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Sigmoid kinetics of protein crystal nucleation

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ABSTRACT

A non-linear differential equation expressing the new phase nucleation rate in the different steps of the process (non-stationary and stationary nucleation and in the plateau region) is derived from basic principles of the nucleation theory. It is shown that one and the same sigmoid (logistic) function describes both nucleation scenarios: the one according to the classical theory, and the other according to the modern two-stage mechanism of protein crystal formation. Comparison to experimental data on both insulin crystal nucleation kinetics and on bovine β-lactoglobulin crystallization indicates a good agreement with the sigmoidal prediction. Experimental data for electrochemical nucleation and glass crystallization obey the same sigmoid time dependence, and suggest universality of this nucleation kinetics law.

1. Introduction

One of nucleation theory's main objectives is to provide expressions for the nucleation rate, dn/dt, which is the number of the new phase nuclei, n, appearing in unit volume (1 cm^3) per unit time, t=1 s. Since nucleation rate cannot be measured directly, plots of experimentally determined number densities of nuclei formed per unit time are used. Such determinations are done by separating nucleation and growth stages (see Section 3.1).

Experimental studies under constant supersaturation, e.g. [1–2] have indicated that after some initial time-lag, the early crystal nucleation stage is slow, then speeds up gradually (nearly exponential upside), and slows down by approaching the final stage where saturation is established, i.e. a nucleation rate of zero. Many processes in nature (e.g. plant growth), human society and diverse inorganic and bio-systems, including amyloid fibril formation [3] run in a similar way. Such S-shaped progressions that have slow beginnings, then accelerate and over time reach a climax are described quantitatively by sigmoid (logistic) functions [4–6].

It is the aim of this paper to elucidate theoretically the increase in number densities of new phase nuclei, n, formed under constant supersaturation during nucleation time, t. It is worth noting that when nucleation is effectively arrested, e.g. using the classical nucleation-growth-separation principle, NGSP (in which the nucleation time is set short, and the growth of the nuclei during this stage is negligible, Section 3.1), the consideration is much simpler than in Kolmogorov–Johnson–Mehl–Avrami theory [7–11].

2. Theoretical

According to the nucleation theory, when supersaturation is established in a system, cluster size distribution changes first from the previous equilibrium distribution to a new distribution corresponding to the metastable state; this is the physical reason for the so-called *induction time* or *non-stationary time lag* in nucleation. Due to the random density fluctuations in the mother phase, the larger the cluster, the longer it takes for it to emerge, and hence, the new distribution of clusters, smaller than the critical size has to be formed before the first critical nucleus appears; it is the latest to appear. However, the gradual accommodation to the cluster size distribution corresponding to the metastable state condition continues even after the first nucleus appears, i.e. throughout the entire initial non-steady-state nucleation period. Thus, the number of nuclei precursors' increases with time, and augments the basis for (accelerated) nucleation. It is worth noting that according to the classical nucleation theory (and to Szilard chain) critically sized clusters form through attachment of one molecule to subcritical clusters of size (i^*-1), i^* being the number of molecules constituting the critical cluster. However, according to Frenkel's "chemical" approach (and some more recent ideas),

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monomer attachment may not be the sole nucleation scenario; critical and/or supercritical nuclei can form by cluster coalescence events as well. Somehow or other, the nucleation rate dn/dt initiates proportionally to the critical nuclei number density, n, multiplied by the "birth" frequency, k: $dn/dt=kn$. Thus, starting with a single nucleus, the nucleation process advances with time in an exponential manner: $n=\exp(kt)$.

The initial non-steady-state period is followed by a stationary nucleation, lasting for a limited time only; afterwards, dn/dt decreases continuously, until becoming zero in the plateau region of the n vs. t dependences. This is a typical example of a self-limiting increase where deceleration can be attributed to exhaustion of particles (and/or centers), which are active for nucleation; known generally as nucleants, such particles are always present in protein solutions. The nucleants are gradually occupied by nuclei (generated by rate kn) and ingested by local *nucleation exclusion zones*, which form around growing nuclei by rate ωn. Because the two decelerating factors act in parallel, the probability for their simultaneous action is ωkn^2, ω being a constant: $dn/dt=kn-\omega kn^2$. The condition to have a plateau in the n vs. t dependence is $dn/dt=0$. Hence, $\omega=1/n_s$, where n_s is the saturated nuclei number density, and

$$\frac{dn}{dt}=kn\left(1-\frac{n}{n_s}\right) \tag{1}$$

This is a first-order non-linear differential equation that can be solved exactly. The integral of this equation shows a sigmoidal dependence of n on t (Fig. 1). Note however that the overall supersaturation does not change with time for short nucleation times only (e.g. as by using NGSP); the reason is the negligible amount of solute, which is included in the extremely small nuclei.

Nucleation acceleration and deceleration tendencies have to equilibrate. This occurs at the point when the maximum nucleation rate is reached. Hence, at this point the second derivative is $d^2n/dt^2=0$, and $n/n_s=0.5$. Putting this value in Eq. (1), we obtain the maximum nucleation rate

$$(dn/dt)_{\max}=kn_s/4 \tag{2}$$

It is seen that k models the maximum nucleation rate.

Eq. (2) is used to calculate the time t_c, when the maximum nucleation rate is reached, and $n=n_s/2$. With this end in view, we draw a straight line, which is tangential to the sigmoid point $n=n_s/2$ (the dashed line in Fig. 1); its linear equation is

$$n=a+kn_st/4 \tag{3}$$

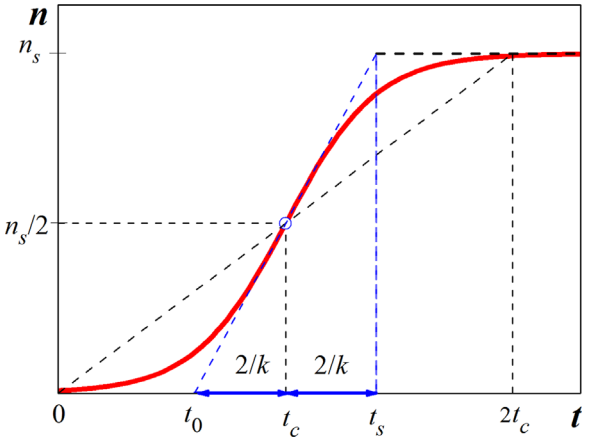

Fig. 1. Sigmoid curve with characteristic points (see text); the nucleation induction time lies in the negative part of abscissa.

For $t=t_c$ at $n=n_s/2$, it results in

$$a=n_s(1-kt_c/2)/2 \tag{4}$$

And with this $a-$value the tangential line equation transforms in

$$n=\frac{n_s}{2}\left(1-\frac{kt_c}{2}\right)+\frac{kn_s}{4}t \tag{3.1}$$

Now, denoting the intersection point of the tangential straight line with the abscissa by t_o (Fig. 1), we obtain

$$t_0=t_c-\frac{2}{k},\ \text{and}\ t_c=t_o+2/k \tag{5}$$

It should be noted that t_o is related to the induction time τ (see Section 3.3).

The symmetry of the sigmoid curve shows that n_s is reached at time $t_p=2t_c$ (Fig. 1). With the above parameters, and after some mathematical transformations, the integral of Eq. (1) results in the following logistic (sigmoid) function

$$n=\frac{n_s}{1+\exp\left[-k(t-t_c)\right]} \tag{6}$$

Sigmoid's midpoint is reached at $t=t_c$, because $\exp[-k(t-t_c)]=1$; and when $\exp[k(t-t_c)]>>1$, $n\to n_s$, which is the final nucleation stage.

The coefficient k is the rate determining constant (it has dimension of reciprocal time), and is of special interest in the physics of new phase nucleation [12]. Eq. (1) shows that at the beginning of the nucleation process, when $n=1$, $dn/dt=k(n_s-1)/n_s$; and with $n_s>>1$, $dn/dt\approx k$. However, there are more practical ways to determine k. Firstly, by fitting experimental data in Eq. (6) the independent parameters k and t_c are obtained, e.g. Table 1 (and the comments in Section 3.2). Still another possibility of evaluating k is by using the time t_s, when the saturated nuclei number density n_s is reached (see Fig. 1), according to the tangential line equation (3.1)

$$t_s=t_c+2/k \tag{7}$$

which combined with Eq. (5) renders (also see Fig. 1)

$$k=4/(t_s-t_o) \tag{8}$$

Besides the simultaneous action of the two decelerating factors, there are two more possibilities to consider. The first one assumes an exceptionally pure system (e.g. vapor phase) where no nucleants are present and the homogeneous nucleation is decelerated solely because of the nucleation excluded zones arising around the nuclei; increasing in number, such zones may decrease noticeably the volume where nucleation can still proceed; an exponential increase in nuclei number density is expected to occur at constant supersaturation. But system's overall supersaturation is constant for sufficiently short nucleation time only; soon or latter, it drops

Table 1
Data calculated using Eq. (6) for insulin crystal nucleation in bulk solution, on glass surface, at the air/solution interface and in the three-phase angle air/solution/glass.

$\ln(c/c_e)$	Bulk insulin crystal nucleation				On-glass	Air/solution	3-phase angle
	n_s	t_c [s]	$k\times10^4$ [s^{-1}]	R^2	$k\times10^4$ [s^{-1}]	$k\times10^4$ [s^{-1}]	$k\times10^4$ [s^{-1}]
2.99	4921	6262	6.5	0.971	–	–	–
3.22	39536	4416	7.74	0.990	5.23	–	–
3.31	52768	3305	12.42	0.986	–	–	–
3.40	74609	2700	19.94	0.988	–	–	–
3.48	104624	1380	20.5	0.951	–	9.05	–
3.55	110987	1403	23.4	0.984	35.9	12.77	–
3.69	149132	1462	20.79	0.986	31.87	14.38	15.5