CHAPTER II

EXPERIMENTAL

Materials

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

1. Model Drug –Diclofenac sodium (Bromine free) (Lot.No.DS 0010 / 230 Yung Zip Chemical Ind. Co.,Ltd., Taiwan)

2. Additives
   - Ammonio methacrylate copolymer type A and sodium carboxymethylcellulose
     : Eudragit ® RD 100 (Lot.No. 0400456061 Rohm Pharma, Germany)
   - Ammonio methacrylate copolymer type A
     : Eudragit ® RL 30 D (Lot.No. 0481016192 Rohm Pharma, Germany)
   - Colloidal silica
     : Aerosil ®200 (Wacker Chemie GMBH Germany)
   - Nymcel
     : Sodium carboxymethylcellulose (Lot.No. E 3103/294, Germany)

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, France)

Equipments

- Analytical balance (Model A200S, Sartorious, Germany)
- Dissolution apparatus (Model SR-2, Hanson Research, USA)
- Fourier transform infrared spectrometer (Model 1760X, 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)
- Mac-Lab and MacIntosh (Model LC 4758/160, Singapore)
- pH meter (Model PHI 50, Beckman , USA)
- Pneumatic pump (Model 505 S , Watson - Marlow, England)
- Rheology Viscometer (Model RI:2:M-H, Rheology (International) Shannon Ltd., Ireland)
- Scanning electron microscope (Model JSM-5410 LV, Joel, Japan)
- Spray dryer (Mobile Minor Unit, Niro Atomizer, Denmark)
- Surface area determination equipment (Model Flowsorb 230 FC, Micrometrics Instrument Corporation, USA.)
- Thermal analyzer (Model NETZSCH DSC 200 , NETZSCH – Geratebau GmbH, Germany)
- Ultraviolet / visible spectrophotometer (Model UV –160A, Shimadzu, Japan)
- X-ray diffractometer (Model JDX – 8030, Jeol, Japan)

Methods

A. Preparation of Spray Dried Powder

1. Formulation of Spray Dried Suspension

1.1 Preliminary study

The aqueous solubility of diclofenac sodium in deionized water was reported to be higher than 9 mg/ml (Adeyeye and Li, 1990). However, it could not be completely dissolved in the dispersing medium (deionized water) in presence of polymers due to the polymers needed the deionized water to dissolve so the amount of the deionized water was not sufficient to dissolve the drug. Thus, the feed used was in a suspension form. Eudragit®RD100, Eudragit®RL 30D and NaCMC were used as polymeric matrices; the amount used was varied by trial and error ranging from 1-5 % w/w. The total solid content (polymer and drug) of the suspension feed was kept approximately 10% w/w. The compositions of spray dried suspension are presented in Table 8.

From the preliminary study (Formulation A-J), it was found that the amount of polymer should not be more than 4 % w/w. Formulation I and J could not be sprayed because the suspension was too viscous. Formulation A - H could be sprayed and gave satisfactory production yield. In these formulations, the suspension was not too viscous to be sprayed therefore obstruction in the spray nozzle did not occur. The adherence of the powder to the spray drying chamber was also lower. Most spray dried powders adhered to the walls of the drying chamber and cyclone collector which were hard t harvest.

Table 8 Formulations of spray dried suspension feed for preliminary study.

Formulation	Diclofenac Sodium [1] (%w/w)	Eudragit® RD 100 [2] (%w/w)	Eudragit ®RL30D and NaCMC [3] (%w/w)	DI water qs.to (g)
A	9	1	-	1000
B	9	-	1	1000
C	8	2	-	1000
D	8	-	2	1000
E	7	3	-	1000
F	7	-	3	1000
G	6	4	-	1000
H	6	-	4	1000
I	5	5	-	1000
J	5	-	5	1000

[1+ 2 and 1+ 3] The total solid content (polymer and drug) of suspension feed.

[3] A powder combination of 91 parts of Eudragit®RL100 and 9 parts of NaCMC, Eudragit® RL30D calculated on dry basis was used instead of Eudragit ® RL100.

1.2 Preparation of diclofenac sodium spray dried powder

The solid content in the feed suspension of each formulation was in the range of 10%w/w . Approximately 0-4 % w/w of Eudragit ® RD100 or Eudragit ® RL30D and NaCMC was used as polymer matrices. The amount of Aerosil ® 200 was varied in the range of 0-30% w/w of the content of drug and polymer in the formulation.

The amount of ingredients used in each formulations depended on the ratio of drug to polymer and the amount of Aerosil ®200 used. The compositions are presented in Table 9.

Table 9 Composition of suspension feed for spray dried powder.

Formulation	Diclofenac sodium (g)	Eudragit® RD 100 (g)	Eudragit® RL30D * (g)	NaCMC (g)	Aerosil 200 (g)	DI water qs.to (g)
1	100	-	-	-	-	1000
2	100	-	-	-	-	1000
3	90	10	-	-	-	1000
4	90	-	9.1	0.9	-	1000
5	80	20	-	-	-	1000
6	80	-	18.2	1.8	-	1000
7	70	30	-	-	-	1000
8	70	-	27.3	2.7	-	1000
9	60	40	-	-	-	1000
10	60	-	36.4	3.6	-	1000
11	60	40	-	-	15	1000
12	60	-	36.4	3.6	15	1000
13	60	40	-	-	30	1000
14	60	40	-	-	30	1000
15	60	40	-	-	30	1000
16	60	-	40.0	-		1000

* In this study Eudragit® RL 30D calculated on dry basis was used instead to Eudragit®RL 100.

2. Preparation of Spray Dried Suspension

Diclofenac sodium powder was sieved through a sieve No. 80 (180 micrometers opening) before used in every formulations. The medium for the spray dried suspension was deionized water. The procedures for preparation of spray dried suspension were as followed:

2.1 Diclofenac sodium with Eudragit ® RD100

Diclofenac sodium and Eudragit®RD100 and Aerosil®200 were individually weighed and separately dispersed in deionized water and then mixed together. The dispersion was then homogenized with an aid of a homogenizer. After the dispersion was homogeneously mixed, the resulting dispersion was adjusted to volume by deionized water.

2.2 Diclofenac sodium with Eudragit ®RL30D and NaCMC

Diclofenac sodium, Eudragit®RL 30 D and NaCMC, Aerosil®200 were individually weighed. Diclofenac sodium and Eudragit®RL 30 D were mixed together in a beaker. Aerosil®200 was seperately dispersed in deionized water, then were added to the polymeric drug mixture and mixed together. NaCMC was dissolved in deionized water to form solution and subsequently added together. The dispersion was then homogenized with the aid of a homogenizer. After the dispersion was homogeneously mixed. The resulting dispersion was adjusted to volume by deionized water.

3. Spray Drying Process

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

3.1 Preliminary study

The conditions of spray drying process in Table 10 were varied and adjusted to obtain the satisfactory physical properties of spray dried powder such as maximum percentage yield and good flowabilities.

Table 10 Parameters of spray drying process for preliminary study.

Parameter	Condition
Inlet temperature	130° C
Outlet temparature	75-90° C
Feed Rate (rpm)	12*
Atomizing pressure	2 bar

*12 rpm = 20 ml/min

Table 11 The spray drying conditions and polymer to drug ratio used through the study.

Formulation	Polymer: Drug Ratio	Inlet Air Temp. (°C)	Feed Rate (rpm)	Atomizing Pressure (bar)	Total Vol. (g)
1	0:1	130	12	2	1000
2	0:1	160	12	2	1000
3	RD1:9	130	12	2	1000
4	RL+NaCMC1:9	130	12	2	1000
5	RD1:4	130	12	2	1000
6	RL+NaCMC1:4	130	12	2	1000
7	RD 1:2.33	130	12	2	1000
8	RL+NaCMC1:2.33	130	12	2	1000
9	RD 1:1.5	130	12	2	1000
10	RL+NaCMC 1:1.5	130	12	2	1000
11*	RD 1:1.5	130	12	2	1000
12*	RL 1:1.5	130	12	2	1000
13**	RD 1:1.5	130	12	2	1000
14**	RD 1:1.5	160	12 [1]	2	1000
15**	RD 1:1.5	160	9 [2]	2	1000
16***	RL 1:1.5	130	12	2	1000

* Formulation 11-12 Aerosil200 =15% w/w of content of drug and polymer.

** Formulation 13-15 Aerosil200 =30% w/w of content of drug and polymer.

*** Formulation 16 = no NaCMC in the formulation [1] = 20 ml/min, [2] = 15 ml/min

From the preliminary study the formulation was adapted and the condition of process for spray drying was modified. The processing variables of spray drying technique such as polymer to drug ratio of suspension, inlet temperature and feed rate were varied according to Table 11. The outlet temperature could not be directly controlled. It was depended on the inlet air temperature and feed rate of suspension.

In order to study the effect of polymer to drug ratio on physical properties, the polymer to drug ratio was varied while the inlet temperature, feed rate and atomizing pressure were fixed. In the case to study the effect of feed rate or inlet temperature, other parameters were fixed.

3.2 Effect of polymer to drug ratio of suspension

The polymer to drug ratios used were varied at 1:9, 1:4, 1:2.33, 1:1.5 while the inlet temperature, feed rate and atomizing pressure were kept at 130°C, 20 ml/min and 2 bars respectively. Physical properties of spray dried powder obtained were evaluated for the optimum polymer to drug ratio that gave the best results such as maximum percentage yield, spherical and smooth surface, ease to spray because the suspension did not have viscous property.

3.3 Effect of inlet temperature

The inlet temperature used were 130°C and 160°C while feed rate was 20 ml/min and the atomizing pressure was maintained at 2 bars. The optimum polymer to drug ratio used was from the result of 3.1. The physicochemical properties of the obtained powders were evaluated similarly as in 3.1.

3.4 Effect of feed rate

Feed rate used were 20 and 15 ml /min while the inlet temperature used was 160°C and the atomizing pressure was maintained at 2 bars. The optimum

polymer to drug ratio used was from the result of 3.2. The physicochemical properties of the obtained powders were evaluated similarly as in 3.2.

B. Evaluation of Physicochemical Properties of Spray Dried Powders

1. Powder Morphology

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

2. Particle Size Distribution

Size distributions of granules were determined by using sieve analysis. Approximately 25 gm of granules were accurately weighed and put on the top of sieve series ranging from sieve No. 40, 60, 80, 100 to 120 , respectively (425, 250, 180, 150 to 106 μm). The sieves were placed on the sieve shaker for 20 minutes. The results were calculated in percentage of granules retained on each sieve size.

3. Flow Rate

The flow rate of spray dried powder was determined using a Mc- Lab and MacIntosh with funnel and clamp . About 5 g of sample powder was accurately weighed and filled in the funnel (internal diameter = 1.5 cm) which was placed on clamp. The height from the tip of funnel to surface of a cup on top of a paper that connected to a transducer of Mc- Lab was about 5 cm. The powder was filled to the top of funnel. The tip was closed by coverglass. The coverglass was then taken out of the tip of funnel, thus a round heap of powder was produced on the cup. The slope of the curve presented the flow rate. The time (x axis) and the weight (y axis) were recorded by the Mc-Lab computer until the powder passed the tip completely. The weight was recorded in grams and the time was recorded in seconds. Therefore the

unit of flow rate by Mc- Lab was recorded by gram per second (gram / sec). The results were averaged from six determinations.

4. Angle of Repose

The angle of repose was determined by cylinder method. Adequate amount of powder was weighed and fill into the cylinder (height =5.2 cm, radius = 2.4 cm) which was placed on a graph paper. After filling to the top of the cylinder, the cylinder was slowly turned upside down and lifted in the vertical direction thus produced a round heap of powder. The height and the radius of the heap powder on the paper graph were recorded in centimeters. The results were averaged from three determinations. Each angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} H/R$$

where α was the angle of repose, H was the height of the heap and R was the radius of the heap.

5. Bulk, Tapped Density and Percent Compressibility

Approximately 30 gm of the spray dried powder was accurately weighed and carefully transferred into a 100 ml graduate cylinder, then the bulk volume was recorded. Division of weight by bulk volume presented the bulk density.

$$\text{Bulk Density (g/ml)} = \frac{\text{Weight of the powder}}{\text{Bulk volume}} \quad (1)$$

The tapped density was performed by dropping graduated cylinder filled with powder on a hard surface from a 5 cm height, until the volume was stable. Division of the weight by this constant volume presented the tapped density.

$$\text{Tapped Density (g/ml)} = \frac{\text{Weight of the powder}}{\text{Volume after tapped}} \quad (2)$$

The percent compressibility of the powder was calculated from bulk and tapped densities by the following equation.

$$\text{Percent Compressibility} = \frac{(T - B)}{T} \times 100 \quad (3)$$

B and T were bulk and tapped density, respectively. Both densities and percent compressibility were averaged from three determinations.

6. Viscosity

The viscosity of the suspension feed was measured by a viscometer at room temperature. The instrument was calibrated by the sample with known viscosity (i.e., sodium carboxymethylcellulose). Each sample was measured using ASTM spindle No.1 for 30 seconds and the triplicate observations of each sample were measured.

7. 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.

8. The Infrared Spectroscopy

Infrared spectroscopy was used to study the change in the functional groups of substances and products after spray drying process by observing the positions and intensities of IR peaks.

The infrared spectroscopy of all powders was recorded using a KBr disc method. The dried sample was mixed with potassium bromide in agate mortar and pestle by geometric dilution technique. The mixture was then transferred to a hydraulic press to a thin disc. The KBr disc was then measured within the wave numbers of 4000-400 cm^{-1}.

9. The Powder X- Ray Diffraction Analysis

The X – ray diffractometry was used to determine the diffraction angles of the substances which showed crystallinity and interplanar spacing of the crystal planes and compared with the products after interactions with the components from spray dried process.

The samples for X- ray diffractions studies were firmly packed into a cavity of a thin rectangular metal plate using two glass slides attached to metal plate with adhesive tapes. The first glass slide was then removed, and the prepared sample was taken to expose to the X- ray diffraction radiation. The X- ray diffraction patterns were recorded from 5 to 60 in terms of 2θ angle.

10. The Differential Scanning Calorimetry Study

Thermal analysis is the most common approach to study physicochemical interactions of two or more component systems.

- The Differential Scanning Calorimetry

The thermograms of the spray dried powders prepared from various formulations were examined by differential scanning calorimeter (DSC). The differences in thermal energy patterns between the original substances and their products were evaluated before and after spray drying process.

Approximately 3-5 mg of spray dried powder was accurately weighed into the DSC pan . Then it was crimped with the sealed pan and immediately made a few holes for determinations. The pan filled with spray dried powder was placed in the equipment beside the reference pan made by the same method except without powder. All thermal runs were controlled at a heating rate of 10° C per minute and in the range of 35 °C to 300 °C.

11. Determination of Diclofenac Sodium Content of Spray Dried Powders

11.1 Calibration curve of diclofenac sodium content

Standard diclofenac sodium of 50 mg was accurately weighed into 100 ml volumetric flask through the aid of a glass funnel. The powder was rinsed off the funnel by absolute methanol. The final concentrations of the obtained standard solutions were 5, 10, 15, 20 and 25 μg / ml, respectively.

The absorbances of the standard solutions were determined by a UV spectrophotometer at 283 nm with absolute methanol as a blank reference (Christainah, 1990). The absorbance and the calibration curve of diclofenac sodium are presented in Table 22 and Figure 69, respectively. The result of each concentration of the standard solution was averaged from three determinations.

11.2 Assay of diclofenac sodium content in spray dried powder.

The spray dried powder equivalent to drug of 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 transferred into a 50 ml volumetric flask. Methanol was added to volume and mixed. Finally, the solution was determined spectrophotometrically at 283 nm (Christainah, 1990). Diclofenac sodium content was calculated from calibration curve of diclofenac sodium in methanol. The result of each formulation of the spray dried powder was averaged from three determinations.

C. Spray Dried Powder Evaluation

1. Dissolution Studies

In this study, the pH change dissolution system was performed. This

system was specified in the delay release dissolution test, in the USP dissolution test (modified USP 24) by using apparatus I.

In this dissolution model with pH change, pH of the medium was kept at 0.1 N. HCL (pH 1.2) for two hours. Then pH was increased to 6.8 by adding 4.4064 g of NaOH and 6.125 g of KH_2PO_4 which were dissolved in 100 ml of 0.1 N HCl in a beaker and added up into dissolution flask. All fluid was boiled to deaerate before used.

Nine hundred milliliters of medium was placed in a glasss vessel specified in the USP dissolution test and equilibrated at 37 ± 0.5 ^{0}C. Spray dried product equivalent to 100 mg of diclofenac sodium was filled into a capsule. Three capsules of each formulation were evaluated. Each capsule was placed in a basket 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 were evaluated. In this experiment Voltaren SR tablet 100 mg was used as commercial products.

At the time interval of 1, 2, 3, 6, 9, 12, 15, 18, 24 hours, ten milliliters of the specimen were withdrawn and the medium was added immediately in the same quantity after the sampling to keep the volume of the medium constant during the experiment .

Each sample was diluted to a suitable concentration if necessary. 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.

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

2. Calibration Curve of Diclofenac Sodium

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 a stock solution.

The stock solutions of 1 , 2 , 3 , 4 and 5 ml were individually 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 absorbance of known drug concentration was determined by a UV/visible spectrophotometer in a 1 cm cell at 275 nm. The 0.1 N HCl was used as a reference solution. Each concentration was determined in three determinations. The calibration curve of diclofenac sodium in 0.1 N HCl are shown in Table 23 and Figure 70, respectively.

2.2 In phosphate buffer of pH 6.8 solution

Diclofenac sodium 50 mg was accurately weighed into a 100 ml volumetric flask and dissolved with phosphate buffer of 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 individually pipetted into 100 ml volumetric flask and then diluted to volume with phosphate buffer pH 6.8. The final concentration of each solution was 5, 10, 15, 20 and 25 μg/ml, respectively.

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 of pH 6.8 was used as a reference solution. Each concentration was determined in three determinations. The calibration curve of diclofenac sodium in phosphate buffer pH 6.8 are shown in Table 24 and Figure 71, respectively.