CHAPTER IV
RESULTS AND DISCUSSION

4.1 Physical Properties of Andrographolide Microcapsules

Visual and microscopic characteristics of andrographolide microcapsules are summarized in Table 4-1. The results indicate that the microcapsules, which were prepared by acacia (formulations no. 1, 9, 12, and 13), resulted in a grey powder-like form as shown in Figure 4-1 and the rest (formulations no. 7, 8, 10 and 14) occurred as an aggregate small granule-like form. The microcapsules, which were prepared using sodium alginate and xanthan gum (formulations no. 2 and 5, respectively), appearing as a sheet-like form.

The scanning electron micrograph of andrographolide is shown in Figure 4-2. It was rectangular-shaped crystal. The lower core to wall ratios (1:1 and 1:2) gave a smoother surface due to the excess of wall material in the systems. However, the microencapsulation of andrographolide was rectangular-shaped particles in all cases (formulations no. 1, 9, 12 and 13). The results on the morphology of microcapsules under SEM are shown in Figures 4-3, 4-4, 4-5 and 4-6.

Table 4-1 Summary of physical properties of andrographolide microcapsules

Fomulation no.	Visual appearance		Microscopic morphology
	Color	Observation	
1	Grey	powder-like	rectangular
2	White	sheet-like	irregular
3	Grey	aggregate	irregular
4	*	*	*
5	Grey	sheet-like	irregular
6	Grey	aggregate	irregular
7	Grey	aggregate granule-like	rectangular
8	Grey	aggregate granule-like	rectangular
9	Grey	powder-like	rectangular
10	Grey	aggregate granule-like	rectangular
11	*	*	*
12	Grey	powder-like	rectangular
13	Grey	powder-like	rectangular
14	Grey	small granule-like	rectangular

* The microcapsules could not be obtained.

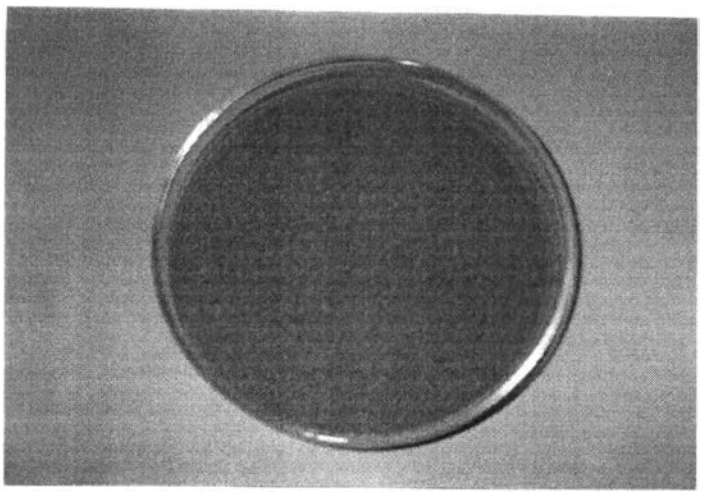

Figure 4-1 Photograph of grey powder-like microcapsules which were prepared by acacia

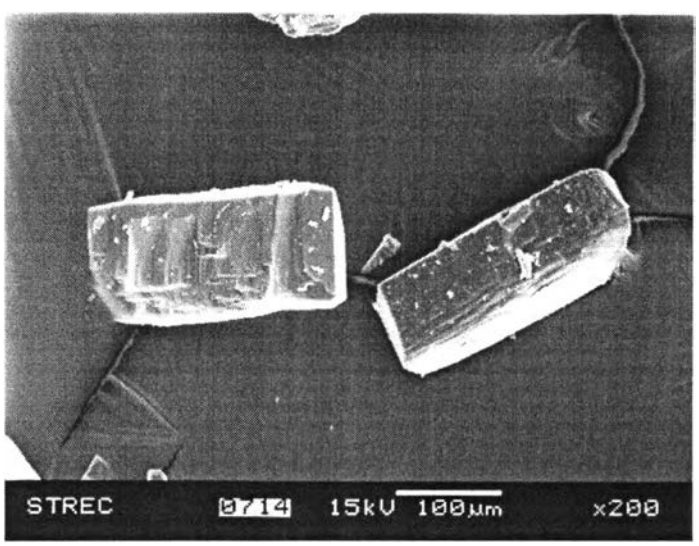

Figure 4-2 Scanning electron micrograph of andrographolide (Magnification 200x and scale bar 100 µm)

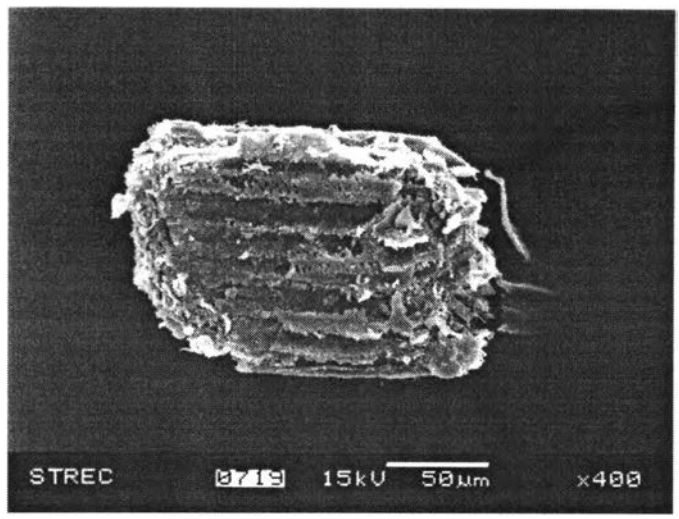

Figure 4-3 Scanning electron micrograph of formulation no. 1 microcapsules (Magnification 400x and scale bar 50 µm)

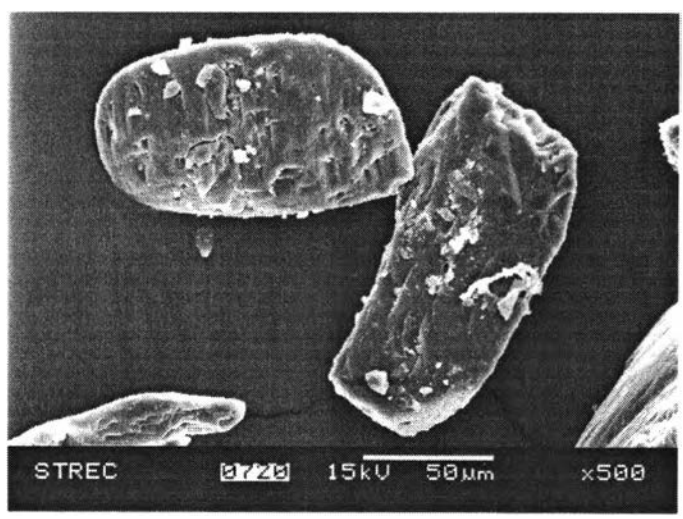

Figure 4-4 Scanning electron micrograph of formulation no. 9 microcapsules (Magnification 500x and scale bar 50 µm)

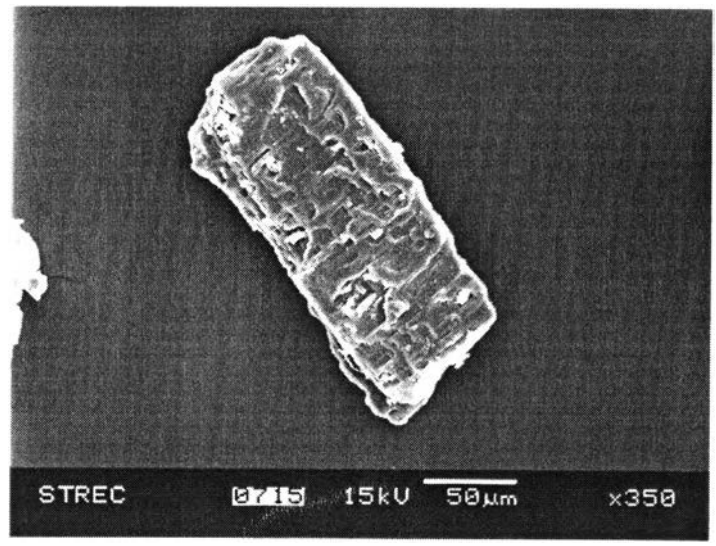

Figure 4-5 Scanning electron micrograph of formulation no. 12 microcapsules (Magnification 350x and scale bar 50 µm)

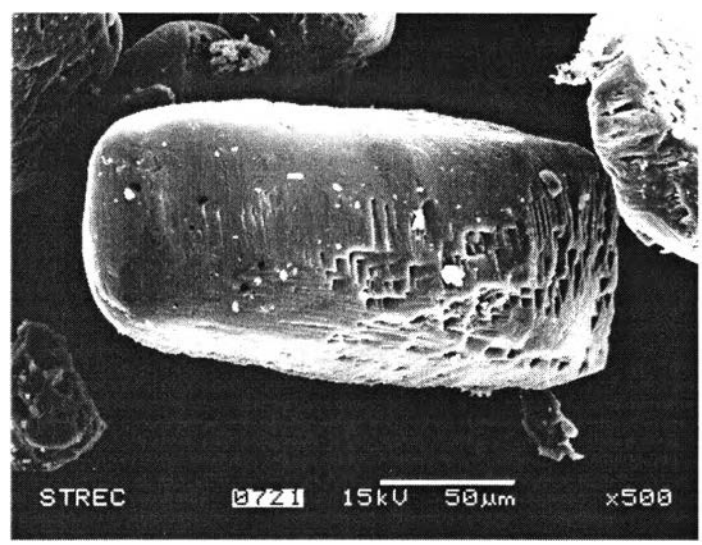

Figure 4-6 Scanning electron micrograph of formulation no. 13 microcapsules (Magnification 500x and scale bar 50 µm)

The optical microscope allows the view of the actual particles and it should be possible to use the optical microscope for particle size measurement in the range of 0.2 μm to about 100 μm. To measure the microcapsule size, the ocular scale was previously calibrated with an objective micrometer which has a division of 10 μm. Since 100 divisions in the ocular scale are equal to 40 divisions in an objective micrometer at the magnification of 40x, each division in ocular scale is then equal to 4 μm [34].

For the particle size, only the powder-like microcapsules (formulations no.1, 9, 12 and 13) were studied. The microcapsules were aligned on the ocular scale and their size was determined by counting the number of the divisions in the ocular scale and calculating into μm unit. The results of the particle size determination of microcapsules for each formulation are presented in Appendix II. The summary of the average and standard deviation (SD) of particle size of andrographolide, which were prepared by each formulation, is shown in Table 4-2.

Table 4-2 Particle size of andrographolide microcapsules

Formulation no.	Size (SD) (μm)
1	50.60 (2.57)
9	50.51 (2.43)
12	50.41 (2.69)
13	50.43 (2.56)

From Table 4-2, the particle size of microcapsules, which were prepared by all formulations, was not significantly different, therefore the studied factors might not influence the size of andrographolide microcapsules. Nixon and Nouh [12] reported that the stirring speed had some effect on the microcapsule size of benzaldehyde microcapsules (oil-containing microcapsules), which were prepared by gelatin-acacia complex coacervation. In this study, the andrographolide microcapsules were

prepared at the same stirring speed for each batch, therefore the microcapsule might be controlled to the same size.

4.2 Preparation of Standard Curve for Andrographolide

Andrographolide can absorb UV light in the wavelength between 223 and 254 nm [8]. The wavelength that was used to analyze andrographolide in this study was 224 nm. At this wavelength, it was initially observed that there were no interferences by the solvent (methanol). The standard curve was plotted between the absorbances at 224 nm and the concentrations of andrographolide standard solutions and fitted using linear regression analysis. The results are shown in Table 4-3 and Figure 4-7. The straight line was obtained with a good correlation coefficient (r^2) of 0.9998. The equation for the line is

$$y = 0.3609x + 0.0198 \quad (13)$$

where y is the absorbance and x is the concentration of andrographolide solution in mg%. This equation was then used to calculate amount of andrographolide in the prepared microcapsules in terms of the percentage of drug content, drug entrapped, drug released and drug remaining.

Table 4-3 Standard calibration of andrographolide

Standard sol^n no.	Concentration (mg%)	Absorbance at 224 nm
1	0.8	0.3069
2	1.0	0.3794
3	1.2	0.4538
4	1.4	0.5287
5	1.6	0.5988
6	1.8	0.6688
7	2.0	0.7384

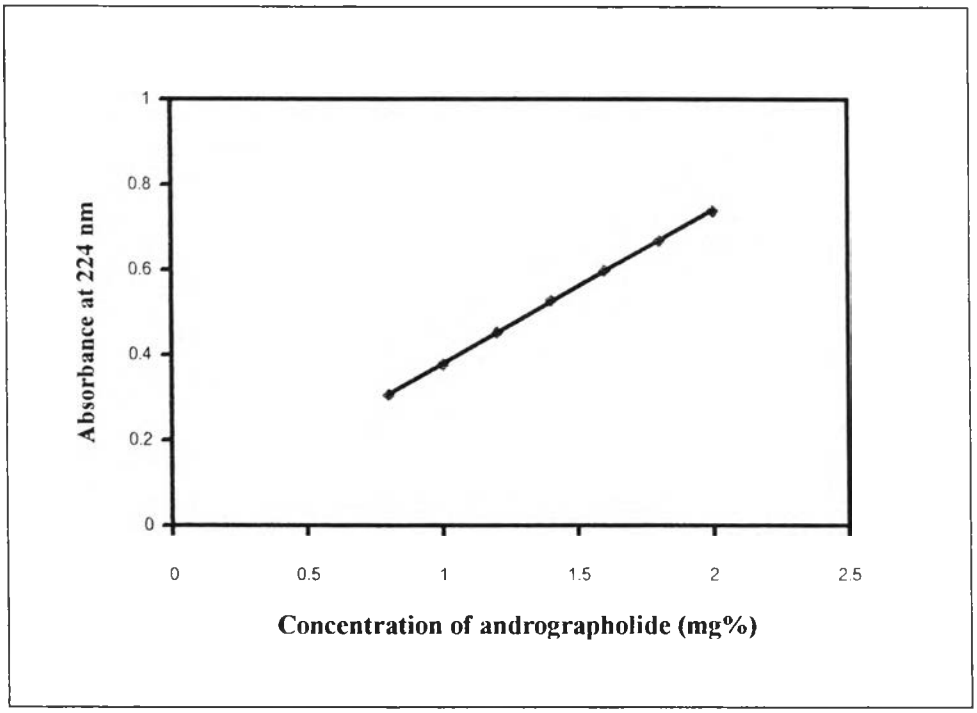

Figure 4-7 Standard curve of andrographolide assayed by UV/Visible spectrophotometer ($r^2 = 0.9998$)

4.3 Yield of Andrographolide Microcapsules

The yield of andrographolide microcapsules is shown in Table 4-4. It gave high yield (90.58%). The yields of microcapsules prepared with various formulation variables were not different. Therefore, the study of the effects on formulation variables did not influence the amount of produced microcapsules.

Table 4-4 Yield of andrographolide microcapsules

Formulation no.	% Yield (SD)
1	89.72 (0.54)
2	88.33 (0.04)
3	87.98 (0.06)
4	*
5	88.50 (0.08)
6	87.71 (0.08)
7	88.39 (0.23)
8	87.93 (0.15)
9	90.58 (0.10)
10	90.58 (0.33)
11	*
12	90.29 (0.02)
13	90.17 (0.12)
14	90.21 (0.01)

* The microcapsules could not be obtained.

4.4 Evaluation of Factors and Conditions for Preparation of Andrographolide Microcapsules

4.4.1 Effect of negative charge polymer

From Table 4-1, the characteristics of microcapsules which were prepared using acacia (formulation no. 1) resulted in a powder-like form. Sheet-like characteristics were obtained when the microcapsules were prepared using sodium alginate and xanthan gum (formulations no. 2 and 5, respectively) while that of microcapsules which were prepared using carragenan and locust bean gum (formulations no. 3 and 6, respectively) appearing as aggregate form. The wall material could not be obtained when pectin was used.

4.4.2 Effect of Gelatin to Acacia Ratio

From Table 4-1, the characteristics of microcapsules which were prepared using 40:60 and 60:40 gelatin to acacia ratios (formulations no. 7 and 8, respectively), resulted in aggregate granule-like forms, while that of microcapsules which were prepared using 50:50 gelatin to acacia ratio (formulation no. 1) occurring as a powder-like form. The aggregation of microcapsules that were prepared using unequal amounts of gelatin and acacia may be due to the adhesion of excess colloid presented during the extraction stage. Table 4-5 shows the results of the percentage drug content and drug entrapped in andrographolide microcapsules that were prepared using various ratios of gelatin and acacia. Since the percentages of drug entrapped in all three formulations were no significant difference, the suitable gelatin to acacia ratio, which was selected according to the appearance of the prepared microcapsules, was 50:50 gelatin to acacia ratio. This result agrees with Nixon and Agyilirah who indicated that the condition of equal amounts of gelatin and acacia produced the best microcapsules [35]. In addition, Deasy explained about complex coacervation that when complex coacervation occurred, colloid-rich droplets usually contained an approximate 1:1 ratio of the colloid [11].

Table 4-5 Effect of gelatin to acacia ratio on the properties of andrographolide microcapsules

Formulation no.	Gelatin : acacia ratio	%Drug content (SD)	%Drug entrapped (SD)
7	40 : 60	28.70 (0.34)	86.10 (0.34)
1	50 : 50	30.88 (0.05)	92.63 (0.15)
8	60 : 40	28.05 (0.23)	84.16 (0.70)

4.4.3 Effect of Core to Wall Ratio

Three ratios of core and wall; i.e, 1:1, 1:2 and 1:3, were used to prepare

andrographolide microcapsules while other factors were controlled. When 1:3 core to wall ratio (formulation no. 10) was used, the system tended to become lumpy and difficult to filter and there was a tendency to form the aggregation of the microcapsules, possibly because of the adhesive effect of excess polymers. Ease in filtration and powder-like characteristics were obtained when the microcapsules were prepared using 1:1 and 1:2 core to wall ratios (formulations 1 and 9, respectively). From Table 4-6, the results indicated that the percentage of drug content and drug entrapped, with 1:2 core to wall ratio gave a significantly higher percentage of drug entrapped than with 1:1 core to wall ratio. Therefore, 1:2 core to wall ratio was a good condition to prepare andrographolide microcapsules. This result might be due to the highest affinity of the core and coating was promoted at this condition.

Table 4-6 Effect of core to wall ratio on the properties of andrographolide microcapsules

Formulation no.	Core : wall ratio	%Drug content (SD)	%Drug entrapped (SD)
1	1 : 1	30.88 (0.05)	92.63 (0.15)
9	1 : 2	32.66 (0.18)	97.98 (0.54)
10	1 : 3	31.63 (0.21)	94.88 (0.21)

4.4.4 Effect of Hardening Time

Andrographolide microcapsules were prepared using various hardening times, i.e. 60, 120 and 180 min (formulations no. 11, 9 and 12, respectively). The microcapsules could be obtained only when 120 and 180 min of hardening time were used. Ease in filtration and powder-like characteristics of microcapsules, which were prepared using these two hardening times, were similar. Theoretically, there were two steps in the mechanism of reaction of gelatin with formaldehyde; i.e. reaction of formaldehyde with amino groups of gelatin and crosslinking between molecules of the product [11]. The 60 min of hardening time might not be enough to promote the crosslinking of polymers on the core surface which causes the microcapsules to be

gelled on cooling and which could not be separated by vacuum filtration. Table 4-7 shows the percentage yield, drug content and drug entrapped and indicates that there was no significant difference between the results obtained when 120 and 180 min of hardening times were used. Even though hardening times of 120 and 180 min gave the same results, the hardening time of shorter time, 120 min, was preferred since it might be possible that the microcapsule wall could be deteriorated if the microcapsules were left to harden for a long time [13]. Therefore, the 120 min of hardening time should be enough and suitable to harden the andrographolide microcapsules wall.

Table 4-7 Effect of hardening time on the properties of andrographolide. microcapsules

Formulation no.	Hardening time (min)	%Drug content (SD)	%Drug entrapped (SD)
9	120	32.66 (0.18)	97.98 (0.54)
11	60	*	*
12	180	29.93 (0.16)	89.80 (0.48)

*The microcapsules could not be obtained.

4.4.5 Effect of Amount of Hardening Agent

Microcapsules, which were prepared under controlled conditions to evaluate the effect of three different amounts of hardening agent, i.e. 5,10 and 15 ml of formaldehyde solution (formulations no. 9, 13 and 14, respectively), were typically grey, powder-like and small granule-like as shown in Table 4-1. Takenaka et al., found that as more hardening agent was used (5 to 50 ml of formaldehyde sol[n]), there was an increase in the size of microcapsules (19.1 to 22.0 μm) because the hardened wall might prevent the shrinkage that occurred during the dehydration and drying process [36]. However, the results on difference in particle size of andrographolide microcapsules, which were prepared using various amounts of formaldehyde solution in this study, were not significant (see Table 4-8).

During prepared the process. It was found that the amount of hardening agent could effect the difficulty in filtration of microcapsules. More amounts of formaldehyde solution used caused more viscous vehicle, and thus, the filtration was difficult. The rise in viscosity of the vehicle might be due to the crosslinking between gelatin in the equilibrium colloid-poor layer and excess formaldehyde [11]. The results on percentage drug content and drug entrapped are shown in Table 4-8. Percentage of drug content and drug entrapped seems to be the highest when 5 ml of formaldehyde solution was used as a hardening agent. Additionally, the viscosity of the coacervate mixture in this condition was low and ease in filtration. Therefore, 5 ml of formaldehyde solution was most suitable to prepare andrographolide microcapsules.

Table 4-8 Effect of amount of hardening agent on the properties of andrographolide microcapsules

Formulation no.	Amount of hardening agent (ml)	%Drug content (SD)	%Drug entrapped (SD)
9	5	32.66 (0.18)	97.98 (0.54)
13	10	27.13 (0.21)	88.16 (0.73)
14	15	27.13 (0.40)	81.38 (1.19)

4.5 Release Characteristics

The Higuchi equation is a good fitting equation to define the release of drug from andrographolide microcapsules. 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 shown in Appendix II.

The powder-like microcapsule formulations were selected for further study of the drug release from microcapsules. 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 1- 15 min. The effect of core to wall ratio in drug release from the microcapsules (formulations no. 1 and 9) is shown in Figure 4-8. The release rate of andrographolide from the microcapsules increases significantly with the increasing core to wall ratio, since the greater core to wall ratio yielded a thinner film coating of polymer on the drug particles. The effects of hardening time and amounts of hardening agent on the drug release from the microcapsules (formulations no. 12 and 13) are shown in Figure 4-9. The amounts of hardening time and hardening agent do not affect the drug release rate significantly.

4.6 Stability of Andrographolide Microcapsules

4.6.1 Physical Stability

The physical characteristics of freshly prepared microcapsules were observed and compared with those after 3 months storage at room temperature. The results are summarized in Table 4-9.

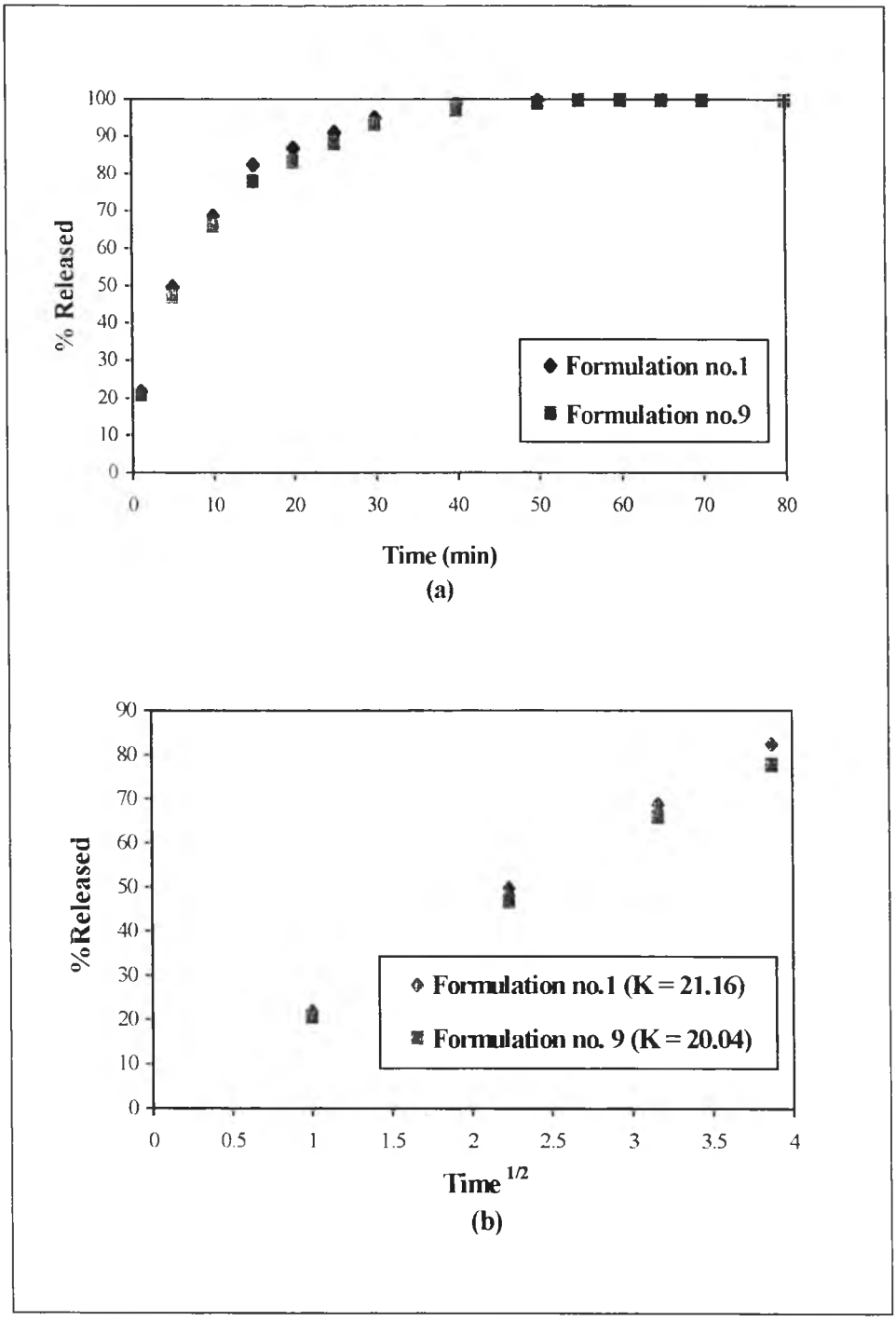

Figure 4-8 Release profiles (a) and Higuchi release plots (b) of the andrographolide microcapsules with 1:1 and 1:2 core to wall ratios (formulations no. 1 and 9, respectively)

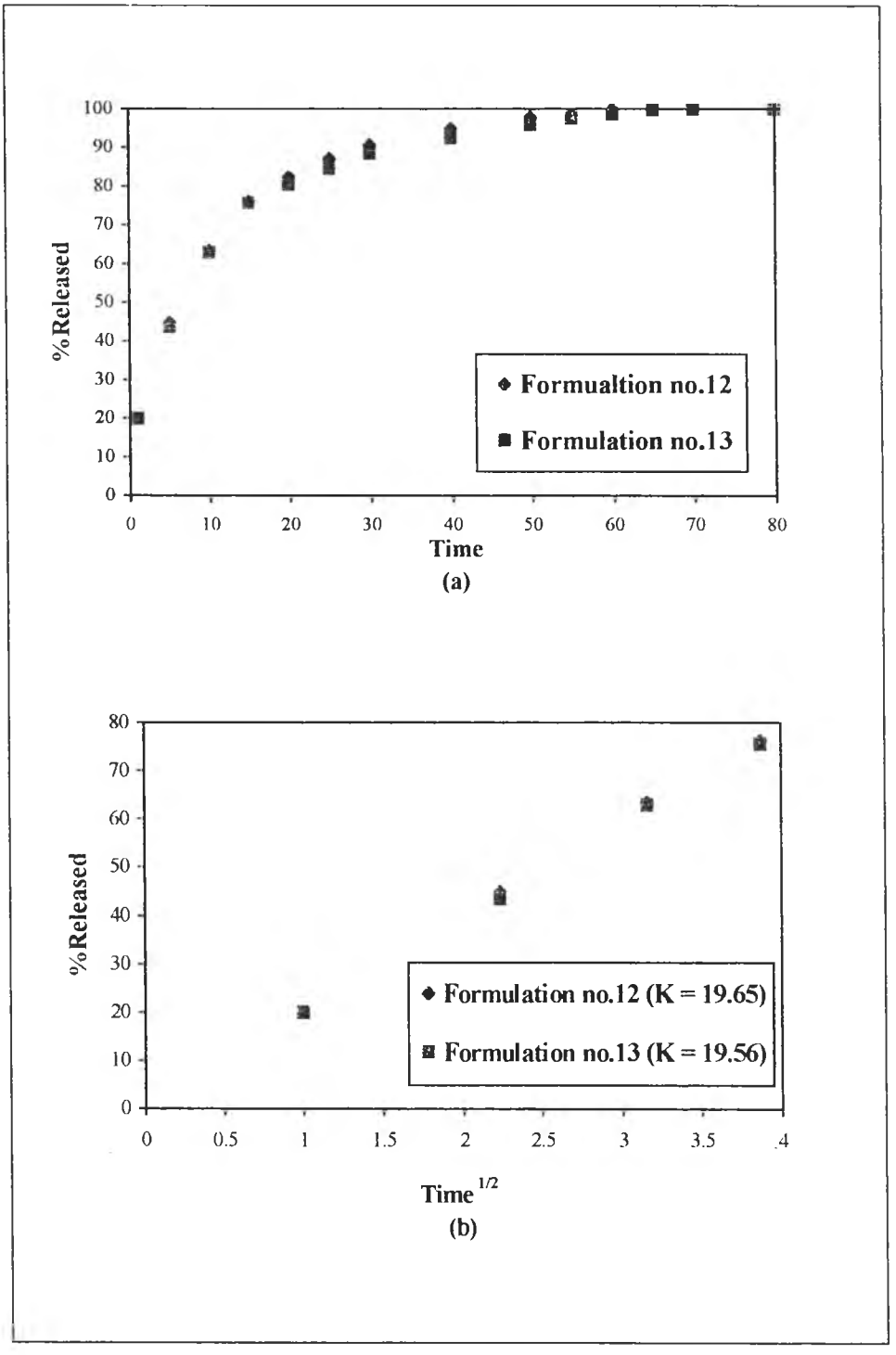

Figure 4-9 Release profiles (a) and Higuchi release plots (b) of the andrographolide microcapsules with 120 and 180 min of hardening time and 10 and 5 ml of hardening agent(formulations no. 13 and 12, respectively)

Table 4-9 Comparison of physical characteristics between freshly prepared andrographolide microcapsules and those after 3 months storage

Formulation no.	Physical characteristics	
	Freshly prepared	After 3 months
1	Grey and powder-like	Dark grey and powder-like
9	Grey and powder-like	Dark grey and powder-like
12	Grey and powder-like	Dark grey and powder-like
13	Grey and powder-like	Dark grey and powder-like

It could be seen from Table 4-9 that the physical characterisstics of andrographolide microcapsules in all formulations changed its color to dark grey after 3 months storage at room temperature. Nonprotected from light (clear-glass) and in protected from light (amber-glass) condition was similar, that in the nonprotected from light condition seemed to occur faster. This indicated that the prepared microcapsules were not stable under the storing conditions.

4.6.2 Chemical Stability

The percentages of andrographolide remaining in the microcapsules were plotted against time (days) to be stored in protected from light (amber-glass) condition and in nonprotected from light (clear-glass) condition shown in Figures 4-10, 4-11 and 4-12, respectively.

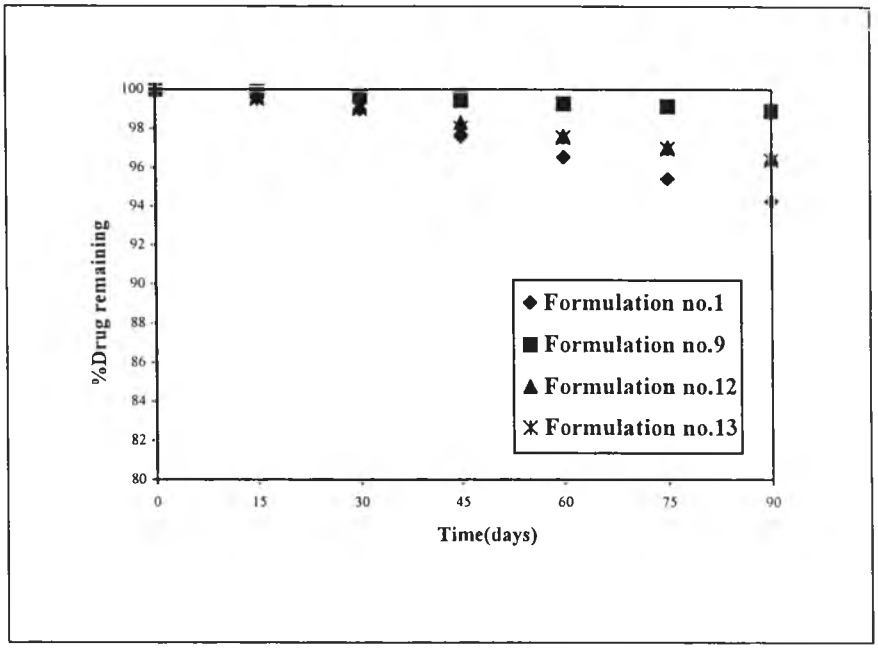

Figure 4-10 The percentage of andrographolide remaining in protected from light (amber-glass) condition

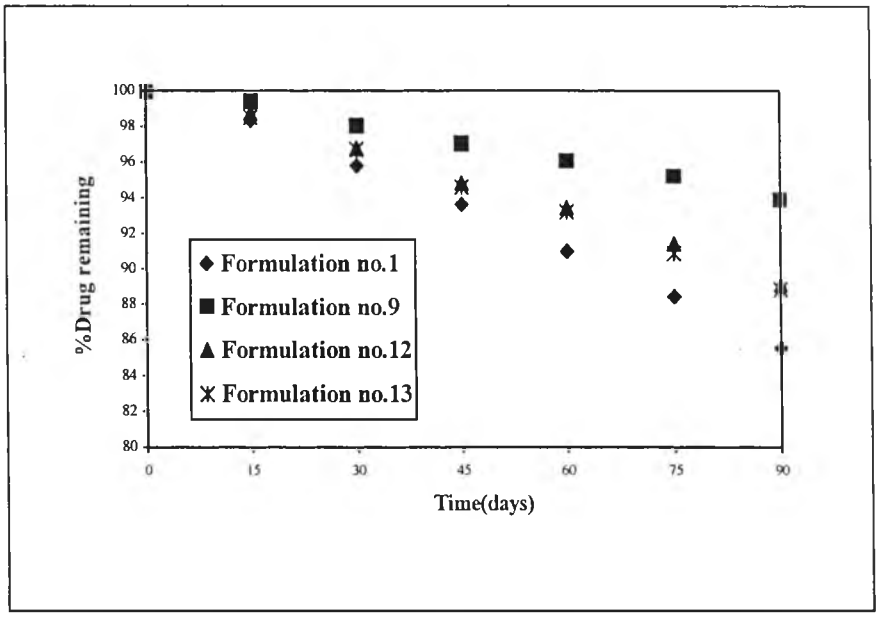

Figure 4-11 The percentage of andrographolide remaining in nonprotected from light (clear-glass) condition

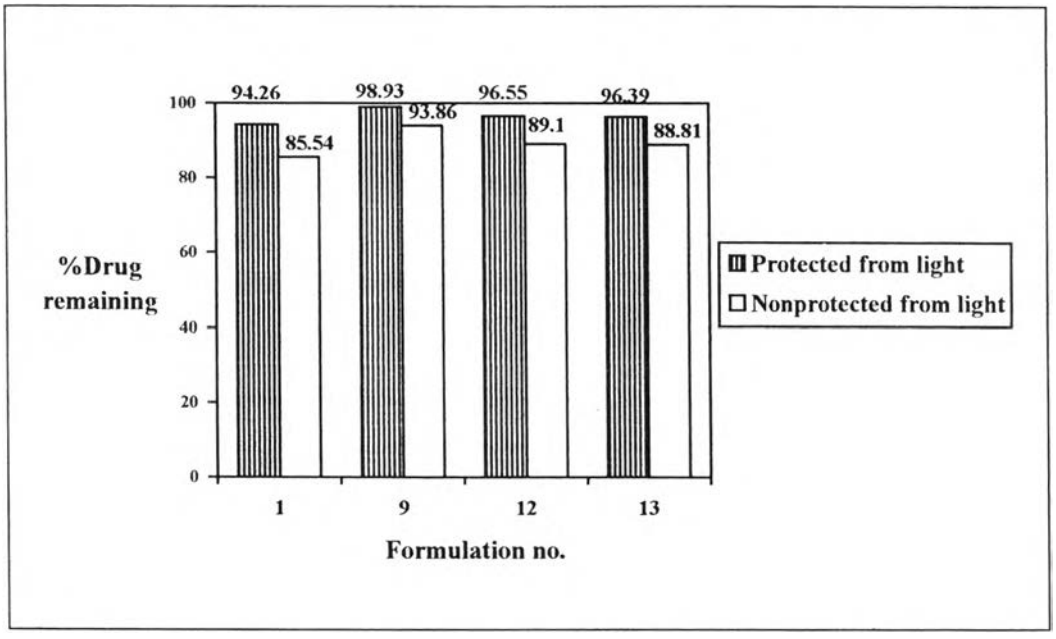

Figure 4-12 Comparison between the percentage of andrographolide remaining in protected from light (amber-glass) condition and nonprotected from light (clear-glass) condition after 3 months

In this study, it was found that the percentage of remaining andrographolide was reduced to 93.86% for the nonprotected from light condition and to 98.93% for the protected from light condition (formulation no. 9) after the microcapsules were stored for 3 months. The stability data is shown in Appendix II. The percentage of drug remaining in the nonprotected from light condition is less than that in the protected from light condition, but it was still stable. Microencapsulation of andrographolide in gelatin-acacia wall by a complex coacervation technique was very stable.