



CHAPTER II

MATERIALS AND METHODS

Materials

1. Drug

- Hydrochlorothiazide B.P., USP (Marsing & Co., Ltd., Denmark) Batch No. M0802

- USP Hydrochlorothiazide RS

2. Carriers

- PEG 4000 (BASF, West Germany)

- PEG 6000 (BASF, West Germany) Batch No. 54-0197

- PEG 20000 (Chemische Werke Hüls AG., West Germany)

- PVP K-17 (BASF, West Germany) Batch No. 80-7502

- PVP K-30 (BASF, West Germany) Lot No. 88-7376

- PVP K-90 (BASF, West Germany) Lot No. 82-4195

- Urea (Merck, Germany) Lot No. 1020286

3. Others

- Acetone, AR (Merck, Germany) Lot No. 2751014

- Methanol, AR (Merck, Germany) Lot No. 4184709

- di-Sodiumhydrogen phosphate anhydrous (Merck, Germany)

Lot No. 133786

- Sodium hydroxide, AR (Merck, Germany) Lot No. 2717966

- Calcium chloride dihydrate (Merck, Germany) Lot No.

176581

Apparatus

- Analytical balances (August Sauter KGD7470, West Germany and Mettler PC440, Switzerland)
- Blender (Moulinex, Type Standard 2-241, France)
- Desiccators and Desiccator with hygrometer
- Differential Thermal Analyzer (Shimadzu, Model DT-30)
- Dissolution apparatus (72 RL, Hanson Reserch Corp., U.S.A.)
- Incubator (Memmert, Type B40, West Germany)
- Infrared spectrophotometer (Shimadzu, Model IR-440)
- Rotary evaporator (Rotavapor RE 120, Buchi, Switzerland)
- U.S. Standard sieve No. 60 mesh (W.S. Tyler Co., U.S.A.)
- UV-VISIBLE spectrophotometer (Spectronic 2000, Bausch & Lomb, U.S.A.)
- X-ray Powder diffractometer (Philips, Type PW1730, Holland)

Methods

1. Preparation of Solid Dispersions

Solid dispersions of HCTZ were prepared in various ratios (weight by weight) between HCTZ and various carriers by two methods.

1.1 Melting Method

The physical mixtures of required amount of HCTZ and carriers (PEG 4000, PEG 6000, PEG 20000, and UREA) were melted in beakers with continuous stirring on a sand bath until the mixtures were completely melted. The melts were quickly solidified by pouring on to stainless steel plates, kept for 24 hours in a freezer, and kept for another 24 hours in a dessicator at ambient temperature.

The products were then scraped off the plates, powdered by using a blender, sieved through a 60 mesh sieve, and stored in a desiccator at ambient temperature for further studies.

1.2 Solvent Method

The physical mixtures of required amount of HCTZ and carriers (PVP K-17, PVP K-30, PVP K-90, PEG 4000, PEG 6000, PEG 20000, and urea) were dissolved in minimum volume of solvent (equal parts of methanol and acetone), then placed in the flask of a rotary evaporator. Evaporation was carried out under vacuum in a water bath at 40°C until dryness. The coprecipitates were scraped off the flasks and total elimination of the remaining solvent was achieved by storing in an incubator at 40°C until constant weight obtained. After that the coprecipitates were powdered, sieved, and stored as above.

2. Preparation of Physical Mixtures

HCTZ and various carriers (sub 60 mesh sieved) were weighed accurately in various portions, mixed thoroughly by trituration in mortar. The physical mixtures were sieved through 60 mesh sieve, and stored in a desiccator at ambient temperature for further studies.

3. Standard Curves of Hydrochlorothiazide Solutions

Standard HCTZ was dried at 105°C for 1 hour, accurately weighed (50 mg), dissolved and adjusted to volume of 1000 ml with CO₂-free deionized water. Appropriate dilutions were made with CO₂-free deionized water to obtain standard solutions of known

concentrations between 0-14 and 0-90 mcg/ml for standard curve at 273 and 317 nm, respectively. The UV absorbances at 273 and 317 nm of each concentration were determined by using a Spectronic 2000 spectrophotometer.

4. Assay of Hydrochlorothiazide, Hydrochlorothiazide in Solid Dispersions and Hydrochlorothiazide in Physical Mixtures

The amount of HCTZ alone, HCTZ in solid dispersions and HCTZ in physical mixtures were determined according to the USP XXI method (17) as followed:

- **Standard preparation** A suitable quantity of USP hydrochlorothiazide RS was dried at 105 °C for 1 hour, accurately weighed, and dissolved in sodium hydroxide solution (1 in 125). A solution obtained had a known concentration of about 10 mcg/ml.

- **Assay preparation** The samples were accurately weighed equivalent to 50 mg of HCTZ, transferred to 50-ml volumetric flasks, dissolved in each 20 ml of sodium hydroxide solution (1 in 125) and adjusted volume to 50 ml with the same solution. All carriers were dissolved in the solvent so that the solutions of all samples were clear. One ml of each assay preparation was pipetted, transferred to 100-ml volumetric flask, diluted with CO₂-free deionized water to volume. In the systems containing urea, two ml of each assay preparation was pipetted, transferred to 25-ml volumetric flask, diluted with CO₂-free deionized water. Absorbances were determined at 273 nm for non-urea containing systems and at 317 nm in the systems containing urea. All carriers except urea presented in the systems did not interfere with determination of HCTZ at 273

nm while urea did not interfere at 317 nm.

The quantities of HCTZ in mg presented in the solid dispersions and physical mixtures were calculated by using the formula $5C(A_U/A_S)$, in which C is the concentration, in mcg/ml of USP Hydrochlorothiazide RS in the standard preparation, and A_U and A_S are the absorbances of the solution from the assay preparation and the standard preparation, respectively.

4. Dissolution Test

The dissolution rates were determined by using the USP XXI dissolution apparatus type II (paddle) method. Stirring rates of the paddles were 50 ± 1 r.p.m. The medium was CO_2 -free deionized water, 900 ml, maintained at $37 \pm 0.5^\circ\text{C}$ in a water bath.

After each sample equivalent to 50 mg of HCTZ was introduced into the medium, a 5 ml-aliquot of the solution was taken through a stainless steel filter at 0, 2, 5, 10, 15, 20, 25, 30, 45, 60, 90 and 120 minutes, and was replaced with the equal volume of dissolution medium after each sampling. The samples were then assayed spectrometrically at 317 nm for HCTZ content. All carriers did not interfere with the determination at this wavelength. The samples concentrations were calculated from the standard curve. All dissolution studies were carried out in triplicate.

6. Powder X-ray Diffraction Study

Powder X-ray diffractometer with Ni-filtered, $\text{Cu-K}\alpha$ radiation (voltage 40 kv, current 30 mA). The rate of counting was 2×10^4 pulses/second. Diffraction spectra were run 1°/minute in term

of 2θ angle.

7. Infrared (IR) Absorbtion Study

IR spectra were measured by using a Shimatzu Infrared Spectrometer. The measurements were made by the KBr disc method.

8. Differential Thermal Analysis (DTA)

DTA thermograms were measured with a Shimatzu Thermal Analyzer, with a heating rate 20°C/minute, range ±50mV, and N₂ 30 ml/minute.

9. Study on Aging Effect

In the study on aging effect, the chemical stability and dissolution rate of the selected solid dispersion with the fastest dissolution rate according to the former study were compared with those of pure HCTZ after storage under the following conditions:

9.1 Dry Storage The solid dispersion and pure HCTZ were kept in a desiccator with silica gel at ambient temperature for 0, 10, and 30 days.

9.2 Accelerated Storage The solid dispersion and the pure HCTZ were stored in an air-tight glass containers in an incubator at 40°C for 0, 10, and 30 days.

9.3 Moist Storage The solid dispersion and pure HCTZ were kept in a desiccator, which the bottom was filled with a saturated aqueous solution of sodium dihydrogen phosphate to maintain 75±5% relative humidity (R.H.), at ambient temperature for

0, 5, 10, 20, and 30 days.

In order to correct the weight change caused by absorption of moisture during the moist storage, the stored samples were dried under reduced pressure at room temperature for 6 hours in a calcium chloride desiccator before the further studies. For the samples stored at 40°C in air-tight glass containers, the moisture absorption was negligible.

After that, all stored samples were assayed for HCTZ contents and dissolution tests were performed within 24 hours. X-ray diffractograms, IR spectra and DTA thermograms were also determined.