CHAPTER III

MATERIALS AND METHODS

Materials

1. Drug

-Diclofenac sodium (Batch DFSH 045, CFS PTE Ltd., Switzerland)

2. Carriers

-Ethylcellulose (Ethocel 10 cps, Dow Chemical Company, U.S.A.)
-Chitosan (Unicord PCL Ltd., Thailand)
-Carbomer (Carbopol 934, Batch No. A701031, distributed by S. Tong Chemicals Co. Ltd., Thailand)
-Methacrylic acid copolymer (Eudragit RS 100, Rohm Pharma, Germany)
-Hydroxypropyl methylcellulose (Methocel E4M Premium, Batch No. MM89120413E, Dow Chemical Company, U.S.A.)

3. Additives

-Spray dried rice starch (Era-Tab, Lot No. T910118, Erawan Pharmaceutical Research and Laboratory Co., Ltd., Thailand)

-Croscarmellose sodium, USP XX/NF XV (Ac-Di-Sol, Lot No. T934, AMC Co., U.S.A.)

-Aerosil (Wacker Chemie GMBH, Germany)

-Magnesium stearate (Lot No. MaF01, Italy)

4. Others

-Absolute ethanol (Merck, Germany)

-Concentrated hydrochloric acid (Merck, Germany)

-Glacial acetic acid (Merck, Germany)

-Sodium phosphate dodecahydrate (Lot No. A278487, Merck, Germany)

Equipment

-Analytical balance (Sartorius, Model A200S, Germany)

-Sieve No. 40 (Retsch GmbH & Co. KG, Germany)

-Spray dryer (Buchi Co., Buchi 190 Mini Spray Dryer, Switzerland)

-pH meter (Schott Co., Model CG 840, Germany)

-Single punch tabletting machine equipped with strained gauge (Viuhang Engineering, Thailand)

-Strain amplifier (Tokyo Sokki Kenkyujo Co., Ltd., DA 12 A Strain Meter, Japan)

-Hardness tester (Dr. Schleuniger Co., Type THP-4M, Switzerland)

-Friabilator (K.S.L. Engineering Co., Ltd., Erweka type, Thailand)

-Disintegration apparatus (K.S.L. Engineering Co., Ltd., USP type , Thailand)

-Dissolution apparatus (Pharma Test Co., Model TW II, Germany)

-Spectrophotometer (Milton Roy Company, Spectronic 3000 Array, U.S.A.)

-Scanning electron microscope (Jeol, JSM-35 CF, Japan)

-Differential thermal analyzer (Shimadzu, Model DT-30, Japan)

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

Methods

1. Preparation of Diclofenac Sodium Solid Dispersions

1.1 Preparation of 3:1 Diclofenac Sodium:Polymer Solid Dispersions

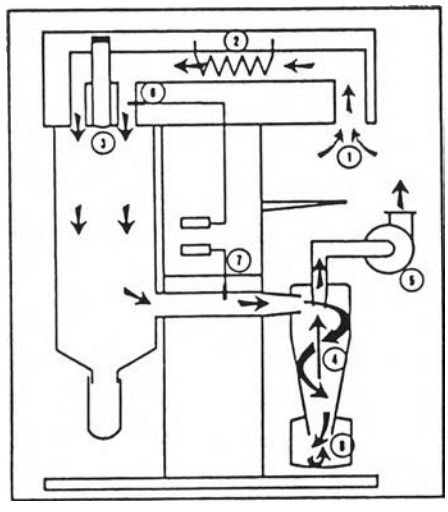
In this study various diclofenac sodium solid dispersion systems were developed utilizing different types of polymers as carriers. The polymers being used were insoluble polymers, ethylcellulose (EC) and methacrylic acid copolymer (Eudragit), and swellable polymers, hydroxypropyl methylcellulose (HPMC), carbomer and chitosan. Since the dose of sustained release diclofenac sodium is quite high as 100 mg in a commercial product (Reynolds, 1989), therefore only the limited amounts of the polymers can be used as carriers in preparing diclofenac sodium controlled release solid dispersions otherwise the size of the dosage form will be too large. For this reason the ratio of 3:1 diclofenac

sodium:polymer was chosen as the starting point in preparing the diclofenac sodium controlled release solid dispersions.

In order to prepare the 3:1 drug:polymer solid dispersions the accurate amounts of diclofenac sodium and polymers were weighed. Table 7 lists the types and amounts of various polymers including the amount of the drug being used in preparation of diclofenac sodium solid dispersions. The drug and each polymer were dissolved separately in absolute ethanol except chitosan which was dissolved in 1% acetic acid. To prepare solid dispersion, the polymer solution was added into the diclofenac sodium solution. The resulting solution or colloidal dispersion was adjusted to final volume by water or 1% acetic acid and spray-dried using a spray feeding rate of 10 ml per minute and inlet temperature between 110 to 130^{0}C. Figure 2 shows the detail of the spray dryer being utilized in this study. The conditions of spray drying for each preparation are also shown in Table 7. The 3:1 diclofenac sodium:polymer solid dispersions were then kept in a desiccator.

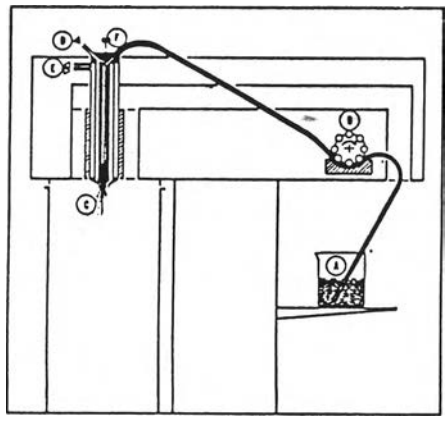
1.2 Preparation of Diclofenac Sodium Solid Dispersion Systems Using Ethylcellulose and Chitosan as Single and Combined Carriers

Ethylcellulose as an insoluble carrier and chitosan as a swellable carrier were selected to be used as combined carriers in preparing diclofenac sodium controlled release solid dispersions. To investigate the role of ethylcellulose and chitosan as combined carriers in preparing the diclofenac sodium controlled release solid dispersion, the 10:(0.95+0.05) diclofenac sodium:(EC+chitosan) solid dispersion was



1 Air-intake
2 Heating
3 Flow-stabilized entrance into the drying chamber
4 Cyclone. Here the product is separated from the air stream.
5 Aspirator
6 Temperature feeler "air inlet"
This temperature can be regulated with the heating.
7 Temperature feeler "air outlet"
The optimum selection of the temperature difference between the "inlet" and "outlet" temperatures is one of the most important aspects of spray drying. The outlet temperature cannot be set as desired since it results from a combination between the inlet temperature – aspirator setting and Product-feed pump performance.
8 Receiving vessel for the final product.

Figure 2.1. Diagram of flow of drying air.



A Solution, emulsion or dispersion of the product
B Peristaltic feed pump
C Product channel.
D Connection for spray flow (pressurized air or inert gas)
E Connection for cooling water
F Nozzle needle with twist channel. The needle can be used to clear the nozzle should it become clogged by the product. For difficult products, the nozzle needle can be operated automatically. This device is available as an accessory.

Figure 2.2. Diagram of flow of product and of spray nozzle.

Figure 2. Diagram showing detail of Buchi 190 Mini Spray Dryer.

Table 7. Formulations and Spray Drying Conditions for Preparing 3:1 Diclofenac Sodium :Polymer Solid Dispersions.

Formulation	EC	Eudragit	HPMC	Carbomer	Chitosan
Diclofenac Sodium (g)	7.50	7.50	7.50	7.50	7.50
Polymer (g)	2.50	2.50	2.50	2.50	2.50
Absolute Ethanol (ml)	280	280	280	200	200
Water (ml)	120	120	120	600	-
1% Acetic Acid (ml)	-	-	-	-	400
Total Volume (ml)	400	400	400	800	600
Pump Feed Rate (ml / min)	10	10	10	10	10
Spray flow rate (normliter / hr)	450	450	450	450	<100
Inlet Temperature (°C)	110	110	110	130	110

prepared and its dissolution profile was compared with those of 10:1 drug:EC and 10:0.1 drug:chitosan solid dispersions.

Solid dispersions of 10:1 diclofenac sodium:EC, 10:0.1 diclofenac sodium:chitosan, and 10:(0.95+0.05) diclofenac sodium: (EC+chitosan) were prepared according to Table 8. To prepare the three solid dispersions, the predetermined amounts of diclofenac sodium and ethylcellulose were dissolved separately in absolute ethanol while accurately weighed chitosan of one gram was dissolved in 100 ml of 1% acetic acid in a volumetric flask to yield 1% stock chitosan solution. Then the ethylcellulose solution or 10.00 ml of 1% stock chitosan solution was added to the diclofenac sodium solution. The resulting solution or colloidal dispersion was adjusted to final volume by water and spray-dried to yield the required diclofenac sodium solid dispersions. In order to prepare 10:(0.95+0.05) diclofenac sodium:(EC+chitosan) solid dispersion five milliters of 1% stock chitosan solution was added to the mixture of drug and ethylcellulose in absolute ethanol. The resulting colloidal dispersion was adjusted to final volume by water and spray-dried. The solid dipersions were then kept in a desiccator.

1.3 Preparation of Diclofenac Sodium:(EC+chitosan) Solid Dispersions According to Hadamard Matrix H [8]

In order to search for the optimum ratio of ethylcellulose and chitosan being utilized as combined carriers and the optimum conditions of spray drying, an experimental design using Hadamard matrix H[8] as shown in Table 9 was generated. Four parameters were studied for their main effects on dissolution profiles of the diclofenac

Table 8. Formulations and Spray Drying Conditions in Preparing 10:1 Diclofenac Sodium:EC, 10:0.01 Diclofenac Sodium:Chitosan and 10:(0.95+0.05) Diclofenac Sodium:(EC+chitosan) Solid Dispersions.

Formulation	EC	Chitosan	EC:Chitosan (0.95:0.05)
Diclofenac Sodium (g)	10.00	10.00	10.00
Ethylcellulose (g)	1.00	-	0.95
Chitosan (g)	-	0.10	0.05
Absolute Ethanol (ml)	280	280	280
Water (ml)	120	110	115
1% Acetic Acid (ml)	-	10	5
Total Volume (ml)	400	400	400
Pump Feed Rate (ml/min)	10	10	10
Spray flow rate (normliter / hr)	450	450	450
Inlet Temperature (^{O}C)	110	110	110

Table 9. Experimental Design by Hadamard Matrix H[8] for Preparing Diclofenac Sodium:(EC+chitosan) Solid Dispersions.

Experiment	Diclofenac Sodium (g)	Feeding Volume (Reduced Variable)	Absolute Ethanol (Reduced Variable)	EC Content (Reduced Variable)	Chitosan Content (Reduced Variable)
I	10.00	1	1	1	1
II	10.00	-1	1	-1	1
III	10.00	1	-1	-1	1
IV	10.00	-1	-1	1	1
V	10.00	1	1	1	-1
VI	10.00	-1	1	-1	-1
VII	10.00	1	-1	-1	-1
VIII	10.00	-1	-1	1	-1

Experiment	Diclofenac Sodium (g)	Feeding Volume (ml)	Absolute Ethanol fraction	EC Content (g)	Chitosan Content (g)
I	10.00	500	0.70	3.00	0.10
II	10.00	200	0.70	1.00	0.10
III	10.00	500	0.30	1.00	0.10
IV	10.00	200	0.30	3.00	0.10
V	10.00	500	0.70	3.00	0.02
VI	10.00	200	0.70	1.00	0.02
VII	10.00	500	0.30	1.00	0.02
VIII	10.00	200	0.30	3.00	0.02

sodium:(EC+chitosan) solid dispersions. Those parameters were the amount of ethylcellulose, the amount of chitosan, the fraction of absolute ethanol employed, and the spray feeding volume. The last parameter, the spray feeding volume, was an indication of the feeding liquid concentration. Increasing feeding volume would result in decreasing concentration of the feeding liquid and vice versa. By varying these variables, eight diclofenac sodium:(EC+chitosan) solid dispersions were achieved.

Diclofenac sodium:(EC + chitosan) solid dispersions were prepared by spray drying using various amounts of ethylcellulose and chitosan as shown in Table 10. The volume of absolute ethanol, water, and feeding solution employed in each preparation also were varied. To prepare each solid dispersion the accurate volume of 1% stock chitosan solution was added to the mixture of diclofenac sodium and ethylecellulose in absolute ethanol. The resulting colloidal dispersion was adjusted to final volume by water and spray-dried. The resulting solid dispersions were kept in a desiccator.

2. Dissolution Studies of Diclofenac Sodium Solid Dispersions

Dissolution studies of diclofenac sodium solid dispersions, each equivalent to 100 mg drug, as compared to 100 mg diclofenac sodium powder were conducted according to Method A described under Drug Release in USP XXII & NF XVII (The United States Pharmacopeil Convention, 1990). The dissolution medium was maintained at 37±1°C. The dissolution tests were run by the USP type II dissolution apparatus using a stirring rate of 50 rpm. The powdered drug or diclofenac sodium

Table 10. Formulation and Spray Drying Conditions in Preparing Diclofenac Sodium:(EC+chitosan) Solid Dispersions.

Formulation	I	II	III	IV	V	VI	VII	VIII
Diclofenac Sodium (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Ethylcellulose (g)	3.00	1.00	1.00	3.00	3.00	1.00	1.00	3.00
Chitosan (g)	0.10	0.10	0.10	0.10	0.02	0.02	0.02	0.02
Absolute Ethanol (ml)	350	140	150	60	350	140	150	60
Water (ml)	140	50	340	130	148	58	348	138
1% acetic acid (ml)	10	10	10	10	2	2	2	2
Total Volume (ml)	500	200	500	200	500	200	500	200
Pump Feed Rate (ml/min)	10	10	10	10	10	10	10	10
Spray Flow Rate (normliter/hr)	450	450	450	450	450	450	450	450
Inlet Temperature (^{O}C)	110	110	110	110	110	110	110	110

solid dispersion was dissolved firstly in 0.1 N HCl for 2 hours and later in pH 6.8 phosphate buffer solution for 10 hours. Five milliters of sample solutions were withdrawn at predetermined time intervals. The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium. The dissolution studies were performed on six samples obtained from each solid dispersion system and from diclofenac sodium powder.

Assay - The sample solutions in acid and buffer media were assayed spectrophotometrically at 275 nm and 278 nm for diclofenac sodium content, respectively. Figure 3 and 4 illustrate the ultraviolet spectra of diclofenac sodium in dissolution media, 0.1 N HCl and pH 6.8 phosphate buffer solutions. The wavelengths of maximum absorption for the two solvents were 275 and 278 nm, respectively. The sample concentrations were calculated from standard curves.

Standard curve - Standard curves of diclofenac sodium in 0.1 N HCl and pH 6.8 phosphate buffer solutions, were obtained between 0-50 mg/ml and 0-80 mg/ml, respectively. Absorbance at 275 nm and 278 nm versus concentration plots, as presented in Figure 5 and 6, revealed that Beer's law were followed. Table 11 and 12 show the relationships between the concentrations of diclofenac sodium in the two dissolution media and the absorbances at 275 nm and 278 nm, respectively. Linear regression was applied to build the relationship between the obtained absorbances and drug concentrations. The equations represented these relationships at 275 and 278 nm then were derived.

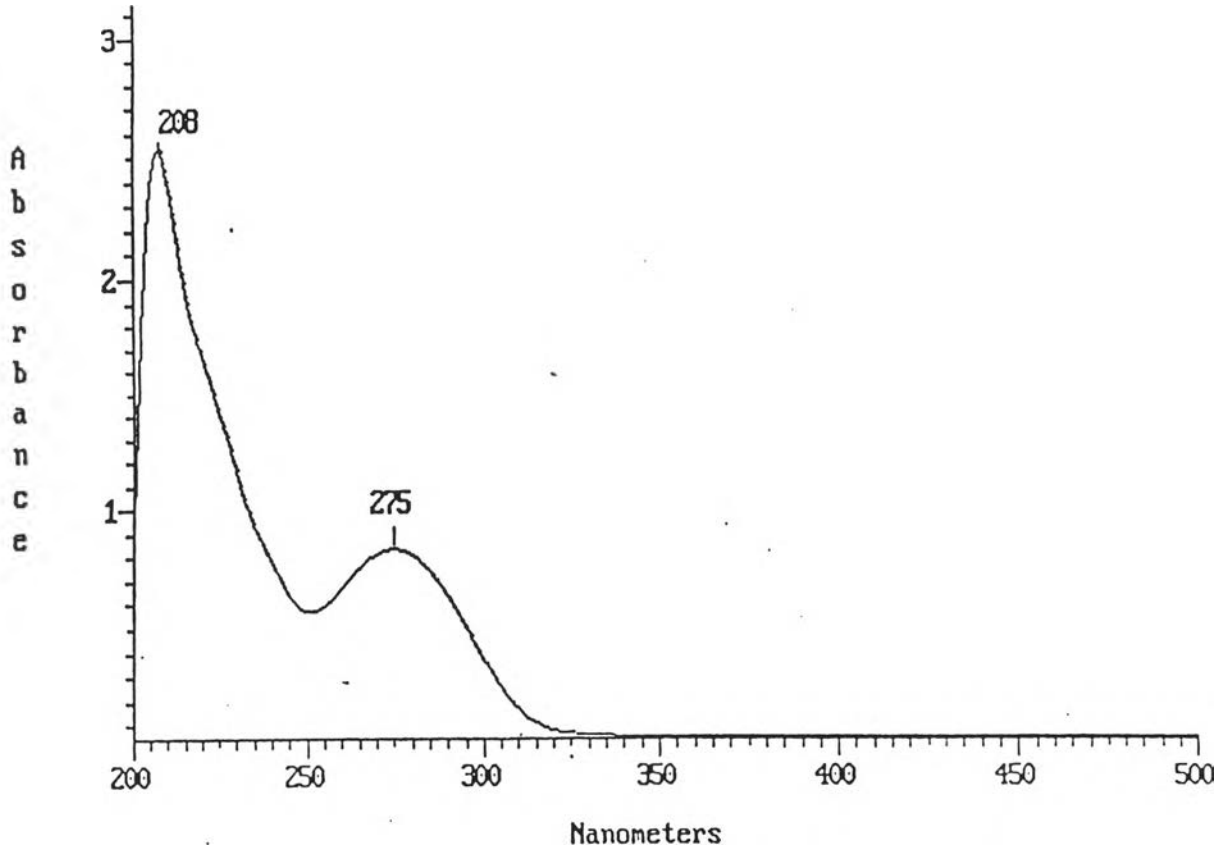


Figure 3. Ultraviolet spectrum (200-400 nm) of diclofenac sodium in 0.1 N HCl.

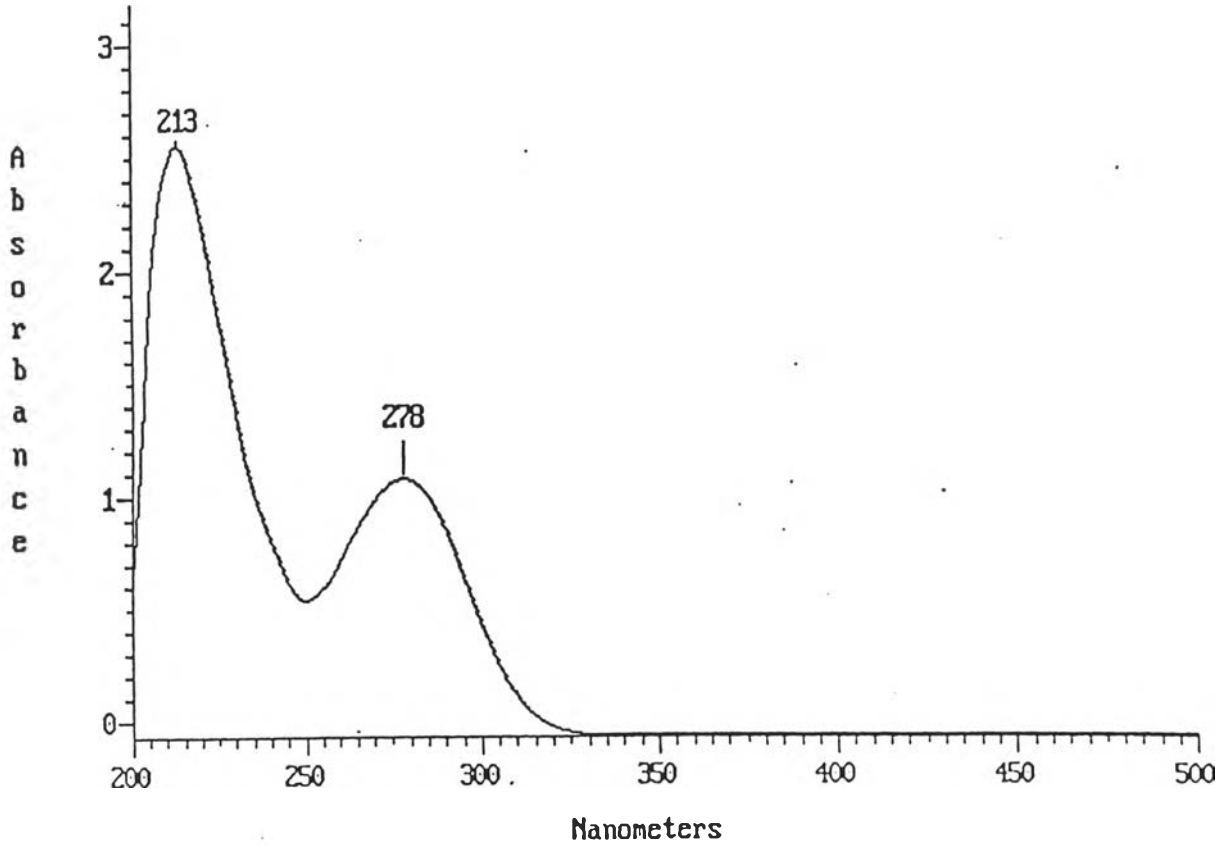


Figure 4. Ultraviolet spectrum (200-400 nm) of diclofenac in pH 6.8 phosphate buffer.

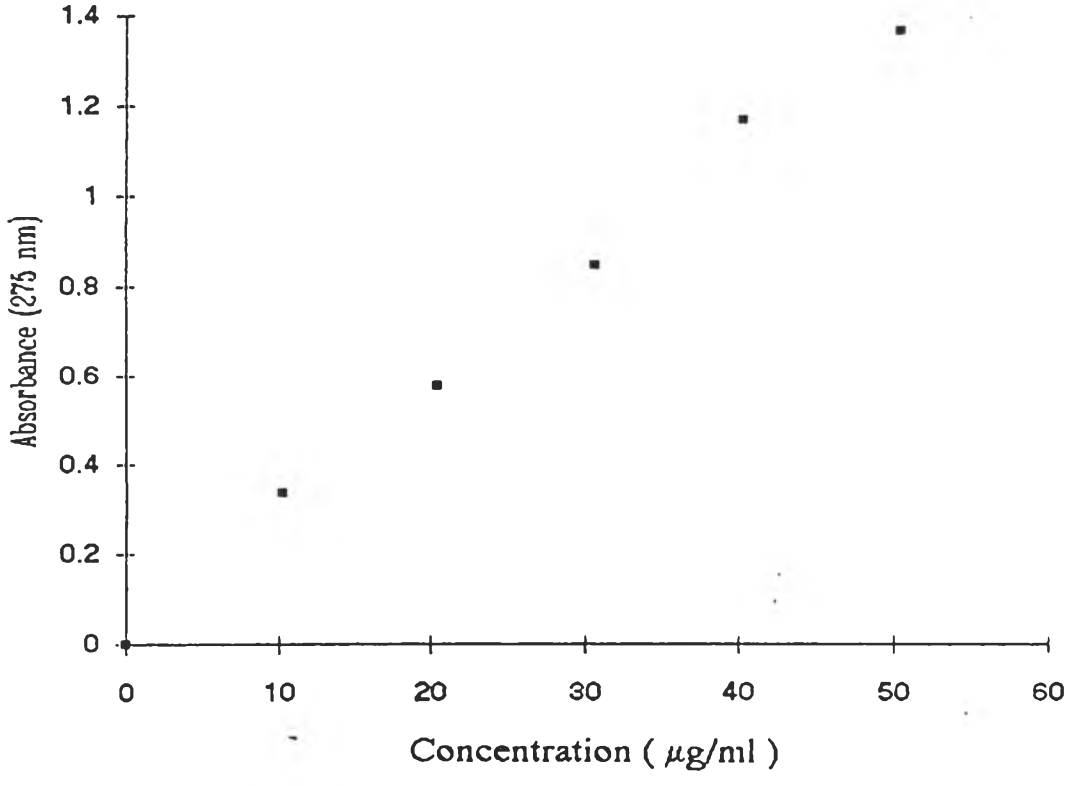


Figure 5. Standard curve plotting the concentration of diclofenac sodium versus absorbance at 275 nm.
($r^2 = 0.996204$)

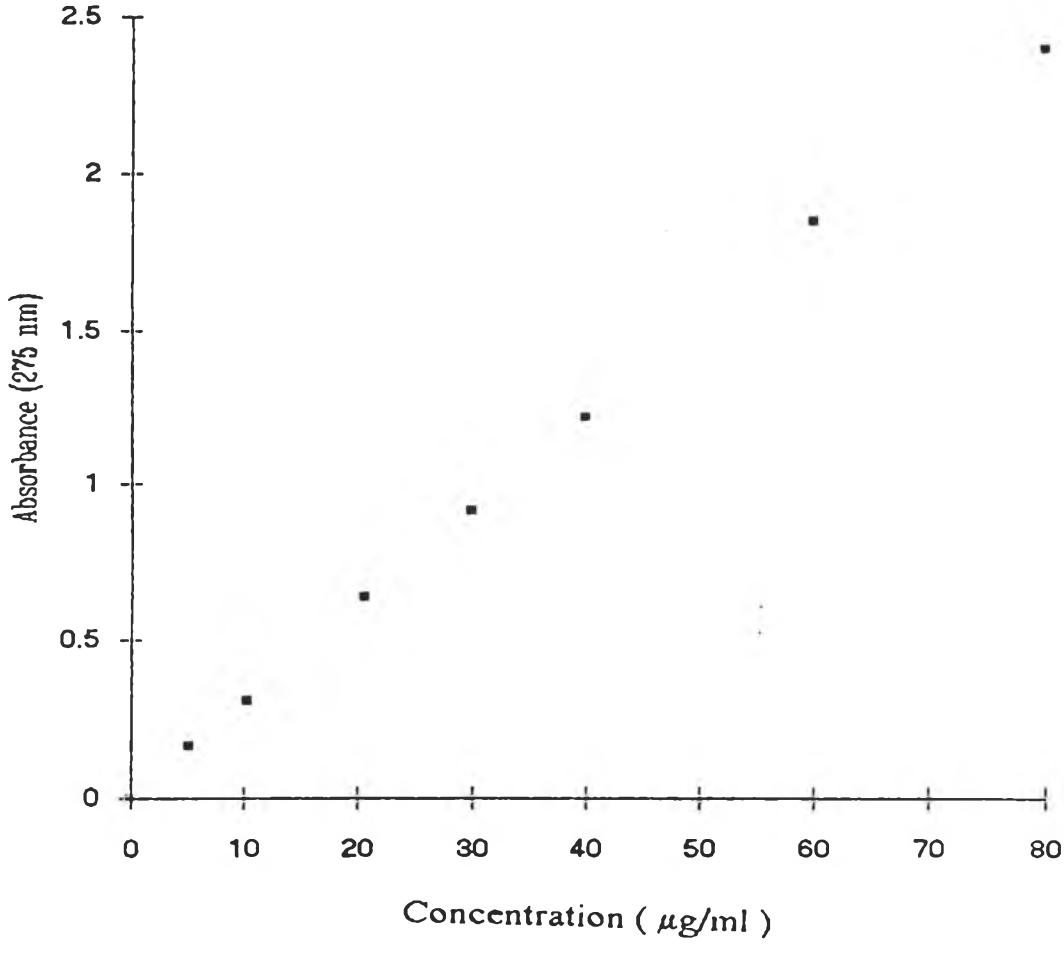


Figure 6. Standard curve plotting the concentration of diclofenac sodium versus absorbance at 278 nm.
($r^2 = 0.999565$)

Table 11. Relationship between Concentrations of Diclofenac Sodium in 0.1 N HCl and Absorbances at 275 nm.

Concentration (μg/ml)	Absorbance[a] (275 nm)
0.0	0.000
10.2	0.335
20.4	0.578
30.6	0.847
40.2	1.167
50.5	1.366

[a] Average of two determinations

Table 12. Relationship between Concentrations of Diclofenac Sodium Phosphate Buffer pH 6.8 and Absorbances at 278 nm.

Concentration (μg /ml)	Absorbance[a] (278 nm)
0.0	0.000
5.1	0.162
10.3	0.309
20.6	0.638
29.9	0.913
39.9	1.217
59.9	1.847
79.8	2.3875

[a] Average of two determinations

3. Validation of Optimized Solid Dispersion

The optimized 10:(2.5+0.02) diclofenac sodium: (EC+chitosan) controlled release solid dispersions was prepared by spray drying using optimum ratio and conditions obtained from optimization of diclofenac sodium controlled release solid dispersions in 1.3. The conditions of spray drying are demonstrated in Table 13. To prepare the solid dispersion the predetermined amounts of diclofenac sodium and ethylcellulose were dissolved in absolute ethanol while accurately weighed chitosan of one gram was dissolved in 100 ml of 1% acetic acid in a volumetric flask to yield 1% stock chitosan solution. Then the 2.00 ml of 1% stock chitosan solution was added to the mixture of diclofenac sodium and ethylcellulose in absolute ethanol. The resulting colloidal dispersion was adjusted to final volume by water and spray-dried. The prepared solid dispersion was kept in a dessicator. Its dissolution profile was studied by the same procedure as in 2.

4. Preparation of Diclofenac Sodium Controlled Release Solid Dispersion Tablets

The optimized 10:(2.5+0.02) diclofenac sodium: (EC+chitosan) solid dispersion was incorporated into direct compressed tablets. Table 14 lists the information of diclofenac sodium tablet formulations being employed in tablet production. An orthogonal central composite design was applied for studying the effects of four parameters: the compression force, the amount of spray-dried rice starch (Era-Tab), the amount of cross carmellose sodium (Ac-Di-Sol), and the amount of magnesium stearate being utilized in tablet production, on tabet

Table 13. Formulation and Spray Drying Conditions for Preparing Optimized Diclofenac Sodium:(EC+chitosan) Solid Dispersion.

Formulation	IX
Diclofenac Sodium (g)	10.00
Ethylcellulose (g)	2.50
Chitosan (g)	0.02
Absolute Ethanol (ml)	140
Water (ml)	58
1% Acetic Acid (ml)	2
Total Volume (ml)	200
Pump Feed Rate (ml / min)	10
Spray Flow Rate (normliter / hr)	450
Inlet Temperature (^{o}C)	110

Table 14. Formulation of Diclofenac Sodium Controlled Release Tablets.

Ingredient	Amount Per Tablet
Diclofenac Sodium Solid Dispersion	125.2 mg
Era-Tab	166.52, 174.8, 194.8, 214.8, 223.08 mg
Ac-Di-Sol	1.1%, 1.5%, 2.5%, 3.5%, 3.9%
Magnesium Stearate	0.15%, 0.25%, 0.50%, 0.75%, 0.85%
Aerosil	1%

properties. The detail of the experimental design is demonstrated in Table 15. The half fractional factorial was used in this design therefore the total of 17 formulations were obtained.

The number of experiments was calculated from:

$$N = 2^{k-F} + 2k + C$$

where

k = number of variables

F = fraction of the full factorial

C = number of centerpoint replicates.

In this study the half-fractional factorial-based central composite design of four variables was empolyed resulting in the values of k, F, and C of 4, 1, and 1, respectevely. Therefore the number of experiments was 17.

The tablets were prepared by direct compression by the following procedure. Firstly, all the tablet ingredients were screened through a 40 mesh sieve. Then the required quantity of the solid dispersion was mixed with the required amounts of Era-Tab, Ac-Di-Sol, magnesium sterarate, and aerosil by tumbling action for 5 minutes. The obtained mixture was directly compressed into tablets using a single punch tabletting machine equipped with a strain guage. The tablets were prepared to have a diameter of 12 mm and were stored in a desiccator for the further study.

Table 15. Experimental Design by Central Composite Design.

Formulation	Compression Force (psi)	Era-Tab (mg)	Ac-Di-Sol (%)	Magnesium Stearate (%)
I	560	174.8	1.5	0.25
II	840	174.8	1.5	0.75
III	560	214.8	1.5	0.75
IV	840	214.8	1.5	0.25
V	560	174.8	3.5	0.75
VI	840	174.8	3.5	0.25
VII	560	214.8	3.5	0.25
VIII	840	214.8	3.5	0.75
IX	900	194.8	2.5	0.50
X	500	194.8	2.5	0.50
XI	700	223.08	2.5	0.50
XII	700	166.52	2.5	0.50
XIII	700	194.8	3.914	0.50
XIV	700	194.8	1.086	0.50
XV	700	194.8	2.5	0.8535
XVI	700	194.8	2.5	0.1465
XVII	700	194.8	2.5	0.50
Formulation	X1	X2	X3	X4
I	-1	-1	-1	-1
II	1	-1	-1	1
III	-1	1	-1	1
IV	1	1	-1	-1
V	-1	-1	1	1
VI	1	-1	1	-1
VII	-1	1	1	-1
VIII	1	1	1	1
IX	1.414	0	0	0
X	-1.414	0	0	0
XI	0	1.414	0	0
XII	0	-1.414	0	0
XIII	0	0	1.414	0
XIV	0	0	-1.414	0
XV	0	0	0	1.414
XVI	0	0	0	-1.414
XVII	0	0	0	0

5. Study of Tablet Properties

The prepared diclofenac sodium controlled release tablets were investigated for tablet weight variation, friability, hardness, disintegration and dissolution.

5.1 Tablet Weight Variation

The tablet weight variation of each formualtion was evaluated on 20 tablets on an electronic analytical balance.

5.2 Tablet Friability

Friability was determined on 20 tablet samples obtained from each formulation in a friabilator rotating at the rate of 25 rpm for 4 minutes.

5.3 Tablet Hardness

Tablet hardness was measured on 5 tablets of each formulation using a Schleuniger hardness tester. The average value was then calculated.

5.4 Tablet Disintegration

Disintegration time studies were conducted on test tablets according to USP XXII & NF XVII (The United States Pharmacopeil Convention, 1990) using USP type disintegration apparatus. The average disintegration time was obtained from 6 tablets.

5.5 Tablet Dissolution

Dissolution studies of the diclofenac sodium controlled release solid dispersion tablets were run by the same procedure in 2. The dissolution studies were conducted on 6 tablets obtained from each formulation.

6. Validation of Optimized Diclofenac Sodium Controlled Release Tablet

The optimized diclofenac sodium controlled release tablet was prepared by direct compression according to an optimum formulation obtained from optimization of diclofenac sodium controlled release tablets in 4 and 5. Table 16 lists the detail of the tablet formulation. The prepared optimized tablet was studied for tablet weight variation, friability, hardness, disintegration and dissolution by the same procedures as in 5.

7. Scanning Electron Microscope Study

The diclofenac sodium powder and the optimized 10:(2.5+0.02) diclofenac sodium:(EC+chitosan) solid dispersion were investigated and photographed by a scanning electron microscope. The samples were coated with gold using ion sputtering before they were examined. The obtained pictures were compared in order to comfirm the formation of diclofenac sodium solid dispersion.

Table 16. Formulation of Optimized Diclofenac Sodium Controlled Release Tablet.

Ingredient	Amount Per Tablet (mg)
Diclofenac Sodium Solid Dispersion	125.2
Era-Tab	194.8
Ac-Di-Sol	6.4 (2.0%)
Magnesium Stearate	1.6 (0.5%)
Aerosil	3.2 (1%)
Compression Force	700 psi

8. Differential Thermal Analysis Study

The diclofenac sodium, polymer, and optimized 10:(2.5+0.02) diclofenac sodium:(EC+chitosan) solid dispersion powders were analyzed for their melting points by a differential thermal analyzer. Powder was accurately weighed and put into the equipment using a given condition.

Heating rate = 10°C per minute
Sensitivity = ± 50 or ± 100 μV
Atmosphere = static air
Chart speed = 10 mm per minute

9. X-ray Diffraction Study

The diclofenac sodium, polymer, and optimized 10:(2.5+0.02) diclofenac sodium:(EC+chitosan) solid dispersion powders were evaluated by an X-ray diffractometer which used target Cu, voltage 45.0 ku and scanning from 5 - 90° with 2θ.