12. Crystallization

Crystallization

- **Crystallization**
  - The process of producing crystals from a homogeneous phase
  - Arrangement of molecules in space lattice
- **Crystallization vs. precipitation**

Description	Crystallization	Precipitation
Solubility	Wide range, medium to high	Sparingly soluble
Relative supersaturation	Low	High
Product morphology	Well-defined	Ill-defined
Product crystal size	Large	Small
Nucleation mechanism	Secondary	Primary
Nucleation rate	Low	High
Growth rate	Wide range	Low
Controllability	Controllable	Difficult to control

Applications of Crystallization

- **Polishing**
  - The process needed to put the bioproduct in its final form for use
  - E.g. antibiotics
- **Purification**
  - Crystallization can generated products at very high purity (99.9%)
- **X-ray chrystallography**
  - Need a small number of crystals with good sixe (0.2-0.9 mm) and internal quality
  - For determination of protein structure

1. Crystallization Principles

Crystals

- **Structure**
  - Polyhedrons
  - 7 crystals systems
    - Defined by angles of crystals
    - Same material generates crystals with the same angles
  - Crystal habit
    - Difference shapes of a crystal system
    - Different relative sizes of the faces
    - Depending on the conditions of crystallization
      - Impurities
      - Solvents

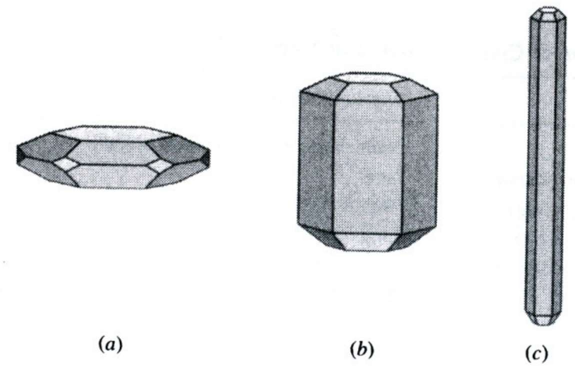


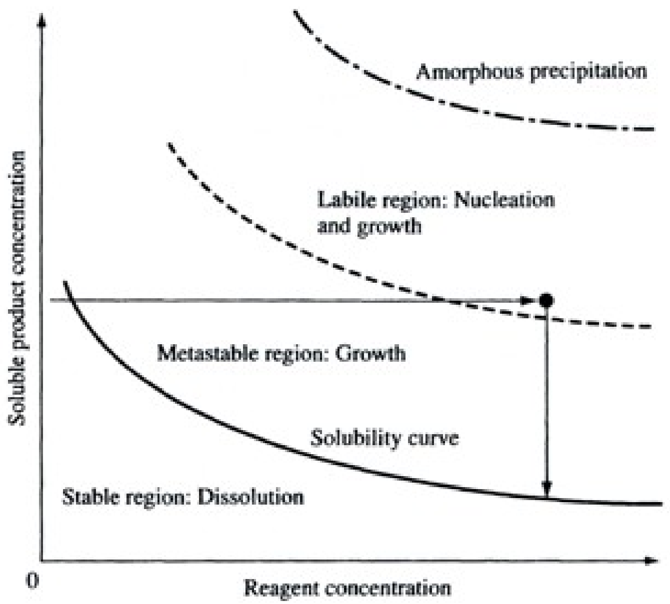
Figure 9.1 Crystal habit illustrated for hexagonal crystals: (*a*) tabular, (*b*) prismatic, and (*c*) acicular.

Nucleation

- **Types**
  - Primary nucleation
    - Occurs in the absence of crystals
  - Secondary nucleation
    - Influences by the existing crystals
    - Usually predominates during industrial cryatallization
- **Primary nucleation**
  - Types
    - Homogeneous nucleation
      - No foreign particles are present
    - Heterogeneous nucleation
      - Presence of foreign particles during nucleation
  - Rate of primary nucleation
    - $B = dN/dt = k_n(c-c^*)^n$
      - B : the number of nuclei formed/ V/t
      - N : the number of nuclei / V
      - K_n : the rate constant
      - c^* : Solute concentration at saturation
      - $c-c^*$: Supersaturation
      - n : typically 3~4, upto 10

Nucleation

- **Secondary nucleation**
  - Types
    - Shear nucleation
      - Occurs as a result of fluid shear on growing crystal faces
    - Contact nucleation
      - By collision between crystals or with internal surfaces
  - Rate of secondary nucleation
    - $B = dN/dt = k_1 M_T^j (c-c^*)^b$
      - M_T : Suspension density
      - b : mostly 2, up to 5
      - j : mostly 1, up to 1.5



Crystal Growth

- **Crystal growth**
  - Addition of molecules in solution to the surface of existing crystals
- **The rate of mass deposition (R)**
  - $R = 1/A \cdot dW/dt = k_G (c-c^*)^g$ **(1)**
    - W : Mass of crystals / volume of solvent
    - A : the surface area of crystals / volume of solvent
    - k_G : Overall mass transfer coefficient
      - – Depending on temperature, crystal size, hydrodynamic conditions, the presence of impurities
    - g : 0~2.5, near 1 is the most common
- **The Overall linear growth rate (G)**
  - $G = dL/dt = k_g(c-c^*)^g$
    - L : A characteristic single dimension of the crystal (Length)
    - Delta L law
      - – Geometrically similar crystals of the same material grow at the rate G independent of crystal size
- **Two steps for crystal growth**
    - Diffusion : $R = k_d(c-c_i)^d$ **(2)**
    - Surface integration : $R = k_r(c_i-c^*)^r$ **(3)**
    - C_i : Concentration at the interface between the liquid and solid phase
- **(1) + (2) + (3)**
  - $1/ k_G = 1/ k_d + 1/ k_r$
  - If $k_r \gg k_d,\ k_G \approx k_d$

Crystallization Kinetics from Batch Experiments

- **Moment analysis**
  - Mass balance on the number of crystalline particles within L and L +ΔL
  - The population balance equation for size independent growth in a perfectly mixed, constant volume batch crystallizer

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

    - The population density distribution function *n(L)* Ξ *dN/dL*
    - Liner growth rate G = *dL/dt*
    - The total number of particles within two particle diameters ∫ *n(L) dL*
  - The k^{th} moment of the population density distributions in L about the origin, obtained by moment transformation with respect to size is defined as

$$\mu_k \equiv \int_0^\infty n(L)L^k\,dL$$

  - If the moments of the experimental population density function can be determined at t and Δ t
    - The average values of the nucleation rate $\bar{B} = \frac{\Delta\mu_0}{\Delta t}$
    - The average values of the growth rate $\bar{G} = \frac{\Delta\mu_1}{\bar{\mu}_0\Delta t}$

Crystallization Kinetics from Batch Experiments

- **Average size**

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

- **The meaning of *k*th moment**

TABLE 9.2
Moment Analysis of Crystal Size Distribution

Moment	k of moment [Equation (9.2.9)]	Meaning of moment	Mathematical definition	Average value for spherical crystals
μ_0	0	Number of crystals	$\int_0^\infty n(L)\,dL$	
μ_1	1	Size of crystals	$\int_0^\infty n(L)L\,dL$	$\frac{\mu_1}{\mu_0}$
μ_2	2	Area of crystals[a]	$\int_0^\infty n(L)L^2\,dL$	$\pi\left(\frac{\mu_2}{\mu_0}\right)$
μ_3	3	Mass of crystals[b]	$\int_0^\infty n(L)L^3\,dL$	$\frac{\pi\rho}{6}\left(\frac{\mu_3}{\mu_0}\right)$

[a] Multiply moment by area shape factor relating area to L^2 to obtain area of crystals per unit volume (area shape factor $= \pi$ for spheres).
[b] Multiply moment by crystal density ρ and by a volume shape factor relating volume to L^3 to obtain mass of crystals per unit volume (volume shape factor $= \pi/6$ for spheres).

- **Fraction of total number of crystals between size 0 and L**

$$L = \frac{\int_0^L n(L)\,dL}{\int_0^\infty n(L)\,dL}$$

2. Crystallization

Crystallizers

- **Types**
  - Continuous
  - Batch
    - Extensively used in the pharmaceutical industry to produce biochemicals

- **Methods to achieve supersaturation in batch crystallizers**
  - Dilution (Salting out if the diluent contains a salt)
    - Reduction of solubility of the solute to be crystallized
    - Constant diluent addition rate
    - Constant rate of diluent concentration change
    - Constant level of supersaturation
  - Chemical reaction to from a less solubel product

Crystallization of Proteins

- **Cystallization for protein purification**
  - Relatively inexpensive than chromatography
  - Proteins crystals are stable at low temperature for long storage
- **Methods**
  - Adjust pH to PI
  - Addition of organic solvents
  - Salting out
  - Addition of nonionic polymers
  - Reduction of ionic strength (reverse of the salting in effect)
- **Procedure**
  - Very slow movement of the system to reach the state of minimum solubility of the desired protein
  - High protein concentration : 10-100 mg/ml