The Influence of Crystallization Conditions on the Morphology of Lactose Intended for Use as a Carrier for Dry Powder Aerosols

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Abstract

Lactose has been widely used as a carrier for inhalation aerosols. The carrier morphology is believed to affect the delivery of the drug. The aim of this study was to investigate the effects of crystallization conditions on the morphology of α-lactose monohydrate intended for use as the carrier for dry powder aerosols.

The crystallization of lactose was carried out from aqueous solutions at different supersaturations, temperatures, different stages of crystallization and in the presence of different water-miscible organic solvents. The majority of lactose crystals were found to be either tomahawk-shaped or pyramidal after crystallization at an initial lactose concentration between 33–43% w/w, but these became prismatic if the lactose concentration was increased to 50% w/w. A further increase in the lactose concentration to 60% w/w led to the preparation of elongated cuboidal crystals. Higher initial lactose concentrations tended to result in the crystallization of more elongated particles. Crystallization at 40°C was shown to prepare lactose crystals with a more regular shape and a smoother surface than those crystallized at 0°C. Lactose particles generated during the later stage of crystallization were found to be more regular in shape with a smoother surface than those prepared in the earlier stage. The addition of 10% (v/v) methanol or ethanol or acetone to the mother liquor increased the growth rate of lactose particles whereas addition of propanol or glycerine inhibited the rate of crystal growth. Lactose crystals prepared in the presence of glycerine were more regularly shaped with a smoother surface than those prepared in the presence of ethanol or acetone. All the resultant crystals were shown to comprise α-lactose monohydrate.

Lactose crystals could be prepared with a precisely defined morphology by means of carefully controlling the crystallization conditions.

Lactose, 4-(β-D-galactosido-)-D-glucose, can be obtained in either two basic isomeric forms, α and β-lactose, or as an amorphous form. α-Lactose monohydrate has been observed in a wide variety of shapes, depending on the conditions of crystallization. The principal factor governing the crystal habit of lactose is the supersaturation, i.e. the ratio of actual concentration to the solubility at the specific temperature (Herrington 1934). At higher supersaturations, crystallization is forced to occur more rapidly and more elongated crystals form. As the supersaturation decreases, the dominant crystal shape changes to diamond-shaped plates and then to pyramids, resulting from an increase in the thickness of the diamond. With slow crystallization, lactose crystals usually exhibit the most common characteristic shape of tomahawks (Clydesdale et al 1996).

The crystallization of lactose has received the most attention of all sugars especially in the dairy industry and is the subject of some excellent reviews (e.g. Hartel & Shastry 1991). However, most of the studies have focused on the crystallization of lactose from commercial cheese whey, since this is the primary source of refined lactose. The principal reasons for the study of lactose crystallization, in the dairy industry, are either with a view to inhibiting crystal growth of lactose so as to prevent 'sandiness' in some food products such as ice cream (Nickerson 1962) or maximizing the

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gross yield of lactose crystals. Although different crystal forms of lactose have been commercially available to the pharmaceutical industry, few studies have been carried out to investigate the effects of crystallization conditions on the particle morphology of lactose, such as particle size, particle shape and surface texture. These morphological features of lactose crystals are of great importance in determining their physical properties and in particular, they play a crucial role in determining the surface interaction of lactose particles with other adhered particles such as drug powders. For example, α-lactose monohydrate has been employed most frequently as the carrier for dry powder aerosols and it is usually designed to have a size between 63–90 μm for this purpose (Timsina et al 1994). The surface smoothness, particle size and polymorphic forms of lactose all have been shown to affect the dispersion and deaggregation of drugs (Ganderton 1992; French et al 1996; Larhrib et al 1998). Therefore, we have investigated the effects of some preparative conditions on the morphology of lactose particles. This was carried out with a view to preparing lactose particles of favourable size, shape and surface texture to act as a carrier in dry powder formulations designed to deliver drugs to the lower airways.

Materials and Methods

One-step crystallization from aqueous solutions
A predetermined amount of lactose (Lactochem, Borculo Whey Ltd, Chester, UK) was dissolved in 100 mL distilled water at approximately 80°C. After filtration through a Whatman filter paper ($<0.45\,\mu$m), the solution was transferred to a 150-mL glass beaker which had been placed in either an ice bath (0°C) or a water bath at 40°C. The solution was stirred at 500 rev min^{-1} (Heidolph Overhead Stirrer, Fisons Laboratory Instruments, UK) with a four-blade (1 × 3 cm) stirrer which was situated 2 cm above the bottom of the container. After the crystallization had been allowed to continue for a predetermined period of time, the crystals were filtered and washed sequentially with 60% (v/v) and absolute ethanol. The crystals were allowed to dry at room temperature overnight before drying in a vacuum oven at 70°C for 3 h. A small amount of sample (approximately 0.5 g) was taken from each batch of lactose for the measurement of particle size, shape and surface smoothness. The remaining lactose crystals were then poured into a 90-μm sieve that had been placed upon a 63-μm sieve. The particles were then sieved manually into three size fractions (<63, 63–90 and $>90\,\mu$m), which were collected and weighed separately. The lactose fractions thus obtained were transferred separately to sealed vials and placed into a desiccator over silica gel until required for further investigation. Eleven batches of lactose were prepared under the conditions listed in Table 1.

Two-step crystallization
Lactose (200 g) was dissolved in 200 mL distilled water at approximately 90°C. After filtration through Whatman filter paper (0.45 μm), the solution was transferred to a 500-mL glass beaker and then stirred for 2.5 h under the conditions described above. The first batch of crystals was filtered and the mother liquor was placed back into the beaker and stirred for a further 16 h to obtain the second

Table 1. The conditions used to crystallize lactose and the resultant particle size and distribution of the prepared lactose crystals.

Batch no.	C_L (% w/w)	T_c (°C)	t_c (h)	Diameter $\pm$ s.d. (μm)	% Particle (μm)		
					<63	63–90	>90
1	33	40	12	83.6 $\pm$ 12.8	13.9	45.8	40.3
2	33	40	24	115.8 $\pm$ 14.6	5.6	15.1	79.3
3	33	0	12	63.7 $\pm$ 9.4	33.0	40.0	26.8
4	33	0	24	100.3 $\pm$ 18.9	15.2	17.2	67.6
5	43	0	5	94.4 $\pm$ 13.4	19.6	21.8	56.6
6	43	0	12	104.5 $\pm$ 14.8	14.9	23.2	61.9
7	43	40	5	103.8 $\pm$ 20.6	14.4	21.6	64.0
8	43	40	12	100.6 $\pm$ 15.3	24.5	17.9	57.6
9	50	40	3	88.8 $\pm$ 13.8	27.5	31.9	40.6
10	60	40	0.3	76.4 $\pm$ 15.7	33.8	46.3	19.9
11	60	40	1.5	91.8 $\pm$ 17.9	26.3	27.6	46.1

C_L, initial lactose concentration; T_c, crystallization temperature; t_c, crystallization time.

batch of crystals. Both batches were washed separately with 60% (v/v) and absolute ethanol, respectively. After drying at room temperature overnight, both batches of crystals were classified separately into two size fractions by sieving manually. The sub-batches obtained from the first and second batch were designated batches A and B with a sieved particle size of 63–90 μm or a' and b' with a sieved particle size <63 μm. Each size fraction of crystals was dried in a vacuum oven at 70°C for 3 h before transferring to a sealed vial and placed in a desiccator over silica gel.

Crystallization in the presence of small amounts of organic solvents

Lactose (75 g) was dissolved in 100 mL distilled water at approximately 90°C. After filtration through a Whatman filter paper while hot, the solution was transferred to a 250-mL beaker and allowed to cool to room temperature without any disturbance. It was then stirred under the conditions described above and a predetermined amount of a water-miscible organic solvent was added dropwise to the solution such that the final concentration of the solvent in the solution was 10% (v/v). During the crystallization process, the crystals were sampled at different time points for monitoring the particle size and morphology using an optical microscope. The stirring was continued until the majority of the crystals were grown to a size between 63–90 μm. The crystals were filtered and washed with 60% and absolute ethanol. Finally the crystals were allowed to dry at room temperature before being stored in a desiccator over silica gel.

Measurement of particle size and shape by optical microscopy image analysis

A small amount of lactose particles was scattered on a microscope slide using a small brush ensuring that the particles deposited separately. The slide was then mounted on an optical microscope (Labophot-2, Nikon, Japan) and the images of the particles were transferred to an IBM-compatible computer through a Nikon camera. Particle images were analysed automatically using analySIS 2.0 (SIS Image Analysis GmbH, Germany). The size of each individual particle was calculated as the diameter of a spherical particle that produces a projected image of the same area to the measured particle. At least 300 particles were measured for each batch of lactose and the surface-volume mean diameter (d_{sv}) recorded.

The morphology of lactose crystals was quantified by a combination of different descriptors,

derived from the length (L), width (W), perimeter (P) and area (A). These descriptors included the elongation ratio (L/W) and shape factor ($4\pi \times A/P^2$).

Density measurement

The true density of lactose was measured using a Beckman Air Comparison Pycnometer (Model 930, Beckman Instrument, Inc., Fullerton, US). Each sample was measured in triplicate and the mean was taken.

Measurement of specific surface area

The surface area of the various lactose fractions was determined by an air permeation method employing a Fisher sub-sieve sizer, which measures the ability of air to flow through a packed powder bed. A mass of powder equal to its true density was compressed to different porosities in the cell of the Fisher sub-sieve sizer. The flow rate of air through the bed at a fixed pressure differential was transformed by the instrument to an average particle diameter (d_a). The minimum diameter under low porosity was considered to be the true diameter and employed to calculate the specific surface area (A_a) of the particles according to the following equation:

$$A_a = \frac{6 \times 10^4}{d_a \rho} \, | \, (cm^2 \ g^{-1}) \qquad (1)$$

where ρ is the true density (g cm^{-3}) and d_a is the average particle diameter (μm). Each sample was measured in duplicate and the mean particle size was taken to calculate the specific surface area of the particles.

The specific surface area obtained by optical microscopy (A_m) was calculated by replacing d_a in equation 1 with surface volume mean diameter (d_{sv}).

Calculation of rugosity of lactose particles

The rugosity (R_s) of a particle is defined as the ratio between the actual specific surface area, A_a, obtained by the air permeation method and the specific surface area A_m, obtained by the microscopic method (Carstensen 1980).

Characterization of particle morphology by scanning electron microscopy (SEM)

Double-sided adhesive tape was placed on an aluminium stub and after stripping off the upper side of the adhesive, a small amount of particles was

scattered on the stub and dispersed by tapping lightly on the edge of the stub with a spatula to break agglomerates. The particles were then coated with approximately 15–20 nm gold using a sputter coater (Polaron E5100, Polaron Equipment Ltd, Watford, UK) using an electrical potential of 2·0 kV, 20 mA. A Philips SEM501B scanning electron microscope (Einhoven, Holland) was used to produce several photomicrographs, scanning fields selected randomly at a number of different magnifications.

Characterization of polymorphic forms
A small amount of lactose crystals (4–5 mg) was placed in an open aluminium pan. This was then placed in the sample chamber of an STA 625 Differential Scanning Calorimeter (Rheometrics Scientific Ltd, Surrey, UK). An empty matched aluminium pan was used as the reference for all measurement. The instrument was calibrated using tin, indium and gallium as standard materials. Thermal gravimetric analysis and differential scanning calorimetry of the sample were carried out concomitantly at a heating rate of $10°C \, min^{-1}$ from ambient temperature to 280°C under N_2 flowing at $50 \, mL \, min^{-1}$.

The X-ray powder diffraction pattern of lactose was measured at room temperature with a Philips X'Pert Dual Goniometer (Philips Analytical, Holland). The X-ray source was a copper-K_α operated at a voltage of 40 kV and with a current of 50 mA. Samples were back-filled into 16-mm holders. Results were recorded over a range of 2–35° (2θ) with a step size of 0·04° and a count rate of $1 \, step \, s^{-1}$.

Results and Discussion

One-step crystallization from aqueous solutions
Lactose particles of different size and shape were prepared under different crystallization conditions (Table 1). To evaluate the relationship between the particle size of lactose and the specific conditions of crystallization, the following empirical equation was generated by 'step-wise forward algorithm' in multiple regression using Minitab for Windows (Version 10.2, Minitab Inc. USA).

$$Mean \ diameter \ (\mu m) = 1·50C_L + 0·13T_c + 2·46t_c$$

$$r = 0·80$$

$$(2)$$

where C_L is the initial lactose concentration (% w/w), T_c is the crystallization temperature (°C) and t_c is the time period (h) for crystallization.

According to equation 2, the particle size of lactose increases with an increase in the initial concentration of lactose or crystallization temperature or the time period of crystallization. The increased size of the final lactose crystals can be attributed to an increased growth rate of lactose crystals at higher initial concentrations. The effect of temperature on crystal growth is more complicated than that due to lactose concentration. Temperature influences two important aspects of the crystallization process, namely, supersaturation and the crystallization rate constant including rate of diffusion, rate of mutarotation and rate of orientation of lactose molecules into the crystal lattice, all of which will increase with temperature. However, supersaturation decreases with temperature. If the supersaturation is not substantially reduced, then increasing the temperature would be expected to accelerate crystal growth by decreasing viscosity and increasing kinetic activity of lactose molecules. For example, increasing the temperature from 30 to 50°C was shown to increase the rate of lactose crystal growth but no further increases in growth were observed between 50 and 70°C (Jelen & Coulter 1973). It is obvious that the longer the time for crystal growth, the larger the final particles will be. Since lactose particles employed as a carrier for dry powder aerosols are usually required to have a size between 63–90 μm, the time period of crystallization is one of the major operational parameters to be controlled in order to obtain the desired particle size of lactose crystals. However, lactose crystals prepared under all the conditions investigated exhibited a wide range of particle size distribution with relative standard deviation (s.d./mean) over 15%, resulting in a relatively small portion of particles with a size range between 63–90 μm (Table 1). Crystal size distribution was attributed to the inherent different rates of growth between crystals (Janse & deJong 1976), leading to some crystals growing faster than others.

The various batches of lactose crystals exhibited different shapes, which appear to be dependent upon the initial concentrations of lactose (Figure 1). At lower initial lactose concentrations (33% and 43%, w/w), the majority of the crystals obtained were either tomahawk- or pyramid-shaped (batches 1–8). When the concentration of lactose was increased to 50% (w/w), most of the resultant crystals were prismatic in shape (batch 9). When the concentration was increased to 60%, elongated particles were produced (batches 10 and 11). The change in crystal shape with the initial concentration is in agreement with a previous report (Nickerson 1962). Different shapes of lactose crystals
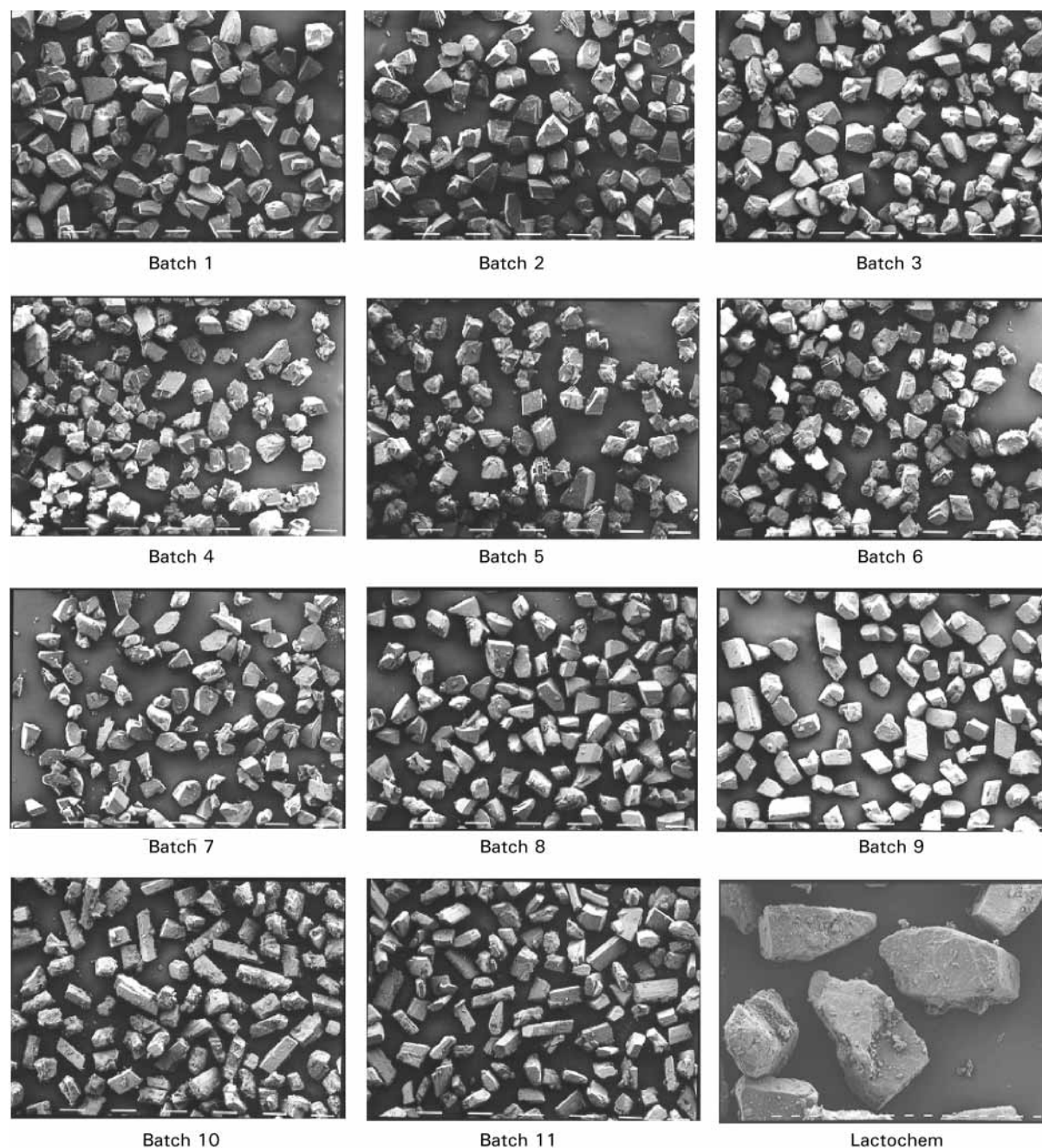
Figure 1. Scanning electron micrographs of different batches of lactose. Scale represents 100 μm. For conditions of crystallization see Table 1.

have been reported to be due to the different relative growth rates on the crystal faces at different supersaturations (van Kreveld & Michaels 1965).

The crystals prepared in an ice bath (batch 4) possessed rougher surfaces and less regular shapes than particles (batch 1) prepared at an elevated temperature (40°C). It was also observed microscopically that the crystals tended to aggregate when prepared by rapid cooling using an ice bath. There are two opposite mechanisms involved in crystallization. The first is the transfer of molecules

from the solution onto the surface of a crystal, resulting in the growth of the crystal. The second is the re-dissolving of the molecules from the crystals into the surrounding solution. If the former exceeds the latter, then the crystal will grow. However, if the latter dominates over the former, the crystal will start to dissolve. During crystallization under mechanical stirring, crystal defects are unavoidable. These defects, such as unwanted hills and edges on the crystals, occur owing either to the misarrangement of molecules in the crystal lattice

or to the fracture of intact crystals by mechanical stirring. These distortions are more likely to dissolve in the surrounding solutions than the crystal surfaces themselves. An increased temperature would accelerate the dissolution of these surface distortions, causing the production of smoother surfaces. Therefore, crystallization at elevated temperatures would appear to favour production of regular crystal shape with improved surface smoothness. However, too high a temperature of crystallization of lactose may result in a change in the ratio of α- to β-form of the final products, which may in turn influence the bulk properties of lactose particles.

The majority of lactose crystals showed a true density from 1·52 to 1·55 g cm^{-3} (Table 2), similar to the value of 1·54 g cm^{-3} for α-lactose monohydrate (Wade & Weller 1994). Most of the crystallized lactose particles showed a rugosity value higher than that of Lactochem lactose. Rugosity is a term that combines the macroscopic shape and surface texture. Only a sphere with a perfectly smooth surface will have a rugosity value of 1. A non-spherical particle will always have a rugosity value greater than 1 even though the particle may have a perfectly smooth surface. The more anisometric the particle is, the higher the rugosity value and vice versa. For example, a more elongated particle will have a higher rugosity value than a less elongated particle even if these two particles have a similar surface texture.

Rugosity has been used interchangeably with surface roughness in some previous reports (Kassem 1990). However, this is true only when the selected particles have similar shapes. For example, from the scanning electron micrographs, lactose

crystals prepared at 40°C (batches 1, 2, 7 and 8) had a similar shape to the particles prepared in an ice bath (batches 3–6). Hence, the generally lower rugosity values for the former batches would indicate a smoother surface as compared with the latter batches and this appears to be justified qualitatively by a visual examination of the scanning electron micrographs (Figure 1). The exceptionally low rugosity value measured for lactose batch 3 (1·84), which might not be anticipated on the basis of the scanning electron micrograph of the same batch (Figure 1), might be due to the less elongated shape of these particles as compared with the rest of the batches. Lactose batch 9 had the lowest rugosity value (1·50) and this finding is supported by the scanning electron micrograph of these particles (Figure 1). However, the high rugosity values of batches 10 and 11 (2·31 and 2·54, respectively) may be primarily due to their elongated forms rather than to rougher surfaces. Therefore, caution should be taken when rugosity is used to compare the surface roughness of crystals with different shapes. In order to represent the macroscopic and microscopic shape of a particle, rugosity should be employed in combination with other shape factors such as those obtained by image analysis.

Similar to the rugosity, the shape factor, as defined, is also a combination of particle shape and surface textures. A spherical particle with a smooth surface will have a shape factor of 1 whilst irregularly shaped particles have shape factor values less than 1. The more irregular the shape and/or rougher the surface, the smaller the shape factor. Elongation ratio is a shape descriptor, the higher the elongation ratio then the more elongated the particle. In general, more elongated particles

Table 2. The density, specific surface area measured by optical microscopy (A_m) and an air permeation method (A_a), and some shape descriptors of lactose particles.

Batch no.	Density (g cm^{-3})	Specific surface area (cm^2 g^{-1})		Rugosity	Shape factor	Elongation ratio
		A_m	A_a			
Lactochem	1·53	442·1	834·4	1·89	0·74 ± 0·09	1·68 ± 0·36
1	1·54	416·3	829·0	1·99	0·74 ± 0·11	1·39 ± 0·28
2	1·53	409·3	834·4	2·04	0·74 ± 0·10	1·39 ± 0·25
3	1·55	420·3	774·2	1·84	0·74 ± 0·99	1·34 ± 0·25
4	1·55	421·7	1138·5	2·70	0·60 ± 0·14	1·28 ± 0·22
5	1·53	447·7	1153·4	2·58	0·68 ± 0·10	1·29 ± 0·19
6	1·54	441·2	974·0	2·21	0·72 ± 0·09	1·30 ± 0·23
7	1·54	410·5	927·6	2·26	0·69 ± 0·12	1·64 ± 0·33
8	1·53	427·7	817·0	1·91	0·72 ± 0·12	1·37 ± 0·22
9	1·52	406·1	607·3*	1·50	0·78 ± 0·06	1·63 ± 0·29
10	1·52	427·7	986·8	2·31	0·68 ± 0·11	2·08 ± 0·61
11	1·54	403·3	1025·3	2·54	0·73 ± 0·08	1·71 ± 0·44

Values are mean ± s.d., n > 100. *Estimated value because the upper limit of the Fisher subsieve sizer was exceeded.

(higher values of elongation ratio) have smaller shape factor values. The combination of shape factor and elongation ratio provides a detailed description of the particle shape and surface smoothness. For example, the lactose crystals of batch 4 have the lowest values for elongation ratio (1·28) and shape factor (0·60), suggesting that this batch of crystals are the least elongated with the highest surface roughness. Lactose crystals from batch 10 have a low shape factor value (0·68) but high values of elongation ratio (2·08), indicating that they are elongated particles with rough surfaces.

A relationship between rugosity and shape factor was derived as:

$$\text{Rugosity} = -6 \cdot 8 \times \text{Shape factor} + 6 \cdot 9$$
$$r^2 = 0.85 \tag{3}$$

As might be expected from the equations defining rugosity and shape factor, particles with higher rugosity values have smaller values of shape factor. Higher rugosity represents either a rougher surface or more irregular shape and in either case, the value of shape factor will decrease as a result of an increase in particle perimeter. Such a relationship between the 2-dimensional shape factor and the 3-dimensional rugosity value suggests that different faces of the lactose crystals may have similar surface textures.

Both crystallization temperature and initial concentration of lactose affect the shape of lactose crystals, as expressed by the elongation ratio. Crystallization from a higher initial concentration of lactose tends to produce more elongated lactose crystals (higher value of elongation ratio) regardless of the temperature of the mother liquor. For example, an initial lactose concentration of $\geq 50\%$ (w/w) produced lactose crystals (batches 9, 10 and 11) with a significantly higher ($P<0.001$) value ($1·78 \pm 0·48$, n $= 208$) of elongation ratio than the crystals ($1·39 \pm 0·28$, n $= 371$) prepared from an initial lactose concentration of 43% w/w (batches 5–8). Crystallization at a higher temperature also appeared to produce more elongated lactose crystals. For example, the combination of all lactose crystals prepared at 40°C (batches 1, 2, 7, 8–11) have an elongation ratio of $1·60 \pm 0·42$ (n $= 484$), which is significantly higher ($P<0.001$) than the elongation ratio ($1·30 \pm 0·22$, n $= 373$) of the combined lactose crystals prepared at 0°C (batches 3–6). As found previously, lactose crystals were confirmed to grow faster at 40°C than at 0°C. Although the crystals prepared at 40°C had higher values of elongation ratio than the crystals prepared at 0°C, the former crystals still had a significantly

higher ($P<0.01$) value for the shape factor ($0·73 \pm 0·11$, n $= 766$) than the latter ($0·69 \pm 0·12$, n $= 541$). These results suggest that crystals prepared at 40°C have a smoother surface as compared with those prepared at 0°C. Any defects formed on the crystal surface will be more likely to dissolve at 40°C as compared with those formed at 0°C. Furthermore, from microscopy observation during crystallization, the crystals prepared at 0°C are more likely to form aggregates than the crystals prepared at 40°C. These aggregates may eventually reduce the uniformity of particle shape and surface smoothness. Therefore, crystallization at a higher temperature may be more favourable to produce elongated particles with a smoother surface.

Higher initial concentrations of lactose lead to higher rates of crystal growth, resulting in the production of more elongated crystals. However, the effect of initial lactose concentration on the surface smoothness of lactose is more complicated, depending on many other factors such as the crystallization temperature, stirring rate, etc. In general, crystallization from an initial lactose concentration of 43% w/w appears to produce lactose crystals with a smoother surface in comparison with the crystals prepared from an initial lactose concentration of 33% w/w. Crystallization from an initial lactose concentration of 33% w/w usually took a longer period of time (>12 h) for the crystals to grow to the desirable size ($63–90 \, \mu$m) than crystallization from an initial lactose concentration of 43% w/w. Since the crystallization solution was subject to constant mechanical stirring, some crystals were likely to be fractured by the stirrer and this can be seen from the scanning electron micrographs that show some fractured faces on the crystals. The longer the crystals are subjected to mechanical stirring, the more crystal faces will be fractured. These may be the reasons for crystals prepared from an initial lactose concentration of 33% w/w generally having more surface asperities than crystals prepared from 43% w/w.

Two-step crystallization
The crystals obtained from the same stage of crystallization had similar rugosity values whilst the crystals prepared at the later stage of crystallization had smaller rugosity values than the crystals obtained at the initial stage of crystallization (Table 3). The crystals prepared during the first stages of crystallization (batch A or a′) appeared to have a similar value of elongation ratio to those with the same sieved size but prepared at the second stages (batch B or b′). However, there was a

Table 3. The density, surface-volume mean diameter (d_{sv}), specific surface area measured by optical microscopy (A_m) and measured by air permeation (A_a), elongation ratio, shape factor and rugosity values of lactose crystals prepared from different stages of the crystallization process (n > 100). Sub-batches A and B contained particles within a sieved size range of 63–90 μm whilst the size of particles within sub-batches a′ and b′ was <63 μm.

Properties	First stage		Second stage	
	Batch A	Batch a′	Batch B	Batch b′
d_{sv} (μm)	104·7	68·6	93·0	65·3
Density (g cm^{-3})	1·55	1·53	1·54	1·52
A_m (cm^2 g^{-1})	369·8	571·7	405·9	604·6
A_a (cm^2 g^{-1})	921·7	1400·6	865·8	1233·6
Shape factor	0·65 ± 0·08	0·65 ± 0·10	0·69 ± 0·12	0·72 ± 0·11
Elongation ratio	1·79 ± 0·31	1·55 ± 0·30	1·81 ± 0·45	1·54 ± 0·33
Rugosity	2·49	2·45	2·13	2·04

significant difference ($P<0·001$) between the elongation ratio of different size fractions of lactose particles from the same stage of crystallization. For example, batch A had a higher value of elongation ratio than batch a′ whilst batch B had a higher elongation ratio than batch b′. The elongation ratio of lactose particles was dependent upon the particle size ($P<0·01$) but independent of the crystallization stage ($P = 0·808$). The larger size fraction (63–90 μm) had a higher value of elongation ratio than the smaller size fraction (<63 μm). Therefore, it can be concluded from these results that larger lactose particles are more elongated than smaller lactose particles. It was reported that due to different faces of lactose crystals having different growth rates, lactose crystals will grow along their longitudinal axis (van Kreveld & Michaels 1965; Twieg & Nickerson 1968), resulting in the larger particles being more elongated than the smaller particles.

Statistical analysis of the results shows that the shape factor of lactose particles is determined by the stage of crystallization ($P<0·01$) but is independent of the particle size fractions ($P = 0·595$). Crystals prepared during the final stages of crystallization (batches B and b′) had a higher value of shape factor ($P<0·001$) than crystals obtained from the conditions prevalent at earlier stages of the crystallization process (batches A and a′). These results suggest that batches B and b′ had a more regular shape with a smoother surface than batches A and a′. The initial crystallization may act to 'purify' or 'stabilize' the aqueous solution, leading to subsequent crystallization being carried out from a more homogeneous solution. Further, the initial crystallization is accompanied by a rapid drop in temperature and an increase in viscosity. At an initial lactose concentration of 43%, the crystallization usually starts before the temperature

reaches room temperature. Therefore, the crystals grow in an environment of a changing temperature and viscosity, both of which are known to affect the growth of lactose crystals (Hartel & Shastry 1991). With a drastic change in temperature and/or viscosity, the initial crystallization may be carried out in a more 'chaotic' solution than the final stages of crystallization when the temperature tends towards a constant value. This might be the major reason why lactose crystals prepared during the later stages of the crystallization process (batches B and b′) have a more regular shape with a smoother surface than those prepared during the first stages of crystallization (batches A and a′). Visser (1980) reported that due to most lactose solutions being acidic (pH 5) even pharmaceutical-grade lactose contains some impurities. These impurities have been identified as di-lactose phosphate complexes that are incorporated into the crystal lattice (Visser 1984). Di-lactose phosphate complexes inhibit the growth of lactose crystals by adsorbing onto several of the lactose crystal faces, thereby inhibiting the incorporation of α-lactose molecules into the crystal lattice (Visser et al 1988). Therefore, it would be reasonable to assume that some of the impurities are incorporated into crystals prepared during the initial stage of crystallization, leading to a relatively pure solution being generated for subsequent crystallization. This may further improve shape and surface smoothness of lactose crystals prepared after the initial crystallization.

Crystallization in the presence of small amounts of organic solvents
A majority of the organic solvents, with the exception of glycerine and propanol, markedly increased the growth rate of lactose crystals from aqueous solutions (Figure 2). The use of propanol
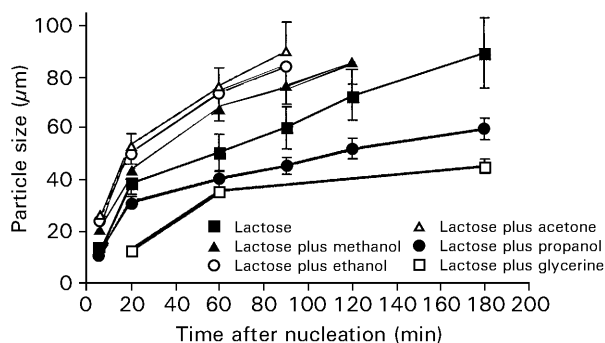
Figure 2. The effects of some organic solvents on the growth of lactose crystals. Error bars denote standard deviation, n = 50.

Table 4. The surface-volume mean diameter (d_{sv}), density, specific surface area measured by microscopy (A_m) and air permeation (A_a) as well as some shape factors of lactose crystals prepared in the presence of different organic solvents.

Properties	*Batch C	*Batch D	*Batch E
d_{sv} (μm)	93·7	98·7	103·7
Density (g cm^{-3})	1·57	1·57	1·55
A_m (cm^2 g^{-1})	407·86	387·20	373·29
A_a (cm^2 g^{-1})	770·86	1057·05	709·24
Shape factor	0·76±0·12	0·54±0·09	0·74±0·12
Elongation ratio	1·25±0·19	1·35±0·23	1·82±0·27
Rugosity	1·89	2·73	1·90

*Batches C, D and E were prepared in the presence of ethanol, acetone and glycerine, respectively.

and glycerine slowed crystal growth. The effects of organic solvents on particle growth rates may be due to three mechanisms. First, since lactose is a hydrophilic compound, its aqueous solubility is reduced in the presence of these water-miscible organic solvents. This will lead to an increased supersaturation and hence an increased rate of lactose crystallization (Jelen & Coulter 1973). Although acetone, ethanol and methanol are all water-miscible, they have different hydrophilicities, the order being methanol > ethanol > acetone. Such differences in hydrophilicity may partly account for the different effects of the solvents on crystal growth. Second, the solvents may reduce the surface tension between crystal faces and their surrounding solution and thus facilitate the transfer of lactose molecules to the crystal surfaces and increase the crystal growth. Although alcohol promotes spontaneous nucleation, and this may be another factor involved in increasing the crystallization of lactose, such an effect might not be detected by measuring the individual particle size as employed in the current work. The inhibited growth rate of lactose crystals by propanol and glycerine might be due to the increased viscosity of the solution in the presence of these solvents. Increasing viscosity might reduce the molecular mobility and thus, retard the transfer of lactose molecules from the surrounding solution to the crystal surface.

Lactose particles prepared in the presence of different organic solvents appear to have different shape and surface texture (Table 4). Particles prepared in the presence of ethanol (batch C) and acetone (batch D) were shown to have a significantly smaller elongation ratio ($P<0.001$) than particles prepared in the presence of glycerine (batch E) and particles prepared from solutions without any added organic solvents. Addition of 5% w/w ethanol to the mother liquor was reported to accelerate the growth rate of face (0Ì1) by three times (18), face (110) by 1·4-times and faces (1Ì0) and (100) by 1·3-times (van Kreveld 1969). Therefore, ethanol would appear to accelerate the growth of width and thickness more than the length of the particles. This leads to the production of 'rounder' particles, resulting in the lower elongation ratio of this batch of lactose particles. However, there is no significant difference in the values of shape factors and rugosity (Table 4) between batches C and E. Given the lower value of elongation ratio for batch C, it would be reasonable to assume that lactose batch E had a smoother surface than batch C. This is verified by examination of the scanning electron micrographs (Figure 3) where crystals from lactose batch E appear to be more elongated and have smoother surfaces, as compared with particles from batch C. Furthermore, batch C tended to contain more aggregates than batch E. Batch D had a significantly ($P<0.001$) higher rugosity value but a significantly ($P<0.001$) lower
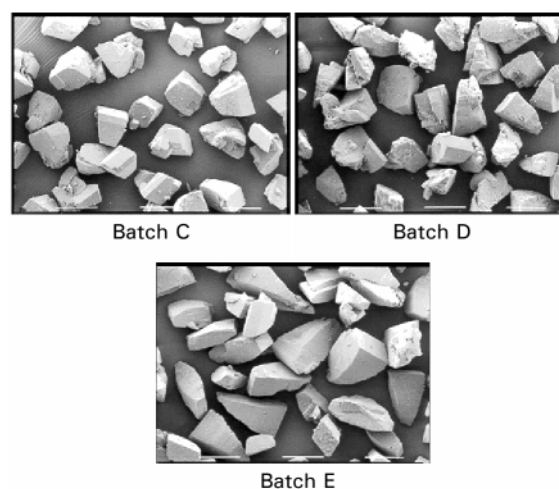

Figure 3. The scanning electron micrographs of lactose prepared in the presence of different organic solvents. Scale represents 100 μm.
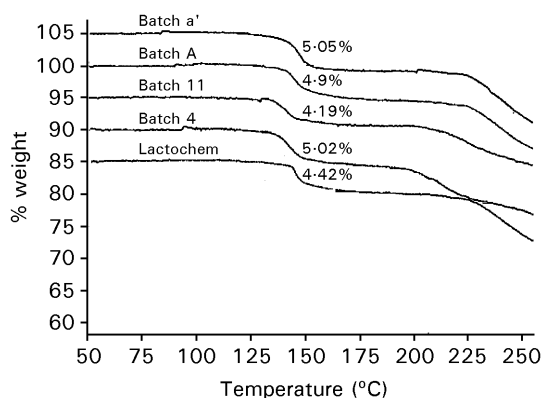
Figure 4. Thermogravimetric analysis data of some batches of lactose.

Figure 5. Differential scanning calorimetry traces of some batches of lactose crystals.

shape factor value than either batch C or batch E. Batch D contained particles with a more irregular shape with more surface asperities than batches C and E (Figure 4).

The more irregular shape and rougher surfaces observed for batches C and D, in comparison with batch E, may be due to the faster growth rate of lactose crystals, which may result in more crystal imperfections being formed. These imperfections may lead to the production of irregularly shaped lactose with rougher surfaces. The improved crystal morphology observed for batch E may have been due to the slower crystal growth rate when glycerine was added. Thus, in order to obtain lactose crystals of regular shape with smooth surfaces, organic solvents, which accelerate crystal growth rate, should be avoided in the mother liquor. However, addition of a small amount of viscous solvents to the mother liquor may improve the crystal shape and surface texture of lactose crystals.

All crystallized lactose had thermal gravimetric analysis data (Figure 4), which were similar to that of α-lactose monohydrate. Weight loss between approximately 120–190°C was due to the dehydration of crystallization water instead of the vaporization of free water, which occurred at approximately 100°C. Weight loss at 200–250°C was due to decomposition of lactose. All batches of lactose showed a dehydration of 4·5–5·5% w/w, suggesting that they contained approximately 1 mol water of crystallization (mol anhydrous lactose)$^{-1}$.

Each batch of lactose showed the differential scanning calorimetry-curve (Figure 5), typical of α-lactose monohydrate (Lerk et al 1984a). The endothermic transition starting at approximately 130°C corresponds to the dehydration of crystallization water whereas the endothermic peak at approximately 217°C is the melting endotherm of α-lactose monohydrate. A small exothermic peak was observed at about 177°C, which can be
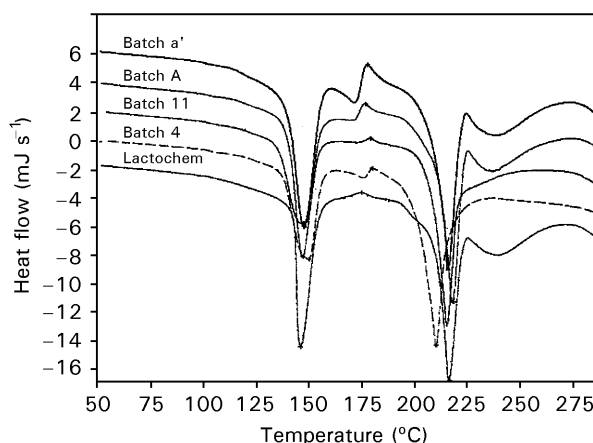
attributed to the crystallization of amorphous lactose (Lerk et al 1984b). Different batches of lactose had different intensities of this peak, in decreasing order of batch a′ (9·67 J g^{-1}) > batch A (2·77 J g^{-1}) > batch 4 (2·03 J g^{-1}) > batch 11 (1·88 J g^{-1}) ≥ Lactochem (1·82 J g^{-1}) lactose. Since the heat of crystallization is directly proportional to the concentration of amorphous lactose (Saleki-Gerhardt et al 1994), the re-crystallized lactose except for batch a′ would be expected to have a similar amorphous content to the commercial Lactochem lactose. Lactose batch a′ clearly possessed a higher amorphicity, due to its higher heat of crystallization of amorphous lactose, than the other batches of crystalline lactose although this batch of lactose did not undergo any size-reduction processing, which is known to introduce amorphous regions (Ward & Schultz 1994). As mentioned previously, batches A and a′ were two size fractions from the same batch of lactose crystals. Batch A had a mean diameter of 104·7 μm whilst batch a′ had a mean diameter of 68·6 μm (Table 3). It is possible that the higher apparent amorphicity of batch a′ compared with batch A may be a consequence of the smaller size and hence higher specific surface area of the particles. The reasons for this are not known but it is possible that the larger lactose particles may have a higher degree of crystallinity as a consequence of the crystal growth, which occurred subsequent to their initial precipitation from solution.

All batches of lactose had similar X-ray powder diffraction patterns (Figure 6) to α-lactose monohydrate (Brittain et al 1991), further confirming that the re-crystallized lactose existed in this form.

In conclusion, the results from this study have identified many of the factors that affect the morphology of α-lactose monohydrate during the crystallization process. A range of α-lactose
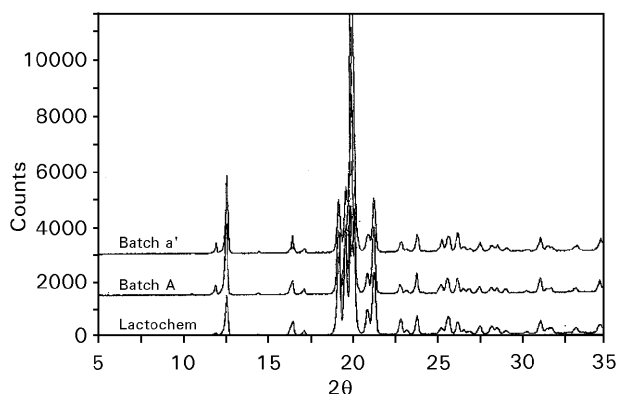
Figure 6. The X-ray powder diffraction patterns of some batches of lactose crystals.

monohydrate crystals with precisely defined morphologies can be produced by careful control of the crystallization conditions.

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