CHAPTER 2

LITERATURE REVIEW

N-(2-propylpentanoyl) urea

N-(2-propylpentanoyl) urea (Valproyl urea, VPU) is a new acylurea derivative which was synthesized by Boonardt Saisorn and co-workers (4). The structure of VPU modeled partially on barbiturate ring and Valproic acid (VPA) in the same molecule (Figure 2) (4,23,24).

The synthesis of VPU is composed of two steps. First, VPA was allowed to react with thionyl chloride to yield 2-propylpentanoyl chloride. Then, VPU was synthesized by the reaction of 2-propylpentanoyl chloride and urea in dry benzene. The synthetic pathway of VPU is shown in Figure 3.

The anticonvulsant activity of VPU in mice and rats has been studied by Thongchai Sooksawate (1). The study was demonstrated that, in comparison to VPA, VPU exerted higher potency, greater safety margin and lower unwanted side effects. Further studies by Boonyong Tantisira and co-workers (2,6) and Tipsuchon Chunngam (7) confirm a good prospect of being a potent broad spectrum antiepileptic drug with higher anticonvulsant activity and higher relative safety margin than its parent compound, VPA.

Boonyong Tantisira and co-workers (6) have studied anticunvulsant activity, lethality and neurotoxicity of VPU in comparison to VPA. The study demonstrated that VPU possessed a higher broad spectrum anticonvulsant activity in maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) induced convulsions in mice. Intraperitoneally administered VPU exhibited median effective dose (ED_{50}) of MES and PTZ tests of 66 and 57 mg/kg, while those of VPA are 242 and 95 mg/kg. Orally administered VPU demonstrated an ED_{50} approximately 6 times higher than its ED_{50} by the intraperitoneal route. VPU, demonstrating by median lethal dose (LD_{50}) of 1553 mg/kg, also possesses a greater margin of safety (LD_{50}/ED_{50}) than did VPA. The median neurotoxic dose (TD_{50}) as measured by rotorod test was 625 mg/kg for intraperitoneally given VPU. This finding results in higher protective index ($PI = TD_{50}/ED_{50}$) of VPU (PI = 9.5) than that of VPA (PI = 1.1) implying that, in therapeutic dose, VPU may produce less neurological side effects than did VPA.

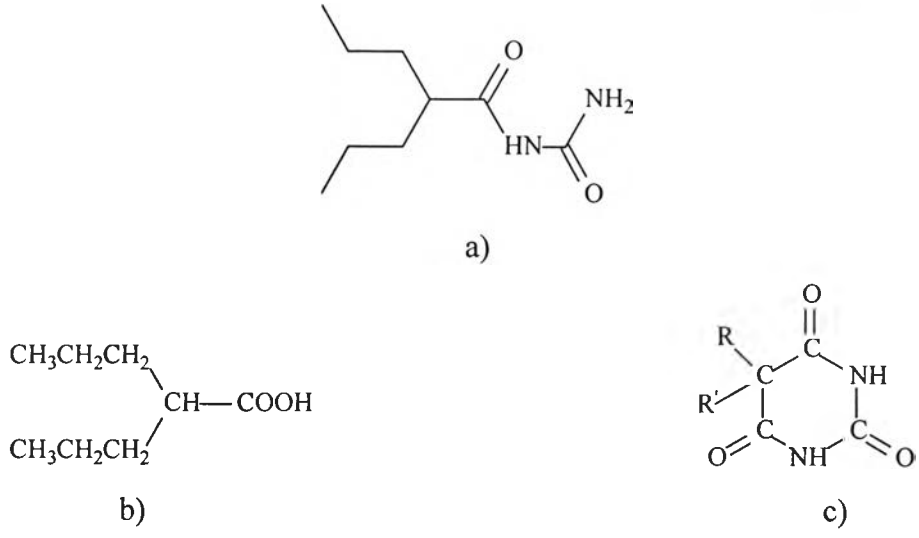
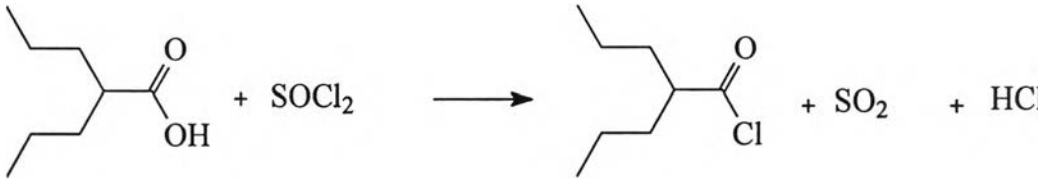


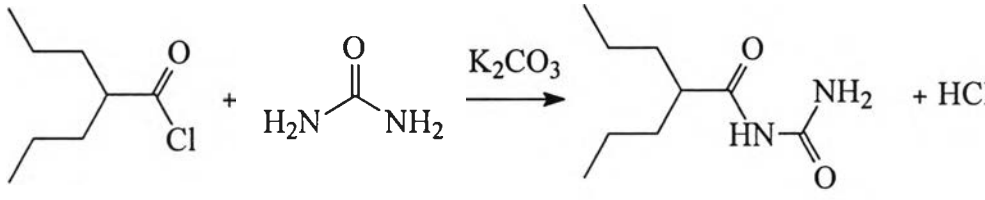
Figure 2: Chemical structures of a) N-(2-propylpentanoyl) urea,
b) Valproic acid, c) Barbiturate

Synthesis of valproyl chloride



valproic acid thionyl chloride valproyl chloride

Synthesis of valproyl urea



valproyl chloride urea valproyl urea

Figure 3: Synthesis pathway of N-(2-propylpentanoyl) urea

Embryotoxicity in rats has been conducted by Roongruedee Meesomboon and co-workers (8). The study, regarding to effects on axial rotation and embryonic growth, suggested that VPU produced less developmental toxicity than VPA did.

Studying by Watcharaporn Patchamart shows that VPU exhibited hepatotoxic effects, including an increase of transaminase activities and a depletion of hepatic total glutathione at high dose of administration in an in vitro study using isolated hepatocyte cells (25).

PREFORMULATIONS

A new drug substance should undergo extensive pharmacological and toxicological studies before human trials begin. However, there are several other factors that contribute to the therapeutic success of a drug, e.g., shelf life, reproducibility of absorption, and economical manufacture of the dosage form. It is important to gain knowledge of the physicochemical, physicomechanical, and biopharmaceutical properties of the compound at an early stage of drug development. Therefore, when a new synthesized drug shows sufficient pharmacologic promise in animal models, preformulation should be commenced (9,26,27).

Preformulation involves numerous investigations on a drug substance in order to produce useful information for subsequent formulation of a physicochemically stable and biopharmaceutically suitable drug dosage form. The physicochemical and physicomechanical parameters include chemical stability, solubility, dissolution rate, dissociation constant, partition coefficient, crystallinity, polymorphism, solvate, and particle size. The compatibility, that is drug-excipient interaction, is also investigated and the results are useful for dosage form design. Preliminary in vivo animal studies of drug substance absorption, metabolism, protein binding, distribution, and elimination may be performed during this process. A thorough understanding of these properties may ultimately provide a rationale for formulation design (9,10).

One of the most important parts of preformulation research is solid state characterization (28). Many pharmaceutical solid can exist in several internal structures, such as polymorph, amorphous, solvates and hydrates. Such forms may present different physicochemical properties that could affect product performance in

solid-state dosage forms (12,13,14). The internal structure alteration may affect solubility (15,16,17) dissolution rate (15,17,18) and solid state stability (19,20). Solid drug substances display a wide and largely unpredictable variety of solid state properties. Therefore, increased understanding of solid state properties allows a rationale for formulation design and processing (21,22)

CLASSIFICATION OF SOLIDS

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. Thorough understanding of solid state property not only ensure uniformity of the materials used throughout the clinical trials but also fully resolve solid state issues before the critical stages of drug development. Further benefit of these studies is the development of a meaningful set of solid state specifications, which critically describe the solid form of the drug substance (21).

Solid chemical compound can be divided following its differential habit and crystal chemistry (12) as shown in Figure 4. Habit is the description of the outer appearance of a crystal, whereas the internal structure is the molecular arrangement within the solid (9).

Crystal Habits

According to the definition of Haleblian (12), habit is the outer appearance of a crystal. If the environment of a growing crystal affects its external shape without changing its internal structure, a different habit results. These alterations are caused by the interference with the uniform approach of crystallizing molecules to the different faces of the crystal.

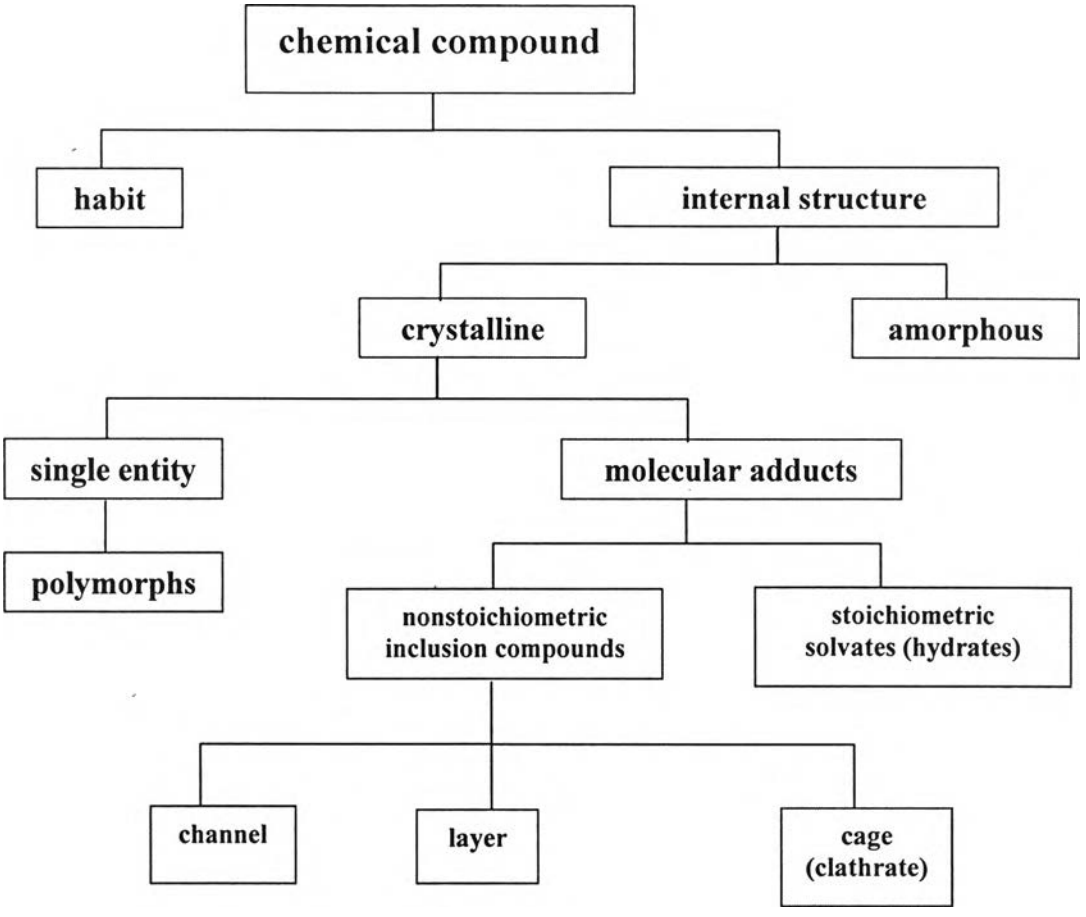


Figure 4: Outline differentiating habit and crystal chemistry of a chemical compound

Crystal growth may be impeded by adjacent crystals growing simultaneously or contacting container walls. As a result, the development of plane faces may be inhibited or, in the case of late crystallizing crystals, an irregularly shaped crystal may occur since it is constrained to occupy only the spaces left between substances already crystallized. Such irregularly shaped crystals are described as anhedral or allotriomorphic; those bound by plane faces are termed euhedral or idiomorphic as shown in Figure 5 (29). Anhedral crystals, although irregularly shaped, have a regular arrangement of building units, which may be proved by x-ray diffraction (12).

Classification of euhedral crystals are described in Table 1 and morphology shown in Figure 6 (29).

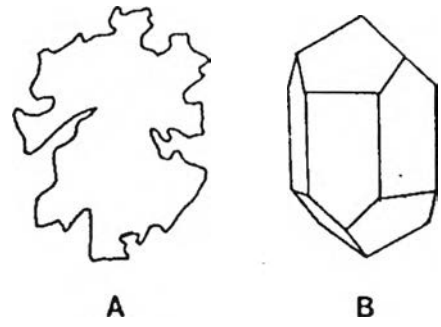


Figure 5: Morphology of anhedral (A) and euhedral (B)

Table 1: Classification of crystal habits

Descriptor	Description
A. Tabular crystal	moderate development of a pair of parallel faces, at the expense of the others, produces a tabular crystal.
B. Platy crystal	excessive development of the parallel faces as described in the tabular habit produces a platy crystal.
C. Prismatic crystal	crystal has a columnar form.
D. Acicular crystal	prism is elongated so much as to be needle like.
E. Bladed crystal	acicular crystal is flattened.

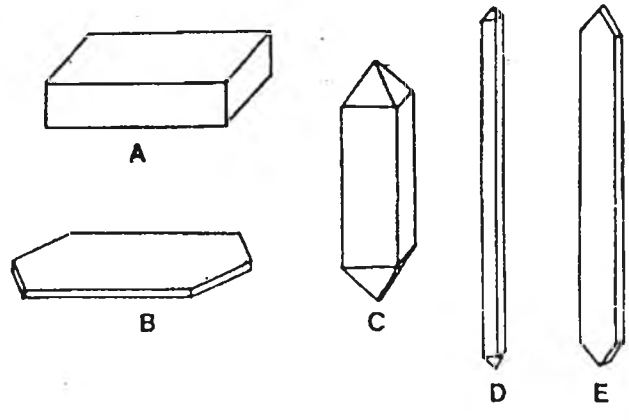


Figure 6: Different habits of crystals

(A-tabular, B-platy, C-prismatic, D-acicular, and E- bladed)

There are many factors that may affect crystal habits (12,30) including solvent effect. The interaction between the solute and the solvent is important in controlling the crystal habit. For example, resorcinol crystallizes from benzene into fine needles, but it crystallizes from butyl acetate into squat prisms. Similarly, iodoform crystallizes as hexagonal bipyramids from aniline and as prisms from cyclohexane. This difference is due to the affinity of a solvated solvent to be adsorbed on certain crystal faces and thus to inhibit the growth of those particular crystalline faces (31).

Furthermore, the addition of any new substance in a crystallizing medium may resulted in changing of habit of the crystal formed (12). In addition, supersaturation may affect the crystal habits.

As supersaturation is increased, the crystal form tends to change from granular to needle like. A thin needle or dendrite loses less heat by conduction than a thicker crystal, so it grows faster.

Internal structure of solid

According to concept of internal structure of solids, in general, solids are divided into two categories: (a) amorphous, where there is no regularity in the structure, and (b) crystalline, where the atoms are arranged in regular arrays. The crystalline form is the more frequent of the two and includes the metals and most minerals (12).

Noncrystalline solids-amorphous form

Many pharmaceutical solids can exist in an amorphous form, which, because of its distinctive properties, is sometimes regarded as polymorphs. However, unlike true polymorphs, amorphous forms are not crystalline (12,13,32). In fact, amorphous solids consist of disordered arrangements of molecules and therefore possess no distinguishable crystal lattice nor unit cell and consequently have zero crystallinity. In amorphous forms, the molecules display no long-range order, although the short-range intermolecular forces give rise to the short-range order typical of that between nearest neighbors (Figure 7). Thermodynamically, the absence of stabilizing lattice energy causes the molar internal energy or molar enthalpy of the amorphous form to

exceed that of the crystalline state. The absence of long-range order causes the molar entropy of the amorphous form to exceed that of the crystalline state. Furthermore, the lower stability and greater reactivity of the amorphous form indicates that its molar Gibbs free energy exceeds that of the crystalline state (14).

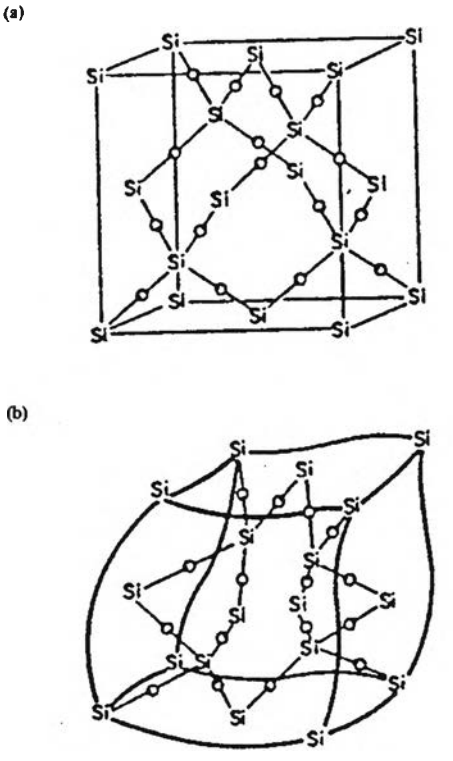


Figure 7: Two forms of silica: (a) crystobalite crystalline and (b) glass amorphous

The high internal energy and specific volume of the amorphous state relative to the crystalline state can lead to enhanced dissolution and bioavailability, but can also create the possibility that during processing or storage the amorphous state may spontaneously convert back to the crystalline state (32).

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 (21). As shown in Figure 8 (33), amorphous form exhibits the classical diffuse "halo" x-ray powder diffraction pattern rather than the sharp peaks observed in the pattern of a crystalline substance (34).

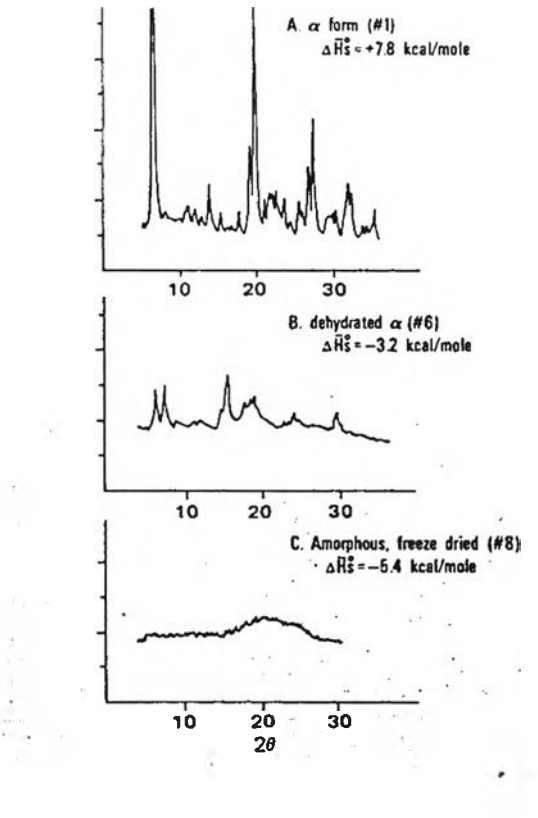


Figure 8: X-ray diffraction and heats of solution for crystalline, weakly crystalline, and amorphous forms of cefazolin sodium

Crystallinity of β-lactam antibiotics was studied by Pikal et al. (33). The XRD pattern obtaining from crystalline and amorphous forms of cefazolin sodium is shown in Figure 8, XRD pattern of amorphous form exhibit the halo pattern. Amorphous form of cefazolin sodium was energy rich and had more exothermic heat of solution than crystalline form.

Amorphous forms usually much more soluble than their crystalline (21). Solubility advantage of amorphous solids was studied by Hancock and Parks. (16) Amorphous solids are markedly more aqueous soluble than their crystalline counterparts, however, their experiment solubility advantage is typically less than that predicted from simple thermodynamic considerating.

Thermal decomposition rates for amorphous and crystalline cefoxitin sodium samples were determined by Oberholtzer and Brenner (19). Amorphous cefoxitin sodium was considerably less stable than its corresponding crystalline form.

In considering the importance of the amorphous state in pharmaceutical systems we must direct our attention to two main situations. In the first, a material may exist intrinsically in the amorphous state or it may be purposefully rendered amorphous and we would like to take advantage of its unique physical chemical properties. Under these circumstances we usually want to develop strategies to prevent physical and chemical instability of the amorphous sample. In the second case, we may be dealing with a crystalline material that has been inadvertently rendered amorphous during processing (32).

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 (21).

Single entity crystalline solids - polymorph

Many pharmaceutical solids exhibit polymorphism, which is frequently defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice (12-14). Thus, in the strictest sense, polymorphs are different crystalline forms of the same pure substance in which the molecules have different arrangements and/or different conformations of the molecules. As a result, the polymorphic solids have different unit cells and hence display different physical properties, including those due to packing, and various thermodynamic, spectroscopic, interfacial, and mechanical properties, as discussed below (12,13,14).

For example, acetaminophen can exist as a monoclinic form, which is thermodynamically stable under ambient conditions. The compound can also be

obtained as a less stable orthorhombic form, which more suitable for direct compressing (34). Carbamazepine can exist in monoclinic or trigonal form (36).

The various polymorphs of a substance can exhibit a variety of different physical properties as listed in Table 2. Because of differences in the dimensions, shape, symmetry, capacity, and void volumes of their unit cells, the different polymorphs of a given substance have different physical properties (34).

There are several researches studied on different physical properties among various polymorphs, e.g. molar volume and density (36), dissolution rate (15,17,18,37), solubility (15,16,17,38), solid state stability (19,20), physicochemical stability (39), habit (36), tableting (35), solution calorimetry (28,33,40), solid state Nuclear Magnetic Resonance spectroscopy (15,28) and Fourier Transform Infrared Spectroscopy (28,36,41,42)

Stoichiometric adducts-solvates

During crystallization from a solution, crystals separating may consist of a pure component or be a molecular compound. Molecular compounds may contain two or more constituents that have completely satisfied classical "valence forces" and are crystallized together as a new single crystalline entity. Solvates are molecular complexes that have incorporated the crystallizing solvent molecule in their lattice. When the solvent incorporated in the solvate is water, it is called a hydrate.

Leung et al. (37) found that commercial form of aspartame hemihydrate (Form II) converts to hemihydrate form I by heating to 160°C in the presence of steam or by ball-milling.

In addition, a complex molecule can exhibit solvate forms such as hydrate form of trimethoprim-sulfamethoxypyridazine 1:1 molecular complex (43).

Various publications demonstrated physicochemical property difference of solid in hydrate and anhydrous form such as Diclofenac N-(2-hydroxyethyl) pyrrolidine anhydrate and dihydrate forms (44), Paclitaxal (45) and AG 337 antitumor drug (46). Difference physicochemical property between Nedocromil bivalent metal salt octahydrate and heptahydrate was studied by Zhu et al. (47).

Table 2 : List of physicochemical properties that differ among various polymorphs

1. Packing properties
   a) Molar volume and density
   b) Refractive index
   c) Conductivity, electrical and thermal
   d) Hygroscopicity
2. Thermodynamic properties
   a) Melting and sublimation temperatures
   b) Internal energy (i.e., Structural energy)
   c) Enthalpy (i.e., Heat content)
   d) Heat capacity
   e) Entropy
   f) Free energy and chemical potential
   g) Thermodynamic activity
   h) Vapor pressure
   i) Solubility
3. Spectroscopic properties
   a) Vibrational transitions (i.e., infrared absorption spectra and Raman spectra)
   b) Rotational transitions (i.e., far infrared or microwave absorption spectra)
   c) Nuclear spin transitions (i.e., nuclear magnetic resonance spectra)
4. Kinetic properties
   a) Dissolution rate
   b) Rates of solid state reactions
   c) Stability
5. Surface properties
   a) Surface free energy
   b) Interfacial tensions
   c) Habit (i.e., shape)
6. Mechanical properties
   a) Hardness
   b) Tensile strengh
   c) Compactibility, tableting
   d) Handling, flow, and blending

Nonstoichiometric adducts-clathrates

Clathrates are inclusion compounds. They were given this name by Powell since the guest is enclosed or protected by crossbars of a grating. According to one source, a clathrate is a single-phased solid with two distinct components; the host and the quest. The quest is retained in closed cavities provided by the crystalline structure of the host. Generally, a cage and its enclosed molecule(s) are taken as a unit cell.

GENERATION OF POLYMORPHS, HYDRATES, SOLVATES, AND AMORPHOUS SOLIDS

Methods employed to obtain unique polymorphs, solvates, hydrate and amorphous forms including of sublimation, crystallization from a single solvent, evaporation from a binary mixture of solvents, vapor diffusion, thermal treatment, crystallization from the melt, rapidly changing solution pH to precipitate acidic or basic substances, removal of solvent from crystalline solvates or hydrates, growth in the presence of additives, spray drying, solidification of the melt, lyophilization and grinding (34). Some method commonly used is described below.

Crystallization from a single solvent

The solvents selected for recrystallization should include any with which the compound will come into contact during synthesis, purification, and processing, as well as solvents having a range of boiling points and polarities. Examples of solvents routinely used for such work are listed in Table 3 together with their boiling points (34).

Table 3 Solvents often used in the preparation of polymorphs

Solvent	Boiling point (°C)
Dimethylformamide	153
Acetic acid	118
Water	100
1-Propanol	97
2-Propanol	83
Acetonitrile	82
2-Butanone	80
Ethyl acetate	77
Ethanol	78
Isopropyl ether	68
Hexane	69
Methanol	65
Acetone	57
Methylene chloride	40
Diethyl ether	35

The process of solution mediated transformation can be considered the result of two separate events, (a) dissolution of the initial phase, and (b) nucleation/growth of the final, stable phase.

If two polymorphs differ in their melting point by 25-50°C, for monotropic polymorphs the lower melting, more soluble, form will be difficult to crystallize.

A commonly used crystallization method involves controlled temperature change. Slow cooling of a hot, saturated solution can be effective in producing crystals if the compound is more soluble at higher temperatures; alternatively, slow warming can be applied if the compound is less soluble at higher temperatures. Sometimes it is preferable to heat the solution to boiling, filter to remove excess solute, quench cool using an ice bath or even a dry ice-acetone bath.

The reason for using crystallization solvents having varying polarities is that molecules in solution often tend to form different types of hydrogen-bonded aggregates, and that these aggregate precursors are related to the crystal structures that develop in the supersaturated solution (48).

Crystal structure analysis of acetanilide shows that a hydrogen-bonded chain of molecules is aligned along the needle axis of the crystals. This pattern is characteristic of secondary amides that crystallize in a trans conformation so that the carbonyl acceptor group and the –NH hydrogen bond donor are anti to one another. The morphology of acetanilide crystals can be controlled by choosing solvents that promote or inhibit the formation of this hydrogen-bond chain. Hydrophobic solvents such as benzene and carbon tetrachloride will not participate in hydrogen-bond formation, so they will induce the formation of rapidly growing chains of hydrogen-bonded amides. Crystals grown by evaporation methods from benzene or carbon tetrachloride are long needles. Solvents that are proton donors or proton acceptors inhibit chain formation by competing with amide molecules for hydrogen-bonding sites. Thus acetone inhibits chain growth at the –NH end, and methanol inhibits chain growth at the carbonyl end of the chain. Both solvents encourage the formation of rod-like acetonilide crystals, while mixtures of benzene and acetone give hybrid crystals that are rod-shaped, with fine needles growing on the ends (49).

Some solvents favor the crystallization of a particular form or forms because they selectively adsorb to certain faces of some polymorphs, thereby either inhibiting their nucleation or retarding their growth to the advantage of others. Among the factors affecting the types of crystal formed are (a) the solvent composition or polarity, (b) the concentration or degree of supersaturation, (c) the temperature, including cooling rate and the cooling profile, (d) additives, (e) the presence of seeds, (f) pH, especially for salt crystallization, and (g) agitation (48).

Form I of dehydroepiandrosterone was obtained by recrystallization from warm ethyl acetate, acetone, acetonitrile, or 2-propanol. Form II was obtained by rapid evaporation, using a vacuum from solutions in dioxane, tetrahydrofuran, or chloroform (which are higher boiling, less polar solvents) (18).

Chlordiazepoxide Form I was obtained by recrystallized from ethanol, which is the solvent, used in the original preparation, while Form II was recrystallized from methanol (28). Trigonal polymorph of carbamazepine was obtained by crystallization from a number of solvents (36).

Evaporation from a binary mixture of solvents

If single-solvent solutions do not yield the desired phase, mixtures of solvents can be tried. Multicomponent solvent evaporation methods depend on the difference in the solubility of the solute in various solvents. In this approach, a second solvent in which the solute is sparingly soluble is added to a saturated solution of the compound in a good solvent (34).

Tolbutamide Form III was prepared by dissolving tolbutamide in ethanol at 60°C, slowly adding warm water, and allowing the resulting solution to stand at room temperature (15).

Thermal treatment

Aspartame hemihydrate Form II was obtained by heating Form I to 160°C in the presence of steam (37).

Crystallization from the melt

In accordance with Ostwald's rule, the cooling of melts of polymorphic substances often first yields the least stable modification, which subsequently rearranges into the stable modification in stages. Since the metastable form will have the lower melting point, it follows that supercooling is necessary to crystallize it from the melt. After melting, the system must be supercooled below the melting point of the metastable form, while at the same time the crystallization of the more stable form or forms must be prevented. Quench cooling a melt can sometimes result in formation of an amorphous solid that on subsequent heating undergoes a glass transition followed by crystallization (34). The metastable form of Thymitaq was melted at 213°C, followed by crystallization of stable form (46).

Methods employed to obtain hydrate forms

Hydrates can be prepared by recrystallization from water or from mixed aqueous solvents. They can also result, in some instances, from exposure of crystal solvates (such as methanolates or ethanolates) to an atmosphere containing water vapor (34).

Typically, hydrates are obtained by recrystallization from water. Diclofenac N-(2-hydroxyethyl) pyrrolidine hydrate was prepared by dissolving the anhydrous form in distilled water at 40°C and recrystallizing the solid by slow evaporation at room temperature (44).

Hydrates can sometimes be obtained by simply suspending the anhydrous material in water, whereupon a form of Ostwald ripening occurs (34). Paclitaxel hydrate was prepared by suspending the anhydrous form in distilled water for 24 hours (45).

Simply exposing an anhydrous powder to high relative humidity can often lead to formation of a hydrate. Tolbutamide Form II was prepared by storing Form IV at 60°C, 75% RH for 10 minute (15).

The techniques used to obtain solvates are generally similar to the solvent methods used to obtain polymorphs, i.e. crystallization from a single solvent, from

mixed solvents, or by vapor diffusion (34). Solvated form of Stanozolol was obtained by recrystallized from ethanol, methanol and 2-propanol (38).

Methods employed to obtain amorphous materials

Amorphous solids can be precipitated from solution or obtained from melts of compounds by carrying out the solidification in such a way as to avoid the thermodynamically preferred crystallization process. They also can be prepared by disrupting an existing crystal structure. Excess free energy and entropy are incorporated into solids as they are converted into the amorphous state, since solidification occurs without permitting the molecules to reach their lowest energy states (34).

Amorphous form of Quinapril hydrochloride obtained from grinding of crystalline form or by solvent evaporation (20).

Amorphous indomethacin was prepared by melting indomethacin in an aluminum weighing pan at 165°C, followed by quenching with liquid nitrogen. Preparation of amorphous sodium indomethacin was carried out by three procedures: freeze-drying, grinding and precipitation (50).

Solidification of the melt

Amorphous solids are often created by rapidly cooling a liquid so that crystallization nuclei can neither be created nor grow sufficiently, whereupon the liquid then remains in the fluid state well below the normal freezing point (34).

METHODS FOR THE CHARACTERIZATION OF POLYMORPHS, HYDRATES, SOLVATES, AND AMORPHOUS SOLIDS

Characterization of solid forms involves verifying that the solid is the expected chemical compound, characterizing the internal structure, and then, describing the habit of the crystal (9).

The most important aspect relating to an understanding of polymorphic solid and solvate species is the range of analytical methodology used to perform the characterization studies (51). Byrn et al. have provided a series of useful definitions that concisely give the characteristics of the various solid forms that can be found for a given drug substance (21).

Of all the methods available for the physical characterization of solid materials, it is generally agreed that crystallography, microscopy, thermal analysis, solubility studies, vibrational spectroscopy, and nuclear magnetic resonance are the most useful for characterization of polymorphs and solvates. However, it cannot be overemphasized that the defining criterion for the existence of polymorphic types must always be a nonequivalence of crystal structures. For compounds of pharmaceutical interest, this ordinarily implies that a nonequivalent x-ray powder diffraction pattern is observed for each suspected polymorphic variation. All other methodologies must be considered as sources of supporting and ancillary information; they cannot be taken as definitive proof for the existence of polymorphism by themselves alone (51).

X-ray powder diffraction (XRPD)

Diffraction is a scattering phenomenon. When x-rays are incident on crystalline solids, they are scattered in all directions. In some of these directions, the scattered beams are completely in phase and reinforce one another to form the diffracted beams. Bragg's law describes the conditions under which this would occur. It is assumed that a perfectly parallel and monochromatic x-ray beam, of wavelength λ, is incident on a crystalline sample at an angle θ. Diffraction will occur if

$$n\lambda = 2d\sin\theta$$

where d = distance between the planes in the crystal, expressed in angstrom units, and n = order of reflection (an integer) (52, 53).

Qualitative XRPD analysis

To measure a powder pattern, a randomly oriented sample is prepared so as to expose all the planes of a sample and is irradiated with monochromatic x-ray radiation. The scattering angle θ is determined by slowly rotating the sample and using a scintillation counter to measure the angle of diffracted x-rays with respect to the angle of the incident beam. Alternatively, the angle between sample and source can be kept fixed, and the detector moved along a proscribed path to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, the spacing between the planes (identified as the d-spacings) is calculated using Bragg's law.

The XRPD pattern will therefore consist of a series of peaks detected at characteristic scattering angles. These angles, and their relative intensities, can be correlated with the computed d-spacings to provide a full crystallographic characterization of the powdered sample. Since every compound produces its own characteristic powder pattern owing to the unique crystallography of its structure, powder x-ray diffraction is clearly the most powerful and fundamental tool for a specification of the polymorphic identity of an analyze (51,52). The USP general chapter on x-ray diffraction states that identity is established if the scattering angles of the ten strongest reflections obtained for an analyze agree to within ±0.20 degrees with that of the reference material, and if the relative intensities of these reflections do not vary by more than 20 percent (54).

Degree of crystallinity

The term degree of crystallinity is useful in attempts to quantify these intermediate states between crystalline and amorphous states (52). Often the degree of crystallinity is determined using the following approximate expression (53):

$$X_{cr} = \frac{I_c \times 100}{I_c + I_a}$$

where X_{cr} = degree of crystallinity

I_c = crystalline intensity

I_a = amorphous intensity

The use of an internal standard will permit measurement of relative intensities and will eliminate the need for the measurement of absolute intensities.

Ryan (55) developed a method to determine the relative degree of crystallinity (RDC) used this relationship

$$RDC = \frac{I_{sa}}{I_{max}}$$

where I_{sa} = peak intensity of the sample under investigation

I_{max} = peak intensity at the same angle for the sample with the highest intensity

Quantitative XRPD analysis

Quantitative XRPD analysis using an internal standard was described by Suranarayanan (52,53). Alexander and Klug (53) derived a general equation for a mixture of several components. This mixture can be regarded as being composed of just two components: component J (which is the unknown), and the sum of the other components (which is designated the matrix). Following the addition of an internal standard (component S) to the sample in known amount, the integrated intensities of line I of component J, I_{iJ}, and line k of component, S, I_{kS}, are determined. The weight fraction of the unknown component is the original sample, x_J, is given as

$$x_J = kI_{iJ}/I_{kS}$$

where k is a constant. When the internal standard is added in a constant proportion, the concentration of component J is a linear function of the intensity ratio, I_{iJ}/I_{kS}.

Thermal methods of analysis

Measurement of thermal analysis are conducted for the purpose of evaluating the physical and chemical changes that may take place in a heated sample, requiring that the operator interpret the events noted in a thermogram in terms of plausible reaction processes. Thermal reactions can be endothermic (melting, boiling, sublimation, vaporization, desolvation, solid-solid phase transitions, chemical

degradation, etc.) or exothermic (crystallization, oxidative decomposition, etc.) in nature (51).

Thermogravimetric analysis (TGA)

Thermogravimetry is a measure of the thermally induced weight loss of a material as a function of the applied temperature. TG analysis is restricted to transitions that involve either a gain or a loss of mass, and it is most commonly used to study desolvation processes and compound decomposition. TG analysis is a useful method for the quantitative determination of the total volatile content of a solid and can be used as an adjunct to Karl Fischer titrations for the determination of moisture. As such it readily permits the distinction between solvates and the anhydrous forms of a given compound (51,56).

Desolvation processes or decomposition reactions must be accompanied by weight changes, and they can be thus identified by a TG weight loss over the same temperature range. On the other hand, solid-liquid and solid-solid phase transformations are not accompanied by any loss of sample mass and would not register in a TG thermogram. The TG analysis of compound decomposition can also be used to compare the stability of similar compounds. In general, the higher the decomposition temperature of a given compound, the greater would be its stability (51).

The entire utility of TG analysis is in the differentiation and characterization of solvate species.

Differential scanning calorimetry (DSC)

In the DSC method, the sample and reference materials are maintained at the same temperature, and the heat flow required to keep the equality in temperature is measured. DSC plots are therefore obtained as the differential rate of heating (in units of W/s, cal/s, or J/s) against temperature. The area under a DSC peak is directly proportional to the heat absorbed or evolved by the thermal event, and integration of these peak areas yields the heat of reaction (in units of cal/s.g or J/s.g).

Differential scanning calorimetry can be used to establish the melting points of polymorphic species, investigate phase transformation, determine kinetics of solid state transformation, characterize of hydrate and solvate system, etc (51).

Scanning Electron Microscope (SEM)

Evaluation of the morphology of a pharmaceutical solid is of extreme importance, since this property exerts a significant influence over the bulk powder properties of the material (57). Both optical and electron microscopies have found widespread use for the characterization of polymorphs and solvates. Although optical microscopy is more limited in the range of magnification suitable for routine work. Electron microscopy work can be performed at extraordinarily high magnification levels (up to 90,00x on most units), and the images that can be obtained contain a considerable degree of three-dimensional information. The two methods are complementary in that each can provide information not obtainable by the other. With judicious use of these techniques, one can obtain substantial characterization of a polymorphic system (51).

Electron microscopy yields excellent topographic and shape information and is most useful in forensic situations involving trace evidence characterization and identification (51).

Infrared absorption spectroscopy (IR)

Infrared spectroscopy is typically used as an identification assay for various compounds. Most solid state investigations of bulk drug material involve the identification and quantitation of polymorphic and pseudopolymorphic systems. Since different polymorphic forms of a drug substance exhibit different three-dimensional structures, the vibrational motion for each polymorphic form is potentially different, whence the ability to investigate polymorphism by vibrational spectroscopy techniques (58).

The solid-state FTIR spectra of many polymorphic systems often are found to be only slightly different, indicating that the pattern of molecular vibrations is not grossly affected by differences in crystal structure (51).

When solvent molecules are incorporated in a crystal lattice, the new structure is often sufficiently different from that of the anhydrous phase so that many of the molecular vibrational modes are altered. As a result, water acts as the solvation agent, observations within the OH stretching region (3100-3600 cm^{-1}) are most fruitful for identification purposes. Therefore, if systems can form multiple anhydrate, hydrate, and solvate phases, the use of infrared spectroscopy can be extremely valuable. It is clear that vibrational spectroscopy has considerable use beyond the identification of polymorphs and solvates (51).

Thin-layer Chromatography

Thin-layer Chromatography (TLC) is used for separation and identification of compounds. It is a method of chromatography in which a mobile phase moves by capillary action across a uniform thin layer of finely divided stationary phase bound to the plate (59).

$$R_f = \frac{\text{Distance the substance travels from the origin}}{\text{Distance the solvent front travels from the origin}}$$

SOLID STATE STABILITY

The stability of drugs in solid dosage forms is the most important, since solid dosage forms are more common than the other types, and secondly because the first clinical trials are usually carried out in this type of dosage form (60-62).

Solid state reaction can be categorized either as chemical reaction or as physical transformations (37). The physical transformations such as polymorphic transitions, solvations and desolvations are affects the pharmaceutically important physical properties such as solubility, flow and tableting behavior (12-14). The solid state stabilities of aspartame hemihydrate both chemical degradation reaction and physical transformations was determined by Leung, et al (37,63). The chemical reaction was observed at 186-202°C with the liberation of water to yield cyclic compound (63). While, the polymorphic transitions was occurred by treatment of samples with ball-milling and elevated temperature (37). Hydration and dehydration

of crystalline and amorphous forms of Raffinose was determined by Saleki-Gerhardt, et al (64). Effect of grinding on thy physicochemical properties of indomethacin was examined, the α and γ forms of indomethacin were converted to noncrystalline solid durint grinding at 4°C (65).

Kinetics of Solid State Transformation

The kinetics of physical transformations are concerned (60). The investigations conducted by Shefter, et al (66) demonstrated that theophylline monohydrate transforms directly to a crystalline anhydrous form with apparent zero-order kinetics and the loss of water from ampicillin trihydrate results in amorphous state (66).

Kinetics study on the isothermal transition of the tolbutamide and mefenamic acid at high temperatures shown that, the transition of form B to form A of tolbutamide proceeds by the mechanism of three-dimensional diffusion, and the transition of form I to form II of mefenamic acid appeared to follow the zero-order mechanism (67).

By using equations which best fit the plot of rate versus time and eventually applying the Arrhenius equation, the activation energy was obtained. Initially, kinetic studies produce a plot of fraction decomposed, α, versus time, t. To determine rate constants, kinetic equations are chosen to fit the initial data. These kinetic equations are largely dependent on the mechanisms, which control the reactions (68). It is not unusual to find that several kinetic equations may give excellent fits to the data; such as different dehydration mechanisms were found for sulfaguanidine monohydrate when the data was fitted using various kinetic equations (69). Byrn (70) stated that "The fact that more than one equation fits the data indicates that solid-state kinetic data cannot be used to prove the mechanism of solid-state reaction. Nevertheless it is important for the determination of the activation energy to select the proper kinetic equation". There are many kinetic equations derived from different reaction models and concepts. They are currently widely used in assessing kinetic parameters, especially to determine the activation energies of solid-state reactions.

Kinetic Equations Based on Reaction Orders

Order of reactions have been used extensively to analyzed data from reactions in solution. In solid state reactions, however, the molecularity concept is not as well understood as for solution reactions. Nevertheless, these kinetic equations are, in some cases, useful in analysing solid state reaction data (70).

1. Zero-order equation

$$1 - \alpha = kt$$

where α = fraction decomposed

The reaction rate is independent of the concentration of the reacting species and only dependent on the time. The transition of form I to form II of mefenamic acid and transition of theophylline monohydrate to a crystalline anhydrous form appeared to follow the zero-order mechanism (66, 67).

2. First-order equation

$$\ln(\alpha) = kt$$

This reaction order is frequently found in many solid-state reactions. The rate of the reaction is proportional to the concentration of only one of the reacting compound. First-order kinetics was observed in thermal decomposition of several amorphous β-lactam antibacterials, such as, cephalothin sodium, cefamandole nafate and cefamandole sodium (71).

3. Second-order equation

$$\frac{1}{(1-\alpha)} = kt$$

The rate of reaction is proportional to the square of a concentration of a single reacting species.

Reactions Controlled by Contracting Geometries

The reaction is not controlled by nucleation but by the progression of phase boundaries from the outside to the inside of the crystal (62,70).

1. One Dimensional Growth of a Phase Boundary

The reaction is assumed to proceed along one direction. The rate is a function of time. Thus, a zero-order rate equation is valid.

$$1-\alpha = kt$$

2. Two Dimensional Growth of a phase boundary

The reaction is assumed to grow from the surface of a circular disk or a cylinder.

$$1-(1-\alpha)^{1/2} = kt$$

3. Three Dimensional Growth of a Phase Boundary

The reaction is assumed to advance from the surface of a sphere inward.

$$1-(1-\alpha)^{1/3} = kt$$

Reactions Controlled by Nucleation

1. The Prout-Tompkins Equation

The rate of the reaction is presumably controlled by nuclei that grow linearly, branch into chains and are terminated when the chains of the growing crystals come in contact with one another (70).

$$\ln\left(\frac{1}{1-\alpha}\right) = kt$$

2. Avrami-Erofeev Equation

The rate of the reaction is presumably controlled by random nuclei that grow in three dimensional directions and are able to ingest other nuclei (70).

$$[-\ln(1-\alpha)]^n = kt$$

where n = 1/4, 1/3, 1/2, 2/3, and 1.

Reactions Controlled by Diffusion

The reactions are controlled by a diffusion process. Only gaseous reactants or products should be considered for application of these equations (70).

1. One Dimensional Diffusion

The rate of the reaction is controlled by a one dimensional diffusion process (70).

$$\alpha^2 = kt$$

2. Two Dimensional Diffusion

The rate of reaction is controlled by diffusion from the surface of a circular disk or a cylinder which is a two dimensional diffusion (70).

$$(1-\alpha)\ln(1-\alpha)+\alpha = kt$$

3. Three Dimensional Diffusion

The reaction is controlled by diffusion from the surface of a sphere (70).

$$\left[1-(1-\alpha)^{1/3}\right]^2 = kt$$

Power-Law Equation

There is no theoretical basis for the power-law equation, but is has been found to be appropriate in several cases of solid state reactions (70).

$$\alpha^2 = kt$$

where n = 1/4, 1/3, 1/2, and 1

After all the kinetic equations are fitted, only the one with the best correlation coefficient should be chosen for the determination of the activation energy (62,70). The activation energy can then be used to calculate the rate constant at the labeled storage conditions. This information enables the manufacturer to approximate the expiration date or the stability of the drug when stored at that labeled condition. The activation energy can be obtained by using the Arrhenius equation.

$$k = Ae^{-E_T/RT}$$

where k is the rate constant, R is the gas constant, T is temperature in Kelvin and A is proportionality constant. By plotting the logarithm of the rate constants at different temperatures versus reciprocal time, the activation energy can be calculated from the slope of the plot.