CHAPTER II

EXPERIMENTAL

MATERIALS

1. Terbutaline sulphate (USP, Batch No.52/92-93, India)

2. Lactose hydrous (USP/NF/BP/EP, The Lactose Company of New Zealand, New Zealand)

3. Microcrystalline cellulose (Avicel PH101^{R}, Lot No A4, Asahi Chemical Industry Co., Ltd., Japan)

4. Hydroxypropyl methylcellulose (Methocel E-15LV^{R}, Premium grade, The Dow Chemical Company, USA)

5. Hydroxypropyl cellulose (HPC-L^{R}, Lot No.BC-101, Nippon Soda Co., Ltd., Japan)

6. Hydroxypropyl cellulose (HPC-M^{R}, Lot No.BD-191, Nippon Soda Co., Ltd., Japan)

7. Methyl cellulose (Methocel A4M^{R}, Premium grade, The Dow Chemical Company, USA)

8. Ethyl cellulose 10 cps. (Batch No. MM 910307-2 The Dow Chemical Company ,USA)

9. Propylene glycol, USP XIX (Distributed by Srichand United Dispensary Co.,Ltd., Thailand)

10. Ethyl alcohol 95 % (Ayuthaya Spirit Factory, Excise Department, Thailand)

11. Chloroform (Farmitalia Carlo Erba S.P.A.,Italy

12. Potassium dihydrogen phosphate (131 A 592673,E. Merck, Germany)

13. Sodium hydroxide (Nobel Industries, Sweden)

14. Hydrochloric acid (2 M 196302 M, Farmitalia Carlo Erba, USA.)

15. Ortho - Phosphoric acid 85 % (709 K 4247473, E . Merck, Germany)

16. Sodium 1 - heptanesulfonate (No.70-A, Tokyo Kasei Kogyo Co., Ltd., Japan)

17. Methanol (HPLC Grade, J.T. Baker Inc., USA)

18. Pindolol (Lot. 55 F 0749, Sigma Chemical Co.,Ltd. USA.)

19. Bricanyl^{R} Durules 5 mg (Batch .SK 1085, Mfg. 10-92, Exp.11-95, Astra, Sweden)

EQUIPMENTS

1. Analytical balance (A 200 S, Sartorious, Germany)

2. Planetary mixer (EB 20 F Gypto - Peerless Ltd., England)

3. Extruder (Pharmaceutical and Medical Supply Co.,Ltd., Thailand)

4. Spheronizer (Pharmaceutical and Medical Supply Co.,Ltd., Thailand)

5. Hot air oven (Mammertt, Germany)

6. Scanning Electron Microscope (JEOL, JSM - T 220, Japan)

7. Sieve shaker (Nr 995941, Hessenwerk Darmstadt, Western Germany)

8. US sieve standard (Laboratory test sieve ASTM E 11, Endecotts. Ltd. USA)

9. Cube mixer (Type MU 5 - H JIS A-1-1, Kasuga E.W. Ltd., Japan)

10. Fluidized bed air suspension (Uni-Glatt,Glatt^{R} GmbH, Germany)

11. Magnetic stirrer (Model SP46920-26, Barnstead/Thermolyne, USA)

12. pH meter (Model 292 Pye Unicam, England)

13. Dissolution apparatus (Hanson Research Model SR-2, USA)

14. Ultraviolet spectophotometer (Backman DU-68, USA)

15. High Pressure Liquid Chromatography

Pump : Shimadzu LC-3A ,Japan

Injector : Rheodyne 7125 ,USA

Column : Lichrosphere^{R} 100 RP -18 (5 um), 125x4 mm (Lichro CART ^{R}, Merck 50943, USA)

Detector : Fluorometric detector (Fluoro Monitor^{Tm} 4100 LDC Analytical,USA)

Integrator : Shimadzu C - R1A, Chromatopac, Japan

METHOD

1 Preparation of Placebo Pellets

1.1 Formulation

The amount of ingredients used in each formulation and conditions for preparation are represented in Tables 3 and 4.

1.2 Step for Preparation

1.2.1 Dry Mixing

Lactose and Avicel PH101^{R} were weighted and mixed in a planetary mixer.

1.2.2 Wetting

Binder solution was poured onto the powder and the mixture was mixed until wet mass was obtained.

1.2.3 Extrusion

The wet mass was transferred to extruder and screened through a 1 mm sieve at 26 rpm of extruder speed. Extrudates were obtained.

Table 3 Amount of ingredients and conditions employed to prepare pellets

Formulation	Percent of each ingredient (% w/w)		
	Lactose	Avicel PH101^{R}	Binder (%)
1	59.20	39.47	Methocel E-15LV^{R} 1.33%
2	59.00	39.33	Methocel E-15LV^{R} 1.67%
3	58.80	39.20	Methocel E-15LV^{R} 2.00%
4	59.20	39.47	HPC-L^{R} 1.33%
5	59.00	39.33	HPC-L^{R} 1.67%
6	58.80	39.20	HPC-L^{R} 2.00%
7	59.80	39.87	Methocel A4M^{R} 0.33%
8	59.60	39.73	Methocel A4M^{R} 0.67%
9	59.20	39.47	HPC-M^{R} 1.33%
10	59.00	39.33	HPC-M^{R} 1.67%
11	58.80	39.20	HPC-M^{R} 2.00%
12	58.60	39.07	HPC-M^{R} 2.33%

Remark : Each formulation contained 40 % of water content base on dry basis with 414 rpm and 951 rpm of spheronizer speed and each spheronizer speed prepared with 5, 10 and 15 min of spheronization time

Table 4 Amount of water content base on dry basis and conditions employed to prepare pellets

Formulation	% of water base on dry basis
13	35%
14	42%
15	44%
16	50%

Each formulation contained

Lactose	58.80% w/w
Avicel PH101^{R}	39.20% w/w
HPC-M^{R}	2.00% w/w

Various percent of water is presented in the Table 4.

Spheronizer speed was 951 rpm.

Spheronization time was 15 min.

1.2.4 Spheronization

The extrudates were transferred to spheronizer. The various conditions of spheronizer were setted and operated. Finally, placebo pellets were obtained.

1.2.5 Drying

The placebo pellets were dried in hot air oven at 60°C for 12 hours.

2 Placebo Pellets Evaluation

2.1 Determination of Pellet Appearance

Photomicrographs of pellet samples were taken with scanning electron microscope. The samples were coated with gold prior to the microscopic examination using ion sputtering.

2.2 Particle Size Distribution

Particle size distribution was determined by sieve analysis. The 100.0 g of pellets were put on the top sieve of a sieve with series of opening ranging from 1.41, 1.00, 0.84, 0.41, to 0.20 mm, respectively. The nest of sieve was placed on the sieve shaker for 10 minutes. The results, which averaged from two determinations, were reported as percentage of weight retained on each sieve size. The

average pellet size was given corresponding to 50% size on the cumulative percentage undersize axis.

2.3 Bulk Density and Tapped Density

The bulk density and tapped density were determined from the weight of 40.0 g sample, carefully charged into a 100 ml graduated cylinder and the volume was recorded. The pellets were tapped from the height of 5 cm until a constant volume was obtained. Both densities were averaged from three determinations.

2.4 Flow Rate and Angle of Repose

An amount of 40.0 g of pellets was filled in a glass funnel with 6 mm internal stem diameter fixed on a clamp. The time was recorded when the pellets start to flow until finish. Flow rate was calculated in g/min and angle of repose was calculated from the following equation.

$$\text{alpha} = \tan^{-1} \frac{H}{R}$$

Where alpha is the angle of repose: H and R are the height and radius of the pellet pile, respectively. The results were averaged from three determinations.

2.5 Percent Friability

Pellet friability determination method was modified from Kavee Chanpaparp,1989. Pellets retained on 14/20 mesh cut and five stainless spheres (each sphere weight 1.06 g and diameter 6.35 mm) were filled into the polyvinylchloride container 10.6 cm in lenght and 7.3 cm in diameter. The container was firmly closed with the cap,put on the cube mixer and rotated for 5 minutes. The pellets finer than 20 mesh was sieve off. The percent friability, which averaged from two determinations, was calculated as percentage of weight loss.

3 Preparation of Terbutaline Sulphate Pellets

3.1 Formulation

The amount of ingredients used in the formulation are presented below.

Formulation

Terbutaline sulphate	1.80% w/w
Avicel PH101^{R}	38.61% w/w
Lactose	57.92% w/w
HPC-M^{R}	1.67% w/w
Water	40.00% w/w by dry weight of powder

3.2 Step for Preparation

3.2.1 Dry Mixing

Terbutaline sulphate, Avicel PH101^{R} and lactose were weighed and mixed by geometric dilution. The powder was transferred to a planetary mixer.

3.2.2 Wetting

Binder solution was poured onto the powder and the mixture was mixed in the planetary mixer until wet mass was obtained.

3.2.3 Extrusion

The wet mass was transferred to extruder and screened through a 1 mm sieve at 26 rpm of extruder speed to obtain the extrudates.

3.2.4 Spheronization

The extrudates were transferred to spheronizer. The spheronizer speed of 1,010 rpm and 15 minutes of spheronization time were used to prepare pellets. Finally, terbutaline sulphate pellets were obtained.

3.2.5 Drying

The terbutaline sulphate pellets were dried in hot air oven at 50 ^{o}C for 12 hours.

4 Evaluation of Terbutaline Sulphate Pellets

4.1 Physical Properties of Terbutaline Sulphate Pellets

Physical properties of terbutaline sulphate pellets were determined as the same as 2.1 to 2.5.

4.2 Standard Curve of Terbutaline Sulphate

Weight accurately about 100 mg of terbutaline sulphate was dissolved in 0.01 N hydrochloric acid.The solution was then adjusted to 100.0 ml with 0.01 N hydrochloric acid in 100.0 ml volumetric flask and used as stock solution.

The stock solution was individually pipetted 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml into a 50.0 ml volumetric flask and was diluted to volume with 0.01 N hydrochloric acid.The final concentration of each solution was 0.02, 0.04, 0.06, 0.08, 0.10 and 0.12 mg/ml,respectively.

The absorbance of known drug concentration was determined by a single beam spectrophotometer in a 1-cm cell at 278 nm. The 0.01 N hydrochloric acid was used as a blank solution. Each concentration was determined in triplicate.

Preparation of 0.01 N hydrochloric acid

The amount of 1.0 g of 36.5% w/w hydrochloric acid was

diluted to 1000.0 ml with distilled water.

4.3 Measurement of Terbutaline Sulphate Content in Terbutaline Sulphate Pellets

Weight accurately about 300 mg of 14/20 mesh cut of terbutaline sulphate pellets (equivalent to 5.4 mg of terbutaline sulphate) was transferred to 50.0 ml volumetric flask. Then 30 ml of 0.01 N hydrochloric acid was added into the flask. The mixture was stirred for 30 minutes, added to volume with 0.01 N hydrochloric acid and mixed. The mixture was filtrated and the filterred was collected.

The absorbance of the filtrate was determined by single beam spectrophotometer in a 1-cm cell at 278 nm.The 0.01 N hydrochloric acid was used as a blank solution. Content of terbutaline sulphate in 300 mg pellets in each batch of preparation was averaged from ten determinations.

5 Preparation of Film Coated Terbutaline Sulphate Pellets

5.1 Film Coating Formulation

The amount of ingredients used in the film coating formulation are represented in Table 5.

5.2 Film Coated Terbutaline Sulphate Pellets Preparation

Table 5 Amount of ingredients employed to prepare film coated solution for terbutaline sulphate pellets

Ingredient (% w/v)	Formulation				
	1	2	3	4	5
Ethylcellulose 10cps	2.5	2.5	2.25	2.0	1.75
HPC-M[R]	–	–	0.25	0.5	0.75
Propylene glycol	0.25	0.5	0.25	0.25	0.25
Ethanol 95%	20	20	20	20	20
Chloroform qs to.	100	100	100	100	100

The 500 g of 14/20 mesh cut of terbutaline sulphate pellets were placed in the bottom spraying of fluidized bed coating machine (Wurster type). The film coating solution was sprayed to the pellets under conditions of inlet air temperature 50-55 ^{o}C, outlet air temperature 48-50 ^{o}C, inlet air volume 45, spraying pressure 1.5 bar, spraying rate 10 ml/min and partition height 2.5 cm.

For formulation 1, the film thickness was varied from 0 %, 1.1 %, 1.5 %, 3.2 % and 5.4 % coating level based on the percentage weight increased. For formulation 2, the film thickness was about 5.4 % and formulation 3, 4, 5, the film thickness was about 3.2 % weight increased. Three batches of the selected formulation were prepared.

6 Evaluation of Film Coated Terbutaline Sulphate pellets

6.1 Terbutaline Sulphate Content in Film Coated Terbutaline Sulphate Pellets

Method of determination was similar to that of terbutaline sulphate pellets but added chloroform approximately 30 ml to dissolve the film out before added 0.01 N hydrochloric acid for continuing assay of the drug as previously mentioned in 4.3. But, the mixture was heated about 60^{o}C to get rid of chloroform before the mixture was filtered.

Although the final weight of terbutaline sulphate pellets before and after coating was equal. However, the coating of

terbutaline sulphate will be deducted to obtained the true assay value. The film was calculated by the following equation.

$$F = \frac{(U-C)}{C} \times 100$$

Where F is the percent film based on weight increased; U and C are the amount of the drug in 300 mg pellets of uncoated and coated terbutaline sulphate pellets, respectively.

6.2 The Release of Terbutaline Sulphate from Film Coated Terbutaline Sulphate Pellets

6.2.1 Standard Curve of Terbutaline Sulphate

Weight accurately about 100 mg of terbutaline sulphate was transferred to 50.0 ml of volumetric flask, and adjusted to volume with water. The solution was used as stock solution. Weight accurately about 25 mg of pindolol was transferred to 250.0 ml volumetric flask, and adjusted to volume with methanol. The pindolol solution was used as an internal standard.

The stock solution of terbutaline sulphate was diluted to 0.001, 0.002, 0.004,0.008, 0.010 and 0.012 mg/ml with water.

Standard concentration 1 ml was diluted with 0.02 M potassium phosphate monobasic with the pH adjusted to 3.6 with

1.77 % phosphoric acid. Pindolol, which the final concentration was 1 ug/ml, was added to each standard concentration before they were adjusted to 5.0 ml with phosphate buffer in 5.0 ml volumetric flask.

6.2.2 Dissolution Study of Film Coated Terbutaline Sulphate Pellets

Dissolution test was determined using dissolution apparatus II (paddle) according to USP XXII. The averaged of terbutaline sulphate released was calculated from three determinations.

Five hundred milliliters of dissolution medium was equilibrated to 37 $\pm$ 0.5 ^{o}C. The dissolution medium consisted of 0.05 M potassium phosphate monobasic to pH 1.2 with concentrated hydrochloric acid, which was adjusted after one hour to pH 7.5 using 50% NaOH solution. The apparatus was operated at the speed of 50 rpm. Weight accurately about 300 mg of film coated terbutaline sulphate pellets (equivalent to about 5 mg of terbutaline sulphate) was added to each dissolution vessel. Samples were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 hours, respectively. Five milliliters of the sample was taken at that time. After the sample was taken, 5 ml of fresh dissolution medium was returned to the dissolution vessels to maintained a constant volume. One milliliter of each sample taken was diluted with 0.02 M potassium phosphate monobasic with the pH adjusted to 3.6 with 1.77 % phosphoric acid. Pindolol, which the final concentration was 1 ug/ml, was added to each sample before they were adjusted to 5.0 ml with the phosphate buffer in 5.0 ml of volumetric flask.

Standard concentration solution and these samples were assayed by HPLC and the peak area ratios of terbutaline sulphate and pindolol were determined.Terbutaline sulphate concentration in each sample solution was calculated from standard curve. Percent cumulative released was calculated.

(*)

6.2.3 Assay Procedure

All standards and samples were assayed by high performance liquid chromatography (HPLC) using fluorometric detector.

The mobile phase consisted of 35 % v/v of HPLC grade methanol, 0.0025 M of 1-heptanesulfonate sodium and 0.02 M potassium phosphate monobasic with the pH adjusted to 3.6 with 1.77 % phosphoric acid. A flow rate of 1.0 ml/min was used. Terbutaline sulphate was detected using an excitation wavelength (λex) of 280 nm and an emission wavelength (λem) of 310 nm. The retention times of terbutaline sulphate was 2 minutes and pindolol was 5 minutes.

6.3 Microscopic Determination of Film Coated Terbutaline Sulphate Pellets at Before and After Dissolution Testing

Photomicrographs of the pellets samples were taken with scanning electron microscope as the same as 3.2.1.

(*) Remark : Formulations of terbutaline sulphate pellets for released profile study

- Uncoated terbutaline sulphate pellets
- 5.4 % by increased weight of formulation 2
- 1.1 %, 1.5 %, 3.2 % and 5.4 % increased weight of formulation 1
- 3.2 % increased weight of formulation 3,4,5
- mixture of 1.1 % or 1.5 % increased weight of formulation 1 and uncoated pellets.
- Bricanyl^{R} Durules
- Three batches of the selected formulation