CHAPTER III

MATERIALS AND METHODS

Materials

- Minocycline hydrochloride (Lot no. MLD-00-004 & 005, supplied by Wyeth-Ayerst, Thailand)
- Poly (L-lactide) MW. 85,000-160,000 (Lot no. 99H0670, SIGMA, Germany)
- Poly (DL-lactide) MW. 106,000 (Lot no. 98H0951, SIGMA, Germany)
- Poly (DL-lactide-co-glycolide) 75 : 25 MW. 90,000-126,000 (Lot no. 31K1589, SIGMA, Germany)
- Poly (DL-lactide-co-glycolide) 50 : 50 MW. 59,000 (Lot no. 40K0731, SIGMA, Germany)
- Sodium carboxymethylcellulose (MV) (Lot no. E3103/294, Wendt Cheme, Germany)
- Hydrolysed polyvinyl alcohol MW. 125,000 (Lot no. 29791, BDH Chemicals Ltd., England)
- Dichloromethane (Lot no. K20245050 340, Merck, Germany)
- Boric acid (Lot no. 547 K 1033265, Merck, Germany)
- Di-sodium tetraborate decahydrate (Lot no. 203 A 612308, Merck, Germany)
- Sodium chloride (Lot no. K 28555404, Merck, Germany)
- N,N-Dimethylformamide (Analysis no. 346411/1 595, Fluka, Switzerland)
- Distilled water

Instruments

- Stirrer (RW 10 R, IKA-RHURWERK, Germany)
- Vacuum pump (DOA-V130-BN, Waters, USA)
- pH meter (Orion Model 420A, Orion Research Inc., USA)
- UV spectrophotometer (Milton Roy Spectronic 3000 ARRAY, Item 33560, Milton Roy Company, USA)
- Water bath shaker (DK-3450, Heto Lab Equipment, Allerod, Denmark)
- Scanning electron microscope (Model JSM-6400, Jeol Co., Ltd., Japan)
- Laser particle size analyzer (Mastersizer-s long bed Ver. 2.19, Malvern Instruments Ltd., UK)
- Gas chromatography (HP 5890 Series II, Hewlett Packard, USA)

Methods

1. UV Analysis

The UV spectrophotometric method used to determine amount of minocycline hydrochloride was validated under the following condition (Gorog, 1995).

1.1 Specificity

The specificity of the UV spectrophotometric method was evaluated by comparing the spectrum of vehicle used to prepare standard solutions, minocycline hydrochloride and other components of a combined formulation. The maximum absorbance peak of drug must not be interfered by those of other compounds.

1.2 Linearity

The linearity was determined by plotting the standard curve between absorbance of minocycline hydrochloride prepared to contain 2, 4, 9, 14, 19, and 24 μg/mL in isotonic borate buffer pH 7.5 ± 0.1 and concentration in μg/mL of minocycline hydrochloride. Then the standard curve was fitted using linear regression analysis. The coefficient of determination (R^2) and the equation for the line were calculated.

1.3 Accuracy

The determination of accuracy of minocycline hydrochloride assayed by UV spectrophotometric method was done by analyzing the percent recoveries of three sets of 2, 12, and 22 μg/mL minocycline hydrochloride solution. The percent recovery of each set was calculated by dividing the estimated concentration from a calibration curve by the known concentration. The mean, standard deviation, and percent coefficient of variation (%C.V.) were determined.

1.4 Precision

1.4.1 Within-run precision

The determination of within-run precision of minocycline hydrochloride assayed by UV spectrophotometric method was done by analyzing three sets of each concentration in the same day. The mean, standard deviation (SD), and percent coefficient of variation (%C.V.) of the absorbance of minocycline hydrochloride of each concentration were determined.

1.4.2 Between-run precision

The determination of between-run precision of minocycline hydrochloride assayed by UV spectrophotometric method was done by comparing the absorbance of minocycline hydrochloride of three sets of each concentration for three different days. The mean, standard deviation (SD), and percent coefficient of variation (%C.V.) of each concentration were determined.

1.5 Limit of Quantitation

The determination of limit of quantitation was done by analysing the lowest amount of minocycline hydrochloride in a sample that can be determined with acceptable precision and accuracy.

2. Preparation of Minocycline Hydrochloride Microcapsules by Solvent Evaporation Technique

The microcapsules of minocycline hydrochloride were prepared by w/o/w solvent evaporation technique modified from a method described by Esposito et al. (1997). Poly (L-lactide) was dissolved in 15 mL of dichloromethane. Minocycline hydrochloride 0.2 g was dissolved in 15 mL of sodium carboxymethylcellulose (SCMC)

solution and then dispersed into the polymer solution. The dispersion was emulsified into 150 mL of hydrolysed polyvinyl alcohol (PVA) solution (0.25% w/v) and maintained under mechanical stirring at a controlled stirring rate of 300 rpm at room temperature. The stirring was continued for 3 hours. Dichloromethane was completely removed by evaporation and the microcapsules were separated from the solution by vacuum filtration. The filtered microcapsules were then washed twice with 20 mL of distilled water. The microcapsules were then collected, dried at elevated temperature not more than 40 °C overnight and stored in a desiccator for further studies.

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

2.1 Effect of Polymer Type and Stabiliser Concentration

Microcapsules of minocycline hydrochloride were prepared by the same method with varying types of polymer and stabiliser concentrations, using poly (L-lactide) (L-PLA), poly (DL-lactide) (DL-PLA), poly (DL-lactide-co-glycolide) 75:25 (PLGA 75:25), and poly (DL-lactide-co-glycolide) 50:50 (PLGA 50:50) and 0, 0.05, 0.10, and 0.20 % w/v sodium carboxymethylcellulose (SCMC) in water. The other parameters were fixed, i.e., 1:5 core to wall ratio, 0.25% w/v PVA, and stirring rate of 300 rpm. The parameters involved are shown in Table 5.

The appropriate concentration of SCMC was selected from the preparation with the highest core entrapment for each coating polymer. Results were evaluated using split-plot design and randomized block design. John Tukey's Hornestly significant difference method was used to test multiple comparison.

Table 5 The parameters used in the preparation of minocycline hydrochloride microcapsules with varying types of polymer and stabiliser concentrations

Coating polymer	SCMC concentration (%w/v)	Core to wall ratio	PVA concentration (%w/v)	Stirring rate (rpm)
L-PLA	0%	1:5	0.25	300
	0.05%	1:5	0.25	300
	0.10%	1:5	0.25	300
	0.20%	1:5	0.25	300
DL-PLA	0%	1:5	0.25	300
	0.05%	1:5	0.25	300
	0.10%	1:5	0.25	300
	0.20%	1:5	0.25	300
PLGA 75:25	0%	1:5	0.25	300
	0.05%	1:5	0.25	300
	0.10%	1:5	0.25	300
	0.20%	1:5	0.25	300
PLGA 50:50	0%	1:5	0.25	300
	0.05%	1:5	0.25	300
	0.10%	1:5	0.25	300
	0.20%	1:5	0.25	300

2.2 Effect of Core to Wall Ratio

Microcapsules of minocycline hydrochloride were prepared by the same method with varying core to wall ratios for each type of polymer. The other parameters were fixed, i.e., stabiliser concentration (from the result of 2.1), 0.25% w/v PVA, and stirring rate of 300 rpm. The parameters involved are shown in Table 6.

Table 6 The parameters used in the preparation of minocycline hydrochloride microcapsules with varying core to wall ratios

Coating polymer	SCMC concentration (%w/v)	Core to wall ratio	PVA concentration (%w/v)	Stirring rate (rpm)
L-PLA	from the result of 2.1	1:5	0.25	300
	from the result of 2.1	1:1	0.25	300
	from the result of 2.1	5:1	0.25	300
DL-PLA	from the result of 2.1	1:5	0.25	300
	from the result of 2.1	1:1	0.25	300
	from the result of 2.1	5:1	0.25	300
PLGA 75:25	from the result of 2.1	1:5	0.25	300
	from the result of 2.1	1:1	0.25	300
	from the result of 2.1	5:1	0.25	300
PLGA 50:50	from the result of 2.1	1:5	0.25	300
	from the result of 2.1	1:1	0.25	300
	from the result of 2.1	5:1	0.25	300

The percent yield, percent minocycline hydrochloride content, core entrapment, morphology, particle size and the release characteristics of microcapsules were characterised. The appropriate core to wall ratio and coating polymer were selected

from these characteristics. A randomized block design was used to test the significant differences of the results.

3. Physicochemical Characterization of Minocycline Hydrochloride Microcapsules

3.1 Yield of Microcapsules

The prepared microcapsules were accurately weighed. The percent yield of microcapsules was determined from equation 8.

$$\% \text{ Yield} = \frac{\text{Wt. of microcapsules (g)} \times 100}{\text{Theoretical wt. of microcapsules (g)}} \quad (6)$$

where,

Theoretical wt. of microcapsules (g) = Wt. of core (g) + Wt. of polymer (g)

3.2 Percent Content and Percent Core Entrapment

The percent content and percent entrapment of minocycline hydrochloride were determined using UV spectrophotometric method at 244 nm. Triplicate samples of microcapsules approximately 40 mg were accurately weighed, then dissolved in 2 mL of dichloromethane using vortex mixer for about 15 minutes. After completely dissolved, 2 mL of isotonic borate buffer pH 7.5±0.1 was used to extract minocycline hydrochloride using votex mixer for about 5 minutes. The mixture was centrifuged for 5 minutes at 5,000 rpm. The aqueous phase was transferred into a 10 mL flask. Five successive extractions were collected, diluted to volume and then assayed by UV spectrophotometric method at 244 nm. The amount of minocycline hydrochloride was determined from the standard curve. The percent content and entrapment of minocycline hydrochloride were calculated using equations (7) and (8), repectively.

$$\% \text{ Observed content} = \frac{\text{Assayed amount of minocycline hydrochloride (g) x 100}}{\text{Amount of weighed microcapsules (g)}} \quad (7)$$

$$\% \text{ Entrapment} = \frac{\% \text{ Observed content x 100}}{\% \text{ Theoretical content}} \quad (8)$$

where,

$$\% \text{ Theoretical content} = \frac{\text{Wt. of minocycline hydrochloride (g) x 100}}{\text{Wt. of minocycline hydrochloride (g) + Wt. of polymer (g)}} \quad (9)$$

3.3 Morphology

The morphology of the microcapsules was observed by scanning electron microscopy (SEM). The sample was coated with gold by ion sputtering under a high vacuum and high voltage. The coated samples were examined under SEM. The surface characteristics and shape of microcapsules were studied.

3.4 Particle Size and Size Distribution

The particle size and size distribution of the microcapsules were measured triplicately by laser particle size analyzer. The mode size was obtained.

3.5 The Release of Minocycline Hydrochloride from Microcapsules

The release studies of minocycline hydrochloride from microcapsules were modified from a method described by Esposito et al. (1997). Approximate weight of minocycline hydrochloride microcapsules containing 2 mg minocycline hydrochloride was transferred into 20 mL of isotonic borate buffer pH 7.5 $\pm$ 0.1 which was maintained at 37 $\pm$ 0.1°C and shaked at 10 rpm in a water bath shaker. Five milliliter samples were

withdrawn at predetermined time intervals and replaced with fresh medium for 48 hours. The samples were assayed by UV spectrophotometer at 381 nm.

The percent release of minocycline hydrochloride was plotted against time (hours) to obtain the release profile. The drug release profile was plotted according to zero order, first order and Higuchi plots. The release rate constant (k), coefficient of determination (R^2), and intercept of graph were calculated from release profile at 11-48 hours for elucidate the release kinetics of minocycline hydrochloride from microcapsules. Each release profile was determined with 6 units.

3.6 Determination of Residual Dichloromethane Content in the Microcapsules

Determination of residual dichloromethane content in the microcapsules was modified from a method described by Spenlehauer, Veillard, and Benoit (1986). A weighed quantity of microcapsules (22 mg) was dissolved in 2 mL of dimethylformamide. The solution was injected into a gas chromatography. Gas chromatographic conditions for determination of dichloromethane were as follows:

Column: HP OV-101 (100/120 mesh), 6Ft

Carrier gas: Nitrogen, flow rate 15 mL/min

Detector: FID

Hydrogen: Flow rate 35 mL/min

Air: 350 mL/min

Injector temperature: 150 °C

Oven temperature program: Initial temp. 40 °C, initial time 7 min, rate 45 °C/min, final temp. 200 °C, final time 5 min

The amount of residual dichloromethane was determined from the standard curve. The standard curve was plotted between peak area of dichloromethane which prepared to contain 5,10,15, and 20 ppm in dimethylformamide and concentration in ppm of dichloromethane.