CHAPTER II

EXPERIMENTAL

Materials

The following materials were obtained from commercial sources. Deionized water was used throughout this study.

1. Model Drug
   - Diclofenac Sodium (Batch No. 930705 , Bright Trade Ltd., Hong Kong)

2. Additives
   - Methocel E 15 LV (Premium EP grade , Batch No. MM94110921 E , The Dow Chemical Company , USA)
     : Hydroxypropylmethylcellulose
   - Aquacoat$^{(R)}$ (Type ECD - 30 , Batch No. J 5102, FMC Corporation , USA)
     : Ethylcellulose Aqueous Dispersion
   - Chitosan (Kyowa Technos Co., Ltd., Japan supplied by G.T. Chemical ,Thailand)
   - Citric Acid Anhydrous (Supplied by Srichand United Dispensary Co., Ltd., Thailand)

3. Dissolution Medium
   - Potassium Dihydrogen Phosphate , Analytical grade (E. Merck , Germany)

- Sodium Hydroxide , Analytical grade
  (J. T. Baker Inc., USA)
- Hydrochloric Acid , Analytical grade
  (BDH Laboratory, England)

4. Solvents - Methyl Alcohol Anhydrous , Analytical grade
   (Mallinckrodt Chemical , Franch)

Equipment

Analytical balance (Sartorius , Germany)

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

Fourier transform infrared spectrometer (Model 1760 X , Perkin Elmer , USA)

Homogenizer (Model Ultra - Turrax T 50 DPX , IKA , Germany)

Magnetic stirrer (Model MR 3001 , Heidolph , Germany)

Mechanical sieve shaker (Josef Deckelmann , Aschaffenburg , Germany)

Moisture determination balance (Model 6100 H, Ohaus , USA)

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

Pneumatic pump (Model 505 S, Watson - Marlow , England)

Scanning electron microscope (Model JSM - T220A , Jeol , Japan)

Spray dryer (Mobile Minor Unit , Niro Atomizer , Denmark)

Surface area equipment (Model 2300 FC, Micromeritics , USA)

Thermal analyzer (Shimadzu , Japan)

Ultraviolet / visible spectrophotometer (Spectronic 3000 Array, Milton Roy , USA)

X-ray diffractometer (Model JDX-8030, Jeol, Japan)

Methods

A. Preparation of Spray Dried Powders

1. Formulation of Spray Dried Solution

The solid content in spray dried solution in the range of 10-15% was used. The compositions of spray dried solution employed for preliminary investigation on the effects of processing variables are presented in Table 7.

Table 7 Formulation of spray dried solution for preliminary investigation.

Ingredients	Amount of Solid
Diclofenac Sodium	% w/w
Methocel E 15 LV	% w/w
Deionized Water q.s. to	1500 ml

The amounts of ingredients used in each formulation are presented in Tables 8 to 9. The polymer to drug ratios of solution are shown in Table 10. The formulation of diclofenac sodium with both Aquacoat(R) and chitosan was not investigated in this study because Aquacoat(R) aqueous dispersion was found to be potentially incompatible with chitosan solution. There was an interaction between the amino groups of chitosan and the sulfate groups of sodium lauryl sulfate in Aquacoat(R) dispersion.

Table 8 Formulation of spray dried solution for each capsule.

Ingredients	Amount per Capsule (mg)
Diclofenac Sodium	100
Matrix Additives	% w/w*

* Percentage of polymer used in each formulation is presented in Table 9.

Table 9 The percentage of polymer in each preparation.

Formulation	% w/w Polymer		
	HPMC*	EC**	CT***
Blank	no polymer material		
1	33.33	-	-
2	20.00	-	-
3	14.29	-	-
4	-	47.37	-
5	-	32.14	-
6	-	19.57	-
7	-	14.06	-
8	-	-	27.13
9	-	-	17.55
10	-	-	12.98
11	16.36	16.36	-
12	9.89	9.89	-
13	7.09	7.09	-
14	10.61	6.82	-
15	13.24	6.62	-
16	6.57	13.14	-
17	15.00	-	15.00
18	9.38	-	9.38
19	6.82	-	6.82
20	12.77	-	6.38
21	3.53	-	7.06

* Methocel E 15 LV
** Aquacoat(R)
*** To be dissolved in 2% citric acid solution

Table 10 The polymer to drug ratios of solution.

Formulation	Polymer : Drug (w/w)				
	HPMC:DS	EC:DS	CT:DS	(HPMC:EC):DS	(HPMC:CT):DS
1	1:2	-	-	-	-
2	1:4	-	-	-	-
3	1:6	-	-	-	-
4	-	1:1	-	-	-
5	-	1:2	-	-	-
6	-	1:4	-	-	-
7	-	1:6	-	-	-
8	-	-	1:2	-	-
9	-	-	1:4	-	-
10	-	-	1:6	-	-
11	-	-	-	(1:1):4	-
12	-	-	-	(1:1):8	-
13	-	-	-	(1:1):12	-
14	-	-	-	(1.5:1):12	-
15	-	-	-	(2:1):12	-
16	-	-	-	(1:2):12	-
17	-	-	-	-	(1:1):4
18	-	-	-	-	(1:1):8
19	-	-	-	-	(1:1):12
20	-	-	-	-	(2:1):12
21	-	-	-	-	(1:2):12

2. Preliminary Studies of Spray Drying Process

The spray drying apparatus used is a laboratory type, with drying chamber of 80 cm. in diameter, 60 cm. in cylindrical height and conical base. The cone angle is 60°. The suspension was atomized into a drying chamber by rotating centrifugal wheel atomizer.

The processing variables of spray drying technique such as polymer to drug ratio of solution, inlet air temperature, feed rate and atomizing air pressure were varied according to Table 11. The outlet temperature cannot

be directly controlled, but is a function of the dryer inlet air temperature and the solution feed rate, whereas in this study a range of 70-95 °C was used.

Table 11 Parameters of spray drying process variables.

Polymer : Drug (w/w)	1 : 2	1 : 4	1 : 6
Inlet Air Temperature (^{0}C)	130	150	170
Feed Rate (ml/min)	14	20	26
Atomizing Air Pressure (bar)	2	3	4

In order to study the effect of each variable in spray drying process, it was necessary to keep the other variables constant and varied only the desired variable. To study the effect of polymer to drug ratio on physical properties, the polymer to drug ratio was varied while the temperature, feed rate and atomizing pressure were fixed. In the case of the effect of temperature, feed rate and atomizing pressure, the experiments were carried out in the same way.

2.1 Effects of Polymer to Drug Ratio of Solution

The polymer to drug ratios used were varied at 1:2 , 1:4 and 1:6 while the temperature; feed rate and pressure were kept at 150 ^{0}C, 20 ml./min., 3 bars respectively. Physical properties of spray dried powder obtained were evaluated and the polymer to drug ratio that gave the best results, i.e., maximum percentage yield, biggest size, highest and lowest angle of repose (highest flowability) , was selected to be the optimum polymer to drug ratio to be used later.

2.2 Effects of Inlet Air Temperature

Temperatures used were 130, 150 and 170 ^{0}C while feed rate was 20 ml./min.. The atomizing pressure was maintained at 3 bars, and the optimum polymer to drug ratio was from the study in 2.1. The spray dried products were evaluated in the same way as described earlier, and the optimum temperature was selected based on the same criteria.

2.3 Effects of Feed Rate

Experimental feed rates used were 14, 20 and 26 ml./min.. Pressure was set at 3 bars; the polymer to drug ratio and temperature were the same as the study in 2.2. The products were evaluated and the optimum feed rate was selected in the same way as described earlier.

2.4 Effects of Atomizing Air Pressure

Atomizing pressure at 2, 3 and 4 bars were used, along with the optimum polymer to drug ratio, optimum temperature and optimum feed rate obtained earlier. The optimum atomizing pressure was selected as previously described.

3. Preparation of Spray Dried Solution

Diclofenac sodium powder was sieved through a sieve no. 80 (180 μm.) before being used in every formulation. The procedures for preparation of spray dried solution were as follows:

3.1 Diclofenac Sodium with Methocel E 15 LV

Diclofenac sodium and hydroxypropylmethylcellulose were weighed. HPMC was dissolved in deionized water to form solution. Then diclofenac sodium was added with the aid of a homogenizer. After the suspension was mixed homogeneously, it was adjusted to volume with deionized water. The suspension was subsequently spray dried under suitable conditions.

3.2 Diclofenac sodium with Aquacoat$^{(R)}$

Diclofenac sodium and Aquacoat$^{(R)}$ were individually weighed and mixed together in a beaker. Deionized water was added to the mixture with the aid of a homogenizer. After the suspension was mixed homogeneously, it was adjusted to volume with deionized water. The suspension was subsequently spray dried under suitable conditions.

3.3 Diclofenac Sodium with Chitosan

Diclofenac sodium, chitosan and citric acid were weighed. Chitosan was dissolved in citric acid solution. Then diclofenac sodium was added with the aid of a homogenizer. After the suspension was mixed homogeneously, it was adjusted to volume with deionized water. The suspension was subscquently spray dried under suitable conditions.

3.4 Diclofenac Sodium with Methocel E 15 LV and Aquacoat$^{(R)}$

Diclofenac sodium, HPMC and Aquacoat$^{(R)}$ were weighed. HPMC was dissolved in deionized water to form solution and Aquacoat$^{(R)}$ was

mixed. Then diclofenac sodium was added with the aid of a homogenizer. After the suspension was mixed homogeneously, it was adjusted to volume with deionized water. The suspension was subsequently spray dried under suitable conditions.

3.5 Diclofenac Sodium with Methocel E 15 LV and Chitosan

Diclofenac sodium, HPMC, chitosan and citric acid were weighed. Chitosan was dissolved in citric acid solution. HPMC was dissolved in deionized water to form solution and chitosan solution was mixed. Then diclofenac sodium was added with the aid of a homogenizer. After the suspension was mixed homogeneously, it was adjusted to volume with deionized water. The suspension was subsequently spray dried under suitable conditions.

4 . Spray Drying Process

After completion of the preliminary investigation, the suitable condition of spray drying process for producing the most satisfactory spray dried powders was selected. So, only the polymer to drug ratio of each polymer was further investigated to improve drug release rate from the matrices. The polymer-diclofenac sodium suspension was spray dried under various conditions as in Table 12.

Table 12 Spray drying condition during the polymer to drug ratio variable studied .

Inlet Air Temperature (^{0} C)	170
Feed Rate (ml/min)	20
Atomizing Air Pressure (bar)	3

B. Evaluation of Physicochemical Properties of Spray Dried Powders

1. Powder Morphology

Morphology of powder samples was determined with scanning electron microscopy. The samples were coated with gold prior to the microscopic examination using ion sputtering. Size, shape and surface topography of the spray dried powders were observed.

2. Particle Size Distribution

Particle size distribution was determined by using sieve analysis. Approximately 25 g. of powder was put on the top of a sieve series ranging from 425, 250, 180, 150 to 106 μm. respectively. The nest of sieve was placed on the sieve shaker for 20 minutes. The results from two determinations were averaged and reported as percentage of weight retained on each sieve size.

3. Bulk Density Determination

The bulk density was determined from the weight of about 30 g. (accurate weight recorded), carefully transferred into a 100 ml. graduated cylinder and the bulk volume was recorded. Division of weight by bulk volume yields bulk density. The results were averaged from three determinations.

4. True Density Determination

True density was determined by using a 10 ml. pycnometer bottle. Sample of 0.5 g. was accurately weighed and transferred into a pycnometer. An amount of a low surface tension solvent, (water, hexane, acetone and

petroleum benzin) in which the powder was not soluble, was added to filled up the pycnometer, and the sample - solvent mixture was shaken. The whole bottle was accurately weighed and the true density was calculated.

5. Angle of Repose

Each angle of repose was determined by the cylinder method. An appropriate amount of powder was carefully filled into a cylinder (height = 5.2 cm., radius = 2.4 cm.) placed on the graph paper. When the powder was filled to the top of the cylinder, the cylinder was slowly lifted in a vertical direction, thus producing around heap of powder. The result was averaged from three determinations and was reported. Each angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} \frac{H}{R} \qquad (24)$$

where α is the angle of repose

H is the height of heap

R is the radius of heap.

6. Moisture Determination

The moisture content of powder was determined by using moisture determination balance. About 1 g. of sample was exposed to an IR lamp until constant weight was obtained. The percentage of moisture content was calculated automatically. Results were obtained from the average of three determinations.

7. Porosity Determination

Total pore volume of powder was determined by using surface area equipment. The method for determination was based on the American Society for Testing Material method D4567-86. Total pore volume was calculated.

8. Infrared Absorption Study

The Infrared spectra of all powders were obtained by an infrared spectrophotometer in a range of 4000-450 $cm.^{-1}$.

9. Powder X-ray Diffraction Study

The crystallinity of diclofenac sodium in the spray dried powder was examined by X-ray diffractometry. The samples for X-ray diffraction studies were firmly packed into a cavity of a thin rectangular metal plate using two glass slides attached 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 5^0 to 65^0 in terms of 2 θ angle.

10. The Differential Scanning Calorimetry Study

Thermal analysis is the most common approach to study physicochemical interactions of two or more component systems. Several modified techniques utilizing the principle of change in thermal energy as a function of temperature are: cooling curve method, thawment method, thermomicroscopic method, differential thermal analysis (DTA), differential scanning calorimetry (DSC).

DSC is an effective thermal method to study the equilibrium phase of either a pure compound or a mixture. Different effects, associated with physical or chemical changes, are registered as a function of temperature or time as the substance is heated at a uniform rate.

The thermograms of spray dried powders in different ratios of polymer and diclofenac sodium were recorded on thermal analyzer. All thermal runs were carried out at a heating rate of 10^0C/min and the temperature between 35^0C and 300^0C.

11. Determination of Diclofenac Sodium Content of Spray Dried Powder

A portion of powder samples equivalent to 100 mg. of diclofenac sodium was accurately weighed and transferred into a 100 ml. volumetric flask. The powder was dissolved in methanol and the solution was adjusted to volume. Then, it was mixed and filtered through Whatman(R) filter paper no. 1. The stock solution (1 ml.) was individually pipetted to a 50 ml. volumetric flask. Methanol was added to volume and the solution was mixed. The absorbance of the solution was determined by a UV/visible spectrophotometer in 1 cm. cell at 283 nm., using methanol as the blank. This absorbance was compared with that of standard solution of diclofenac sodium at comparable concentrations in methanol. Percentage of drug content was calculated accordingly. Each sample was determined in triplicates.

C. Matrix Evaluation

1. Dissolution Study

Oral controlled release tablets are supposed to pass the entire upper gastrointestinal tract, it would be ideal when the release of drug was constant over a wide range of pH values (from 1 to about 7). Therefore, an in vitro test for controlled release tablets should at least cover at this pH range. For this reason, pH change method was used in this study (Jonkman , Berg and De Zeeuw , 1983).

In the dissolution model with pH range, pH of the medium was kept at 0.1 N. HCl for two hours. Then pH was increased to 6.8 by adding 4.4064 g. of NaOH and 6.125 g. of $KH_2 PO_4$ dissolved in a few ml. of 0.1 N. HCl. All fluid was boiled to deaerate before used.

Nine hundred millilitres of medium was placed in a glass vessel specified in the USP dissolution test and equilibrated at 37 ± 0.5 ^{0}C. One capsule containing 100 mg. of diclofenac sodium 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. Six capsules were evaluated.

Five millilitres of specimen was withdrawn at the time intervals of 1, 2, 3, 4, 6, 9, 12, 15, 18 and 24 hours. The same quantity of medium was replaced immediately after each sampling to keep the volume of the medium constant through the experiment.

Each sample was diluted to a suitable concentration. The absorbance was spectrophotometrically determined in a 1 cm. cell at 275 nm. for 0.1 N. HCl and 277 nm. for phosphate buffer pH 6.8.

Each amount of diclofenac sodium released at each time interval was calculated from the calibration absorbance-curve. A cumulative correction was made for the previously removed sample to determine the total amount of drug released.

2. Calibration Curve of Diclofenac Sodium

2.1 In Acidic Solution

Diclofenac Sodium 50 mg. was accurately weighed and transferred into a 100 ml. volumetric flask. The drug was dissolved in methanol; the solution was adjusted to volume, and was used as stock solution.

The stock solution was individually pipetted (1, 2, 3, 4, and 5 ml.) into a 100 ml. volumetric flask and diluted to volume with 0.1 N.HCl. The final concentration of each solution was 5, 10, 15, 20, or 25 μg./ml. accordingly.

The absorbance of known drug concentration was determined by a UV/visible spectrophotometer in a 1 cm. cell at about 275 nm. The 0.1 N.HCl was used as blank . Each concentration was determined in three determinations.

2.2 In Buffer Solution

Diclofenac sodium 25 mg. was accurately weighed and transferred into a 100 ml. volumetric flask. The drug was dissolved in phosphate buffer pH 6.8; the solution was adjusted to volume, and used as stock solution.

The stock solution was individually pipetted (1, 2, 3, 4 and 5 ml.) into a 100 ml. volumetric flask, and diluted to volume with phosphate buffer pH 6.8. The final concentration of each solution was 2.5, 5, 7.5, 10 or 12.5 μg./ml. accordingly.

The absorbance of known drug concentration was determined by a UV/visible spectrophotometer in a 1 cm. cell at about 277 nm. The phosphate buffer was used as blank. Each concentration was determined in three determinations.