CHAPTER II

EXPERIMENT

Materials

The following materials were obtained from commercial sources. Deionized water was used throughout the experiment.

1. Model Drug
   - Diclofenac sodium (Bromine free) (Lot .No. DCS R-86429 -194 Yung Zip Chemical Ind. Co.,Ltd., Taiwan)

2. Additives
   - 3 grades of chitosan (Pronova Biopolymer, Norway)
     - :- Seacure ® 143 (Lot.No. 731727G)
     - :- Seacure ® 243 (Lot.No. 731127G)
     - :- Seacure ® 343 (Lot.No. 731517G)
   - Colloidal silicon dioxide
     - :- Aerosil ® 200 (CH - 59 / 75 - DG, Degussa, Germany)
   - Glycerin USP XXII (Lot.No. 20-98, Cusson (Thailand) Co.Ltd., supplied by Srichand United Dispensary Co.,Ltd., Thailand)
   - Lactose hydrous USP/NF/BP/EP 200 mesh (Lot.No. 7091802 - 109, Wyndale, New Zealand)
   - Microcrystalline cellulose, NF/BP
     - :- Avicel ® PH-101 (Lot.No. 1764, Asahi Chemical Ind. Co., Ltd., Japan)
   - Propylene Glycol USP XXII (Lot.No. PL 70/611, Arco Co.Ltd. Singapore, supplied by Srichand United Dispensary Co.,Ltd., Thailand)

3. Chemicals
- Acetic acid, glacial 100% (E. Merck, Germany)
- Hydrochloric acid 37 %, sp.gr. 1.18, AR grade (BDH Laboratory Supplies, England)
- Sodium hydroxide pellet, AR grade (Eka Nobel AB, Nobel Industries, Sweden)
- Tri - sodium phosphate, ACS - AR grade (Carlo ERBA Reagent, Italy)
- Methyl alcohol anhydrous, absolute (low acetone), AR grade (Mallinckrodt Chemical, France)

Equipment

Analytical balance (Model A200S, Sartorius GmbH, Germany and Model PB3002 Mettler, Switzerland)

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

Differential scanning calorimeter (Model DSC 3100, Mac Science, Japan)

Extruder (Model EXKS-1, Fuji Paudal Co., Ltd., Japan)

Fourier transform infrared spectrometer (Model SP2000, Perkin Elmer Ltd., England)

Homogenizer (Model Ultra-Turrax T 50 DPX, Janke & Kunkel GmbH & Co. Kg., IKA labortechnik, Germany)

Hot air oven (Model UL80, Memmert, Germany)

Hydraulic-punch for tabletting (Department of Pharmaceutics , Chulalongkorn University, Thailand)

Magnetic stirrer (Model SP 46920-26, Cimarec 2, Thermolyne, USA)

Moisture determination balance (Model 6100-H, Ohaus Marca Reg., USA)

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

Paddle stirrer (DT, Erweka GmbH, Germany)

Planetary mixer (Model A701A, Kenwood Mfg. Ltd., England)

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

Scanning electron microscope (Model JSM-6400 LV, Jeol Ltd., Japan)

Sieve shaker (Josef Deckehnann Aschaflenberg, Germany)

Spheronizer (Model S320, Aeromatic-Fielder, England)

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

Tablet hardness tester (Model TBH-30 MD, Erweka GmbH, Germany)

Tablet thickness tester (Teclock Corp., Japan)

Thermal gravimeter - differential thermal analyzer (Model TG-DTA 2000, Mac Science, Japan)

Ultrasound transonic digital sonicator (Model T900, Elma , Germany)

Ultraviolet - visible recording spectrophotometer (Model UV-160 A, Shimadzu Corp., Japan)

X-ray powder diffractometer (Model JDX-3530, Jeol Ltd., Japan and Rigaku Denki [Miniflex], Japan)

Methods

1. 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. Rotating centrifugal wheel atomizer was used to spray and atomize the suspension through the spraying head nozzle into small droplets which were immediately dried in spray drying chamber .

In the preliminary study, the condition was adjusted to obtain the satisfactory physical properties of spray dried powder i.e., maximum percentage yield, lowest moisture content and good flowability.

Table 7 The spray drying conditions used throughout the study.

Parameter	Condition
Inlet air temperature (C)	160
Outlet air temperature (C)	75
Feed rate (ml/min)	15
Atomizing air pressure (bar)	4
Dilution Medium	1% acetic acid solution

The processing variables which affected the physical properties of the spray dried products were the inlet air temperature, feed rate and atomizing air pressure. Whereas, the outlet air temperature could not be controlled by this machine type, it depended on the inlet air temperature, the suspension feed rate and the operating time.

The inlet air temperature of 160°C gave percentage yield about 6% higher than that of 170°C. This temperature also minimized the drying and obstructions of the suspension at the spraying head nozzle. Higher outlet air temperature of more than 75°C gave lower moisture content in the spray dried powder, but took longer to reach the preferred temperature. Therefore the temperature of 75°C was chosen. The lower feed rate and higher atomizing air pressure prevented the obstruction of the suspension feed at the spraying head nozzles caused by the viscous and sticky characteristics chitosan solution that was observed in the preliminary test, especially when the high viscosity grade and high amount of chitosan in the suspension was used. The spray drying conditions used in this experiment are summarized in Table 7.

2. Preparation of Diclofenac Sodium Spray Dried Powders

2.1 Formulation of Spray Dried Powders

The solubility of diclofenac sodium in deionized water was higher than 9 mg/ml (Adeyeye and Li, 1990). It meant that the drug could not be completely dissolved in 1% acetic acid solution which was the preferred dispersing medium for chitosan solution because of its acidity. Thus it was used as a suspension feed.

In this study, chitosan in three different grades of Seacure® were used, whereas Seacure®143 was cut off during the test. Viscosity was the main difference between the three grades of Seacure®. Each 1% Seacure® solution (dry weight basis) in 1% acetic acid solution at 20°C showed different viscosity depended on the molecular weight of the polymer. The higher molecular weight chitosan (longer chain length) had higher viscosity. The properties of different grade of Seacure® are presented in Table 8.

Table 8 The properties of different grade of Seacure (Pronova Biopolymer).

Grade	Visocosity*	% Deacetylation
Seacure 143	lower than 20	> 80
Seacure 243	20 - 200	> 80
Seacure 343	200 - 800	> 80

* The viscosity of 1% Seacure in 1% acetic acid solution (mPa.s).

The spray dried suspension was diluted by the smallest amount of 1% acetic acid solution. In such manner, the suspension was not too viscous to be sprayed in order to avoid obstructions in the spray head nozzle. The adherence of the powder to the spray drying chamber was also lower. The final volume of the spray dried suspension directly affected the time used for spray drying process. Thus the smallest amount of the final volume was used to save time of experiment, when the constant feed rate was fixed at 15 ml/min. The final volume of the spray dried suspension, depended on the grade of polymer and polymer to drug ratio, as shown in Table 9.

The solid content in the spray dried suspension in each formulation was in the range of 10 -15% w/w. Approximately 3 - 15% w/w of chitosan was used as a polymer to sustain the release of drug. The amount of Aerosil® 200 used as anti-adherent for spray dried suspension was 5% w/w of the content of drug and polymer in the formulation. The compositions of spray dried suspension is presented in Table 10.

The amount of ingredients used in each formulation depended on the ratio of drug to polymer and the amount and type of plasticizer used. The compositions of each formulation are presented in Table 11 and 12.

Table 9 The dependent of the final volume of spray dried suspensions on the grade of polymer and polymer to drug ratio used.

Grade	Polymer to drug ratio	The final volume (ml)
Seacure®243	1 : 5	1,200
	1 : 10	900
	1 : 15 , 1 : 20 and 1 : 30	600
Seacure®343	1 : 5	1,500
	1 : 10	1000
	1 : 15 , 1 : 20 and 1 : 30	800

Table 10 The compositions of spray dried suspensions.

Ingredients	Amount of solid (% w/w)
Diclofenac sodium	78 - 92
Chitosan	3 - 17 *
Aerosil® 200	5 **
1% Acetic acid solution q.s.	

* Percent of polymer used in dry substance.

** Percent by weight of the drug and polymer content.

Table 11 Formulations of spray dried suspension feed for each preparation.

Ingredients	Amount per formulations
Diclofenac sodium	100 mg
Chitosan	3 -15 % *
Aerosil® 200	5 % **
Plasticizers	10 - 40 % ***

* Percent of polymer used (calculated on the dry basis).

** Percent by weight of drug and polymer.

*** Percent of plasticizers used (calculated on the weight of drug and polymer).

Table 12 The percentage and type of polymer and plasticizer in each formulation and polymer to drug ratio of spray dried suspensions.

Formulation	Diclofenac sodium	Seacure 243 *	Seacure 343 *	PG*** (%)	Glycerine*** (%)	Aerosil 200 (%)
A1	79.17	15.83 (1:5)**	.	.	.	5.00
A2	86.36	8.64 (1:10)	.	.	.	5.00
A3	89.06	5.94 (1:15)	.	.	.	5.00
A4	90.48	4.52 (1:20)	.	.	.	5.00
A5	91.94	3.06 (1:30)	.	.	.	5.00
B1	79.17	.	15.83 (1:5)	.	.	5.00
B2	86.36	.	8.64 (1:10)	.	.	5.00
B3	89.06	.	5.94 (1:15)	.	.	5.00
B4	90.48	.	4.52 (1:20)	.	.	5.00
B5	91.94	.	3.06 (1:30)	.	.	5.00
A1 P2	62.50	12.50 (1:5)	.	19.00 (20)	.	5.00
A1 P3	53.04	10.61 (1:5)	.	31.35 (33)	.	5.00
A1 G2	62.50	12.50 (1:5)	.	.	19.00 (20)	5.00
A1 G3	53.04	10.61 (1:5)	.	.	31.35 (33)	5.00
A2 P1	77.73	7.77 (1:10)	.	9.50 (10)	.	5.00
A2 P2	69.09	6.91 (1:10)	.	19.00 (20)	.	5.00
A2 P3	57.86	5.79 (1:10)	.	31.35 (33)	.	5.00
A2 P4	51.82	5.18 (1:10)	.	38.00 (40)	.	5.00
A2 G1	77.73	7.77 (1:10)	.	.	9.50 (10)	5.00
A2 G2	69.09	6.91 (1:10)	.	.	19.00 (20)	5.00
A2 G3	57.86	5.79 (1:10)	.	.	31.35 (33)	5.00
B2 P1	77.73	.	7.77 (1:10)	9.50 (10)	.	5.00
B2 P2	69.09	.	6.91 (1:10)	19.00 (20)	.	5.00
B2 P3	57.86	.	5.79 (1:10)	31.35 (33)	.	5.00
B2 P4	51.82	.	5.18 (1:10)	38.00 (40)	.	5.00
B2 G1	77.73	.	7.77 (1:10)	.	9.50 (10)	5.00
B2 G2	69.09	.	6.91 (1:10)	.	19.00 (20)	5.00
B2 G3	57.86	.	5.79 (1:10)	.	31.35 (33)	5.00
A3 P1	80.16	5.34 (1:15)	.	9.50 (10)	.	5.00
A3 P2	71.25	4.75 (1:15)	.	19.00 (20)	.	5.00
A3 P3	59.67	3.98 (1:15)	.	31.35 (33)	.	5.00
B3 P1	80.16	.	5.34 (1:15)	9.50 (10)	.	5.00
B3 P2	71.25	.	4.75 (1:15)	19.00 (20)	.	5.00
B3 P3	59.67	.	3.98 (1:15)	31.35 (33)	.	5.00
B3 G1	80.16	.	5.34 (1:15)	.	9.50 (10)	5.00
B3 G2	71.25	.	4.75 (1:15)	.	19.00 (20)	5.00
B3 G3	59.67	.	3.98 (1:15)	.	31.35 (33)	5.00

* Used 1% acetic acid solution as a medium.

** Polymer to Drug Ratio.

*** Percent of the plasticizers used (calculated on the weight of drug and polymer).

2.2 Preparation of Spray Dried Suspension

Diclofenac sodium powder was sieved through a sieve No. 80 (180 μm) before used in each formulation. The medium for the spray dried suspension was 1% acetic acid solution prepared by diluting glacial acetic acid with deionized water. The procedures for preparation of spray dried suspension were as follows :

2.2.1 Diclofenac Sodium with Chitosan

Diclofenac sodium, chitosan and Aerosil® 200 were individually weighed. Chitosan was dispersed in 1% acetic acid solution by stirring overnight until clear solution was obtained. The solution was filtered through gauze cloth to separate the undissolved portion of chitosan. This solution was used as a medium for the spray drying process.

Diclofenac sodium was then gradually added with the aid of a homogenizer. Acetic acid solution of 1% was used to dilute if the suspension was too viscous. Aerosil® 200 was subsequently added. The suspension was homogenized again until completely dispersed.

2.2.2 Diclofenac Sodium with Chitosan and Plasticizers

The formulations containing plasticizers were prepared by the same procedures as described above, except plasticizer was added into the filtered chitosan solution prior to the addition of diclofenac sodium. The plasticizer and chitosan solution were mixed thoroughly by a magnetic stirrer.

The final volume of the suspension was not fixed. It depended on the viscosity of the suspension which was influenced by the amount of chitosan used as listed in Table 9. The resulting suspension was continuously stirred and subsequently sprayed into the spray drying chamber under suitable conditions.

3. Evaluations of Physiochemical Properties of Spray Dried Powders

3.1 Powder Morphology

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

3.2 Bulk, Tapped Density and Percent Compressibility

About 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 that 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.

3.3 Angle of Repose

The angle of repose was determined by the cylinder method. Adequate amount of powder was weighed and filled into the cylinder (height = 5.2 cm, radius = 2.4 cm) which was placed on graph paper. After filling the powder 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 millimeters. The result was averaged from three determinations. Each angle of repose was calculated from the following equation.

$$\alpha = \tan^{-1} H / R \qquad (4)$$

Where α is the angle of repose, H is the height of the heap and R is the radius of the heap.

3.4 Moisture Determinations

The loss on drying value of powder was determined using a moisture determination balance. About 1 gm of sample powder was accurately weighed and exposed to an IR lamp until a constant weight was obtained. The percent moisture content was calculated from the average of three determinations.

3.5 The Infrared Spectroscopy

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

The IR spectra of the diclofenac sodium and additives in the spray dried powders were examined using the potassium bromine disc (KBr) method by an infrared spectrophotometer in the range of 4000 - 400 cm^{-1}. The plasticizers used in this study were not examined by this evaluation, because they were in the liquid forms that could not be tested by the KBr disc method.

3.6 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 between the components in spray drying process.

The crystallinity of diclofenac sodium and additives in the spray dried powders, except the plasticizers, was examined by X-ray diffractometer.

Each sample powder was packed into a thin rectangular quartz slide by the other cover slide. After firmly packed, the cover slide was taken off and the sample-packed slide was exposed to the X-ray beam in the X-ray diffraction chamber. The X-ray diffraction pattern was recorded at the speed of 20° per minute from 3° to 40° 2θ angle.

3.7 The Thermal Analysis

Thermal analysis is the most common evaluation method to study physicochemical properties of the interactions between two or more components systems. The thermal analysis has several techniques such as : DSC, TG-DTA, etc.

3.7.1 The Differential Scanning Calorimetry

The thermograms of the spray dried powder 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 after spray drying process.

About 3 - 5 mg of spray dried powder was accurately weighed into the DSC pan. Then it was crimped with the hermetically scaled 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° to 350°C.

3.7.2 The Thermal Gravimetry and the Differential Thermal Analysis

The thermograms of spray dried powder prepared from various formulations were examined by thermal gravimeter and differential thermal analyzer. The differences in exotherm, endotherm and percent weigh loss between the original substances and their products were detected, when the same heating conditions were given.

The sample preparation was made by the same method in differential scanning calorimetry, except the nitrogen gas was given instead of air throughout the process. All thermal runs were controlled at the heating rate of 10°C per minute and in the range of 40° to 450°C.

3.8. Determination of Diclofenac Sodium Content of Spray Dried Powders

3.8.1 Calibrations 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, then dissolved and adjusted to volume with absolute methanol. This solution was used as a standard stock solution.

The standard stock solution of 1, 2, 3, 4 and 5 ml was individually pipetted into the 100 ml volumetric flask and diluted to volume with absolute methanol. The final concentrations of the obtained standard solutions were 5, 10, 15, 20 and 25μg/ml, respectively.

The absorbances of the standard solutions were determined by a UV/visible spectrophotometer at 281 nm with absolute methanol as a blank reference. The absorbance and the calibration curve of diclofenac sodium are presented in Table 29 and Figure 72, in Appendix A, respectively. The result of each concentration of the standard solution was averaged from three determinations.

3.8.2 Assay of Diclofenac Sodium Content in Spray Dried Powder.

A portion of sample powder equivalent to 100 mg of diclofenac sodium was accurately weighed into a 100 ml volumetric flask. The powder was dissolved with absolute methanol by the aid of sonicator about 30 minutes, then adjusted to volume with absolute methanol and mixed thoroughly. The solution was filtered through Whatman® filter paper No. 1 and used as a stock solution.

One milliliter of the stock solution was individually pipetted into a 50 ml volumetric flask, then adjusted to volume with absolute methanol and mixed.

The resulting solution was determined as the standard by UV/visible spectrophotometry at 281 nm with absolute methanol as a reference blank. Diclofenac sodium content was calculated from the calibration curve of diclofenac sodium in absolute methanol.

4. Preparation of Diclofenac Sodium Matrix Tablets

The spray dried powder was compressed into matrix tablet by the use of a hydraulic punch tabletting machine. In the preliminary test, 100, 300 and 500 pounds compression forces were used and the hardness and dissolution profiles of the matrix tablets were compared. The result showed that the suitable compression force was 300 pounds.

About 5 gm of each formulation of diclofenac sodium spray dried powder was mixed with 1% w/w of Aerosil® 200 as a lubricant for tabletting in a small bottle for 5 minutes. After thoroughly mixed, the powder was accurately weighed equivalent to 100mg of diclofenac sodium. A 6.5 mm diameter round flat faced punch and die were used. The compression force was maintained at 300 pounds for 10 seconds and quickly released. The punch and die were lubricated with grease and cleaned off before production of each tablet. The matrix tablets were weighed again and recorded as a correct weight for further study.

5. Matrix Tablets Evaluations

5.1 Morphology of Matrix Tablets

The morphology of spray dried diclofenac sodium matrix tablets was examined both before and after dissolution testing by scanning electron microscopy.

The matrix tablets were coated with gold prior to microscopic observations using ion sputtering method. Inner, outer and side surface of the matrix tablets were observed and compared with those of commercial Voltaren® SR tablet.

5.2 Thickness

The thickness of the matrix tablet was measured by using a tablet thickness tester in millimeters. The average thickness was calculated from eight determinations.

5.3 Diameter and Hardness

The diameter and hardness of the tablet were measured by using tablet hardness tester in millimeters and in kilopounds, respectively. The average diameter, the mean and standard deviation were obtained from three determinations.

5.4 Weight per Tablets

The weight of the matrix tablet was measured again after tabletting as a correct weight. The mean and standard deviation were calculated from eight determinations.

6. Preparation of Diclofenac Sodium Pellets

In the preliminary study of pelletization, only microcrystalline cellulose (Avicel® PH101) and lactose were chosen as extrusion-spheronization aids for pellets. Chitosan solution was prepared with the same method in the spray drying process and used as a binder for pelletization.

The pelletization process was initiated by weighing diclofenac sodium, lactose and Avicel® PH101. Then all dry components were mixed together with the aid of planetary mixer. After thoroughly mixed, chitosan solution was gradually added and mixed until damp mass occurred. The damp mass was transferred to the extruder and immediately rolled into solid spheres on the spinning friction plate of a spheronizer at different spheronization times and speeds. The solid spheres or pellets were dried by hot air oven at 60°C for 2 hours.

The factors affecting pelletization were the initial load of extruded mass, spheronization speed and spheronization time. All factors had to be controlled throughout the experiments. The pelletization conditions are presented in Table 13.

The amount of ingredients used in each formulation depended on the ratio of the drug to extrusion-spheronization aids and percent of the polymer. The formulations using one or two diluents with the aid of polymer solution as a binder were used in this study. The formulations of pellets composed of drug, polymer and diluents at different percentage are shown in Table 14. The ratio of drug to diluents and percentage of all components used in each pellet formulation are presented in Table 15.

Table 13 The pelletization conditions for preliminary testing and suitable conditions.

Factors	Pelletization conditions	
	Preliminary testing	Suitable conditions
Extruded mass load (gm)	250 - 300	more than 300
Spheronization speed (rpm)	500 or 900	500
Spheronization time (min)	5, 10, 15, 20, 25 and 30	15

Table 14 Formulations of diclofenac sodium matrix pellets of each preparation.

Ingredients	Amount of solid (% w/w)
Diclofenac sodium	20 - 50 *
Chitosan	1.5 - 3.5 **
Avicel pH 101	32.5 - 72.5
Lactose	0 - 35
Deionized water q.s.	(as a water in chitosan solution)

* Diclofenac sodium equivalent 100 mg / preparation.

** Percent of polymer used in dry substance.

Table 15 The ratio of drug to diluents and percentage of all components used in each pellet formulation.

Formulation	Ratio of drug to diluents			Percent of all components				
	DS	*AV*	*LC*	DS	AV	LC	*S 243	S 343
L1	1	.	1	cc**	.	cc	cc	cc
L2	1	.	1.5	cc	.	cc	cc	cc
L3	1	.	2	cc	.	cc	cc	cc
AA1	1	1	.	48.92	48.92	.	2.16	.
AA2	2	1	.	cc	cc	.	cc	cc
AA3	3	1	.	cc	cc	.	cc	cc
AA4	1	2	.	32.27	64.54	.	3.19	.
AA5	6	10	.	36.27	60.45	.	3.28	.
BA1	1	1	.	48.81	48.81	.	.	2.38
BA2	1	1.5	.	39.00	58.50	.	.	2.50
BA3	1	2	.	32.47	64.94	.	.	2.59
BA4	1	2.5	.	27.74	69.34	.	.	2.92
BA5	1	3	.	24.18	72.53	.	.	3.29
AAL1	1	1.25	1.25	27.87	34.90	34.90	2.33	.
BAL1	1	1.25	1.25	28.06	35.15	35.15		1.64
BAL2	1	1	1	32.76	32.76	32.76	.	1.72

* Percent of all components were calculated from the total dry weight of the pellet formulations.

** cc = could not be calculated.

DS = Diclofenac sodium, AV = Avicel PH 101, LC = Lactose, S243 = Seacure 243 and S343 = Seacure 343.

7. Evaluations of Physiochemical Properties of Pellets

7.1 Pellet Morphology

The morphology of diclofenac sodium pellets was determined by scanning electron microscopy. The pellets were coated with gold about 2 - 3 times to cover all of the spherical surface, then the coated pellets were examined using ion sputtering method. Size, shape and surface topography of the pellets were observed.

7.2 Sieve Analysis

Size distributions of pellets were determined by using sieve analysis. Approximately 50 gm of pellets was accurately weighed and put on the top of sieve series ranging from sieve No. 14, 18, 20 to 25, respectively (1400, 1000, 900 to 700 μm). The sieves were placed on the sieve shaker for 30 minutes. The results was calculated in percentage of pellets retained on each sieve size.

8. Evaluation of Diclofenac Sodium Products

8.1 Dissolution Studies

In this study, the pH-change dissolution system was specified in the delay-release dissolution test, method A, in USP XXIII by using apparatus I. The medium for testing was placed in a glass vessel and equilibrated at 37 ± 0.5°C. One product was placed in the dry basket at the beginning of each test and at 2.5 cm above the bottom of the vessel. The speed of the dissolution apparatus was controlled at 100 rpm.

Seven hundreds and fifty milliliters of 0.1 N HCl solution (pH about 1.3) was filled first into each vessel and operated at 37 ± 0.5°C for 2 hours. Then 250 milliliters of 0.20 M tri-basic sodium phosphate which was equilibrated at 37 ± 0.5°C was

filled into the vessel. After this had mixed, pH of the medium was increased to 6.8. In case that the pH of the medium did not reach the requirement, 2N HCl or 2N NaOH solution was added to adjust the pH within the range of 6.8 ± 0.05.

In both the spray dried powder and matrix tablet preparations, a ten milliliters of the specimen in each vessel was withdrawn at the time intervals of 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12, 16, 20 and 24 hours and the time intervals of 1, 2, 3, 4, 6, 9, 12 and 24 hours for pellet preparations. The same volume of the medium at that time was added immediately after each sampling to keep the constant volume of the medium in the vessel until the end of the experiment.

The specimen was diluted, if necessary, to the range of 5 - 30 μg/ml and was determined by UV/visible spectrophotometry 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 was calculated from the calibration curve for each medium. A cumulative correction was made for the previously removed sample to determine the total amount of the drug release.

8.1.1 Dissolution Studies of Spray Dried Powder

Spray dried powder equivalent to 100 mg of diclofenac sodium was accurately weighed and filled into a capsule shell No.1. The evaluation for each formulation was done on three determinations.

8.1.2 Dissolution Studies of Spray Dried Matrix Tablet

Spray dried powder equivalent to 100 mg of diclofenac sodium was accurately weighed, tabletted by the hydraulic punch tabletting machine and then was weighed again. The physical properties of the matrix tablets were evaluated before dissolution tests. The evaluation for each formulation was done in triplicate.

8.1.3 Dissolution Studies of Diclofenac Sodium Pellets

Each of three different size of pellets was accurately weighed equivalent to 100 mg of diclofenac sodium and filled into a capsule shell No.1. The evaluation for each formulation was done in triplicate. The chosen sizes were selected from the top three highest percentage yield of the pelletization process in each preparation.

8.2 Calibration Curves of Diclofenac Sodium

8.2.1 In 0.1 N HCl Solution

Diclofenac sodium of 100 mg was accurately weighed into a 100 ml volumetric flask, then dissolved and adjusted to volume with absolute methanol. This solution was used as the first stock solution. Ten milliliters of the first stock solution was pipetted into 50 ml volumetric flask and adjusted to volume as the second stock solution.

The 1, 2, 3, 4, and 5 ml of second stock solution were individually pipetted into 50 ml volumetric flasks and 1, 3, and 5 ml. into 25 ml volumetric flasks. All solutions were adjusted to volume with 0.1 N HCl. The final concentration of each solution was 5, 10, 15, 20, 25, 30, 40 and 50 µg/ml of diclofenac sodium, respectively.

The final solution was assayed spectrophotometrically at 275 nm. The absorbance and the calibration curve of diclofenac sodium in 0.1 N HCl are presented in Table 30 and Figure 73 in Appendix A, respectively. Each concentration was determined in tripicate.

8.2.2 In Phosphate Buffer pH 6.8 Solution.

Diclofenac sodium of 125 mg was accurately weighed into a 200 ml volumetric flask, then dissolved and adjusted to volume with absolute methanol. This solution was used as the first stock solution. Ten milliliters of the first stock solution was pipetted into 50 ml volumetric flask and adjusted to volume as the second stock solution.

The 1, 2, 3, 4, and 5 ml of second stock solution were individually pipetted into 50 ml volumetric flasks and 1, 3, and 5 ml. into 25 ml volumetric flasks. All solutions were adjusted to volume with phosphate buffer pH 6.8. The final concentration of each solution was 2.5, 5, 7.5, 10, 12.5, 15, 20 and 30 μg/ml of diclofenac sodium, respectively.

The final solution was assayed spectrophotometrically at 277 nm. The absorbance and the calibration curve of diclofenac sodium in phosphate buffer pH 6.8 are presented in Table 31 and Figure 74 in Appendix A, respectively. Each concentration was determined in triplicate.