Journal of Crystal Growth 470 (2017) 8–14

Contents lists available at ScienceDirect

Journal of Crystal Growth

journal homepage: www.elsevier.com/locate/crys

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

D.A. Barlow

Dept. of Natural Sciences, Santa Fe College, Gainesville, FL 32606, USA

ARTICLE INFO

Article history:
Received 9 March 2017
Received in revised form 29 March 2017
Accepted 31 March 2017
Available online 3 April 2017
Communicated by T.F. Kuech

Keywords:
A1. Growth models
A1. Nucleation
A2. Growth from solutions
B1. Proteins

ABSTRACT

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

1. Introduction

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

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

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

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

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

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

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

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

E-mail address: d.alderman.barlow@gmail.com

http://dx.doi.org/10.1016/j.jcrysgro.2017.03.053
0022-0248/

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

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

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

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

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

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

2. Solution of the governing equations

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

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

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

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

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

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

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

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