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Transformation of lactulose trihydrate into anhydrous lactulose by fluidized bed drying and its characterization

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Abstract—Stable anhydrous lactulose was produced from lactulose trihydrate by stepwise heating on a fluidized bed. The processes were performed on stable powder forms. The anhydrous lactulose was characterized by an opaque white appearance, a coarse surface structure with random cracks and indentations, a high degree of crystallization, stability under humid conditions, and by X-ray powder diffraction, differential thermal analysis, and differential thermogravimetry. Those characteristics were different from those of the original trihydrate, which was transparent, had a smooth surface and a higher degree of crystallization, was stable under humid conditions and had different X-ray powder diffraction, differential thermal analysis, and thermogravimetric characteristics. The transformation was enhanced when the inlet temperature was 45–55 °C or when the temperature of the fluidized bed was over 40 °C. At these cutoff temperatures, both crystalline forms were observed.

Keywords: Lactulose trihydrate; Anhydrous lactulose; Dehydration; X-ray powder diffraction; Differential thermal analysis

1. Introduction

Lactulose was first produced in 1930 as anhydrous lactulose crystallized from an alcohol solution.[1] Lactulose trihydrate was first described in the literature in 1992[2] and in a patent filed in 1991 but not issued as a patent until 1994.[3] It was crystallized from aqueous solution,[2,3] and its physicochemical properties[4] and solubility profile[5] were reported. Crystalline lactulose is normally marketed in the form of anhydrous lactulose, dehydrated lactulose trihydrate obtained by vacuum drying was reported to be amorphous, not to exhibit a sharp ^{13}C cross-polarization magnetic angle spinning (CPMAS) NMR spectrum,[2] and to be highly hygroscopic.[6] Patents also described that removing the water of crystallization from the trihydrate by a fluidized bed drying method[7] and other methods[6] would produce stable anhydrous lactulose. The present study investigated the process of transforming the trihydrate into the anhydrous form using fluidized bed drying, from a physicochemical viewpoint.

2. Results and discussion

2.1. Temperature control of fluidized bed drying

The temperature profiles recorded during the stepwise heat processing by fluidized bed drying are listed in Table 1. The inlet temperature was controlled and resultant temperatures at the fluidized bed and the outlet were monitored. The sample of lactulose trihydrate was heat processed up to an inlet temperature of 70 °C. The temperature difference between the inlet and the fluidized bed increased with increasing temperature, because of increasing heat loss from the equipment. The temperature differences between the inlet temperature and the fluidized bed temperature at inlet temperatures of 50

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Table 1. Temperature profiles during transformation from lactulose trihydrate to anhydrous lactulose by fluidized bed drying

Controlled inlet temperature (°C)	Temperature at sampling (°C)			Temperature difference (°C)[a]
	Inlet	Fluidized bed	Outlet	
25	24.5	24.4	24.4	−0.1
35	34.2	33.3	32.6	−0.9
40	39.8	37.5	37.1	−2.3
45	44.9	41.0	40.2	−3.9
50	50.7	44.2	43.3	−6.5
55	54.7	48.1	46.1	−6.6
60	59.2	52.8	51.6	−6.4
65	65.8	58.3	56.0	−7.5
70	70.5	61.3	59.1	−9.2
Cooled to 50	50.5	45.0	46.0	−5.5

[a] Temperature difference = (fluidized bed temperature) − (inlet temperature).

and 55 °C were especially large compared with those at other temperatures. These temperatures corresponded to those of the largely decreasing water of crystallization described later. Therefore, these large temperature differences were considered to represent the latent heat of evaporation of water of crystallization.

2.2. Water changes and stability versus humidity

The changes in water content as measured by titration, free water lost on silica gel, and stability at 52% relative humidity associated with the transformation from the trihydrate to the anhydrous form are shown in Figure 1. The water content of samples at an inlet temperature of not more than 40 °C was similar to those of the trihydrate (13.6%, theoretical level), whereas samples at an inlet temperature of at least 60 °C showed the low water content (0.3–0.4%) of the anhydrous form, with a gradual transition between these levels at an inlet temperature of 45–55 °C. The amount of free water in all samples was very low (0.04–0.13%) after drying in a desiccator with silica gel at 25 °C for 120 h.

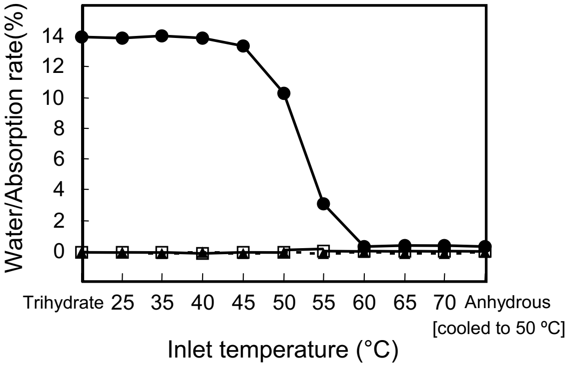

Figure 1. Changes in water content and stability under humid conditions associated with the transformation from lactulose trihydrate to anhydrous lactulose by fluidized bed drying. ●: water by titration, ▲: free water, □: absorption rate at 52% RH at 24.5 °C for 24 h.

Under conditions of 52% relative humidity at 24.5 °C for 24 h, none of the samples absorbed moisture; instead, they released a very small amount (0.15–0.07%). In contrast, the trihydrate dehydrated by vacuum drying (containing 0.5% water by titration) was extremely hygroscopic, as indicated by the amount of water absorbed (10.7% weight increase) and evidence of partial dissolution.

A reference sample of anhydrous lactulose crystallized from methanol solution showed 0.2% water, 0.04% free water, and a 0.04% weight decrease under the humid conditions.

All the samples with different water contents, along with the anhydrous lactulose prepared from the trihydrate by this method, contained little free water, and exhibited stability under humid conditions. This suggests that the water of crystallization exists uniformly until just before its release from the crystals.

2.3. Surface structure and appearance

Typical changes in appearance produced by the processing, as observed by light microscopy, are shown in Figure 2a. Lactulose trihydrate appears as colorless and clear crystals, whereas anhydrous lactulose dehydrated by fluidized bed drying appears white and opaque. The changes associated with the processing consisted of four stages: (1) clear crystals of the trihydrate at an inlet temperature of 25 °C (indicated in Fig. 2a), (2) a change from clear crystals to a slightly cloudy powder observed at an inlet temperature of 35–45 °C, (3) a change to a higher level of whiteness and opacity observed at an inlet temperature of 50 °C, and (4) the full whiteness and cloudiness observed in anhydrous lactulose at and over an inlet temperature of 55 °C. In comparison, a reference sample of anhydrous lactulose crystallized from methanol solution appeared colorless and clear (data not shown).

Typical changes in the surface structure of powders associated with the processing, as observed by scanning electron microscopy, are shown in Figure 2b. The flat surface of lactulose trihydrate changed to a coarse surface with random cracks and indentations of anhydrous lactulose. These changes in surface structure could also be divided into four stages: (1) flat surfaces of the trihydrate at an inlet temperature of 25 °C, (2) an appearance of a partly coarse surface at inlet temperatures of 35–40 °C, (3) an increase in cracks and indentations at inlet temperatures of 45–50 °C, and (4) the full fine scars in anhydrous lactulose observed at and over an inlet temperature of 55 °C. In comparison, the surface of a reference sample of anhydrous lactulose appeared smooth (data not shown).

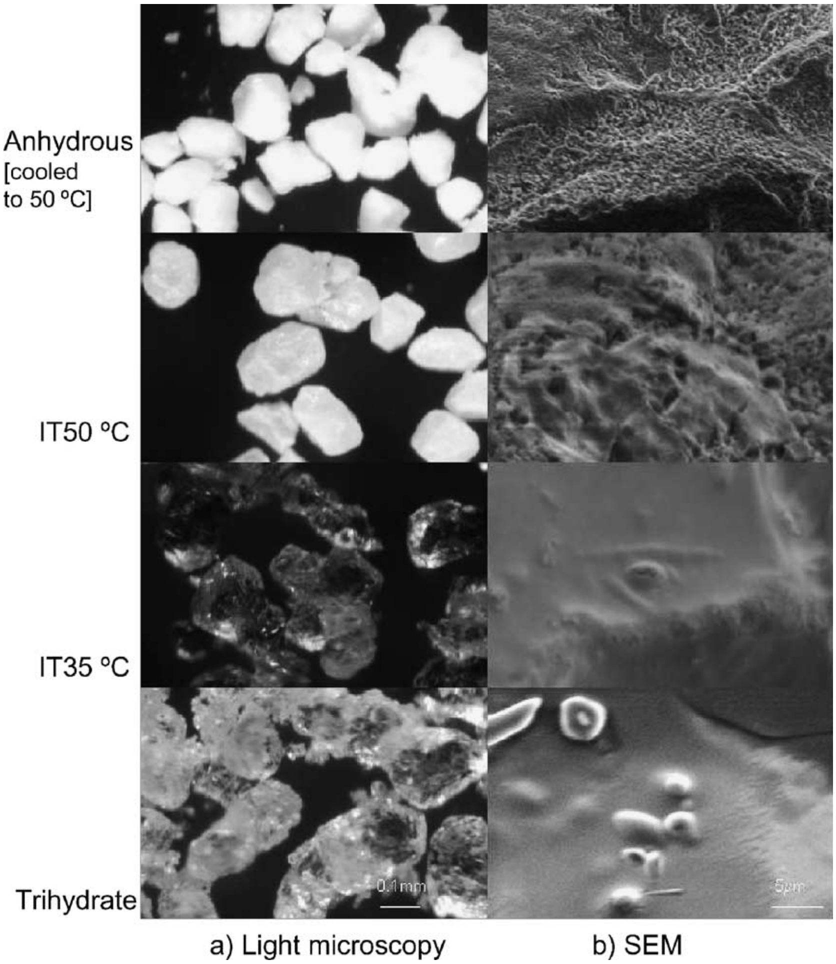

Figure 2. Changes in appearance of samples observed by (a) light microscopy and (b) scanning electron microscopy (SEM) associated with the transformation from lactulose trihydrate to anhydrous lactulose by fluidized bed drying. IT: inlet temperature.

2.4. X-ray powder diffraction spectrum and degree of crystallization

Typical X-ray powder diffraction spectra of the samples are shown in Figure 3. An X-ray pattern of lactulose trihydrate maintained up to an inlet temperature of 45 °C (Fig. 3) and another X-ray pattern of anhydrous lactulose fully transformed at an inlet temperature of not less than 60 °C are shown. Samples obtained for an inlet temperature of 50 and 55 °C exhibited a mixture of the two patterns shown. The main peak angle and the unique peak angles for the trihydrate were $2\theta = 21.9°$ and $2\theta = 9.1°$, 12.9°, 16.5°, 19.3°, and 20.5°, respectively, and $2\theta = 15.2°$ and $2\theta = 10.4°$, 20.0°, 20.9°, 22.3°, and 25.9° for the anhydrous form, as indicated in Figure 3 by the arrows.

Degrees of crystallization of the samples calculated from X-ray powder diffraction spectra are listed in Table 2. The approximate degree of crystallization of the trihydrate was 89%, decreasing to 80% for the anhydrous form. The samples from inlet temperatures of 45–55 °C showed transitional values. The value of transformed anhydrous lactulose was the same as that for a reference sample of anhydrous lactulose.

2.5. Differential thermal analysis and differential thermogravimetry

Typical differential thermal analysis (DTA) and differential thermogravimetry (DTG) analyses for the samples are shown in Figure 4. Peak temperatures in DTA, heats calculated from DTA peaks corresponding to those of lactulose trihydrate and anhydrous lactulose, and weight loss before and after the peak in DTG corresponding to the trihydrate are listed in Table 3. From these data, the changes from trihydrate to anhydride can be divided into four stages on the basis of inlet temperature: (1) 25 °C (similar to the trihydrate), (2) 35–45 °C, (3) 50–55 °C, and (4) $\geqslant 60$ °C (similar to the anhydrous form). The peak corresponding to anhydrous lactulose first appeared in the DTA graph at an inlet temperature of 35 °C (Fig. 4). At an inlet temperature of 50–55 °C, the peak corresponding to the trihydrate decreased in height, and at an inlet temperature of $\geqslant 60$ °C the peak

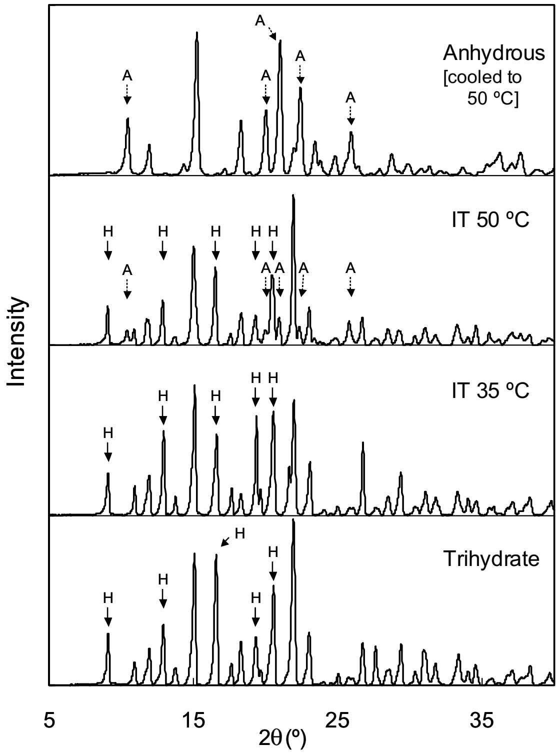

Figure 3. Changes in X-ray powder diffraction spectra associated with the transformation from lactulose trihydrate to anhydrous lactulose by fluidized bed drying. H and A arrows: unique peaks to trihydrate and anhydrous form, respectively. IT: inlet temperature.

Table 2. Degree of crystallization calculated from X-ray powder diffraction spectra associated with the transformation from lactulose trihydrate to anhydrous lactulose by fluidized bed drying

Sample and inlet temperature (°C)	Degree of crystallization (%)
Lactulose trihydrate	88.8
25	89.8
35	88.3
40	90.2
45	92.0
50	83.9
55	77.7
60	80.8
65	81.0
70	80.0
Anhydrous lactulose [cooled to 50 °C]	79.6
Lactulose RS[a]	80.9

[a] Lactulose RS: reference sample of anhydrous lactulose prepared from methanol solution.

corresponding to the trihydrate disappeared. Hence, both peaks—corresponding to the trihydrate and anhydrous lactulose—were evident in DTA graphs for an inlet temperature of 35–55 °C.

The peak temperature corresponding to the trihydrate lowered from 71 to 63 °C for an inlet temperature of at least 35 °C, where the peak corresponding to anhydrous lactulose was also observed in the same DTA graph. However, pressurization of the samples for an inlet temperature of 35 and 50 °C changed the peak corresponding to the trihydrate into a main peak with a small shoulder peak at a higher temperature, with this higher peak approaching the level of the original trihydrate (Table 3). The peak temperature corresponding to anhydrous lactulose was 171–172 °C, higher than the 164.5 °C determined for the reference sample of anhydrous lactulose. However, with pressurization, the temperature decreased and approached the level of the reference sample of anhydrous lactulose. Scanning electron microscopy showed that the anhydrous lactulose had a porous surface structure. The shifts in peak temperature corresponding to the trihydrate and the anhydrous form observed by pressurization were attributed to cancellation of a thermal insulation effect of this porous surface structure of anhydrous lactulose.

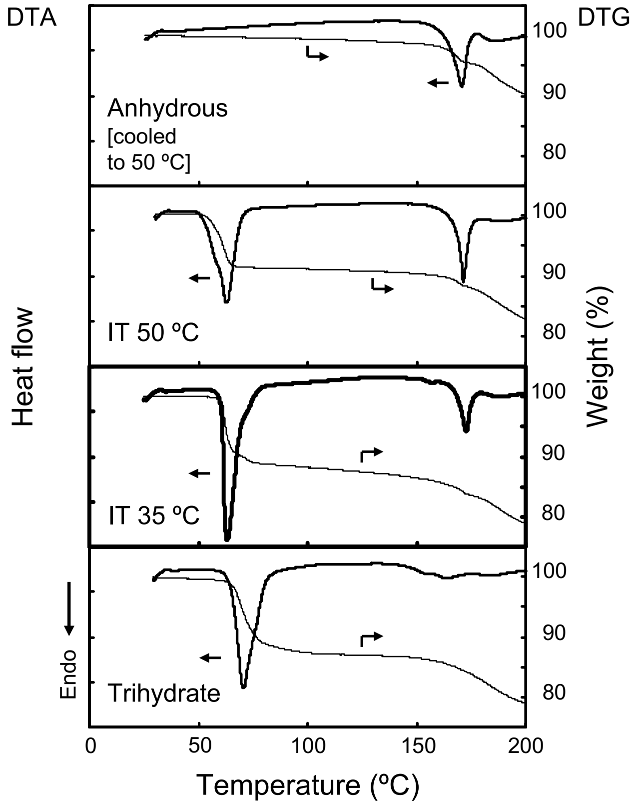

Figure 4. Changes in differential thermal analysis (DTA) and differential thermogravimetry (DTG) graphs associated with the transformation from lactulose trihydrate to anhydrous lactulose by fluidized bed drying. IT: inlet temperature.

The DTA analysis of the trihydrate suggests that a temperature of about 50 °C initiates its melting, but this temperature is different from the initiation inlet temperature (45 °C) that initiates drastic changes on fluidized bed drying. However, the temperature that initiates melting of the trihydrate in the sample at an inlet temperature of 35–55 °C in DTA shifted to the lower side, as

Table 3. Peak temperature, heat changes, and weight loss in differential thermal analysis (DTA) and differential thermogravimetry (DTG) associated with the transformation from lactulose trihydrate to anhydrous lactulose by fluidized bed drying

Sample and inlet temperature (°C)	Peak temperature in DTA originated from		Heat calculated from DTA peak corresponding to		Weight loss before and after trihydrate peak in DTG (%)
	Trihydrate (°C)	Anhydrous (°C)	Trihydrate (kJ/g)	Anhydrous (kJ/g)	
Trihydrate	70.6	—	−3.62	—	12.3
25	71.0	—	−3.61	—	12.0
35	63.3	172.5	−3.32	−0.873	11.4
40	63.2	171.2	−3.48	−0.839	11.6
45	63.0	171.8	−3.45	−0.939	11.5
50	62.9	171.5	−2.71	−0.990	9.0
55	62.7	171.3	−0.846	−1.07	2.7
60	—	171.4	—	−1.17	—
65	—	171.1	—	−1.23	—
70	—	170.7	—	−1.14	—
Anhydrous [cooled to 50 °C]	—	170.7	—	−1.21	—
35 [pressed]	64.1, 71.3	167.4	−3.95	−1.42	13.2
50 [pressed]	58.9, 67.6	170.9	−2.86	−0.989	10.1
Anhydrous [cooled to 50 °C, pressed]	—	168.1	—	−1.01	—
Lactulose RS[a]	—	164.5	—	−1.07	—

[a] Lactulose RS: reference sample of anhydrous lactulose prepared from methanol solution.

shown in Figure 4. The latter temperature corresponds to the inlet temperature that initiated the drastic changes from the trihydrate to the anhydrous form on fluidized bed.

The heat output based on calculations from the DTA peak corresponding to the trihydrate was −3.6 kJ/g, and about 90–95% of this was at the inlet temperature of 35–45 °C. The heat outputs at inlet temperatures of 50 and 55 °C showed intermediate values of 75% and 23% of that of lactulose trihydrate, respectively, representing the decrease in the proportion of trihydrate. The heat output calculated from the DTA peak corresponding to the anhydrous form at inlet temperatures of 35–45 °C was about 75% of that of the sample of it cooled to 50 °C, which had been fully transformed into the anhydrous form. The combined heat output associated with the peaks corresponding to the trihydrate and anhydrous lactulose at the inlet temperature of 35–45 °C was 160–165% of those of pure samples. This value of over 100% was attributed to the anhydrous crystals being produced secondarily during the heating process of the DTA analysis by concentration of molten trihydrate and crystallization of the anhydrous form seeded with a small amount of this form, which was produced initially by fluidized bed drying. Therefore, the values calculated from DTA peaks corresponding to anhydrous lactulose are considered unrepresentative of its real quantitative state. The conversion rate of trihydrate to anhydrous form might be typically represented by the value of [100 minus the rate of the heat output corresponding to trihydrate in the processed powder to that of pure trihydrate]. The calculated conversion rates for the inlet temperature of 25, 35, 40, 45, 50, and 55 °C were 0.7%, 8.5%, 4.7%, 5.0%, 25%, and 77%, respectively.

The weight losses before and after the trihydrate peak in DTG at inlet temperatures of 25–45 °C were 11.5–12.3%, about 2% lower than the theoretical value (13.6%) or the measured water by titration (13.4–14.1%). These might represent insufficient evaporation of melted water of crystallization through the formation of a thick syrupy surface. The levels were slightly lowered at an inlet temperature of at least 35 °C, where the anhydrous form peak appeared in DTA. The weight losses at inlet temperatures of 50 and 55 °C showed intermediate values, representing the decreases in the proportion of trihydrate.

Substantial weight losses were observed at the peak of the anhydrous form in DTG, although it does not contain water of hydration. These represent some degradation and decomposition processes, as confirmed by the browned appearance of samples after measurements (data not shown).

The DTA and DTG data in Table 3 also suggest that the first appearance of the anhydrous form at an inlet temperature of 35 °C was limited, and did not progress until the inlet temperature reached 50 °C.

2.6. Transformation of lactulose trihydrate to stable anhydrous lactulose

It has been reported that the dehydration of carbohydrates having water of crystallization can result in several forms of the solid phase, depending on the conditions used. α-Lactose monohydrate was transformed into unstable anhydrous α-lactose in a vacuum at 100 °C and into stable anhydrous α-lactose by water vapor at 150 °C[8] or by refluxing in methanol[9]. Trehalose dihydrate was transformed into anhydrous polymorphic forms or an amorphous phase.[10]

Lactulose trihydrate was transformed into amorphous anhydrous lactulose by vacuum drying at 57 °C.[2] The dehydrated trihydrate prepared by vacuum drying in the present study, which had the same ^{13}C CPMAS-NMR spectrum (data not shown) as that reported for amorphous dehydrated trihydrate,[2] was unstable and highly hygroscopic. In contrast, the dehydrated trihydrate prepared by stepwise heating on a fluidized bed described in this study released the water of crystallization and showed stability, even under humid conditions. This indicates that the anhydrous lactulose prepared in this study was different from amorphous, vacuum-dried trihydrate. Since the anhydrous lactulose prepared in this study showed a clear and fixed X-ray diffraction pattern, a high degree of crystallization, and a clear peak in DTA, it was considered to have a fixed crystalline conformation of stable anhydrous lactulose.

The transformation from trihydrate to stable anhydrous form was observed using several indexes, and the changes were shown to occur in a stepwise manner. The first stage was the original trihydrate, and the change to the second stage was first observed at an inlet temperature of 35 °C, at which the sample showed changes in surface structure and appearance, and the presence of a peak corresponding to the anhydrous form in DTA. The sample properties remained stable until an inlet temperature of 40 °C was reached. Large changes were observed during the third stage at an inlet temperature of 50–55 °C, at which the sample showed distinct changes in surface structure and appearance, a decrease in water content (as measured by titration), a decrease in the peak area corresponding to trihydrate and an increase in the peak corresponding to anhydrous lactulose in DTA analysis, mixed X-ray diffraction spectra of both forms, and a change in the degree of crystallization. The final stage occurred at an inlet temperature of 60 °C, after which the physicochemical data did not change. The peaks corresponding to trihydrate in DTA and in the X-ray diffraction spectrum were diminished at the stage. The water content of the anhydrous form was very low (0.3%), and the degree of crystallization was about the same as that of a reference sample of anhydrous lactulose. At an inlet temperature of 45 °C, the sample exhibited minor transitional changes in surface structure, water content, and the degree of crystallization.

The foregoing observations suggest that the change from trihydrate to anhydrous was initiated and limited at the surface of the trihydrate, as indicated by the changes in surface structure and the DTA analyses, but not in X-ray diffraction spectra nor water content.

The changes of trihydrate to stable anhydrous form appear to occur in the following manner: partially melted trihydrate is subjected to vapor diffusion and concentration, to crystallization of the anhydrous form in the liquid phase, and then to full dehydration. These changes may develop partially and proceed from the surface to the center of the powder by heat flow from the fluid bed air. The reduction in the volume of anhydrous compared with trihydrate caused by recrystallization with the loss of water of crystallization would produce a coarse surface with random cracks and a white and cloudy appearance.

The data also suggest that the transformation of trihydrate to anhydrous occurred via a stable powder form.

An anhydrous crystalline lactulose powder [MLC-A (Morinaga Milk Industry)] has been manufactured commercially in accordance with the method described here and marketed for food and pharmaceutical uses. It shows the stability required for pharmaceuticals and handling for commercial uses.

3. Experimental

3.1. Lactulose trihydrate

Lactulose trihydrate for this study was prepared by recrystallization in water, in which trihydrate (obtained as a process sample crystallized from lactulose solution in the commercial production of MLC-A [crystalline anhydrous lactulose powder, Morinaga Milk Industry]) was dissolved at a concentration of 70% total solids and crystallized at 5 °C. The separated crystals were washed with water, dried and kept in a desiccator with constant relative humidity of 73% at 20 °C.

3.2. Transformation from lactulose trihydrate into anhydrous lactulose by fluidized bed drying and sampling

One kilogram of lactulose trihydrate was placed in the chamber of a fluidized bed dryer (Glat WSD 5/1, Okawara, Shizuoka), fluidized with ambient air flowing at room temperature (25 °C), heated stepwise to inlet temperatures of 35, 40, 45, 50, 55, 60, 65, and 70 °C (each step held for 30 min), and then cooled to 50 °C to produce anhydrous lactulose. The temperatures at the fluidized bed and its outlet were also monitored. Just before each temperature increase, about 20 g of the sample was taken, packed and sealed in an aluminum bag, and then frozen rapidly by dipping in a dry ice–ethanol system. The samples were kept in a freezer (−35 °C), and thawed at room temperature one day before analysis.

3.3. Reference samples

3.3.1. Dehydrated lactulose trihydrate. Dehydrated lactulose trihydrate was prepared by vacuum drying at 57 °C for 24 h, and then stored in a vacuum desiccator.[2]

3.3.2. Pressed samples. Pressed samples corresponding to inlet temperatures of 35 and 50 °C, and of anhydrous lactulose [cooled to 50 °C] were prepared by gradual compression to 6 ton/1.33 cm^2 under a vacuum, and then powdered in a triturator.

3.3.3. Reference sample of anhydrous lactulose. A reference sample of anhydrous lactulose was prepared by crystallization of the trihydrate from MeOH solution.

3.4. Analyses

X-ray powder diffraction spectra were recorded using a Shimazu device (XRD-6000, Shimazu, Kyoto). The data were processed and the degree of crystallization calculated using Shimazu software with an intensity ratio of 1.000 of the reflection from the amorphous part to that of the crystalline part.

DTA and DTG were performed using open cells, a sample weight of 3 mg, and a temperature increase of 3 °C/min using a Shimazu device (DTG-50, Shimazu).

Water content was assayed using a Karl Fischer titrator (Hiranuma AQV-55, Ibaragi).

The surface structure was observed using a scanning electron microscope SM-200 (Topcon, Tokyo) in high-vacuum mode at a magnification of 3000 times, after coating the sample with gold by ion sputtering. The samples were also observed with a light stereomicroscope at a magnification of 64×.

Loss on drying in a desiccator with silica gel at 25 °C for 120 h was used as an indicator of free water.

The weight increase rate (%) in a desiccator at 52% relative humidity and 24.5 °C for 24 h in a constant humidity system, using satd aq $Mg(NO_2)_2 \cdot 6H_2O$ as a solid phase, was used as an indicator of stability under humid conditions.

References

1. Montgomery, E. M.; Hudson, C. S. *J. Am. Chem. Soc.* **1930**, *52*, 2101–2106.
2. Jeffrey, G. A.; Huang, D. E.; Pfeffer, P. E.; Dudley, R. L.; Hicks, K. B.; Nitsch, E. *Carbohydr. Res.* **1992**, *226*, 29–42.
3. Tomita, M.; Shimamura, S.; Tamura, Y.; Mizota, T.; Nakano, S.; Suzawa, I. US Patent 5,304,251, 1994; *Chem. Abstr.*, 1994, *121*, 9923c.
4. Tamura, Y.; Mizota, T.; Shimamura, S.; Tomita, M. *Bull. IDF* **1993**, *289*, 43–53.
5. Mizota, T.; Suzawa, I.; Seki, N.; Tamura, Y.; Shimamura, S. *Carbohydr. Res.* **1994**, *263*, 163–166.
6. Tomita, M.; Shimamura, S.; Tamura, Y.; Mizota, T.; Nakano, S. J. Patent 2,977,654, 1999; *Chem. Abstr.*, 1993, *119*, 119903n.
7. Shimamura, S.; Tamura, Y.; Mizota, T.; Suzawa, I.; Seki, N. J. Patent 3,022,699, 2000; *Chem. Abstr.*, 1994, *121*, 231265y.
8. Walstra, P.; Jenness, R. Carbohydrates. In *Dairy Chemistry and Physics*; A Wiley-Interscience Publication: New York, 1984; pp 27–41.
9. Buma, T. J.; Wiegers, G. A. *Neth. Milk Dairy J.* **1967**, *21*, 208–213.
10. Nagase, H.; Endo, T.; Ueda, H.; Nakagaki, M. *Carbohydr. Res.* **2002**, *337*, 167–173.