CHAPTER III

EXPERIMENTAL

1) Materials

The following materials obtained from commercial sources were used as received

1.1) Model drug

- Diclofenac Sodium BP (Bromine Free) (Batch NO. DS/0010/2301, supplied by Amoli Organics Ltd. Thailand)

1.2) Additives

- Spray – dried lactose (Ludipress®) (Lot NO.R1-44/00394, BASF, Germany) (supplied by Government Pharmaceutical Organization, Thailand)
- Lactose Anhydrous (Lot NO. R1 44/00780, Wyndale, New Zealand) (supplied by Government Pharmaceutical Organization, Thailand)
- Corn Starch (Lot NO R1-43/06458, Wide Trade Co. Ltd., Thailand) (supplied by Government Pharmaceutical Organization, Thailand)
- Tapioca Starch (Lot NO R1-40/00512, Wide Trade Co. Ltd., Thailand) (supplied by Government Pharmaceutical Organization, Thailand)
- Era – tab (Lot NO R1-40/00286, Wide Trade Co. Ltd., Thailand) (supplied by Government Pharmaceutical Organization, Thailand)
- Croscarmellose Sodium (Lot NO. 5610296249, JRS, Germany) (supplied by Government Pharmaceutical Organization, Thailand)
- Hydroxypropylmethylcellulse (Methocel E4M) (Lot NO. ll86121121, The Dow Material Company, USA)
- Ethylcellulose (Ethocel 10 cps.) (Lot NO A10710, FMC Corporation, USA)
- Magnesium Stearate (Lot NO. R1-42/11431, BASF, Germany) (supplied by Government Pharmaceutical Organization, Thailand)
- Aerosil (Lot NO R1-42/01485, Wacker Chemie GHBH, Germany) (supplied by Government Pharmaceutical Organization, Thailand)

1.3) Dissolution Medium

- ❖ Monobasic Potassium Phosphate (E. Meick, Germany)
- ❖ Sodium Hydroxide, AR grade (J.T. Baker Inc., USA.)
- ❖ Hydrochloric Acid (BOH Laboratory, England)

1.4) Solvents

- ❖ Purified Water
- ❖ 96% Ethyl Alcohol (Lot NO. R1/43/45210) (supplied by Government Pharmaceutical Organization, Thailand)
- ❖ Methyl Alcohol Anhydrous, AR grade (Mallinckrodt Chemical, Franch)

2) Equipment

- ❖ Analytical Balance (Mettler AM 50, Switzerland)
- ❖ Planetary Mixer (Model K 45, The Hobart Mfg. Co. Troy., USA.)
- ❖ Hand Sieving #10 ,30 ,60 (S.Y. pattana Co. Ltd., Thailand)
- ❖ The Rotary Tabletting Machine (Manesty F3 single-punch tabletting machine, Germany)
- ❖ Stirring Hot Plates (Double Ring Co. Ltd., China)
- ❖ Hardness Tester (Scheuniger-2E, Germany)
- ❖ Friability Tester (Pharmatest, PTFR-A, Germany)
- ❖ Disintegration Tester (Erwaka ZT 34, Germany)
- ❖ Dissolution Apparatus (Model SR-2, Hanson Research, USA.)
- ❖ Mechanical Sieve Shaker (Josef Deckelmann, Ashaffenburg, Germany)
- ❖ Moisture Content Analyzer (Mettler HR 73, Switzerland)
- ❖ Ultraviolet/Visible Spectrophotometer (Model Shimudzu UV – 1601A ,Japan)
- ❖ Scanning Electron Microscope (Model JSM – 5410LV ,Japan)
- ❖ X – ray Diffractometer (Model JDX-8030, Jeol, Japan)
- ❖ Thermal Analysis (Model NETZSCH DCS200, NETZSCH-Geratebau GmbH, Germany)
- ❖ Infrared Spectrometer (Model FT-IR 1760X, Perkin-Elmer, USA.)

- ❖ Surface Area Determination Equipment (Model Flowsorb 2300 FC, Micrometrics Instrument Corporation, USA)

3) Methods

3.1) Preliminary studies of diclofenac sodium powders

3.1.1) Evaluation of physical properties of diclofenac sodium

1) Morphology

Photomicrographs of diclofenac sodium powders were taken under scanning electron microscope. The sample was coated with gold prior to the microscopic examination using ion sputtering. The size, shape, and topography of diclofenac sodium powder were observed.

2) The Infrared spectroscopy

The IR spectra of diclofenac sodium powders were examined by milling the samples with KBr at the ratio of 1 :100. Then, it was compressed with 1000 psi. pressure and was detected with an infrared spectrophotometer in a range of wavelength of 4000-450 cm^{-1}. The resolution was 8.0.

3) The X – ray diffraction

The crystallinity of diclofenac sodium powders was examined by X-ray diffractometry. The sample for X-ray diffraction studies was firmly packed into the cavity of a thin rectangular metal plate using two glass slides, which were fastened to the metal plate with adhesive tape. The first glass slide was then removed, and the prepared sample was taken to expose to the X – ray diffraction chamber. The X – ray diffraction patterns were recorded from 5°–60° in terms of 2θ angle.

4) The differential scanning calorimetry (DSC)

The thermograms of diclofenac sodium powders were recorded on a thermal analyzer. About 3-4 g of sample was put onto the aluminum pan. The sample was taken into the condition that had been pierced with liquid nitrogen gas. All thermal runs were carried out at a heating rate of 10° C/min and the temperature between 35° and 300°C.

3.1.2) Preliminary investigation on compressibility of diclofenac sodium powders.

Before proceeding with the preparation of diclofenac sodium microtablets, preliminary investigation of microtabletting process was carried out to determine optimum original compressible properties of diclofenac sodium powders. During the feasibility trial, the batch sizes of 20 g were used. The investigation was performed on three types of diclofenac sodium powders.

1) Untreated diclofenac sodium powder.
2) Ground diclofenac sodium powder by milling with mortar and pestle for 2 minutes.
3) Sieved diclofenac sodium powder through #30 mesh.

3.1.3) Preliminary investigation on flowability of suitable diluent for the preparation of diclofenac sodium microtablets

The free - flowing property of powders was necessary for compression into microtablets. Therefore, five diluents were examined by homogeneously mixing the active ingredient with various diluents at the same quantity. Then, twenty grams of the mixed powders were sampling for examining the flow properties of the mixed powder. The two parameters that indicated flowability were the flow rate and the angles of repose. After that, the mixed powders were mixed with croscarmellose sodium and magnesium stearate, respectively for 5 minutes or until homogeneously. Then, it was compressed to be microtablets with the single-punch tabletting machine, which had three small concave punches per punch holder. The physical properties were investigated. They were hardness and % friability.

The composition of DS microtablet formulations for choosing the most suitable diluent in preliminary investigation are presented in Table 4

Table 4 Composition of diclofenac sodium microtablet in preliminary investigation

Formulation / Component	D1 (mg)	D2 (mg)	D3 (mg)	D4 (mg)	D5 (mg)
Diclofenac sodium	0.5	0.5	0.5	0.5	0.5
Diluent**	9.0	9.0	9.0	9.0	9.0
Croscarmellose sodium	0.25	0.25	0.25	0.25	0.25
Magnesium stearate	0.25	0.25	0.25	0.25	0.25
Total weight/ tablet	10	10	10	10	10

****Diluent : lactose, corn starch, Ludipress, tapioca starch, Era-tab, respectively (D1-D5)**

3.2) Formulation and preparation of sustained – release diclofenac sodium powder nmixtures and granules

Capsule was chosen as a dosage form containing the sustained - release DS microtablets. This preparation could be prepared by filling the microtablets into the capsule. The dose of DS per capsule was equal to 75 mg similarly to that of the commercial product (Voltaren SR 75 mg). Therefore, the amount of drug per microtablet was depended on the capsule size. The capsule of NO.3 and NO.2 were selected due to ease of consumption. The total weight of each microtablet was 10 mg and the thickness was 3.5 mm. The maximum amount of eighteen and twenty-five microtablets could be filled into the capsule NO.3 and NO.2, respectively by flowing pass through the funnel . The amount of drug per microtablet was calculated as the following :

The amount of drug per microtablet was equal to the total dose of the preparation was divided to the amount filled microtablet into capsule. Therefore, the

amount of drug per microtablet for capsule NO.3 and NO.2 were 4.2 and 3 mg, respectively. Table 5-8 list the formulation of sustained release diclofenac sodium powder mixtures and granules for capsule NO.3 and NO.2.

Two techniques for preparing preparation of sustained-release DS microtablets for capsule NO. 3 and NO.2 were used.

- ***Preparation of powder mixtures by direction compression***
- ***Preparation of granules by wet granulation***

Preparation of powder mixtures by direction compression

Required amount of DS in the powder mixtures of each formulation were presented in Tables 5 and 6 were mixed manually with the suitable diluent from preliminary investigation for 5 minutes in the plastic bag. Then, the sustained release materials (hydroxypropylmethylcellulose (Methocel E4M) and ethylcellulose (Ethocel 10 cps.)).were added and mixed for 5 minutes. After that, magnesium stearate and Aerosil were added and mixed manually in the plastic bag until homogeneously. The powder mixtures were stored in tight light – resistant and dry containers.

Table 5 The composition of the diclofenac sodium powder mixtures for direct compression microtablet at the dose of 4.2 mg per microtablet

Formulation / Component	DLHC (mg)	DLEC (mg)	DSHC (mg)	DSEC (mg)
Diclofenac sodium	4.2	4.2	4.2	4.2
Ludipress	3.0	3.0	-	-
lactose	-	-	3.0	3.0
Methocel E4M	2.4	-	2.4	-
Ethocel 10 cps.	-	2.4	-	2.4
Mg Stearate	0.8	0.8	0.8	0.8
Aerosil	0.2	0.2	0.2	0.2

Total weight /Tablet : 10 mg/tablet with a total batch size of 100 grams

Each component showed that in mg

D : Direct compression L : Ludipress S : Lactose HC : Methocel EC : Ethocel

Table 6 The composition of the diclofenac sodium powder mixtures for direct compression microtablet at the dose of 3 mg per microtablet

Formulation / Component	DHC (mg)	DEC (mg)
Diclofenac sodium	3.0	3.0
Ludipress	3.0	3.0
Methocel E4M	3.0	-
Ethocel 10 cps.	-	3.0
Mg Stearate	0.8	0.8
Aerosil	0.2	0.2

Total weight /Tablet : 10 mg/tablet with a total batch size of 100 grams

D : Direct compression L : Ludipress S : Lactose HC : Methocel EC : Ethocel

Preparation of granules by wet granulation for capsule NO.3

Granules containing 42 % w/w of diclofenac sodium were prepared by means of wet granulation. Fractions of drug and diluent were mixed in a Hobart mixer for 5 minutes and wet granulated using different % w/w of aqueous HPMC solution of and alcoholic EC solution as a binder. All of HPMC was used to be 20 % w/w aqueous solution as a binder in the formulation WGHCL. In contrast, the amount of HPMC in the formulation WGHCH was divided to equal as a diluent and binder. The binder was used as 18 % w/w aqueous solution. For the wet mixing process of the formulation WGEC was similar to the formulation WGHCH but HPMC was replaced to EC at the same quantity. But EC was used as 18 %w/w alcoholic solution that using 96% ethyl alcohol. The damp mass was passed through a 10 mesh sieve, then oven - dried at 60°C for 1 hour. The dried granules were passed through sieve #30 and #60 respectively. After that, magnesium stearate and Aerosil were added and mixed manually in the plastic bag until homogeneously. The obtained granules were stored in tightly light – resistant and dry containers. The granule composition is presented in Table 7.

Table 7 The composition of diclofenac sodium by wet granulation at the dose of 4.2 mg per microtablet

Formulation / Component	WGHCL (mg)	WGHCH (mg)	WGEC (mg)
Diclofenac sodium	4.2	4.2	4.2
Lactose	3.6	2.0	2.0
Methocel E4M	2.0	3.6	-
Ethocel 10 cps.	-	-	3.6
Mg Stearate	0.8	0.8	0.8
Aerosil	0.2	0.2	0.2

Solvent : deionized water for hydroxypropylmethylcellulose ; 96% ethanol for ethylcellulose
Total weight /tablet : 10 mg/tablet with a total batch size of 100 grams
WG : wet granulation HC : Methocel EC : Ethocel H : high content L : low content

Preparation of granules by wet granulation for capsule NO.2

Granules containing 30 % w/w of diclofenac sodium were prepared by means of wet granulation. Fractions of drug and diluent were mixed in a Hobart Mixer for 5 minutes. Hydroxypropylmethylcellulose and ethylcellulose were added and mixed in a Hobart mixer. Then, the solvents of both cellulose derivatives were sprayed through the nozzle on the drug-diluent mixture while continuous mixing. The damp mass was passed through a 10 mesh sieve, then oven – dried at 60°C for 1 hour. After that, the dried granules were screened through sieve #30 and #60, respectively. Magnesium stearate and Aerosil were then added and mixed manually in the plastic bag until homogeneously. The granules were stored in tightly light – resistant and dry containers. The granules composition are presented in Table 8.

Table 8 The composition of diclofenac sodium by wet granulation at the dose of 3 mg per microtablet

Formulation / Component	WHCL (mg)	WHCH (mg)	WECL (mg)	WECH (mg)
Diclofenac sodium	3.0	3.0	3.0	3.0
Lactose	3.0	2.0	3.0	2.0
Methocel E4M	3.0	4.0	-	-
Ethocel 10 cps.	-		3.0	4.0
Mg Stearate	0.8	0.8	0.8	0.8
Aerosil	0.2	0.2	0.2	0.2

Solvent : deionized water for hydroxypropylmethylcellulose ; 96% ethanol for ethylcellulose
Total weight /tablet : 10 mg/tablet with a total batch size of 100 grams
W : wet granulation HC : Methocel EC : Ethocel H : high content L : low content

3.3) Evaluation of the powder mixtures and granules

3.3.1) Moisture Determination

The moisture content of the powder mixtures and granules were determined by using a hydrogen moisture analyzer. About 5 g of samples were spread uniformly in thin layer of aluminium plate. The accurately weight was recorded. Then, it was exposed to an infrared lamp at 4 watts until constant weight was obtained. The percentage of moisture content was calculated based on the following equation.

$$\% \text{ Moisture Content} = \{(\text{wet weight} - \text{dry weight})/\text{wet weight}\} \times 100 \quad \ldots\ldots\ldots (11)$$

3.3.2) Determination of angle of repose

The angle of repose was determined by the cylinder method (Fassihi et al., 1986). An appropriate amount of powder was carefully filled into a cylinder which was placed on the graph paper until it was filled at the top of cylinder. Then the cylinder was slowly lifted in the vertical direction, thus producing around heap of powder. Each angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} H/R \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

where α, H and R are the angle of repose, height and radius of the heap respectively. The result was averaged from three determinations.

3.3.3) Determination of flow rate

Accurate weight of about 5 g of granules or the powder mixtures were filled in a glass funnel with 8 mm internal stem diameter fixed on the clamp. The time was recorded when the granules started to flow until finished. The flow rate averaged from six determinations was reported in term of g/second.

3.3.4) Bulk density, tapped density and carr's compressibility index.

The bulk and tapped densities were determined by pouring 30 g of the powder mixtures and granules into a graduated cylinder. The bulk volume was recorded and bulk density was calculated. Tapped density was performed by dropping graduated cylinder on a hard surface from height of 5 cm until a constant volume was obtained. Then, tapped volume was divided by weight to attain tapped density. Both densities were averaged from three determinations. The Carr's compressibility was calculated from the following equation.

$$\% \text{ Carr's compressibility} = \{(T-B) / T)\times 100\} \quad (13)$$

where T and B are Tapped density and Bulk density, respectively.

3.3.5) Particle size distribution

Particle size distribution was determined by sieve analysis. Approximately 50 g of powder was put on the top sieve series that the pore of sieve was ranged from 250, 180, 150, 125, and 106 μm respectively. The nest of sieve was placed on the sieve shaker for 20 minutes. The results were reported as percentage of weight retained on each of the nest of sieve.

3.3.6) The IR spectroscopy

The IR spectra of the powder mixtures and granules were examined by milling the samples with KBr at the ratio of 1 :100. Then, it was compressed with 1000 psi. pressure. It was detected with an infrared spectrophotometer in a range of wavelength of 4000-450 cm^{-1}. The resolution was 8.0.

3.3.7) The Differential scanning calorimetry (DSC)

The thermograms of the powder mixtures and granules were recorded on thermal analyzer. About 3-4 g of sample was put onto the aluminum pan. The sample was taken into the condition that had been pierced with liquid nitrogen gas. All thermal runs were examined in duplicate determinations. But they were carried out at the different condition. The first condition used the heating rate of 10°C/min and the temperature between 35° and 300°C. And, the heating rate of 20°C/min and the temperature between 35° and 400°C were used in the second condition.

3.3.8) The X-ray diffraction

The crystallinity of the powder mixtures and granules that contained various amount of cellulose derivatives and diluents were examined by X-ray diffractometry. The samples for X-ray diffraction studies were firmly packed into the cavity of a thin rectangular metal plate using two glass slides, which were fastened to the metal plate with adhesive tape. The first glass slide was then removed, and the prepared sample was taken to expose to the X – ray diffraction chamber. The X – Ray diffraction patterns were recorded from 5° – 60° in terms of 2θ angle.

3.4) Preparation of microtablets

Each formulation of the powder mixtures and granules at various dose of drug, which were 4.2 mg per microtablet and 3 mg per microtablet were mixed with magnesium stearate and Aerosil until homogeneously as shown in Tables 9 and 10. The single punch tabletting machine compressed the powder mixtures and granules with special punches and dies into microtablets. The punch station was equipped with a punch holder containing three small concave punches, each with a diameter of 2.5 mm in parallel position. The special punches and dies are shown in Figures 9.

Table 9 Composition of microtablet at the dose of 4.2 mg per microtablet

Formulation / Component	DLHC (mg)	DLEC (mg)	DSHC (mg)	DSEC (mg)	WGHCL (mg)	WGHCH (mg)	WGEC (mg)
Diclofenac Na	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Ludipress	3.0	3.0	-	-	-	-	-
Lactose	-	-	3.0	3.0	3.6	2.0	2.0
Methocel E4M	2.4	-	2.4	-	2.0	3.6	-
Ethocel 10 cps.	-	2.4	-	2.4	-	-	3.6
Mg Stearate	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Aerosil	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total/tab	10	10	10	10	10	10	10

Total weight /tablet : 10 mg/tablet with a total batch size of 100 grams
D : Direct compression L : Ludipress S : Lactose HC : Methocel EC : Ethocel

WG : wet granulation HC : Methocel EC : Ethocel H : high content L : low coctent

Table 10 Composition of microtablet at the dose of 3 mg per microtablet

Formulation / Component	DHC (mg)	DEC (mg)	WHCL (mg)	WHCH (mg)	WECL (mg)	WECH (mg)
Diclofenac Na	3.0	3.0	3.0	3.0	3.0	3.0
Ludipress	3.0	3.0	-	-	-	-
Lactose	-	-	3.0	2.0	3.0	2.0
Methocel E4M	3.0	-	3.0	4.0	-	-
Ethocel 10 cps.	-	3.0	-	-	3.0	4.0
Mg Stearate	0.8	0.8	0.8	0.8	0.8	0.8
Aerosil	0.2	0.2	0.2	0.2	0.2	0.2
Total/ tab	10	10	10	10	10	10

Total weight /tablet : 10 mg/tablet with a total batch size of 100 grams
D : Direct compression S : Lactose HC : Methocel EC : Ethocel

W : wet granulation HC : Methocel EC : Ethocel H : high content L : low coctent

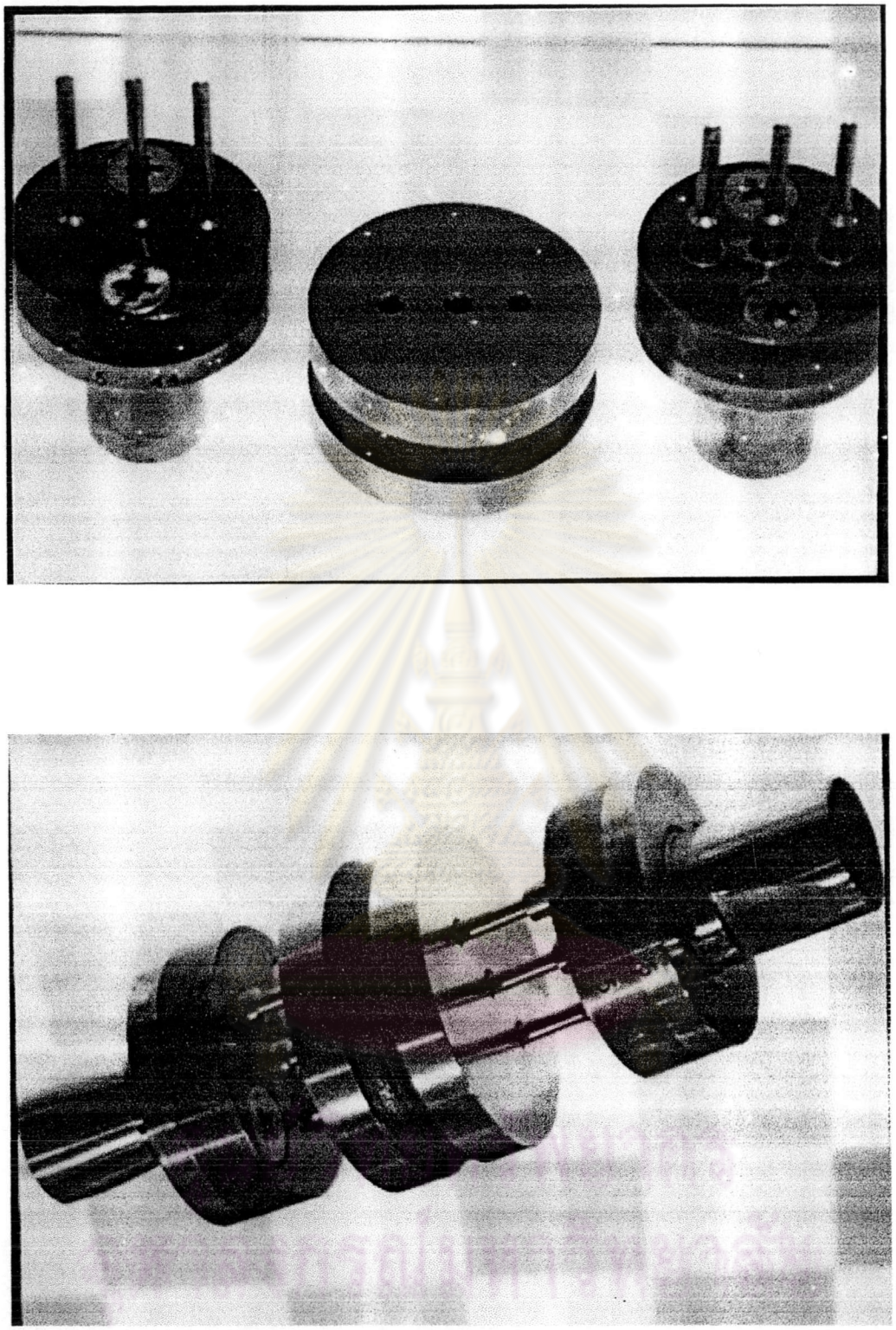

Figure 9 Special punches and dies for microtablet production

3.4.1) Evaluation of microtablets

1) Hardness

The hardness of each microtablet was measured using the Schleuniger - 2E hardness tester and the mean and standard deviation were averaged from five determinations.

2) Friability test

The friability of microtablet was determined by a friabilator. Six grams of microtablets were weighed by an analytical balance which was "w_0" Then, the weighed microtablets were poured into the friability tester. It was rotated for 5 minutes. Their microtablets were weighed again after the dust was eliminated. The second weight was "w". The percent of friability was calculated based on the following equation. The results were obtained from the average of three determinations.

$$\% \text{ Friability} = \{(w_0-w)/w_0\}\times 100 \quad \text{.....(14)}$$

3) Weight variation

The weight variation of microtablets was determined by an analytical balance. Twenty microtablets were individually weighed. The mean and standard deviation were averaged from twenty determinations.

4) Morphology

The microtablets were examined under a scanning electron microscope (SEM) for morphological evaluation. The shape and surface topography of microtablets were determined. The microtablets were also cross-sectioned for observation of the density into the microtablets. The samples were prepared by gold sputtering technique before SEM examination.

5) The X-ray diffraction

The crystallinity of microtablets were examined by X - ray diffractometry. The samples for X-ray diffraction studies were milled and were firmly packed into the cavity of a thin rectangular metal plate using two glass slides, which were fastened to the metal plate with adhesive tape. The first glass slide was then removed, and the prepared sample was taken to expose to the X – Ray diffraction chamber. The X – Ray diffraction patterns were recorded from 5° – 65° in terms of 2θ angle.

6) The IR spectroscopy

The IR spectra of the microtablets were examined by milling the samples with KBr at the ratio of 1 :100. Then, it was compressed with 1,000 psi. pressure. It was detected with an infrared spectrophotometer in a range of wavelength of 4000-450 cm^{-1}. The resolution was 8.0.

7) The differential scanning calorimetry (DSC)

The thermograms of the microtablets were recorded on a thermal analyzer. About 3-4 g of milled sample was put onto the aluminum pan. The sample was taken into the condition that had been pierced with liquid nitrogen gas. All thermal runs were examined in duplicate determinations. But they were carried out at the different condition. The first condition used the heating rate of 10°C/min and the temperature between 35° and 300°C. And, the heating rate of 20°C/min and the temperature between 35° and 400°C were used in the second condition.

8) Measurement of surface area

The surface area of DS microtablet was examined with BET method by a surface area analyzer. The samples were taken into a holder of the surface area analyzer. The moisture into the microtablets was eliminated by heating at the temperature of 100°C for 30 minutes. Then, the amount of nitrogen gas was released into the system at 5%, 12%, 18%, and 24%, respectively. The principal of surface area measurement was nitrogen gas adsorption on the surface area of testing material. Thereby, the resultant record would be shown in term m^2 per gram of DS microtablets.

9) Determination of diclofenac sodium content in microtablets

The method for determining DS content used in this study was modified from previous work (Y.K. Aghawal, V.P. Upadhyay and S.K. Menon, 1998).

The standard preparation, Approximately 20 mg of DS was accurately weighed and dissolved in 70% methanol in a 50 ml volumetric flask. Then, the solution was adjusted to volume and mixed thoroughly that used as stock solution. Three ml of the stock solution was pipetted into a 100 ml volumetric flask. The 70% methanol was added to volume and mixed. The final concentration of solution was 0.012 mg/ml.

The sample preparation, Twenty microtablets were accurately weighed together and calculated to average weight per microtablet. Then, they were milled to be powder with mortar and pestle. The powder was accurately weighed that equal to the weight of DS in the standard preparation. Then, it was dissolved with 70% methanol in a 50 ml volumetric flask and shaked with the mechanical shaker for 30 minutes. The solution was adjusted to volume and mixed thoroughly that used as stock solution. The stock solution was filtered through paper filter (Whatman® NO 1). The first one militer of filtrate was discarded. And then, 3 ml of the stock solution was pipetted into a 100 ml volumetric flask and diluted to volume with 70%

methanol. Finally, the standard and sample solutions were determined spectrophotometrically in a 1-cm cell at 280 nm. Each sample was determined in triplicate.

Calibration curve of diclofenac sodium

DS 50 mg was accurately weighed into a 100 ml volumetric flask and dissolved with methanol, then adjusted to volume. The solution was used as standard stock solution.

The 1, 2, 3, 4, and 5 ml standard stock solution was individually pipetted into a 100 ml volumetric flask and diluted to volume with methanol. The final concentration of each solution was 5.0, 10.0, 15.0, 20.0, and 25.0 μg/ml respectively.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at 280 nm. For standard solution, each concentration was determined in triplicate.

The absorbance of standard solution was determined by a UV/visible spectrophotometer in a 1-cm cell at 280 nm. The absorbance and the calibration curve of DS are shown in Table 26 and Figure 62, respectively, in Appendix A. Each concentration was determined in triplicate.

10) The uniformity of content of diclofenac sodium in microtablets

In this study, special attention for oral sustained – release dosage forms were to the uniformity of content, which affected to uniform release rate of the drug. The processes for preparing the standard preparation were similar to the processes of the determination of diclofenac sodium content. Finally, the final concentration of solution was 0.012 mg/ml.

The sample preparation, About five microtablets was taken by random sampling. Each microtablet was filled into a 50 ml volumetric flask. Then, they were dissolved with 70% methanol in a 50 ml volumetric flask and shaked with the

mechanical shaker for 30 minutes. Each solution was adjusted to 50 ml with 70% methanol used as the individual stock solution. Ten ml of the stock solution was pipetted into a 50 ml. volumetric flask. Each solution was adjusted to volume and mixed thoroughly. Finally, the standard and sample solution were determined spectrophotometrically in a 1-cm cell at 280 nm. Each sample was determined triplicate.

11) Dissolution studies of hard capsule containing diclofenac sodium microtablets

Dissolution of DS from microtablets were studied by using modified USP XXIV with basket method. In this study, a special attention was paid to the effect of pH of dissolution medium on the release of DS from microtablets. Therefore, the 2 dissolution systems, 0.1N HCl and phosphate buffer pH 6.8 system were studied.

Nine hundred milliliters 0.1N HCl or phosphate buffer pH 6.8 were placed in a glass vessel specified in the USP dissolution test. The medium was equilibrated to 37±0.5^{0}C. The amount of microtablets equivalent to 75 mg of DS was filled into one capsule and was placed in a dry basket. At the beginning of each test as, specified in the compendium. The basket was then placed at the center of the vessel and at 2.5 cm above the bottom of the vessel. The dissolution apparatus was operated at the speed of 50 rpm. Three capsules of each formulation were evaluated.

Ten milliliters of specimen in 0.1N HCl were withdrawn at the time intervals of 30, 60, 90, and 120 minutes. The same quantity of medium was replaced immediately after each sampling to keep the volume of the medium constant during the experiment.

In the dissolution model with pH change, the pH of the medium was kept by 0.1N HCl for two hours, then the pH was increased to 6.8 by transferring the basket to the another vessel which was contained with nine hundred milliliters of phosphate buffer pH 6.8.

The phosphate buffer pH 6.8 was prepared by dissolving accurate weight of 27.22 g of monobasic potassium phosphate in purified water. The solution was then adjusted to 1000 ml with purified water. Then 8.0 g of NaOH was accurately weighed and dissolved in purified water. The solution was then adjusted to 1000 ml with purified water. After that, 448 ml of NaOH solution was poured into monobasic potassium phosphate solution and mixed until homogeneously. The solution was then adjusted to 4000 ml with purified water.

Ten milliliters of specimen in phosphate buffer pH 6.8 were withdrawn at the time intervals of 30, 60, 90, 120 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 20, 21, 22, 23, and 24 hours. The same quantity of medium was replaced immediately after each sampling to keep the volume of the medium constant during the experiment.

Each sample was filtered through paper filter (Whatman® NO.1). The first one milliliters of filtrate were discarded and was diluted to suitable concentration which gave the absorbance between 0.2-0.8. The absorbance was spectrophotometrically determined in a 1-cm cell at 276.5 nm for 0.1N HCl and phosphate buffer pH 6.8.

Each amount of DS release at each time interval was calculated from the calibration absorbance – curve was made for the previously removed sample to determine the total amount of drug release.

Calibration curve of diclofenac sodium

DS 50 mg was accurately weighed and dissolved in methanol. The solution was then adjusted to 100 ml with 0.1N HCl or phosphate buffer pH 6.8 used as stock solution.

The 1, 2, 3, 4, and 5 ml stock solution was individually pipetted into a 100 ml volumetric flask and diluted to volume with 0.1N HCl or phosphate buffer pH 6.8. The final concentration of each solution was 5.0, 10.0, 15.0, 20.0, and 25.0 μg/ml, respectively.

The absorbance of known drug concentration was determined by a UV/visible spectrophotometer in a 1-cm cell at 276.5 nm. The 0.1N HCl or phosphate buffer pH 6.8 was used as blank. Each concentration was determined in three determinations.

The absorbance and calibration curve of DS in 0.1N HCl or phosphate buffer pH 6.8 are presented in Tables 27, 28 and Figures 63, 64 respectively, in Appendix A.