Nucleation and Crystallization Kinetics of Hydrated Amorphous Lactose above the Glass Transition Temperature

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Abstract □ The crystallization kinetics of amorphous lactose in the presence and absence of seed crystals were investigated at 57.5% relative humidity. Isothermal crystallization studies were conducted gravimetrically in an automated vacuum moisture balance at several temperatures between 18 and 32 °C. The crystallization rate constants were then determined from Johnson–Mehl–Avrami (JMA) treatment and isothermal activation energies were obtained from Arrhenius plots. Based on microscopic observations, a reaction order of 3 was used for JMA analysis. The nonisothermal activation energies were determined by differential scanning calorimetry using Kissinger's analysis. Isothermal activation energies for amorphous lactose with and without seed crystals were 89.5 (±5.6) kJ/mol and 186.5 (±17.6) kJ/mol, respectively. Nonisothermal activation energies with and without seed crystals were 71 (±7.5) kJ/mol and 80.9 (±8.9) kJ/mol, respectively. The similarity of the isothermal and nonisothermal activation energies for the sample with seeds suggested that crystallization was occurring by growth from a fixed number of preexisting nuclei. Markedly different isothermal and nonisothermal activation energies in the absence of seeds suggested a site-saturated nucleation mechanism, and therefore allowed calculation of an activation energy for nucleation of 317 kJ/mol.

Introduction

Increases in the dissolution rate and bioavailability of poorly soluble drugs can often be achieved by using metastable amorphous preparations.[1−4] Nevertheless, the use of amorphous oral dosage forms, such as solid dispersions, has seen very limited commercial application. One reason for this limited use is undoubtedly the physical instability of such formulations. Since the amorphous state is thermodynamically unstable compared to the crystalline state, there is a tendency for conversion to the crystalline form. When the conversion occurs during manufacturing or normal storage, product failure can result from the reduced dissolution rate of the crystalline form. If the advantages of metastable amorphous drugs in solid oral dosage forms are to be fully utilized, it is vital to understand the crystallization process, develop methods to stabilize the amorphous phase, and evaluate potential crystallization inhibitors in a timely manner.

The crystallization of amorphous materials can also be detrimental to parenteral products. For example, in protein lyophilization, disaccharides have been shown to stabilize the tertiary structure during the removal of water.[5] One requirement for this stabilizing effect is that the disaccharide form an amorphous phase with the dried protein. It follows that crystallization of the amorphous disaccharide would result in increased protein denaturization. Thus, understanding the transition from amorphous to crystalline states and the stabilization of amorphous phases is also important for parenteral product development.

Spray-dried lactose is widely used in solid dosage forms, and it is probably the most commonly used metastable amorphous material in the pharmaceutical industry. The crystallization of amorphous lactose, particularly during tableting operations[6] and storage of micronized powders,[7] has been reported as an industrial problem. Recent reports in the pharmaceutical literature have discussed amorphous lactose crystallization; however, these studies have not addressed the kinetics.[8−10] This may be a result of the complicating issue of mutarotation between the α- and β-forms of lactose and the ensuing crystal form issues.

Sucrose, another disaccharide, is much less complicated and fairly well studied. Over the years, the crystallization of amorphous sucrose alone and with additives has been studied by various isothermal and nonisothermal techniques including X-ray diffraction,[11] gravimetry,[12−14] and differential scanning calorimetry.[11,15] It is somewhat surprising that other than the statistical approach taken by Van Scoik and Carstensen,[13] crystallization rate constants, and the separation of kinetic parameters for nucleation and growth, are not readily obtained from the literature. Johnson−Mehl−Avrami theory has been used for the determination of crystallization rate constants of materials in frozen solution[16] and for the crystallization of amorphous lactose[17] and amorphous indomethacin;[18] however, none of these studies focused on the separation of nucleation and growth processes.

Determination of the kinetic parameters associated with crystallization of amorphous lactose with and without seed crystals is the focus of the current investigation. The use of isothermal and nonisothermal techniques, combined with microscopic observations, allowed elucidation of the nucleation mechanisms and calculation of activation energies associated with nucleation and growth. The methods and results reported here for lactose form the foundation for later studies on potential crystallization inhibitors.

Theoretical Section

Johnson−Mehl−Avami (JMA) theory describes many solid-state reactions that occur through a process of nucleation and growth such as crystallization.[19−23] For isotropic growth in m dimensions, the general form of the JMA equation is:

$$\alpha(t) = 1 - \exp[-\int_0^t g[\int_\tau^t Y(\Theta)\mathrm{d}\Theta]^m I(\tau)\mathrm{d}\tau] \qquad (1)$$

where α is the fraction crystallized, $Y(\Theta)$ represents the growth rate for all m dimensions of growth, g is a geometric

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Table 1—Summary of the Meanings of Isothermal and Nonisothermal Apparent Activation Energies from Woldt[26] [a]

nucleation mechanism	reaction order (n)	isothermal apparent E_a	nonisothermal apparent E_a
continuous	$m+1$	$(E_a^N + mE_a^G)/(m+1)$	$(E_a^N + mE_a^G)/(m+1)$
fixed number	m	E_a^G	E_a^G
site saturated	m	$(E_a^N + mE_a^G)/m$	E_a^G

[a] The superscripts N and G refer to nucleation and one-dimensional growth, respectively.

constant, and $I(\tau)$ is the nucleation rate. Equation 1 can be reduced to the following simplified form:[24−26]

$$\alpha = 1 - e^{-(k(t - t_o))^n} \quad (2)$$

where, k is the crystallization rate constant (time^{-1}), t is time, t_o is the induction time, and n is the reaction order which depends on the nucleation mechanism and the number of dimensions in which growth is occurring. After assigning the appropriate order, the linearized form of eq 2

$$[-\ln(1 - \alpha)]^{1/n} = k(t - t_o) \quad (3)$$

can be used for determining isothermal induction times and rate constants.

If nucleation and growth rates are functions of temperature only (i.e. not position or time dependent), then three general mechanisms of nucleation can be considered: continuous (which reduces to constant rate under isothermal conditions), fixed number of nuclei, and site-saturated nucleation.[26] Continuous nucleation means that nuclei continue to form and grow throughout the transformation. Fixed number nucleation occurs when growth proceeds from a fixed number of preexisting nucleation sites. The number of nuclei is independent of the experimental conditions. The term site-saturated nucleation is a hybrid of the previous two cases. In this situation all nuclei are present at the beginning of the isothermal process, and additional nuclei do not form during the transformation, but the absolute number of nuclei depend on the temperature. One example of this would be if the rate of growth was much greater than the rate of nucleation.

Starting with eq 1 and assuming only Arrhenius dependence of growth and nucleation rates, Woldt derived the meanings of activation energies determined from isothermal and nonisothermal experiments.[26] After corrections for the differences in the units of k between Woldt's representation and eq 3, the meanings of reaction orders and apparent activation energies determined isothermally and nonisothermally can be summarized as shown in Table 1. Thus, if growth occurs in the same number of dimensions, the apparent E_a obtained isothermally and nonisothermally should be identical under fixed number and continuous nucleation. A more interesting situation arises if nucleation occurs through a site-saturated mechanism. In this case different apparent activation energies will be obtained under isothermal and nonisothermal conditions, and the underlying activation energies for nucleation and growth, E_a^N and E_a^G, can be determined. Therefore, the combination of isothermal and nonisothermal methods of determining activation energies for crystallization can, in theory, provide insight into the underlying mechanism of crystallization.

Experimental Section

Materials—Anhydrous lactose was purchased from Quest International (Norwich, NY). Lactose monohydrate was purchased from Van Waters and Rogers (Bedford Park, IL).

Crystallization Conditions—The International Conference on Harmonization (ICH) has recommended 60 ± 5%RH as a humidity condition for long-term stability testing of pharmaceutical products.[27] Since 57.5% RH is easily achieved at 25 °C using saturated aqueous solutions of NaBr, this was the humidity condition chosen for crystallization studies. Preliminary studies showed that amorphous lactose absorbed ca. 14% water at 57.5% RH, resulting in a glass transition temperature (T_g) for the hydrated material of ca. 5 °C. For comparison, the T_g of dry amorphous lactose is ca. 114 °C. Since all crystallization experiments were conducted at least 13 °C above the T_g, the assumption of Arrhenius dependence of nucleation and growth rates employed in Woldt's analysis should be valid.

Preparation and Characterization of Amorphous Lactose—All amorphous samples were prepared by spray-drying (Buchi mini spray dryer, model B-191) freshly prepared aqueous solutions of anhydrous lactose (10% w/v). The inlet air temperature was set to 150 °C and the other parameters were adjusted to give an outlet air temperature of ca. 80 °C. Spray drying was started exactly 4 min after addition of water to the lactose powder and lasted for 12.5 min. The amorphous nature of the product was assessed by X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC). The amorphous product was then observed by polarized light microscopy (PLM) at a magnification of 332× to check for the presence of seed crystals. One batch of amorphous lactose showed no evidence of crystals by any of the techniques. A second batch appeared amorphous by XRPD and DSC, but small crystallites could be observed by PLM indicating that nucleation occurred during processing stage. Therefore, two batches of amorphous lactose were studied: one which had previously nucleated designated as "with seeds" and one which did not show evidence of nucleation designated as "without seeds".

Polarimetry—The proportion of α and β diastereomers in the raw material, spray-dried product, and recrystallized amorphous material was estimated by polarimetry (Jasco DIP-370 digital polarimeter). Briefly, a standard curve was constructed from the literature values for specific rotation of pure α and β forms.[28] Solutions (1% w/v) were prepared and optical rotation measurements made as a function of time. The specific rotation values were plotted versus time and the y-intercept, which gave the specific rotation at $t = 0$, was used in the standard curve to estimate the α/β content of the solid materials.

Differential Scanning Calorimetry—The DSC experiments employed a Mettler DSC 30 (Mettler-Toledo Inc., Highstown, NJ) or a TA Instruments model 2920 DSC (TA Instruments, Inc. New Castle, DE). Both instruments used aluminum sample pans and were purged with dry nitrogen at 50 mL/min. The DSC cell constants were calibrated with indium and temperature calibrations were performed using indium (single point) or indium, lead and zinc (three point). For nonisothermal crystallization studies, amorphous lactose samples (ca. 2−3 mg) were weighed into sample pans and stored over a saturated aqueous solution of NaBr. After exactly 2 h, the pans were hermetically sealed and immediately scanned in the DSC. The nonisothermal E_a was determined by Kissinger analysis[29,30] at scanning rates of 7.5, 10, 15, 20, and 25 °C/min.

A comparison of the crystallization products from isothermal and nonisothermal crystallization was also performed by DSC. Crystals obtained from isothermal crystallization were scanned from 25 to 300 °C at rates between 7.5 and 25 °C/min using DSC pans with a pinhole. The pinhole was used so that any water could be removed in a reproducible manner and not interfere with the melting transitions. The nonisothermal crystallizations were conducted at rates of 7.5−25 °C/min in hermetically sealed pans as used in Kissinger analysis. However, immediately after the crystallization exotherm a pinhole was placed in the pan and the scan continued to observe the dehydration and melting behavior of the product. The composition of the crystallization products obtained during Kissinger analysis and gravimetric analysis were then compared by using the magnitudes of the α-monohydrate dehydration peak at ca. 145 °C and the α-anhydrous melting peak at ca. 215 °C.

Gravimetry—Crystallization kinetics were determined gravimetrically at 57.5% RH using a vacuum moisture balance (VTI Corporation, Hialeah, FL). The balance was calibrated prior to each run, and the accuracy of the relative humidity was periodically checked by measuring the water uptake of PVP K-90 at 80% RH. Samples weighing approximately 15 mg were used for all but

Table 2—Results of Solution Polarimetry Studies Used to Estimate α/β Composition of Solid Materials

sample	specific rotation[a] (deg/g/dm)	calcd % α
lactose, monohydrate	89	99
starting material	46	20
spray dried	48	24
recrystallized @ 57.5%RH	51	29

[a] Specific rotation values are calculated on an anhydrous lactose basis.

one experiment with seeds at 29 °C which used a 6 mg sample to check for possible sample size effects. All samples were dried under vacuum at 50 °C until a constant weight was maintained. After the instrument reached the desired experimental temperature, the humidity was rapidly increased to 57.5% and maintained at that level for the duration of the experiment. Sample weight was monitored as a function of time, and the weight loss associated with crystallization was used for the determination of crystallization kinetics. The first step in data reduction was transforming the raw data into fraction crystallized (α) versus corrected time as follows:

$$\alpha = \left(\frac{\mathrm{Wt}_{\max} - \mathrm{Wt}_t}{\mathrm{Wt}_{\max} - \mathrm{Wt}_{\mathrm{final}}}\right), \quad t_{\mathrm{corrected}} = t - t_{\mathrm{drying}} \qquad (4)$$

The α and $t_{\mathrm{corrected}}$ values in the region between $\alpha = 0.15$ and $\alpha = 0.85$ were then fit using eq 3. Crystallization rate constants determined between 18 and 32 °C were used to calculate the apparent activation energies of samples with and without seed crystals.

Microscopy Studies—The crystallization process was observed with a polarized light microscope fitted with a video camera, VCR, and video printer. Constant humidity chambers were constructed using a standard microscope slide, a cover slip, and an O-ring as follows: A greased O-ring was placed on a slide and a small drop of a saturated salt solution placed inside the O-ring. A thin layer of amorphous lactose was pressed on the inside surface of the cover slip, which was then placed on top of the O-ring forming a closed constant humidity chamber suitable for microscopic analysis. The crystallization at room temperature and 57.5% RH was then recorded on the VCR at magnifications of 166× and 332×.

Results and Discussion

Polarimetry—The extrapolated specific rotation values and calculated α/β compositions of various types of lactose are given in Table 2. An increase in α content of 4% is observed on going from crystalline anhydrous lactose to the spray-dried amorphous product. Crystallization of the amorphous material at 57.5% RH results in another 5% increase in α content. Errors in the determination of α content primarily result from different moisture contents of the samples which, based on prior experience, we estimate to be on the order of ca. 2%. The equilibrium distribution of α and β anomers in aqueous lactose solutions has been reported as 37.3% α and 62.7% β at 20 °C.[28] The data in Table 2 suggest that exposure of lactose samples to liquid water or 57.5% RH results in a drift toward the equilibrium composition. However, the increase in α content during isothermal crystallization is small and therefore its impact on the kinetic parameters is considered to be insignificant.

Microscopy—The crystallization of amorphous lactose was observed using polarized light microscopy. For the sample without seeds, visible crystallites formed after a short induction time. Subsequent crystal growth occurred from these "nuclei" with no new "nuclei" appearing in the amorphous regions. Similar observations were made in the presence of seed crystals. Since new crystals should constantly form during the growth phase with continuous nucleation, these findings served to rule out the possibility

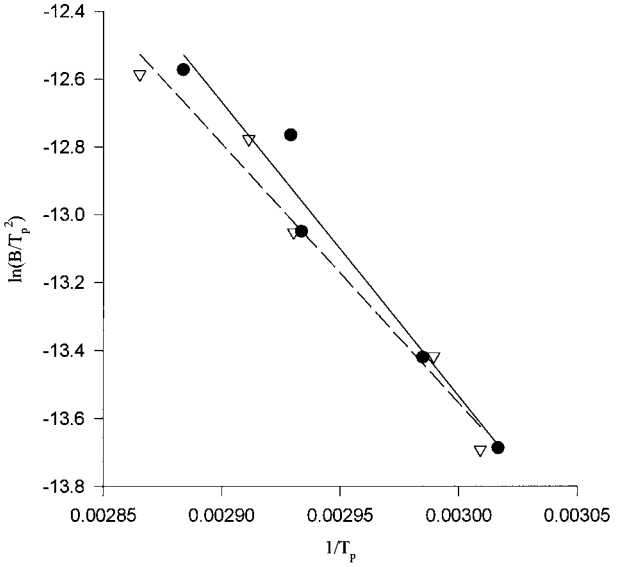

Figure 1—Kissinger plot of the nonisothermal crystallization of amorphous lactose equilibrated at 57.5% RH. Circles, solid line = without seeds; triangles, dashed line = with seeds.

of this mechanism. Table 1 shows that for both fixed number and site-saturated nucleation mechanisms the reaction order is equal to the dimensions of growth. We assume three-dimensional crystal growth and therefore use an order (n) of 3 for fitting gravimetric data to eq 3. Combining this information with the analysis described in the theoretical section should allow elucidation of the nucleation mechanisms in the presence and absence of seed crystals.

Differential Scanning Calorimetry—In Kissinger's analysis, E_a is determined by measuring peak crystallization temperature (T_p) at several heating rates (β), in K/s. The slope of a plot of $\ln(\beta/T_p^2)$ versus $1/T_p$ is $-E_a/R$. Figure 1 shows representative Kissinger plots for lactose at 57.5% RH. Each point in Figure 1 represents a single DSC experiment. The nonisothermal activation energies were 71 (±7.5) and 81 (±8.9) for samples with and without seed crystals, respectively. The reported values represent the averages of three or four Kissinger plots, and the uncertainties are the standard deviations.

As discussed in the theoretical section, the actual meaning of this activation energy may be elucidated by comparing the isothermal and nonisothermal determinations. However, it is possible that effects such as changes in the relative humidity inside the DSC pan during heating could affect the process. Therefore, before a comparison can be made we must have some assurance that the same process is occurring under isothermal and nonisothermal conditions. To support this requirement, DSC thermograms of isothermally recrystallized amorphous lactose and nonisothermally recrystallized amorphous lactose are shown in Figure 2. Both recrystallized lactose samples show an endotherm at ca. 145 °C which is indicative of the dehydration of α-lactose monohydrate. Integration of these endotherms gives values of 55.4 and 58.7 J/g for the isothermal and nonisothermal crystallization products, respectively. A comparison of the previous dehydration endotherms to that obtained for pure α-lactose monohydrate under identical experimental conditions indicates that approximately 33−35% of the sample crystallized as α-lactose monohydrate under both isothermal and nonisothermal conditions.[31] This is in excellent agreement with the proportion of α-lactose in the crystallized product as estimated by polarimetry (29%). The small endotherm at ca. 215 °C represents melting of the α-anhydrous crystal and is

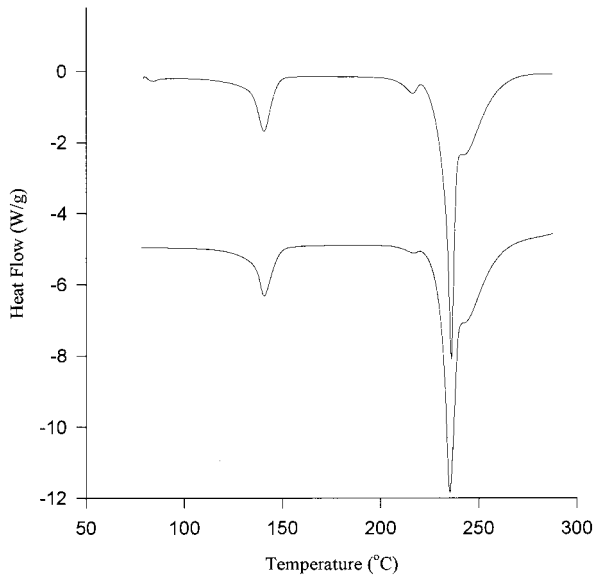

Figure 2—DSC thermograms of amorphous lactose crystallized at 57.5% RH under isothermal and nonisothermal conditions.

slightly larger in the nonisothermal crystallization products. Using a method similar to that described for estimating the amount of α-lactose monohydrate it is estimated that less than 2% α-anhydrous is formed isothermally and that slightly more, but less than 5%, is formed nonisothermally. The DSC-estimated composition of the crystallization products did not depend on the presence of seeds or the heating rate used during nonisothermal crystallization. Given that the difference in crystal composition between isothermal and nonisothermal crystallizations is only on the order of a few percent a significant affect on the activation energy determinations is not expected.

Gravimetry—Isothermal crystallization processes can be followed by techniques including X-ray powder diffraction, isothermal calorimetry, and gravimetry. The large difference in hygroscopicity between the amorphous and crystalline lactose at 57.5% RH made gravimetry an acceptable technique for this study. Other methods such as isothermal calorimetry or X-ray powder diffraction may be better suited for more hydrophobic drug molecules where the difference in hygroscopicity between amorphous and crystalline states is negligible. A representative plot of the raw moisture balance output obtained at 25 °C is shown in Figure 3a. The raw data transformed into fraction crystallized as a function of time shown in Figure 3b give the typical sigmoidal curve expected for reactions occurring through nucleation and growth. Figure 3c shows the experimental data and fit of eq 3 with $n = 3$ in the region $0.15 < \alpha < 0.85$.

One important assumption when determining crystallization kinetics from gravimetric data is that the weight loss is crystallization rate limited, and not limited by evaporation. This assumption was tested by comparing crystallization rate constants at 25 °C and 52.5, 57.5, and 62.5% RH. Since evaporation will occur at a slower rate at higher RH, the observed crystallization rate constants should decrease with increasing RH if weight loss is evaporation limited. Table 3 shows that the rate constants increased markedly with RH as is expected for crystallization rate limited weight loss. The similarity of rate constants obtained for two different sample sizes (Figure 4, 29 °C with seeds) also supports crystallization limited weight loss and suggests sample size has minimal effects on the observed rate constants.

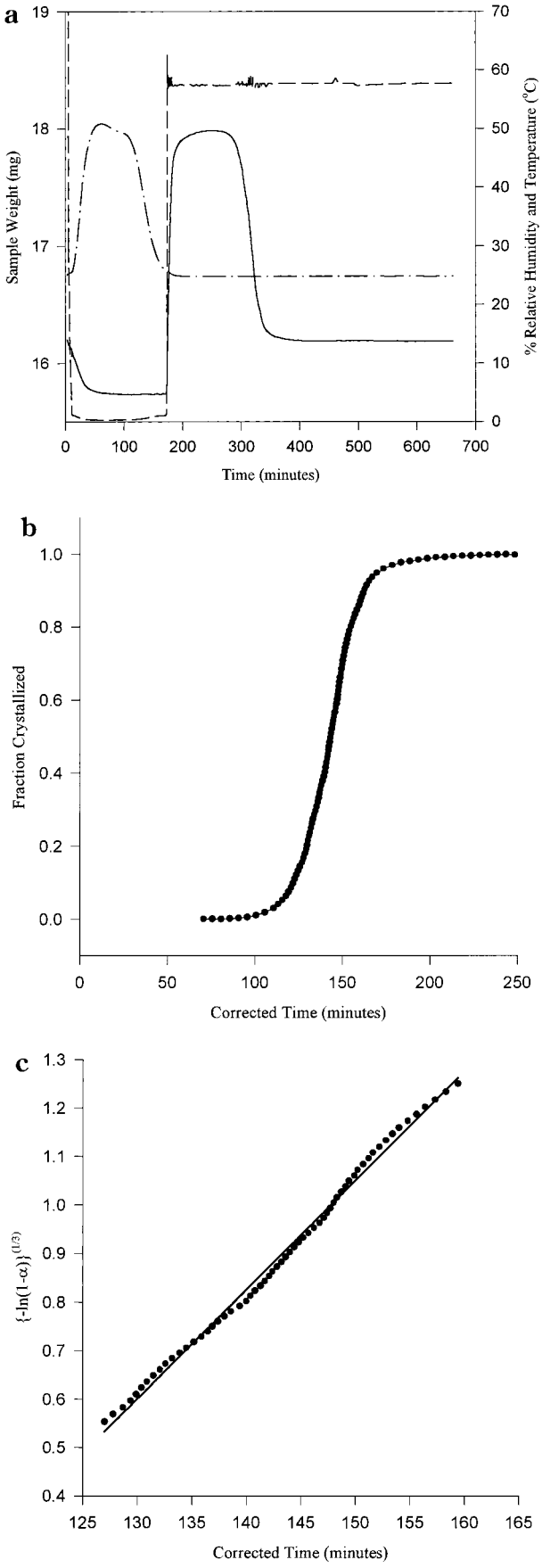

Figure 3—(a) Plot of typical gravimetric data showing sample temperature (dash–dot), sample weight (solid), and percent relative humidity (dashed) as a function of time. (b) Plot of transformed raw data. (c) JMA plot of transformed gravimetric data.

Table 3—Crystallization Rate Constants Determined at 25 °C as a Function of Relative Humidity

% relative humidity	k (min^{-1})
52.5	0.0056
57.5	0.0155, 0.0224
62.5	0.0466

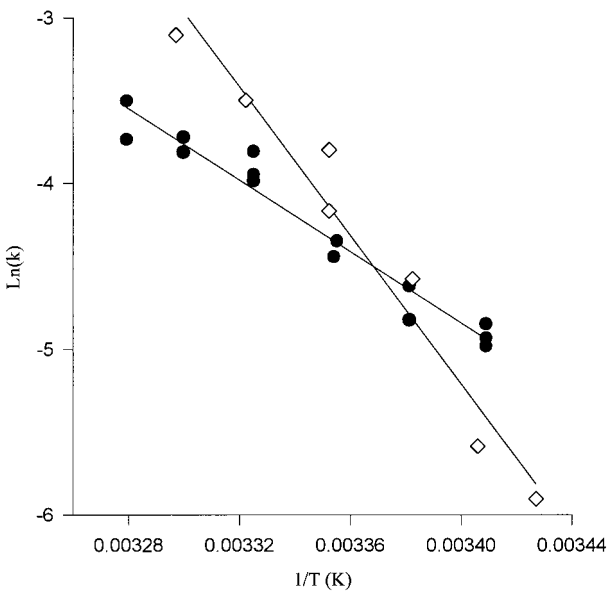

Figure 4—Arrhenius plots of JMA rate constants determined gravimetrically at 57.5% relative humidity. Solid circles = with seeds; open squared = without seeds. The solid lines show the least squares linear fit.

Arrhenius plots of the crystallization rate constants (Figure 4) gave isothermal activation energies with and without seeds of 89.5 (±5.6) and 187 (±17.6) kJ/mol, respectively. The uncertainties represent one estimated standard error obtained from regression analysis.

Comparison of Isothermal and Nonisothermal Activation Energies—Table 4 shows that amorphous lactose containing seed crystals gives essentially the same activation energies by isothermal and nonisothermal techniques. Remembering that continuous nucleation has been ruled out by microscopy, reference to Table 1 reveals that equivalent isothermal and nonisothermal activation energies are expected for growth from a fixed number of preexisting nuclei. Under the fixed number nucleation mechanism, the activation energy determined isothermally or nonisothermally is that for growth. In the absence of seed crystals there is a marked difference between activation energies obtained from isothermal and nonisothermal experiments (Table 4). As shown in Table 1 this result supports a site-saturated nucleation mechanism. Both fixed number and site-saturated nucleation mechanisms should give an activation energy for growth under nonisothermal conditions, and the results obtained with and without seeds (71 ± 7.5 and 81 ± 8.9, respectively) support this assertion.

The process of crystal growth can be separated into bulk diffusion and surface integration steps.[32] It has been shown experimentally that for sucrose crystallization from solution, the activation energies for diffusion are on the order of 25−33 kJ/mol while activation energies for surface incorporation are 67−84 kJ/mol.[33] The activation energies for lactose crystal growth determined here compare very favorably with the range cited for surface incorporation. This comparison suggests that under the conditions studied the crystal growth rate of lactose is limited by surface incorporation rather than diffusion.

Table 4—Comparison of Isothermal and Nonisothermal Activation Energies Determined in the Presence and Absence of Seed Crystals

sample	isothermal E_a (kJ/mol)	nonisothermal E_a (kJ/mol)
seeds present	89.5 ± 5.6	71 ± 7.5
seeds absent	187 ± 18	81 ± 8.9

With site-saturated nucleation and three-dimensional growth, the E_a values obtained isothermally and nonisothermally are given by $(E_a^N + 3E_a^G)/3$ and E_a^G, respectively. Using the experimentally determined apparent E_a values for samples without seeds we can calculate the activation energy for nucleation as $E_a^N = 317$ kJ/mol. The relative value of this activation energy compared to that obtained for growth seems intuitively correct; however, no literature values are available for a direct comparison of the magnitude. By separating the activation energies for nucleation and growth it may be possible to ascertain how various excipients affect nucleation and growth processes during crystallization. This knowledge could allow directed inhibition of nucleation, growth, or both during product development.

Another method for determining an apparent E_a^N involves using the reciprocal of the induction time in an Arrhenius plot.[34] When the induction time data calculated from the intercept and slope of the JMA plots were analyzed in this manner, apparent activation energies of 112 (±15) and 104 (±7.5) kJ/mol were obtained. The fact that equivalent activation energies were obtained with and without seeds seriously calls into question the use of this apparent activation energy for evaluating nucleation. These results may suggest that activation energies determined from induction times may be related to the slow growth of initial nuclei, so-called germ nuclei, into growth nuclei rather than the actual nucleation step as recently discussed by Jacobs.[35]

Conclusions

Under the crystallization conditions used in this report, amorphous lactose appears to give a mixed product consisting of ca. 30% α-monhydrate, 70% β-anhydrous, and possibly a small amount of α-anhydrous crystal forms. Growth rates appear to be limited by surface incorporation rather than diffusion, and in the absence of seed crystals nucleation appears to occur though a site-saturated mechanism. Gravimetry was shown to be useful method for determining crystallization rate constants and induction times of lactose. The combination of isothermal and nonisothermal activation energies allowed investigation of both crystal growth and nucleation mechanisms and led to the separation of activation energies for nucleation and growth. Finally, the relationship between induction-time-based activation energies and nucleation has been questioned. Additional studies employing additives are needed to determine if the separation of activation energies for nucleation and growth allows a more mechanistic understanding of the role of additives in stabilizing or destabilizing amorphous phases.

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