CHAPTER III
MATERIALS AND METHODS

Materials

1. Andrographolide (Faculty of Pharmaceutical Science, Chulalongkorn University).
2. Gelatin (Srichand United Dispensary Co., LTD., Thailand).
3. Acacia (Srichand United Dispensary Co., LTD., Thailand).
4. Sodium alginate (Srichand United Dispensary Co., LTD., Thailand).
5. Carragenan (System Bio-Industries Co., Thailand).
6. Pectin (System Bio-Industries Co., Thailand).
7. Xanthan gum (System Bio-Industries Co., Thailand).
8. Locust bean gum (System Bio-Industries Co., Thailand).
9. Glycerine (Srichand United Dispensary Co., LTD., Thailand).
10. Formaldehyde solution 37% w/w (Lab-Scan).
11. Hydrochloric acid (HCl) (BDH Chemicals Ltd.).
12. Isopropanol (IPA) (Merck).
13. Methanol (MeOH) (Merck).
14. Deionized water.

Equipment

1. A variable-speed stirring motor fitted with a four-blade stirring shaft (Model RW20DZM, GmbH&Co., Germany).
2. Hotplate magnetic stirrer (Model 34532, Snijderes, Japan).
3. Centrifuge (Model 4206, ALC, Italy).
4. Vacuum pump (Model DOA-V130-BN, Waters, USA).
5. Optical microscope (Model KHC 211409, Olympus, Japan).
6. Ocular piece (Model Ocular P7X Micro, Olympus, Japan).

7. Objective micrometer (Olympus, Japan).
8. Microscope equipped with camera (Leitz, Germany).
9. Scanning electron microscope (JEOL JSM-5800LV, Japan).
10. UV/Visible spectrophotometer (Model 7800, printer Model PTL-3965, Jasco, Japan).
11. pH meter (Model 420A, ORION, USA).
12. Hot air oven (Memmert, Germany).

Methods

3.1 Preparation of Andrographolide Microcapsules

The microcapsules of andrographolide were prepared by a complex coacervation technique. The solution of andrographolide in 2% w/w acacia solution was emulsified in the 50 ± 1°C, 2% w/w gelatin solution, in which its pH was previously adjusted to 4 by the gradual dropwise addition of HCl solution while stirring was maintained at 1500 rpm for 45 min. After 45 min, the mixture was cooled to 5°C in an ice bath while maintaining agitation. Formaldehyde solution was added to the mixture while cooling and maintaining agitation for various hardening times. The microcapsules were then separated by vacuum filtration using a Buchner funnel and washed with IPA to dehydrate. Finally, the prepared microcapsules were dried. The schematic of the method of preparation is illustrated in Figure 3-1.

The following microcapsules were prepared using the above procedure to investigate the effects of formulation variables on properties of andrographolide microcapsules.

3.1.1 Effect of Negative Charge Polymer

The microcapsules of andrographolide were prepared using various negative charge polymers; i.e. acacia, sodium alginate, carragenan, pectin, xanthan gum and locust bean gum while other factors were controlled. The controlled factors

used were 1:1 core to wall ratio, 120 minutes of hardening time, and 5 ml of formaldehyde solution. The microencapsulation was performed in the same manner as 3.1. The effect of negative charge polymer providing a powder-like form of andrographolide microcapsules was selected to evaluate the effect of gelatin to negative charge polymer ratio in 3.1.2.

3.1.2 Effect of Gelatin to Negative Charge Polymer Ratio

The microcapsules of andrographolide were prepared using various weight ratios of gelatin and negative charge polymer; i.e. 40:60, 50:50 and 60:40 while other factors were controlled. The microencapsulation was performed in the same manner as 3.1. The gelatin to negative charge polymer ratio providing the highest yield of andrographolide microcapsules was selected to evaluate the effect of core to wall ratio in 3.1.3.

3.1.3 Effect of Core to Wall Ratio

The microcapsules of andrographolide were prepared using various weight ratios of andrographolide (core material) to gelatin and negative charge polymer (wall material), i.e. 1:1, 1:2 and 1:3 by varying the amount of wall material and keeping the amount of core material constant. The other factors were controlled and the procedure was continued in the same manner as 3.1. The core to wall ratio providing the highest yield of andrographolide microcapsules was selected to evaluate the effect of hardening time in 3.1.4.

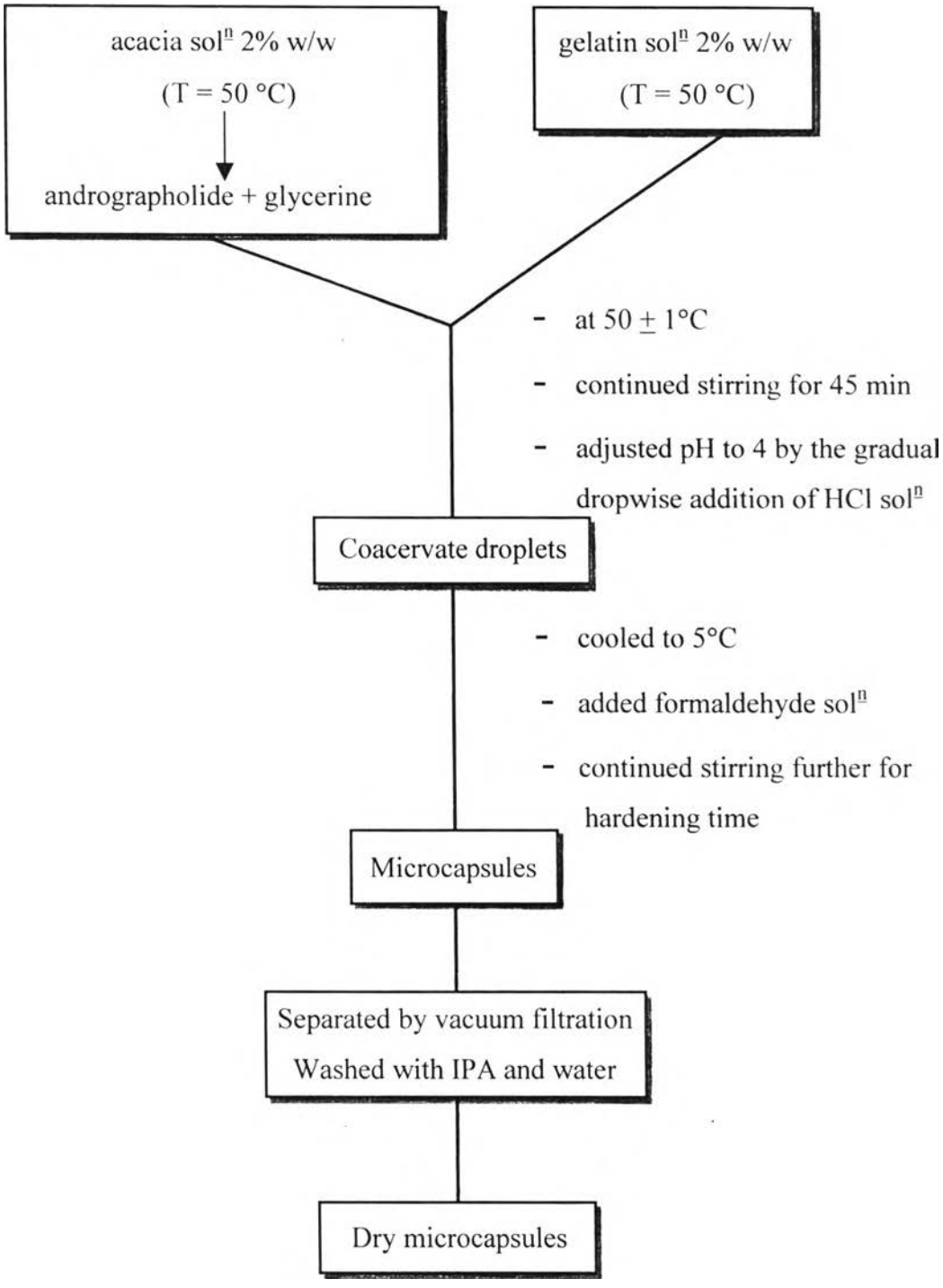

Figure 3-1 Schematic of complex coacervation technique for the microencapsulation of andrographolide microcapsules

3.1.4 Effect of Hardening Time

The microcapsules of andrographolide were prepared using various hardening time; i.e. 60, 120 and 180 min while other factors were controlled. The microencapsulation was performed in the same manner as 3.1. The hardening time providing the highest yield of andrographolide microcapsules was selected to evaluate the effect of amount of hardening agent in 3.1.5.

3.1.5 Effect of Amount of Hardening Agent

The microcapsules of andrographolide were prepared using various amounts of hardening agent; i.e. 5, 10 and 15 ml while other factors were controlled. The microencapsulation was performed in the same manner as 3.1.

The summary of the formulations that were used to prepare microcapsules of andrographolide according to 3.1.1, 3.1.2, 3.1.3, 3.1.4 and 3.1.5 are shown in Tables 3-1.

3.2 Evaluation of Andrographolide Microcapsules

3.2.1 Physical Properties

3.2.1.1 The microcapsules were visually observed for their physical appearances. The color and aggregation of particles were recorded.

3.2.1.2 The morphology of the microcapsules was observed under microscope with camera and scanning electron microscope (SEM). The microcapsules of andrographolide were electrically non-conductive specimen, therefore, before observed under SEM, their surface must be subjected to gold coating by the use of an ion sputtering device after fixed on the specimen stub. Then, photographs of coated samples were taken under the SEM.

Table 3-1 Formulation of andrographolide microcapsules

No.	Types of negative charge polymer	Gelatin : negative - charge polymer ratio	Core : wall ratio	Hardening time (min)	Amount of hardening agent (ml)
1	acacia	50 : 50	1 :1	120	5
2	sodium alginate	50 : 50	1 : 1	120	5
3	carragenan	50 : 50	1 : 1	120	5
4	pectin	50 : 50	1 :1	120	5
5	xanthan gum	50 : 50	1 : 1	120	5
6	locust bean gum	50 : 50	1 : 1	120	5
7	acacia	40 : 60	1 : 1	120	5
8	acacia	60 : 40	1 : 1	120	5
9	acacia	50 : 50	1 : 2	120	5
10	acacia	50 :50	1 : 3	120	5
11	acacia	50 : 50	1 : 2	60	5
12	acacia	50 : 50	1 : 2	180	5
13	acacia	50 : 50	1 : 2	120	10
14	acacia	50 : 50	1 : 2	120	15

3.2.1.3 The particle size of microcapsules was measured using an optical microscope with an ocular scale that has already been calibrated with an objective micrometer. One hundred particles were determined for each sample.

3.2.2 Preparation of Standard Curve

About 10 mg of andrographolide was accurately weighed, dissolved and diluted with methanol in 100 ml volumetric flask. Appropriate dilutions were then made with methanol to obtain standard solutions of 0.8,1.0, 1.2, 1.4, 1.6, 1.8 and 2 mg%. The absorbances of these solutions were determined at the wavelength of 224 nm, the λ_{max} of andrographolide, with UV/Visible spectrometer, using methanol as a

blank. The relationship between absorbance and concentration was fitted using linear regression analysis.

3.2.3 Yield

An amount of the produced microcapsules was weighed and % yield was calculated according to equation (7).

$$\% \text{ Yield} = \frac{\text{Wt of produced microcapsules (g) x 100}}{\text{Theoretical Wt of microcapsule (s)}} \quad (7)$$

Where

$$\text{Theoretical wt (g)} = \text{Wt of andrographolide (g)} + \text{Wt of gelatin (g)} + \text{Wt of negative charge polymer (g)} \quad (8)$$

3.2.4 Drug Content and Drug Entrapment

In order to determine the total drug content of the microcapsules, the method of assay for andrographolide was adapted. About 10 mg of andrographolide microcapsules was accurately weighed and crushed with methanol in the mortar. The extraction was then filtered to separate the shell fragments and diluted with methanol to appropriate concentration. The dilution was measured at an absorbance of 224 nm with UV/Visible spectrometer, using methanol as a blank. The amount of andrographolide was determined from the standard curve and the percentage of drug content and drug entrapped were calculated using equations 9 and 10, respectively.

$$\% \text{ Drug content} = \frac{\text{Amount of andrographolide x 100}}{\text{Amount of microcapsules}} \quad (9)$$

$$\% \text{ Drug Entrapped} = \frac{\% \text{ Drug content x 100}}{\% \text{ Theoretical content}} \quad (10)$$

where

$$\% \text{ Theoretical content} = \frac{\text{Wt of andrographolide (g)} \times 100}{\text{Wt of andrographolide (g)} + \text{Wt of gelatin (g)} + \text{Wt of negative charge polymer (g)}} \quad (11)$$

3.2.5 Release Characteristics

An aqueous solution of HCl solution (pH = 5) was used as the dissolution medium. About 200 mg of microcapsules were accurately weighed and transferred into 750 ml of dissolution medium, which was maintained at 37 ± 0.1°C and stirred at a constant stirring rate of 300 rpm. Five-milliliter samples were withdrawn at appropriate time intervals and immediately replaced with fresh dissolution medium. The samples were then filtered and diluted with the dissolution medium to an appropriate concentration. The concentrations of andrographolide were then determined using a UV spectrophotometer at a wavelength of 224 nm. The mean percentage of andrographolide released, which was based on the total drug in the sample determined after each dissolution test as 100%, and standard deviation were computed. The mean percentage of andrographolide released was plotted against time (min) to obtain release profiles. Finally, the drug release profiles were fitted to Higuchi square root of time model according to equation (6). The correlation coefficient (r) and release rate constant (K) were calculated from an appropriate portion of the plots. Each release determination was performed in triplicate.

3.3 Stability of Andrographolide Microcapsules

3.3.1 Physical Stability Evaluation

The physical properties of microcapsules. which included the color and aggregation of particles, were observed when the microcapsules were stored at room temperature for 3 months in both protected from light condition (in amber-glass bottle) and nonprotected from light condition (in clear-glass bottle).

3.3.2 Chemical Stability Evaluation

The microcapsules were stored at room temperature for 3 months in both protected from light condition (in amber-glass bottle) and nonprotected from light condition (in clear-glass bottle). The sample was drawn and assayed occcasionally using the technique according to 3.2.4. The percentage of drug remaining was calculated using equation 12.

$$\% \text{ Drug remaining} = \frac{\% \text{ Drug content at time t x } 100}{\% \text{ Drug content at initial time}} \qquad (12)$$