A Stochastic Model for Nucleation Kinetics Determination in Droplet-Based Microfluidic Systems

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Received July 18, 2009; Revised Manuscript Received April 9, 2010

ABSTRACT: The measured induction times in droplet-based microfluidic systems are stochastic and are not described by the deterministic population balances or moment equations commonly used to model the crystallization of amino acids, proteins, and active pharmaceutical ingredients. A stochastic model in the form of a Master equation is formulated for crystal nucleation in droplet-based microfluidic systems for any form of nucleation rate expression under conditions of time-varying supersaturation. An analytical solution is provided to describe the (1) time evolution of the probability of crystal nucleation, (2) the average number of crystals that will form at time \( t \) for a large number of droplets, (3) the induction time distribution, and (4) the mean, most likely, and median induction times. These expressions are used to develop methods for determining nucleation kinetics. Nucleation kinetics are determined from induction times measured for paracetamol and lysozyme at high supersaturation in an evaporation-based high-throughput crystallization platform, which give low prediction errors when the nucleation kinetics were used to predict induction times for other experimental conditions. The proposed stochastic model is relevant to homogeneous and heterogeneous crystal nucleation in a wide range of droplet-based and microfluidic crystallization platforms.

1. Introduction

Numerous studies have been carried out to obtain a better fundamental understanding of nucleation mechanisms. More recently, high-throughput microfluidic crystallization systems have been used to crystallize a variety of organic compounds including amino acids, proteins, and active pharmaceutical ingredients. The goals of such studies include the identification of conditions to produce protein crystals for X-ray and neutron crystallography, the search for polymorphs for pharmaceutical compounds, and exploration of the behavior of nucleation and crystallization kinetics at high supersaturation. Such applications have the potential to impact structure-function analysis, pharmaceutical design, bioseparations, controlled drug delivery, treatment of protein condensation diseases, and study of human degenerative conditions. Estimates of the nucleation kinetics can be used to revise crystallization conditions to produce higher quality protein crystals, for the design of kinetic processes such as separations, and for investigations into the fundamentals of nucleation. Microfluidic crystallization systems enable the measurement of a large number of induction times for various experimental conditions using only micrograms of starting material, and many researchers have looked into the estimation of nucleation kinetics from such data.

Techniques for the estimation of nucleation kinetics in macroscale crystallizers (e.g., see the reviews) are poorly applicable at the microscale. Because of the small volumes, fluctuations in measured induction times at the microscale can be large. Several methods have been used to estimate nucleation kinetics in droplets. The deterministic population balance models and moment equations used by various researchers to estimate nucleation kinetics do not capture this stochastic behavior, which has been characterized in a series of high-throughput constant-supersaturation experiments.

Nucleation kinetics have been fit to average induction times, to the proportion of drops not containing crystals as a function of time, and to the average number of crystals per drop at long times. The Poisson distribution provided a good fit to distributions in the number of lysozyme and lactose crystals nucleated at constant supersaturation in droplets using temperature jump techniques. Izmailov et al. developed the induction time statistics for the classical nucleation rate expression by assuming that the induction times could be fit by a gamma distribution. Knezic et al. employed electrodynamic levitation and a two-step model to analyze cluster size fluctuations and cluster rearrangements to study nucleation kinetics at high supersaturation.

The aforementioned papers do not fully characterize the distribution of crystal nuclei in droplets in cases where supersaturation varies with time. Time-varying supersaturation occurs, for example, in an evaporation-based microwell crystallization platform (see Figure 1) designed to force a phase change in every experiment. An alternative microfluidic crystallization system utilizes microchannels to form droplets of varying concentrations. In either system, the supersaturation in each droplet can be monotonically increased in a known way as the evaporation proceeds. In Section IIa of this paper, a stochastic model in the form of a Master equation is formulated for any form of nucleation rate expression under conditions of time-varying supersaturation. An analytical solution is given for the Master equation to describe the (1) time evolution of the probability of crystal nucleation, (2) the average number of crystals that will form at time \( t \) for a large number of droplets, (3) the induction time statistics, and (4) the mean, most likely, and median induction times. Section IIb describes how to apply these expressions to estimate the nucleation kinetics from experimental data collected from droplet-based microfluidic systems. Section III estimates kinetic parameters in the classical nucleation expression by fitting mean induction times and induction time distributions for the nucleation...
The time evolution of the probability \( P_n(t) \) of having exactly \( n \) crystals in the system is given by

\[
\frac{dP_n(t)}{dt} = \kappa(t)(P_{n-1}(t) - P_n(t)), \quad P_n(0) = 0, \quad n = 1, 2, \ldots
\]

(1b)

where the differential equations for \( n \geq 1 \) assume that earlier nuclei do not grow fast enough to significantly deplete solute from the solution. This latter assumption was explicitly made by Dombrowski et al.\(^{28}\) and implicitly made by other researchers (e.g., ref 25), and is reasonable provided that the crystals observed in each droplet have approximately the same size. The differential equations (1) describe a nonstationary Poisson process\(^{33}\) and can be solved recursively or by defining a probability-generating function (see Supporting Information) to give

\[
P_0(t) = e^{-\int_0^t \kappa(s) \, ds}
\]

(2a)

\[
P_n(t) = \frac{1}{n!}\left[\int_0^t \kappa(s) \, ds\right]^n e^{-\int_0^t \kappa(s) \, ds}, \quad n = 1, 2, \ldots
\]

(2b)

Figure 2 shows the time evolution of the probabilities for droplets in which the overall nucleation rate \( \kappa \) is constant:

\[
P_n(t) = \frac{1}{n!}(Kt)^n e^{-Kt}, \quad n = 0, 1, 2, \ldots
\]

(3)

which has been used to fit experimental data for nucleation in droplets of constant supersaturation.\(^{25,35,36}\) The shapes of the curves in Figure 2b are the same as those observed experimentally for nucleation of lysozyme crystals in droplets under constant supersaturation (see Figure 4 of ref 25).

Consider a large number of droplets in a high-throughput microfluidic device in which each droplet moves from undersaturated to saturated to supersaturated conditions. The mean number of crystals at time \( t \) (averaged over a sufficiently large number of droplets) is\(^{37}\)

\[
E\{N(t)\} = \bar{N}(t) = \sum_{n=0}^{\infty} nP_n(t)
\]

\[
= \sum_{n=0}^{\infty} \frac{t^n}{n!} \left[\int_{t_{\text{sat}}}^{t} \kappa(s) \, ds\right]^n e^{-\int_{t_{\text{sat}}}^{t} \kappa(s) \, ds}
\]

\[
= \int_{t_{\text{sat}}}^{t} \kappa(s) \, ds
\]

so that the time when the mean number of crystals is equal to 1 is

\[
\int_{t_{\text{sat}}}^{t_{\text{mean}}} \kappa(s) \, ds = 1
\]

(5)

where the subscript \( n \) indicates that this induction time is an average over the number of crystals.

Hence, the most likely time \( t_{n,\text{ml}} \) for there to be exactly one crystal in the system is equal to the time for the mean number of crystals to be equal to 1. At this time, the probability distributions are \( P_n = 1/(n!e) \) and the variance in the number of crystals \( n \) is

\[
\text{Var}[n] = \sum_{n=0}^{\infty} (n - \bar{N})^2 P_n = \frac{1}{e} \sum_{n=0}^{\infty} \frac{(n - 1)^2}{n!} = 1
\]

(7)

The large value for the variance indicates a high probability in a particular experiment that there is either no nucleus or multiple nuclei at \( t = t_{\text{mean}} = t_{n,\text{ml}} \). This is also seen by the low probability for having exactly 1 crystal in the system at the mean induction time, \( P_1 = 1/e \). In general, it can be shown that the variance in the number of crystals \( n \) is

\[
\text{Var}[n(t)] = \int_{t_{\text{sat}}}^{t} \kappa(s) \, ds
\]

(8)

which is monotonically increasing for experiments with positive supersaturation (i.e., \( \kappa(t) \geq 0 \)). These results hold for any time evolution of the overall nucleation rate \( \kappa(t) \) and hence any time evolution of the supersaturation.

The cumulative distribution function (CDF) for the time \( T_n \) when at least \( n \) crystals have nucleated is

\[
P(T_n \leq t) = F(t) = 1 - \frac{1}{t_{\text{sat}}} \sum_{m=0}^{n-1} \left[\int_{t_{\text{sat}}}^{t} \kappa(s) \, ds\right]^m e^{-\int_{t_{\text{sat}}}^{t} \kappa(s) \, ds}
\]

(9)

The corresponding probability distribution function (PDF) is\(^{38,39}\)

\[
f(t) = \frac{dF(t)}{dt} = \frac{\kappa(t) \left[\int_{t_{\text{sat}}}^{t} \kappa(s) \, ds\right]^{n-1}}{(n-1)!} e^{-\int_{t_{\text{sat}}}^{t} \kappa(s) \, ds}
\]

(10)

whose integral gives the probability of \( T_n \) lying within a particular interval, \( \int_{t_1}^{t_2} f(t) \, dt = P(t_1 \leq T_n \leq t_2) \). These induction time
distributions can be used to compute a variety of induction times that can be compared to experiments. For the induction time defined by the appearance of at least 1 nucleus (\( n = 1 \)):

\[
P(T_i \leq t) = F(t) = 1 - e^{-\int_{0}^{t} \kappa(s) \, ds}
\]

\[
f(t) = \frac{dF(t)}{dt} = -\frac{d}{dt} e^{-\int_{0}^{t} \kappa(s) \, ds} = \kappa(t)e^{-\int_{0}^{t} \kappa(s) \, ds}
\]

(11)

For time-varying nucleation rate \( \kappa(t) \), the mean time for the appearance of at least \( n \) crystals is

\[
t_{\text{mean}} = \int_{0}^{\infty} t \kappa(t) \left( \int_{0}^{t} \kappa(s) \, ds \right)^{n-1} \frac{1}{(n-1)!} e^{-\int_{0}^{t} \kappa(s) \, ds} \, dt
\]

(12)

with the mean induction time for the nucleation of at least 1 crystal being

\[
t_{\text{mean}} = \int_{0}^{\infty} t \kappa(t) e^{-\int_{0}^{t} \kappa(s) \, ds} \, dt
\]

(13)

This analytical expression corresponds to the induction time that is normally experimentally reported in the literature.\(^{40}\) The variance of the distribution of times about the mean time for the appearance of at least \( n \) crystals is

\[
\text{Var}(t_{\text{ind}}) = \int_{0}^{\infty} t^2 \kappa(t) \left( \int_{0}^{t} \kappa(s) \, ds \right)^{n-1} \frac{1}{(n-1)!} e^{-\int_{0}^{t} \kappa(s) \, ds} \, dt - t_{\text{mean}}^2
\]

(14)

which for \( n = 1 \) is

\[
\text{Var}(t_{\text{ind}}) = \int_{0}^{\infty} t^2 \kappa(t) e^{-\int_{0}^{t} \kappa(s) \, ds} \, dt - t_{\text{mean}}^2
\]

(15)

The maximum likelihood (most likely) induction time \( t_{\text{ml}} \) must occur at a time \( t \) that satisfies \( df/dt = 0 \) for \( f \) in eq 11, which occurs if and only if

\[
\frac{dk}{dt}(t_{\text{ml}}) - k^2(t_{\text{ml}}) = 0
\]

(16)

That is, \( t_{\text{ml}} \) can be computed from all (typically 1 or 2) of the finite positive roots of the above expression:

\[
t_{\text{ml}} = \arg \max_{t_k} \left\{ f(t_k) \left( \frac{dk}{dt}(t_k) - k^2(t_k) \right) = 0 \right\}
\]

(17)

The median induction time \( t_{\text{median}} \) is given for the nucleation of at least 1 crystal as

\[
P(T_i \leq t_{\text{median}}) = 0.5 \iff -\int_{0}^{t_{\text{median}}} \kappa(s) \, ds = \ln 2
\]

(18)

which is smaller than the induction times (5)–(6) but has a similar value when the overall nucleation rate \( \kappa(t) \) increases rapidly right before a crystal nucleates, which typically occurs in an evaporation-based crystallization platform with constant or increasing evaporation rate.\(^{29}\)

**B. Determination of Nucleation Kinetics.** The overall nucleation rate \( \kappa(t) \) could be experimentally estimated from the ratio of the proportion of droplets not containing nuclei to its derivative as a function of time in eq 1A, but taking derivatives of data is inaccurate. Least-squares estimation is an alternative approach that first parametrizes \( \kappa(t) \) in terms of nucleation parameters and then fits these parameters to experimental data.\(^{41}\) For example, the overall nucleation rate is given by \( \kappa(t) = J(S(t))V(t) \) for homogeneous nucleation in droplets, where \( S(t) = (C(t) - C_{\text{sat}})/C_{\text{sat}} \) is the relative supersaturation determined from a material balance on the solute,\(^{42}\) \( C(t) \) is the concentration (g/L) of solute to be crystallized at time \( t \), \( C_{\text{sat}} \) (g/L) is the solubility, \( J(S) \) is the number of crystals formed per unit area per unit unit \((\#/s-L)\), and \( V(t) \) is the volume (L) of the droplet determined from the evaporation rate and a material balance on the solvent.\(^{13}\) The nucleation parameters \( A \) and \( B \) in the classical homogeneous nucleation expression\(^{43-46}\)

\[
J(S) = AC \exp(-B/(\ln(S+1))^{2})
\]

(19)

can be determined by numerical solution of

\[
\min_{A,B} \sum_{i=1}^{n_{\text{exp}}} \sum_{k} \left( \ln P_{0i,k} + \int_{0}^{t_{\text{measured}}} J(S_{i}(s))V_{i}(s) \, ds \right) ^{2}
\]

(20)

which minimizes deviations from eq 2A, summed over all experimental conditions \( i \) and times \( t_{k} \) in which the proportion of droplets without crystals, \( P_{0i,k} \), are measured. As eq 20 was derived based only on eq 1A, its application does not require the assumption that the growth of any crystals does not significantly deplete solute from the solution.

An alternative formulation fits a mean or most likely induction time \( t_{\text{ind}} \in \{ t_{\text{ml}} \, t_{\text{mean}} \, t_{\text{median}} \, t_{\text{measured}} \} \) to

\[
\psi = \min_{A,B} \sum_{i=1}^{n_{\text{exp}}} (t_{\text{ind}}, i - t_{\text{measured}}, i)^{2}
\]

(21)
A number of experiments are used to assess accuracy of the model predictions. 

Supporting Information.

κ described by inserting modifications to the overall nucleation rate κ.

F_t induction time

The above approaches for the determination of nucleation kinetics also apply to heterogeneous nucleation, with suitable modifications to the overall nucleation rate k(t). For example, consider droplets attached to a solid surface but otherwise surrounded by humid air. If the crystals nucleate at the solid—liquid interface, then this heterogeneous surface nucleation is described by inserting k(t) = J_{net}S(t)κ into the above expressions (1)–(18), where A(t) is the contact area between the droplet and the solid surface. If the nucleation occurs at the liquid—air interface, then A(t) would be defined as the contact area between the liquid and humid air. If nucleation occurs at the interface between aqueous droplets surrounded by an oil, then A(t) would be the contact area between the two liquids.

The next section applies two of the above approaches to estimate nucleation kinetics from several sets of measured induction times, both to validate the statistical model for nucleation in droplets and for illustration purposes.

### III. Results and Discussion

This section applies the above analyses to experimental data for the nucleation of organic crystals in aqueous droplets in an evaporation-based microfluidic platform (for details on the experimental system and procedures, see ref 13). Computational fluid dynamics indicated that natural convection was sufficient to ensure that the solutions were spatially uniform. It was observed during the experiments that the crystals did not stick to the glass slips, so the applications assume that the nucleation was homogeneous.

#### A. Prediction of Mean Induction Time for Different Experimental Conditions

The mean time in which paracetamol crystals were first observed in an aqueous droplet was recorded for each of 11 experimental conditions with varying evaporation rates and initial solution concentrations (see Table 1). The nucleation parameters A and B for the classical nucleation expression 19 were obtained by numerical solution of eq 21 for the odd-numbered experimental conditions based on the mean induction time t_{mean}. Even-numbered experiments are used to assess accuracy of the model predictions. An example calculation of the maximum likelihood induction time t_{ind} in Supporting Information.

\[ t_{ind} = \text{minimum} \left( t_{ind} \in \{ t_{n,ml}, t_{n,mean} \} \right) \]

with F(t) given in eq 11.

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### Table 1. Induction Times \{ t_{ind}, t_{n,mean}, t_{n,dn}, t_{n,mean} \} from eqs 5, 6, 13, and 18 for Paracetamol in Water for 11 Experimental Conditions with Different Evaporation Rates and Initial Solute Concentrations

<table>
<thead>
<tr>
<th>experimental condition #</th>
<th>evaporation rate (µg/h)</th>
<th>C_0 (g/kg water)</th>
<th>t_{mean} (h)</th>
<th>t_{n,mean},t_{n,ml} (h)</th>
<th>t_{median} (h)</th>
<th>t_{ml} (h)</th>
<th>t_{ind,measured} (h)</th>
<th>t_{ml,lower} (h)</th>
<th>t_{upper} (h)</th>
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<td>13.2</td>
<td>13.5</td>
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</tr>
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<td>17.4</td>
<td>17.8</td>
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<td>5</td>
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<td>17.9</td>
<td>18.2</td>
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<td>25.7</td>
<td>24.2</td>
<td>26.6</td>
</tr>
</tbody>
</table>

* Each measured induction time is the time in which one or more crystals was observed averaged over multiple droplets and t_{ml,lower}, t_{upper} are 90% confidence limits. The nucleation parameters A and B were fit in eq 21 for the odd-numbered experimental conditions based on the mean induction time t_{mean}. Even-numbered experiments are used to assess accuracy of the model predictions. An example calculation of the maximum likelihood induction time t_{ind} in Supporting Information. t_{mean} is determined from \( \int_0^{t_{mean}} f(t) dt = 0.05 \) and t_{upper} from \( \int_0^{t_{upper}} f(t) dt = 0.95 \) where f(t) is the induction time distribution (11).
The solubility of \(C_{\text{solv}}(t) = 1.5694 \times 10^{-7}\) g/L was incorporated into the time-varying supersaturation to correctly capture the change in solubility. The nucleation parameters \(A\) and \(B\) were fit in eq 22 for Experimental Condition \#1. Induction times for Experimental Condition \#2 were used to assess accuracy of model predictions.

The goodness of fit of the cumulative distribution function \(F(t)\) in eq 11 to the experimentally measured induction times was also evaluated using the Kolmogorov–Smirnov statistic
\[
D_n = \sup_{t} |F_N(t) - F(t)|
\] (23)
where the null hypothesis is that \(F(t)\) in eq 23 is the distribution 11. The statistical analysis, with details in Supporting Information, accepted the distribution function 11 as appropriate for describing the empirical distribution function at an alpha level of 0.1.

### IV. Conclusion

In this study, a stochastic model was presented that characterizes the statistics of crystal nucleation in microfluidic systems with time-varying supersaturation and for any form of nucleation rate expression. The analytical solution for this model is applicable to microfluidic crystallization platforms in which supersaturation changes with time due to changes in solute/precipitant concentration, temperature, pH, etc., and can be used to identify nucleation kinetics by fitting the measured average crystal number vs time (eq 4), the proportion of droplets that do not contain crystals vs time (eq 20), induction times (eq 21), or induction time distributions (eq 22). The kinetic parameters for the nucleation of paracetamol in aqueous droplets obtained by fitting the mean induction times (13) to six experimental conditions gave predicted induction times with an average deviation <1/2 h for six other experimental conditions. The nucleation kinetic parameters in lysozyme–NaCl–water droplets determined by fitting the cumulative induction time distribution (11) at one experimental
condition accurately predicted the cumulative induction time distribution at another experimental condition. The Komogorov–Smirnov goodness-of-fit test indicated that the measured induction time distribution at each experimental condition is consistent with the theoretical distribution (11). The stochastic model is widely applicable to crystal nucleation in a wide range of droplet and microfluidics-based devices including levitated droplet systems, continuous-flow plug-based crystallization, and patterned substrate-based systems.

As discussed in Section II, the model should also be applicable to heterogeneous nucleation at solid—liquid, liquid—vapor, and liquid—liquid interfaces.

Acknowledgment. Financial support is acknowledged from the National Science Foundation (Grant No. 0426328), the National Institutes of Health (R21 EB004513), 3M, and the Singapore Agency for Science, Technology and Research.

Supporting Information Available: (a) The derivation of the analytical solution of the Master equation, (b) an example calculation of the maximum-likelihood induction time, (c) a description of the quantification of uncertainty in kinetic parameters, and (d) a description of Kolmogorov–Smirnov statistics. This information is available free of charge via the Internet at http://pubs.acs.org/.

References

(2) ten Wolde, P. R.; Frenkel, D. Science 1997, 277, 1975.
function of time for each drop as computed from mass and/or energy conservation equations.
(49) Because of the low number of parameters, Eq 21 was solved by gridding over the model parameters $A$ and $B$.
(50) The mean induction time (13) was numerically computed using the Matlab *quad* problem to find the value of inner integral and *trapz* to find the value of the outer integral, as well as by solving an equivalent system of two ordinary diffusion equations (ODEs) using *ode45*; one ODE for the inner integral and the second ODE for the outer integral. The same numerical method used for finding the mean can be used to determine the variance (15).
(51) For experiments at constant temperature such as for the specific experiments described in this paper, the value of $A = 2\pi(kT)^{1/2}/h$ has only a weak dependence on the solute properties and assuming a constant value for $A$ is a very accurate approximation. The value of $B = -16\pi\sigma^2v^2\Delta G_a/k^4T^4$ depends more strongly on solute properties, but was kept constant due to a lack of data available on the values of the surface energy $\sigma$ and the diffusion activation energy $\Delta G_a$ as a function of the solute concentration.