

CHAPTER II

EXPERIMENTAL

MATERIALS

The following materials obtained from commercial sources were used as received. Deionized water was used through out this study.

1. Ac-di-sol[®] (FMC Corporation, USA, Lot no. T934)
2. Ammonium metavanadate (Farmitalia Carlo Erba, Italy, Code 420184)
3. Ammonium molybdate (J.T. Baker Inc., USA, Lot no. B20337)
4. Corn starch (Mc.Garrett. Peter Rich & Co., Holland)
5. Dibasic calcium phosphate dihydrate (Emcompress[®], Edward Mendell Co, Inc., USA)
6. Hydrochloric acid, AR grade (BDH Laboratory, England)
7. Magnesium stearate (Peter Greven Fett-Chemie, Germany, Lot no. CU 1952)
8. Methyl alcohol anhydrous, AR grade (Mallinckrodt Baker, Inc. Franch, Lot no. 3016 KVDD)
9. Nitric acid (Searle, England)
10. Nymcel[®] ZSD-16 (Metsa - Serla Chemical B.V., Netherlands, Lot no. B 4809)
11. Paracetamol (Mallinckrodt Chemical, Inc., Lot no. 6088996 K840)
12. Potassium dihydrogen phosphate (E. Merck, Germany, Lot no. 131 A 592673)
13. Primojel[®] (AVEBE, Holland, Batch no. 3952)

14. Silver nitrate (E. Merck, Germany, Lot no. K 20368112)
15. Sodium carbonate anhydrous (Carlo Erba, Italy, Lot no. 5 F526145G)
16. Sodium carboxymethylcellulose (DKS international, Inc., Japan, Lot no. 253630)
17. Sodium carboxymethylcellulose (Thai cellulose product Ltd., Thailand, Lot no. 512 PBL 002X, 601 PBL 002X / 611 PBL 004X, 510 PBL 006X)
18. Sodium hydroxide, AR grade (J.T. Baker, Inc., USA)
19. Sodium trimetaphosphate (Sigma Chemical Co., USA, Lot no. 103H 0282)
20. Spray dried lactose (Tablettose[®], The lactose company of New Zealand Ltd., New Zealand, Batch no. 2031104)
21. Talcum (Haicheng, Thailand, Code 21943-010323)
22. Zinc acetate (Carlo Erba, Italy, Lot no. 5E 038315E)

APPARATUS

1. Analytical balance (Sartorius, Model A-200S, Germany)
2. Disintegration Tester (Erweka, Type ZT31, Germany)
3. Dissolution Tester (Erweka, Type DT6R, Germany)
4. Fourier transform infrared spectrometer (Model 1760 X, Perkin Elmer, USA)
5. Hardness tester (Erweka, Germany)
6. Hot air oven (Mettler, Type UL 80, Germany)
7. Hydraulic press
8. Laser diffraction (Mastersizer, Marvern, UK)
9. Magnetic stirrer (Thermolyne, Model no. SP 46920-261)
10. Mixer, Oscillator (Erweka Type AR400, Germany)
11. Moisture analyzer (Sartorius MA 30, Germany)

12. Muffle furnace
13. pH meter
14. Refrigerated centrifuge (Hitachi, SCR 20B, Japan)
15. Scanning electron microscope (Jeol, JSM-353F, Japan)
16. Single punch tableting machine (Vihang Engineering, Thailand)
17. Spectrophotometer (Bausch & Lomb, Spectronic-2000, USA)
18. Tablet - Abrasion and Friability Tester (Erweka, Type TAR:10, Germany)
19. Thermal analyzer (Perkin Elmer DSC 7, England)
20. Top load balance (Sartorius, Model 1264MP, Germany)
21. Viscometer (Brookfield Engineering Laboratories Inc., USA)

METHODS

1. Preparation of Modified Sodium Carboxymethylcellulose

The method of preparation was modified from Kerr et al.'s method (1957). Formulation of the modification of sodium carboxymethylcellulose are shown in Table 2. The specification of sodium carboxymethylcellulose used in this study are shown in Table 3. Eighty grams of sodium carboxymethylcellulose was dispersed in 840 ml of methanol. 2.4 g of sodium trimetaphosphate, 25.44 g of sodium carbonate anhydrous and 5 g of sodium hydroxide were dissolved in 360 ml of water and then heated to 50°C. Add this solution to sodium carboxymethylcellulose dispersion with continuous mixing. The reaction was held at 50°C for 3 hours with agitation. After the reaction had proceeded to the desired degree, the pH of resultant sodium carboxymethylcellulose dispersion was adjusted to 6.5 with 37% hydrochloric acid, washed several times with 80% methanol, filtered and the product was dried at 50°C, 8 hours. Collected the dried powder and screened through 60 mesh sieve.

In order to remove unreacted sodium trimetaphosphate an extensive washing step after the reaction was necessary. To identify phosphate in the filtrate, 5 ml of silver nitrate solution R1 was added to 5 ml of filtrate, a yellow precipitate was formed (European Pharmacopoeia, 1980). The product was washed with 80% methanol until the yellow precipitate was not appeared in the filtrate.

Table 2 Formulation of the modification of sodium carboxymethylcellulose

Material	Amount
sodium carboxymethylcellulose	80 g
sodium trimetaphosphate	2.4 g
sodium carbonate anhydrous	25.44 g
sodium hydroxide	5 g
methanol*	840 ml
water*	360 ml

* The ratio of methanol : water = 70 : 30

Table 3 The specification of sodium carboxymethylcellulose used in this study

Material	Degree of substitution	Viscosity (cps)	NaCl content (%)
NaCMC ₁ ^(a)	0.65-0.75	20-50	0.27
NaCMC ₂ ^(b)	0.63	1730	0.32
NaCMC ₃ ^(a)	0.65-0.75	500-800	0.30
NaCMC ₄ ^(a)	0.65-0.75	1400-1800	0.30

(a) = Supply from Thai cellulose product , Ltd.

(b) = Supply from DKS international, Inc.

1.1 Effect of Ratio of Methanol to Water on Crosslinking Reaction between Sodium Carboxymethylcellulose and Sodium Trimetaphosphate

The procedure given above was repeated with varying in the ratio of methanol to water, that was 50:50 , 60:40 , 70:30 and 80:20. Sodium carboxymethylcellulose used in this study was NaCMC₂.

1.2 Effect of Time on Crosslinking Reaction between Sodium Carboxymethylcellulose and Sodium Trimetaphosphate

The procedure given above was repeated with the ratio of methanol to water equal 70:30. The reaction time was varied at the range of 3, 6, 12 and 24 hours. Sodium carboxymethylcellulose used in this study was NaCMC₂.

1.3 Effect of Viscosity of Sodium Carboxymethylcellulose on Crosslinking Reaction between Sodium Carboxymethylcellulose and Sodium Trimetaphosphate

Sodium carboxymethylcellulose used in this study was low, medium and high viscosity grade (20-50, 500-800, 1730 and 1400-1800 cps). The procedure given above repeated with the ratio of methanol to water was 70:30 and the reaction was held for 3 hours.

2. Detection Crosslinking Reaction in Modified Sodium Carboxymethylcellulose

Phosphate content in modified sodium carboxymethylcellulose was determined by UV-visible spectrophotometry (Smith, 1967). The crosslinking reaction was confirmed by IR-spectrophotometry, differential scanning calorimetry and evaluation of their viscosity.

2.1 Percent Phosphate

Five grams of modified sodium carboxymethylcellulose was accurately weighed into 100-ml evaporating dish, 10 ml of 10% zinc acetate solution was added, distributing the solution uniformly through the sample by adding water if necessary. The sample was evaporated to dryness on a steam bath, heated on a hot plate until thoroughly charred, and then ignited 2 hours in a muffle furnace at 550°C. The dish was cooled to room temperature, and the residue was wetted by cautious addition of 3 ml of 29% nitric acid. The sample was again evaporated to dryness on a steam bath and dehydrated completely by brief heating on a hot plate, and the dish was returned to the muffle furnace at 550°C for about 30 minutes.

After cooling to room temperature, the sides of the dish were washed down with 10 ml of 29% nitric acid followed by 15 ml of water. The dish was covered with a watch glass; the residue solution was heated to incipient boiling and held 10 minutes at that temperature. After cooling to room temperature, the solution was quantitatively filtered through Whatman no.1 paper into 50-ml volumetric flask. The transfer was completely by washing the dish and filter paper with small portion of water, and the solution was diluted to volume and mixed thoroughly.

An aliquot selected to contain not more than 2.5 mg of phosphorus was pipetted into a 100-ml volumetric flask, and 25 ml of water was added to another 100-ml volumetric flask serving for the reagent blank. The following reagents were added to both flasks, in the order stated, mixing after each addition; 10 ml of 29% nitric acid, 10 ml of 0.25% ammonium vanadate solution, and 10 ml of 5% ammonium molybdate solution. Both solutions were diluted to volume with water, mixed thoroughly, and allowed to stand 10 minutes. Using the reagent blank in a 1-cm cuvette as the reference at zero absorbance, the absorbance of the sample solution at 500 nm was determined in a 1-cm matching cuvette. The phosphorus concentration

(mg/100 ml) of the sample solution was read from the calibration curve in Figure 25 (It obeyed Beer's law in the range of concentration of 0-6 mg/100ml), and data are shown in Table 25 (Appendix I) that was prepared as follows:

Potassium dihydrogen phosphate 4.3947 gm was accurately weighed into a 1000-ml volumetric flask and dissolved in water. The solution was diluted to volume with distilled water and used as stock solution. The stock solution was pipetted 2, 4, 6, 8 and 10 ml into separate 100-ml volumetric flask, and 25 ml of water was added to another flask serving for the reagent blank. The following reagents were added to each flask, in the order stated, mixing after addition: 10 ml of 29% nitric acid, 10 ml of 0.25% ammonium vanadate solution and 10 ml of 5% ammonium molybdate solution. The solutions were diluted to volume with water, mixed thoroughly, and allowed to stand 10 minutes.

The absorbance of known potassium dihydrogen phosphate concentration was determined using the reagent blank in UV absorption spectrophotometer in a 1-cm cell at 500 nm. Each concentration was determined in triplicate.

Phosphorus content of sample solution were ascertained by reference thereto.

$$\text{Percent Phosphorus} = \frac{P \times \text{Dilution volume (ml)} \times 100}{\text{Aliquot volume (ml)} \times \text{Sample weight (g)} \times 1000}$$

in which P = Phosphorus content (mg/100ml) determined from calibration curve

$$\text{Percent Phosphate} = \text{Percent Phosphorus} \times 3.065$$

2.2 IR Spectrophotometry

The infrared spectra of sodium carboxymethylcellulose, modified sodium carboxymethylcellulose and the physical mixture of sodium carboxymethylcellulose and sodium trimetaphosphate were recorded using a KBr disc method by an infrared spectrophotometer in a range of 4000-450 cm^{-1} .

2.3 Differential Scanning Calorimetry

The thermograms of sodium carboxymethylcellulose, modified sodium carboxymethylcellulose and the physical mixture of sodium carboxymethylcellulose and sodium trimetaphosphate were recorded on thermal analyzer. All thermal runs were carried out at a heating rate of 20°C/min and the temperature between 50°C and 300°C.

2.4 Viscosity

The viscosity was determined in 1% aqueous solution at 25°C using Brookfield viscometer with spindle LV series at the speed of 30 and 60 rpm. (Brookfield Engineering Laboratories Inc., USA).

3. Physical Properties of Modified Sodium Carboxymethylcellulose

3.1 Powder Morphology

Morphology of powder samples were determined with scanning electron microscope. The sample was coated with gold prior to the microscopic examination using ion sputting. Size, shape and surface topography of powders were observed, and then photographed at appropriate magnification.

3.2 Size Distribution and Specific Surface Area

Samples of modified sodium carboxymethylcellulose were examined for size distribution, cumulative size distribution, specific surface area and average diameter of particle by laser diffraction (Mastersizer S long bed Ver 2.11, Malvern, UK).

3.3 Bulk Density, Tapped Density and Percent Compressibility

Bulk density was performed by pouring an accurate weight of each sample (about 20 g) into a 100-ml graduated cylinder and measuring the bulk volume. Tapped density was performed by dropping the graduated cylinder containing the sample onto a hard wood surface from a height of 5 cm until the powder attained a constant tapped volume.

The bulk and tapped densities were calculated by dividing the weight of sample by its bulk volume and tapped volume respectively. The results were presented as an average of three trials. The compressibility was calculated from the following equation.

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

T = Tapped density

B = Bulk density

3.4 Moisture Determination

One gram of sample was accurately weighed on a pan of the moisture analyzer (Sartorius MA30, Germany), dried until constant weight was obtained. The result was shown as percent moisture content.

4. Evaluation of Physical Properties of Modified Sodium Carboxymethylcellulose Compared with Sodium Carboxymethylcellulose Used as Starting Materials and Commercial Disintegrants

4.1 Water Uptake

The amount of water uptake of powders were determined by the apparatus which modified from Nogami's apparatus as shown in Figure 1. Five hundred milligrams of powders sample was carefully filled in the sample tube (made by 5 ml plastic syringe, cut the end, and close one side with Whatman filter paper No.1). After the sample was filled, the sample tube was tapped for 20 times to made equally packing of particles in all experiments. A sample tube contained particles of disintegrant was placed onto a sinter-glass filter No.3 and the penetration of water into the sample through the sinter-glass filter was determined using a measuring pipet. The evaporation of water was prevented by closing the small free air space over the sinter-glass filter with a petri-disk. The volume of liquid taken up into the sample was observed simultaneously at 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 10.00, 15.00, 20.00, 25.00, 30.00, 40.00, 50.00 and 60.00 minutes. Deionized water was used as testing media and the experiment was performed at a constant temperature of $37\pm 1^{\circ}\text{C}$. The mean of three determinations was calculated (Nogami et al., 1969; Rudnic et al., 1982).

4.2 Swelling of Particles

Swelling of particles in water was studied by comparing the particle size distribution of disintegrant powder dispersed in water to that in a less polar solvent, methanol, using laser diffraction (Mastersizer, Marvern, UK). The data was collected at various time intervals of 1, 2, 3, 5, 10, 15 and 20 minutes (Caramella et al., 1984; Pesonen, Paronen and Ketolainen, 1989).

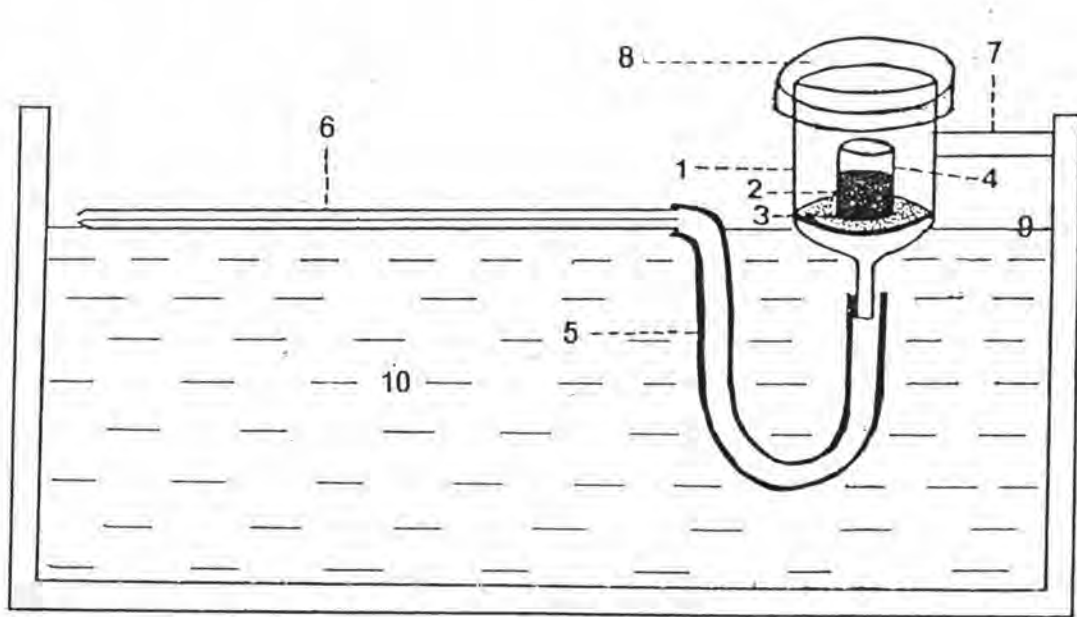


Figure 1 Diagrammatic representation of apparatus for determination of water uptake of disintegrants

- | | | |
|--|-----------------------|---------------------|
| 1 sinter-glass filter | 2 disintegrant powder | 3 filter paper |
| 4 sample tube | 5 rubber tube | 6 measuring pipette |
| 7 clamp | 8 petri-disk | 9 water level |
| 10 thermostated at $37\pm 1^{\circ}\text{C}$ | | |

4.3 Sedimentation Volume

The sedimentation volume was determined by using cylindrical method. Five grams of sample was suspended in distilled water and transferred to a 100-ml graduated cylinder with the aid of water. Then the volume was adjusted to 100 ml with water. The cylinder was stoppered and inverted for three times. It was allowed to stand at $37\pm 1^{\circ}\text{C}$ for 24 hours and the sediment was measured. The sedimentation volume was calculated in ml per gram. The result was the mean of three determinations.

4.4 Hydration capacity

A sample of 100 mg of each disintegrant was placed into a 10-ml centrifuge tube tared with stopper. Five milliliters of water was added, and the tube was stoppered and shaken vigorously to suspend the sample thoroughly. The suspension was allowed to stand for 10 minutes. During this time, it was mixed by inverting three times at the end of 5 and 10 minutes. The stopper was then removed and the tube was centrifuged for 15 minutes at 1000×g, 20°C using refrigerated centrifuge (Hitachi, SCR 20B, Japan). The centrifuge was allowed to stop without braking. The supernate was carefully decanted and the tube was inverted to allow draining. The tube was then restoppered and the contents were weighed (Kornblum and Stoopak, 1973). The hydration capacity was calculated in the following manner:

$$\text{Hydration capacity} = \frac{(\text{weight of tube} + \text{wet sediment}) - (\text{weight of tube})}{\text{sample weight (dry basis)}}$$

Hydration capacity presented as the mean of three determinations.

5. Evaluation of Disintegrating Property of Modified Sodium Carboxymethylcellulose Compared with Sodium Carboxymethylcellulose Used as Starting Materials and Commercial Disintegrants

5.1 Preperation of tablets

Tablets were manufactured by direct compression method using two types of directly compressible diluents, α -lactose monohydrate (Tablettose[®]) and dibasic calcium phosphate dihydrate (Emcompress[®]) which were water soluble and water insoluble excipients, respectively. The study of disintegrating efficacy of modified sodium carboxymethylcellulose was made in comparison with sodium carboxymethylcellulose which used as a starting material and the following disintegrants: Ac-di-sol[®], Nymcel[®], Primojel[®] and corn starch. The concentration of disintegrants used were 2%, 4% and 6%.

The compositions of the tablet formulations used in this study were presented in Table 4.

Table 4 Tablet compositions for evaluation of disintegrating property

Ingredient	%w/w per tablet			
<u>Series 1 (Water soluble diluent)</u>				
Tabletose [®]	96	94	92	90
Disintegrant *	0	2	4	6
Talcum	3	3	3	3
Magnesium stearate	1	1	1	1
<u>Series 2 (Water insoluble diluent)</u>				
Emcompress [®]	96	94	92	90
Disintegrant *	0	2	4	6
Talcum	3	3	3	3
Magnesium stearate	1	1	1	1

* NaCMC₁, M₁, NaCMC₂, M₂, NaCMC₃, M₃, NaCMC₄, M₄, Ac-di-sol[®], Nymcel[®], Primojel[®] or corn starch

in which, M₁ = NaCMC₁ cross-linked with sodium trimetaphosphate
M₂ = NaCMC₂ cross-linked with sodium trimetaphosphate
M₃ = NaCMC₃ cross-linked with sodium trimetaphosphate
M₄ = NaCMC₄ cross-linked with sodium trimetaphosphate

All disintegrants, magnesium stearate, talcum and the diluents; dibasic calcium phosphate dihydrate and α -lactose monohydrate were passed through 60-mesh handle screen sieve, then all excipients were dried in hot air oven at 60°C (Mettler, Germany) for 30 minutes. A batch of 500 g

of each formulation was prepared by mixing the diluent, disintegrant and talcum in a laboratory scale cube mixer (Erweka Type AR 400, Germany) for 10 minutes at rotation speed of 20 rpm, magnesium stearate employed as lubricant was then added to the mixer, mixing for 3 minutes. The materials were then compressed on hydraulic press equipped with 1/4 inch round flat faced punch. The weight of tablets for each formulation was 350 mg. Each tablet formulation was compressed at three different compressional forces: 3000, 3500 and 4000 pounds.

5.2 Disintegration

Disintegration time of tablets were determined using the disintegration tester (Erweka Type ZT31, Germany) with water at $37\pm 1^\circ\text{C}$ as a disintegration fluid. The test was performed without disk and the average disintegration time was calculated from six determinations.

6. Evaluation of Modified Sodium Carboxymethylcellulose as Tablet Disintegrant in Paracetamol Tablets

6.1 Preparation of Paracetamol Tablets

From the all of modified sodium carboxymethylcellulose, M_4 was selected for evaluation of disintegration property in paracetamol tablets. Although the water uptake of M_4 was inferior to that of M_2 and the disintegration property in soluble and insoluble system was comparable to the others modified sodium carboxymethylcellulose, M_4 showed the highest swelling. And NaCMC_4 was used to compared with M_4 as the starting material.

Table 5 lists the general formulation used for preparation of the paracetamol tablets

Table 5 General formula for paracetamol tablets

Ingredient	Composition (%w/w)	Amount per tablet (mg)
Paracetamol	80	500
PVP 30K	4	25
Lactose	8	50
Disintegrant * - intragranular	2	12.50
- extragranular	2	12.50
Talcum	3	18.75
Magnesium stearate	1	6.25
Total	100	625

* NaCMC₄, M₄, Ac-di-sol[®], Nymcel[®], Primojel[®], corn starch

These materials in formulation were passed through a 60-mesh screen prior to mixing. The intragranular disintegrant and lactose were geometrically diluted with paracetamol before dry blending. Dry blending was performed in a mixer (Erweka Type AR 400, Germany) for 15 minutes. Polyvinylpyrrolidone 30K (4%w/w of the tablet formulation), dissolved in water to give a 15%w/v solution, was added gradually to the mixing bowl during wet mixing for 5 minutes. To ensure even and complete granulation, the mixing blades and the bowl were scraped well with a spatula before allowing the granulation to mix for an additional 5 minutes. The wet mass was pass through a 16-mesh and then dried in a hot air oven at 60°C, 1 hours. The dried granulation was passed through a 18-mesh screen using an oscillating granulator (Erweka Type AR 400, Germany).

The milled granulation was mixed with the required amount of extragranular disintegrant and talcum in the cube mixer (Erweka Type AR

400, Germany) for 5 minutes. The final blend was then mixed with magnesium stearate for 2 minutes.

The tablets were compressed with a single punch machine equipped with a 3/8 inch, round, flated-faced bevelled edge punch and die to a targeted hardness of 6 ± 1 kp. and to a tablet weight of 625 mg.

6.2 Evaluation of Tablets

6.2.1 Weight Variation

Twenty tablets of each batch were individually weighed, using an analytical balance (Satorious, Model A-200S, Germany). The average weight and standard deviation were determined.

6.2.2 Thickness

The thickness of tablets were determined by using motorized hardness tester (Erweka, Germany) which is simultaneously to measured during hardness testing and expressed in millimeter. The thickness value was an average of ten determinations.

6.2.3 Hardness

The tablet hardness was determined immediately after compression on a motorized hardness tester (Erweka, Germany). Ten tablets were tested for each batch and then the mean and standard variation were calculated. Hardness was measured in kilopound (1 kilopound = 9.8 newtons = 1.4 Strong-Cobb units; S.C.U.).

6.2.4 Friability

Twenty previously weighed tablets were placed in the tablet-abrasion and friability tester (Erweka, Germany) and then rotated at 25 rpm for 4 minutes to subject the tablets to 100 drops. The tablets were then

weighed and the weight loss was calculated in terms of “percent friability” (Kornblum and Stoopak, 1973).

6.2.5 Disintegration time

Disintegration time of tablets were determined, using the disintegration tester (Erweka, Type ZT31, Germany) with purified water at $37 \pm 2^\circ\text{C}$ as a disintegration fluid. The test was performed without disk and the average disintegration time was calculated from six determinations.

6.2.6 Dissolution

The dissolution test of tablets were carried out according to USP dissolution type II method using the dissolution tester (Erweka, Type DT6R, Germany). A 900 ml of phosphate buffer pH 5.8 was used as dissolution medium which maintained the temperature at $37 \pm 0.5^\circ\text{C}$ during the test. The paddle was rotated at the speed of 50 rpm, and then a tablet was immersed in the vessel.

Five milliliters of sample solution was withdrawn at various time intervals of 1, 2, 5, 10, 15, 20, 25 and 30 minutes, then the equal volume of phosphate buffer pH 5.8 was added immediately to maintain constant volume of dissolution medium. The aliquot was diluted with phosphate buffer pH 5.8 to a suitable volume and the absorbance was measured in a 1-cm cell at the maximum absorbance wavelength of 243.4 nm with a UV-visible spectrophotometer (Bausch&Lomb, Spectronic 2000, USA) using phosphate buffer pH 5.8 as a blank. The amount of drug dissolved in each sample was calculated from the standard calibration curve in Figure 26 and data are shown in Table 26 (Appendix I). Dissolution profile was obtained from the average of three determinations.