CHAPTER III

MATERIALS AND METHODS

Materials

1. Brij®30 (Polyoxyl 4 lauryl ether, The East Asiatic, Thailand)
2. Brij®52 (Polyoxyl 2 cetyl ether, The East Asiatic, Thailand)
3. Brij®72 (Polyoxyl 2 stearyl ether, The East Asiatic, Thailand)
4. Brij®98 (Polyoxyl 2 cetyl ether, The East Asiatic, Thailand)
5. Cholesterol (Lot. No. 111H8488, Sigma, USA)
6. Simulsol® M52 (Lot. No. T40351, Seppic, France)
7. 95% Ethanol (Lot. No. 46967, obtained from the Government Pharmaceutical Organization, Thailand)
8. Acetonitrile HPLC grade (Lab-Scan, Thailand)
9. Methanol HPLC grade (Lab-Scan, Thailand)
10. Dextrose (Lot. No. AD-0045, Purechem, Thailand)
11. Lactose, anhydrous 60 mesh grade (Lot. No. ET070200, New Zealand)
12. Sorbitol (Lot. No. M430197, Merck, Germany)
13. Gelatin capsules No. 0 (distributed by Samchai chemicals, Thailand)
14. Saquinavir mesylate (SQV, Lot. No. SM0040802, Hetero, India)

Equipment

1. Analytical balance (Model A200s, Satorius GmbH, Germany)
2. Flask shaker (Model SF1, Struart Scientific, UK)
3. Heating bath (Model B-490, Buchi, Switzerland)
4. Rotary evaporator (Model Rotarvapor R-205, Buchi, Switzerland)
5. Vacuum controller (Model B-721, Buchi, Switzerland)
6. Shaking incubator (LabTech, England)
7. Centrifuge (Model 5810, Eppendorf, Germany)
8. Ultracentrifuge (Model L-80, Beckman, USA)
9. Hot-air oven (Memmert, Germany)

10. Dissolution apparatus (Model VK7000, Varian , USA)
11. Optical light microscope (Model Eclipse E400, Nikon, Japan) equipped with an attached camera (Model FX-35DX, Nikon, Japan), a hot stage (Model FP82HT, Mettler Toledo, Switzerland) and a central processor (Model FP90, Mettler Toledo, Switzerland)
12. Laser diffraction particle size analyzer (Model Mastersizer 2000, Malvern Instruments, England)
13. Photon correlation spectrophotometer (Model ZetaPlus, Brookhaven Instruments, USA)
14. pH meter (Model 210A^{+}, Thermo Orion, Germany)
15. Moisture analyzer (Model HR83, Mettler Toledo, Switzerland)
16. Sieve shaker (Josef Deckelmann Aschaflenberg, Germany)
17. Tapped density tester (Model SVM22, Heusenstamm, Germany)
18. UV-visible spectrophotometer (Model V-530, Jasco, Japan)
19. High performance liquid chromatography (Model SCL-10A VP, Shimadzu, Japan) :
    - Degasser (Model DGU-14A, Shimadzu, Japan)
    - Pump A, B liquid chromatography (Model LC-10AD, Shimadzu, Japan)
    - Auto injector (Model SIL-10A, Shimadzu, Japan)
    - Column oven (Model CTD-10AS, Shimadzu, Japan)
    - UV-VIS detector (Model SPD-10A, Shimadzu, Japan)
    - System controller (Model SCL-10A, Shimadzu, Japan)
    - Analytical column (Hypersil®, 25 cm × 4.6 mm, C18, 5µm)
20. Scanning electron microscope (JSM-5410LV, Jeol, Japan)
21. Transmission electron microscope (Model JEM-200CX, Jeol, Japan)
22. Differential scanning calorimeter (Model DSC822^{e}, Mettler Toledo, Switzerland)
23. X-ray powder diffractrometer (Model D8 Discover, Bruker AXS)
24. Infrared spectroscopy (Model FT-IR 1760X, Perkin Elmer, Germany)

Methods

1. Characterization of saquinavir mesylate (SQV) and recrystallized SQV(R-SQV)

The physicochemical properties of SQV powder, a model lipophilic drug was characterized. In addition, the process variables, such as heat and solvent in niosome or proniosome preparation were also investigated for their effects on SQV properties. R-SQV was prepared by dissolving a 5 g of SQV in 100 ml ethanol. The mixture was then heated and sonicated to be a clear solution. Consequently, solvent was evaporated at 70°C under vacuum using a rotary evaporator for 1 h. R-SQV was then identified and examined the morphology, solid state morphology and thermal property.

1.1 Morphological study

SQV and R-SQV were dispersed in mineral oil and observed under a hot stage microscope with the magnification of ×10.

SQV and R-SQV were gold coated in a sputter coater and the surface morphology was observed using a scanning electron microscope with the magnification of ×75 and ×2000.

1.2 Particle size and size distribution

Size and size distribution of SQV was measured by a laser diffraction particle size analyzer. The measurement was performed in triplicate.

1.3 Flowability

The flow rate of SQV powder was measured by a flow through orifice method. Accurately weighed 10 g of SQV was placed in a glass funnel with 1.2-cm internal diameter of lower aperture. The powder was allowed to flow through the aperture. The time taken for the total amount of powder flows through the aperture at a fixed 10-cm height was recorded. The rate of flow was calculated in term of gram per second. The test was carried out in triplicate.

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1.4 Identification

SQV and R-SQV were identified to confirm the functional groups using infrared spectroscopy. Potassium bromide (KBr) disk method was selected in this study. The small amount of sample was mixed with dried KBr in a agate mortar and directly compressed to form a clear disk. The disk was then placed on the holder and subjected into the IR beamline. The IR spectrum was detected in the range of 4000 - 500 cm^{-1} at 150 KV accelerating voltage and recorded.

1.5 The solid state morphology

The solid state morphology of SQV and R-SQV was examined by a powdered X-ray diffractrometer. The pattern was recorded from 5° - 40° terms of 2-theta angle.

1.5 Thermal property

Thermal property of SQV and R-SQV was investigated using a differential scanning calorimetry (DSC) technique. An accurately weighed 5 mg of drug was placed into a 40-μl pan. The thermogram was recorded as the temperature was raised with the rate of 10°C/ min, starting from 25°C to 300°C.

1.6 Solubility

SQV solubility in different media, i.e. water, 0.1 N hydrochloric acid and phosphate buffer pH 6.8 solution was determined at 37°C. Excess amount of SQV (approx. 1 g) was weighed into 50 ml-test tubes, and a 30 ml of each medium was then added. The tubes were incubated in a shaking incubator at 37°C ± 0.5°C with a shaking speed of 120 rpm. The supernatant of 3 ml was periodically withdrawn and filtered. The drug concentration of the filtrate in all media was analyzed by a UV-spectrophotometer at the maximum wavelength of 240 nm, excepted for that in 0.1N hydrochloric acid was analyzed by a UV-spectrophotometer at the maximum wavelength of 242 nm. The experiment was carried out until the constant drug concentration measured was obtained. The solubility of the drug in each medium was determined in triplicate.

2. Preparation of niosomes

Niosomes were prepared by hand shaking method (Pillai and Salim, 1999). In general, a nonionic surfactant was co-dissolved with or without cholesterol and Simulsol®M52 in 95% ethanol. Ethanol was then evaporated at 70°C for 1 h under vacuum using a rotary evaporator. The dried film was then rehydrated with aqueous medium and shaken for 30 min at 70°C or 1 h at 37°C. The total lipid/ surfactants concentration was 60 mM.

First attempt was made to investigate the effects of hydrophobic head groups and hydrophilic side chains of series of polyoxyethylene alkyl ether surfactants, i.e. Brij®30, Brij®52, Brij®72 or Brij®98 on niosome formation. Niosomes were prepared in 5 ml of water at above phase transition temperature, 70°C. The mole ratios of nonionic surfactant: cholesterol: Simulsol®M52 were 60:30:10, 45:45:10 and 30:60:10. Then the morphology and particle size and size distribution were studied. The nonionic surfactants that could form niosomes at 70°C were selected to form niosomes at 37°C, a body temperature.

To investigate the possibility to prepare niosomes for oral delivery, niosomes with given mole ratio of nonionic surfactant: cholesterol: Simulsol®M52 (45:45:10) were prepared by hydrating dried films with 15 ml of water, 0.1N hydrochloric acid or phosphate buffer pH 6.8 at 37°C, mimicking gastrointestinal fluid. Niosomes were prepared in various media using a flask shaker for 1 h and then the niosome dispersions were stored at room temperature, 4°C and 45°C for 2 weeks to observe their physical stability. The thermal property of freshly prepared niosomes dispersed in water was examined. The morphology and particle size distribution of freshly prepared niosomes and niosomes after storage for 1 week and 2 weeks were also investigated. A surfactant which gave particle size of niosomes which was able to be taken-up by Peyer's patches less than 10μm (Eldridge et al., 1990), was chosen for further study.

Encapsulation of saquinavir mesylate (SQV)

In order to encapsulate the drug, niosomes were prepared at 37°C by hand shaking method. Chosen nonionic surfactant, cholesterol and Simulsol®M52 were dissolved in ethanol. Two hundred-milligrams of SQV were then added to the lipid/ surfactants ethanolic solution. The clear mixed solutions were solvent evaporated at 70°C for 1 h under vacuum. The dried film was hydrated with 15 ml of water with a flask shaker for 1 h at 37°C.

When effects of lipid/ surfactants mole ratio on SQV solubility enhancement were studied, the total lipid/ surfactant concentration was constant at 60 mM. SQV niosomes which were prepared with the given mole ratios of 45:45:10, 90:0:10, 80:0:20 and 70:0:30 were dispersed in water at 37°C. The particle size and size distribution, total amount of solubilized SQV in niosomal dispersions and entrapment efficiency of niosomes prepared with different mole ratios of lipid/ surfactants were investigated. The mole ratio of lipid/ surfactants which could enhance entrapment efficiency to the greatest extent was chosen to study the effects of lipid/ surfactant concentration.

Effects of lipid/ surfactants concentration on SQV solubility enhancement was studied by varying lipid/ surfactants concentrations of niosomes. SQV niosomes were prepared from chosen nonionic surfactant: cholesterol: Simulsol®M52 with the selected mole ratio that gave greatest entrapment efficiency of niosomes. The various concentrations of lipid/ surfactants, i.e. 60, 120, 180 and 300 mM were used to prepare niosomes in water at 37°C. The amount of SQV entrapped in niosomes was investigated. According to the results of SQV solubility enhancement, the niosomal system, which required minimum amount of lipid surfactant and provided the greatest ratio of encapsulated drug to free drug, was chosen for further study.

To study the effects of hydrating medium on formation of niosomes, the dry film were hydrated with various media, i.e. water, 0.1N hydrochloric and phosphate buffer pH 6.8 at 37°C. The selected lipid/ surfactant mole ratio and concentration from previous studies was employed. SQV niosomes were prepared by direct adding

405 mg of SQV to ethanolic solution of lipid/ surfactants. Then ethanol was evaporated at 70°C under vacuum. The dried film was hydrated with 90 ml different media. All samples were left overnight at 4°C before characterization.

3. Characterization of niosomes

3.1 Morphological study

Niosomal dispersions were mounted on a glass slide and observed under an optical microscope at 2.5 × 40 magnification.

The dispersions of niosomes dispersed in water at 37°C were also stained with 1% phosphotungstic acid on a grid and allowed to dry. Niosomes were then viewed under a transmission electron microscope at 100 KV.

3.2 Particle size and particle size distribution

Size and size distribution of niosomes containing cholesterol (45:45:10 mole ratio) were determined by a laser diffraction particle size analyzer. Non-cholesterol niosomes (90:0:10, 80:0:20 and 70:0:30) were analyzed for size and size distribution using a photon correlation spectrophotometer because a laser diffraction particle size analyzer has the lower limit of 0.2 μm in measurement. The samples were redispersed before measured. The measurement was performed in triplicate.

3.3 Phase transition temperature

Phase transition temperature of niosomal dispersion prepared in water at 37°C was studied by a differential scanning calorimeter. Accurately weighed 10 mg of niosomal dispersion prepared in water to a 40-μl pan. The temperature of the pans of niosomal dispersions was raised from 0°C to 300°C, at a rate of 10°C/ min. Where appropriate, the temperature of the pan was raised from -20°C to 150°C.

3.4 SQV entrapment efficiency

The excess saquinavir mesylate (SQV) powder was separated from niosomal dispersion by centrifugation at 4000 rpm at ambient temperature for 30 min. The niosomal dispersion was then ultracentrifuged at 65000 rpm at 4°C for 6 h in order to separate the encapsulated SQV from SQV dissolved in the supernatant. The amount

of SQV in supernatant of water and phosphate buffer pH 6.8 was analyzed by UV-spectroscopy at 240 nm, while that of 0.1N hydrochloric acid was detected at 242 nm. The retained pellets were disrupted by addition of ethanol. The solution was analyzed for the amount of niosomally entrapped SQV by UV-spectroscopy at the maximum wavelength of 240 nm, except when 0.1N hydrochloric acid was used as medium.

Percentage of saquinavir mesylate (SQV) entrapped in niosomes of each preparation was determined by equation (2):

$$\%Entrapment = \frac{SQV_{pellets}}{SQV_{dispersion}} x100 \qquad (2)$$

$SQV_{pellets}$ = amount of SQV in pellets

$SQV_{dispersion}$ = total amount of solubilized SQV in niosomal dispersion (drug dissolved in supernatant + drug encapsulated in niosomal pellets)

Entrapment efficiency was defined as the mole fraction of saquinavir mesylate (SQV) in pellets devided by the certain amount of lipid/ surfactants used. Entrapment efficiency was expressed in terms of mole of encapsulated SQV per mole of total lipid/ surfactants as in equation (3):

$$Entrapment \ \ efficiency = \frac{mole_{pellets}}{mole_{lipid / surfac \tan ts}} \qquad (3)$$

$mole_{pellets}$ = mole of SQV in pellets

$mole_{lipid / surfac \tan ts}$ = mole of total lipid/ surfactants in niosomal dispersion

Both percent entrapment and entrapment efficiency were averaged from three measurements.

The percent drug recovery was also examined. The total amount of drug dissolved in niosomal pellets and clear supernatants was compared to the amount of the drug added into the systems.

3.5 Stability of SQV niosomes

The stability profiles of SQV niosomes prepared with nonionic surfactant: cholesterol: Simulsol®M52 with the mole ratio of 70:0:30 were studied in three different media: water, 0.1N hydrochloric acid and phosphate buffer pH 6.8. Ninety milliters of niosomal dispersions containing excess amount of SQV was prepared. The undissolved SQV was separated from dispersions by centrifugation at 4000 rpm at ambient temperature for 30 min. Then, 10 ml of niosomal dispersions were pipetted into 8 separate test tubes. The samples were incubated in a shaking incubator at 37°C with a shaking speed of 120 rpm for 12 h. At each time interval (0, 0.5, 1, 2 ,4, 6, 8 and 12 h), 6 ml aliquot from one tube was withdrawn and ultracentrifuged at 4°C, 65000 rpm for 6 h. The clear supernatant was withdrawn and analyzed for the amount of SQV released by UV-spectroscopy at 240 nm, except when 0.1N hydrochloric acid was as medium. The release test was examined in triplicate.

4. Preparation of proniosomes

Proniosomal granules were prepared by mixing 60 ml niosomes (70:0:30 nonionic surfactant: cholesterol: Simulsol®M52) with a water soluble carrier, i.e. lactose, dextrose and sorbitol. The mixture was then dried in a hot air oven at 70°C for 24 h. The dried samples were screened through a 30-mesh screen to obtain proniosomal granules. The selected formulation was the one requiring the least amount of lactose to form dried granules with the moisture content less than 2%.

The possibility to form proniosomal granules was investigated by mixing of niosomes with varied amount of lactose used as a carrier. The amount of lactose used was 15, 20, 25, 30 and 35 folds of the amount of dissolved SQV, i.e. total amount of free and niosomally entrapped SQV, in niosomal dispersion which was determined by the methods described in 3.4. The amount of niosomal dispersion was constant (1ml) in all formulations (**Table 3**). In addition, dextrose or sorbitol was also used as

carriers in a weight ratio of SQV: carrier 1:25, in order to compare with proniosomes using lactose as a carrier.

Table 3. Proniosomes obtained by mixing plain niosomal dispersion with lactose and subsequent oven drying

Formulation	Ratio of dissolved SQV : lactose
NL15	1 : 15
NL20	1 : 20
NL25	1 : 25
NL30	1: 30
NL35	1 : 35

SQV = Saquinavir mesylate

Preparation of SQV proniosomal granules

An appropriate amount of lactose was wetted with 1ml of niosomal dispersion 60 mM of 70:0:30 mole ratio of nonionic surfactant: cholesterol: Simulsol®M52 with loaded SQV (formulation SQV-NL) or without SQV, i.e. plain niosomal dispersion (formulation NL). The mixture of these formulations was then oven dried to form proniosomes. The final concentrations of SQV and lipid/surfactants in dried proniosomes were 2.66% and 30.77% w/w, respectively.

In order to investigate the effects of lipid/ surfactants incorporating method on the formation of SQV proniosomal granules, proniosomal granules were also prepared by wetting of lactose with a clear alcoholic solution of lipid/ surfactants with SQV (formulation SQV-AL) or without SQV (formulation AL), as described in **Table 4.** The final concentration of SQV and lipid/surfactants was kept constant as described for previous method. The mixture of niosomes or lipid/surfactant solution and lactose with/without SQV was oven dried at 70°C for 24 h. The dried mass was then screened through a 30 mesh sieve.

Table 4. The compositions of proniosomal granules

Formulation	Description
AL	Alcoholic solution of lipid/ surfactants mixed with lactose
SQV-AL	Alcoholic solution of SQV and lipid/ surfactants mixed with lactose
NL	Plain niosomal dispersion mixed with lactose
SQV-NL	SQV niosomal dispersion mixed with lactose

SQV = Saquinavir mesylate

5. Characterization of proniosomes

5.1 Morphological study

Morphology of proniosomes was investigated by a scanning electron microscopy. Proniosomal granules were gold coated in a sputter coater and the surface morphology was observed using a scanning electron microscope with the magnification of ×2000.

Proniosomes rehydrated with various media, i.e. water, 0.1N hydrochloric acid or phosphate buffer pH 6.8 were observed for their transformation of proniosomes to niosomes. The samples were observed under a light microscope with the magnification of × 100 and a transmission electron microscope at 100 KV.

The proniosomal granules about 10 mg were mounted on a glass slide and suspended with a drop of different media. Niosome formation was observed under an optical microscope at 2.5×40 magnification. On the other hand, diluted proniosomal dispersion in various media was performed to observe the existence of niosome formation from proniosome granule under diluted condition. Accurately weigh 312 mg of proniosome granules were directly suspended in 1000 ml of testing media at 37°C with the aid of continuous stirring. The diluted samples were withdrawn and transferred onto glass slide and then the photomicrographs were recorded by a light microscope. Moreover, the dilute samples were also viewed and recorded their morphology under a transmission electron microscope at 100 KV.

5.2 Particle size and size distribution

Size and size distribution of proniosomal granules was determined by sieve analysis. Eighty grams of granules was placed on the top of sieve set (aperture of 1.41, 1 and 0.595 mm), which was assembled on a shaker. The sieves were allowed to shake for 30 min. The amounts of granules retained on each sieve were weighed and calculated as percentage based on total granule weight (80 g).

5.3 Flowability

The flow rate of proniosomal granules was measured by a flow through orifice method. Accurately weighed 10 g of granules was filled into a glass funnel having the lower aperture with 1.2-cm internal diameter. The granules were allowed to flow through the aperture. The time taken for the total amounts of granules flowed through the aperture for a fixed 10-cm height was recorded. The test was carried out in triplicate.

5.4 Bulk density and tapped density

Ten grams of proniosomal granules were weighed into a 25 ml-graduated cylinder. The cylinder was then tapped by tapped density tester. The volume of proniosomes before and after tapping was recorded as bulk volume and tapped volume, respectively. The bulk density (ρ_b) and tapped density (ρ_t) were calculated in triplicate. The Carr's compressibility, which expresses the flow property as presented in **Table 5**, was calculated using the following equation. The measurement was done in triplicate.

$$\text{The Carr's compressibility} = \frac{(\rho t - \rho b)}{\rho t} x100 \qquad (4)$$

, where ρt is bulk density and ρb is tapped density

Table 5. Classification of flowability by Carr's Indices (Davies, 2001)

Carr's Index (%)	Flow
5-12	Free flowing
12-16	Good
18-21	Fair
23-33	Poor
35-38	Very poor
>40	Extremely poor

5.5 Loss on drying

Moisture content of proniosomal granules was measured in duplicate by a moisture analyzer. Percentage of moisture content was calculated in terms of percent loss on drying.

5.6 Identification

Proniosomal granules were identified to confirm the functional groups using infrared spectroscopy. Potassium bromide (KBr) disk method was selected in this study. The small amount of sample was mixed with dried KBr in agate mortar and directly compressed to form a clear disk. The disk was then placed on the holder and subjected into the IR beamline. The IR spectrum was detected in the range of 4000 - 500 cm^{-1} at 150 KV accelerating voltage and recorded.

5.7 Solid state morphology

The interaction of proniosomal components was examined by using an X-ray diffractrometer. The X-ray pattern was recorded from 5°- 40° in terms of 2-theta angle.

5.8 Thermal property

Thermal property of proniosomes was investigated using differential scanning calorimetry technique. An accurately weighed 10 mg of proniosomes was placed into a 40-µl pan. The temperature was raised from 25°C to 300°C with the rate of 10°C/ min. The DSC thermogram of sample were compared with those of SQV , R-SQV, lactose, Brij®30, Simulsol®M52 and the physical mixture of SQV and lipid/ surfactants.

5.9 Drug content

An exact amounts of various proniosomes **(Table 6)** containing 12 mg of SQV was weighed and filled in gelatin capsules No. 0.

The drug content of saquinavir mesylate in capsules was analyzed by mean of absorption peak area from HPLC method. The analytical method validation of analysis of saquinavir mesylate by HPLC is described in **Appendix (II).**

Table 6. Capsules filled with various proniosome formulations or physical mixture of powder

Formulation	Description
A	SQV mixed with lactose
B	R-SQV mixed with lactose
SQV-C	SQV niosomal dispersion mixed with lactose (SQV-NL)
SQV-D	Alcoholic solution of SQV and lipid/ surfactants mixed with lactose (SQV-AL)

SQV = Saquinavir mesylate

5.10 HPLC conditions

The chromatographic conditions were slightly modified from HPLC condition adapted by Armbruster et al. (2001).

Column	:	Hypersil®, 25 cm × 4.6 mm, C18, 5μm
Detector	:	UV detector, detected at 240 nm
Flow rate	:	1 ml/min
Injection volume	:	20 μl
Mobile phase	:	0.15 M potassium phosphate: acetonitrile (volume ratio of 55: 45, pH 3.45)

Preparation of mobile phase

Potassium phosphate 20.4106 grams was dissolved in 1000 ml ultrapure water. The solution was mixed with acetonitrile (volume ratio 45:55) and pH adjusted to 3.45 with dropwise addition of hydrochloric acid. The solution was filtered through a 0.45 μm membrane filter and degassed by sonication for 1 h prior to use.

5.10.1 Calibration curve of SQV

The stock solution of 500 μl/ml saquinavir mesylate was prepared by dissolving 25 mg of saquinavir mesylate in methanol and volume was adjusted to 50 ml with methanol.

Standard solutions were prepared by pipetting 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml into 10 ml-volumetric flasks and diluted and volume adjusted with methanol. The final concentrations of standard solutions were 5, 10, 20, 30, 40 and 50 μl/ml, respectively.

5.10.2 Assay of SQV content in capsules

Twenty capsules of each formulation in **Table 6** were randomly collected and the capsule shells were removed. Proniosome granules were mixed and 13 mg of granules which was equivalent to 1 mg of saquinavir meylate accurately weighed into 50-ml volumetric flask. The granules were dissolved in methanol, sonicated for 1 h and filtered through a 0.45 μm membrane. The concentration of the sample was 20 μg/ml. Saquinavir mesylate content was analyzed in triplicate.

5.10.3 SQV released from proniosomal granules

5.10.3.1 Calibration curve of SQV

Calibration curves of saquinavir mesylate in water, 0.1N hydrochloric acid and phosphate buffer pH 6.8 solution were performed. Saquinavir mesylate 25 mg was accurately weighed into a 50 ml-volumetric flask and dissolved with ethanol. The solution was sonicated for 30 min, adjusted to volume with ethanol and was used as standard stock solutions. The standard stock solutions were pipetted and diluted to the final concentration range of 4-12 μg/ml with various media as mentioned above.

Standard solutions of all media were analyzed for the amount of saquinavir mesylate by UV-spectrophotometer at the maximum wavelength of 240 nm, excepted for those of 0.1N hydrochloric acid which were analyzed at 242 nm. The relationship between absorbances and concentrations was determined by linear regression analysis.

5.10.3.2 Dissolution profiles

The dissolution of SQV proniosome capsules was studied by using USP 27 standard apparatus II (paddle) at 37°C ± 0.5°C with a rotation speed of 50 rpm. The release of SQV from proniosome capsules **(Table 6)** was studied in three media, i.e. 1000 ml of deionized water, 0.1 N hydrochloric acid and phosphate buffer pH 6.8 solution.

The dissolution medium of 10 ml was withdrawn at the time intervals of 10 min, 20 min, 30 min, 1, 2, 4, 6, 8 and 12 h, except for 0.1 N hydrochloric acid, the medium was withdrawn in the first 4 h. The percentage of drug release in triplicate was determined by UV- spectrophotometer at the maximum wavelength of 240 nm.

In addition, the dissolution study of the physical mixture of SQV or R-SQV and lactose filled in capsules **(Table 6)** was performed as the same condition.

5.10.3.3 Data analysis

The pattern of drug release possibly exhibit in many patterns, but the most often used release profiles are categorized into three models as follow:

Zero-order model

An ideal controlled release device which can deliver the drug with a constant rate until the device is exhausted of drug has release pattern as zero-order kinetics. The equation of zero-order model is given as:

$$\frac{dM_t}{dt} = k \qquad (5)$$

, where M_t is the percentage of active ingredient released in time t, t is time and k is the zero-order constant.

First-order model

The release rate in first order model is proportional to active ingredient remained in the device. The rate is given as:

$$\frac{dQ_t}{dt} = k(Q_0 - Q_t) \qquad (6)$$

, where Q_0 is the initial amount of drug, Q_t is the amount of drug release and k is the first-order rate constant.

First-order model can be predicted by equation (6) by plotting the log of the drug remained against time (Schwartz et al, 1968). The initial curvature can be attributed to the presence of surface drug and can be ignored.

$$\log Q = \frac{kt}{2.303} + \log Q_0 \qquad (7)$$

, where Q is the amount of drug left, Q_0 is the initial amount of drug, k is the first-order rate constant and t is time.

Higuchi model

Higuchi model is the one of common release pattern referred to as the square root of time release, in accordance with following equation (Higuchi, 1963).

$$Q = \frac{[D \in (2A - \in C_s)C_s t]^{\frac{1}{2}}}{\tau} \qquad (8)$$

Where Q = the amount of drug released per unit surface area

D = the diffusion coefficient of the drug in the release medium

$\in$ = the porosity of the device

A = the total amount of drug in the device per unit volume

C_s = the solubility of drug in the release medium

t = time

In general Higuchi equation is presented as a following equation.

$$Q = kt^{1/2} \qquad (9)$$

, where Q is the percentage of drug released in time t, t is time and k is the release rate constant.

The dissolution data were fitted according to zero-order, first-order or Higuchi model. The percentage of dissolved drug (%Q) or log of remained drug in the system were plotted as a function of time (t) for zero order and first order release kinetic respectively. Meanwhile the relationship between %Q versus square root of time ($t^{1/2}$) was employed in the case of Higuchi model. The linear regression method was used to determine the suitable drug release kinetics. Straight line of the above relationship occurred in the appropriate kinetic model and thus provided the correlation of determination (r^2) value that is closed to 1.

The extent of drug release may be compared using area under the curve (AUC), calculated by the trapezoidal rule using the following equations.

$$\text{Total AUC} = \text{sum of } \left[\frac{1}{2} \times (\text{\%drug release at lower time interval} + \text{\% drug released at higher time interval}) \times \text{time interval}\right] \quad (10)$$

5.10.4 SQV Stability study

The SQV-C capsules, capsule filled with the dried mixture of SQV niosomal dispersion and lactose, were kept at room temperature and 45°C ± 2°C with 75 ± 5% RH for 4 months (จุไรรัตน์ รักวาทิน, 2547). The capsules were examined for saquinavir mesylate content at the time intervals of 1, 2, 3 and 4 months.

Twenty capsules of each formulation were randomly collected and the capsule shells were removed. Proniosome granules were mixed and accurately weighed (13 mg) into 50-ml volumetric flask which was equivalent to 1 mg of saquinavir mesylate. The granules were dissolved in methanol, sonicated for 1 h and filtered through a 0.45 µm membrane. Saquinavir mesylate content was analyzed in triplicate.