CHAPTER II

MATERIALS AND METHODS

Materials

1. Model drug

- Diltiazem hydrochloride (Lot No. DIL/M- 021/01, Supported by Siam Pharmaceutical Industry Co., Ltd., Thailand)

2. Additives

- Carnauba wax (Lot No. CGAA 12/823, Japan, Supplied by Srichand United Dispensary Co.,Ltd., Thailand)
- Ethylcellulose 10 cps. (Lot No. MM960117-1, The Dow Chemical Company, USA)
- Glyceryl behenate (Compritol 888 ATO® , Lot No.24230, Gattefosse, France)
- Glyceryl monostearate (flake) (Lot No. 00918, Belgium , Supplied by Srichand United Dispensary Co.,Ltd., Thailand)
- Hydroxypropylcellulose (HPC-M® ,Lot No. CE-211 Nippon Soda Co.,Ltd., Japan)
- Lactose (DMV International, Holland)
- Microcrystalline cellulose (Avicel PH 101 ® Lot No. 10CO , ASAHI Chemical Industry Co.,Ltd.,

Japan)

- Triethyl citrate (Analysis No. 387266/1 34398, Fluka, Switzerland)

3. Chemicals

- Chloroform (Samchai Chemical , Thailand.)
- Hydrochloric acid (Lot No. H613KVAH, Mallinckrodt AR , USA)
- Sodium chloride (Lot No.7581MTVV , Mallinckrodt , USA)
- Trisodiumphosphate dodecahydrate (EEC.No. 231-509-8, Carloerba reagenti)

4. Equipment

- Analytical balance (Model A200S, Satorious, GmbH, Germany)
- Differential scanning calorimeter (Model DSC-7, Perkin Elmer, England)
- Dissolution apparatus (Model DT-6R, Erweka® , USA)
- Extruder (Model EXKS-1, Fuji paudal Co.,Ltd., Japan)
- Fluidized bed coater (Model STREA1, Niro-Aeromatic AG, Switzerland)
- Friabilator (Erweka TAR 20, Germany)
- Fourier transform infrared spectrometer (Model

SP2000, Perkin Elmer Ltd.,England)

- GC (Model GC Autosystem XL, Perkin Elmer Ltd.,England)
- Hot air oven (Model UL 80, Mammertt,Germany)
- Magnetic stirrer (Model SP46920-26, Cimarec 2, Thermolyne, USA)
- Moisture analyzer (MA30 satorious, Germany.)
- Peristaltic pump (Model 1B.1003/R, Roto consulta,Germany)
- pH meter (Model 292, Pye Unicam Ltd., England)
- Planetary mixer (EB 20 F Gypto Peerless Ltd., England)
- Scanning electron microscope (Model JSM-6400 LV, Jeol Ltd., Japan)
- Sieve shaker (Nr995941, Hessenwerk Darmstadt, Westerm, Germany)
- Spheronizer (Model S320, Niro Fielder, England)
- Ultraviolet spectrophotometer (UV-160A, Shimadzu, Japan)
- X-ray powder diffractometer (Model JDX-3530, Jeol Ltd., Japan)

Methods

Pelletization process

1. Preparation of DTZ HCl Pellets

Initially, core pellets without drug were prepared from the previous research of Padungkwan Chitropas,1995, by using extruder and spheronizer. The core pellets composed of lactose and Avicel PH 101® , which were also called diluent of the formulation. In addition , HPC-M® was used as a binder. The formulation of the core pellets is presented in Table 4

Table 4 Formulation of core pellets

Ingredients	%w/w
Lactose	58.8
Avicel PH 101®	39.2
HPC-M®	2.0
Water (base on dry basis)	40.0

The pelletization process was performed according to the following chart

Mix dry ingredients in planetary mixer

↓

Add water, until the mixture becomes damp mass

↓

Extruding into cylindrical segments

↓

Immediately roll into solid spheres on a spinning friction plate of spheronizer

↓

Dried in a hot air oven at 60 °C for 6 hours

The spheronizer was a laboratory type, which consisted of a friction plate 1.2 mm in height (H),1.8 mm in width (W), 3 mm in length (L) (Niro-Fielder, Model S320).The spheronizer speed was set at 900±10 rpm, the spheronization time were operated and optimum time chosen at 10 min.

The formulation and condition for DTZ HCl pellets were prepared from the previous research of Surachet Wattana, 1999, which composed of the ingredients and pelletization conditions as shown in Tables 5 and 6.

Table 5 Formulation of DTZ HCl pellets

Ingredients	%w/w
DTZ HCl	60.0
Avicel PH 101®	38.5
HPC-M®	1.5
Water (base on dry basis)	25.0

Table 6 The pelletization conditions

Factors	Pelletization conditions
Spheronization speed (rpm)	900±10
Spheronization time (min)	10
Mass load (g)	300

2. Evaluations of Uncoated DTZ HCl Pellets

The following physical properties of pellets were evaluated:

2.1 Morphology

Photomicrographs of pellet samples were taken with scanning electron microscope (SEM). The samples were coated with gold prior to microscopic examination using ion sputtering. As a result, the shape and surface of the core pellets were determined. The core pellets were also cross-sectioned for observation of the internal texture.

2.2 Particle size distribution

Particle size distribution of various formulations were determined by sieve analysis. The 100.0 g of pellets were put on the top of sieve series which have opens ranging from 1.40, 1.00, 0.84, 0.71 to 0.61 mm (14, 18, 20, 25 and 30 mesh) , respectively. The sieves were placed on the sieve shaker and shaken for 20 minutes. The results, which averaged from five determinations, were reported as percentage of weight retained on each sieve size.

2.3 Bulk density and tapped density

The bulk density and tapped density were determined from the weight of 40.0 g sample, carefully charged into a 100 ml graduated cylinder. The bulk volume was recorded and the bulk density was calculated. The pellets were tapped from the height of 3 inches

until a constant volume was obtained and tapped volume was recorded. Then, tapped volume was divided by weight to attain tapped density. Both densities were average from three determinations. The Carr's compressibility was calculated from the following equation

$$\% \text{ Carr's compressibility} = \frac{(T_d - B_d) \times 100}{T_d} \quad (7)$$

Where T_d and B_d are tapped and bulk density, respectively.

2.4 Flow rate and Angle of repose

The weight of 40.0 g pellets were filled in a glass funnel with 6 mm internal stem diameter, fixed on the clamp at 4.0 cm height. The time was record when the pellets start to flow until finish. Flow rate was calculated in g/min and angle of repose was calculated from the following equation.

$$Alpha = \tan^{-1} \frac{H}{R} \quad (8)$$

Where *Alpha* is the angle of repose : H and R are the height and radius of pellet pile, respectively. The results were average from three determinations.

2.5 Percent friability

Ten grams of core pellets retained on 14/20 mesh cut and five stainless spheres (each sphere weight 1.06 gm and diameter 6.35 mm), to increase the mechanical stress on the pellets, were filled into the PVC container. The container was firmly closed with the cap and rotated at 25 RPM for 4 minutes. After that, pellets finer than 20 mesh were sieved

out. The percent friability, average from two determinations, was calculated as percentage of weight loss from the following equation.

$$\text{Friability} = \frac{\text{weight loss}}{\text{Initial weight}} \times 100 \quad (9)$$

2.6 Moisture content

The moisture content of DTZ HCl pellets was determined by using Moisture Analyzer Ma30 Sartorious. The 7 g pellets were accurately weighed and uniformly spread in a thin layer of aluminium plate. Then, it was exposed to high temperature at 100 °C until constant weight was obtained. The results were obtained from the average of two determinations. The percentage of moisture content was calculated based on the following equation.

$$\% \text{ Moisture content} = \frac{(W_w - W_d)}{W_w} \times 100 \quad (10)$$

Where W_d is weight after drying and W_w is weight before drying

2.7 Determination of DTZ HCl content of core pellets

Weighed accurately about 3.0 g of 14/20 mesh cut of DTZ HCl pellets. Grind the contents thoroughly, and transferred an accurately weighed portion, equivalent to about 150 mg of DTZ HCl , to a 100 mL volumetric flask. Add approximately 60 mL of water , and sonicated the solution for 30 minutes. Shaked the resulting solution for 10 minutes to complete extraction. Adjusted with water to volume, and mix. Dilute the mixture to a

suitable concentration for UV spectrometry determination. The mixture was filtered and the filtrated was collected.

The absorbance of the filtrate was determined at 237 nm by using double beam UV-VIS spectrophotometer. Deionized water was used as a reference solution. The content of DTZ HCl pellets was calculated from a standard curve, triplicate assays were performed.

Coating process

1. Preparation of DTZ HCl Coated Pellets

1.1 Preparation suitable concentration and coating condition of the waxes coating

In preliminary study, Compritol 888 ATO® was chosen as a representative to collect the suitable concentration of the waxes coating solution by dissolving in chloroform and mixed for 10 min. The Compritol 888 ATO® solution were prepared at 2%, 5% and 7% w/w, respectively. Known weight of pellets (100 g) was transferred into the fluidized-bed coating apparatus (Model STREA 1, Niro Aeromatic), using bottom-spray coating process with Wurster column. The suitable concentration of the coating solution was adjusted by observing pellets appearance and minimized problems during the coating process.

A coating conditions were performed to obtain an optimal coating conditions such as inlet air temperature, outlet air temperature, atomizing air pressure, fan capacity, air volume, partition height and feed rate of coating solution. The maximum inlet air temperature which did not show agglomeration problems and achieve uniform waxes

distribution on the surface. Trials were performed using 40, 45 and 50 °C inlet air temperature (base on the waxes melting temperature). The 40 and 45 °C showed a wet and sticky pellets. The 50 °C pellets showed uniform smooth coated surface, therefore 50 °C was set as the inlet air temperature. A suitable setting of coating conditions are presented in Table 7. On completion of coating, pellets were continually fluidized for another 5 minutes to ensure complete removal of chloroform and complete drying.

Table 7 The coating conditions using bottom spray method

Coating conditions	
Inlet air temperature	50 ° C
Outlet air temperature	48-50 ° C
Atomizing air pressure	1.5 bar
Fan capacity	7
Air volume	125-130 m^3/h
Partition height	2 cm
Feed rate of coating solution	15 ml/min

1.2 Coating solution

The compositions of various kind of waxes and ethyl cellulose as the coating solution are presented in Table 8. A 5% w/w was selected to prepare the coating solution containing various amounts of waxes and ethyl cellulose with triethyl citrate (30% weight of polymer). The coating agents were dissolved in chloroform about 10 minutes and spray to 100 g of pellets. These formulations (F1-F16) were used for the study of the effect of percentage of the coating levels on the release profiles of DTZ HCl from the coated pellets

Table 8 The compositions of coating solution of DTZ HCl in preformulation studied.

Ingredients	Amount (g)											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Carnauba wax	5	5	5	-	-	-	-	-	-	-	-	-
GMS	-	-	-	5	5	5	-	-	-	-	-	-
Compritol88ATO®	-	-	-	-	-	-	5	5	5	5	5	5
Chloroform	95	95	95	95	95	95	95	95	95	95	95	95
% of coating levels	10	15	20	10	20	30	10	20	30	40	50	60

Table 8 The compositions of coating solution of DTZ HCl in preformulation studied (cont.)

Ingredients	Amount (g)			
	F13	F14	F15	F16
Ethylcellulose (EC)	5	5	5	5
Triethyl citrate (%of polymer)	3 0	30	30	30
Chloroform	95	95	95	95
% of coating levels	5	7	9	11

From preliminary study, when pellets were coated with Compritol 888 ATO® alone , it was observed that immediate release of the drug occurred from 10 and 20% coating levels. Hence, lead to further development to mix Compritol 888 ATO® with EC at various ratios according to the formulation shown in Table 9. Different percentage of coating levels of each ratio of the mixture was approximately equal to 10, 15 and 20% of pellets weight. The method to prepare the coating solutions involved:

1. Dissolving Compritol 888 ATO® in chloroform and mix for 10 minutes
2. Dissolving EC and triethyl citrate in chloroform and mix for 10 minutes

Mixing the solution from step 1 and step 2 for 10 minutes. The Compritol 888 ATO® and EC ratios in this study range from 1:1 to 4:1 in the final solution.

Table 9 The compositions of coating solution of DTZ HCl with the mixture of Compritol 888 ATO® and EC (1:1).

Ingredients (g)	Ratio of Compritol 888 ATO® : EC (1:1)		
	C1	C2	C3
Compritol 888 ATO®	5	7.5	10
EC	5	7.5	10
Triethyl citrate (% of polymer)	30	30	30
Chloroform (g)	190	285	380
% of coating levels	10	15	20

Table 9 The compositions of coating solution of DTZ HCl with the mixture of Compritol 888 ATO® and EC (2:1) (cont.).

Ingredients (g)	Ratio of Compritol 888 ATO® : EC (2:1)		
	C4	C5	C6
Compritol 888 ATO®	6.67	10	13.33
EC	3.33	5	6.67
Triethyl citrate (% of polymer)	30	30	30
Chloroform (g)	190	285	380
% of coating levels	10	15	20

Table 9 The compositions of coating solution of DTZ HCl with the mixture of Compritol 888 ATO® and EC (3:1) (cont.).

Ingredients (g)	Ratio of Compritol 888 ATO® : EC (3:1)		
	C7	C8	C9
Compritol 888 ATO® EC Triethyl citrate (% of polymer) Chloroform (g)	7.5 2.5 30 190	11.25 3.75 30 285	15 5 30 380
% of coating levels	10	15	20

Table 9 The compositions of coating solution of DTZ HCl with the mixture of Compritol 888 ATO® and EC (4:1) (cont.).

Ingredients (g)	Ratio of Compritol 888 ATO® : EC (4:1)		
	C10	C11	C12
Compritol 888 ATO® EC Triethyl citrate (% of polymer) Chloroform (g)	8 2 30 190	12 3 30 285	16 4 30 380
% of coating levels	10	15	20

All the coating processes were performed using fluidized bed coater under the conditions shown in Table 7.

2. Evaluation of Physicochemical Property of Cast Films

In order to study the physicochemical property, the dry films were prepared by blending Compritol 888 ATO® and EC in organic system. The following ratios of Compritol 888 ATO® to EC of 1:1, 2:1, 3:1, 4:1 were prepared as the total solid content of 5% w/w in chloroform. Then all of the mixtures were stirred constantly with a magnetic stirrer about 10 minutes. The mixtures were carefully poured onto the glass petridish and dried at 50 °C. The dried film was then removed from the petridish and kept in a desiccator at ambient temperature prior to use. The physicochemical property of dry film was determined using The powder x-ray diffraction analysis, infrared spectroscopy and differential scanning calorimetry.

2.1 The powder X-ray diffraction analysis

The different ratios of Compritol 888 ATO® and EC were prepared as previously described. X-ray diffractograms were obtained by using x-ray diffractometer model JDX-3530 (JEOL, Japan) with Ni-filtered CuK_{α} radiation, a voltage of 45 kV, and a current of 35 mA. The scanning speed was 6 ° /min over a 2θ range of 5-40°.

2.2 Infrared spectroscopy

The same ratio of the mixture of Compritol 888 ATO® and EC as described in section 2 were tested with infrared spectrophotometer. The IR spectra of the mixture of films were examined by using the potassium bromide disc (KBr) method in the range of 4000-400 cm^{-1}.

2.3 Differential scanning calorimetry

Thermal analysis was performed on the Compritol 888 ATO® alone and the mixture of Compritol 888 ATO® and EC at ratios of 1:1, 2:1, 3:1 and 4:1. These samples were tested using a Perkin Elmer DSC-7 differential scanning calorimeter. Aluminum pans and lids were used for samples and temperature calibration was performed using cyclohexane and indium as standards. An empty pan, sealed in the same way as the sample, was used as reference. Samples of 2 mg were accurately weighed into aluminum pans and then sealed. The samples were tested under nitrogen gas purge at a heating rate of 20 °C/min, in the temperature range from 40 to 250 °C.

3. Evaluation of the DTZ HCl Coated Pellets

3.1 Morphology

A scanning electron microscope was used to examine the structure of the coated pellets. The coated pellets were cut with scalpel for cross-section observation. All samples were coated with gold by using an ion sputter coater under vacuum. The shape and surface topography of the coated pellets were examined.

3.2 Determination of DTZ HCl content of coated pellets

The method of determination was similar to that of DTZ HCl uncoated core pellets as described in section 2.7. The film was calculated by the following equation.

$$F = \frac{U-C}{C} \times 100$$

Where F is the percent film based on weigh increased; U and C are the amount of the drug in 150 mg pellets of uncoated and coated DTZ HCl pellets, respectively.

3.3 Dissolution studies

Dissolution tests were performed according to Diltiazem Hydrochloride Extended Release Capsule USP 24 (for products labeled for dosing every 12 hours,Test 1) to measured a drug release and release characteristics of DTZ HCl coated pellets. The DTZ HCl coated pellets of each formulation were hand filled into hard gelatin capsules (fill weight 150 mg), and used for dissolution study with the USP Paddle Device. The USP paddle dissolution method was modified because the pellets had a tendency to float in the dissolution medium that cause variation for the dissolution profiles. To minimize this effect in preformulation studies, the pellets containing a total of 150 mg DTZ HCl were placed inside 3×2 cm diameter stainless-steel minibaskets with 40 mesh openning. The position of the basket was set and not interfering with the flow pattern of the medium. The paddle constantly stirred the dissolution medium at 100 rpm. The medium was 900 ml of deionized water and the temperature was maintained at 37±0.5 °C. Ten milliliters of specimen was withdrawn at the time interval of 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10 and 12 hours. The same quantity of medium was added immediately after each sampling to keep the volume of medium constant throughout the experiment. The release amount of DTZ HCl at any time interval was calculated from a calibration curve by UV-VIS spectrophotometer. A cumulative amount of drug release as a function of time was determined. Tolerances for Test 1 is expressed as the labeled amount of DTZ HCl($C_{22}H_{26}N_2O_4S \cdot HCl$) dissolved at the specified times that conform to acceptable criterion which are given below

Time (hours)	Amount dissolved
3	between 10% and 25%
9	between 45% and 85%
12	not less than 70%

Further dissolution study was performed to determine the effect of continuous pH change as a function of time on the release of the preparations. When the drug release study in purified water medium was completed some of DTZ HCl coated pellets formula that follow a criteria in the Diltiazem Hydrochloride Extended Release Capsule USP 24 (for products labeled for dosing every 12 hours, Test 1) was selected to examine in pH change study and mechanical impact study.

In the dissolution model with pH change method, hydrochloric acid buffer pH 1.2 (at first 2 hours) was prepared by following direction of preparing HCl acid standard buffer solutions pH 1.2 in USP 24 and after that adjust to pH 6.8 by adding trisodium phosphate $\cdot 12\ H_2O$ of 18.5 g. The operation was continued until completing 12 hours.

To evaluate the effect of mechanical impact on drug release from the coated pellets, the dissolution test apparatus used was the same as that described above. The release of DTZ HCl was examined in deionized water containing 20 polystyrene beads (diameter 6.35 mm, specific gravity, 1.05 g/cm^3). The sampling method and assay of released DTZ HCl concentration were performed as described above.

3.4 Calibration curve for determination of the drug dissolved

Calibration curves of DTZ HCl in various media were constructed to determine the amount of drug dissolved during determination of drug content and dissolution testing. Those curves are presented in the Appendix A.

The 200 mg DTZ HCl was accurately weighed and transferred to 100 ml volumetric flask, then adjusted to volume with water. This solution was used as stock solution 1 (concentration 2,000 mcg/ml) . Stock solution 1 was dilute to 20 mcg/ml and 40 mcg/ml for preparing stock solutions number 2 and 3. Stock solution 2 was diluted to 2, 4, 6, 8, 10 mcg/ml and stock solution 3 was dilute to 12 and 16 mcg/ml with water, respectively. The absorbances of dilute solutions 2-16 mcg/ml were determined at wavelength of 237 nm. In the case of other dissolution medium, construction of calibration curve by the same method but adjusting the volume and made blank solution with its medium.

3.5 Membrane thickness determination

The membrane thickness of the coated pellets were determined by obtaining the difference between the coated diameter and core pellets. Photographs of each samples were taken using a digital camera. Image enhancements and measurements were made using the system software (SemAfore computer program). Diameters of 300 pellets were measured for each sample, and the average diameter was calculated.

3.6 GLC analysis of residual chloroform in coated pellets

The procedure for detecting residual chloroform in coated pellets, grind 1 g of sample with 4 ml of ethanol, the suspension filtered through Whatman no. 40 filter paper, and 1 μl of the filtrate injected into a gas ghrematograph. The calibration curves of chloroform in ethanol were prepared using concentrations between 14.81 and 44.43 × 10^{-2} mg/ml. Gas chromatographic conditions for quantitation of chloroform used a Perkin-Elmer Model GC Autosystem XL gas chromatograph equipped with a flame ionization detector, Stabilwax® (0.25 mm × 30 m) capillary column containing packing phase G16, 0.25 μm. The flow rate was about 1.0 ml/min, injector temperature was 250 °C, detector temperature was 250 °C. Temperature programming: initial set point at 50 °C up to 100 °C with rate about 10 °C/min, after that up to 220 °C with rate about 20 °C/min, end time 11 mins.