



Chapter II

Experimental

1. Materials

The following materials were used without further purification except tablet additives which were dried at 60°C for three hours before using and deionized water was used throughout this study.

1.1 Model Drug

- Ergoloid mesylate

1.2 Carriers

- PVP K-30 (BASF, West Germany)
- PVP K-90 (BASF, West Germany)
- PEG 4000 (Nippon Oil & Fats., Japan)
- PEG 6000 (BASF, West Germany)
- Poloxamer 188 (Pluronic F- 68^(R), BASF, West Germany)

1.3 Tablet Additives

- Anhydrous lactose (Die Melkindustrie Veghel, UK.)
- Sodium-starch glycolate (Explotab^(R), Edward Mendel, USA.)
- Magnesium stearate (supplied by Pharmaceutical Sciences, Bangkok, Thailand)

1.4 Others

- Five commercial 1 mg. ergoloid mesylate tablet products
- Absolute ethyl alcohol, AR (Merck, Germany)
- Chloroform, AR (Merck, Germany)
- Hydrochloric acid, AR (Merck, Germany)

2 Methods

2.1 Preparation of ergoloid mesylate solid dispersions

Ergoloid mesylate and carriers (PVP K-30, PVP K-90, PEG 4000, PEG 6000, poloxamer 188 and mixture of 3% poloxamer in PVP K-30) were accurately weighed in the ratios as shown in Table 2. The ergoloid mesylate was dissolved completely in suitable volume of 1:1 chloroform-ethanol solvent system and the carrier was added and mixed thoroughly. The solution was spread in a thin layer onto the silicon paper and the solvent was subsequently removed under vacuum in a desiccator at room temperature (about 27^o C). After evaporation was completed, the resulting coprecipitate was scraped from the silicon paper using a microspatula. It was kept in another desiccator, using silica gel as adsorbent, until constant weight was obtained. The dry dispersion was pulverized at low temperature (about 15^o C) in a glass mortar with a pestle and passed through a 80-mesh sieve. The dispersion was kept in the desiccator for further studies.

Table 2 Weight of drug and carrier used in preparing dispersion systems

Preparation (w/w)	Weight of (g)	
	ergoloid mesylate	carrier
Ergoloid mesylate:PVP K-30 (1:1)	0.5000	0.5000
Ergoloid mesylate:PVP K-30 (1:3)	0.5000	1.5000
Ergoloid mesylate:PVP K-30 (1:5)	0.5000	2.5000
Ergoloid mesylate:PVP K-30 (1:7)	0.5000	3.5000
Ergoloid mesylate:PVP K-90 (1:1)	0.5000	0.5000
Ergoloid mesylate:PVP K-90 (1:3)	0.5000	1.5000
Ergoloid mesylate:PVP K-90 (1:5)	0.5000	2.5000
Ergoloid mesylate:PVP K-90 (1:7)	0.5000	3.5000
Ergoloid mesylate:PEG 4000 (1:1)	0.5000	0.5000
Ergoloid mesylate:PEG 4000 (1:3)	0.5000	1.5000
Ergoloid mesylate:PEG 4000 (1:5)	0.5000	2.5000
Ergoloid mesylate:PEG 4000 (1:7)	0.5000	3.5000
Ergoloid mesylate:PEG 6000 (1:1)	0.5000	0.5000
Ergoloid mesylate:PEG 6000 (1:3)	0.5000	1.5000
Ergoloid mesylate:PEG 6000 (1:5)	0.5000	2.5000
Ergoloid mesylate:PEG 6000 (1:7)	0.5000	3.5000
Ergoloid mesylate:poloxamer 188 (1:1)	0.5000	0.5000
Ergoloid mesylate:poloxamer 188 (1:3)	0.5000	1.5000
Ergoloid mesylate:poloxamer 188 (1:5)	0.5000	2.5000
Ergoloid mesylate:poloxamer 188 (1:7)	0.5000	3.5000
Ergoloid mesylate:3%poloxamer 188 in PVP K-30 (1:1)	0.5000	A* B*
Ergoloid mesylate:3%poloxamer 188 in PVP K-30 (1:4)	0.5000	0.0150+0.4850 A* B*
Ergoloid mesylate:3%poloxamer 188 in PVP K-30 (1:6)	0.5000	0.0600+1.9400 A* B*
Ergoloid mesylate:3%poloxamer 188 in PVP K-30 (1:6)	0.5000	0.0900+2.9100

* A: poloxamer 188 B: PVP K-30

2.2 Preparation of ergoloid mesylate physical mixtures

All ingredients (ergoloid mesylate and carriers) were pulverized and sieved individually through a 80-mesh sieve. Ergoloid mesylate and carriers were accurately weighed in certain ratios as shown in Table 2. The physical mixture was prepared by gently triturating in a glass mortar with a pestle and mixed for 5 minutes. The mixture was then sieved through a 80-mesh sieve and kept in a desiccator, using silica gel as absorbent, for further studies.

2.3 Preparation of treated ergoloid mesylate

Ergoloid mesylate was dissolved completely in 1:1 chloroform-ethanol solvent system and the solution was spread in a thin layer onto the silicon paper. The solvent was removed under vacuum in desiccator. After evaporation was complete, the treated ergoloid mesylate was kept in another desiccator using silica gel as adsorbent. The dry treated drug was pulverized and sieved as described in preparation of ergoloid mesylate solid dispersion. The treated drug was kept in desiccator for further studies.

2.4 Assay for the content of ergoloid mesylate, treated ergoloid mesylate and ergoloid mesylate in solid dispersions.

The amount of ergoloid mesylate alone, treated ergoloid mesylate and ergoloid mesylate in solid dispersions were determined using the method described by Hunt et al. (1981).

2.4.1 Calibration curve of ergoloid mesylate

About 40 mg., accurately weighed, of ergoloid mesylate was dissolved in 200 ml. of 0.1 N. HCl in deionized water and then adjusted to volume of 200 ml. with the same solution. Appropriate dilutions were made with 0.1 N. HCl to obtain standard solutions of known concentration at the 15, 12.5, 10, 7.5, 5, 2.5, 1, and 0.5 mcg. per ml. The final solutions were filtered through a disposable glass filter. The amount of ergoloid mesylate was determined in a spectrofluorophotometer (Shimadzu-RF 520, Japan) at an excitation wavelength at 290 nm. and an emission wavelength at 360 nm. were determined, using 0.1 N.HCl as a blank. Fluorescences obtained versus known concentrations of the drug were fitted to a straight line using linear regression.

2.4.2 Analytical method

Pure ergoloid mesylate, treated ergoloid mesylate and all ergoloid mesylate solid dispersions containing equivalent amount of the pure drug 50 mg. were individually dissolved and diluted with 0.1 N.HCl to obtain the concentration of solution at the 10 mcg./ ml.. The solution was filtered and determined the concentration by spectrofluorophotometer as described in 2.4.1. At this wavelength carriers did not interfere with the measurement.

2.5 Dissolution studies

Dissolution tests of ergoloid mesylate, treated ergoloid mesylate and ergoloid mesylate solid dispersion were carried out in 500 ml. of 0.1 N.HCl equilibrated at $37\pm 0.5^{\circ}\text{C}$, using the USP. XXII dissolution apparatus type II (paddle) at the rate of 50 ± 1 rpm. (Hanson Research Corp., USA.). Ergoloid mesylate 50 mg., physical mixture and solid dispersion (passed through no. 80-mesh) containing equivalent amount of the pure drug, were introduced in each vessel. Five milliliters of samples were collected, at the time interval of 1, 3, 5, 7, 9, 11, 13, and 15 minutes, then filtered through glass filter and analyzed for drug content by the spectrofluorophotometer as described in 2.4.1. The volumes withdrawn each time were then replaced by equivalent amount of temperature equilibrated 0.1 N.HCl to maintain a constant volume of dissolution medium during the course of the test. The amount of ergoloid mesylate dissolved was calculated from the calibrated concentration fluorescence curve as described in 2.4.1. All carriers did not interfere with the determination at these wavelength. Each solid dispersion system was run at least in triplicate. The dissolution profile was obtained by plotting the percent of ergoloid mesylate dissolved against time.

2.6 Determination of physicochemical properties of solid dispersion

The following physicochemical properties of the selected solid dispersions and physical mixtures were determined

and compared with the pure ergoloid mesylate and carriers.

2.6.1 Infrared (IR) absorption studies

IR spectra were measured using an infrared spectrometer (Shimadzu-IR 440, Japan). The measurements were made by the KBr disc method.

2.6.2 Differential thermal analysis (DTA)

DTA Thermograms were obtained on a thermal analyser, with a heating rate $10^{\circ}\text{C}/\text{minute}$, sensitivity ± 50 μV ., and opened cell in static air (Shimadzu, Japan).

2.6.3 Scanning electron microscopy (SEM)

Electron photomicrographs of powder samples were taken with scanning electron microscope (JSM-T 220 A, JEOL, Japan). The samples were coated with gold prior to the microscopic examination using ion sputtering. Size, shape, and surface topography of the powder samples were observed.

2.6.4 Powder x-ray diffraction studies

The crystallinity of ergoloid mesylate in the solid dispersions was examined by x-ray diffractometry (JDX-8030, JEOL, Japan). The samples for x-ray diffraction studies were firmly packed into the cavity of a thin rectangular metal plate using two glass slides which were fastened to the metal plate with adhesive tape. The first glass slide was then removed, before taken the prepared sample to expose the x-ray in

the x-ray diffraction chamber. The x-ray diffraction patterns were recorded at the rate of 80° per minute from 5° to 105° in the term of 2θ angle.

2.7 Preparation of ergoloid mesylate tablets

From the dissolution studies of all solid dispersions, it was appeared that two carriers, PVP K-30 and poloxamer 188, seemed to be more advantageous than other carriers. Consequently, they were selected for further investigation on preparation of tablets products.

2.7.1 Method of tablet manufacture

Four procedures of tablet manufacture were employed: procedure A is traditional wet granulation method; procedure B is modified solid dispersion of wet granulation method; procedure C is traditional direct compression method; and procedure D is solid dispersion of direct compression method.

The composition of all formulations for 500 tablets was as follows:

Ergoloid mesylate	0.50	gm.
PVP K-30	2.50	gm.
Anhydrous lactose	78.00	gm.
Sodium starch glycolate	0.65	gm.
Magnesium stearate	0.40	gm.

In procedure A, ergoloid mesylate and anhydrous lactose were dry-mixed and wetted with the 10%

alcoholic PVP solution and wet-massed through a 16-mesh sieve before drying under vacuum for 24 hours at room temperature. The mass was then dry-sieved with a 14 mesh sieve to produce proper granulations. Sodium starch glycolate was mixed with dried-granules. The granules were lubricated with 0.5% magnesium stearate before compression.

In procedure B, the ergoloid mesylate was dissolved in the 10% alcoholic-PVP solution and then wet-massed with anhydrous lactose. The damp mass was sieved through a 16 mesh sieve. Then, proceed as directed in procedure A .

In procedure C, the ergoloid mesylate and PVP K-30 were mixed and remixed with anhydrous lactose. The magnesium stearate was added before compression.

In procedure D, the solid dispersion between drug and PVP K-30 was prepared as described in 2.1 prior to dry-mix with anhydrous lactose and sodium starch glycolate. The mixtures were lubricated with 0.5% magnesium stearate before compression.

In addition, the procedure E was carried out. In this procedure, the tablets were prepared as described in procedure D but the solid dispersions of drug-poloxamer 188 (1:5) were used.

All granulation were compressed into tablet on a sigle punch tablet machine (Viuhang Engineering, Thailand), using flat-faced punches, 8 mm. diameter.

2.7.2 Tablet evaluations

The physical properties of prepared tablets as well as five commercial ergoloid mesylate tablet products were evaluated as follows:

2.7.2.1 Weight variation

Twenty tablets were individually weighed. The average weight and standard deviation of ergoloid mesylate tablets was calculated.

2.7.2.2 Tablet hardness

The hardness was measured using the Schleuniger-2E hardness tester (Switzerland) and the mean of ten determinations was calculated.

2.7.2.3 Tablet thickness

The thickness was measured by using micrometer (Teclock-SM 112, Japan). The mean was averaged from ten determinations.

2.7.2.4 Disintegration time

Disintegration time was determined according to USP. XXII method. One tablet was placed in each of six tubes of the basket. The apparatus (Hanson Research-QC 21, USA.) was operated, using water maintained at $37 \pm 2^{\circ}\text{C}$. The average was calculated from six determinations.

2.7.2.5 Content uniformity

Each tablet, which was triturated individually to fine powder, was transferred to a 250 ml. volumetric flask, diluted with 100 ml. of water, shaken for 15 minutes and added sufficient water to produce 250 ml. Mixed, filtered through glass filter and stepwise diluted with water until the concentration of ergoloid mesylate was about 4 mcg. per ml.. Fluorometric method was employed to determine the amount of ergoloid mesylate at an excitation wavelength at 290 nm. and an emission wavelength at 360 nm.. The content was calculated from calibration curve as described in 2.4.1 except that the concentrations were changed to 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mcg. per ml..

2.7.2.6 Dissolution studies

The dissolution behaviors of ergoloid mesylate tablet was determined in compliance with USP. XXII monograph. The dissolution profile was the average of six determinations.

The dissolution profile of single tablet was measured in 500 ml. of deionized water at $37 \pm 0.5^\circ\text{C}$ as the dissolution medium. The paddle was rotated at 50 ± 1 rpm.. Sample of 5.0 ml. were withdrawn periodically at 2, 5, 10, 15, 20, 30, and 60 minutes interval. The volume taken was substituted by an equal volume of prewarm deionized water. The sample was assayed spectrophotometrically and the concentration was calculated from calibration curve as described in 2.7.2.5.