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Nucleation of Crystals from Solution: Classical and Two-Step Models

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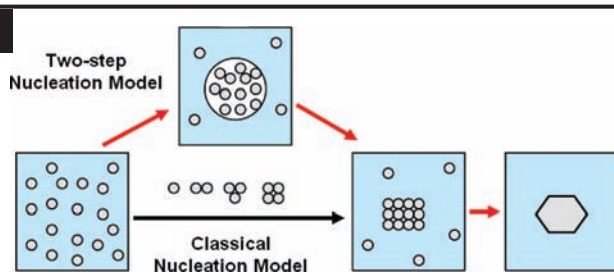

Crystallization is vital to many processes occurring in nature and in the chemical, pharmaceutical, and food industries. Notably, crystallization is an attractive isolation step for manufacturing because this single process combines both particle formation and purification. Almost all of the products based on fine chemicals, such as dyes, explosives, and photographic materials, require crystallization in their manufacture, and more than 90% of all pharmaceutical products contain bioactive drug substances and excipients in the crystalline solid state. Hence control over the crystallization process allows manufacturers to obtain products with desired and reproducible properties. We judge the quality of a crystalline product based on four main properties: size, purity, morphology, and crystal structure. The pharmaceutical industry in particular requires production of the desired crystal form (polymorph) to assure the bioavailability and stability of the drug substance.

In solution crystallization, nucleation plays a decisive role in determining the crystal structure and size distribution. Therefore, understanding the fundamentals of nucleation is crucial to achieve control over these properties. Because of its analytical simplicity, researchers have widely applied classical nucleation theory to solution crystallization. However, a number of differences between theoretical predictions and experimental results suggest that nucleation of solids from solution does not proceed via the classical pathway but follows more complex routes. In this Account, we discuss the shortcomings of classical nucleation theory and review studies contributing to the development of the modern two-step model.

In the two-step model that was initially proposed for protein crystallization, a sufficient-sized cluster of solute molecules forms first, followed by reorganization of that cluster into an ordered structure. In recent experimental and theoretical studies, we and other researchers have demonstrated the applicability of the two-step mechanism to both macromolecules and small organic molecules, suggesting that this mechanism may underlie most crystallization processes from solutions. Because we have observed an increase in the organization time of appropriate lattice structures with greater molecular complexity, we propose that organization is the rate-determining step.

Further development of a clearer picture of nucleation may help determine the optimum conditions necessary for the effective organization within the clusters. In addition, greater understanding of these processes may lead to the design of auxiliaries that can increase the rate of nucleation and avoid the formation of undesired solid forms, allowing researchers to obtain the final product in a timely and reproducible manner.

1. Introduction

The primary goal of solution crystallization is to generate particles with desired size, shape, crystal form, and chemical purity in a reproducible manner, because these individual characteristics can affect the physical and chemical properties of

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the solid. To accomplish this goal, one has to establish control over crystallization, which is a complex process completed in several stages. The first stage is the formation of supersaturated solutions since the spontaneous appearance of a new phase occurs only when a system is in a nonequilibrium condition. In the next stage, molecules dissolved in solution begin to aggregate (concentration fluctuations), which eventually leads to the formation of nuclei that can act as centers of crystallization. A nucleus can be defined as the minimum amount of a new phase capable of independent existence. The birth of these small nuclei in an initially metastable phase is called nucleation, which is a major mechanism of first-order phase transition. The growth stage, which immediately follows the nucleation, is governed by the diffusion of particles, called the growth units, to the surface of the existing nuclei and their incorporation in the structure of the crystal lattice.

The early stages of solution crystallization play a decisive role in determining the crystal properties, mainly the crystal structure and size distribution. Thus, higher levels of control over crystallization cannot be achieved without understanding the fundamentals of nucleation. However, an accurate description of the process is still missing, and the design of crystallization processes is more often than not done on an empirical basis. The essential difficulty of studying nucleation and developing an accurate description of the process arises from the fact that the critical nucleus sizes typically fall in the range of 100−1000 atoms, which is hardly accessible to most of the current experimental methods.[1] Even if they are detected by microscopic techniques, the structure may not be distinguished due to their small size.[2] Furthermore, they exist for extremely short times and freely move throughout the available volume of solution, reducing the change of their appearance in the volume being examined. Nevertheless, experimental and theoretical studies of supersaturated solutions over the last two decades were able to provide valuable information on structures of these solutions, which encouraged re-evaluation of classical nucleation theory (CNT), which was developed 80 years ago and still widely used to describe solution crystallization due to its analytic simplicity. This Account discusses the major assumptions and the shortcomings of CNT and reviews studies that contributed to the development of modern two-step theory, which potentially presents a more accurate description of nucleation mechanisms in supersaturated solutions.

Classical Nucleation Theory (CNT)

CNT is the simplest and most widely used theory that describes the nucleation process. Even though CNT was originally derived for condensation of a vapor into a liquid, it has also been employed "by analogy" to explain precipitation of crystals from supersaturated solutions and melts. The thermodynamic description of this process was developed at the end of the 19th century by Gibbs, who defined the free energy change required for cluster formation (ΔG) as sum of the free energy change for the phase transformation (ΔG_v) and the free energy change for the formation of a surface (ΔG_s). In terms of crystallization from solution, the first term describes the spontaneous tendency of a supersaturated solution to undergo deposition. Since the solid state is more stable than the liquid, ΔG_v becomes negative and thus decreases the Gibbs free energy of the system. On the other hand, introduction of a solid/liquid interface increases the free energy by an amount proportional to the surface area of the cluster. As a result, the growth of clusters depends on the competition between a decrease in ΔG_v, which favors growth, and an increase in ΔG_s, which favors dissolution (Figure 1). The positive surface free energy ΔG_s term dominates at small radii, which causes an increase in the total free energy change initially. Thus the smallest clusters in solution typically dissolve. As cluster size increases total free energy goes through a maximum at a critical size (r_c), above which the total free energy decreases continuously and growth becomes energetically favorable, resulting in the formation of crystal nuclei.

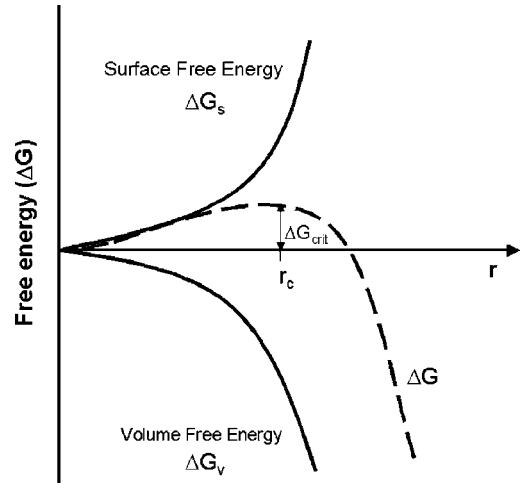

FIGURE 1. Free energy diagram for nucleation.

In the kinetic theory developed based on Gibb's formalism, the steady-state rate of nucleation (J), which is equal to the number of nuclei formed per unit time per unit volume, is expressed in the form of the Arrhenius reaction rate equation as

$$J = A\exp\left(-\frac{\Delta G_{crit}}{kT}\right) \quad (1)$$

where k is the Boltzmann's constant and A is the pre-exponential factor. The theoretical value of the pre-exponential factor is given as 10^{30} cm^{-3} s^{-1}; however, it is very difficult to measure in practice.[3] This kinetic factor is related to the rate of attachment of molecules to the critical nucleus, thus depends on the molecular mobility.[4] Since the molecular mobility changes rapidly with temperature, the temperature dependence of the pre-exponential factor can be quite significant.

CNT is based on major assumptions, which simplify the description of the process but at the same time restrict its applications to certain cases:

(1) The clusters are modeled as spherical droplets having uniform interior densities and sharp interfaces (droplet model). The density of the droplet is independent of the droplet size and equal to the macroscopic density of the bulk condensed phase. For crystallization from solution, this assumption implies that the building blocks are ordered; thus, the molecular arrangement in a crystal's embryo is identical to that in a large crystal.

(2) The surface tension of a liquid droplet is equal to the respective value of this quantity for a stable coexistence of both phases at an infinite planar interface (capillarity approximation).[5] In other words, the curvature (or size) dependence of the surface tension is neglected. In addition, the surface energy is assumed to be temperature-independent.[3]

(3) The growth of clusters takes place by addition of one monomer at a time. Furthermore, collisions between more than two particles, as well as two pre-existing clusters, and break-off of pre-existing clusters into two or more smaller clusters are ignored. Instead, clusters are at rest, and they do not undergo translational, vibrational, or rotational motion.

(4) The stationary distribution of subcritical solute clusters is established instantaneously after the onset of supersaturation. The nucleation rate is time-independent, that is, the features of the process are considered in terms of steady-state kinetics.

(5) The clusters are incompressible and the vapor surrounding them is an ideal gas with a constant pressure. Thus, the formation of clusters does not change the vapor state.

3. Shortcomings of Classical Nucleation Theory

Given that all input parameters of CNT are known with a high accuracy for condensation experiments, even a few orders of magnitude difference between the predicted and measured nucleation rates for single-component fluids can be an indication of the inadequacy of the classical theory. Even for condensation of water, CNT predicts nucleation rates 1−2 orders of magnitude higher than the rates inferred from expansion cloud chamber experiments.[6] For condensation of *n*-alcohol vapors, the experimental and theoretical rates were found to differ significantly, with the ratio between two values ranging from about 10^{-10} for methanol to 10^{7} for *n*-hexanol.[7] Furthermore, even though the dependence of the nucleation rate on supersaturation was correctly described by CNT, the predicted temperature dependences significantly disagreed with the experimental findings. These results suggested that a multiplicative temperature-dependent correction was required to make classical theory agree with experiment. Because of these deviations, it is accepted that CNT gives qualitatively reasonable but quantitatively incorrect results for the gas−liquid transition of single-component nonpolar fluids.

Even though classical theory has practical success for single-component nucleation, the classical binary nucleation theory used for the mixed nucleus is oversimplified. In CNT, composition is assumed to be uniform throughout the droplet. However, it is widely believed that the surface of alcohol−water and acetone−water clusters can have a considerably different composition than bulk due to the surface enrichment effects. For example, at low ethanol concentrations, there exists a very significant surface enrichment of ethanol.[8] For these systems, the surface energy and nucleation rate depend sensitively on the composition of the critical nucleus. Therefore CNT fails both quantitatively and qualitatively to describe binary nucleation in water-rich aqueous alcohol or acetone mixtures since the composition of nucleus is incorrectly predicted.[9]

The steady-state nucleation rate in the CNT is calculated for the condition that the size distribution of clusters does not change in time. This leads to a constant nucleation rate, that is, a linear increase of the number of nuclei with time.[10] This assumption fails at the very beginning of the nucleation process since a certain time, called the transition time, is required to establish the steady-state distribution of subcritical clusters.[11] Furthermore, in some experiments the relaxation process into steady state takes a much longer time than the

characteristic lifetime of the supersaturated system; hence a steady-state nucleation does not exist.

CNT cannot predict the absolute nucleation rates given that the pre-exponential factor in the kinetic equations remains undetermined. Rather, the kinetic factor, as well as the surface free energy, is adjusted to fit the experimental nucleation rate data to the theory. For the calculation of kinetic factors at various temperatures, it is necessary to neglect not only the size dependence but also the temperature dependence of the surface energy.[3] Even so, when the thermodynamic driving force of the transformation was calculated as the difference of the chemical potentials in the respective macroscopic phases, large discrepancies were observed between the experimental and predicted kinetic factors.[12] In some cases, the experimental factor exceeded the theoretical one by ~130 orders of magnitude.[3] These significant deviations may be caused by one of the assumptions made in CNT that neglects the movement of clusters since the pre-exponential factor is related to the molecular mobility.

There has been a long-lasting debate concerning whether a macroscopic thermodynamic description of a liquid drop can be used to model small clusters containing only a few of tens of molecules.[9,13] It was stated that this assumption fails for nuclei containing only 20−50 molecules, which are small enough that the center is not in the thermodynamic limit and the interface is sharply curved, changing its free energy.[14] The failure of the droplet model in describing small particles is evident from the fact that the work of formation of monomers differs from zero.[15] Since the properties of small clusters cannot be divided into volume and surface characteristics, the concept of surface tension seems to be artificial as applied to these clusters. According to Yau and Vekilov,[2] the surface tension is ill-defined for clusters smaller than 100 molecules, and the nucleus shape cannot be approximated with a sphere. They showed, using atomic force microscopy, that the critical nucleus of apoferritin contains between 20 and 50 molecules arranged identically to those found in crystals and consists of planar arrays of one or two monomolecular layers, which makes the nucleus to look like a raft. This disagrees with the droplet model, which states that spherical molecules, such as apoferritin, should form a spherical nucleus, like a tiny ball cut out from the bulk crystal lattice.[16] If the critical nuclei are not spherical, CNT can no longer be valid. In fact, it was stated that a critical nucleus cannot be a smooth sphere since cubes or polyhedra can represent better lattice forming shapes.[17] If one assumes a cubic instead of a spherical shape, the number of molecules present in the critical nucleus would be expected to be twice as large. In addition, critical clusters consisting of only 1−10 lysozyme molecules contradict the assumption that the molecular arrangement in near-critical clusters is identical to that in crystals since clusters so small cannot have the structure of a tetragonal lysozyme crystal.[18] In fact, theoretical studies showed that the properties of the critical nucleus can differ drastically from the eventual new phase in composition and structure.[14] For instance, the precritical nucleus of a Lennard-Jones solid, whose structure is known to be face-centered cubic, was found to be predominantly body-centered cubic ordered.[19]

While CNT allows one to estimate the size of critical nucleus and nucleation rate, it does not provide any information about the structure of aggregates or pathways leading from the solution to the solid crystal.[20] Perhaps the most significant shortcoming of the classical theory is that size is accepted to be the only criterion to whether the aggregates become nuclei or not. Since the CNT was developed for condensation of vapor to liquid, it does not distinguish between organized clusters and aggregates where the orientation of the molecules does not correspond to the orientation in the resulting crystal. Consequently, local density is the only order parameter that differs between the two phases.[21] This picture is not complete for crystallization from solutions, where at least two order parameters, for example, density and periodic structure, are necessary to sufficiently distinguish between old and new phases.[18] The classical theory assumes simultaneous fluctuations along both density and structure order parameters, that is, the molecules get together in ordered arrays. In reality, these two parameters for transition need not to go together; one can dominate the critical nucleation and serve as a prerequisite for the other one.[22] CNT is limited in that sense since it cannot identify the different pathways to crystallization when various order parameters do not all change at the same time.[21] In the past decade, a line of simulations, theories, and experimental studies, including those reported by our group, suggested an alternative mechanism of crystal nucleation where the structure fluctuation follows and is superimposed upon density fluctuations, called the two-step mechanism (Figure 2), which will be discussed in detail shortly.

4. Classical Nucleation Theory in Perspective of Polymorphism

The classical theory assumes that the clusters evolve in size by attachment of single molecules and neglects the collision between two clusters. However, based on MD computer experiments, it was suggested that cluster−cluster interactions may

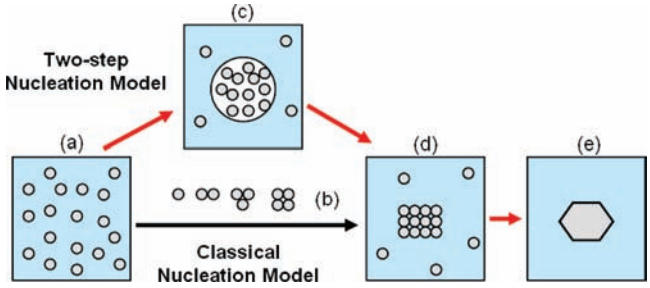

FIGURE 2. Alternative pathways leading from solution to solid crystal: (a) supersaturated solution; (b) ordered subcritical cluster of solute molecules, proposed by classical nucleation theory; (c) liquid-like cluster of solute molecules, dense precursor proposed by two-step nucleation theory; (d) ordered crystalline nuclei; (e) solid crystal.

have a considerable influence on the process of nucleation and large monomer concentrations are not enough to guarantee validity of the monomer addition approximation.[23] In fact, theoretical and experimental studies imply that the nucleation may involve the assembly of preformed clusters. For instance, simulation of crystallization in a solute−solvent system consisting of atoms of two noble gases showed that the solute particles aggregate into small clusters first, then these small clusters come together to form a single large cluster, which eventually nucleates to the final crystalline phase.[24] Many crystals are known to have growth units other than monomers and it is highly possible that the formation of prenucleation clusters may proceed through successive aggregation of these preassembled growth units, rather than the monomer addition model suggested by classical theory.[25] In addition, many studies revealed the fact that clusters of molecules exist even in undersaturated solutions. Since the time evolution of prenucleation clusters in a supersaturated solution is superimposed on the change that occurs in an undersaturated solution, the exact and full understanding of the latter process is required for that of the former. The formation of clusters in the undersaturated state is usually governed by the ability of the solvent to disturb or promote particular hydrogen-bonding networks, which was shown by numerous groups to control the polymorphic outcome of the crystallization. For instance, MD simulations on 5-fluorouracil demonstrated the formation of cyclic dimers in dry nitromethane, which was consistent with the crystallization of the doubly bonded ribbon structure of form 2 from this solvent. On the other hand, strong binding of water to 5-fluorouracil molecules hindered the formation of cyclic dimers, consequently promoted the crystallization of form 1 from aqueous solutions.[26] Similarly, application of FTIR spectroscopy to concentrated solutions of tetrolic acid showed that the chloroform solutions rich in dimers were biased toward the nucleation of the α polymorph, which was based on a classic dimer motif, while the ethanolic solutions in which dimer formation was hindered produced the β-form, which was built of H-bonded chains.[27] Small-angle X-ray scattering (SAXS) studies by our group revealed the existence of dimers in aqueous solutions and monomers in acetic acid−water mixtures.[28] Since the aqueous solution favored the formation of the α-form built of cyclic dimers and acidic solution produced the γ-form built of polar chains, results supported the direct link between the initial association of solute molecules in solution and the crystallized solid form. However, since classical theory is valid only for supersaturated solutions and ignores growth units other than monomers, it cannot provide any information about the nature of clusters formed in undersaturated solutions and accordingly cannot account for the impact of solvent-induced molecular self-association in solution on the polymorphic outcome.

5. Two-Step Nucleation Theory

One of the first computational works supporting the two-step mechanism was reported by ten Wolde and Frenkel,[29] who studied homogeneous nucleation in a Lennard-Jones system of short-range attraction by the Monte Carlo technique and confirmed that away from the fluid−fluid critical point ($T > T_c$ or $T < T_c$), fluctuations along density and structure order parameters occur simultaneously, similarly to the classical viewpoint. On the other hand, large density fluctuations were observed around the critical point, which caused a striking change in the pathway for crystal nucleation: the formation of a highly disordered liquid droplet was followed by the formation of a crystalline nucleus inside the droplet beyond a certain critical size. Furthermore, proximity to the critical point decreased the free energy barrier for crystallization and consequently increased the nucleation rate by many orders of magnitude. The presence of an intermediate phase in the form of a high concentration liquid was shown to be a generic feature for substances that interact through sufficiently short-range of interactions, such as proteins.[30] Following this work, a molecular dynamics simulation on a system consisting of 50 widely separated acetic acid molecules within a box of 1659 CCl_4 molecules showed formation of a liquid-like micelle of acetic acid as the concentration of solute was increased by removing solvent molecules from the system.[31] It was suggested that the formation of a microemulsion of liquid-like particles was the first step of crystal nucleation. Brownian dynamics simulations on the phase separation of colloidal particles revealed a metastable colloid vapor−liquid phase coex-

istence region in which the colloid fluid was metastable with respect to the equilibrium crystal phase.[32] The metastable phase separation resulted in regions of high colloid density, out of which nucleation of crystal phase was observed to proceed rapidly. Additionally, a molecular dynamics study on nucleation of AgBr in water showed that stable prenucleation clusters as large as $Ag_{18}Br_{18}$ were disordered, which provided further support to the idea that the initial step in nucleation from solution involves the formation of disordered clusters.[33] On the other hand, clusters as small as Ag_4Br_4 were found to exist in an ordered configuration in vacuo, which indicated that the interaction with solvent was responsible from the disorder within clusters.

In addition to computational simulations, theoretical studies have also provided evidence for the two-step nucleation mechanism. Density functional theory was applied to the study of crystal nucleation from solution, and the nature of nucleation was found to change qualitatively near the metastable critical point, with nucleation rates increasing by several orders of magnitude.[22] At temperatures higher than the critical temperature, nucleation was found to proceed through the formation of crystal-like clusters, while at lower temperatures, liquid-like clusters with an extended wetting layer were favored. These results suggest that close to the critical point, the first step toward the critical nucleus is the formation of a liquid-like droplet, followed by the nucleation of crystal in this droplet at a certain critical size. This mechanism was particularly proposed for nucleation of colloids and globular proteins from solution. Recent density functional theory calculations demonstrated the validity of two-step nucleation theory for simple atomic fluids modeled with Lennard-Jones interaction, suggesting that crystallization involving passage through a metastable disordered state may be a generic phenomenon.[34] It was stated that the lack of experimental evidence for two-step nucleation in the simple fluids could be explained by the short lifetime of the metastable phase in these systems. By calculating the parameters that characterize the concentration fluctuations in solutions, prenucleation clusters for various salts were shown to have an amorphous structure with diffuse boundaries.[35] Based on this finding, it was concluded that nucleation is, at least, a two barrier process in terms of the thermodynamic potential, in which the first barrier necessary for cluster formation is lower than the main barrier necessary for the transformation of the already formed cluster into a stable crystalline nucleus. In addition, calculations of concentration profile and Gibbs free energy of the interface between protein crystal and aqueous solution confirmed the two-step nucleation mechanism.[36] Such a mechanism with small activation energy for each step was expected to be faster than a one-step mechanism with a larger activation barrier. For lysozyme, the formation of liquid droplets with high protein concentration, that is, the first step, was found to be the rate-determining step of the nucleation process. Recently, a simple phenomenological model of protein crystallization via an intermediate liquid state was developed, which showed that the rate-determining step in the nucleation mechanism is the formation of an ordered cluster within the dense liquid intermediate.[37] This emphasizes the role of viscosity within the dense liquid drop in the kinetics of nucleation of ordered solid phases.

Additional support for the two-step nucleation mechanism has been provided by various experimental studies. Dynamic and static light scattering studies on nucleation of lysozyme crystals showed that the monomers rapidly aggregate in the diffusion-limited aggregation regime to form fractal clusters in the initial stages of crystallization, which progressively restructure into compact structures at the later stages of aggregation.[38] Numerous small-angle scattering studies on nucleation of proteins and colloidal particles also suggested that the first observable nuclei in solution are droplet-like or fractal aggregates that subsequently rearrange to form more compact structures.[39] Differential scanning calorimetry analysis of the supersaturated lysozyme solution revealed that an unstable structure formed just after the preparation of the solution transforms into a more structured, probably ordered aggregate just before the end of the induction period.[40] Perhaps the most important among the studies contributed to the development of the two-step mechanism were those presented by Vekilov and co-workers.[18] It was demonstrated that dense liquid droplets facilitate the nucleation of deoxy-hemoglobin S polymers by serving as centers for nucleation, which was attributed to the higher hemoglobin concentration in the droplets.[41] These results allowed presenting a two-step nucleation mechanism where a structure fluctuation occurs within a region of higher density of molecules existing for a limited time due to a density fluctuation. It was concluded that there exists a density fluctuation with optimal size and density that provides the highest probability of occurrence of structure fluctuation in the droplet. In other words, structure fluctuations do not require large density fluctuations or long-lifetime droplets, such as those existing below the liquid−liquid separation line, in order to become crystalline nuclei. More recently, it has been shown that the structuring of the dense liquid precursor into an ordered cluster, that is, the second step, determines the rate of crystal nucleation because the crystal

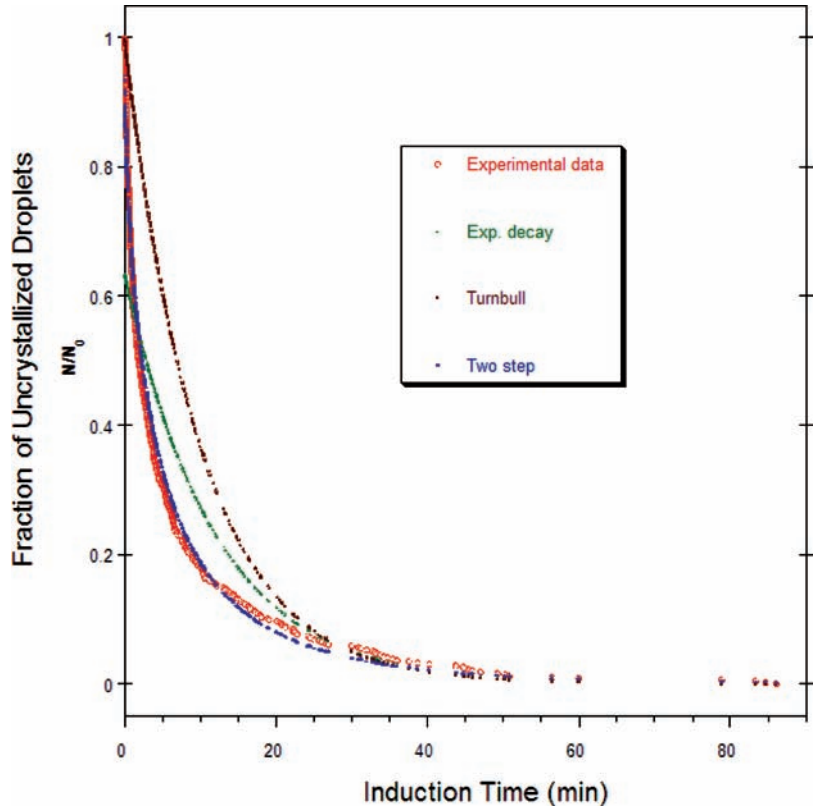

FIGURE 3. Nucleation induction time of lysozyme in levitated aqueous solution droplets. Experimental data revealed significant deviation from the classical Turnbull's model and was best described by the two-step nucleation model. Reproduced with permission from ref 11. Copyright 2004 American Chemical Society.

nucleation was found to be 8−10 orders of magnitude slower than the nucleation of dense liquid droplets.[42]

Experimental studies performed by our group have also provided support for the two-step mechanism. The nucleation induction time measurements on lyzoyme using electrodynamic levitation of single solution droplets showed that the two-step nucleation model described the behavior of the experimental data better than the analysis based on the classical model (Figure 3).[11] It was found that the second step did not happen instantaneously, which implied that the bulky protein molecules required some degree of orientation prior to forming a stable nucleus capable of further growth. During nonphotochemical laser-induced nucleation (NPLIN) studies, supersaturated solutions of small organic molecules that were exposed to the laser light were found to nucleate much faster than control solutions.[43] This was explained by electric-field-induced alignment of the molecules in existing prenucleation clusters in the solution and consequent reduction of the entropic barrier for ordered lattice formation. If the nuclei would form by successive aggregation of molecules in an ordered manner as proposed in CNT, induced alignment of the molecules would not cause a significant change in the structure of already ordered clusters; hence any drastic deviation between the induction times of the lased and control solutions would not be observed. Moreover, freshly prepared supersaturated solutions that were exposed to the laser light did not go through NPLIN, and the solutions had to be aged before the laser could induce nucleation. This implied that the laser-induced organization of molecules within a cluster would lead into nucleation only if that cluster was of sufficient size. This concept supported the two-step nucleation theory, because once the laser light encountered sufficiently large clusters, it would reduce the induction time through the organization step by aligning the molecules within the clusters. It was also demonstrated that two different polymorphs of glycine can be crystallized from aqueous solutions depending on the laser polarization state, circular polarization producing the α-form and linear polarization generating the γ-form.[44] This was explained by different efficiencies of linearly and circularly polarized lights in aligning the distinct building blocks of the two polymorphs, which lent further support to the proposed NPLIN mechanism, consequently providing strong evidence for the two-step nucleation model. More recently, small-angle X-ray scattering was utilized to directly study the nucleation of glycine from aqueous solutions, and results indicated that glycine dimers were engaged in mass fractal aggre-

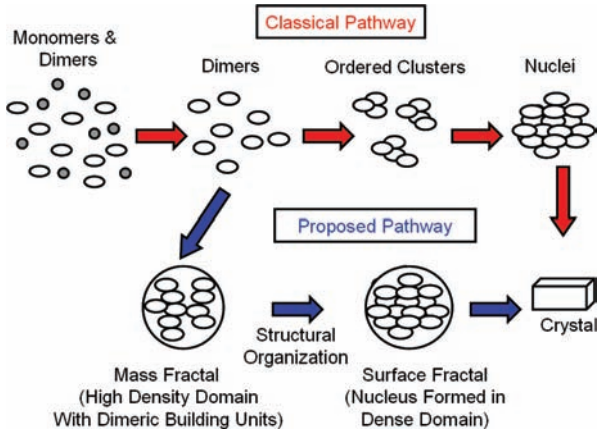

FIGURE 4. Proposed pathway of the nucleation of glycine from aqueous solution via transformation of mass fractal aggregates into surface fractal structures in accordance with the two-step nucleation model, observed by small-angle X-ray scattering.

gates in supersaturated solutions, which transformed into surface fractal structures prior to nucleation.[45] This transformation was attributed to the organization of liquid-like clusters into ordered lattice structures, in accordance with the two-step nucleation model (Figure 4).

6. Summary

The shortcomings of classical theory discussed herein suggest that nucleation of solids from solution does not proceed via the classical pathway but follows much more complex routes. The alternative two-step model, where the first step is the formation of a sufficient size cluster of solute molecules and the second step is the reorganization of such a cluster into an ordered structure, was initially proposed for crystallization of proteins. Recent experimental and theoretical studies, including the ones reported by our group, demonstrated the applicability of the two-step mechanism not only to macromolecules but also to small organic molecules, suggesting that this mechanism may underlie most crystallization processes from solutions. The organization step was proposed as the rate-determining step, which is consistent with the observation that the nucleation from solution takes a longer time as the complexity of molecules increases since it would be more difficult for more complex molecules to arrange themselves in the appropriate lattice structures due to their high degree of conformational flexibility. Further work is needed, and these recent developments are promising and may lay the groundwork for future studies. Developing a clearer picture of nucleation may help determine the optimum conditions necessary for the effective organization within the clusters or lead to the design of auxiliaries that can accelerate this stage (i.e., increase the rate of nucleation) and as a result avoid the formation of oil and amorphous materials or undesired solid forms and obtain the final product in a timely and reproducible manner.

BIOGRAPHICAL INFORMATION

Deniz Erdemir received her B.S. degree in Chemical Engineering in 1999 from Middle East Technical University, Turkey. Her Ph.D. work at the Illinois Institute of Technology under the direction of Allan S. Myerson focused on understanding the mechanism of nucleation from solutions. She is currently employed by Bristol-Myers Squibb as Research Investigator in Process Research and Development.

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Allan S. Myerson is the Philip Danforth Armour Professor of Engineering in the Department of Chemical and Biological Engineering at the Illinois Institute of Technology. His research interests are in the general area of crystallization from solution with an emphasis on nucleation and prenucleation phenomena, polymorphism, impurity crystal interactions, and industrial applications of crystallization. Dr. Myerson serves as Associate Editor of the ACS journal *Crystal Growth and Design*.

FOOTNOTES

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