CHAPTER II

EXPERIMENTAL

Materials

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

1. Model drug   - Diclofenac sodium (Lot.No. DCS 0022-1

Yung Zip Chemical Ind. Co., Ltd., Taiwan)

2. Additives   - Poly (ethylacrylate methylmethacrylate)

: Eudragit® NE 30 D (Lot. No. 1260112014

Rohm Pharma, Germany)

- Ammonio methacrylate copolymer type A

: Eudragit® RL 30 D (Lot. No. 0450316165

Rohm Pharma, Germany)

- Ammonio methacrylate copolymer type B

: Eudragit® RS 30 D (Lot. No. 00450918221

Rohm Pharma, Germany)

- Colloidal silica

: Aerosil® 200 (Wacker Chemie GMBH, Germany)

- Dibutyl phthalate (Electron Microscopy Sciences, USA)

3. Dissolution medium - Potassium dihydrogen phosphate, AR grade

(E.Merck, Germany)

- Sodium hydroxide, AR grade (J.T. Baker Inc., USA)

- Hydrochloric acid, AR grade (BDH Laboratory, England)

4. Solvent   - Methyl alcohol anhydrous, AR grade
                                   (Mallinckrodt Chemical, Franch)

<u>Equipment</u>

Analytical balance (Model A200S, 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 (Model PHI 50, Beckman, USA)

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

Scanning electron microscope (Model JSM-5410 LV, Jeol, Japan)

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

Surface area determination equipment (Model Flowsorb 2300 FC, Micromeritics Instument Corporation, USA)

Thermal analyzer (Model NETZSCH DSC 200, NETZSCH-Geratebau GmbH, Germany)

Ultraviolet / Visible spectrophotometer (Model UV-160 A, Shimadzu, Japan)

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

Method

1. Preparation of Spray Dried Powders

1.1 Formulation of spray dried solution

The aqueous solubility of diclofenac sodium in deinonized water was higher than 9 mg/ml. However, it could not be completely dissolved in the dispersing medium (deionized water), thus the feed was a suspension feed.

In preliminary study, Eudragit® NE 30D was used. The amount used was varied by trial and error ranged from 0-20 %w/w. The total solid content (polymer and drug) of the suspension feed was kept approximately 10 %w/w. The amount of aerosil was varied in the range of 0-30 %w/w of polymer. The composition of spray dried suspension feed for preliminary study on the effect of formulation variables is represented in Table 2.

Table 2   Formulation of spray dried suspension feed for preliminary study.

Ingredients	Amount of Solid
Diclofenac Sodium	80-100 % w/w
Eudragit® NE 30 D	0-20 % w/w*
Aerosil	0-30 % w/w**
Deionized Water q.s. to	1500 ml

    *   Percent of polymer used in dry substance
    **   Percent by weight of polymer

From the preliminary study, it was found that the amount of polymer should not be more than 20 % w/w because most spray dried powders adhered to the walls of the drying chamber and cyclone collector, which was hardly harvested. The addition of aerosil into the formulation could decrease the adhesiveness of the obtained particles. By visual observation, the flow property of the formulation

contained aerosil was better than that without aerosil. The optimal amount of aerosil was 30 % by weight of polymer.

After preliminary experiments, the amount of ingredients used in each formulation are presented in Table 3 to 4.

Table 3   Formulation of spray dried suspension feed for each capsule.

Ingredients	Amount per capsule (mg)
Diclofenac Sodium	100
Polymer Material	5-20 % w/w *
Aerosil	30 % w/w **
Dibutyl Phthalate	10 % w/w ***

   * Percent of polymer used (calculated on dry polymer basis) in each preparation is presented in Table 4
  ** Percent by weight of polymer
 *** Percent by weight of polymer, used as plasticizer, as required for Eudragit® RL 30 D and Eudragit® RS 30 D, but not for Eudragit® NE 30 D

Table 4   The percentage of polymer material in each preparation.

Formulation	Inlet Air Temperature					
	150°C	170°C	190°C	210°C		
	% w/w polymer					
	NE	NE	NE	NE	RS	RL
Blank	no polymer material					
1	10					
2	8.33					
3	6.25					
4		20				
5		14.29				
6		10				
7		8.33				
8		6.25				
9			10			
10			8.33			
11			6.25			
12				10		
13				8.33		
14				6.25		
15					8.33	
16						8.33
17					4.16	4.16

1.2 Preparation of Spray Dried Solution

Diclofenac sodium powder was sieved through a sieve no. 80 (180 μm.) prior to preparation. The spray dried solution was prepared as follows :

Diclofenac sodium, polymer material and Aerosil® were individually weighed. Diclofenac sodium and Aerosil® were separately dispersed in deionized water and mixed together. The dispersion was then homogenized with the aid of a homogenizer. After the dispersion was homogeneously mixed, the polymer or dibutyl phthalate-added polymer was added and mixed slowly under gentle stirring. The resulting dispersion was adjusted to volume by deionized water and subsequently sprayed into the chamber of spray dryer under suitable condition. Because it was a suspension feed, thus gentle stirring had to be performed throughout spray drying process.

2. Spray Drying Process

The spray dryer used was a laboratory type, having drying chamber of 80 cm. in diameter, 60 cm. in cylindrical height and conical based. The cone angle was 60°. The solution was atomized into a drying chamber by a rotating centrifugal atomizer.

In the previous study (Pearnchob, N., 1996), it was found that the optimal spray drying conditions for diclofenac sodium with HPMC, EC or chitosan spray-dried powders were : the drying inlet air temperature of 170°C, the feed rate of 20 ml/min and the atomizing air pressure of 3 bars, which showed the most satisfactory physical properties of spray dried powder. Therefore, in this preliminary study, the aforementioned conditions were used. The spray drying conditions for preliminary study were fixed as follow :

- Inlet Air Temperature : 170°C

- Atomizing Air Pressure : 3 bars

- Feed Rate : 20 ml/min

- Outlet Air Temperature : 85-95°C

From these spray drying conditions, unsatisfactory shape and surface topography of particles were obtained. The particles were not spherical and seemed to partially collapsed.

The processing variables which could be regulated were the atomizing pressure, the rate of feed solution and the inlet air temperature. Whereas, the outlet air temperature could not be directly controlled. It is depended on the inlet air temperature and the rate of feed solution.

In this study, the inlet air temperatures were varied between 150-210°C to determine the optimum condition for the spray drying of diclofenac sodium with polymer material. The spray drying conditions used in preparation of controlled released diclofenac sodium with polymer material are shown in Table 5.

Table 5   The spray drying conditions used in preparation of controlled released diclofenac sodium with polymer material.

Conditions	Formulation			
	1-3	4-8	9-11	12-17
Inlet Air Temperature (^{o}C)	150	170	190	210
Feed Rate (ml/min)	20			
Atomizing Air Pressure (bar)	3			
Outlet Air Temperature (^{o}C)	85-105			

3. Evaluation of Physicochemical Properties of Spray Dried Powders

3.1 Powder Morphology

The morphology of powder samples was examined by using 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.

3.2 Particle Size Distribution

Particle size distribution was determined by using sieve analysis. Approximately 25 g of powder was accurately weighed and put on the top of sieve series ranging from 425, 250, 180, 150 to 106 μm respectively. The sieves were 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.

3.3 Angle of Repose

Each angle of repose was determined by the cylinder method. An appropriate amount of powder was carefully filled into a cylinder, which was 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. Each angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} \frac{H}{R} \tag{18}$$

where α is the angle of repose, H is the height of heap, R is the radius of heap

3.4 Moisture Determination

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

3.5 Porosity Determination

The specific surface area and the total pore volume of powder were determined by BET adsorption method using a surface area analytical equipment. The specific surface area and the total pore volume were calculated automatically.

3.6 The Infrared Spectroscopy

The IR spectra of all powders were recorded using a KBr disc method by an infrared spectrophotometer in a range of 4000-450 cm^{-1}.

3.7 The Powder X-ray Diffraction Analysis

The crystallinity of diclofenac sodium in the spray dried powders 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 expose to the X-ray beam in the X-ray powder diffraction chamber. The X-ray diffraction patterns were recorded at the rate of $60°$ per minute from $5°$ to $80°$ in the term of 2θ angle.

3.8 The Differential Scanning Calorimetry

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

3.9 Determination of Diclofenac Sodium Content of Spray Dried Powder

3.9.1 Calibration curve of diclofenac sodium content

Diclofenac sodium 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 standard stock solution of 1, 2 ,3 ,4 and 5 ml was individually pipetted into 100 ml volumetric flasks and then diluted to volume with methanol. The final concentration of each standard solution was 5, 10, 15, 20 and 25 μg/ml, respectively.

The absorbance of standard solution was determined by a UV/visible spectrophotometer at 283 nm. The absorbance and the calibration curve of diclofenac sodium are shown in Table 14 and Figure 68, respectively, in Appendix A. Each concentration was determined in triplicate.

3.9.2 Assay of diclofenac sodium content in spray dried powder

The spray dried powder approximately 100 mg was accurately weighed and dissolved with methanol in a 100 ml volumetric flask, then the solution was adjusted to volume and mixed thoroughly. The solution was filtered through a Whatman® filter paper no.1 and used as stock solution. One ml of this stock solution was pipetted

and transfered into a 50 ml volumetric flask. Methanol was added to volume and mixed. Finally, the solution was determined spectrophotometrically at 283 nm. Diclofenac sodium content was calculated from calibration curve of diclofenac sodium in methanol. Each formulation was determined in triplicate.

4. Spray Dried Powder Evaluation

4.1 Dissolution Studies

The aqueous solubility of diclofenac sodium is dependent on pH; solubility is poor at low values of pH but when the pH rises above the pKa (pKa in water is 4), rapid increases in solubility occur (Lund, 1994). For drugs that exhibit pH-dependent solubility and dissolution behaviours, dissolution screening at various pH media should be performed (Khan, 1996)

As controlled release tablets were 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 this pH range (Jonkman, Berg and De Zeeuw, 1983)

In this study, a special attention was paid to the effect of pH of dissolution medium on the release of diclofenac sodium from spray dried products, therefore, the 2 dissolution systems, pH-change and phosphate buffer pH 6.8 system were studied.

Spray dried product equivalent to 100 mg of diclofenac sodium was filled into a capsule. Three capsules for each formulation were evaluated.

Nine hundred milliliters of 0.1 N HCl (pH change system) or one thousand milliliters of phosphate buffer pH 6.8 (phosphate buffer pH 6.8 system) was placed in a glass vessel specified in the USP dissolution test, using apparatus I. The medium was equilibrated to 37±0.5°C. One capsule was placed in a dry basket, specified in the compendium, and immersed in the medium at the center of the vessel and at 2.5 cm above the bottom of the vessel. The apparatus was operated at a speed of 50 rpm.

In the dissolution model with pH change, the pH of the medium was kept at pH 1.2 using 0.1 N HCl for two hours, then the pH was increased to 6.8 by adding 4.4064 g of NaOH followed by 6.125 g of KH_2PO_4 dissolved in a few milliliters of 0.1 N HCl. All fluids were deaerated before use by boiling.

At the time interval of 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 18 and 24 hours, ten milliliters of the specimen was withdrawn and the medium was added immediately in the same quantity after each sampling to keep the volume of the medium constant during the experiment.

The absorbance of each sample was spectrophotometrically assayed at 274 nm for 0.1 N HCl and 276 nm for phosphate buffer pH 6.8. The sample was diluted to a suitable concentration, if necessary.

The amout of diclofenac sodium release at any time interval was caluculated from the calibration curve. A cumulative correction was made for the previously removed sample to determine the total amount of drug release.

4.2 Calibration curve of diclofenac sodium

4.2.1 In 0.1 N HCl solution

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

The stock solution of 1, 2, 3, 4 and 5 ml was individualy pipetted into 100 ml volumetric flask and then diluted to volume with 0.1 N. HCl. The final concentration of each solution was 5, 10, 15, 20 and 25 µg/ml, respectively.

The solution was assayed spectrophotometrically at 274 nm. The absorbance and the calibration curve of diclofenac sodium in 0.1 N. HCl are shown in Table 15 and Figure 69, respectively, in the Appendix A. Each concentration was determined in triplicate.

4.2.2 In phosphate buffer pH 6.8 solution

Diclofenac sodium 50 mg was accurately weighted into a 100 ml volumetric flask and dissolved with phosphate buffer pH 6.8, then adjusted to volume. The solution was used as stock solution.

The stock solution of 1, 2, 3, 4 and 5 ml was individualy pipetted into 100 ml volumetric flasks and then diluted to volume with phosphate buffer pH 6.8. The final concentration of each solution was 2.5, 5, 7.5, 10 and 12.5 µg/ml, respectively.

The solution was assayed spectrophotometrically at 276 nm. The absorbance and the calibration curve of diclofenac sodium in phosphate buffer pH 6.8 are shown in Table 16 and Figure 70, respectively, in Appendix A. Each concentration was determined in triplicate.