CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of microcapsules

1.1 Temperature Induced Coacervation Technique

Ascorbic acid is a water soluble solid that is sensitive to moisture. In this study, the temperature induced coacervation technique employed an organic liquid, i.e. cyclohexane, in which the drug is insoluble but ethylcellulose used as a coating polymer is soluble under temperature above 80-81°C which was found to be suitable for preparation. The temperature of the ethylcellulose-cyclohexane mixture is raised to 80°C to form homogeneous solution. Ethylcellulose is deposited around ascorbic acid and hardened when the system is cooled to 20°C (Deasy, 1984). Therefore, the temperature induced coacervation technique could be used in the microencapsulation of ascorbic acid. The stirring speed which provided the highest yield of the prepared microcapsules was 600 rpm. The lower stirring speed was not satisfactory because the produced microcapsules were aggregate markedly and at the higher speed, excessive splashing occurred that resulted in a low yield of the prepared microcapsules. The concentration of ethylcellulose in cyclohexane that provided the highest yield of microcapsules was 1%w/v. Hexanes was the organic solvent used to wash the produced microcapsules since it gave less aggregate microcapsules than cyclohexane.

1.2 Solvent Evaporation Technique

In this study, ethylcellulose was used as the coating polymer. Acetone chosen as the solvent for ethylcellulose is immiscible with light liquid paraffin which was used as the continuous phase. Hexanes used to remove the oil adhering to the microcapsule surface does not dissolve ethylcelluloase and ascorbic acid. Under this condition ascorbic acid was slightly soluble in acetone and insoluble in light liquid paraffin. The emulsion has been formed before solvent diffusion occurs (Palomo et al., 1996). Since acetone has a slow diffusion, small microcapsules would be formed. The proper volumes of acetone and light liquid paraffin were 80 and 220 ml, respectively. These volumes were optimum to the stirring speed selected. The stirring speed selected was 900 rpm since this speed provided proper dispersion and gave the highest yield of the produced microcapsules. The lower and higher speeds provided lower yields of the produced microcapsules.

2. Standard Curve and Validation of Ascorbic Acid Assay

2.1 UV/Visible spectrophotometer

In aqueous solutions, ascorbic acid can absorb UV light at the wavelength range of 260-265 nm. For concentration ranging between 0.5 and 2.5 mg%, Beer's law holds with sufficient exactitude to permit the use of spectrophotometric measurements for quantitative predictions of concentrations (Al-Meshal and Hassan, 1982). Ascorbic acid in an aqueous solution can be rapidly degraded by oxidation that

is catalyzed by metal ions. Therefore, EDTA was added as a chelating agent and ascorbic acid was stable in this medium over the experimental period.

The wavelength used to analyze ascorbic acid in this study was 265 nm since it was the λ_{max} of ascorbic acid in this medium. At this wavelength, there was no interference by other ingredients used to form microcapsule wall and degradation products of ascorbic acid (dehydroascorbic acid and oxalic acid), as illustrated in figure 16. The spectrum of ascorbic acid released from the microcapsules (figure 16(a)) was similar to that of ascorbic acid in 5mM EDTA aqueous solution (figure 16(b)). Figures 16(c), 16(d), and 16(e) show the spectra of 5mM EDTA aqueous solution, dehydroascorbic acid, and oxalic acid in 5mM EDTA aqueous solution, respectively. The vehicle and the degradation products of ascorbic acid had no significant absorption at 265 nm. Thus, the UV/Visible spectrophotometry technique could be used to assay the amount of ascorbic acid released from the prepared microcapsules.

Figure 17 shows the spectra of ascorbic acid standard solutions at various concentrations. A standard curve was plotted between the ascorbic acid absorbance at 265 nm and its concentrations, and was fitted using linear regression analysis. The results are presented in table 10 and figure 18. A straight line is obtained with a coefficient of determination (r^2) of 0.9995. The regression equation of this line is

$$y = 0.0145 + 0.0649x \qquad (13)$$

where y is the absorbance of ascorbic acid and x is the concentration of ascorbic acid solution in μg/ml. This equation was then used to calculate amount of ascorbic acid released from the prepared microcapsules and percentage of drug released.

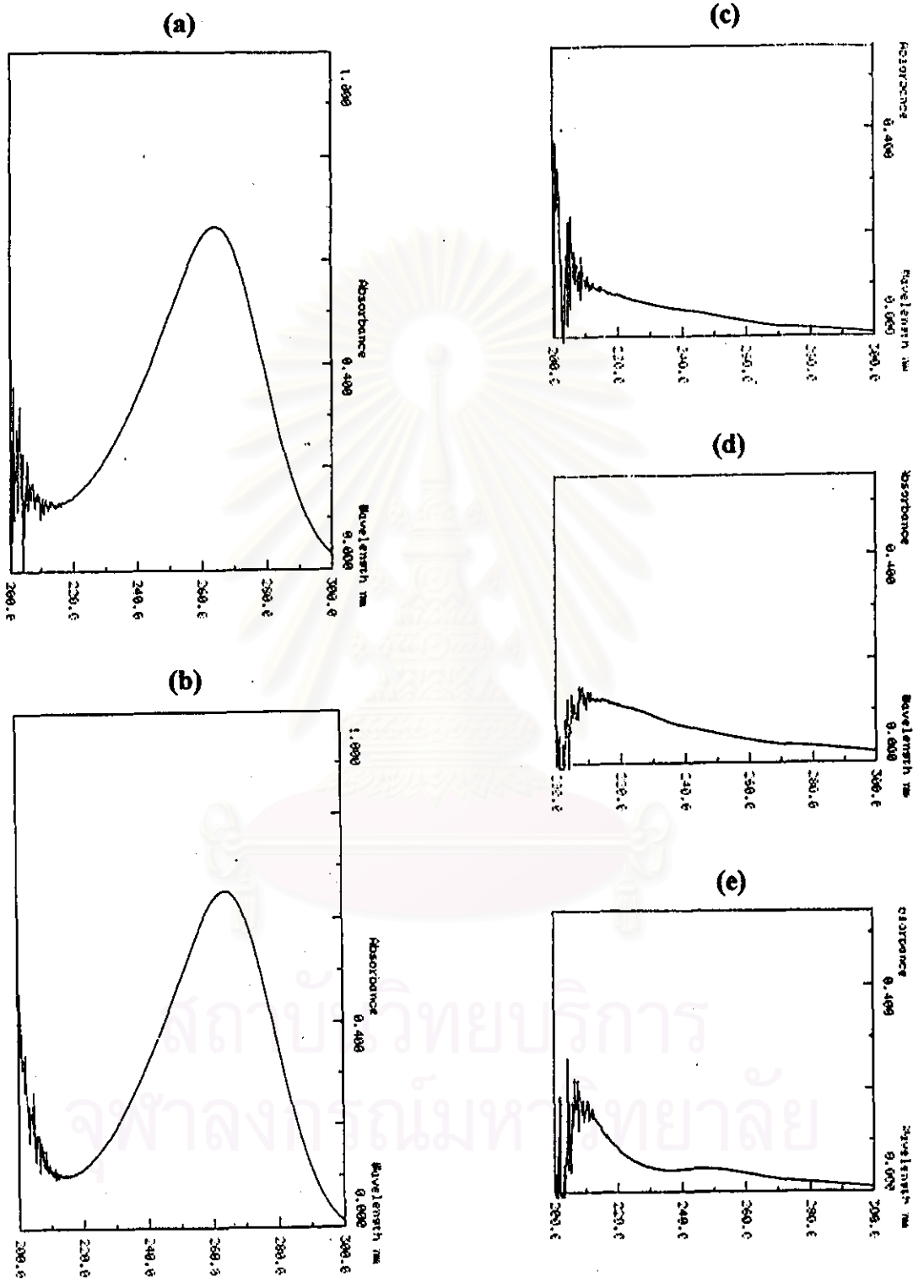


Figure 16. Spectra of ascorbic acid released from the microcapsules (formulation 9) (a); 9.12 μg/ml ascorbic acid in 5mM EDTA aqueous solution (b); 5mM EDTA aqueous solution (c); 20 μg/ml dehydroascorbic acid in 5mM EDTA aqueous solution (d); 21.2 μg/ml oxalic acid in 5mM EDTA aqueous solution (e).

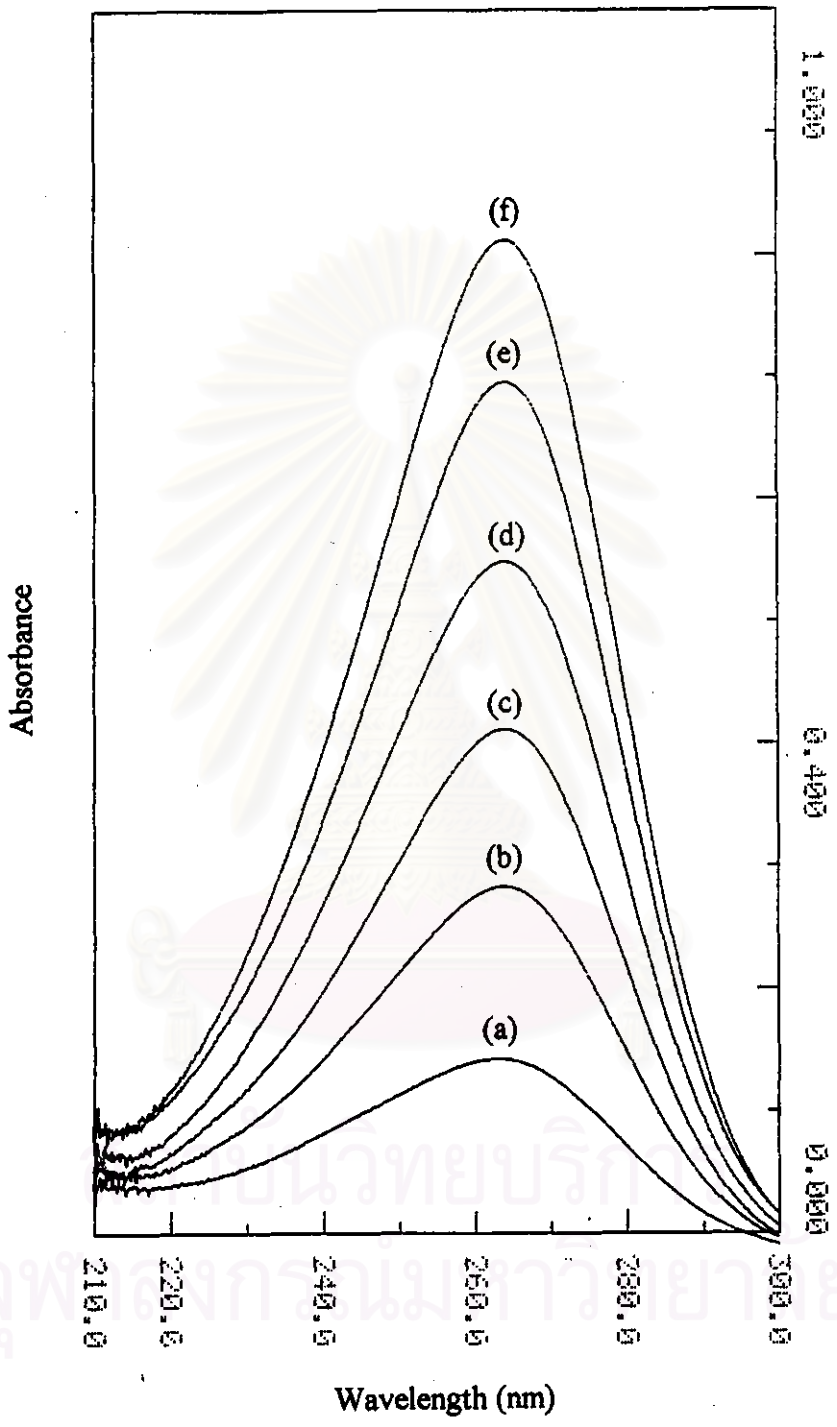


Figure 17. Spectra of standard solutions of ascorbic acid at the concentrations of 2.094 (a), 4.188 (b), 6.282 (c), 8.376 (d), 10.470 (e), and 12.564 (f) µg/ml, respectively.

Table 10. Calibration curve data of ascorbic acid assayed by UV/Visible spectrophotometer.

Standard solution no.	Concentration (μg/ml)	Absorbance at 265 nm
1	2.094	0.149
2	4.188	0.293
3	6.282	0.425
4	8.376	0.571
5	10.470	0.700
6	12.564	0.827

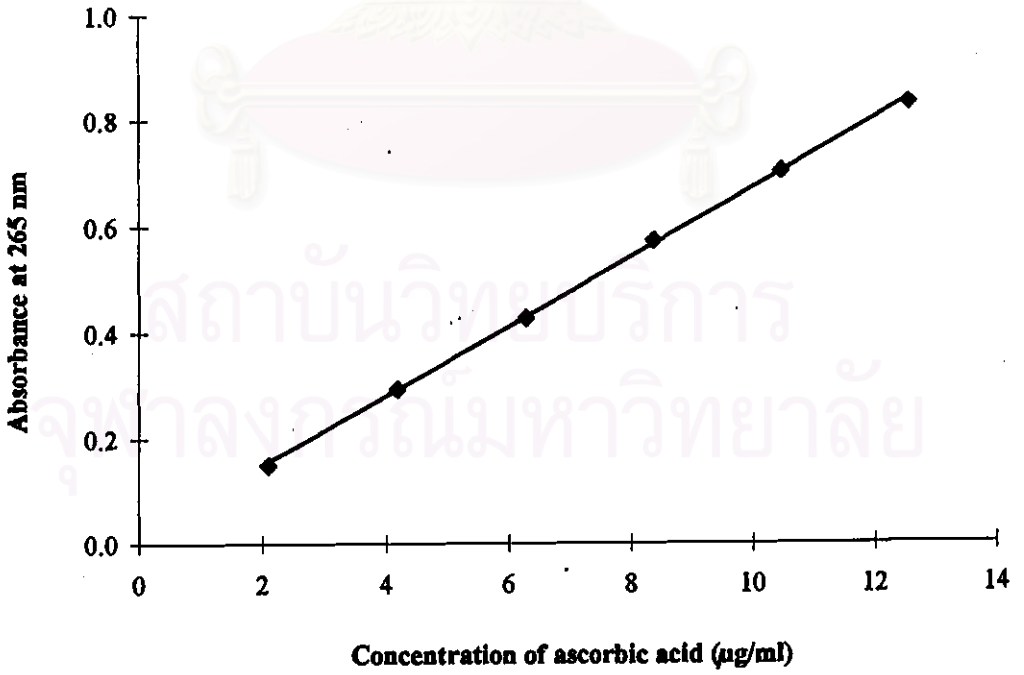


Figure 18. Calibration curve of ascorbic acid assayed by UV/Visible spectrophotometer ($r^2 = 0.9995$).

2.2 HPLC Method

The ascorbic acid content of the prepared microcapsules could be determined by HPLC assay with UV detection which was developed from the method described by Haralpanhalli et al. (1993). The wavelength used to analyze ascorbic acid in this study was 243 nm which was the λ_{max} of ascorbic acid in this mobile phase. The validation of the HPLC method used are presented as follows:

2.2.1 Specificity

Figure 19 shows the chromatograms of ascorbic acid, its vehicle, its degradation products, and other ingredients used to form microcapsule wall. Retention time of ascorbic acid and sodium urate (internal standard) were at 4.8-4.9 and 11.2-11.4 min, respectively. There was no interference to the ascorbic acid and sodium urate peaks.

2.2.2 Linearity

The chromatograms of standard solutions are shown in figure 20. Retention times of ascorbic acid and sodium urate were about 4.8-4.9 and 11.2-11.4 min, respectively. The standard curve was plotted between the peak area ratios of ascorbic acid to sodium urate and the concentrations of ascorbic acid in μg/ml, and was fitted using linear regression analysis. The results are shown in table 11 and figure 21. A straight line is obtained with a coefficient of correlation (r) of 0.99997. The equation for this line is

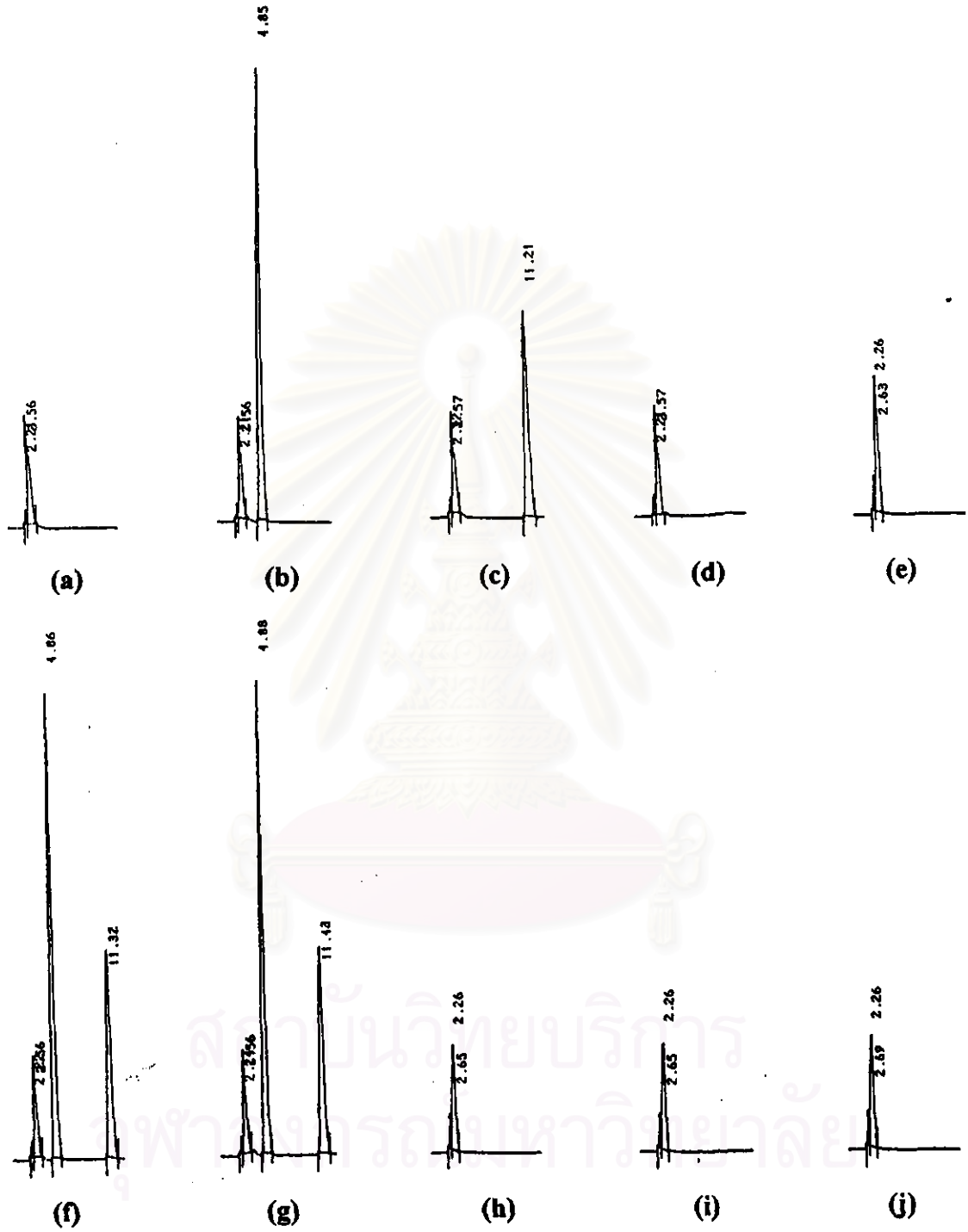


Figure 19. Chromatograms of 5mM EDTA aqueous solution (a); 10 μg/ml ascorbic acid (retention time = 4.85 min) (b); sodium urate (retention time = 11.21 min) (c); 20 μg/ml dehydroascorbic acid (d); 21.2 μg/ml oxalic acid (e); ascorbic acid and sodium urate (f); ascorbic acid, sodium urate, and dehydroascorbic acid (g); triacetin (h); triethyl citrate (i); and tween80 (j).

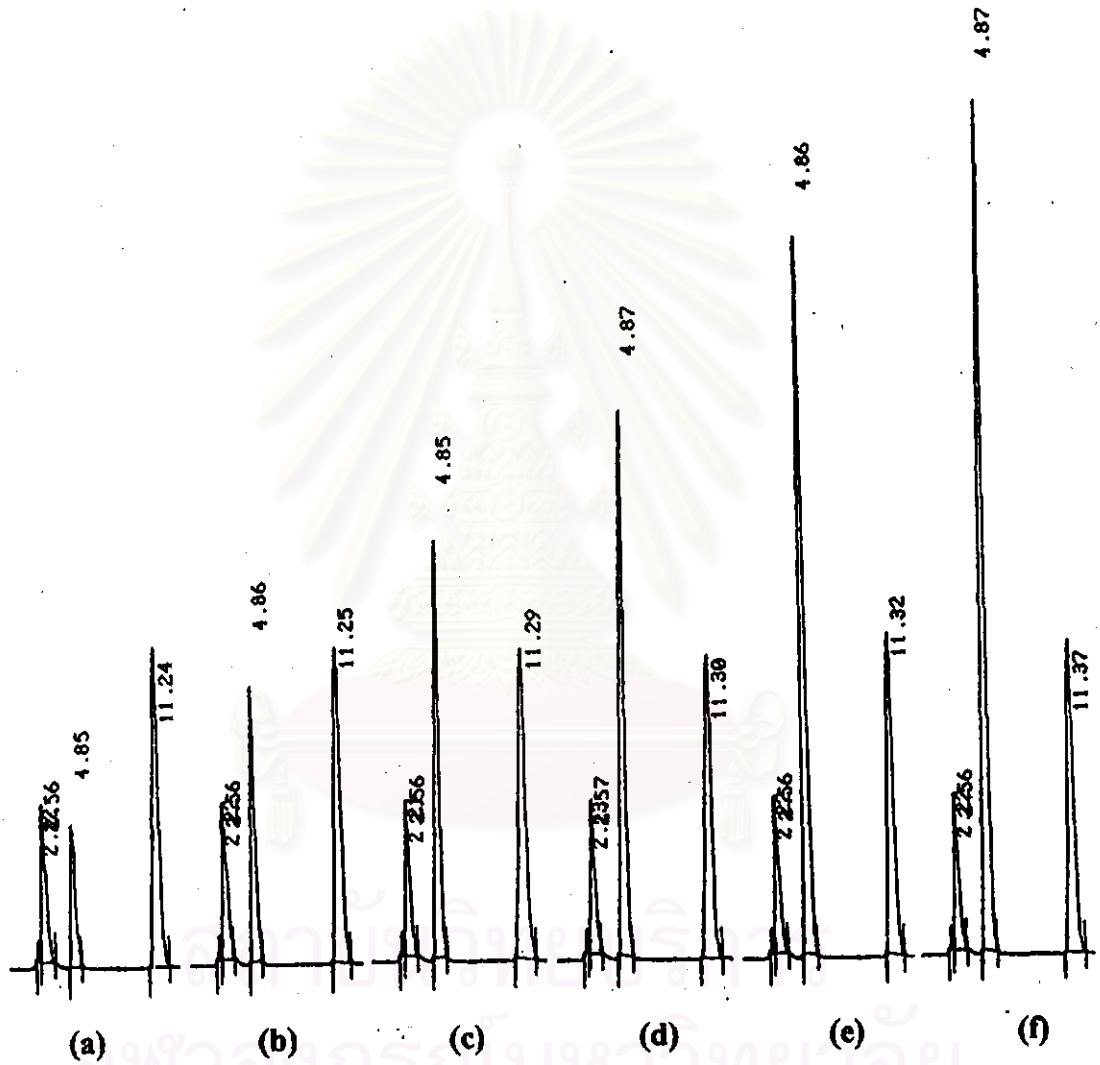


Figure 20. Chromatograms of standard solutions of ascorbic acid at the concentrations of 2.17 (a), 4.34 (b), 6.51 (c), 8.68 (d), 10.85 (e), and 13.02 (f) μg/ml, respectively. Retention times of ascorbic acid and sodium urate are at 4.8 and 11.3 min, respectively.

Table 11. Calibration curve data of ascorbic acid assayed by HPLC technique.

Standard solution no.	Concentration (μg/ml)	Peak area ratio of ascorbic acid to sodium urate
1	2.17	0.2724
2	4.34	0.6236
3	6.51	0.9645
4	8.68	1.3169
5	10.85	1.6669
6	13.02	2.0001

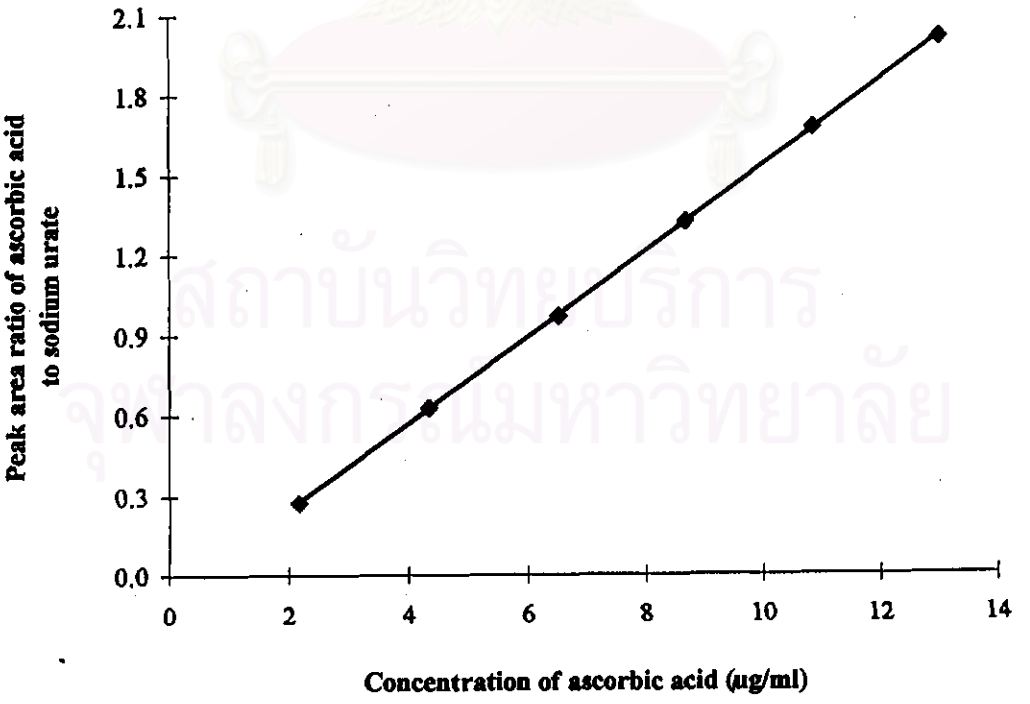


Figure 21. Calibration curve of ascorbic acid assayed by HPLC technique (r^2 = 0.99994).

$$y = -0.07137 + 0.15959x \quad (14)$$

where y is the peak area ratio of ascorbic acid to sodium urate and x is the concentration of ascorbic acid in μg/ml.

2.2.3 Accuracy

The accuracy data is presented in table 12. The %CV values of %recovery were very low (0.55) which indicated that the HPLC method could be used to accurately determine ascorbic acid content within the concentration range studied.

Table 12. Accuracy data of ascorbic acid assayed by the HPLC method.

Ascorbic acid conc. (μg/ml)	Fitted conc. (μg/ml)	% Recovery
4.34	4.35	100.23
4.34	4.39	101.15
4.34	4.39	101.15
8.68	8.70	100.23
8.68	8.71	100.35
8.68	8.71	100.35
13.02	12.98	99.69
13.02	12.99	99.77
13.02	13.16	101.08
	Mean	100.45
	SD	0.55
	%CV	0.55

2.2.4 Precision

Tables 13 and 14 show data of within-run precision and between-run precision of ascorbic acid assayed by the HPLC method, respectively. The %CV values of peak area ratios of both precisions were low which indicated that the HPLC method could be used to determine the amount of ascorbic acid over a period of time studied.

In conclusion, there were specificity, linearity, accuracy, and precision in the assay of ascorbic acid by the HPLC method. Therefore, the HPLC assay could be used to determine ascorbic acid content in the microcapsules which were stored at 40°C, 75% R.H. over the period of time studied.

Table 13. Data of within-run precision of ascorbic acid assayed by the HPLC method.

Ascorbic acid conc. (μg/ml)	Peak area ratio of ascorbic acid to sodium urate					
	Injection #1	Injection #2	Injection #3	Mean	SD	%CV
2.17	0.2724	0.2795	0.2747	0.2755	0.0036	1.32
4.34	0.6236	0.6293	0.6284	0.6271	0.0031	0.49
6.51	0.9645	0.9658	0.9660	0.9654	0.0008	0.08
8.68	1.3169	1.3193	1.3184	1.3182	0.0012	0.09
10.85	1.6669	1.6669	1.6476	1.6605	0.0111	0.67
13.02	2.0001	2.0019	2.0296	2.0105	0.0165	0.82

Table 14. Data of between-run precision of ascorbic acid assayed by the HPLC method.

Ascorbic acid conc. (µg/ml)	Peak area ratio of ascorbic acid to sodium urate					
	Day #1	Day #2	Day #3	Mean	SD	%CV
2.17	0.2724	0.2733	0.2801	0.2753	0.0042	1.5294
4.34	0.6236	0.6141	0.6298	0.6225	0.0079	1.2703
6.51	0.9645	0.9456	0.9685	0.9595	0.0122	1.2747
8.68	1.3169	1.3512	1.3414	1.3365	0.0177	1.3219
10.85	1.6669	1.6869	1.6957	1.6832	0.0148	0.8768
13.02	2.0001	1.9999	2.0255	2.0085	0.0147	0.7330

3. Characteristics of Ascorbic Acid Microcapsules

3.1 Scanning Electron Microscope (SEM)

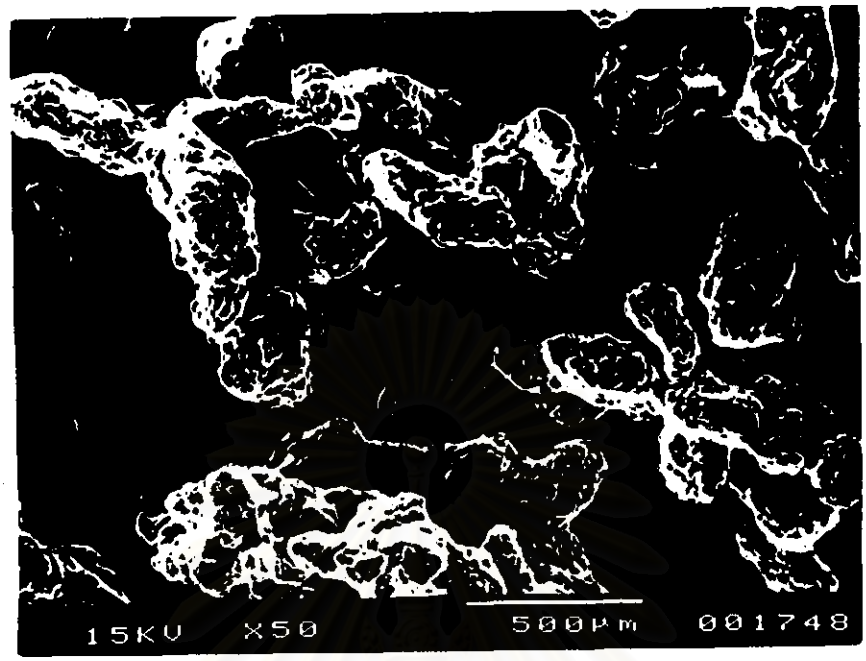
3.1.1 Temperature Induced Coacervation Technique

Physical characteristics of all formulations of the ascorbic acid microcapsules prepared by the temperature induced coacervation technique were pale yellow, free-flowing, and granule-like aggregates except for the case of 1:2 core to wall ratio (formulation 1) where microcapsules could not be prepared; this may be due to the high proportion of polymer, and a lump of polymer and drug was resulted.

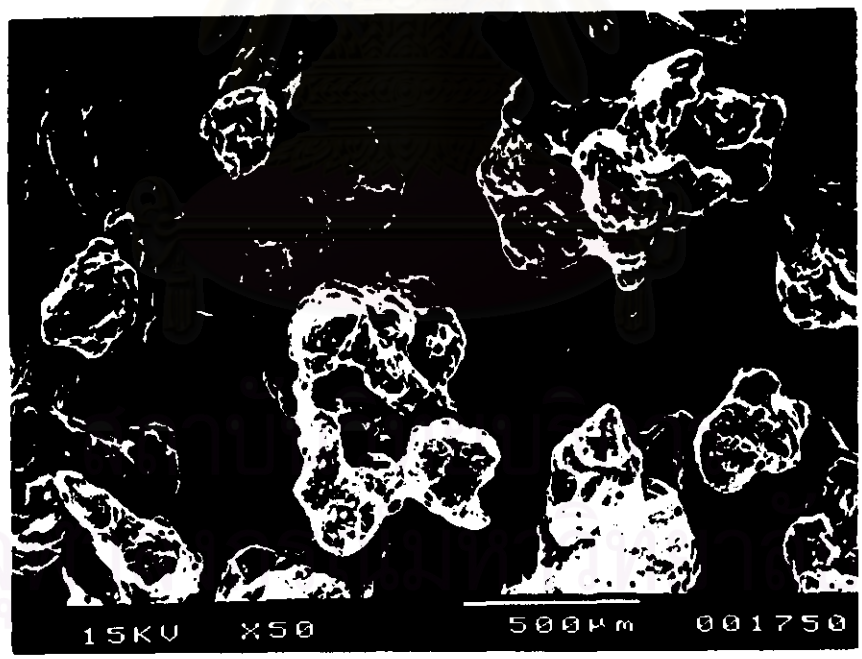
From the scanning electron micrographs illustrated in figure 22, the temperature induced coacervation technique yielded aggregate and irregular-shaped microcapsules with rough surface and pores on the surface. The SEM micrograph of a cross-sectioned microcapsule revealed that this technique gave multinucleated microcapsules (containing more than one drug particle) with porous structure inside (figure 23). This result is consistent with previous studies by Jalsenjak et al. (1976), and Sveinsson and Kristmundsdottir (1992). The aggregates of microcapsules were composed of the smaller ones. During the drying process at room temperature, the microcapsules fused together to produce larger and aggregate particles. The rough surface of microcapsules was probably due to ascorbic acid crystals, which were coated with polymer, adhering at the surface of microcapsules. The pore-forming process of ethylcellulose film was discussed by Narisawa et al. (1993). As polymer lose solvent, a large number of coacervation droplets appear, and then gradually grow into contact with each other until they form a gel-like coagulation phase. The gel phase gradually turns into the solid-state and finally yields a xerogel with numerous opening pores. Gelation should be the most important process in porous film formation because the precipitated gel phase almost decides the geometrical dimension of the resultant film. After gelation, since the reduction in volume is considerably hindered due to the structural rigidity of the gel phase, the subsequent solvent evaporation from the surface and the inside would make the film more porous.

3.1.2 Solvent Evaporation Technique

Physical characteristics of the ascorbic acid microcapsules prepared by the solvent evaporation technique are summarized in table 15. All formulations gave yellow microcapsules due to the color of surfactants. The microcapsules prepared by

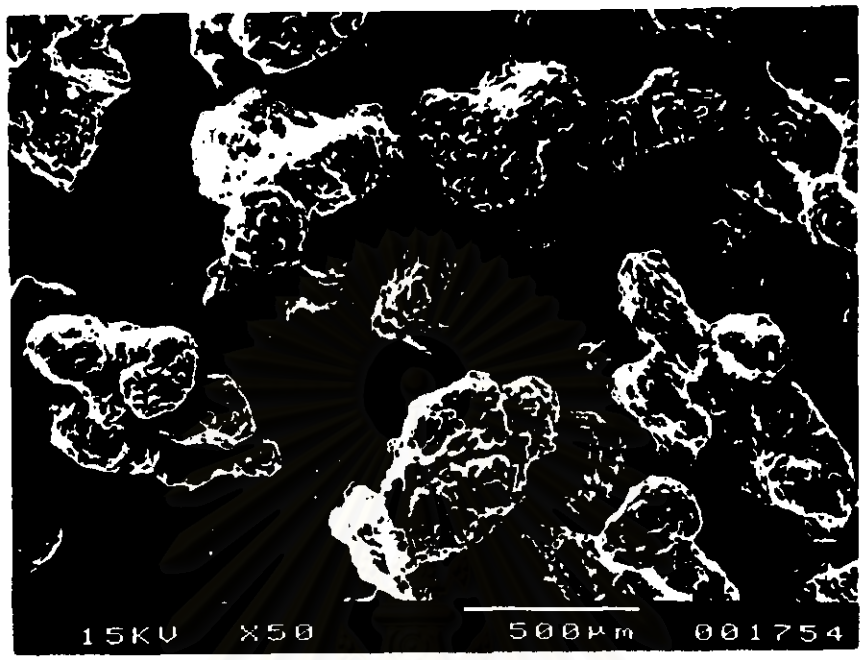


(a) Formulation 2: a core to wall ratio of 1:1.

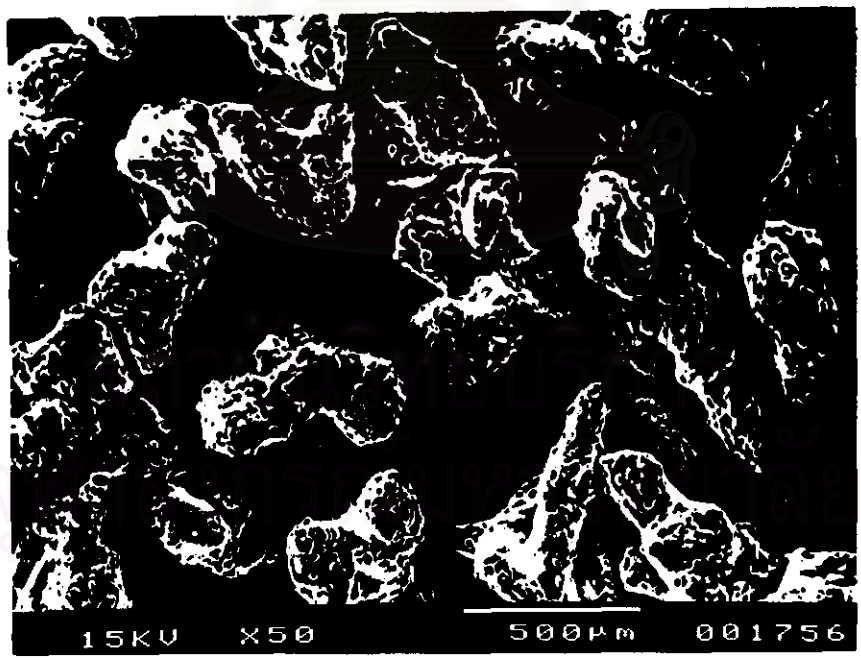


(b) Formulation 3: a core to wall ratio of 3:2.

Figure 22. Scanning electron micrographs of the ascorbic acid microcapsules prepared by the temperature induced coacervation technique with various formulation variables. Magnification 50x. Scale bar 500 µm.



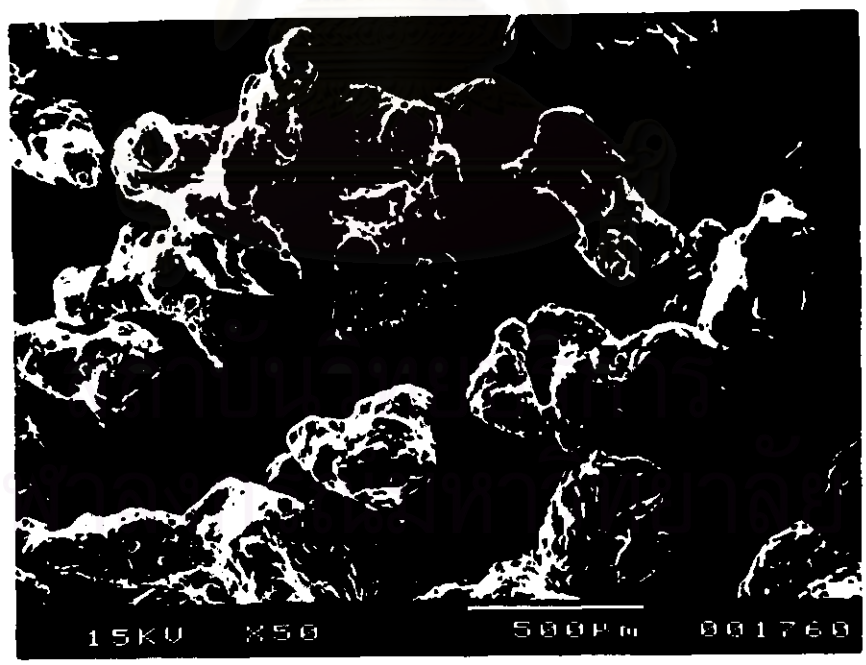
(c) Formulation 4: a core to wall ratio of 1:1, 20% Triacetin.



(d) Formulation 5: a core to wall ratio of 1:1, 30% Triacetin.

Figure 22. Continued.

(e) Formulation 6: a core to wall ratio of 1:1, 20% Triethyl citrate.



(f) Formulation 7: a core to wall ratio of 1:1, 30% Triethyl citrate.

Figure 22. Continued.

(g) Formulation 8: a core to wall ratio of 1:1, 20% Dibutyl sebacate.

(h) Formulation 9: a core to wall ratio of 1:1, 30% Dibutyl sebacate.

Figure 22. Continued.

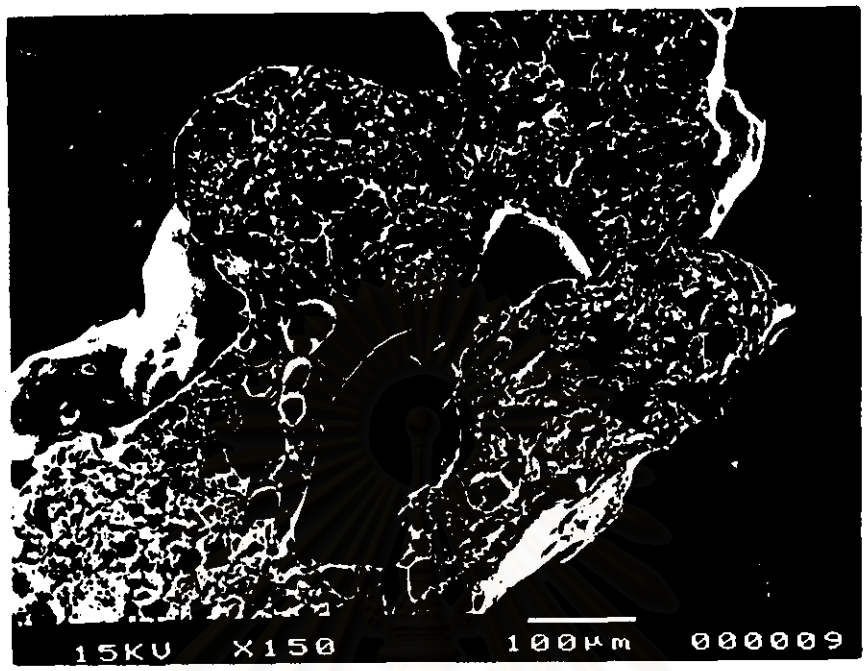


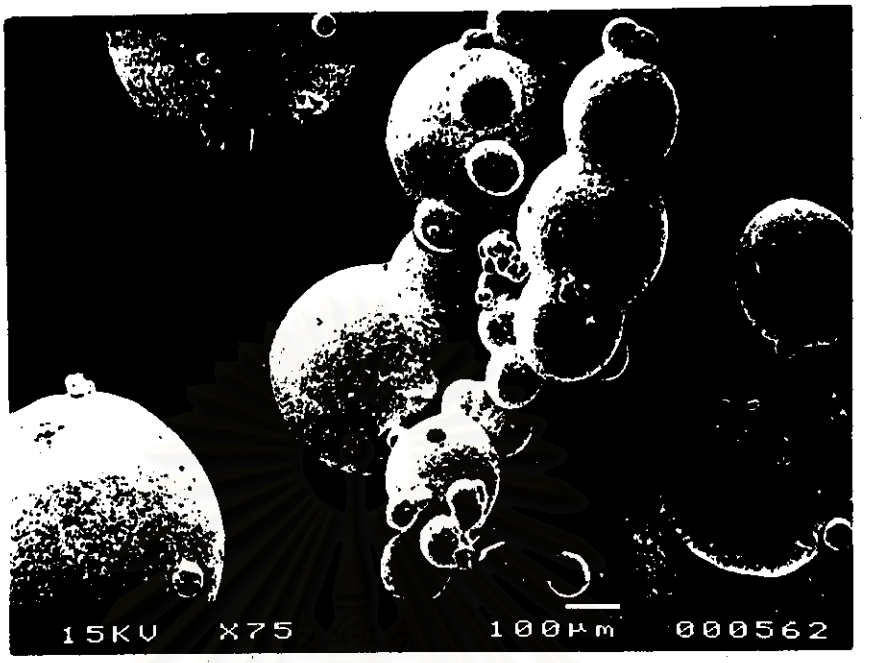
Figure 23. Scanning electron micrograph of a cross-section of ascorbic acid microcapsules (formulation 9) prepared by the temperature induced coacervation technique. Magnification 150x. Scale bar 100 µm.

this technique were free-flowing. Their shapes could be visually observed due to their large sizes.

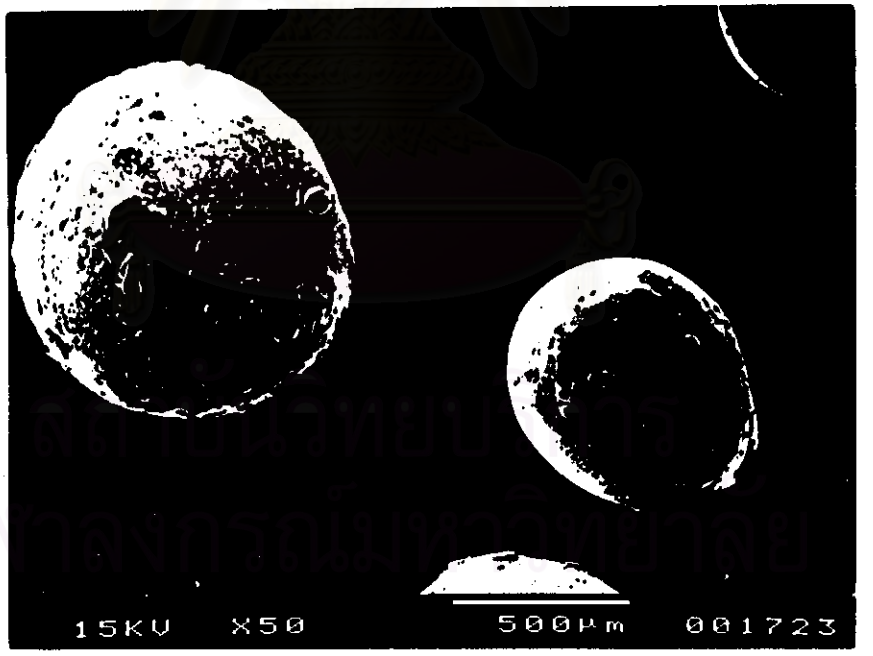
Figure 24 illustrates the scanning electron micrographs of the microcapsules prepared by the solvent evaporation technique with various formulation variables. The lower ethylcellulose concentration (4% EC) with an excess of solvent provided the aggregate microcapsules since small soft microcapsules joined together to form aggregates during stirring (figure 24(a)). This result agrees with a previous study of Sanghvi and Nairn (1991). With higher concentrations of ethylcellulose (5 and 6%), the microcapsules prepared with 1.0% Span80 were spherical in shape (figures 24(b)

Table 15. Physical appearances of the ascorbic acid microcapsules prepared by the solvent evaporation technique with various formulation variables.

Formulation no.	Variables			Physical appearances
	% EC (w/v)	Core to wall ratio	% Surfactant (w/v)	
10	4	1:2	1.0% Span80	yellow, free-flowing, aggregate
11	5	1:2	1.0% Span80	yellow, free-flowing, spherical
12	6	1:2	1.0% Span80	yellow, free-flowing, spherical
13	6	1:1	1.0% Span80	pale yellow, free-flowing, spherical
14	6	3:2	1.0% Span80	pale yellow, free-flowing, spherical (some rod-like)
15	6	1:2	-	pale yellow, free-flowing, aggregate
16	6	1:2	0.5% Span80	pale yellow, free-flowing, spherical
17	6	1:2	1.5% Span80	yellow, free-flowing, spherical
18	6	1:2	0.5% Tween80	yellow, free-flowing, aggregate
19	6	1:2	1.0% Tween80	yellow, free-flowing, irregular and aggregate
20	6	1:2	1.5% Tween80	yellow, free-flowing, irregular

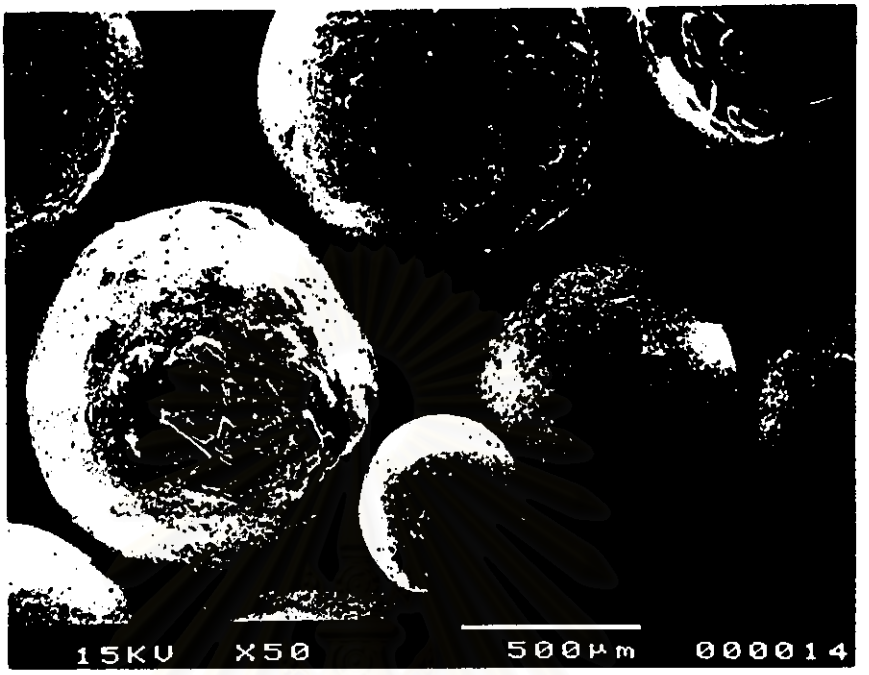


(a) Formulation 10: 4% EC, a core to wall ratio of 1:2, 1.0% Span80.

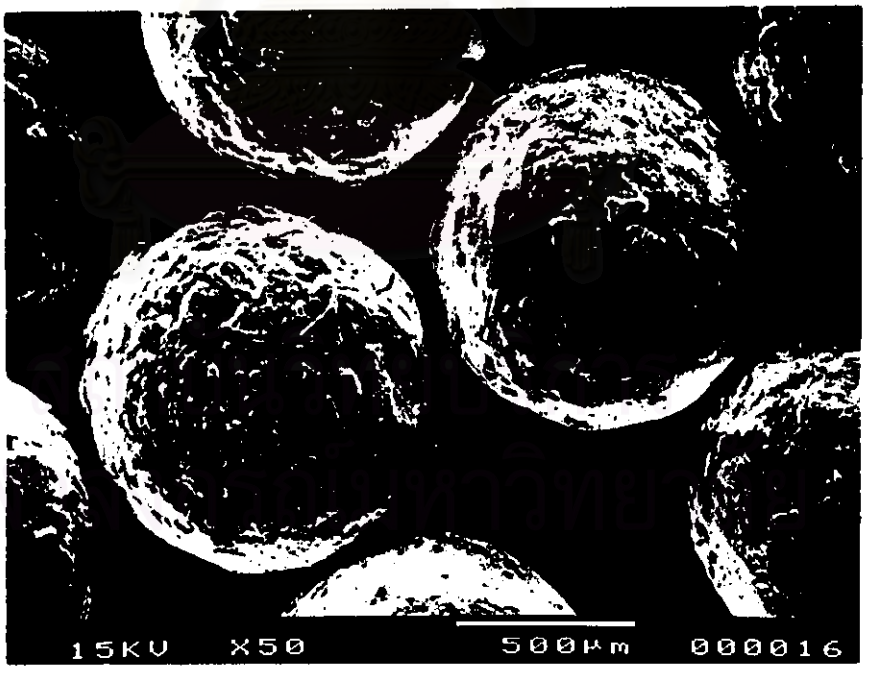


(b) Formulation 11: 5% EC, a core to wall ratio of 1:2, 1.0% Span80.

Figure 24. Scanning electron micrographs of the ascorbic acid microcapsules prepared by the solvent evaporation technique with various formulation variables. (a) Magnification 75x. Scale bar 100 µm. (b)-(k) Magnification 50x. Scale bar 500 µm.



(c) Formulation 12: 6% EC, a core to wall ratio of 1:2, 1.0% Span80.



(d) Formulation 13: 6% EC, a core to wall ratio of 1:1, 1.0% Span80.

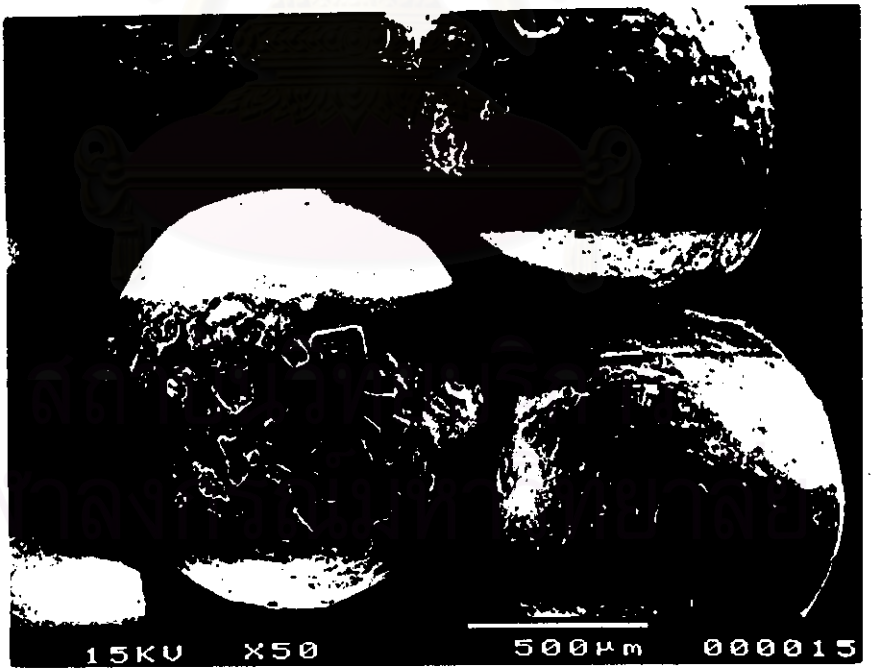
Figure 24. Continued.

(e) Formulation 14: 6% EC, a core to wall ratio of 3:2, 1.0% Span80.

(f) Formulation 15: 6% EC, a core to wall ratio of 1:2, no emulsifier.

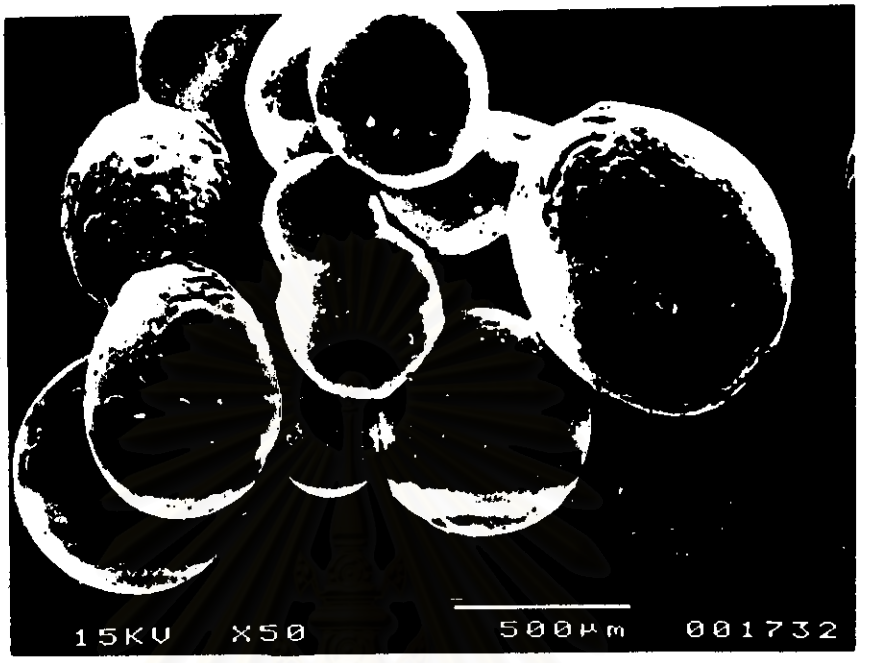
Figure 24. Continued.

(g) Formulation 16: 6% EC, a core to wall ratio of 1:2, 0.5% Span80.



(h) Formulation 17: 6% EC, a core to wall ratio of 1:2, 1.5% Span80.

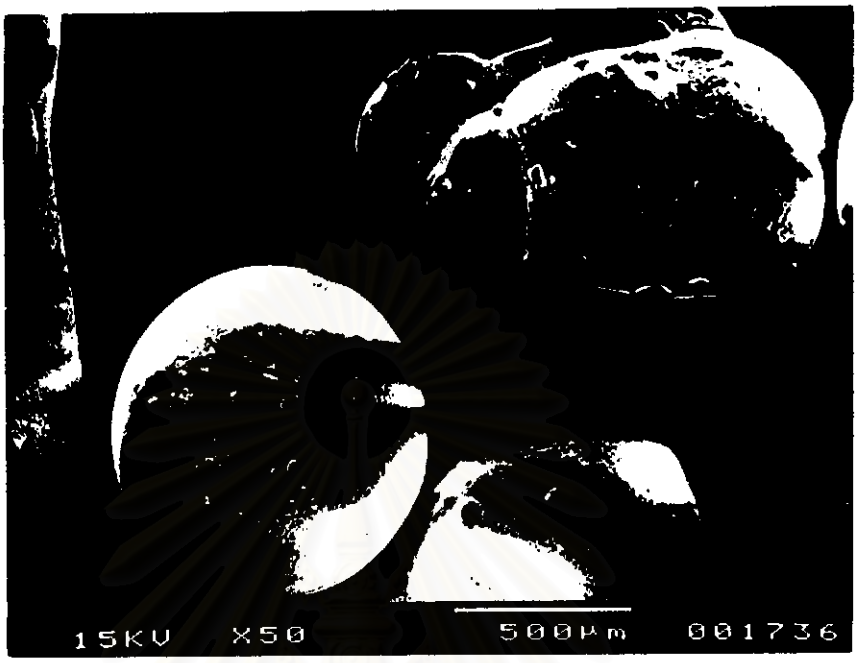
Figure 24. Continued.



(i) Formulation 18: 6% EC, a core to wall ratio of 1:2, 0.5% Tween80.

(j) Formulation 19: 6% EC, a core to wall ratio of 1:2, 1.0% Tween80.

Figure 24. Continued.



(k) Formulation 20: 6% EC, a core to wall ratio of 1:2, 1.5% Tween80.

Figure 24. Continued.

and (c). The higher core to wall ratios (1:1 and 3:2) gave some drug crystals on the surface of microcapsules due to the excess of drug in the systems (figures 24(d) and (e)). The microcapsules prepared without any emulsifier showed some aggregations (figure 24(f)). Roles of an emulsifier in microencapsulation by solvent evaporation technique are both facilitation of the emulsification process and stabilization of the suspended polymer droplets to prevent aggregation and coalescence of the microcapsules (Watts et al., 1990). The microcapsules prepared using Span80 as the emulsifier with 6% ethylcellulose were spherical in shape and had a few pores on their surface (figures 24(c), (d), (e), (g), and (h)), whereas Tween80 gave irregular-shaped microcapsules with smoother surface (figures 24(i)-(k)). Span80 may provide a protective sheath around polymer droplets and help to prevent the droplets from

coalescence during the manufacturing process (Huang and Ghebre-Sellassie, 1989). The microcapsules prepared with higher Span80 concentrations had more drug crystals adhering on the microcapsule surface (figure 24(h)) similar to the case of higher core to wall ratios. Tween80 yielded aggregate microcapsules but the greater amount gave the less aggregate microcapsules. This aggregation was due to the fact that Tween80, which has a relatively high HLB value (15.0), is immiscible with the microencapsulating vehicle (light liquid paraffin), which has a relatively low HLB value (about 10). Thus, Tween80 preferred to partition into polymer-acetone droplets and acted as a plasticizer for the polymer; the polymer became soft and fused. However, the lower amount of Tween80 was not enough for acting as the emulsifying agent. When Tween80 concentration was increased, the amount increased was enough to act both as the plasticizer and the emulsifier, therefore the microcapsules were less aggregate. The microcapsule prepared with Tween80 had a relatively dense internal structure (figure 25) compared with that prepared with Span80 (figure 26).

Consequently, Span80 was selected as the emulsifier for microencapsulation by this technique since it provided spherical microcapsules.

3.2 Size and Size Distribution of Microcapsules

The optical microscope allows the observer to view the actual particles. To measure the microcapsule size, the ocular scale have been previously calibrated with an objective micrometer which has a division of 10 μm. Since 100 divisions in ocular scale are equal to 40 divisions in objective micrometer at the magnification of 40X, each division in ocular scale is then equal to 4 μm. At the magnification of 10X, accordingly, each division in ocular scale is equal to 16 μm.

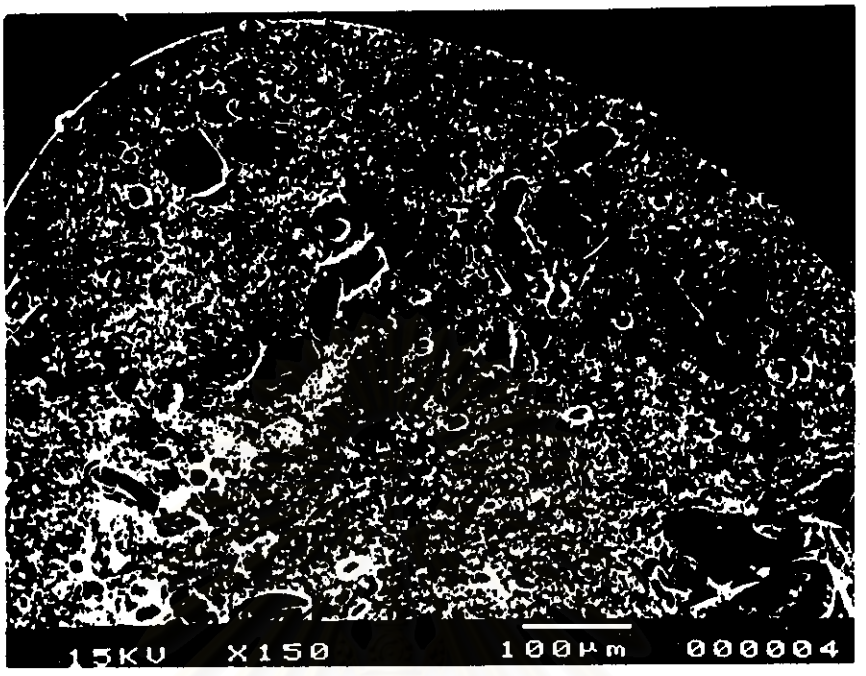


Figure 25. Scanning electron micrograph of a cross-section of the ascorbic acid microcapsules with 1.5% Tween80 (formulation 20) prepared by the solvent evaporation technique. Magnification 150x. Scale bar 100 µm.

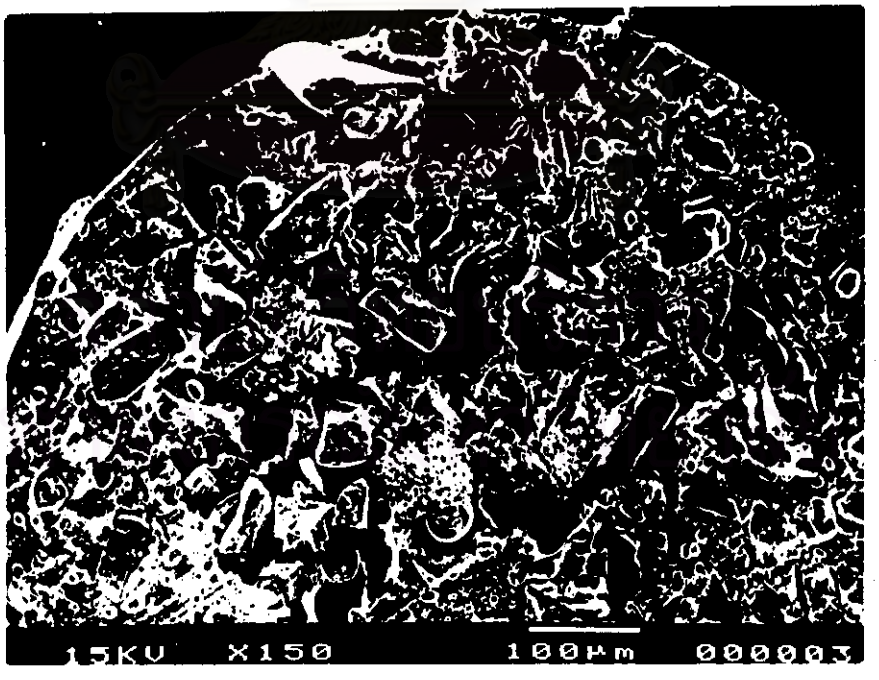


Figure 26. Scanning electron micrograph of a cross-section of the ascorbic acid microcapsules with 1.5% Span80 (formulation 17) prepared by the solvent evaporation technique. Magnification 150x. Scale bar 100 µm.

The longest side of the microcapsules was aligned on the ocular scale, then the sizes were determined by counting the number of the divisions in the ocular scale and calculated into μm unit. The sizes of microcapsules were recorded into frequency distribution table. The size mean and standard deviation were calculated from the frequency distribution table presented in Appendix II.

3.2.1 Temperature Induced Coacervation Technique

The effect of the core to wall ratio on the size distribution of ascorbic acid microcapsules prepared by temperature induced coacervation technique is depicted in figure 27. The size distribution of microcapsules with the high core to wall ratio shifted to the smaller sizes. The decrease in size as the core to wall ratio increased was attributed to the low proportion of polymer which resulted in the reduced aggregation (less microcapsules attached to one another by linkages) of the prepared microcapsules (see the SEM shown in figures 22(a) and (b)).

The effects of various types and amounts of plasticizer on the size distribution of ascorbic acid microcapsules prepared by temperature induced coacervation technique are displayed in figures 28, 29, and 30 for triacetin, triethyl citrate, and dibutyl sebacate, respectively. The mean sizes of the microcapsules prepared by various types and amounts of plasticizer were not different.

3.2.2 Solvent Evaporation Technique

For the solvent evaporation technique, the effect of the ethylcellulose concentration on the size distribution of the microcapsules is displayed in figure 31. From the SEM shown in figure 24(a), the low ethylcellulose concentration (4%)

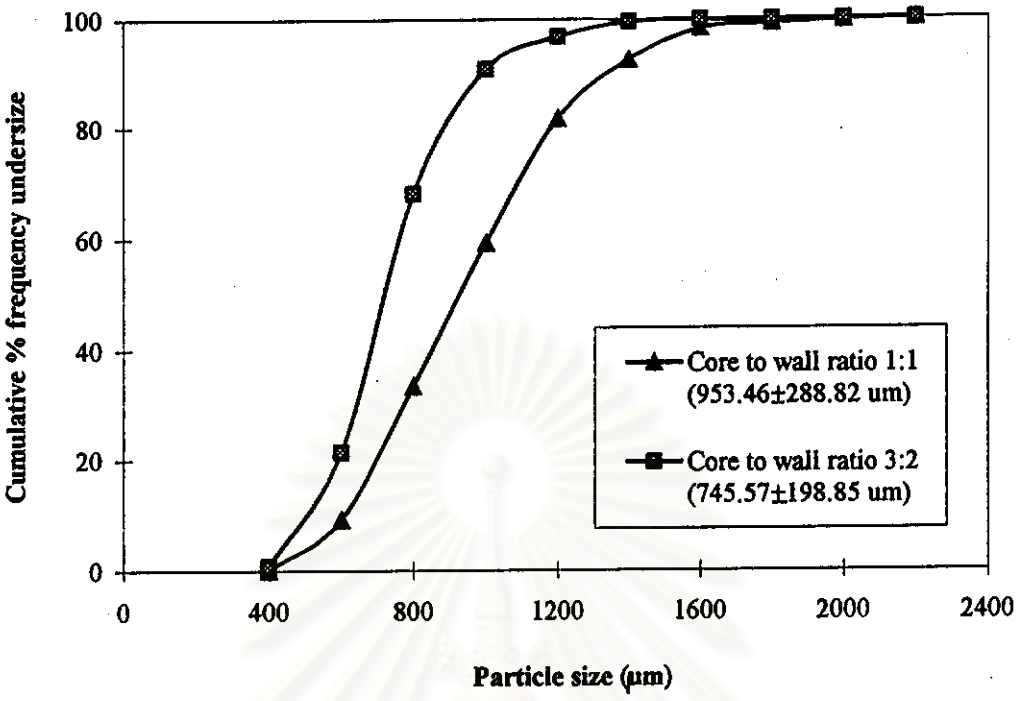


Figure 27. Cumulative % frequency undersize of the ascorbic acid microcapsules with 1:1 and 3:2 core to wall ratios prepared by the temperature induced coacervation technique.

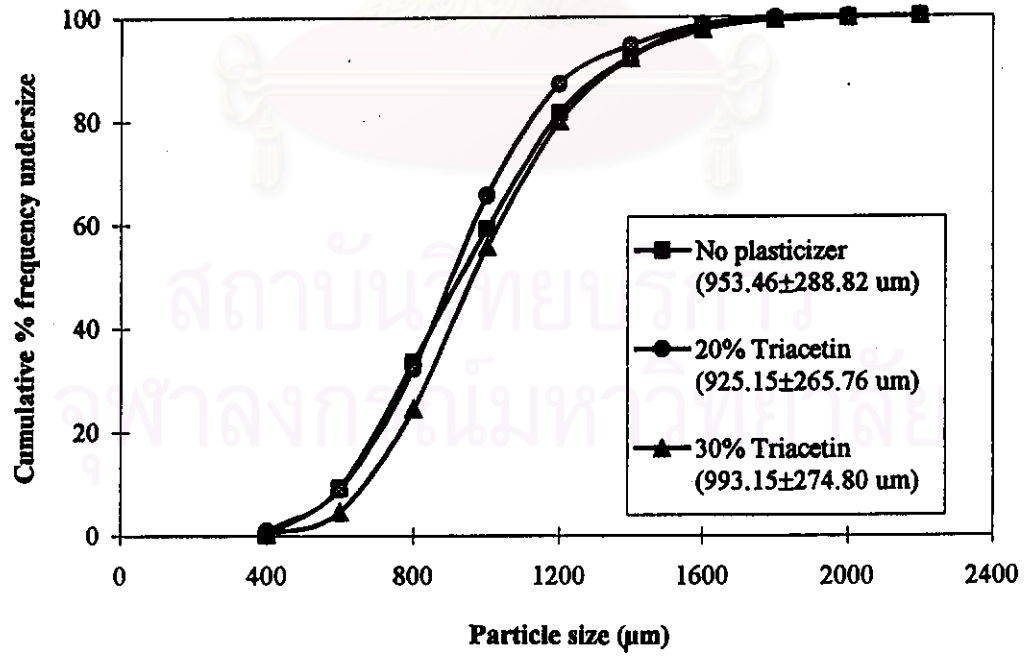


Figure 28. Cumulative % frequency undersize of the ascorbic acid microcapsules with 0, 20, and 30% triacetin (with a core to wall ratio of 1:1) prepared by the temperature induced coacervation technique.

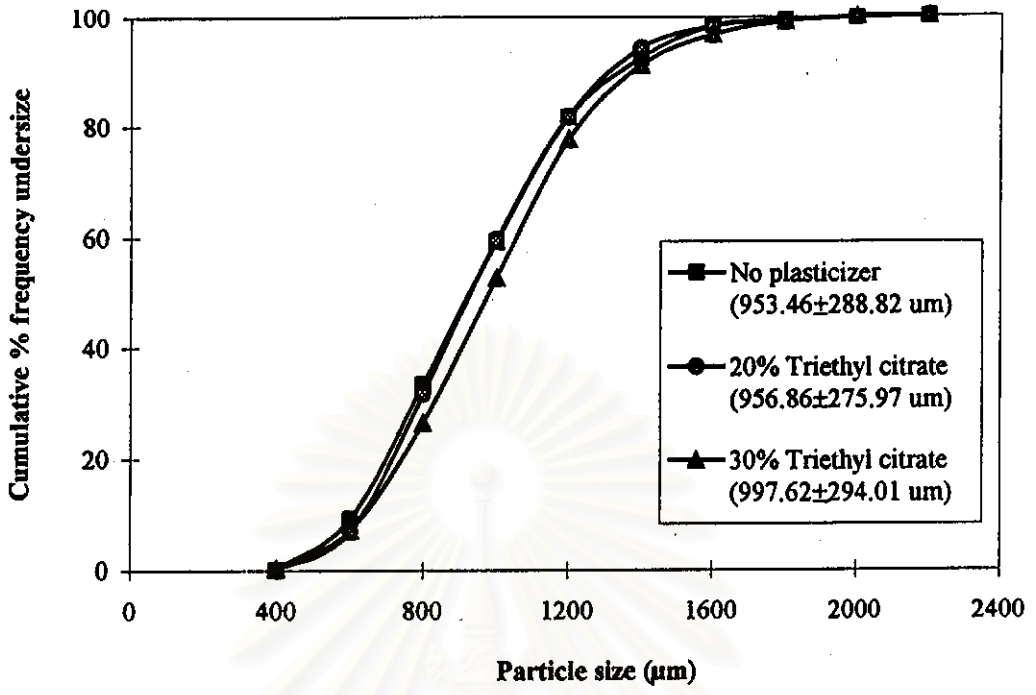


Figure 29. Cumulative % frequency undersize of the ascorbic acid microcapsules with 0, 20, and 30% triethyl citrate (with a core to wall ratio of 1:1) prepared by the temperature induced coacervation technique.

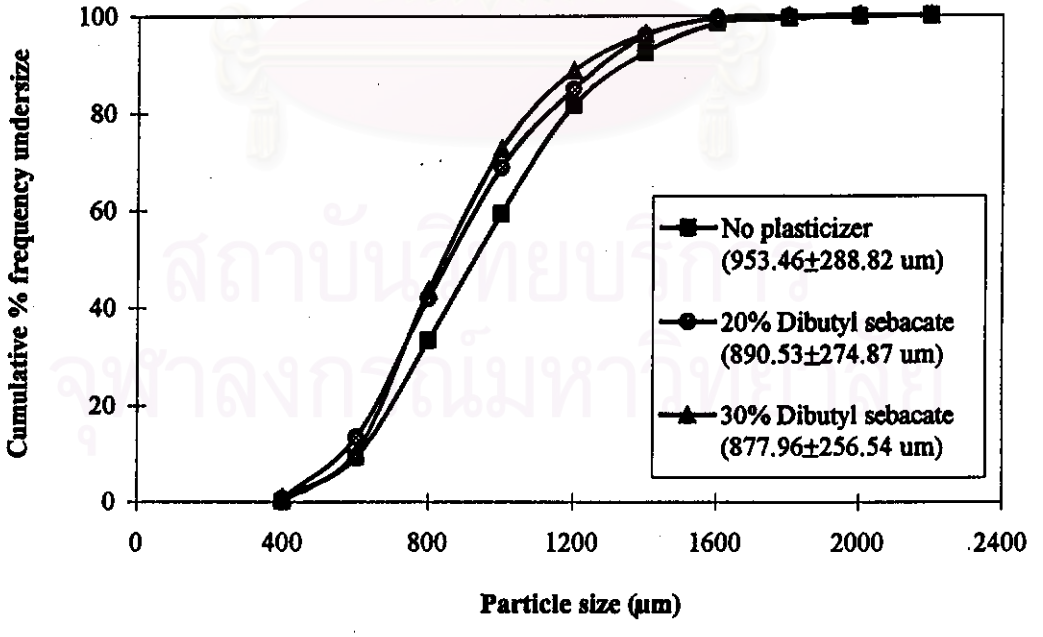


Figure 30. Cumulative % frequency undersize of the ascorbic acid microcapsules with 0, 20, and 30% dibutyl sebacate (with a core to wall ratio of 1:1) prepared by the temperature induced coacervation technique.

provided aggregate microcapsules resulting in the larger mean size. There was no difference between the mean size of the microcapsules prepared with 5 and 6% ethylcellulose concentrations.

The effect of core to wall ratio on the size distribution of the microcapsules prepared by the solvent evaporation technique is depicted in figure 32. As the core to wall ratio increased, the curve of cumulative % frequency undersize shifted to the larger sizes meaning that the increase in core to wall ratios resulted in the larger mean microcapsule sizes. The core to wall ratios were varied by varying amounts of the drug. Amounts of the polymer and other factors were kept constant. Consequently, the increase in the microcapsule size with increasing the core to wall ratio was attributed to the higher consistency of the internal phase due to the high concentration of drug in the polymer solution leading to more difficulty in dispersing it into the external phase during evaporation. This is in agreement with results obtained by Pongpaibul and Whitworth (1986), and Ruiz, Sakr and Sprockel (1990).

Abu-Izza, Contreras, and Lu (1996) discussed the effect of drug loading on particle size. Upon evaporation of the solvent, the polymer starts to shrink due to its desolvation, and the size of the forming microcapsules decreases gradually as a result. The phenomenon is more pronounced at the lower drug content, where the polymer constitutes the major part of the solids in the droplet. This results in the formation of smaller microcapsules as the core to wall ratio decreases.

The effects of type and amount of emulsifier on the size distribution of ascorbic acid microcapsules with 1:2 core to wall ratio prepared by the solvent evaporation technique are shown in figures 33 and 34 for Span80 and Tween80,

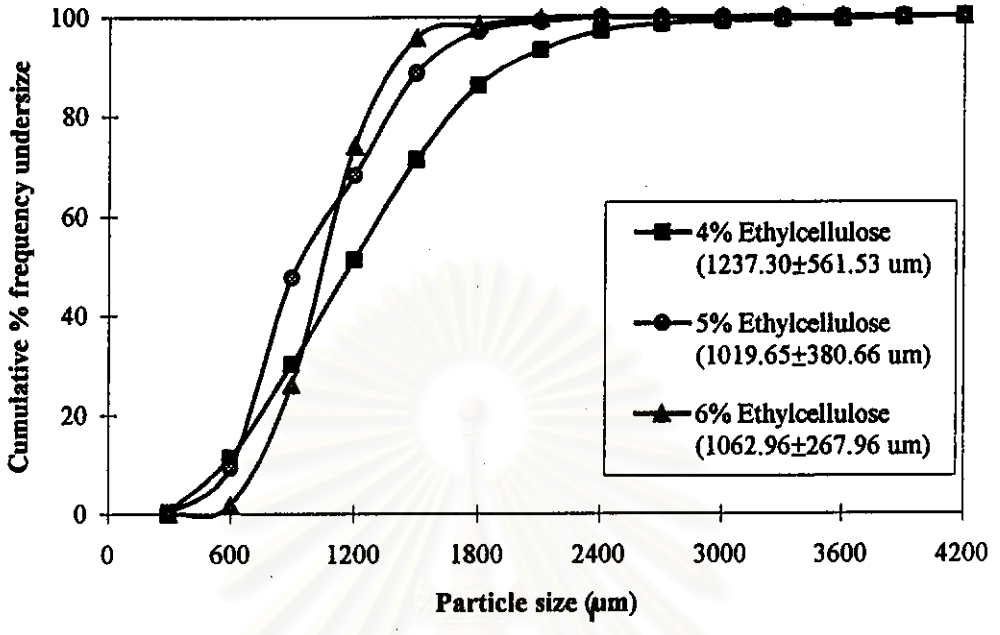


Figure 31. Cumulative % frequency undersize of the ascorbic acid microcapsules with 4, 5, and 6% ethylcellulose (with a core to wall of 1:2 and 1.0% Span80) prepared by the solvent evaporation technique.

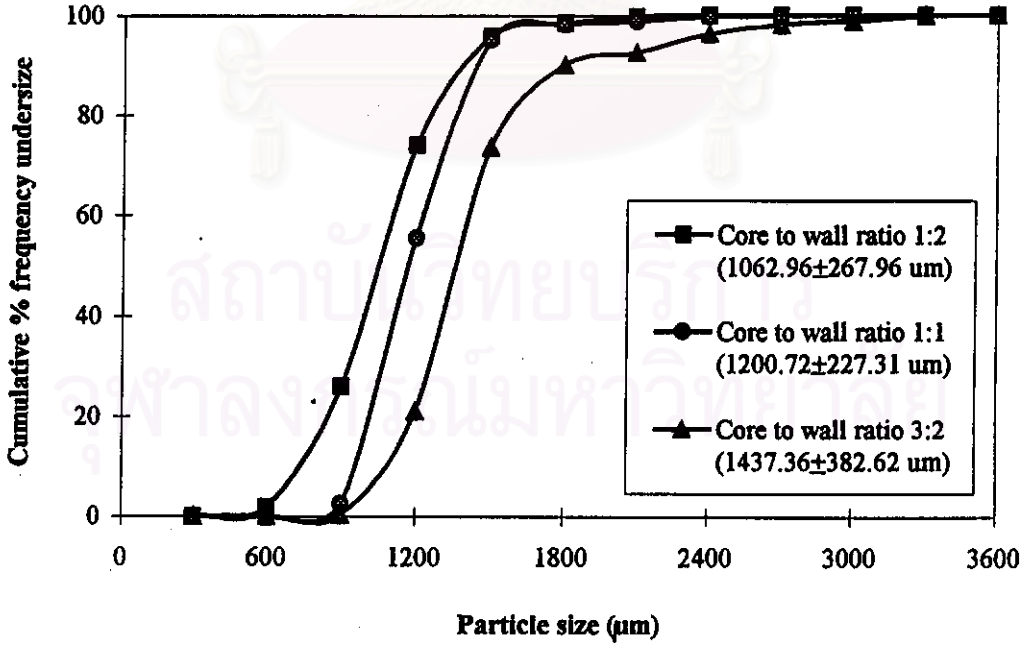


Figure 32. Cumulative % frequency undersize of the ascorbic acid microcapsules with 1:2, 1:1, and 3:2 core to wall ratios (with 6% ethylcellulose and 1.0% Span80) prepared by the solvent evaporation technique.

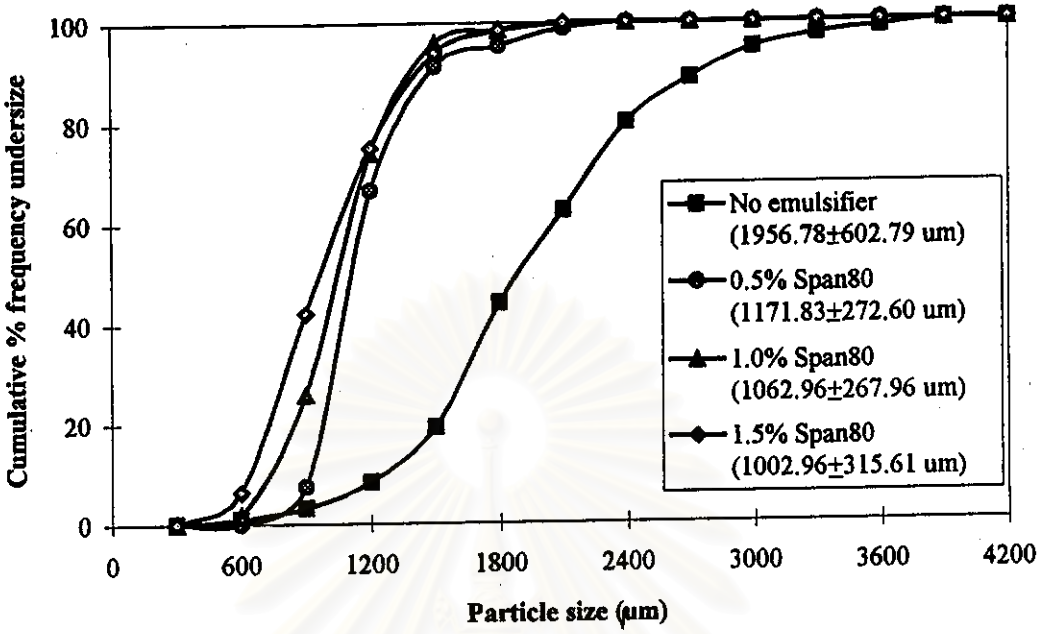


Figure 33. Cumulative % frequency undersize of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Span80 (with 6% ethylcellulose and a core to wall ratio of 1:2) prepared by the solvent evaporation technique.

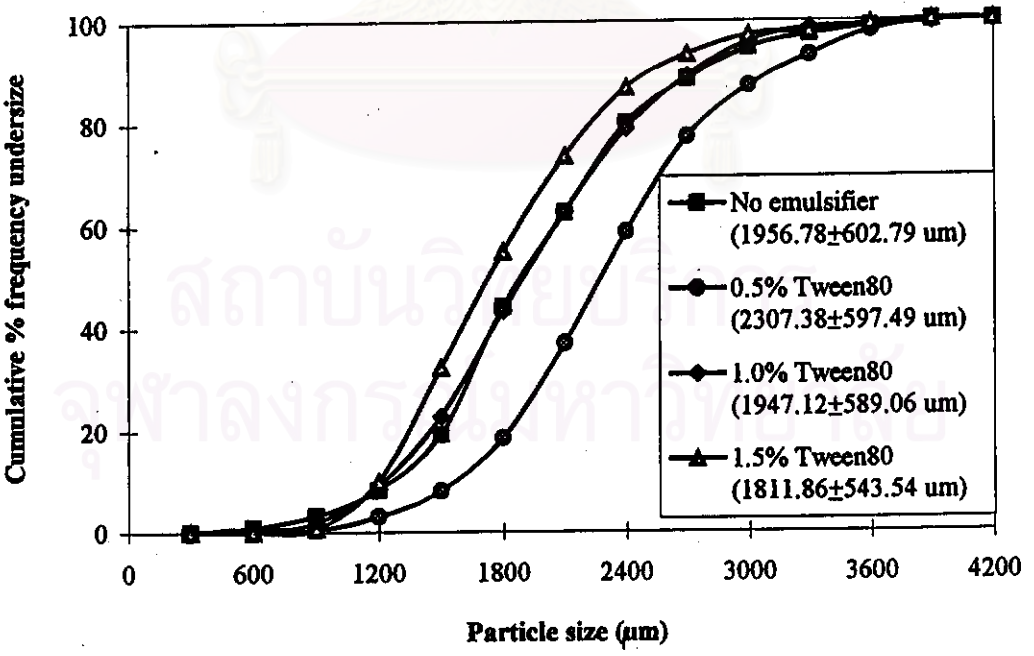


Figure 34. Cumulative % frequency undersize of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Tween80 (with 6% ethylcellulose and a core to wall ratio of 1:2) prepared by the solvent evaporation technique.

respectively. When Span80 (a surfactant with a low HLB value) was used to stabilize the non-aqueous emulsion system, the mean microcapsule size decreased slightly with increasing the concentration of Span80. This may be due to the effect of Span80 in the reduction of interfacial tension which resulted in the smaller volume of the initial droplets in the non-aqueous emulsion. When there was no Span80 inclusion, the mean microcapsule size was much larger than the mean size when Span80 was included. The aggregation of microcapsules when Span80 was not included was responsible for the large mean size. When the concentrations of Tween80 were increased from 0.5 to 1.0 and 1.0 to 1.5%, the mean sizes decreased due to less aggregation of the microcapsules. When the mean sizes of microcapsules prepared using Span80 and Tween80 were compared, the mean sizes of the microcapsules prepared using Span80 were smaller than that using Tween80 because Tween80 gave aggregate microcapsules. It could be proposed that Span80 gave a better reduction of the interfacial tension and enhancement of the stability of droplets than Tween80 did.

3.3 Yield of Microcapsules

3.3.1 Temperature Induced Coacervation Technique

The yield of ascorbic acid microcapsules prepared by the temperature induced coacervation technique is presented in table 16. This technique gave high yields (95%), and the yields of microcapsules prepared with various formulation variables were not different. Therefore, the studied core to wall ratios and plasticizers did not influence the amount of produced microcapsules.

Table 16. Yield of the ascorbic acid microcapsules prepared by the temperature induced coacervation technique.

Formulation no.	Variables		Yield (%)
	Core to wall ratio	Plasticizer (%w/w)	
2	1:1	-	95.00
3	3:2	-	95.74
4	1:1	20% TA	95.35
5	1:1	30% TA	95.68
6	1:1	20% TEC	95.47
7	1:1	30% TEC	95.37
8	1:1	20% DBS	94.89
9	1:1	30% DBS	94.92

3.3.2 Solvent Evaporation Technique

For the solvent evaporation technique, the yields of microcapsules prepared with various formulation variables are shown in figures 35-38.

As depicted in figure 35, the yield of microcapsules increased with an increase in the ethylcellulose concentration. At the same stirring speed, the low ethylcellulose concentration with an excess of solvent caused the excessive splashing which resulted in the loss of prepared microcapsules. Therefore, 6 %w/v ethylcellulose in acetone was the most suitable concentration for further evaluation of the effect of other formulation variables.

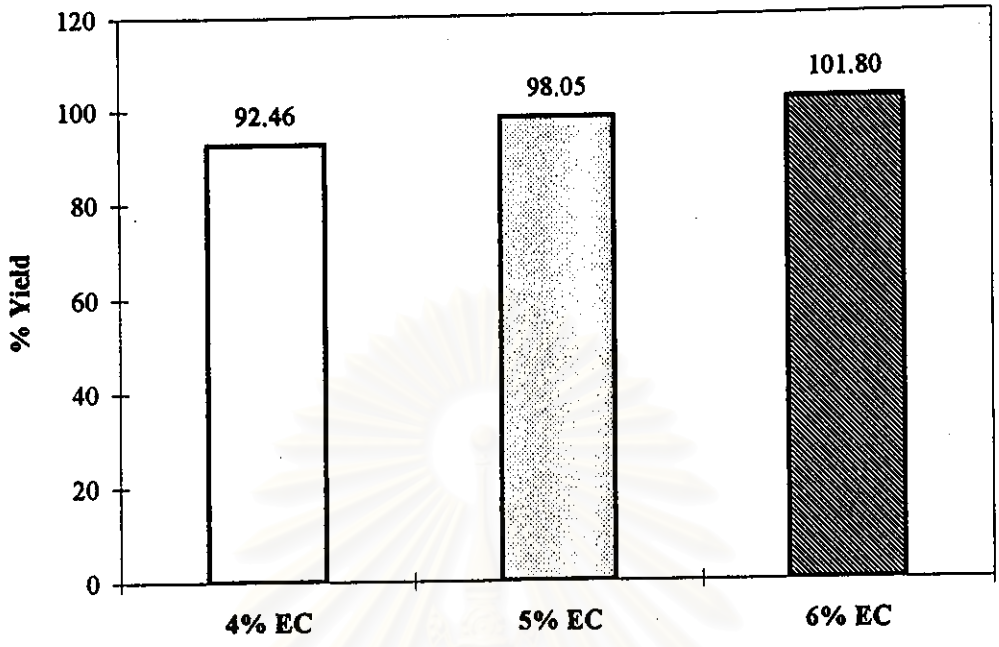


Figure 35. Percent yield of the ascorbic acid microcapsules with 4, 5, and 6% ethylcellulose (with a core to wall ratio of 1:2 and 1.0% Span80) prepared the solvent evaporation technique.

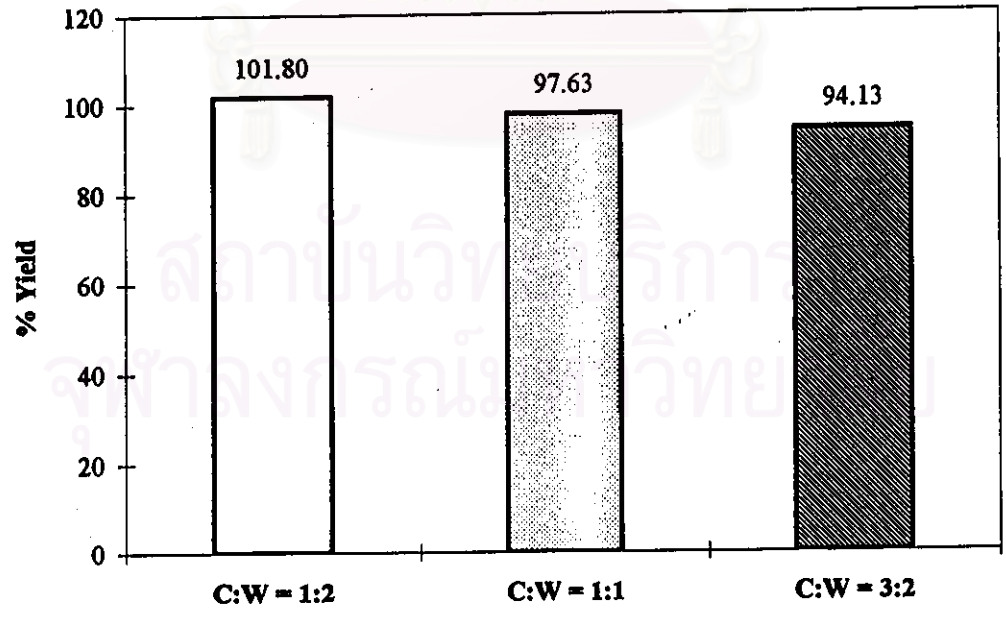


Figure 36. Percent yield of the ascorbic acid microcapsules with 1:2, 1:1, and 3:2 core to wall ratios (with 6% ethylcellulose and 1.0% Span80) prepared the solvent evaporation technique.

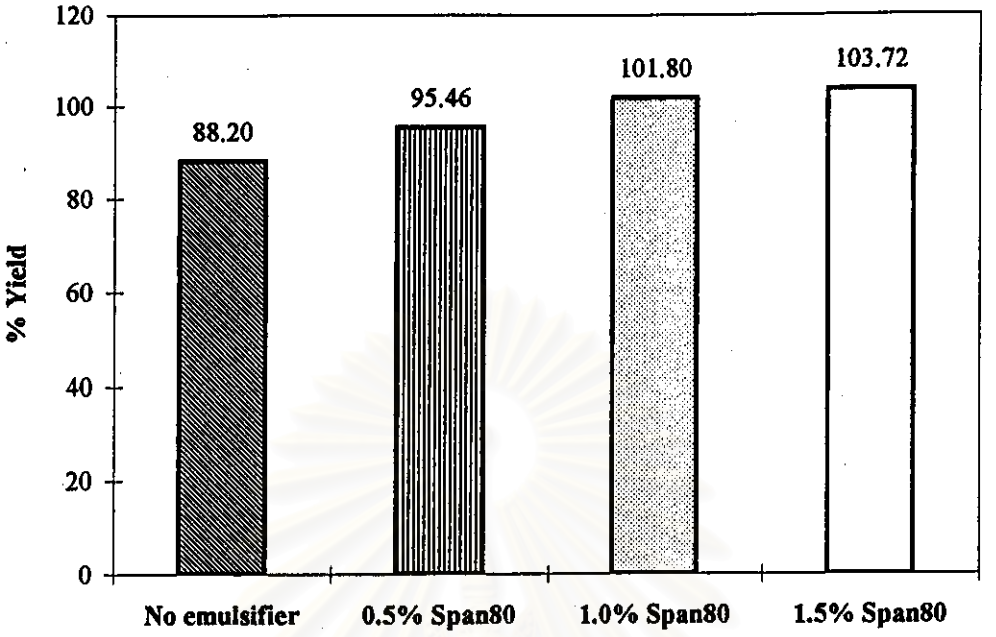


Figure 37. Percent yield of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Span80 (with 6% ethylcellulose and a core to wall ratio of 1:2) prepared the solvent evaporation technique.

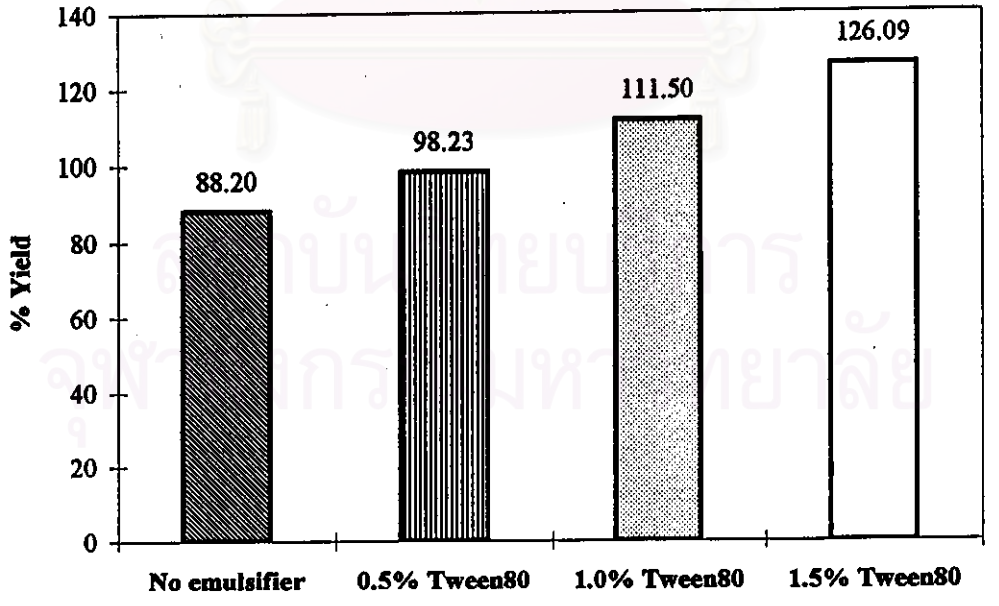


Figure 38. Percent yield of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Tween80 (with 6% ethylcellulose and a core to wall ratio of 1:2) prepared the solvent evaporation technique.

Figure 36 shows that the yield of prepared microcapsules decreased with an increase in the core to wall ratio at a constant concentration of Span80 (1.0 %w/v). The effect of core to wall ratio on the yield was not clear due to the unknown amount of emulsifier included into the microcapsules. The core to wall ratio chosen to investigate the effect of emulsifier was 1:2 since it gave spherical and smoother surface microcapsules.

From figure 37, the yield of produced microcapsules increased with increasing Span80 concentration. The positive deviations of the yield from the theoretical value (100%) were attributed to the inclusion of Span80 into the polymer matrix. When the higher amount of Span80 was added to the oil phase, the greater amount was partitioned into the microcapsules. However, it could not partition entirely into microcapsules and remained partly in the microencapsulating vehicle since Span80 is miscible with the microencapsulating vehicle (light liquid paraffin). The effect of Tween80 concentration on the yield of the microcapsules (figure 38) was similar to the effect of Span80 but Tween80 increased the yield more than Span80 did. Since Tween80 is immiscible with light liquid paraffin, the amount of Tween80 partitioned into the microcapsules was more than that of Span80.

3.4 Drug Content and Drug Entrapment of Ascorbic Acid Microcapsules

3.4.1 Temperature Induced Coacervation Technique

For the ascorbic acid microcapsules prepared by the temperature induced coacervation technique, the drug entrapment was typically 100-104% as presented in table 17. The positive deviations of the drug entrapment from the theoretical value

(100%) could be explained by the fact that some of the polymer used were lost during the stirring and in the formation of empty coacervates (microcapsules without the drug) which had been removed during the washing and decantation processes.

Table 17. Drug entrapment of the ascorbic acid microcapsules prepared by the temperature induced coacervation technique.

Formulation no.	Variables		Drug entrapment (%) (SD)
	Core to wall ratio	Plasticizer (%w/w)	
2	1:1	-	101.57 (0.70)
3	3:2	-	100.67 (0.75)
4	1:1	20% TA	100.72 (2.20)
5	1:1	30% TA	101.12 (0.56)
6	1:1	20% TEC	104.25 (1.58)
7	1:1	30% TEC	102.61 (1.60)
8	1:1	20% DBS	101.63 (1.45)
9	1:1	30% DBS	103.87 (0.18)

3.4.2 Solvent Evaporation Technique

For the solvent evaporation technique, the drug entrapment of the ascorbic acid microcapsules with various formulation variables is displayed in figures 39-42. The drug entrapment of the ascorbic acid microcapsules prepared by this technique were in the range of 55-93%. Since ascorbic acid was slightly soluble in the

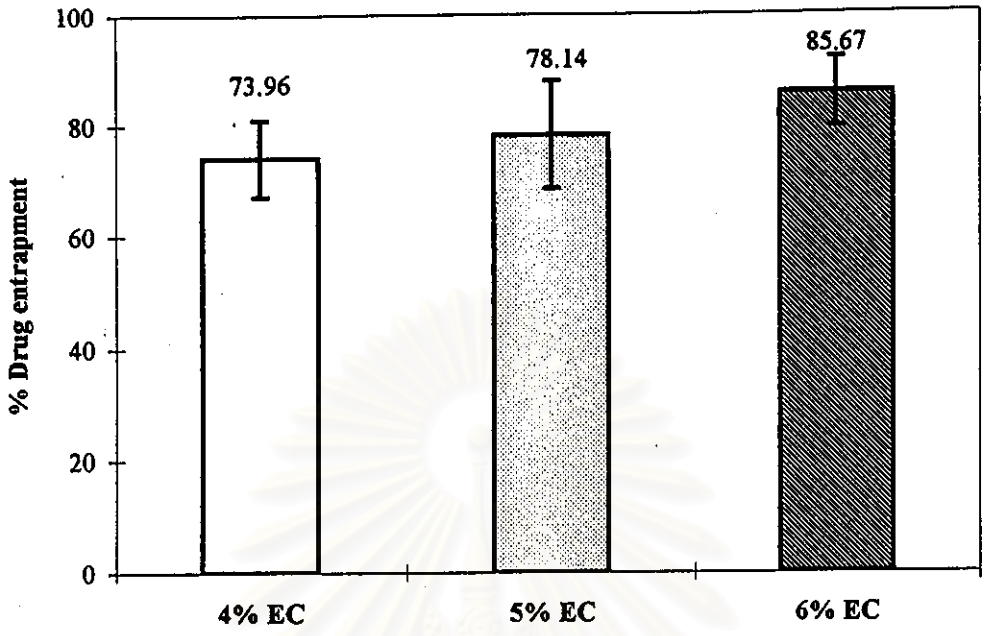


Figure 39. Drug entrapment of the ascorbic acid microcapsules with 4, 5, and 6% ethylcellulose (with a core to wall of 1:2 and 1.0% Span80) prepared by the solvent evaporation technique.

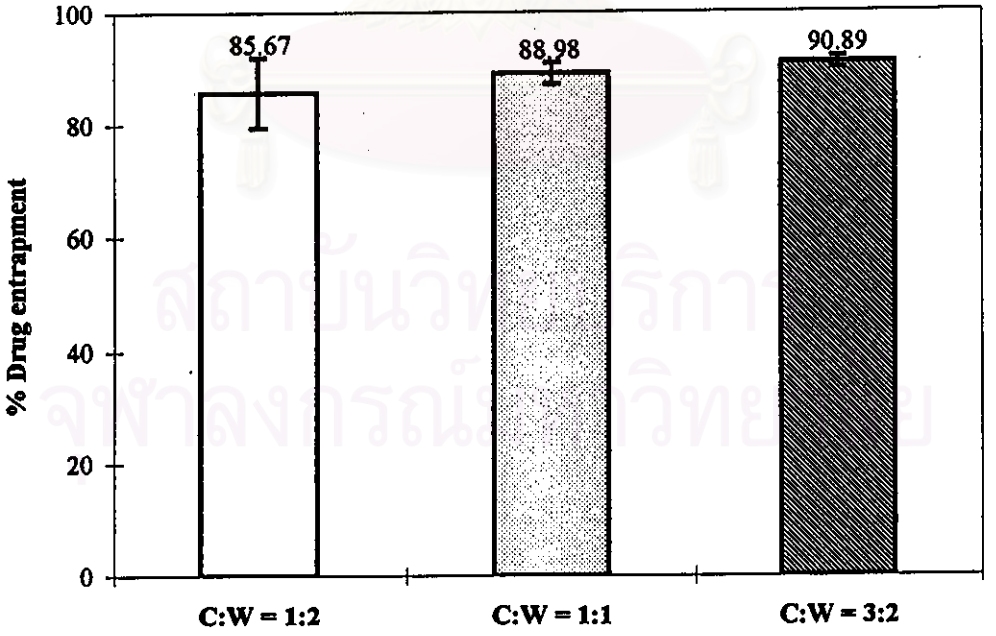


Figure 40. Drug entrapment of the ascorbic acid microcapsules with 1:2, 1:1, and 3:2 core to wall ratios (with 6% ethylcellulose and 1.0% Span80) prepared by the solvent evaporation technique.

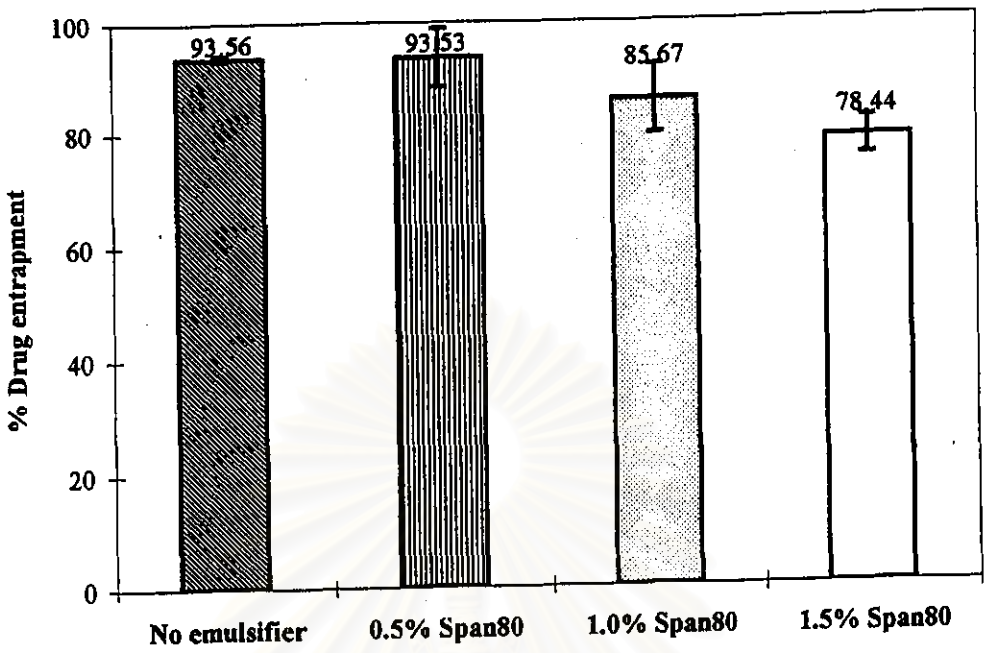


Figure 41. Drug entrapment of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Span80 (with 6% ethycellulose and a core to wall ratio of 1:2) prepared by the solvent evaporation technique.

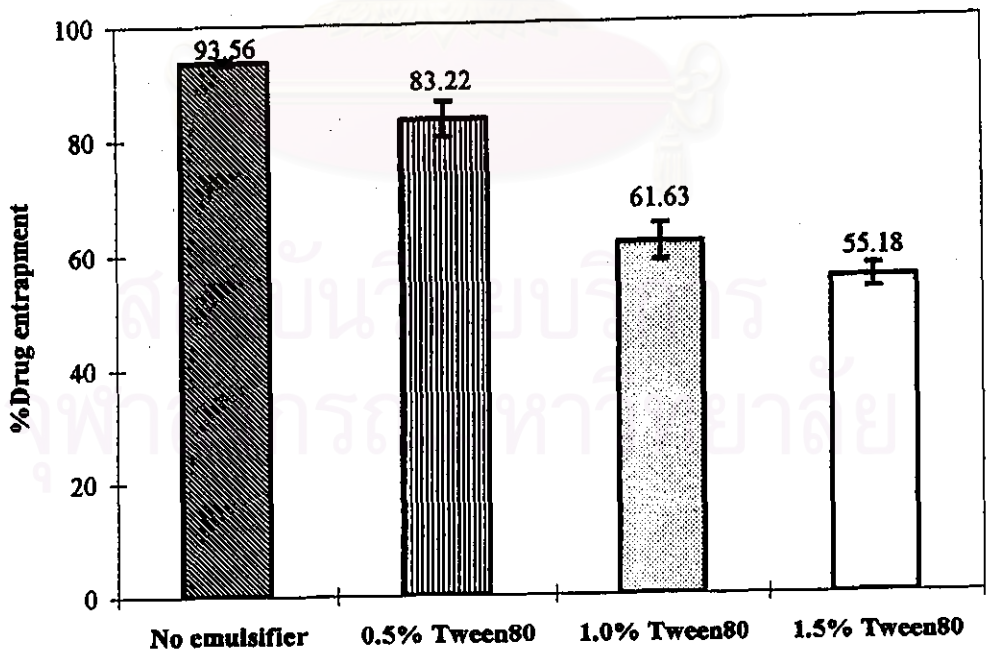


Figure 42. Drug entrapment of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Tween80 (with 6% ethycellulose and a core to wall ratio of 1:2) prepared by the solvent evaporation technique.

polymer solution, some drug was lost when this mixture was poured into the mineral oil phase and subsequently emulsified. The other reason was that the theoretical drug content was calculated by multiplying 100 with the ratio of the amount of drug to the amount of drug plus polymer (eq. 11); the emulsifier weight was not included in the denominator. This calculation would give a high theoretical content value. In practice, however, the unknown amount of emulsifier could be included in the microcapsules which resulted in the higher amount of the prepared microcaspule in eq. (9) than the amount of drug plus polymer in eq. (11). This caused the lower observed drug content of the microcapsules than the theoretical content which resulted in the lower drug entrapment.

Figure 39 shows the increases in drug entrapment with the increments of ethylcellulose concentration. If the theoretical drug content was calculated by including the amount of emulsifier in the denominator of eq. (11), the theoretical contents of microcapsules with 4, 5, and 6% EC would be 22.86, 24.39, and 25.53%, respectively and the drug entrapments would therefore be 107.84%, 106.77%, and 111.84% for 4%, 5%, and 6% EC, respectively. However, the highest polymer concentration (6% EC) gave the greatest drug entrapment because the precipitation of the polymer at the droplet surface and the transition from liquid droplets to solidified microcapsules occurred more rapidly with more concentrated systems. The drug loss across the solidified microcapsule surface was therefore impeded (Bodmeier et al., 1994). This also resulted in an increase in yield as 6% ethylcellulose was used.

The effect of the core to wall ratio on drug entrapment was similar to the effect of the ethylcellulose concentration (figure 40). The drug entrapment increased with increasing the core to wall ratio. If the theoretical drug content was calculated by including the amount of emulsifier, the drug entrapment of the microcapsules would

decrease with increasing the core to wall ratio (111.84%, 109.37%, and 107.55% for core to wall ratio of 1:2, 1:1, and 3:2, respectively). Since the amount of emulsifier included in the microcapsules was unknown, the effect of the core to wall ratio on drug entrapment was not clear.

Figure 41 shows that the ascorbic acid entrapment of the microcapsules decreased with increasing the Span80 concentration in the oil phase. The effect of Span80 concentration on the yield of microcapsules indicated that the higher Span80 concentration, the greater amount of Span80 included into the microcapsules which resulted in the lower drug content and drug entrapment. The other reason for reduction in the drug entrapment was the loss of drug during the processing. The effect of Tween80 concentration on the drug entrapment of the microcapsules (figure 42) was similar to the effect of Span80 but Tween80 decreased the drug entrapment more than Span80 did because the amount of Tween80 partitioned into the microcapsules was more than that of Span80 as discussed in 3.3.2. Tween80 enhanced the solubilities of ascorbic acid and acetone in the oil phase resulting in a more favorable partitioning of the drug into the oil phase and hence lowering drug contents with increasing the Tween80 concentrations.

3.5 Release Characteristics

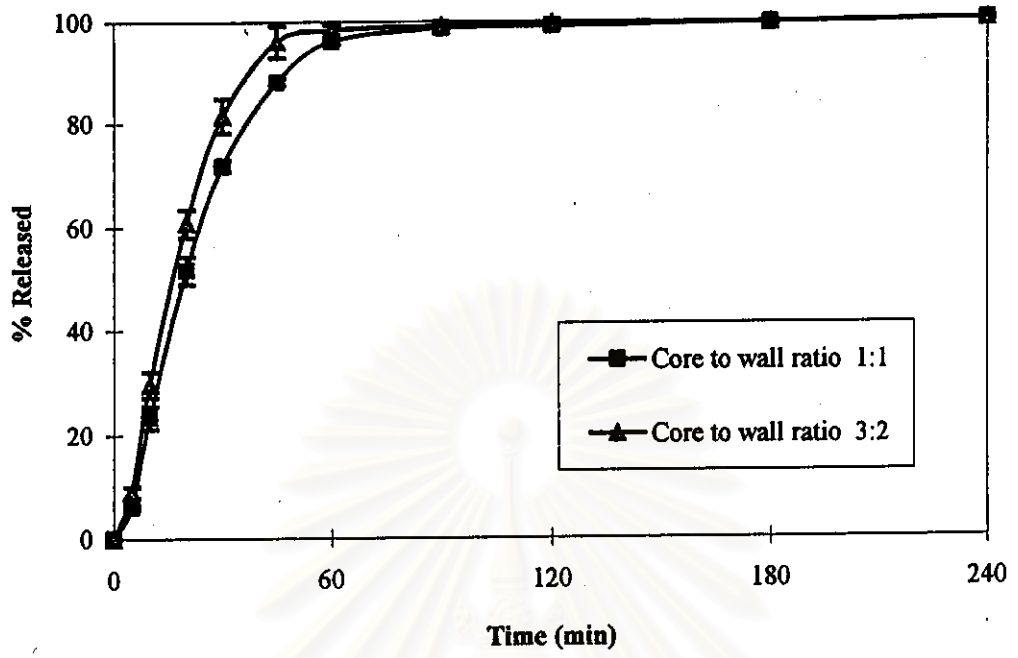
Microencapsulation by both temperature induced coacervation and solvent evaporation techniques yielded matrix-type products. Water-soluble drugs permeate through ethylcellulose microcapsules mainly by diffusion through water filled pores or water channels existing in the microcapsule membrane (Deasy, 1984; Koida et al., 1987). These pores are filled with solution when the microcapsules come into contact with an aqueous medium; the liquid penetrates into the microcapsules through the

pores and dissolves the drug which then diffuses out into the bulk solution (Washington, 1990). The Higuchi equation is a good fitting equation to define the release of drug from ethylcellulose microcapsules (Jalsensak et al., 1976, 1980; Chemtob, Chaumeil, and N'Dongo, 1986). The release rate of drug from microcapsules was influenced by the formulation variables. The assessment of formulation variables affecting drug release from the microcapsules could be investigated by comparing the slopes or release rate constants (K) of the plots of the percentage of drug released versus square root of time (Higuchi plot) as presented in Appendix II. Analysis of Covariance (ANCOVA) was used for comparing the drug release rates from the formulations studied and performed by using SPSS 7.5 program (Bryman and Carmer, 1997; SPSS® advance statistics 7.5, 1997). A null hypothesis, i.e. there is no difference in the release rates compared, was tested against an alternative hypothesis, i.e. at least one pair of the release rates is not equal. The results are presented in Appendix III.

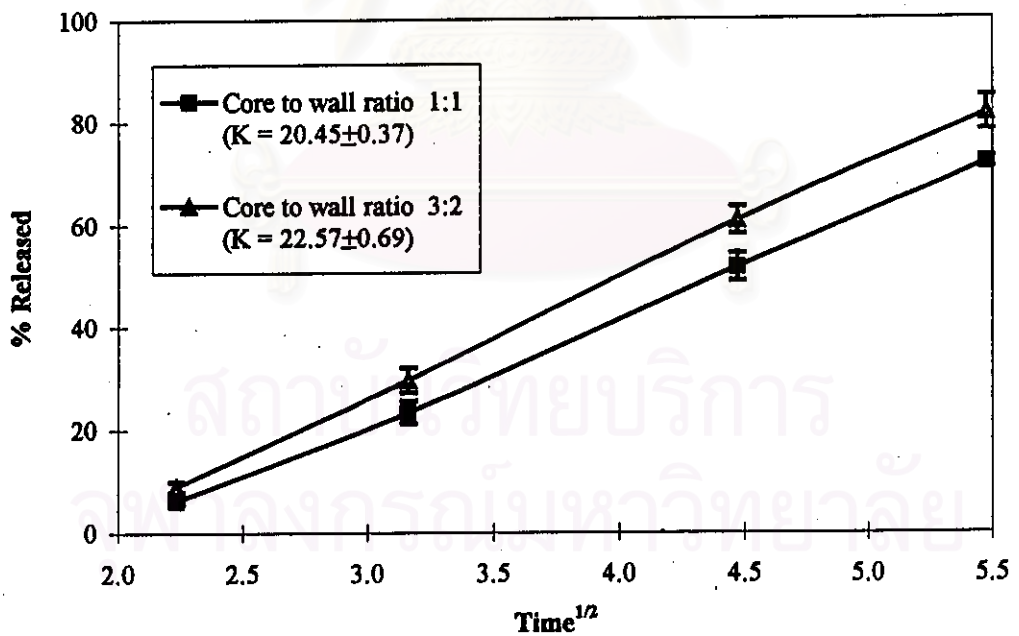
3.5.1 Temperature Induced Coacervation Technique

For the temperature induced coacervation technique, the Higuchi model provides a good fit to the release data of the microcapsules with a correlation coefficient of more than 0.99 during a time period of 5-30 min. The effect of core to wall ratio on drug release from the microcapsules is illustrated in figure 43. The release rate of ascorbic acid from the microcapsules increases significantly with increasing the core to wall ratio ($P = 0.019$) since the greater core to wall ratio yielded a thinner film coating of polymer on the drug particles.

The effects of amount of triacetin, triethyl citrate, and dibutyl sebacate on the drug release from the microcapsules are depicted in figures 44, 45, and 46,

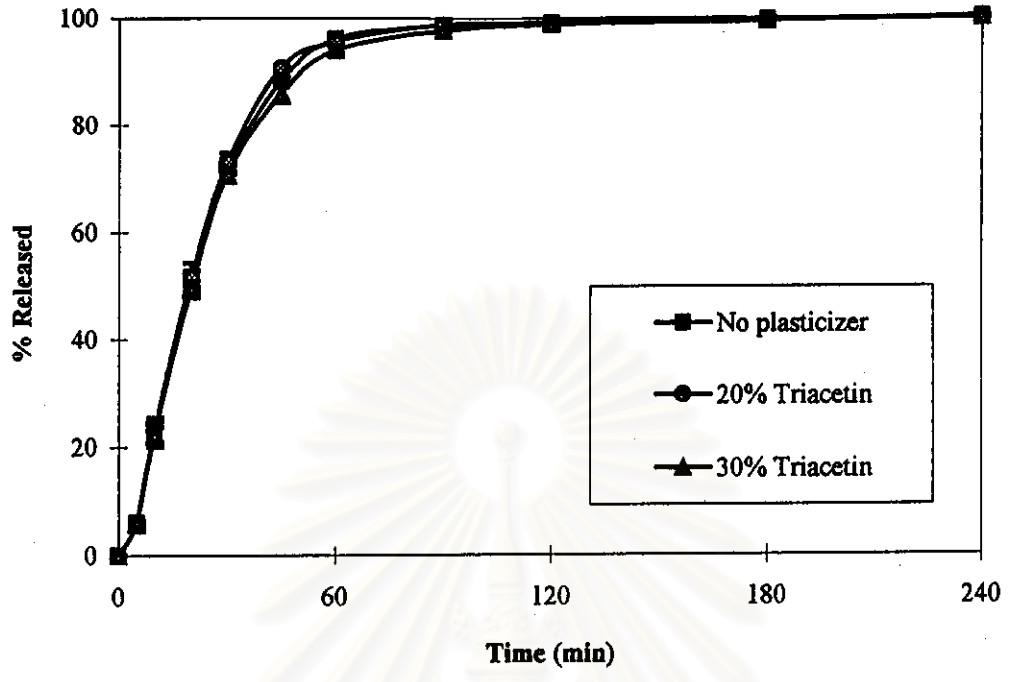


(a)

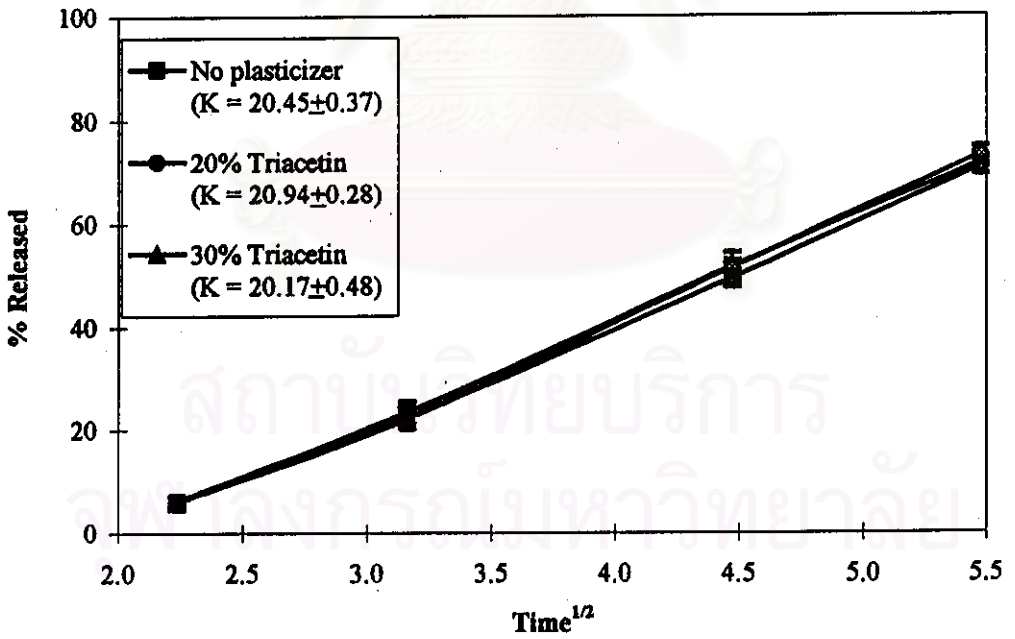


(b)

Figure 43. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 1:1 and 3:2 core to wall ratios prepared by temperature induced coacervation technique.

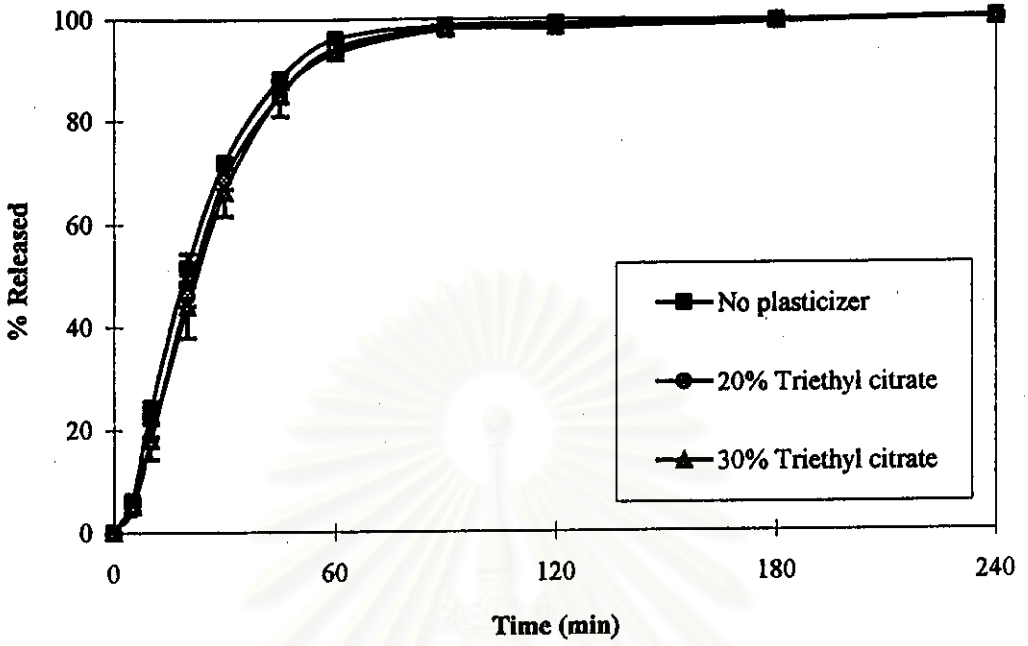


(a)

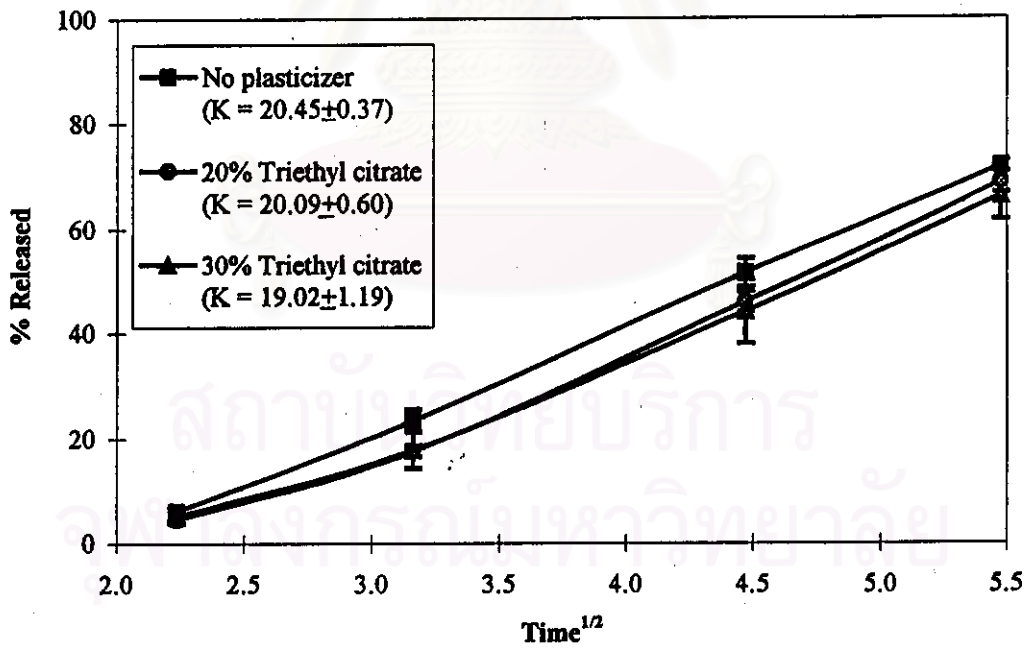


(b)

Figure 44. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 0, 20, and 30% triacetin (with a core to wall ratio of 1:1) prepared by temperature induced coacervation technique.

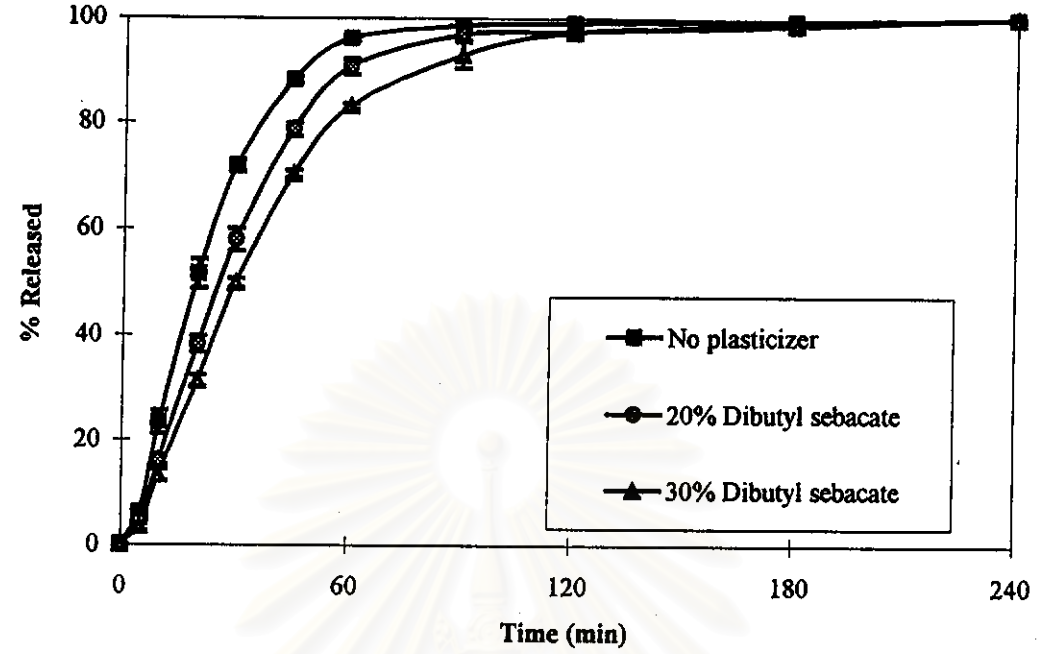


(a)

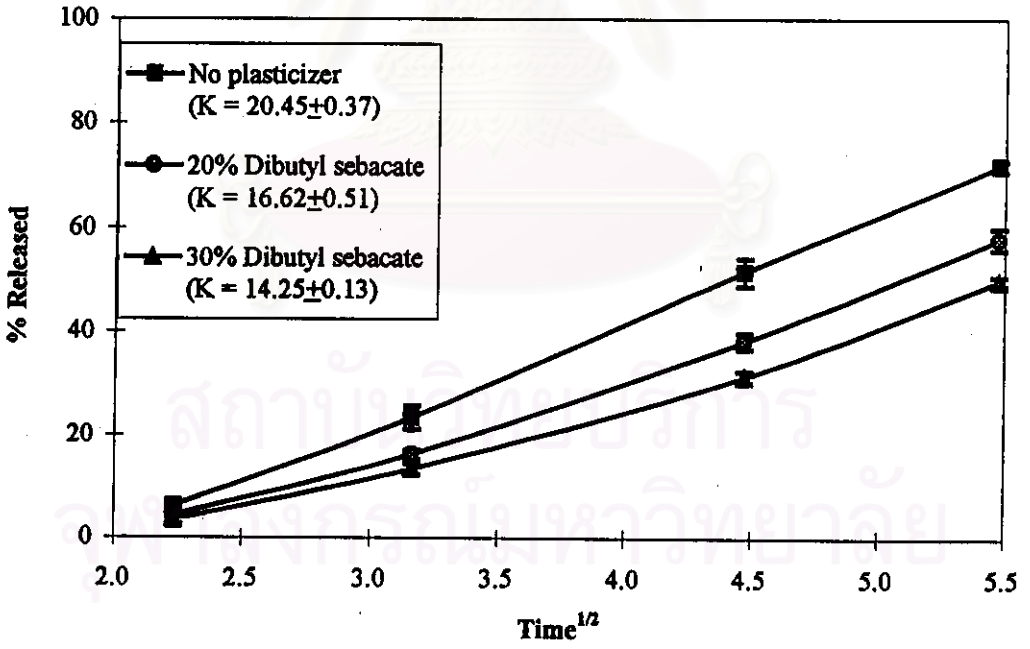


(b)

Figure 45. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 0, 20, and 30% triethyl citrate (with a core to wall ratio of 1:1) prepared by temperature induced coacervation technique.



(a)



(b)

Figure 46. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 0, 20, and 30% dibutyl sebacate (with a core to wall ratio of 1:1) prepared by temperature induced coacervation technique.

respectively. The amounts of TA (P = 0.573) and TEC (P = 0.598) do not affect the drug release rate significantly. The P-value of 0.005 indicates the statistical significance of the drug release rates from microcapsules prepared using 0, 20, and 30% DBS. With its poor water solubility and long-chain molecules (see Appendix I), DBS penetrates the polymer chains better than the more spherical TEC and branched TA which can be soluble partly in water (Hyppola, Husson, and Sundholm, 1996). As plasticizers interpose itself between the polymer chains and interact with the forces which hold the chains together, a secondary effect of these compounds is that they alter the permeability of the coating (Deasy, 1984). The water permeability through the coating is related to plasticizer solubility in water (Banker, 1966). A soluble plasticizer tends to make the coating more permeable to aqueous media and an insoluble plasticizer limits water permeation through the film which is required to produce an enteric or slow release coating. Water vapor permeability has also been shown to be dependent on the relative polarity of the polymer. The less polar films have less affinity for moisture and water sorption. Consequently, dibutyl sebacate in the amount of 30% by weight of polymer was the most efficient plasticizer to produce the slow release ethylcellulose microcapsules. This result is consistent with previous reports described by McGinity (1989) and Hyppola et al. (1996).

An incorporation of a plasticizer is necessary to obtain an effective coating without defects such as cracks, edging or splitting. The decrease in the incidence of cracking causes a decrease in permeability. Plasticizers are added to polymeric solutions to increase the flexibility or distensibility, improve flow, and reduce brittleness of the polymeric material. These effects are caused by a decrease in the cumulative intermolecular forces along the polymer chains (reduction in cohesion) which generally decreases tensile strength, lowers the softening temperature, and decreases the glass transition temperature (Banker, 1966; McGinity, 1989). The

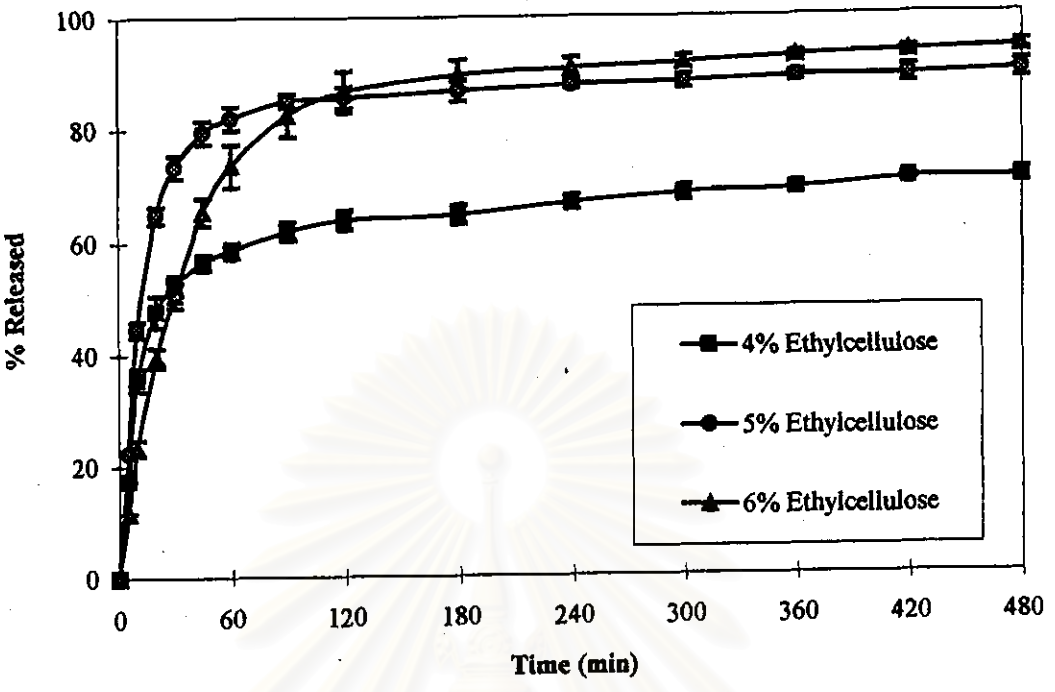
degree of plasticization of a polymer is dependent to a large extent on the amount of plasticizer in the film and the interactions between the plasticizer and the polymer. For a plasticizer to be effective, the plasticizer must be able to diffuse and interact between the polymer chains and has minimal or no tendency for migration, exudation, or volatility (Gutierrez-Rocca and McGinity, 1994).

3.5.2 Solvent Evaporation Technique

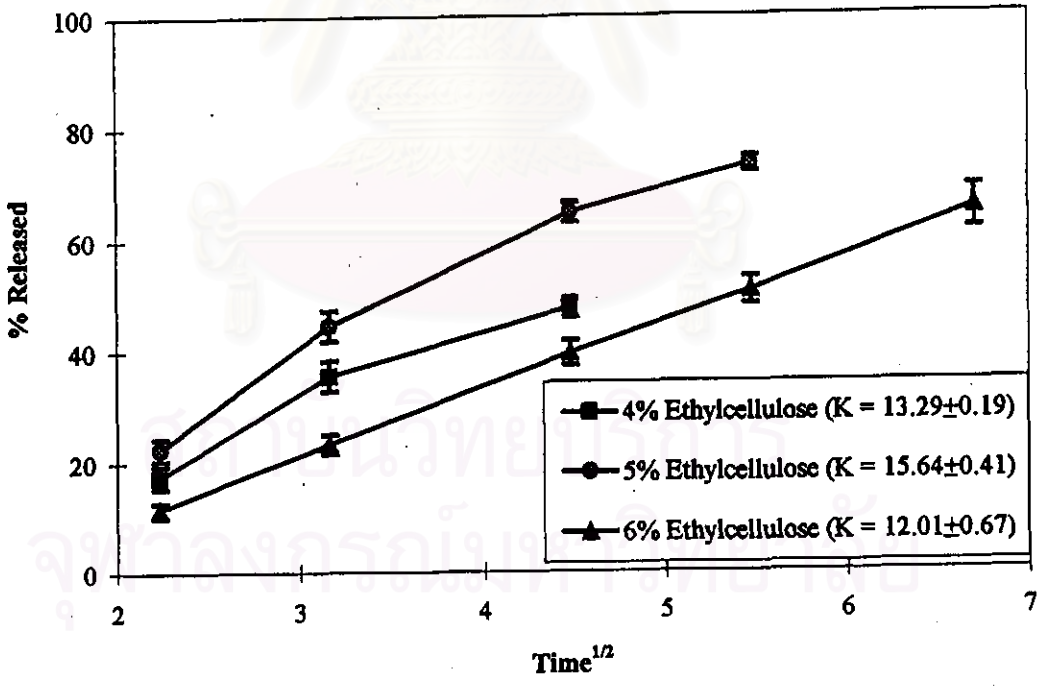
The release profiles of most formulations prepared by the solvent evaporation technique were linear. A time period providing a linear Higuchi plot increased with decreasing the core to wall ratio (figure 48(b)), decreasing the amount of Span80 (figure 49(b)), and increasing the amount of Tween80 (figure 50(b)).

The effect of ethylcellulose concentration on drug release from the microcapsules is illustrated in figure 47. Five percent ethylcellulose concentration gave higher release rate than 6% ethylcellulosee. Since the lower concentration caused a decrease in viscosity of internal phase, the solvent evaporated more rapidly which resulted in the more defects. However, 4% EC concentration gave lower initial release rate than 5% and lower %drug released than 5% and 6% EC. The lower release rate and % released may be due to aggregation of the microcapsules prepared with 4% EC which resulted in larger size. However, the difference between the release rate constants of the microcapsules prepared with various ethylcellulose concentrations is not statistically significant (P = 0.211).

Figure 48 shows the effect of core to wall ratio on the release characteristics of the ascorbic acid microcapsules. Ascorbic acid released from the microcapsules with a core to wall ratio of 3:2 was faster than those with core to wall

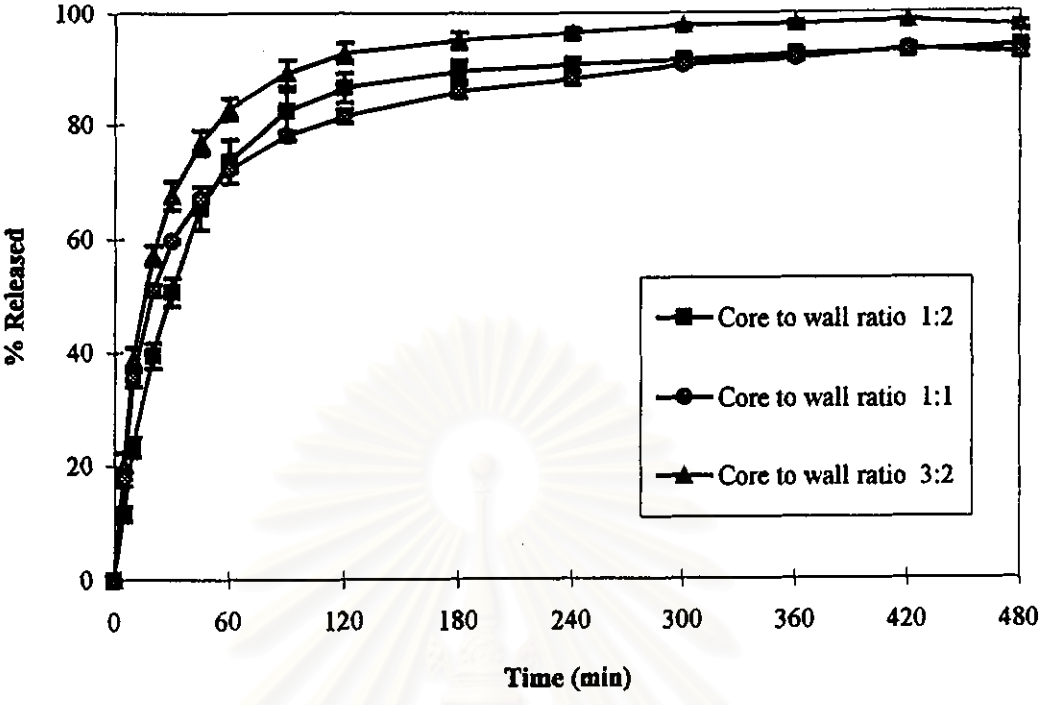


(a)

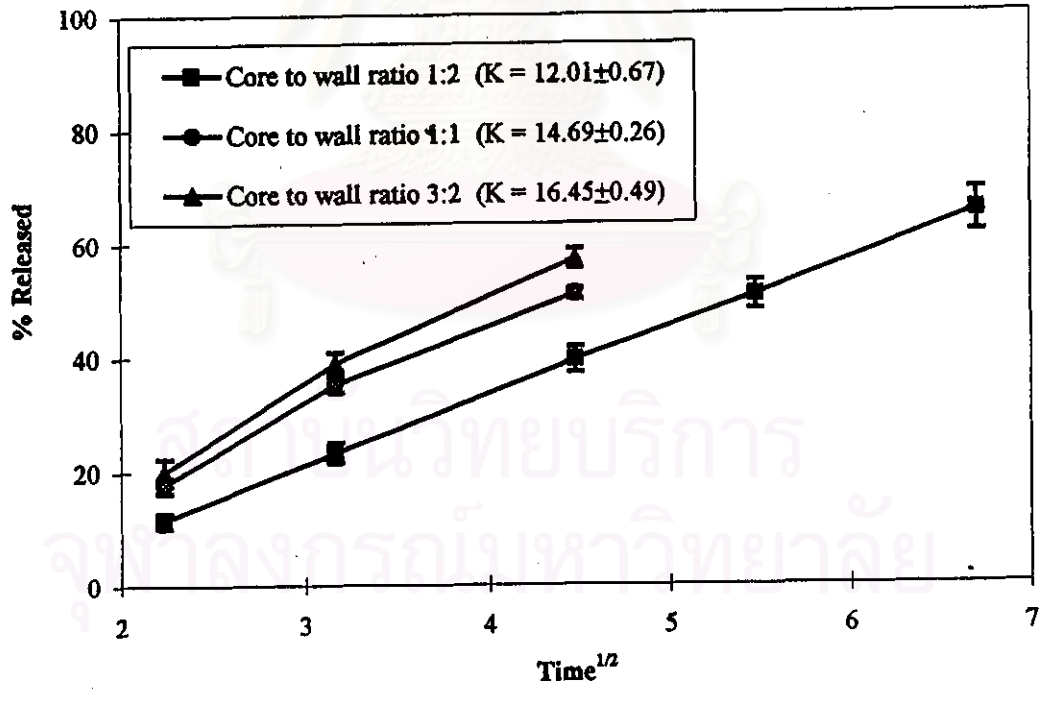


(b)

Figure 47. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 4, 5, and 6% ethylcellulose (with a core to wall ratio of 1:2 and 1.0% Span80) prepared by the solvent evaporation technique.

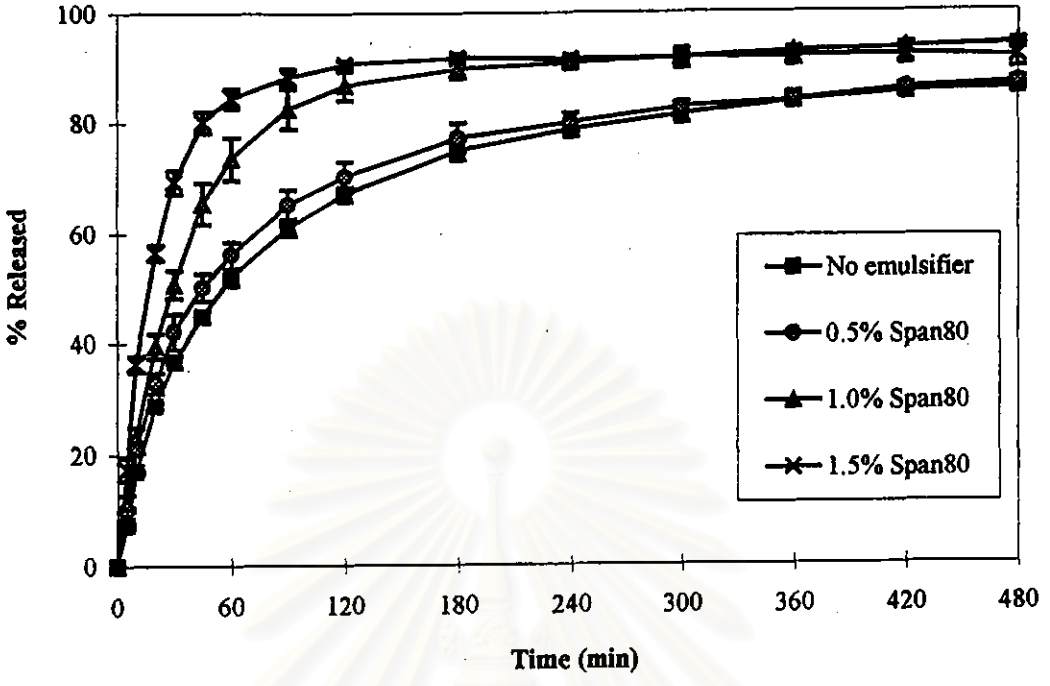


(a)

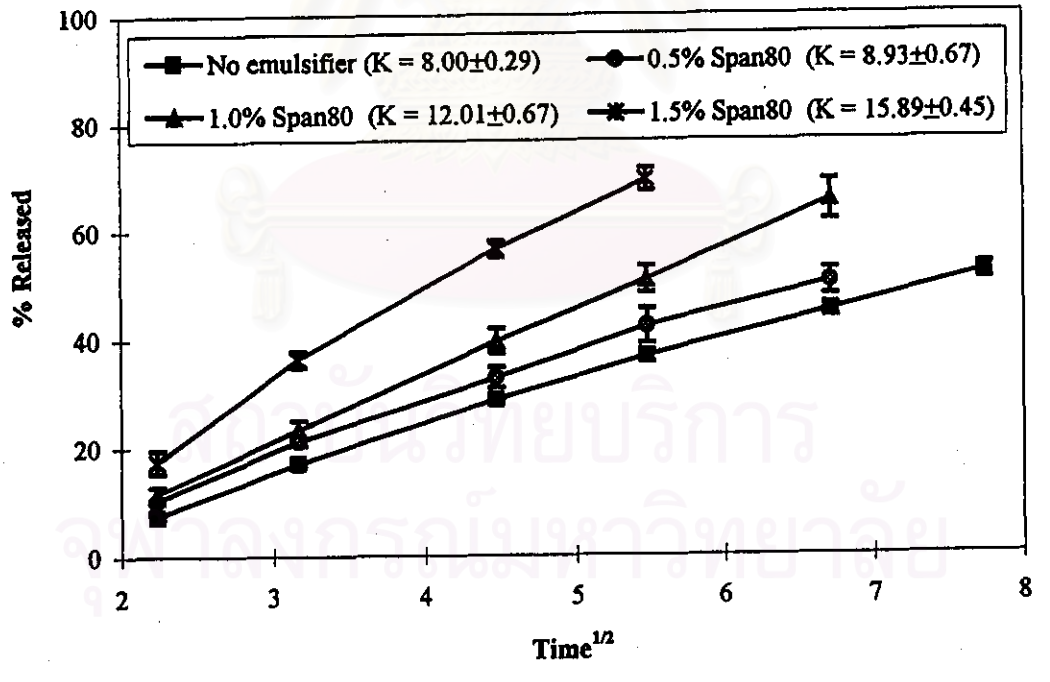


(b)

Figure 48. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 1:2, 1:1, and 3:2 core to wall ratios (with 6% ethylcellulose and 1.0% Span80) prepared by the solvent evaporation technique.

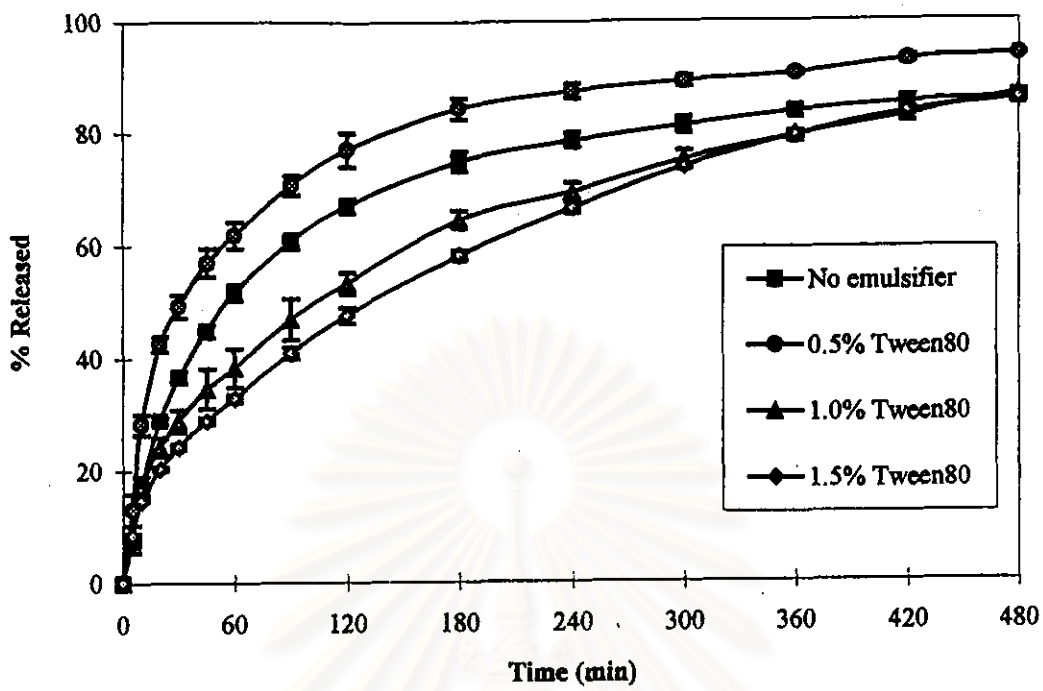


(a)

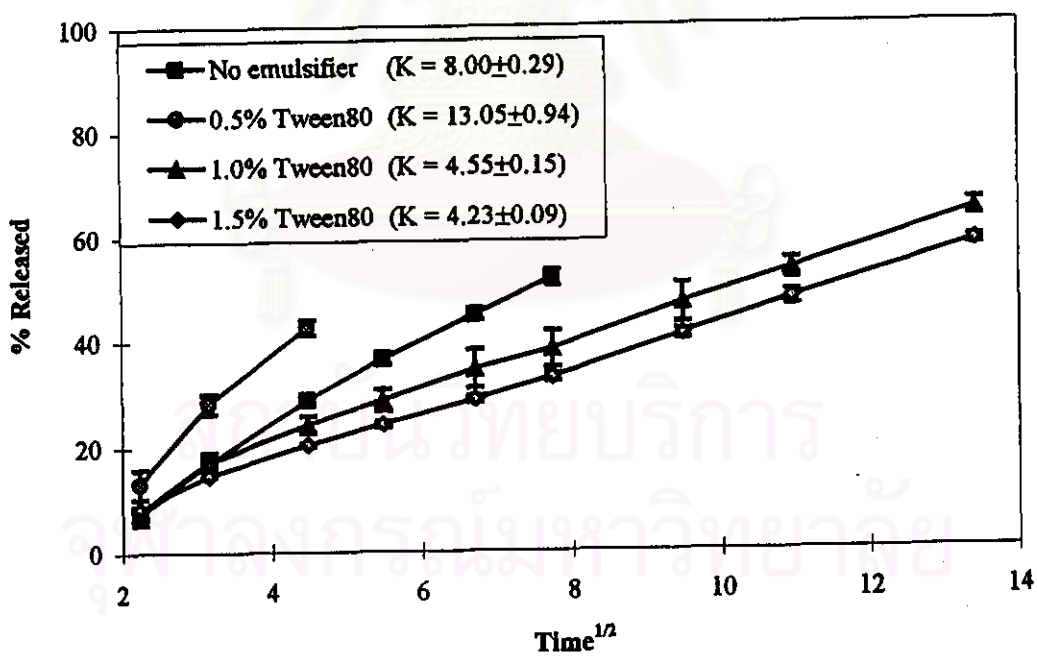


(b)

Figure 49. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Span80 (with 6% ethylcellulose and a core to wall ratio of 1:2) prepared by the solvent evaporation technique.



(a)

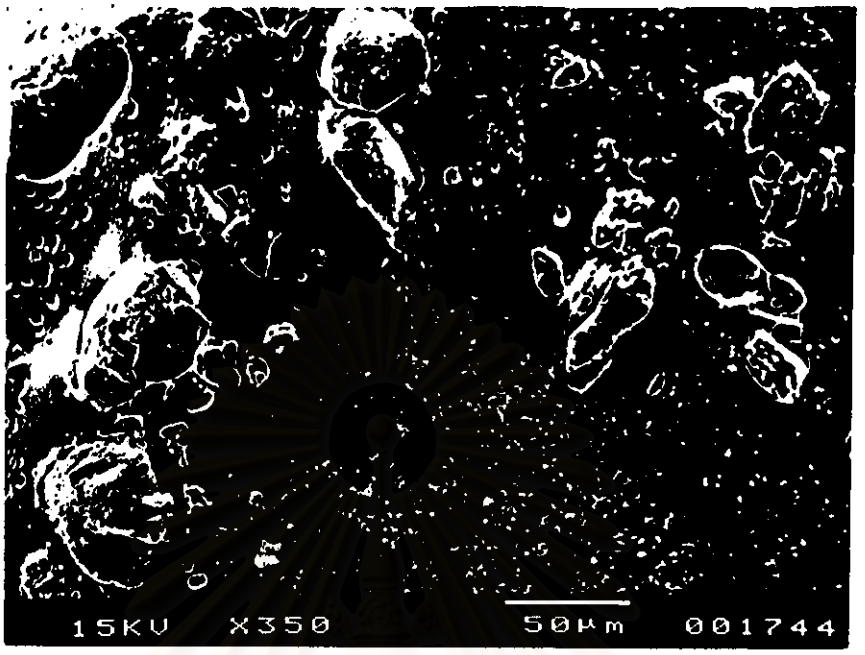


(b)

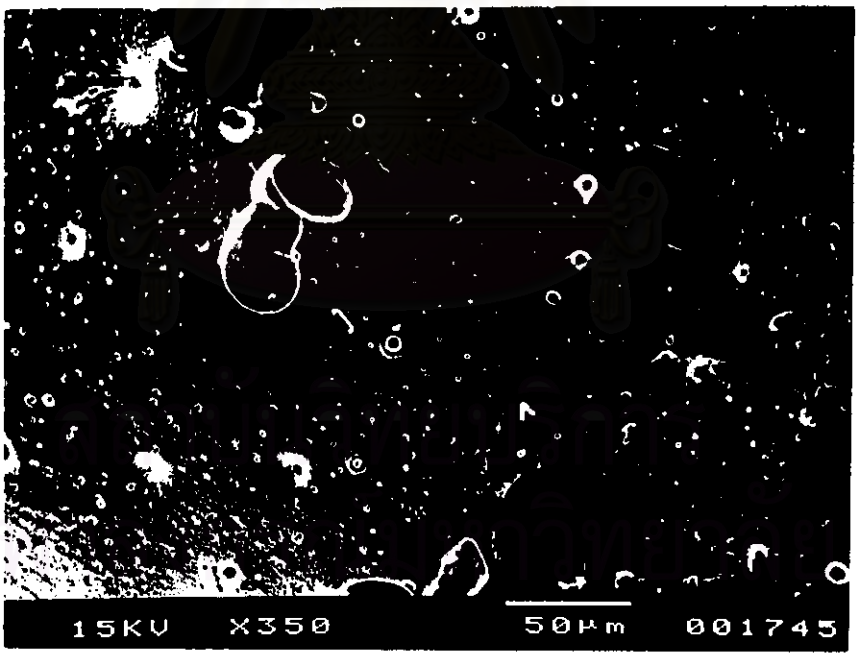
Figure 50. Release profiles (a) and Higuchi release plots (b) of the ascorbic acid microcapsules with 0, 0.5, 1.0, and 1.5% Tween80 (with 6% ethylcellulose and a core to wall ratio of 1:2) prepared by the solvent evaporation technique.

ratios of 1:1 and 1:2. The higher release rate was due to the thinner coating of polymer. Their release rates were also correlated with the presence of the free drug crystals embedded in the surface of the microcapsules, as shown in figure 24(c)-(e). The dissolution of the drug crystals created many pores which were easily accessible to solvent (figure 51). Therefore, an increase in the core to wall ratio not only reduced the relative amount of polymer material as a diffusional barrier but also increased the pores of the film coating. This is consistent with a previous study of Bodmeier and McGinity (1987b) on poly(dl-lactide) microspheres.

Figure 49 depicts the effect of Span80 concentration on the release characteristic of the ascorbic acid microcapsules prepared by the solvent evaporation technique with a constant core to wall ratio of 1:2. The drug release rate increased with increasing the Span80 concentration. The release rate constant of microcapsules prepared without emulsifier was close to that with 0.5% Span80. The microcapsules prepared without emulsifier showed slightly slower release effect due to the larger sizes of aggregate microcapsules as shown in figure 24(f). The increase in release rate constants associated with the presence of drug crystals on the surface of the microcapsules were observed. The amounts of drug crystals on the microcapsule surface were increased with increasing the concentrations of Span80 (figures 24(c),(g), and (h)). This result is consistent with previous studies reported by Bodmeier et al. (1994) and Radwan, Price, and Tackett (1995). The increase in the amount of drug crystals on the microcapsule surface may be a result of drug solubility decreased by the emulsifier. Since the HLB value of Span80 is lower than light liquid paraffin, an increase in amount of Span80 would result in a reduction of polarity of the system and this caused a decrease in drug solubility.



(a)



(b)

Figure 51. Scanning electron micrographs of the ascorbic acid microcapsule with a core to wall ratio of 1:1 and 1.0% Span80 before the release study (a), and after the release study (b). Magnification 350x. Scale bar 50 µm.

Mechanism of the growth of drug crystals on the microcapsule surface was described by Dubernet, Benoit, and Puisieux (1991). The presence of drug crystals on the surface of microcapsules prepared by the solvent evaporation method is explained by the formation of nuclei in a non-stirred layer surrounding the emulsified droplets during solvent evaporation and they stick onto the surface of semi-solid microcapsules at the end of the process. The ability of droplets to adsorb crystals without dissolving them will be more pronounced if the organic phase is initially very concentrated with drug. In this case, the sticking of crystals may occur at an earlier stage because the formation of crystal nuclei in the non-stirred layer occurs earlier than with lower drug content, when the polymer medium at the interface is still fluid.

The effect of Tween80 concentration on the release characteristic of the microcapsules is displayed in figure 50. The increasing of Tween80 concentrations resulted in slower release rates of ascorbic acid. However, the microcapsules with the lowest Tween80 concentration (0.5%) gave faster release rate than those without emulsifier. Since the larger microcapsules with 0.5% Tween80 were aggregates of smaller microcapsules and Tween80 is miscible with water, the microcapsules prepared with 0.5% Tween80 had faster release rates. The release rate of ascorbic acid decreased when Tween80 was added in a greater amount because higher concentrations of Tween80 yielded the microcapsules with dense internal structure as shown in figure 25.

4. Chemical Stability of Ascorbic Acid Microcapsules

The microcapsule formulations that showed low release rates were chosen for further study of the stability of ascorbic acid in microcapsules. The degradation rate

constants were also compared with the degradation rate constant of ascorbic acid powders which were stored under the same condition (40°C, 75% R.H.) for 5 months. In this study, the formulations chosen were the microcapsules with 30% DBS prepared by the temperature induced coacervation technique (formulation no. 9), the microcapsules with 0.5% Span80 (formulation no. 16) and 1.5% Tween80 (formulation no. 20) prepared by the solvent evaporation technique.

The percentage of ascorbic acid remaining in the microcapsules was plotted against time (days) as illustrated in figure 52. Since the drug degradation during the time period studied was very slight, the zero order degradation rate constant was used to compare the stability of ascorbic acid in the powder form and in the formulations studied. Since the values of correlation coefficient of the rate constant of formulation no. 9 and 16 are not satisfactory, the k values obtained are not certain (table 18). However, the plots show similar degradation of ascorbic acid from the dry powder form, formulation no. 9, and formulation no. 16. It was assured that ascorbic acid in the microcapsules with 1.5% Tween80 degraded the fastest. Tween80 is a surfactant which is miscible with water, and it may also directly promote sorption of water resulting in the rapid degradation of ascorbic acid which is a moisture-sensitive drug. The stability data shown in Appendix II indicate that the microencapsulation by temperature induced coacervation technique with suitable plasticizer (30% DBS) might improve stability of ascorbic acid. If the stability of ascorbic acid were studied at a longer period of time, a significant effect of microencapsulation might be concluded.

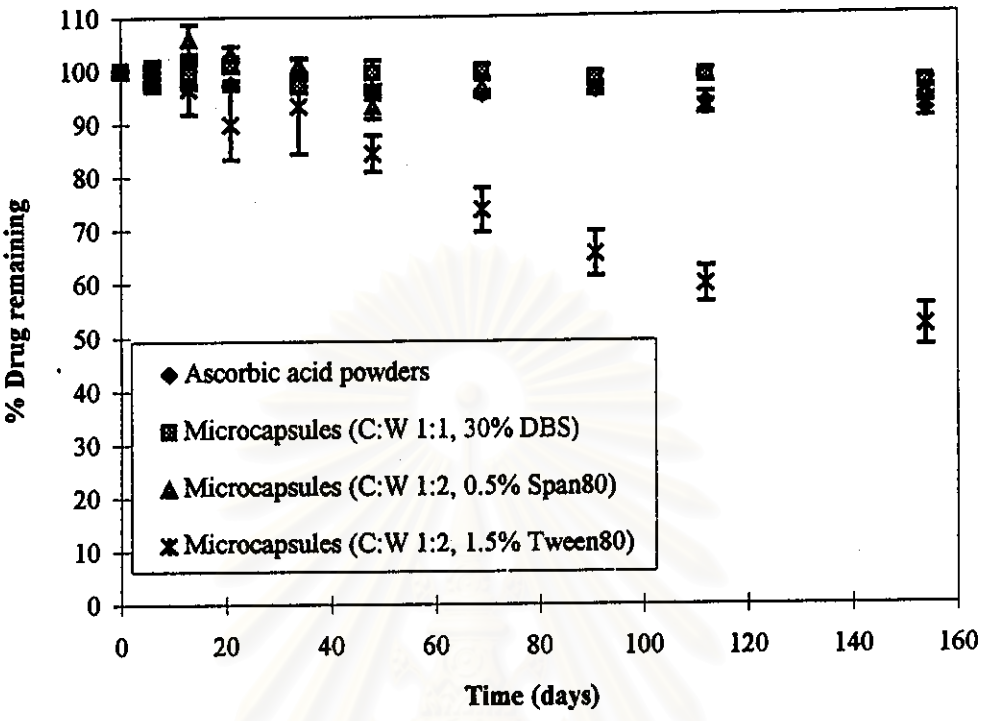


Figure 52. Zero order plot of ascorbic acid degradation.

Table 18. Zero order degradation rate constant (k) of ascorbic acid.

Formulation	k (% days^{-1})	r
Ascorbic acid powder form	0.052	0.9452
Formulation no. 9	0.012	0.4739
Formulation no. 16	0.058	0.4697
Formulation no. 20	0.340	0.9717