CHAPTER II

EXPERIMENT

Materials

All materials employed in this study were obtained from commercial sources.

1. Model Drug

- Propranolol hydrochloride (Batch 010425, Jintan Pharmaceutical Factory, China)

2. Tablet Excipients

- Lactose hydrous (Lot 00087527 Wyndale, Hawera, New Zealand)
- Magnesium stearate (Asia Pacific PTE Ltd., Australia)
- Polyvinylpyrrolidone K30 (Lot 03100065640 supplied by ISP Technologies, Inc.,Thailand)

3. Film Formers

- Chitosan (MW of 50,000-100,000 dalton Lot 1F507, Kyowa Tecnos Co., Ltd., Japan)
- Chitosan (MW of 37,000 dalton, Seafresh Co., Ltd., Thailand)
- Chitosan (MW of 100,000 dalton, Seafresh Co., Ltd., Thailand)

4. Additives

- Castor oil (Lot OAF62/1194 supplied by Srichand United Dispensary Co., Bangkok, Thailand)
- Kingcol Brilliant Blue FCF (Batch 109212, supplied by Nutrition Limited Partnership)

5. Chemicals for Preparation of Dissolution Medium

- Citric acid anhydrous (Lot 620354 supplied by Srichand United Dispensary Co., Bangkok, Thailand)

- Disodium hydrogen phosphate anhydrous (Farmitalia Carlo Erba, Milano, Milan, Italy)
- Hydrochloric acid (Batch 03020186, Labscan Asia Co., Ltd., Bangkok, Thailand
- Sodium chloride (Batch No F2C273, Asia Pacific Specialty Chemicals Ltd., Australia)

Equipment

- Analytical balance (Satorius, model A200 S, Germany)
- Computer program (Image-Pro Plus Version 4.5 for Window, Media Cybernetics, Inc., USA)
- Differential scanning calorimeter (Model DSC 7, Perkin-Elmer, USA)
- Disintegration tester (Erweka GmbH, type ZT31, Heosemstamm, Germany)
- Dissolution apparatus (Hanson Research, model SR2, USA.)
- High liquid performance chromatography (Model SCL-10 A VP, Shimadzu, Japan)
- Hobart mixer (model EB20F 154682, Crypto-peerless Ltd., London, England)
- Hot air oven (Memmert, type UL80, Germany)
- Infrared spectrometer (Model FT-IR 176OX, Parkin Elmer, Germany)
- Micrometer (Teclock SM-112, Japan)
- Oscillating granulator (Viuheng Engineering, Bangkok, Thailand)
- Pan coater (type MBBIO75A, Fuji Electric Co. Ltd., Japan)
- Peristaltic pump (Uni Glatt Labolatory unit, Germany)
- Scanning electron microscope (Model JSM-6400LV, Joel Ltd., Japan)
- Single punch tabletting machine (Viuheng Engineering, Bangkok, Thailand)
- Spray nozzle (Uni Glatt Labolatory unit, Germany)
- Stereo microscope (Meiji EMZ-TR, Japan)
- Tablet hardness tester (Model 2E/205, Schleuniger, Switzerland)
- Ultraviolet/visible spectrophotometer (Model V-530, Jasco, Japan)
- V-shape mixer (Kan Seng Lee Machinery Ltd., Bangkok, Thailand)
- X-ray diffractometer (Model JDX-8030, Jeol, Japan)

Methods

1. Preparation of Propranolol HCl Core Tablets

The formulations of core tablets are presented in Table 2. Core tablets containing a model drug, propranolol HCl, with the amount of 80 mg/tab were prepared by wet granulation method.

Table 2 The formulations of propranolol HCl core tablets

Substance	Formulation (mg)				
	Core 1	Core 2	Core 3	Core 4	Core 5
Propranolol HCl	80.00	80.00	80.00	80.00	80.00
Sodium chloride	0.00	50.00	100.00	150.00	200.00
Lactose	202.00	152.00	102.00	52.00	2.00
PVP K30	12.00	12.00	12.00	12.00	12.00
Magnesium stearate	6.00	6.00	6.00	6.00	6.00
Total weight (mg)	300.00	300.00	300.00	300.00	300.00

Propranolol HCl, sodium chloride and lactose were individually passed through 40-mesh screen. Required amount of each material was weighed and then thoroughly mixed together. Ethanolic PVP K30 solution was utilized as binder. The wet mass was later granulated through a high speed granulator. The granules were tray dried in a hot air oven at the temperature of 60°C for 1 hour. The dry granules were screened through an oscillating granulator with sieve no. 18 mesh. Then the obtained granules were mixed with magnesium stearate in V-shape blender for 5

minutes. The lubricated granules were compressed into 300 mg tablets on a single punch tableting machine. The compression force was controlled in order to obtain the tablet hardness of 10±2 kps.

2. Evaluation of Propranolol HCl Core Tablets

The following properties of the core tablets were investigated.

2.1 Average Weight and Weight Variation

The weight variation of core tablets was determined by an analytical balance. Each of twenty tablets was accurately weighed. The average weight and standard deviation were calculated.

2.2 Friability

The friability of core tablets was determined by a friabilator. A sample of twenty tablets was weighed on an analytical balance "w_o". A sample was tested with a friabilator at a fixed speed of 25 rpm for 4 minutes. The tablets were reweighed again after the dust was eliminated, "w". The percent of friability was calculated based on the following equation. The results were obtained from the average of three determinations.

$$\%\text{Friabilation} = \{(w_o - w)/ w_o\} \times 100 \qquad \text{(Eq.28)}$$

2.3 Diameter and Thickness

Each of ten tablets was subjected to the micrometer. Diameter and thickness was expressed in millimeter unit. Mean and standard deviation were calculated.

2.4 Tablet Hardness

Each of ten propranolol HCl tablets was sampled and individually subjected to a hardness tester. The tablet hardness was expressed in kilopounds unit. Mean and standard deviation of the tablet hardness were determined.

2.5 Uniformity of Dosage Units

Ten Propranolol HCl tablets were sampled and individually transferred to a 50-ml volumetric flask. Twenty ml of methanol was added. The volumetric flask-containing tablet was sonicated until the tablet was disintegrated. After that, it was diluted with methanol to volume and mixed.

A portion of mixture was later filtered through a filter paper. An accurately measured volume of the clear solution was further quantitative and diluted with methanol to obtain a solution containing about 30 µg of propranolol HCl per ml

The absorbance of the solution was measured spectrophotometrically (USP 26).

Preparation of Calibration Curve

An accurately weighed portion about 100 mg of propranolol HCl was transfered to a 100 ml volumetric flask. The drug was then dissolved, diluted with methanol to volume and thoroughly mixed, in order to obtain the stock solution containing about 1 mg of propranolol HCl per ml.

An accurately measured aliquot of the stock solution was further quantitatively diluted with methanol to provide the solutions within the concentration range of about 10-50 µg/ml. The absorbances of these known drug concentration solutions were determined with the spectrophotometer (USP 26). Each concentration was also determined in triplicate.

2.6 Percentage of Labeled Content

The content of propranolol HCl tablet was determined with high performance liquid chromatography (HPLC), using the isocratic reversed phase technique (USP 26). The ultraviolet absorption detector set at 220 nm was selected. Degassed ultra-purified water was utilized for HPLC assay. The mixture between phosphate buffer and acetonitrite (650:350) was utilized as mobile phase. The diluting solvent was the mixture between water and acetonitrite (650:350). The flow rate was 1 ml/min and the injection volume was 20 µl. The peak area of propranolol HCl was calculated. Each formulation was determined in triplicate.

2.6.1 Validation of HPLC Method

The analytical parameters used for the assay validation were specification, accuracy, precision and linearity.

a) Specificity

Under the chromatographic condition used, the peak of propranolol HCl had to be completely separated from and not be interfered by the peak of other components in the sample. The chromatograms were evaluated by comparing with the standard solution of propranolol HCl.

b) Accuracy

Three sets of standard solutions of propranolol HCl having concentration 1-6 µg/ml were prepared and injected. The percentage of the analytical recovery of each standard solution was calculated.

c) Precision

With Run Precision, The within run precision was determined by analyzing three sets of three standard solution of propranolol HCl in the same day. Peak areas of propranolol HCl were compared and percentage coefficients of variation (%CV) of each concentration were determined.

Between Run Precision, The between run precision was determined by comparing each concentration of propranlol HCl solutions that were prepared and injected on different days. The percentage coefficients of variation (%CV) of propranolol HCl peak areas from the three sets of standard solutions having the same concentration were determined.

d) Linearity

Propranolol HCl standard solutions ranging from 1.2 to 6 µg/ml were prepared and analyzed. Linear regression analysis of peak area versus their concentrations was performed.

2.6.2 Preparation of Calibration Curve

An accurately weighed portion about 30 mg of propranolol HCl was transferred to a 50-ml volumetric flask. The drug was then dissolved; dilute with methanol to volume and thoroughly mixed, in order to obtain solution containing about 600 µg/ml. The certain volume was quantitatively diluted with diluting medium to obtain the stock solutions with the concentration of about 12 µg/ml.

An accurately measured aliquot of the stock solution was further quantitatively diluted with diluting solvent to provide the solution within the concentration range of about 1-6 µg/ml. Each concentration was also determined in triplicate.

2.6.3 Assay of Preparation

Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 30 mg of propranolol HCl, was transferred to a 50-ml volumetric flask. Forty ml of methanol was added. The mixture was sonicated for about 1 h and then diluted with methanol to volume and mixed.

A portion of the mixture was later filtered through a filter paper. An accurately measured volume of the clear filtrate was quantitatively and stepwisely diluted with

diluting solvent in the final step to obtain a sample solution having a concentration of about 3.6 µg of propranolol HCl per ml.

2.7 Disintegration Time

Disintegration time was measured from six tablets using disintegration apparatus. Deionized water, maintained at the temperature of 37± 2° C throughout the experiment, was used as an immersion fluid. The test was performed with disk. Mean and standard deviation were calculated.

2.8 Drug Release

The drug release tests were based on the monograph of Propranolol Hydrochloride Extended Release Capsules USP 26 that using dissolution apparatus method I.

One thousand ml of pH 1.2 buffer solution which was prepared by dissolving 2 g of sodium chloride in water, adding 7.0 ml of hydrochloric acid, diluting with water to 1 liter, and mixing, was used as dissolution medium during the acid stage. The dissolution medium was allowed to equilibrate and maintain at temperature of 37± 0.5° C throughout the experiment. The tablet was placed in the apparatus and operated at a rotating speed of 100 rpm. For the buffer stage, 1,000 ml of pH 6.8 buffer solutions, prepared by dissolving 21.72 g of dibasic sodium phosphate and 4.94 g of citric acid in water, diluting with water to 1 liter, and mixing, equilibrating to the temperature of 37± 0.5° C, was then substituted for the acid solution in the vessel. A portion of the solution under test was assayed spectrophotometrically at the wavelength of maximum absorbance at 320 nm

Preparation of Calibration Curve

– In Phosphate Buffer pH 6.8 Solution

Standard propranolol hydrochloride 100 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 solution. This solution was used as a stock solution.

The stock solutions of 1, 2, 3, 4, 5 ml, respectively, were individually pipetted into 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 solution was assayed spectrophotometrically at 288 nm. The absorbance and the calibration curve of propranolol hydrochloride in phosphate buffer pH 6.8 solutions are presented in Table 2A and Figure 2A (Appendix A). Each concentration was determined in triplicate.

– In pH 1.2 Buffer Solution

Standard propranolol hydrochloride 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 pH 1.2 buffer solution. This solution was used as a stock solution.

The stock solutions of 1, 2, 3, 4, 5 ml, respectively, were individually pipetted into the 100 ml volumetric flasks, diluted and adjusted to volume with pH 1.2 buffer solutions. The final concentrations of each solution were 10, 20, 30, 40 and 50 µg/ml, respectively.

The final solution was assayed spectrophotometrically at 288 nm. The absorbance and the calibration curve of propranolol hydrochloride in pH 1.2 buffer solutions are presented in Table 2A and Figure 2A (Appendix A). Each concentration was determined in triplicate.

3. Preparation of Osmotic Pump Tablets

3.1 Preparation of Film Coating Formulation

Three grades of chitosan were examined. Some initial properties of these materials are given in Table 3

Table 3 Some properties of chitosan A, B and C

Chitosan	MW	% deacetylation	Source
A	50,000-100,000	90.7	Kyowa Japan
B	37,000	90	Seafresh Thailand
C	100,000	95	Seafresh Thailand

The film coating formulations employed in this investigation were prepared as aqueous system of dispersions. Chitosan was used at a concentration of 5%w/w based on coating component. The acetic acid was utilized as solubilizer in mole ratio of acid: glucosamine unit (1.2:1). The mixture was mixed and left standing for about 20 h. The mixture was later filtered through a polyester cloth. Castor oil, brilliant blue and magnesium stearate was then added into the filtrate at concentrations of 15, 0.05 and 45 %w/w based on chitosan, respectively. The mixture was homogenized until smooth, fine dispersion was obtained.

3.2 Tablets Coating Process

Core tablets were loaded into a conventional coating pan. A batch size of 500 g was coated with coating formulation in the coating pan coupling with an air-atomized spray nozzle. Prior to coating the core tablets were warmed with drying air at temperature of 60-65°C. The atomizing air pressure was 2 bars. The coated tablets were allowed to be dried in the pan with drying air for 10 minutes. The coated tablets were then kept in a desiccator before evaluation.

3.3 Preparation of Passageway

The passageway on the face of each osmotic pump tablet was obtained by using a high precision-drilling machine called microdrills with diameters of 200, 400, 600 μm.

4. Evaluation of Osmotic Pump Tablets

The properties of the osmotic pump tablets were similarly investigated as of the core tablet in 2.1 to 2.8.

4.1 Dissolution Data Analysis

The dissolution data were fitted according to the following well-known exponential equation given below, which is often used to describe the drug release behavior.

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{(Eq. 29)}$$

where M_t / M_∞ is the fraction of drug released, t is the release time, k is a kinetic constant (with units of t^{-n}) incorporating structural and geometric characteristics of the release device and n is the release exponent indicative of the mechanism of release. This equation can be used to analyzed the first 60% of a release profile where the release profile is linearly related to t^{-n}, regardless of geometric shape (Pappas, 1985). Values for n and k for each formulation were obtained by plotting the logarithm of the fractional release against the logarithm of time. The slope of the line is n while log k is the intercept. The drug release data were plotted using values of M_t / M_∞ within the range of 0-0.60.

Although the constant k is one of the measures of the drug release rate, it should not be used for comparison because different kinetics is usually involved in different test conditions (Talukdar et al., 1996). Therefore, to characterize the drug release rate in different experimental conditions, relative dissolution time (RDT) was calculated from dissolution data by using following equation (Brockmeier and Hattingberg, 1982).

$$\text{RDT} = \frac{ABC}{M_\infty} \qquad \text{(Eq. 30}$$

4.2 Surface Topography

Propranolol HCl tablet was coated with gold using ion sputtering prior to the microscopic examination with scanning electron microscope. The gold-coated tablet was imaged using a 15-20 kv electron beam and then photographed at an appropriate magnification. The morphology of surface and cross section area of film-coated tablets was observed.

4.3 Physicochemical Properties of Film Coat

The film coats were carefully peeled off from core tablet and then measured for Fourier transform infrared (FT-IR) spectra, X-ray powder diffractograms and differential scanning calorimeter.

4.3.1 Powder X-ray Diffraction Studies

Powder X-ray diffractometry was used to determine the diffraction angles of substances that showed crystallinity and interplannar spacing of the crystal planes. This mode was used to study the change of crystallinity of polymer and other additive after preparation which could explain some physicochemical properties of coated film.

The power X-ray diffractograms were recorded at room temperature using an X-ray diffractometer. The X-ray source was nickle-filtered Cu Kα radiation generated at 30 Kv and 30 Ma. The target element was Cu-λ = 1.54 A°. The dried film was carefully placed on a glass plate and scanned from 2° to 90°2θ using reflection of a glass plate as blank.

4.3.2 Fourier Transform Infrared (FT-IR) Studies

Fourier transform infrared spectrophotometry was used to study the change in functional groups of the polymer and additive in the coated film.

The FT-IR spectra were performed using an IR spectrometer by KBr disc method. Prior to measurement, the chitosan films were cut and then ground by a small vibrating mill before grinding with KBr.

4.3.3 Differential Scanning Calorimetry Studies

Differential scanning calorimetry was used to determine the thermograms of polymers and other additive. The differences in thermal energy patterns between the original substances and their products were evaluated. This method was used to study interaction between components after preparation.

DSC curves were recorded using a differential scanning calorimetry analyzer. A powderous sample was encapsulated in a pierced lid aluminum pan prior to test. A heating rate of 10°C/min and a temperature range of 40-400°C were selected for scanning in the N_2 gas atmosphere with a flow rate of 15 cm^3/min.

4.4 Tablet Swelling and Change of Passageway during Dissolution

The shape and size of osmotic pump tablets and passageway were detected from the radian changed. Three tablets of each formulation were tested in deionized water under the same condition with the dissolution study. At an appropriate time intervals (6, 12 and 24 h), the tablets were taken out and then individually photographed by using stereomicroscope at the magnification of 0.7 and 4.5. The radian size of tablets and passageway were measured with Image-pro Plus® program. The measurement was made in triplicate.