# Chapter II

# Experimental

## Materials

The following materials obtained from commercial source were used .

### Model drug

- Propranolol hydrochloride BP., Batch No 931030 (China National Chemicals Im & Exp Corp., China)

### Additives

- Lactose hydrous (Hawera, New Zealand)
- PVP K30 (GAF, Singapore)
- Crosslinked carboxymethylcellulose sodium (Ac-Di-Sol®), (FMC corporation, USA)

### Lubricant

-Magnesium stearate (Supplied by Pharmaceutical Sciences, Thailand)

### Film formers

- Chitosan L, Lot UCPL-L01; Chitosan M, Lot UCPL-M01 and Chitosan H, Lot UCPL-H01, (Unicord Plc., Bangkok, Thailand)

### Plasticizers

- Propylene glycol USP.XX, Lot PL 07 (Supplied by Srichana United Dispensary Ltd, Bangkok, Thailand)
- Polyethylene glycol 400 (PEG 400), Lot PID 09/4 (Supplied by Srichana United Dispensary Ltd, Bangkok, Thailand)
- Glyceryl triacetate (triacetin), Lot 43 H3404 (Sigma Chemical Co, MO, USA)

**Commercial drug product**

- Inderal®, Lot 16250 (F.E. Zuellig (Bangkok) Ltd., Bangkok, Thailand)

**Miscellaneous**

- Absolute ethanol (E. merck, Darmstadt, Gernamy)
- Sodium chloride (E. merck, Darmstadt, Gernamy)
- Glacial acetic acid (E. merck, Darmstadt, Gernamy)
- Hydrochloric acid (BDH Laboratory Suppliers, England)
- Methanol AR (BDH Laboratory Suppliers, England)

**Equipments**

Analytical-balance (Satorius model A200 S, Germany)

Balance (Berkel AG, Zurich, Switzerland)

Differential thermal analyzer (Model DT-30, Shimadzu, Japan)

Disintegration apparatus (Hanson Research model QC-21, USA)

Dissolution apparatus (Hanson Research model SR2, USA)

Fitz mill (Kan Seng Lee Factory Ltd., Part, Bangkok, Thailand)

Harvard trip balance (Ohaus Florham Park, NJ., USA)

Hot air oven (Memmert type UL 80, Germany)

Kenwood mixer (Havant, Hants model A701 A, England)

Micrometer (Teclock Co., Japan)

Oscillating granulator (Viuheng Engineering, Bangkok, Thailand)

Oswald viscometer ( USA)

Overhead projector (Elmo Model HP-A290 , Elmo Co. LTD., Japan)

Pan coater (Fuji Electric Co. LTD., Japan)

Peristaltic pump (Uni Glatt Laboratory unit, Germany)

pH meter (Hanson Research model HI 8417, USA)

Scanning-electron microscope (JSM-T220A, Japan)

Single punch tabletting machine (Viuheng Engineering, Bangkok, Thailand)

Spectrophotometer (Spectronic 2000, Bausch and Lomb, NY., USA)

Spray nozzle (Uni Glatt Laboratory unit, Germany)

Tablet friability tester (Erweka-Apparatebau GmbH, Heusenstamm Kr, Germany)

Tablet hardness tester (Schleuniger model 2E/205, Switzerland)

Thermolyne stirrers and stirring hot plate (Nuova 7 model No. SP-18420, Thermolyne Sybron Corp., Dubuque, IA., USA)

Universal tensile tester (Instron 4301, USA)

V-shape mixer (Kan Seng Lee Machinery Ltd., Bangkok, Thailand)

Viscometer (Haake Rotovisco RV20 equipped with Haake Rheocontroller RC 20 and computerized system) (Haake Mess-Technik GmbH, Karlsruhe, Germany)

X-ray deffractometer (Philips, model PW 1130/90, Netherland)

**Methods**

1. Preparation and evaluation of propranolol HCl core tablets.

1.1 The composition of propranolol HCl core tablets.

Core tablets containing a model drug, propranolol HCl, with the amount of 40 mg/tab were prepared by using the compositions shown in Table 8. Propranolol HCl, lactose, PVP K 30 and Ac-Di-Sol were separately passed through an oscillating granulator with a 30-mesh sieve. Magnesium stearate was passed through an oscillating granulator with a 80-mesh sieve. Then all materials were dried at 60 $^{\circ}$c for 1.5 hours before used.

**Table** 8 The compositions of propranolol HCl core tablet.

| Substance | mg/tab |
|---|---|
| Propranolol HCl | 40.0 |
| Lactose | 195.0 |
| PVP K30 | 7.5 |
| Ac-Di-Sol | 7.5 |
| Magnesium stearate | 2.5 |
| **Total** | **252.5** |

1.2 Preparation of propranolol HCl core tablets

Propranolol HCl, lactose and Ac-Di-Sol were weighed and then thoroughly mixed together in a V-shape blender for 10 minutes. PVP K30 solution (10% w/v in absolute ethanol) was gradually added to the dry mixture in a Kenwood mixer and agitated with a fixed speed of No 1 until wet mass was obtained. The wet mass was granulated by pass it through a 18-mesh sieve. The granulates were dried at 60 $^{o}$c for 1 hr and then sieved through the oscillating granulator with a 20-mesh sieve to obtain uniform-size granules. Dried granules were weighed and mixed with magnesium stearate in a V-shape blender for 5 minutes and the lubricated granules were compressed into 250 mg tablets using 8.6 mm in diameter, round standard concave punch on a single punch tabletting machine. The compression force as well as tablet weight were controlled in order to obtain the tablet hardness within the acceptable range of 7 $\pm$ 2 Kp.

1.3 Evaluation of propranolol HCl core tablets

The properties of core tablets were investigated :

1.3.1 Average weight and weight variation

Each of 20 tablets was accurately weighed on an analytical balance. The average weight and standard deviation were calculated.

1.3.2 Tablet hardness

Each of 10 tablets was subjected to the hardness tester which expressed the tablet hardness in

kilopounds (Kp) unit. Mean and standard deviation of the tablet hardness were determined.

1.3.3 Tablet thickness

Each of 10 tablets was subjected to the micrometer.The thickness was expressed in micrometer (μm) unit. Mean and standard deviation were determined.

1.3.4 Tablet friability

A sample of 20 tablets was weighed on an analytical blalnce. A sample was tested with a friabilator at a fixed speed of 25 rpm for 4 minutes. The tablet friability was reported in percentage.

1.3.5 Disintegration time

Disintegration time was measured from 6 tablets using a disintegration apparatus. Both immersion fluids, deionized water and dilute HCl (1 in 100) solution were maintained at the temperature of 37 $\pm$ 2 °c throughout the experiment. The test was performed with disk. Disintegration time was measured in seconds. Mean and standard deviation were calculated.

1.3.6 Drug dissolution

The procedure for studying the release of propranolol HCl tablets was based on the USP XXIII, using dissolution apparatus method 2 as following :

One thousand millilitre of dilute HCl ( 1 in 100) solution was prepared and used as the dissolution medium. It was maintained to equilibrium at temperature of 37 $\pm$ 0.5° c throughout the experiment. The tablet was placed in the apparatus and operated for 30 minutes at a rotation speed of 100 rpm. A 5 ml aliquot of dissolution medium was withdrawn and simultaneously filtered at various predetermined time interval. The volume withdrawn at each time interval was replaced by the same quantity of the dissolution medium at 37° c.

A portion of a solution under test was assayed, using an ultraviolet spectrophotometer at wavelength of 289 nm. The amount of propranolol HCl released was then calculated from absorbance-concentration calibration curve.

Calibration curve of propranolol HCl was prepared as following procedure :

One gram of propranolol HCl was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved, diluted with the dissolution medium to volume and thoroughly mixed. This was used as stock solution containing 1 mg of propranolol HCl per ml. An accurately measured volume of the stock solution was then quantitatively pipetted and diluted to volume with the dissolution medium. The final concentrations of each solution were 10, 15 , 20, 25, 30, 35 and 40 μg/ml.

The absorbance of known drug concentration was determined by a spectrophotometer at 289 nm. The dissolution medium was used as a blank solution. Each concentration was determined in triplicate. The calibration curve of propranolol HCl in dilute HCl (1 in 100) solution was illustrated in Figure 80 in the Appendix 1.

1.3.7 Assay of active ingredient

The procedure for studying the drug content was based on the BP 1993 as following :

Twenty tablets were sampled, weighed and powdered. A quantity of the powder containing 20 mg of propranolol HCl was shaked with 20 ml of water for 10 minutes, added 50 ml of methanol and then shaked for a further 10 minutes. The sufficient methanol was added to produce 100 ml and the solution was filtered. Then an accurately measured volume, 10 ml, of the filtrate was diluted to 50 ml with methanol. The absorbance of the resulting solution was measured at the maximum at 290 nm. Each concentration was determined in triplicate.

Calibration curve of propranolol HCl in methanol was prepared in the same way as described in 1.3.6 using methanol as a blank solution. The absorbance was

determined by a spectrophotometry at 290 nm. The calibration curve of propranolol HCl in methanol was illustrated in Figure 81 in the Appindix I.

1.3.8 Surface topography

Surface topography of propranolol HCl core tablet was observed by scanning electron microscope at the magnifications of 75 and 2000 using 15-20 KV electron beam and SEM photomicrographs were taken.

2. Preparation and evaluation of film coating formulations

2.1 Preliminary study of the properties of chitosan solutions

2.1.1 Preparation of chitosan solutions.

Chitosan L M and H were examined. Some initial properties of these materials are given in Table 9.

**Table 9** Some properties of chitosan L, M and H.

| Chitosan | % deacetylation | % moisture | color |
|---|---|---|---|
| L | 97.2 | 12.9 | Yellowish |
| M | 76.4 | 11.8 | White |
| H | 79.6 | 11.4 | White |

Three grades of chitosan were individually pulverized with a Fitz mill and passed through a 80-mesh screen. Required quantity of chitosan was gradually dispersed in half of the required volume of water for 15 minutes until all particles were thoroughly wetted. The amount of glacial acetic acid giving 1 % w/w acid in the final solution was dissolved in another part of water and then added into the previous dispersion. The mixture was mixed with a magnetic stirrer for 18-20 hrs and then passed the solution through the polyester cloth before used.

### 2.1.2 Evaluations of chitosan solutions

#### 2.1.2.1 The determination of molecular weight of chitosan

To determine viscosity average molecular weight of chitosan, Oswald viscometer was used to determine the time required for a given volume of solutions to flow through a capillary. Chitosan powder was dissolved in solution of sodium chloride(0.2 M) and acetic acid(0.2 M). The concentration of chitosan solutions was 0.05, 0.075, 0.100, 0.250, 0.500 g/100ml. The densities of prepared solutions were measured by using a pycnometer. The measurement was made at room temperature at about 28°c using deionized water as relative solution. The time in second, for liquid to flow from the upper mark to the lower mark in the capillary tube was recorded. The relative viscosity($\eta_{rel}$) was obtained by

$$\eta_{rel} = \rho_1 t_1 / \rho_0 t_0$$

when $\rho_1$ was a density and $t_1$ was an efflux time of a prepared solution,$\rho_0$ was a density and $t_0$ was an efflux time of a relative solution.

The intrinsic viscosity was the intercept of the plot between ln $\eta_{rel}$ /conc and conc. According to the Mark-Houwink equation:

$$[\eta] = Km_v^a$$

,the viscosity average molecular weight of polymer(Mv) could be calculated from this relationship, where the proportionality constant (K) of chitosan=$1.8*10^{-3}$ $cm^3/g$ and the shape factor(a) of chitosan =0.93 (Qurashi, Blair and Allen, 1992).

#### 2.1.2.2 Viscosity determination

A cup and bob type viscometer, Haake viscometer (model RV 20 Rotovisco), was used for this propose. In all measurements, 9 ml of chitosan solutions at the concentration of 0.25, 0.50, 0.75, 1.50, 2.00 and 3.00% by weight of chitosan L and M, and 0.25, 0.50, 0.75, 1.50 and 2.00 % by weight of chitosan H were individually investigated. The reported data were averaged from three determinations. Each

viscosity of the solutions was then plotted against the concentration to observe the viscosity-concentration relationship.

#### 2.1.2.3 pH determination

Chitosan solutions were prepared as the method in 2.1.1 with the concentration as described in 2.1.2.2 They were individually measured by a pH meter. The results were the means of three determinations.

## 2.2 Preparation of film coating solutions

### 2.2.1 The formulations of film coating solution

The concentrations of chitosan solutions providing the viscosity of 125 mPa.s were selected to be prepared as coating solutions. These selected solutions were easily sprayed and used for this experiment. Plasticizers were also added to study the effect of plasticization at different concentrations as shown in Table 10.

**Table 10** Formulations of film coating solutions.

| Chitosan | Plasticizer | Conc.of plasticizer (%w/w of polymer) | Code |
|---|---|---|---|
| L | - | - | L0 |
| | Propylene glycol | 10,20 and 30 | LA10, LA20, LA30 |
| | PEG 400 | " " | LB10, LB20, LB30 |
| | Triacetin | " " | LC10, LC20, LC30 |
| M | - | - | M0 |
| | Propylene glycol | 10,20 and 30 | MA10, MA20, MA30 |
| | PEG 400 | " " | MB10, MB20, MB30 |
| | Triacetin | " " | MC10, MC20, MC30 |
| H | - | - | H0 |
| | Propylene glycol | 10,20 and 30 | HA10, HA20, HA30 |
| | PEG 400 | " " | HB10, HB20, HB30 |
| | Triacetin | " " | HC10, HC20, HC30 |
| LH | - | - | LH0 |
| | Propylene glycol | 10,20 and 30 | LHA10, LHA20, LHA30 |

2.2.2 Preparation of film coating solutions

The film coating formulations were prepared by the method described in 2.1.1. Plastcized film coating solutions were prepared by dissolving the required plasticizers in the dilute acetic acid solution portions before adding into the mixture. The combined chitosan solutions were prepared by mixing the chitosan solutions of L and H in ratio 1:3 by weight .

3. Propranolol HCl tablets coating and evaluation

3.1 Propranolol HCl tablets coating

Conventional pan-spray method was used for this purpose. The film coated tablets were obtained by following procedure.

A batch size of 500 g of core tablets was loaded into the conventional coating pan equipped with 4 baffles. An air-atomized spray nozzle was attached and adjusted on the upper of the tablet bed about 4 inches. The core tablets were warmed by using the drying air at 60-65 °c, the exhaust air and the atomizing air pressure of 1.75 bars, for about 15 minutes. Then the film coating solution was applied with intermittent spray at feed rate No. 0.25 using a peristaltic pump. The amount of coating solution applied to each batches was determined from a percentage coating level increased at about 1.50 % by weight. The coated tablets were allowed to be dried with dried air and heat on for 10 minutes. A batch size of coated tablets was kept in the desiccator.

3.2 Evaluation of propranolol HCl coated tablets

The average weight and weight variation, hardness, thickness, friability, disintegration time, drug release test and the surface topography were examined as the method described in 1.3.1-1.3.6. The drug release of Inderal® tablets was also eximined and compared to the drug releases of coated tablets prepared from this experiment.

The color and gloss of coated tablets were observed with naked eye. The coated tablet defects (cracking,

splitting and picking) were estimated by checking 100 coated tablets samples.

4. Preparation and evaluation of free films

4.1 Preparation of free films

The thin free films (50-100 μm) prepared by evaporating the coating solutions on the glass plates were taken by following procedure. The solutions in 2.2.2 were spreaded onto the clean surface glass plates with a diameter of 15 cm. The glass plates containing film coating solutions were then dried at 60 °c ( Chitosan solutions of L and M using the drying time for 24-26 hrs and 48-50 hrs for chitosan solution of H) in hot air oven.

Dried films were consequently removed from the glass surface and stored in the desiccator.

4.2 Evaluation of free films

4.2.1 Physical appearances

Color, transparency and bleeding of the free films were visually observed. Ease of detachment from glass plates was also observed.

4.2.2 The substance characterization

Infrared spectrometry, X-ray diffraction and differential thermal analysis were used to characterize the substances in free films.

4.2.2.1 Infrared spectrometry

Infrared spectra were examined by using a Fourier transform infrared spectrometer. Very thin free films about 10-20 μm were prepared as the method described in 3.4.1. The obtained films were directly examined. These spectra were compared to the spectra which were taken from initial chitosan powders using KBr disc and the spectra of pure plasticizers which were taken by using multiple internal reflection (MIR) technique.

4.2.2.2 Powder X-ray diffraction

The powder X-ray diffractograms from chitosan films were examined by the reflexion method with nickle-filtered CuKα radiation of Jeol diffractometer operated in the ω - 20 scanning mode between 4° and 50.°

4.2.2.3 Differential thermal analysis

DTA curves of chitosan powders and chitosan films were obtained by using a thermal analyzer with a heating rate of 10 °c /min, sensivity ± 50 μv and chart speed 10 mm/min in static air atmosphere (aluminium sealed cell).

4.2.3 Moisture sorption

The determination of the moisture sorption of the strip films was taken by following procedure.

The free films which were carefully cut into rectangular size 5 x 7 $cm^2$ were placed on the watch glassed and kept in the desiccator which filled with silica gels for 1 week at room temperature, about 28 °c. Then the first step was to determine the initial dry weight (Wo) of the strip films and these strip films were stored in a securely closed desiccator (6 inches diameter) containing the saturated sodium chloride solution in the well, at room temperature, about 28 °c and about 75% relative humidity. The hygrometer was also used to re-examine the relative humidity. Then the weights after contacted the moistrue ($W_1$) were measured at 7, 10 and 15 days.

The percentage of the moisture sorption was calculated by the following equation and all means and standard deviation were calculated from three determinations

$$\text{moisture sorption} = \frac{W_1 - Wo}{Wo} \times 100\ \%$$

Where Wo = the initial weight of the strip film
$W_1$ = the weight of the strip film after exposed to the moisture

### 4.2.4 The swelling of free films

The simple method to determine the swelling of chitosan free films was modified from the technique of Wan and Prasad (1987). The swelling values were detected from the change of volume and weight of films at before and after submersion in deionized water and in dilute HCl (1 : 100) solution at rome temperature. The data of films swelling were obtained by following procedure.

The film specimens were carefully cut into 2.5 cm x 2.5 cm strips and kept in the desiccator which filled with silica gels for 1 week at room temperature, about 28 $^{o}$c. The initial weight (Wo) of each strips was measured. The thickness of each film strips was taken from the mean value of five separate measurements. Then the initial film strip volume (Vo) was calculated.

The circular dish with 15 cm in diameter and 1.70 cm in depth was filled with deionized water up to a level of 1.00 cm and placed on the glass plate of the overhead projector. Two transparent scales graded in mm division were placed under the dish to measure the dimension of the film strip image which was held on the screen magnetically.

Each film strips was forced to immerse into the medium. At 5 minutes after, the swollen film strip image was projected on the screen and measured its dimensions. The swollen film strip was picked up and placed on the filter paper. Excess water on the swollen film strip was then removed by careful blotching of the strip with filter paper. Then the thickness of the swollen film strip was taken from the mean value of five separate measurements, and the swollen film strip volume ($V_1$) was calculated and the weight of the swollen film ($W_1$) was measured. Then the obtained film strips were placed on the watch glassed and dried at 60 $^{\circ}$c for 20 hrs in a hot air oven. The dried film strips were kept in the desiccator for 3 days before the final wight ($W_2$) was measured.

The swelling of film strips was performed in triplicate and calculated by the following equations.

Weight swelling index(w) $= (W_1 - Wo)/W_0$

Volune swelling volume(v) $= (V_1 - Vo)/V_0$

4.2.5 Tensile properties

The ultimate tensile strength and percentage of elongation were examined by using an universal testing machine equipped with 100 N tension load cell. The relative humidity of the laboratory for testing was about 55% and temperature was $25 \pm 1°$ c. The data of tensile properties were obtained by following procedure.

Film specimens were cut into small strips by using a standard knife. The thickness of each strips was the mean value of five separate measurements taken along the length of the sample using a micrometer. The accurate 2 cm in length was marked in the middle section of each strips. Then the strip was carefully clamped by an upper and lower pneumatic flat-faced grip and was extended by the test machine at speed 10.0 mm/min until it was certainly ruptured. The breaking force from the digital display and the change in the length at the moment of rupture were recorded. The acceptable data were only ones obtained from the strip that ruptured at the bilateral section.

The larger film strip with 0.57 cm of the width of L0, M0 and H0 cut by using a bigger standard knife were also tested to study the tensile stress-strain curve of these materials.

The ultimate tensile strength and percentage of elongation were determined by the following equations.

The ultimate tensile strength = breaking force/ cross section area.

The percentage of elongation $= \Delta L / Lo$ ; $\Delta L$ = the difference of length; Lo = the initial length

The mean and standard deviation of both values were obtained from 3 determinations.

7. Effect of accelerated condition on the properties of propranolol HCl coated tablets

The coated tablet were kept in a securely closed desiccator containing a saturated sodium chloride solution in the well. This desiccator was placed in the incubator setting the temperature at 45 °c for 1 week and inside desiccator had about 75% relative humidity. The treated tablets were examined for the various properties as same as the coated tablets in 3.2 and uniformity of content in 1.3.7. These data were compared to the data of coated tablets which were examined for the properties after coating and kept at room temperature for 1 week.