CHAPTER II
RESEARCH DESIGNS AND METHODS EXPERIMENTAL

MATERIALS AND METHODS

2.1 Materials

The following materials obtained from commercial source were used in this experiment. Chlorpheniramine maleate (CPM) was produced by Supriya Chemicals, Mumbai, India lot no. SC/C0304159 used as a water-soluble model drug. Diazepam (DZP) was manufactured by Changzhou, China, batch number C200605010 which provided by Atlantic Laboratories Bangkok Thailand used as poorly soluble model drug. Absolute ethyl alcohol (AR grade, Merck, Germany) and dichloromethane (AR grade, Fisher scientific, England) was used as solvents for drug solution. A direct compression tablet included dibasic calcium phosphate dihydrate (DCP dihydrate direct compression grade, USP 24 standard, lot no. T27B, Sudeep Pharma Ltd. India), Ac-Di-Sol® (Croscarmellose sodium, lot no. T651N, FMC, Belgium) and magnesium stearate (lot no. 1812, Akcros chemicals, Netherland). Pharmatose® (lactose monohydrate, lot no. 10025939, DMV International) and PVP K30 (polyvinyl pyrrolidone, Serva, USA) were used as a diluent and a binder, respectively and purified talcum BP (Haichen), magnesium stearate as glidant and lubricant, respectively. SEA CHLOR® (SEA PHARM CO., LTD., Lot No. 5374C) and diazepam tablet (Government Pharmaceutical Organization, GPO Thailand, Lot No. T490361) were the commercial chlorpheniramine maleate, 4 mg tablet and diazepam, 5 mg tablet respectively.

2.2 Equipments

- Instrumented single-stroke tableting machine (Fette® KO, Germany)
- Hot air oven (Binder 240/E2, Germany)

- Microsyringe 50 µl size (Hamilton # 80500, USA)
- Analytical balance (readability: 0.0001 g, Sartorius, Germany)
- Hardness Tester (Pharma Test, PTZ1, Germany)
- Friabilator (Pharma Test, Germany)
- Micrometer (Fowler, USA)
- Accupyc 1330 pycnometer (Micromeritics, USA)
- Disintegration time Tester (Pharma Test, Germany)
- Digital camera (Olympus: model IR 300)
- Scanning Electron Microscope (JEOL, JSM-5910LV, Japan)
- Powder X-ray diffractometer (Diffraktometer Siemens D500, Germany)
- X-ray monochromator (single crystal) (modified JEOL, JDX 8030; Siemens D500, Germany)
- Differential scanning calorimeter (DSC 7, Perkin Elmer, USA)
- UV-visible spectrophotometer (Spekol 1200, Germany)
- Dissolution apparatus (Hanson Research, USA)

2.3 Methods

2.3.1 Preparation of blank tablet

Direct compression (DC) blank tablet

DC blank tablet was the tablet without active ingredient prepared by direct compression method. DCP as a water-insoluble filler and Ac-Di-Sol® as a superdisintegrant were mixed thoroughly for five minutes. Subsequently, the homogeneous powder was combined with magnesium stearate as lubricant for two minutes. The formulation was shown in Table 2.1. The mixed powder was then compressed on instrumented single-stroke tableting machine with 1000, 1400 and 1800 kg compression force (CF). The obtained blank tablets had a flat surface with a diameter of 10 mm and 500 mg tablet weight.

Table 2.1 The DC blank tablet formulation

Ingredients	Weight per batch
DCP dihydrate	1500 g
Ac-Di-Sol® 2 % w/w	30 g
Magnesium stearate	0.75% w/w

Wet granulation (WG) blank tablet

WG blank tablet was the tablet without active ingredient prepared by wet granulation method. Lactose monohydrate as a hydrophilic diluent and polyvinyl pyrrolidone as a binder, in solution form with the concentration 5% in isopropyl alcohol (IPA), were wet-mixed and sieved through 14 mesh. The granule received was dried at 60° C in hot air oven overnight and then passed through a 16 mesh-size sieve. The dried granule was mixed with Ac-Di-Sol® as a superdisintegrant and then with of magnesium stearate as lubricant and talcum as glidant for two minutes. The formulation was shown in Table 2.2. The mixed granule was compressed to form 350 mg tablet, using the same set of compression tool to provide compression forces as DC blank tablet.

Table 2.2 The WG blank tablet formulation

Ingredients	Weight per batch
Pharmatose®	1500 g
Ac-Di-Sol® 2 % w/w	30 g
5% PVP K90 in isopropyl alcohol	qs.
Magnesium stearate	2 % w/w
Talcum	3 % w/w

2.3.2 Preparation of drug tablet by conventional method

CPM tablet by direct compression (CPM DC) and wet granulation (CPM WG)

CPM DC tablet and CPM WG tablet were the tablets with CPM prepared by direct compression and wet granulation method respectively. The excipients and methods were the same as in the preparation of DC and WG blank tablets, except for having CPM 4 mg per tablet incorporated during the dry-mixing.

Diazepam tablet by direct compression (DZP DC) and wet granulation (DZP WG)

DZP DC tablet and DZP WG tablet were the tablets with diazepam prepared by direct compression and wet granulation method respectively. The excipients and methods were the same as in the preparation of DC and WG blank tablets, except for having diazeapm 5 mg per tablet incorporated during the dry-mixing.

2.3.3 Evaluation of blank tablet properties

The properties of DC and WG blank tablet at 1000, 1400 and 1800 kg of compression force were examined by the following methods.

2.3.3.1 Weight variation

The following weight variation investigations provide limits for the permissible variations in the weights of individual tablets expressed in term of the allowable deviation from the average weight of a sample. The method follows weight variation test of USP. Weigh individually twenty whole tablets by an analytical balance, and calculate the average weight, relative standard deviation.

2.3.3.2 Hardness test

The hardness of a blank tablet was expressed as that force required breaking the tablet. It was measured by Pharma Hardness Tester in Newton unit force. The mean and standard variations were calculated from ten determinations.

2.3.3.3 Friability

The friability tester was used as a device to determine tablet friability. The instrument was designed to measure the wearing qualities of tablets. Twenty dustless blank tablets were weighed by an analytical balance, the weight (W_1) was obtained. The weighed tablets were placed in the tumbling apparatus and then turn on the friability tester. After 25 rpm of rotations for 4 minutes, the tablets were weighed after the dusts were removed to get W_2. The tablet friability was calculated from the weight loss due to the friability as a percentage of the initial weight by using Equation 2.1. The loss in weight was a measure of the ability of the tablets to withstand this type of wear identity transportation handling. The friability was not more than 1% according to BP 1998.

$$\% \text{ friability} = (1 - \frac{W_2}{W_1}) \times 100 \qquad \text{.....(2.1)}$$

2.3.3.4 Tablet porosity

The porosity of the tablet was calculated using Equation 2.2

$$\% \text{ porosity} = (1 - \frac{V_t}{V_a}) \times 100 \qquad \text{.....(2.2)}$$

Whereas the tablet apparent volume (V_a) was calculated from the diameter and thickness of tablet measured with a micrometer. The tablet true volume (V_t) was determined using Accupyc 1330 pycnometer by measuring the amount of displaced helium gas into the tablet porosity. The data given are the mean of fifteen tablet measurements.

2.3.3.5 Disintegration test

The disintegration time was measured by using disintegration apparatus Tester. Place 1 tablet in each of the six tubes of the basket, add a disk to each tube and operate the apparatus, using water maintained at 37 $\pm$ 2 ^{o}C as the immersion fluid. The time that the tablets required to disintegrate completely was

recorded. The data given are the mean of six tablets determinations. The disintegration time is within 30 minutes as stated in USP 30 <2040>. If 1 or 2 tablets fail to disintegrate completely, repeat the test on 12 additional tablets: not less than 16 of the total of 18 tablets tested disintegrate completely.

2.3.4 Preparation of drug-solution-dropping tablet

CPM and DZP was used as water soluble and poorly water soluble drug model respectively. In this study the absolute alcohol was firstly chosen to be the solvent for CPM and diazepam but the drug could not be soluble in small amount of alcohol. The reason is the amount of the drug solution dropping on the surface of the blank tablet should be as less as possible for no effect on the tablet property. The mixture of absolute alcohol and dichloromethane in a ratio of 1:3 by volume was used instead of the pure absolute alcohol. Each drug was dissolved in a mixed solvent of absolute alcohol and dichloromethane in a ratio of 1:3 by volume to obtain a solution concentration of 100 micrograms per microliter. The portion of 40 and 50-µL of each prepared solution (4 mg of CPM and 5 mg of DZP) was dropped on the surface of DC blank tablet by using 50 µL-microsyringe (Hamilton # 80500). For WG blank tablet, it was found the effect of the drug solution using a mixed solvent on the tablet surface. Then only dichloromethane was chosen as the solvent for this case. WG blank tablet was treated in the same manner but using only dichloromethane as a solvent for preparing drug solution. All of treated tablets were dried at 50^{o} C in hot air oven for one hour after leaving solvent evaporation in open air one hour.

2.3.5 Study of the penetration depth of drug solution

The penetration of drug-solution was inspected when it was dropped on the surface of DC blank tablet of 1000 kg CF by using color solution., 0.1% fast green FCF in alcohol. The color solution was dropped 40 and 50-µl, the same quantity as CPM solution and DZP solution on the surface of blank tablet. The pictures of the flat surfaces of no dropped and dropped areas of dye solution including the side of these tablets were taken by using digital camera. The objective of this study was to determine how deep the solution could penetrate through the blank tablet.

2.3.6 Tablet characterization studies

The drug-solution-dropping tablets (DSDT) prepared from DC and WG blank tablet and their particles from the surface were characterized by the following studies:

2.3.6.1 Morphology study by digital camera

The surface of DSDT prepared from DC and WG blank tablet were taken by digital camera with high performance. The comparison between the flat surfaces of no dropped and dropped including the side of tablet was studied to analyze the tablet surface after dropping drug solution.

2.3.6.2 Morphology study by scanning electron microscopy (SEM)

SEM with various magnifications was used to study more detail of the top and deeper surface of DSDT. Particle morphology from the top and deeper surface of the tablet was also revealed by SEM. The accelerating voltage of SEM was set at 15 kV and magnification at 100X, 1000X and 1500X to characterize the whole of size, shape and surface of the powder. The sample was in the aluminium stub with two-faced glue paper and coated with gold before SEM analysis. The excipients in DC and WG blank tablet, CPM and diazepam powder, including the powder from surface of DC blank tablet and WG blank tablet were also compared to the powder from surface of DSDT.

2.3.6.3 X-ray powder diffraction (XRPD)

Powdered X-ray patterns were carried out to determine the sample crystalline behavior by using Powder X-ray diffractometer and X-ray monochromator (single crystal). Samples were exposed to Cu Kα radiation 20 kv. The patterns were shown in the range of the diffraction angles (2-theta) 5° and 30°. The sample were the excipients in blank tablet such as DCP, lactose including CPM, DZP and the powder from the tablet surface and the scratched powder from DC blank tablet, WG blank tablet before and after dropping drug solution.

2.3.6.4 Differential scanning calorimetry (DSC)

The differential scanning calorimeter was used to show the thermal behavior of CPM, DZP and another excipient in the tablet, including the powder from surface of DSDT. The powder sample 3-5 milligram was packed in a

sealed aluminum pan, 40 microliter size. The running temperature was 75°C to 200°C with the heating rate of 10°C/min.

2.3.7 Assay of the active ingredients

The amount of CPM and DZP in DSDT prepared from DC blank tablet was determined by using the analytical method as stated in the monograph of USP 30 and BP 2007 respectively to confirm the proposed preparation meet requirement (standard). CPM tablets USP contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of CPM. Content of DZP in diazepam tablets BP is 92.5 to 107.5% of the stated amount.

2.3.8 Content Uniformity Testing

Any drug product recognized in the Pharmacopoeia should comply with the Pharmacopoeia requirement for content uniformity if such requirement is included in the monograph for the drug. The product must comply with the specifications for individual dosage unit assay and for relative standard deviation. Prepared CPM tablets were tested following the requirements of uniformity of dosage units, content uniformity, in USP 30 monograph while the prepared DZP tablets were followed under uniformity content in BP 2007 monograph.

2.3.9 Dissolution Profiles Studies

A blank solvent without drug was dropped on the surface of CPM-solution-dropping tablet and DZP-solution-dropping tablet with the same volume of drug solution. The blank solvent dropping was repeated 2, 4 or 6 times which expected to increase the penetration of drug. The effect of times of solvent dropping on the dissolution rate of these DSDT was evaluated.

The dissolution profiles of the CPM-solution-dropping tablets prepared from DC and WG blank tablet, CPM DC, CPM WG tablet, CPM tablet from market, DZP-solution-dropping tablets prepared from DC and WG blank tablet, DZP DC, DZP WG tablet and diazepam tablet from market were performed in dissolution apparatus. The tester was apparatus type 2 for CPM tablet and type 1 for diazepam

tablet according to USP 30 and BP 2007, respectively. The speed of the apparatus was 50 rpm and 500 mL of 0.01 N hydrochloric acid was used as medium for tablet of CPM, and tablet of diazepam, 100 rpm and 900 mL of 0.1 N hydrochloric acid were used. The medium solutions were collected at 1, 3, 5, 7, 9, 15, 20, 25 and 30 minutes and were analyzed by using a spectrophotometer at the absorbance of 265 nm for CPM and 284 nm for diazepam. Quantity of drug released was calculated from standard curve of reference standard drug solution.

The statistical significant differences in the result of dissolution tests of CPM and diazepam tablets and their DSDT in this study were performed by comparisons of two independent groups with unknown variance (Bolton, 1997).

2.3.10 Stability Studies

The effect of long-term storage on the properties of DSDT and conventional tablet preparation were evaluated. The DSDT were subjected to stability study at room temperature storage condition. The drug content and dissolution profile were analyzed using as in assay of the active ingredients and dissolution profile study after 1, 2, and 3 months of storage.