

CHAPTER II

MATERIALS AND METHODS

1. Materials

1.1 Model drug

- Diltiazem HCl (Lot No.0690798, support by Siam Pharmaceutical)
- Herbesser[®] 90 SR. (Lot No. XB644, Tanabe, Japan)

1.2 Additives

- Castor oil (Lot No. OAF 11/5, Srichand united dispensary Co., Ltd., Thailand)
- Diethylphthalate (Analysis No. 325384/1 393, Fluka, Switzerland).
- Ethylcellulose 10 cps. (Lot No. MM 960117-1, The Dow Chemical Company, USA.)
- Hydroxypropylcellulose (HPC-M[®], Lot No. BD-191, Nippon Soda Co. Ltd., Japan)
- Microcrystallinecellulose (Avicel[®] PH101, Lot No. 1871, ASAHI Chemical industry Co., Ltd., Japan)
- Polyethyleneglycol 400 (Lot No. PID 24/796, England, Srichand united dispensary co.,ltd.)
- Triethylcitrate (Analysis No. 387266/1 34398, Fluka, Switzerland)

1.3 Solvents

- Ethyl alcohol anhydrous (Lot V569 MVDY, Mallinckrodt, USA)
- Methylene Chloride (Lot No. 9324-03, J.T.Baker, USA)

1.4 Dissolution medium

- Hydrochloric acid (Lot no. H613 KVBC, Mallinckrodt AR, USA)
- Potassium chloride (Merck, Germany)
- Sodium chloride (Lotno. K20420804, E.merck, Germany)
- Sodium sulphate (Lot no. TA518349843, E.merck, Germany)
- Trisodiumphosphate dodecahydrate (EEC.No. 231-509-8, Carloerba reagenti)

2. Equipments

- Analytical balance (A 200 S, Satorious, Germany)
- Analytical balance (Metler Toledo AG 204)
- Cryoscopic osmometer (Model Osmomat[®] O30-D, Gonotec, Germany)
- Dissolution apparatus (Hanson Research Model SR-2, USA)
- Extruder (Model EXKS-1, Fuji Paudal Co., Ltd., Japan)
- Fluidized bed air suspension (Model STREA1, Niro-AeromaticAG, Switzerland)

- Friabilator (Erweka TAR 20, Germany)
- Hot air oven (Mammertt, Germany)
- Image analyzer (Microscope, Video camera and program KS.400V2.00)
- Magnetic stirrer (Model SP46920-26, Barnstead/ hermolyne, USA)
- Moisture analyzer (MA 30 Sartorius, Germany)
- Peristaltic pump (Model 1B.1003/R, Roto Consulta, Germany)
- pH meter (Model 292 Pye Unicam , England)
- Planetary mixer (EB 20 F Gypto – Peerless Ltd., England)
- Scanning electron microscope (Model S2360N, Hitachi, Ltd., Japan)
- Sieve shaker (Nr 995941, Hessenwerk Darmstadt, Western Germany)
- Spheronizer (Model S320, Niro Fielder, England)
- Tensometer (Model 4301, Instron Corp., USA)
- Ultraviolet spectrophotometer (UV-160A Shimadzu, Japan)
- US standard sieve (Laboratory test sieve ASTM E 11, Endecotts. Ltd. USA)

3. Methods

3.1 Formulation of Core Pellets

The formulation and condition for DTZ HCl pellets preparation were modified from the previous research of Padungkwan Chitropas, 1995, which composed of the ingredients showed in the Table 4.

Table 4. The original placebo core pellets formulation.

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

However, the amount of lactose was substituted by DTZ HCl then the amount of water and binder concentration used were optimized to provide the best characteristic pellets. The suitable formulation of DTZ HCl 90 mg pellets was chosen and used as the model to produce the other doses of diltiazem hydrochloride pellets (i.e., 60, 45 and 30 mg pellets). The pellets were prepared under the conditions of spheronization speed of 900 ± 10 rpm, spheronization time at 15 min. The ingredients used in each formulation are presented in the Table 5.

3.2 Preparation Processes

Extrusion-spheronization process involve with five steps, these consist of dry mixing, granulation, extrusion, spheronization and drying. Details of preparation mentioned below.

DTZ HCl and Avicel[®] PH 101 were weighed and transferred to a planetary mixer, then carefully mixed for 5 minutes. Binder solution was poured onto the powder and the mixture was mixed until wet mass was obtained. The wet mass was transferred to extruder and screened through a 1.0 mm sieve at 26 rpm extruder speed to obtain extrudates. Then extrudates were transferred to spheronizer. The spheronizer consisted of a frictional 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. In preliminary study, the various spheronization times were operated and optimum time was chosen at 15 min then DTZ HCl pellets in each concentration were obtained. Finally, The obtained pellets were dried in a hot air oven (Mettler[®], Germany) at 60° C for 6 hours.

4. Evaluation of Pellets

4.1 Determination of Pellets Appearance

Photomicrographs of pellet samples were taken with scanning electron microscope (S.E.M.). The samples were coated with gold prior to the microscopic examination using ion sputtering.

Table 5. The ingredients and amount of water used in each formulation.

No.	DTZ HCl	Avicel [®] PH 101	HPC-M [®]	Water base on dry basis
1	60% ¹	38.0%	2.0%	40%
2	60% ¹	38.0%	2.0%	30%
3	60% ¹	38.0%	2.0%	25%
4	60% ¹	38.0%	2.0%	20%
5	60% ¹	38.5%	1.5%	25%
6	60% ¹	39.0%	1.0%	25%
7	60% ¹	39.5%	0.5%	25%
8	60% ¹	40.0%	0.0%	25%
9	40% ²	59.5%	0.5%	q.s.to 40%
10	30% ³	69.5%	0.5%	q.s.to 55%
11	20% ⁴	79.5%	0.5%	q.s.to 70%

¹ is equivalent to DTZ HCl 90 mg / 150 mg pellets; ² is equivalent to DTZ HCl 60 mg / 150 mg pellets; ³ is equivalent to DTZ HCl 45 mg / 150 mg pellets; ⁴ is equivalent to DTZ HCl 30 mg / 150 mg pellets.

4.2 Sphericity of Pellets

The sphericity of prepared pellets was determined by image analyzer. One hundred sample pellets of each formulation were analyzed by software program KS.400V2.00 of Image analyzer. The data from image analyzer e.g. area, perimeter and Feret's diameter (max, min) of sample pellets were calculated to get the parameters indicating shape of pellet as follows: circularity, roundness, elongation, pellites, rectangle and modelx. Evaluate these parameters models as described by Hellen et al., 1993c, to obtain the most spherical pellet formulation as compare to the photomicrograph from S.E.M.

4.3 Particle Size Distribution

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

4.4 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.

$$\%Carr's\ compressibility = \frac{Td - Bd}{Td} \times 100 \quad (15)$$

Where Td and Bd are tapped and bulk density, respectively.

4.5 Flow Rate and Angle of Repose

The weight of 40.0 g pellets was 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.

$$Alpha = \tan^{-1} \frac{H}{R} \quad (16)$$

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.

4.6 Percent Friability

Ten grams of core pellets retained on a sieve size of 25 mesh (0.71 mm) and five spherical metallic balls with a diameter of 0.67 cm and 1.25 gm per ball were filled in plastic cylindrical container which has diameter of 4.69 cm and height 5.95 cm. Then rotating was performed in Erweka TAR20 Friabilator for 200 cycles. The

percent weight loss of pellets after screening on 25 mesh sieve size were determined at 2 determinations.

4.7 Moisture Content

The moisture content of DTZ HCl pellets was determined by using Moisture Analyzer Ma 30 Sartorius. 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 equation 17.

$$\% \text{Moisture content} = \frac{W_w - W_d}{W_w} \times 100 \quad (17)$$

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

4.8 Determination of DTZ HCl Content of Core Pellets

Weighed accurately about 3.0 g of 14/20 mesh cut of DTZ HCl pellets. Grinded the contents thoroughly, and transferred an accurately weighed portion, equivalent to about 150 mg of DTZ HCl, to a 100 ml volumetric flask. Added 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 mixed. Diluted the mixture to suitable concentration for UV determinations. The mixture was filtered and the filtrate was collected.

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

5. Preparation and Evaluation of Film Casting.

Ethylcellulose film at 5% concentrations were prepared by film casting method. The amount of ingredients that have been used for determining tensile testing were represented in the Table 6. Each formulation was weighed about 7 g per plate for 40-80 μm film thickness and dried at 50 °C for 4 hrs, then left for 2 days before testing.

5.1 Determination of Ultimate Tensile Strength, Percent Elongation at Break and Toughness of Tested Film.

The tensile tester was used as an apparatus for measuring ultimate tensile strength, percent elongation at break and toughness of tested film. A film specimen was cut into rectangular shape 0.5 cm in width and 4 cm in length. The thickness of each specimen was the average value of 5 separate measurements along the length of the specimen. The cross-section area of the test film was calculated by multiplying the mean thickness with gauge width. The tested was clamped by an upper and lower grip then tensile tester was operated. The samples were tested and average of the results by six determinations. The ultimate tensile strength, percent elongation at break and toughness were calculated from the following equations.

$$T_s = \frac{Bl}{Sc} \quad (18)$$

Where T_s is ultimate tensile strength, B_l is breaking load and S_c is cross-section area of the test specimen.

$$\%E = \frac{L_s - L_o}{L_o} \quad (19)$$

Where E is elongation at break, L_s is length at breaking point and L_o is original length of test specimen.

Toughness or area under stress strain curve is function of the work done (force x displacement) in breaking the film.



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Table 6. Film casting formulations.

Ingredients (%w/v)	Formulations										
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
EC ¹ 10 cps.	5	5	5	5	5	5	5	5	5	5	5
PEG ¹ 400	-	10	-	-	-	-	-	-	-	-	-
TEC ¹	-	-	10	20	30	-	-	-	-	-	-
DEP ¹	-	-	-	-	-	10	20	30	-	-	-
Castor oil	-	-	-	-	-	-	-	-	10	20	30
ETOH anh ¹ .	50	50	50	50	50	50	50	50	50	50	50
MeCl ₂ ¹	50	50	50	50	50	50	50	50	50	50	50

¹ EC, PEG, TEC, DEP, ETOH anh. and MeCl₂ are represented for Ethylcellulose, Polyethyleneglycol, Triethylcitrate, Diethylphthalate, anhydrous Ethylalcohol and Methylene chloride, respectively.

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6. Preparation of Film Coated DTZ HCl Pellets

6.1 Film Coating Formulation

Compositions of film coating formulation are presented in Table 7. The solution was made by dissolving polymer in solvent mixture of anhydrous ethylalcohol and methylene chloride in a ratio of 1:1, and then plasticizer was added and mixed for 30 minutes. The formulations C1 – C3 and formulations C3 – C4 were used for studying the effect of plasticizer type and also concentration of plasticizer on released profile of DTZ HCl from coated pellets, respectively.

Table 7. Amount of ingredients employed to prepare film coated solution for coating DTZ HCl pellets.

Ingredients (%w/v)	Formulations				
	C1	C2	C3	C4	C5
EC ¹ 10 cps.	5	5	5	5	5
Castor oil	20	-	-	-	-
DEP ¹	-	20	-	-	-
TEC ¹	-	-	20	10	30
ETOH anh ¹ .	50	50	50	50	50
MeCl ₂ ¹	50	50	50	50	50

¹ EC, PEG, TEC, DEP, anh. ETOH and MeCl₂ are represented for ethylcellulose, polyethyleneglycol, triethylcitrate, diethylphthalate, anhydrous ethylalcohol and methylene chloride, respectively.

6.2 Coating Conditions

The 200 g of 18/20 mesh cut of DTZ HCl pellets were placed in the bottom spraying of fluidized bed coating machine (Wurster type). Various factors in coating condition such as inlet/outlet air temperature, fan capacity, atomizing air pressure and feed rate of coating solution were adjusted to the most optimal condition in preliminary study. A suitable setting was presented in Table 8.

Table 8. The coating conditions using Wurster type bottom spray method.

Coating conditions	
Inlet air temperature	$45 \pm 2^{\circ}\text{C}$
Outlet air temperature	$40 \pm 2^{\circ}\text{C}$
Atomizing air pressure	1.0 bar
Fan capacity	7
Air volume	125-130 m ³ /h.
Partition height	2.0 cm
Feed rate of coating solution	8-10 rpm

Pellets were coated at low and high coating level, 2.5 and 5.0 % based on the percentage of weight increased, in preliminary study, to collect the suitable release profile for further development when compare to commercial product and 7.5 % coating level is the result of final development.

7. Evaluation of Film Coated DTZ HCl Pellets

7.1 DTZ HCl Content in Film Coated Pellets

The method of determination was similar to that of DTZ HCl uncoated core pellets as mentioned before.

7.2 The Release of DTZ HCl from Film Coated Pellets

7.2.1 Calibration Curve of DTZ HCl

Calibration curves of DTZ HCl in various media were constructed to determine an 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 weighed accurately about and transferred to 100 ml volumetric flask, then adjusted to volume with water. This solution was used as stock solution 1 (concentration 2,000 $\mu\text{g/ml}$). Stock solution 1 was diluted to 20 $\mu\text{g/ml}$ and 40 $\mu\text{g/ml}$ for preparing stock solution 2 and 3. Stock solution 2 was diluted to 2, 4, 6, 8, 10 $\mu\text{g/ml}$ and stock solution 3 was diluted to 12 and 16 $\mu\text{g/ml}$ with water, respectively. The absorbances of dilute solutions 2-16 $\mu\text{g/ml}$ were determined at a wavelength of 237 nm. In the case of other dissolution mediums, preparation of calibration curve by the same method but adjusted to volume and made blank solution with its medium.

7.2.2 Preparation of Dissolution Medium

In this study, the various types of medium were used in dissolution testing for studying the mechanism of release as follows: hydrochloric acid buffer pH 1.2 (for pH changed studies), phosphate buffer pH 6.8 (for pH changed and kinetics studies), sodium chloride 0.8, 1.0 and 1.2 osmol/kg, and sodium sulphate 0.8, 1.0 and 1.2 osmol/kg, respectively (for mechanism studies).

7.2.2.1 Hydrochloric acid Buffer pH 1.2

Prepared by following direction of preparing HCl acid standard buffer solutions pH 1.2 in USP XXIII.

7.2.2.2 Buffer pH 6.8

Adjust pH from HCl acid buffer pH 1.2 by adding trisodium phosphate 12 H₂O to pH 6.8. For 900 ml HCl acid buffer pH 1.2 used trisodium phosphate·12 H₂O 18.5 g (Kao C. C., Chen, S. C. and Sheu, M. T. 1997.).

7.2.2.3 Sodium Chloride and Sodium Sulphate 0.8, 1.0 and 1.2 osmol/kg

Dissolved 23.4, 29.2 and 35.0 g of sodium chloride in 1 kg of water. These were sodium chloride 0.8, 1.0 and 1.2 osmol/kg solution, respectively. In the case of sodium sulphate solution was prepared by dissolving 37.88, 47.35 and 56.82

g of sodium sulphate in 1 kg of water. These were sodium sulphate 0.8, 1.0 and 1.2 osmol/kg solution, respectively.

7.2.3 Dissolution Study of Film Coated DTZ HCl Pellets

An accurate weight portion of the coated pellets equivalent to dose of prepared DTZ HCl pellets were filled in capsule for dissolution study. Dissolution test was determined using apparatus II (paddle) according to USP XXIII in comparing release profile to commercial product, however, for release mechanism study, apparatus I (basket) was used. The average of DTZ HCl released was calculated from three determinations.

In development of product, the dissolution medium was DI water and pH changed medium, pH 1.2 (at first 2 hours) and adjusted to 6.8 after that. In the case of released kinetic and mechanism studied, phosphate buffer pH 6.8 was employed, in addition, solution that can create osmotic pressure was also used as a medium. Nine hundred milliliters of dissolution medium was equilibrated to 37 ± 0.5 °C. In development of product study, Herbesser[®] 90 SR was also tested for drug release using the same procedure as described above. The release characteristics of developed formula were compared to this commercial product.

A ten milliliters of specimen was withdrawn at predetermined time interval of 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 hours. The same quantity of medium was added after each sampling in order 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. A cumulative amount of drug release as a function of time was determined.

DTZ HCl 90 mg pellets was coated with C1, C2 and C3 formulations at 5 % w/w coating level for effect of plasticizer types studying. In the case of studying about the effect of plasticizer amount, each DTZ HCl 30, 45, 60 and 90 mg pellets was coated with C3, C4 and C5 formulas at 7.5% w/w coating level, respectively.

8. Solubility of DTZ HCl in various media at 37 °C

Excess DTZ HCl powder was dissolved in following medium: water, sodium-chloride solution (0.8,1.0 and 1.2 osmol/kg) and sodium sulphate solution (0.8,1.0 and 1.2 osmol/kg). Three samples were set for each medium. Placed the samples in rolling tube water bath for 2 days. The temperature was controlled at 37°C and speed of rolling tube was 60 rpm. Collected the sample at the determining time. Those samples were filtered through 0.45 µm filter paper before diluted in each medium to suitable concentration for determining with UV spectrophotometer at 237 nm. Calculated solubility from calibration curve in each medium was performed.

9. Swelling Characteristics of Avicel PH 101[®] and HPC-M[®]

10 g of Avicel PH 101[®], which composed of HPC-M[®] 0.5 % w/w, were poured into a 100 ml volumetric cylinder and the bulk volume was measured. Next 90 ml of deionized water was added and the dispersion was mixed for 5 minutes. Water was added up to 100 ml. The sedimentation volume was read after the dispersion was allowed to stand for 24, 48 and 72 hr, respectively. The percentage of swelling volume was calculated by dividing the different values between bulk and sedimentation volume by bulk volume and multiplied by 100 to make percentage. The results were averaged from 3 determinations. (Herman, Remon and DeVilder, 1989.)