

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: <http://ees.elsevier.com/ajps/default.asp>

Original Research Paper

A practical guide to pharmaceutical polymorph screening & selection



Eun Hee Lee*

College of Pharmacy, Korea University, 2511 Sejong-ro, Sejong 339-700, Republic of Korea

ARTICLE INFO

Article history:

Received 1 April 2014

Received in revised form

29 April 2014

Accepted 3 May 2014

Available online 16 May 2014

Keywords:

Polymorphs

Polymorph screening

Polymorph selection

Polymorph preparation

ABSTRACT

Prevalence and importance of polymorphism occurring in pharmaceutical compounds are well recognized. It is of great importance to prepare and select the right form from the beginning during drug discovery and development. This review introduces the basic concepts of “What is polymorphism?”, addresses a fundamental question of “Why do polymorphs form?”, and provides practical guidelines of “How to prepare polymorphs?”, “How to evaluate the relative thermodynamic stability between polymorphs?”, and “How to analyze polymorphs?”. Moreover, case studies of pharmaceutically important polymorphs are provided.

© 2014 Shenyang Pharmaceutical University. Production and hosting by Elsevier B.V. All rights reserved.

1. Introduction

The importance of polymorphism in pharmaceutical, pigment, electrical industry is mainly due to two reasons. The first one is because the existence of polymorphism is inevitable. In other words, polymorphism unavoidably occurs during discovery, development, and/or manufacturing process. As Ostwald stated in 1899, “Almost every substance can exist in two or more solid phases provided the experimental conditions are suitable.” The other reason is because one may want it to happen. In other words, one can change the physicochemical properties of a given compound by using different

polymorphs. Jean-Paul Garnier, CEO of GlaxoSmithKline said that “About 50 % of drug candidates that enter clinical trials fail due to efficacy and safety concerns, and the remaining 40% fizzle due to patent concerns and issues like solubility and drug interaction.” [1,2] This statement emphasizes the importance of manipulating the desired physicochemical properties during drug development.

Improvements in physicochemical properties can be achieved by altering the physical forms of a given compound such as polymorphs, solvates, amorphous, salts, cocrystals, and/or hydrates, etc. [3] Singhal et al. well summarized the physicochemical properties shown by different polymorphs with examples [4]. The physicochemical properties that can be

* Corresponding author. Tel.: +82 44 860 1620; fax: +82 44 860 1606.

E-mail address: eunheelee@korea.ac.kr.

Peer review under responsibility of Shenyang Pharmaceutical University



Production and hosting by Elsevier

<http://dx.doi.org/10.1016/j.ajps.2014.05.002>

1818-0876/© 2014 Shenyang Pharmaceutical University. Production and hosting by Elsevier B.V. All rights reserved.

Table 1 – Properties that can be altered by choosing different polymorphic forms [7–9,12].

Thermodynamic properties	Kinetic properties
<ul style="list-style-type: none"> Melting and sublimation temperatures, and vapor pressure Enthalpy, entropy, and heat capacity Free energy, chemical potential, and solubility 	<ul style="list-style-type: none"> Dissolution rate Rates of solid state reaction Physical/chemical Stability Rate of nucleation/crystal growth
Packing properties	Surface properties
<ul style="list-style-type: none"> Molar volume and density Conductivity, electrical and thermal Refractive index Particle morphology Hygroscopicity Color 	<ul style="list-style-type: none"> Surface free energy Interfacial tensions Habit
	Mechanical properties
	<ul style="list-style-type: none"> Hardness Tensile strength Compactibility and tableting Handling, filtration, flow and blending Cleavage

altered by choosing different polymorphs are summarized in Table 1. The most important properties during drug discovery and development include solubility, dissolution, bioavailability, and physical/chemical stability. The best known polymorphs showing a significantly different bioavailability in human study are chloramphenicol palmitate [5] and mefenamic acid [6]. As the amount of the metastable form B present in suspension formulation increases, the peak plasma concentration of chloramphenicol palmitate in human increases linearly.

Over the past two decades, the importance of pharmaceutical polymorphism has been arising in scientific community and industry as well as regulatory agencies. Numerous reviews, book chapters, and literatures related to pharmaceutical polymorphism have been published [1,4,7–13]. This review introduces basic concepts of “What is polymorphism?”, address fundamental questions of “Why do polymorphs form?”, and provide practical guidelines of “How to prepare polymorph?”, “How to evaluate the relative stability between polymorphs?”, and “How to analyze polymorphs?”. Moreover, case studies of pharmaceutically important polymorphs are provided.

2. What is polymorphism?

Polymorphism occurs when a chemical compound crystallizes with different internal structures. ICH Q6A defines polymorphism as “some new drug substances exist in different crystalline forms which differ in their physical properties. Polymorphism may also include solvation or hydration products (also known as pseudopolymorphs) and amorphous forms.” [14] In a strict sense, polymorphs have to retain the same chemical composition and therefore, solvates or hydrates are defined as pseudopolymorphs. Polymorphs can be categorized into two subtypes: (1) conformational polymorphism and (2) packing polymorphism. Conformational polymorphs occur when conformationally different molecules exist in the crystalline

state. Since pharmaceutical compounds generally contain flexible moieties in their molecular structures, there are numerous examples of conformational polymorphs. In packing polymorphism, the molecules share the same molecular conformations, but are packed differently in three-dimensional space. Form I and II of acetaminophen are the example of the most well known packing polymorphs [15,16].

In many known cases, several polymorphs crystallize concomitantly. Concomitant polymorphism is headache for pharmaceutical industry which seeks for polymorphic purity or blessing for those who seeks for several polymorphic forms. There are numerous examples of showing concomitant polymorphism. Extensive study of concomitant polymorphism was conducted by Bernstein et al. [17].

Another interesting and frustrating phenomenon related to polymorphism is “disappearing polymorphism.” [18–20] It is generally believed that “any authentic crystal form should be capable of being re-prepared, although selection of the right conditions may require some time and trouble.” [21] In contrast to the previous statement, “disappearing polymorphism” refers to a situation where the previously prepared form no longer appears after obtaining the more stable form. There are quite a few examples showing disappearing polymorphism including benzyldiene-dl-piperitone, benzocaine picrate, mannose, etc. [18] “God-only knows where” seeds may play a role in crystallization of disappearing polymorph similar to Dr. Breed case for seeding phenomena [22].

3. Why do polymorphs form?

System tends to move toward thermodynamically equilibrated state. In other words, the system eventually transforms to the most stable state. However, the routes to the final state depend on kinetics as well as other factors as shown below. Several mechanisms were proposed why the metastable form appears first.

3.1. Ostwald’s rule of stages

Ostwald in 1897 stated, “...beim Verlassen irgend eines Zustandes und dem Übergang in einen stabileren nicht der unter den vorhandenen Verhältnissen stabilste aufgesucht wird, sondern der nächstliegende,” and “die Form, welche unter möglichst geringem Verlust an freier Energie erreicht werden kann.” [23] When the system leaves any state, the transition occurs to a more stable one, not the most stable one but the nearest one under a given condition (Fig. 1). According to the theory, one has to observe all metastable forms before one finally observes the stable form. However, it is not always true. We often observe the direct crystallization of the stable form from a solution.

3.2. Kinetic nucleation theory

In this theory, the rate of nucleation of metastable form and the stable form are compared. In classical nucleation theory, the rate of nucleation is derived using Arrhenius equation [24].

$$J_{\text{meta}} = A_{\text{meta}} \exp\left(-\frac{\Delta G_{\text{meta}}}{kT}\right) \quad \text{Eq. 1}$$

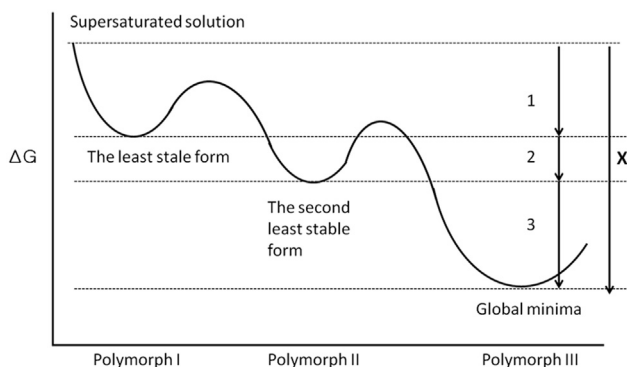


Fig. 1 – Hypothetical diagrams of energy states of polymorphs explaining Ostwald's rule of stages.

$$J_{\text{stable}} = A_{\text{stable}} \exp\left(-\frac{\Delta G_{\text{stable}}}{kT}\right) \quad \text{Eq. 2}$$

where k is the Boltzmann constant, A , the rate of attachment frequency, and ΔG , the activation energy for nucleation. The possible scenarios follow: (1) $A_{\text{meta}} > A_{\text{stable}}$, (2) $A_{\text{meta}} = A_{\text{stable}}$, (3) $A_{\text{meta}} < A_{\text{stable}}$, (4) $\Delta G_{\text{meta}} > \Delta G_{\text{stable}}$, (5) $\Delta G_{\text{meta}} = \Delta G_{\text{stable}}$, (6) $\Delta G_{\text{meta}} < \Delta G_{\text{stable}}$. ΔG term includes the degree of supersaturation, temperature of crystallization, and interfacial tension. The rate of nucleation is expressed as:

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

where γ is the interfacial tension, T , temperature, S , degree of supersaturation, and v molecular volume.

On the basis of classical nucleation theory, the rate of nucleation of two polymorphic forms depends on the frequency factor, temperature, interfacial tension, and supersaturation (Eq. (3)). One can assume that the melting point of the stable form is higher than that of metastable form and therefore, the degree of supersaturation is higher for the stable form at the same crystallization temperature [25]. In general, the attachment frequency of the metastable form will be higher than that of the stable form since entropy of the metastable form is higher than that of the stable form. The effects of the attachment frequency and the supersaturation by supercooling oppose to each other. It is likely that the rate of nucleation for the stable form is higher at low temperature

(high degree of supersaturation) and the transition occurs near the melting temperature (low degree of supersaturation) since the contribution of degree of supersaturation will diminish near the melting temperature.

According to Ostwald's rule of stages, the rate of nucleation of the metastable form is always higher than that of the stable form over all temperature ranges. However, kinetic nucleation theory suggested that the rate of nucleation of the metastable form is never higher over the entire supersaturation ranges (Fig. 2).

3.3. Cross nucleation

Cross nucleation occurs when a polymorph nucleates on another polymorph. Cross nucleation have been observed for small organic molecules as well as polymers from a solution or from the melt [26–30]. During cross nucleation process, several different polymorphs nucleate on each other. The polymorphic form with a fastest growth rate will be eventually observed regardless of the rate of nucleation. It is also noted that a certain polymorph selectively nucleates on a specific polymorph: in the case of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY) compound, form YN specifically nucleates on R while, R and R05 nucleate on Y04 [27]. Cross nucleation does not occur following the thermodynamic stability, a less or a more stable polymorph can nucleate on the surface of the existing polymorph. In a certain case, new forms of ROY, R and R05, were discovered via cross nucleation. They had never been spontaneously nucleated before.

The mechanistic study of cross nucleation showed that the initial polymorphs formed, the growth rate, the nucleation rate, thermodynamic stability, defects, lattice matching, crystallographic orientation, and formation of interfacial transition layers all play major roles in inducing cross nucleation [31,32].

In the case of D-mannitol, α form seed always induces the crystallization of the α form, but the β polymorph seed yields the β or α form whereas, the γ form seed yields the α form [28]. As we mentioned previously, the final polymorph is the one with the fastest growth rate irrespective of the initial nuclei formed during crystallization. From this, one can assume that the α form grows the fastest among the three polymorphs. However, the β polymorph can nucleate on the α form when the solution is quench-cooled. Since growth rate can change as a function of the degree of supersaturation, temperature, or interfacial tension, the final polymorphic form originating

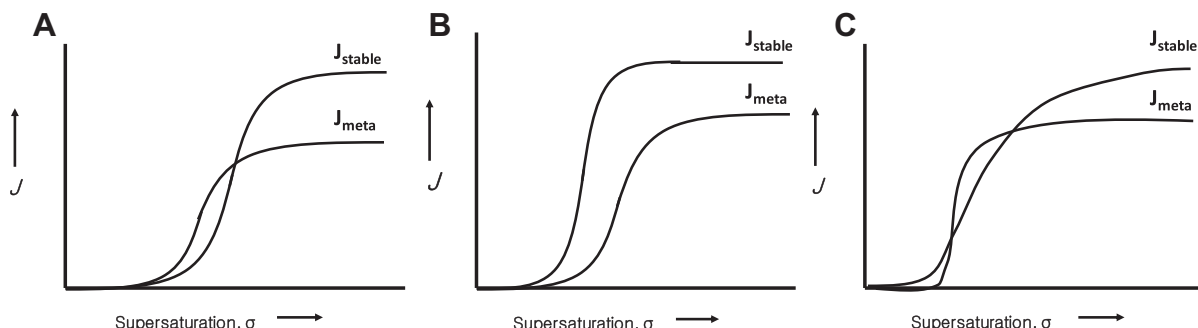


Fig. 2 – The rate of nucleation between two polymorphs as a function of supersaturation.

from the same initial form can vary depending on the crystallization conditions. Polymorph selectivity of highly polymorphic ROY compounds via cross nucleation was further conducted by Yu et al. Yu et al. explained that growth rate, the rate of heterogeneous nucleation of the new polymorph, and defects play major roles in determining the final polymorph obtained via cross nucleation [27]. However, they concluded that the relative stability or lattice matching is insufficient to explain polymorph selectivity by cross nucleation.

The selectivity of polymorphic form resulting from cross nucleation was also investigated using molecular dynamics [33,34]. Molecular dynamics (MD) study showed that cross nucleation occurs only when the free energy of two polymorphs are almost equivalent and confirmed that the polymorph with the fastest growth rate will appear in the end. MD study also showed that common lattice planes between two cross nucleated polymorphs at the interface are necessary. An interfacial transition layer is required to induce cross nucleation between polymorphs that do not share structural matching at the interface [33].

3.4. Heterogeneous two-dimensional nucleation: chemotaxy, ledge-directed epitaxial growth, and two-dimensional lattice matching

According to the classical nucleation theory, homogeneous two-dimensional nucleation requires less energy for molecules to nucleate than three-dimensional nucleation.

On the basis of the classical nucleation theory, the energy required for homogeneous three-dimensional/two-dimensional nucleation follows (Eq. (4)):

$$\Delta G = a\gamma + v\Delta G_v \quad \text{Eq. 4}$$

where a and v are the area and volume of the nucleus.

When we consider the attachment of adsorption layer on the surface of the crystal as homogeneous two-dimensional nucleation/growth, the energy required for two-dimensional nucleation follows (Eq. (5)):

$$\Delta G_{2D} = 2\pi rh\gamma + \pi r^2 h \Delta G_v \quad \text{Eq. 5}$$

where the circular disc of radius is r , height, h , interfacial tension, γ .

$$\Delta G_{3D} = 4\pi r^2 \gamma + \frac{4}{3}\pi r^3 \Delta G_v \quad \text{Eq. 6}$$

When compared to the energy requirement for three-dimensional nucleation (Eq. (6)), one can intuitively know that two-dimensional nucleation is energetically favorable than three-dimensional nucleation and therefore, lower degree of supersaturation is enough for two-dimensional nucleation to occur as compared to three-dimensional nucleation. However, it is noted that the heterogeneous two-dimensional nucleation can be different from homogeneous two-dimensional nucleation in a sense that the interfacial tension between two layers from different crystals contributes to the energy required for the attachment of adsorption layer.

When heterogeneous two-dimensional nucleation is considered, the interfacial tension is an important factor

controlling the nucleation process. The factors controlling the interfacial tension between two layers include chemical interaction [35], formation of monolayer between deposit and substrate [36], and/or geometric configurations including molecular conformations at the interface between layers, ledge-directed epitaxy [37,38], or two-dimensional lattice matching [39–42].

Ledge-directed epitaxy is a specific form of two-dimensional heterogeneous nucleation where a material nucleates and grows on a substrate with a specific geometric match. Interplanar dihedral angles between two close planes of ledge site and two close planes of prenuclear aggregates are crucial factors leading to the ledge-directed epitaxial growth. Organic molecules nucleated on organic single crystal substrates with small free energy via ledge-directed epitaxy [37]. The contributions from chemical interaction between deposit and substrate is small while, topographic structure, lattice parameters at ledge sites, symmetry constraints, and molecular composition play a major role in determining the nucleation of organic crystals on substrates.

Another geometric configurations leading to two-dimensional nucleation is two-dimensional lattice matching between overlay plane and the substrate [39]. Two-dimensional lattice matching can be evaluated using the epitaxy score. The epitaxy score is calculated using Geometrical GRACE (global real-space analysis of crystal epitaxy) that is available and free for academic purpose [43]. Discovery and stabilization of YN polymorph of ROY compound was explained by using two-dimensional lattice matching [39].

3.5. Additive-induced polymorph selection

Molecular recognition at the interface has been well recognized in fields of nucleation/growth [44,45], supramolecular chemistry [46], and resolution of enantiomer [47–53]. Tailor-made additives or structurally related impurities inhibit or enhance nucleation/growth process of parent crystals [54–56]. Carefully designed additives can inhibit the nucleation by being incorporated into the prenuclear aggregates/crystals or by binding to nuclei/crystals. There are three scenarios possible for inhibition of nucleation/crystal growth by additives. First, additives prevent prenuclear aggregates of a particular form from growing into a nucleus with a critical radius by creating defects in the aggregates. As a result, prenuclei redissolve back into the solution. In this respect, additives can decrease the rate of nucleation by increasing the critical supersaturation for nucleation and/or interfacial tension. Second scenario is that additives can favorably attach to prenuclei of a certain form of polymorph or enantiomers. However, other polymorph remains unaffected by additives. As a result, the unaffected prenuclei grow into the nuclei with a critical radius size, and become resulted crystals. The third scenario is that additives attach to the fastest growing face of the stable polymorph. The inhibition of growth of the fastest growing face prevents the stable form from growing. As a result, the metastable polymorph crystallizes.

The same analogy applies to inhibition of crystal growth by additives. Certain additives such as structurally related additives, can easily attach to a certain face of crystals as host molecules do. However, additives will inhibit the attachment

of next layers of the host molecules to the additive layers due to steric hindrance. Growth inhibition of a certain form results in growth of unaffected form such as different polymorph [57,58]. Inhibition of growth of a specific faces will result in changes in morphology, dissolution rate, and/or polymorph selection [54].

4. How to prepare polymorphs?

Considering the importance of polymorphism in pharmaceutical industry, polymorph screening is an essential part of drug discovery and development process. As McCrone stated in 1965, “It is at least this author’s opinion that every compound has different polymorphic forms, and that, in general, the number of forms known for that compound is proportional to the time and money spent in research on that compound.” [59] After a decade, Kuhnert-Brandstatter stated that “probably every substance is potentially polymorphous. The only question is, whether it is possible to adjust the external conditions in such a way that polymorphism can be realized or not.” [60] On the basis of the above statements, it is clear that polymorphs can be obtained when external conditions are adjusted. How extensive should polymorph screening be conducted during early development process is controversial and depends on decision of the company. While the facts that the attrition rate of drug candidates during early development process is very high, conducting an exhaustive polymorph screening is time and money consuming. However, the appropriate polymorph selection during drug development process to address desired stability and physicochemical property issues should not be shadowed. The methods to prepare polymorphs are summarized in Table 2. Some important and/or newly developed methods are discussed in detail.

4.1. Crystallization from a single or mixed solvent

Generally, polymorph screening is conducted by crystallizing substances from a single or mixed solvent via cooling crystallization, evaporation, or antisolvent crystallization. Selection of appropriate solvents is challenging. Gu et al. have well summarized the physicochemical properties of 96 solvents which are often used for polymorph screening [61]. The solvents were classified into 15 categories using cluster statistical analysis where solvent parameters such as hydrogen-bond acceptor/donor propensity, polarity/dipolarity, dipole moment, dielectric constant, etc. are variables. One can select a solvent from each category for initial polymorph screening and mix solvents from different categories.

In addition, heating and cooling rates, crystallization temperature, evaporation rate, the degree of supersaturation, the rate of agitation, pH of the media are variables which can affect crystallization process and thus, polymorphs formed. Considering the number of organic solvents and the combinations of solvents as well as factors affecting crystallization, thousands of crystallization experiments need to be conducted with small amounts of drug candidate during polymorph screening. Attempts to obtain polymorphs using solution crystallization can be made by high-throughput screening technology [62–66].

Table 2 – Methods of obtaining polymorphs.

Method of obtaining polymorphs	
1	Crystallization from a single or mixed solvents/HTS [62–66]
2	Thermal activation of the solid substrates [67]
3	Crystallization from the melt [73–75]
4	Desolvation/dehydration of solvates/hydrates by heat or by reslurry [85,91]
5	Crystallization in nano-confined structures [96,98,100]
6	Seeding/pseudoseeding [101,102]
7	Solution mediated polymorphic transformation/slurry conversion method [103–106]
8	Solid-state polymorphic transformation [85,107]
9	Mechanical activation of the solid substance [11,108]
10	Crystallization in a capillary tube [109,110]
11	Exposure to vapor at high or low humidity [111–113]
12	Exposure to organic vapor [90]
13	Directed crystallization on molecular substrates [37–42]
14	Crystallization in the presence of tailor-made additives [44–58]
15	Laser induced crystallization [114,115]
16	Crystallization from a supercritical fluid [116–118]
17	Structure prediction [119–123]

4.2. Thermal activation of the solid substrates

Before we discuss thermal activation method to obtain a desired polymorph, we need to know the thermodynamic relationships between polymorphs. Any given two polymorphs can be either **monotropic** or **enantiotropic**. Monotropic relationship occurs when one of polymorphs is stable over entire temperature range (Fig. 3a). In the case of enantiotropic system, the transition temperature at which the free energy between two polymorphs is equal occurs below melting point (Fig. 3b). In other words, one form is stable below the transition temperature, and the other form is stable above the transition temperature.

In an enantiotropic system, the metastable form at room temperature can be obtained by heating the stable form above the **transition temperature**. In a monotropic system, the stable form at room temperature can be obtained by heating the metastable form at any temperature. The rate of transformation can be facilitated by heating the metastable form at high temperature. If one starts with the stable form, it is impossible to obtain the metastable form by thermal activation method in a monotropic system. For example, flufenamic acid I which is metastable form at room temperature can be obtained easily by heating flufenamic acid III above 105 °C [67]. The transition temperature can be estimated by observing the **transition events** during DSC measurements [68], by calculating the Gibbs free energy difference between polymorphs over temperature ranges via direct heat capacity measurement [69], by constructing the van Hoff’s plot via solubility measurements of polymorphs over temperature ranges available [6,70], or by slurrying the mixtures of polymorphs over temperatures available, etc [71,72].

4.3. Crystallization from the melt

Crystallization from the melt is similar to crystallization from amorphous material. Since amorphous is a

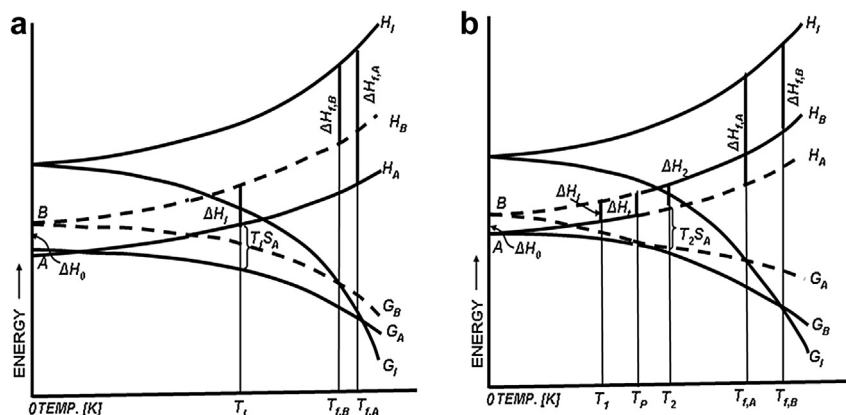


Fig. 3 – Energy/temperature diagrams of dimorphic systems (a) monotropic systems, (b) enantiotropic systems (T_p : transition point; T_f : fusion point; H: molar enthalpy; G: molar free energy; S: molar entropy; A, B: crystalline modifications; l: liquid phase).

thermodynamically unstable form as compared to corresponding crystalline materials, amorphous materials tend to crystallize. There are numerous papers describing crystallization/devitrification behavior from amorphous materials. Generally, crystallization from amorphous material is problematic since crystallization from amorphous alters physico-chemical properties of a drug compound. However, one can use crystallization from amorphous as a technique to grow desired polymorphic forms. Depending on the external stress applied to amorphous, crystallization from the melt generates different polymorphic forms with different kinetics and mechanism [73–75]. It is reported that crystallization from the melt is the only method to generate metastable forms, II (or β) and III (or γ) of nifedipine, [76,77] as well as the metastable form III of acetaminophen [78].

4.4. Desolvation/dehydration of solvates/hydrates by heat or by reslurry

As the number of experiments during screening process increases with an advance in high-throughput screening and polymorphic screening methods, the number of solvates/hydrates discovered during screening process also increases [62,63,79–85]. When desolvation or dehydration occurs, solvates/hydrates can undergo a phase transition and thus, form non-solvated/anhydrous polymorph [86,87] or can lose crystallinity and thus, form amorphous [88]. In other cases, isomorphic desolvates/dehydrates form, meaning that solvent/water molecules leave crystals without affecting the crystal structures of solvates/hydrates [89,90]. Generally, desolvation/dehydration process is conducted at low relative humidity or low organic vapor pressure. Recently, it has been shown that desolvation using reslurrying solvates in a solvent with poor solubility at low or high temperature yields pharmaceutically relevant polymorphs [91].

4.5. Crystallization in nano-confined structures

We do not define nano-materials just by measuring the size of materials, although the definition of nano-materials is still controversial. However, it is at least clear that nano-materials

show size-dependent properties such as optical [92], electronic [93], or magnetic properties [94] which are different from macro-sized crystals. Nano-materials show an extremely high surface-to-volume ratio compared to macro-sized materials. As a result, the surface effects become increasingly important as the size of materials decreases [95–97].

A clear example of abnormal property of nano-materials is the melting point depression as a function of size [98,99]. The relationship can be written according to the Gibbs-Thomson equation:

$$T_m(d) = T_m^{\text{bulk}} - \frac{4T_m^{\text{bulk}}\sigma}{d\Delta H_m^{\text{bulk}}\rho} \quad \text{Eq. 7}$$

where ΔH_m^{bulk} is the equilibrium heat of melting and T_m^{bulk} the equilibrium melting temperature of bulk crystals, ρ density of bulk crystal, d the pore diameter of cylindrical nano-confined structures. σ , the effective surface energy of nano-sized crystals confined in the cylindrical pores can be obtained by plotting the melting point depression as a function of diameter of nano-sized crystals. The diameter of nano-sized crystals is assumed to be the same as pore size of nano-confined structure. On the basis of the Eq. (7), it is clear that the size of crystals affects the total free energy of the nano-sized crystal. The Gibbs free energy equation can be rewritten including the contribution of the effective surface energy of nano-sized crystals:

$$\Delta G_{\text{CA}}^{\text{nano}} = \Delta G_{\text{CA}}^{\text{bulk}} - \left(\frac{A}{V}\right) \left(\frac{\sigma_c^{\text{nano}}}{\rho_c^{\text{bulk}}}\right) \quad \text{Eq. 8}$$

where $\Delta G_{\text{CA}}^{\text{nano}}$ is the Gibbs free energy difference between liquids and nano-sized crystals, $\Delta G_{\text{CA}}^{\text{bulk}}$ the Gibbs free energy difference between liquids and bulk crystals, A the surface area, V the volume, σ_c^{nano} the effective surface energies of nano-sized crystals, ρ_c^{bulk} the density of bulk crystals.

The relative stability of polymorphs also changes as a function of crystal size (pore size) as well as temperature. By combining Eq. (7) and Eq. (8), the relative stability between two nano-sized polymorphs can be obtained and plotted as a function of temperature. Crystals can be grown in nano-sized pores to reverse the relative thermodynamic stability between

polymorphs [96,97], and to improve the physical stability of the metastable polymorph in case of acetaminophen form III [98], to obtain unknown polymorphs such as δ -pimelic acid, β -suberic acid, and β -coumarin or to crystallize the stable forms such as mefenamic acid, glycine, and ROY that are not easily obtainable under normal crystallization conditions [97–100].

4.6. Seeding

Seeding facilitates the crystallization process via heterogeneous or secondary nucleation. Seed crystals can be exactly the same form of interest, a pseudo-seeds that are structurally compatible to the desired form of interest but not exactly the same, or the one that is not related to the form of interest [24]. Seeding with a desired polymorphic form is a commonly used technique for controlling polymorphic form during industrial crystallization [101]. Pseudoseeding is often used when the seed of interest is not available [102]. It is usually expected that the polymorphic form of seeds will crystallize out. However, it should not be forgotten that the seeding can also facilitate the crystallization of some undesired form(s)/another polymorphic form(s) when those seeds serve as templates for cross nucleation or epitaxial growth. In those cases, the form that grows faster than the seed crystals will eventually crystallize out.

4.7. Solution mediated polymorphic transformation/slurry conversion method

Solution mediated polymorphic transformation (SMPT) is a fast, easy, and reliable method to obtain the stable polymorph. If one is fortunate, one may obtain the series of polymorphs following the Ostwald's rule of stage. SMPT occurs via the dissolution of the metastable form followed by nucleation/growth of another form which is more stable than the previous form. The thermodynamic driving force for SMPT is the solubility difference between polymorphs. The kinetic factors governs the rate of solution-mediated polymorphic transformation including solubility difference between polymorphs in a given solvent, the dissolution rate of the

metastable form, and the rate of nucleation/growth of the stable form [71,103]. In addition, if dissolution rate limited SMPT occurs, particle size will play an important role in determining the rate of SMPT.

SMPT is also an easy and simple method for obtaining solvates/hydrates of a given compound. Generally, solvate/hydrate is the most stable form in a given solvent if a compound shows solvate forming tendency. When specific solvates/hydrates are needed, solvent-free crystals can be slurried in the given solvent for an extended period of time. When two solvent-free polymorphs are considered, the thermodynamic stability of two polymorphs is determined by the Gibbs free energy difference at a given temperature and pressure. When hydrate/anhydrous forms are considered, the water activity in the solvent contributes to the relative physical stability and thus, the resulting form [104,105]. It was found that the water activity in a solvent mixture plays a major role in determining the resulting crystalline forms of theophylline regardless of the corresponding organic solvent [106].

5. How to evaluate the relative thermodynamic stability between polymorphs?

The relative thermodynamic stability between polymorphs are of great interest due to the physical stability and solubility issues and play a decisive role in determining the polymorphic form to be used in formulation. Table 3 summarizes the most re-known "the thermodynamic rules" to determine the relative thermodynamic stability and the thermodynamic relations of a dimorphic system by Burger and Ramberger [124,125].

The thermodynamic relationships can be experimentally determined by calculating the Gibbs free energy difference between polymorphs over temperature ranges via direct heat capacity measurement, by constructing the van Hoff's plot via solubility measurements of polymorphs over temperature ranges available, or by slurrying the mixtures of polymorphs over temperatures available, etc. similar to those which were described in "How to Prepare Polymorphs?" section (Fig. 4).

Table 3 – Thermodynamic rules to determine the relative thermodynamic stability and the thermodynamic relations of a dimorphic system. [127,128].

Heat of transition rule	Polymorphs are enantiotropically related if endothermic heat of transition from low melting form to high melting form is observed Polymorphs are monotropically related or the transition temperature is higher if exothermic transition is observed
Heat of fusion rule	Polymorphic pairs are enantiotropically related if the low melting form has the higher heat of fusion, otherwise they are monotropically related
Entropy of fusion rule	Polymorphs are enantiotrope if the high melting form has the lower entropy of fusion, otherwise they are monotrope
Heat capacity rule	Polymorphic pairs are enantiotropically related if the high melting form has higher heat capacity than the low melting form at a given temperature
Density rule	One polymorph with a higher density can be assumed to be more stable at 0 K than the other polymorph with a lower density
Infrared rule	One polymorph with a higher absorption band in the infrared spectrum of a hydrogen-bond molecular crystal may be assumed to have larger entropy than the other with a lower absorption band
Solubility rule	If the higher melting form has higher solubility above the transition temperature, polymorphs are enantiotropically related. In a monotropic system the higher melting form always has a lower solubility

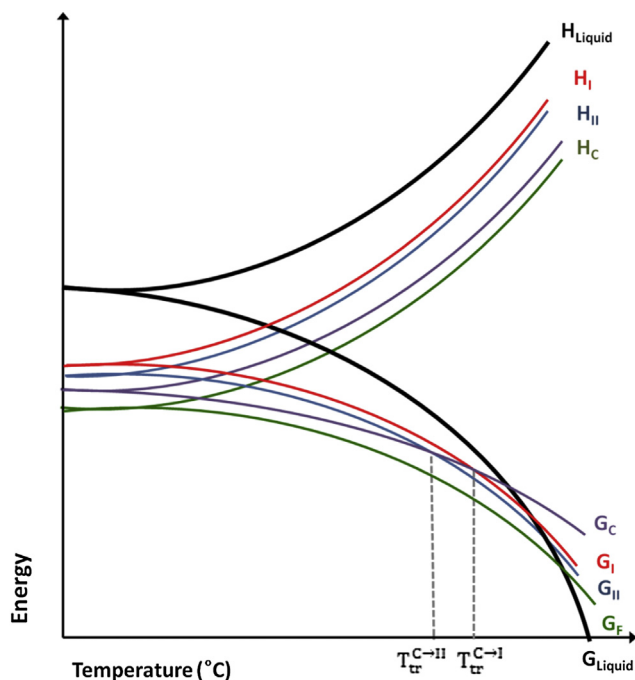


Fig. 4 – Schematic energy-temperature phase diagram of donepezil polymorphs I, II, C, and F. Adapted from Ref. 69.

6. How to analyze polymorphs?

Most of the solid state characterization techniques except single crystal X-ray analysis are complimentary. Table 4 shows some characteristic features of different techniques in terms of destructiveness, sample amounts, analysis time, etc. Powder X-ray diffraction (PXRD) is the front-line technology to analyze polymorphs. PXRD patterns between polymorphs are normally very distinct as compared to FTIR/Raman spectra. It can be

used for quantitative analysis of polymorph mixtures, and for an analytical tool for high-throughput screening with some modifications. However, when the reference powder patterns of pure forms are not available, it is often difficult to realize if the powder pattern is from the mixture or pure material. This is where single crystal X-ray analysis technique is necessary. Single crystal X-ray analysis is a definitive tool to obtain the crystal structural information of polymorphs and can be used for obtaining the calculated powder patterns. However, it is often very difficult to obtain a single crystal suitable for single crystal X-ray analysis, especially metastable forms. Crystal structure information can also be deduced from the powder patterns. However, it is difficult to obtain a good quality data suitable for structure elucidation using laboratory powder X-ray diffractometer equipped with Cu K α radiation. Therefore, it is often necessary to obtain the data from synchrotron X-ray radiation source.

7. Examples of polymorphism of pharmaceutically important compounds

7.1. Aspirin

Aspirin was synthesized by Felix Hoffmann in 1897 for the first time, and has been on the market since 1899. Since then, aspirin has been one of the most widely used drugs in the world, and it is apparent that it has been crystallized repeatedly for a long time. With such a long history, aspirin was thought to be one of the compounds that did not show polymorphism. Then, came the debate regarding the existence of new polymorphic forms of aspirin [138]. But the crystal structure information of a new polymorphic form (form II) obtained from single crystal structure analysis was not revealed until 2005 [139]. Even after the crystal structure of aspirin form II was discovered, the existence of polymorphic form is still

Table 4 – Some practical information regarding the solid state characterization method of polymorphs.

Techniques	Analysis time	Sample (mg)	Destructiveness	Preparation	Quantitation	
PXRD [112,126,127]	3–8 min	10–30	X	Simple	O	Difficult to differentiate the mixtures, first-line to analyze polymorphs, HTS
DSC [128–130]	20–30 min	2–4	O	Simple	O	Easy to detect the mixtures
TGA	20–30 min	~10	O	Simple	X	Thermodynamic relationships
Single crystal X-ray	1–2 day	Single crystal	O	Difficult	X	Existence of solvates/hydrates
FTIR (Pellet)	10–20 min	3~ (pellet)	O	Difficult	O	Idea of how strong solvents interact with molecules
FTIR (ATR)	10–20 min	10~	X	Simple	O	Definitive tool
FTIR (Probe)	3 s	10~	X	Simple	O	Molecular interactions
FT-Raman [131,132]	~20 min	10~	O	Simple	O	No sample preparation
FT-Raman (Probe) [133]	3 s	3~	X	Simple	O	On-line monitoring
HSM [134]	20–30 min	2–3	X	Simple	X	Molecular interactions, HTS
ssNMR [135–137]	1 h	20–30	O	Difficult	O	On-line monitoring

DSC – differential scanning calorimetry; TGA – Thermogravimetry analysis; HSM – Hot-stage microscopy; HTS – High-throughput screening; O = Yes X = No.

controversial due to unique intergrowth phenomenon seen between two polymorphic domains [140,141]. However, it is clear that the two polymorphic forms show significantly different solid state properties such as dissolution rate, mechanical properties, crystal habit, melting point, and pKa.

7.2. Acetaminophen

Acetaminophen is a widely used analgesic and antipyretic drug. Acetaminophen shows three polymorphic forms, I, II, and III [15,16,142–144]. Form I and II are known to show packing polymorphism where molecular conformations are the same, but the crystal packings are different. Commercial form of acetaminophen polymorph is form I. Acetaminophen form II is slightly more soluble than form I and suitable for direct compression, but is less stable and susceptible to transforming to form I during compression and storage [145]. Form II can be obtained by crystallizing solids in benzyl alcohol at high temperature, by cooling the melt, by adding carboxylic acid additives, or by using evaporation method. Form III is known to be highly unstable, and obtained by cooling the melt. It undergoes solid state polymorphic transformation to form II within hours [146]. Recently, it was shown that form III can easily crystallize in nano-confined structures with a pore size ranging from 10 to 103 nm [98].

7.3. Atorvastatin

Atorvastatin calcium is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and is used for lowering blood cholesterol. Atorvastatin was developed and marketed by Pfizer under the trade name Lipitor. It was the number one selling drug in US until its patent expired at 2011. At least 60 polymorphic forms/solvates/hydrates have been patented and/or published [147]. Surprisingly, none of single crystal structure information has been reported on CSD (Cambridge Structural Database, 2013). It will be very interesting to know what makes atorvastatin calcium highly polymorphic.

7.4. Ritonavir

Ritonavir is an antiretroviral drug belonging to protease inhibitor class and is used to treat HIV-1 infection. Ritonavir was marketed in 1996, but had to be withdrawn from the market in 1998 due to a sudden appearance of the stable and less soluble form II in Norvir semi-solid capsules [163]. The metastable form is more than five times soluble than the stable form II in a mixture of water and ethanol. It was reported that degraded impurity provided seeds for heterogeneous nucleation of the stable form. Later, three more forms including two solvates and one unsolvated form were discovered by high-throughput screening [64]. Almost 2000 experiments were conducted with 2 g of the API. Form II is reported to be the stable form among the five polymorphic forms isolated so far.

7.5. Highly polymorphic form, axitinib

Axitinib is a tyrosine kinase inhibitor that targets the vascular endothelial growth factor and is known to interrupt tumor angiogenesis and thus, prevent the growth of cancer cells. 60

solvates, polymorphs of solvates, and five anhydrous forms were discovered [91]. Conventional crystallization methods did not lead to the discovery of the most stable polymorph. The stable anhydrous form was obtained via reslurrying the solvates at high temperature, and used in commercial formulation under trade name Inlyta [164]. Understanding of the desolvation pathway was critical for obtaining the most stable polymorph of axitinib.

8. Summary

Polymorph screening, selections, and controls play parts in determining the success of new drug discovery and development. With the advancements in understanding of solid state chemistry along with new analytical techniques, realization of crystal engineering and molecular level design of pharmaceutical solids with desired physicochemical properties are now closer. It has been recognized that molecular level understanding of nucleation of polymorphs is necessary to control crystallization of polymorphs and comprehend the issues relevant to physical stability. Thermodynamics, kinetics, and supramolecular chemistry via molecular recognition are crucial factors in understanding the nucleation of polymorphs. Appropriate manipulation of factors affecting the nucleation of polymorphs is the key for the formation of new polymorphic forms which might have the desired physicochemical properties.

We have described some important factors affecting nucleation processes of polymorphs in terms of kinetics, thermodynamics, and molecular recognition and summarized some pharmaceutically important and/or newly developed methods of preparing polymorphs. Some of the pharmaceutically important polymorphs were also introduced. Although a certain polymorphic form is still obtained serendipitously [139–165], it is hoped that this review will promote the understanding of polymorphism in molecular level and make ease of undertaking polymorph screening and selections.

Acknowledgments

This research was supported by a grant (14172MFDS189) from Ministry of Food and Drug Safety in 2014 and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2014R1A1A1006429).

REFERENCES

- [1] Colin RG, Christopher TW, Örm A. Drugs as materials: valuing physical form in drug discovery. *Nat Rev* 2004;3:926–934.
- [2] GlaxoSmithKline on track to launch 11 drugs by Dec. 2003. *Dow Jones Newswires* 8 September.
- [3] Grant DJW. [Chapter 1]. Theory and origin of polymorphism. Polymorphism in pharmaceutical solids. In: Brittain Harry G, editor. Marcel Dekker, Inc; 1999.
- [4] Dharmendra S, William C. Drug polymorphism and dosage form design: a practical perspective. *Adv Drug Deliv Rev* 2004;56:335–347.

- [5] Aguiar AJ, Krc J, Kinkel AW, et al. Effect of polymorphism on the absorption of chloramphenicol from chloramphenicol palmitate. *J Pharm Sci* 1967;56:847–853.
- [6] Aguiar AJ, Zelmer JE. Dissolution behavior of polymorphs of chloramphenicol palmitate and mefenamic acid. *J Pharm Sci* 1969;58:983–987.
- [7] Byrn SR, Pfeiffer RR, Stowell JG. Solid-state chemistry of drugs. 2nd ed. SSCI, Inc; 1999.
- [8] Hilfiker Rolf, editor. Polymorphism: in the pharmaceutical industry. Wiley-Vch Verlag GmbH&Co, KGaA; 2006.
- [9] Brittain Harry G, editor. Polymorphism in pharmaceutical solids. 2nd ed. CRC Press; 2009.
- [10] Rodríguez-Spong B, Price CP, Jayasankar A, et al. General principles of pharmaceutical solid polymorphism: a supramolecular perspectives. *Adv Drug Deliv Rev* 2004;56:241–274.
- [11] Braga D, Grepioni F, Maini L. The growing world of crystal forms. *Chem Commun* 2010;46:6232–6242.
- [12] Lee AY, Erdemir D, Myerson AS. Crystal polymorphism in chemical process development. *Annu Rev Chem Biomol Eng* 2011;2:259–280.
- [13] Desiraju GR. Crystal engineering: from molecule to crystal. *J Am Chem Soc* 2013;135:9952–9967.
- [14] Specification: Test procedures and acceptance criteria for new drug substances and new drug products: chemical substances Q6A, part of U. S. Food and Drug Administration. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q6A/Step4/Q6Astep4.pdf (accessed 03.10.14).
- [15] Haisa M, Kashino S, Maeda H. The orthorhombic form of p-hydroxyacetanilide. *Acta Crystallogr* 1974;B30:2510–2512.
- [16] Haisa M, Kashino S, Kawai R, et al. The monoclinic form of p-hydroxyacetanilide. *Acta Cryst* 1976;B32:1283.
- [17] Bernstein J, Davey RJ, Henck JO. Concomitant polymorphs. *Angew Chem Int Ed* 1999;38:3440–3461.
- [18] Dunitz JD, Bernsterin J. Disappearing polymorphs. *Acc Chem Res* 1995;28:193–200.
- [19] Lancaster RW, Karamertzanis PG, Hulme AT, et al. The polymorphism of progesterone: stabilization of a 'disappearing' polymorph by co-crystallization. *J Pharm Sci* 2007;96:3419–3431.
- [20] Lancaster RW, Harris LD, Pearson D. Fifty-year old samples of progesterone demonstrate the complex role of synthetic impurities in stabilizing a metastable polymorph. *Cryst Eng Comm* 2011;13:1775–1777.
- [21] Woodard GD, McCrone WC. *J Appl Cryst* 1975;8:342.
- [22] Vonnegut Kurt. Cat's cradle; 1963.
- [23] Ostwald Von W. Studien über die Bildung und Umwandlung fester Körper. 1. Abhandlung: Übersättigung und Überkaltung. *Zeltschrift f. physic. Chemie* 1897;XXII:289–330.
- [24] Mullin JW. Crystallization. 4th ed. Butterworth-Heinemann; 2001.
- [25] Davey R, Goldbeck-Wood G. General Discuss Faraday Discuss 1993;95:145–172.
- [26] Lian Y. Nucleation of one polymorph by another. *J Am Chem Soc* 2003;125:6380–6381.
- [27] Chen S, Xi H, Yu L. Cross-nucleation between ROY polymorphs. *J Am Chem Soc* 2005;127:17439–17444.
- [28] Tao J, Jones KJ, Yu L. Cross-nucleation between D-mannitol polymorphs in seeded crystallization. *Cryst Growth Des* 2007;7:2410–2414.
- [29] Yu L. Survival of fittest polymorph: how fast nucleator can lose to fast grower. *Cryst Eng Comm* 2007;9:847–851.
- [30] Cavallo D, Gardella L, Portale G, et al. Kinetics of cross-nucleation in isotactic poly(1-butene). *Macromolecules* 2014;47:870–873.
- [31] Nguyen AH, Jacobson LC, Molinero V. Structure of the clathrate/solution interface and mechanism cross-nucleation of clathrate hydrates. *J Phys Chem C* 2012;116:19828–19838.
- [32] Nguyen AH, Molinero V. Cross-nucleation between clathrate hydrate polymorphs: assessing the role of stability, growth rate, and structure matching. *J Chem Phys* 2014;140(084506):1–10.
- [33] Desgranges C, Delhommelle J. Molecular mechanism for the cross-nucleation between polymorphs. *J Am Chem Soc* 2006;128:10368–10369.
- [34] Bolhuis PG, Frenkel DMSC, Huse DA. Entropy difference between crystal phases. *Nature* 1997;388:235–237.
- [35] Carter PW, Ward MD. Directing polymorph selectivity during nucleation of anthranilic acid on molecular substrates. *J Am Chem Soc* 1994;116:769–770.
- [36] Leunissen ME, Grawinkel WS, van Enckevort WJP, et al. Epitaxial nucleation and growth of n-alkane crystals on graphite (0001). *Cryst Growth Des* 2004;4:361–367.
- [37] Carter PW, Ward MD. Topographically directed nucleation of organic crystals on molecular single-crystal substrates. *J Am Chem Soc* 1993;115:11521–11535.
- [38] Bonafede SJ, Ward MD. Selective nucleation and growth of an organic polymorph by ledge-directed epitaxy on a molecular crystal substrate. *J Am Chem Soc* 1995;117:7853–7861.
- [39] Mitchell CA, Yu L, Ward MD. Selective nucleation and discovery of organic polymorphs through epitaxy with single crystal substrates. *J Am Chem Soc* 2001;123:10830–10839.
- [40] Frincu MC, Sharpe RE, Swift JA. Epitaxial relationships between cholesterol crystals and mineral phases: implication for human disease. *Cryst Growth Des* 2004;4:223–226.
- [41] Seaton CC, Parkin A, Wilson CC, et al. Growth of an organic co-crystal upon a component subphase. *Cryst Growth Des* 2008;8:363–368.
- [42] Lee EH, Boerrigter SXM, Byrn SR. Epitaxy of a structurally related compound on the (100) faces of flufenamic acid form I and III single crystals. *Cryst Growth Des* 2010;10:518–527.
- [43] Ward MD. Software GRACE: Geometric Real-Space Analysis of Crystal Epitaxy. Chemical Engineering & Materials Science, University of Minnesota and New York University; 2003. <http://www.nyu.edu/fas/dept/chemistry/wardgroup/software.html>.
- [44] Addadi L, Berkovitch-Yellin Z, Weissbuch I, et al. Growth and dissolution of organic crystals with "tailor-made" inhibitors-implications in stereochemistry and materials science. *Angew Chem Int Ed Engl* 1981;24:466–485.
- [45] Addadi L, Weiner S. Control and design principles in biological mineralization. *Angew Chem Int Ed Engl* 1992;31:153–169.
- [46] Leiserowitz L, Nader F. The molecular packing modes and the hydrogen-bonding properties of amide: dicarboxylic acid complexes. *Acta Cryst* 1977;B33:2719–2733.
- [47] Addadi L, Lahav M. Toward the planning and execution of an "absolute" asymmetric synthesis of chiral dimmers and polymers with quantitative enantiomeric yield. *Pure Appl Chem* 1979;51:1269–1284.
- [48] van Mill J, Gati E, Addai L, et al. Useful impurities for optical resolutions. 1. On the crystallization of photopolymerizing dienes in the presence of their chiral topochemical products. *J Am Chem Soc* 1981;103:1248–12449.
- [49] Addadi L, van Mil J, Lahav M. Useful impurities for optical resolutions. 2. Generality and mechanism of the rule of reversal. *J Am Chem Soc* 1981;103:1249–1251.
- [50] Addadi L, Gati E, Lahav M. Useful impurities for optical resolutions 3. An improved Pasteur-type resolution of conglomerates and a new empirical method for assignment

- of absolute configuration. *J Am Chem Soc* 1981;103:1251–1252.
- [51] Addadi L, van Mil J, Lahav M. Engineering of chiral crystals for asymmetric ($2_{\pi}+2_{\pi}$) photopolymerization. Execution of an “absolute” asymmetric synthesis with quantitative enantiomeric yield. *J Am Chem Soc* 1982;104:3422–3429.
 - [52] van Mil J, Addadi L, Gati E, et al. Attempted amplification of optical activity by crystallization of chiral crystals of photopolymerizing dienes in the presence of their topochemical products. *J Am Chem Soc* 1982;104:3429–3434.
 - [53] Addadi L, Weinsein S, Gati E, et al. Resolution of conglomerates with the assistance of tailor-made impurities. Generality and mechanistic aspects of the “rule of reversal”. A new method for assignment of absolute configuration. *J Am Chem Soc* 1982;104:4610–4617.
 - [54] Weissbuch I, Popovitz-Biro R, Lahav M, et al. Understanding and control of nucleation, growth, habit, dissolution and structure of two- and three-dimensional crystals using ‘tailor-made’ auxiliaries. *Acta Cryst* 1995;B51:115–148.
 - [55] Weissbuch I, Lahav M, Leiserowitz L. Toward stereochemical control, monitoring, and understanding of crystal nucleation. *Cryst Growth Des* 2003;3:125–150.
 - [56] Davey RJ, Blagden N, Potts GD, et al. Polymorphism in molecular crystals: stabilization of a metastable form by conformational mimicry. *J Am Chem Soc* 1997;119:1767–1772.
 - [57] Lee EH, Byrn SR, Carvajal MT. Additive-induced metastable single crystal of mefenamic acid. *Pharm Res* 2006;23:2375–2380.
 - [58] Mattei A, Li T. Polymorph formation and nucleation mechanism of tolfenamic acid in solution: an investigation of pre-nucleation solute association. *Pharm Res* 2012;29:460–470.
 - [59] McCrone WC. Polymorphism. In: Fox D, Labes MM, Weissberger M, editors. *Physics and chemistry of the organic solid state*, vol. 2. New York: Wiley Interscience; 1965. p.725.
 - [60] Kuhner-Brandstatter M. Polymorphie bei Arzneistoffen. *Pharm Unserer Zeit* 1975;4:131–137.
 - [61] Gu C, Li H, Gandhi RB, et al. Grouping solvents by statistical analysis of solvent property parameters: implication to polymorph screening. *Int J Pharm* 2004;283:117–125.
 - [62] Florence AJ, Johnston A, Price SL, et al. An automated parallel crystallization search for predicted crystal structures and packing motifs of carbamazepine. *J Pharm Sci* 2006;95:1918–1930.
 - [63] Almarson Ö, Hickey MB, Peterson ML, et al. High-throughput surveys of crystal form diversity of highly polymorphic pharmaceutical compounds. *Cryst Growth Des* 2003;3:927–933.
 - [64] Morrisette SL, Soukasene S, Levinson D, et al. Elucidation of crystal form diversity of the HIV protease inhibitor ritonavir by high-throughput crystallization. *Proc Natl Acad Sci* 2003;100:2180–2184.
 - [65] Morrisette SL, Almarsson Ö, Peterson ML, et al. High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids. *Adv Drug Deliv Rev* 2004;56:275–300.
 - [66] Leterson ML, Morrisette SL, McNulty C, et al. Iterative high-throughput polymorphism studies on acetaminophen and an experimentally derived structure for Form III. *J Am Chem Soc* 2002;124:10958–10959.
 - [67] Krc Jr J. Crystallographic properties of flufenamic acid. *Microscope* 1976;25:31–45.
 - [68] Behme RJ, Brooke D. Heat of fusion measurement of a low melting polymorph of carbamazepine that undergoes multiple-phase changes during differential scanning calorimetry. *J Pharm Sci* 1991;80:986–990.
 - [69] Park Y, Lee J, Lee SH, et al. Crystal structures of tetramorphic forms of donepezil and energy/temperature phase diagram via direct heat capacity measurements. *Cryst Growth Des* 2013;13:5450–5458.
 - [70] Higuchi WI, Lau PK, Higuchi T, et al. Polymorphism and drug availability. *J Pharm Sci* 1963;52:150–153.
 - [71] Cardew PT, Davey RJ. The kinetics of solvent-mediated phase transformation. *Proc R Soc Lond A* 1985;398:415–428.
 - [72] Davey RJ, Cardew PT, McEwan D, et al. Rate controlling processes in solvent-mediated phase transformations. *J Cryst Growth* 1986;79:648–653.
 - [73] Wu T, Yu L. Origin of enhanced crystal growth kinetics near Tg probed with indomethacin polymorphs. *J Phys Chem B* 2006;110:15694–15699.
 - [74] Sun Y, Xi H, Chen S, et al. Crystallization near glass transition: transition from diffusion-controlled to diffusionless crystal growth studied with seven polymorphs. *J Phys Chem B* 2008;112:5594–5601.
 - [75] Zhu L, Wong L, Yu L. Surface-enhanced crystallization of amorphous nifedipine. *Mol Pharm* 2008;5:921–926.
 - [76] Burger A, Koller KT. Polymorphism and pseudopolymorphism on nifedipine. *J Pharm Sci* 1996;64:293–301.
 - [77] Gunn E, Guzei IA, Cai T, et al. Polymorphism of nifedipine: crystal structure and reversible transition of the metastable β polymorph. *Cryst Growth Des* 2012;12:2037–2043.
 - [78] Burger A. Zur interpretation von polymorphie-untersuchungen. *Acta Pharm Technol* 1982;28:1–20.
 - [79] Brittain HG. Polymorphism and solvatomorphism 2007. *J Pharm Sci* 2005;96:705–728.
 - [80] Brittain HG. Polymorphism and solvatomorphism 2008. *J Pharm Sci* 2006;97:3611–3636.
 - [81] Brittain HG. Polymorphism and solvatomorphism 2009. *J Pharm Sci* 2007;98:1617–1642.
 - [82] Brittain HG. Polymorphism and solvatomorphism 2010. *J Pharm Sci* 2008;99:3648–3664.
 - [83] Brittain HG. Polymorphism and solvatomorphism 2011. *J Pharm Sci* 2009;100:1260–1279.
 - [84] Brittain HG. Polymorphism and solvatomorphism 2012. *J Pharm Sci* 2010;101:464–484.
 - [85] Albers D, Galgocsi M, King D, et al. Characterization of the polymorphic behavior of an organic compound using a dynamic thermal and X-ray powder diffraction technique. *Org Process Res Dev* 2007;11:846–860.
 - [86] Yamauchi M, Lee EH, Otte A, et al. Contrasting the surface and bulk properties of anhydrate and dehydrated hydrate materials. *Cryst Growth Des* 2011;11:692–698.
 - [87] Mirza S, Miroshnyk I, Rantanen J, et al. Solid-state properties and relationship between anhydrate and monohydrate of baclofen. *J Pharm Sci* 2007;96:2399–2408.
 - [88] Chen LR, Young Jr VG, Lechuga-Ballesteros D, et al. Solid-state behavior of cromolyn sodium hydrates. *J Pharm Sci* 1999;88:1191–1200.
 - [89] Lee EH, Smith DT, Fanwick PE, et al. Characterization and anisotropic lattice expansion/contraction of polymorphs of tenofovir disoproxil fumarate. *Cryst Growth Des* 2010;10:2314–2322.
 - [90] Lee J, Boerrigter SXM, Jung YW, et al. Organic vapor sorption method of isostructural solvates and polymorph of tenofovir disoproxil fumarate. *Eur J Pharm Sci* 2013;50:253–262.
 - [91] Campeta AM, Chekal BP, Abramov YA, et al. Development of a targeted polymorph screening approach for a complex polymorphic and highly solvating API. *J Pharm Sci* 2010;99:3874–3886.

- [92] Lopez R, Feldman LC, Haglund Jr RF, et al. Size-dependent optical properties of VO₂ nanoparticle array. *Phys Rev Lett* 2004;93:177403-1–177403-4.
- [93] Viosin C, Christofilos D, Del Fatti N, et al. Size-dependent electron-electron interactions in metal nanoparticles. *Phys Rev Lett* 2000;85:2200–2203.
- [94] Lau JT, Föhlisch A, Nietubyc R, et al. Size-dependent magnetism of deposited small iron clusters studied by X-ray magnetic circular dichroism. *Phys Rev Lett* 2004;89:057201-1-4.
- [95] Wang CX, Yang GW. Thermodynamics of metastable phase nucleation at the nanoscale. *Mater Sci Eng* 2005;49:157–202.
- [96] Rengarajan GT, Enke D, Steinhart M, et al. Size-dependent growth of polymorphs in nanopores and Ostwald's step rule of stages. *Phys Chem Chem Phys* 2011;13:21367–21374.
- [97] Ha JM, Hamilton BD, Hillmyer MA, et al. Phase behavior and polymorphism of organic crystals confined within nanoscale chambers. *Cryst Growth Des* 2009;9:4766–4777.
- [98] Beiner M, Rengarajan GT, Pankaj S, et al. Manipulating the crystalline state of pharmaceuticals by nanoconfinement. *Nano Lett* 2007;7:1381–1385.
- [99] Alcoutlabi M, McKenna GB. Effects of confinement on material behavior at the nanometer size scale. *J Phys Condens Matter* 2005;17:R461–524.
- [100] Ha JM, Wolf JH, Hillmyer MA, et al. Polymorph selectivity under nanoscopic confinement. *J Am Chem Soc* 2004;126:3382–3383.
- [101] Beckmann W, Nickisch K, Budde U. Development of a seeding technique for the crystallization of the metastable A modification of abecarnil. *Org Process Res Dev* 1998;2:298–304.
- [102] Friščić T, MacGillivray LR. Engineering cocrystal and polymorph architecture via pseudoseeding. *Chem Commun*; 2009:773–775.
- [103] Gu CH, Young Jr V, Grant DJW. Polymorph screening: influence of solvents on the rate of solvent-mediated polymorphic transformation. *J Pharm Sci* 2001;90:1878–1890.
- [104] Qu H, Louhi-Kultanen M, Kallas J. Solubility and stability of anhydrate/hydrate in solvent mixtures. *Int J Pharm* 2006;321:101–107.
- [105] Zhu H, Grant DJW. Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 2. Ampicillin. *Int J Pharm* 1996;139:33–43.
- [106] Zhu H, Yuen C, Grant DJW. Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 1. Theophylline. *Int J Pharm* 1996;135:151–160.
- [107] Lee EH, Byrn SR. Stabilization of metastable flufenamic acid by inclusion of mefenamic acid: solid solution or epilayer? *J Pharm Sci* 2010;99:4013–4022.
- [108] Lin SY. An overview of famotidine polymorphs: solid-state characteristics, thermodynamics, polymorphic transformation and quality control. *Pharm Res*; 2014. <http://dx.doi.org/10.1007/s11095-014-1323-5>. in press.
- [109] Chyall LJ, Tower JM, Coates DA, et al. Polymorph generation in capillary spaces: the preparation and structural analysis of a metastable polymorph of nabumetone. *Cryst Growth Deg* 2002;2:505–510.
- [110] Hilden JL, Reyes CE, Kelm MJ, et al. Capillary precipitation of a highly polymorphic organic compound. *Cryst Growth Des* 2003;3:921–926.
- [111] Otsuka M, Ofusa T, Matsuda Y. Effect of environmental humidity on the transformation pathway of carbamazepine polymorphic modifications during grinding. *Colloids Surf B* 1999;13:263–273.
- [112] Matuda Y, Tatsumi E, Chiba E, et al. Kinetic study of the polymorphic transformations of phenylbutazone. *J Pharm Sci* 1984;73:1453–1460.
- [113] Terada K, Kurobe H, Ito M, et al. Polymorphic and pseudomorph transformation behavior of acyclovir based on thermodynamics and crystallography. *J Therm Anal Calorim* 2013;113:1261–1267.
- [114] Garetz BZ, Aber JE, Goddard NL, et al. Nonphotochemical, polarization-dependent, laser-induced nucleation in supersaturated aqueous urea solutions. *Phys Rev Lett* 1996;77:3475–3476.
- [115] Zaccaro J, Matic J, Myerson AS, et al. Nonphotochemical, laser-induced nucleation of supersaturated aqueous glycine produces unexpected gamma-polymorph. *Cryst Growth Des* 2001;1:5–8.
- [116] Pasquali I, Bettini R, Giordano F. Supercritical fluid technologies: an innovative approach for manipulating solid-state of pharmaceuticals. *Adv Drug Deliv Rev* 2008;60:399–410.
- [117] Tozuka Y, Kawada D, Oguchi T, et al. Supercritical carbon dioxide treatment as a method for polymorph preparation of deoxycholic acid. *Int J Pharm* 2003;263:45–50.
- [118] Bettini R, Bonassi L, Castoro V, et al. Solubility and conversion of carbamazepine polymorphs in supercritical carbon dioxide. *Eur J Pharm Sci* 2001;13:281–286.
- [119] Mooij WTM, van Eijck BP, Kroon J. Ab initio crystal structure predictions for flexible hydrogen-bonded molecules. *J Am Chem Soc* 2000;122:3500–3505.
- [120] Price SL. The computational prediction of pharmaceutical crystal structures and polymorphism. *Adv Drug Deliv Rev* 2004;56:301–319.
- [121] Ouvrard C, Price SL. Toward crystal structure prediction for conformationally flexible molecules: the headaches illustrated by aspirin. *Cryst Growth Des* 2004;4:1119–1127.
- [122] Nowell H, Frampton CS, Waite J, et al. Blind crystal structure of a novel second polymorph of 1-hydroxy-7-azabenzotriazole. *Acta Cryst* 2006;B62:642–650.
- [123] Braun DE, Karamertzanis PG, Price SL. Which, if any, hydrates will crystallize? Predicting hydrate formation of two dihydroxybenzoic acids. *Chem Commun* 2011;47:5443–5445.
- [124] Burger A, Ramberger R. On the polymorphism of pharmaceuticals and other molecular crystals. I. *Mikrochim Acta* 1979;II:259–271.
- [125] Burger A, Ramberger R. On the polymorphism of pharmaceuticals and other molecular crystals. II. *Mikrochim Acta* 1979;II:273–316.
- [126] Tozuka Y, Ito A, Seki H, et al. Characterization and quantitation of clarithromycin polymorphs by power X-ray diffractometry and solid-state NMR spectroscopy. *Chem Pharm Bull* 2002;50:1128–1130.
- [127] Dong Z, Munson EJ, Schroeder SA, et al. Neotame anhydrate polymorphs II: quantitation and relative physical stability. *Pharm Res* 2002;19:1259–1264.
- [128] Yu L. Inferring thermodynamic stability relationship of polymorphs from melting data. *J Pharm Sci* 1995;84:966–974.
- [129] Hsieh DS, Sarsfield BA, Davidovich M, et al. Use of enthalpy and Gibbs free energy to evaluate the risk of amorphous formation. *J Pharm Sci* 2010;99:4096–4105.
- [130] Yu L, Stephenson GA, Mitchell CA, et al. Thermochemistry and conformational polymorphism of a hexamorphic crystal system. *J Am Chem Soc* 2000;122:585–591.
- [131] Skorda D, Kontoyannis CG. Identification and quantitative determination of atorvastatin calcium polymorph in tablets using FT-Raman spectroscopy. *Talanta* 2008;74:1066–1070.

- [132] Kojima T, Onoue S, Murase N, et al. Crystalline form information from multiwall plate salt screening by use of Raman microscopy. *Pharm Res* 2006;23:806–812.
- [133] Luner PE, Mehrens S. Application of Raman spectroscopy in establishing solid form integrity for a highly polymorphic API. *Am Pharm Rev* 2013;16:5.
- [134] Burger A, Henck JO, Hetz S, et al. Energy/temperature diagram and compression behavior of the polymorphs of d-mannitol. *J Pharm Sci* 2000;89:457–468.
- [135] Tishmack PA, Bugay DE, Byrn SR. Solid-state nuclear magnetic resonance spectroscopy-pharmaceutical applications. *J Pharm Sci* 2003;92:441–474.
- [136] Suryanarayanan R, Weidmann TS. Quantitation of the relative amounts of anhydrous carbamazepine ($C_{15}H_{12}N_2O$) and carbamazepine dehydrate ($C_{15}H_{12}N_2O \cdot 2H_2O$) in a mixture by solid-state nuclear magnetic resonance (NMR). *Pharm Res* 1990;7:184–187.
- [137] Padden BE, Zell MT, Dong Z, et al. Comparison of solid-state ^{13}C NMR spectroscopy and powder X-ray diffraction for analyzing mixtures of polymorphs of neotame. *Anal Chem* 1999;71:3325–3331.
- [138] Payne RS, Rowe RC, Roberts RJ, et al. Potential polymorphs of aspirin. *J Comput Chem* 1999;20:262–273.
- [139] Vishweshwar P, McMahon JA, Oliveira M, et al. The predictably elusive form II of aspirin. *J Am Chem Soc* 2005;127:16802–16803.
- [140] Bond AD, Boese R, Desiraju GR. On the polymorphism of aspirin: crystalline aspirin as intergrowths of two “polymorphic” domains. *Agnew Chem Int Ed* 2007;46:618–622.
- [141] Varughese S, Kiran MSRN, Solanko KA, et al. Interaction anisotropy and shear instability of aspirin polymorphs established by nanoindentation. *Chem Sci* 2011;2:2236–2242.
- [142] Thomas LH, Wales C, Zhao L, et al. Paracetamol form II: an elusive polymorph through facile multicomponent crystallization routes. *Cryst Growth Des* 2011;11:1450–1452.
- [143] Martino PD, Conflant P, Drache M, et al. Preparation and physical characterization of forms II and III of paracetamol. *J Therm Anal* 1997;48:447–458.
- [144] Szelagiewicz M, Marcolli C, Cianferani S, et al. In situ characterization of polymorphic forms. The potential of Raman techniques. *J Therm Anal Cal* 1999;57:23–43.
- [145] Martino PD, Gyuot-Hermann A-M, Conflant P, et al. A new pure paracetamol for direct compression: the orthorhombic form. *Int J Pharm* 1996;128:1–8.
- [146] Peterson ML, Morrisette SL, McNulty C, et al. Iterative high-throughput polymorphism studies on acetaminophen and an experimentally derived structure for form III. *J Am Chem Soc* 2002;124:10958–10959.
- [147] Inventor: McKenzie AT, Applicant: Warner-Lambert Company. Form III crystalline (R-(R*,R*)-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methyl-ethyl)-3-phenyl-4-phenylamino)carbonyl)-1H-pyreol-1-heptanoic acid hemi calcium salt (Atorvastatin). 1997. WO97/03958.
- [148] Inventor: Briggs CA, Wade RA, Harasawa K, et al. Applicant: Warner-Lambert Company. Crystalline [R-(R*,R*)-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methyl-ethyl)-3-phenyl-4-phenylamino)carbonyl)-1H-pyreol-1-heptanoic acid hemi calcium salt (Atorvastatin). 1997. WO97/03959.
- [149] Inventor: Mathew J, Ganesh S. Applicant: Biocon India Limited. Form V crystalline [R-(R*,R*)-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyreol-1-heptanoic acid hemi calcium salt (Atorvastatin). 2002. WO02/057229 A1.
- [150] Inventor: Teva Pharmaceutical Industries, Inc. Applicant: Teva pharmaceutical Industries, Ind. Atorvastatin hemi-calcium form VII. 2002. WO02/41834 A2.
- [151] Inventor: Byrn SR, Coates D, Andrew G, et al. Applicant: Warner-Lambert Company. Crystalline forms of ‘(R-(R*,R*)-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methyl-ethyl)-3-phenyl-4-phenylamino)carbonyl)-1H-pyreol-1-heptanoic acid calcium salt (2:1) (Atorvastatin). 2003. WO03/004470 A1.
- [152] Inventor: Reddy M. Satyanarayana N, Chakilam S, et al. Applicant: Dr. Reddy’s Laboratories Ltd. Crystalline forms VI and VII of atorvastatin calcium. 2003. WO03/011826 A1.
- [153] Inventors: Faustamann J, Jegorov A. Applicants: Invax Corporation. Crystalline [R-(R*,R*)-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyreol-1-heptanoic acid calcium salt (2:1). 2003. WO03/050085 A1.
- [154] Inventors: Tessler L, Aronhimer J, Lifshitz-Liron R, et al. Novel crystal forms of atorvastatin hemi-calcium and processes for their preparation as well as novel processes for preparing other forms. 2003. US2003/0212279 A1.
- [155] Inventors: Gogulapati B, Panakala R, Chavakula R, et al. Applicant: Matrix Laboratories Ltd. 2007. WO2007/096903 A2.
- [156] Inventors: Aronhime J, Lidor-Hadas R, Niddam-Hildesheim V, et al. Novel crystal forms of atorvastatin hemi-calcium and processes for their preparation as well as novel processes for preparing other forms. 2007. US2007/0265456 A1.
- [157] Inventors: Kumar Y, Kumar S, Madhava D, et al. Applicant: Ranbaxy Laboratories Limited. Crystalline form of atorvastatin hemi calcium. WO2005/090301 A1.
- [158] Inventors: Blatter F, Szelagiewicz M, Van der Schaaf PA. Applicants: Teva Pharmaceuticals Industries Ltd. Crystalline Form. WO2004/040618 A2.
- [159] Inventors: Levi S, Lifshitz-Liron R, Avahar-May-Dan. Applicant: Teva Pharmaceutical Industries Ltd. Crystalline forms of atorvastatin. 2008. WO2008/002655 A2.
- [160] An S-G, Sohn Y-T. Crystal forms of atorvastatin. *Arch Pharm Res* 2009;6:933–936.
- [161] Pandu Ranga Rao V, Somannavar YS, Senthil Kumar N, et al. Preparation of stable new polymorphic form of atorvastatin calcium. *Der Pharm Lett* 2011;3:48–53.
- [162] Jin YS, Ulrich J. New crystalline solvates of atorvastatin calcium. *Chem Eng Technol* 2010;33:839–844.
- [163] Bauer J, Spanton S, Henry R, et al. Ritonavir: an extraordinary example of conformational polymorphism. *Pharm Res* 2001;18:859–866.
- [164] http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002406/WC500132190.pdf.
- [165] Rafilovich M, Bernstein J. Serendipity and four polymorphic structures of benzidine, $C_{12}H_{12}N_2$. *J Am Chem Soc* 2006;128:12185–12191.