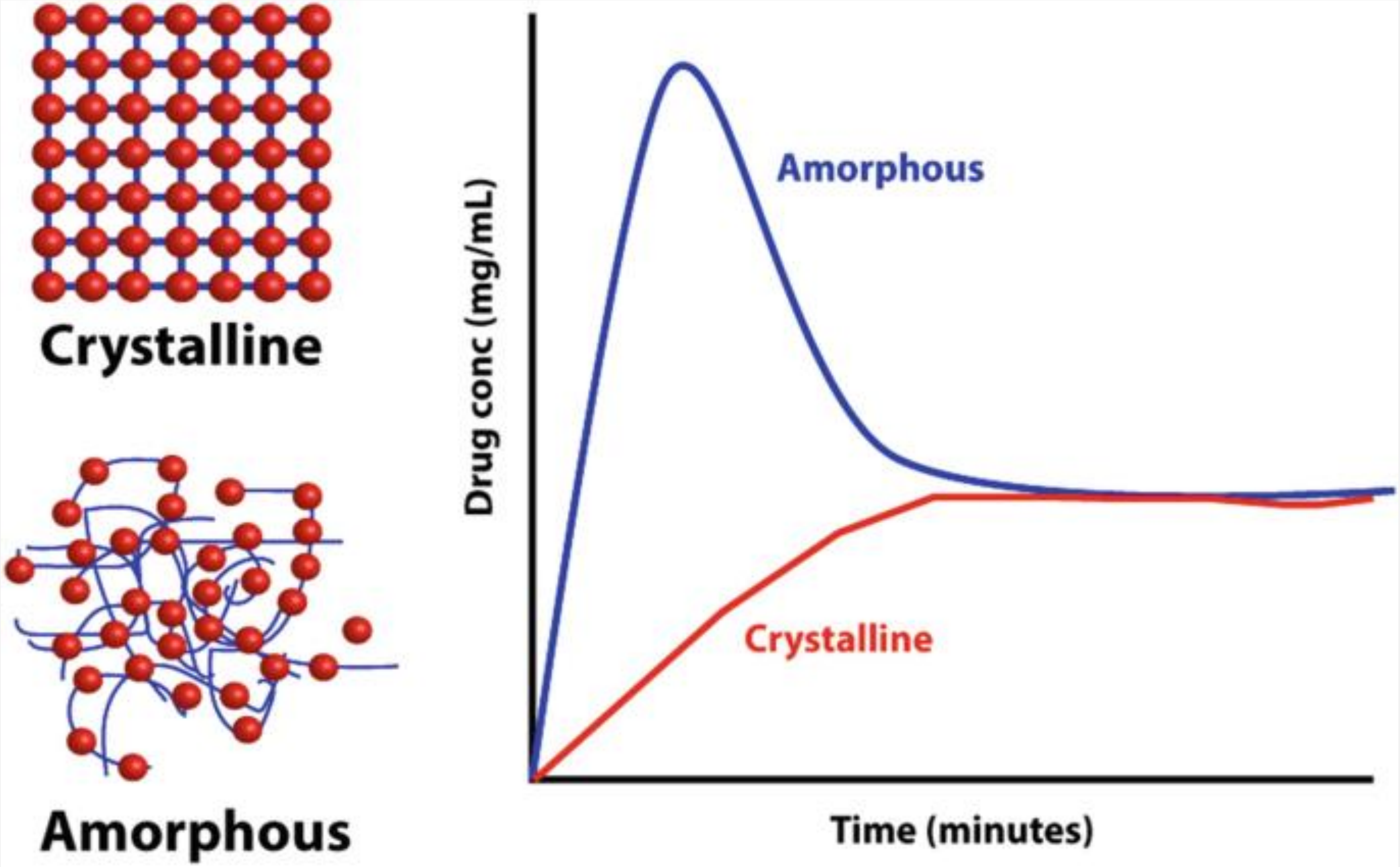
PRE-FORMULATION

By

By Assistant Prof. Dr Ghaidaa S. Hameed


Crystalline
Amorphous
Drug conc (mg/mL)
Amorphous
Crystalline
Time (minutes)

- **MOLECULAR PROPERTIES**
- Initial Physicochemical Characterization
- pKa Determinations
- The Partition and Distribution Coefficients
- **INITIAL SOLUBILITY INVESTIGATIONS**
- Effect of Ionization on Solubility
- **INITIAL STABILITY INVESTIGATIONS**
- Solution Stability
- Kinetics of Degradation
- **THE ORGANIC SOLID STATE**
- Crystalline States and Structural Assessment
- Polymorph Production Methods
- Salts and Cocrystals
- Solvates
- Hydrates
- Amorphous Phases

INITIAL PHYSICOCHEMICAL CHARACTERIZATION

- Nuclear magnetic resonance (NMR),
- Mass spectra, and
- Elemental analysis

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PKA DETERMINATIONS

- Potential CDs that possess **ionizable groups**, as either weak acids or bases, can be exploited to vary biological and physical properties such as:
- binding to **target enzyme or receptor**,
- binding to **plasma proteins**,
- gastrointestinal (GI) **absorption,**
- central nervous system **(CNS) penetration**,
- **solubility, and rate of dissolution**

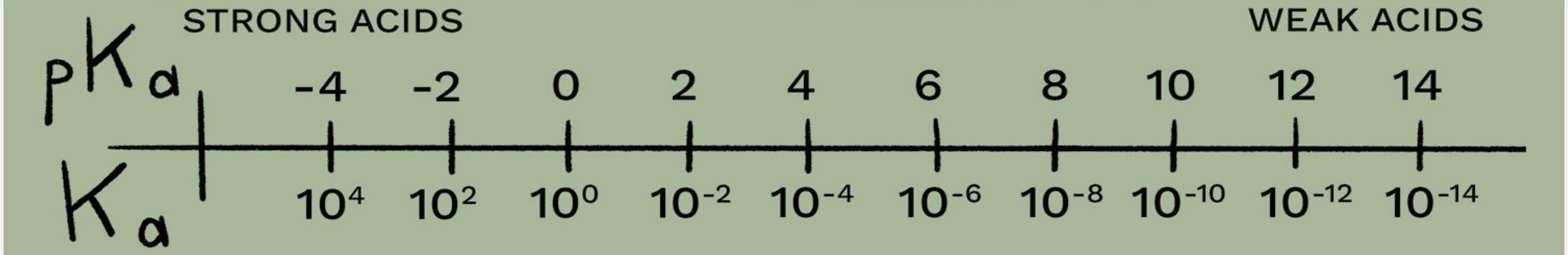
Therefore, one of the most important initial determinations carried out prior to their development is the **pKa or ionization constant(s).**

What Is pK_a in Chemistry?

The pKa value is the negative base-10 logarithm of the acid dissociation constant (K_a) of a solution. →

$$pK_a = -\log_{10} K_a$$

The smaller the value of pK_a, the stronger the acid.



STRONG ACIDS → WEAK ACIDS

pK_a	-4	-2	0	2	4	6	8	10	12	14
K_a	10^{4}	10^{2}	10^{0}	10^{-2}	10^{-4}	10^{-6}	10^{-8}	10^{-10}	10^{-12}	10^{-14}

ThoughtCo.

- If the compound contains an **ultraviolet (UV)** chromophore that changes with the **extent of ionization**, then a method involving UV spectroscopy can be used. This method involves measuring the UV spectrum of the compound as a function of pH. Mathematical analysis of the spectral shifts can then be used to determine the pKa(s) of the compound. This method is most **suitable for compounds where the ionizing group is close to or actually within an aromatic ring**, which usually results in **large UV shifts upon ionization**. The UV method requires only 1 mg of compound, and the potentiometric method around 3 mg of compound.
- Another method of determining pKa is the **pH indicator titration** described by Kong et al. (2007). This appears to be quite a novel approach insofar that it utilizes a **universal indicator solution** with **spectrophotometric detection** for the determination of the pKa instead of a pH electrode. The method works by calculating the **pH from the indicator spectra** in the **visible region** and then obtaining the spectra in the UV. Favorable results were obtained from a test set of **five compounds**.
- **Prediction of pKa**

The pKa of a compound may be estimated using a number of software packages, for example, **PALLAS, MARVIN, ACDpKa, and SPARC** (Meloun and Bordovska´ , 2007). These authors looked at the accuracy of the pKa data generated by these packages and concluded that **ACDpKa provided the most accurate prediction** of this value. In addition, ACDpKa also contains a large database of measured pKa data

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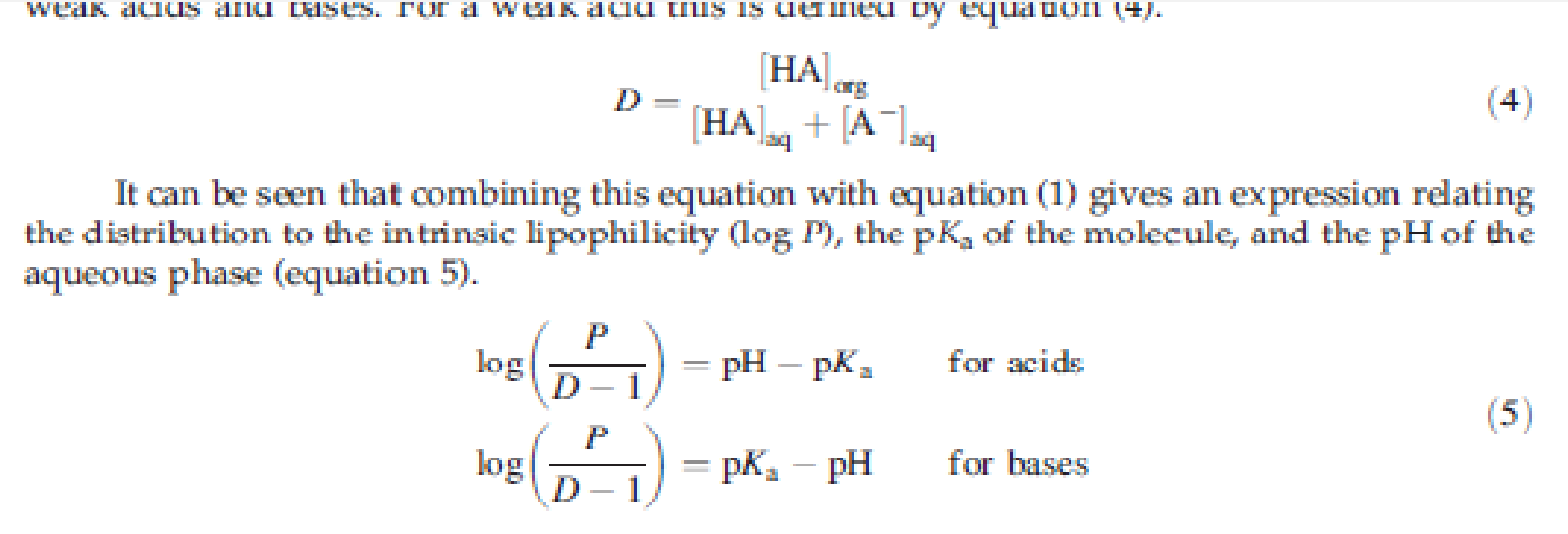
THE PARTITION AND DISTRIBUTION COEFFICIENTS

- They are important in the **solubility, absorption potential, membrane permeability, plasma protein binding, volume of distribution, and renal and hepatic clearance.**
- *P* =unionized compound org/unionized compound aq
- According to Lipinski (1997), log P values of less than **5** are best from a drug-like perspective. Generally compounds with **log P** values between **1 and 3 show good absorption**, whereas those with **log P** values **greater than 6 or less than 3 often have poor transport characteristics.**
- Highly **lipophilic molecules** have a preference to reside in the lipophilic regions of membranes, and **very polar compounds** show **poor bioavailability** because of their inability to penetrate membrane barriers. Thus, there is a parabolic relationship between log P and drug transport such that drug that exhibit a **balance between** these two properties will probably show the best oral bioavailability.

- The partition coefficient refers to the **intrinsic lipophilicity** of the drug in the context of the equilibrium of **unionized drug** between the **aqueous and organic phases**. However, if the drug has **more than one ionization center**, the distribution of species present will **depend on the pH**. The concentration of the **ionized drug in the aqueous phase** will therefore have an effect on the overall observed partition coefficient. This leads to the definition of the distribution coefficient (log D) of a compound, which takes into account the dissociation of weak acids and bases. For a weak acid this is defined by equation (4).



weak acids and bases. For a weak acid this is defined by equation (4).

$$D = \frac{[\mathrm{HA}]_{\mathrm{org}}}{[\mathrm{HA}]_{\mathrm{aq}} + [\mathrm{A}^-]_{\mathrm{aq}}} \qquad (4)$$

It can be seen that combining this equation with equation (1) gives an expression relating the distribution to the intrinsic lipophilicity (log P), the pK_a of the molecule, and the pH of the aqueous phase (equation 5).

$$\begin{aligned} \log\left(\frac{P}{D-1}\right) &= \mathrm{pH} - \mathrm{p}K_a \qquad \text{for acids} \\ \log\left(\frac{P}{D-1}\right) &= \mathrm{p}K_a - \mathrm{pH} \qquad \text{for bases} \end{aligned} \qquad (5)$$

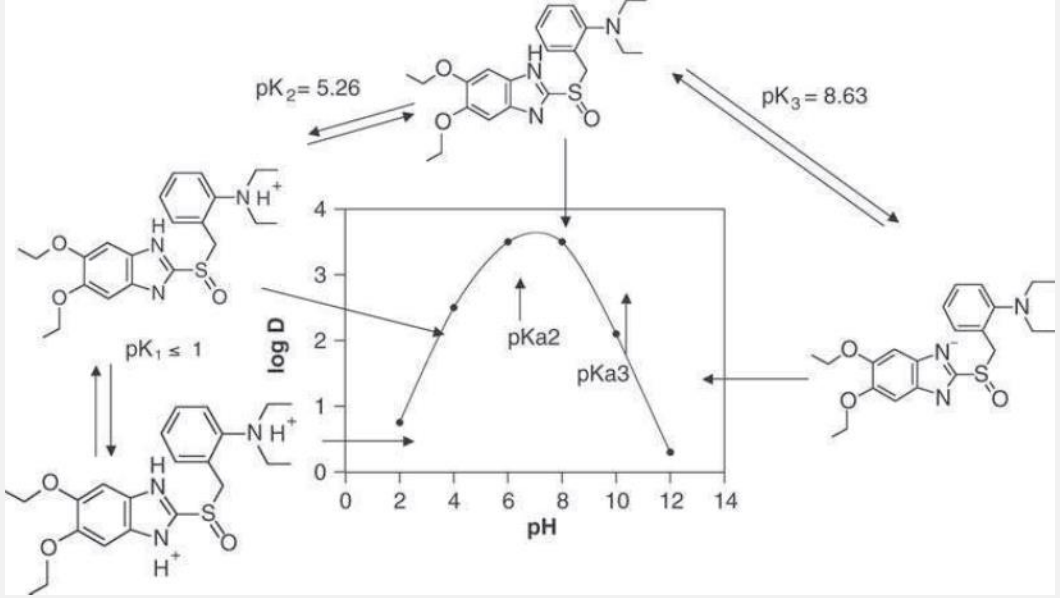


Figure 1 shows the effect of ionization on the partitioning of a proton pump inhibitor compound. This compound has a log P of 3.82 and three pKa values, that is, ,<1, 5.26, and 8.63.

At low pH, both the **benzimidazole and diethylamine nitrogens** are protonated, and hence the tendency of the compound is to reside in the aqueous phase.

As the pH increases, deprotonation of the protonated nitrogen of the **benzimidazole** takes place, and as the compound is **less ionized,** the compound resides more in the octanol phase.

At neutral pH, **deprotonation** of the **diethylamine** nitrogen renders the molecule neutral and hence its **lipophilicity is a maximum**.

A further increase in the pH results in **deprotonation of the second nitrogen** to form an anion, which, being ionized, is more **hydrophilic** and hence causes a **decrease in log D**

As a consequence of the pH effect on the log P value of a compound, Bhal et al. (2008) have argued that log D is a better descriptor in the context of Lipinski's rule, since it more physiologically relevant, that is, it takes into account the ionizable nature of many pharmaceutical molecules.

METHODS FOR DETERMINING LOG P AND LOG D

The most common technique for determining partition and distribution coefficients is the **shake-flask method**. In this method, the compound is equilibrated with an **octanol-aqueous buffer mixture for 30 minutes**, and the resulting emulsion is centrifuged to separate the two constituent phases. Once separated, the concentration of each layer is determined by high performance liquid chromatography (HPLC) and log D/log P is calculated.

Prediction of Log P and Log D

Computer methods have been devised to calculate these values. The first approach is where the molecule is broken down into fragments of known lipophilicity, and the log P is calculated using various computer routines. Alternatively, there are atom-based methods, and the lipophilicity is calculated by summing the atom-type values.

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INITIAL SOLUBILITY INVESTIGATIONS

- Effect of Ionization on Solubility
- The solubility of a compound, at a given pH, is a function of the solubility of the **ionized form** and the **limiting solubility of the neutral molecule**

$$\mathrm{pH} - \mathrm{p}K_\mathrm{a} = \log\left(\frac{S - S_0}{S_0}\right) \text{ for weak acids}$$

$$\mathrm{pH} - \mathrm{p}K_\mathrm{a} = \log\left(\frac{S_0}{S_0 - S}\right) \text{ for weak bases}$$

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INITIAL STABILITY INVESTIGATIONS

Knowledge about the chemical and physical stability of a drug in the **solid and liquid** state is extremely important in drug development for a number of reasons. In the longer term, the stability of the formulation will dictate **the shelf life of the marketed product**; however, to achieve this formulation, careful **preformulation work** will have characterized the compound such that a rational choice of conditions and excipients are available to the formulation team.

The amount of **impurities** allowed from product storage, and for most drugs the allowable levels of a single impurity permissible without toxicological cover **is much less than 1%.** In early development, **accelerated stability** therefore needs to be undertaken to assess how the drug will stand up to a longer term.

To elucidate their stability with respect to, for example, **temperature, pH, light, and oxygen**, a number of experiments need to be performed. The major objectives of preformulation team are therefore to **identify conditions** to which the compound is sensitive and identify degradation profiles under these conditions. The main routes of drug degradation in solution are via **hydrolysis, oxidation, or photochemical** means. In their book, Connors et al. (1986) have dealt with the physical chemistry **involved in the kinetic analysis of degradation of pharmaceuticals** very well, and the reader is referred there for a detailed discussion.

Although hydrolysis and oxidation constitute the main mechanisms by which drugs can decompose, **racemization** is another way in which the compound can change in solution. For example, Yuchun et al. (2000) reported the kinetics of the **base-catalyzed racemization of ibuprofen enantiomers**. Although ibuprofen is **highly resistant to racemization in acids**, it will racemize in basic solutions through a keto-enol mechanism.

SOLUTION STABILITY

Hydrolysis

- Mechanistically, hydrolysis takes place in two stages. In the first instance, **a nucleophile, such as water or the OH– ion adds** to, for example, **an acyl carbon to form an intermediate** from which the **leaving group** then breaks away. **The structure of the compound** will affect the rate at which this reaction takes place, and the stronger the **leaving conjugate acid, the faster the degradation reaction will take place**. Degradation by hydrolysis is affected by a number of factors, of which solution **pH, buffer salts, and ionic strength** are the most important. In addition, the presence of **cosolvents, complexing agents, and surfactants** can also affect this type of degradation.

- As noted, **solution pH** is one of major determinants of the stability of a compound. **Hydroxyl ions are stronger nucleophiles than water, thus degradation reactions are usually faster in alkaline solutions than in water**, that is, **OH– ions** catalyze the reaction. In solutions of **low pH, H+ can also catalyze hydrolysis reactions**. In this case, catalysis by H+ and OH– is termed specific acid base catalysis. Of course, H+ and OH– ions are not the only ions that may be present during an experiment or in a formulation. It is well known that **buffer ions such as acetate or citrate can catalyze degradation,** and in this case the effect is known as general **acid-base degradation**. Therefore, although it is prudent to adjust the pH to the desired value to optimize stability, this should always be done with **the minimum concentration necessary**.

EXAMPLES

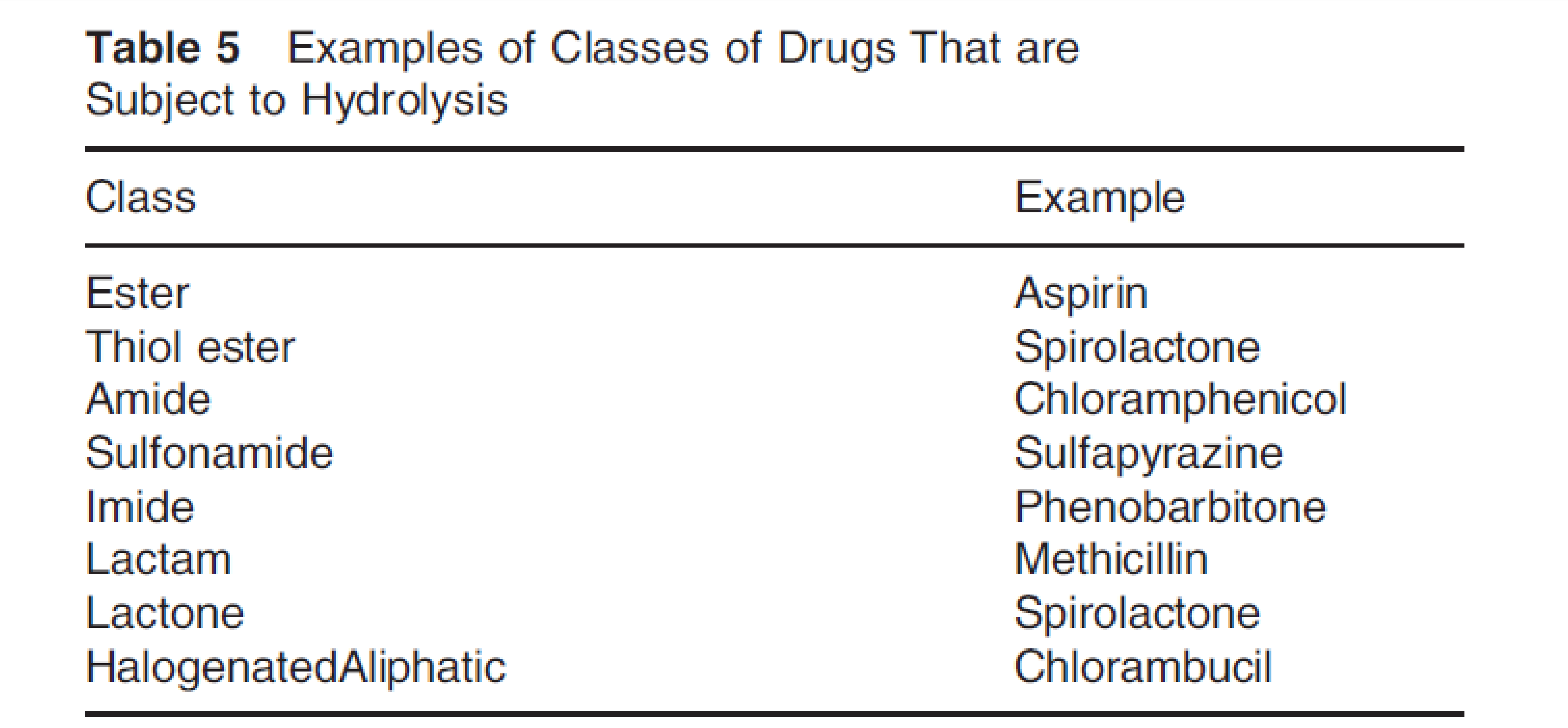

Table 5 Examples of Classes of Drugs That are Subject to Hydrolysis

Class	Example
Ester	Aspirin
Thiol ester	Spirolactone
Amide	Chloramphenicol
Sulfonamide	Sulfapyrazine
Imide	Phenobarbitone
Lactam	Methicillin
Lactone	Spirolactone
HalogenatedAliphatic	Chlorambucil

OXIDATION

- The second most common way a compound can decompose in solution is via **oxidation.** Reduction/oxidation (redox) reactions involve one of the following processes: (1) **transfer of oxygen or hydrogen atoms** or (2) **transfer of electrons**. Oxidation is promoted by the presence of oxygen, and the reaction can be initiated by the action of **heat, light, or trace metal ions** that produce organic free radicals. These radicals propagate the oxidation reaction that proceeds until inhibitors destroy them or by side reactions that eventually break the chain.

KINETICS OF DEGRADATION

- Essentially we must determine the **amount of the compound remaining with respect to time** under the conditions of interest. Alternatively, the appearance of degradation product could also be used to monitor the reaction kinetics.
- Thus, the rate of a reaction can be defined as the rate of change of concentration of one of the reactants or products. For a simple drug decomposition, therefore, the following situation holds
- Drug $\longrightarrow$ product(s)
- The concentration of the compound will decrease with time; equation (15) relating to these quantities

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THE ORGANIC SOLID STATE

- Solid phases or molecular solids are defined in thermodynamic terms as states of matter that are uniform throughout in chemical composition and also in physical state (Wunderlich, 1999). **Molecular solids** can exist as **crystalline** or noncrystalline **(amorphous)** phases depending on the **extent of three-dimensional** order and the **relative thermodynamic stability** hierarchy. Crystalline states can be described as a periodic array of molecules within a **three-dimensional framework**. Whereas noncrystalline materials **lack significant three-dimensional order**, but may exhibit lower-dimensional short-range order.

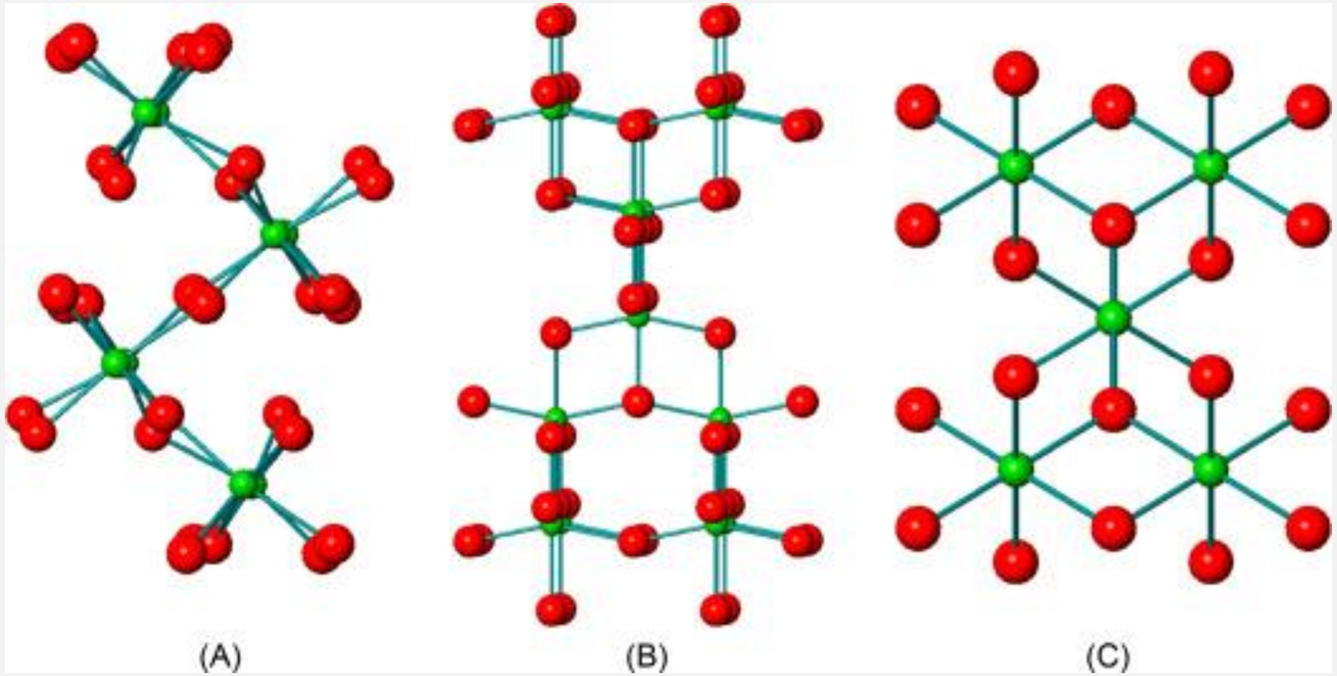
A significant interest in molecular crystals originates from the ability to use molecular level “crystal engineering” strategies to **rationally design crystal packing to control specific physical properties.**

Polymorphism and Related Phenomena

In 2002, Bernstein pointed out that structural diversity is present in almost every facet of nature, and crystal polymorphism is one manifestation of this diversity. Polymorphism, in a chemical sense, is a solid-state phenomenon where the **crystal structures** of a chemical entity **are different**, but correspond to identical liquid and vapor states.

A variation in crystal structure is brought about by **differences in molecular packing and intermolecular interactions within the three-dimensional framework of the crystalline state**. The way the molecules pack is defined in part by the molecular structure itself, and there is also the possibility of forming stable intermolecular interactions such **as H-bonds**, giving rise to structures with differences in **density.** Consequently, polymorphs will have **different lattice energies**, which in turn govern the physical properties and behaviors of the material.

POLYMORPHISM



- Polymorphism is a common phenomenon in **small organic molecules**, and the occurrence of polymorphs has been documented extensively (Borka and Haleblain, 1990; Byrn et al., 1999; Bernstein, 2002). Specifically, in the area of pharmaceutical material selection, polymorphs are selected on the **basis of physical and chemical stability, behavior to processing and formulation, and biopharmaceutical properties as an assessment of in vivo performance.**

- Knowledge of the relative behavior of the polymorphs with respect to the properties outlined above allows a rationalized selection. For instance, differences in **solubility and dissolution rate** between polymorphs can have a pronounced impact on the **oral bioavailability** (i.e., dissolution and absorption from the GI tract) of pharmaceuticals as exemplified by investigations of formulations of **tolbutamide.**

- Many approaches can be taken to induce **polymorphic changes** to explore its occurrence. These include **solution-mediated** transitions such **as recrystallization and solution maturation studies** (Cardew and Davey, 1985) and **thermally induced** (Giron, 1995) and **mechanical/pressure-induced** changes such as those exhibited by **chlorpropamide** (Wildfong et al., 2005). Other solvent-free methods of isolating polymorphs involve **quenching from the molten liquid or gaseous state** (**sublimation experiments**), as are used to isolate polymorphs of **venlafaxine hydrochloride** (Roy et al., 2005).`

- In addition to simple variations in hydrogen bonding, polymorphism can also be induced by conformational differences, that is, the existence of different conformers of the same molecule in different polymorphic modifications. When a molecule is conformationally flexible with a number of energetically accessible conformations

INTERCONVERSION OF POLYMORPHS

- Recrystallization
- Role of impurities
- In most industries in which polymorphism plays an important role in materials and their properties, there are several business drivers for **polymorph characterization and selection**.
- **Firstly**, there is a need to understand the **external effect** on structural behavior, enabling the selection of a robust and stable material that will not interconvert to a less desirable polymorph upon **storage or processing**.
- **Secondly**, it is important to have as much of the polymorph "hypersurface" mapped to ensure that all **plausible low-energy structures**, which could represent developable forms, are isolated and characterized. Information on structural relationships and the ease of interconversion (exploring both **kinetics and mechanisms**) allow the **selection of the most optimum or developable form.**

POLYMORPH PRODUCTION METHODS

1. **Crystallization from different solvents under variable conditions**, for example, different agitation speeds and temperatures Therefore, it is important to screen a **variety of solvents** that cover a diversity of physicochemical parameters. Although the solvent of crystallization can be critical in producing a particular polymorph, Getsoian et al. (2008) showed that **for carbamazepine** varying the crystallization. temperature and level of supersaturation was sufficient to produce three of the four known polymorphs from a single solvent.

2. **Precipitation by, for example, addition of an antisolvent** to a solution containing **the drug or by pH** adjustment of solutions of weak acids or bases (Bosch et al., 2004). An interesting example of this phenomenon is the quasi-emulsion precipitation of a number of polymorphic compounds using **PEG300** as the solvent and water as the antisolvent (Wang et al., 2005). In this system, the intensity of mixing appeared to control the polymorphic form because of increased viscosity of the PEG300-water solution.

3. **Concentration or evaporation**. Capes and Cameron (2007) reported that a metastable form was obtained from the periphery of an evaporating solution. The resultant metastable form that was left free of the solvent was unable to transform to the more stable polymorph via a solvent-mediated phase transformation.

4. **Formation of polymorphs from solvate desolvation**. Nicolai et al. (2007) showed that forms I of **spironolactone** could be obtained by desolvation of its ethanol solvate.

5. **Crystallization from the melt**, assuming that melting is not accompanied by thermal degradation. Schmidt et al. (2006) found that modification I of **salicaine HCl** crystallized from the melt above 1108C and modification II crystallized from the melt below this temperature.

6. **Grinding and compression**. Trask et al. (2005a) have reported that polymorphic conversions **of anthranilic acid** (ortho-aminobenzoic acid), for example, could be induced by dry grinding (neat powder) and also in the presence of a small amount of solvent. Linol and Coquerel (2007) have also demonstrated that high-energy milling could be used to accelerate (relative to slurry experiments) the polymorphic conversion between the **monoclinic and orthorhombic forms of 5-methyl- (40-methyl phenyl)hydantoin.**

7. **Lyophilization (freeze-drying)** can induce polymorphism, as exemplified by **pentamidine isethionate** where various polymorphs can be obtained by altering the freeze-drying conditions (Chongprasert et al., 1998). Often, however, the compound is rendered amorphous by the freeze-drying process (Zhu and Sacchetti, 2002).

8. **Spray-drying**. Amorphous **ursodeoxycholic acid** can be prepared by spray-drying (Ueno et al., 1998).

9. **Crystallization from supercritical fluids**. Park et al. (2007) used a supercritical antisolvent (SAS) process to produce different forms of **fluconazole.**

10. **Potentiometric cycling**. Llina`s et al. (2007) used this method to produce polymorphic forms of **sulindac**.

11. **High pressure**. **Piracetam** has been crystallized in a number of polymorphic forms using the high pressures (0.07–0.4 GPa) generated using a diamond anvil cell (Fabbiani et al., 2005).

12. **Sublimation.** Exploiting the vapor phase as a solvent-free method of crystallizing polymorphs, as shown by Liu et al. (2008).

SALT

- If a compound possesses an **ionization center**, then this opens up the possibility of forming a salt. The majority of drugs administered as medicines are salts of the active pharmaceutical ingredient (Stahl and Wermuth, 2002). Therefore, salt evaluation should be an integral part of the prenomination phase and is usually carried out to modulate the physicochemical properties of the **free acid or base**. Properties that can be altered by salt formation include solubility, dissolution, bioavailability (Gwak et al., 2005), hygroscopicity, taste, physical and chemical stability (Farag Badawy, 2001), or polymorphism (Stahl and Wermuth, 2002; Serajuddin, 2007).
- Alternative salt proposed by the generic company **may have to undergo toxicological testing** in addition to bioequivalence testing before it would be accepted by the regulatory authorities as an acceptable alternative.
- The aqueous solubility of the remaining salts are determined to ascertain whether there may be dissolution or bioavailability problems. In the final tier, the stability of the final candidate salts are then investigated under accelerated conditions, (**temperature, humidity, and presence of excipients**). If desired, compatibility testing with excipients may be conducted at this stage. Consideration of ease of synthesis, analysis, potential impurities, and so on must also be undertaken.

- To comply with the concept that in preformulation studies minimal amounts of compound are used, an in situ salt-screening technique for basic compounds has been developed by Tong and Whitesell (1998). **Firstly**, the protocol for **basic drug compounds is based on only using counterions with a pKa value that is at least two pH units from that of the drug**. **Secondly, solubility studies should be performed on the base in solutions of the chosen acid counterions.**
- The concentration of the acid should account for an excess after the formation of the salt. It was recommended that the **amount of base added** should be accurately recorded because of its effects on the amount of **acid consumed** in preparing the salt and the **pH** of the final solution recorded.
- Finally, the solids formed (both wet and dry) should then be analyzed using the standard techniques, for example, **DSC, thermogravimetric analysis (TGA), and XRPD.** By using this protocol, there was good agreement between the solubilities of salts prepared by conventional means and the solubility of the base in the in situ technique in all cases **except for the succinate**. This was probably due to the fact that, as prepared, it was **a hydrate** and highlighted a potential drawback of the technique. Indeed, it was stated that the in situ technique should not replace traditional salt selection techniques. Rather, it should be used as salt-screening tool to rule out those salts that have **poor solubility characteristics**, thus obviating the need for their synthesis.

METHOD OF PREPRATION

- Dissolve drug in methanol or other suitable standard solvent
- Add drug solution to 96-well plate using, for example, a multipipette
- Add counterion solutions (2 of each)
- Evaporate slowly, normally subambient
- Select crystalline (crossed polarizers) particles and store their x, y, z coordinates
- Collect Raman spectra (batch job)
- Repeat procedure in different solvents

COCRYSTALS

- In contrast to salt formation, which is accompanied **by proton transfer from an acid to a base**, cocrystals are crystalline materials where two or more **neutral molecules** are hydrogen bonded to each other. Childs et al. (2007) have argued that both salts and cocrystals are both part of a continuum of multicomponent crystals linked by the extent of proton transfer. Indeed there is a gradation of properties such that, for example, **norfloxacin** forms both salts (succinate, malonate, and maleate) and a cocrystal (isonicotinamide) (Basavoju et al., 2006).

- However, as **with salt formation, the goal of cocrystal formation** is to alter or improve the properties of the parent compound; thus, the choice of whether to take a salt or cocrystal into development should not represent a concern so long as the cocrystal meets the criteria set for selection (Aakero¨y et al., 2007). For example, cocrystal formation was used **to control the hygroscopicity** of **caffeine with the oxalic acid cocrystal** showing complete stability even at high relative humidities (Trask et al., 2005b). Similarly, the same group showed that the physical stability **of theophylline**, with regard to moisture uptake, could be enhanced through cocrystal formation (Trask et al., 2006). Moisture uptake as **a method** of producing co-crystals has been reported by Jayasankar et al. (2007). By using a variety of substances (e.g., **carbamazepine-saccharin)**, they formed the cocrystal by exposing it to very high levels of RH, which **caused deliquescence** followed by cocrystal formation. Microscopically, it was observed that after excessive moisture uptake the compounds dissolved to form a solution from which the cocrystal underwent nucleation and growth.

- In vivo studies comparing the bioavailability of anhydrous carbamazepine with its saccharin cocrystal showed that the cocrystal had the **same chemical stability, superior suspension stability, and bioavailability comparable to an immediate-release tablet (Hickey et al., 2007a)**. A glutaric acid cocrystal with a development compound (a potential sodium channel blocker) was shown to improve its **bioavailability** (MacNamara et al., 2006). As noted above, cocrystals **can contain more than two components**, and as an example Karki et al. (2007) have reported the cocrystal between **theophylline, citric acid, and water** in the ratio 1:1:1. In production, cocrystals have been produced by a variety of means, **for example, dry grinding and solvent-assisted grinding are popular. Zhang et al. (2007) have communicated a slurry/suspension method for screening cocrystal formers**.

SOLVATES

Solvates are materials where solvent or water molecules are **incorporated in the crystal lattice** or in **interstitial voids or channels**. It has been described by some authors by the term **pseudopolymorphism**; however, there has been some debate about whether this is the meaningful description and its use should now be avoided (Desiraju, 2004; Seddon, 2004). Generally speaking, four main roles are fulfilled by solvents in crystal structures (van der Sluis and Kroon, 1989). These are:

(1) participation as acceptors and/or donors **in hydrogen-bonding schemes**,

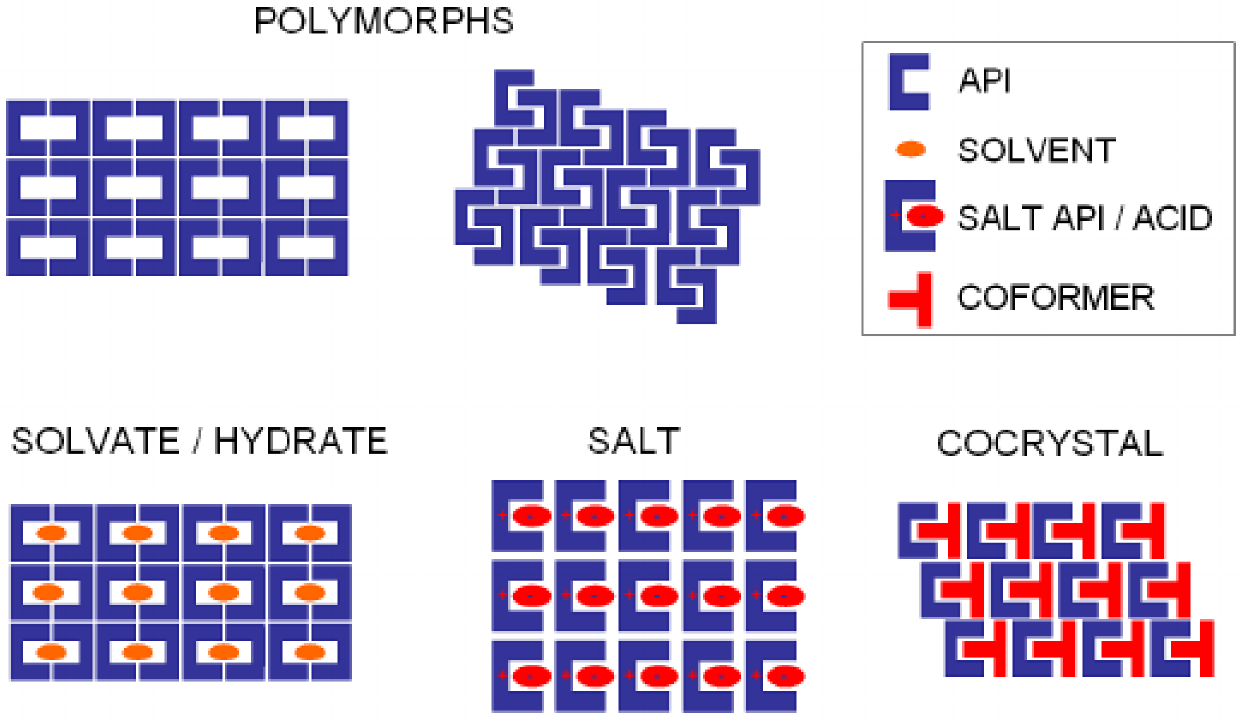
(2) filling **void spaces**,

(3) completing **coordination** around metal ions, and

(4) bridging **polar and nonpolar regions** in the crystal.

Specific interactions can take place through hydrogen bonding, and Bingham et al. (2001) have classified the space filling solvates as inclusion phases and the hydrogen-bonded species as cocrystals. Another type of inclusion solvate, known as **clathrate,** has been defined where the solvent is located in isolated lattice sites with **no significant interaction to the host molecule**; for example, Kemperman et al. (2000) have reported the **clathrated b-naphthol structures of a range of cephalosporin antibiotics.** Furthermore, Sheth et al. (2002) have pointed out that these guest molecules lie trapped in closed, three dimensional cavities formed by the crystalline structure of the host. By using this definition and a determination of the crystal structure, they showed that the material supplied as“**warfarin sodium clathrate**” (with 2-propanol) was in fact a solvate.

- The crystal has large empty **crystallographic channels or holes**, their nature will determine which solvent will be included and the structure of the resulting solvate. From a structural point of view, the inclusion of a variety of solvates can show regularity. For example, Hosokawa et al. (2004) have reported the isostructurality of the **2:1 benzene, cyclohexane, 1,4 dioxin, tetrahydrofuran, tetrachloromethane, and chloroform** solvates of **phenylbutazone**.
- In thermodynamic terms, Senthil Kumar et al. (1999) have described the formation of solvates in the following terms: “If solute-solvent interactions are unusually important, say because of multipoint recognition, the entropic advantage associated with solvent expulsion into the bulk may be overridden by these additional **enthalpic factors, resulting in retention of some solvent in the crystal.**”
- Although solvates **can show higher solubilities and dissolution rates** compared to nonsolvated species (Stoltz et al., 1988; Suleiman and Najib, 1989), solvates cannot normally be used in the pharmaceutical arena because of the **intrinsic toxicity** of the solvent itself.


POLYMORPHS
API
SOLVENT
SALT API / ACID
COFORMER
SOLVATE / HYDRATE
SALT
COCRYSTAL

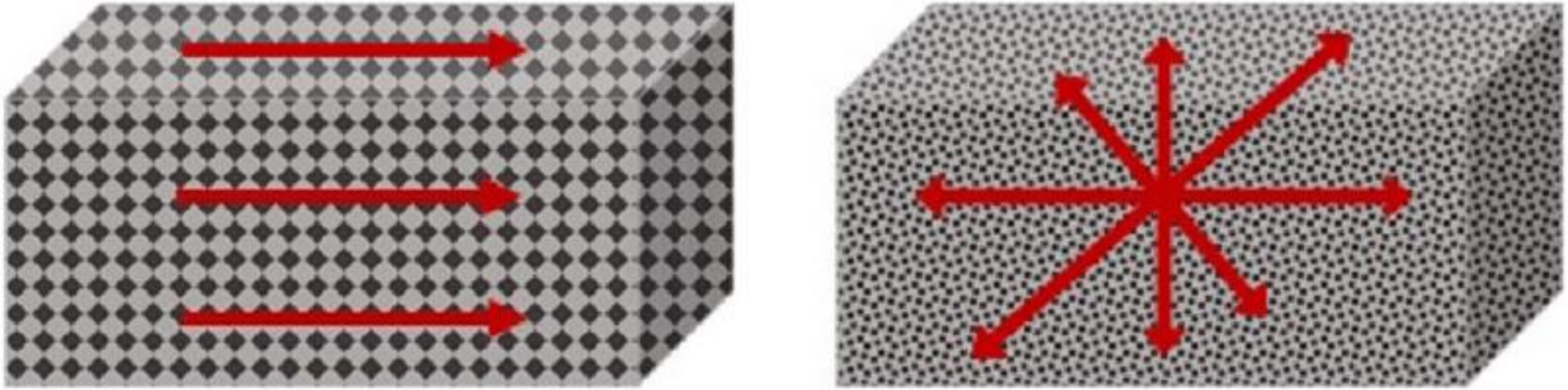
When the solvent is removed from the crystal lattice, which retains its three-dimensional order, a so-called **isomorphic desolvate** is created (Stephenson et al., 1998).

The desolvated structure is **highly energetic and reduces this situation by simply taking up moisture from the atmosphere or undergoing a certain degree of structural collapse to reduce the unit cell volume**.

Petit et al. (2007) have investigated the mechanism of a range of **cortisone acetate solvates**.

They identified two main mechanisms by which the solvent was lost.

- **The dihydrate and the tetrahydrofuran solvate lost their solvent anisotropically**, which was followed by a cooperative structural rearrangement to an anhydrous polymorph.
- In contrast the dimethylformamide and dimethylsulfoxide solvates desolvated via a **partial dissolution** of the internal part of the crystals.



(a) **Anisotropic layered structure**

(b) **Isotropic uniform structure**

HYDRATES

- The most common case of solvation is the incorporation of water molecules, and they are almost always involved in **hydrogen bonding**. Indeed, it is the hydrogen-bonding network that contributes to the coherence of the crystal, such that they usually show, for example, **slower dissolution** rates compared with the corresponding anhydrates. Byrn (1982) has illustrated the importance of this topic by stating that there are more than 90 hydrates listed in the USP. It should be noted that not only the APIs but excipients also have the potential to form hydrates, for example, magnesium stearate (Bracconi et al., 2003). As shown by Salameh and Taylor (2006), excipients can also have an effect on the stability of hydrates. For example, **when PVP12 was mixed with theophylline monohydrate and carbamazepine dihydrate, it dehydrated both hydrates.**

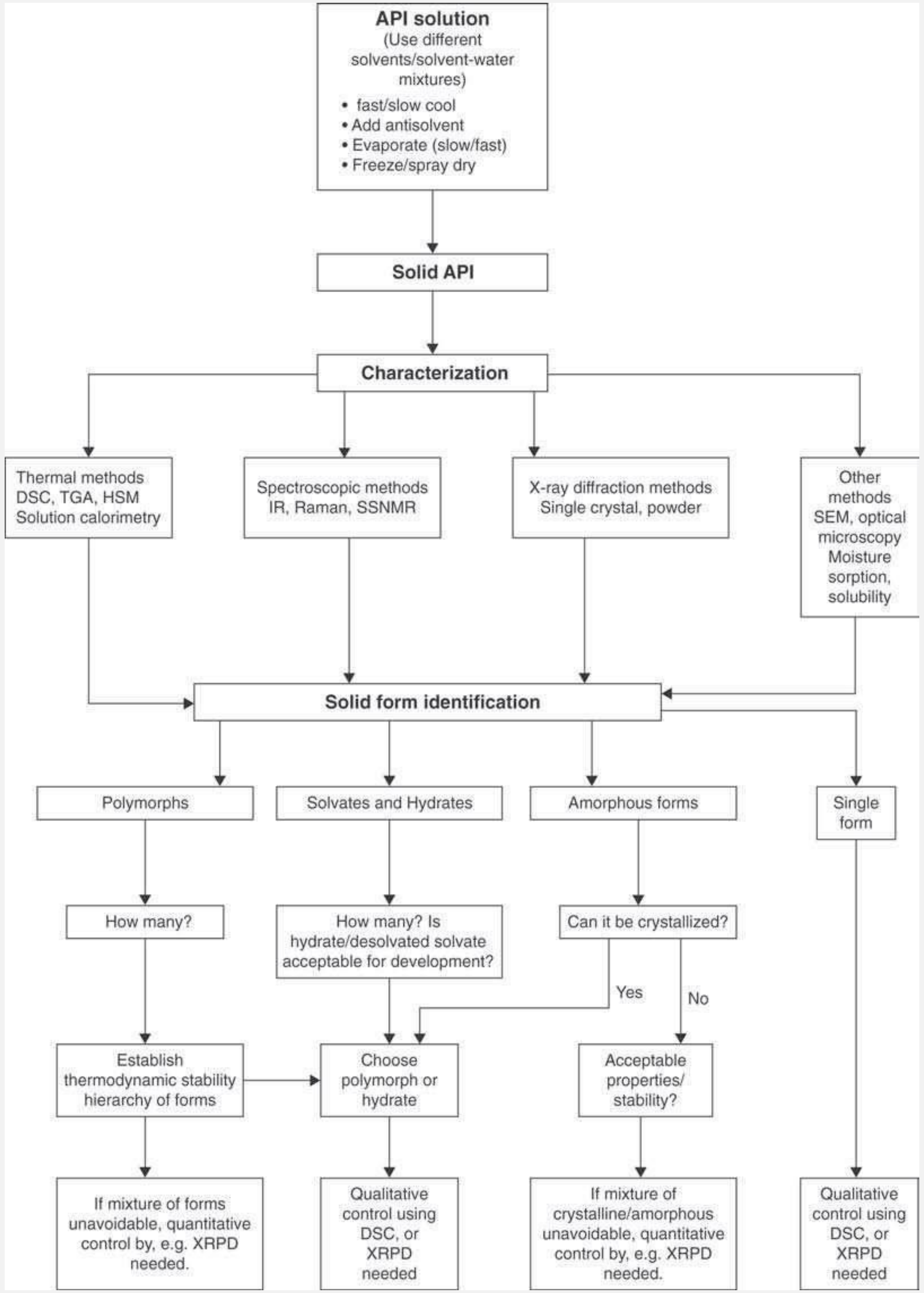
- In instances for which water plays a crucial role in maintaining the crystal structure via the formation of a hydrogen-bonding network, dehydration can often lead to complete structural collapse, giving rise to an **amorphous anhydrate**, as observed with **eprosartan mesylate dihydrate** (Sheng et al., 1999). In this particular case, the water of crystallization forms a hydrogen-bonding framework directly to the parent drug and the salt counterion.
- **Dehydration** results in an **amorphous material**, which becomes annealed upon heating, giving rise to a crystalline hydrate. **Such hydrates are considered to be very stable and represent developable materials**. Hydrates in which water acts as a "space filler" occupying voids or crystallographic channels can dehydrate to give isomorphous anhydrates or undergo a change of structure to give a more **densely packed arrangement**. Examples of these types of hydrates include **cephalexin (Kennedy et al., 2003), erythromycin A, and cefaclor** (Stephenson et al., 1997), all of which give rise to isomorphous anhydrates as determined by XRPD analysis

- Hydrates can also **exhibit polymorphism**. For example**, amiloride hydrochloride dihydrate is present in two polymorphic forms**. By milling or compressing both forms, it was shown that form A was more stable than form B. Moreover, it was shown that the anhydrate rapidly rehydrated to form A **dihydrate on exposure to atmospheric RH** (Jozwiakowski et al., 1993).
- **Niclosamide also exists as two monohydrated forms and an anhydrated phase** (van Tonder et al., 2004). It was found that suspension formulations of the anhydrate readily converted to **thick suspensions of monohydrate**. **Rapid solution-mediated conversion to a hydrate** can often result in the formation of an unusable thixotropic formulation. In addition to this complication, in situ formation of a hydrated phase may modify **dissolution and hence bioavailability** of the material. For these reasons, it is important to assess the propensity for hydrate formation in formulation vehicles as part of the material selection program.

AMORPHOUS PHASES

- Amorphous phases are noncrystalline materials that possess **no long-range order**, but can exhibit a certain degree **of short-range order** (Yu, 2001; Bhugra and Pikal, 2008). Amorphous phases represent **highly energetic, unstable materials** largely because of this lack of three dimensional or long-range order found in crystalline materials. This lack of three-dimensional periodicity is reflected in the materials' inability to diffract **X rays constructively**; as such analysis of an amorphous sample by XRPD gives rise to a **diffuse profile** devoid of the **characteristic peaks observed for crystalline phases**. However, it must be borne in mind that there are some samples that when analyzed by XRPD also give rise to a diffuse profile, but are shown by other methods (such as the presence of a **discrete melting event**) to be crystalline. Such phases are termed X-ray amorphous and represent micro- or nanocrystalline structures.
- The amorphous phase can be thought of as a **frozen or supercooled liquid**, but with the thermal fluctuations present in a liquid frozen to a greater or lesser extent, leaving only largely static structural disorder (Elliot et al., 1986). According to the USP, the degree of crystallinity depends on the fraction of crystalline material in the mixture, and this is termed the two-state model. Another way of viewing this situation is that the crystallinity can have a value that ranges from **100% for perfect crystals (0 entropy) to 0% (noncrystalline or amorphous**); this is known as the one-state model. Yu (2001) has reviewed the characteristics and significance of the amorphous state with regard to pharmaceuticals.

- The amorphous state can be characterized **by the glass transition temperature (Tg),** where the molecular motion is faster above and slower below this transition (Zhou et al., 2002). The Tg can be thought of as similar to a **second-order phase transition**, but the glassy state is regarded as far from being in thermal equilibrium. Hancock and Shamblin (2001) addressed the question of how the **molecular mobility** of the material influences its **performance**. By using DSC, they argued that most pharmaceutical glasses should have a similar relaxation time around the Tg.
- Methods for the production of the amorphous state include **quenching the melt of a compound, rapid precipitation from solution by, for example, addition of an antisolvent, freeze and spray-drying (Ueno et al., 1998), dehydration of crystalline hydrates, and grinding/milling** (Wildfong et al., 2006; Chieng et al., 2008). One consequence of a disordered structure is that amorphous phases are **thermodynamically unstable, and, therefore, they are the most energetic forms of a compound. The tendency of amorphous phases is thus to revert to a more stable, crystalline form**


API solution
(Use different solvents/solvent-water mixtures)
• fast/slow cool
• Add antisolvent
• Evaporate (slow/fast)
• Freeze/spray dry
Solid API
Characterization
Thermal methods DSC, TGA, HSM Solution calorimetry
Spectroscopic methods IR, Raman, SSNMR
X-ray diffraction methods Single crystal, powder
Other methods SEM, optical microscopy Moisture sorption, solubility
Solid form identification
Polymorphs
Solvates and Hydrates
Amorphous forms
Single form
How many?
How many? Is hydrate/desolvated solvate acceptable for development?
Can it be crystallized?
Yes
No
Establish thermodynamic stability hierarchy of forms
Choose polymorph or hydrate
Acceptable properties/ stability?
If mixture of forms unavoidable, quantitative control by, e.g. XRPD needed.
Qualitative control using DSC, or XRPD needed
If mixture of crystalline/amorphous unavoidable, quantitative control by, e.g. XRPD needed.
Qualitative control using DSC, or XRPD needed

ASSESSMENT OF THE ORGANIC SOLID STATE

- X-ray diffraction (XRD) (powder and single crystal) (Stephenson, 2005)
- Microscopy [optical (Nichols, 1998); electron (Tian et al., 2006); and atomic force (Hooton, et al. 2006)]
- Thermal analytical techniques, for example, DSC, TGA [with MS or Fourier transform infrared (FTIR) for effluent gas analysis (Rodriguez and Bugey, 1997)], and hot-stage microscopy (HSM) (Vitez et al., 1998)
- Isothermal microcalorimetry (Phipps and Mackin, 2000)
- Solution calorimetry (Gu and Grant, 2001)
- Mid- and near-IR spectroscopies (Threlfall and Chalmers, 2007)
- Raman spectroscopy (Fini, 2004)
- Cross polarization magic angle spinning (CP MAS) solid-state NMR (Harris, 2007)
- Hygroscopicity measurements (Kawakami et al., 2005)
- Phase solubility analysis (Sheikhzadeh et al., 2007)
- Intrinsic dissolution rates (Pereira et al., 2007)

SINGLE CRYSTAL STRUCTURE DETERMINATION

- Molecular identity
- Basic crystal information such as bond lengths and angles and space group
- Molecular conformations
- Absolute configuration of chiral molecules (R or S)
- Molecular disorder
- Hydrogen-bonding motifs

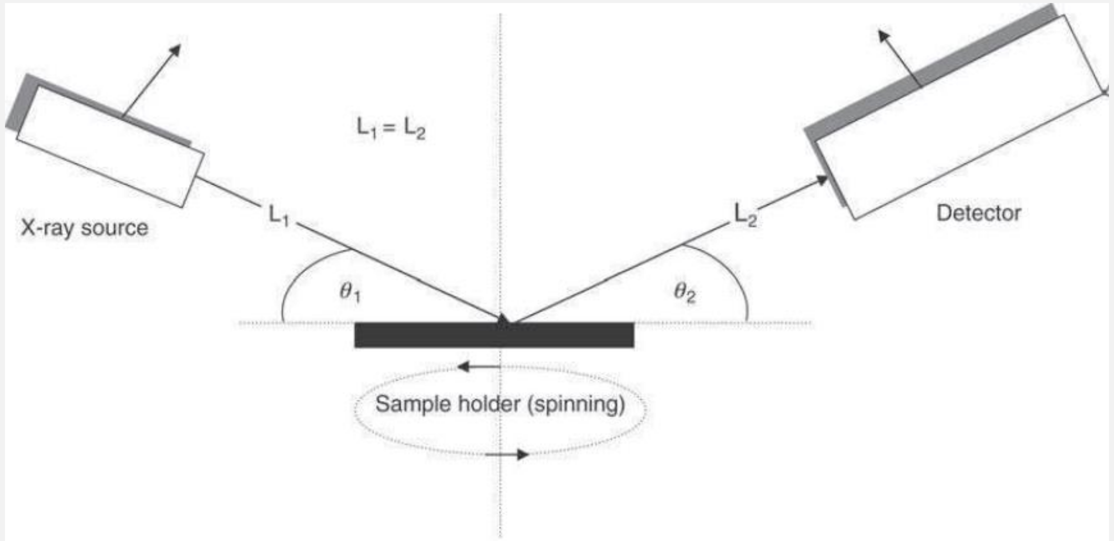
- Other data can be derived from single-crystal data, such as the **true density**, the calculated **XRPD pattern** and the **morphology**. Together these data provide an absolute proof of structure that can be presented in investigational new drug (IND) and new drug applications (NDAs).
- Brittain (2000b) has presented an introductory paper on the use of single-crystal XRD (SXRD) to study **polymorphism and psuedopolymorphism**, and a good introductory text is that by Glusker et al. (1994). Clegg's (1998) primer on the subject is also a good starting place for the novice. Datta and Grant (2004) have reviewed the advances in the determination, prediction, and engineering of crystal structures. **X rays are short wavelength, high-energy beams of electromagnetic radiation between 0.1 and 100A** ° . X rays are generated when a beam of **electrons are accelerated against (usually) a copper target (anode) where the electrons are stopped by the electrons of the target element** and a **broad band of continuous radiation is emitted** (bremsstrahlung—braking radiation-white radiation), superimposed on which are discrete wavelengths of varying intensity (X rays). The former is due to **collisions between the electrons and the target**, and the latter is due to **ionization of the metal atoms, which lose an inner shell electron**.

XRPD

- X rays are **part of the electromagnetic spectrum** lying between UV and X rays, and they are expressed in **angstrom units (A°). Diffraction is a scattering** phenomenon, and when X rays are incident on **crystalline solids**, they are **scattered** in all directions. Scattering occurs due to **the radiation wavelength being in the same order of magnitude as the interatomic distances within the crystal structure**.
- **Bragg's law** describes the conditions under which **diffraction** will occur. Diffraction will occur if a perfectly parallel and monochromatic X-ray beam of wavelength l is incident on a crystalline sample at an angle y that satisfies the Bragg equation (equation 27)
- $$n\lambda=2d\sin\Theta$$
- where n is the order of reflection (an integer, usually 1), l the wavelength of X ray, d the distance between **planes in crystal (d-spacings), and ϴthe angle of incidence/reflection.**

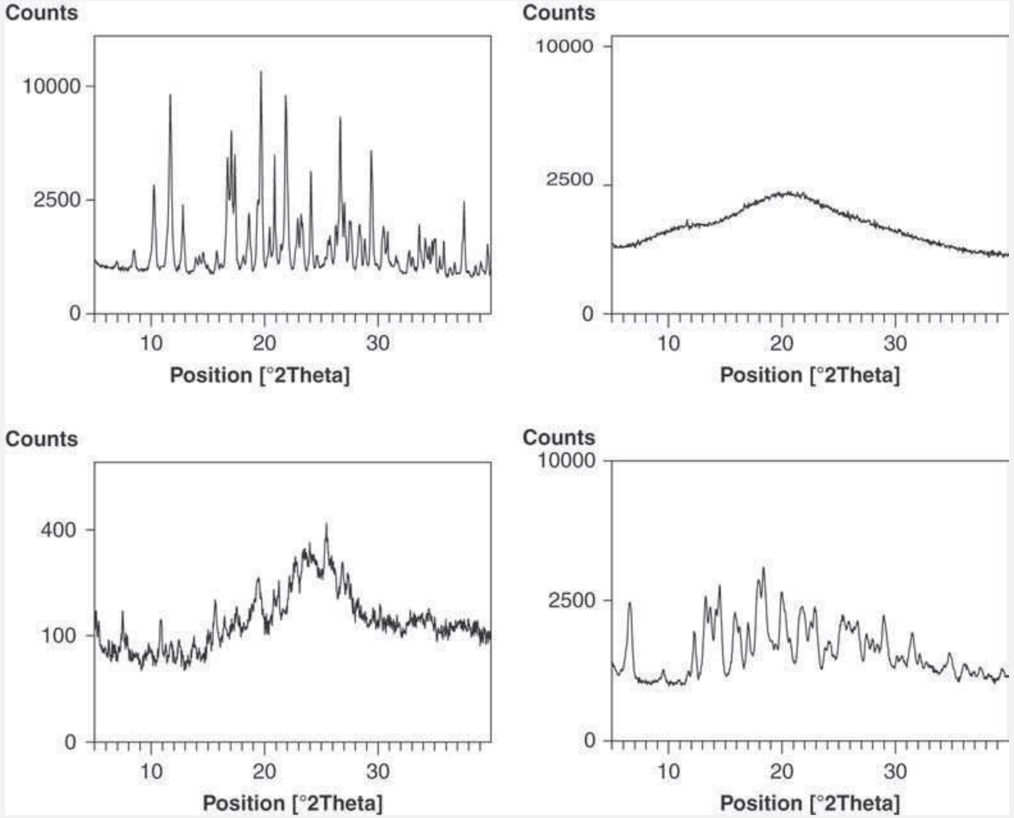
An X-ray diffractometer is made up of an X-ray tube generating X rays from, for example, Cu K α or Co source and a detector. The most common arrangement in pharmaceutical powder studies is the **Bragg-Bentano reflection**Θ - Θor Θ-2Θ configurations (Fig. 20).

The powder pattern consists of a series of peaks that have been collected at various **scattering angles**, which are related to d-spacings such that unit cell dimensions can be determined. In most cases, measurement of the d-spacings will suffice to positively **identifying a crystalline material**. If the sample does not **show long-range order, that is, it is amorphous**, the X rays are incoherently scattered leading to the **so-called halo pattern.**



Bragg–Bentano geometry used in XRPD experiments. Abbreviation: XRPD, X-ray powder diffraction

In addition to amorphous phases of a compound, the existence of disordered nanocrystalline phase should be included (Bates et al., 2006). Those samples that exhibit continuous peak broadening are nanocrystalline in contrast to amorphous phases that do not show this effect. The observation of glass transition during DSC analysis of a sample would be sufficient confirmation to distinguish it from a nanocrystalline sample



Typical XRPD patterns of crystalline, amorphous, and partially crystalline forms.

EXPLORING SHORT- AND MEDIUM-RANGE ORDER SPECTROSCOPIC TECHNIQUES

- Mid-Infrared Spectroscopy
- Near-IR Spectroscopy
- Terahertz Spectroscopy
- Solid-State Raman Spectroscopy
- Solid-State NMR

SOLID-STATE RAMAN SPECTROSCOPY

- Raman spectroscopy, **as with IR spectroscopy, provides information on quantized vibrational energy levels in a molecule, with subjected incident radiation being scattered by the sample**. Raman spectroscopy relies on **molecular polarizability**; a bond must be anisotropically polarizable for the vibration of the bond to be Raman active.
- As with IR spectroscopy, the Raman spectrum provides **a unique fingerprint** of the compound being studied. Since the method is based on the **vibrations** of the molecule, it is sensitive to small changes in the molecular structure (such as conformations) that affect vibrational behavior. As an example of its use, Variankaval et al. (2000) examined the **polymorphs of b-estradiol**. A and B were easily distinguishable; however, C and D were almost identical. Maherns et al. (2005) have provided a method of analysis based on descriptive statistics and analysis of variance (ANOVA) methods to address these subtle differences in the Raman spectra of polymorphs.

- Usually the Raman spectrum is obtained by collecting backscattered laser light from a powder or formulation. Bolton and Prasad (1981) listed the advantages (griseofulvin and its chloro- and bromoform solvates) as follows:

1. No special sample preparation is involved.
2. **Small** amounts of material are required.
3. Short data collection **time** period is required.
4. Thermal, for example, **desolvation** studies can be carried out.
5. Low-level laser light can be used that permits work on **highly colored** species.

CHARACTERIZATION

- **XRPD**
- **SPECTROSCOPIC**
- NMR
- IR
- Raman……….
- **CRYSTAL MORPHOLOGY ASSESSMENT**
- **THERMAL**

- Please look at

Preformulation Investigations using Small Amounts of Compound as an Aid to Candidate Drug Selection and Early Development Gerry Steele and Talbir Austin

AstraZeneca R&D Charnwood, Loughborough, Leicestershire, U.K.

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