# CHAPTER IV

# RESULTS AND DISCUSSION

## Preparation of Cimetidine Polymorphs A and B

The results of the crystallization experiments are presented in Table 3. Crystallization from acetonitrile gave the transparent crystals of polymorph A and from aqueous solution gave the opaque crystals of polymorph B. The yields were about 83 % in both cases. Crystallizations from other solvents, such as absolute alcohol, absolute methanol, isopropanol, and water at various solute/solvent ratios, gave mixtures of polymorphic forms. The reproducibility of these results could not be warranted. It seemed that minor changed in the cooling system could cause the formation of mixtures.

## Identification of Cimetidine Polymorphs A and B

There are many techniques used to identify the different polymorphs, such as Microscopy, Hot-Stage Methods, X-Ray Powder Diffraction, Infrared Spectroscopy, and Thermal Methods. In this experiment, Infrared spectroscopy and Thermal methods were used. The IR spectra of polymorphs A and B, shown in Figure 6, 7 and 8 were coincided with form I and form IV reported by Prodic-Kojic et al. (7) respectively.

The characteristic IR absorption bands of polymorphs A and B, were listed in Table 3. (In Fig.6(b) and 7(b); the bands at 1470 $cm^{-1}$ and 1380 $cm^{-1}$ were nujol bands). The Differential Thermal Analysis (DTA) curves in Figure 9, showed the sharp endothem of polymorphs A and B at 140°C and 145°C, respectively. The melting points of polymorphs A and B determined by the Electrothermal Melting Point Apparatus were 139-142°C and 142-145°C, respectively (Table 3).

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

The IR spectra of polymorphs A and B recorded at different manual grinding time were presented in Figure 10 and 11, respectively. The IR spectrum of polymorph A showed no sign of alteration at 10-minute-grinding time. No alteration occurred when polymorph B was ground manually for 3 and 5 minutes. At 7-minute-grinding time some changes were observed in the IR spectrum of polymorph B. The absorption band at 1205 $cm^{-1}$, which is characteristic of polymorph A, was obviously seen. This band as well as another at 1155 $cm^{-1}$ was increased with increasing grinding time.

Figure 12 and 13 showed the IR spectra of polymorphs A and B obtained from the grinding in the vibration grinder. The changes were observed in the region of 1230-600 $cm^{-1}$ of the spectra of the two polymorphs. None of the changes in the IR spectra of polymorphs A and B were corresponded to the characteristic bands of polymorphs B and A, respectively.

Since it was observed that the method of grinding with KBr, both manually in an agate mortar and mechanically in the vibration grinder, caused some changes in polymorphic forms of cimetidine. Therefore the potassium bromide disc method was not suitable for quantitative determination of the polymorphs. No such a phenomenon was observed when using the nujol mull technique. Therefore the later was the method of choice for the IR analysis of the mixture of cimetidine polymorphs.

Quantitative Determination of Cimetidine Polymorph B in the Mixture of Polymorphs A and B using Standard Curve I.

The IR spectra of pure polymorphs A and B were recorded over the range of 1300-1000 $cm^{-1}$, as shown in Figure 8. Two characteristic absorption bands of polymorph A appeared at 1205 $cm^{-1}$ and 1155 $cm^{-1}$ which were well separated from the band characteristic of polymorph B at 1180 $cm^{-1}$. The intensity of the band at 1205 $cm^{-1}$ of polymorph A was greater than that at 1155 $cm^{-1}$. Therefore the former was chosen for the quantitative determination of polymorph A. Obviously the band at 1180 $cm^{-1}$ was used for the determination of polymorph B.

Figure 14 showed the IR spectra of a series of mixtures of polymorphs A and B. The absorption band at 1180 $cm^{-1}$ belongs to polymorph B, the intensity of this band was increased when the content of polymorph B was increased while the intensity of the band at 1205 $cm^{-1}$ (polymorph A) was decreased. The absorbance ratios were summarized in Table 4 and the standard curve thus obtained was presented in Figure 15,

the curve was non linear. When the natural logarithm of the absorbance ratios were plotted graphically against the polymorph B content in percent, a linear standard curve within the range of 0-60 percent of polymorph B as shown in Figure 16 was obtained.

Precision and Accuracy of the Determination Based on Standard Curve I

Ten determinations were made on each of the standard cimetidine containing 5.00, 10.0 and 15.0 % of polymorph B, the results obtained are summarized in Table 6. The determinations based on the nonlinear standard curve, Figure 15, gave the relative standard deviations of 17.6, 7.47 and 5.34 % and the relative errors of -9.00, -5.00 and -2.67 % respectively, when the determinations based on the linear standard curve, Figure 16, it gave the relative standard deviations of 17.2, 6.43 and 4.53 % and the relative errors of -13.0,-2.00 and -1.33 % respectively. The results showed that the determinations based on the linear standard curve, which was constructed by plotting the natural logarithm of the absorbance ratios against the content of polymorph B were more reproduced than the determinations based on the nonlinear standard curve. From Table 6, the results showed that when the polymorph B in the mixture was less than 10.0 % the reproducibility of the method was not good but when the content of polymorph B in the mixture was more than 10.0 % the reproducibility was fairly good. At the concentration of 5.0 % polymorph B the intensity of the absorption band at 1180 $cm^{-1}$ (Fig. 14 b) was too low to measured accurately.

Determination of Cimetidine Polymorphs A and B in Commercial Tablets using the Standard and Curve II.

There are many excipients in a tablet formulation such as lactose, corn starch and magnesium stearate. Figure 17 showed the IR spectra of lactose (a), corn starch (b), magnesium stearate (c), the mixture of the excipients in the formula I (d), the cimetidine raw material of tablet sample 5(e), and tablet sample 5(f). No significant absorption band is observed in spectra a, b and d the spectrum c shows a weak but sharp band at about 1100 $cm^{-1}$. A major change at the baseline is observed in the spectrum f when compared to the spectrum d. The sharp band at about 1100 $cm^{-1}$ due to the excipients has some effects on the cimetidine peaks at this frequency. The baseline could not be drawn between the same two minima as those in the spectrum of cimetidine itself, it had to be drawn between the minima at 1210 and 1100 $cm.^{-1}$ Therefore standard curve I could not be used for the analysis of tablets. To overcome this difficulty, a new standard curve (II) was constructed by adding a mixture of certain excipients to the standard mixtures of polymorphs A and B. Figure 18 shows the spectra of the standard mixtures with added excipients, the results are summarized in Table 5. The absorbance raitos were calculated over the concentration range of 0-60 % polymorph B. When the content of polymorph B was higher than 60 % the baseline at 1210-1100 $cm^{-1}$ could not be drawn. (Fig. 18 1). When the standard curve II was used in the quantitative estimation of the polymorphs in tablet samples, the interference from the tablet excipients was concelled.

From Table 5, the plot of the relative absorbance ratios against the content of polymorph B in per cent gave a nonlinear standard curve (Fig. 19) whereas the plot against the natural logarithm of the absorbance ratios gave a linear standard curve (Fig. 20). The linearity of the curve was obtained over the concentration range of 0-40 %.

Precision and Accuracy of the Determinations Based on standard Curve II

Table 7 shows the results of ten determinations on each of the three standard mixtures of the two polymorphs, containing 5.00, 10.0 and 15.0 % of polymorph B, in the formula I. The relative standard deviations based on the nonlinear standard curve in Figure 19 were 19.1 6.70 and 4.68 % and their relative errors were -12.0,-0.00 and -6.27 % respectively and the determinations based on the linear standard curve in Figure 20, gave the relative standard deviations of 13.4 , 4.70 and 3.08 % and their relative errors were -6.00, -2.00 and -8.66 % respectively. The results showed that the determinations based on the linear standard curve were more reproduced than those based on the nonlinear standard curve. When polymorph B in the mixture was less than 10.0 % the reproducibility of the method was not good. When the content of polymorph B in the mixture was more than 10.0 %, the results were reproducible.

Quantitative Determination of Cimetidine Polymorph B in Experimentally Formulated Tablet

Results of the determinations of the polymorph B content in experimentally formulated tablets, Formula II and Formula III, are

summarized in Table 9. The quantitative estimation of the results was based on the standard curve II although there were some slight differences of these two formulae from Formula I, which was used to construct the standard curve II, regarding the type and proportion of each excipient. The recovery of the polymorph B content was acceptable, range of recovery 100-105 % for Formula II and 100-110 % for Formula III, it indicated that this method is useful for determining the content of polymorph B in tablet provided that the quantitative estimation is based on a suitable standard curve.

Determination of Cimetidine Polymorphs A and B in Commercial Raw Materials and Tablets

Ten samples of commercial cimetidine raw material and of their respective formulated tablets from ten manufacturers were determined by infrared spectrophotometry. The results obtained are shown in Table 8. Only one sample of raw material and it respective tablet (sample 5) were found to contain about 12 % of polymorph B, their IR spectra are shown in Figure 17e and 17 f. Quantitative estimations of these raw materials and tablets were based on the standard curves presented in Figure 19 and 20, respectively. The formula of sample 5 was similar to that of the formula I in this study.

The results shown in Table 8 revealed that no alteration of the polymorphic form of cimetidine occurred during the manufacturing process.