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RESEARCH ARTICLE

Stability test for amorphous materials in humidity controlled 96-well plates by near-infrared spectroscopy

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Abstract

The purpose of this research is to apply near infrared spectrometry (NIR) with chemoinformetrics to predict the change of crystalline properties of indomethacin (IMC) amorphous under various levels of relative humidity storage conditions. Stability test for amorphous and meta-stable polymorphic forms was performed in humidity controlled the modified 96-well quartz plates containing various kinds of saturated salt solutions (0–100% of relative humidity (RH)) by NIR spectroscopy. Amorphous form was obtained melt product to pour into liquid nitrogen and after then ground. Samples were stored at 25°C in the 6-well plates at various levels of RH. The spectra of the powder samples were measured by the reflectance FT-NIR spectrometer. The second derivative spectra of form α showed specific absorption peaks at 4980, 6036, 7296 and 8616 cm^{-1} and that of form γ showed those at 5020, 5028, 7344, 7428 and 8436 cm^{-1}. After storage at less than 50% RH, the peak intensities at 5020, 5028, 7344, 7428 and 8436 cm^{-1} of the amorphous solid increased with increasing of storage time. However, the peak intensity at 4980, 6036 and 7296 cm^{-1} increased at more than 50% RH Please check and confirm the edit. The results suggested that at lower humidity, the IMC amorphous solid transformed into form γ, but it transformed into form α at more than high humidity. It is possible that crystalline stability of the pharmaceutical preparations could be predicted by using humidity controlled 96-well plates and reflectance NIR-chemoinformetric methods.

Keywords: Near-infrared spectroscopy, chemoinfometrics, polymorph, indomethacin, humidity condition, 96-well plate

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. However, in the case for drugs with limited solubility, more than one crystalline form or solvate could exist. Polymorphs exhibit different physicochemical stability, processing characteristic, dissolution rate, etc. Dissolution rate of the undissolved drugs may be affected on drug absorption for oral dosage form in the gastrointestine, resulting in the variation of bioavailability for those pharmaceutical compounds[1–4]. 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[5], differential scanning calorimetry[6], thermal gravimetric analysis, microcalorimetry[7], infrared spectroscopy[8,9], Raman spectroscopy[10] and dissolution kinetics[11]. However, these methods are too time-consuming in the preparation of samples and/or their measurements.

In contrast, NIR spectroscopy is simple to do because the method required nondestructive sample preparation and no contact to measure. NIR 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 ultra-violet, NIR spectroscopy, chromatography, mass spectrometry and NMR[12] spectra of multi-component samples. A number of chemoinfometric and statistical techniques are employed in NIR quantitative and

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qualitative analysis, because these approaches have been proved successful in extracting the desired information from unprocessed NIR spectra. Calibration methods such as multiple linear regressions (MLR), principal component analysis/principal component regression (PCA/PCR) and partial least squares regression (PLS) are commonly used[14–21]. Many invesitigaters[22–25] reported that quantitative analysis of polymorphs in powder mixtures based on their NIR spectra by MLR and PLS. The purpose of this research is to apply NIR with chemoinformetrics, that by nondestructive method to predict the change of crystalline properties of indomethacin (IMC) amorphous during various storage conditions. Stability test for the amorphous and meta-stable polymorphic forms was performed in humidity controlled 96-well plates containing various kinds of saturated salt solutions by NIR spectroscopy. It is possible that crystalline stability of the pharmaceutical preparations could be predicted without contact using humidity controlled 96-well plates and reflectance NIR-chemoinformetric methods.

Experimental

Materials

Bulk powder of IMC was obtained from Yashiro Co., Japan. The form α of IMC was prepared by recrysatallization from ethanol solution[6]. The form γ of IMC was prepared by recrystallization from ethyl ether at room temperature[6].

Amorphous form was obtained melt product to pour into liquid nitrogen. The mass was ground in an agate mortar and pestle.

Storage conditions

Samples were stored at 25°C in the modified 96-well quartz plates containing various kinds of saturated salt solutions at 0, 11, 21, 42, 53, 75, 82 and 100% RH as shown in Figure 1.

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 (i) scan mode-step scan, (ii) target-Cu, (iii) filter-Ni, (iv) voltage-20 kV, (v) current-20 mA, (vi) receiving slit-0.1 mm, (vii) time constant-1 s, and (viii) scan width-0.1 degree/step.

Fourier transform near infrared (FT-NIR) spectroscopy

FT-NIR spectra were taken using an NIR spectrometer (NIRFlex™ N-400, Buchi AG., Flawil, Swaziland). Briefly, a fiber-optic probe was applied to the bottom of the quartz 96-well sample holder containing about 5 mg of the sample powder, and five scans per sample were recorded in the spectral range of 4000–10000 cm^{-1}. A ceramic (Coor's Standard) reference scan was taken for each set of samples. FT-NIR spectra of six sample sets were recorded 5 times at each measurements times at 0, 1, 2, 3, 4, 5, 6 and 7 days with the NIR spectrometer. A total

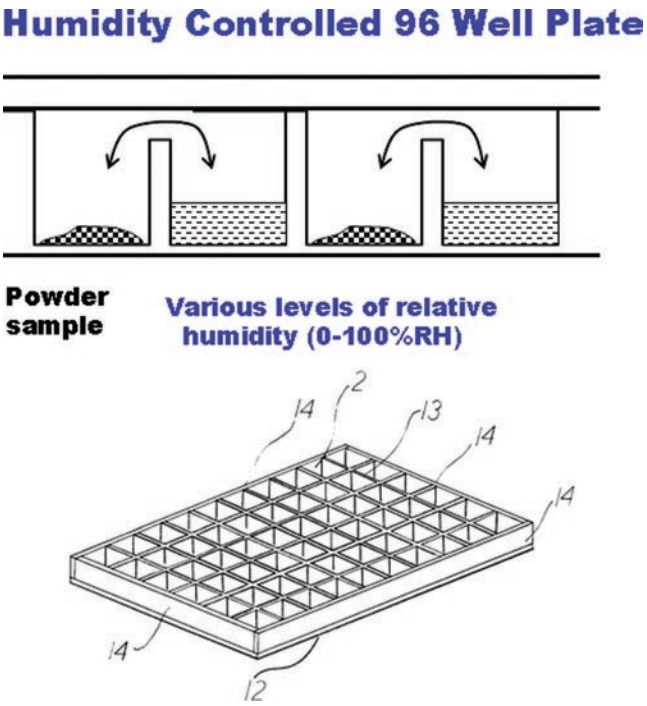

Figure 1. Humidity controlled quartz 96 well.

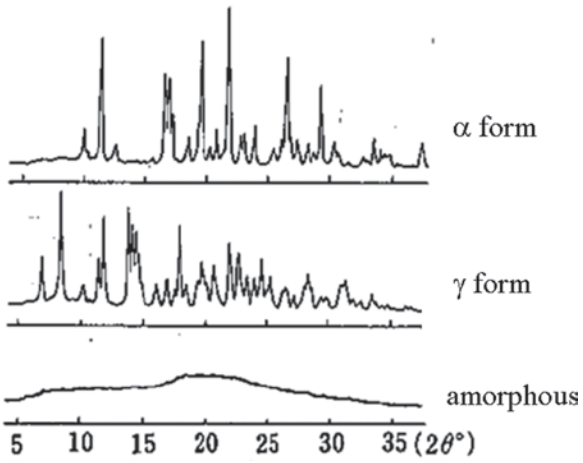

Figure 2. X-ray powder diffraction patterns of form α, form γ and indomethacin amorphous solid.

of 320 spectral data were transformed to remove particle size effect by various functions, such as the multiplicative scatter correction (MSC) and the second derivative, and then, the spectra data sets were used to establish an analytical model by PCA. Chemometric analysis was performed using the PCR program associated with the Pirouette software (InfoMetrix Co., USA). The best conditions were determined to minimize the standard error of cross-validation (SEV).

Results and discussion

Characterization of form α, form γ and amorphous solid IMC

Figure 2 shows the powder X-ray diffraction profiles of the pure form α, form γ and amorphous solid IMC. 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 previously[5]. In contrast, the diffraction profile of amorphous solid had no diffraction peak. The result suggested that the pure form α, form γ and amorphous solid IMC used in the present study were highly purified.

Effect of various humidity conditions on NIR spectra of the amorphous solids of IMC

Figure 3 shows the effect of humidity conditions on polymorphic transformations of amorphous solid were measured by NIR spectroscopy. The forms α and γ, and amorphous solid IMC showed significant NIR spectral peaks. The NIR absorption peaks of IMC were identified[26]. The absorption peak at 4656, 5780, 5850, 7280, 8432, 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 the 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. Form γ had a peak attributable to COOH group at 5380 cm^{-1}, but not for the α form. The result indicated that form γ was the dimmer form, and form α was a monomer as reported in X-ray diffraction results[27].

After storage, the amorphous solid at lower humidity conditions in Figure 3, the base line of NIR spectral peaks were shifted to lower reflectance with elapse of the time, and the peak at 5250 cm^{-1} was disappeared. In contrast, at high humidity conditions, the base lines were shifted and the whole spectral peaks were transformed into broad spectra, and the peak intensity at 5250 cm^{-1} increased. The spectral pattern of the sample at lower humidity was shifted into that of form γ, and that at higher was into that of form α respectively. Since the peak at 5250 cm^{-1} is attributable to adsorbed water, and the base line shift may be also attributable to change of particle size of the samples, the spectra change suggested that the new fine crystals were recrystallized out from the solid surface, the base lines of the sample spectra were shifted. It is well known that amorphous solid of IMC[28] were transformed into form γ at lower humidity and into form α at high humidity. In the previous study, the powder X-ray diffraction profiles of amorphous solids after storage at below and above 50% RH for 7 days showed forms γ and α, respectively.[28] The current NIR result was consistent with the reference data.

Chemoinformetric analysis for polymorphic transformation processes of amorphous solid of IMC under various RH conditions

In order to understand polymorphic transformation of IMC amorphous solid at various levels of relative humidity, PCA was applied in this study, since PCA is useful to understand for relationship between the objective parameters and principle component in the spectra.

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 a score matrix **T** times a loading matrix **P** plus a residual matrix E (equation 1)[19]

$$X = t1p'1 + t2p'2 + + E = TP' + E \quad (1)$$

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**.

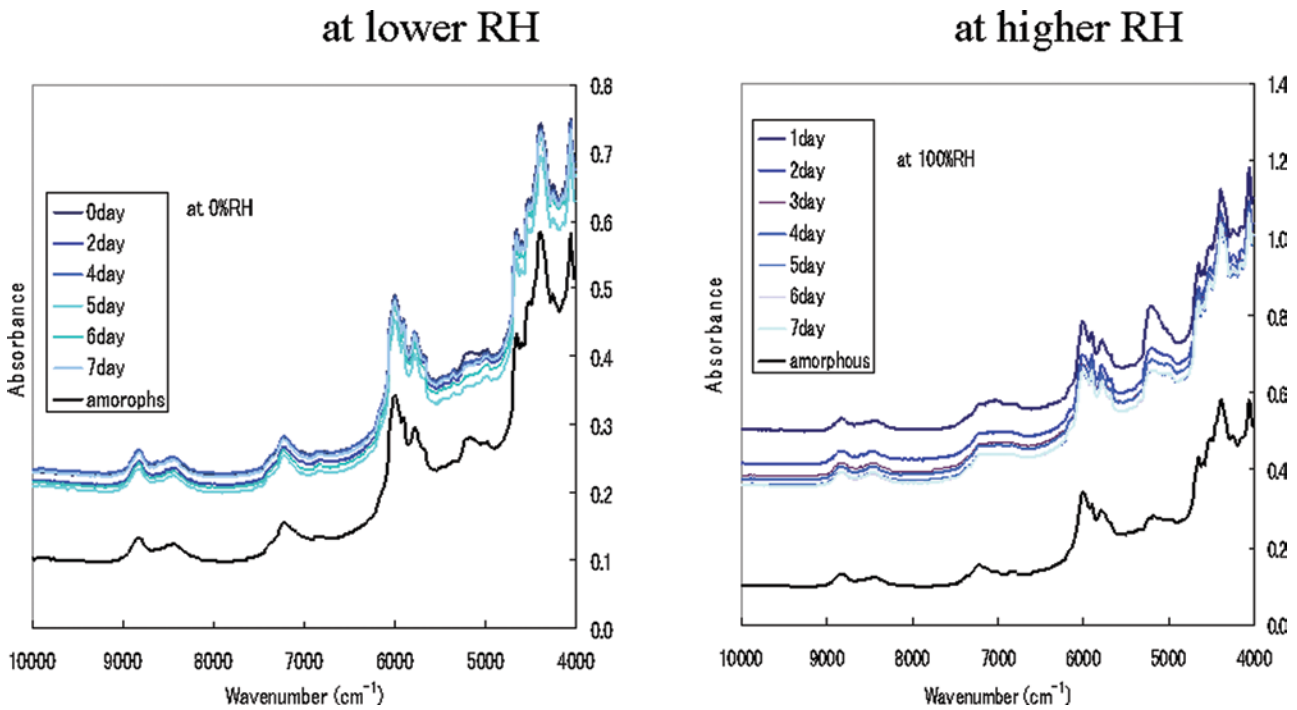

Figure 3. Change of NIR spectra of amorphous solid at lower and higher humidity conditions.

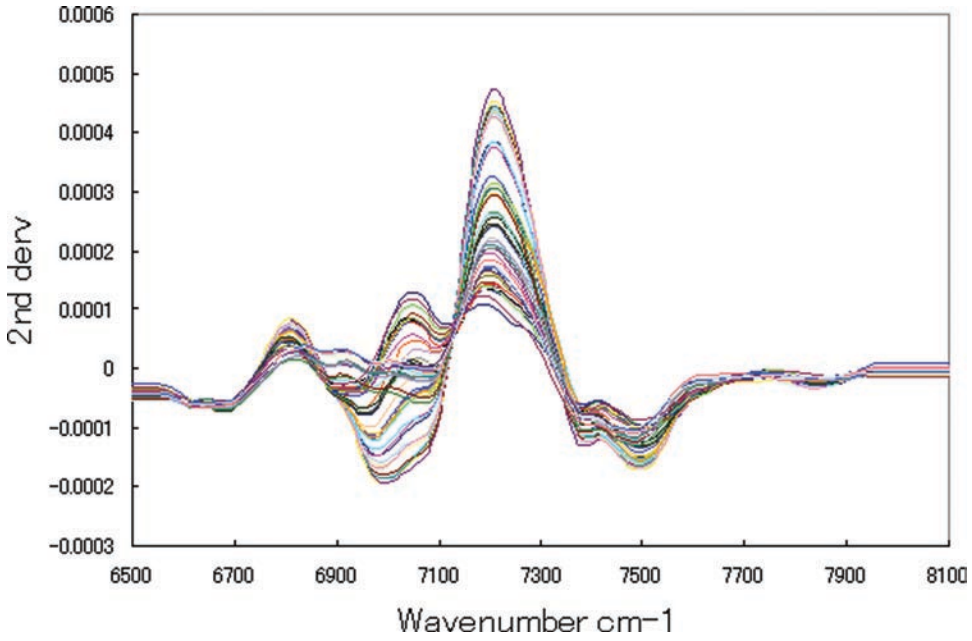

Figure 4. Second derivative NIR spectra of indomethacin amorphous solid at lower and higher humidity conditions.

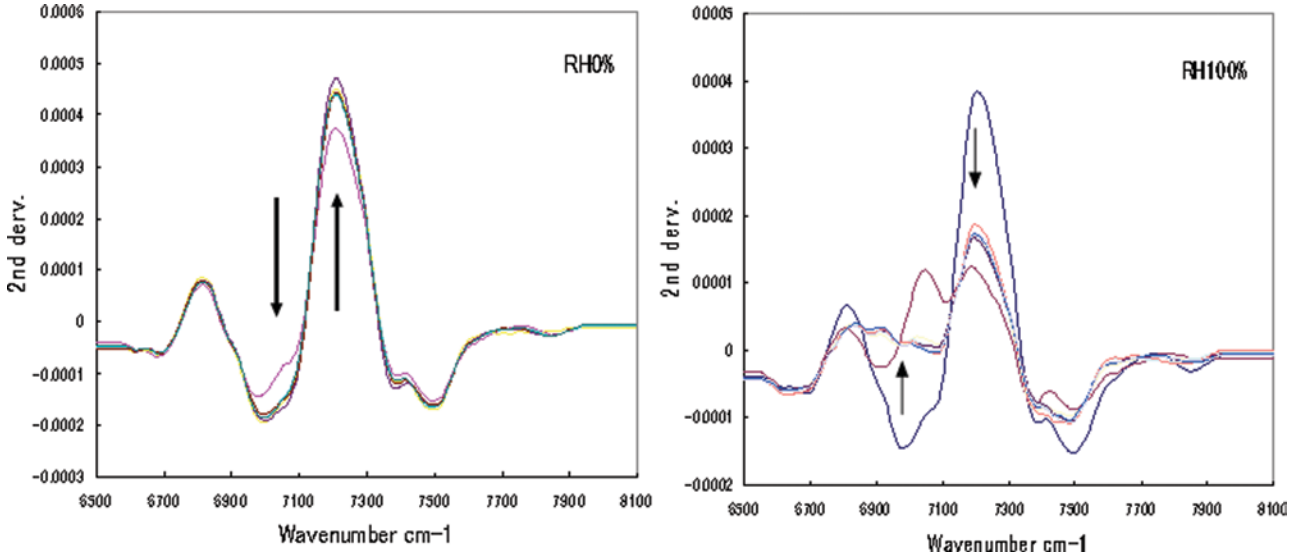

Figure 5. Second derivative NIR spectra of indomethacin amorphous solid at 0% RH and 100% RH.

In this study, the NIR spectra between 6400 and 8100 cm^{-1} were attributable to C-H band in methyl group.

Figures 4 shows MSC and 2nd derivative NIR spectra of the sample powder after storage for 1–7 days at various RHs. As shown in Figure 3 after storage in various humidity conditions, the base-line of the NIR spectra was shifted by change of sample physical property, since the sample solids transformed into fine crystalline particles. The MSC and 2nd derivative functional treatments were effectively removed the particle effect on the NIR spectra. The peaks at 7200 and 6970 cm^{-1} due to C-H in methyl group are significantly changed depended on polymorphic transformation under various humidity conditions.

Figure 5 shows the NIR spectral change at lower and higher storage conditions. The peak intensity at 6970 cm^{-1} of the sample at 0%RH decreased, but that at 7200 cm^{-1} increased. In contrast, the peak intensity at 6970 cm^{-1} at 100%RH increased, but that at 7200 cm^{-1} decreased. The result suggested that molecular interaction due to methyl group had tight relationship with crystalline forms. The X-ray diffraction data also suggested that the amorphous solids were transformed into form γ and α at lower and higher RH conditions, respectively, as reported previously[28].

The NIR spectra of the amorphous solid in Figure 4 were analyzed by PCA. Figure 6 shows three dimensional plots of scores of PC 1, 2 and 3 based on NIR spectra calculated by PCA. This result suggested that the data group at lower humidity could be clearly separated from those at higher humidity in the three dimensional space by PCA. Norris et al.[20] reported that dynamic polymorphic form conversion process evaluated by using PC1 and PC2 score plot.

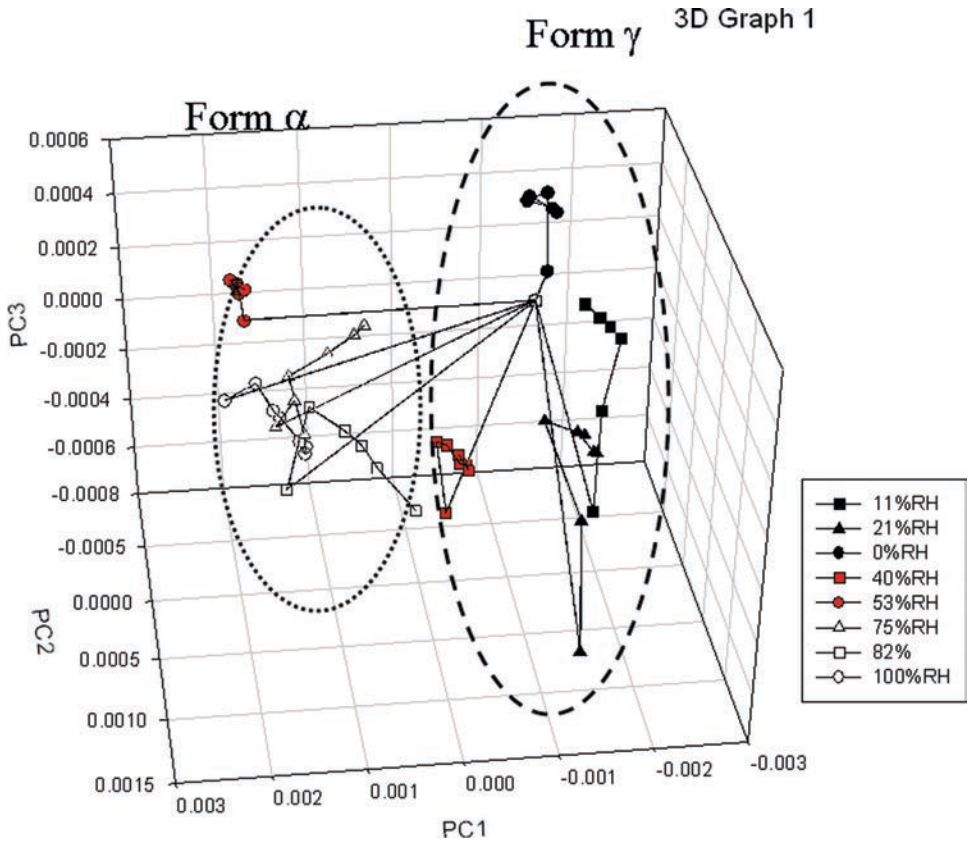

Figure 6. The score plots of PC 1 and 2 based on NIR spectra calculated by PCA.

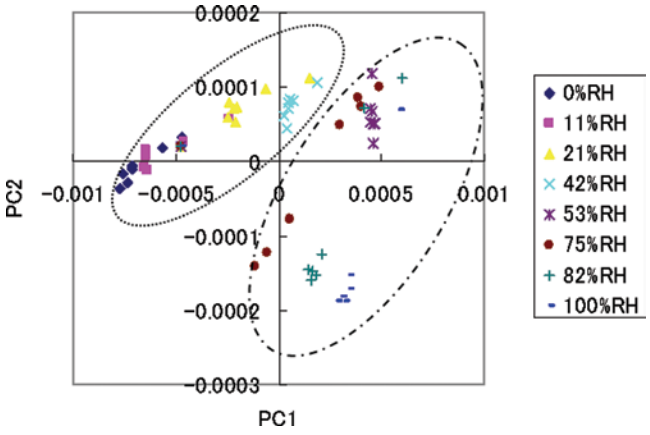

Figure 7. Three dimensional plots of scores of PC 1, 2 and 3 based on NIR spectra calculated by PCA.

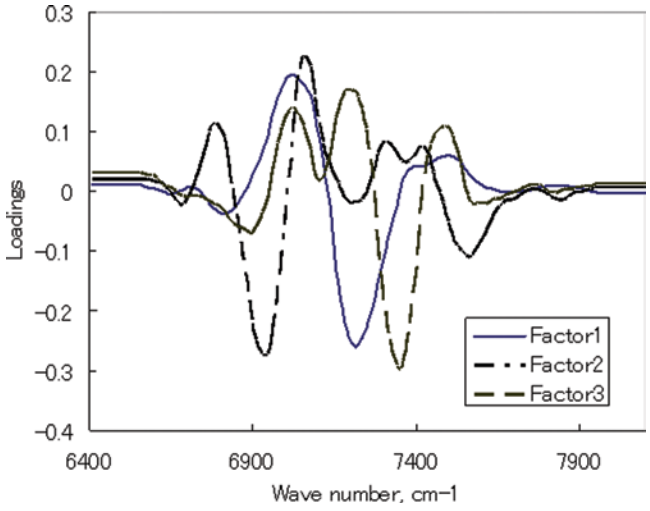

Figure 8. Loading vectors of the PC 1, 2 and 3 based on NIR spectra calculated by PCA.

They theoretically explained the process and could obtain their accurate end-point by the method. Even in Figure 7, there are two spectral data groups; one is that at lower RH and the other is that at higher RH; so, it is possible to separate these two groups. This result indicated that the NIR method (no contact and nondestructive method) could be separated in to two groups, forms γ and α of the amorphous IMC after storage under various RH conditions.

Figure 8 shows loading vectors corresponded to the principal component (PC), respectively. The peak at 7020 cm^{-1} was the highest value, and the peaks at 7212 cm^{-1} were the lowest on PC1. The peak at 7056 cm^{-1} was the highest value, and the peaks at 6936 cm^{-1} were the lowest on PC2. The variance of PC1, PC2 and PC3 were 92.6, 4.95 and 1.16, respectively. The peak change at 6936 cm^{-1} might be suggested that PC2 loadings related with transformation of form α at higher RH, but PC1 loading related with that of from γ at lower RH.

Figure 9 shows modeling power of PCA. The modeling powder suggested the spectral band between 6900–7500 cm^{-1} had useful information to identify crystalline forms of IMC, such as forms α and γ, and the amorphous solid, in this analysis.

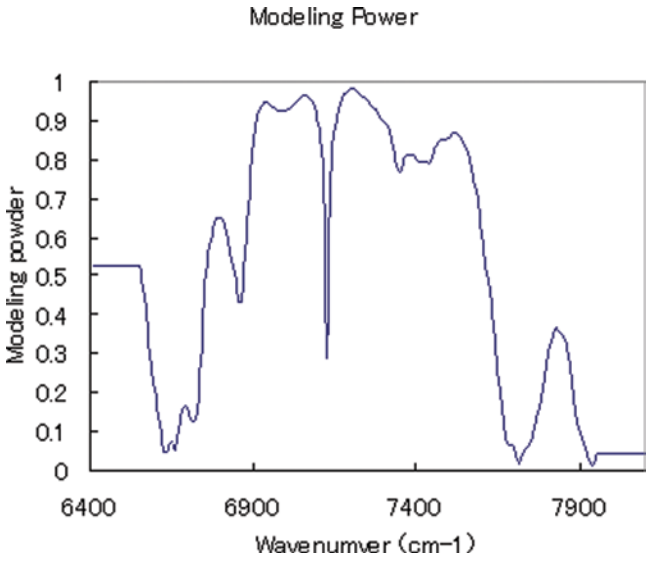

Figure 9. Modeling powder for hydration analysis of indomethacin amorphous solid by PCA.

Conclusions

It is possible that crystalline stability of the pharmaceutical preparations could be predicted by using humidity controlled 96-well plates and reflectance NIR-chemoinformetric methods. It is also possible to evaluate drug stability against humidity by using small amount of bulk powder, because this humidity controlled system is useful to candidate drugs that have limited the bulk drug resource.

Qualitative evaluation of IMC polymorphs by NIR spectroscopy with PCA method was proven to be significantly advantageous than conventional powder X-ray diffractometry. This method is expected to provide a rapid quality analysis of polymorphs at preparations, as characterized by the simple, nondestructive and high sensitivity nature of the method.

Declaration of interest

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