Comparative Evaluation of the Degree of Indomethacin Crystallinity by Chemoinfometrical Fourie-Transformed Near-Infrared Spectroscopy and Conventional Powder X-Ray Diffractiometry

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ABSTRACT A chemoinfometrical method for evaluating the degree of crystallinity based on fourie-transformed near-infrared (FT-NIR) spectroscopy was established and compared with the conventional powder X-ray diffraction method. Powder X-ray diffraction profiles and FT-NIR spectra were recorded for 11 kinds of standard materials with various degrees of crystallinity obtained by physically mixing crystalline and amorphous indomethacin (IMC). Chemoinfometric analysis was performed on the FT-NIR spectral data sets by multiple linear regression (MLR) (MLR-Set-Up Search program). The crystalline and amorphous forms showed significant NIR spectral peaks. MLR analysis was performed based on normalized NIR spectra sets for standard samples of known crystallinity. A calibration equation was determined to minimize the root mean square error of prediction. The predicted crystallinity values were reproducible and had a smaller standard deviation. The values of crystallinity predicted by X-ray powder diffractometry and FT-NIR spectrometry suggested a satisfactory correlation between the 2 techniques. The results indicated that FT-NIR spectroscopy provides for an accurate quantitative analysis of crystallinity compared with conventional X-ray diffractometry.

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INTRODUCTION

Recrystallization, grinding, compaction, and freeze drying are frequently used in the pharmaceutical industry to obtain a desirable crystalline form of bulk powder and excipients. These processes affect not only the surface area, but also the crystalline disorder of the powder materials. Because both these parameters may affect the bioavailability of a drug through the rate of dissolution (1,2), it is necessary to control the conditions under which the pharmaceutical drug powders are produced. The extent of disorder in a crystalline solid may induce the hygroscopicity of the drug in addition to the flow, mechanical properties, and chemical stability. Because the qualities of a pharmaceutical preparation depend on the characteristics of the bulk powders and excipients, controlling the production process is important. An amorphous solid-state powder may determine the bioavailability of a slightly water-soluble drug because the property affects solubility and hence absorption of the drug in the gastrointestinal tract. However, the amorphous form has problems regarding stability and hygroscopicity, resulting in transformation to a more stable crystalline form during preservation.

Therefore, in order to control the quality of pharmaceutical solid dosage products, techniques for the evaluation of crystallinity of the bulk powders and/or excipients are needed. X-ray diffraction (3-5), differential scanning calorimetry (6), FT-Raman spectroscopy (7), and micro-calorimetry (8) are currently the most widely used methods to evaluate crystallinity.

Near-infrared (NIR) spectroscopy is becoming an important technique for pharmaceutical analysis. NIR spectroscopy is simple and easy because no sample

preparation is required and samples are not destoyed. In the pharmaceutical industry, NIR spectroscopy has been used to determine several pharmaceutical properties, and a growing literature exists in this area. Berntsson et al (9) determined the moisture content in hard gelatin capsules by NIR. Buckton et al (10) reported on the crystalline state of amorphous and crystalline lactose. Buchanan et al (11) and Bianco et al (12) reported on coating of tablets analyzed using the NIR method. Franke et al (13) determined the particle size of lactose by chemoinfometrical methods. Morisseau and Rhodes (14) evaluated tablet hardness by the NIR method.

A variety of chemoinfometric and statistical techniques have been used to extract pharmaceutical information from raw spectroscopic data. Calibration models generated by multiple linear regression (MLR) analysis, principal component analysis, and partial least squares regression analysis have been used to evaluate various parameters (15).

On the other hand, many investigators (16-18) have reported on amorphous and polymorphous characteristics of indomethacin (IMC) and have evaluated crystalline content in the solid, because a meta-stable form transformed into a stable form during production and storage.

In this study, we established a chemoinfometrical method based on FT-NIR spectroscopy to evaluate degree of crystallinity, and we compared the chemoinfometrical method with the conventional powder X-ray diffraction method.

MATERIALS AND METHODS

A bulk powder of IMC, in γ-crystal form (13), was obtained from Yashiro Co., Japan. The crystallinity of this material was assumed to be 100%. Amorphous IMC (6) was obtained by cooling in liquid nitrogen after melting the bulk powder at 165°C for 5 minutes. The crystallinity of this material was assumed to be 0%.

X-Ray Powder Diffraction Analysis

X-ray powder diffraction profiles were taken with an X-ray diffractometer (XD-3A; Shimadzu Co., Japan). The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 5 mA; receiving slit, 0.1 mm; time constant, 1 second; scanning speed, 4° 20 min^{-1}. The X-ray powder diffraction profiles were measured as follows: briefly, known quantities of standard mixtures were obtained by physically mixing γ form crystalline and amorphous IMC powders at various ratios (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 wt/wt% crystal content) in an agate mortar and pestle without grinding. About 80 mg of each sample powder was carefully loaded in a glass holder without particle orientation using a spatula and glass plate. After the powder X-ray diffraction profiles of samples were measured under the above conditions, the intensity values were normalized to the intensity of silicon powder (20 = 28.4°C) as an external standard. The calibration curves for quantification of crystal content were based on the total relative intensity of 3 diffraction peaks (20 = 11.5, 19.4, and 21.7°C), for the γ form crystal. All data were averages of 5 runs.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed with a Type 3100 instrument (Mac Science Co., Japan). The operating conditions, in an open pan system, were as follows: sample weight, 5 mg; heating rate, 10°C min^{-1}; N_2 gas flow rate, 30 mL min^{-1}.

Fourier Transform Near Infrared (FT-NIR) Spectroscopy

FT-NIR spectra were taken using an NIR spectrometer (InfraProver, Bran + Luebbe Co., Norderstedt, Germany) as follows: A fiber-optic probe was inserted into the sample powder (2 g) in a 20-mL glass bottle, and 32 scans per sample were recorded in the spectral range of 4500 to 10,000 cm^{-1}. A ceramic (Coor's Standard) reference scan was taken for each set of samples. FT-NIR spectra of 11 calibration sample sets were recorded 5 times with the NIR spectrometer. A total of 55 spectral data sets

were transformed to remove particle size effect (19) by the normalized function, and chemoinfometric analysis was performed using the MLR-Set-Up Search program contained in SESAMI software (Bran + Luebbe Co.).

Quantitative Analysis of Unknown Samples

Samples (20 kinds) of unknown crystallinity were obtained by storage of various mixtures of IMC powder samples at 25 ± 2ºC, 75 ± 5% RH (NaCl saturated solution) for 3, 6, 10, 12 or 14 days, after physically mixing γ form crystalline and amorphous IMC powders at various ratios (20, 40, 60, and 80 wt/wt% crystal content). The degree of crystallinity of these unknown samples was determined by both chemoinfometrical FT-NIR and conventional powder X-ray diffraction methods.

RESULTS AND DISCUSSION

Characterization of Crystalline and Amorphous IMC

Figure 1 shows the X-ray powder diffraction profiles of the standard material samples with 0%, 50%, and 100% crystallinity.

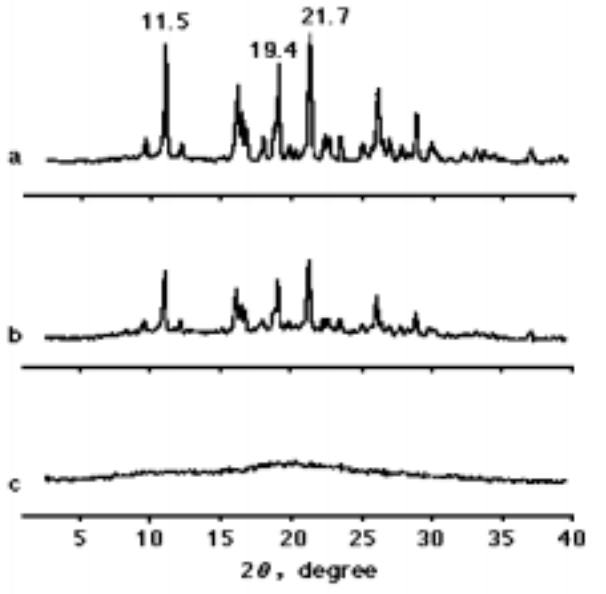

Figure 1. X-ray powder diffraction profiles of crystalline and amorphous indomethacin (IMC). (a) 100% crystallinity (γ form IMC); (b) 50% crystallinity; (c) 0% crystallinity (amorphous form).

The profile of the amorphous form showed a halo pattern and exhibited no diffraction peaks. In contrast, the profile of the crystalline form had specific diffraction peaks at 11.5, 19.4, and 21.7° (2θ) attributable to γ form IMC (6), meaning that the bulk powder was a high-quality crystalline powder.

Figure 2 shows the DSC profiles of the crystalline and amorphous samples.

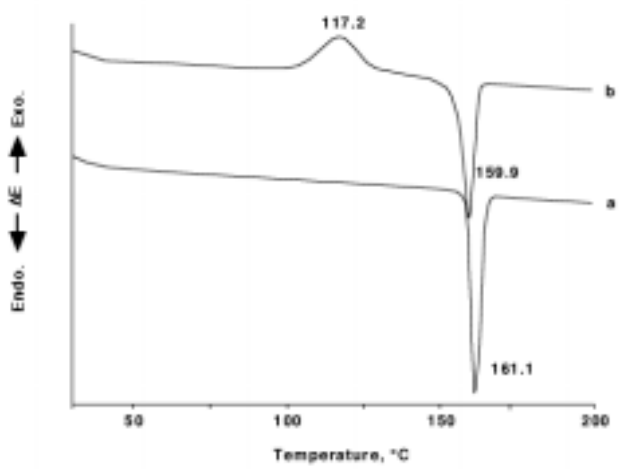

Figure 2. DSC curves of crystalline and amorphous IMC. (a) 100% crystallinity (γ form IMC); (b) 50% crystallinity; (c) 0% crystallinity (amorphous form).

The DSC curve of the crystalline γ form showed an endothermic peak at 161.1°C attributable to melting. However, the curve of the amorphous form (16) showed an exothermic peak at 117.2°C attributable to crystallization and a subsequent endothermic peak at 159.9°C attributable to melting of the γ form. These results suggested that the crystalline and amorphous IMC used in the present study were high-quality solids free from impurities.

Measurement of Crystallinity by Conventional X-Ray Powder Diffractometry

The calibration curve for measuring the crystalline content by conventional X-ray diffraction methods was based on the total intensity of the 3 specific diffraction peaks. Figure 3 shows a plot of the relation between the actual crystallinity and total intensity.

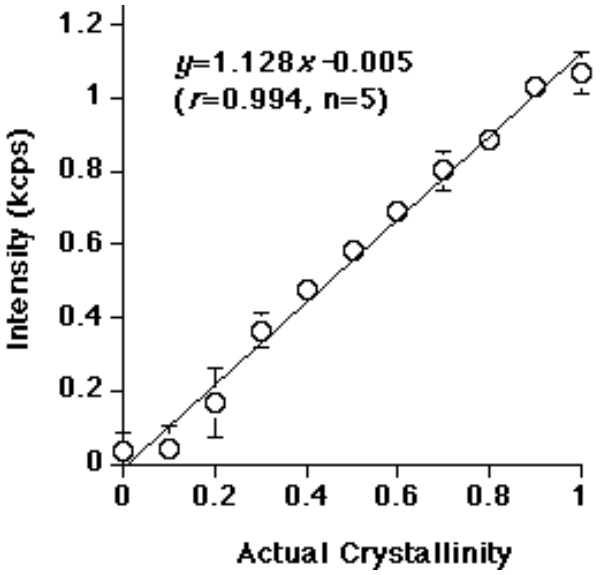

Figure 3. Calibration curve for various IMC standard samples obtained by conventional X-ray powder diffractometry. Bars represent standard deviation.

This plot can be fitted as a straight line with a slope of 1.128, an intercept of -0.005, and a correlation coefficient of 0.994. Figure 4 shows a plot of the calibration data illustrating the relation between the actual and predicted crystallinity.

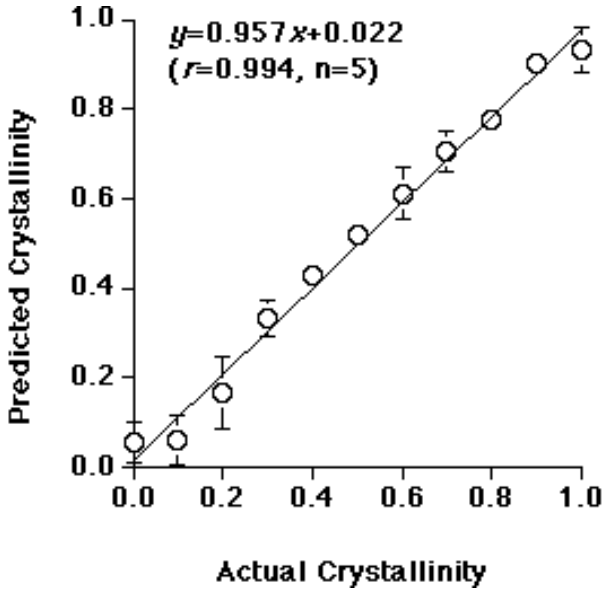

Figure 4. Relation between predicted and actual crystallinity of IMC standard samples determined by conventional X-ray powder diffractometry. Bars represent standard deviation.

This plot has a slope of 0.957, a value-fluctuated intercept of 0.022, and a correlation coefficient of 0.994. The lower crystallinity may be attributable to difficulty in separation of the smaller diffraction peaks from the background, as shown in Table 1.

Table 1. Predicted crystallinity of IMC samples

Xc	FT-NIR	±sd	XRD	±sd
1.000	0.992	0.008	0.933	0.049
0.900	0.891	0.011	0.902	0.027
0.800	0.811	0.008	0.779	0.016
0.700	0.709	0.010	0.707	0.046
0.600	0.600	0.016	0.613	0.059
0.500	0.507	0.004	0.521	0.021
0.400	0.383	0.011	0.431	0.023
0.300	0.295	0.014	0.334	0.040
0.200	0.213	0.012	0.166	0.080
0.100	0.093	0.010	0.060	0.055
0.000	0.042	0.014	0.054	0.045

Xc is degree of crystallinity, XRD is conventional X-ray diffraction method, sd is standard deviation (n=5).

However, the result suggested that conventional X-ray diffractometry was still useful for evaluating the degree of crystallinity of IMC.

Measurement of Crystallinity by Chemoinfometric FT-NIR Spectroscopy

Figure 5 shows the FT-NIR spectra of the standard samples with crystallinity of 0%, 50%, and 100%.

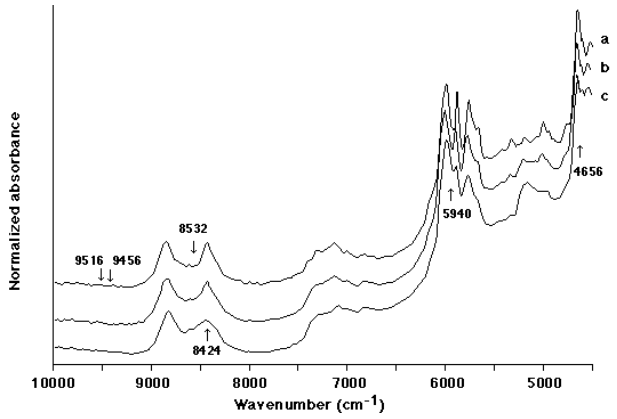

Figure 5. FT-NIR spectra of crystalline and amorphous IMC. (a) 100% crystallinity (γ form IMC); (b) 50% crystallinity; (c) 0% crystallinity (amorphous form).

The crystalline and amorphous forms of IMC showed significant NIR spectral peaks. Table 2 shows the assignment of functional groups in the NIR spectrum of IMC (19). The peak at 5900 cm^{-1} is attributable to an aromatic ring that broadened as the degree of crystallinity decreased.

In order to evaluate the degree of crystallinity based on FT-NIR spectra, MLR was used for the calibration. The base equation was as follows:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3,,+a_nX_n \quad \textit{(Equation 1)}$$

where Y is crystallinity, X_n is absorbance at wave number n, and a_n is the regression coefficient. The NIR spectrum consisted of 460 data points between 4500 and 10,000 cm^{-1}. Eleven batches of IMC standard samples with various degrees of crystallinity were prepared, and 4 spectra were collected per batch. Thus, a total of 44 spectra were selected for the calibration. MLR analysis was performed based on a spectra set of 44 normalized NIR standard samples. For each calibration equation, 2 to 6 wave numbers were chosen automatically and, by an interactive procedure, the best combinations of wavelength and absorbance were determined to minimize the root mean square error of prediction (RMSEP; Equation 2):

$$RMSEP = \sqrt{\frac{\sum (y_p - y_r)^2}{n}} \quad \textit{(Equation 2)}$$

where y_p is the predicted value, y_r the actual property value, and n number of experiments.

The minimized RMSEP of the calibration curve was calculated for 6 parameters based on the wavelengths; therefore, the degree of crystallinity of IMC could be evaluated based on the following equation:

$$Y = -1326.1 + 1235.2X4656 + 377.1X5940 - 1083.9X8424 + 654.8X8532 + 579.3X9456 - 644.4X9516 \quad \textit{(Equation 3)}$$

Figure 6 shows a plot of the calibration data illustrating the relation between actual and predicted crystallinity.

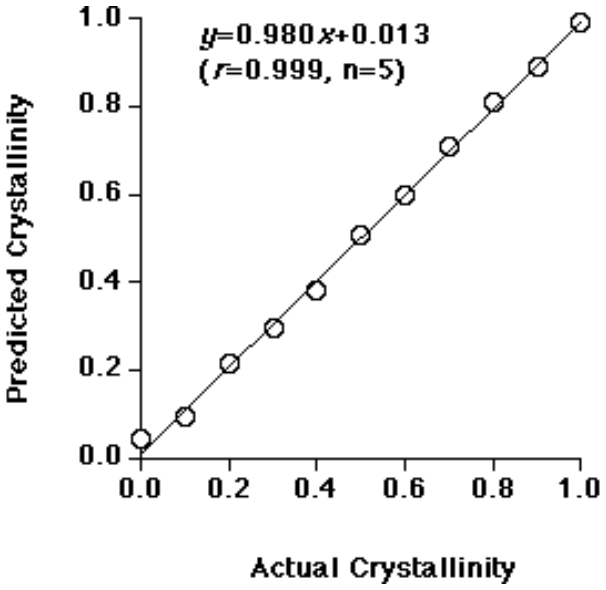

Figure 6. Relation between predicted and actual crystallinity of IMC standard samples determined by chemoinfometrical FT-NIR spectrometry. Bars represent standard deviation.

The predicted values were reproducible and had a smaller standard deviation, as shown in Table 1. The multiple correlation coefficient, the standard error of estimate, and the standard error of prediction were evaluated to be 0.999, 0.017, and 0.023, respectively.

Because the purpose of this study is to compare the accuracy of the chemoinfometrical FT-NIR method with that of conventional powder X-ray diffraction, the mean bias and the mean accuracy were determined by Equations 4 and 5, respectively.

$$B_m = \frac{\sum_{i=1}^{n} \frac{(X_c - X_t)}{X_t}}{n} \times 100 \quad \textit{(Equation 4)}$$

$$A_m = \frac{\sum_{i=1}^{n} \frac{|X_c - X_t|}{X_t}}{n} \times 100 \quad \textit{(Equation 5)}$$

B_m is percentage mean bias, A_m is percentage mean accuracy, X_c is predicted value of crystallinity, X_t is true value of crystallinity, and n is number of experiments.

The mean bias for the FT-NIR and X-ray diffraction methods were calculated to be 0.364% and 3.92%, and the mean accuracy was 2.46% and 9.29%, respectively. These results indicate that the FT-NIR assay provides an accurate quantitative analysis of crystallinity compared with conventional X-ray diffractometry.

Comparative Evaluation of Conventional Powder X-Ray Diffraction and Chemoinfometric FT-NIR Methods

Figure 7 compares predicted crystallinity values for unknown IMC samples obtained by X-ray diffractometry with those obtained by FT-NIR spectrometry.

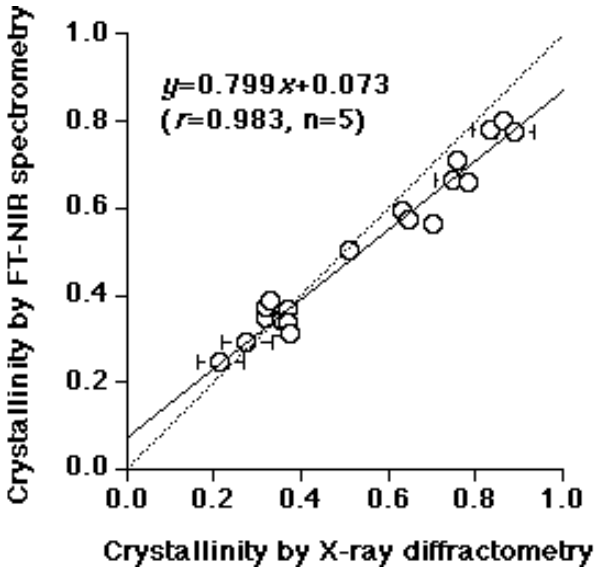

Figure 7. Relation between predicted crystallinity of unknown IMC samples obtained by conventional X-ray powder and chemoinfometircal FT-NIR method. Bars represent standard deviation.

Because X-ray diffraction profiles of unknown samples exhibited diffraction peaks due to γform, but no peak due to α form (20 = 7 and 8.5º), the degree of crystallinity of the samples was evaluated based on γ form and amorphous form. The plot of theoretic values distributes on a line with a slope of 1.0, but the plot of measured values has a slope of 0.799. This is probably because the accuracy of the X-ray diffraction method in the lower crystallinity range was lower than that of the FT-NIR method, as shown in Table 1; however, the line represents a satisfactory correlation between the 2 predicted values of crystallinity. Thus FT-NIR spectroscopy is an effective method for the evaluation of crystallinity of pharmaceutical products.

Prediction of Molecular Structure of Amorphous IMC

Figure 8 shows the absorbance of physical mixtures of amorphous and crystalline IMC at different wave numbers.

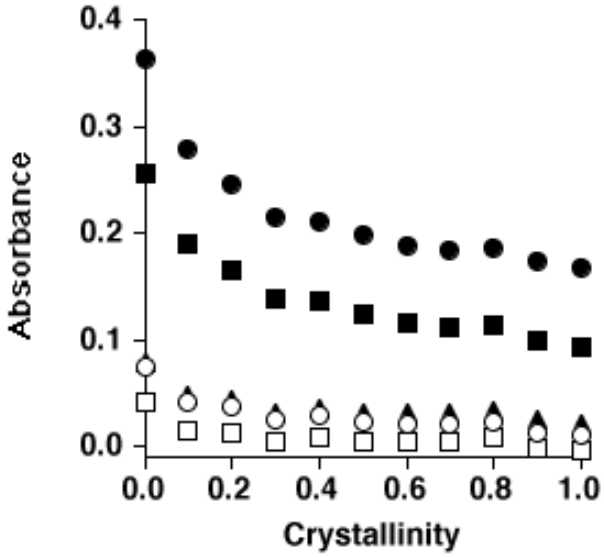

Figure 8. Change in absorbance of FT-NIR of IMC standard samples. Closed circle, 4656 cm^{-1} (HC = CH); closed square, 5940 cm^{-1} (aromatic ring); closed triangle, 8532 cm^{-1}; (CH_3); open circle, 8432 cm^{-1} (HC = CH); open square, 9456 cm^{-1} ($R\text{-}NH_2$); open triangle, 9516 cm^{-1} (CH_2)(The symbol for CH_2, open triangle, overlaps the open square symbol).

Functional group assignments for IMC based on NIR spectra are shown in Table 2. In this plot, absorbance decreased as crystallinity increased, especially at 4656 and 5940 cm^{-1} (attributed to C = C and aromatic ring, respectively). This result suggested that amorphous and crystalline γ forms differed

significantly in terms of interaction at the aromatic ring.

Table 2. Functional group assignment for IMC based on NIR spectra

Functional group	Wave number (cm^{-1})
HC=CH	4656
Aromatic ring	5940
CH_3	8532
HC=CH	8432
R-NH_2	9456
CH_2	9516

Figure 9 shows the molecular structure of the crystalline γ form IMC by X-ray diffraction analysis (20) and predicted the structure of the amorphous form based on the changes of absorbance shown in Figure 8.

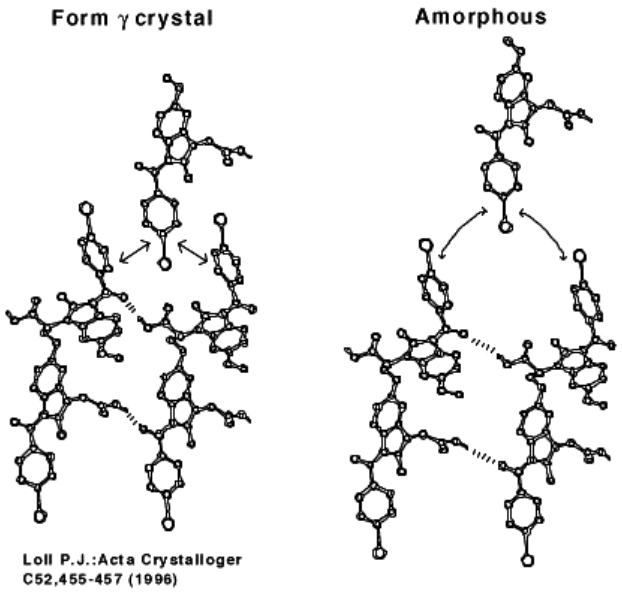

Figure 9. Crystalline structure of crystalline and amorphous solids.

In the crystalline state, IMC forms a dimer via an intermolecular hydrogen bond between H3 of the carboxylic acid and O1 of the amid, and the dimer molecules form a tight arrangement. In general, an amorphous solid is less dense than a crystalline solid, because molecular mobility in the amorphous state is increased in a solid (21). Therefore, with the reduction in crystallinity, it seems that the molecular mobility in a portion of the aromatic ring was increased in the amorphous state, and this resulted in a decrease in absorbance at the aromatic ring of IMC.

CONCLUSIONS

FT-NIR spectroscopy with chemoinfometrical techniques could be useful in evaluating the degree of crystallinity of IMC. The crystallinity of unknown samples obtained by chemoinfometrical FT-NIR spectrometry was consistent with that obtained by conventional X-ray powder diffractometry and was more accurate. According to the change in NIR absorbance for noncrystallization of IMC, the solid structure of amorphous IMC was significantly different from that of the crystalline form.

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