CHAPTER II

EXPERIMENTAL

Materials

1. Model drug

- Propranolol Hydrochloride
  (Lot.No. ARB7149, Zhejiang medicines and health products Imp. & Exp. Corp., China)

2. Additives

- Ethylcellulose 10 cps.
  (Lot.No. MM 960117-1, Rama Product, Thailand)
- Eudragit®RS100
  (Lot.No. 0841208165, Rohm GmbH, Germany)
- Eudragit®RS30D
  (Lot.No. 0480318065, Rohm GmbH, Germany)
- Surelease® (Type E-7-19010)
  (Lot.No. F8ABC0514, A gift from Colorcon Inc., USA)
- Lactose
  (Lot.No. 8111902 117, The lactose company of newzeland LTD., Newzeland)
- Corn Starch
  (Srichand United Dispensary Co., Ltd., Thailand)
- Microcrystalline Cellulose
  (Lot.No. 1824, AMC corporation Ltd., Thailand)
- Talcum
  (Lot.No. 45SC96019, Srichand United Dispensary Co., Ltd., Thailand)

- Magnesium Stearate
  (Lot.No. MAF 07, Srichand United Dispensary Co., Ltd., Thailand)
- Dibutyl Phthalate
  (Lot.No. 61219343, Merck, Germany)
- Triethyl Citrate
  (Lot.No. 2010707, Fluka AG., Switzerland)
- Triacetin
  (Lot.No. 43H3404, Fluka AG., Switzerland)

3. Chemicals

- Hydrochloric acid 37%, sp.gr 1.18, AR grade
  (Malinckrodt, USA)
- Anhydrous Dibasic Sodium Phosphate
  (Carlo ERBA reagent, Italy)
- Sodium Chloride
  (Merck, Germany)
- Citric Acid Monohydrate
  (Fluka, Switzerland)
- Methyl Alcohol
  (Malinckrodt, USA)
- Isopropyl Alcohol
  (J.T. Baker, USA)
- Acetone
  (Merck, Germany)

Equipments

- Analytical balance (Model A200S, Sartorius GmbH, Germany)

- Dissolution apparatus (Model SR-2, Hanson Research, USA)
- Differential scanning calorimeter (Model DSC 7, Perkin Elmer, Germany)
- Extruder (Model EXKS-1, Fuji Paudal Co., Ltd., Japan)
- Fluidized bed coater (Model STREA 1, Niro-Aeromatic AG, Switzerland)
- Fourier transform infrared spectrometer (Model SP2000, Perkin Elmer Ltd., England)
- Hot air oven (Model UL80, Memmert, Germany)
- Magnetic stirrer (Model SP 46920-26, Cimarec 2, Thermolyne, USA; Model MR 3001, Heidolph, Germany)
- Peristaltic pump (Model 1B.1003/R, Roto Consulta, Germany)
- pH meter (Model 292, Pye Unicam, England)
- Planetary mixer (DT, Erweka GmbH, Germany)
- Scanning electron microscope (Model JSM-6400 LV, Jeol Ltd., Japan)
- Sieve shaker (Josef Deckehnann Aschaflenberg, Germany)
- Spheronizer (Model S320, Aeromatic-Fielder, England)
- Tensiometer (Instron model 5565, Instron Corp., Canton, MA, USA)
- Ultrasound transonic digital sonicator (Model T680/H, Elma, Germany)
- Ultraviolet-visible recording spectrophotometer (Model UV-160 A, Shimadzu Corp., Japan)
- X-ray powder diffractometer (Model JDX-3530, Jeol Ltd., Japan and Rigaku Denki (Miniflex), Japan)

Methods

1. Preparation of Propranolol Hydrochloride Pellets

A extrusion-sphronization technique was utilized to prepare pellets, using the same formulation of Coowanitwong (1997) as described below.

Table 2 Formulation of propranolol hydrochloride pellets

Ingredients	Amount of solid (%w/w)
propranolol hydrochloride	42.5
lactose	11.5
corn starch	23
microcrystalline cellulose (Avicel pH 101)	23
water qs. (125 ml.)	

The pelletization process was initiated by weighing propranolol hydrochloride, corn starch, lactose and Avicel pH 101. Then all of dry components were mixed together in planetary mixer. After thoroughly mixed, water was gradually added and mixed until damp mass occurred. The damp mass was transferred to the extruder and immediately rolled into solid spheres on the spinning friction plate of a spheronizer at definite spheronization times and speeds. The pellets were dried for 4 hours by hot air oven at 50 °C. The spheronizing conditions are presented in Table 3.

Size distributions of pellets were classified by using sieve method. The pellets passed through sieve No. 14 (1400 μm) and retained on sieve No. 18 and No. 20 (1000 and 850 μm) were selected and used in this experiment.

Table 3 The spheronizing conditions.

Factors	Values
Extruded mass load (gm)	300
Spheronization speed (rpm)	800
Spheronization time (min)	15

2. Evaluations of Core Pellets

2.1 Morphology

The morphology of core pellets was observed by scanning electron microscope (SEM). The pellets were coated with gold prior to microscopic examination using ion sputtering method. The size, shape, and surface topography of the core pellets were determined.

2.2 Determination of propranolol hydrochloride content of core pellets

Approximately 376 mg of (approximately equivalent to propranolol hydrochloride of 160 mg) core pellets (n = 10) was accurately weighed into a 100 ml volumetric flask. The pellets were dissolved with methanol by the aid of sonicator about 60 minutes, then adjusted to volume with methanol and mixed thoroughly. The solution was filtered through Whatman® filter paper No.1 and used as a stock solution. One milliliter of the stock solution was individually pipetted into a 50 ml volumetric flask, then adjusted to volume with methanol and mixed. The resulting solution was determined at 289 nm by UV/visible spectrophotometry with methanol as a reference. Propranolol hydrochloride content was calculated from the calibration curve of propranolol hydrochloride in methanol.

3. Preparations of Coating Mixtures

3.1 Polymer organic solution

The composition of coating solution is presented in Table 4. The amount of ingredients used in each formulation depended on the ratio of ethylcellulose (EC) to Eudragit®RS100 (EuRS100) and the amount and type of plasticizers used. The different formulations used for coating the pellets are given in Table 5.

Talcum and magnesium stearate were sieved through a sieve No.80 (180 microns) before used in each formulation. The polymer organic solution was made by dissolving polymer in solvent mixture of acetone and isopropyl alcohol in a ratio of 1:1 until clear solution was obtained. After that, talcum and magnesium stearate were dispersed in solution as antiadherent. The plasticizer was added to the polymer organic solutions. Then all the components of coating solution were homogeneously blended by a magnetic stirrer and stirring was continued for two hours before starting the coating process.

3.2 Polymer aqueous dispersion

In the preliminary study of mixing polymer aqueous dispersions, both Aquacoat® and Surelease® were tested and blended with Eudragit®RS30D. The blends of Aquacoat® and Eudragit®RS30D in each ratio produced very viscous dispersion similar to a gelatinous solution, so Surelease® was chosen in this experiment instead of Aquacoat®. The compositions of coating dispersion are given in Table 6. The ratios of polymers and the amount and type of plasticizers used in aqueous dispersion are presented in Table 7. Each coating dispersion contains 10% w/w polymer solids.

Table 4 The compositions of polymer organic solution

Ingredients	%w/w
Polymer*	7.69
Talcum	5.38
Magnesium stearate	1.54
Plasticizers**	0.77 or 1.54
Acetone : isopropyl alcohol (1:1) qs.	100

* ethylcellulose or Eudragit®RS100 or the mixture of ethylcellulose and Eudragit®RS100

** Plasticizer used : dibutyl phthalate (DBP) and triethyl citrate (TEC)

Table 5 The ratio between ethylcellulose and Eudragit®RS100 and the amount and type of ingredients in each formulation.

Formulation	Ratio of	Composition of coating solutions (gm)							Plasticizer
	EC:EuRS100	EC	EuRS100	DBP	TEC	talcum	Mg.stearate	solvent	(%)
A1	10:0	22.50	-	2.25	-	15.76	4.50	247.59	10
A2	10:0	22.50	-	4.50	-	15.76	4.50	245.34	20
A3	0:10	-	22.50	2.25	-	15.76	4.50	247.59	10
A4	0:10	-	22.50	4.50	-	15.76	4.50	245.34	20
A5	5:5	11.25	11.25	2.25	-	15.76	4.50	247.59	10
A6	5:5	11.25	11.25	4.50	-	15.76	4.50	245.34	20
A7	3:7	6.75	15.75	2.25	-	15.76	4.50	247.59	10
A8	3:7	6.75	15.75	4.50	-	15.76	4.50	245.34	20
A9	7:3	15.75	6.75	2.25	-	15.76	4.50	247.59	10
A10	7:3	15.75	6.75	4.50	-	15.76	4.50	245.34	20
A11	10:0	22.50	-	-	2.25	15.76	4.50	247.59	10
A12	10:0	22.50	-	-	4.50	15.76	4.50	245.34	20
A13	0:10	-	22.50	-	2.25	15.76	4.50	247.59	10
A14	0:10	-	22.50	-	4.50	15.76	4.50	245.34	20
A15	5:5	11.25	11.25	-	2.25	15.76	4.50	247.59	10
A16	5:5	11.25	11.25	-	4.50	15.76	4.50	245.34	20
A17	3:7	6.75	15.75	-	2.25	15.76	4.50	247.59	10
A18	3:7	6.75	15.75	-	4.50	15.76	4.50	245.34	20
A19	7:3	15.75	6.75	-	2.25	15.76	4.50	247.59	10
A20	7:3	15.75	6.75	-	4.50	15.76	4.50	245.34	20

Table 6 The compositions of polymer aqueous dispersion

Ingredients	%w/w
Polymer*	10
Talcum	7
Plasticizers**	1 or 1.5
Deionized water qs.	100

* Surelease® or Eudragit®RS30D or the mixture of Surelease® and Eudragit®RS30D

** Plasticizer used : triethyl citrate (TEC) and triacetin (TRC)

Table 7 The ratio between Surelease® and Eudragit®RS30D and the amount and type of ingredients in each formulation

Formulation	Ratio of	Composition of coating dispersions (gm)						Plasticizer
	SR:EuRS30D	SR	EuRS30D	TEC	TRC	talcum	H_2O	(%)
B1	10:0	90	-	2.25	-	15.75	117.00	10
B2	10:0	90	-	3.38	-	15.75	115.87	15
B3	0:10	-	75	2.25	-	15.75	132.00	10
B4	0:10	-	75	3.38	-	15.75	130.87	15
B5	5:5	45	37.5	2.25	-	15.75	124.50	10
B6	5:5	45	37.5	3.38	-	15.75	123.37	15
B7	3:7	27	52.5	2.25	-	15.75	127.50	10
B8	3:7	27	52.5	3.38	-	15.75	126.37	15
B9	7:3	63	22.5	2.25	-	15.75	121.50	10
B10	7:3	63	22.5	3.38	-	15.75	120.37	15
B11	10:0	90	-	-	2.25	15.75	117.00	10
B12	10:0	90	-	-	3.38	15.75	115.87	15
B13	0:10	-	75	-	2.25	15.75	132.00	10
B14	0:10	-	75	-	3.38	15.75	130.87	15
B15	5:5	45	37.5	-	2.25	15.75	124.50	10
B16	5:5	45	37.5	-	3.38	15.75	123.37	15
B17	3:7	27	52.5	-	2.25	15.75	127.50	10
B18	3:7	27	52.5	-	3.38	15.75	126.37	15
B19	7:3	63	22.5	-	2.25	15.75	121.50	10
B20	7:3	63	22.5	-	3.38	15.75	120.37	15

Commericially available Surelease® contains 25% w/w solids and Eudragit®RS30D contains 30% w/w solids. Each product was prepared by diluting to attain a solid content of 10% before blending the other ingredients. After mixing two polymers together, talcum was then dispersed to avoid a tendency to stick. Various levels and types of plasticizers were added to the polymer aqueous dispersions, and the mixtures were stirred constantly for two hours prior to use.

4. Preparation of Coated Pellets

Known weight of pellets (150 gm) was transferred into the fluidized-bed coating apparatus (Model STREA 1, Niro Aeromatic), using bottom-spray coating process with Wurster column, and coated with the various coating solutions until the desired film weight was increased to 15% coating level. A spray gun with nozzle diameter about 1.1 mm and 2 mm diameter stainless steel tubing was used to apply the film coating mixture. The Wurster air suspension technique was selected for the preparation of the controlled release pellets due to advantages in efficiency, applicability and versatility. During the coating operations, the mixtures were stirred continuously to prevent sedimentation of the insoluble particles. The coating conditions of organic and aqueous systems are presented in Table 8.

After the coating process, the pellets were dried in the coating chamber for another 15 minutes at the same temperature and air flow.

5. Evaluations of Propranolol Hydrochloride Coated Pellets

5.1 Morphology

A scanning electron microscope was used to examine the structure of the coated pellets. The coated pellets were cut with scalpel for cross-section observation.

Table 8 The coating conditions using bottom spray method

Parameter	Organic system	Aqueous system
Pre-heating time (minutes)	5	5
Inlet air temperature (°C)	40 ± 2	50 ± 2
Outlet air temperature (°C)	35 ± 2	40 ± 2
Feed rate of coating mixture (ml/min)	8 – 10	8
Pneumatic spraying pressure (bar)	1.8	1.8
Total spraying time (minutes)	100	60

All samples were coated with gold by using an ion sputter coater under vacuum. The size, shape and surface topography of the coated pellets were examined.

5.2 Determination of propranolol hydrochloride content of coated pellets

About 440-460 mg of coated pellets (n=3), was precisely weighed into a 100 ml volumetric flask. The other steps used were the same as previously described in section 2.

5.3 Dissolution studies

Dissolution tests were performed according to USP XXIII standard by using apparatus I (basket) at 37 °C ± 0.5 °C with a stirring rate of 100 rpm. The acid buffer pH 1.2 and phosphate buffer pH 6.8 of 900 ml were used as dissolution medium in order to determine the influence of pH on drug release from coated pellets. An accurate weight portion of the coated pellets equivalent to 160 mg of propranolol hydrochloride

was placed in the dry basket at the beginning of each test and at 2.5 cm above the bottom of the vessel. The dissolution tests were performed in triplicate.

The samples of 10 milliliters were withdrawn at predetermined intervals of 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 hours. The same volume of the medium was added immediately after each sampling to keep the volume of medium constant until the end of the experiment.

The samples were assayed spectrophotometrically at a wavelength of 289 nm either directly or after appropriate dilution with the release medium to the range of 10-40 μg/ml. The amount of propranolol hydrochloride released at any time was calculated from the calibration curve for each medium. A cumulative correction was made for the previously removed sample to determine the total amount of the drug release.

5.4 Calibration curves 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. Propranolol hydrochloride of 250 mg was exactly weighed into a 100 volumetric flask, then dissolved and adjusted to volume with methanol or acid buffer pH 1.2 or phosphate buffer pH 6.8 depending on which one was used as the solvent in experiment. Then 10 ml of the solution was pipetted into 100 ml volumetric flask and adjusted to volume with the same solvent and used as the stock solution. Then the stock solutions were further diluted with proper amount of the solvent to obtain final concentrations of each solution at 10, 15, 20, 25, 30 and 40 μg/ml, respectively.

The concentration of the final solution was determined from the absorbance at 289 nm by using a double beam spectrophotometer. Each concentration was determined in triplicate. The absorbance and the calibration curve of propranolol hydrochloride in various media are presented in Tables 17-19 and Figures 157-159 in Appendix B.

6. Evaluations of Physico-chemical and Mechanical Properties of Casting Films.

6.1 Mechanical evaluation of free films

In order to study the physico-chemical and mechanical properties, the dry films were prepared by blending two kinds of polymers both in organic and aqueous systems. All of the formulations in Tables 5 and 7 were operated. The following ratios of ethylcellulose/Eudragit®RS100 : 10:0, 0:10, 5:5, 3:7, 7:3 were prepared as the total solid content of 7.69 % w/w in solvent mixture of acetone and isopropyl alcohol. In the part of aqueous system, the mixtures of Surelease® and Eudragit®RS30D were prepared in the identical ratio as described above. Surelease® and Eudragit®RS30D were separately diluted with water until 10 % w/w based on polymer solids before mixing together. Then all of these organic or aqueous mixtures were stirred constantly with a magnetic stirrer about 15 minutes. After homogeneously mixing the two polymers, antiadherent was dispersed in the mixtures. Thereafter each film formula was prepared by adding the plasticizer to the mixture and stirring for 2 hours at a fixed speed.

The polymer mixture was carefully poured onto the glass petridish covered the inner side by non-stick paper. The film was placed onto the levelled surface and dried overnight at 40 °C for organic system and 50 °C for aqueous system. The amount of solution needed to obtain a film with a thickness of 100-200 μm varied from 5-6 gm depending on the ratio of two polymers.

The dried film was peeled off from the petridish and allowed to equilibrate to room temperature, sealed in a double plastic bag, and stored in a desiccator for a minimum of 1 week prior to testing. The thickness of dry films was determined using a micrometer at different five points of film sheet.

Before the measurement of mechanical properties, film specimens were cut into 5 x 40 mm and examined visually for physical defects. Special care was exercised during cutting to avoid jagged edges. Each strip was measured at five points with a micrometer to ensure the thickness of 100-200 μm.

The point-at-break test was performed on a tensiometer which was interfaced to a computer (Instron model 5565, 1 kN load detecting transducer). The specimen was clamped using an upper and a lower pneumatic grip. The distance between the grips, and therefore the effective length of the film under stress, was kept constant at 40 mm. Proper alignment of the film specimen between the upper and the lower grip was checked before initiation of the test. The cross-head speed was set at 5 mm/min.

Six parallel measurements were made for each type of films. Typical test parameters such as % strain at break, stress at break, Young's modulus and toughness were automatically computed by the Instron Series IX material testing software. However, it was impossible to obtain accurate values for the thickness of the dry films because of non-uniform spread of film over the entire surface.

6.2 The powder X-ray diffraction analysis

The polymer mixtures in two systems without antiadherents were prepared and studied under X-ray diffraction analysis. Three different ratios of two polymers : 10:0, 5:5, 0:10 in both systems were investigated. The mixture films in

organic system were plasticized by triethyl citrate and the mixture films in aqueous dispersion system were plasticized by triacetin. The polymer mixtures in these ratios were prepared as previously described with a stirring time for 2 hours. About 10 gm of the mixtures was poured into glass petridishes covered by non-stick paper to allow a better peeling of the dry films. The film was casted onto the levelled surface in an oven, preset at a desired coalescense temperature overnight. The dried film was then removed from the petridish and was kept at room temperature in a desiccator over silica gel prior to use. Moreover, the same formulations in Table 5 and Table 7 plasticized by 15% and 20% level of plasticizers were also prepared by the same procedures. These samples were likewise tested with X-ray diffractometer.

The polymer films without antiadherent and some parts of the blended films in aqueous system were cut in a rectangular size so that can be put into the sample holder by using removable adhesive to fix them before testing with X-ray diffractometer (Jeol JDX-3530). In the part of the mixed films in organic system, films were cut up and packed into a thin rectangular quartz slide by the other cover slide. After firmly packed, the cover slide was taken off and the sample holder was placed into the powder X-ray diffraction apparatus (Rigaku Denki). A measurement was run with a scanning speed of 6° 2θ per minute over a range of 5-40 $^{\circ}$ 2θ.

6.3 The infrared spectroscopy

The same ratios of two polymers as described in section 6.2 without antiadherents and plasticizer were tested with infrared spectrophotometer. Additionally, mixed films in the ratio of 3:7 and 7:3 without additives in both systems were also tested.

The IR spectra of the blended polymer films and antiadherent powder were examined by using the potassium bromide disc (KBr) method by an infrared spectrophotometer. The film should be cut in small pieces. Approximately 1 part of the

sample was triturated with 100 parts of dried, finely powdered KBr. The mixture was thoroughly ground with an agate mortar and pestle to obtain a uniform mixture, spread it in a die with diameter of 7 mm and compressed with the Qwik Handi-Press. The scanning range used was in the range of 4000-400 cm^{-1}.

6.4 The differential scanning calorimetry

Thermal analysis was performed on the different pure polymers alone, the blended polymer in the ratio of 5:5 and the selected formulations from Table 5 and Table 7. These samples were tested by a Perkin Elmer DSC-7 differential scanning calorimeter. Aluminum pans and lids were used for samples and temperature calibration was performed using cyclohexane and indium as standards. An empty pan, sealed in the same way as the sample, was used as reference. Film samples of 10-14 mg were accurately weighed into aluminum pans and then sealed. The samples were tested under nitrogen gas purge at a heating rate of 10 $^{\circ}$C/min. Thermal analysis were performed at temperature ranging from –20 to 150 $^{\circ}$C.