CHAPTER II

MATERIALS AND METHODS

Materials

Raw materials were used as received without further purification.

1 **Drug subtances**

Indomethacin (Merkin Limited. Hong Kong)

Piroxicam (supplied by Rotar Lab., manufactured by Milano lab. Italy)

Piroxicam (supplied by Asian Drug and Chemical, Source from Hong Kong)

2 **Carriers**

β-cyclodextrin (Ringdex®, supplied by Rama Production Co., Ltd. Thailand) with water content of 13.0 % w/w

3 **Excipients**

Lactose (The Lactose Company. Newzealand Co., Ltd.)

Stearic acid (supplied by Srichand United Dispensary Co., Ltd. Thailand)

Starch (supplied by Bhaesach Panich, Thailand)

Sodium starch glycolate (Explotab®, Edward Mandel, U.S.A.)

PVP Kollidon 30, (BASF, West Germany)

Magnesium stearate (supplied by Pharmaceutical Science, Thailand)

Talcum (supplied by Pharmaceutical Science, Thailand)

Sodium lauryl sulfate (Farmitalia Carlo Erba, Italy)

4 **Reagents**

Potassium dihydrogen phosphate (Merck, Germany)

Sodium hydroxide (Merck Germany)

Disodium hydrogen phosphate (Merck Germany)

Phosphoric acid (Farmitalia Carlo Erba, Italy)

Hydrochloric acid (Farmitalia Carlo Erba Italy)

Citric acid (Farmitalia Carlo Erba, Italy)

Sodium chloride (E. Merck, Germany)

Methanol (HPLC grade J.T. Baker U.S.A.)

Capsule No. 2 (Hiap Heng Pharmacy Co., Ltd. Thailand)

Methods

1 Phase Solubility Analysis

Solubility measurements were carried out according to Higuchi and Conners. Excess amount of drug (100 mg) was added to an aqueous solution containing various concentrations of β-cyclodextrin($0 - 14 \times 10^{-1}$ M) The mixture was shaken in the sealed ampoules with a constant temperature water bath (shaking water bath, CGA Pricision Scientific) controlled at 35±1°C and equilibrated for 24 hours. After equilibrium was reached the suspensions were filtered through 0.45 μm membrane filter. The filtrates were analyzed spectrophotometrically (Perkin Elmer Lamda 15) at about 320 nm for indomethacin and 352 nm for piroxicam using water as a blank. The effect of β-cyclodextrin on the absorption was negligible. The β-cyclodextrin showed no absorbance at the wavelength measured.

Phase solubility curves were constructed by plotting the concentration of β-cyclodextrin versus concentration of the dissolved drug. Apparent solubility constants, K_C, were calculated from the straight line of the solubility diagrams according to the equation.

$$K_C = \frac{\text{Slope}}{\text{Intercept (1-slope)}}$$

2 Preparation of Wet Kneaded Mixtures

2.1 Indomethacin and β-Cyclodextrin Systems

Indomethacin and β-cyclodextrin were accurately weighed in certain ratios as shown in Table 6. The preparations were prepared in a mortar with pestle. First β-cyclodextrin was suspended in certain volumes of water as shown in Table 6. Small portions of indomethacin were then added and continuously kneaded with pestle for 30, 60 and 90 minutes respectively, or until the mixtures were turned to be harden pastes. (While kneading, the mixtures were going to be harden) The creamy products were dried and passed through No.25 mesh screen. The granules were dried in the oven at 60 °C for 3 hours. (Heraeus instruments) The dried granules were screened through No.30 mesh screen and kept in close containers for further studies. The wet kneaded mixtures were prepared both at ambient temperature and at 60 °C of water bath

2.2 Piroxicam and β-Cyclodextrin Systems

Piroxicam and β-cyclodextrin were wet kneaded in the same manner as indomethacin and β-cyclodextrin systems. Piroxicam and β-cyclodextrin were accurately weighed in certain ratios and wet kneaded

with volumes of water as shown in Table 7. The colors of wet kneaded mixtures were changed from off-white to yellowish pastes due to tautomerism of piroxicam. The mixtures were dried in the oven and passed through No.25 mesh screen. The granules were dried at 60 °C for 3 hours. After drying, the color of granules changed from yellowish to creamy granules. The granules were kept in closed containers for further studies. The wet kneaded mixtures were prepared both at ambient temperature and at 60 °C of water bath.

Table 6 Indomethacin and β-Cyclodextrin Systems

For 1:1 molar ratio

indomethacin (gm)	β-cyclodextrin (gm)	vol. of water (ml)	kneading time (minutes)
5	18.23	11.6 (50 %w/w)	30, 60, 90
5	18.23	23.2 (100 %w/w)	30, 60, 90
5	18.23	34.8 (150 %w/w)	30, 60, 90

For 1:2 molar ratio

indomethacin (gm)	β-cyclodextrin (gm)	vol. of water (ml)	kneading time (minutes)
5	36.46	20.7 (50 % w/w)	30, 60, 90
5	36.46	41.4 (100 % w/w)	30, 60, 90
5	36.46	62.1 (150 % w/w)	30, 60, 90

Table 7 Piroxicam and β-Cyclodextrin Systems

For 1:1 molar ratio

piroxicam (gm)	β-cyclodextrin (gm)	vol. of water (ml)	kneading time (minutes)
4	15.74	9.9 (50 % w/w)	30, 60, 90
4	15.74	19.8 (100 % w/w)	30, 60, 90
4	15.74	29.7 (150 % w/w)	30, 60, 90

For 1:2 molar ratio

piroxicam (gm)	β-cyclodextrin (gm)	vol. of water (ml)	kneading time (minutes)
4	31.48	17.7 (50 % w/w)	30, 60, 90
4	31.48	35.4(100 % w/w)	30, 60, 90
4	31.48	53.1(150 % w/w)	30, 60, 90

3 Preparation of Ground Drugs

Indomethacin and piroxicam were separately kneaded in a mortar with pestle in the same manner as wet kneaded procedure but excluded β-cyclodextrin and water. The ground drugs were kneaded for 90 minutes.

4 Preparation of Physical Mixtures

The physical mixtures of drug and β-cyclodextrin were prepared by simple blending in a mortar with pestle without the aid of water. The ground indomethacin, piroxicam (powder from 3) and β-cyclodextrin were accurately weighed to have weights listed in Table 6 and 7. Separately

mixed the ground drug with β-cyclodextrin in the proportion of both drugs: β-cyclodextrin 1:1 and 1:2 molar ratio. The mixtures were blended for 10 minutes and kept in closed containers for further studies.

5 Preparation of Ground Drugs with 1.0 %w/w Sodium Lauryl Sulfate

The ground indomethacin (powder from 3) were mixed with 1.0 % w/w sodium lauryl sulfate by using mortar and pestle. The sodium lauryl sulfate was used both in solution and in powder forms. For solution form, the ground indomethacin were mixed with solution of sodium lauryl sulfate with mortar and pestle.

The ground piroxicam (powder from 3) were mixed with 1.0 % w/w sodium lauryl sulfate prepared by dissolving sodium lauryl sulfate in aqueous PVP K 30 solution.

6 Preparation of Indomethacin, Piroxicam Freeze-dried Inclusion Complex

Indomethacin-β-cyclodextrin and piroxicam-β-cyclodextrin inclusion complexes were prepared as a comparative study for the characterization of the wet kneaded mixtures. The freeze-dried method was used in the preparation.

Method of preparation inclusion complex

Dissolved exact amount of β-cyclodextrin (as shown in Table 8) in about 25 ml. of water, then warmed the suspension to about 60 °C until β-cyclodextrin was totally dissolved. Exact amounts of drugs (Table 8) were added and mixed on magnetic stirrer. Ammonium hydroxide solution

was added drops by drops until drugs were dissolved (Kurozumi, M. et al. 1975; Bettinetti, G.P. 1988 and Pasini, M. 1990). The solutions were then freeze-dried. (Triphilizer FTS, U.S.A.)

Table 8 Indomethacin, Piroxicam freeze-dried inclusion complex

Indomethacin-β cyclodextrin inclusion complex		
materials	1:1 molar ratio	1:2 molar ratio
indomethacin β-cyclodextrin	100 mg 365 mg	100 mg 730 mg
Piroxicam-β-cyclodextrin inclusion complex		
materials	1:1 molar ratio	1:2 molar ratio
piroxicam β-cyclodextrin	80 mg 315 mg	80 mg 630 mg

Preparation of Indomethacin Capsules

The wet kneaded mixtures of indomethacin-β-cyclodextrin (mixtures from 2.1), the physical mixtures (mixtures from 4), the mixtures of ground indomethacin with 1.0 % w/w sodium lauryl sulfate both in solution and in powder forms (mixture from 5) were formulated into capsules with the compositions as shown in Table 9 and 10.

Table 9 The Compositions of Indomethacin Capsules:

For indomethacin: β-cyclodextrin

Ingredients	1:1 molar ratio	1:2 molar ratio
indomethacin*	25.0 mg	25.0 mg
β-cyclodextrin*	91.0 mg	182.0 mg
lactose	140.0 mg	50.0 mg
stearic acid	2.0 mg	2.0 mg

*the mixtures prepared according to (2.1)

Table 10 Indomethacin ground drug with 1.0 % w/w sodium lauryl sulfate

Indomethacin*	25.0 mg
sodium lauryl sulfate*	0.25 mg
Lactose	240.0 mg
Stearic acid	2.0 mg

*the mixtures prepared according to (5)

(The formulation of mixture of indomethacin ground drug and lactose was the same as that of mixture of indomethacin and 1.0 % w/w sodium lauryl sulfate except without sodium lauryl sulfate.)

Method of preparation of indomethacin capsules

The required quantities of the wet kneaded mixtures, the physical mixtures and the ground indomethacin were mixed with lactose in closed container for 5 minutes. Then stearic acid was added and mixed altogether for another 3 minutes. The final mixtures were then filled in capsule No. 2

using semiautomatic capsule filling machine. (Panviv A 01, Union Chemical and Surgical). In case of using 1.0 % w/w sodium lauryl sulfate solution, firstly kneaded the ground indomethacin with sodium lauryl sulfate solution, then sieved through No.30 mesh screen, dried in the oven at 60 °C for 3 hours. The granules were mixed with lactose and stearic acid in the same manner as mentioned above and filled in capsule No. 2. Concomitant prepared the capsules using 1.0 % w/w sodium lauryl sulfate in powder form by mixing ground indomethacin with sodium lauryl sulfate in a motar with pestle and then formulated into capsules in the same manner as above. The capsules were evaluated for their properties, weight variation, content and dissolution.

Preparation of Piroxicam Tablets

The compositions of piroxicam tablets were shown in Table 12. Wet granulation method was used in the preparation of piroxicam tablets. Piroxicam-β-cyclodextrin wet kneaded mixtures (mixtures from 2.2), physical mixtures (mixtures from 4), and ground piroxicam were separately mixed with starch, lactose and half portion of sodium starch glycolate in a mortar. PVP K 30 aqueous solution, a granulating agent, were added and mixed with pestle for 10 minutes. The mixtures were sieved through No.16 mesh screen, dried the granules in the oven at 60 °C for 3 hours. The dried granules were sieved through No.18 mesh screen. Another portion of sodium starch glycolate, magnesium stearate and talcum was added to the dried granules and mixed together in plastic bag for 10 minutes, then compressed into tablets using single punch tabletting machine equipped with flat-faced punch 10 cm. in diameter. For the preparation of piroxicam tablets using 1.0 % w/w sodium lauryl sulfate as wetting agent, the tablets

were prepared in the same manner as before but PVP K 30 solution was mixed with sodium lauryl sulfate before using as a granulating agent. The prepared tablets were evaluated for their properties, weight variation, content, disintegration and dissolution.

Piroxicam tablets, preparing from the ground piroxicam powder mixed with starch and lactose, were formulated as previous. But replacing β-cyclodextrin with the mixture of starch and lactose in the proportion of 1:2 w/w (starch 80 mg: lactose 160 mg per tablet)

Table 11 Formulation of Indomethacin Capsules and Preparation Conditions

Rx	molar ratio	vol. of water (ml)	kneading time (min.)	kneading condition
I-1	1:1	11.6 (50 %)	30	ambient
I-2	1:1	11.6 (50 %)	60	ambient
I-3	1:1	11.6 (50 %)	90	ambient
I-4	1:1	23.2 (100 %)	30	ambient
I-5	1:1	23.2 (100 %)	60	ambient
I-6	1:1	23.2 (100%)	90	ambient
I-7	1:1	34.8 (150 %)	30	ambient
I-8	1:1	34.8 (150 %)	60	ambient
I-9	1:1	34.8 (150 %)	90	ambient
I-10	1:1	11.6 (50 %)	20	water bath 60 °C
I-11	1:1	23.2 (100 %)	30	water bath 60 °C
I-12	1:1	23.2 (100 %)	55	water bath 60 °C
I-13	1:1	34.8 (150 %)	30	water bath 60 °C

Table 11(cont.) Formulation of Indomethacin Capsules and Preparation Conditions

Rx	molar ratio	vol. of water (ml)	kneading time (min)	kneading condition
I-14	1:2	20.7 (50 %)	30	ambient
I-15	1:2	20.7 (50 %)	60	ambient
I-16	1:2	20.7 (50 %)	90	ambient
I-17	1:2	41.4 (100 %)	30	ambient
I-18	1:2	41.4 (100 %)	60	ambient
I-19	1:2	41.4 (100 %)	90	ambient
I-20	1:2	62.1 (150 %)	30	ambient
I-21	1:2	62.1 (150 %)	60	ambient
I-22	1:2	62.1 (150 %)	90	ambient
I-23	1:2	20.7 (50 %)	15	water bath 60 °C
I-24	1:2	41.4 (100 %)	30	water bath 60 °C
I-25	1:2	62.1 (150 %)	30	water bath 60 °C
I-26	1:2	62.1 (150 %)	50	water bath 60 °C

The following formulas were prepared as a comparative study	
I-27(p)	indomethacin ground drug mix with 1.0 % w/w sodium lauryl sulfate (powder)
I-27(s)	indomethacin ground drug mixes with 1.0 % w/w sodium lauryl sulfate (solution)
I-28	indomethacin: β-cyclodextrin physical mixture 1:1 molar ratio
I-29	indomethacin: β-cyclodextrin physical mixture 1:2 molar ratio
I-30	indomethacin ground drug mix with lactose

Table 12 The Composition of Piroxicam Tablets:

For piroxicam : β-cyclodextrin

Ingredients	1:1 molar ratio	1:2 molar ratio
piroxicam*	20.0 mg	20.0 mg
β-cyclodextrin*	78.7 mg	157.4 mg
starch	55.0 mg	30 .0 mg
lactose	110.0 mg	60.0 mg
sodium starch glycolate	22.3 mg	22.3 mg
PVP K30	3.0 mg	3.0 mg
talc	3.0 mg	3.0 mg
magnesium stearate	1.5 mg	1.5 mg

*the mixtures prepared according to (2.2)

Table 13 Formulation of Piroxicam Tablets and Preparation Conditions

Rx	molar ratio	vol.of water (ml)	kneading time (minutes)	kneading condition
P-1	1:1	9.9 (50 %)	30	ambient
P-2	1:1	9.9 (50 %)	60	ambient
P-3	1:1	9.9 (50 %)	90	ambient
P-4	1:1	19.8 (100%)	30	ambient
P-5	1:1	19.8 (100%)	60	ambient
P-6	1:1	19.8 (100%)	90	ambient
P-7	1:1	29.7 (150%)	30	ambient
P-8	1:1	29.7 (150%)	60	ambient

Table 13(cont.) Formulation of Piroxicam Tablets and Preparation Conditions

Rx	molar ratio	vol.of water (ml)	kneading time (minutes)	kneading condition
P-9	1:1	29.7 (150%)	90	ambient
P-10	1:1	9.9 (50 %)	7	water bath 60 °C
P-11	1:1	19.8 (100%)	17	water bath 60 °C
P-12	1:1	29.7 (150%)	25	water bath 60 °C
P-13	1:2	17.7 (50 %)	30	ambient
P-14	1:2	17.7 (50 %)	60	ambient
P-15	1:2	19.8 (100%)	30	ambient
P-16	1:2	35.4 (100%)	60	ambient
P-17	1:2	35.4 (100%)	90	ambient
P-18	1:2	53.1 (150%)	30	ambient
P-19	1:2	53.1 (150%)	60	ambient
P-20	1:2	53.1 (150%)	90	ambient
P-21	1:2	17.7 (50 %)	10	water bath 60 °C
P-22	1:2	35.4 (100%)	18	water bath 60 °C
P-23	1:2	53.1 (150%)	30	water bath 60°C
P-24	1:2	53.1 (150%)	50	water bath 60 °C
The following formulas were prepared as a comparative study				
P-25	piroxicam: β-cyclodextrin physical mixture 1: 1 molar ratio			
P-26	piroxicam: β-cyclodextrin physical mixture 1: 2 molar ratio			
P-27	piroxicam ground drug mix with 1.0 % w/w sodum lauryl sulfate			
P-28	piroxicam ground drug mix with lactose			

The piroxicam tablets, using the piroxicam raw material supplied from Asian Drug and Chemical, source from Hong Kong were prepared in the same manners according to the formulas P-1, P-13, P-25, P-27, P-28

Characterization of The Mixtures

1. Scanning Electron Microscope

Electron photomicrographs of the ground drugs, β-cyclodextrin, the wet kneaded mixtures and physical mixtures were observed by using scanning electron microscope. (Jeol JSM-T 220A, Japan) The samples were coated with gold using ion sputtering before microscopic examination.

2. Infrared Spectrophotometer

Infrared spectra of the ground drugs, β-cyclodextrin, the wet kneaded mixtures and physical mixtures were measured using infra-red spectrophotometer. (Perkin Elmer 654) All samples were in the form of potassium bromide discs. The scanning time was 6 minutes.

3. Differential Scanning Calorimeter

DSC Thermograms were obtained from Mettler Thermal Analyzer. (Mettler TA 3000 equipped with DSC 20) The instrument was calibrated with indium. All measurements were carried out using 3-7 mg of samples placed in aluminium pan and crimp. Scanning was performed at the temperature range of 50-250 °C with the scanning rate 20 °K/min.

4. X-ray Diffractometer

Samples were investigated by X-ray diffractometer. (JDX-8030, Japan) All diffraction spectra were scanned from 5 - 40 ° in term of the

2 θ angle.

5. Wettability

The method used in the wettability studied was an upward migration of water in a small glass column packed with 1.0 gm. of samples. The ground drugs, physical mixtures and wet kneaded mixtures were filled in glass columns. The upward migration of colored water through the capillary ending was measured in a function of time.

Tablets and Capsules Evaluations

1 Weight variation

Twenty units of capsules and tablets were individual weighed. The average weight and standard deviation were calculated.

2 Hardness

The hardness of indomethacin tablets was controlled in the range of 3-6 Kp. The hardness was measured using hardness tester. (Schleuniger, Switzerland) Mean of five determinations was calculated.

3. Friability

Twenty tablets were weighed and inserted into a friabilator (Erweka friabilator), revolving 100 times in 4 minutes. At the end of the period of time, tablets were reweighed and the percentage of friability was calculated.

4 Disintegration

Disintegration was determined according to USP method. The tablets were placed in each tube of the apparatus. The apparatus was

operated using water as the medium and maintained at 37± 1 °C. The time was recorded when all tablets (6 determinations) were totally disintegrated.

5 Content of Indomethacin

The content of indomethacin was spectrophotometrically assayed. Accurately weighed and dissolved about 50 mg of indomethacin in 10 millilitres of water, allowed to stand for 10 minutes and swirled occasionally. Added 75 millilitres of methanol, shook well, added sufficient methanol to produce 100.0 millilitres and filtered. To five millilitres of the filtrate added sufficient volume of a mixture of equal volumes of methanol and phosphate buffer pH 7.2 to produce 100.0 millilitres. Measured the maximum absorbance of the resulting solution at 318 nm. The content of indomethacin was calculated from the standard curve prepared in the same manner.

6 Content of Piroxicam

The content of piroxicam was spectrophotometrically assayed. Accurately weighed and dissolved about 50 mg of piroxicam and dissolved in 70 millilitres of 0.01 N methanolic hydrochloric acid solution, shook for 30 minutes and diluted to 100.0 millilitres with the same medium, filtered if necessary. Diluted two millilitres of the filtrate with the same solution to 100.0 millilitres and measured the absorbance of the resulting solution at 333 nm. The content of piroxicam was calculated from the standard curve prepared in the same manner.

7 Dissolution of Indomethacin Capsules

The dissolution of indomethacin capsules was determined according to the USP method, using rotating basket. The dissolution medium was 750 millilitres of phosphate buffer pH 7.2:water=1:4, warmed at 37±0.5 °C. The baskets were rotated at 100 rpm. At time interval 5, 10, 15, 20, 25 and 30 minutes, successive portions of 5.0 millilitres of sample solution were withdrawn and analyzed for indomethacin content by spectrophotometer at maximum absorbance at 318 nm. Five millilitres of prewarmed dissolution medium was replaced after each sampling to maintain constant volume of dissolution medium during the assay. The amount of indomethacin dissolved was calculated from the standard curve prepared with the same medium. The dissolution profile of drug dissolved was obtained by plotting the percentage of drug dissolved versus time.

(Phosphate buffer pH 7.2 : Placed 50 millilitres of 0.2 M potassium phosphate monobasic solution in a 200 millilitres flask. 0.2 M. Sodium hydroxide solution, 37.4 millilitres, was added and adjusted to volume with water)

8. Dissolution of Piroxicam Tablets

The dissolution of piroxicam tablets was determined by the USP method, using rotating basket. The dissolution medium was 900 millilitres of simulated gastric fluid without pepsin*, warmed at 37±0.5 °C. The baskets were rotated at 50 rounds per minute. At time intervals 5, 10, 15, 25, 35, 45 and 55 minutes., successive portions of 5.0 millilitres of sample solution were withdrawn and analyzed for piroxicam content by spectrophotometer at maximum absorbance at 333 nm. Five millilitres of prewarmed dissolution medium were replaced after each sampling to

maintain constant volume of dissolution medium during the assay. The amount of piroxicam dissolved was calculated from the standard curve prepared with the same medium. The dissolution profile of drug dissolved was obtained by plotting the percentage of drug dissolved versus time.

(* Simulated gastric fluid without pepsin: Dissolved 2 gm. of sodium chloride in 7.0 millilitres of hydrochloric acid and added sufficient water to make 1000 millilitres. The solution has pH about 1.2)

9. Effect of Aging

Indomethacin capsules and piroxicam tablets were kept in loosely closed bottles, stored at room temperature and at 45°C/ 75% relative humidity for 3 months. The dissolution and stability of the dosage forms were performed and compared to the freshly prepared dosage forms. The dissolution was performed in the same manner as above. The stability of each drug was analyzed using high performance liquid chromatography. (Spectra Physics SP 4000) The high performance liquid chromatographic method for indomethacin and piroxicam were modified from the United States Pharmacopoeia XXII. The sample preparations of indomethacin capsules and piroxicam tablets were prepared by dissolving in the mobile phase to obtain concentration as described below.

HPLC condition for testing stability of indomethacin capsules

Mobile phase	: Methanol: 0.1 % v/v Phosphoric acid 75:25
Column	: Novapak C 18, 4µ Water Associated
	3.9x150 mm. Spherical
Detection	: UV 240
Flow rate	: 1.0 millilitre/minute.
Chart speed	: 0.5 cm/minute.

Injection volume : 10 µl

Sample concentration : 50 µg/millilitre in mobile phase.

HPLC condition for testing stability of piroxicam tablets

Mobile phase : Methanol: Phosphate-citrate buffer*, 70:30

Column : Spherisorb 10 ODS, 4.6x250 mm, Phenomenex

Detection : UV 254

Flow rate : 1.5 millilitres /minute.

Injection volume : 10 µl

Sample concentration : 50 µg/ millilitres in mobile phase

*Phosphate-citrate buffer : Separately dissolved 7.72 gm of anhydrous citric acid in 400 millilitres of water, 5.35 gm of dibasic sodium phosphate in 100 millilitres of water. Added the phosphate solution to the citric acid solution, diluted with water to make 1000 millilitres and mixed.