Isomerization of Lactose in Solid-state by Mechanical Stress During Grinding

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Abstract—Isomerization during grinding of solid-state α- monohydrate, α-anhydrate and β-anhydrate of lactose was investigated. Samples were ground in an agate centrifugal ball-mill at 270 rev min^{-1} at room temperature (20°C). The crystallinity of ground lactose was measured by Hermans' method from the powder X-ray diffraction profiles. The α- and β-lactose content of the ground lactose was obtained from the specific rotation measured by using angular rotation spectrophotometry. The crystalline lactose samples were transformed into non-crystalline solids by mechanical stress during grinding. After grinding, the water content of all ground lactose samples increased, and the samples had about 2 mol water per lactose after 10 h grinding. After 10 h grinding of α-monohydrate and α-anhydrate, 10 and 15%, respectively, of α-lactose was transformed into β-lactose by the mechanical treatment. After 10 h grinding of β-lactose, 20% of β-lactose was transformed into α-lactose. The results suggest that crystalline lactose was transformed into a non-crystalline solid, and water was adsorbed on the non-crystalline lactose. The non-crystalline solids of α- or β-lactose were then transformed into their counterparts by the mechanochemical effects of grinding.

Lactose has been widely used as a diluent in tablets because of the favourable characteristics of the bulk powder. Commercial grades may vary in their content of crystalline (α-monohydrate, α-anhydrate, β-anhydrate) and amorphous forms (Norris & Greenstreet 1958; Goulden 1958; Itoh et al 1977, 1978) and many grades of lactose are thus available, including crystalline, anhydrate, spray-dried, or granulated products. However, there are few reports of the physicochemical properties of lactose and its pharmaceutical consequences. Lerk et al (1983) reported that the binding properties of α-lactose monohydrate increased with increasing dehydration of the solid. Crystalline α-lactose monohydrate has been transformed into a non-crystalline lactose during grinding (Morita et al 1984), and the hardness of tablets made from the ground lactose increased with increase of grinding time (Sagawa 1983). We have reported mechanochemical effects on the physicochemical properties of several pharmaceutical bulk powders (Otsuka & Kaneniwa 1986). We have investigated the physicochemical stability of various kinds of crystalline lactose during manufacture of pharmaceutical preparations, and found isomerization of lactose in the solid-state during grinding. In the present study we have investigated the physicochemical properties of various kinds of crystalline lactose after isomerizaton brought about in the grinding process.

Materials and Methods

Materials

Crystalline α-lactose monohydrate (Wako Pure Chem. Ind. Ltd) was of Pharmacopeia Japonica XI grade. α-Anhydrate was recrystallized from methanol (Lerk et al 1983) and β-anhydrate was recrystallized from distilled water at 100°C (Itoh et al 1978).

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Mechanical treatment

A sample of lactose powder (10 g) was ground in an agate centrifugal ball-mill (Fritsch Co. Ltd) with a capacity of 250 mL. The diameters and numbers of balls were: 10 mm × 20, 15 mm × 10, 20 mm × 4. Speed was 270 rev min^{-1}. The grinding was performed at room temperature (20°C), at about 60% relative humidity. The mill was opened and ground samples (500 mg) removed at 1, 2, 4, 7 and 10 h. These samples were stored in closed containers at −35°C.

Powder X-ray diffraction analysis

Powder X-ray diffraction was measured at room temperature with a type 11 PA diffractometer (Nihon Denshi Co. Ltd). The measurement conditions were: target Cu; filter Ni; voltage 30 kV; current 7·5 mA; time constant 1 s; step slit 0·03°; counting time 0·5 s; measured from $2\theta = 5°$ to $2\theta = 40°$.

Measurement of infrared (IR) spectra

The IR spectra were measured as a mull in Nujol on an IR-2 infrared spectrophotometer (Nihon Bunko Co. Ltd).

Determination of crystallinity

(i) *Hermans' method.* Crystallinity was estimated by Hermans' method (Hermans & Weidinger 1948). The degree of crystallinity of an intact lactose sample was regarded as 100%. The crystalline peaks were separated from the diffuse scattering due to non-crystalline material and lattice imperfections by the usual free-hand method, and the integrated intensities of crystalline and non-crystalline regions were determined by weighing cut-outs of photocopies of the X-ray diffraction profiles as reported previously (Otsuka & Kaneniwa 1983).

(ii) *Diffraction intensity method.* Standard samples with various degrees of crystallinity were prepared by physically mixing intact α-monohydrate or intact β-anhydrate with the non-crystalline lactose otained by grinding for 10 h. Plots of

X-ray diffraction peak area at $2\theta = 12 \cdot 6^\circ$ due to α-monohydrate or at $2\theta = 10 \cdot 6^\circ$ due to β-anhydrate against the degree of crystallinity of standard samples were straight lines. The crystallinities of α-monohydrate or β-anhydrate were calculated from the X-ray diffraction intensities from the calibration lines.

Thermal analysis

Differential thermal analysis (DTA) curves and thermogravimetry (TG) curves were measured with a type DT-20 DTA instrument and a type TG-30 TG instrument (Shimadzu Seisakusho Co. Ltd), respectively. The measurement conditions were: sample weight 3 mg for DTA and 5 mg for TG; heating rate $10^\circ C$ min^{-1} N$_2$ gas flow 30 mL min^{-1}; sample cell aluminium crimp cell.

Determination of the water content of lactose

The water content of lactose was measured by the TG method as described in the previous section.

Determination of the α- and β-lactose content

Content of α- and β-lactose were estimated from the specific rotation data as follows: a sample of powder (250 mg) was dissolved in 50 mL of 0·01 M phthalate buffer (pH standard solution #100-4, Horiba Ltd, pH 4·00). The sample weight was corrected by the water content values as measured by the TG method. The angular rotation was measured (DIP-140 angular rotation spectrometer, Nihon Bunko Co. Ltd) at suitable times (3, 8, 13, 18 and 23 min) at $20 \pm 2^\circ C$, and the specific rotation was calculated from the measured angular rotation. The specific rotation at 0 min was calculated by the least-squares method from a plot of the log (specific rotation) against time. Standard samples with various ratios of α- and β-lactose content were obtained by physically mixing α-lactose and β-lactose. Plots of log (specific rotation) against time for the standard samples were straight lines. The plot of specific rotation at 0 min against α- and β-lactose content was also a straight line estimated by the least-squares method as follows:

$$Y = 0 \cdot 5165X + 36 \cdot 07 \qquad (r = 0 \cdot 9976)$$

where Y is the value of specific rotation at 0 min, X is the percent content of α-lactose and r is the correlation coefficient. The α-and β-lactose content of a sample was obtained from the specific rotation at 0 min.

Measurement of equilibrium specific rotation

A small amount of concentrated ammonia solution was added to the sample solutions, and after 2 h at room temperature the equilibrium specific rotation of the samples was measured. The equilibrium specific rotation of ground lactose was almost the same as that of intact lactose, as shown in Table 1, suggesting that the lactose samples did not decompose during grinding.

Table 1. Effect of grinding on the equlibrium specific rotation of various kinds of crystalline lactose.

Sample	Specific rotation ($^\circ$)	
	Intact	Ground for 10 h
α-Monohydrate	+53·0	+52·6
α-Anhydrate	+51·6	+52·6
β-Anhydrate	+52·0	+53·2

Results

Change of the physicochemical properties of lactose during grinding

Fig. 1 shows changes of the X-ray diffraction profiles of crystalline lactose during grinding. The diffraction peak
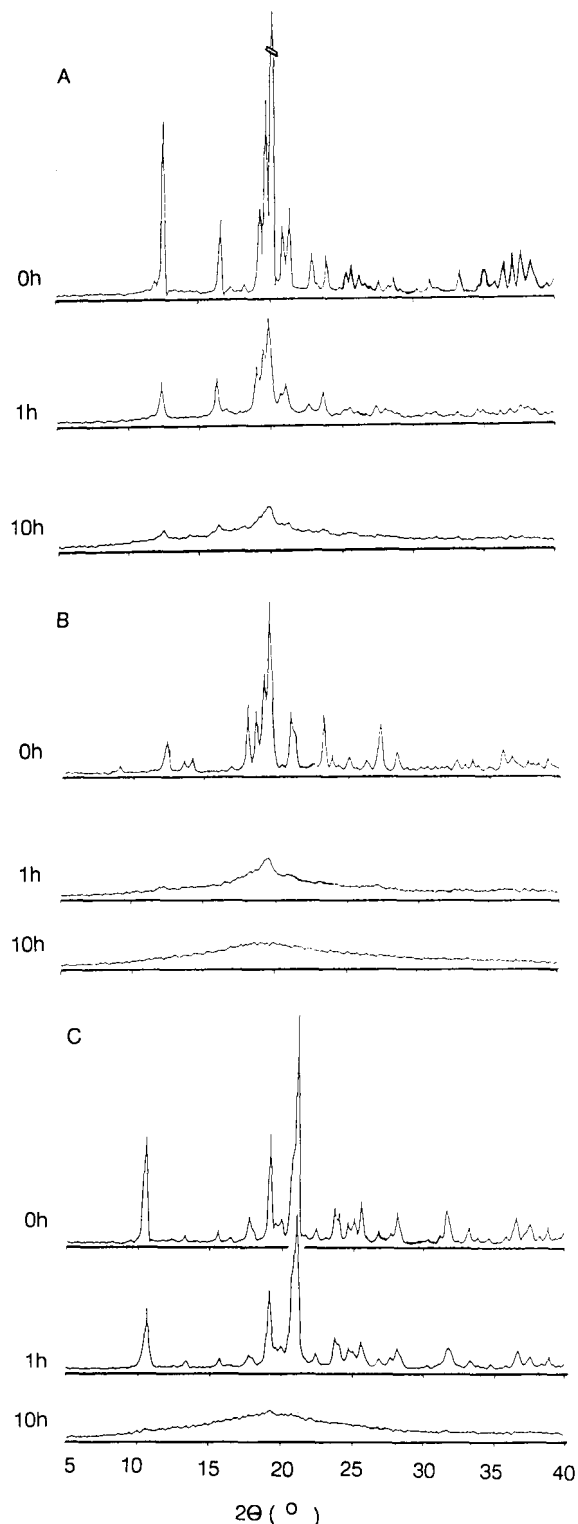

FIG. 1. Change of X-ray diffraction profiles of various kinds of crystalline lactose during grinding. (A), α-monohydrate; (B) α-anhydrate; (C), β-anhydrate.

intensity of all samples decreased with increased grinding time. The X-ray differentiation profiles of α-anhydrate and β-anhydrate had halo patterns after 10 h grinding but the profile of α- monohydrate had several small diffraction peaks even after 10 h grinding.

The infrared (IRF) spectra of crystalline lactoses were explained as follows: the bands at 3600–3200 cm^{-1} were due to stretching vibration of the hydroxyl group, the weak band at 1650 cm^{-1} was due to bending vibration of the hydroxyl groups of crystal water, and the band at 1200–1070 cm^{-1} was attributed to asymmetric stretching vibration of C–O–C in the glucose and galactose (Nakanishi 1960). The IR spectra of α-monohydrate and α-anhydrate had a specific band at 920 cm^{-1} but the spectrum of β-monohydrate did not (Nakanishi 1960). After grinding for 10 h, the IR spectra of all ground lactose samples had broader absorption peaks than the intact samples. The bands at 920 cm^{-1} of the ground α-monohydrate and the ground α-anhydrate decreased with increased grinding time, suggesting that the α-lactose content had decreased. Bands at 920 cm^{-1} appeared in the spectrum of the ground β-lactose and increased with grinding time. Bands of the ground α- and β-anhydrate at 3600–3200 cm^{-1} and 1650 cm^{-1} increased with increased grinding time.

Figs 2 and 3 show DTA and TG curves of the ground lactose samples. The DTA curve of intact α-monohydrate had an endothermic peak at 154°C with 5% weight loss in the TG curve attributed to dehydration of 1 mol of crystal water, an exothermic peak at 170°C was attributed to transformation to α- anhydrate, and an endothermic peak at 221°C was attributed to partial degradation of α-lactose (Itoh et al 1977). After 10 h grinding of α-monohydrate, the DTA curve had an endothermic peak at 120–150°C with 3·1% loss of weight in the TG curve, and a sharp exothermic peak at 160°C with no weight loss attributed to crystallization, an endothermic peak at 220°C was attributed to partial degradation of α-lactose, and a new endothermic peak at 230°C was attributed to partial degradation of β-anhydrate (Itoh et al 1978). The TG curve of α-monohydrate ground for 10 h had a 5·2% weight loss at 30–100°C with no thermal peak in the DTA curve and 3·1% loss at 100–150°C. We conclude that the water dehydrated below 100°C was the adsorbed water and the water dehydrated above 100°C was crystal water. After 10 h grinding of α-monohydrate, the adsorbed water content increased and the crystal water content decreased.

The DTA and TG curves of intact α-anhydrate had endothermic peaks at 221°C with weight loss attributed to partial degradation of α-lactose (Lerk et al 1983). The DTA curve of α-anhydrate ground for 10 h had endothermic and exothermic peaks at 60–120°C with 8·5% weight loss in the TG curve, endothermic and exothermic peaks at 130–170°C with 2·8% weight loss in the TG curve, and two endothermic peaks at 220 and 230°C attributed to partial degradation of α-lactose and β-lactose, respectively.

The DTA curve of intact β-anhydrate had endothermic peaks at 238°C with weight loss attributed to partial degradation of β-lactose (Itoh et al 1978). The DTA curve of β-anhydrate ground for 10 h had a very broad peak at 120–170°C with 2·9% weight loss and an endothermic peak at 220–230°C. The endothermic peak attributed to α- and β-lactose was divided into two peaks at 220 and 225°C. The TG

curve of β-anhydrate ground for 10 h had 8·7% weight loss at 30–100°C with no thermal peak in the DTA thermogram attributed to adsorbed water.
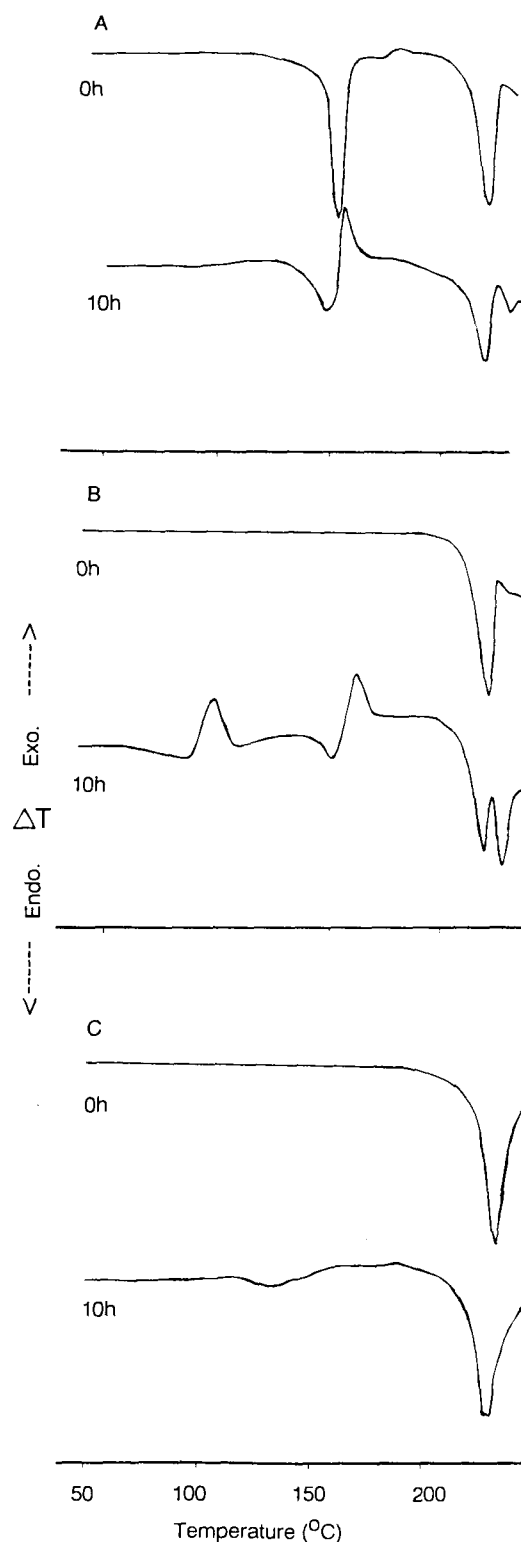

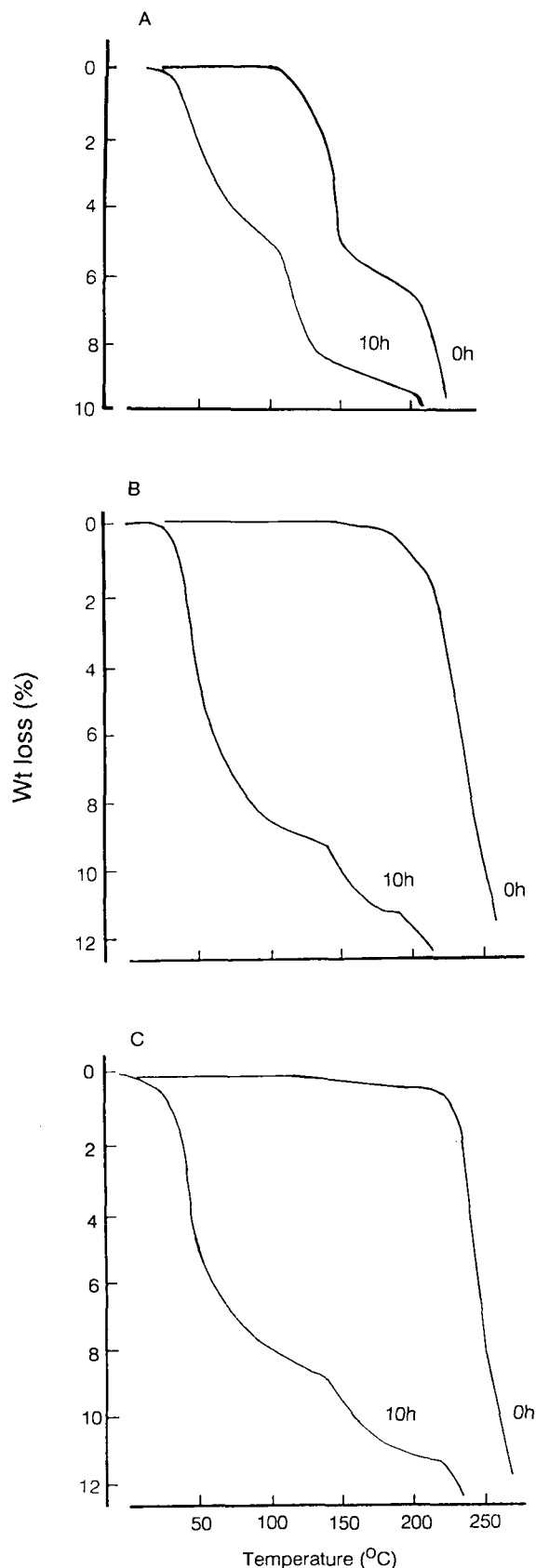FIG. 2. Change of DTA curves of various kinds of crystalline lactose during grinding. (A). α-monohydrate: (B) α-anhydrate: (C), β-anhydrate.
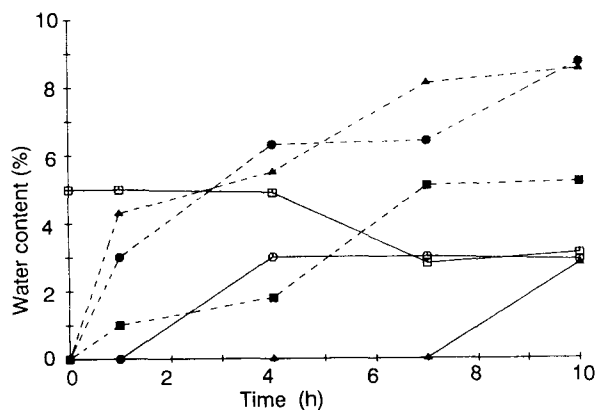
FIG. 4. Change of water content of various kinds of crystalline lactose during grinding. —□—, crystal water for α-monohydrate; —△—, for α-anhydrate; —O—, for β-anhydrate; ---■---, adsorbed water for α-monohydrate; ---▲---, for α-anhydrate; ---●---, for β-anhydrate.

Change of lactose water content during grinding

From the results of TG analysis it was assumed that the weight loss below 100°C was adsorbed water and the weight loss above 100°C was crystal water. The change of water content in various kinds of lactose during grinding is shown in Fig. 4. The adsorbed water of ground α-monohydrate increased with increased grinding time, but the crystal water decreased, and the product ground for 10 h had 5·2% (1·04 mol mol^{-1}) adsorbed water and 3·1% (0·62 mol mol^{-1}) crystal water; the crystal water of α-monohydrate was converted into adsorbed water by mechanical stress, and the total water content increased during grinding. The adsorbed water of α-anhydrate increased with increased grinding time. After grinding for 1 h, it had 4·3% (0·86 mol mol^{-1}) adsorbed water and product ground for 10 h had 8·5% (1·7 mol mol^{-1}) adsorbed water and 2·8% (0·56 mol mol^{-1}) crystal water. The water content of β-anhydrate increased with increased grinding time; the product ground for 1 h had 3·0% (0·5 mol mol^{-1}) adsorbed water, and product ground for 10 h had 8·7% (1·74 mol mol^{-1}) adsorbed water and 2·9% (0·58 mol mol^{-1}) crystal water. This suggested that the water content of α- and β-anhydrates increased during grinding.

FIG. 3. Change of TG curves of various kinds of crystalline lactose during grinding. (A), α-monohydrate; (B), α-anhydrate; (C), β-anhydrate.
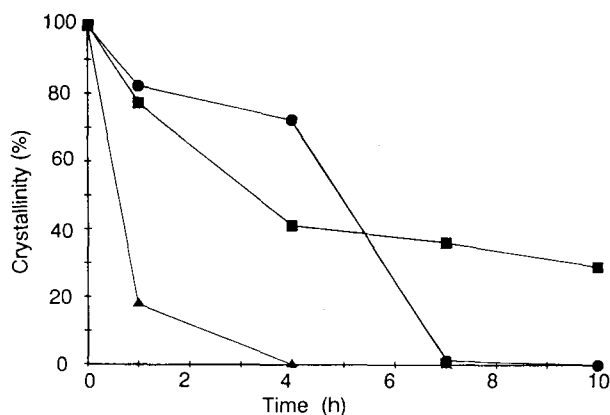

FIG. 5. Change of crystallinity of various kinds of crystalline lactose during grinding. ■, α-monohydrate; ▲, α-anhydrate; ●, β-anhydrate.

Change in crystallinity of crystalline lactose during grinding
Fig. 5 shows the change of crystallinity (Hermans & Weidinger 1948) of various kinds of crystalline lactose during grinding. The crystallinity of α-monohydrate decreased with increased grinding time, and after 10 h the ground product had 30% crystallinity. In the case of α-anhydrate, the product ground for 1 h had 18% crystallinity, and after grinding for more than 4 h, it had 0% The β-anhydrate ground for 4 h had 75% crystallinity, and after grinding for more than 7 h it had 0%.

Change of crystallinity of ground lactose during storage
Fig. 6 shows the change of X-ray diffraction profiles of ground lactose after 21 days at 60 ± 10% relative humidity, 20 ± 5°C. After storage of the α-monohydrate ground for 10 h and α-anhydrate ground for 10 h, the X-ray diffraction profiles had main diffraction peaks due to α-monohydrate. The diffraction profiles of the β-anhydrate ground for 10 h had diffraction peaks due to α-monohydrate and β-anhydrate.

The crystallinity of the storage ground lactose was estimated by the diffraction intensity method based on peak

Table 2. Change of crystallinity of ground lactose during storage.

Sample	Crystallinity (%)	
	Fresh ground product	After 21 days
α-Monohydrate	26·0 (α-monohydrate)	45·6 (α-monohydrate)
α-Anhydrate	0·0	44·0 (α-monohydrate)
β-Anhydrate	0·0	61·0*

* (37·6% of β-anhydrate and 23·4% of α-monohydrate).

FIG. 7. Change of specific rotatory power of α-monohydrate lactose during grinding. □, intact α-monohydrate; △, product ground for 1 h; ○, for 2 h; ☒, for 4 h; ×, for 7 h; +, for 10 h.
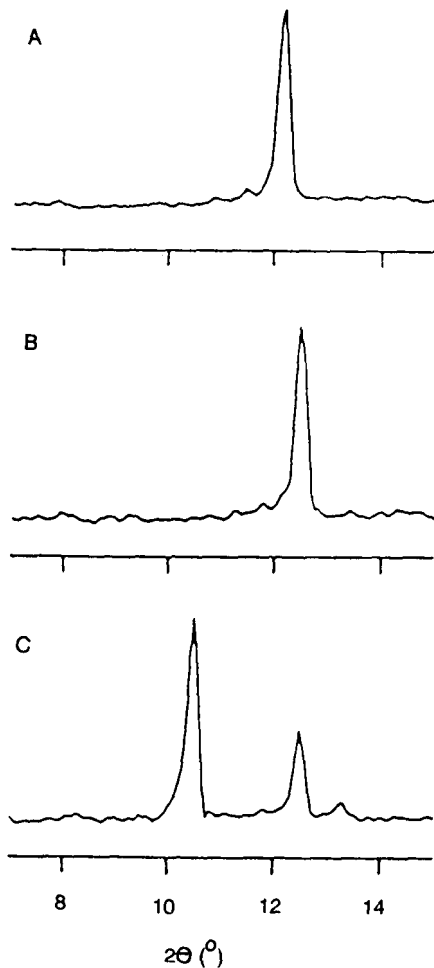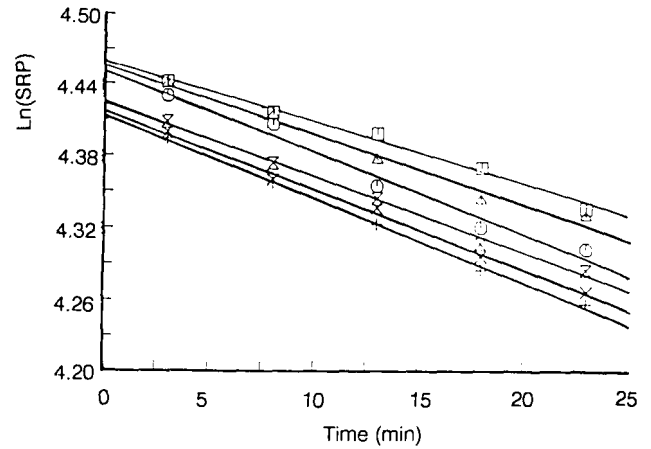

FIG. 6. Change of X-ray diffraction profiles of ground lactose after 21 days storage. (A), The ground product of α-monohydrate; (B), of α-anhydrate; (C), of β-anhydrate.

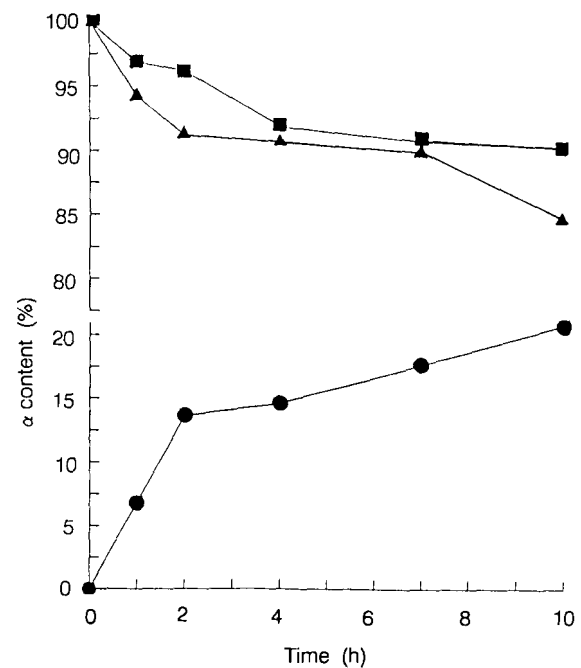FIG. 8. Change of α and β-lactose content of various kinds of crystalline lactose during grinding. ■, α-monohydrate; ▲, α-anhydrate; ●, β-anhydrate.

intensity, because the crystallinity of 3-component samples (i.e. α-monohydrate, β-anhydrate and non-crystalline solid) could not be estimated by Hermans' method. The results are summarized in Table 2. The crystallinity of ground α-monohydrate increased 19·6%, and the ground α-anhydrate was 44·0% transformed into α-monohydrate. The ground β-anhydrate was 23·4% transformed into α-monohydrate and was 37·6% recrystallized to β-anhydrate.

Change of specific rotation and α- and β-lactose content during grinding

Fig. 7 shows a typical change of specific rotation profiles of α-monohydrate lactose due to grinding. The changes of α- and β- lactose content of various kinds of lactose during grinding are shown in Fig. 8. The α-lactose content of ground α-monohydrate decreased with increased grinding time. After 10 h grinding, about 10% of α-lactose was transformed into β-lactose. The α-lactose content of ground α-anhydrate also decreased with increased grinding time; after 10 h grinding 16% of α-lactose was transformed into β-lactose. In the case of β-anhydrate, the α-lactose content increased with increased grinding time, after 10 h grinding 20% of β-lactose was transformed into α-lactose.

Discussion

Water adsorption on crystalline and non-crystalline lactose

Various kinds of crystalline lactose were transformed into non-crystalline lactose by mechanical stress during grinding (Fig. 1), in agreement with the findings of Morita et al (1984) with the α-monohydrate of lactose. The adsorbed water of the ground products of all kinds of lactose increased as a consequence of decreased crystallinity of the samples. This suggests that non-crystalline lactose is hygroscopic, so the adsorbed water increased with an increase of non-crystalline lactose (Fig. 4). Lactose molecules pack in the crystal by hydroxyl group hydrogen bonds (Noordik et al 1984). The hydrogen bonding network of lactose is destroyed by mechanical force, and water is adsorbed on the active hydrogen binding site on the new surface of the ground lactose, which contains disordered crystals or non-crystalline parts. We suggest that the amount of adsorbed water of crystalline lactose increases as a consequence of decreased crystallinity of ground lactose.

Isomerization of lactose by the mechanochemical effect during grinding

The change of α- and β-lactose content of various kinds of lactose during grinding suggests the isomerization of lactose by mechanochemical energy as shown in Fig. 8. The results of IR spectra, DTA and TGB curves (Figs 2, 3) of the ground lactose samples and the crystal content of ground lactose after storage (Table 2) supported the idea of isomerization in the solid-state during grinding. In general, α- or β-lactose in

aqueous solution is transformed rapidly and the α- and β-lactose content reach constant values, as shown in Table 1. However, the isomerization of lactose does not proceed in the solid-state under normal storage conditions (0–40°C, 0–75% relative humidity). Therefore, the isomerization of lactose in the solid state must occur only during grinding. The active solid state for chemical reaction during grinding has been called the triboplasma state (Senna 1985). The results as shown in Fig. 8 suggest that the isomerization rate of α-anhydrate lactose is more than that of the α-monohydrate. The rationale for this is that the ground α-monohydrate is still partly crystalline with crystal water; even samples ground for 10 h had about 30% crystalline α-monohydrate and 0·62 mol mol^{-1} of crystal water. Thus about 30% of the total water is crystal water which is unable to initiate isomerization of lactose. On the other hand, the ground α-anhydrate had low crystallinity and high content of adsorbed water which is active for solid state isomerization.

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