

## CHAPTER II

### MATERIALS AND METHODS

#### Materials

The following materials were purchased from commercial sources except low-molecular weight chitosan (LMCS). Deionized water was used through out this study.

#### 1. Model drug

Hydrochlorothiazide (Batch No. CU0201, Bright Trade E company, Hong Kong)

#### 2. Carriers

Chitin (Kyowa Technos Co., Ltd., Japan, distributed by G.T. Chemical Bangkok Thailand)

Chitosan (Kyowa Technos Co., Ltd., Japan, distributed by G.T. Chemical, Bangkok, Thailand)

Chitosan (Unicord co. Ltd., Bangkok, Thailand)

Low molecular weight chitosan (C-13, lot no E0 11-2, kindly supplied by Kurita Water Industries Ltd., Kanagawa, Japan)

Polyvinylpyrrolidone K30 (Batch no 303861, GAF, Singapore)

Polyethylene glycol 4000 (Batch No 70115, Nippon Oil & Fat, Japan)

#### 3. Tablet additives

Magnesium stearate (Peter Greven Fett-Chemic GmbH, Germany)  
Spray dried Lactose (Super-tab<sup>R</sup>, Batch No. 0231104, Wyndale, New Zealand)

Sodium starch glycolate (Explotab<sup>R</sup>, Edward Mendel, USA.)

#### 4. Others

Absolutes ethyl alcohol, analytical grade (E. Merck, Germany)  
 Acetonitrile, HPLC Grade (May & Baker Ltd, England)  
 Ethanol 95% (Liquor division, The excise department, Thailand)  
 Hydrochloric acid analytical grade ( Farmitalia Carloerba,  
 Barcelona, Spain)  
 Hydrochlorothiazide tablets (Dichlortide<sup>R</sup>, lot no.510843, M&H  
 manufacturing Co.,Ltd., Thailand ; brand A )  
 Hydrochlorothiazide tablets (Hydrochlorothiazide<sup>R</sup>, lot no.  
 411308, T.O. chemicals Ltd., Thailand ; brand B)  
 Sodium hydroxide, Puriss (Eka Nobel, Sweden)

#### Apparatus

Analytical balance (Sartorius, Germany)  
 Hot air oven (Mettler, type ULSO, Germany)  
 Fizeau mill (Kan Seng Lee factory Ltd., Thailand)  
 Magnetic stirrer ( Model SP-18420, Nuova 7 Stir-Plate , Sybron  
 Thermolyne, USA)  
 Vortex Genie 2 (Scientific Industries Inc, New York, USA)  
 Mechanical sieve shaker (Josef Deckelmann, Aschattenburg,  
 Western Germany)  
 Sonicator (Bransonic 321, Smithkline, USA)  
 Rotavapor (Type RE-111, Büchi, Switzerland)  
 Ball mill (The Pascall Engineering co., Ltd., England)  
 Vibrating Mill (Model No.118104, Shimadzu corporation, Japan)  
 Swinging Mill Broyeur Oscillant (Model HSM 100-H, Herzog  
 Maschinenfabrik GmbH, Co., Ltd., Germany)  
 Carver press (Model C, Perkin Elmer, USA)  
 Hardness tester (Schleuniger, Germany)  
 Disintegration Apparatus (Hanson-Research, USA)  
 Dissolution Apparatus (Hanson-Research , USA)  
 Spectrophotometer (Spectronic 2000, Bauch & Lomb, USA)  
 Fourier Transform Infrared spectrometer ( Model 1760x, Perkin  
 Elmer, USA)  
 X-ray Diffractometer (Philips, Model PW1130/90, Netherland)  
 Differential Thermal Analyzer (Model DT-30, Shimadzu, Japan)  
 Scanning electron microscope (Model JSM-T 22A, Jeol, Japan)

## Methods

### A. Preparation of Powder Dispersion.

Different dispersion techniques were conducted to obtain powder dispersion. HCTZ was dispersed by four techniques of dispersion consisting of physical (PM), kneading (KM), solvent and solvent deposition (SM,SMD), and ball milling (BM) techniques using different carrier of chitin (CT), chitosan (CS,CSU), low molecular weight of chitosan (LMCS), PVP K30 and PEG 4000 at three ratios of drug : carrier of 1:1, 1:2, 1:3. All of prepared dispersions were illustrated in Table 1.

#### 1. Preparation of HCTZ Physical Mixture

The physical mixture of HCTZ and each carrier of CT, CS, CSU, LMCS, PVP and PEG, in a weight ratio as shown in Table 23 (Appendix II), were prepared by mixing both drug and carrier in mortar with a pestle for 5 minutes as illustrated in Figure 6. The mixture was then screened through a 30-mesh sieve and stored in a desiccator, containing silica gel as absorbent, for further studies.

#### 2. Preparation of HCTZ Kneaded Mixture

The kneaded mixtures of HCTZ and each carrier of CT, CS, CSU, LMCS, PVP and PEG were prepared by kneading method. The scheme of kneading method was illustrated in Figure 7. HCTZ and carrier in a weight ratio as shown in Table 23 (Appendix II) was individually weighed and placed in a mortar. The mixture was kneaded with deionized water in the amount of 1.2 times of powder weight. For PEG, the amount of deionized water was reduced to 0.1-time. The kneaded mixture was passed through a 30-mesh sieve and then the drying process was carried out in an incubator(45°C) overnight except the mixture of PEG was incubated at 25°C . The kneaded mixture of LMCS and PVP had to be dried first before being passed through a sieve due to the overwet mixtures during the preparation. The fraction that was pulverized, passed through a 30-mesh sieve and then kept in a desiccator.

#### 3. Preparation of HCTZ Solvent Dispersion

HCTZ solvent dispersion was prepared by two methods : solvent method and solvent deposition method. The scheme of preparation was illustrated in Figure 8.

Table 1 Classification of all HCTZ prepared dispersions.

CARRIER	RATIO (DRUG/CARRIER)	METHOD			
		PHYSICAL (PM)	KNEADING (KM)	SOLVENT (SM/SMD)	MILLING (BM)
-	1 : 0	HCTZ PM	HCTZ KM	HCTZ SM	HCTZ BM
CHITIN (CT)	1 : 1 1 : 2 1 : 3	1 : 1 CT PM 1 : 2 CT PM 1 : 3 CT PM	1 : 1 CT KM 1 : 2 CT KM 1 : 3 CT KM	1 : 1 CT SMD 1 : 2 CT SMD 1 : 3 CT SMD	1 : 1 CT BM 1 : 2 CT BM 1 : 3 CT BM
CHITOSAN (CS)	1 : 1 1 : 2 1 : 3	1 : 1 CS PM 1 : 2 CS PM 1 : 3 CS PM	1 : 1 CS KM 1 : 2 CS KM 1 : 3 CS KM	1 : 1 CS SMD 1 : 2 CS SMD 1 : 3 CS SMD	1 : 1 CS BM 1 : 2 CS BM 1 : 3 CS BM
CHITOSAN UNICORD (CSU)	1 : 1 1 : 2 1 : 3	1 : 1 CSU PM 1 : 2 CSU PM 1 : 3 CSU PM	1 : 1 CSU KM 1 : 2 CSU KM 1 : 3 CSU KM	1 : 1 CSU SMD 1 : 2 CSU SMD 1 : 3 CSU SMD	1 : 1 CSU BM 1 : 2 CSU BM 1 : 3 CSU BM
LOW MOLECULAR WEIGHT CHITOSAN (LMCS)	1 : 1 1 : 2 1 : 3	1 : 1 LMCS PM 1 : 2 LMCS PM 1 : 3 LMCS PM	1 : 1 LMCS KM 1 : 2 LMCS KM 1 : 3 LMCS KM	1 : 1 LMCS SM/SMD 1 : 2 LMCS SM/SMD 1 : 3 LMCS SM/SMD	1 : 1 LMCS BM(*) 1 : 2 LMCS BM(*) 1 : 3 LMCS BM(*)
PVP K30	1 : 1 1 : 2 1 : 3	1 : 1 PVP PM 1 : 2 PVP PM 1 : 3 PVP PM	1 : 1 PVP KM 1 : 2 PVP KM 1 : 3 PVP KM	1 : 1 PVP SM 1 : 2 PVP SM 1 : 3 PVP SM	1 : 1 PVP BM 1 : 2 PVP BM 1 : 3 PVP BM
PEG 4000	1 : 1 1 : 2 1 : 3	1 : 1 PEG PM 1 : 2 PEG PM 1 : 3 PEG PM	1 : 1 PEG KM 1 : 2 PEG KM 1 : 3 PEG KM	1 : 1 PEG SM 1 : 2 PEG SM 1 : 3 PEG SM	- - -

\* Vibrating mill and Swinging mill

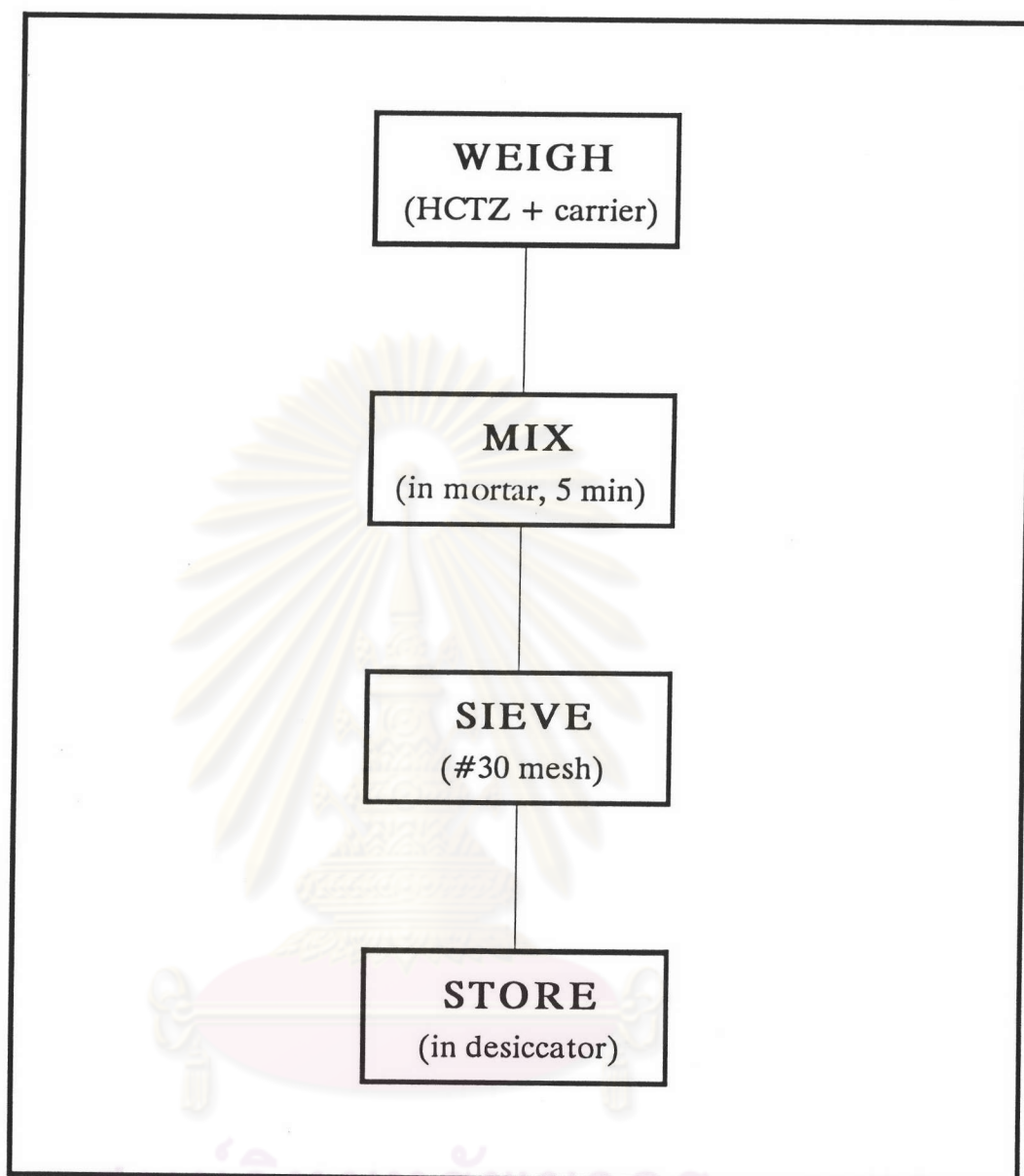


Figure 6 A schematic diagram for preparing HCTZ dispersions by physical method

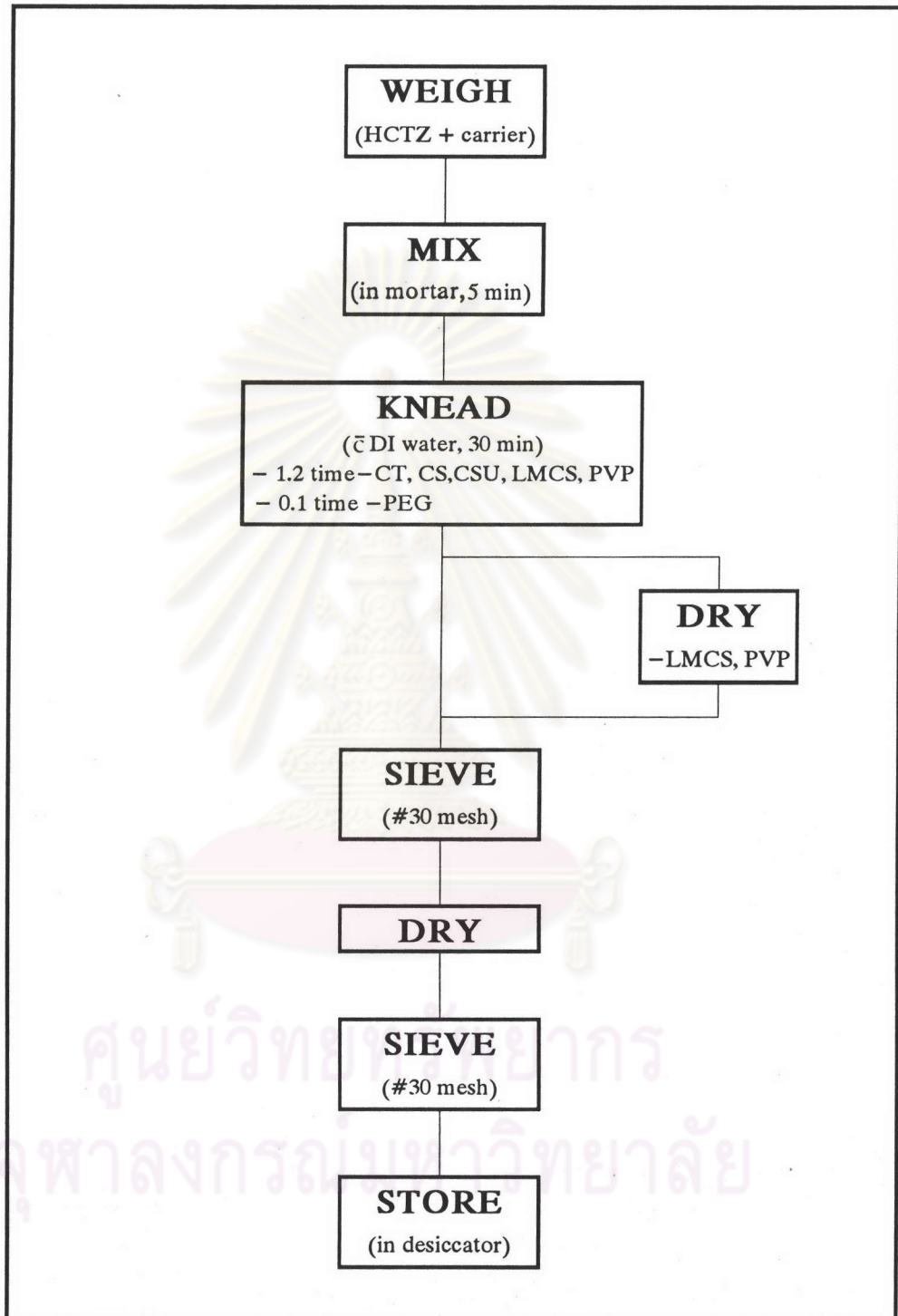


Figure 7 A schematic diagram for preparing HCTZ dispersions by kneading method

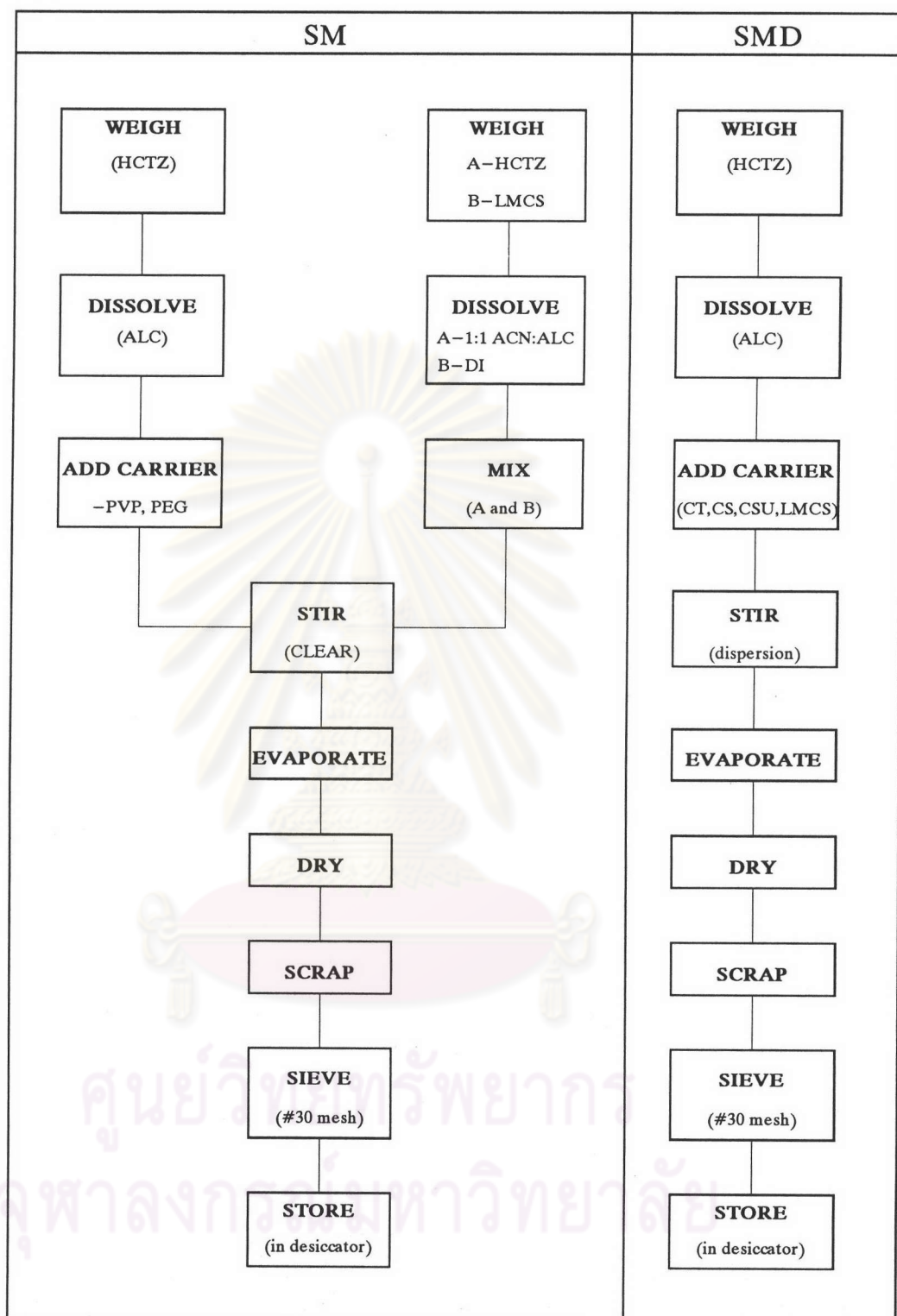


Figure 8 A schematic diagram for preparing HCTZ dispersions by solvent (SM) and solvent deposition (SMD) method.

### 3.1 Solvent method.

HCTZ coprecipitate was prepared by completely dissolving 1gm of HCTZ in 85 ml of 95% ethanol. Then PVP or PEG was subsequently added and stirred until the mixture was cleared. For LMCS, the carrier must be dissolved in deionized water prior to adding into the clear solution of HCTZ in 1:1 acetonitrile: absolute ethanol. After the solvent was allowed to evaporate continuously under rotavapor, the resulting coprecipitate was placed in an incubator overnight, then the drug coprecipitate was grounded and screened through a 30-mesh sieve and kept in a desiccator.

### 3.2 Solvent deposition method.

HCTZ solvent deposited mixture was prepared by completely dissolving HCTZ in 95% ethanol. CT, CS, CSU or LMCS was incorporated and stirred with a magnetic stirrer. While the mixture was in dispersion, the solvent was allowed to evaporate by a rotavapor and kept in an incubator overnight. The resultant was passed through a 30-mesh sieve and stored in a desiccator.

## 4. Preparation of HCTZ Ball Milling Mixture

The ball-milled mixture of HCTZ with CT, CS, CSU or PVP was prepared by using a pascall engineering ball mill, while milled mixture of LMCS was prepared by vibrating mill oscillant and/or swinging mill due to low amount of the supplied carrier. The scheme of preparation was illustrated in Figure 9.

4.1 Each ingredient of a milled mixture of CT, CS, CSU or PVP was weighed and transferred into a ceramic pot of 12.5 cm. in diameter and in 159.53 cm<sup>3</sup> capacity. The mill contained 255 ceramic balls of three sizes: 31 were 17mm; 69 were 12 mm; and 155 were 8 mm in diameter. The ball-mill was continuously revolved at the rate of 250 rpm for 24 hours. The ball-milled mixture of PEG was too sticky that the preparation could not be conducted.

4.2 Each ingredient of a milled mixture of LMCS was weighed and transferred to the vibrating container of a vibration mill which had 1.0 cm in diameter and 3.0 cm in length with one 0.44 gm weight of a stainless steel ball. The grinding operation time was 9 minutes. Another ball milling mixture was additionally prepared by a swinging mill, of 7.9 cm in diameter, and 218.0



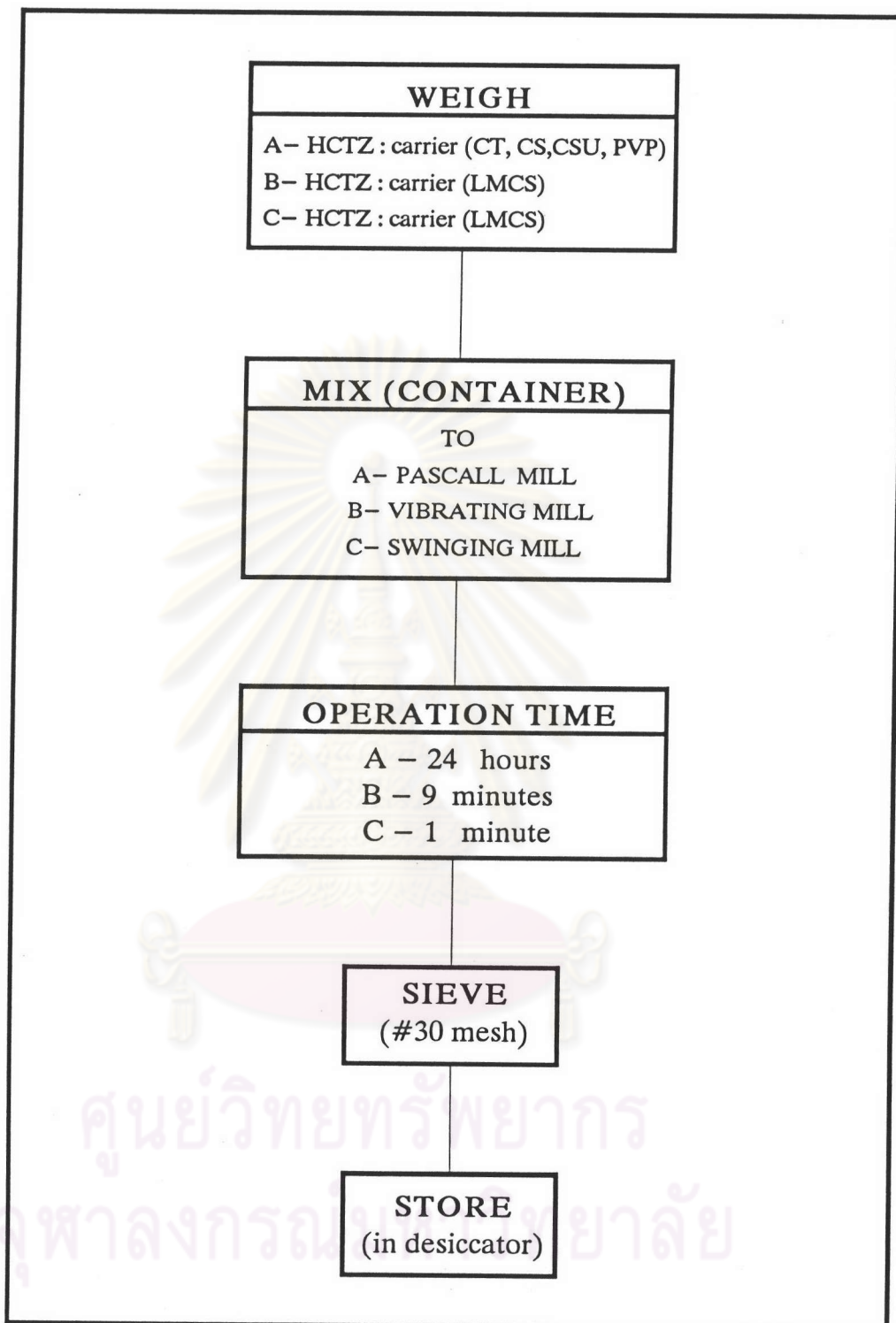


Figure 9 A schematic diagram for preparing HCTZ dispersions by ball milling method

cm<sup>3</sup> in capacity with one 1600 gm weight of a cylindrical steel. The operation time was 1 minute.

All the ball-milled samples were passed through a 30-mesh sieve and kept in the desiccator for further studies.

## 5. Preparation of Treated HCTZ

The treated HCTZ was prepared by using the same procedure as in the preparation of physical method, kneading method, solvent method and ball milling method without addition of carriers.

### B. Preparation of HCTZ Tablets

The preparation of dispersions that showed good dissolution profiles were selected for further studies. Only the ratio of 1:1 drug:carrier was formulated into direct compressed tablet. The composition of all formulations was as follow

Table 2 The composition of HCTZ tablet.

Ingredients	Amount per tablet (mg)
1. HCTZ with carrier (ratio 1:1, 50 mg HCTZ)	100.00
2. Spray dried lactose (qs)	103.75
3. Sodium starch glycolate	10.75
4. Magnesium stearate	1.08

Fifty tablets were prepared for all formulations except tablets of LMCS which twenty five tablets were prepared.

In the procedure, ingredients 1,2 and 3 were triturated in a mortar for 5 minutes, ingredient 4 was added and mixed, then directly compressed into tablets using a Carver press with 8 mm diameter, flat-faced, and bevel - edged

punch, under the pressure of 500 lbs. The prepared tablets were kept in an amber glass container and stored in a desiccator for further studies.

## **C. In - Vitro Evaluation**

### **1. Analysis and Calibration Curve of HCTZ**

HCTZ was accurately weighed and dissolved in 0.1 N hydrochloric acid medium. The solution was then adjusted to 500 ml in a volumetric flask with the same medium and was used as stock solution. Appropriate dilutions were made from the stock solution to obtain the solution of known concentrations between 0-14 µg/ml calibration. The UV absorbances at 272 nm wavelength of each concentration was determined by using a double beam spectrophotometer in a 1-cm cell spectronic 2000 spectrophotometer .

### **2. Dissolution Study**

#### **2.1 Dissolution studies of powder preparations**

Dissolution of HCTZ from each powder preparation was studied by using USP XXII paddle method with 900 ml dissolution medium of 0.1 N hydrochloric acid (pH 1.2) maintained at 37°C. Each preparation containing 50 mg HCTZ was transferred directly into the dissolution medium, which was stirred with a stainless steel paddle at 100 ±1 rpm. Five ml of sample solution was withdrawn periodically at 2, 4, 6, 8, 10, 15, 20, 30, 45 and 60 minutes interval through a membrane filter (pore diameter, 0.45 µm) and immediately replaced with an equal volume of the test medium. The sample solutions were assayed spectrophotometrically at 272 nm and the concentrations were calculated from the calibration curve.

#### **2.2 Dissolution studies of HCTZ tablets**

Dissolution of HCTZ from different tablets were tested in compliance with USP XXII dissolution test apparatus, Method I (Rotating basket method). The procedure was similar to that described in dissolution studies of powder preparations except rotating basket was used instead of stainless steel paddle. A tablet containing 50 mg HCTZ was used and the dissolution profile was the average of six determinations.

### **3. Disintegration Test**

Disintegration time of tablets was studied using a Hanson-Research disintegration tester according to the method in USP XXII. Water

maintained at  $37 \pm 2^\circ\text{C}$  was used as the test fluid. Experiments were conducted and the mean values were obtained from six determinations.

#### **4. Hardness**

Hardness of tablets was measured using Schleuniger Hardness Tester. Experiments were conducted and the mean value was obtained from three determinations.

#### **5. Scanning Electron Microscopy Study**

Electron photomicrographs of powder samples were taken with a scanning electron microscope (JEOL, model JSM-J 220 A)

The samples were coated with gold prior to the microscopic examination using ion sputtering. The selection sample of pure drug, treated drug, pure carriers, and preparations of ratio 1:3 were observed by scanning electron microscope at a low magnification (X50) first and then at a higher magnification (X500).

#### **6. Powder X - ray Diffraction Study**

Powder x - ray diffractometry was carried out by using a Philips X - ray diffraction model PW 1130/90, with a scanning goniometer supply model PW 1050/70, a channel analyzer model PW 1390, and a recorder model PM8203. The X - ray diffraction patterns were scanned from  $5^\circ$  to  $60^\circ$  in the term of  $2\theta$  angle.

#### **7. 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	=	$\pm 50 \mu\text{v}$
Atmosphere	=	static air
Chart speed	=	5 mm per min.

## 8. Infrared Absorbtion Study

The Infrared spectrum was measured by using a Perkin Elmer model 1760X infrared spectrophotometer in the range of 4000-400  $\text{cm}^{-1}$ . Assignments for the characteristic bands were observed and the samples examined were pure HCTZ, CT, CS, CSU, LMCS and dispersion mixture at ratio 1:1 of CT BM,CS BM, LMCS BM , and at 1:2 ratio of LMCS SM.



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย