Thermodynamic and Kinetic Levers in Cooling Crystallization for Nanoscale APIs

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Abstract. Poor solubility limits the bioavailability of Active Pharmaceutical Ingredients (APIs), motivating research on nanocrystal formations with enhanced dissolution rate. Cooling crystallization is widely used in bulk API production, though its application to nanocrystal design is limited. Classical Nucleation Theory has shown that high supersaturation is required to produce nanoscale crystalline structures. However, such a labile zone operation may induce poor reproducibility, broad Particle Size Distribution (PSD), and crystal defects. This review examines the three major process levers — cooling rate, seeding, and agitation — that govern the balance between nucleation and crystal growth. While effective for bulk API production, they cannot be used alone to deliver reproducible PSD. Hybrid intensification techniques, such as antisolvent-cooling crystallization, are employed to create a localized supersaturation burst for controlled nucleation. Computational tools, including Process Analytical Techniques (PATs) with population balance models (PBMs), extend the use of cooling crystallization to predict PSD under dynamic conditions. At the same time, molecular simulations clarify possible non-classical nucleation pathways. Thus, understanding of cooling crystallization provides a theoretical basis, but requires hybrid intensification strategies and modeling techniques for nanoscale API production.

Keywords: Classical nucleation theory (CNT); cooling crystallization; nanocrystal APIs; metastable zone width (MSZW); antisolvent crystallization.

1. Introduction

Poor solubility of Active Pharmaceutical Ingredients (APIs) is a significant barrier to effective oral drug delivery, as it directly impacts the API’s ability to dissolve gastrointestinal fluids and thereby limits its bioavailability. Such a challenge is particularly acute in central nervous system (CNS) targeting drugs, where achieving a therapeutically effective dosage requires not only absorption of API but also penetration of API through the blood-brain barrier [1]. More than 40% of new chemical entities are practically insoluble in water, which inevitably leads to inefficient API absorptions. Meanwhile, around 70% of pharmaceutical agents fall under Biopharmaceutics Classification System class II, which refers to products with low solubility, high permeability [2, 3]. This leads to inefficient absorption and dose escalation.

In the pharmaceutical realm, nanocrystalline usually refers to nano-scale crystals of approximately 1000 nm in size [4]. For systemic bioavailability, nanocrystal technology has emerged as one of the most promising solutions. Because dissolution rate rises exponentially with surface area, as defined by Noyes-Whitney, therefore, nanocrystalline structures are advantageous [3]. Among all preparation techniques, cooling crystallization is an attractive bottom-up approach. Lowering the temperature reduces the equilibrium solubility of solid [c(T)], raising the supersaturation [$S = c/\ c(T)$], the driving force for nucleating of crystalline structure. However, depending on the design, both the Crystal Size Distribution (CSD) and the mean crystal size will vary.

This essay aims to explore the theoretical basis of cooling crystallization through Classical Nucleation Theory (CNT). It discusses how its kinetic principles underpin both conventional bulk API production and the development of nanocrystal processes.

2. Classical Nucleation Theory – Primary Nucleation

2.1. Free Energy Balance

The first-order phase transition from parent phase to daughter phase would occur instantaneously or uniformly across the system [5]. Instead, it begins with a random, localized formation of small, ordered molecular structures, known as clusters. CNT provides a thermodynamic framework for understanding the stability of these clusters, whether they diffuse back into the mother liquid or form a stable nucleus that allows for further growth. This is quantified by the Gibbs free energy change (ΔG) associated with their formation, which reveals the energy barrier that must be overcome for the formation of a stable crystal.s

$$\Delta G(r) = \Delta G_S + \Delta G_V = 4\pi r^2\gamma + \frac{4}{3}\pi r^3 \underline{\Delta G_V} \tag{1}$$

As stated in equation 1 [6], r is the radius of the spherical nuclei, γ is the surface tension between the liquid-solid interface, and (ΔG_V) is the free energy change of transformation per unit volume.

The free energy change (ΔG) for spherical cluster formation of radii (r) is the sum of two competing terms: the volume contribution term (ΔG_V), and surface free energy term (ΔG_S). Where ΔG_V expresses the free-energy driving force that directs the crystallization process, it measures the energy released during the formation of metastable liquid to a stable, ordered solid state. It is a negative quantity, as such a transformation is thermodynamically favorable. ΔG_S represents the energy penalty for the creation of an interface between two phases. Therefore, this term is unfavorable (positive) and proportional to the cluster's surface area.

Therefore, the competition between bulk free energy gain and surface penalty explains why nanoscale nuclei require high supersaturation, clarifying why bulk C.C. seldom accesses stable nuclei without additional intensification.

2.2. Critical Nucleus Size

The stationary point between instability and stability is marked by the critical nucleus size, r^*. This is the size of the crystal at which the free energy of the cluster, ΔG, reaches its maximum. Cluster of size r^* is in equilibrium, the addition of a single molecule would cause it to irreversibly grow, while the loss of a single molecule would cause it to redissolve into the solution.

Mathematically, r^* is found by differentiating $\Delta G(r)$ with respect to r, and setting the derivative to zero [6]:

$$\begin{gathered}\frac{dG(r)}{dr} = \frac{d}{dr}\left(4\pi r^2\gamma + \frac{4}{3}\pi r^3\underline{\Delta G_V}\right) = 8\pi r\gamma + 4\pi r^2\underline{\Delta G_V} = 0 \\ (where\ r^* = -\frac{2\gamma}{\underline{\Delta G_V}},\ and\ \underline{\Delta G_V} < 0)\end{gathered} \tag{2}$$

By plugging equation 2 into equation 1, we can obtain the critical free energy change of transformation ΔG_{crit} , as shown in equation 3:

$$\Delta G_{crit} = \frac{4\pi\gamma r_c^2}{3} = \frac{16\pi\gamma^3}{3\,(\Delta G_V)^2} \tag{3}$$

The notion of ΔG_{crit} is fundamental in CNT, reflecting the essential energy barrier that the nuclei must surpass to achieve stability. During the cooling crystallization process, the ΔG_V is directly modified by the degree of supersaturation, which is controlled by temperature [6]. Lowering the temperature increases S, which in turn decreases both ΔG_V and ΔG_{crit}, thereby increasing the probability of nucleation.

For pharmaceutical nanocrystals, which are generally of size ~1000 nm, the clusters of interest are well validated under the capillary approximation, which is a set of assumptions outlined in CNT [4]. Through manipulations of process parameters (i.e., cooling rate), which influence supersaturation and thus ΔG_V and r^*, CNT provides a foundational framework for designing crystallization processes to achieve targeting Particle Size Distribution (PSD).

Since $r \propto 1/\ln(S)$, where only large supersaturation would lead to nanoscale crystals. In practice, achieving such high ln(S) uniformly in bulk solvent is unstable, which is why often a localized or hybrid approaches are needed, instead of purely relying on bulk cooling.

2.3. Nucleation Rate and the Role of Supersaturation

Nucleation of crystalline structure is only possible if the free energy of transformation, ΔG, surpasses the critical free energy change, ΔG_{crit}. However, to understand the rate of crystal formation, which is crucial for understanding the final particle population and size, we must transfer from thermodynamics to kinetics. The nucleation rate, J, is the direct measure of the number of nuclei formed per unit volume per time. Such expression is given by Arrhenius reaction velocity equation (equation 4) [6]:

$$J = A\exp\left(-\frac{\Delta G_{crit}}{kT}\right) \quad (4)$$

By plugging equation 3 into equation 4, we can obtain:

$$J = A\exp\left(-\frac{16\pi\gamma^3 v^2}{3k^3T^3(\ln S)^2}\right) \quad (5)$$

Equation 5 is the cornerstone of CNT for cooling crystallization design. It indicates the nucleation rate of a given supersaturated system is governed by three major factors. The interfacial tension, γ; Figure 1 shows the exponential dependence of nucleation rate, J, in terms of the supersaturation degree, S. The x-axis starts from one, meaning physically no nucleation would occur at such value, due to exceptionally large ΔG_{crit}. It is not until S reaches the critical supersaturation value, S_{crit}, around 4, that the spontaneous nucleation of crystal would occur [6].

Figure 1 also showed the primary limitation of the CNT model, where it fell short in determining the kinetic factors of the system. The theory relies on the capillary approximation, treating a cluster as a macroscopic object with well-defined properties and surface energy, which essentially breaks down when modeling clusters composed of a few molecules. Despite these simplifications, CNT provides an invaluable perspective in understanding the effect of supersaturation, as the driving force for the nucleation process, on the production of nuclei.

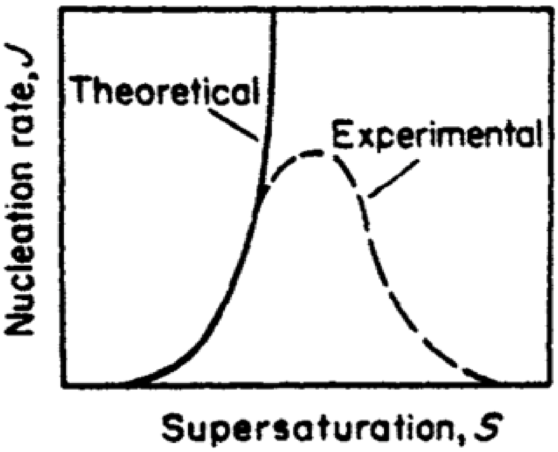

Fig. 1 Nucleation rate as a function of Supersaturation.

2.4. Crystal Growth

Though Classical Nucleation theory describes a strong foundation for understanding the nucleation process of cooling crystallization, it does not account for the subsequent growth of nuclei, which is essential towards obtaining ~1000 nm APIs.

Although there is no complete agreement on the crystal growth mechanism, the diffusion-reaction theory provides a valuable perspective [6], which is essentially a two-step process. First, the solutes would diffuse across the solution boundary layer to the crystal surface, driven by a concentration gradient. Followed by a first order ‘reaction’, where the solutes on the surface would incorporate themselves onto the active sites present, such as kinks or steps. Such a relation can be expressed as:

$$\frac{dm}{dt} = K_G A(c - c^*)^g \tag{6}$$

Where K_G is the overall growth rate constant; A is the surface area of the crystal; Δc is the supersaturation; g = the growth order, determined by the rate-limiting step.

Therefore, the final CSD is the direct reflection of the competition between nucleation and growth. Where nucleation increases exponentially with supersaturation, while growth follows a power-law dependence. Because the nucleation process is far more sensitive, high supersaturation favors the production of a large quantity of small clusters, which is the basis of nanocrystal production [6].

3. Three Key Process Parameters – From CNT Perspective

3.1. The Metastable Zone

The cooling crystallization pathway is primarily determined by the solution's state relative to the solubility curve [7]. The metastable zone is the region where the supersaturation is insufficient for spontaneous nucleation. Therefore, crystallization must be initiated by seeking. By contrast, the labile zone lies above the metastable limit, where the system would spontaneously nucleate a large quantity of small clusters.

As illustrated by Figure 2, the solution at saturation (point A) can follow two distinct cooling pathways. A controlled cooling path that remains at the metastability limit (route A→D); with seeding, this avoids nucleation and allows for the formation of larger crystals with controlled morphology. By contrast, a more aggressive cooling route (route A→B) would cross the metasolubility limit into the labile zone, where nucleation dominates, and a large quantity of small crystals would form. While this kinetic pathway highlights the sensitivity of nucleation towards supersaturation, labile zone operation is rarely applied in cooling crystallization due to poor reproducibility, though it may be exploited by different techniques (eg, Antisolvent cooling crystallization).

The metastable zone width (MSZW), unlike the solubility curve, is not a fixed property of the solute but depends on process parameters such as cooling rate, additives, and agitation. These effects shall be discussed in the following section.

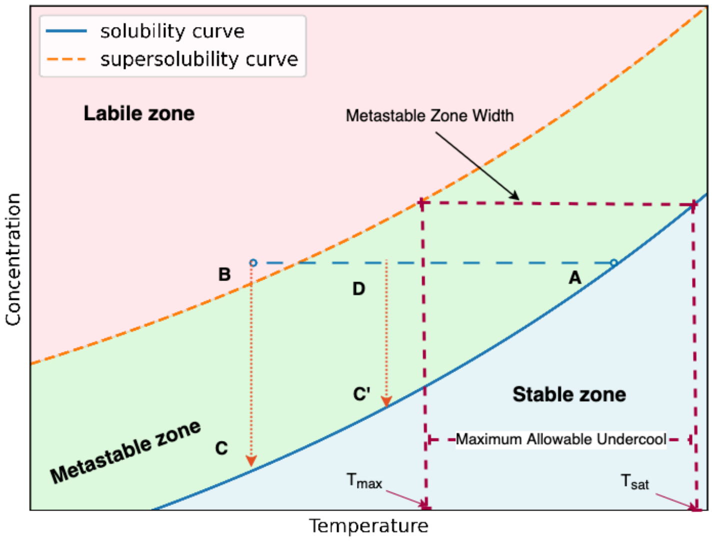

Fig. 2 Metastable Zone illustration

3.2. Cooling Rate: Kinetic Parameter

Increasing the cooling rate reduces Maximum Allowable Undercooling (MAU) in both seeded and unseeded solutions. MAU is the maximum temperature undercooling allowable without spontaneous primary nucleation (ie, labelled in Figure 2). A higher cooling rate narrows the metastable zone width (MSZW), thereby increasing the risk of entering the labile zone [6]. Labile zone nucleation is uncontrollable and hinders the downstream processing, showing why cooling alone cannot deliver reproducible nanocrystals.

In bulk API production, rapid cooling is generally avoided. However, the same kinetic principle explains why intensified processes yield smaller particles, which are suitable for nanocrystal design [8]. For example, continuous antisolvent crystallization of carbamazepine dihydrate, carried out by Honavar et al., demonstrated that increasing feed supersaturation led to a marked increase in nucleation rate and a reduction in mean CSD [9]. This illustrates how different techniques utilize rapid supersaturation generation, which functionally resembles labile zone conditions, although it is usually achieved by methods such as antisolvent addition rather than cooling crystallization alone.

3.3. Seeding: Manual Control Parameter

For cooling crystallization, crystals can be either spontaneously formed from a clear solution (primary nucleation) or under the assistance of a seeding crystal (secondary nucleation). Section II has described how CNT explains primary nucleation, but in practice, it is often avoided, as it produces non-reproducible particle size distributions (PSD) and may lead to crystal defects or impurity inclusions [10]. Seeding is therefore used to suppress primary nucleation.

In seeded crystallization within metastable zone, both secondary nucleation and growth occur simultaneously. The stable seed surface provides kinks for molecular attachment, which lowers the nucleation barrier (ΔG_{crit}), thus creating a competition between nucleation and growth, such competition would yield two distinct outcomes [11]. Industrial seeding strategies aim at growth-dominated crystallization for generations of large and uniform crystals, whereas nanocrystal strategies deliberately utilize a secondary-nucleation-dominated pathway to generate submicron particles with enhanced dissolution and solubility. Seeding provides control by avoiding operations in labile zones. When manipulated near the metastable limit, it can also bias towards secondary nucleation, offering a lever for smaller particle sizes.

3.4. Agitation: Kinetic Lever

Agitation strongly influences crystallization outcomes by altering both supersaturation homogeneity (ie. mixing) and nucleation. Since, secondary nucleation rate increases with impeller speed, suspension density, and supersaturation [11]. Increased agitation would result in enhanced collisions between crystal, wall, and impeller, which lowers MSZW and promotes the formation of fines alongside crystal growth. Thus, agitation is not only a way to encourage mixing but also a kinetic lever that shifts the balance between nucleation and growth.

From a pharmaceutical perspective, agitation is a critical factor for determining final PSD. Researchers have found that while increasing agitation may improve overall supersaturation homogeneity, narrowing the MSZW would directly result in uncontrolled fines and broaden the PSD [12]. For bulk API production, moderate agitation is applied to promote mixing while maintaining reproducibility. By contrast, intensified agitation is deliberately used in nanocrystal strategies to promote nucleation and generate submicron particles that enhance dissolution.

4. Future Perspectives

4.1. Limitations of Cooling Crystallization for Nanocrystals

Cooling crystallization is one of the most widely applied techniques in bulk API manufacturing, though its direct application for producing nanocrystals is limited [11]. The primary reason is that the industrial crystallization process is designed to achieve reproducible, narrow PSD, with maximized crystals, thereby simplifying the downstream processing, such as filtration and drying. To achieve such objectives, it requires operations within a metastable limit, with conditions such as cooling profile, seeding, and agitation, chosen to suppress primary nucleation deliberately. Industrial crystallization processes are therefore optimized to avoid excessive fines, making cooling crystallization poorly suitable for the direct production of nanocrystals [13].

From the CNT perspective, reaching nanoscale crystals requires near or within labile zone conditions (eg, under rapid cooling) for the bursting of nucleation to exceed growth. However, this region of operation is generally avoided in industry because it induces crystal defects, broader PSD, and impurity entrapment, all of which are incompatible with pharmaceutical quality standards. Moreover, the supersaturation generated by bulk cooling is limited by the heat-transfer efficiency and spatially non-uniform, so nucleation bursts occur at different locations and times, further widening PSD. Fine crystals generated under such conditions typically have a high surface-to-volume ratio, which leads to poor flowability and filterability, posing significant challenges for scale-up and industrial applications. For these reasons, attempts to rely solely on cooling crystallization to produce nanoscale crystals are undesirable due to poor reproducibility and low industrial viability.

4.2. Hybrid and Emerging Technologies

Cooling crystallization provides the theoretical foundation for particle size control, although it is not suitable for controlled bursts of nucleation formation, as supersaturation in the bulk is not developed uniformly. Therefore, an effective solution is maintaining the control of solubility via temperature, while introducing an intensification step to create a short, uniform supersaturation burst, followed immediately by measures to suppress subsequent growth. From a CNT perspective, the intensifier raises supersaturation ($S=C/C^*$) rapidly, producing large values of $\ln(S)$, which reduces the critical nucleus size ($r \propto 1/\ln(S)$), and lowers the free energy barrier for nucleation ($\Delta G_{crit} \propto 1/(\ln(S))^2$). This would give it the special advantage of increasing supersaturation, as a driving force for nucleation, while maintaining it in a localized and temporally controlled manner, rather than a completely uncontrolled burst as in bulk cooling crystallization near the labile zone.

One common hybrid approach is to combine cooling with antisolvent addition, where the antisolvent decreases the solubility abruptly and produces a surge in supersaturation without excessive cooling [13]. According to CNT, such conditions would be expected to have nucleation over growth, potentially shifting the system towards a smaller particle size. However, experiments demonstrated that product outcomes depend strongly on mixing and process configuration. In continuous antisolvent crystallization of carbamazepine dihydrate, while increasing supersaturation of the feed increased the nucleation rate, the mean CSD remained unchanged, as the process is stabilized under steady-state conditions [9]. This highlights that although supersaturation can theoretically drive the size reduction, the outcome also depends on process mode.

4.3. Computational Modeling in Crystallization

Computational modeling provides special advantage over lab-scale experiments, where it allows to predict and control crystallization, each suited for distinct purposes. At process level, Population Balance Models (PBMs) are typically employed to describe how PSD evolve through nucleation and growth under defined operating conditions. When integrated with Process Analytical Technology (PAT), essentially are set of in-line sensors, for example ATR-FTIR for solute concentration, to provide real time data without sampling [13]. Integration of PAT with PBMs improved the parameter estimation and accuracy of particle formation prediction, thereby reducing the reliance on trial-and-error experiment approach [13].

Application of PBMs is essential for prediction of particle-size outcomes based on process parameters in pharmaceutical crystallization [12]. They showed how controlling cooling profile, agitation and seeding strategies determines the balance of nucleation and growth, therefore, the final PSD. PBMs serves as a mathematical tool that essentially links critical process parameters (CPPs) such as impeller speed or cooling rate to critical quality attributes (CQAs) including crystal width distribution and final crystal size. This made PBMs an essential element of the rational design of the crystallization process.

Recent molecular dynamic studies have applied enhanced sampling methods to map free-energy barriers and critical nuclei in solution crystallization, which is not observable at the reactor scale [14]. These simulations uncovered possible alternative routes for crystallization, from prenucleation clusters to a two-step nucleation process, that moves beyond the CNT. Although not yet feasible for process-scale applications, it provides a possible future outlook for improved PBM in industrial crystallization.

5. Conclusion

Cooling crystallization is a fundamental technique in pharmaceutical API manufacturing, yet its industrial optimization is commonly designed to favor the growth of large, uniform crystals with narrow PSD, which in turn simplifies the downstream processing steps. From a CNT perspective, nanocrystal production requires high supersaturation to reduce the critical nucleus size and shift kinetics toward nucleation-dominated regimes. However, this is often avoided in practice, as bulk cooling crystallization generates broad PSDs, crystal defects, and reproducibility issues.

Analysis of key process levers, including cooling rates, seeding, and agitation, helps to understand these limitations. Where controlled cooling suppresses primary nucleation in favor of growth, yielding larger crystals. Seeding narrows the PSD and enhances reproducibility, although near metastable limit operations can be tuned to favor secondary nucleation. Agitation promotes mixing, yet increasing the agitation rate may result in increasing fines due to collision events. Though undesirable in bulk API production, it can be exploited in nanocrystal strategies. While these levers provide strong kinetic control over the cooling crystallization process, this process is fundamentally limited in nanocrystal production.

Alternative hybrid techniques can be employed to resolve this mismatch. Antisolvent with cooling crystallization, for example, creates rapid, localized supersaturation bursts, enabling a controlled operation window of nucleation bursts without destabilizing the system. Computational tools, such as a combination of PAT and PBMs, further extend the utility of cooling crystallization. Where PAT would give processing information to PBMs in time, allowing for predictive analysis of PSD. Whereas molecular simulations clarify the nonclassical nucleation pathway, with a quantified nucleation barrier.

In summary, cooling crystallization alone is poorly suited for reproducible nanocrystal production, but it provides a strong theoretical basis for understanding hybrid techniques and modeling. The future of nanocrystal API production will likely rely on integrating hybrid techniques, alongside dynamic simulation to meet the pharmaceutical performance requirements.

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