CHAPTER II

MATERIALS AND METHODS

1. Materials

The following substances were obtained from commercial sources

1.1 Model drug - Paracetamol BP. (Rhodia, France.)

1.2 Additives - Lactose BP. (Vehgel, Holland.)

- Microcrystalline cellulose(Avicel PH101, FMC Corporation, Japan.)
- Magnesium stearate USP. (Durham, England.)
- Talc BP. (Shin Industrial, Korea.)
- Corn starch (C.P., England.)
- Polyvinylpyrrolidone(PVP K30,British Oxygen Co, England.)

1.3 Dissolution medium - Hydrochloric acid, analytical grade (Mallincrodt, USA.)

2. Preparation of Drug-Additive Mixtures for Dissolution Studies

All the materials were passed through 60-mesh sieve to break up any agglomerate and dried at 60° for 6 hours before used. The mixtures, composed of varying quantities of additives, were

prepared according to the following procedures :-

2.1 Drug-additive mixtures

2.1.1 The amount of lactose, microcrystalline cellulose, talc, and magnesium stearate employed in the formulation together with paracetamol are listed in Table 1. The appropriate quantities of paracetamol and additives in the table were weighed and mixed throughly by manual bottle tumbling method.

2.1.2 The composition of paracetamol-binder mixtures are shown in Table 2 (amounts of binder listed in the table were the amount of dry mass of binder).

Starch paste were in the concentration of 10 % w/w in water. The concentration of corn starch in a mixture was changed by varying the amount of starch paste added.

Polyvinylpyrrolidone solution was prepared by dissolving the required quantity in 12.5 ml of water.

The binders were gradually mixed with paracetamol powder in the mortar and the damp mass was passed through 12-mesh sieve and dried in hot air oven at 60° for 6 hrs. After drying the mixture was again passed through 12-mesh sieve.

Polyvinylpyrrolidone was also added dry and mixed throughly with paracetamol in the mortar, then 12.5 ml of water was gradually added. Wet mixture was screened through 12-mesh sieve and after drying at 60° for 6 hours rescreened through 12-mesh sieve.

Table 1. Composition of Paracetamol-Diluent and Paracetamol-Lubricant Mixtures.

Quantity, % w/w							
D*-Lactose Mixtures		D -Microcrystalline Cellulose Mixtures		D -Talc Mixtures		D -Magnesium Stearate Mixtures	
D	Lactose	D	Microcrystalline Cellulose	D	Talc	D	Magnesium Stearate
90	10	90	10	99	1	99.5	0.5
80	20	80	20	97	3	99	1
70	30	70	30	95	5	97	3
				93	7	95	5

D* = Paracetamol.

Table 2. Composition of Drug-Binder Mixtures.

Quantity, % w/w					
D* - Starch Paste(10 % w/w) Mixtures		D - Polyvinylpyrrolidone (in solution) Mixtures		D - Polyvinylpyrrolidone (dry adding) Mixtures	
D	Corn Starch	D	Polyvinylpyrrolidone	D	Polyvinylpyrrolidone
99	1	97	3	97	3
98	2	96	4	96	4
97	3	95	5	95	5
96	4				

D* = Paracetamol.

2.2 Granules-additive mixtures

The granule was made to contain 68 % w/w of paracetamol, 30% w/w of lactose, and starch paste 10 % w/w used as the binder.

The granule was prepared by blending paracetamol and lactose in the mortar, the binder was then added gradually. Damp mass was screened through 12-mesh sieve and dried at 60° for 6 hours before dry screening through 12-mesh sieve.

The granule was mixed with disintegrant and lubricants as following:

2.2.1 The granules were mixed with dry corn starch, talc, and magnesium stearate by manual bottle tumbling. The amounts of talc and magnesium stearate were maintained constant at the concentration of 3 % and 1 % of granules, respectively, while the amounts of dry corn starch were varied to be 1,3,5,7,9% of granules respectively.

2.2.2 Factorially designed experiment was carried out to study the effect produced by the interaction of talc and magnesium stearate. The variation formulation included two level of talc(3 and 5 %), two level of magnesium stearate(0.3 and 1 %), and two level of compressional forces (2,000 and 3,000 lb), The granules were blended with high and low level of lubricants as shown in Table 3 and the mixtures were compressed at 2,000 and 3,000 lb respectively.

Table 3. Combination of Talc and Magnesium Stearate Mixed with Granules.

Concentration, % of Granule	
Talc	Magnesium Stearate
3.0	0.3
5.0	0.3
3.0	1.0
5.0	1.0

3. Dissolution Studies

3.1 Rotating disk assembly

The specially designed compression die assembly served as the disk holder being similar to the previously described by Wood, et al.[47] and Prakongpan, et al.[48], was fabricated with a slight modification from stainless steel. Fig. 3 shows the schematic drawing and dimensions of compression die assembly. It consists of a die(a), punch(b), plunger(c), and rotating shaft(d). The surface area of the die hole is 0.61 cm^2.

3.2 Preparation of disks

The materials to be studied must be compressed under vacuum to eliminate the effect of the entrapped air. Fig. 4 shows the vacuum casing(e) for insertion the die(a) and could be exhausted during compression. After the die was inserted into the vacuum casing, a given amount of sample was introduced into the die hole. The two plastic-O-ring(f) were installed between the vacuum casing, die, and the top plate(g) to make air tight inside the vacuum casing. The top plate was tightened to the body of vacuum casing with the three bolts(h). Then the punch and plunger were inserted into the die hole respectively.

The completed assembly was attached to the hydraulic press equipped with gauge (Carver Press, Model C). The vacuum pump (Arthur H. Thomas, Pa., USA.) was attached to the pipe of

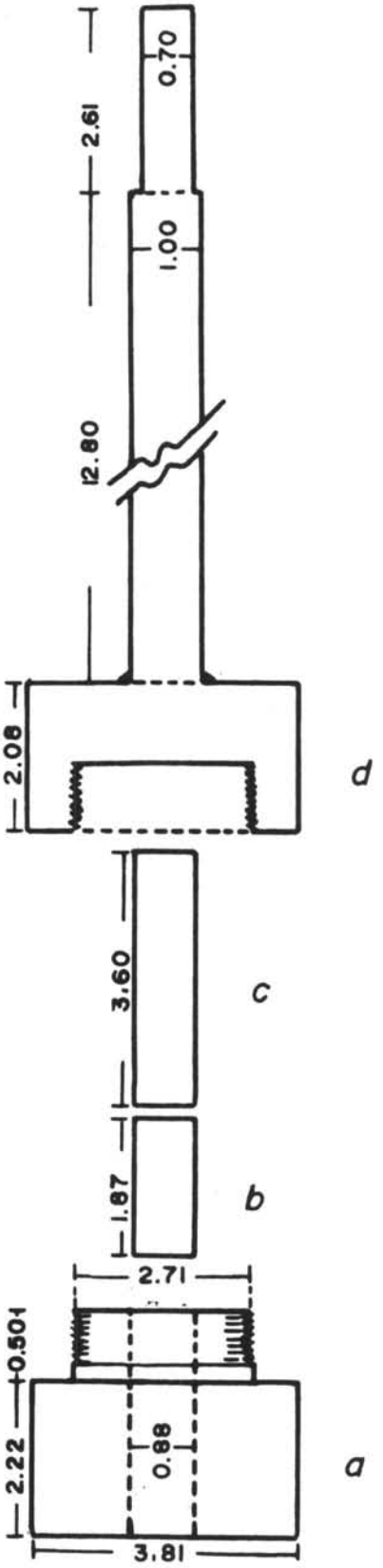

Figure 3. The schematic drawing and dimension in cm of compression die assembly.

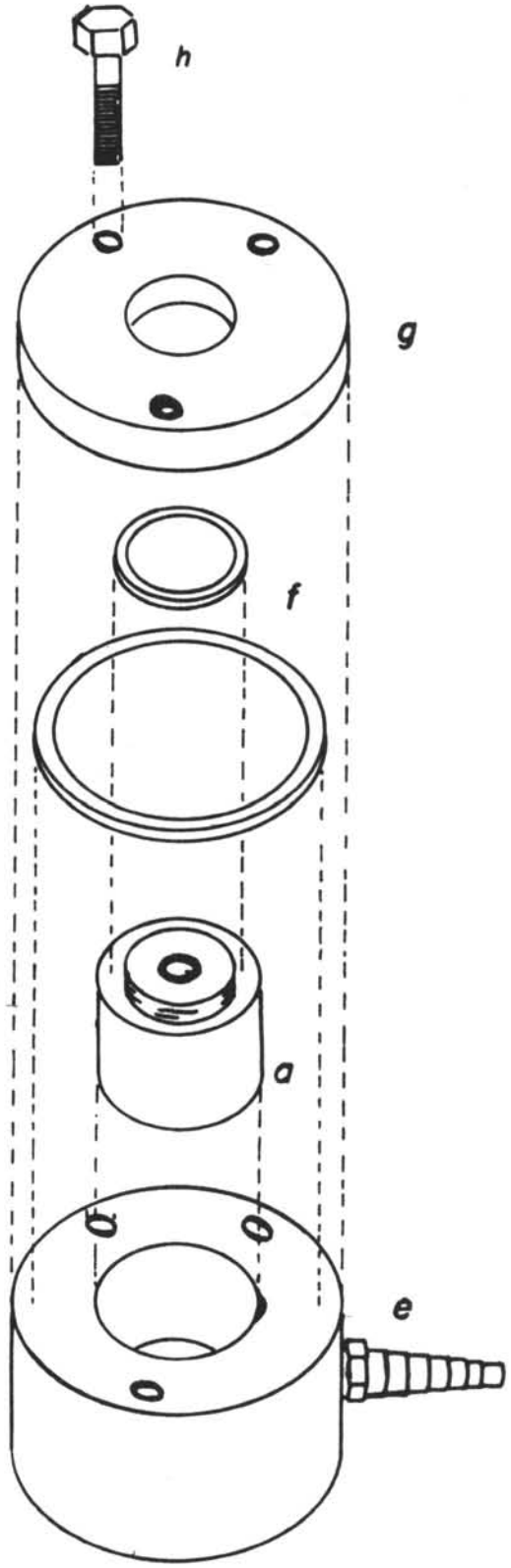

Figure 4. The schematic drawing of outer casing assembly.

vacuum casing to exhaust the air inside and gradually apply the load until the required force was attained. Reproducible dwell time was achieved for each tablet by applying the force to a specific load, and at this point the hydraulic valve was immediately release to reduce the pressure.

3.3 Determination of dissolution rate

3.3.1 Apparatus -Fig. 5 shows a schematic representation of rotating disk dissolution apparatus. It consists of the following : resin flask(k, used in USP dissolution apparatus) contained an amount of dissolution medium. The vessel was placed in a suitable water bath(m) which maintained constant temperature and agitation(p)

Compression die assembly(s) for use in rotational dissolution was mounted with constant speed motor(u) and it was immersed in dissolution medium at the center of the vessel, the distance of 2.0 cm between the bottom of the vessel and the bottom face of the disk was kept.

3.3.2 Procedure -250 ml of 0.1 N HCl was placed in the vessel and permitted to equilibrate to 37°. After the compressed disk of 300 mg was prepared by using compression die assembly as described in section 3.2. The compression die assembly was attached to the constant speed motor and immersed to dissolution medium. During immerssion,the careful attention

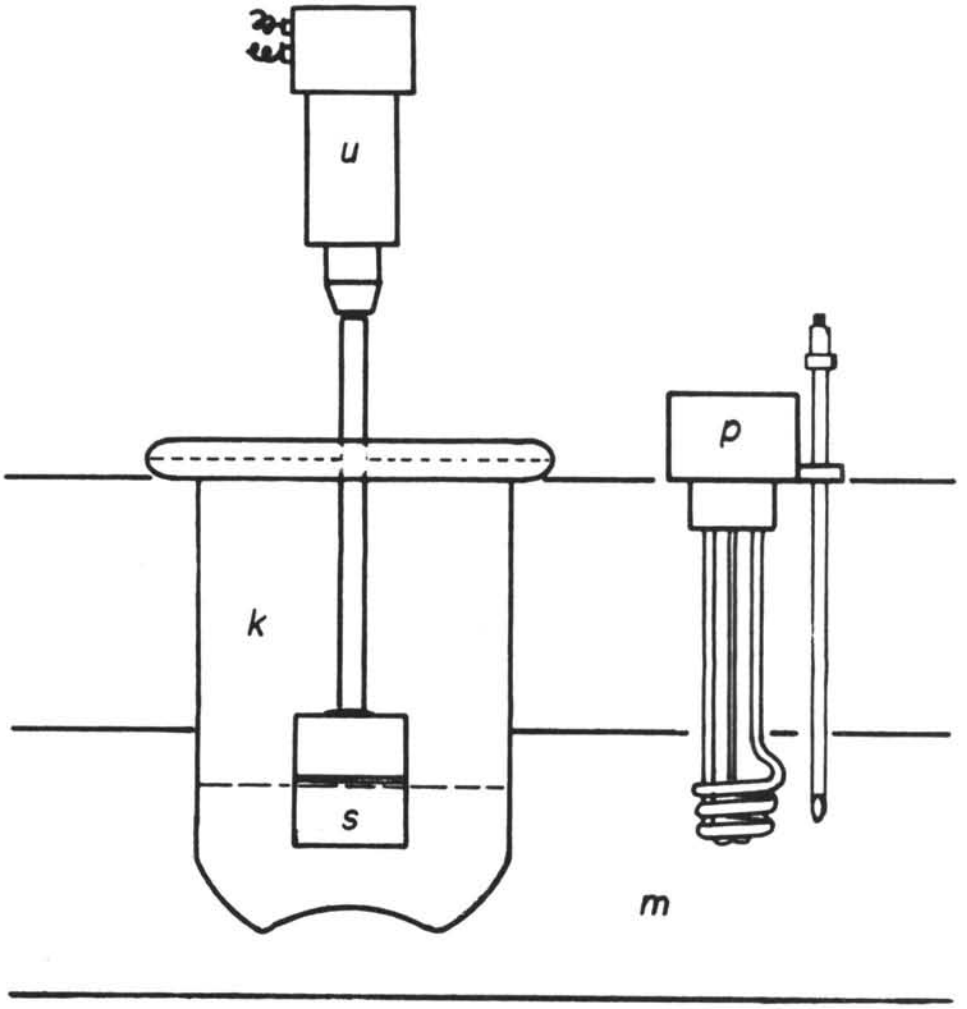

Figure 5. The schematic representation of dissolution apparatus.

should be paid on the surface of the disk in order that air bubbles would not attach. At the moment of contact between disk and the solvent, the motor and the timer were started simultaneously. The compression die assembly was rotated at the speed of 50 rpm.

The dissolution experiments were conducted over a period of 30 minutes. Every 5 minutes interval, 2 ml of sample was withdrawn by a sampling pipette. The same quantity of 0.1 N HCl was added immediatly after each sampling to keep the volume of dissolution medium constant during the course of the test.

The amount of drug in each sample was analyzed spectrophotometrically as described later. When the amounts dissolved were plotted as a function of time, a straight line should result. Dissolution rate was calculated from the slope of this plot divided by the constant surface area of compressed disk.

3.3.3 Analytical method -The standard concentration-absorbance curve of paracetamol was constructed by preparing the standard solution at the concentration of 0.25, 0.50, 0.75, 1.00, 1.50, and 2.50 mg %. The absorbance was measured at 244 nm by Pye-Unicam Spectrophotometer.

2 ml. of sample solution described above, was diluted with water to 10 ml, the absorbance was measured spectrophotometrically at 244 nm by using mixture of 0.1 N HCl and water as

blank. The concentration of the samples were determined by comparison with a standard curve and the amount dissolved at the specified time interval was calculated. A cumulative correction was made the previouly removed sample in determined the total amount dissolved,

4. Solubility Determinations

The saturated concentration of paracetamol in 0.1 N HCl at 37° was measured by the rotating disk method of Nogami et al.[18] Disk of 1 gm and 1.5 cm in diameter were prepared by precompression the drug at 10,000 lb, then the slug was broken into small granules and later they were recompressed with the same force.

The disk was sticked to the holder with chlorovinyl adhesive and covered the side of the disk with epoxide resine so that the only a flat surface of the disk was exposed to the dissolution medium. The holder with the disk was attached to the motor of the dissolution apparatus as previorly described. The dissolution was determined in 50 ml of 0.1 N HCl at 37° and rotational speed of 200 rpm. Samples were removed at 15 minutes interval for 2 hours and analyzed spectrophotometrically. The concentration time curve under non-sink condition was obtained by plotting the concentration versus time. If C_1 and C_2 are the contentration at time t and t + 15 minutes respectively, the plot of C_2 versus C_1 gives a straight line. The intersection between

this plot and the line of $C_1 = C_2$ gives saturated concentration value, C_s

5. Particle Sizes Measurments

The particle size of materials were measured by microscopic method. The glass slides used is a hemocytometer(Spencer, Bright-Line Hemacytometer, A.O. Instrument Co., NY.) that has a grid of lines of known distance. So that the particles can be measured. In microspopic method one makes a dilute suspension of particles in a liquid in which the materials is not soluble. Mineral oil was used as a disperse medium for paracetamol and lactose. Water was used for microcrystalline cellulose, talc, magnesium stearate, and corn starch.