CHAPTER II

EXPERIMENTAL

Materials

The following materials were obtained from commercial sources and deionized water was used throughout the experiment.

1. Model drugs

- Propranolol hydrochloride (Lot No. 990508, Zhejiang Medicines and Health Products, China)
- Diclofenac sodium (Lot No. DS/0006/111, Amoli Organics Ltd., Gujarat, India)

2. Additives

- Microcrystalline cellulose, NF/BP (Avicel® PH-101, Lot No. 1871, Asahi Chemical Ind. Co., Ltd., Japan)
- Lactose hydrous USP/NF/BP/EP 200 mesh (Lot No. 7091802-109, Wyndale, New Zealand)
- Glyceryl monostearate (flake) (Lot No. GCB19, Belgium, supplied by Srichand United Dispensary Co., Ltd., Thailand)
- Hydrogenated vegetable oil (Lubritab®, Lot No. K9100L, Mendell, USA)
- Glyceryl behenate (Compritol 888 ATO®, Lot No. 25638, Glattefosse, France)
- Cab-O-Sil® (Colloidal Silica, Eingetragenes Warenzeichen, Bundesrepublik, Dentschland)
- Talcum (Fineness 325 mesh, China)

3. Chemicals

- Hydrochloric acid, AR grade (Lot No. K27703017 015, Merck KGaA, Germany)
- Chloroform, AR grade (Lot No. 2K 080052, Lab-Scan Analytical Science, IRELAND)

- Methanol, AR grade (Lot No. 2k 100054, Lab-Scan Analytical Science, IRELAND)
- Citric acid monohydrate, AR grade (Lot No. K 20379444 349, E. Merck, Germany)
- Anhydrous dibasic sodium phosphate, AR grade (Lot No. 91706290B, Carlo Erba Reagenti)
- Sodium Chloride (crystals), AR grade (Lot No. 7581 MTVV, Mallinckrodt, USA)
- Polyethylene glycol 1450 (Lot No. 27 Mar 1996, Pharmaceutical Traders Co., Ltd., USA)
- Tween 80 (Lot No. 807870, B.L. Hua and Co., Ltd., Japan)

4. Equipment

- Analytical balance (Model A200s, Sartorius GmbH, Germany and Model PB3002 Mettler, Switzerland)
- Dissolution apparatus (Model DT-6R, Erweka®, Germany)
- Differential Scanning Calorimeter (Model NETZCH DSC 200, Germany)
- Extruder (model EXKS-1, Fuji Paudal Co., Ltd., Japan)
- Fourier transform infared spectrometer (Model 1760X, Perkin Elmer, Germany)
- Fluidized bed air suspension (Model STREA1, Nitro-Aeromatic AG, Switzerland)
- Friabilator (Erweka TAR 20, Germany)
- Hot air oven (Model UL80, Memmert, Germany)
- Hydraulic equipment (Model C, Carver Laboratory Press, USA)
- Image analyzer (×6.5, resolution 0161 mm/pixel, Buecher)
- Magnetic stirrer (Model SP 46920-26, Cimarec 2, Thermolyne, USA)
- pH-meter (Model 292, Pye Unicam, England)
- Planetary Mixer (Model A701A, Kenwood MFG.Ltd., England)
- Scanning electron microscope (Model JSM 5410LV, Joel Ltd., Japan)
- Sieve shaker (Josef Deckehnann Aschaflenberg, Germany)
- Spheronizer (Model S320, Aeromatic-Fielder, England)
- Ultrasound transonic digital sonicator (Model T680/H, Elma, Germany)

- US Standard sieve (Laboratory test sieve ASTM E11, Endecotts, Ltd., USA)
- UV-visible spectrophotometer (Model UV-1601, Shimadzu)
- X-Ray powder diffractometer (Model JDX-3530, Jeol Ltd., Japan and Rigaku Denki [Miniflex], Japan)

Methods

1. Pelletization Process

The preliminary formulation of core for pellets as prepared in Table 6 were investigated for the suitable condition of pelletization process. Glyceryl monostearate was used as glyceride material in this preliminary formulation.

Table 6 Formulation of core pellets.

Ingredients	% w/w
Glyceryl monostearate (GMS)	40
Lactose	40
Microcrystalline cellulose (MCC)	20
Water qs 100% w/w	

In the preparation of drug containing pellets, the amount of GMS was substituted by propranolol hydrochloride and diclofenac sodium. Then the amount of water used were optimized to provide the best characteristics pellets.

The procedure for preparing of pellets as follows. The glycerides was melted in the beaker by controlling temperature above its melting point about 10 °C. When the glycerides was completely melted, chloroform was added and stirred by using magnetic stirrer until the solution was homogeneous. Filler (lactose) and extrusion aids (MCC) were mixed together with the aid of planetary mixer for 5 minutes. After thoroughly mixed, glyceride solution was gradually added and mixed until its congealed. The congealed mass was first transferred to the extruder to produce powdered mass. The powdered mass was again placed into planetary mixer. Water was gradually added and mixed until damp mass was obtained. The damp mass was transferred to the extruder again and extruding into the cylindrical segments. The extrudate was immediately rolled into solid sphere on the spinning friction plate of spheronizer at a speed 700 ± 10 rpm, and spheronization time at 10 minutes. The pellets were dried in a hot air oven at 45 °C for 4 hours.

Effect of Glyceride Concentration

In the case of glyceride concentration effect study, the amount of propranolol hydrochloride and diclofenac sodium in the formulation was kept constant. Then the amounts of glyceride were varied between 40 and 50 % according to the formulations that are presented in Table 7.

Table 7 Composition of propranolol hydrochloride and diclofenac sodium matrix pellets at different percentage of wax concentration.

Ingredients (%)	Formulation											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
PL	40	40	40	40	40	40	-	-	-	-	-	-
DS	-	-	-	-	-	-	40	40	40	40	40	40
MCC	20	10	20	10	20	10	20	10	20	10	20	10
GMS	40	50	-	-	-	-	40	50	-	-	-	-
Lubritab®	-	-	40	50	-	-	-	-	40	50	-	-
Compritol®	-	-	-	-	40	50	-	-	-	-	40	50
$CHCl_3$* (ml)	50	25	50	25	50	25	50	25	50	25	50	25
Water	14	14	28	24	24	24	34	28	46	42	52	50

*$CHCl_3$ is used for preparing wax solution and will be evaporated during drying process

Effect of Loading Dose

The amounts of glycerides in the formulation were kept constant. Then the amount of propranolol hydrochloride and diclofenac sodium was studied at concentrations of 20 and 30 % as presented in Table 8.

Effect of Additive

The amounts of glycerides and diclofenac sodium in the formulation were kept constant. Then one part of lactose were substituted with PEG 1450 and Tween 80 which varied from 0.2 % to 1 % in the formulation and are presented in Table 9.

Table 8 Composition of propranolol hydrochloride and diclofenac sodium matrix pellets at different percentage of loading dose.

Ingredients(%)	Formulation											
	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24
PL	20	30	20	30	20	30	-	-	-	-	-	-
DS	-	-	-	-	-	-	20	30	20	30	20	30
MCC	10	10	10	10	10	10	10	10	10	10	10	10
GMS	60	60	-	-	-	-	60	60	-	-	-	-
Lubritab®	-	-	60	60	-	-	-	-	60	60	-	-
Compritol®	-	-	-	-	60	60	-	-	-	-	60	60
Lactose	10	-	10	-	10	-	10	-	10	-	10	-
$CHCl_3$* (ml)	50	50	50	50	50	50	50	50	50	50	50	50
Water	10	8	24	24	24	24	20	18	38	40	44	44

*$CHCl_3$ is used for preparing wax solution and will be evaporated during drying process

Table 9 Composition of diclofenac sodium matrix pellets at different percentage of PEG 1450 and Tween 80.

Ingredients(%)	Formulation						
	F25	F26	F27	F28	F29	F30	F31
DS	40	40	40	40	40	40	40
MCC	10	10	10	10	10	10	10
GMS	40	40	40	40	40	40	40
PEG 1450	5	3	1	1	1	0.5	0.2
Tween 80	-	-	-	3	1	0.5	0.2
Lactose	5	7	9	6	8	9	9.6
$CHCl_3$* (ml)	50	50	50	50	50	50	50
Water	**	**	22	**	16	22	26

*$CHCl_3$ is used for preparing wax solution and will be evaporated during drying process

**Cannot to formulate because the damp mass was sticky

Effect of Curing Temperature and Time on Propranolol Hydrochloride Matrix Pellets

Formulations of propranolol hydrochloride matrix pellets were selected to investigate the effect of curing temperature on drug release by using fluidized bed dryer.

The fluidization conditions were as follow:

Pellets load	10 g
Inlet air temperature	50-58 °C, 55-65 °C, 60-75 °C
Out let temperature	50-58 °C, 55-65 °C, 60-75 °C
Holding time	30, 60, 90 minutes

Time and temperature of curing for each formulation of propranolol hydrochloride matrix pellets are presented in Table 10.

Table 10 Curing condition for propranolol hydrochloride matrix pellets.

Formulation	PL 40 % GMS 40 %	PL 40% Lubritab 40 %	PL 40 % Compritol 40%
-	50°C 30 min	55°C 30 min	65°C 30 min
-	50°C 60 min	55°C 60 min	65°C 60 min
-	50°C 90 min	55°C 90 min	65°C 90 min
-	55°C 30 min	60°C 30 min	70°C 30 min
-	55°C 60 min	60°C 60 min	70°C 60 min
-	55°C 90 min	60°C 90 min	70°C 90 min
-	58°C 5 min*	65°C 60 min*	75°C 7 min*
+ Cab-O-Sil®** 1%	58°C 5 min*	65°C 5 min*	75°C 7 min*
+ Talcum** 1%	58°C 20 min*	65°C 20 min*	75°C 7 min*

* Time used depended on the sticky of wax to the screen

** Cab-O-Sil® and talcum was added at temperature curing condition to prevent sticking of glycerides pellets to fluid bed dryer

2. Evaluations of Matrix Pellets

2.1 Pellet Morphology

Morphology of matrix pellets was characterized using scanning electron microscopy. The pellets were coated with gold about 2-3 times to cover the entire spherical surface prior to microscopic examination using ion-sputtering method. Size, shape, and surface topography were observed. The matrix pellets were also cross-sectioned for observation of internal texture.

2.2 Size Distribution of the Pellets

Size distribution of pellets were determined using sieve analysis (Millili and Schwartz, 1990), consisted of a set of US standard sieves, ranging from sieve No.14, 18, 20, 25, 30, respectively and a collector pan (1400, 1000, 900, 700, 500 μm). One hundred grams of pellets were accurately weighed and put on the top of sieves. The sieves were placed on the sieve shaker and shook for 20 minutes. The pellets retained on each sieve size were weighed and calculated in percent of total weight.

2.3 Bulk, Tapped Density, and Percent Compressibility

Fifty grams of the matrix pellets were accurately weighed and carefully poured into a 100 ml graduated cylinder. Graduated cylinder was dropped two seconds interval on hard surface for 3 times at one-inch height (Gupta et al., 2001). The bulk volume was recorded. Division of weight by bulk volume presented bulk density.

$$\text{Bulk density (g/ml)} = \frac{\text{weight of pellets (g)}}{\text{bulk volume (ml)}}$$

Tapped density was performed by dropping graduated cylinder filled with pellets on a hard surface from one inch height until the volume was constant. Division of weight by the constant volume presented the tapped density.

$$\text{Tapped density (g/ml)} = \frac{\text{weight of pellets (g)}}{\text{tapped volume (ml)}}$$

The % compressibility of the pellets were established by the following equation

$$\% \text{ Compressibility} = (T\text{-}B) \times \frac{100}{T}$$

B and T were bulk and tapped densities, respectively. All of these factors were calculated from three determinations.

2.4 **Angle of Repose** (Gupta et al., 2001)

Each angle of repose was determined by the cylinder method. An appropriate amount of pellet was carefully filled into a cylinder, which was placed, on the graph paper. When the pellet was filled to the top of the cylinder, the cylinder was slowly lifted in a vertical direction, thus producing a round heap of pellet. The result was averaged from three determinations. Each angle of repose was calculated from the following equation:

$$\alpha = \tan^{-1} \frac{H}{R}$$

where α is the angle of repose, H is the height of heap, R is the radius of heap

2.5 **Percent Friability** (Millili and Schwartz, 1990)

Ten grams of matrix pellets retained on 14/20 mesh cut and five stainless spheres (each sphere weight 1.06 gm and diameter 6.34 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 was sieved off. The result averaged from two determinations, reported as percentage of weight loss.

2.6 **Sphericity of Pellets**

The sphericity of prepared pellets was determined by using image analyzer. Thirty sample pellets of each formulation were analyzed by software program Ominent of Image analyzer. Longest diameter or Feret maximum (R_1) and smallest diameter or Feret minimum (R_2) of pellets were divided to perform the aspect ratio that referred to the sphericity of pellets and also show the average size of pellets.

2.7 Surface Area Analyzer

The specific surface area and the total pore volume of pellet were determined by BET adsorption method using the surface area analytical equipment. The specific surface area was automatically calculated.

2.8 Infrared Spectroscopy

Infrared spectroscopy was used to confirm the change in the functional groups of the substances and products after pelletization process by observing the positions and intensities of IR peaks.

The IR spectra of propranolol hydrochloride, diclofenac sodium, and diluents in the matrix pellets were examined using the potassium bromide disc (KBr) method with an infrared spectroscopy in the range of 4000-400 cm^{-1}.

2.9 Powder X-Ray Diffraction Analysis

The X-ray diffractometer was used to determine the diffraction angle of the substance, which showed crystallinity and interplanar spacing of the crystal planes and determines the position, each component in mixing and pelletization process.

The crystallinity of propranolol hydrochloride, diclofenac sodium, and diluents in the pellets were examined by X-ray diffractometer.

The pellets were ground in the mortar and firmly packed in the cavity of a thin rectangular quartz slide by the other glass slide. The glass slide was taken off and the prepared sample was exposed to the X-ray beam in the X-ray diffraction chamber. The X-ray diffraction patterns were recorded at the speed of 0.04° per minute from 5° to 60° in the term of 2θ angle.

2.10 Differential Scanning Calorimetry

The thermograms of pellets prepared from various formulations were examined by differential scanning calorimeter (DSC). The differences in thermal energy patterns between the original substances and their products were evaluated after pelletization process.

An about 5-mg sample of single material was accuratcly weighed, and mixtures of drugs-various waxes were melted in the DSC pan. Then, it was crimped with the hermetically sealed pan and immediately made a few holes for determinations. The pan filled with mixtures was placed in the equipment beside the reference pan made by the same method using Perkin Elmer DSC 7 for analyzing the thermograms. Nitrogen was used as a carrier gas at a flow rate of 10 mL/min throughout running conditions with a heating rate of 20 °C per minute and in the temperature range of 35 °C to 300 °C.

2.11 Determination of Propranolol Hydrochloride and Diclofenac Sodium Content in Matrix Pellets

2.11.1 Calibration Curve of Propranolol Hydrochloride and Diclofenac Sodium

A. Calibration Curve of Propranolol Hydrochloride Content

Standard propranolol hydrochloride of 300 mg was accurately weighed into 100 ml volumetric flask through the aid of glass funnel. The powder was dissolved and adjusted to volume with absolute methanol. This solution was used as the first stock solution. Ten milliliters of the first stock solution was pipetted into a 100-mL volumetric flask and adjusted to volume as the second stock solution.

The 2, 3, 4, 5, 6, and 7 mL of second stock solution were individually pipetted into the 50-mL volumetric flask. All solutions were adjusted to volume with absolute

methanol. The final concentrations of each solution were 12, 18, 24, 30, 36, and 42 μg/mL, respectively.

The final solution was assayed spectrophotometrically at 290 nm. The absorbance and the calibration curve of propranolol hydrochloride in absolute methanol are presented in Table 1A, and Figure 1A (Appendix A). Each concentration was determined in triplicate.

B. Calibration Curve of Diclofenac Sodium Content

Standard diclofenac sodium of 50 mg was accurately weighed into a 100-mL volumetric flask through the aid of a glass funnel. The powder was dissolved and adjusted to volume with absolute methanol. And used as a stock solution.

The standard stock solution of 1, 2, 3, 4, and 5 mL were individually pipetted into the 100 mL volumetric flask, diluted and adjusted to volume with absolute methanol. The final concentrations of each solution were 5, 10, 15, 20, and 25 μg/mL, respectively.

The final solution was assayed spectrophotometrically at 283 nm. The absorbance and the calibration curve of diclofenac sodium in absolute methanol are presented in Table 4A and Figure 4A (Appendix A). Each concentration was determined in triplicate

2.11.2 Assay of Propranolol Hydrochloride and Diclofenac Sodium Content in Matrix Pellets

Pellets of 150 mg were accurately weighed into a 50-mL volumetric flask. The pellets was extracted with absolute methanol by the aid of sonicator for about 1 hr, then adjusted to volume with absolute methanol and mixed thoroughly. The solution was filtered through filter paper and used as stock solution. 1-mL of the stock solution was individually pipetted into a 50-mL volumetric flask. then adjusted to volume with absolute methanol and mixed.

The final solution of propranolol hydrochloride and diclofenac sodium was determined by UV/Visible spectrophotometry at 290 and 283 nm, respectively, and used absolute methanol was used as a reference blank. Amount of drugs content was calculated from the calibration curve of each drug in absolute methanol.

3. Compaction of Propranolol Hydrochloride and Diclofenac Sodium Matrix Pellets

The pellets were compacted into matrix tablets using a hydraulic press equipped with punch-die assembly. Glycerides matrix tablets were prepared by direct compression of pellets matrix equivalent to 80 and 100 mg for propranolol hydrochloride and diclofenac sodium, respectively.

The hydraulic pressed equipped with ¼ inch diameters round flat-faced punch and dies, the compression force of 1000 psi was used.

4. Evaluation of Propranolol Hydrochloride and Diclofenac Sodium Products

4.1 Calibration Curve of Propranolol Hydrochloride and Diclofenac Sodium

4.1.1 Calibration Curve of Propranolol Hydrochloride

A. In 0.1 N HCl Solution

Standard propranolol hydrochloride of 100 mg was accurately weighed into a 100-mL volumetric flask through the aid of a glass funnel. The powder was dissolved with 10-mL of absolute methanol and adjusted to volume with 0.1 N HCl solution. This solution was used as a stock solution.

The stock solution of 1, 2, 4, 6, and 8 mL, respectively, were individually pipetted into a 200-mL volumetric flask, diluted and adjusted to volume with 0.1 N HCl solutions. The final concentration of each solution was 5, 10, 20, 30, and 40 μg/mL, respectively.

The final solutions were assayed spectrophotometrically at 289 nm. The absorbance and the calibration curve of propranolol hydrochloride in 0.1 N HCl solutions are presented in Table 2A and Figure 2A in (Appendix A). Each concentration was determined in triplicate.

B. In Phosphate Buffer pH 6.8 Solution

Standard propranolol hydrochloride of 100 mg was accurately weighed into a 100-mL volumetric flasks through the aid of a glass funnel. The powder was dissolved and adjusted to volume with pH 6.8 phosphate buffer solution. This solution was used as a stock solution.

The stock solution of 1, 2, 3, 4, and 5 mL, respectively, were individually pipetted into the 100 ml volumetric flasks, diluted and adjusted to volume with pH 6.8 phosphate buffer solution. The final concentrations of each solution were 10, 20, 30, 40, and 50 μg/mL, respectively.

The final solutions were assayed spectrophotometrically at 289 nm. The absorbance and the calibration curve of propranolol hydrochloride in phosphate buffer pH 6.8 solutions are presented in Table 3A and Figure 3A (Appendix A). Each concentration was determined in triplicate.

4.1.2 Calibration Curve of Diclofenac Sodium

A. In 0.1 N HCl Solution

Standard diclofenac sodium of 50 mg was accurately weighed into a 100-mL volumetric flask through the aid of a glass funnel. The powder was dissolved with 10 mL of absolute methanol and adjusted to volume with absolute methanol solution. This solution was used as a stock solution.

The stock solution of 1, 2, 3, 4, and 5 mL, respectively, were individually pipetted into 100-mL volumetric flask, diluted and adjusted to volume with 0.1 N HCl

solutions. The final concentrations of each solution were 5, 10, 45, 20, and 25 μg/mL, respectively.

The final solutions were assayed spectrophotometrically at 273 nm. The absorbance and the calibration curve of diclofenac sodium in 0.1 N HCl solutions are presented in Table 5A and Figure 5A (Appendix A). Each concentration was determined in triplicate.

B. In Phosphate Buffer pH 6.8 Solution

Standard diclofenac sodium of 25 mg was accurately weighed into a 100-mL volumetric flask through the aid of a glass funnel. The powder was dissolved and adjusted to volume with pH 6.8 phosphate buffer solution. This solution was used as the stock solution.

The standard stock solution of 1, 2, 3, 4, 5, and 6 ml, respectively, were individually pipetted into a 100-mL volumetric flask, diluted and adjusted to volume with pH 6.8 phosphate buffer solution. The final concentrations of each solution were 2.5, 5, 7.5, 10, 12.5, and 15 μg/mL, respectively.

The final solutions were assayed spectrophotometrically at 275 nm. The absorbance and the calibration curve of diclofenac sodium in phosphate buffer pH 6.8 solutions are presented in Table 6A and Figure 6A, (Appendix A). Each concentration was determined in triplicate.

4.2 Dissolution Studies

The dissolution study was performed by modified from USP XXIV. As an oral controlled release pellets were supported to pass the entire upper gastrointestinal tract. It would be ideal when the release of drug was constant over a wide range of pH value (pH 1-7). So, in vitro test for controlled release products should at least cover this pH range. The acid buffer pH 1.2 and phosphate buffer pH 6.8 were individually used to test not only the release of drug from the matrix pellets, but also the effect of the dissolution medium.

In this study, accurate weight of matrix pellets was filled in the capsule for release studies. The 900 ml of 0.1 N HCl solutions and pH 6.8 phosphate buffer were employed as dissolution media, and filled in a glass vessel. The dissolution apparatus type I (Model DT6R, Erweka, Germany) was used with basket rotating of 100 rpm. The dissolution data was evaluated from three capsules of each formulation.

In the dissolution model with pH-change, the pH of the medium was kept at pH 1.2 using 0.1 N HCl for two hours, then the pH was increased to 6.8 by adding sodium hydroxide 3.6 g, monobasic potassium phosphate 3.06 g and dibasic sodium phosphate 4.005 g dissolved in a few milliliters of 0.1 N HCl. The operation was continued until completing 12 hours.

The sample of 10 mL were withdrawn at the time intervals of 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hrs. The same volume of the medium at that time was added immediately after each sampling to keep the constant volume of the medium in the vessel throughout the experiment.

Each sample was filtered through filter paper. The filtrate was diluted in suitable concentration in order to determined by UV/visible spectrophotometry at the maximum wavelength of each dissolution medium.

The amounts of propranolol hydrochloride and diclofenac sodium released at any times were calculated from the calibration curve for each dissolution medium. A cumulative correction was achieved for the previously removed sample to determine the total amount of the drug release. Each of the dissolution values reported was based on an average of three determinations of each formulation.