CHAPTER IV

RESULTS AND DISCUSSION

1.Methods of quantitative analysis of retinyl palmitate for entrapment studies

1.1 UV spectrophotometer

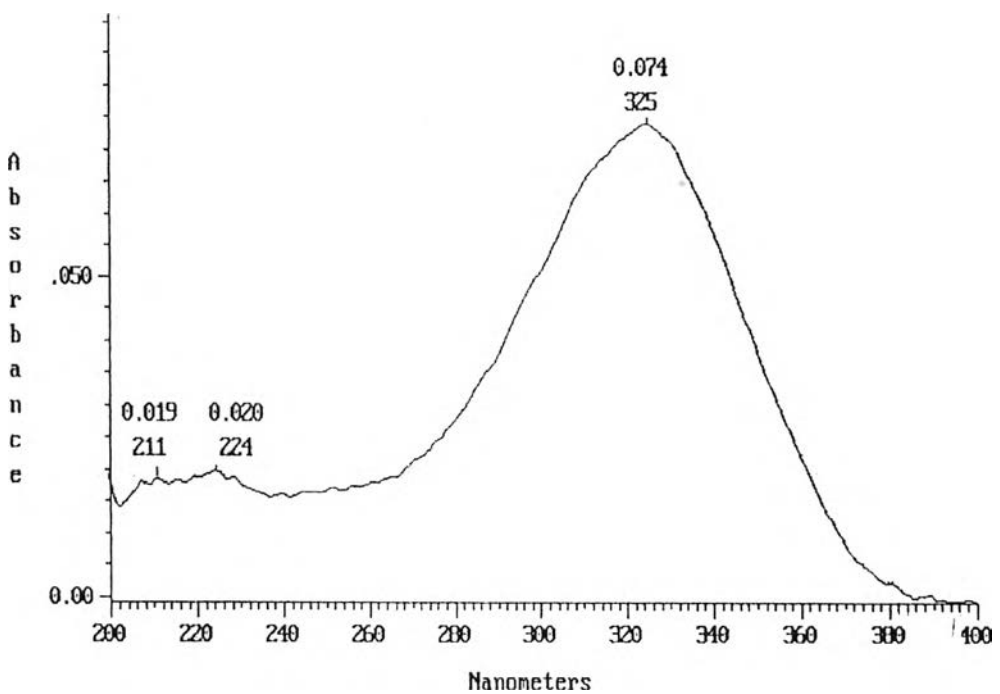


Figure 14. The U.V. spectrum of retinyl palmitate

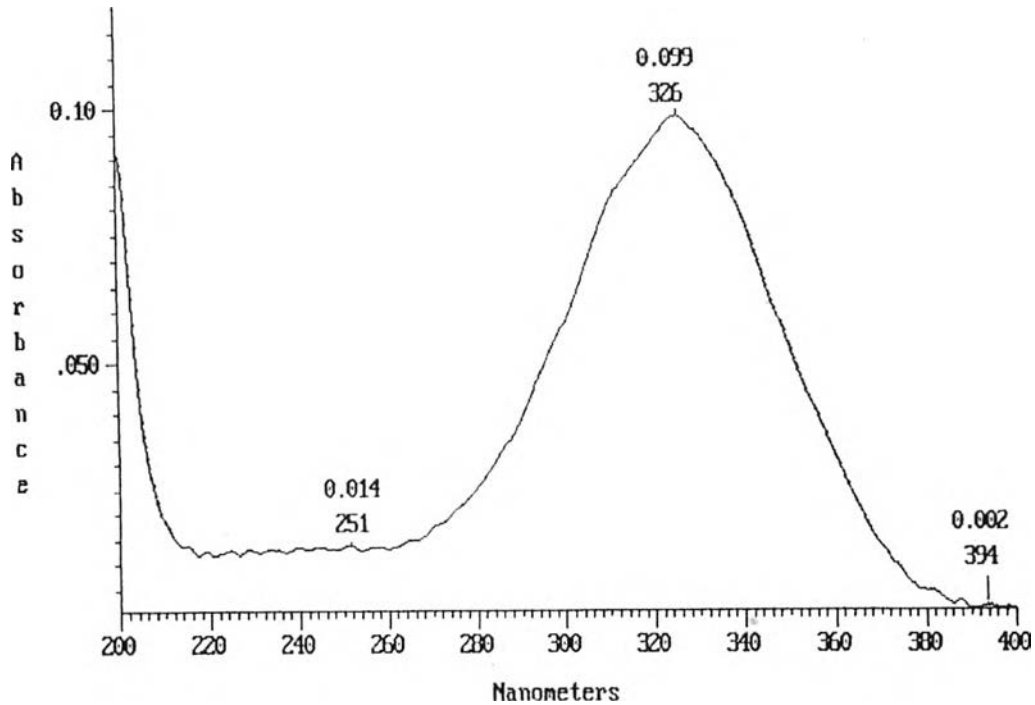


Figure 15. The U.V. spectrum of retinyl acetate

Both retinyl palmitate and retinyl acetate show the maximum absorption wavelength at 325 nm as shown in figure 14,15. Therefore, the UV detector of HPLC was set at 325 nm in order to obtain the accurately quantitative analysis of retinyl palmitate for entrapment study and permeation study.

1.2 HPLC assay for retinyl palmitate analysis

The analytical method for retinyl palmitate was performed and validated for its accuracy, precision, specificity and linearity.

Accuracy

Tables 2,3,4 show the percentage of analytical recovery of three groups of niosome suspension. The average percentage of recovery for niosome preparation prepared by Span 40 and Span 60 were approximately 100% while that from Span 85 was 106%. Therefore, retinyl palmitate was accurately determined from Span 40 and Span 60 niosomes.

Table 2. Accuracy of retinyl palmitate in niosome suspension prepared from Span 40.

Initial amount (mg)	Analytical amount (mg)	%Recovery
5.10	4.94	96.86
5.30	5.34	100.78
5.70	5.90	103.50
		mean = 100.40
		SD = 3.33

Table 3. Accuracy of retinyl palmitate in niosome suspension prepared from Span 60

Initial amount (mg)	Analytical amount (mg)	%Recovery
5.70	5.68	99.64
5.70	5.70	100.00
5.70	5.78	101.40
		mean = 100.30
		SD = 0.93

Table 4. Accuracy of retinyl palmitate niosome suspension prepared from Span 85

actual amount	total amount	%recovery
5.80	5.86	101.03
5.80	5.88	101.37
5.80	6.20	106.89
		mean = 103.90
		SD = 3.29

Precision

Tables 5,6 show the peak area ratio of retinyl palmitate and retinyl acetate analyzed by HPLC both in the same day and different days. The percentage of coefficient of varration (% CV) of each concentration of standard solutions was in the range of 2.0 μg/ml to 8.0 μg/ml.

For within-run analysis, the %CV were within the range of 0.062-0.344%

Table 5. Within run precision data

Concentration (μg/ml)	peak area ratio			mean	SD	%CV
	N_1	N_2	N_3			
2.0	1.414	1.422	1.422	1.419	0.004	0.344
4.0	2.888	2.880	2.872	2.880	0.007	0.274
6.0	4.432	4.429	4.426	4.429	0.002	0.062
8.0	5.714	5.711	5.960	5.707	0.009	0.166

N=sample No.

Table 6. Between run precision data

concentration (μg/ml)	peak area ratio			mean	SD	%CV
	day_1	day_2	day_3			
2.0	1.403	1.409	1.409	1.407	0.003	0.217
4.0	2.881	2.846	2.847	2.858	0.020	0.699
6.0	4.424	4.413	4.411	4.416	0.007	0.163
8.0	5.701	5.664	5.699	5.691	0.015	0.267

The %CV from between run analysis were in the range of 0.163-0.699%. Therefore, the analysis of retinyl palmitate in niosome preparation was precise enough to be used.

Specificity

Figure16,17 and 18 show the chromatograms of retinyl palmitate and retinyl acetate in standard solution , entrapped RP in pellet and unentrapped RP . The retention times for retinyl palmitate and retinyl acetate were identical in all solution. They were approximately 5.68-5.79 and 2.12-2.13 minutes, respectively. The chromatograms show no interference from the constituents in the preparation. Therefore, the conditions of HPLC for quantitative analysis of retinyl palmitate were appropriately applicable to the experiment without the interference.

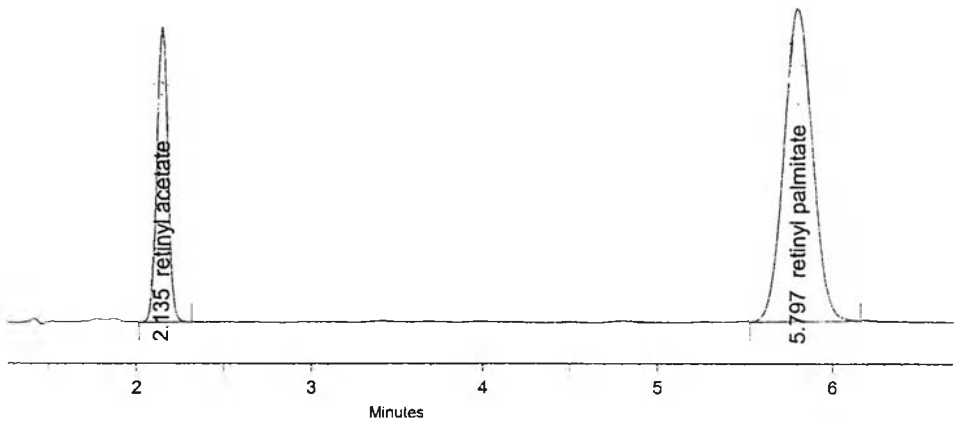


Figure 16. The chromatogram of retinyl palmitate in standard solution

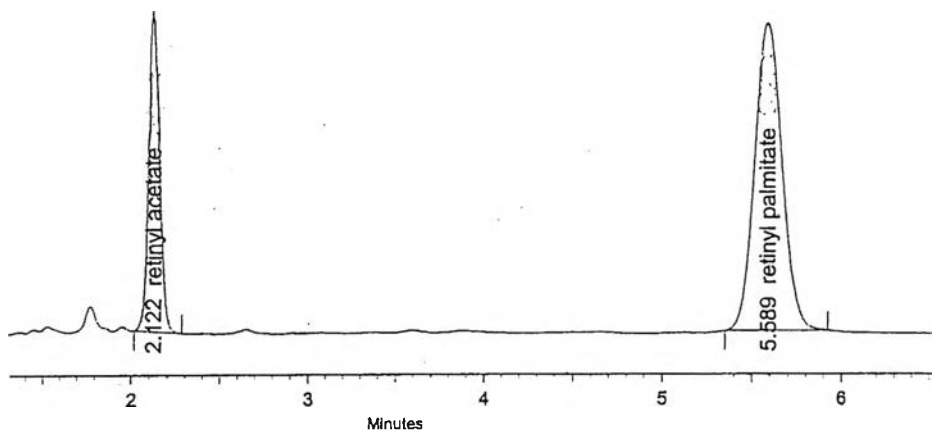


Figure 17. The chromatogram of retinyl palmitate in the pellet

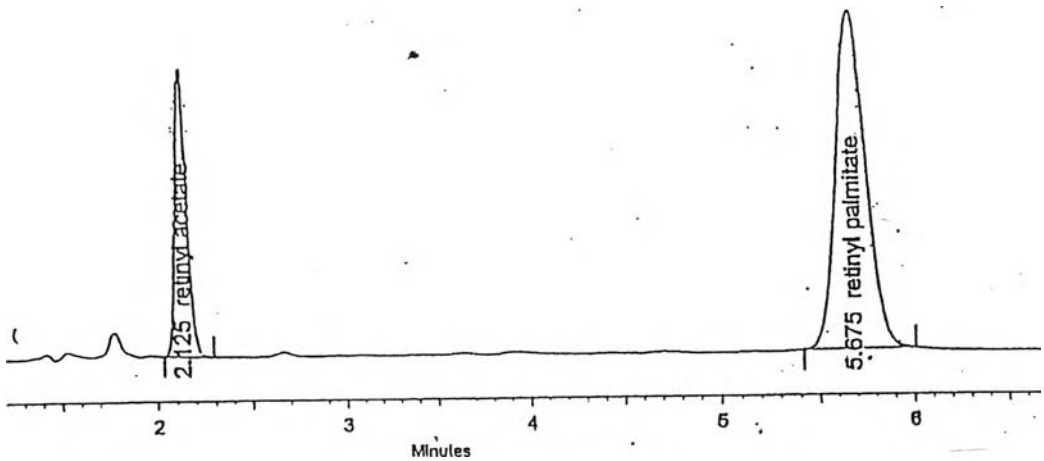


Figure 18. The chromatogram of unentrapped retinyl palmitate

Linearity

Table 7 shows the peak area ratio of standard solutions. Linear regression analysis of the peak area ratio was performed in the concentration range of 2.0-8.0 μg/ml with a coefficient of determination (R^2) of 0.9985.

Figure 19 shows the calibration curve of retinyl palmitate. It was obviously seen that the relationship between the peak area ratio and the concentration of retinyl palmitate was linear. So the quantitative analysis of retinyl palmitate was considerably corresponding to its mathematical formula.

In conclusion, the method for quantitative analysis of retinyl pamitate in niosome suspensions was successfully validated to confirm its accuracy, precision,

specificity and linearity.The peak area ratio was greatly proportionate to the concentration of retinyl palmitate.

Table 7. the peak area ratio of standard solutions

concentration (μg/ml)	peak area ratio
2.0	1.404
4.0	2.881
6.0	4.424
8.0	5.702

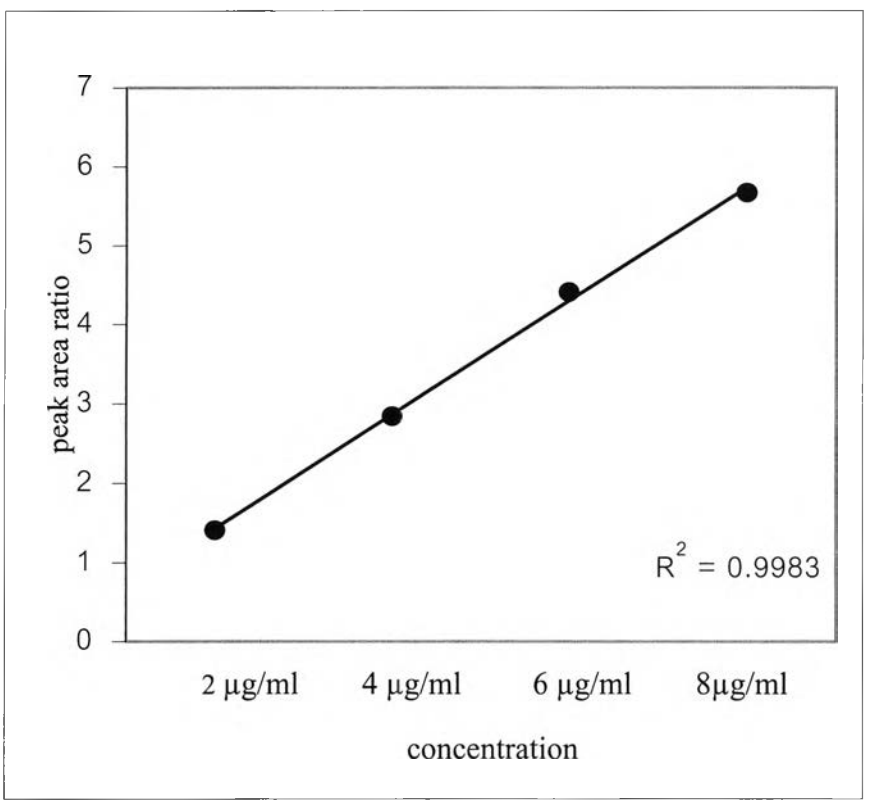


Figure 19. The representative calibration curve for retinyl palmitate

2. Preparation of retinyl palmitate niosomes

All processes of retinyl palmitate niosome preparation were conducted in a dark room to prevent from photodegradation. Retinyl palmitate, lipophilic compound, could incorporate in the membranes of vesicle. It was essential to prepare the vesicle at a temperature above the gel-liquid phase transition temperature of nonionic surfactants; Span 60 has the highest phase transition temperature of about 50 °C (Yoshioka, 1994). Therefore, all vesicle preparations were carried out at 60 °C or above.The excessive organic solvent should be removed by the drier for few minutes.

3.Characterization of retinyl palmitate niosomes prepared by hand-shaking method

3.1 Optical microscopy

The formation of vesicle was proved by using of an optical microscope. Not only the spherical shape but also tubular shape was found in the preparation. The greater spherical shape showed many layers of membrane in vesicles.

Figure 20 show the retinyl palmitate niosomes prepared by Span 40: cholesterol: Solulan C-24 (45:45:10), Span 60: cholesterol: Solulan C-24 (45:45:10)

and Span 85: cholesterol: Solulan C-24 (45:45:10) possess spherical vesicles with lamellae. The vesicles appeared under the light microscope to be varied in size. The smaller vesicles appeared many lamellae whereas the larger one showed few lamellae.

In addition, the size and the shape of retinyl palmitate niosomes prepared by Span 40: cholesterol: Solulan C-24 (45:45:10), Span 60: cholesterol: Solulan C-24 (45:45:10) and Span 85: cholesterol: Solulan C-24 (45:45:10) were the same average diameter and shape.

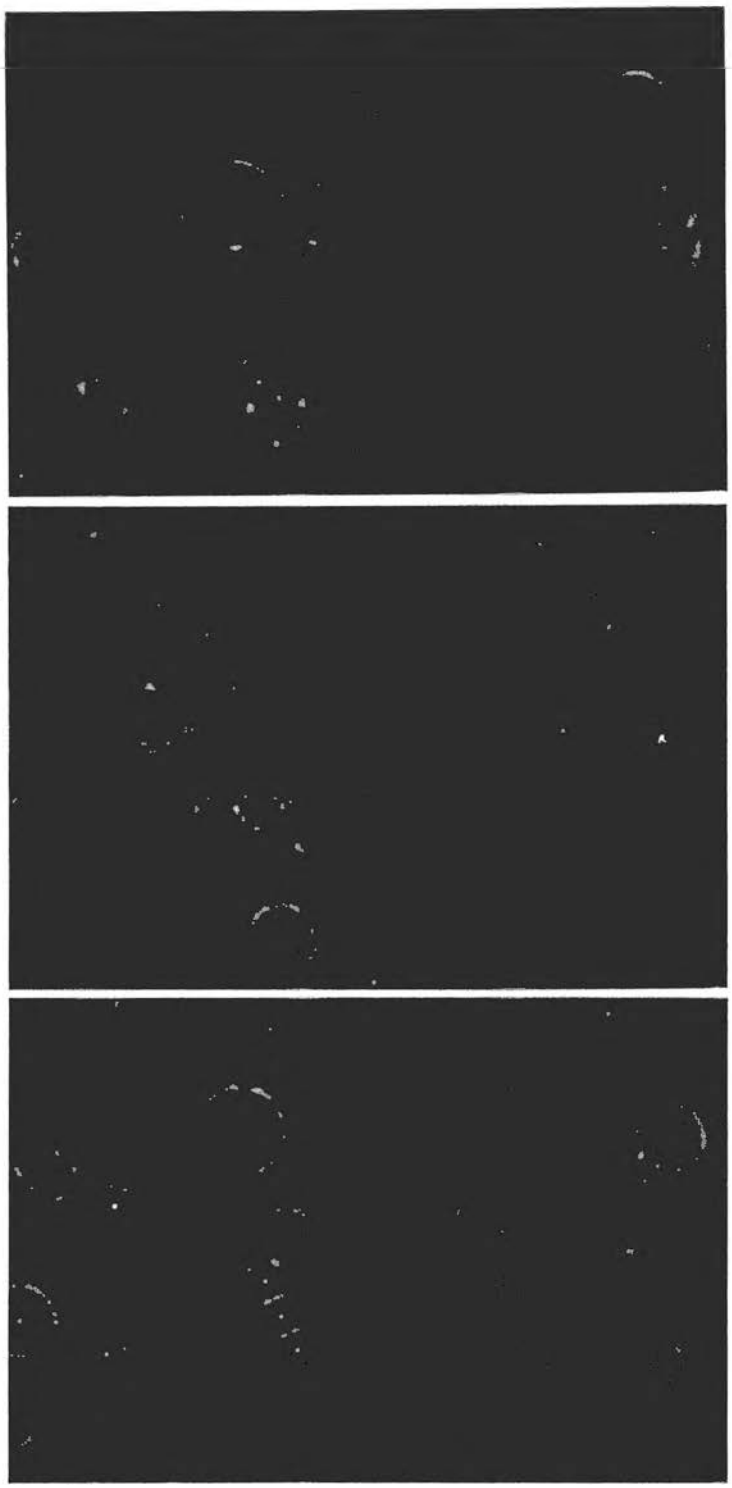
3.2 Scanning electron microscopy

Figure 21 shows morphology of vesicles by scanning electron microscopy. The retinyl palmitate niosomes were spherical shape and possessed smooth surface with varying in size. There was some aggregation of niosomes

3.3 Determination of size and size distribution

In general, the mean of particle size of retinyl palmitate niosomes prepared by Span 40: cholesterol: Solulan C-24 (45:45:10) was approximately 10 μm. The size distribution of this preparation was rather wide. Eventhough the prepared niosomes was sonicated for a few minutes, the size range was appeared in micron. Similarly, the retinyl palmitate niosomes prepared by Span 60: cholesterol: Solulan C-24 (45:45:10)

had the mean of particle size of nearly 11 μm while those prepared by Span 85: cholesterol: Solulan C-24 (45:45:10) had a lower mean of particle size of 10 μm as shown in Figure 22. However the mean particle size of among three niosome preparations was not significantly different ($P>0.05$) to one another.

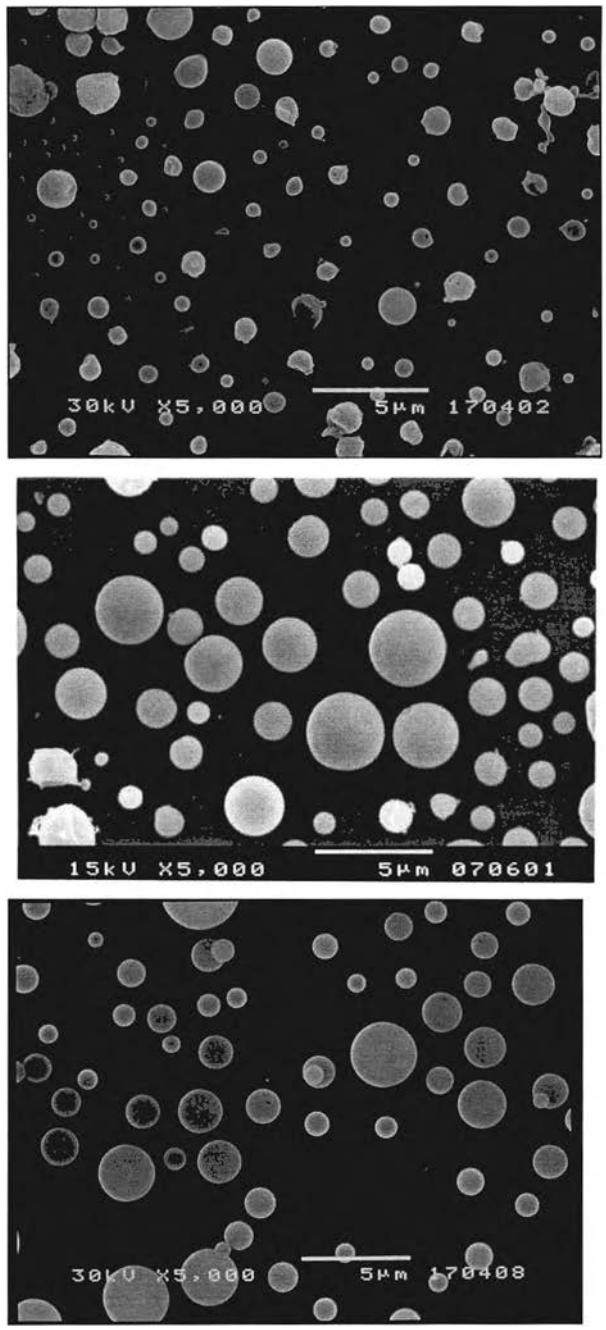


(a)

(b)

(c)

Figure 20. Photographs of niosomes prepared.by (a) span 40: cholesterol : solulan C-24 (45 : 45 : 10) (b) span 60: cholesterol : solulan C-24 (45 : 45 : 10) and (c) span 85: cholesterol : solulan C-24 (45 : 45 : 10) Magnification 40x10



(a)

(b)

(c)

Figure 21. Scanning electron microscope of retinyl palmitate niosomes prepared by

(a) Span 40 (b) Span 60 and (c) Span 85 Magnification x5,000

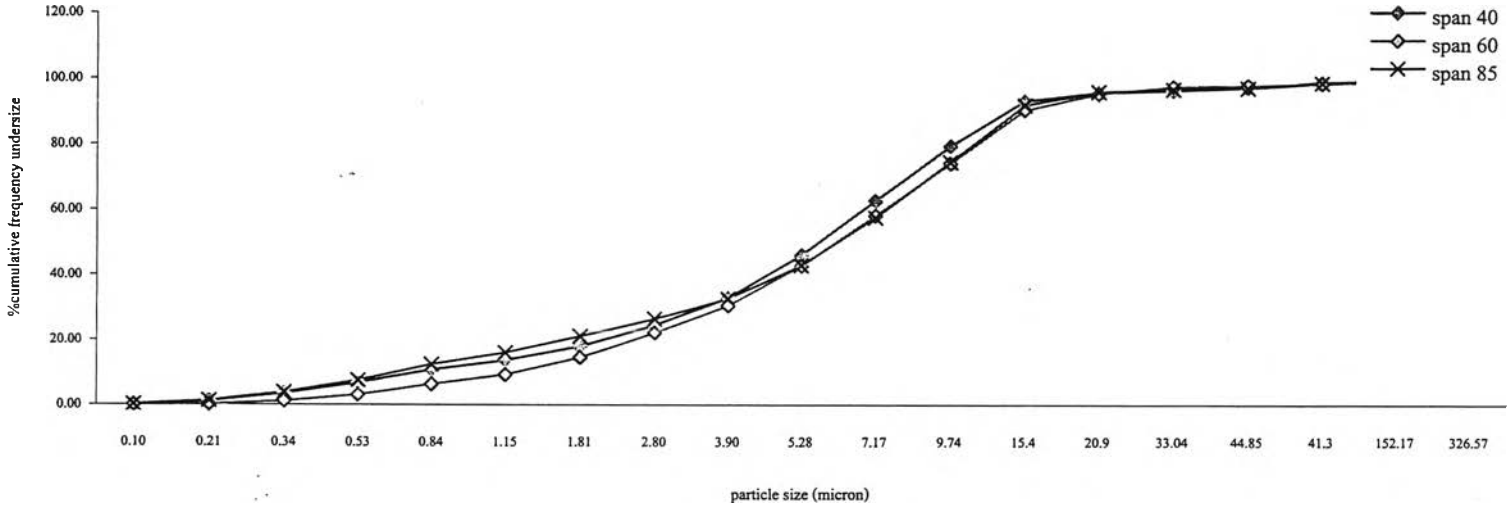


Figure 22. The percentage cumulative frequency undersize of retinyl palmitate niosomes prepared by span 40, span 60 and span 85

4.Determination of retinyl plamitate entrapment efficiency

4.1 Effect of surfactants and drug loading on entrapment efficiency

The experiment was set by varying the amount of retinyl palmitate added in niosome suspensions for those three sets of niosome preparation with varing surfactant Span 40, Span 60 and Span 85. The entrapped retinyl palmitate and unentrapped retinyl palmitate were analyzed after separation by ultracentrifugation. There were three compartments of niosome suspensions i.e. the upper and the lower compartment locating at tube contain white pellets and the yellow material that is unentrapped retinyl palmitate is stick at the upper part of tube. The amount of entrapment and unentrapment of retinyl palmitate in niosomes suspensions and the percent entrapment efficiency were shown in Table 8,9 and 10. The comparative entrapment of different loading retinyl palmitate in each surfactant were shown in Figure 23,24 and 25. The entrapment efficiency of retinyl palmitate was shown in Figure 26,27 and 28

Table 8. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 40:cholesterol:solulan C-24 with varying drug loading

Total amount of RP added in niosome (mg)	Unentrapped RP (mg)	Entrapped RP				% Entrapment	%Entrapment efficiency
		Upper part (mg)	Lower part (mg)	Total (mg)	Total (μmole)		
4.99	0.00	3.11	1.88	4.99	9.50	100.00	3.16
4.93	0.00	2.36	2.57	4.93	9.39	100.00	3.13
5.02	0.00	2.92	2.10	5.02	9.56	100.00	3.18
4.98±0.04	0.00±0.00	2.79±0.38	2.18±0.35	4.98±0.04	9.48±0.08	100.00±0.00	3.15±0.02
8.57	0.90	4.19	3.48	7.67	14.61	89.49	4.87
8.55	0.90	4.16	3.49	7.65	14.57	89.47	4.85
8.55	0.90	4.17	3.48	7.65	14.57	89.47	4.85
8.55±0.01	0.90±0.00	4.17±0.01	3.48±0.00	7.65±0.01	14.58±0.02	89.47±0.01	4.85±0.01
10.02	0.78	7.24	2.00	9.24	17.60	92.21	5.86
10.27	0.89	7.38	2.00	9.38	17.86	91.33	5.95
10.54	0.89	7.65	2.00	9.65	18.38	91.55	6.12
10.27±0.20	0.85±0.06	7.42±0.20	2.00±0.00	9.42±0.20	17.61±0.24	91.69±0.45	5.97±0.13

Table 9. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 60:cholesterol:solulan C-24 with varying drug loading.

Total amount of RP added in niosome (mg)	Unentrapped RP (mg)	Entrapped RP				% Entrapment	%Entrapment efficiency
		Upper part (mg)	Lower part (mg)	Total (mg)	Total (μmole)		
5.04	0.00	2.89	2.11	5.04	9.60	100.00	3.20
4.91	0.00	3.14	1.77	4.91	9.35	100.00	3.11
4.79	0.00	2.61	2.18	4.79	9.12	100.00	3.04
4.91±0.12	0.00±0.00	2.88±0.26	2.02±0.21	4.91±0.12	9.35±0.24	100.00±0.00	3.11±0.08
8.23	0.95	3.83	3.45	7.28	13.86	88.45	4.62
8.43	0.95	3.92	3.47	7.39	14.07	88.61	4.69
8.35	0.95	3.95	3.45	7.40	14.09	88.62	4.69
8.33±0.10	0.95±0.00	3.90±0.06	3.45±0.01	7.35±0.06	14.00±0.12	88.56±0.09	4.66±0.04
9.94	1.77	5.49	2.68	8.17	15.56	82.19	5.18
10.02	1.77	5.50	2.75	8.25	15.71	82.33	5.23
9.70	1.77	5.52	2.41	7.93	15.10	81.75	5.03
9.88±0.16	1.77±0.00	5.50±0.01	2.61±0.17	8.11±0.16	15.45±0.31	82.09±0.30	5.14±0.10

Table 10. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by span 85:cholesterol:solulan C-24 with varying drug loading.

Total amount of RP added in niosome (mg)	Unentrapped RP (mg)	Entrapped RP				% Entrapment	%Entrapment efficiency
		Upper part (mg)	Lower part (mg)	Total (mg)	Total (μmole)		
5.31	0.00	5.15	0.16	5.31	10.11	100.00	3.37
5.51	0.00	5.01	0.50	5.51	10.49	100.00	3.49
5.45	0.00	5.16	0.29	5.45	10.38	100.00	3.46
5.42±0.10	0.00±0.00	5.10±0.08	0.31±0.17	5.42±0.10	10.32±0.19	100.00±0.00	3.44±0.06
8.33	2.54	5.08	0.71	5.79	11.02	69.50	3.67
8.16	2.20	5.24	0.72	5.96	11.35	73.03	3.78
8.16	2.17	5.27	0.72	5.99	11.40	73.40	3.80
8.21±0.09	2.30±0.20	5.19±0.10	0.71±0.00	5.91±0.10	11.25±0.20	71.97±0.07	3.75±0.07
11.22	4.52	5.90	0.80	6.70	12.76	59.71	4.25
11.24	4.52	5.92	0.80	6.72	12.80	59.78	4.26
11.23	4.51	5.92	0.80	6.72	12.80	59.78	4.26
11.23±0.01	4.51±0.00	5.91±0.01	0.80±0.00	6.71±0.01	12.78±0.02	59.75±0.04	4.25±0.00

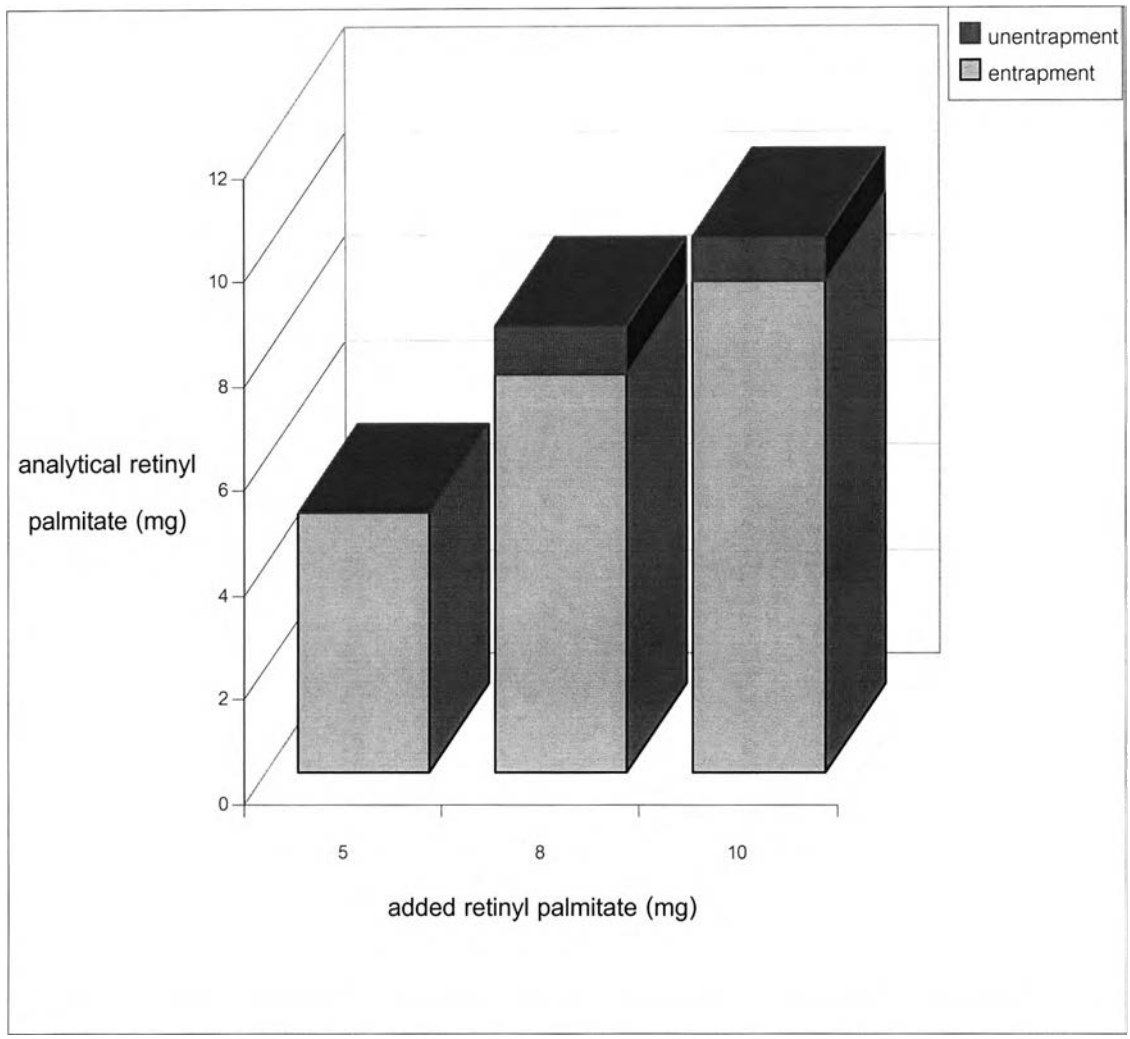


Figure 23. The entrapment of retinyl palmitate in niosomes prepared by

Span 40:cholesterol:Solulan C-24.(45:45:10)

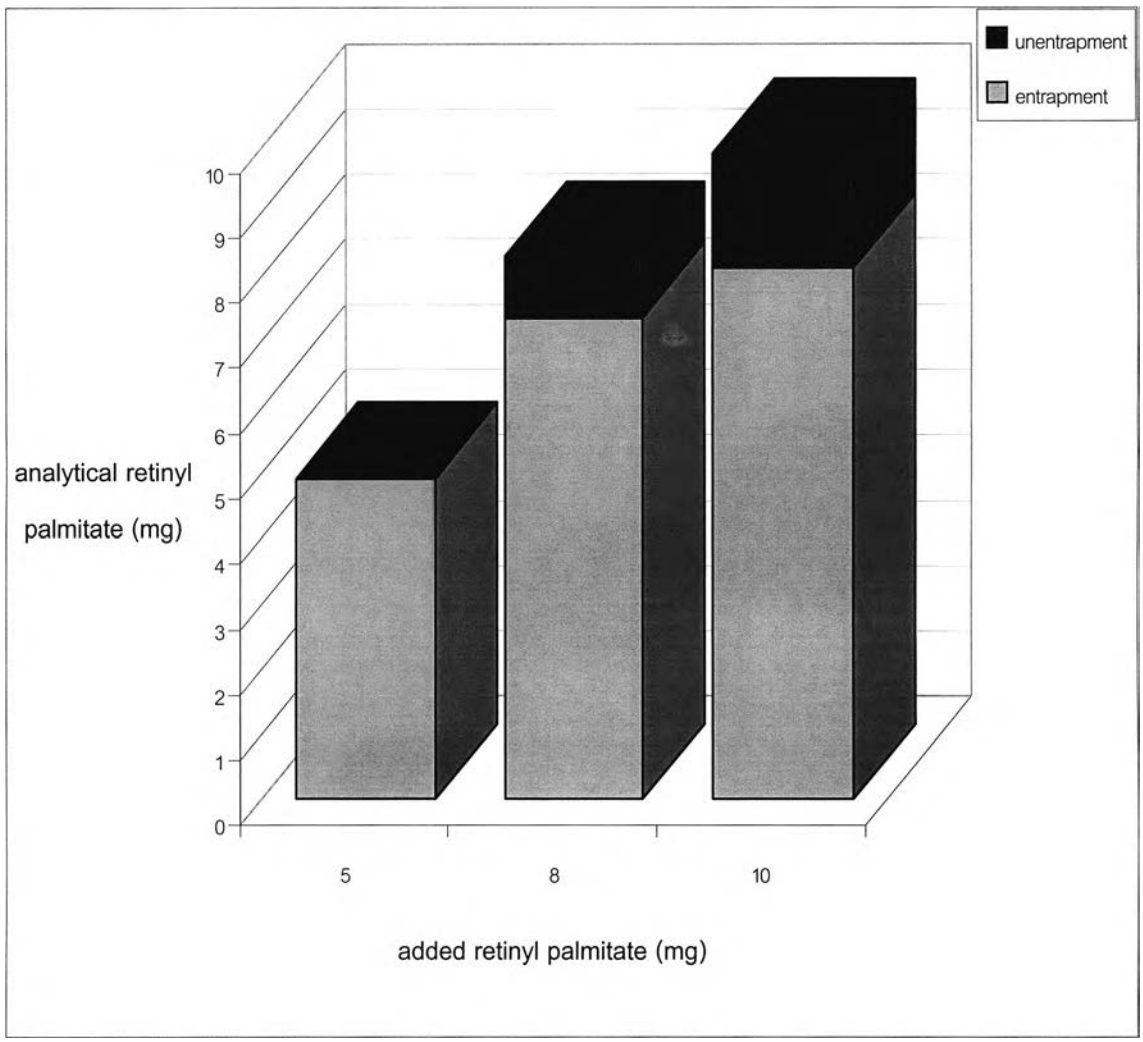


Figure 24. The entrapment of retinyl palmitate in niosomes prepared by

Span 60:cholesterol: Solulan C-24 (45:45:10)

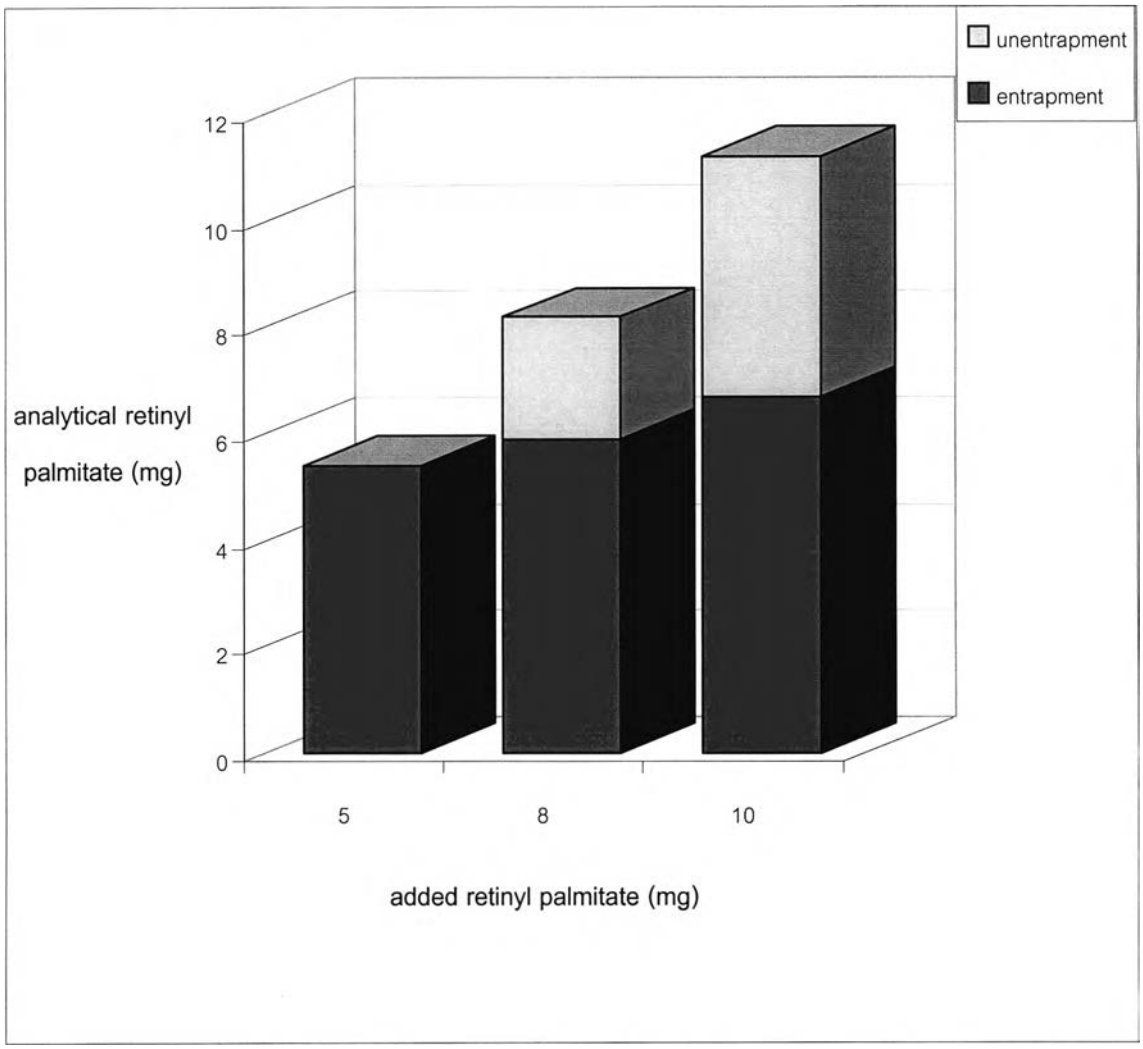
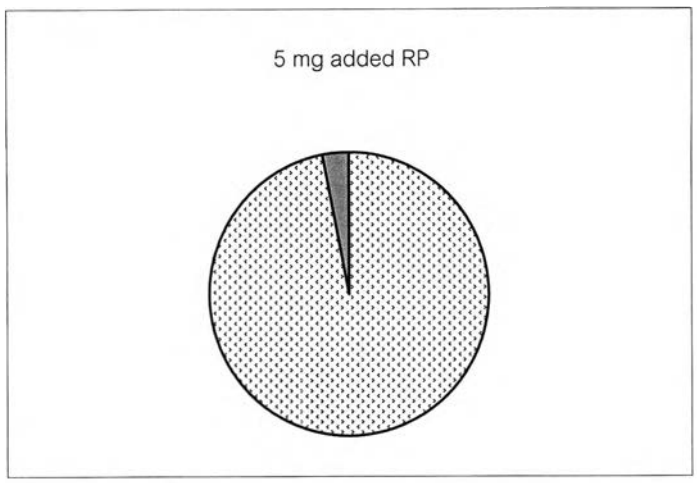


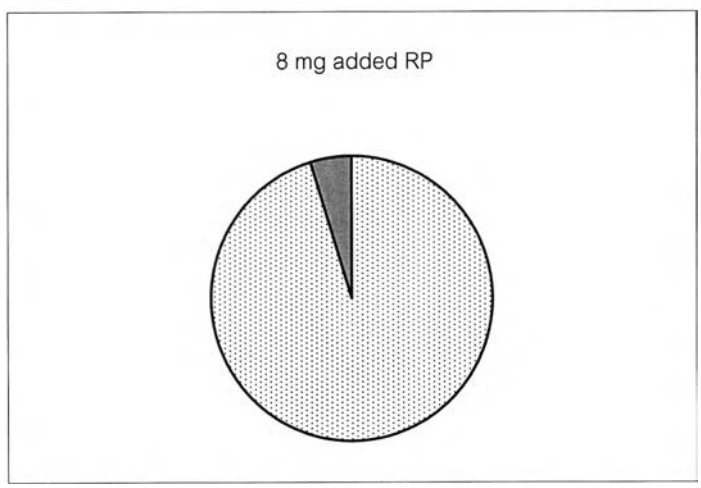
Figure 25. The entrapment of retinyl palmitate in niosomes prepared by

Span 85: cholesterol: Solulan C-24 (45:45:10)



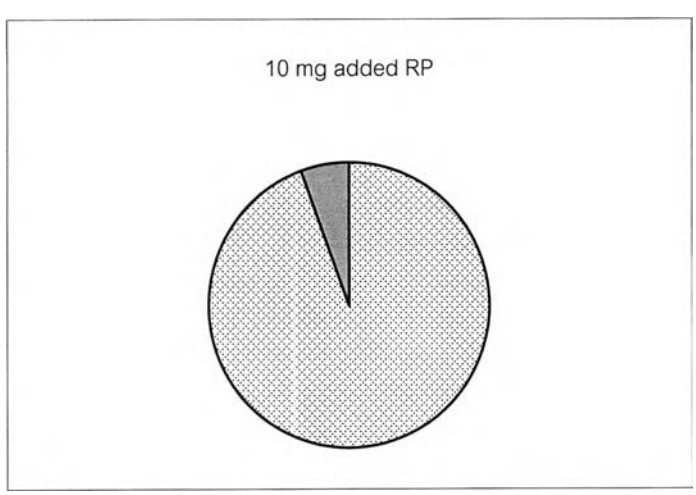
Entrapment efficiency

0.03



Entrapment efficiency

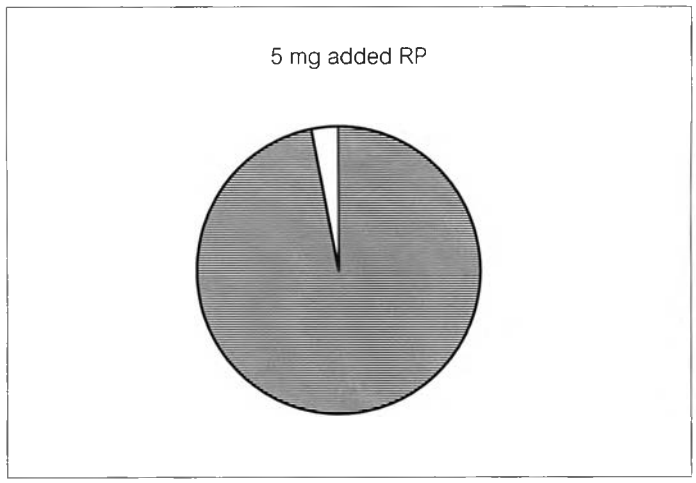
0.05



Entrapment efficiency

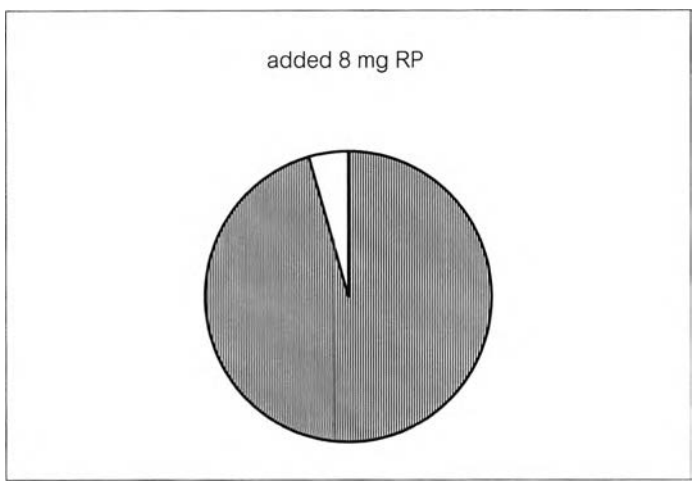
0.06

Figure 26. The entrapment efficiency of retinyl palmitate in 300 μmole of total lipid/ surfactant from Span 40:cholesterol:Solulan C-24 (45:45:10)



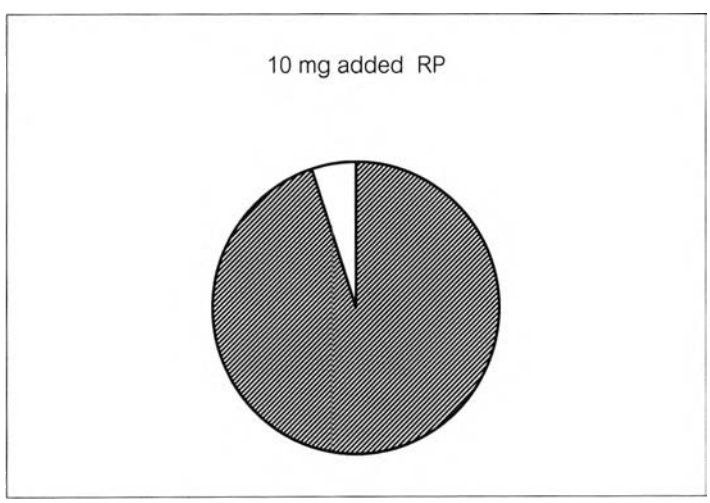
Entrapment efficiency

0.03



Entrapment efficiency

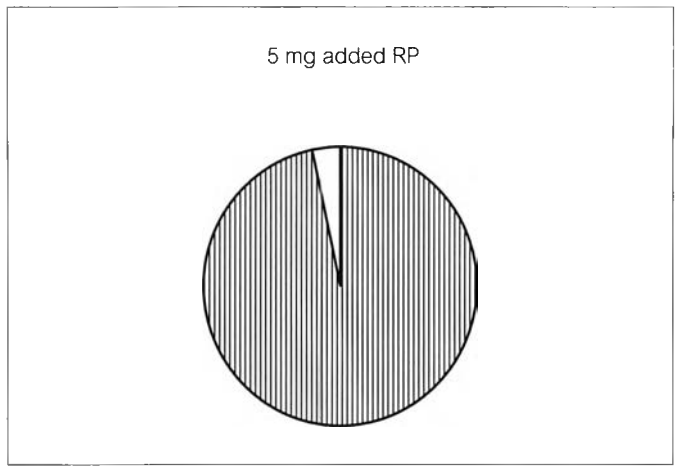
0.05



Entrapment efficiency

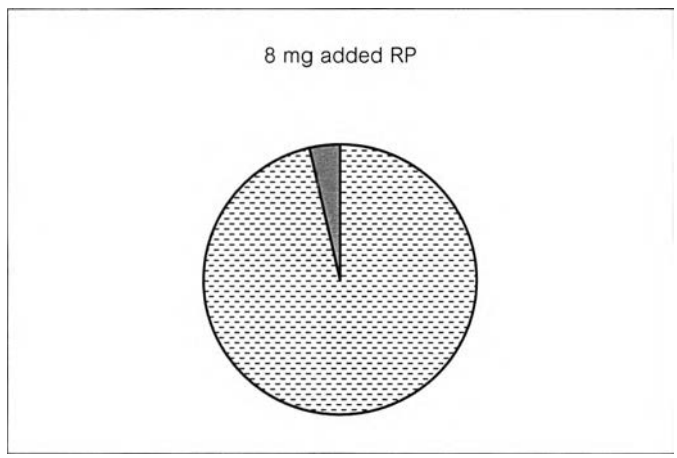
0.05

Figure 27. The entrapment efficiency of retinyl palmitate in 300 μmole of total lipid/ surfactant from Span 60:cholesterol:Solulan C-24 (45:45:10)



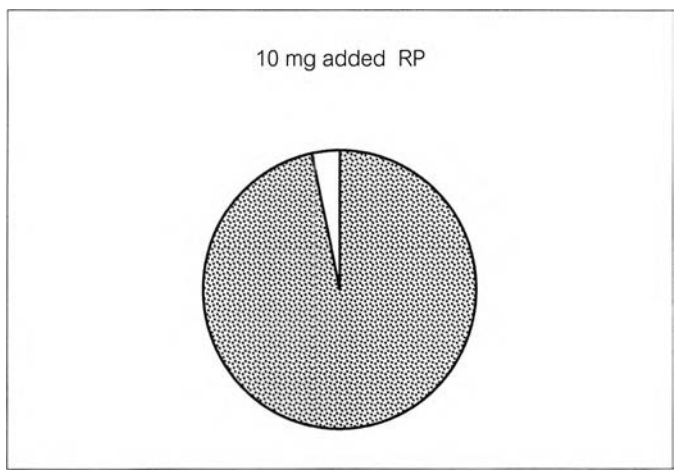
Entrapment efficiency

0.03



Entrapment efficiency

0.04



Entrapment efficiency

0.04

Figure 28. The entrapment efficiency of retinyl palmitate in 300 μmole of total lipid/ surfactant from Span 85:cholesterol:Solulan C-24 (45:45:10)

Retinyl palmitate loading of 5 mg could be entrapped completely in all kinds of niosome suspensions prepared by 300 μmole of Span:cholesterol:Solulan C-24 (45:45:10). The increment of adding retinyl palmitate in niosome suspensions resulted in the more entrapment of retinyl palmitate in vesicles. However retinyl palmitate could not be entrapped completely. The more the amount of retinyl palmitate added in niosome formulation, the more the amount of unentrapped retinyl palmitate was. Comparatively, the amount of unentrapped retinyl palmitate found in Span 85 niosomes was greater than those found in both Span 40 and Span 60 niosomes. It was possible that the phase transition temperature of Span 85 was lower than that of Span 40 and Span 60 leading to the more amount of unentrapped retinyl palmitate in this kind of niosome suspension. At 25°C, Span 85 is at liquid state while Span 40 and Span 60 are at solid state(Yoshioka,1994).

Due to the liquid state of Span 85, retinyl palmitate was easily moved from the membrane of Span 85 vesicles. Also, the disorder of bilayer fluidity might result in the enhancement of bilayer permeability to solutes(Fang,2001)

Figure 26-28 show the entrapment efficiency of retinyl palmitate when it was added in niosome suspensions in the range amount of 5 mg to 10 mg. The amount of 5 mg amd 10 mg of retinyl palmitate are equivalent to 9.50 μmole and 19.05 μmole of

retinyl palmitate, respectively. Therefore, 9.50 μmole of retinyl palmitate could entrapped in 300 μmole of total lipid/surfactant vesicles without unentrapped retinyl palmitate in niosome suspension. Accordingly, the entrapment efficiency was 0.03. However the percentage of entrapment efficiency of retinyl palmitate niosomes prepared by Span 85 was rather lower than that prepared by Span 40 and Span 60. Retinyl palmitate niosomes from Span 85 showed the entrapment efficiency in the range of 0.03 to 0.04 whereas those from Span 40 showed the entrapment efficiency in the range of 0.03 to 0.06 as well as those from Span 60 showed the entrapment efficiency in the range of 0.03 to 0.05.

In conclusion, the entrapment efficiency for retinyl palmitate niosomes prepared by Span 40, Span 60 and Span 85 was somewhat low. In this experiment, it was just about 9 μmole –15 μmole for 300 μmole of total lipid/surfactant vesicles.

4.2 Effect of cholesterol on drug entrapment.

The experiment was set by varying the mole ratio of Span to cholesterol with fixed Solulan C-24 mole ratio to investigate the effect of cholesterol on retinyl palmitate entrapment efficiency.

The ratios of Span: cholesterol was varied from 10:80 to 90:0. The cholesterol was a membrane additive for niosome formation which can influence on physical

nature of vesicles. Figure 29-31 show cholesterol content that can effect on the amount of entrapped retinyl palmitate in niosomes. There was a significant difference among the different mole ratio of total lipid at $\alpha = 0.05$ for each kind of niosome preparation.

Table 11. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by Span 40: cholesterol: Solulan C-24 in different ratios with differences in cholestrol

Mole ratio	Total amount (mg)	Unentrapped RP (mg)	Entrapped RP				% Entrapment	Entrapment efficiency
			Upper part (mg)	Lower part (mg)	Total (mg)	Total (μmole)		
10:80:10	7.62	0.09	5.17	2.36	7.53	14.34	98.81	0.05
	7.66	0.09	5.21	2.36	7.57	14.41	98.83	0.05
	7.66	0.09	5.20	2.37	7.57	14.41	98.82	0.05
Mean± SD	7.64±0.02	0.09±0.00	5.19±0.02	2.36±0.00	7.55±0.02	14.38±0.04	98.82±0.01	0.05±0.00
45:45:10	8.57	0.90	4.19	3.48	7.67	14.60	89.49	0.05
	8.55	0.90	4.16	3.49	7.65	14.57	89.47	0.05
	8.55	0.90	4.17	3.48	7.65	14.57	89.47	0.05
Mean±SD	8.55±0.01	0.90±0.00	4.17±0.01	3.48±0.00	7.65±0.01	14.58±0.01	89.47±0.01	0.05±0.00
65:25:10	8.06	0.15	4.12	3.79	7.91	15.06	98.13	0.05
	8.03	0.15	4.14	3.74	7.88	15.00	98.13	0.05
	8.04	0.15	4.13	3.76	7.89	15.02	98.13	0.05
Mean±SD	8.04±0.01	0.15±0.00	4.13±0.01	3.76±0.02	7.89±0.01	15.02±0.03	98.08±0.18	0.05±0.00
90:0:10	7.59	0.05	0.00	7.54	7.54	14.36	99.34	0.05
	7.59	0.05	0.00	7.54	7.54	14.36	99.34	0.05
	7.63	0.05	0.00	7.58	7.58	14.43	99.34	0.05
Mean±SD	7.60±0.02	0.05±0.00	0.00±0.00	7.55±0.02	7.55±0.02	14.38±0.04	93.86±0.28	0.05±0.00

Table 12. The amount of entrapped and unentrapped retinyl palmitate in niosome suspensions prepared by Span 60: cholesterol: Solulan C-24 in different ratio with difference in cholesterol

Mole ratio	Total amount (mg)	Unentrapment (mg)	Entrapped RP				% Entrapment	Entrapment efficiency
			Upper part (mg)	Lower part (mg)	Total (mg)	Total (mole)		
10:80:10	7.78	0.35	3.72	3.22	7.43	14.15	95.50	0.05
	7.78	0.35	3.73	3.21	7.43	14.15	95.50	0.05
	7.79	0.36	3.73	3.21	7.43	14.15	95.50	0.05
Mean±SD	7.78±0.00	0.35±0.00	3.72±0.00	3.21±0.00	7.43±0.00	14.15±0.00	95.00±0.00	0.05±0.00
45:45:10	8.23	0.95	3.83	3.45	7.28	13.86	88.45	0.05
	8.34	0.95	3.92	3.47	7.39	14.07	88.61	0.05
	8.35	0.95	3.95	3.45	7.40	14.09	88.62	0.05
Mean±SD	8.30±0.06	0.95±0.00	3.90±0.06	3.45±0.01	7.35±0.06	14.00±0.12	88.56±0.09	0.05±0.00
65:25:10	8.95	0.38	3.55	5.02	8.57	16.32	95.75	0.05
	8.94	0.38	3.57	4.99	8.56	16.30	95.74	0.05
	8.94	0.38	3.58	4.98	8.56	16.30	95.74	0.05
Mean±SD	8.94±0.00	0.38±0.00	3.56±0.01	4.99±0.02	8.56±0.00	16.3±0.01	95.74±0.00	0.05±0.00
90:0:10	9.36	0.57	2.88	5.91	8.79	16.74	93.91	0.06
	9.34	0.56	2.87	5.91	8.78	16.72	94.00	0.06
	9.32	0.57	2.87	5.88	8.75	16.66	93.88	0.06
Mean±SD	9.34±0.02	0.56±0.00	2.87±0.00	5.90±0.01	8.77±0.02	16.70±0.04	93.93±0.06	0.06±0.00

Table 13. The amount of entrapped and unentrapped retinyl palmitate in niosome suspension prepared by Span 85: cholesterol: Solulan C-24 in different ratios with differences in cholesterol

Mole ratio	Total amount (mg)	Unentrapped RP (mg)	entrapped RP				% Entrapment	Entrapment efficiency
			upper part (mg)	lower part (mg)	Total (mg)	Total (μmole)		
10:80:10	7.62	1.20	5.65	0.77	6.42	12.22	84.25	0.04
	7.57	1.20	5.67	0.70	6.37	12.13	84.14	0.04
	7.56	1.21	5.65	0.70	6.37	12.09	83.99	0.04
Mean±SD	7.58±0.03	1.20±0.00	5.65±0.01	0.72±0.04	6.38±0.03	12.14±0.06	84.12±0.13	0.04±0.00
45:45:10	8.33	2.54	5.08	0.71	5.79	11.02	69.50	0.04
	8.16	2.20	5.24	0.72	5.96	11.35	73.04	0.04
	8.16	2.17	5.27	0.72	5.99	11.40	73.40	0.04
Mean±SD	8.21±0.09	2.30±0.20	5.19±0.10	0.71±0.00	5.91±0.10	11.25±0.20	71.98±2.15	0.04±0.00
65:25:10	8.29	2.79	4.87	0.63	5.50	10.47	66.34	0.03
	8.32	2.82	4.87	0.63	5.50	10.47	66.10	0.03
	8.35	2.79	4.93	0.63	5.56	10.59	66.58	0.03
Mean±SD	8.32±0.03	2.80±0.01	4.89±0.03	0.63±0.00	5.52±0.03	10.51±0.06	66.34±0.24	0.03±0.00
90:0:10	8.49	1.36	6.76	0.37	7.13	13.58	83.98	0.04
	8.51	1.36	6.78	0.37	7.15	13.61	83.63	0.04
	8.53	1.35	6.81	0.37	7.18	13.67	84.17	0.04
Mean±SD	8.51±0.02	1.35±0.00	6.78±0.02	0.37±0.00	7.15±0.02	13.62±0.04	83.92±0.27	0.04±0.00

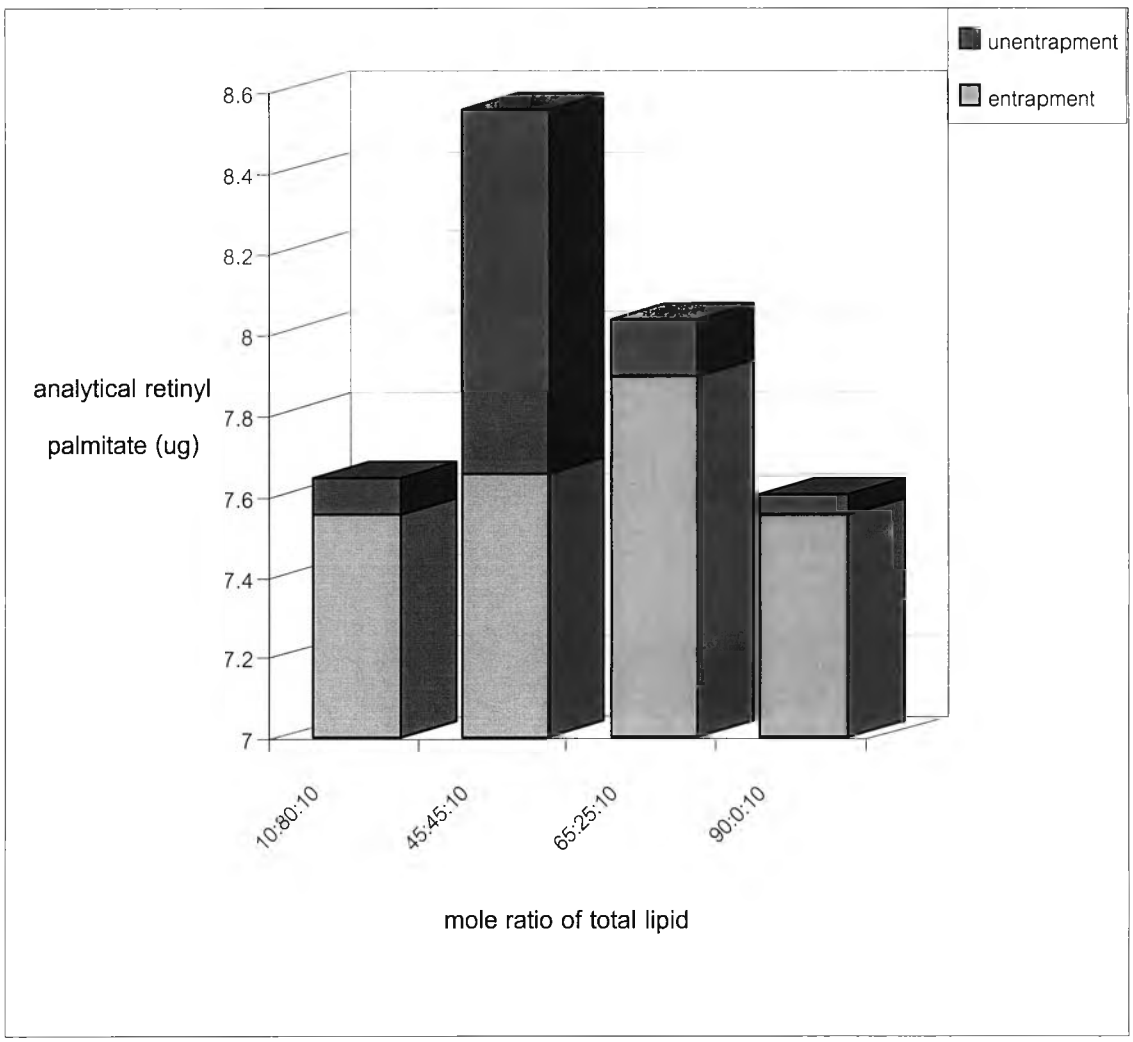


Figure 29. The entrapment of retinyl palmitate entrapped in niosomes prepared by Span 40 with different mole ratio of total lipid/surfactant

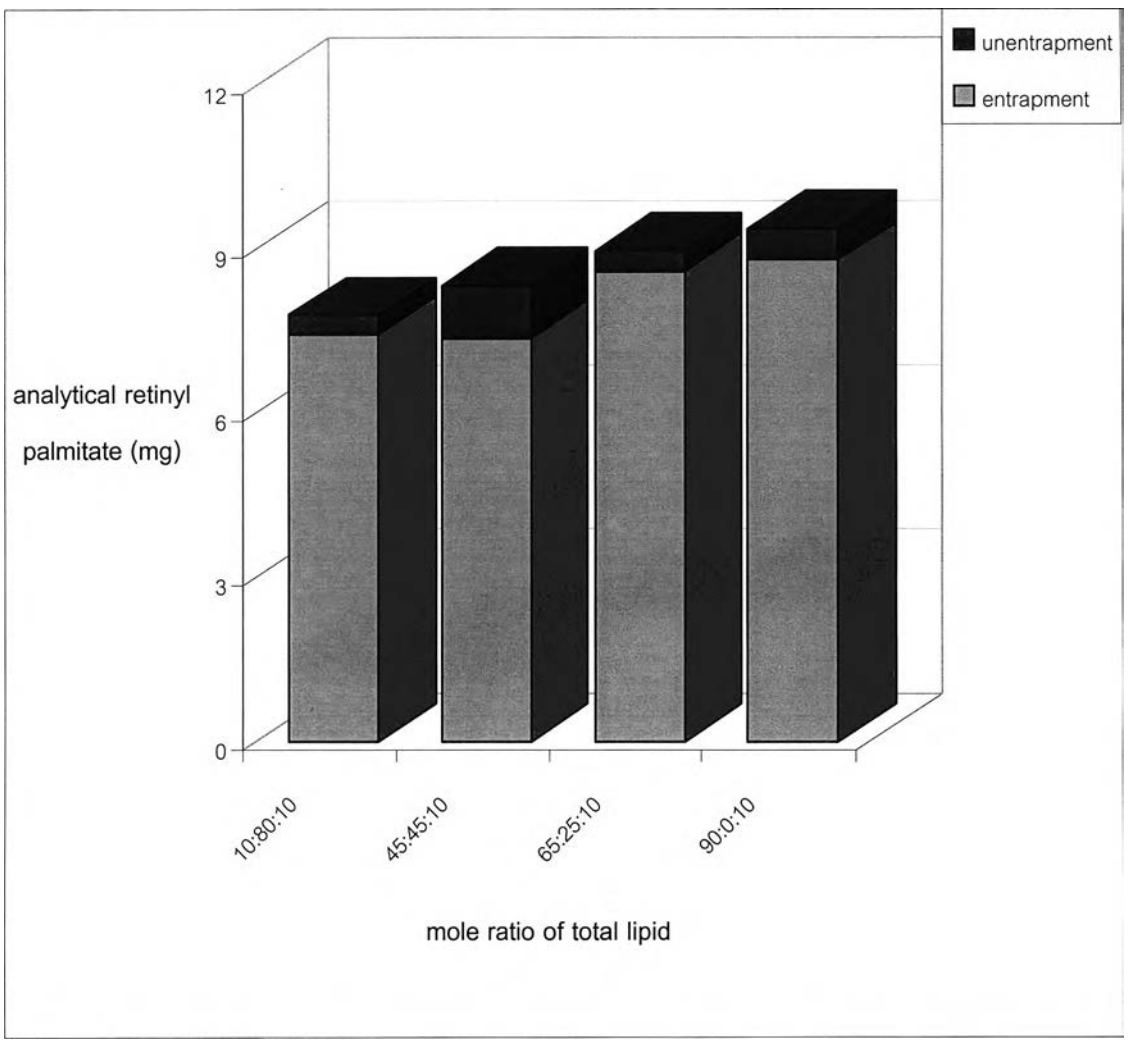


Figure 30. The entrapment of retinyl palmitate in niosomes prepared by Span 60 with different mole ratio of total lipid/surfactant

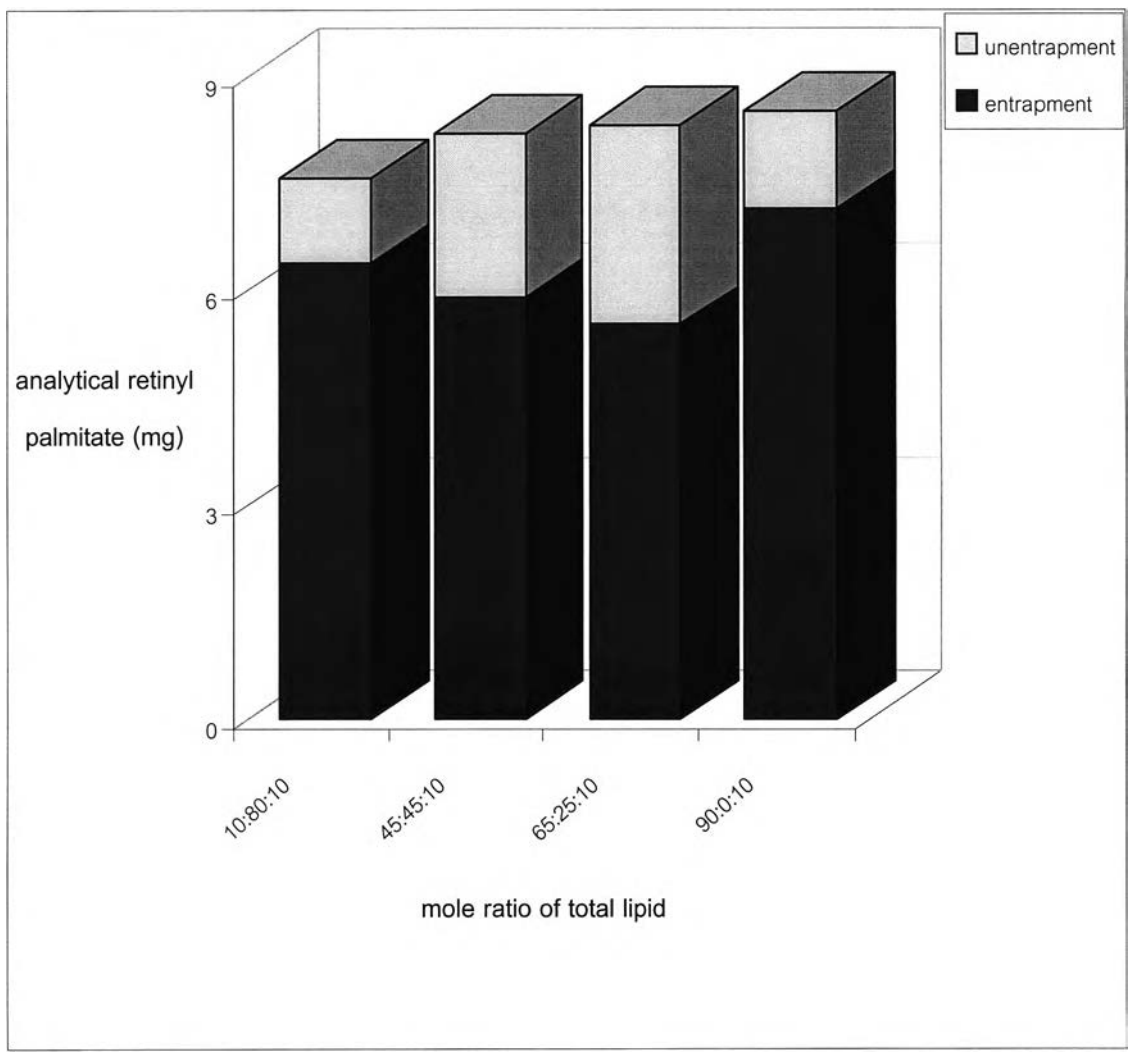


Figure 31. The entrapment of retinyl palmitate in niosomes prepared by Span 85 with different mole ratio of total lipid/surfactant

From figure 29-31, the first column stood for 80% of cholesterol mole ratio, the second column stood for 45% of cholesterol mole ratio and the third column stood for 25% of cholesterol mole ratio. For niosomes prepared by Span 40: cholesterol: Solulan C-24, the reduction of cholesterol mole ratios from 80% to 25 % resulted in an increase of the amount of entrapped retinyl palmitate. However if there was no cholesterol, the amount of entrapped retinyl palmitate was not significantly different at α= 0.05 from 80%of cholesterol ratio of total lipid. Therefore, the presence of cholesterol with the optimum ratio was more likely to affect the entrapment of retinyl palmitate in niosomes prepared by Span 40: cholesterol: Solulan C-24.

For niosomes prepared by Span 60: cholesterol: Solulan C-24, the cholesterol ratio decreased from 80% to 25 % of total lipid mole ratio resulted in the increase of the amount of entrapped retinyl palmitate. So the reduction of cholesterol content resulted in the reduction of entrapped retinyl palmitate as the same as Span 40 Nevertheless, retinyl palmitate could be entrapped more in niosomes prepared by only Span 60 and Solulan C-24 without the presence of cholesterol. The cholesterol ratio between 45 and 80 did not affect on the entrapment of retinyl palmitate. There was no significant difference at α= 0.05 in the amount of entrapped retinyl palmitate in those ratios. The lowest in the percentage of retinyl palmitate entrapment was found in

niosomes prepared by both Span 40: cholesterol: Solulan C-24 with the ratio of 45:45:10 and Span 60:cholesterol: Solulan C-24 with the ratio of 45:45:10. The other mole ratio of total lipid/surfactant for both kinds of prepared niosomes represented the higher percentage of retinyl palmitate entrapment.

For niosomes prepared by Span 85: cholesterol: Solulan C-24. The amount of entrapped retinyl palmitate was significantly different with varying the cholesterol mole ratio at $\alpha = 0.05$. The amount of entrapped retinyl palmitate was apparently lower than that found in niosomes prepared by both Span 40: cholesterol: Solulan C-24 and by Span 60: cholesterol: Solulan C-24.

In addition, this kind of prepared niosomes represented the highest amount of unentrapped retinyl palmitate. The high amount of unentrapped retinyl palmitate might be a result from Span 85. It is at the liquid state at 25°C (Yoshioka, 1994). Nevertheless, the cholesterol content might not affect directly on the entrapment of retinyl palmitate. Although the presence of cholesterol leaded to the higher amount of entrapped retinyl palmitate in niosomes prepared by Span 85: cholesterol: Solulan C-24. It was the fact that the higher cholesterol content might lead to the membrane of vesicle more rigid (Uchegbu, 1998). The addition of cholesterol caused the greater percentage of retinyl palmitate entrapment.

For niosomes prepared by Span 40: cholesterol: Solulan C-24 as well as by Span 60: cholesterol: Solulan C-24, both represented the percentage entrapment efficiency of retinyl palmitate in the range of 88-99%. Similarly, retinyl palmitate could be entrapped in vesicles from approximately just above 14 μmol to 16 μ mole per 300 μmol of total lipid. On the other hand, the entrapped retinyl palmitate was from 7.6mg to 8.7 mg. For niosomes prepared by Span 85: cholesterol: Solulan C-24, the entrapment efficiency of retinyl palmitate in niosome was in the range of 66-84%. Eventhough the amount of retinyl palmitate added in niosome preparation was high, the entrapped retinyl palmitate was somewhat lower than the other prepared niosome preparations. The entrapment efficiency of retinyl palmitate in vesicle was from around 10.5 μmol to 13.6 μmol per 300 μmol of total lipid/surfactant.

In conclusion, the higher the cholesterol mole ratio was the lower the quantity of retinyl palmitate intercalated in the membrane of vesicles. This relation of the cholesterol effect and retinyl palmitate entrapment was true in niosomes prepared by both Span 40: cholesterol: Solulan C-24 and Span 60: cholesterol: Solulan C-24. In contrast, the higher cholesterol ratio caused the higher amount of entrapped retinyl palmitate for niosomes prepared by Span 85: cholesterol: Solulan C-24.

5. Drug Permeation Study

The experiment was designed for the study of the permeation enhancer activity of all three series of Span, Span 40, Span 60 and Span 85. Therefore, retinyl palmitate niosomes prepared by three series of Span was investigated for the one that could pass through the snake skin membrane leading to the appearance of retinyl palmitate in receptor fluid. Typically, niosomes prepared by Span: cholesterol: Solulan C-24 (45:45:10) with 5 mg of retinyl palmitate. The concentration of retinyl palmitate niosomes in donor compartment was 33.33 mM. Each of preparation employed 5 pieces of snake skin membranes. The wholely receptor fluid was colllected every three hours. The cumulative amount of retinyl palmitate in niosomes found in the receptor compartment was investigated. The entrapped retinyl palmitate in vesicles was analyzed using isopropanol to destroy vesicles before the analysis of retinyl palmitate.

In permeation study of retinyl palmitate niosomes prepared by Span 40, it was found that the minimum amount of retinyl palmitate found in the receptor fluid could also be analyzed by HPLC at approximately three hours. It could pass through shed snake skin within the range of 1.18-2.87 μg. At the sixth hour, the amount of retinyl

palmitate was so little that some examples couldn't be analyzed by HPLC but some could be analyzed. However niosomes would be entrapped in lipid barrier of shed snake skin which interfere the penetration through model membrane. If there was higher amount of niosomes in shed snake skin, it would result in positive gradient to push niosomes pass through the model membrane.

Table 14. The cumulative amount of retinyl palmitate from niosomes prepared by Span 40: cholesterol: Solulan C-24 (45:45:10)

Span 40					Flux μg/hr.cm^2
Sample No.	Cumulative amount ofretinyl palmitate in receptor compartment (μg)				
	3Hr	6Hr	9Hr	12Hr	
1	1.18	UN	2.60	2.60	0.10
2	1.10	UN	1.10	1.10	0.04
3	1.34	UN	2.95	2.95	0.12
4	2.08	5.31	6.33	6.33	0.26
5	2.87	4.95	6.72	6.72	0.27
mean	1.71	2.05	3.94	3.94	0.16
SD	0.75	2.81	2.46	2.46	0.10

UN=undetectable

In Table 14, the retinyl palmitate could not be found in sample No 1, 2 and 3 but it could be found in sample No 4 and 5 at the sixth hour. The penetration could

perform to the nineth hour only. The retinyl palmitate could not be found at the twelfth hour. The flux was 0.16 μg/cm^2.hr

Table 15. The cumulative amount of retinyl palmitate from niosomes prepared by Span 60: cholesterol: Solulan C-24 (45:45:10)

Span 60				
Sample No.	Cumulative amount of retinyl palmitate in receptor compartment (μg)			
	3Hr	6Hr	9Hr	12Hr
1	UN	UN	UN	UN
2	UN	UN	UN	UN
3	UN	UN	UN	UN
4	UN	UN	UN	UN
5	UN	UN	UN	UN

UN=undetectable

Table 16. The cumulative amount of retinyl palmitate from niosomes prepared by Span 85: cholesterol: Solulan C-24 (45:45:10)

Span 85					Flux μg/hr.cm^2
Sample No.	Cumulative amount of retinyl palmitate in receptor compartment (μg)				
	3Hr	6Hr	9Hr	12Hr	
1	UN	UN	UN	UN	-
2	UN	UN	UN	UN	-
3	UN	UN	UN	UN	-
4	UN	1.81	2.64	2.64	0.10
5	UN	1.45	2.51	2.51	0.10
mean	-	0.65	1.03	1.03	0.04
SD	-	0.90	1.41	1.41	0.05

UN=undetectable

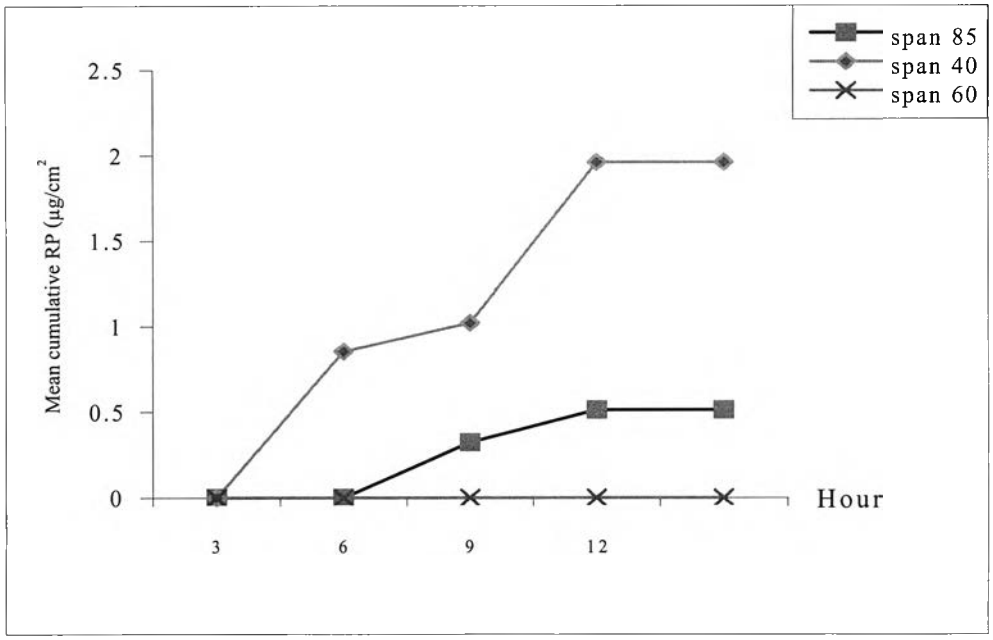


Figure 32. Drug permeation profile of retinyl palmitate niosomes prepared by Span 40: cholesterol: Solulan C-24.(45:45:10), Span 60: cholesterol: Solulan C-24. (45:45:10) and Span 85: cholesterol: Solulan C-24.(45:45:10)

In permeation study of retinyl palmitate niosomes prepared by Span 60, it was found that no retinyl palmitate could be detected in receptor fluid in Table 15. It was possible that niosomes were entrapped in lipid barrier of shed snake skin.

In permeation study of retinyl palmitate niosomes prepared by Span 85, it was found that there was no retinyl palmitate detected in sample 1,2 and 3. However it could be detected in sample 4 and 5. The reason was the same as discussed above, while retinyl palmitate came across shed snake skin, it was entrapped by lipid barrier

until obtaining high concentration gradient which resulted in retinyl palmitate transportation through the lipid barrier. Apparently, there was no retinyl palmitate within 3 hours of the experiment by sample No 4 and 5 but it could be seen at the sixth and ninth hours while it was not seen at the twelfth hour. Retinyl palmitate niosomes from Span 85 showed the flux at 0.04 $\mu g/hr.cm^2$

Figure 32 shows the permeation profile of retinyl palmitate niosome prepare by Span 40, Span 60 and Span 85. It was shown that the flux of retinyl palmitate from niosomes prepared by Span 40 was the highest.

Based on this experiment, retinyl palmitate niosomes prepared by Span 60 could not pass through stratum corneum of shade snake skin while niosomes arising from Span 40 and Span 85 could pass through stratum corneum.In addition, Span 40 and Span 85 have already been investigated for penetration enhancing activity (French,1993). The amount of permeable retinyl palmitate carried by ones from niosomes using Span 40 was greater than that carried by ones prepared from niosomes using Span 85 at α=0.1 (p=0.0513). So niosomes from Span 40 was more likely to present penetration activity through stratum corneum than ones from Span 85. Niosomes from Span 60 could not allow retinyl palmitate pass through the snake skin because Span 60 had the highest phase transition temperature of about $50^{\circ}C$ (Yoshioka, 1994). The high phase transition temperature was responsible for its low

permeable nature.(Vora, 1998). It was the fact that the higher the phase transition temperature was, the lower the permeability of surfactant into the skin was. So Span 60 was the most rigidity because it was in gel state at 32°C. The bilayer fluidity could result in the enhancement of bilayer permeability to solutes. So niosomes from Span 85 should allow retinyl palmitate passing through the shade snake skin. Niosomes from Span 40 show the highest amount of retinyl palmitate pass through the shade snake skin. The possible explanation was niosomes prepared by Span 40 could entrap retinyl palmitate more than those from Span 85 could. So retinyl palmitate niosome prepared from Span 40 had the higher thermodynamic activity resulted in the driving force for lipophilic drugs transport across the skin.

Additionally, Guenin (1995) evaluated the skin permeation of retinyl palmitate from nonionic surfactant vesicles. The researchers used 50 μg radiolabeled retinyl palmitate niosome application in order to permeate across 300 μm thick human skin with 0.636 cm^2 cell surface. It was shown that the radiolabeled retinyl palmitate containing in human stratum corneum was 2.5 $\mu g/cm^2$ and the viable tissue was 0.55 $\mu g/cm^2$ at the twelfth hour. It was implied that niosome formulation might be important in prolonging effect to applied lipophilic molecules. (Guenin, 1995).

In this experiment, retinyl palmitate niosomes prepared by both Span 40: cholesterol: Solulan C-24 (45:45:10) and Span 85: cholesterol: Solulan C-24 (45:45:10) had the feasibility of topically controlled drug delivery for further investigation *in vivo.* The retinyl palmitate niosomes prepared by Span 40: cholesterol: Solulan C-24 (45:45:10) showed higher penetration and higher flux with significantly different at $\alpha = 0.1$.