Drug Development and Industrial Pharmacy, 2010; 36(1): 72–80

informa
healthcare

ORIGINAL ARTICLE

Determination of the crystallinity of cephalexin in pharmaceutical formulations by chemometrical near-infrared spectroscopy

Yuya Fukui[1] and Makoto Otsuka[2]

[1]*Kobe Pharmaceutical University, Motoyamakitamachi, Higashinada, Kobe, Japan and* [2]*Research Institute of Pharmaceutical Sciences, Musashino University, Shinmachi, Nishi-Tokyo, Japan*

Abstract

Background: Near-infrared (NIR) spectroscopy has gained wide acceptance in the pharmaceutical industry as a rapid and non destructive method for drug identification and the determination of the drug content of preparations. *Aim*: The crystallinity of cephalexin (CEX) in microcrystalline cellulose (MCC) was determined using a nondestructive NIR reflectance spectroscopic technique. The molecular interaction of a ground amorphous solid of CEX was investigated by the method. *Method*: Six kinds of standard material with various degrees of crystallinity were prepared by the physical mixing of crystalline, amorphous CEX, and MCC. X-ray powder diffraction profiles and NIR spectra were recorded for standard samples. A chemometric analysis of the NIR spectral data sets was conducted using principal component regression (PCR). *Results*: The correlation between the actual crystallinity of CEX and that predicted using the conventional X-ray diffraction method showed a straight line with a slope of 1.000, an intercept of -2.071×10^{-5} and a correlation coefficient of determination (R^2) of 0.974. The NIR spectrum of amorphous CEX showed significantly different peaks at 1176 and 1206 nm because of the CH_3 group from those of CEX. PCR was performed on various kinds of pretransformed NIR spectral data sets of standard samples of CEX. To minimize the SE of cross-validation (SECV), the spectral data sets were subjected to the leave-one-out method. The second derivative treatment in the range of 1176–1206 nm yielded the lowest SECV values. Based on a two-component model, a plot of the calibration data between the actual crystallinity of CEX and that predicted by the NIR method was obtained. The plot showed a straight line ($Y = 0.995X + 0.117$ and $R^2 = 0.994$; $n = 18$). The mean bias for the NIR and X-ray powder diffraction methods was calculated to be 1.52% and 2.26%, and mean accuracy was 3.06% and 7.14%, respectively. *Conclusion*: NIR spectral changes of crystalline CEX during grinding suggested that the intermolecular hydrogen bonds between the amino and carboxyl groups are destroyed and the binding of methyl groups is heightened by the resonance effect of carboxyl groups, and the crystals are transformed into amorphous CEX.

Key words: *Amorphous solid; cephalexin; chemometrics; crystallinity; microcrystalline cellulose; near-infrared reflectance spectroscopy; principal component regression*

Introduction

Polymorphic and amorphous changes are widely observed in the pharmaceutical industry and continue to be important to drug development because of their impact on the physicochemical properties of drugs. Particularly, changes in both the surface area and the crystallinity of bulk powders during mechanical processes such as grinding[1], mixing with an excipient[2], granulation[3], and compression[4] could affect the bioavailability of a drug through the rate of dissolution. In some instance, an amorphous solid-state powder can be used to improve the absorption of a drug in the gastrointestinal tract. The level of disorder in a crystalline solid may affect the hygroscopicity of the drug, resulting in a change in the powder flow, compaction properties, and chemical stability of preparations. X-ray diffraction (XRD)[5–7], differential scanning calorimetry (DSC)[8], Raman

Address for correspondence: Makoto Otsuka, PhD, Research Institute of Pharmaceutical Sciences, Musashino University, Shinmachi 1-1-20, Nishi-Tokyo 202-8585, Japan. Tel: +81 0424 68 8670, Fax: +81 0424 68 8670. E-mail: motsuka@musashino-u.ac.jp

(Received 26 Feb 2009; accepted 2 Jun 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040903092327

spectroscopy[9], and micro-calorimetry[10] are currently the methods most widely used to evaluate crystallinity.

Since the introduction of process analytical technology (PAT) guidelines by the Food and Drug Administration, online, real-time analyses as a tool to monitor and control manufacturing processes have become increasingly accepted in the pharmaceutical industry[11]. Online analyses hold the promise of reducing or eliminating reworked batches, increasing manufacturing efficiency, decreasing the burden of finished product testing, and ensuring product quality throughout the manufacturing process. Substantial time is saved and sampling difficulties are minimized by following reactions and processes in situ rather than collecting samples and analyzing them in the laboratory using conventional approaches.

Near-infrared (NIR) spectroscopy has gained wide acceptance in the pharmaceutical industry as a rapid and nondestructive method for drug identification and the determination of the drug content of preparations[12]. Sample preparation is much simpler for NIR than for alternative methods. NIR spectroscopy is, therefore, the most effective current method for the monitoring of quality parameters during a pharmaceutical manufacturing process. This is especially true because of the recent interest in PAT[11]. Furthermore, it has been widely used to determine several pharmaceutical properties, such as mean or median particle size, degree of crystallinity, tablet hardness, and moisture content, in the pharmaceutical industry. The number of research publications related to this technique is growing as well. There are many examples of applications of NIR spectroscopy to the pharmaceutical process.

Rantanen et al.[13] measured the moisture content during granulation by diffuse reflectance NIR. Buckton et al.[14] reported the crystalline state of amorphous and crystalline lactose. Buchanan et al.[15] and Blanco et al.[16] examined the drug content of coated tablets using the NIR method. Frake et al.[17] determined the particle size of lactose and Morisseau and Rhodes[18] evaluated tablet hardness using chemometrical methods[19]. There are several reports for PAT concerning the quantitative analysis of polymorphic and amorphous contents of bulk powders using the chemometrical NIR method[20–23]. However, there are a few reports explaining molecular interactions of the solids. This research group also reported the degree of crystallinity in a bulk powder of cephalexin (CEX), an antibiotic, based on molecular interaction examined by chemometrical NIR spectroscopy[24]. Because the carboxyl group of CEX binds with the amino group through crystalline water, intermolecular hydrogen bonds are formed between the carboxyl or amino groups and the crystalline water. After the hydrogen bonds are destroyed by mechanical energy during grinding, amorphous CEX increases with the increase in free amino groups. Thus, it is very likely that the band at 1620 nm in the NIR spectrum, which is because of the hydrogen-bonded amino group, decreases whereas that at 1530 nm, which is assigned to the free amino groups, increases. In the study, they found that CEX amorphization during grinding is consistent with molecular interaction between the carboxyl groups, the amino groups, and the crystalline water. However, for a drug to be used as a pharmaceutical preparation, its characterization in multipowder formulations is necessary. It is not easy to measure the characteristics of a bulk powder in mixed formulation by nondestructive means, as key bands overlap with bands because of inactive ingredients.

The purpose of this study is establishing a calibration model to evaluate the crystallinity of bulk powders in a mixed system by the chemometric NIR method. A mixture of 50% CEX monohydrate in microcrystalline cellulose (MCC) was used as a model, and an NIR method was developed based on a stoichiometrical chemical model.

Experimental

Materials

A bulk powder of CEX, Japanese Pharmacopoeia XIV, monohydrate form[25], was obtained from Nippon Bulk Pharmaceutical Co. Ltd. (lot no. LAID705P; Osaka, Japan). The crystallinity of this material was assumed to be 100%. MCC, Avicel PH-101, was obtained from Asahikasei Co. Ltd. (lot no. 1263; Tokyo, Japan). All other chemicals used were of analytical grade.

Preparation and evaluation of standard samples

Amorphous CEX[26] was prepared by grinding a bulk powder (10 g) in an agate centrifugal ball mill (Fritsch Co. Ltd., Albisheim, Germany; 300-mL capacity, number and diameter of balls were as follows: 10, 10 mm in diameter; 5, 15 mm in diameter; 2, 20 mm in diameter) at 360 rpm for 15 hours at room temperature. As the X-ray powder diffraction profile of ground CEX had a typical halo pattern, the crystallinity of the ground material was assumed to be 0%[26]. Standard samples with varying degrees of crystallinity were prepared by physically mixing monohydrate crystalline, amorphous (noncrystalline, NC) CEX, and MCC, see Table 1 for

Table 1. Formulation of the CEX–MCC standard.

	Crystallinity (%)					
Formulation (mg)	0	20	40	60	80	100
CEX	0	100	200	300	400	500
NC-CEX	500	400	300	200	100	0
MCC	500	500	500	500	500	500

formulations, in an agate mortar with a pestle without changes in crystal content.

X-ray powder diffraction analysis

The X-ray powder diffraction profiles were measured with a powder X-ray diffractometer (RINT-ULTIMA, Rigaku Co., Akishima, Japan). The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 20 kV; current, 20 mA; receiving slit, 0.1 mm; time constant, 1 second; scanning speed, 2° 2θ min^{-1}. About 50 mg of each sample powder was accurately weighed and carefully loaded in a glass holder without particle orientation using a spatula and a glass plate. About 25 mg of MCC was measured using the same method. The CEX peak was separated by subtracting the MCC peak from the sample peak using the multiple peaks and subtract program contained in JADE Ver. 5.0 software (Rigaku Co.). The calibration curves for the quantification of crystal content were based on the relative intensity of diffraction peaks, 2θ = 7.2°, 16.5°, 17.4°, and 22.2° for CEX monohydrate. All data were averages of three runs.

Near-infrared spectroscopic analysis

NIR spectra were obtained using a diffuse reflectance NIR spectrometer (model NH1100; Sectron Tech Co., Seoul, South Korea) as follows: a fiber-optic probe was inserted into the sample powder (1 g) in a 20-mL glass bottle. This probe carries the light energy to the sample, which is centered on the quartz glass window above the tip of the fibers. On reaching the sample, the light penetrates the material of interest and the nonabsorbed energy is collected by an InGaAs (indium, gallium, and arsenide) detector. Thirty scans per sample were recorded in the spectral range of 1100–1750 nm at 2-nm intervals. Spectra of the six calibration sample sets were recorded three times with the NIR spectrometer. A total of 18 data sets were transformed to remove the effect of particle size by various functions, such as normalization, second derivative, and multiplicative scattering correlation (MSC) and then were used to establish a calibration model to predict crystallinity by principal component regression (PCR) analysis. The best conditions were determined to minimize the SE of cross-validation (SECV) by the leave-one-out method. Chemoinfometric analysis was performed using the PCR program contained in Pirouette Ver. 2.6 software (Infometrix Co., Woodenville, WA, USA).

Results and discussion

Measurement of the degree of CEX crystallinity by conventional X-ray powder diffractometry

Figure 1a and b shows the XRD profiles of pure crystalline CEX and MCC, respectively. The main XRD peaks of CEX were at 2θ = 7.2°, 16.5°, 17.4°, and 22.2° for CEX monohydrate, as reported by Otsuka et al. and Kaneniwa et al.[25–30]. Figure 2a shows XRD profiles of the CEX–MCC mixture. XRD profiles of CEX only (Figure 2b) were obtained to subtract the MCC peak in profiles of the CEX–MCC mixture, because of large noise in the range of 10–35° in Figure 2b. The calibration curve for measuring the crystallinity of CEX by the conventional XRD method was based on the intensity of one specific diffraction peak, 2θ = 7.2°. There are two main causes of fluctuation in the determination of crystallinity using the XRD method. One is a fluctuation in the intensity of the X-ray's direct beam during measurement. The other is the orientation of crystals when the sample is loaded

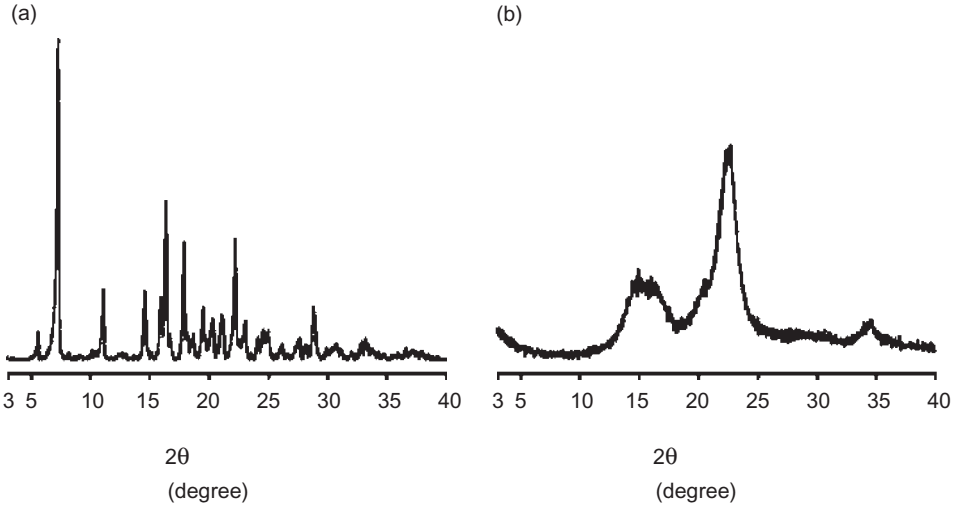

Figure 1. X-ray diffraction profiles of (a) pure crystalline CEX and (b) microcrystalline cellulose.

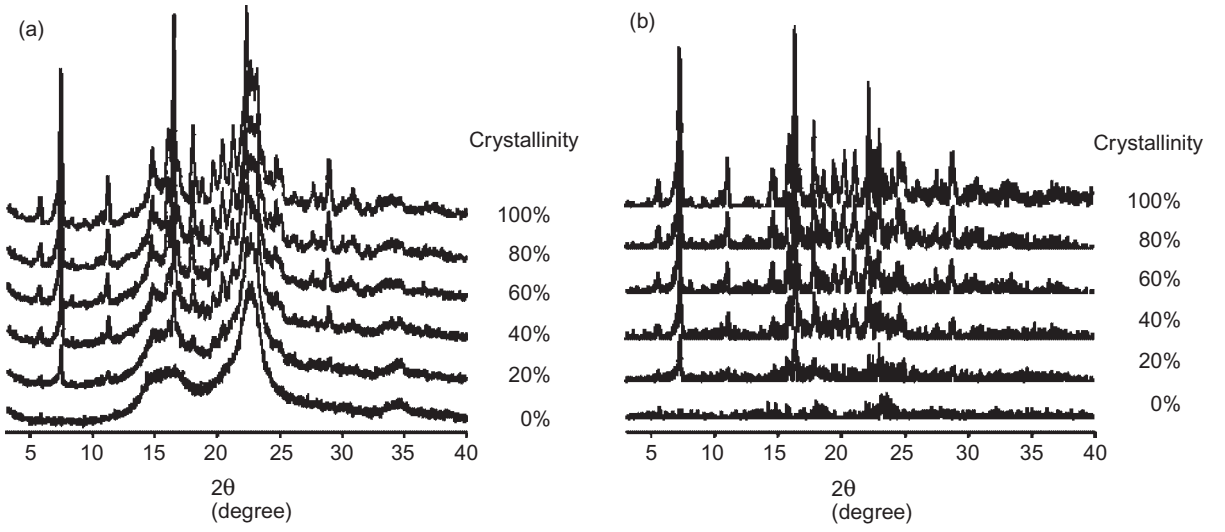

Figure 2. X-ray diffraction profiles of the CEX–MCC mixture before (a) and after (b) treatment.

into the holder. To avoid fluctuations of beam intensity, the peak at 2θ = 28.8° for silicon powder was measured as an external standard for the correction of crystalline content. On the whole, as the number of diffraction peaks increases, the effect of crystal orientation decreases. However, when noise in XRD profiles grows larger, the accuracy of the calibration curve becomes lower. Therefore, crystalline content is evaluated using the profile fitted area under the peak, 2θ = 7.2°. A plot of the relationship between peak area intensity (y, cps) and the measured fraction of crystallinity (x) was fitted as a straight line ($Y = 21.46X - 65.56$; $R^2 = 0.974$). The degree of crystallinity of the sample powder was calculated using this calibration curve.

Figure 3 shows the plot of the relation between the actual and the predicted crystallinity of CEX measured using the conventional XRD method. This plot shows a straight line with a slope of 1.000, an intercept of -2.071×10^{-5}, and an R^2 of 0.974 and had sufficient 95% confidence levels for the prediction of individual y-values and 95% confidence intervals of regression.

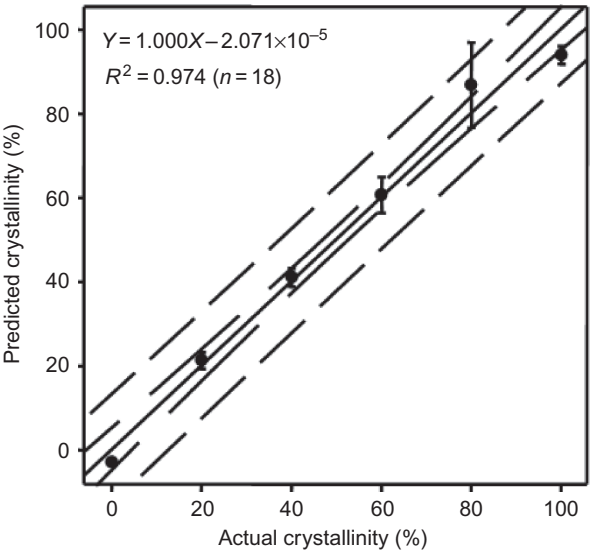

Figure 3. Relationship between actual crystallinity and that predicted using X-ray diffraction. Each bar represents the mean ± SD ($n = 3$).

Measurement of the degree of crystallinity by chemometric NIR spectroscopy

Figure 4 shows the NIR spectra of CEX–MCC standard samples. The spectra were treated by MSC to correct spectral baseline offset because of particle size. A band at 1480 nm is associated with the first overtone of ν_{OH} of the MCC-derived OH group and a band at 1684 nm is associated with the first overtone of ν_{CH} of the CEX-derived aromatic C−H group. These spectra were transformed for normalization at 1684 nm and second derivative (Figure 5a) because NIR bands of the CEX–MCC mixture did not identify a specific spectral change.

The NIR spectrum of amorphous CEX differed from that of crystalline CEX from 1176 to 1206 nm. A band at 1195 nm is associated with the second overtone of ν_{CH} of the CH_3 group derived from CEX. The band at 1185 nm (Figure 5b) increased with the decrease in the degree of crystallinity of CEX, but the band at 1195 nm decreased, indicating that the shifts at 1185 and 1195 nm are because of changes in the degree of crystallinity. Otsuka et al.[26–27] investigated the amorphization of CEX by means of mid-infrared spectroscopy and powder X-ray diffractometry. The mechanism was explained as follows: as the carboxyl group binds with the amino group

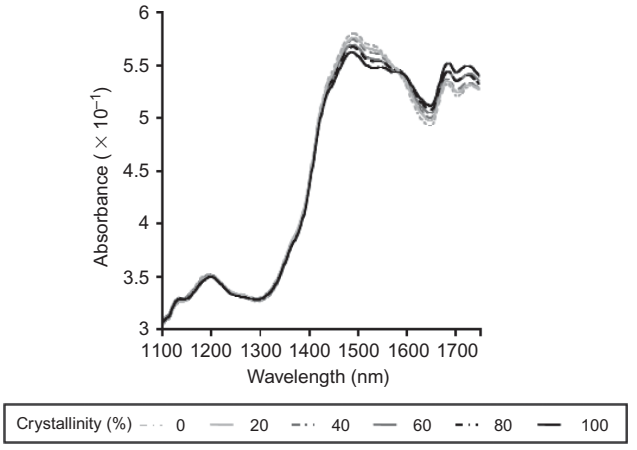

Figure 4. NIR spectra of the CEX–MCC mixture at various levels of crystallinity.

through crystalline water, intermolecular hydrogen bonds are formed between the carboxyl or amino groups and the crystalline water as shown in Figure 6. As the hydrogen bonds are destroyed by mechanochemical energy during grinding, amorphous CEX heightens the strength of CH bonds by a resonance effect (Figure 7). The relationship between frequency and spring constant is shown in Equation (1). When the strength of CH bonds increased, frequency shifted toward high (wavelength shift toward long).

$$\upsilon = \frac{1}{2\pi}\sqrt{\frac{\kappa}{\mu}} \tag{1}$$

where υ is the number of vibrations, κ is the spring constant, and μ is reduced mass.

As the observed NIR spectral shifts were consistent with the degree of crystallinity, a chemometric method could be used to evaluate the degree of crystallinity based on the spectral change because of the CEX resonance effect.

PCR is a multivariate data analysis tool, which aims to regress dependent Y values against independent X variables. PCR is presented as the regression of Y on selected principal components of X. Properties of PCR are given below together with a discussion on the selection of eigenvectors. As PCR is useful for understanding the relationship between the objective parameters and the principal components in the spectra, it was applied to the present NIR data set. A spectrum, including n spectral data, can be seen as a point in an n-dimensional space. In the multivariate data analysis, PCR decomposes X into a score matrix, T, times a loading matrix, P, plus a residual matrix, E (Equation (2)).

$$X = t_1 p_1 + t_2 p_2 + \cdots + E = TP' + E \tag{2}$$

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.

Spectral pretreatment was performed for the NIR spectra to reduce the effect of physical properties and minimize experimental sample packing error by MSC, normalize, and second derivative. The best conditions were determined to minimize the SECV by the leave-one-out method (Table 2) and to reduce the decrease in variance (Table 3). The NIR spectral data set for the standard CEX samples with various degrees of crystallinity was subjected to a chemometrical analysis by PCR. Table 2 shows SECV profiles calculated based on the spectral

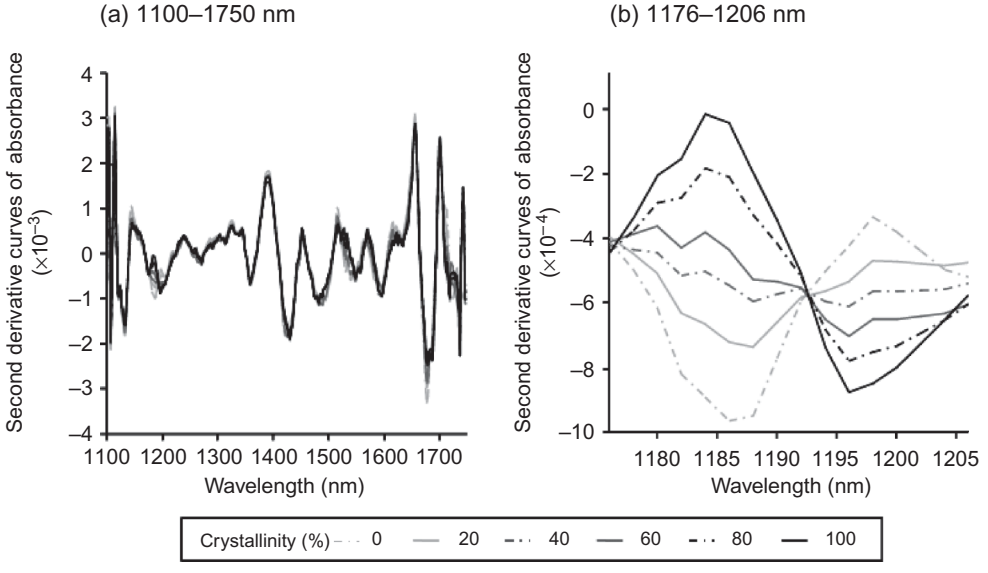

Figure 5. Second derivative of NIR spectra of CEX–MCC standard samples (a) 1100–1750 nm and (b) 1176–1206 nm.

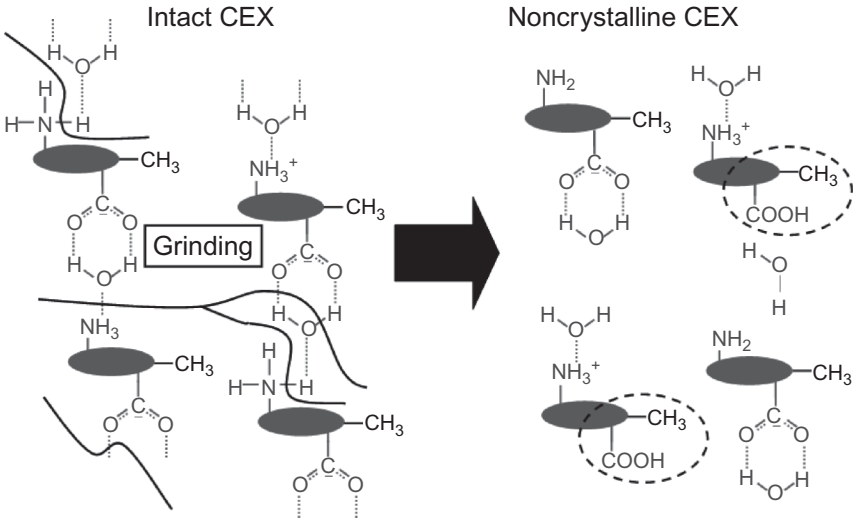

Figure 6. Possible mechanism for grinding effect on chemical structure of CEX into MCC.

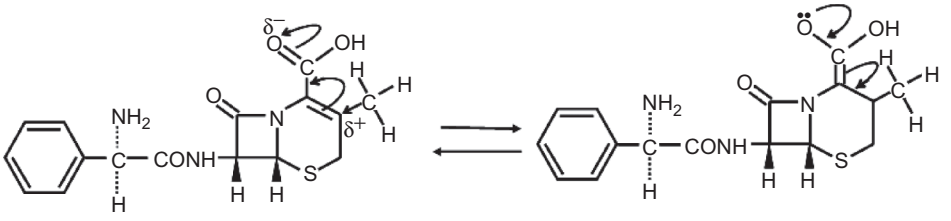

Figure 7. Resonance effect of CEX by mechanical grinding.

Table 2. Effect of number of PC on variance and SE of cross-validation for evaluation of the crystallinity of CEX by the NIR chemometrical method.

	SECV				
Transformation	PC1	PC2	PC3	PC4	PC5
No pretreatment	28.51	13.23	6.87	1.31	0.55
MSC	5.61	3.90	2.17	1.59	1.13
Normalize	38.15	3.79	3.27	0.38	0.33
Normalize & MSC	5.60	3.90	2.18	1.59	1.14
Second derivative (1100–1750 nm)	12.02	6.16	6.80	7.38	5.55
Second derivative & MSC	6.83	5.34	4.03	4.33	2.12
Second derivative (1176–1206 nm)	3.51	2.68	2.82	2.73	1.31

Table 3. Effect of number of PC on variance for evaluation of trypsin activity by the NIR chemometrical method.

	Variance (%)				
Transformation	PC1	PC2	PC3	PC4	PC5
No pretreatment	99.43	0.52	0.05	—	—
MSC	95.14	4.32	0.39	0.08	0.05
Normalize	81.72	17.98	0.19	0.09	0.02
Normalize & MSC	95.14	4.32	0.39	0.08	0.05
Second derivative (1100–1750 nm)	78.17	15.42	3.18	0.98	0.84
Second derivative & MSC	77.27	11.59	5.58	2.05	1.54
Second derivative (1176–1206 nm)	98.87	0.73	0.24	0.15	0.02

data corrected by pretransformation. The minimal SECV value based on the original indicates the calibration based on a two-principal component model. Figure 8 shows the plot of the calibration data obtained using the NIR method between the actual and the predicted CEX crystallinity based on the one-component model and using the combination of a second derivative in the range from 1176 to 1206 nm pretreatment. The plot shows a straight line ($Y = 0.995X + 0.117$ and $R^2 = 0.994$; $n = 18$).

Figure 9 shows the loading vector corresponding to the first principal component (PC1) and that the variance of PC1 was 98.9%. The peak at 1186 nm shows the lowest value whereas that at 1196 nm gives the highest value on PC1-loading vectors. Note that the maximum and minimum peaks of PC1-loading vectors are in good agreement with the decrease and increase in peaks of CEX in Figure 5b. The result suggests that the loading vectors reflect the spectral variation because of the CH_3 group caused by the change in crystallinity.

Figure 10 shows a plot of the relationship between scores of PC1 and predicted crystallinity obtained by the NIR method. The plot yields a straight line ($Y = 1.896 \times 10^{-5}X - 0.948 \times 10^{-3}$ and $R^2 = 0.992$; $n = 18$). The degree of crystallinity was evaluated based on one PC-loading vector. This result indicates that the CEX amorphization during grinding is consistent with molecular interaction

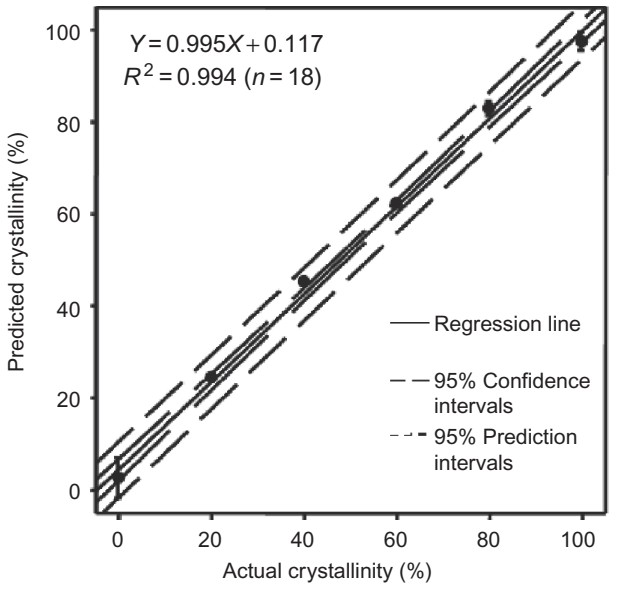

Figure 8. Relationship between actual crystallinity and that predicted using NIR spectroscopy. Each bar represents the mean ± SD ($n = 3$).

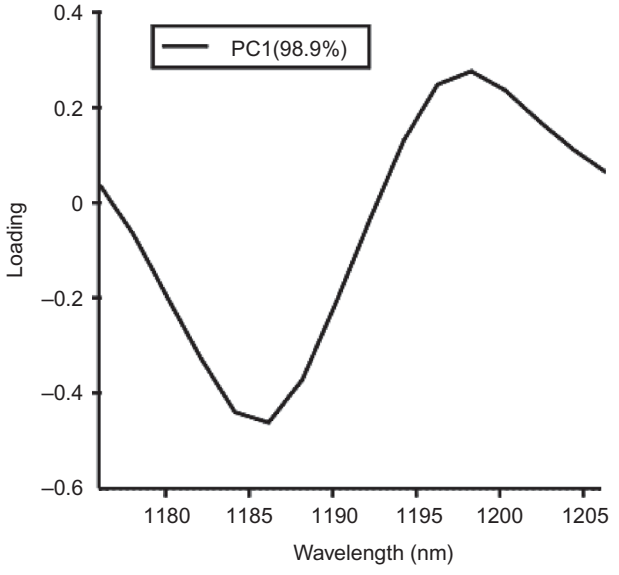

Figure 9. PC1-loading vector of calibration model for crystallinity of CEX.

between the carboxyl and amino groups and the crystalline water, as shown above.

Comparison of conventional X-ray diffraction and chemoinfometric NIR methods in the evaluation of crystallinity

One of the purposes of this study was to compare the accuracy in the evaluation of crystallinity between the chemometrical NIR method and the conventional XRD

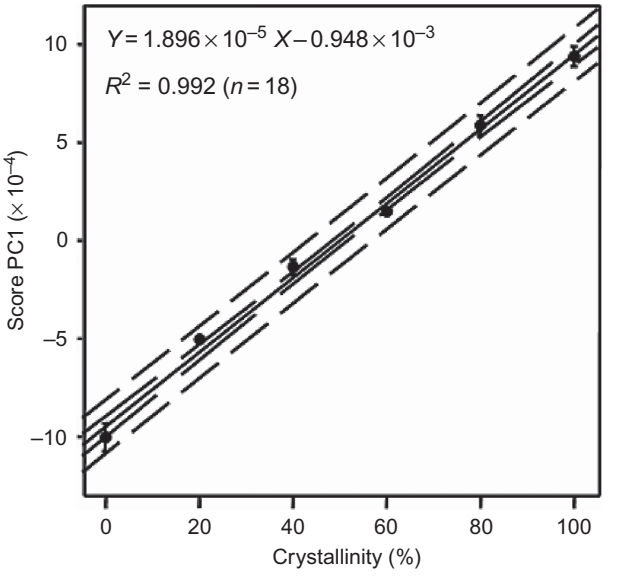

Figure 10. Relation between crystallinity of CEX and score of PC1. Each bar represents the mean ± SD ($n = 3$).

method. The accuracy of the NIR method using a second derivative in the range from 1176 to 1206 nm pretreatments (Figure 8) was compared to that of the XRD method (Figure 3), using the mean bias and the mean accuracy as determined for the test set samples by Equations (3) and (4), respectively.

$$B_{\mathrm{m}} = \frac{\sum_{i=1}^{n} (X_{\mathrm{c}} - X_{\mathrm{t}})/X_{\mathrm{t}}}{n} \times 100 \tag{3}$$

$$A_{\mathrm{m}} = \frac{\sum_{i=1}^{n} |X_{\mathrm{c}} - X_{\mathrm{t}}|/X_{\mathrm{t}}}{n} \times 100 \tag{4}$$

B_{m} is a percentage of mean bias, A_{m} is a percentage of mean accuracy, X_{c} is the predicted crystallinity of CEX, X_{t} is the actual crystallinity of CEX, and n is the number of experiments.

Table 4 shows a comparison between mean bias, mean accuracy, and measurement time for the XRD and the NIR methods using CEX (100%)[24] and CEX–MCC mixture (50%). Previously we reported that the mean bias for the NIR and XRD methods was calculated to be 3.40% and –1.58% and the mean accuracy was 7.70% and 5.76%, respectively, when the sample used was CEX (100%). On the other hand, the mean bias for the NIR and X-ray powder diffraction methods was calculated to be 1.52% and 2.26% and the mean accuracy

Table 4. Comparison of X-ray diffractometry with NIR spectroscopy.

	X-ray		NIR[a]	
Evaluation item	100%	50%	100%	50%
Bias	−1.58	2.26	3.40	1.52
Accuracy	5.76	7.14	7.70	3.06
Measurement time[b] (h)	12	15	1	1

[a]Pretransformed NIR at 100 and 50% of CEX concentration were normalize and MSC (region from 1100 to 1750 nm), and second derivative (region from 1176 to 1206 nm), respectively.
[b]Involved a preparation time of calibration curve.

was 3.06% and 7.14%, respectively, when the sample used was the CEX–MCC mixture (50%). The difference between NIR and XRD in accuracy and bias was greater for the CEX–MCC mixture (50%) than for CEX (100%).

It takes about 30 minutes to measure the XRD profile of a sample whereas it takes less than 1 minute to obtain an NIR spectrum. The sample preparation is also much simpler using NIR than for XRD. As a result, to determine the degree of crystallinity of CEX, it takes approximately 15 hours using the conventional XRD method but only 1 hour using the NIR method.

Conclusions

The crystallinity of CEX was evaluated based on clear scientific evidence because of the molecular interaction and resonance effects in the calibration model by chemometrical analysis. The results show that chemometric NIR spectroscopy can be used to evaluate crystallinity with greater accuracy than the conventional X-ray method and provide a rapid quantitative analysis of a mixed powder during preparation. In summary, the combination of NIR spectrometry and a chemometrical method is simple, nondestructive, and highly sensitive.

Acknowledgment

This work was supported by HAITEKU (2004–2008) from the Ministry of Education, Culture, Sports, Sciences and Technology, Japan.

Declaration of interest

The authors report no conflicts of interest.

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