Crystallization is the process of producing crystals from a homogeneous phase. For biochemcials, the homogeneous phase from which crystals are obtained is always a solution. Crystallization is similar to precipitation in that solid particles are obtained from a solution. However, precipitates have poorly defined morphology, while in crystals the constituent molecules are arranged in three-dimensional arrays called space lattices. In comparison to crystallization, precipitation occurs at much higher levels of supersaturation and rates of nucleation but lower solubilities. These and other differences between crystallization and precipitation are highlighted in Table 9.1. Because of these differences and because the theory of crystallization that has been developed is different from that for precipitation, crystallization is considered separately from precipitation.

Crystallization is capable of producing bioproducts at very high purity (say, 99.9%) and is considered to be both a polishing step and a purification step. Polishing refers to a process needed to put the bioproduct in its final form for use. For some bioproducts, such as antibiotics, this final form must be crystalline, and sometimes it is even necessary that a specific crystal form be obtained. In some instances, the purification that can be achieved by crystallization is so significant that other more expensive purification steps such as chromatography can be avoided.

There are actually two very different applications of crystallization in biotechnology and bioproduct engineering: crystallization for polishing and purification, and crystallization for crystallography. In the latter case, the goal is a small number of crystals with good size (0.2–0.9 mm) and internal quality. Although it has become common to crystallize proteins for characterization of their three-dimensional structure by x-ray diffraction, this is performed only at small scale in the laboratory, and the knowledge about how to crystallize proteins at large scale in a production process is less developed. However, many antibiotics and other small biomolecules are routinely crystallized in production scale processes.

This chapter is oriented toward the use of crystallization in processes that can be scaled up. The principles of crystallization are discussed first, including information about crystals, nucleation, and crystal growth. This is followed by discussions of batch crystallizers, which are widely used in the pharmaceutical industry. Strategies
for the crystallization of proteins from solution are outlined. The chapter concludes with a discussion of crystallizer scale-up and design.

### 9.1 INSTRUCTIONAL OBJECTIVES

After completing this chapter, the reader should be able to do the following:

- Explain the differences between crystallization and precipitation.
- Utilize power law kinetics in the primary and secondary nucleation of crystals.
- Calculate crystal nucleation and growth rates from crystal size distribution data taken from batch experiments.
- Perform engineering analysis of a batch crystallizer.
- Outline strategies for crystallizing proteins.
- Scale up a crystallization process.

### 9.2 CRYSTALLIZATION PRINCIPLES

To understand crystallization, it is important to know what crystals are and how they form and grow. This understanding can then be used as a basis for the appropriate design and operation of crystallizers.

#### 9.2.1 Crystals

When crystals are allowed to form freely, they appear as polyhedrons, or solids formed by plane faces. Although the relative sizes of the faces of the same material may differ considerably, the *angles* made by the corresponding faces of the same material do not vary—they are characteristic of that substance. These angles are the basis of the classification of seven crystal systems.
The relative sizes of the faces of a crystal in a particular system can vary considerably, resulting in a variety of crystal shapes. This variation is called a modification of *habit*. For example, if the crystals grow much more rapidly in one direction, needle-shaped crystals can result. This variation is illustrated in Figure 9.1 for hexagonal crystals. Crystal habit is influenced by the conditions of crystallization, particularly by the impurities present and by the particular solvent or solvents used. Impurities have been found to stunt the growth of a crystal in certain directions.

### 9.2.2 Nucleation

The generation of submicron particles in the process of nucleation is the sum of contributions by *primary nucleation* and *secondary nucleation*. Primary nucleation occurs in the absence of crystals, while secondary nucleation is attributed to the influence of existing crystals.

Primary nucleation can be either homogeneous or heterogeneous. For homogeneous nucleation, no foreign particles are present, while there are foreign particles present during heterogeneous nucleation. Primary nucleation is usually heterogeneous in actual practice. The rate of primary nucleation, either homogeneous or heterogeneous, has been modeled by the following power law expression [1]:

\[
B = \frac{dN}{dt} = k_n(c - c^*)^n
\]

where \(B\) is the number of nuclei formed per unit volume per unit time, \(N\) is the number of nuclei per unit volume, \(k_n\) is a rate constant, \(c\) is the instantaneous solute concentration, and \(c^*\) is the solute concentration at saturation. The \((c - c^*)\) term is called the *supersaturation*. The exponent \(n\) can range up to 10 but typically is in the range of 3 to 4.

Experimental data from industrial crystallizers have indicated that secondary nucleation usually predominates. Two types of secondary nucleation are shear
nucleation and contact nucleation [1]. Shear nucleation occurs as a result of fluid shear on growing crystal faces, and contact nucleation happens because of crystals colliding with each other and with the impeller and other vessel internal surfaces. The most widely used relation for the rate of secondary nucleation in crystallization is the following [1, 2]:

\[
B = \frac{dN}{dt} = k_1 M_T^j (c^* - c)^b
\]

where \( k_1 \) is a rate constant, and \( M_T \) is the suspension density. The exponent \( b \) can range up to 5 but has a most probable value of 2. The exponent \( j \) ranges up to 1.5, with 1 being the most probable value.

As in precipitation, the solution must be supersaturated in order for particles to form in crystallization, which is reflected in Equations (9.2.1) and (9.2.2). However, as indicated in the phase diagram in Figure 9.2, the supersaturation must be above a certain value before nucleation will begin. In a part of the phase diagram called the metastable region, the supersaturation is so low that nucleation will not start. Once the supersaturation has been raised enough to be in the labile region of the phase diagram, nucleation can begin. At this point, crystals begin to grow, and the supersaturation decreases. A possible way of carrying out a crystallization is indicated by the lines with arrows in Figure 9.2. If the supersaturation becomes too high, the nucleation rate will be too great, and an amorphous precipitate will result.

FIGURE 9.2 Typical phase diagram. The components in solution consist of the product (ordinate) and the precipitating reagent (abscissa). The lines with arrows outline one possible way of performing the crystallization.
9.2.3 Crystal Growth

Crystal growth is the postnucleation process in which molecules in solution are added to the surface of existing crystals. For engineering purposes in crystallizer design, the most useful relationship describing the rate of mass deposition $R$ during crystal growth is [1, 2]

$$R = \frac{1}{A} \frac{dW}{dt} = k_G (c - c^*)^g$$

where $W$ is the mass of crystals per volume of solvent, $A$ is the surface area of crystals per volume of solvent, and $k_G$ is an overall mass transfer coefficient. The order, $g$, of the overall growth process is usually between 0 and 2.5, with an order near unity being the most common. The overall mass transfer coefficient $k_G$ in general depends on temperature, crystal size, hydrodynamic conditions, and the presence of impurities.

It is also sometimes convenient to express the overall linear growth rate as

$$G = \frac{dL}{dt} = k_g (c - c^*)^g$$

where $L$ is a characteristic single dimension of the crystal, such as length. It has been shown that geometrically similar crystals of the same material grow at the rate described by Equation (9.2.4), independent of crystal size [3]. This is known as the delta L law. Although a few exceptions to this law have been found (e.g., when the crystals are very large), it is reasonably accurate in numerous crystallizations of industrial importance [4].

Crystal growth is actually a process that consists of two steps in series. Solute molecules must reach the crystal surface by means of diffusion through a boundary layer. At the surface, the solute must be integrated into the crystal lattice. These two processes may be modeled as [1]

$$\begin{align*}
\text{(9.2.5) Diffusion:} & \quad R = k_d (c - c_i)^d \\
\text{(9.2.6) Surface integration:} & \quad R = k_r (c_i - c^*)^r 
\end{align*}$$

where $c_i$ is the concentration at the interface between the liquid and solid phase, and $k_d$ and $k_r$ are mass transfer coefficients. When the exponents are unity, combining Equations (9.2.3), (9.2.5), and (9.2.6) gives

$$\frac{1}{k_G} = \frac{1}{k_d} + \frac{1}{k_r}$$

Thus, if surface integration is very fast compared with bulk diffusion, then $k_r >> k_d$, and $k_G \equiv k_d$. 
9.2.4 Crystallization Kinetics from Batch Experiments

Estimates of nucleation and growth rates during crystallization can be obtained from batch experiments by a method known as moments analysis [1]. By making a mass balance on the number of crystalline particles within a given size range between \( L \) and \( L + \Delta L \), as was done for precipitate particles (Chapter 8), the population balance equation for size-independent growth in a perfectly mixed, constant-volume batch crystallizer with negligible attrition and agglomeration becomes

\[
\frac{\partial n(L)}{\partial t} + G \frac{\partial n(L)}{\partial L} = 0
\]  

(9.2.8)

The \( k \)th moment of the population density distribution in \( L \) about the origin, obtained by moment transformation of Equation (9.2.8) with respect to size, is defined as

\[
\mu_k \equiv \int_0^\infty n(L)L^k dL
\]  

(9.2.9)

If the moments of the experimental population density function can be determined at two times that differ by a small time interval \( \Delta t \) over which the linearity of the model can be assumed, then the average values of the nucleation and growth rates can be expressed as

\[
\bar{B} = \frac{\Delta \mu_0}{\Delta t}
\]  

(9.2.10)

and

\[
\bar{G} = \frac{\Delta \mu_1}{\mu_0 \Delta t}
\]  

(9.2.11)

Here, \( \Delta \) represents the difference in values of a quantity at two different times and the bar an arithmetic average of the quantities. These equations are derived by moment transformation of the population balance equation with respect to size [Equation (9.2.8)]. Therefore, the average nucleation rate \( \bar{B} \) and the average overall linear growth rate \( \bar{G} \) may be calculated from two experimental population density plots obtained from a batch crystallization experiment at times \( t \) and \( t + \Delta t \). In addition, the average size \( \bar{L} \) at each time can be obtained as follows from the moments:

\[
\bar{L} = \frac{\int_0^\infty n(L)L dL}{\int_0^\infty n(L) dL} = \frac{\mu_1}{\mu_0}
\]  

(9.2.12)

The meaning of each \( k \)th moment in the crystal population is given in Table 9.2.
Fractional moments can also be useful. For example,

\[
\text{Fraction of total number of crystals between size 0 and size } L
\]

\[
= \frac{\int_0^L n(L) dL}{\int_0^\infty n(L) L dL}
\]

Fractional moments can also be applied to the number, area, and mass of crystals.

### 9.3 BATCH CRYSTALLIZERS

Batch crystallizers are extensively used in the pharmaceutical industry to produce biochemicals such as antibiotics. Often, the final step of purification of an antibiotic is crystallization. Batch crystallizers have the advantages of being flexible to operate and requiring less capital investment than continuous crystallizers. A widely employed technique for achieving supersaturation in batch crystallizers is the method of dilution, also called the “salting-out” method if the diluent contains a salt. The addition of a diluent reduces the solubility of the solute that is desired to be crystallized. For example, antibiotics have been crystallized from aqueous solution by the slow addition of a miscible organic solvent such as ethanol. Another way of
generating supersaturation in batch crystallizers is by chemical reaction to form a less soluble product; the reaction can vary from the addition or removal of a proton to the coupling of two molecules. In this section, the dilution method of batch crystallization is analyzed.

9.3.1 Analysis of Dilution Batch Crystallization

In dilution batch crystallizers, operation can be by several different modes, which include a constant diluent addition rate, a constant rate of diluent concentration change, and a constant level of supersaturation. Since the process includes the addition of a third component (the diluent), the crystallizer volume varies with time, although in some cases the diluent volume required may be relatively small, and thus the volume change may be neglected. The addition of a diluent causes the solubility of the solute being crystallized to be lowered. A commonly used expression for the dependence of the solubility of the solute upon the concentration of the diluent being added is the following [5]:

\[ c^* = c_o^* \exp(-k_p c_d) \]  

where \( c_d \) is the diluent concentration and \( k_p \) is a constant.

Since the working volume of a batch crystallizer may vary with time, it is convenient to define the population density function, \( \hat{n} \), based on the total solvent volume at any time as

\[ \hat{n}(L) = n(L)V \]  

The resulting population balance equation for a perfectly mixed batch crystallizer with negligible attrition and agglomeration is [6]

\[ \frac{\partial \hat{n}(L)}{\partial t} + \frac{\partial \hat{n}(L)G}{\partial L} = 0 \]

where \( G \) is the overall linear growth rate, previously defined by Equation (9.2.4). To simplify the solution of this equation, a new variable, \( y \), is defined such that

\[ y = \int_0^t G(t)dt \]

so that

\[ t = \int_0^y \frac{dy}{G(y)} \]
The variable $y$ represents the size of a crystal at any time $t$, that originally nucleated at time $t = 0$. With $y$ as a variable, the population balance for a crystal system where the growth rate is independent of size becomes

\[ \frac{\partial \hat{n}}{\partial y} + \frac{\partial \hat{n}}{\partial L} = 0 \]  

(9.3.6)

The boundary conditions ($y = 0$) for the nuclei population density can be represented by the equation [1]

\[ \hat{n}(t,0) = \hat{n}^0 = \frac{\hat{B}}{G} \]

(9.3.7)

To average the distribution with respect to the internal coordinate properties, a transformation of the population balance equation [Equation (9.3.6)], leads to the following moment equations [6], where the hat symbol ($\hat{\cdot}$) represents quantities based on total solvent volume:

\[ \frac{d\hat{N}}{dy} = \hat{n}(0,y) \]

(9.3.8)

\[ \frac{d\hat{L}}{dy} = \hat{N} \]

(9.3.9)

\[ \frac{d\hat{A}}{dy} = 2k_a \hat{L} \]

(9.3.10)

\[ \frac{d\hat{W}}{dy} = \frac{3k_a \rho}{k_a} \hat{A} \]

(9.3.11)

where $\hat{N} =$ number of crystals

$\hat{L} =$ crystal size (length)

$\hat{A} =$ crystal area

$\hat{W} =$ mass of crystals

$k_a, k_o =$ surface and volume shape factors

$\rho =$ crystal density

These relationships can be combined to predict the time dependence of crystal size in a batch crystallization process, as in the following example.
EXAMPLE 9.1

Batch Crystallization with Constant Rate of Change of Diluent Concentration

Derive the relationship between $y$ and $t$ for an unseeded batch dilution crystallizer with a constant rate of change of diluent concentration, given the following information [6, 7]:

System kinetics: $G = 10^{-6} \Delta c \text{ m/s}; B = 10^6 \Delta c^2 \text{ number (liter of solvent)}^{-1} \text{s}^{-1}$, where $\Delta c = c - c^*$

Physical parameters: $\rho = 2 \times 10^3 \text{ kg/m}^3; k_v = 0.5; k_a = 3.5; c_0^* = 0.25 \text{ kg of solute/liter solvent}; k_p = 10 \text{ liters (solvent and diluent)/liter of diluent}; V_0 = \text{initial volume of solvent} = 1.0 \text{ liter}; \Delta c_0 = 0.01 \text{ kg of solute per liter of solvent};$ and $k_m = 10^{-6} \text{ liter of diluent (liter of solvent)}^{-1} \text{s}^{-1}$, where

$$\frac{dc_d}{dt} = k_m = \text{rate of change of diluent concentration}$$

Solution

We need to determine how $y$ depends on $t$ in this dilution crystallizer. Assume that the nuclei population density based on the initial supersaturation level is constant throughout the volume of the batch. From Equation (9.3.7) at $t = 0$,

\[ \hat{n}(0,0) = \hat{n}_0^0 = \frac{\hat{B}}{G} = \frac{10^6 \Delta c^2 V_0}{10^{-6} \Delta c_0} = 10^{12} \left( \frac{0.01 \text{ number m liter}^{-1}}{10^0 \text{ liter}} \right) (1.0 \text{ liter}) = 10^{10} \text{ number m}^{-1} \]

Assuming the nuclei population density to be constant at this value, after integration of Equations (9.3.8), (9.3.9), and (9.3.10) and substitution into Equation (9.3.11), we obtain

\[ \frac{d\hat{W}}{dy} = k_v \rho \hat{n}_0^0 y^3 = 0.5 (2 \times 10^3)(10^{10}) y^3 = 10^{13} y^3 \text{ kg m}^{-1} \]

Since the change in solute concentration leads to crystal formation, we can write the mass balance for the crystallization as

\[ d\hat{c} + d\hat{W} = 0 \]

where $\hat{c} = Vc$ and $\hat{W} = V \hat{W}$. From the definition of supersaturation ($\Delta \hat{c} = \hat{c} - Vc^*$), the mass balance can be rewritten as

\[ \frac{d\Delta \hat{c}}{dy} + \frac{dVc}{dy} + \frac{d\hat{W}}{dy} = 0 \]
which is called the “supersaturation balance equation.”

We now evaluate the first two terms of the foregoing mass balance equation, with the objective of obtaining a term involving \( \frac{dt}{dy} \) that would allow us to determine \( y \) as a function of \( t \). To evaluate the term \( dVc^*/dy \), we first differentiate the solubility \( c^* \), as given by Equation (9.3.1), with respect to \( t \):

\[
\frac{dc^*}{dt} = -c_0^* k_p \frac{dc_d}{dt} \exp(-k_p c_d) = -k_p c^* \frac{dc_d}{dt}
\]

Substituting for \( dc_d/dt \) gives

\[
\frac{dc^*}{dt} = -k_p k_m c^*
\]

Assuming the initial concentration of diluent to be zero, the solution volume increases according to

\[
V = \frac{V_0}{1 - k_m t}
\]

Differentiating \( V \) with respect to time gives

\[
\frac{dV}{dt} = \frac{V_0 k_m}{(1 - k_m t)^2} = \frac{V^2}{V_0} k_m
\]

The term \( dVc^*/dt \) can be expanded to give

\[
\frac{dVc^*}{dt} = V \frac{dc^*}{dt} + c^* \frac{dV}{dt}
\]

Substituting for \( dc^*/dt \) and \( dV/dt \) yields

\[
\frac{dVc^*}{dt} = c^* k_m V \left( \frac{V}{V_0} - k_p \right)
\]

or,

\[
\int dVc^* = c^* k_m V \left( \frac{V}{V_0} - k_p \right) dt
\]

Next, we assume that volume and solubility changes are negligible (i.e., \( V \equiv V_0 \) and \( c^* \equiv c_0^* \), and that changes in the supersaturation are negligible with respect to \( y \) (i.e., \( d\Delta c/dy = 0 \)). This last assumption is realistic because of the inherent
self-regulating character of batch crystallizers [6]. Substituting for $dV \cdot c^*$ in the supersaturation balance equation gives

$$[c_0^* k_m V_0 (k_p - 1)] \frac{dt}{dy} = \frac{d\hat{W}}{dy}$$

Substituting the result obtained previously for $d\hat{W}/dy$ and rearranging leads to

$$\frac{dt}{dy} = \frac{10^{13} y^3}{(0.25 \times 9)10^{-6}} = 4.44 \times 10^{18} y^3 \, \text{s/m}$$

Integrating with $y = 0$ at $t = 0$ gives

$$y = 3.08 \times 10^{-5} t^{0.25} \, \text{m}$$

This is a useful result that gives an estimate of how long the crystallization must be carried out to obtain crystals of a desired size. Note the modest time dependence of $y(t)$. Also note that this derivation is for a specific case and must be modified when different cases have different kinetic power functions.

### 9.4 PROCESS CRYSTALLIZATION OF PROTEINS

Because crystals of bioproducts tend to be quite pure, the use of crystallization in a purification process often results in great simplification of the process. For the purification of proteins, there are some strong reasons for utilizing crystallization in the process: (1) one or more expensive chromatography steps possibly can be eliminated; (2) in comparison to chromatography, crystallization is relatively inexpensive to carry out, since costly adsorbents are not required; and (3) proteins crystals often can be stored for long periods at low temperatures without being degraded or denatured after the addition of stabilizing agents such as ammonium sulfate, glycerol, or sucrose.

The methods that have been used to crystallize proteins include those used for precipitation: adjustment of the pH to the isoelectric point (isoelectric precipitation), the addition of organic solvents, the addition of salts (salting out), and the addition of nonionic polymers. Another crystallization method that has proven useful for proteins is the reduction of ionic strength by dialysis or diafiltration, which relies on the limited solubility of many proteins at low ionic strength (the reverse of the “salting-in” effect described in Chapter 8, Section 8.2.3).

Besides the method chosen for the protein crystallization, there are important factors to consider about how the method is performed, which include the rate of addition of the precipitation agent, the protein concentration and purity, precipitant concentration, pH, and temperature. A key strategy for crystallization is to move the
system very slowly to a state of minimum solubility of the desired protein until a limited degree of supersaturation is reached. High protein concentrations, in the range of 10 to 100 mg/ml, are recommended for crystallization, and seeding with small crystals of the desired protein is often beneficial [8]. While the protein purity needs to be 99% and greater for the crystallization for structural studies, it can be as low as 50-60% for large-scale crystallization of industrial enzymes such as alkaline phosphatase [9]. In a study of the effect of impurities on the crystal growth of lysozyme, this effect varied from no effect to a significant crystal face specific effect leading to growth cessation, while little effect was observed on solubility or crystal purity [10]. Growth cessation of crystal faces is frequently observed in protein crystal growth. Proteins are typically crystallized over a narrow pH range (< 1 pH unit) and at either 4°C or at ambient temperature [9]. Since the growth rate of protein crystals is slowed at low temperature, crystallization at ambient temperature is common at large scale if protein stability is not affected.

The following studies of protein crystallization illustrate the different approaches that can be taken:

- To crystallize rituximab, a chimeric murine/human monoclonal antibody, a procedure originally used at the microscale with vapor diffusion drops was scaled up. A rituximab solution was added to an equal volume of buffer containing polyethylene glycol, and the mixture was seeded with rituximab crystals made at the microscale. After overnight mixing, rituximab crystals in the form of needle clusters formed. The yield was 85%, and the biological activity of the antibody was fully retained [11].

- Alcohol oxidase, originally contained in *Pichia pastoris* yeast broth grown in a 100-liter pilot plant fermentor, was crystallized by lowering the ionic strength by diafiltration with deionized water; crystallization was preceded by lysis of the yeast cells, diafiltration with a microfilter to obtain the alcohol oxidase in the permeate, and concentration and then diafiltration with an ultrafiltrator [12]. The yield was 208 g of pure enzyme.

- Ovalbumin was crystallized in the presence of conalbumin and lysozyme by the addition of 2.5 µm seed crystals to a solution (600 ml) that had been made supersaturated by slowly adding ammonium sulfate solution [13]. The supersaturation was kept in the metastable region to avoid nucleation (see Figure 9.2). The growth rate of ovalbumin crystals was found to have a second-order dependence on the ovalbumin supersaturation, and the presence of the other two proteins did not affect the growth rate constant \( k_g \) in Equation (9.2.4).

- A fungal lipase was crystallized at low conductivity by slowly lowering the pH with formic acid [14]. The solution for crystallization was prepared by removing the cells by centrifugation (the lipase had been secreted from the cells), concentration by ultrafiltration, and diafiltration to lower the conductivity. An analysis of the crystal size distribution data gave a power dependency of the nucleation rate and growth rate on the lipase supersaturation of 10.8 and 6.4,
respectively. These high powers were attributed to possible underestimation of the supersaturation.

- Recombinant human insulin was commercialized by Eli Lilly in 1982, and crystallization was the final step in the purification process. The crystallization process for insulin is discussed in Chapter 12 with the other steps in the production process.

### 9.5 CRYSTALLIZER SCALE-UP AND DESIGN

Since the behavior of each biochemical to be crystallized is unique, the development of a large-scale crystallization process must be based on experimental data. Thus, data are taken first in the laboratory and are followed by data from pilot plant equipment, which form the basis of the scale-up calculations and plant process design.

#### 9.5.1 Experimental Crystallization Studies as a Basis for Scale-up

Experimental work to serve as a basis for scale-up can be divided into three stages: (1) gathering basic data on solubility and metastable zone width, (2) developing the conditions in a laboratory scale batch crystallizer to obtain a product that meets required specifications at a satisfactory yield, and (3) pilot plant crystallizations that use optimal laboratory conditions as a starting point.

Data on the solubility of the solute to be crystallized should be obtained in the starting solvent as a function of temperature. These solubility data are the upper limit of the concentration of the solute at the start of the crystallization. The metastable zone width is the increase in concentration above the equilibrium solubility before primary nucleation occurs (see Figure 9.2). The metastable zone width can be determined by slowly increasing the supersaturation in a solution free of crystals until primary nucleation occurs, then noting the equilibrium solubility level at nucleation conditions [15].

A convenient size of a batch crystallizer for carrying out initial tests of crystallization is around 1 liter, assuming that enough material is available [15]. Protein equilibrium data, however, have been obtained using as little as 10 µl. A 1-liter volume is generally enough to allow for sampling during the experiment, generating of enough product slurry to obtain filtration rate data, and determining the effect of washing on crystal purity. Typically, the experiment starts with a crystal-free undersaturated solution, and for the proposed end point of the crystallization to be reached in about 2 h, supersaturation is generated by using the chosen method at an approximately linear rate. The point at which primary nucleation occurs is noted and compared with the earlier previous measurements of the metastable zone width. When crystallization has started, it is useful to measure the solution concentration as a function of time. At the end of the generation of supersaturation, the crystal slurry should be agitated for an additional hour or two, continuing to measure the solution concentration to determine the approach to equilibrium. After this holding period, the crystal slurry can be filtered to obtain filtration rate data. If there is sufficient slurry, the effect of
different types of washing on crystal purity can be determined. After drying, the 
crystals should be characterized for particle size distribution and examined under an 
optical microscope, which often gives important information about crystal structure. 

Problems with the final crystals not meeting specifications or with low yield 
should be addressed at the laboratory scale before the scale of testing is increased. 
One such problem that can be difficult to solve occurs when the particle size distribu-
tion is out of range, resulting in low filtration rates of the crystal slurry. In order to 
change the crystal size distribution, the relative rates of nucleation and growth must 
be changed, which can be accomplished by adjusting the supersaturation history. 
In general, the nucleation rate increases more than the growth rate with increasing 
supersaturation, as can be seen from the exponent of the supersaturation in Equations 
(9.2.1) and (9.2.3). Therefore, operating at low supersaturation favors growth of 
large crystals, while high supersaturation close to the metastable limit tends to give 
many more nuclei and smaller crystals. One method that may increase crystal size is 
the addition of seed crystals to a just-supersaturated solution.

The production of crystals with a purity that is lower than that required is another 
problem that can occur in crystallization. An impurity that is the cause of the prob-
lem can be in the following locations in the crystal sample [15]:

1. Deposited on crystal surfaces due to incomplete removal of impure mother 
   liquor
2. Trapped within voids between separate crystals in materials that agglomerate 
3. Contained in inclusions of mother liquor within individual crystals 
4. Distributed throughout the crystals by molecular substitution at the lattice sites 

An obvious approach to eliminating the impurity from the crystal surface is to 
improve the solid-liquid separation and washing procedures. For crystals that agglom-
erate, it may be possible to reduce agglomeration by changing the crystallization condi-
tions (such as using a different crystallization solvent). Reducing the supersaturation 
level to reduce both the nucleation and growth rates should help to minimize the prob-
lem of impurities in inclusions of mother liquor. The problem of the impurity being 
distributed throughout the crystals is difficult to solve for an individual crystallization 
and many require that the crystals be recrystallized. Recrystallization, which is widely 
used, is done by dissolving the crystals in fresh solvent and performing the crystalliza-
tion again. Purer crystals can result if the impurity is more soluble in the solvent than the 
main product. Complex fractional recrystallization schemes, requiring more than one 
recrystallization [5], have been developed but are not used frequently at the plant scale. 

Once satisfactory operating conditions have been obtained at the labora-
tory scale, it is often advisable to test the crystallization at the pilot plant scale. 
Scale-up ratios of 100 to 1000 from laboratory to pilot plant appear reasonable 
[1]. Common scale-up rules are outlined in the next section. The same types of 
measurement made in the laboratory crystallizations should also be made in the 
pilot plant crystallizations.
9.5.2 Scale-up and Design Calculations

The challenge in scale-up is to generate conditions at the large scale that are similar to those at the small scale. As with precipitation (Chapter 8), the key problem is in the scale-up of mixing to control the supersaturation level both locally and globally throughout the crystallizer and to ensure that the crystals are carried throughout the volume of the crystallizer under controlled conditions of frequency and intensity of crystal-crystal interactions [15]. Crystallizer scale-up ratios of 50 to 500 from pilot plant to commercial scale are reasonable [1].

As with precipitation, it is often recommended that geometric similarity and constant power per volume be used in scaleup of crystallizers [1, 16]. For turbulent flow in vessels (Reynolds number > 10,000), constant power per volume means that [17]

\[
P V \propto N_i^3 d_i^2
\]

where \( N_i \) is the impeller rotation rate and \( d_i \) is the impeller diameter. For an agitated tank, the Reynolds number is given by [17]

\[
Re = \left( \frac{d_i^2 N_i \rho}{\mu} \right)
\]

where \( N_i \) is in revolutions per unit time, \( \rho \) is the fluid density, and \( \mu \) is the fluid viscosity.

Two additional strategies are also sometimes used in scale-up of crystallizers [16]:

1. Maintaining constant impeller tip speed, which implies that

\[
N_i d_i = \text{constant}
\]

2. Scale-up at the minimum speed required for particle suspension, which has been shown to require [18]

\[
N_i d_i^{0.85} = \text{constant}
\]

This last criterion should give both secondary nucleation and crystal growth rates that are more or less independent of the scale of operation.

From an analysis of the data for the operation of the crystallization at the laboratory and pilot plant scales, the scale-up equation or equations that prove to be the most accurate in describing the laboratory to pilot plant scale-up should be used in the design of the plant crystallizer. However, flexibility should be designed into the plant unit so that agitation rates and flow rates can be varied, which may be necessary because of error in the scale-up to the larger scale and because of the effect that changes in the concentration of impurities in the starting solution may have on the operation.
EXAMPLE 9.2

Scale-up of Crystallization Based on Constant Power per Volume

It is desired to scale-up a batch crystallization of an antibiotic based on experiments with a 1-liter crystallizer. The use of a 3 cm diameter impeller at a speed of 800 rpm led to good crystallization results. For maintaining power per volume constant upon scale-up to 300 liters, what should be the diameter and speed of the large-scale impeller? The solvent has the same density and viscosity as water.

Solution

For the scale-up, we assume geometric similarity. Thus, the volume \( V \) scales with the impeller diameter \( d \) as

\[
V \propto d^3
\]

Letting subscripts 1 and 2 denote the small and large scales, respectively,

\[
\frac{V_2}{V_1} = \left( \frac{d_{i,2}}{d_{i,1}} \right)^3
\]

Solving for \( d_{i,2} \),

\[
d_{i,2} = d_{i,1} \left( \frac{V_2}{V_1} \right)^{1/3} = 3 \left( \frac{300}{1} \right)^{1/3} = 20.1 \text{ cm}
\]

From Equation (9.5.1), for power per volume to be constant,

\[
N_{i,1}^3 d_{i,1}^3 = N_{i,2}^3 d_{i,2}^3
\]

This equation is valid for turbulent flow in the crystallizer. To verify that the flow is turbulent, we use Equation (9.5.2) to calculate the Reynolds number for the lab crystallizer:

\[
Re = \left( \frac{d_i^2 N_i \rho}{\mu} \right)_1 = \frac{(3\text{ cm})^2 \times 800}{\text{min}} \times 1.00 \frac{\text{g}}{\text{cm}^3} \times \frac{1\text{ min}}{60\text{ s}} = 12,000
\]

This Reynolds number indicates that the flow is turbulent. Solving for \( N_{i,2} \),

\[
N_{i,2} = N_{i,1} \left( \frac{d_{i,1}}{d_{i,2}} \right)^{2/3} = 800 \left( \frac{3}{20.1} \right)^{2/3} = 225 \text{ rpm}
\]

Thus, the rotation rate is reduced considerably compared with the laboratory scale.
9.6 SUMMARY

The process of producing crystals from a homogeneous phase is called crystallization. Bioproducts can be obtained at very high purity (say, 99.9%) by crystallization. This process is considered to be both a polishing step and a purification step, and, in some instances, the purification that is achieved by crystallization is so significant that other more expensive purification steps such as chromatography can be avoided.

- Crystallization differs primarily from precipitation in that when a substance comes out of solution as a solid it also forms a regular lattice of molecules, or crystal.
- Crystals nucleate either by primary or secondary nucleation. Primary nucleation occurs in the absence of crystals, while secondary nucleation is attributed to the influence of existing crystals. Secondary nucleation dominates in industrial crystallizers. Two types of primary nucleation are possible: homogeneous and heterogeneous, where heterogeneous nucleation requires the presence of foreign particles for nucleation to occur and homogeneous nucleation does not.
- The rate of increase in the concentration of nuclei is proportional to a power of the supersaturation, $c - c^*$, where $c^*$ is the concentration at equilibrium ("saturation"). The power has a most probable value of 3 to 4 for primary nucleation and 2 for secondary nucleation.
- The crystal growth rate is also proportional to a power of the supersaturation, with a power of unity being the most common.
- The differential equation for a material balance on a crystal size interval can be subjected to a moment transformation. The zeroth moment is the number of nuclei (crystals), the first moment is the linear size, the second moment is proportional to the area, and the third moment is proportional to the mass (or volume). The crystal nucleation and growth rates can be determined from the moments of the experimental population density function.
- Batch crystallizers can be analyzed by the population balance equation.
- Scale-up of crystallizers, like scale-up of precipitation units, emphasizes mixing. The mixing parameters that can be held constant are power input per volume, impeller tip speed, and agitation sufficient to keep crystals in suspension. All three of these variables cannot be held absolutely constant upon scale-up at the same time, because each depends differently on the impeller diameter. Therefore, scaling priorities are also based on experimental measurements and plant scale design for flexibility.

NOMENCLATURE

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>surface area of crystals per volume of solvent (cm² liter⁻¹)</td>
</tr>
<tr>
<td>$B$</td>
<td>nucleation rate during crystallization (number liter⁻¹ s⁻¹)</td>
</tr>
<tr>
<td>$c$</td>
<td>concentration of solute (M, or g liter⁻¹)</td>
</tr>
<tr>
<td>$c_d$</td>
<td>concentration of diluent (liter liter⁻¹)</td>
</tr>
<tr>
<td>$c_i$</td>
<td>concentration of solute at interface between liquid and solid phases (g liter⁻¹)</td>
</tr>
</tbody>
</table>
\(d_i\)  impeller diameter (cm)
\(G\)  overall linear growth rate of crystals (cm s\(^{-1}\))
\(P/V\)  power input per volume of liquid (W liter\(^{-1}\))
\(k_a\)  surface shape factor (dimensionless)
\(k_d\)  mass transfer coefficient for bulk diffusion [Equation (9.2.5)] [g cm\(^{-2}\) s\(^{-1}\) (g/liter)]\(^{-\alpha}\)]
\(k_g\)  overall mass transfer coefficient for linear crystal growth [Equation (9.2.4)] [cm s\(^{-1}\) (g/liter)]\(^{-\alpha}\)]
\(k_G\)  overall mass transfer coefficient for crystal growth [Equation (9.2.3)] [g cm\(^{-2}\) s\(^{-1}\) (g/liter)]\(^{-\alpha}\)]
\(k_1\)  rate constant for secondary nucleation [Equation (9.2.2)] [number liter\(^{-1}\) s\(^{-1}\) (g/liter)]\(^{b-j}\)]
\(k_m\)  rate of change of diluent concentration (liter liter\(^{-1}\) s\(^{-1}\))
\(k_n\)  rate constant for primary nucleation [Equation (9.2.1)] [number liter\(^{-1}\) s\(^{-1}\) (g/liter)]\(^{-n}\)]
\(k_p\)  constant in equation for solubility of solute as a function of diluent concentration [Equation (9.3.1)] (liter liter\(^{-1}\))
\(k_r\)  mass transfer coefficient for surface integration [Equation (9.2.6)] [g cm\(^{-2}\) s\(^{-1}\) (g/liter)]\(^{-\gamma}\)]
\(k_v\)  volume shape factor (dimensionless)
\(L\)  characteristic dimension of crystal (cm, or cm liter\(^{-1}\))
\(M_T\)  crystal suspension density (g liter\(^{-1}\))
\(n\)  population density distribution function (number liter\(^{-1}\) cm\(^{-1}\))
\(N\)  number concentration of nuclei (number liter\(^{-1}\))
\(N_i\)  impeller rotation rate (revolutions s\(^{-1}\))
\(Re\)  Reynolds number \(d_i^2N_i\rho / \mu\) (dimensionless)
\(R\)  rate of mass deposition on crystals during growth (g cm\(^{-2}\) s\(^{-1}\))
\(t\)  time (s)
\(V\)  solvent volume (liter)
\(W\)  mass of crystals per volume of solvent (g liter\(^{-1}\))
\(y\)  size of crystals at time \(t\) [Equation (9.3.4)] (cm)

**Greek Letters**

\(\mu\)  fluid viscosity (g cm\(^{-1}\) s\(^{-1}\))
\(\mu_k\)  \(k\)th moment of the population density distribution (units vary)
\(\rho\)  crystal or fluid density (g liter\(^{-1}\))

**Subscripts**

\(f\)  final
\(0\)  initial

**Superscripts**

\(*\)  saturation or equilibrium
\(^\wedge\)  quantities based on total solvent volume
\(^-\)  average quantities
PROBLEMS

9.1 Modeling of Crystal Growth  The data in Table P9.1 have been measured for the growth of crystals of an antibiotic. From this data set, obtain an expression for the relationship between $\frac{dL}{dt}$ and the supersaturation $c - c^*$.  

<table>
<thead>
<tr>
<th>$c - c^*$ (g/liter)</th>
<th>$\frac{dL}{dt}$ (μm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>0.35</td>
<td>0.45</td>
</tr>
<tr>
<td>0.67</td>
<td>0.90</td>
</tr>
<tr>
<td>1.25</td>
<td>1.80</td>
</tr>
<tr>
<td>2.05</td>
<td>3.30</td>
</tr>
<tr>
<td>2.75</td>
<td>5.00</td>
</tr>
</tbody>
</table>

9.2 Nucleation and Growth Rates from Crystal Size Distribution Data  Crystal size distribution (CSD) data were obtained with a multichannel Coulter counter during a crystallization experiment in a batch agitated vessel with isothermal conditions. The results of two CSD samples taken at different times differing by a small time interval are shown in Table P9.2. Use the method of moments analysis to determine the average nucleation and growth rates. At each time, also determine the population average crystal size. (Data from N. S. Tavare, *Industrial Crystallization*, p. 180, Plenum Press, New York, 1995.)

<table>
<thead>
<tr>
<th>Channel number</th>
<th>$\bar{L}$ (μm)</th>
<th>$\Delta L$ (μm)</th>
<th>$n(L) \times 10^{-10}$ (number m$^{-1}$ kg$^{-1}$) At $t_1 = 3600$ s</th>
<th>At $t_2 = 4500$ s</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>17.4</td>
<td>4.0</td>
<td>180.9</td>
<td>176.3</td>
</tr>
<tr>
<td>5</td>
<td>21.9</td>
<td>5.0</td>
<td>43.6</td>
<td>55.7</td>
</tr>
<tr>
<td>6</td>
<td>27.6</td>
<td>6.4</td>
<td>24.5</td>
<td>32.1</td>
</tr>
<tr>
<td>7</td>
<td>34.8</td>
<td>8.0</td>
<td>21.8</td>
<td>23.4</td>
</tr>
<tr>
<td>8</td>
<td>43.8</td>
<td>10.1</td>
<td>13.8</td>
<td>17.6</td>
</tr>
<tr>
<td>9</td>
<td>55.2</td>
<td>12.7</td>
<td>13.9</td>
<td>18.1</td>
</tr>
<tr>
<td>10</td>
<td>69.6</td>
<td>16.0</td>
<td>6.4</td>
<td>9.3</td>
</tr>
<tr>
<td>11</td>
<td>87.7</td>
<td>20.2</td>
<td>3.8</td>
<td>4.8</td>
</tr>
<tr>
<td>12</td>
<td>110.5</td>
<td>25.4</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>13</td>
<td>139.2</td>
<td>32.0</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>175.4</td>
<td>40.3</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>15</td>
<td>221.0</td>
<td>50.8</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

9.3 Batch Crystallizer with Constant Rate of Diluent Addition  For a solute being crystallized by batch crystallization with a constant rate of change of diluent concentration, predict the effect on the crystal size at any given time for each of the changes in the variables given. Justify your answers based on an expression for the crystal size as a function of these variables.
(a) An increase in the nucleation rate
(b) An increase in the growth rate
(c) An increase in the supersaturation, for \( b \) [Equation (9.2.2)] greater than \( g \) [Equation (9.2.3)]
(d) An increase in \( k_m \) in the equation \( dc_d/dt = k_m \), where \( c_d \) is the diluent concentration
(e) An increase in \( k_p \) [Equation (9.3.1)]

9.4 Estimate of Particle Size for an Unseeded Batch Crystallizer  
For an unseeded batch crystallization of a biochemical using a constant rate of diluent concentration, the following is known:

\[
G = 10^{-7} \Delta c^2 \text{ m/s} \\
B = 10^6 \Delta c^3 \text{ number (liter of solvent)}^{-1} \text{ s}^{-1} \\
\rho = 2.5 \times 10^3 \text{ kg/m}^3 \\
k_v = 0.6 \\
k_a = 3.0 \\
c_*^0 = 0.05 \text{ kg of solute per liter of solvent} \\
k_p = 5 \text{ liters (solvent and diluent) per liter of diluent} \\
V_0 = \text{initial volume of solvent} = 1.0 \text{ liter} \\
\Delta c_0 = 0.005 \text{ kg of solute per liter of solvent} \\
k_m = 2 \times 10^{-5} \text{ liter of diluent (liter of solvent)}^{-1} \text{ s}^{-1}, \text{ where}
\]

\[
\frac{dc_d}{dt} = k_m
\]

Estimate the average size of the crystals 20 min from the start of nucleation. State all the assumptions necessary for the calculation.

9.5 Comparison of Three Scale-up Methods for a Crystallization  
A batch crystallization of an antibiotic was performed using a volume of 750 ml in the laboratory with a 3.5 cm diameter impeller at a speed of 600 rpm, the minimum speed required to fully suspend the crystals. Estimate the size of the impeller and the impeller speed for scale-up to 250 liters for each of the following three assumptions as a basis for scale-up: (1) constant power per volume, (2) constant impeller tip speed, and (3) full suspension of crystals (at minimum speed).

9.6 Deduction of Scale-up Equations for a Crystallization  
The batch crystallization of a biochemical resulted in virtually identical properties when conducted either at the laboratory scale (1 liter) or the plant scale (1000 liters). The lab crystallizer had an impeller of 3 cm diameter operating at 750 rpm, while the plant crystallizer had a 30 cm impeller with a speed of 77 rpm. What scale-up equations can be used to approximately characterize the scale-up of this crystallization? What property or properties are being assumed constant upon scale-up?

9.7 Bulk Volume Too High for Crystalline Erythromycin  
The bulk volume of dry, crystalline erythromycin (an antibiotic) produced in a plant batch crystallizer is out of specification (too high). Erythromycin free base is crystallized by the slow addition of dilute sodium hydroxide to a solution of an erythromycin salt dissolved in water-acetone. You have been asked to study this problem in the laboratory and
recommend a solution based on your results. Outline the experiments you would conduct in the lab, the results of which would serve as a basis for recommendations for how to change the operation of the plant crystallizer.

**9.8 Primary versus Secondary Nucleation** Why is it generally more desirable to have secondary nucleation rather than primary nucleation in crystallization?

**REFERENCES**