CHAPER II
EXPERIMENTAL

Materials

The following materials were obtained from commercial sources. Deionized water was used throughout the experiment.

1. **Model drug**

   - Propranolol HCl (Lot. No. 961206, 970604, Zhejiang Medicines and Health Products, China)

2. **Additives**

   - Microcrystalline cellulose, NF/BP

     :- (Avicel® PH-101, Lot. No. 1741, Asahi Chemical Ind.Co., Ltd., Japan)

   - Lactose hydrous USP/NF/BP/EP 200 mesh (Lot. No. 7091802-109, Wyndale, New Zealand)
   - White beeswax (Lot. No. BC30/657, BS33/496, Japan, supplied by Srichand United Dispensary Co., Ltd., Thailand)
   - Carnauba wax (Lot. No. SCB01995, Japan, supplied by Srichand United Dispensary Co., Ltd., Thailand)
   - Glyceryl monostearate (flake) (Lot. No. GCB18 ,Belgium, supplied by Srichand United Dispensary Co., Ltd., Thailand)
   - Hydrogenated vegetable oil

     :- (Lubritab®, Lot. No. I9100L, Mendell, USA)

   - Glyceryl behenate

     :- (Compritol 888ATO®, Lot. No. 20718, Gattefosse, France)

- Glyceryl palmitostearate

:- (Precirol ATO5®, Lot. No. 20569, Gattefosse, France)

- C_{12-18} Glycerides fatty acid

:- (Gelucire 50/02®, Lot. No. 18453, Gattefosse, France)

3. Chemicals

- Hydrochloric acid (Lot. No. H613KVBC, Mallinckrodt, USA)
- Carbon tetrachloride (Lot. No. 5004410SD, Farmitalia Carlo Erba, Italy)
- Anhydrous Methanol, AR grade (Lot. No. 3016KVDD, Mallinckrodt, USA)
- Citric acid monohydrate (Lot. No. 4A258284D, Farmitalia Carlo Erba, Italy)
- Anhydrous dibasic sodium phosphate (Lot. No. 4I599084M, Farmitalia Carlo Erba, Italy)
- Sodium chloride (Lot. No. K23679633-705, BDH laboratory supplies, England)
- Monobasic potassium phosphate (Lot. No. 612612, Ajax chemicals, Australia)

Equipments

- Analytical balance (Model A200S, Sartorius GmbH, Germany and Model PB3002 Mettler, Switzerland)
- Dissolution apparatus Model DT-6R, Erweka®, USA)
- Differential scanning calorimeter (Model DSC-7, Perkin Elmer, England)
- Extruder (Model EXKS-1, Fuji Paudal Co., Ltd., Japan)

- Fourier transform infrared spectrophotometer (Model SP 2000, Perkin Elmer Ltd., England)
- Hot air oven (Model UL 80, Memmert, Germany)
- Hydraulic press
- Magnetic stirrer (Model SP 46920-26, Cimarec 2, Thermolyne, USA)
- PH meter (Model 292, Pye Unicam Ltd., England)
- Paddle stirrer (DT, ErwekaGmbH, Germany)
- Planetary Mixer (Model A701A, Kenwood Mfg. Ltd., England)
- Scanning electron microscope (Model JSM-6400LV, Leol Ltd., Japan)
- Sieve shaker (Josef Deckehnann Aschaflenberg, Germany)
- Single punch tabletting machine
- Spheronizer (Model S320, Aeromatic-Fielder, England)
- Tablet hardness tester (Model TBH-30MD, Erweka GmbH, Germany)
- Tablet thickness tester (Telcock Corp., Japan)
- Ultrasound transonic digital sonicator (Model T900, 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. Pelletization Process

The extruder and spheronizer used was a laboratory type. The extruder was screw-feeding extruder. It has single radial screw that rotates along the horizontal axis and hence transports the material horizontally. The spheronizer consisted of a friction plate 1.2 mm in height (H), 1.8 mm in width (W), 3 mm in length (L) (Niro-fielder, Model S320, Figure 12)

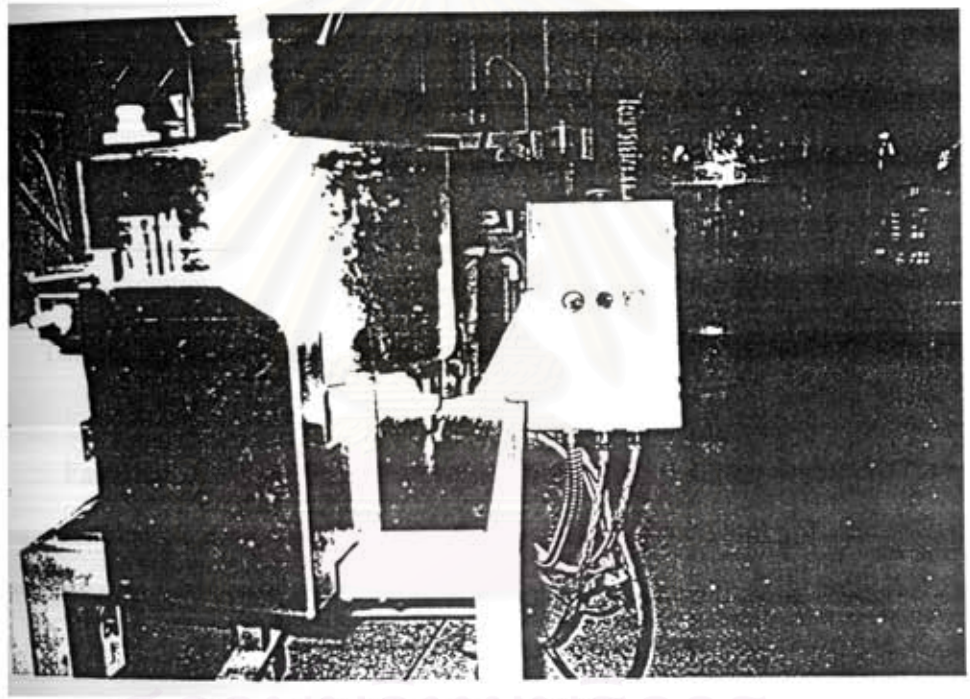

Figure 12 Niro-fielder, Model S320

In the preliminary study of pelletization is to investigate the possibility and suitable conditions for pellet preparation containing wax material in the formulation. Only glyceryl monostearate and lactose were used to prepare blank core pellets without drug. In addition, microcrystalline cellulose (Avicel® PH 101) was chosen as an extrusion and spheronization aid. The spheronization speed, time, and mass load were varied from 500-900 RPM, 5-20 min, and 200-300 gm, respectively.

The factors affecting pelletization were the initial load of extruded mass, spheronization speed, spheronization time. All factors, which were 700 RPM of spheronization speed, 10 min of spheronization time, and 250 gm loading had to be controlled throughout the experiments. The formulation of blank core pellet and pelletization conditions are presented in Tables 3 and 4, respectively.

Table 3 Formulation of core pellets.

Ingredients	% w/w
Glyceryl monostearate	40
Lactose	40
Microcrystalline cellulose	20
Water	qs

Table 4 The pelletization conditions for preliminary study and suitable conditions.

Factors	Pelletization conditions	
	Preliminary study	Suitable condition
Spheronization speed (RPM)	500 – 900	700
Spheronization time (min)	5, 10, 15, and 20	10
Mass load (gm)	200, 250, and 300	250

Prior to transferring the materials into the extruders, the wax was melted in the beaker by controlling temperature about 10 ^{0}C above its melting point. When the wax was completely melted, carbon tetrachloride was added and stirred by using magnetic stirrer until the solution was homogenous. Filler and extrusion aids were mixed together with the aid of planetary mixer for 5 minutes. After thoroughly mixed, wax solution was gradually added and mixed until it congealed. The congealed

mass was firstly transferred to the extruder for producing powdered mass. The powdered mass was placed into planetary mixer. Water was gradually added and mixed until damp mass occurred. The damp mass was transferred to the extruder again and extruding into the cylindrical segment. The extrudate was immediately rolled into solid spheres on the spinning friction plate of spheronizer at different spheronization time and speed. The pellets were dried by hot air oven at 45 ^{0}C for 4 hours.

After the suitable condition was established, some of lactose in the formulation was then substituted by propranolol HCl. The amount of ingredients used in each formulation depend on the ratio of drug to extrusion – spheronization aids and the percent of the wax. The composition of pellets consisted of drug, wax, and diluent at different percentage are presented in Table 5

Table 5 Compositions of propranolol HCl matrix pellets at different percentage of wax content.

Ingredients (gm)	Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
Propranolol HCl	100	100	100	100	100	100	100	100
Avicel PH 101	50	50	50	25	50	50	50	25
GMS	**50**	**75**	**100**	**125**	-	-	-	-
Lubritab®	-	-	-	-	**50**	**75**	**100**	**125**
Beeswax	-	-	-	-	-	-	-	-
Carnauba wax	-	-	-	-	-	-	-	-
Lactose	50	25	-	-	50	25	-	-
CCl_4*	100	75	50	25	100	75	50	25
Water (gm)	85	87.5	80	85	75	80	85	70

* CCl_4 is used for preparing wax solution and will be evaporated during drying process.

Table 5 (Continued.)

Ingredients (gm)	Formulation							
	F9	F10	F11	F12	F13	F14	F15	F16
Propranolol HCl	100	100	100	100	100	100	100	100
Avicel	50	50	50	25	50	50	50	25
GMS	-	-	-	-	-	-	-	-
Lubritab®	-	-	-	-	-	-	-	-
Beeswax	**50**	**75**	**100**	**125**	-	-	-	-
Carnauba wax	-	-	-	-	**50**	**75**	**100**	**125**
Lactose	50	25	-	-	50	25	-	-
CCl_4*	100	75	50	25	100	75	50	25
Water (gm)	105	105	115	95	100	112	130	110

Table 5 (Continued.)

Ingredients (gm)	Formulation										
	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27
Propranolol	100	100	100	100	100	100	100	100	100	100	100
Avicel	50	50	50	25	50	50	50	25	50	50	50
Compritol®	**50**	**75**	**100**	**125**	-	-	-	-	-	-	-
Precirol®	-	-	-	-	**50**	**75**	**100**	**125**	-	-	-
Gelucire®	-	-	-	-	-	-	-	-	**75**	**100**	**125**
Lactose	50	25	-	-	50	25	-	-	50	25	-
CCl_4*	100	75	50	25	100	75	50	25	75	50	25
Water (gm)	60	55	66	68	70	60	65	60	50	43	-

In the case of loading dose effect study, the amount of wax in the formulation was kept constant, then the amount of propranolol HCl was varied between 30-50% in the formulations that are presented in Table 6.

Table 6 Compositions of propranolol HCl matrix pellets at different percentage of loading dose.

Ingredients (gm)	Formulation											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Propranolol	**50**	**75**	**125**	**50**	**75**	**125**	**50**	**75**	**125**	**50**	**75**	**125**
Avicel	50	50	25	50	50	25	50	50	25	50	50	25
GMS	100	100	100	-	-	-	-	-	-	-	-	-
Lubritab®	-	-	-	100	100	100	-	-	-	-	-	-
Beeswax	-	-	-	-	-	-	100	100	100	-	-	-
Carnauba	-	-	-	-	-	-	-	-	-	100	100	100
Lactose	50	25	-	50	25	-	50	25	-	50	25	-
CCl_4*	50	50	50	50	50	50	50	50	50	50	50	50
Water (gm)	30	30	25	85	85	65	60	60	55	100	100	90

Table 6 (Continued.)

Ingredients (gm)	Formulation								
	F13	F14	F15	F16	F17	F18	F19	F20	F21
Propranolol	**50**	**75**	**125**	**50**	**75**	**125**	**50**	**75**	**125**
Avicel	50	50	25	50	50	25	50	50	25
Compritol®	100	100	100	-	-	-	-	-	-
Precirol®	-	-	-	100	100	100	-	-	-
Gelucire®	-	-	-	-	-	-	100	100	100
Lactose	50	25	-	50	25	-	50	25	-
CCL4*	50	50	50	50	50	50	50	50	50
Water (gm)	80	80	60	65	60	65	30	28	25

2. Evaluations of Propranolol HCl Matrix Pellets.

2.1 Pellet Morphology

Morphology of matrix pellets was photographed using scanning electron microscopy. The pellets were coated with gold about 2 – 3 times to cover all of the spherical surface prior to microscopic examination using ion sputtering method. Size, shape, and surface topography were observed. The matrix pellets were also cross-sectioned for observation of internal texture.

2.2 Size Distribution of the Pellets

Size distribution of pellets were determined using sieve analysis, consisted of a set of US standard sieves, ranging from sieve No. 14, 18, 20, 25, 30 and a collector pan respectively (1400, 1000, 900, 700, 500 μm). One hundred grams of pellets were accurately weighed and put on the top of sieves. The sieves were placed on the sieve shaker and shaken for 20 minutes. The pellet retained on each sieve size were weighed and calculated in percent of total weight.

2.3 Bulk, Tapped Density and Percent Compressibility

Fifty grams of the matrix pellets were accurately weighed and carefully poured into a 100 ml graduated cylinder. Graduated cylinder was dropped two seconds interval on hard surface for 3 times at one inch height. The bulk volume was recorded. Division of weight by bulk volume presented bulk density

$$\text{Bulk density (g/ml)} = \frac{\text{weight of pellets (g)}}{\text{bulk volume (ml)}}$$

Tapped density was performed by dropping graduated cylinder filled with pellet on a hard surface from one inch height until the volume was constant. Division of weight by the constant volume presented the tapped density.

$$\text{Tapped density (g/ml)} = \frac{\text{weight of pellets (g)}}{\text{Tapped volume (ml)}}$$

The % compressibility of the pellets was established by the following equation.

$$\% \text{ Compressibility} = \frac{(T - B) \times 100}{T}$$

B and T were bulk and tapped densities, respectively. All of these factors were calculated from three determinations.

2.4 Angle of Repose and Flow Rate

The angle of repose was determined by the funnel method. Adequate amount of pellets was weighed and filled in the funnel (internal diameter = 0.8 cm) which was placed on clamp. The height of the tip's funnel was far from surface about 5 cm. While filling the pellet to the top of funnel, closing the tip by the finger. Taking the finger out of the tip of funnel thus produced a round heap of powder on the paper graph. The time was recorded until the pellets passed the tip completely. The height and the radius of the heap pellet were recorded in millimeters. The result was averaged from three determinations.

Angle of repose and flow rate was calculated from the following equation.

$$\propto = \tan^{-1}(H/R)$$

$$\text{Flow rate (g/sec)} = \frac{\text{weight of pellets}}{\text{time}}$$

where $\propto$ is the angle of repose, H is the height of the heap and R is the radius of the heap.

2.5 Percent Friability

Ten grams of matrix pellets retained on 14/20 mesh cut and five stainless spheres (each sphere weight 1.06 gm and diameter 6.35 mm), to increase the mechanical stress on the pellets, were filled into the PVC container. The container was firmly closed with the cap and rotated at 25 RPM for 4 minutes. After that, pellets finer than 20 mesh were sieved off. The percent friability, averaged from two determinations, was calculated as percentage of weight loss from the following equation.

$$\text{Friability (\%)} = \frac{\text{weight loss}}{\text{initial weight}} \times 100$$

2.6 Sphericity of Pellets

Sphericity of 20 pellets with a specific size was analyzed using an image analyzer. The image analyzer consisted of a computer system linked to video

camera and a stereomicroscope. Each individual pellet was inspected and data were processed automatically. The features parameters, longest diameter or Feret maximum (R1), smallest diameter or Feret minimum (R2), area and perimeter were determined. Aspect ratio and form factor which gave a measure of the degree of pellet sphericity were derived from those four basic parameters and could be calculated by the following equations

$$\text{Aspect ratio} = \frac{\text{longest diameter}}{\text{smallest diameter}} = R1/R2$$

$$\text{form factor} = \frac{4\pi\,[\text{area}]}{[\text{perimeter}]^2}$$

These two value of unity describe a perfect circle.

2.7 Infared Spectroscopy

The infared spectrophotometry was used to study the change in the functional groups of our products after pelletization process by observing the positions and intensities of IR peaks.

The IR spectra of the propranolol HCl and diluents in the matrix pellets were examined using the potassium bromide disc (KBr) method with an infared spectrophotometer in the range of 4000 – 400 cm^{-1}.

2.8 The X-ray Diffraction Analysis

The X-ray diffractometry was used to determine the diffraction angle of the substances, which showed crystallinity and interplanar spacing of the crystal planes and determines the interaction between each components in mixing and pelletization process.

The crystallinity of propranolol HCl and additives in the pellets were examined by X-ray diffractometer.

The pellet were ground in the motar and firmly packed in the cavity of a thin rectangular quartz slide by the other glass slide. The glass slide was taken off and the prepared sample was exposed to the X-ray beam in the X-ray diffraction chamber. The X-ray diffraction patterns were recorded at the speed of 6^0 2θ per minute from 5^0 to 60^0 2θ angle.

2.9 Differential Scanning Calorimetric (DSC) Study

The thermograms of the pellet prepared from various formulations were examined by differential scanning calorimeter (DSC). The differences in thermograms between the original substances and their products were evaluated after pelletization process.

About 5 mg samples of single material were accurately weighed. Mixtures of propranolol- various waxes (1:1) were melted in a DSC pan. Then, it was crimped in the hermetically sealed pan and immediately made a few holes for determinations. The pan filled with mixtures was placed in the equipment beside the reference vacanted pan made by the same method. Perkin Elmer DSC 7 was used for analyzing the thermograms. Nitrogen was used as a carrier gas at a flow rate of 50 ml/min. A heating rate of 10^0 C per minute was used throughout running conditions and in the range of 40^0 to 280^0 C.

2.10 Determination of Propranolol HCl Content in Pellets.

2.10.1 Calibration Curve of Propranolol Content

Standard propranolol HCl of 0.3 gm was accurately weighed into 100 ml volumetric flask through the aid of glass funnel. The powder was dissolved and adjusted to volume with absolute methanol or acid buffer pH 1.2 or phosphate buffer pH6.8 depending on which one was used as the medium in experiment. The solution was filtered through paper filter (Whatman, NO 1). Then, a 10 ml filtrate was pipetted and adjusted to 100 ml volumetric flask with the same solvent and used as a stock solution.

The standard stock solution of 2, 3, 4, 5, 6, and 7 ml was individually pipetted into the 50 ml volumetric flask, diluted and adjusted to volume with the same solvent. The final concentration of the obtained standard solutions were 12, 18, 24, 30, 36, 42 mcg/ml, respectively.

The absorbance of known drug concentration was determined by a UV/VIS spectrophotometer at 289 nm against blank solution. Each concentration was determined in triplicate. The absorbance and the calibration curve of propranolol HCl are presented in Tables 27-29 and Figures 106-108, in Appendix A.

2.10.2 Assay of Propranolol HCl Content in Matrix Pellet

Pellets of 150 mg was accurately weighed into a 50 ml volumetric flask. The pellet was extracted with absolute methanol by the aid of sonicator about 1 hours, then adjusted to volume with absolute methanol and mixed thoroughly. The solution was filtered through filter paper and used as stock solution.

One ml of the stock solution was individually pipetted into 50 ml volumetric flask, then adjusted to volume with absolute methanol and mixed.

The absorbance of final solution was determined by UV/VIS spectrophotometry at 289 nm with absolute methanol as a blank solution. Propranolol HCl content was converted from the calibration curve of propranolol HCl in absolute methanol.

3. Compaction of Propranolol HCl Matrix Pellets.

The pellets were compacted into matrix tablet using a hydraulic press equipped with punch-die assembly. In the preliminary study, 500, 1000, 1500 psi compression forces were employed and compression force that provided the optimum hardness of the compact was selected. The result showed that the suitable compression force was 1500 psi.

The pellets were accurately weighed equivalent to 80, 120, 160, 200 mg of propranolol HCl depending on the formula. The hydraulic press equipped with a 3/8 inch diameter round flat faced punch and die. Each compact consisted of about 400 mg of material that was held at the designated compaction pressure at the period of 10 seconds. Comparisons were based on samples prepared at constant pressure (1500 psi). The punch and die were cleaned off before production of each tablet. The matrix tablets were reweighed and recorded as a correct for further study.

After completion of tablet dissolution studies, the formula which gave the dissolution profile within the USP XXIII was selected and wax matrix pellets of those was compacted with single punch tabletting machine again. The machine equipped with a inch diameter round flat faced punch and die. The maximum pressure

was used until obstruction did not occurred during compaction. The matrix tablets were reweighed and recorded as a correction for further study.

4. Evaluations of Matrix Tablets.

4.1 Morphology of Matrix Tablets.

The morphology of propranolol HCl wax matrices were examined both before and after dissolution testing by scanning electron microscopy. The matrix tablets were coated with gold prior to microscopic observations using ion sputtering method. Outer surface and cross section of the matrix tablets were observed.

4.2 Weight per Tablets.

The weight of tablet after compacting was measured again as a correct weight. The mean and standard deviation were calculated from six determinations.

4.3 Diameter and Hardness.

The diameter and hardness of the tablet were measured by tablet thickness tester and hardness tester in millimeters and in kilopounds, respectively. The mean and standard deviation were obtained from six determinations.

4.4 Thickness.

The thickness of the tablet was measured by using a tablet thickness tester in millimeters. The average thickness was calculated from six determinations.

4.6 Friability.

Twenty tablets were filled into the Erweka friabilator. The friabilator rotated for 4 minutes at 25 RPM. The percent friability was calculated as percentage of weight loss.

5. Evaluation of Propranolol HCl Products.

5.1 Dissolution Studies

The monograph for studying dissolution of oral controlled release propranolol HCl was pH change method. But in this study was concentrated to pH of dissolution medium. As an oral controlled release pellets were supposed to pass the entire upper gastrointestinal tract. It would be ideal when the release of drug was constant over a wide range of pH value (pH 1 – 7). So, in vitro test for controlled release products should at least cover this pH range. The acid buffer pH 1.2 and phosphate buffer pH 6.8 were individually used to test not only the release of drug from the matrix pellet and matrix tablet, but also the effect of the dissolution medium.

In this study, accurate weights of matrix pellets were filled in the capsule or compacted into the tablets for release studies. A system specified for dissolution test followed the USP XXIII dissolution method for extended release propranolol HCl capsule. The acid buffer pH 1.2 and phosphate buffer pH6.8 were filled in a glass vessel. The dissolution apparatus type I (Model DT 6R, Erweka, Germany) was used at basket rotation of 100 RPM. The dissolution data was evaluated from three capsules or tablets of each formulation.

A ten milliliters of the specimen in each vessel was withdrawn at the time intervals of 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours. The same

quantity of the medium at that time was added immediately after each sampling to keep the constant volume of the medium in the vessel throughout the experiment.

Each sample was filtered through filter paper. The filtrate was diluted, if necessary, to the range of 12–42 mcg/ml and examined by UV/VIS spetrophotometry at 289 nm for both acid buffer pH 1.2 and phosphate buffer pH6.8.

The amount of propranolol HCl released at any time interval 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. Each of the dissolution values reported was based on an average of three determinations of each formulation.

5.2 pH Change Dissolution Studies

After completion of the release studies of all formulation, proper formula was selected from various factors such as release profile, physical characteristics of product, possibility and feasibility to develop the product in large scale. The chosen formula of wax matrix pellets was compacted with both hydraulic press and single punch tabletting machine to investigate the feasibility of continuous tabletting process and release profile from different machine. The selected formula was then evaluated by the dissolution study with the pH change method of USP XXIII for 12 hours of propranolol HCl extended release capsules.

In this part, Inderal® LA 160 was also evaluated by using the same method as described below. The release profile between developed formula and commercial product was compared to perform release characteristic and mechanism.

In the dissolution model with pH change method, the pH of the medium was kept by 0.1 N HCl for one and a half hours. The pH was increased to 6.8 by adding sodium hydroxide 3.6 gm, monobasic potassium phosphate 3.06 gm and dibasic sodium phosphate 4.005 gm. The operation was continued until completing 12 hours after additions of dry buffer. The apparatus was operated at a speed of 100 RPM using basket method.

Ten milliliters of specimen was withdrawn at each time interval of 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours. The same quantity was added immediately after each sampling to keep the volume of medium constant throughout the experiment. Each sample was filtered through paper filter. The absorbance of the filtrate was determined spectrophotometrically at 289 nm for both acid buffer pH 1.2 and phosphate buffer pH 6.8.

The amount of propranolol HCl release at any time interval was calculated from the calibration curve. A cumulative correction was made for the previously removed sample to determine the total amount of drug released.