The Qualitative and Quantitative Analysis of Chlorpropamide Polymorphic Mixtures by Near-Infrared Fourier Transform Raman Spectroscopy

Andrew M. Tudor,[1] Steven J. Church,[3] Patrick J. Hendra,[2] Martyn C. Davies,[1,4] and Colin D. Melia[1,4]

Received June 26, 1992; accepted May 24, 1993

We analyzed binary mixtures of polymorphs A and B of chlorpropamide ((1-[4-chlorobenzenesulphonyl]-3-propyl urea)) by near-infrared Fourier transform Raman spectroscopy (FTRS). The individual polymorphs were prepared and characterized by differential scanning calorimetry (DSC), Fourier transform infrared (FT-IR) microscopy, and physical appearance. The FTR spectra of the two polymorphs showed distinct differences which result from "crystal splitting" effects. A series of 13 different mixtures of polymorph A and B was prepared by geometric mixing and their FTR spectra statistically analysed by factor analysis programming. Predictions of the A/B polymorphic composition of mixtures were made and compared with the theoretical values. The results demonstrate that FTRS combined with factor analysis programming may be successfully applied to the *in situ* monitoring of the A/B polymorphic nature of a chlorpropamide sample.

KEY WORDS: Fourier transform-Raman; polymorphism; chlorpropamide; factor analysis.

INTRODUCTION

The importance of drug polymorphism in pharmaceuticals has been widely documented (1). Different polymorphs of the same drug may have different solubilities and dissolution rates, which, particularly if the drug possesses poor solubility, may result in variable absorption and bioavailability problems *in vivo*. As a result, changes in the polymorphic nature of a drug may alter the therapeutic efficacy of a formulation, and there is therefore a need to characterize the polymorphic nature and composition of such drugs in both the quality control of the raw drug material and the final dosage form.

Chlorpropamide (1-[4-chlorobenzenesulfonyl]-3-propyl urea) is a hypoglycemic agent (2), with three major polymorphic forms (A, B, and C), which have been extensively characterized and described in the literature (3). Matsumoto *et al.* describe (4) the polymorphic transformation of form A to C of chlorpropamide during compression processes by temperature and pressure. Form A and B were selected for this study due to their ease of manufacture in their pure form.

The technique of near-infrared Fourier transform Raman spectroscopy (FTRS) has been described in detail elsewhere (5) and is finding extensive application in chemical analysis (6,7). It has been successfully applied to the analysis of drugs (8,9) and drugs in polymers (10), and very recently, some work on drug ploymorphism has been described (11,12). Interferometry has considerably reduced spectral acquisition times and use of a near-infrared source has addressed the problem previously found in resonance Raman spectroscopy of sample-induced fluorescence and photodecomposition.

In this work we describe the use of FTRS to distinguish between two major polymorphs of chlorpropamide (A and B) and how the combination of FTRS and a factor analysis program may be used to evaluate quantitatively the polymorphic composition of binary mixtures of the two. Factor analysis is a form of chemometrics which facilitates interpretation and correlation of spectral information and highlights trends or patterns which may subsequently be used to predict the composition of unknowns (13–15). The program used in this study utilizes principal-component regression, where the known properties of interest of each sample (in this case the percentage of polymorphs A and B) is related to changes in intensities and/or band shifts in their Raman spectra. In this process, those spectral characteristics which are associated with each polymorphic mix are identified, and can be used to predict the A/B composition of an unknown sample from its spectrum.

MATERIALS AND METHODS

Chlorpropamide (1-[4-chlorobenzenesulfonyl]-3-propyl urea) was used as received from Sigma Chemicals (Poole, Dorset, UK). All reagents were of Analar grade.

Preparation of Polymorphs A and B

Preparation methods for chlorpropamide polymorphs were based on a procedure described previously (3) and summarised here.

Chlorpropamide Polymorph A. Chlorpropamide powder (5.0418 g) was dissolved in hot ethanol (7 mL). Water (1 mL) was added dropwise and the resultant solution allowed to recrystallize overnight at room temperature. The crystals were then filtered, washed with cold ethanol/water (1:1), and dried and stored in a vacuum desiccator. A yield of 61.98% was obtained.

Chlorpropamide Polymorph B. Chlorpropamide powder (9.007 g) was dissolved in hot benzene (16 mL) and recrystallized overnight at room temperature. The resulting crystals were filtered, washed with benzene, dried, and stored in a vacuum desiccator. The yield was 96.39%.

Polymorphic Mixtures. Thirteen different binary mixtures were prepared by geometric mixing of appropriate amounts of the two polymorphs (Table I), based on a total weight of 200 mg. The mixtures were stored in a vacuum desiccator at room temperature.

[1] Department of Pharmaceutical Sciences, University of Nottingham, Nottingham, NG7 2RD U.K.
[2] Department of Chemistry, University of Southampton, Southampton, SO9 5NH U.K.
[3] Courtaulds Research, Lockhurst Lane, Coventry, CV6 5RS U.K.
[4] To whom correspondence should be addressed.

0724-8741/93/1200-1772$07.00/0

Table I. The Theoretical Percentage Composition of Chlorpropamide Polymorphs A and B in the Sample Mixtures

Percentage	
A	B
100.00	00.00
87.74	12.26
78.46	21.54
74.46	25.54
68.75	31.25
61.47	38.53
51.85	48.15
39.91	60.09
27.85	72.15
24.93	75.07
20.00	80.00
11.68	88.22
00.00	100.00

Characterization

Differential Scanning Calorimetry (DSC). DSC data on each polymorph were obtained at a scanning rate of 5°/min over the range 370 to 420 K, using a Perkin–Elmer DSC 2.

IR Spectroscopy. Infrared spectra of the polymorphs as prepared, were acquired over 200 scans at a resolution of 4 cm^{-1} using a Bruker FT-IR microscope system (Bruker Spectrospin, Coventry, UK) with a 50-μm aperture spot.

FTR Spectroscopy

Raman spectra of both individual polymorphs and binary mixtures were obtained using a Bruker FRA 106 FTR spectrometer (Bruker Spectrospin, Coventry, UK), an IFS 88 optics bench, and a Nd/YAG diode pumped laser (Adlas Lasers, Lubeck, Germany) at 1.064 μm as the near-infrared source. For qualitative analysis of the individual polymorphs, spectra were acquired over 100 scans at 2-cm^{-1} resolution and a laser operating power of 260 mW. For quantitative analyses spectra were acquired on precisely weighed (in the range 2–4 mg) portions of samples, over 50 scans at 4-cm^{-1} resolution and a laser power of 150 mW. Total analysis time per sample was of the order of 4 min. Factor analysis was undertaken using commercially available software ("Q Factor," Bruker Spectrospin, Coventry, UK).

RESULTS AND DISCUSSION

Identification and Characterization of the Polymorphs

Physical Appearance

The two polymorphs were readily differentiated by their physical appearance. Polymorphic B was a fine white powder, while polymorph form A is in the form of characteristic white "needles," approximately 1 mm in length (3).

DSC

The two polymorphs were readily distinguished by DSC. Polymorph A (analysis weight, 13.71 mg) produced a biphasic thermogram of two peaks over the range 384.38–406.10 K (onset, 400.5 K), the overall ΔH for this transition being 24.81 Cal/g. Polymorph B (analysis weight, 5.07 mg) produced a triphasic thermogram of a main peak with two preceding shoulders over a range of 372.5–401.1 K (onset, 396.8 K). The overall ΔH for the process was 21.29 Cal/g. The thermograms were typical of those reported previously for polymorphs A and B (3).

IR Spectroscopy

The IR spectra showed good correspondence with those published elsewhere for chlorpropamide polymorphic forms A and B (3). The characterization data above suggested that forms A and B were of the desired polymorphic form and were pure.

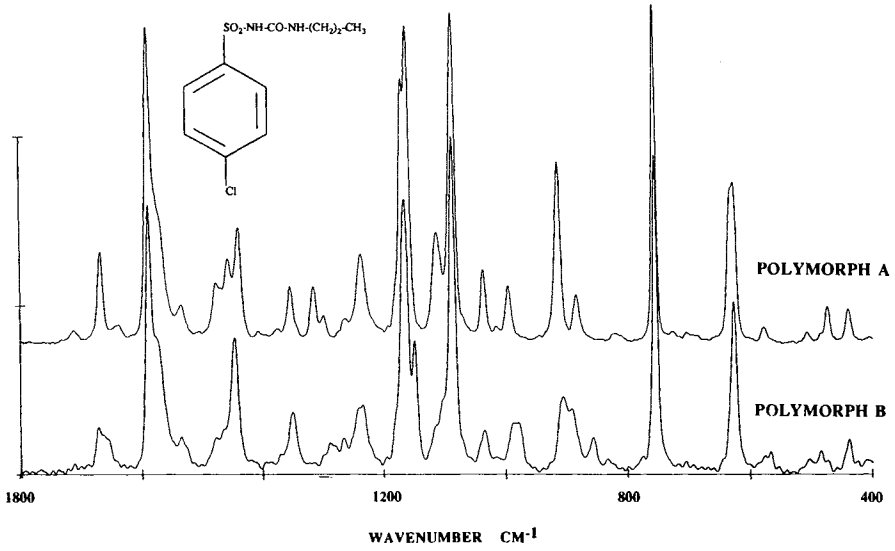

Fig. 1. Comparison of the FTR spectra of polymorphs A and B over the region 1800–400 cm^{-1}.

Table II. Notable Band Splittings in the FTR Spectra of Polymorphs A and B Between 1400 and 800 cm^{-1}

1400–1300 cm^{-1}	Polymorph
	A, 2 bands
	B, singlet
1300–1200 cm^{-1}	Polymorph
	A, singlet
	B, doublet
1100–1000 cm^{-1}	Polymorph
	A, 2 bands
	B, singlet plus doublet
1000–800 cm^{-1}	Polymorph
	A, sharp doublet
	B, broad doublet and low-intensity triplet

FTR Spectroscopy of Polymorphs A and B

FTR spectra for both polymorphs showed good signal-to-noise ratio and no fluorescence. They possessed two distinct regions of interest and are discussed in two sections, region I (1800–400 cm^{-1}) and region II (3200–2800 cm^{-1}). No bands were detected in either spectra between 2500 and 2000 cm^{-1}. Within the regions of interest, the differences between the polymorphs appeared to be manifested in the form of band or peak splitting. The spectra of A and B were also compared with those of the solvents used in recrystallization, ethanol/water (7:1) and benzene, respectively. The spectral differences observed did not appear to originate from these solvents.

Region I, 1800–400 cm^{-1} (Fig. 1)

The peak assignments below are based on literature values or were inferred from IR data. The carbonyl band in the spectrum is manifest as a sharp peak at 1668 cm^{-1} in polymorph A, in comparison with a broader and less intense doublet at 1671/1654 cm^{-1} seen in the spectrum of polymorph B. The peaks around 1450 cm^{-1} (possibly CH_2 bending) are distinctive, with a sharp band (1446 cm^{-1}) with shoulders in the spectrum of B but a low-intensity triplet (1478/1457/1439 cm^{-1}) in polymorph A. It is evident from a close inspection of Fig. 3 that there are many other differences of a similar type throughout region I, and the most notable of these are listed in Table II.

Region II, 3200–2800 cm^{-1} (Fig. 2)

This region is concerned primarily with the C–H stretching vibrations of the methyl and methylene groups and the aromatic ring. Both polymorphs show an intense, sharp doublet associated with aromatic C–H stretching at 3074/3067 cm^{-1} (form A) and 3080/3073 cm^{-1} (form B). In the region 3000–2800 cm^{-1}, the bands associated with CH_2/CH_3 symmetric and C–H asymmetric stretching show marked differences between the polymorphs and the distinctive peak splitting. In polymorph A the spectral region shows five bands (2878, 2922, 2933, 2961, and 2978 cm^{-1}), whereas in polymorph B there are only three (2878, 2940, and 2970 cm^{-1}). These bands may arise as a result of overtones from other vibrational modes or from crystal splitting effects.

Spectra over the region 3000–2800 cm^{-1} for the two polymorphs is compared with that of a mixture (51.85% A and 48.15% B) in Fig. 3. Visual inspection shows that the spectrum of the mixture exhibits characteristics typical of both component polymorphs and in shape appears to be midway between the two.

Quantification of Binary Polymorphic Mixtures by FTRS and Factor Analysis

Instrumental Reproducibility

The FTR spectra of the polymorphs and mixtures were acquired in triplicate and were normalized for differences in sample weight and corrected for background black body emission prior to factor analysis. The spectral windows selected for the analysis were 3200–2800 and 1738–400 cm^{-1} and the corrected spectra over these regions were used to set up a calibration within the factor analysis program. The region 2800–1738 cm^{-1} was excluded, as there is little spectral

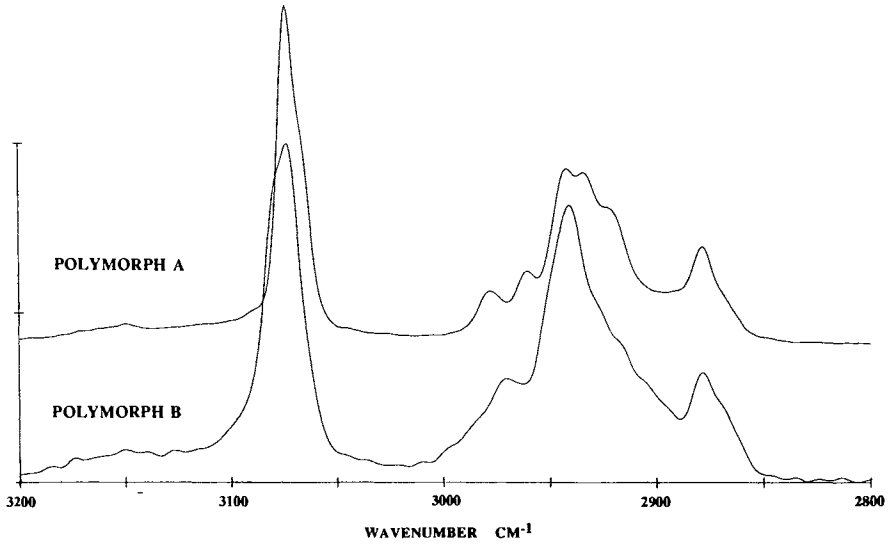

Fig. 2. Comparison of FTR spectra of polymorphs A and B over the region 3200–2800 cm^{-1}.

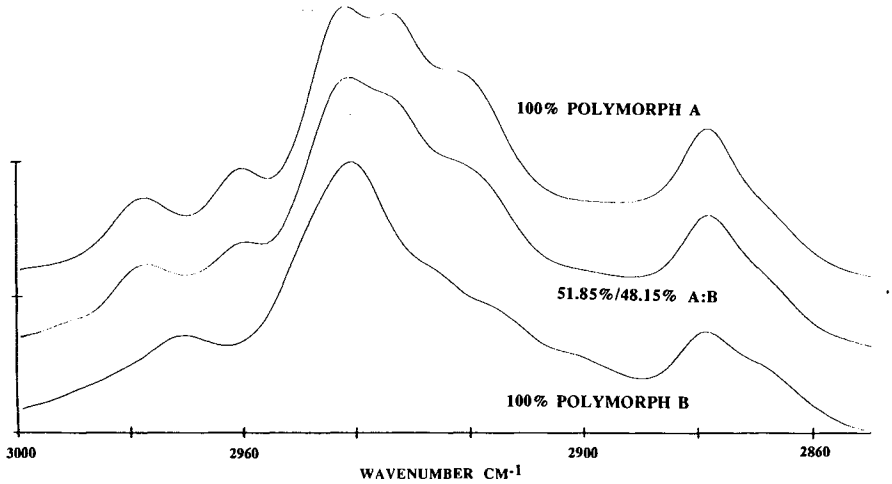

Fig. 3. Comparison of FTR spectra of polymorphs A and B with a 51.85:48.15 A:B mixture over the region 3000–2800 cm^{-1}.

information and the additional baseline noise would reduce the efficiency. Measurements on each sample were repeated three times (to measure instrument response variability), and the polymorphic composition of each individual spectrum was then predicted by factor analysis. Plots of theoretical composition against predicted values are shown in Fig. 4. The standard deviation bars (σ_{n-1}) give an indication of the within-sample precision of the method. Linear regression analysis gave slopes of 0.97 and 0.97, intercept values of 1.2 and 1.9%, and correlation coefficients of $r = 0.988$ and $r = 0.987$ for polymorphs A and B, respectively.

Sample Reproducibility

Three different sample portions from each mixture were analyzed and their FTR spectra examined using factor analysis after being normalised and background corrected as above. The spectral windows were as used previously. Plots of theoretical composition against mean values predicted by the factor analysis are shown in Fig. 5. Linear regression analysis of the data gave slopes of 0.92, intercept values of 3.8 and 3.9%, and correlation coefficients of $r = 0.99$ and for both polymorph A and polymorph B.

In conclusion, we have shown that FTR spectroscopy distinguishes between the chlorpropamide polymorphs A and B, and with factor analysis, may be used to quantify the composition of binary mixtures of the two. The principal benefits of FTRS include being able to analyze samples in their native state (i.e., without the need for sample preparation) and provided there are groups present that are good

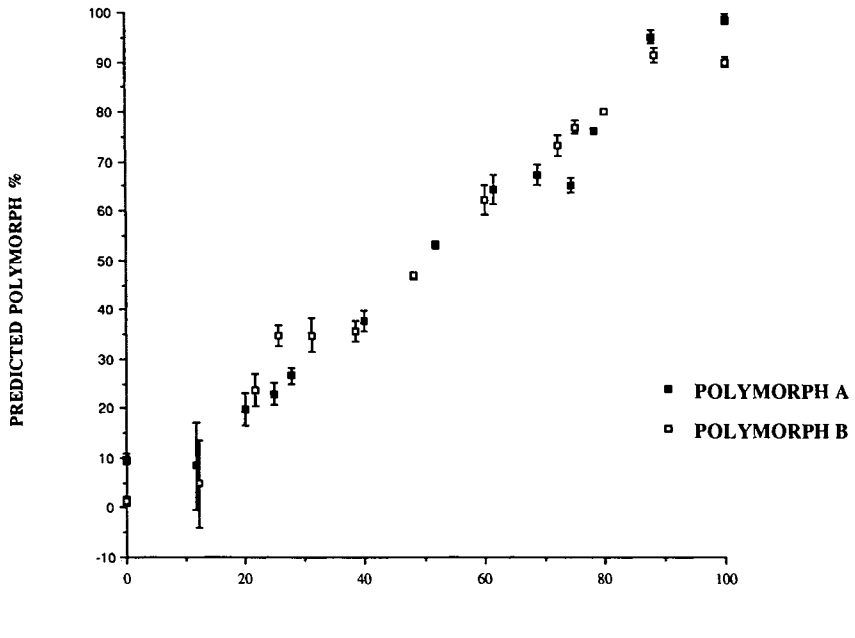

Fig. 4. Plot of values predicted by factor analysis against theoretical polymorph composition, for instrumental reproducibility experiment.

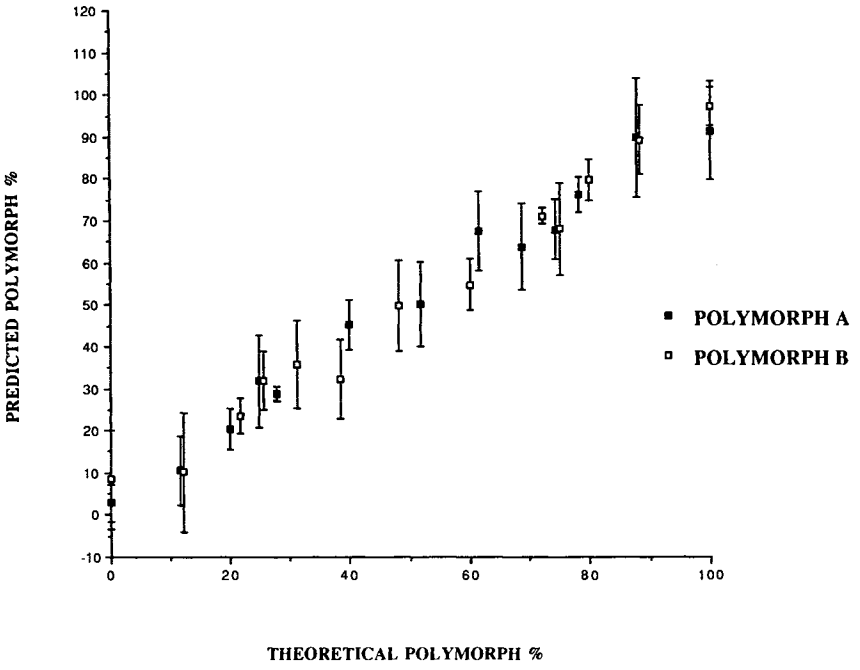

Fig. 5. Plot of values predicted by factor analysis against theoretical polymorph composition, for the between-sample reproducibility experiment.

Raman scatters, and a speed of data acquisition. In addition, providing the material is not unduly sensitive to the Raman laser beam, the same sample can subsequently be analyzed by other techniques to support the Raman data. Automated FTRS equipped with factor analysis would allow samples to be analyzed directly within a manufacturing procedure, in order to monitor any changes in the desired polymorphic ratio.

ACKNOWLEDGMENTS

The authors wish to thank the SERC and Courtaulds for sponsoring AMT and Dr. Paul Turner at Bruker Spectrospin, Coventry, for his help and assistance with the work.

REFERENCES

1. J. K. Haleblian. Characterisation of habits and crystalline modification of solids and their pharmaceutical applications. *J. Pharm. Sci.* 64(8):1269–1288 (1975).
2. *Pharmaceutical Codex,* 11th ed., Pharmaceutical Press, London, 1979.
3. D. L. Simmons, R. J. Ranz, and N. D. Gyanchandani. Polymorphism in pharmaceuticals. II. Chlorpropamide. *Can. J. Pharm. Sci.* 8(4):125–127 (1973).
4. T. Matsumoto, N. Kaneniwa, S. Higuchi, and M. Otsuka. Effects of temperature and pressure during compression on polymorphic transformation and crushing strength of chlorpropamide tablets. *J. Pharm. Pharmacol.* 43:74–75 (1991).
5. D. B. Chase. Fourier transform Raman spectroscopy. *Anal. Chem.* 59:88A (1987).
6. G. Ellis, P. J. Hendra, C. M. Hodges, T. Jawhari, C. H. Jones, P. Le Barazer, C. Passingham, I. A. M. Royaud, A. Sanchez-Blazquez, and G. M. Warnes. Routine analytical Fourier transform Raman spectroscopy. *Analyst* 114:1061 (1989).
7. I. W. Levin and E. N. Lewis. Fourier transform Raman spectroscopy of biological materials. *Anal. Chem.* 62(21):1101A (1990).
8. A. M. Tudor, C. D. Melia, J. S. Binns, P. J. Hendra, S. Church, and M. C. Davies. The application of Fourier transform Raman spectroscopy to the analysis of pharmaceuticals and biomaterials. *J. Pharm. Biomed. Anal.* 8:717–720 (1990).
9. M. C. Davies, J. S. Binns, C. D. Melia, and D. Bourgeois. Fourier transform Raman spectroscopy of polymeric biomaterials and drug delivery systems. *SpectroChim. Acta* 46A:277–285 (1990).
10. P. J. Watts, A. M. Tudor, S. Church, P. J. Hendra, P. Turner, C. D. Melia, and M. C. Davies. Fourier transform Raman spectroscopy for the qualitative and quantitative characterization of sulfasalazine containing polymeric microspheres. *Pharm. Res.* 8(10):1323–1328 (1991).
11. A. M. Tudor, S. J. Church, P. J. Hendra, D. C. Lee, C. D. Melia, R. C. Mitchell, and M. C. Davies. The application of near-infrared Fourier transform Raman spectroscopy to the analysis of polymorphic forms of cimetidine. *SpectroChim. Acta* 47A:1389–1393 (1991).
12. S. O. Paul, C. J. H. Schutte, and P. J. Hendra. The Fourier transform Raman and infrared spectra of naphthazarin. *SpectroChim. Acta* 46A:323–330 (1990).
13. D. C. Lee, P. I. Harris, D. Chapman, and R. C. Mitchell. Determination of protein secondary structure using factor analysis of infrared spectra. *Biochemistry* 29(39):9185–9193 (1990).
14. P. M. Fredericks, J. B. Lee, P. R. Osborn, and D. A. J. Swinkels. Materials characterization using factor analysis FT-IR spectra. 1. Results. *Appl. Spect.* 39(2):303–310 (1985).
15. P. Turner and J. Gronholz. Quantitative analysis of mixtures using factor analysis. Bruker FT-IR Application Note 38.