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The kinetics of homogeneous and two-step nucleation during protein crystal growth from solution

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Abstract

Many experimental reports for the kinetics of crystal nucleation and growth, from an isothermal solution, point to a sigmoidal-like behavior for the process. Here we consider three different nucleation models from the literature and show that all lead to sigmoidal or sigmoidal-like behavior for the kinetics of nucleation. A two-step nucleation process is known to occur within certain supersaturated protein solutions, and it is demonstrated in this report how the sigmoidal law yields kinetic information for the two-step and homogeneous nucleation modes. We propose here that two-step solute-rich associates form in the solution around seed nuclei that are already present at or near the point in time when the solution is prepared. Using this hypothesis, we are able to model the time-dependent volume of the two-step phase per unit volume of solution and show that this compares well with reported experimental data. A kinetic model is given for the proposed process, which leads to a sigmoidal rate law. Additionally, a relation between the initial and final nuclei densities and the induction time is derived. As a result of this study, the conclusion is that two-step activity increases with increasing initial supersaturation or increasing salt concentration.

KEYWORDS

crystal growth, nucleation, nucleation kinetics, proteins, two-step nucleation

1 | INTRODUCTION

Events leading to the first seeds for crystal growth, in addition to their particular structure, within the bulk of a supersaturated solution, continue to be somewhat of a mystery. Early pioneers in the field like Becker and Döring equated the process to that of the condensation of water droplets from the vapor as previously considered by Gibbs and Volmer (Ref. 1, p. 175). From this model, a thermodynamic criteria for the formation of crystal seeds or so-called critical nuclei can be established. Henceforth, in this report, the words nucleus or nuclei are taken to be synonymous with critical nucleus or critical nuclei. This approach has come to be known as the classical model for homogeneous nucleation. With a thermodynamic requirement for nuclei formation established, a temperature dependence and rate of nuclei formation are then obtained by assuming an Arrhenius dependence. Finally, using the Gibbs-Thomson relation the supersaturation can be related to the free energy of nuclei formation, thus yielding the nucleation rate J, that is the number of nuclei forming per unit volume per unit time, in terms of the solute concentration c:

$$J = A \exp\left[\frac{-16\pi\gamma^3 v_m^2}{3k_B{}^3T^3(\ln c/c_o)^2}\right], \tag{1}$$

here T is absolute temperature, k_B Boltzmann's constant, γ the interfacial tension between nuclei and solvent, c_o the solubility, v_m the molecular volume, and A a constant.

The crystal growth process for a supersaturated solution can be divided into three stages: induction, intermediate, and ripening. In the first stage, a period of time elapses before the

appearance of crystals and thus the first detectable decrease in a solute concentration. We take the view here given by Mullin, and let the *induction period* be the sum of a relaxation and nucleation time as well as an additional time period for the nuclei to grow to detectable size (Ref. 1, p. 194). Next, the *intermediate stage* begins with the onset of crystal growth and a detectable decrease in the supersaturation. The intermediate stage ends when the solute concentration equals its solubility concentration. Finally, in the *ripening stage* while the solute concentration remains essentially constant there can be an energetically favorable transfer of material from small to larger crystals.

Equation (1) is sometimes referred to as the model of Weber-Volmer-Frenkel-Zel'dovich.[2] Another frequently used model for the nucleation rate is the power law expression often attributed to Meir: $J = \beta(c - c_o)^p$.[2] Here β is a constant and p a positive integer. In both cases, the time dependence at constant temperature comes through the solute concentration, that is, $c = c(t)$, where t is time. Beginning with a kinetic model, Katz and Donohue derived a result similar to Equation (1) with the concentrations being replaced by forward and backward attachment rate coefficients so that the time dependence for the nucleation rate depends upon behavior of these coefficients.[3]

A rigorous treatment of the time dependence of the nucleation phenomenon comes from the kinetic model of Szilard and Farkas.[4,5] This complete, subsequent monomer addition mechanism has been studied theoretically by many workers. However, after a thorough search of the literature on protein aggregation Morris et al. found that "…the resulting mathematics and kinetic equations corresponding to the complete mechanism are, however, not routinely used to fit experimental data".[6] More recently, several phenomenological theories for the nucleation rate have been considered where time is an explicit independent variable in the governing equations. All are very useful for comparison with experimental results. Nanev and Tonchev proposed a rate law for the cumulative density of nuclei in a supersaturated protein solution.[7,8] Here the nuclei density n at time t are governed by the logistic differential equation,[9]

$$\frac{dn}{dt} = kn - \omega k n^2, \tag{2}$$

where ω and k are constants. The solution for Equation (2) is the sigmoid function for $n(t)$. Many examples of this kinetic behavior for cluster formation are found in the experimental literature.[10–14] Nanev and Tonchev find this kinetic behavior specifically for nucleation within supersaturated insulin solutions for seven different initial supersaturations.[7] Morris et al. used the Finke-Watzky two-step kinetic model to study over 10 cases of protein aggregation from the literature.[6] This model, which has a step for nucleation and another for aggregation, leads to a sigmoidal equation for the concentration of protein converted to aggregate. Here two-step refers to the steps in the kinetic model not to a two-step nucleation process. In the Appendix, we present a kinetic model exclusively for nuclei from which Equation (2) can be derived.

Another approach involves use of a Fokker-Planck equation for continuity coupled with an integral equation for mass balance. Larson and Randolph showed that a similar system could be solved for the case of continuous crystallization.[15] Later, the same theory was modified by Buyevich and Mansurov for the study of batch crystallization where a description of the crystal size was included via a size distribution function.[16] In this form, the system is nonlinear and an approximate solution was given. Since the time of Buyevich and Mansurov's paper, variants of this model have been used to study a variety of crystal growth systems.[2,17–23] The governing equations are

$$\frac{\partial f(t,r)}{\partial t} + \frac{\partial G(t) f(t,r)}{\partial r} = 0, \tag{3}$$

$$\frac{ds(t)}{dt} = -K \int_0^\infty r^2 G(t) f(t,r) dr, \tag{4}$$

where s is the normalized supersaturation, r the crystal radius or some other characteristic length, $G = dr/dt$ the linear crystal growth rate, f the size distribution function, and K a constant. A standard approach in dealing with the system above is to select a model for the growth rate G and the nucleation rate J, where $J = Gf(t,0)$. Barlow showed that by assuming the distribution function to be separable, along with a model for the growth rate, the system given by Equations (3) and (4) can be solved for the distribution function.[18] Therefore, the nucleation rate is determined by this solution and need not be modeled beforehand. This result for the nucleation rate is shown here to be equivalent to the result arrived at by Nanev and Tonchev through the solution of Equation (2). As a result, the parameters from the expression for the nucleation rate determined by Ref. 18 can be related to those of Ref. 7.

A third model was recently considered by Kashchiev and co-workers.[24] Here the nuclei are presumed to appear as part of a two-step nucleation mechanism—a phenomenon that has been suspected to occur in certain cases of crystal growth and/or protein aggregation.[10,25–33] Rather than forming homogeneously, it is proposed that nuclei form within solute-rich clusters throughout the host solution. Henceforth in this report, we will refer to these clusters as *associates*. As the growth process proceeds, these associates can either develop into stable crystals, dissolve back into the solution, or be directly consumed by nearby growing crystals. If the final density of nuclei that will form during the growth process is given by n_0 then Kashchiev and co-workers give the time-dependent density of two-step nuclei, n_{ts}, forming in

the solution as a generalization of the Johnson-Mehl-Avrami-Kolmogorov (JMAK) equation (Ref. 5, p. 375)

$$n_{ts} = n_0\left\{1 - \exp\left[-\int_o^t j_c(t')v(t-t')dt'\right]\right\}. \tag{5}$$

These associates form at time t', where $t' <= t$. The volume of an associate is given by v, whereas the rate of nucleation within the two-step associate is given by j_c. Kashchiev and co-workers go on to model the function v with an Avrami-type expression and then use Equation (5), and other similar expressions, to study steady-state two-step protein nucleation from solution.

In addition to demonstrating that the nucleation rate determined by the solution of Equation (2) is equivalent to that as found from the system of Equations (3) and (4), it is shown that under certain conditions Equation (5) yields sigmoidal-like results for the kinetics of nucleation, that is, when the integrand in Equation (5) is a function of t.

Two-step nucleation, of some sort, is suspected to be active for certain cases of protein crystal growth from solution and has been confirmed recently for the crystallization of β-lactoglobulin.[10] We suggest then that the sigmoidal rate law describes both nucleation via the two-step method and the homogeneous one as well. By comparing this claim with experimental reports, we demonstrate how the theory describes cases where homogeneous nucleation is dominant and situations when two-step nucleation is also present. These results suggest that the two-step associates form around seed nuclei that exist essentially at the instant of solution creation. Then, as time proceeds, the two-step nuclei dissolve or are transferred directly into growing crystals formed around homogeneous seeds. Based on the reports studied here, it appears that for batch protein crystallization as the initial supersaturation or salt concentration increases the two-step mechanism becomes more active. Additionally, an estimate is given for the volume of a two-step associate as a function of time for a reported case of protein crystal growth where two-step activity was confirmed by X-ray diffraction. Finally, an expression for the induction period is generalized here so that it is written in terms of the rate constant and the initial and final nuclei densities. All applications of these models in this report were for supersaturated batch growth from solution at constant temperature.

2 | ANALYSIS

The nucleation rate determined by solving Equation (2) is shown here to be equivalent in form to the nucleation rate given in Ref. 18 for the solution of Equations (3) and (4). We let Equation (2) have the initial and final conditions that at $t = 0$, $n = n_1$ and as $t \to \infty$, $n \to n_0$. With these, Equation (2) can be separated and integrated to yield

$$n = \frac{n_0}{1 + \frac{(n_0 - n_1)}{n_1}e^{-kt}}. \tag{6}$$

Now, using this result we find the nucleation rate $J = dn/dt$:

$$J = \frac{kn_0\left(\frac{n_0 - n_1}{n_1}\right)e^{-kt}}{\left(1 + \frac{n_0 - n_1}{n_1}e^{-kt}\right)^2}. \tag{7}$$

Starting with Equations (3) and (4), Ref. 18 gives a nucleation rate, which after rearrangement leads to

$$J = \frac{Ck^2e^{-kt}}{\lambda b^2\left[1 + (1/b)e^{-kt}\right]^2}, \tag{8}$$

where the parameters λ, C, and b are defined in Ref. 18. By inspection, it is seen that Equations (7) and (8) are of the same form. Further, we find parameters between the two relate as

$$\frac{1}{b} = \frac{n_0 - n_1}{n_1}, \qquad \frac{Ck}{\lambda} = \left(\frac{n_1}{n_0 - n_1}\right)n_0. \tag{9}$$

Now, consider the third model mentioned above, Equation (5). This expression is a generalization of the original JMAK equation used to study the fraction or extent of crystallization over time. It implies a first-order growth law. If the rate coefficient is time dependent, then the model can yield sigmoidal-like behavior. For example, for the fraction of crystallization, α, Kashchiev gives

$$\alpha(t) = 1 - \exp\left(\frac{-t^p}{B}\right), \tag{10}$$

where B and p are positive valued parameters (Ref. 5, p. 378). In using the model here for the study of nucleation, the time dependence for the rate coefficient will be determined by the integral in Equation (5). In the next section, it is shown that a variant of Equation (5) can be used to model the kinetics for a two-step nucleation process and thus the time dependence for the volume of the two-step associate can be estimated.

A first-order decay/growth law with a time-dependent rate coefficient, which yields sigmoidal-like behavior, is typically associated with chemical reactions where there is a precatalytic phase and thus an induction period. In the past, certain groups have suggested that the system given by Equations (3) and (4) be restricted to an analysis of the intermediate stage of growth.[16,18,22] However, the more general view taken here is that the sigmoid law can be used to describe both the intermediate stage and induction period less any relaxation time. That is, it includes an initial time period where though the supersaturation decays, this decay is imperceptibly small. Thus we take the induction period τ to be given by $\tau = t_n + t_g$, where t_n is the nucleation time, the time required for the first stable

nucleus to appear and t_g the time required for a nucleus to grow to a crystal of detectable size.

Previously, it has been proposed that the time span for the induction period τ, be given in terms of the sigmoid rate constant k, and the time t_c when the sigmoidal nucleation rate is a maximum, as[7,34]

$$\tau = \left(\frac{6}{\pi^2}\right)\left(t_c - 2/k\right). \tag{11}$$

Using Equation (7), this can be written in terms of the initial and final nuclei densities. From Equation (7), we find for t_c:

$$t_c = \frac{1}{k}\ln\left(\frac{n_0 - n_1}{n_1}\right), \tag{12}$$

and thus

$$\tau = \left(\frac{6}{\pi^2 k}\right)\left[\ln\left(\frac{n_0 - n_1}{n_1}\right) - 2\right]. \tag{13}$$

Interestingly, Equation (13) predicts that the induction period decreases as the ratio n_1/n_0 increases and becomes zero when $n_1/n_0 = 1/(e^2 + 1) \approx 0.119$. Values for τ are computed using these results for three different experimental cases and listed in the following section.

3 | COMPARISON WITH EXPERIMENT

Since two-step and homogeneous nucleation have been reported for or are suspected to be involved in the nucleation and crystallization of the proteins, insulin, lysozyme, and β-lactoglobulin,[7,10,26] and the sigmoid function has been shown to accurately describe the kinetics of protein nucleation,[7] we propose here that Equation (6) describes both the two-step and homogeneous nucleation process. To reveal this notion, we note that Equation (6) can be rearranged and separated into two terms as follows:

$$\frac{n_0 e^{kt}}{\frac{(n_0-n_1)}{n_1} + e^{kt}} = \frac{n_0}{\frac{(n_0-n_1)}{n_1} + e^{kt}} + \frac{n_0 e^{kt} - n_0}{\frac{(n_0-n_1)}{n_1} + e^{kt}}. \tag{14}$$

It is proposed that the first term on the right of Equation (14) describes the kinetics of two-step nucleation whereas the second term gives the homogeneous nucleation time dependence. Therefore, it follows that there must have been n_1 initial nuclei. These critical seeds are likely hard to detect and are available as soon as the solution is prepared. However, when n_1 is small the second term dominates for all times t. In this case, nucleation occurs mostly through the homogeneous mechanism. These relationships are further clarified by using Equation (14) to describe three sets of experimental data.

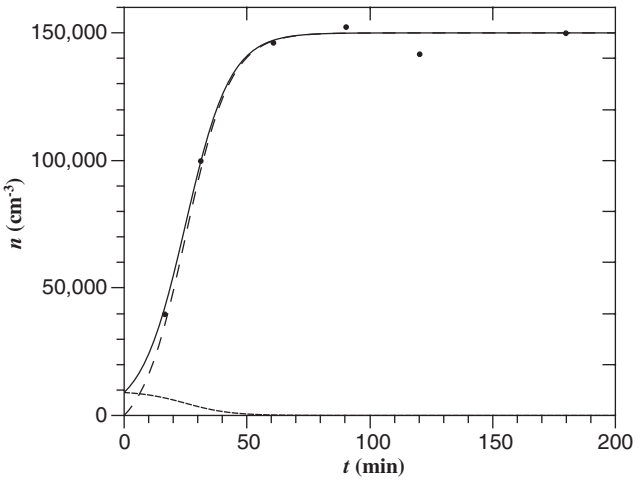

FIGURE 1 Data for nuclei density versus time reported by Nanev and Tonchev[7] for a relative supersaturation of 3.69. Equation (14) is fitted to these data. The solid curve is for the total nuclei density, that is the left side of Equation (14). The long-dashed curve gives the predicted homogeneous nuclei density, whereas the finely dashed curve gives the predicted two-step nuclei density which decays as nuclei are transferred to growing crystals. $k = 0.11\ \text{min}^{-1}$, $n_0 = 150,000\ \text{cm}^{-3}$, $n_1 = 9000\ \text{cm}^{-3}$, $n_1/n_0 = 0.06$, $T = 303$ K

Nanev and Tonchev[7] have reported experimental nucleation data for insulin crystal growth from batch solution for several different initial supersaturations. These data were collected using the *nucleation-growth-separation principle* (NGSP) technique. Equation (14) is fitted to data from this report for the highest initial supersaturation given. This is depicted in Figure 1. $n(t)$ overall, that is, Equation (6), is fitted to the data, and then the two terms on the right of Equation (14) are also separately shown. In this higher supersaturation case, the initial nuclei n_1 are a significant fraction of the final nuclei n_0. From the curve given by the first term on the right of Equation (14), it is seen that the initial nuclei n_1 are used to initiate the two-step process and are eventually consumed directly or indirectly into crystals growing from homogeneous seeds. The curve for the second term on the right of Equation (14) indicates the generation of homogeneous nuclei, which were not present in the solution at the time of preparation.

It should be noted that when using the NGSP technique to determine n, the behavior of any two-step nuclei in the solution at the time of separation could be altered. That is, they might dissolve back into solution or go on to form into small crystals. So there is some ambiguity as to whether the NGSP method accounts for only homogeneous nuclei or both homogeneous and two-step nuclei. Even if the results account for only homogeneous nuclei, as we suspect, the resulting curve fits in Figure 1 go largely unaltered.

In the low supersaturation case (Figure 2), it can be seen that nucleation is predicted to be almost entirely of the homogeneous type, that is the ratio of n_1/n_0 is smaller than for the

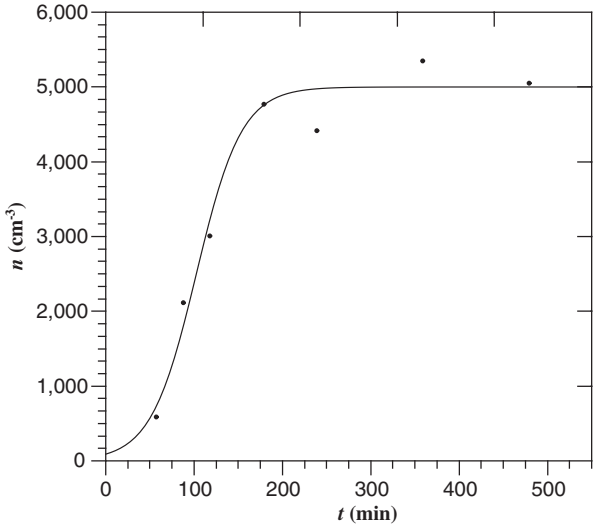

FIGURE 2 Data for nuclei density versus time reported by Nanev and Tonchev[7] for the lower relative supersaturation of 2.99. Equation (14) is fitted to these data. Only the overall nucleation density curve is shown fitted here as the nuclei are predicted to be mostly of the homogeneous type. $k = 0.039$ min^{-1}, $n_0 = 5,000$ cm^{-3}, $n_1 = 90$ cm^{-3}, $n_1/n_0 = 0.018$, $T = 303$ K

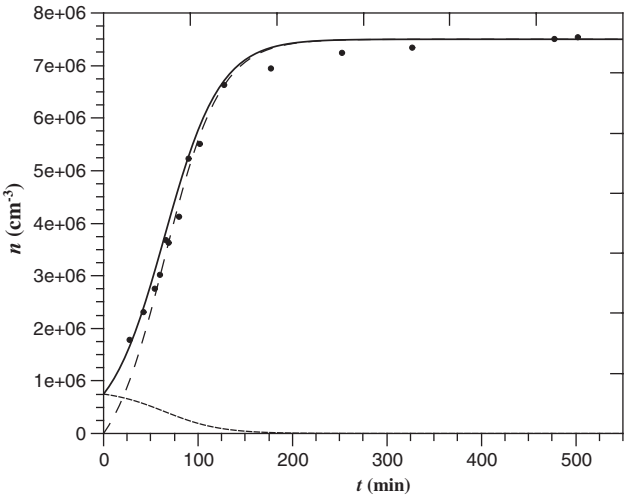

FIGURE 3 Estimated crystal density versus time data based on data reported by Sauter et al.[10] for 15 mM $CdCl_2$ in 20 mg/mL β-lactoglobulin solution—the higher salt concentration of the two cases given in their figure 4. (Data were reported in number per area. An assumption for the thickness involved, that is 50 μm, was used to convert the data into number per unit volume for this work. This was done for consistency and does not alter the value of k nor the ratio n_1/n_0.) For purposes of discussion, Equation (14) is fitted to these data. The solid curve is for the total nuclei density, that is the left side of Equation (14). The long-dashed curve gives the predicted homogeneous nuclei density,whereas the finely dashed curve gives the predicted two-step nuclei density which decays as nuclei are transferred to growing crystals. $k = 0.034$ min^{-1}, $n_0 = 7.5 \times 10^6$ cm^{-3}, $n_1 = 0.75 \times 10^6$ cm^{-3}, $n_1/n_0 = 0.1$, $T = 293$ K

higher supersaturation case. These results, along with other reports from the literature,[25,28] lead us to speculate that two-step nucleation activity in protein batch growth increases as the initial supersaturation increases.

β-Lactoglobulin crystallization and growth was studied recently by Sauter et al.[10] We focus here on information given for two growth runs at different salt concentrations and different initial supersaturations, that is, their figure 4. Data for crystal density versus time were listed in this report for the higher salt concentration case. Though the crystal density does not necessarily equate with the nuclei density n at a particular time, it is however, following the description given in the insulin cases above, likely to give a slightly time-shifted representation of the homogeneous nuclei density. Fitting $n(t)$ with these data was done previously by Nanev and Tonchev.[7] They report a rate constant from this fitting of 0.036 min^{-1}. The process is repeated here with both terms on the right of Equation (14) fitted as well. Our fit leads to $k = 0.034$ min^{-1} and is depicted in Figure 3. Here, as in Figure 1, the curve fit points to the presence of initial seed nuclei indicating two-step activity. Unfortunately, crystal density versus time was not reported for the low salt concentration case but from the experimenter's description of this run, that is it having a longer induction period and no evidence of two-step activity, we suspect that it to could be fitted to Equation (14) and reveal characteristics similar to those of Figure 2. From these results, we are lead to suggest that increasing salt concentration during batch protein crystal growth increases two-step activity. Additionally, Sauter et al.[10] carried out X-ray diffraction measurements during the two batch growth runs mentioned above. These results indicate the presence of two-step activity, what they refer to as an intermediate phase, in the high salt concentration case but not in the lower concentration case.

Expression (5) can be used to model the kinetic behavior of the volume of an associate affiliated with a seed nuclei governed by the first term on the right of Equation (14). We employ the first-order decay analogue of Equation (5) and equate this to the two-step term on the right of Equation (14):

$$n_{ts}(t) = \frac{n_0}{\frac{(n_0 - n_1)}{n_1} + e^{kt}} = n_1 \exp\left(\int_o^t j_c(t')v(t-t')dt'\right). \tag{15}$$

Here $j_c < 0$ and v is a function of $t - t'$. t', the time of birth for the two-step associate, is set to zero so that all two-step nuclei are formed at the start of the run. With these conditions, the integral in expression (15) becomes $j_0 v(t)t$, where j_0 is the initial two-step nucleation rate. Solving for $v(t)$ leads to

$$v(t) = \frac{1}{|j_0|t}\left\{\ln\left[\frac{e^{kt} + \left(\frac{n_0}{n_1} - 1\right)}{n_0/n_1}\right] - \frac{n_1}{n_0}kt\right\}. \tag{16}$$

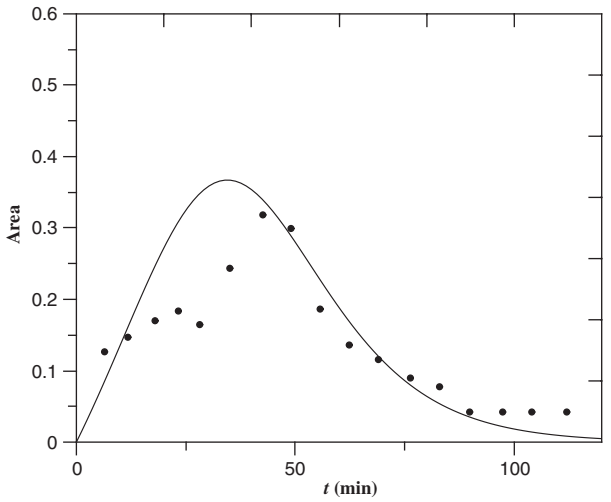

FIGURE 4 Fitting of Equation (17) to X-ray diffraction data reported in Ref. 10. $|j_o|$ and k were used as adjustable parameters. $k = 0.071\ \text{min}^{-1}$, $|j_o| = 1.37 \times 10^4\ \text{cm}^{-3}$. The quantity of area is indicative of the presence of the two-step (intermediate) phase. The area was computed from fitting X-ray data to a Gaussian curve, and interested readers are encouraged to see Ref. 10 for further details

Here since the two-step associate was a critical nucleus at $t = 0$, we require that $v(0) = 0$. This initial condition was used to arrive at $v(t)$ in the form given above. Using this model for v, along with the two-step nuclei density as given by the first term on the right of Equation (14), we write the total two-step associate volume, V_T, in 1 cm^3 of solution as a function of time as

$$V_T = \frac{n_0 v(t)}{\frac{(n_0 - n_1)}{n_1} + e^{kt}}. \tag{17}$$

Recently, Sauter et al. reported X-ray diffraction data taken during the crystallization of β-lactoglobulin from solution.[10] Their data indicate that solute-rich two-step associates (an intermediate phase) are initially not present but then grow as the run proceeds and then eventually disappears as the crystallization process goes to completion. Equation (17) is fitted to X-ray diffraction data reported for the growth case depicted in Figure 3. From Figure 3, we get n_1 and n_0. k and j_0 are left as adjustable parameters. This curve and data are depicted in Figure 4. This fit yields $k = 0.071\ \text{min}^{-1}$. Since the crystal density data shown in Figure 3 are likely to be a time-shifted picture of the nuclei density, it is not surprising that k obtained from Figure 4, where the kinetics of two-step nuclei were studied, is larger than that of Figure 3. That is, the crystal density at some point in time must correspond to an equivalent nuclei density at an earlier time.

The ratio n_1/n_0 is given for each case considered here in Figures 1–3. For the insulin nucleation cases studied here, in Figures 1 and 2, the larger initial supersaturation case of Figure 1 has the larger n_1/n_0 ratio of the two and thus the shorter induction period. For the case of β-lactoglobulin given in Figure 3, we see that the value for the ratio is very near the maximum value allowed by Equation (13) and thus predicts a relatively short induction period. This is consistent with what was reported for this growth run; the solution became cloudy after preparation, and protein aggregates formed quickly after sample preparation.[10]

TABLE 1 Induction periods and nucleation times computed using Equations (13) and (18) for the cases depicted in Figures 1–3

	Figure 1	Figure 2	Figure 3
τ	4.16 min	31.2 min	1.67 min
t_n	60.6 ms	17.0 s	1.01 ms

n_1, n_0, and k for each case were listed with the figures. For the β-lactoglobulin data, given in Figure 3, k arrived at from the curve fit of Figure 4 was used.

An estimate can now be given for the nucleation time t_n. We let this be the time when the homogeneous nuclei density equals 1.0 cm^{-3}. Using the far right term of Equation (14), one arrives at

$$t_n = \frac{1}{k} \ln\left(\frac{n_0 + 1 + n_0/n_1}{n_0 - 1}\right) \approx \frac{1}{k} \ln\left(1 + 1/n_1\right). \tag{18}$$

Using Equations (13) and (18), induction periods and nucleation times are estimated for the cases depicted in Figures 1–3. These data are listed in Table 1.

4 | CONCLUSION

In this report, it has been shown how three separate theoretical schemes lead to sigmoidal behavior for the kinetics of crystal nucleation from solution. This result gives credence to the suggestion of Nanev and Tonchev[7] for the possibility of there being a universal behavior for the kinetics of nucleation in organic and inorganic systems. Assuming this type behavior, the resulting expression for n versus t should then encompass details for two-step and homogeneous nucleation simultaneously. By separating the expression into two terms, it is proposed that one gives the two-step process whereas the other describes homogeneous activity. This in turn predicts that two-step associates grow around seed nuclei that are already present or are formed immediately after solution preparation.

The total associate volume grows during the initial phase of the batch run and then dissipates as their nuclei and related material are transferred either directly or indirectly into crystals growing from homogeneous nuclei. Experimental evidence for the transfer of matter from a solute-rich associate directly into a growing protein crystal has been reported for β-lactoglobulin,[10] lysozyme,[26,35] and phosphoglucomatase.[31] Additionally, recent work by Allahyarov et al. provides evidence for a similar type of behavior with heterogeneous

seeds which are present at the start of the crystallization process.[36]

The sigmoid expression is compared with three different protein batch crystal growth runs. The results indicate that two-step activity increases with increasing initial supersaturation or increasing salt concentration. Other reports from the literature suggest that two-step activity also increases with decreasing temperature.[26,28] The value for the rate constant k also likely depends upon initial supersaturation, salt concentration, and temperature. However, an interesting consequence of Equations (6) and (13) is that as $n_1 \to 0$ nuclei are not predicted to form for any k and the induction period becomes infinite.

It should be noted that the crystallization experiments studied here were likely performed such that concentrations and/or temperature placed the process near a liquid-liquid phase boundary—often a situation conducive to the protein crystal growth process. Therefore, the trends suggested above should be understood in light of this fact.

A known expression for the induction period was rewritten in terms of the initial and final nuclei densities. It predicts that as the ratio of initial to final nuclei density increases the induction time decreases and reaches zero when the ratio is ≈0.119.

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APPENDIX

Here a simple high-level kinetic reaction model is given for the formation of nuclei in a supersaturated protein solution. The scheme leads to the logistic differential equation and thus a sigmoidal description of the nuclei density versus time.

Let a critical nucleus be given by N and a protein monomer by P. Let the number of monomers in the nucleus be i. Then, consider the following two-step process:

$$P_{i-1} + P \longrightarrow N \text{ (homogeneous nucleation) (i)}$$

$$N + N \longrightarrow product \text{ (initial seed nuclei consumption) (ii)}$$

Reaction (i) gives

$$\frac{d[N]}{dt} = k_1[P_{i-1}][P]. \tag{A1}$$

Reaction (ii) leads to

$$-\frac{d[N]}{dt} = k_2[N]^2. \tag{A2}$$

So the total nucleation rate is then

$$\frac{d[N]}{dt} = k_1[P_{i-1}][P] - k_2[N]^2. \tag{A3}$$

We use the approximation that $[P_{i-1}] \approx [N]$ and also assume that $[P] = [P]_0 - a[N]$, where $[P]_0$ is the initial protein concentration and a is a positive constant. Making these substitutions into Equation (A3), we arrive at

$$\frac{d[N]}{dt} = k_1[P]_0[N] - (ak_1 + k_2)[N]^2. \tag{A4}$$

With the substitutions $k = k_1[P]_0$ and $\omega = (ak_1 + k_2)/k_1[P]_0$, we arrive at the Nanev-Tonchev equation for the rate of critical nuclei density.

$$\frac{d[N]}{dt} = k[N] - k\omega[N]^2. \tag{A5}$$