The Quantitative Analysis of Crystallinity Using FT-Raman Spectroscopy

Lynne S. Taylor[1] and George Zografi[1,2]

Received December 8, 1997 accepted February 8, 1998

Purpose. To establish if FT-Raman spectroscopy can be used to quantitate the degree of crystallinity in a model compound.
Methods. Mixtures containing different proportions of amorphous and crystalline indomethacin were prepared. Using the peak intensity ratio 1698 cm^{-1} (crystalline) to 1680 cm^{-1} (amorphous), a correlation curve was prepared. This correlation curve was validated by testing further samples of known composition. Partially crystalline indomethacin was prepared by milling crystalline indomethacin.
Results. A linear correlation curve was obtained across the entire range of 0–100% crystallinity. Using this method, it was possible to detect down to either 1% amorphous or crystalline content. The largest errors were found to result from inhomogeneities in the mixing of the calibration and validation samples. The spectra of the mechanically processed samples were similar to the spectra of the calibration samples, and the degree of crystallinity could be estimated in these samples.
Conclusions. FT-Raman spectroscopy is a potentially useful method to complement existing techniques for the quantitative determination of crystallinity.

KEY WORDS: FT-Raman spectroscopy; indomethacin; crystallinity; quantitation.

INTRODUCTION

Many pharmaceutical processing operations including milling and compaction can introduce disorder into crystalline materials. The extent of this disorder can range from local surface disorder to complete disruption of the lattice. The creation of amorphous material may be problematic resulting in increased solubility and hygroscopicity in addition to changes in mechanical and flow properties and a decrease in chemical stability. However, in some instances, these properties that the amorphous state confer may be advantageous and it is often desirable to produce and maintain the amorphous state. Thus the increased initial solubility can enhance bioavailability of poorly water soluble drugs whilst the addition of sugars as lyoprotectants during the freeze-drying of proteins requires the additive to be in the same amorphous phase as the protein in order to be effective.

Many techniques may be utilised to detect, quantitate and characterise the amorphous state (1,2). For quantitative measurements, x-ray diffraction, moisture sorption and microcalorimetry are currently the most widely utilised methods (2–5). Techniques which enable the detection and quantitation of low percentages of amorphous material (less than 5%) are of particular interest as are those which are amenable to *in situ* and on-line testing.

FT-Raman spectroscopy has recently been successfully used to quantitate mixtures of polymorphs, utilising distinct spectral peaks characteristic of each form (6) or factor analysis techniques (7,8). This method has been advocated for such studies since the analysis time can be very rapid and no sample preparation is required. In theory, it can be applied to the quantitation of amorphous content if the spectra of the amorphous and crystalline phases are sufficiently different, as has been recently suggested (6).

Previous work in this laboratory has involved the spectroscopic characterisation of the crystalline and amorphous forms of indomethacin (9). Indomethacin, a poorly water soluble hydrophobic molecule, can easily be prepared as the amorphous phase by quench cooling of the melt. The amorphous and stable crystalline γ polymorph can be distinguished by FT-Raman spectroscopy and it was thus of interest to determine if this method could be used for the quantitation of amorphous or crystalline content. In this work, mixtures of amorphous and γ crystalline indomethacin (hereafter referred to as crystalline indomethacin) were prepared in known ratios and an equation which allowed calculation of the percentage crystallinity from measured spectral intensity ratios was generated. This equation was used to estimate the extent of crystallinity in validation mixtures and in milled samples.

MATERIALS

Indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, as the γ crystal form, was obtained from Sigma Chemical Co. This material was in the form of a fine powder which could be passed through a 150 μm sieve and it was assumed that this material was 100% crystalline. Amorphous indomethacin was prepared by melting crystalline indomethacin at 165°C for 5 minutes and quench cooling in liquid nitrogen. The amorphous material was warmed to room temperature under vacuum to prevent atmospheric moisture condensation on the sample, lightly pulverised to a fine powder (<150 μm) and stored over phosphorous pentoxide at −20°C.

METHODS

X-ray Powder Diffraction

X-ray powder diffraction patterns of crystalline and amorphous indomethacin were obtained using a PadV scintag scanning x-ray powder diffractometer (Scintag Inc., Santa Clara, California) as described in more detail previously (2). A step size of 0.05 degrees 2θ with a count time of 1.5 seconds was used over the 2θ range 10–40°.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry measurements were carried out in aluminum pans with a pinhole lid using a Seiko model SSC5200 instrument (Seiko Instruments, Horsham, PA) at a heating rate of 20°C min^{-1} under a dry nitrogen gas purge.

[1] School of Pharmacy, University of Wisconsin-Madison, 425 North Charter Street, Madison, Wisconsin 53706.
[2] To whom correspondence should be addressed. (e-mail: gzografi@facstaff.wisc.edu)

0724-8741/98/0500-0755$15.00/0

Mixing of Samples

Known ratios of amorphous and crystalline indomethacin were prepared by geometrically mixing a total weight of 500 mg in a Wig-L-Bug (Crescent Dental Mfg. Co., Lyons, IL) stainless steel mixing capsule without the ball. This procedure was not found to influence the crystallinity of the crystalline material as monitored by comparing the enthalpy of fusion of the crystalline material before and after agitating in the mixing capsule. The entire sample was transferred to a sealed glass vial of approximately 1 cm diameter and stored at −20°C at 0% RH until analysis was performed.

Milling of Samples

Milled samples were prepared by grinding 500 mg of crystalline indomethacin in the Wig-L-Bug stainless steel mixing capsule with a metal milling ball for 5, 15 and 30 minutes.

FT-Raman Spectroscopy

FT-Raman spectra were collected on a Bruker RFS 100 FT-Raman system with a near infrared Nd:YAG laser operating at 1064 nm. The laser was focused on the sample as an approximately 1 mm spot with a power of around 450 mW and a liquid nitrogen-cooled germanium detector was used. Back scattered radiation at an angle of 180° was collected and the Stokes scattering is reported. 100 scans over the wavenumber range 4000–50 cm^{-1} at a resolution of 4 cm^{-1} were averaged for each sample. The powders were analysed in sealed thin-walled glass vials.

Estimation of Assay Errors

The following parameters were investigated in order to evaluate potential sources of error and are based in part upon those suggested by Bugay et al. who investigated errors associated with the quantitation of polymorphs using diffuse reflectance infrared spectroscopy and x-ray powder diffraction (10).

(a) Laser power—three consecutive scans were obtained from a 55% crystalline sample at laser powers of 225, 450 and 900 mW.

(b) Instrument variability—a sample composed of 55% crystalline indomethacin was placed in the instrument and 10 consecutive scans were obtained without removing the sample.

(c) Sample homogeneity—samples composed of 55% crystalline indomethacin was placed in the instrument, scanned, removed and shaken prior to rescanning. A total of 5 scans were obtained for each of 6 samples. The range in the crystallinity measured for the 30 samples was determined. It should be noted that there is a contribution from sample positioning to the error determined for sample homogeneity.

(d) Overall method error—6 independent mixtures containing 55% crystalline indomethacin were prepared and each scanned 5 times, shaking after each analysis. The relative standard deviation of the method RSD(%) at this composition was calculated from:

$$\mathrm{RSD}(\%) = \frac{\mathrm{SD}*100}{\bar{x}} \qquad (1)$$

where SD is the standard deviation and $\bar{x}$ is the mean.

RESULTS

Characterisation of the Reference Materials

Figure 1 shows the X-ray powder diffraction patterns for the samples of indomethacin used in the study and taken to represent 100% amorphous and 100% crystalline materials. The X-ray diffraction pattern of the amorphous material shows no evidence of crystalline peaks. The diffraction pattern for the crystalline γ polymorph of indomethacin is in good agreement with that published previously (11).

On analysis by DSC, the amorphous material exhibited a glass transition temperature at 45°C. Crystalline indomethacin showed no evidence of a glass transition and underwent fusion at 161°C (peak temperature) with a fusion enthalpy of 109 J/g. These results are in excellent agreement with previous studies on the characterisation of indomethacin (11,12) and confirm the essentially amorphous and crystalline nature of the reference materials.

Construction of a Correlation Curve

The Raman spectra of crystalline and amorphous indomethacin are shown in Figure 2. The differences in the spectra have been discussed previously (9). For quantitative analysis it is necessary to identify peaks characteristic of each form; the benzoyl carbonyl vibrations fulfill this criteria as can be seen from Figure 3. Crystalline indomethacin has a carbonyl peak at 1698 cm^{-1} whilst that of amorphous indomethacin appears at 1680 cm^{-1}. These peaks were used for quantitative analysis.

The two peaks are not completely resolved as a consequence of the broadening of the carbonyl peak in the amorphous spectrum. Therefore the contribution of the amorphous peak to the crystalline peak intensity and vice versa must be taken into account. Kontoyannis *et al.* (13) recently developed a method

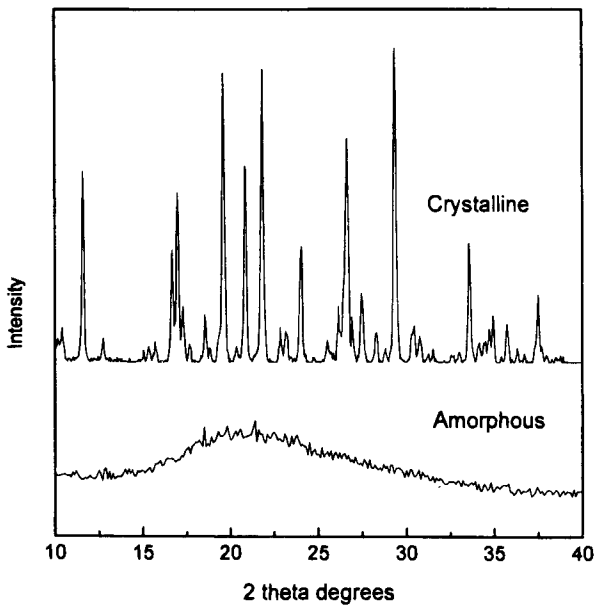

Fig. 1. X-ray diffraction patterns of crystalline and amorphous indomethacin taken to represent 100% crystalline and 100% amorphous starting materials.

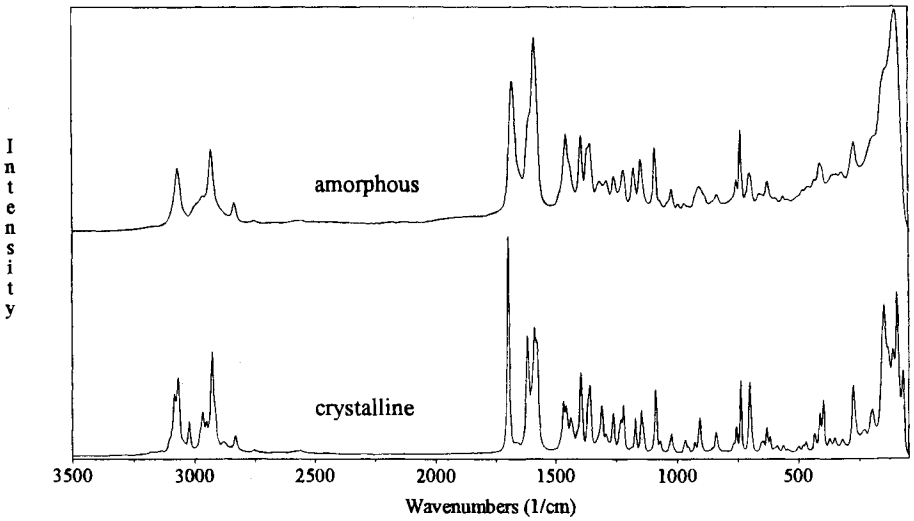

Fig. 2. FT Raman spectra of crystalline and amorphous indomethacin.

for the quantitative analysis of calcium oxalate hydrates using Raman spectroscopy, where the characteristic peaks of each hydrate are not completely resolved. This method can be applied to the quantitation of the amorphous-crystalline system under investigation, as follows. The measured intensity of a Raman line, $I(\nu)$ can be given by equation 2:

$$I(\nu) = I_0 K(\nu) C \tag{2}$$

where I_0 is the intensity of the excitation laser line, ν is the Raman shift, $K(\nu)$ is a factor which includes the overall spectrophotometer response, the self absorption of the medium and molecular scattering properties of the medium and C is the concentration of the species of interest. The ratios of the K factors can be considered to be dependent only on the scattering parameter associated with each band (13).

The ratio of the intensities of the crystalline and amorphous peaks, IR, is described by:

$$I_R = \frac{I_c^{1698}}{I_a^{1680}} = \frac{x_c K_c^{1698} + x_a K_a^{1698}}{x_c K_c^{1680} + x_a K_a^{1680}} \tag{3}$$

where the contribution of the amorphous peak to the measured intensity of the crystalline peak and vice versa is taken into account. The subscript denotes the form of the material (c for crystalline and a for amorphous), x is the mole fraction of each form present.

The ratios K_c^{1680}/K_c^{1698} and K_a^{1698}/K_a^{1680} were calculated from the spectra of crystalline and amorphous indomethacin, respectively, and values reported represent the arithmetic mean of 10 measurements. K_c^{1680}/K_c^{1698}; was calculated to be 0.052 and K_a^{1698}/K_a^{1680} to be 0.401. The ratio K_c^{1680}/K_c^{1698} represents the contribution of the crystalline component to the region of the spectrum where the amorphous peak intensity is measured. Since this value is only 0.052 for the pure crystalline material, the contribution to the measured peak intensity of the amorphous component will be relatively minor in the mixtures reflecting the narrow half-height width of the crystalline peak and that little spectral overlap occurs. In contrast the higher value of the analogous ratio for the amorphous material, K_a^{1698}/K_a^{1680}, indicates that the contribution of the broad amorphous peak to the observed crystalline peak in mixtures will be larger.

These values can be substituted into equation 4 (derived from equation 3) which can then be used to calculate the ratio K_c^{1698}/K_a^{1680}:

$$\frac{K_c^{1698}(I_R * 0.052 - 1)}{K_a^{1680}(I_R - 0.401)} = 1 - \frac{1}{x_c} \tag{4}$$

The IR values were determined from mixtures containing known ratios of crystalline and amorphous indomethacin over the composition range 0–100% crystalline. A plot of $(x_c - 1)$ $(I_R - 0.401)$ versus $x_c(0.052 * I_R - 1)$ is shown in Figure 4. The

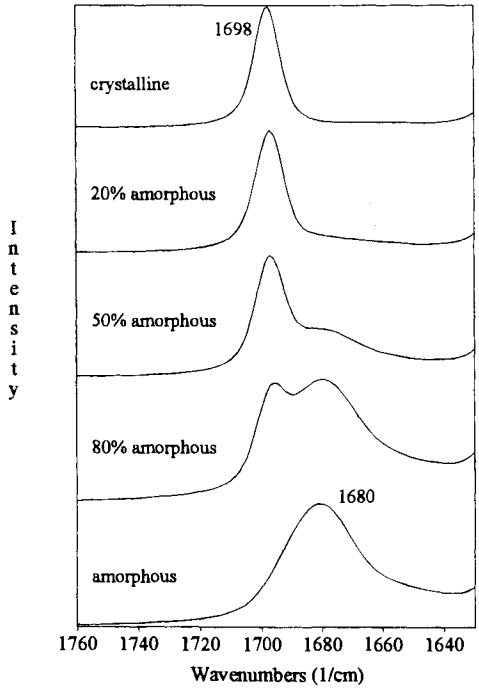

Fig. 3. FT Raman spectra of the carbonyl stretching region of mixtures of amorphous and crystalline indomethacin.

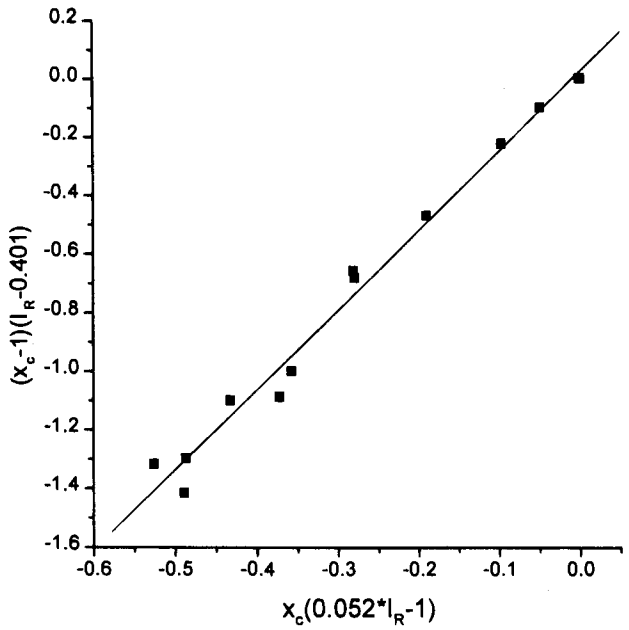

Fig. 4. Plot of $(x_c - 1)(Ir-0.401)$ vs. $x_c(0.052*I_r-1)$ where x_c represents the mole fraction of crystalline component and I_r is the intensity ratio of the Raman band at 1698 cm^{-1} to that at 1680 cm^{-1}.

data were fitted using least squares analysis and the slope of the line (correlation coefficient 0.993), equal to K_c^{1698}/K_a^{1680}, was determined to be 2.72. This value can be used to rewrite equation 4 as follows:

$$x_c = \frac{I_R - 0.401}{0.859I_R + 2.316} \quad (5)$$

Equation 5 thus enables the percentage crystallinity to be determined from a knowledge of the intensity ratio of the amorphous and crystalline peaks. Figure 5 shows a plot of the calibration data illustrating the relationship between the calculated and known crystallinity. This plot can be fitted by least squares analysis to a straight line which has a slope of 1.01, an intercept of −0.01 and a correlation coefficient of 0.9996, demonstrating the validity of equation 5.

Validation of the Correlation Curve

One way of determining the validity of the calibration data is to test samples of known composition which were not used in the calculation of the values in equation 5. It is also of interest to probe the ability of this method to measure small percentages of both crystalline and amorphous components. The relationship between the calculated crystallinity, determined using equation 5, and the actual crystallinity for the validation samples across the entire composition range is shown in Figure 6. The data are linear with a slope of 1.008 and an intercept close to zero at −0.0139, indicating that the assay is reasonably rugged and that the calculated and input crystallinity values are in good agreement. These data are presented in more detail in Table 1. Here the mean values, standard deviations and range of the data are shown. It is apparent that it is possible to detect and quantitate both small percentages of amorphous and of crystalline material in addition to mid-range compositions. The range of the data is quite large indicating that several measurements must be averaged in order to accurately predict the composition. Thus there is an improvement in accuracy of the measured crystallinity value on increasing the number of measurements from 5 (one sample) to 15 (three independent samples) or even 30 (six independent samples). The source of these errors are explored in more detail below.

One State Versus Two State Model

It has been pointed out that the use of physical mixtures of amorphous and crystalline materials represent a two state model such that each particle is entirely amorphous or entirely

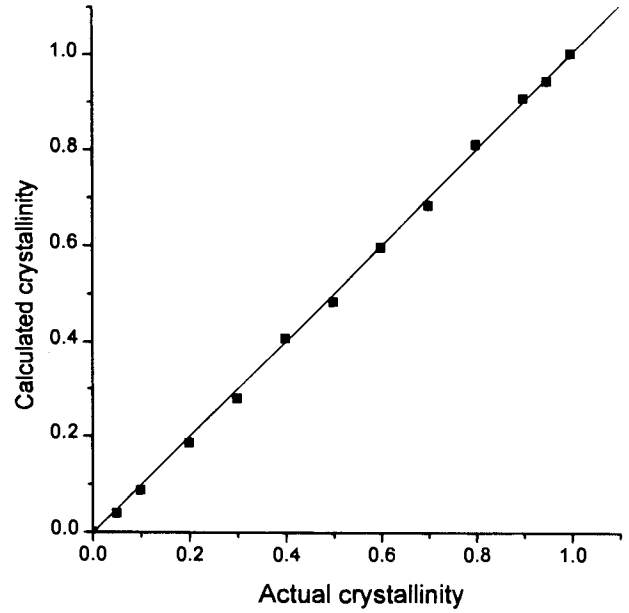

Fig. 5. Correlation curve for mixtures of crystalline and amorphous indomethacin.

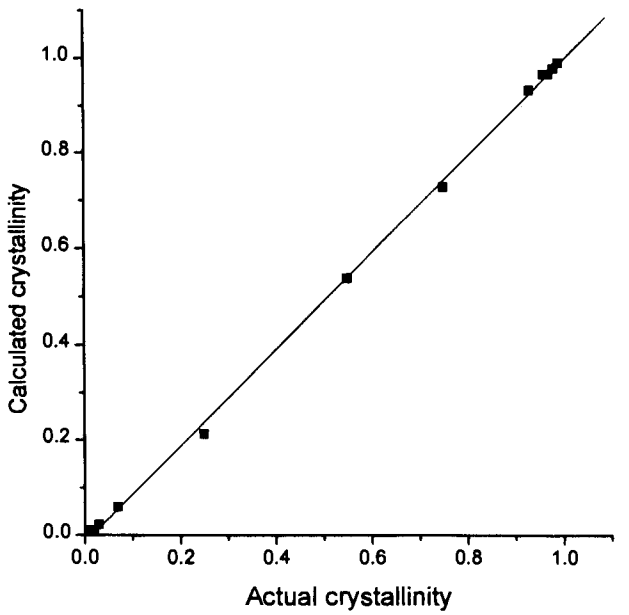

Fig. 6. Validation curve for mixtures of crystalline and amorphous indomethacin.

Table 1. Comparison of Known Crystallinity and Measured Crystallinity for Samples Across the Range of 99-1% Crystallinity Illustrating the Standard Deviation (Parentheses) and Range of the Data

Actual Crystallinity	Measured Crystallinity	Experimental Range
99	98.7 (0.3)[a]	99.2–97.9
98	97.7 (0.6)[a]	98.6–96.1
97	96.5 (1.2)[a]	97.7–94.0
93	93.5 (0.9)[a]	94.5–91.8
96	96.3 (0.9)[b]	97.1–94.8
75	73.6 (2.3)[b]	75.9–70.1
55	54.0 (4.0)[c]	62.5–44.8
25	21.3 (1.5)[b]	22.9–19.1
7	5.9 (0.6)[b]	6.8–5.3
3	2.2 (0.4)[b]	2.6–1.6
2	1.0 (0.4)[b]	1.35–0.05
1	0.9 (0.2)[b]	1.05–0.64

[a] 3 samples, 5 runs each.
[b] 1 sample, 5 runs.
[c] 6 samples, 5 runs each.

crystalline, whereas in mechanically processed samples, materials are likely to exist in an intermediate, partially crystalline state, termed the one state model (14). It is therefore of interest to investigate the ability of Raman spectroscopy to detect the degree of order in mechanically processed samples. Following the milling of crystalline indomethacin, the intensity at 1680 cm^{-1} increased indicating that amorphous material had been formed as a result of the milling process. After milling for 5, 15 and 30 minutes, the resultant FT-Raman spectra were comparable to those obtained from the calibration samples with no changes in peak positions noted. It should be noted that milling reduces the particle size of the sample and this can also influence the intensity measurements. This effect of particle size was not evaluated in this study. The degree of crystallinity, estimated using equation 5, was observed to decrease with increased milling time and was estimated to be equivalent to binary mixtures containing 85.0% (85.3–84.8), 77.5% (78.5–76.0) and 63.5% (61–65.5) crystalline component after 5, 15 and 30 minutes of grinding, respectively. The data are the arithmetic mean of 5 determinations and the values in parentheses represent the range of the data.

Estimation of Detection and Quantitation Limits

The limit of detection of amorphous material in a crystalline sample was estimated by calculating 3 standard deviations of the crystallinity value determined using equation 5 for pure crystalline sample (15). The standard deviation for 10 measurements of pure crystalline indomethacin was 0.2% giving a lower limit of detection of 0.6%. The limit of quantitation can likewise be estimated by calculating 10 standard deviations resulting in a value of around 2%. The standard deviation for the measurement of the pure amorphous component was also 0.2% so similar values for the detection and quantitation levels of crystalline material can be estimated. From the experimental data shown in Table 1 a limit of detection of around 1% amorphous or crystalline content would seem reasonable and is fairly close to the estimated value, however the variation in the data obtained for the mixtures suggests that the estimate of the limit for quantitation is too low.

Sources of Error

It was found that little error was introduced into the measurement by changing the laser power with values over the range 54.4–56.6% crystallinity being determined. Although the peak height ratios change very little the absolute intensities do increase with an increase in laser power. Instrument fluctuations were measured by comparing consecutively acquired spectra and found to vary between 55.8 and 56.3% crystallinity. Sample heating is a well known problem associated with laser Raman spectroscopy. The reproducibility of the results both on raising the laser power and on scanning the same spot 10 times consecutively in a mixture of amorphous and crystalline indomethacin suggests that in this instance any heat supplied to the sample by the laser does not result in crystallisation of the amorphous component. It should be noted that errors associated with the measurement of the peak heights contribute to all of the errors discussed.

The largest source of error appears to be in the homogeneity of mixing during the preparation of samples with the crystallinity values measured for 30 samples ranging between 44.8 and 62.5%. This can be seen in more detail from Table 2 which compares mean values and standard deviations obtained from repeat scans on the same sample, shaken in between analyses, with those obtained from independently prepared samples. The mixtures were 55% crystalline. The variation in results from analysing different regions of one sample 5 times is comparable to that obtained from analysing 6 samples once and the mean values are also comparable. Thus the source of variation can be attributed to non homogeneities of mixing within a sample rather than non-reproducibilities in the preparation of the independent samples. The overall method error was estimated from the data in Table 2. This error includes any error in the sample preparation as well in the analysis of the samples. The mean of the 30 data points in Table 2 was calculated to be 54% with a standard deviation of 4% resulting in an overall method RSD value of 7.3% at this composition. However, it can be seen from Table 1 that the RSD will be dependent on the composition of the sample since the standard deviations show no discernible trends with the crystallinity.

DISCUSSION

The ability of this method to quantitate the degree of order in the model system examined suggests that Raman spectroscopy would be a useful technique to complement existing methods. The other techniques measure various properties of the amorphous or crystalline states with the lower limits of detection varying from method to method. For example in X-ray diffraction, the average degree of crystalline order is measured with a lower limit of detection for amorphous content of around 10% (2). In contrast techniques such as water vapor sorption and isothermal microcalorimetry rely on the higher energy state associated with the disordered phase and can detect as low as 1% disorder (2,16). Raman spectroscopy, like IR, provides molecular information about both the crystalline and the amorphous phases as are, with no phase transformation being required for analysis. Previous studies have found a good correlation between the degree of crystallinity measured by infra red spectroscopy and X-ray diffraction (4). It appears from the results of this study that it is possible to measure not only mid range levels of crystallinity but also low percentages of both

Table 2. Inter and Intra Sample Variation for a Nominally 55% Crystalline Sample

	A	B	C	D	E	F	Mean	SD
1	59.7	50.0	52.2	54.0	55.5	46.6	53.0	4.5
2	52.7	58.0	53.0	55.4	44.8	54.6	3.1	4.5
3	53.7	53.5	62.5	54.5	54.5	52.5	55.2	3.7
4	57.5	53.8	56.9	52.2	55.2	59.6	55.9	2.7
5	53.8	47.7	55.7	52.2	49.5	59.1	53.0	4.1
Mean	55.5	52.6	56.1	53.8	51.9	54.5	54.0	1.6
SD	3.0	4.0	4.1	1.4	4.6	5.3	1.4	

Note: The letters A–F represent 6 independently prepared samples whilst the numbers 1–5 represent the scan number for scans obtained from the same samples where the sample is shaken in between scans. SD refers to the standard deviation of the data.

crystalline and amorphous components using Raman spectroscopy.

Raman spectroscopy has both potential advantages and disadvantages as a technique for measuring the degree of disorder. One advantage is that no sample preparation is necessary, thus the likelihood of inducing phase changes through processing is reduced. However, the laser can cause heating of the sample which could result in crystallisation if care is not taken. Accessories which cool the sample may be used if this is found to be a problem.

In this study, inhomogeneities in mixing were found to be the largest source of error in quantitating the degree of crystallinity. The preparation of homogeneously mixed standards for the construction of a calibration curve is obviously a necessity for any quantitative method. However, different methods will be sensitive to inhomogeneities over different scales. The area of the powder bed sampled in Raman spectroscopy can be small and can lead to sub-sampling. In this study, the laser beam was defocused to the largest spot size which is approximately 1 mm for the commercial instrument used. Both particle size and the homogeneity of mixing will influence how representative the scan is of the overall mixture. It is easy to envisage non-representative sampling in powders composed of large particles (close to dimensions of the laser sampling area), containing a wide range of particle sizes, or containing clusters of one component. Bugay *et al.* (10) utilised an acetone slurry technique to improve the mixing of two hydrates, however caution must be applied when dealing with amorphous materials which may sorb solvents, altering the material properties. Another approach is detailed by Langkilde *et al.* (6) who devised a rotating sample holder, allowing several areas of the powder bed to be sampled during the course of analysis. This device was found to provide a spectrum more representative of the overall composition, in addition to decreasing the likelihood of sample heating. In this study several spectra were obtained for each mixture to try and ensure representative sampling. Each spectrum was obtained after re-mixing the powder by agitation. However this is not an efficient method compared with a rotating sample holder and the problems with homogeneity are reflected in the relatively high standard deviations of the data. It is therefore envisaged that use of a device which enabled averaging of data obtained from different parts of the sample would improve this method substantially and reduce the variation in results. This in turn would improve the limits of detection and quantitation since these are dependent on the variation in the data.

For samples which have been processed so that amorphous character has been induced, if each particle has the same degree of disorder, the small sampling area would be not be problematic. However, it is more likely that a distribution of disorder is introduced depending on the extent of the particle size reduction of the individual particles so the above considerations apply.

To date, the influence of particle size on the Raman signal intensity has not been examined in detail. Initial studies on inorganic materials indicate that the intensity increases with a decrease in particle size with changes of the order of 100 μm resulting in observable differences (17). It would therefore be cautious to ensure that the particle size distribution of the calibration standards is similar to that of the test samples or that the influence of particle size variations is evaluated. The particle sizes of the two powders used to make binary calibration standards should also be controlled.

There is a great deal of interest currently in utilising analytical techniques which enable non destructive *in situ* testing of raw materials and products in addition to on-line monitoring of pharmaceutical processes. Near infrared spectroscopy is receiving a great deal of attention in this capacity (18,19). The possibilities of using FT-Raman spectroscopy for the analysis of the active ingredient in an intact dosage form have also been pointed out; polymeric excipients are generally poor Raman scatters relative to drug substances (20,21). Since Raman spectroscopy can be used in conjunction with fibre optic probes there are also good possibilities for remote sampling. It is thus conceivable that this technique could find application for the on-line analysis of pharmaceutical processes, for example to assess the impact of a milling stage on the polymorphic form or crystallinity of a drug.

CONCLUSIONS

A quantitative, non-destructive FT-Raman spectroscopic method has been developed for the analysis of mixtures of crystalline and amorphous indomethacin. A correlation curve was constructed which was linear across the entire composition range. Using this method, low levels of both amorphous and crystalline material could be detected in the mixtures. The loss of crystallinity in mechanically processed samples was also estimated. This preliminary study suggests Raman spectroscopy would be a useful method for the quantitation of the degree of crystallinity and possible advantages and disadvantages have been discussed. Further investigations are needed to establish correlations with existing techniques for processed samples

where the measured response may depend on the property of the amorphous/crystalline state to which each technique is sensitive. Evaluation of the errors associated with this assay indicates that the largest source of variation in measurements arises from inhomogeneous mixing of the amorphous and crystalline components in the powder blends. It is considered that the method would be improved and the variations in data reduced by utilising an alternative sampling technique which averaged data from different regions of the sample.

ACKNOWLEDGMENTS

The authors wish to thank the sponsors of the Purdue/ Wisconsin Joint Program on Molecular Mobility and Solid-State Properties for financial support of this project. Dr. R. Atalla and Dr. U. Agarwal of the USDA Forest Products Laboratory, Madison, Wisconsin are thanked for their helpful discussions and use of the FT-Raman apparatus. We are most grateful to Dr. K.A. Connors of the School of Pharmacy, University of Wisconsin-Madison for assistance with data analysis. We would like to thank the anonymous reviewers for helpful comments and suggestions.

REFERENCES

1. B. C. Hancock and G. Zografi. Characteristics and significance of the amorphous state. *J. Pharm. Sci.* **86**:1–12 (1997).
2. A. Saleki-Gerhardt, C. Ahlneck, and G. Zografi. Assessment of disorder in crystalline solids. *Int. J. Pharm.* **101**:237–247 (1994).
3. H. P. Klug and L. E. Alexander. *X-Ray diffraction procedures for polycrystalline and amorphous materials.* Wiley, New York, 1974.
4. D. B. Black and E. G. Lovering. Estimation of the degree of crystallinity in digoxin by X-ray and infrared methods. *J. Pharm. Pharmacol.* **29**:684–687 (1977).
5. T. Sebhatu, M. Angberg, and C. Alhneck. Assessment of the degree of disorder in crystalline solids by isothermal microcalorimetry. *Int. J. Pharm.* **104**:135–144 (1994).
6. F. W. Langkilde, J. Sjöblom, L. Tekenbergs-Hjelte, and J. Mrak. Quantitative FT-Raman analysis of two crystal forms of a pharmaceutical compound. *J. Pharm. Biomed. Anal.* **15**:687–696 (1997).
7. C. M. Deeley, R. A. Spragg, and T. L. Threlfall. A comparison of Fourier transform infrared and near-infrared Fourier transform Raman spectroscopy for quantitative measurements: an application in polymorphism. *Spectrochimica Acta* **47A**:1217–1223 (1991).
8. A. M. Tudor, S. J. Church, P. J. Hendra, M. C. Davies, and C. D. Melia. The qualitative and quantitative analysis of chlorpropamide mixtures by near-infrared Fourier transform Raman spectroscopy. *Pharm. Res.* **10**:1772–1776 (1993).
9. L. S. Taylor and G. Zografi. Spectroscopic characterization of interactions between PVP and indomethacin in amorphous molecular dispersions. *Pharm. Res.* **14**:1691–1698 (1997).
10. D. E. Bugay, A. W. Newman, and P. Findlay. Quantitation of cefepime 2 HCl in cefepime 2HCl monohydrate by diffuse reflectance IR and powder X-ray diffraction techniques. *J. Pharm. Biomed. Anal.* **15**:49–61 (1996).
11. M. Otsuka, T. Matsumoto, and N. Kaneniwa. Effect of environmental temperature on polymorphic solid-state transformation of indomethacin during grinding. *Chem. Pharm. Bull.* **34**:1784–1793 (1986).
12. M. Yoshioka, B. C. Hancock, and G. Zografi. Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. *J. Pharm. Sci.* **83**:1700–1705 (1994).
13. C. G. Kontoyannis, N. C. Bouropoulos, and P. G. Koutsoukos. Use of Raman spectroscopy for the quantitative analysis of calcium oxalate hydrates: application for the analysis of urinary stones. *Appl. Spectrosc.* **51**:64–67 (1997).
14. R. Hüttenrauch, S. Fricke, and P. Zielke. Mechanical activation of pharmaceutical systems. *Pharm. Res.* **2**:302–306 (1985).
15. J. C. Miller and J. N. Miller. Statistics for Analytical Chemistry. Ellis Horwood, New York 1993, pp. 101–139.
16. L. E. Briggner, G. Buckton, K. Bystrom, and P. Darcy. The use of isothermal microcalorimetry in the study of changes in crystallinity induced during the processing of powders. *Int. J. Pharm.* **105**:125–135 (1994).
17. M. V. Pellow-Jarman, P. J. Hendra, and R. J. Lehnert. The dependence of Raman signal intensity on particle size for crystal powders. *Vibrational Spectroscopy* **12**:257–261 (1996).
18. P. A. Hailey, P. Doherty, P. Tapsell., T. Oliver, and P. K. Aldridge. Automated system for the on-line monitoring of powder blending processes using near-infrared spectroscopy Part I. System development and control. *J. Pharm. Biomed. Anal.* **14**:551–559 (1996).
19. D. J. Wargo and J. K. Drennen. Near-infrared spectroscopic characterization of pharmaceutical powder blends. *J. Pharm. Biomed. Anal.* **14**:1415–1423 (1996).
20. M. C. Davies, J. S. Binns, C. D. Melia, P. J. Hendra, D. Bourgeois, S. P. Church, and P. J. Stephenson. FT Raman spectroscopy of drugs in polymers. *Int. J. Pharm.* **66**:223–232 (1990).
21. P. J. Hendra. Fourier transform-Raman spectroscopy in pharmaceutical analysis and research. *American Laboratory* **28**:17–24 (1996).