CHAPTER II

EXPERIMENTAL

Materials

The following materials were obtained from commercial sources. Deionized water was utilized throughout this study.

1. **Model drug**

•Propranolol HCl (Batch No.941002, China National Chemicals Imp.&Exp. Corp., China)

2. **Film Former**

•Chitosan (M.W. of 40,000 dalton Lot 5j519, Kyowa Technos Co., Ltd., Japan, supplied by G.T. Chemicals Co., Bangkok, Thailand).

3. **Organic Acid**

•Citric acid anhydrous (Lot ACF29, supplied by Srichand United Dispensary Co., Bangkok, Thailand)
• DL-malic acid (Lot SM1G1225, Haamann&Relmer Corp., Elkhart, IN, USA.)
•Formic acid (Lot K17806164, Merck, E. Merck, Darmstadt, Germany)
•Glacial acetic acid (Merck, E. Merck, Darmstadt, Germany)
•Glycolic acid (Farmitalia Carlo Erba, Milano, Milan, Italy)
•Lactic acid (Lot K20704866, Merck, E. Merck, Darmstadt, Germany)
•Propionic acid (Lot 1887F00, Farmitalia Carlo Erba, Milano, Milan, Italy)

4. **Tablet Excipients**

•Crosslinked carboxymethylcellulose sodium (Ac-Di-Sol®) (Lot T934, FMC Corp.USA.)
•Lactose hydrous (Wyndale, Hawera, New Zealand)
•Magnesium stearate (Batch 41060980, Lek Pharm and Chem Work, Yugoslavia)
•Polyvinyl pyrrolidone K30 (Lot 40709, GAF, Singapore)

5. **Additives**

•Castor oil (supplied by Srichand United Dispensary Co., Bangkok, Thailand)

•Colorants (Brilliant blue (C.I. No. 42090), erythrosine (C.I.No.45430), ponceau 4R (C.I.No. 16255), sunset yellow (C.I.No.15985) and tartrazine (C.I.No. 19140) (Government Pharmaceutical Organization, Bangkok, Thailand); (green FS) (Butterfield Food Ingredients Ltd., Norfolk, England)

•D-Sorbitol (Lot R01956, Fluka Bio Chemika, AG CH-9470 Buchs, Switzerland)

•Diethyl phthalate (Fluka Chemie AG CH-9470 Buchs, Switzerland)

•Glycerin (Lot GB03/28, supplied by Srichand United Dispensary Co., Bangkok, Thailand)

•Polyethylene glycol 1450 (Pharmaceutical Traders Co., Bangkok, Thailand)

•Polyethylene glycol 400 (Lot PID 09/4, supplied by Srichand United Dispensary Co., Bangkok, Thailand)

•Polyethylene glycol 6000 (Pharmaceutical Traders Co., Bangkok, Thailand)

•Potassium bromide (for IR spectroscopy, Lot G07029, Fluka Chemie AG CH-9470 Buchs, Switzerland)

•Potassium dihydrogen phosphate (Lot 227 A679473, Merck, E. Merck, Darmstadt, Germany)

•Propylene glycol USP.(Lot PL70/611, supplied by Srichand United Dispensary Co., Bangkok, Thailand)

•Stearic acid (Lot ACL06, supplied by Srichand United Dispensary Co., Bangkok, Thailand)

•Titanium dioxide (Lot 877, Nam Siang Co., Bangkok, Thailand)

•Triacetin (Lot 43H3404, Sigma Chemical Co., St Louis, MO 63178, USA.)

•Triethyl citrate (Lot AG CH9470 Fluka Chemical, Buch, Switzerland)

•Urea (Lot R0382, Fluka Chemika Chemie AG, CH-9470 Buchs, Switzerland)

6. **Chemicals for Preparation of Dissolution Medium**

•Citric acid anhydrous (Lot ACF29, supplied by Srichand United Dispensary Co., Bangkok, Thailand)

•Disodium hydrogen phosphate anhydrous (Lot 127286 628, Merck KGaA, Darmstadt, Germany)

•Hydrochloric acid (Lot 510602, Univar, Auburn, Australia)

•Sodium chloride (Lot K20420804, Merck, E. Merck, Darmstadt, Germany)

•Sodium hydroxide (Lot 080882, Eka Nobel, Bohus, Sweden)

•Glucose (Lot RB12719, Fluka Bio Chemica AG CH-9471, Buchs, Switzerland)

7. **Solvent**

•Absolute ethanol (Merck, E. Merck, Darmstadt, Germany)

•Acetonitrite (Lot 9017-03, J.T. Baker, Phillipsburg, USA.)

•Methanol AR (BDH Laboratory Suppliers, England)

•Methanol(HPLC) (Batch 97 10 0008, Lab Scan, Bangkok, Thailand)

8. **Commercial Product**

•Inderal LA-80 (Lot MH803B MADe 9 1995, distributed by The East Asiatic Co., Bangkok, Thailand, made in United Kingdom)

Equipment

•Analytical balance (Satorius, model A200 S, Germany)
•Computor program (Scientist®, Salt Lake City, Utah, USA.)
•Differential scanning calorimeter (Netzsch DSC 200, Germany)
•Disintegration tester (Erweka®GmbH, type ZT31, Heosemstamm, Germany)
•Dissolution apparatus (Hanson Research, model SR2, USA.)
•Fitz mill (Kan Seng Lee Factory Ltd., Bangkok, Thailand)
•Friability tester (Erweka, type TA3, Heusenstamm, Germany)
•FT-IR spectrometer (Spectrum 2000, model SP 2000, Perkin Elmer, USA.)
•Hardness and diameter tester (Erweka TBH30, type TBH30 MD, Erweka®G mbH, Heusenstamm, Germany)
•Hobart mixer (model EB20F 154682, Crypto-peerless Ltd., London, England)
•Hot air oven (Memmert, type UL80, Germany)
•HPLC (Millipore Waters Chromatography Division, Milford, Massachusetts, USA.) composed of:-
:Model 600E multisolvent delivery system
:Water 746 data module
:Water 484 tunable absorbance detector
:Model 712 Waters Intelligent Sample Processor (WISP™)
•HPLC column (Merck 50943, LiChro CART®125-4, LiChrospher®100RP-18 (5 μm), 253385, E. Merck, Darmstadt, F.R.Germany
•Micrometer (Teclock Co., Japan)
•Microprocessing PC computor (CPU 486DXA-100, O.A. Tech Co., Ltd., Bangkok, Thailand)
•NMR spectrometer (Bruker model DPX-300, Switzerland)
•Oscillating granulator (Viuheng Engineering, Bangkok, Thailand)
•Osmometer (Osmomat 030-DM, Gonotec GMBH, Berlin, Germany)
•Pan coater (type MBBIO75A, Fuji Electric Co. Ltd., Japan)
•Peristaltic pump (Uni Glatt Labolatory unit, Germany)
•pH meter (model PHI 32, Beckman Instruments, USA.)
•Roto stator (Ultra-Turrax T50 DPX, IKA-Labortechnik, Germany)
•Scanning-electron microscope (JSM-T220A, Jeol, Tokyo, Japan)
•Shaker (type TBV S-01, Heto (TBVS HETOMIX, Japan)
•Single punch tabletting machine (Viuheng Engineering, Bangkok, Thailand)
•Spray nozzle (Uni Glatt Labolatory unit, Germany)
•Thermolyne stirrers and stirring hot plate (Nuova 7 model No. SP-18420, Thermolyne Sybron Corp., Dubuque, IA, USA.)
•Universal tensile tester (Instron 5565, USA.)
•UV-vis spectrophotometer (Spectronic 3000 Array, Milton Roy Co., USA.)
•V-shape mixer (Kan Seng Lee Machinery Ltd., Bangkok, Thailand)

•Vibrating mill (Shimadzu, Japan)

•Viscometer (Brookfield viscometer, model RVTDCP, Brookfield Engineering Labolatories, Inc., USA.)

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

Methods

Chitosan having molecular weight of 40,000 derived from chitin of crab shell was utilized. The N-deacetylation degree was 85.91±0.75%, experimentally determined by colloidal titration (Hayes, 1978) as presented in Table *83*, Appendix A. Chitosan flakes were previously ground by using a Fitz mill and passed through sieve No.80 mesh before used. A 5 % w/w of chitosan solutions were prepared by using carboxylic acids (acetic, citric, formic, glycolic, lactic, malic and propionic acid) as solubilizer by varying the mole ratio of glucosamine unit of chitosan : acid to determine the suitable mole of acid utilized to obtain clear solution.

1. Evaluation of Chitosan Solution

Chitosan solutions at concentration of 5% w/w were prepared by dissolving chitosan powder in acid (acetic, citric, formic, glycolic, lactic, malic and propionic) solutions by varying the mole ratio of glucosamine unit : acid from 1:0.5 to 1: 1.5. The mole ratio at which all investigated acids could solubilize chitosan to obtain clear solution was selected.

1.1 Physical Appearance

The physical appearances such as color and the presence of insoluble matter after mixing between chitosan and acids at different mole ratios of the glucosamine unit : acid in deionized water were visually observed.

1.2 pH

The pH value of 5% w/w chitosan in acid solutions at 24 hours after dissolution at selected mole ratio was determined by a pH meter. The experiments were performed in triplicates.

1.3 Viscosity Measurement

The viscosity of chitosan solutions solubilized with different acids during 24 hours was measured by using a viscometer. The measurement was conducted with cone No. 41 at a speed of 100 rpm (shear rate of 200 sec^{-1}) using 9 ml of each solution.

2. Evaluation of Physicochemical Characteristic of Chitosan Films

2.1 Preparation of Chitosan Film

Five % w/w of chitosan solutions were prepared by dissolving chitosan powder in acid solution of mole ratio glucosamine unit : acid 1:1.2 with constant stirring for 14 hours and then filtering through polyester clothe. Chitosan films were obtained by casting the filtrate on glass petri dish and then drying on a leveled surface for 6 hours at a temperature of 60°C. The films thickness was about 80 μm. The obtained films were stored in a desiccator at ambient temperature for 48 hours prior to further treatment.

Dried chitosan films were then exposed to moist heat at 60°C 75% RH, exposed to heat at 60°C and 130°C in hot air ovens and kept in vacuum at ambient temperature. At various time intervals, these films were characterized. The neutralised films with 0.5 N sodium phosphate solution were also prepared and characterized.

2.2 Percent Water Sorption (WS) and Dissolution of Film

To determine the amount of water absorbed, films of 2x2 cm^2 were carefully cut, weighed (W_1) and then immersed in three media (deionized water pH 5.6, hydrochloric buffer solution pH 1.2 and phosphate buffer solution pH 6.8). After 24 hours, the film remnants were wiped off excess surface water using filter paper and weighed (W_2). The swollen films were dried at 60°C for 48 hours and kept in desiccator for 48 hours prior to reweigh (W_3). The water sorption and the extent of dissolution were calculated using formula:

$$\text{Water sorption(ws)(\%)} = (W_2-W_3)/W_3 \times 100 \quad \text{(Eq. 15)}$$

$$\text{Dissolution(\%)} = (W_1-W_3)/W_1 \times 100 \quad \text{(Eq. 16)}$$

Six samples were used for each measurement.

2.3 Fourier Transform Infrared (FT-IR) Studies

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.

2.4 Powder X-ray Diffraction Studies

The powder 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 5 to 50°2θ using reflection of a glass plate as blank.

2.5 Differential Scanning Calorimetry Studies

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.

Chitosan powder, pure acids, and physical mixture of chitosan powder and solid acids (mole ratio glucosamine unit : acid 1:1.2) were also instrumentally characterized, except the pure liquid acids which were not tested for X-ray diffractogram and DSC curve. In case of neutralised chitosan films, since they were to be shrinkage and wrinkle, they had to be cut and ground with a small vibrating mill before testing with powder X-ray diffraction study.

2.6 Solid-State ^{13}Carbon Nuclear Magnetic Resonance Spectroscopy (^{13}C CP-MAS NMR spectroscopy)

Solid-state ^{13}C NMR experiment was carried out on an NMR spectrometer by means of the cross polarization-magic angle spinning (CP-MAS) method at a resonance frequency of 300 MHz for ^{13}C nuclei at room temperature (23±1°C). The ^{13}C chemical shifts were calibrated through the use of adamantane as reference. Powderous chitosan, untreated and heat treated chitosan films were loaded into a zirconium oxide rotor. These specimens were washed with 1 N NaOH solution before measurement. The peak picking and integration were recorded for analysis.

2.7 UV-VIS Absorption Studies

The UV-VIS absorption spectra of chitosan film was directly carried out by using a standard UV-VIS spectrophotometer in an absorption mode between 200-900 nm.

3. Compatibility of Chitosan Solution, and Plasticizers and Colorants

Five %w/w of chitosan solution was prepared by dissolving chitosan powder in acetic, citric, malic and propionic acid solutions (mole ratio glucosamine unit of chitosan:acid 1:1.2) with constant stirring for 14 hours and then filtering through the polyester clothe. Chitosan solution was individually incorporated with plasticizers; glycerin, propylene glycol and PEG 6000 at concentrations of 5, 15, 25, 35 and 45 %w/w, and PEG 400, PEG 1450, triacetin and triethyl citrate at concentration of 5, 15, 25 and 35 %w/w. Substances which could be compatible with chitosan and there was no phase separation would be selected as plasticizer for chitosan.

A suitable plasticizer was selected and incorporated into chitosan solution and then this solution was tested for compatibility with some water soluble dyes: brilliant blue (C.I. No. 42090), erythrosine (C.I.No.45430), green FS, ponceau 4R (C.I.No.16255), sunset yellow (C.I.No.15985) and tartrazine (C.I.No. 19140). One % w/w of dye solution was mixed with plasticized chitosan citrate solution to obtain the final dye

concentration of 0.02, 0.05, 0.20, 0.50 and 1.00% w/w of chitosan. After mixing, the physical appearance of the mixtures was visually observed as in 1.1. The substances which provided enough tinctorial strength and could be miscible with chitosan solution would be selected for further study.

4. An Incorporation of Pigment and Preparation of Cast Film

A 5 %w/w chitosan solution was prepared by dissolving chitosan powder in citric and malic acid solutions (mole ratio glucosamine unit of chitosan: acid 1:1.2) with constant stirring overnight and then filtering through the polyester clothe. Each selected plasticizers at concentration of 5, 15, 25, 35 and 45 % w/w was individually incorporated with chitosan citrate and malic solutions. Selected water soluble dye was individually dissolved in plasticized chitosan solution at concentration of 0.5 %w/w of polymer. Pigment (talcum and titanium dioxide) after passed through sieve no. 80 mesh was individually incorporated in plasticized and colored chitosan solution at 10, 20 and 30 % w/w of chitosan. Cast films of unplasticized chitosan citrate and malate and those after mixing with plasticizers (propylene glycol or glycerin), colorants (brilliant blue or green FS) and pigments (talcum or titanium dioxide) were prepared with the method described in 2.1.

5. Evaluation of Cast Film

Cast films of unplasticized chitosan citrate and malate and those after mixing with plasticizers (propylene glycol or glycerin), colorants (brilliant blue or green FS) and pigments (talcum or titanium dioxide) were evaluated.

5.1 Physical Appearance

Physical appearances such as color, transparency, glossiness, precipitation, stickiness, flexibility and bleeding were visually examined. The film additives which could provide desired characteristics were selected.

5.2 Mechanical Properties

Mechanical properties of cast films were performed using a universal tensile tester at room temperature (25-28°C). Cast films were cut into 5 x 0.2 cm strips. Each strip was then measured for thickness using a micrometer. Thickness was determined five positions along the length of the strip. The mean thickness of each strip was calculated. The mechanical measurements were performed using a 10 N load cell with an initial gauge length of 0.5 cm and a cross speed of 1 mm/min. Tensile strength, % strain at break, modulus of elasticity (Young's modulus) and toughness were automatically calculated from stress-strain curve. Total of six specimens were examined and the mean of the results was taken in all cases. The plasticized cast films that showed the high toughness would be chosen for pigmentation.

5.3 Moisture absorption

Cast films were carefully cut into square size of 4 x 4 cm^2 and accurately weighed. They were then stored in securely closed desiccator containing saturated sodium chloride solution in the well, at room temperature and 75% RH. The moisture uptake was measured after 15 days. Six samples were utilized for each measurement.

5.4 Fourier Transform Infrared (FT-IR) Studies

The determination was followed as the method described in 2.3.

5.5 Powder X-ray Diffraction Studies

The determination was followed as the method described in 2.4.

5.6 Differential Scanning Calorimetry Studies

The determination was followed as the method described in 2.5.

6. Preparation and Evaluation of Propranolol Hydrochloride Core and Coated Tablets

6.1 Preparation and Evaluation of Propranolol Hydrochloride Core Tablets

The 8.6 mm. in diameter of core tablets containing a model drug, propranolol hydrochloride 40 and 80 mg/tab for consequently preparing fast release and extended release coated tablets, respectively were prepared by wet granulation method. The composition of these core tablets are shown in Tables 2A and B. Ethanolic PVP K30 solution was utilized as binder. The wet mass was screened through sieve No.18 mesh prior to drying at 60C 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 using 8.6 mm in diameter biconcave punch on a single punch tableting machine. The compression force was controlled in order to obtain the tablet hardness of 10±2 Kps.

Table 2. The composition of propranolol HCl core tablets.

A. The composition of propranolol HCl core tablet for extended release.

Substance	mg/tab
Propranolol HCL	40
Lactose	233
PVP K30	12
Ac-Di-Sol®	9
Magnesium stearate	6
Totai	300

B. The composition of propranolol HCl core tablet for extended release.

Substance	mg/tab
Propranolol HCL	80
Lactose	202
PVP K30	12
Magnesium stearate	6
Total	300

6.2 Evaluation of Core Tablet

6.2.1 Surface Topography

The morphology of surface and cross section area of core tablets was studied under a scanning electron microscope at the magnifications of 200 and 1000.

6.2.2 Weight Variation and Friability

Each of 20 tablets was accurately weighed on an analytical balance. The average weight and standard deviation were calculated. The weight variation of tablets should be less than 7.5% as notified in USP XXIII. A sample of 20 tablets was weighed and tested with a friability tester at a fixed speed of 25 rpm for 4 minutes. The tablet friability was reported in percentage.

6.2.3 Hardness and Diameter

Each of 10 tablets was subjected to the hardness and diameter tester which expressed the hardness in kilopounds (Kp) unit and the diameter in milimetre. Mean and standard deviation of the tablet hardness and diameter were determined.

6.2.4 Disintegration Time

Disintegration testing of six tablets was performed using standard USP testing method without disk. Disintegration fluid used was deionized water and the temperature was maintained at 37±1°C. Results were reported as the time required for complete disintegration of the tablets.

6.2.5 Uniformity of Dosage Units and Assay of Propranolol HCl

Uniformity of dosage units and assay of this drug were undertaken based on the monograph of propranolol HCl extended-release capsules USP XXIII. Determination of uniformity of dosage units was undertaken spectrophotometrically at the wavelength as shown in Table *84* (Appendix A). The limit of USP XXIII for the uniformity of dosage units was the drug content 85-115% and %CV ≤ 6%. For assay procedure, pindolol was used as an internal standard and ultraviolet absorption detector set at 220 nm was selected. Degassed ultra-purified water was utilized for the 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 1.2 ml/min and the injection volume was 20 µl. The attenuation was 64 and the chart speed was 0.25 mm/mim. The ratio between peak area of propranolol HCl and pindolol was calculated. Each formulation was determined in triplicate. The calibration curve and representative chromatogram was shown in Figures *247* and *248* Appendix A respectively. The percentage of labeled content was calculated from the calibration curve.

6.2.6 Drug Release Studies

The drug release tests were based on the USP XXIII using dissolution apparatus basket method at 100 rpm and 37°C. Dilute HCl (1 in 100) solution pH 1.2 and phosphate buffer pH 6.8 were utilized as dissolution fluids for tablets containing propranolol HCL 40 mg whereas HCl buffer pH 1.2 and phosphate buffer pH 6.8 were used as dissolution fluids for tablets containing propranolol HCl 80 mg. A portion of dissolution medium at various time intervals was assayed spectrophotometrically at the wavelength as shown in Table *84* (Appendix A). The cumulative amount of medicament release was then calculated from the absorbance-concentration calibration curve. Six tablets of each formulation were determined. To study the effect of pH of dissolution medium on release behavior, the drug release in phosphate buffer pH 6.8 was also tested.

7. Preparation and Evaluation of Coated Tablet

7.1 Preparation of Coated Tablet

Film coating formulations as evaluated in section 5 which were containing or without plasticizer, colorant and pigment were prepared. A batch size of 500g core tablets was coated with coating formulation in a coating pan coupling with an air-atomized spray nozzle. The core tablets were warmed with drying air at temperature of

60-65°C, the atomizing air pressure was 2 bars. The coating component was applied with intermittent spray at spray rate of 7.5 ml/min using a peristaltic pump. Various coating levels were prepared. The coated tablets were allowed to be dried in the pan with drying air for 15 minutes. The coated tablets were then kept in a desiccator before evaluation.

To investigate the effect of heat treatment on properties of coated tablet, the coated tablets were then exposed to moist heat at 45°C 75% RH, 60°C 75% RH or dry heat at 60°C. At various time intervals, these treated coated tablets were evaluated. In order to investigate the effect of %RH of moist heat treatment, the coated tablet was incubated in enclosed desiccator containing desiccant (silica gel) or saturated solution of sodium chloride, potassium nitrate and potassium sulphate for maintaining the %RH at 60 °C of 0, 75, 85 and 96% respectively. These %RH's at 60°C were calculated by the method as described by Lide (1992).

In order to briefly describe the component in coating formula used in this study, the abbreviations representing the chemical which was employed were described in Table *3*.

Table 3 Abbreviations used to represent the chemical utilized in film coating component

Polymer	Acid	Plasticizer	Other Additives
Chitosan (C)	Acetic (A)	Castor oil (Cas)	Brilliant blue (B)
	Citric (C)	Diethyl phthalate (Dp)	Green FS (G)
	Malic (M)	Glycerin (G)	Magnesium stearate (M)
	Propionic (P)	PEG 6000 (E)	Talcum (Ta)
		Propylene glycol (P)	Titanium dioxide (Ti)
		Triacetin (Tri)	Urea (U)

The first alphabet was used to represent the chitosan used at a concentration of 5% w/w based on coating component and the second alphabet was used to represent the organic acid utilized as solubilizer used in mole ratio of acid : glucosamine unit (1.2 : 1). The next alphabet represented the plasticizer or the additive experimentally added into coating component. The number after the third and next alphabets indicated to the amount of that substance incorporated in coating component (%w/w based on the amount of chitosan). For example, CC P25 B0.5 was the tablet coated with chitosan citrate film plasticized and colored with propylene glycol at concentration of 25 % w/w based on chitosan and brilliant blue at concentration of 0.5 % w/w based on chitosan respectively. CA M45 CAS15 U5 was the tablet coated with chitosan acetate film containing magnesium stearate, castor oil and urea at concentration of 45, 15 and 5 %w/w based on chitosan respectively.

7.2 Coated Tablet Evaluation

7.2.1 Physical Appearance

Physical appearances of coated tablets were examined. The structural defects: cracking, splitting, peeling, incomplete edge covering and pinhole were visually detected by checking one hundred coated tablet samples.

7.2.2 Surface Topography

The in-depth morphology of surface and cross section area of film coated tablets was studied under a scanning electron microscope at the magnifications of 200 and 1000.

7.2.3 Weight Variation and Friability

The determinations were followed as the methods described in 6.2.2

7.2.4 Hardness, Diameter and Film Thickness

The hardness and diameter were measured as method in 6.2.3. Film thickness was calculated from an increased diameter of coated tablet compared to that of core tablet. Mean and standard deviation of film thickness were determined.

7.2.5 Disintegration Time

The determination was followed as the method described in 6.2.4.

7.2.6 Uniformity of Dosage Units and Assay of Propranolol HCl

The determination was followed as the method described in 6.2.5.

7.2.7 Adhesion between Film Coat and Core Surface

The adhesion between chitosan film to core surface was also assessed using universal tensile tester. Before measurement, the film around the edge of the tablet was removed by a sharp knife. The tablet was mounted onto the lower part of a material testing apparatus. The half surface of peeled film was fixed with the upper grip of the instrument. Adhesion strength measurement was performed using a 10 N load cell and a cross speed of 1 mm/min. The half surface area on the segment of tablet was calculated as method described in Figure *249* Appendix A. Total of six specimens were examined and the mean of the results was taken in all cases.

7.2.8 Physicochemical Properties of Film Coat

The film coats were peeled off from core tablet and then ground using small vibrating mill before measurement for Fourier Transform Infrared (FT-IR)

spectra as the method described in 2.3, X-ray Powder Diffractograms as the method described in 2.4, DSC curves as the method described in 2.5.

The dispersion containing magnesium stearate 2.25% w/w in 1.85% w/w of acetic acid solution was incubated by stirring at room temperature, 40°C and 60°C for 45 minutes. This dilute acetic acid was prepared at the same concentration used to solubilize chitosan and the amount of magnesium stearate was 45% w/w based on chitosan, but chitosan was not added in prepared dispersions. These incubated dispersions without chitosan were subsequently filtered through the filter paper, washed with deionized water several times, dried at 45°C for 2 hours and kept in a desiccator before analyzing with FT-IR spectroscopy, powder X-ray diffractometry and differential scanning calorimetry. The samples prepared from the dispersions of magnesium stearate in deionized water incubated at room temperature and 60°C for 45 minutes were used as the control.

7.2.9 Drug Release Studies

In vitro drug release was performed under monograph of propranolol HCl tablet in USP XXIII for fast release and propranolol HCl extended-release capsules by pH change method in USP XXIII Drug Release Test 1 for extended release. For the dissolution test with pH change method, the pH of the medium was HCl buffer of pH 1.2 for one and half hours. The pH was enhanced to 6.8 by adding sodium hydroxide 4.6 g., monobasic potassium phosphate 3.06 g. and dibasic sodium phosphate 4.005 g. The operation was continued until completing 24 hours. The criteria for drug dissolution of fast release was not less than 75% of drug dissolved in 30 minutes using dilute HCl (1 in 100) as dissolution fluid. For the extend release product, the percentages of the labeled amount of drug dissolved at the times specified were to conform to the criteria in Table *4*.

Table 4. The tolerances of drug release from propranolol HCL extended-release capsules

Time(hours)	Amount dissolved
1.5	not more than 30%
4	between 35% and 60%
8	between 55% and 80%
14	between 70% and 95%
24	between 81% and 110%

8. Stability of Coated Tablets

The coated tablets after storage at room temperature for one year were retested as methods in 7.2.1, 7.2.3, 7.2.4, 7.2.5, 7.2.6 and 7.2.9 to evaluate their stability. The coated tablets after direct exposure to accelerated condition (45°C 75%RH) for 7 days or after storage in bottle under accelerated condition for 7 or 30 days were also evaluated. The drug content in dosage form as mentioned in USP XXIII was not less than 90% and not more than 110% of label amount.

9. Elucidation the Drug Release Mechanism

9.1 Equilibrium Drug Solubility

Determination of the equilibrium drug solubility was undertaken at 37±0.5 °C in deionized water, HCl buffer pH 1.2, phosphate buffer pH 6.8 and glucose solutions (1.22, 2.06 and 3.13 molal). In each determination, the test tubes containing excess of propranolol HCl in each medium were incubated in thermoset shaking apparatus. After 48 hours, an aliquot of each sample was collected, filtered through filter paper, diluted and spectrophotometrically assayed.

9.2 Viscosity of Dissolution Medium Measurement

The viscosity of deionized water, HCl buffer pH 1.2, phosphate buffer pH 6.8 and glucose solutions (1.22, 2.06 and 3.13 molal) was measured using a viscometer as described in 1.3.

9.3 pH of Dissolution Medium Measurement

The pH of each dissolution medium was measured using a pH meter.

9.4 Osmotic Pressure Determination

The osmolality's of dissolution fluids (deionized water, HCl buffer pH 1.2, phosphate buffer pH 6.8 and glucose solutions (1.22, 2.06 and 3.13 molal)) were measured by using a freezing point osmometer. Glucose solutions were previously diluted ten times before this measurement. The concentrate core component was prepared by dispersing core tablets 15 g in 10 g of dionized water and was stirred for 24 hours. This concentrate component was then diluted 250 times before measurement.

The osmotic pressure was calculated using Morse equation

$$\pi = mRT \qquad \text{.....(Eq. 17)}$$

where π is the osmotic pressure in atm, m is the concentration of the solute in molality or osmolality, R is the gas constant equal to 0.0821 liter atm/mole deg and T is the absolute temperature

The osmolality value measured from osmometer is in osmolal/kg. The osmotic pressure difference between various dissolution media and concentrate core component also calculated.

9.5 Drug Release Studies

In vitro drug release was performed in deionized water and glucose solutions (1.22, 2.06 and 3.13 molal) compared to the drug release in HCl buffer pH 1.2 and phosphate buffer pH 6.8.

9.6 Measurement of Weight Gain of Coated Tablets During Dissolution Test

The coated tablets were tested for their weight gain in HCl buffer pH 1.2, phosphate buffer pH 6.8 and pH change system under the same condition with the dissolution study. After prefixed time intervals, the coated tablets were moved and botched their excess surface water and then individually weighed with analytical balance. After finishing the experiment, the coated tablets were immediately returned to the dissolution medium. The experiments were performed in triplicate.

10. Data Fitting to The Selected Models

The conventional release equations and developed model expressions were selected to fit with the release data using the Scientist® program. There was some sequences on running with Scientist® program. Scientist® program provided the calculation of statistics and parameter values. Using the commands in the Menu bar, Tool bar, and Status line, it could perform all of the steps necessary to fit models to data, generate statistics reports and plot results. The normal sequence of events that was used in Scientist® was to open or create a model file, open, create or generate a data set, open or create a parameter set, perform the desired fitting operation, generate a statistics report, and plot the result of fitting.

Equation

The model parser processes the equations from the top of the model to the bottom and therefore it could only properly define equation when all of their defining variables having previously been assigned. For differential equations, the symbol (simple apostrophe) was used to indicate the derivative of variable. For example

F'=.....

Independent variables, dependent variables and parameters would be included in the equation. Parameters were those variables whose values had to be changed during least squares fitting, or which had to be held constant for fitting of a single data set, but varied between sets of data.

Initial conditions

For the solution of differential equation, initial conditions and initial estimates for parameter values should be entered in the model file. For example of initial condition,

T=0

F=0

These values would be used as the initial conditions for the solution of differential equations if the variables for which the values given were defined by a differential equation or as the initial values for parameters when performing simulation or

fitting. Variables that were not parameters or defined by differential equations should not be assigned initial values since the model parser might hold that variable fixed at the specified value. Initial condition had to be defined below the differential equations to which they applied.

Constraints

Constraints on values might also be entered in a Scientist model. Constraints were entered using the '<' symbol. For example,

0.0<A/B<1.00

The manner in which constraints were used depended upon which type of variable was being constrained. If the variable was a parameter, the limits represented the lower and upper bounds over which the parameter could vary during fitting.

If functions

There were the functions in this program the implement an If-Then-Else operator such as IFGEZERO. The syntax for the use of these functions was the same, e.g.,

Y= IFGEZERO(A,B,C)

assigned the value of B to Y if A was greater than or equal to zero, otherwise Y was assigned to the value of C. The other IF functions worked in a similar manner except that condition being tested for was that A was equal to zero, greater than zero, less than or equal to zero, less than zero, or not equal to zero as indicated by the names of the functions.

Simulation

Simulation allowed the user to generate data using the selected model, parameters, and data (if available). Simulation would be performed only if compilation of the model was completed successfully.

Least squares fitting

This procedure was performed using a modified Powell algorithm to obtain the minimum sum of squared deviates between observed data and model calculations. The sum of squares value was given by:

$$S=S_{min}(1+ (N_{fit}/(N_{obs}-N_{fit}))*F_{1-\alpha} (N_{fit},N_{obs}-N_{fit})),$$

where S_{min} was the minimum sum of squares value; N_{obs} was the number of observed data points; N_{fit} was the number of parameters for which the joint confidence region was being calculated; F_{α} was the significance point of the F distribution with N_{fit} and N_{obs}-N_{fit} degrees of freedom; and $(1-\alpha)$ is the confidence level desired. Note that in the case of

nonlinear parameters, these confidence ranges were approximations based on the assumption that the parameters behave linearly near S_{min}

Goodness-of-fit statistics

There were several statistics parameters that might be used as indicators of goodness-of-fit. For this study, coefficient of determination was chosen to indicate the goodness-of-fit. Curve fitting which could provide the high value of coefficient of determination signified that the experimental data could be fitted by the tested model expression. The coefficient of determination was defined by the formula:

$$\frac{\sum_{i=1}^{n} w_i(Y_{obs\,i}-\bar{Y}_{obs})^2 - \sum_{i=1}^{n} w_i(Y_{obs\,i}-Y_{cal\,i})^2}{\sum_{i=1}^{n} w_i(Y_{obs\,i}-\bar{Y}_{obs})^2} \qquad \text{.....(Eq. 18)}$$

where n is the number of points, w_i is the weight applied to each point and Y_{obs} is the weighted mean of the observed data. The coefficient of determination was a measure of the fraction of the total variance accounted for by the model. This was an appropriate measure of the goodness-of-fit than either correlation or R-squared.

The model files of selected equations and developed model expressions for this study are shown as followed,

```
// MicroMath Scientist Model File(HIXSON AND
CROWELL CUBE ROOT)
IndVars: T
DepVars: F
Params: K,TI
1-((1-F)^(1/3))=K*(T-TI)
***
// MicroMath Scientist Model File(FIRST ORDER)
IndVars: T
DepVars: F
Params: K,TI
F=1-EXP(-K*(T-TI))
***
// MicroMath Scientist Model File(HIGUCHI's)
IndVars: T
DepVars: F
Params: K,TI
F=K*((T-TI)^(1/2))
***
// MicroMath Scientist Model File(KGT1)
IndVars: T
DepVars: F
Params: A,B,C,TI
F3=(1-F)^(1/3)
F'=C/(A-B*(T-TI)+(1-F3)/F3)
//Initial conditions
F=0
T=0
***
// MicroMath Scientist Model File(KGT2)
IndVars: T
DepVars: F
Params: A,B,C,TI,K
TD=(T-TI)-(A/B)
S=IFGEZERO(TD,1,0)
F3=(1-F)^(1/3)
F'=(1-S)*C/(A-B*(T-TI)+(1-F3)/F3)+S*K*(F3^2)
//Initial conditions
F=0
T=0
***
// MicroMath Scientist Model File(KGT3)
IndVars: T
DepVars: F
Params: A,C,TI
((3/2)*(1-((1-F)^(2/3))))+((A-1)*F)=C*(T-TI)
//Constraints
0.0<F<1
***
// MicroMath Scientist Model File(POWER LAW
EXPRESSION)
IndVars: T
DepVars: F
Params: K,TI,N
F=K*((T-TI)^N)
***
// MicroMath Scientist Model File(WEIBULL)
IndVars: T
DepVars: F
Params: A,B,TI
F=(1-EXP(-((T-TI)^B)/A))
***
// MicroMath Scientist Model File(ZERO ORDER)
IndVars: T
DepVars: F
Params: K,TI
F=K*(T-TI)
***
```