Chapter II

Experimental

1. Materials

- Sodium hydroxide, commercial grade (Thai Asali Chemical, Ltd. Thailand)
- Chitin (Unicord, Thailand)
- Acetic acid glacial 100% (Merck, Germany)
- Conc. hydrochloric acid (Merck, Germany)
- Sodium hydroxide, analytical grade (Riedel-de Haen AG, Germany
- Propranolol hydrochloride (China, Supplied by Poltco, Thailand)
- Citric acid (Archer Daniel midland, USA)
- Dibasic calcium phosphate (Budenhiem, Germany)
- Magnesium stearate (Akcros Chemical V.O.F, Netherland)
- Aerosil 200 (Cabot, Germany)
- Salicylamide (Polfa, Poland)
- Sodium chloride (The Government Pharmaceutical Organization, Thailand)
- Disodium hydrogen phosphate (Monsanto, USA)
- Potassium dihydrogen phosphate (Merck, Germany)
- Sodium hexanesulfonate (Fisons Scientific Equipment, England)

- Hydroxymethylcellulose
  - Methocel E4M (Premium grade, The Dow Chemical Company, USA)

2. Equipments

- Fitz mill (Kan Seng Lee Factory Ltd., Thailand)
- Mechanical sieve shaker (Josef Deckelman, Germany)
- Magnetic stirrer (Thermolyne Corporation, USA)
- Hot air oven (Heraeus, Germany)
- Analytical balance (Mettler, Swizerland)
- Moisture determination balance (Mettler LP16, Swizerland)
- Spray Dryer (Niro Atomizer, Denmark)
- Homoginizer (Silverson, England)
- Scanning electron microscope (model JSM-35CF, Jeol, Japan)
- X-ray diffractometer (model JDX 8030, Jeol, Japan)
- Infrared Spectrometer (model 1760x, Perkin Elmer, USA)
- High liquid performance chromatography
  - uv/vis spectrophotometer (LDC Analytical, USA)
  - pump (LDC Analytical, USA)
  - column (Bondclone 10 um C_{18} 300*3.9 mm.,Phenomenex, USA)
- Carver press (model C, Perkin Elmer, USA)
- Thickness and hardness tester (model PTB311, Pharmatest, Germany)
- Disintegration tester (model PTZ1, Pharmatest, Germany)

- Dissolution tester
  - dissolution (Sotax AT7, Swizerland)
  - uv/vis spectrophotometer (model DU 60, Beckman, USA)
- Brookfield viscometer(model LVT-DUII, Brookfield Engineering Laboratories, Inc.,USA)

3. Methods

3.1 Preparation of Chitosan

Chitosan is the deacetylated product of chitin. Chitin from shrimp hulls was used as the dry starting material. It was pulverized with fitz-mill (Kan Seng Lee Factory Ltd., Thailand) and sized through 60/200 mesh. Chitin was deacetylated by following procedure.

1. Sodium hydroxide solution 50%(w/v) was prepared. First, 650 grams of sodium hydroxide (commercial grade) were placed in a 2000 ml pyrex beaker that was placed on a magnetic stirrer (Thermolyne Corporation,USA). After that, deionized water was added to dissolve and to make solution volume of 1,300 ml. The heater-controller was adjusted to make reaction temperature of 110°C.

2. Chitin of 100 grams were added into the solution and then the beaker was covered with aluminium foil to prevent the evaporation of water . Stirring speed was set at No.8. The reaction temperature was checked periodically.

3. The reaction time was varied to produce chitosan in different percentage of degree deacetylation.

4. After reaching the reaction time as required, the suspension of chitosan was transferred from the beaker into a sintered-glass filter No.1 that connected with erlenmyer flask and vacuum pump. The process of suction and washing chitosan with deionized water was repeated until the filtrate was neutral to pH paper.

5. Chitosan was removed from sintered-glass filter and dried in hot air oven (Hereus, Germany) at 70°C for 7 hours.

6. Dried chitosan was sieved through No.60 mesh screen and stored in tight glass-bottle in desiccator.

3.2 Evaluation of Physicochemical Characteristics

3.2.1 Determination of degree of deacetylation of chitosans

3.2.1.1 Colloidal titration

Degree of deacetylation of chitosans were determined according to the method described by Hayes (1978).

Preparation of chitosan hydrochloride

Chitosan hydrochloride was obtained by following procedure.

1. About 2.5 grams of chitosan were weighed and dissolved in 400 ml of 10% acetic acid by stirring at 1250 rpm

for about 15 minutes

2. Undissolved particles were removed by suction filtration using polyester cloth as a filter.

3. Concentrated hydrochloric acid (~20 ml) was added slowly to the chitosan solution with rapidly stirring until no further precipitation of chitosan hydrochloride was obtained.

4. The precipitate was filtered through polyester cloth. The solid was made into a slurry with 100 ml of methanol and refiltered. This operation was repeated until the washing was free of chloride ion (test with 0.1% silver nitrate solution).

5. The chitosan hydrochloride precipitate was dried in a hot air oven at 50 °C over night. The dried chitosan hydrochloride was light brown in color.

Titration of chitosan hydrochloride

1. Accurate weight (about 1 gm) of dried chitosan hydrochloride was dissolved in water and the solution was diluted to 250 ml in volumetric flask.

2. The solution of 50.0 ml was transferred to 125 ml erlenmeyer flask. Next, the solution was titrated with standard sodium hydroxide solution using phenolphthalein as an indicator.

3. The volume of sodium hydroxide solution used was recorded as millilitre and percentage of degree of

deacetylation of each chitosan sample could be calculated. The results were the means of three determinations.

3.2.1.2 Infrared spectrometry

Infrared spectra were examined by using a Fourier transform infrared spectrometer (model 1760x, Perkin Elmer, USA) and KBr disc.

3.2.2 Viscosity measurement

A Brookfield viscometer (model LVT DVII, Brookfield Engineering Laboratories, Inc., USA) with small sample adapter (spindle No.31) was used for this purpose. In all measurements, 50 ml of chitosan solution was prepared in 2% acetic acid at 10 g/l concentration. Measurements were made in triplicate at speed No.6 on solution at 25±1°C

3.3 Preparation of Co-spray Dried Powder of Propranolol Hydrochloride and chitosan

3.3.1 Formulation of co-spray dried solution

The amount of ingredients used in each formulation were presented in Table 4. The different reaction time of chitosan used in each formulation were presented in Table 5.

Table 4 Formulation for each matrix

Ingredients	Amount per Matrix
	(mg)
Propranolol hydrochloride	40
Chitosan	30
Citric acid	120
Dibasic calcium phosphate	110
Total	300

Table 5 The different reaction time of chitosan in each Formulation

Formulation	Chitosan	Reaction Time (hour)
I	CS2*	2
II	CS3.5	3.5
III	CS7	7
IV	CS10	10

*CS was abbreviated from chitosan, the number indicated reaction time using

3.3.2 Preparation of Co-Spray Dried Solution

The procedures for preparation of co-spray dried solution were as follows. Citric acid was dissolved in the water then chitosan and propranolol hydrochloride were dissolved respectively. The solution was added to dibasic calcium phosphate. The mixture was sprayed into the spray dryer in a suitable condition.

3.3.3 Spray Drying Technique

The spray drying apparatus used was a laboratory type one (Niro Atomizer Mobile Minor Unit, Denmark), having drying chamber of 80 cm. in diameter, 60 cm.in cylindrical height and conical based. The cone angle was 60^{o}. The solutions were atomized into a drying chamber by a rotating centrifugal wheel atomizer.

Before proceeding with the preparation of co-spray dried powder, preliminary investigation of spray-drying process was carried out to determine optimum condition. The conditions of spray dry process , e.g., inlet air temperature, feed rate, and air pressure were represented as below.

Spray drying conditions used in preparation of co-spray dried powder

Condition	
Inlet air temperature (°C)	155
Feed rate (ml/min)	24
Compression air (bar)	4

3.4 Evaluation of Physical Properties of Co-spray Dried Powder

3.4.1 Determination of Powder Characteristics

Photomicrographs of powder samples were taken with scanning electron microscope (model JSM-T 220A, Jeol, Japan). The sample were coated with goal prior to the microscopic examination using ion sputtering. Size, shape, and surface topography of the co-spray dried powder were observed.

3.4.2 Moisture Determination

The moisture content of powder was determined by using the Mettler LP16 integrated with Mettler PM100 (Mettler Instrument AG, Swizerland).About 1 gram of co-spray dried powder was exposed to an IR lamp until constant weight was reached. The percent moisture content was calculated by this apparatus. These values were obtained from the average of three determinations.

3.4.3 Particle Size Distribution

Particle size distribution was determined by sieve analysis. The approximately 10 grams of powder was put on the top sieve series ranging from 212, 150, 106, 75, to 45 μm respectively. The nest of sieves (Endecotts Ltd., England) were placed on the sieved shaker (Jasef Deckelman, Germany) for 20 minutes. The results were reported as percentage of weight retained on each sieve size.

3.4.4 Determination of Propranolol Hydrochloride Content in Co-Spray Dried Powder

The method for determining propranolol hydrochloride content used in this study was high-performance liquid chromatography (HPLC) that was modified from USP XXII and Henry, et al. (1986)

3.4.4.1 Chromatographic condition and instrumental settings

Column : bondclone C_{18}, 10 um, 300*3.9 mm.

Mobile phase : 0.01 M sodium hexanesulfonate in 1% acetic acid and methanol (50:70)

Flow rate : 1 ml/min

Detector : UV at 290 nm

Integrator : BDS software

Injection volume :20 µl

Temperature : ambient

3.4.4.2 Mobile phase preparation

Sodium hexanesulfonate 1.88 grams was dissolved in 1% acetic acid solution and diluted to final volume of 1000 ml with the same solvent to prepare 0.01 M sodium hexanesulfonate in 1% acetic acid. The solution was mixed with methanol to obtain the solvent ratio of 50:70. The mobile phase was filtered through a PTFE membrane filter (pore size 0.45 µm) and then sonicated for 30 minutes.

3.4.4.3 Internal standard solution

The internal standard, salicylamide 250 mg was dissolved in 50% methanol in 100 ml volumetric flask and diluted to volume with the same solvent to give the final concentration of 2.5 mg/ml.

3.4.4.4 Standard solution of propranolol HCl

Propranolol hydrochloride 40 mg was accurately weighed and was dissolved in 50% methanol in 100 ml volumetric flask. 5.0 ml of internal standardsolution was added to the solution and was diluted to volume with 50% methanol.

3.4.4.5 Assay preparation

Accurately weigh the co-spray dried powder, equivalent to 40 mg of propranolol hydrochloride, to a 100 ml volumetric flask and then 70 ml of 50% methanol was added. The mixture was sonicated for 10 minutes and 5 ml of internal standard solution was added Dilute with 50% methanol to volume, mix and filter through a filter through a fiter paper (Whatman No.1).

3.4.5 The IR Spectroscopy

IR spectra of all powders were recorded on a KBr disc with an infrared spectrophotometer (Shimadzu IR440, Japan).

3.4.6 X-ray Diffraction

The crystallinity of propranolol hydrochloride in the co-spray dried powder were examined by x-ray diffractometry (model JDX-8030, Jeol, Japan). The sample for x-ray diffraction studies were firmly packed into the cavity of a thin rectangular metal plate using two glass slides which was fastened to the metal plate with adhesive tape. The first glass slide was then removed, and the prepared sample was taken to expose to the x-ray diffraction chamber. The x-ray diffraction patterns were recorded at the rate of 80^{o} per minute from 5^{o} to 105^{o} in the term of 2θ angle.

3.5 Preparation of Matrix

The powder was compressed at compressional pressure of 3000 pounds by Carver Laboratory Press (model C, Perkin Elmer,USA) using 9 mm. flatfaced, circular punch. The punch and die were lubricated with magnesium sterate prior using. The compression pressure was maintained for 10 seconds and quickly released.

3.6 Matrix Evaluation

3.6.1 Matrix Thickness

The thickness was measured by using Pharmatest PTB311. The mean and standard deviation were obtained from six determinations.

3.6.2 Matrix Hardness

The hardness was measured by using Pharmatest PTB311. The mean and standard deviation of six determinations were calculated.

3.6.3 Disintegration Time

The disintegration time was determined according to USPXXII method. The average was calculated from six determinations. The disintegration time was measured using the USPXXII apparatus

(model PTZ1 Pharmatest, Germany) with 0.1N HCl at 37±2°C as disintegration fluid. The test was performed with disks.

3.6.4 Dissolution Studies

In this study, special attention was paid to pH of dissolution medium. As oral controlled release tablets were suppose to pass the entire upper gastrointestinal tract, it would be ideal when the release of drug was constant overa wide range of pH values (from 1 to about 7) therefore, an *in vitro* test for controlled release tablets should at least cover this pH range. The studies can be devided into two steps; first,to study the effect of buffer pH 1.5 and buffer pH 6.8 on the release profiles, then the satisfactory preparation was selacted for further study by pH change method (USP XXII).

Nine hundred milliliters of buffer pH 1.5 or buffer pH 6.8 were placed in a vessel specified in the USP dissolution test and the medium was equilibrated to 37±0.5°C . One Tablet was immersed in the vessel and the paddle, 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 (Sotax AT7, Swizerland) was operated at speed of 100 rpm. Six tablets of each formulation were evaluated.

Dissolution device is connected to the dissolution testing accessory, composed of a dissolution programs, an auto 7-

sample, seven flow cells, tubings, and an eight channel pump. The dissolution program are used for instrumental control and calculations The auto 7-sample is a seven position transport which is installed in the sample compartment of the spectrophotometer (model DU series 60, Beckman USA).It is designed to hold a reference solution and six sample flow cells. The reference solution is placed in cell positon zero, which is closest to the front of the sample compartment. The six sample flow cells are placed in cell position one to six, numbering from the front of the sample compartment. If the reference solution is a blank, the blank is read on each cycle, before the sampl , and the sample values are corrected for the blank reading. The tubin is designed to go from vessel to the pump, to the flow cell and back to the vessel. The absorbance of the sample was determined spectrophotometrically in a 1-cm cell at 280 nm for buffer pH 1.5 and buffer pH 6.8 at the time interval every 30 minutes until 12 hours or propranolol hydrochloride was completely released.

In the dissolution model with pH-change , the pH of the medium was kept by buffer pH 1.5 for 1.5 hours, then the pH was increased to 6.8 by adding 2.345 grams of NaOH followed by 6.125 grams of KH_2PO_4 dissolved in a few ml of buffer pH 1.5. The sampling time were every 30 minutes until 12 hours.

The amount of propranolol hydrochloride released at any time interval was calculated from the calibration absorbance-concentration curve by dissolution program.

3.6.5 Calibration Curve of Propranolol Hydrochloride

Propranolol hydrochloride 250 mg. was accurately weighed and dissolved in bufffer pH 1.5 or buffer pH 6.8. The solution was then adjusted to 1000 ml with buffer pH 1.5 or buffer 6.8 and used as stock solution.

The stock solution was individually pipetted 2, 3, 4, 5, 6, 7, 8 and 9 ml. into a 50 ml volumetric flasks and diluted to volume with buffer pH 1.5 or buffer pH 6.8. The final concentration of each solution was 10, 15, 20, 25, 30, 35, 40, and 45 ug/ml respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at 290 nm. for buffer pH 1.5 or buffer pH 6.8. The buffer pH 1.5 or buffer pH 6.8 was used as a blank solution. Each concentration was determined in duplicate.

The concentration versus absorbance of propranolol hydrochloride in buffer pH 1.5 and buffer pH 6.8 at 290 nm. were presented in Table 13 and 14 and showed a linear relationship with the correlation coefficient of 0.999967 and .999973 respectively. The standard curve of propranolol hydrochloride after regression analysis was illustrated in Figure 32 according to the equation $y = 51.43822x - .09444$ and $y = 51.23451x + .122281$, respectively.

3.6.6 Preparation of Co-Spray-Dried Propranolol HCl-Chitosan -HPMC and its Matrix

Following the drug release profile studies of the Formulation are discussed . Only the matrices containing chitosan which have reaction time two hours showed promising result for futher investigations on the release rate modifications by incorporating HPMC into the formulation . The amount of ingredients used in each formulation were represented in Table 6 and 7.

Table 6 Formulations for each matrix

Ingredients	Amount per Matrix (mg)
Propranolol hydrochloride	40
Chitosan (CS2)	30
Citric acid	120
HPMC (Methocel E4M)	%
Dibasic calcium phosphate q.s	300

Table 7 Formulations for spray dried

Formulation	% HPMC
V	2
VI	3.5
VII	5