### Reactive Crystallization: A Review

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Reactive Crystallization: A Review

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Abstract

Reactive crystallization is not new, but there has been recent growth in its use as a means of improving performance and sustainability of industrial processes. This review examines phenomena and processes in which reaction and crystallization are coupled in the production of a desired chemical species. Coverage includes fundamental phenomena, such as solubility, supersaturation, crystal nucleation and growth, and chemical kinetics. Systems examined are divided into two groups, those best described as undergoing ionic reactions (including neutralizations), which have near instantaneous rates and result in the formation of ionic bonds, and those undergoing covalent reactions in which the key step occurs at measurable rates and results in the formation of covalent bonds, Discussion of the latter category also includes the impact of catalysis. Examples of a variety of reactions and applications are enumerated, and special attention is given to the utility of reactive crystallization in chiral resolution. Integration of reactive crystallization into process design, including both batch and continuous operations, and the development and efficacy of modeling, monitoring and control are reviewed. Finally, a perspective addressing needs to advance the usefulness and applications of reactive crystallization is included.

Introduction

Crystallization is used in the production of a wide variety and quantity of products and intermediates, perhaps more than any other separation technique. In different applications,
crystallization can separate, concentrate, or purify a specific species or it may be part of diagnostic or analytical procedures. In most applications the most important function of crystallization is to generate a product in a specific solid form. Many medicines, synthetic materials, food ingredients, and specialty and commodity chemicals require crystallization during the transformation from raw materials to product. The process by which chemical species are crystallized often impacts their properties, such as purity, morphology, mean size, and size distribution. Such properties can affect therapeutic capabilities and dissolution profiles of pharmaceuticals, the efficacy of agricultural chemicals, and a variety of material properties.

While the use of crystallization is older than the chemical industry, there are aspects of the process that remain poorly understood. Furthermore, the diversity of processes utilizing crystallization and the chemical and physical variations of species crystallized has led to many different methodologies for conducting this operation. The present review focuses on reactive crystallization from solution: that is, those processes in which a chemical reaction produces a specific crystallizable species in solution and, thereby, generates a driving force for the formation of a crystalline product. Clearly, the chemical reaction must produce sufficient amounts of the crystallizing species to exceed solubility. While reactive crystallization may fulfill the same functions of crystallization cited earlier, there also are two other important roles that can be played by reactive crystallization: (1) If a reaction is controlled by equilibrium, removal of the reaction product from solution by crystallization pulls the reaction towards the product as dictated by Le Chatelier’s principle. (2) Suppose the desired product is an intermediate in a larger reaction network and the yield of that product is reduced by its subsequent reaction. Then the yield can be increased by operating under conditions causing crystallization of the product. As solids, species are stabilized and less likely to undergo subsequent solution-phase reactions.

Besides being generated by reaction, the driving force for crystallization can be created or enhanced by solvent removal (for example, by evaporation or transport through a membrane), adjustment of pH, change in temperature, or addition of a nonsolvent. The presence of multiple species in solution complicates the system but is frequently encountered in industrial processes. In a reactive crystallization process, there are necessarily multiple species present—that is, the reactants and products of the reaction—that may impact the solubility of each species, the pH and temperature of the solution, and the kinetics of the reactions and the crystallization.
As the chemical industry continues to strive towards ambitious goals of process intensification, efficiency enhancements and sustainability, reactive crystallization can be a helpful tool in achieving such goals. Thermal separations dominate the chemical industry and have a huge energy cost; on the other hand, reactive crystallizations can be operated without expenditure of the thermal energy that is required for cooling crystallization, evaporative crystallization, or many other separation processes.¹ The combination of reaction and crystallization can also reduce production time and hold-up of intermediates, thereby reducing the number of operations and eliminating the need to transfer materials between vessels. Use of the same solvent for reaction and crystallization can additionally reduce waste production and the need for wastewater treatment, and it may even eliminate the need to use additional solvents.

Reactive crystallization encompasses several fundamental phenomena. Reactant mixing is limited by convection and diffusion, as are the reaction products as they are transported through the bulk liquid to the surface of a growing crystal. New crystals are formed by either primary or secondary nucleation, thereby generating fresh surfaces for growth. The rates at which these kinetic phenomena occur are influenced by mixing, catalyst design, and other engineering decisions, and they determine the size distribution, yield, and other aspects of crystal quality. Moreover, such fundamental considerations work their way into the design, implementation, and control of processes in which reactive crystallization plays the key role.

The aims of this review are to (1) describe fundamental thermodynamic and kinetic phenomena important in reactive crystallization, (2) examine reaction types and provide tabulations of references for specific reaction systems, (3) examine the design and control of systems using reactive crystallization, and (4) identify areas for future research. Only crystallization from solution is considered and there is an emphasis on processes involving high-value products such as pharmaceuticals and specialty chemicals. A condensed summary of fundamentals of crystallization and reactive processes is followed by a compilation, with commentary, of recent studies of processes utilizing reactive crystallization. Processes are categorized based on reaction type and relative rates of reaction and crystallization. Finally, the future of reactive crystallization processes is discussed, including what needs to be accomplished for more widespread adoption of this intensified process.

Fundamentals
**Solubility.** Solid-liquid equilibrium thermodynamics determine solute solubility: that is, the maximum mole fraction (or other measure) of the solute in a solution at a specific set of conditions, including temperature, pressure, pH, and solution composition. The governing requirement for solid-liquid equilibrium is that the chemical potential of each component distributed between the two phases is the same in the solid and liquid phases; that is \( \mu_i^s = \mu_i^l \).

Proceeding from this fundamental expression, a thermodynamic framework for cases involving complex liquid mixtures, multi-component solids, and polymorphs can be developed.\(^2\) Thermodynamics also may determine the state of the solid, which may be anhydrous, a hydrate or solvate, a salt, or one of a family of polymorphs.\(^*\) When the system involves a chemical reaction, the thermodynamics must include interactions of reactants, products, and by-products, all of which greatly complicate the system behavior and make it difficult to formulate working expressions for solute solubility.

In reactive crystallization, system conditions usually are selected so that reactants and byproducts\(^‡\) remain in solution, and the driving force for crystallization of the reaction product is created by its synthesis. This was the goal outlined by Encarnacion-Gomez et al.\(^3\) and McDonald et al.\(^4\) who compared the effect of pH value on solubilities of a product, ampicillin, to those of the reactant, 6-aminopenicillanic acid, and byproduct, phenylglycine, to guide selection of the pH value at which to run the reaction and crystallization. The approach was extended to include systems in which primary products included amoxicillin and cephalexin and corresponding reactants and byproducts.\(^5\)

In many instances, empirical relationships, which often are based on simplifying assumptions regarding the fundamental thermodynamics, are used to relate solubility as a function of temperature and composition. Such approaches require experimental measurements of solubilities, and the resulting correlations provide a means of interpreting and interpolating the

---

\(^*\) Hydrates and solvates are sometimes referred to as pseudopolymorphs, but strictly speaking they are distinct chemical entities. Polymorphs, on the other hand, are all the same species, but have different packing structures.

\(^‡\) Reactants are chemical species that are consumed by a reaction; byproducts are produced by a reaction but are not the desired species. Products are generated by a chemical reaction, but the term may also be used to indicate the output from the overall process.
data. As an illustration, consider the findings of Hu et al. on the solubility of an important intermediate compound, methyl D-(-)-4-hydroxy-phenyl glycinate, C$_9$H$_{11}$NO$_3$, in the production of certain β-lactam antibiotics. Over a pH range of 1-13 they showed that the solubility was lowest at the isoelectric point,* which is discussed below. Solubilities increased with solvent polarity, except when water was the solvent, and it was asserted that water was an outlier because of the dominance of the hydrophobic groups in the solute molecule. Temperature had the most significant effect on solubility in both pure and mixed solvents, which was correlated by the Apelblat equation

$$\ln x_1 = A + \frac{B}{T} + C \ln T$$

(1)

where $x_1$ is the mole fraction of solute 1 and $A$, $B$ and $C$ are constants fit to data for each solvent or solvent mixture ($T$ in Kelvin).

The rationale for the Apelblat equation was developed by Cuevas-Valenzuela et al. and it has since been cited in over 400 publications, with each describing its use for a variety of solutes and solvents. In some instances, the third term in the equation can be omitted to obtain the classic van't Hoff relationship

$$\ln x_1 = A' + \frac{B'}{T}$$

(2)

where $A'$ and $B'$ are fitted parameters, and $B'$ is often referred to as the apparent heat of solution ($T$ in Kelvin).

* The isoelectric point (pK$_I$) is the pH at which the species carries no net charge. For a molecule with two proton-labile moieties, like many amino acids, the isoelectric point commonly is taken to be the average of the acid dissociation constant (pK$_A$) of each moiety.
Solubilities of amphoteric species, such as amino acids, are lowest at their isoelectric point. At this condition, the solid in equilibrium with the solution is a neutral zwitterion. Addition of acid or base to move the pH value away from the isoelectric point initially causes modest increases in solubility, but then much greater increases occur as pH moves further from the isoelectric point. The distribution of acidic, neutral, and basic forms of serine are shown in Figure 1 and illustrate the general behavior of amino acids.\(^8\)

The composition of the solid in equilibrium with a coexisting solution of an amphoteric species can vary with pH. Over the pH range that includes the isoelectric point (see Figure 1), the coexisting crystal is the zwitterion (neutral species), but as pH moves towards the lower pK\(_{A1}\) the solid species may change from the zwitterion to an acid salt (for example, leucine hydrochloride when HCl is used to reduce pH). Kempkes and van Enckevort\(^9\) presented \textit{in situ} micrographs showing both glutamic acid hydrochloride and glutamic acid crystals coexisting in a 1:1 solution of glutamic acid and HCl in water. Alternatively, addition of a base to a solution near the isoelectric point moves pH towards the higher pK\(_{A2}\) and the coexisting solid formed may be a basic salt: for example, sodium leucinate (or leucine sodium) when sodium hydroxide is added to a leucine solution near its upper pK\(_A\). At pH extremes, solubilities of acid or basic salts may be lower than those of the zwitterion and used to enhance recovery of the species of interest. For example, Sano\(^10\) describes the early production of L-glutamic acid hydrochloride by contacting vegetable proteins with HCl, and then processing the recovered acid salt to become monosodium glutamate.
Tseng et al.\textsuperscript{11} measured solubilities of five amino acids over a pH range from 2 to 10 and used an NRTL model\textsuperscript{*} to describe activity coefficients of the non-ideal solutions. Figueiredo et al.\textsuperscript{12} developed a methodology for estimating activity coefficients using UNIFAC methods and the Debye-Hückel equation that they tested successfully against literature data. Additional data on the effect of pH on the aqueous solubility of a number of amino acids can be found in various sources.\textsuperscript{13-16}

The presence of co-solutes and/or impurities can impact the solubility of a species of interest. McDonald et al.\textsuperscript{17} showed that during crystallization of cephalexin, the reactants used to make cephalexin inhibited complete consumption of supersaturation relative to the pure cephalexin system, by effectively increasing the drug solubility. Hu et al.\textsuperscript{6} showed that increasing concentrations of ammonium chloride or D-4-hydroxyphenylglycine methyl ester hydrochloride increased the solubility of methyl D-(−)-4-hydroxy-phenyl glycinate. This is particularly important because the ester is a reactant in the synthesis of the glycinate product. Co-solutes can also decrease solubility; Isakov et al.\textsuperscript{22} showed that a compound with a valine-to-isoleucine ratio of 2:1 was formed from solutions containing the two amino acids and the compound formed a third separate solid phase with lower solubility that contaminated the pure-component solid phases. The presence of electrolytes in solutions of amphoteric species such as amino acids also affect the species’ solubility. For example, the addition of sodium chloride to neutral solutions decreased glycine solubility at low sodium chloride concentrations, but then increased solubility as concentration was increased.\textsuperscript{23} The researchers hypothesized that such behavior is based on ions of the electrolyte shielding the hydrophobic characteristics of the amino acid. Steendam et al.\textsuperscript{24} examined how the difference in solubilities of two structurally similar impurities in solutions of paracetamol (acetaminophen) impacted the properties of paracetamol crystallized from those solutions. Further work with these species demonstrated that despite their significant impact on paracetamol crystal properties, the impurities had little influence on paracetamol solubility at the prescribed impurity concentrations.\textsuperscript{25}

\textsuperscript{*} The NRTL model (non-random two-liquid model) is an expression used to correlate activity coefficients of species in solution with the composition (expressed as mole fractions) of the solution. Activity coefficients are used to account for solution nonidealities, which usually represent deviations from Raoult’s law. UNIQUAC and UNIFAC are group-contribution techniques for predicting activity coefficients.
Solubility is a function of solvent composition. For example, Granberg and Rasmuson measured solubilities of paracetamol in 26 solvents and determined ideal solubilities and activity coefficients in the saturated solutions. Also, Jiang and Ni considered how compositions of water-acetic acid mixtures influenced paracetamol solubilities and crystal morphology. Solvent effects on the solubilities of amino acids also have been investigated; the effects of ethanol on aqueous solubilities of twenty amino acids were determined and found to be related to the side chain on the amino acid.

The relationship of solubility to the form of a co-existing solid at equilibrium has been observed in the work of Zhang et al. who found that hydrogen-bonding ability was a key factor in determining solubility and polymorph formation of clopidogrel hydrogen sulfate (CHS). They used two different experimental procedures to determine solubilities of CHS Forms I and II in five alcohols, two ketones and two acetates. The van’t Hoff relationship was used to correlate solubilities in the nine solvents for the two different polymorphic forms. Solubilities in ethyl acetate of Forms I and II, along with that of an amorphous form, were also determined by Lu et al. Additionally, temperature may determine which of two polymorphs is more soluble in a given solvent, with the one having lower solubility being more stable. In enantiotropic systems the form that has the lowest solubility changes with temperature. While there do not appear to be any studies on reactive crystallization of enantiotropic species, several well-known enantiotropes can be crystallized by reactive crystallization (e.g. p-aminobenzoic acid).

Supersaturation. The difference between a system at a given state and at equilibrium represents a driving force for change, which in crystallization is referred to as supersaturation. The formal definition of supersaturation is the difference in chemical potential of a solute at the existing conditions ($\mu_i$) and at equilibrium ($\mu_i^*$): that is, $\mu_i - \mu_i^* = RT \ln \left( \frac{a_i}{a_i^*} \right)$, where $a_i$ and $a_i^*$ are activities of solute $i$ in the solution at the existing state and at equilibrium, $T$ is absolute temperature and $R$ is the gas constant. Activity can be expressed as the product of an activity coefficient ($\gamma_i$), mole fraction* and reference-state fugacity; choosing the same reference state for the existing and saturated solutions (for example, pure supercooled liquid at the system conditions) gives

* Activities based on other expressions of composition such as concentration (mol/L) can be used.
\[ \Delta \mu_j = RT \ln \frac{\gamma_j x_j}{\gamma_j^* x_j^*} \]  

Unless system conditions produce a significant difference between \( \gamma_j \) and \( \gamma_j^* \), this equation reduces to the simple dimensionless expression

\[ \frac{\Delta \mu_j}{RT} = \ln \frac{x_j}{x_j^*} \]  

If the ratio of mole fractions is less than about 1.2, there is less than 10% error in substituting \( \left[ \left( x_j/x_j^* \right) - 1 \right] \) for \( \ln \left( x_j/x_j^* \right) \). The dimensionless relative supersaturation \( \sigma_i \) then becomes

\[ \sigma_i = \frac{x_j - x_j^*}{x_j^*} = S_i - 1 \]

where \( S_i \) is the ratio of mole fractions and is referred to as the supersaturation ratio. Expressions for systems involving hydrates, partially dissociated electrolytes and mixtures of electrolytes have been developed by Sohnel and Mullin.33

Mass balances and other operations in crystallization are often more convenient when compositions are expressed in terms of ratios of mass of solute \( (w_i) \) per unit mass of solvent \( (w_s) \):

\[ X_i = w_i/w_s \]  
The relative supersaturation and supersaturation ratio can be expressed as

\[ \sigma_i = \frac{X_i - X_i^*}{X_i^*} = S_i - 1 \]

provided \( \sum (w_i/M_i) \ll 1 \) where \( w_i \) is the mass of solute \( i \) in solution and \( M_i \) is the molecular weight of \( i \); \( w_s \) and \( M_s \) are, respectively, the mass of solvent and the solvent molecular weight.

Another important way of expressing supersaturation is in terms of concentrations, \( c_i \) (mol/L):

\[ \sigma_i = \frac{c_i - c_i^*}{c_i^*} \]

which is valid if the solution molar densities at system conditions and at saturation are approximately the same. Mullin34 provides an example illustrating a violation of this assumption with mixtures of sucrose, where differences between system conditions and saturation are substantial.
It may be useful at this point to contrast how supersaturation is generated in reactive crystallization with how it is developed in other settings: here a chemical reaction creates the desired solute. If the system is isothermal and solvent has constant composition and is not being removed, $\sigma$ can be created only as a species is formed by a chemical reaction. If the system is operating as a batch unit, with reactants added at the start of the process, the concentration of the product, $c_i$, would typically increase from an initial value of zero. Upon exceeding the solubility, $c_i^*$, the supersaturation ratio, $S_i$, becomes greater than one, and crystallization can begin to occur and proceed as long as the reaction maintains supersaturation in the system ($S_i > 1$).

Reactive crystallization necessarily occurs in the presence of multiple components, in addition to the product. Accordingly, while the product solubility in pure solvent may provide a first approximation to behavior in the reaction solution, such an assumption may be incorrect in describing complicated interactions in the system with multiple species. As described earlier, the effects of other solutes may alter (increase or decrease) product solubility, $c_i^*$, and therefore supersaturation, and if solubility is increased, there may no longer be a driving force for crystallization. As the objective is to recover pure product, the supersaturation ratio of other species formed in the reaction system must remain below their metastable limits (described in the next section). Otherwise, subsequent separation of species simultaneously crystallized will be required and detract from the efficiency of the process.

**Nucleation and Growth Kinetics.** The kinetics of crystallization are defined by nucleation and growth phenomena and play a central role in determining the characteristics of a crystalline product, such as crystal size distribution. In this section, nucleation is discussed from the perspective of mechanisms leading to formation of crystals, and growth is recognized as how crystals increase in mass (and size). Kinetics of nucleation and growth are given for several crystallization systems to give context for crystallization kinetics in reactive systems.

*Nucleation*, in the context of this manuscript, is formation of a solid crystalline phase from a liquid solution, which often sets the character of the process and is a critical factor in determining product crystal size distributions. Classical nucleation theory (CNT) is based on

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* Agglomeration and breakage, two additional kinetic phenomena that can affect crystal size distribution, are covered by Randolph and Larson, Lewis et al., Ochsenbein et al., and Salvatori and Mazzotti.
homogeneous and heterogeneous mechanisms, both of which describe formation of crystals through a process of sequentially combining constituent units to form larger and larger entities until a stable nucleus is produced.\textsuperscript{39,40} Both heterogeneous and homogeneous mechanisms are referred to as primary nucleation because existing crystals play no direct role in the nucleation mechanism. Supersaturation has a highly nonlinear relationship to primary nucleation rate as illustrated by the following equation from classical nucleation theory:\textsuperscript{36}

\[ J = AS \exp \left( -\frac{\phi 16 \pi \gamma_{sl}^3 v^2}{3(kT)^3 (\ln S)^2} \right) \]  

where \( J \) is the primary nucleation rate, \( A \) is a preexponential term, \( \gamma_{sl} \) is interfacial energy between solid and liquid, \( v \) is molecular volume, \( k \) is the Boltzmann constant, \( T \) is absolute temperature, and \( S \) is the supersaturation ratio as defined earlier. The term \( \phi \) is an empirical parameter whose value is 1 for homogeneous nucleation (heterogeneous surfaces play no role in the nucleation event) and between 0 and 1 for heterogeneous nucleation (the presence of heterogeneous surfaces lowers the energy barrier to nucleation). Paxton \textit{et al.}\textsuperscript{41} showed the effect of \( \phi \) in distinguishing between homogeneous and heterogeneous nucleation of chicken egg-white lysozyme. Because the effect of supersaturation in the exponential term is much greater than in the preexponential, the equation is often written as:\textsuperscript{34}

\[ J = A' \exp \left( -\frac{\phi 16 \pi \gamma_{sl}^3 v^2}{3(kT)^3 (\ln S)^2} \right) \]  

Alternative mechanisms for primary nucleation have been proposed, including a two-step process in which a metastable liquid phase is formed prior to formation of solid. These are reviewed in various sources but are beyond the scope of this review.\textsuperscript{36,42}

It is generally recognized that solutions can maintain supersaturation without primary nucleation taking place over modest observation timescales. Classical nucleation theory shows that formation of a stable nucleus at low supersaturations is rare, but then substantial nucleation occurs beyond a threshold referred to as a metastable limit. The region of a phase diagram between solubility and nucleation is referred to as a metastable zone; at a given solute concentration, the difference between the saturation temperature and the temperature at which nucleation occurs is known as the metastable zone width (MSZW). Figure 2 illustrates behavior...
of mixtures of DL-glutamic acid in water by showing solubility and metastable limits obtained when initially undersaturated solutions were cooled at different rates. Clearly, metastable limits are not thermodynamic quantities.

![Solubility and metastable limits for glutamic acid](image)

*Figure 2. Solubility and metastable limits for glutamic acid. Adapted from the work of Svang-Ariyaskul.*

Primary nucleation in the metastable zone is unlikely but not impossible. More than 550 references emerged from a recent search using key words nucleation and metastable zone, demonstrating the effort that has gone into measuring how the metastable limit varies according to the system in which it is measured. The width of the metastable zone has been measured to be as wide as 55 °C for citric acid (σ ≈ 1.4) with a cooling rate of 0.05 K/min. Kashchiev et al. and Kashchiev and van Rosmalen provide a formula for estimating the width of metastable zones. In cooling crystallization, the MSZW increases with increased cooling rate (see Figure 2). Ma et al. found the same result for reactant addition rate; they showed that in the reactive crystallization of lithium carbonate, increasing the addition rate led to a larger MSZW. Generally, the MSZW increases as the rate of supersaturation generation increases. Boukerche et al. found that adding heterogenous solids reduced metastable zone widths and facilitated primary nucleation of different polymorphs. Sparging of inert gases was found to shrink the MSZW for several systems and lessen the induction time.
Primary nucleation rates are often linked to induction time, which is defined as the elapsed time between a solution becoming supersaturated and observation of nuclei formation. An estimate of this quantity frequently assumes that the time to form a nucleus is much greater than the time required for that nucleus to grow to observable size. The stochastic nature of primary nucleation contributes to variability in induction-time measurements, which have been correlated to probability distribution functions.\textsuperscript{51-55} Interestingly, most, if not all, of the studies observing stochastic outcomes are in small volumes (frequently 1 mL). Noting this, other researchers have pointed out that as system volume increases, the observed stochasticity in both induction time and MSZW is diminished.\textsuperscript{56, 57} Kadam \textit{et al.}\textsuperscript{56} described experiments on paracetamol nucleated from clear 1-mL and 1-L solutions. In the former, MSZWs in many experiments ranged from 7.2 °C to 33.8 °C. Variations in the 1-L experiments were low, having MSZWs between 7.0 °C and 7.5 °C: in other words, near the lower end of the range for 1-mL measurements. Evolving from such observations, Kadam \textit{et al.}\textsuperscript{56} proposed what they called the Single Nucleus Mechanism, which postulates that at a given supersaturation a single nucleus forms and ultimately leads to secondary nucleation. Kadam \textit{et al.}\textsuperscript{52} extended this analysis by suggesting that MSZW is a volume-dependent stochastic property in which the stochastic nature of primary nucleation is dominant in systems of small volume; however, increasing system volume enhances the probability of primary nucleation, making it deterministic at sufficiently large volumes.

Uncertainties associated with primary nucleation lead to the common practice of seeding, which is intentionally adding pre-existing crystals of the desired species into the system expected to produce product crystals. This shift in the controlling phenomenon from primary to secondary nucleation is especially useful in industrial settings where reproducibility of outcomes is a paramount consideration. Moreover, after startup, continuous crystallizers maintain a slurry of crystals which serves to repopulate itself through secondary nucleation. By this mechanism, a metastable polymorph may be produced continuously. Kollges and Vetter predicted by modeling and demonstrated with metastable β-L-glutamic acid that by tuning the residence time of the reactor to the secondary nucleation kinetics (or adjusting the secondary nucleation kinetics by milling) a population of the metastable form could be sustained indefinitely.\textsuperscript{58, 59} Similar results were found by Agnew \textit{et al.}\textsuperscript{60} for Form II of paracetamol.
Any mechanism involving existing crystals in the formation of new crystals is referred to as secondary nucleation. In the late 1960s and early 1970s, considerable research identified this form of nucleation as an important aspect of crystal formation, especially in industrial settings. This early work was summarized in a foundational review by Garside and Davey and a later review provided by Agrawal and Paterson. Among several mechanisms of secondary nucleation, contacts or collisions of existing crystals with other crystals, crystallizer internals, circulation pumps and impellers, which is known as contact nucleation or collision breeding, is considered most important. Contact nucleation is characterized by a low-order dependence on supersaturation (compared to primary nucleation) and absence of a metastable region. The latter factor means that substantial secondary nucleation can occur at low supersaturation. Although secondary nucleation is often considered to be a form of attrition, it need not produce macroscopic damage to parent crystals (those from which secondary nuclei are produced).

Secondary nucleation kinetics have been examined extensively for stirred-tank crystallizers, with some of the early proposed correlations provided by Grootscholten et al., Ploss and Mersmann, and others. Lewis et al. summarize such relationships in two related power-law equations that link nucleation rate to variables such as impeller speed, power input, and mass of crystals per unit volume:

\[
B_{sec} = k N G^i N^k M^j T
\]

\[
B_{sec} = k' S^a P^b M^j T
\]

where \(B_{sec}\) is the secondary nucleation rate [number/volume·time], \(G\) is crystal growth rate, \(N\) is rotational speed, \(M_T\) is a measure of the crystal concentration in the stirred-tank system (usually mass of crystals/volume of either slurry or solvent), \(S\) is supersaturation ratio, \(P\) is specific power input, and the other quantities are fitted parameters. Such correlations overlook some important process variables, such as type of impeller and existence of baffles or a draft tube and are likely to be most useful in scaling up between similar crystallizer geometries.

The roles of primary and secondary nucleation in a batch operation depend on whether seeding is used. Beginning with a clear solution (unseeded) means primary nucleation is an

* In this context, attrition is taken to mean removal of small parts of a crystal by abrasion, collision, or friction.
essential step in crystal formation. Even so, it is likely that crystals resulting from primary
nucleation grow and then serve as stimuli for secondary nucleation, analogous to the Single
Nucleus Mechanism.\textsuperscript{52} Li et al.\textsuperscript{73} used experiments in which paracetamol was crystallized from
clear solutions in ethanol to evaluate models, and then used these models to demonstrate that
only a small fraction of the final crystals per batch originated from primary nucleation. The
modeling results indicate that the foremost role of primary nucleation is to produce a small
number of crystals, which then serve to stimulate secondary nucleation. The overall rate of
crystallization in seeded batch and continuous operations is generally dominated by secondary
nucleation.

\textit{Crystal growth} rates depend on how they are measured, and they may be expressed in
terms of those measurements. For example, both the linear advance rate of a specific crystal face
and the rate of change in a characteristic dimension (which, again, can be determined in a variety
of ways) are measures of growth kinetics. The rates of change in the mass of a crystal or a
population of crystals also provide growth rates. These seemingly different measures are related
through crystal shape and density:

\[
\frac{dm_{\text{crys}}}{dt} = 3\rho \frac{k_v}{k_a} a_{\text{crys}} G
\]

where \(m_{\text{crys}}\) and \(a_{\text{crys}}\) are the mass and interfacial area of a crystal, \(\rho\) is its density, \(k_a\) and \(k_v\) are
area and volume shape factors (quantities that relate crystal surface area and volume to a selected
characteristic dimension), and \(G\) is rate of change in the characteristic crystal dimension.

At least two resistances in series must be overcome for growth to occur: (1) transport (by
diffusion or convection) of a solute species though solution to the crystal face and (2)
incorporation of solute into a crystal lattice. The rates at which the two processes occur must be
equal at steady state, and the growth rate may be expressed in terms of transport coefficients and
driving forces associated with each:

\[
G = k_d (c - c_{\text{int}})
\]

\[
G = k_r (c_{\text{int}} - c)^r
\]

where \(r, k_d,\) and \(k_r\) are fitted parameters, \(c\) is solute concentration and \(c_{\text{int}}\) is solute concentration
at the crystal-solution interface. Should the dominant resistance to growth lie in surface
incorporation, \( c \rightarrow c_{\text{int}} \) and \( G = k_r (c - c^*) \); alternatively, if the dominant resistance is transport to the crystal surface, \( c_{\text{int}} \rightarrow c^* \) and \( G = k_d (c - c^*) \).

Concepts of how molecules or ions incorporate into a growth face generally are based on either birth-and-spread or screw-dislocation theories. Mechanistic descriptions of these approaches, along with primary sources, are provided in several references.\(^{34,36}\) An empirical power-law expression can be used to relate growth kinetics to supersaturation by fitting growth-rate data:

\[
G = k_G A_G \sigma^g
\]  

(14)

where \( k_G \) is a rate coefficient and \( A_G \) is the surface area of the crystals. The exponent \( g \) is a fitted parameter with typical values between 1 and 2. This range of values for \( g \) encompasses those resulting from the cited theories, but, as demonstrated by Soto and Rasmuson,\(^{74}\) distinguishing among the birth-and-spread and spiral growth theories is difficult; the empirical power-law is often sufficient.

Empirical expressions used to correlate nucleation and growth kinetics should be developed in systems similar to those for which the expressions are to be used. Reactive crystallizations present challenges in that regard because of the inherent presence of species other than a primary crystalline product; in other words, reactants, byproducts, and other species can impact crystallization kinetics of product crystals. The effect can result from changing either the solubility (as discussed previously) or nucleation and/or growth kinetics. Illustrations of the effect on growth kinetics are provided by Capellades et al.\(^{75}\) who found that impurities from upstream operations impacted the growth rate of the antibiotic ciprofloxacin in a continuous crystallizer, while having little effect on nucleation kinetics. McDonald et al.\(^{17}\) found that during the reactive crystallization of cephalixin (which is from an antibiotic family that is different from ciprofloxacin) the presence of reactants inhibited crystal growth in a mechanism dependent on both the reactant concentration and cephalixin supersaturation. Neither of these two studies found an effect of co-solutes on nucleation kinetics. Kubota\(^{76}\) presents a broad review of the impact of co-solute species on crystal growth.

**Chemical Reactions.** Two broad categories of reactions are considered in this review: ionic reactions and covalent reactions. Both will be covered in sections that provide specific examples of such systems.
Ionic reactions are defined by bonds formed from electrostatic attractions between oppositely charged ions; for example, a positive sodium ion and negative chloride ion in sodium chloride (NaCl). Ionic reactions tend to be very fast; formation of the bond requires only displacement of solvent molecules from the solvation shell and the interaction is governed principally by coulombic forces. Computational work on aqueous NaCl crystallization suggests that the rate limiting step in this reaction between Na\(^+\) and Cl\(^-\) is the removal of water from the chloride attachment site on the NaCl crystals.\(^{77}\) One caveat to these results is the low supersaturations studied. The ability of water to transport protons at a rate faster than expected by diffusion suggests that in neutralizations (see below) the solvent may not pose the same barrier as in inorganic ionic systems like the NaCl system.\(^{78}\)

Neutralizations are a subset of ionic reactions that involve the addition or removal of a proton from a proton-labile functional group such as an acid, amine, or alcohol. Neutralization reaction rates are often considered instantaneous in the reactive crystallization literature.\(^{3, 79-81}\) The rates are governed by the collision frequency of the reactants; every collision between reactants results in the product being formed, regardless of collision energy or orientation. Therefore, these reactions are often treated as mixing-limited or diffusion-limited. Many studies investigate means of mixing reactants to ensure a uniform reaction,\(^{81-85}\) with a minireview by Teychene et al.\(^{86}\) The equilibrium composition of these reactions is highly pH-dependent in aqueous systems; in organic solvents equilibrium composition depends on the species as well as the proton capacity of the solvent.

Covalent reactions involve forming bonds requiring the sharing of electrons between atoms. These reactions range from very slow to fast and often require a catalyst; both the flow timescale of the reactor and diffusion in and out of the catalyst (if a heterogeneous catalyst is used) may impact the effective reaction rates and overall conversion. While many covalent reactions require a catalyst to proceed at appreciable rates, the presence of the catalyst does not change the reaction equilibrium, which may favor reactants or products, depending on the specific system. Many covalent reactions involve networks or series of reactions, which further complicate reaction equilibria since the products of one reaction are reactants in another. Covalent reactions may also be most amenable to reactive crystallization, as they may benefit from shifts in equilibrium, enhancements in rate and selectivity, isolation of intermediates, and many of the other motivators for implementing a reactive crystallization process.
The rate of reaction is described by an equation that expresses the rate of reactant consumption as a function of reactant concentration and reaction conditions (such as temperature, pressure, etc.). The function can be derived empirically, from insight into the reaction mechanism, or through first-principle approaches in computational chemistry. For the simple reaction \( A \rightarrow B \), the elementary rate equation could be \( r = -\frac{dc_A}{dt} = k_1c_A^n \) where the rate constant \( k_1 \) is typically a function of reactor conditions (e.g. temperature), \( c_A \) is the concentration of \( A \) (mol/L), and the exponent \( n \) is the reaction order. If the reverse reaction \( B \rightarrow A \) also occurs then the overall rate equation for \( A \) (assuming a first order reaction so that \( n = 1 \)) would be \( r = k_1c_A - k_{-1}c_B \) where the subscript -1 indicates the reverse of the forward reaction.\(^87\)

Reaction equilibrium occurs when the rates of the forward and reverse reactions are equal and therefore the overall rate is zero; that is, \( \frac{dc_A}{dt} = 0 \).\(^*\) Such conditions are reached when sufficient product has accumulated for the forward (desired) and reverse reactions to have equal rates. The ratio of the forward and reverse rate constants, \( k_1 \) and \( k_{-1} \), is the equilibrium constant, \( K_{eq} = c_B/c_A \). Equilibrium for a reaction system involving multiple reactions (for example, \( A \rightleftharpoons B \rightleftharpoons C \)) means that the concentrations of all species are constant and the reaction equilibrium constant for the overall system is the product of the individual equilibrium constants: \( K_{overall} = K_1K_2 \). If the concentration of a compound, such as species \( B \) in the above system, is elevated above its solubility, then crystallization removes the species from solution and pulls the reaction further towards its production. Overall equilibrium of the system is reached when there is no driving force for reactions, crystal nucleation, growth, or dissolution (i.e. the chemical potential of each species is the same in all phases, and temperature and pressure are uniform).

In reactive crystallization, elementary kinetic expressions may be unknown, in which case empirical relations can be derived. Take for example the reactive crystallization of a crop-protection agent, \( Z \), considered by Bhamidi et al.\(^88\) The agent is synthesized in the reaction \( 2A + M \rightarrow Z \) with \( Z \) crystallizing from the water/methanol reaction solvent. It was empirically determined that consumption of \( M \) followed the rate equation \( -\frac{dc_M}{dt} = k_1c_Ac_M \) where \( k_1 \) is the rate constant, and \( c_A \) and \( c_M \) are the concentrations of \( A \) and \( M \) respectively. An Arrhenius

\(^*\) This situation is analogous to solid-liquid equilibrium where the rates of crystallization and dissolution are equal.
relationship accounted for the dependence of \( k_1 \) on temperature, \( k_1 = k_0 \exp(-E_A/RT) \) where \( k_0 \) is the frequency factor, \( E_A \) is the activation energy, \( R \) is the universal gas constant, and \( T \) is the temperature. They were able to determine the heat of reaction as well as the activation energy and frequency factor using calorimetry. The relatively simple expression used here was sufficient for an economic analysis of the homogeneous uncatalyzed reaction to produce \( Z \).

Complicated expressions are often required to describe complex reactions, especially those requiring catalysts. Uncatalyzed reactions may only have one or two local energy minima along their reaction pathway; catalyzed reactions, on the other hand, often have a more complicated energy pathway with several transition states and energy minima corresponding to different interactions between the catalyst and reacting species. Each of these steps has the potential to be a rate-limiting transition state, leading to complex rate equations.

For example, enzymes, which represent an important class of catalysts, were used in a reactive crystallization leading to deracemization of amino acids.\(^{89}\) The authors of that study confirmed that in the reactive crystallization the enzyme D-amino acid oxidase followed Michaelis-Menten kinetics:

\[
r = c_{enz} \frac{k_{cat} c_A}{K_M + c_A}
\]

where \( c_{enz} \) is the concentration of the enzyme, \( c_A \) is the concentration of the reactant, \( k_{cat} \) is the catalytic rate constant, and \( K_M \) is the Michaelis constant. When the reactant concentration is large (\( c_A \gg K_M \)), as is often the case, the reaction rate varies only with the enzyme concentration, rendering catalyst concentration an important design variable.\(^{87}\) When \( c_A \ll K_M \) the rate is sensitive only to the reactant concentration.

Catalyzed reactions involving two reactants require rate equations of even greater complexity; at different concentration regimes the order of the reaction may appear to change as the rate-limiting step shifts from one state to another. An example is palladium-catalyzed hydrogenolysis, involving hydrogen and a second reactant, which is found in several reactive crystallization systems.\(^{90-93}\) Yap et al.\(^{94}\) found that these reactions follow the mechanism

\[
A + S \rightleftharpoons AS \\
H_2 + 2 S \rightleftharpoons 2 HS \\
AS + HS \rightarrow B + 2 S
\]
where A is the species undergoing hydrogenolysis, B is the product, and S denotes a catalytic surface adsorption site; AS and HS indicate adsorbed reactant and hydrogen, respectively. They formulated the rate equation using Langmuir-Hinshelwood kinetics as

$$r = k c_S \frac{K_A c_A \left( K_{H_2} c_{H_2} \right)^{1/2}}{\left[ 1 + K_A c_A + (K_{H_2} c_{H_2})^{1/2} \right]^2}$$  \hspace{1cm} (17)$$

where \( c_A \) and \( c_{H_2} \) represent concentrations of reactants A and H\(_2\), \( c_S \) is the surface concentration of active sites S, and \( K_A \), \( K_{H_2} \), and \( k \) represent the surface adsorption equilibrium constants for the first reaction, the second reaction, and the rate constant of the third reaction, respectively. The number of reactants is not limited to one (as in the above enzyme example) or two (as in the above palladium example) reactants; in general, increasing the number of species increases the complexity of the overall rate equation unless simplifying assumptions can be made.

Catalysts may be homogeneous (dissolved in solution) or they may be heterogeneous; in the latter case, the active material often is bound to a porous, inert support. When a catalyst is a solid, for example platinum supported on ceramic or an enzyme immobilized on a polymer, both the rate of reactant consumption at the catalyst surface and the rate of reactant replenishment by diffusion or convection (from the bulk fluid to the surface) may affect the overall reaction kinetics. Solid catalysts are evaluated by their effectiveness factor \( \eta \), which is defined as the ratio of the observed reaction rate \( r_{obs} \) to the reaction rate with rapid mass transfer \( r \). An effectiveness factor close to unity indicates good utilization of the active catalytic material (i.e. the palladium or enzyme on the solid support). An effectiveness factor \( \ll 1 \) indicates inefficient use of the catalytic material; a re-engineering of the catalyst may increase the effectiveness factor, possibly by changing the size or morphology of the catalyst surface, the catalyst loading, the pore size of the inert support, or other aspects of the catalyst. A similar concept for crystal growth involving resistances in series was described in the previous section, although usefulness of the effectiveness factor for crystal growth is limited.

**Combined Crystallization and Reaction Kinetics.** Taken together, the kinetics of nucleation, growth, and reaction, along with process configuration, determine the quality of the crystal product. For illustrative purposes, four different kinetic scenarios are examined for the first-order reversible reactive crystallization \( A ⇌ B \rightarrow B_{(s)} \) (with \( K_{eq} = 1 \)) taking place in a batch, isothermal, unseeded system: scenario (1) slow reaction and slow crystallization kinetics (1×),
(2) fast reaction and fast crystallization kinetics ($5 \times$), (3) slow reaction and fast crystallization kinetics, and (4) fast reaction and slow crystallization kinetics. For each of the four cases, three different relative rates of nucleation and growth were examined, where the primary and secondary nucleation rates ($A'$ and $k_N$ from Equations 9 and 10, respectively) were increased or decreased by a factor of five. The growth rate was varied to match the concentration profile while compensating for the change in nucleation rate, as is observed experimentally in many well-mixed crystallizers. Details of the simulation are available in the supplementary material.

For each case, the concentrations of reactant A and product B$_{(S)}$ over time and endpoint normalized population densities (a representation of the more general crystal size distribution) are shown in Figure 3. Experimental observations of the concentration profile and end-point population density can be used to fit nucleation and growth kinetics. As can be seen, depending on the relative rates of reaction, nucleation, and growth, a batch reactive crystallization can yield a variety of population densities, some of which may not meet product specifications. Bimodal or skewed population densities may be particularly problematic.

Figure 3. Quadrant plot showing solution concentrations (normalized by the saturation concentration) of reactants (dashed blue curves) and products (solid red curves) during the first order reaction $A \leftrightarrow B \rightarrow B_{(S)}$ for baseline reaction and crystallization kinetics (gray, lower left), fast reaction baseline crystallization kinetics (pink, lower right), fast crystallization baseline reaction kinetics (orange, upper left) and fast reaction and crystallization kinetics (white, upper right). The insets depict the population density calculated at baseline nucleation rate (gray), fast nucleation (yellow), and slow nucleation (green); for the different nucleation rates the growth rate was varied
The concentrations in Figure 3 have been normalized by the solubility of the product, rendering the solid curve the product supersaturation. As expected, with slower crystallization kinetics a larger supersaturation accumulates before crystal growth can match the rate of the reaction. Crystallization is helping to pull the reaction towards the product, as illustrated by the faster consumption of reactant in cases with faster crystallization kinetics. Sustained higher supersaturations lead to large amounts of primary nucleation and bimodal crystal population densities. If the product B were consumed by a second reaction the sustained higher supersaturation would be expected to further decrease yield and productivity. When the rates of reaction and crystallization are scaled together, comparable population density functions are obtained, as can be seen in the similarity between the bottom left and top right insets in Figure 3.

In generalizing Figure 3 one realizes that in reactive crystallization systems the rate of crystallization will always lag the reaction, a consequence of the sequential nature of the process. Enhancing the rate of crystallization (and the benefits of reactive crystallization) can be accomplished by increasing the rate of the reaction, as can be seen comparing the bottom two panes of Figure 3; the faster reacting system spends less time at an elevated supersaturation and reaches a higher peak supersaturation. Alternatively, the kinetics of crystallization can be sped up by techniques such as milling, which is discussed in a later section, to improve overall yield and size distribution. A detailed understanding of the process kinetics is indispensable for producing material with the desired properties in a process with the desired performance.

**Types of Reactions**

**Crystallization and Ionic Reactions.** Ionic compounds utilized in applications ranging from pharmaceutical additives to polymer fillers can be produced via reactive crystallization. Processes involving reactive crystallization can be used for removing contaminants, such as heavy metals, from water, or for separating a product, such as lithium carbonate, from solution. Ionic reactions include synthesis of inorganic compounds, formation of salts, and neutralization of organic ions.

Reactive crystallization of ionic compounds is based on the reaction between an anion and a cation in solution. The solutions are typically aqueous, but other solvents, such as supercritical CO₂, have been used. The solubility of the target compound is often substantially
lower than that of precursors supplying the reacting ions. For sparingly soluble ionic species, solubility is usefully defined in terms of a solubility product. For example, for the species $A_aC_c$ having an anion $A$ with a negative charge $c^-$ and a cation $C$ with a positive charge $a^+$, the solubility product $K_{sp}$ is given by

$$K_{sp} = [A_c^-][C_a^+]$$

(18)

where the bracketed terms represent the concentrations of ions at equilibrium in mol/L. The relative supersaturation $\sigma$ for the compound $A_aC_c$ may be defined as:

$$\sigma = \frac{[A_c^-][C_a^+] - K_{sp}}{K_{sp}}$$

(19)

$[A^-]$ and $[C^+]$ are the anion and cation concentrations at system conditions in the same units used for $K_{sp}$. The time course of ion concentrations during a process can be measured using an ion-selective electrode, a conductivity sensor, or a spectrophotometric probe, such as a UV-vis probe, an IR probe, or a Raman probe. In systems with high ionic strength, Equations 18 and 19 should be modified by replacing each concentration with an activity, which is defined as the product of a concentration and an activity coefficient. In systems at low concentration, ions do not interact with each other and the activity coefficient can be assumed to be unity, otherwise, the activity coefficient for each species can be estimated using the Debye-Hückel theory. Note that in some cases (for example, the production of calcium phosphate), the compounds may have several dissociation states, depending on pH value, and the solubility product should be corrected to take that feature into account.

A common theme in many ionic reactive crystallization studies is that the reaction between the anion and cation is considered instantaneous. The dynamic behavior of the system is governed by crystal nucleation, growth, and mixing. Fast reaction kinetics contrast with those undergoing covalent reactions such as enzymatic synthesis and crystallization of ampicillin, where the reaction step limits the overall timescale of the process. Rapid reaction kinetics, in addition to the formation of a practically insoluble compound, may lead to very high levels of supersaturation. In such cases, the mixing strategy, especially in fed-batch systems, can have a significant impact on process attributes such as crystal size distribution and shape. Mixing will be discussed further in the process design sections.
Examples of inorganic compounds produced by reactive crystallization are hydroxides such as nickel\(^{85}\) and aluminum hydroxide,\(^{115}\) carbonates such as lithium\(^{98, 106}\) and calcium carbonate,\(^{116}\) phosphates such as calcium phosphate,\(^{105}\) and sulfates such as barium sulfate.\(^{79}\)

Table 1 summarizes some of the representative inorganic ionic reactive crystallization studies and precursor materials used in each. These systems have been examined from different viewpoints, including developing kinetic models for crystal nucleation and growth,\(^{98}\) prediction and control of particle size distribution,\(^{108}\) the effect of process parameters such as stirring rate on product size and morphology,\(^{116, 117}\) and the effect of different additives on polymorph formation.\(^{118}\)

Gas-liquid reactive crystallizations result from contacting liquid and gas phases containing reactants whose combination produces a crystalline product. Many of these systems involve synthesis of carbonates through direct injection of CO\(_2\) gas into the crystallizer.\(^{117, 119, 120}\)

The gaseous CO\(_2\) gets absorbed by the liquid phase to form the carbonate ion:\(^{120}\)

$$\text{CO}_2(g) + \text{H}_2\text{O} \rightarrow \text{CO}_3^{2-} + 2\text{H}^+ \tag{20}$$

The carbonate ions then react with cations to produce solid species. Several groups have studied the effect of different gas-injection variables such as bubble size and CO\(_2\) mole fraction on the structural and chemical properties of the crystal product. For instance, Matsumoto et al.\(^{117}\) controlled the polymorphic form of calcium carbonate crystals by manipulating the CO\(_2\)-to-N\(_2\) ratio of the inlet gas. Varma et al.\(^{120}\) used the same method with different dispersion agents, including citrate ions and polyacrylic acid, for producing calcium carbonate nanocrystals. These systems can be used both for the recovery of metals from solutions and potential removal of CO\(_2\) from industrial gas streams.\(^{120, 121}\)

A similar approach was proposed and illustrated for production of hydroxides by injecting ammonia gas into the crystallization solution to produce hydroxide ions:\(^{101}\)

$$\text{NH}_3(g) + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^- \tag{21}$$

In these gas-liquid-solid cases, the absorption of gaseous reactant into the liquid phase can affect the supersaturation and kinetics of the process. Attempts have been made to model this transport limitation, such as proposing a double-film theory-based mass-transfer model.\(^{122-124}\)

Table 1 highlights some of the studies of reactive crystallization in the types of inorganic systems discussed above. The studies include continuous and batch processes with various
reactor configurations. In inorganic systems the focus tends to be crystallization-centric, as seen from the last column in the table. The listed works either give insight into a specific crystallization phenomenon, such as growth mechanism, size control, or polymorph control, or are case studies on process designs for recovery of a certain species, such as nickel from wastewater or CO₂ from flue gas. In the coming sections it will be shown that many desirable features of reactive crystallization, such as equilibrium modification and intermediate isolation, are more common in organic systems.

Table 1. A list of representative studies on the reactive crystallization of ionic compounds with the precursors used and the focus of the work. MgDS₂ stands for dodecyl sulfate, FB for fluidized bed, and CSD for crystal size distribution.

<table>
<thead>
<tr>
<th>Product</th>
<th>Reference No.</th>
<th>Reactant 1</th>
<th>Reactant 2</th>
<th>Focus</th>
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<tbody>
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<td>CO₂</td>
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<td>LiCl</td>
<td>CO₂</td>
<td>Growth mech./product characterization</td>
</tr>
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<td>LiCl</td>
<td>Na₂CO₃</td>
<td>Effect of additives on shape/size</td>
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<tr>
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<td>Crystal polymorph control</td>
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</table>

Examples of organic ionic products from reactive crystallizations include calcium¹⁴¹ and magnesium¹⁴² carboxylates, amines with carboxylic acid anions,⁸⁰ and amphoteric molecules
such as amino acids. Together these reactions are termed neutralizations because they remove acids or bases from solution. They are highly pH-dependent as many species only possess the required charge in a specific pH range. Some neutralizations are fermentation-based where the primary reactant is glucose, although many other nutrients are needed by the fermenting microbes. The motivation for each of the cited works on organic ionic reactions is different, and ranges from demonstration of continuous reactive crystallization in a chemical plant to yield enhancement by product sequestration to crystal size optimization. Applications of organic ionic reactive crystallizations include pharmaceutical development, carbon capture, and production of chemicals from renewable platforms.

Specially tailored amines are used to remove carboxylates or other anions selectively from solution. Custelcean et al. engineered a \( m \)-benzene-(bis-iminoguanidine) (\( m \)-BBIG) anion for crystallization of an amino-carbonate salt for direct air capture and sequestration of carbon dioxide. A much different application was described by Sturm et al. who used diphenylamine to crystallize the pharmaceutical compound cefdinir from a solution of impurities. In addition to carboxylates, specialty amines can be used in reactively crystallizing other highly soluble anions. Custelcean et al. synthesized urea functionalized amines to remove sulfate from a nuclear-waste simulant and examined the competition between accelerated reaction kinetics and increased solubility as the system temperature is increased. In these cases, engineered amines were used to remove specific anions by reactive crystallization.

Amines can also be removed from solution through reactive crystallization. However, unlike the cases for carboxylic acids, simple ammonium salts tend to have high solubility, which means complex anions are needed to form salts with low solubility. Aakeroy et al. screened 105 potential reactive crystallizations of amines with carboxylates and found 30 combinations in which crystal products resulted, although the kinetics of these reactions and the possible role of solvent evaporation are unclear. Quon et al. developed a continuous approach to crystallize the amine drug aliskiren as the hemifumarate salt. Cole et al. produced the amine drug prexasertib as the lactate salt. While many amines are crystallizable as carboxylate salts, the applications are mostly limited to specialty and pharmaceutical chemicals produced in low volumes. General guidance for producing solid amines takes advantage of the reduced solubility of the free amine compared to their salts, reacting the amine salt with a hydroxide to crystallize the free amine.
Reactive crystallization may be used to control the pH in a fermenter, as in the synthesis of carboxylic acids such as citric, lactic, gluconic, and itaconic acids. Synthesis of the acids decreases the pH in the fermenter, and at high concentration can halt the fermentation. Addition of a neutralizing base such as one of the calcium compounds Ca(OH)$_2$, CaO, or CaCO$_3$ stabilizes pH and causes many carboxylic acids to crystallize as calcium salts, which may further benefit the fermentation by sequestering inhibitory products. Magnesium compounds are also used, but to a lesser extent. Reactive crystallization with a calcium neutralizing agent is not always the most economical approach to separating carboxylic acids.

Figure 4 illustrates several alternative operating procedures for reactive crystallization in the production of carboxylic acids. Line AB' represents neutralization with a calcium compound that produces a saturated solution at B' while Line AB represents neutralization with a soluble base such as sodium hydroxide. The need for neutralization can be avoided by engineering acid-tolerant strains of the fermenting microorganism, in which case the operating line of the fermentations may be represented by Line AC.

Changes in solubility with pH often are exploited to isolate carboxylic acids. For example, after microbial production by fermentation, cinnamic acid, furan dicarboxylic acid, and fumaric acid is recovered by lowering the pH of the fermentation broth. Paths of
acidification are illustrated in Figure 4, which represent a case in which either seed crystals are added to initiate nucleation (curve BCD) or primary nucleation at the metastable limit is followed (curve BC'D). Acidification after fermentation and continuous neutralization with Ca^{2+} are two competing methods to isolate the product acid from a fermentation broth. The same process is used to isolate amino acids, although amino acids with multiple acid and base moieties have more complex solubility curves than shown for the monoprotic acid in Figure 4.\textsuperscript{161}

The inverse of acidification, deprotonation of an amine by a strong base, is also feasible. However, there are fewer studies of these systems as solid amines are typically limited to specialty chemicals and pharmaceuticals. For example, Diab et al.\textsuperscript{162} optimized the continuous production of nevirapine with a final reactive crystallization step between sodium hydroxide and nevirapine hydrochloride.

Acidification of an organic acid by a strong mineral acid is at the boundary between ionic and covalent reactions; the product itself is covalent (e.g. RCOOH), but the reactants are ionic (e.g. RCOO\textsuperscript{-} + H\textsuperscript{+}), and the reaction kinetics are fast (~10\textsuperscript{10} mol/sec), which is why acidification is discussed in this section. The organic acid, after receiving a proton from the dissociated strong acid, crystallizes from solution, as exemplified by the chemical equation

$$6\text{-APA}^- + \text{HCl} \rightarrow 6\text{-APAH}_{(s)} + \text{Cl}^- \quad (22)$$

Typically, though not always, the neutral form of organic acids (or bases) tends to be less soluble than the negatively (or positively) charged deprotonated (or protonated) form in aqueous solution. Ferreira et al.\textsuperscript{163} exploit this feature to produce the beta-lactam antibiotic precursor 6-aminopenicillanic acid (6-APA). 6-APA is produced enzymatically from penicillin G at neutral pH where it exists primarily in a soluble, dissociated state. After complete conversion of penicillin G to 6-APA the solution is acidified to the isoelectric point, pH 3, and the zwitterionic 6-APA crystallizes.

McDonald et al.\textsuperscript{17} used the extremely fast kinetics of acid/base reactions to study crystallization kinetics in the reactive crystallization of cephalexin. Cephalexin, which can be produced by a slow enzymatic reactive crystallization,\textsuperscript{164} was instead crystallized by reacting HCl with a solution of cephalexin sodium. The reaction is mass-transfer controlled and, with sufficient agitation, can be considered instantaneous. By adding the reactants of the enzymatic system to the cephalexin sodium solution, cephalexin nucleation and growth could be studied in
a manner representative of the enzymatic process without needing to deconvolute the enzyme reaction kinetics and crystallization kinetics.

Table 2 lists reactive crystallizations involving organic acids and bases and their salts. The diversity of listed compounds is much greater than was the case for inorganic reactive crystallizations. The cited studies are also more wide ranging as these species tend not to be the model compounds used to study phenomena but are industrial products or intermediates with economic motivators for an intensified process.

<table>
<thead>
<tr>
<th>Product</th>
<th>Reference No.</th>
<th>Reactant 1</th>
<th>Reactant 2</th>
<th>Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic Salts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aliskiren hemifumarate</td>
<td>80</td>
<td>Aliskiren free base</td>
<td>Fumaric acid</td>
<td>Optimization of purity and yield</td>
</tr>
<tr>
<td>Aliskiren hemifumarate</td>
<td>144</td>
<td>Aliskiren free base</td>
<td>Fumaric acid</td>
<td>Control of crystallization in an integrated continuous plant</td>
</tr>
<tr>
<td>Amino acid bicarbonates</td>
<td>143</td>
<td>K and Na salts of amino acids</td>
<td>CO₂</td>
<td>Found enhanced carbon capture using precipitating CO₂ absorbing solvents</td>
</tr>
<tr>
<td>m-BBIG carbonate</td>
<td>147</td>
<td>m-BBIG (see text for abbr.)</td>
<td>CO₂</td>
<td>Improved ligand for reversible crystallization for CO₂ direct air capture</td>
</tr>
<tr>
<td>Sodium cefuroxime</td>
<td>146, 165</td>
<td>Cefuroxime</td>
<td>Sodium acetate</td>
<td>Control of mixing and particle size distribution, stability of product</td>
</tr>
<tr>
<td>Ca citrate</td>
<td>166</td>
<td>Citric acid</td>
<td>CaO</td>
<td>Large amount of gypsum byproduct, from <em>A. niger</em> fermentation</td>
</tr>
<tr>
<td>Ca gluconate</td>
<td>167</td>
<td>Glucose</td>
<td>Ca(OH)₂, CaCO₃</td>
<td>Crystallization during fermentation in <em>A. niger</em> inhibits oxygen transfer</td>
</tr>
<tr>
<td>Mg 6-hydroxynicotinate</td>
<td>168</td>
<td>Nicotinic acid</td>
<td>MgO</td>
<td>Hydroxylated by <em>A. xylosoxidans</em>. Improved yield</td>
</tr>
<tr>
<td>Ca lactate</td>
<td>145</td>
<td>Glucose</td>
<td>Ca(OH)₂</td>
<td><em>B. coagulans</em> fermentation. 75% yield increase, 1.7x productivity increase</td>
</tr>
<tr>
<td>Mg lactate</td>
<td>142</td>
<td>Glucose</td>
<td>MgO</td>
<td>Reduction in water use by 40% and nutrient use by 43%</td>
</tr>
<tr>
<td>Ca malate</td>
<td>169</td>
<td>Ca fumarate</td>
<td></td>
<td>Used overexpressed fumarase, better kinetics with solubilized Ca fumarate</td>
</tr>
<tr>
<td>Ca malonate</td>
<td>141</td>
<td>Glucose</td>
<td>Ca(OH)₂</td>
<td>Fermentation with <em>P. kudriavzevii</em></td>
</tr>
<tr>
<td>1-PEA DPPA salt</td>
<td>170</td>
<td>iPA-DPPA</td>
<td>Acetophenone</td>
<td>Conversion increase from 19% to 91% by shifting equilibrium (see text for abbreviations)</td>
</tr>
<tr>
<td>Pyridinium salts</td>
<td>151</td>
<td>2-aminopyridine derivative</td>
<td>Carboxylic acids</td>
<td>Screening for new salts and co-crystals, 105 pyridine/carboxylate pairs tested</td>
</tr>
<tr>
<td>Ca succinate</td>
<td>171</td>
<td>Glucose</td>
<td>Ca(OH)₂</td>
<td>Review of several fermentation technologies</td>
</tr>
<tr>
<td>NH₄ succinate</td>
<td>155</td>
<td>Glucose</td>
<td>Ammonia</td>
<td>Future directions discussed, enhanced regeneration (succinate back to succinic acid) with ammonium salt.</td>
</tr>
<tr>
<td>TREN-tris-urea sulfate</td>
<td>149</td>
<td>1,1',1''- (nitrilotris(ethane-2,1-diyl))tris(3-(pyridin-3-yl)urea</td>
<td>Na₂SO₄</td>
<td>Sulfate recovery from nuclear waste by crystallization with engineered ligands, kinetic and equilibrium study</td>
</tr>
<tr>
<td><strong>Acids/Bases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-amino-penicillanic acid</td>
<td>163</td>
<td>6-APA NH₄ salt</td>
<td>HCl</td>
<td>Growth and solubility in presence of precursor and byproduct</td>
</tr>
</tbody>
</table>
Crystallization and Covalent Reactions. Covalent reactions are inherently more complex than ionic reactions; the bonding moieties tend to be bulky with a variety of characteristics (polarity, hydrophobicity, size, etc.) playing a role in the nature and strength of bonds formed.

This section addresses that complexity by dividing covalent systems into three broad categories: non-catalytic, catalytic, and biocatalytic reactions. Lastly, reactive crystallization for chiral resolution, an application with enormous industrial importance and unique operating considerations, is discussed.

Dividing covalent reactive crystallization according to the use and nature of catalysts assists in comparing the different process conditions each reaction type requires. Uncatalyzed reactive crystallization is accomplished by controlling only the reactant concentrations and crystallizer conditions (e.g. solvent composition, temperature, etc.). However, catalyzed
processes can be adjusted with variation of catalyst properties and loading. Reactive crystallization utilizing traditional metal catalysts may have wider operating ranges than uncatalyzed processes as the catalyst helps decouple reaction rates from important crystallization conditions, such as temperature and solvent composition. Biocatalytic processes are constrained by limited biocatalyst stability, but reactive crystallization is often applied to biocatalytic processes as a means of overcoming other catalyst deficiencies, for example poor selectivity. Chiral resolution by reactive crystallization may be accomplished with any of the listed catalytic strategies provided the reaction, which racemizes the enantiomers, is much faster than the crystallization and prevents nucleation of the undesired form. Chiral resolution is given its own section because of this unusual (in the context of the other examples) operating requirement and industrial importance. Reactive crystallization enables chiral resolution by diastereomeric resolution, preferential crystallization, enantiomeric enrichment, and attrition enhanced deracemization.

Uncatalyzed covalent reactive crystallization. The literature has relatively few examples of covalent reactive crystallizations that do not use a catalyst. However, one prominent class involves synthesis of amides by coupling amines with acid chlorides. The amines and acid chlorides often have higher solubility than the resulting amides, making these products good candidates for reactive crystallization. Liu et al. used a fed-batch system as the rate of reactant addition provided adequate control over the rate of reaction and rate of generation of supersaturation. As is typical of most crystallization processes, decreased supersaturation suppressed nucleation and increased mean crystal size. Covalent reactions typically cannot be considered instantaneous, and their rate is a strong function of temperature. In the system studied by Liu et al., raising the temperature was shown to have a stronger effect on increasing product solubility compared to the impact on reaction rate; the outcome was an overall lower supersaturation at higher process temperatures and, concomitantly, larger mean crystal size. While this result is important to note, the competition between increasing reaction rate and solubility with temperature is system-specific and results from a single system cannot be used to predict the outcome for other chemistries. As another example of uncatalyzed covalent reactive crystallization, Jiang and Ni studied the synthesis of paracetamol from 4-aminophenol and acetic anhydride. The same authors investigated several different types of reactors, including batch and continuous
oscillating-baffle reactors and concluded that combining reaction and crystallization improved yield by limiting the extent of further paracetamol reactions. It was also found that the growth mechanism for paracetamol was different in an aqueous solvent from that in a predominately acetic acid solvent. Crystal shape also depended on the solvent composition, in qualitative agreement with the observed change in growth kinetics.

Reactive crystallization can provide a framework to understand biological assembly, supporting a means for chemical selection and evolution. For example, the assembly of peptide-like polymers into paracrystalline assemblies is driven by uncatalyzed polycondensation reactions. Thioesters of racemic amino acids undergo polymerization and then beta-sheet assembly, providing a selection for isotactic peptides. In a more recent study, peptide aldehyde monomers first polymerize, driving a liquid-liquid phase separation from which beta-sheet crystals nucleate and grow. The resulting peptides are highly monodisperse, supporting a secondary nucleation mechanism for templated polymerization.

It is difficult to adjust the rate of reaction independent of the rate of crystal growth in uncatalyzed reactive crystallizations; both are sensitive to temperature, composition, and concentration. While the lack of a catalyst makes the process simpler, it may complicate production of a specified size, shape, and form of crystal if the reaction rate cannot be adequately controlled.

*Inorganic catalyzed covalent reactive crystallization.* Combining catalysis with reactive crystallization results in complex but useful processes. For example, hydrogenolysis is a commonly encountered reaction that takes place on metal catalysts. Hansen et al. published a workup of a BACE (beta-site amyloid precursor protein cleaving enzyme) inhibitor, with potential as an anti-Alzheimer’s drug, involving hydrogenolysis of a precursor by hydrogen gas in an aqueous environment with a palladium-on-carbon catalyst. The API (active pharmaceutical ingredient) crystallized in the reaction environment, which made reclaiming the solid catalyst difficult. Rather than pursue a solid-solid separation, acid was added to the reaction solvent to increase the API solubility and allow the catalyst to be filtered off. After catalyst recovery the API was deprotonated by reaction with sodium hydroxide and crystallized based on the acidification mechanism described in the ionic reaction section.

Reactive crystallization to produce terephthalic acid (TPA), a precursor to the ubiquitous polymer polyethylene terephthalate, by oxidizing 1,2-dichlorobenzene can improve the impurity profile of
the resulting product.\textsuperscript{193} Species formed during the oxidation, which is catalyzed by soluble cobalt/manganese catalysts with bromine promoter, are shown in Figure 5. Incomplete oxidation leads to the formation of 4-carboxybenzaldehyde (4-CBA), which can incorporate into the terephthalic acid crystals. Rejection of 4-CBA during crystallization of terephthalic acid is paramount as 4-CBA terminates polymerization. Wang \textit{et al.}\textsuperscript{193} found that 4-CBA was incorporated to a greater extent when the TPA growth rate was faster and seed crystals were not used. They developed a process with slow feed rate and higher temperature to minimize impurity incorporation, while maintaining the same mean crystal size.

![Chemical structure](image)

\textit{Figure 5. Successive oxidation in the conversion of p-xylene (left) to TPA (right). CBA (second from right) can incorporate into TPA crystals at high concentration, requiring tuning reaction conditions to prevent buildup of CBA while maintaining slow formation of TPA for crystal size control.}

\textit{Biocatalytic covalent reactive crystallization.} In this section, biocatalytic reactions are described that crystallize products formed from specific reactants. There has already been some discussion on reactive crystallization as it pertains to fermentation, which can be considered a highly non-selective form of biocatalysis (glucose is converted into a myriad of products). In the biocatalysis community reactive crystallization is sometimes referred to as \textit{in situ} product crystallization, ISPC (a subset of \textit{in situ} product removal, ISPR). Hulsewede \textit{et al.}\textsuperscript{194} provide a minireview on ISPC; here ISPC is discussed in the general framework of reactive crystallization.

Biocatalytic processes are well-positioned to benefit from reactive crystallization. Many biocatalysts, such as whole live cells, are poisoned by high concentrations of products, which can be reduced by reactive crystallization.\textsuperscript{195} Other biocatalysts, such as resting or whole dead cells, may catalyze undesired reactions with the desired product as reactant, but utilization of reactive crystallization can insulate that product from further reaction.\textsuperscript{196} Purified enzymes, while highly specific and more resistant to poisoning, often catalyze reactions with equilibrium coefficients on the order of unity leading to low yields; reactive crystallization can shift equilibrium towards products.\textsuperscript{197}

Synthesis of beta-lactam antibiotics is a well-studied example of a biocatalytic reactive crystallization. Ferreira \textit{et al.}\textsuperscript{198} have demonstrated good recyclability of an immobilized
penicillin G acylase (PGA) for production of ampicillin in saturating conditions. The three main reactions catalyzed by PGA are shown in Figure 6 for ampicillin; they are synthesis (desired, top), reactant hydrolysis (undesired, left), and product hydrolysis (undesired, right). Using PGA entrapped in agarose gel particles afforded only slight mass transfer resistance in the catalyst particle. After some time, the solution saturated and ampicillin crystallized because the reactants, phenylglycine methyl ester (PGME) and 6-APA, are both more soluble than ampicillin on a molar basis. Once again, the crystallization made reclaiming the catalyst difficult and so the product was dissolved after filtration, leaving behind the immobilized enzyme for recycling.

Another study used a soluble version of the same enzyme to increase the selectivity towards ampicillin by >50% by sequestering the product from enzymatic hydrolysis by crystallization.4

Figure 6. PGA-catalyzed synthesis of ampicillin (desired, top), hydrolysis of phenylglycine methyl ester, PGME (undesired, left), and hydrolysis of ampicillin (undesired, right).

The value of the biocatalyst (relative to the value of the product) often dictates whether the catalyst is immobilized on a solid, which enables catalyst recovery and reuse, or is soluble, enabling product recovery by filtration. In the case of ampicillin, the product is less valuable than the enzyme, which must be recycled repeatedly for economic sustainability; since solid-solid separations are difficult, the product crystals may be re-dissolved to enable recovery of the enzyme in solid form. With the simplified recovery of a solid biocatalyst comes the challenge of catalyst engineering: choosing a particle size and material to avoid mass-transfer limitations and efficiently utilize the active material, for example PGA in Figure 6. 184 When catalyst recovery is a nonissue, and trace catalyst does not impact downstream quality, e.g. heavy metal toxicity, a reactive crystallization with a dissolved catalyst may be preferable.

Recently, a biocatalytic process with reactive crystallization to produce the HIV drug islatravir has been described. 199 The reactive crystallization involves four dissolved enzymes.
The three final reactions to islatravir are reversible, so crystallization helps shift the equilibrium towards the product, and the fourth enzyme catalyzes a reaction to consume a byproduct and further improve the yield of islatravir (see Figure 7). The high value of the product favors its recovery by filtration and use of fresh enzyme for each batch. Upstream of the reactions shown in Figure 7 product recovery by filtration is not favorable, and five immobilized enzymes are used. The starting solution for the reactive crystallization is the filtrate from the reactions catalyzed with immobilized enzyme. The relative harmlessness of enzymes (compared to heavy metals) and the value of the reaction products favor reactive crystallization processes. Furthermore, the strong specificity of enzymes enables coupling many reactions to transform soluble reactants to the desired insoluble product. A more recent study of the same system used crystallization of the byproduct (previously consumed by the fourth enzyme) as a calcium salt to drive the reaction further; the improvement in reaction yield (82% versus 76%) outweighed the difficulty of recrystallization.²⁰⁰

**Figure 7.** Enzymatic synthesis of islatravir by three enzymes in a single vessel from an alkyne precursor (also produced enzymatically). Islatravir crystallization helps drive the reaction to the right; consumption of the phosphate byproduct by (top) reaction with calcium and crystallization of calcium phosphate or (bottom) enzymatic reaction with sucrose pulls the reaction further towards the product. Adapted from Huffman et al.¹⁹⁹

Many chemistries that do not seem plausible with biocatalytic processes could be implemented with biocatalytic reactive crystallization. For example, equilibrium favors removal of CO₂ from carboxylic acids, not carboxylation by addition of CO₂. However, Ren et al.²⁰¹ overcame the unfavorable reaction equilibrium when they carboxylated phenols with a decarboxylase enzyme (which, as the name suggests, typically catalyzes removal of CO₂) by crystallizing the products as quaternary ammonium salts, improving the yield from 40% to 97%.

Table 3 lists several examples of the three types of covalent reactive crystallizations discussed, non-catalytic, catalytic, and biocatalytic. The product, reactants, and focus of the study are listed for each entry in Table 3. The focuses are varied, reflecting the wide-ranging applications of covalent reactive crystallization.
Table 3. A list of representative studies on covalent reactive crystallization processes, divided based on catalyst usage and type.

<table>
<thead>
<tr>
<th>Product</th>
<th>Reference No.</th>
<th>Reactant 1</th>
<th>Reactant 2</th>
<th>Focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncatalyzed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>189</td>
<td>4-aminophenol</td>
<td>Acetic anhydride</td>
<td>Impurity content, crystal shape, growth and nucleation kinetics</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>190</td>
<td>4-aminophenol</td>
<td>Acetic anhydride</td>
<td>Continuous oscillating baffled reactor</td>
</tr>
<tr>
<td>2,4,6-triamino-1,3,5-trinitro-benzene</td>
<td>122</td>
<td>2,4,6-trichloro-1,3,5-trinitro-benzene</td>
<td>Ammonia</td>
<td>Gas, liquid, solid phase. Effect of feed rate and temperature on particle size in bubble column reactor</td>
</tr>
<tr>
<td>Dithiocarbamate (DTC)</td>
<td>88</td>
<td>DTC-precursor</td>
<td>Formaldehyde</td>
<td>Narrow CSD, avoid oiling out and spherulites, optimize productivity</td>
</tr>
<tr>
<td>Amides</td>
<td>84</td>
<td>Acid chlorides</td>
<td>Amines</td>
<td>Plug flow reactor crystallizer, examined fouling</td>
</tr>
<tr>
<td>Inorganic catalyst</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terephthalic acid (TPA)</td>
<td>193</td>
<td>p-xylene</td>
<td>Oxygen</td>
<td>Intermediate impurity incorporation, Co/Mn catalyst, Br promoter</td>
</tr>
<tr>
<td>TPA</td>
<td>202</td>
<td>p-xylene</td>
<td>Oxygen</td>
<td>Evaporative cooling of exothermic reaction to avoid fouling</td>
</tr>
<tr>
<td>TPA</td>
<td>203</td>
<td>p-xylene</td>
<td>Oxygen</td>
<td>Two reactive crystallizers in series to eliminate intermediate impurity</td>
</tr>
<tr>
<td>BACE inhibitor</td>
<td>92</td>
<td>Isoxazidoline derivative</td>
<td>Hydrogen</td>
<td>Difficulty separating Pd/C catalyst from solid product, redissolved</td>
</tr>
<tr>
<td>Relebactam</td>
<td>204</td>
<td>Carboxybenzyl relebactam</td>
<td>Hydrogen, silylating agent</td>
<td>Pd/C, DABCO then HOAc. In situ protect/deprotect with crystallization</td>
</tr>
<tr>
<td>Akt kinase inhibitor</td>
<td>91</td>
<td>Amine precursor</td>
<td>Methyl phenylacetate</td>
<td>Cs2CO3 catalyst, impurity rejection</td>
</tr>
<tr>
<td>Hedgehog pathway inhibitor</td>
<td>93</td>
<td>Carboxybenzyl protected API</td>
<td>Hydrogen</td>
<td>Solids formed on Pd/C catalyst, form HCl salt instead</td>
</tr>
<tr>
<td>Biocatalytic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>4</td>
<td>6-amino penicillanic acid (6-APA)</td>
<td>Phenylglycine methyl ester (PGME)</td>
<td>Improved enzymatic yield with crystallization</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>173</td>
<td>6-APA</td>
<td>PGME</td>
<td>Reactive crystallization of product and byproduct, phenylglycine</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>198</td>
<td>6-APA</td>
<td>PGME</td>
<td>Taylor-Couette flow reactor to suspend slurry with low shear</td>
</tr>
<tr>
<td>Ampicillin, Amoxicillin, Cephalexin</td>
<td>205</td>
<td>6-APA, 7-amino-desacetoxy-cephalosporanic acid (7-ADCA)</td>
<td>Phenylglycine amide, 4-hydroxy-phenylglycine amide</td>
<td>Used supersaturated reactants for three different pen G acylase catalyzed reactions</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>206</td>
<td>6-APA</td>
<td>Phenylglycine amide</td>
<td>Fed batch, solid reactants dissolving, solids purity versus conversion</td>
</tr>
<tr>
<td>Amoxicillin, Cephalexin</td>
<td>5, 113</td>
<td>6-APA, 7-ADCA</td>
<td>PGME</td>
<td>Continuous reactive crystallization modeling, size, purity, yield</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>207</td>
<td>6-APA</td>
<td>Phenylglycine nitrile</td>
<td>Complex product with 1,5-dihydroxynaphthalene (reduced sol.)</td>
</tr>
<tr>
<td>Ilatravir</td>
<td>199</td>
<td>2-Ethynyl-glyceraldehyde-3-phosphate</td>
<td>2-F-adenine</td>
<td>Three reactions in series, crystallization pulls equilibrium right</td>
</tr>
<tr>
<td>Methyl trans-3-(4-methoxy-phenyl) glycidate</td>
<td>208</td>
<td>Racemate</td>
<td></td>
<td>Lipase immobilized on membrane to facilitate enzyme recovery from crystals. Deracemization</td>
</tr>
<tr>
<td>Levodione</td>
<td>154, 195</td>
<td>4-oxoisophorone</td>
<td></td>
<td>Reduction by live S. cerevisiae, crystallization reduce over-reduction</td>
</tr>
<tr>
<td>Reaction</td>
<td>Product</td>
<td>Reactionants</td>
<td>Auxiliary</td>
<td>Remarks</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>--------------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>3-cyano pyridine</td>
<td>water</td>
<td>Avoid overhydration to nicotinic acid by crystallization</td>
<td></td>
</tr>
<tr>
<td>Methionine, phenylalanine</td>
<td>Racemate</td>
<td>Ammonia borane, oxygen</td>
<td>Deracemization</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>Aspartic acid</td>
<td>Deracemization, whole cell (dead) Pseudomonas dacunhae catalyst</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3- and 2,6-dihydroxybenzoate</td>
<td>Resorcinol, catechol</td>
<td>KHCO₃</td>
<td>Use of quaternary ammonium salts to increase yield from 40% to 97%</td>
<td></td>
</tr>
<tr>
<td>Allo-threonine</td>
<td>Threonine</td>
<td>Isomerase reaction, solid reactant and product, constant liquid composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z-aspartame</td>
<td>Carboxybenzyl aspartate, phenylalanine methyl ester</td>
<td>Two enzymes tested, reaction kinetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Homo-phenylalanine</td>
<td>2-oxo-4-phenylbutyric acid</td>
<td>L-aspartate</td>
<td>Fed batch to overcome substrate inhibition</td>
<td></td>
</tr>
<tr>
<td>L-phenylglycine</td>
<td>Phenylglyoxylate, L-glutamate</td>
<td>Thermophilic enzyme, crystallization shifted equilibrium to products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptides</td>
<td>Amino acid with protected amine, unprotected amino acid</td>
<td>Improving yield, conversion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the carboxylation example by Ren et al., the product formed is an organic salt, but it is discussed in the covalent section of this review because the rate-limiting step is the formation of covalent bonds (carboxylation). The kinetics of this process are more representative of those found for other covalent and biocatalytic reactive crystallizations. The same behavior is observed in fermentation with continuous neutralization by calcium hydroxide; the rate-limiting step is not the reaction between carboxylate and neutralizing base, but rather production of the acid by the microorganism. The product, a dissociated acid, is ionic, but the rate-limiting reaction that produced the acid forms covalent bonds. Neutralization in batch at the end of the fermentation may also be practiced, in which case the ionic reaction between Ca²⁺ and the carboxylate is rate-limiting. The use of a second species, such as Ca²⁺ for carboxylates, to form a less-soluble complex with the product is a common application of reactive crystallization.

Enzymatic peptide synthesis is thermodynamically unfavorable in aqueous solutions, but reactive crystallization enables even thermodynamically-controlled reactions to achieve high yields, especially when using a second species to promote crystallization. For example, the thermolysin-catalyzed synthesis of the artificial sweetener aspartame (L-asp-L-phe-OMe) is used in a 10,000 ton/year-scale process with 96% yield in the enzymatic step. Carboxybenzyl L-aspartate (Z-asp) is coupled with equimolar L-phenylalanine methyl ester (L-phe-OMe) at neutral pH in purely aqueous solution. The anion generated at neutral pH values, Z-aspartame (Z-asp-L-phe-OMe), forms an insoluble salt with cationic D-phe-OMe, Z-asp-L-phe-OMe:D-phe-OMe. Racemic DL-phe-OMe is employed in 2:1 molar ratio with Z-aspartate such that the L-enantiomer is consumed in the reaction and the D-enantiomer promotes crystallization.
Eichhorn et al.\textsuperscript{217} demonstrated that reactive crystallization of thermolysin-catalyzed dipeptide couplings leads to high yields with a wide range of amino acids and their derivatives. In a high-solids medium (only about 10% aqueous solution), at the kilogram scale, mixing limits the reaction rate. In theoretical work on peptide synthesis, Erbeldinger et al.\textsuperscript{216} and Ulijn et al.\textsuperscript{215} showed that high yield is the consequence of reactive crystallization and that above a threshold of product solubility, yield switches from low values to values of almost 100%.

The work of Hulsewede et al.\textsuperscript{170} described a nearly 5-fold increase in the yield of 1-phenylethylamine (1-PEA), synthesized by a transaminase enzyme, by reactively crystallizing the 1-PEA. Starting with acetophenone and isopropylammonium-3,3-diphenylpropionate (iPA-DPPA), the enzyme transfers the amine group from iPA to acetophenone and the resulting 1-PEA crystallized as a salt with DPPA. The crystal product was a salt, but the rate-limiting step is the enzyme-catalyzed covalent reaction. The DPPA was present in stoichiometric quantity throughout the reaction since the iPA was fed as the DPPA salt. It was found that low reaction equilibrium (19%) could be overcome by coupling to the favorable crystallization of 1-PEA-DPPA and 92% conversion could be reached. The general process, illustrated in Figure 8, is also applicable to similar chemistries.\textsuperscript{222}

Depending on the nature of the interactions between the product and the second species, the resulting crystals may be called salts (coulombic interactions) or cocrystals.\textsuperscript{223-225} In the previous example, 1-PEA is crystallized as a salt of DPPA, but numerous examples can be found where the final solid is a cocrystal. As an illustration, the yield of cephalosporin antibiotics can
be increased by reactive crystallization with an aromatic species to isolate the antibiotic product, which is an intermediate in an enzymatic cascade. The process has been demonstrated for cefaclor (yield increased from 57% to 80%),\textsuperscript{226} cephalexin (42% to 67%),\textsuperscript{227} and cephradine (yield not evaluated).\textsuperscript{228} Recent published work on cocrystallization has focused on discovery and/or prediction of cocrystal-forming systems,\textsuperscript{229, 230} which could make reactive crystallization practical for a wider range of products if co-formers\textsuperscript{*} promoting crystallization can be identified for more products. Other applications of cocrystallization include: improved solids handling,\textsuperscript{231} solubility,\textsuperscript{232} stability,\textsuperscript{233} and pharmaceutical activity,\textsuperscript{234} Karimi-Jafari \textit{et al.} reviewed the entire topic.\textsuperscript{235}

Carbamazepine has been a favorite model compound for cocrystal research and is the only compound for which cocrystallization kinetics have been published. Gagniere \textit{et al.}\textsuperscript{236} qualitatively examined the rates of cocrystallization in the carbamazepine-nicotinamide system while Rodriguez-Hornedo \textit{et al.}\textsuperscript{237} used Raman spectroscopy to monitor the nucleation and growth of carbamazepine-nicotinamide from a slurry of the individual solids. Kudo and Takiyama\textsuperscript{238} have worked out much of the thermodynamics of reactive cocrystallization in the carbamazepine-saccharin system. Each of these studies highlights the difficulties designing reactive crystallizations with multiple small molecules, namely complex phase diagrams with at least three solids (more with chiral compounds and if cocrystals with several stoichiometries exist) and uncertain driving forces for complexation.

\textit{Chiral Resolution.} Enantiomers are prevalent in pharmaceutically active compounds, with one enantiomer typically being responsible for clinical activity and, in some cases, the mirror compound having deleterious effects. Both left and right enantiomers have the same solubility, vapor pressure, partition coefficients, etc. (in achiral solvents), making their separation by traditional methods very difficult. Many non-biological reactions are agnostic of chirality, the products are racemic mixtures that, by definition, contain equal amounts of the two enantiomers. Producing a compound with a single chirality often involves a deracemization step. Provided a racemizing reaction (a reversible reaction interconverting the two enantiomers) occurs with

\textsuperscript{*} The FDA defines a co-former (or coformer) as one of the two different molecules in the same crystal lattice, forming a co-crystal. More information can be found at \url{https://www.fda.gov/media/81824/download}
appreciable speed, the left- and right-hand enantiomers ($S$ and $R$) will exist in a 1:1 ratio in
solution, irrespective of removal of one of the enantiomers from solution by crystallization.

Conglomerate-forming systems are ones in which each of the two enantiomers form
enantiomerically pure crystals that may be produced as a physical mixture if crystallized
together. Racemate-forming systems produce crystals in a single lattice structure with a 1:1 ratio
of the two enantiomers. Conglomerate-forming systems are more amenable to resolution by
reactive crystallization, and are estimated to comprise 5-20% of all enantiomeric systems with
racemates making up the remainder.\textsuperscript{239, 240} Reviews of several techniques for enhancing
enantiomeric purity, some involving reactive crystallization, are provided by Lorenz and Seidel-
Morgenstern,\textsuperscript{241} Palmans,\textsuperscript{242} and Brands and Davies.\textsuperscript{185} In the following discussion, special
emphasis is given to a class of covalent reactive crystallizations used to recover enantiomerically
pure products.

Crystallization can result in deracemization in four ways: (1) by formation of a
diastereomeric salt with a chiral resolving agent, (2) by preferential crystallization, (3) by
selectively subjecting the undesired enantiomer to racemization with an asymmetric reaction,
thereby enriching the solution in the preferred enantiomer and facilitating its crystallization, and
(4) by attrition-enhanced deracemization of conglomerate-forming enantiomers.

Diastereomeric resolution, or crystallization with a resolving agent, sometimes referred to
as “classical resolution,” takes advantage of differences in interactions between two chiral
molecules. In this process, two enantiomers of a single species are reacted with a single
enantiomer of another compound (resolving agent) to form two diastereomers that have different
properties, including solubilities. The two diastereomers may then be separated by crystallizing
the less soluble of the two. The desired free enantiomer is then recovered by reversing the
reaction that formed the diastereomer.

An illustration of diastereomeric resolution is the production of the unnatural amino acid
$R$-($\rightarrow$)-2-phenylglycine, $R$-PG, mentioned previously in reference to certain beta-lactam
antibiotics. In the commercial process, a racemic mixture of phenylglycine ($RS$-PG) is reacted
with ($1S$)-$(+)$-camphor-10-sulfonic acid, $S$-CS, to produce two diastereomeric salts, $RS$-PG-CS
and $SS$-PG-CS. The former has a lower aqueous solubility (5.75 g/100 g at 25 °C) than the latter
($>150$ g/100g, 25 °C), which facilitates separation.\textsuperscript{243} Racemization of $RS$-PG in the presence of
one equivalent of S-CS and half an equivalent of hydrochloric acid (to keep zwitterionic PG from crystallizing) leads to greater than 90% yield of R-PG from RS-PG.

The simultaneous use of multiple resolving agents has been shown to enhance resolution, likely by inhibiting nucleation of the undesired diastereomeric salt. Brands and Davies list many systems for which diastereomeric salt resolution has proven successful. The utility of this method is demonstrated by its use in the production of large quantities of (S)-naproxen, (R)-phenylglycine, and (R)-4-hydroxyphenylglycine. Diastereomeric resolution can be used to resolve individual enantiomers of conglomerate-forming and racemate-forming compounds. Figure 9 represents the resolution of the S enantiomer of a conglomerate forming compound by addition of resolving agent $S^\dagger$. The apex represents pure solvent, while the lower left vertex is pure $S$ or $SS^\dagger$ and the lower right vertex represents pure $R$. Using process analytical technology (PAT, discussed further in the process design section) one would observe the solution composition following the solid black curve from Point A to Point B, with movement up and to the right due to crystallization and movement left due to racemization.

Figure 9. Isothermal ternary phase diagram for diastereomeric resolution. The phase equilibrium before addition of the resolving agent is symmetrical, shown by bold gray curves, while after addition the resulting diastereomer $SS^\dagger$ phase equilibrium is asymmetric, shown by dashed gray curves, enabling crystallization of only the desired enantiomer. During the process, the solution moves from Point A (undersaturated) to B upon addition of $S^\dagger$ with concurrent racemization in solution.

** Preferential crystallization utilizes the addition of seed crystals to solutions of enantiomers in the metastable zone to initiate nucleation and subsequent growth of a single enantiomer from a racemic solution. The methodology is useful for conglomerate-forming systems. Seed crystals of the desired enantiomer are added, and the supersaturation is held within...
the metastable zone. The seed crystals grow and breed new crystals of the desired chirality while
the undesired enantiomer remains in solution; primary nucleation must not occur as it would
initiate crystallization of the undesired enantiomer. Simultaneously subjecting the solution to a
racesimization reaction enhances the yield of the seeded species. Yoshioka\textsuperscript{245} summarized several
eamples of preferential crystallization of amino acids, both proteogenic and unnatural, with
racemization by catalytic salicylaldehyde. Petrusevska-Seeback \textit{et al.} give an example of
combining preferential crystallization with enzymatic racemization.\textsuperscript{246} Supersaturation may be
generated by cooling, pH adjustment or other means. Figure 10 shows the preferential
crystallization of \textit{S} by acidification reactive crystallization, where \(pH_1 > pH_2 > pH_3\) and the
solubility decreases with decreasing pH value.

\textbf{Figure 10. Ternary phase diagram for preferential crystallization of S by addition of S seed crystals.}

Supersaturation is generated by decreasing the pH from \(pH_1\) to \(pH_3\) while a racemization reaction converts excess \textit{R}
in solution to restore a 1:1 ratio of \textit{R}:\textit{S} in solution. Again, the bold black curve from Point A to Point B represents
the solution composition observed by PAT, while the blue arrows represent movement due to crystallization and the
red arrows movement due to racemization.

\textbf{Enantiomeric enrichment} followed by crystallization is especially valuable if an
enantiomerically pure product is to be recovered from a racemate-forming system. Enrichment in
the present context means that the relative proportion of the desired enantiomer in solution is
increased to an extent that crystallization of enriched pure enantiomer is feasible. Enrichment of
a specific enantiomer can be accomplished in several ways. For example, Johnson \textit{et al.}\textsuperscript{247} used
an asymmetric palladium catalyst in route to an API, producing the desired enantiomer at a
solution enantiomeric excess of 95%; crystallization was used to upgrade the enantiomeric
excess of the solid to >99%. Encarnacion-Gomez \textit{et al.}\textsuperscript{210} used a chemo-enzymatic
stereoinversion reaction system to enrich the concentration of a desired enantiomer to appropriate concentrations for crystallization of the pure enantiomer. They demonstrated the process using racemic mixtures of phenylalanine and of methionine and recovered crystals of the desired pure enantiomer from each system. Harriehausen et al. suggested several process configurations that combined enantioselective chromatography with enzymatic racemization to recover pure enantiomer with nearly complete extinction of the undesired enantiomer. Figure 11 shows enantiomeric enrichment in a racemate forming system by reacting R to S followed by crystallization in a racemate-forming system. The middle cone-shaped region identifies conditions corresponding to equilibrium between crystals of the racemate and the solution. The objective of enantiomeric enrichment is to move from the central region to the yellow region, thereby facilitating crystallization of the desired enantiomer.

Figure 11. The ternary phase diagram for enantiomeric enrichment in a racemate-forming system. The enantiomeric enriching reaction occurs under supersaturated conditions, but high conversion ensures only the desired enantiomer is crystallized. Again, the bold black curve from Point A to Point B represents the solution composition observed by PAT, while the blue arrow represents movement due to crystallization and the red arrow movement due to an enantioselective isomerization reaction.

Attrition-enhanced deracemization, sometimes referred to as “Viedma ripening,” was observed originally by Viedma and explored further by Viedma and Blackmond. The original observations were on aqueous solutions of sodium chlorate, a species that is achiral in solution but which, on crystallization, produces chiral crystals. Experiments included seeding with mixtures of R and S chiral crystals that had been subjected to attrition before being added to the system and which were subject to continuous attrition by the presence of glass beads that were added to the stirred system. After stirring for a sufficiently long period, the resulting
crystals were found to be enantiomerically pure $R$ or $S$. Later work found similar behavior for conglomerate-forming enantiomers that underwent rapid racemization reactions and, therefore, maintained the ratio of enantiomers in solution near 1:1. Examples of such systems include: an amino acid derivative N-(2-methylbenzylidene)-phenylglycine amide;\textsuperscript{253} aspartic acid, one of two conglomerate forming proteogenic amino acids;\textsuperscript{250} and an imine that is the key component in clopidogrel.\textsuperscript{254} Spix \textit{et al.}\textsuperscript{255} converted the racemate-forming amino acids alanine and phenylalanine to conglomerate-forming salts alanine 4-chlorobenzene sulfonic acid and phenylalanine 2,5-xylenesulfonic acid. After deracemization of the salts, the free amino acids were recovered by neutralizing the salts with alkali.

\textbf{Reactive Crystallization Process Design}

Development of reactive crystallization processes for use at the industrial scale is a challenging multi-objective problem. The generation of a given product by reactive crystallization involves simultaneous fundamental thermodynamic and kinetic phenomena, including reaction, mass transfer, and crystal nucleation and growth, all coupled with the engineering aspects of reactor design, configuration, control, and product recovery. While each of these has been well-studied in the literature, decisions for process design, optimization, and control are often unique to the system of interest.

Reactive crystallization processes generate supersaturation by synthesizing a product through a reaction, concomitantly increasing the concentration of the product and leading to crystallization. The rates of the reaction and crystallization steps guide strategies for reactor design and control. In cases where fixed operating conditions favor both reaction and crystallization, reactive crystallization can be performed in a single vessel. For example, many of the ionic reactions mentioned earlier produce a sparingly soluble compound in an instantaneous reaction and fall into this category. Other cases may require the steps to be temporally or spatially separated, several configurations of which are illustrated in Figure 12. Temporally separated processes include well-mixed batch or semi-batch designs in which synthesis reactions occur, followed by a shift in temperature or pH to promote subsequent crystallization of the product. Processes that are spatially separated utilize multiple vessels or different segments of a vessel—first performing the reaction to generate supersaturation and then feeding the mixture into a second vessel for crystallization. Process intensification in the form of combining both reaction and crystallization steps into a single vessel is likely to lead to savings in capital and
operating costs as the technology matures, but like many aspects of design problems, the potential benefit is system-dependent.

Figure 12. (a) Temporal separation of reaction and crystallization favoring conditions in a well-mixed batch reactor; (b) Spatial separation of reaction and crystallization favoring conditions in continuous well-mixed reactors (above) and tubular reactors (below).

Key factors in process design for reactive crystallization include the presence of multiple components (sometimes with more than one crystallizing), effects of mixing on crystallization kinetics, the necessity of maintaining mutually beneficial operating conditions for reaction and crystallization, and the need for producing crystals that can be readily separated from a reaction slurry. In instances where a heterogeneous catalyst is used to facilitate a desired reaction, separating the catalyst from product crystals represents an additional challenge. As crystal shape and size distributions are strongly influenced by supersaturation, process control is also a unique challenge due to the generation of supersaturation from a reaction rather than by temperature manipulations, evaporation, or anti-solvent addition. In the following discussion, challenges unique to reactive crystallization process design and control that focus on the aforementioned issues are discussed in more detail.

**Reactor Design.** Design and implementation of reactive crystallization systems rely heavily on reaction and crystallization kinetics. A strong distinction between the operation of a reactive crystallizer and a traditional crystallizer is that reactive crystallization conditions must
satisfy both the reaction and the crystallization. For example, an enzymatic reaction usually
requires benign temperatures (20 to 37 °C), aqueous conditions, and near-neutral pH values. If
these conditions promote crystallization of the desired product, then reaction and crystallization
may be conducted in the same vessel. If the conditions for reaction do not permit crystallization
of the product, then using multiple vessels may need to be investigated. Alternatively, the
conditions within the vessel may be varied over the time course of the reactive crystallization or
across the volume of the reactor (specifically for tubular reactors). For example, Jiang et al.\textsuperscript{190}
varied the temperature across a tubular reactor to allow for enhanced reaction kinetics in the
upstream section and improved crystallization kinetics in the downstream section.

Configurations of vessels in which reactive crystallizations are conducted usually are
similar to those of non-reactive crystallizers. The perfectly mixed continuous crystallizer, which
is often referred to as a mixed-suspension mixed-product removal (MSMPR) crystallizer,\textsuperscript{*} is an
idealized version of a continuous stirred-tank crystallizer. A similar vessel may serve as a batch
crystallizer with feed added at time zero and product removed at a designated endpoint. In a
semi-batch (or fed-batch) unit, one or more feed streams is added during the course of a run and
product removed at the designated endpoint. More details on these generic configurations, which
often have to do with mixing, are available in general references on industrial crystallization.\textsuperscript{36, 258}

Most applications of reactive crystallization described in the literature focus on batch or
semi-batch systems. While these are often relatively simple to design and operate, continuous
reactive crystallization processes offer several benefits. Advantages of continuous systems have
been outlined specifically for pharmaceuticals manufacturing by Lee et al.\textsuperscript{259} and Acevedo et
al.\textsuperscript{260} Some of these benefits include higher process capacity and productivity, more efficient use
of raw materials and energy, production of fewer intermediate compounds, and more robust
control. The design and use of various arrangements of reactive crystallization equipment for
batch, semi-batch and continuous operation are covered in the following discussion.

\* The MSMPR crystallizer is a useful model for continuous stirred-tank crystallizers. The assumptions of perfect
mixing and uniformity of residence time distributions of solvent and crystals of all sizes are approximations often
approached in actual systems.
Batch and semi-batch systems. The use of a batch reactor allows for adjustment of process parameters during the time course of the reactive crystallization, such as decreasing the temperature or adjusting the pH value towards the end of a batch so as to crystallize more product and increase the overall process yield. For example, in the enzymatic synthesis of beta-lactam antibiotics shown in Figure 6, the solution pH value typically decreases as the reaction progresses due to the formation of acidic products. The lower pH value further decreases the solubility of the product, improving product crystallization and yield.\textsuperscript{4,261}

Batch and semi-batch reactive crystallization processes are operated in well-mixed reactors that allow for implementation of on-line PATs, are robust, and are more resistant to clogging and encrustation issues than tubular reactors. Also, these well-mixed reactors are often highly modular, allowing for the inclusion of baffles, a draft tube,\textsuperscript{262} or different impeller types to enhance mixing.\textsuperscript{263} Cao \textit{et al}.\textsuperscript{85} used an airloop-lift reactor (ALR) to improve mixing for the batch reactive crystallization of Ni(OH)\textsubscript{2} over traditional impeller stirring. Another alternative to traditional impeller-stirred reactors is the Taylor-Couette reactor used by Ferreira \textit{et al}.,\textsuperscript{198} which uses a combination of inner rotating and outer stationary cylinders to gently mix the slurry of crystals and catalyst particles in the region between the cylinders (Figure 13). The design is used to protect the catalyst particles, which are susceptible to the shear stress induced by an impeller in a conventionally mixed reactor. Often, traditional impeller stirring may be adequate for a system, but for very fast reactions, the various parameters involving mixing and reactant addition rate or novel techniques of mixing may need to be investigated to achieve a desirable crystal size distribution.

In the case of fast reactions in semi-batch systems, mixing and the method and rate of reactant addition significantly impacts the product size distribution. Åslund and Rasmuson\textsuperscript{264} studied the effect of stirring rate, impeller type, reactant concentration, reactant feed rate, and feed position (e.g. surface level, bulk liquid, next to impeller feeds) on the size distribution of benzoic acid crystals formed via reactive crystallization. The rate of addition of the reactant hydrochloric acid, reactant concentration, and stirring rate significantly influenced the product size distribution. As the stirring rate was increased, the size of resulting crystals increased up to a maximum where crystal size began to decrease; the type of impeller and feed position had a lesser effect on the product size distribution, especially at higher stirring rates. Chen \textit{et al}.\textsuperscript{114} investigated mixing during reactive crystallization of barium sulfate. Three separate scales of
mixing, Kolmogorov, turbulent, and convective, were proposed in a new mixing model composed of viscous deformation and molecular diffusion in a slab element. The model was used to give accurate predictions of the effect of stirring speed, feed location, and viscosity on particle sizes. Zauner and Jones\textsuperscript{265, 266} investigated the effect of feed rate, feed concentration, feed tube diameter, impeller type and stirring rate on particle size for the reactive crystallization of calcium oxalate and calcium carbonate. Overall, the results of the aforementioned mixing studies suggest that poor mixing conditions promote a higher rate of primary nucleation, leading to a larger number of nuclei and a size distribution of smaller final crystals.

\textit{Continuous systems.} As in batch systems, the stirred tank is the most commonly-used vessel in continuous systems, but alternative geometries have also been proposed and implemented. Several examples of alternatives to the stirred-tank crystallizer are shown in Figure 13. These include Taylor-Couette, impinging jet, airlift loop, continuous flow inversion, and oscillatory baffled crystallizers. Examples (a), (b), and (c) in Figure 13 approximate MSMPRs, while (d) and (e) are used to provide near plug-flow behavior.

\textbf{Figure 13.} Summary of novel reactive crystallizers; (a) Taylor-Couette,\textsuperscript{198, 267} (b) Impinging jet mixer,\textsuperscript{165} (c) airlift loop,\textsuperscript{85} (d) continuous flow inverter – Reprinted with permission from Kurt et al.\textsuperscript{268} copyright 2017, Elsevier, (e) continuous oscillatory baffled.\textsuperscript{190}
For reactions of short timescales, often a reactor that is nearly perfectly mixed is used due to its robust operation and the ease of implementing process analytical technologies (which are discussed later and may include in situ microscopy, Raman and IR spectroscopy, and particle size analysis). Well-mixed reactors such as an MSMPR are also used when high supersaturation is not desired, such as when high supersaturation promotes the formation of an undesired polymorph or a finer crystal size distribution. For reactions in which high reactant concentrations are preferred, a single well-mixed reactor may not be the best design because it operates at outlet conditions; that is, conditions throughout the vessel correspond to those at the outlet. However, the use of a plug-flow reactor may introduce clogging concerns. To address this issue, Hu et al. utilized multiple MSMPR reactors in series to approach plug-flow behavior for a second-order reaction in which high reactant concentration was needed to achieve high conversions.

When the reaction proceeds much faster than crystallization (both nucleation and growth), techniques such as wet-milling or sonocrystallization can promote nucleation and more rapid consumption of supersaturation. Yang et al. and Acevedo et al. showed that wet milling in a continuous crystallizer increased the yield, operating as a promoter of both primary and secondary nucleation for paracetamol in two different solvents. Use of wet-milling has also been shown to enable deracemization of conglomerates by combining the principles of preferential crystallization and Viedma ripening. Sonocrystallization, or the application of ultrasound to a crystallizer, has been shown to hasten crystallization in batch. Hatakka et al. used sonocrystallization to selectively produce a single polymorph during the batch reactive crystallization of L-glutamic acid while experiments without sonication resulted in a mixture of polymorphs. The authors concluded that the polymorphic purity results from reduced supersaturation due to enhanced nucleation by sonication. Wet-milling and sonocrystallization can both lead to narrower, finer size distributions as a result of increased nucleation.

Slower reactions may require the use of multiple vessels in series to achieve higher conversions. Mo and Jensen demonstrated the use of a micro-CSTR cascade for two separate solid-forming reactions: (1) the reaction of glyoxal and cyclohexylamine to form the practically insoluble N,N'-dicyclohexylethylenediimine and (2) the sulfonylation of 2-octanol which produced the sparingly soluble side product triethylamine hydrochloride. For the first reaction, a six-unit CSTR cascade (15-minute total residence time) was needed to reach nearly 100% conversion while the second reaction only required three CSTRs to achieve 100% conversion.
Effective control of process parameters such as temperature and pH, often important variables in determining the reaction and crystallization rates, is typically achieved in well-mixed reactors with the inclusion of probes, a thermal jacket, and addition of acid or base. Process control will be discussed in more detail below.

Mixing intensity may be an issue in continuous reactive crystallization. Like batch crystallizers, if the reaction utilizes a catalyst that is susceptible to high shear, a Taylor-Couette style reactor may be operated with a feed and continuous product removal. Aggregation of mixing-induced nuclei may also be a concern, leading to a wide crystal size distribution (CSD). Liu et al. found that incorporation of an impinging jet mixer in the continuous reactive crystallization of the antibiotic sodium cefuroxime resulted in a narrower CSD and improved product stability compared to a traditional impeller-stirred reactor. Jung et al. found a similar decrease in the size distribution using a Taylor-Couette reactor for calcium hydroxide production because the more gentle mixing discouraged agglomeration.

Continuous tubular reactors such as plug flow reactors (PFRs) are frequently used in the chemical industry. Higher overall concentrations of reactants across the length of the reactor lead to higher conversions for positive-order reactions. For reactive crystallization, unlike in a well-mixed tank, temperature or pH value may be varied along the length of the reactor, allowing for the enhancement of reaction or crystallization kinetics in different sections. Jiang and Ni varied the temperature across a continuous oscillatory baffled crystallizer (COBC), a type of continuous tubular reactor designed to achieve plug flow at low fluid velocities, such that higher temperatures in the initial segments favored the reaction and lower temperatures towards the outlet improved the rate of crystallization. Kurt et al. achieved near plug-flow behavior of slurry by designing and operating a coiled flow inverter (CFI) crystallizer for the reactive crystallization of calcium carbonate. The inverting flow and helically coiled tubing enhanced mixing via formation of Dean and Taylor vortices, which increased secondary flow perpendicular to the primary flow direction. Others have physically segmented plugs of slurry by injecting inert gas spacers. All of these crystallizers enhance mixing and slurry suspension by achieving a more plug-flow-like flow profile than conventional PFRs. Increased turbulence using these novel designs permits the use of reactors with larger diameters and shorter lengths to achieve the same residence times. In general, using small-diameter tubing to achieve adequate turbulence and slurry suspension is a concern in PFRs because of clogging. Polster et al.
attempted to design a continuous reactive crystallization process for a BACE inhibitor using a PFR, but eventually settled on an MSMPR due to clogging inside the reactor tubing. Oroskar and Turian developed the following correlation to estimate the critical slurry velocity, which is defined as the velocity required to suspend particles and prevent their deposition in a tube or pipe:

\[
\frac{v_c}{\sqrt{gd(s-1)}} = 1.85C^{0.15}(1-C)^{0.36}\left(\frac{d}{D}\right)^{-0.38}\left(\frac{D\rho_l\sqrt{gd(s-1)}}{\mu}\right)^{0.90}x^{0.30}
\]

All dimensional variables are in SI units and \(v_c\) represents the critical velocity, \(d\) the equivalent spherical particle size, \(C\) the fractional slurry density, \(D\) the pipe diameter, \(\rho_l\) the liquid density, \(\mu\) the liquid viscosity, and \(s\) the ratio of the liquid-to-solid density. Here, \(x\) is a correlation factor estimated using the fraction of particles above the critical velocity generally approximated to be unity. This correlation may be used to estimate the minimum diameter of a PFR to avoid clogging keeping in mind that the minimum diameter, while providing the most turbulence, must be large enough to accommodate the largest crystals.

Another downside of using continuous tubular reactors is that the composition of the suspension cannot be easily manipulated after the inlet. Thus, controlling the pH can be difficult (especially for reactions involving acids and bases), which may lead to unintended changes in reaction or crystallization kinetics across the reactor. Controlling pH is a special challenge in working with enzymes as biocatalysts typically work in very narrow, well-regulated pH environments. As a solution, Jiang and Ni used multiple sampling ports across the length of a tubular reactor to learn more about the slurry by off-line analysis. Another issue arises when a continuous tubular reactor requires seed crystals to initiate crystallization; either the reactor must be fed seed crystals or a recycle stream must be incorporated to introduce crystals at the inlet, complicating the design and operation of the reactor. In contrast, a well-mixed reactor (for example, an MSMPR unit) does not face this issue as it operates with a suspension of crystals that participate in secondary nucleation and foster crystal breeding during continuous operation.

**Modelling, Monitoring, and Process Control.** A control scheme for reactive crystallization processes depends on the nature of the species being produced and its desired characteristics. Various factors such as temperature, pH, and the presence of additives can dictate the final crystal properties in specific processes. More generally, control of
supersaturation and seeding have the greatest effect on crystal size distribution, crystal shape, and polymorphic form. Compared to crystallization alone, supersaturation may be more difficult to control in a reactive crystallization process because it can only be manipulated indirectly via adjusting the reaction rate. Extreme examples are near-instantaneous reaction rates, typical of ionic systems, where the only manipulation of the reaction rate is through the supply of reactants. Slow-reacting systems are also difficult to control as the solution stays near saturation (S ≈ 1.0). Reactive crystallization models that couple the kinetics of the three governing phenomena of chemical reaction, nucleation, and crystal growth are constructed to narrow an operating window that will provide a desired CSD, crystal shape, and polymorph. From models, the development of process control algorithms for reactant flow rate or seed-crystal addition can be tuned to optimize robust production of the desired crystal properties.

One of the earliest and most general models for semi-batch reactive crystallization processes focused on developing reactive phase equilibrium equations and diagrams based on reaction equilibria where the products are non-soluble and instantly form a solid. While useful for identifying reaction conditions and simulating reactor performances that generate only solid products, this model focused on hypothetical examples and did not include the kinetics of nucleation and growth that determine CSD and crystal shape. Kelkar and Ng expanded this model to include nucleation and growth kinetics for MSMPR crystallizers. Their model included the steps for reactions in solution generating supersaturation, nucleation, and linear growth rate of crystals from supersaturated product in solution. Applying their model for process design, they studied the reaction rate under different reactor throughput conditions, dissolution rates, and mass transfer rates. The effect of different reaction rates, nucleation rates, and growth rates on the product weight fraction distribution was also predicted.

The previously discussed models primarily focus on using hypothetical kinetic parameters to simulate reactor performance, while only a few studies have been directed towards process optimization. Choong and Smith further expanded semi-batch reactive crystallization models by including a population balance to allow for the optimization of CSD. Assuming perfect mixing, the initial amount of reactants, feed addition time, and number of feeds were considered to be design variables for two optimization problems: (1) maximizing crystal size with a constraint of maximum coefficient of variation of the size distribution and (2) minimizing the coefficient of variation subject to a constraint of minimum average crystal size. A stochastic
optimization framework was then used to circumvent traditional pitfalls such as nonconvergence or suboptimal solutions in highly nonlinear systems.\textsuperscript{284} The model neglected secondary nucleation with the rate of nuclei generation being defined by Equation (9). Within that limitation, their model allowed for devising nonlinear control policies that could be implemented in a semi-batch reactive crystallization process.

While the studies discussed above are not exhaustive, they have developed models that present the primary features of reactive crystallization processes and strategies for their optimization. Other reactive crystallization models include more specific studies on modelling and control of enantiomer and polymorph crystallization,\textsuperscript{285} multi-objective optimization and generation of Pareto-optimal solutions for desired crystal properties,\textsuperscript{286} incorporation of seeding strategies,\textsuperscript{287} resistances in double-film mass transfer models for gas-liquid systems,\textsuperscript{121} and effects of macro- and micromixing regimes on crystal size distributions.\textsuperscript{114} System-specific models to predict control profiles for optimal mean crystal size and narrow CSD have also been developed for ampicillin,\textsuperscript{288} barium carbonate,\textsuperscript{108} aluminum hydroxide,\textsuperscript{115} and 2,4,6-triamino-1,3,5-trinitrobenzene.\textsuperscript{122} Similar models for continuous reactive crystallization have also been developed but focus primarily on instantaneous reaction kinetics\textsuperscript{287} or are specific to a given system\textsuperscript{5, 113} and examples in the literature are still quite limited in the literature.

Reactive crystallization process modeling and process evaluation would not be robust without in-line monitoring using PAT and sensors to obtain qualitative and quantitative data on the evolution of the solution and crystal phases.\textsuperscript{289} The use of PAT is important for process monitoring, which in turn allows for analyses of reaction and crystallization mechanisms and process control. A summary of PATs common to reactive crystallization processes is shown in Figure 14. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) coupled with multivariate models is often used to analyze the liquid-phase composition during the process, as it has low solid-phase sensitivity.\textsuperscript{29, 290, 291} Raman spectroscopy can be used to quantify solution or crystal composition for molecules that can undergo large polarization changes and thus exhibit strong Raman scattering. Raman measurements are especially useful in systems with co-crystallization or multiple polymorphs to distinguish different solids.\textsuperscript{180, 237, 290, 291} Focused beam reflectance measurement (FBRM) estimates the evolution of chord-length distributions of crystals (a proxy for, but fundamentally different from, the crystal size distribution) in real time.\textsuperscript{84, 98, 146, 173, 270, 292} Coupled with system-dependent algorithms, chord-length distributions
can be directly related to crystal size distributions; however, substantial work is required to transform chord-length distributions for comparison with size distributions obtained by laser diffraction or optical imaging.\textsuperscript{293, 294} Particle vision and measurement (PVM) can provide \textit{in situ} estimates of crystal size and shape by observing crystals in a crystallizer or a reactor as transformations take place. PVM has been shown to be especially useful in observing polymorph shifts,\textsuperscript{295} agglomeration,\textsuperscript{88, 296} and crystal growth under different process conditions.\textsuperscript{292} Other monitoring techniques include HPLC for offline solution composition measurements and laser diffraction particle size analyzers coupled with image analysis to collect information about particle size and shape. Crystal structure can be further characterized offline with x-ray diffraction (XRD) and x-ray powder diffraction (PXRD). For products that undergo color changes upon degradation, a stability chamber and transmittance measurements using UV to obtain color-grade data can be used to evaluate purity of the final product. These methods are performed offline after crystal isolation but they do provide metrics for confirmation and calibration of online measurements or process stability.\textsuperscript{297} PATs are often combined to give a more complete view of the reaction and crystallization phenomena.
Control of final product properties can be achieved without modelling the system of interest but requires a detailed understanding of how different process parameters affect qualities of the solution and crystal phases. Polster et al.\textsuperscript{84} developed a continuous reactive crystallization process for production of an API for clinical trials and studied experimentally how crystallizer residence time, temperature, solvent composition, and recycle configuration impacted size distribution, final blend flow function coefficients, yield, and tapped density. The control strategy then focused on feedback control of reactant flow rates and pH adjustments.

Figure 14. Common PAT used for characterizing composition and concentration of species in solution as well as crystal properties. Representative data outputs from each PAT are shown.
In any control application, model-free control is simpler and easier to implement compared to model-based optimal control, but the latter can provide superior performance if an accurate model is available. A representative example for how PAT can assist in modelling, monitoring, and optimal control during the development of a large-scale process is the reactive crystallization of glutamic acid from monosodium glutamate and sulfuric acid. A common control strategy in batch antisolvent or cooling crystallizations is to determine the optimal supersaturation trajectory for the process as a function of some manipulated variable such as temperature or anti-solvent flow rate and to design a feedback control system to maintain the optimal state using ATR-FTIR for direct measurement of solution concentrations. Direct concentration control commonly used in antisolvent or cooling crystallizations is often difficult to employ in reactive crystallizations due to strong process nonlinearities that can arise from coupled reaction, dilution, and crystallization dynamics. For instance, the desired supersaturation trajectory in the reactive crystallization of glutamic acid is dome-shaped, and conventional concentration control cannot be used at many operating points because it would require a reduction in volume to hit the desired trajectory. However, volume is constantly increasing from the startup of the system due to the addition of reactants. A variation of direct concentration control known as Just-in-Time-Learning, JITL, can be employed to build a nonlinear model-predictive control strategy based on extended predictive self-adaptive control that accounts for nonlinearities. This method is used to identify a set of empirical state-space models along the desired process trajectory. JITL has also been coupled with system models to include batch-to-batch model predictive control strategies.

Glutamic acid crystallization is further complicated by the existence of two polymorphs; the desired α form is thermodynamically metastable and eventually transforms into the undesired but stable β form. Obtaining the metastable α form requires a detailed monitoring and control process. Alatalo et al. showed that ATR-FTIR measurements coupled with thermodynamic modeling in a multivariate partial least squares model were effective in accurately measuring glutamic acid concentrations in solution over the course of the reaction. By using coupled ATR-FTIR and Raman spectroscopy measurements, Qu et al. determined that formation of the stable β polymorph was favored at high supersaturation. They speculated that this was due to the β form having a higher barrier to nucleation than the α form. The in-line monitoring techniques allowed discovery of the formation of the β polymorph at conditions of high global
supersaturation and in regions of poor mixing, especially those where feed was introduced above the liquid surface in the crystallizer. Alatalo et al.\textsuperscript{298,303} further extended this work to the design of a closed-loop feedback control algorithm based on the ATR-FTIR measured concentration of glutamic acid in solution and adjustment of the sulfuric acid feed rate with a PID controller. Such an arrangement allowed control of supersaturation and the formation of the $\alpha$ polymorph in a 50-L reactor.

**Perspectives and Future Directions for Reactive Crystallization**

Reactive crystallization provides opportunities for process intensification and product improvements, which could be favorable to product economics. In this section the necessary developments needed for reactive crystallization to advance to new applications are outlined, with emphasis on the need for more data, improved PAT, advanced crystallizer design, and better understanding of reaction and crystallization mechanisms. Brief guidance for adaptation of existing processes or creation of new processes for reactive crystallization is given. Finally, possible future enhancements centered on reactive crystallization, primarily continuous manufacturing and hybrid systems, are outlined.

**Kinetics: distinguishing reactive crystallization from other separation techniques.**

Utilizing crystallization, and therefore reactive crystallization, in industrial processes presents unique challenges with respect to product quality: namely those associated with meeting criteria on crystal size, size distribution, purity and form. Such challenges must be met while also satisfying the usual process requirements of yield and economics. Advancing reactive crystallization to where it can increase the yield of a product, while at the same time meeting quality criteria, is a great challenge. Environmental and economic sustainability add urgency that new manufacturing processes are implemented and matured.

Some specific studies of how reactive crystallization can fit into a useful process have already been published.\textsuperscript{84,113} However, relating reactive crystallization to meet final product specifications including purity, size distribution, morphology (including polymorphic form and crystal shape), and more, remains a difficult task. For example, a few authors have undertaken the task of optimizing simulated reactive crystallization processes,\textsuperscript{79,282,284} but bypassed the significant effort required to characterize the reaction kinetics and crystallization kinetics in the combined reactive crystallization environment.
Several authors have shown that the kinetics of crystal growth and nucleation, which together with system configuration determine product crystal size distribution, can vary in the presence of other species such as reactants and byproducts. Elucidating reaction and crystallization kinetics in complex environments requires large volumes of data, which can come from either high-throughput or data-rich experiments. High-throughput experimentation involves measuring reaction and crystallization (that is, nucleation and growth) rates across a large sampling of process conditions and compositions to predict complex process features such as crystal size distribution. Data-rich experimentation, on the other hand, uses a smaller number of experiments, but with collection of high-dimensional data, such as crystal size distribution, which can be used to work backwards and determine reaction and crystallization kinetics. Both approaches enable the required level of description for designing new reactive crystallization processes, and substantial progress has been made towards both ends; however, each approach also comes with its own hurdles and challenges.

Reactive crystallizations typically take place in more complex solutions than cooling, antisolvent, or evaporative crystallization; PAT tools that are effective in such complex solutions would enable more accurate modeling and precise control of reactive crystallization processes. Taking ATR-FTIR as an example, each dissolved species contributes to the overall IR spectrum, and through careful construction of calibration models the concentration of each species can be extracted from the overall spectrum. However, the presence of uncharacterized species such as impurities, intermediates, byproducts, or catalysts, can break calibration models, and the work required to characterize all species and their combination may be too laborious or even impossible (e.g. non-isolatable intermediates). Improved baselining algorithms and regression techniques may make calibration model construction more manageable, such as recent work by Maggioni et al. based on blind source separation and independent component analysis requiring only single point calibration with robustness against unknown species. The methodology was demonstrated on mixtures of simple oxyanions and did not perform as well when the anions’ spectra overlapped. For many reactive crystallizations the reactants, intermediates, and products have similar functional groups and IR spectra; continued chemometric development is required for general solution phase monitoring by spectroscopic methods. Additionally, many species may only be present at low concentration compared to the reactants and products. In the production of terephthalic acid the intermediate 4-CBA has a
maximum concentration of <10% of the total concentration of aromatics, leading to 1% 4-CBA in the final product. Using control to minimize the 4-CBA concentration is difficult as improved spectroscopic analysis would be needed to quantify the small amount of 4-CBA in such a complex solution (see Figure 5). Likewise, in the enzymatic production of beta-lactam antibiotics the undesired byproduct phenylglycine (see Figure 6) may crystallize and contaminate the antibiotic slurry. Detecting the nucleation of phenylglycine amounts to detecting a change in phenylglycine concentration of <10 mmol/L in a solution with >100 mmol/L 6-APA and PGME, both of which have IR spectra with significant overlap with phenylglycine, and has proven to be very challenging with ATR-FTIR.

Improvement in the characterization of solids will also benefit reactive crystallization process design and process control. In the case of beta-lactams the ability to distinguish between antibiotic crystals and byproduct crystals *in situ* could circumvent the challenges associated with solution-phase monitoring. FBRM has been used to indicate polymorph transformation in paracetamol and in-situ microscopy with machine learning has been demonstrated for classifying individual crystals versus agglomerates. Both FBRM and *in situ* microscopy are restricted to use in slurries of low solids concentration. Reactive crystallization would benefit considerably from the development of techniques that can identify new particle types with minimal training data and calibration effort, especially since many systems involve solid reactants dissolving, reacting, and crystallizing. A promising development for these systems, where solution concentration cannot quantify reaction conversion, are composite PAT arrays and crystallization informatics. An array of complimentary sensors combined with process expertise has been shown to enable quantification of different solids concentrations in other solution-mediated solids transformations, such as polymorph transformations and hydrate-to-anhydrate transformations; application to a reactant-to-product transformation follows naturally.

**Determining the applicability and utility of reactive crystallization.** Heuristics to identify when a reactive crystallization could enhance a process do not exist and should be created. It is often assumed that the product must be less soluble than the reactants, however fed-batch and continuous stirred-tank reactors (with independent solution and solids residence times) can enable reactive crystallization even when the product is more soluble. Use of co-formers can decrease the solubility of the product below that of the reactants, also enabling productive
reactive crystallization. A brief guide to how reactive crystallization can improve a process is presented in Table 4, below.

Table 4. A list of useful heuristics for recognizing when reactive crystallization is advantageous and/or feasible.

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Reactive crystallization almost always possible, preferable with sparingly soluble compounds, mixing control key to supersaturation control and size control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic</td>
<td>Usually possible with appropriate counter-ion. Preferable for temperature-sensitive compounds and hydrates. Ideal for isolating intermediates, overcoming reaction equilibrium. Requires modest aqueous solubility in charged state</td>
</tr>
<tr>
<td>Organic Ionizable</td>
<td>Should be evaluated on a case by case basis. Examples of successful processes include covalent reactions eliminating charged groups in aqueous solution (e.g. amide bond formation) or creating charged groups in nonpolar solvents (e.g. hydrogenolysis)</td>
</tr>
<tr>
<td>Organic Nonionic</td>
<td>Reactive crystallization ideal for improving yields and selectivity by shifting equilibrium, isolating inhibitory products, protecting reactive intermediates</td>
</tr>
<tr>
<td>Critical Quality and Process Attributes</td>
<td>Complex composition in reaction mixture can increase number of possible impurities, may change form preference from pure solution, reaction solvents may limit solvate options</td>
</tr>
<tr>
<td>Crystal Size Distribution</td>
<td>Fast reactions, i.e. neutralizations and acidifications, will create a fines-dominated CSD. Slow reactions and seeding typically give large crystals. Reaction rate modification by catalyst engineering can enable fine-tuning of CSD</td>
</tr>
</tbody>
</table>

Methods of Reactive Crystallization

| Neutralization | Reactive crystallization by neutralization ideal for intermediate process steps, e.g. isolating an intermediate as a calcium salt before dissolving salt for further processing. Limited by neutralizing agents, usually calcium, magnesium, and ammonium |
| Acidification  | Ionizable compounds typically least soluble in protonated (neutrally charged) form, ideal for removing acids/bases from complex mixtures of nonionic species |
| Covalent reaction | System specific, often catalyst dependent. Least generalizable but most opportunity for process improvement. Requires that the catalyst be in a form that can easily be separated from the crystal product |

Continuous manufacturing. The end-to-end integrated continuous manufacturing paradigm is becoming increasingly important in the pharmaceutical industry, due in large part to the potential benefits of lower costs, shorter supply chains, smaller footprints, and better quality
monitoring and controls with PAT.\textsuperscript{259,318,319} In this relatively new paradigm in pharmaceutical manufacturing, reactive crystallization processes should be designed to be adapted to the whole process, rather than considered as an independent unit operation. For example, traditional batch operations may allow operational flexibility over a range of conditions, such that the reaction time can be shortened or extended based on the extent of reaction; such alterations are not easily made in continuous reactors.

Control of raw materials to an integrated reactive crystallization is important, as: (1) raw materials often include insoluble particles requiring a clarification bypass system beforehand,\textsuperscript{320} (2) variability in raw materials (like differences in purity or water content) could result in lower yield or higher impurity profile. Batch processes have more flexibility to handle raw material variability; different control strategies for continuous reactive crystallization may be more or less amenable to mitigating the risks of variability in raw material composition, based on the frequency and extent of the irregularity.

**Novel reactive crystallization process designs.** Process intensification has been cited as one of the primary reasons for implementing a reactive crystallization process. Further intensification can be found with examples of evaporative reactive crystallization,\textsuperscript{202} cooling reactive crystallization,\textsuperscript{321} membrane-assisted reactive crystallization,\textsuperscript{322,323} liquid-liquid extraction reactive crystallization,\textsuperscript{181} and chromatography-assisted reactive crystallization.\textsuperscript{241,324} In each case multiple techniques may be applied simultaneously: for example, a reaction occurring in one liquid phase, crystallization occurring in a second liquid phase, and rapid mass transfer between phases;\textsuperscript{322} or sequentially: for example, a reactive crystallization occurring at high temperature with fast reaction kinetics followed by cooling to reduce solubility and generate supersaturation while sacrificing reaction speed.\textsuperscript{89}

Most complex chemical processes require catalysts to control reactions and produce desired products. Using stoichiometric amounts of catalysts is often undesirable from the standpoints of process economics as well as purification. Catalysts must be recovered for sustainability and product purity. Separating solid catalysts from a crystal slurry can be prohibitively difficult; several examples of reactive crystallization followed by dissolution for the purpose of catalyst recovery have already been discussed.\textsuperscript{92,198} Alternatively a soluble catalyst can be used and retained/recovered via ultrafiltration, which may work well for dissolved biocatalysts as bio-macromolecules can be separated from small molecules with
Nanofiltration, which has proven useful for removing impurities during crystallization, may be useful for recovery of non-biologic soluble catalysts. However, membranes are subject to fouling and are not compatible with all solvents, substrates, and catalysts, in which case the pursuit of solid-solid separations based on size, density, or other properties may be preferable. Examples of such solid-solid crystal-catalyst separators include hydrocyclones, elutriation, and sieves.

Further research into the fundamentals of crystallization in complex environments, i.e. with multiple solutes, solvents, and surfaces, will enable future synthesis and separation processes with improved yield and sustainability.

Concluding remarks

As process designers continue to experiment with novel methods of increasing economic and environmental sustainability, reactive crystallization has become increasingly attractive as a means to those ends. Reactive crystallization is not particularly new, as ionic compounds have been synthesized by reactive crystallization since the beginning of the chemical industry. However, new applications of reactive crystallization promise to make possible new processes and improve existing ones. New applications of ionic reactive crystallization include capturing greenhouse gases as carbonates, recovering minerals from wastewater, and enabling fermentation routes to platform chemicals. Covalent reactive crystallization, while less developed, has more promise as a tool to enhance existing reactions or overcome the kinetic and equilibrium limitations of formerly untenable reactions. Biocatalytic processes could benefit the most, as those systems are most sensitive to accumulations of products and intermediates. Additionally, crystallization plays a dominant role in processes to separate enantiomers, where the combination of a racemization reaction and crystallization can lead to enantiomeric excess greater than 99%. The design of reactive crystallization systems follows closely the design of crystallizers in general. Crystallization is a highly nonlinear process; controlling crystallization coupled with a reaction is difficult but many control strategies and reactor designs have proven effective for specific cases. The future of reactive crystallization rests on collection of more data and generalization of findings from disparate case studies, which is the primary aim of this review. While this review may not be exhaustive, it should serve as a starting point for the design of reactive crystallization processes for any type of compound across many scales of industry.
Conflicts of Interest

The authors have no conflicts to declare.

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