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Research Paper

Evaluation of the Microcrystallinity of a Drug Substance, Indomethacin, in a Pharmaceutical Model Tablet by Chemometric FT-Raman Spectroscopy

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Purpose. To establish a chemometric method for the precise evaluation of the microcrystallinity of indomethacin (IMC) in a pharmaceutical model tablet, based on FT-Raman spectroscopy.
Methods. Standard sample powders of homogeneous mixtures of amorphous and crystalline IMC were prepared in various proportions. A calibration model for the crystallinity of IMC was constructed by partial least-square (PLS) analysis based on the multiplicative scatter correction (MSC) + second-derivative transformed Raman spectra. A calibration model for the crystallinity of IMC in a model pharmaceutical product (IMC/mannitol = 1:9 wt/wt) was also constructed using homogeneous standard sample powders of various degrees of crystallinity of IMC.
Results. This technique was validated to detect to 2% an amorphous or crystalline material in IMC contained in the model product (0.2% of the total mass of the tablet). Using this technique, not only pressure-induced amorphization but also the difference in microcrystallinity of IMC at the surface and interior of a model product tablet was elucidated after compaction of the tablet.
Conclusions. The established technique is ideally suited for precise quantification of microanalysis of drug substances and drug products, particularly at the surface and interior of the tablet.

KEY WORDS: chemometrics; compaction; crystallinity; indomethacin; Raman spectroscopy.

INTRODUCTION

Recrystallization, grinding, compaction, and freeze-drying are frequently used in the pharmaceutical industry to obtain a desired crystalline form of a bulk powder or excipient. These processes affect not only the surface area, but also the crystalline disorder of the powder materials. Because either of these parameters may affect the bioavailability of a drug through the dissolution rate (1,2), it is necessary to control the conditions under which pharmaceutical drug powders are produced and preserved. The extent of disorder in a crystalline solid may induce hygroscopicity, a decrease in chemical stability, or changes in mechanical and powder flow properties. However, in some instances, an amorphous solid-state powder may be used to improve absorption of the drug in the gastrointestinal tract. In that case, temperature, humidity, or mechanochemical factors may result in undesirable transformation to a more stable solid-state crystal or hydrate. These changes in solid-state powders are not necessarily homogeneous phenomena, as those in liquids are.

Therefore, to control the quality of pharmaceutical compounds that include solid dosage products, analytical methods that accurately evaluate the degree of crystallinity of drug substances or excipients in an anisotropic solid-state are needed. However, such a precise investigation has not been reported so far, other than FT-Raman spectroscopy by Taylor and Zografi (3), although many kinds of quantitative techniques to measure crystallinity, such as X-ray powder diffraction (4–6), differential scanning calorimetry (7), microcalorimetry (8), infrared (IR) spectroscopy (4), near-infrared (NIR) spectroscopy (9), FT-Raman spectroscopy (3), and solid-state nuclear magnetic resonance (NMR) (10) are known.

FT-Raman spectroscopy has the following desirable characteristics: no sample preparation, nondestructive, rapid measurement, and high spatial resolution. Moreover, since excipients used for pharmaceutical dosage products are poor Raman scatters relative to drug substances (11,12) this technique has the possibility of being a powerful tool in the analysis of active ingredients in intact dosage form (13,14).

Taylor and Zografi investigated a quantitative method of crystallinity of indomethacin (IMC) using FT-Raman spectroscopy, and reported the potential usefulness of this technique through a challenge to measure small amounts down to 1% amorphous or crystalline component of IMC (3). In the study, the authors said that the greatest source of error was attributed to inhomogeneity of standard sample powders used for calibration curve generation. This seems to be the result of backfire in the high spatial resolution in this

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ABBREVIATIONS: DSC, differential scanning calorimetry; IMC, indomethacin; MSC, multiplicative scatter correction; NIR, near-infrared; NMR, nuclear magnetic resonance; PLS, partial least square; XRD, X-ray powder diffraction.

technique, as typically FT systems focus the laser to around 50-several hundreds micrometers and thus the area of sample being excited is small. However, this suggests that FT-Raman spectroscopy may enable precise quantitative analysis of local areas in intact pharmaceutical tablet, through the high spatial resolution and high specificity for Raman active components, as long as homogeneity of calibration samples can be achieved so that calibration curves with enough precision and accuracy can be generated. So far, such a precise quantification based on FT-Raman spectroscopy has not been reported, although the techniques extracting quantitative information from Raman spectra are well established (15). Provided that the small variations of the crystallinity (amorphicity) of active ingredient at the surface and interior of an intact pharmaceutical tablet are quantitatively evaluated, it will be possible to promote more precise control of solid-state properties of drug products, and more intensive investigation into formulation development.

In our previous work, a method for preparing multiple mixtures of originally differently shaped powders without causing polymorphic transition or decreases in crystallinity, homogeneously and accurately on a several-gram scale, was established (16). In addition, chemometrical spectroscopy based on a whole spectrum region characteristic to the components, rather than a few specific peak intensities/ratios, was developed for constructing a calibration model for crystallinity or polymorphic contents (9,17).

In this study, FT-Raman spectroscopy was applied to evaluate the degree of crystallinity of standard sample powders or intact tablet, using IMC as a model compound and IMC–mannitol (1:9 wt/wt) as a model pharmaceutical product. Using the above standard sample preparation method, small amounts of crystalline or amorphous powder mixtures were prepared homogeneously and accurately, and a reliable calibration model was constructed using chemometrics based on the obtained FT-Raman spectra. This calibration model was used to evaluate not only pressure-induced amorphization but also small differences in microcrystallinity of IMC in a model product tablet on the surface and in cross section, from the measurement of the degree of crystallinity of IMC (10% wt) in mannitol-blended tablet samples produced at various tabletting pressures. Such a high spatial and quantitative microanalysis would be difficult to achieve by means of other than FT-Raman spectroscopy.

MATERIALS

IMC bulk powder in γ crystal form was obtained from Sumitomo Pharmaceutical 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. The crystallinity of this material was assumed to be 0%. Mannitol was supplied by Wako Chemicals Co. (Japan).

METHODS

Preparation of Standard Mixtures for Quantitative Analysis of Crystallinity

In accordance with published methods (16), standard mixtures with known percentages of crystallinity were obtained as follows: First, crystalline and amorphous powders were independently micronized in a tabletop jet mill (A–O Jet Mill; Seishin Enterprise Co., Japan). A total of 2 g of micronized crystalline and amorphous powders were physically mixed at various ratios in a vibrating mill (model TI-100; CMT Co., Japan) containing three urethane rubber balls (diameter, 20 mm; weight, 4–5 g; Tigers Polymer Co., Japan) for 3 min. These IMC powders of various crystallinity were mixed with mannitol at 1:9 wt/wt (IMC/mannitol) in the same way. These mixtures were then used as standard samples for quantitative analysis of crystallinity of IMC in the pharmaceutical model product, as detailed below.

IMC Standard Samples

IMC standard samples were prepared at various ratios (0, 1, 2, 3, 5, 10, 20, 40, 60, 95, 98, and 100% wt/wt) of crystallinity. These were divided into a calibration set (0, 2, 5, 20, 60, 95, and 100%) for constructing the calibration model and a validation set (1, 3, 10, 40, and 98%) for validating the model.

IMC/Mannitol Mixture Standard Samples (1:9 wt/wt)

IMC/mannitol standard samples were prepared at various ratios (0, 2, 5, 10, 20, 40, 60, 80, 90, 95, 98, and 100% wt/wt) of IMC crystallinity. These were also divided into a calibration set (0, 5, 20, 60, 90, 95, 100%) for constructing the calibration model and a validation set (2, 10, 40, 80, 98%) for validating the model.

Compaction of Samples

The micronized IMC crystalline powder samples and the intact mannitol were physically mixed in a vibrating mill containing rubber balls as described previously, at the ratio of 1:9 wt/wt (IMC/mannitol). Then, 125 mg of the homogeneous powder mixture was compressed in a tabletting tester (Sankyo Paioteku Co., Japan) at various compressions (20, 49, 98, 196 MPa). The tablets obtained were R-type, 7 mm in diameter and about 3 mm in thickness.

X-Ray Powder Diffraction Analysis

X-ray powder diffraction (XRD) profiles were recorded using an X-ray diffractometer (Rint 2500 V, Rigaku Co., Japan). The measurement conditions were as follows: radiation, Cu$K\alpha$; power, 50 kV × 300 mA; automatic monochromator; divergence slit, 0.5°; scattering slit, 0.5°; receiving slit, 0.15 mm; scan mode, continuous mode (Note: counting was done with scanning at constant speed); scan range, 2–40°; scan rate, 2.0°/min; scan step, 0.02°.

About 200 mg of each sample powder was loaded in a glass holder of diameter 240 mm and depth 0.5 mm (Rigaku Co. Japan). The sample holder was rotated in a plane parallel to its surface at 120 rpm during the measurement. Measurements of each sample were repeated for refills three times.

Differential Scanning Calorimetry (DSC)

DSC was performed with a Type DSC6200 calorimeter (Seiko, Co., Japan) in open aluminum pans under operating

conditions as follows: sample weight, 5 mg; heating rate, 5°/min; N_2 gas flow rate, 50 ml/min.

FT-Raman Spectroscopy

FT-Raman spectra were collected on a Nicolet Nexus 870 instrument equipped with an FT-Raman module (Thermo Electron K. K., Japan) with air-cooled diode laser excitation (1064 nm), CaF_2 beam splitter, and InGaAs detector, under operating conditions as follows: About 100-mg powder samples were put in 5-mm diameter NMR tubes (Shigemi Co., Japan), and the laser was focused on three separate spots per powder sample (spot size, several hundred micrometers; power, approximately 450 mW). Over the spectral range of 3700–100 cm^{-1} at a resolution of 4 cm^{-1} in a 180° scattering arrangement, 100 scans were made and averaged for each spot. Compacted tablet samples were directly irradiated on different local areas of the tablet; the center of surface, edge of surface, and cross-sectional surface. Two or three tablets were used for three separate measurements at 100 scans each.

RESULTS AND DISCUSSION

Characterization of the Reference Materials

Figure 1 shows the X-ray powder diffraction profiles of the standard materials used in this study and taken to represent 100% amorphous IMC and 100% crystalline IMC, which were assumed to be of 0% and 100% crystallinity, respectively. 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 11.6°, 16.8°, 19.6°, 21.9°, and 26.7°(2θ) attributable to γ-form IMC as reported previously (9), meaning that the bulk powder was a high-quality crystalline powder. As for the X-ray diffraction method used for qualifying standard materials, the detection limit for crystalline material in the amorphous standard are estimated to be about 0.3%, by calculating 3 standard deviations of the crystallinity value determined using the X-ray diffraction calibration curve described in the section, "Construction of Calibration Curve by X-Ray Powder Diffractometry" for pure amorphous standard. The detection limit for amorphous material in the crystalline standard is estimated likewise to be about 20%, by calculating 3 standard deviations of the crystallinity value for pure crystalline standard.

X-ray powder diffraction profiles of the mannitol and of crystalline IMC/mannitol (1:9 wt/wt) mixture samples are also showed in Fig. 1. A diffraction peak that was distinguishable from those of mannitol was selected as specific to IMC (2θ = 11.6°), since this peak exhibited the best sensitivity and the best correlation with the crystallinity of IMC of all the distinguishable peaks. This is described in the section, "Characterization of the Reference Materials."

The DSC curve of the amorphous form of IMC showed an exothermic peak at 106°C, attributable to crystallization, and subsequent endothermic peaks at 155°C and 160°C, attributable to melting of the α form and γ form, respectively. However, the curve of the crystalline IMC showed an endothermic peak at 162°C, attributable to melting of the γ-form. These results suggested that the crystalline and amorphous IMC used in the present study were consistent with the reported data (9,17), and high-quality solids free from impurities.

Figure 2 shows the FT-Raman spectra of γ-form crystalline IMC, amorphous IMC, and the samples of those mixed with mannitol. The γ-form of crystalline and amorphous IMC has a characteristic peak region at 1500–1750 cm^{-1}. The peaks at 1680–1697 cm^{-1} were attributable to carbonyl groups and the peaks at 1577–1618 cm^{-1} were attributable to C=C in indole and C–H in aromatic compounds. As mannitol has no apparent peaks on this spectral region, crystalline and amorphous forms of IMC seemed to be distinguishable even when IMC was diluted in mannitol.

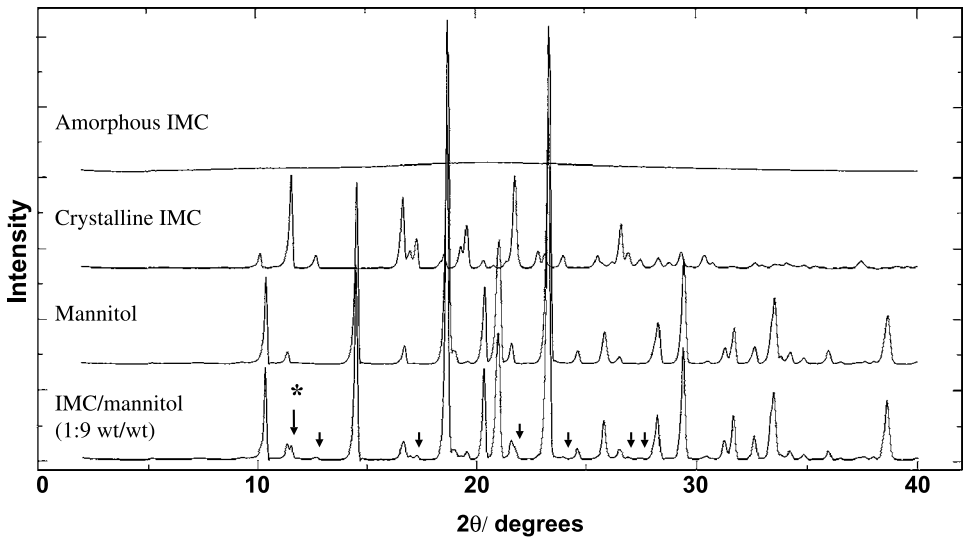

Fig. 1. X-ray powder diffraction profiles of amorphous indomethacin (IMC), crystalline IMC, mannitol, and of crystalline IMC/mannitol (1:9 wt/wt) mixture. Arrows show intrinsic peaks of IMC. Asterisked peak (2θ = 11.6°) was used for developing a calibration curve of IMC crystallinity.

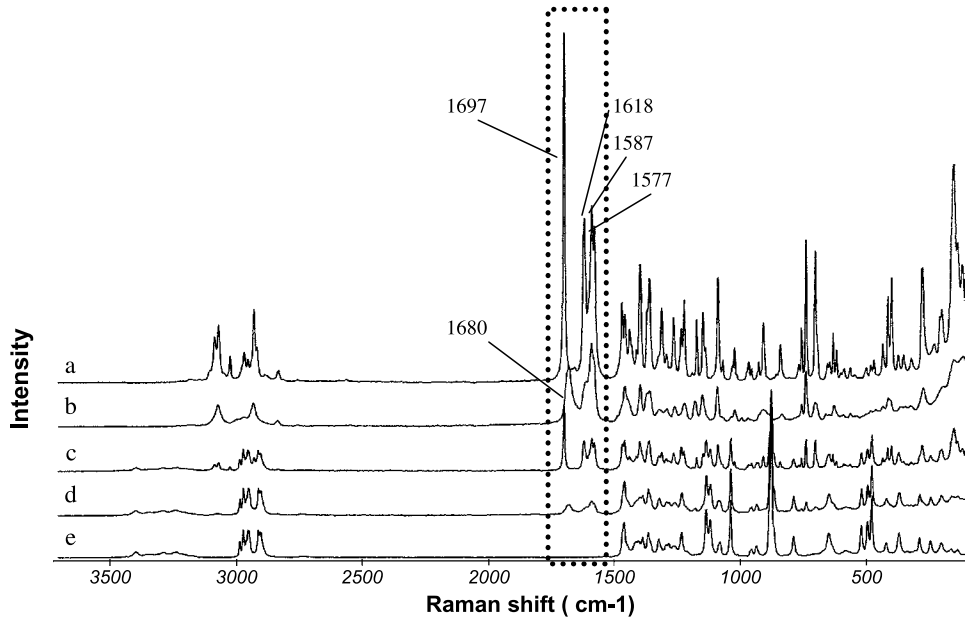

Fig. 2. FT-Raman spectra of indomethacin (IMC) and the samples those blended with mannitol. (a) Crystalline IMC (crystallinity 100%). (b) Amorphous IMC (crystallinity 0%). (c) Mannitol-blended IMC (crystallinity 100%). (d) Mannitol-blended IMC (crystallinity 0%). (e) Mannitol. Rectangle dotted line region was used for developing calibration curves of crystallinity of IMC.

Measurement of Crystallinity of IMC

Construction of a Calibration Model by FT-Raman Spectroscopy and Validation of the Model

Three spectra were collected per calibration set standard sample. A total of 21 spectra were taken. Figure 3 shows the second derivative Raman spectra after multiplicative scatter correction (MSC) pretreatment, which is one of the baseline correction methods (18), for various standard mixtures in the 1520–1730 cm^{-1} regions. As seen, spectral differences between crystal and amorphous forms are significantly enhanced. The partial least-squares (PLS) calibration models were constructed for this spectral region, followed by cross-validation using the leave-one-out method. The number of factors was selected based on the PRESS (prediction residual error sum of squares) plot (19). Table I shows the PRESS and the RMSECV (root-mean-square error of cross-validation) for each factor. This indicates that a two-factor model is most appropriate. Figure 4 shows the correlation between the actual and predicted crystallinity. The multiple correlation coefficient and the root-mean-square error of prediction were evaluated to be 0.9999 and 0.9487, respectively. As the result of measuring the validation set standard samples, the root-mean-square error of prediction was evaluated to be 0.6407 for the validation set samples. Therefore, the calibration model was verified to have good accuracy and precision.

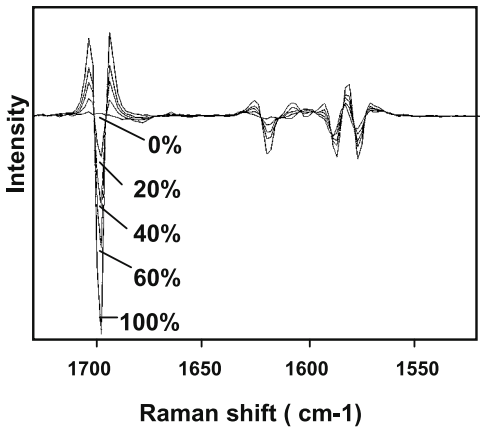

Fig. 3. Overlaid of second derivative of the MSC transformed spectra of indomethacin samples of various crystallinity. Percentages show the degree of indomethacin crystallinity in the sample.

Construction of Calibration Curve by X-ray Powder Diffractometry (XRD)

To validate the FT-Raman method, a conventional X-ray powder diffraction method was developed for the crystallinity of IMC. Table II shows the result of X-ray diffraction measurements of IMC standard samples (peak $2\theta = 11.6°$).

Table I. PRESS and RMSECV of Correlation Calculated by PLS Based on the Number of Factors, for Crystallinity of IMC

Number of factors	PRESS	RMSECV
1	78.7	1.94
2	28.1	1.16
3	52.7	1.58
4	40.1	1.38
5	36.2	1.31
6	34.9	1.29
7	35.1	1.29
8	34.9	1.29

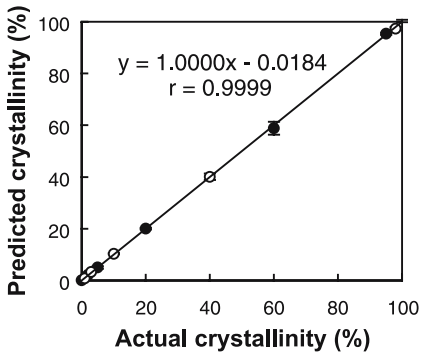

Fig. 4. Correlation between the actual and predicted crystallinity of IMC standard samples determined by chemometric FT-Raman spectroscopy. Linear line: regression curve for the calibration set; closed circle: calibration set; open circle: validation set. Bars represent standard deviations (n = 3).

The calibration curve was constructed based on a plot of diffraction peak height intensity ($2\theta = 11.6°$) *vs.* known percentage of crystallinity as prepared. The obtained calibration curve has good linearity, with a slope of 280.64, an interval of 1085.07, and a correlation coefficient of 0.9986. The standard deviations for the slope and the intercept are small: 4.65 and 249.21, respectively. These results indicate that the calibration samples were prepared, homogeneously and accurately, while causing little change in crystallinity, and were of sufficiently high quality for the purposes of this study.

Comparison of FT-Raman Spectroscopy with XRD

Table III shows the predicted crystallinity of the validation set standard samples by XRD and FT-Raman spectroscopy. The result elucidated that FT-Raman spectroscopy is a more precise quantification method for evaluating low levels of both amorphous and crystalline material, of around 0% and 100% degree of crystallinity. It is also confirmed that this chemometrical method has a practical use, because of the smaller standard deviation than in the previous method in which peak intensity ratio was used (3); moreover, the accuracy of the FT-Raman method was compared to that of the X-ray powder diffraction, using the mean bias and the mean accuracy as determined for the validation set samples by Eqs. (1) and (2), respectively.

Table II. XRD Measurements of IMC Standard Samples

Xc (%)	Peak intensity[a] (cps)	SD (cps)	RSD (%)
0	1510	18	1.2
1	1802	58	3.2
2	1962	56	2.8
3	2212	30	1.4
5	2629	92	3.5
10	3540	163	4.6
20	5847	458	7.8
40	11,192	1450	13.0
60	18,160	954	5.3
95	27,267	909	3.3
98	29,630	1346	4.5
100	29,065	1928	6.6

[a] Peak height, mean of three refill measurements ($2\theta = 11.6°$).
RSD, relative standard deviation; SD, standard deviation; Xc, known crystallinity.

Table III. Predicted Crystallinity (%) of IMC Samples

Xc	FT Raman	SD	XRD	SD
1	0.8	0.0	2.6	0.2
3	3.3	0.1	4.0	0.1
10	10.3	0.3	8.7	0.6
40	40.1	1.3	36.0	5.2
98	97.3	0.6	101.7	4.8

SD, standard deviation (n = 3); Xc, known crystallinity.

$$\mathrm{Bm} = \frac{\sum_{i=1}^{n} (\mathrm{Xp} - \mathrm{Xt})/\mathrm{Xt}}{n} \times 100; \quad (1)$$

$$\mathrm{Am} = \frac{\sum_{i=1}^{n} |\mathrm{Xp} - \mathrm{Xt}|/\mathrm{Xt}}{n} \times 100; \quad (2)$$

where Bm is the percentage mean bias, Am is the percentage mean accuracy, Xp is the predicted value of crystallinity of IMC, Xt is the true value of crystallinity, and n is the number of experiments. The Bm for the FT-Raman and XRD methods were calculated to be −1.9% and 34.1%, and the Am was 7.5% and 44.0%, respectively. These results indicate that the FT-Raman assay provides an accurate quantitative analysis of crystallinity compared with conventional X-ray powder diffractometry.

Measurement of Crystallinity of IMC in a Pharmaceutical Model Product

Construction of a Calibration Model by FT-Raman Spectroscopy and Validation of the Model

The high-precision quantitative analytical method of chemometric FT-Raman spectroscopy using homogeneous standard mixtures described earlier was performed for a pharmaceutical model product consisting of IMC and mannitol (1:9 wt/wt). The crystalline or amorphous content of IMC to be measured in the analyte was 1/10 wt% in the case of IMC only, since the ratio of IMC content in the model product was 10 wt%. As with the measurement of IMC crystallinity, a total of 21 spectra were taken for the calibration set standard samples. Prior to PLS analysis, MSC treatment and second-derivative spectral treatment were performed for each Raman spectrum. Figure 5 shows the significant correlation in the 1520–1730 cm^{-1} regions between the transformed spectra and the crystallinity of IMC in the model product. PLS calibration models were constructed in this spectral region where any Raman peaks from mannitol were not detected, as shown in Fig. 2. A three-factor model was selected based on the PRESS plot, as indicated in Table IV.

Figure 6 shows the correlation between the actual and predicted crystallinity of IMC in the model product. The multiple correlation coefficient and the root-mean-square error of prediction were evaluated to be 1.000 and 0.5719,

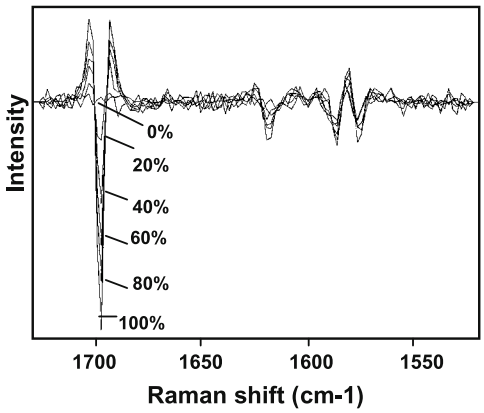

Fig. 5. Overlaid of second derivative of the MSC transformed FT-Raman spectra of IMC/mannitol mixtures of various degrees of IMC crystallinity. Percentages show the degree of IMC crystallinity in the sample.

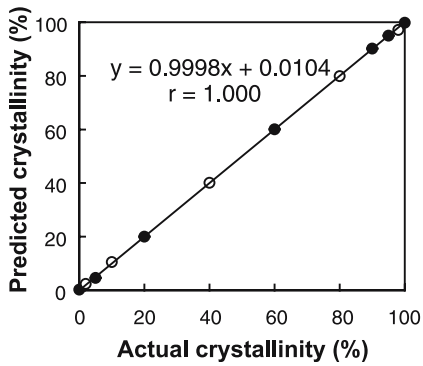

Fig. 6. Correlation between the actual and predicted crystallinity of IMC in standard mixtures of pharmaceutical model samples determined by chemometric FT-Raman spectroscopy. Linear line: regression curve for the calibration set; closed circle: calibration set; open circle: validation set. Bars represent standard deviations (n = 3)

respectively. As a result of measuring the validation set standard samples, the root-mean-square error of prediction was evaluated to be 1.376 for the validation set samples. Therefore, the calibration model was verified to have good accuracy and precision.

Construction of Calibration Curve by XRD

To validate the FT-Raman method, a conventional X-ray powder diffraction method was also developed for the crystallinity of IMC in the pharmaceutical model product. Table V shows the result of X-ray diffraction measurements of IMC/mannitol mixture samples (peak 2θ = 11.6°). The diffraction peak of IMC (2θ = 11.6°) was not detected for the samples with smaller degrees of IMC crystallinity (less than 10%). This may be because the content of crystalline IMC is proportionately reduced in sample mixtures and therefore the peak intensity of IMC was reduced to about one tenth that of IMC only, or it may be attributable to high measurement error due to the close proximity between the IMC peak used for quantification and the mannitol peaks. The calibration curve was constructed based on a plot of diffraction peak height intensity (2θ = 11.6°) *vs.* the known percentage of crystallinity as prepared. The obtained calibration curve has a correlation coefficient of 0.9789, a slope of 29.24, and an intercept of −171.52. The standard deviations for the slope and the intercept are not negligible levels: 2.31 and 170.04, respectively. Experimental error and sensitivity are apparently worse than those of IMC because of the limitation of the potential X-ray diffractometry; however, the good correlation in calibration curve was considered to be due to the fact that the prepared standard mixture samples are of sufficiently high quality for the purposes of this study, since the accuracy and homogeneity of the mixed powders prepared by the standard sample preparation method has already been verified in the above investigations with IMC only.

Table IV. PRESS and RMSECV of Correlation Calculated by PLS Based on the Number of Factors, for Crystallinity of IMC in a Pharmaceutical Model Product

Number of factors	PRESS	RMSECV
1	180.9	2.93
2	150.8	2.68
3	132.4	2.51
4	111.0	2.30
5	121.4	2.40
6	127.9	2.47
7	128.0	2.47
8	125.4	2.44
9	123.8	2.43
10	123.9	2.43

Comparison of FT-Raman Spectroscopy with XRD

Table VI shows the crystallinity of IMC mixed with mannitol in validation set standard samples predicted by X-ray powder diffractometry (XRD) and FT-Raman spectroscopy. The mean bias (Bm) and mean accuracy (Am) were also determined for the validation set samples. The Bm for the FT-Raman and XRD methods were calculated to be 4.7% and 117.6%, and the Am was 7.1% and 129.7%, respectively. The large Bm and Am values in X-ray

Table V. XRD Measurements of IMC–Mannitol Standard Mixture Samples

Xc (%)	Peak intensity[a] (cps)	SD (cps)	RSD (%)
0	ND	—	—
2	ND	—	—
5	ND	—	—
10	317	91	28.7
20	429	93	21.8
40	889	92	10.4
60	1454	129	8.9
80	1819	82	4.5
90	2340	243	10.4
95	2646	305	11.5
98	3088	287	9.3
100	2813	293	10.4

[a] Peak height, mean of three refill measurements (2θ = 11.6°).
ND, not detected; RSD, relative standard deviation;
SD, standard deviation; Xc, known crystallinity.

diffractometry came mainly from the poor accuracy in the measurements at less than 10% of crystallinity. The large error in the measurement results leads to the conclusion that X-ray diffractometry might not be a practical method for quantitating the degree of IMC crystallinity in a pharmaceutical product. On the other hand, the FT-Raman method was confirmed to be useful in quantifying the content variation of small amounts of crystalline (near 0% crystallinity) or amorphous (near 100% crystallinity) IMC, with only small measurement error. Although the precision seems rather reduced compared to that of IMC only, this method has remarkable precision and accuracy considering that IMC content is only 1/10 of the model product.

Evaluation of Microcrystallinity of IMC in Compaction Samples

The FT-Raman method established in this study was applied to mannitol-blended analyte samples that were compressed at various pressures. Conventional XRD was not applied to these compressed samples since it was difficult to measure crystallinity within a small area on a tablet by this method. There was no other new peak on the obtained Raman spectra. This indicates that no polymorph transition has occurred from γ form to α form during the compaction. This is also confirmed by the FT-Raman spectra and XRD profiles of IMC bulk powder crumbed after compaction at 196 MPa, as these polymorphs are easily distinguishable with high sensitivity (16,17).

The crystallinity values of the compressed samples were predicted based on the calibration model constructed from the standard samples of IMC mixed with mannitol. The results are shown in Table VII. A decline in the degree of the crystallinity of IMC with increased compaction pressure was observed. This seems to indicate the so-called pressure-induced amorphization. Marked amorphization normally observed at larger than 1 GPa has been well investigated, especially in the fields of inorganic chemistry and mineral material science (20–23). For the 196 MPa compaction, local variations in crystallinity on the tablet were evaluated. Among the three areas evaluated, the center of surface, edge of surface, and cross-sectional surface, the edge of surface was significantly decreased in the crystallinity (t-test, $p < 0.05$). This suggests that the edge of surface was compressed with higher pressure than the center of surface or cross-sectional surface of the tablet. This is in good accordance with a report published previously by D. Train (24), in which powder compaction experiments performed with a punch and die set revealed that higher pressure was imposed on the shoulder area than the surface area during the compaction, that is, higher shear forces at the die walls.

Table VI. Predicted Crystallinity (%) of IMC in IMC–Mannitol Mixed Samples

Xc	FT-Raman	SD	XRD	SD
2	2.4	0.8	ND	—
10	10.5	0.5	16.7	3.1
40	40.1	1.6	36.3	3.2
80	79.9	2.7	68.1	2.8
98	97.1	1.4	111.5	9.8

ND, not detected; SD, standard deviation ($n = 3$); Xc, known crystallinity.

Table VII. Predicted Crystallinity of Compressed Samples of IMC–Mannitol Mixtures

Sample	Compaction pressure MPa	Crystallinity (%)	SD[a] (%)
1[b]	20	94.3	0.7
2[b]	49	92.7	1.6
3[b]	98	90.5	0.8
4[b]	196	87.6	1.7
5[c]	196	81.1	2.0
6[d]	196	85.9	1.6

[a] $n = 3$: three independent spots were irradiated.
[b] Sample 1–4: irradiated on the center of the tablet surface.
[c] Sample 5: irradiated on an edge of the tablet surface.
[d] Sample 6: irradiated on a cross-sectional surface of the tablet.

The value of the FT-Raman method as a quantitative microanalysis technique for this pharmaceutical product has been established in this study. Commercially available FT-Raman spectrometers can focus the laser beam down to 50 μm in aperture, and hence more precise quantitative analysis would be possible for local areas on the surface or inside of a tablet. In this study, a quantitative method was established for the degree of crystallinity using standard mixtures of crystalline and amorphous materials; however, this technique could also be easily applied to the quantitative analysis of polymorphs in pharmaceutical products, so long as the FT-Raman spectra of the polymorphs are distinct and the distinct region is free from excipients peaks. This technique would surely promote more precise control of solid-state properties of drug products, and more intensive investigation into formulation development.

CONCLUSION

A chemometrical FT-Raman spectroscopic method was established for determination of small variations of the crystallinity of IMC in IMC only (= putative drug substance) and in IMC/mannitol mixtures containing 10 wt% IMC (= putative drug product). This method was verified to be useful for the quantitative analysis of the microcrystalline composition of a pharmaceutical product, particularly at the surface and interior of the tablet.

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REFERENCES

1. FDA Paper. Guideline for submitting supporting documentation in drug applications for the manufacture of drug substances. February 1987.
2. M. Otsuka and Y. Matsuda. Polymorphism: pharmaceutical aspects. In J. Swarbrick and J. C. Boylan (eds.), *Encyclopedia of Pharmaceutical Technology*. Vol. 12, Marcel Dekker, New York, 1995, pp. 305–326.

3. L. S. Taylor and G. Zografi. The quantitative analysis of crystallinity using FT-raman spectroscopy. *Pharm. Res.* **15**:755–761 (1998).
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. S. Fuchs, T. Schmidt, and J. Jerosch. Change in crystalliniry by sterilization and processing of ultrahigh molecular polyethylene used in endoprosthetics. *Unfallchirung* **101**:382–387 (1998).
6. H. Yoshino, Y. Hagiwara, S. Kobayashi, and M. Samejima. Estimation of polymorphic transformation degree of pharmaceutical raw materials. *Chem. Pharm. Bull.* **32**:1523–1536 (1984).
7. M. Otsuka and N. Kaneniwa. A kinetic study of the crystallization process of noncrystalline indomethacin under isothermal conditions. *Chem. Pharm. Bull.* **36**:4026–4032 (1988).
8. H. Ahmed, G. Buckton, and D. A. Rawlins. The use of isothermal microcalorimetry in the study of small degrees of amorphous content of a hydrophobic powder. *Int. J. Pharm.* **130**:195–201 (1996).
9. M. Otsuka, F. Kato and Y. Matsuda. Comparative evaluation of the degree of indomethacin crystallinity by chemoinfometrical fourie-transformed near-infrared Spectroscopy and conventional powder X-ray diffractometry. *AAPS Pharmsci.* **2**(1) article 9 (2000) (http://www.pharmsci.org/).
10. M. J. Gidley and S. M. Bociek. Molecular organization in starches: a 13C CP/MAS NMR study. *J. Am. Chem. Soc.* **107**:7040–7044 (1985).
11. 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).
12. P. J. Hendra. Fourier transform-Raman spectroscopy in pharmaceutical analysis and research. *Am. Lab.* **28**:17–24 (1996).
13. I. Jedvert, M. Josefson, and F. Langkilde. Quantification of an active substance in a tablet by NIR and Raman spectroscopy. *J. Near Infrared Spectrosc.* **6**:279–289 (1998).
14. L. S. Taylor and F. W. Langkilde. Evaluation of solid-state forms present in tablets by Raman spectroscopy. *J. Pharm. Sci.* **89**:1342–1353 (2000).
15. M. J. Pelletier. Quantitative analysis using Raman spectrometry. *Appl. Spectrosc.* **57**:20A–42A (2003).
16. T. Okumura and M. Otsuka. A novel standard sample powder preparation method for quantitative analysis of polymorphs. *J. Pharm. Sci.* **94**:1013–1023 (2005).
17. M. Otsuka, F. Kato, and Y. Matsuda. Determination of indomethacin polymorphic contents by chemometric near-infrared spectroscopy and conventional powder X-ray diffractometry. *Analyst* **126**:1578–1582 (2001).
18. I. S. Helland, T. Nacs, and T. Isaksson. Related versions of the multiplicative scatter correction method for preprocessing spectroscopic data. *Chemom. Intell. Lab. Syst.* **29**:233 (1995).
19. H. Martens and T. Næs. *Multivariate Calibration*, John Wiley & Sons, New York, 1989.
20. E. V. Boldyreva. Molecules in strained environment. *NATO Science Series, II: Mathematics, Physics and Chemistry Chem.* **140**(High-Pressure Crystallography):495–512 (2004).
21. A. K. Arora. Pressure-induced amorphization. *Proc. Int. Sch. Phys.* "*Enrico Ferm*" **147**(High Pressure Phenomena):545–565 (2002).
22. T. Yamanaka, T. Nagai, and T. Tsuchiya. Mechanism of pressure-induced amorphization. *Zeitschr. Kristall.* **212**:401–410 (1997).
23. S. M. Sharma and S. K. Sikka. Pressure induced amorphization of materials. *Prog. Mater. Sci.* **40**:1–77 (1996).
24. D. Train. Transmission of forces through a powder mass during the process of pelleting. *Trans. Inst. Chem. Eng.* **35**:258–266 (1957).