Application study of chemometric near infrared spectroscopy in the pharmaceutical industry

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Introduction

The polymorphs of pharmaceutical solids exhibit different physicochemical stability, processing characteristic, dissolution rate, etc. Particularly, dissolution rate may be affected which, in return, may significantly affect drug absorption for oral dosage form in the gastro-intestine resulting in a variation of bioavailability for the pharmaceutical compounds.[1–4] Therefore, an accurate assessment of polymorphism and solvate of bulk materials are required for reproducible preparation of pharmaceutical products. There are various analytical methods for polymorph determination, including 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 are too time-consuming in the preparation of samples and/or their measurements. In contrast, near infrared (NIR) spectroscopy is simple due to its method of non-destructive sample preparation. Consequently, NIR spectroscopy is fast becoming an important technique used for pharmaceutical analysis in the industry.

On the other hand, chemometrics provides an ideal means of extracting quantitative information from UV-vis, IR and NIR spectroscopy, chromatography, mass spectrometry and NMR[11,12] spectra of multi-component samples. A number of chemometric and statistical techniques are employed in NIR quantitative and qualitative analysis because these approaches have been proved 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 in common use.[13–18] Norris *et al*. and the others[19–22] reported that polymorphism of pharmaceuticals were evaluated based on their NIR spectra by MLR, PCR and PLS.

The purpose of this study is to investigate the application of the PCR method on analysing NIR spectroscopy for the quantitative determination of indomethacin (IMC) polymorphism. A direct comparison with the accuracy and experimental advantages with the conventional powder X-ray diffraction method was also explored.

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Experimental

Materials

The bulk powder of IMC was obtained from the Yashiro Co., Japan. The α form of IMC was prepared by using the following method. Ten 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 a vacuum at room temperature. The γ form of IMC was prepared by recrystallisation from ethyl ether at room temperature.[6]

X-ray powder diffraction analysis

X-ray powder diffraction profiles were obtained using X-ray diffractometer (XD-3A, Shimadzu Co., Japan). The measurement conditions include (1) scan mode: step scan, (2) target: Cu, (3) filter: Ni, (4) voltage: 20 kV, (5) current: 20 mA, (6) receiving slit: 0.1 mm, (7) time constant: 1 s, (8) scan width: 0.1 degree/step. The X-ray powder diffraction profiles were measured using the following method. Known quantities of standard mixtures were obtained by physically mixing α and γ forms of IMC powders at various ratios (0, 20, 40, 60, 80 and 100 w/w% γ form content) in a V-type mixer for one hour. About 80 mg of each sample powder were carefully loaded in a glass holder without particle orientation using a spatula and glass plate. After the powder X-ray diffraction profiles of samples had been measured under the above conditions, the intensity values were normalised against the intensity of silicon powder ($2\theta = 28.8°$) which was the external standard. The calibration curves for quantification of crystal content were based upon the total relative intensity of four diffraction peaks, $2\theta = 11.6$, 19.6, 21.8, 26.6°, of the γ form crystal. All data were reported as the average of five runs.

Fourier transform near infrared (FT-NIR) spectroscopy

FT-NIR spectra were taken using an 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 of 4500 to 10000 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 chemometric analysis was performed using the PCR program associated with the SESAMI software (Bran+Luebbe Co.).

Results and discussion

Characterisation of α and γ forms of IMC

The results of powder X-ray diffraction profiles and the DSC profiles of the pure α and γ forms of IMC suggested that the main X-ray diffraction peaks of the α form were at 8.4, 14.4, 18.5, 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 prevously.[5] 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 suggested that the α and γ forms of IMC used in the present study were highly purified.

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

The calibration curve for measuring the content of the γ form by conventional X-ray diffraction method was based on the total intensity of the four specific diffraction peaks. The X-ray diffraction profiles showed two main causes in fluctuation in the determination of crystal content, one is a inten-

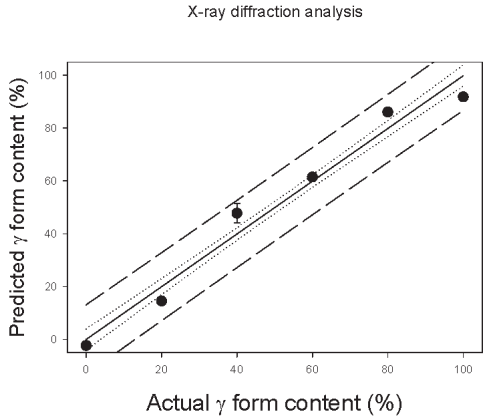

Figure 1. Relation between the actual and predicted content of form γ IMC obtained by conventional X-ray powder diffractometry. Bars present standard deviation. The symbols and error bars present average and standard deviation (*n* = 5). The solid line, long dash line and dotted line are represented a regression line, 95% predicted interval and 95% confidential interval, respectively.

sity fluctuation of the X-ray direct beam during measurement and the other is crystal orientation when the sample powder was loaded in the sample holder. In order to avoid fluctuation of direct beam intensity, the peak at $2\theta = 28.8°$ of silicon powder was measured as an external standard for correction of the value of crystalline content. The four diffraction peaks with the highest intensity were measured to minimise a systematic error due to crystal orientation.

Figure 1 shows a plot of the relationship 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 relationship. 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, indicating that the X-ray diffraction method has relatively low accuracy in the determination of crystalline content.

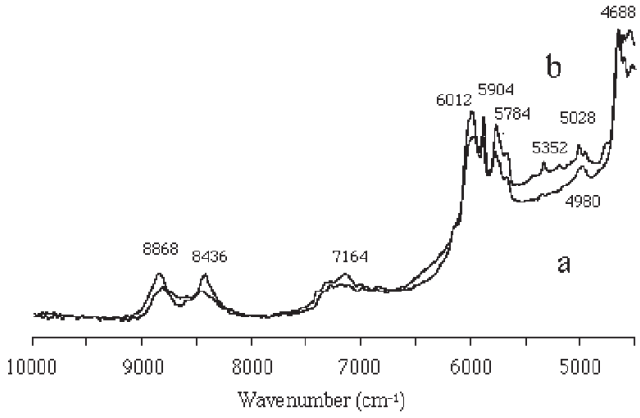

Figure 2. FT-NIR spectra of form α and form γ indomethacin. (a) form α; (b) form γ.

Measurement of content of the γ form by chemometric FT-NIR spectroscopy

Figure 2 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]

In this study, the NIR spectra consist of 459 data points between 4500 to 10000 cm^{-1} at intervals of 12 cm^{-1}. Even batches of standard samples with various content of the γ form of IMC were prepared; four spectra were collected per batches. A total of 24 spectra were selected for the calibration (calibration set) and six spectra were removed and used for prediction of the calibration (prediction set). A pre-treatment was performed on the NIR spectra of the samples to minimise experimental error by using transformations of absorbance, normalised absorbance and second derivative. The best conditions were determined to minimise the root mean squared error of prediction (*RMSEP*, Equation 1).

$$RMSEP = \sqrt{\frac{\sum (y_p - y_r)^2}{n}} \quad (1)$$

Table 1 shows *RMSEP* of the correlation curves were calculated based on the spectral data corrected by normalisation. The *RMSEP* value decreased with an increase in the number of principal component factors, but this was almost constant after three PCs. Table 2 shows *RMSEP* of the correlation curves, which were calculated based on the spectral data corrected by three transformations. As a result, the minimum *RMSEP* value was the normalised NIR spectra based on a three-principal component model, so, the three-principal component model based on normalised NIR spectra was taken for the later analysis.

Figure 3 shows loading vectors corresponding to the principal components (PCs), respectively. The peak at 4560 cm^{-1} was the highest value and the peaks at 6048, 5772, 5352, 8836 and 8486 cm^{-1} were lower on PC1, because there were large spectral intensity differences between α and γ forms at the peaks. The loading vector of PC1 was similar to that of PC2, but not to that of PC3. The result suggested that the loading vectors reflected the spectral difference between α and γ forms.

Figure 4 shows a plot of the calibration data obtained by the NIR method between the actual and predicted contents of the γ form of IMC. The predicted values were reproducible and had a smaller standard deviation. The multiple correlation coefficient, the standard error of estimate (*SEE*) and the *RMSEP* were evaluated to be 0.998 2.559, 3.507, respectively. Since the purpose of this study is to compare the accuracy of the chemometric NIR method with that of conven-

Table 1. *RMSEP* of correlation calculated by PCR based on number of PC.

Transfomation	Number of PC	*RMSEP*
Abs.	2	7.401
Abs. + Nor.	3	3.507
Abs. + 2nd deriv.	2	5.680

Abs: Absorbance
Nor: Normalise
2nd deriv: second derivative.

Table 2. *RMSEP* of correlation calculated by PCR based on various transformations.

Number of factor	*SEP*
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

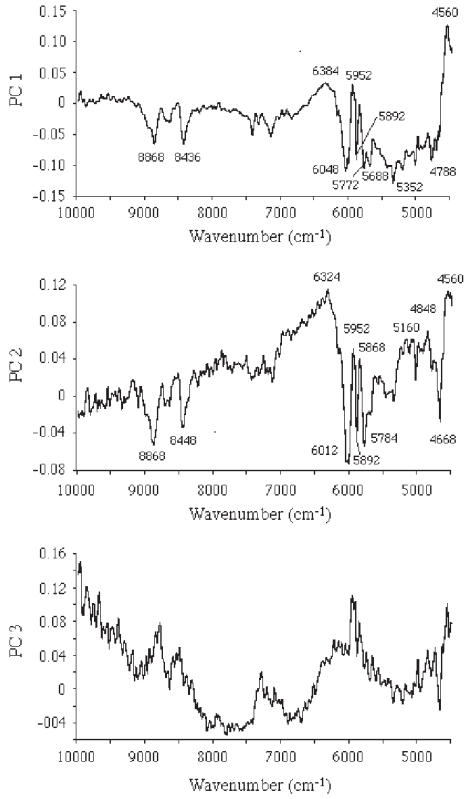

Figure 3. Loading vectors of PCs 1, 2 and 3 based on normalised NIR spectra calculated by PCR.

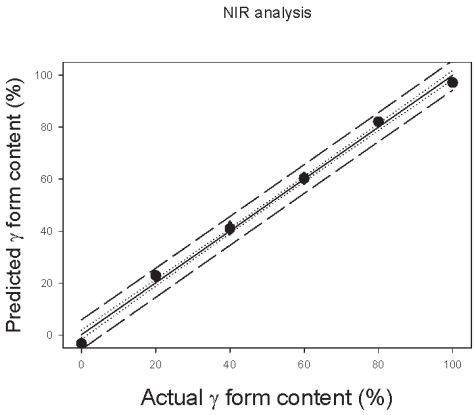

Figure 4. Correlation between actual and predict content of form γ IMC obtained by FT-NIR spectroscopy. The symbols and error bars present average and standard deviation (n = 5). The solid line, long dash line and dotted line are represented a regression line, 95% predicted interval and 95% confidential interval, respectively.

tional X-ray powder diffraction, the mean bias and the mean accuracy were determined by Equations 2 and 3, respectively.

$$B_m = \frac{\sum_{i=1}^{n} \frac{(X_c - X_t)}{X_t}}{n} \times 100 \quad (2)$$

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

B*m* is percentage mean bias, A*m* is percentage mean accuracy, *Xc* is the predicted value of content of the γ form of IMC, *Xt* is actual value of content of the γ form of IMC and *n* is number of experiments.

The mean bias for the NIR and X-ray powder diffraction method were calculated to be 2.95% and –0.94% and the mean accuracy 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 that for using the conventional X-ray method, but the result was consistant with the X-ray method. These results indicate that the NIR method was more accurate than the X-ray method. Thus, this assay is found to have significant advantages for quantitative analysis of IMC polymorphs.

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