CHAPTER IV
RESULTS

1.Evaluation of homogenization process

Fat emulsion containing 5% soybean oil and 2% poloxamer 407 was prepared as a model to evaluate the efficiency of a homogenizer. In the primary step, pre-emulsion was prepared by a high speed homogenizer. The stirring speed was fixed at 4,080 rpm while the stirring time was varied in 4 levels: 5, 10, 20 and 30 minutes. It was found that the stirring time of 5 minutes was not long enough to obtain good pre-emulsion. The oil droplets were found on the surface of emulsion. When the stirring time of 10 minutes or more was used, oil droplets on the surface disappeared. Thus, stirring time of 10 minutes was selected for preparing pre-emulsion.

The size of pre-emulsion was reduced by a high pressure homogenizer. Pre-emulsion was homogenized at pressure between 4000 and 12000 psi and between 1 and a maximum of 10 homogenization cycles. The transmittances of emulsion are illustrated in Figure 8 and appendix D. It was found that the transmittance increased with increasing pressure and cycles of homogenization. However, at homogenization pressure of 12000 psi a slight decrease of transmittance at cycle 10 was found.

When the homogenization pressure was increased from 4000 to 6000, 8000, and 10000 psi, respectively, significant differences between the transmittance of different pressure in each cycle of homogenization were evident (α=0.05). No significant differences were found when the pressure was increased from 10000 to 12000 psi and the cycle of homogenization of more than 1 cycle was used.

Considering the cycles of homogenization, it was shown that there were no statistically differences in transmittance between 5 and 6 cycles at the pressures of 4000, 6000 and 12000 psi. At homogenization pressure of 8000 psi there was no significant difference between 6 and 7 cycles, while at homogenization pressure of 10000 psi there was no significant difference between 4 and 5 cycles. Moreover, higher cycles of homogenization were also no significant differences in transmittance.

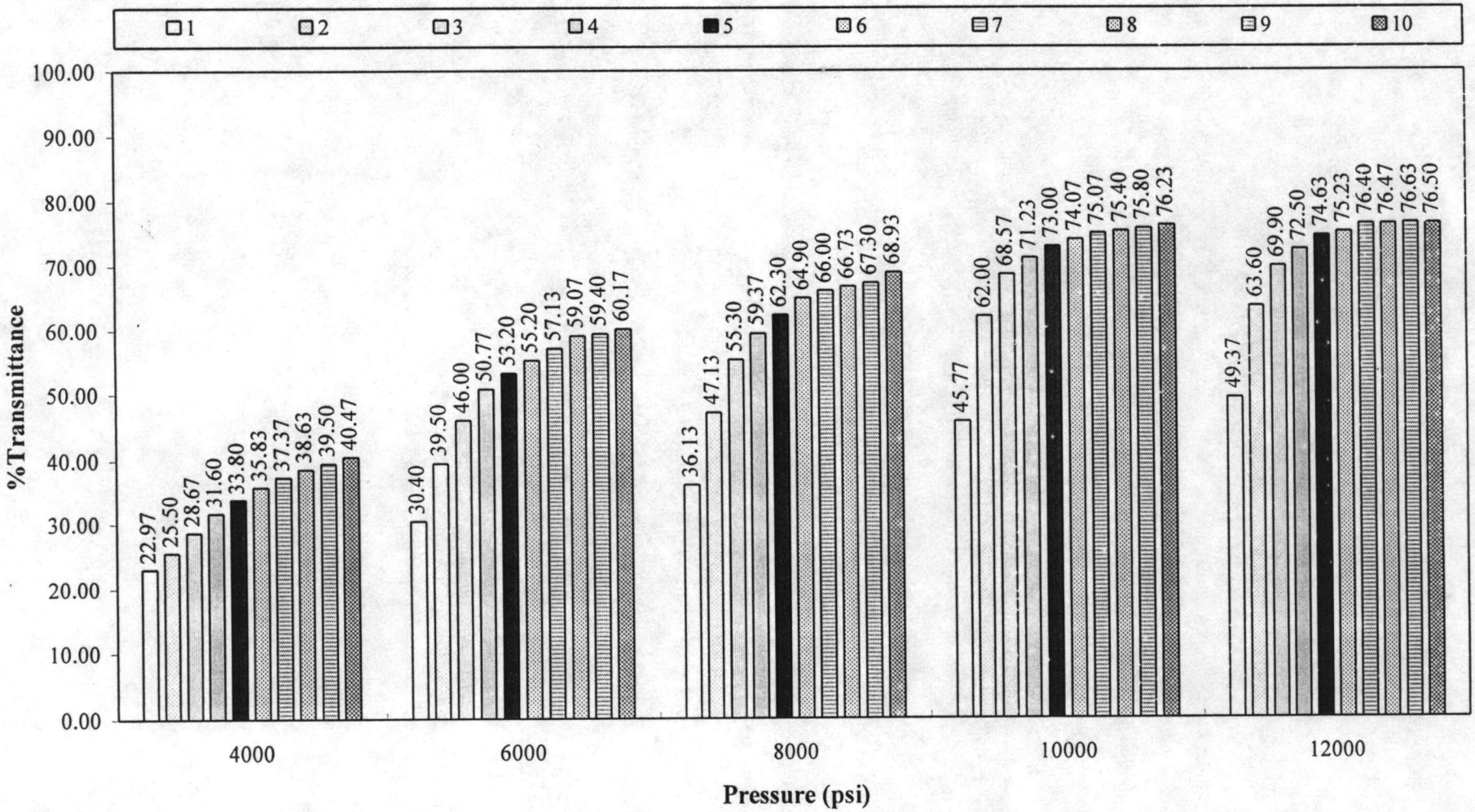

Figure 8. Percentage of transmittance of soybean oil in water emulsion as a function of pressure (4,000–12,000 psi) and cycle of homogenization (1-10 cycles) prepared by Emulsiflex® C5

From the data obtained it was indicated that the homogenization pressure of 10000 psi and approximately 5 cycles were the optimum condition for preparing fat emulsion. Thus, this condition was used to prepare SLN throughout this study.

2.Preparation and characterization of drug free SLN

2.1 Effect of stabilizers

2.1.1 Physical appearance

The physical appearances of the dispersions of SLN both before and after sterilization by autoclaving are listed in Table 3. All preparations were prepared by hot melt homogenization method. When 1% tween 80 was used as a stabilizer, there were large particles of cooled lipid and occlusion at the orifice occurred during cycle 1 and 2 of homogenization.

Poloxamer 407 of 1-5% could stabilize dispersions of SLN both before and after autoclaving. White fluid dispersions were observed. Tween 80 of 1-5% could also produce SLN in good physical appearance. Higher concentration to 3-5 % produced a smaller particle and bluish white fluid dispersions were observed before autoclaving. However, after autoclaving all preparations precipitated after storage for 1 month at room temperature but could be easily redispersed, except when 3% tween 80 was used. Gel forming was found upon cooling.

SLN containing egg lecithin of 1-5% could also be prepared. Brown fluid dispersions were observed due to the color of egg lecithin. These preparations could be sterilized by autoclaving. No separation of oily and aqueous phase was observed. However, increasing the concentration of egg lecithin to 3-5% gel like dispersions were obtained.

Table 3. The physical appearances of the dispersions of SLN containing various types and amounts of stabilizer.

Formulation	Macroscopic observation	
	Before autoclaving	After autoclaving
5TP+1P407	White fluid dispersion	White fluid dispersion; stable on storage
5TP+2P407	White fluid dispersion	White fluid dispersion; stable on storage
5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
5TP+4P407	White fluid dispersion	White fluid dispersion; stable on storage
5TP+5P407	White fluid dispersion	White fluid dispersion; stable on storage
5TP+1T80	White fluid dispersion	White fluid dispersion; sediment after storage
5TP+2T80	White fluid dispersion	White fluid dispersion; sediment after storage
5TP+3T80	Bluish white fluid dispersion	Gel formation after cooling
5TP+4T80	Bluish white fluid dispersion	White fluid dispersion; sediment after storage
5TP+5T80	Bluish white fluid dispersion	White fluid dispersion; sediment after storage
5TP+1EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage
5TP+2EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage
5TP+3EL	Brown fluid dispersion	Gel formation after cooling
5TP+4EL	Brown fluid dispersion	Gel formation after cooling
5TP+5EL	Brown fluid dispersion	Gel formation after cooling

2.1.2 Particle size

The particle sizes of SLN determined by laser particle sizer (Master sizer®) are listed in Table 4. The d(v,0.5) is used for comparison the mean size in each preparation.

For preparations containing poloxamer 407 of 1-5%, particles in the size range of nanometer were obtained. D(v,0.5) was below 1 μm both before and after autoclaving (Figure 9). There was no significant difference of d(v,0.5) between before and after autoclaving tested by paired-samples T test, 2 tailed ($p=0.180$; $\alpha=0.05$). The optimum concentration of 3% could produce the smallest particles. The percentage of particles larger than 1μm was the least. It also remained in the nanometer size when storage for 6 months at room temperature. After 12

months, its d(v,0.5) still existed in the nanometer size, but higher amount of particle larger than 1μm was observed.

Tween 80 of 1-5% produced the d(v,0.5) below 1 μm before autoclaving. However, the growth of particles occurred after autoclaving. Their d(v,0.5) were more than 1 μm (Figure 10). All preparations showed high amount of particle of larger than 1 μm after autoclaving (82.32-94.23%). After storage for 6 months at room temperature, the d(v,0.5) of preparations containing 1-2% tween 80 was decreased to nanometer size again, but slightly higher than their original size.

Increasing the concentration of egg lecithin from 1% to 5% could decrease their particle size. Only preparations containing 4-5% egg lecithin could obtain the d(v,0.5) lower than 1 μm. After autoclaving preparations of 1-3% egg lecithin showed decrease in particle size but slight increase in preparations of 4-5% (Figure 11). All preparations showed the high amount of particle larger than 1 μm after autoclaving. After storage for 6 months at room temperature, the preparations of 1-2% egg lecithin maintained the size higher than 1 μm.

2.1.3 pH

As shown in Figure 12, the type and amount of stabilizers effected the pH of preparations. All preparations were weakly acidic. Poloxamer 407 produced the highest pH value while egg lecithin gave the lowest pH value. Increasing the amount of poloxamer 407 could slightly increase the pH, while increasing the amount of tween 80 and egg lecithin could slightly decrease it.

Table 4. Particle sizes of SLN containing various types and amounts of stabilizer before and after autoclaving, and after storage for 6 and 12 months at room temperature.

Formulation	Volume particle size (μm)							%Particle larger than		
	d(v,0.1)	d(v,0.5)	d(v,0.9)	d(4,3)	d(3,2)	span	uniformity	1μm	5μm	10μm
5TP+1P407(a)	0.29	0.64	8.89	4.07	0.59	13.34	5.69	34.17	18.51	8.74
5TP+1P407(b)	0.26	0.57	2.66	1.25	0.50	4.23	1.55	23.47	4.59	0.68
5TP+2P407(a)	0.26	0.46	0.95	1.34	0.43	1.48	2.17	8.83	2.46	1.11
5TP+2P407(b)	0.25	0.45	0.92	0.62	0.42	1.49	0.65	8.12	1.14	0.00
5TP+3P407(a)	0.23	0.39	0.75	0.49	0.36	1.25	0.53	3.52	0.59	0.01
5TP+3P407(b)	0.23	0.40	0.77	0.49	0.37	1.34	0.49	4.36	0.28	0.00
5TP+3P407(c)	0.13	0.30	0.71	1.30	0.25	1.96	3.74	6.22	2.72	2.13
5TP+3P407(d)	0.13	0.30	49.14	10.21	0.26	163.85	33.5	20.15	19.03	18.19
5TP+4P407(a)	0.22	0.42	1.08	0.62	0.39	2.05	0.80	11.39	1.07	0.02
5TP+4P407(b)	0.22	0.39	0.96	0.64	0.36	1.94	0.95	9.65	1.50	0.03
5TP+5P407(a)	0.22	0.38	0.88	0.59	0.36	1.74	0.83	8.51	0.99	0.00
5TP+5P407(b)	0.24	0.37	0.60	0.52	0.36	0.97	0.61	4.30	0.74	0.00
5TP+1T80 (a)	0.26	0.40	0.68	2.50	0.39	1.05	5.51	6.71	5.01	3.95
5TP+1T80 (b)	0.48	15.63	39.84	19.14	2.10	2.52	0.80	84.27	79.84	66.42
5TP+1T80 (c)	0.16	0.41	1.16	0.60	0.31	2.47	0.88	13.66	0.65	0.16
5TP+2T80 (a)	0.24	0.38	2.07	0.75	0.38	4.86	1.23	13.82	1.77	0.00
5TP+2T80 (b)	2.64	13.31	34.24	18.05	5.01	2.37	0.84	94.23	83.94	63.12
5TP+2T80 (c)	0.19	0.59	4.18	2.06	0.41	6.74	2.95	32.78	8.52	3.58
5TP+3T80 (a)	0.19	0.33	0.61	0.46	0.31	1.25	0.66	4.84	0.23	0.00
5TP+3T80 (b)	0.44	2.73	6.61	3.23	1.27	2.26	0.68	82.32	20.81	1.54
5TP+4T80 (a)	0.14	0.28	0.57	0.42	0.24	1.56	0.82	4.49	0.34	0.00
5TP+4T80 (b)	1.28	6.03	15.78	7.96	2.46	2.40	0.85	94.01	87.48	26.04
5TP+5T80 (a)	0.17	0.31	0.66	0.52	0.29	1.55	0.93	6.78	0.62	0.00
5TP+5T80 (b)	1.23	4.30	11.30	6.15	1.92	2.34	0.93	93.20	43.72	13.50
5TP+1EL (a)	19.40	57.11	128.34	69.69	10.77	1.91	0.64	97.89	97.34	95.61
5TP+1EL (b)	17.87	50.59	108.41	57.84	12.43	1.79	0.55	98.34	97.95	95.95
5TP+1EL (c)	7.79	37.96	88.12	52.62	3.23	2.12	0.86	92.76	91.39	88.57
5TP+2EL (a)	11.44	43.94	93.77	49.08	4.49	1.87	0.57	94.59	93.57	90.90
5TP+2EL (b)	0.38	20.90	48.94	23.48	1.69	2.32	0.72	81.85	79.51	72.72
5TP+2EL (c)	7.49	22.15	48.45	27.61	3.87	1.85	0.67	94.97	92.82	85.15
5TP+3EL (a)	0.39	22.35	52.23	24.98	1.81	2.32	0.70	83.34	80.76	74.49
5TP+3EL (b)	2.18	9.40	24.81	11.99	3.45	2.41	0.75	92.48	76.90	46.90
5TP+4EL (a)	0.24	0.37	0.63	0.54	0.36	1.05	0.66	4.46	0.90	0.13
5TP+4EL (b)	0.31	2.55	11.03	5.55	0.80	4.20	1.94	55.63	34.56	12.16
5TP+5EL (a)	0.21	0.34	0.58	0.68	0.33	1.08	1.23	5.19	2.90	1.10
5TP+5EL (b)	0.26	0.42	10.53	3.01	0.45	24.68	6.48	23.86	18.73	10.69

Note: (a):before autoclaving, (b):after autoclaving,

(c):after autoclaving and storage for 6 months at room temperature

(d):after autoclaving and storage for 12 months at room temperature

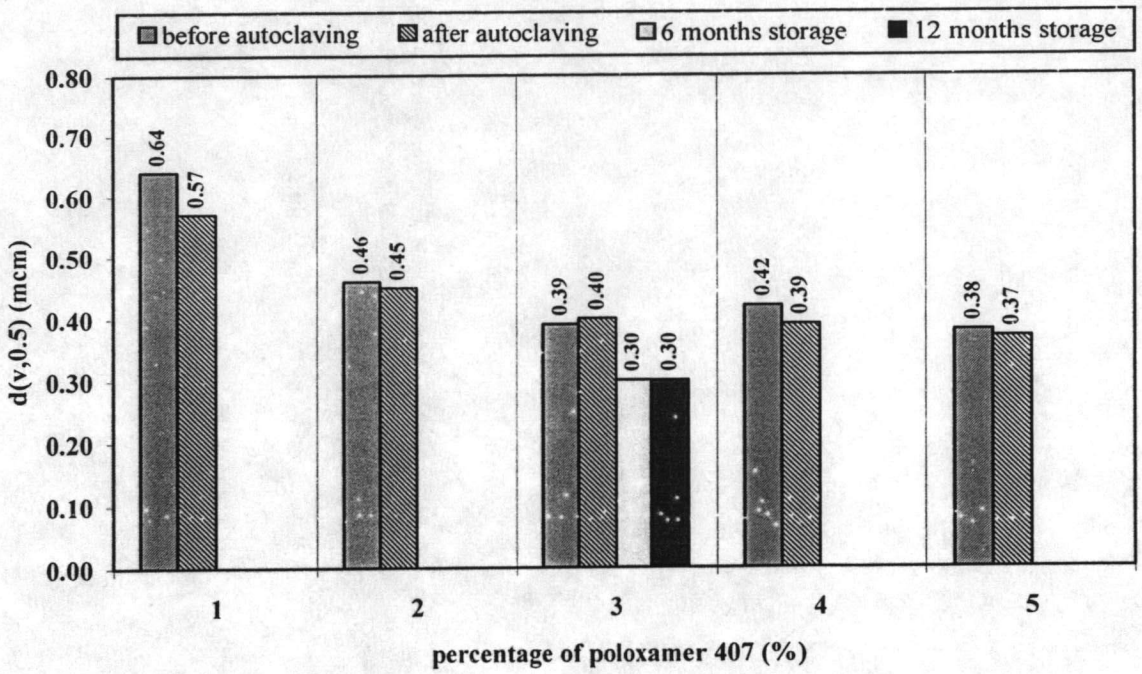

Figure 9. The d(v,0.5) of SLN containing 1-5% poloxamer 407.

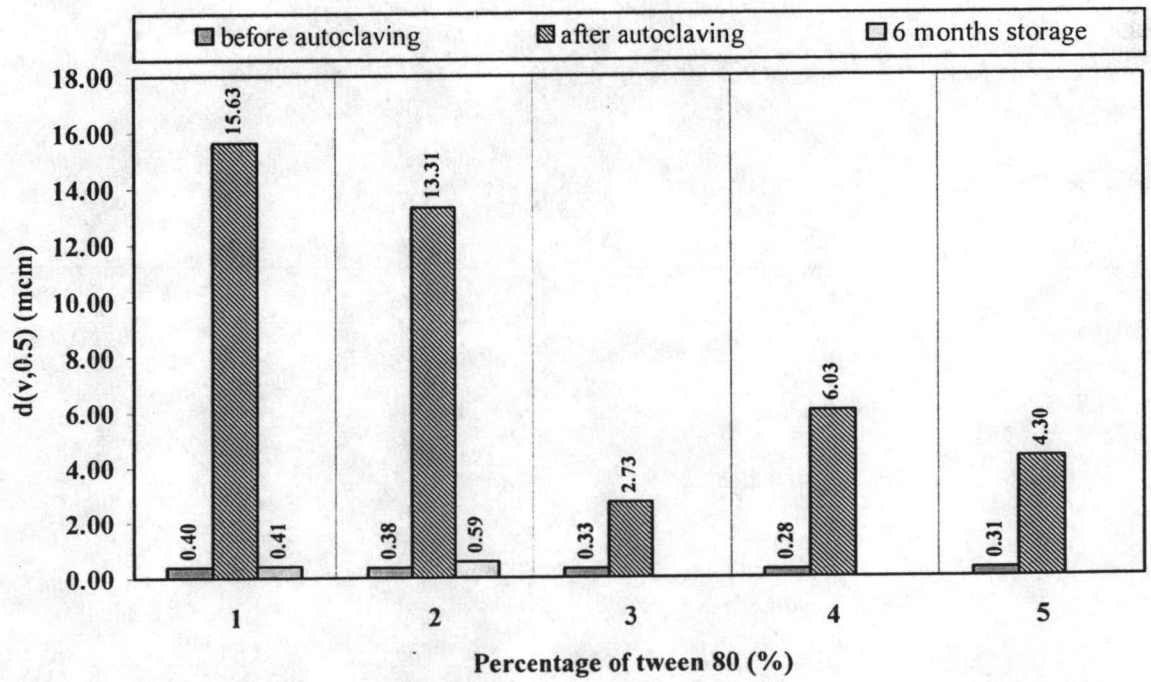

Figure 10. The d(v,0.5) of SLN containing 1-5% tween 80.

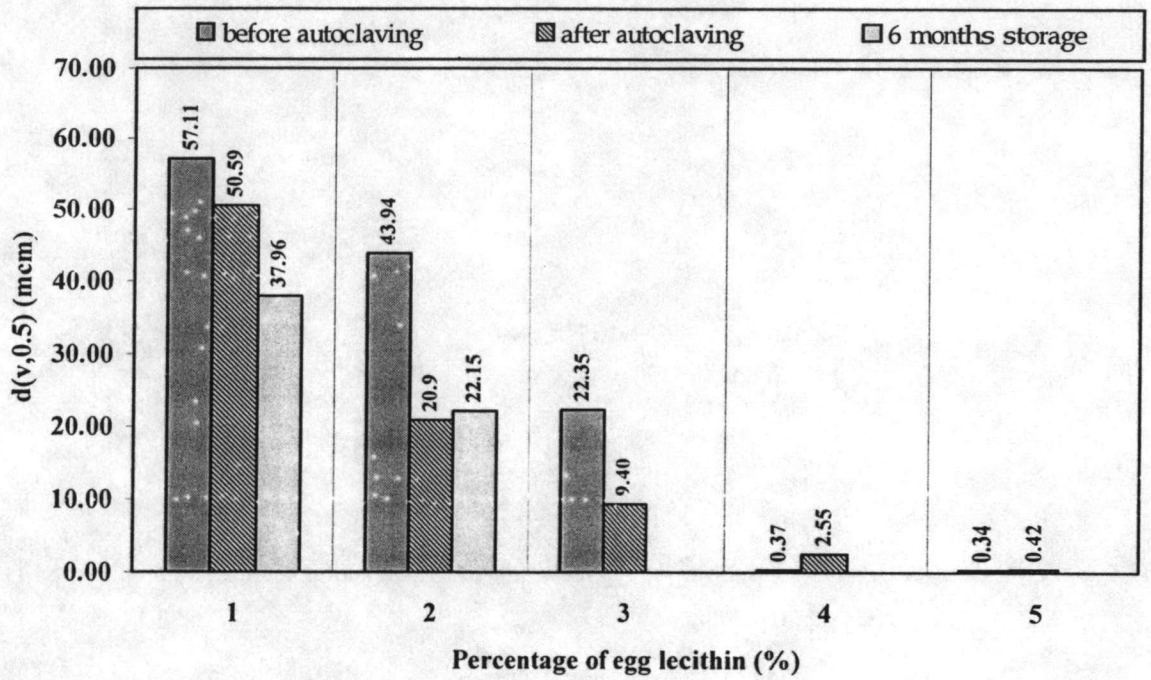

Figure 11. The d(v,0.5) of SLN containing 1-5% egg lecithin.

2.1.4 Osmolality

The osmolality was slightly effected by the composition of SLN. Egg lecithin exerted higher osmolality than the other two stabilizers. Increasing the percentage of stabilizers could slightly increase the osmolality (Figure 13). However, all data showed very low osmolality of these preparations compared with the isotonic solution of 0.9% sodium chloride which had the osmolality of 0.280 osmole/kg.

2.1.5 Zeta potential

All preparations showed the negative charge of zeta potential. For SLN containing poloxamer 407 and egg lecithin, the zeta potential value increased with the increasing amount of stabilizer in the preparations. Egg lecithin gave higher negatively zeta potential than poloxamer 407. While increasing the

amount of tween 80 could decrease the zeta potential (Figure 14). However, all preparations showed the zeta potential lower than –40 millivolts which was the optimum zeta potential for good stability as follows in Table f1 (Zeta meter system 3.0 manual).

2.1.6 Viscosity

The viscosities of preparations of SLN containing various types and amounts of stabilizer are shown in Figures 16-21. However, their viscosity curves showed two types of liquid rheology; Newtonian and non-Newtonian systems. Thus the viscosity at 1000 s^{-1} was selected for comparison between various preparations as shown in Figure 15. SLN containing poloxamer 407 showed the lowest viscosity. Their viscosities were slightly higher than water which had the viscosity of 1 mPa·s. Increasing its concentration slightly increased the viscosity. Preparations of 2-5% poloxamer 407 were Newtonian systems which shear stress directly and proportionally increased with shear rate. Only preparation containing 1% poloxamer 407 produced the thixotropy type as the down-curve was displaced to the left of the up-curve as shown in Figures 16-17.

Both 1-2% tween 80 and egg lecithin produced low viscosity. Higher amount of 3-5% produced higher viscosity. Preparation of 3% had the highest viscosity for both stabilizers. Preparations of both stabilizers were of difference patterns in each level. Only preparations containing either 1-2% tween 80 or 1% egg lecithin were Newtonian systems. Their viscosities were very low and constant in all shear rates. For preparations of 3-5% tween 80 and 2-5% egg lecithin, the rheology patterns were different. Negative thixotropies were shown. For preparations of 4-5% tween 80 and 2% egg lecithin, the up-curve was dilatant flow which slightly increased in higher shear rate, and the down-curve were plastic flows which the increasing of viscosity occurred when shear rate decrease. While preparations of 3% tween 80 and 3-5% egg lecithin both up- and down-curves were plastic flows. The viscosity decreased with higher shear rate and they had the yield point at rest. The down-curve had the viscosity lower than up-curve to be thixotropy systems (Figures 18-21).

Table 5. The pH, osmolality, zeta potential, and viscosity of dispersions of SLN containing various types and amounts of stabilizer.

Formulation	pH	Osmolality	Zeta potential (millivolts) (±SD)	Viscosity at the shear rate of $1000s^{-1}$ (mPa•s) (±SD)
5TP+1P407	5.65	0.007	-22.373 (± 2.514)	2.082 (± 0.475)
5TP+2P407	5.72	0.009	-23.167 (± 2.832)	2.089 (± 0.092)
5TP+3P407	5.96	0.012	-23.901 (± 2.607)	2.295 (± 0.096)
5TP+4P407	6.06	0.017	-24.880 (± 2.699)	2.562 (± 0.154)
5TP+5P407	6.14	0.024	-25.617 (± 3.035)	3.180 (± 0.069)
5TP+1T80	5.63	0.007	-36.006 (± 2.606)	1.532 (± 0.095)
5TP+2T80	5.23	0.009	-30.675 (± 2.577)	2.877 (± 0.073)
5TP+3T80	5.18	0.011	-25.361 (± 2.288)	22.862 (± 1.813)
5TP+4T80	5.12	0.013	-19.414 (± 2.255)	6.496 (± 0.305)
5TP+5T80	4.72	0.015	-15.377 (± 1.955)	7.284 (± 0.323)
5TP+1EL	4.34	0.015	-27.496 (± 1.625)	4.675 (± 0.356)
5TP+2EL	3.61	0.029	-30.353 (± 1.625)	9.275 (± 0.512)
5TP+3EL	3.40	0.040	-32.317 (± 1.709)	54.942 (± 1.730)
5TP+4EL	3.28	0.049	-34.851 (± 1.557)	42.174 (± 1.653)
5TP+5EL	3.18	0.075	-36.898 (± 1.745)	43.641 (± 1.614)

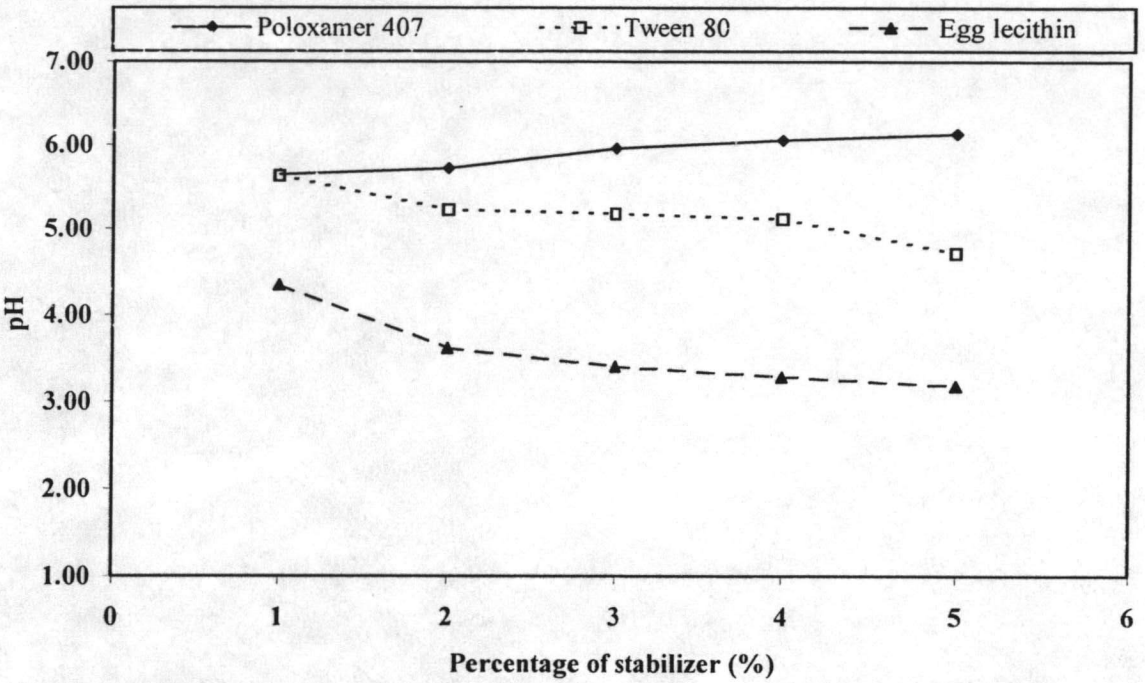

Figure 12. The pH of dispersions of SLN containing various types and amounts of stabilizer.

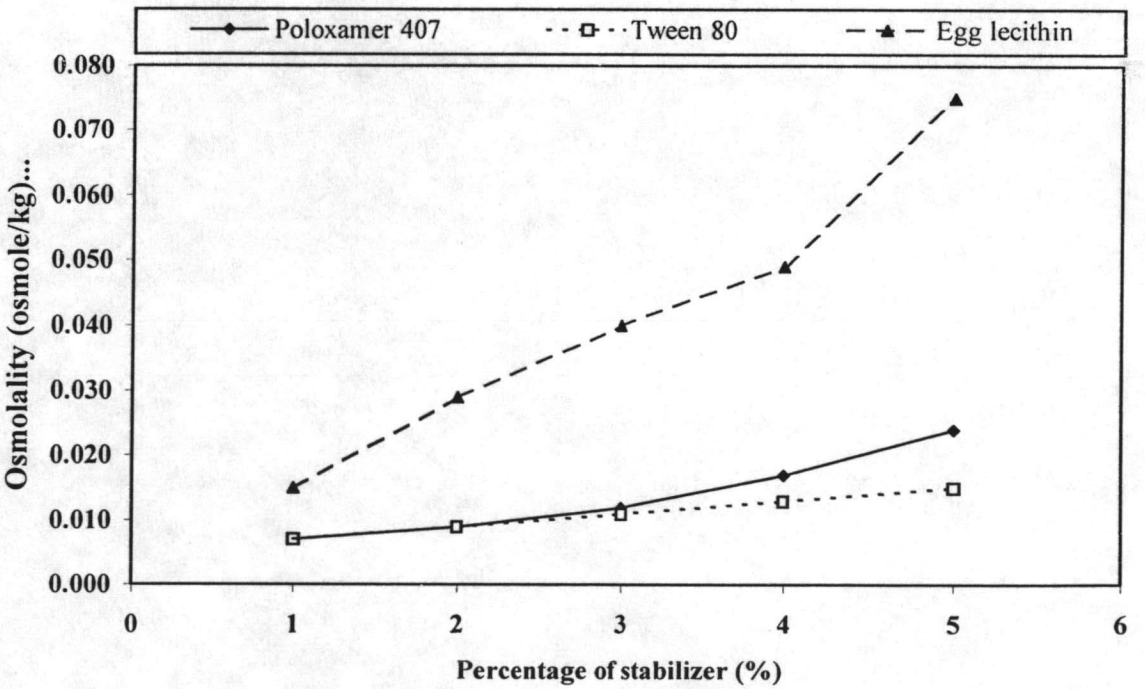

Figure 13. The osmolality of dispersions of SLN containing various types and amounts of stabilizer.

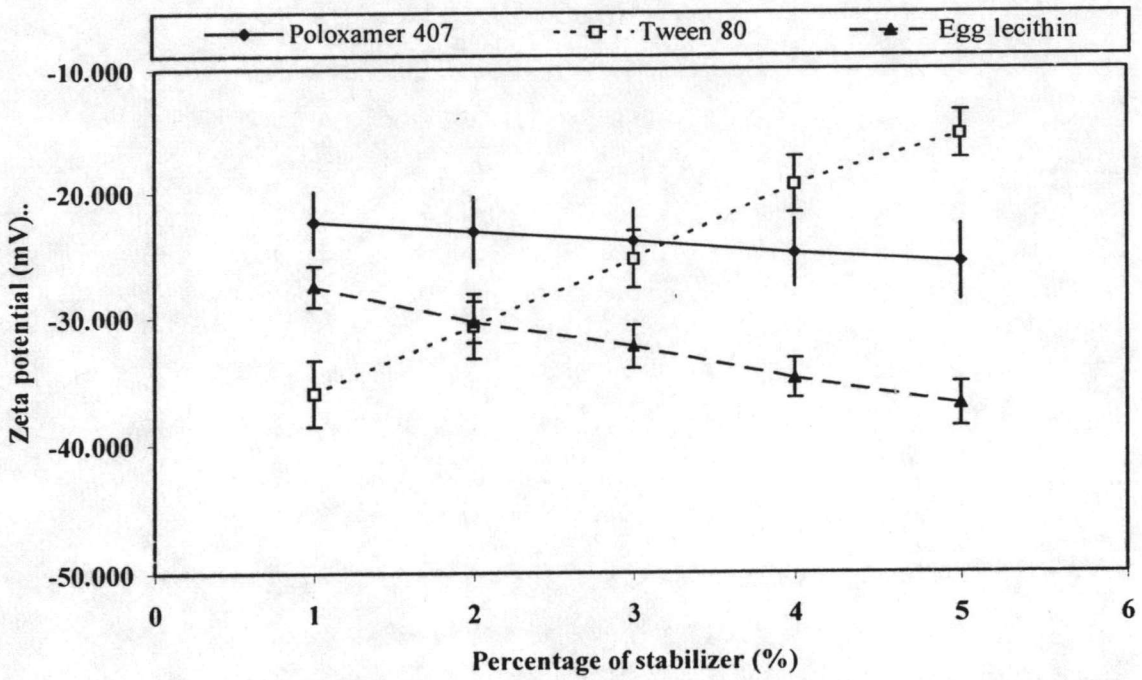

Figure 14. The zeta potential of dispersions of SLN containing various types and amounts of stabilizer.

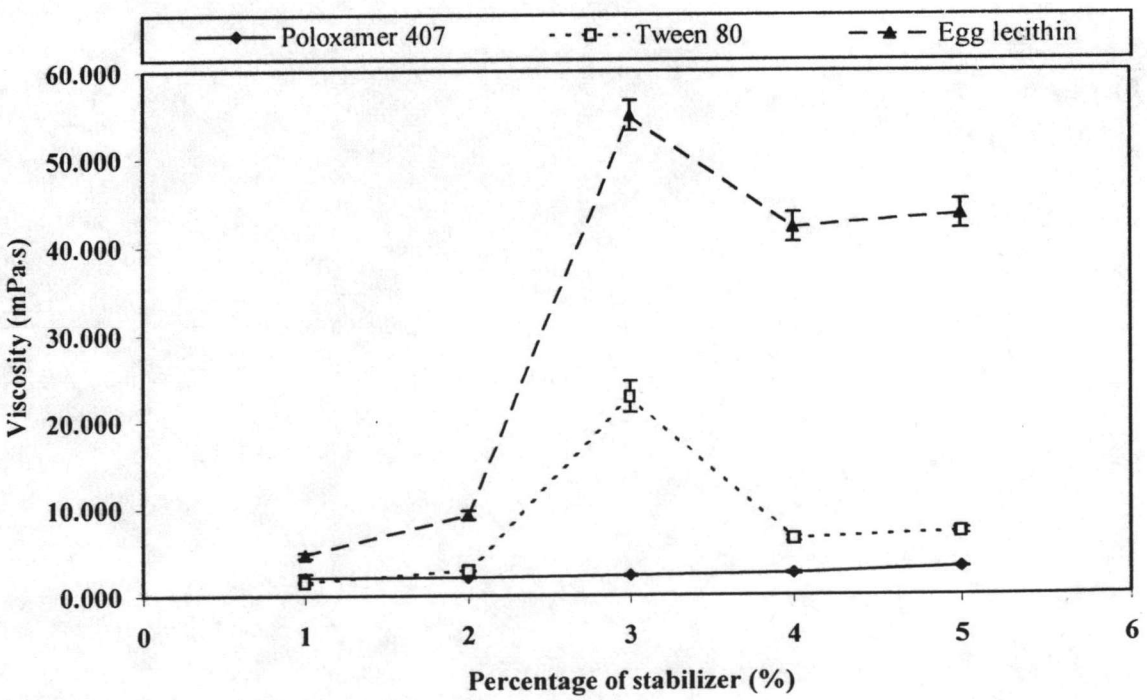

Figure 15. The viscosity at shear rate of 1000 s^{-1} of dispersions of SLN containing various types and amounts of stabilizer.

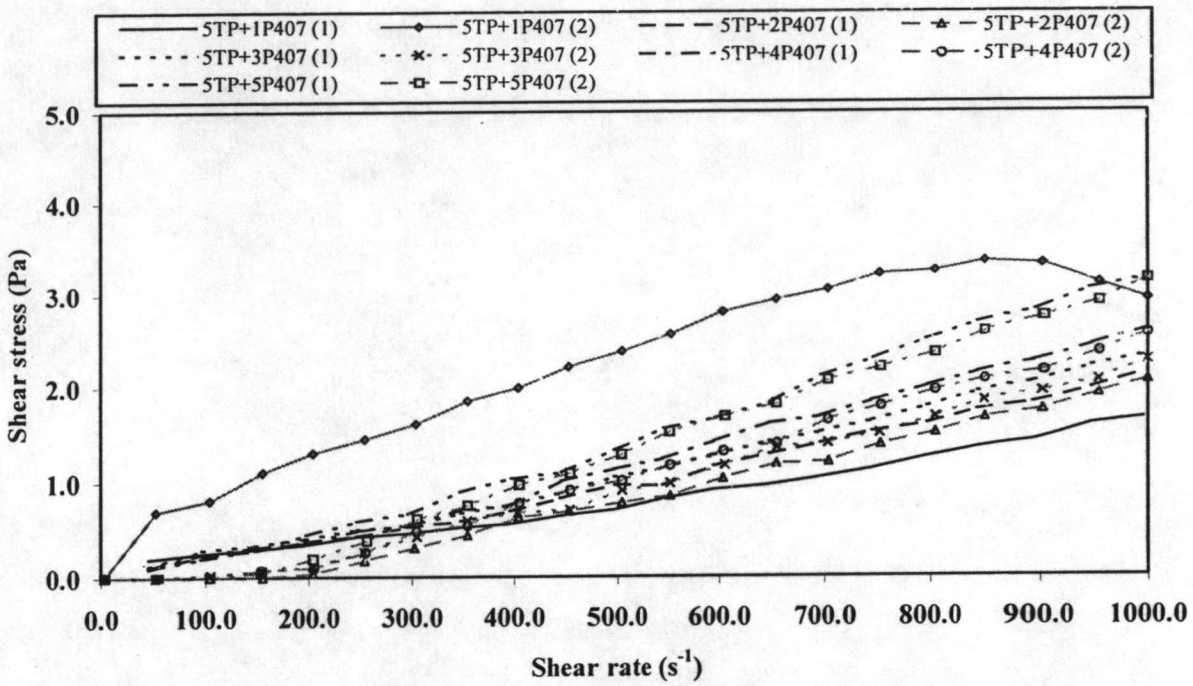

Figure 16. Flow curves of dispersions of SLN containing 5% tripalmitin and 1-5% poloxamer 407 ((1) up-curve and (2) down-curve).

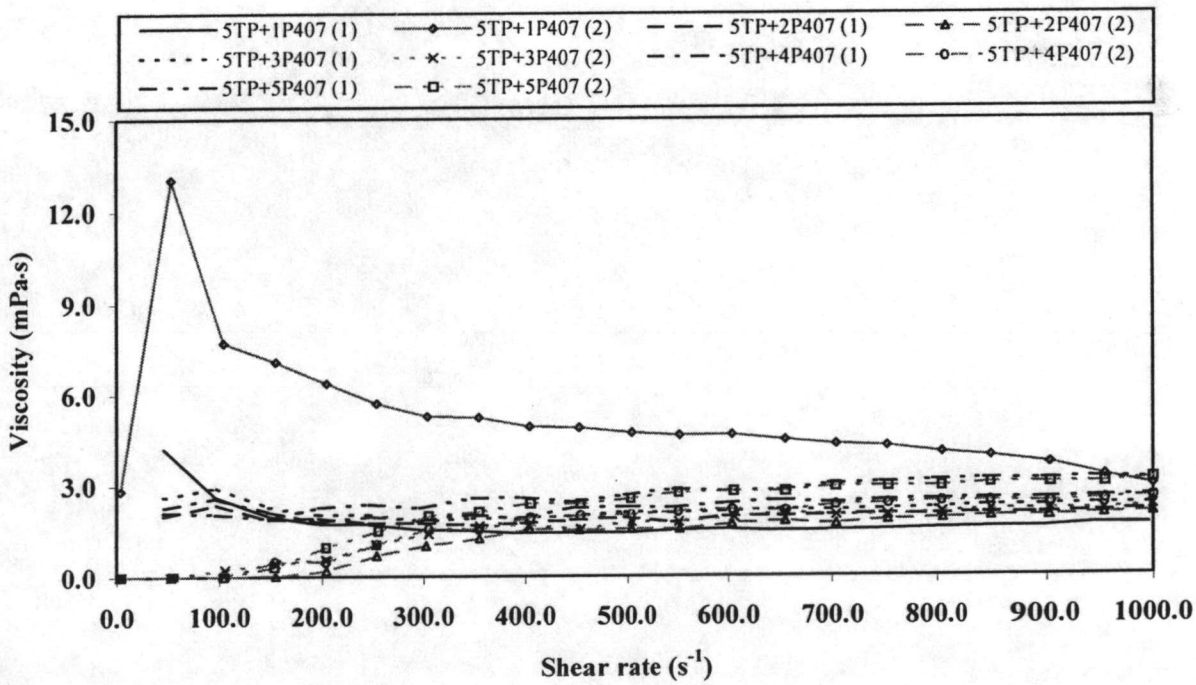

Figure 17. Viscosity curves of dispersions of SLN containing 5% tripalmitin and 1-5% poloxamer 407 ((1) up-curve and (2) down-curve).

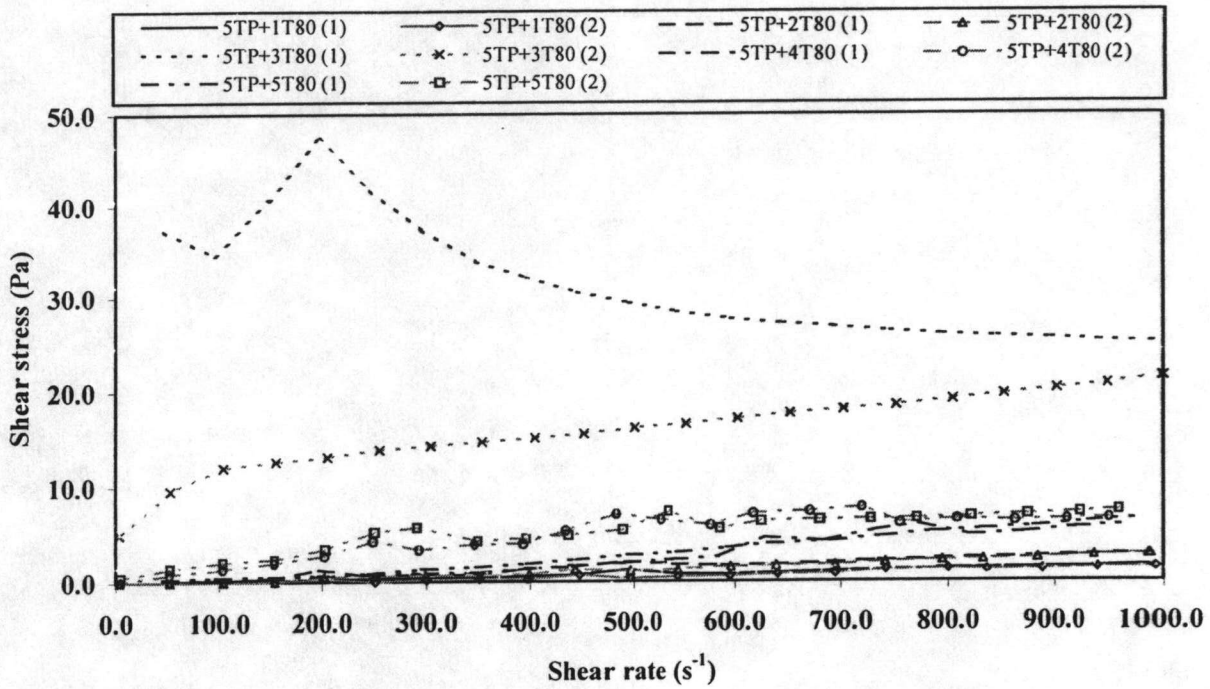

Figure 18. Flow curves of dispersions of SLN containing 5% tripalmitin and 1-5% tween 80 ((1) up-curve and (2) down-curve).

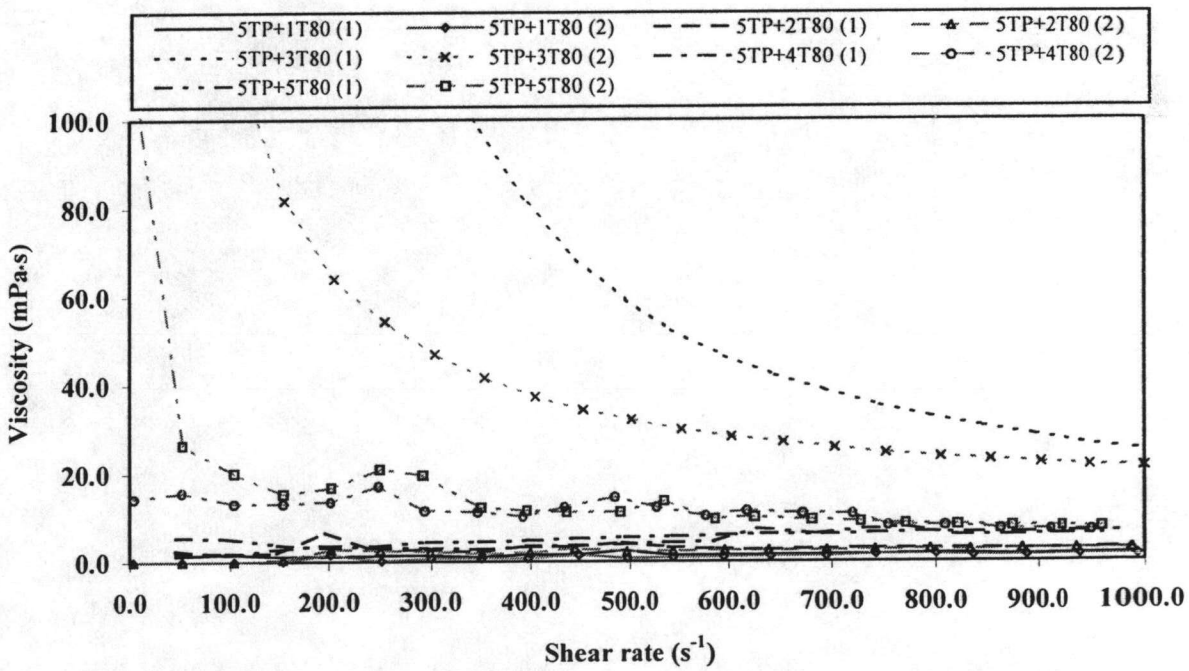

Figure 19. Viscosity curves of dispersions of SLN containing 5% tripalmitin and 1-5% tween 80 ((1) up-curve and (2) down-curve).

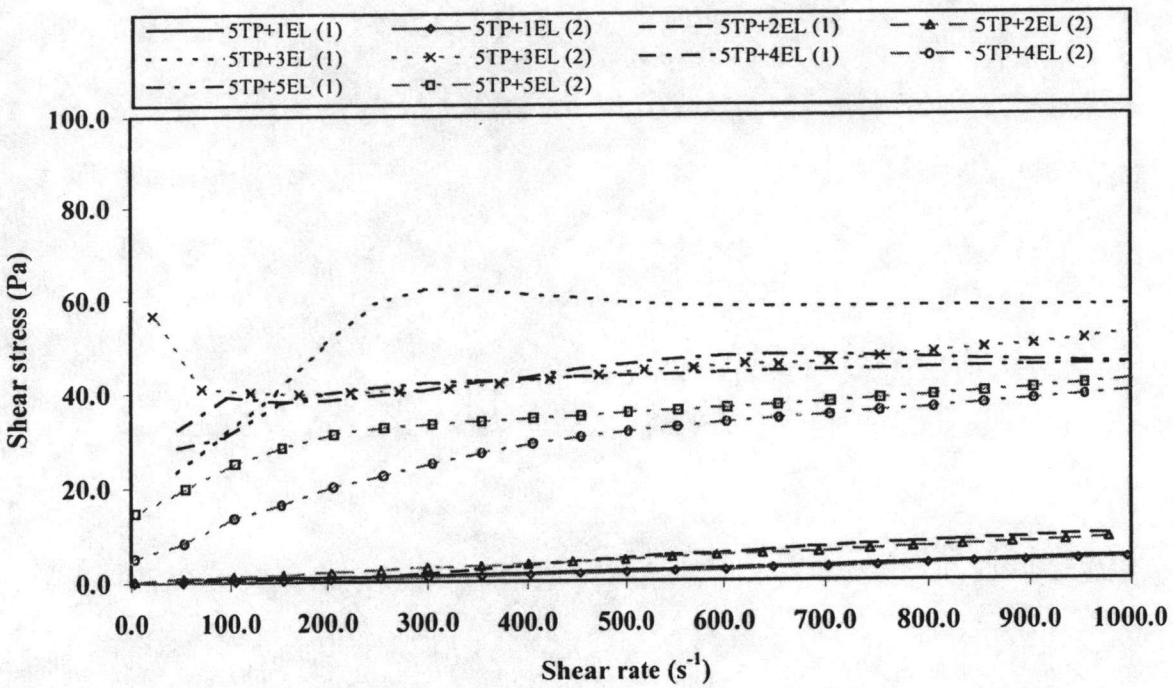

Figure 20. Flow curves of dispersions of SLN containing 5% tripalmitin and 1-5% egg lecithin ((1) up-curve and (2) down-curve).

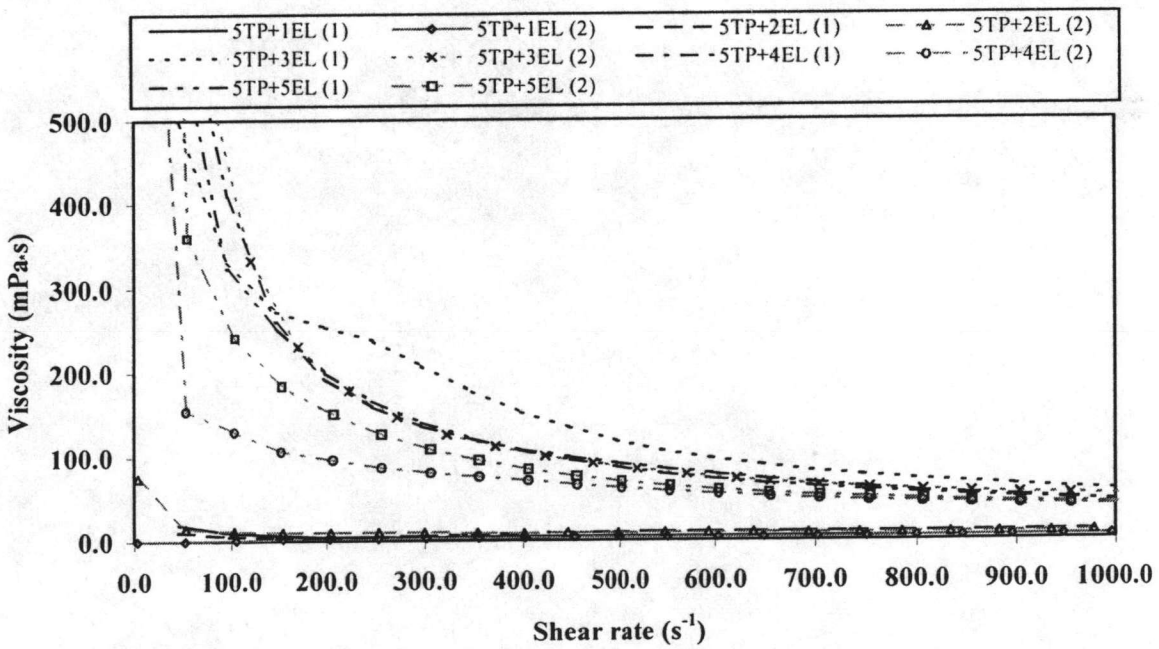

Figure 21. Viscosity curves of dispersions of SLN containing 5% tripalmitin and 1-5% egg lecithin ((1) up-curve and (2) down-curve).

2.2 Effect of lipids

2.2.1 Physical appearance

The physical appearances of dispersions of SLN both before and after sterilization by autoclaving are listed in Table 6. All preparations could be prepared by hot melt homogenization method and sterilized by autoclaving. White fluid dispersions were observed both before and after autoclaving, and were stable upon storage. For preparation of stearic acid, pearly white dispersion was observed due to the color of lipid, and the sedimentation occurred after storage for 2 months at room temperature.

2.2.2 Particle size

Table 7 shows the particle size of SLN containing various types and amounts of lipid. The d(v,0.5) of SLN containing 3-7% tripalmitin was below 1 μm both before and after autoclaving, and still existed in the nanometer size after storage for 6 months at room temperature. There were no significant differences of d(v,0.5) between before and after autoclaving, and after storage at room temperature tested by paired-samples T test, 2 tailed ($p=0.108$ and 0.172; $\alpha=0.05$, respectively) (Figure 22).

Different types of lipid affected their particle sizes. The preparation of SLN containing trimyristin obtained the smaller particle size than tripalmitin, tristearin, and stearic acid, respectively. There was no particle size larger than 1 μm before and after autoclaving. The d(v,0.5) of SLN of trimyristin and tripalmitin were below 1 μm both before and after autoclaving, and still existed in the nanometer size after storage for 6 months at room temperature. While the d(v,0.5) of SLN containing tristearin was below 1 μm before autoclaving but slightly increased to higher than 1 μm after autoclaving, and decreased to the nanometer size after storage for 6 months at room temperature. Only SLN of stearic acid produced the d(v,0.5) above 1 μm, and increased to the higher size after autoclaving (Figure 23).

Table 6. The physical appearances of the dispersions of SLN containing various types and amounts of lipid.

Formulation	Macroscopic observation	
	Before autoclaving	After autoclaving
3TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
4TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
6TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
7TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
5TM+3P407	White fluid dispersion	White fluid dispersion; stable on storage
5TS+3P407	White fluid dispersion	White fluid dispersion; stable on storage
5SA+3P407	Pearly white fluid dispersion	Pearly white fluid dispersion; sediment after storage

2.2.3 pH

The pH of preparations of SLN containing tripalmitin was no difference when increasing its percentage in the range of 3-7%. Moreover, increasing the chain of lipids in triglyceride molecule from C-14 triglyceride to C-18 triglyceride showed to decrease the pH. Preparation of SLN containing stearic acid was also produced a weakly acid preparation due to its acidic property (Figure 24).

2.2.4 Osmolality

The amount of tripalmitin in preparations did not affect their osmolalities. There were no differences between their various amounts. Furthermore, various type of lipids also did not affect their osmolality. These values were very low in all preparations as shown in Figure 25. However, preparation of SLN containing stearic acid was higher osmolality than preparations of triglyceride SLN.

Table 7. Particle sizes of SLN containing various types and amounts of lipid before and after autoclaving, and 6 months storage at room temperature.

Formulation	Volume particle size (μm)							%Particle larger than		
	d(v,0.1)	d(v,0.5)	d(v,0.9)	d(4,3)	d(3,2)	span	uniformity	1μm	5μm	10μm
3TP+3P407(a)	0.17	0.38	3.25	1.04	0.34	8.20	2.11	21.32	4.56	0.27
3TP+3P407(b)	0.25	0.44	4.22	1.31	0.46	8.94	2.24	25.00	6.76	0.11
3TP+3P407(c)	0.12	0.29	2.38	0.86	0.25	7.71	2.32	14.33	4.88	0.35
4TP+3P407(a)	0.22	0.38	0.75	0.51	0.36	1.38	0.62	5.31	0.42	0.00
4TP+3P407(b)	0.24	0.41	0.77	0.58	0.39	1.31	0.69	6.28	0.88	0.00
4TP+3P407(c)	0.21	0.48	0.63	0.69	0.33	0.89	0.99	4.86	1.85	0.59
5TP+3P407(a)	0.23	0.39	0.75	0.49	0.36	1.25	0.53	3.52	0.59	0.01
5TP+3P407(b)	0.23	0.40	0.77	0.49	0.37	1.34	0.49	4.36	0.28	0.00
5TP+3P407(c)	0.13	0.30	0.71	1.30	0.25	1.96	3.74	6.22	2.72	2.13
6TP+3P407(a)	0.24	0.41	0.78	0.52	0.39	1.30	0.53	4.84	0.41	0.00
6TP+3P407(b)	0.23	0.42	1.01	0.64	0.39	1.86	0.82	10.21	1.17	0.00
6TP+3P407(c)	0.18	0.33	0.72	0.54	0.30	1.63	0.90	6.35	1.23	0.08
7TP+3P407(a)	0.23	0.39	0.71	0.50	0.37	1.24	0.51	3.81	0.42	0.00
7TP+3P407(b)	0.23	0.39	0.71	0.50	0.37	1.24	0.54	4.01	0.45	0.00
7TP+3P407(c)	0.21	0.35	0.66	0.53	0.33	1.31	0.79	5.75	0.98	0.09
5TM+3P407(a)	0.26	0.36	0.53	0.38	0.35	0.74	0.23	0.00	0.00	0.00
5TM+3P407(b)	0.25	0.36	0.52	0.38	0.35	0.75	0.23	0.00	0.00	0.00
5TM+3P407(c)	0.23	0.32	0.48	0.38	0.31	0.77	0.36	1.19	0.25	0.03
5TS+3P407(a)	0.27	0.86	5.46	2.12	0.61	6.03	2.01	48.80	12.37	0.85
5TS+3P407(b)	0.27	1.66	5.97	2.43	0.66	3.44	1.17	54.76	15.63	0.96
5TS+3P407(c)	0.16	0.43	7.42	2.51	0.38	16.90	5.29	42.06	18.43	5.35
5SA+3P407(a)	0.41	3.82	16.99	6.64	1.23	4.33	1.39	74.60	43.05	22.70
5SA+3P407(b)	0.40	11.75	64.76	23.91	1.45	5.48	1.77	77.79	63.05	52.79

Note: (a): before autoclaving, (b): after autoclaving;

(c): after autoclaving and storage for 6 months at room temperature

2.2.5 Zeta potential

As shown in Figure 26, the zeta potentials were not different in preparations of SLN containing 3-7% tripalmitin. All preparations had the negative charge in the range of –23.348 to –24.034 millivolts. SLN of trimyristin and tristearin had the zeta potential close to tripalmitin, while SLN of stearic acid had the zeta potential higher than the other preparations, but osmolalities of all preparations were not high enough to stabilize the dispersion.

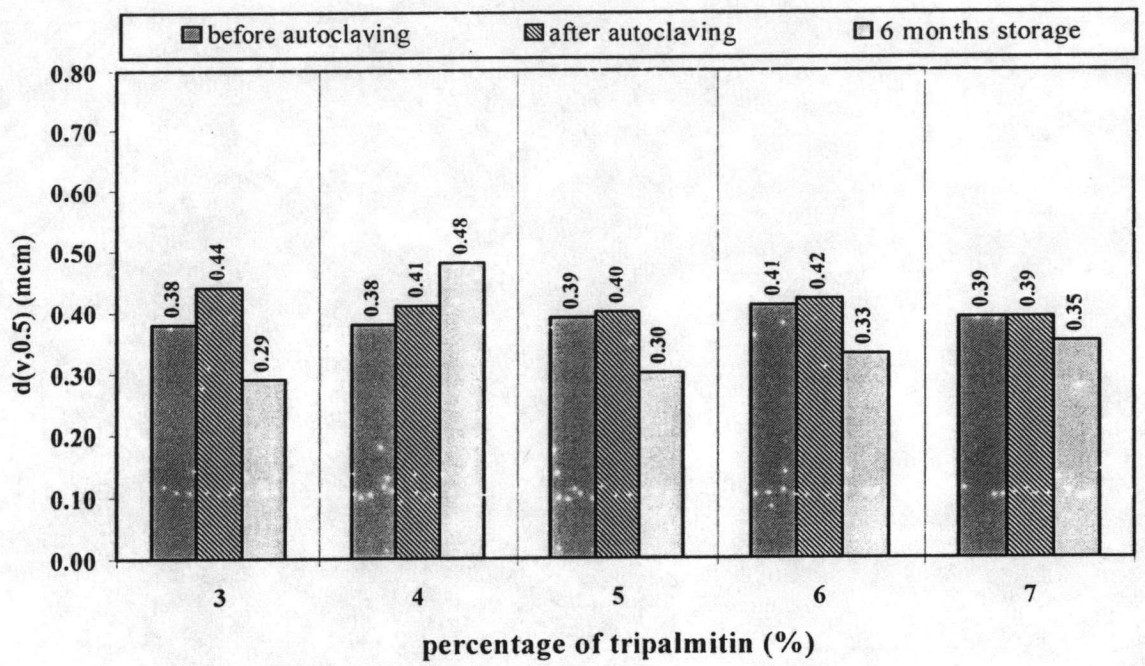

Figure 22. The d(v,0.5) of SLN containing 3-7% tripalmitin.

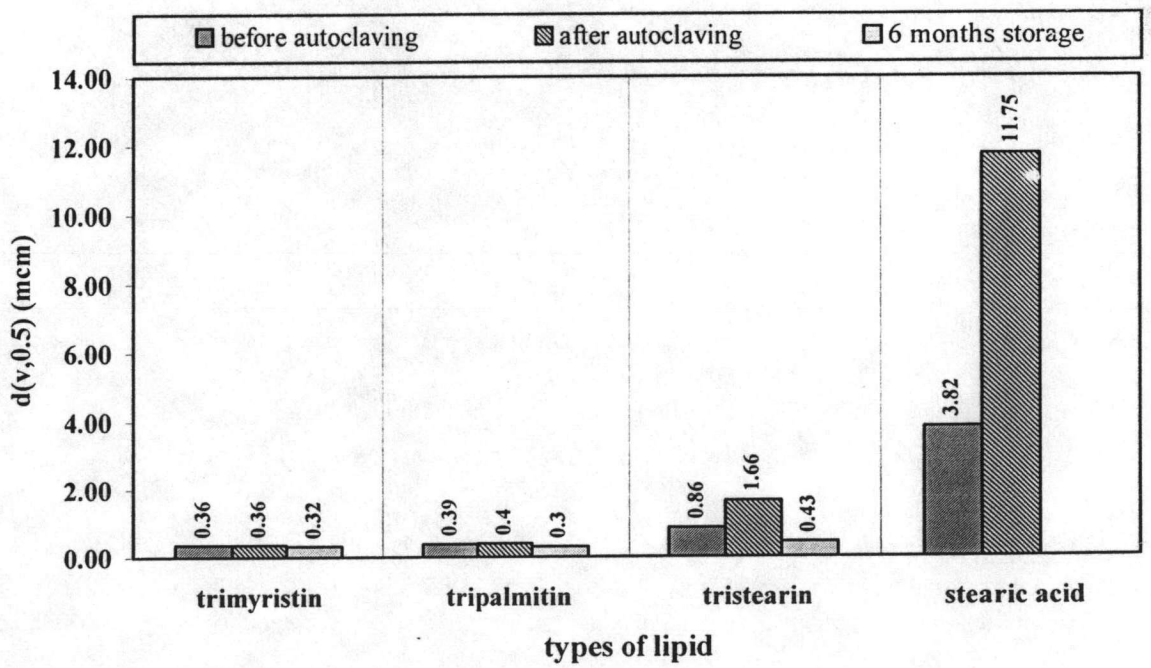

Figure 23. The d(v,0.5) of SLN containing various types of 5% lipid.

Table 8. The pH, osmolality, zeta potential, and viscosity of dispersions of SLN containing various types and amounts of lipid.

Formulation	pH	Osmolality	Zeta potential (millivolts) (±SD)	Viscosity at the shear rate of $1000s^{-1}$ (mPa·s) (±SD)
3TP+3P407	5.98	0.013	-23.348 (± 2.828)	1.562 (± 0.070)
4TP+3P407	5.97	0.012	-23.722 (± 2.900)	1.817 (± 0.103)
5TP+3P407	5.96	0.012	-23.901 (± 2.607)	2.295 (± 0.096)
6TP+3P407	5.88	0.011	-24.034 (± 1.939)	2.758 (± 0.079)
7TP+3P407	5.81	0.011	-23.945 (± 1.903)	2.935 (± 0.069)
5TM+3P407	6.51	0.012	-21.533 (± 2.352)	1.321 (± 0.059)
5TS+3P407	5.28	0.011	-21.563 (± 1.934)	2.584 (± 0.135)
5SA+3P407	5.65	0.016	-32.393 (± 1.763)	1.496 (± 0.146)

2.2.6 Viscosity

Figures 27-31 show flow curves of preparations at the viscosity of 1000 s^{-1}, and viscosity curves of preparations of SLN with different type and amount of lipids. The SLN of tripalmitin showed the Newtonian flow. The viscosity was constant in each shear rate and did not change after decreasing shear force. Higher lipid content slightly increased the viscosity. However, all preparations still showed low viscosity.

The longer chain of fatty acid in triglyceride showed slightly higher viscosity. Preparation of trimyristin produced the lowest viscosity. However, Newtonian systems were observed in dispersions of SLN containing all three triglycerides. Moreover, preparation of SLN of stearic acid showed fluctuating viscosity.

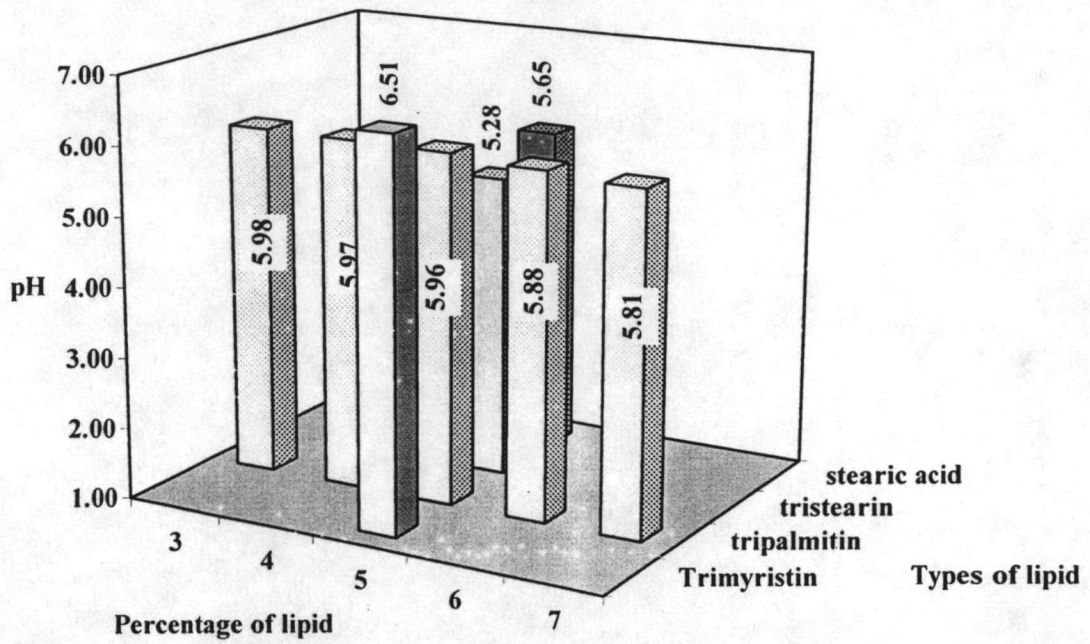

Figure 24. The pH of dispersions of SLN containing various types and amounts of lipid.

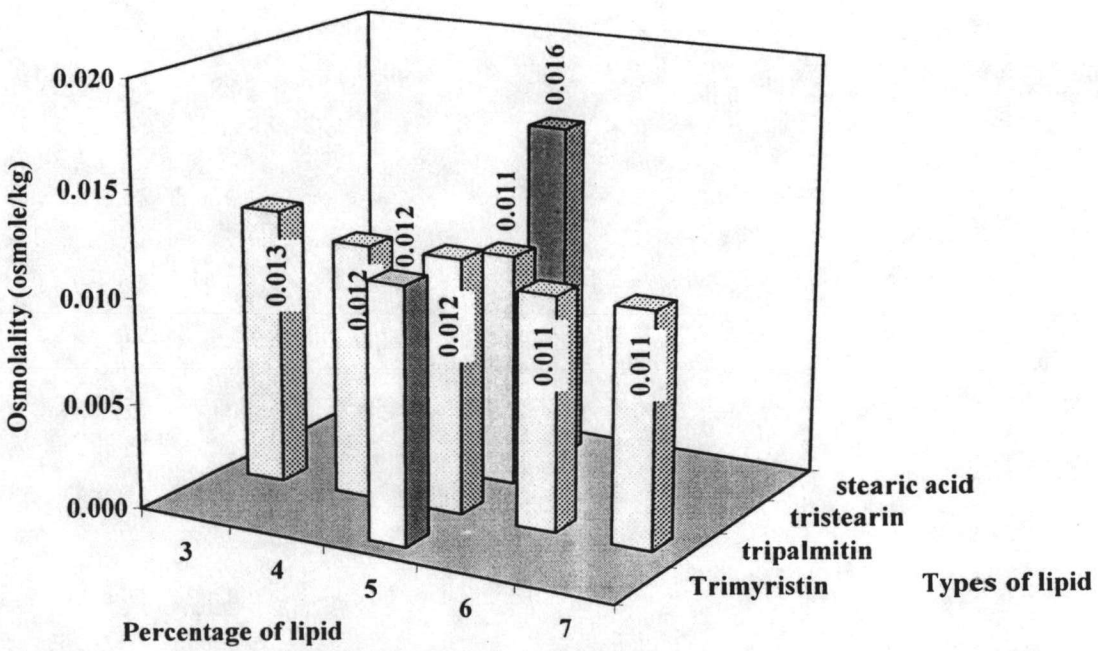

Figure 25. The osmolality of dispersions of SLN containing various types and amounts of lipid.

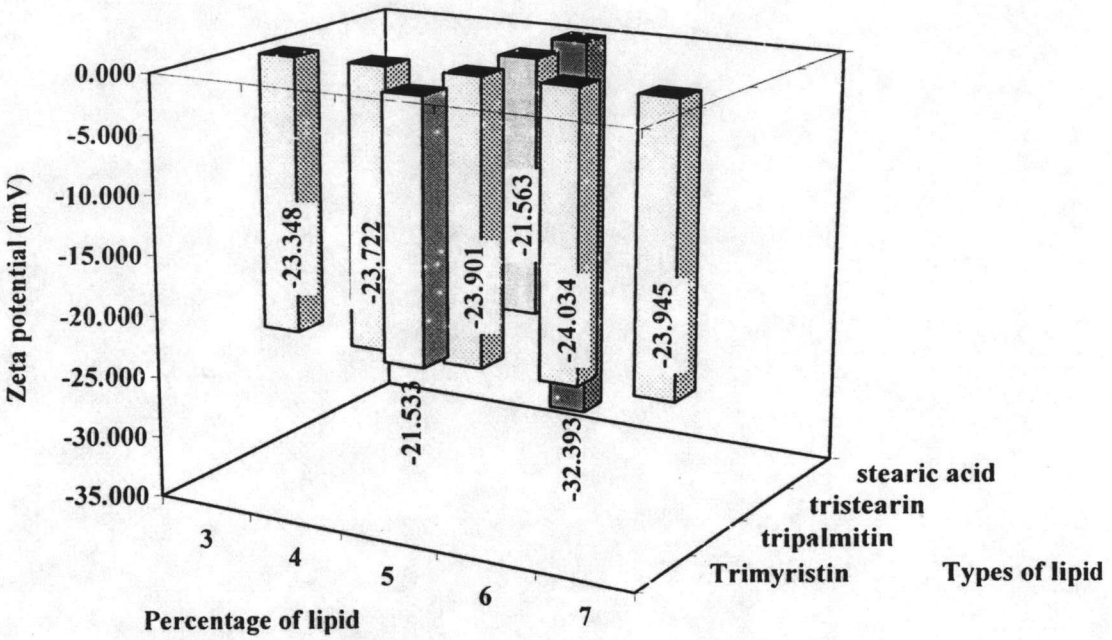

Figure 26. The zeta potential of dispersions of SLN containing various types and amounts of lipid.

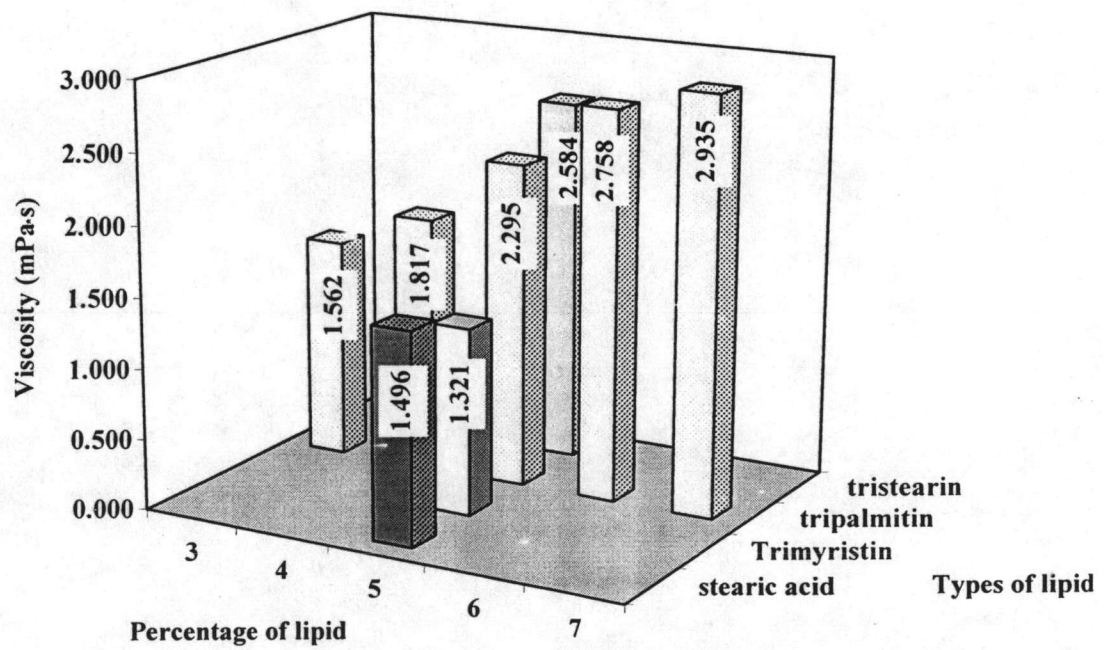

Figure 27. The viscosity at shear rate of 1000 s^{-1} of dispersions of SLN containing various types and amounts of lipid.

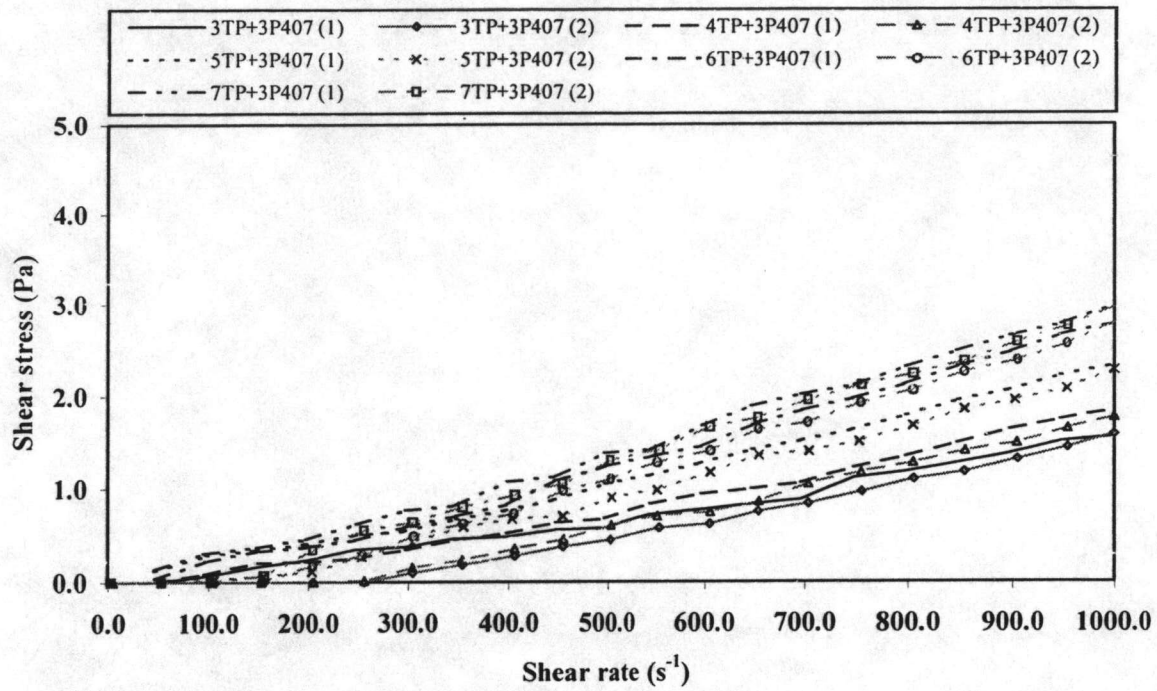

Figure 28. Flow curves of dispersions of SLN containing 3-7% tripalmitin and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

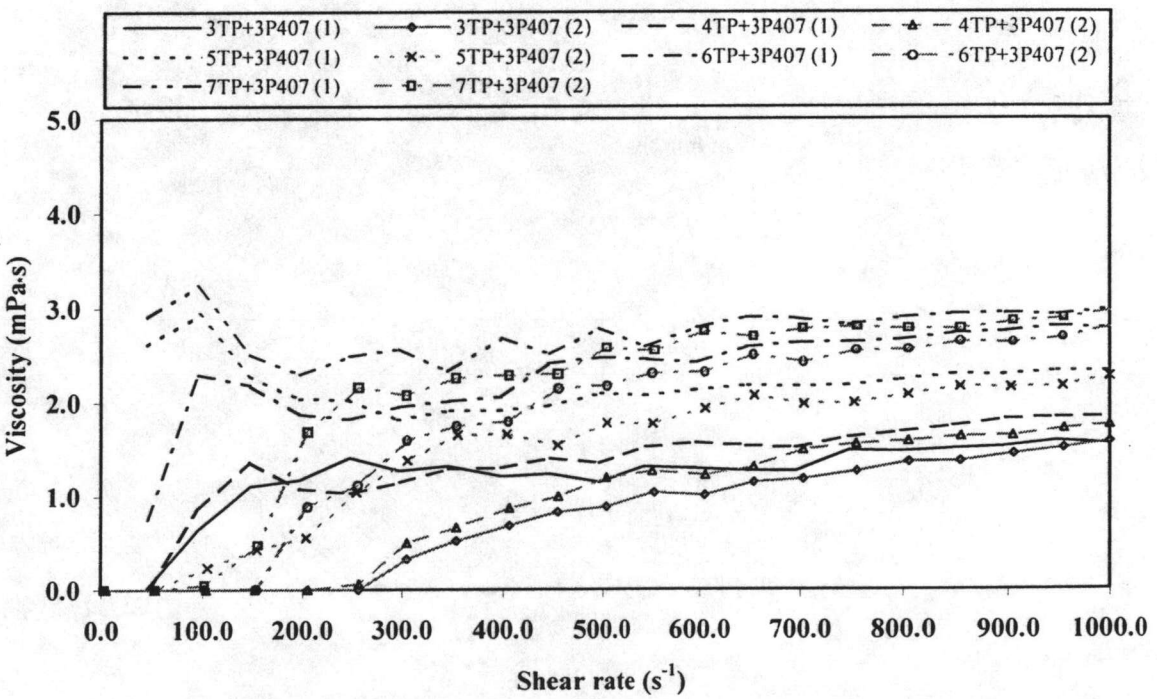

Figure 29. Viscosity curves of dispersions of SLN containing 3-7% tripalmitin and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

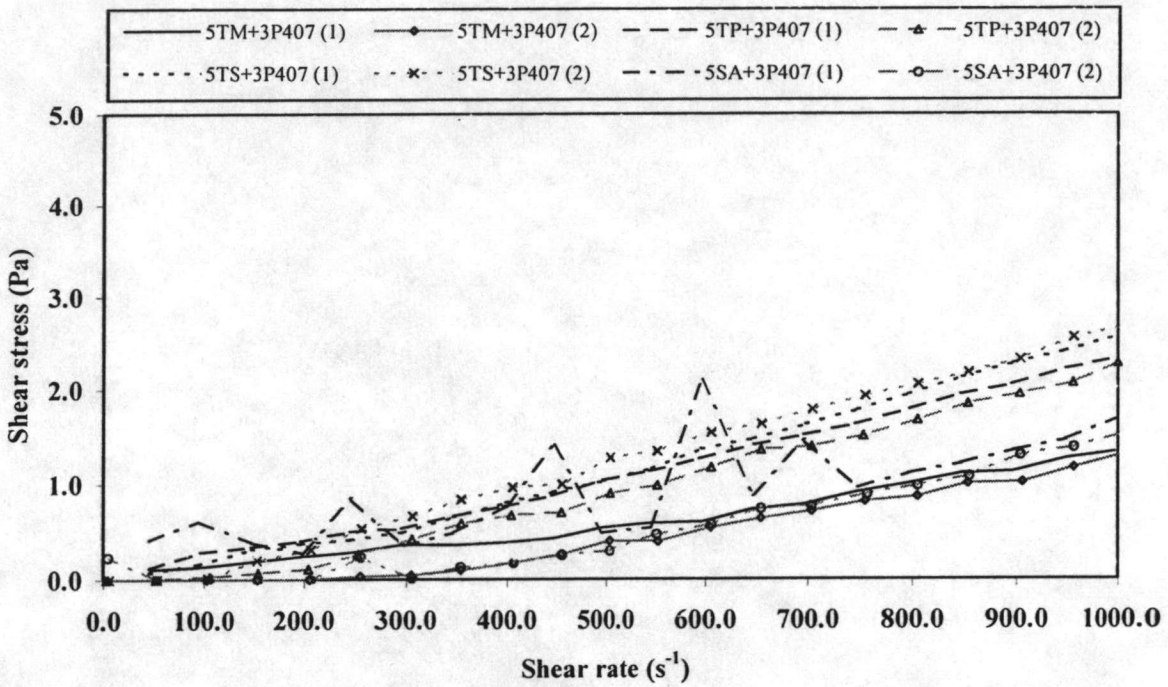

Figure 30. Flow curves of dispersions of SLN containing various types of 5% lipid and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

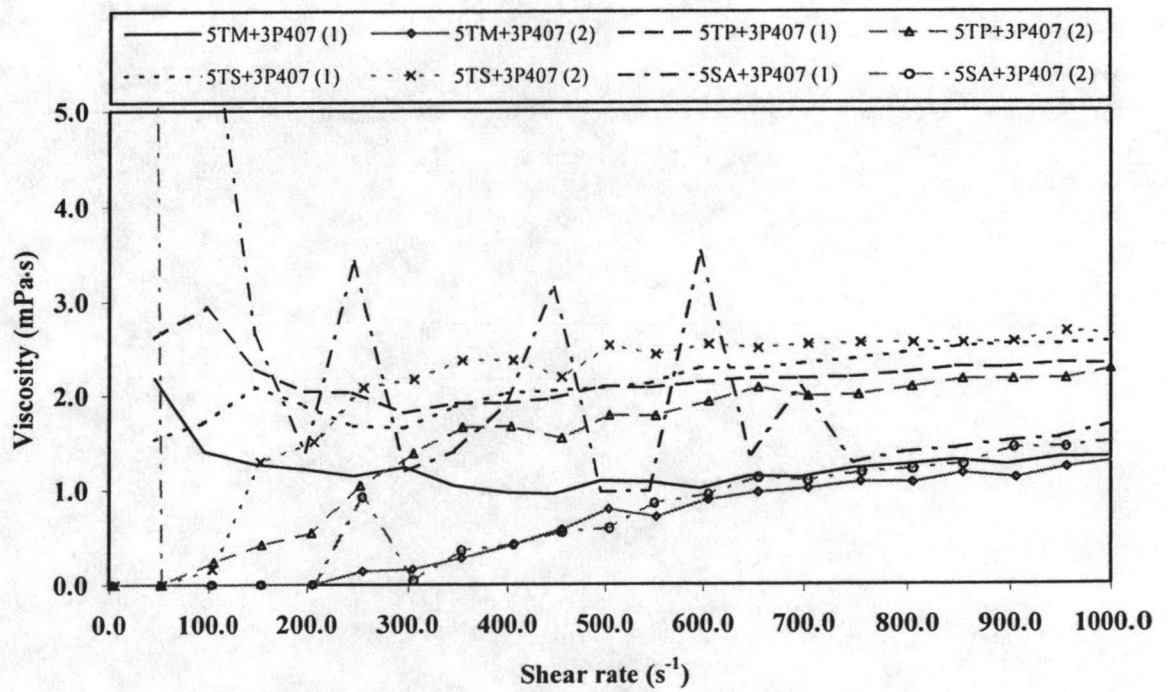

Figure 31. Viscosity curves of dispersions of SLN containing various types of 5% lipid and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

2.2.7 Particle shape

The shape and surface morphology was observed by cryo-scanning electron microscopy. Figure 32 shows the photomicrograph of preparation containing 5% tripalmitin and 3% poloxamer 407. Various sizes of spherical particles were observed in agglomerate patterns. All particles were smaller than 1 μm , and smooth of particles surface was observed.

2.2.8 Infrared spectra

The IR spectra of tripalmitin, poloxamer 407, and solid lipid prepared from the ultracentrifugation of SLN containing 5% tripalmitin and 3% poloxamer 407 are shown in Figure 33. The principle peaks of tripalmitin were observed at the wavenumbers of 718, 1179, 1472, 1736, 2850, and 2917 cm^{-1}. The sharp peaks at 2850 and 2917 cm^{-1} were CH_2 symmetric and CH_2 asymmetric of aliphatic C-H stretching, respectively. The peaks of 1179 and 1472 cm^{-1} and small peak between both wavenumbers were CH_2 bending vibrations. The peak of 712 cm^{-1} was CH_2 rocking vibration. The distinguished peak at 1736 cm^{-1} was the C=O stretching.

The IR spectrum of poloxamer 407 showed the sharp peak of C-O-C stretching at 1111 cm^{-1} and the CH_2 symmetric stretching at 2889 cm^{-1}. The small peaks at 1242-1469 cm^{-1} showed the CH_2 bending vibrations.

The IR spectrum of SLN containing 5% tripalmitin and 3% poloxamer 407 showed the peaks of both tripalmitin and poloxamer 407. The sharp peaks at 718, 1112, 1472, 1736, 2850, and 2917 cm^{-1} were observed from the combination of both spectra. No new peak was observed from its mixture.

2.2.9 Differential scanning calorimetry

The DSC thermograms of tripalmitin, poloxamer 407, and solid lipid prepared from the ultracentrifugation of SLN containing 5% tripalmitin and 3%

poloxamer 407 are shown in Figure 34. The thermogram of tripalmitin presented two endotherms. The small endotherm began from 31.85°C to 51.95°C which had the peak at 45.25°C. The dominant endotherm began from 51.44°C to 67.42°C which had the peak at 63.52°C. While the thermogram of poloxamer 407 showed one endotherm between 31.85°C and 60.20°C which had the peak at 53.16°C.

The thermogram of solid lipid of SLN containing 5% tripalmitin and 3% poloxamer 407 showed three endotherms. Two small peaks between 25.67°C to 54.02°C which had the peak at 45.92°C and a very small shoulder at approximately 50°C was observed. The dominant endotherm presented between 53.50°C to 66.90°C which had the peak at 63.33°C. All three endotherms were the combination of endotherms from both tripalmitin and poloxamer 407. No new endotherm was observed from its mixture.

2.2.10 Powder X-ray diffraction

The X-ray diffractograms of tripalmitin, poloxamer 407, and solid lipid prepared from the ultracentrifugation of SLN containing 5% tripalmitin and 3% poloxamer 407 are shown in Figure 35. Tripalmitin was crystalline and showed the characteristic peaks at 6.50°, 19.28°, 23.02°, and 24.10° and small peaks at 16.50° and 17.02°. While poloxamer 407 had two high peaks at 19.00° and 23.32°.

The diffractogram of solid lipid of SLN containing 5% tripalmitin and 3% poloxamer 407 showed the characteristic similarly to the diffractogram of tripalmitin. The crystalline of SLN was obtained. The sharp peaks at 6.58°, 19.30°, 23.02°, and 24.12° were observed with the small peaks at 16.52° and 17.06°. The intensities of the diffraction peaks were slightly weaker than that of pure tripalmitin. However, the intensity of the peak at 23.02° was increased compared with the peak at 24.10° due to the reinforcement of the peak at 23.32° of poloxamer 407. No new diffractogram was observed from its mixture.

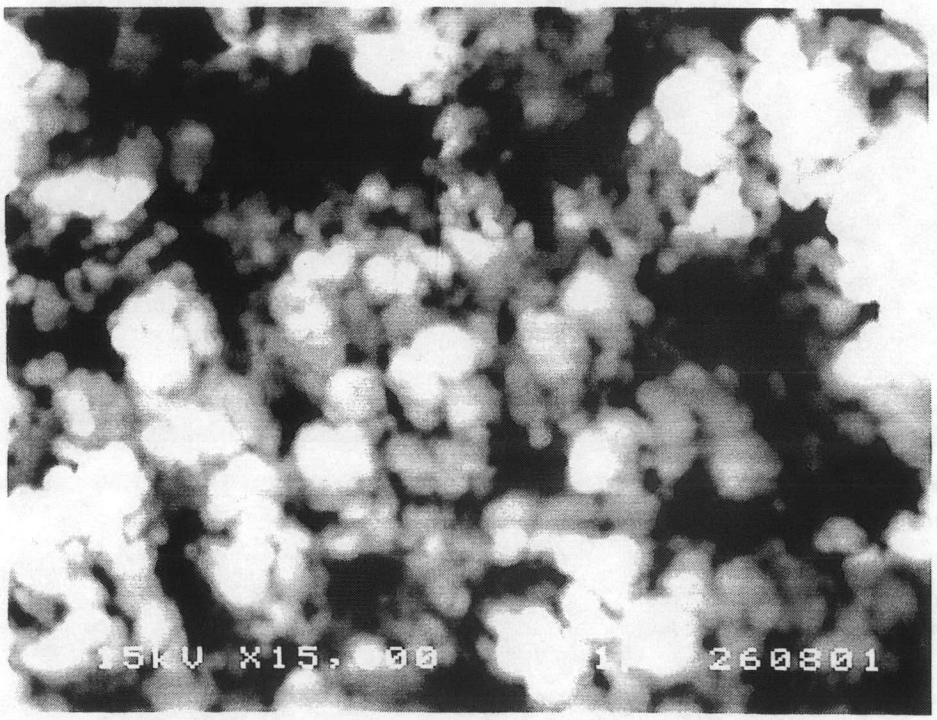

Figure 32. Cryo-scanning electron photomicrograph of SLN containing 5% tripalmitin and 3% poloxamer 407.

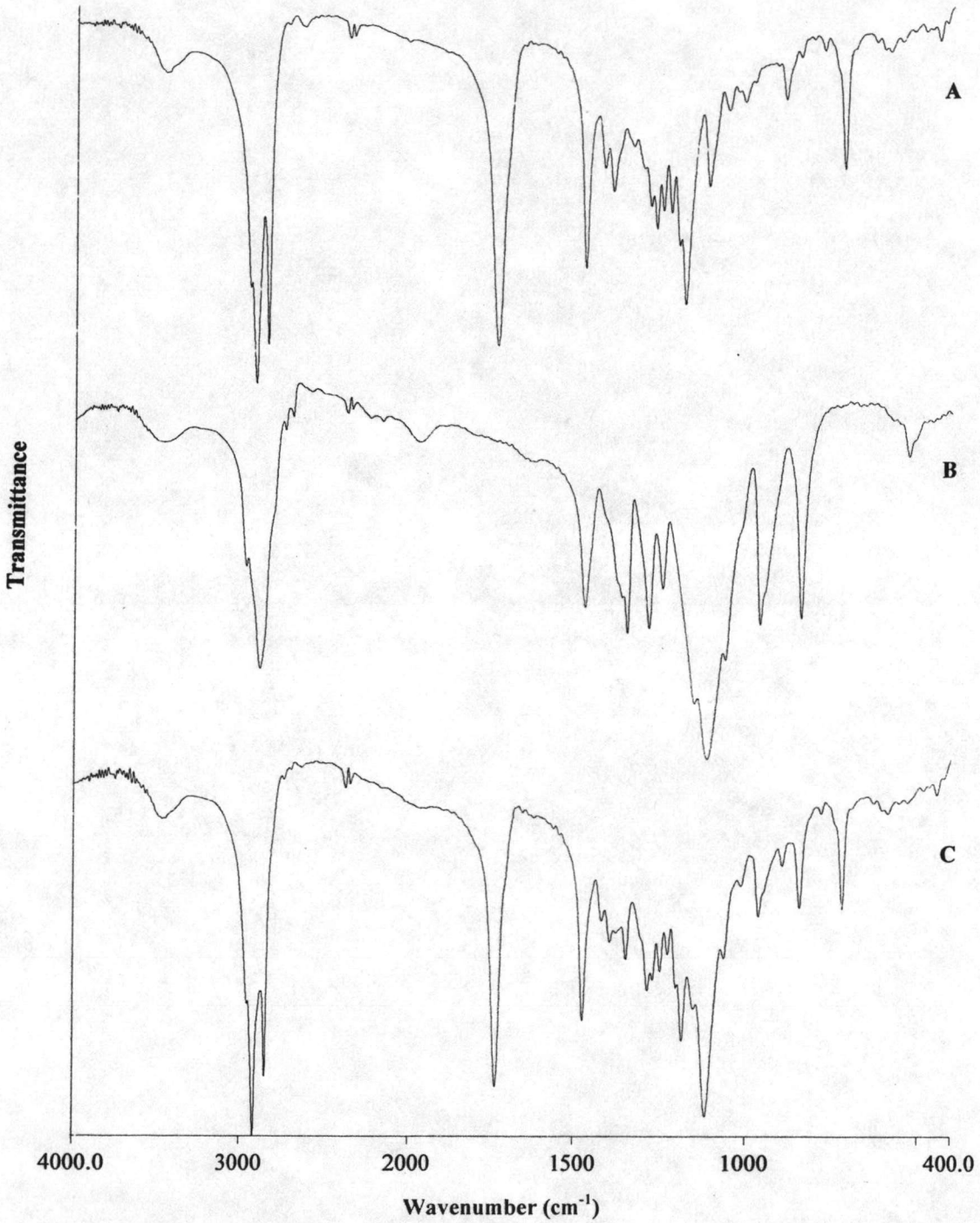

Figure 33. IR spectra of (A) tripalmitin; (B) poloxamer 407; and (C) SLN containing 5% tripalmitin and 3% poloxamer 407.

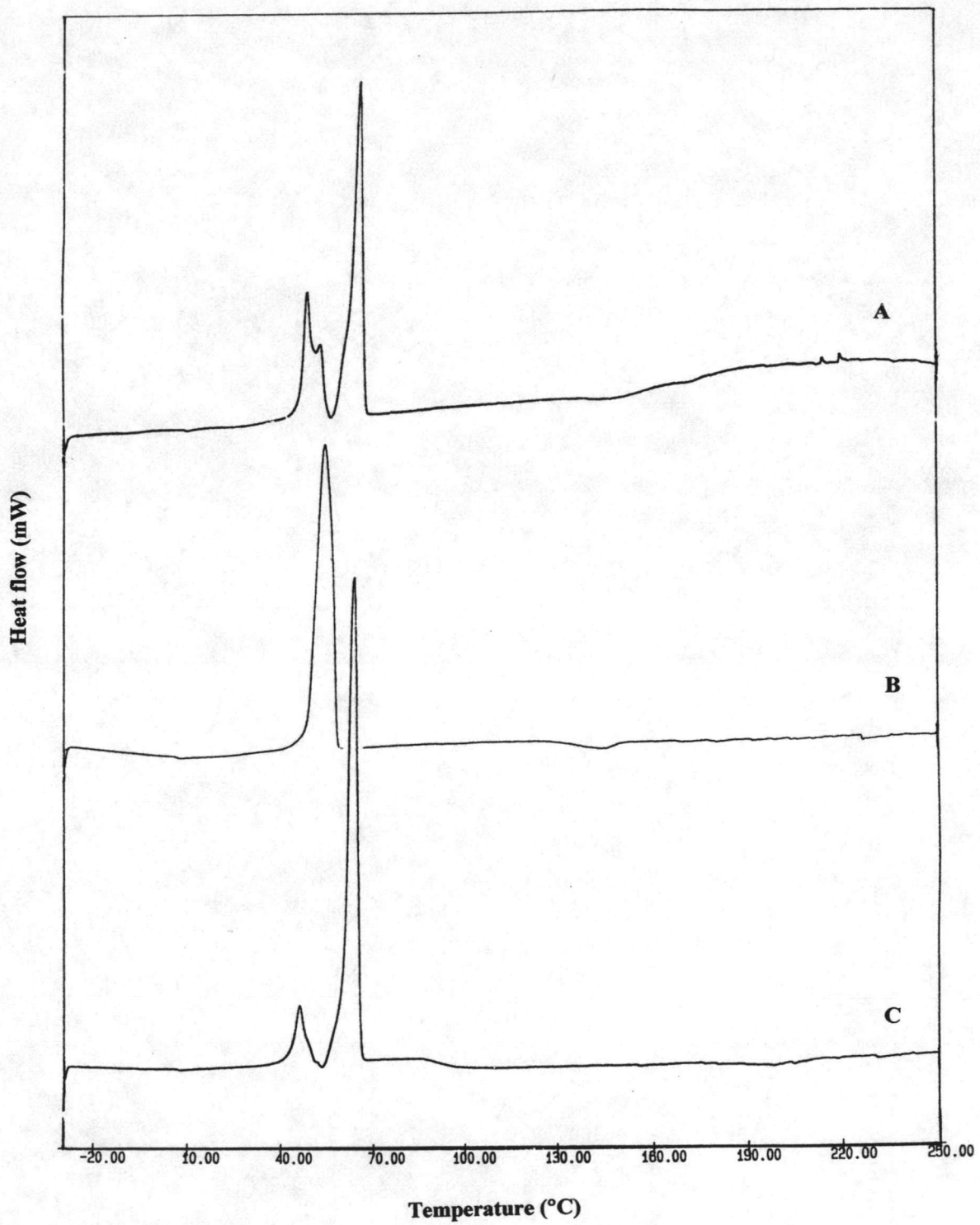

Figure 34. DSC thermograms of (A) SLN containing 5% tripalmitin and 3% poloxamer 407; (B) poloxamer 407; and (C) tripalmitin.

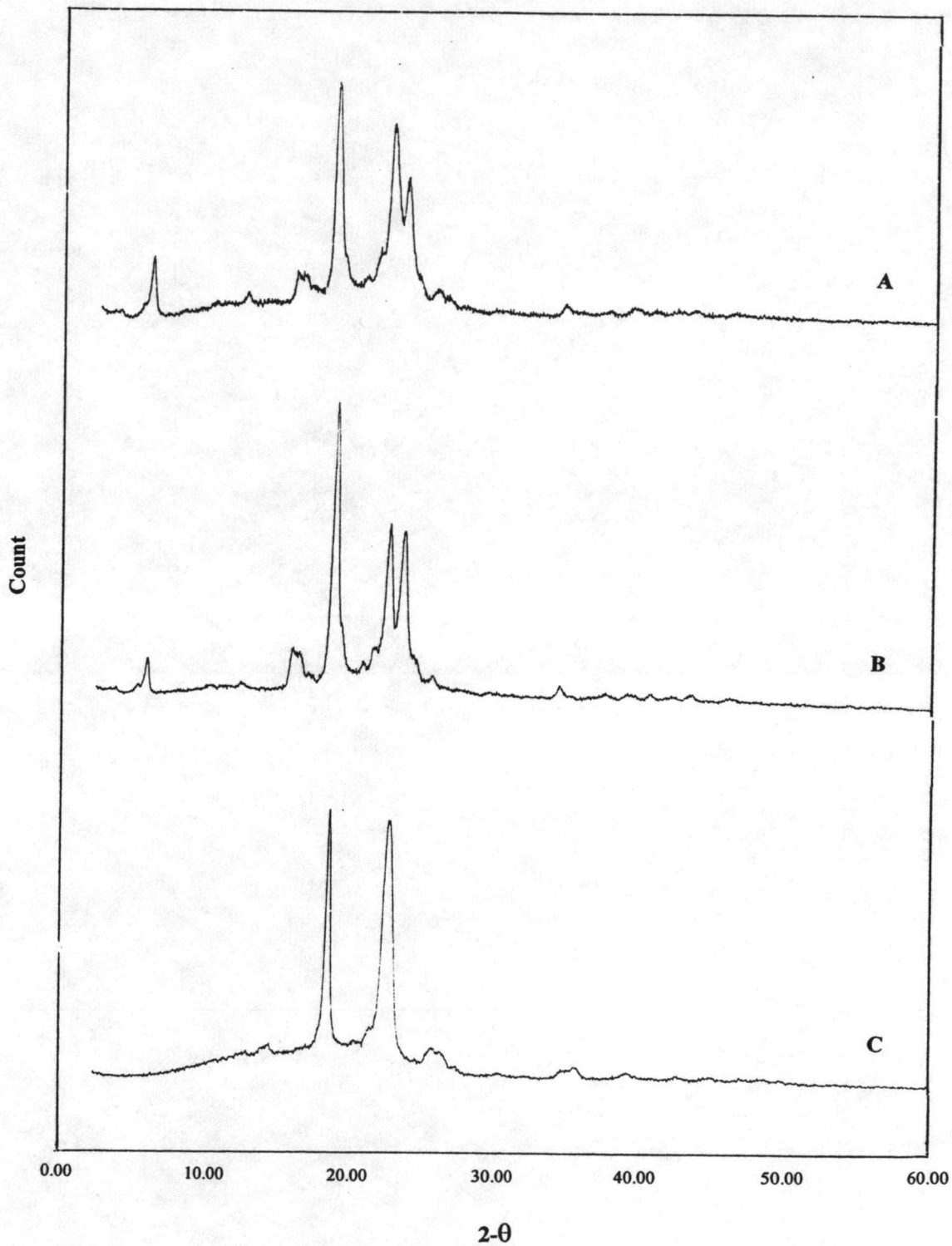

Figure 35. X-ray diffractograms of (A) SLN containing 5% tripalmitin and 3% poloxamer 407; (B) tripalmitin; and (C) poloxamer 407.

3.Preparation and characterization of drug loaded SLN

3.1 Diltiazem hydrochloride loaded SLN

3.1.1 Physical appearance

The physical appearances of the dispersions of SLN containing diltiazem hydrochloride both before and after sterilization by autoclaving are listed in Table 9. Preparations of SLN containing 0.5-1.5% diltiazem hydrochloride could be prepared using 3% poloxamer 407 as stabilizer. White fluid dispersion was observed with both water and phosphate buffer pH 7 dispersion medium. Good stability was obtained both before and after autoclaving. Preparations using tween 80 and egg lecithin as stabilizer, however, were not stable after autoclaving. Separation of oily phase as the layer of solid lipid occurred at the surface when 2-3% tween 80 and 1-2% egg lecithin were used.

3.1.2 Particle size

The particle sizes of SLN containing diltiazem hydrochloride are shown in Table 10. SLN containing 0.5-1.5% drug could be produced using water as dispersion medium in the size range higher than drug free preparation. Their d(v,0.5) were higher than 1 μm. The particles of larger than 1 μm were observed at high percentage (63.33-72.35%). When phosphate buffer pH 7 was used instead of water, the d(v,0.5) of lower than 1 μm was obtained in preparations of 0.5-1.0% drug. This size was slightly smaller than the preparations in water dispersion medium with the same composition. The value was higher than 1 μm when containing 1.5% drug. Increasing the concentration of drug could slightly increase the d(v,0.5). Their d(v,0.5) after autoclaving in Figure 36 showed that the preparation containing 0.5% diltiazem hydrochloride in buffer pH 7 had the smallest size and could be reproduced.

Table 9. The physical appearances of dispersions of SLN containing diltiazem hydrochloride.

Formulation	Macroscopic observation	
	Before autoclaving	After autoclaving
0.5Dil+5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
1.0Dil+5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
1.5Dil+5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
0.5Dil+5TP+3P407(pH7)	White fluid dispersion	White fluid dispersion; stable on storage
1.0Dil+5TP+3P407(pH7)	White fluid dispersion	White fluid dispersion; stable on storage
1.5Dil+5TP+3P407(pH7)	White fluid dispersion	White fluid dispersion; stable on storage
0.5Dil+5TP+2T80	White fluid dispersion	Separation; solid lipid floating on the surface
1.0Dil+5TP+2T80	White fluid dispersion	Separation; solid lipid floating on the surface
1.5Dil+5TP+2T80	White fluid dispersion	Separation; solid lipid floating on the surface
0.5Dil+5TP+3T80	Bluish white fluid dispersion	Separation; solid lipid floating on the surface
1.0Dil+5TP+3T80	Bluish white fluid dispersion	Separation; solid lipid floating on the surface
1.5Dil+5TP+3T80	Bluish white fluid dispersion	Separation; solid lipid floating on the surface
0.5Dil+5TP+1EL	Brown fluid dispersion	Separation; solid lipid floating on the surface
1.0Dil+5TP+1EL	Brown fluid dispersion	Separation; solid lipid floating on the surface
1.5Dil+5TP+1EL	Brown fluid dispersion	Separation; solid lipid floating on the surface
0.5Dil+5TP+2EL	Brown fluid dispersion	Separation; solid lipid floating on the surface
1.0Dil+5TP+2EL	Brown fluid dispersion	Separation; solid lipid floating on the surface
1.5Dil+5TP+2EL	Brown fluid dispersion	Separation; solid lipid floating on the surface

3.1.3 pH

The pH of dispersions of drug loaded SLN would be affected by the concentration of drug in preparations. Figure 37 shows the pH of preparations containing drug. It was evident that diltiazem hydrochloride could slightly decrease the pH of preparation. Increasing the concentrations would decrease this value. Nevertheless, the pH could increase to be between pH 6.4-6.8 when phosphate buffer pH 7 was used as dispersion medium instead of water. Furthermore, increasing the concentration of drug would also slightly decrease the pH. Reproduction of dispersion containing 0.5% diltiazem hydrochloride could be obtained with same pH in all three batches.

Table 10. Particle sizes of SLN containing diltiazem hydrochloride after autoclaving.

Formulation	Volume particle size (μm)							%Particle larger than		
	d(v,0.1)	d(v,0.5)	d(v,0.9)	d(4,3)	d(3,2)	span	uniformity	1μm	5μm	10μm
0.5Dil+5TP+3P407	0.18	2.29	4.92	2.32	0.50	2.07	0.71	63.33	9.54	0.00
1.0Dil+5TP+3P407	0.27	2.72	5.65	2.82	0.81	1.97	0.63	72.35	15.22	0.19
1.5Dil+5TP+3P407	0.27	2.36	5.96	2.71	0.75	2.41	0.79	67.01	16.05	1.07
0.5Dil+5TP+3P407(pH7)#1	0.16	0.31	1.44	0.71	0.28	4.09	1.57	10.05	2.94	0.15
0.5Dil+5TP+3P407(pH7)#2	0.21	0.32	2.62	0.81	0.33	7.42	1.72	14.24	2.98	0.00
0.5Dil+5TP+3P407(pH7)#3	0.23	0.31	0.45	0.33	0.30	0.70	0.21	0.00	0.00	0.00
1.0Dil+5TP+3P407(pH7)	0.19	0.35	4.54	1.41	0.35	12.38	3.32	29.15	7.83	0.12
1.5Dil+5TP+3P407(pH7)	0.23	2.16	6.08	2.56	0.55	2.71	0.95	56.52	17.05	0.92

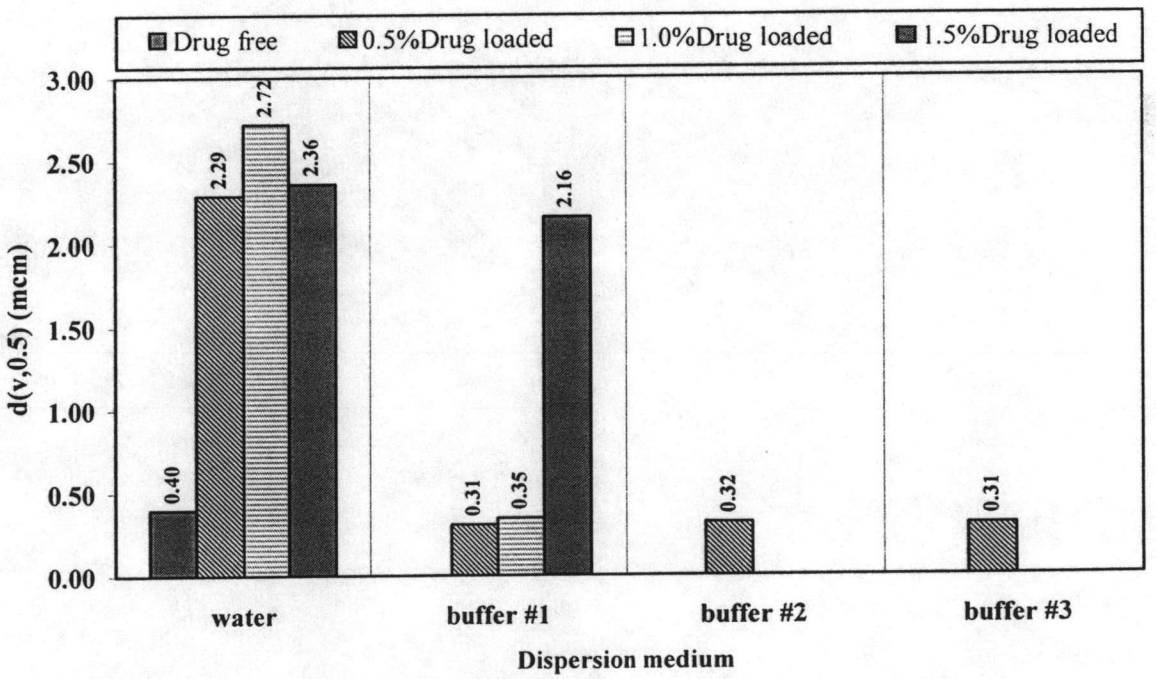

Figure 36. The d(v,0.5) of SLN containing diltiazem hydrochloride after autoclaving.

Table 11. The pH, osmolality, zeta potential, and viscosity of dispersions of SLN containing diltiazem hydrochloride after autoclaving.

Formulation	pH	Osmolality	Zeta potential (millivolts) (±SD)	Viscosity at the shear rate of $1000s^{-1}$ (mPa s) (±SD)
0.5Dil+5TP+3P407	4.64	0.029	-6.588 (± 1.449)	2.340 (± 0.139)
1.0Dil+5TP+3P407	4.37	0.044	-6.088 (± 1.421)	2.459 (± 0.169)
1.5Dil+5TP+3P407	4.38	0.060	-5.559 (± 1.281)	2.367 (± 0.220)
0.5Dil+5TP+3P407(pH7)#1	6.72	0.148	-19.998 (± 2.208)	1.205 (± 0.053)
0.5Dil+5TP+3P407(pH7)#2	6.74	0.149	-19.905 (± 2.071)	1.844 (± 0.042)
0.5Dil+5TP+3P407(pH7)#3	6.74	0.148	-19.819 (± 2.564)	1.960 (± 0.045)
1.0Dil+5TP+3P407(pH7)	6.53	0.164	-15.215 (± 1.600)	1.311 (± 0.049)
1.5Dil+5TP+3P407(pH7)	6.41	0.185	-13.030 (± 1.434)	1.474 (± 0.071)

3.1.4 Osmolality

As shown in Figure 38, diltiazem hydrochloride loaded SLN could affect the osmolality of preparations. Their preparations had higher osmolality than drug free preparation. Increasing the concentration of drug would increase these values. For preparations using buffer pH 7 as the dispersion medium, the osmolality was higher than preparations in water medium, but stilled lower than the value of isotonic solution. Reproduction of dispersion of SLN containing 0.5% diltiazem hydrochloride in buffer pH 7 gave the same osmolality in all three batches.

3.1.5 Zeta potential

All preparations containing diltiazem hydrochloride had negative charge as shown in Figure 39. Diltiazem hydrochloride could decrease the zeta potential of SLN. When water was used as dispersion medium, the zeta potential was very low compared to that of drug free preparation. Increasing the concentration of drug could decrease the negatively zeta potential to nearly zero. The values of –6.588 to –5.559 millivolts were observed. When buffer pH 7 was used instead of water, a slight decrease of zeta potential though higher than when using water was observed. Increasing drug concentration could also decrease the zeta potential.

Reproduction of dispersion of SLN containing 0.5% diltiazem hydrochloride in buffer pH 7 gave similar values in all three batches. The zeta potential values between –19.819 to –19.998 millivolts were obtained.

3.1.6 Viscosity

The viscosity of preparations of SLN containing diltiazem hydrochloride was not different from drug free preparation. Their viscosities were very low. Figure 40 shows that the viscosities of preparations in buffer pH 7 at shear rate of 1000 s^{-1} were lower than the other preparations. The drug concentration did not affect the viscosity. There were no differences in different concentration.

As shown in Figures 41-46, all preparations had the flow patterns that were similar to drug free preparation. Newtonian system was observed. Reproduction of dispersion of SLN containing 0.5% diltiazem hydrochloride in buffer pH 7 obtained similar viscosity patterns in all three batches.

3.1.7 Particle shape

The scanning electron photomicrograph of preparation of SLN containing 0.5% diltiazem hydrochloride in buffer pH 7 is shown in Figure 47. Small agglomerate particles were observed. Their shapes were likely to be sphere though not clear due to their agglomeration and had very small crystal fixed on their surfaces. In addition, no large particle could be seen.

3.1.8 Infrared spectra

Figure 48 shows diltiazem hydrochloride vibrational features in the 4000 to 400 cm^{-1}. The strong peaks appeared at 1680 cm^{-1} of lactam C=O stretching, 1744 cm^{-1} of acetate C=O stretching, and broad peak at 2391 cm^{-1} of amine HCl salt N-H stretching. There were small peaks at 781 and 839 cm^{-1} of substituted aromatic C-H out-of-plane deformation. Moreover, the aromatic C=C skeleton stretching showed the peaks at 1512, 1583, and 1608 cm^{-1}.

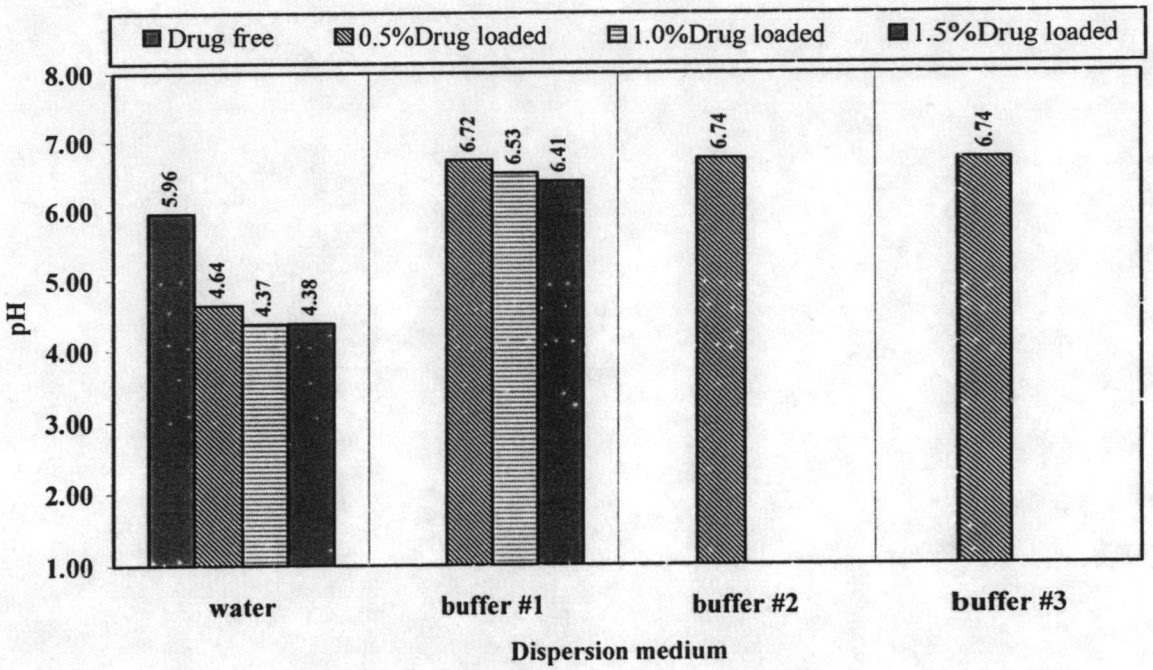

Figure 37. The pH of dispersions of SLN containing diltiazem hydrochloride after autoclaving.

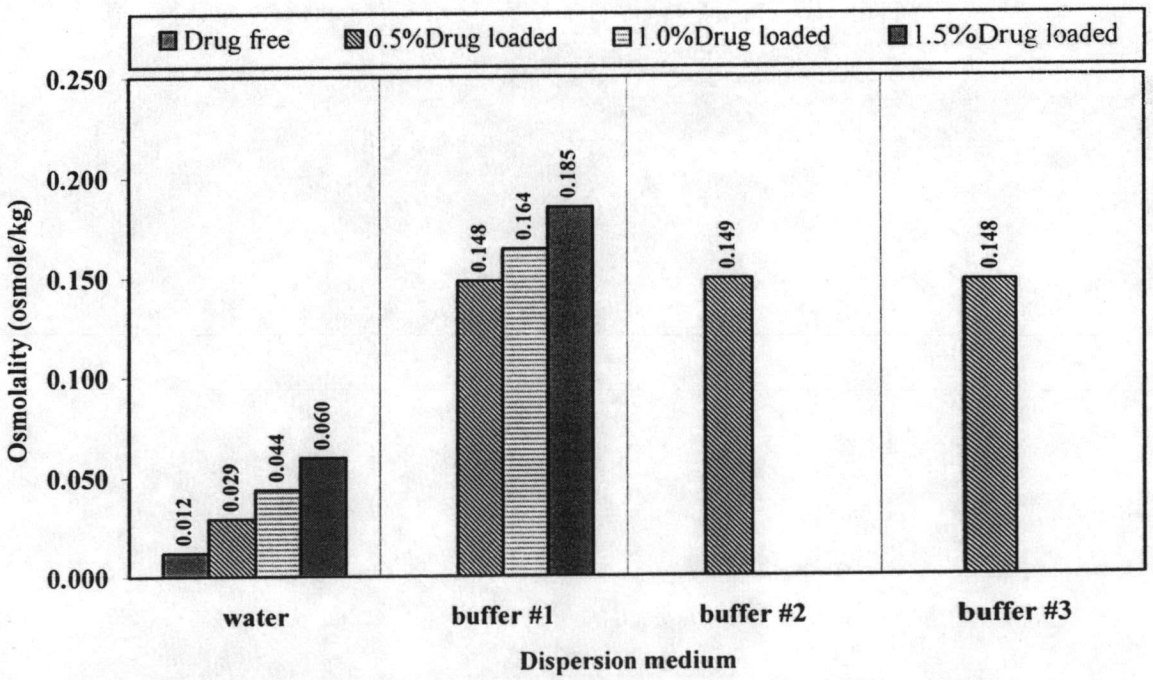

Figure 38. The osmolality of dispersions of SLN containing diltiazem hydrochloride after autoclaving.

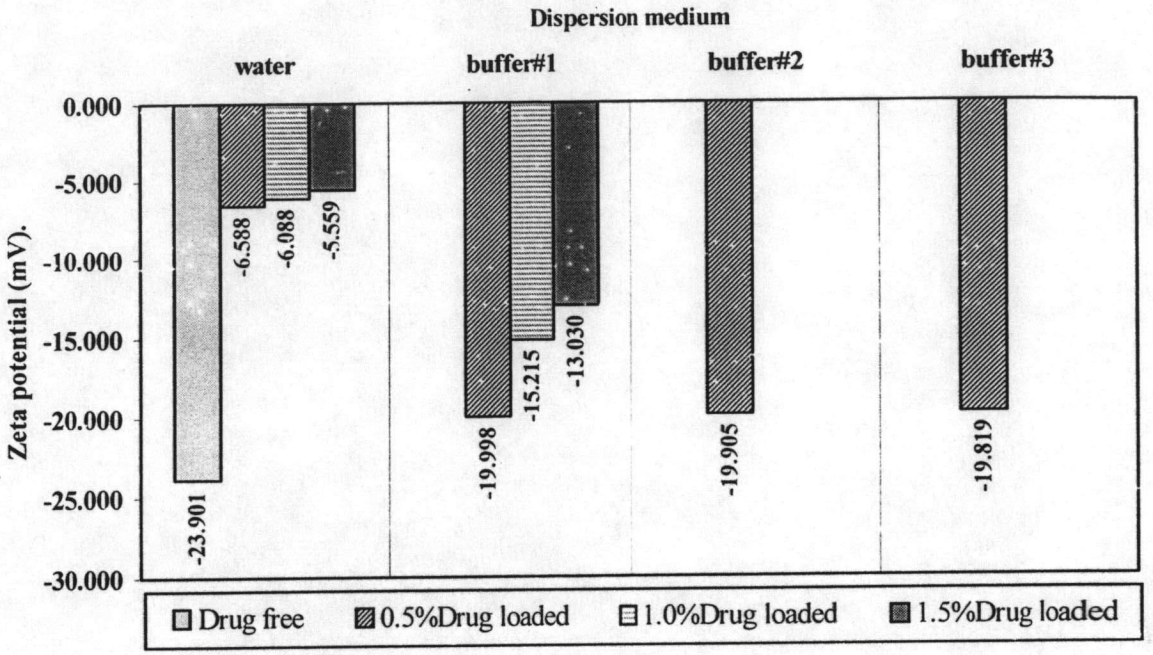

Figure 39. The zeta potential of dispersions of SLN containing diltiazem hydrochloride after autoclaving.

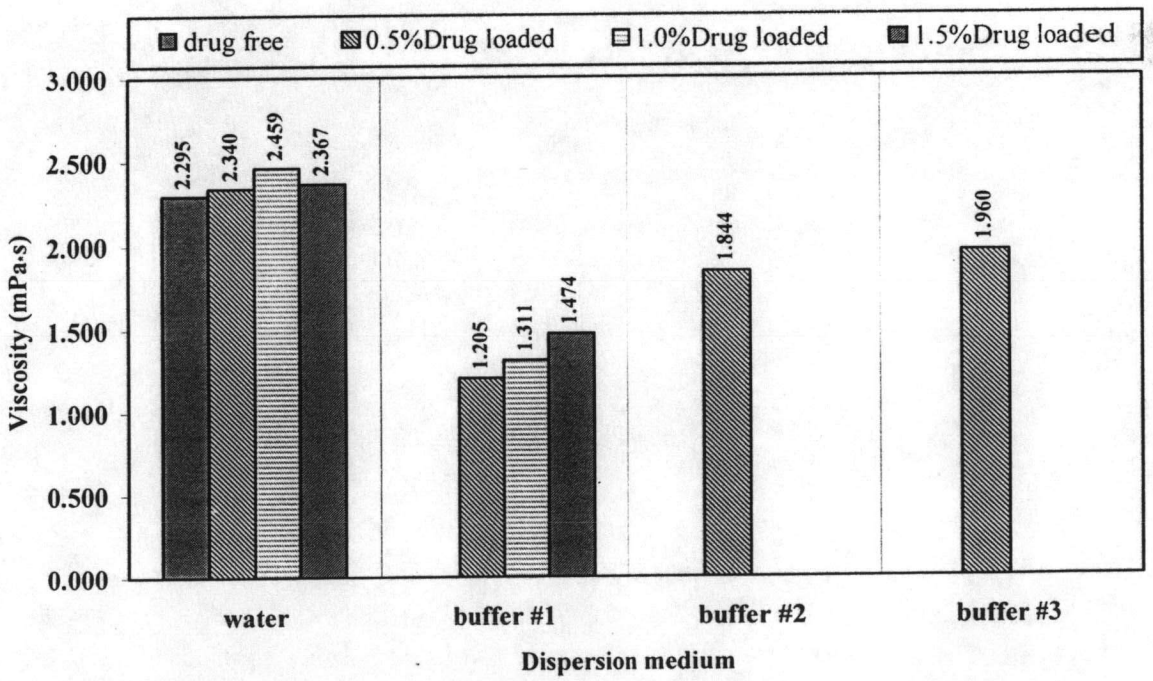

Figure 40. The viscosity at shear rate of 1000 s^{-1} of dispersions of SLN containing diltiazem hydrochloride after autoclaving.

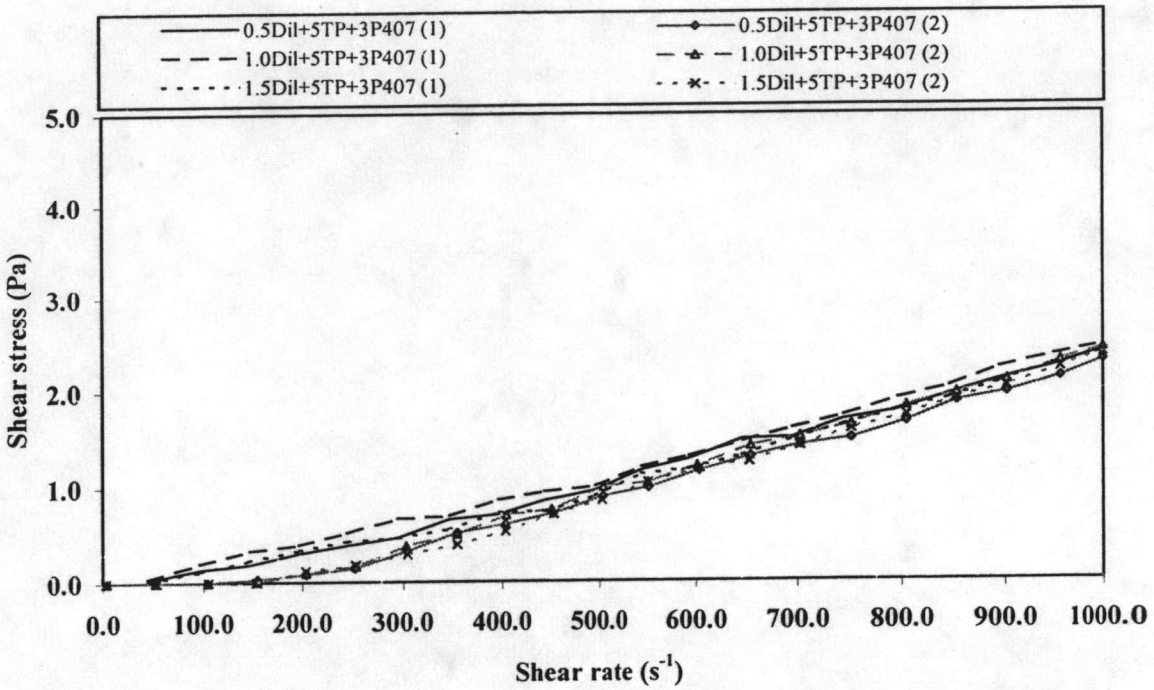

Figure 41. Flow curves of dispersions of SLN containing 0.5-1.5% diltiazem hydrochloride and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

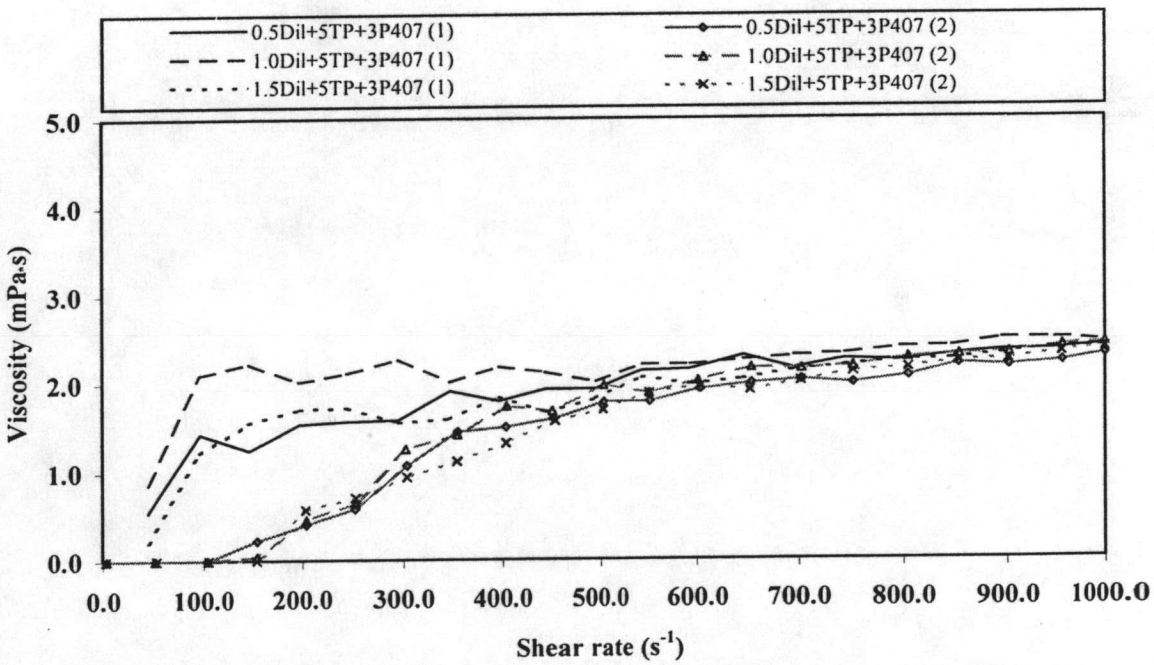

Figure 42. Viscosity curves of dispersions of SLN containing 0.5-1.5% diltiazem hydrochloride and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

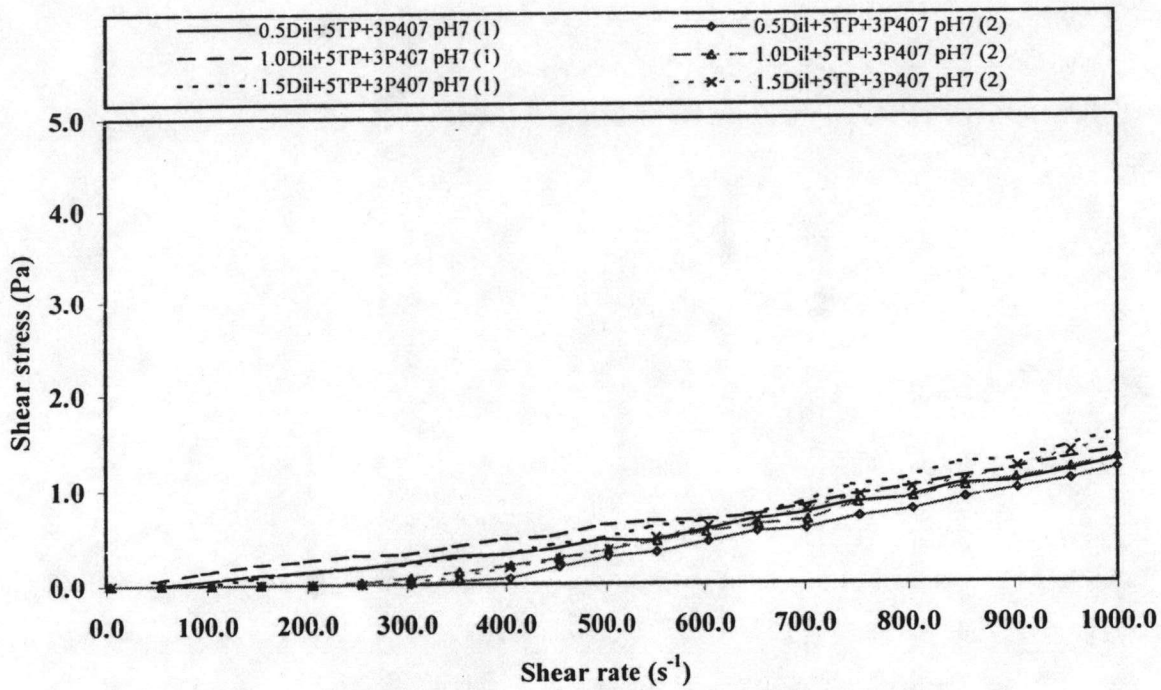

Figure 43. Flow curves of dispersions of SLN containing 0.5-1.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7 ((1) up-curve and (2) down-curve).

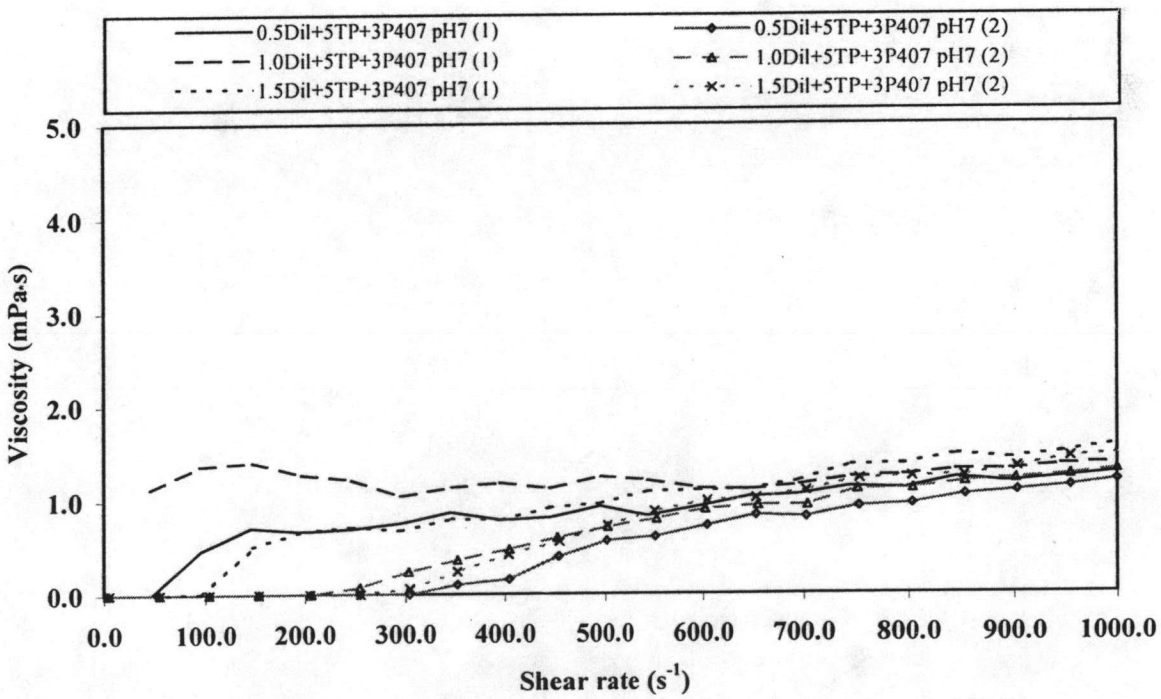

Figure 44. Viscosity curves of dispersions of SLN containing 0.5-1.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7 ((1) up-curve and (2) down-curve).

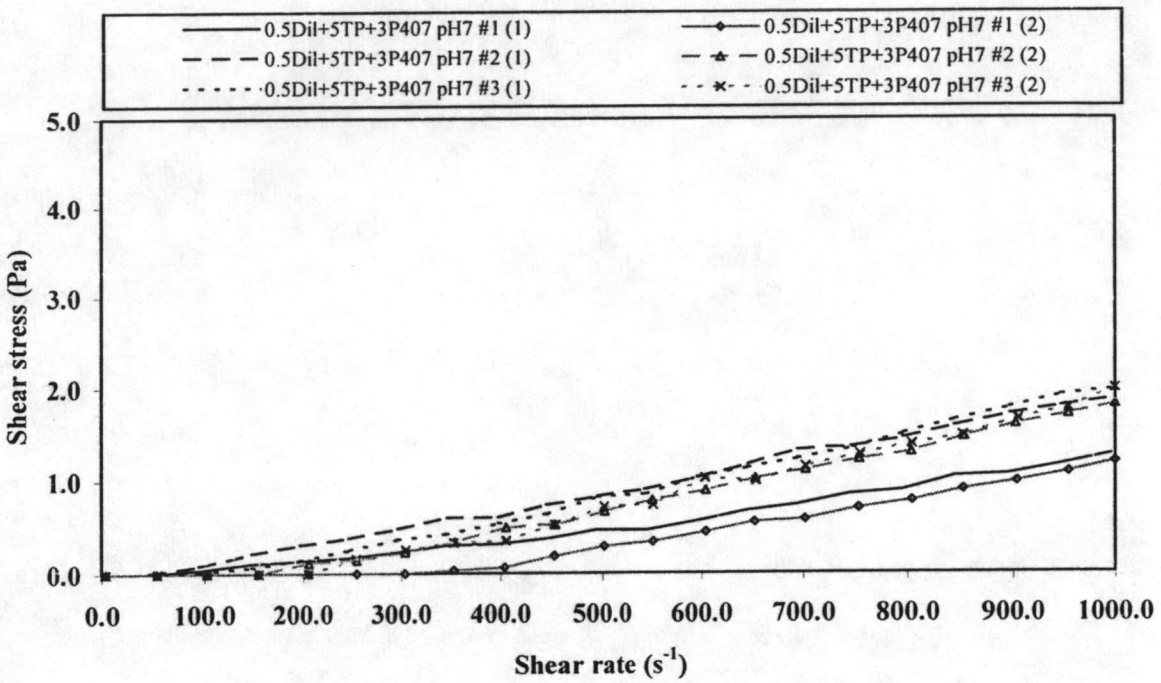

Figure 45. Flow curves of three batches of preparation containing 0.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7 ((1) up-curve and (2) down-curve).

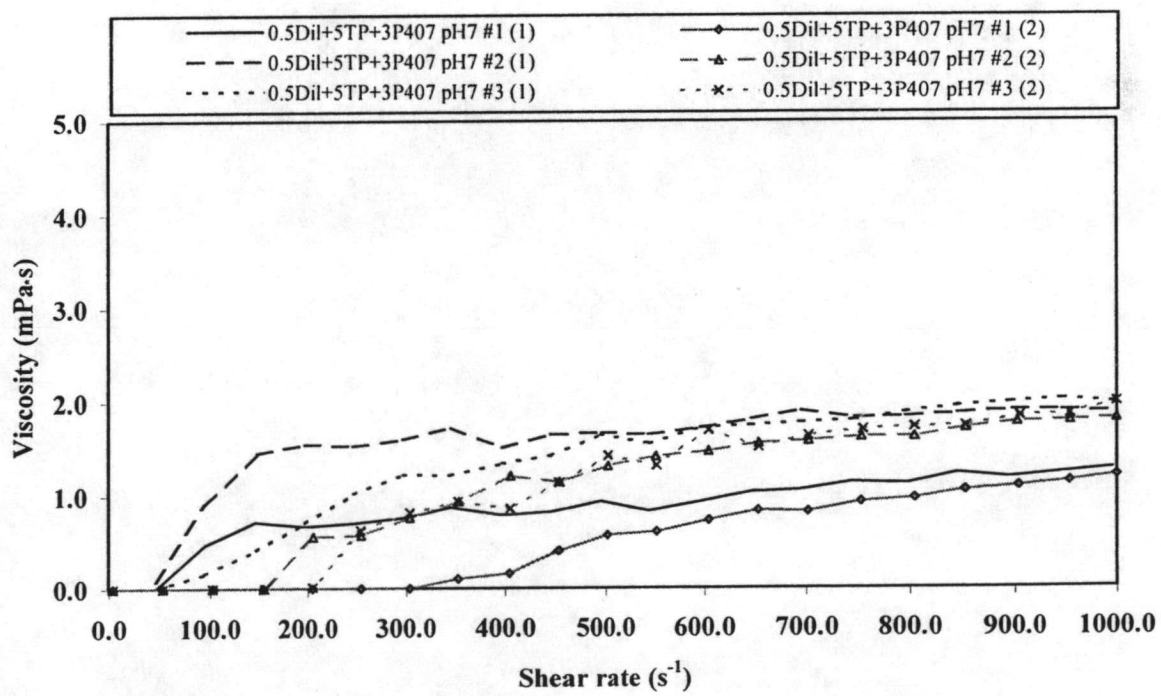

Figure 46. Viscosity curves of three batches of preparation containing 0.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7 ((1) up-curve and (2) down-curve).

Only preparation containing drug and poloxamer 407 were stable after autoclaving. Their IR spectra showed the basic peaks similar to pure tripalmitin as shown in Figures 49-50. Very small peaks of drug appeared at 1512, 1583, 1608, and 1681 cm^{-1}, and their intensities were higher when increased the drug concentration in the preparations. Moreover, the peak of poloxamer 407 was also appeared at 1113 cm^{-1}. No new peak was observed from the mixture.

The solid lipid floated on the surface of preparation containing tween 80 and egg lecithin was examined by infrared spectroscopy. Figure 51 shows the peaks of preparations containing drug and tween 80 that were similar to pure tripalmitin. The peaks of diltiazem hydrochloride and tween 80 were not shown in the spectra of solid lipid which meant no strong interaction between tripalmitin and other components. These other components were very soluble in dispersion medium, thus they did not partition into the lipid. The spectra of preparations containing drug and egg lecithin are shown in Figures 52-53. It was found that there were the basic peaks similar to pure tripalmitin, and showed the small peaks of drug at 1512, 1583, 1608, and 1681 cm^{-1}, and the broad heap peaks between 2731-2368 cm^{-1}. Their intensities were higher when increasing the concentration of drug and egg lecithin in preparations. No new peak was observed from its mixture. It showed that there was not interaction between tripalmitin and other components, and drug could be loaded into matrix.

3.1.9 Differential scanning calorimetry

The DSC thermograms of diltiazem hydrochloride, and solid lipid sedimented by ultracentrifugation of preparations containing drug are shown in Figure 54. Diltiazem hydrochloride showed the endotherm from 192.96°C to 216.40 °C with a peak at 210.51°C. This peak was not seen in the preparation of SLN containing drug. Thermogram of preparation of SLN containing 0.5% drug and poloxamer 407 in buffer pH 7 showed two endotherms in the range of 24.29°C to 52.11°C with the peak of 46.15°C and 52.11°C to 68.08°C with the peak at 62.98°C. These ranges were slightly lower than those from drug free solid lipid. The

thermogram of preparation of SLN containing 1.5% drug and poloxamer 407 in buffer pH 7 showed two endotherms in the range of 7.29°C to 51.59°C with the peak at 45.11°C and 51.08°C to 67.56°C with the peak of 62.98°C.

3.1.10 Powder X-ray diffraction

The X-ray diffractograms of diltiazem hydrochloride, and solid lipid sedimented by ultracentrifugation of preparations containing drug are shown in Figure 55. Diltiazem hydrochloride was crystalline and showed the peaks at 4.200°, 8.400°, and 16.820°, and the other small peaks between 9.960° to 38.400°. However, these peaks were not appeared when loaded into the solid lipid preparations. The diffractogram of preparation of SLN containing 0.5-1.5% drug and 3% poloxamer 407 in buffer pH 7 was similar to drug free preparation. Only crystalline of tripalmitin and small peaks of poloxamer 407 were observed.

3.1.11 Entrapment efficiency

Table 12 and Figure 56 show the entrapment efficiency of diltiazem hydrochloride in SLN. Diltiazem hydrochloride, the highly water soluble drug, could be loaded into solid lipid particles in low level. Increasing the percentage of drug would slightly decrease the entrapment efficiency. About 16.58, 15.74, and 14.69% of drug could be loaded into the particles of preparations containing 0.5%, 1.0%, and 1.5% drug, respectively. However, the entrapment efficiency could be improved by increasing the pH of preparation. When the phosphate buffer pH 7 was used as dispersion medium instead of water, the pH of preparations was increased (as shown in Figure 37) and the entrapment efficiency was higher than those of preparations in water medium. Increasing the percentage of drug loading, however, would slightly decrease the entrapment efficiency similarly as with water dispersion medium. About 53.15, 45.46, and 35.88% could be loaded into the particles of preparations containing 0.5%, 1.0%, and 1.5% drug, respectively. The reproduction of SLN containing 0.5% diltiazem hydrochloride in buffer pH 7 gave the entrapment efficiency of no difference in all three batches. They were between 53.15 to 54.48%.

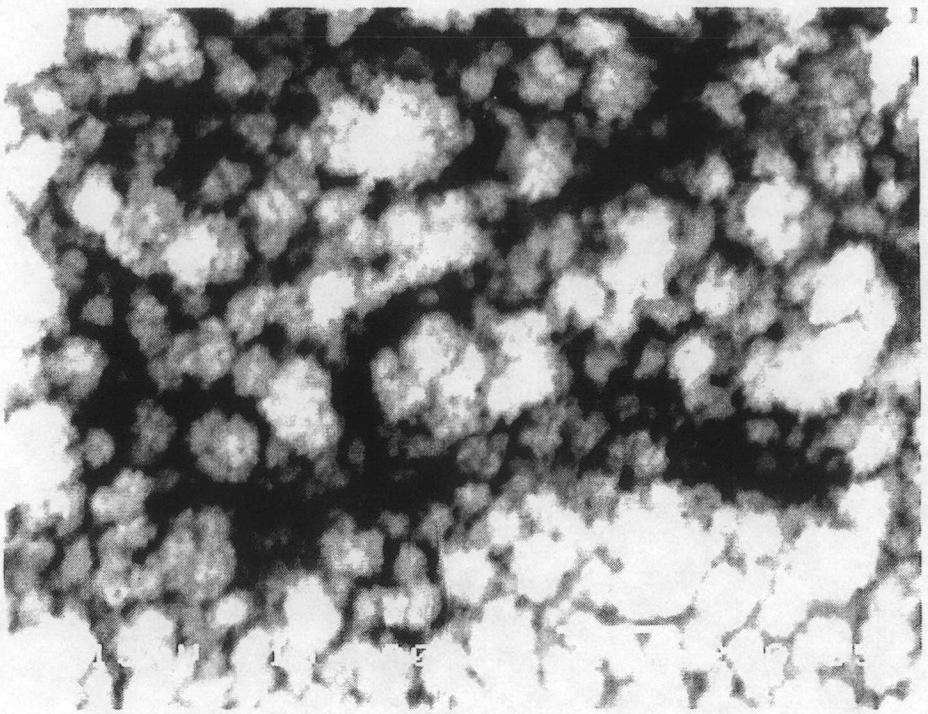

Figure 47. Cryo-scanning electron photomicrograph of SLN containing 0.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7.

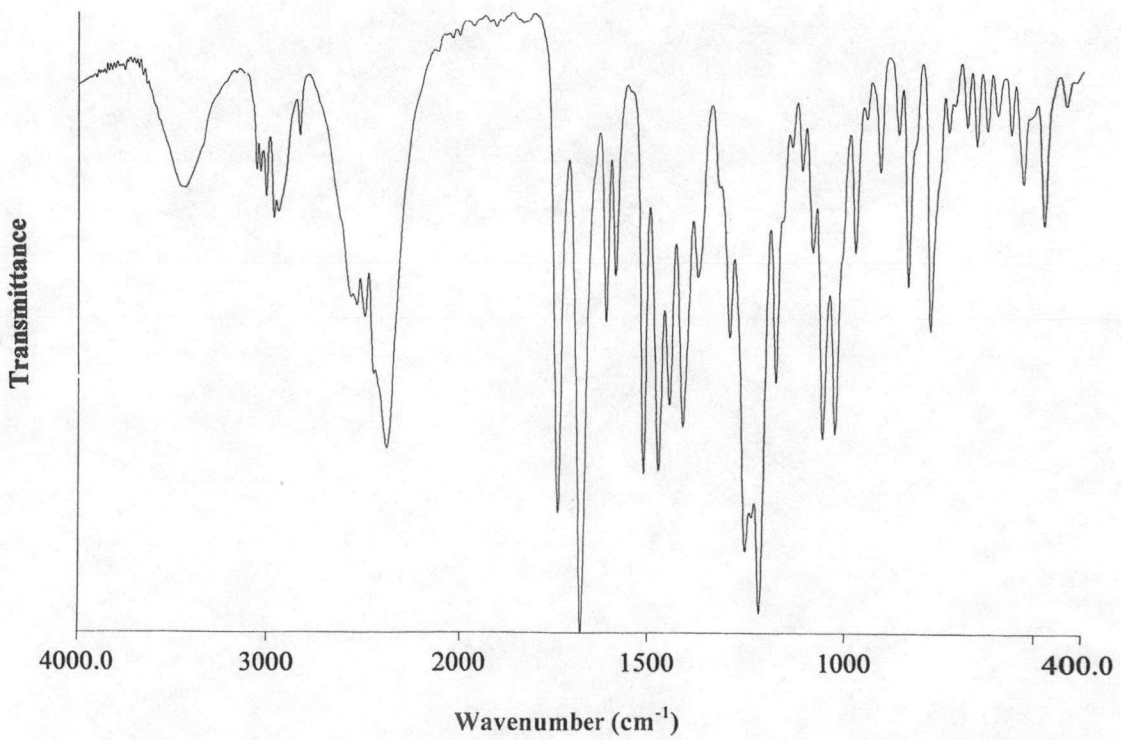

Figure 48. IR spectrum of diltiazem hydrochloride.

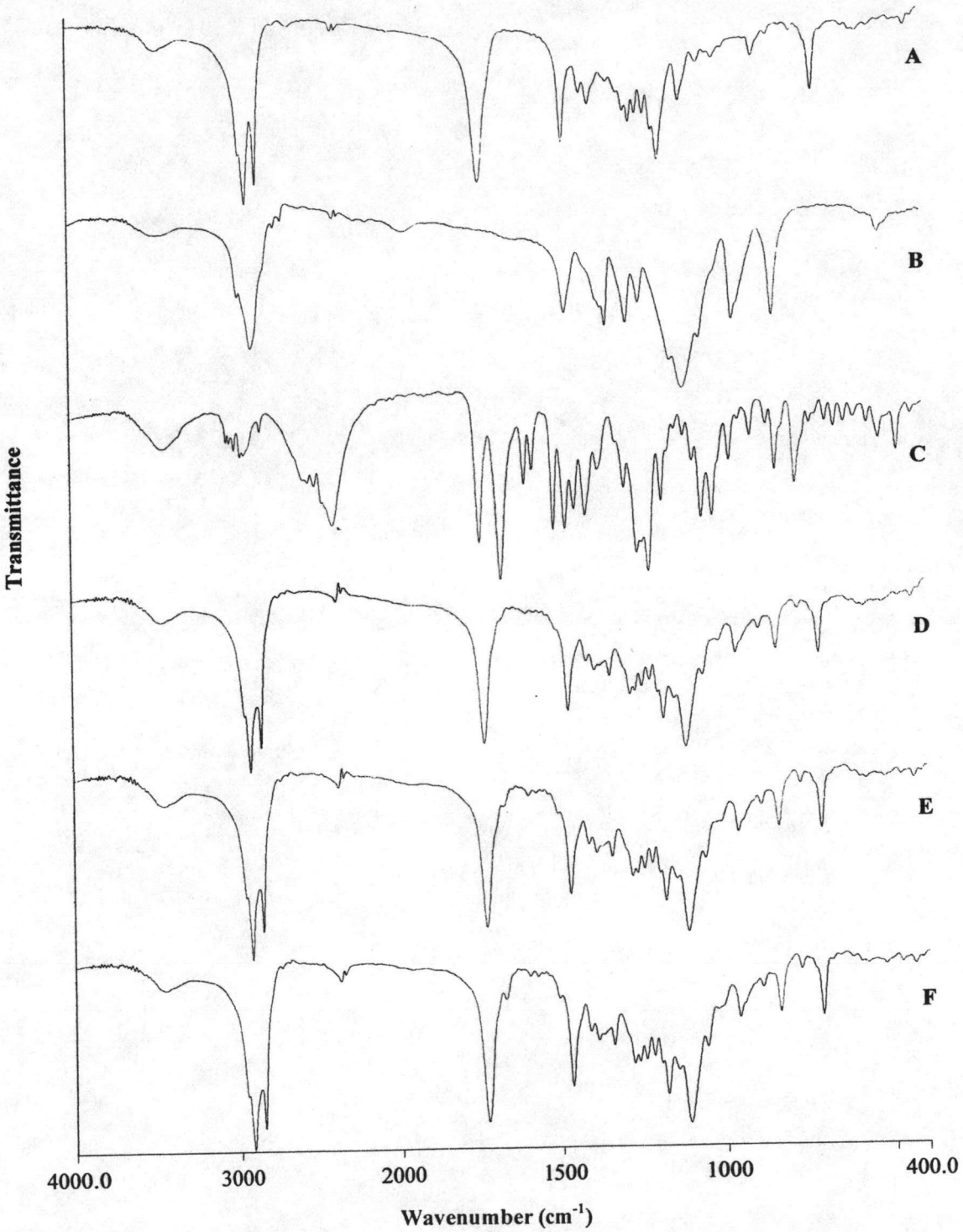

Figure 49. IR spectra of (A) tripalmitin; (B) poloxamer 407; (C) diltiazem hydrochloride; and lipid matrices of preparations containing (D) 0.5%,(E) 1.0%, and (F) 1.5% diltiazem hydrochloride and 3% poloxamer 407.

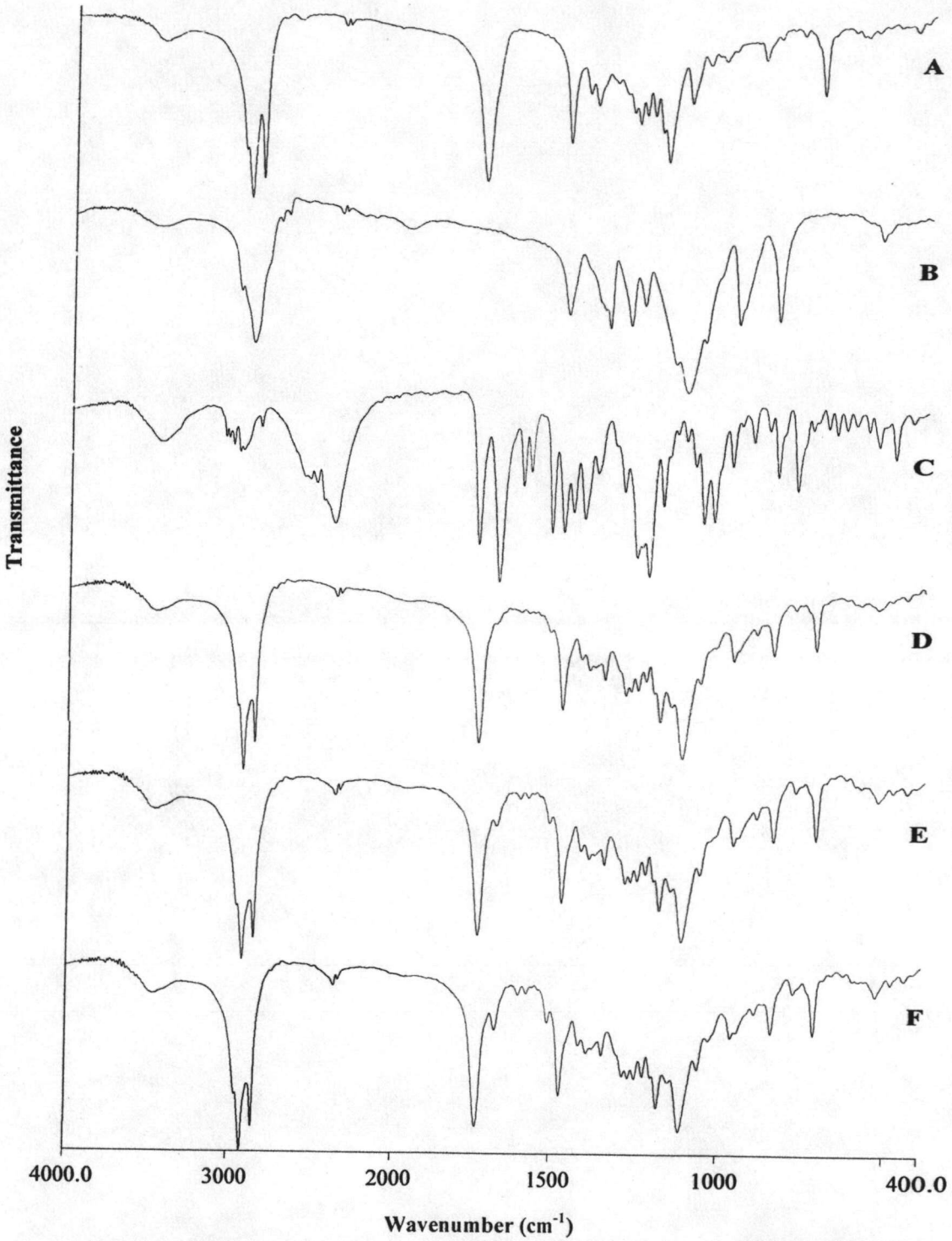

Figure 50. IR spectra of (A) tripalmitin; (B) poloxamer 407; (C) diltiazem hydrochloride; and lipid matrices of preparations containing (D) 0.5%,(E) 1.0%, and (F) 1.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7.

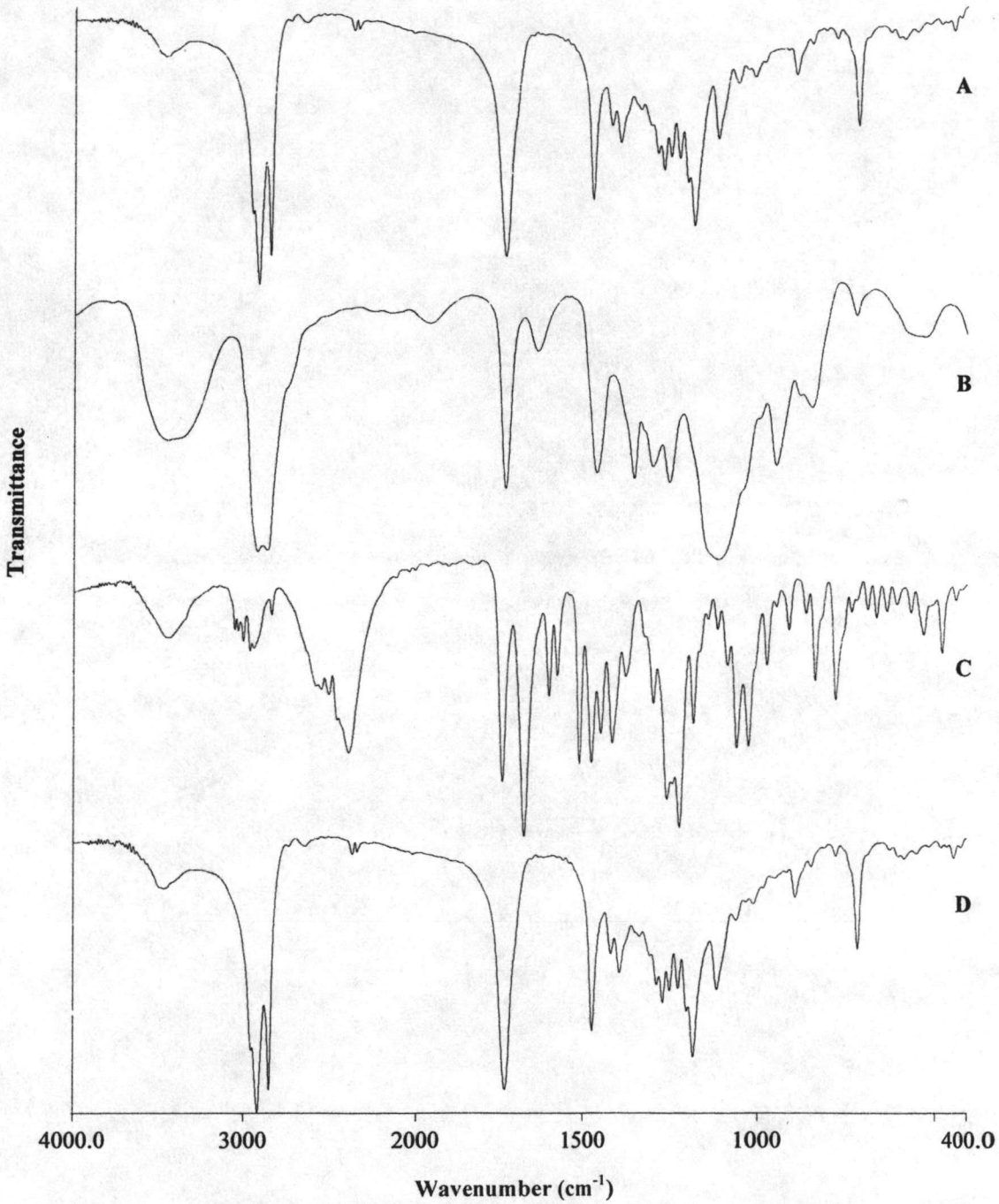

Figure 51. IR spectra of (A) tripalmitin; (B) tween 80; (C) diltiazem hydrochloride; and (D) lipid matrix of preparation containing 0.5% diltiazem hydrochloride and 2% tween 80.

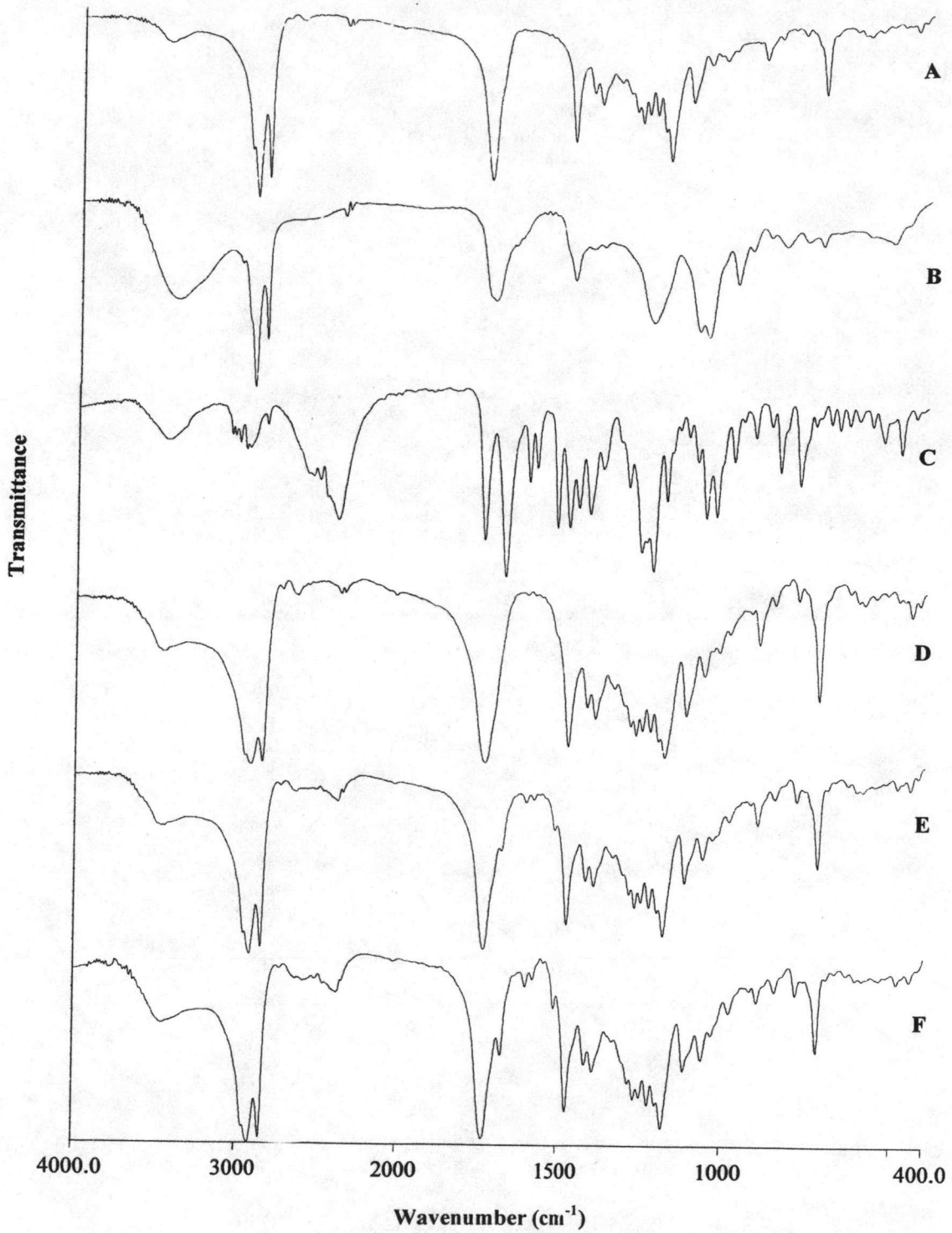

Figure 52. IR spectra of (A) tripalmitin; (B) egg lecithin; (C) diltiazem hydrochloride; and lipid matrices of preparations containing (D) 0.5%, (E) 1.0%, and (F) 1.5% diltiazem hydrochloride and 1% egg lecithin.

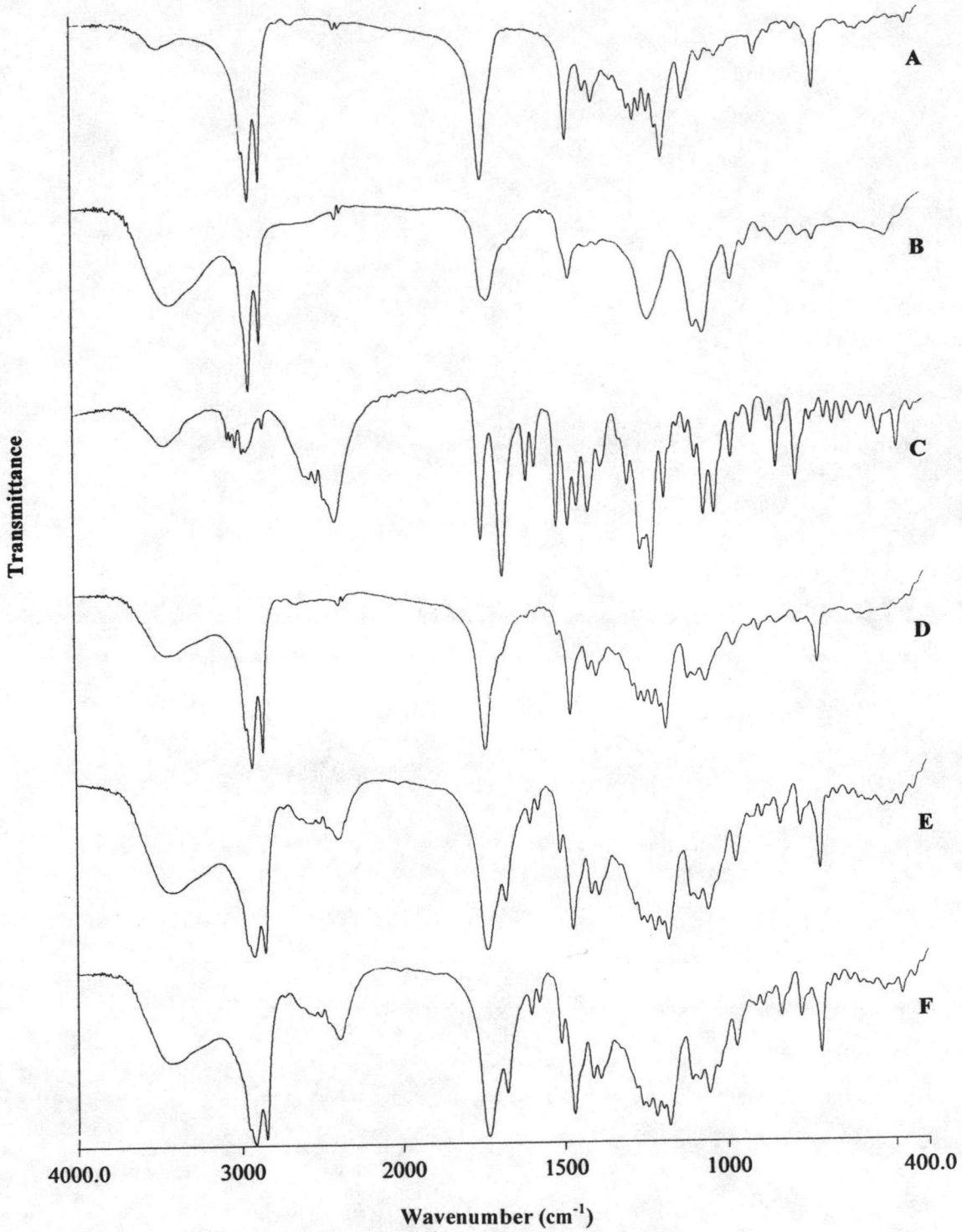

Figure 53. IR spectra of (A) tripalmitin; (B) egg lecithin; (C) diltiazem hydrochloride; and lipid matrices of preparations containing (D) 0.5%, (E) 1.0%, and (F) 1.5% diltiazem hydrochloride and 2% egg lecithin.

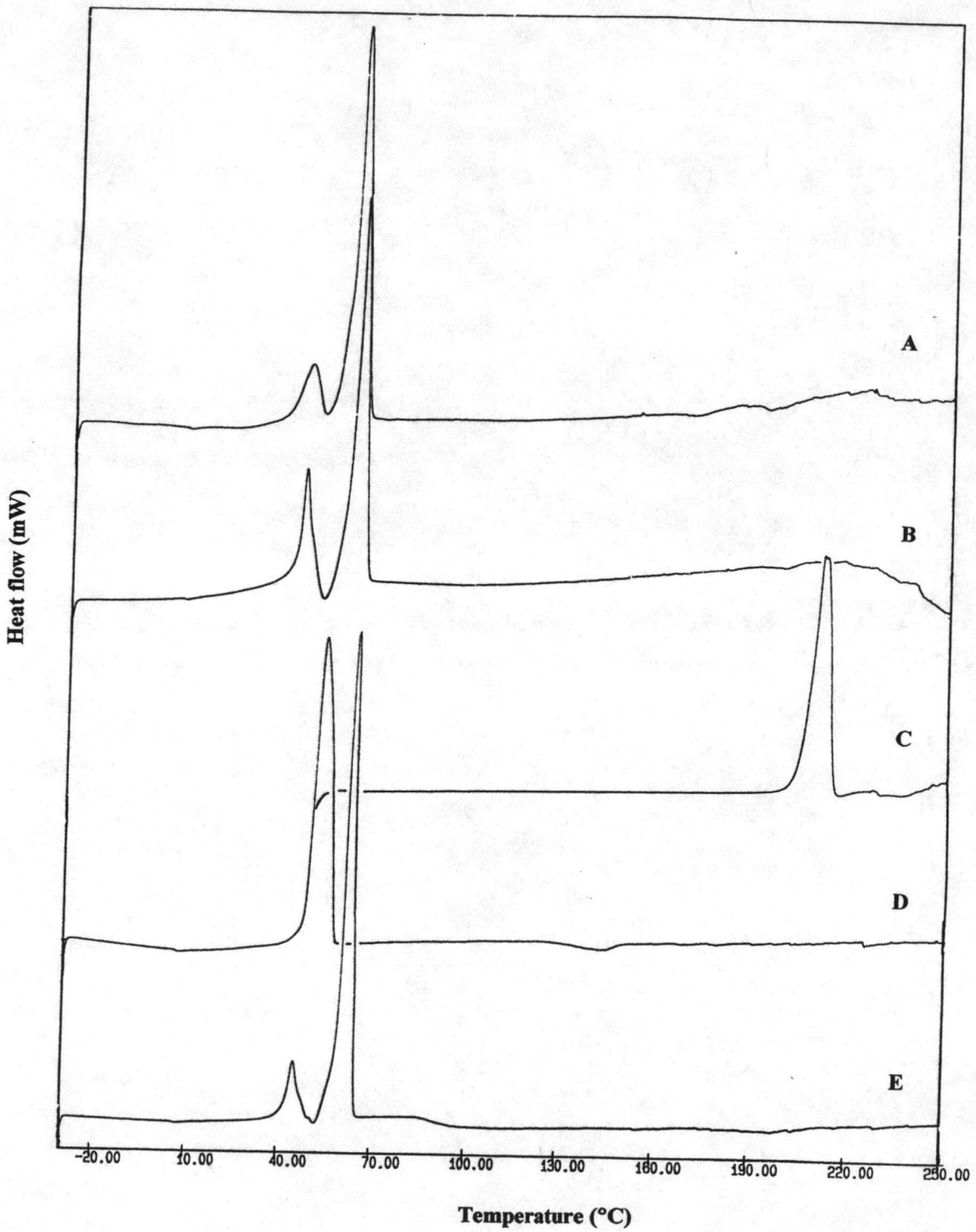

Figure 54. DSC thermograms of lipid matrices of preparations containing (A) 0.5%, (B) 1.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7; (C) diltiazem hydrochloride; (D) poloxamer 407; and (E) tripalmitin.

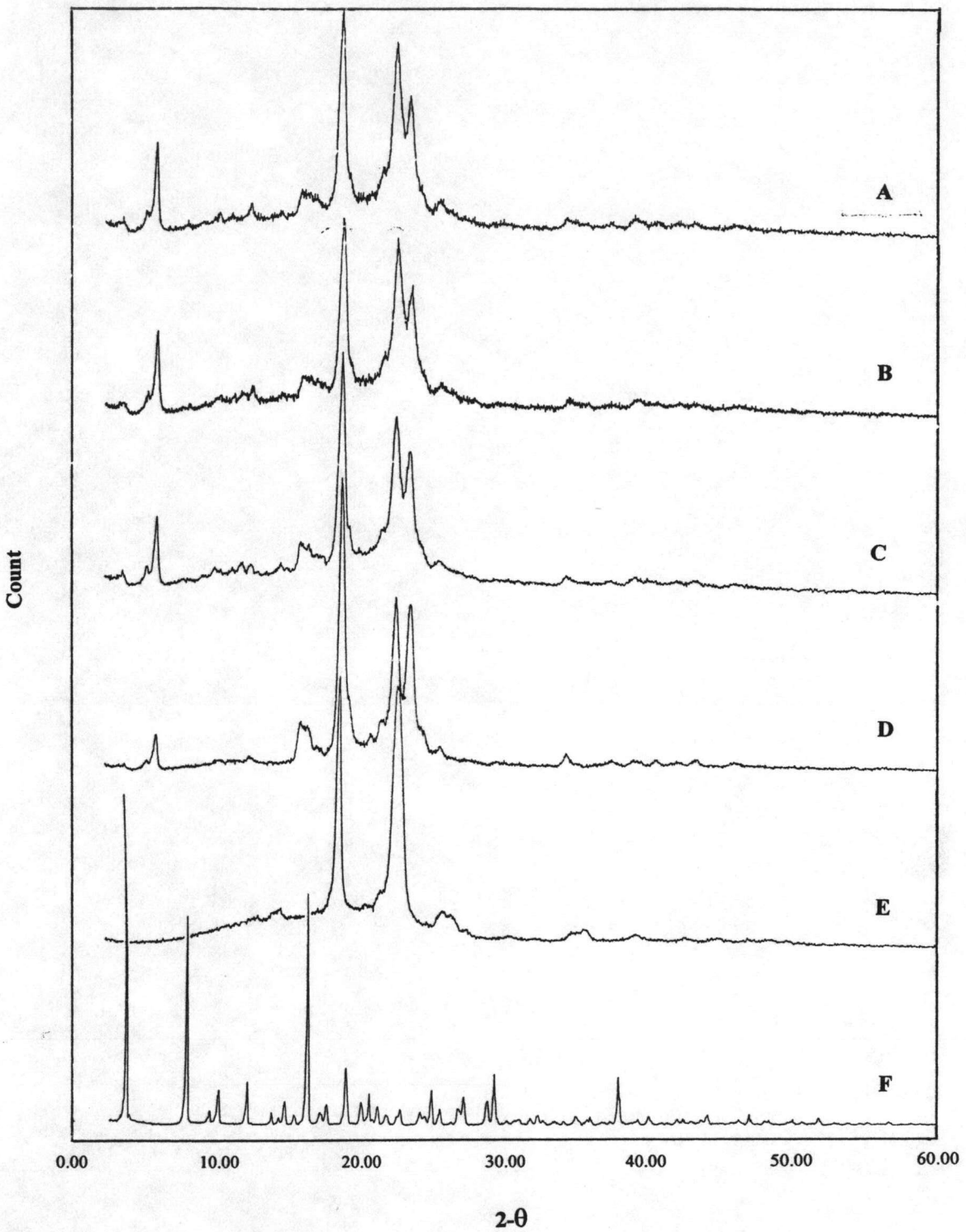

Figure 55. X-ray diffractograms of lipid matrices of preparations containing (A) 0.5%, (B) 1.0%, and (C) 1.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7; (D) tripalmitin; (E) poloxamer 407; and (F) diltiazem hydrochloride.

Table 12. Entrapment efficiency of diltiazem hydrochloride loaded into SLN after autoclaving.

Formulation	Percentage drug entrapment of SLN					
	No.1	No.2	No.3	mean	SD	%CV
0.5Dil+5TP+3P407	16.83	16.46	16.46	16.58	0.21	1.26
1.0Dil+5TP+3P407	16.28	15.38	15.56	15.74	0.48	3.05
1.5Dil+5TP+3P407	14.65	14.89	14.53	14.69	0.18	1.26
0.5Dil+5TP+3P407 (pH 7) batch 1	53.87	53.15	52.42	53.15	0.73	1.37
0.5Dil+5TP+3P407 (pH 7) batch 2	55.33	51.33	53.51	53.39	2.00	3.75
0.5Dil+5TP+3P407 (pH 7) batch 3	54.60	54.60	54.24	54.48	0.21	0.38
1.0Dil+5TP+3P407 (pH 7)	45.70	45.70	44.98	45.46	0.42	0.92
1.5Dil+5TP+3P407 (pH 7)	35.84	35.84	35.96	35.88	0.07	0.19

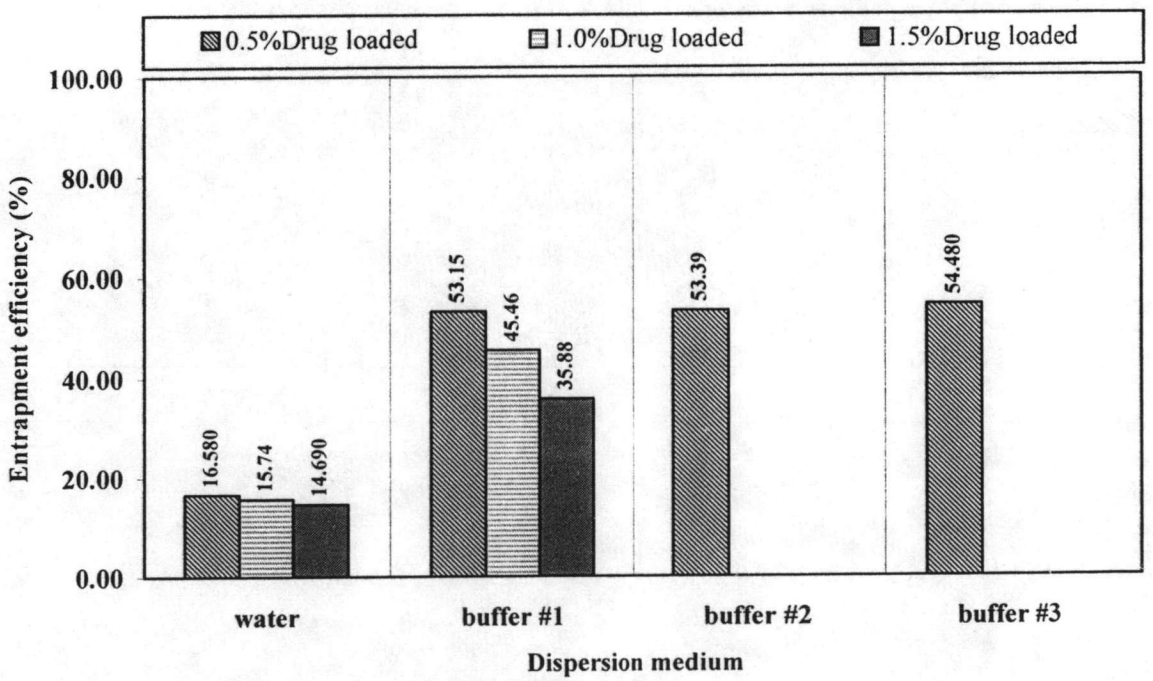

Figure 56. The entrapment efficiency of diltiazem hydrochloride loaded into SLN after autoclaving.

3.1.9 Drug release

In this study, five models of release kinetics: zero order, first order, Higuchi model, power expression model, and cube root model were used to elucidate the drug release model. For zero order model, the percentages of drug release were plotted with times. The first order model was plotted between logarithm of fraction of drug release and initial drug amount against times. While the Higuchi model was plotted between the percentages of drug release and the square root of times. The power expression model was plotted between logarithm of fraction of drug release and total drug against the logarithm of times. And the cube root model was plotted between cube root of the initial weight of drug minus cube root of weight remained against times. Therefore, the plots of these kinetic models of each sample were constructed. The higher coefficient of determination (R^2) was accepted as a model of drug release. Their rate constants were obtained from the correlation constant (k) of each equation, and could be used to compare in different preparations.

The equations for elucidating the drug release model are as follows:

Zero order model: $$Q = kt \quad (3)$$

First order model: $$\ln(Q / Q_0) = kt \quad (4)$$

Higuchi model: $$Q = kt^{1/2} \quad (5)$$

Power expression model: $$Q = kt^n \quad (6)$$

or $$\ln Q = \ln k + n \ln t \quad (7)$$

Cube root model: $$Q_0^{1/3} - Q_t^{1/3} = kt \quad (8)$$

Where Q was the amount of drug released at time t;

Q_o was the initial amount of drug;

k was the correlation constant; and

n was the power of expression model

The concentration of diltiazem hydrochloride saturated solution used in drug release studied was 480.30 mg/ml. As shown in Figure 57, the drug release of diltiazem hydrochloride saturated solution was sustained. Only 51.79% of drug was released within 24th hours. However, it was found that its drug release rate from saturated solution was the fastest. Its release kinetic was fitted with both zero order and power expression models.

As shown in Figure 58, the release profiles of both supernatants and dispersions of SLN containing diltiazem hydrochloride in water were higher than saturated solution. The drug releases from both supernatants and dispersions were very fast and came to the plateau states at 8th-10th hours. After the 10th hour, the drug was slowly released but slightly higher than the supernatant profile. Increasing percentage of drug loading showed no difference in the drug release profiles (as shown in Figure 58).

The dispersions of SLN containing 0.5-1.5% diltiazem hydrochloride in buffer pH 7 show the release profiles in Figure 59. The release profiles of both supernatants and dispersions were also higher than saturated solution. However, the release rate of saturated solution was the highest due to its high concentration in donor compartment. And the release rate of SLN with high drug concentration was higher than the lower drug concentrations. These release rates of both supernatant and dispersion of SLN containing drug are shown in Figure 61. The drug releases from supernatants were very fast and came to the plateau states at the 8th-10th hours. Whereas the drug release profiles of dispersions were not different from their supernatants within 4 hours. After 4 hours, drug release profiles from dispersions were higher than their supernatants. So data of drug release after 4 hours were used to elucidate the drug release model. However, total drug release data was also elucidated the drug release model to consider the release profile of drug from both supernatants and dispersions of SLN. Slightly higher drug release profile was observed with higher drug concentration in the first period, and inclined to be the

same at the 24th hour. Dispersion of SLN containing 0.5% diltiazem hydrochloride in buffer pH 7 showed the lowest drug release profile. It could be reproducible at the same physicochemical properties, and had the same release profiles as shown in Figure 60.

Tables 13 and 14 show the coefficient of determination of each preparation calculated from total drug release data and data after the 4th hour, respectively. Only dispersions of SLN in buffer pH 7 were elucidated because they had high entrapment efficiency and could sustain release beyond 24 hours. These data were average from three determinations. It was found that diltiazem hydrochloride was released from SLN followed both Higuchi and power expression models. The coefficients of determination both two models were not different in all preparations and were nearly integral.

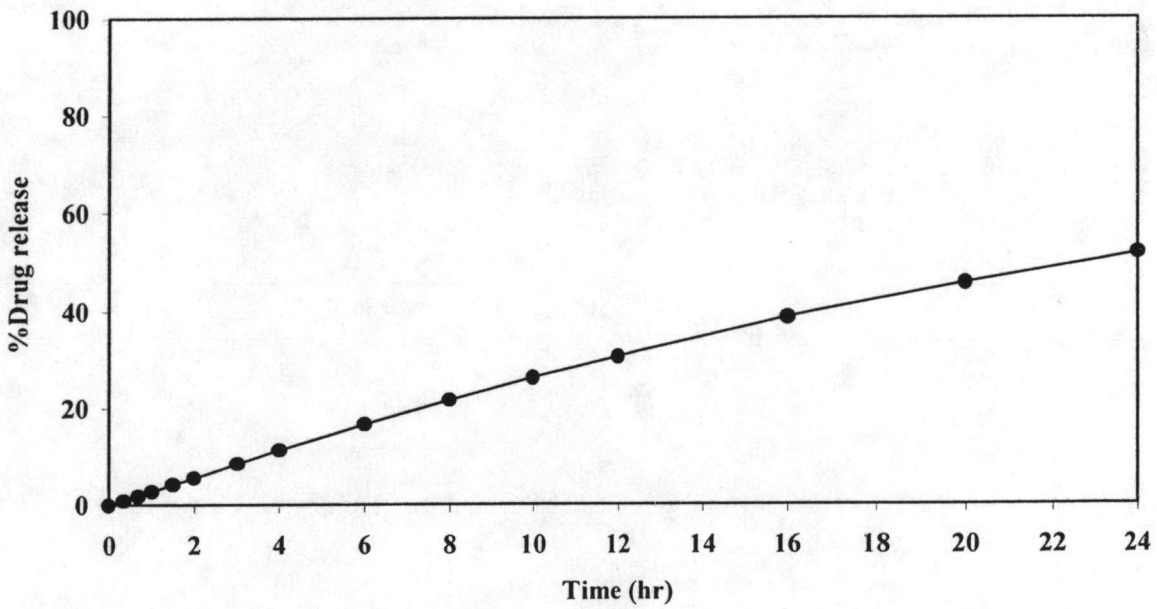

Figure 57. The release profile of diltiazem hydrochloride saturated solution.

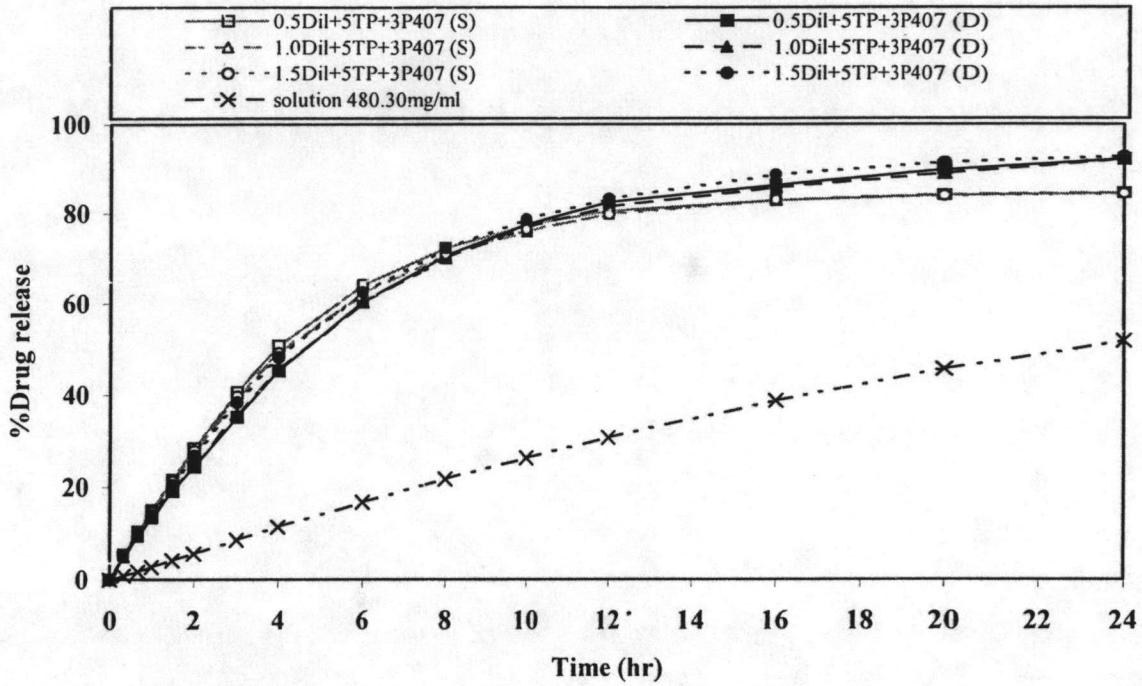

Figure 58. The release profiles of preparations containing 0.5-1.5% diltiazem hydrochloride and 3% poloxamer 407 ((S) supernatant and (D) dispersion).

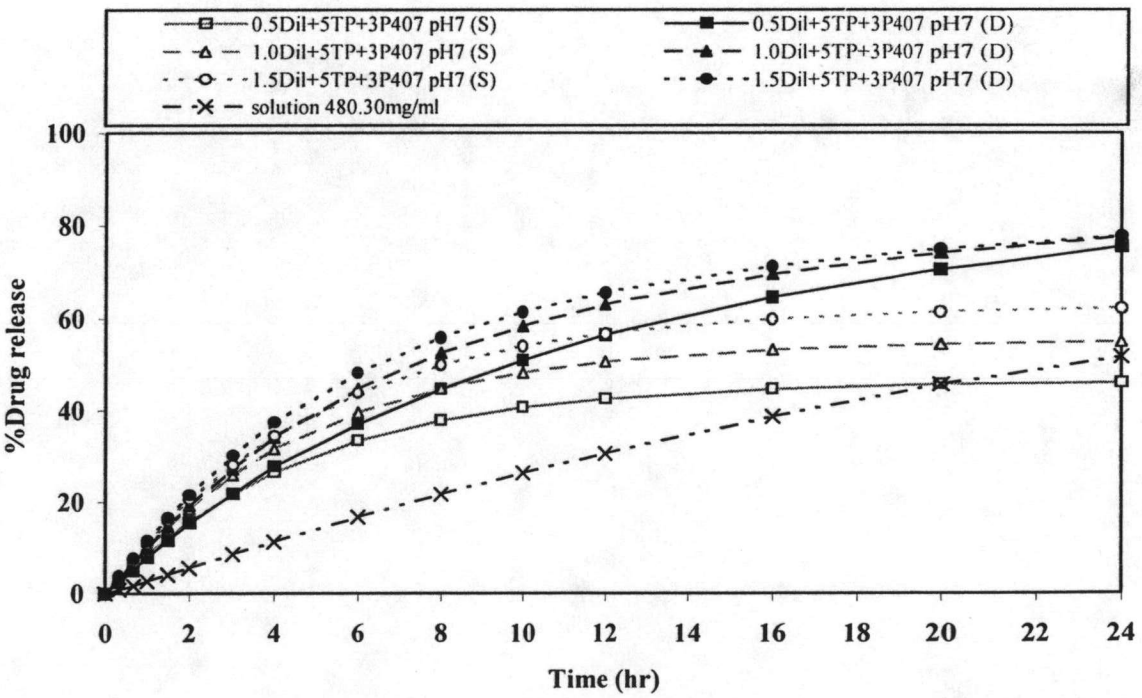

Figure 59. The release profiles of preparations containing 0.5-1.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7 ((S) supernatant and (D) dispersion).

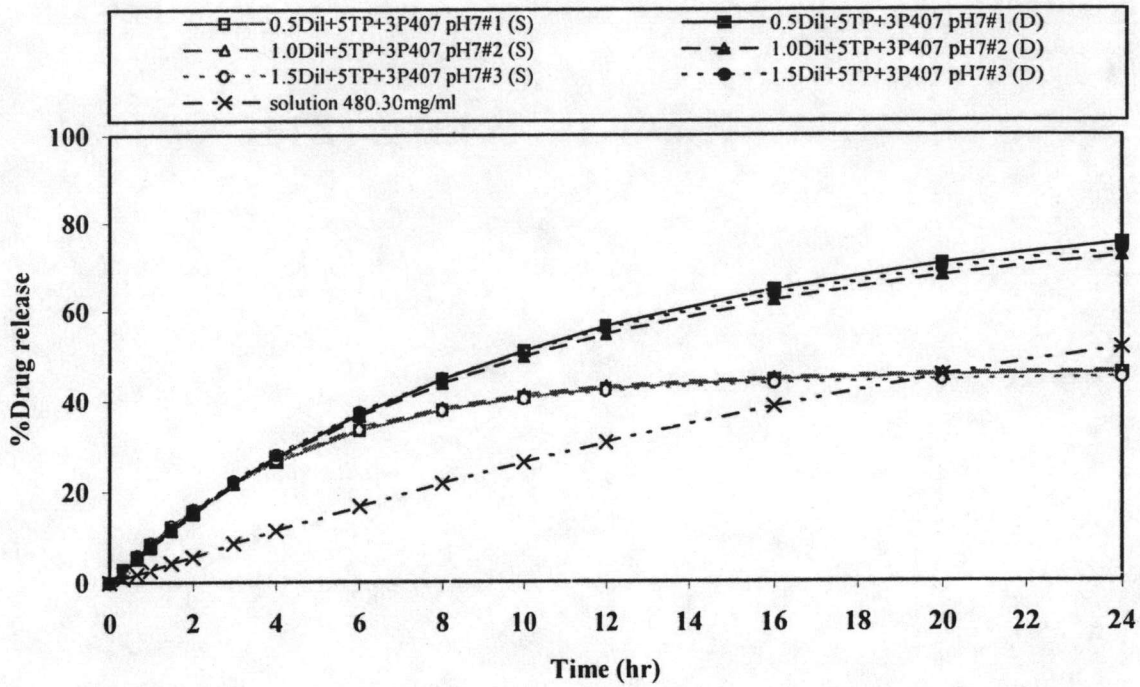

Figure 60. The release profiles of three batches of preparation containing 0.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7 ((S) supernatant and (D) dispersion).

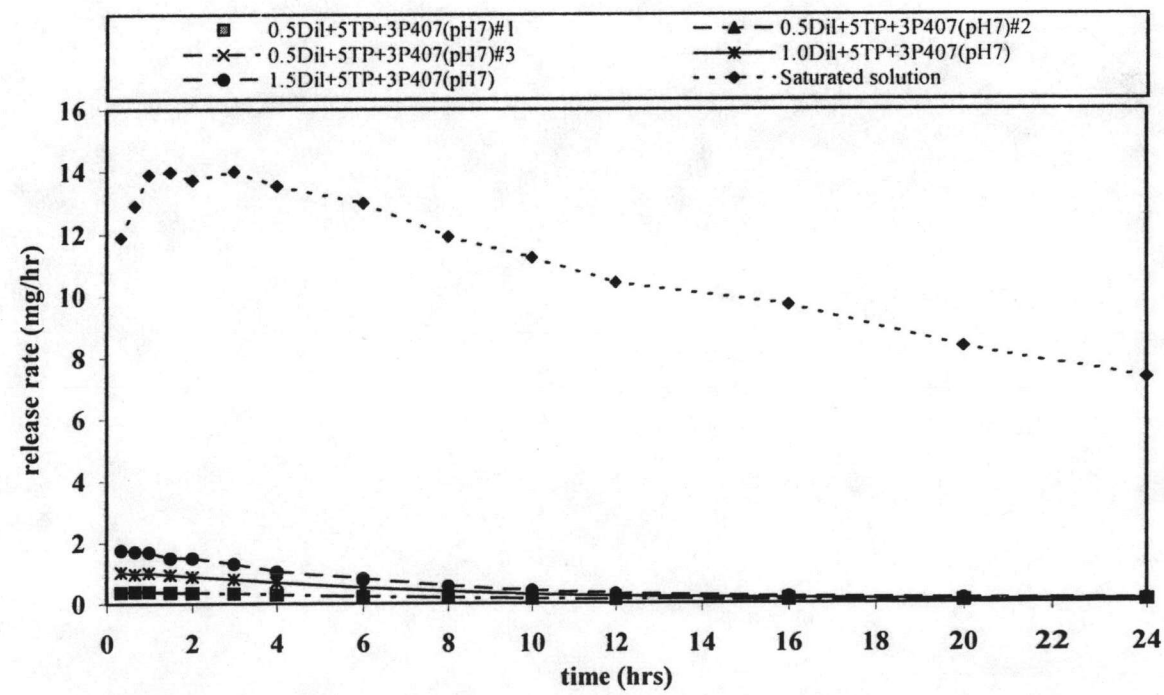

Figure 61. The release rate profiles of diltiazem hydrochloride saturated solution and dispersions containing 0.5-1.5% diltiazem hydrochloride and 3% poloxamer 407 in buffer pH 7.

Table 13. The coefficients of determination of dispersions and saturated solution of diltiazem hydrochloride in various drug release kinetics calculated from total drug release data.

Formulation	Coefficient of determination (R^2) (±SD)				
	Zero order model	First order model	Higuchi model	Power expression	Cube root model
Diltiazem hydrochloride saturated solution	0.9897 (±0.0020)	0.7369 (±0.0052)	0.9835 (±0.0016)	0.9971 (±0.0007)	0.9473 (±0.0034)
0.5Dil+5TP+3P407 (pH7) #1	0.9254 (±0.0069)	0.6665 (±0.0069)	0.9936 (±0.0015)	0.9859 (±0.0015)	0.8994 (±0.0046)
0.5Dil+5TP+3P407 (pH7) #2	0.9189 (±0.0031)	0.6599 (±0.0031)	0.9921 (±0.0007)	0.9843 (±0.0007)	0.8951 (±0.0020)
0.5Dil+5TP+3P407 (pH7) #3	0.9173 (±0.0077)	0.6597 (±0.0074)	0.9917 (±0.0019)	0.9843 (±0.0014)	0.8948 (±0.0046)
1.0Dil+5TP+3P407 (pH7)	0.8773 (±0.0092)	0.6317 (±0.0079)	0.9775 (±0.0036)	0.9709 (±0.0016)	0.8603 (±0.0037)
1.5Dil+5TP+3P407 (pH7)	0.8484 (±0.0068)	0.6122 (±0.0074)	0.9643 (±0.0029)	0.9584 (±0.0009)	0.8332 (±0.0013)

Table 14. The coefficients of determination of dispersions and saturated solution of diltiazem hydrochloride in various drug release kinetics calculated from drug release data after the 4^{th} hour.

Formulation	Coefficient of determination (R^2) (±SD)				
	Zero order model	First order model	Higuchi model	Power expression	Cube root model
Diltiazem hydrochloride saturated solution	0.9915 (±0.0021)	0.9092 (±0.0036)	0.9987 (±0.0004)	0.9971 (±0.0007)	0.9473 (±0.0034)
0.5Dil+5TP+3P407 (pH7) #1	0.9482 (±0.0045)	0.8684 (±0.0049)	0.9881 (±0.0022)	0.9859 (±0.0015)	0.8994 (±0.0046)
0.5Dil+5TP+3P407 (pH7) #2	0.9445 (±0.0021)	0.8650 (±0.0023)	0.9865 (±0.0011)	0.9843 (±0.0007)	0.8951 (±0.0020)
0.5Dil+5TP+3P407 (pH7) #3	0.9437 (±0.0048)	0.8645 (±0.0048)	0.9860 (±0.0024)	0.9843 (±0.0014)	0.8948 (±0.0046)
1.0Dil+5TP+3P407 (pH7)	0.9091 (±0.0045)	0.8320 (±0.0032)	0.9660 (±0.0028)	0.9709 (±0.0016)	0.8603 (±0.0037)
1.5Dil+5TP+3P407 (pH7)	0.8805 (±0.0017)	0.8069 (±0.0020)	0.9470 (±0.0012)	0.9584 (±0.0009)	0.8332 (±0.0013)

3.2 Theophylline loaded SLN

3.2.1 Physical appearance

The physical appearances of dispersions of SLN containing theophylline both before and after sterilization by autoclaving are shown in Table 15. When using poloxamer 407 of 3% as stabilizer, only the preparations of 0.25-0.75% drug were stable both before and after autoclaving, and after storage at room temperature. Stable preparation of higher concentrations could be produced and sterilized by autoclaving, but the drug crystallization occurred after cooling down the preparation. When tween 80 of 2-3% was used good dispersions could also be produced both before and after autoclaving. However, these preparations precipitated after storage for 1 month. When using egg lecithin of 2% as stabilizer, theophylline of 0.25-0.75% could be loaded to give good preparations both before and after autoclaving. However, only 0.25% drug could be loaded when 1% egg lecithin was used. The preparations of 0.50-0.75% drug separated after autoclaving, the oily phase floated on the surface.

3.2.2 Particle size

The particle sizes of SLN containing theophylline are shown in Table 16. These data showed high percentage of particle larger than 1 μm in all preparations. When using poloxamer 407 as stabilizer, the d(v,0.5) of preparations of drug were slightly higher than drug free preparation. The particle sizes decreased with increasing the drug concentrations. When using egg lecithin as stabilizer, the increasing of particle sizes occurred with increasing the drug concentrations. These preparations produced particles larger than the preparations containing poloxamer 407. Increasing the concentration of egg lecithin in the formulation could decrease the particle size. Tween 80 as stabilizer, however, did not affect the particle size. The particle sizes of preparations of 0.25-0.75% theophylline using tween 80 of 2% as stabilizer were lower than drug free preparation, but still higher than 1 μm which were not suitable for intravenous administration.

Table 15. The physical appearances of dispersions of SLN containing theophylline.

Formulation	Macroscopic observation	
	Before autoclaving	After autoclaving
0.25Theo+5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
0.50Theo+5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
0.75Theo+5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
1.0Theo+5TP+3P407	White fluid dispersion	Drug recrystallization after cooling
5.0Theo+5TP+3P407	White fluid dispersion	Drug recrystallization after cooling
0.25Theo+5TP+2T80	White fluid dispersion	White fluid dispersion; sediment after storage
0.50Theo+5TP+2T80	White fluid dispersion	White fluid dispersion; sediment after storage
0.75Theo+5TP+2T80	White fluid dispersion	White fluid dispersion; sediment after storage
0.25Theo+5TP+3T80	Bluish white fluid dispersion	White fluid dispersion; sediment after storage
0.50Theo+5TP+3T80	Bluish white fluid dispersion	White fluid dispersion; sediment after storage
0.75Theo+5TP+3T80	Bluish white fluid dispersion	White fluid dispersion; sediment after storage
0.25Theo+5TP+1EL	Brown fluid dispersion	Brown fluid dispersion
0.50Theo+5TP+1EL	Brown fluid dispersion	Separation; solid lipid floating on the surface
0.75Theo+5TP+1EL	Brown fluid dispersion	Separation; solid lipid floating on the surface
0.25Theo+5TP+2EL	Brown fluid dispersion	Brown fluid dispersion
0.50Theo+5TP+2EL	Brown fluid dispersion	Brown fluid dispersion
0.75Theo+5TP+2EL	Brown fluid dispersion	Brown fluid dispersion

3.2.3 pH

The pH of dispersions of SLN containing theophylline was not different with drug free SLN with all stabilizers as shown in Figure 63. Preparations containing egg lecithin gave the lower pH than the preparations of other two stabilizers. Drug concentrations did not affect their pH. These results showed that type of stabilizers showed more dominant effect to the pH of preparations than the drug concentrations.

Table 16. Particle sizes of SLN containing theophylline after autoclaving.

Formulation	Volume particle size (μm)							%Particle larger than		
	d(v,0.1)	d(v,0.5)	d(v,0.9)	d(4,3)	d(3,2)	span	uniformity	1μm	5μm	10μm
0.25Theo+5TP+3P407	0.19	2.01	5.48	2.46	0.58	2.62	0.84	67.81	12.81	0.62
0.50Theo+5TP+3P407	0.13	0.93	4.66	1.80	0.37	4.87	1.60	49.06	8.32	0.48
0.75Theo+5TP+3P407	0.13	0.76	4.64	1.74	0.35	5.93	1.93	46.92	8.03	0.10
0.25Theo+5TP+2T80	0.63	2.64	11.02	4.69	1.51	3.93	1.34	76.17	33.90	12.24
0.50Theo+5TP+2T80	1.24	5.50	13.10	6.75	3.20	2.15	0.71	94.22	54.72	19.34
0.75Theo+5TP+2T80	1.54	3.98	8.02	4.42	3.05	1.63	0.51	98.46	36.21	3.48
0.25Theo+5TP+1EL	8.15	30.62	68.43	37.08	5.45	1.97	0.66	95.46	92.62	88.03
0.25Theo+5TP+2EL	1.21	11.82	25.79	13.14	2.18	2.08	0.61	90.50	80.83	58.47
0.50Theo+5TP+2EL	6.94	31.72	75.41	39.44	4.91	2.16	0.71	94.96	91.65	87.01
0.75Theo+5TP+2EL	7.46	36.99	84.62	44.85	4.41	2.09	0.68	93.62	91.52	88.26

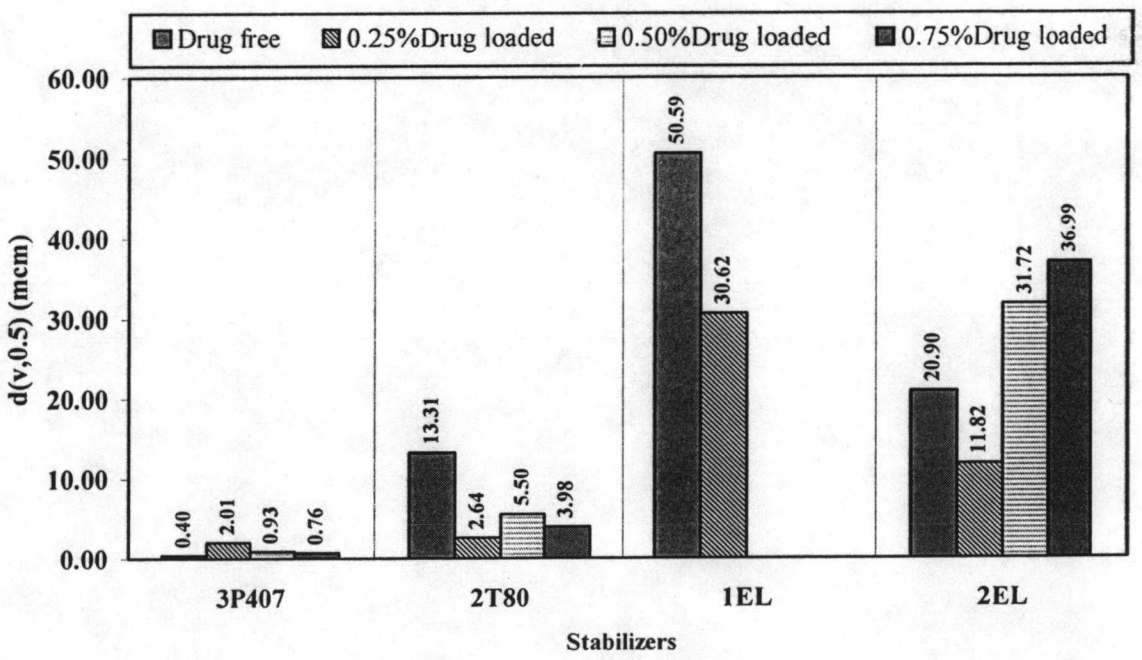

Figure 62. The d(v,0.5) of SLN containing theophylline after autoclaving.

Table 17. The pH, osmolality, zeta potential, and viscosity of dispersions of SLN containing theophylline after autoclaving.

Formulation	pH	Osmolality	Zeta potential (millivolts) (±SD)	Viscosity at the shear rate of $1000s^{-1}$ (mPa s) (±SD)
0.25Theo+5TP+3P407	5.22	0.021	-24.064 (± 1.949)	2.947 (± 0.082)
0.50Theo+5TP+3P407	5.49	0.031	-23.549 (± 2.396)	2.298 (± 0.053)
0.75Theo+5TP+3P407	5.53	0.038	-23.166 (± 1.995)	2.625 (± 0.085)
0.25Theo+5TP+2T80	5.59	0.017	-24.485 (± 1.770)	23.676 (± 0.748)
0.50Theo+5TP+2T80	5.55	0.027	-24.066 (± 1.657)	22.410 (± 0.592)
0.75Theo+5TP+2T80	5.23	0.034	-23.606 (± 1.914)	18.254 (± 0.578)
0.50Theo+5TP+3T80	5.55	0.027	-22.373 (± 2.514)	18.441 (± 1.533)
0.25Theo+5TP+1EL	3.62	0.024	-28.760 (± 1.877)	5.099 (± 0.240)
0.25Theo+5TP+2EL	3.65	0.031	-30.216 (± 2.283)	15.848 (± 0.802)
0.50Theo+5TP+2EL	3.86	0.037	-29.342 (± 1.913)	10.474 (± 1.439)
0.75Theo+5TP+2EL	3.67	0.045	-28.996 (± 1.667)	12.627 (± 0.973)

3.2.4 Osmolality

Figure 64 shows the osmolality of dispersions of SLN containing theophylline. It was found that theophylline increased the osmolality of preparations. These values of all preparations containing drug were higher than drug free preparations. Furthermore, increasing its concentration could increase these osmolalities. However, their osmolalities were lower than that of isotonic solution.

3.2.5 Zeta potential

Theophylline did not affect the zeta potential. The zeta potential of dispersions of SLN containing theophylline was not different in each drug concentration. Furthermore, these values were similar to that of drug free preparations. About –22.373 to –30.216 millivolts of the charge were obtained in all preparations containing drug as shown in Figure 65.

3.2.6 Viscosity

When using poloxamer 407 as stabilizer, the viscosity of preparations of SLN containing 0.25-0.75% theophylline was not different from drug free preparations. The low viscosity in the range of 2.298-2.947 mPa·s, which was a Newtonian flow, was observed and shown in Figures 67-68. The drug concentration did not affect their viscosity.

Preparations containing 2% tween 80 increased the viscosity and higher than drug free preparation. Plastic flow was obtained. These were shown in Figures 69-70. The dispersions showed very high viscosity with yield point, and then decreased when further increasing shear force. The preparation containing 3% tween 80 also produced similar flow pattern with high viscosity. Moreover, their viscosities were higher than preparations containing poloxamer 407.

When using 1% egg lecithin as stabilizer, only the preparation of 0.25% theophylline could be prepared to be stable. Its viscosity was quite low, but still higher than preparations containing poloxamer 407. It showed the plastic flow as in Figures 71-72. Increasing the concentration of egg lecithin to 2% produced preparations of 0.25-0.75% drug. However, higher viscosity was observed and their flows were inclined to be the plastic systems.

3.2.7 Infrared spectra

The IR spectrum of theophylline is shown in Figure 73. It showed the dominant N-H stretching with the placement of the methyl groups on the purine ring between 2610-3122 cm^{-1}. Moreover, the identical peaks between 1200 and 2000 cm^{-1} were observed. The dominant peaks of C=O stretching at 1668 and 1716 cm^{-1}, C=C stretching at 1566 cm^{-1}, C=N stretching at 1486 cm^{-1} and C-N and C-O vibration at 1187-1314 cm^{-1}, and C-H bending at 1486 cm^{-1}. Their fingerprint regions between 650-1300 cm^{-1} could be used for differentiation.

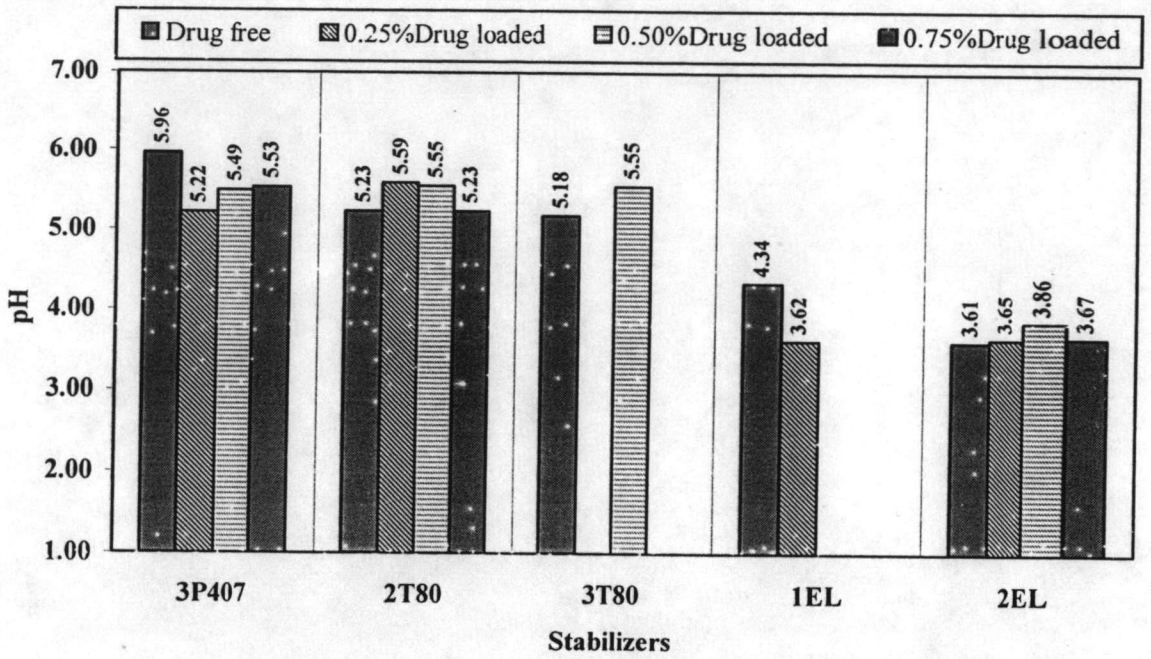

Figure 63. The pH of dispersions of SLN containing theophylline after autoclaving.

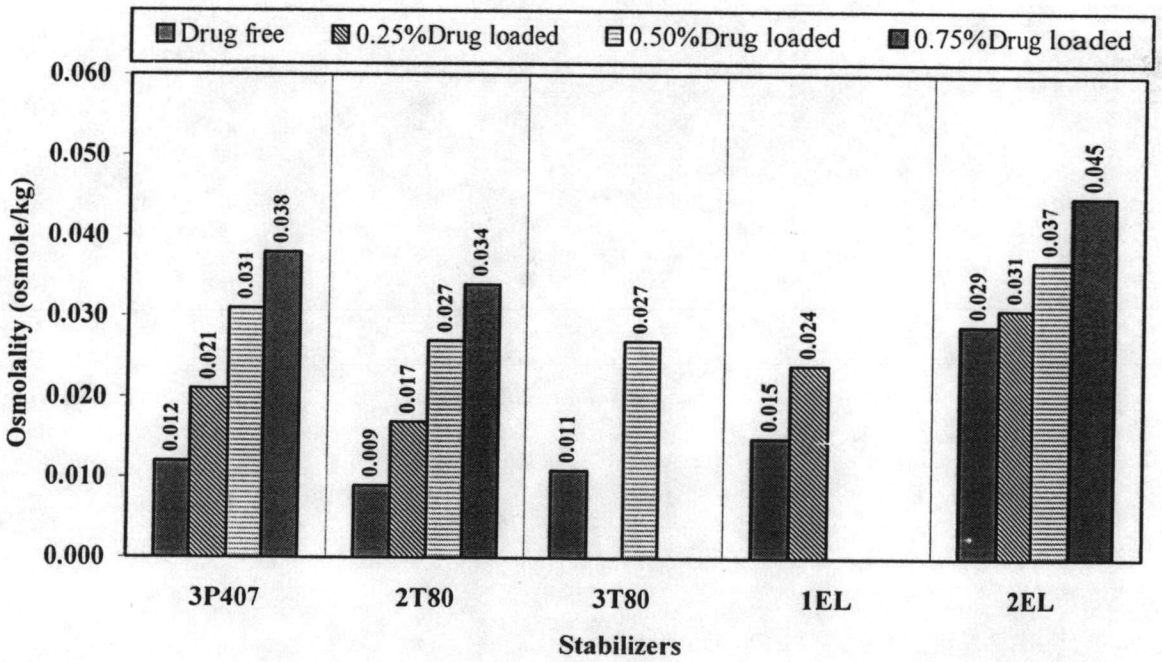

Figure 64. The osmolality of dispersions of SLN containing theophylline after autoclaving.

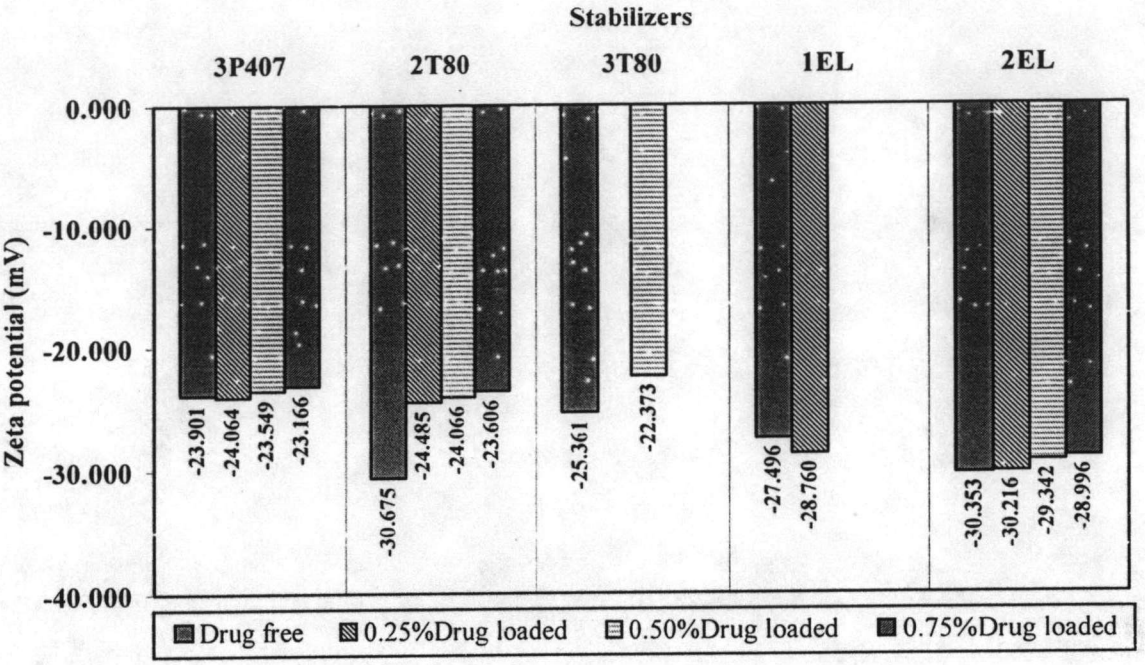

Figure 65. The zeta potential of dispersions of SLN containing theophylline after autoclaving.

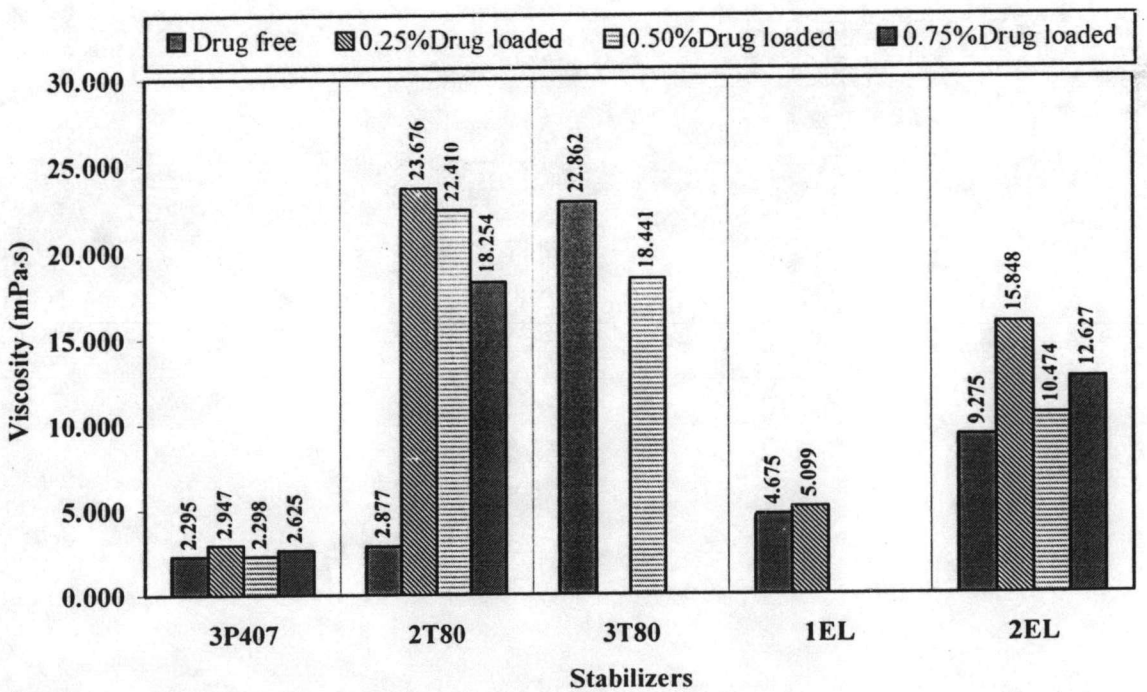

Figure 66. The viscosity at shear rate of 1000 s^{-1} of dispersions of SLN containing theophylline after autoclaving.

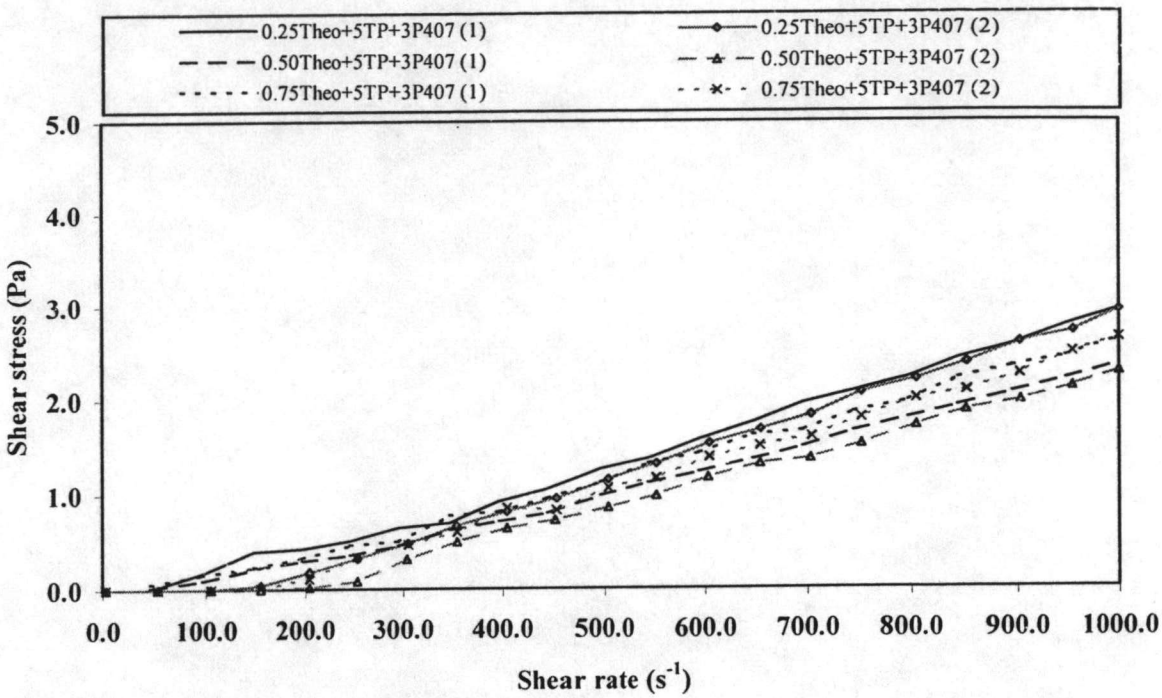

Figure 67. Flow curves of dispersions of SLN containing 0.25-0.75% theophylline and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

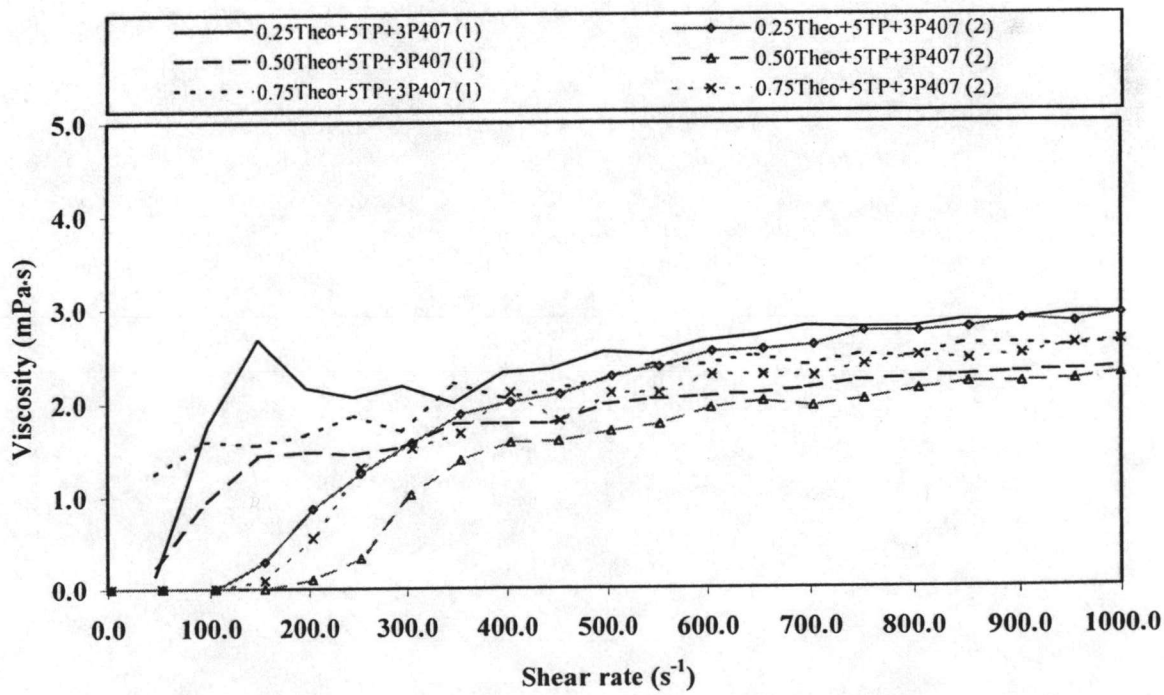

Figure 68. Viscosity curves of dispersions of SLN containing 0.25-0.75% theophylline and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

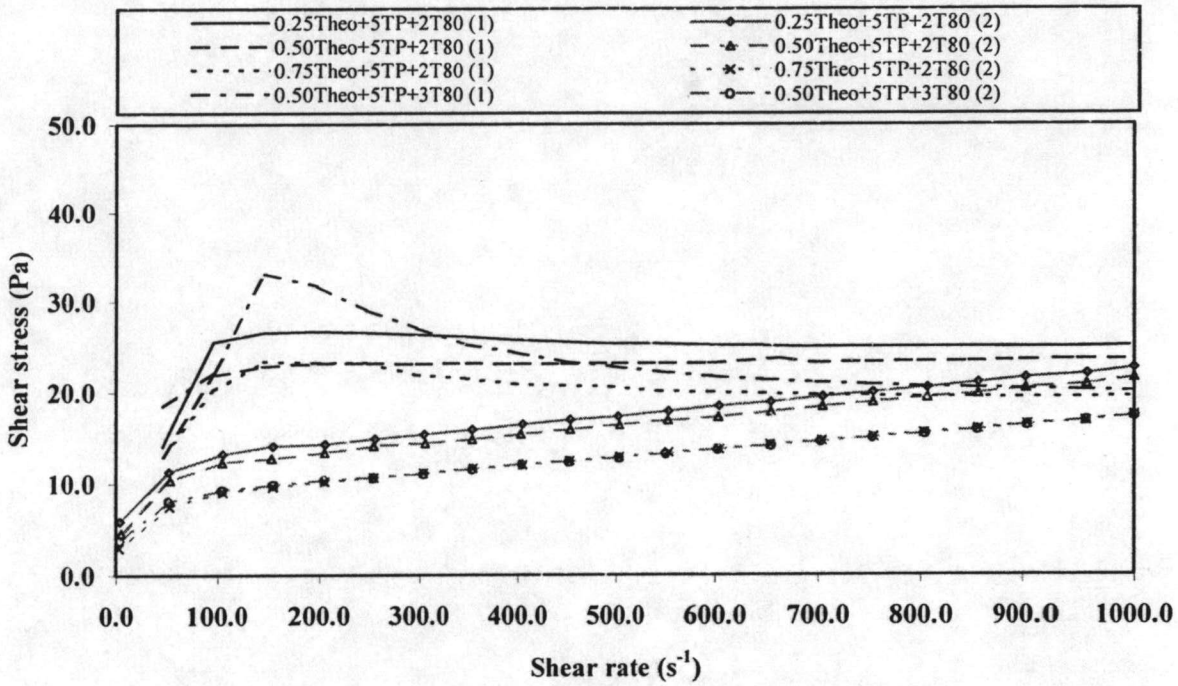

Figure 69. Flow curves of dispersions of SLN containing 0.25-0.75% theophylline and 2-3% tween 80 ((1) up-curve and (2) down-curve).

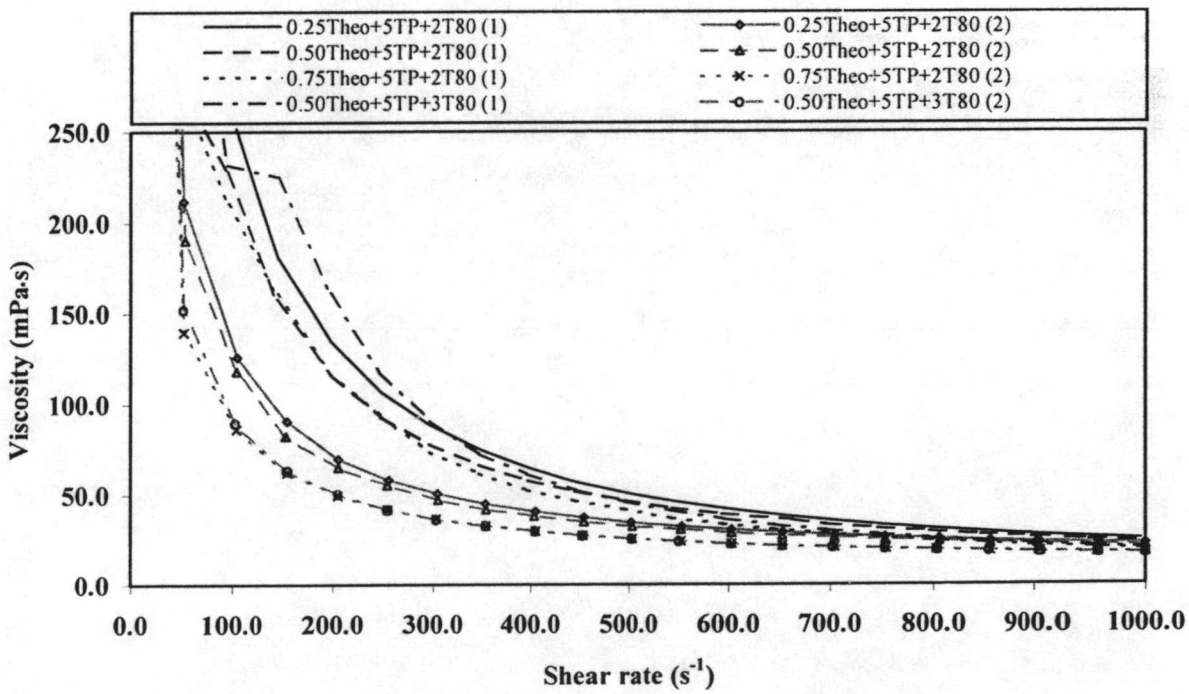

Figure 70. Viscosity curves of dispersions of SLN containing 0.25-0.75% theophylline and 2-3% tween 80 ((1) up-curve and (2) down-curve).

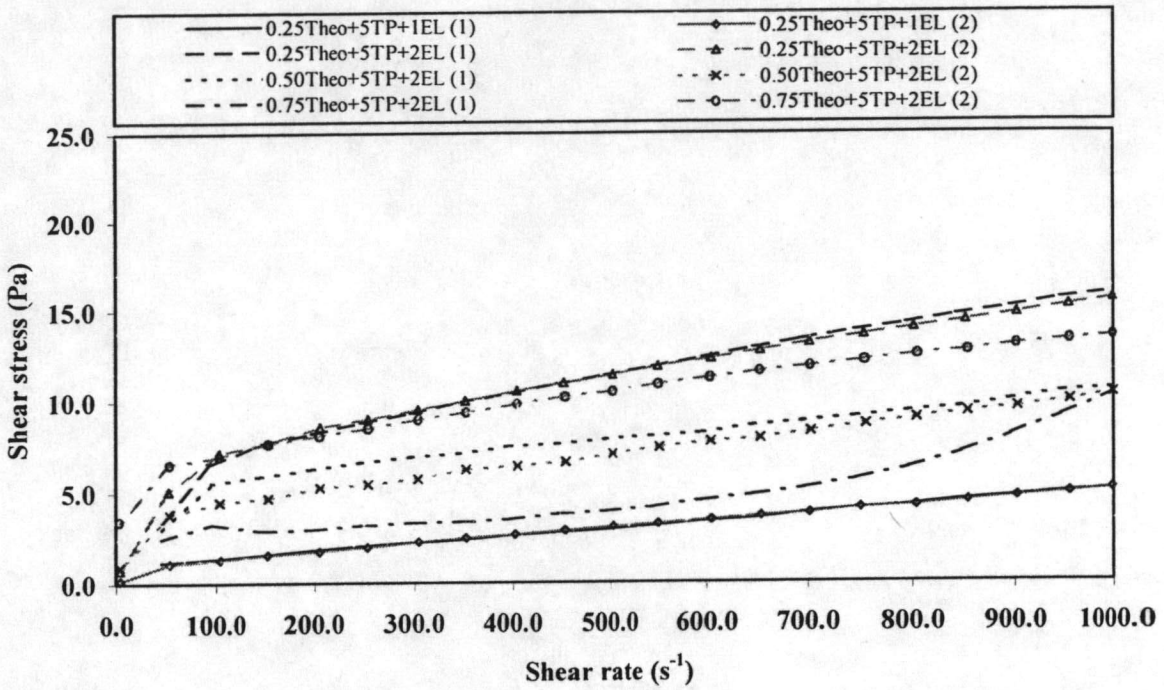

Figure 71. Flow curves of dispersions of SLN containing 0.25-0.75% theophylline and 1-2% egg lecithin ((1) up-curve and (2) down-curve).

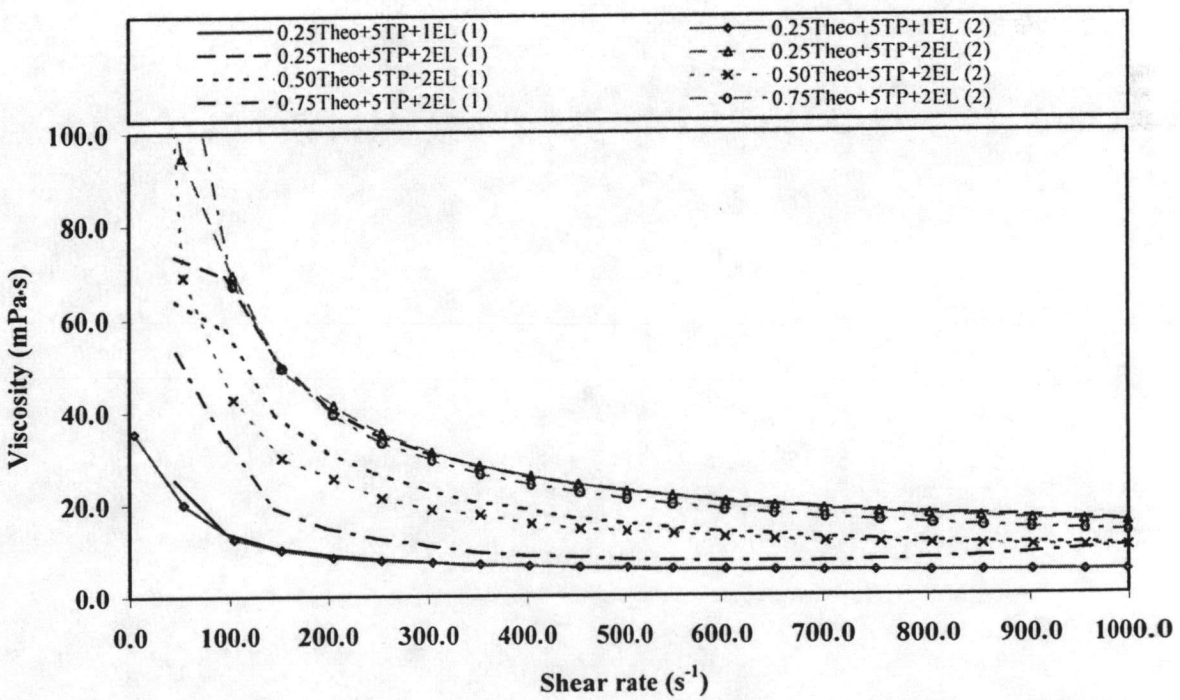

Figure 72. Viscosity curves of dispersions of SLN containing 0.25-0.75% theophylline and 1-2% egg lecithin ((1) up-curve and (2) down-curve).

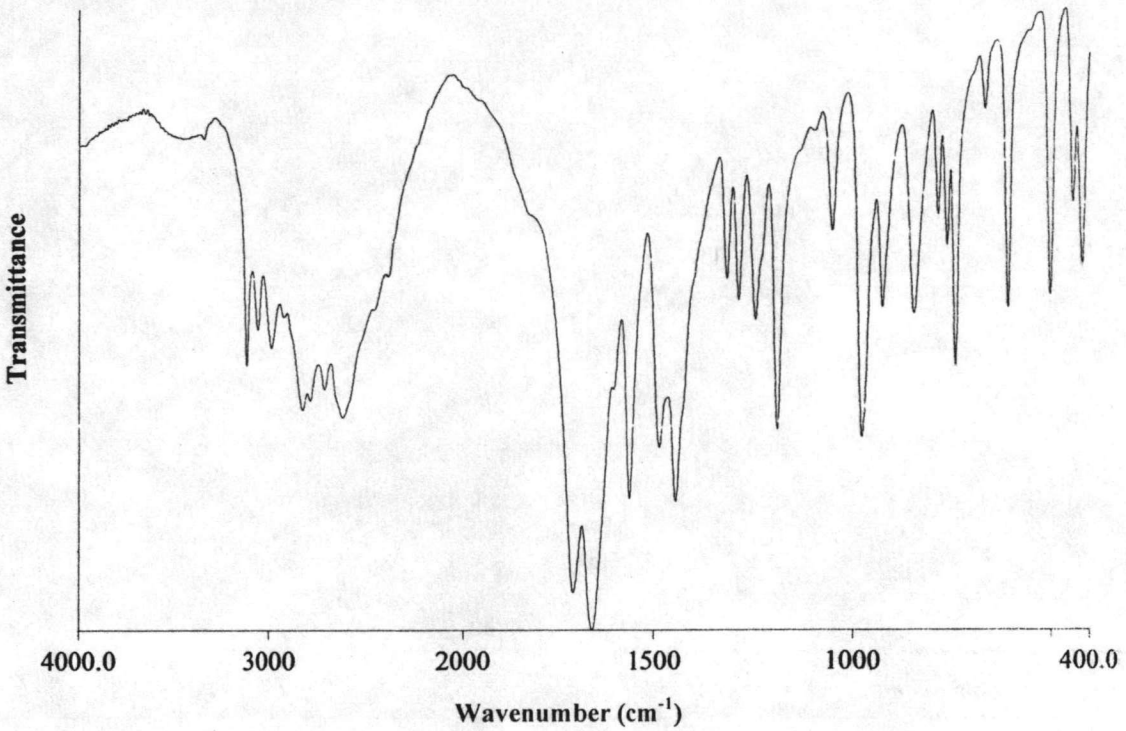

Figure 73. IR spectrum of theophylline.

The SLN containing 0.25-0.75% theophylline showed the dominant spectra similar to the spectra of tripalmitin as shown in Figure 74. However, very small peaks of drug at 1669 and 1180 cm^{-1} were observed. The spectra did not shift which indicated no strong interaction of tripalmitin and the other components. These peaks were also observed in preparations containing tween 80 and egg lecithin as shown in Figures 75-76.

3.2.8 Entrapment efficiency

The entrapment efficiency of theophylline, a sparingly water soluble drug, in SLN is shown in Table 18 and Figure 77. Preparations of SLN containing 0.25-0.75% theophylline using either poloxamer 407 or tween 80 as stabilizers showed low entrapment efficiency. Increasing drug concentrations could increase the entrapment efficiency. However, increasing upto 1% caused the drug

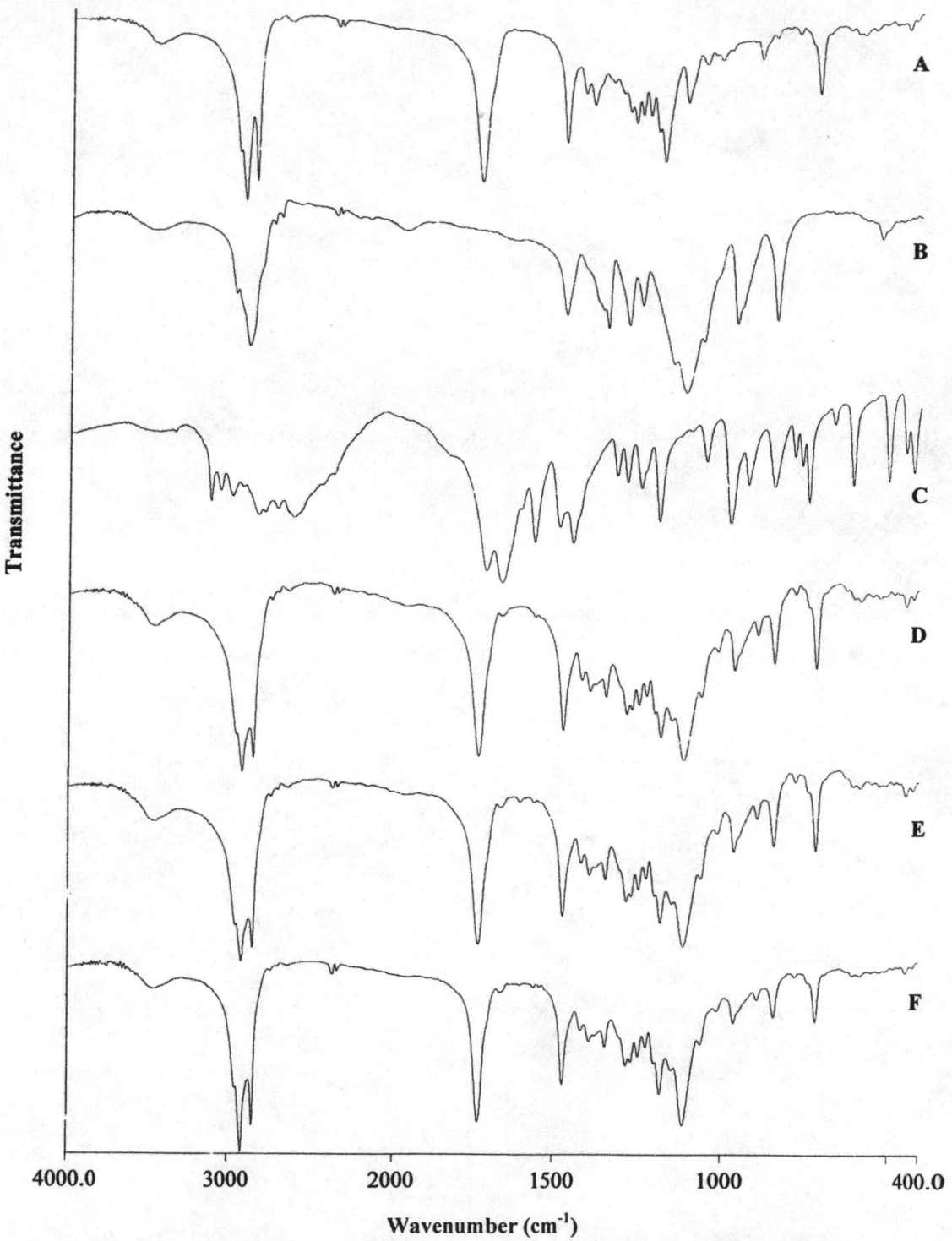

Figure 74. IR spectra of (A) tripalmitin; (B) poloxamer 407; (C) theophylline; and lipid matrices of preparations containing (D) 0.25%, (E) 0.50%, and (F) 0.75% theophylline and 3% poloxamer 407.

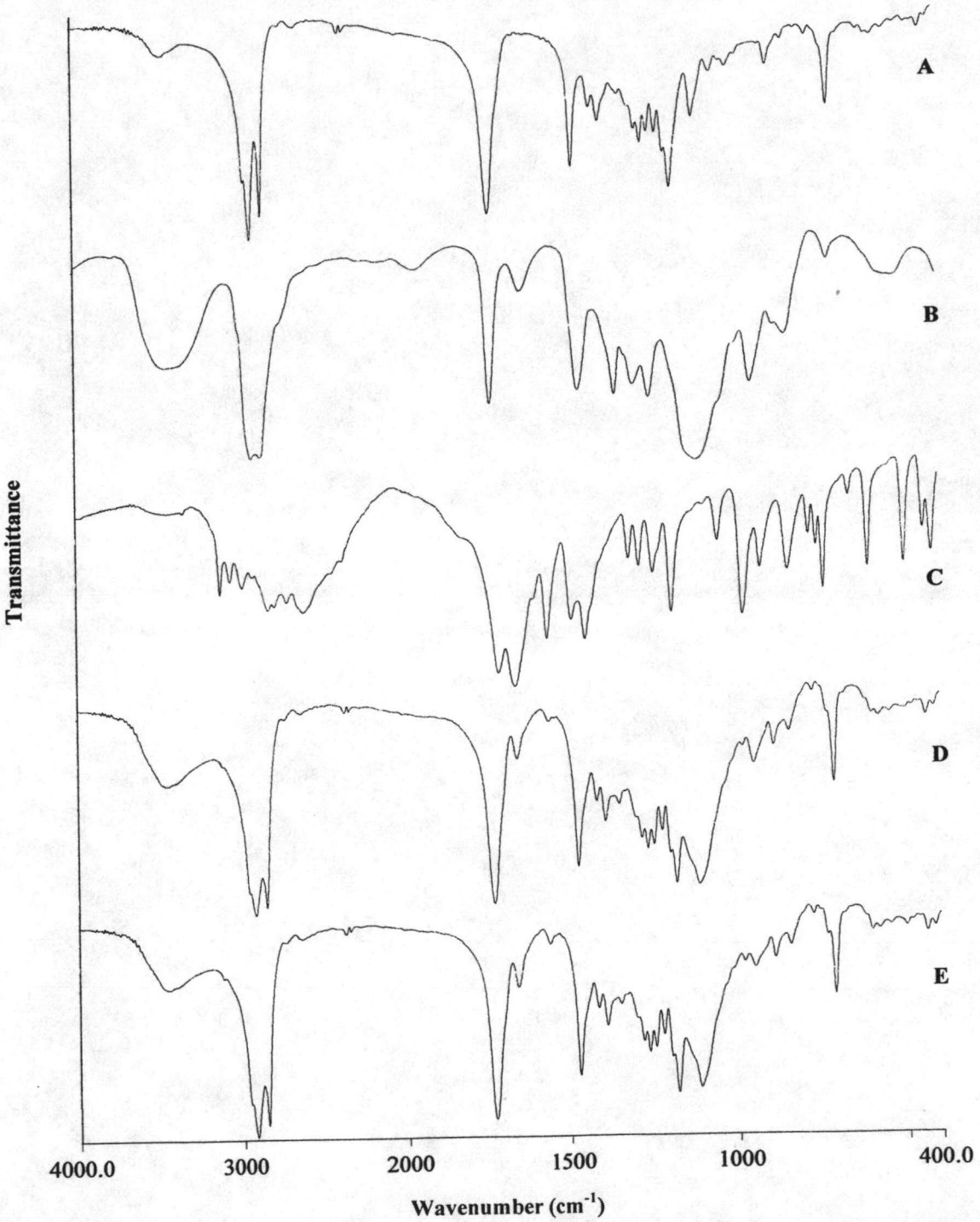

Figure 75. IR spectra of (A) tripalmitin; (B) tween80; (C) theophylline; and lipid matrices of preparations containing (D) 0.25%, and (E) 0.50% theophylline and 2% tween 80.

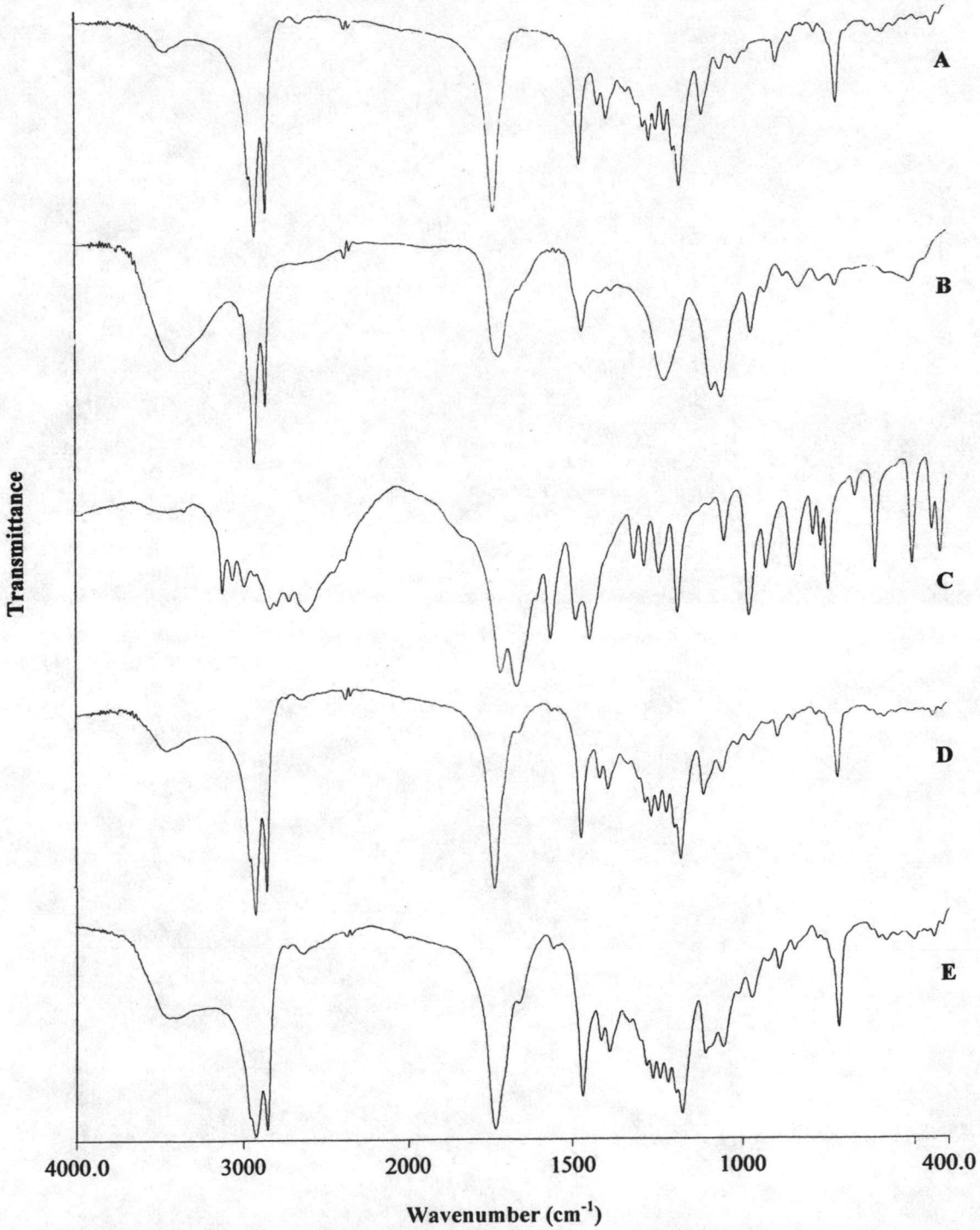

Figure 76. IR spectra of (A) tripalmitin; (B) egg lecithin; (C) theophylline; and lipid matrices of preparations containing 0.25% theophylline and (D) 1%, and (E) 2% egg lecithin.

Table 19. Entrapment efficiency of theophylline loaded into SLN after autoclaving.

Formulation	Percentage drug entrapment of SLN					
	No.1	No.2	No.3	mean	SD	%CV
0.25Theo+5TP+3P407	6.94	6.27	6.94	6.72	0.39	5.80
0.50Theo+5TP+3P407	9.30	9.64	8.63	9.19	0.52	5.61
0.75Theo+5TP+3P407	27.40	27.62	27.84	27.62	0.22	0.81
0.25Theo+5TP+2T80	6.27	6.94	6.94	6.72	0.39	5.80
0.50Theo+5TP+2T80	10.99	10.99	10.99	10.99	0.00	0.00

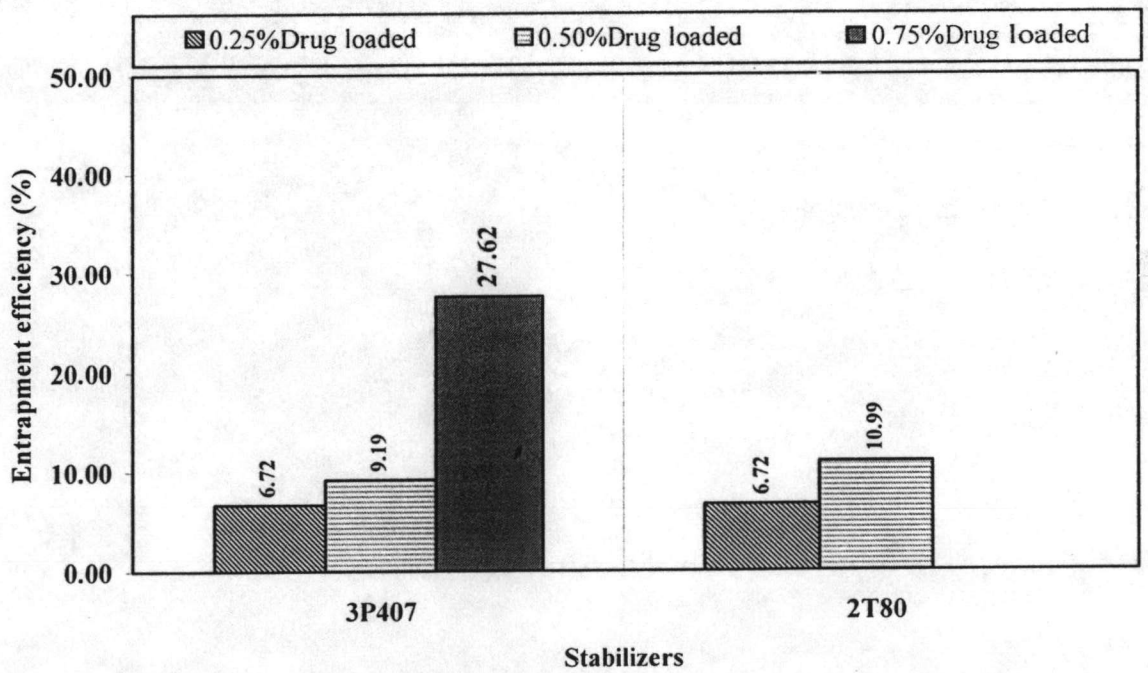

Figure 77. The entrapment efficiency of theophylline loaded into SLN after autoclaving.

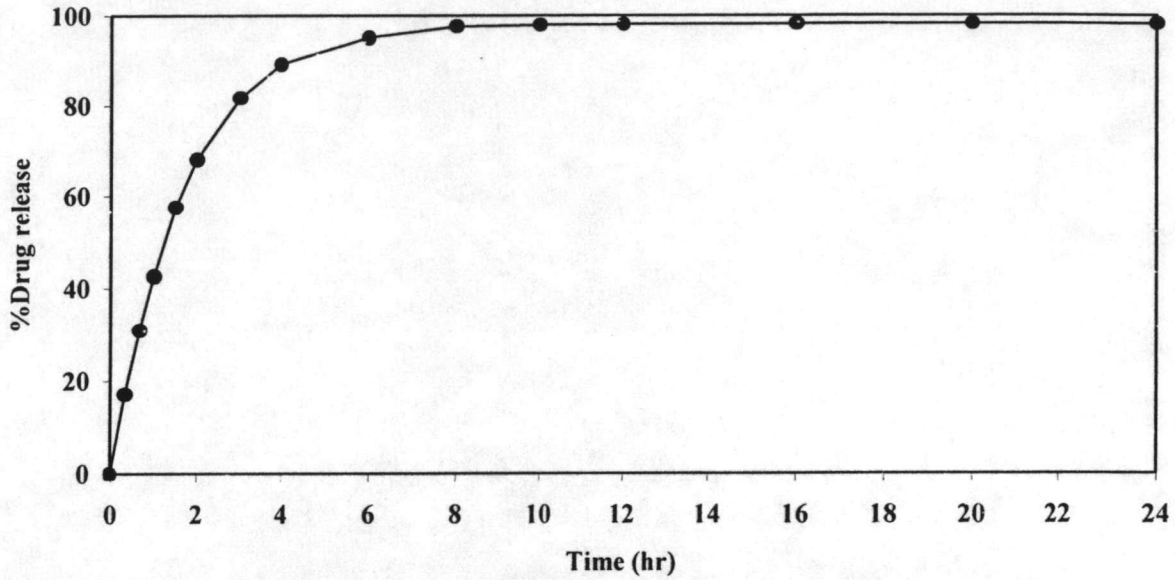

Figure 78. The release profile of theophylline saturated solution.

precipitation. The highest entrapment efficiency of 27.62% was observed in preparation of SLN containing 0.75% theophylline and 3% poloxamer 407.

3.2.9 Drug release

Theophylline saturated solution was rapidly released through the dialysis membrane. More than 80% of drug was obtained in the receptor compartment within 3 hours. It reached to the plateau before the 8th hour. This result indicated the applicability of dialysis membrane for studying the drug release from the preparations.

Preparations of SLN containing theophylline and poloxamer 407, however, could not sustain the drug release. Figure 79 showed rapidly release of the drug from preparations, supernatant, and saturated solution. It was found that there was no difference in the release profiles. However, preparations with tween 80 could sustain the drug release more than those with poloxamer 407 as shown in Figure 80.

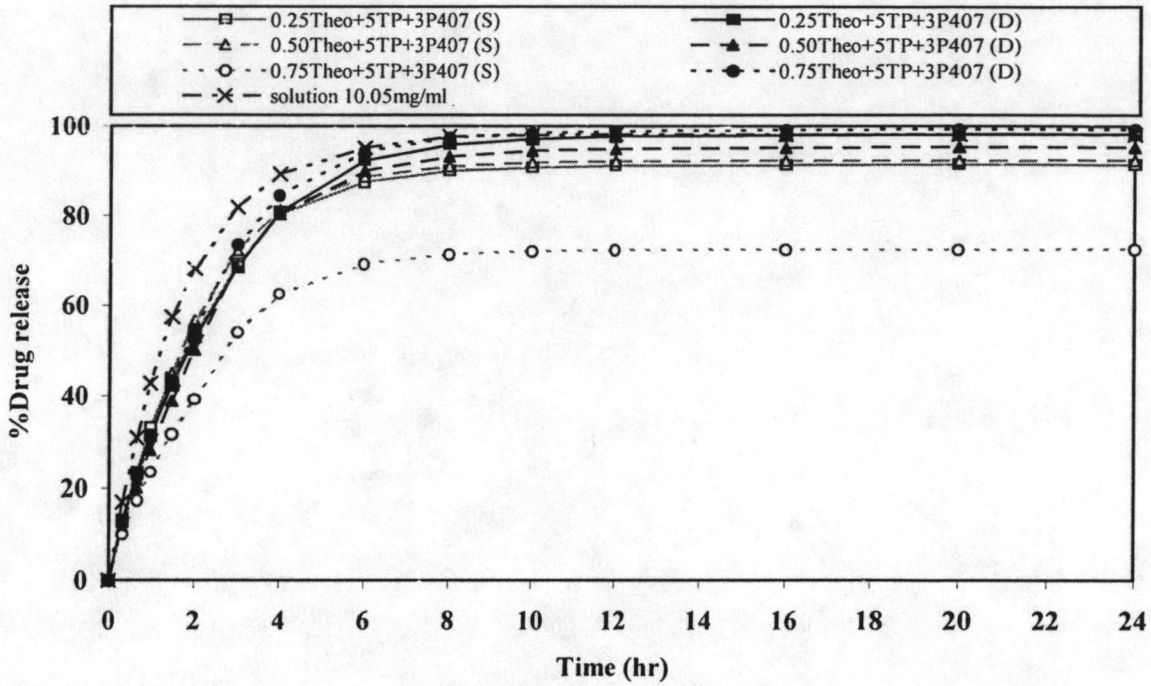

Figure 79. The release profiles of dispersions containing 0.25-0.75% theophylline and 3% poloxamer 407 ((S) supernatant and (D) dispersion).

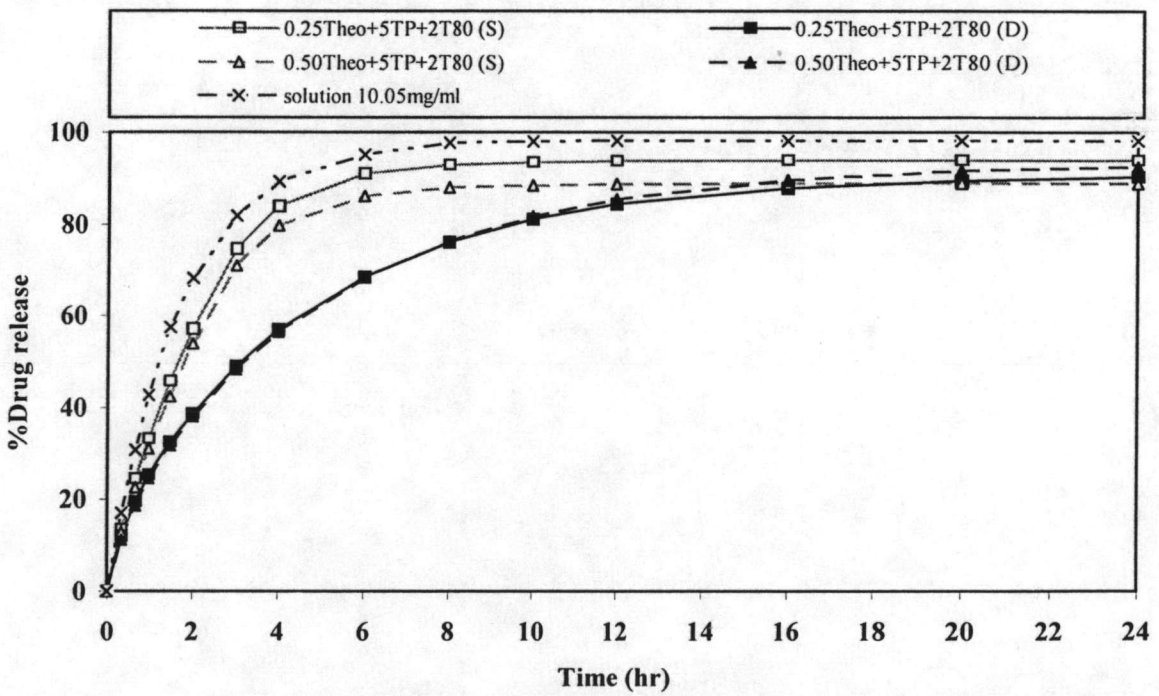

Figure 80. The release profiles of dispersions containing 0.25-0.50% theophylline and 2% tween 80 ((S) supernatant and (D) dispersion).

3.3 Piroxicam loaded SLN

3.3.1 Physical appearance

Dispersions containing piroxicam could not be produced as stable preparations. Table 19 shows the physical appearance of each preparation. About 0.1-5.0% piroxicam were loaded in preparations of SLN containing tripalmitin and poloxamer 407. Drug could dissolve in the melted lipid at 75°C, and could be prepared as good preparations. Yellow-white fluid dispersions were observed. However, these preparations were not stable. After autoclaving and cooling to room temperature, drug recrystallization occurred. After 1 week the yellow precipitated matter was increasingly obtained with the white fluid dispersion.

3.3.2 Infrared spectra

For elucidating the piroxicam precipitation in preparations of SLN, the infrared spectroscopy was used to study. The spectra of precipitated matter and solid lipid dispersion were compared with raw materials.

The IR spectrum of piroxicam is shown in Figure 81. Piroxicam generally exists in two different interconvertible crystal polymorphs (Mihalic´ et al., 1986). In this study, the principle peak was observed at 3393 cm^{-1} of N-H and O-H stretching. This peak indicated the polymorph of needle form. The other characteristic peaks presented at the different wavenumbers as following: 1641 cm^{-1} of C=O amide carbonyl stretching, 1531 cm^{-1} of C-N secondary amide band stretching, 1438 cm^{-1} of CH_3 asymmetric stretching and Aryl-C=C- stretching, 1354 cm^{-1} of CH_3 symmetric stretching, 1181 and 1153 cm^{-1} of $-SO_2$-N- stretching, 776 and 734 cm^{-1} of ortho-substituted phenyl.

Figures 82 and 83 show the spectra of lipid matrices of preparations containing 1-5% piroxicam of and 3% poloxamer 407 prepared by ultracentrifugation and their precipitated matters. For solid lipid the dominant peaks were similar to tripalmitin. It was also found the spectra of poloxamer 407 at 1113,

Table 18. The physical appearances of dispersions of SLN containing piroxicam.

Formulation	Macroscopic observation	
	Before autoclaving	After autoclaving
0.1Pirox+5TP+3P407	Yellow-white fluid dispersion	Drug recrystallization after cooling
0.2Pirox+5TP+3P407	Yellow-white fluid dispersion	Drug recrystallization after cooling
0.3Pirox+5TP+3P407	Yellow-white fluid dispersion	Drug recrystallization after cooling
0.5Pirox+5TP+3P407	Yellow-white fluid dispersion	Drug recrystallization after cooling
1.0Pirox+5TP+3P407	Yellow-white fluid dispersion	Drug recrystallization after cooling
5.0Pirox+5TP+3P407	Yellow-white fluid dispersion	Drug recrystallization after cooling

1282, and 1345 cm^{-1}. The piroxicam peaks were slightly observed at 1180 cm^{-1} in solid lipid matrix. However, there was new peak at the wavelength of 1609 cm^{-1}. Whereas there were many new peaks in the spectra of precipitated matter. The spectra were different from raw materials both two concentrations of drug. Broad peaks were easily observed at 3700-3000 cm^{-1} which indicated their inter- and intra-molecular hydrogen bonding at high and low frequency, respectively. Moreover, these wavenumbers showed two peaks at 3450 and 3097 cm^{-1} of O-H and N-H stretching. The other new peaks occurred at 1600, 1552, and 1329 cm^{-1} that were the spectra of C=C–Aryl conjugated double bond stretching, the C-N secondary amide band stretching that shift from 1531 cm^{-1}, and the CH_3 symmetric stretching that shift from 1354 cm^{-1}, respectively. Furthermore, some piroxicam peaks were higher intensity, such as 1239, 1158, 1099, 1040, 770, and 575 cm^{-1}.

For studying the piroxicam-tripalmitin compatibility, dispersion containing 0.2% piroxicam and 5% tripalmitin were dispersed in water at 75°C. The stabilizer was not incorporated. After cooling, there was three separated parts of preparation: the solid lipid floated on the surface, water, and precipitated matter. Two solid parts were tested by infrared spectroscopy. The both two spectra are shown in Figure 84. In solid lipid floated matter, the dominant tripalmitin peaks were observed. However, small peak at 1600 cm^{-1} was observed. This peak was also presented in precipitated matter and similar to the new peak of preparations containing 1-5% piroxicam and 3% poloxamer 407 which was previously described.

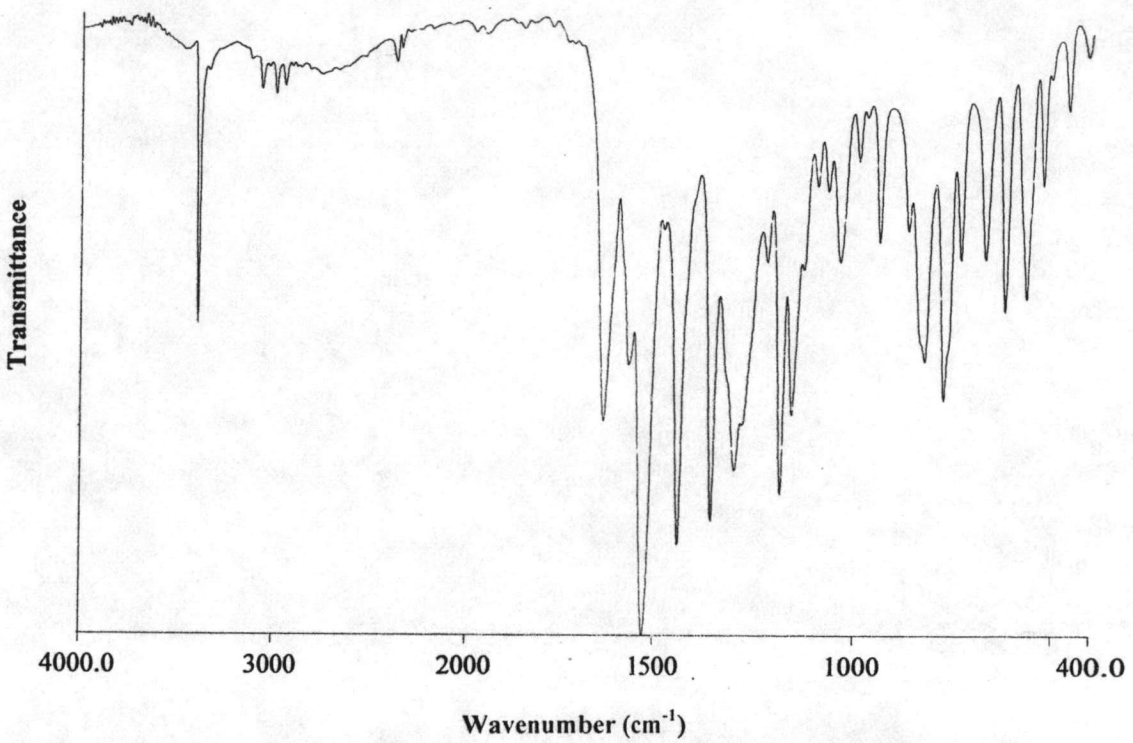

Figure 81. IR spectrum of piroxicam.

The incompatibility of piroxicam and tripalmitin was additionally studied. Piroxicam was dissolved in the melted tripalmitin at 75°C. This mixture was cooled to be solid lipid at room temperature and then tested by infrared spectroscopy. It was found that the obtained matrix was intense yellow colour which difference from its raw material. Its IR spectra are shown in Figure 85. The dominant tripalmitin peaks were observed with the small peaks of piroxicam. Unfortunately, the new peak was observed at 1630 cm^{-1} with the small shoulder at the lower frequency which was the C=O amide carbonyl stretching that shift from 1641 cm^{-1}. Some tripalmitin peaks were slightly shifted from the original wavenumbers. Moreover, The N-H stretching at 3393 cm^{-1} was also shift to 3339-3345 cm^{-1}.

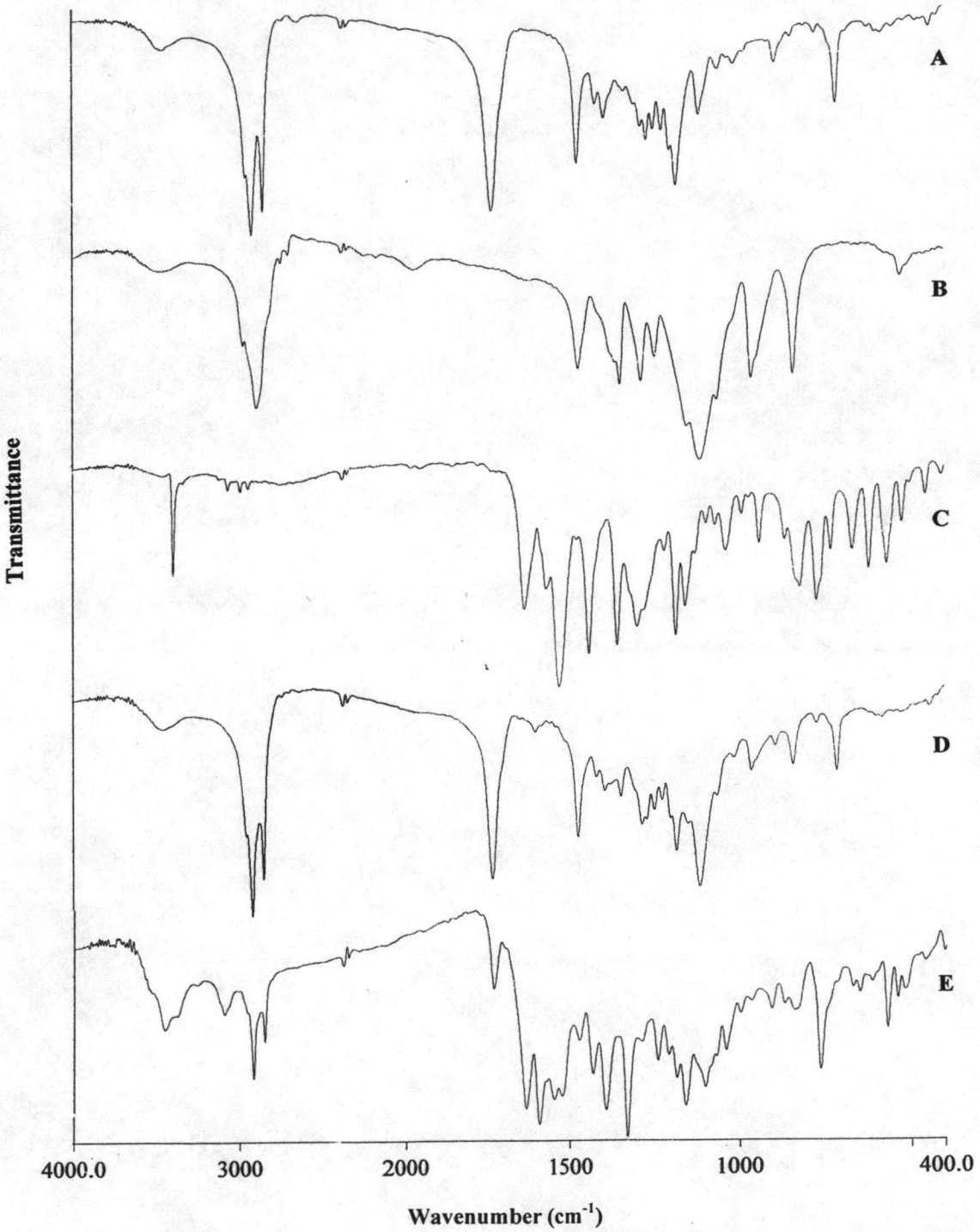

Figure 82. IR spectra of (A) tripalmitin; (B) poloxamer 407; (C) piroxicam; (D) lipid matrix of preparation containing 1.0% piroxicam and 3% poloxamer 407; and (E) its precipitated product.

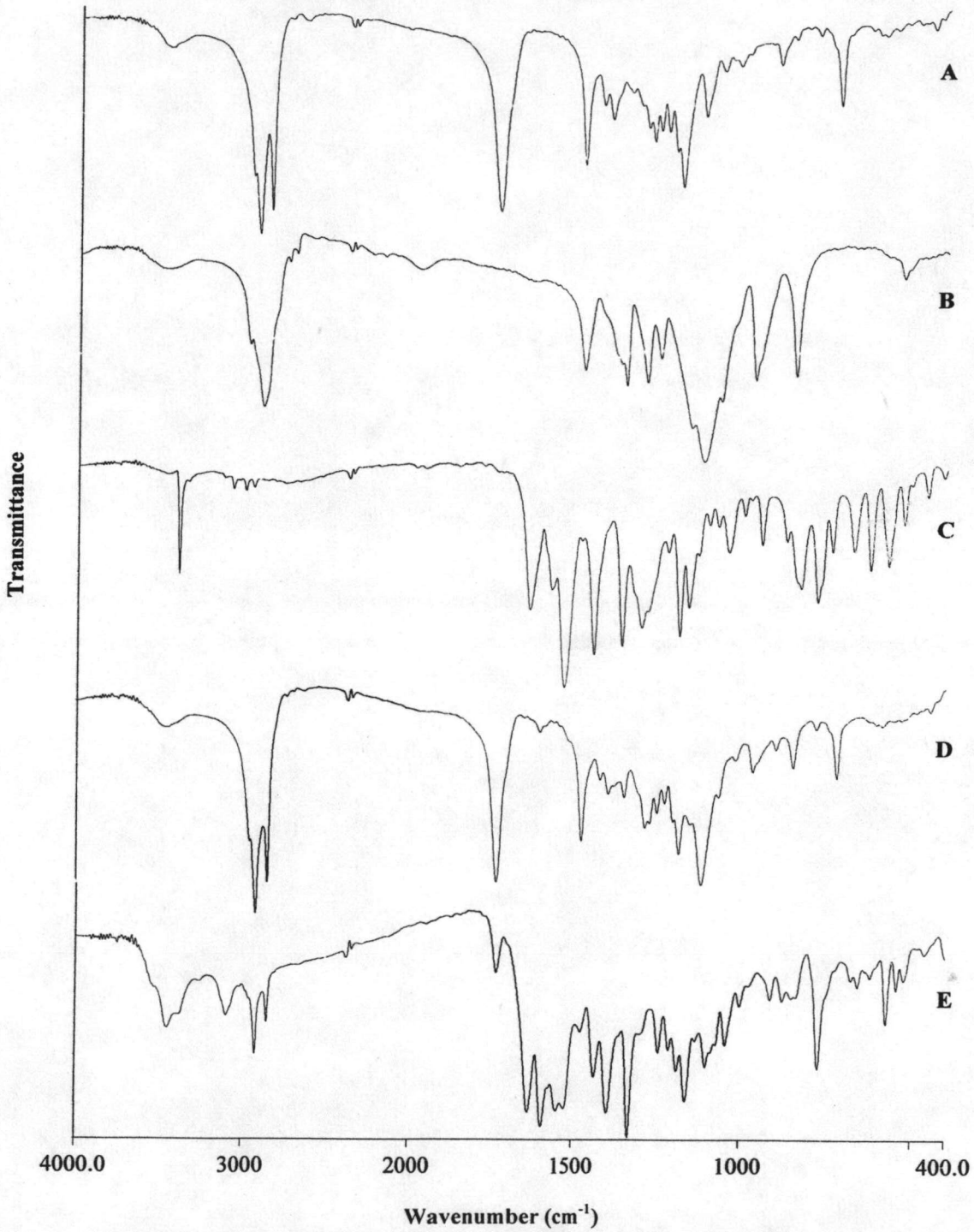

Figure 83. IR spectra of (A) tripalmitin; (B) poloxamer407; (C) piroxicam; (D) lipid matrix of preparation containing 5.0% piroxicam and 3% poloxamer 407; and (E) its precipitated product.

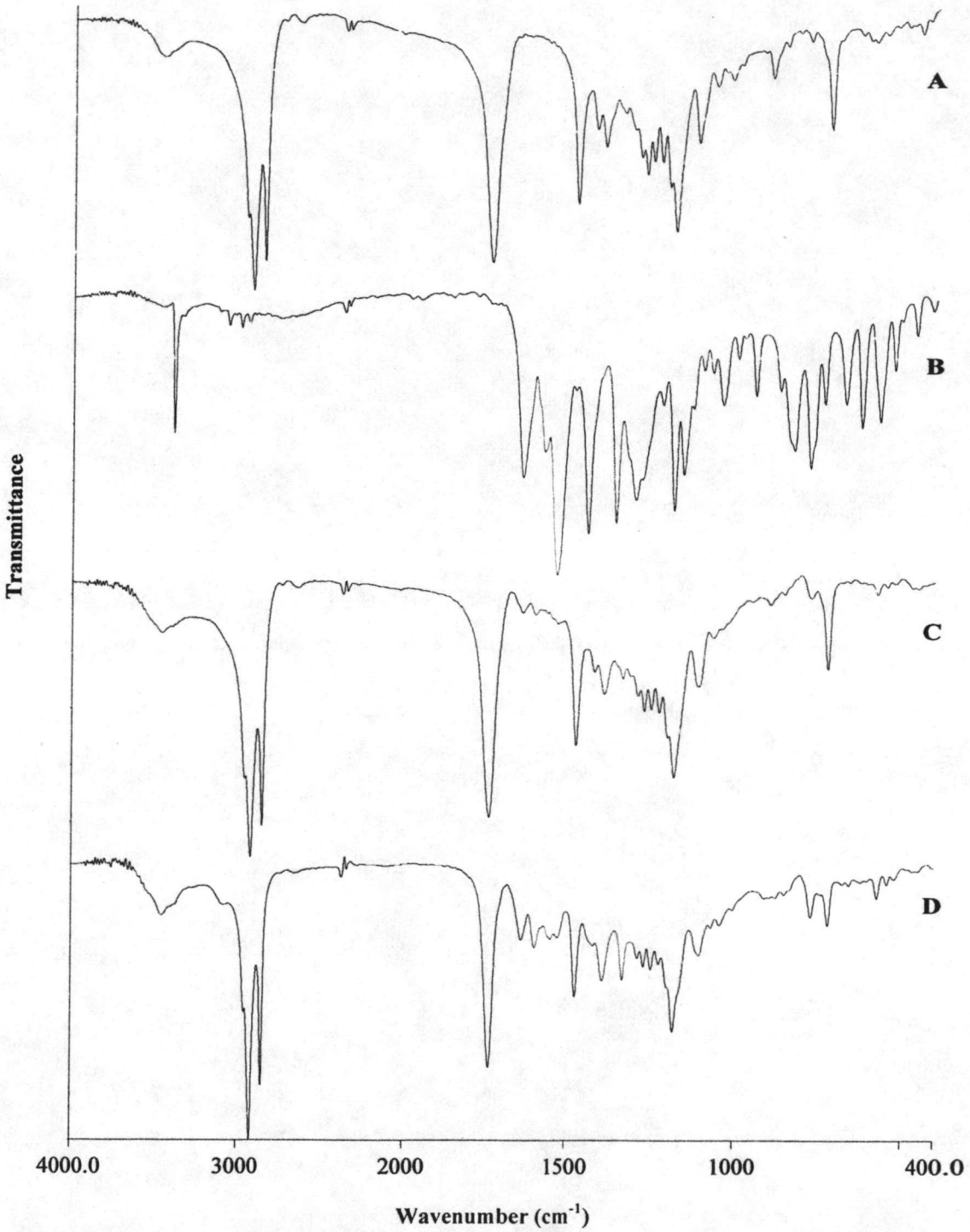

Figure 84. IR spectra of (A) tripalmitin; (B) piroxicam; (C) floated product of preparation containing 0.2% piroxicam and 5% tripalmitin without stabilizer; and (D) its precipitated product.

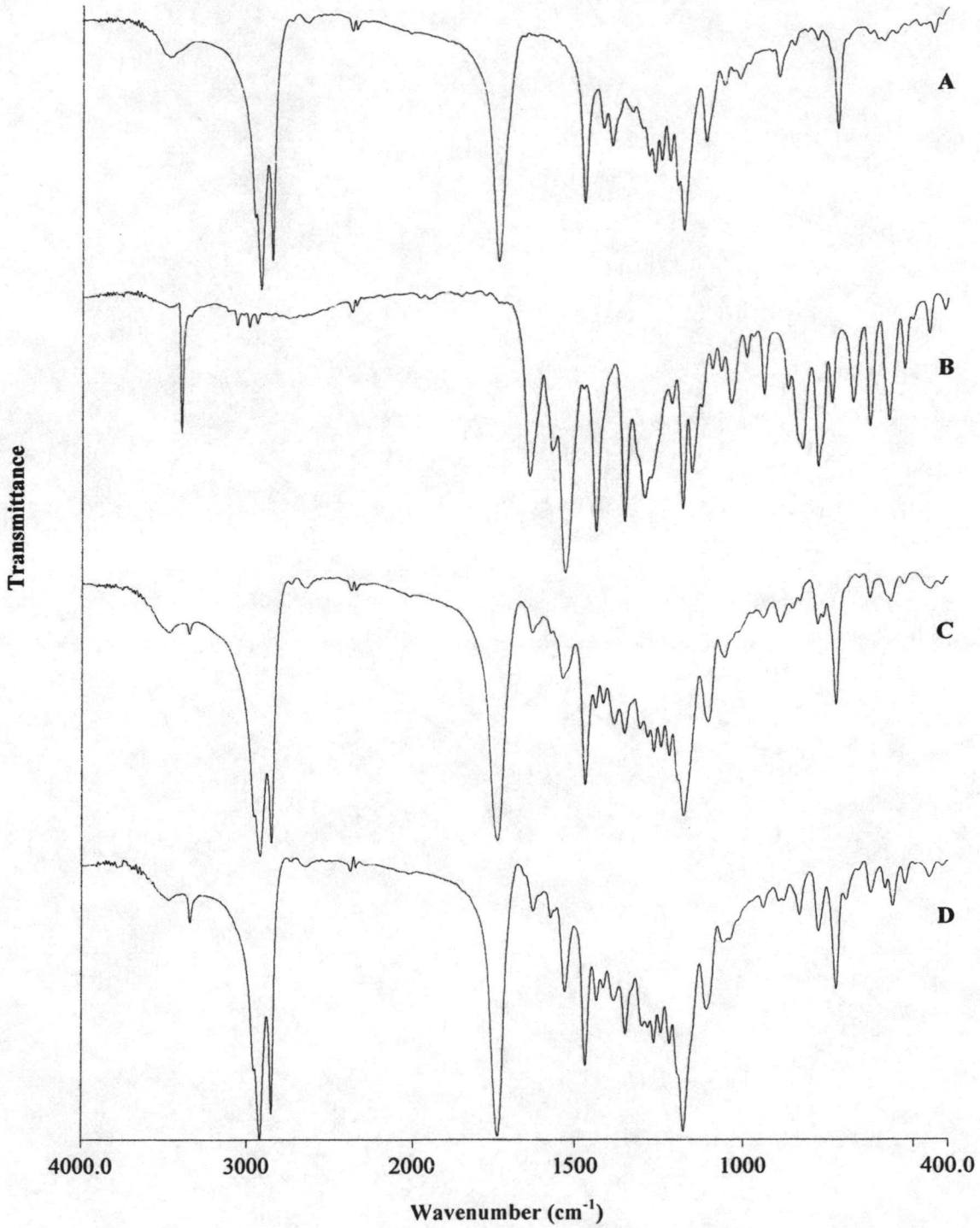

Figure 85. IR spectra of (A) tripalmitin; (B) piroxicam; the mixture of tripalmitin and piroxicam in the ratio of (C) 1:0.06,and (D) 1:0.12 prepared by melted and mixed at 75°C.

3.4 Ibuprofen loaded SLN

3.4.1 Physical appearance

The physical appearances of dispersions of SLN containing ibuprofen both before and after sterilization by autoclaving are shown in Table 20. When using poloxamer 407 of 3% as stabilizer, only preparation of 0.5% ibuprofen could be prepared to be stable both before and after autoclaving. White fluid dispersion was obtained. For preparation of 1.0% ibuprofen, phase separation was observed after autoclaving but could be redispersed. However, this preparation was not stable. The precipitation occurred after storage for 2 months at room temperature. The oily phase of preparation of 1.5% drug also separated out after autoclaving and could not be redispersed.

Both 2% and 3% tween 80 could not sufficiently stabilize the preparations. The preparations of 0.5-1.5% ibuprofen could be first produced with the good appearance. After autoclaving, the oily phase separated out and floated on the surface and could not be redispersed. Contrarily, egg lecithin could stabilize preparations containing ibuprofen to be stable. Brown fluid dispersions were observed. They could also be sterilized by autoclaving.

3.4.2 Particle size

The particle size of SLN containing ibuprofen is shown in Table 21. When using poloxamer 407 of 3% as stabilizer, the particle size lower than 1 μm was obtained from preparation of 0.5% ibuprofen. Its d(v,0.5) was about 0.31-0.33 μm in 4 batches reproduction. These values were smaller than those of drug free preparations. For preparation containing 1% ibuprofen, the d(v,0.5) was slightly higher than 1 μm. However, there was no particle size higher than 5 μm. Its particle size distribution was very narrow.

Table 20. The physical appearances of dispersions of SLN containing ibuprofen.

Formulation	Macroscopic observation	
	Before autoclaving	After autoclaving
0.5Ibu+5TP+3P407	White fluid dispersion	White fluid dispersion; stable on storage
1.0Ibu+5TP+3P407	White fluid dispersion	Separation; could be redispersed into dispersion
1.5Ibu+5TP+3P407	White fluid dispersion	Separation; solid lipid floating on the surface
0.5Ibu+5TP+2T80	White fluid dispersion	Separation; solid lipid floating on the surface
1.0Ibu+5TP+2T80	White fluid dispersion	Separation; solid lipid floating on the surface
1.5Ibu+5TP+2T80	White fluid dispersion	Separation; solid lipid floating on the surface
0.5Ibu+5TP+3T80	Bluish white fluid dispersion	Separation; solid lipid floating on the surface
1.0Ibu+5TP+3T80	Bluish white fluid dispersion	Separation; solid lipid floating on the surface
1.5Ibu+5TP+3T80	Bluish white fluid dispersion	Separation; solid lipid floating on the surface
0.5Ibu+5TP+1EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage
1.0Ibu+5TP+1EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage
1.5Ibu+5TP+1EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage
0.5Ibu+5TP+2EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage
1.0Ibu+5TP+2EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage
1.5Ibu+5TP+2EL	Brown fluid dispersion	Brown fluid dispersion; stable on storage

Surprisingly, the preparations of SLN containing ibuprofen using egg lecithin as stabilizer showed the particle size lower than drug free preparation, especially in high drug and egg lecithin concentrations. Increasing drug concentration decreased the particle size to lower than 1 μm. The d(v,0.5) lower than 1 μm was produced from 1.0-1.5% and 0.5-1.5% drug in preparations of 1% and 2% egg lecithin, respectively.

3.4.3 pH

The ibuprofen loaded SLN dispersions were weakly acidic as shown in Figure 87. When using poloxamer 407 as stabilizer, the pH of dispersions of SLN containing drug was lower than that of drug free preparation. Increasing drug concentration could slightly decrease its pH. The pH of dispersions containing egg lecithin was slightly lower than preparations of poloxamer 407. These values were

Table 21. Particle sizes of SLN containing ibuprofen after autoclaving.

Formulation	Volume particle size (μm)							%Particle larger than		
	d(v,0.1)	d(v,0.5)	d(v,0.9)	d(4,3)	d(3,2)	span	uniformity	1μm	5μm	10μm
0.5Ibu+5TP+3P407 #1	0.18	0.31	0.52	0.33	0.27	1.12	0.33	0.00	0.00	0.00
0.5Ibu+5TP+3P407 #2	0.21	0.33	0.54	0.35	0.31	1.01	0.30	0.00	0.00	0.00
0.5Ibu+5TP+3P407 #3	0.22	0.31	0.43	0.32	0.30	0.68	0.21	0.00	0.00	0.00
0.5Ibu+5TP+3P407 #4	0.23	0.33	0.53	0.42	0.33	0.89	0.44	4.69	0.00	0.00
1.0Ibu+5TP+3P407 #1	0.76	1.15	1.57	1.16	1.07	0.70	0.20	69.34	0.00	0.00
1.0Ibu+5TP+3P407 #2	0.77	1.19	1.61	1.19	1.10	0.70	0.21	72.66	0.00	0.00
0.5Ibu+5TP+1EL	10.67	35.51	86.93	44.94	7.13	2.15	0.71	96.59	94.40	90.63
1.0Ibu+5TP+1EL	0.20	0.53	23.09	9.37	0.44	43.49	17.25	40.68	32.15	23.24
1.5Ibu+5TP+1EL	0.21	0.35	2.11	2.30	0.35	5.37	5.78	11.16	7.82	5.36
0.5Ibu+5TP+2EL	0.21	0.43	8.41	5.55	0.42	19.18	12.35	36.15	17.04	8.12
1.0Ibu+5TP+2EL	0.21	0.34	1.83	1.30	0.34	4.76	3.05	10.89	4.74	2.26
1.5Ibu+5TP+2EL	0.23	0.36	0.67	1.01	0.35	1.23	2.08	6.51	3.05	1.53

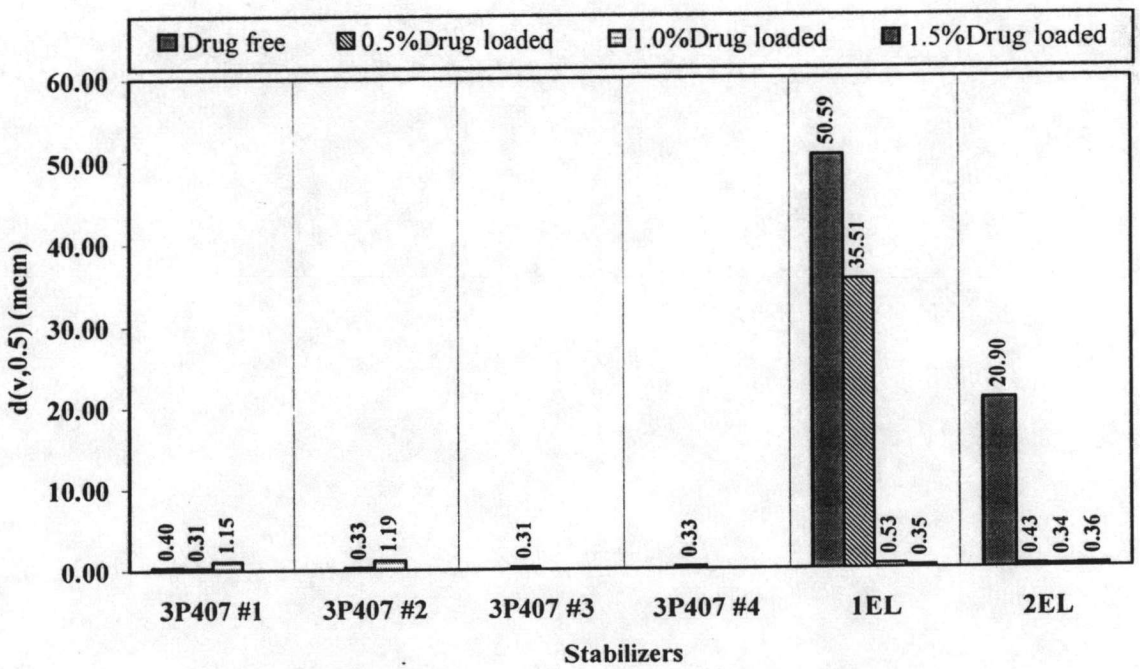

Figure 86. The d(v,0.5) of SLN containing ibuprofen after autoclaving.

Table 22. The pH, osmolality, zeta potential, and viscosity of dispersions containing ibuprofen after autoclaving.

Formulation	pH	Osmolality	Zeta potential (millivolts) (±SD)	Viscosity at the shear rate of $1000s^{-1}$ (mPa s) (±SD)
0.5Ibu+5TP+3P407 #1	4.45	0.019	-13.610 (± 1.659)	0.940 (± 0.056)
0.5Ibu+5TP+3P407 #2	4.82	0.014	-13.435 (± 2.109)	1.156 (± 0.045)
0.5Ibu+5TP+3P407 #3	4.10	0.015	-13.491 (± 1.872)	1.194 (± 0.062)
0.5Ibu+5TP+3P407 #4	3.99	0.015	-13.405 (± 1.964)	1.246 (± 0.066)
1.0Ibu+5TP+3P407 #1	4.03	0.020	-14.665 (± 1.566)	1.144 (± 0.062)
1.0Ibu+5TP+3P407 #2	4.57	0.015	-14.548 (± 1.621)	1.166 (± 0.081)
0.5Ibu+5TP+1EL	3.64	0.018	-24.504 (± 2.069)	3.648 (± 0.384)
1.0Ibu+5TP+1EL	3.69	0.017	-29.259 (± 2.270)	7.485 (± 0.613)
1.5Ibu+5TP+1EL	3.70	0.016	-27.128 (± 2.533)	9.046 (± 0.555)
0.5Ibu+5TP+2EL	3.50	0.028	-26.759 (± 1.978)	6.376 (± 0.072)
1.0Ibu+5TP+2EL	3.45	0.032	-28.031 (± 2.399)	18.119 (± 0.806)
1.5Ibu+5TP+2EL	3.43	0.031	-24.747 (± 2.478)	21.569 (± 0.483)

also lower than drug free preparations. However, increasing drug concentration did not affect its pH. The preparations of 1% and 2% egg lecithin showed the pH in the range of 3.64-3.70 and 3.43-3.50, respectively.

3.4.4 Osmolality

As shown in Figure 88, loading of ibuprofen did not affect the osmolality of preparations. The osmolality was very low, and was not different from that of drug free preparation. Moreover, drug concentrations also had no effect. The highest osmolality was obtained in preparation of 2% egg lecithin. However, all values were very low compared with the isotonic solution.

3.4.5 Zeta potential

Figure 89 shows the zeta potential of dispersions of SLN containing ibuprofen. All preparations showed the negative charge. For preparations

of poloxamer 407, the charge of preparations containing drug was decrease compared to drug free preparation. However, the drug concentration did not affect their charges. Reproduction of preparations containing 0.5% and 1% ibuprofen using 3% poloxamer 407 as stabilizer gave the values that were not different in all batches. About –13.405 to –13.610 and –14.548 to –14.665 millivolts were obtained in preparations of 0.5% and 1.0% drug, respectively. When using egg lecithin as stabilizer, higher negative charge was reported. The drug concentration did not affect their charges in both preparations of 1% and 2% egg lecithin.

3.4.6 Viscosity

The viscosities of dispersions of SLN containing ibuprofen are shown in Figure 90. Very low viscosities were observed in preparations of poloxamer 407. Moreover, these viscosities were lower than that of drug free preparation. There was no difference between preparations of 0.5% and 1% ibuprofen. Both preparations showed the Newtonian flow patterns with slightly negative thixotropic systems as shown in Figures 91-92. A slight decrease was observed in the down curves. The reproduced preparations also shown the similar viscosity.

For preparations containing egg lecithin, higher viscosity products were observed. The viscosity at shear rate of 1000 s^{-1} of preparation containing 0.5% ibuprofen was slightly decreased compared with drug free preparation, but increased in preparations of 1.0% and 1.5% drug. Increasing drug concentration increased their viscosity. Figures 95-98 show the flow and viscosity patterns of preparations of egg lecithin. These preparations showed high viscosity in low shear force but the viscosity decreased in high shear force. Only preparation containing 0.5% ibuprofen and 1% egg lecithin showed the plastic flow in up-curve and likely Newtonian flow in down-curve. The other preparations showed the plastic flow in both up- and down-curves.

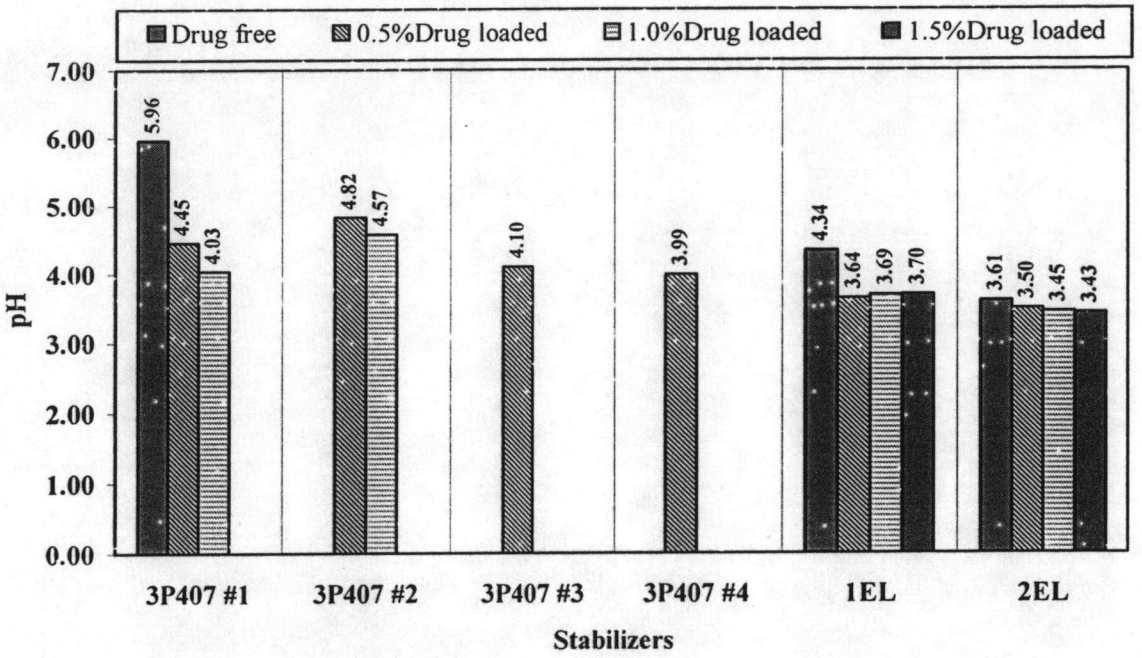

Figure 87. The pH of dispersions of SLN containing ibuprofen after autoclaving.

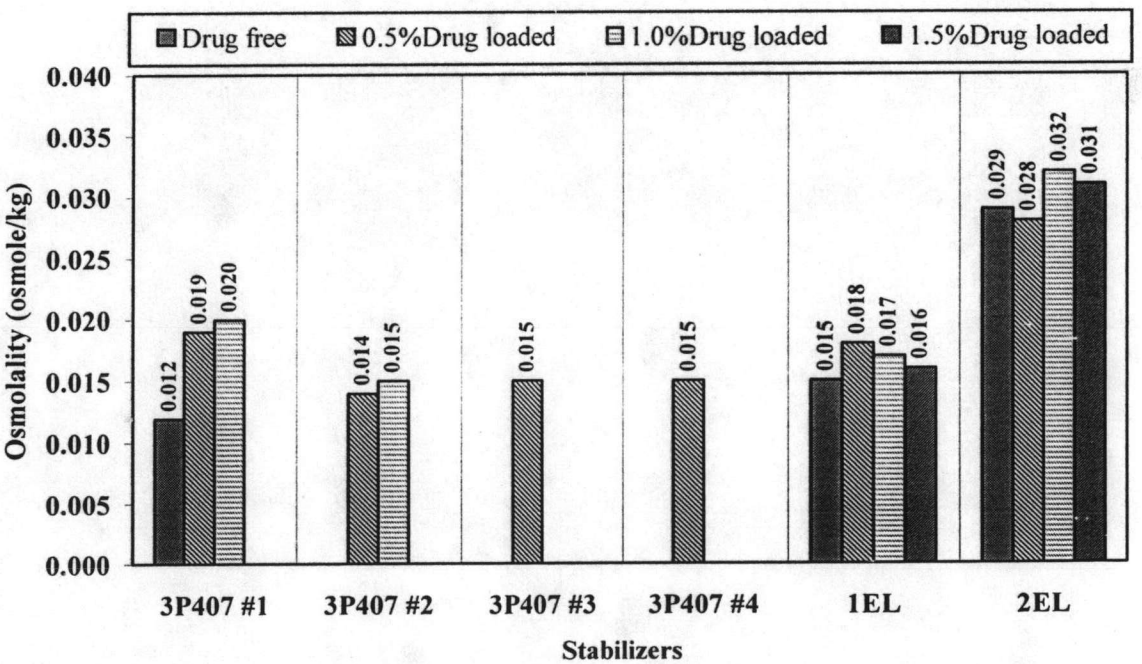

Figure 88. The osmolality of dispersions of SLN containing ibuprofen after autoclaving.

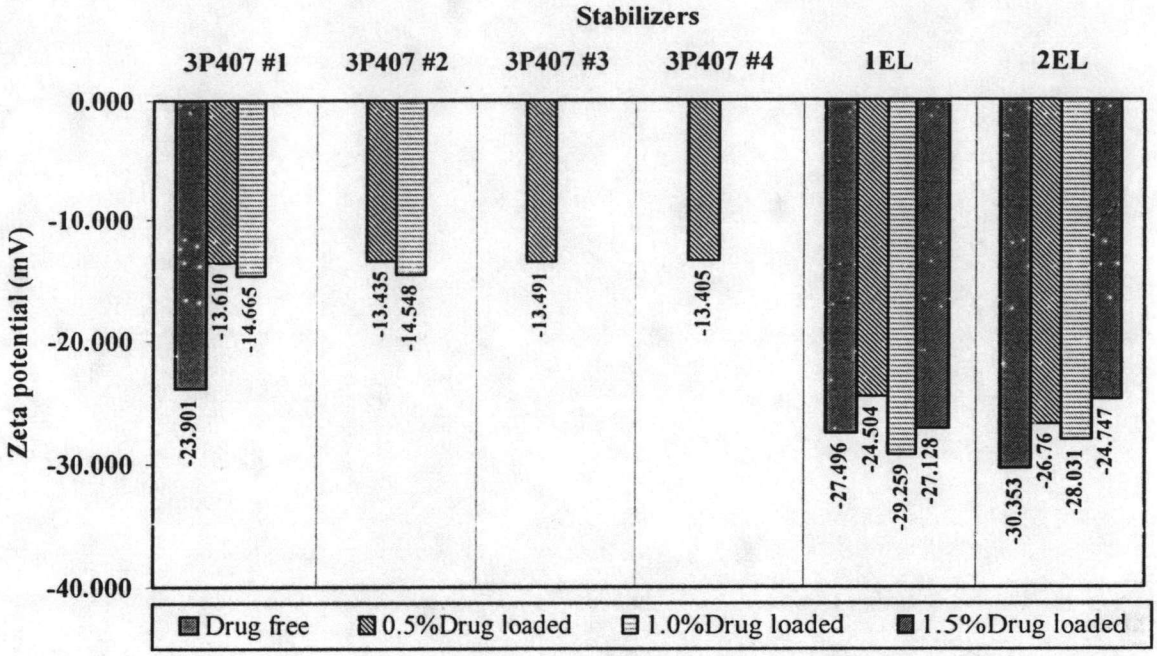

Figure 89. The zeta potential of dispersions of SLN containing ibuprofen after autoclaving.

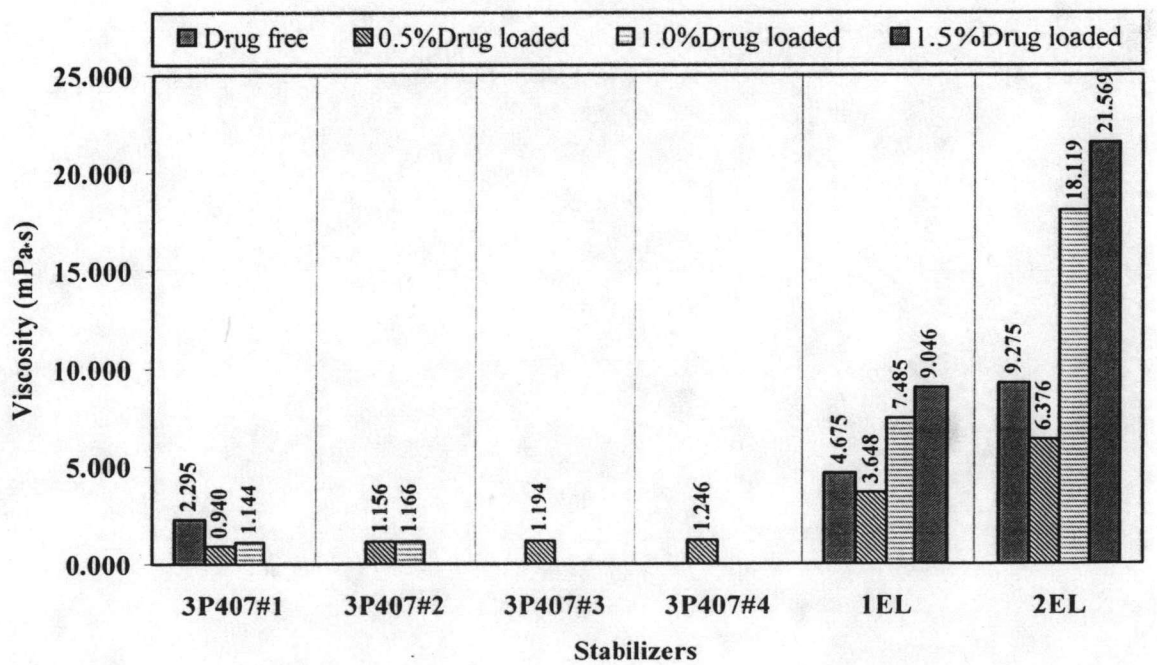

Figure 90. The viscosity at shear rate of 1000 s^{-1} of dispersions of SLN containing ibuprofen after autoclaving.

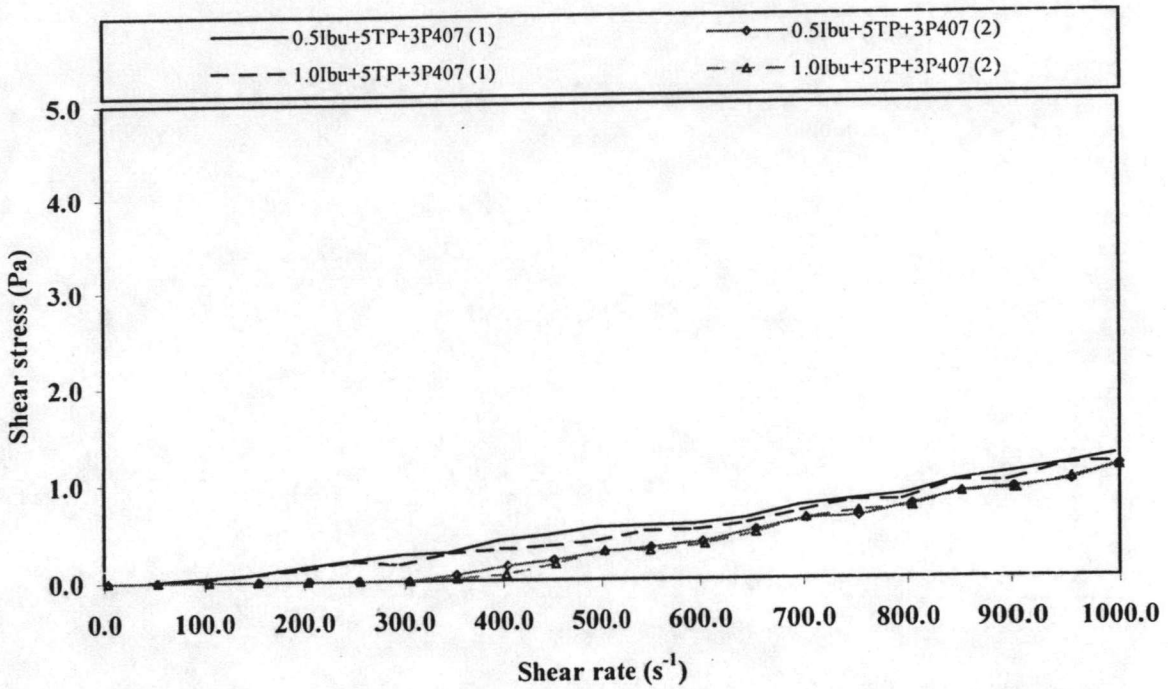

Figure 91. Flow curves of dispersions of SLN containing 0.5-1.0% ibuprofen and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

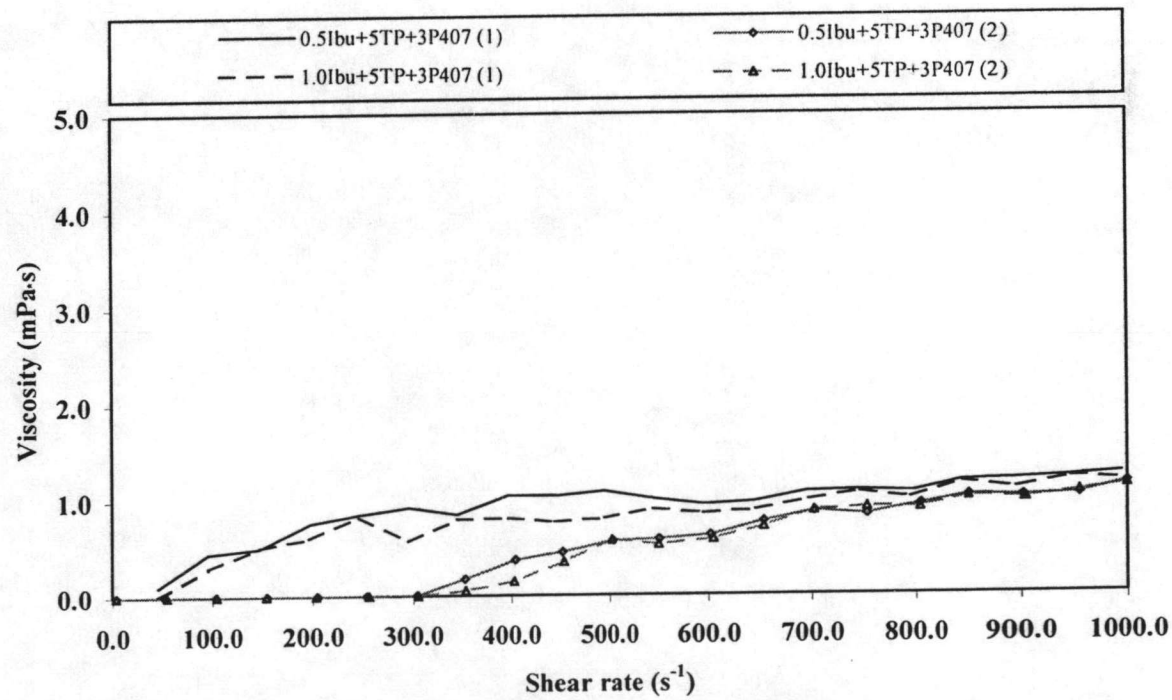

Figure 92. Viscosity curves of dispersions of SLN containing 0.5-1.0% ibuprofen and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

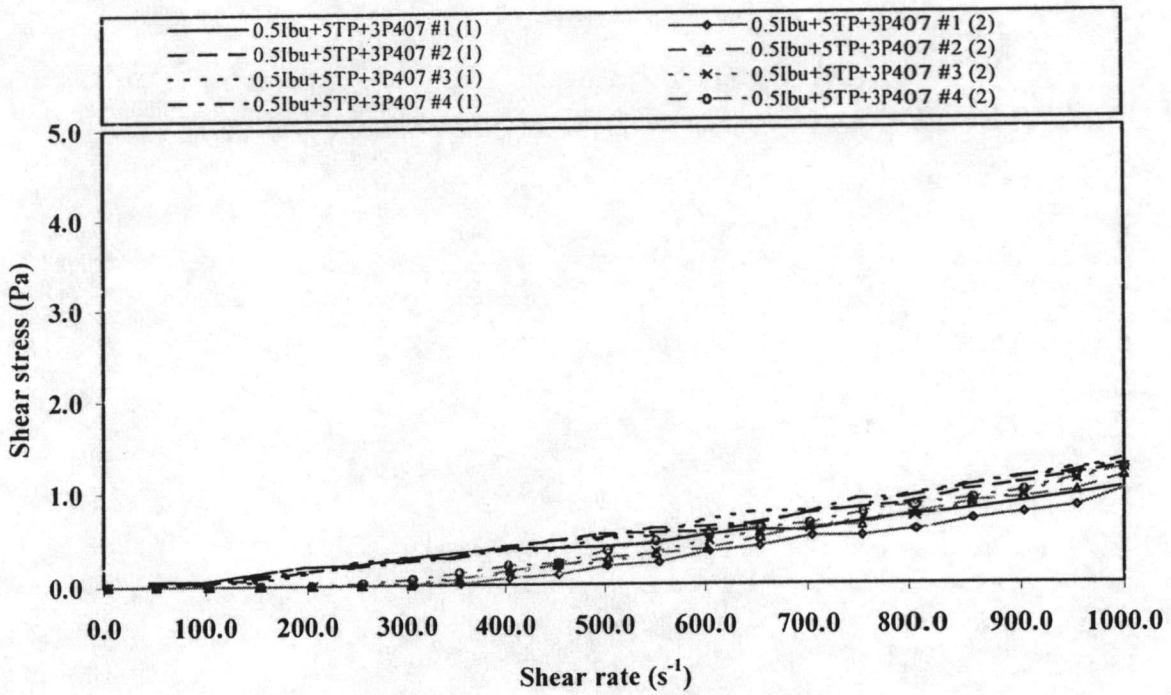

Figure 93. Flow curves of four batches of preparation containing 0.5% ibuprofen and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

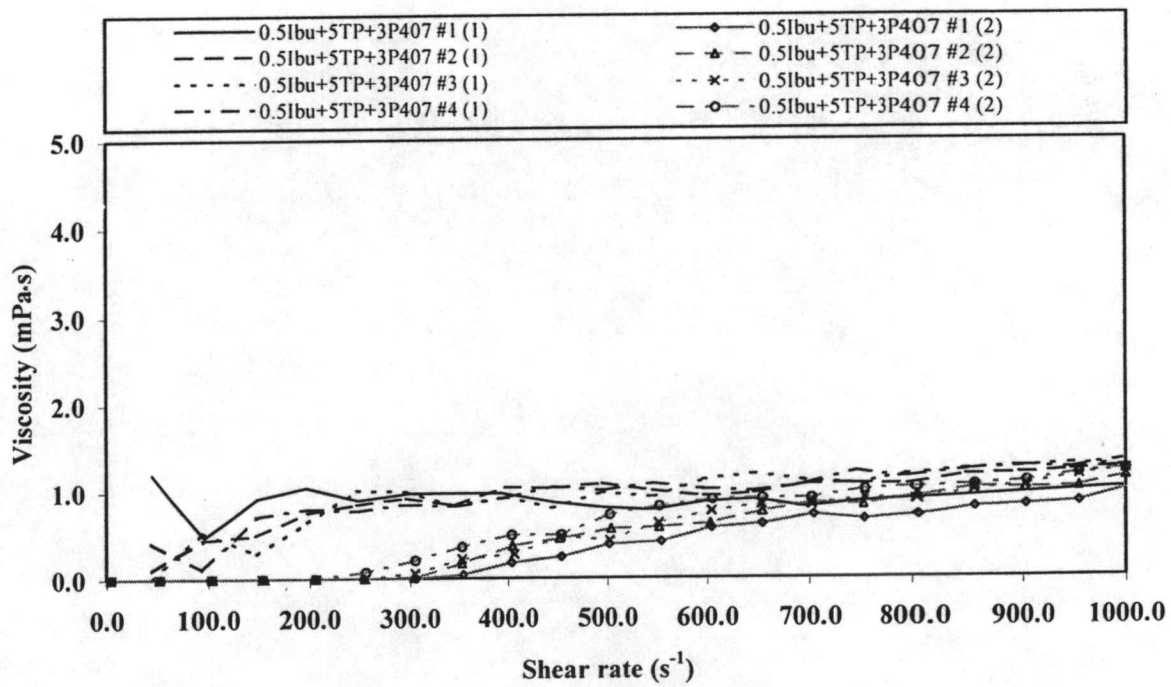

Figure 94. Viscosity curves of four batches of preparation containing 0.5% ibuprofen and 3% poloxamer 407 ((1) up-curve and (2) down-curve).

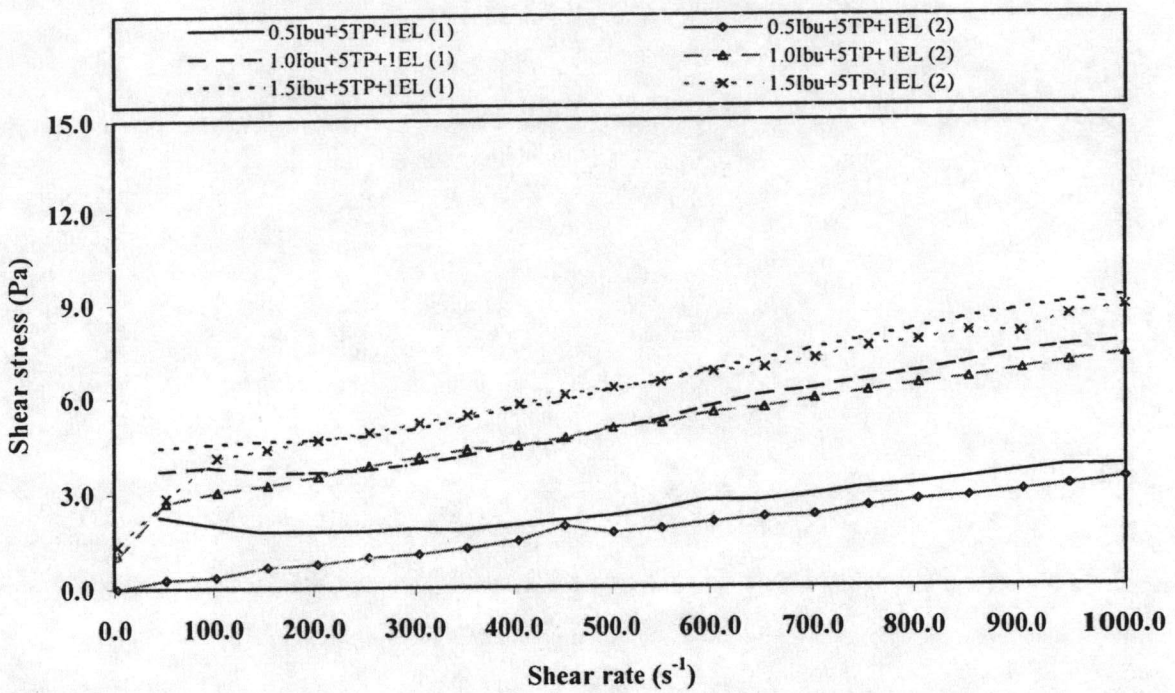

Figure 95. Flow curves of dispersions of SLN containing 0.5-1.5% ibuprofen and 1% egg lecithin ((1) up-curve and (2) down-curve).

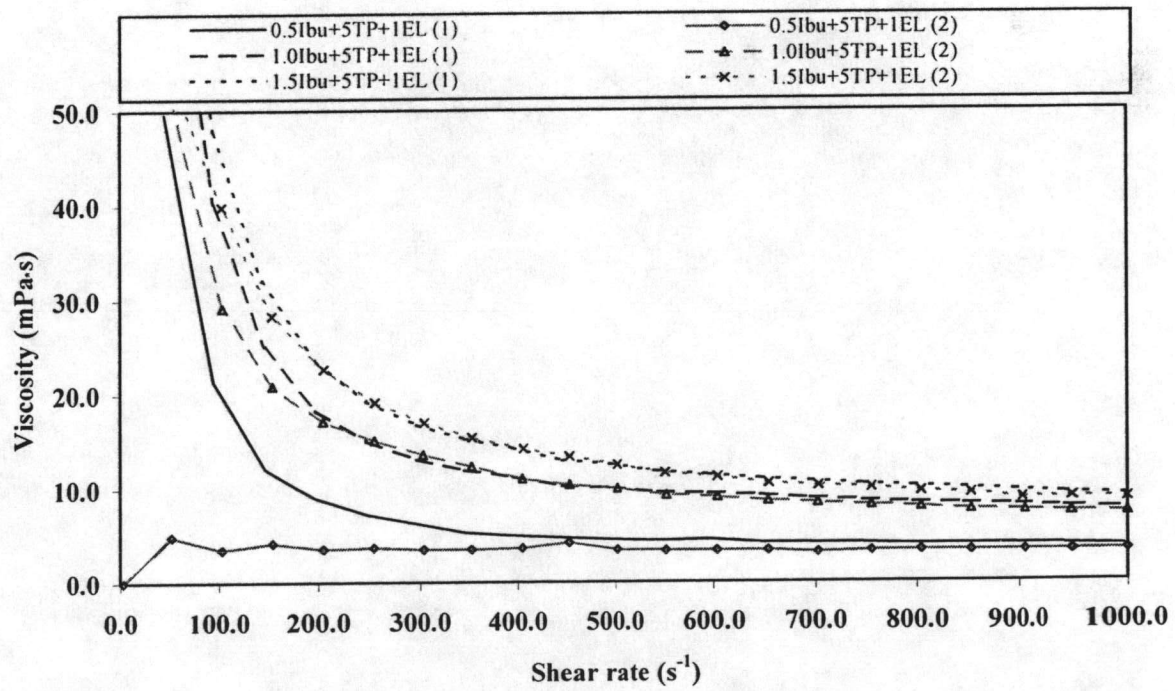

Figure 96. Viscosity curves of dispersions of SLN containing 0.5-1.5% ibuprofen and 1% egg lecithin ((1) up-curve and (2) down-curve).

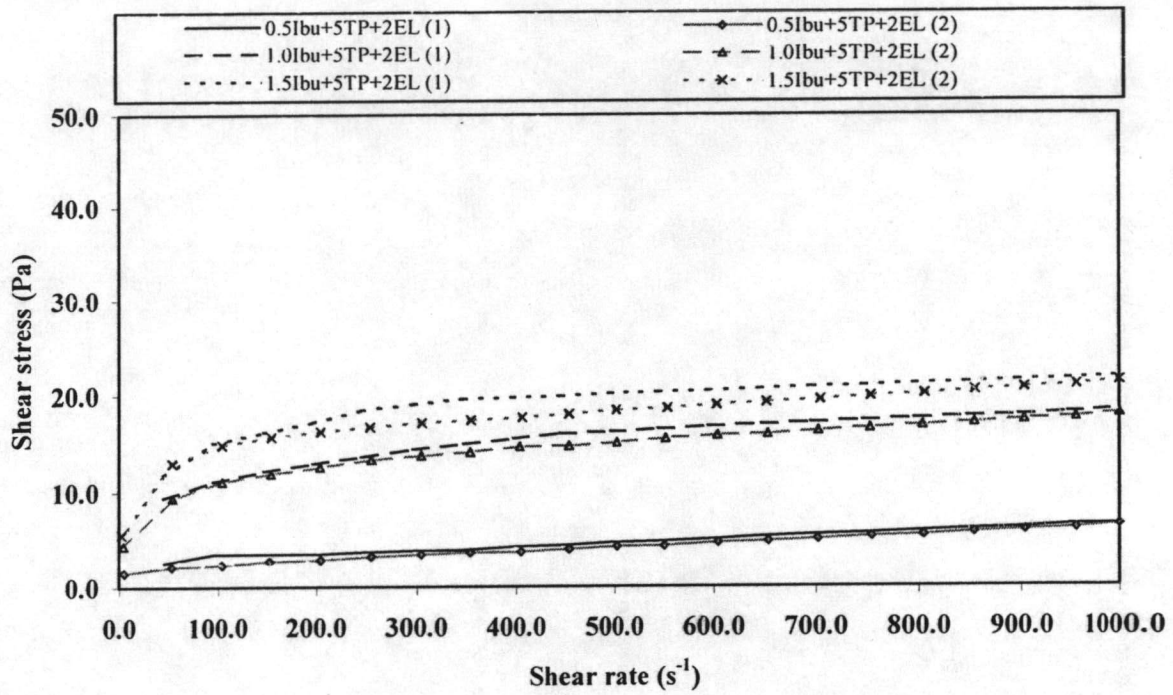

Figure 97. Flow curves of dispersions of SLN containing 0.5-1.5% ibuprofen and 2% egg lecithin ((1) up-curve and (2) down-curve).

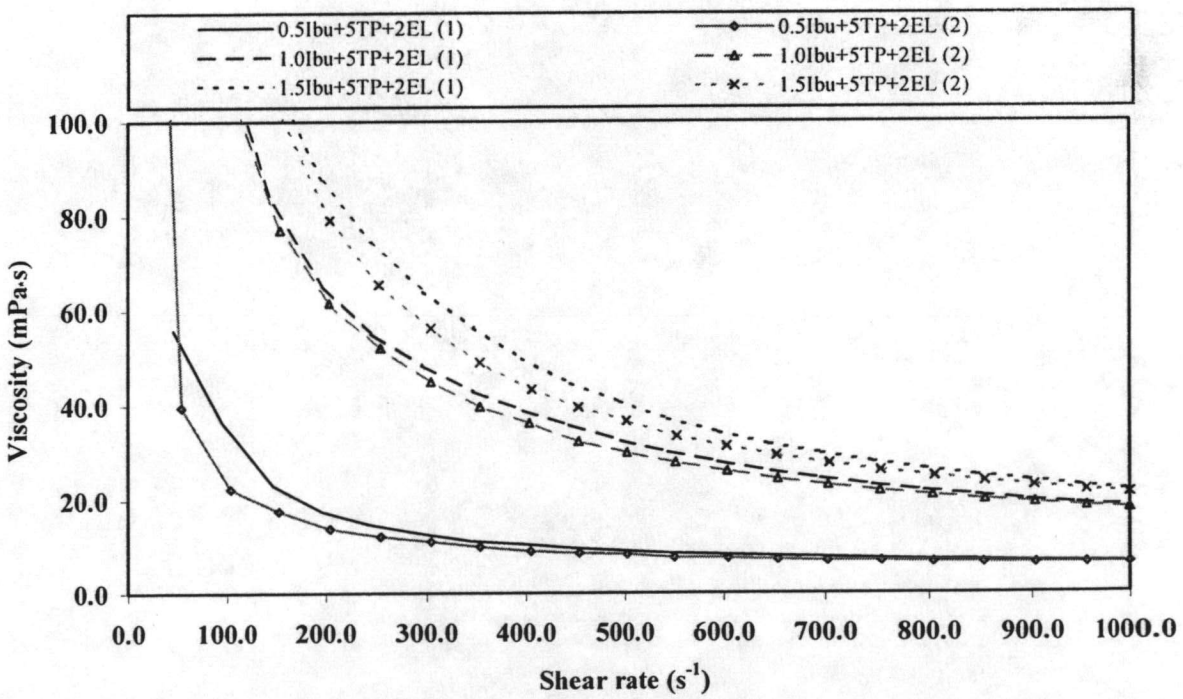

Figure 98. Viscosity curves of dispersions of SLN containing 0.5-1.5% ibuprofen and 2% egg lecithin ((1) up-curve and (2) down-curve).

3.4.7 Particle shape

The scanning electron photomicrograph of preparation of SLN containing ibuprofen of 0.5% and poloxamer 407 of 3% showed the spherical particles as in Figure 99. Their sizes varied, but always lower than 1 μm. Some smaller crystals were found on their surfaces.

3.4.8 Infrared spectra

Figure 100 shows the spectrum of ibuprofen. The dominant spectra were the broad peak of O-H stretching at 2500-3000 cm^{-1}, the carbonyl C=O stretching at 1721 cm^{-1}, O-H in plane bending at 1420 cm^{-1} and out of plan bending at 936 cm^{-1}, and the para-substituted benzene ring at 866 cm^{-1}.

The solid lipid matrices of ibuprofen were tested by infrared spectroscopy. Figures 101-103 show their spectra compared with raw materials. It was found that the basic spectra were similar to tripalmitin. Only very small peaks of drug appeared at 1508, 1420, 1227, 936 cm^{-1}, and the broad peak at 3000 cm^{-1}. These peaks were clearly distinguishable in egg lecithin stabilized preparations and at higher drug concentration. No new peak was observed from its mixture.

3.4.9 Differential scanning calorimetry

The DSC thermograms of lipid matrices of ibuprofen were studied between -30°C and 150°C. These thermograms were compared with their raw materials as shown in Figure 104. Ibuprofen showed the endotherm from 72.85°C to 83.83°C with the peak at 79.08°C. This was its melting point, and lack of the peak in SLN showed that the drug mixed well with tripalmitin during the process for preparing the SLN. For the solid lipid matrix of preparation containing 0.5% ibuprofen and 3% poloxamer 407, there were two endotherms in the range of 10.48°C to 51.53°C with the peak at 45.00°C, and 51.25°C to 65.59°C with the peak at 61.98°C. And the solid lipid matrix of preparation containing 1.0% ibuprofen and

3% poloxamer 407 also showed two endotherms in the range of 10.61°C to 49.97°C with the peak at 43.72°C, and 49.69°C to 66.00°C with the peak at 62.26°C. These results showed that ibuprofen was not in crystalline form when loaded into the solid lipid matrix.

3.4.10 Powder X-ray diffraction

The X-ray diffractograms of ibuprofen, tripalmitin, poloxamer 407, and the solid lipid matrices from ibuprofen loaded preparations are shown in Figure 105. Ibuprofen was the crystalline and showed the peaks at 6.020°, 12.100°, 16.580°, 17.620°, 18.780°, 19.060°, 19.980°, 20.120°, 22.220°, and the small peaks between 22.700° to 38.580°. After loaded into the lipid matrices, their crystallinities did not appear. Only the crystalline peaks of lipid were presented in the preparations. However, there was the very small peak of drug in preparation containing 1.0% ibuprofen and 3% poloxamer 407 at 6.200° and 16.540° due to the drug crystallization in drying process.

3.4.11 Entrapment efficiency

High entrapment efficiency was obtained in preparations of SLN containing ibuprofen. Table 23 and Figure 106 show the entrapment efficiency of their preparations. It was shown that ibuprofen, the water insoluble drug, could be loaded into SLN at high levels. The percentage of entrapment efficiency of higher than 85% was observed in all preparations. Especially in preparations of egg lecithin , more than 97% was entrapped into lipid matrices. It was found that increasing the drug concentration would increase the entrapment efficiency. Four different batches of preparation containing 0.5% ibuprofen and 3% poloxamer 407 yielded the entrapment efficiency values that were not different.

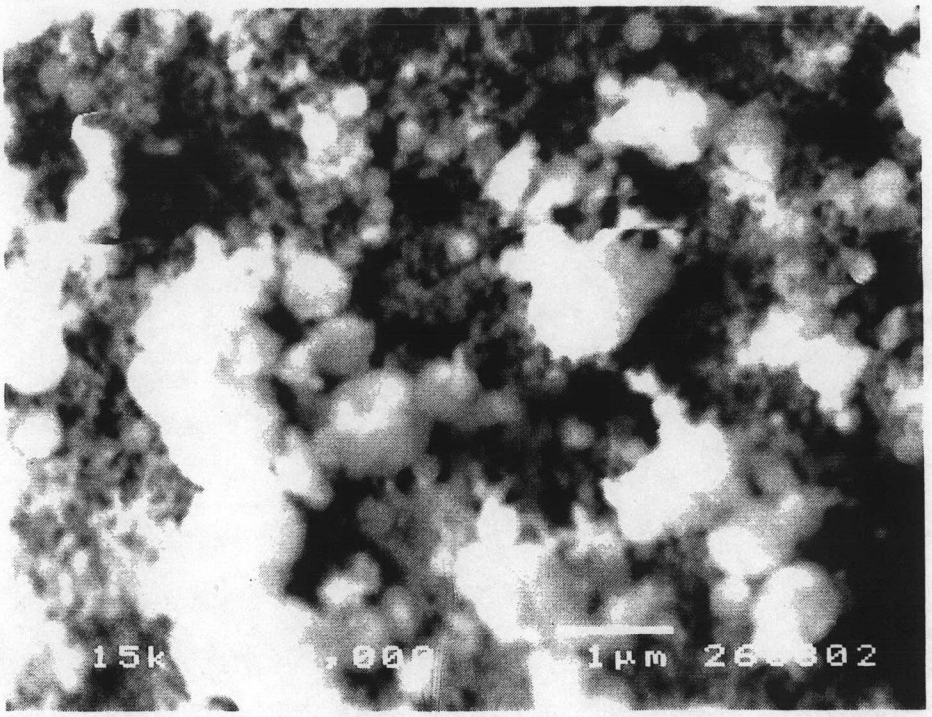

Figure 99. Cryo-scanning electron photomicrograph of SLN containing 0.5% ibuprofen and 3% poloxamer 407.

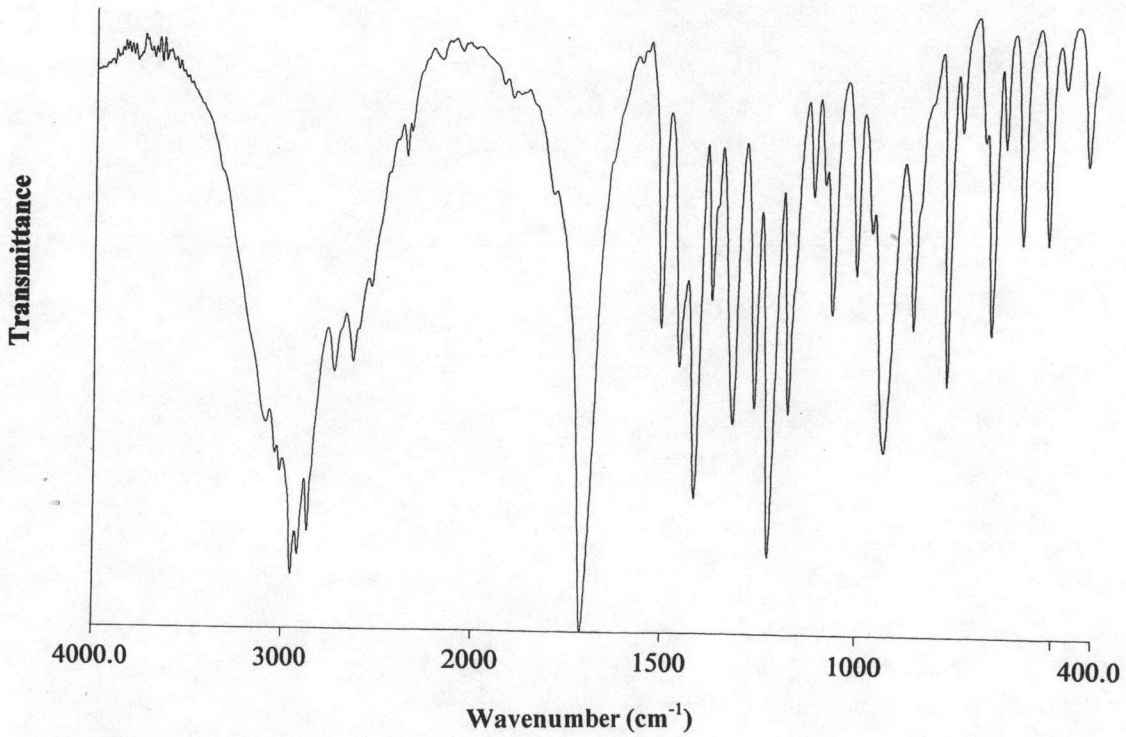

Figure 100. IR spectrum of ibuprofen.

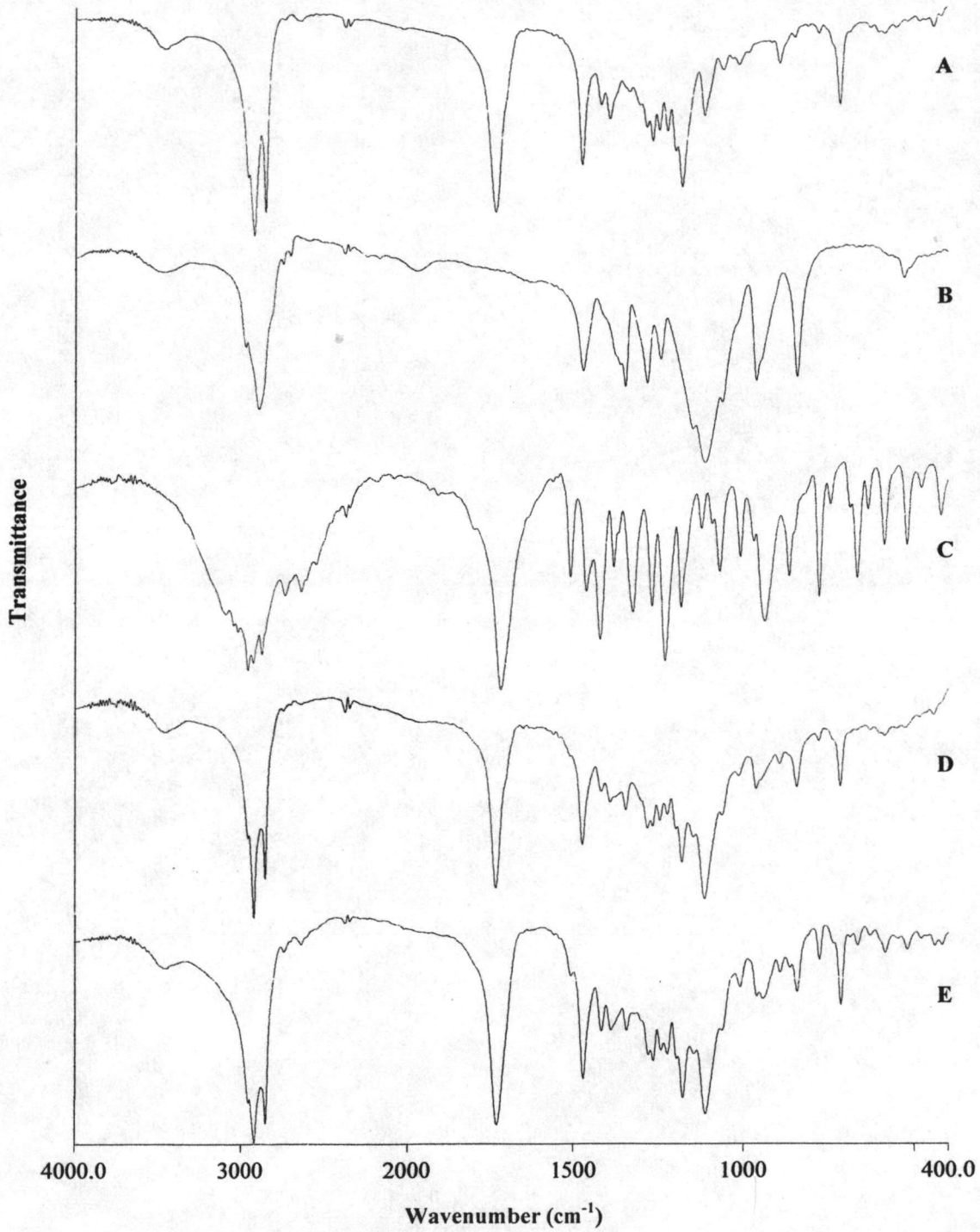

Figure 101. IR spectra of (A) tripalmitin; (B) poloxamer407; (C) ibuprofen; and lipid matrices of preparations containing (D) 0.5%, and (E) 1.0% ibuprofen and 3% poloxamer 407.

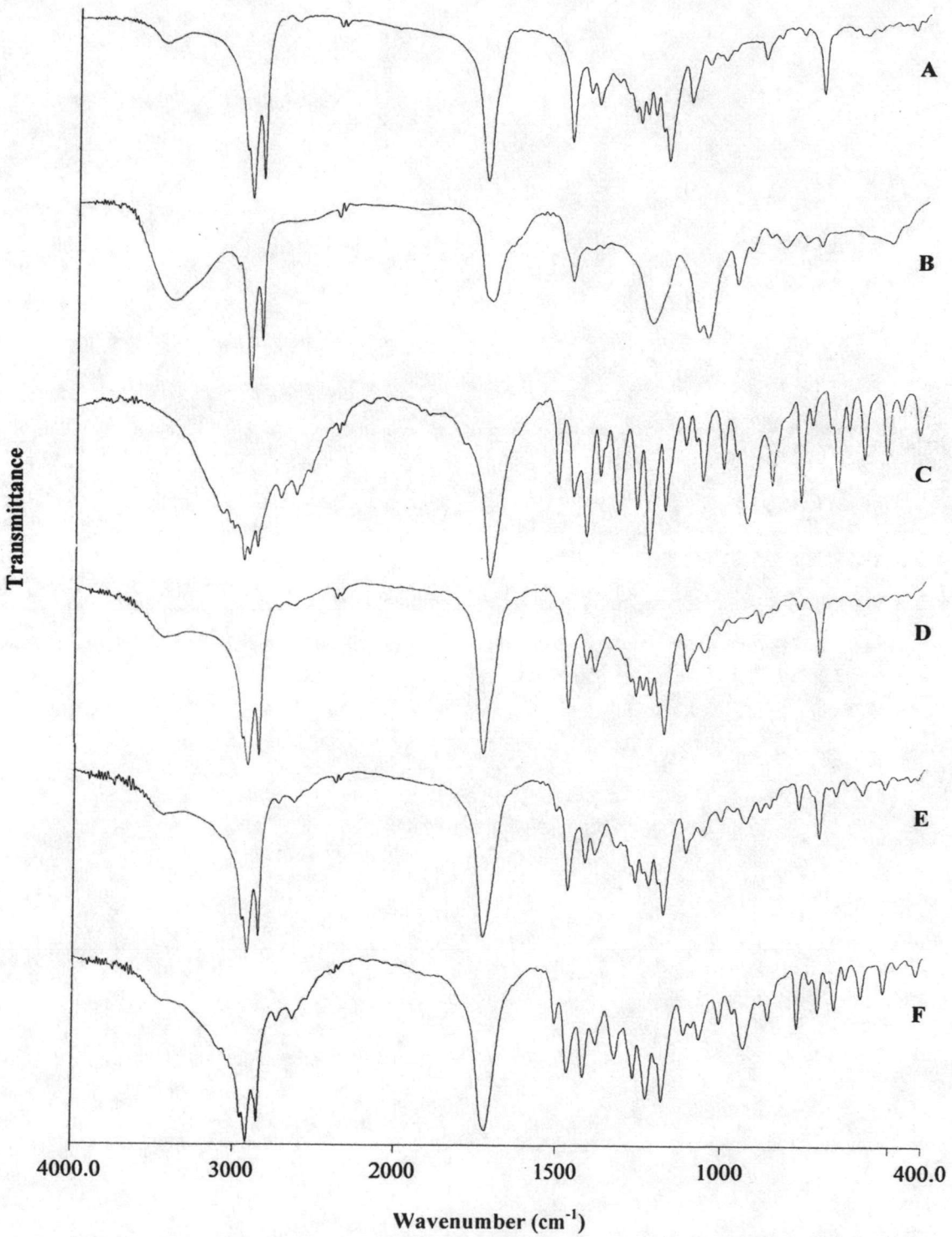

Figure 102. IR spectra of (A) tripalmitin; (B) egg lecithin; (C) ibuprofen; and lipid matrices of preparations containing (D) 0.5%, (E) 1.0%, and (F) 1.5% ibuprofen and 1% egg lecithin.

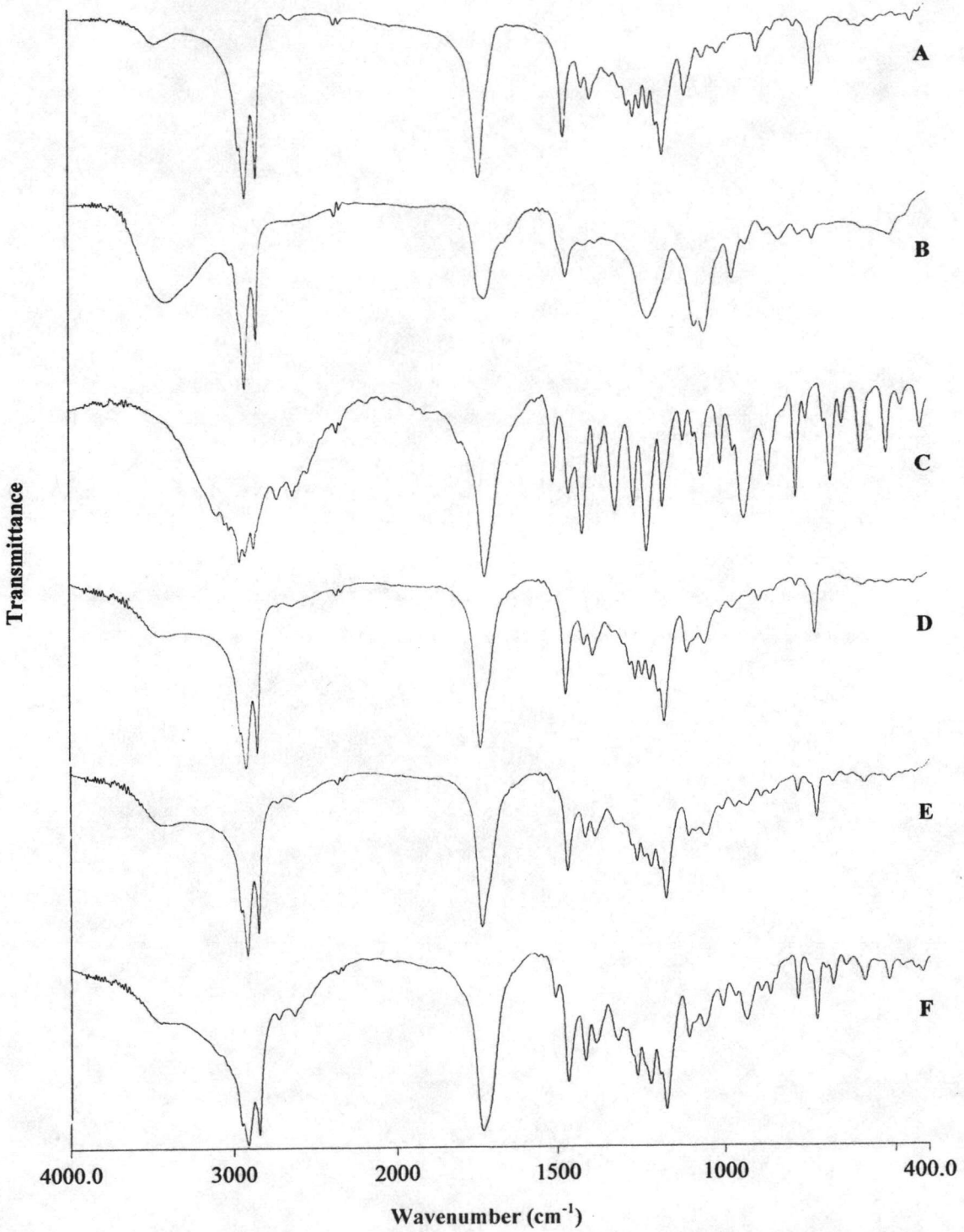

Figure 103. IR spectra of (A) tripalmitin; (B) egg lecithin; (C) ibuprofen; and lipid matrices of preparations containing (D) 0.5%, (E) 1.0%, and (F) 1.5% ibuprofen and 2% egg lecithin.

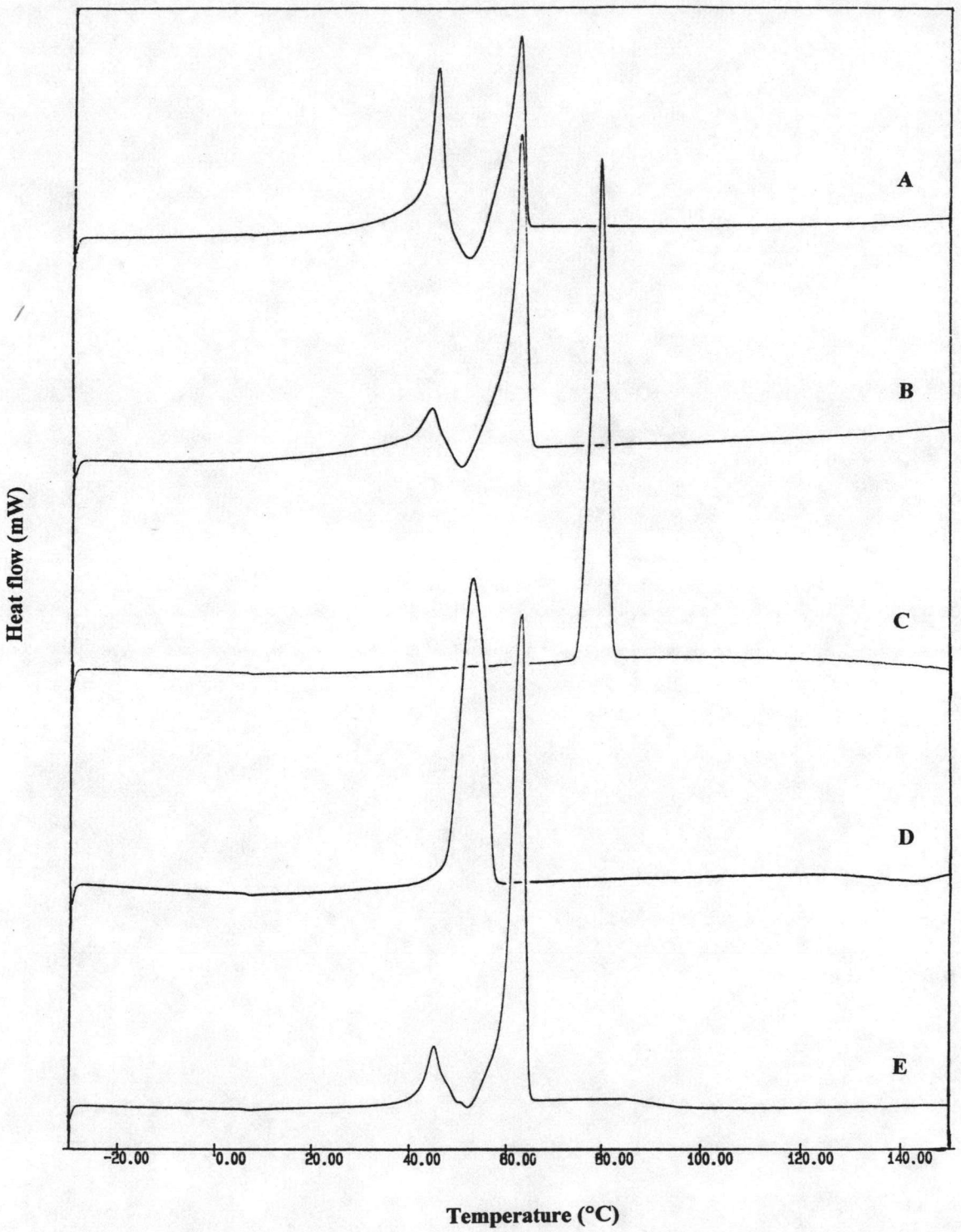

Figure 104. DSC thermograms of lipid matrices of preparations containing (A) 0.5%, and (B) 1.0% ibuprofen and 3% poloxamer 407; (C) ibuprofen; (D) poloxamer 407; and (E) tripalmitin.

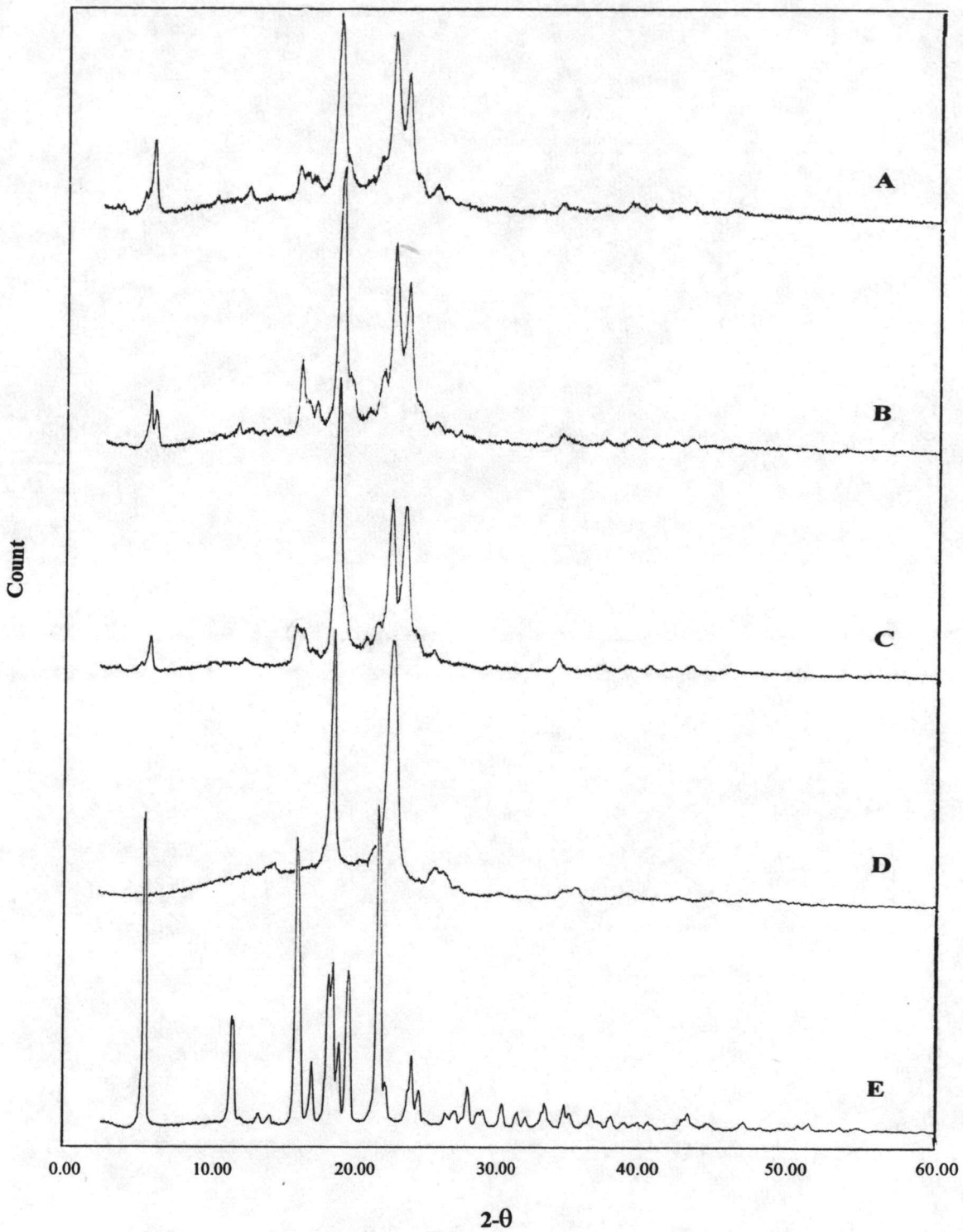

Figure 105. X-ray diffractograms of lipid matrices of preparations containing (A) 0.5%,and (B) 1.0% ibuprofen and 3% poloxamer 407; (C) tripalmitin; (D) poloxamer 407; and (E) ibuprofen.

Table 23. Entrapment efficiency of ibuprofen loaded into SLN after autoclaving.

Formulation	Percentage drug entrapment of SLN					
	No.1	No.2	No.3	mean	SD	%CV
0.5Ibu+5TP+3P407 (batch 1)	86.93	86.87	86.96	86.92	0.04	0.05
0.5Ibu+5TP+3P407 (batch 2)	85.26	85.29	85.26	85.27	0.02	0.02
0.5Ibu+5TP+3P407 (batch 3)	86.24	86.24	86.19	86.22	0.03	0.03
0.5Ibu+5TP+3P407 (batch 4)	85.20	85.30	85.37	85.29	0.09	0.10
1.0Ibu+5TP+3P407 (batch 1)	91.14	91.14	91.12	91.13	0.01	0.02
1.0Ibu+5TP+3P407 (batch 2)	90.70	90.60	90.83	90.71	0.11	0.12
0.5Ibu+5TP+1EL	97.77	97.62	97.61	97.67	0.09	0.09
1.0Ibu+5TP+1EL	99.02	99.04	99.03	99.03	0.01	0.01
1.5Ibu+5TP+1EL	99.54	99.55	99.58	99.55	0.02	0.02
0.5Ibu+5TP+2EL	97.52	97.46	97.49	97.49	0.03	0.03
1.0Ibu+5TP+2EL	99.10	99.10	99.10	99.10	0.00	0.00
1.5Ibu+5TP+2EL	99.31	99.32	99.34	99.32	0.02	0.02

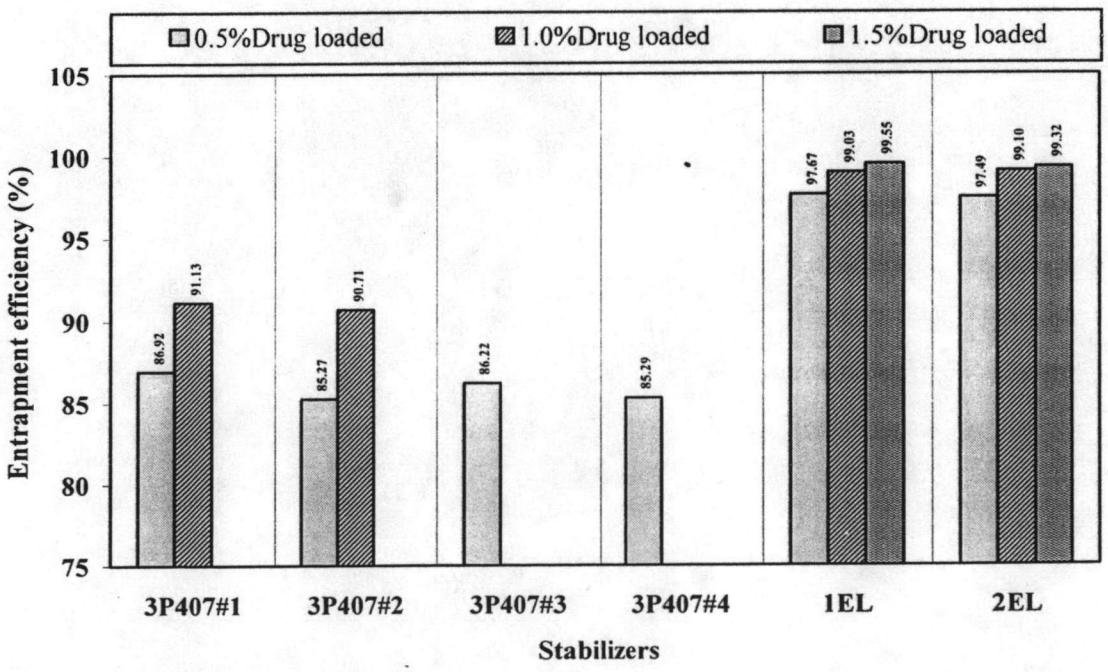

Figure 106. The entrapment efficiency of ibuprofen loaded into SLN after autoclaving.

3.4.12 Drug release

Ibuprofen saturated solution rapidly diffused through the dialysis membrane. About 100% of drug was determined in the receptor part within 6 hours. However, it reached to the plateau more than 110% in 12 hours. These results were repeated in triplicate, and the similar results were obtained.

Ibuprofen loaded SLN could slowly release the drug for more than 7 days. Figures 108-109 showed the release profiles of preparations of SLN containing 0.5% and 1.0% ibuprofen using 3% poloxamer 407 as stabilizer, respectively. It was found that the supernatant of preparations was also rapidly release similar to that of saturated solution. It reached to the plateau within 12-24 hours. These showed the releasing of soluble drug in dispersion medium could be released completely within 12-24 hours. Then the drug was prominently released from solid lipid matrix. So data of drug release after 12 hours were used to elucidate the drug release model. However, total drug release data was also used to elucidate the drug release model of the release profile of drug from both supernatant and SLN.

The preparations of SLN containing ibuprofen could effectively sustain the drug release. Preparations of 0.5% and 1.0% ibuprofen could release approximately 70%, and 50% of drug at the 7^{th} day, respectively. Furthermore, it could be reproduced and gave similar release profiles with different batches. The elucidations of drug release kinetic are shown in Tables 23-24. Ibuprofen was released from SLN following Higuchi and power expression models. The coefficient of determination of nearly an integral was obtained in all release profiles. These values were not different in all preparations.

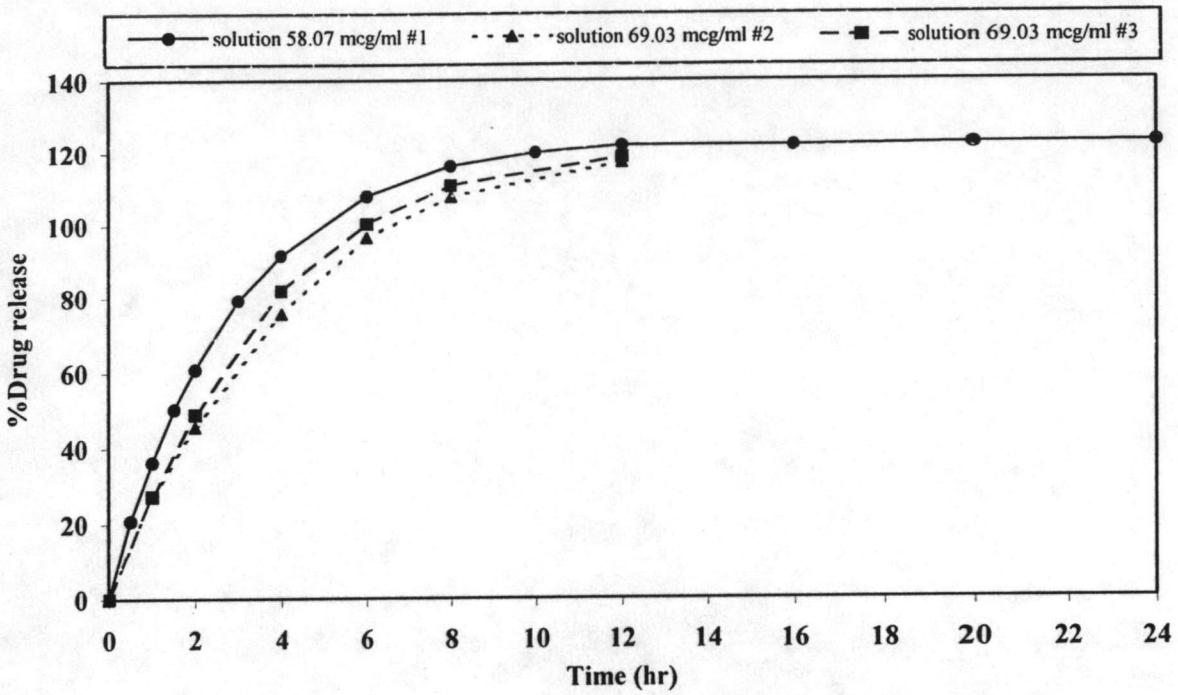

Figure 107. The release profiles of ibuprofen saturated solution.

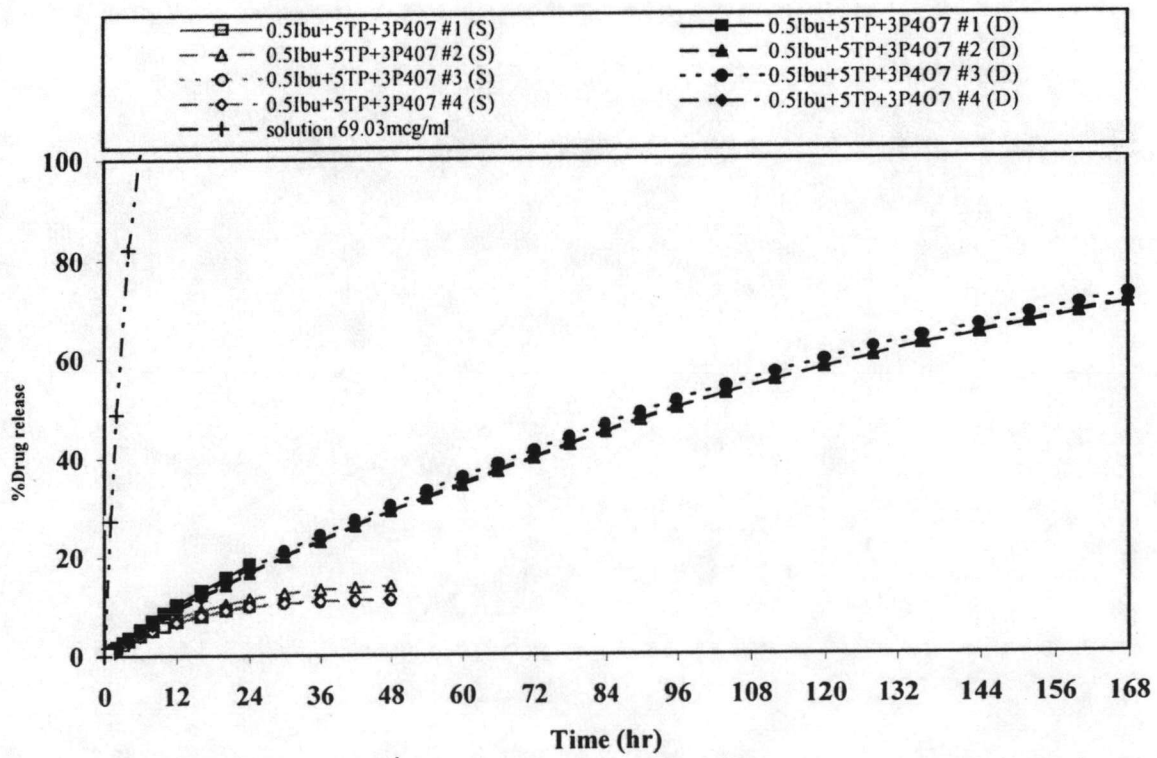

Figure 108. The release profiles of preparation of SLN containing 0.5% ibuprofen and 3% poloxamer 407 ((S) supernatant and (D) dispersion).

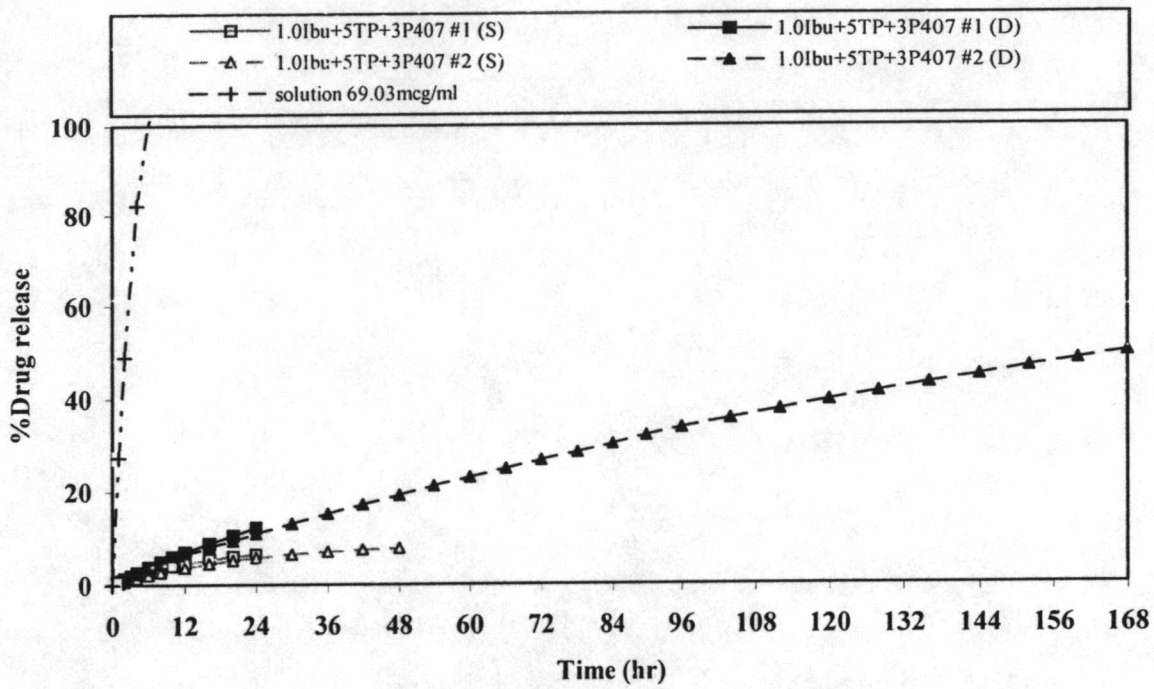

Figure 109. The release profiles of preparation of SLN containing 1.0% ibuprofen and 3% poloxamer 407 ((S) supernatant and (D) dispersion).

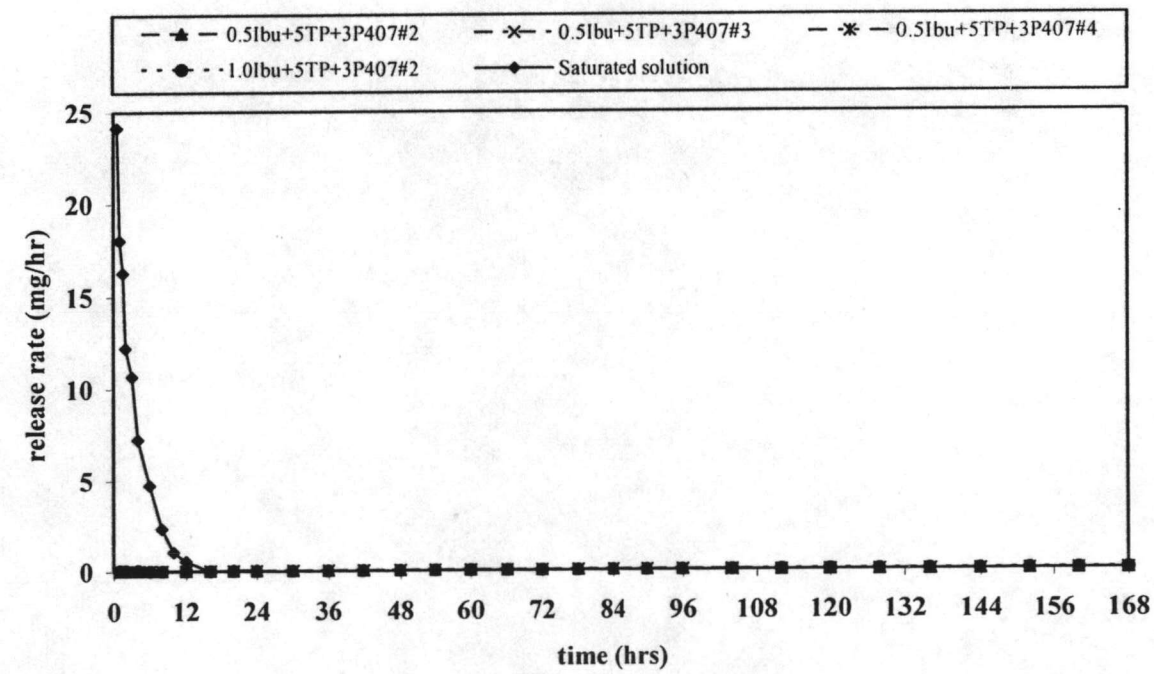

Figure 110. The release rate profiles of preparations of SLN containing 0.5-1.0% ibuprofen and 3% poloxamer 407.

Table 24. The coefficients of determination of preparations containing ibuprofen in various drug release kinetics calculated from total drug release data.

Formulation	Coefficient of determination (R^2) (±SD)				
	Zero order model	First order model	Higuchi model	Power expression	Cube root model
0.5Ibu+5TP+3P407 #2	0.9806 (±0.0020)	0.7007 (±0.0040)	0.9905 (±0.0010)	0.9985 (±0.0003)	0.9233 (±0.0029)
0.5Ibu+5TP+3P407 #3	0.9776 (±0.0029)	0.6920 (±0.0032)	0.9923 (±0.0014)	0.9975 (±0.0004)	0.9173 (±0.0032)
0.5Ibu+5TP+3P407 #4	0.9792 (±0.0017)	0.6896 (±0.0021)	0.9914 (±0.0009)	0.9974 (±0.0002)	0.9178 (±0.0012)
1.0ibu+5TP+3P407 #2	0.9883 (±0.0008)	0.7175 (±0.0026)	0.9854 (±0.0007)	0.9995 (±0.0001)	0.9358 (±0.0005)

Table 25. The coefficients of determination of preparations containing ibuprofen in various drug release kinetics calculated from drug release data after the 12^{th} hours.

Formulation	Coefficient of determination (R^2) (±SD)				
	Zero order model	First order model	Higuchi model	Power expression	Cube root model
0.5Ibu+5TP+3P407 #2	0.9849 (±0.0019)	0.8715 (±0.0034)	0.9975 (±0.0004)	0.9985 (±0.0003)	0.9233 (±0.0029)
0.5Ibu+5TP+3P407 #3	0.9827 (±0.0023)	0.8631 (±0.0029)	0.9985 (±0.0005)	0.9975 (±0.0004)	0.9173 (±0.0032)
0.5Ibu+5TP+3P407 #4	0.9838 (±0.0013)	0.8626 (±0.0016)	0.9984 (±0.0002)	0.9974 (±0.0002)	0.9178 (±0.0012)
1.0Ibu+5TP+3P407 #2	0.9918 (±0.0007)	0.8844 (±0.0008)	0.9948 (±0.0003)	0.9995 (±0.0001)	0.9358 (±0.0005)