CHAPTER II

MATERIALS AND METHODS

Materials

The following substances were obtained from commercial sources.

1. Model drug

Indomethacin (lot no 850602 China National - Chemical Imp. Exp. Corp. China)

2. Carriers

Mannitol (Vidhayasom, Thailand)

Polyethylene Glycol 4000 (Nippon oil & Fat, Japan)

Polyvinylpyrrolidone K 30 (BASF, West Germany)

Sodium Lauryl Sulfate (Pasach Panich, Thailand)

3. Capsule excipients

Lactose (Rama Production, Thailand)

Corn starch (Pasach Panich, Thailand)

Stearic acid (Pasach Panich, Thailand)

4. Others

Absolute ethanol AR grade (Merck, Germany)

Sodium hydroxide AR grade (Merck, Germany)

Dihydrogen potassium phosphate AR grade (Merck, Germany)

Capsule number 2 (Lab manter, Thailand)

Methanol AR grade (Merck, Germany)

All materials were used without further purification and deionized water was used throughout this study.

Apparatus

Analytical balance (Sartorius, Germany)

Water bath and hot plate

Blender (Molinex, type 241, France)

Hot Air Oven (Memmert, type ULSO, Germany)

U.S. standard sieve series no. 40,60 mesh (Endecotts Ltd. London, England)

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

Dissolution Apparatus (Hanson Research, U.S.A.)

Spectrophotometer (Spectronic 2000, Bauch & Lomb, U.S.A.)

Scanning Electron Microscope (Jeol, JSM-35 CF, Japan)

Differential Thermal Analyzer (Shimadzu, Model DT-30, Japan)

IR Spectrophotometer (Shimadzu, Model 1R 440, Japan)

X-ray Diffractrometer (Jeol, Japan)

Sonicator (Bransonic 321, Smithkline, U.S.A.)

Oswald viscometer (Thomas, CAT. No. 7162, U.S.A.)

Tensiometer K 6 (Kruss, Germany)

Glass tube with 2.5 mm diameter and 5 cm length

Pycnometer (EXELO, BS733)

Methods

1. Preparation of IDM coprecipitates

IDM coprecipitates were prepared by dissolving quantities of IDM and carriers, presented in Table 7, in 60 ml of absolute ethanol. The solution was mixed thoroughly and the solvent was allowed to evaporate continuously under air stream. The resulting coprecipitates were placed in an incubator overnight. Then, the drug coprecipitates were ground and screened through a # 40 mesh sieve and kept in a desiccator.

2. Preparation of IDM physical mixture

The required amounts of IDM and carrier, shown in Table 7, were thoroughly mixed in a closed cylindrical container for 5 minutes. Thereafter, the mixture was sieved through a # 40 mesh sieve and stored in a desiccator.

Table 7 The amount of IDM and carriers in a given preparation

ratio	1:1	1:5	1:10
Material	weight (gm)	weight (gm)	weight (gm)
IDM	2.5	2.5	2.5
Mannitol	2.5	12.5	25
PEG 4000	2.5	12.5	25
PVP K30	2.5	12.5	25
SLS*	0.25	1.25	2.5

*only for the ratios of SLS were 1:0.1, 1:0.5 and 1:1.0 respectively

3. Preparation of treated IDM

The treated IDM was prepared by using the same procedure as in the preparation of IDM coprecipitates but excluded the carrier.

4. Preparation of IDM capsules

Each IDM coprecipitates, IDM physical mixture, pure IDM or treated IDM was incorporated into capsules by the following procedure.

The required quantity of coprecipitate, physical mixture, pure IDM or treated IDM was mixed geometrically with the required lactose, in a closed cylindrical container for 5 minutes. Corn starch and stearic acid were then added into and mixed together for another 5 minutes. The final mixture was passed through a # 40 mesh sieve and was filled into number 2 capsules.

The composition of each formula was appeared in Table 8 The prepared capsules were kept in amber glass containers and stored in a desiccator for further studies.

5. Calibration curve of IDM

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

The stock solution was precisely pipetted into a volumetric flask and then diluted to volume with 1:4 phosphate buffer of pH 7.2 : deionized water. 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. The 1:4 phosphate bufer of pH 7.2 : deionized water was used as a blank solution. Each concentration was determined in triplicate.

6. Content uniformity test

IDM capsule was weighed and then put into a volumetric flask and adjusted to 100 ml with 1:4 phosphate buffer of pH 7.2 : deionized water. The solution was filtered through a paper filter. The filtrate was assayed spectrophotometrically at the wavelength of 318 nm. Six capsules were evaluated.

7. Disintegration test

The disintegration test of IDM capsule was studied using USP Disintegration Apparatus. The medium was 37 degree celcius water. The time was recorded when all capsule completely disappeared.

8. Dissolution Studies

8.1 Dissolution studies of IDM, treated IDM, IDM coprecipitates, and IDM physical mixturs.

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 used as dissolution medium. The paddle was adjusted to rotate at 100 rpm. Five milliliters of dissolution medium were pipetted out at the interval of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 minutes and passed through membrane filter of 0.45 um. The volume withdrawn at each time interval

Table 8 The composition of IDM capsule.

IDM (as pure IDM, treated IDM, IDM in coprecipitatd* and IDM in physical mixture*)	25 mg
Lactose	80 mg
Corn starch	30 mg
Stearic acid	5 mg

*The only ratio 1:1 of IDM-Mannitol, IDM PEG 4000, and IDM-PVP K30 was investigated. Furthermore, the ratio of 1:0.1 of IDM-SLS was studied.

was replaced by the same quantity of fresh dissolution medium at 37°C.

The sample solutions were assayed spectrophotometrically at 318 nm. The sample concentrations were calculated from the calibration curve (Figure 73, page 189)

8.2 Dissolution studies of corresponding IDM capsules

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

9. Particle Appearance

The appearances in powder form of IDM, treated IDM, all ratios of IDM coprecipitates and all ratios of IDM physical mixtures, including pure carriers were studied by using an Electron Microscope. The samples were coated with gold using ion sputtering before they were examined, then photographed at appropriate magnification scale by a Scanning Electron Microscope.

10. Differential Thermal Analysis Study

Each powder was investigated for its melting point by a Differential Thermal Analyzer. Powder was accurately weighed, and put into the equipment using a given condition.

Heating rate = 10°C per min
Sensitivity = ± 100 μv
Atmosphere = static air
Chart speed = 10 mm per min

11. Powder X-ray Diffraction Study

IDM, treated IDM, carriers and only the ratio of 1:1 of IDM coprecipitates were evaluated by an X-ray Diffractrometer which uses target Cu, voltage 45.0 ku and scanning from 5 - 40° with 2θ.

12. Solubility study

Each powder was weighed accurately of 5 gm and put into a 100 ml glass vial with twenty milliliters of 1:4 phosphate buffer of pH 7.2 : deionized water. The suspension was shaken before putting in a sonicator. This suspension was sonicated for seven hours, then was moved into an incubator at 37°C and stored for twelve hours. The suspension was withdrawn and filtered through a membrane of 0.45 μm, then assayed for IDM spectrophotometrically at 318 nm. After forty-eighth

hour, the suspension was again withdrawn and the previous process was repeated.

13. Infrared (IR) Absorption Study

IR spectra were measured by using a Shimatzu Infrared Spectrometer. The measurements were made by the KBr disc method. The samples that were examined were IDM, treated IDM, pure carrier. In addition the coprecipitate and physical mixture of the 1:1 IDM-carrier were also investigated, except IDM-SLS that was used only the 1:0.1 system.

14. Wettability Study

All types of powders were investigated for their wettabilities. Saturated solution of each powder was to measure of its surface tension and viscosity which were consequently to determine its wettability.

Powder was accurately weighed and put in a glass tube for wettability test. The glass tube was marked the point of starting and ending of solvent front. Then, the powder was compressed into constant volume and put the tube in the horizontal way. The saturated solution was put over the top of compressed powder, and the time that the solvent front moved from the starting point to the ending point was recorded. Data was calculated by using Washburn-Rideal equation (equation 2).

14.1 Viscosity Measurement

The viscosity of the saturated solution of powder was examined by using an ostwald viscometer, (Appendix II). In this case, the relative value was determined. The relative value was compared with standard solvent, deionized water.

14.2 Surface tension study

The tensiometer K9 was used for measuring the surface tension of all solutions. Tensiometer K9 is a ring type method. A platinum ring connected to a balance beam is immersed into a liquid. In order to obtain absolute values of surface tension, the result of the measured interfacial tension was be multiplied by a correction factor (Appendix II).

14.3 Density Study

The saturated solution was used as a sample for density measurement. The pycnometer was used as an instrument for measuring the density. The volume of the pycnometer was 10 milliliters. The solution and pycnometer was accurately weighed by an Analytical balance.

15. The Nuclear Magnetic Resonance Study

The Nuclear Magnetic Resonance was the equipment that used for examining the microviscosity around the drug particle. The relaxation times from all IDM-carrier systems were recorded. Deuterium water (D_2O) was the solvent for dissolving the powder.