Chapter 2

Experimental

Materials and Methods

1. Materials

1.1 Model drug

-Propranolol Hydrochloride
Batch No. 950915
(China National Chemical, Imp&Exp Corp., China.)

1.2 Additives

-Ethylcellulose 10 cps.
(Supplied by Rama Product, Thailand)
-Eudragit®RL100, RS100
(Rohm Pharma, Germany)
-Lactose
(DMV International, Holland)
-Corn Starch
(Supplied by Pharmaceutical Sciences Co., Ltd. Thailand)
-Microcrystalline Cellulose
(Avicel PH101, ASAH1 Chemical Industry Co., Ltd.)
-Dibutyl Phthalate
(Electron Microscopy Sciences, USA)
-Talcum
(Osmanthus Brand, China)
-Magnesium Stearate
(Peter Greven Fett-chemie GmbH., Germany)

1.3 Dissolution medium -Hydrochloric Acid
(E. Merck, Germany)
-Anhydrous Dibasic Sodium Phosphate
(E. Merck, Germany)
-Sodium Chloride
(Ajax Chemicals, Australia)
-Citric Acid Monohydrate
(E. Merck, Germany)

1.4 Solvent -Anhydrous Methanol, AR grade
(J.T. Baker Inc., USA.)
-Isopropyl Alcohol
(Shell Chemical, Thailand)
-Acetone
(Shell Chemical, Thailand)

2. Equipments

-Planetary Mixer
(Crypto-Peer Less LTD., England)
-Oscillating Granulator
(Viuhang Engineering, Thailand)
-Spheronizer
(Model S320, Niro Fielder, England)
-Fluidized Bed Coater
(Model STREA 1, Niro-Aeromatic AG, Switzerland)
-Peristaltic Pump
(Model 1B.1003/R, Roto Consulta, Germany)
-Magnetic Stirrer
(Heidolph, MR3001, Germany)
-Dissolution Tester
(Model DT6R, Erweka, Germany)

-pH Meter
(Model 292, Pye Unicam, England)
-Spectrophotometer
(Spectronic3000 Array, Milton Roy, USA.)
-Analytical Balance
(Sartorius, Model A200S, Germany)
-Sieve Shaker
(Josef Deckehnann Aschaffenburg, Western Germany)
-Scanning Electron Microscope
(Model S2360N, Hitachi, Ltd., Japan)
-Friabilator
(Erweka, Germany)

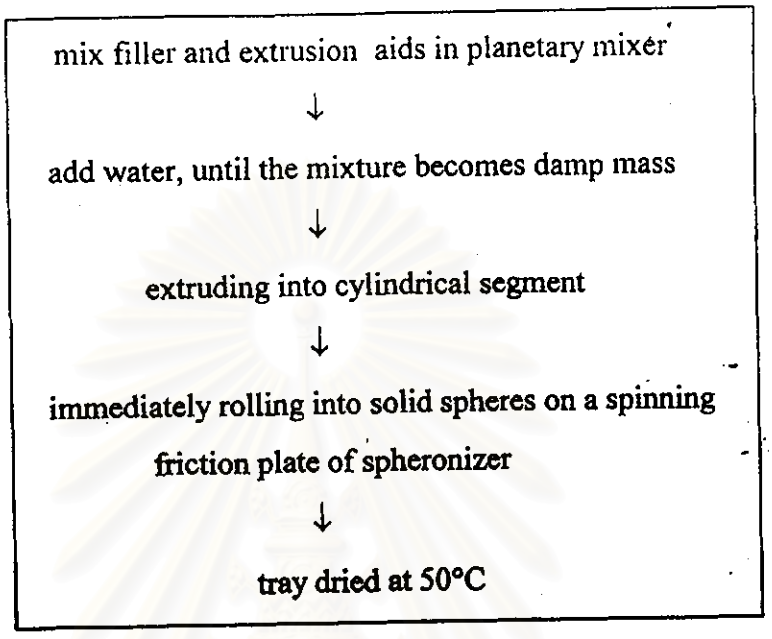
3. Preparation of Propranolol Hydrochloride Pellets.

Initially, blank core pellets without drug were prepared by using oscillating granulator and spheronizer in order to investigate suitable conditions for pellet preparations. The blank core pellets composed of lactose and corn starch, which were also called diluent of the formulation. In addition, microcrystalline cellulose (Avicel PH101) was used as an extrusion aid and a binder. The composition of the blank core pellets is presented in Table 4.

Table 4 Formulation of core pellets.

Ingredient	% w/w
lactose	54
corn starch	23
microcrystalline cellulose	23
water qs.	

The pelletization process was performed according to the following flow chart:



The spheronizer was a laboratory type, which consisted of a friction plate 1.2 mm in hight (H), 1.8 mm in width (W), 3 mm in length (L) (Niro-fielder, Model S320, Figure 10). In order to test a variety of spheronizing conditions, various factors such as load of extruded mass, speed, and rolling time were adjusted. A suitable setting of spheronizing conditions was presented in Table 5.

Table 5 The spheronizing conditions.

spheronizing condition	value
load of extruded mass	500 g
spheronizer speed	500-520 rpm
spheronization time	2 min

After the suitable condition was established, part of lactose in the formulation was then substituted by propranolol hydrochloride and final composition of propranolol hydrochloride pellets was shown in Table 6.

Table 6 Formulation of propranolol hydrochloride pellets.

ingredient	% w/w
propranolol hydrochloride	42.5
lactose	11.5
corn starch	23
microcrystalline cellulose	23
water qs.	

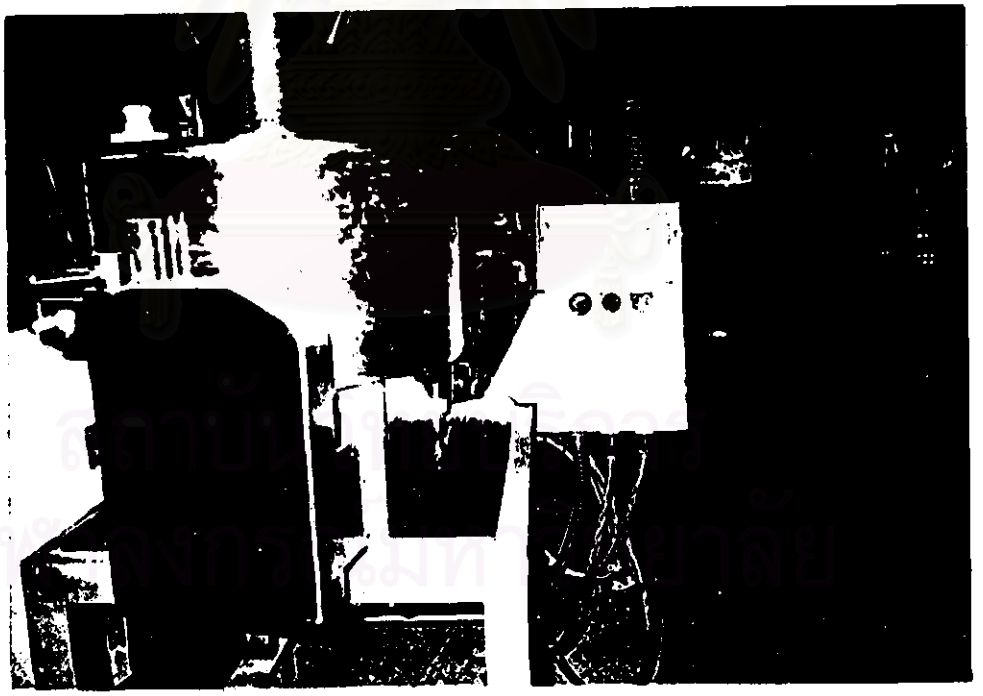


Figure 10 The photograph of spheronizer (Model S320, Niro Fielder, England).

4. Evaluation of Core Pellets.

The following physical properties of core pellet were evaluated:

4.1 Morphology

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

4.2 Density

The bulk and tapped density were determined by pouring 50 grams of the cored pellets 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 5 cm hight until a constant volume was obtained. Then, tapped volume was devided by weight to attain tapped density. Both densities were average of three determinations. The Carr's compressibility was calculated from the following equation.

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

T and B are tapped and bulk density, respectively.

4.3 Friability

Ten grams of core pellets retained on sieve size 40 mesh (420μm) and eight spherical metallic balls with a diameter of 0.5 cm were filled in a closed 30 ml bottle. Then, rotating was performed in Roche Friabilator for 100 cycles. The percentage

weight loss of pellet after screening on 40 mesh sieve size were determined. The results were averages of the two determinations.

4.4 Moisture Content

The moisture content of propranolol hydrochloride pellets was determined by using Ohaus moisture determination balance. About 7 g of pellets were spread uniformity as a thin layer and accurately weighed on a pan. And heat intensity was set at 4 watts until constant weigh was obtained. The percent moisture content was calculated based on the following equation.

$$\% \text{ moisture content} = \frac{\text{weight before drying - weight after drying}}{\text{weight before drying}} \times 100$$

4.5 Determination of Propranolol Hydrochloride Content of Core Pellets.

Approximately 150 mg of crushed sample was accurately weighed and dissolved in methanol. Then adjusted to 50 ml in a volumetric flask and filtered.

Pipet 1.0 ml of filtrate and transfer to 50 ml volumetric flask. Adjusted methanol to volume and mixed. The absorbance of resulting solution was determined at 289 nm using double beam spectrophotometer. In this procedure, methanol was used as a blank. Finally the content was calculated from a standard curve, triplicate assays were performed.

5. Pellets Coating Process.

5.1 Coating Solution

The composition of coating solution was presented in Table 7. The solution was made by dissolving polymer in solvent mixture of acetone and isopropyl alcohol in a ratio of 1:1. Dibutyl phthalate was added together with talcum and magnesium stearate. The amount of polymer mentioned in the coating solution implied the mixture of ethylcellulose and Eudragit®RL100 or ethylcellulose and Eudragit®RS100 according to the formulation shown in Table 8-9. Different percent of coating levels of each polymer mixtures were implemented in order to have different thickness of the coating films.

Table 7 Composition of coating solution in preliminary investigation.

Ingredient	% w/v
polymer	7.692
talcum	5.385
magnesium stearate	1.539
dibutyl phthalate	0.769
acetone	42.308
isopropyl alcohol	42.308

Table 8 Ratio between Eudragit®RL 100 and ethylcellulose.

Formulation	Ethylcellulose (%)	Eudragit®RL100 (%)	% Coating level (w/w)
1	0	100	5
2	0	100	10
3	0	100	15
4	0	100	20
5	20	80	5
6	20	80	10
7	20	80	15
8	20	80	20
9	40	60	5
10	40	60	10
11	40	60	15
12	40	60	20
13	60	40	5
14	60	40	10
15	60	40	15
16	60	40	20
17	80	20	5
18	80	20	10
19	80	20	15
20	80	20	20
21	100	0	5
22	100	0	10
23	100	0	15
24	100	0	20

Table 9 Ratio between Eudragit®RS 100 and ethylcellulose.

Formulation	Ethylcellulose (%)	Eudragit®RS100 (%)	% Coating level (w/w)
25	0	100	10
26	0	100	15
27	0	100	20
28	20	80	10
29	20	80	15
30	20	80	20
31	40	60	10
32	40	60	15
33	40	60	20
34	60	40	10
35	60	40	15
36	60	40	20
37	80	20	10
38	80	20	15
39	80	20	20

5.2 Coating Condition

The fluidized bed coater was a laboratory type (Model STREA 1, Niro Aeromatic, Figure 11) with bottom spray attachment. A spray gun of nozzle diameter is about 1.10 mm and 2 mm diameter stainless steel tubing were used. In order to test on a variety of coating conditions, various factors such as inlet air temperature, outlet air temperature, spray air pressure, feed rate of coating solution, and post coating drying were adjusted. A suitable setting of coating conditions was presented in Table 10. The batch size of the coating core was 250 g and coating time was about 1-1.5 hours.

Table 10 The coating conditions using Wurster spray method.

coating condition	value
inlet air temperature	45° C
outlet air temperature	40° C
spray air pressure	2 bar
feed rate of coating suspension	20 ml/min
post coating drying	15 min at 40°C

Figure 11 The photograph of fluidized bed (Model STREA 1, Niro-Aeromatic AG, Switzerland).

6. Evaluation of the Propranolol Hydrochloride Coated Pellets.

The following physical properties of finished coated pellets such as morphology, density, friability, moisture content, drug content, and release characteristics were evaluated. The method used were the same as previously described in section 3.2. The release study proceeded as the method described below.

In this study, a special attention was paid to pH of dissolution medium. As an oral controlled-release pellets, it was supposed 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). Therefore, in vitro test for controlled-release pellets should at least cover this pH range. The release of the coated pellet of formulation 1-39 were tested in acid buffer pH 1.2 and phosphate buffer pH 6.8 to observe the effect of the dissolution medium.

An accurate weight portion of the coated pellets equivalent to 160 mg of propranolol hydrochloride were filled in a capsule for release studies. A procedure employed for dissolution test followed the USP XXIII dissolution method for extended release propranolol hydrochloride capsule. The acid buffer pH 1.2 or phosphate buffer pH 6.8 of 900 ml was used as a medium and the buffer medium was equilibrated to 37±0.5°C. One capsule, immersed in a basket and vessel specified in the compendium, was placed at the center of the vessel and at 2.5 cm above the bottom of the vessel. The apparatus (Model DT6R, Erweka, Germany) was operated at a speed of 100 rpm. Three capsules of each formulation were evaluated.

Ten milliliters of specimen was withdrawn at the time interval of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours. The same quantity of medium was added immediately after each sampling to keep the volume of medium constant throughout the experiment.

Each sample was filtered through paper filter (Whatman, No. 1). The absorbance of the filtrate was determined spectrophotometrically in a 1-cm cell at 289.0 nm for both acid buffer pH 1.2 and phosphate buffer pH 6.8.

The release amount of propranolol hydrochloride at anytime interval was calculated from a calibration absorbance-concentration curve. A cumulative correction was made to determine total amount of drug released.

After completion of the release studies of formulation 1-39, the release profiles of each formulation were observed. Proper formula were selected, and combined to adjust release of the drug to comply with the USP XXIII for 24 hours propranolol hydrochloride extended release capsules in term of release testing as followed:

Time(hours)	Amount dissolved
1.5	not more than 30 %
4	between 35 % and 60 %
8	between 55 % and 80 %
14	between 70 % and 95 %
24	between 81 % and 110 %

The dissolution test procedure was the same as described in the USP. A 900 ml of simulated gastric fluid without pepsin (acid buffer pH 1.2) was prepared and employed as a dissolution medium for the first 1.5 hour of dissolution study. At the end of gastric dissolution, the test was temporarily stopped to change the dissolution medium to 900 ml simulated intestinal fluid (phosphate buffer pH 6.8). The dissolution in the intestinal fluid was run for the next 22.5 hours. The apparatus was operated at a speed of 100 rpm using basket method.

Ten milliliters of specimen was withdrawn at the time interval of 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 14, 18 and 24 hour. The same quantity of medium was added immediately after each sampling to keep the volume of medium constant

throughout the experiment. Each sample was filtered through paper filter (Whatman No. 1). The absorbance of the filtrate was determined spectrophotometrically in a 1-cm cell at 289.0 nm for both acid buffer pH 1.2 and phosphate buffer pH 6.8.

The amount of propranolol hydrochloride released at anytime interval was calculated from the calibration absorbance-concentration curve. A cumulative correction was made for the previously removed sample to determine the total amount of drug released.

In this part, Inderal®LA 160 was also tested for drug release using the same procedure as described above. The release characteristics of developed formula was compared with the release behaviors of this commercial products.

7. Calibration Curve for Determination of the Drug Dissolved.

Calibration curves of propranolol hydrochloride in various media were constructed to determine an amount of the drug dissolved during determination of drug content and dissolution testing.

One gram of propranolol hydrochloride was accurately weighed and dissolved in methanol or acid buffer pH 1.2 or phosphate buffer pH 6.8 depending on which one was used as the solvent in experiment. The solution was adjusted to 50 ml in a volumetric flask. The solution was also filtered through paper filter (Whatman, No.1). Then, a 2.0 ml filtrate was pipetted and adjusted to 50 ml volumetric flask with the same solvent and used as a stock solution. The stock solution was pipeted at volume of 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml into 50 ml volumetric flask, diluted, and adjusted to volume with the same solvent. The final concentrations of each solution were 20, 24, 28, 32, 36, and 40 μg/ml, respectively.

The stock solution in methanol was pipetted at the volumes of 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 ml into 50 ml volumetric flask, diluted, and adjusted to volume with

methanol. The final concentrations of each solution were 0, 12, 16, 20, 24, 28, and 32 μg/ml, respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in 1-cm cell at 289.0 nm against blank solution. Each concentration was determined in triplicate. The calibration curves were shown in Figure 184-186 (Appendix C).