CHAPTER II

MATERIALS AND METHODS

Materials

The following materials were obtained from commercial sources except for α-,δ- and heptakis-2,6-di-O-methyl-β-cyclodextrins (DIMEB) were kindly donated by Chiba University (Japan) and used as received. All other materials and solvents were of analytical reagent grade. Deionized water was used throughout this study.

1. Model Drug

Indomethacin (Batch No. 850201, China National Chemicals Imp & Exp Corp., China)

2. Carriers

α-cyclodextrin (Lot No. Unknown, Nakarai Tesque, Co., Ltd., Japan)

β-cyclodextrin (Lot No. 23102, Rama Production Co., Ltd., Thailand)

δ-cyclodextrin (Lot No. Unknown, Mercian, Co., Ltd., Japan)

DIMEB (Lot No. Unknown, Toshin Chemical, Co., Ltd., Japan)

3. **Capsule Excipients**

Lactose (Lot No. Unknown, Wyndale, New Zealand)

Corn Starch (Lot No. Unknown, Pasach Panich, Thailand)

Stearic acid (Lot No. ACL06, Srichand United Dispensary Co., Ltd.,)

4. **Miscellaneous**

Absolute ethanol (Merck, Germany)

Sodium hydroxide (Merck, Germany)

Potassium dihydrogen phosphate (Merck, Germany)

Methanol (Merck, Germany)

Capsule number 2 (Hiap Heng Pharmacy Ltd., Part., Thailand)

Equipments

1. Analytical Balance (Sartorius, Model A200s, Germany)
2. Hot Air Oven (Memmert, Type UL80, Germany)
3. US. Standard Sieve No.40,60 mesh (Endecotts Ltd., London, England)
4. Water Bath and Hot Plate
5. Rotary Evaporator (CH-9230, Büchi Laboratoriums, Technik AG, Switzerland)
6. Dissolution Apparatus (Hanson Research, Model SR2, U.S.A.)
7. Spectrophotometer (Bausch & Lomb, Spectronic-2000, U.S.A.)
8. Automatic Voltage Stabilizer (Quasar, Model AVS-4002B, U.S.A.) Equip with Spectronic-2000
9. Corn Wall Syringe (IPAS, Thailand)

10. Vortex mixer (Vortex Genie-2, Model G-500E, Scientific Industries Inc., Bohermia, New York, U.S.A.)

11. Disintegration Apparatus (Hanson Research, U.S.A.)

12. Shaking Water Bath (JULABO D763B, SW-1, West Germany)

13. Capsule Filling Machine (Model PANVIV A01 No. 230, Union Chemical and Surgical, Thailand)

14. Ultrasonic Bath (Branson B321, Swithkline Co., U.S.A.)

15. Microprocessor Bench pH Meter (HI 8417, Hanna Instruments, U.S.A.)

16. Scanning Electron Microscope (JEOL JSM-T220A, Japan)

17. Differential Thermal Analysis (Shimadzu, Model DT-30, Japan)

18. IR Spectrophotometer (Perkin Elmer, 1760X, U.S.A.)

19. IR Spectrophotometer (Perkin Elmer, Model 283, U.S.A.)

20. X-ray Diffractometer (JDX-8030, Japan)

Methods

1. **Preparation of grounded mixtures**

IDM and carriers were accurately weighed in certain ratios as shown in Table 7 . The grounded mixture was prepared by gently trituating in a glass mortar with a pestle and mixed for 10 minutes. Then the mixture was kept in an amber glass bottle and stored in a desiccator.

2. **Preparation of kneaded mixtures**

IDM and carriers were accurately weighed in the ratios as shown in Table 7 . The kneaded mixture was prepared by gently trituating in a glass mortar with a pestle and mixed for 10 minutes. Then water (0.4 times of total weight) was added and kneaded for another 10 minutes to obtain homogeneous paste. During the process, an appropriate quantity of water was added to the mixture in order to maintain a suitable consistency. The paste was passed through a 30-mesh sieve and dried in an incubator at 45 °C overnight. Then, the kneaded mixture was grounded and screened through a 40-mesh cut and collected the size of 40/60-mesh sieve in an amber glass bottle and stored in a desiccator for further studied.

3. **Preparation of solvent mixtures**

α-CD, β-CD and δ-CD solvent mixtures were prepared by solvent deposition method. DIMEB solvent mixtures were prepared by

Table 7 The ratios of IDM : Carriers used in the preparations.

Type of carriers	Method of preparations		
	GM*	KM*	SM*
α-CD	1:0.5	1:0.5	-
	1:1	1:1	1:1
	1:2	1:2	1:2
	1:3	1:3	1:3
β-CD	1:0.5	1:0.5	-
	1:1	1:1	1:1
	1:2	1:2	1:2
	1:3	1:3	1:3
	1:6	1:6	-
	1:10	1:10	-
γ-CD	1:0.5	1:0.5	-
	1:1	1:1	1:1
	1:2	1:2	1:2
	1:3	1:3	1:3
DIMEB	1:0.5	1:0.5	-
	1:1	1:1	1:1
	1:2	1:2	1:2
	1:3	1:3	1:3

* GM = Grounded mixtures

KM = Kneaded mixtures

SM = Solvent mixtures

coevaporation method.

3.1 Solvent deposition method

IDM-carrier mixtures were prepared by dissolving IDM in sufficient absolute ethanol and uniformly wetting carriers in the quantity as shown in Table 7 in a mortar for 10 minutes. The wet mass was passed through a 30-mesh sieve and dried in a desiccator (27 °C) overnight, then pulverized and passed through a 40-mesh sieve to collect the size of 40/60 mesh cut in an amber glass bottle and stored in a desiccator.

3.2 Coevaporation method

IDM and DIMEB was mixed in a mortar with a pestle for 10 minutes, then the mixture was dissolved in 60 ml of absolute ethanol to obtain a clear solution. The solvent was removed under vacuum in a rotary evaporator. After the evaporation was completed, the resulting coevaporated mixture was kept in the desiccator at 45 °C until obtained the constant weight. Thereafter, the coevaporated mixture was pulverized and screened through a 40-mesh sieve and selected the size of 40/60 cut sieve in an amber glass bottle and stored in a desiccator.

4. Preparation of grounded drug, kneaded drug and treated drug

The grounded drug, kneaded drug and treated drug were

prepared by using the same procedure as for the preparation of grounded mixtures, kneaded mixture and coevaporation method respectively but excluded the carrier.

5. Preparation of IDM Capsules

Selected grounded mixture, kneaded mixture and coevaporate mixture including pure IDM, grounded drug, kneaded drug and treated drug was filled into capsules by the following procedure.

The composition of each formula was represented in Table 8 . The required quantity of grounded mixture, kneaded mixture, coevaporate mixture, pure IDM, grounded drug, kneaded drug and treated drug was each mixed geometrically with the required lactose and corn starch in a plastic bag for 5 minutes. Then stearic acid was added in and mixed together for another 3 minutes. The final mixture was filled into number 2 capsule by Capsule Filling Machine. The prepared capsules were kept in an amber glass containers and stored in a desiccator for further investigations.

Table 8 The composition of IDM capsules.

Ingredients	Weight(mg/capsule)
IDM*	25
lactose	180
corn starch	30
stearic acid	5

* as pure IDM, grounded drug, kneaded drug, treated drug, grounded mixture, kneaded mixture and coevaporated mixture.

6. Calibration curve of IDM

IDM was accurately weighed and dissolved in 5 ml of methanol, then the solution was adjusted to 100 ml with phosphate buffer solution of pH 7.2 and used as stock solution.

The stock solution was precisely pipetted into a volumetric flask and diluted to volume with a mixture of phosphate buffer solution of pH 7.2 : deionized water in ratio 1:4. The final concentration of each solution was recorded.

The absorbance of known drug concentration was determined by a double beam spectrophotometer in a 1-cm cell at the wavelength of 318 nm and used a mixture of 1:4 phosphate buffer of pH 7.2 : deionized water as a blank solution. Each concentration was determined in triplicate.

The calibration curve using in the analysis of content uniformity of capsule was prepared in the same procedure as the calibration curve of IDM but used phosphate buffer of pH 7.0 instead of phosphate buffer of pH 7.2 and substituted a mixture of 1:4 phosphate buffer of pH 7.2 : deionized water to a mixture of 1:1 phosphate buffer of pH 7.0 : Methanol to meet the procedure according to USP XXII. Each concentration was determined in triplicate.

7. Content Uniformity Test

IDM capsule was weighed and transferred the content into a volumtric flask. Added 10 ml of water and allowed to stand for 10 minutes with occasionly swirled. Then 60 ml of methanol was added and allowed to stand another 10 minutes. The solution was adjusted to 100 ml with methanol and filtered. The filtrate was pipetted and diluted with 1:1 of phosphate buffer solution of pH 7.0 : methanol. Thereafter, the solution was assay spectrophotometrically at the wavelength of 318 nm. Ten capsules were evaluated.

8. Dissolution studies

Dissolution studies of the powders were performed by using USP XXII paddle method. Seven hundred and fifty milliliters of solution of 4:1 deionized water and phosphate buffer of pH 7.2 solution was employed as a dissolution medium. The paddle was adjusted to rotate at 100 rpm. Five milliliters of dissolution medium were pipetted out at the predetermined interval of 2,4,6,8,12,16,20,25,30,35,40,50 and 60 minutes and passed through membrane filter of 0.45 um. The equivalent volume (5 ml) of fresh dissolution medium at 37 $^{\circ}$C was replaced at each time interval of withdrawal.

The solutions were assayed spectrophotometrically at 318 nm. The sample concentrations were calculated from the calibration curve (Fig. 75 ,page 169) the records came from six determinations.

The dissolution test of IDM capsules were performed according to the dissolution of IDM capsule, USP XXII basket method. The procedure was similar to that described in dissolution studies for powders.

9. Disintegration test

The disintegration test of IDM capsule was studied by using USP Disintegration Apparatus. The medium was deionized water kept at 37 °C. The time was recorded when all capsule completely disappeared. The data was the average of six determinations.

10. Solubility studies

Solubility studies was performed according to the method of Higuchi and Connors (Higuchi and Connors, 1965). Excess amount of IDM was added in 1:4 of phosphate buffer of pH 7.2 : deionized water containing various concentrations of α-,β-,δ-CD and DIMEB. The concentrations of α-, δ-CD were 0,0.02,0.04,0.06,0.0,0.1 M and β-CD, DIMEB were 0,0.004,0.008,0.012,0.016,0.02 M. These mixtures were shaken at 37 °C and equilibrated for 48 hours (The preliminary test shown that the equilibrium was obtained about 36 hours). Then the solution was passed through 0.45 um FH type filter and pipetted into a volumetric flask. After diluted to volume with phosphate buffer solution of pH 7.2 : deionized water (1:4), the solution was analyzed spectrophotometrically at the UV maximum (318 nm) of IDM, as the effect of cyclodextrins on the absorbance of IDM was negligible, two trials were determined.

11. Particle Appearance

The size and appearance in powder form of IDM, grounded drug, kneaded drug, treated drug, 1:3 ratio of grounded mixtures, kneaded mixtures, solvent mixtures including pure carriers were studied by using an Scanning Electron Microscope. The samples were coated with gold using ion sputtering before they were examined, then photographed at appropriate magnification scale.

12. Differential Thermal Analysis study

The powders were investigated for its melting point by a Differential Thermal Analyzer. Powder was accurately weighed, and put into the equipment using a given conditions.

Sample size = 7-8 mg

Heating rate = 10 °C per minute

Sensitivity = ± 50 μv

Atmosphere = N_2, 30 ml per minute

Chart speed = 2.5 mm/min

13. Infrared (IR) Absorption study

IR spectra were measured by the KBr disc method. The samples were IDM, treated IDM, 1:3 ratio of grounded mixtures, kneaded mixtures, solvent mixtures and pure carriers. The quantity of samples were about 10 mg and KBr was 1 g

14. Powder X-Ray Diffraction study

IDM, treated IDM, DIMEB, 1:3 ratio of kneaded mixture and coevaporate of IDM:DIMEB were investigated by the X-Ray Diffratrometer which used target Cu, voltage 45.0 ku and scanning from 5-40° with 2θ.