CHAPTER III

MATERIALS AND METHODS

Apparatus

1. Infrared Spectrophotometer, Perkin-Elmer 283
2. Laboratory Press, Corver Model C
3. Vibration grinder, Grescent Dental M/G
4. Thermal Analyzer, Shimadzu DT-30
5. Electrothermal Melting Point

Materials

1. Cimetidine
2. Nujol, Perkin Elmer 1862302
3. Potassium Bromide, Perkin-Elmer 990-5927
4. Acetonitrile(Fluka) Analytical grade
5. Talcum B.P.
6. Corn starch B.P.
7. Magnesium stearate B.P.
8. Lactose B.P.

Methods

1. Crystallization of Cimetidine Polymorph A

Cimetidine(2.0)g was dissolved in acetonitrile(60 ml)by warming to 60°C to a clear solution. This solution was set aside at

room temperature (25 ± 2°C) in a cover beaker and allowed to cool. After 6 hours, the precipitated solid was collected by filtering through a filter paper (Whatman No. 1), washed with 2-3 ml of pure acetonitrile, and dried overnight in a vacuum oven over phosphorous pentoxide.

2. Crystallization of Cimetidine Polymorph B

Cimetidine (2.0 g) was dissolved in tridistilled water (30 ml) by heating to 90°C to a clear solution. This solution was placed inside a cover beaker and allowed to evaporate slowly at room temperature (25 ± 2°C). After 3-4 weeks, the precipitated solid was collected by filtering through a Büchner funnel (vacuum filtration), washed with 2-3 ml of water, and dried overnight in a vacuum oven over phosphorous pentoxide.

3. Identification of Cimetidine Polymorphs A and B

3.1 Spectroscopy

Infrared spectra of the crystalline polymorphs A and B were recorded, using Nujol Mull and Potassium Bromide disc techniques, on a Perkin-Elmer 283 Grating Infrared Spectrophotometer. The KBr infrared spectra of cimetidine polymorphs A and B were compared with those respective infrared spectra reported previously (7,8).

3.2 Physical constant

3.2.1 Melting point

A few milligrams of sample were ground in an agate mortar, and the fine powder was filled into a capillary tube which was sealed at one end. The sample tube was put into the melting point

apparatus and the temperature was increased at a rate of 4-5° C per minute and then 1°C per minute at the melting point range.

3.2.2 Differential thermal analysis:-

The differential thermal analysis curves of the two crystalline polymorphs in seal cells, in air atmosphere, were recorded on Thermal Analyzer.

4. Effect of Grinding on the Polymorph Alteration in Potassium Bromide Disc Technique

4.1 Grinding in an agate mortar

A few milligrams of cimetidine polymorph A were mixed with 250 mg of potassium bromide, and ground by hand in an agate mortar for about 1 minute. The finely ground powder was pressed into a thin disc under the total force of 8 tons, and the IR spectrum was recorded over the range of 4000-400 cm^{-1}.

The procedure was repeated by increasing the gringing-time of about 3, 5, 7 and 10 minutes.

The cimetidine polymorph B was treated in a similar manner.

4.2 Grinding in the vibration grinder

A few milligrams of cimetidine polymorph A were mixed with 250 mg of potassium bromide in an agate capsule with one agate ball mill and ground machanically in the vibration grinder for exactly 10 seconds. The powder was pressed into a thin disc under the total force of 8 tons and the infrared spectrum was recorded over the range of 4000-400 cm^{-1}.

The procedure was repeated by increasing the grinding-time to exactly 1, 2 and 4 minutes.

The cimetidine polymorph B was treated in a similar manner.

5. Quantitative Determination of Cimetidine Polymorphs A and B

5.1 General procedure

A few milligrams of sample (or standard) were mulled with 1-2 drops of nujol in an agate mortar until a uniform consistency was obtained. The mull was placed in the center of one of the potassium bromide plate. The other plate was gently put on the mull and the plates were slowly squeezed together to spread the mull uniformly. The two plates were clamped firmly together in a metal cell holder. The assembled cell was examined by holding it up to the light. It should appear smooth and free of any air bubbles and when placed in the instrument it should give a per cent transmittance of 35 to 45 per cent at 1300 cm^{-1}. The cell was placed in the infrared spectrophotometer and the absorption spectrum was recorded from 1300 to 1000 cm^{-1}.

The general procedure was used throughout the experiment.

5.2 Treatment of spectra

The recorded spectrum was inspected, and the exact wavenumbers of minimum absorption at approximately 1260 and 1090 cm^{-1} (1210 and 1100 cm^{-1} in the spectrum of tablet formulation) were determined. The exact wavenumbers of maximum absorption at approximately 1205 and 1180 cm^{-1}, the characteristic absorption bands of polymorphs A and B respectively, were also determined by inspection.

A straight baseline was drawn between the minimum occurring at 1260 and 1090 cm^{-1} (1210 and 1100 cm^{-1} in the spectrum of tablet

formulation). Straight lines at 1205 and 1180 cm^{-1} were drawn intersecting both the recorded spectrum and the baseline. The corrected absorbances at 1205 and 1180 cm^{-1} were obtained and the absorbance ratio was calculated as follows:

$$\text{absorbance ratio} = \frac{a1205\ cm^{-1}}{a1180\ cm^{-1}} = \frac{B_{1205}-S_{1205}}{B_{1180}-S_{1180}}$$

$a1205\ cm^{-1}$ = Corrected absorbance value at 1205 cm^{-1};

S_{1205} = Absorbance value of recorded spectrum at 1205 cm^{-1};

B_{1205} = Absorbance value at point of intersection of the 1205-cm^{-1} line with the base line;

$a1180\ cm^{-1}$ = Corrected absorbance value at 1180 cm^{-1};

S_{1180} = Absorbance value of recorded spectrum at 1180 cm^{-1};

B_{1180} = Absorbance value at point of intersection of the 1180 cm^{-1} line with the base line.

5.3 Preparation of standard curve I (for raw material)

Following the general procedure and the treatment of spectra described above, a series of standard mixture containing 0.00 (pure A), 5.00 10.0, 15.0....100 % of polymorph B in polymorph A were prepared and the IR spectra were recorded by the nujol mull technique. The absorbance ratios were calculated, and plotted graphically against the polymorph B content in per cent. This plot was used as the standard curve.

5.4 Precision and accuracy of the determinations based on standard curve I

Three mixtures of polymorphs A and B containing 5.00, 10.0and 15.0 % of polymorph B were prepared. Ten determinations were performed on each of the three mixtures, using the general procedure. From the absorbance ratios obtained the contents of the polymorph B could be read from standard curve I and the standard deviations of the results were calculated.

5.5 Preparation of standard curve II (for tablet)

A standard curve for the determination of cimetidine polymorphs A and B in tablet was prepared, using a tablet formulation (Formula I) consisting of cimetidine and excipients.

A series of standard mixtures containing 0.0 (pure A), 5.00, 10.0, 15.0, 20.0, 30.0...., 100 % of polymorph B in polymorph A, were mixed with the tablet excipients according to the following formula:

Formula I (It was similar to tablet sample 5)

Each tablet contains

Lactose	60 mg
Corn starch	30 mg
Magnesium stearate	10 mg
Cimetidine	200 mg

The sufficient amounts of the excipients were first mixed together, and 33.0 mg aliquot of the mixed excipients was mixed with 66.0 mg of each of the standard mixtures. The resulting preparations were subjected to the general procedure, the absorbance ratios were calculated and plotted graphically against the polymorph B content in per cent.

5.6 Precision and accuracy of the determinations based on standard curve II

Three mixtures of polymorphs A and B containing 5.0, 10.0 and 15.0 % of polymorph B were prepared. Each of them was mixed with the excipients as described in 5.5. Ten determinations were performed on each of the resulting preparations, using the general procedure. From the absorbance ratios obtained, the contents of the polymorph B could be read from standard curve II and the standard deviations of the results were calculated.

5.7 Determination of cimetidine polymorph B in experimentally formulated tablet

Two experimentally formulated tablets were developed as follows:

Formula II

Each tablet contains:

Lactose	30 mg
Corn starch	50 mg
Talcum	10 mg
Magnesium stearate	4 mg
Cimetidine	200 mg

Formula III

Each tablet contains:

Corn starch	57 mg
Lactose	40 mg
Magnesium stearate	3 mg
Cimetidine	200 mg

In each formula a standard mixture of cimetidine polymorphs A and B, containing 10 % of polymorph B, was mixed with the tablet excipients. The general procedure was used to obtain the IR spectra. Three determinations were performed on each formula and the quantitative estimations were based on standard curve II.

5.8 Determination of cimetidine polymorphs A and B in commercial raw materials and tablets

A sample of cimetidine raw material and of its formulated tablet obtained from each of ten different local manufacturers were subjected separately to the general procedure. From the spectra obtained, the absorbance ratios were calculated and the polymorph B contents in raw material and in its formulated tablet were read from standard curves I and II, respectively.

Note The identity of crystalline polymorph A of the cimetidine raw material was confirmed by melting point and IR spectrum.

6. Statistical Evaluation of the Results

The reproducibility of the analysis was evaluated by the determination of the relative standard deviation (RSD) as follows:

$$\bar{X} = \frac{\Sigma X_i}{n}$$

$$SD = \sqrt{\frac{\Sigma (X_1 - \bar{X})^2}{n - 1}}$$

$$RSD = \frac{SD}{\bar{X}} \times 100 \ \%$$

$$\text{Relative error} = \frac{O - T}{T} \times 100 \ \%$$

$\bar{X}$ = Mean

X_i = The value of the i^{th} observation;

n = The number of observations;

SD = The standard deviation

RSD = The relative standard deviation.

O = Observed values

T = True values