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Effects of Grinding on the Physicochemical Properties of Cephalexin Powder[1)]

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The effects of grinding on the physicochemical properties of cephalexin (CEX) were studied by means of water content measurements, differential thermal analysis (DTA), differential scanning calorimetry (DSC), X-ray diffraction analysis and scanning electron microscopy (SEM). The water content of CEX which had been ground for 4 h and converted to a noncrystalline state was proportional to relative humidity (RH) in the range of 0—62% RH, and was about 2 mol/mol at 62—82% RH. The water content of intact crystal phase IV was about 1 mol/mol at 20—75% RH, and about 2 mol/mol at 82—95% RH. The X-ray diffraction data indicated that ground CEX remains in the noncrystalline state until it has absorbed more than about 2 mol of water per mol. At 62% RH, the water content of phase IV increased with increasing grinding time due to the conversion to noncrystalline state.

The dehydration point of the ground CEX was depressed by up to about 25 °C with increasing grinding time, and the decomposition point of the ground product was depressed by up to about 30 °C. In general, the physicochemical properties changed rapidly when the crystallinity of the ground product fell below about 25%.

The activation energy and mechanism of dehydration were determined by using non-isothermal kinetic analysis (Criado's method). The dehydration of the product ground for 4 h (noncrystalline solid) followed first-order kinetics and its activation energy and latent heat were calculated to be 14.83 kcal/mol and 8.31 kcal/mol, respectively. The product ground for 4 h was about 2.5 times more soluble than intact phase IV. The dehydration behavior and solubility of the product ground for 4 h were different from those of the freeze–dried product, even though both products are in a noncrystalline state.

Keywords——cephalexin; hygroscopicity; grinding; thermal behavior; nonisothermal kinetic analysis; activation energy of dehydration; noncrystalline state

Some work has been done on the relationship between hygroscopicity and crystalline phase,[2,3)] but there are few reports on the effect of grinding on the hygroscopicity of drugs. In the previous paper, we reported on the effect of grinding on the degree of crystallinity of cephalexin (CEX) powder.[4)] In the present paper, the effects of grinding on the physicochemical properties *i.e.* hygroscopicity, dehydration and decomposition of CEX powder were studied.

Experimental

Materials——CEX used in the present study was prepared as follows.

(1) Crystalline CEX (Phase IV): Phase IV was the monohydrate, and was obtained by recrystallization as described in a previous paper.[3)]

(2) Noncrystalline CEX (NC–H_2O): 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 a previous paper.[4)]

Mechanical Treatment——Phase IV (5 g) was ground in an agate shaker mill; a sample of phase IV (1 g) was also ground in an agate mortar and pestle as described in a previous paper.[4)]

Thermal Measurement——The differential thermal analysis (DTA) and the differential scanning calorimetry (DSC) curves were measured with DTA (DT-20B; Shimadzu Seisakusho Co., Ltd.) and DSC (SC-20B; Shimadzu

Seisakusho Co., Ltd.) instruments, respectively. The measurement conditions were as follows: sample weight, about 3 mg (DTA) or about 5 mg (DSC); sample cell, an aluminium crimp cell having a cell cover with five holes for stainless steel hypodermic needles (JIS cord H) for gas flow; N_2 gas flow, 30 ml/min. The values of latent heat, dehydration point and decomposition point are averages of 3 runs of DSC or DTA.

Measurement of Water Content——The samples were stored in desiccators with saturated salt solutions of various RH values (0—95% RH) at 35±1 °C, and when the samples reached constant weight, the water content was determined by the Karl–Fischer method (type MK-S instrument; Kyoto Denshi Kogyo Co., Ltd.) and by measuring the weight loss after heating to 130 °C by DSC (each value of water content is the average of 3 runs) (Fig. 2).

Measurement of X-Ray Diffraction——The X-ray diffraction was measured with a type JDX-7E diffractometer (Nihon Denshi Co., Ltd.) as described in a previous paper.[4]

Measurement of Crystallinity——The degree of crystallinity was measured by the X-ray diffraction internal standard method as described in a previous paper.[4]

Solubility Measurement——Measurement of concentration was done in the manner reported previously.[5]

Scanning Electron Microscopy (SEM)——An SEM (HS-9; Hitachi Seisakusho Co., Ltd.) was used at an accelerating voltage of 20 kV.

Results and Discussion

Change in Water Content of CEX by Grinding

Phase IV was changed to a noncrystalline solid by grinding for 4 h in a shaker mill as described in a previous paper,[4] and Fig. 1 shows the water content-RH diagram after storage of the product ground for 4 h at various RH values at 35 °C for 2 weeks. The mol water content of the product ground for 4 h at 20% RH was about 1 mol/mol, and that of the product at 62—82% RH was about 2 mol/mol; the amount of water was proportional to RH in the range of 20—62% RH. The water content of the product ground for 4 h at 95% RH was much larger than that of the product at 82% RH. After storage for 2 weeks under various RH values at 35 °C, the X-ray diffraction patterns of the product ground for 4 h at up to 82% RH showed no diffraction peaks, but that of the product stored at 95% RH showed several diffraction peaks which coincided with those of phase II.[3] This result suggests that the product ground for 4 h having up to about 2 mol of water remains in the noncrystalline state, but crystallizes when it has absorbed more than about 2 mol of water.

As reported in a previous paper,[3] the mol water content of phase IV was about 1 mol/mol at 20—75% RH, and that at 95% RH was about 2 mol/mol, but the mol water content of the noncrystalline product (NC) obtained by freeze–drying was about 1 mol/mol at 20—32% RH and about 2 mol/mol at 43—66% RH and NC crystallized when it had absorbed more than 2 mol of water at 75—95% RH.[6] These results suggest that the product ground for 4 h

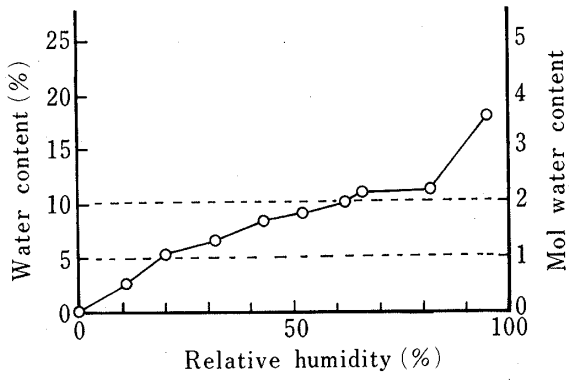


Fig. 1. Changes of Water Content of Ground CEX under Various RH Values at 35 °C

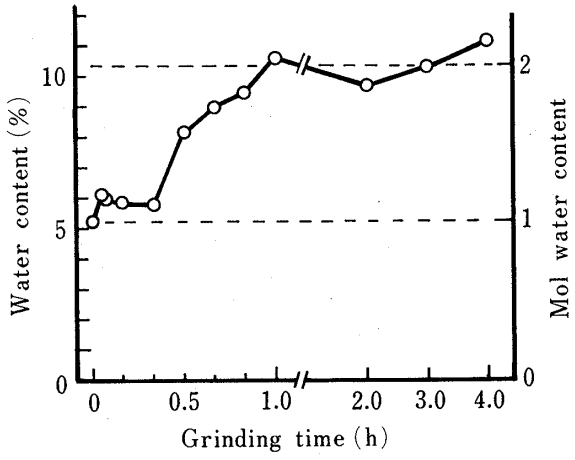


Fig. 2. Effect of Grinding Time on the Water Content of CEX

After storage at 62% RH at 35 °C for 2 weeks.

has higher hygroscopicity than phase IV, but is more stable against moisture than NC under high RH conditions.

Figure 2 shows the effect of grinding time in a shaker mill on the water content of CEX after storage at 62% RH at 35 °C. The mol water content of CEX increased with increasing grinding time; after 30 min it was about 1.5 mol/mol, and after 1 h it was about 2 mol/mol. This result suggests that the hygroscopicity of CEX increases with increasing grinding time at 62% RH.

Figure 3 shows the effect of grinding time on the latent heat of dehydration of CEX after storage for 2 weeks at 62% RH. The value of latent heat of the product ground for 1 h was about twice that of phase IV. However, its value thereafter stayed approximately constant at about 45 cal/g, even after prolonged grinding, and the value of latent heat for the ground dihydrate was closer to that of phase II[3] than to that of NC dihydrate.[6]

These results suggest that crystalline CEX is converted to a noncrystalline form with increasing grinding time, and the hygroscopicity of the ground product increases with increasing content of noncrystalline form at 62% RH, but its mol water content stays approximately constant at 2 mol/mol.

Change in Dehydration and Decomposition Points by Grinding

Figure 4 shows the change in DTA curve of ground CEX after storage for 2 weeks at 20% RH. The dehydration peak became broader, and the temperature of the decomposition peak became lower with increasing grinding time.

Figure 5 shows the change in dehydration point on the DTA curve caused by grinding (from Fig. 4). The dehydration point of the ground product fell with increasing grinding time; it was 25 °C after grinding for 1 h, but its value thereafter stayed approximately constant at about 23 °C, even after prolonged grinding.

Figure 6 shows the change in the decomposition point on the DTA curve caused by grinding (from Fig. 4). The decomposition point of the ground product fell rapidly at the beginning of grinding, remained approximately constant at 177 °C during grinding for 10—30 min, and then fell to 160.2 °C after 1 h. However, it thereafter stayed at approximately

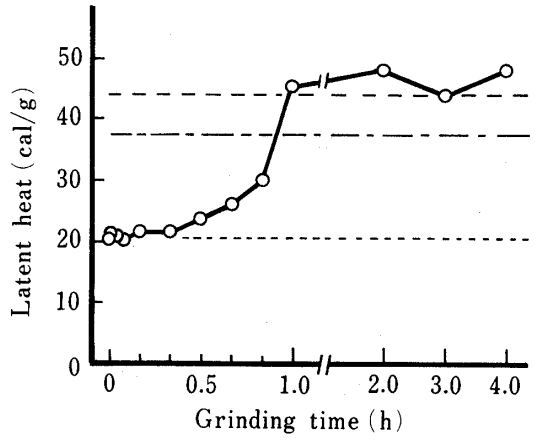


Fig. 3. Effect of Grinding Time on the Latent Heat of Dehydration of CEX

After storage at 62% RH at 35 °C for 2 weeks.
----, phase II; - - - -, NC–$2H_2O$; ------, phase IV.

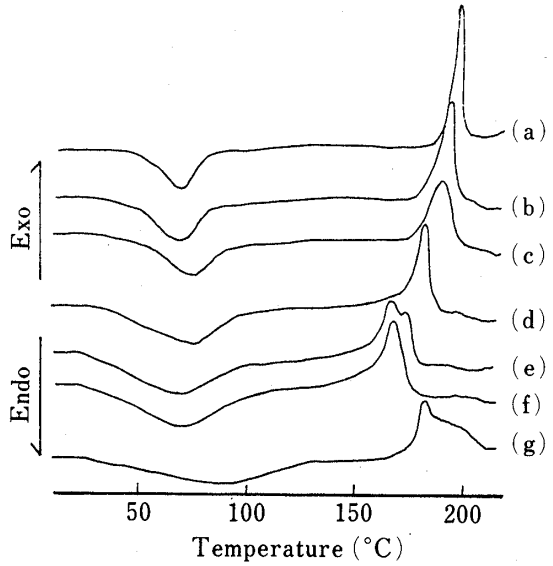


Fig. 4. Change in DTA Curve of CEX by Grinding

(a) intact phase IV; (b) ground for 3 min; (c) 5 min; (d) 30 min; (e) 1 h; (f) 4 h; (g) NC–H_2O.
After storage at 20% RH at 35 °C for 2 weeks.

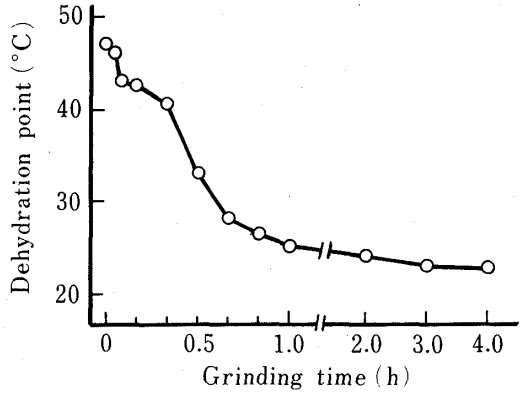


Fig. 5. Change in Dehydration Point of CEX by Grinding

After storage at 20% RH at 35 °C for 2 weeks.

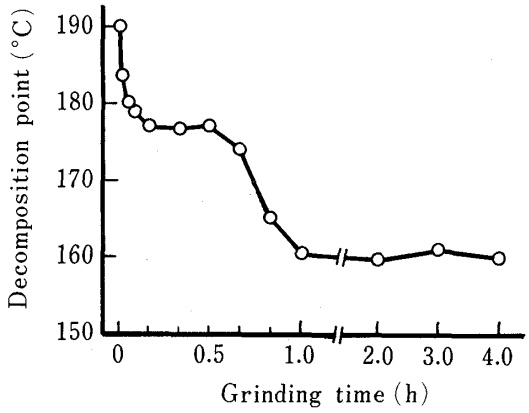


Fig. 6. Change in Decomposition Point of CEX by Grinding

After storage at 20% RH at 35 °C for 2 weeks.

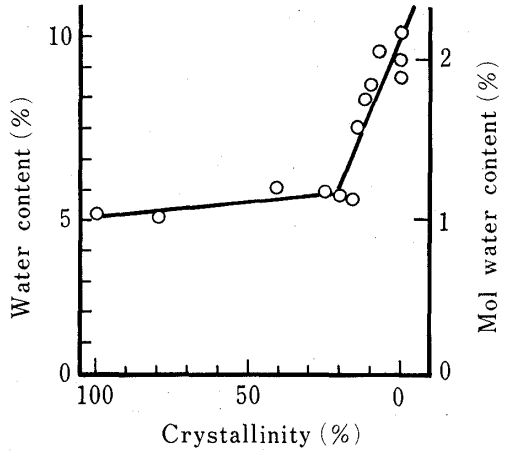


Fig. 7. Relation between the Degree of Crystallinity and Water Content of Ground CEX

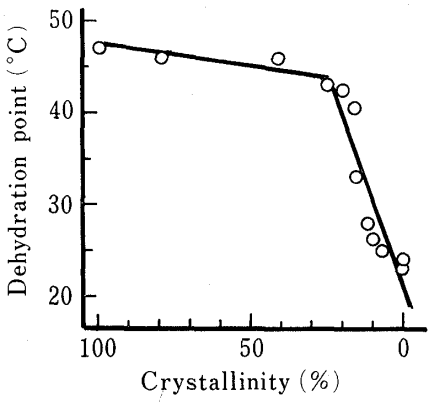


Fig. 8. Relation between the Degree of Crystallinity and the Dehydration Point of Ground CEX

160 °C, even after prolonged grinding. The decomposition point of NC–H_2O was 173 °C,[6] despite its noncrystalline state. This result suggests that the noncrystalline product obtained by freeze–drying is more stable at high temperature than that obtained by grinding.

Relation between the Degree of Crystallinity and Physicochemical Properties

The crystallinity of CEX decreased with increasing grinding time,[4] and the relation between the crystallinity obtained by the X-ray internal standard method and the change of physicochemical properties of ground CEX was studied.

Figure 7 shows the relation between the degree of crystallinity and water content of ground CEX stored at 62% RH and 35 °C. The water content of the ground products increased slightly with decreasing crystallinity in the range of 100—25% (the product ground for 0—20 min, shown in Fig. 2), but its value increased rapidly when the crystallinity decreased below about 25%, and the water content of the product with about 0% crystallinity was about 2 mol/mol (the product ground for 1—4 h, shown in Fig. 2).

Figure 8 shows the relation between the degree of crystallinity and dehydration point of ground CEX. The dehydration point of the ground product fell rapidly when the crystallinity of the product was less than about 25% (grinding time less than about 20 min, shown in Fig.

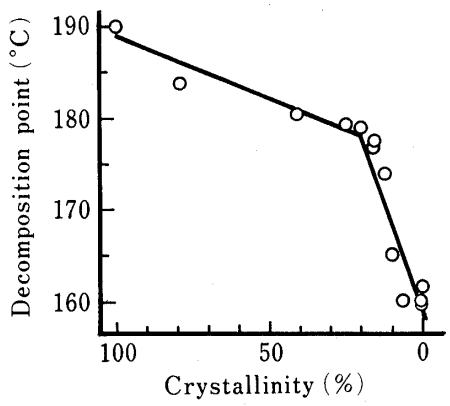


Fig. 9. Relation between the Degree of Crystallinity and the Decomposition Point of Ground CEX

5), and the dehydration point of the product with 0% crystallinity (the product ground for 1—4 h, shown in Fig. 5) was about 25 °C lower than that of phase IV.

Figure 9 shows the relation between the degree of crystallinity and decomposition point of ground CEX. The decomposition point of the ground product fell gradually with decreasing value of crystallinity, but its value fell rapidly when the crystallinity decreased below about 20% (grinding time about 30 min, shown in Fig. 6), and the decomposition point of the ground product changed to the noncrystalline state was about 30 °C lower than that of phase IV.

These results suggest that the physicochemical properties of ground CEX change rapidly when the crystallinity decreases below about 25% (grinding time about 30 min).

Change in Activation Energy of Dehydration by Grinding

We previously reported a thermal kinetic study of CEX hydrate,[7] and in the present work, we investigated the effect of grinding on dehydration under nonisothermal conditions using a DSC instrument. Criado[8] *et al.* summarized 9 kinetic equations in solid-state decomposition and derived the so-called Criado equation:

$$\ln g(x) - 2\ln T = \ln(AR/Ea) - E/RT \qquad (1)$$

where $g(x)$ is the integrated form of the kinetic equation, x is the fraction of dehydration at time t, A is the frequency factor, R is the gas constant, a is the heating rate, E is the activation energy and T is temperature (Kelvin).

Phase IV was stored in a 43% RH desiccator and NC–H_2O, the product ground for 30 min, and the product ground for 4 h were stored in a 20% RH desiccator for 2 weeks; the water contents of these samples were almost the same at 1 mol/mol.

The most linear Criado plots for CEX phase IV, the product ground for 30 min, the product ground for 4 h and NC–H_2O under nonisothermal conditions (heating rate 10 °C/min) are shown in Fig. 10. The activation energy and the frequency factor were calculated from the plots by the least-squares method and the results are shown in Table I. The dehydration of phase IV and the product ground for 4 h followed first-order kinetics (F_1), and the latent heat and the activation energy of the product ground for 4 h were almost the same as those of phase IV. The latent heat of the product ground for 4 h and which had absorbed 2 mol of water (at 62% RH) was 16.75 $\pm$0.75 kcal/mol (shown in Fig. 3), and its value was twice as large as that of the product which had absorbed 1 mol of water, but the frequency factor of the product ground for 4 h was about 10 times larger than that of phase IV.

The dehydration of the product ground for 30 min followed three-dimensional diffusion kinetics (Jander equation) (D_3), but the product was a mixture of crystalline phase IV and noncrystalline solid, since its X-ray diffraction profile showed several broad peaks and the infrared (IR) spectrum showed separate β-lactam carbonyl bands at 1770 and 1750 cm^{-1}.[4]

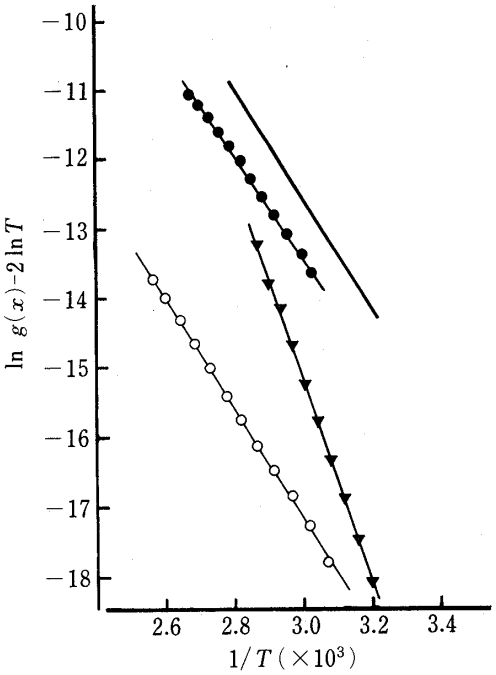


Fig. 10. Criado Plot of Ground CEX (Non-isothermal Conditions)

(——), intact phase IV; ▼, ground for 30 min; ●, 4 h; ○, NC–H_2O.

TABLE I. Thermal Dynamic Parameters of Dehydration of Ground CEX

Sample	Latent heat (kcal/mol)	Activation energy (kcal/mol)	Frequency factor (min^{-1})	Most linear model
Phase IV	$7.13 \pm 0.20^{a)}$	15.67	5.432×10^9	F_1
Grinding for 30 min		29.37	6.828×10^{17}	D_3
Grinding for 4 h	$8.31 \pm 0.43^{a)}$	14.83	5.984×10^8	F_1
NC–H_2O	$5.07 \pm 0.24^{a)}$	15.81	6.331×10^7	D_4

a) Standard deviation ($n=3$).

The dehydration of NC–H_2O followed three-dimensional diffusion kinetics (Ginstiling–Brounshtein equation) (D_4).

These results suggest that the dehydration mechanism of the product ground for 4 h is different from that of NC–H_2O, even though both products are noncrystalline as determined from the results of X-ray diffraction analysis and polarizing microscopy. However, in the IR spectrum of NC–H_2O the β-lactam carbonyl band was at 1760 cm^{-1}, whereas that of the product ground for 4 h was at 1750 cm^{-1}.[4] These results may reflect intermolecular hydrogen bonding interactions between CEX and water.

Effect of Grinding on the Solubility of CEX

Figure 11 shows the concentration–time curves for ground CEX in distilled water at 10 °C. The concentration of the product ground for 4 h then stored at 62% RH for 2 weeks was about 2.5 times larger than that of phase IV initially, but rapidly decreased, and became constant at about 20 mg/ml. It seems likely that there are very fine crystal particles in the product ground for 4 h, and the particles acted as seeds for crystallization of the non-crystalline product. However, NC–$2H_2O$ was about 3 times more soluble than the product ground for 4 h, even though both products are in a noncrystalline state.

Effect of Grinding in a Mortar

Figure 12 shows the effect of grinding in an agate mortar and pestle on the dehydration

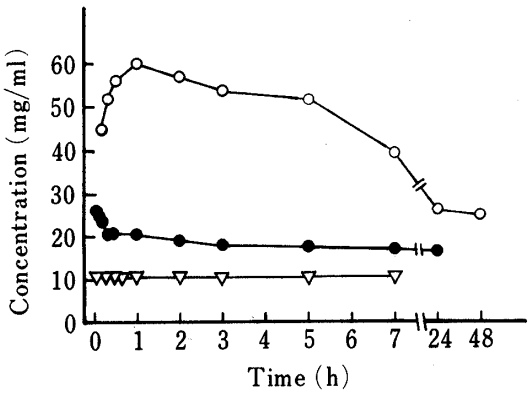


Fig. 11. Concentration–Time Curves for Dissolution of Ground CEX in Distilled Water at 10 °C

▽, intact phase IV; ●, ground for 4 h; ○, NC–$2H_2O$.[6]

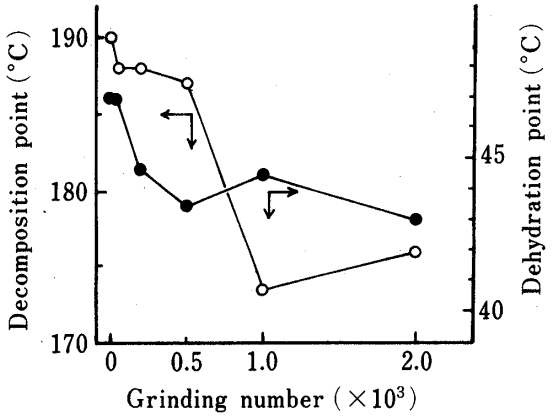


Fig. 12. Effect of Grinding in a Mortar on the Dehydration and Decomposition Points of Ground CEX

●, dehydration point; ○, decomposition point.

and decomposition points of the ground CEX. The dehydration and decomposition points fell with increasing grinding number. It was also found that the depressions of the dehydration and the decomposition points of the product ground in the mortar corresponded well to those of the product ground in the shaker mill at grinding time up to 20 min (Figs. 5 and 6). In a previous paper,[4] the crystallinity of the product ground in the mortar also corresponded well to that of the product obtained with the shaker mill at grinding time up to 20 min. The grinding efficiencies in the mortar and in the shaker mill as estimated from the change of the physicochemical properties in this study were all consistent with those estimated from the crystallinity in a previous paper,[4] though mortar grinding is not more efficacious than shaker mill grinding.

Effect of Grinding on the Surface Characteristics of CEX

Figure 13 shows SEM photographs of ground CEX. On grinding in the mortar, the particle size decreased with grinding number. The particle size after grinding 1000 times was less than 1 μm, and it was shown that the primary particles aggregated (Fig. 13(d)). On grinding in a shaker mill, the particle size of CEX ground for 30 min was smaller than that of the product ground for 4 h (Fig. 13(f)). The small particles produced by grinding for 30 min appeared to aggregate during grinding for more than 30 min, and the particle size increased, but the aggregated particles were in the noncrystalline state, as shown in a previous paper.[4]

Conclusions

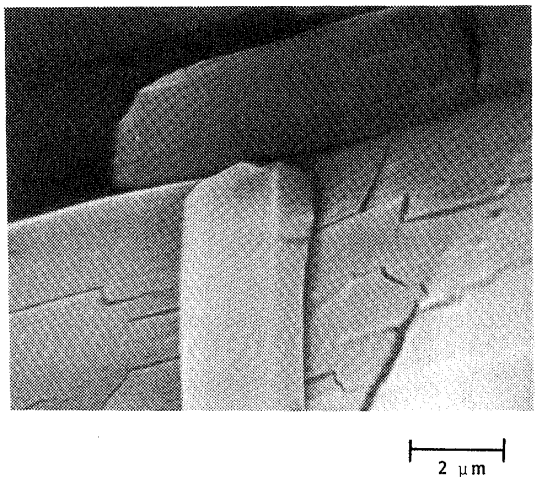
1) The noncrystalline product obtained by grinding was more stable to moisture than that obtained by freeze–drying under high RH conditions.

2) The water content and latent heat of CEX increased with increasing grinding time due to the conversion to a noncrystalline state at 62% RH at 35 °C.

3) The dehydration and decomposition points of CEX fell with increasing grinding time; those of the product ground for 4 h were about 23 and 160 °C, respectively.

4) In the relation between the degree of crystallinity and the physicochemical properties (water content, dehydration point and decomposition point), the physicochemical properties changed gradually with decreasing crystallinity in the range of 100—25%, but these properties changed rapidly when the crystallinity decreased below about 25%.

5) The dehydration behavior and solubility of the ground product were different from those of the freeze–dried product, even though both products were in the noncrystalline state as determined by X-ray diffraction analysis and polarizing microscopy.



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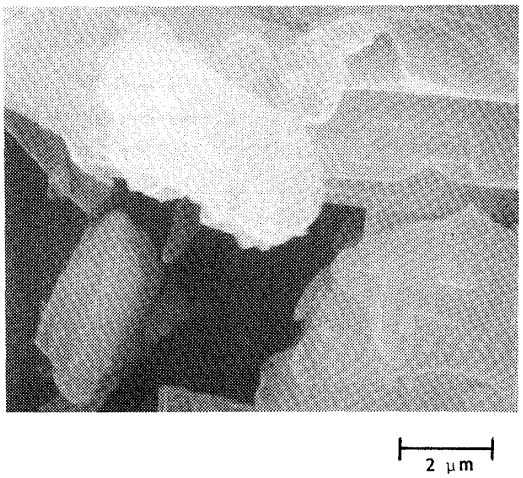

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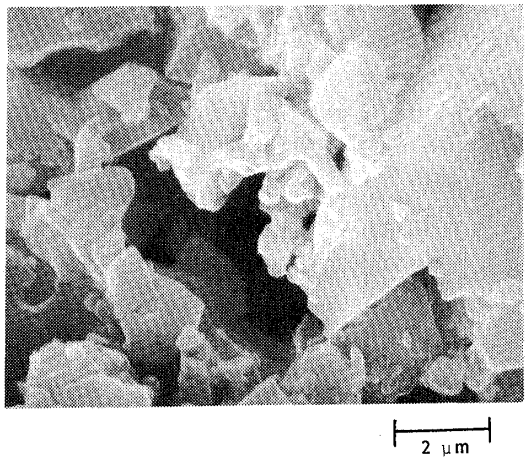

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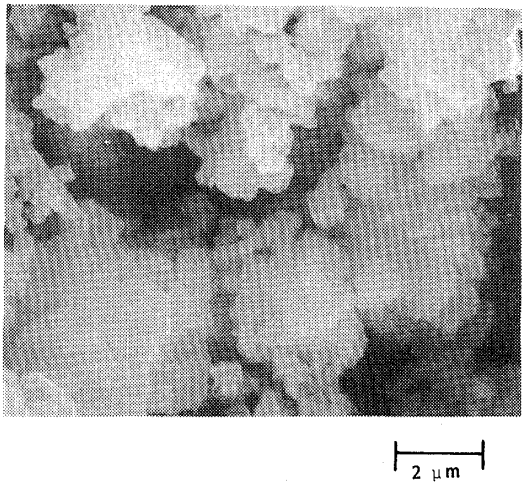

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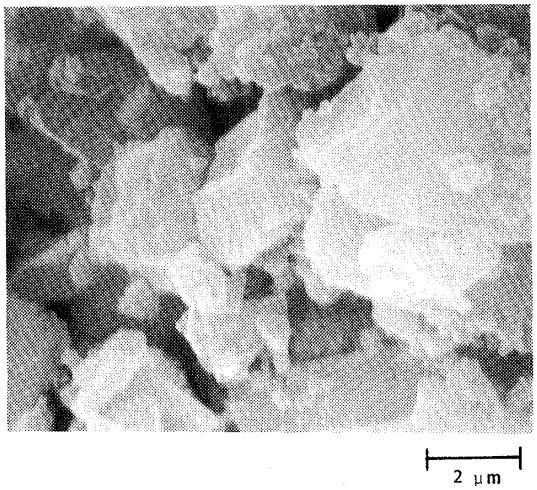

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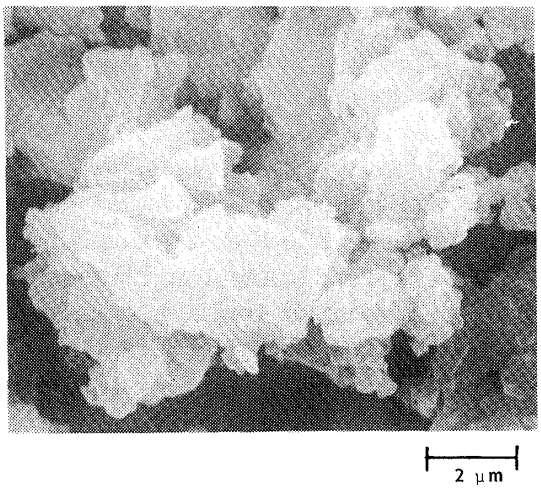

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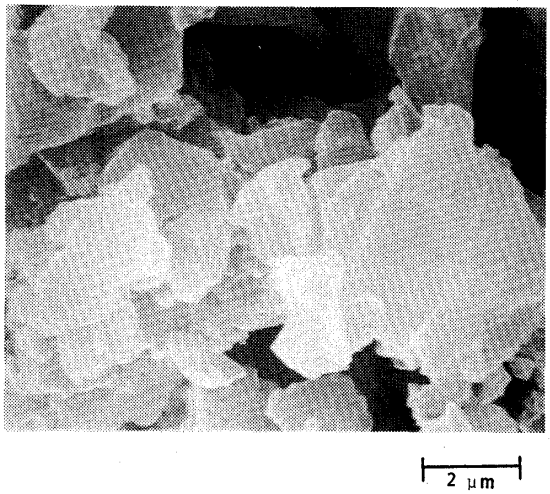

(g)

Fig. 13. Effect of Grinding on the Surface Characteristics of Ground CEX (SEM Photographs, × 10000)

(a) intact phase IV; (b) 50 times in mortar; (c) 300 times in mortar; (d) 1000 times in mortar; (e) 5 min in shaker mill; (f) 30 min in shaker mill; (g) 4 h in shaker mill.

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