Polymorphism: An Overview

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Keywords
Polymorphism, phase transition, intermolecular interactions, monotropes, enantiotropes.

It is quite common for the same material to crystallize in different arrangements of the molecules in the crystal. This phenomenon is referred to as polymorphism and it happens for elements, pharmaceuticals, biological compounds and proteins. In this article we present a brief review of polymorphism, stressing its physical aspects, types and applications. We discuss why it is important to understand the phenomenon, and the need to develop ways of controlling it. We also outline some legal issues related to it.

The gross property differences between graphite and diamond is known even to a high school student: In fact, this example is taught as a typical case of how structure determines properties. Chemists were cognizant of many such examples of structure – property relationships even while chemistry was in its incipient stage. Today, most students of science would have deeply imbibed this sentence "Graphite and diamond are allotropes". They are aware that elements have allotropes and this phenomenon is defined as allotropism. What about compounds? The present article discusses the phenomenon in compounds, scientifically referred to as polymorphism.

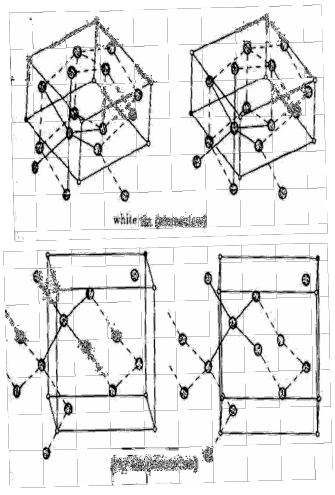
'Polymorphism' comes from the Greek word, *Polus* = many and *morph* = shape. Thus it is defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements or conformations of the molecules in the crystal lattice. It essentially means that in different polymorphs, the same molecule exists in different ways. If this difference is because of packing, it is termed as *packing polymorphism* and if it is due to difference in conformation, it is called *conformational polymorphism*. As a result of polymorphism, molecules have different arrangements in the unit cell of its crystal and thus display different physical properties. These include different packing

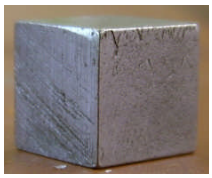
properties, thermodynamic properties such as solubility, free energy, melting point, etc., spectroscopic properties, kinetic properties such as dissolution rate, stability, and mechanical properties such as hardness, compatibility, tableting, tensile strength, etc. Polymorphism is very important in those areas of chemical research where full characterization of a material has a pivotal role in determining its ultimate use, e.g., in pharmaceutical, pigment, agrochemical, explosive and fine chemical industries. It is interesting to note that polymorphism has left an impression even on the history of our world (see *Box* 1).

The particular advantage of polymorphism is that the chemical identity of the material remains unchanged from one polymorph to another, so that a direct correlation between activity and solid state structure may be made. Why does the same chemical, say paracetamol, exist in different ways in the solid state? The answer is not simple but it is definite that their free energy difference cannot be huge. In fact, it is just 0.5 to a maximum of about 8 kcal/mol. So some forms

Box 1. Phase Transition in Tin

Polymorphism has a link with Napoleon Bonaparte, the French military and political leader. In the chilling winter of 1812, the highly decorated and shining buttons on the uniform of Napoleon's soldiers crumbled to dirty grey and the soldiers believed that it is the wrath of God; their morale became so low that they faced a pathetic defeat at the gates of Moscow.





β-Tin or 'white' tin stable above 18° C.
Tetragonal, $I4_1/amd$
a = b = 5.832, c = 3.182 Å
Metallic

Phase transition

α-Tin or 'grey' tin stable below 18° C.
Cubic, Fd3m
a = b = c = 6.489Å
Non-metallic

The scientific reason for the crumbling of the buttons is interesting. At subzero temperatures of Moscow, the metallic white tin underwent a polymorphic transition to the stable but nonmetallic grey tin, thus reducing the decorum of the mighty soldiers.

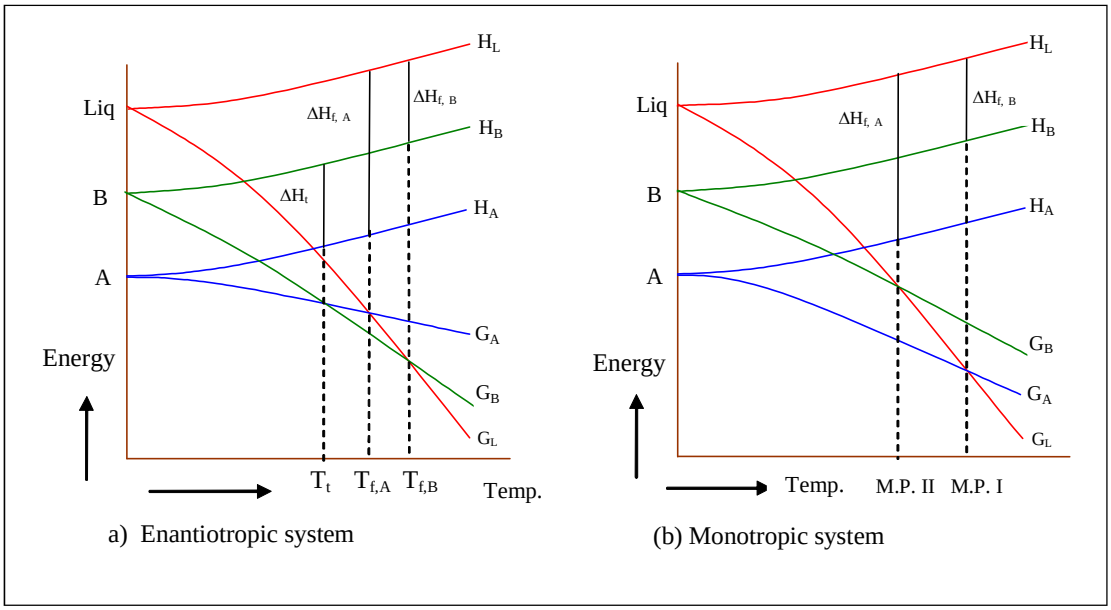
are slightly unstable compared to others and we can have a functional classification based on this factor.

Types of Polymorphism

Polymorphs are categorized into two types, monotropes and enantiotropes, depending upon their stability with respect to the range of temperatures and pressures. If one of the polymorphs is stable (i.e., has lower free energy content and solubility) over a certain temperature range and pressure, while the other polymorph is stable over a different temperature range and pressure, then the two polymorphs are said to be enantiotropes (*Figure* 1a). On the other hand, sometimes only one polymorph is stable at all temperatures below the melting point, with all the other polymorphs being unstable. These polymorphs are said to be monotropes (*Figure* 1b).

Both types of polymorphs can be easily understood by looking at the graphs. In *Figure* 1a, polymorph A is stable below temperature T_t (transition temperature), having lesser free energy G_A than polymorph B. But as the temperature increases and becomes more than T_t, free energy G_B of polymorph B becomes less than G_A; thus polymorph B becomes more stable than polymorph A. This gives rise to an enantiotropic system of solid phases. For an enantiotropic system, a reversible transition can be observed at a definite transition temperature at which the

Figure 1. Variation of energy with temperature for enantiotropic and monotropic systems. Curves H_A, H_B and H_L are for enthalpy, whereas $\Delta H_{f,A}$ and $\Delta H_{f,B}$ represent enthalpy of fusion and ΔH_t represents enthalpy of transformation.



free energy curves cross, before the melting point is reached. Whereas in *Figure* 1b, the polymorph A has its free energy less than the other polymorphs throughout the range below the melting point. For a monotropic system, the free energy curves do not cross, and therefore no reversible transition can be observed below the melting point; the polymorph with higher free energy curve and solubility is the unstable polymorph. Generally, it is possible to distinguish between monotropes and enantiotropes from their heats of fusion. An endothermic polymorphic transition indicates enantiotropes whereas an exothermic one indicates monotropes. Other than Differential Scanning Calorimetric (DSC) analysis, there are a number of efficient ways to characterize polymorphs (see *Box* 2).

We all know that millions of molecules must aggregate to form a crystal. In this process, if there are energetically viable alternate pathways, molecules may choose any of these ways depending

Box 2. Characterization of Polymorphs

Characterization of polymorphs is of utmost importance and there are different analytical methods ranging from simple melting point determination to complete structural determination through single crystal X-ray crystallography. Other procedures like studying the morphology of crystals by microscopic methods, observing changes in crystal forms with temperature, phase transition by thermal methods, interpreting molecular motion and chemical environment by the use of vibrational spectroscopy and solid state NMR are used depending upon the information sought. Crystallographic methods include both single crystal X-ray diffraction as well as powder X-ray diffraction. A successful single crystal X-ray diffraction study can provide unambiguous atomic positions and complete structural information, but obtaining a single crystal suitable for this study becomes often the bottleneck. In such cases, powder X-ray diffraction studies using microcrystalline samples become a major tool. In fact, it has become routine to take powder diffractograms to ascertain the solid state nature and purity of every batch of synthetic drugs.

Another quick and efficient method is to study the crystal morphology by optical microscopy. As unit cell repetition leads to crystal formation, this feature is reflected in the outer appearance of crystals that can be observed by simple hand lens or microscope. Further, a detailed study can be performed using polarizing optical microscopy, electron microscopy and thermal microscopy. The third important method, which is widely used in pharmaceutical industries for characterization of polymorphism, solvation, purity, degradation and drug compatibility, is thermal analysis, which includes Thermogravimetry, Differential Thermal Analysis (DTA) and Differential Scanning Calorimetry (DSC). The study of molecular motions by use of vibrational spectroscopy is also sometimes employed in the characterization of polymorphs. This method includes infrared absorption spectroscopy and Raman spectroscopy. Nowadays solid state NMR is also used for characterization. It studies the chemical environment of the nuclei which is different in polymorphs because of magnetic non-equivalence. Resonance peaks for the magnetically nonequivalent nuclei will differ in different polymorphs and can yield very useful information.

upon the crystallization conditions This leads to the different polymorphs. Thus formation of a large enough aggregate (i.e., nucleation) is an important step in the formation of a polymorph.

Nucleation of Polymorphs

Consider the simple case of evaporation of a sugar solution kept in a beaker. As time passes, more and more molecules of water leave the beaker progressively concentrating the solution and at a stage (very near to saturation but above it) many sugar molecules come closer to form aggregates. These will have their characteristic curve of change in Gibbs free energy vs. aggregate size as shown in *Figure* 2. The Gibbs free energy difference between the new phase (that is, the aggregate) and the old phase (that is, the sugar solution) is composed of two terms: (1) The difference in Gibbs free energy between the two phases in macroscopic quantities (usually called the volume term, ΔG_v), and (2) the difference in Gibbs free energy caused by the interfacial energy (called the surface term, ΔG_s). The total free energy cost, ΔG_{tot}, of forming a nucleus of a spherical crystal of radius r in a liquid (here sugar solution) is the sum of these two:

$$\Delta G_{tot} = \Delta G_v + \Delta G_s = \frac{4}{3}\pi r^3 \rho \Delta\mu + 4\pi\rho^2\gamma,$$

where γ is the interfacial free energy, ρ is the density of the saturated solution and $\Delta\mu$ is the chemical potential difference between the new phase and the old phase. The volume term, ΔG_v, varies as r^3 and is negative. The surface term, ΔG_s, is always positive and varies as r^2. Examination of *Figure* 2 shows that till a critical size r^* is reached, the Gibbs free energy change increases, and then onwards ΔG decreases as a result of which an aggregate with $r > r^*$ (i.e., a nucleus) can spontaneously grow. However molecules can also leave the aggregate leading to complete dissolution of the aggregate (both r and ΔG decrease). The surviving aggregates may not be similar, depending upon how the molecules arrange themselves; they can be of different

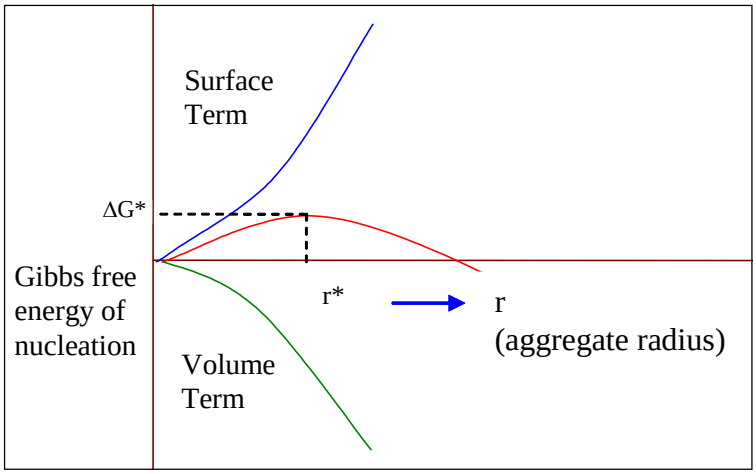


Figure 2. Plot of Gibbs free energy G of molecular aggregates vs. size of the aggregates. The red curve represents total free energy. The activation free energy ΔG* is the free energy at r*.

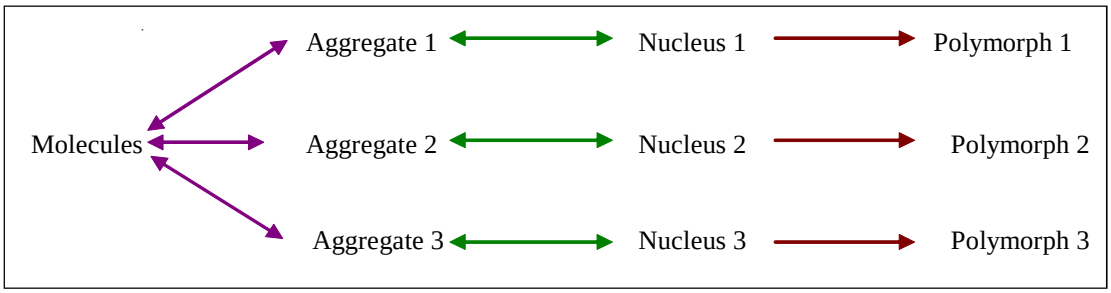


Figure 3.

types, which compete among themselves to grow in size and to give a particular nucleus. The complete event may be visualized as in *Figure* 3.

The aggregate present in largest numbers will form the nucleus that grows further to give a particular polymorph. It is this primary crystallization process involving formation of critical nuclei which gives rise to polymorphs. This is a general mechanism that explains the usual situation of crystallization in which polymorphs are crystallized depending upon the conditions prevailing.

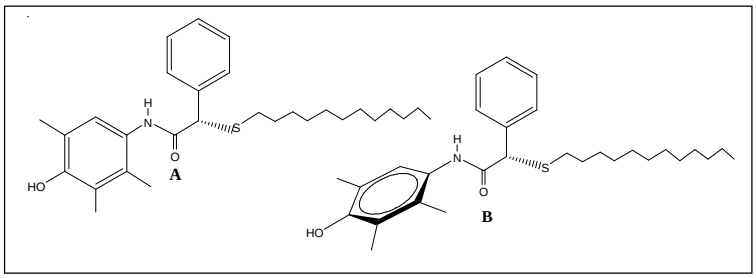
We have learned that "who (aggregates) survives succeeds". What are the factors that determine such a survival? There are many. Molecular conformations, hydrogen bonding, packing arrangements and lattice energies are a few to cite. But, in forming an aggregate which grows and to a nucleus then to a specific polymorph, the role of intermolecular interactions is paramount.

Role of Intermolecular Interactions

The intermolecular interactions like van der Waals interactions, coulombic interactions, hydrogen bonding, and steric repulsions play an important role in determining the arrangement of molecules in a crystal. Even the cooperative action of very weak interactions like C-H…O hydrogen bonding, π…π, X…X (X = halogen), and C-H…π interactions can significantly contribute towards stabilizing a specific molecular arrangement in a crystal. These weak interactions are strong enough to cause changes in torsion angles, thereby giving different conformations and a particular molecular conformation which is near the most stable equilibrium conformation is often stabilized. As these interactions are weak and various molecular conformations can have nearly the same energy, the molecules can crystallize in different crystal forms.

Molecules capable of possessing torsional degrees of freedom give rise to different conformations that may be preserved in different crystal forms. The existence of different conformations

Structure 1. Two polymorphs of eflucimibe: In Form A, the –OH bearing the aromatic ring is in the plane of the paper, whereas in Form B it is out of the plane.



of the same molecule in different polymorphic modifications is termed conformational polymorphism. Among polymorphic modifications, conformational polymorphs have the major share. Sometimes polymorphs crystallize simultaneously when conformations are very close energetically and structurally, and nucleation rates are of the same order of magnitude. If two or more polymorphs crystallize under identical conditions, then it is known as concomitant polymorphism.

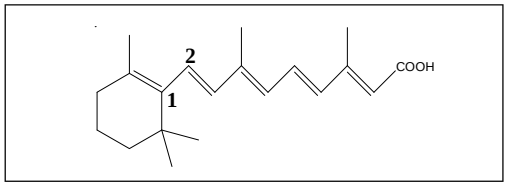
Although there is a plethora of examples of conformational polymorphism, two of them, which are shown here, are biologically active compounds. One of the recent examples is 'Eflucimibe' a new acyl-coenzyme A: Cholesterol O-acyl transferase (ACAT) inhibitor drug. This drug crystallizes simultaneously in two polymorphic forms A and B which differ by a conformational change of the phenol group in the crystal lattice, that occurs through a rotation of the bond connecting the amide nitrogen and aromatic ring (*Structure* 1).

A second example is that of the Vitamin A acid, a member of the family of visual pigments. It has been reported in two crystalline forms: triclinic and monoclinic.

In Vitamin A acid, the exocyclic bond can acquire two conformations, S-*cis* and S-*trans* by rotation about the bond connecting atoms 1 and 2 (*Structure* 2). Indeed, it is observed that the triclinic form exhibits S-*cis* conformation about the exocyclic bond and has a non-planar conformation, whereas the monoclinic form is S-*trans* about the exocyclic bond and is much more planar in nature.

It is also worth mentioning that so far the number of polymorphs of a given compound has reached double digits. The maximum of ten polymorphs for 5-methyl-2-[(2-nitrophenyl) amino]-3-thiophenecarbonitrile commonly known as ROY (*Figure* 4) has been obtained. The name ROY comes from the Red, Orange and Yellow color of its polymorphs.

Structure 2. Vitamin A acid: S-trans isomer.



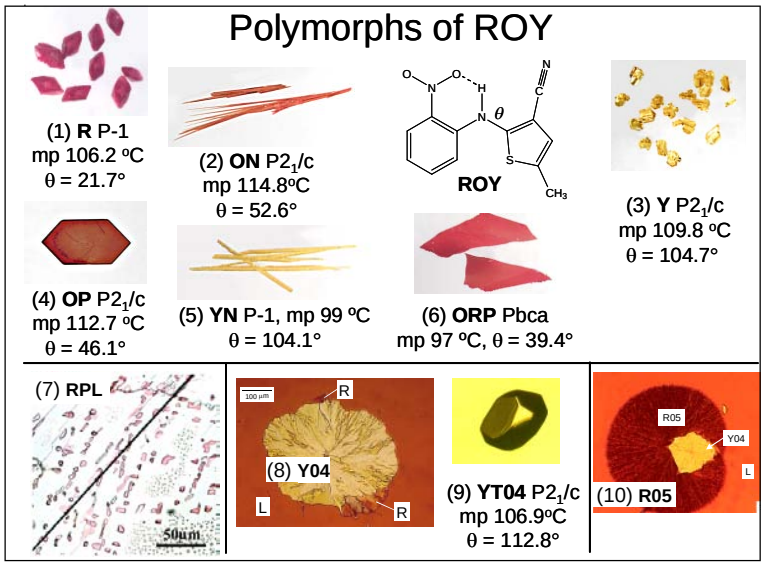


Figure 4. 5-Methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile has been crystallized in ten polymorphs: The structure of the molecule is shown in the figure. The system has been named ROY for its red, orange, and yellow crystal colors. The different polymorphs are named as, yellow prisms (Y), red prisms (R), orange needles (ON), orange plates (OP), yellow needles (YN), orange-red plates (ORP), and red plates (RPL). The figure is reproduced with permission from the web pages of Prof. Lian Yu of University of Wisconsin-Madison (see: http://www.pharmacy.wisc.edu/SOPDir/PersonDetails.cfm?&ID=32)

In the following we discuss polymorphism exhibited by various inorganic, pharmaceutical and biological compounds.

Polymorphism in Elements and Inorganic Materials

Polymorphism in the case of elements is termed as 'Allotropism'. The simplest example, as discussed in the beginning, is that of carbon to which the new entrants are fullerenes and carbon nanotubes. Other examples include phosphorous (P_4) in cubic (white), monoclinic (purple) and orthorhombic (black) forms; Sb in rhombohedral (grey), cubic (grey), hexagonal (metallic) forms. Boron exists in different allotropes such as tetragonal, rhombohedral, orthorhombic, monoclinic and hexagonal forms. A large number of other elements like actinides and molecular elements exist in polymorphic forms which interconvert with change of conditions like temperature and pressure. A typical example is that of uranium:

$$\text{Uranium (U)orthorhombic} \rightarrow \text{(U)tetragonal (941 K)} \rightarrow \text{(U)bcc (1047 K)}.$$

Polymorphism is also abundant in inorganic minerals that are known by their common names,

e.g., ZnS is known in forms referred to as wurtzite and sphalerite; $CaCO_3$ as calcite, aragonite and valerite; TiO_2 is known as rutile, brookite and anatase.

Polymorphism in Pharmaceutical Compounds

Most drugs are formulated and marketed in crystalline form. Many of the drug molecules are highly functionalized and can self-organize in several ways in the solid state with nearly the same lattice energies. Often solvent may be incorporated in the crystal lattice. These features and the conformational flexibility of molecules are the primary driving forces for the existence of crystal polymorphism in active pharmaceutical ingredients (APIs). This makes the study of polymorphism and crystallization of pharmaceutical compounds highly important. Nowadays, research on polymorphism and material properties of active drug compounds and excipients (excipients are ingredients included in a pharmaceutical preparation for the purpose of improving its physical qualities) is an integral part of drug development. The knowledge of solid-state properties in an early stage of drug development helps to avoid manufacturing problems, to fine-tune the performance of drugs and provides space for innovations.

Drugs that were previously known to exist only in a single form are now shown to have various polymorphic forms. This has perplexed pharmaceutical companies and now they have to investigate crystal polymorphism in order to optimize the physical properties of a pharmaceutical solid before the drug development. A large number of pharmaceutical compounds show polymorphism and books like *Polymorphism in Pharmaceutical Compounds* [1] are written to disseminate the knowledge within the scientific community. Some of the important examples are cited here to show its ubiquity in this area.

Paracetamol, also known as acetaminophen, is the most widely used antipyretic (fever suppressant) and analgesic (pain killer) in the world. Though the drug seems to be simple, it has been shown to exist in two polymorphic forms. One is monoclinic Form-I ($P2_1/n$), which is marketed whereas Form-II is orthorhombic (Pbca). Similarly, Famotidine which is an excellent histamine H_2 receptor antagonist is also found to exist in two different polymorphic forms, metastable polymorph B and stable polymorph A. Piroxicam, a nonsteroidal, anti-inflammatory drug widely prescribed all over the world exists in three forms I, II and III.

Another important example is Ritonavir. It is a novel protease inhibitor for Human Immunodeficiency Virus (HIV), the causative agent of Acquired Immune Deficiency Syndrome (AIDS). This drug is marketed as Norvir. Launched in 1996 and distributed for about 18 months without trouble, its manufacturing company Abbott observed an unexpected occurrence. The final product did not show dissolution and the drug was precipitating. After considerable investigations it was revealed that this was because of a new thermodynamically more stable and less

soluble polymorph Form-II. Surprisingly, the company's attempt to formulate Form-I thereafter turned out very difficult (perhaps the exact conditions could not be reproduced) and put the company almost into a market crisis. The drug is now often quoted as an example in pharmaceutical industries to show the importance of polymorphism in this field.

The last example discussed here is of Norfloxacin. It is the widely used synthetic broad-spectrum antibacterial fluoroquinolone for the treatment of prostate and urinary tract infections. This drug exists in two anhydrous polymorphs (A and B), an amorphous form and several hydrated forms. Of the two anhydrous polymorphs, Form B is the most stable at room temperature. But the commercial sample of norfloxacin used is the Form A, which is metastable at room temperature.

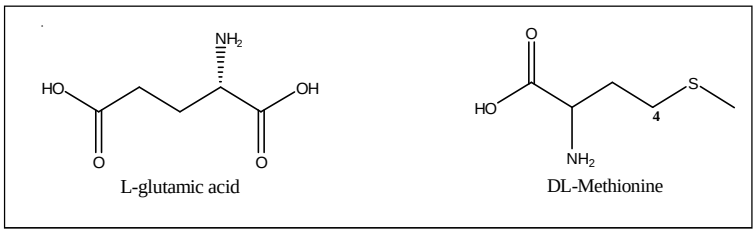
All these examples clearly show that it is highly important to make the required polymorphic form, as the other form may not show the desired effects. Usually the form that is most stable is preferred in market formulation as the metastable form may transform to other stable forms. But it is a universally accepted rule that the metastable form has higher solubility than the stable form and this form converts into the stable form as a result of spontaneous change but the reverse never happens. Thus, whenever possible, metastable forms having high solubility that can survive for years without changing to the stable form are selected for formulation. This means that, forms that have considerable activation barrier in moving from metastable state to stable state would be selected. That is, careful evaluation of both thermodynamic parameters (tendency toward formation of more stable polymorphs) and kinetic parameters (which lead to formation of metastable polymorphs) is of high importance in crystallization process of such compounds.

Polymorphism of Proteins and Other Biological Compounds

Polymorphism is not confined to the small molecule regime; large molecules like proteins can also exist in polymorphic forms. Proteins have tertiary and quaternary structures which show various interactions and because of which they have a particular structure. Proteins can crystallize in different crystal forms as they can have different conformations of peptide units. One of the classic examples is lysozyme. It is known to crystallize in six different forms that differ in water content, nature and amount of anions and difference in packing arrangements. It exists in triclinic, monoclinic, orthorhombic, trigonal, tetragonal and hexagonal forms. Another important example is hemoglobin, which is known in monoclinic, orthorhombic and tetragonal modifications. Many other protein molecules are also known to exist in different forms, like glutathione-S-transferase from rat liver (three polymorphic forms).

Not only proteins, but its monomer amino acid units also show polymorphism. As these amino

acid molecules can have different conformational preferences, they crystallize in different forms e.g., L-glutamic acid is known in two forms, *á* and *â*. Both the forms are orthorhombic ($P2_12_12_1$) and crystals have distinct rhombic and needle-like morphologies.



Structure 3.

Similarly, DL-methionine crystallizes in two forms, *á* and *â*, which differ in torsion angle about the biologically important C(4)-S bond. Even the simplest amino acid glycine has three polymorphs i.e., *á*, *â* and *ã*. The thermodynamically stable *ã*-form crystallizes in $P3_1$, whereas the less stable *á*-form in $P2_1/n$ space group and *â*-form appears in $P2_1$ space group.

From these discussions it is clear that polymorphism has affected many research/commercial avenues. As this field is growing and expanding, especially in drug and pharmaceutical fields, legal issues have also cropped up (see *Box* 3).

Control of Polymorphism

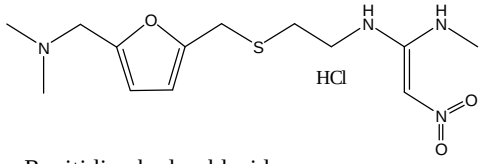
Sometimes an undesired polymorphic form persistently appears, (like the case of Norvir) and because of this, the commercial form may not be available causing great loss. Therefore, it becomes necessary to develop methodologies to get the commercially preferable form and prevent the appearance of the other one. Crystal engineering can provide answers to this question of control of polymorphism. It is the application of concepts of supramolecular chemistry to the solid state to engineer the molecules in a desired way. In a crystal, molecules are held together by a composite interplay of various interactions and understanding them both by experiment and theory would also provide the means to control polymorphism. In addition, by studying the polymorphic crystal structures of a compound, various additives can be selected which mimic the conformation of a given molecule. These additives can be used to control polymorphism by inhibiting the growth of undesired polymorphic forms, thus crystallizing the required one. For example, trimesic acid and transglutonic acid, which conformationally mimic *â*-form of glutamic acid, selectively inhibit the crystallization of the *â*-form and stabilize the metastable *á*-form of glutamic acid.

While some chemists are finding means to suppress polymorphism, to many others, the search for new polymorphs with novel properties is a fascinating though arduous task. Either way, new insights are gathered in this fascinating field and accedes with the view of McCrone who

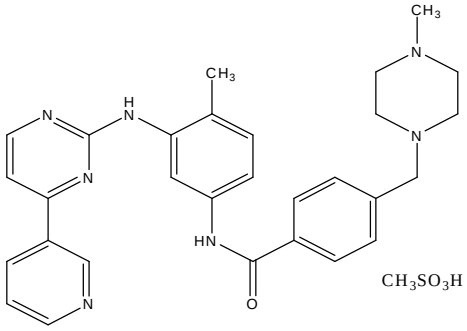
Box 3. Legal Aspects Associated with Polymorphism

The commercial arena, especially the pharmaceutical companies, has recently faced many complex legal issues arising from polymorphism. Most of these issues are concerned with patent cases.

The most famous case is the Zantac patent case, which is concerned with the solid-state form of Glaxo's major drug, ranitidine hydrochloride, for the treatment of peptic ulcers. This is a polymorphic drug capable of adopting two crystal structures. A process resulting in the crystallization of Form I was patented in 1978; two years later a more stable crystalline Form II appeared which was also patented and which subsequently became the active ingredient for Zantac formulations. When the patent expired in 1995, other generic companies also came in this field and subsequent legal battles resulted.



Ranitidine hydorchloride



Imatinib mesylate

The second case is of Novartis patent case, which was dealt in Madras High Court. This case is about a life-saving cancer drug 'Gleevac' containing imatinib mesylate. Gleevac offers a cure to the life threatening form of the cancer 'chronic myeloid leukemia'. Novartis invented imatinib in 1992 and patented it in 1993 in US and other countries. The company applied for a patent in India in 1998 for β-crystalline form of imatinib mesylate, which led to a legal scrutiny of Patents Act 1970.

suggested that virtually 'every compound has different polymorphic forms and that, in general, the number of forms known for a given compound is proportional to the time and energy spent in research on that compound'.

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