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Effect of Grinding on the Degree of Crystallinity of Cephalexin Powder[1)]

MAKOTO OTSUKA and NOBUYOSHI KANENIWA*

*School of Pharmaceutical Sciences, Showa University,
Shinagawa-ku, Tokyo 142, Japan*

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The effect of grinding on the degree of crystallinity of cephalexin (CEX) powder was investigated. The intensities of the X-ray diffraction peaks of crystalline CEX decreased with increasing grinding time in an agate shaker mill, and after 4 h showed the same halo pattern as the noncrystalline solid (NC) obtained by the freeze-drying method.

The crystallinity of CEX ground for various times was determined by two X-ray diffraction methods, that is, by the internal standard method using lithium fluoride as the internal standard, and by Hermans' method, as well as by the infrared (IR) spectral method, for which standard samples were obtained by physically mixing crystalline monohydrate (phase IV) and NC-H_2O. The degree of crystallinity determined by Hermans' method was considerably larger than that determined by the X-ray internal standard method for short grinding times (up to about 1 h), but CEX ground for 3-4 h showed 0% crystallinity by both methods. The crystallinity determined by the IR-spectral method accorded quite well with that determined by the X-ray internal standard method for grinding times up to about 30 min, but remained approximately constant at about 15% for longer grinding times. Thus, the state of noncrystalline CEX produced by grinding differs from that of the material obtained by freeze-drying.

Keywords——cephalexin; crystallinity; X-ray diffraction; Hermans' method; grinding; noncrystalline state

The authors have reported previously on the different crystalline phases of cephalexin (CEX) and their hygroscopicity,[2,3)] as well as on the effect of compression on the physicochemical properties of crystalline CEX.[4)] It was concluded that compression destroyed the cystalline structure and converted CEX into a noncrystalline state.

Nakai *et al.*[5)] described the effect of grinding on the crystallinity of microcrystalline cellulose determined by Hermans' method.[6)] In the present paper, the crystallinity of CEX was estimated by three kinds of methods, that is, two X-ray diffraction methods (the internal standard method and Hermans' method) and the infrared (IR)-spectral method in order to investigate the effect of grinding on the crystallinity of CEX.

Experimental

Materials——The crystalline and noncrystalline CEX used in the present study were prepared as follows. Other chemicals used were of reagent grade.

(1) Crystalline CEX (Phase IV): Phase IV was the monohydrate, which was obtained by recrystallization as described in the previous paper.[2)]

(2) Noncrystalline CEX (NC): NC-H_2O containing 1 mol of absorbed water was obtained by storage of NC under 20% relative humidity (RH) at 35 °C after freeze-drying as described in the previous paper.[3)]

(3) Standard Samples: Standard samples with various degrees of crystallinity were obtained by physically mixing phase IV and NC-H_2O, regarding the degrees of crystallinity of phase IV and NC-H_2O as 100 and 0%, respectively. Standard samples were stored under 20% RH at 35 °C.

Mechanical Treatment——(1) Grinding in a Shaker Mill: Phase IV (5 g) was ground in an agate shaker mill (capacity 350 ml; diameter and numbers of balls, 10 mm × 20, 15 mm × 10 and 20 mm × 4; 200 rpm).

(2) Grinding in a Mortar: Phase IV (1 g) was ground in an agate mortar and pestle (60 rpm by hand). Ground samples were stored under about 20% RH at room temperature.

X-Ray Diffraction Analysis——The X-ray diffraction was measured at room temperature with a type JDX-7E diffractometer (Nihon Denshi Co., Ltd.) using a Geiger Muller (G.M.) counter. The measurement conditions were as follows: target Cu, filter Ni, voltage 20 kV, current 10 mA, receiving slit 0.2 mm, time constant 2 s, counting range 6×10^4 cpm, scanning speed 1°/min.

(1) Internal Standard Method: Fifty percent of lithium fluoride (LiF) was mixed with samples as an internal standard, and about 80 mg of the mixed sample was loaded with a micro spoon into a glass holder without introducing a preferred orientation of the crystals.

(2) Hermans' Method: About 80 mg of sample was loaded with a micro spoon into a glass holder, and measurements were made in the range of $2\theta = 3°$ and $2\theta = 45°$. The degree of crystallinity of intact phase IV was regarded as 100%. The crystalline peaks were separated from the diffuse scattering due to noncrystalline material and lattice imperfections by the usual freehand method, and the integrated intensities of crystalline and noncrystalline regions were determined by weighing cut-outs of photocopies of the X-ray diffraction profiles.

IR Spectra——IR spectra were recorded as mulls in Nujol on an IR-2 infrared spectrophotometer (Nihon Bunko Co., Ltd.). The concentration of mulls was adjusted to give almost the same absorption intensity of the key band at each measurement.

Polarizing Microscopy——A polarizing microscope (Olympus Model POS) was used.

Results

Estimation of the Degree of Crystallinity by X-Ray Diffraction Analysis

Count Losses of the G.M. Counter——The count losses of the detector are an important factor in quantitative X-ray diffraction analysis. Now, it is known that the true intensity of primary X-rays is directly proportional to the current in the X-ray tube at constant voltage.[7] The count losses of the G.M. counter were therefore determined by measuring the diffraction intensity of silicon powder ($2\theta = 38.7°$) as the current of the X-ray tube was changed at 30 kV. A plot of the observed counting rate *versus* the current in the X-ray tube is shown in Fig. 1. The observed counting rate was directly proportional to the current up to about 6×10^4 cpm. Therefore, quantitative X-ray diffraction analysis with the G.M. counter can be carried out under the condition that the observed counting rate is less than about 6×10^4 cpm.

Effect of Grinding in a Shaker Mill on the X-Ray Diffraction Profile——Figure 2 shows

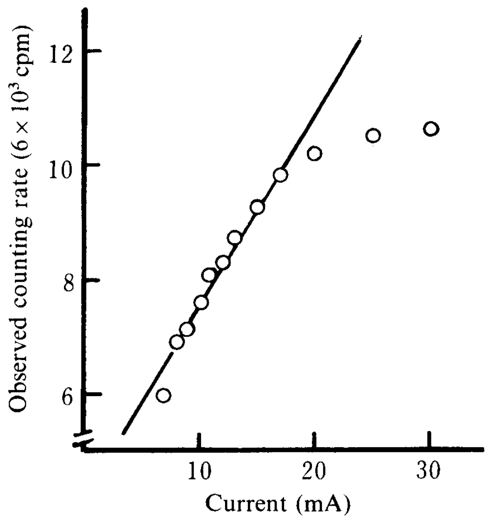

Fig. 1. Plot of Counting Rate as a Function of X-Ray Tube Current

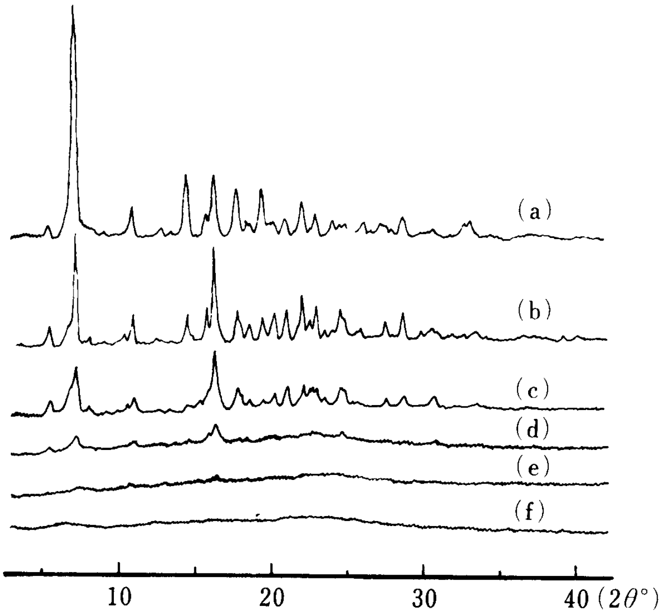

Fig. 2. Effect of Grinding on the X-Ray Diffraction Profile of Cephalexin

(a) intact crystals (phase IV), (b) 5 min, (c) 30 min, (d) 1 h, (e) 4 h, (f) NC-H_2O.

the changes in the X-ray diffraction profile of phase IV during grinding in an agate shaker mill. The peak intensities of the profiles of phase IV decreased with increasing grinding time; the profile after 1 h was very broad (Fig. 2(d)), and after 4 h the same halo pattern as for NC–H_2O obtained by the freeze–drying method was seen, as shown in Fig. 2(e, f). The product ground for 4 h also showed no polarization. These results suggest that phase IV was converted to a noncrystalline state by the mechanical treatment.

Internal Standard Method——Figure 3 shows a plot of $I_{7.2}$ *versus* I_{all}, where $I_{7.2}$ is the ratio of the X-ray diffraction intensity of phase IV at $2\theta = 7.2°$ to that of LiF at $2\theta = 38.7°$, and I_{all} is the ratio of intensity of all the X-ray diffraction peaks of phase IV to that of LiF at $2\theta = 38.7°$. A plot of $I_{7.2}$ *versus* I_{all} gave a straight line for standard samples with 20, 40, 60, 80 and 100% crystallinity.

Figure 4 shows a plot of $\int I_{7.2}(\theta)d\theta / \int I_{38.7}(\theta)d\theta$ *versus* the degree of crystallinity of the standard samples. The plot gave a straight line with a slope of $k = 2.232$ and a correlation coefficient of $r = 0.9959$ by the least-squares method. The degree of crystallinity (X_{cr}) of a

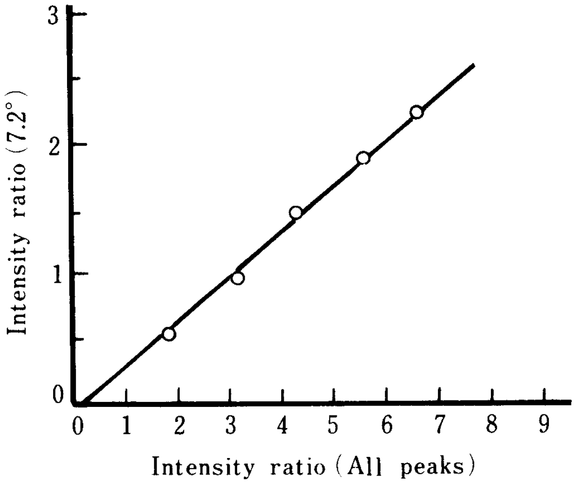

Fig. 3. Relation between the Intensity Ratio at $2\theta = 7.2°$ and That for All Peaks of the X-Ray Diffraction Profile of Cephalexin with Respect to LiF Internal Standard (See the Text)

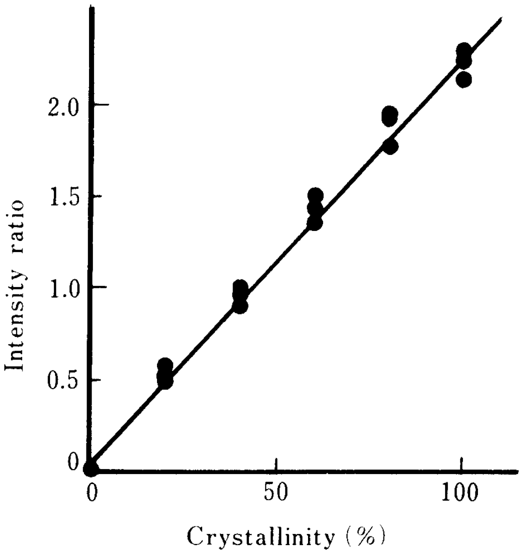

Fig. 4. Relation between the Degree of Crystallinity and the X-Ray Diffraction Intensity Ratio of Cephalexin ($2\theta = 7.2°$) with Respect to LiF Internal Standard ($2\theta = 38.7°$)

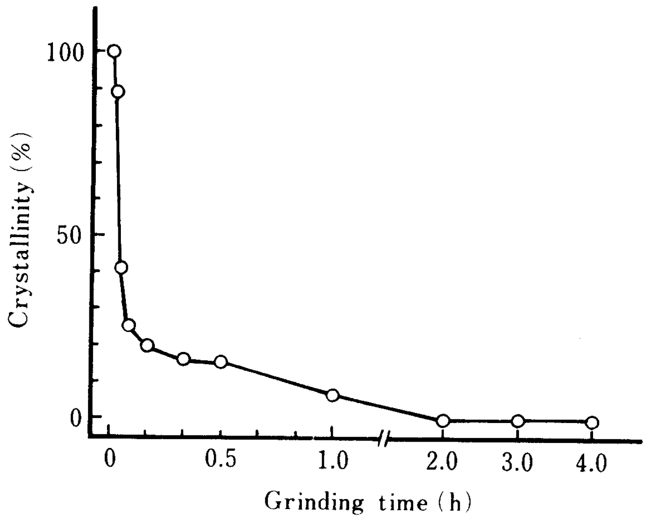

Fig. 5. Effect of Grinding on Crystallinity (X-Ray Internal Standard Method)

sample was thus obtained by substituting the observed intensity of phase IV, $\int I_{7.2}(\theta)d\theta$, and that of LiF, $\int I_{38.7}(\theta)d\theta$, into Eq. (1).

$$X_{cr}=[\int I_{7.2}(\theta)d\theta/k\int I_{38.7}(\theta)d\theta]\times 100 \quad (1)$$

Figure 5 shows the effect of grinding in a shaker mill on the degree of crystallinity as determined by the internal standard method. Each value of crystallinity is the average of 3 runs. The X_{cr} of phase IV decreased with increasing grinding time; after 10 min it was about 20%, and after 2 h it was 0%, that is, phase IV had been converted to a completely noncrystalline state.

Hermans' Method——In the present study, the X-ray diffraction angles were confined to the region between $2\theta=3$ and $2\theta=45°$. The integrated intensities of the X-ray diffraction from the crystalline and the noncrystalline regions, $\int I_c(\theta)d\theta$ and $\int I_n(\theta)d\theta$, respectively, in the above range are plotted against each other in Fig. 6, where (a) is the result for the standard samples, and (b) is the result for samples ground in the shaker mill. These plots each gave a straight line, and the slopes (k_c/k_n) of (a) and (b) were calculated to be 0.5646 and 0.6967, respectively, by the least-squares method, where k_c and k_n are crystalline and noncrystalline constants. Thus, the degree of crystallinity (X'_{cr}) is given by Eq. (2).

$$X'_{cr}=[\int I_c(\theta)d\theta/\{\int I_c(\theta)d\theta+(k_n/k_c)\int I_n(\theta)d\theta\}]\times 100 \quad (2)$$

The degrees of crystallinity of the standard samples (physical mixtures of phase IV and NC–H_2O) were estimated by Hermans' method, on the basis of both the above values of

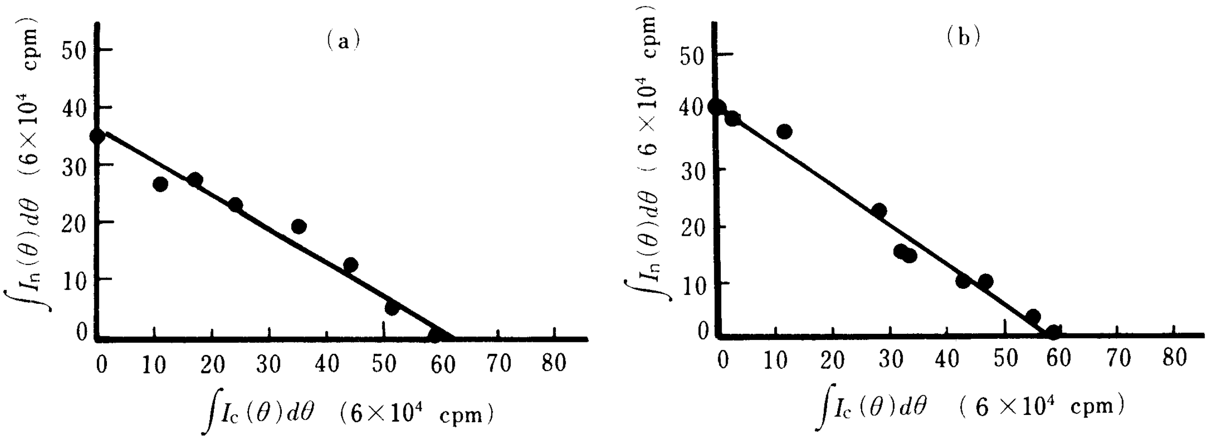

Fig. 6. Regression Lines for Physical Mixtures and Ground Cephalexin Samples with Different Crystallinity Obtained by Hermans' Method

(a) physical mixtures; (b) ground samples.

Table I. Crystallinity of Standard Samples Estimated by Hermans' Method

Crystallinity of standard samples (%)	Crystallinity (%) calculated on basis of: k_c/k_n for physical mixtures (Fig. 6(a))	Crystallinity (%) calculated on basis of: k_c/k_n for ground samples (Fig. 6(b))
80	86.2	88.5
60	66.6	71.1
40	50.7	55.9
30	37.5	42.5
20	26.5	30.8
10	19.3	22.7

(k_c/k_n), and the results are shown in Table I. It is apparent that X'_{cr} is larger than the true crystallinity.

Figure 7 shows the effect of grinding on the degree of crystallinity as determined by Herman's method. X'_{cr} decreased with increasing grinding time, and the value after 10 min based on the value of k_c/k_n from Fig. 6(a) was about 60%, while that after 3 h was 0%, that is, phase IV had been converted to a completely noncrystalline state.

Estimation of the Degree of Crystallinity by the IR-Spectral Method

Marrelli reported that the IR spectral bands of cephalexin at 1760 and 1600 cm^{-1} (broad) were due to the β-lactam $\nu_{C=O}$ and the carboxyl $\nu_{C=O}$, respectively.[2,8] Figure 8 shows the changes of IR spectrum of phase IV during grinding in an agate shaker mill. The peak intensities of the β-lactam $\nu_{C=O}$ at 1750 cm^{-1} and the carboxyl $\nu_{C=O}$ at 1580 cm^{-1} of phase IV

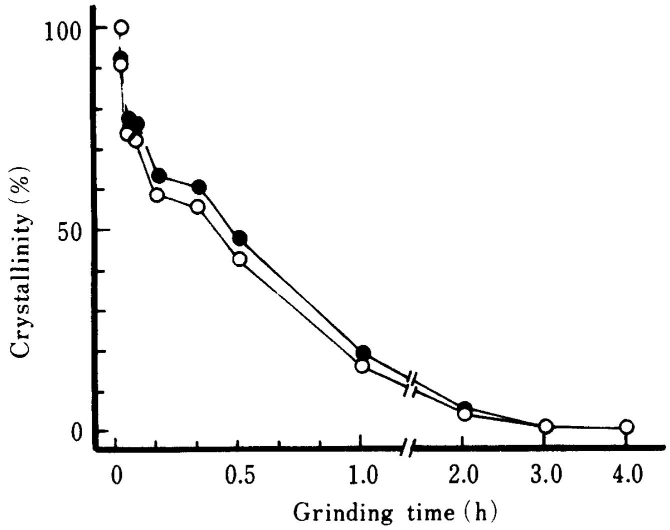

Fig. 7. Effect of Grinding on Crystallinity (Hermans' Method)

○, based on k_c/k_n for physical mixtures (Fig. 6(a)); ●, based on k_c/k_n for ground samples (Fig. 6(b)).

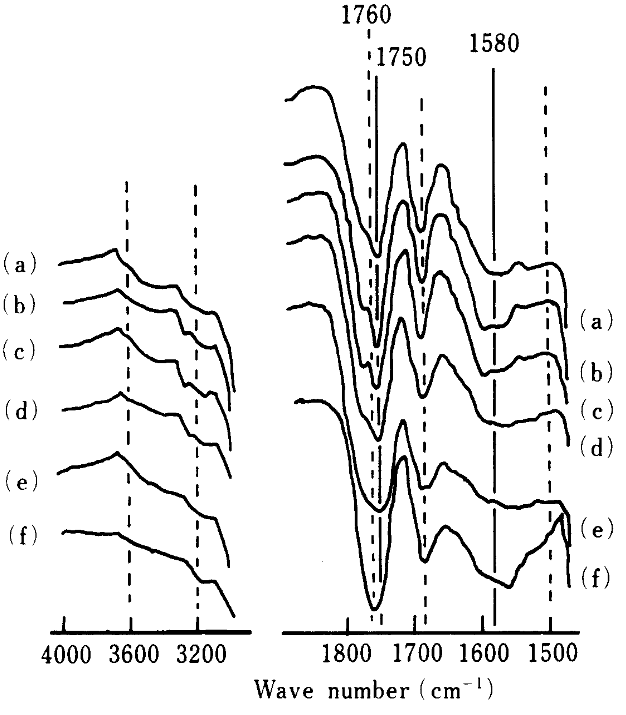

Fig. 8. Effect of Grinding on the IR Spectrum of Cephalexin

(a) intact phase IV; (b) 5 min; (c) 30 min; (d) 1 h; (e) 4 h; (f) NC–H_2O.

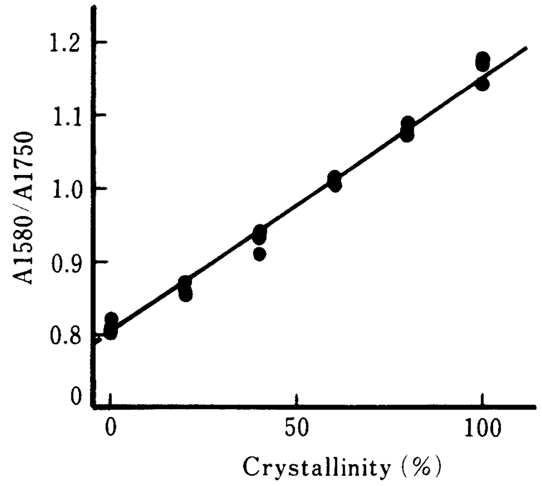

Fig. 9. Relation between the Degree of Crystallinity of Cephalexin and Absorbance Ratio in the IR Spectra (See the Text)

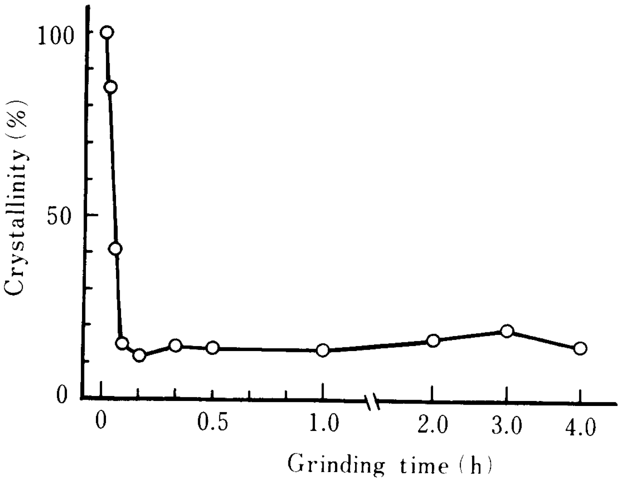

Fig. 10. Effect of Grinding on Crystallinity (IR-Spectral Method)

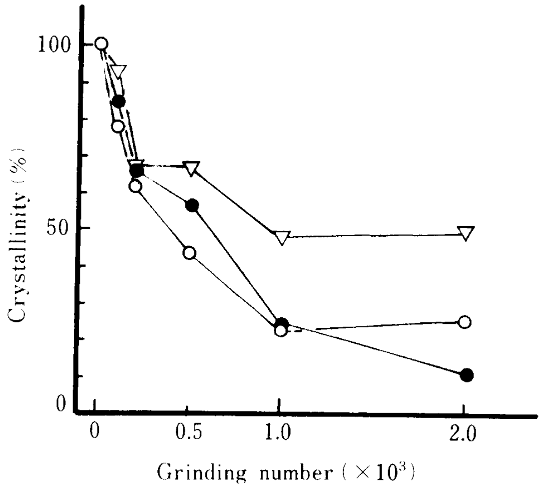

Fig. 11. Effects of Grinding in an Agate Mortar and Pestle on Crystallinity of Cephalexin

▽, Hermans' method; ○, internal standard method; ●, IR spectral method.

changed with increasing grinding time. On the other hand, in the IR spectrum of NC–H_2O (Fig. 8(f)) obtained by the freeze–drying method, the peak intensity of the β-lactam $\nu_{C=O}$ at 1760 cm^{-1} was larger than that of the carboxy $\nu_{C=O}$. In order to utilize these differences to calculate the percent crystallinity,[9] the peak heights were measured from a base line drawn between the minima at about 880 and 1830 cm^{-1}. The IR spectra of the standard samples were measured, and the absorbance ratio of the peaks at 1580 and 1750 cm^{-1} was calculated. Figure 9 shows a plot of the absorbance ratio *versus* the degree of crystallinity. The plot gave a straight line with a slope of $k=0.3564$ and a correlation coefficient of $r=0.9935$ by the least-squares method. Thus, the degree of crystallinity (X''_{cr}) of a sample was obtained by substituting the absorbance ratio into Eq. (3).

$$X''_{cr}=[\{(A_{1580}/A_{1750})-0.7954\}/0.3564]\times 100 \qquad (3)$$

Figure 10 shows the effect of grinding on the degree of crystallinity as determined by the IR-spectral method. Values of X''_{cr} are averages of 3 measurements. The value of X''_{cr} of phase IV decreased rapidly during grinding, and was about 15% after 10 min. However, its value thereafter stayed approximately constant at 15%, even after prolonged grinding.

Effect of Grinding in a Mortar

Figure 11 shows the effect of grinding in a mortar on the crystallinity of phase IV as determined by the X-ray internal standard method (X_{cr}), Hermans' method (X'_{cr}) based on the standard sampes and the IR-spectral method (X''_{cr}). X_{cr} agreed reasonably well with X''_{cr}, but X'_{cr} was larger than X_{cr} and X''_{cr}.

Discussion

It is well known that various errors can affect the results of quantitative X-ray diffraction analysis, for example, the existence of preferred orientation of sample crystals, statistical error, count losses of the G.M. counter, *etc.* In the X-ray internal standard method, LiF added as the internal standard acts as a diluent and resists preferred orientation of sample crystals; in the present experiments, the X-ray diffraction profiles were reproducible. Count losses of the G.M. counter used here were discussed in a previous section. Thus, the results obtained here by the X-ray internal standard method are probably quite accurate.

On the other hand, in Hermans' method, the observed crystallinity was larger than the true one, probably because of the lack of corrections for Lorentz factor and polarization factor in Hermans' method (the degree of crystallinity determined by Hermans' method was about 3 times larger than that determined by the X-ray internal standard method after grinding for 10 min). Further, the error for a low crystallinity sample was larger than that for a high crystallinity sample, as can be seen in Table I. One reason for this may be that in Hermans' method very small diffraction peaks must be measured, so that statistical errors increase. From the results of estimation of the crystallinity of standard samples (Table I) and the effect of grinding on the crystallinity (Fig. 7), the crystallinity determined by the use of the k_c/k_n value for physically mixed standard samples was closer to the true one than that obtained by the using of the value for ground samples (see Fig. 6). As equal weights of samples were used in both cases in order to avoid effects of sample density differences, the difference of the apparent crystallinities may be a result of surface differences, for example X-ray absorption factor and X-ray reflection factor.

In the IR-spectral method (Fig. 10), the change of crystallinity was in accord with that of the X-ray internal standard method for grinding times up to 30 min, but the apparent crystallinity remained approximately constant at about 15% on further grinding. In this case, the β-lactam $\nu_{C=O}$ of the material ground for 4 h remained at 1750 cm^{-1}, whereas that of NC–H_2O appears at 1760 cm^{-1} (Fig. 8(f)). This finding suggests that the ground CEX retains some sort of order. Thus, the completely noncrystalline state determined by X-ray diffraction methods differed from that determined by the IR-spectral method; the peaks of β-lactam $\nu_{C=O}$ may reflect intermolecular hydrogen bonding interactions.

It was also found that grinding in a mortar (Fig. 11) produced changes of crystallinity corresponding well to those obtained with the shaker mill at grinding times up to 20 min. This result suggests that mortar grinding is not more efficacious than shaker mill grinding.

Conclusion

Crystalline CEX was converted to a noncrystalline form by grinding, and became completely noncrystalline after grinding for more than 3 h as determined by two X-ray diffraction methods. However, the results of the IR-spectral method indicated that the state of the noncrystalline solid obtained by grinding differed from that of the material obtained by the freeze–drying method.

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