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P-2018/15824

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES
E. S. D.

Re: Expediente No. **NC2020/0001111**
Solicitud de patente a nombre de **AMGEN INC.**

Yo, CAROLINA DAZA MONTALVO identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, obrando como apoderada de la sociedad AMGEN INC., domiciliada en Thousand Oaks, California, Estados Unidos de América, solicitante de la patente de la referencia, en respuesta al oficio No. **19767** notificado electrónicamente el 14 de diciembre de 2021, relacionado con los requerimientos de fondo de la solicitud, atentamente me permito presentar la siguiente información:

1. Nivel Inventivo

El examinador establece que las reivindicaciones 1 a 6 carecen de nivel inventivo a la luz de las enseñanzas descritas en la publicación WO2011153509 (D1) y el documento POLYMORPHISM IN PHARMACEUTICAL SOLIDS (D2).

A este respecto, me permito decir que el artículo 18 de la Decisión 486 establece que "*Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica*".

Lo anterior significa que el análisis de nivel inventivo **NO** es una comparación de los objetos de las anterioridades y la solicitud en estudio. El análisis de nivel inventivo **debe demostrar** la obviedad de la invención y establecer cómo ésta no contiene un salto cualitativo, que demuestre su altura inventiva, teniendo en cuenta que se hace **necesario demostrar** que la invención analizada carece de nivel inventivo y hubiese sido obvia para una persona versada en la materia.

De acuerdo con lo anterior, se solicita tener en cuenta los siguientes aspectos sobre el arte previo.

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D1 no describe, enseña o sugiere la forma cristalina anhidra del Compuesto A, y mucho menos las excelentes propiedades de la forma cristalina anhidra ilustrada a continuación.

Por su parte, el documento D2 no soluciona las deficiencias de D1, aunque enseña técnicas comunes para preparar polimorfos.

Como se demuestra a continuación, los expertos en la técnica no conseguirían fácilmente la invención reclamada.

Como se muestra en la Tabla 1A a continuación, el solicitante probó diferentes solventes y concentraciones para la cristalización del Compuesto A sin éxito, dando como resultado solo el Compuesto A amorfo.

Masa del Compuesto A (mg)	Volumen del solvente (mL)	Composición del solvente	Condición	Observación
7.5	1.0	Agua/etanol (90/10 v/v))	Suspensión a temperatura ambiente	Amorfo por XRPD después de 2 meses
8.0	1.0	Agua/dimetilformamida (90/10 v/v))	Suspensión a temperatura ambiente	Amorfo por XRPD después de 2 meses
8.7	1.0	Heptano/tolueno (98/2 v/v))	Suspensión a temperatura ambiente	Amorfo por XRPD después de 2 meses
8.7	1.0	Heptano/metil-t-butiléter (98/2 v/v))	Suspensión a temperatura ambiente	Amorfo por XRPD después de 2

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				meses
9.5	1.0	Ciclohexano/tolueno (98/2 v/v))	Suspensión a temperatura ambiente	Amorfo por XRPD después de 27 días
10.5	1.0	Ciclohexano/metil-t-butiléter (98/2 v/v))	Suspensión a temperatura ambiente	Amorfo por XRPD después de 27 días

Dicha dificultad se demostró adicionalmente en el Ejemplo de la descripción:

> El compuesto amorfo realizado en el procedimiento anterior (Síntesis C) se usó en un tamiz polimorfo de alto rendimiento (HT). Se observó que el material de partida era amorfo por XRPD. En el experimento de cribado de forma, de **192** condiciones analizadas, se observó solo 1 muestra cristalina que representaba una forma mostrada en la Figura 5 (Forma 2 del compuesto A cristalino). La forma identificada por el cribado HTS no es coherente con el compuesto A cristalino anhidro (Ver página 55, primer párrafo de la solicitud PCT, énfasis añadido por nosotros).

En otras palabras, el uso de diferentes solventes y combinaciones de los mismos, y los métodos de cristalización conocidos no dan como resultado la invención reclamada. El solicitante obtuvo finalmente la forma cristalina anhidra del Compuesto A a partir del etanolato del Compuesto A (página 62, último párrafo y página 63, Tabla 3 de la solicitud PCT) no a partir del Compuesto A amorfo. Dicho descubrimiento es sin duda inventivo.

Imprevisibilidad del polimorfismo

El Examinador opina que D2 enseña técnicas comunes para la obtención de polimorfos, y tales técnicas comunes en combinación con D1 llegarían a la invención reivindicada. El solicitante respetuosamente no está de acuerdo.

La suposición subyacente del examinador es contraria al conocimiento común en la técnica sobre el polimorfismo. Las referencias adjuntas en su idioma original y de las

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cuales se resumen a continuación, describen la imprevisibilidad del polimorfismo de un compuesto conocido.

1. En general, sigue siendo imposible predecir la estructura, incluso de la forma cristalina más simple a partir del conocimiento de su composición química (Nature, vol. 335, 1988, p. 201).

2. Si bien puede no ser sorprendente que se haya encontrado que muchos materiales farmacéuticamente importantes son polimórficos, o que cualquier compuesto en particular puede resultar polimórfico, cada compuesto es esencialmente una situación nueva, y el conocimiento y la comprensión más avanzados de la El fenómeno del polimorfismo sigue siendo tal que no podemos predecir con ningún grado de confianza si un compuesto es polimórfico, prescribir cómo hacer posibles polimorfos (desconocidos) o predecir cuáles pueden ser sus propiedades (Polymorphism in Molecular Crystals, 2002, p. 241) .

3. Como resultado, hay muy poca información disponible para guiar de manera general la predicción de la estabilidad de los compuestos en estado sólido, y mucho menos en sus diferentes formas sólidas o diferentes formulaciones (Chemical Master, vol. 6, 1994, pág. 1149).

4. Las sustancias farmacológicas sólidas muestran una variedad amplia y en gran parte impredecible de propiedades de estado sólido (Pharmaceutical Research, vol. 12, 1995, p. 945).

Como se describe en las referencias anteriores, se sabe que se pueden obtener varios polimorfos cristalinos dependiendo de varios disolventes y condiciones. Sin embargo, no se sabe ni es obvio obtener realmente un polimorfo cristalino de un compuesto conocido debido a la imprevisibilidad de las propiedades físicas del compuesto como lo evidencian las referencias citadas antes mencionadas.

Además, para ejemplos como se evidencia en la referencia adjunta (Chemical Master, vol. 6, 1994, p. 1150), varios factores pueden afectar la formación de polimorfos. Dichos factores son la composición o polaridad del disolvente, la concentración o el grado de sobresaturación, la temperatura, incluida la velocidad de enfriamiento y el perfil de enfriamiento, los aditivos, las semillas y la presencia de semillas, el pH y la agitación. Por ejemplo, se puede obtener un tipo de polimorfo cristalino enfriando soluciones saturadas calientes, y se puede obtener un tipo diferente de polimorfo cristalino

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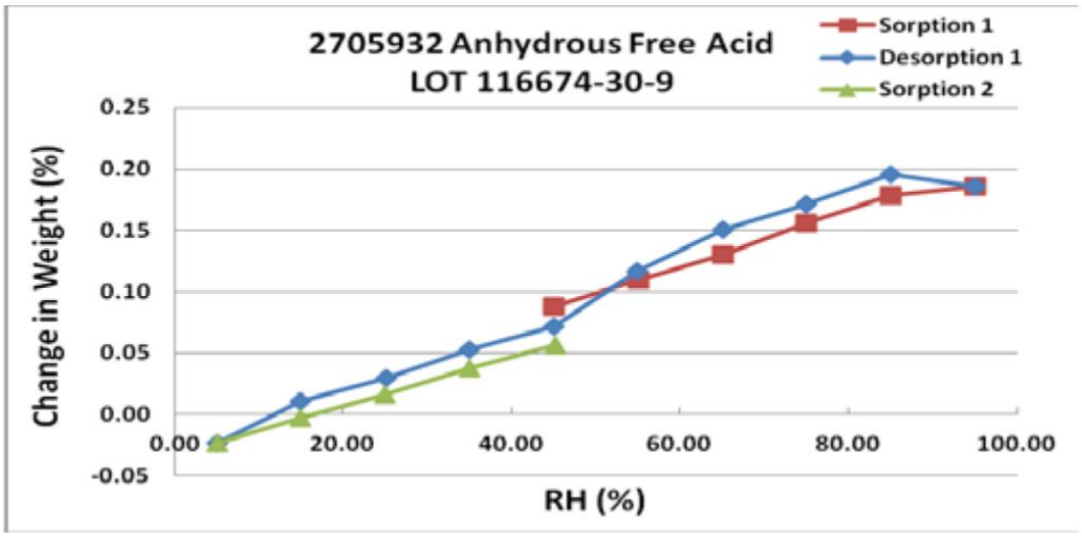
evaporando parcialmente una solución saturada clara, incluso si se usa el mismo solvente en ambos métodos. Dados esos diversos factores que contribuyen a la formación de un polimorfo cristalino, existe un número infinito de combinaciones posibles. Por lo tanto, la formación de una determinada forma de polimorfo cristalino requiere un esfuerzo inmenso y la superación de obstáculos desafiantes e impredecibles, que no deberían considerarse obvios para una persona con conocimientos ordinarios en la técnica.

Propiedades ventajosas de la forma cristalina anhidra del Compuesto A

A continuación, se presentan las siguientes propiedades ventajosas del cristalino anhidro del Compuesto A.

Higroscopicidad

El cristalino anhidro del Compuesto A no es higroscópico con un cambio del 0.2% en peso entre 0 y 95% de humedad relativa a 25°C, como se muestra en los datos siguientes.



Estabilidad de estado sólido

Como se muestra en los datos de estabilidad de sólidos a continuación, la forma cristalina anhidra del Compuesto A es estable en condiciones aceleradas (plato abierto; 25°C / 60% RH, 40°C / 75% RH y 60°C / RH ambiente) para al menos 6 semanas, sin degradación química ni cambios en la forma de los cristales. Por lo tanto, se espera que el cristalino anhidro del Compuesto A sea estable cuando se almacena en condiciones ambientales.

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Condición	Vial Abierto/Cerrado	Tiempo (semanas)	Estabilidad
25C / 60% RH	Ambos	6	no se detectaron degradantes sin cambios en la cristalinidad
40C / 75% RH	Ambos	6	no se detectaron degradantes sin cambios en la cristalinidad
60C	ambos	6	no se detectaron degradantes sin cambios en la cristalinidad

Estabilidad de la solución

Como se muestra en los datos de estabilidad a continuación, la forma cristalina anhidra del Compuesto A es estable en varias soluciones bajo diferentes condiciones. No se detectan degradantes después de 14 días a 40°C.

Media*	Temperatura (°C)	Tiempo (días)	Estabilidad
0.3M HCl	40	14	Estable, no se detectaron degradantes
9.5mM amortiguador de fosfato, pH 7	40	14	Estable, no se detectaron degradantes
9.5mM amortiguador de fosfato, pH 12	40	14	Estable, no se detectaron degradantes
0.25mM de óxido de hierro (II), 0.15 H2O2	40	14	Estable, no se detectaron degradantes
0.95mM amortiguador de fosfato, pH 7	2X ICH		Estable, no se detectaron degradantes

*Contiene 50% de acetonitrilo, ~50ug/mL 2705932

Adecuado para el desarrollo de fármacos

Debido a la forma sólida ventajosa y la estabilidad de la solución, incluida la higroscopicidad, el cristalino anhidro del Compuesto A es adecuado para un mayor desarrollo de fármacos para el tratamiento del cáncer en humanos, como lo demuestran los siete ensayos clínicos en humanos descritos en http://www.clinicaltrials.gov, los cuales se adjuntan al presente memorial.

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En resumen, el solicitante descubre el cristalino anhidro del compuesto A a través de una extensa investigación. Las propiedades ventajosas del cristalino anhidro del Compuesto A conducen a un mayor desarrollo para uso humano en el tratamiento del cáncer. Por consiguiente, la invención reclamada es inventiva.

De esta forma, teniendo en cuenta que los documentos D1 y D2 **no mencionan, describen o sugieren**, en forma individual o en combinación, todas las características técnicas reclamadas en el nuevo capítulo reivindicatorio presentado por el solicitante, **las nuevas reivindicaciones presentadas son novedosas, e inventivas sobre el estado del arte**.

En este sentido, la presente invención tal y como se encuentra definida en las reivindicaciones 1 a 6, tiene diferencias con el estado del arte e implicó un nivel inventivo durante su desarrollo, por lo cual debe ser considerada como novedosa e inventiva según las disposiciones de los artículos 16 y 18 de la Decisión 486.

2. Conclusión

Con las modificaciones introducidas y los argumentos presentados puede concluirse que las reivindicaciones que se encuentran en trámite son patentables sobre D1 y D2, cumpliendo así con los requerimientos del Artículo 18 de la Decisión 486.

Por tanto, atentamente me permito solicitar a ese Despacho otorgar el privilegio de patente solicitado a la solicitud en estudio, toda vez que cumple a cabalidad con los requerimientos de la Decisión 486/00.

En consecuencia, solicitamos a ese Despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

3. Anexos

- Lu J. & Rohani S. (2009) *Polymorphism and Crystallization of Active Pharmaceutical Ingredients.*
- Pharmaceutical_Research
- Byrn S., et al. (1995) *Pharmaceutical Solid : A strategic approach to Regulatory Considerations*. Pharmaceutical Research, Vol 12, No. 7, 1195.

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- Moddix J. (1988) *Crystals from first principles*. Nature Vol 335, 15 September 1988.
- Byrn S., et al. (1994). Solid-State Pharmaceutical Chemistry. Chem Mater. 1994, 6, 1148-1158.

Señor Director,

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 Current Medicinal Chemistry, 2009, *16*, 884-905

Polymorphism and Crystallization of Active Pharmaceutical Ingredients (APIs)

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Abstract: Active pharmaceutical ingredients (APIs), frequently delivered to the patient in the solid-state as part of an approved dosage form, can exist in such diverse solid forms as polymorphs, pseudopolymorphs, salts, co-crystals and amorphous solids. Various solid forms often display different mechanical, thermal, physical and chemical properties that can remarkably influence the bioavailability, hygroscopicity, stability and other performance characteristics of the drug. Hence, a thorough understanding of the relationship between the particular solid form of an active pharmaceutical ingredient (API) and its functional properties is important in selecting the most suitable form of the API for development into a drug product. In past decades, there have been significant efforts on the discovery, selection and control of the solid forms of APIs and bulk drugs. This contribution discusses the thermodynamics and kinetics of polymorphic systems, the characterization of polymorphs, and the transformation between polymorphs. The major techniques for polymorph discovery and control developed in the past years are discussed as well.

Keywords: Crystallization, polymorphism, pseudopolymorphism, active pharmaceutical ingredients (APIs), discovery, control.

1. INTRODUCTION

Active pharmaceutical ingredients (APIs) are frequently delivered to the patient in the solid-state as part of such dosage forms as tablets, capsules, granules, powders, etc [1]. No matter whether as pure drug substances or in formulated products, APIs can exist in various solid forms, such as polymorphs, pseudopolymorphs (solvates and hydrates), salts, co-crystals and amorphous solids. Each form may possess its own unique mechanical, thermal, physical and chemical properties that can remarkably affect the solubility, bioavailability, hygroscopicity, melting point, stability, compressibility and other performance characteristics of the drug [2]. Hence, a thorough understanding of the relationship between the particular solid form of an API and its functional properties is crucial for selecting the most suitable form of the API for development into a drug product.

Crystallization refers to the formation of solid particles from a vapor, the solidification from a liquid melt or the formation of dispersed solids from a solution. Crystallization, incorporating wider definition to include precipitation and solid-state transitions, is a major technological process for particle formation in pharmaceutical industry [3]. It is estimated that over 70% of all solid materials are produced by crystallization. As a matter of fact crystallization plays a key role in defining the physicochemical properties of solid APIs and their final dosage forms.

An understanding of the solid state leads to a full acquirement of the drug properties, which is critical for many of the activities of the pharmaceutical industry. In this review, we focus on the polymorphism of active pharmaceutical ingredients. The identification of polymorphic system and the characterization of polymorphs are emphasized. The development in polymorph-selective crystallization technologies has been presented in detail. The thermodynamic and kinetic factors that can affect the polymorph of final product are discussed. The polymorphic transformation and the computational approaches for predicting crystal structures are also included.

2. SOLID FORMS OF ACTIVE PHARMACEUTICAL INGREDIENTS

The solid forms in which an API may present include polymorphs, solvates, hydrates, salts, co-crystals and amorphous solids, as shown in Fig. (**1**). Discovery and identification of the solid forms of the active pharmaceutical ingredient provide various options to develop it into the drug product. The choice and design of the preferred form should be based on a comparison of the physico-chemical properties, of which the desired properties may lie on the mode of delivery (e.g., oral, pulmonary, nasal, rectal, transdermal, etc.). Therefore the preferred solid form may differ for each optimized dosage form [1].

2.1. Polymorphs

Polymorphism may be defined as the ability of a compound to exist in two or more crystalline forms in which the molecules have different arrangements (packing polymorphism) and/or conformations (conformational polymorphism) in the crystal lattice [4,5]. In other words, polymorphs have the same chemical composition, different lattice structures and/or different molecular conformations [6-10]. Polymorphism is a widespread phenomenon observed for more than half of all active pharmaceutical ingredients [4,11]. So far, only a small quantity of medicinally active substances can be considered for a practical purpose to be non-polymorphic, e.g., aspirin [12,13]. There still is a theoretical possibility that those non-polymorphic organic compounds may have potential polymorphs [1].

Polymorphs generally have different physical and chemical properties resulting in different stability and bioavailability of drug products [14]. Mebendazole, a kind of broad-spectrum anthelmintic drug against the infestations by as-

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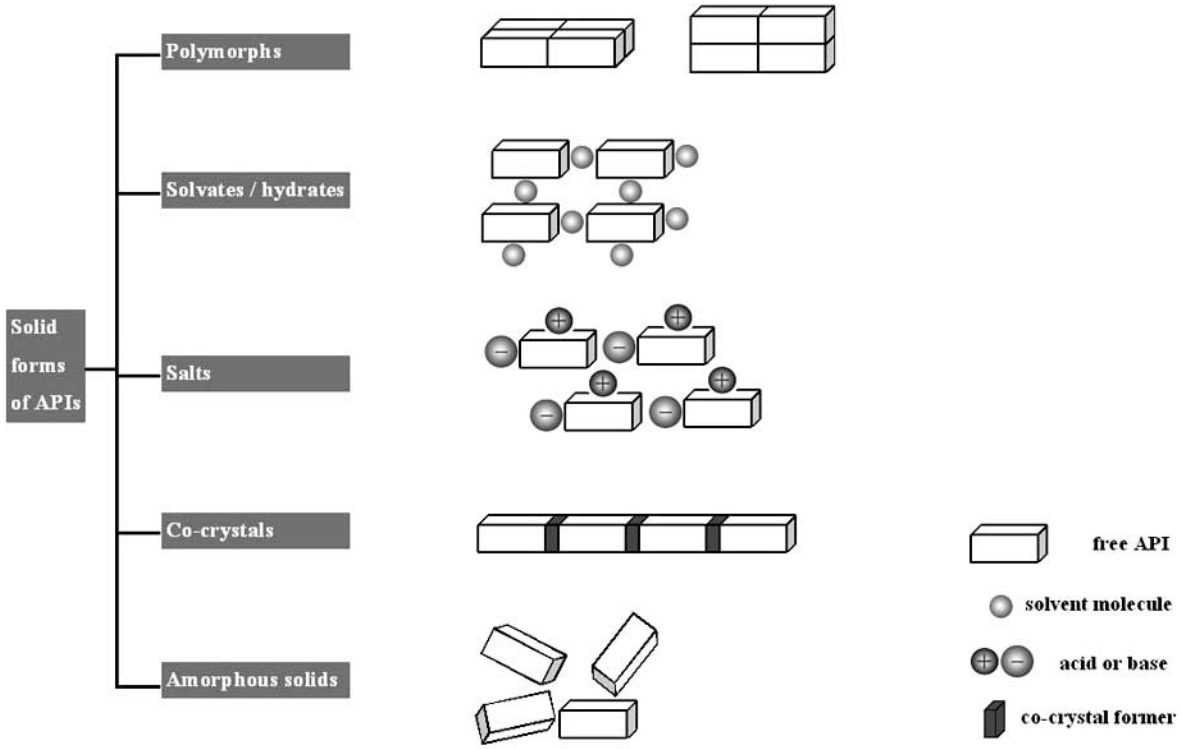

Fig. (1). Schematic representation of the structures of solid forms of APIs.

caris, threadworms, hookworms and whipworms, has been found to have three polymorphic forms (A, B, C) displaying solubility and therapeutic differences [15]. The polymorphs differ with respect to their spectral and thermal properties as well. The solubility of the three polymorphs of mebendazole in 0.03M hydrochloric acid is in the order A, C, B. Solubility studies and clinical trials have shown that polymorph C is therapeutically favored [16]. As to the two polymorphs of sulfamerazine, Sun and Grant [17] have demonstrated that polymorph I possesses greater plasticity, compressibility and tabletability than polymorph II.

One of the most well-known examples of the evolution of polymorphic APIs into the marketed drug products is Ritonavir (marketed as Norvir® by Abbott Laboratories). Since the original Norvir® capsule entered into the market, a previously unknown but thermodynamically more stable polymorph (form II) of Ritonavir was discovered. This new form was approximately 50% less soluble in the hydroalcoholic formulation vehicle than form I. Then the original Norvir® capsule was eventually withdrawn from the market [18], and a new formulation of Norvir® using form II was launched [19].

2.2. Pseudopolymorphs

Pseudopolymorphism may be defined as crystalline forms of a compound in which solvent molecules are included as an integral part of the structure [20,21]. Pseudopolymorphs (solvates and hydrates) can be stoichiometric or nonstoichiometric in nature [22]. The propensity of an API molecule to form pseudopolymorphs is deemed to be relevant to molecular structures, hydrogen bonding ability, and crystal packing [23-26].

Pseudopolymorphs generally show different solubilities, dissolution rates, mechanical behavior, stability and bioavailability from their unsolvated counterparts [27]. For example, caffeine hydrate is much less soluble in water than anhydrous caffeine, but the hydrate is much more soluble in ethanol than anhydrous caffeine [28]. The monohydrate of theophylline possesses higher mechanical strength than anhydrous theophylline, resulting from a large number of intermolecular hydrogen bonds in its crystal structure [29]. The weaker mechanical strength of the anhydrate makes it more brittle than the monohydrate. Meanwhile, Liu *et al.* [30] have further demonstrated that there exist crystallinity differences between the anhydrous and hydrated forms of caffeine and theophylline.

The propensity of solvents to be included in molecular crystals closely links with their ability to effectively participate in hydrogen bonding. Multi-point recognition with strong and weak hydrogen bonds between solvent and solute molecules can facilitate the retention of organic solvents in crystals [20]. During a crystallization process the pre-nucleation solute–solvent aggregates contain solute–solute, solute–solvent and solvent–solvent interactions. Strong solute–solvent interactions shall result in the nucleation of solvated crystals [6]. The water molecule, because of its small size, activity and ability to act as both a hydrogen bond donor and acceptor, is found to be more capable of linking to drug molecules to form new crystal structures than any other solvent. Approximately one-third of active pharmaceutical ingredients can form crystalline hydrates [31]. Based on the location of water in their structures, crystalline hydrates can be categorized into three categories [32,33]: (i) isolated lattice site hydrates, e.g. cephadrine dehydrate [34]; (ii) lattice channel hydrates, e.g. theophylline monohydrate [35]; (iii)

metal-ion coordinated hydrates, e.g. dihydrate and trihydrate of disodium adenosine 5′-triphosphate [36]. Owing to the multiple roles of water, some hydrates can be classified into more than one category [37].

Solvates and hydrates may be either final or intermediate products of crystallization, and can transform into higher (or lower) solvates or anhydrous "desolvated" forms [3]. Choice of development of solvated or unsolvated form of an API shall depend upon its pharmaceutical properties, how long and under what conditions it can survive [6].

2.3. Salts

During the selection of the most suitable form of the active pharmaceutical ingredient for development into a drug product, the desirable solid form is generally its thermodynamically most stable crystalline form [2]. But the stable crystal form frequently presents insufficient solubility or dissolution rate leading to a poor bioavailability. In this case, alternative solid forms should be considered. As for those ionizable compounds, preparing their salt forms by use of pharmaceutically acceptable acids or bases is a universal approach to modulate solubility (or dissolution rate) [38], to increase chemical stability [39], to improve bioavailability [40] or to enhance manufacturability [1].

Several strategies for performing the salt selection process have been developed, such as in-situ salt screening technique for ranking the solubility of salts [41], the multi-tier approach developed by Morris *et al.* [42] for the selection of optimal salt form for a new drug candidate. Moreover, the selection process of pharmaceutical salts can be facilitated by use of the pKa and pH–solubility curve of the drug [43], as the type and number of salts that will be formed can thus be greatly narrowed down [6,38].

The presence of ions shall greatly influence the physicochemical properties of the crystals of formed salts, including solubility, dissolution rate, hygroscopicity, degree of crystallinity, crystal habit, and physical and chemical stability [44,45]. On the basis of these properties, their suitability for development can be assessed. It is worth noting that, like their parent compounds, pharmaceutical salts may exist in several polymorphic, solvated and/or hydrated forms [1]. For example, ranitidine hydrochloride (RAN-HCl) has been found to have two polymorphic forms and tautomerism is considered as the main reason of structural differences in the solid state of RAN-HCl [46]. Theoretically, RAN-HCl can have three tautomers (enamine, nitronic acid and imine) in the solution. Mirmehrabi and Rohani [47] have demonstrated that solvents that are strong hydrogen bond donors such as methanol and water interact with nitro group of nitroethenediamine moiety and favor the formation of nitronic acid tautomer, and nitronic acid is the predominant tautomer of form 2 crystals. On the other hand, form 1 contains the enamine tautomer, and weak hydrogen bond donor solvents or aprotic solvents favor formation of enamine tautomer and subsequently form 1.

Sertraline HCl, the active ingredient in the antidepressant Zoloft®, is an example of a salt form that is highly polymorphic and prone to solvate formation. So far 28 forms of sertraline HCl have been disclosed by several companies, including 17 polymorphs, 4 solvates, 6 hydrates and the amorphous solid. Almarsson *et al.* [48] and Remenar *et al.* [49] have applied high-throughput (HT) crystallization technique into the salt screening for sertraline HCl. They have demonstrated that seemingly minor differences in salt former can have profound effects on the number of polymorphs and solvates that can be found in the corresponding salts.

2.4. Co-Crystals

Co-crystals consist of two or more components that are solid at room temperature. The primary difference between co-crystals and salts is that in salts a proton is transferred from the acidic to the basic functionality of the crystallization partner, or vice versa, whereas in co-crystals no such transfer occurs [50]. On the other hand, the main difference between co-crystals and solvates is the physical state of the separate pure components at room temperature: if one component is in liquid state, the crystals are designated as solvates; if both components are in solid state, the crystals are designated as co-crystals [1]. So far co-crystals have been increasingly recognized as an attractive alternative for solid forms of drug products [51]. Formation of co-crystals using an API with the excipient [52], or with other component [53,54], can provide an opportunity to design drug delivery systems at the molecular level and to improve some pharmaceutical properties of the API [6,55].

Co-crystals containing APIs represent a new type of pharmaceutical materials. In addition to potential improvements in solubility, bioavailability, and stability, co-crystals may enhance a large number and variety of essential properties, including hygroscopicity, compressability, and flowability [56]. A well-documented case is itraconazole, a poorly water-soluble antifungal drug, which can form co-crystals with various pharmaceutically acceptable acids, such as fumaric acid, succinic acid and L-, D- or DL-tartaric acid. Different carboxylic acid co-crystals exhibit a higher solubility and a faster dissolution rate than the free itraconazole [5]. The co-crystals of aspirin, *rac*-ibuprofen and *rac*-flurbiprofen have been prepared by disrupting the carboxylic acid dimers using 4,4V-bipyridine [57].

Co-crystals can be prepared by melt-crystallization [58], grinding [59] and recrystallization from solvents [60]. When solution crystallization is employed, the co-crystals' domain of existence can be described by the ternary phase diagram (solvent, molecule, co-crystal former), and the solvent for the co-crystals must dissolve all components, but must not interfere with the interactions necessary for co-crystals' formation. The screening of co-crystals is thus performed within the given co-crystals' domain of existence [61]. It is worth noting that co-crystals can also form solvates and exhibit polymorphism.

2.5. Amorphous Solids

Amorphous solids consist of disordered arrangements of molecules and do not possess a distinguishable crystal lattice [62]. Amorphous solids lack the three-dimensional long-range order of molecular packing or well-defined molecular conformation if the constituent molecules are conformationally flexible, but may have short-range order [63, 64].

Amorphous solid states of an API are far from equilibrium than its crystalline counterparts, and possess higher energy. Amorphous solids normally have desirable pharmaceutical properties such as higher solubility [65], faster dissolution rate [66], improved bioavailability [67,68] and mechanical properties [69] compared to their crystalline counterparts. One distinguished example of the applications of amorphous APIs is the formulation of insulin suspensions. Various proportions of amorphous, crystalline and complexed forms of insulin have been marketed to achieve short, intermediate and long acting. However, amorphous solids are less physically and chemically stable than their crystalline counterparts: (i) very apt to crystallize at a later stage during product shelf life [70]; (ii) often reactive and unstable to mechanical and thermal stresses [71]; and (iii) extraordinarily sensitive to water sorption [72]. The instability is the major reason that amorphous pharmaceutical solids are not marketed as widely as the crystalline forms [5,6,62].

Amorphous solids can be prepared by the following processes: (a) rapid precipitation by antisolvent addition [73,74]; (b) quenching a melt by rapid cooling [75]; (c) freeze-drying [76]; (d) spray-drying [77]; (e) fast evaporation of solvent in liquid solution [78]; (f) introduction of impurities [79]; (g) milling or grinding crystalline solids at low temperatures [80]; (h) desolvation of crystalline materials [81]; and (i) production by solid-dispersion [82]. For example, dehydration of crystalline hydrates has been demonstrated as a feasible and "gentle" route to the amorphous state of organic solids [62]. The amorphous form of raffinose and carbamazepine can be produced by the dehydration of their pentahydrate [83] and dihydrate [84], respectively. In general, production of amorphous solids is compound-specific. Relatively large and/or flexible molecules tend to form a disordered state even at mild crystallization conditions [3].

The mechanism of the formation of amorphous solids is still not quite clear [3]. Current research in the crystallization and stabilization of amorphous solids focuses on: (i) the understanding of crystallization kinetics of the amorphous state (amorphous crystallization); (ii) the stabilization of labile substances during processing and storage by use of additives; (iii) the interactions between APIs and excipients; and (iv) the selection of appropriate storage conditions under which amorphous solids are stable [62].

3. THERMODYNAMICS

As for a polymorphic system, different crystallization operating parameters normally result in different crystalline forms. Hence the control of polymorphic form *via* crystallization requires a full understanding of the nucleation, crystal growth, and phase transformation in the crystallization sequence. The knowledge about thermodynamic and kinetic properties of the polymorphic system is essential for the control of polymorphic crystallization.

3.1. Polymorphic Nature and Thermodynamic Stability

The Gibbs free energy change ΔG_C of a crystallization process at constant temperature and pressure is

$$\Delta G_C = \Delta H_C - T\Delta S_C \quad (1)$$

where ΔH_C, ΔS_C are the enthalpy change and the entropy change of the crystallization process respectively. From the thermodynamic viewpoint, for a polymorphic system, the minimization of ΔG_C is the classical thermodynamic driver, which leads to the formation of stable form, whilst the maximization of the rate of entropy production is the driver in irreversible thermodynamics, which will lead to the formation of less stable form. The equilibrium composition of the polymorphic mixture will depend on the rate at which excess energy is applied to the system [85].

Meanwhile, the relative thermodynamic stability of polymorphs and the driving force for a transformation at constant temperature and pressure is also determined by the difference in Gibbs free energy between the polymorphs ΔG_T and has

$$\Delta G_T = \Delta H_T - T\Delta S_T \quad (2)$$

where ΔH_T, the enthalpy difference between the polymorphs, reflects the lattice or structural energy differences and the entropy difference; ΔS_T, the entropy difference between the polymorphs, is related to the disorder and lattice vibrations. When $\Delta G_T < 0$, the transformation can occur spontaneously; $\Delta G_T = 0$, the free energy of the two phases is the same; $\Delta G_T > 0$, the spontaneous transformation is not possible under the specific conditions.

According to the corresponding thermodynamic relationships, polymorphs can be classified as either enantiotropes or monotropes, depending on whether or not one form can transform reversibly to another [86–89]. As for a single-component and dimorphic system, three types of Gibbs free energy versus temperature phase diagram are schematized in Fig. (**2**). Fig. (**2a**) represents a monotropic system, in which the liquidus line intersects the curves at temperatures lower than the thermodynamic equilibration temperature. In other words, one of the forms is always stable below the melting points of both forms. As illustrated in Fig. (**2a**), the free energy of form A is always lower than that of form B at all temperatures below $T_{m,\,A}$. Consequently, form B can undergo a spontaneous exothermic transformation to form A at any temperature. Furthermore, in this case, crystallization of the two forms is possible, when the rate of the solid-state transformation is lower than that of the crystallization.

As shown in Fig. (**2b**) and (**2c**), the liquidus line intersects the curves at temperatures greater than the thermodynamic equilibration temperature, thus the system possesses the enantiotropic nature. Below $T_{t,\,A\text{-}B}$, form A is stable because the free energy of form A is lower than that of form B, and form B can undergo spontaneous exothermic transformation into form A. Above $T_{t,\,A\text{-}B}$, form B is the stable solid phase because its free energy is lower than that of form A, and form A can undergo spontaneous endothermic transformation into form B. It is worth noting that, as shown in Fig.

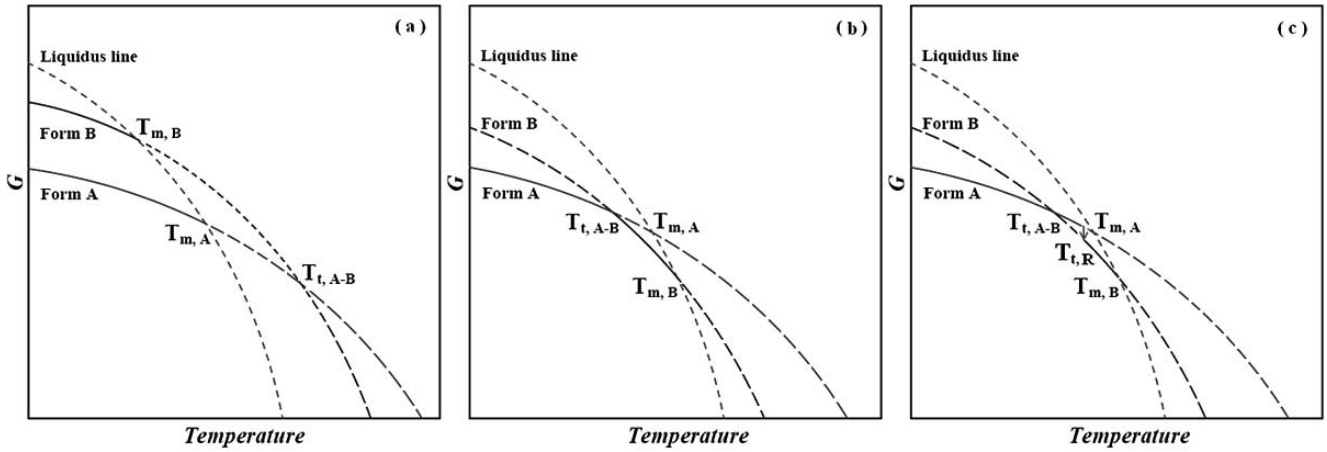

Fig. (2). Gibbs free energy curves for dimorphic systems: (**a**) monotropic; (**b**) and (**c**) enantiotropic. Melting points, T_m, for the crystalline phases are shown by the intersection of the curves for the crystalline and liquidus states. Thermodynamic equilibration temperature, $T_{t,A-B}$, of the forms A and B are shown by the intersection of the curves for two crystalline states. $T_{t,R}$ is the real transition temperature [90].

(**2c**), the solid-state transformation sometimes is hindered by steric hindrance. In this case, the real transition temperature $T_{t,R}$ is different from the thermodynamic equilibration temperature (i.e. theoretical transition temperature) $T_{t,A-B}$, and simultaneous occurrence of the two forms during crystallization is also possible [91].

3.2. Prediction Rules

As shown in Eq. (2) and Fig. (**2**), the relative stability of polymorphs depends on their free energies, such that a more stable polymorph has a lower free energy [92,93]. Under a specific condition, only one polymorph has the lowest free energy. This polymorph is designated as the thermodynamically stable form and the other polymorphs are termed as metastable forms. However, the exact course of *G* isobars cannot be followed experimentally, since the entropy cannot be determined. Therefore, a series of rules have been developed for predicting the relative thermodynamic stability of polymorphs and the nature of polymorphic system [8,89].

Heat-of-Transition Rule

This rule generalizes that, if an endothermic enthalpy of phase transition between two crystal forms is observed at a specific temperature, so then there is a transition point below this temperature and the two polymorphs are enantiotropically related. If an exothermic enthalpy of phase transition is observed at a particular temperature, and there is no transition at higher temperatures, the two polymorphs may be monotropically related [86,87].

Heat-of-Fusion Rule

The heat-of-fusion rule indicates that in an enantiotropic system the higher-melting polymorph of a pair will have the lower enthalpy of fusion. If the higher-melting polymorph also has the higher enthalpy of fusion, the two polymorphs are monotropically related. The rule will be valid so long as the Gibbs energy profiles of dimorphic systems can be described as Fig. (**3**) [89].

Entropy-of-Fusion Rule

The melting point is defined as the temperature at which the liquid is in equilibrium with the solid so that the difference in Gibbs free energy between the two phases in zero. The entropy of fusion ΔS_f can then be expressed as

$$\Delta S_f = \frac{\Delta H_f}{T_f} \tag{3}$$

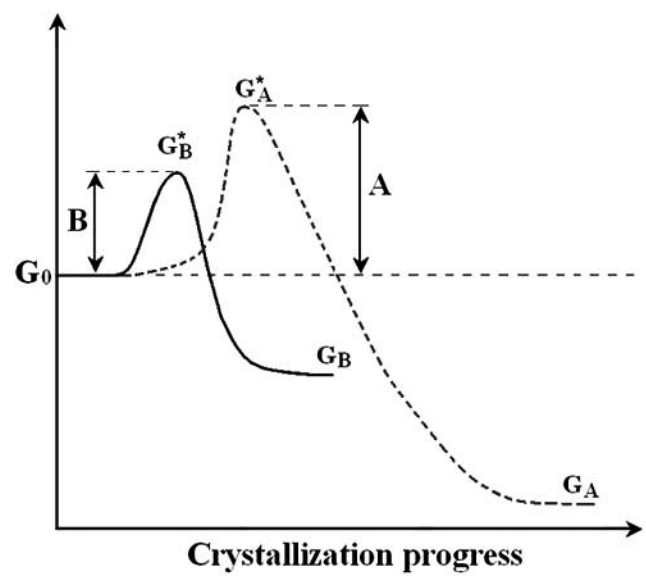

Fig. (3). Schematic diagram for the crystallization progress in a dimorphic system from the initial state, G_0, to two different polymorphs A or B. [6,8].

According to the rule, if a polymorph has the higher melting point but has the lower entropy of fusion, the two polymorphs are enantiotropically related. Monotropism is inherent if the lower melting polymorph has the lower entropy of fusion [94].

Heat-Capacity Rule

At a given temperature, if one polymorph has both the higher melting point and the higher heat capacity than another polymorph, these two polymorphs are enantiotropically related. For a pair of polymorphs, if the polymorph with the higher melting point also has a higher heat capacity at a given temperature, there exists an enantiotropic relationship between them. Otherwise, the system is monotropic [89,92].

Enthalpy-of-Sublimation Rule

If the polymorph with the higher melting point has the lower enthalpy of sublimation, the two polymorphs are enantiotropic. Monotropism is realized if the lower melting form shows the lower enthalpy of fusion [8].

Density Rule

The most energetically stable structure is expected to correspond to the one that has the most efficient packing. The two polymorphs are monotropically related if the polymorph with higher melting point possesses the higher density. Otherwise they are enantiotropically related [8]. This rule is quit general for ordered molecular solids that are dominated by van der Waals interactions. Exceptions such as acetazolamide [95], are not unexpected when other interactions, such as hydrogen bonds, dominate the packing, since some energetically favourable hydrogen-bond dominated packing arrangements can lead to large voids in the crystal structure with correspondingly lower density [86].

Infrared Rule

The rule is normally for the hydrogen-bonded crystals. For the highest frequency infrared absorption band in polymorphic structure containing strong hydrogen bonds. The formation of strong hydrogen bonds is associated with a reduction in entropy and an increase in the frequency of the vibrational modes of those same hydrogen bonds. The hydrogen-bonded polymorphic structure with the higher frequency in the bond stretching modes may be assumed to have the larger entropy [96].

Solubility Rule

Since the solubility is directly proportional to the free energy of a polymorph, determination of solubility is the most reliable method of assessing ΔG_T between polymorphs. Generally the stable form has a lower solubility. It is important to note that although the absolute solubility of a polymorph will be solvent dependent, the relative solubility of different forms will not depend on the solvent used [8].

If one form with a higher melting point has a higher solubility at temperatures above the transition temperature, polymorphs are enantiotropic. When the polymorphs are monotropic, the solubility of one form with the higher melting point is always lower than another form with the lower melting point [89].

4. KINETIC CONSIDERATION ON POLYMORPHIC CRYSTALLIZATION

4.1. Nucleation of Polymorphs

Crystallization is a complicated process of molecular assembly with a precise packing arrangement. In the classical nucleation theory [97], the nucleus is assumed to be spherical, and then the homogeneous nucleation rate is given by [98]

$$J = A_n \exp\left[\frac{-\Delta G^*_{\text{hom}}}{RT}\right] = A_n \exp\left[-\left(\frac{16\pi M^2\gamma^3 N_A}{3R^3T^3\rho^2}\right)\ln^{-2} S\right] \quad (4)$$

where ΔG^*_{hom} is the energy barrier for homogeneous nucleation, J is the nucleation rate, A_n is the collision factor, γ is the interfacial energy, S is the supersaturation ratio, N_A is the Avogadro's Number.

The radius R^* of the critical nuclei for homogeneous nucleation is given by:

$$R^* = \frac{2\Omega\gamma}{\Delta\mu} \quad (5)$$

$$\Delta\mu \approx kT \ln S \quad (6)$$

where Ω is the molar volume, k is the Boltzmann's constant.

Heterogeneous nucleation on a surface is generally considered to be energetically less demanding than homogeneous nucleation due to lowering of the surface energy of the nucleus on the substrate upon interfacial contact [99]. Hence

$$\Delta G^*_{het} = \phi \Delta G^*_{\text{hom}} \quad (7)$$

where ϕ is the ratio of the Gibbs free energy of heterogeneous nucleation to homogeneous nucleation.

The energy-reaction coordinate diagram of a classical dimorphic crystallization process can be schematically presented by Fig. (**3**). Starting from a supersaturated solution of which the free energy per mole of a solute is termed as G_0, form A or B can nucleate. In Fig. (**3**), form A is more stable and less soluble than form B. The energy barrier for the nucleation of form A ($G^*_A - G_0$) is greater than that for form B, ($G^*_B - G_0$), and the supersaturation with respect to form B (simplified as $G_0 - G_B$) is lower than $G_0 - G_A$ for form A. According to the classical theory of nucleation from homogeneous solutions, the size of critical nuclei (critical size) is dependent on the level of supersaturation. The higher the level of supersaturation, the smaller this size is. Based on these considerations either form A or form B can nucleate. When form B is kinetics favorable under specific conditions, form B will preferably nucleate and transform to form A.

The polymorph of final product will depend on both crystallization and transformation rates.

As to a dimorphic system with the monotropic nature (Fig. **4**), when a solution equilibrated at A_0 is cooled and nucleates at A_1, only stable form A can nucleate. The polymorph of metastable form B preferably nucleates when nucleation points A_2 or A_3 are located upper the metastable limit of polymorph B. If the transformation rate from polymorph B to polymorph A is far lower than the crystallization rate of polymorph B, e.g. at point A_3, pure polymorph B can be collected. Otherwise the final product may be the mixture of both polymorphs.

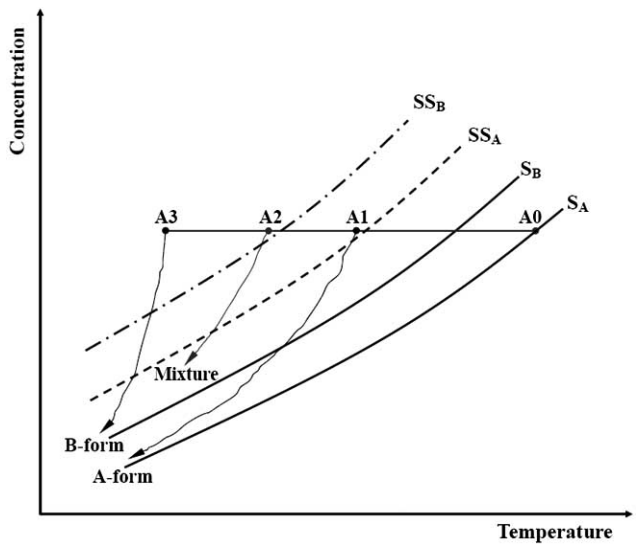

Fig. (4). Polymorphic system of two monotropically related polymorphs A and B. Full lines S_A and S_B are solubility curves respectively; dashed lines SS_A and SS_B are metastable limits (supersaturation).

Furthermore, it is necessary to take account of the structural elements of molecular assembly processes that determinate the formation of nuclei and how they are controlled. From the viewpoint of structure, the polymorph that will nucleate preferentially from a melt or solution may be the one whose energy barrier is smallest so as to be most easily formed or the one whose structural organization is most readily derived from the molecular arrangement in the melt or solution. It is suggested that the metastable form sometimes appears to have a structure which most closely resembles that of the melt or solution [100].

4.2. Ostwald Law of Stages and Its Validity

In experimental and industrial practice, it has been commonly observed that the metastable form appears first and then transfer into a more stable structure. From this phenomenon, Ostwald concluded that "when leaving a metastable state, a given chemical system does not seek out the most stable state, rather the nearest metastable one that can be reached without loss of free energy". That is, in a crystallization from the melt or from solution, the solid first formed will be that which is the least stable of the polymorphs, the one with the largest Gibbs free energy [101]. Ostwald law of stages can be explained by that the limit of metastable zone is closer to the solubility curve for the metastable form than for the stable form, as shown in the Fig. (**4**). Besides, the least stable form normally possesses the highest volume free energy and the lowest specific step free energy, and thus has the highest average step velocity and crystal growth rate [102,103]. As a result, the crystallization of the least stable form is expected to predominate at high levels of supersaturation. Meanwhile this effect is also dependent upon the solvent-solute interactions [3].

Although it is a useful indicator of a possible sequence of production of crystalline forms, Ostwald law of stages is not as universal and reliable [4]. This is because the appearance and evolution of solid forms are determined by the thermodynamics and the kinetics of nucleation, growth and transformation under the specific experimental conditions

Table 1. Analytical Techniques for Characterizing Polymorphs [111]

Category	Techniques	Information
X-ray diffraction	Powder X-ray diffraction	Structure, crystallinity, chemical and phase composition, molecular weight, etc.
	Single-crystal X-ray diffraction	
Vibrational spectroscopy	Raman spectroscopy	Structure, molecular conformation, chemical and phase composition, hydrogen bonding, etc.
	Infrared spectroscopy	
Microscopy	Optical microscopy	Crystal size and habit, etc.
	Scamming electron microscopy	
	Atomic force microscopy	Surface properties, interactions between particles, etc.
Thermal methods	Hot-stage Microscopy	Melting point, transitions, etc.
	Thermogravimetric analysis	Melting point, transitions, etc.
	Differential thermal analysis	Thermal transitions.
	Differential scanning calorimetry	Melting point, transitions, heat capacity, crystallinity, etc.
	Isothermal calorimetry	Heat and rate of transition, crystallinity, etc.
Nuclear magnetic resonance spectroscopy	Solid-state nuclear magnetic resonance spectroscopy	Chemical and phase composition, structure, crystallinity, intermolecular interaction, conformational change, etc.

[104,105], and by the link between molecular assemblies and crystal structure [6,106–108].

5. CHARACTERIZATION OF POLYMORPHS

In the pharmaceutical industry, identification of polymorphism during early stage development is critical, as unanticipated polymorphic changes of a drug substance can affect chemical and physical stability, solubility, morphology, hygroscopicity, and, ultimately, bioavailability [109,110]. A number of analytical techniques have been employed for characterizing polymorphs and in-situ monitoring the formation and transformation of polymorphs during the process, as listed in Table **1**.

5.1. X-Ray Diffraction

Generally only X-ray crystallographic methods (i.e. X-ray powder or single-crystal diffraction) can provide definitive proof of the existence of polymorphism [112]. Single crystal X-ray crystallography is still the most powerful technique for the three-dimensional structure determination for small molecules and macromolecules as it can provide detailed pictures of the structure of the molecule in the crystal lattice. However, high-quality single crystals for X-ray crystallography are sometimes difficult to obtain. On this account powder X-ray diffraction (PXRD) is more popular in the identification of different polymorphs. The positions of the peaks in a powder X-ray diffraction pattern correspond to periodic spacings of atoms in the solid state; namely, different lattice constants will lead to different peak positions (Fig. **5**).

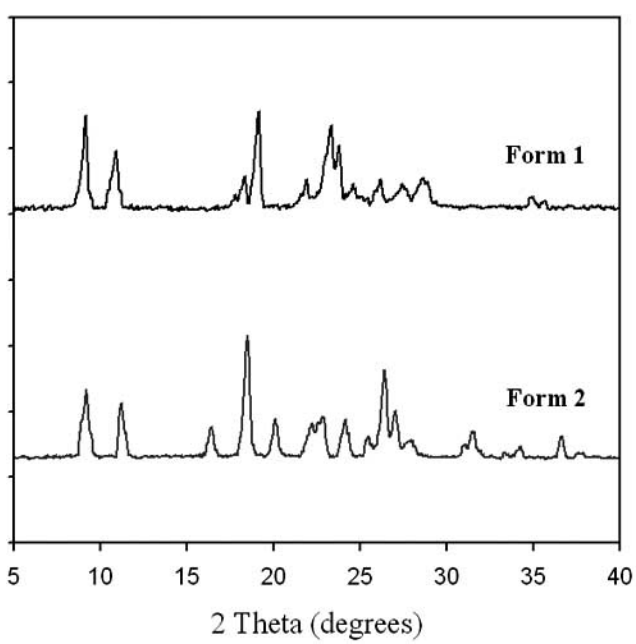

Fig. (5). Powder X-ray diffraction patterns of forms 1 and 2 of stavudine crystals.

Furthermore, the generally good separation between peaks in the diffractogram allows for quantitative analysis of mixtures of polymorphs using PXRD [2]. For example, let us assume that polymorphs A and B have characteristic diffraction peaks at θ_A and θ_B, respectively. The fractions of the A and B polymorphs in a sample can be calculated from the ratio of the area under their characteristic peaks using the following equations respectively [113]

$$\text{Fraction of A polymorph} = \frac{\text{peak area of the peak at } \theta_A}{\text{sum of peak areas of peaks at } \theta_A \text{ and } \theta_B} \quad (8)$$

$$\text{Fraction of B polymorph} = \frac{\text{peak area of the peak at } \theta_B}{\text{sum of peak areas of peaks at } \theta_A \text{ and } \theta_B} \quad (9)$$

High quality PXRD data sometimes can be employed to obtain complete crystal structures and structure determination from powder diffraction (SDPD) is currently one of the exciting frontiers in structural chemistry [114,115]. David *et al.* [116] determined the crystal structure of capsaicin, thiothixene and promazine hydrochloride from their powder diffraction data.

When PXRD is used for the characterization of polymorphs, both differences in particle size and preferred orientation [117], together with peaks' overlap [118], can affect the sensitivity of PXRD assays. When samples are prepared for analysis, the particle size must be closely controlled because preferred orientation effects can vary the relatively intensity of the diffraction lines. Furthermore, PXRD does not allow any correlation of individual diffraction peaks with specific structural features. To achieve good quantitative analysis for polymorphic composition, a standard curve must be prepared from pure polymorphic forms, which may or may not be available. Because of these factors, much research has focused on alternative techniques for the determination of crystal form [112].

5.2. Infrared Spectroscopy

Vibrational spectroscopic methods for polymorph identification such as infrared and Raman spectroscopy can provide information on structure and molecular conformation in the solid forms by probing vibrations of atoms [6]. Besides, the subtle spectroscopic changes arising from intermolecular (hydrogen) bonding can also be observed [119]. Both techniques can be used in estimating polymorphic composition rapidly and in confirming results obtained from PXRD analysis.

Infrared spectroscopic techniques are economical and easily available, and have already been applied to polymorph characterizations simply by inspection of spectral differences [112]. Spectroscopic differences have resulted in the identification of absorbance bands useful for the quantitative analyses of polymorph mixtures. The applied infrared spectroscopic techniques include diffuse reflectance Fourier transform infrared (DRIFT-IR) [120], attenuated total reflection Fourier transform infrared (ATR-FTIR) [121,122] and near infrared (NIR) [123]. As shown in Fig. (**6**), to construct the calibration curve for quantitative analysis, the character-

istic peaks at W_1 cm^{-1} and W_2 cm^{-1} are selected for pure A form and pure B form, respectively. The reference peak at W_0 cm^{-1}, common to both forms, is selected as an internal standard. The following equation can be employed for calibration [124,125].

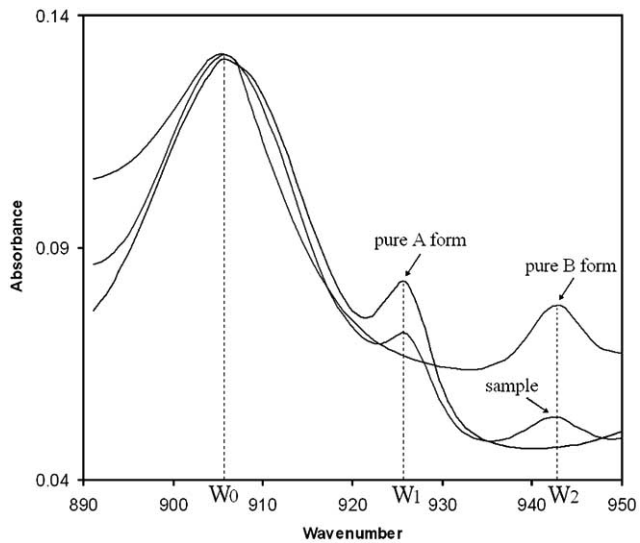

Fig. (6). FTIR spectra of the two pure polymorphs and polymorphic mixture of famotidine [126] illustrating the quantitative analysis of polymorphic composition in the mixture.

$$Y = \frac{a'bc'}{ab'c + a'bc'} \tag{10}$$

where a and a' are the intensities of the reference peak at W_0 cm^{-1} of pure A form and pure B form, respectively. b and b' are the intensities of the peak at W_1 cm^{-1} of pure A form and the sample, respectively. c and c' are the intensities of the peak at W_2 of pure B form and the sample. Y is the calculation factor. The calibration curve can be obtained by use of plotting the calculation factor Y against the concentration of form B in a series of standard samples. The standard samples shall be prepared as the mixtures of the two polymorphs in various mass fractions of form B in the mixture.

Although infrared spectroscopy is quite simple and convenient, it is worth noting that the infrared spectra of different polymorphs of some APIs may be almost identical, e.g. the polymorphs I and II of tropine phenylacetate [127]. Besides, the difference in infrared spectra may be resulted from the difference in purity, crystal size and preparation process of the samples. Sample preparation may also introduce changes to the observed absorbance.

5.3. Raman Spectroscopy

Raman spectroscopy provides similar chemical information to IR spectroscopy [119]. When it is applied to investigate polymorphism, the Raman spectroscopy can be sensitive to polymorphic changes, and thus can be used for the quantitative characterization of polymorph transitions in the solid state if the compound under study does not exhibit background fluorescence. When Raman spectroscopy is employed to analyze fluorescent samples, the wavelength of the excitation laser can be changed to the near-IR to reduce fluorescence of problematic samples.

At lower frequency vibrational bands, the Raman spectrum arises largely due to lattice vibrations that are very sensitive to structural changes in the solid state. Differences in the spectral patterns in this region between polymorphs may also be due to differences in intermolecular interactions (e.g. hydrogen bonding) and differences in crystal symmetry in the polymorphs. The bulk of the chemical structure details can be obtained from the region at higher frequency vibrational bands.

Raman spectroscopy with a microscope can focus on small crystalline samples. Information about drug dispersal in the formulated tablet and polymorphic changes occurring during formulation can thus be analyzed off-line. However, when spectra are acquired from the surface with a relatively powerful laser, the surface will be heated which may cause polymorphic transitions. On the other hand, Raman spectroscopy has been widely used for in-situ studies of polymorphism because it can perform measurements both behind glass and in solution. For example, by use of in-situ Raman spectroscopy, Ono *et al.* [128] conducted in-situ quantitative measurement of the polymorphic fraction of different polymorphs in the batch crystallization process of L-glutamic acid. They demonstrated that Raman spectroscopy could be a powerful tool for measuring the polymorphic fraction in suspension.

5.4. Microscopy

Microscopy was first applied in the chemical analysis in 1833 by Raspail [129]. In case polymorphs differ in morphology, optical or scanning electron microscopy can be used to identify polymorphs or to monitor the crystallization process of a polymorphic system. It is obvious that microscopy alone cannot be used to study polymorphism, as there is no inherent relationship between the morphology and the structure of crystals.

The atomic force microscope (AFM) is a very high-resolution type of scanning probe microscope with nanometer resolution. Since it was invented by Binnig *et al.* [130] in 1986, AFM has become one of the foremost tools for imaging the topography of a variety of surfaces at a range of resolutions from the micrometers to the molecular scale [131] and for direct measurement of discrete intermolecular forces [132]. An example of applying AFM to polymorphism is the study conducted by Danesh *et al.* [133] who applied tapping mode atomic force microscopy to distinguish and characterize the polymorphs of drug cimetidine. The atomic force microscope was also used to investigate the interaction forces between individual particles qualitatively as well as quantitatively [134].

5.5. Thermogravimetric Analysis

Thermal analysis includes differential scanning calorimetry (DSC), thermal-modulated DSC (TMDSC), differential

thermal analysis (DTA), thermogravimetric analysis (TGA), hot-stage microscopy (HSM), etc. Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by measuring the weight change that occurs as a sample is heated. The measurement is normally carried out in air, nitrogen, helium or argon, and the weight is recorded as a function of increasing temperature. TGA can thus be used to analyze the processes of decomposition or sublimation, and to determine the fraction of solvent in hydrates and solvates.

5.6. Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measures the amount of energy absorbed (endothermal event) or released (exothermal event) by a sample either during continuous heating/cooling experiments or during isothermal experiments. Such thermodynamic data as melting point, heat capacity and heat of fusion as well as polymorphic transitions can be obtained by use of DSC. When DSC is employed for the characterization of polymorphs, unknown thermal events such as possible decomposition and recrystallization often occur. Other analytical methods such as PXRD, TGA or HSM must be carried out to identify these events. Moreover, like other most widely used methods for solid-state characterization such as PXRD, FTIR spectroscopy and NIR spectroscopy, normal DSC is not sufficiently sensitive for detecting relatively low levels (<5%) of polymorphic impurity. Tong *et al.* [135] have developed a modified approach to quantify trace levels of polymorphic form II impurity in form I samples of salmeterol xinafoate using a standard DSC instrument.

5.7. Hot-stage Microscopy

As a type of thermomicroscopy, hot-stage microscopy (HSM) is developed as an analytical technique combing the properties of microscopy and thermal analysis to enable the characterization of the physicochemical properties of materials as a function of temperature. Varying the temperature of a specimen and simultaneous viewing it under a microscope can provide plentiful information on melting and/or recrystallization behaviors. Besides, in case polymorphic transitions are accompanied by a change in a crystal's birefringence, HSM can be applied for studying the polymorphic transitions during the processes of heating and cooling. This technique also offers the detection of solvates by observing the evolution of a gas or liquid from a crystal. Therefore, HSM has been widely used for the solid-state characterization of bulk drugs, evaluation of crystal forms and hydrates, and other physicochemical properties [136].

When polymorphs of some APIs (e.g. cimetidine) have close melting points and similar bulk thermal behaviors, normal thermal methods such as DSC or thermomicroscopy cannot be used to identify polymorphs or elucidate the relative stability between polymorphs [137]. Sanders *et al.* [138] have combined scanning thermal microscopy and localized thermal analysis to distinguish the polymorphs of cimetidine in terms of that various polymorphs have different thermal conductivity.

5.8. Solid-State Nuclear Magnetic Resonance Spectroscopy

As a nondestructive and noninvasive analytical technique, solid-state nuclear magnetic resonance (SS-NMR) spectroscopy can be used to analyze drug formulations, to study inclusion compounds, and to examine host–guest interactions [139]. In addition, SS-NMR spectroscopy can be employed to investigate polymorphism and pseudopolymorphism by probing the environments of atoms in the solid state. Non-equivalent nuclei will resonate at different frequencies and these chemical shift differences may be associated with changes in conformation or chemical environment of the API molecules in different solid forms [6]. That is, SS-NMR spectroscopy can detect various structures resulting from different packings, conformational changes and hydrogen bonding. Apart from most often used ^{13}C NMR spectroscopy, many other nuclei (e.g., ^{19}F, ^{15}N, ^{23}Na, ^{31}P, ^{17}O, or ^{2}H) can be used, depending upon the sample to be studied [140-142]. As it is quantitative and selective, SS-NMR spectroscopy can quantify mixtures of polymorphic crystalline forms, or of crystalline and amorphous materials, so that polymorphic transformations and molecular motion in the solid can be investigated. All analyses can be performed without the need for pure forms or a standard curve [143].

Although it has unique advantages over other analytical techniques, SS-NMR spectroscopy has some disadvantages. For example, it generally demands much expertise in the technique to run it properly. Its sensitivity is often insufficient so that a large quantity of sample should be provided to generate an adequate spectrum when using low natural abundance nuclei such as ^{13}C. Analysis times of SS-NMR experiments may be another problem as they can range from a few minutes to several days or more, depending upon the sample and the type of NMR experiment used. Moreover, peak assignment in the SS-NMR spectrum sometimes seems a thorny subject because multiple peaks could be present for a single nuclear site or the presence of overlapping peaks [144].

6. POLYMORPH DISCOVERY AND CONTROL

Various methods have been employed to produce different polymorphs of an API, such as cooling or quenching of melts [145], deposition (desublimation) [146], solvent drop grinding [147], solution crystallization from single or mixed solvents [148], etc. The crystallization process of polymorphs is consisted of competitive nucleation, growth, and the transformation from a metastable to a stable form. To selectively crystallize polymorphs, the mechanism of each elementary step in the crystallization process need be revealed with relation to the operational conditions and the key controlling factors. It is recognized that the nucleation process is the most important to the control of the polymorphic crystallization.

6.1. Traditional Methods

A large number of factors can influence the outcome of polymorphic crystallization, including supersaturation, temperature, solution concentration, cooling rate, solvent, agitation, pH, additive, impurity, seeding, interface, etc. Kitamura [149] has grouped the controlling factors for polymorphic crystallization into two groups, where the relative importance of each controlling factor depends on systems and crystallization methods. The primary factors, the basic important factors in the operation of the polymorphic crystallizations, include supersaturation, temperature, seeds, the stirring rate, the addition rate of antisolvent, the cooling rate, the mixing rate of reactant solutions, etc. On the other hand, such factors as solvents, additives, interfaces, etc. are grouped in the secondary factor group. Both primary and secondary factor groups impart thermodynamic and kinetic effects on polymorphic crystallization. Nevertheless, the mechanism of the effect and the quantitative relationship between the operational factors and the crystallization characteristics of polymorphs is not clearly understood yet.

Control of Supersaturation Level

According to Eq. 4, the nucleation of polymorphs is dependent upon supersaturation level. Fig. (**7**) schematically shows three of possible competitive nucleation types of dimorphic systems. For example, the nucleation of form A can be higher than that of form B in whole range of supersaturation ratio (Fig. **7a**), at high supersaturation ratio (Fig. **7b**), or at both low and high supersaturation ratio (Fig. **7c**). For some polymorphic systems the polymorphs can be selectively obtained by careful control of the level of supersaturation.

Control of Nucleation Temperature

The temperature normally affects the intermolecular interactions, solubility, supersaturation, the collision frequency between molecules, etc. Therefore, the temperature is one of the predominant operational factors that affect the nucleation, growth and transformation of polymorphs. In the process of crystallization from solution, the effect of crystallization temperature sometimes may be overshadowed by other factors, particularly deliberate or adventitious seeds. Obviously, the effect of temperature has both thermodynamic and kinetic implications, particularly for enantiotropically related polymorphs that change relative stability order near the transition temperature [3].

Solvent Screening

The most active pharmaceutical ingredients are purified and isolated by crystallization from an appropriate medium during the final step in the synthetic process. As to the crystallization of polymorphic APIs, the discovery of possible polymorphs typically starts with crystallization of APIs from a number of solvents; the appropriate choice of solvent can have a dramatic effect on the polymorphic outcome of a crystallization process. For example, forms II and III of drug sulfathiazole can be crystallized in water, two other forms I and IV are obtained from acetone, whilst *n*-propanol gives only form I [150].

Crystallization of polymorphs from solvent may be under kinetic or thermodynamic control depending on the conditions. In the latter case the nature of the solvent will have no relationship with the polymorph produced [105]. Whereas in the former case, the selectivity of solvent upon polymorphs is related to kinetic rather than thermodynamic mechanisms, e.g. selective adsorption of solvent molecules on crystal faces followed by inhibition of nucleation and growth of particular polymorphic forms [3], the solvent-solute interactions, etc. [108]. The solution-solute interactions can affect nucleation, crystal growth and solution-mediated polymorph transformation [151], which consequently affect the appearance of polymorphs. Besides, such bulk properties of solvent as viscosity, surface tension, etc. may also affect the crystallization kinetics and the occurrence domain of polymorphs [97].

In order to address the effect of solvent on polymorphism, the ability of hydrogen bonding of solvent is analyzed [152]. Hydrogen bonding can occur between solute–solute, solvent–solvent, and solvent–solute molecules. A solvent molecule that has greater ability to donate or accept hydrogen bonding than the solute molecule will establish hydrogen

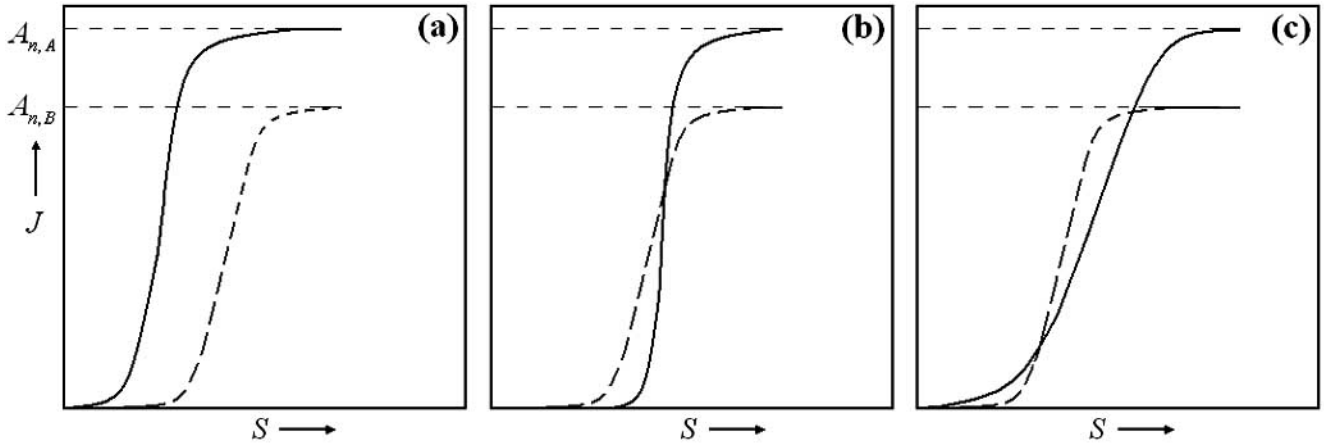

Fig. (7). Schematic illustration of the effect of supersaturation level on the nucleation rates of two polymorphs [8].

bonding with the solute molecules and may not allow the other solute molecules approach the same site. This will affect the final outcome of crystal structure and may even lead to a solvate formation [106].

To a certain extent supercritical fluid can act as a crystallization medium. Crystallization from the supercritical fluid can enhance molecular diffusion and ease of solubility. The control of temperature, pressure and solvents provides a means to carefully control the occurrence of polymorphs [3]. Kordikowski *et al.* [153] used supercritical CO_2 to control the polymorphs of sulfathiazole. Pure polymorphs of sulfathiazole were obtained at different temperatures and flow rate ratios of CO_2/solvent. With methanol forms I, III, and IV and their mixtures could be crystallized. With acetone form I or a mixture of form I and amorphous sulfathiazole was obtained. Moribe *et al.* [154] found that during the rapid expansion of supercritical solutions (RESS) process the stable form of phenylbutazone could transfer to the metastable form. They [155] recently have contributed a review on the application of supercritical carbon dioxide for polymorphic control and for complex formation of APIs.

Seeding Technology

Seeding is frequently used to control the product crystal polymorph. This control strategy is effectively utilized during the nucleation phase by adding seeds of the desired form and thus overriding spontaneous nucleation. The effectiveness of seeding is greatly related to the amount of solid surfaces introduced and crystallization rate. It is expected that more solid surfaces should be introduced when higher crystallization rate is conducted.

The drug substance hydroxytriendione exists in three modifications, forms I, II and III. The form II, thermodynamically stable form at room temperature, is chosen as the solid-state form for the active pharmaceutical ingredient. Spontaneous nucleation will lead to either of the two other forms. Beckmann *et al.* [156] have developed a seeding process to ensure the reproducible crystallization of the desired form II. Zhou *et al.* [157] have optimized and controlled the polymorphic crystallization of etoricoxib by seeding with the desired polymorph at a moderate supersaturation condition. As for the polymorphic crystallization of chiral compound, Courvoisier *et al.* [158] have investigated the effect of the polymorphic form of the seeds on the preferential crystallization. The 5(4'-methylphenyl)-5-methylhydantoin is known to crystallize in two polymorphic forms, orthorhombic and monoclinic forms. Seeded isothermal preferential crystallization method was applied to perform the separation of the enantiomers at a 2-L scale with two types of seeds, monoclinic and orthorhombic. However, orthorhombic form was never obtained in the crops but the physical parameters of the crystals produced (size, form, etc.) were strongly affected by the nature of the polymorph seeds.

Tao *et al.* [159] seeded the liquid of D-mannitol with its polymorphs (alpha, beta, and delta) and revealed several cases of cross-nucleation between polymorphs. When seeds of the alpha polymorph alone was added, the same polymorph grew, whereas at small undercoolings seeds of the delta polymorph yielded the nucleation of the alpha polymorph. The surfaces of metastable anhydrous phases of theophylline [160] and carbamazepine [161] have been reported to facilitate the nucleation of the stable hydrate forms during dissolution. In the case of cross-nucleation, the polymorphic selectivity of seeding cannot be anticipated.

Introduction of Additives

Adding additives sometimes causes a dramatic effect on the outcome of crystallization [162]. Through influencing the processes of nucleation and crystal growth, additives often influence the product's structure, shape, crystal size distribution, purity, bioavailability, stability and shelf life, filterability; caking/agglomeration property, etc. Commonly used additives for the crystallization of small molecules and proteins are listed in Table **2**.

To date, how additives affect crystallization is still poorly understood. Methods for choosing additives for crystallization are still largely empirical. Probable mechanisms of the effect of additives on crystallization include: (a) altering solubility and the width of the metastable zone, solution structure or thermodynamic phase behavior; (b) varying adsorbent layer around crystals, but not incorporate into crystal lattice; (c) being adsorbed on the surface of crystals, and blocking growth; (d) incorporating into crystal lattice of product when additive having similar lattice; (e) changing surface energy of the crystals, etc.

If a compound, which is similar in character to the forming nuclei, is attached to a surface, the crystal growth rate may be altered. In solution, structurally similar additives

Table 2. Additives Frequently Used in the Crystallization Process

Crystallization of proteins	Crystallization of small molecules
Metal ions: Co^{+}, Cs^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, etc, from 0.05M ~ 0.1M	Metal ions: Cr^{3+}, Fe^{3+}, Al^{3+}, Cu^{+}, Cd^{2+}, Mn^{2+}, etc, from ppm ~ large quantity
Surfactants: non-ionic and ionic surfactants, non-ionic detergents	Surfactants: sodium dodecyl benzenesulfonate (SDBS), surfactant gels, etc.
Organic compounds: dimethyl sulfoxide (DMSO), urea, glycine, alditols, phenol, diols, glycerol, polyethylene glycols (PEGs), polysaccharides, etc.	Salts: sodium chloride, ammonium chloride, etc. Organic compounds: urea, oxalate, alcohols, amino acids, etc.

may influence crystal structure by selectively binding the faces of a growing crystal. When the crystal structures of polymorphs are already known then the design of the promoters or inhibitors for nucleation or crystal growth is feasible by use of tailor-made additives [6]. Flufenamic acid (FFA) is a kind of nonsteroidal anti-inflammatory drug. Its metastable polymorph V can undergo a rapid interface-mediated polymorphic transformation. Lee *et al*. [163] used a salt additive ammonium chloride to induce crystallization of the metastable polymorph V of FFA. In addition, reaction byproducts and other impurities sometimes can influence the polymorphic outcome and crystal properties [1]. 4-Methyl-2-nitroacetanilide has three polymorphs; He *et al.* [164] have investigated the effects of the isomorphic molecules 4-chloro-2-nitroacetanilide and 2-nitro-4-trifluoromethyl-acetanilide on the crystallization of 4-Methyl-2-nitro-acetanilide. At the same doping level the percentage of 4-chloro-2-nitroacetanilide incorporated into the crystal lattice of polymorph A is much higher than that of 2-nitro-4-trifluoromethyl acetanilide. Mirmehrabi and Rohani [165] found that the impurities thymine and thymidine had significant effects on the crystal habit and crystal bulk density of stavudine but had no influence on the polymorphic structure.

Polymer-Induced Heteronucleation

When the surface introduced can decrease the free energy for nucleation, heterogeneous nucleation occurs much faster. One kind of approach to controlling polymorphism that directly targets nucleation is the method of polymer-induced heteronucleation. In this method APIs are crystallized in the presence of polymeric nucleants that mimic the pharmaceuticals by solvent evaporation, cooling, sublimation or other traditional crystallization techniques. Crystallization in the presence of polymer heteronuclei controls formation of different polymorphs presumably through a selective nucleation process mediated by the polymer surface. The first application of polymer heteronuclei for crystalline polymorph selection was conducted by Lang *et al.* [166]. In their work, the presence of certain polymers including nylons, isotactic polypropylene, chlorinated polyethylene, poly(tetrafluoroethylene), poly(2,3,5-tribromostyrene), and poly(vinyl chloride) was found to cause the growth of the orthorhombic polymorph of acetaminophen from evaporation of aqueous solution. Whereas, other polymers such as ethyl cellulose, polycarbonate, poly(vinyl acetate), etc. favored the monoclinic form of acetaminophen. The orientation of crystal growth from the polymer surface varied between polymer types, indicating an important role for stabilization of the crystal faces by the polymers through specific interactions.

This technique has also been applied for other polymorphic systems, such as carbamazepine, sulfamethoxazole and 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophene carbonitrile (ROY) [167]. Briefly, the polymer can act as an additional diversity element to affect the crystallization outcome. Polymer-induced heteronucleation is expected to control the formation of established forms and to discover unknown polymorphs without prior knowledge of solid-state structure [6].

Capillary Crystallization

Counterdiffusion crystallization in capillary primarily directed toward protein crystallization. Its principles are derived using well-known concepts coupling the ideas of precipitation and diffusion mass transport in a restricted geometry [168], where high supersaturation can be produced and, turbulence and convection are reduced. Recently capillary crystallization techniques have been applied for polymorph generation. For example, Chyall *et al.* [169] obtained a metastable polymorph of nabumetone (4-(6-methoxy-2-naphthalenyl)-2-butanone), by evaporation of a 1:3 water-acetone solution at room temperature inside a 1.0 mm capillary tube. In addition to providing high levels of supersaturation the evaporation of a solution in a capillary is nonturbulent and convection is minimized, which provides quiescent growth environments and may promote the generation of crystal forms not easily attainable using other methods. The metastable polymorph (form B) of metformin hydrochloride was also obtained by capillary crystallization [170].

Apart from the preparation of new polymorphs of organic compounds, a particular advantage of capillary crystallization is that the produced crystals can be directly analyzed by PXRD or single crystal X-ray diffraction without removal from the crystallization vessel [6].

Nucleation Confined in Nanopores

The unique characteristics of nucleation suggest that nucleation in specific pores possessing dimensions near the size of critical nuclei typically in nanometer scale can be regulated through adjustment of the pore size. This adjustment may provide a possibility that polymorphs can selectively nucleate in these pores, as polymorphs may have different critical nuclei sizes. Recently, Ha *et al.* [171] reported a novel approach to polymorph selectivity under nanoscopic confinement. They found that, the form III of anthranilic acid crystallized in the pores of 55-nm CPG (controlled pore glass), whereas the form II became evident in 23-nm CPG and was clearly predominant in 7.5-nm CPG.

Heteronucleation on Substrates

A variety of substrates have long been employed in controlling polymorphism in crystallization process. Selective growth of a thermodynamically less preferred polymorph of the salt $(DMTC^{+})(TMO^{-})\cdot CHCl_3$ has been achieved from $CHCl_3$ solutions by nucleation on single-crystal succinic acid substrates [99]. This can be explained by a ledge-directed epitaxy mechanism in which the dihedral angle between two close-packed planes of a substrate ledge site matches that of two close-packed planes of a prenucleation aggregate corresponding to the observed polymorph. By extending this method, a combinatorial library of inorganic and organic crystal surfaces has been used as substrates for the polymorph discovery of many compounds by the epitaxial mechanism [172]. Another impressive example on this subject is that selective nucleation and discovery of polymorphs of ROY were accomplished through the epitaxial growth on single-crystal substrates. ROY has six structurally characterized conformational polymorphs. The yellow needle polymorph of ROY can be collected through the sublimation of ROY on the (101) face of a pimelic acid single crystal. Whereas the sublimation of ROY on the (010) face of a succinic acid single crystal resulted in the concomitant formation of three polymorphs: yellow needles, orange needles, and red plates. The red plates were designated as the seventh form of ROY [173].

An alternative strategy for polymorph control by use of surfaces is to introduce self-assembled monolayers (SAMs) into the crystallization solution which can selectively promote or inhibit the heterogeneous nucleation of a specific polymorph. Hiremath *et al.* [174] have demonstrated that functionalized SAMs can be used as substrates for the selective growth of a less stable polymorph of 2-iodo-4-nitroaniline. Orthorhombic and triclinic crystals of 2-iodo-4-nitroaniline grow concomitantly from supersaturated ethanol solutions, but the less stable orthorhombic form can be selectively grown on 3'-X-4-mercaptobiphenyl (X=NO_2, I) self-assembled monolayer templates.

Laser-Induced Nucleation

External fields such as ultrasonic field, electric field and magnetic filed have been attempted to influence the crystallization process of small molecules and proteins. However, the mechanism of accelerating or suppressing nucleation by these fields is still ambiguous.

Nonphotochemical, laser-induced nucleation (NPLIN) has been suggested as a crystallization technique that can effectively affect nucleation process and thus the outcome of crystallization. Garetz *et al.* [175] firstly reported an experiment in which nucleation of supersaturated solutions of urea in water might be induced when exposed to intense, plane-polarized pulses of near-infrared laser light. A photochemical mechanism for the process has been proposed, i.e., the electric field may induce the alignment of urea molecules in preexisting clusters of randomly oriented urea molecules, which can help them to organize to form a crystallite. In other investigations by the same group, it was demonstrated that the technique could provide polymorphic selectivity. When supersaturated aqueous solutions of glycine exposed to intense pulses of plane-polarized laser light at 1.06 μm, the least stable γ form of glycine unexpectedly crystallized. Whereas the most stable α form was always produced when the same solutions was not exposed to the laser. This result suggests that NPLIN may be a promising approach to polymorph control and discovery [176].

6.2. New Approaches

Recent advances in computational chemistry, combinational technologies, experimental techniques, hardware and software systems have triggered some new approaches to the solid forms' discovery for APIs, such as high-throughput crystallization, crystal structure prediction, etc.

High-Throughput Crystallization

To explore an API's polymorphs, hydrates, solvates, salts, co-crystals and amorphous states, a huge number of experimental conditions should be carried out, which is quite difficult and time-consuming by conventional experimental methods. As the concepts of high-throughput (HT) screening and combinatorial synthesis are applied to the development of drugs, high-throughput (HT) crystallization systems have recently been developed and are emerging as an accelerator for the discovery and screening of solid forms of an API [177].

In a fully integrated HT system, crystallization is performed at small scale using a combinatorial approach, where a large variety of parameters of composition and process conditions are investigated in parallel. The API can be dispensed in the solid state with appropriate powder-handling systems, or as a solution followed by solvent removal. Then combinations of solvents and/or additives are added to each crystallization vessel. Liquid transfers can be achieved using liquid-handling robots or multi-channel pipettors. The most used crystallization methods include cooling crystallization, antisolvent crystallization, evaporative crystallization, precipitation, etc. Crystals produced in the vessels are analyzed by a combination of optical image analysis, Raman spectroscopy and/or PXRD to differentiate polymorphs. Because the number of crystallization trials under different experimental conditions is greatly increased by an HT approach, the probability of discovering new forms is increased. After a particular form at small scale is identified in the HT screening, scale-up studies will be conducted to further optimize the process for laboratory scale production [1,6].

The HT crystallization has been applied to discover solid forms for a series of APIs, such as acetaminophen, sulfathiazole, etc. Peterson *et al.* [177] discovered the highly unstable form III of acetaminophen by use of the HT crystallization technique. Another example is that solid forms of the salts of sulfathiazole were explored through HT crystallization technique by use of varying stoichiometric ratios of pharmaceutically acceptable organic and mineral bases [178]. The screen resulted in the rapid identification and characterization of 10 salt forms and showed that the salts exhibited a range of melting points depending on the counter-ion type and stoichiometric ratio. Briefly, HT crystallization technology can provide rapid and comprehensive discovery of solid forms of APIs, which thus can facilitate selecting the optimal physical or chemical form of a given API for development into a drug product.

Crystal Structure Prediction

The ultimate goal of crystal structure prediction is to predict the crystal structure from the molecular structure of a compound. Although various research groups have successfully predicted the structures of some small, relatively rigid organic molecules with few functional groups in last decades, it remains troublesome to predict the crystal structure of a compound from its chemical composition [179]. To date, it is still extremely difficult to predict the influence of subtle conformational changes and weak interactions between adjacent molecules on the crystalline structure [1]. Even if a crystalline form can be predicted, its relative stability to other crystalline forms is difficult to predict with accuracy. In addition, the crystal structure prediction for either multi-component (e.g., solvates, hydrates, co-crystals) or ionic systems is not yet possible [180,181].

The theory of Kitaigorodsky is typically employed for the crystal structure prediction [182]. Interactions between molecules are assumed to be weak and lacking in directionality. In addition, it is assumed that all interactions taper off at longer distances in roughly the same way. In such an isotropic model, close packing dominates the formation of crystal structures, thus hypothetical crystal structures that approximately satisfy these close-packing conditions can be generated by computer programs. Among these calculated

Table 3. Examples of Polymorphic Active Pharmaceutical Ingredients in the Literature

Compound	Forms	Crystallization methods	Factors investigated	Refs.
Abecarnil	A, B, C	▪ Cooling ▪ Supercritical antisolvent	▪ Supersaturation ▪ Impurity ▪ Temperature ▪ Solvent	[186]
Acetaminophen	I, II, III, trihydrate	▪ Cooling ▪ Evaporation	▪ Polymer heteronuclei ▪ Excipient	[167] [187]
p-Aminobenzoic acid	α, β	▪ Cooling	▪ Solvent ▪ Cooling rate	[188]
Carbamazepine	α, β, γ, 2 solvates	▪ Solution-mediated transformation	▪ Surfactant	[161]
Cimetidine	A, B, C, D, monohydrate	▪ Evaporation ▪ Cooling	▪ Solvent ▪ Cooling rate	[137]
Diflunisal	I, II, III, IV, 2 solvates	▪ Crystal structure prediction	▪ Hydrogen-bonding	[185]
Eflucimibe	A, B	▪ Cooling	▪ Cooling rate ▪ Seeding ▪ Initial concentration	[189]
Estradiol	A, B, 2 solvates	▪ Evaporation ▪ Melt	▪ Solid-state characterization	[190]
Ethylenediammonium 3,5-dinitrobenzoate	Monoclinic, Triclinic	▪ Reactive	▪ pH	[191]
Famotidine	A, B	▪ Cooling	▪ Solvent ▪ Temperature ▪ Cooling rate ▪ Seeding ▪ Initial concentration	[126]
Fluconazole	I, II	▪ Supercritical antisolvent	▪ Solvent	[192]
Fluticasone propionate	1, 2	▪ Supercritical antisolvent	▪ Structure determination through powder X-ray diffraction	[193]
(*R*,*R*)-Formoterol tartrate	A, B, C	▪ Reactive	▪ Impurity	[194]
L-glutamic acid	α, β	▪ Cooling	▪ Amino acid additive	[195]
Hydroxytriendione	I, II, III	▪ Cooling ▪ Evaporation	▪ Seeding ▪ Solvent	[156]
Indomethacin	α, β, γ	▪ Cooling	▪ Substrate	[196]
Mebendazole	A, B, C	▪ Cooling	▪ Solid-state characterization	[16]
Metformin hydrochloride	A, B	▪ Capillary	▪ Solvent	[170]
Nabumetone	I, II	▪ Evaporation	▪ Solid-state characterization	[197]
Nifedipine	I, II, III, 5 solvates	▪ Evaporation	▪ Solid-state characterization	[198]
Oxybuprocaine hydrochloride	I, II, III	▪ Molecular modeling.	▪ Hydrogen-bonding	[199]
Phenylbutazone	α, β, δ, ε, γ	▪ Cooling	▪ Supersaturation	[113]
Progesterone	1, 2	▪ Crystal structure prediction	▪ Solvent ▪ Hydrogen-bonding	[200]
Ranitidine hydrochloride	1, 2	▪ Antisolvent	▪ Solvent	[47]
Ribavirin	I, II	▪ Cooling ▪ Melt	▪ Solid-state characterization	[135]
Ritonavir	I, II	▪ Evaporation ▪ Solution-mediated transformation ▪ High-throughput	▪ Solvent	[201] [202]

(Table 3). Contd…..

Compound	Forms	Crystallization methods	Factors investigated	Refs.
Salmeterol xinafoate	I, II	▪ Supercritical antisolvent	▪ Temperature ▪ Pressure	[203]
Sertraline hydrochloride	28	▪ High-throughput	▪ Solvent ▪ Salt former	[49]
Stavudine	I, II, 1 hydrate	▪ Cooling	▪ Solvent ▪ Agitation ▪ Supersaturation ▪ Impurity	[165]
Stearic acid	A, B, C, D, E	▪ Cooling	▪ Solvent	[204]
Sulfamerazine	I, II	▪ Solution-mediated transformation	▪ Solvent ▪ Additive (impurity)	[108] [205]
Sulfamethoxazole	I, II, III, IV	▪ Evaporation	▪ Polymer heteronuclei ▪ Solvent	[167]
Sulfathiazole	I, II, III, IV, V	▪ Cooling ▪ Evaporation ▪ SEDS	▪ Solvent ▪ Template ▪ Supercritical CO_2	[206] [207] [153]
Sulindac	I, II, III, 3 solvates	▪ Cooling	▪ Solvent	[208]
Thiazole derivative, BPT	A, B, C, 2 solvates	▪ Antisolvent	▪ Solvent ▪ Addition rate ▪ Initial concentration	[149]
Tolbutamide	I, II, III, IV	▪ Cooling ▪ Supercritical antisolvent	▪ Additive (cyclodextrin) ▪ Additive (metal ion) ▪ Solvent	[209] [210] [211]
Venlafaxine hydrochloride	1, 2, 3, 4, 5, 6	▪ Solid-to-solid transformation	▪ Temperature	[212]

structures some may not be observed in experimental studies. For example, approximately half of the energetically feasible predicted crystal structures of carbamazepine exhibit the C=O···H–N $R_2^2(8)$ dimer motif, and have been observed in the known polymorphs. Whereas the other energetically feasible structures with the C=O···H–N C(4) chain hydrogen bond motif have not been observed yet. This is attributed to that the kinetics favored the nucleation of crystal structures based on the $R_2^2(8)$ dimer motif [183]. Recently some research groups have incorporated the kinetic factor during crystallization into the overall prediction process [5,184].

Crystal structure prediction is beneficial to solid form discovery for active pharmaceutical ingredients. Some hydrogen-bonding motifs or molecular layer types are frequently observed in the predicted structures, which can be used to assist the design of crystallization experiments. Cross *et al.* [185] combined crystal structure prediction with targeted experimental crystallization to screen the solid forms of diflunisal. Graph set analysis was used to analyze and classify the structural predictions, and crystallization solvents were selected to promote the formation of crystals containing the most commonly predicted hydrogen-bonding motifs. Four new crystal structures of diflunisal were discovered, including two solvates and two new polymorphs.

In spite of more than a century of great efforts, the fundamental mechanisms and molecular properties that drive molecular crystal form diversity, specifically the nucleation of polymorphic forms, are not well understood [106]. As a result, the prediction for assessing polymorphic behavior of active pharmaceutical ingredients remains a great challenge. It is expected that the on-going development of theoretical methods coupled with validation of the predictions through extensive crystallization screening will bring about better models and computational methods [1].

In summary, multifarious techniques have been developed for the discovery and control of the polymorphs of active pharmaceutical ingredients. A list of examples of polymorphic systems that have been systematically studied is presented in Table **3**. Nevertheless, among those existing techniques there is still no method to guarantee the production of desired form even the most thermodynamically stable form of an API [6]. There remains a lack of fundamental understanding of the nucleation process and those factors that may contribute to crystallization of diverse forms of a compound. In the cause of polymorph discovery and control, the mechanisms of the effects of crystallization conditions on the nucleation, crystal growth and transformation of polymorphs needs to be further clarified.

7. POLYMORPHIC TRANSFORMATION

When an API exhibits polymorphism, the knowledge of the relative stability and the transformation kinetics between polymorphs is essential to the development of its drug product and to the appropriate storage condition. Many pharmaceutical crystals, such as theophylline [213], chlorpropamide [214], carbamazepine [215], etc., are known to undergo a

variety of polymorphic transformations during their processing and formulation. These polymorphic transformations shall affect the stability and the bioavailability of drug products.

Polymorphic transformations proceed generally *via* the solid-solid or the solution-mediated mechanism [209]. The kinetics of polymorphic transformation of drugs can be affected by such variables as the structure of the product, imperfections within the crystal structure and sample history [215]. The process of polymorphic transformation can be monitored by in-situ or off-line analytical techniques.

7.1. Solid-Solid Transformation

The solid-solid transformation is dependent on internal rearrangements or conformational changes of the molecules in crystals [209]. Stress-induced transformation refers to that solid-solid transformations of APIs are caused by mechanical stress. This kind of phase transformation is more frequently associated with the formation of metastable forms or amorphous states [6]. Betamethasone acetate has three polymorphic forms (form II, Iα, Iβ) and a hydrate. When the three polymorphic forms were subjected to the ball-milling in an agate centrifugal mill, the crystallinity of all three forms decreased with time during the grinding and gradually turned to the amorphous state [216].

Temperature-induced transformation is another widespread phenomenon of solid-solid transformation. The form II of betamethasone acetate was completely converted to the form Iβ when heated at 160 ℃ for 15 min. The form Iα at 95 ℃ for 7 days transformed to the form Iβ partially, but the same treatment did not cause any change of the form Iβ [216]. By use of terahertz pulsed spectroscopy and differential scanning calorimetry, Zeitler *et al.* [217] found forms III, IV, and V of sulfathiazole converted to form I *via* a solid–solid phase transition at temperatures below 450 K.

7.2. Solution-Mediated Transformation

The solution-mediated transformation is controlled by differences in solubility of stable and metastable forms, where a metastable form possesses higher solubility [209]. If the starting point lies within the metastable zone of the stable phase, the transformation starts only after elapse of the corresponding induction period. In the case when the starting point is located outside the metastable zone of the stable solute, the transformation proceeds without any delay [218]. When the temperature is increased and/or the stable form is introduced as "seed", the transformation process normally shall be accelerated.

Skrdla *et al.* [112] have systematically investigated the solution-mediated transformation of a pharmaceutical compound (2R,3S)-2-({(1R)-1-[3,5-bis(trifluoromethyl)phenyl] ethyl}oxy)-3-(4-fluorophenyl) morpholine hydrochloride from metastable form II to stable form I, by use of FTIR. The time-course of transformation is found to be in accordance with the Prout-Tompkins model. Based on the Arrhenius plot consisting of the natural logarithm of the reaction rate constants plotted against their corresponding reciprocal temperatures, the energy of activation for the transformation from form II to form I is obtained as 42 kJ/mol.

Because of its tremendous advantages over other techniques, in-situ Raman spectroscopy has recently been widely used to monitor the solution-mediated polymorphic transformation. By use of Raman spectroscopy coupled with an immersible fiber optic probe, Wang *et al.* [219] have studied the solution-mediated polymorphic transformation from form II to form I of progesterone.

8. SUMMARY

In the last decades, great progress has been made in the understanding of the mechanisms of polymorphism, which leads to the molecular level design of pharmaceutical solids and better control of the desired forms. Polymorph control has advanced from being a mysterious process to a meaningful tool to solve the problems occurring in the process of drug development. It is recognized that the interplay between molecular structures, intermolecular interactions, thermodynamics, and kinetics may affect the processes of molecular assemblies and thus can determine the crystallization outcomes. This review has introduced the mechanisms of polymorphism and recent advances in the technologies for the characterization, discovery and control of polymorphs. It is expected that new crystal forms with novel properties and for novel drug products will explosively expand with the progresses in high-throughput crystallization and crystal structure prediction techniques.

ABBREVIATIONS

AFM	=	Atomic force microscope
API	=	Active pharmaceutical ingredient
ATR-FTIR	=	Attenuated total reflection Fourier transform infrared
CPG	=	Controlled pore glass
DRIFT-IR	=	Diffuse reflectance Fourier transform infrared
DSC	=	Differential scanning calorimetry
DTA	=	Differential thermal analysis
FFA	=	Flufenamic acid
HSM	=	Hot-stage microscopy
HT	=	High-throughput
NIR	=	Near infrared
NPLIN	=	Nonphotochemical, laser-induced nucleation
PXRD	=	Powder X-ray diffraction
RAN-HCl	=	Ranitidine hydrochloride
RESS	=	Rapid expansion of supercritical solutions
ROY	=	5-methyl-2-[(2-nitrophenyl)amino]-3-thiophene carbonitrile
SAM	=	Self-assembled monolayer
SDPD	=	Structure determination from powder diffraction

SS-NMR = Solid-state nuclear magnetic resonance

TGA = Thermogravimetric analysis

TMDSC = Thermal-modulated DSC

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Review

Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations

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Purpose. This review describes a conceptual approach to the characterization of pharmaceutical solids. ***Methods.*** Four flow charts are presented: (1) polymorphs, (2) hydrates, (3) desolvated solvates, and (4) amorphous forms. ***Results.*** These flow charts (decision trees) are suggested as tools to develop information on pharmaceutical solids for both scientific and regulatory purposes. ***Conclusions.*** It is hoped that this review will lead to a more direct approach to the characterization of pharmaceutical solids and ultimately to faster approval of regulatory documents containing information on pharmaceutical solids.

KEY WORDS: polymorph; hydrate; amorphous form; desolvated solvate.

Interest in the subject of pharmaceutical solids stems in part from the Food and Drug Administration's (FDA's) drug substance guideline that states "appropriate" analytical procedures should be used to detect polymorphic, hydrated, or amorphous forms of the drug substance. These guidelines suggest the importance of controlling the crystal form of the drug substance. The guideline also states that it is the applicant's responsibility to control the crystal form of the drug substance and, if bioavailability is affected, to demonstrate the suitability of the control methods.

Thus, while it is clear that the New Drug Application (NDA) should contain information on solid state properties, particularly when bioavailability is an issue, the applicant may be unsure about how to scientifically approach the gathering of information and perhaps what kind of information is needed. This review is intended to provide a strategic approach to remove much of this uncertainty by presenting concepts and ideas in the form of flow charts rather than a set of guidelines or regulations. This is especially important because each individual compound has its own peculiarities which require flexibility in approach. The studies proposed herein are part of the Investigational New Drug (IND) process.

Solid drug substances display a wide and largely unpredictable variety of solid state properties. Nevertheless, application of basic physicochemical principles combined with appropriate analytical methodology can provide a strategy for scientific and regulatory decisions related to solid state behavior in the majority of cases. By addressing *fundamental* questions about solid state behavior at an early stage of drug development, both the applicant and the FDA are in a better position to assess the possible effects of any variations in the solid state properties of the drug substance. The resulting early interaction of the parties with regard to this area would not only tend to ensure uniformity of the materials used throughout the clinical trials but also fully resolve solid state issues before the critical stages of drug development. A further benefit of these scientific studies is the development of a meaningful set of solid state specifications which critically describe the solid form of the drug substance. These specifications would thus also facilitate the approval of a change in supplier or chemical process.

Our approach in this review is to suggest a sequence for collecting data on a drug substance that will efficiently answer specific questions about solid state behavior in a logical order. In "difficult" cases, perhaps where mixtures of forms must be dealt with, or other unusual properties are encountered, the suggested sequences would still have to be followed as a first stage in this investigation.

We have chosen to present this approach in the form of a series of decision trees, or flow charts (algorithms), one for each of the most common solid state forms. The charts are accompanied by examples from the literature representing the kind of data that would be useful in supporting the various decisions.

Decision trees provide conceptual frameworks for understanding how the justification for different crystal forms might be presented in the drug application. Industry may wish to use these decision trees as a strategic tool to organize the gathering of information early in the drug development process. Put another way, these decision trees provide a thought process that will lead to development of the most

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appropriate analytical controls. One should also note that it is the responsibility of the industry to select the appropriate test or tests to identify the phase of the solid and determine its relevant pharmaceutical properties. This approach is superior to simply performing a broad range of tests without regard to their relevance.

We should point out that, from a regulatory standpoint, if a company can establish a specification/test to ensure production of a well defined solid form of the drug substance, then it is not necessary to do all of the physical/chemical testing outlined in the decision trees. From a scientific standpoint, however, such an approach is risky since new forms may appear unpredictably during various stages of the development process. The appearance of these new forms usually slows the drug approval process and makes planning difficult.

Four decision trees are described in the sections that follow: Polymorphs; Hydrates (Solvates); Desolvated Solvates; and Amorphous Forms. Polymorphs exist when the drug substance crystallizes in different crystal packing arrangements all of which have the same elemental composition (Note that hydrates can exist in polymorphs). Hydrates exist when the drug substance incorporates water in the crystal lattice in either stoichiometric or nonstoichiometric amounts. Desolvated solvates are produced when a solvate is desolvated (either knowingly or unknowingly) and the crystal retains the structure of the solvate. Amorphous forms exist when a solid with no long range order and thus no crystallinity is produced. It is apparent that the appropriate flow chart can only be determined after the solid has been characterized using some of the tests described in the first decision point of the decision trees/flow charts (i.e. X-ray powder diffraction, elemental analysis, etc.). If there is no interest in marketing or producing an amorphous form or desolvated solvate at any stage in the process, then the corresponding flow charts do not need to be addressed. As already mentioned, it is advisable to investigate the drug substance for the existence of polymorphs and hydrates since these may be encountered at any stage of the drug manufacturing process or upon storage of the drug substance or dosage form.

All of the flow charts end (see for example Figure 1) with an indication of the types of controls which will be required based on whether a single morphic form or a mixture will be produced as the drug substance. Although this ending provides a simplistic view of a very complicated process of selecting appropriate controls, it is included to illustrate the consequence of the decisions made with regard to the drug substance. The reader should realize that the actual selection of the appropriate control could be the subject of another review which might contain another set of flow charts or decision trees.

POLYMORPHS

The flow chart/decision tree for polymorphs is shown in Figure 1. It outlines investigations of the formation of polymorphs, the analytical tests available for identifying polymorphs, studies of the physical properties of polymorphs and the controls needed to ensure the integrity of drug substance containing either a single morphic form or a mixture.

A. Formation of Polymorphs—Have Polymorphs Been Discovered?

The first step in the polymorphs decision tree is to crystallize the substance from a number of different solvents in order to attempt to answer the question: Are polymorphs possible? Solvents should include those used in the final crystallization steps and those used during formulation and processing and may also include water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, hexane and mixtures if appropriate. New crystal forms can often be obtained by cooling hot saturated solutions or partly evaporating clear saturated solutions. The solids produced are analyzed using X-ray diffraction and at least one of the other methods. In these analyses, care must be taken to show that the method of sample preparation (i.e. drying, grinding) has not affected the solid form. If the analyses show that the solids obtained are identical (e.g. have the same X-ray diffraction patterns and IR spectra) then the answer to the question "Are polymorphs possible?" is "No",

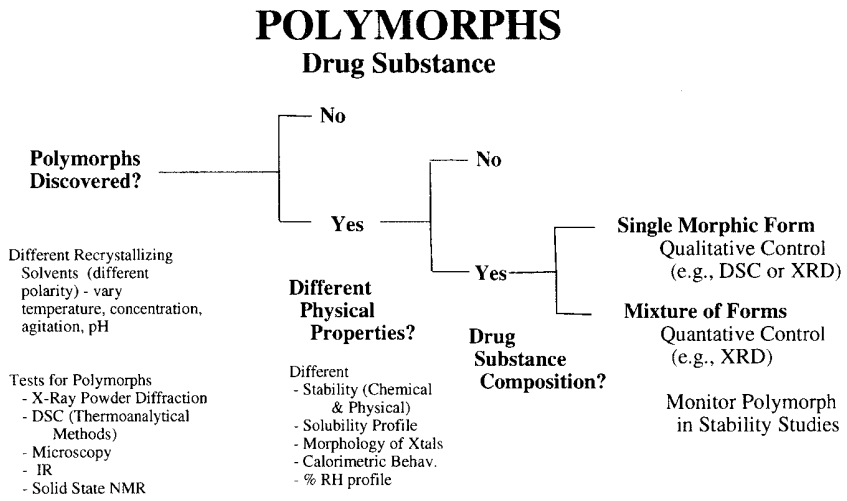

Figure 1. Flow chart/decision tree for polymorphs.

and further research is not needed. The work of Miyamae *et al.* serves as a good example of solid state studies of a drug substance which exists as polymorphs (1). Powder diffraction showed that there were two crystal forms (see Figure 2).

These workers also carried out single crystal analysis of the two crystal forms of the compound. The structures are shown in Figure 3. While such studies are not required, and indeed sometimes not possible, they provide an unequivocal confirmation of the existence of polymorphs. Moreover, once the single crystal structure of a phase has been determined, it is possible to calculate the corresponding X-ray powder pattern. This provides an irrefutable standard for identifying the phase by that method.

The DSC thermal curves of the two forms are slightly different, as shown in Figure 4 and thus may not be the preferred way of differentiating these polymorphs.

The IR spectra of the two polymorphs are quite similar(1), and IR does not appear to be a powerful method for differentiating the crystal forms in this case. Thus, for 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine, powder diffraction appears to be the best method for differentiating the two forms.

Solid-state NMR is another powerful technique for analyzing different crystal forms (2,3). Figure 5 shows the solid-state C-13 NMR spectra of Forms I and II of prednisolone. Differences in the positions of the two resonances in the 120 ppm range clearly differentiate the two forms. In principle, solid state NMR is an absolute technique in which the signal intensity is proportional to the number of nuclei provided appropriate conditions are met. In addition, solid state NMR is a bulk technique which is not very sensitive to surface changes. This method appears to be very sensitive and will undoubtedly be used more often in the future as a tool to detect different crystal forms. However, with present technology, errors in solid-state quantitative studies may be rather large.

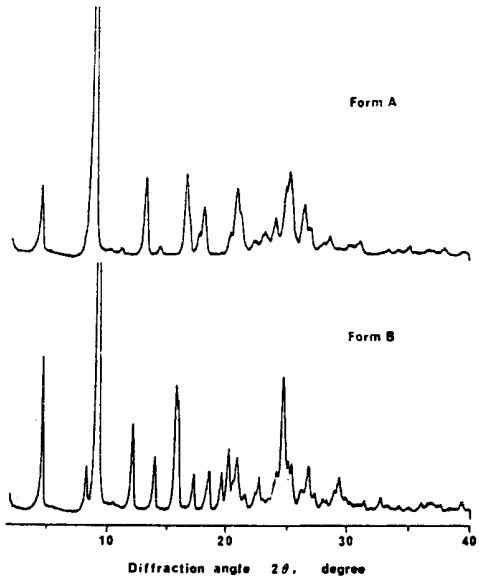

Figure 2. Powder X-ray diffraction patterns of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine (1).

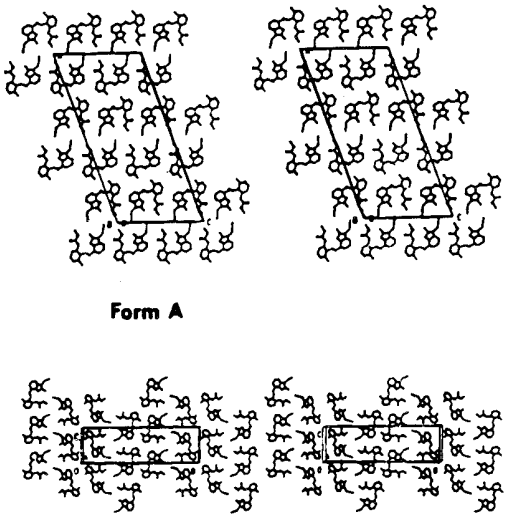

Figure 3. Stereoscopic drawings of the crystal packing of both polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine viewed along the shortest axis (Form A, b-axis; Form B, a-axis) (1).

B. Do the Polymorphs Have Different Physical Properties?

If polymorphs exist then it is necessary to examine the physical properties of the different polymorphs that can affect dosage form performance (bioavailability and stability) or manufacturing reproducibility. The properties of interest are solubility profile (intrinsic dissolution rate, equilibrium solubility), stability (chemical and physical), and crystal morphology (including both shape and particle size), calorimetric behavior, and %RH profile. If there are no discernible differences between these physico-chemical properties, then the answer to the second question in the decision tree, "Different physical properties?" is "No."

The variable physical properties of several drugs with different polymorphs are reported in the literature. For example, the dissolution profiles of the polymorphs of chloramphenicol are significantly different (4). In addition, van't Hoff solubility analysis has been used to elucidate the dif-

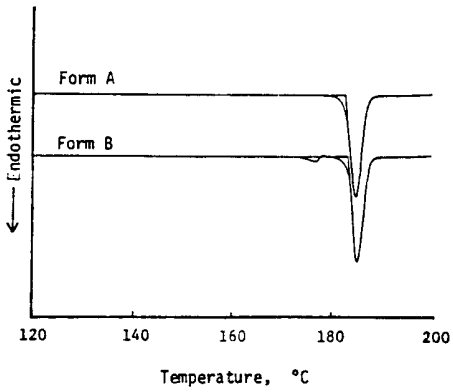

Figure 4. DSC thermal curves of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine (1). These curves show that Form A melts whereas Form B undergoes a small endothermic transition and then melts at the same temperature as Form A.

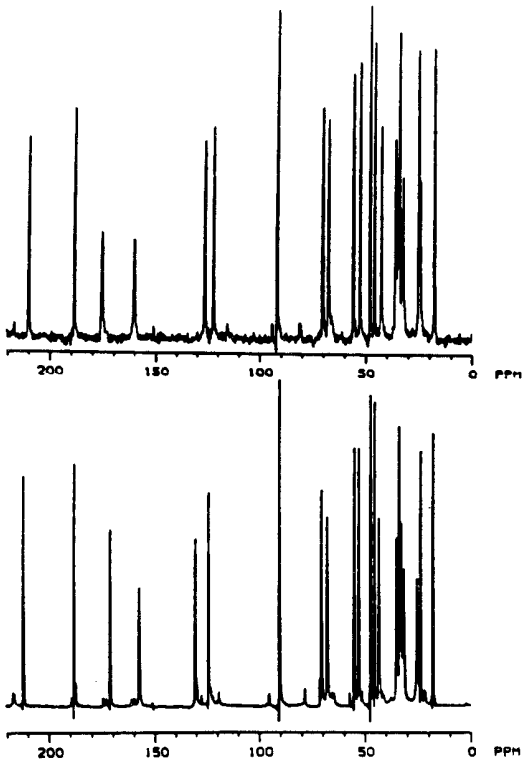

Figure 5. Solid state NMR of the two Crystal Forms of Prednisolone (2).

ferent solubilities of two polymorphs of methyl prednisolone(5). This method involves determining the equilibrium solubility of each polymorph at various temperatures. The log of the equilibrium solubility is then plotted vs 1/T. This should give straight lines for each polymorph and the temperature at which the curves intersect is the transition temperature. This technique does not work if the polymorphs interconvert.

For balance, it is important to point out that there are also cases where polymorphs exist but they have virtually identical dissolution properties(6).

C. Drug Substance Control

The important question lies in the properties that differ among polymorphs and whether those properties affect the dosage form performance (i.e., quality or bioavailability). If they do then from a regulatory standpoint it is appropriate to establish a specification/test (e.g. powder X-ray diffraction or IR) to ensure the proper form is produced. From a production standpoint, it is important to develop a process that reproducibly produces the desired polymorph.

If mixtures of forms cannot be avoided, then quantitative control is needed to ensure that a fixed proportion of forms is obtained. Furthermore, the method of analyzing for the proportion of forms would have to be validated. Also, the proportion of forms would have to remain within stated limits through the retest date of the drug substance and potentially throughout the shelf life of the product; a difficult requirement if the forms interconvert. Thus, the way to avoid a substantial amount of work in this area is to select a single solid form for production. Usually, this would be the most physically stable form when their bioavailabilities are not significantly different. Selection of the most stable from would, of course, insure that it there would be no conversion into other forms.

Powder diffraction is often a useful method to determine the percentages of polymorphs in a mixture; however, the detection limit is variable from case to case and can be as high as 15%. Matsuda (7) carried out a mixture analysis of phenylbutazone polymorphs. Diffraction lines disappear and appear as the ratio of the crystal forms change. Some of these calibration curves developed from this analysis are almost horizontal, meaning that any given mixture gives the same line intensity in this mixture range. However, other calibration curves are sloped and would appear to allow a reasonable analysis. It is fair (although Matsuda did not carry out an estimate) to estimate the errors in this analysis as ±15%.

Tanninen and Ylirussi (8) used computer curve fitting to carry out a mixture analysis of prazosin. In this particular case, they reported a highly accurate analysis, and, in fact, showed a calibration curve that could detect 0.5% of one form in another. This is obviously a highly accurate mixture analysis by powder diffraction and shows the power of this method for some applications. However, this analysis required extreme care in sample preparation and may be more difficult to carry out in a production setting where particle size may not be controlled. Similar comments apply to the analysis of mixtures by IR, where the accuracy and precision may also vary considerably from case to case. Given the analytical problems in dealing with mixtures of forms, it may generally be simpler to develop a method to prepare only one crystal form.

In summary, it is important to determine whether polymorphs are present and to solve any problems before pivotal clinical studies are initiated.

D. Determination of the Polymorph Present in the Drug Product

In cases where stability or bioavailability issues exist, the solid form present in the drug product should be investigated, if possible.

For bulk drug substances, X-ray powder diffraction and other techniques can identify the polymorph; however, solid state NMR appears to be the best method for the study of the drug substance in the dosage form (2, 3). Solid-state NMR study of three commercial products containing prednisolone showed that the products A and B contain Form I, whereas product C contains Form II.. This analysis was possible even though these tablets contain approximately 95 mg of excipients and 5 mg of drug. There are numerous cases, often involving complex mixtures or low dose products, where solid state NMR (and, in fact, any technique) will not be sensitive enough to identify the polymorph present in the drug product. However, the safety and efficacy is, of course, controlled by the potency assays and by the physical tests (e.g., dissolution).

HYDRATES (SOLVATES)

The flow chart/decision tree for hydrates (solvates) is shown in Figure 6. It outlines investigations of the formation

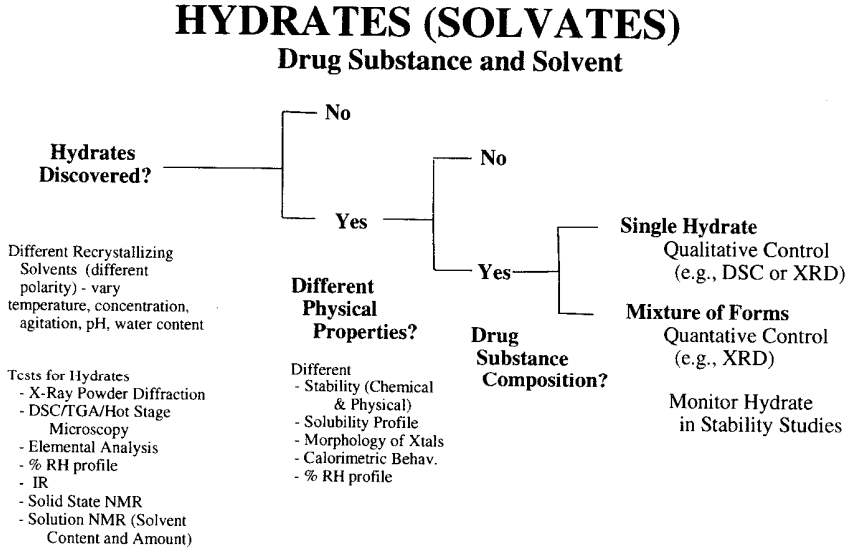

Figure 6. Flow chart for solvates or hydrates.

of hydrates (solvates), the analytical tests available for hydrates (solvates), studies of the physical properties of hydrates (solvates) and the controls needed to ensure the integrity of drug substance containing either a single morphic form or a mixture.

A. Have Hydrates (Solvates) Been Discovered?

The flow chart for hydrates (solvates) (Figure 7) is applied after the preliminary crystallizations have been completed. These are essentially the same as in the polymorph decision tree but, in addition, should include solvent-water mixtures in order to maximize the chance for hydrate formation. These experiments can be guided by the moisture uptake (% RH) studies. Any solids that indicate a significant change in water content as indicated by the % RH-moisture profile should also be examined. The resulting solid phases are preferably characterized by a combination of methods—two for phase identity and two to reveal composition and stoichiometry.

With a very few exceptions, the structural solvent contained in marketed crystalline drug products is water. It is nevertheless often desirable to characterize other solvated crystalline forms of a drug for several reasons: they may be the penultimate form used to crystallize the final product and thus require controlled characterization; they may form if the final crystallization from solvents, especially mixed solvents, is not well controlled; they may be the actual crystallized form of a final product that is desolvated during a final drying step; they may be the form used in recovery for subsequent rework. The relevance of these points will vary from case to case, but for the present discussion we shall treat the subject of solvates in its broadest form.

Examples taken from the literature serve to illustrate the kind of data that proves useful in characterizing solvated crystal forms. For example, a recent report from our laboratory showed that IR and solid state NMR was useful for the identification of the different crystal forms of dirithromycin(9). TGA is another powerful method for the analysis of solvates. For example, one early study showed that TGA could differentiate three different hydrated salts of fenoprofen(10). Combined with IR or other methods, TGA is an unequivocal method for the verification of the existence of solvates. In addition, TGA is a good method for looking at mixtures of solvated and unsolvated crystal forms, and probably can be developed into an analytical method for determining the ratios of solvated and unsolvated forms.

DSC is also a good method for detecting solvates since there is usually heat change involved in desolvation, especially for hydrates(11). Specifically, DSC by itself does not prove the existence of a solvate, but once other analytical

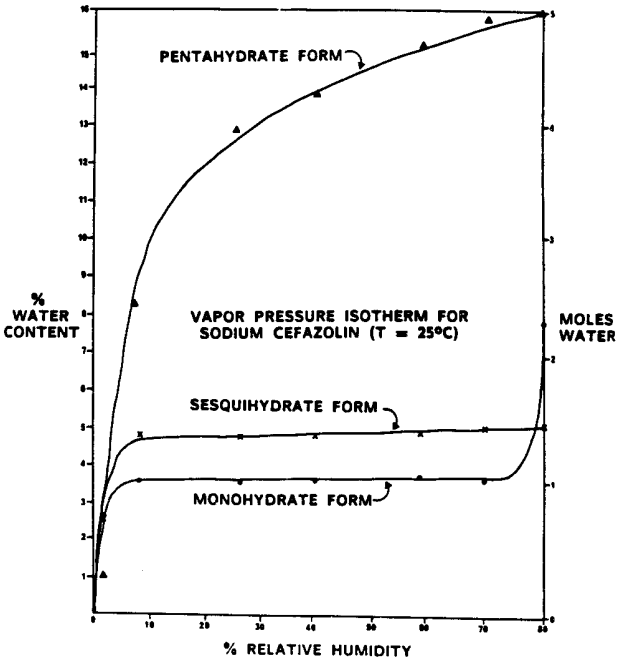

Figure 7. Water uptake vs percent relative humidity for sodium cefazolin.

data from TGA, NMR, etc. are available, DSC becomes a good method for analyzing solvates and determining a percentage of solvates present.

The three solvates of ethynylestradiol (0.5 acetonitrile, 1.0 methanol, 0.5 water) provides another interesting example (12). These solvates have different cell parameters and are crystallographically completely distinct materials. The hemihydrate was obtained from an organic solvent which is not completely miscible with water but was saturated with water. In fact, it is known that crystallization from water-immiscible solvents containing small but slightly different proportions of water can produce different hydrates of a substance.

The DSC/TGA of the three ethynylestradiol solvates(12) are unique and in this case it may be possible to develop DSC/TGA into an analytical procedure for determining the proportions of each solvate. The DSC in some of these traces appears to show a melt and recrystallization corresponding to the loss of solvent of crystallization. However, the exact interpretation of this is not possible without either a DSC microscope or interrupting the tracing to analyze the sample at various temperatures. The methanolate appears to lose solvent in two equal steps, indicating that there may also be a hemimethanolate of this compound. Again, confirmation of this would require interrupting the heating and analyzing the substance after the first solvent loss has occurred. In addition, the DSC/TGA traces suggest that all of the forms are converted to an anhydrous form which then melts at a higher temperature. Thus, interrupting any one of these thermal curves just prior to the final melt could reveal a new form that gives the powder pattern for the anhydrate. Unfortunately, no data of this type is provided in the case cited.

DSC analysis of solvates should be carried out using either an open pan or a pan with a pin-prick; otherwise, unusual and variable results will be obtained because the solvent is not provided a way of escape from the pan. One advantage of using an open pan for DSC is that it reproduces the conditions under which the TGA is performed.

Comparison of the ethinylestradiol powder diffraction patterns clearly establishes that these solvates are different crystal forms as would be expected from the single crystal data(12). In summary, DSC, TGA, and powder diffraction are all good methods for analysis of the different crystal forms of ethinylestradiol.

Figure 7 shows a percent relative humidity versus water uptake study of the type recommended by the USP committee on water(13) In this case, there are two hydrates which are relatively well behaved insofar as they are completely hydrated at about 10% relative humidity and remain uniformly hydrated throughout a wide humidity range. On the other hand, the so-called pentahydrate, which really is only a pentahydrate at very high humidity, changes water content considerably as the relative humidity is changed. The USP committee on moisture specifications recommended that moisture uptake vs relative humidity studies should be routinely performed on all drug substances and excipients (13).

B. Do the Hydrates (Solvates) Have Different Physical Properties?

The physical properties of hydrates are often quite different from the anhydrate form. Figure 8 shows the dissolution profile of theophylline hydrate and anhydrate. This figure shows that the anhydrate reaches a much higher solubility in water, and on extended exposure recrystallizes to the less soluble hydrate. Such differences must be further examined for possible effects on bioavailability.

In our laboratory we have described the different crystal forms of hydrocortisone-21-tertiary butylacetate(14). Our studies showed that the nonstoichiometric ethanolate is oxygen-sensitive and, of course, would show different physical properties from the stoichiometric ethanolate and the other solvates. Prednisolone tertiary-butylacetate also exists as a nonstoichiometric hydrate which is oxygen sensitive(15). Thus, these are cases where different crystal forms have different chemical stability, although there may be no significant differences in solubility.

C. Mixtures of Polymorphs and Hydrates

Other drug substances exist as both polymorphs and solvates. For example, furosemide exists in two polymorphs, two solvates, and an amorphous form (16, 17). The polymorphs are enantiotropically related, which means that at one temperature one polymorph is more stable, but at a different temperature the other polymorph is physically more stable. That is, plots of solubility versus temperature cross for the two polymorphs. In addition, the different crystal forms have different photostability (chemical stability in light) and moreover have different dissolution rates. Thus, there are significant differences in both chemical and physical properties.

The five different forms, or modifications of furosemide, give clearly different powder patterns. Thus, powder diffraction is a good method for analysis of these different forms. There are similarities between the IR spectra of the five different forms but there are also some significant differences, and expansion and careful analysis could lead to an FT/IR method for analysis of these different forms. IR would probably be a useful method for analysis at least for pairs of these compounds. However, it is not clear whether IR could be used to determine the percentages of several different forms in a more complex mixture. The DSC and TGA of the different forms are significantly different. As expected, the solvates show weight loss in the TGA.

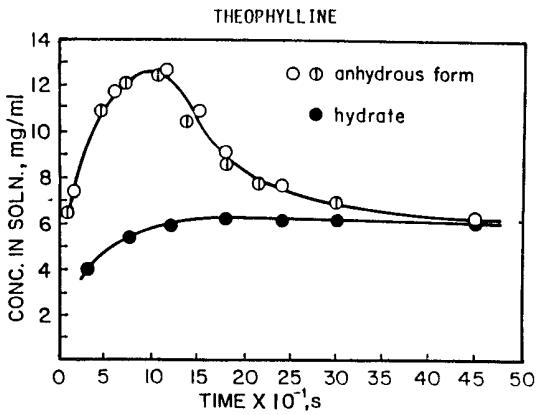

Figure 8. The dissolution-time curves for anhydrous and hydrated theophylline in water at 25°. The two types of open circles represent successive experiments (18).

The interconversion of the different forms of furosemide have been analyzed and a diagram constructed. Such a diagram can be experimentally difficult when so many pairs of crystal forms must be studied for possible interconversions and under different conditions. It is clear from this diagram that many of the forms of furosemide can be converted to form I. This study is one of the most complete reports of solvates and polymorphs available in the literature and serves as a model for studies of such systems for regulatory submissions.

D. Determination of the Hydrate Present in the Drug Product

Another important area is the analysis of the material which is produced after wet granulation of a substance which can form hydrates. We are aware of cases where the bulk drug substance is manufactured and stored as the anhydrate. However, upon wet granulation, there is a conversion (either partial or complete) to a hydrate. Subsequent drying is sometimes not adequate to convert the substance back to the anhydrate, and a hydrate or a mixture of hydrate and anhydrate remain. The formation of a hydrate and its subsequent drying can result in a change in particle size of the drug substance (19). It may also be possible to cause transformations during other processing steps. It is thus recommended that if wet granulation or processing that subjects the drug to even brief changes in temperature or pressure (e.g. milling or compression) is contemplated, then extensive studies of the ability to convert the drug substance to a new crystal form be carried out by mimicking the processing step in the laboratory.

DESOLVATED SOLVATES

The term "desolvated solvates" refers to compounds that are crystallized as solvates but undergo desolvation prior to analysis. Often these "desolvated solvates" retain the structure of the solvate with relatively small changes in the lattice parameters and atomic coordinates, but no longer contain the solvent. In addition, desolvated solvates are apt to be less ordered that their crystalline counterparts. These forms are particularly difficult to characterize properly since analytical studies indicate that they are unsolvated materials (anhydrous crystal forms) when, in fact, they have the structure of the solvated crystal form from which they were derived. Several observations may give clues that one is dealing with a desolvated form: (1) The form can be obtained from only one solvent; (2) On heating, the form converts to a structure known to be unsolvated; and (3) The form has a particularly low density compared to other forms of the same substance. Experiments that help to clarify whether an apparently solvent free modification is a desolvated form or a true anhydrate include: (1) Single crystal X-ray structure determination in the presence of mother liquor from the crystallization; (2) comparison of the X-ray powder diffraction patterns and solid state NMR spectra of the solvated and desolvated crystal forms; and (3) determination of the vapor pressure isotherm by varying the vapor pressure of the specific solvent involved. A desolvated form will often take up stoichiometric amounts of the relevant solvent. In addition, crystals of the form directly isolated from the crystallizing medium will show a plateau in their isotherm as the vapor pressure of the solvent is reduced.

Figure 9 shows the flow chart used to address regulatory issues involving desolvated solvates. It is similar to the polymorphs flow chart except that the first question involves determining whether a solvate was formed initially and then desolvated, perhaps by "air drying." The remaining questions are identical to the polymorphs flow chart.

Figure 10 shows the behavior of three different crystal forms of the same antibiotic. One crystal form takes up about two waters of hydration and further analysis indicated that it was the dimethanolate which had been desolvated by drying. The second crystal form takes up approximately one water and was originally the monomethanolate which had been desolvated by drying. The third crystal form also takes up

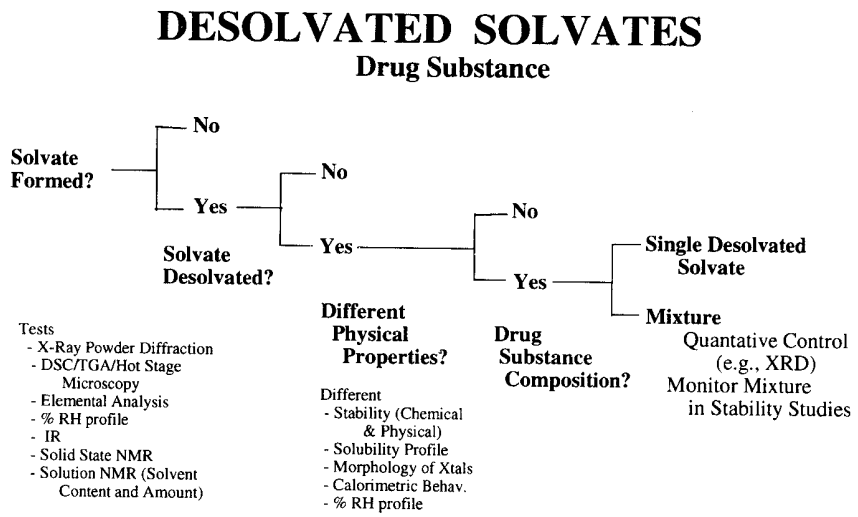

Figure 9. Flow chart for desolvated solvates.

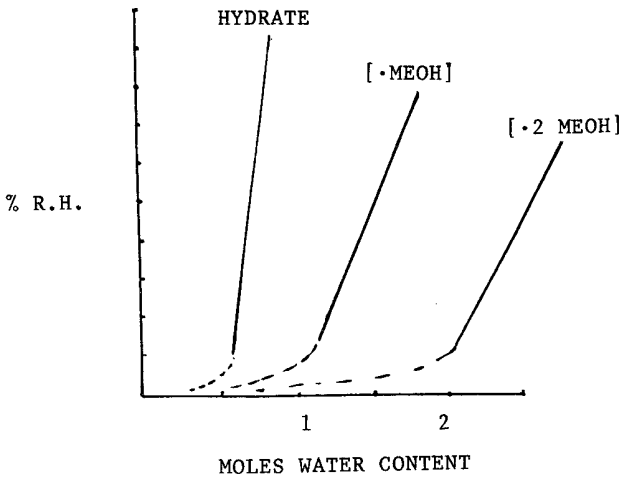

Figure 10. Water sorption by three crystal forms of cephaloridine. The brackets indicate the crystal form produced by desolvating the designated methanolate.

about one molecule of water and is the 0.75 hydrate typically obtained from water solution.

AMORPHOUS FORMS

Amorphous forms are of substantial interest because they usually are much more soluble than their crystalline counterparts. Indeed, there are cases where the amorphous forms is the only solid form that has adequate bioavailability. The initial question with this flow chart (Figure 11) is similar to the previous ones: "Are amorphous forms possible?" Amorphous forms can be prepared in different ways, for example, by spray drying or by freeze drying. One can test whether an amorphous form has been produced by using one of the methods listed. X-ray powder diffraction and microscopy are the two primary methods for determining whether an amorphous form has been produced. Powder diffraction is an excellent method for determining the existence of an amorphous form since they usually exhibit a broad hump between 2 and 20° 2θ. An amorphous form is expected to have no peaks in the powder diffraction pattern. The USP test for the presence of an amorphous form involves determining, by microscopy, whether the material lacks birefringence. IR and solid-state NMR may be useful for detecting amorphous forms since the amorphous nature of the solid sometimes results in broad lines, or in NMR, altered relaxation times. The next question on the flow chart is: "Do the amorphous forms have different physical properties?" The answer to this question will almost certainly be "Yes." Three differences from crystalline forms may generally be expected: 1) Amorphous forms would have greater solubility, 2) Amorphous forms take up water more extensively, and 3) Amorphous forms are sometimes less chemically stable. Another key question for an amorphous form is: "Does it crystallize, and how and when?" This question is very important since inadvertent crystallization can greatly affect the solubility and dissolution rate, and lead to other failures in formulation. Attempts to purposely cause amorphous forms to crystallize can provide information on the parameters involved in crystallization of amorphous forms. Specific questions include: (1) "Does the amorphous form crystallize upon exposure to heat and/or humidity?," and (2) "What other factors (e.g. mechanical pressure and seeding) can lead to the crystallization of the amorphous forms?"

The amorphous form of any substance can be partly characterized by the glass transition temperature, T_g (11). When heated to a temperature above T_g, the solid transforms from a glassy state to a more fluid-like rubbery state. The corresponding increased molecular mobility greatly raises the likelihood of two adverse events: (1) Crystallization and subsequent decreased solubility; and (2) Reduced chemical stability in the more reactive amorphous solid. Amorphous solids are also often prone to absorb moisture and this water sorption reduces the glass transition temperature further. The weight of water required to reduce the glass transition

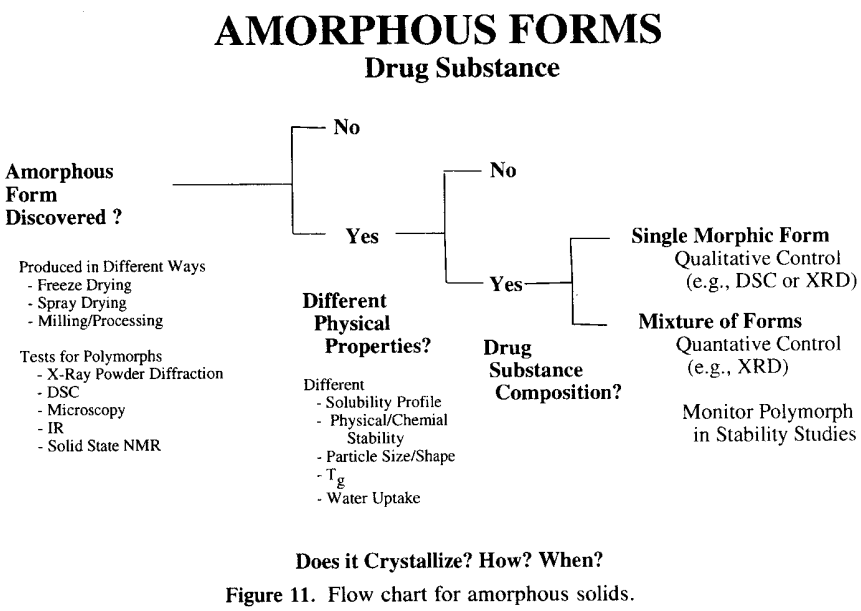

Figure 11. Flow chart for amorphous solids.

temperature to room temperature is of obvious interest and is termed W_g. Table I shows a series of interesting studies on amorphous forms of some common pharmaceuticals.

The table compares the glass transition temperatures (T_g) of a number of pharmaceutical solids with the melting temperatures (T_m). It is interesting that the average ratio of the glass transition temperature to the melting temperature is about 0.70. This table provides a simple rule of thumb which allows the prediction of the glass transition temperature of pharmaceuticals from the known melting point. Crystallization and other solid-state phenomena, such as degradation reactions, as we have said, would be more likely to occur at temperatures above the glass transition temperature. For stability, one might, therefore, wish to prepare amorphous forms only for drugs which have a T_g well above room temperature.

Amorphous indomethacin crystallizes upon standing at room temperature(Figure 12). Obviously, formulations containing amorphous indomethacin are at significant risk to crystallize and thus become less soluble. This has to some extent hampered preparing more bioavailable indomethacin dosage forms.

Quantitative analysis of mixtures of amorphous and crystalline forms provides some challenges. Cefixamine trihydrate is the subject of some early research in this area. This antibiotic, upon grinding, became a mixture of crystalline and amorphous forms. A calibration curve based upon analyzing the height of a selected powder X-ray peak was constructed and used to determine the crystallinity versus grinding time for this system. It is clear that powder diffraction provides a way to estimate the amount of amorphous cefiximine. These studies show that milling and other similar processing steps can create amorphous material and that this process may be detectable. As with wet granulation where transitions to hydrated forms can occur, processing of the drug substance can promote the formation of amorphous drug.

Pikal has compared the analysis of mixtures of crystalline and amorphous forms of several antibiotics by powder diffraction and calorimetry (20). His studies indicate that calorimetry can be a more accurate method for analysis of percent crystallinity but are complicated by water sorption. Zografi and co-workers (unpublished results) have developed a powerful method for the determination of low percentages of amorphous material based on the general propensity of amorphous materials to sorb moisture.

Table I. Pharmaceuticals Forming Glasses Above Room Temperature (21)

Pharmaceutical	T_g (K)	T_m (K)	T_g/T_m
Cholecalciferol	296	352	0.84
Sulfisoxazole	306	460	0.67
Stilbestrol	308	439	0.70
Phenobarbital	321	443	0.72
Quinidine	326	445	0.73
Salicin	333	466	0.71
Sulfathiazole	334	471	0.71
Sulfadimethoxine	339	465	0.73
Dehydrocholic acid	348	502	0.69
17β-Estradiol	351	445	0.80

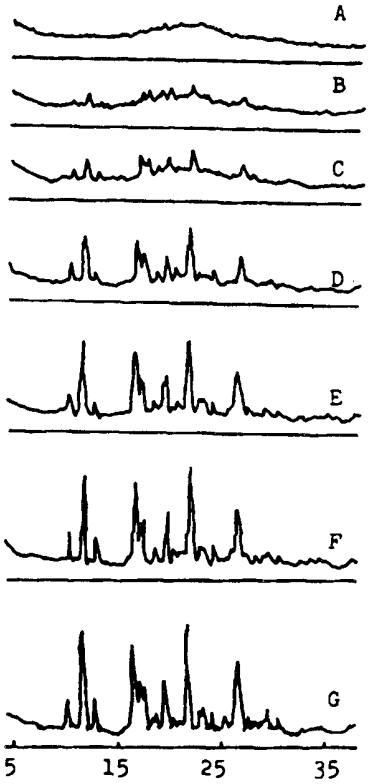

Figure 12. Behavior of amorphous indomethacin upon standing: A, at start; B 24 h; C, 48 h; D, 7d; E, 14d; F, 30d; G, 67d (22).

SUMMARY

Four flow charts which describe approaches to regulatory issues involving pharmaceutical solids have been developed. These flow charts are for the different types of solids generally encountered (polymorphs, solvates, desolvated solvates, and amorphous forms). It is hoped that these flow charts will guide the solid-state research needed to prepare a comprehensive regulatory submission on the physicochemical properties of a pharmaceutical. It is also hoped that this review has provided enough information to allow the generation of results and information necessary to prepare a drug substance submission that will be quickly approved.

ACKNOWLEDGMENTS

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Crystals from first principles

A new calculation of the polymorphs of silica appears to have broken new ground in deriving crystal structure from chemical composition. But X-ray crystallographers need not worry — yet.

ONE of the continuing scandals in the physical sciences is that it remains in general impossible to predict the structure of even the simplest crystalline solids from a knowledge of their chemical composition. Who, for example, would guess that graphite, not diamond, is the thermodynamically stable allotrope of carbon at ordinary temperature and pressure? Solids such as crystalline water (ice) are still thought to lie beyond mortals' ken.

Yet one would have thought that, by now, it should be possible to equip a sufficiently large computer with a sufficiently large program, type in the formula of the chemical and obtain, as output, the atomic coordinates of the atoms in a unit cell.

That time may not yet have arrived, but S. Tsuneyuki, M. Tsukada and H. Aoki from the University of Tokyo, with Y. Matsui from Okayama University, have brought it a good step nearer. Starting with the information that the polymorphic forms of pure silica consist exclusively of SiO_2, they first calculate, from first principles, the force constants in clusters of the atoms and then, by molecular dynamics, the lattice constants of the four common polymorphs of silica (α-quartz, α-cristobalite, coesite and stishovite — the two last first recognized in the debris from meteoritic impacts).

The authors thank two computer centres in Japan for access to computing machinery, although they do not say how many Cray-equivalent hours their calculations occupied (*Phys. Rev. Lett.* **61** 871; 1988).

The calculation is not merely a taste of things to come but a pointer to the obstacles that remain, as well as being, in its own right, a considerable achievement. What the group has done is to invert the usual procedure in which force constants are calculated empirically from the properties of crystals. Their strategy is instead to calculate the force constants from first principles, for which purpose they must calculate the electronic structure of a complex of silicon and oxygen atoms.

The snag is that, even with the the price of Cray machines and their equivalents falling year by year, it remains beyond the capacity of computation to calculate the properties of a real crystal. So Tsuneyuki *et al.* start with the simplest of all relevant structures — the tetrahedral cluster SiO_4^{4-}.

But why SiO_4^{4-}, not SiO_4? Because Si readily surrenders electronic charge to oxygen atoms — the authors estimate 0.7 of an electron charge for every SiO bond, or close on two electron charges for those O atoms bound to two Si atoms. So, to ensure that their calculated structures are electrically neutral, the authors place electronic charges at points 1.65 Å along the four tetrahedral axes of the cluster, neatly simulating in the process the electrostatic effects of the neighbouring oxygen ions found in structures such as quartz.

It would be wrong to say that, from this point, everything depends on what the programmers have done, but that is how the article reads. Tsuneyuki *et al.* essentially calculate the electronic structure of the cluster for various departures of the atoms from their mean positions. They use pre-calculated atomic orbitals in a Hartree–Fock self-consistent field procedure (one of the means of allowing for electron–electron interactions in many-electron atoms).

In principle, there is every reason why the energy of the system should be quite a complicated function of the atomic positions. The surprise is that the parts of the energy surface so far calculated can be accurately represented by potential functions depending only on the distances between pairs of atoms in the cluster. Not surprisingly, one of these is a Coulomb term, another corresponds to the van der Waals attractive potential proportional to r^{-6} where r is the separation.

The molecular dynamics is made to seem just routine, no doubt because of the careful development of the appropriate programs by Japanese colleagues. Pressure and temperature are determined by the size of a repeating three-dimensional unit in a periodic lattice and by total kinetic energy respectively. The known structures of the four polymorphs of silica are each dealt with separately. In each case, the object of the exercise is to tell whether the polymorphs are dynamically stable, or that they are not spontaneously converted into each other, which all four prove to be. This is taken as an explanation of why they are all apparently stable under ordinary conditions even though, thermodynamically, they are metastable.

Given previous difficulties in calculating from first principles the properties of real solids, the results are extremely good. Unit cell dimensions turn out to be within 5 per cent of their measured values, bulk properties such as density and bulk modulus within 10 per cent. The agreement between calculated energy (enthalpy) of the polymorphs and the few measurements available is even better (1 per cent), but the authors say they could probably do even better if they allowed for the band structure of these polymorphs and the extent to which the bands are filled at different temperatures.

Several refinements remain to be explored. The chances are that three-body potentials and even more complicated variations of energy with configuration can be demonstrated by calculation and by comparison, for example, with measured specific heats. Evidently the treatment of charge transfer between Si and O, while effective, is somewhat artificial and does not allow for cooperative configurations extending over more than one SiO_4^{4-} tetrahedron.

In themselves, of course, these results are not a fulfilment of the goal of calculating from first principles the crystal structure of a compound of which nothing is known except chemical composition. But that cannot be far away.

On the face of things, the most obvious artificially introduced variables are the known crystal structures of the polymorphs of silica, used as a starting point for the molecular dynamics. It should not be beyond the wit of Tsuneyuki and his colleagues, with the help of a few computer centres, to select from a listing of all possible structures of an arbitrary material those that can be considered plausible on crystallographic and other grounds.

Perhaps the most striking feature of this laconic paper is the contrast it presents with the state of the art of calculating the properties of solids as recently as a decade ago, when it seemed that even the use of force constants derived empirically from the measured properties of crystals did not regenerate, on calculation, the authentic properties of those same crystals. But the practical importance of the new calculation could be considerable, especially with the need to learn something of the crystal structure of materials that are either very small ('microdot' zero-dimensional quantum wells, for example) or which vary in composition over distances smaller than the limits of X-ray crystallography (such as doped semiconductors). In applying calculations to these needs, a demonstration of success can rank, psychologically, with the example set by those who first climbed Everest.

John Maddox

Solid-State Pharmaceutical Chemistry

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Solid-state pharmaceutical chemistry encompasses a wide range of studies on pharmaceutical solids including (1) determination of the physical properties of polymorphs and solvates, (2) physical transformations between polymorphs and solvates, (3) chemical reactions in the solid state, and (4) solid–solid reactions which occur in pharmaceutical preparations. Recent advances in this field include improved understanding of crystallization processes, improved understanding of the need for characterization of polymorphs and solvates for both control and regulatory purposes, and a better understanding of the mechanisms of solid-state degradations and solid–solid reactions. This review will briefly describe recent advances in the following areas: (1) crystallization and the properties of crystals of pharmaceutical solids; (2) characterizations of crystal forms of drugs using solid-state NMR spectroscopy.

The study of the solid-state chemistry of drugs not only encompasses many scientific disciplines but also impinges on virtually all phases of the pharmaceutical industry, from discovery to successful marketing. It is clear that an understanding of the molecular structure of the solid state can lead to better design and control of drug performance.

The mission of those working in the field of solid-state pharmaceutical chemistry is to provide each drug in a solid form that has optimum performance in a given application. Pursuit of this mission requires recognition of several general, interrelated points: (1) Drugs can exist in a number of solid forms, each having different properties of pharmaceutical importance, including stability and bioavailability; the number and properties of these forms are largely unpredictable and vary considerably from case to case. (2) The forms of a drug may interconvert under various conditions. (3) Once a solid form is chosen for a product, methods for analysis and control of the form must be devised.

Let us briefly review each of these points with regard to their status in current practice and to some associated scientific challenges that remain.

(1) The most common solid forms that may be found for a given drug substance are as follows: **crystalline polymorphs**, forms having the same chemical composition but different crystal structures and therefore different densities, melting points, solubilities, and many other important properties; **solvates**, forms containing solvent molecules within the crystal structure, giving rise to unique differences in solubility, response to atmospheric moisture, loss of solvent, etc. Sometimes a drug product may be a **desolvated solvate**, formed when solvent is removed from a specific solvate while the crystal structure is essentially retained—again, many important properties are unique to such a form; finally, **amorphous** solid forms that have no long-range molecular order (i.e., no crystallinity) and which tend to be more soluble, more prone to moisture uptake, and less chemically stable than their crystalline counterparts (pharmaceutical processing operations may produce solids of low crystallinity intermediate between that of a crystalline solid and an amorphous solid).

To be sure, differentiating among the various solid forms of a substance is generally a routine matter. A number of analytical methods, used together, make this possible. Various familiar methods reveal the chemical composition of the solid and reveal the presence of solvent. The physical form is further characterized by X-ray powder diffraction, infrared and solid-state NMR spectroscopy, differential scanning calorimetry, and microscopy. The single most valuable piece of information about a crystalline solid, although not always available, is the molecular structure, determined by single-crystal X-ray diffraction.

Perhaps the chief challenge in managing the phenomenon of multiple solid forms of drugs is our inability to predict how many forms can be expected in a given case: too often costly delays are encountered when a less soluble solid form suddenly appears late in a development program. Progress along these lines awaits analysis and quantification of the myriad intermolecular forces within any proposed crystal structure as well as the ability to postulate the likely packing modes for a given molecule in all its configurations. Further research similar to that of Margaret Etter and co-workers, reviewed in section A, will doubtless lead to better success in predicting alternative solid forms of new drugs.

A second challenge relates to the strikingly different reactivity of different solid forms of many substances, whether it be oxidation, dehydration, decarboxylation, or other chemical reactions. Kinetics involving the solid state, in which specific contacts (or noncontacts) between reactive groups are dictated by the structure of a given solid form, are apt to be more complex than kinetics in solution, where the corresponding molecular encounters are much more random. Much the same can be said about the relative difficulty of elucidating mechanisms in these two states. Moreover, reactions in the solid state may be further complicated by other, often unknown factors such

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as nucleation of a reaction product phase, presence of various amounts of amorphous component, strain or disorder in the crystal structure, and multiplicity of reaction products. As a result, there is very little information available to guide in any general way the prediction of the stability of compounds in the solid state, let alone in their different solid forms or different formulations. Thus, the stability of all but the most rugged products is generally characterized empirically at actual storage temperatures, and the value of studies at elevated temperatures must be assessed case by case. Elucidation of the kinetics and mechanisms of solid-state reactions is pursued in only the most pressing cases.

(2) A dictate of formulation technology is that the physical form of the drug substance, after being defined and verified, should not change once the product has been manufactured. Therefore, in addition to identifying various solid forms of a drug, an understanding of the specific factors that may bring about *transformations* between the forms is also essential.

Rates of solid-to-solid transformations in drugs are affected by one or more of the following variables: temperature, solubility in a given liquid phase, and vapor pressure of solvent. At a minimum, the investigator must therefore first identify and obtain certain physicochemical data on each relevant solid form:

- transition temperature(s) between polymorphs
- solubility of the drug in all solvents and solvent mixtures used in preparation of the final drug substance and throughout the formulation process
- equilibrium water vapor pressure *vs* composition isotherm

This information reveals which solid form is the most physically stable (least soluble) under specific conditions of temperature and composition; thus, if that form is already the one present, no transformation will occur. If, however, a less physically stable (more soluble) form is present, the *direction* of any transformation can now be predicted for given conditions. One might well ask why the most stable form is not always selected and, indeed, that is usually the case. There are however, situations where the peculiar properties of a less stable solid form are required for a product's performance—achieving higher solubility, for example. In such cases, the manufacturer has the added burden of demonstrating the *lack* of transformation to a more stable solid form throughout the life of the product. As discussed in the previous section, this task requires dealing with the problems of solid-state kinetics and must usually be approached empirically.

(3) Two main challenges to the analysis of pharmaceutical solids are dealing quantitatively with mixtures of forms in the drug substance and identifying the solid form of the active ingredient in the formulated product, particularly when the drug is a minor component in the presence of numerous other materials (excipients).

With this background on the current status of solid-state pharmaceutical chemistry, we can now turn to a number of recent advances in this field.

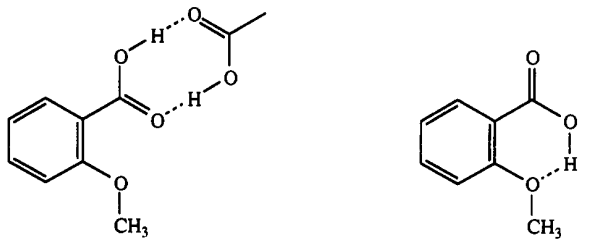
The work of Margaret Etter and co-workers, treated in section A, is an example of how present research addresses some of the issues of structure prediction raised above.

The topics discussed in sections B–D have significant bearing on many of the issues discussed in all three of the introductory subsections.

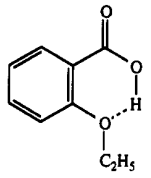
A. Forces Holding Crystals Together

Two main types of forces are responsible for holding drug crystals together: nonbonded interactions and hydrogen bonding. Nonbonded interactions occur in all crystals while hydrogen bonding is important in many compounds, especially pharmaceuticals. Etter has reviewed the extent and types of hydrogen bonding which can exist in organic solids.[1,2]

Carboxylic acids have been a particularly useful class of compounds for investigating alternative hydrogen bonding possibilities. For example, in *o*-alkoxybenzoic acids both dimerization and formation of intramolecular hydrogen bonding are observed.[3] In *o*-anisic acid, dimers are observed in the solid state while intramolecular hydrogen bonds are observed in dilute solution. However,



in *o*-ethoxybenzoic acid, only intramolecular hydrogen bonds are observed in the solid state and in solution.



The Etter group studied the hydrogen bonding in salicylamide derivatives and pointed out that two types of hydrogen bonding are possible in these compounds.[4] One type involves an intramolecular NH···O hydrogen bond and an intermolecular C═O···HO— hydrogen bond and the other an intramolecular C═O···HO— hydrogen bond and an intermolecular NH···O hydrogen bond.

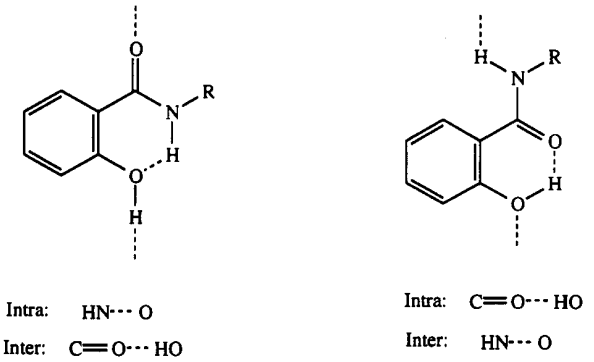


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Table 1. Reliable and Occasional Hydrogen-Bond Donors and Acceptors

type	functional group involved
reliable donor	–OH,[a] –NH_2, –NHR, –$CONH_2$, –CONHR, –COOH
occasional donor	–COH, –XH, –SH, –CH
reliable acceptors	–COOH, –CONHCO–, –NHCONH–, –CON< (1–3°), >P=O, >S=O, –OH
occasional acceptors	>O, –NO_2, –CN, –CO, –COOR, –N<, –Cl

[a] Not COH.

Etter, MacDonald, and Bernstein developed a graph-theory-based approach to classifying and symbolically representing the different types of hydrogen bonds that can be formed.[5]

Etter also developed rules governing hydrogen bonding in solids. These rules require a classification of hydrogen bond donors and acceptors into "reliable" hydrogen-bond donors and acceptors and "occasional" donors and acceptors (Table 1). Using these classifications, three rules were devised: (1) All (or as many as possible) good proton donors and acceptors are used in hydrogen bonding. (2) Six-membered ring intramolecular hydrogen bonds form in preference to intermolecular hydrogen bonds. (3) The best proton donors and acceptors remaining after intramolecular hydrogen bond formation will form intermolecular hydrogen bonds. These rules apply quite well to hydrogen bonding of small molecules. However, in some larger molecules, e.g., erythromycins, steric factors make it impossible to satisfy all of the possible hydrogen bonded interactions, and some donors and acceptors are not involved in any hydrogen bonds.

Cocrystals. An important aspect of research into hydrogen bonding involves the realization that cocrystals can be obtained from certain solutions containing more than one molecular species. Cocrystals can also be formed by mixing or grinding two solids together. Cocrystals are usually formed between a hydrogen-bond donor molecule and a hydrogen-bond acceptor molecule. The nature of hydrogen bonding in cocrystals can also be described using the above rules. The cocrystals observed by Etter's group include numerous ureas with ketones, carboxylic acids with 2-aminopyridine, and also adenine or cytosine combined with many acidic organic compounds[6] such as carboxylic acids and *N*-acylamino acids. Other classes of cocrystals investigated by Etter's group are

pyrimidine, pyridines: carboxylic acids

pyridine-N-oxides: acids, alcohols, amines

phosphine oxides: acids, amides, alcohols, ureas sulfonamides, amines, water

carboxylic acids: other carboxylic acide, amides

m-dinitroureas: acids, ethers, phosphine oxides, sulfoxides, nitroanilines

imides: other imides, amides

The formation of cocrystals may be quite important in explaining solid–solid interactions in many fields including those of pharmaceuticals.

This elegant work of Etter and co-workers has greatly increased our understanding of the hydrogen-bonding interactions of molecules in both the solid state and in solution.

B. How Crystals Form

Crystallization and the factors controlling the formation of crystals is an extremely important area in solid-state pharmaceutical chemistry. Dr. Margaret Etter made an extremely important observation when she pointed out that molecules in solution often tend to form different types of hydrogen-bonded aggregates and hypothesized that these aggregate precursors are related to the crystal structures that form from the supersaturated solution.[2] This concept helps to explain the many different hydrogen-bonding motifs seen in different solids.

A number of factors can affect the crystal formed either by influencing the hydrogen-bonded aggregate precursors in solution or influencing one of the many other factors involved in crystallization. These include (1) solvent composition or polarity, (2) concentration or degree of supersaturation, (3) temperature including cooling rate and the cooling profile, (4) additives, (5) seeds and the presence of seeds, (6) pH (pH is important for crystallization of salts), and (7) agitation.

The composition of the solvent used is known to influence crystallizations either directly or by influencing the temperature at which the crystallization is initiated. For example, in the mannitol system, the α-polymorph is formed by evaporation of 100% ethanol while the β-polymorph is formed by crystallization from aqueous ethanol.[7] In a study of inosine, Suzuki showed that crystallization from water gave the α-form whereas crystallization from 70% DMSO gave the β-form.[8]

A second important factor influencing crystallization is the degree of supersaturation—the ratio of the concentration of the solution to that of a saturated solution. In his study of the polymorphism of cimetidine, Sudo showed[9,10] that in isopropyl alcohol at high supersaturation ratios (greater than 3.6) form A crystallized spontaneously or in the presence of seeds of either form A or form B, whereas in lower supersaturation ratios (less than 2) form A crystallized if there were A seeds and B crystallized if there were B seeds.

Temperature can have a very significant effect on the polymorph produced. Studies by Kitamura[11] on the crystallization of L-glutamic acid showed that at 45° the α-form nucleates slowly resulting in β-form growth, whereas at 25 °C the α-form nucleates rapidly causing α-form growth.

The effect of additives on crystallization has been of interest for many years. Early work by Simonelli indicated that polymeric additives could prevent the crystallization of certain phases.[12] Significantly, studies in recent years by Lahav and co-workers[13] have shown that additives (as

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little as 0.03%) can inhibit nucleation and crystal growth of a stable polymorph, thus favoring the growth of a metastable polymorph. They also showed that it is possible to design crystal nucleation inhibitors to control polymorphism.

In addition, Grant et al. have shown the effects of additives on the properties of adipic acid, acetaminophen (paracetamol), and (*R*,*S*)-(−)-ephedrinium-2-naphthalenesulfonate, a chiral drug. The effects on each of these crystalline solids will now be considered in turn.

When adipic acid is crystallized from water containing traces of *n*-alkanoic acids[14] or oleic acid,[15] the additive is taken up by the crystals, while the very small water content is not significantly affected. Changes in crystal habit and in the crystal growth kinetics are consistent with a structural model in which the added *n*-alkanoic acid molecules occupy lattice sites at the crystal surfaces.[16] The incorporated additive also changes the thermodynamic properties of the crystal. Notably, the crystal energy and entropy are increased, as measured by reductions in the enthalpies of fusion and solution, while the melting point is little affected.[14,15] In addition, the Gibbs free energy of the crystal increases, as measured by the dissolution rate and the specific surface area, and the density of the crystals is changed. Furthermore, the compaction properties, as measured by Hiestand's indices of tableting performance of the crystals are also modified.[17] Low levels of incorporated *n*-octanoic acid produce an increase in lattice strain, reducing the energy required for plastic deformation leading to improved tableting performance. At higher levels of incorporated *n*-octanoic acid, the tableting performance again approaches that of the pure crystal, indicating a reduction in plasticity. The above results with adipic acid may be explained by the impurity defects and attendant dislocations introduced by incorporated additive, resulting in increased lattice strain at low concentrations of additive and in reversal of the effects at higher concentrations. These proposed changes in lattice strain have recently been confirmed by corresponding increases and decreases in the mosaic spread of the Laue diffraction pattern when single crystals are irradiated by white X-radiation from a synchrotron source.[18]

When acetaminophen is crystallized from water containing the structurally related synthetic impurity, *p*-acetoxyacetanilide (PAA), the additive is incorporated and the crystals become acicular (needle-shaped). Increasing concentrations of PAA lead to increasing uptake of PAA to a maximum constant value (suggesting a saturated solid solution), to a decrease in water content, and to an increase in the length/width ratio.[15] Higher concentrations of PAA cause the length/width ratio and the water content to return to nearly the initial values. The maximum length/width ratio and the minimum in water content correspond approximately to maxima in the enthalpy and entropy of fusion and in the intrinsic dissolution rate of the crystals, the melting point being little affected. The physical properties of the acetaminophen crystals mentioned above were measured under 29 different crystallization conditions, defined by the initial concentration of PAA, the initial supersaturation of acetaminophen, and the rate of stirring of the crystallization solution.[19] Statistical analysis of the properties of these crystals, crystallized and analyzed in triplicate, showed strong correlations between the length/width ratio and the concentration of PAA taken up by the crystal and between the intrinsic dissolution rate and the length/width ratio, the thermodynamic quantities playing a minor role.[20] These results demonstrate the significance of additive-induced changes in crystal habit in influencing the intrinsic dissolution rate of acetaminophen crystals. Thus, whereas the behavior of doped crystals of adipic acid may be attributed to differences in lattice strain, the behavior of doped acetaminophen crystals may be attributed to differences in crystal habit.

In the above examples, adipic acid and acetaminophen exist as achiral molecules and crystallize in achiral space groups. The question arises as to the effect of traces of the opposite enantiomer on the crystal properties of a chiral drug. To help answer this question, the chiral drug (*R*,*S*)-(−)-ephedrinium 2-naphthalenesulfonate [(−)-EN] was crystallized from aqueous solutions containing traces of the opposite enantiomer (*S*,*R*)-(+)-ephedrinium 2-naphthalenesulfonate [(+)-EN].[21] Crystals of (−)-EN took up the opposite enantiomer with an appreciable segregation coefficient (0.153), while the water content and melting point of the crystals remained constant. Uptake of the opposite enantiomer led to changes in the thermodynamic properties and intrinsic dissolution rate. These changes are similar to those observed when adipic acid incorporated *n*-alkanoic acid from the crystallization solution. The similar behavior suggests an analogous molecular mechanism in the solid state, implying that doping with the opposite enantiomer produces changes in crystal properties that are analogous to doping of a foreign molecule of different, but related structure. Interestingly, (−)-EN and (+)-EN when heated together give a phase equilibrium diagram with a eutectic (together with limited solid solution formation) between each enantiomer and the racemic compound. This observation suggests that the observed changes in the thermodynamic properties and intrinsic dissolution rate of (−)-EN may be attributed to doping of the crystals with [(+)-EN/(−)-EN] molecular pairs or clusters rather than simply with the added (+)-EN molecules.

Seeding is used extensively to control crystal form and also to control the extent of nucleation (i.e., final particle size). In almost all cases, seeding can be used to control the desired crystal form. For example, Suzuki et al.[8] showed that the α-form of inosine could be obtained by crystallization from water whereas to obtain the β-form, seeds of the β-form must be used. An interesting study of the effect of seeding was reported by Konapudi, and the mechanism of crystallization was elucidated by McBride and Carter.[22] Sodium chlorate crystallizes in both a chiral and a racemic form. Since sodium chlorate is not chiral in solution, crystallization from aqueous solution produces equal numbers of L and D crystals.

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(21) Duddu, S. P.; Fung, F. K. Y.; Grant, D. J. W. *Int. J. Pharm.* **1993**, *94*, 171–179.
(22) McBride, J. M.; Carter, R. L. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 293–295.

Surprisingly, crystallization of an aqueous solution with stirring gives mostly crystals of one chirality, either L or D. Investigation showed that this effect was due to the fact that once a particular crystal (either L or D) has formed, when that initial crystal collides with the stirrer and is broken into many small seeds which then nucleate further crystallization of that hand crystal (either L or D). This observation supports the idea that one nucleating seed can produce a single crystalline form. It also suggests that in this particular crystallization, if one added a seed of either L or D crystals then one would obtain entirely that crystal form. This is one of the best examples of the concept: one seed, one crystal.

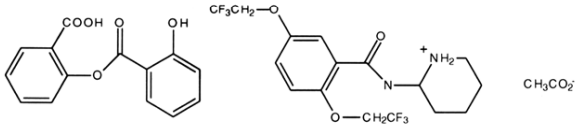
McCrone, in a letter to the editor of the *Journal of Applied Crystallography*, suggested that nucleation by seeding was the best explanation for the situation in which one unexpectedly obtains a new, more stable crystal form and then finds it is difficult or impossible to obtain the older less stable crystal form.[23] In response to this letter Jacewicz et al.[24] suggested that it is not impossible to produce the earlier form. They stated that the original crystal form should be capable of being produced but that the selection of the right conditions may require some time and trouble.

C. Disorder in Crystals of Pharmaceutical Solids

Another solid-state phenomenon which has important implications for pharmaceutical solids is disorder. Just as crystals of chiral and racemic drugs may have different physical properties, the presence of disorder in a crystal may also affect the physical properties of a crystal. One method of classifying disorder is to distinguish between cases involving static and dynamic disorder. Because single-crystal X-ray techniques give results which are averages over a long time period relative to molecular motion, X-ray crystallography at one temperature is usually unable to distinguish between static and dynamic disorder. Static and dynamic disorder can often be distinguished by comparing X-ray thermal parameters (ADPs) for two different temperatures. If the disorder results in observable resonance peaks in solid-state NMR spectra, then clearly this technique can be used to study this disorder.

In the crystallographic literature there are numerous examples of disorder which are caused by a molecule crystallizing in a single conformation but in a different orientation relative to other molecules in the crystal. There are also examples where the disorder is due to molecules crystallizing in different conformations.

Two such examples of conformational disorder are the crystal structures of nonsteroidal antiinflammatory (NSAID) drug salicylsalicylic acid (SALSALATE)[25,26] and the antiarrhythmic compound flecainide (TAMBOCOR).[27]



SALSALATE TAMBOCOR

(23) McCrone, W. C. *J. Appl. Crystallogr.* **1975**, *8*, 342.
(24) Jacewicz, V. W.; Nayler, J. H. C. *J. Appl. Crystallogr.* **1979**, *12*, 396–397.

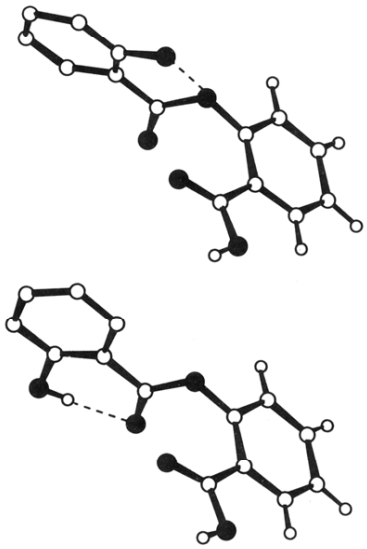


Figure 1. The two geometries of the SALSALATE molecule which are incorporated into the disordered crystal.

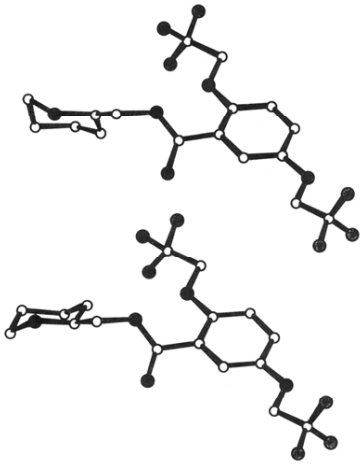


Figure 2. The two geometries of the TAMBOCOR which are incorporated into the disordered crystal.

The salsalate molecule can crystallize in two different conformations, each of which is intramolecularly hydrogen-bonded. Presumably, these two different intramolecular modes of hydrogen bonding are roughly equivalent energetically and the gross shape of the molecule in both conformations is similar so that both conformations can be incorporated into a single crystal as shown in Figure 1.

The other interesting example is the antiarrhythmic compound flecainide which is disordered in the solid state. The form of the compound which exhibits this disorder is actually the acetate salt, which was originally used for therapeutic purposes. Here the piperidinium ring takes on two alternate chair conformations as shown in Figure 2.

What are the consequences of such disorder? At the level of processing, the difficulty in reproducing material with the same properties may be responsible for different

(25) Frink, N.; Gleason, W. B.; Sweeting, L. M. In *IVth Midwest Organic Solid-State Chemistry Symposium*; Lincoln, NE, 1992.
(26) Frink, N.; Beaurline, J.; Gleason, W. B. In *Am. Cryst. Assoc. Annual Meeting*; Pittsburgh, PA, 1992.
(27) Gleason, W. B.; Bannit, E. H. In *Am. Cryst. Assoc., Annual Meeting*, 1987; p PE18.

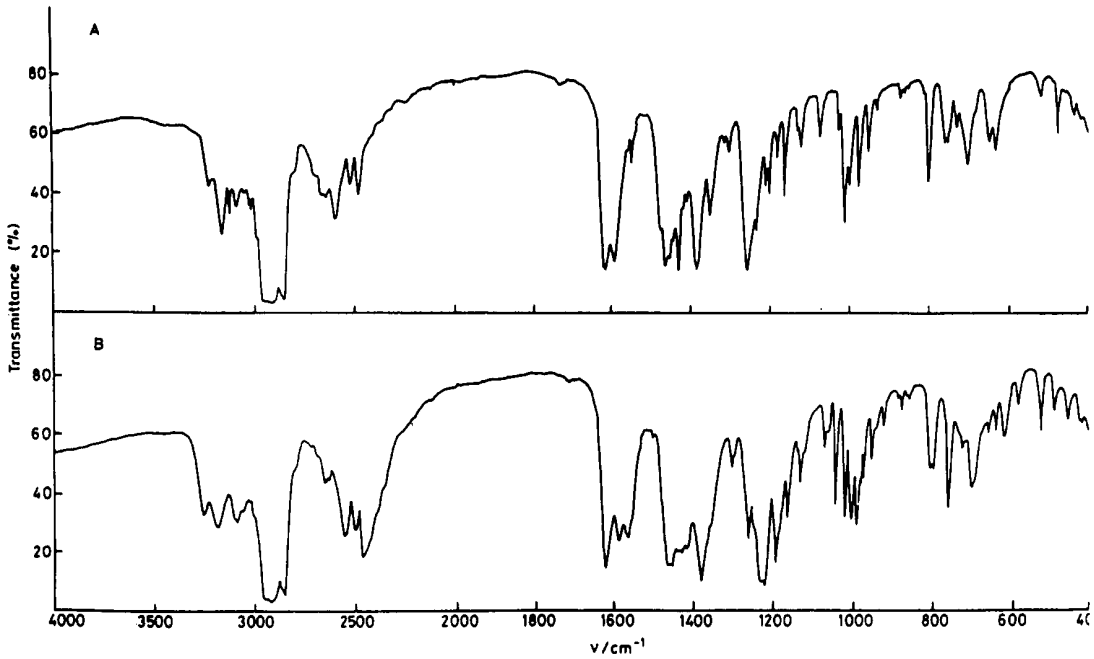


Figure 3. IR spectra of Nujol mulls of the two polymorphs of ranitidine hydrochloride: A, form 1; B, form 2.[29]

batches having dissimilar behavior. Batch-to-batch variability may be caused by differences in the ratio of the different conformations of molecule in the bulk sample.

Although disorder is a well-known phenomenon in the crystallography of small organics, the consequences of disorder in drug molecules appears not to have been widely considered. Just as crystals of chiral and racemic drugs may have different physical properties, the presence of disorder in a crystal may also affect the physical properties of the crystal. The amount of disorder (overall ratio of high occupancy to low occupancy conformations in the batch sample) found in a material may be highly dependent on the exact crystallization conditions. In turn the physical properties of individual batches may range from free-flowing powders to flocculent precipitates suffering badly from the effects of static electricity.

In addition, dynamic disorder reflects enhanced molecular mobility in the solid state. This enhanced molecular mobility may lead to enhanced chemical reactivity as suggested by the four-step mechanism of solid-state reactions first advanced by Paul and Curtin.[28] In this mechanism the first step is molecular loosening. Obviously, crystals possessing dynamic disorder are relatively loosely packed (at least near the site of disorder) and thus may be expected to be more reactive. At Purdue University, we are presently investigating cases in which the disorder of the system may relate to enhanced reactivity.

Are disordered samples with different amounts of disorder distinguishable? One method to distinguish this is discussed later in this review, solid-state NMR spectroscopy. Another approach is powder diffraction. Whether a particular sample will give a powder diffraction line which will change as a function of the disorder is a function of the scattering power of atoms in the alternate conformations and their contribution to scattering for a particular scattering plane. Computer programs are available for the calculation of powder diffraction patterns from single-crystal X-ray diffraction data. In one case we have examined, that of salicylsalicylic acid, our calculations indicate that certain diffraction lines are sensitive to the ratio of high occupancy to low occupancy conformations present in the salicylsalicylate sample. Thus the ratio of the intensities calculated for the two strongest lines in the powder pattern (311/202) varies from 0.80 to 1.75 as the amount of the "major conformer" is varied from 30% to 95%. Even though one must always be concerned with the effect of preferred orientation upon intensities for a powder sample, this calculation is encouraging for the possibility of using powder diffraction as a monitor for disorder in a material intended for pharmaceutical, or other use.

Salicylsalicylic acid crystallizes in Fdd2, an acentric space group, even though the molecule itself is not chiral.[25,26] This compound, as other salicyl esters, also exhibits strong triboluminescence. It is also disordered in the solid. The exact crystallization conditions for this compound can lead to solid material with a broad range of physical behavior. The variability in physical properties of this material may be tied to disorder in the crystalline material and may be a function of the amount of disorder found in the bulk sample.

D. Characterization of Pharmaceutical Solids

In this section the characterization of pharmaceutical solids will be briefly reviewed. This section is intended to provide the reader with a picture of the structure and properties of the various solid forms of pharmaceuticals.

The clearest indication of the existence of polymorphs and solvates comes from X-ray crystallographic examination of single crystals of various samples that are known to have the same composition but which may be suspected of being polymorphic. If single crystals of these materials have different unit-cell parameters then they may be polymorphs. Often, however, X-ray powder diffraction is sufficient to establish the existence of polymorphs. Thus polymorphs can be uniquely identified by their X-ray powder diffraction pattern and their structure completely elucidated by single-crystal X-ray diffraction.

(28) Paul, I. C.; Curtin, D. Y. *Acc. Chem. Res.* 1973, 7, 223.

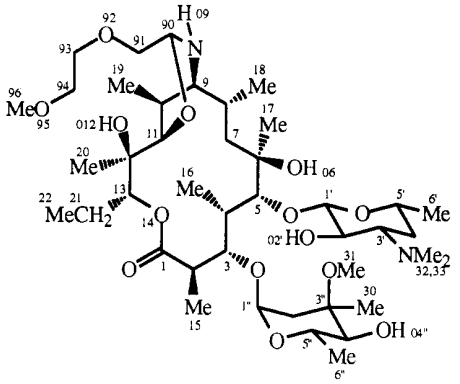
Knowledge of the polymorphs, solvates, and habits in which a drug crystallizes can lead to a dramatic improvement in understanding the stability, bioavailability, and ease of processing of pharmaceutical solids.

1. Infrared Spectroscopy of Solids. Infrared spectroscopy is quite useful for the analysis of solids, because it can be performed on small amounts of the solid substance. The method involves grinding the sample and measuring its infrared spectrum in one of several types of cells available. Infrared spectra of solids can also be obtained after suspending the sample in Nujol or grinding the sample with KBr and compressing this mixture into a disc. FT-IR microscopy can be used to measure the infrared spectrum of a single crystal or group of crystals. If care is taken to study only crystals of the same morphology, this method minimizes the possibility of obtaining infrared spectra of mixtures of crystal forms which is a distinct advantage over approaches that use powdered samples.

The infrared spectrum is extremely sensitive to the structure and conformation of a compound and, thus, can be used to compare the structure and conformation of the compound in different solids or in solid and solution. Infrared spectroscopy is thus a powerful method for the determination of polymorphs.

Cholerton et al.[29] reported the use of infrared spectroscopy to differentiate form 1 and form 2 of ranitidine hydrochloride (Figure 3). This is a classic example of the power of infrared analysis in polymorph studies.

Infrared spectroscopy is also useful for the investigation of solvates, as demonstrated by a study of erythromycin derivative (I).[30] Figure 4 shows the spectra of forms I and



II and the amorphous form of this compound. Clearly these three forms have different spectra especially in the carbonyl region. Note that form I shows two carbonyl absorptions, whereas the amorphous form and form II show only a single carbonyl absorption. Figure 5 shows the infrared spectra of three of the many solvates of this erythromycin derivative. These spectra are fairly similar except for the obvious C≡N stretching absorption in the acetonitrile·$3H_2O$ solvate. There are also slight differences among these three solvates in the fingerprint region. Figure 6 shows the infrared spectra of six solvates which are isomorphous (have the same crystallographic unit cell). These spectra are fairly similar as might be expected since they crystallize in the same unit cell. In cases where the solvents contain keto groups (e and f) the ketone absorption is obvious as a second carbonyl absorption in the spectrum.

(29) Cholerton, T. *J. Chem. Soc., Perkin Trans 2* **1984**, 1761.
(30) Stephenson, G. Unpublished observations, 1993.

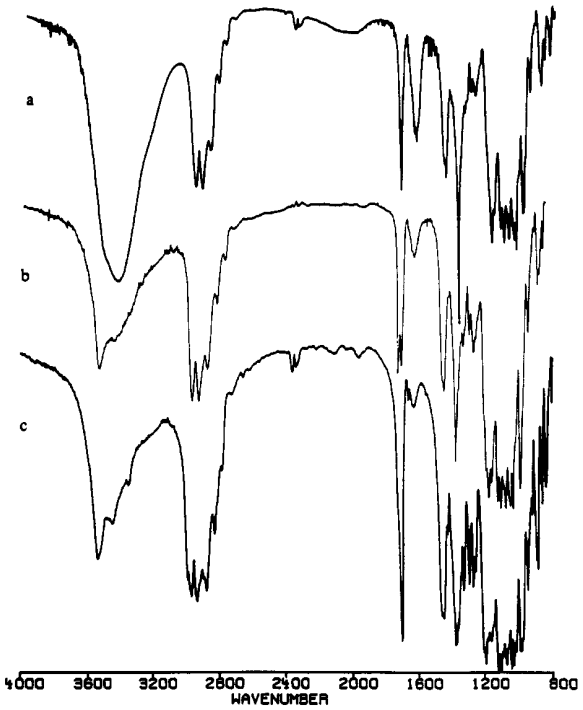


Figure 4. Infrared spectra of the amorphous form (a) and two anhydrous forms, I and II, (b and c) of an erythromycin derivative.[30]

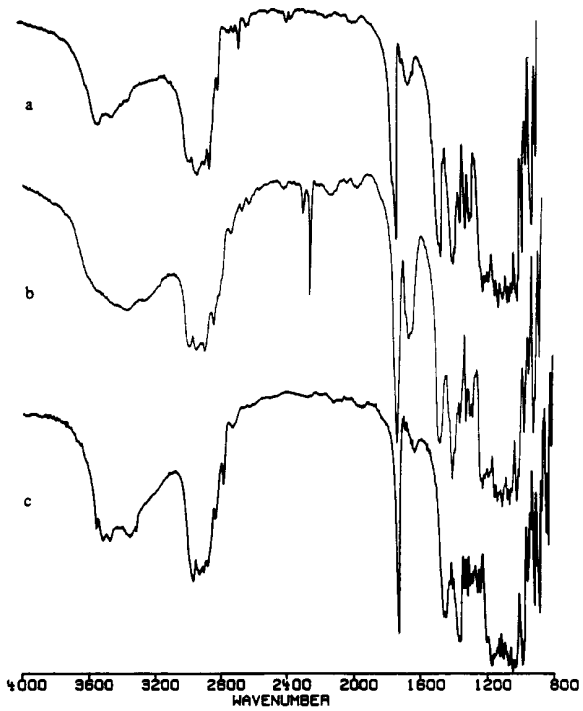


Figure 5. Infrared spectra of three nonisomorphic solvates of an erythromycin derivative: (a) tricyclohexane, (b) acetonitrile·$3H_2O$, and (c) *tert*-butyl alcohol solvate.[30]

Figure 7 shows an enlarged view of the ester carbonyl region of the spectrum in three of the crystal forms of the erythromycin derivative. These spectra suggest that the *n*-propanol solvate contains non-hydrogen-bonded carbonyl groups, that form II contains hydrogen-bonded carbonyl groups, and that form I contains both hydrogen-bonded and non-hydrogen-bonded carbonyl groups. These suggestions have been confirmed by the crystal structures

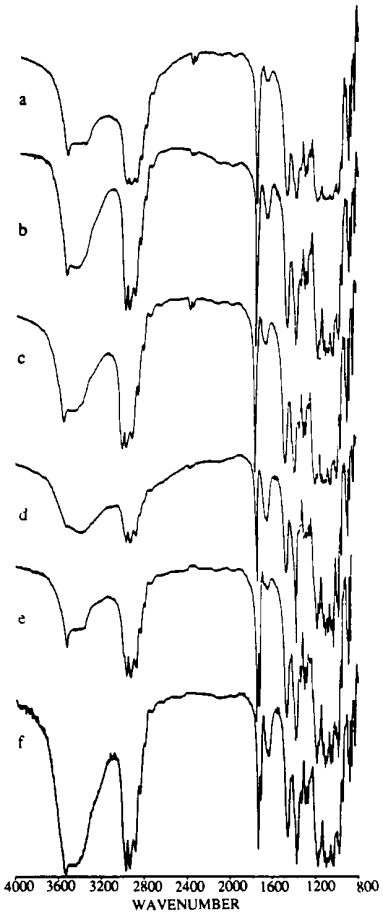


Figure 6. Infrared spectra of the isomorphic solvates of an erythromycin derivative: (a) ethanol, (b) *n*-propanol, (c) isopropyl alcohol, (d) *n*-butanol, (e) acetone, (f) methyl ethyl ketone.[30]

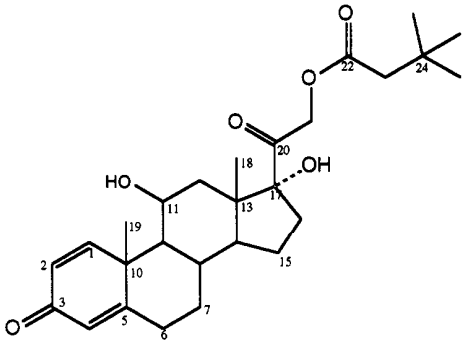
of the *n*-propanol solvate and hydrogen-bonded form II. Unfortunately, the crystal structure of form I could not be determined. Solid-state NMR confirms these suggestions as shown in Figure 8. Again, form I, which shows two carbonyl stretching vibrations in the IR also shows two carbonyl resonances in the solid-state NMR spectrum and the hydrogen bonded form shows a shift to lower field as expected based on model studies (see below).

2. Solid-State NMR Spectroscopy of Pharmaceutical Solids. Solid-state ^{13}C CP/MAS NMR offers significant advantages for the study of solid drugs.[31,32] Figure 9 shows the solid-state ^{13}C NMR spectra of two crystal forms of prednisolone and shows that they can be differentiated on the basis of differences in these spectra. Apparently changing the crystal packing results in significantly different magnetic environments for some of the carbon nuclei. This observation has considerable practical value: Figure 10 shows the ^{13}C CP/MAS NMR spectra of three prednisolone tablets from different vendors. It is clear that one can still observe resonances due to prednisolone, although these tablets contain only 5 mg of prednisolone in 95 mg of excipients. Comparison of Figures 9 and 10 shows that in tablets A and B form I exists, whereas in tablet C form II exists.

(31) Saindon, P. A.; Cauchon, N. S.; Sutton, P. A.; Chang, C. J.; Peck, G.; Byrn, S. R. *Pharm. Res.* **1993**, *10*, 197.
(32) Byrn, S. R.; Sutton, P. A.; Tobias, B. *J. Am. Chem. Soc.* **1988**, *110*, 1609–14.

Other reports show that different polymorphs of cellulose show different ^{13}C CP/MAS NMR spectra.[33] Moreover, solid-state ^{13}C CP/MAS NMR spectroscopy also offers the possibility of the analysis and characterization of amorphous forms. A particularly important question in this area is, What is the mobility of the molecule in amorphous forms? This question can be addressed by comparing the T_1 relaxation times of carbon atoms in the crystalline and amorphous state. In addition, the technique can be used to examine conformational and dynamic changes which occur in solid drugs by determining the coalescence temperature and associated rate constants for the process. In addition, because the time-scale of solid-state NMR spectroscopy is much shorter than X-ray crystallography, it offers the potential for analysis of a range of dynamic processes which cannot be studied by X-ray crystallography. These facts could lead to the widespread use of solid-state NMR spectroscopy in the pharmaceutical industry.

In our laboratory, we have used solid-state NMR to study the different crystal forms of prednisolone *tert*-butylacetate.[32] These studies show that the different crystal forms of prednisolone *tert*-butylacetate have different



Prednisolone 21-*tert*-butylacetate

solid-state NMR spectra. This is no doubt due to different crystal packing. Even forms I and II which have very similar unit cells have slightly different chemical shifts. However, the biggest difference between forms I and II is the position of the solvent peaks. In form I the ethanol solvent shows resonances at 18.1 and 58.2 ppm; in form II the propanol solvent shows resonances at 26.3 and 64.3 ppm. The solid-state NMR spectra of forms III, IV, and V are much different from those of form I and II and from each other. The biggest difference involves carbon 1 which shows chemical shifts which differ by up to 8 ppm from each other in the different crystal forms. Several of the other carbon atoms also show fairly large chemical shift differences. Figure 11 shows a plot of the difference between the solid state and solution chemical shift of carbon 1 vs the hydrogen-bond distance to the O3 carbonyl oxygen which is part of the α,β-unsaturated ketone system which contains carbon 1. It is important to note that this hydrogen bond to O3 is intermolecular and one of the forces responsible for holding the crystals of this steroid together. As can be seen from this plot (Figure 11), there is a more or less gradual shift to larger changes in ppm as the hydrogen-bond distance gets shorter and shorter. This observation is consistent with other studies of hydrogen bonding and also with solution studies

(33) Atalla, R. H.; Gast, J. C.; Sindorf, D. W.; Bartuska, V. J.; Maciel, G. E. *J. Am. Chem. Soc.* **1980**, *102*, 3249.

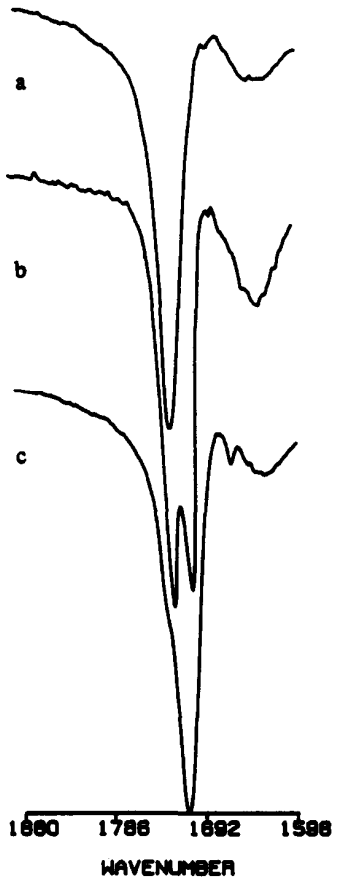
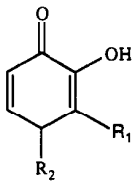


Figure 7. Ester carbonyl region of the *n*-propanol solvate (a), form I (b), and form II (c).[30]

where the stronger the hydrogen bonding the greater the chemical shift changes in ppm.

This type of correlation for different polymorphs and solvates shown in Figure 11 may also apply to members of a series of closely related compounds. Figure 12 shows that the $^{13}C(4)$ chemical shift difference between solid state and solution is a negative linear function of the crystallographic O(4)···O hydrogen bond distance in the crystal structures of a series of 1,2-dialkyl-3-hydroxy-4-pyridones (DAHPs) whose molecular structures are shown below:[34]



EMHP	$R_1 = CH_3$	$R_2 = C_2H_5$
PMHP	$R_1 = CH_3$	$R_2 = C_3H_7$
BMHP	$R_1 = CH_3$	$R_2 = C_4H_9$
MEHP	$R_1 = C_2H_5$	$R_2 = CH_3$
DEHP	$R_1 = C_2H_5$	$R_2 = C_2H_5$
EMHP	$R_1 = C_2H_5$	$R_2 = C_3H_7$

The DHAPs are iron chelators some of which may be promising drugs for oral administration in the treatment of diseases associated with iron overload. Although the

(34) Ghosh, S. Ph.D. Thesis, University of Minnesota, 1993.

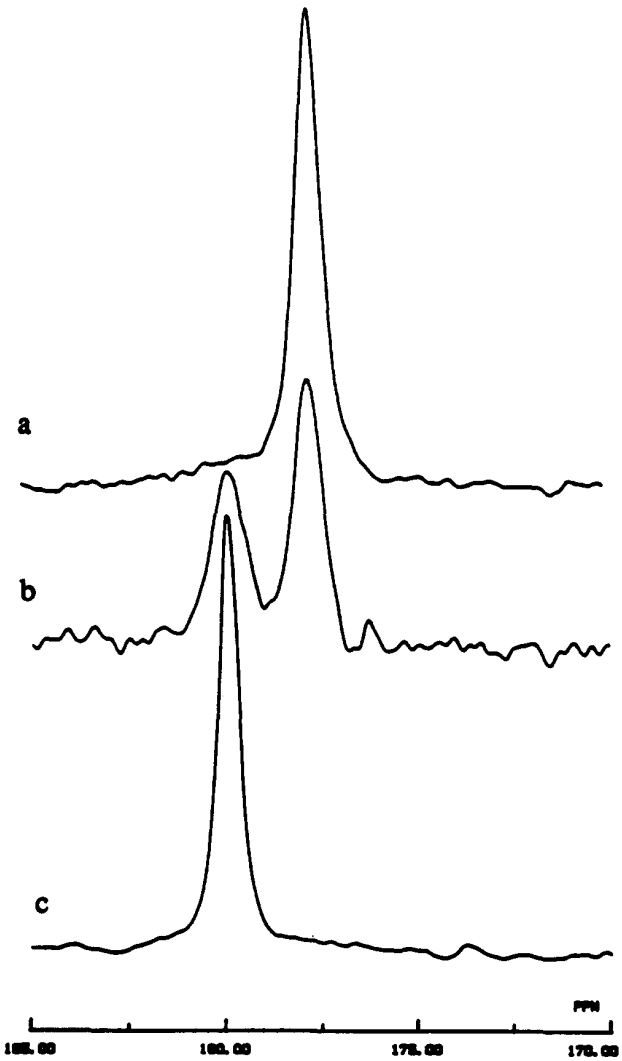


Figure 8. Solid-state NMR spectra of the ester carbonyl carbon: (a) *n*-propanol solvate, (b) form I, and (c) hydrogen-bonded form II.[30]

crystal structure of the compound BMHP could not be determined, this compound provided an excellent ^{13}C NMR spectrum. The measured $^{13}C(4)$ chemical shift of BMHP might enable the O(4)···O hydrogen bond distance to be predicted using Figure 12, if this correlation is generally applicable.

Solid-state NMR is also useful for the study of mobility in solids. The rate of relaxation of the carbon spins toward equilibrium is characterized by the spin lattice relaxation time, T_1.[35] In this process the excess energy from the spin system is transferred to "the lattice". Interactions of randomly fluctuating magnetic fields at the Larmor frequency of the nucleus stimulate these transitions. Such fields arise from motions of other nuclear magnetic moments such as protons. Spin–lattice relaxation is thus most efficient when these motions are near the Larmor frequency of the nucleus being observed. Studies of relaxation times can give valuable information about fast motions in the megahertz range such as methyl group rotations. Numerous studies of molecular motion using

(35) Fukushima, E.; Roeder, S. B. W. *Experimental Pulse NMR*; AddisonWesley: Reading, MA, 1988.

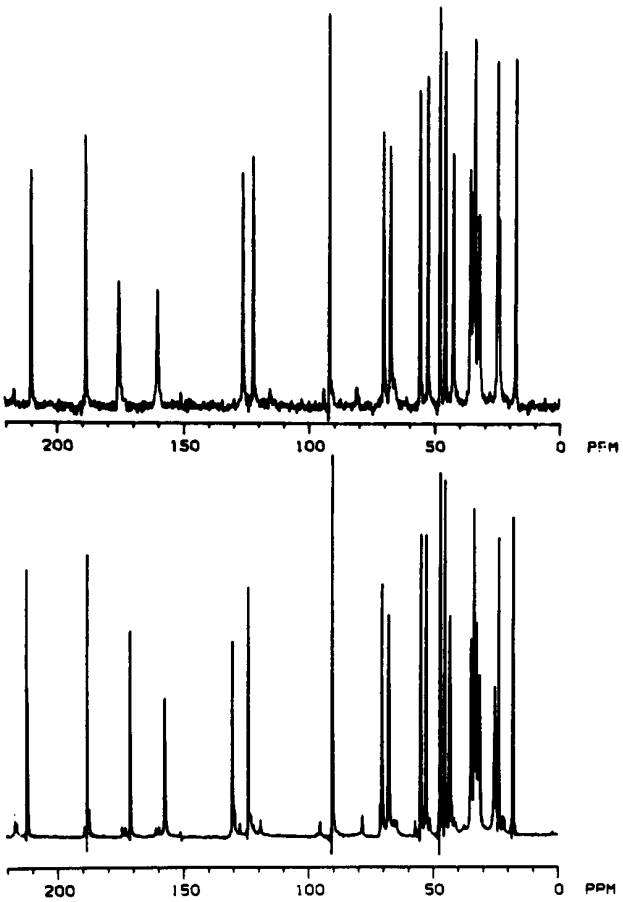


Figure 9. Solid-state ^{13}C CP/MAS NMR spectra of two polymorphs of prednisolone.[31] The top spectrum is form 1 and the bottom spectrum is form 2.

relaxation times have been published.[36] In particular, applications in the field of polymer science have been widespread. For example, solid-state NMR has been used to study both main chain and side group motion in polymers.[37-40] In addition, studies have been made of the relationship between polymer crystallinity and relaxation parameters including T_1, $T_{1\rho}$, and T_2.[41-43]

The mobility of functional groups in solids has been of interest for many years. An understanding of this mobility can lead to insight into the mobility of molecules, the forces responsible for conformational interconversions, and the factors responsible for solid-state reactions. In 1973, Paul and Curtin[28] suggested that molecular loosening or molecular mobility is the first step in a solid-state reaction, and, in our laboratory, we have suggested that solid-state degradations of pharmaceuticals are related to molecular mobility.[32,44,45] In addition, Zografi has suggested that water absorption enhances the molecular mobility of amorphous pharmaceutical solids, perhaps explaining the

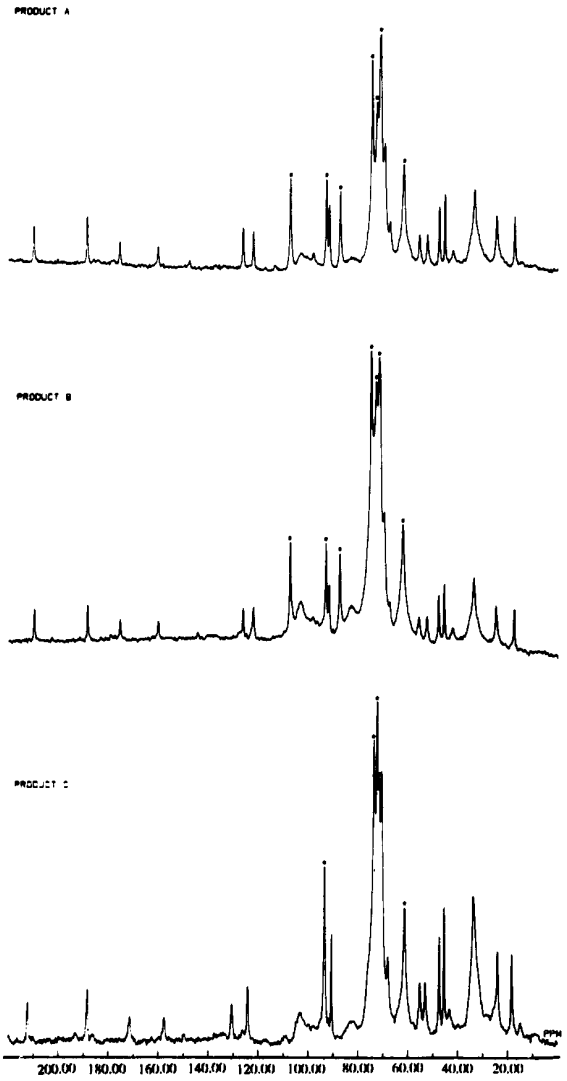


Figure 10. Solid-state ^{13}C CP/MAS NMR spectra of prednisolone tablets from three different vendors.[31]

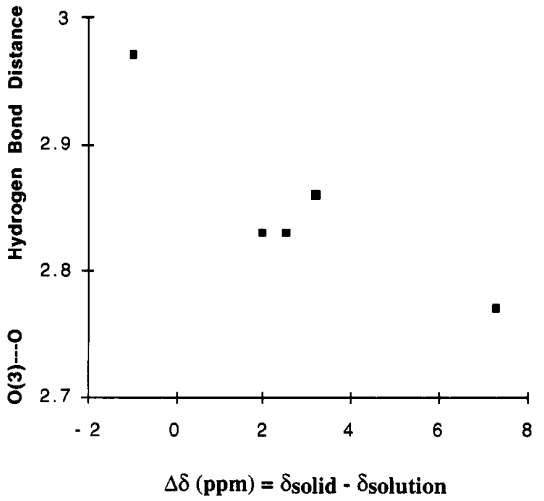


Figure 11. Plot of the difference in chemical shift for carbon 1 between the solid state and solution and the O(3)···O hydrogen bond distance.[32]

(36) Fyfe, C. A. *Solid-state NMR for chemists*; CFC Press: Toronto, Ontario, 1983.
(37) Lyerla, J. R. *Contemporary Topics in Polymer Science*; Shen, M., Ed.; Plenum Press: New York, 1979; Vol. 3, pp 143–213.
(38) Garroway, A. N.; Moniz, W. B.; Resing, H. A. In *Carbon-13 NMR in Polymer Science*; Pasika, W. M., Ed.; American Chemical Society: Washington, DC, 1979; pp 67–86.
(39) Tonelli, A. E.; G., M. A.; Tanaka, H.; Schilling, F. C.; Cozine, M. H.; Lovinger, A. J.; Bovey, F. A. *ACS Adv. Chem. Ser.*; Craver, C. D., Provder, T., Eds.; American Chemical Society: Washington, DC, 1990; Vol. 227, pp 409–451.
(40) Axelson, D. E. *Carbon-13 NMR in Polymer Science*; Pasika, W. M., Ed.; American Chemical Society: Washington, DC, 1979; pp 181–214.
(41) Menger, E. M.; Veeman, W. S.; de Boer, E. *Macromolecules* **1982**, *15*, 1406.
(42) Kitamara, R.; Horii, F.; Murayama, K. *Macromolecules* **1986**, *19*, 636–643.
(43) Earl, W. L.; VanderHart, D. L. *Macromolecules* **1979**, *12*, 762.

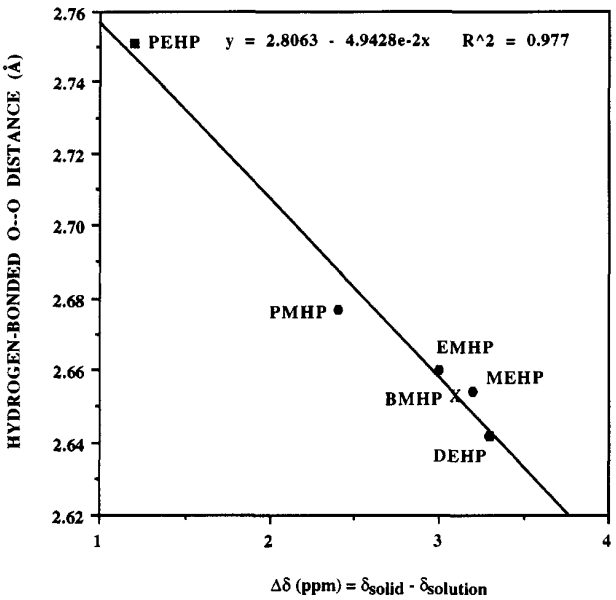


Figure 12. Plot of the hydrogen bonded O···O distances against the differences ($\Delta\delta$) between the ^{13}C chemical shifts of the pyridone carbonyl (O4) and the solid state (δ_{solid}) and the solution state ($\delta_{solution}$) of DAHPs. Although the crystal structure of BMHP is unknown, its hydrogen-bonded O···O distance can be estimated from the plot using the NMR chemical shift data (point X).[34]

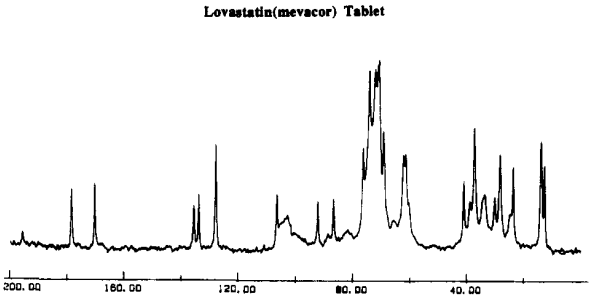
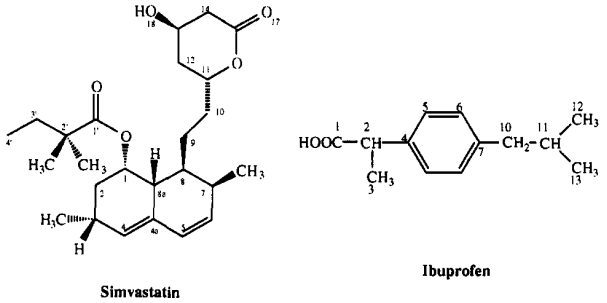


Figure 13. Solid-state CP/MAS NMR spectrum of a lovastatin tablet.

enhanced chemical reactivity of these materials in the presence of water.[46] Further study of the relationship between molecular mobility and solid-state reactivity requires the development of new approaches to determine molecular mobility especially in mixtures such as pharmaceutical dosage forms where single-crystal X-ray methods cannot be used.

Solid-state NMR offers several approaches to studying the molecular mobility of solids. These include (1) determination of the activation energies for T_1 relaxation of individual carbon atoms using variable-temperature solid-state NMR, (2) study of processes which result in peak coalescence of solid-state NMR resonances using variable-temperature solid-state NMR, (3) use of interrupted decoupling to detect methylene and possible methine groups with unusual mobility, and (4) comparison of solid-state MAS spectra measured with and without cross polarization.

Recent research at Purdue University is using all of these approaches but especially the first one to study the relative mobility of methyl groups in a number of drugs which contain multiple methyl groups including simvastatin, lovastatin, ibuprofen, and phenacetin.[47-49] The results of these studies will be published shortly.



In addition, Maverick and Dunitz[50] have suggested that solid-state NMR activation energies and X-ray thermal parameters are related. Therefore, solid-state NMR appears to be a good method for study of the mobility of methyl groups in solids. Because solid-state NMR can be used to study pharmaceutical dosage forms and other mixtures,[31] this powerful method will find increased application in the determination of the molecular mobility of drugs in mixtures and dosage forms.

Surprisingly little research has been done on the solid-state NMR spectra of pharmaceutical dosage forms. To our knowledge, prior to the paper by Saindon et al.,[31] solid-state NMR has been used to study only two tablets both consisting almost entirely of pure drug: aspirin[51,52] and acetaminophen.[53] This paper shows that solid-state NMR is useful for the study of more complex pharmaceutical products.[31]

For example, Figure 13 shows the solid-state NMR spectrum of lovastatin tablets, which contain a rather large excess of excipients. The excipients, which have resonances in the 50–100 ppm range obscure resonances of lovastatin which appear at the same chemical shift but have little effect on resonances at other chemical shifts. Similar results were obtained for tablets of another closely related HMG-CoA reductase inhibitor, simvastatin.

E. Summary

In this review, areas of particular interest to Peggy Etter and her group were discussed including crystallization, hydrogen bonding, solvates and solid-state NMR. It is clear that her pioneering work has tremendously important implications for solid-state pharmaceutical chemistry.

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