FULL PAPER
THE ANALYST
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Determination of indomethacin polymorphic contents by chemometric near-infrared spectroscopy and conventional powder X-ray diffractometry

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Received 18th April 2001, Accepted 21st June 2001
First published as an Advance Article on the web 30th July 2001

A chemoinfometric method for the quantitative determination of the crystal content of indomethacin (IMC) polymorphs, based on Fourier-transform near-infrared (FT-NIR) spectroscopy, was established. A direct comparison of the data with those collected using the conventional powder X-ray diffraction method was performed. Pure α and γ forms of IMC were prepared using published methods. Powder X-ray diffraction profiles and NIR spectra were recorded for six kinds of standard material with various contents of the γ form of IMC. Principal component regression (PCR) analyses were performed on the basis of the normalized NIR spectral sets of standard samples with known contents of the γ form of IMC. A calibration equation was determined to minimize the root mean square error of the prediction. The predicted γ form contents were reproducible and had a relatively small standard deviation. The values of the γ form contents predicted by the two methods were in close agreement. The results indicated that NIR spectroscopy provides an accurate quantitative analysis of crystallinity in polymorphs compared with the results obtained by conventional powder X-ray diffractometry.

Introduction

In order to ensure the manufacture of safe and efficacious pharmaceutical products, the validation of the production process is required in order to meet regulatory requirements. However, for drugs with limited solubilities, more than one crystalline form or solvate may exist. These polymorphs exhibit different physicochemical stabilities, processing characteristics, dissolution rates, *etc*. In particular, the dissolution rate may be affected which, in turn, may significantly affect the absorption of orally administered drugs in the gastrointestine, resulting in a variation in the bioavailability of pharmaceutical compounds.[1–4] Therefore, an accurate assessment of the polymorphism and solvate of bulk materials is required for the reproducible preparation of pharmaceutical products. Analytical methods for the determination of polymorphs include powder X-ray diffraction,[5] differential scanning calorimetry (DSC),[6] thermal gravimetric analysis (TGA), microcalorimetry,[7] infrared (IR) spectroscopy,[8] Raman spectroscopy[9] and dissolution kinetics.[10] However, these methods involve time-consuming sample preparation and/or measurements. In contrast, near-infrared (NIR) spectroscopy is simple due to its non-destructive sample preparation method. Consequently, NIR spectroscopy is fast becoming an important technique for pharmaceutical analysis in industry.

Chemoinfometrics provides an ideal means of extracting quantitative information from UV/VIS, IR and NIR spectroscopy, chromatography, mass spectrometry and NMR[11,12] spectroscopy of multi-component samples. A number of chemoinfometric and statistical techniques have been employed in NIR quantitative and qualitative analysis because these approaches have proved to be successful in extracting the desired information from unprocessed NIR spectra. Calibration methods, such as multiple linear regression (MLR), principal component analysis/principal component regression (PCA/PCR) and partial least-squares regression (PLS) are commonly used.[13–18] Norris *et al*.[19] reported that polymorphic transformations of trovafloxacin mesylate in suspension could be evaluated on the basis of their NIR spectra by PCA. Sarver *et al*.[20] reported the quantitative determination of delavirdine mesylate polymorphic forms based on IR spectra by PCR. Patel *et al*.[21] and Blanco *et al*.[22] reported the quantitative analysis of polymorphs in powder mixtures based on their NIR spectra by MLR and PLS.

In a previous study,[13] we applied chemoinfometric methods to evaluate the quality of bulk powders of pharmaceutical products. The degree of crystallinity of indomethacin (IMC) bulk powder was evaluated using the MLR method. NIR spectroscopic data were consistent with those obtained by the conventional powder X-ray diffraction method. However, the MLR method utilized only a few spectral data points to evaluate the properties. The rest of the spectral data were unused. In contrast, the PCR method utilizes all of the data to determine the properties, and is therefore more accurate than MLR. The purpose of this study was to investigate the application of the PCR method in the analysis of NIR spectroscopy for the quantitative determination of IMC polymorphism. Direct comparison with the conventional powder X-ray diffraction method, in terms of accuracy and experimental advantages, was also performed.

Experimental

Materials

The bulk powder of IMC was obtained from Yashiro Co., Japan. The α form of IMC was prepared by the following method: 10 g of IMC bulk powder was dissolved in 10 ml of ethanol at 80 °C; the undissolved drug was filtered off; then, 20 ml of distilled water at room temperature was added to the IMC-saturated ethanol solution at 80 °C; the precipitated crystals were removed by filtration using a glass funnel and then dried under

 DOI: 10.1039/b103498g

vacuum at room temperature. The γ form of IMC was prepared by recrystallization from ethyl ether at room temperature.[6]

X-Ray powder diffraction analysis

X-Ray powder diffraction profiles were obtained using an X-ray diffractometer (XD-3A, Shimadzu Co., Japan). The measurement conditions were as follows: scan mode, step scan; target, Cu; filter, Ni; voltage, 20 kV; current, 20 mA; receiving slit, 0.1 mm; time constant, 1 s; scan width, 0.1° per step. The X-ray powder diffraction profiles were measured using the following method. Known quantities of standard mixtures were obtained by physically mixing α form and γ form IMC powders in various ratios (0, 20, 40, 60, 80 and 100% w/w γ form) in a V-type mixer for 1 h. 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 the samples had been measured under the above conditions, the intensity values were normalized against the intensity of silicon powder (2 = 28.8°) which was the external standard. The calibration curves for the quantification of the crystal content were based upon the total relative intensity of four diffraction peaks, 2 = 11.6°, 19.6°, 21.8°, 26.6°, of the γ form crystal. All data were reported as the average of five runs.

Thermal analysis

DSC was performed with a Type 3100 instrument (MacScience 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 obtained using a NIR spectrometer (InfraProver™, BRAN + LUEBBE Co., Norderstedt, Germany). Briefly, a fibre-optic probe was inserted into the sample powder (2 g) in a 20 ml glass bottle. Five scans per sample were recorded in the spectral range 4500–10 000 cm^{-1}. A ceramic (Coor's Standard) reference scan was taken for each set of samples. FT-NIR spectra of six calibration sample sets were recorded five times with the NIR spectrometer. A total of 30 spectral data were analysed by the various methods, and chemoinfometric analysis was performed using the PCR program associated with the SESAMI software (BRAN + LUEBBE Co.)

Quantitative analysis of unknown samples

Unknown samples were obtained. The pure α form of IMC (5 g) was dissolved in 50 ml of ethanol in a 100 ml glass beaker. The temperature was maintained in a water bath at 36 or 40 °C. Samples were withdrawn at appropriate time intervals, filtered and dried under vacuum at room temperature.[5] The contents of the α and γ forms were determined using X-ray powder diffraction and FT-NIR methods.

Results and discussion

Characterization of the α and γ forms of IMC

Fig. 1 shows the powder X-ray diffraction profiles of the pure α and γ forms of IMC. The main X-ray diffraction peaks of the α form were at 8.4°, 14.4°, 18.5° and 22.0° (2θ), and those of the γ form were at 11.6°, 16.8°, 19.6°, 21.9° and 26.7° (2θ), as reported previously.[5]

Fig. 2 shows the DSC profiles of the pure α and γ forms of IMC. The DSC curves of the α and γ forms showed corresponding endothermic peaks at 155 and 162 °C, respectively, which are attributable to sample melting. These results suggest that the α and γ forms of IMC used in the present study were of high purity.

Measurement of the polymorphic content of the γ form of IMC by conventional X-ray powder diffractometry

The calibration curve for the measurement of the content of the γ form of IMC by conventional X-ray diffractometry was based on the total intensity of the four specific diffraction peaks. The X-ray diffraction profiles showed two main reasons for the fluctuation in the determination of the crystal content: one is the intensity fluctuation of the direct X-ray beam during measurement, and the other is the crystal orientation when the sample powder is loaded into the sample holder. In order to avoid fluctuation of the direct beam intensity, the peak at 2 = 28.8° of silicon powder was measured as an external standard for correction of the crystalline content. The four diffraction peaks with the highest intensity were measured to minimize the systematic error due to crystal orientation.

Fig. 3 shows a plot of the relation between the actual and predicted polymorphic contents of the γ form of IMC measured using the X-ray diffraction method. This plot shows a linear relation. It has a slope of 0.9983, an intercept of 0.7739×10^{-3} and a correlation coefficient of 0.9699. However, it has slightly higher 95% confidence levels for the prediction of individual y values and 95% confidence intervals of regression than NIR methods, indicating that the X-ray diffraction method has relatively low accuracy in the determination of crystalline content.

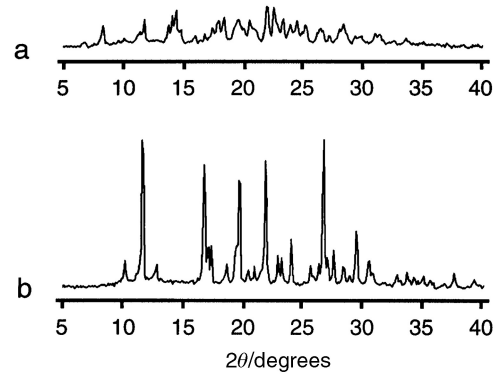

Fig. 1 X-Ray powder diffraction patterns of the α (a) and γ (b) forms of indomethacin.

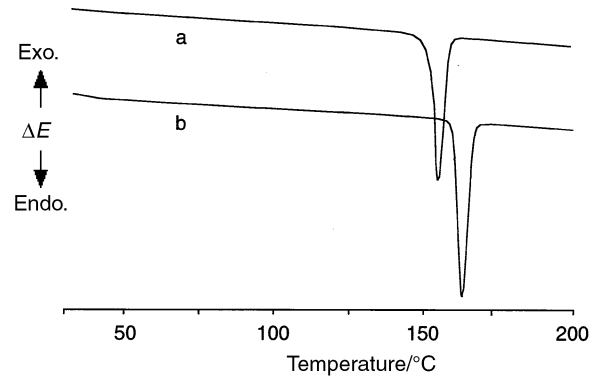

Fig. 2 Differential scanning calorimetry curves of the α (a) and γ (b) forms of indomethacin.

Measurement of the content of the γ form of IMC by chemoinfometric FT-NIR spectroscopy

Fig. 4 shows the FT-NIR spectra of the α and γ forms of IMC. The α and γ forms of IMC showed significant NIR spectral peaks. The NIR absorption peaks of IMC were identified.[23] The absorption peaks at 4656, 5780/5850, 7280, 8432 and 8860 cm^{-1} are associated with the C=O moiety of the carboxyl group, $–CH_2–$ group, methyl group, CH group and HC=CH group of the benzene ring, respectively. All of the peak intensities of the γ form were stronger than those exhibited by the α form, except for the peak at 4580 cm^{-1} which was attributable to the C=O group. The γ form exhibited a peak attributable to the COOH group at 5380 cm^{-1}, but this was not observed for the α form. The results indicated that the γ form was a dimer and the α form was a monomer, as reported in X-ray diffraction results.[24]

PCR is presented as the regression of y on selected principal components of x. The properties of PCR are given, together with a discussion on the selection of eigenvectors. Since PCR is useful for the determination of the relationship between objective parameters and principal components in the spectra, PCR was applied to the present study.

A spectrum including n spectral data can be seen as a point in n-dimensional space. In multivariate data analysis, PCA/PCR of a spectral data matrix **X** is a basic tool. PCA/PCR decomposes **X** into a score matrix **T** times a loading matrix **P** plus a residual matrix **E**:[18]

$$\mathbf{X} = \mathbf{t}_1\mathbf{p}'_1 + \mathbf{t}_2\mathbf{p}'_2 + \ldots + \mathbf{E} = \mathbf{TP}' + \mathbf{E} \quad (1)$$

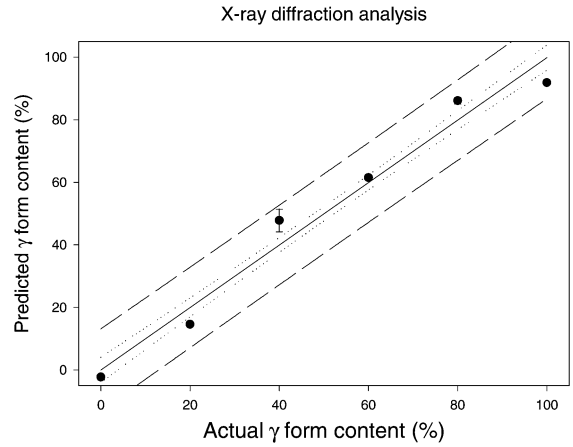

Fig. 3 Relation between the actual and predicted content of the γ form of indomethacin obtained by conventional X-ray powder diffractometry. The filled circles and error bars represent the average and standard deviation (n = 5), respectively. The full line, long broken line and dotted line represent the regression line, 95% predicted interval and 95% confidence interval, respectively.

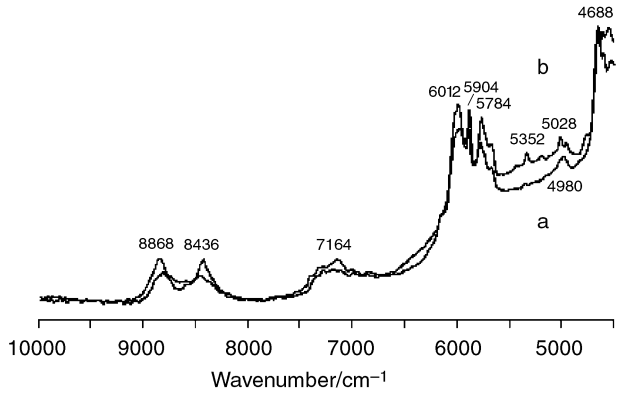

Fig. 4 Fourier-transform near-IR spectra of the α (a) and γ (b) forms of indomethacin.

This decomposition is particularly useful for converting **X** to a few information plots (score plots and loading plots) and for modelling the systematic structure in **X**.

In this study, the NIR spectra consist of 459 data points between 4500 and 10 000 cm^{-1} at intervals of 12 cm^{-1}. Even batches of standard samples with various contents of the γ form of IMC were prepared; four spectra were collected per batch. A total of 24 spectra were selected for the calibration (calibration set) and six spectra were used for the prediction of calibration (prediction set). For the NIR spectra of the samples, a pre-treatment was performed to minimize the experimental error by using transformations of absorbance, normalized absorbance and second derivative. The best conditions were determined to minimize the root-mean-square error of prediction (RMSEP):

$$\mathrm{RMSEP} = \sqrt{\frac{\Sigma(y_p - y_r)^2}{n}} \quad (2)$$

The RMSEP values of the correlation curves were calculated on the basis of the spectral data corrected by normalization and are summarized in Table 1. The RMSEP value decreased with increasing number of principal component factors, but was almost constant after three principal components. Table 2 shows the RMSEP values of the correlation curves calculated on the basis of the spectral data corrected by three transformations. The minimum RMSEP value was calculated for the normalized NIR spectra based on a three principal component model. Therefore, the three principal component model was used for subsequent analysis.

Fig. 5 shows the loading vectors corresponding to the principal components (PC). The peak at 4560 cm^{-1} was the highest value for PC1, but the peaks at 6048, 5772, 5352, 8836 and 8486 cm^{-1} were lower, because there were large spectral intensity differences between the α and γ forms at the peaks. The loading vector of PC1 was similar to that of PC2, but not to that of PC3. This result suggests that the loading vectors reflect the spectral differences between the α and γ forms.

Fig. 6 shows the correlation between the actual and predicted contents of the γ form of IMC obtained by the NIR method. The predicted values were reproducible and had a smaller standard deviation than the X-ray diffraction method. The multiple correlation coefficient, the standard error of the estimate (SEE) and the RMSEP were evaluated to be 0.998, 2.559 and 3.507,

Table 1 Root-mean-square error of prediction (RMSEP) of correlation calculated by principal component regression based on the number of principal components (PC)

Number of PC	RMSEP
0	35.642
1	12.802
2	6.027
3	3.208
4	2.665
5	3.281
6	2.282
7	2.476
8	2.510
9	2.349
10	2.361

Table 2 Root-mean-square error of prediction (RMSEP) of correlation calculated by principal component regression based on various transformations

Transformation	Number of PC	RMSEP
Absorbance	2	7.401
Absorbance + normalization	3	3.507
Absorbance + second derivative	2	5.680

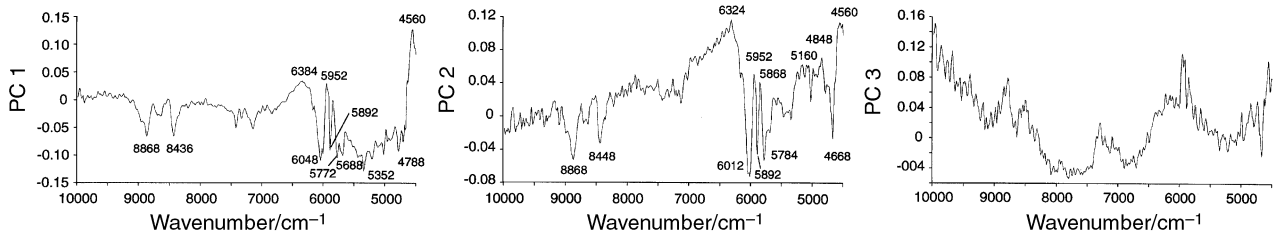

Fig. 5 Loading vectors of the principal components PC1, PC2 and PC3 based on the normalized near-IR spectra calculated by principal component regression.

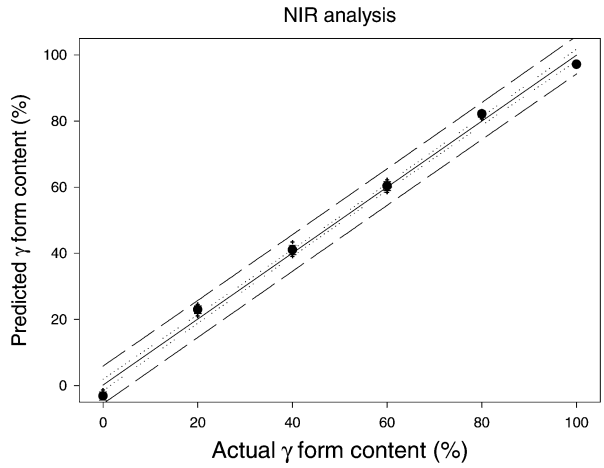

Fig. 6 Correlation between the actual and predicted contents of the γ form of indomethacin obtained by Fourier-transform near-IR spectroscopy. The filled circles and error bars represent the average and standard deviation (n = 5), respectively. The full line, long broken line and dotted line represent the regression line, 95% predicted interval and 95% confidence interval, respectively.

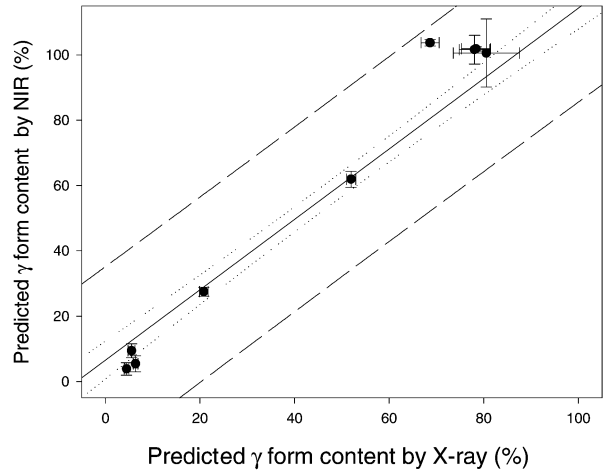

Fig. 7 Correlation between the predicted γ form content measured by X-ray diffractometry and that measured by the near-IR method. The filled circles and error bars represent the average and standard deviation (n = 5), respectively. The full line, long broken line and dotted line represent the regression line, 95% predicted interval and 95% confidence interval, respectively.

respectively. As the purpose of this study was to compare the accuracy of the chemoinfometric NIR method with that of conventional X-ray powder diffraction, the mean bias and the mean accuracy were determined by eqns. (3) and (4), respectively:

$$B_m = \frac{\sum_{i=1}^{n} (X_c - X_t) / X_t}{n} \times 100 \quad (3)$$

$$A_m = \frac{\sum_{i=1}^{n} \left| X_c^n - X_t \right| / X_t}{n} \times 100 \quad (4)$$

where B_m is the percentage mean bias, A_m is the percentage mean accuracy, X_c is the predicted content of the γ form of IMC, X_t is the actual content of the γ form of IMC and n is the number of experiments.

The mean bias values for the NIR and X-ray powder diffraction methods were calculated to be 2.95 and −0.94% and the mean accuracies were 4.29 and 10.80%, respectively. The confidence levels for the prediction of individual y values for the NIR method were much narrower than those using the conventional X-ray method, but the results were consistent with those obtained with the X-ray method. These results indicate that the NIR method was more accurate than the X-ray method. Thus, this assay has significant advantages for the quantitative analysis of IMC polymorphs.

Comparative evaluation of conventional powder X-ray diffraction and chemoinfometric NIR methods

Fig. 7 shows the correlation between the predicted γ form content measured by X-ray diffractometry and that measured by the NIR method. The plot has a slope of 1.296, an intercept of 1.109 and a correlation coefficient of 0.992. The line represents a satisfactory correlation between the two predicted values of the γ form content. Thus NIR spectroscopy is an effective method for the quantitative evaluation of polymorphs in pharmaceutical products.

Conclusions

The quantitative evaluation of IMC polymorphs by NIR spectroscopy using the PCR method was demonstrated to have a significant advantage over conventional powder X-ray diffractometry. This method is expected to provide a rapid quantitative analysis of polymorphs during preparations due to the simplicity, non-destructive nature and high sensitivity of the method.

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