CHAPTER 3

EXPERIMENTAL

MATERIALS AND METHOD

3.1 Chemicals

1. Pentoxifylline Powder (Ph Eur/BP/JP), Laboratoric Chimico Internazionale SPA (LABOCHIM®, Italy)
2. Hydroxyethyl Cellulose WP40, Aldrich Chemical Company, Inc., USA
3. Hydroxyethyl Cellulose QP 52000, Aldrich Chemical Company, Inc., USA
4. Hydroxyethyl Cellulose (M_v 90000), Aldrich Chemical Company, Inc., USA
5. Hydroxypropyl Methylcellulose E4M, Colorcon, USA
6. Hydroxypropyl Methylcellulose F4M, Colorcon, USA
7. Lactose Anhydrous BP/USP, Meggle, Germany
8. Polyvinyl pyrrolidone K15, M_r ~ 10000, Fluka Chemika, Switzerland
9. Polyvinyl pyrrolidone K25, M_r ~ 24000, Fluka Chemika, Switzerland
10. Polyvinyl pyrrolidone K30, M_r ~ 40000, Fluka Chemika, Switzerland
11. Karl Fischer Reagent 5, Merck, Germany

3.2 Equipments

1. Dissolution Apparatus (Hanson Research, SR2 Dissolution test station. Hanson Research Corporation, USA.)
2. Spectrophotometer (Milton Roy Spectronic 1001 plus, USA.)
3. Single – punch Tableting Machine
4. Hardnesss Tester (Pharma Test, type PTB311,Germany)
5. Hot air oven (Mermert type UM-500,Germany)
6. Sieve No.12 - 120 (U.S.A. Standard Sieve Testing)

7. Mixer (Kitchen Aid Mixer)

8. Sonicator (Branson 8200, USA.)

9. Sieve Analysis Apparatus (Retsch Apparatus, Germany)

10. Stamp Volumeter (JEL STAV 2003, Germany)

11. Karl Fisher Titrator (Metrohm 701 KF Titrino, Germany)

3.3 Methods

3.3.1 Selection of Polymers for Pentoxifylline Tablets

Swelling of Polymers

The swelling property of polymer was determined by adding 0.2 g of polymer in 10 ml cylinder and 10 ml of water was slowly added into the cylinder. The sample were kept at room temperature for 24 hours and the swelling volume of polymers was measured.

3.3.2 Physical Characteristics of Granules

Granules Bulk and Tapped Density

The bulk density of each batch of granules was determined by pouring an accurately weighed 50 gram sample through a funnel into a 100 ml graduated cylinder. The volume was then read as a bulk volume. The density was then reported as the weight of the sample divided by bulk volume. The tapped density of each batch was then determined by subjecting the previously described graduated cylinder system to 500 taps from a height of approximately 2 cm by Stamp Volumeter (JEL STAV 2003, Germany). The tapped density was also reported as weight per volume.

Compressibility Ratio

The bulk density and tapped density were used to calculate the compressibility ratio as follow :

$$\text{Compressibility Ratio (\%)} = \frac{\text{Tapped Density - Bulk Density}}{\text{Tapped Density}} \times 100$$

The Compressibility Ratio was used to indicate the flow ability of granules as shown in Table 3.1

Table 3.1 : Relationship of Compressibility Ratio and Flow ability (45)

Compressibility Ratio Percent	Flow ability
5 – 12	Excellent
12 – 16	Good
16 – 21	Fair
21 – 35	Poor
Over 35	Very Poor

Repose Angle (α)

The pentoxifylline granules was weighed, about 50 mg, then poured through the fixed funnel onto the known accurate weight (w) and area (a) paper that 10 cm laid under the tip of funnel stem. The height of the granules heap (h) was read. The circumscription granules heap on the paper was cut then weighed and calculated the radius of circle (r). The repose angle (α) was calculated as follow :

$$\text{Tan}(\alpha) = h/r$$

Granules Size Distribution

The granule size distribution was analyzed by sieve analysis. A nest of standard sieves (10, 20, 30, 40, 50, 60, 80, 100 and 120 mesh) were attached with the sieve analysis apparatus (Retsch Apparatus, Germany). The 50 g of drug granule was poured into the upper sieve and allowed to shake for 20 min. The fractions of granule remaining on each sieve were weighed then the median granule sizes were calculated.

Moisture Content of Pentoxifylline Granules

The moisture content of pentoxifylline granule was determined by using Karl Fisher Titrator (Metrohm 701 KF Titrino, Germany). The Karl Fischer Reagent 5 (Merck, Germany) was used as titer. About 100 mg of granule was used in each determination.

3.3.3 Preparation of Pentoxifylline Tablets

Pentoxifylline tablets were prepared according to the typical formulation shown in Table 3.2

Table 3.2 : Typical Formulation of Pentoxifylline Matrix Tablet

Substance	Quantity (mg)
Pentoxifylline	400
Polymers*	191
Talcum	6
Magnesium Stearate	3
Total	600

Note : 1) Water or ethanol was used as polymer in wet granulating step.

2) *Various polymers see Table 3.3

Table 3.3 : Polymers Used in Tablet and Designated Formulation Number

Polymers	**Granulating Liquid**	
	Ethanol (1) Formulation	Distilled water (2) Formulation
Hydroxyethyl Cellulose wp40 (A)	A1	A2
Hydroxyethyl Cellulose QP 52000 (B)	B1	B2
Hydroxyethyl Cellulose M_v 90000 (C)	C1	C2
Hydroxypropyl Methylcellulose E4M (D)	D1	D2
Hydroxypropyl Methylcellulose F4M (E)	E1	E2
Lactose (F)	F1	F2

Pentoxifylline powder and polymer were mixed in Kitchen Aid Mixer. The mixed powder was granulated by wet granulation method with either distilled water or ethanol as granulating solution. The wet mass was sieved through No.12 US. Standard Sieve and wet granules were dried in hot air oven at temperature between 55-60 $^{\circ}$C for 8 hours. The dried granules were sieved (US. Standard Sieves No.20) and then mixed with talcum and magnesium stearate. The granules were compressed into a 600 mg oblong tablet on a single punch tabletting machine.

3.3.4 Physical Characteristic of Pentoxifylline Tablets

Weight variation. The weight of 10 - 20 pentoxifylline tablets were accurately weighed individually, and the average weight and standard diviation were obtained

Hardness. The hardness of pentoxifylline tablets was measured by Hardness Tester (Pharma Test®).

3.3.5 Pentoxifylline Content

Standard Curves of Pentoxifylline in simulated gastric fluid USP without enzyme (SGF), in simulated intestinal fluid USP without enzyme (SIF), in dichloromethane, in distilled water and in phosphate buffer pH 5.8, 7.0 and 8.0

The maximum absorption wavelength of pentoxifylline in SGF, SIF, dichloromethane, distilled water and in phosphate buffer pH 5.8, 7.0 and 8.0 were determined using UV spectrophotometer. The wavelength of the maximum absorption was found to be 273 nm for all the solutions above.

Standard curve of pentoxifylline

1 mg/ml stock solution of pentoxifylline in SGF, SIF, dichloromethane, distilled water, and phosphate buffer pH 5.8, 7.0 and 8.0 were prepared. Triplicate dilution of each stock solution were made with SGF, SIF, dichloromethane, distilled water, and phosphate buffer pH 5.8, 7.0 and 8.0 to obtain standard solutions of 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.010, 0.015, 0.020, 0.025 and 0.030 g/ml. The absorbance of these solutions were measured by using UV spectrophotometer at 273 nm wavelength and a standard curve was prepared. The data was optimized with Linear Regression by Excel, the computer program, then the equation was obtained.

Solubility of Pentoxifylline

The solubility of pentoxifylline was determined as follow: four grams of pentoxifylline powder was put into the L-shaped tube. 10 ml of each solution of phosphate buffer pH 5.8, 7.0, 8.0, distilled water, SGF, SIF and dichloromethane was added in each tube. The tube was fixed on the sample holder in a controlled water bath (37.0 °C) and shaken for 24 hours. After 24 hours, the solution were filtered through a 0.2 μm membrane filter. The absorbance of samples were measured at 233 nm wavelength. The solubilities of pentoxifylline were calculated. This process was performed in triplicate and the suitable solvent was chosen for determination of pentoxifylline content in the matrix tablets.

Validation of the assay method

Place accurate weight of pentoxifylline powders (about 10 – 15 mg) and hydroxypropyl methylcellulose E4M (about 10 – 15 mg) and dichloromethane into 50 ml volumetric flask. Sonicate for 30 minutes then add dichloromethane to make 50 ml solution. The solution was diluted with dichloromethane and measured the absorbance by UV spectrophotometer at 273 nm. The content of pentoxifylline that dissolved in dichloromethane and % recovery of pentoxifylline were calculated.

Determination pentoxifylline content

The pentoxifylline tablet was ground in a mortar. A known weight of the powders about 10 – 15 mg was placed in a volumetric flask. Dichloromethane was added and the mixture was sonicated for 30 minutes to dissolve the drug. Add dichloromethane to make 50 ml solution. The solution was diluted with dichloromethane and the quantity of pentoxifylline in the solution was measured by UV spectrophotometer at 273 nm. % recovery of pentoxifylline was used for correction of pentoxifylline quantity, then labeled amount of drug was calculated.

3.3.6 Release Studies

Three tablets of each formulation were evaluated by using dissolution apparatus (Hanson Research, SR2 Dissolution test station, Hanson Research Corporation, USA.). The study was conducted in both SGF and SIF. 900 milliliters of dissolution medium was filled in dissolution vessel and the temperature was kept at 37 ± 0.5 °C. Each tablet was inserted in the sinker before dropping into the dissolution vessel. The paddle was adjusted so that the distance between paddle and tablet was 2.0 cm. The paddle was rotated at a speed of 50 rpm.

The dissolution experiment was conducted over a period of 3 hours for SGF, and 8 hours for SIF. A 5 ml sample was withdrawn at 10, 20, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes, and replaced with 5 ml of fresh dissolution medium after each

sampling to maintain a constant volume. The samples were analyzed by UV spectrophotometer (Milton Roy Spectronic 1001 plus, USA.) at 273 nm for the content of pentoxifylline. Correction for sample volume withdrawn was taken into account in the calculations of pentoxifylline released.

Figure 3.1 : Sinker used in dissolution test

3.3.7 Effect of Some Additives on Release Profiles of Pentoxifylline

The pentoxifylline matrix tablet formulation D2 (Pentoxifylline in HPMC E4M) was chosen to modify the formulation by adding lactose or polyvinyl pyrrolidone K15 or K25 and K30. Each of the formulations is shown in Table 3.4. The tablet hardness and weight variation as well as release profile were also obtained the same method as previously described.

Table 3.4 : Additives Added into the HPMC E4M-Pentoxifylline Matrix Tablet and Designated Formulation Number

Formulation / Substance	Quantity (mg)					
	D2	D3	D4	D5	D6	D7
Pentoxifylline	400	400	400	400	400	400
HPMC F4M	191	131	131	131	131	161
PVP K15	0	60	0	0	0	0
PVP K25	0	0	60	0	0	30
PVP K30	0	0	0	60	0	0
Lactose	0	0	0	0	60	0
Talcum	6	6	6	6	6	6
Magnesium Stearate	3	3	3	3	3	3
Total	600	600	600	600	600	600

3.3.8 Reproducibility Studies

The pentoxifylline matrix tablet formulation D7 (Pentoxifylline, PVP K25 and HPMC E4M) was chosen to undergo the reproducibility test. Three batches of the pentoxifylline matrix tablet formulation were prepared. The physical characteristic of tablet and release profile were determined.