4.1 Introduction

Crystalline solids have regular arrangements of molecules that repeat in three dimensions, whereas amorphous solids lack the long-range order present in crystals. These differences in the long-range periodicity of the molecules result in substantially different physical and chemical properties of crystalline and amorphous solids in pharmaceutical substances.\textsuperscript{1} Although amorphous solids often have desirable properties such as faster dissolution rates than their crystalline counterparts, they are not marketed as widely as crystalline forms because of their lower physico-chemical stability and their innate tendency to crystallize.\textsuperscript{1} Most of the marketed pharmaceuticals are preferred in their crystalline stable forms. The arrangement of the molecules in a crystal determines its physical and chemical properties, i.e. physicochemical profile of the particular drug. At the supramolecular level, further variation in the crystalline forms of a solid compound is possible. They may either exist as single molecular entities or multi-component species. As single molecular entities, molecular solids can show polymorphism, which is defined as the ability of a substance to exist in two or more crystalline phases that have different arrangements and/or conformations of molecules in the crystal lattice without undergoing changes in its chemical composition.\textsuperscript{2} Multi-component crystals include salts, hydrates, solvates and cocrystals of the drug molecule where a second component (counter-ion, water, solvent or a cocrystal former respectively) is incorporated into the crystal lattice. Sometimes multi-component crystals can also exhibit polymorphism as discussed in chapter 5.

A crystalline phase is created as a consequence of molecular aggregation processes in solution that lead to the formation of nuclei, which achieve a certain size during the nucleation phase to enable its growth into macroscopic crystals. The factors influencing the rate and mechanism by which crystals are formed are solubility, supersaturation, diffusivity, temperature, and reactivity of the surfaces towards nucleation. The various forces responsible for holding the organic crystalline solids together are mainly hydrogen bonds and other non-covalent interactions. The subtle differences in the interplay of
these interactions may lead to the formation of polymorphs. According to Ostwald’s rule\textsuperscript{3}, the system moves to equilibrium from an initial high-energy state through minimal changes in free energy. Therefore the structure that crystallizes first is one which has the lowest energy barrier (highest energy, kinetic, metastable form). This form would then transform to the next lower energy polymorph and so on until a thermodynamically stable state is achieved. The kinetics under which this transformation occurs, however, are system specific. Therefore, the existence of a more stable polymorph does not necessarily imply that a metastable polymorph cannot be produced. This can be easily understood by considering the allotropes or polymorphs of elemental carbon i.e. graphite and diamond. The former is the thermodynamically preferred crystalline form, but kinetic factors in particular high energy activation barrier make the rate of transformation from diamond to graphite infinitely slow.\textsuperscript{4} Furthermore, the structural analysis of various polymorphs at different stages would help us to understand the nature and the extent of self-assembly process that take place during crystallization.\textsuperscript{5} In this context crystallization may be considered as a supramolecular reaction and like all chemical reactions it is controlled by kinetic and thermodynamic factors.\textsuperscript{5d}

The structural differences between polymorphs originate through two mechanisms, namely, packing polymorphism and conformational polymorphism (Figure 1).\textsuperscript{6,12b} Packing polymorphism is a mechanism by which molecules that are conformationally rigid can be packed into different three-dimensional structures. For example, the anti-bacterial Nitrofurantoin is a dimorphic substance.\textsuperscript{7,8} The form 1 contains amide dimer N–H···O hydrogen bonds, whereas form 2 arises from different packing arrangements of molecules connected by infinite chain of N–H···O hydrogen bonds or an amide catemer. Two polymorphs of pyrazine-N,N\textsuperscript{1}-dioxide discussed in the chapter 3 are packing polymorphs. Pyrazinamide, Carbamazepine, Paracetamol are other examples of packing polymorphism.\textsuperscript{7} Conformational polymorphism (name coined by Corradini)\textsuperscript{9} on the other hand arises from different conformers that can pack into different crystal structures. For example, Ritonavir\textsuperscript{10} was previously known to exist in form I with anti amide conformation. After two years of the launch of the product, a second form II with syn amide conformer was accidentally crystallized. This is presumed to be obtained due to an increase in the level of carbamate impurity which has syn amide conformer (Figure 2a and 2b). ROY molecule,\textsuperscript{11a,11b} 4,4-diphenyl-2,5-cyclohexadienone,\textsuperscript{11c} Fuchsones,\textsuperscript{11d}
Benzidine,\textsuperscript{11e} Venlafaxine hydrochloride,\textsuperscript{11f} Bis\((p\text{-}toly)\) ketone \(p\)-Tosylhydrazone,\textsuperscript{11g} Phenobarbital,\textsuperscript{11h} and Barbital\textsuperscript{7} are some examples of conformational polymorphs. Two recent reviews by Nangia\textsuperscript{12} are exclusively dedicated to the subject of conformational polymorphism. In general, the differences in packing arrangements invariably affect the molecular geometry and, conversely, the differences in molecular geometry causes the molecules to pack differently. As a result, most examples of polymorphism in organic crystals have a mixed origin and exhibit differences in both the conformation and packing arrangement of the constituent molecules.

**Figure 1.** Schematic representation of polymorphs \(i\) and \(ii\) for a rigid molecule, (b) a conformationally flexible molecule has a greater number of packing arrangements, \(iii\text{-}vi\), and (c) two symmetry independent molecules \((Z' > 1)\) in conformational isomorph \(vii\). Figure taken from A. Nangia, *Acc. Chem. Res.* \textbf{2008}, \textit{41}, 595.
Figure 2a. The syn and anti amide conformers of ritonavir in form I and II. The accidental discovery of form II with syn amide conformer is presumed to be obtained due to an increase in the level of carbamate impurity which has fixed syn amide conformer.

Figure 2b. Ritonavir form I contains non-hierarchic hydrogen bonds (amide NH to carbonyl, alcohol OH to N of thiazole ring), whereas all the strong hydrogen bond donors and acceptors are matched in form II (alcohol OH to carbonyl and amide dimer).

Various analytical methods\(^1\) are being currently used to characterize the crystalline form of a drug. The most valuable piece of information about the crystalline solid is the molecular structure, which is determined by single crystal X-ray diffraction. Powder X-ray diffraction provides a “fingerprint” of the solid phase which is used to differentiate between various crystalline phases and used for the determination of crystal structure at high resolution.\(^{11\text{th}}\) Other spectral methods, such as Fourier Transform Infrared spectroscopy (FT-IR), Fourier Transform Raman scattering spectroscopy (FT-Raman), Solid-State Nuclear Magnetic Resonance spectroscopy (SSNMR) are used for further characterization. Of special significance are thermal methods\(^{13}\) to establish the kinetic/thermodynamic stability relationships between the polymorphs. These are Differential Scanning Calorimetry (DSC), Thermo Gravimetric Analysis (TGA), and Hot Stage Microscopy (HSM).
There has been substantial research activity in the field of polymorphism driven by both fundamental scientific discovery and their importance across a wide range of industries like agrochemicals, dyes, foodstuffs and especially in the pharmaceutical industry. Recent books by Hilfiker, Brittain, Bernstein and Byrn highlight the importance of polymorphism in pharmaceuticals. There are numerous special issues of journals dedicated to this particular topic and several annual reviews and patents are published in the last decade. Despite its potential implications, the phenomenon of polymorphism is not always well understood, and even after many decades of research there is no consensus on what type of molecules exhibit polymorphism. Some molecules easily form polymorphs but some molecules like Benzoic acid do not show signs of polymorphism even after extensive research. In 2006 a new crystal form of Maleic acid was discovered during attempted co-crystallization experiments with Caffeine, 124 years after the first crystal form was studied. Similarly a second polymorph of Aspirin was obtained in the presence of an additive Levetiracetam. These experiments show that additives can induce the appearance of polymorphic forms. It has been reported that changing the crystallization conditions such as solvents and temperature, pseudoseeding, adding impurities, additives or polymer have yielded novel polymorphs in the literature. Of these methods, the induced nucleation or pseudoseeding with the adequate seed crystals of the form to be reproduced is the most straightforward and promising to get the desired polymorph by inhibiting the nucleation or the crystal growth of other undesired polymorphs. McCrone’s statement that ‘the number of forms known for a given compound is proportional to the time and energy spent in research on that compound’ appears to be prophetic today and validated by the discovery of many new polymorphs.

Since different inter and intramolecular interactions such hydrogen bonds, halogen interactions, $\pi$-stacking and van der Waals interactions are present in different crystal structures, different polymorphs will have different free energies and therefore exhibit different physical properties such as solubility, dissolution rate, density, heat capacity, crystal habit, melting point, thermal conductivity, optical activity, and particle morphology. These differences impact on drug formulation and processing, dissolution rates and ultimately bioavailability of the drug. Furthermore, stability presents a special concern. Because energy differences between polymorphs are usually small, form
inter-conversion is common. The phase transformations from a metastable to more stable form can affect the bioavailability and stability of the product drug.\textsuperscript{14} For example, about two years after FDA approval, the HIV protease inhibitor Ritonavir\textsuperscript{10} appeared as a previously unknown, thermodynamically more stable polymorph II with a different conformer and more number of stronger hydrogen bonds than the metastable form I (Figure 2b). The new polymorph caused the existing capsule product to fail its regulatory specifications, thus forcing removal of the capsule from the market until the product could be reformulated to meet the necessary performance criteria. The estimated loss incurred due to the accidental appearance of new polymorph and its post effects is nearly 250 $ million dollars to the Abbott laboratories.\textsuperscript{10} Therefore risks of marketing a drug product without awareness and recognition of the thermodynamically most stable form are very high.

In the absence of solvents and humidity, the thermodynamically stable polymorph is the only one that is guaranteed not to convert into another polymorphic form and it is most often chosen for the drug development. The disadvantage of the thermodynamically stable form is that it is the least soluble polymorph and as a result has the least bioavailability. The differences in the solubility of various polymorphs are typically lower than a factor of two.\textsuperscript{17c} Therefore, choosing the stable form with lower solubility is a small price to pay for the very large advantage gained with its absolute kinetic stability. In some cases a metastable crystalline phase may be purposely developed for the drug development when there is a significant increase in solubility and dissolution rate of metastable form (5 to 10 times) and thereby improving the absorption and/or bioavailability over the stable polymorph. Metastable form I is preferred than the stable form II of Ritonavir.\textsuperscript{10}

Another important issue with respect to polymorphism is that different crystal forms can be considered as a patentable invention.\textsuperscript{1,14,15} As such, Generic pharmaceutical companies are increasingly devoting their time and effort in searching for novel crystal forms in order to allow them to gain an early access into the market place, while the Innovator companies are equally giving their best to patent on all the polymorphs of a particular drug so as to extend their monopoly in pharmaceutical industry and to protect their product from generic competitors attack.\textsuperscript{30} The Food and Drug Administration
Polymorphism in…

(FDA) has strengthened regulation of the drug development process, requiring the identification and characterization of all possible polymorphs of a particular drug. Consequently, polymorph screening\(^{21}\), characterizing all the possible crystalline forms of drug and identification of the stable form\(^{22}\) has been regarded as an indispensable step in the early stages of drug development. Indeed, such an exhaustive polymorph screening led to the discovery of the thermodynamically stable form of anti-depressant Venlafaxine hydrochloride\(^{11f}\) in our laboratory by solid to solid phase transition at high temperature, which can not be obtained from usual solution crystallization techniques. Traditional methods to generate polymorphs include recrystallization from various solvents, changing the temperature and pressure, melt and sublimation crystallization, slurry conversion, and thermal microscopy. Recently, high-throughput (HT) polymorphism screening\(^{23}\) has been developed with the aim of comprehensively addressing form diversity.

A recent review\(^{24}\) of the Cambridge Structural Database (CSD) showed that only 5% of compounds are polymorphic. But its occurrence is more pronounced in drug molecules (30-50% in drug substances of \(<600 \text{ g mol}^{-1}\) molecular weight\(^{1,14,17f}\) as they contain functional groups that are good hydrogen bond donors/acceptors and are also conformationally flexible molecules. This combination makes for a good drug but it also leads to polymorphism. Polymorphs of antitumour drug Temozolomide (TMZ) and loop diuretic Furosemide (FMD) are chosen to study the influence of conformer changes on hydrogen bonding and crystal packing. The single bond rotations of amide moiety in TMZ and sulphonamide and furan ring torsions in FMD render conformational flexibility to the chosen molecules. As polymorphism in TMZ\(^{25b,c}\) and FMD\(^{26b,c}\) is well established in the literature by PXRD patterns and other spectroscopy techniques, current study was undertaken to characterize them by single crystal XRD. The main advantage with single crystal X-ray diffraction study is that the detailed information about conformer changes, hydrogen bonding and molecular packing is accurately known. A thorough polymorphic screening\(^{21}\) was carried out with the intent of obtaining novel polymorphs.
4.2 Polymorphs of Temozolomide

Scheme 1. Conformers of Temozolomide via rotation about the C_{amide}–C_{imidazole} bond.

Temozolomide (8-carbamoyl-3-methylimidazo[5,1-\textit{d}]-1,2,3,5-tetrazin-4(3\textit{H})-one, TMZ, 6) is an antitumor prodrug, active against malignant melanoma, which acts by water-assisted tetrazinone ring opening and DNA alkylation of the incipient cytotoxic form.\textsuperscript{25a} Only one crystal structure of TMZ is reported\textsuperscript{25b} in the space group \textit{P}2\textsubscript{1}/\textit{c} with unit cell parameters \(a = 17.332(3)\), \(b = 7.351(2)\), \(c = 13.247(1)\) \(\text{Å}\), \(\beta = 109.56(1)^\circ\). Nine unsolvated TMZ forms were disclosed in a recent US patent.\textsuperscript{25c} However, the structural origins of polymorphism in TMZ are not known because the various polymorphic forms were characterized and differentiated by powder X-ray diffraction (PXRD) and IR spectroscopy. As mentioned earlier, the accurate information about hydrogen bonding and molecular packing in crystal structures is available from single crystal X-ray diffraction. The presence of carboxamide and N-heterocyclic functional groups (tetrizine, imidazole) in the same molecule are believed to be favourable structural features for polymorphism because of additional possibility for N–H···N hydrogen bonding in addition to the usual N–H···O bond of the amide group. For example, tetramorphs of pyrazinamide\textsuperscript{27a} and dimorphs of isonicotinamide\textsuperscript{27b} display differences in N–H···O and N–H···N hydrogen bond synthons. In comparison, temozolomide has a more complex molecular structure than pyrazinamide and isonicotinamide and expected to be polymorphic. TMZ can undergo rotation about the C_{amide}–C_{imidazole} bond to give two conformers, \textit{A} and \textit{B}, both of which are stabilized by intramolecular N–H···N interaction with different N acceptor atom (Scheme 1).
4.2.1 Structural description and PXRD patterns of TMZ polymorphs

Polymorphs of temozolomide are named as polymorph 1, 2, 3, whereas they are designated as form I, II, III for furosemide polymorphs, in the order in which they are obtained. Crystallization of TMZ from common solvents such as EtOH, acetone and CH$_3$CN gave single crystals that matched with the unit cell of form 1 reported at room temperature (refcode DIPGIS10). TMZ polymorphs 2 and 3 were obtained during attempted co-crystallization experiments with carbamazepine (CBZ) and pyridine-N-oxide partners.

**TMZ polymorph 1, 6a:** It crystallizes in the space group $P2_1/c$ with two symmetry-independent molecules ($Z' = 2$). Both molecules adopt conformation $A$ that has an intramolecular N$_{amide}$–H···N$_{imidazole}$ bond of 5-member ring (Scheme 1). TMZ molecules hydrogen bond in the crystal structure via amide dimer synthon (N1–H1A···O3: 2.03 Å, 3.024(2) Å, 167.7°; N7–H7A···O1: 1.84 Å, 2.855(2) Å, 177.3°) between symmetry independent TMZ molecules and extend as helices down the [010] direction via N7–H7B···O4 (2.02 Å, 2.997(2) Å, 160.7°) and C6–H6B···O1 (2.28 Å, 3.279(2) Å, 151.5°) H bonds (Figure 3). Weak C–H···O, N–H···N and C–H···N interactions complete the crystal packing.

**TMZ polymorph 2, 6b:** Co-crystallization of TMZ with CBZ or 3-hydroxypyridine-N-oxide, with the intent of obtaining a 1:1 cocrystal, gave a second polymorph of TMZ in the space group $P2_1/n$. The crystal conformer is $A$ ($Z' = 1$). The amide dimer between inversion related TMZ molecules (N1–H1A···O1: 1.84 Å, 2.848(1) Å, 170.1°) extends via C4–H4···O2 (2.14 Å, 3.208(1) Å, 165.0°) dimers as a one-dimensional tape parallel to the [120] direction (Figure 4). There are helices of C6–H6A···O1 (2.42 Å, 3.442(1) Å, 155.3°) and C6–H6B···N2 (2.61 Å, 3.276(1) Å, 118.8°) interactions. Surprisingly the amide $anti$ N–H is not involved in conventional intermolecular hydrogen bonding in this crystal structure.
Figure 3. (a) The carboxamide dimer of crystallographic unique molecules (colored differently) in TMZ form 1. *Anti* N–Hs are involved in N7–H7B···O4 and N1–H1B···N11 bonds. (b) Helices mediated via N–H···O and C–H···O hydrogen bonds along the *b*-axis are connected by the amide dimer of symmetry-independent molecules.

Figure 4. (a) N–H···O and C–H···O dimers assemble in a tape motif in form 2. The *anti* NH of CONH$_2$ makes only intramolecular hydrogen bond in this crystal structure. (b) Helical arrangement of TMZ via C6–H6A···O1 interaction along the *b*-axis. (c) A larger helix is present via N1–H1A···O1 and C6–H6B···N2 hydrogen bonds.
**TMZ polymorph 3, 6c:** Co-crystallization of TMZ and CBZ gave block-like crystals that matched with previously discussed two monoclinic crystal structures (same unit cell parameters) and a few irregular morphology crystals. This latter crystal was found to be a third polymorph of TMZ. Now there are two conformers A and B ($Z' = 2$, Scheme 1) in the space group $P\overline{1}$. The presence of different conformers of the same molecule in crystal structures, or conformational isomorphism due to $Z' > 1$ (Figure 1), is an interesting chemical occurrence that is as such not so common among polymorphic sets.\(^{28}\) The amide group flips over to make an intramolecular $N_{\text{amide}}$–H···N$_{\text{tetrazine}}$ bond in conformer B (6-member ring). Although $Z' = 2$ in both polymorphs 1 and 3, the two crystallographic unique molecules have the same conformation in polymorph 1 ($\tau = -0.5$, $-3.5^\circ$) but different conformations in polymorph 3 ($\tau = 5.1$, $178.3^\circ$). Figure 5 shows the amide dimer between conformers A and B ($N1$–H1A···O3: 2.11 Å, 3.114(4) Å, 169.3\(^\circ\); $N7$–H7A···O1: 1.86 Å, 2.863(5) Å, 171.7\(^\circ\)) extends as 1D tapes via C6–H6A···O4 interaction (2.44 Å, 2.989(5) Å, 109.4\(^\circ\)). Such parallel tapes are connected by N1–H1B···N8 (1.98 Å, 2.986(5) Å, 174.0\(^\circ\)) and C4–H4···O2 dimer (2.27 Å, 3.327(5) Å, 164.6\(^\circ\)) to form a 2D layer. Monoclinic polymorphs 1 and 2 are helical structures whereas triclinic polymorph 3 is layered. The serendipitous discovery of new polymorphs during attempted co-crystallization is known in the literature.\(^{11e,18}\)

**Figure 5.** Amide N–H···O dimer of conformers A and B (capped-stick and ball-and-stick). N–H···O and C–H···O dimers extend the 1D tapes into 2D sheet in polymorph 3.
**Powder X-ray diffraction (PXRD) patterns:** The known polymorph 1 ($P2_1/c$)\textsuperscript{25b} was found to be identical with patented form III.\textsuperscript{25c} PXRD of polymorph 2 ($P2_1/n$) compares well with form IX of the same patent. Polymorph 3 however ($P\bar{T}$) is novel and does not match with any of the nine forms reported in the patent (Figure 6).

![Simulated PXRD from TMZ polymorph 1 crystal structure](image1)

![PXRD pattern of form III reported in US Patent 2005/0187206 A1](image2)

![Simulated PXRD from TMZ polymorph 2 crystal structure](image3)

![PXRD pattern of form IX reported in US Patent 2005/0187206 A1](image4)

![Simulated PXRD from TMZ polymorph 3 crystal structure](image5)

![This powder pattern does not match with any of the nine powder patterns reported in US Patent 2005/0187206 A1.](image6)

**Figure 6.** (a1) Simulated PXRD from TMZ polymorph 1 crystal structure. (a2) PXRD pattern of form III reported in US Patent 2005/0187206 A1. (b1) Simulated PXRD from TMZ polymorph 2 crystal structure. (b2) PXRD pattern of form IX reported in US Patent 2005/0187206 A1. The crystal structures of TMZ polymorphs 1 and 2 match with forms III and IX (of the patent). (c) Simulated PXRD from TMZ polymorph 3 crystal structure. This powder pattern does not match with any of the nine powder patterns reported in US Patent 2005/0187206 A1.
4.2.2 Grinding experiments and phase transitions of TMZ polymorphs

The grinding experiments,\textsuperscript{29} solid-solid phase transitions\textsuperscript{14,15,16c,16d} and slurry conversion studies or solvent mediated transformations\textsuperscript{30} are very important in establishing the kinetic/thermodynamic stability of the polymorphs. As discussed earlier in chapter 3, metastable pyrazine-\(N,N'\)-dioxide form 2 converted to more stable form 1. Further grinding on PYZNO form 1 did not show any phase transition indicating that form 1 is thermodynamically stable polymorph. To carry out similar solid-state grinding experiments, sufficient quantities of the TMZ polymorphs are required. TMZ polymorph 1 is the mostly obtained form in almost all the crystallization batches, except in CH\(_3\)NO\(_2\), DMSO and supersaturated solution of CH\(_3\)CN which yielded solvated forms of temozolomide. TMZ polymorph 2 was obtained by co-crystallization experiments in small quantities of a few crystals. Therefore there is a need to stabilize this TMZ polymorph 2. We were successful in preparing bulk-material in a controlled way by desolvating the solvated forms of temozolomide. As these solvates are obtained easily, they were desolvated at the room temperature over a period of time to yield TMZ polymorph 2, which will be discussed in detail along with other structural characterization of cocrystals in chapter 6. Crystals of TMZ polymorph 3 obtained in an early experiment could not be reproduced in subsequent batches; hence further experiments could not be carried out on this sample. A reason for difficulties in crystallizing this particular form is due to the presence of the higher energy conformer B in the asymmetric unit of the crystal lattice will be discussed next.

Phase changes have been characterized by PXRD and IR patterns. There are substantial differences in the IR frequencies (range 3500-3000 cm\(^{-1}\)) indicating that hydrogen bonding of amide NH group is different for both the polymorphs of TMZ in the solid-state as shown figure 7. Ball mill grinding or manual grinding of pure TMZ polymorph 2 for 15 min in mortar/pestle did not show any changes in IR or PXRD pattern, indicating that there is no phase transformation. Addition of CH\(_3\)CN solvent during the grinding also did not have any affect. This could be due to the high activation energy required for phase transformation. Sometimes such a barrier can be easily surmounted with the seeds of the stable polymorph 1 or pseudoseeding.\textsuperscript{19b} With this intention, a mixture of TMZ polymorphs (25 mg each of polymorph 1 and 2) was subjected to grinding for 15 min,
adding 1-2 mL of CH$_3$CN solvent. IR pattern on the resulting material matches nicely with the TMZ polymorph 1 (Figure 8), indicating that polymorph mixture has converted to the stable polymorph 1.

**Figure 7.** IR spectra of TMZ polymorphs (KBr, cm$^{-1}$). Polymorph 1(blue) and polymorph 2 (red) are shown in different colours.

**Figure 8.** IR spectra of TMZ polymorphs mixture before grinding (Blue), after 15 min grinding (magenta). Resulting material (Magenta) nicely matches with the TMZ polymorph 1(red).

In order to confirm that TMZ polymorph 1 is the thermodynamically stable form, slurry grinding experiments are carried out. These slurry or aging experiments rely on the fact that a saturated solution of any metastable polymorph is supersaturated with respect to
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the most stable polymorph. Given sufficient time, the most stable polymorph will crystallize in order to establish thermodynamic equilibrium and remove supersaturation. 30 100 mg of TMZ polymorph 2 is added to 10 mL of CH₃CN and stirred for 2 days at RT. The IR and PXRD patterns confirmed that it has completely converted to TMZ polymorph 1. Further stirring for a week yielded polymorph 1, establishing that the polymorph 1 is the thermodynamically most stable form and polymorph 2 is a metastable polymorph.

The metastable nature of polymorph 2 is ascribed to hydrogen bonding differences in the two crystal structures. The amide syn NH makes N–H···O hydrogen bonds in both structures. The anti NH forms N–H···N bond in polymorph 1 whereas the donor is not intermolecularly hydrogen bonded in form 2 (Figure 3). Further C=O accepts hydrogen bonds from imidazole CH and forms C–H···O dimer even though a better hydrogen bond amid anti NH donor is available, which is against the rule of hierarchy in hydrogen bond formation in crystals. 31 During the phase transition, molecular reorganization takes place and the unused anti N–H donor in polymorph 2 moves by half a molecular length translation to make intermolecular N–H···O bond in form 1 as shown in figure 9. The C=O acceptor now accepts hydrogen bonds from amide anti NH donor in accordance with hierarchic in hydrogen bonding. The helical architecture persists before and after reorganization. And the fact that both these polymorphs were obtained concomitantly during attempted co-crystallization experiments with CBZ point out that they have similar crystallization pathway. In summary, the phase transition of TMZ polymorph 2 to 1 during grinding and slurry experiments may be understood by the stabilization from more number of hydrogen bonds in the product. All the unused hydrogen bonds in metastable polymorph 2 are utilized in the stable polymorph 1. Dimorphs of the pigment 2,9-dichloro-5,12-dihydroquino(2,3-b)acridine-7,14-dione typify a similar situation. Two unused N–H donors and C=O acceptors in the black polymorph (–50.26 kcal mol⁻¹, Cerius²) make N–H···O bonds in the red form (–59.93 kcal mol⁻¹).

The identification of unused hydrogen bond donors/acceptors in crystal structures has attracted special mention. 33 Etter 31 proposed hydrogen bonding rules in molecular crystals. The first rule states that “all good proton donors and acceptors are used in
hydrogen bonding.” Thus, molecules with strong donors, such as O—H, N—H, and C=O acceptor are expected to form conventional hydrogen bonds. Alloxan is the archetype amide with strong NH donors and C=O acceptors, but these groups hardly engage in any conventional hydrogen bonds.\cite{33c,33d} When such strong hydrogen bonds are absent the acceptor may seek out C–H donors to form weak hydrogen bonds.\cite{33a} Carbamazepine polymorphs are sustained by the persistent amide dimer but the \textit{anti} NH donor is unused due to geometric constraint of the dibenzapine ring\cite{33c} and the absence of other heteroatom acceptors in the molecule. The trimorph cluster of bis(p-tolyl) Ketone \textit{p}-Tosylhydrazone\cite{11g} is a rare example with SO$_2$NH functional group but do not utilize its strong hydrogen bond donors/acceptors in two of its polymorphs. However other polymorph contains conventional hydrogen bonds i.e. sulfonamide dimer. Interestingly thermodynamically stable form is the one without hydrogen bonds. The reason for the presence of hydrogen bonding or lack of it in these crystal forms is attributed to the molecular conformation.

Figure 9. Hydrogen bond reorganization of TMZ polymorph 2 (a) to polymorph 1 (b). The unused amide \textit{anti} N–H in form 2 (a, thin arrows) moves by half a molecular length translation to make intermolecular N–H···O bond in form 1 (b).
4.2.3 Conformer and lattice energy calculations

Conformer energies were calculated in Gaussian 03 (DFT, B3LYP/6-31G (d,p), Table 1) by fixing the main torsion angles to the experimental values but bond distances were allowed to relax at the nearest local minima. Conformer B is 1.44 kcal mol\(^{-1}\) higher in energy than conformer A. The difference in the energy of A and B conformers is due to electrostatics. Electron density at the imidazole and tetrazine N atoms (N2 and N6) flanking the amide group is quite different. ESP charges at the electronegative N atoms were computed in a putative perpendicular amide conformation C (Scheme 2, Figure 10) because intramolecular hydrogen bonding lowers the negative potential at the acceptor N atoms by about 20 kcal mol\(^{-1}\) (ESP charge at the same N atom in conformer A and B vs. C is compared in Table 1). The electrostatic potential at the imidazole N2 is greater than tetrazine N6 in conformer C (−40.41 vs. −32.46 kcal mol\(^{-1}\), figure 10 for ESP maps). Hence, intramolecular N–H···N\textsubscript{imidazole} interaction (in 5-member ring) of conformer A should be stronger than N–H···N\textsubscript{tetrazine} interaction (in 6-member ring) of conformer B. Secondly, amide O–imidazole N repulsion in conformer B (N8···O3 2.82 Å) would be marginally more severe than amide O–tetrazine N repulsion in conformer A (N6···O1 2.96 Å). The marginally higher repulsion in conformer B could also be due to better overlap of lone pair lobes of heterocycle N and amide O atoms.

Table 1. Conformer energy computed in Gaussian 03 (DFT, B3LYP/6-31G (d,p)). ESP charges at imidazole N2 and tetrazine N6 were calculated in Spartan 04 (RHF/6-31G**).

<table>
<thead>
<tr>
<th>Conformer</th>
<th>(E_{\text{conf}}) [kcal mol(^{-1})]</th>
<th>N2–C2–C1–N1 (\tau) [(^{\circ})]</th>
<th>ESP charge N2 (imidazole) [kcal mol(^{-1})]</th>
<th>ESP charge N6 (tetrazine) [kcal mol(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.00</td>
<td>3.3</td>
<td>−22.83</td>
<td>---- ([a])</td>
</tr>
<tr>
<td>B</td>
<td>1.44</td>
<td>−178.3</td>
<td>---- ([a])</td>
<td>−10.01</td>
</tr>
<tr>
<td>C</td>
<td>7.38</td>
<td>−90.8</td>
<td>−40.41</td>
<td>−32.46</td>
</tr>
</tbody>
</table>

\([a]\) Overlap with the electron density of amide O that lies adjacent to the ring N gives unrealistic high value of −65.16 (N2) and −59.14 (N6) kcal mol\(^{-1}\).
Scheme 2. Conformers A, B and C of TMZ. Conformer A (N–H···Nimidazole bond) was taken from the X-ray structure of polymorph 1 and conformer B (N–H···Ntetrazine) was extracted from polymorph 3. The putative conformer C (perpendicular amide group) was generated computationally. Conformer energies are listed in Table 1.

**Figure 10.** Electrostatic surface map of TMZ conformers A, B and C. The charges at amide O and heterocyclic N atoms are shown.

**Energy relationships:** It is difficult to measure the melting point of TMZ polymorphs 1 and 2 (as an indicator of solid form stability) because temozolomide decomposes upon heating. Therefore the stability of polymorphs 1, 2 and 3 is verified through energy computations, phase transitions, density as well as packing fraction. Crystal density (\(\rho_{\text{calc}}\)) and packing fraction follows the order polymorphs 1 > 2 > 3 (1.669, 1.626, 1.573 g cm\(^{-3}\); 74.9 %, 72.7 %, 70.5% respectively). Lattice energy calculations (Cerius\(^{2}\), COMPASS) are consistent with polymorph 1 as the stable polymorph (−33.19 kcal mol\(^{-1}\)) followed by polymorph 2 (−32.04 kcal mol\(^{-1}\)) as shown in table 2. TMZ polymorph 3 crystals of TMZ obtained in an early experiment could not be reproduced in the subsequent batches. The disappearing nature\(^{19b}\) of polymorph 3 is due to destabilization from a strained conformer B which is 1.44 kcal mol\(^{-1}\) higher in energy than conformer A. Although crystal lattice energy of polymorph 3 is lower than that of 1 and 2 (Table 2), the strained conformer B destabilizes the total crystal energy of form 3. This situation, namely a strained conformer being stabilized in a lower crystal energy environment, is found to be quite general in conformational polymorph sets\(^{12b}\) is discussed in detail in the subsequent sections.
**Table 2.** Crystal lattice energy $U_{\text{latt}}$ of TMZ polymorphs computed in Cerius$^2$ (COMPASS force field).

<table>
<thead>
<tr>
<th>Crystal structure (Data T)</th>
<th>Conformer</th>
<th>$U_{\text{latt}}$ kcal mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form 2 (100 K)</td>
<td>$A$</td>
<td>$-32.04$</td>
</tr>
<tr>
<td>Form 1 (100 K)</td>
<td>$A$</td>
<td>$-33.19$</td>
</tr>
<tr>
<td>Form 1 (298 K)</td>
<td>$A$</td>
<td>$-33.84$</td>
</tr>
<tr>
<td>Form 3$^a$ (298 K)</td>
<td>$A + B$</td>
<td>$-34.17$</td>
</tr>
</tbody>
</table>

$^a$The crystal structure of metastable polymorph 3 could not be determined at 100K because it is disappearing polymorph.$^{19b}$

**4.3 Polymorphs of Furosemide**

![Furosemide structure]

Furosemide or frusemide (5-(aminosulfonyl)-4-chloro-2-[(2-uranyl methyl) amino] benzoic acid, FMD) is an anthranilic acid derivative, which is a loop diuretic marketed under the brand name Lasix.$^{26a}$ The name lasix is derived from lasts Six (hours)-referring to the duration of action. The therapeutic effect is obtained after 30 to 60 min following an oral dose with a bioavailability of approximately 43–69%. It acts on the ascending loop of Henle in the kidney and causes the kidneys to get rid of unneeded water and salt from the body into the urine. It is also used to treat patients with high blood pressure. The molecular structure gives wealth of information especially for the conformationally flexible molecules in predicting whether it can exhibit polymorphism or not. The rigid portion of the molecule is the anthranillic acid moiety and the flexible portions are single bond rotation of sulfonamide group and the furan ring. An intramolecular N–H···O hydrogen bond renders rigidity to the anthranillic acid moiety; as a result C–C bond rotation of carboxylic moiety is not permitted. The sulfonamide and furan ring torsions may lead to different conformers in the solid state, thereby increasing the chances of polymorphism.
The first crystal structure was reported by Fronckowiak\textsuperscript{34a} in 1976 (refcode FURSEM) in the triclinic setting with one molecule in the asymmetric unit ($Z' = 1$). 3D coordinates were not reported but it was mentioned in the CSD that furfuryl group is disordered. Lamotte \textit{et al.} in 1978 (FURSEM01)\textsuperscript{34b} reported another crystal structure with two molecules in the asymmetric unit ($Z' = 2$), also in triclinic setting. There is no disorder with furfuryl group. The $a$-axis is doubled when compared with the previous one (10.467 and 5.251 Å, Table 3). These are named as big unit cell (FURSEM01) with doubled $a$-axis and small unit cell (FURSEM). In 1983 another crystal structure was deposited in the CSD by Shin & Jeon (FURSEM02)\textsuperscript{34c} with unit cell parameters matching the small unit cell. Even though the simulated powder patterns are found to be almost identical as shown in figure 11a, large differences in unit cell parameters between small and big unit cell structures created an ambiguity,\textsuperscript{26,35,36} whether they relate to the same polymorph or two different polymorphs. A recent article by Hursthouse\textsuperscript{36} entitled "Further errors in polymorph identification: furosemide and finasteride" in 2006 suggested that the reported crystal structures in the CSD are not different polymorphs, but relate to only one polymorph.

Table 3. Unit cell parameters reported for furosemide crystal structures in the CSD are compared with the crystal data of FMD form I discussed in this chapter.

<table>
<thead>
<tr>
<th></th>
<th>FURSEM</th>
<th>FURSEM01</th>
<th>FURSEM02</th>
<th>AN867*</th>
<th>AN727*</th>
<th>AN734*</th>
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<tr>
<td>$a$ [Å]</td>
<td>5.251</td>
<td>10.467(12)</td>
<td>9.584</td>
<td>5.234(3)</td>
<td>5.2336(8)</td>
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<td>$c$ [Å]</td>
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<td>9.584(10)</td>
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<td>15.948(15)</td>
<td>14.987(2)</td>
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<td>$a$ [°]</td>
<td>101.77</td>
<td>71.87</td>
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<td>103.68(12)</td>
<td>78.097(3)</td>
<td>93.505(9)</td>
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<td>$β$ [°]</td>
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<td>89.130(3)</td>
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<td>1332.842</td>
<td>666.577</td>
<td>666.50(7)</td>
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<td>yes</td>
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<td>11</td>
<td>9.35</td>
<td>7.16</td>
<td>6.68</td>
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<td>big</td>
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<td>ordered</td>
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<td>298</td>
<td>298</td>
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<td>298</td>
<td>100</td>
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</tbody>
</table>

\* AN867 (small unit cell at 298K), AN727(big unit cell at 298K) and AN734 (big unit cell at 100K) are recollected for form I crystal of furosemide.
Hursthouse in their paper reported that one of the crystallization experiments from methanol yielded plate like crystals on the walls and needle like crystals on the floor of the flask. Unit cell determination was carried out on both morphologies. Plate crystal matches small unit cell structure while the needle matches big unit cell structure (No data was collected; only routine unit cell checks were done). When the arrangement of molecules in the two crystal structures were analyzed from the data available from CSD, it is apparent that the symmetry independent molecules in the big unit cell structure lie alternately in analogous orientations along the $a$-axis except that they differ in the furan orientations only (Figure 11b). As a result the $h$-odd X-ray reflections receive contributions mainly from the atoms of the furan groups and are generally weak. Consequently in the cell determination of the small crystal, these were not picked up, so the halved cell was obtained.

Figure 11a. (a) Simulated PXRD patterns from the big unit cell (FURSEM01) (b) Simulated PXRD patterns from the small unit cell (FURSEM02). Powder patterns are identical.

Figure 11b. A diagram showing relative orientation of the asymmetric units of small and big unit cell crystal structures of furosemide. The small unit cell has disorder in the furan ring.
We have also observed two types of crystals one plate like (very few) and needles to block in one of our crystallization experiment from \( n \)-propanol. Data were collected on both morphologies and \( hkl \) reflections were viewed in the RLATT program to confirm the points raised by Hursthouse. As suggested small unit cell crystal structure has few and very weak \( h \)-odd X-ray reflections shown as green spots in the RLATT (Figure 12a), are not considered in the cell determination, reduction and crystal structure solving. Therefore a single molecule is obtained in the asymmetric unit with furan ring disordered over two sites with equal occupancy. In comparison \( h \)-odd reflections in the big unit cell crystal structures are as many as \( h \)-even reflections and all the reflections are considered during cell determination (Figure 12b), resulting a big unit cell with two symmetry independent molecules with different furan ring orientations. As more number of reflections are considered for crystal structure solution, \( R \)-factor is lower for big unit cell structure. The choice of axes and angles for any particular crystal setting is based on the default settings that are encoded into most of the diffractometer software packages, which led to the gross differences between big and small unit cell crystal structures. Therefore both the crystal structures are not polymorphs but relate to same form I of furosemide.

\[ \text{Figure 12. (a) Reflections of small unit cell crystal structure viewed in RLATT program. The } h \text{-odd x-ray reflections are few in number (green color) and are not considered in unit cell determination therefore } a \text{-axis is } 5.241\text{Å. (b) Reflections of big unit cell crystal structure with equal number of } h \text{-odd and } h \text{-even reflections. Consequently } a \text{-axis is doubled. i.e. } 10.472\text{Å.} \]

Apart from FMD form I crystal structure in CSD, two papers\(^{26b,c} \) have discussed about the polymorphism in furosemide. Totally six crystalline forms and one amorphous form have been discussed and characterized by PXRD, IR, DSC, SS-NMR, of which four are
true polymorphs (I, II, III and VI) and two are solvates, DMF (IV) and dioxane (V). A high temperature form VI is known to be obtained from phase transformations of form I, II and III. The PXRD pattern of form I matches the simulated powder pattern of the crystal structure reported in the CSD. In this chapter other polymorphs of furosemide are characterized by single crystal XRD and their stability relationships are established. The effect of conformational flexibility of furosemide on the hydrogen bonding and crystal packing is discussed.

4.3.1 Structural description and PXRD patterns of furosemide polymorphs

Crystallization from most of the solvents like methanol, ethanol, n-propanol, i-propanol, acetonitrile have always yielded form I. The formation of form II is solvent dependent as it was obtained exclusively from anhydrous methanol and some times from anhydrous ethanol. Form III is obtained only as few single crystals along with form I from acetic acid solvent. As the quantity of form III is less, its presence can not be detected by conventional PXRD and IR techniques. Single crystal XRD is the only method to identify and characterize this particular form. Experimental conditions mentioned for obtaining form III in the literature have always yielded form I only. Polymorphs of FMD are designated as form I, II, and III whereas TMZ polymorphs were designated as polymorph 1, 2 and 3.

FMD form I, 7a: This crystal structure is already reported in the CSD (FURSEM01, big unit cell structure) at RT. It is recollected at 100K for a better comparison with other polymorphs. It crystallized in the triclinic space group $P\overline{1}$ with two symmetry independent molecules of FMD in the asymmetric unit ($Z' = 2$). As mentioned earlier, two conformers are identical except in the orientation of furan ring. The secondary amine NH forms an intramolecular N–H···O with the carbonyl acceptor (N2–H2···O3: 1.91 Å, 2.744(5) Å, 137.6°; N5–H5···O8: 1.92 Å, 2.747(4) Å, 136.8°), which is common in all the polymorphs. Carboxylic acid dimer is formed between two symmetry independent molecules in the asymmetric unit (O4–H4···O8: 1.68 Å, 2.672(5) Å, 179.9°; O9–H9···O3: 1.65 Å, 2.635(5) Å, 173.1°). Such 1D growth units of acid dimer molecules extend into 2D corrugated sheet like structure parallel to (031) plane via weak C–H···O interactions (C8–H8B···O1: 2.54 Å, 3.155(6) Å, 114.7°; C11–H11···O6: 2.48 Å,
3.452(5)Å, 148.6°; C20–H20A···O6, 2.44 Å, 3.217(6) Å, 127.3°; Figure 13a). The NH donor and S=O acceptors of sulphonamide point up and down-wards from the 2D layer (Figure 13b) and form centro-symmetric dimers using one sulfonamide NH donor (N1–H1A···O2: 1.98 Å, 2.978(5) Å, 170.6°; N3–H3A···O7: 1.99 Å, 2.999(5) Å, 172.2°). The second NH donor connects centrosymmetric sulphonamide dimer by N–H···O hydrogen bonds (N1–H1B···O7: 2.28 Å, 3.099(6) Å, 137.2°; N3–H3B···O2: 1.99 Å, 2.926(6) Å, 151.9°; Figure 13c) and forms three dimensionally packed stair-case like structure. Sulfonamide dimer can be represented by a graph-set notation $R_2^2(8)$ which is of similar origin to commonly observed 5.1 Å carboxamide packing. The crystal structure is dissected into 2D corrugated sheets and 3D packing to discuss all the hydrogen bonds in crystal structure.

![Figure 13](image)

**Figure 13.** (a) Arrangement of molecules in a corrugated 2D sheet of FMD form I. (b) The sulfonamide O atoms and NH protons point up and downwards to form sulfonamide dimer in the third dimension. (c) 3D packing of the crystal structure.

**FMD form II, 7b:** It crystallized in the monoclinic space group $P2_1/c$ with one furosemide molecule in the asymmetric unit ($Z' = 1$). The furan ring is disordered over two sites with 75 % and 25 % site occupancy at the 298K as shown in figure 14a.
Disorder is settled at 100K (Figure 14b). This is a dynamic disorder arises due to the wagging of the flexible furan ring which is removed on lowering the temperature as the molecules are virtually frozen at 100K. Disorder in this form II should not be confused with furan ring disorder of the small unit cell structure of form I which arises due to the lack of $h$-odd reflections; hence disorder persists even at 100K.

Figure 14. (a) The asymmetric unit of form II at 298K. Furan ring is disordered over two sites with 75 % and 25 % site occupancy. (b) The asymmetric unit of form II at 100K. ORTEP is drawn at 50 % probability of non-hydrogen atoms and there is no disorder.

Figure 15. (a) Infinite chain of N–H···O hydrogen bonds between screw-related molecules, which further lead to square-network of sulfonylamine synthons as shown in (b). The carboxylic acid dimer connects this square-net work of sulfonylamine synthons as shown in (c).
Form II is a three dimensional architecture made of N–H···O hydrogen bonds. Unlike previous form I, sulphonamide dimer is not present; instead four molecules form a tetrameric sulphonamide synthon. Each furosemide molecule is connected to its two-fold screw related molecule via infinite chain of N–H···O bonds involving one of its sulfonamide NH donor and S=O acceptor (N1–H1A···O1: 2.17 Å, 3.113(4) Å, 154.4º; figure 15a). Such parallel infinite N–H···O chains are connected by other sulphonamide NH donor to form an infinite square-network of tetrameric sulphonamide synthons via N–H···O H bonds (N1–H1B···O2: 1.97 Å, 2.960(4) Å, 164.1º; figure 15b). The graph set notation of tetrameric sulfonamide synthon is $R_{4}^{2}(14)$. Carboxylic acid dimer synthon (O4–H4···O3: 1.65 Å, 2.640(3) Å, 178.0º) connects these parallel square-net work of molecules as shown figure 15c. Various other C–H···O interactions and close packing of furan ring complete the crystal packing.

FMD form III, 7c: It also crystallized in the triclinic space group $P\overline{1}$ like form I. Asymmetric unit contains one molecule of furosemide ($Z' = 1$). Apart from the common intramolecular hydrogen bond, another intramolecular hydrogen bond with adjacent chlorine atom is present (N1–H1B···Cl1: 2.66 Å, 3.252(4) Å, 117.0º). There is a centro-symmetric carboxylic acid dimer (O4–H4···O3: 1.67 Å, 2.644(3) Å, 168.5º) with inversion related molecule. These carboxylic acid dimer aggregates form zig-zag tapes via C–H···O dimer of sulfonamide (C3–H3···O2: 2.52 Å, 3.610(4) Å, 175.3º). Such tapes fill 2D corrugated sheet via close packing of the furan ring (Figure 16a). Sulfonamide NH and O atoms that point up and down-wards from the 2D layer form centro-symmetric dimers using one NH donor (N1–H1A···O2: 2.05 Å, 3.028(4) Å, 161.0º). Other NH donor and O acceptor join sulfonamide dimers by N–H···O H bonds (N1–H1A···O2: 2.35 Å, 2.911(4) Å, 113.9º; N1–H1B···O1: 2.37 Å, 3.178(4) Å, 136.2º; figure 16b). These two sulfonamide synthons are represented as $R_{2}^{2}(8)$ and $R_{2}^{2}(6)$ synthons. Even though sulfonamide synthons are different in these three polymorphs, the carboxylic acid group however forms similar acid dimer or $R_{2}^{2}(8)$ synthon (Figure 13, 15 and 16).
Polymorphism in... 111

Figure 16. (a) Arrangement of molecules in a corrugated 2D sheet of form III. (b) Sulfonamide forms a skewed dimer in its 3D packing.

**Powder patterns:** Simulated powder patterns of all the crystal structures of furosemide are compared with PXRD patterns reported in the literature. All FMD forms I, II and III are found to be identical with earlier reported patterns as shown in figure 17.

Figure 17. Simulated powder patterns from crystal structures (blue), which are compared with PXRD patterns reported in the literature (black). (a) Form I. (b) Form II. (c) Form III. All of them match nicely except for few minor differences after $2\theta > 20^\circ$ in form III crystal structure as shown in (c).
Table 4. Geometrical parameters of hydrogen bonds in furosemide polymorphs.

<table>
<thead>
<tr>
<th>Polymorph</th>
<th>Interaction</th>
<th>H···A/ Å</th>
<th>D···A/ Å</th>
<th>∠D–H···A/º</th>
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</thead>
<tbody>
<tr>
<td>FMD form I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7a</td>
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<td>2.672(5)</td>
<td>179.9</td>
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<tr>
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<td>2.635(5)</td>
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</table>

4.3.2 IR characterization of FMD polymorphs

Many peaks in the IR spectrum of furosemide form I exhibited marked differences in the respective positions of furosemide form II (Figure 18), indicating that hydrogen bonding of sulfonamide group is different in both the polymorphs in the solid-state. Form I has three characteristic peaks, 3400.0 cm⁻¹ (asymmetric sulfonamide NH stretch), 3351.0 cm⁻¹ (secondary amine NH vibration), 3285.6 cm⁻¹ (symmetric sulfonamide NH stretch). In comparison form II has only two peaks in this region, 3347.4 cm⁻¹ (secondary amine NH vibration) and 3253.9 cm⁻¹ (symmetric sulfonamide NH stretch). Asymmetric stretch of sulfonamide of form II has disappeared perhaps due to the symmetrical environment of sulfonamide NH in the tetrameric sulfonamide synthon in the crystal structure of
form-II. The polymorphic mixture of form I and II obtained contains peaks of both forms i.e. 3399.9, 3350.2, 3285.4 and 3254.5 cm$^{-1}$.

**FMD form I**
- 3400.0, 3351.0
- 3285.6 cm$^{-1}$

**FMD form II**
- 3347.4, 3285.9 cm$^{-1}$

**Figure 18.** IR spectrum (KBr, cm$^{-1}$) of furosemide polymorphs. Form I (red) with three sharp peaks while the form II (magenta) has only two peaks.

### 4.3.3 Grinding experiments and phase transitions of furosemide polymorphs

Forms I and II are obtained in sufficient quantities to carry out solid-state grinding experiments. Phase changes have been characterized by PXRD and IR patterns. Form III is obtained only as few single crystals during the crystallization from acetic acid and the material is not sufficient for grinding and slurry conversion experiments.

**FMD mixture I + II, green**
- 3399.9, 3350.2, 3285.4, 3254.5 cm$^{-1}$

**After 30 min, blue**
- 3400.6, 3351.6, 3284.7, 3255.9 cm$^{-1}$

**After 60 min, magenta**
- 3401.0, 3351.9, 3284.7 cm$^{-1}$

**After 120 min, red**
- 3400, 3351, 3285.6 cm$^{-1}$, FMD form I

**Figure 19.** IR spectrum (KBr, cm$^{-1}$) recorded at regular intervals of grinding polymorph mixture (green colour spectra). After 30 min (blue), 60 min (magenta), 120 min (red). The polymorphic mixture converted to stable FMD form I after 60 min as shown by disappearance of one peak in the IR spectrum and the material showed no further change for further grinding for another 1h.
Mechanical grinding experiments on pure FMD form I and II in two different set of experiments showed no phase conversions. However when the polymorphic mixture (forms I + II) was subjected to grinding in mortar/pestle using of CH$_3$CN solvent, phase conversion occurred to form I in 1hr. Further grinding for another 1h showed no change in IR peaks (Figure 19) and in the relative positions of PXRD pattern (form I recovered), but increased the amorphous content in the material. The polymorphic mixture obtained was also subjected to slurry conversion which converted to form I completely in 2 days, indicating that form I is thermodynamically more stable form.

4.3.4 Thermal analysis of FMD polymorphs

The Differential Scanning Calorimetry (DSC) thermograms of form I showed two endotherms (Figure 20). The first endotherm occurs in the range 129-135 °C with peak temperature at 133°C, indicating a phase transition. The Thermo Gravimetric Analysis (TGA) curve shows no weight loss over this temperature range suggesting that the first endotherm does not arise due to desolvation or decomposition of the compound. When the sample was allowed to cool after heating up to 150 °C, an exothermic peak observed at 133.5 °C suggesting that the phase transition is reversible and both the phases are enantiotropically related (black colour curve in figure 20). The second endotherm is a melting endotherm. It is a very small endotherm at 207 °C, immediately followed by the compound decomposition to 2-chlorosulfonyl anthranillic acid with sharp exothermic peak in the range 207-217 °C. The physical and morphological changes undergone by the samples during the process heating were monitored using Hot Stage Microscopy (HSM). Interestingly there are no morphological changes of crystals observed in the first endotherm temperature range as shown in figure 21. The compound did not melt however there was gradual degradation in the range 200-215 °C indicated by the blackening of the sample. The DSC curve of form II lacks the first endotherm. The decomposition of furosemide occurs at temperature lower than form I (Tonset is 201 °C for form II and 207 °C for form I). Similar observations were noted in the HSM snapshots of form II crystals (figure 22). Unlike form I, form II showed a clear melting of the compound followed by decomposition. Apart from the thermal decomposition of furosemide at high temperature, photolytic degradation is also observed in the literature as shown in scheme 3 which is evident from the change in colour from white
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to yellow on long storage. Form I crystals are observed to be relatively more stable and resistant to degradation than form II crystals.

Figure 20. DSC curves of form I (red) and II (blue) of furosemide polymorphs. Heat-cool-heat cycle of form I is shown in black colour.

Scheme 3. Decomposition of Furosemide to CSA and other by-products.

Figure 21. HSM snapshots recorded at different temperature for FMD form I crystals. There is no phase change observed at 135°C. There is a gradual degradation of compound at higher temperature indicated by blackening of crystals.
Figure 22. HSM snapshots recorded at different temperature for FMD form II crystals. Unlike form I, form II showed a clear melting of the compound at 200°C followed by decomposition.

Figure 23. HSM snapshots recorded at different temperature for FMD form III. Crystals get cracked in the temperature range of 125-130 °C, followed by decomposition after 200°C.

The advantage with HSM techniques is that wealth of information is known even with 1 or 2 crystals. This is illustrated using form III crystals for which DSC, TGA, PXRD, IR was not recorded due to the lack of sample in sufficient quantity. These crystals were seen to undergo an abrupt physical change resulting in the break up of the crystals in the
temperature range 125-130 °C, which may be due mechanical stress caused due to the
arrangement of molecules in the crystal lattice or a phase transition (Figure 23).

4.3.5 Lattice and conformer energy calculations

Lattice energies for furosemide polymorphs are calculated in Cerius² using DREIDING
2.21 and COMPASS force fields. The energy values obtained from DREIDING do not
appear to be reliable because of the large differences between them (form I, −87.83; form
II, −65.549; form III, −71.873 kcal mol⁻¹) and the energy values obtained from
COMPASS force field are too close to infer any stability relationships amongst the
polymorph sets (form I, −41.653; form II, −41.778; and form III, −41.533 kcal mol⁻¹).
Generally the lattice energy values are less than 1 kcal mol⁻¹ for concomitant polymorph
systems due to which they appear in the same flask during the crystallization. Several
crystallization experiments on furosemide yielded concomitant mixture of form I + II
from methanol, form I + III from acetic acid, and form II + III from n-propanol solvent,
suggesting that all the crystal structures in principle should have almost close lattice
energy values. Crystal density and packing fraction follow the stability order: Form I >
form II > form III (\(D_c = 1.700, 1.637 \text{ and } 1.622 \text{ g/cm}^3\); Packing fraction = 74.0 %, 71.1%
and 70.6 %) in accordance with the stability order from phase transitions and slurry
conversion studies as discussed in the previous sections. Form I is the
thermodynamically most stable polymorph at room temperature and form II and III are
metastable forms.

There are four different conformers of furosemide molecule obtained from three
polymorphs analyzed (two in form I and one each in form II and III). The overlay of all
four conformers is shown in figure 24b. The conformational flexibility is due to torsions
of sulfonamide and furfuryl ring (Figure 24a). Conformer energies were calculated in
Gaussian 03 (DFT, B3LYP/6-31G (d,p), Table 5) by fixing the main torsion angles to the
experimental values but bond distances were allowed to relax at the nearest local
minima. The two symmetry independent molecules in form I are almost identical except
in the furfuryl ring torsions attached to secondary amine moiety. The energy difference
between these molecules is 0.1 kcal mol⁻¹ (Table 5) which concludes that the furfuryl
ring torsions have minimal effect on the conformer energy of the molecule. These two
conformers are 4.447 and 4.551 kcal mol$^{-1}$ higher in energy than the global minima conformer or stable conformer present in the form III. The sulfonamide torsion ($\tau_1$) in the stable conformer of form III is 55.7$^\circ$, whereas it is $-166.0$ and 163.2$^\circ$ for higher energy conformers of form I. In form II, the torsion ($-79.9^\circ$) is few degrees greater than the stable conformer (55.7$^\circ$), consequently its energy is marginally higher than form III stable conformer by 0.71 kcal mol$^{-1}$. In order to see how the conformer energy varies with different sulfonamide torsions with respect to adjacent chlorine, we have scanned a half a circle of the torsion with 10 deg increments at each step, starting from 0 to 180 deg, and their energy values are plotted against the torsion angle changes. Other half of the torsion map is generated symmetrically as the molecule is planar. Furosemide molecule is truncated to N-methyl instead of N-furfuryl for faster number crunching and moreover the furfuryl group is observed to have almost no effect on the conformer energy (Figure 25).

\[ \text{Table 5. Conformer energy computed in Gaussian 03 (DFT, B3LYP/6-31G (d,p)).} \]

<table>
<thead>
<tr>
<th>Conformer</th>
<th>$\tau_1$</th>
<th>$\tau_2$</th>
<th>$\tau_3$</th>
<th>Energy kcal mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form I_A</td>
<td>-166.0</td>
<td>83.9</td>
<td>-68.2</td>
<td>+4.447</td>
</tr>
<tr>
<td>Form I_B</td>
<td>163.2</td>
<td>-61.4</td>
<td>-57.6</td>
<td>+4.551</td>
</tr>
<tr>
<td>Form II</td>
<td>-79.9</td>
<td>-166.4</td>
<td>-78.2</td>
<td>+0.713</td>
</tr>
<tr>
<td>Form III</td>
<td>55.7</td>
<td>91.3</td>
<td>-59.9</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ \text{Figure 24. (a) Molecular diagram of furosemide with three torsion angles. (b) Overlay of four conformers observed in three polymorphs of furosemide. Form I (pink and blue), form (yellow) and form (red).} \]
The sulfonamide torsion renders the furosemide molecule with two energy minima as evident from the torsion map as shown in figure 25. The global minima occurs in the torsion range 50-80°, and any conformer in this torsion range is likely to have energy difference of only 1 kcal mol⁻¹ from the stable conformer at 60°. The second minima which is higher than the first minima by ~4 kcal mol⁻¹ occurs in the range 160-180°. Energy of various conformers with different torsion angles observed experimentally in furosemide polymorphs are compared from the calculated energy-torsion map (Figure 25). Their energy values are in the expected ranges for all three forms and are shown on the torsion map. The CSD⁷ was then searched for all structures that contain sulfonamide moiety adjacent to chlorine atom. There are 53 structures that match the required criteria and their sulfonamide torsion angles verified. Except for 3 entries (FURSEM01, RESRIR and REZXEZ), all of them have values in the range 50-80°, suggesting that most of the crystal structures have conformers in the first energy minima. Hydrochlorothiazide³⁹ is a furosemide derivative used as diuretic is worthy to mention in this context. There are 10 crystal structures (two polymorphs and eight solvated forms) of this drug in the CSD and interestingly all of them have torsion angles between 50-80° i.e. in the first energy minima.

Figure 25. Furosemide molecule is truncated to its N-methyl derivative. Energy values are plotted against sulfonamide torsion angle. The energy values correlated from the torsion map are in the expected range: Form I (□), form II (○) and form III (△).
4.4 Systematic effects in conformational polymorphs

In general conformational flexibility introduces two potential complications to the crystallization process which are not encountered by rigid molecules. Firstly, a greater number of structural options are available for crystallization, giving rise to polymorphs that differ not only in the mode of packing but also in molecular conformation. \textsuperscript{11} Secondly, the rate of crystallization may be significantly reduced by conformational flexibility as it has to select the right conformer from the wrong ones. \textsuperscript{40} 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile (ROY, because of its red, orange and yellow colored forms) \textsuperscript{11a,b} is a conformationally flexible molecule known to exist in nine polymorphs, of which single crystal X-ray structures are reported for seven of these forms with different conformers in their crystal structures. Earlier studies of conformational polymorphism have shown that the bond lengths and angles do not differ significantly between polymorphs because of high energy penalty for bond stretching, compression or distortion. However rotation about C–C single bonds or single bond torsions have energy requirements of 0.5-3 kcal mol$^{-1}$, which is comparable to the lattice energy differences observed between polymorphs (< 4 kcal mol$^{-1}$). \textsuperscript{12} This means that the energy penalty in molecular conformation can be compensated by the lattice energy gained through intermolecular interactions and close packing. In a classical example, the flat conformation of ortho-H biphenyl is favoured in the solid state although it lies ~1.5 kcal mol$^{-1}$ above the lowest (gas-phase) energy conformation, which has an inter-ring twist angle of 44°. The herringbone T-motif stabilizes the metastable planar conformation in biphenyl crystal structures. Brock and Minton\textsuperscript{41} referred to the stabilization of strained conformers by crystal packing forces as a systematic effect. Thus, molecular conformation and hydrogen bonding can influence each other and in turn the overall crystal packing. An excellent review by Nangia\textsuperscript{12b} showed that these systematic effects are common in conformationally flexible molecular crystals and found to occur in 16 out of 23 conformational polymorphic systems analyzed.

This intra and intermolecular energy compensation reduces total crystal energy differences, which increases the likelihood of polymorphism. Normally for rigid molecules, the total energy difference between the polymorphs is given by crystal lattice energy i.e. $\Delta E_{\text{total}} = \Delta U_{\text{latt}}$. But for conformationally flexible molecule, destabilization
energy from the higher energy conformer should be added to the lattice energy i.e. \( \Delta E_{\text{total}} = \Delta U_{\text{latt}} + \Delta E_{\text{conf}} \) which decreases the total energy difference between the polymorphs and thereby increasing the polymorphs promiscuity. This observation is of significant relevance to pharmaceutical industry because typical drug molecules represent a confluence of conformational mobility and functional complexity. This is illustrated by furosemide and temozolomide molecules with sufficient conformational flexibility and functional diversity. The two high energy conformers of furosemide form I (4.447 and 4.551 kcal mol\(^{-1}\) higher in energy than the global minima conformer) are stabilized in the stable crystal lattice of form I due to better hydrogen bonding and other non-covalent forces. Similarly the metastable conformer \( B \) of temozolomide which is 1.44 kcal mol\(^{-1}\) higher in energy than conformer \( A \), is stabilized in the lower crystal lattice of form 3. However there are few examples where the stable conformer is present in the stable crystal lattice. One such example is ROY molecule,\(^{11a}\) where the stable perpendicular conformation is present in the thermodynamic yellow crystal form. The role of solvent in conformer stabilization and crystal nucleation are surely important during the crystallization of conformationally flexible molecules.\(^{5b,19a}\)

4.5 Synthon Polymorphism in molecular crystals

Supramolecular synthon-based polymorphism or synthon polymorphism\(^{42}\) arises due to different hydrogen bond synthons in different polymorphs. Polymorphic crystal structures of hydrogen bonding functional groups may be analyzed at two levels: the primary level of cyclic synthon or strong hydrogen bonds and the secondary level of single hydrogen bonds or weak interactions. When there is a substantial difference in the hydrogen bond synthons between polymorphs as in acid dimer and catemer or acid–acid and acid–pyridine synthons, they belong to the primary level synthon polymorph category. However in many crystal structures, the most important or principal hydrogen bond synthon remains same in all the polymorphs and the differences are only due to other hydrogen bonds. These are called as secondary level synthon polymorphs illustrated by tetramorphic pyrazinamide. All four forms have similar amide dimer motifs and they differ only in their \textit{ anti } N–H hydrogen bonding. A few common examples of primary synthon polymorphs are sulfathiazole,\(^{5b}\) isonicotinamide,\(^{27b}\) and tetrolic acid\(^{42}\) as shown in figure 26.
Figure 26. Different hydrogen bond synthons in tetrolic acid polymorphs. (a) Carboxylic acid dimer. (b) Carboxylic acid catemer.

Polymorphs 1, 2 and 3 of TMZ exhibit secondary level synthon polymorphism. They have the same amide dimer, indicated by graph-set notation $R_2^2(8)$, but the anti NHs make different intermolecular interactions: N–H···O and N–H···N in TMZ polymorph 1 (6a), the donor is intermolecularly not bonded in polymorph 2 (6b), and N–H···Ns are present in polymorph 3 (6c) as shown in figure 27. On the contrary, FMD polymorphs exhibit primary level synthon polymorphism. The interacting sulfonamide is differently hydrogen bonded in three forms: sulfonamide dimer, $R_2^2(8)$, in FMD form I (7a), sulfonamide tetramer, $R_4^1(14)$, in form II (7b) and a modified sulfonamide dimer, $R_2^2(8)$ and $R_2^2(6)$, in form III (7c) as shown in figure 28. The sulfonamide moiety is similar to carboxamide except that it has an extra S=O acceptor. It can either prefer to use a single acceptor or use both acceptors in hydrogen bond formation with donor N–H groups. FMD form I synthon is similar to the commonly observed carboxamide 5.1 Å motif which uses one of its one S=O acceptor and two sulfonamide N–H donors in synthon formation. On the other hand, form II and III utilizes both acceptors and donors in the sulfonamide synthons, and make different synthons. The carboxylic acid in all these forms is involved in the acid dimer which is at the secondary level of polymorphism.

The subtle differences in the conformations can have a drastic effect on the crystal packing. The anti-depressant drug Venlafaxine hydrochloride is one such example with flexible –OCH$_3$ group which can undergo rotation about C–O bond, is known to exist in three anhydrous forms. It is either present on the same side of the –OH group (cis conformer) or on the opposite side of –OH group (trans conformer) as shown in figure 29. Form 1 contains crystal packing via trans conformer, whereas form 2 contains cis
conformer only. Interestingly the newly discovered stable third polymorph contains both conformers present in the asymmetric unit.\textsuperscript{11f}

**Figure 27.** Polymorphs 1, 2 and 3 of TMZ exhibit secondary level synthon polymorphism. They have the same amide dimer, $R_2^2(8)$, but the \textit{anti} NHs make different intermolecular interactions.

**Figure 28.** FMD polymorphs exhibit primary level synthon polymorphism. The sulfonamide is differently hydrogen bonded in three forms: sulfonamide dimer in form I, sulfonamide tetramer in form II and a modified or skewed sulfonamide dimer in form III. Curved arrow shows movement of SO$_2$NH group to give different synthons.

**Figure 29.** Conformers of Venlafaxine drug via C–C bond rotation of –OCH$_3$ group.
**Figure 30.** Form I shown in red colour has torsion angles $\tau_1 = 166^\circ$ and $163.2^\circ$. Form III is shown as green colour has $\tau_1 = 55.7^\circ$. Hydrogen bond motifs in form I and III are almost identical because of similar orientation of sulfonamide NH donors and O acceptors for dimer H-bonding, even as there are large differences in their torsion angles.

![Image of molecular structure]

**Figure 31.** Different hydrogen bonded synthons of BIXGIY polymorphs arising from different conformers. Phenyl ring C11–C6–N2–C1 torsion angles are indicated along with refcodes.

The change of the sulfonamide moiety conformation in FMD form III ($\tau_1 = 55.7^\circ$) to II ($\tau_1 = 79.9^\circ$) has severely affected the hydrogen bond motifs which resulted in a distorted sulfonamide dimer in form III, whereas it is a sulfonamide tetramer in form II. Interestingly the hydrogen bond motifs in form I and III are almost identical, although there is a large difference in their torsion angle ($\tau_1 \approx 165^\circ$ in form I, 55.7° in form III). This is because conformers in form I and III have similar orientation of the sulfonamide.
NH donor and S=O acceptor, except that they are in opposite direction, i.e. sulfonamide NH away from chlorine atom (red conformer) in form I and towards chlorine atom in form III (green conformer) as shown in figure 30. Hence both the conformers can still form sulfonamide dimer with minor differences. Different conformers leading to different hydrogen bonded synthon is noted in analgesic and anti inflammatory drug 2-(2-Methyl-3-chloroanilino)-nicotinic acid (refcode BIXGIY)\textsuperscript{44} which contains four forms that differ in the phenyl ring torsion attached to secondary amine: carboxylic acid dimer in BIXGIY02 (–22.2°) and BIXGIY03 (–0.6°), carboxylic acid–pyridine synthon with proton located on carboxylic acid in BIXGIY (–111.9°), and carboxylate–pyridinium synthon with proton migrated from carboxylic acid to pyridine moiety in BIXGIY04 (42.6°) as shown in figure 31.

4.6 Conclusions

Polymorphs of antitumour drug Temozolomide and loop diuretic Furosemide are characterized by single crystal XRD to study the influence of conformer changes on hydrogen bonding and crystal packing. These are observed to be conformational polymorphs, having different conformations in different crystal structures. TMZ can undergo rotation about the C\textsubscript{amide}–C\textsubscript{imidazole} bond to give two conformers, A and B. Conformer B is 1.44 kcal mol\textsuperscript{−1} higher in energy than conformer A. The stable conformer A is present in TMZ polymorphs 1 (6a) and 2 (6b) whereas both conformers crystallized in TMZ polymorph 3 (6c). Solid-state phase transitions, slurry conversions, lattice energy calculations, packing fraction and density values showed that the original polymorph 1 is the most stable polymorph followed by polymorphs 2 and 3. The metastable nature of TMZ polymorphs 2 and 3 is due to the presence of unused hydrogen bond donors/acceptors and strained conformer B respectively. Polymorph 3 crystals of TMZ obtained in an early experiment could not be reproduced in subsequent batches perhaps due to destabilization by strained conformer B.

Three polymorphs of Furosemide were characterized. Conformational flexibility and variations were examined at two places: important one is the orientation of sulfonamide group towards adjacent chlorine atom and minor one is the orientation of N-methyl furan rings torsions. Totally there are four conformers present in three polymorphs: FMD form
I (7a) has two molecules in the asymmetric unit whereas form II (7b) and III (7c) contain one molecule each. Systematic effects\textsuperscript{12} are analyzed, where in high energy metastable conformers (4.44 and 4.55 kcal mol\textsuperscript{-1}) are compensated in the stable crystal lattice of FMD form I (7a), while the stable conformer is present in the metastable crystal lattice of form III. Similarly metastable conformer B of Temozolomide is also compensated in the stable crystal lattice of form 3 (6c). We conclude that conformational flexibility in organic molecules increases the likelihood of polymorphism. (1) Several conformers are available in the crystallization milieu (solution or melt phase) to form different hydrogen bond synthons and close-packing motifs. (2) The intra- and intermolecular energy compensation reduces total crystal energy differences, which increases the likelihood of polymorphism.

Polymorphism in both these systems is based on differences in conformations and hydrogen bond synthons. Interestingly different conformations of furosemide lead to different hydrogen bonded sulfonamide synthons: sulfonamide dimer in form I, tilted or skewed dimer in form III, and a tetrameric sulfonamide synthon in form II referred to primary level synthon polymorphs. Although the conformers in form I and III are largely different, they can still form sulfonamide dimer with minor differences. In comparison, all TMZ polymorphs are secondary synthon polymorphs in that the primary amide dimer synthon is the same but the secondary N–H···N/ N–H···O hydrogen bonds are different.

4.7 Experimental Section

**Preparation of Polymorphs:** Temozolomide was supplied by Dabur Research Foundation, India. Crystallization of TMZ from common solvents such as EtOH, \textit{i}-PrOH, acetone and CH\textsubscript{3}CN by slow evaporation resulted in single crystals of the known monoclinic polymorph 1. Attempted co-crystallization with carbamazepine (CBZ) in a 1:1 molar ratio (TMZ, 25 mg, 0.127 mmol + CBZ, 30 mg, 0.127 mmol) by dissolving the components in 5 mL EtOH and slow evaporation of the solvent gave single crystals of TMZ. Needle shaped crystals had a monoclinic unit cell (Polymorph 2) and a few irregular shaped crystals had the triclinic setting (polymorph 3). Attempts to reproduce polymorph 3 (disappeared polymorph)\textsuperscript{19b} gave a concomitant mixture of polymorph 1 (plate or block morphology, dominant) and polymorph 2 (needle shaped, minor) but no
Polymorphism 3. Polymorph 2 was also obtained by TMZ and 3-hydroxypyridine-N-oxide co-crystallization in MeOH. No cocrystals were obtained in these experiments. Furosemide was extracted from commercial Lasix tablets using methanol solvent. NMR, IR and PXRD confirmed purity of the compound. Crystallization from most of the solvents like methanol, ethanol, n-propanol, i-propanol, acetonitrile always yielded FMD form I. Very fine needles of form II for single crystal data were obtained on slow evaporation of 50 mg of compound dissolved in 5 mL of anhydrous MeOH. Form III crystals were obtained concomitantly with form I when 50 mg of compound in 2 mL acetic acid or 75 mg in 4-5 mL of n-propanol.

**X-ray crystal structures:** Reflections on single crystals of TMZ and FMD polymorphs were collected on Bruker SMART-APEX CCD X-ray diffractometer (Mo-Kα radiation, \( \lambda = 0.71073 \) Å). SMART was used for collecting frames, indexing reflections, and determining lattice parameters. The integration of reflections intensity and scaling was carried out in SAINT. Absorption correction was done in SADABs\(^{45} \) and SHELX-TL\(^{46} \) was used for space group determination, structure solution, and least-squares refinements on \( F^2 \) to give satisfactory \( R \)-factor. Structures were solved by the direct methods. RLATT was used to visualize \( hkl \)-reflections for small and big unit cell structures of form I of furosemide. X-Seed\(^{47} \) was used to prepare figures and packing diagrams.

**Solid-state grinding and slurry conversion:** 75-100 mg of TMZ polymorph 2 was ground in a ball-mill for 60 min. There is no change in PXRD and IR patterns. However when the mixture of polymorph 1 and 2 (25 mg of each) are ground in mortar/pestle for 15 min using 1 mL of CH\(_3\)CN solvent, the resulting IR and PXRD matches polymorph 1. These seeds of stable polymorph 1 are not required for phase transition in slurry conversion experiments. 100 mg of polymorph 2 is stirred in 5-8 mL of CH\(_3\)CN. Some material was filtered after 2 days. IR and PXRD patterns of the filtered material match with TMZ polymorph 1 indicating phase transition from polymorph 2 to 1. No further change observed when the stirring was continued for a week. Similar experiments are carried on furosemide. Mixture of FMD form I and II is obtained when 500 mg of the compound is dissolved in 50-75 mL of MeOH, and evaporated slowly in an open beaker. This mixture was used for grinding and slurry conversion experiments. 200 mg of this polymorph mixture was shaken in a Wig-L-Bug type mixer mill equipped with 5 mL stainless steel grinding jar and balls of 4 mm diameter. Grinding was performed at 20 Hz
oscillation rate. IR and PXRD patterns are recorded at regular intervals, 30 min, 60 min and 120 min. Phase conversion of mixture to form I was complete with in 1h and further grinding for 1h more showed no change. In a different experiment, 100 mg of the mixture was made slurry in 5-8 mL of CH3CN and stirred for 2 days. Mixture was completely converted to form I. Further stirring on the slurry for a week only recovered form I, suggesting that FMD form I is the stable form. Powder X-ray diffraction patterns was recorded on a PANalytical 1830 (Philips Analytical) diffractometer using Cu-Kα X-radiation (λ = 1.54056 Å) at 35 kV power and 25 mA current over the 2θ range 5-50° at scan rate of 1° min^{-1}. Fourier transform IR (FT-IR on Nicolet 6700) spectra are recorded in the mid IR region (4000-400 cm^{-1}).

**Computations:** Conformer energies were calculated in Gaussian 03 (DFT, B3LYP/6-31G (d,p)). Since the observed conformation in the crystal structure is usually different from the gas phase minimized conformer and often higher in energy, constrained optimization of the crystal conformer was carried out by keeping the main torsion angles fixed but allowing bond distances and angles to relax at the nearest local minima (E_{conf}). Lattice energies were computed in Cerius² using the COMPASS and DREIDING 2.21 force field. Crystal structures were minimized (U_{lat}) by allowing small variations in cell parameters but not gross differences between the calculated and experimental crystal lattice. The electrostatic potential map of the three conformers A, B and the perpendicular amide conformation C are computed in Spartan 04, HF/6-31G**.

### 4.8 References

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