CHAPTER III

MATERIALS AND METHODS

Materials

1. The fruits of *Garcinia mangostana* (purchased in Bangkok, Thailand in July, 2003)

2. Clotrimazole (S. Tong Chemicals Co., Ltd., Thailand, lot no. 20010605)

3. Disodium hydrogen orthophosphate (Merck, Germany, lot no. F974786 538)

4. Ethanol absolute, AR grade (Merck, Germany, lot no. K32751783 349)

5. Ethyl acetate, AR grade (Labscan Asia, Thailand, lot no. 04070073)

6. Glyceryl monooleate (Donated from Hong Huat Co., Ltd., Thailand, lot no. 601)

7. Hexane, AR grade (Labscan Asia, Thailand, lot no. 03030145)

8. Lutrol F127 (BASF, Germany, lot no. WPHY615B)

9. Methanol, HPLC grade (Labscan Asia, Thailand, lot no. 04070120)

10. Mueller Hinton agar (Oxoid, England, CM0337)

11. n-Octanol (Asia Pacific Specialty Chemicals Ltd., Australia, lot no. H1F223)

12. Olive oil (Bertolli, Italy, lot no. L014BS)

13. Polyamide membrane filter 47 mm, 0.45 μm (Satorius AG, Germany, lot no. 0503 25006 0340093)

14. Potassium dihydrogen orthophosphate (Merck, Germany, lot no. A262673 045)

15. Sesame oil (Thai China Flavours & Fragrances Industry Co., Ltd., Thailand, lot no. 4607113/0807)

16. Sodium chloride, AR grade (Merck, Germany, lot no. K28555404 049)

17. Soybean oil (Thai Vegetable Oil Public Co., Ltd., Thailand, lot no. 310546)

18. *Streptococcus mutans* KPSK$_2$ (Department of Microbiology, Faculty of Dentistry, Chulalongkorn University, Thailand)

19. Syringe filter cellulose acetate 13 mm, 0.45 μm (Chrom Tech Inc., USA, lot no. 100301)

20. TLC Alumina sheet silica gel 60F 254 20 × 20 cm (Merck, Germany, lot no. OB290814)

21. Whatman filter paper No.1, 150 mm (Whatman International Ltd., England, lot no. A815891)

Equipment

1. Analytical balance (Model AG285, Mettler Toledo, Switzerland)

2. Botanical grinder (Retsch GmbH SK1, Germany)

3. Cone and plate viscometer (Brookfield viscometer, Scientific Industries, Inc., USA)

4. CO_2 water jacketed incubator (Forma Scientific, Inc., USA)

5. Differential scanning calorimeter (DSC822^e, Mettler Toledo, Switzerland)

6. Digital camera (Coolpix 5400, Nikon, Japan)

7. High performance liquid chromatography
    - Auto Injector (SIL-10A, Shimadzu, Japan)
    - Communications bus module (CBM-10A, Shimadzu, Japan)
    - Liquid chromatograph pump (LC-10AD, Shimadzu, Japan)
    - UV-VIS detector (SPD-10A, Shimadzu, Japan)
    - Column (BDS Hypersil C18, 5 μm, 250 × 4.6 mm, Thermo Electron Corporation, England, lot no.6596)
    - Precolumn (μBondapack C18, 10 μm, 125A°, Water Corporation, Ireland, lot no. W2336B1)

8. Hot air oven (Memmert, Germany)

9. Laminar air flow (BH 2000 series, Clyde-Apac, Australia)

10. Microscope (Eclipse E200, Nikon, Japan)

11. Modified Franz diffusion cells (Crown Glass Company, USA)

12. pH Meter (Model 420A, Orion, USA)

13. Refrigerated incubator (FOC 225I, VELP Scientifica, Italy)

14. Rotary evaporator (Rotavapor RE-120, Buchi, Switzerland)

15. Ultrasonicator (Crest Ultrasonics, Malaysia)

16. UV-visible spectrophotometer (UV-1601, Shimadzu, Japan)

17. Vacuum pump (CB169 Vacuum system, Buchi, Switzerland)

18. Vortex mixer (Vortex Genies-2, Scientific Industries, Inc., USA)

Methods

A. Extraction of Active Constituents from *Garcinia mangostana*

The fruit hulls (2 kg) of *Garcinia mangostana* were cut into small pieces about 1×1 inch, and then dried at the temperature 45 ± 0.5 °C in hot air oven. The dried fruit hulls were ground into powder by using a botanical grinder and extracted by maceration method with hexane at room temperature overnight. Then, the marc was extracted with ethyl acetate by the same method. The ethyl acetate extract was filtered and evaporated using a rotary evaporator at 45 ± 0.5 °C. The crude extract was concentrated and crystallized. The yield was kept in a desiccator and used for further studies (Hiranras, 2001).

B. Determination of Active Constituents from *Garcinia mangostana*

1. Differential Scanning Calorimetric (DSC) Method

The DSC thermogram was determined by using differential scanning calorimeter (DSC822^e, Mettler Toledo, Switzerland) to detect the melting point. An accurately weighed amount of 3-5 mg of the crude extract was placed in an aluminum crucible. Then the aluminum crucible was sealed with the lid under the pressure of the plunger by using crucible sealing press. After sealing, the aluminum crucible was placed on a DSC sensor in the furnace. The scan rate was performed at 10 °C/min over the temperature range of 0-250 °C under a nitrogen atmosphere.

2. Thin Layer Chromatographic (TLC) Method

TLC is the most versatile and flexible chromatographic method. It is rapid and gives highest sample throughput because many samples and standards can be applied to a single plate and separated at the same time.

The extract was dissolved with ethyl acetate and spotted on a TLC alumina sheet, compared with standard mangostin. After that the alumina sheet was placed in a closed chamber saturated with the vapor of mobile phase, ethyl acetate : hexane (3:1). When the mobile phase had moved to an appropriate distance, the alumina sheet was removed and dried. The alumina sheet was detected under UV light at the wavelength of 254 nm (Hiranras, 2001). The basic parameter used to describe the migration is the Rf value, where

$$Rf \quad = \quad \frac{\text{distance moved by solute}}{\text{distance moved by mobile phase front}}$$

3. High Performance Liquid Chromatographic (HPLC) Method

The determination of active constituents from *Garcinia mangostana* was performed by HPLC method because of specificity and high sensitivity. The condition for HPLC analysis was developed from that of Hiranras (2001).

3.1 HPLC Condition

The HPLC conditions for the analysis of mangostin were as follows:

Column	:	BDS Hypersil C18, 5 μm, 250 × 4.6 mm
Precolumn	:	μBondapack C18, 10 μm, 125A°
Mobile phase	:	methanol : water (85:15)
Injection volume	:	20 μl
Flow rate	:	1 ml/min
Detector	:	UV detector 243 nm
Temperature	:	ambient
Run time	:	20 min
Internal standard	:	clotrimazole

The mobile phase was prepared by using methanol and water with the ratio of 85:15 v/v. The solution was thoroughly mixed, filtered through 0.45 μm membrane filter and then degassed by sonication for 30 min prior to use.

3.2 Standard Solutions for HPLC

From the preliminary study, clotrimazole was used as an internal standard due to its appropriate retention time and optimal resolution from mangostin peak. A stock solution of clotrimazole was prepared by accurately weighing 125 mg of clotrimazole into a 100 ml volumetric flask. Fifty percent of ethanol was added to dissolve and the solution was adjusted to the final volume. The final concentration of clotrimazole was 1.25 mg/ml.

A stock solution of mangostin was prepared by accurately weighing 25 mg of mangostin into a 10 ml volumetric flask. Absolute ethanol was added to dissolve and the solution was adjusted to the final volume. This stock solution had a final concentration of 2.5 mg/ml. Then 5.0 ml of the stock solution was transferred into a 100 ml volumetric flask and diluted with 50% v/v ethanol. The final concentration of this solution was 125 µg/ml. Standard solutions of mangostin were prepared by pipetting 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ml of the above solution into a 25 ml volumetric flask, respectively. Then 5.0 ml of clotrimazole stock solution was added into each of these volumetric flasks. The solutions were adjusted to volume with 50% v/v ethanol so that the concentrations of mangostin were 5, 10, 20, 30, 40 and 50 µg/ml, respectively and 0.25 mg/ml clotrimazole. These standard solutions were prepared for each HPLC run. As a result, the standard curve of mangostin between concentration and peak area ratio was plotted.

3.3 Sample Solutions for HPLC

The sample stock solution was prepared by accurately weighing 25 mg of *Garcinia mangostana* extract into a 10 ml volumetric flask. Absolute ethanol was added to dissolve and the solution was adjusted to the final volume. This stock solution had a final concentration of 2.5 mg/ml. Then 1.0 ml of the stock solution was transferred into a 25 ml volumetric flask and diluted with 50% v/v ethanol. The final concentration of this solution was 100 µg/ml. Then 5.0 ml of the above solution was transferred into a 25 ml volumetric flask and 5.0 ml of clotrimazole stock solution was added into this flask. The solutions were adjusted to volume with 50% v/v

ethanol so that the concentrations of *Garcinia mangostana* extract and clotrimazole were 20 μg/ml and 0.25 mg/ml, respectively.

3.4 Validation of HPLC Method

The analytical parameters used for validation of the HPLC method were specificity, linearity, accuracy and precision.

3.4.1 Specificity

Under the chromatographic conditions used, the peak of mangostin must be completely separated from and not be interfered by the peaks of other components in the sample.

3.4.2 Linearity

Three sets of six standard solutions were prepared and analyzed. Linear regression analysis of the peak area ratios versus their concentrations was performed. The linearity was determined from the coefficient of determination (R^2).

3.4.3 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was determined from the percentage of recovery. Five sets of three standard solutions (low, medium, high) were prepared and analyzed. The percentage of recovery of each concentration was calculated from the ratio of inversely estimated concentration to known concentration multiplied by 100.

3.4.4 Precision
a) Within Run Precision

The within run precision was determined by analyzing five sets of three standard solutions (low, medium, high) in the same day. Peak area ratios of mangostin to clotrimazole were calculated and the percent coefficient of variation (%CV) of each concentration was determined.

b) Between Run Precision

The between run precision was determined by analyzing three standard solutions (low, medium, high) on five different days. The percent coefficient of variation (%CV) of mangostin of each concentration was determined.

Acceptance criteria:

For accuracy, the percentage of recovery should be within 98-102% of each nominal concentration, whereas the percent coefficient of variation for both within run precision and between run precision should be less than 2%.

C. Preparation of Monoglyceride-Based Drug Delivery System

1. Monoglyceride-Based Drug Delivery System

Monoglyceride-based drug delivery system is based on the ability of mixture of monoglyceride and triglyceride to form liquid crystals when in contact with water. Monoglyceride-based drug delivery systems were developed by using glyceryl monooleate, triglycerides and water. Triglycerides used in this study were sesame oil, soybean oil and olive oil.

Monoglyceride-based drug delivery systems were prepared on a weight by weight (w/w) basis by weighing the three components in screw-capped tubes and vigorously mixed using vortex mixer for 5 min. The samples were heated at 45 ± 0.5 °C and occasionally mixed for 30 min. The samples were stored at room temperature for 1 week before further studies.

2. Physical Characterization

2.1 Polarized Light Microscopy

A drop of samples was placed on a glass slide and examined for the liquid crystalline phases under the polarized light microscope (Eclipse E200, Nikon, Japan). Anisotropic liquid crystalline phases, such as hexagonal, lamella and reversed

hexagonal phases, are optically birefringent. This property can be used for studying such phases with polarizing microscopy. On the other hand, isotropic phases (e.g. micellar and reversed micellar solutions and cubic phases) are nonbirefringent and generate a dark background when investigated under the polarizing microscope.

2.2 Physical Stability

The samples that could form reversed hexagonal phases were evaluated for their physical stability by heating-cooling cycle. The samples were stored in a hot air oven at 45 ± 0.5 °C for 48 hr, and then placed in a refrigerator at 4 ± 0.5 °C for 48 hr. The heating-cooling cycle was repeated for 6 cycles.

D. Formulation of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System

The most stable samples from the study in topic C 2.2, with the equilibrium water content, were selected. Since a mixture of glyceryl monooleate and oil with drug comes into contact with water, hydration progresses and the formulation turns into a liquid crystalline state with high viscosity. The equilibrium water content means the maximum water uptake without causing phase separation. Monoglyceride-based drug delivery systems were prepared by weighing the three components and 2% *Garcinia mangostana* extract in screw-capped tubes and then followed method as described in C1.

E. Characterization of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System

1. Determination of Physicochemical Properties

The physicochemical properties of formulations before and after incorporating the *Garcinia mangostana* extract were determined as follows:

1.1 Physical Appearances

The physical appearances of formulations such as color, clarity and phase separation were observed.

1.2 pH Measurement

The pH of formulations was measured by using pH meter (Model 420A, Orion, USA). The measurements were done in triplicate.

1.3 Viscosity Measurement

The viscosity of formulations was determined by using cone and plate viscometer (Brookfield viscometer, Scientific Industries, USA.). The determination of the viscosity was performed by applying about 0.5 ml of sample to the lower plate of the viscometer. The cone CP-40 was used. The measurements were performed in triplicate.

1.4 Polarized Light Microscopy

A drop of samples was placed on a glass slide and examined for the liquid crystalline phases under polarized light microscope. The polarized light photomicrographs were recorded by digital camera (Coolpix 5400, Nikon, Japan) to compare the liquid crystalline phases before and after adding the extract.

2. Determination of Injectability through the Syringe

The injectability through the syringe was performed to ensure that the formulations could be administered by syringe into a periodontal pocket. Since the high-viscous formulations had stiffness and could not be administered by syringe, the low-viscous formulations were used in this study. When they are in contact with the fluid in the oral cavity, the formulation turns into a liquid crystalline state and changes to a semi-solid at the injection sites.

The water-free formulations with low viscosity were evaluated for injectability by administering through a syringe with 23-gauge tip needle. The viscosity of formulations was also determined by using cone and plate viscometer in triplicate.

3. *In vitro* Liquid Crystalline Phase Formation Study

This study was performed to ensure that the formulations could form liquid crystalline phases with high viscosity upon dilution with water. The study was modified from Scherlund et al. (2001). The test was conducted within a screw-capped tube at the temperature 37 ± 0.5 ℃ using water bath. The water-free formulations 0.5 ml was added into 2 ml of water then the liquid crystal formation was detected by polarized light microscopy.

4. *In vitro* Release Study

4.1 Solubility Study of the Receiver Fluid

Selection of an appropriate receiver fluid is important to maintain sink condition during the release study. Ethanol was used to enhance drug solubility in receiver fluid. Excess amount of the extract was put into each screw-capped tube containing 5 ml of pH 7.4 phosphate buffer and ethanol in pH 7.4 phosphate buffer ranging from 0 to 35%. The tubes were continuously rotated at 37 ± 0.5 ℃ for 48 hr to achieve equilibrium. Then the suspensions were centrifuged and the supernatants were withdrawn and filtered through 0.45 µm cellulose acetate syringe filters. The filtrates were appropriately diluted and analyzed by UV spectrophotometer (UV-1601, Shimadzu, Japan). All solubility determinations were carried out in triplicate.

4.2 *In vitro* Release Study

The *in vitro* release study was performed using modified Franz diffusion cell which consists of a donor compartment and a receiver compartment (Figure 18). The cellophane membrane was placed between two compartments of modified Franz diffusion cell. The membrane was soaked in the medium before use.

The receiving compartment contained 14 ml of 35% v/v ethanol in pH 7.4 phosphate buffer which was maintained at 37 ± 0.5 °C by a circulating water jacket. The receptor fluid and membrane were equilibrated to the desire temperature for 1 hr before the release study. After equilibration, 0.1 g of the sample was carefully placed into the donor compartment using a syringe and then covered with paraffin film to prevent evaporation. The receptor fluid was continuously mixed by magnetic stirring bar at 600 rpm throughout the time of release study. Any air bubbles formed under the preparation had necessarily been removed before the experiment was started. Four specimens of each formulation were examined.

Samples of 5 ml were taken from the receiver medium at certain time intervals (1, 3, 6, 9, 12, 24, 36 and 48 hr) via the sampling port of diffusion cell. The receiver medium was removed by using a syringe and the receiver compartment was replaced with the same amount of medium to keep the constant volume during the experiment. All receiver solutions taken were analyzed concentrations of mangostin in the medium by using UV spectrophotometer at 243 nm.
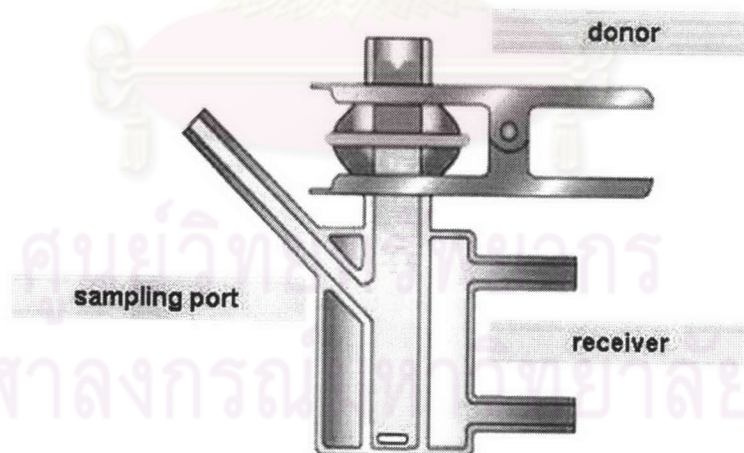

Figure 18 A schematic illustration of the two compartment of Franz diffusion cell

The amount of drug release was calculated by multiplying the drug concentration with the receiver volume. The percentage of drug release was calculated by the following equation:

$$\% \text{ drug release} = (A_t / A_0) \times 100$$

where A_t is the cumulative released amount of drug at a particular time; A_0 is the initial amount of drug.

4.3 Assay of *In vitro* Release

The determination of amount of mangostin release was performed by UV spectrophotometry due to rapidity and convenience.

4.3.1 Standard Solutions for UV

A stock solution of mangostin was prepared by accurately weighed 12 mg of mangostin into a 10 ml volumetric flask. Absolute ethanol was added to dissolve and the solution was adjusted to the final volume. Then 1.0 ml of this solution was transferred into a 100 ml volumetric flask, diluted and adjusted to volume with 35% v/v ethanol in pH 7.4 phosphate buffer. This solution had a final concentration of 12 μg/ml. Standard solutions of mangostin were prepared by pipetting 2.0 to 8.0 ml of this solution into a 10 ml volumetric flask, diluted and adjusted to volume with the same solvent. The final concentrations of the solution were 2.4, 3.6, 4.8, 6.0, 7.2, 8.4 and 9.6 μg/ml, respectively.

The standard solutions were analyzed by UV spectrophotometer at 243 nm. Thirty-five percent of ethanol in pH 7.4 phosphate buffer was used as blank. The standard curve was plotted between concentration and absorbance.

4.3.2 Validation of UV Spectrophotometric Method

The analytical parameters used for the assay validation were specificity, linearity, accuracy and precision.

4.3.2.1 Specificity

Under the condition selected for *in vitro* release study, the absorbance of mangostin must not be interfered by the absorbance of other components in the sample.

4.3.2.2 Linearity

Three sets of seven standard solutions of mangostin ranging from 2.4 to 9.6 μg/ml were prepared and analyzed. Linear regression analysis of the absorbances versus their concentrations was performed. The linearity was determined from the coefficient of determination (R^2).

4.3.2.3 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was determined from the percentage of recovery. Five sets of three standard solutions (low, medium, high) were prepared and analyzed. The percentage of recovery of each concentration was calculated from the ratio of inversely estimated concentration to known concentration multiplied by 100.

4.3.2.4 Precision

a) Within Run Precision

The within run precision was determined by analyzing five sets of three standard solutions (low, medium, high) in the same day. The percent coefficient of variation (%CV) of each concentration was determined.

b) Between Run Precision

The between run precision was determined by analyzing three standard solutions (low, medium, high) on five different days. The percent coefficient of variation (%CV) of each concentration was determined.

Acceptance criteria:

For accuracy, the percentage of recovery should be within 98-102% of each nominal concentration, whereas the percent coefficient of variation for both within run precision and between run precision should be less than 2%.

5. Differential Scanning Calorimetric (DSC) Method

The DSC thermogram of samples was determined by using differential scanning calorimeter (DSC822^e, Mettler Toledo, Switzerland). A highly sensitive ceramic sensor in DSC instrument is used to measure the difference between the heat flows to the sample and reference crucibles. The standard type of pan for DSC measurement is aluminum crucible standard 40 µl. An accurately weighed amount of 3-5 mg of the samples was placed in an aluminum crucible. Then the aluminum crucible was sealed with the lid under the pressure of the plunger by using crucible sealing press. After sealing, the aluminum crucible was placed on a DSC sensor in the furnace. Samples were heated from -20 to 250 °C at a heating rate of 10 °C/min. All tests were performed under a nitrogen atmosphere.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

1 Heat shield
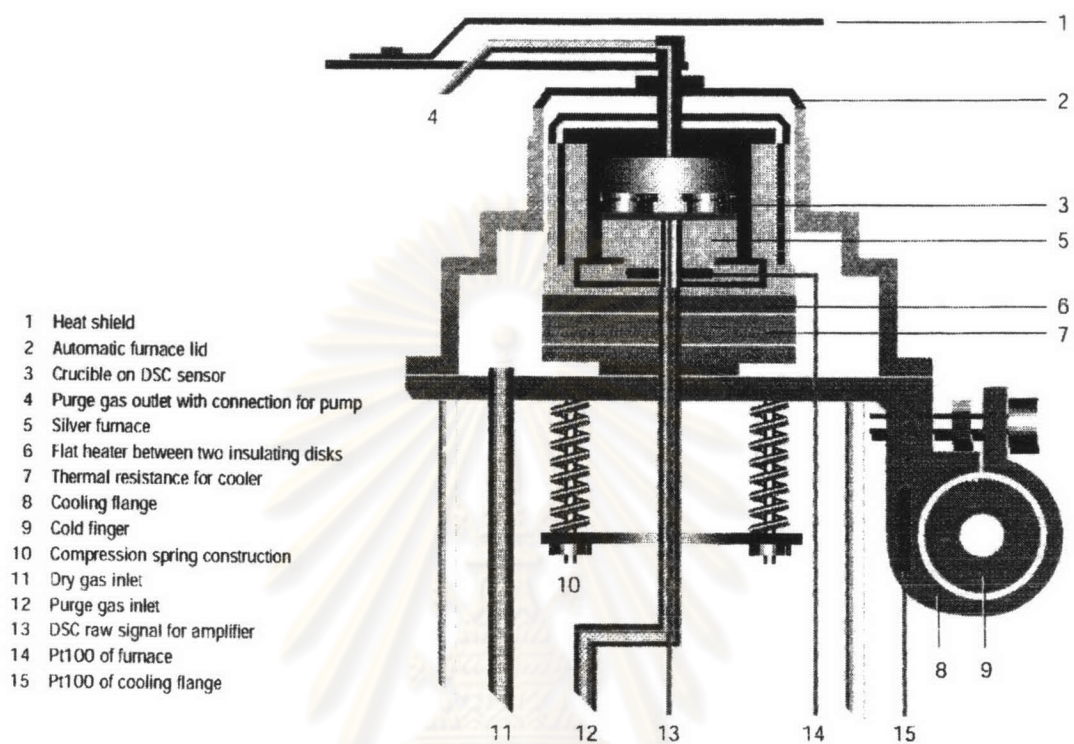2 Automatic furnace lid
3 Crucible on DSC sensor
4 Purge gas outlet with connection for pump
5 Silver furnace
6 Flat heater between two insulating disks
7 Thermal resistance for cooler
8 Cooling flange
9 Cold finger
10 Compression spring construction
11 Dry gas inlet
12 Purge gas inlet
13 DSC raw signal for amplifier
14 Pt100 of furnace
15 Pt100 of cooling flange

Figure 19 A schematic illustration of differential scanning calorimeter (DSC822[e], Mettler Toledo, Switzerland)
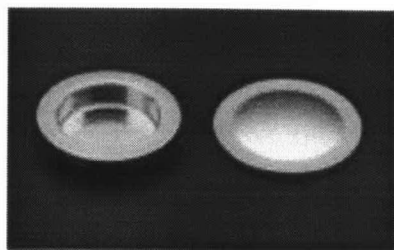
Figure 20 Aluminum crucible standard 40 µl

Figure 21 Differential scanning calorimeter (DSC822^e, Mettler Toledo, Switzerland)

6. Determination of Antimicrobial Activity of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System

Streptococcus mutans KPSK$_2$ is a representative of bacteria in the dental plaque. The effectiveness of formulations against *Streptococcus mutans* KPSK$_2$ was evaluated using agar diffusion method. The efficacy was determined by the diameter of inhibition zone.

The principle of agar diffusion method is dependent upon the inhibition of the growth of bacteria on the surface of an inoculated agar plate, by the antimicrobial agent that diffuses into the surrounding medium.

Mueller Hinton Agar medium was prepared by adding 19 g of the agar to 500 ml of distilled water and boiled to dissolve the medium completely. After that the sterilization was performed by autoclaving at 121$\pm$0.5 °C for 15 min. Each agar plate was prepared by pouring 30 ml of the medium to Petri dish with a diameter of 10 cm and allowed to solidify at room temperature. The depth of agar was approximately 4 mm. Then the agar plates were perforated by using a 5 mm diameter cylindrical tube to make a hole for filling the samples.

The inoculum was prepared by picking the isolated 48 hr colonies of *Streptococcus mutans* KPSK$_2$ into broth and letting the inoculum grow for 12 hr. Then 5 ml of the inoculum was transferred to new broth (100 ml) and grown for 4 hr to reach mid-exponential growth phase. The inoculum was compared for the turbidity equivalent to the McFarland No.0.5 standard (1.5×10^8 CFU/ml) to provide an appropriate growth of the microorganism for testing the antimicrobial activity. The method for determining the turbidity of McFarland No.0.5 standard was done by using spectrophotometer. The cuvette of inoculum was placed in the spectrophotometer and read the absorbance at 600 nm, the absorbance of the inoculum should be approximately 0.015. If the suspension was too dense, the inoculum was diluted with additional sterile broth. The standardized inoculum suspensions should be used within 15 min of preparation.

After adjusting the turbidity of the inoculum suspension, a sterile swab was dipped into the suspension and rotated the swab several times with a firm pressure on

the inside wall of the flask above the fluid level to remove excess inoculum from the swab. The inoculum was swabbed over the entire agar surface in three different directions to ensure an even distribution of the inoculum. The plate was allowed to dry at room temperature for 5-10 min. The formulations were placed into the holes of agar about 4-5 holes/plate. Each formulation was performed in triplicate. Then the plates were incubated at 37 ± 0.5 °C for 24 hr in CO_2 water jacketed incubator (Forma Scientific, Inc., USA). After incubation, the diameters of the zones produced by antimicrobial inhibition of bacterial growth were measured using Vernier caliper (Komsri, 1997; Mahon and Manuselis, 2000).

F. Determination of Partition Coefficient of *Garcinia mangostana* Extract

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water

$$P_{ow} = \frac{C_{n\text{-octanol}}}{C_{water}}$$

The partition coefficient therefore is the quotient of two concentrations and is usually given in the form of its logarithm to base ten (log P).

The most common method for determining partition coefficient is the shake flask method. In this technique, the candidate drug is shaken between n-octanol and water layers, from which an aliquot is taken and analyzed.

Before the partition coefficient was determined, the two solvents were mutually saturated by shaking at the room temperature. Two stock bottles were shaken for 24 hr on a mechanical shaker, one containing n-octanol and a sufficient quantity of water, and the other containing water and sufficient quantity of n-octanol. Then the two bottles were let to allow the phase separation.

The test solution was prepared in n-octanol with a concentration of 5 mg/ml of mangostin. Five millilitres of the two solvents prepared as described above were filled

into screw-capped tubes. The tubes were continuously rotated at room temperature for 48 hr to achieve equilibrium. Then the tubes were let to allow the phase separation. The partition coefficient was determined by analyzing the concentrations of mangostin in both phases by using UV spectrophotometer at 243 nm. All partition coefficient determinations were carried out in triplicate (OECD 107, 1995).

G. Stability Study of *Garcinia mangostana* Extract Monoglyceride-Based Drug Delivery System

The stability study was performed by heating-cooling cycle. The samples were placed in a closed container and stored in a hot air oven at 45 ± 0.5 °C for 48 hr, and then placed in a refrigerator at 4 ± 0.5 °C for 48 hr. The heating-cooling cycle was repeated for 6 cycles. The physicochemical properties were determined in the same method as described in E1 and the determination of amount of mangostin was performed by HPLC method. The analysis of samples followed the method described in B3. The amount of mangostin was determined in triplicate at the initial and after the stability study.

H. Statistical Analysis

The data of viscosity, percentage of drug release, coefficient of determination of the release kinetic, the Higuchi release rate constant and amount of mangostin remaining after the stability study were analyzed by one-way analysis of variance (ANOVA). When a significant difference ($P<0.05$) was indicated, the data were subjected to multiple comparison by Fisher's least significant difference (LSD) test to compare the difference. The statistical package for the social sciences (SPSS) program version 12.0 was used in this study.