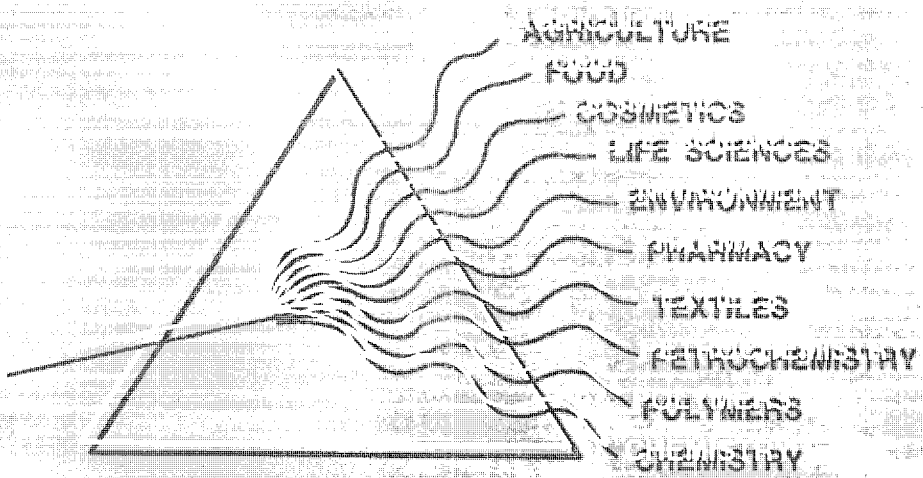
일본의 근적외선분광법에 대한 제약회사 응용 및 현황

Makoto Otsuka (일본 Kobe대학)



Application Study of Chemoinfometrical Near-Infrared Spectroscopic Method to Evaluate for Polymorphic Content of Pharmaceutical Powders

Makoto Otsuka
Department of Pharmaceutical Technology,
Kobe Pharmaceutical University,
Higashi-Nada, Kobe 658-8558, Japan.

***, Address correspondence to: Makoto Otsuka, Ph. D.,**
Department of Pharmaceutical Technology,
Kobe Pharmaceutical University,
Motoyama-Kitamachi 4-19-1, Higashi-Nada,
Kobe 658-8558, Japan.
e-mail: m-otsuka@kobepharma-u.ac.jp

Abstract

A chemoinfometrical method for quantitative determination of crystal content of indomethacin (IMC) polymorphs based on fourie-transformed near-infrared (FT-NIR) spectroscopy was established. A direct comparison of the data with the ones collected from 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 materials with various content of γ form IMC. The principal component regression (PCR) analyses were performed based on normalized NIR spectra sets of standard samples of known content of IMC γ form. A calibration equation was determined to minimize the root mean square error of the prediction. The predicted γ form content values were reproducible and had a relatively small standard deviation. The values of γ form content predicted by two methods were in close agreement. The results were indicated that NIR spectroscopy provides for 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 manufacturing of safe and efficacious pharmaceutical products, the production process validation is required in order to meet regulatory requirements. 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 and polymorphism of the powder materials. Since both these parameters might be affect the bioavailability of a drug through the rate of dissolution,[1)] it is necessary to control the conditions under the pharmaceutical drug powders are produced. The extent of disorder in a crystalline solid and/or polymorphism may induce the hygroscopicity of the drug in addition to the flow, mechanical and dissolution properties, and chemical stability.[1-3)]. Therefore, an accurate assessment of polymorphism and solvate of bulk materials are required for reproducible preparation of pharmaceutical products. Analytical methods for polymorph including powder X-ray diffraction[4)], differential scanning calorimetry (DSC) [5)], thermal gravimetric analysis (TGA), microcalorimetry[6)], infrared (IR) spectroscopy[7)], Raman spectroscopy[8)] and dissolution kinetics[9)]. However, these methods are too time-consuming in the preparation of samples and/or their measurements. In the 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, chemoinfometrics provides an ideal means of extracting quantitative information from UV-VIS, IR and NIR spectroscopy, chromatography, mass spectrometry and NMR[10-11)] spectra of multi-component samples. A number of chemoinfometric 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 liner regression (MLR), principal component analysis/principal component regression (PCA/PCR) and partial least squares regression (PLS) are commonly used [12-17)]. Norris et al.[18)] reported that polymorphic transformations of trovafloxacin mesylate in suspension were evaluated based on their NIR spectra by PCA. Sarver et al.[19)] reported that quantitative determination of delavirdine mesylate polymorphic forms based on IR spectra by PCR. Patel et al.[20)] reported that quantitative analysis of

polymorphs in additive powder mixtures based on their NIR spectra by MLR and PLS.

We applied, therefore, chemoinfometrical methods[12, 13] to evaluate quality of pharmaceutical products. PCR method utilizes NIR spectral data to determine the indomethacin (IMC) bulk powder, which is anti-inflammation drug. The purpose of this study is to investigate the application of PCR method on analyzing NIR spectroscopy for the quantitative determination of IMC polymorphism. A direct comparison with the accuracy and experimental advantages with the conventional powder X-ray diffraction method was also explored.

Measurement of polymorphic content of form γ IMC by conventional X-ray powder diffractometry[13]

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

The DSC curves of the form α and form γ (Figure 2) showed corresponding endothermic peaks at 155 and 162 °C, respectively, which are attributable to sample melting. These results suggested that the form α and form γ of IMC used in the present study were highly purified.

The calibration curve for measuring the content of form γ 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 intensity fluctuation of X-ray direct beam during measurement and the other is crystals orientation when sample powder was loaded in the sample holder. In order to avid fluctuation of direct beam intensity, the peak at 2θ=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 minimize a systematic error due to crystal orientation.

The plot of the relation between the actual content of form γ and total diffraction intensity can be fitted as a straight line with a slope of 0.023, an intercept of 0.131 and a correlation coefficient of 0.986. The polymorphic content of the sample powders were calculated based on the calibration curve.

The relation between the actual and predicted polymorphic contents of form γ

IMC measured using the X-ray diffraction method is shown in Figure 3. This plot shows a linear relation. It has slope of 0.023, an intercept of 0.131 and a correlation coefficient of 0.986. 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.

Measurement of content of form γ by chemoinfometric FT-NIR spectroscopy[13)]

FT-NIR spectra of the form α and form γ IMC is shown in Figure 4. The form α and form γ IMC showed significant NIR spectral peaks. The NIR absorption peaks of IMC were identified [21)]. The absorption peak at 4656 cm^{-1}, 5780 & 5850 cm^{-1}, 7280 cm^{-1}, 8432 cm^{-1}, and 8860 cm^{-1} are associated with C=O group of carboxyl group, -CH2- group, methyl group, CH group, and HC=CH group of benzene ring, respectively. All of peak intensities of γ form were stronger than those exhibited in α form except for the peak at 4580 cm^{-1} which was attributable to C=O group. The γ form had a peak attributable to COOH group at 5380 cm^{-1}, but not for the α form.. The result indicated that γ form was the dimmer form and α form was a monomer as reported in X-ray diffraction results[22)].

To predict value y from a suite of other measurement x_j (where j=1, 2,.., $y = m$), we must first establish a relationship between two sets of measurements. If we assume that y is linearly related to x and write:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + ... + \beta_m x_m + f \qquad (1)$$

then the • specify the relationship, and f contains the error in describing this relationship. For a set of n samples (i=1,2,.. n):

$$y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + ... + \beta_m x_{im} + f_i \qquad (2)$$

In matrix format, this becomes

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{f}$$

The error vector, **f**, is included because it is unlikely that **y** can be expressed exactly in terms of the **X** block; fi is the y residual for the i^{th} sample. The determination of the vector of regression coefficients allows future values to be predicted from future X block measurements. Thus, finding the • vector is described as creating a regression model.

PCR is presented as regression of y on selected principal components of X.

Properties of PCR are given together with a discussion on selection of eigenvectors.

A spectrum including n spectral data can be seen as a point in an n-dimensional space. In multivariate data analysis, PCA/PCR of a spectral data matrix **X** is a basic tool. PCA/PCR decomposes **X** into three matrices (equation 3) [17]

$$\mathbf{X} = \mathbf{USV}^{\mathbf{T}} \qquad (3)$$

This decomposition is particularly useful for converting **X** to a few information plots (score plots and loading plots) and for modeling the systematic structure in **X**. The **U** matrix hold the eigenvectors of the row space, the **V** matrix holds eigenvectors of the column space, **S** is a diagonal matrix whose diagonal elements are the singular values, **T** is a scores matrix, and **L** is a loadings matrix.

$$\mathbf{L} \equiv \mathbf{V} \qquad (4)$$

$$\mathbf{T} \equiv \mathbf{US} \qquad (5)$$

$$\mathbf{y} = (\mathbf{USV}^{\mathbf{T}})\boldsymbol{\beta} + \mathbf{f} \qquad (6)$$

The solution then becomes:

$$\boldsymbol{\beta} = \mathbf{VS}^{-1}\mathbf{U}^{\mathbf{T}}\mathbf{y} \qquad (7)$$

where this b term is the regression vector. Predicting y from a new x form from:

$$\mathbf{y}_{new} = \mathbf{x}_{new}\boldsymbol{\beta} = \mathbf{x}_{new}\mathbf{VS}^{-1}\mathbf{U}^{\mathbf{T}}\mathbf{y} \qquad (8)$$

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 form γ IMC were prepared; four spectra were collected per batches. A total of 24 spectra were selected for the calibration (calibration set) and removed 6 spectra were used for prediction of calibration (prediction set). The NIR spectra for samples were performed a pre-treatment to minimize 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, equation 9).

$$RMSEP = \sqrt{\frac{\sum (y_p - y_r)^2}{n}} \qquad (9)$$

Table 1 shows RMSEP of the correlation curves were calculated based on the spectral data corrected by three transformations. As the result, the minimum RMSEP value was calculated normalized NIR spectra based on three-principal component model. The RMSEP value calculated based on three-principal component model after normalization. The RMSEP decreased with increasing of number of factors, but it did not after number of three, so, the three-principal component model was taken for the later analysis.

The loading vectors corresponded to the principal component (PC), respectively are sown in Figure 5. 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 was 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 was reflected the spectral difference between α and γ forms.

The calibration data obtained by NIR method between the actual and predicted contents of form γ IMC is shown in Figure 6. The predicted values were reproducible and had a smaller standard deviation. The multiple correlation coefficient, the standard error of estimate (SEE) and the standard SEP were evaluated to be 0.998 2.559, 3.507, respectively. Since the purpose of this study is to compare the accuracy of the chemoinfometrical NIR method with that of conventional X-ray powder diffraction, the mean bias and the mean accuracy were determined by equations 10 and 11, respectively.

$$B_m = \frac{\sum_{i=1}^{n} \frac{(X_c - X_t)}{X_t}}{n} \times 100 \qquad (10)$$

$$A_m = \frac{\sum_{i=1}^{n} \frac{|X_c - X_t|}{X_t}}{n} \times 100 \qquad (11)$$

Bm is percentage mean bias, Am is percentage mean accuracy, *Xc* is predicted value of content of form γ IMC, *Xt* is actual value of content of form γ 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 more narrower than that for using the conventional X-ray method, but the result was consisted with the X-ray method. These results indicate that the NIR method was more accurate than X-ray method. Thus, this assay is found to be superior for quantitative analysis of IMC polymorphs.

Comparative evaluation of conventional powder X-ray diffraction and chemoinfometric NIR methods[13]

The relatioship between the predicted transformation rate (%) of form α IMC to form γ as measured by X-ray diffractometry and those as measured by NIR method is shown in Figure 7. 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 form γ IMC content. Thus NIR spectroscopy is an effective method for the evaluation to the pharmaceutical products of quantitative of polymorph.

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Fig. 1 X-ray powder diffraction patterns of form α and form γ indomethacin.
a; form α, b; form γ.

Fig. 2 DSC curves of form α and form γ indomethacin.
a; form α, b; form γ.

Fig. 3 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.

Fig. 4 FT-NIR spectra of form α and form γ indomethacin.
a; form α b; form γ

Fig. 5 Loading vectors of the PC 1, 2 and 3 based on normalized NIR spectra calculated by PCR.

Fig. 6 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.

Fig. 7 Relation between predicted transformation rate (%) of form α indomethacin to form γ obtained by powder X-ray diffractometry and 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.

Table 1 RMSEP of correlation calculated by PCR based on various transformations.

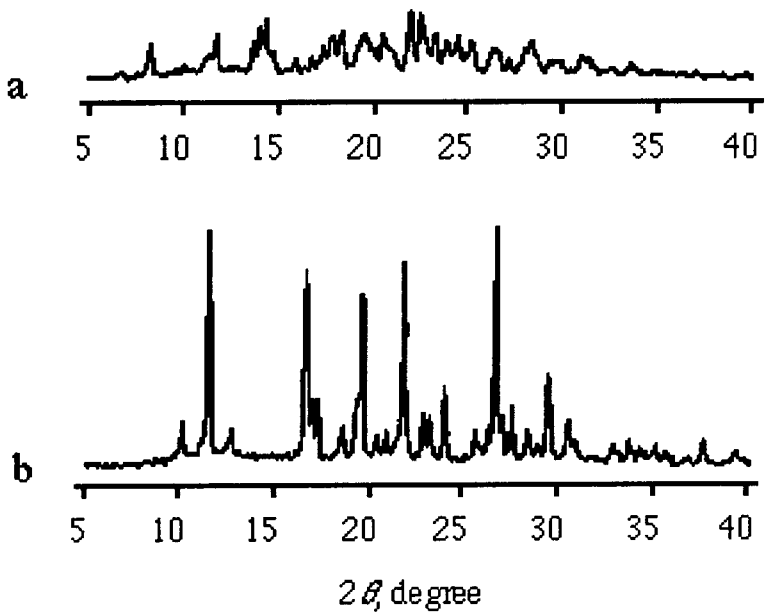

Fig. 1

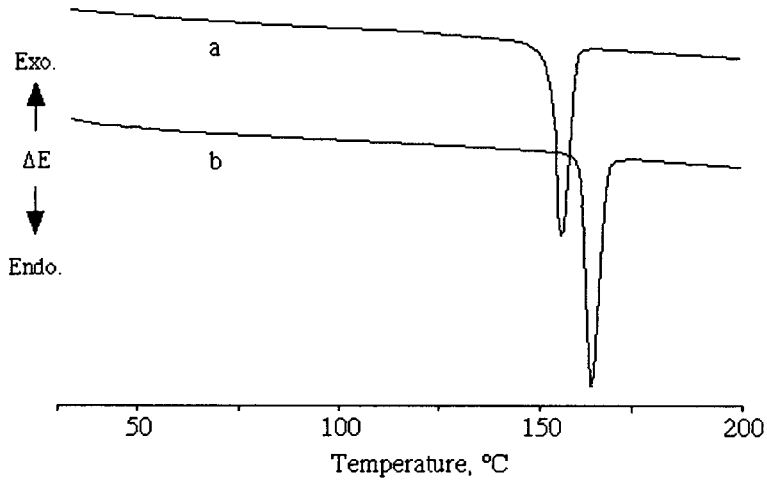

Fig. 2

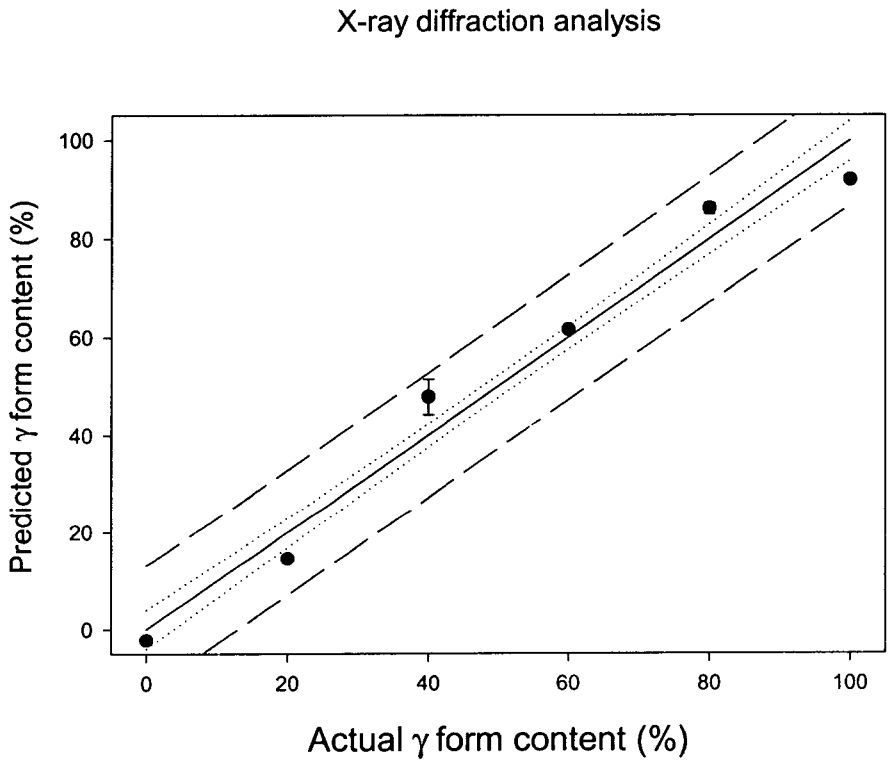
X-ray diffraction analysis
Predicted γ form content (%)
100
80
60
40
20
0
0
20
40
60
80
100
Actual γ form content (%)

Fig. 3

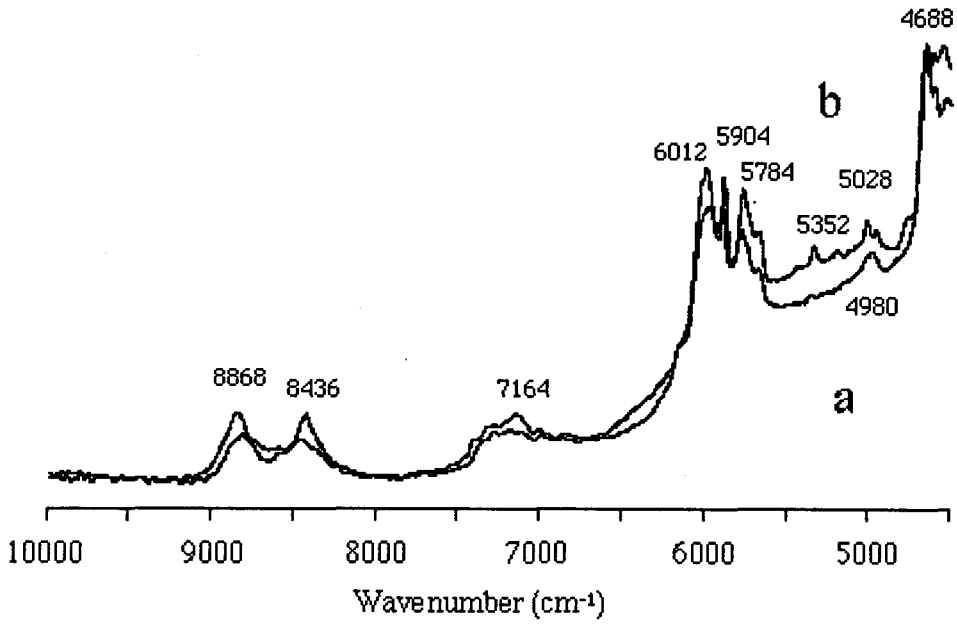
4688
b
6012
5904
5784
5028
5352
4980
8868
8436
7164
a
10000
9000
8000
7000
6000
5000
Wavenumber (cm-1)

Fig. 4

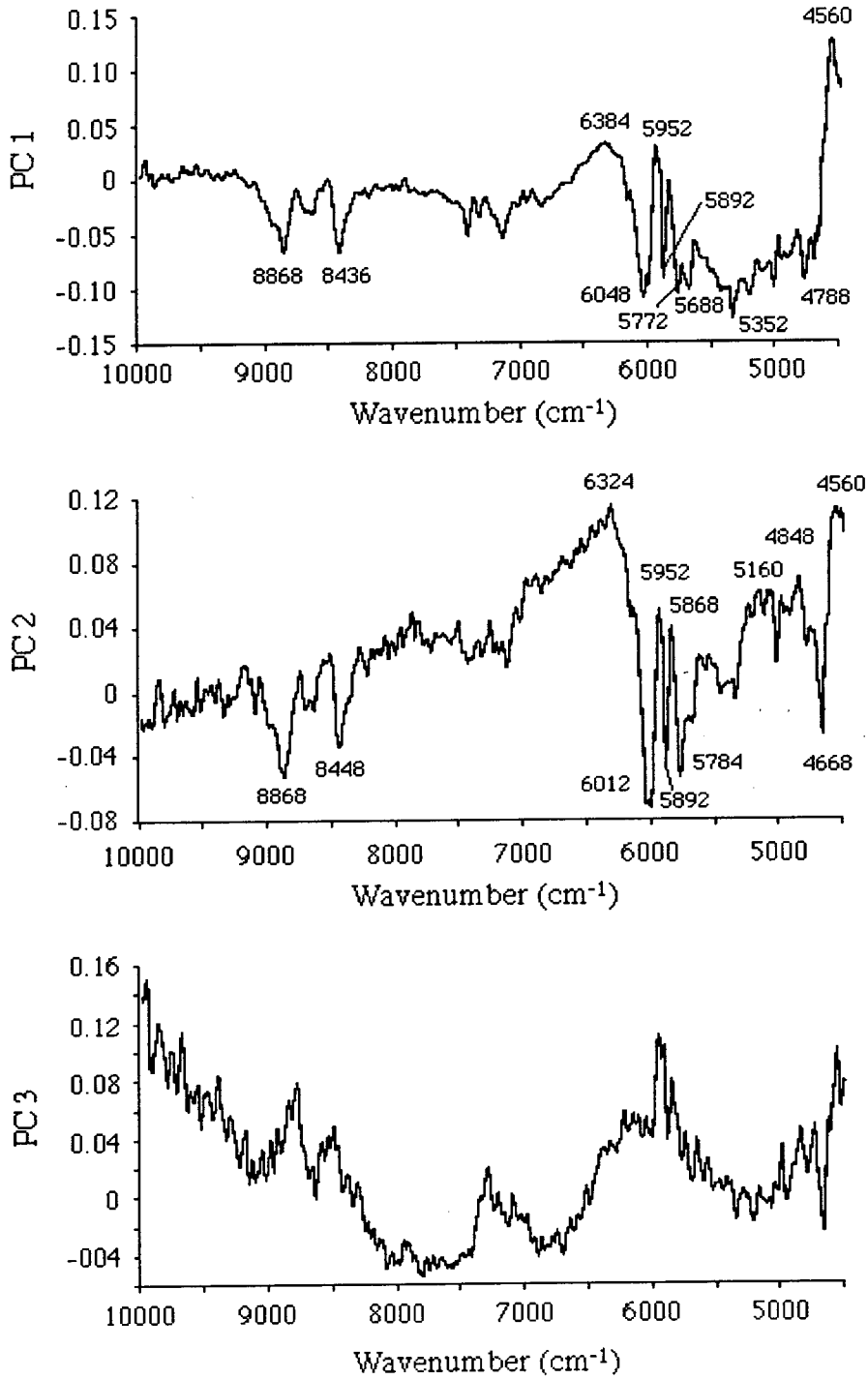
PC 1
0.15
0.10
0.05
0
-0.05
-0.10
-0.15
4560
6384
5952
5892
8868
8436
6048
5772
5688
5352
4788
10000
9000
8000
7000
6000
5000
Wavenumber (cm-1)
PC 2
0.12
0.08
0.04
0
-0.04
-0.08
6324
4560
4848
5952
5160
5868
8868
8448
6012
5892
5784
4668
10000
9000
8000
7000
6000
5000
Wavenumber (cm-1)
PC 3
0.16
0.12
0.08
0.04
0
-004
10000
9000
8000
7000
6000
5000
Wavenumber (cm-1)

Fig. 5

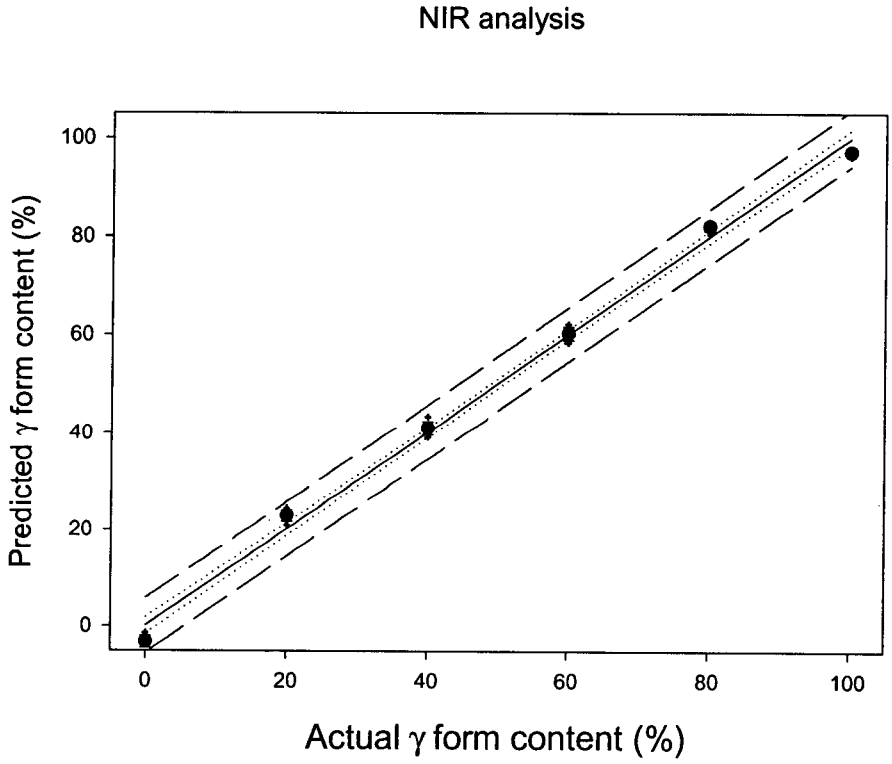
NIR analysis
Predicted γ form content (%)
Actual γ form content (%)
0
20
40
60
80
100

Fig. 6

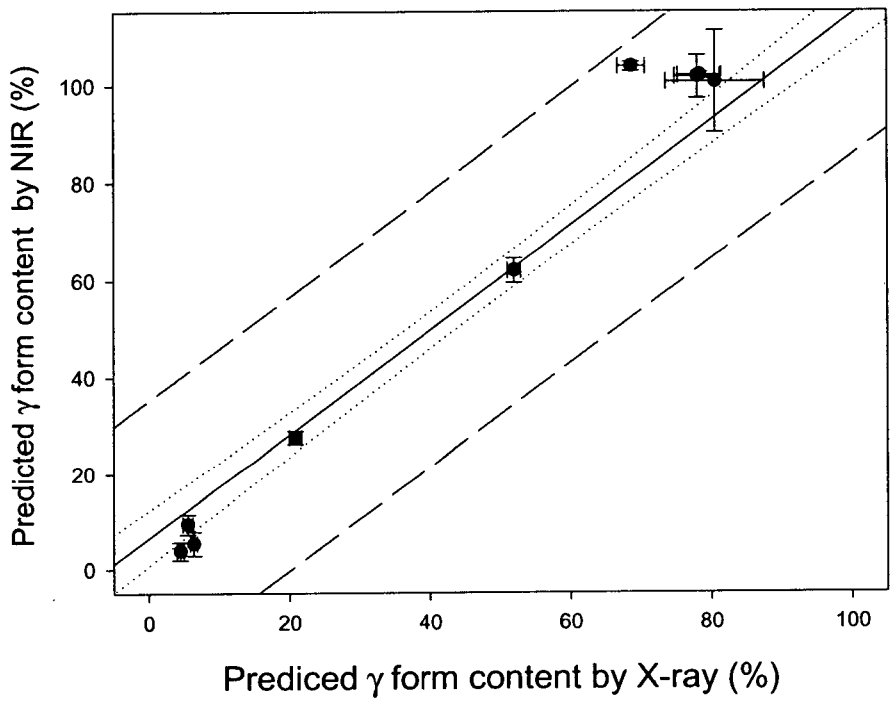
Predicted γ form content by NIR (%)
0
20
40
60
80
100
Prediced γ form content by X-ray (%)
0
20
40
60
80
100

Fig. 7

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

Abs. ; Absorbance
Nor. ; Normarize
2nd deriv. ; second derivative

Table 1