Crystal Engineering Studies: polymorphs and co-crystals

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Abstract. We review the key topics of one of the areas with the biggest impact of the last years in the chemical and pharmaceutical industry that is Crystal Engineering. The relevance of polymorphs and co-crystals from different points of view is been highlighted and broadly illustrated by means of several recent examples of studies carried out in this field. In addition, the most suitable instrumental techniques and the intellectual property implications are reviewed.

1. Introduction

Crystal engineering is the rational design of functional molecular solids from neutral or ionic building blocks, using intermolecular interactions in the design strategy [1]. This field has its origins in organic chemistry and in physical chemistry. The expansion of crystal engineering during the last years as a research field has gone parallel with a significant interest in the origin and nature of intermolecular interactions and their use in the design and preparation of new crystalline structures.

Active pharmaceutical ingredients (APIs) represent a particularly great challenge to crystal engineers because of both fundamental and applied reasons. APIs are inherently predisposed for self-assembly since their utility is normally the result of the presence of one or more supramolecular synthons. The crystalline materials obtain their fundamental physical properties from the molecular arrangement within the solid, and altering the placement and/or interactions between these molecules can have a direct impact on the properties of a particular solid. Usually, solid-state scientists call upon a variety of different strategies when trying to modify the chemical and physical solid-state properties of APIs, namely, the formation of polymorphs, hydrates, solvates, salts and co-crystals [2] (Fig. 1).

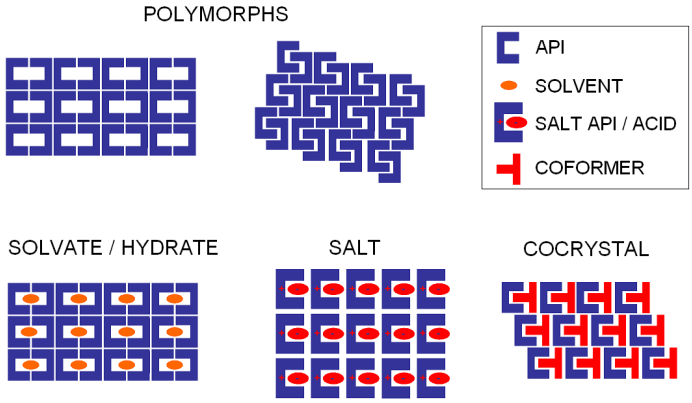


Figure 1. Different solid forms of an API: polymorphs, solvates/hydrates, salts and co-crystals.

Polymorphism is the ability of a substance to crystallize in different crystal modifications, each of them having the same chemical structure but different arrangements or conformations of the molecules in the crystal lattice. Polymorphs can differ in their chemical, physical and biological properties. There are many cases where insufficient exploration of possible crystallization and interconversion conditions caused serious delays in market launch and losses of revenue. Hence, the understanding and control of polymorphism and polymorphic behaviour is of considerable fundamental and practical importance. Moreover, a metastable polymorph is sometimes accepted for example to provide better handling/processing properties [3].

Crystallizing the API as a multicomponent crystal has been another accepted approach to generating form and physical property diversity. Hydrate and salt formation are the most common applications of this concept. Pharmaceutical hydrates are important due to the presence of water molecules in so many pharmaceutical drugs. On the other hand, it is estimated that over half of the medicines on the market are administered as salts. However, a major limitation within this approach is that the API must possess a suitable (basic or acidic) ionisable site. In comparison, the emerging field of pharmaceutical co-crystals (multicomponent assemblies held together by freely reversible, noncovalent interactions) offer a different pathway, where any API regardless of acidic, basic or ionisable groups, could potentially be co-crystallized [4].

2. Methodology and Applications

2.1. Relevance of polymorphs and co-crystals in the pharmaceutical and agrochemical industry
Crystal polymorphism is a common phenomenon in the pharmaceutical industry because the solid-state form is a significant quality attribute of a crystalline drug compound. The control of the desired crystal form during the manufacturing, storage or transport of a pharmaceutical substance is a key issue and losing this control can have serious economical effects due to the infringement of the intellectual property [5].

In addition, intrinsic properties of a drug substance candidate like its solubility or its chemical stability can be affected by its solid state. Poor physical properties of drug substances may be improved by using different crystal forms. Pharmaceutical formulations are developed in order to affect and improve solid-state properties of the drug substance in the final product [6].

Although polymorphism is a less frequent issue in agrochemicals, some important compounds, including pesticides and herbicides such as metazachlor exist in different polymorphic modifications [7] and their different solubility can affect the efficiency and toxicity of the pesticide.

Finally, co-crystals of APIs represent a class of multi-component crystalline forms that are of interest for their advantageous physical properties and for intellectual property implications [8]. In the last years, a great number of patents have been issued involving new co-crystals of APIs together with pesticides, herbicides and fungicides.

2.2. Polymorphs and co-crystals screening
During the last decades, high-throughput methodologies have been developed to look for new polymorphs and co-crystals of relevant drug compounds. Initially, the design of screening methods was based on solution growth. However, other methods such as solvent-drop grinding, slurring and the use of experimentally-determined phase diagrams have been developed in order to screen as much as possible the polymorphic landscape of a target compound. Due to the fact that all these screening methods are very time consuming and expensive, modern computational virtual screening methodologies for co-crystals have appeared recently with very promising results [9].

2.3. Analytical Techniques
Polymorphs and co-crystals represent different crystal structures of a compound. In principle, any physical or chemical property can a priori vary along the crystalline landscape of a compound and thus, practically any analytical technique able to measure properties of a crystalline material is suitable to study the solid state behaviour of a given compound. In this sense, the most useful techniques are:

2.3.1. Differential Scanning Calorimetry (DSC). This technique measures the difference in the amount of heat required to increase the temperature of a sample and a reference as a function of temperature. Pure crystalline forms are characterized by their melting process and the polymorphic behaviour can be studied through the observed onset and enthalpy of any thermal phenomenon which can be accurately measured. Endotherms represent phenomena such as desolvation, phase transitions and melting. On the other side, exotherms are associated with crystallization, phase transitions and decomposition reactions.

2.3.2. Thermogravimetry (TGA). Thermogravimetric analysis records the mass changes of a sample as a function of time and temperature under a controlled atmosphere. Although this technique does not give direct information about the existence of polymorphism, since all polymorphs have the same mass, it is crucial in determining the existence of solvates and the determination of their stoichiometries. During polymorph screening of drugs, TGA helps to confirm or discard the appearance of a new polymorph.

2.3.3. X-ray Diffraction (XRD). This technique is probably the most useful in the detection of a new polymorph, co-crystal and solvate. There exist two different methodologies of application

depending on the sample: powder diffraction for those polycrystalline samples and single crystal diffraction when a single crystal can be isolated. Usually powder methods are used to characterize qualitatively a new crystal phase while the single crystal method is used to determine the crystal structure. However, recently powder methods are being applied also to solve the crystal structure of microcrystalline solids.

2.3.4. Thermomicroscopy. Hot stage microscopy consists of a hot stage and a microscope equipped with a digital camera which provides useful information about thermal events, including meltings, solid-solid transitions, crystallizations, sublimations, desolvations and co-crystallizations. Although the visual observation of a polymorphic transition or a melting onset is subjective, this technique can provide very useful qualitative information about the polymorphic system under study. In addition, the observation of changes in size, shape, colour, etc. during hot stage experiments can be useful to detect the existence of polymorphism in a rapid screening study.

2.3.5. Other Techniques. Spectroscopic techniques such as Infrared, Raman and solid-state Nuclear Magnetic Resonance (NMR) can also provide useful information about different crystalline forms of the same organic compound and can be used to identify a pure crystal form and quantify a mixture of two forms.

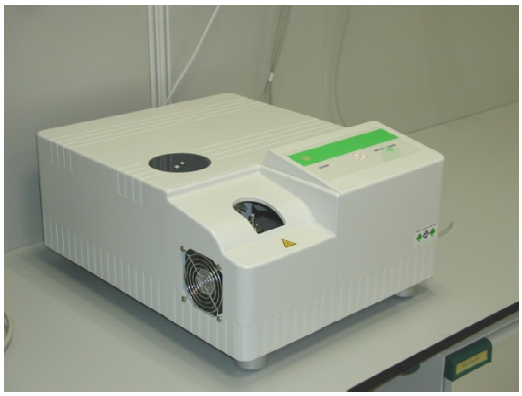


Figure 2: Differential Scanning Calorimeter.

Figure 3: Thermomicroscope.

2.4. Crystal Engineering and intellectual property

Polymorphs and co-crystals of a drug can be protected by patents since they represent different crystal forms of the same compound with different properties. The relevance of this issue in the pharmaceutical industry is extreme because the discovery of a new polymorph can be translated into the filing of a new patent application with important economical consequences. In this sense, a new polymorph (or a co-crystal) with improved properties such as stability or solubility could be marketed and displace other commercial polymorphs of the same API. Many examples in recent years, such as the Zantac or Cefadroxil cases (involving huge amounts of money) highlight the importance of having a deep knowledge of the polymorphic landscape during the development of a new drug. This is particularly relevant since generics started to be commercialized.

3. Examples of applications

In this section, we show some case studies conducted in our facilities which briefly illustrate the key topics of crystal engineering in reference to the pharmaceutical industry and new materials.

3.1. Norfloxacin

This API is a synthetic broad antibacterial fluoroquinolone compound used in the treatment of gonorrhea, prostate and urinary tract infections [10].

3.1.1. Anhydrous forms of Norfloxacin. The polymorphism screening on Norfloxacin conducted in our laboratory revealed the existence of up to three different anhydrous forms [11]. These three forms were characterized by means of X-ray Powder Diffraction (XRPD) (Fig. 4) and DSC (Fig. 5).

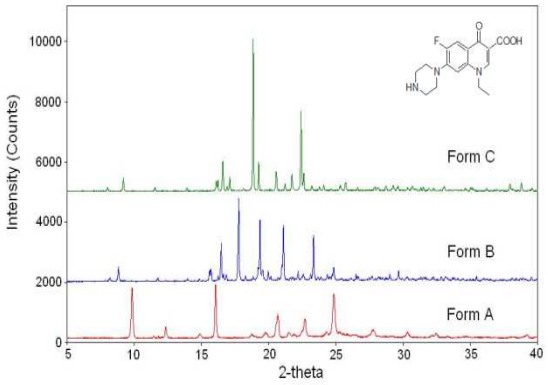


Figure 4: XRPD of forms A, B and C of Norfloxacin

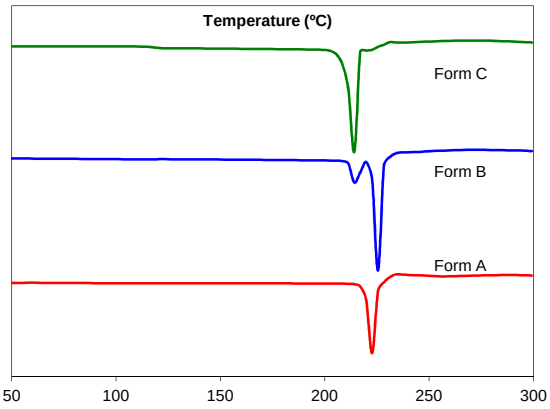


Figure 5: DSC thermograms of forms A, B and C of Norfloxacin

Form A is the higher melting form (219ºC) and form C melts at 207ºC. On the other hand, the DSC thermogram of form B shows the melting endothermic process of form B followed by an exothermic process (crystallization of the higher melting form A, the most stable at melting temperature) which happens simultaneously, being the net heat flow smaller than the expected for the melting of form B. Finally, melting of crystallized form A occurs. Occasionally, samples of pure polymorph B show endothermic solid-solid transition of form B to form A, followed by melting of remaining form B and subsequent melting of form A (Fig. 6).

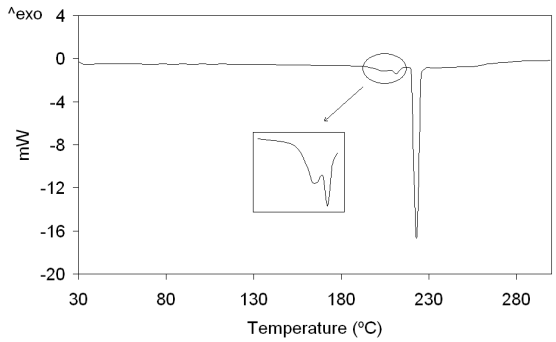


Figure 6: DSC of form B showing rare occasions when the sample underwent partly a solid-phase conversion at 196 ºC before melting

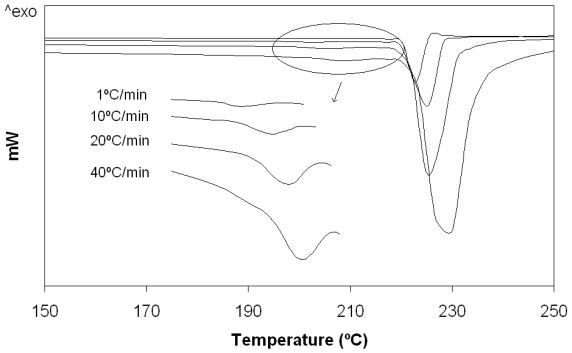


Figure 7: DSC of form A mixed with small amounts of form B, recorded at the heating rates of 1-40 ºC/min

When polymorph A is mixed with small amounts of polymorph B, an endothermic phenomenon is observed at 196 ºC in a DSC experiment at 10 ºC/min, followed by melting of form A. Heating rate influences the onset temperature of the first endotherm whereas the onset temperature of the melting of form A is not affected (Fig. 7), thus confirming that the first endotherm is a solid-solid transition.

It is of practical interest to know the relative thermodynamic stability of all forms. The main questions to solve are whether two polymorphs are monotropically (one form is more stable than the other at any temperature) or enantiotropically (a transition temperature exists, below and above which the stability order is reversed) related, and for an enantiotropic system, where transition

temperature lies. According to the "heat of transition rule" of Burger and Ramberger [12], forms A and B are enantiotropically related. Based on physicochemical data, a semi-schematic energy/temperature diagram was constructed in order to display the thermodynamic relationship of the three polymorphs at different temperatures (Fig. 8).

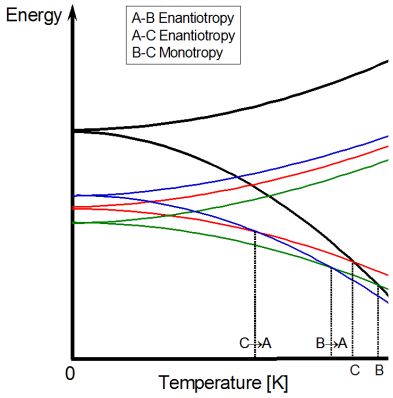


Figure 8. Semi-schematic energy/temperature diagram of Norfloxacin.

3.1.2. Hydrates of Norfloxacin. Pharmaceutical hydrates are important due to the presence of water molecules in so many pharmaceutical drugs, which affects to a variety of physical and chemical properties such as the stability, solubility and dissolution rate. Usually, hydrates are less soluble than their anhydrous forms because the interaction between the compound and the water molecules confers an extra thermodynamical stability [13]. Norfloxacin is an example which contradicts that general rule because the hydrated forms are more soluble than the anhydrous one. This implies that the hydration process plays an important role in influencing the bioavailability of Norfloxacin and it has made its hydrates an interesting object of study.

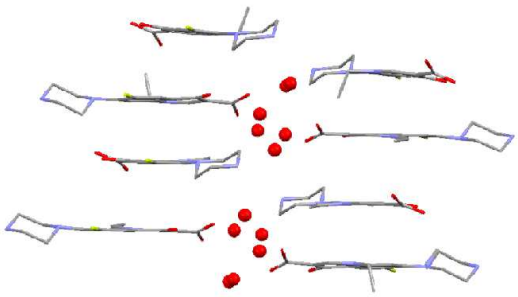


Figure 9: Water molecules forming channels in the crystal structure of form II

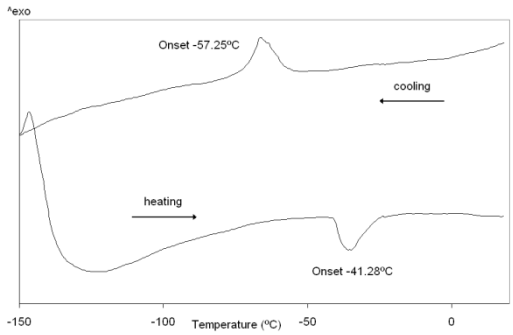


Figure 10: Cooling-heating DSC experiment, where the reversibility of the solid-solid transition of NF sesquihydrate can be observed

During this study, two polymorphs of Norfloxacin sesquihydrate were discovered and their crystal structures solved, one of them at low temperature [14] (Fig. 9). Taking into account that there could be a possibility for a phase transition while cooling, we decided to perform a DSC experiment cooling from room temperature to -150ºC and heating from -150ºC to room temperature. As it can be seen in Figure 10, a reversible solid-solid transition was observed at -57ºC while cooling and at -41ºC while heating. This experiment allows us to establish the relative thermodynamic stability between both forms, which is a matter of great relevance. According to the heat of transition rule forms I and II are enantiotropically related, being form I the most stable at room temperature and form II the most stable under approximately -40ºC. It was also possible to study the reversible phase transition of form I to form II by means of thermomicroscopy (Fig. 11).

Moreover, the variable temperature XRPD experiment demonstrates that form I of NF sesquihydrate undergoes a phase transformation into form II while cooling down (Fig. 12).

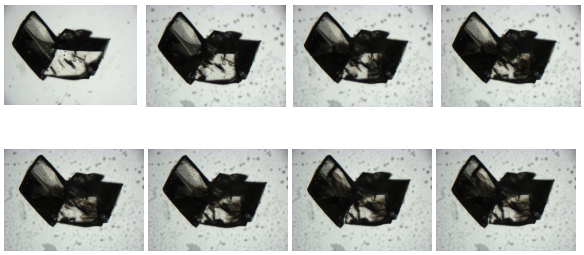


Figure 11: Photomicrographs of NF Sesquihydrate in polarized light.

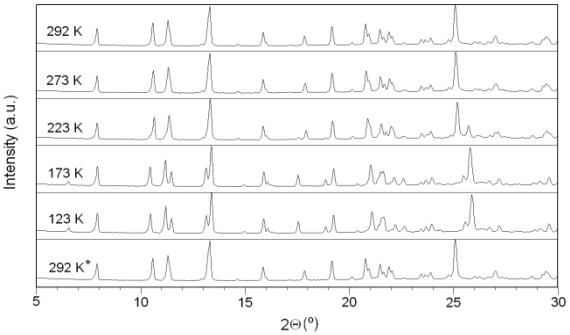


Figure 12: Variable XRPD of NF sesquihydrate showing the phase transition from form I to form II

ChT.1

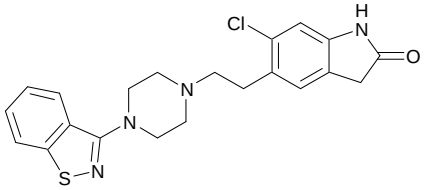
3.2. Ziprasidone

Ziprasidone is a poorly soluble drug in water, a factor that unfavorably affects its bioavailability [15]. Ziprasidone, an antipsychotic agent useful for the treatment of psychotic disorders of the schizophrenic types [16], is marketed under the name GEODON as an oral capsule and as an injectable drug. GEODON capsules contain the monohydrate hydrochloride salt of ziprasidone, whereas GEODON for injection contains ziprasidone mesylate trihydrate. The need for improved water soluble forms of ziprasidone has been recognized during the last years. Salts are usually employed for increasing aqueous solubility [17].

It is commonly accepted that the formation of a stable salt requires a difference of at least three units between the pK_a value of the ionisable group and that of the counterion [18]. So, we selected a range of acids according to this information. A microscale screening allowed us to discard some acids from the selected list before completion of the study because no salt was isolated from these acids. Next, a screening at a larger scale was developed with the remaining acids. The obtained salts were characterized by DSC, XRPD, IR and ^{1}H-NMR. Additionally, the aqueous solubility of these salts was determined in order to compare the data obtained with the previously known solubilities of hydrochloride and mesylate salts [19]. Results are reported in table 1.

Table 1 Aqueous solubilities of ZP salts by HPLC at 25ºC

Salt	Aqueous solubility (μg/mL)[a]
Free base	0.3
Hydrochloride	80
Mesylate	1000
Phosphate	28
Citrate	30
Fumarate	55
Oxalate	236
Isethionate	1121
Malate	475



[a]Solubility values indicate the weight in μg of Ziprasidone calculated as the free base, per mL of water.

As it can be seen, isethionate salt is the one with the highest solubility. However, the appearance of coloured impurities during its isolation together with the poor recovery forced us to reject this salt. Then, our efforts were directed towards the malate salt as the selected target for further studies due to its good solubility. Moreover, malic acid is generally regarded as safe (GRAS) by the Food and Drug Administration (FDA) and has previously been used in FDA-approved marketed drugs.

The malate salt of ZP was found to exist in three anhydrous crystalline forms. Each form was characterized by means of DSC, XRPD, IR and ^{1}H-NMR spectroscopy. The aqueous solubilities of the ZP malates were also evaluated (table 2). Interestingly, forms B and C show an improved, highly solubility value of 989 and 1084 µg/mL respectively, if compared to malate form A. In addition, these solubility values are in the same range as that of mesylate. In this sense, malate salts of ZP are good candidates for further pharmaceutical development.

Table 2 Aqueous solubilities of the malate salts of ZP by HPLC

Salt	Aqueous solubility (µg/mL)[a]
Malate form A	475
Malate form B	989
Malate form C	1084

[a]Solubility values indicate the weight in µg of ziprasidone calculated as the free base, per mL of water

ChT.1

3.3. Triphenylglycol

(*S*)-triphenylglycol is a chiral auxiliary reagent which has found wide application in asymmetric aldol additions and ester enolate imine condensations. It has been used also in synthesis of natural products and biological active compounds. Furthermore it has applications as a chiral solvating agent in NMR spectroscopy [20]. In order to explore the possible polymorphic behaviour of triphenylglycol, we decided to study by thermal analysis the amorphous form obtained from quenching the melt, instead of performing classical crystallizations methods [21]. Interestingly, the heating of the amorphous form in a DSC experiment shows a collection of crystallization and melting phenomena demonstrating the complex polymorphic system (Fig. 13). From the glassy state (glass transition at 32 ºC) form D crystallizes first and melts (mp 72 ºC) with crystallization of other phases. With continuous heating, forms E (mp 110 ºC), F (mp 116 ºC), B (mp 119 ºC) and A (mp 127 ºC) melt successively. It has been found that a heating rate of 10 ºC/min must be applied in order to observe the maximum number of polymorphs in a DSC experiment. Lower heating rates lead to the crystallization of fewer forms whereas higher heating rates are not suitable due to a loss in the resolution. The cooling rate during quenching seems to have no influence on the result.

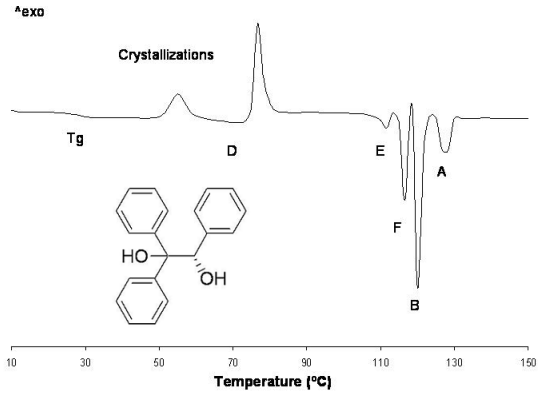


Figure 13: DSC of a quenched from the melt sample.

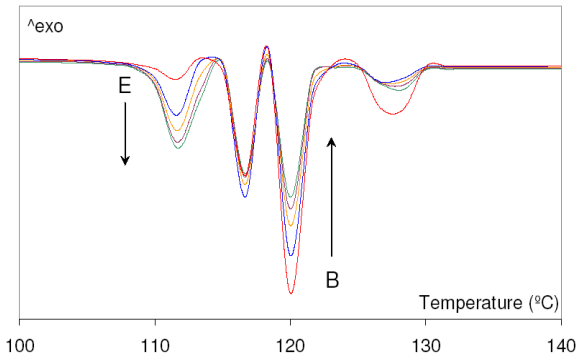


Figure 14: DSC of five successive heating/cooling cycles.

Since we were not able to isolate forms D, E and F by conventional crystallization methods, we explored alternative approaches in view of the observed thermal behaviour by DSC. After some heating/cooling cycles (Fig. 14) starting from form A in a DSC (heating rate 10 ºC/min), form E grows at expenses of form B. Crystallization of the various polymorphic forms are time and temperature dependent, and a thermodynamic/kinetic competition in the crystallization of the different forms from the melt is observed. This can be explained by some kind of kinetical amplification process leading to the metaestable form E. After four cycles, the relative intensities of

all forms remain invariable. This suggests that form E is the first crystallizing form which transforms into the other three forms by crystallization from the melt during heating.

3.4. Dibenzylsquaramide

Secondary squaramides are a family of synthetic compounds that exhibit interesting and useful properties as supramolecular synthons. Previous studies of secondary squaramides in solution demonstrate that these compounds can exist in several conformations due to the partially restricted rotation of the C-N bond (anti/anti and anti/syn conformers) [22]. We have studied the solid state behaviour of secondary squaramides in order to establish relations between the aforementioned properties and their possible polymorphism.

In this example we have chosen dibenzylsquaramide (DBZSQ) as the model compound to explore its possible polymorphic behaviour [23]. In order to obtain as many crystal forms as possible a polymorphic screening was carried out. DBZSQ is only soluble in polar media such as dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO). Therefore different combinations of those solvents with polar and non polar antisolvents were tested at several concentrations and temperatures, with variable cooling rates, in both thermodynamic and kinetic conditions, revealing a polymorphic system consisting of three polymorphs according to their different X-ray patterns.

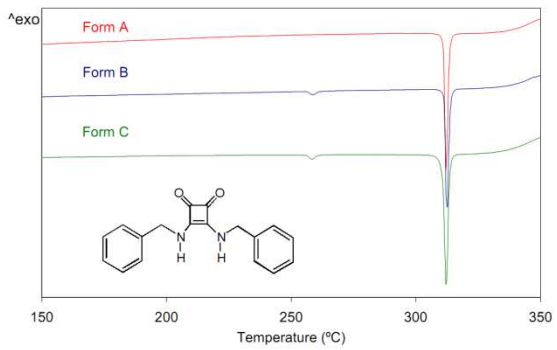


Figure 15: DSC of a quenched from the melt sample.

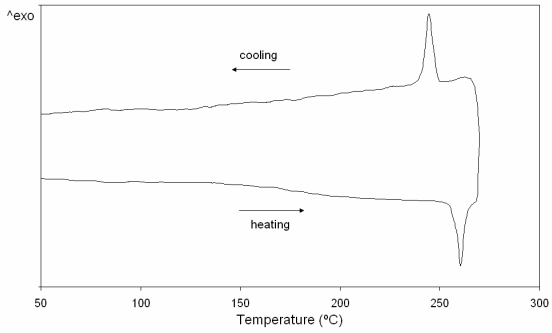


Figure 16: DSC of five successive heating/cooling cycles.

The DSC analysis of the three forms show the same sharp endothermic phenomenon at 311ºC but two of them also show a low intensity broad endothermic phenomenon at 257ºC (Fig. 15). Variable heating rates confirm that the first phenomenon is a solid-solid transition, with the second one being a melting process. This can be interpreted in the following manner: two forms (B and C) transform during the DSC analysis in the same polymorph (A) which melts at 311ºC.

Curiously, the transition temperature is observed around the same value in all the cases. Although several polymorphs can present the same melting value (e.g. conformational polymorphism), to the best of our knowledge this is the first time that two forms share the same transition temperature. Therefore, a misleading interpretation of this system could be concluded if based only on the DSC analysis.

According to the heat of transition rule [12] forms B and C are enantiotropically related to form A. This means that form A is a metastable form at room temperature. In addition, the reversibility of the solid transition could be observed in both cases by a heating-cooling DSC experiment (Fig. 16). Both forms B and C return to form B after cooling (confirmed by XRPD).

All the examples reviewed highlight the necessity of a multidisciplinary approach to solve a polymorphic problem or to design new crystal structures. Scientists from diverse areas such as physical-organic chemistry, supramolecular chemistry, physics or X-ray diffraction spectroscopy usually work together in ambitious research projects looking for new crystalline solids with improved properties.

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