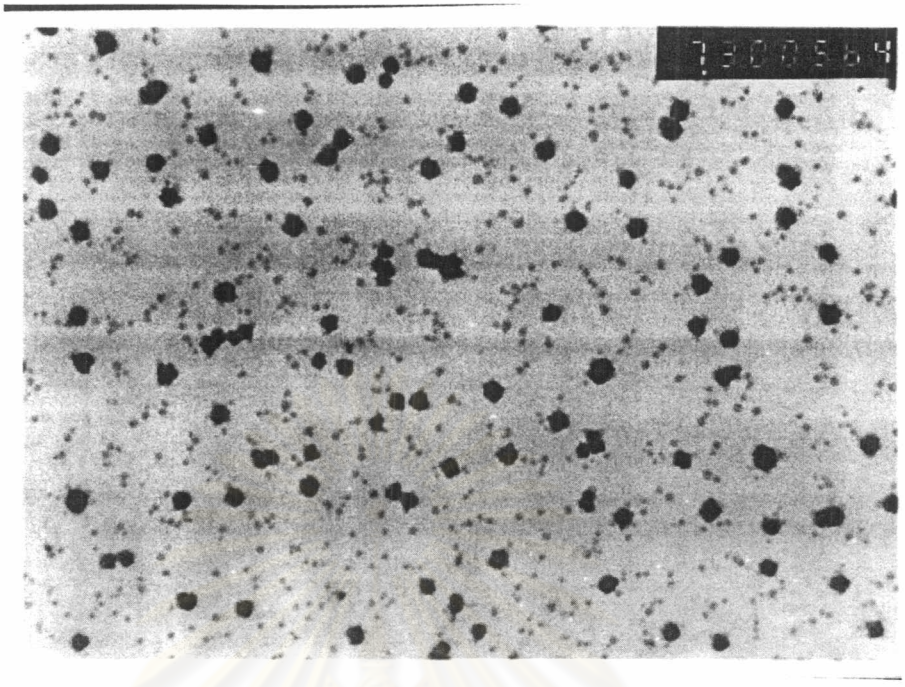

Figure 92 The TEM photomicrograph of the Formulation T1 G1 O4 D5 after stability testing (1 mm from picture equivalent to 33.33 nm).

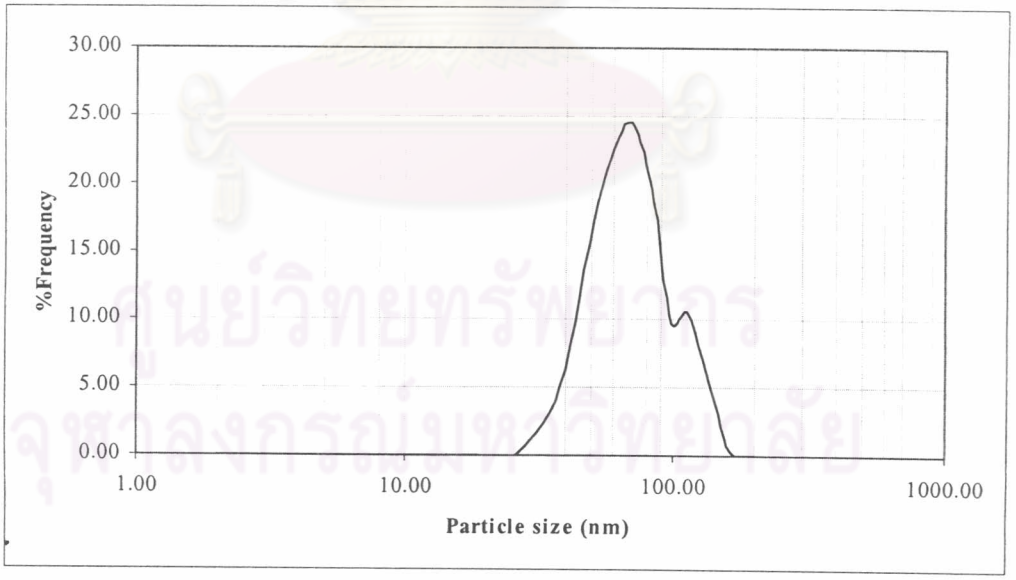

Figure 93 Particle size distribution of Formulation T1 G1 O4 D5 after stability testing.

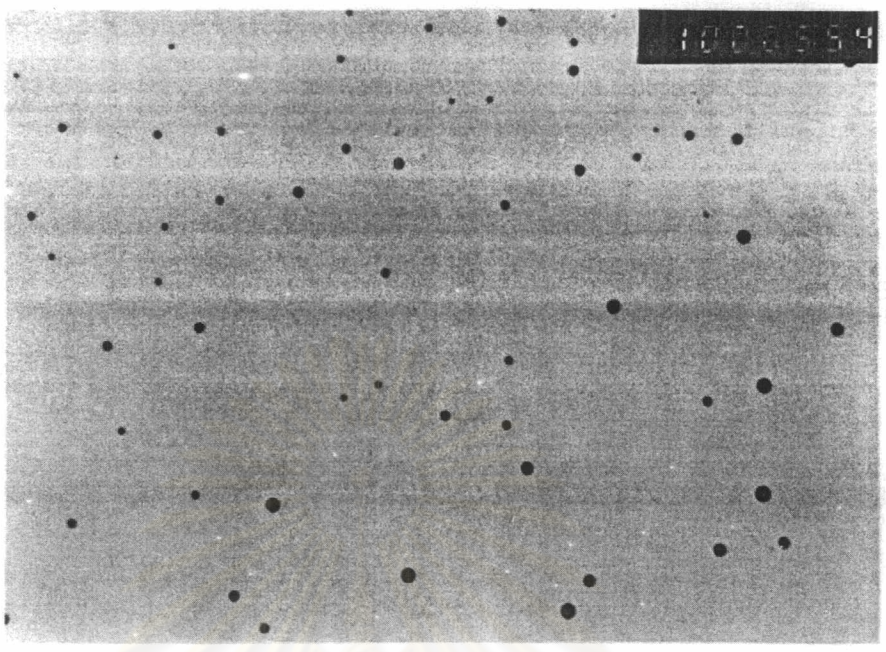

Figure 94 The TEM photomicrograph of the Formulation T1 G1 O4 D10 after stability testing (1 mm from picture equivalent to 33.33 nm).

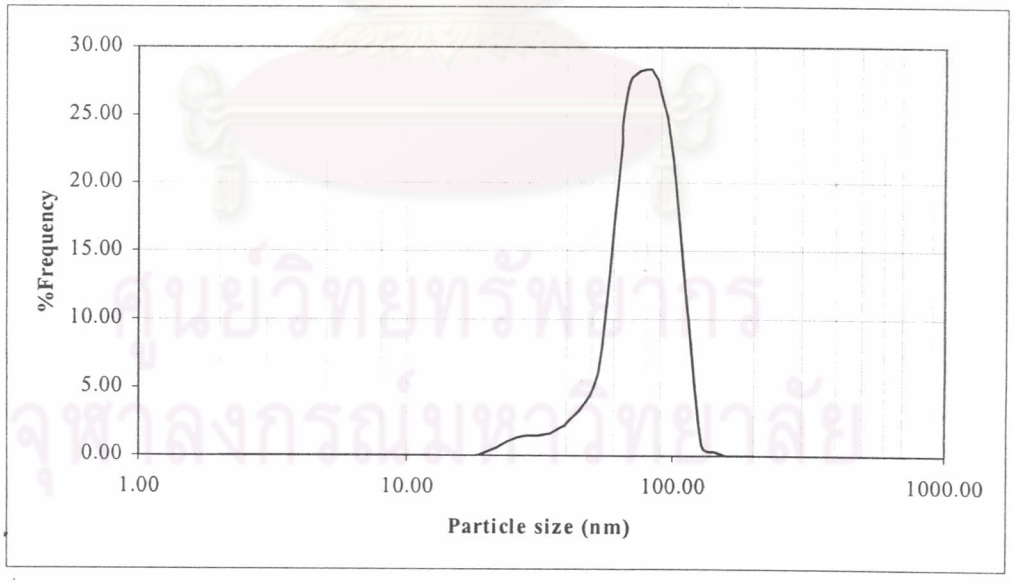

Figure 95 Particle size distribution of Formulation T1 G1 O4 D10 after stability testing.

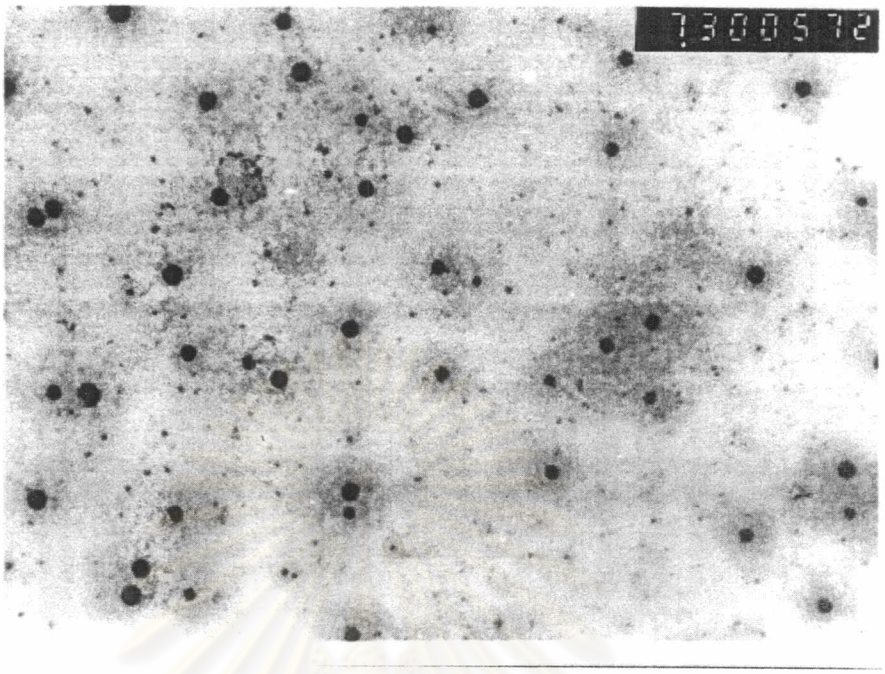

Figure 96 The TEM photomicrograph of the Formulation T1 P0.7 O4 D5 after stability testing (1 mm from picture equivalent to 33.33 nm).

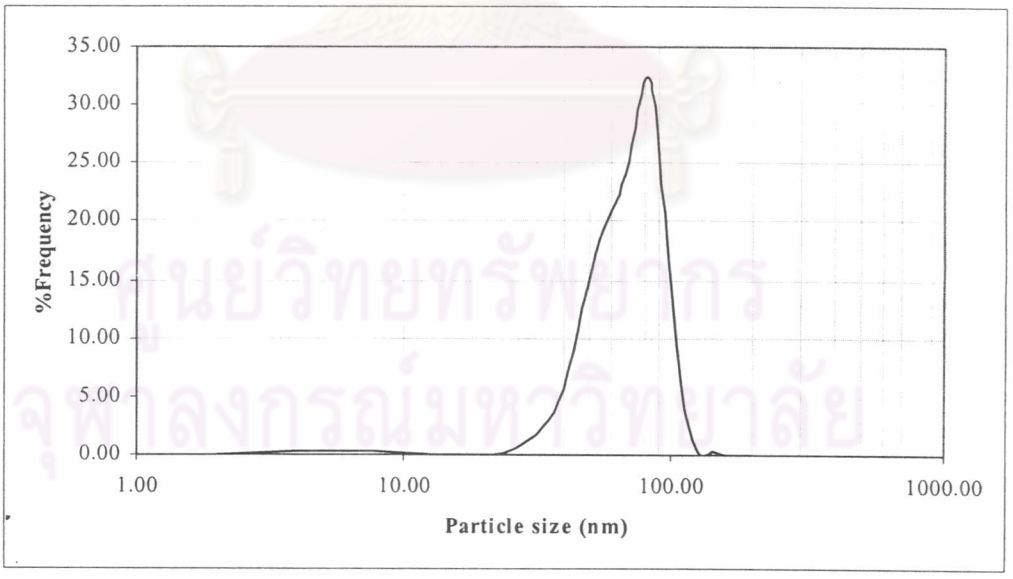

Figure 97 Particle size distribution of Formulation T1 P0.7 O4 D5 after stability testing.

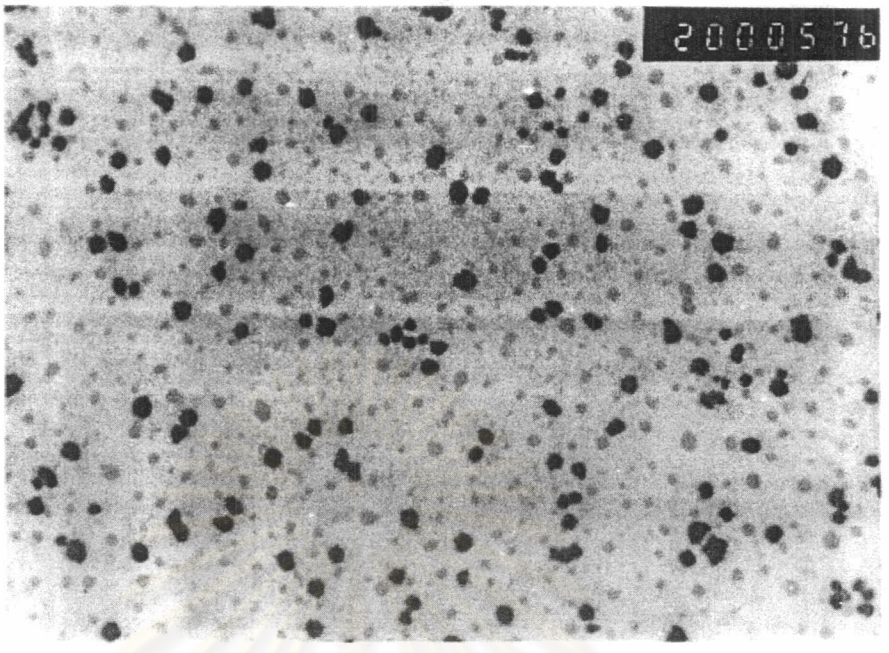

Figure 98 The TEM photomicrograph of the Formulation T1 P0.7 O4 D10 after stability testing (1 mm from picture equivalent to 33.33 nm).

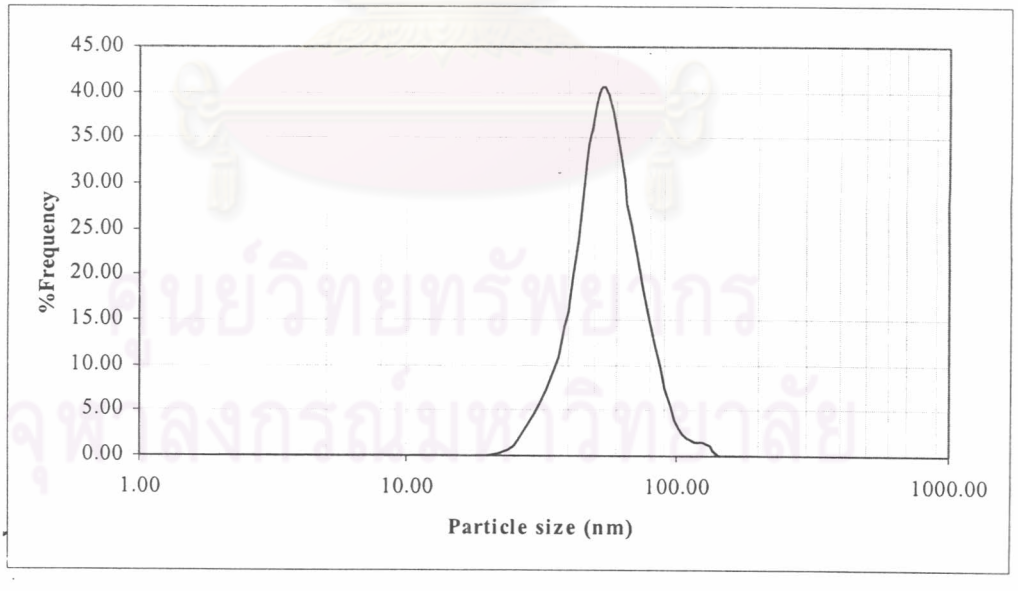

Figure 99 Particle size distribution of Formulation T1 P0.7 O4 D10 after stability testing.

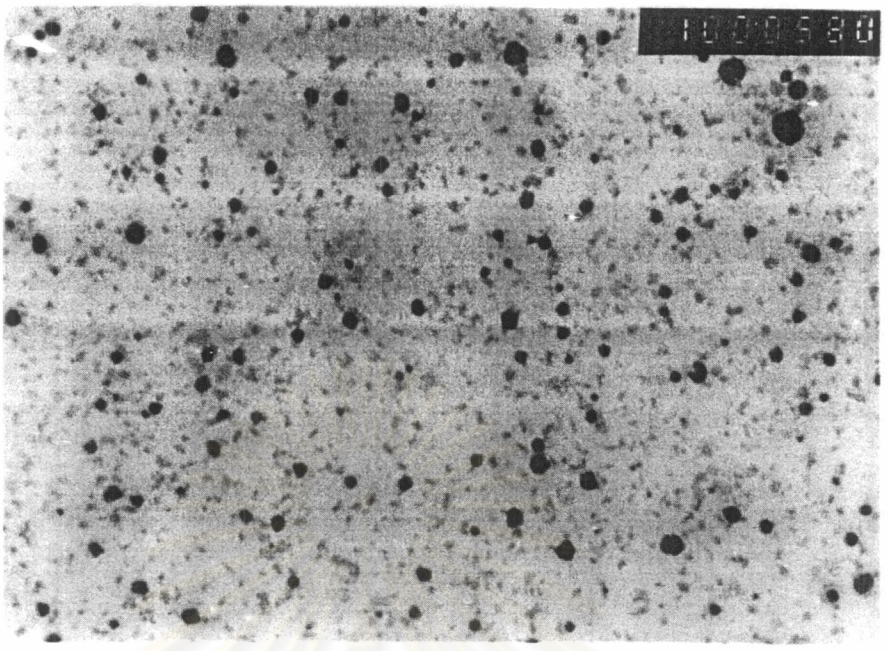

Figure 100 The TEM photomicrograph of the Formulation T1 P0.5 O4 D5 after stability testing (1 mm from picture equivalent to 33.33 nm).

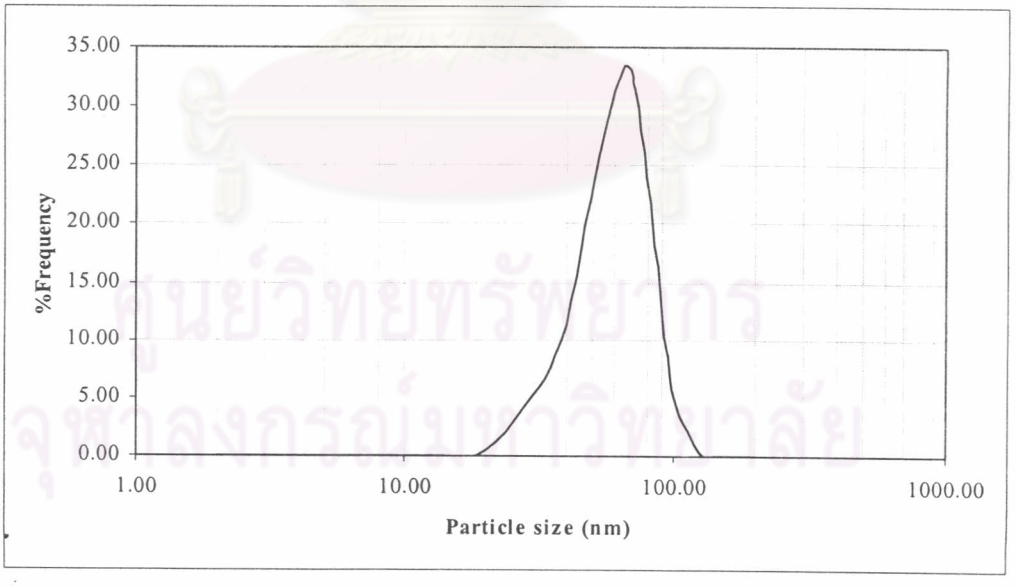

Figure 101 Particle size distribution of Formulation T1 P0.5 O4 D5 after stability testing.

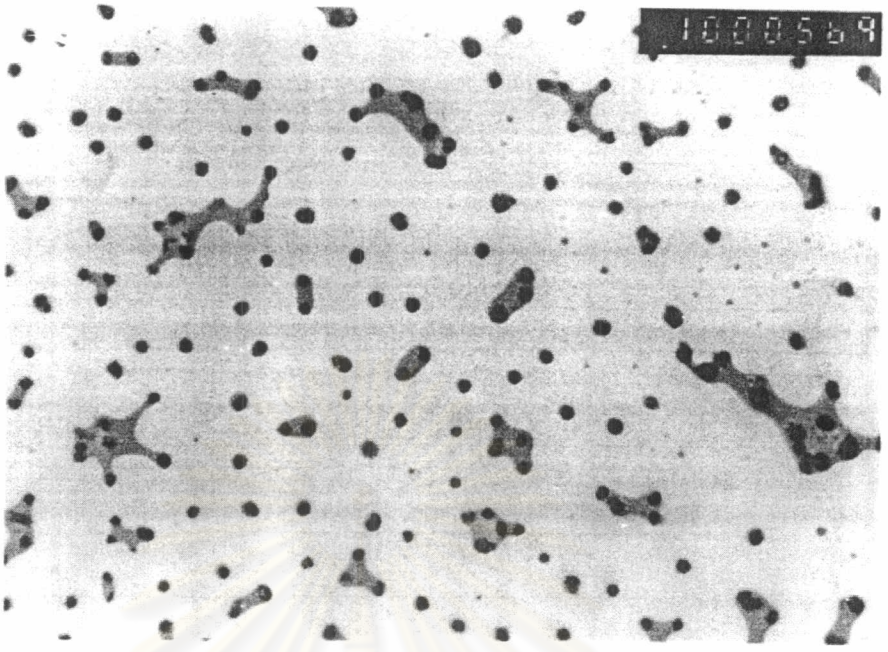

Figure 102 The TEM photomicrograph of the Formulation T1 P0.5 O4 D10 after stability testing (1 mm from picture equivalent to 33.33 nm).

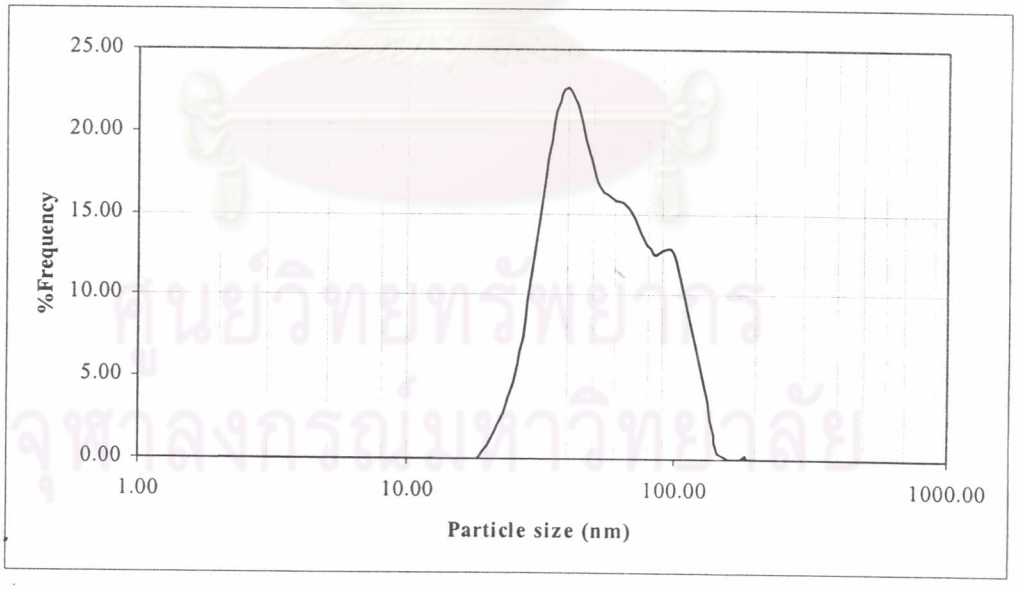

Figure 103 Particle size distribution of Formulation T1 P0.5 O4 D10 after stability testing.

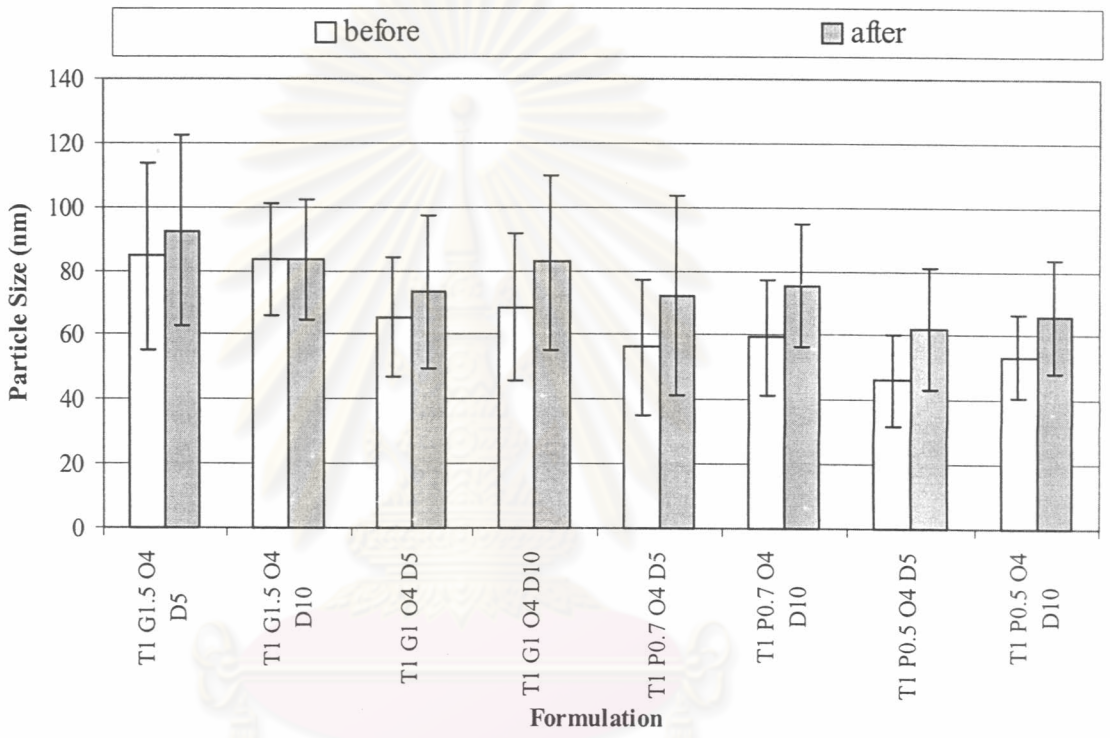

Figure 104 Comparison of the mean particle size of microemulsions between before and after stability testing.

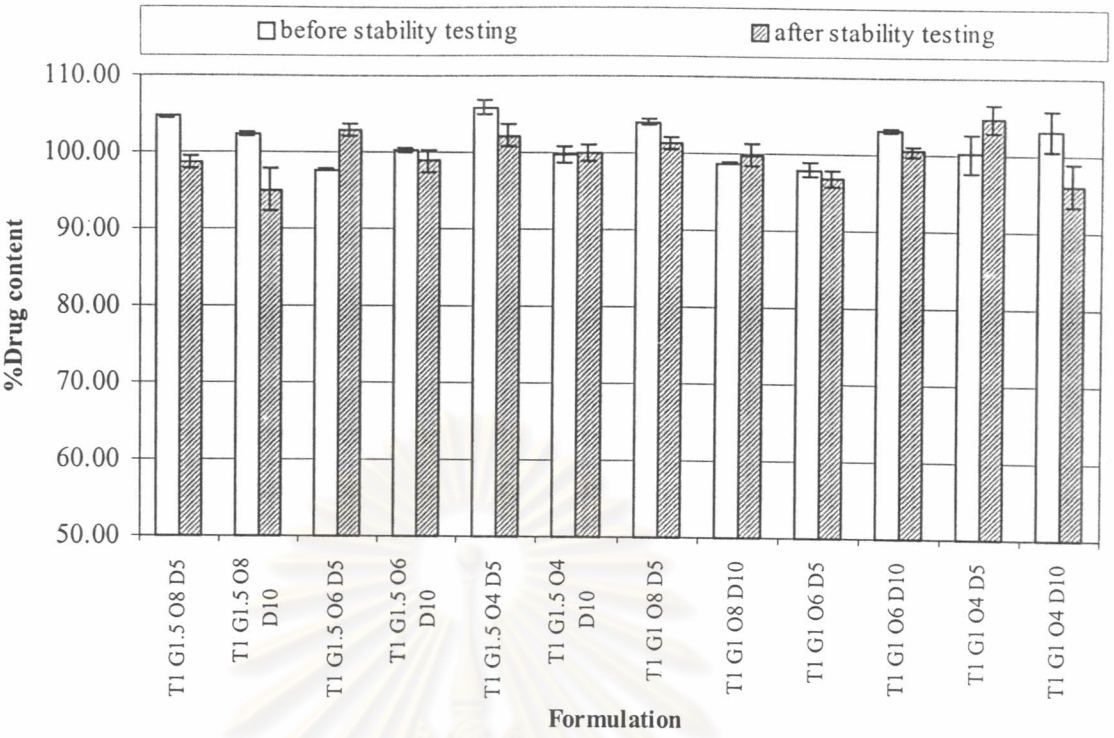

Figure 105 The content of diazepam in microemulsions in tween80:glycerin systems before and after stability testing.

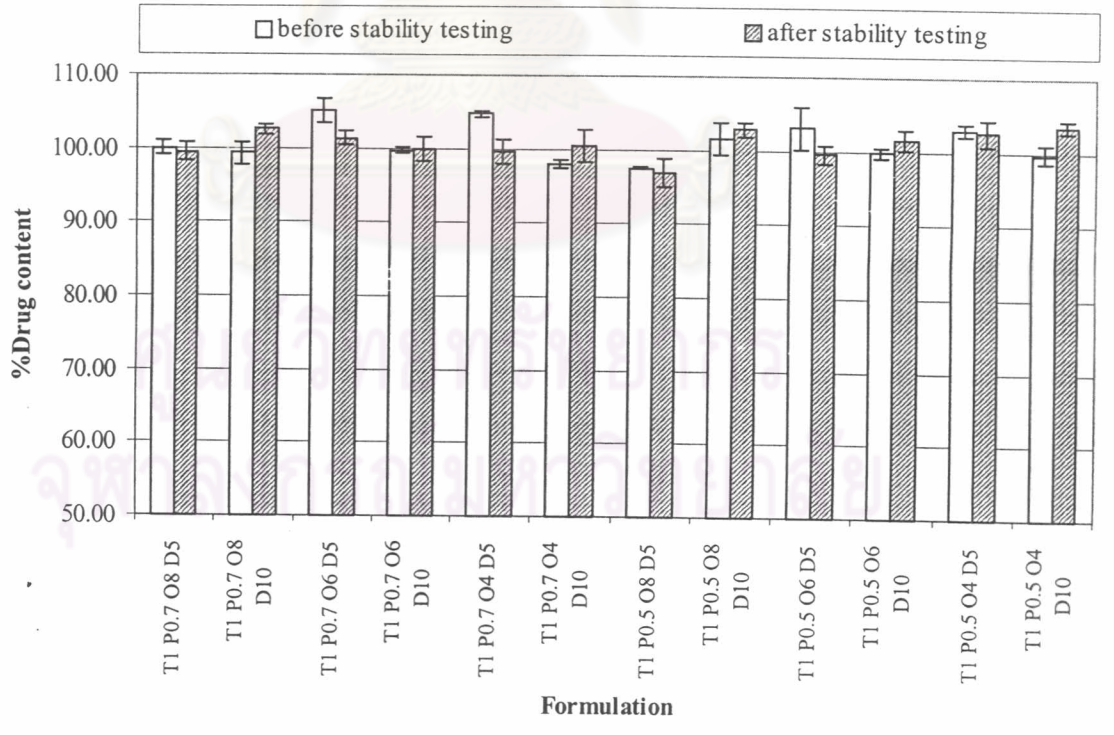

Figure 106 The content of diazepam in microemulsions in tween 80:polyethylene glycol 400 systems before and after stability testing.