CHAPTER II

EXPERIMENTAL

MATERIALS AND METHODS

1. Materials

1.1 Model drug - Propranolol Hydrochloride
Batch No. 911210
(China National Chemical ,
Imp & Exp Corp., China.)

1.2 Beads core - Sugar crystal
(Mitrpol Company., Thailand)
- Spheroidal pellet
(Supplied by Pharmaceutical and
Medical Supply Co.Ltd., Thailand)

1.3 Additives - Ethylcellulose 10 cps.
(Rama Production, Thailand)
- Hydroxypropylmethylcellulose
(Rama Production, Thailand)
- Glycerylmonostearate
(Srichand United Dispensary,
Thailand)
- Castor oil
(Srichand United Dispensary,
Thailand)
- Polyethylene Glycol 4000
(Pharmaceutical Traders,
Thailand)
- Aerosil (Degussa , Germany)

1.4 Dissolution media

- Sodium Hydroxide
  (Eka Novel, Sweden)
- Dihydrogen Potassium Phosphate
  (E.Merck, Germany)
- Hydrochloric acid
  (E.Merck, Germany)
- Dibasic sodium phosphate
  (E.Merck, Germany)
- Monobasic potassium phosphate
  (E.Merck, Germany)

1.5 Solvent

- Methanol, AR grade
  (BDH Laboratory Supplies, England)
- 95% Ethanol
  (Liquor Division, The Excise Department, Thailand)
- Methylene chloride
  (May & Baker Ltd., England)
- Chloroform
  (May & Baker Ltd., England)

1.6 High Performance Liquid Chromatography Analysis

- Pindolol Lot. NO.55F0749
  (Sigma Chemical, USA)
- Methanol HPLC grade
  (J.T Baker Inc, USA)
- Acetonitrile
  (May & Baker Ltd., England)

2. Equipment

- Fluidized Bed Coater
  (Uni-Glatt Laboratory Units, Western Germany)
- Dissolution Tester
  (Hanson Research Corporation SR2, USA)
- pH meter
  (Hanna Instruments 8417, USA)
- Spectrophotometer
  (The Bausch & Lomb Spectronic 2000, USA)
- Peristaltic Tube Pumps
  (Verder Type VRX 88, Germany)
- Centrifuge (Sartorius Analytic Type A 200 g, Germany)
- Balance, Top Load
  (Sartorius 1264 MP, Germany)
- Sieve Shaker (Josef Deckehnann Aschaffenburg, Western Germany)
- Scanning Electron Microscope
  (Model JSM-T 220 A, Jeol, Japan)
- Stirring Hot Plates
  (Thermolyne Corporation, USA)
- Friabilator (Erweka, Western Germany)

3. Preparation of Propranolol Hydrochloride Sustained Release Beads

3.1 Preliminary Investigation for Suitable Coating Condition and Coating Solution

Top spray method of fluidized bed coating was used in preliminary investigation by trial and error for suitable coating conditions and the uniformity of coating film.

The coating solution which coated on size classified sugar crystal or pellets has the composition as presented in Table 2. Coating solution was made by dissolving ethylcellulose in ethanol. Then, erythrosine dye was added and the solution was mixed homogeneously by homogenizer. The coloring agent was used as an indicator to observe the uniform coating property during preliminary study on coating conditions.

The coating conditions were gradually adjusted by varying inlet air temperature, outlet air temperature, spraying air pressure, spraying rate to have uniformity of coating by visual observation of coated sugar.

Table 2 Composition of coating solution in preliminary investigation

Ingredient	% w/v
Ethylcellulose	5
Erythrosine dye	0.05
Ethanol qs	100

After the suitable coating condition was established, erythrosine dye was substituted by propranolol hydrochloride.

3.2 Beads core for coating

3.2.1 Sugar crystal

Sucrose crystals from commercial source were classified by sieving to have the size range of 20/40 mesh (420-840 microns) and 40/60 mesh (250-420 microns).

3.2.2 Spheroidal pellets

Pellets, 0.5 mm in size, composed of lactose, corn starch, microcrystalline cellulose (Avicel pH 101®) and polyvinyl pyrrolidone (Kollidon 30®) were produced by extrusion and spheronization process.

3.3 Preparation of coated beads

3.3.1 Formulation of coating solution

The general composition of coating solution was presented in Table 3. During studying , the components of coating formulations were varied in order to modify the release characteristics such as propranolol hydrochloride, ethylcellulose and plasticizer. The plasticizers were glycerylmonostearate, castor oil and PEG 4000 in various concentrations of 1%, 2% and 3%.

The coating solution was freshly prepared in the amount of 2400 ml as this volume could provide required drug content in final product and used shortest coating time.

Table 3 General composition of coating solution

Ingredient	% w/v
Propranolol HCl	5
Ethylcellulose	5
Plasticizer (Glycerylmonostearate or Castor oil or PEG 4000)	1 or 2 or 3
Ethanol qs	100

3.3.2 Preparation of coating solution

Ethylcellulose was weighted and dissolved in ethanol, then propranolol hydrochloride was added. After, the solution was mixed homogeneously, then the plasticizer was added and adjusted to 2400 ml with ethanol.

3.3.3 Preparation of outercoating solution

In some formulations of the coated beads, the outercoating was performed to have thicker film of coated beads in order to decrease drug release rate from the coated beads. The outercoating solution formulations were similar to the coating solution as shown in Table 3 but the drug was not added. The preparation of outercoating solution was the same method as previously described in coating solution but the volume was used only 1200 ml.

3.3.4 Fluidized-Bed Coating Process

The fluidized-bed apparatus used was a laboratory type (Uni-Glatt), setting the coating condition according to Table 4 which was based on the conditions previously found in preliminary investigation. The batch size of the coating core was 150 g and coating time was about 3-4 hrs.

Table 4 The coating conditions using top spray method

Coating condition	value
Inlet air temperature	50 C
Outlet air temperature	40 C
Spraying air pressure	2 bar
Feed rate of coating solution	20 ml/min.

4. Evaluation of the Coated Beads

4.1 Beads Morphology

Morphology of beads were observed using scanning electron microscopy(SEM). The samples were coated with gold prior to the microscopic examination using ion sputtering. Size, shape and surface topography of the beads were determined. The beads were also cross-sectioned for observation of the coated film.

4.2 Particle Size Distribution

Particle size distribution was determined using sieve analysis method. The approximately 30 g of coated beadlets was put on the top of a seive series ranging from 840, 420, 250 and 177μm. The nest of sieves were placed on the sieve shaker for 20 minutes. The results averaged from two determinations were reported as percentage of weight of coated beads retained on each sieve size. The geometric mean diameter was determined.

4.3 Bulk, Tapped Density and Carr's Compressibility index.

The bulk and tapped density were determined by pouring 30 grams of the coated beads into a 100 ml. graduated cylinder. The bulk volume was recorded and bulk density was calculated. Tapped density was performed by dropping graduated cylinder on a hard surface from height 5 cm. until a constant volume was obtained. 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.

$$\% \text{ Carr's compressibility} = \frac{(T - B) \times 100}{T}$$

T and B are tapped and bulk density, respectively.

4.4 Friability of Coated Beads

Ten grams of coated beads which were retained on sieve size 40 mesh(420 μm)and ten spherical metalic ball diameter

0.5 cm. filled in a closed 60 milliliters bottle. Then,rotated in Roche Friabilator for 4 minutes at the speed of 25 rpm.The result averaged from two determination were reported as percentage of weight of coated beads passed sieve size 40 mesh.

4.5 Determination of Propranolol HCl content of coated beads.

The approximately 150 mg. of sample was accurately weighed and dissolved in methanol then adjusted to 50.0 ml volume and filtered.

Pipet 1.0 ml of filtrate and transfer to 50.0 ml volumetric flask. Adjusted methanol to volume and mixed. The absorbance of resulting solution was determined at 289.8 nm using double beam spectrophotometer. The methanol was used as a blank. The content was calculated from the standard curve as Figure 100.

4.6 Dissolution studies

The dissolution study was performed by pH change method using USP dissolution test apparatus (basket method). As oral controlled release products were supposed to pass the entire upper gastrointestional tract, it would be ideal when the release of drug was constant over a wide range of pH values, so an in vitro test for controlled release products should at least cover the pH range between 1-7.

In the dissolution model with pH change method, the pH of the medium was kept by 0.1 N HCl for one and a half hours. 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. The operation was continued until completing 24 hrs after addition of dry buffers.

Nine hundred milliliters of medium were placed in a glass vessel specified in the USP XXII dissolution test and equilibrated at 37 ± 0.5 °C. The basket was operated at the speed of 100 rpm.

Five milliliters of dissolution medium were with drawn at the time interval of 15, 30 minuters, 1, 1.5, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hrs. The same quantity of medium was added immediately after each sampling to keep the volume of the medium constant during the experiment.

Each sample was diluted to suitable concentration which gave the absorbance between 0.2-0.9. The absorbance was determined spectrophotometrically at 288.6 nm. for 0.1 N HCl and 286.7 nm. for phosphate buffer pH 6.8

Each of the dissolution values reported was based on an average of three determinations of each formulation. The amount of propranolol release at anytime interval was calculated from the calibration absorbance concentration curve. (see Figure 101 and 102 in Appendix B).

5. Standard Calibration Curve

One gram of propranolol HCl was accurately weighed and dissolved in methanol or 0.1 N HCl or phosphate buffer pH 6.8 depending on whichone was used as the solvent in the experiment. The solution was adjusted to 200 ml. with each solvent and used

as stock solution. The stock solution was individually pipeted at the volume of 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ml into 50.0 ml. volumetric flask, diluted and adjusted to volume with each solvent. The final concentration of each solution was 10.0, 15.0, 20.0, 25.0, 30.0 and 35.0 μg/ml, respectively.

The absorbance of known durg concentration was determined by a double beam spectrophotometer in a 1 cm. cell at 289.8 nm. for methanol, 288.6 nm for 0.1 N HCl and 286.7 nm for phosphate buffer pH 6.8 against blank solution. Each concentration was determined in duplicate. The calibration curve was shown in Figure 100-102 (Appendix B).

6. Stability Study of Propranolol Hydrochloride in Coating Solution and Coated Beads.

The stability of propranolol hydrochloride was studied in both coating solution and in the coated beads. The stability of the drugs in coating solution was determined in the period of 4 hrs. as it was the total time spent for coating process. The finished coated beads were analysed for the drugs to confirm that the drugs remained unchanged after finishing coating process (see Appendix C).

6.1 Construction of Calibration Curve.

The HPLC method was used to determine the amount of the drug as follow :

Propranolol hydrochloride 10.0 mg was accurately weighed and dissolved in methanol HPLC grade. The solution was adjusted to 100.0 ml with methanol HPLC grade and used as a stock solution at the concentration about 100 µg/ml.

As internal standard, Pindolol 10.0 mg was accurately weighed and dissolved with methanol HPLC grade. The solution was adjusted to 100.0 ml with methanol HPLC grade and used as internal standard stock solution with concentration about 100 µg/ml.

Propranolol HCl stock solution was individually pipeted 2.5, 5.0, 7.5, 10.0 and 12.5 ml. into 25.0 ml. volumetric flask and pipeted 1.0 ml internal standard stock solution filled in all flasks and adjusted with diluting agent to volume. The final concentration of each solution was 10.0, 20.0, 30.0, 40.0 and 50.0 µg/ml, respectively and the concentration of internal standard was about 4 µg/ml in all flasks.

The ratio between peak area of propranolol HCl and internal standard was determined by HPLC method at absorbance of 272 nm. Each concentration was determined in duplicate. The calibration curve and representative chromatogram was shown in Figures 103 and 104 (Appendix B).

6.2 Assay of Propranolol Hydrochloride in finished product

The content of the five capsules of propranolol HCl beads were grinded. The accurately weighted powder which contained propranolol HCl about 10.0 mg was filled into 100.0 ml volumetric

flask and dissolved with methanol (HPLC grade). It was finally adjusted with methanol to have the concentration of 100 µg/ml.

Pipet 5.0 ml of propranolol solution and 1.0 ml of internal standard (Pindolol) filled into another volumetric flask 25.0 ml, then adjusted with dilutingagent solution. The concentration of propranolol hydrochloride was determined by HPLC method at absorbance of 272 nm and calculated with calibration curve in Figure 103 (Appendix B). The chromatogram was shown in Figure 108 (see Appendix C).

6.3 Assay of Propranolol Hydrochloride in Coating Solution

The propranolol HCl in coating solution after keeping for 4 hrs at room temperature was assayed as follow :

Pipet 1.0 ml of coating solution at time interval 0,1,2,3 and 4 hrs. filled into 50.0 ml volumetric flask, diluted and adjusted with methanol HPLC grade. Then pipeted of 1.0 ml of this solution and 2.0 ml of internal standard solution filled in to 50.0 ml volumetric flask, adjusted with diluting agent solution. The results averaged from two determinations were assayed by HPLC method. The representative chromatogram was shown in Figure 107 (see Appendix C).