CHAPTER II

EXPERIMENT

Materials

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

1. Model drug

- Propranolol Hydrochloride
  (Batch No.010425 , Jintan Pharmaceutical Factory, China)

2. Capsule shell

- Gelatin Capsule , Conisnap Type (CAPSUGEL®)
  (International Capsule , Thailand)
- HPMC Capsule , Conisnap Type (VCAPS®)
  (International Capsule , Thailand)

3. Film forming agent

- Cellulose acetate (acetyl 39.8%) , CA-398-10 NF
  (Lot No. AC-0433 NF , Eastman Chemical , USA)
- Diethyl phthalate
  (Lot No. 325384/1-393 , Fluka Chemika , Germany)
- Polyethylene glycol 400
  (Lot No. 403351/1-54699 , Fluka Chemika , Germany)
- Hydroxypropylmethylcellulose (METHOCEL® E5LV PREMIUM EP)
  (Rama Production , Thailand)

- Propylene glycol
  (Lot No. PL45/363 , Srichand United Dispensary , Thailand)

4. Solvents

- Methylenechloride
  (Liquor Division , The Excise Department , Thailand)
- Ethanol
  (Liquor Division , The Excise Department , Thailand)
- Methanol
  (Liquor Division, The Excise Department , Thailand)
- Isopropyl alcohol
  (Liquor Division, The Excise Department, Thailand)

5. Osmotic agents

- Lactose
  (Lot 00087527 NZMP , New Zealand)
- Sodium Chloride
  (Lot No. F2C273 , Asia Pacific Specialty Chemicals , Australia)
- Sucrose
  (Lot No. F3D103 , Ajax Finechem , New zealand)
- Potassiumchloride
  (Lot No.F4E074, Ajax Finechem, New zealand)

6. Dissolution media

- Deionized water
- Hydrochloric acid 37% , AR grade
  (Lot No.03020186 , Lab-scan Analytical Sciences , Ireland)

- Monobasic potassium phosphate
  (Lot No. A315973-127 , Merck , Germany)
- Sodium Hydroxide , AR grade
  (Lot No. 7708 MVKK , Mallinckrodt , Sweden)

Equipment

- Dissolution apparatus
  (Model VK7000 , Vankel , U.S.A)
- pH meter
  (Model 292 , Pye Unicam , England)
- Scanning electron microscope
  (Model 5410 LV , Jeol , Japan)
- Fluidized bed coater
  (Model Strea1 , Niro-aeromatic , Germany)
- Semiautomatic filling capsule machine
  (Yiewheng , Thailand)
- Osmometer
  (Model Osmomat 031-D , Gonotec , Germany)
- Ultraviolet / visible spectrophotometer
  (Model V-530 , Jasco , Japan)

Methods

1. Preparation of propranolol HCl capsules

Propranolol hydrochloride capsule was prepared to contain propranolol HCl (80 mg) and various amount of sodium chloride or lactose or sucrose or potassium chloride as osmotic agents. Amount of propranolol HCl and sodium chloride / lactose / sucrose / potassium chloride in Table 3(capsule no.1) and Table 4(capsule no.2) were passed through a 80 mesh screen and mixed together. The powder was blended in a V-shape mixer for 15 minutes. The powder mix was filled into capsule no.1(Table 3) and no.2(Table 4) by semiautomatic capsule filling machine. The core capsules were kept in a desiccator prior to coating.

Table 3 : Uncoated capsule formulation (Capsule No.1)

Ingredient (mg/capsule)	C11
Propranolol HCl	80
NaCl	80
Lactose	180
Total weight	340

Remark : Capsule No.1 was used in preliminary study to observe drug release property.

Table 4 : Uncoated capsule formulation (Capsule No.2)

Ingredient (mg/capsule)	Formula							
	C21	C22	C23	C24	C25	C26	C27	C28
Propranolol HCl	80	80	80	80	80	80	80	80
NaCl	28	56	80	135	190	-	-	-
Lactose	162	134	110	55	-	190	-	-
Sucrose	-	-	-	-	-	-	190	-
KCl	-	-	-	-	-	-	-	190
Total weight	270	270	270	270	270	270	270	270
Ratio of drug/osmotic agent	1:0.35	1:0.7	1:1	1:1.69	1:2.38	1:0	-	-

Remark : Capsule No.2, instead of capsule no.1, was used to modify drug release after preliminary study.

2. Preliminary investigation on coating conditions.

The suitable temperature for fluidized bed coater and solvent for coating solution preparation to provide the cellulose acetate film formation on the core capsule were determined. The concentration of 1% w/v of cellulose acetate with PEG 400 in the concentration of 41.18 %w/w of polymer in various solvent mixtures and drying temperatures were tested for suitable film formation. The following solvent mixtures and drying temperatures in Table 5 were investigated. Cellulose acetate was dissolved in solvent indicated in table, 20 milliliters of the solution was poured on the petridish, then placed in the hot air oven using drying temperature as shown until solvent was evaporated completely. Appearance of films were observed.

Table 5 : The solvent systems and drying temperatures.

Solvent mixture	ratio	Temperature(°C)
Methylenechloride : Ethanol	95:5	70
Methylenechloride : Ethanol	95:5	55
Methylenechloride : Ethanol	90:10	70
Methylenechloride : Ethanol	90:10	55
Methylenechloride : Methanol	90:10	60
Methylenechloride : Methanol	90:10	30
Methylenechloride: Isopropyl alcohol	90:10	60
Methylenechloride : Isopropyl alcohol	90:10	30

3. Investigation on suitable coating solutions and coating conditions.

For gelatin capsule, HPMC was used as subcoating layer before coating with cellulose acetate as semipermeable membrane. As without subcoating layer, cellulose acetate could not adhere on the surface of gelatin shell. Only cellulose acetate coating solution was used for coating on HPMC capsule. The concentration of cellulose acetate in coating solution was 1%w/v in the solvent mixture of methylenechloride and ethanol in a ratio of 95:5, and the concentration of HPMC solution was 3% w/v in the solvent mixture of ethanol : H_2O in a ratio of 1:1. The coating solutions were sprayed on the core capsules by fluidized bed coater using inlet air temperature at 55° C, atomizing pressure of 1.4 to 1.6 bar, capacity of fan at level of 11, feed rate in the range of 5-10 rpm. One hundred core capsules were coated in each batch. Before spraying the coating solution, the core capsules were heated to 55°C for 5 minutes. After complete coating, the coated capsules were dried in fluidized bed coater with temperature of 55°C for ten minutes in case of HPMC film coating and five minutes in case of cellulose acetate film coating, and then kept in the desiccator at room temperature.

Table 6 : The formulation of HPMC coating solution.

Ingredients	Amount
HPMC (g)	3
Propylene glycol 400 (g)	3
Ethanol : H_2O (1:1) qs to (ml)	100

Remarks : HPMC coating solution was coated only on gelatin capsules.

In order to obtain suitable amount of coating solution for uniformity of coating. The coating uniformity was indicated by color uniformity that 150 millilitres of HPMC coating solution(per batch) was suitable for uniformity of coating on capsule no.1 and 128 ml. of HPMC coating solution(per batch) for coating on capsule no.2 obtained from calculation of equation 22. The composition of HPMC coating solution is shown in Table 6. The formulation of HPMC coating solution was prepared by dispersing HPMC in a portion of ethanol, part of water was added to obtain clear solution and propylene glycol was then added and mixed. The solution was finally adjusted to required volume with the solvent mixture (ethanol and water).

Table 7 : The formulation of cellulose acetate coating solutions.

Ingredients	Formula				
	CA1	CA2	CA3	CA4	DP1
Cellulose acetate (g)	1	1	1	1	1
PEG 400 (g)	0.3	0.5	0.7	1	-
DEP (g)	-	-	-	-	0.3
Solvent mixture* qs to(ml)	100	100	100	100	100

(* the mixture of methylene chloride and ethanol in a ratio of 95:5)

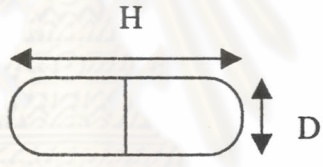
In order to obtain suitable amount of coating solution for uniformity of coating. The coating uniformity was indicated by color uniformity that 335 ml. of CA coating solution was suitable for uniformity of coating on capsule no.1. 286 ml. of CA coating solution(per batch) for capsule no.2 obtained from calculation of equation 23. The composition of various cellulose acetate coating solutions are shown in Table 7.

The formulation of cellulose acetate coating solution was prepared by dissolving cellulose acetate in solvent mixture of methylenechloride and ethanol in the ratio of 95:5. PEG400 or DEP was added and mixed. The solution was adjusted to required volume with the solvent mixture (methylenechloride and ethanol).

The length and diameter of capsule no.1 and no.2 are shown in Table 8. The length and diameter of capsule no.1 and no.2 are substituted in equation 21 to calculate their surface areas.

Table 8 : Length and diameter of capsule no.1 and no.2

Capsule No.	Length of capsule (cm.)	Diameter of capsule.(cm.)
1	1.9120	0.6780
2	1.7820	0.6220



Surface area of capsule = ¶DH (21)

Where D is the diameter of capsule , H is the length of capsule

Result of surface area calculation of both capsules are shown in Table 9.

Table 9 : surface area of capsule no.1 and no.2.

Capsule No.	Surface area (cm^2)
1	4.0730
2	3.4820

Table 10: Calculated amount of HPMC and cellulose acetate solution on capsule no.1 and no.2.

Coating solution	Amount of solution on capsule No.1 (ml)	Amount of solution on capsule No.2 (ml)
HPMC solution	150	128 *
Cellulose acetate solution	335	286 **

* amount of HPMC solution on capsule No.2 calculated using equation (22)

* * amount of CA solution on capsule No.2 calculated using equation (23)

Result from calculation of amounts of HPMC coating solution and CA coating solution on capsule no.2 with equation 22 and 23 are shown in Table 10

$$\text{amount of HPMC coating solution on capsule no.2} = \frac{(\text{amount of HPMC coating solution on capsule No.1} \times \text{surface area of capsule No.2})}{\text{surface area of capsule No.1}} \quad (22)$$

$$\text{amount of CA coating solution on capsule no.2} = \frac{(\text{amount of CA coating solution on capsule No.1} \times \text{surface area of capsule No.2})}{\text{surface area of capsule No.1}} \quad (23)$$

The orifice on coated capsule was fabricated by drilling with driller to have the orifice size of 0.4, 0.6, 0.8 and 1 mm.

4 Evaluation of uncoated capsule

4.1 Weight variation

During filling process, the weight variation was determined by sampling 20 capsules. Individual twenty capsules were weighed. The capsule were opened without losing any part of the shell and the contents were removed completely as possible. The empty shell were weighed. The weight of the contents were the difference between the weighings. The procedure was repeated with another nineteen capsules. The weight variation should conform to BP 2002 specification.

4.2 Assay of content of propranolol HCl

A quantity of the powdered mixed contents of 20 capsules was taken to contain 80 mg. of propranolol hydrochloride and added about 150 ml. of methanol, heated to boil for 2 minutes, removed from the heat, shaked for 20 minutes, cool to 20°C and added sufficient methanol to produce 200 ml. The suspension of propranolol HCl was filtered (Whatman No.1 filter paper) and the first 20 ml. of filtrate was discarded. 10 volumes of the filtrate was diluted to 200 volumes with methanol. The absorbance of the resulting solution was measured at the maximum wavelength of 287 nm. The amount of propranolol hydrochloride was calculated from the calibration absorbance-concentration curve of propranolol hydrochloride in methanol, and the amount of assayed propranolol hydrochloride should conform to USP XXVI specification.

5. Evaluation of coated capsules

5.1 Film thickness

The film thickness was determined using scanning electron microscopy on two capsules of each thickness level. The thicknesses of CA layer were measured at each side of a capsule, i.e., the end of cap, the end of body, both side of cap, both side of body.

5.2 Weight of coating.

The weight of coating among the capsules was observed by determining the weight before and after coating of two layers (HPMC was used as subcoating layer and cellulose acetate was used as semipermeable membrane), using 20 individual capsules. The average weight and standard deviation (SD) were calculated.

5.3 Cellulose acetate film weight.

Weight of the cellulose acetate films were determined from the residue shell after dissolution test for12 hours. (n = 6). The residue shell was depleted after careful washing and drying of the film in hot air oven and then using analytical balance to weigh dry shell.

5.4 The size of orifice.

The size of orifice on the surface of coated capsule was examined by optical microscope. Twenty capsules were drilled in each size of 0.4, 0.6, 0.8 and 1 mm. The size of orifices were measured by optical microscope(n=20). The photographs of each orifice size were also taken by scanning electron microscopy (SEM)

5.5 Film characterization.

The film characteristic of the surface and cross section of the coated capsules that plasticized with various PEG 400 concentration , before and after dissolution test were examined by scanning electron microscopy (SEM).

5.6 Dissolution studies.

5.6.1 Calibration curve for determination of the dissolved drug.

a) Calibration curve of propranolol hydrochloride in deionized water

One hundred milligrams of propranolol hydrochloride were accurately weighed and dissolved in deionized water. The solution was adjusted to volume in a 100 ml volumetric flask with deionized water and used as stock solution. The stock solution was individually pipetted, at the volumes of 1, 2, 3, 4 and 5 ml., and transfered into 100 ml. volumetric flasks, diluted and adjusted to volume with the deionized water. The final concentrations of each solution were 10, 20, 30, 40, 50 μg/ml.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in 1-cm cell at 287 nm. against blank solution. Each concentration was determined in triplicate.

b) Calibration curve of propranolol hydrochloride in methanol

Calibration curve was prepared as above a) except replacement of deionized water with methanol.

c) Calibration curve of propranolol hydrochloride in buffer solution pH 1.2

Calibration curve was prepared as above a) except replacement of deionized water with buffer solution pH 1.2 . The buffer solution pH 1.2 was prepared from dissolving 2 g of sodium chloride in water, adding 7 ml. of hydrochloric acid, diluted with deionized water to 1 liter and mixed.

d) Calibration curve of propranolol hydrochloride in buffer solution pH 6.8

Calibration curve was prepared as above a) except replacement of deionized water with buffer solution pH 6.8. The buffer solution pH 6.8 was prepared by dissolving 21.72 g of anhydrous dibasic sodium phosphate and 4.94 g of citric acid monohydrate in deionized water, diluted with deionized water to 1 liter, and mixed.

e) Calibration curve of propranolol hydrochloride in isotonic buffer solution pH 1.2

Calibration curve was prepared as above c) and adding 3 g of potassium chloride into the solution.

f) Calibration curve of propranolol hydrochloride in isotonic buffer solution pH 6.8.

Calibration curve was prepared as above a) except replacement of water with isotonic buffer solution pH6.8 that was prepared by dissolving 18.238 g of anhydrous dibasic sodium phosphate and 4.148 g of citric acid monohydrate in deionized water and dilution with deionized water to 1 liter, and mixed.

g) Calibration curve of propranolol hydrochloride in isotonic potassium chloride solution (0.1588 M)

Calibration curve was prepared as above a) except replacement of deionized water with isotonic potassium chloride that was prepared by dissolving potassium chloride 11.8421 g in deionized water and adjust to 1 liter with deionized water.

h) Calibration curve of propranolol hydrochloride in 0.5 M potassium chloride solution.

Calibration curve was prepared as above a) except replacement of deionized water with 0.5 M potassium chloride solution that was prepared by dissolving potassium chloride 37.275 g in deionized water and adjusting to 1 liter with deionized water.

i) Calibration curve of propranolol hydrochloride in 1 M potassium chloride solution

Calibration curve was prepared as above a) except replacement of deionized water with 1 M potassium chloride solution that was prepared by dissolving potassium chloride 74.55 g in deionized water and adjusting to 1 liter with deionized water.

j) Calibration curve of propranolol hydrochloride in 2 M potassium chloride solution

Calibration curve was prepared as above a) except replacement of water with 2 M potassium chloride solution that was prepared by dissolving potassium chloride 149.1 g in water, adjusting to 1 liter with deionized water.

5.6.2 Evaluation of the drug release in various medium.

a) Evaluation of the drug release in water.

The USP XXVI dissolution test apparatus I was modified for dissolution study. The dissolution test was performed at 50 rpm in 900 ml. of deionized water at 37 ± 0.5°C. The coated capsules were inserted into the sinker and placed in the baskets. Six capsules of each formulation were evaluated. Ten milliliters of specimen were withdrawn at 0.25, 0.5, 0.75, 1, 1.5, 2.0, 2.5, 3.0, 4, 5, 6, 7, 8, 10, 12 hours. The same volume of medium was added immediately. Each specimen was filtered through filter paper(Whatman, No.1). The absorbance of the filtrate was determined spectrophotometrically in a 1-cm cell at 287 nm. The release amount of propranolol hydrochloride at various time intervals was calculated from calibration of absorbance-concentration curve. A cumulative correction was made to determine total amount of drug released.

b) Evaluation of the drug release in other medium

The same as item (a) except replace deionized water with the other media, buffer solution pH 1.2, buffer solution pH 6.8, isotonic buffer solution pH1.2, isotonic buffer solution pH 6.8. isotonic potassium chloride solution (0.1588 M), 0.5 M potassium chloride solution, 1 M potassium chloride solution and 2 M potassium chloride solution were prepared as described in item 5.6.1 (c -- j)

c) Evaluation of the drug release in pH-change medium

The same as item a) except replace deionized water with the buffer pH 1.2 for the first one hour and changing to the buffer solution pH 6.8 for eleven hours (according to USPXXVI specification).

5.7 Dissolution Data Analysis

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)

$$RDT = \frac{ABC}{M_\infty} \quad (24)$$

The diagrammatic representation of dissolution profile for explaining RDT calculation is illustrated in Figure 5.

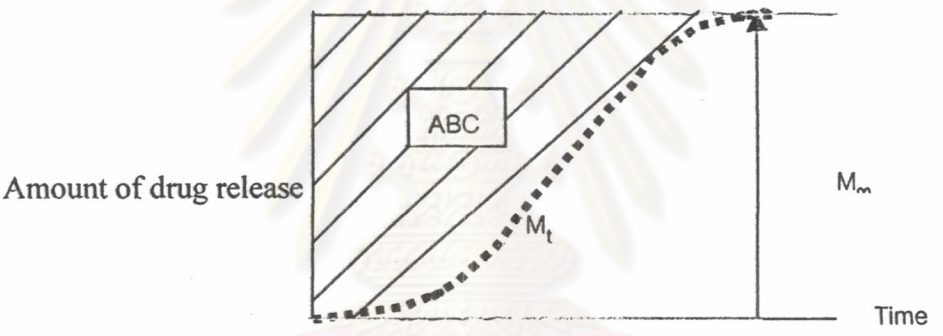


Figure 5 : Diagram of dissolution profile for explaining RDT calculation , ABC is area between upperline (M_∞) and the dissolution curve; M_∞ is maximum drug release at infinite time and M_t is amount of drug release at any time t.

ABC was calculated indirectly by substracting total area (M_∞ multiplied with time function) with area under dissolution curve (AUC). The trapezoidal method was used to calculate AUC.