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Theory of the intermediate stage of crystal growth with applications to insulin crystallization

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ABSTRACT

A theory for the intermediate stage of crystal growth, where two defining equations one for population continuity and another for mass-balance, is used to study the kinetics of the supersaturation decay, the homogeneous nucleation rate, the linear growth rate and the final distribution of crystal sizes for the crystallization of bovine and porcine insulin from solution. The cited experimental reports suggest that the crystal linear growth rate is directly proportional to the square of the insulin concentration in solution for bovine insulin and to the cube of concentration for porcine. In a previous work, it was shown that the above mentioned system could be solved for the case where the growth rate is directly proportional to the normalized supersaturation. Here a more general solution is presented valid for cases where the growth rate is directly proportional to the normalized supersaturation raised to the power of any positive integer. The resulting expressions for the time dependent normalized supersaturation and crystal size distribution are compared with experimental reports for insulin crystallization. An approximation for the maximum crystal size at the end of the intermediate stage is derived. The results suggest that the largest crystal size in the distribution at the end of the intermediate stage is maximized when nucleation is restricted to be only homogeneous. Further, the largest size in the final distribution depends only weakly upon the initial supersaturation.

1. Introduction

The growth of protein crystals from solution continues to be the preferred way to obtain samples for X-ray diffraction investigations. Continuing study of the intermediate crystal growth stage is important for further understanding of the conditions that lead to the creation of high quality crystals of desirable size. The intermediate growth stage commences after the initial induction time period where though the solution is supersaturated there is no detectable decrease in solute concentration and ends when the solute concentration has decayed to the solubility limit. After this stage ripening effects sometime occur where certain crystals in the solution still continue to grow at the expense of the others.

A useful theory for the intermediate stage of batch crystal growth proposes the use of a population-continuity equation for unit volume of solution:

$$\frac{\partial f(t,L)}{\partial t}+\frac{\partial (G(t)f(t,L))}{\partial L}=0 \tag{1}$$

Here f is the distribution of crystal sizes. Constant temperature and pressure are assumed during growth. Consider the density N of spherical crystals of radius L present in the solution at time t, then, $f = dN/dL$. G is the linear growth rate taken to be only dependent upon time, i.e. it is assumed that McCabe's ΔL law holds and that the growth rate is independent of size [1].

Another equality is required to completely define the system, that of mass-balance:

$$\frac{ds(t)}{dt}=-K\int_0^\infty L^2 Gf\,dL \tag{2}$$

Here s is the *normalized supersaturation*. The constant $K = 4\,\pi\,\rho/(c_o - c_s)$, where $4\,\pi$ is an area shape factor, ρ the crystal density, c_o the initial solute concentration and c_s the solubility.

Using a model where the linear growth rate is directly proportional to the normalized supersaturation, and the homogeneous nucleation rate is given by the model of Mier, an approximate solution for the above system was derived [2] and later used to study the von Weimarn crystallization rules for supersaturated solutions [3]. Continuing with the approach taken in Ref. [2], Alexandrov and Malygin [4] derived a complete series solution for the supersaturation as a function of time and for the size distribution function.

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0022-0248/

Assuming that the distribution function is separable and that the growth rate is directly proportional to the normalized supersaturation, a solution was found for Eqs. (1) and (2) which in turn yields the nucleation rate rather than it being given as a boundary condition. These results were then used to describe the kinetics of crystal growth for the proteins lysozyme and canavalin [5]. Recently, modified versions of Eqs. (1) and (2) were used to study the intermediate stage of growth with the added allowance for buoyancy effects and growth rate fluctuations [6,7], issues that are ignored in this study.

In Schlichtkrull's important work on insulin crystallization [8,9] measurements were reported not only for the concentration of insulin in crystalline form at various times during batch growth but also for the distribution of sizes at periodic intervals and at equilibrium, i.e. at the end of the intermediate stage. This data was collected at constant temperature and pressure. These data were given for both bovine and porcine insulin. Reports of this type, where both kinetic data for the supersaturation decay and crystal size distributions are given, are rare in the protein crystal growth literature. In these reports, the author considers the time evolution of the dissolved insulin concentration in solution during crystal growth and through a curve fitting scheme suggested that the linear growth rate was proportional to the square of solute concentration for bovine insulin crystal growth and to the cube of solute concentration for porcine. This motivated us to search for a solution for the above system for the case where the growth rate was proportional to the normalized supersaturation raised to the power of a positive integer greater than one. Assuming growth occurs where the rate limiting step is incorporation and not bulk diffusion, i.e. the *kinetic regime*, we propose here a growth rate in the form of a power law where the exponent is not limited to one as was the case in the previous work with Eqs. (1) and (2). Here a solution is determined for the case where the exponent is any positive integer. This result yields expressions for the time dependent normalized supersaturation, the size distribution function, the nucleation rate and an approximate expression for the largest crystal size at equilibrium.

These expressions are then compared with experimental data. Converting the concentrations during growth from the experimental reports to normalized supersaturation, these data are compared to the theoretical result. We find that the theory describes the kinetics of the reported solute concentration for the case where the growth rate is proportional to the square of the normalized supersaturation for both bovine and porcine insulin. The form of the theoretical size distribution function is equivalent to an expression proposed in the experimental reports. Interpretation of this theoretical distribution function is further clarified by discussion of the possible modes of nucleation.

Schlichtkrull [8,9] reported that in addition to homogeneous nucleation, heterogeneous nucleation was present during the growth runs originating on the container surfaces and faces of the crystals themselves. Through a set of batch growth experiments, where steps were taken to reduce heterogeneous nucleation, so that homogeneous nucleation is the dominant form, it was found that the logarithm of the cumulative distribution in size data versus crystal size lies generally along a straight line of negative slope. Further, these results indicate that when heterogeneous nucleation is present three changes occur relative to the homogeneous nucleation dominated growth case even when both runs had equal initial supersaturation: First, the logarithm of the cumulative distribution data versus L is no longer linear for all L. Secondly, at equilibrium, the total number of crystals per unit volume in the solution are increased. Finally, the largest crystal size in the equilibrium distribution is reduced.

We compare our theoretical results with the two reported insulin growth runs for which data, for the percent of solute converted to solid at a given time, are given: bovine insulin from Ref. [8] and porcine insulin from Ref. [9]. The bovine growth run was from a buffered saline solution while porcine was crystallized from a sodium citrate and acetone solution. Also, data for the cumulative size distribution versus crystal size at equilibrium was given as a plot for both growth runs. This data lies along a straight line of negative slope except for the larger crystal sizes, around 100 μm, were it tends to tail downward. As discussed above, this is apparently due to the influence of heterogeneous nucleation during growth. Since our theory yields a distribution function, the logarithm of which is linear versus L for all L from 0 to the maximum size $L_{\max}$, we suggest that it describes insulin crystal batch growth from solution with only homogeneous nucleation. By comparing our results for $s(t)$ to the data mentioned above, using mass conservation and using the experimental estimate for the initial growth rate, we are able to determine all of the unknown parameters and develop useful expressions for what we entitle an *idealized homogeneous nucleation model* applicable for batch insulin crystal growth from solution. As expected, we find the model predicts the total number of crystals in the solution at equilibrium are less than reported and the largest crystal size is greater than reported. This result leads us to suggest that the largest possible crystal sizes from insulin crystal growth at the end of the intermediate stage are obtained when nucleation is restricted to be of the homogeneous type.

This idealized model leads to an approximate expression for the largest crystal size at equilibrium. According to the proposal above this result gives a theoretical maximum possible crystal size immediately after the intermediate stage of batch growth. In the resulting expression, the largest crystal size at equilibrium is directly proportional to the inverse hyperbolic cosine of the cube root of the initial supersaturation so that beyond a certain size, large changes in the initial supersaturation produce only small changes in the largest maximum size at equilibrium. In the above mentioned experimental reports for bovine and porcine insulin batch crystallization the initial supersaturation in the porcine case was more than double that of the bovine with the largest crystal size at equilibrium in each case being nearly identical.

2. Solution of the governing equations

As suggested in previous work [5,8] we take that the distribution function f is separable in radius L and time t such that

$$f(t,L) = T(t)l(L). \tag{3}$$

Schlichtkrull reported that insulin crystallizes in a rhombohedral shape and reported the diagonal length of the crystal as viewed from above. We take this length to be twice our radius L. Ootaku et al. [10] reported that porcine insulin crystals took the shape of cubes, dodecahedrons and rhombohedrons. We assume that all crystals considered by Schlichtkrull were rhombohedral as depicted in Ref. [10]. Therefore, all crystals have a uniform shape factor and one dimension of the particle, for us L, will characterize the size of all crystals in the assembly.

The linear growth rate, $G = dL/dt$, is assumed of the form given by Christiansen [11]:

$$G = \alpha s^p \tag{4}$$

where p is a positive integer and α is a constant. The time dependence for G comes through s the normalized supersaturation,

$$s(t) = \frac{c(t) - c_s}{c_o - c_s}. \tag{5}$$

$c(t)$ is the time dependent concentration of the solute. It is seen from Eq. (5) that $s = 1$ at the start of the intermediate stage ($t = 0$) and as $t \to \infty$, $s \to 0$. One should not take this limit too literally

since the end of the intermediate stage is when $c(t) - c_s$ first becomes undetectably small. In the experimental work considered here the end of the intermediate stage is taken to be at the time of the last concentration value given in the data set: 1400 min for both bovine and porcine insulin.

Using Eqs. (3) and (4) in Eq. (1) we arrive at the following ordinary differential equation:

$$\frac{1}{T}\frac{dT}{dt}\frac{1}{\alpha s^p} = -\frac{1}{l}\frac{dl}{dL}. \tag{6}$$

Both sides of Eq. (6) equal the same constant λ. The solution for l (L) is,

$$l(L) = Ce^{-\lambda L} \tag{7}$$

where C is a constant. This is identical to the result for the case where $p = 1$, [5], the same form as recommended by Schlichtkrull [8] and a similar expression has also been found to describe the distribution of crystal sizes during continuous crystallization from solution [1].

To show the solution for the temporal side of Eq. (6) we begin by defining the homogeneous nucleation rate J as

$$J = Gf(t, 0). \tag{8}$$

In light of Eqs. (7), (4) and (3) this becomes

$$J = \alpha C s^p T(t). \tag{9}$$

For the case where $p = 1$ it was found previously that the nucleation rate is a function of the normalized supersaturation s, [5]. The proposal here is that J, for any p a positive integer, is a function of the normalized supersaturation s, i.e.

$$J = J(s(t)). \tag{10}$$

With this in Eq. (9) T becomes

$$T = \frac{J}{\alpha C s^p}. \tag{11}$$

The derivative required for the left side of Eq. (6) can now be computed.

$$\frac{dT}{dt} = \frac{1}{\alpha C s^p}\left(\frac{dJ}{ds}\frac{ds}{dt}\right) - \frac{Jp}{\alpha C s^{p+1}}\frac{ds}{dt}. \tag{12}$$

This result can now be used in Eq. (6) along with Eq. (11) and set equal to the separation constant λ.

$$\frac{1}{\alpha J s^p}\left(\frac{dJ}{ds}\frac{ds}{dt}\right) - \frac{p}{\alpha s^{p+1}}\frac{ds}{dt} = \lambda. \tag{13}$$

Now with the use of Eqs. (3) and (11), Eq. (2), can be written as

$$\frac{ds}{dt} = -\gamma K C \alpha s^p T(t). \tag{14}$$

where the integral in Eq. (2) has been set to γ and will be discussed in detail later. Using Eq. (14) in Eq. (13) we arrive at the following first order differential equation:

$$\frac{dJ}{ds} - \frac{pJ}{s} + \frac{\lambda \alpha s^p}{K\gamma} = 0. \tag{15}$$

An integrating factor is s^{-p} so that Eq. (15) can be written as the separable differential equation

$$d[s^{-p}J] = -\frac{\alpha\lambda}{K\gamma}ds. \tag{16}$$

At $t = 0$, $s = 1$ and the initial nucleation rate is J_o so that we integrate Eq. (16) as

$$\int_{J_o}^{s^{-p}J} d[s^{-p}J] = -\int_1^s \frac{\alpha\lambda}{K\gamma}ds. \tag{17}$$

Computing the integrals in Eq. (17) and solving for J we get the nucleation rate for any p a positive integer:

$$J = \left[\frac{\alpha\lambda}{K\gamma}(1 - s) + J_o\right]s^p. \tag{18}$$

An expression for the normalized supersaturation is now determined. Using Eq. (11) in Eq. (14) results in

$$\frac{ds}{dt} = -\gamma k J. \tag{19}$$

Using Eq. (18) for J in Eq. (19) and rearranging leads to the separable differential equation and thus

$$\int_0^t dt = -\int_1^s \frac{ds}{s^p\left[\gamma K\left(\frac{\alpha\lambda}{K\gamma} + J_o\right) - \alpha\lambda s\right]}. \tag{20}$$

We make the following assignments:

$$a = \gamma K\left(\frac{\alpha\lambda}{K\gamma} + J_o\right). \tag{21}$$

$$b = -\alpha\lambda. \tag{22}$$

Eq. (20) becomes

$$t = -\frac{1}{a}\int_1^s \frac{ds}{s^p} + \frac{b}{a}\int_1^s \frac{ds}{s^{p-1}(a + bs)}. \tag{23}$$

Eq. (23) gives the time dependent normalized supersaturation for any p a positive integer. We denote each case as t_p. When $p = 1$, the above reduces to the result previously determined, [5]:

$$t_1 = \frac{1}{a}\ln\frac{a + bs}{s(a + b)}. \tag{24}$$

Setting $p = 2$ in Eq. (23) and integrating leads to

$$t_2 = \frac{1}{a}\left(\frac{1}{s} - 1\right) - \frac{b}{a^2}\ln\left[\frac{a + bs}{s(a + b)}\right]. \tag{25}$$

Continuing for $p > 2$ leads to

$$t_p = \frac{s^{1-p} - 1}{a(p - 1)} - \frac{b}{a}t_{p-1}, \quad \text{for } p = 2, 3, 4, 5, \ldots \tag{26}$$

To assemble the distribution function we first give the initial nucleation rate, J_o, in terms of the parameters involved. The initial nucleation rate J_o is independent of p and from Ref. [5] $T(t)$ was determined for $p = 1$ so that $J_o = \alpha^2\ \lambda C$. Using this with Eqs. (3), (7), (11) and (18) the full distribution function can be written:

$$f = \frac{\lambda}{K\gamma}(1 + \alpha\gamma CK - s)e^{-\lambda L}. \tag{27}$$

3. The kinetics of supersaturation decay

Now the results above can be compared with the kinetic data given in the reports by Schlichtkrull [8,9] for bovine and porcine insulin crystal growth. The percentage of insulin in the crystalline state c_c is given as a function of time. Setting the final percentage, taken to be at the end of the intermediate stage as c_f, we then convert these data to normalized supersaturation s by

$$s = \frac{c_f - c_c}{c_f}. \tag{28}$$

The parameters a and b can be varied and Eq. (25) fit to these data. It is found that $p = 2$ gives the best fit, when compared to $p = 1$ and $p = 3$, for both the bovine and porcine insulin crystallization data sets. Since these data sets seemed to include the induction period, approximations had to be made concerning the start

of the intermediate stage. We take this to be at the 45 min point for the bovine case. Here, virtually no solute decay was reported for the first 50 min. For porcine we take the start to be at 5 min before the first reported non-zero value for c_c. Almost 30 min went by before detection of growth in this case.

The parameters α and λ, which in turn yield b through Eq. (22), can be further understood by considering additional data given in the experimental reports. The distribution of crystal sizes at equilibrium, $f_{\infty,}$ (when $s = 0$), is obtained from Eq. (27):

$$f_\infty = \frac{\lambda}{K\gamma}(1 + \alpha\gamma KC)e^{-\lambda L}. \tag{29}$$

Schlichtkrull reported data points for a cumulative distribution of crystal sizes $F(t, L)$, i.e. the number of crystals per unit volume which are larger than L at time t. This relates to the distribution function f as

$$f(t, L) = -\frac{dF}{dL}. \tag{30}$$

Linearizing F at equilibrium leads to

$$\ln F_\infty = -\lambda L + \ln N_\infty. \tag{31}$$

Here $N_{\infty,}$ is the total number of crystals, per unit volume, in the equilibrium solution. Eq. (31) was fit to the reported data and the slope λ determined. From the vertical intercept one gets $\ln N_\infty$ where

$$N_\infty = \int_0^{L_{max}} f_\infty \, dL. \tag{32}$$

Further, by studying data for F at various times during the intermediate growth stage, Schlichtkrull was able to report that the logarithm of F versus L remained linear with unvarying slope during growth. This implies that the assumption of the rate of crystal growth being independent of crystal size is reasonable here. The logarithm of the cumulative distribution at equilibrium data versus L was linear for up to around 100 μm for both the porcine and bovine runs. The data points then tail downward towards the maximum observed size in the distribution; an effect seemingly due to heterogeneous nucleation. λ, found from a fit to the linear part of the data, was essentially equivalent for the bovine and porcine cases and the largest crystal size, the greatest diagonal length as viewed from above, observed in the final distributions are similar as well, both around 105 μm. This even though the linear fit curve extrapolates to a larger maximum size of about 125 μm. The value found for λ was similar in all the additional experimental insulin crystal growth runs included in Refs. [8,9] thus we take it to be a constant for the two cases we consider and further speculate that it might be a constant for all cases of insulin batch crystal growth from solution.

We now describe how all of the undetermined parameters in the theory can be established. A value for α can be obtained from the experimental report. For α, the kinetic factor in the growth rate, we use a standard model valid for the kinetic regime of growth [12]:

$$\alpha = k(c_o - c_s)/\rho. \tag{33}$$

Here k is a kinetic coefficient. Schlichtkrull gives an initial growth rate for each of the two growth runs. Upon inspection of Eq. (4) it is seen that this initial growth rate is α. With a value for α determined a and b can be varied so that Eq. (25) is fit to the converted experimental data for normalized supersaturation. This is done with λ being equivalent for both cases. These results are depicted in Figs. 1 and 2.

Now, mass conservation can be used to determine L_{max}, the largest crystal size at equilibrium. Since the initial supersaturation is known mass conservation gives:

$$c_o - c_s = \frac{4}{3}\pi\rho \int_0^{L_{max}} L^3 f_\infty \, dL. \tag{34}$$

An expression is required for γ. Consider the integral from Eq. (2) where the upper limit is L_{max}:

$$\gamma = \int_0^{L_{max}} L^2 e^{-\lambda L} \, dL = -\frac{L_{max}^2 e^{-\lambda L_{max}}}{\lambda} + \frac{2}{\lambda}\int_0^{L_{max}} L e^{-\lambda L} \, dL. \tag{35}$$

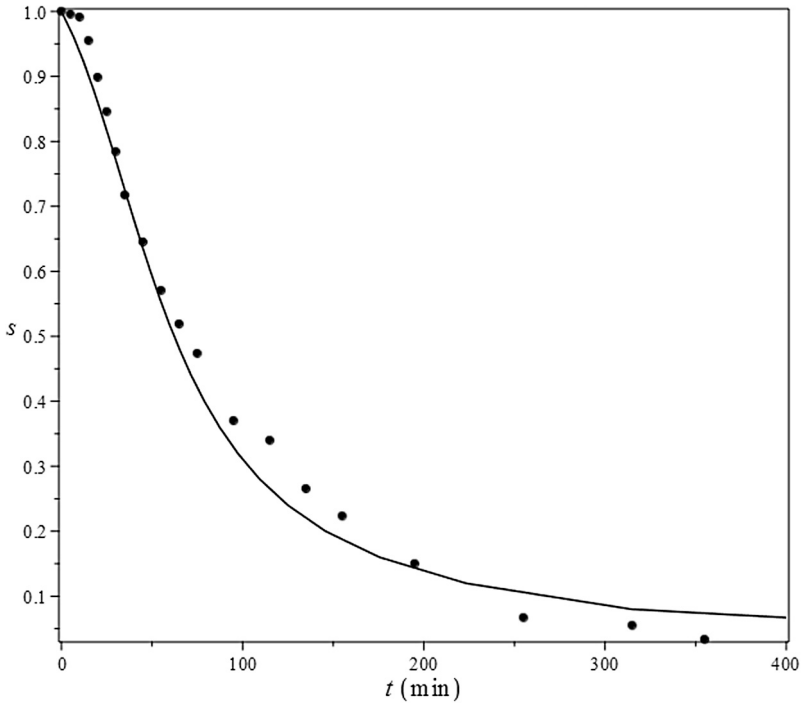

Fig. 1. Normalized supersaturation vs. time given by Eq. (25) fit to data from Ref. [8] for bovine insulin crystallization. $a = 0.05$, $b = -0.045$.

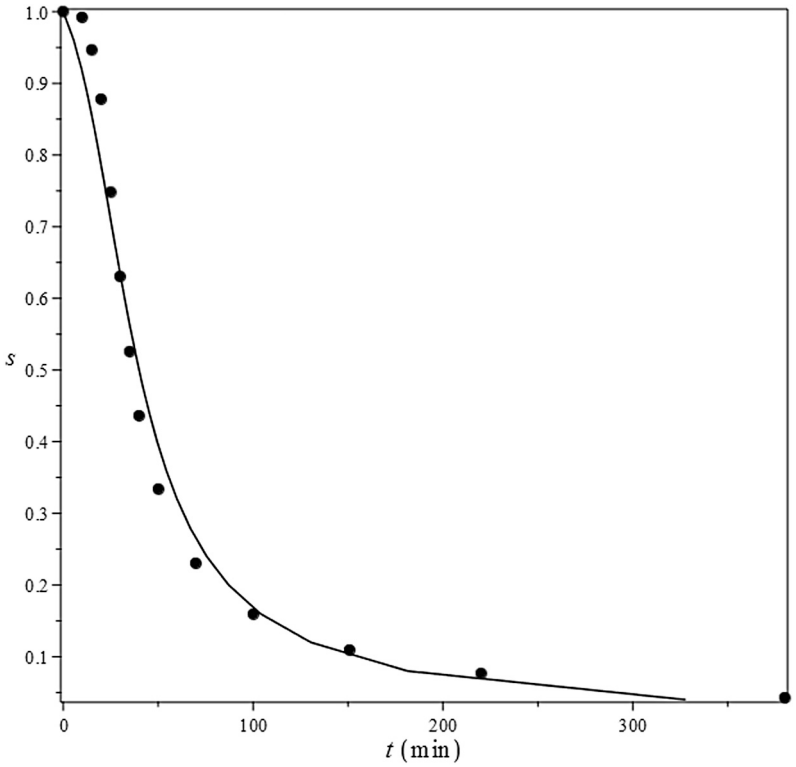

Fig. 2. Normalized supersaturation vs. time given by Eq. (25) fit to data from Ref. [9] for porcine insulin crystallization. $a = 0.09$, $b = -0.084$.

Table 1
Data used and parameters determined in this study. p-porcine, b-bovine. Initial supersaturation, $\Delta c = c_o - c_s$, and initial growth rate α, taken from Refs. [8,9]. Solid state mass density used was $\rho = 1.50$ g/cm^3, taken from Ref. [13]. Molecular weight used for insulin was 5734 Da taken from Ref. [9]. L_{max} values determined from mass balance via Eq. (34). Total density of crystals at equilibrium N_∞ computed using Eq. (32).

$J_o\ \left((\text{ml min})^{-1}\right)$	$\alpha\ \left(\frac{\mu m}{min}\right)$	$C\ \left(\frac{min}{ml\ \mu m}\right)$	K	$\beta\ (\mu m^3 \sqrt[3]{\frac{l}{mmol}})$	$\gamma\ (\mu m^3)$	$\lambda\ (\mu m^{-1})$	$\Delta c\ \left(\frac{mmol}{l}\right)$	$L_{max}\ (\mu m)$	$N_\infty\ \left(\frac{10^4}{ml}\right)$
157.9_b	0.689_b	5120.8_b	5057.6_b	490,000	6558.2_b	0.065	0.65_b	82.0_b	3.4_b
402.8_p	1.29_p	3707.6_p	2185.7_p		6780.1_p		1.50_p	90.0_p	7.2_p
								$53.0^a{}_{b,p}$	$1000^a{}_{b,p}$

[a] From Refs. [8,9].

Further evaluation leads to:

$$\gamma = \frac{2}{\lambda^3} - e^{-\lambda L_{max}}\left(\frac{L_{max}^2}{\lambda} + \frac{2L_{max}}{\lambda^2} + \frac{2}{\lambda^3}\right) \tag{36}$$

$L_{\max}$ is then varied in Eqs. (36) and (34) until we get conservation below 1% for both insulin growth cases. With this final value for γ and $L_{\max}$, J, J_o, C and N_∞ are determined. Relevant data and resulting parameter values are listed in Table 1.

4. The maximum crystal size

An approximation for the maximum size in the final distribution is developed below in which it is found that the maximum size at equilibrium depends only weakly upon the initial supersaturation. To get this expression for $L_{\max}$ we consider a simplified and approximate version of Eq. (36) which retains the general nature of the original expression while being equivalent at $L_{\max} = 0$ and $L_{\max} \to \infty$:

$$\gamma = \frac{2}{\lambda^3}(1 - e^{-\lambda L_{max}}) \tag{37}$$

From the distribution of sizes for insulin given by Schlichtkrull, and from the nature of the distribution function determined here, it must be that the crystals of size $L_{\max}$ originate from the first nuclei generated at the onset of the intermediate stage, those of $L = 0$ at $t = 0$. This is just $f(0,0)$ so that from Eq. (27) comes,

$$f(0,0) = \alpha\lambda C, \tag{38}$$

so that at equilibrium also

$$f(L = L_{max},\ t \to \infty) = \alpha\lambda C. \tag{39}$$

With Eq. (27) at $t \to \infty$ this becomes

$$\frac{1}{\gamma K}(1 + \alpha\gamma CK)e^{-\lambda L_{max}} = \alpha C. \tag{40}$$

Using Eq. (37) for γ in Eq. (40) leads to the following:

$$L_{max} = \frac{1}{\lambda}\cosh^{-1}\left(\frac{\lambda^3}{4\alpha CK} + 1\right). \tag{41}$$

Inserting our definitions for α and K in the above leads to.

$$L_{max} = \frac{1}{\lambda}\cosh^{-1}\left(\frac{\lambda^3}{16\pi Ck} + 1\right). \tag{42}$$

With values listed in Table 1. the largest sizes at equilibrium for the two insulin runs can be computed via Eq. (42). As expected, due to the approximation for γ, Eq. (42) underestimates the value obtained from mass-conservation for the idealized homogeneous nucleation model at 35 μm for bovine and 39 μm for porcine. However, from the computed values for the parameter C and the initial nucleation rates, we find that for the two runs studied here C is inversely proportional to the cube root of the initial supersaturation. With the addition of an appropriate factor, combined within the constant β, Eq. (42) can be converted to a form that gives values for the maximum size at equilibrium to within 2% of those found via mass-conservation:

$$L_{max} = \frac{1}{\lambda}\cosh^{-1}(\beta\lambda^3\sqrt[3]{(c_o - c_s)} + 1). \tag{43}$$

Our value for β is given in Table 1. Now with Eq. (43) we compute a maximum radius for bovine of 84 μm and for porcine, 90 μm. We suggest that these radii are an idealized maximum value, at the end of the intermediate stage, resulting from solely homogeneous nucleation while values from the experimental reports, approximately 53 μm for both, resulted from a mixture of homogeneous and heterogeneous nucleation. It is possible that β and λ are universal constants for all cases of insulin batch crystal growth and that for other proteins undergoing batch growth a similar set of constants might be determined which would enable the use of Eq. (43) for the estimation of $L_{\max}$.

5. Conclusion

A theory for the intermediate stage of crystal growth was used to study the kinetics of supersaturation decay, the homogeneous nucleation rate and the distribution of crystal sizes for bovine and porcine insulin batch crystal growth from solution. A solution was presented for the governing equations where the growth rate is directly proportional to the normalized supersaturation raised to the power of any positive integer. The theory describes the normalized supersaturation decay reported for insulin crystal growth when the linear growth rate is taken to be proportional to the square of the normalized supersaturation. Upon comparing with experimental data the theoretical distribution function underestimates the total density of crystals at equilibrium while overestimating the maximum crystal radius at equilibrium. This leads us to propose that it serves as an idealized homogeneous nucleation model for the insulin growth cases studied here. The resulting expression for the distribution of sizes is equivalent in form to that suggested in the experimental reports. An approximate expression for the largest crystal size at equilibrium is given. With experimental data from the insulin crystal growth studies, we are able to determine values for all parameters in the theory.

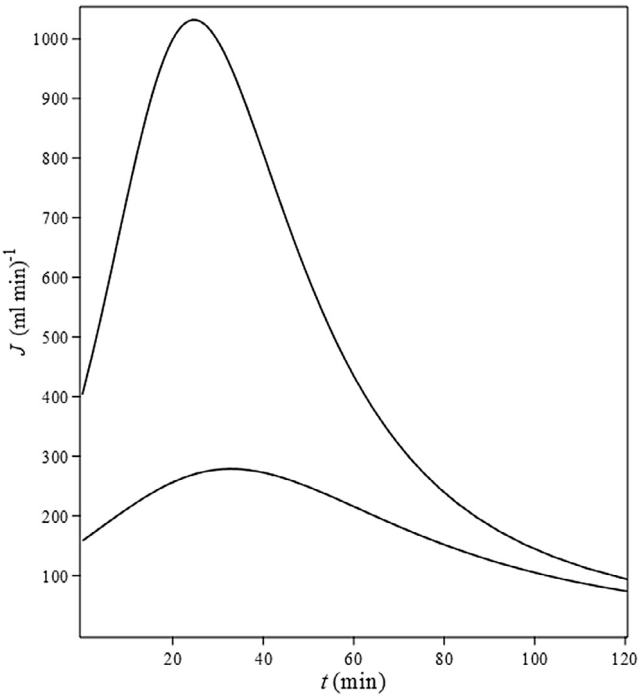

Fig. 3. Plot of the idealized homogeneous nucleation rate vs. time for insulin crystallization cases given in Refs. [8,9]. Lower curve is for bovine upper for porcine.

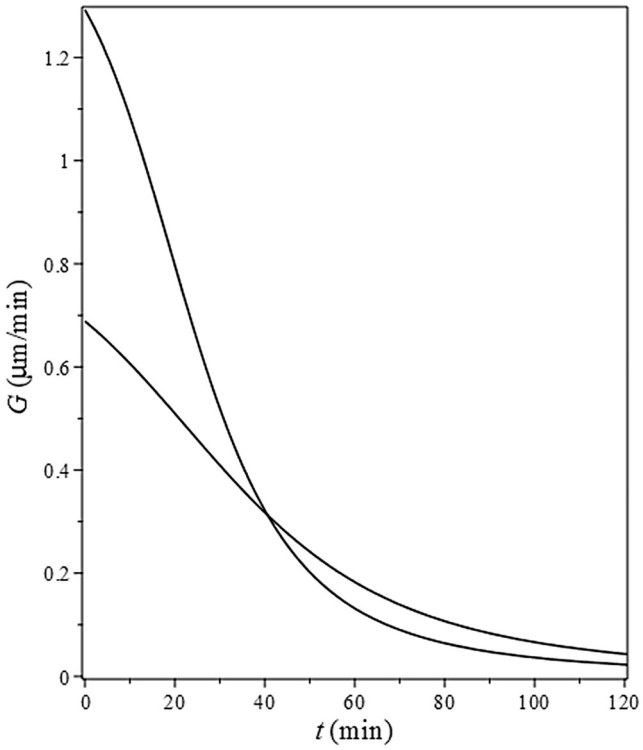

Fig. 4. Plot of the estimated linear growth rate vs. time for the idealized homogeneous nucleation model for insulin crystallization cases given in Refs. [8,9]. Curve with higher initial growth rate is for porcine, lower initial growth rate curve is for bovine.

The homogeneous nucleation rate is estimated for all times during the intermediate stage and is depicted for both insulin types in Fig. 3. The theoretical distribution function, Eq. (27), is at all times exponential with respect to crystal radius L. However, though the distribution function varies with time this change is neither exclusively linear or exponential but of a more exotic nature coming through its dependence upon $s(t)$. The theoretical nucleation rate reaches a maximum in time when the product of the growth rate and the distribution function, for $L = 0$, are a maximum, which is not at $t = 0$. A physical explanation for this effect is that there is some competing process opposing nucleation that is also in some way proportional to the normalized supersaturation. Peculiarities in the homogeneous nucleation rate during protein crystal growth have been reported [14,15]. The theoretical linear growth rate versus time for both insulin types is shown in Fig. 4. Additionally, the total number of crystals per ml of solution at equilibrium, N_∞, was computed for each run using Eq. (32). These values are less than those reported from experiment [8,9] suggesting that heterogeneous nucleation was present thus increasing the final density of crystals. In fact, Schlichtkrull suggested that as N exceeds

10^5 ml^{-1} that a second form of nucleation, on the face of the crystals themselves, becomes dominate until the density of crystals reaches its equilibrium value of approximately 10^7 ml^{-1}.

It seems evident that in the experimental work considered here there was a mixture of homogeneous and heterogeneous nucleation involved in the growth process. After comparing with the theoretical model we suggest that the maximum possible crystal size at the end of the intermediate stage is obtained when nucleation is limited to be only of the homogeneous type. Further, an expression is given that can be used to estimate the largest possible crystal size at the end of the intermediate stage. This relation predicts that for the crystal sizes encountered here, the largest size at equilibrium depends weakly upon the initial supersaturation. These results may be applicable for protein crystal batch growth from solution in general. It is perhaps not a surprise that there exist a restriction on the maximum possible crystal size after the intermediate stage of growth for insulin crystals from solution. Nature has conspired to restrict the size of such crystals. Reports of large volume quality protein crystals seem to indicate final results were achieved via ripening and not by intermediate stage growth alone [10,16].

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