Crystal growth mechanisms of the (010) face of α−lactose monohydrate crystals

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Abstract

The growth rates of the (010) face of α-lactose monohydrate crystals were measured at 30, 40 and 50 °C in the relative supersaturation range 0.55-2.33 in aqueous solutions. The mechanisms of growth were investigated. Spiral growth was found to be the mechanism of growth up to a critical relative supersaturation $(s-1)_{crit}=1.9$ at 30 °C. Above the critical relative supersaturation, the crystal growth mechanisms were predicted to change. All growth models fit equally well to the growth rates. No two-dimensional nucleation was observed above critical supersaturation by AFM. On the other hand increased step height and roughness on the edges of steps were observed. It was concluded that the growth mechanism of the (010) face of α-lactose monohydrate crystal is spiral growth. A parabolic relationship was obtained below critical supersaturation followed by a linear relationship with relative supersaturation.

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1. Introduction

Lactose crystallisation has been investigated extensively under different conditions but there has been only one study on mechanisms of growth. Visser [1] has investigated the growth rates of lactose crystals from non-ionic lactose as a function of supersaturation and temperatures and concluded that at relative supersaturation (s-1) above of 0.35 the growth mechanism changes from spiral growth to the birth and spread model for the (010) and the (100) faces. Large (600-800 μm) single lactose crystals and optical microscopy were employed to measure the growth rates in a stagnant environment. Non-ionic lactose exhibits much faster growth rates than the pharmaceutical grade lactose due to the absence of growth inhibitor lactose phosphate, and thus these results do not apply to typical pharmaceutical grade lactose.

We have recently published the growth rates of four different faces of α-lactose monohydrate crystal at 30, 40 and 50 °C in the relative supersaturation range 0.55-2.33 in aqueous solutions grown in an *in situ* cell in a stagnant solution [2]. A power law was applied to the growth rate data of the (010) face of α-lactose monohydrate crystals and activation energy was calculated to be 9.5 kcal/mol with exponent *n* estimated to be 2.6. The low activation energy indicated that mass transfer was limiting at high temperatures. Growth rates above 0.4 μm/min were found to be influenced by volume diffusion. When the growth rate data in the volume diffusion zone were omitted the exponent n became 2 and the E_{act} was calculated to be 7.8 kcal/mol for the (010) face. Introduction of critical supersaturation lowered the exponent n to 2 and activation energy to 8.9 kcal/mol.

The power law is used to express the growth rate equation and the dependency of growth rate on temperature and supersaturation; it does not explain the mechanisms of crystal growth. In order to investigate the mechanisms of growth, the growth curve needs to be investigated in detail and fit to the equations of different growth mechanisms. In this study, the spiral and two-dimensional growth models were applied to growth rate data of the (010) face. Crystals grown at different supersaturations were imaged by AFM in order to confirm the growth model.

Crystal growth is commonly accepted to occur via two mechanisms. These are spiral growth and two-dimensional growth. At low supersaturations, growth proceeds by addition of growth units to a spiral. At higher supersaturations, if the surface area for growth is not enough for the precipitation pressure, two dimensional nuclei might form on crystal surfaces. There are three

models proposed: Mononuclear, polynuclear and birth and spread models. Growth rate equations describing these models are given in Table 1 [3].

2. Materials and Method

The α-lactose monohydrate used, Sigma Ultra, was purchased from Sigma Chemicals. Supersaturation ratio, *s*, was calculated using the equation given by Visser [4]

$$ s = \frac{C}{C_S - FK_M(C - C_S)} $$ where C is total lactose concentration, g anhydrous lactose/100 g

water, C_S is the final solubility of lactose, g anhydrous lactose/100 g water, F is a temperature dependent factor for depression of solubility of α-lactose by β-lactose and K_M is the β/α ratio of lactose in mutarotation equilibrium at the relevant temperature. and the driving force for crystallisation was expressed as (s-1), relative supersaturation.

An *in situ* crystal growth apparatus developed by Lee and Parkinson [5] was used to measure the growth rates of the (010) face of the α-lactose monohydrate at 30, 40 and 50 °C at eight different relative supersaturations, ranging between 0.55-2.33. 10 µl of the seed solution (40g of α-lactose monohydrate/100 grams of deionised water, filtered and slowly evaporated overnight) was transferred into the *in situ* cell containing 5 ml of supersaturated solution. Seed crystals were 30-100 µ in length in **b** direction, well formed, tomahawk shaped crystals. The increase in the length in **b** direction was measured at certain intervals. As there is no growth observed on the $(0\bar{1}0)$ face, growth rate calculated was the growth rate of the (010) face.

For the AFM investigation, lactose crystals were grown at three relative supersaturations of 0.55, 1.88 and 2.35 at 30 °C. These supersaturations are in the spiral region, transition and two dimensional nucleation regions approximately according to the *in situ* optical growth rate measurements. Required amount of α-lactose monohydrate was dissolved in DI water. After cooling, solution was filtered through 0.45 µm filter paper and kept at 30 °C until sufficient crystals were obtained. Then crystal were filtered and washed with 5 consecutive saturated solutions of ethanol and water (decreasing lactose concentration) in order to obtain crystal surfaces without artefacts. Then crystals were dried at 60 °C in an oven. Selected crystals were mounted on to blue tack to image the selected face of the crystal. Images were obtained using a

Digital Instrument Nanoscope E AFM, operated in the contact mode, in air, using Si_3N_4 200μ triangular tip cantilevers with a spring constant of 0.12 N/m. All the images were collected in the height mode.

Attempts to implement *in situ* AFM investigation were unsuccessful due to spontaneous nucleation observed in the *in situ* cell while imaging. While *in situ* AFM investigation is possible at lower supersaturations the higher levels were necessary to probe the crystal growth mechanisms under relevant conditions. Therefore all the AFM investigation was performed *ex situ*.

3. Results:

Growth rates of the (010) face at 30, 40 and 50 °C at relative supersaturation, ranging between 0.55-2.33 are given in Table 2. Growth rates increase with increasing supersaturation and temperature (Figure 1).

If crystal growth is controlled by diffusion of growth units across the face towards steps on the surface, the growth rate of a face is given by the equation of Burton, Cabrera and Frank [6] . At low supersaturations the BCF equation reduces to $GR = \dfrac{C_S}{\sigma_1}\sigma^2$ and at high supersaturations,

$GR = C_s\,\sigma$ [7]. In other words, the relationship changes from parabolic to a linear growth law as the supersaturation increases. When the growth rate is plotted against (s-1), the parabolic law is obeyed up to a relative supersaturation (critical supersaturation) of 1.88 at 30 °C and it decreases with increasing temperature (1.6 and 1.3 at 40 and 50 °C respectively). The influence of diffusion on crystal growth was analysed by calculating the boundary layer thickness for a natural convection system given by Mullin [8] (page 225). The boundary layer thickness was calculated to be around 23 μm and this resulted in a growth rate of 0.4 μm/min. Growth rates below 0.4 μm/min was found not to be influenced by volume diffusion. Therefore, growth below the critical supersaturation is controlled by surface diffusion only. Van Krevald [9] calculated the growth rate as 0.6 μm/min for his system. Below the critical supersaturation, when ln GR was plotted against ln (s-1) the intercept gives the constant C' and it is proportional to the (-ΔG_{deh}/RT) where ΔG_{deh} is the molar activation free energy for dehydration for entering the surface layer. ΔG_{deh} was calculated by making an Arrhenius plot ln (C') versus 1/T, and the slope of the straight line gives -ΔG_{deh}/R. The activation free energy is calculated to be 7.4 kcal/mol.

The growth rate equation in the spiral growth region can be expressed as: GR= 2.4×10^4 exp (-7.2/RT) $\sigma^{2.0}$

Visser reported activation free energy of 17.2 kcal/mol for the (010) for non-ionic lactose and change of mechanisms around (s-1) of 0.39 where the growth rate observed was 0.13 μ/min. In our study, no measurable growth was observed below a relative supersaturation of 0.47, as previously reported by Shi *et. al.*[10]. Non-ionic lactose does not contain the acidic growth retarder lactose phosphate compounds and thus it grows faster than pharmaceutical grade lactose. Jelen [11] measured growth rates of large lactose crystals to be 40-50 % less than large non-ionic lactose crystals by Visser. In our experience, the growth rate of crystals grown in non-ionic lactose was approximately 10 times faster than with Sigma Ultra when crystals less than 100 μm were used. Visser [12] performed single crystal growth experiment at different lactose phosphate concentration by mixing non-ionic lactose with lactose containing lactose phosphate (Lp) and stated that growth rates decrease with increasing Lp concentration and no further reduction was observed above 33 ppm (s=1.61). At this concentration, about three quarters of the growth sites is covered by HL_P' ions. This leads to a negative electric charge built up on the crystal surface ((010) face) .This has to be neutralised by H^+ or other cations in the solution followed by overgrowth with α-lactose molecules. Recently, Lifran *et al* [13] performed batch growth experiments by adding lactose phosphate to lactose solution at s=1.27 and calculated that above 15 ppm lactose phosphate in solution growth rates plateaus. At this concentration half of the growth sites were calculated to be covered by Lp ions. Lactose used in this research contains lactose phosphate therefore reduced growth rates were observed. Due to the effect of lactose phosphate, similar growth rates were achieved at much higher supersaturations in our system.

Above the critical supersaturations, the spiral growth model predicts that growth rate directly increases with relative supersaturation. At 30°C, growth rate can be expressed as: GR = 0.65 σ - 0.91.

In order to investigate the fit between the 2D growth models and the experimental data, the rate equations were linearised by taking the natural logarithm of the equation, and rearranged [3]. The edge free energies were calculated by taking the average slope of the three lines for three temperatures and rate coefficients for each temperature were calculated. It was found that the mononuclear model fits poorly with the experimental data, giving very low correlation coefficients. The edge free energies calculated for the (010) face with three two-dimensional

growth models are given in Table 3. The edge free energy calculated from the Birth and Spread model is in good agreement with the value calculated by Visser for non-ionic lactose, 4×10^{-21} J/molecule[1]. Edge free energy predicted by the polynuclear model is almost 4 times higher than the birth and spread model. This high surface energy leads to an increase in the radius of the critical nucleus (35 Å (100 unit cells) and 9 Å (7 unit cells) by the polynuclear and birth and spread model respectively). Given the low number of molecules needed to form critical size nuclei, the birth and spread model is more likely the growth model that explains the growth of the (010) face at higher supersaturations. It must be noted that the growth rates above 0.4 μm/min were influenced by volume diffusion.

Figure 2 displays all the growth rate equations applied to the experimental growth rates of the (010) face at 30 °C. At low supersaturations, the spiral growth equation fit the experimental growth rate data better than the two dimensional growth models. However, at high supersaturations, it is impossible to choose between the spiral growth (linear) and the two-dimensional models.

In papers published on mechanisms of growth of other crystal systems, it is often found that when using the least square criterion, any of the relationships represented in the growth rate equation fit the experimental data equally well. This problem is compounded by the spread of data points due to experimental inaccuracy and by phenomena such as growth rate dispersion and influence of additives. Values of constants appearing in the kinetic expressions can therefore rarely be determined with an accuracy of better than ± 30%[14]. Growth rate experiments themselves in the case of lactose crystallisation are not found to be enough to describe the mechanisms of growth observed. The results obtained from the growth rate data needs to be supported by imaging the surface of crystals at high and low supersaturations in order to observe the presence of dislocations and two-dimensional nuclei.

3.1. AFM

α-Lactose monohydrate crystals grown in water display a tomahawk shaped morphology with a large $(0\overline{1}\,\overline{1})$ face and a very small $(0\overline{1}0)$ face [15]. This is due to the inhibitory effect of β-lactose on the $(0\overline{1}0)$ and {0-1-1} faces as β-lactose can easily dock on these surfaces [16]. The galactose end of the lactose molecule lies on the (010) face surface, therefore the β-lactose

molecule is rejected [17]. When the β-lactose content of the solution is low, needle shaped crystals were obtained with fastest growing {0-1-1} faces and equal growth on the (010) and $(0\bar{1}0)$ faces.

The (010) face becomes the fastest growing face of the of α-lactose monohydrate crystals in aqueous solution. This face is bounded by the $(0\bar{1}\bar{1})$, $(0\bar{1}1)$, $(1\bar{1}0)$ and (110) faces. The unit cell height in this direction, **+b**, is 2.1 nm, and the unit cell contains two water and two lactose molecules.

Crystals grown in solutions with (s-1) of 0.55 display one or more double spiral sources and growth occurs by addition of growth units to unit cell high steps emanating from the double spiral. Spirals are elongated in the **c** direction and rotate clockwise. Smaller crystals (less than 300 μm in the **b** direction) with smaller (010) faces usually had one double spiral originating from the centre of the face. Larger crystals (300-1000 μm in the **b** direction) usually had other spirals formed around the central spiral or macrosteps parallel to the (110) face. The number and location of spirals on lactose crystals were found to be different. This might be the reason behind the wide variation in growth rates, hence growth rate dispersion.

Figure 4 shows the central spiral observed on the (010) face of a small α-lactose monohydrate crystal grown at 30 °C and relative supersaturation of 0.55. The mean step distances were larger in the **c** direction, which corresponds to steps parallel to the $\{0\bar{1}\bar{1}\}$ faces, than steps parallel to the $\{1\bar{1}0\}$ faces. The faces at the opposite sides of the spirals are symmetry related but not equal faces and have different step distances indicating different specific free energies. Figure 5 shows the mean step distances of steps in four directions. Chernov [18] reported similar step structures for the (101) face of a lysozyme crystal (monoclinic as well). The mean step distances in the $\{0\bar{1}\bar{1}\}$ and the $\{1\bar{1}0\}$ directions are 596, 550 and 216, 195 nm. BCF model [6] describes step spacing (y_0) of a growth spiral as: $y_0 = \dfrac{19\gamma a}{n\sigma kT}$ where γ is the edge free energy of a growth unit in a step, a is the specific molar volume units, n is the number of cooperating spirals, σ is supersaturation, k is the Boltzmann constant, and T is the crystallisation temperature. This equation assumes that the critical nucleus is circular and radius equal to $x_0/19$. In our case, due to the anisotropic step advancement of four faces, four edge free energies can be calculated. The edge free energies for faces in the $\{110\}$ and the $\{0\bar{1}\bar{1}\}$ directions faces were calculated as 0.32,

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0.29, 0.43 and 0.49 J/m^2, respectively. These edge free energies can be used to calculate the edge free energy of the (010) face. BCF model assumes a growth spiral which is circular in shape, has only one mean step spacing. For the α-lactose monohydrate crystal, the spiral on the (010) face is close to an elliptical shape with four different growth rates of neighbouring faces. The edge free energy of a growth unit at the edge of a spiral on the (010) face growing in the **b** direction has four components to it: molecules attached parallel to the $\{0\bar{1}\bar{1}\}$ faces and molecules attached parallel to the {110} faces. The first approach to calculating the edge free energy of the (010) face could be to average the four step spacing to calculate the average mean step spacing. Using this average mean step spacing the edge free energy of the (010) face is calculated as 0.41 J/m^2. The second approach would be to approximate the spiral into a rectangle. Then, the dimensions of the rectangle can be calculated by using the radius of the critical size nucleus calculated from the mean step spacing of a face. These two dimensions can then be used to calculate the number of molecules that occupy each side of the rectangle. The edge free energies of the molecules at the edge of the rectangle can be calculated by the number of molecules on each face multiplied with its edge free energy. This value divided by the total number of lactose molecules on the edge of the rectangle gives the edge free energy of the growth unit at the edge of a step in the [010] direction. The edge free energy of the (010) face is calculated to be 0.40 J/m^2 by this method. The two methods produced the same number.

The edge free energy of the (010) face calculated from the mean step spacing by AFM can be compared to the value measured experimentally from the *in situ* optical crystal growth experiments. According to the BCF theory, $\sigma_1 = \dfrac{9.5\gamma a}{\varepsilon kT x_S}$ where   a is the distance between two incorporated growth units, ε is the number of incorporating spirals, γ is the edge free energy of the growth unit at the edge of the step, k is the Boltzman constant, T is the temperature and x_s is the mean distance travelled by an adsorbed molecule before it desorbs. When the experimental growth rates of the (010) face at different supersaturations at 30°C obtained *in situ* optical growth rate experiments were fitted to the BCF equation by using non-linear regression analysis, the constants σ_1 and C_S were calculated to be 2.93 and 0.25, respectively. All the numbers required to calculate γ, with the exception of x_s are known. The BCF theory states the

relationship between the distance between two steps of an equidistant step train, y_0, and the mean distance travelled by an adsorbed molecule before it desorbs is given by [3], x_s, as:

$$\frac{\sigma_1}{\sigma} = \frac{y_0}{2x_S}$$

where the σ_1 is the constant in the BCF equation and σ is the relative supersaturation. At 30 °C, σ_1 is calculated to be 2.93. If σ is taken as 0.55, then σ_1/σ becomes 5.3. When the average step distance was taken as 389 nm, then the x_s is calculated to be 36 nm, approximately 56 times the average distance between two growth units (56a). By using this value of x_s, the edge free energy is calculated to be 0.38 J/m^2. The value of x_s is given as $x_s^2 = 4D_s\tau_s$ where the D_s are the solute diffusion coefficient on the surface and τ_s is the mean lifetime of an adsorbed molecule on a surface. For cases where the rate controlling step is surface diffusion, then D_s is usually small and only those molecules which are adsorbed in the immediate vicinity of a kink or step can diffuse to the kink site before desorption [3]. The calculated mean distance travelled by an adsorbed molecule before it is desorbed (x_0) seems to be very high. It might be the case that the lactose molecules have a high mean lifetime on a surface. Since no other work published gives an accurate way of calculating the value of x_s, it is reasonable to calculate the range of the edge free energy value. The edge free energy of the molecule at the step could be between 0.38-0.41 J/m^2.

The derivation of the BCF equation assumes a circular shaped spiral and radius of curvature of spiral and velocity of a spiral step. This step velocity of a spiral is constant. For a circular shaped spiral the size of a critical size spiral is equal to the mean step spacing divided by 19. Since α-lactose monohydrate crystal is in a low symmetry class (P2$_1$), symmetry related faces are unequal and therefore have different growth rates. Therefore, the equations derived for circular shaped spirals does not hold for elliptical spirals. The calculations given in the previous paragraph both assume a circular shaped spiral, and yet the edge free energies calculated by using the mean step spacings and growth rates obtained from an *in situ* optical growth experiment are in very good agreement. To our knowledge, there is no equation derived for elliptical shaped spirals published in the literature. It would be useful to derive the BCF equation for an elliptical shaped spiral in order to calculate the real edge free energy of the growth unit at the edge of a step on the (010) face.

At higher supersaturations, even at (s-1) of 2.35, no two-dimensional nucleation was observed. The (010) surface was found to be covered by macrosteps, up to 20-30 nm high. As growth rate increases with supersaturation, the number of spirals and the step height increases. Larger crystals usually have multiple spirals formed around the central spiral. Combination of increased number of spirals and step height provides enough surface area for growth units to incorporate into edges, therefore no two-dimensional nuclei form. Therefore it can be concluded that the (010) face of the α-lactose monohydrate crystal grows by spiral growth under experimental conditions used.

In aqueous solutions of lactose (concentration less than 60%W/W), the ratio of β/α lactose is independent of supersaturation but decreases with temperature (β/α = 1.64 - 0.0027 T (°C)). At 30 and 50 °C, 61 and 60% of lactose in solution is β-lactose, respectively. β-lactose is a known inhibitor of lactose crystal growth [9, 16, 19-21]. β-lactose inclusion predominantly resides on the {0-1-1} sectors of the crystals and concentration is about 12-15% [22]. As the (010) face is covered by the galactose end of the lactose molecules, β-lactose is rejected [16]. While it can not be completely ruled out that a supersaturation dependent effect of beta-lactose could be causing the results observed here, the fact that beta-lactose is not readily incorporated in the (010) face would suggest that this is unlikely. A more viable hypothesis is that we are observing changes in the crystal growth mechanism as a function of supersaturation.

4. Conclusion

Growth rate experiments themselves in the case of α-lactose monohydrate crystallisation are not found to be enough to describe the mechanisms of growth observed. Growth mechanism was found to be a spiral growth at low supersaturations but all growth models fit equally well at high supersaturations. The results obtained from the growth rate data need to be supported by imaging the surface of crystals at high and low supersaturations in order to observe the presence of dislocations and two-dimensional nuclei.

AFM investigation of the (010) face of α-lactose monohydrate crystals grown under different supersaturation provides very useful information about the crystal growth mechanisms. Growth spirals were observed only on the (010) face of the crystal. Double spirals rotating clock-wise are the constant source of growth, steps emanating from the spirals are 2 nm high (unit cell height in the **b** direction) and spirals were usually observed at the centre of the face and additional spirals

usually formed at steps parallel to the (110) face. The variation in number and location of spirals could be the possible reason behind growth rate dispersion. The double spiral is anisotropic, indicating different step advancement rates in different directions. The mean spacing of the steps parallel to the $(0\bar{1}\bar{1})$ face is larger than those parallel to the (110) face.

No two-dimensional nucleation was observed at high supersaturations. As growth rate increases with supersaturation, the number of spirals and the step height increases. Combination of increased number of spirals and step height provides enough surface area for growth units to incorporate into edges, therefore no two-dimensional nuclei form. Therefore it can be concluded that the (010) face of the α-lactose monohydrate crystal grows by spiral growth under experimental conditions used.

5.   Acknowledgments

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