

The solid state and solvent inclusion properties of gatifloxacin crystal forms

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CHAPTER 1

Solid state and solvent inclusion properties of pharmaceuticals

Introduction

During the development of a new pharmaceutical entity the solid state properties of the drug is an important factor to consider. The pharmaceutical solid can either be crystalline or amorphous. Crystalline solids have molecules that are packed in a specific arrangement. The smallest repeating unit in a crystal, a unit cell, defines this arrangement. The molecules are held together by various forces, including hydrogen bonding and Van der Waals interactions (Newman & Byrn, 2003:898). Amorphous solids are non-crystalline implying that there is no specific arrangement of the molecules in these solids (Newman & Byrn, 2003:898). A distinction should also be made between polymorphs, pseudopolymorphs (solvates), and desolvated solvates. A pharmaceutical solid displays polymorphism when it crystallises into different crystal packing arrangements with the same elemental composition. It exhibits pseudopolymorphism when it forms solvates. This is when the drug substance incorporates a solvent (water in the case of hydrates) into the crystal lattice in either stoichiometric or non-stoichiometric amounts. Desolvated solvates form when a solvate is desolvated and still maintains the initial solvated crystal structure (Byrn *et al.*, 1995:946). All these solid forms will be discussed in more detail later in this chapter.

To ensure that no undesired effects occur during the manufacturing and storage of pharmaceutical products, care should be taken to fully characterise and analyse the crystal forms of the drug and its behaviour. These undesired effects could occur due to polymorphic transition (including desolvation of solvated forms) that could affect the product performance and stability. These undesired effects might include changes in solubility, tableting properties, and chemical stability. A number of factors could lead to polymorphic transition, including grinding, exposure to high relative

humidities, exposure to increased temperatures during storage, or manufacturing processes such as wet granulation or milling (Zhang *et al.*, 2004:377-380).

The solid state properties of active pharmaceutical ingredients (API's) should therefore be studied and fully characterised during preformulation, to prevent stability related problems due to polymorphic transformations. Furthermore, the most appropriate form should be chosen for manufacturing to ensure product stability (polymorphic and chemical stability) during manufacturing and storage of the final dosage form.

1.1 Molecular Basics

1.1.1 The solid state

Most drugs exist as solids at normal room temperature and pressure. These solids consist of molecules held together by intermolecular forces (Buckton, 2002:141).

Pharmaceutical solids can be either *crystalline* or *amorphous*. The molecules in *crystalline* solids are packed in a defined order that is repeated throughout the crystal (Buckton, 2002:142). This repetition of molecules, also called structural units, leads to the formation of a single crystal (Byrn *et al.*, 1999:5).

Amorphous solids have no discernible crystal lattice or unit cell and consist of a disordered arrangement of molecules. Short-range order is possible due to the short-range intermolecular forces. However, no long-range order is possible and consequently the amorphous solid has no crystallinity (Grant, 1999:8). The difference between the molecular packing properties of crystalline and amorphous solids is illustrated in figure 1.1.

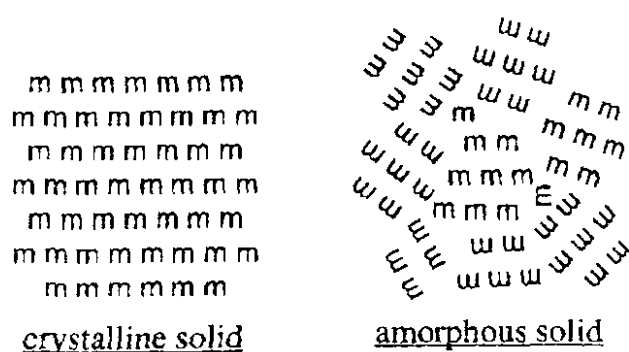


Figure 1.1 Schematic representation of a crystalline and amorphous solid (Yu, 2001:30).

Various methods are available in the pharmaceutical industry to prepare crystalline or amorphous solids (Byrn *et al.*, 1999:15). Some of these methods are listed in table 1.1.

Table 1.1 Common methods for the preparation of solids in the pharmaceutical industry (Byrn *et al.*, 1999:16)

Evaporation (including spray drying and slurry fill)
Cooling a solution
Seeding a supersaturated solution with crystals of the desired form
Freeze drying (including from mixed solvents)
Addition of antisolvents
Salting out
Changing pH
Addition of reagent to produce a salt or new compound
Deliberate phase transitions during slurry, washing or drying steps
Simultaneous addition of two solutions

Factors that may influence the crystallisation process should be considered during the preparation of a pharmaceutical solid. These factors include:

- a. Concentration (degree of supersaturation) of the API/solute.
- b. Temperature and cooling profile of the solution.
- c. Seeding and agitation.
- d. Interconversion of solid forms.

e. Composition, polarity, and pH.

f. Presence of additives and impurities (Byrn *et al.*, 1999:461).

All these factors play a very important role in the crystallisation of a solid and should therefore always be taken into consideration.

Crystal formation usually occurs from a supersaturated solution. This requires the formation of a critical number of ordered molecules, or unit cells, into nuclei. This process is known as primary nucleation. Formation of unit cells below the critical number does not lead to crystal formation, as these unit cells tend to dissolve again. Only assemblies above the critical number lead to growth of a recognisable crystal. The reason for this is that only when an assembly becomes larger, the internal bonds become more significant than the external forces. Thus the forces holding the crystal together become more pronounced than the forces pulling it apart, and crystal growth is possible (Byrn *et al.*, 1999:16-17).

Secondary nucleation may also occur. This is the process where further crystallisation occurs after the initial crystals are formed. This is not always desirable because it often leads to a large quantity of very small crystals (Byrn *et al.*, 1999:17).

If the crystallisation process is not strictly controlled it is possible for the polymorphic system to contain mixtures of crystal forms. This occurs when nuclei with different structures forms and coexists in one system (Byrn *et al.*, 1999:18). This concept is illustrated in figure 1.2, showing that either form I or II of the solid can crystallise individually, or a mixture of the two forms can be obtained. To avoid the formation of an undesired form or a mixture of forms, the abovementioned factors such as seeding, additives and temperature should always be well controlled.

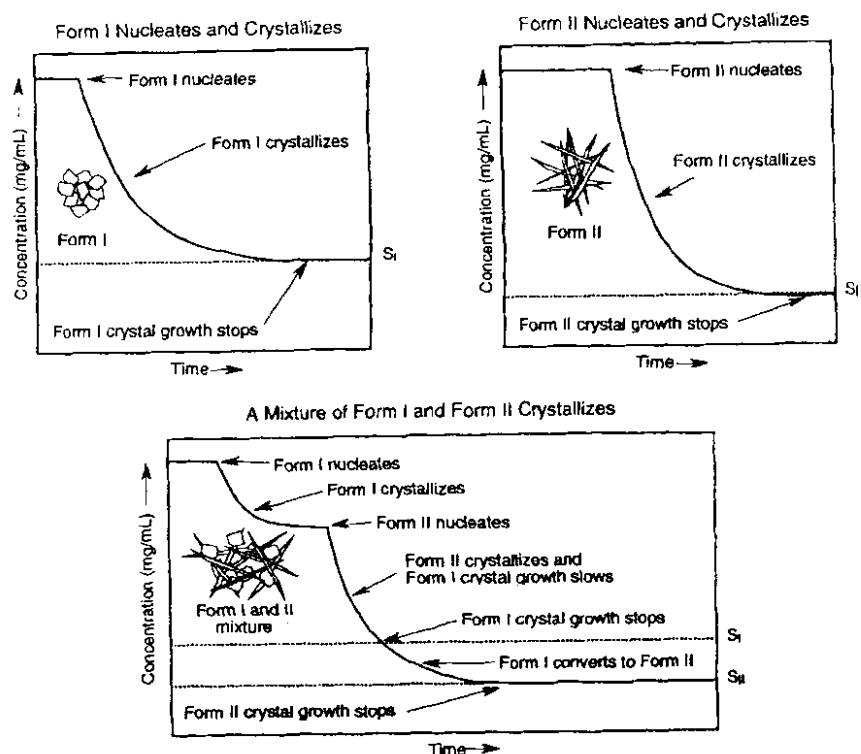


Figure 1.2 Uncontrolled crystallisation in a polymorphic system showing the different polymorphs (top panel) or the mixture of polymorphs (bottom panel) that can result (Byrn *et al.*, 1999:18).

The characterisation of a solid as either crystalline or amorphous refers to the internal structure of the solid, where crystalline solids have a repeating packing order forming a lattice, and the amorphous form have no long range order in its internal structure. However, a further differentiation can be made between the internal and external structure of the crystal. This includes distinguishing between different crystal habits and polymorphism or pseudopolymorphism (Buckton, 2002:148).

1.1.2 Structure of the API

When studying the various properties of a crystal and the influence thereof on product development, it is important to distinguish between the external and internal structure of the drug. Figure 1.3 provides a schematic characterisation tool that may be utilised to classify pharmaceutical solids:

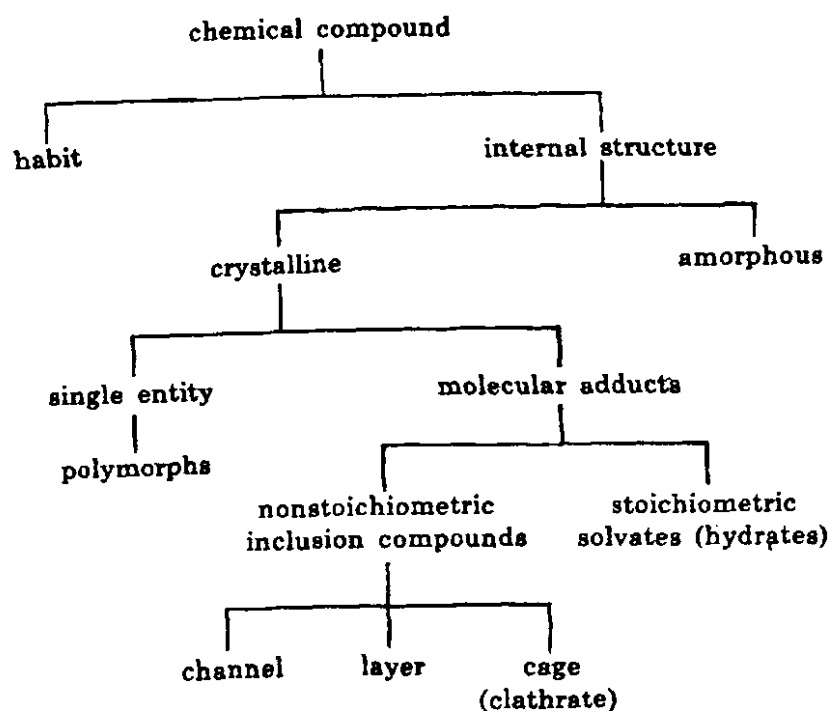


Figure 1.3 Classification of an API according to structure (Haleblian, 1975:1270).

1.1.2.1 External structure – Crystal Habit

The crystals of a solid can vary greatly in their shape and size depending on the solvent and method of recrystallisation used. The habit (external structure) in different samples may resemble needles, rods, plates etc. These differences in shape of the crystals are referred to as differences in *morphology* or *habit*. This could be an indication of polymorphism, although different habits don't necessarily indicate polymorphism. Further studies should be done to determine whether it is just different habits or if polymorphism exists in the system (Byrn *et al.*, 1999:12).

Byrn *et al.* (1999:13) defines different crystal *habits* as samples having the same chemical composition and the same crystal structure (*i. e.*, the same polymorph and unit cell), but displaying different shapes. The crystal shape is a consequence of the rate at which the different faces of the crystal grow. The packing geometry of the molecules in the lattice determines how many polar or non-polar functional groups are exposed on each face of the crystal. The growth on each face will then depend on the relative affinities of the solute for the solvent and faces of the crystal. If, for example, the crystals were grown from an aqueous solution, growth would occur on

the non-polar faces, making the polar faces dominate (Buckton, 2002:148). This concept is illustrated in figure 1.4.

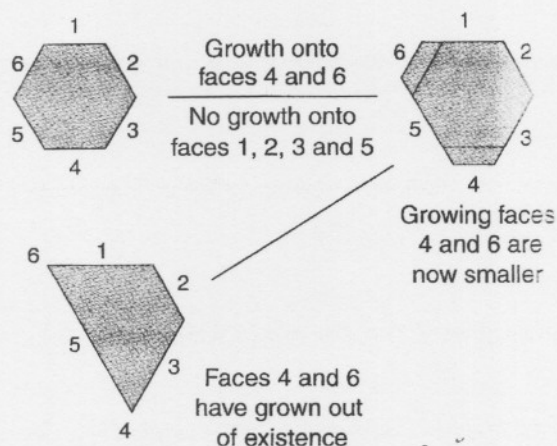


Figure 1.4 Demonstration of growth on faces 4 and 6 of a hexagonal crystal, resulting in the formation of a trapezium shaped crystal (Buckton, 2002:148).

Aspirin is a drug substance that displays different crystal habits. It was recrystallised from various solvents and each product displayed differences in morphology, as can be seen in figure 1.5. However, with further investigation it was revealed that these were all the same polymorph. These differences could be explained as different crystal habits of one polymorph (Byrn *et al.*, 1999:13).

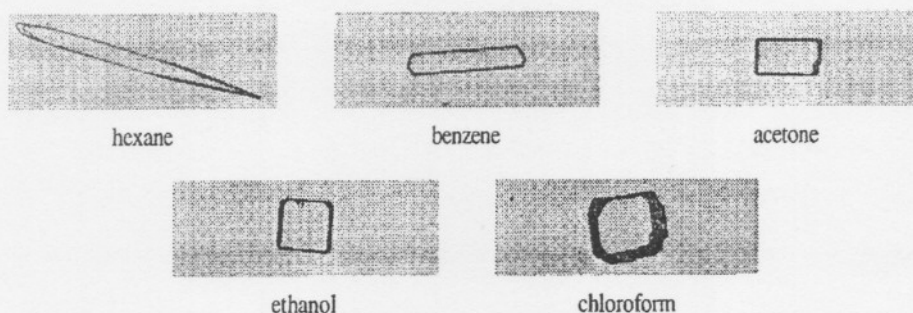


Figure 1.5 Aspirin crystals grown from different solvents (Byrn *et al.*, 1999:13).

Differences in crystal habits usually lead to altered drug properties. Important differences in the dissolution rate of the drug can occur if the surface area to volume ratio is altered. Dissolution rate is directly proportional to surface area, and therefore a needle shaped crystal, for example, would display much faster dissolution than a sphere shaped crystal of the same volume. Other properties such as powder flow can

also be altered by different crystal habits (Buckton, 2002:148). For example, sphere shaped crystals will exhibit better flow properties than needle shaped crystals. It should therefore be stressed that both the internal and external crystal structure should be taken into consideration when selecting the most suitable crystal form for pharmaceutical manufacturing.

1.1.2.2 Internal structure

The internal structure of API's is determined by the orientation and configuration of the molecular units and may thus be classified as: amorphous or crystalline. Crystalline solids may be classified as polymorphs or pseudopolymorphs. The classification of crystalline pharmaceutical solids will now be discussed in greater detail.

1.1.2.2.1 Polymorphism

Many drug substances can exist in various polymorphic forms. Byrn *et al.* (1999:13) defined *polymorphism* as two crystals having the same chemical composition but different internal structure or molecular packing. Figure 1.6 illustrates this concept, showing two polymorphic forms (a) and (b), of a crystal consisting of the same structural unit.

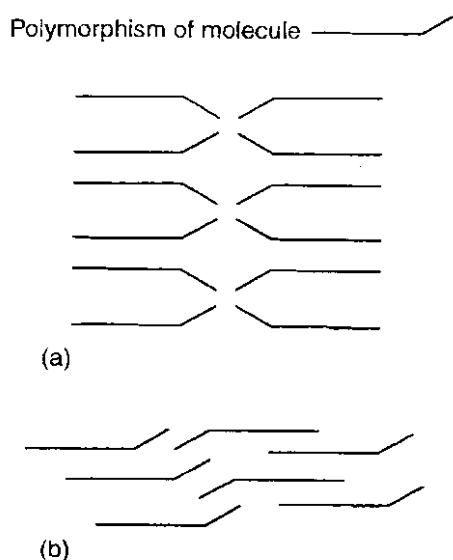


Figure 1.6 Representation of two polymorphic forms [(a) and (b)] of a crystal consisting of the same molecule (Buckton, 2002:142).

The various polymorphs of a drug substance usually exhibit different physical properties. These differences are a result of the different dimensions, shape, symmetry and void volumes of the unit cells of the various polymorphs (Grant, 1999:5-8). A list of some common properties that differ among polymorphs are given in table 1.2.

Table 1.2 List of physical properties that differ among various polymorphs (Grant, 1999:7)

1. Packing properties
a. Molar volume and density
b. Refractive index
c. Conductivity, electrical and thermal
d. Hygroscopicity
2. Thermodynamic properties
a. Melting and sublimation temperatures
b. Internal energy
c. Enthalpy
d. Entropy
e. Thermodynamic activity
f. Solubility
3. Spectroscopic properties
a. Electronic transitions (ultraviolet – visible absorption spectra)
b. Vibrational transitions (infrared absorption spectra)
c. Nuclear spin transitions (nuclear magnetic resonance spectra)
4. Kinetic properties
a. Dissolution rate
b. Rate of solid state reactions
c. Stability
5. Surface properties
a. Surface free energy
b. Habit
6. Mechanical properties
a. Hardness
b. Tensile strength
c. Compactability, tableting
d. Handling, flow

Differences in any of these properties could ultimately lead to differences in product performance and should therefore be identified early in the drug development process.

1.1.2.2.2 Pseudopolymorphism

Solvent molecules can be included into the various crystal forms. This is called *pseudopolymorphism* (Byrn *et al.*, 1999:13). If the solvent that is included into the crystal lattice is water, this crystal form is called a hydrate. If a solvent (other than water) is included, it is called a solvate (Buckton, 2002:144-145). In hydrates water usually reside in specific crystallographic sites in the solid, although it is also possible for the water to be located in tunnels within the crystal. Such hydrates, where the water is included in tunnels within the crystal, are easily influenced by changes in relative humidity and the water content may vary accordingly (Byrn *et al.*, 1999:23-24).

Hydrates or solvates can also exhibit polymorphism. This is true for hydrates or solvates that has solvent incorporated in the same stoichiometric proportion, but displays different crystal structures (Khankari & Grant, 1995:62).

Practically any laboratory solvent can be involved in solvate formation (Byrn *et al.*, 1999:236). Table 1.3 lists some commonly used solvents in solvate formation. Inclusion of specific solvent molecules in a crystal lattice can stabilise the structure. This is achieved by improving either the packing or the intermolecular bonding, especially hydrogen bonding. However, some solvents don't hydrogen bond and these solvents can only serve in a space occupying capacity. Usually the hydrogen bonding capacity of the included solvent molecules are fully exploited (Byrn *et al.*, 1999:234).

Table 1.3 Solvents used in solvate formation with drugs and organic compounds
(Byrn *et al.*, 1999:236)

Water
Methanol, ethanol, 1-propanol, isopropanol, 1-butanol, isobutanol
Acetone, methyl ethyl ketone
Acetonitrile
Diethyl ether, tetrahydrofuran, dioxane
Acetic acid, butyric acid, phosphoric acid
Hexane, cyclohexane
Benzene, toluene, xylene
Ethyl acetate
Ethylene glycol
Dichloromethane, chloroform, carbon tetrachloride
Pyridine
Dimethylsulfoxide

Currently there are various classification schemes available for solvates. Mostly solvates are classified by structure or energetics. One such structural classification scheme is given in table 1.4. Here the crystalline hydrates are divided into three classes, which can be distinguished by the commonly available analytical techniques such as differential scanning calorimetry, thermogravimetric analysis, x-ray powder diffraction and infrared-spectroscopy (Morris, 1999:141). A brief discussion of these classes follows table 1.4.

Table 1.4 Structural classification of crystalline hydrates (Morris, 1999:141)

Class	Description
1	Isolated lattice sites
2	Lattice channels
-a	Expanded channels (non-stoichiometric)
-b	Lattice planes
-c	Dehydrated hydrates
3	Metal-ion coordinated water

According to this scheme, class 1 is the isolated site hydrates. This class represents the structures with water molecules that are isolated from direct contact with other water molecules (Morris, 1999:142).

The channel hydrates form class 2. These hydrates contain water molecules in lattice channels, where they lie next to other water molecules of bordering unit cells, forming "channels" through the crystal (Morris, 1999:145).

The last class in this scheme, class 3, is the ion associated hydrates. These hydrates contain metal ion coordinated water. The most important concern with these hydrates is the effect of the metal-water interaction on the structure of the hydrate. Sometimes this interaction is so strong that dehydration takes place only at relatively high temperatures (Morris, 1999:155).

The inclusion of water or another solvent into the crystal lattice of an API usually alters the thermodynamic activity of the API. The dissolution rate, solubility and physical and chemical stability of the solid is often influenced (Khankari & Grant, 1995:64). Sufficient characterisation of the pharmaceutical hydrates (by means of x-ray powder diffractometry, infrared spectrometry, thermogravimetric analysis, etc.) is necessary to ensure predictable and reproducible behaviour when such a form is used in product development (Morris, 1999:160).

1.1.2.2.3 Amorphous state

As already described in section 1.1.1, the amorphous solid has no discernible crystal lattice and no crystallinity. Amorphous solids have very different properties from its corresponding crystalline form of the same chemical entity. One important difference that can be observed is that crystalline forms have a specific melting point and the amorphous form might not have a melting point. This is due to the fact that an amorphous solid does not have a crystal lattice to break (Buckton, 2002:145). However, amorphous forms have a characteristic temperature at which they undergo a change in properties. This temperature is referred to as the glass transition temperature (T_g). Below this temperature the amorphous form is brittle and in a glassy state. Above T_g it becomes rubbery (Buckton, 2002:146).

Amorphous solids often have higher solubility, higher dissolution rate and better compression properties than its corresponding crystalline form. However, amorphous solids are usually less physically and chemically stable than the crystalline form of the solid (Yu, 2001:28). Therefore amorphous solids are seldom the preferred forms

for inclusion in solid dosage forms, although it may provide the benefit of great solubility in water.

It is also possible for amorphous solids to exhibit a form of polymorphism. This is referred to as *polyamorphism*. Hancock *et al.* (2002:1151) defined *polyamorphism* as the possible existence of two distinct amorphous states of the same chemical entity separated by a clear phase transition. However, the occurrence of true *polyamorphism* is very rare in the pharmaceutical sciences, and therefore it will not be discussed in greater detail for the purpose of this study.

1.1.2.2.4 Desolvated solvates (Isomorphism)

Desolvated solvates are compounds that lose their solvent but retain the same overall molecular packing as the original solvate. This is often referred to as *isomorphism*. These compounds display the same or only subtly shifted x-ray powder diffraction patterns than the solvated forms, even though desolvation caused changes in the solid composition of the drug (Yu *et al.*, 1998:124).

Desolvated solvates are usually unstable, extremely hygroscopic and they easily sorb water even at ambient moisture (Yu *et al.*, 1998:124).

1.1.2.2.5 Clathrates

In order to complete the classification of an API according to its structure, clathrates are briefly mentioned here, although they will not be discussed in detail. According to the Merriam-Webster Online Dictionary (2005), a clathrate is defined as a compound formed by the inclusion of molecules of one kind in cavities of the crystal lattice of another. An example of this phenomenon is warfarin sodium isopropanol clathrate, where the ratio warfarin sodium:isopropanol:water may vary between 8:4:0 and 8:2:2 respectively (Raw *et al.*, 2004:411).

1.2 Solvent inclusion

Solvents may be introduced into the crystal lattice by various methods, such as recrystallisation or moisture absorption on exposure to high relative humidities.

More than one solvent can be incorporated into a crystal structure. These two or more solvent molecules each occupy a different position in the crystal lattice. Byrn *et al.* (1999:234) stated that it is also possible for one solvent to be incorporated in different ratios into the crystal (2:1, 1:1, etc.). These ratios are often stoichiometric, but this is not always the case (Newman & Stahly, 2002:30). Table 1.5 lists some common hydrate ratios.

Table 1.5 Common hydrate ratios (Newman & Stahly, 2002:31)

Ratio of organic:water molecules	Hydrate type
2:1	Hemihydrate
1:1	Monohydrate
2:3	Sesquihydrate
1:2	Dihydrate
1:3	Trihydrate
1:4	Tetrahydrate
1:5	Pentahydrate

Various factors influence solvate formation, including solvent used, degree of saturation and temperature. Low temperatures favour the formation of solvates and also usually lead to inclusion of higher stoichiometric amounts of a given solvent. This is most likely due to the increased strength of the hydrogen bonding at low temperatures (Byrn *et al.*, 1999:234). The influence of various temperatures on the formation of hydrates is illustrated in figure 1.7. It is obvious that a higher stoichiometric amount of solvent is included at lower temperatures (Byrn *et al.*, 1999:239).

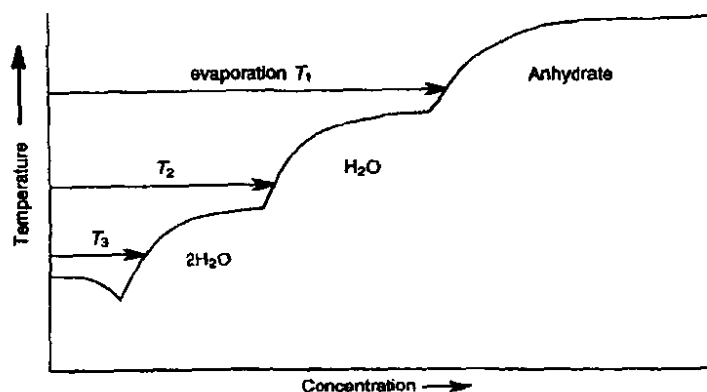


Figure 1.7 Crystal forms produced when evaporations are performed at different temperatures (Byrn *et al.*, 1999:239).

1.2.1 Mechanism of solvent inclusion

Two mechanisms of solvent inclusion, namely recrystallisation and moisture sorption, will be discussed in this section.

1.2.1.1 Recrystallisation

Crystallisation from a single solvent or from a mixture of solvents can lead to solvent inclusion in the crystal lattice. Sometimes one solvent within the crystal lattice is exchanged for another. For example, recrystallisation of a hydrate with dry methanol often leads to formation of a methanol solvate (Guillory, 1999:207).

Many laboratory solvents can be used in recrystallisation, as already mentioned in table 1.3.

1.2.1.2 Moisture sorption

Many crystalline solids sorp water from their immediate atmosphere. These solids are termed hygroscopic. Hygroscopicity is a function of the atmospheric relative humidity. Many solids are hygroscopic in high relative humidity and only a few at low humidity (Byrn *et al.*, 1999:23).

The hygroscopicity of a drug can further be influenced by its surface area and porosity. A large surface area, having more sites for adsorption, will lead to a quicker uptake of moisture (Byrn *et al.*, 1999:23).

Vapour pressure versus relative humidity plots are an easy way to determine how a solid reacts with regard to water sorption. An idealised vapour pressure versus relative humidity plot is given in figure 1.8.

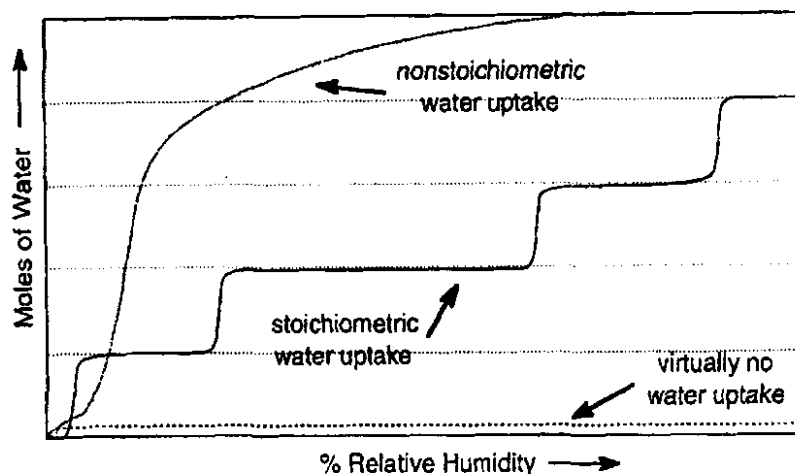


Figure 1.8 An idealised vapour pressure versus relative humidity plot (Byrn *et al.*, 1999:24).

Figure 1.8 illustrated that water uptake can occur through three mechanisms:

1. Virtually *no* water uptake.
2. *Nonstoichiometric* water uptake. This is a steady water uptake that usually occurs with amorphous solids or nonstoichiometric hydrates (hydrates with no specific water molecule to host molecule ratio).
3. "Stair-step" water uptake that occurs with *stoichiometric* hydrates. *Stoichiometric hydrates* are hydrates with a specific water molecule to host molecule ratio (Byrn *et al.*, 1999:24).

1.2.2 Forces and functional groups involved in solvent inclusion

A range of forces is responsible for solvent inclusion and should therefore be included in a discussion on this topic. These forces include hydrogen bonds, Van der

Waals interactions such as ion - dipole forces, dipole - dipole forces, and covalent forces.

Water has a multidirectional hydrogen bonding capability that makes it ideal for connecting many drug molecules into stable crystal structures (Byrn *et al.*, 1999:236). This interaction occurs as follows: the water molecules in hydrates behave as if it has two positive and two negative regions. It interacts on the negative regions with neighbouring molecules through a coordinate covalent bond or by accepting a hydrogen bond. The positively charged regions interact with neighbouring molecules through a donated hydrogen bond (Khankari & Grant, 1995:62).

These neighbouring molecules of a water molecule in a hydrate include electron acceptor groups and electron donor groups. Electron acceptor groups are proton donors such as M^{n+} , R-OH, and R_1R_2NH . Electron donor groups (or proton acceptors) include $R-COO^-$, $R-O^-$ and Cl^- . Other water molecules may also be suitably positioned for hydrogen bonding to a neighbouring water molecule (Khankari & Grant, 1995:62).

A dipole interaction is made possible because of a slight imbalance of charge on one end of a molecule. This makes it behave like a bar magnet. Packing together of the molecules to form a solid leads to alignment of these dipoles, and an attraction occurs between the positive pole of one and the negative pole of another (Buckton, 2002:141).

Induced dipoles do not have this imbalance in the free molecules. However, an imbalance is induced when another molecule is brought into contact with the first (Buckton, 2002:141).

1.3 Influence of different crystal modifications (amorphous, polymorphic & pseudopolymorphic) on the solid state properties of pharmaceutical solids

The hydration-dehydration cycle of the pharmaceutical solid may lead to formation of a metastable or stable form, an amorphous form, or mixtures of various crystalline

forms, including other hydrates or solvates. The product quality can be influenced by any of these changes (Zhang *et al.*, 2004:378). Incorporation of a solvent into the crystal lattice of a solid produces a new unit cell with different physico-chemical properties than the original solid form (Khankari & Grant, 1995:62). These properties include changes in solubility, dissolution, chemical and physical stability. Some of these properties will now be discussed.

1.3.1 Solubility

The hydration state of a solid can change the thermodynamic activity of the solid and this can influence its pharmaceutically important properties, such as solubility, flowability, etc. Changes in solubility usually lead to changes in dissolution behaviour, which can modify the bioavailability and product performance of the drug (Khankari & Grant, 1995:64-65). It is therefore important to understand the influence of the hydration state on the drug, to avoid such complications.

Solubility is defined as the equilibrium concentration of the dissolved solid (the solute) in the solvent medium. This is ordinarily a function of temperature and pressure (Brittain & Grant, 1999:282). At equilibrium concentration the solid does not dissolve or crystallise anymore (Byrn *et al.*, 1999:15).

Usually the anhydrous form is more soluble than the hydrated form (Buckton, 2002:145). The reason for this is that the hydrate has already interacted with the water. The free energy released on crystal dissolution and the further interaction with water is more for the anhydrate than the hydrate (Khankari & Grant, 1995:65). Since solubility is usually a measurement of stability, it can be deduced that the hydrate, which is less soluble than the anhydrate, is usually the most stable form (Byrn *et al.*, 1999:19-20). Various studies, such as the studies on niclosamide and theophylline discussed below, have proved this to be true.

In a study, conducted by Van Tonder *et al.* (2004:426-427), the solubility of niclosamide anhydrate and two monohydrates, H_A and H_B, were compared in water at 25°C. The monohydrate forms were significantly less soluble than the anhydrous form. Monohydrate H_B was the least soluble. The stability of the various forms were determined from the solubility data and confirmed that the anhydrous form was the

least stable form, with monohydrate H_A more stable and monohydrate H_B being the most stable form (Van Tonder *et al.*, 2004:427).

Another drug that displayed improved solubility of the anhydrous form above the hydrated form, is theophylline. Figure 1.9 compares the aqueous solubility of the anhydrate and hydrated forms of theophylline at various temperatures. The anhydrous form has a higher aqueous solubility than the monohydrate at temperatures below 60°C (Rodriguez-Hornedo *et al.*, 1992:153).

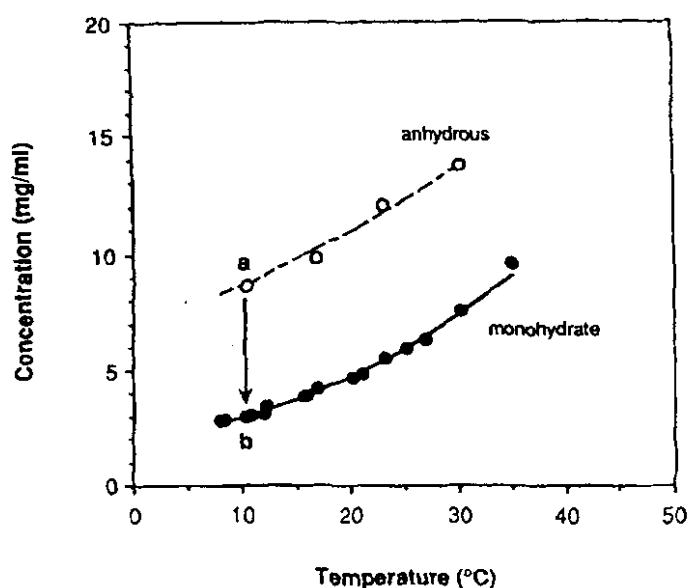


Figure 1.9 Solubility curves for anhydrous and monohydrate theophylline in pH 6 phosphate buffer (Rodriguez-Hornedo *et al.*, 1992:153).

1.3.2 Tableting properties

Various solid crystal forms of a drug can differ in their tableting properties such as compressibility, brittleness and deformation (Zhang *et al.*, 2004:386).

Differences in powder flow are common between polymorphs. This is due to differences in the morphology of the polymorphs. Polymorphs with needle- or rod-shaped particles may exhibit poor flow, while polymorphs with cubic habit or irregular spheres may have better flowability (Singhal & Curatolo, 2004:338).

Direct compression is a fast, easy and very efficient compression process used in tablet manufacturing. For a drug to be incorporated into a tablet by direct compression, it should exhibit certain physico-chemical properties. These properties include that the drug should be free flowing and highly compressible (Fachaux *et al.*, 1995a:123).

The solvation state of a drug could influence these properties of the drug, and could be used to modify these properties of the drug. Paracetamol is a drug with poor flow and compression properties. However, when a dioxane hemisolvate is formed and subsequently desolvated, it produces sintered-like paracetamol crystals (Fachaux *et al.*, 1995b:129). These crystals have a rounded shape and displays good flow properties. Good compressibility is achieved with this sintered crystal structure (Fachaux *et al.*, 1995b:132).

One should always bear in mind that the tableting properties of a drug can be influenced by the hydration / solvation state of the drug. This is especially important when choosing a suitable solid form for development. Attention should be paid to this to avoid a solid form used in tablet manufacturing with inadequate flow and compression properties.

1.3.3 Dissolution properties

Solvent inclusion can often lead to differences in the dissolution profiles of pharmaceutical solids. This is an important aspect to investigate, because differences in dissolution can often lead to differences in bioavailability. Compounds that often display bioavailability problems when tested usually have low aqueous solubility or display low wettability. These include carbamazepine and warfarin and care must be taken to ensure that the intended crystal form of the drug is controlled (Byrn *et al.*, 1999:91).

Carbamazepine is an anticonvulsant drug with a narrow therapeutic index, and transformation of the metastable form to the more thermodynamically stable form in water will lead to a decrease in the concentration of the drug dissolved. This might lead to increased risk of seizures (Murphy *et al.*, 2002:122). Tablets of carbamazepine are formulated with the anhydrous monoclinic form. This form

exhibits a higher solubility and faster dissolution rate than the dihydrate form (Murphy *et al.*, 2002:122). However, the anhydrous monoclinic form undergoes a phase change to the dihydrate during dissolution, as reported by Lowes *et al.* (1987:748-749) and Kobayashi *et al.* (2000:143). This transformation can be observed in the concentration-time profile as a change in slope, as figure 1.10 shows (Murphy *et al.*, 2002:126). The phase transformation is solution-mediated and involves three steps: (a) dissolution of the metastable form, anhydrous monoclinic carbamazepine, (b) nucleation of the stable form, dihydrate carbamazepine, and (c) crystal growth. The rate-determining step in the transformation is the crystallisation of the stable form (Murphy *et al.*, 2002:130).

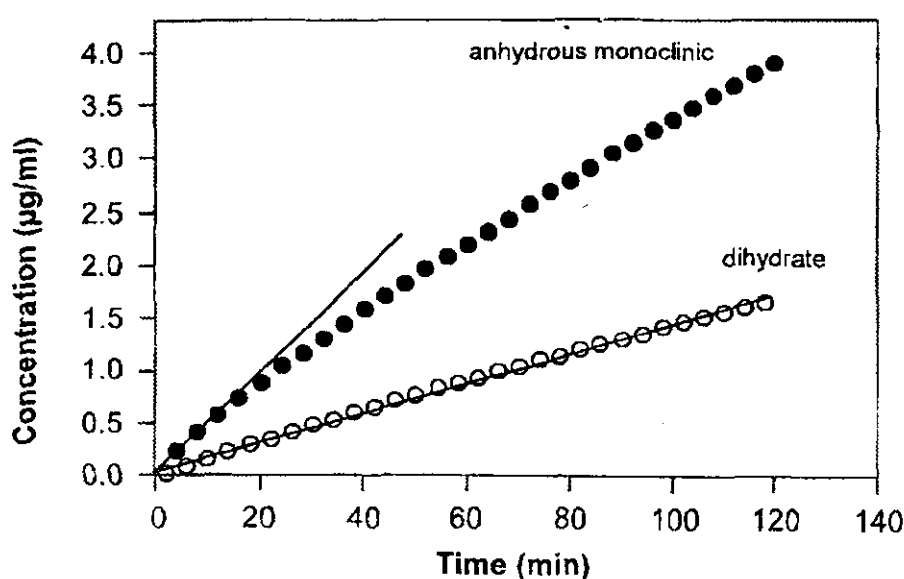


Figure 1.10 Typical concentration profiles during the dissolution of anhydrous monoclinic and dihydrate carbamazepine in water at 25°C (Murphy *et al.*, 2002:126).

Particle size should also be considered when studying the dissolution profiles of pharmaceutical solids. The particle size is directly related to the exposed surface area during the dissolution test and can therefore play an important role in the dissolution behaviour of a solid form (Byrn *et al.*, 1999:92).

1.3.4 Chemical stability

The various crystal forms of a drug have different properties, such as melting points, densities and crystal structures. In the same way, these different crystal forms also have different chemical stabilities (Byrn *et al.*, 1999:222). It is important to control the polymorph or solvate in cases where chemical stability is a problem (Byrn *et al.*, 1999:223).

Amorphous forms are often more reactive than crystalline forms (Byrn *et al.*, 1999:223). This is because of the lack of three-dimensional crystalline lattice, higher free volume and greater molecular mobility (Singhal & Curatolo, 2004:337).

Although many chemical degradation reactions are possible, only oxidation and hydrolysis will be discussed here.

1.3.4.1 Oxidation

Different crystal forms of a solid drug often display different reactivity on exposure to oxygen. The oxidation reaction of a solvate is often preceded by desolvation, although this is not always a necessity for the reaction to occur (Byrn *et al.*, 1999:344).

In a study conducted by Lin *et al.* (1982:2979) various polymorphs and pseudopolymorphs of cortisol *tert*-butylacetate were prepared. Form I was the most common form. It crystallises as a non-stoichiometric solvate from ethanol (the presence of 0.9 mol of ethanol was calculated from elemental analysis). Other solvents that also yielded Form I are propanol, *n*-amyl alcohol and acetonitrile. However, when Form I was recrystallised from these solvents, it contained no solvent from recrystallisation (Lin *et al.*, 1982:2979).

Table 1.6 lists the various crystal forms that were obtained, as well as the solvents used to obtain them.

Table 1.6 Different crystal forms of 21-cortisol *tert*-butylacetate (Lin *et al.*, 1982:2979)

Crystal form	Solvent of recrystallisation	Ethanol content
I	Ethanol, propanol, <i>n</i> -amyl alcohol, acetonitrile	0.9
II	Ethanol	1.0
III	Ethanol, <i>tert</i> -butyl alcohol	-
IV	Pyridine	-
V	Heat forms I, II or III	-

All the forms obtained in this study were tested for oxidation on exposure to UV light. Oxidation of 21-cortisol *tert*-butylacetate produces 21-cortisone *tert*-butylacetate, as illustrated by figure 1.11.

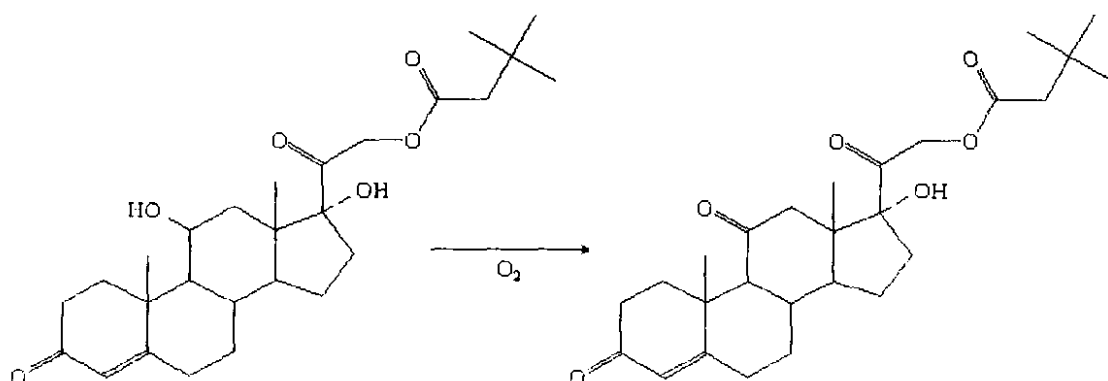


Figure 1.11 Oxidation of 21-cortisol *tert*-butylacetate (Lin *et al.*, 1982:2978).

Forms I and IV were the only forms that underwent oxidation when exposed to ultraviolet light in air. All other forms were inert to irradiation with UV light (Lin *et al.*, 1982:2980). Also, a slight difference in oxidation behaviour could be observed when comparing crystals of form I that have lost ethanol of crystallisation with crystals of form I obtained from *n*-amyl alcohol. The crystals obtained from *n*-amyl alcohol, which contained no solvent from crystallisation, were slightly less reactive than those obtained from ethanol, in which the ethanol has already been lost (Lin *et al.*, 1982:2981).

It was proposed that the reactivity of 21-cortisol *tert*-butylacetate form I towards oxygen was caused by the crystal packing of this form. The packing of this form

allowed penetration of oxygen down the axis of the helix of the crystal. It was also hypothesised by the authors that ethanol of recrystallisation was along this axis. Therefore loss of ethanol from the crystal aided further oxygen penetration. The authors stated that further crystallographic studies are still required to confirm the mentioned hypotheses (Byrn *et al.*, 2001:122).

The only other form that underwent oxidation when exposed to ultraviolet light in air, was form IV, an unstable form recrystallised from pyridine (Lin *et al.*, 1982:2979).

1.3.4.2 Hydrolysis

The reaction of pharmaceutical solids with water vapour is of utmost importance in the pharmaceutical industry. Degradation of various tablets and powders are increased in the presence of high humidities (Byrn *et al.*, 1999:357). This could possibly be due to hydrolysis of the API. The reaction of different crystalline forms of a solid drug with water vapour can differ, and it is therefore necessary to investigate this in preformulation studies.

Etter (1976:5326) crystallised 1-methoxy-1,2-benziodoxolin-3-one in two polymorphs, α and β . Both forms are readily hydrolysed by water vapour. The α -form showed a slow decomposition under ambient conditions. A loss of transparency occurred and a speckled appearance developed after several months. Under similar conditions the β -form hydrolysed within only a week or two (Etter, 1976:5328).

Hydrolysis reactions can lead to formation of crystals with a preferred crystallographic orientation or amorphous solids can be produced (Byrn *et al.*, 1999:363).

1.3.5 Physico-chemical properties

Control during the manufacturing process should be very strict to ensure that batch, as well as batch-to-batch variations do not occur. Precautionary measures may include good manufacturing practices (GMP) and control of the raw materials, processes, equipment, and packaging. Transitions within the solid form of the drug

present in the dosage form are possible, depending on the physical and chemical stability of the API. These phase-transformations can also be induced by excipients in the formulation, as well as the manufacturing method used. Such transitions in the crystal form of the drug within the formulation often results in changes in the quality and performance of the product, and is therefore undesired (Zhang *et al.*, 2004:377). In order to control and prevent product inconsistencies, it is important to understand how the different crystal forms of the drug differ in their physico-chemical properties.

Physico-chemical properties of various crystalline forms may differ. Techniques that can be used to observe these differences include: x-ray powder diffraction (XRPD), infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), hot-stage microscopy (HSM) and scanning electron microscopy (SEM).

1.3.5.1 X-ray powder diffraction (XRPD)

X-ray powder diffraction is a method used for the characterisation of pharmaceutical solids. The diffractograms obtained from this analysis are usually characteristic for each crystal form of the compound and can be considered as a fingerprint of the crystal form. This technique is often used to distinguish between different crystal forms or identify whether a specific form have been developed. Quantitative analysis of a mixture of crystal forms can also be performed to determine the relative amount of each crystal form present in the mixture (Newman & Byrn, 2003:899).

Another useful aspect of XRPD is that it can be used to characterise the (i) solvated versus desolvated and (ii) amorphous versus crystalline forms of a solid, as these forms often exhibit pronounced differences in their powder x-ray diffraction patterns (Suryanarayanan, 1995:216). In the same way different hydrates also exhibit different XRPD patterns. This is illustrated in a study conducted by Zupancic *et al.* (2005:65) where the XRPD patterns of various hydrated forms of pantoprazole sodium was compared. The different hydrates were prepared from different solvents. A monohydrate was obtained from acetone, a sesquihydrate from purified water and two unknown hydrates, resolved as form A and form B, were each prepared from ethyl-acetate and alkaline borate buffer at pH 9 (Zupancic *et al.*, 2005:61). Forms A

and B were further investigated and found to be a hemihydrate and dihydrate respectively (Zupancic *et al.*, 2005:67). The x-ray diffractograms of the various hydrates of pantoprazole sodium are illustrated in figure 1.12.

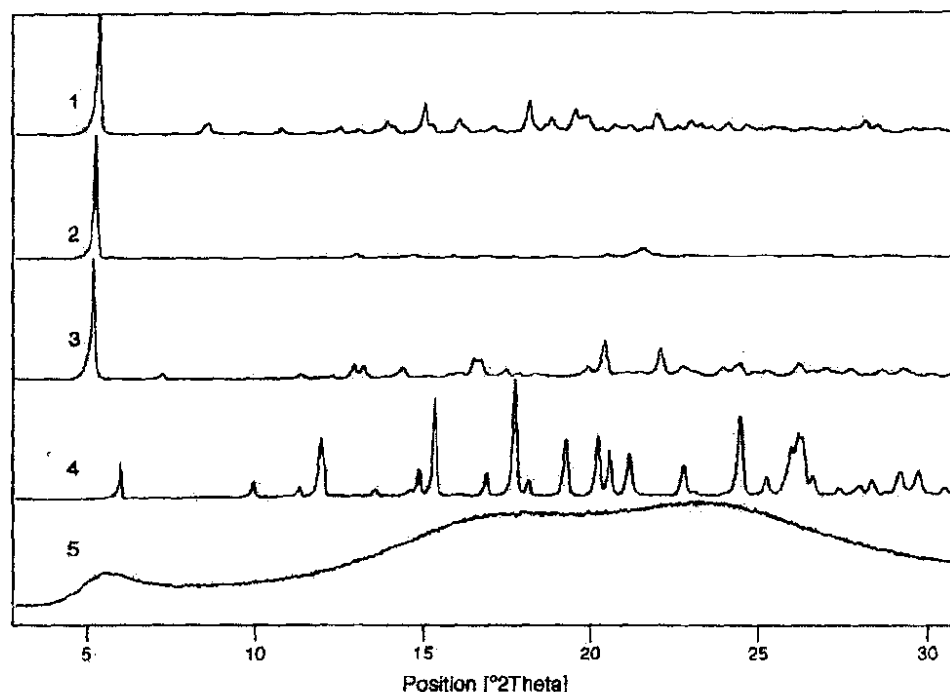


Figure 1.12 X-ray diffractograms of pantoprazole sodium form A (1), monohydrate (2), sesquihydrate (3), form B (4) and an amorphous form (5) (Zupancic *et al.*, 2005:65).

1.3.5.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) can be used to obtain characteristic thermal and melting point data for crystal polymorphs or solvates (McCauley & Brittain, 1995:239). Polymorphs often display different melting points. Also, different solvated forms of a compound will display differences in their desolvation behaviour, which can be observed with DSC, which is illustrated in figure 1.13. Different hydrates of nedocromil sodium were compared by means of differential scanning calorimetry. The dehydration endotherms observed for each hydrate was unique and form-specific (Khankari *et al.*, 1998:1056).

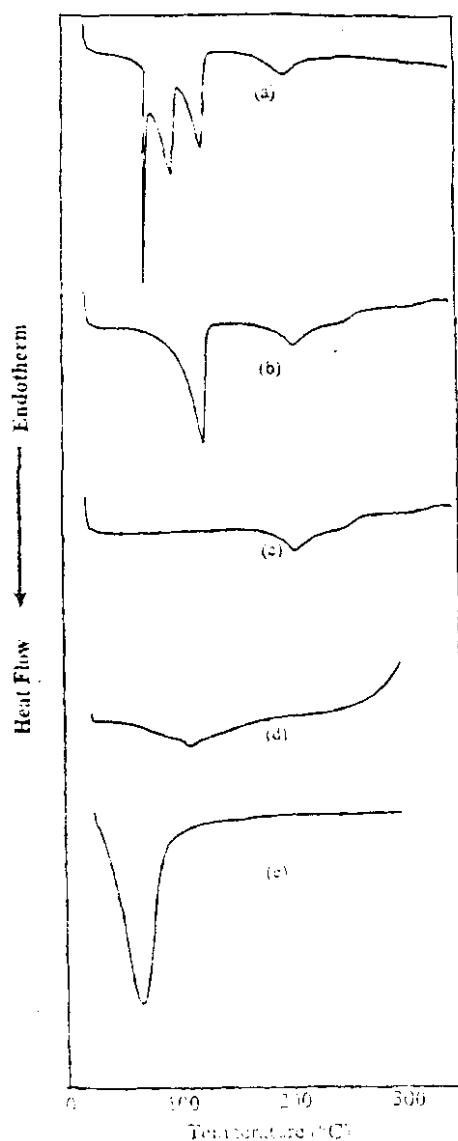


Figure 1.13 Differential scanning calorimetry (DSC) thermograms of the nedocromil sodium hydrates: (a) heptahemihydrate (crimped pan); (b) trihydrate (crimped pan); (c) monohydrate (crimped pan); (d) amorphous phase (crimped pan); (e) heptahemihydrate (open pan) (Khankari *et al.*, 1998:1056).

All the forms were analysed in crimped pans. The heptahemihydrate was further analysed in an open pan. The DSC curve obtained by this method varied greatly from the one obtained from the crimped pan. The reason for this was that the water vapour could not escape readily from the crimped pan. This resulted in a build up of pressure in the closed container, which altered the course of dehydration (Khankari *et al.*, 1998:1056). It should always be specified whether an open or a closed sample pan was used during DSC-thermal analysis. It is also important to use the same type

of sample pan when comparing two forms, to ensure uniformity and eliminate possible differences caused by the use of different sample pans.

However, differences in the DSC thermograms of polymorphs and solvates alone are not sufficient data to distinguish between various forms. Supportive analytical techniques should also be employed for the identification and characterisation of crystal forms (Rocco *et al.*, 1995:20).

1.3.5.3 Thermogravimetric analysis (TGA)

Thermogravimetry is a method of characterisation that is often used in combination with DSC. It is usually used to study the desolvation process of solvated crystal forms. TGA can quantitatively determine the total volatile content of a solid and is used in combination with Karl Fischer titrations to determine sample-moisture. TGA may be utilised to distinguish between solvates and anhydrous forms of API's (Brittain, 1999:246).

TGA curves of the various hydrates of pantoprazole sodium were compared and the difference in weight loss, due to the different proportions of water included in these forms, is obvious from figure 1.14.

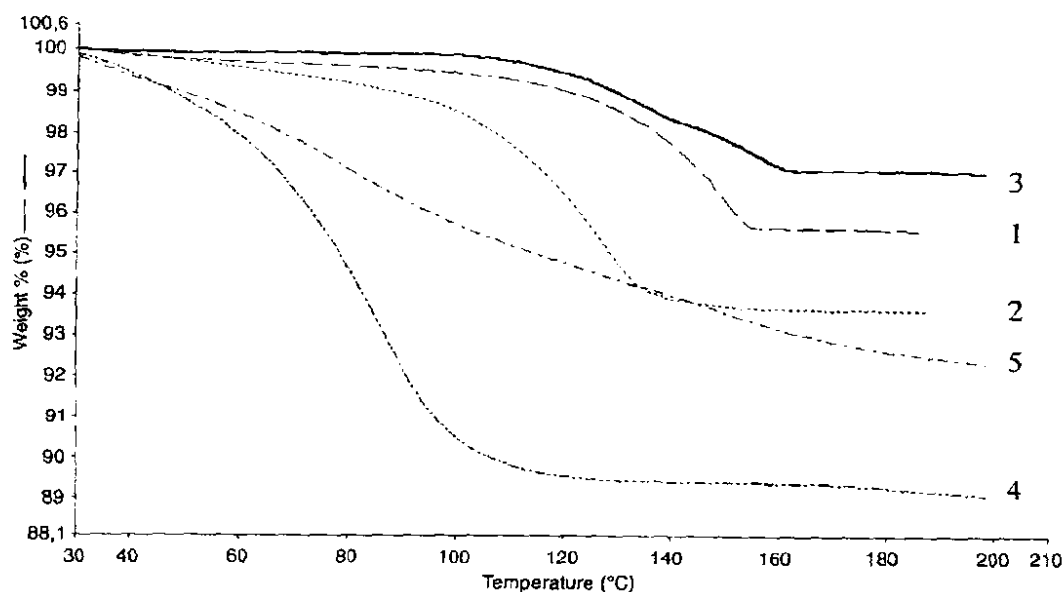


Figure 1.14 TGA curves of pantoprazole sodium monohydrate (1), sesquihydrate (2), form A (3), form B (4) and amorphous form (5) (Zupancic *et al.*, 2005:63).

Differences in the TGA curves of crystal forms of an API should thus be considered when identifying and characterising solvated forms of the API.

1.3.5.4 Fourier-transform infrared spectrophotometry (FT-IR)

Infrared spectrophotometry is a sensitive tool for the evaluation of the structure and conformation of an organic compound. Therefore it is often used for the characterisation and identification of different solid forms of a drug (Byrn *et al.*, 1999:111). Solvent inclusion often leads to differences in the IR spectrums of the hydrated and anhydrous forms and the difference in this physico-chemical property can often be successfully used to distinguish between hydrated and anhydrous forms.

A study conducted by Brits (2003:112) revealed a monohydrate (form H_β) and an anhydrous form (form γ) of venlafaxine HCl, which displayed significant differences in their diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectra. An overlay of the mentioned two forms is illustrated in figure 1.15.

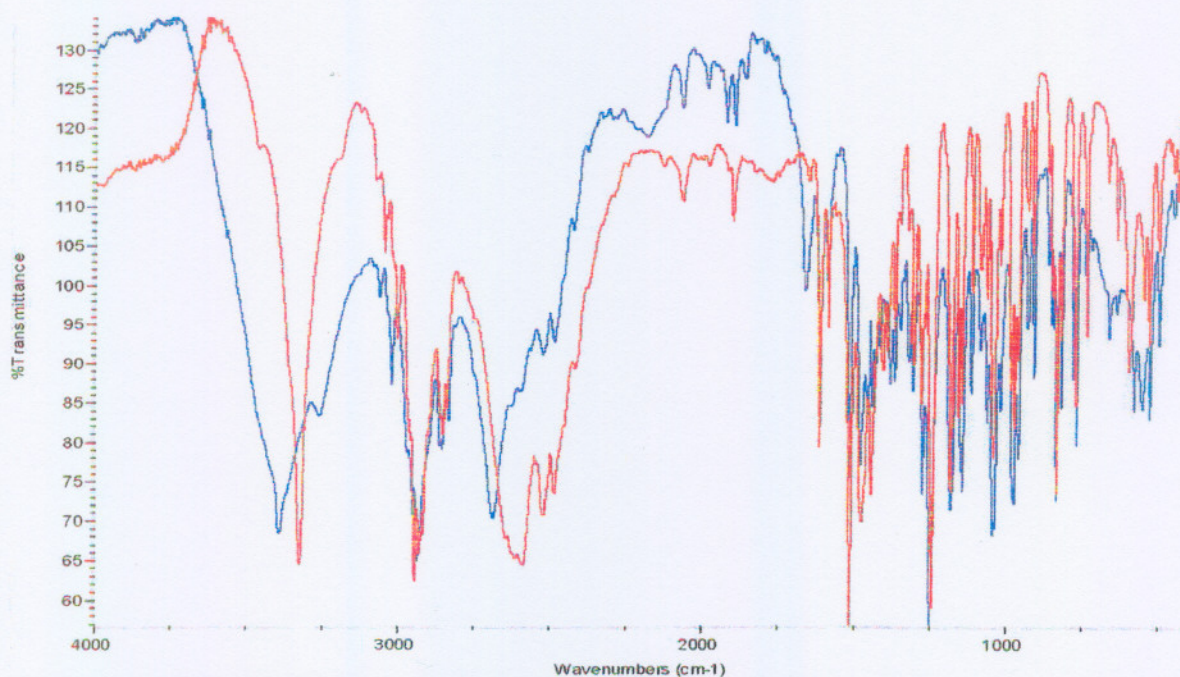


Figure 1.15 DRIFTS spectra of venlafaxine HCl crystal form γ (red) and form H_β (blue) (Brits, 2003:112).

1.4 Stability of various solid forms

The thermodynamic stability of the various crystal forms is usually expressed as the solubility thereof (Byrn *et al.*, 1999:20). In a system where more than one crystal form is possible, each form has a solubility value under certain conditions. These conditions include solvent composition, temperature and pressure (Byrn *et al.*, 1999:18). The dissolution curves for the anhydrous and hydrated forms of theophylline are shown in figure 1.16. From these curves it can be observed that the less stable anhydrous form converts to the more stable (less soluble) hydrated form during dissolution (Shefter & Higuchi, 1963:786). In this case the hydrated form is referred to as the thermodynamically more stable form, and under these conditions it will never convert to the other form (Byrn *et al.*, 1999:19-20).

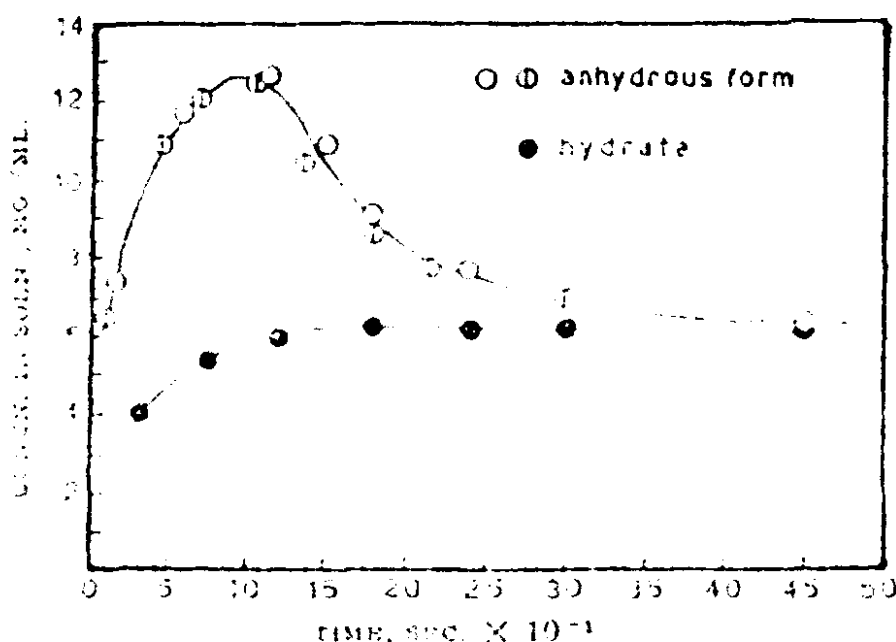


Figure 1.16 Dissolution profiles for the anhydrous and hydrated crystalline forms of theophylline in water at 25°C (Shefter & Higuchi, 1963:785).

It is possible for temperature changes to influence the thermodynamic stability of the various forms. Therefore it is important to distinguish between monotropic and enantiotropic systems. Monotropic systems are defined as systems where a single form is always more stable regardless of the temperature. Enantiotropic systems are defined as systems where the relative stability of the two forms inverts at some

transition temperature (Byrn *et al.*, 1999:20). Figure 1.17 illustrates these different systems graphically.

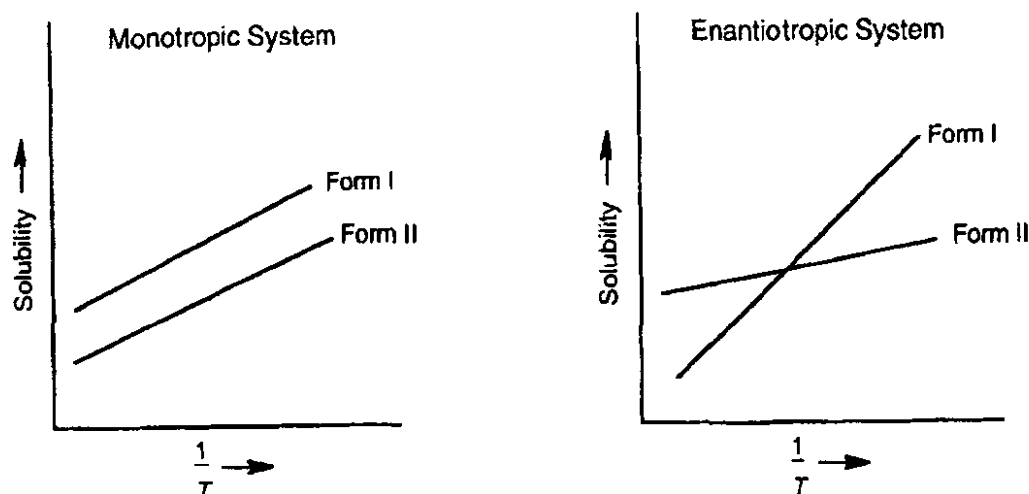


Figure 1.17 Graphic illustration of monotropic and enantiotropic systems (Byrn *et al.*, 1999:20).

Burger and Ramberger (as referred to by Grant, 1999:19) developed four thermodynamic rules to help decide whether two polymorphs are enantiotropes or monotropes. The two rules most often used are the heat of fusion rule and the heat of transition rule.

The heat of transition rule states that the two forms are enantiotropes if an endothermic polymorphic transition is observed. The two forms are monotropes if an exothermic polymorphic transition is observed (Grant, 1999:19).

The heat of fusion rule states that the two forms are enantiotropes if the higher melting polymorph has the lower heat of fusion. Equally, if the higher melting polymorph has the higher heat of fusion, the two forms are monotropes (Grant, 1999:19).

Protecting the solvate from crystal defects and storing it in an atmosphere of the solvent of crystallisation, can ensure that the solvate remains stable. However, sometimes it may be necessary to use another solvent to obtain a stable solvate (Byrn *et al.*, 1999:301).

1.4.1 Factors influencing crystal form stability

Various factors may influence the stability of a crystal form. Changes in the hydrated or solvated state of the solid can occur in response to various environmental conditions, such as temperature, relative humidity and pressure (Vippagunta *et al.*, 2001:17). These are all conditions that the pharmaceutical solid will be exposed to during manufacturing and should therefore be properly investigated. Various energetic steps, such as drying, milling, wet granulation, oven drying and compaction could cause phase transitions in solids. Conditions as harsh as 80°C and 100% RH for up to 12 hours is not unusual during the manufacturing process (Brittain & Fiese, 1999:332). It is thus important to know how a crystal form will react upon exposure to these conditions.

1.4.1.1 Presence of solvent

Changes in the crystal form often occur during wet granulation due to the exposure of the drug to a potential transforming solvent. Wet granulation is used to improve flow and blend homogeneity to allow the high speed compression of tablets. It is used to maintain the distribution of the drug throughout the formulation, even during free flow (Brittain & Fiese, 1999:340).

Wet granulation can be seen as a suspension of the drug in a mixture of solvent and excipients. The most common solvent is water. This exposure to water often results in conversion between anhydrous and hydrated forms, or between different hydrates (Brittain & Fiese, 1999:340).

The moisture-uptake or moisture loss of a compound is best described by a water content versus relative humidity (RH) diagram (Byrn *et al.*, 1999:241).

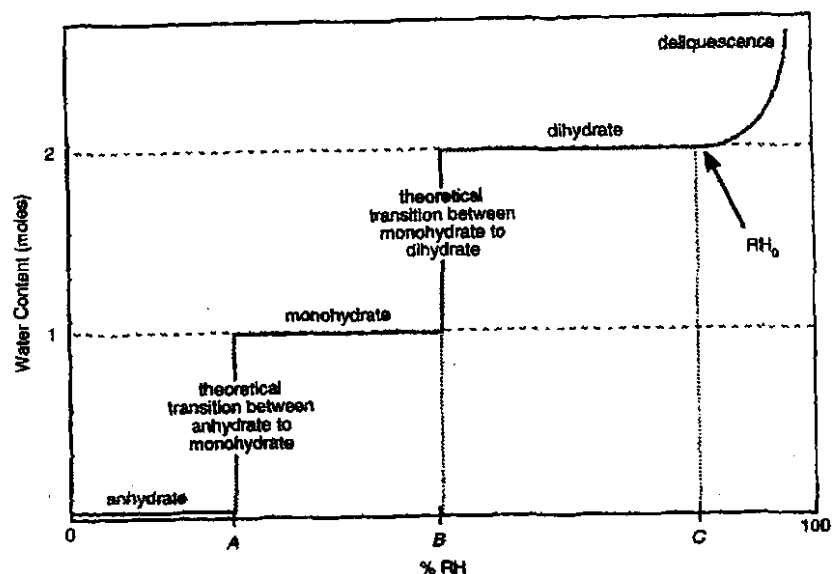


Figure 1.18 Idealised moisture-uptake profile (Byrn *et al.*, 1999:243).

Figure 1.18 illustrates the stepwise changes in moisture content for a drug that can exist as an anhydrate, monohydrate and dihydrate at room temperature. The figure illustrates an anhydrous form at 0% RH that transforms to a monohydrate at point A. Further increase in the percent relative humidity leads to transformation from the monohydrate to the dihydrate form at point B. Increasing the percent relative humidity still further will lead to deliquescence (RH_0) of the sample at point C. Deliquescence of a sample means that it dissolves from the adsorption of atmospheric water. This is only possible under specific conditions, such as above a certain RH value and at a fixed temperature (as can be seen in figure 1.18). These conditions may however vary greatly between different forms (Byrn *et al.*, 1999:243).

Naproxen sodium is a pharmaceutical solid that exhibit pseudopolymorphism. An anhydrous form and a dihydrate were maintained at different relative humidities until equilibrium was reached (Di Martino *et al.*, 2001:297). Figure 1.19 shows the hydration isotherms for both forms.

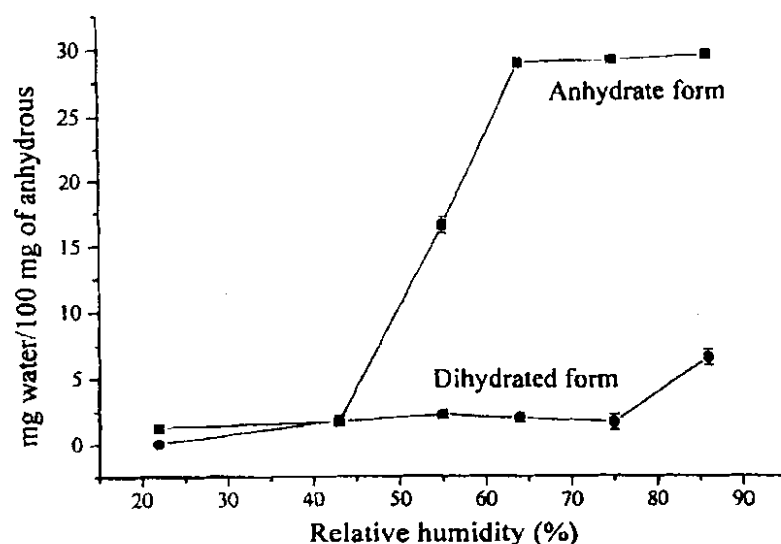


Figure 1.19 Sorption isotherms for naproxen sodium anhydrate and dihydrate after storage at 25°C at different relative humidities (Di Martino *et al.*, 2001:297).

The anhydrate form of naproxen sodium showed low water sorption at relative humidities up to 43%. With this low water sorption no changes in the crystal structure occurred and it remained intact. At a %RH of 55%, hydration occurred. This resulted in the anhydrate converting to the dihydrate. Higher humidities (64, 75 or 86%RH) failed to illustrate further conversion and it remained the dihydrate form. XRPD confirmed the transition to the dihydrate form at 55%RH and remained unchanged at higher humidity levels (Di Martino *et al.*, 2001:297-298).

The dihydrate sorbed low amounts of water at relative humidities of 43%. Larger quantities of water sorption only occurred at higher relative humidities (86%). However, this didn't result in any changes in the crystal structure, which was confirmed to still be the dihydrate. It can therefore be concluded that the increase in water content of the dihydrate is due to an increase in sorbed water and not an increase in bound water. In this case some of the sorbed water forms part of the crystal lattice, but it was possible for the lattice to incorporate further water into non-specific sites, without change in the crystal structure (Di Martino *et al.*, 2001:298).

Solvated compounds respond to changes in vapour pressure in a way similar to how hydrates respond to changes in relative humidity. Changes in vapour pressure can result in weight gain or loss for the solvate. At exposure to air (in other words zero

vapour pressure) an organic solvate will lose solvent. Similarly, solvated crystals will dissolve above a certain vapour pressure (Byrn *et al.*, 1999:245-246).

1.4.1.2 Pressure

Size reduction is often an important part of the manufacturing process, through which improved flow properties, increased surface area, and minimised segregation can be achieved. The main method employed for size reduction is milling. This imparts mechanical stress on the crystals and generates heat, which often leads to phase transition, such as dehydration (Zhang *et al.*, 2004:378). Grinding or milling of a hydrated crystal form often serves to lower the dehydration temperature of the crystal and eases the removal of water from the lattice. This often leads to formation of an amorphous product (Brittain & Fiese, 1999:338). Amorphous formation might be undesirable since the amorphous form is usually hygroscopic and thermodynamically unstable (metastable) compared to the crystalline form. The formed amorphous material converts the normal solid properties of the API and allows better flowing of the powder under the mechanical stress of milling and tableting. Upon heating the amorphous material might become more crystalline as it might be converted at the glass transition temperature. Any changes in crystallinity of the crystal form due to milling should be carefully considered in product formulation (Brittain & Fiese, 1999: 334-335).

Another process in the manufacturing of a drug that also exerts pressure on the solid is the compression during tableting. It is often thought that polymorphic changes due to tableting will not occur. A reason for this hypothesis is that the softer excipients would deform preferentially to the relatively hard crystalline drug and that the exposure time to stress is very little with an increased tableting speed. However, various studies have proven that this is not always true. In many cases polymorphic changes are associated with the compression step (Brittain & Fiese, 1999:348).

An example of a drug that undergoes polymorphic conversion with exposure to pressure is famotidine. The effect of various pressures on the polymorphic form B of famotidine was investigated (Roux *et al.*, 2002:22). The DSC thermograms, obtained for form B after exposure to pressures ranging from 0.1 – 1000 MPa for 15 min, is shown in figure 1.20.

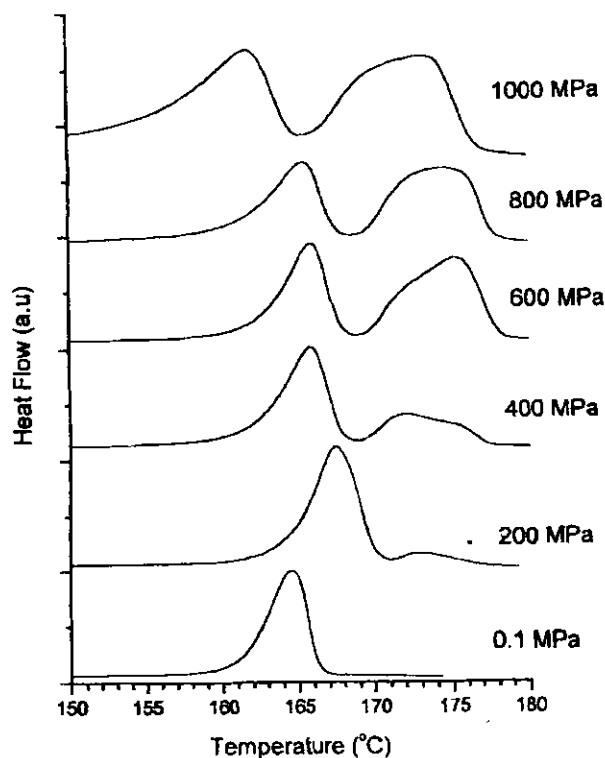


Figure 1.20 DSC thermograms of famotidine form B subjected to pressures of 0.1, 200, 400, 600, 800 and 1000 MPa for 15 min (Roux *et al.*, 2002:22).

An increase in the melting temperature of the samples subjected to 200 MPa was observed. It also exhibited a second broader, smaller endothermic peak at 169.9°C. The thermograms of the samples subjected to pressures from 400–800 MPa showed a progressive change in the second endothermic peak, while the first peak remained relatively unchanged. A pressure of 1000 MPa changed form B entirely, as the thermogram displays. Here a broad endothermic peak was present at lower temperatures, between 144.8 and 165.3°C, with another endothermic peak at 166.0–178.7°C (Roux *et al.*, 2002:22-23). The authors suggested that the changes that occurred in the thermogram were due to the conversion of famotidine form B to another polymorphic form.

1.4.1.3 Temperature

The API is often exposed to elevated temperatures, whether during manufacturing processes, or during storage of the drug. These elevated temperatures often lead to changes in the crystal form, such as polymorphic conversion or desolvation of a

solvate. These changes could alter the properties of the drug and result in undesired effects. Changes occurring in the crystal form due to this increased temperature should therefore be known and avoided if possible.

Olanzapine can exist as various polymorphic and pseudopolymorphic forms. Forms (1) and (2), as described by Polla *et al.* (2005:34), are both anhydrous forms. Olanzapine displayed a partial transformation of form (2) to form (1) when heated to 180°C. The XRPD pattern obtained at 180°C contained characteristic peaks of both forms (1) and (2) (Polla *et al.*, 2005:38). The XRPD patterns of olanzapine form (2) as a function of temperature is shown in figure 1.21.

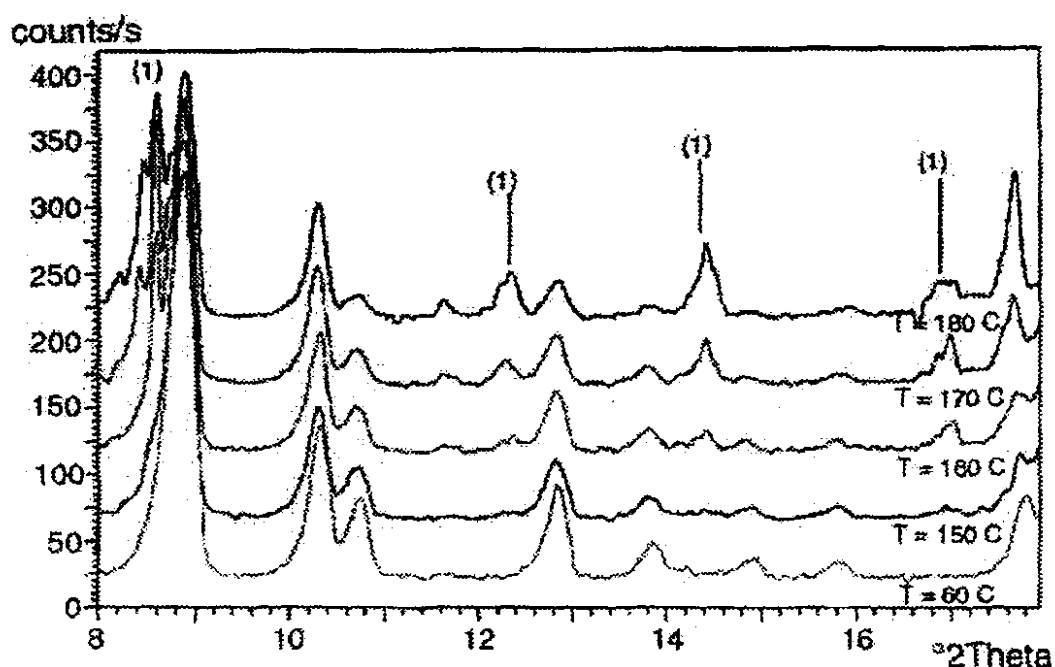


Figure 1.21 XRPD patterns of olanzapine form (2) taken as a function of temperature from 60-180°C. Characteristic peaks of form (1) are indicated (Polla *et al.*, 2005:38).

From this example it is clear that changes in temperature might lead to changes in the polymorphic composition of crystalline solids.

1.4.2 Desolvation / Dehydration reaction

The ease with which a solvate undergoes desolvation varies widely among solvated forms. Some solvates lose solvent readily at room temperatures, while others retain their solvent even at temperatures above the boiling point of the solvent (Byrn *et al.*, 1999:234).

The desolvation reaction can be influenced by many factors. These include atmosphere, nucleation, crystal packing and hydrogen bonding (Byrn *et al.*, 1999:292).

The atmospheric environment can have a great influence on the dehydration or desolvation reaction of a drug. Cephalexin and cephaloglycin formed acetonitrile solvates that desolvated when exposed to a nitrogen atmosphere and rehydrated in the same original stoichiometry on exposure to an acetonitrile rich atmosphere (Byrn *et al.*, 1999:292-293).

Temperature can accelerate the desolvation reaction. Theophylline monohydrate completely dehydrates within 5 days at temperatures between 35-50°C (Byrn *et al.*, 1999:283-284). This dehydration is illustrated in figure 1.22. Initially the desolvation proceeded from the ends of the crystal towards the centre. Only later in the reaction did dehydration also occur from the sides of the crystal (Byrn *et al.*, 1999:284).

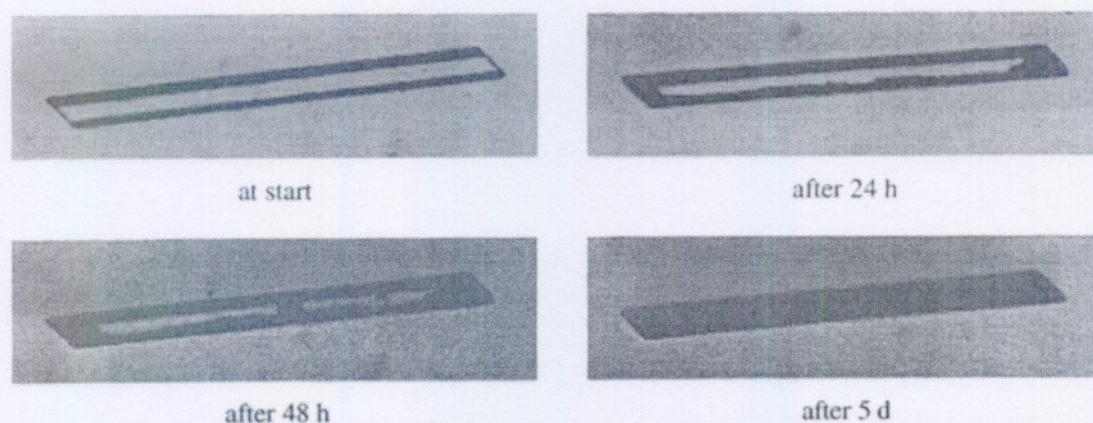


Figure 1.22 Dehydration behaviour of theophylline monohydrate in air at 35°C on a hot stage microscope (Lin & Byrn, as referred to by Byrn *et al.*, 1999:283).

Various crystal-packing factors may influence the desolvation process. Desolvation usually proceeds in the crystallographic direction that offers the least resistance to the escaping solvent molecules. The tunnel size and number of tunnels per unit area are important factors to consider when studying the dehydration process. Larger tunnels provide an easier escape of the solvent molecules, as does more tunnels per unit area. The compactness of the crystal is another factor that might influence the desolvation process. A more compact crystal, thus a more closely packed crystal, will have less solvent loss. The closer the molecules in the crystal are packed, the harder it is for the solvent molecules to escape. Other crystal-packing factors such as the direction of the solvent chain relative to the main plane of the molecules and the position of the solvent molecules relative to the host molecules will also influence dehydration. Hydrogen bonds between the solvent and host molecules hinder desolvation. An increase in the number and strength of the hydrogen bonds between the solvent and host molecules will lead to increased stability of the solvate (Byrn *et al.*, 1999:294).

Defects in the crystal influence the desolvation reaction, as these sites are often the place where desolvation initiates (Byrn *et al.*, 1999:294). Processes that exert mechanical stress on the crystal, such as milling, may accelerate the dehydration reaction as these processes induce surface defects and generate local heat (Zhang *et al.*, 2004:375).

Desolvation can have one of three effects on the crystal structure:

1. The crystal structure undergoes a significant change after desolvation (different XRPD patterns are observed);
2. the crystal structure undergoes only a slight change after desolvation (related XRPD patterns); or
3. the desolvation results in the material becoming amorphous (Byrn *et al.*, 1999:293).

Many solvates exhibit the first type of desolvation reaction. In these cases the XRPD pattern for the product and starting material differ. The XRPD pattern obtained for the product corresponds to the anhydrous form of the drug obtained from

recrystallisation from various solvents. Examples of API's that undergo this type of desolvation reaction include caffeine and 6-mercaptopurine (Byrn *et al.*, 1999:293).

Solvates that undergoes the second type of desolvation reaction loses the solvent, but no change in the crystal structure occurs, this result in a crystal with voids and cavities. Crystals that exhibit this type of desolvation include cephalexin pentahydrate and hydrocortisol 21-*tert*-butylacetate monoethanol solvate (Byrn *et al.*, 1999:293). The desolvated crystals are called *desolvated solvates*. *Desolvated solvates* have such strong interatomic forces holding the crystal lattice together that the crystal maintains its lattice integrity even when the solvent is removed (Byrn *et al.*, 1999:301). This type of internal structure of the crystals has also been discussed in section 1.1.2.2.4.

The third type of desolvation produces an amorphous product. This is an easy way to prepare amorphous materials (Byrn *et al.*, 1999:301).

Desolvation of a solid form can have substantial implications on the dissolution rate, bioavailability, and stability of a solid. The threshold temperature at which desolvation occurs is highly dependent on the crystal packing, hydrogen bonding and crystal defects that occur (Byrn *et al.*, 1999:301).

Conclusion

Various preparation methods can be used to obtain a solid form in the pharmaceutical industry including evaporation, cooling a solution, or seeding a supersaturated solution with crystals of the desired form. These processes are influenced by factors such as the concentration of the solution (degree of supersaturation), temperature and additives and impurities. The crystallisation process should therefore be carefully monitored to ensure that only the desired polymorph (and not mixtures of forms) is obtained.

The different solid forms or solvent inclusions of a drug may influence its solid state properties. Properties that are often altered by different solid forms include solubility, tableting properties, dissolution, chemical stability and other physico-chemical properties. Transition between polymorphs will be influenced by the stability of each

form upon exposure to changes in the relative humidity, pressure and temperature in the immediate environment which the drug is exposed to. These changes could also lead to desolvation of a solvate or solvation of an unsolvated form.

Thorough investigation and characterisation of the various solid forms of API's, whether polymorphic or pseudopolymorphic, is important to ensure manufacturing and maintenance of the most suitable crystal form with optimum properties.

CHAPTER 2

Gatifloxacin: Pharmaceutical and pharmacological background

Introduction

Gatifloxacin is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent with activity against a wide range of gram-negative and gram-positive organisms (Bristol-Myers Squibb Company, 2005).

Gatifloxacin is administered as a racemate with no net optical rotation (Bristol-Myers Squibb, 2005). The two enantiomers exhibit equal antimicrobial activity. Gatifloxacin displays a pH dependent solubility profile and a maximum aqueous solubility occurs in a pH range of 2-5 (Fish & North, 2001:36).

The pharmaceutical and pharmacological properties of gatifloxacin will be discussed in this chapter.

2.1 Description

2.1.1 Nomenclature

Chemical name

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid

Non-proprietary name

Gatifloxacin

Proprietary name

Tequin®

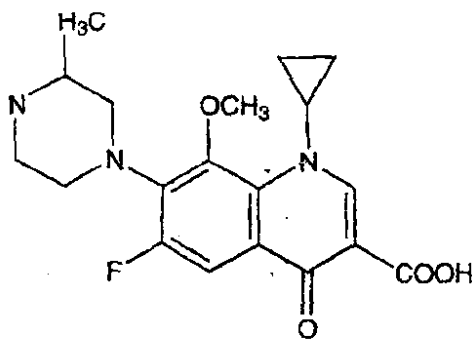
2.1.2 Formulae

Empirical formula

Gatifloxacin: $C_{19}H_{22}FN_3O_4$

Gatifloxacin sesquihydrate: $C_{19}H_{22}FN_3O_4 \cdot 1.5H_2O$

Structural formula of gatifloxacin



2.1.3 Molecular weight

Gatifloxacin: 375.39 g/mol

Gatifloxacin sesquihydrate: 402.42 g/mol

2.1.4 Appearance and colour

Gatifloxacin sesquihydrate is a crystalline powder with a white to pale yellow colour.

2.2 Pharmaceutics of gatifloxacin

Gatifloxacin sesquihydrate is available as Tequin® (gatifloxacin) tablets for oral administration, Tequin® (gatifloxacin) injection and Tequin® (gatifloxacin in 5% dextrose) injection for intravenous administration.

Tequin® (gatifloxacin) tablets are available as 200 mg and 400 mg white, film-coated tablets. The tablets contain gatifloxacin sesquihydrate equivalent to 200 mg or 400 mg gatifloxacin. The 200 mg tablets have "BMS" imprinted on one side and "TEQUIN" and "200" on the other side. The 400 mg tablets have "BMS" imprinted on

one side and "TEQUIN" and "400" on the other side. The tablets are almond shaped and biconvex (Bristol-Myers Squibb, 2005). The intravenous solution is supplied in single-dose vials. The gatifloxacin solution is a clear, light yellow to greenish-yellow solution and contains gatifloxacin in a concentration of 10 mg/ml.

Tequin® tablets and solution for intravenous administration should be stored at 25°C. The tablets can be taken with or without meals, but should be taken at least 4 hours before any aluminium- or magnesium-based antacids, ferrous sulphate, or dietary supplements containing zinc, magnesium, or iron (Bristol-Myers Squibb, 2005). Gatifloxacin has the potential of forming stable coordination compounds with metal ions through chelation with the cations. The potential for chelation decreases in the following order: $\text{Al}^{3+} > \text{Fe}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Mg}^{2+} > \text{Ca}^{2+}$. The formation of nonabsorbable metal-quinolone chelates in the stomach or small intestine could lead to a significant decrease in the bioavailability of the drug. Antacids or dietary supplements containing divalent or trivalent metal cations (such as aluminium or magnesium) should therefore not be taken together with gatifloxacin (Fish & North, 2001:52). The antacid or dietary supplement should be taken 4 hours after administration of gatifloxacin tablets to ensure the proper absorption of the drug (Bristol-Myers Squibb, 2005).

Mild adverse reactions may occur with the use of gatifloxacin, which may include nausea, diarrhoea, headache and dizziness as the most common adverse reactions (RxList, 2005). According to Bristol-Myers Squibb (2005), other adverse reactions that could occur include:

- Allergic reactions.
- Hypertension.
- Hyperglycemia.
- Anxiety, nervousness, vertigo, insomnia and abnormal dreaming.
- Dry skin, rash or sweating.
- Abnormal vision, tinnitus.

These are only a few of the possible adverse reactions that may occur with gatifloxacin administration. Generally all the adverse reactions that may occur are

considered to be mild in nature and gatifloxacin was discontinued due to adverse reactions in only 2.7% of the patients involved in clinical studies (Bristol-Myers Squibb, 2005).

2.2.1 Indications and usage

Gatifloxacin should only be used in the treatment of bacteria known to be susceptible to this agent. This approach ensures the maintained effectiveness of gatifloxacin and reduces the development of drug-resistant bacteria (Bristol-Myers Squibb Company, 2005). According to Bristol-Myers Squibb (2005), gatifloxacin is indicated for the treatment of the following infections, listed in table 2.1 with the causative organisms.

Table 2.1 A list of infections (with the causative organisms) that gatifloxacin is indicated for (Bristol-Myers Squibb, 2005)

Infection	Causative organism
<u>Acute bacterial exacerbation of chronic bronchitis</u>	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Haemophilus parainfluenzae</i> , <i>Moraxella catarrhalis</i> or <i>Staphylococcus aureus</i>
<u>Acute sinusitis</u>	<i>Streptococcus pneumoniae</i> or <i>Haemophilus influenzae</i>
<u>Community-acquired pneumonia</u>	<i>Streptococcus pneumoniae</i> (including multidrug-resistant <i>S. pneumoniae</i>), <i>Haemophilus influenzae</i> , <i>Haemophilus parainfluenzae</i> , <i>Moraxella catarrhalis</i> , <i>Staphylococcus aureus</i> , <i>Mycoplasma pneumoniae</i> , <i>Chlamydia pneumoniae</i> or <i>Legionella pneumophila</i>

Table 2.1 (continued)

Infection	Causative organism
<u>Uncomplicated skin and skin structure infections (simple abscesses, folliculitis and cellulitis)</u>	<i>Staphylococcus aureus</i> (methicillin-susceptible strains only) or <i>Streptococcus pyogenes</i>
<u>Uncomplicated urinary tract infections (cystitis)</u>	<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> or <i>Proteus mirabilis</i>
<u>Complicated urinary tract infections</u>	<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> or <i>Proteus mirabilis</i>
<u>Pyelonephritis</u>	<i>Escherichia coli</i>
<u>Uncomplicated urethral and cervical gonorrhea or acute, uncomplicated rectal infections in women</u>	<i>Neisseria gonorrhoeae</i>

2.2.2 Contraindications and precautions

Gatifloxacin should not be administered to patients with a known history of hypersensitivity to quinolones. The safety and efficacy of gatifloxacin have not been proved for pediatric patients, adolescents, and pregnant or lactating women (Bristol-Myers Squibb, 2005).

Gatifloxacin potentially prolongs the QTc interval of the electrocardiogram in some patients. This could lead to increased risk of ventricular arrhythmias such as torsades de pointes. Gatifloxacin should be avoided in patients with known prolongation of the QTc interval. Patients with uncorrected hypokalemia and patients taking class IA (quinidine, procainamide) or class III (amiodarone, sotalol) antiarrhythmic drugs should avoid taking gatifloxacin. The concurrent use of gatifloxacin with other drugs that prolong the QTc interval as well as in patients with medical conditions such as

bradycardia or acute myocardial ischemia should be done with extreme caution (RxList, 2005).

Diabetic patients should take care when using gatifloxacin since it has been reported to cause blood glucose disturbances in such patients (RxList, 2005).

Fluoroquinolones may cause central nervous system (CNS) effects such as agitation, anxiety, paranoia and insomnia (RxList, 2005).

2.2.3 Dosage and administration

Tequin® is administered once every 24 hours. The recommended dosage depends on the indication for use and is applicable for all patients with a creatinine clearance of ≥ 40 ml/min (Bristol-Myers Squibb Company, 2005). Table 2.2 provides some dosage guidelines for gatifloxacin.

No dosage adjustment according to gender or age is necessary for Tequin® administration, although age related renal impairment should be taken into consideration. A dosage adjustment should be considered for patients with impaired renal function, this includes patients with a creatinine clearance of < 40 ml/min, patients on hemodialysis and patients on continuous peritoneal dialysis (Bristol-Myers Squibb Company, 2005).

No dosage adjustments is recommended for patients with moderate hepatic impairment, since this caused no significant changes in C_{max} , T_{max} , elimination half-life and fraction of unchanged drug excreted in the urine (Rodvold & Neuhauser, 2001:244S).

Patients may switch between oral and intravenous administration without a dose adjustment. The injection should always be administered by intravenous infusion and not by intramuscular, intrathecal, intraperitoneal or subcutaneous administration (Bristol-Myers Squibb, 2005).

Table 2.2 Dosage guidelines for gatifloxacin (Bristol-Myers Squibb Company, 2005)

Infection	Daily dose	Duration
Acute bacterial exacerbation of chronic bronchitis.	400 mg	5 days
Acute sinusitis.	400 mg	10 days
Community-acquired pneumonia.	400 mg	7-14 days
Uncomplicated skin and skin structure infections.	400 mg	7-10 days
Uncomplicated urinary tract infections (cystitis).	400 mg or 200 mg	Single dose or 3 days
Complicated urinary tract infections.	400 mg	7-10 days
Acute pyelonephritis.	400 mg	7-10 days
Uncomplicated urethral gonorrhea in men; Endocervical and rectal gonorrhoea in women.	400 mg	Single dose

2.3 Pharmacology of gatifloxacin

2.3.1 Working mechanism of gatifloxacin

Gatifloxacin is a fluoroquinolone antibacterial agent active against a variety of gram-negative and gram-positive organisms. The fluoroquinolones block bacterial DNA synthesis. This is achieved through the inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV, enzymes essential for the replication of DNA and partitioning of the replicated DNA. DNA gyrase is a tetrameric enzyme that consists of two A and two B subunits and is responsible for the introduction of negative

superhelical twists into bacterial DNA. Topoisomerase IV also consists of four subunits. The enzymes causes negative superhelical twists by breaking the backbone of the DNA and passing a double strand of DNA through the breaks. Gatifloxacin (and other fluoroquinolones) inhibit this process by binding to the enzyme while it is functionally attached to the DNA strand, forming a drug-enzyme-DNA complex. The DNA in these complexes remains broken and causes cell death upon release from these complexes (Fish & North, 2001:36-37). The working mechanism of gatifloxacin is illustrated in figure 2.1.

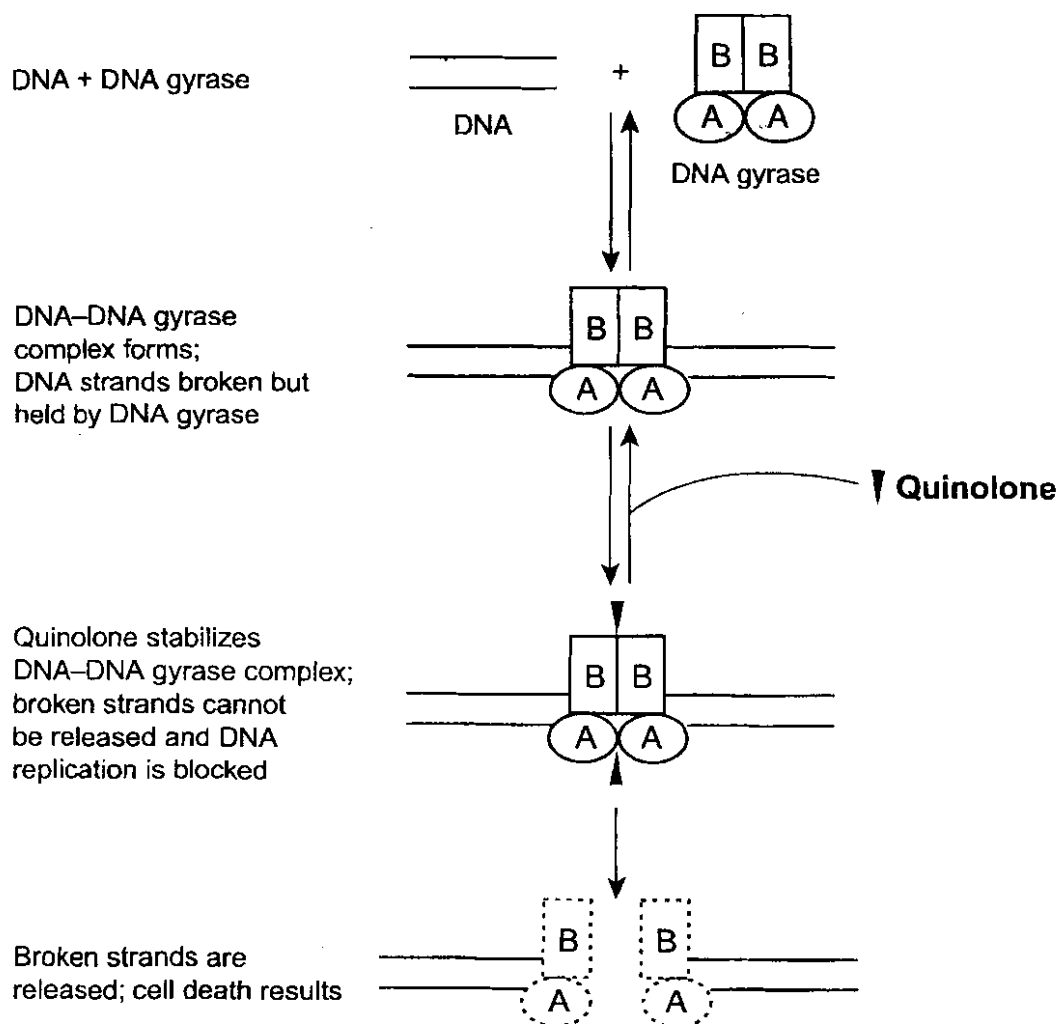


Figure 2.1 Diagrammatic representation of the working mechanism of gatifloxacin (Fish & North, 2001:37).

Another function of topoisomerase IV is also inhibited by fluoroquinolones. This enzyme plays an important role in the separation of replicated chromosomal DNA

into the respective daughter cells. This process is blocked by the inhibition of topoisomerase IV by fluoroquinolones (Chambers, 2001:797).

2.3.2 Pharmacokinetics of gatifloxacin

The pharmacokinetics of gatifloxacin are linear and time-independent for all doses between 200 to 800 mg, administered over a time period of up to 14 days. Steady-state concentrations are reached after the third daily dose of gatifloxacin (Bristol-Myers Squibb, 2005).

2.3.2.1 Absorption

Gatifloxacin is well absorbed after oral administration with a bioavailability of 98%. A peak serum concentration of 3.4 $\mu\text{g/ml}$ is obtained after oral administration (Chambers, 2001:799). Peak serum concentration is reached approximately 1-2 hours after dosing under fasted conditions. The intravenous administration of the drug displays similar serum concentrations to those obtained from oral administration (Bristol-Myers Squibb, 2005). Figure 2.2 compares the mean plasma concentration-time profiles of gatifloxacin following intravenous (IV) and oral (PO) administration of a single 400 mg dose.

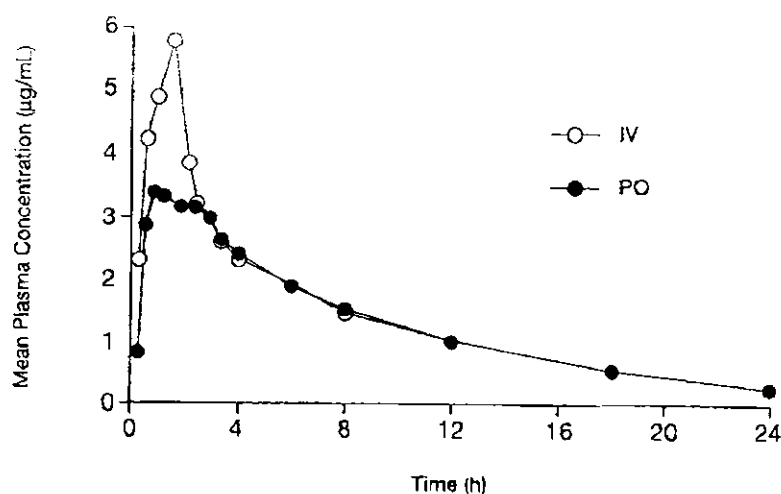


Figure 2.2 Mean plasma concentration-time profiles of gatifloxacin following intravenous (IV) and oral (PO) administration of a single 400 mg dose to healthy subjects (Bristol-Myers Squibb, 2005).

2.3.2.2 Distribution

Gatifloxacin is widely distributed throughout the body and has a low serum protein binding of approximately 20% (RxList, 2005). Serum protein binding is independent of concentration. Gatifloxacin had a steady-state mean volume of distribution ($V_{d_{ss}}$) ranging between 1.5 to 2.0 litre/kg. Gatifloxacin is widely distributed throughout the body and this rapid distribution of gatifloxacin into tissues leads to higher concentrations of gatifloxacin in the target tissues than in serum (Bristol-Myers Squibb, 2005).

2.3.2.3 Metabolism

Gatifloxacin undergoes limited metabolism with less than 1% of the dose excreted in the urine as two metabolites. These metabolites are ethylenediamine and methylethylenediamine (RxList, 2005).

Human and animal studies have indicated that gatifloxacin is not an enzyme inducer and is therefore unlikely to influence the metabolic elimination of itself or other drugs. It has also been determined that gatifloxacin does not inhibit cytochrome P450 (CYP). The pharmacokinetics of drugs metabolised by these enzymes will not be altered by gatifloxacin (RxList, 2005).

2.3.2.4 Elimination

Gatifloxacin is primarily eliminated by renal mechanisms and it is known that gatifloxacin undergoes both glomerular filtration and tubular secretion. Following an oral and intravenous administration of the drug, more than 70% of the administered dose of Tequin® was recovered as unchanged drug in the urine within 48 hours. Gatifloxacin undergoes minimal biliary and intestinal elimination, which resulted in 5% of the unchanged drug being recovered in the faeces. Less than 1% of the dose was excreted in the urine as two metabolites, ethylenediamine and methylethylenediamine (RxList, 2005).

The elimination half-life of gatifloxacin is independent of dose and route of administration. Gatifloxacin has a mean elimination half-life of 7 to 14 hours. Renal

clearance ranges between 124 and 161 ml/min and is not affected by the dose (Bristol-Myers Squibb, 2005).

2.4 Gatifloxacin crystal forms: An intellectual property overview

A number of patents are currently available claiming intellectual property of various crystal forms of gatifloxacin. A list of these patents is given in table 2.3.

Table 2.3 Patent claims on gatifloxacin crystal forms

Patent number
US 2002/0052379
US 2004/0009989
US 2004/0038988
US 2004/0171621
US 2004/0192700
US 5880283
US 6413969
WO 02/22126
WO 03/094919
WO 03/105851
WO 2003/086402
WO 2004/012739
WO 2004/054583
WO 2004/087688
WO 2004/101527
WO 2004/101547

Only the patents containing crystal forms relevant to this study will now be discussed.

2.4.1 Form J (multiple solvates)

Pat. No. WO 03/094919 & US 2004/0009989

Form J is a pseudopolymorph of gatifloxacin that exists as multiple solvates, depending on the solvent used for recrystallisation. Solvated forms of form J include an iso-propanol (IPA) solvate, a methyl ethyl ketone solvate, an acetone solvate, a tetrahydrofuran (THF) solvate and a 1-butanol solvate (1-BuOH) (Niddam-Hildesheim *et al.*, 2003:18).

The characteristic XRPD peaks of form J are at $6.7^{\circ}2\theta$, $11.3^{\circ}2\theta$, $13.8^{\circ}2\theta$ and $16.4^{\circ}2\theta$ ($\pm 0.2^{\circ}2\theta$) regardless of the solvation of form J (Niddam-Hildesheim *et al.*, 2003:18).

Table 2.4 summarises the weight losses (as determined by TGA) and the water content (as determined by KF) for various solvated forms of form J.

Table 2.4 TGA and KF data of form J as some of its solvates (Niddam-Hildesheim *et al.*, 2003:20)

Solvent	Total weight loss % (TGA)	Weight loss step (80-145°C)	Karl Fischer (wt-%)	Corresponding solvate formula
IPA	8.70	4.10	4.01	Gatifloxacin:IPA (4:1)
1-BuOH	10.40	7.60	2.79	Gatifloxacin:1-BuOH (5:2)
IPA	8.70	6.40	2.42	Gatifloxacin:IPA (5:2)
IPA	8.30	4.90	4.84	Gatifloxacin:IPA (3:1)
Acetone	8.90	4.30	3.45	Gatifloxacin:Acetone (3:1)
IPA	11.40	7.80	3.13	Gatifloxacin:IPA (2:1)

A characteristic FT-IR spectrum of form J is shown in figure 2.3.

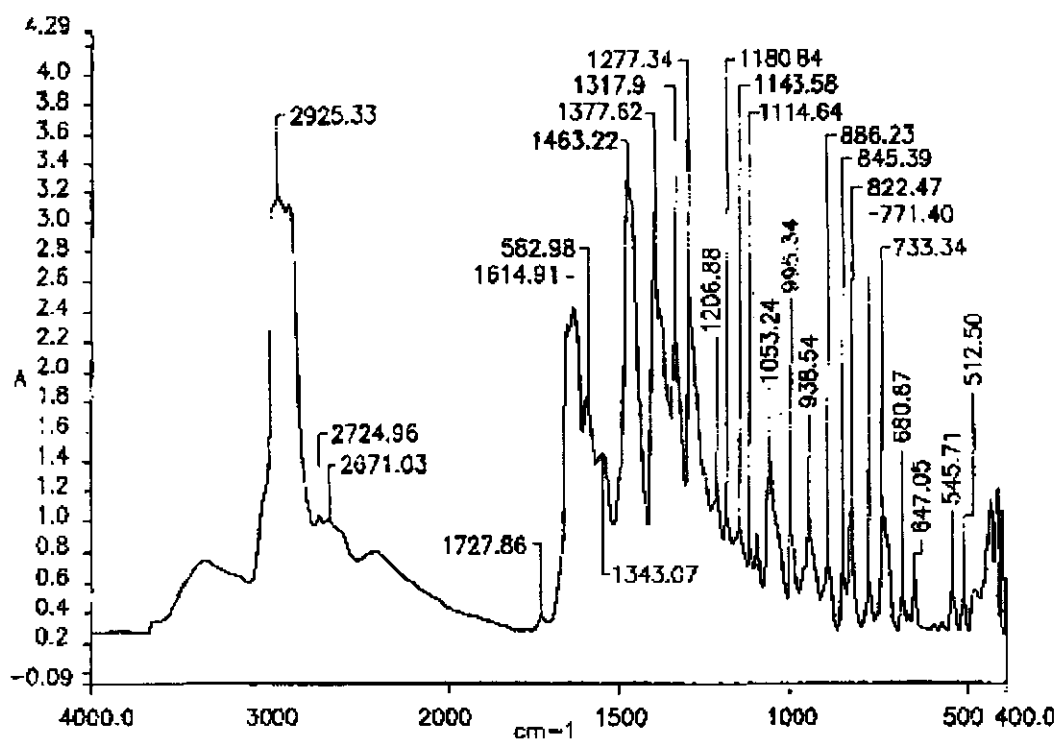


Figure 2.3 Characteristic FT-IR spectra of form J (Niddam-Hildesheim *et al.*, 2003:9).

The characteristic DSC and TGA thermograms of form J are shown in figures 2.4 and 2.5.

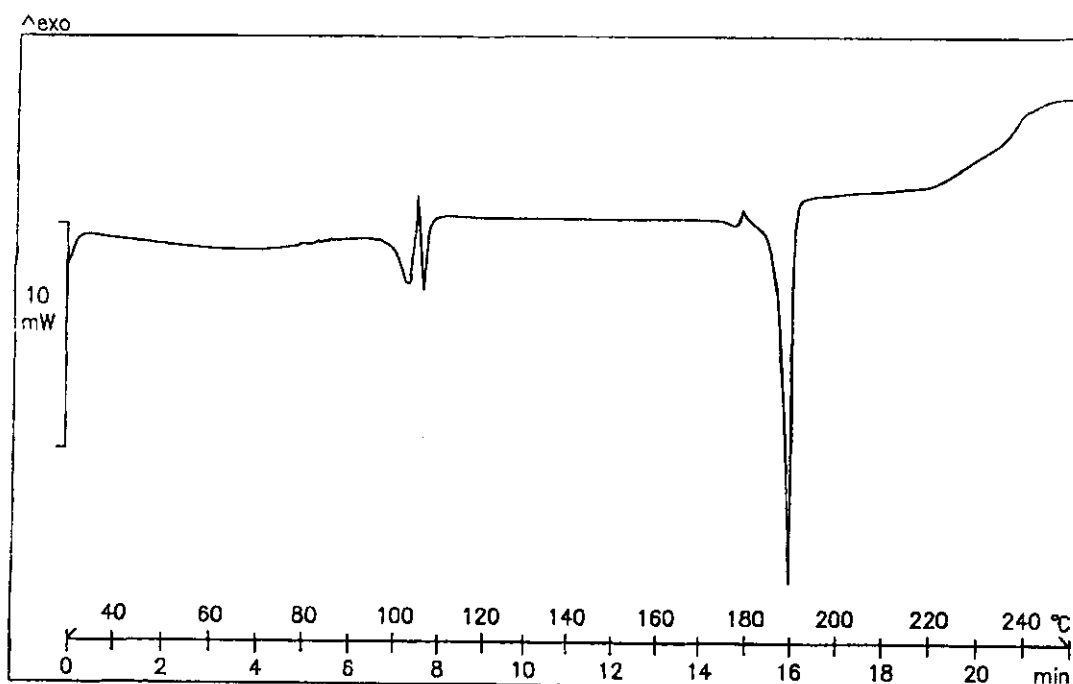


Figure 2.4 DSC thermogram of form J (Niddam-Hildesheim *et al.*, 2003:9).

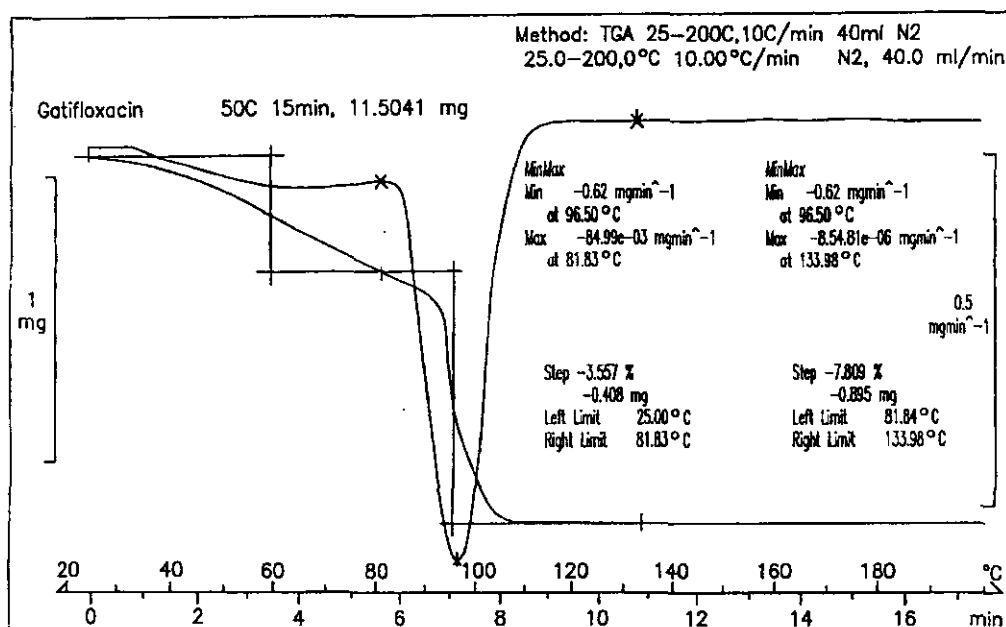


Figure 2.5 TGA thermogram of form J (Niddam-Hildesheim *et al.*, 2003:9).

2.4.2 Form omega

Pat. No. US 2004/0009989, WO 2004/101527 & US 6413969

Vakil *et al.* (2004:1-13) disclosed a method for the preparation of form omega (an anhydrous crystal form). The main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I_0) of form omega are listed in table 2.5. Vakil *et al.* (2004:4-5) disclosed a number of x-ray powder diffractograms of form omega. One of these diffractograms is displayed in figure 2.6.

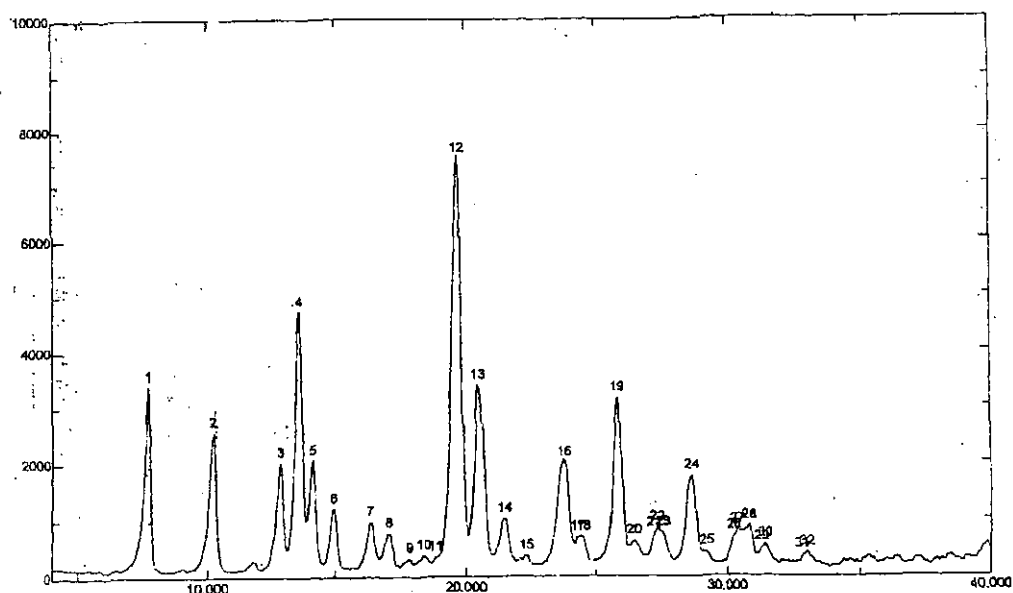


Figure 2.6 XRPD pattern of form omega (Vakil *et al.*, 2004:4).

Table 2.5 The main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I_0) of form omega (Vakil *et al.*, 2004:10)

Main peaks	Form omega	
	($^{\circ}2\theta$)	(I/I_0)
1	7.8	45.0
2	10.2	26.0
3	12.9	37.0
4	13.6	70.0
5	14.1	30.0
6	15.0	17.0
7	16.4	14.0
8	17.0	12.0
9	19.7	100.0
10	20.5	37.0
11	21.5	12.0
12	23.8	25.0
13	24.5	12.0
14	25.9	42.0
15	27.6	11.0
16	28.7	24.0
17	30.4	11.0
18	30.9	11.0
19	31.5	7.0

Niddam-Hildesheim *et al.* (2004:8) disclosed other methods for the preparation of form omega. One such method comprised of the following steps: preparing a filtered solution of gatifloxacin in acetonitrile with a water content of less than 5% at a temperature of approximately 80°C; cooling this solution to a temperature of about 50-56°C; seeding the solution with gatifloxacin at this temperature and maintaining it at this temperature for approximately 30 minutes; cooling the solution preferably to 5°C; and isolating form omega.

2.4.3 Form I

Pat. No. WO 03/086402

Reddy *et al.* (2003:4) disclosed an anhydrous crystal form of gatifloxacin (form I) that is characterised by the XRPD peak angles ($^{\circ}2\theta$) listed in table 2.6. The x-ray powder diffractogram of form I is shown in figure 2.7.

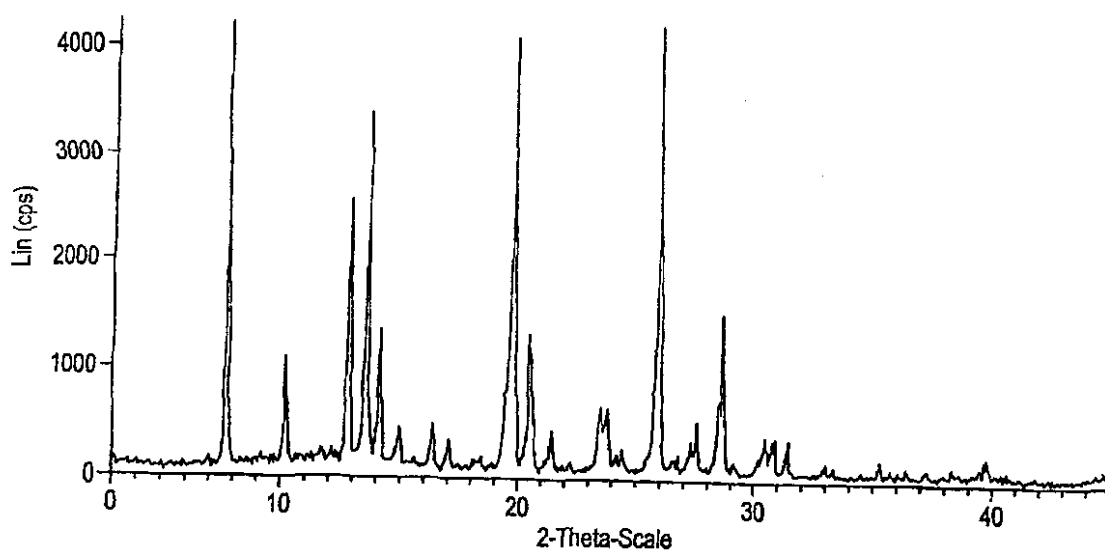


Figure 2.7 X-ray powder diffractogram of form I (Reddy *et al.*, 2003:2).

Table 2.6 Main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values of form I (Reddy *et al.*, 2003:4)

Main peaks	Form I	
	($^{\circ}2\theta$)	(I/I ₀)
1	7.8	100.0
2	10.2	26.8
3	12.9	71.7
4	13.6	89.3
5	14.1	29.3
6	14.9	9.8
7	16.3	12.2
8	17.0	7.2
9	19.7	100.0
10	20.5	35.2
11	21.5	9.7
12	23.6	15.1
13	23.8	14.9
14	24.4	3.7
15	25.9	71.9
16	27.6	10.9
17	28.7	40.9
18	30.5	8.4
19	30.9	6.7
20	31.5	5.6

Figure 2.8 displays the IR spectrum of form I. The characteristic peaks in the IR spectrum of form I are at approximately 3327.7cm^{-1} and 1721.0cm^{-1} .

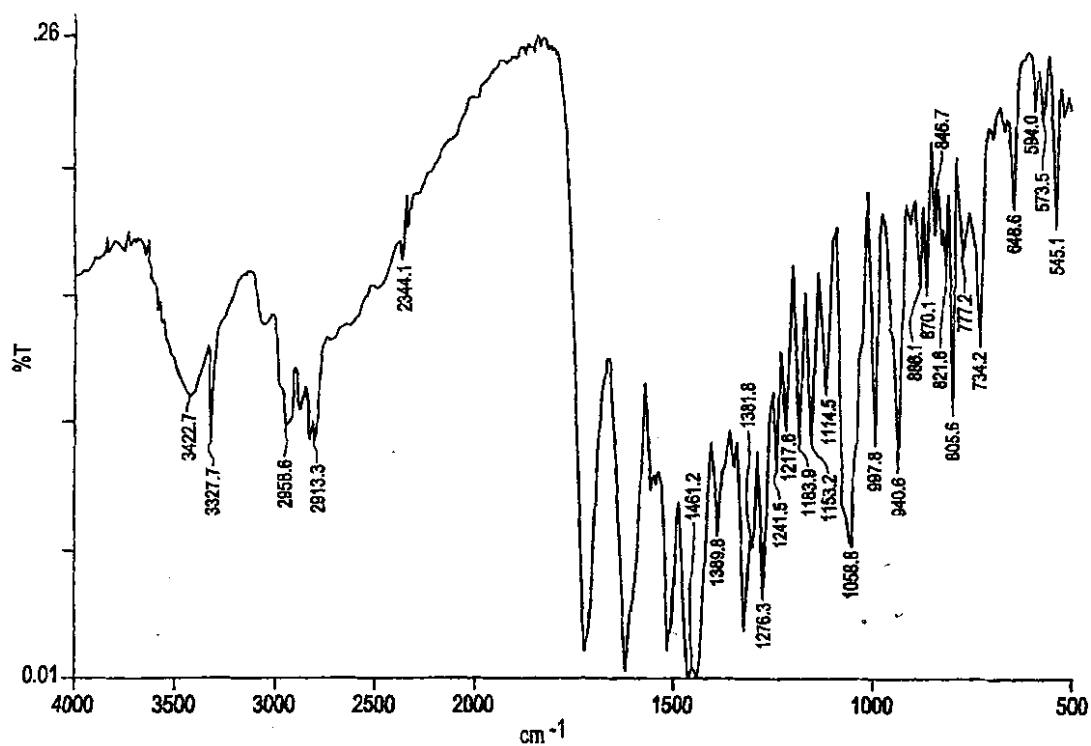


Figure 2.8 IR spectrum of form I (Reddy *et al.*, 2003:3).

The DSC thermogram of form I is shown in figure 2.9. A melting endotherm is present in the thermogram at 188.35°C.

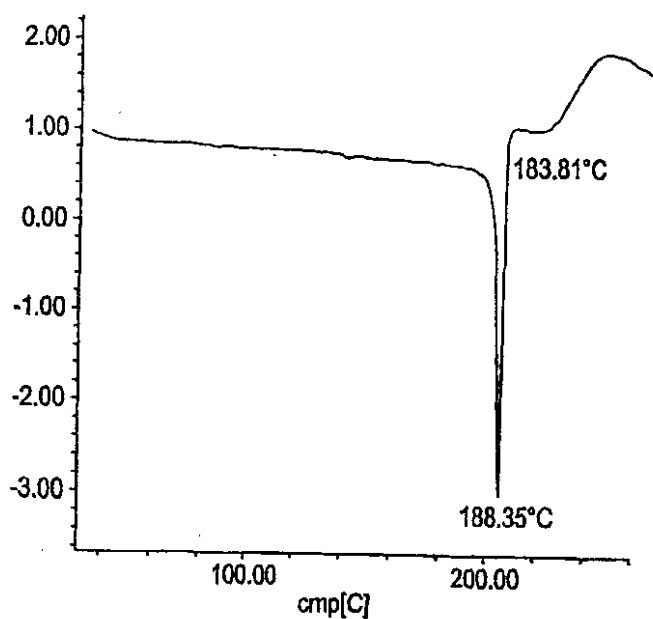


Figure 2.9 DSC thermogram of form I (Reddy *et al.*, 2003:2).

The moisture content of form I may range from 0.05-2.00% when determined by KF and TGA (Reddy *et al.*, 2003:3).

2.4.4 Form T1

Pat. No. US 2004/0192700

Form T1 is a novel crystalline form of gatifloxacin that displays characteristic x-ray powder diffraction peaks at $7.4^{\circ}2\theta$, $8.9^{\circ}2\theta$, $9.6^{\circ}2\theta$, $11.4^{\circ}2\theta$, $12.2^{\circ}2\theta$, $12.9^{\circ}2\theta$, $14.1^{\circ}2\theta$, $21.2^{\circ}2\theta$, $21.8^{\circ}2\theta$, $24.1^{\circ}2\theta$ and $26.0^{\circ}2\theta$ ($\pm 0.2^{\circ}2\theta$) (Niddam-Hildesheim *et al.*, 2004:4). Figure 2.10 displays the XRPD pattern of form T1.

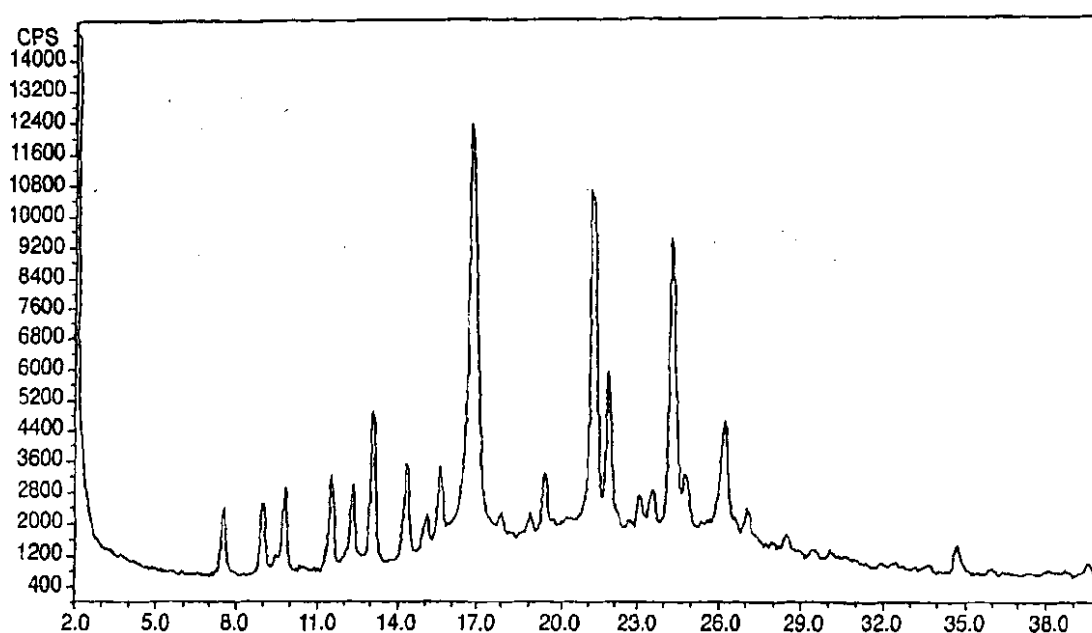


Figure 2.10 XRPD pattern of form T1 (Niddam-Hildesheim *et al.*, 2004:3).

Niddam-Hildesheim *et al.* (2004:4) described a method for the preparation of form T1 which included the following steps: crystallisation of gatifloxacin with acetonitrile; isolation of the gatifloxacin recrystallised; and slurring the gatifloxacin in a lower alkanol preferably having 2 carbon atoms for a time of about 2 hours. Finally the crystalline form of gatifloxacin is isolated from the mixture.

2.4.5 Form H1 (sesquihydrate)

Pat. No. WO 2004/087688

Form H1 is a sesquihydrate crystal form of gatifloxacin that is characterised by x-ray powder diffraction peaks at the following $^{\circ}2\theta$ -positions: $9.2^{\circ}2\theta$, $10.5^{\circ}2\theta$, $12.9^{\circ}2\theta$, $18.4^{\circ}2\theta$, $18.9^{\circ}2\theta$, $19.9^{\circ}2\theta$, $21.2^{\circ}2\theta$, $21.7^{\circ}2\theta$, and $24.0^{\circ}2\theta$ (Parthasaradhi *et al.*, 2004:2). The XRPD pattern of form H1 is displayed in figure 2.11.

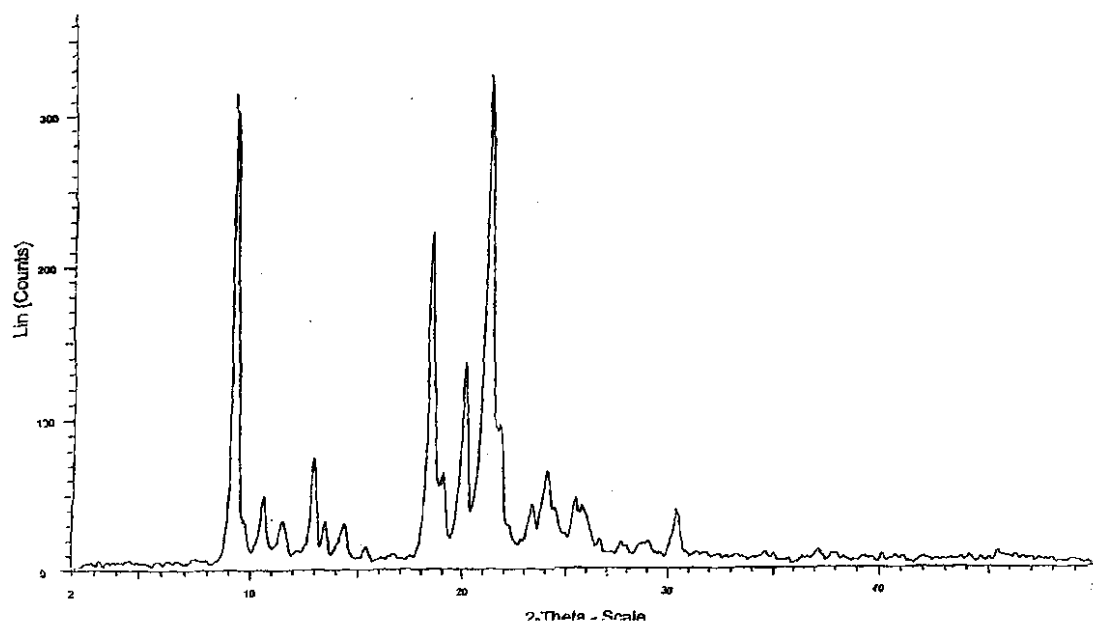


Figure 2.11 XRPD pattern of form H1 (Parthasaradhi *et al.*, 2004:2).

2.4.6 Form H4 (sesquihydrate)

Pat. No. WO 2004/087688

Parthasaradhi *et al.* (2004:3) disclosed a novel crystalline form of gatifloxacin, form H4, which was classified as a sesquihydrate form. Figure 2.12 shows the characteristic x-ray powder diffractogram of form H4 and the characteristic XRPD peak angles ($^{\circ}2\theta$) of form H4 are listed in table 2.7.

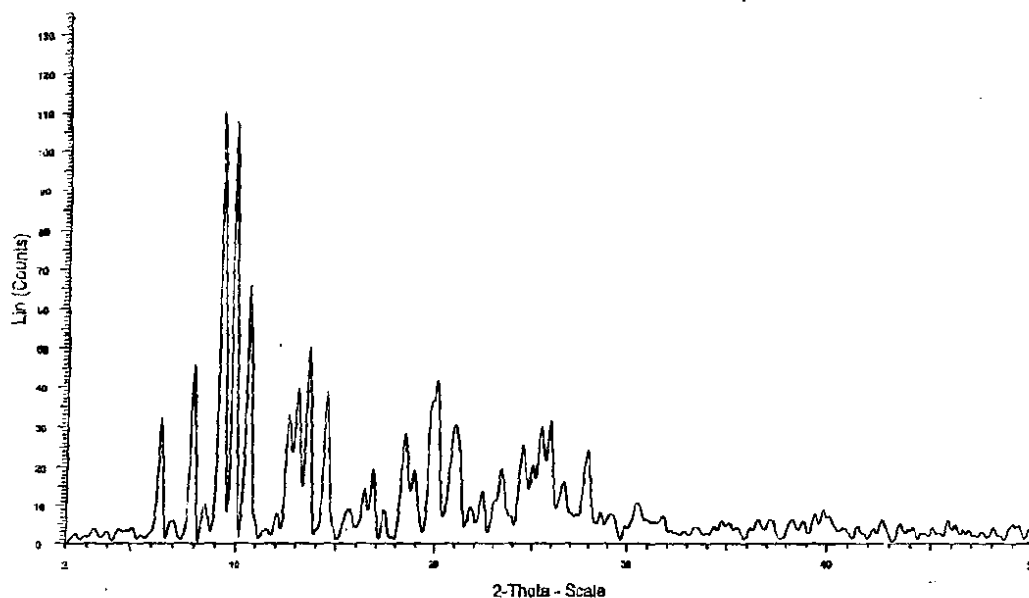


Figure 2.12 X-ray powder diffractogram of form H4 (Parthasaradhi *et al.*, 2004:3).

Table 2.7 Main XRPD peak angles ($^{\circ}2\theta$) of form H4 (Parthasaradhi *et al.*, 2004:3)

Main peaks	Form H4
	($^{\circ}2\theta$)
1	6.3
2	7.8
3	9.2
4	9.8
5	10.6
6	12.6
7	12.9
8	13.5
9	14.4
10	18.4
11	19.8
12	20.0
13	20.9
14	24.4
15	25.4
16	25.9
17	27.9

2.4.7 Form H5 (sesquihydrate)

Pat. No. WO 2004/087688

Form H5 is a sesquihydrate crystal form of gatifloxacin characterised by XRPD peaks at $8.2^{\circ}2\theta$, $13.5^{\circ}2\theta$, $13.9^{\circ}2\theta$, $16.5^{\circ}2\theta$, $17.0^{\circ}2\theta$, $17.9^{\circ}2\theta$, $19.9^{\circ}2\theta$, $21.0^{\circ}2\theta$, $23.3^{\circ}2\theta$, and $24.8^{\circ}2\theta$ (Parthasaradhi *et al.*, 2004:4). The XRPD pattern of form H5 is shown in figure 2.13.

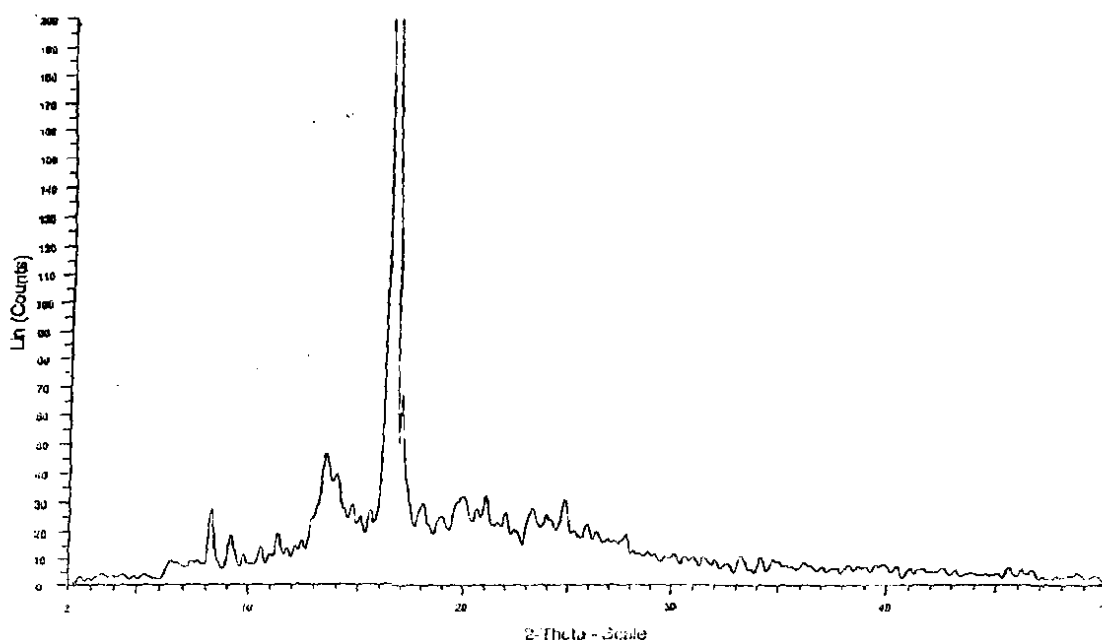


Figure 2.13 XRPD pattern of form H5 (Parthasaradhi *et al.*, 2004:4).

Conclusion

Gatifloxacin is a broad-spectrum fluoroquinolone antibacterial agent. Gatifloxacin is active against gram-positive, gram-negative and atypical organisms (Schaeffer, 2002:49S). Gatifloxacin has an improved pharmacokinetic profile over earlier fluoroquinolones and is often preferred over these agents (Schaeffer, 2002:52S).

Many patents are currently available claiming intellectual property of the various crystal forms of gatifloxacin. These patents describe solvated crystal forms, hydrates and anhydrous forms of gatifloxacin. Insufficient physico-chemical data are provided

in the patents and the aim of this study was to further the knowledge and understanding of the solid state and solvent inclusion properties of gatifloxacin.

Due to the tendency of gatifloxacin to crystallise into various crystal forms, polymorphic studies on this drug (especially during the early development stages of a new dosage form) have become extremely important. Choosing the wrong crystal form for manufacturing of the solid dosage forms could lead to manufacturing and stability problems.

Physico-chemical analytical techniques utilised in this study will be discussed in the following chapter.

CHAPTER 3

Methods for the characterisation and analysis of gatifloxacin crystal forms

Introduction

This chapter describes the various methods of analysis used in the characterisation of gatifloxacin crystal forms. A brief overview of the importance of some of these methods and the reasons for their use in the investigation of gatifloxacin crystal forms is also given.

3.1 Characterisation methods

Various methods and techniques were used in the characterisation of gatifloxacin crystal forms, including thermal methods (such as differential scanning calorimetry and thermogravimetric analysis), x-ray powder diffraction, microscopy, infrared spectrophotometry, solubility and dissolution studies.

3.1.1 Thermal methods

These methods include differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and hot-stage microscopy (HSM). DSC and TGA will be discussed here, as HSM will be discussed in section 3.1.2.1.

3.1.1.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry involves the measurement of the difference in energy (heat flow) between a reference (R) and a sample (S). It can be used to determine the enthalpy changes of a solid state process as well as the melting point of a sample (Byrn *et al.*, 1999:83-84).

Two types of DSC methods are possible, namely power-compensation DSC and heat-flux DSC (Brittain, 1999:253). The difference between these two methods is illustrated in

figure 3.1. Power-compensated DSC uses individualised heating elements to keep the sample and the reference at the same temperature. The difference in power inputs to the two heaters is recorded here (McCauley & Brittain, 1995:237). Heat-flux DSC monitors the heat differential between the sample and the reference material (Brittain, 1999:253). Due to instrument failure all the studies could not be performed on one instrument and therefore both types of DSC methods were used. Three different DSC instruments were used. All three instruments were calibrated with ultra-pure indium standard with a melting point of 156.4°C. Therefore the results obtained using these different calibrated instruments could be viewed as comparable.

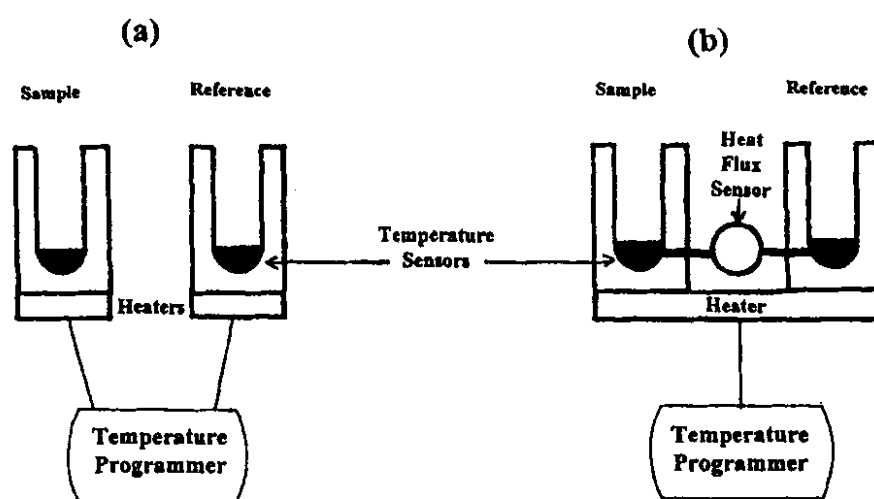


Figure 3.1 Schematic representations of the sample cell configurations for (a) power-compensation and (b) heat-flux modes of DSC detection (McCauley & Brittain, 1995:237).

A heat-flux DSC instrument, namely a Shimadzu DSC-50 (Shimadzu, Kyoto, Japan), was used for most of the samples. Samples weighing 2-4 mg were heated in aluminium crimp cells with pierced lids to allow escape of solvent. The measurements should correlate well with the TGA data, which is also obtained in an open pan. Samples were heated under a nitrogen purge with a flow rate of 35 ml/min. A heating rate of 10°C/min was used and the samples were heated to a maximum temperature of 200°C.

A Perkin Elmer Pyris-1 DSC (Perkin Elmer, USA) was used for some of the samples. This instrument is a power-compensation DSC. Samples weighing 2-4 mg were heated in

aluminium sample holders with pierced lids to a maximum of 200°C. Samples were heated under a nitrogen purge with a flow rate of 20 ml/min at a heating rate of 10°C/min.

A Mettler Toledo DSC (Mettler Toledo, Switzerland) instrument was used for the remainder of the samples. This is a heat-flux DSC instrument. Samples weighing 2-4 mg were heated in aluminium sample pans with pierced lids. Samples were heated from 25-200°C at a heating rate of 10°C/min, under a nitrogen purge with a flow of 30 ml/min.

The thermogram obtained from a DSC analysis, is a plot of ΔT (heat flow/energy difference) versus T (temperature). Endotherms displayed on the thermogram represent processes in which heat is absorbed. These processes include solvent loss, phase transitions or melting. Exotherms represent processes where heat is generated, such as crystallisation or chemical reactions (Byrn *et al.*, 1999:84).

3.1.1.2 Thermogravimetric analysis (TGA)

This method measures the change in the weight of a sample in relation to temperature (Newman & Stahly, 2002:9). TGA is often used to study the desolvation process or compound decomposition. It is an easy method to determine the total volatile content of a sample and is often used in combination with Karl Fischer titrations to determine the moisture content of a sample (McCauley & Brittain, 1995:243).

In this study a Shimadzu TGA-50 instrument (Shimadzu, Kyoto, Japan) was used. The sample weight varied between 5-10 mg each. Samples were weighed in an open platinum cell and the changes in mass upon heating were recorded. The samples were heated at a heating rate of 10°C/min under a nitrogen purge of 35 ml/min. Samples were heated to a maximum temperature of 160°C.

The experimental weight loss, as determined by the TGA, was compared with the theoretical weight loss. The theoretical weight loss was calculated by means of the following equation:

$$\% \text{ Weight loss} = \frac{\text{Molecular weight (solvent)}}{\text{Molecular weight (solvent)} + \text{Molecular weight (gatifloxacin)}} \times 100\%$$

3.1.2 Microscopy

Microscopy is often used in the pharmaceutical industry to assist in the characterisation of polymorphic and pseudopolymorphic solids. Microscopy often provides valuable information regarding the morphology of a solid form and is often used in conjunction with other characterisation methods in the analysis of crystalline solids. Two types of microscopy were used in the investigation of gatifloxacin crystal forms, i.e.: hot-stage microscopy (HSM) and scanning electron microscopy (SEM).

3.1.2.1 Hot-stage microscopy (HSM)

Hot-stage microscopy is often used in combination with DSC and TGA in the characterisation of solid state forms. HSM provides a visual confirmation of thermal events that occurred during DSC and TGA analysis (Byrn *et al.*, 1999:73). Some of these thermal events that can be observed are:

1. Signs of gas evolution (immersed in mineral oil). This is indicative of desolvation.
2. Changes in the outward appearance or transparency. These changes could indicate solid state transformations or chemical reaction.
3. Crystallisation from a melt or solution, indicating phase transformation.
4. Melting behaviour and melting equilibrium, an identifying constant (Byrn *et al.*, 1999:73).

A Nikon Eclipse E400 (Nikon, Japan) thermomicroscope, with a Metrathern 1200d heating unit and a Nikon Coolpix 5400 digital camera (Nikon, Japan) was used. Small amounts of samples were studied both without and emerged in a drop of silicon oil. The

samples were placed on an object plate, covered with a cover plate and observed under the thermomicroscope at a temperature range from 25-200°C.

3.1.2.2 Scanning electron microscopy (SEM)

The particle morphology of pharmaceutical solids is often studied through scanning electron microscopy (SEM) (Newman & Brittain, 1995:140). This type of microscopy can also be used to detect the presence of a mixture of crystal forms, if the forms display different habits.

A FEI Quanta 200 ESEM & Oxford INCA 400 EDS system (FEI, USA) was used to obtain photomicrographs of the various crystal forms of gatifloxacin studied. The samples were adhered to a small piece of carbon tape, mounted onto a metal stud and coated with a gold-palladium film (Eiko Engineering ion Coater IB-2).

3.1.3 Crystallographic methods

X-ray crystallography is very useful in the characterisation of polymorphs as each crystal form has a characteristic diffractogram. As already mentioned in section 1.3.5.1, this diffractogram can be seen as a fingerprint of each specific crystal form, and is often used in the characterisation of solid state forms. X-ray diffraction can be performed on single crystals or powdered samples, and provides structural information on the solid forms (Brittain, 1999:229).

Bragg described the diffraction of a monochromatic x-ray beam striking a plane of atoms in the following way. The diffracted x-ray beams behave as if they were reflected from planes passing through the crystal and the radiation can be viewed as a wave. This diffraction occurs at an angle θ . This angle is the same for the incident and diffracted rays. The three rays, *A*, *B* and *C*, will only reinforce each other if their path lengths differ by precisely one wavelength (λ) (Byrn *et al.*, 1999:53-54). Figure 3.2 illustrates this concept of x-ray diffraction.

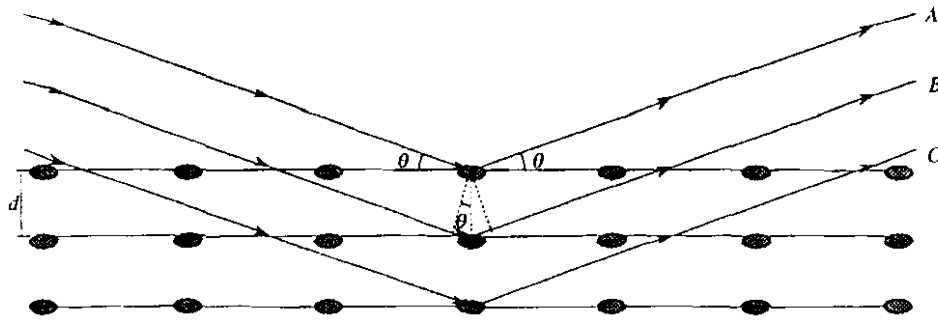


Figure 3.2 X-rays diffracted by planes with spacing d at a diffraction angle of θ (Byrn *et al.*, 1999:54).

The Bragg equation, based on the above, is used in the interpretation of all diffraction patterns (Byrn *et al.*, 1999:53-54).

Bragg equation:

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

Where:

n = multiple

λ = wavelength of the incident beam

d = distance between the planes in the crystal

θ = angle of incidence

The Bragg equation states that a multiple (n) of the wavelength (λ) must equal twice the distance (d) between planes multiplied by the sine of the angle of incidence (Byrn *et al.*, 1999:54). This equation does not give any indication about the intensities of the diffracted radiation and only gives the scattering angles (Brittain, 1999:231).

3.1.3.1 X-ray powder diffraction (XRPD)

X-ray powder diffraction (XRPD) is often used when suitable single crystals of the pharmaceutical solid cannot be obtained. The powdered sample of the drug can be analysed using this method.

A powdered sample will present an entirely random selection of all possible crystal faces at the powder interface. Information regarding all the possible atomic spacings in the crystal lattice is gained from the diffraction off these surfaces. A scintillation counter is used to measure the angle of diffracted x-rays with respect to the angle of the incident beam, of a slowly rotating sample. The scattering angle, $^{\circ}2\theta$, can then be determined from this (Brittain, 1999:235).

The x-ray powder diffraction data for gatifloxacin crystal forms was obtained using a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; antiscatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, $2^{\circ}/\text{min}$ (step size, 0.025° ; step time, 1.0 sec.). An aluminium sample holder was used, and approximately 200 mg of powdered sample was loaded into the sample holder. Care was taken not to introduce a preferential orientation of the crystals.

XRPD analyses at ambient conditions and at increased temperatures, variable temperature x-ray powder diffraction (VT-XRPD), were performed on the crystals. The same conditions were used for both types of x-ray diffraction. For the VT-XRPD an Anton Paar TTK 450, low temperature camera (Anton Paar, Austria), was attached to the Bruker D8 Advance diffractometer (Bruker, Germany). A heating rate of $10^{\circ}\text{C}/\text{cycle}$ was used during determinations and all samples were heated to a maximum temperature of 180°C .

The XRPD pattern obtained from XRPD analysis consists of a series of peaks detected at a characteristic scattering angle (degrees 2θ), with specific relative intensities.

3.1.4 Infrared spectroscopy (IR)

Byrn *et al.* (1999:111) stated that infrared spectroscopy is a powerful method for the characterisation and identification of the various solid forms of a drug. This is due to the extreme sensitivity of this method to the structure and conformation of a chemical entity.

Various IR techniques are available for the characterisation of solid forms. A technique that is often used in the pharmaceutical industry is the diffuse reflectance infrared Fourier transform spectroscopic (DRIFTS) method. In this technique the powdered sample is irradiated by an infrared beam. The particles of the sample cause absorption, reflection and diffraction of the incident radiation. Absorptivity information on the sample can only be obtained from the incident radiation that undergoes diffuse reflectance. The DRIFTS method is often used in polymorphic studies due to the fact that limited sample handling is necessary and the polymorphic character of the sample can be maintained (Bugay & Findlay, 2002:511-512).

A Nicolet Nexus 470-FT-IR spectrometer (Nicolet instrument corporation, Maddison, Wisconsin, USA) was used to record the IR-spectra, over a range of 400-4000 cm^{-1} . The DRIFTS method was used. KBr was used as background. The samples were dispersed in KBr and the IR-spectra measured in a reflectance cell.

3.1.5 Karl Fischer titration

Karl Fischer titration is often used to determine the moisture content in a crystalline solid. This assists in the identification and characterisation of pseudopolymorphs. Karl Fischer is usually used in conjunction with TGA, which determines the total volatile content of a sample, as already mentioned in section 3.1.1.2.

The moisture content of the samples was determined with a Metrohm 701 KF Titrino autotitrator (Metrohm, Switzerland). Calibration of the instrument was performed using a predetermined mass of water and approximately 250 mg of sodium tartrate dihydrate dibasic powder. Approximately 50 mg of each sample was used for the moisture

determination. The experiment was performed in duplicate for each crystal form. The percentage of water (m/m) within the sample was then calculated.

3.1.6 Powder dissolution studies

Powder dissolution studies were performed on all the samples according to the method developed by Lötter *et al.* (1983:59-66).

Particle size is an important factor to consider when performing dissolution studies, seeing that the particle size is directly related to the exposed surface area during the dissolution test (Byrn *et al.*, 1999:92). All the powdered samples of the crystal forms studied were sieved to ensure particle size uniformity. Sieved fractions of 106-250 μm were used in the dissolution studies.

Powder dissolution studies were performed on either a VanKel Vanderkamp 600 or a VanKel 7000 (VanKel Industries, Chatham, New Jersey, USA) dissolution tester. It was equipped with a VanderKamp heater-circulator. The dissolution studies were performed at a constant temperature of $37 \pm 0.2^\circ\text{C}$. The dissolution medium was 0.01 N HCl. Approximately 200 mg of samples was weighed into test tubes. 100 mg of glass beads were added to the test tubes. 5 ml of dissolution medium was added to the samples and each sample was vortexed for 2 minutes. The samples were then rinsed into the dissolution vessels using dissolution medium from each corresponding vessel.

Method 2, Paddle, of the USP 28 (2005:2413), was used for the measurement. Four to five dissolution vessels were used for each crystal form studied. The vessels each contained 450 ml dissolution medium. The rotation speed of the paddles were 50 rpm, and 10 ml samples were withdrawn from the vessels at time intervals of 7.5, 15 and 30 minutes, using 10 ml syringes to which 0.45 μm low protein binding, durapore (PVDF) filters were attached. 3 ml of the withdrawn samples each was diluted to a final volume of 200 ml with dissolution medium.

Approximately 44.44 mg of the sample (reference standard) was weighed for the standard and was dissolved in 20 ml methanol in a 100 ml flask and sonicated for 10

minutes. It was then made up to volume with dissolution medium. 3 ml was withdrawn from this flask and further diluted with dissolution medium to a final volume of 200 ml.

The absorbance of both the standard and the samples were determined using a Beckman DU-650i spectrophotometer (Beckman, USA). Measurements were taken at a wavelength of 293 nm. The % sample that was dissolved at each time interval was calculated using the following equation:

$$\%Dissolved = \frac{(A_{std} \times C_{std} \times DV \times 100\% \times 200 \times M_{theoretical})}{(A_{sample} \times M_{sample} \times 3 \times 0.2)}$$

Where:

- A_{std} = Average absorbance of the standard
- C_{std} = Theoretical concentration of the standard (g/ml)
- DV = Dissolution medium volume (ml)
- $M_{theoretical}$ = Theoretical sample mass (g)
- A_{sample} = Average absorbance of the sample
- M_{sample} = Actual mass of the sample weighed (g)

The dissolution profiles were compared for similarities by using the following equation, described by Moore and Flanner (1996:65):

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n w_t (R_t - T_t)^2 \right]^{-1/5} \times 100 \right\}$$

Where:

- R_t = Reference dissolution value at time point t
- T_t = Test dissolution value at time point t
- n = Number of dissolution time points
- w_t = Optional weight factor

The value for f_2 (similarity factor) varies between 0-100. The reference and test profiles are identical if f_2 is 100 and the similarity between the curves decreases as the value of

f_2 approaches 0. f_2 values greater than 50 indicate similarity between the two dissolution profiles (Moore & Flanner, 1996:65).

3.1.7 Solubility studies

The solubility studies were performed over a 24-hour time period. 0.01 N HCl (pH 2.10-2.25), acetate buffer (pH 4.5), MilliQ water (pH 5.50-5.65) and phosphate buffer (pH 6.8) were used as solvents. All these media were prepared using milliQ water. For each solvent 5 test tubes were used in the determination. 25 mg of sample was weighed into each test tube and 5 ml of solvent was added to each test tube, after which the test tubes were tightly closed. A rotation speed of 65 rpm was used and the temperature in the water bath was controlled at $37 \pm 2^\circ\text{C}$. A Heidolph RZR-2000 rotator (Heidolph, Germany) and a Julabo EM/4 thermostat (Julabo, Germany) were used to obtain the above-specified conditions.

The samples were removed from the water bath after 24 hours and withdrawn from the test tubes using 5 ml syringes, with $0.45 \mu\text{m}$ low protein binding, durapore (PVDF) filters attached to each. 1 ml of each test tube was diluted to 100 ml with the corresponding solvent used. The above-mentioned solutions were then further diluted as follows. 2 ml of each solution was withdrawn and diluted to a final volume of 25 ml with the corresponding solvent. This was done for the 0.01 N HCl, acetate buffer (pH 4.5) and the phosphate buffer (pH 6.8) each. The solutions obtained for the samples tested in milliQ water were further diluted taking 5 ml of each solution, and making it up to a final volume of 25 ml.

The absorption of each of the final dilutions was determined spectrophotometrically using a Beckman DU-650i spectrophotometer (Beckman, USA). A wavelength of 293 nm was used for the measurements.

A standard curve was plotted using various dilutions of gatifloxacin. Theoretical concentrations of these dilutions were plotted against the measured absorbance of each

dilution. Theoretical concentrations and corresponding absorbance are listed in table 3.1.

Table 3.1 Theoretical concentrations and corresponding absorbance of various dilutions of gatifloxacin

Concentration ($\mu\text{g/ml}$)	Absorbance (AU)
0.208	0.0201
0.417	0.0398
0.833	0.0792
1.667	0.1565
3.333	0.3123
6.666	0.6245
13.323	1.2460
26.653	2.3910

The curve obtained from this data is shown in figure 3.3

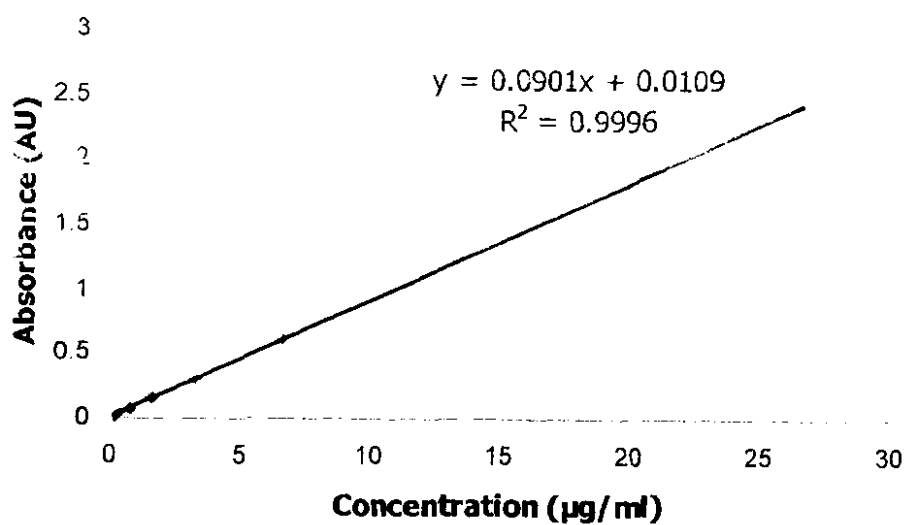


Figure 3.3 Standard curve for gatifloxacin used in spectrophotometric determinations.

The equation of this plot was used to calculate the concentration of the diluted samples. With this knowledge it was possible to calculate the concentration of each of the undiluted samples.

3.1.8 Flowability

The flowability of the pharmaceutical solid is an important factor to consider during product development, seeing that it may affect various processes in the manufacturing of a solid drug. A free-flowing pharmaceutical powder is needed to ensure tablet weight uniformity, and minimise the occurrence of tablet capping due to entrapped air within the powders. This entrapment of air within the powders occurs with uneven powder flow (Staniforth, 2002:197).

Two easy ways to evaluate the flowability of a pharmaceutical solid is the Hausner ratio and Carr's index. The Hausner ratio is:

$$\text{Hausner ratio} = D_f / D_0$$

Where:

D_f = Final density (also known as the tapped density) of the sample (g/ml)

D_0 = Initial density (also known as the poured density) of the sample (g/ml)

This ratio is related to interparticle friction. Hausner found that powders with low interparticle friction had ratios of about 1.2. These are powders such as coarse spheres. A Hausner ratio of greater than 1.6 occurred with more cohesive, less free-flowing powders. Powders that form flakes usually exhibit this kind of flow (Staniforth, 2002:207).

Carr's index determines the % compressibility of a powder and the powder flow can then be calculated from this. Staniforth (2002:207) stated that the % compressibility directly measures the potential powder arch of a powder.

The equation for Carr's index is as follows:

$$\% \text{ Compressibility} = \frac{D_f - D_0}{D_f} \times 100$$

Where:

D_f = Final density (also known as the tapped density) of the sample (g/ml)

D_0 = Initial density (also known as the poured density) of the sample (g/ml)

These two methods were utilised to evaluate the flowability of the samples. A known mass of the sample was poured into a small measuring cylinder and the initial volume of the sample was determined. This, together with the known mass, was used to calculate the initial density of the powder (Density = Mass / Volume). The cylinder was then tapped for 30 seconds after which the volume of the sample was again determined. This final tapped volume was used, together with the mass to calculate the tapped density of the powder. Both values were substituted into the above equations to calculate the Hausner ratio and % compressibility. The % compressibility can be used to measure the flowability of a sample, as shown in table 3.2.

Table 3.2 Relationship between powder flowability and % compressibility (Staniforth, 2002:208)

% Compressibility range	Flow description
5-15	Excellent
12-16	Good
18-21	Fair
23-28	Poor
28-35	Poor
35-38	Very poor
>40	Extremely poor

Conclusion

A drug substance can exist in various solid forms, such as polymorphs and solvates. Comprehensive characterisation of the physico-chemical properties of polymorphs and solvates of an API is an important aspect to consider when developing a solid dosage form.

Various methods can be used in the characterisation of polymorphs and solvates, including crystallography, spectrophotometric studies, thermal analysis and solubility studies. These methods are combined in this study for the identification and characterisation of gatifloxacin crystal forms. The results obtained for the various crystal forms prepared will be discussed in the following chapters.

CHAPTER 4

Solvent inclusion and the effect thereof on the crystal lattice of gatifloxacin

Introduction

The incorporation of a solvent in a crystal lattice may have a profound impact on the solid state properties of the active pharmaceutical ingredients (API's). Minor changes in the solid state properties may lead to problems during the manufacturing of pharmaceuticals. Various actions during the manufacturing process (i.e. wet-granulation, exposure to atmospheric moisture etc.) may lead to the hydration of hygroscopic solids.

The aim of this chapter is to investigate the impact of the hydration status (sesquihydrate) on the physico-chemical properties of gatifloxacin.

4.1 Materials and methods

4.1.1 Materials

Gatifloxacin sesquihydrate raw material was obtained from Xiamen Mchem Pharma (Group) Ltd., China. The details of the raw material used during this study are as follows:

Batch number	: 20031110
Manufacturing date	: November 2003
Expiry date	: November 2006

Table 4.1 is a summary of the analytical results generated prior to the release and distribution of the raw material by the supplier (certificate of analysis, or CoA).

Table 4.1 Certificate of analysis of gatifloxacin sesquihydrate raw material (Xiamen Mchem Pharma (Group) Ltd., China, Certificate of analysis)

Test	Specification	Result
Characteristics of the raw material	White or yellowish crystalline powder, odourless	Complies
Assay by HPLC	$\geq 98.50\%$	99.75%
Impurities detected by HPLC	$\leq 1.00\%$	0.25%
Total water content	4.00-8.00%	6.71%
Residue on ignition	$\leq 0.20\%$	0.05%
Heavy metals	≤ 20 ppm	Complies

Anhydrous gatifloxacin was prepared by drying the raw material for 4 hours at 140°C, 15 mbar in a vacuum oven (Labotec, South Africa) fitted with a vacuum pump (Vacuubrand, Germany).

4.1.2 Methods

Various techniques such as XRPD, IR spectroscopy, DSC, KF, TGA and powder dissolution studies were used to compare the physico-chemical properties of the hydrated and anhydrous forms of gatifloxacin. These techniques have been described in chapter 3.

4.2 Results and discussion

4.2.1 X-ray powder diffraction (XRPD) and variable temperature x-ray powder diffraction (VT-XRPD)

The x-ray powder diffractograms of gatifloxacin sesquihydrate and anhydrate displayed significant differences, indicating that the crystal lattice of the hydrated crystal form differed from that of the anhydrous form. Table 4.2 is a summary of the main peak angles ($^{\circ}2\theta$) and relative intensity values (I/I_0) of the hydrated and anhydrous gatifloxacin.

Table 4.2 The main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I_0) of the hydrated (sesquihydrate) and anhydrous gatifloxacin samples

Main peaks	Samples			
	Gatifloxacin sesquihydrate		Anhydrous gatifloxacin	
	($^{\circ}2\theta$)	(I/I_0)	($^{\circ}2\theta$)	(I/I_0)
1	6.3	5.8	-	-
2	7.8	10.0	7.7	18.7
3	-	-	8.9	6.0
4	9.1	100.0	-	-
5	-	-	9.4	4.5
6	9.7	22.6	-	-
7	-	-	10.2	11.1
8	10.5	16.0	-	-
9	-	-	11.2	11.8
10	11.4	14.6	-	-
11	-	-	11.7	2.7
12	-	-	12.1	9.6
13	12.3	12.7	-	-
14	13.0	33.8	12.9	30.1
15	13.5	18.1	13.6	44.6
16	-	-	14.1	21.8
17	14.3	14.8	-	-
18	14.8	6.9	-	-
19	-	-	15.0	11.5
20	-	-	15.4	11.6
21	15.6	5.3	-	-
22	16.3	6.6	16.4	8.7
23	16.7	5.5	-	-
24	-	-	17.0	6.9
25	17.8	5.2	17.8	3.7
26	18.3	56.5	-	-
27	18.9	14.3	18.9	5.9

Table 4.2 (continued)

Main peaks	Samples			
	Gatifloxacin sesquihydrate		Anhydrous gatifloxacin	
	(°2θ)	(I/I ₀)	(°2θ)	(I/I ₀)
28	19.6	23.2	19.7	58.9
29	20.0	47.4	-	-
30	20.2	18.5	-	-
31	-	-	20.5	22.7
32	20.8	21.2	-	-
33	21.1	37.0	21.2	100.0
34	21.8	15.2	21.7	43.8
35	22.2	7.2	-	-
36	22.9	6.6	-	-
38	-	-	23.8	16.4
39	24.3	22.7	-	-
40	-	-	24.5	36.6
41	-	-	24.9	9.6
42	25.4	22.9	-	-
43	-	-	25.6	7.3
44	25.8	18.1	25.9	26.8
45	26.1	13.0	26.2	14.7
46	26.5	7.9	-	-
47	-	-	27.3	6.3
48	-	-	27.5	6.6
49	27.8	8.8	-	-
50	-	-	28.7	14.2
51	29.0	7.0	-	-
52	29.3	9.4	29.2	3.8
53	30.4	15.4	30.5	6.3
54	-	-	30.9	7.6
55	-	-	31.5	3.7
56	32.3	6.3	-	-
57	-	-	33.1	3.3
58	-	-	34.7	4.3

Differences between the x-ray powder diffractograms of the two samples are highlighted in table 4.2. Some of the most prominent differences include the presence of peaks at $9.1^{\circ}2\theta$, $18.3^{\circ}2\theta$ and $20.0^{\circ}2\theta$, with relative intensities of 100.0%, 56.5% and 47.4% respectively in the XRPD of the sesquihydrate. These peaks were all absent in the XRPD pattern of the anhydrous form. Peaks that were present in the XRPD pattern of the anhydrous form and absent in the XRPD pattern of the sesquihydrate, include peaks at $14.1^{\circ}2\theta$, $20.5^{\circ}2\theta$ and $24.5^{\circ}2\theta$, with relative intensities of 21.8%, 22.7% and 36.6% respectively. The peaks at $19.7^{\circ}2\theta$ and $21.2^{\circ}2\theta$ displayed marked differences in their intensities for the two samples. Figure 4.1 is an overlay of the x-ray powder diffractograms of the two samples.

The XRPD pattern of the sesquihydrate raw material was comparable with that of form H4 (a sesquihydrate form described in section 2.4.6). Most of the characteristic peaks of form H4 were present in the diffractogram of the raw material (see section 2.4.6).

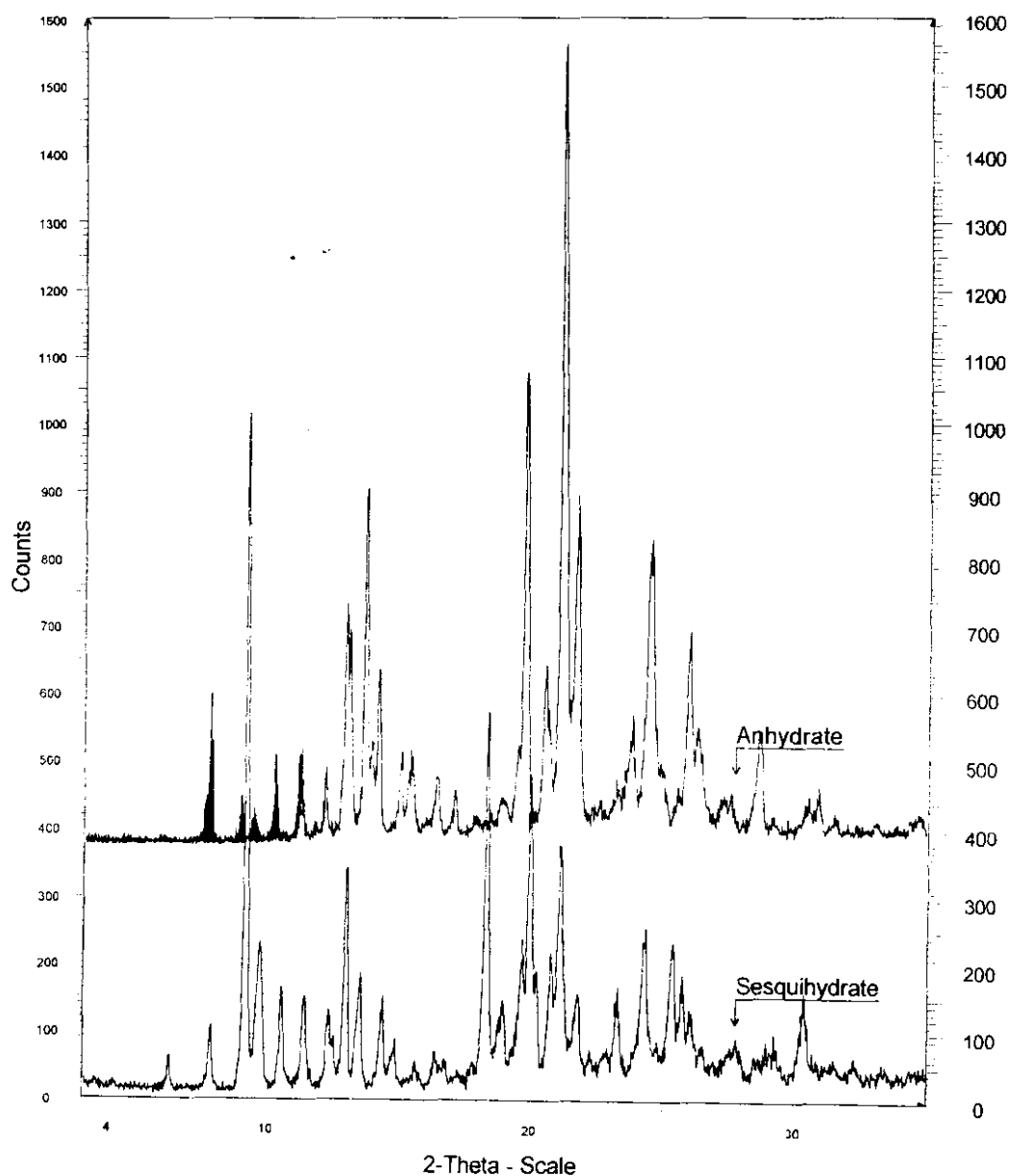


Figure 4.1 X-ray powder diffractogram overlay of gatifloxacin sesquihydrate and gatifloxacin anhydrate.

Variable temperature x-ray powder diffraction (VT-XRPD) was performed on the sesquihydrated form to determine changes that might occur during desolvation. The x-ray powder diffractograms obtained from this study are shown in figure 4.2. The XRPD pattern of the sesquihydrate remained relatively unchanged between 25-75°C with only slight changes occurring in this temperature range. Significant changes were observed at 85°C. Some of the major changes that occurred at 85°C included the following: the peak at 6.3°2 θ decreased in intensity and a new peak was observed at 9.4°2 θ . The peaks at 13.0°2 θ 14.3°2 θ disappeared and the peak at 14.8°2 θ showed an increase in intensity. Other changes included the disappearance of the peak at 18.3°2 θ . The peak at 19.6°2 θ in the diffractogram at 25°C shifted slightly to the right ($\pm$ 19.8°2 θ) and increased in intensity. The peaks between 25-26°2 θ were not present in the diffractogram obtained at 85°C. The diffractogram remained relatively unchanged between 85-95°C, except for the disappearance of the peak at 6.3°2 θ , the appearance of a peak with weak intensity at 13.2°2 θ and the disappearance of the peaks at 23.5°2 θ and 31.4°2 θ . Slight changes were observed in the diffractogram at 110°C such as an increase in intensity of the peak at 13.2°2 θ and the appearance of a new peak at 21.7°2 θ . The changes observed in the diffractogram between 85-110°C were possibly due to a change in the crystal structure caused by dehydration. The diffractogram remained relatively unchanged between 110-120°C. At 150°C slight changes were observed in the diffractogram, including the sharp decrease in the intensity of the peaks at 9.8°2 θ and 11.5°2 θ and the appearance of a small peak at 12.9°2 θ . Other changes that occurred at this temperature included the appearance of peaks at 19.5°2 θ and 19.7°2 θ and the disappearance of the peak at 20.0°2 θ . At 160°C the peaks at 9.8°2 θ and 11.5°2 θ disappeared. Some of the changes that were observed in the diffractogram at 170°C included the appearance of a peak at 11.7°2 θ and the disappearance of the peak at 18.9°2 θ . The peaks between 24-25°2 θ showed a sharp decrease in intensity at 170°C. A characteristic melting diffractogram with no peaks was obtained at 180°C.

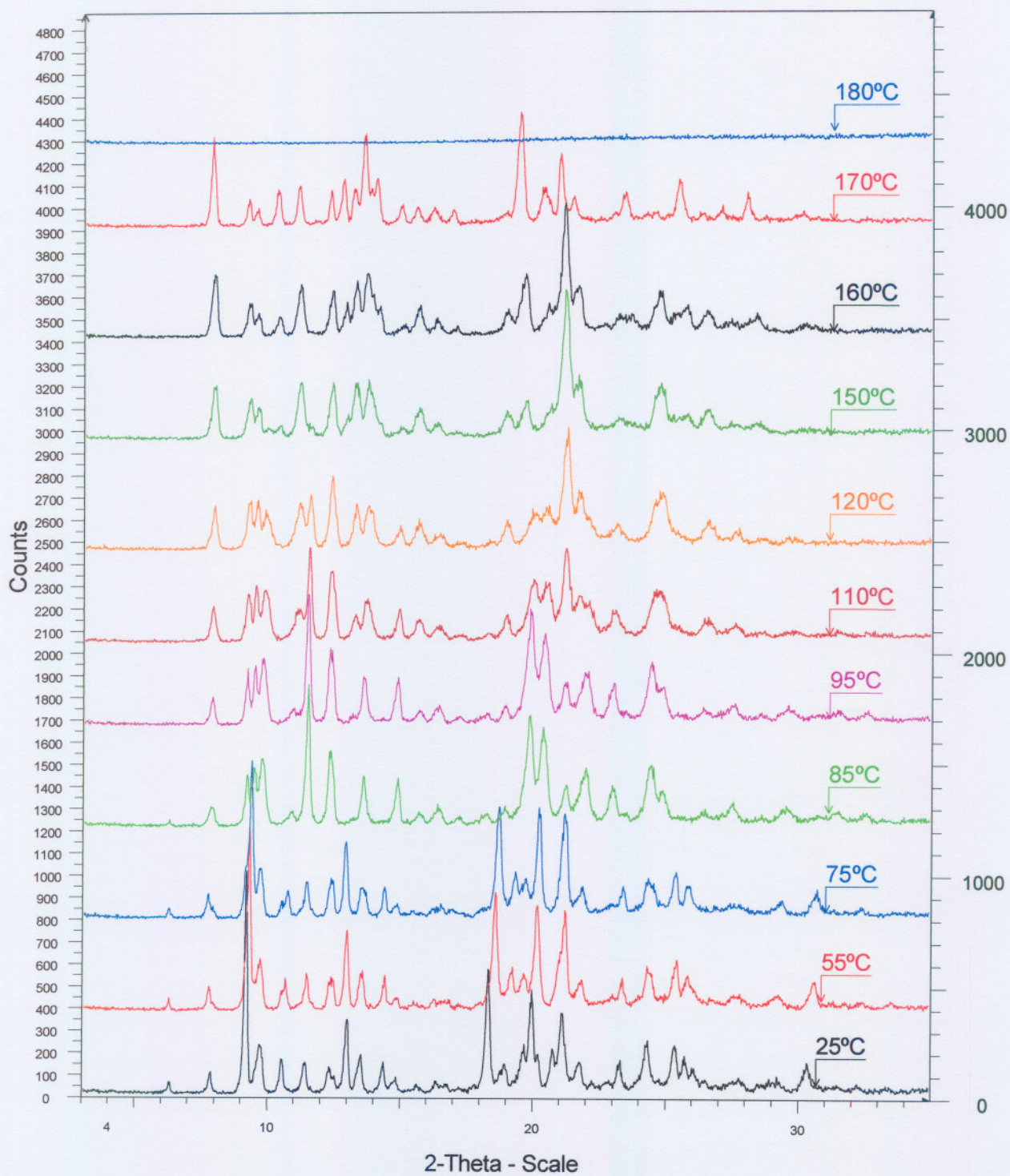


Figure 4.2 Variable temperature x-ray powder diffractograms of gatifloxacin sesquihydrate.

The XRPD pattern of gatifloxacin sesquihydrate displayed significant changes upon heating of the sample, due to the dehydration of the crystals. This indicated that the water incorporated into the crystal lattice played an important role in the stabilisation of the hydrated crystal structure. Dehydration caused changes in the crystal structure resulting in an entirely different XRPD pattern. Thus a different crystal structure was obtained after dehydration of the sesquihydrate.

4.2.2 Infrared spectroscopy (IR)

An overlay of the DRIFT-IR spectra obtained for the two samples is shown in figure 4.3 and the main absorptions are summarised in table 4.3. These spectra displayed significant differences in the main absorptions for the two samples. The most characteristic differences between the sesquihydrate and anhydrate were observed in the region $2400\text{--}3750\text{ cm}^{-1}$.

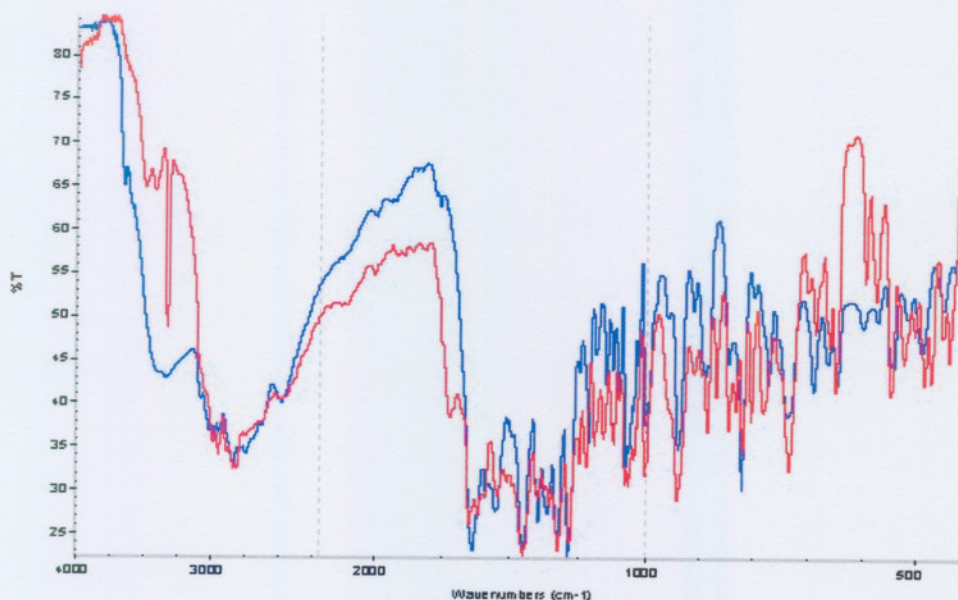


Figure 4.3 Overlay of the DRIFT-IR spectra of gatifloxacin sesquihydrate (blue) and gatifloxacin anhydrate (red).

Table 4.3 Main absorptions in the DRIFT-IR spectra of the hydrated (sesquihydrate) and anhydrous gatifloxacin

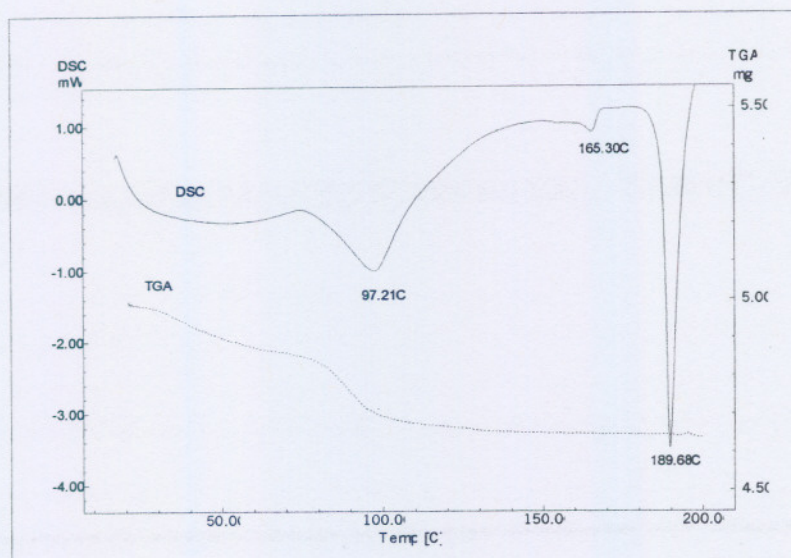
Main absorptions	Wavenumber (cm ⁻¹)	
	Sesquihydrate	Anhydrate
1	-	3490
2	3340	-
3	-	3329
4	2975	-
5	-	2944
6	2842	-
7	-	2813
8	-	1719
9	-	1648
10	1635	-
11	-	1616
12	1551	-
13	-	1542
14	1448	1448
15	1394	1396
16	1359	-
17	-	1351
18	1322	1319
19	1283	-
20	-	1277
21	-	1242
22	-	1217
23	1209	-
24	1179	-
25	-	1185
26	-	1153
27	1143	-
28	1118	1114
29	1095	1094
30	1070	-
31	-	1058
32	1025	-
33	997	1000
34	940	941
35	912	911
36	890	887
37	-	870
38	847	847
39	-	834
40	822	821
41	-	805
42	-	782
43	773	-
44	738	735
45	689	686
46	664	661

Table 4.3 (continued)

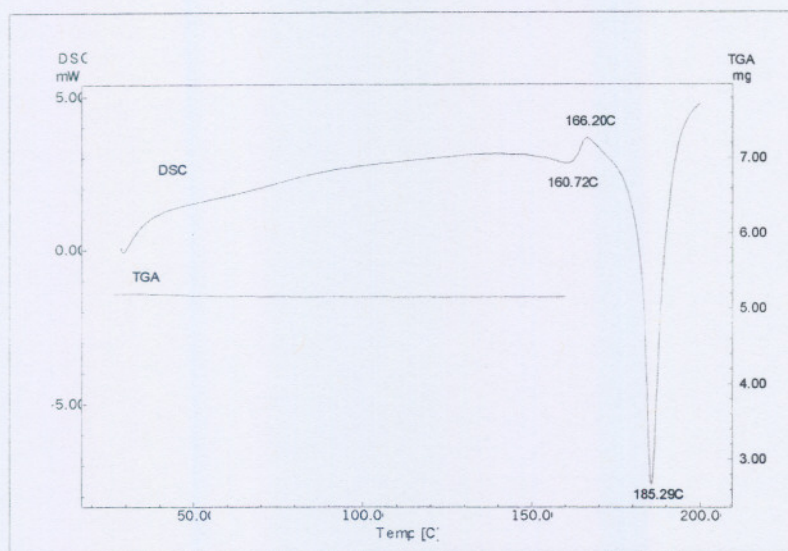
Main absorptions	Wavenumber (cm ⁻¹)	
	Sesquihydrate	Anhydrate
47	647	648
48	599	594
49	-	573
50	542	546
51	-	523
52	514	-
53	-	503
54	490	485
55	-	472

4.2.3 Differential scanning calorimetry (DSC)

Figure 4.4 shows the DSC and TGA thermograms of gatifloxacin sesquihydrate and gatifloxacin anhydrate. The sesquihydrated form showed a dehydration endotherm at 97.21°C. TGA analysis confirmed the dehydration process (with an onset at temperature 74.27°C and an endset temperature of 102.67°C). The anhydrous form displayed no peaks in this area signifying that no dehydration occurred.



(a)



(b)

Figure 4.4 DSC and TGA thermograms of (a) gatifloxacin sesquihydrate and (b) gatifloxacin anhydrate.

The endotherms observed at 160.72°C and 165.30°C for the anhydrate and sesquihydrate respectively, are representative of melting of all the crystals. This

endotherm is immediately followed by a recrystallisation exotherm (confirmed by thermal microscopy in section 4.2.5). This recrystallisation exotherm is more prominent in the thermogram of the anhydrous sample, and less prominent in the thermogram of the sesquihydrate, indicating that the energy needed for the sesquihydrate form to recrystallise might have been below the detection limit of the instrument. A final melting endotherm is observed at 185.29 and 189.68°C for the anhydrate and sesquihydrate respectively.

4.2.4 Thermogravimetric analysis (TGA) & Karl Fischer titration (KF)

Thermogravimetric analysis revealed a total weight loss of 6.09% for the sesquihydrate and 0.99% for the anhydrate. Karl Fischer titrations confirmed these weight losses to be due to the presence of water, with an average moisture content of 6.34% for the sesquihydrate and 0.97% for the anhydrate. These values are comparable to the theoretical moisture content for gatifloxacin sesquihydrate and anhydrate, which is 6.87% and 0.00% respectively. The results of the TGA and KF analysis are tabulated in table 4.4. The TGA and DSC thermograms of the sesquihydrate and anhydrous forms of gatifloxacin are shown in figure 4.4.

Table 4.4 TGA and KF results for the sesquihydrate and anhydrous forms of gatifloxacin

	% Weight loss (TGA)	% Moisture content (KF)
Sesquihydrate	6.09	6.34
Anhydrate	0.99	0.97

4.2.5 Microscopic analysis

Hot-stage microscopy (HSM) was used to examine the thermal behaviour of the samples. Table 4.5 provides a summary of the events observed with HSM of the sesquihydrate form. HSM confirmed the thermal events observed in the DSC thermograms.

Table 4.5 Summary of HSM observations of gatifloxacin sesquihydrate at a temperature range of 25-190°C

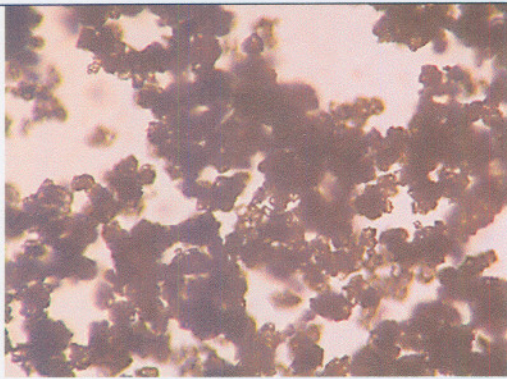
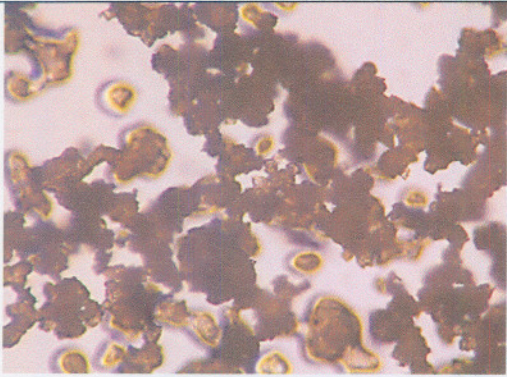
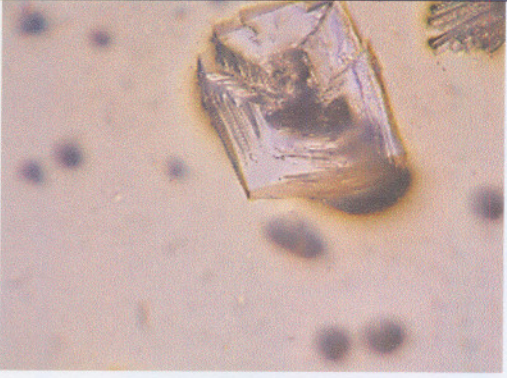
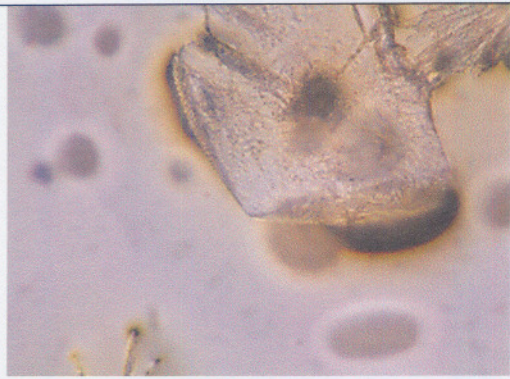
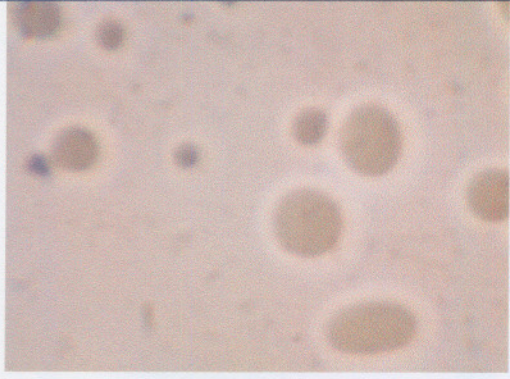
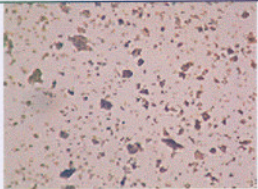
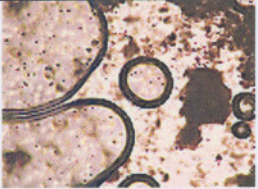
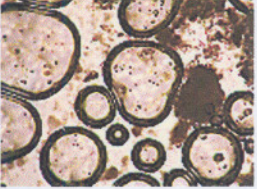
Temperature (°C)	Photomicrograph	Remarks
25		Sesquihydrate form at room temperature.
166		Melting of the crystals started at 166°C.
170		Recrystallisation occurred from the melt at 170°C.

Table 4.5 (continued)

Temperature (°C)	Photomicrograph	Remarks
175		Recrystallisation product.
185		Melting of the recrystallised product started at 185°C.
190		Melting completed.

HSM photomicrographs (table 4.6) of the sesquihydrate (when submerged in silicon oil) revealed gas evolution, which started at 100°C. Gas evolution is indicative of desolvation and confirmed that the endotherm observed at 97.21°C on the DSC thermogram was indeed dehydration.

Table 4.6 HSM observations of gatifloxacin sesquihydrate when submerged in silicon oil

25°C	100°C	110°C
		
Gatifloxacin sesquihydrate crystals at room temperature.	Bubble formation.	Bubble formation.

The thermal microscopic observations of the anhydrous gatifloxacin are shown in table 4.7. Thermal microscopy confirmed the thermal events observed in the DSC thermogram.

Table 4.7 Summary of HSM observations of anhydrous gatifloxacin at a temperature range of 25-185°C when submerged in silicon oil

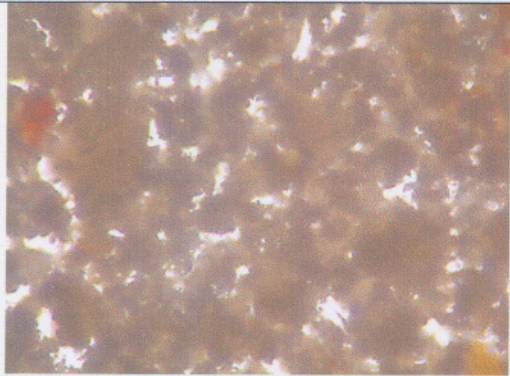
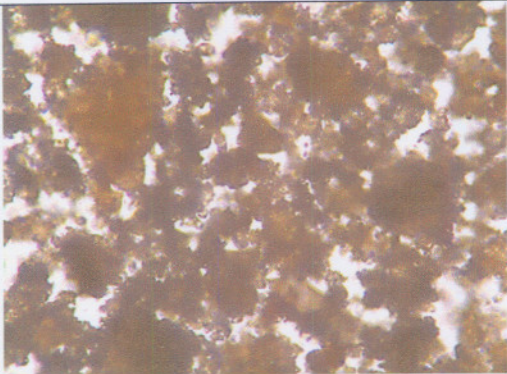
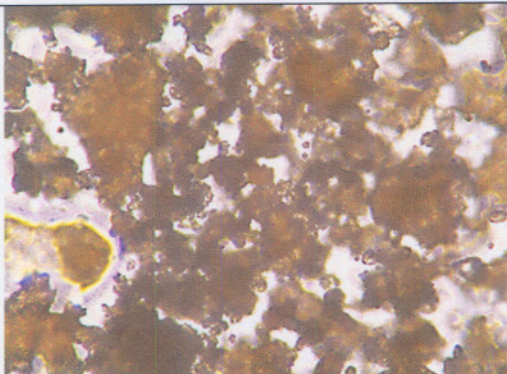
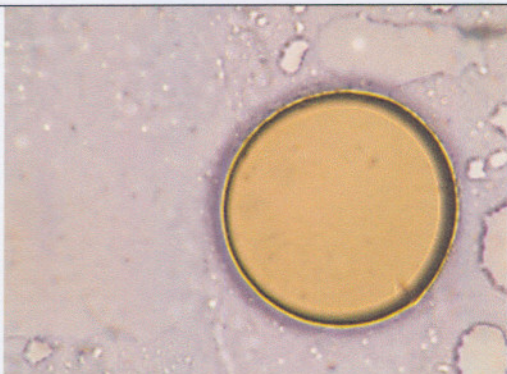
Temperature (°C)	Photomicrograph	Remarks
25		Anhydrous gatifloxacin at room temperature.

Table 4.7 (continued)

Temperature (°C)	Photomicrograph	Remarks
160		Melting at 159°C, immediately followed by recrystallisation at 160-167°C.
170		Melting of the recrystallisation product started at 170°C.
185		Melting of the recrystallised product was completed at 185°C.

The samples were examined for morphological differences by means of scanning electron microscopy (SEM). The SEM photomicrograph of gatifloxacin sesquihydrate is shown in figure 4.5. This sample consisted of a mixture of small and larger crystals that tended to agglomerate. These crystals had no specific shape.

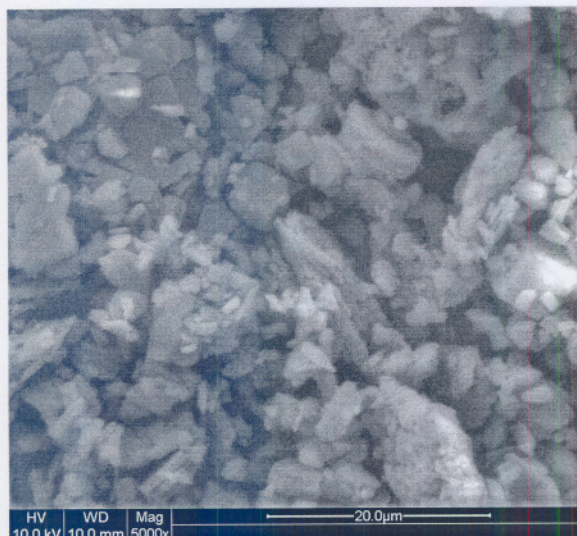


Figure 4.5 SEM photomicrograph of gatifloxacin sesquihydrate.

Figure 4.6 shows the SEM photomicrograph of the anhydrous gatifloxacin sample. The crystals of the anhydrous form were shapeless and tended to agglomerate.

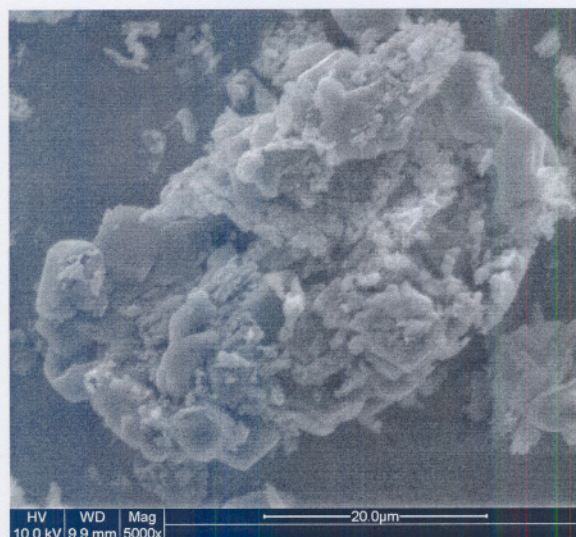


Figure 4.6 SEM photomicrograph of gatifloxacin anhydrate.

4.2.6 Dissolution studies

Powder dissolution studies were performed on the sesquihydrate and anhydrous forms of gatifloxacin. Sieved fractions (106-250 μm) of both forms were used to ensure particle size uniformity.

The dissolution profiles of the two samples are shown in figure 4.7 and the dissolution results obtained are summarised in table 4.8.

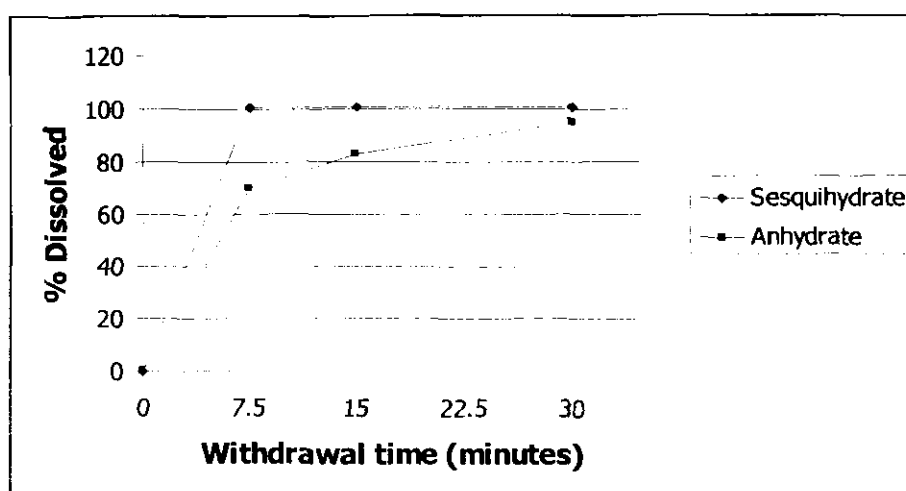


Figure 4.7 Dissolution profiles of gatifloxacin sesquihydrate and gatifloxacin anhydrate in 0.01 N HCl at $37 \pm 0.2^\circ\text{C}$.

After 7.5 minutes 101.0% of the sesquihydrate was already dissolved, whilst only 70.0% of the anhydrous form was dissolved at this time point. After 30 minutes 95.0% of the anhydrous form was in solution.

Table 4.8 Dissolution results of gatifloxacin sesquihydrate and gatifloxacin anhydrate in 0.01 N HCl at 37±0.2°C

Dissolution vessel	% Dissolved					
	7.5 minutes		15 minutes		30 minutes	
	Sesqui-hydrate	Anhydrate	Sesqui-hydrate	Anhydrate	Sesqui-hydrate	Anhydrate
1	99.8	71.2	100.6	82.8	100.6	94.3
2	100.7	73.9	101.0	87.7	101.1	98.2
3	101.1	67.0	101.5	80.9	101.8	91.5
4	100.5	66.0	100.6	82.4	101.0	97.3
Average	101.0±0.54	70.0±3.69	101.0±0.43	83.0±2.95	101.0±0.50	95.0±3.05

The results indicated that differences in the dissolution profiles of the two forms existed. The similarity factor (f_2 value) for the anhydrate was calculated to evaluate whether these differences were significant. The dissolution profile of the sesquihydrate form was used as reference profile. The equation described by Moore and Flanner (1996:65) was used for the calculation:

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n w_t (R_t - T_t)^2 \right]^{0.5} \times 100 \right\}$$

Where:

R_t = Reference dissolution value at time point t

T_t = Test dissolution value at time point t

n = Number of dissolution time points

w_t = Optional weight factor

An f_2 value of >50 indicates that the dissolution profiles of the two forms are similar. The calculated f_2 value (33.88) indicated that the differences between the dissolution profiles could be considered to be significant.

4.2.7 Solubility studies

The solubility of gatifloxacin sesquihydrate and anhydrate was determined in 0.01 N HCl, acetate buffer (pH 4.5), milliQ water and phosphate buffer (pH 6.8) respectively, using the method described in chapter 3. Table 4.9 shows the average solubility (in mg/ml) for both forms in the various media. Figure 4.8 is a bar graph comparing the solubility of the two forms.

• **Table 4.9** Solubility values (in mg/ml) of gatifloxacin sesquihydrate and gatifloxacin anhydrate in various media at $37\pm 2^{\circ}\text{C}$

Medium	pH	Sesquihydrate		Anhydrate	
		Solubility (mg/ml)	% CV	Solubility (mg/ml)	% CV
0.01 N HCl	2.12	4.80	3.72	5.02	0.97
Acetate buffer	4.50	4.50	3.67	4.91	2.33
MilliQ Water	5.50	1.98	3.27	2.68	3.64
Phosphate buffer	6.80	2.64	0.47	2.87	2.14

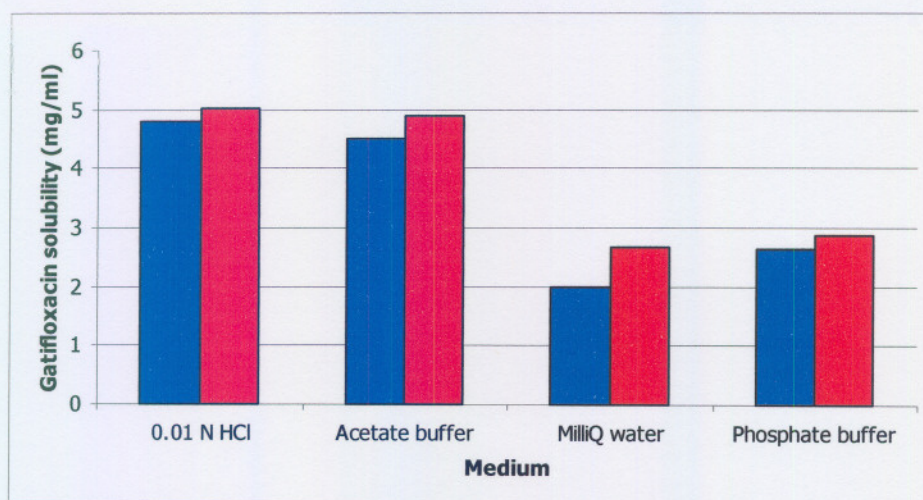


Figure 4.8 Solubility profiles of gatifloxacin sesquihydrate (blue) and gatifloxacin anhydrate (red) in various media at 37±2°C.

The solubility data of the two forms differed slightly. The order of solubility for both forms was observed as: 0.01 N HCl > acetate buffer pH 4.5 > phosphate buffer pH 6.8 > MilliQ water. In media with pH values lower than 5, the solubility of the two forms was significantly higher than that of the more alkaline media.

4.2.8 Flowability

The flowability of the two samples was compared using the Hausner ratio and Carr's index. The results of the measurements are tabulated in tables 4.10 and 4.11.

The Hausner ratio is:

$$\text{Hausner ratio} = D_f / D_0$$

Where:

D_f = Final density (also known as the tapped density) of the sample (g/ml)

D_0 = Initial density (also known as the poured density) of the sample (g/ml)

This investigation revealed no significant differences in the flowability of the two samples. Both samples had a Hausner ratio of more than 1.6, which is characteristic of more cohesive, less free-flowing powders. A Hausner ratio of approximately 1.2 is characteristic of powders with relatively good flow and a ratio above 1.6 is indicative of less free-flowing powders.

The equation for Carr's index is:

$$\% \text{Compressibility} = \frac{D_f - D_0}{D_f} \times 100$$

Where:

D_f = Final density (also known as the tapped density) of the sample (g/ml)

D_0 = Initial density (also known as the poured density) of the sample (g/ml)

The Carr's index interpretation was performed with the assistance of table 3.2. Good flow properties are reflected in a % compressibility of 12-16 and samples with poor flow properties have Carr's index values of 23-35. Carr's index revealed that both the sesquihydrate and the anhydrate had extremely poor flow properties, with a % compressibility of >40.

Table 4.10 Initial density (D_0) and final density (D_f) measurements of gatifloxacin sesquihydrate and anhydrate

Sample	D_0 (g/ml)		D_f (g/ml)	
	Sesquihydrate	Anhydrate	Sesquihydrate	Anhydrate
1	0.27	0.22	0.49	0.38
2	0.27	0.22	0.47	0.38
3	0.27	0.22	0.50	0.38
Average	0.27±0.00	0.22±0.00	0.49±0.02	0.38±0.00

Table 4.11 Hausner ratio and Carr's index of the sesquihydrate and anhydrous forms of gatifloxacin

Sample	Hausner ratio		Carr's index	
	Sesquihydrate	Anhydrate	Sesquihydrate	Anhydrate
1	1.80	1.76	44.44	43.18
2	1.75	1.77	42.86	43.48
3	1.84	1.73	45.71	42.22
Average	1.80±0.05	1.75±0.02	44.34±1.43	42.96±0.66

Conclusion

The aim of this chapter was to describe the potential impact of the hydration status (sesquihydrate) on the physico-chemical properties of the crystal lattice of gatifloxacin. This was examined by comparing the physico-chemical properties of the anhydrous and sesquihydrate forms of gatifloxacin.

The study revealed that the hydration status of gatifloxacin influenced its physico-chemical properties. Differences were observed in most of the physico-chemical properties that were compared. The two forms revealed significantly different x-ray powder diffractograms due to the changes in the crystal lattice caused by desolvation of the crystals. The IR spectra of the two forms differed significantly.

The dissolution profiles of the hydrated and anhydrous forms displayed significant differences. The sesquihydrate had a higher initial dissolution rate compared to that of the anhydrous form. The anhydrous form displayed an overall higher solubility after 24 hours when compared to the sesquihydrate. Both the sesquihydrate and anhydrate exhibited extremely poor flow properties.

Gatifloxacin sesquihydrate is currently the commercially favoured polymorphic form for manufacturing. Possible reasons for this could be due to the good dissolution profile displayed by this crystal form, as this study indicated (section 4.2.6).

Changes in the hydration state of an API could greatly influence its solid state properties, as illustrated in this study. These changes could lead to problems during the manufacturing of pharmaceuticals.

In the next chapter the crystal forms obtained from recrystallisation with the propanolic alcohols are characterised and the influence of solvent inclusion on the physico-chemical properties of these crystal forms is determined.

CHAPTER 5

Solvent inclusion properties: crystals recrystallised from the propanolic alcohols

Introduction

The comparative study in chapter 4 indicated that the inclusion of solvent (water) in the crystal lattice influenced the physico-chemical properties of gatifloxacin. The hydrated (sesquihydrate) and anhydrous forms of gatifloxacin displayed significant differences in their physico-chemical properties. This raised the question of how other solvent inclusions might influence the API's physico-chemical properties. Various recrystallisations were performed to investigate this.

The aim of this chapter was to characterise the crystal forms obtained from recrystallisation with the propanolic alcohols, and to determine the influence of solvent inclusion on the physico-chemical properties of these forms.

5.1 Method

Saturated solutions were prepared by adding gatifloxacin sesquihydrate powder to small volumes of the solvent, while heating the solution to the boiling point of the solvent under constant stirring by means of a magnetic stirrer. The saturated solutions were prepared in glass beakers and covered with Parafilm® containing small holes to allow solvent evaporation at ambient conditions.

Table 5.1 lists the propanolic alcohols that were used, with their boiling points and chemical structures.

Table 5.1 Propanolic alcohols used in recrystallisation studies

Solvent	Molecular weight (g/mol)	Boiling point at room temperature (°C)	Chemical structure	Manufacturer
n-Propanol	60.09	97.20		ACE (Pty) Ltd., South Africa
Iso-Propanol	60.09	82.50		Saarchem, South Africa

The effect of desolvation on the physico-chemical properties of the solvated forms was investigated. The desolvated crystals were obtained by drying the solvated crystals in an Ecotherm drying oven (Labotec, South Africa) for approximately 12 hours at a temperature of 105-130°C, depending on the desolvation temperature of the crystal form. The physico-chemical properties (XRPD, DSC, TGA, KF, powder dissolution studies and solubility studies) were determined and used to study the influence of solvent inclusion on the properties of these forms.

5.2 Characterisation of crystal forms

5.2.1 EP03W and EP03D

Crystal form EP03W was obtained through recrystallisation with n-propanol, and EP03D was obtained by drying EP03W in an Ecotherm drying oven (Labotec, South Africa) at 105°C for 12 hours.

5.2.1.1 X-ray powder diffraction (XRPD) and variable temperature x-ray powder diffraction (VT-XRPD)

The x-ray powder diffractogram of EP03W contained peaks at 11.4°2 θ , 13.9°2 θ , and 16.4°2 θ that were also present in the diffractogram of form J. The peak at 6.7°2 θ in the diffractogram of form J was however a low intensity peak in the diffractogram of EP03W (see chapter 2 section 2.4.1 for a discussion of form J).

The variable temperature x-ray powder diffractograms of EP03W are shown in figure 5.1. The diffractogram remained relatively unchanged between 25-85°C, indicating that no significant change in crystal structure occurred in the mentioned temperature range. At 95°C slight changes were observed in the diffractogram of EP03W, including a decrease in the intensity of the peak at approximately 13.9°2θ, and a significant increase in intensity of the peak at approximately 19.5°2θ. Additional peaks appeared on the diffractogram at 95°C at 25.7°2θ and 28.4°2θ. Significant changes were observed at 100°C, such as the disappearance of a peak at 6.4°2θ, the appearance of a new peak at 7.8°2θ and the disappearance of the peak at 11.3°2θ. Other changes in the diffractogram at 100°C included a slight shifting and a significant decrease in intensity of the peak at 16.4°2θ. An additional peak appeared at 17.0°2θ in the diffractogram at 100°C. The change in crystal structure that occurred at this temperature was due to dehydration and desolvation of the crystals. An increase in the intensities of most peaks was observed, which suggested that the sample became more crystalline after desolvation. The diffractogram remained relatively unchanged between 100-170°C. The diffractogram at 180°C contained only a few peaks and at 190°C an XRPD pattern characteristic of melting (absence of strong diffraction peaks) was obtained.

The XRPD patterns of form omega and form I (anhydrous forms described in the literature) showed strong resemblances, except for the peak present at 23.6°2θ in the diffractogram of form I that is absent in the diffractogram of form omega (see chapter 2 sections 2.4.2 and 2.4.3 for a discussion on these two forms). The x-ray powder diffractogram of EP03D at 25°C (figure 5.2) resembled the diffractogram of form omega. Some other physico-chemical properties such as melting point and characteristic IR absorptions of EP03D resembled that of form I.

The x-ray powder diffractogram of EP03D (figure 5.2) remained relatively unchanged through the entire temperature range from 25-170°C which indicated that after desolvation no significant changes occurred in the crystal structure of this form when exposed to increased temperatures and it can thus be considered to be a thermodynamically stable crystal form. A characteristic melting XRPD pattern was

observed at 180°C. An overlay of the XRPD patterns of EP03D obtained at 25°C, 170°C and 180°C is shown in figure 5.2.

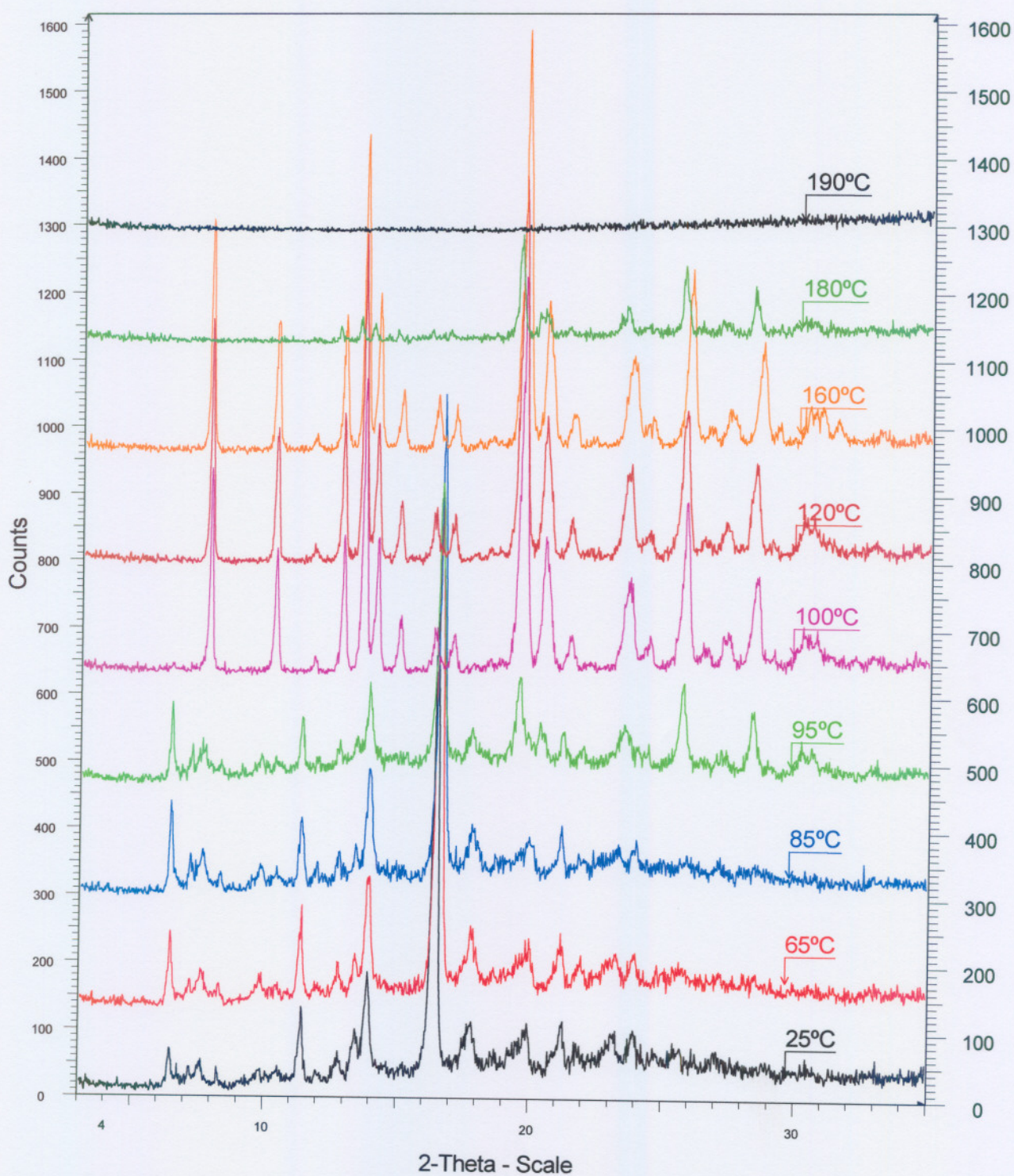


Figure 5.1 Variable temperature x-ray powder diffractograms of EP03W.

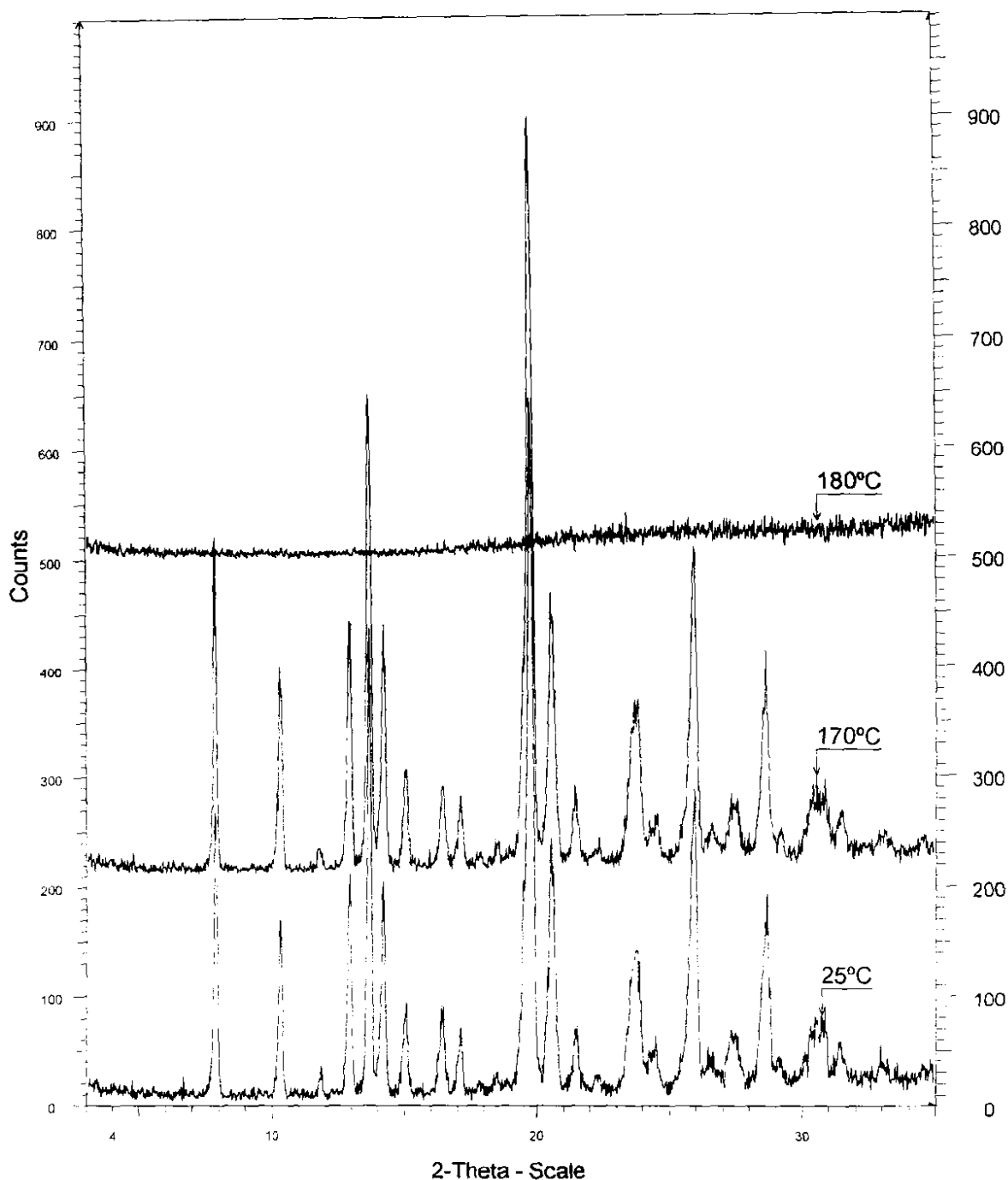


Figure 5.2 Variable temperature x-ray powder diffractograms of EP03D obtained at 25°C, 170°C and 180°C.

The diffractograms of EP03W obtained after desolvation (100-170°C) and that of EP03D were comparable. This indicated that the crystal form obtained after desolvation/dehydration of EP03W, remained unchanged even after exposure to a

temperature of 105°C for 12 hours. An overlay of the diffractogram of EP03W (obtained at 120°C) and EP03D (obtained at 25°C) is shown in figure 5.3.

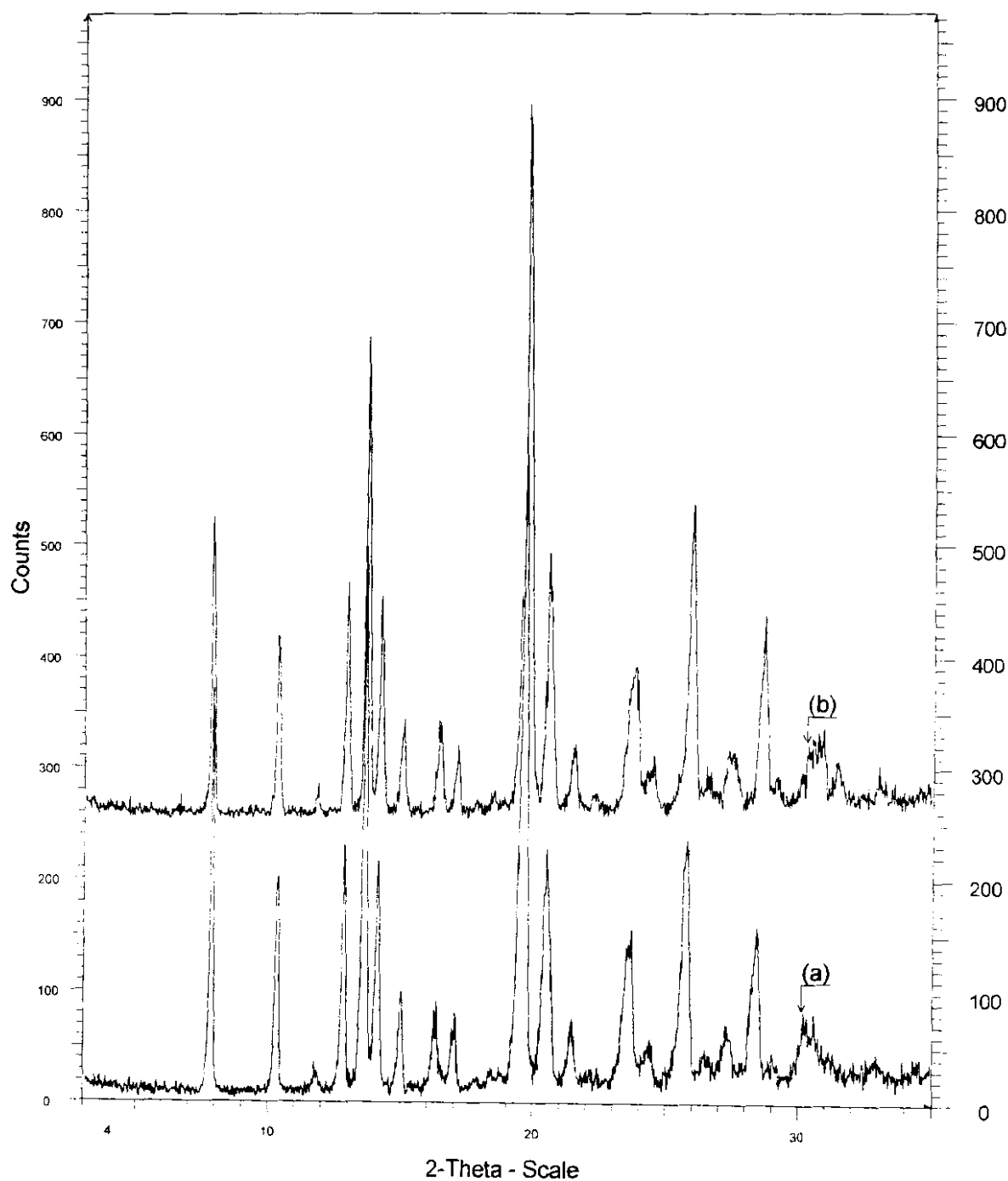


Figure 5.3 Overlay of the x-ray powder diffractograms of (a) EP03W (at 120°C) and (b) EP03D (at 25°C).

5.2.1.2 Infrared spectroscopy (IR)

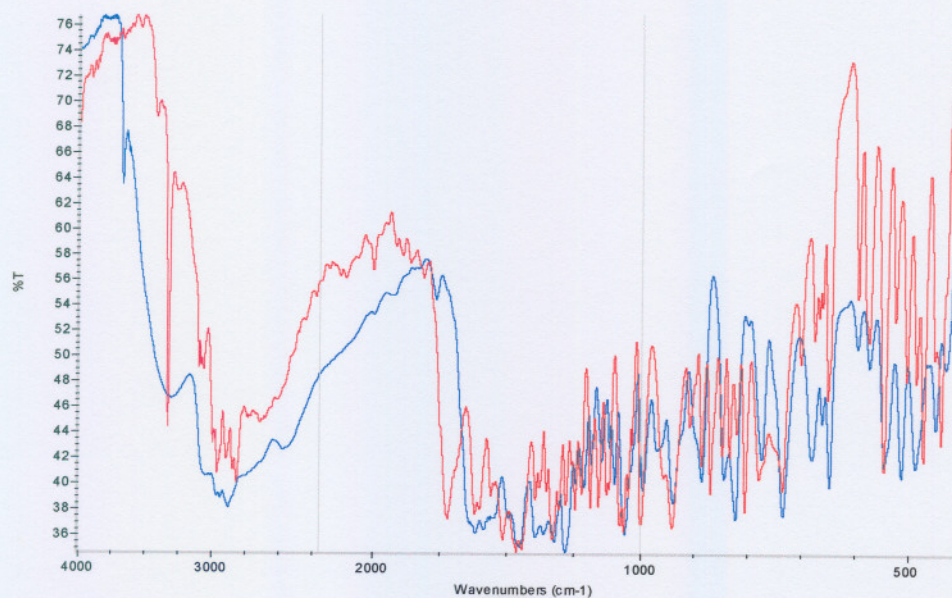
The infrared spectra of EP03W and EP03D displayed significant differences. The main absorption peaks are listed in table 5.2 and an overlay of the DRIFT-IR spectra of the mentioned two forms is shown in figure 5.4. The differences between the spectra of EP03W and EP03D are highlighted in table 5.2. The most characteristic differences between the spectra of EP03W and EP03D were in the region of 2400-3750 cm^{-1} .

Table 5.2 Main absorptions in the DRIFT-IR spectra of EP03W and EP03D

Main absorptions	Wavelength (cm^{-1})	
	EP03W	EP03D
1	3675	-
2	-	3329
3	3303	-
4	-	3098
5	-	3062
6	-	2960
7	-	2893
8	2876	-
9	-	2812
10	-	1999
11	1763	-
12	-	1719
13	1613	1617
14	-	1509
15	-	1460
16	1448	-
17	-	1391
18	-	1349
19	-	1326
20	1319	-
21	1277	1277
22	-	1242
23	-	1218
24	1208	-
25	1181	1185
26	-	1154
27	1144	-
28	-	1125
29	1114	-
30	1094	-
31	1055	1060
32	-	1043
33	996	999
34	970	-
35	940	941
36	-	909
37	886	886
38	-	870
39	844	848
40	-	834

Table 5.2 (continued)

Main absorptions	Wavelength (cm ⁻¹)	
	EP03W	EP03D
41	822	821
42	-	805
43	774	779
44	734	735
45	-	701
46	680	675
47	-	667
48	661	-
49	647	650
50	595	594
51	573	574
52	545	545
53	-	524
54	513	-
55	-	503
56	486	485
57	-	472
58	449	-
59	-	438
60	430	-

**Figure 5.4** Overlay of the DRIFT-IR spectra of EP03W (blue) and EP03D (red).

5.2.1.3 Differential scanning calorimetry (DSC)

The DSC thermogram of EP03W is shown in figure 5.5.

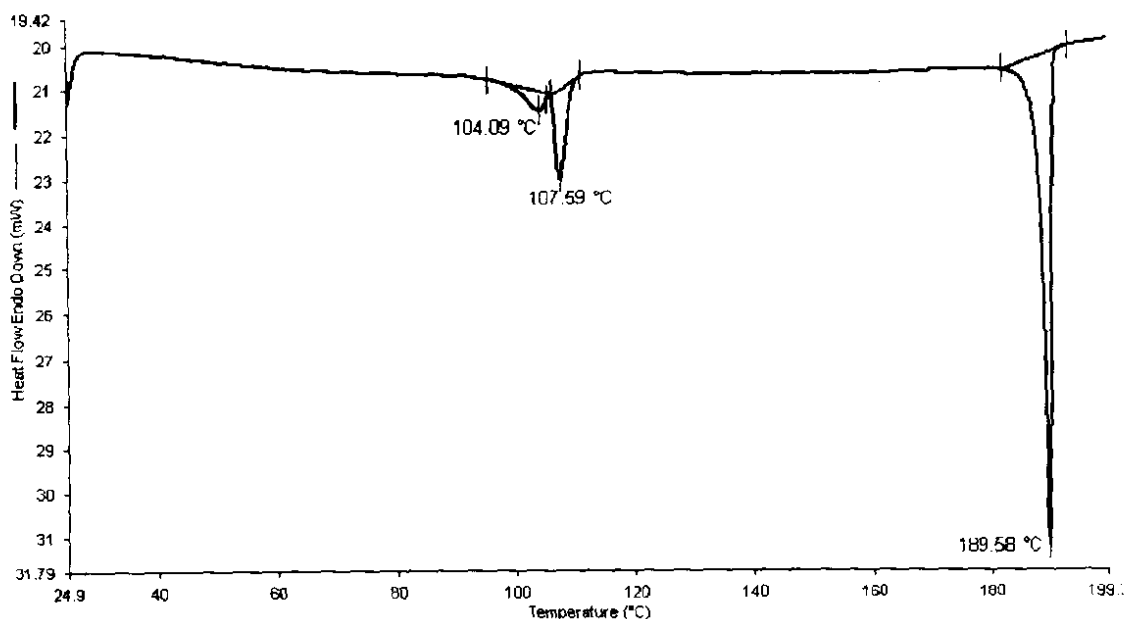


Figure 5.5 DSC thermogram of EP03W.

The thermogram of EP03W displayed three endotherms. The first two occurred at 104.09°C and 107.59°C respectively. These endotherms are representative of dehydration/desolvation and melting respectively. Thermogravimetric analysis (section 5.2.1.4) confirmed that desolvation occurred in this temperature range and the melting endotherm (at 107.59°C) was confirmed by means of thermal microscopy (section 5.2.1.5).

The melting endotherm at 107.59°C was followed by solidification from the melt. This solidification process was clear from the thermal microscopic observations (section 5.2.1.5). The solidification observed with thermal microscopy displayed no exothermic peaks on the DSC thermogram signifying that the energy needed for this process might have been below the detection limit of the instrument. Desolvation of EP03W caused the crystal structure to become unstable and resulted in melting followed by solidification into a more energetically favourable anhydrous crystal form. The endotherm observed at 189.58°C indicated the melting of the desolvated (anhydrous) crystals.

An example of a drug that also displayed this type of behaviour is diclofenac *N*-(2-hydroxyethyl)pyrrolidine dihydrate. Removal of water from the crystal lattice of this drug caused instability in the crystal structure. The crystals melted and recrystallised into a more energetically favourable anhydrate form of the drug (Ledwidge *et al.*, 1996:18).

Figure 5.6 displays the DSC thermogram of EP03D. The DSC thermogram of EP03D showed only one endotherm at 188.49°C, which indicated melting. This correlated well with the final melting point of EP03W, indicating that the melting endotherm observed at 189.58°C on the thermogram of EP03W was the melting point of the desolvated (anhydrous) form.

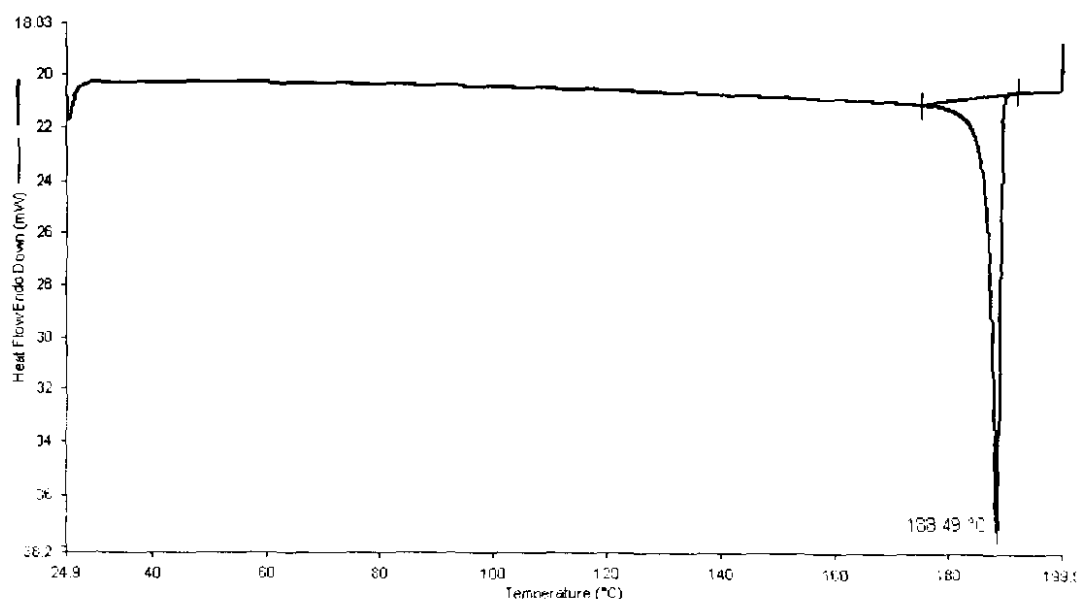


Figure 5.6 DSC thermogram of EP03D.

5.2.1.4 Thermogravimetric analysis (TGA) & Karl Fischer titration (KF)

The TGA thermograms of EP03W and EP03D are shown in figure 5.7. The results obtained from TGA and KF of EP03W and EP03D are tabulated in table 5.3.

The % moisture of EP03W (as determined by KF) correlated well with the theoretical % moisture of a hemihydrate, which is 2.29%. The TGA results indicated a total weight loss of 11.06%, from which it was clear that water was not the only solvent incorporated into the crystal lattice. n-Propanol was also included into the crystal

lattice in an amount of 8.33%. This value was comparable with the theoretical value of a hemi-solvate, which is 6.90%. The difference between the experimental and theoretical values could possibly be due to the presence of surface solvent. EP03W could therefore possibly be classified as a hydrated solvate.

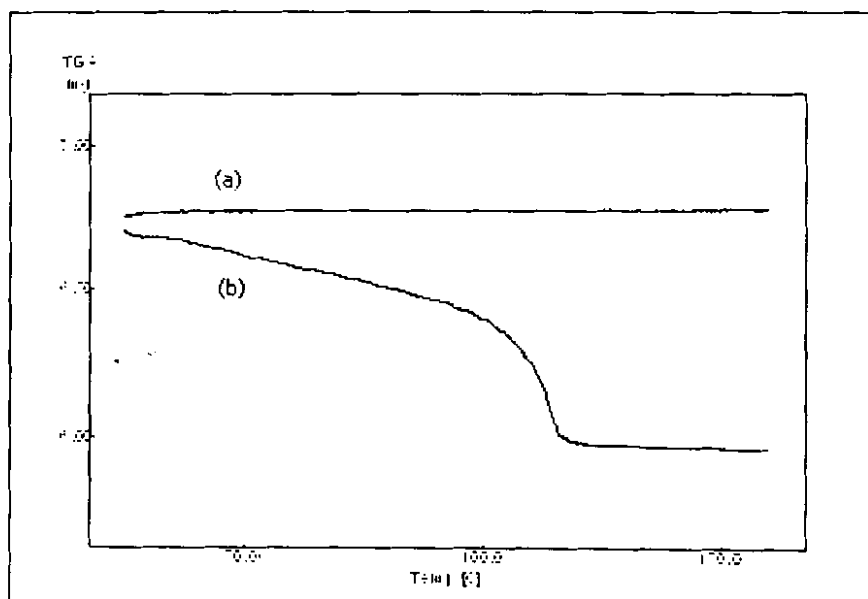


Figure 5.7 TGA thermograms of (a) EP03D and (b) EP03W.

Table 5.3 TGA and KF results of EP03W and EP03D

	% Weight loss (TGA)	% Moisture content (KF)
EP03W	11.06	2.73
EP03D	0.15	0.21

EP03D displayed no significant weight loss with TGA or moisture content with KF as table 5.3 illustrates. EP03D could therefore be regarded as an anhydrous crystal form.

5.2.1.5 Microscopic analysis

Thermal microscopy was used to study the thermal behaviour of the crystals of EP03W and EP03D. A summary of the HSM observations of EP03W is shown in table 5.4.

Table 5.4 Summary of HSM observations of EP03W in the temperature range of 25-191°C

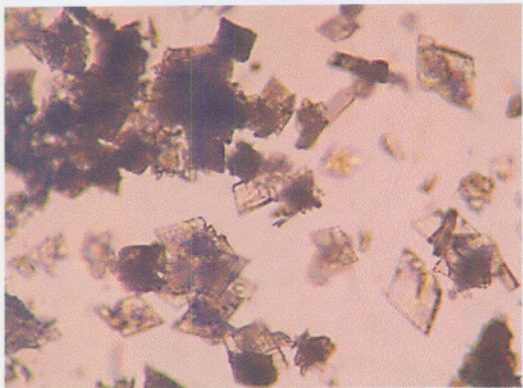
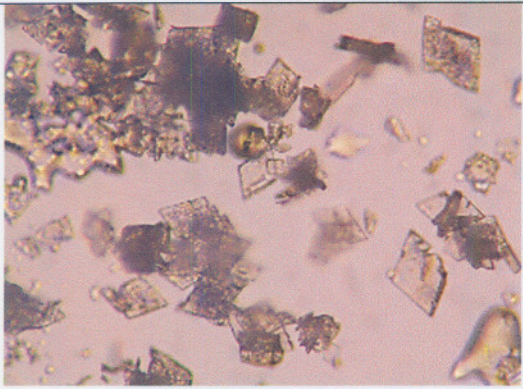
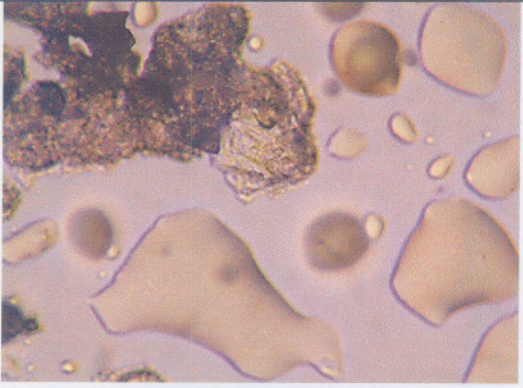
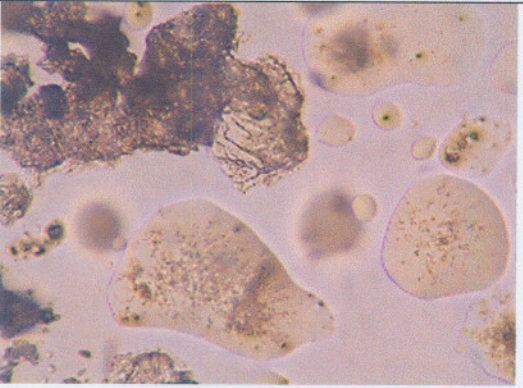
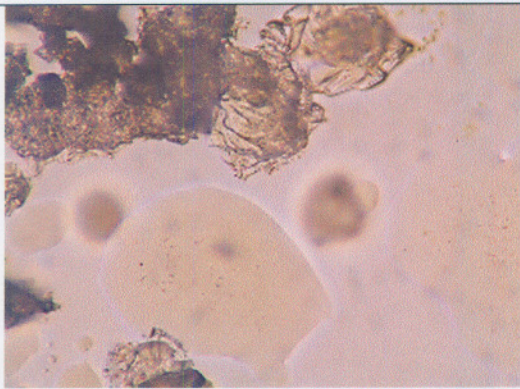
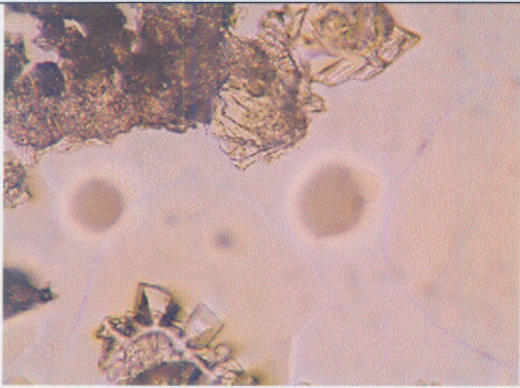
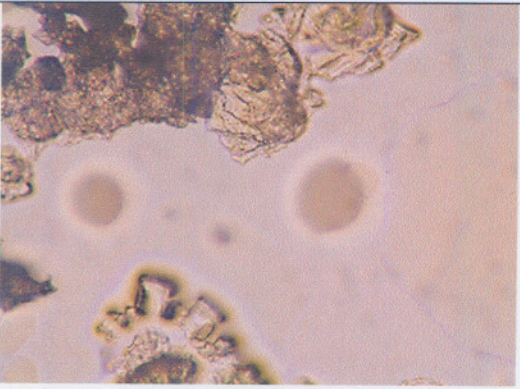
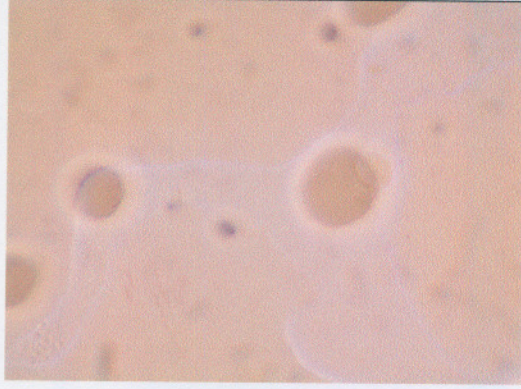
Temperature (°C)	Photomicrograph	Remarks
25		EP03W crystals at room temperature.
111		Melting started.
129		Solidification from the melt started.
145		Solidification product.

Table 5.4 (continued)

Temperature (°C)	Photomicrograph	Remarks
167		Melting of solidification product started at 167°C.
175		Recrystallisation from the melt started at 175°C.
183		Melting of the recrystallisation product started; melting of the rest of the crystals continued.
191		Melting completed.

The thermal microscopic observations confirmed the DSC events present in the thermogram.

A recrystallisation was observed with HSM that did not display any peaks in the DSC thermogram. This is possibly due to the very small recrystallisation enthalpy of this form, which was not detected with differential scanning calorimetry. Literature confirmed that in some cases the change in enthalpy is too small to be detected with DSC (Giron, 1995:28).

EP03W also displayed bubble formation when submerged in silicon oil due to gas evolution. Gas evolution is indicative of the desolvation of the sample. The bubble formation is displayed in table 5.5.

Table 5.5 HSM observations of EP03W when submerged in silicon oil

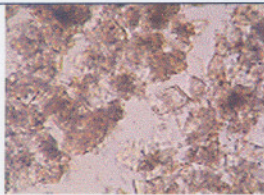
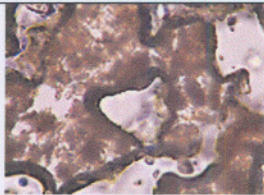
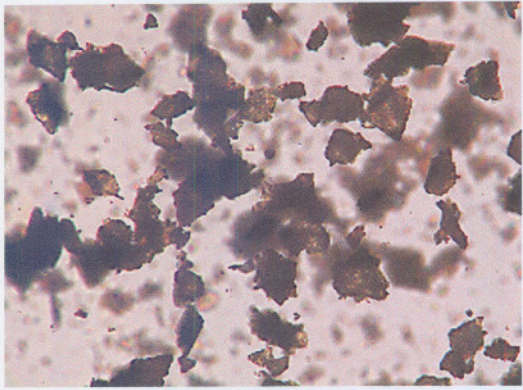
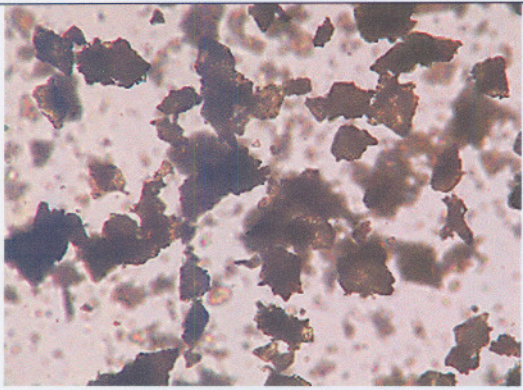
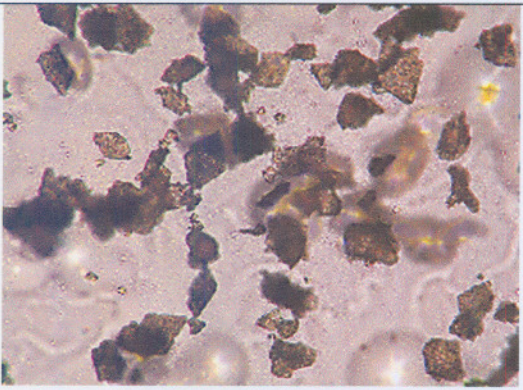
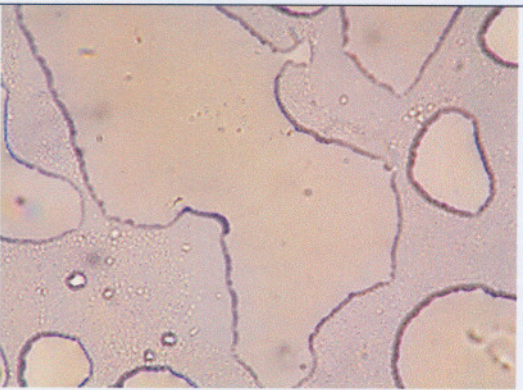
25°C	105°C
	
EP03W crystals at room temperature.	Darkening of the crystals and bubble formation occurred.

Table 5.6 displays the HSM observations of EP03D. The crystals of EP03D underwent no changes upon heating and only melted at 185-188°C. The crystals of EP03D were darker than those of EP03W. This might indicate that EP03D had desolvated.

Table 5.6 Summary of HSM observations of EP03D in the temperature range of 25-188°C

Temperature (°C)	Photomicrograph	Remarks
25		EP03D crystals at room temperature.
110		No change observed.
185		Sample started to melt at 185°C.
188		Melting completed.

Scanning electron microscopy (SEM) was used to study the morphology of the crystals. Figure 5.8 and 5.9 displays the SEM photomicrographs of EP03W and EP03D respectively.

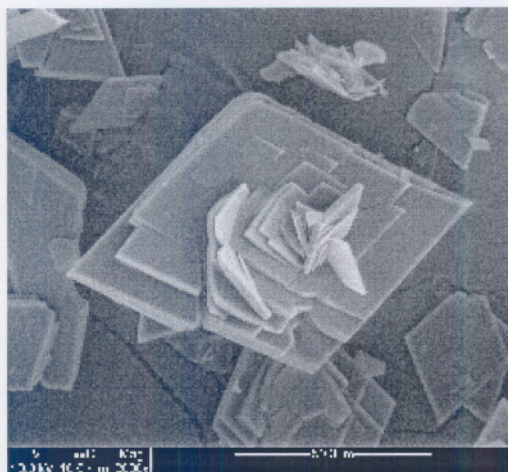


Figure 5.8 SEM photomicrograph of EP03W.

The crystals of EP03W displayed plate-like habit and showed a tendency to agglomerate.

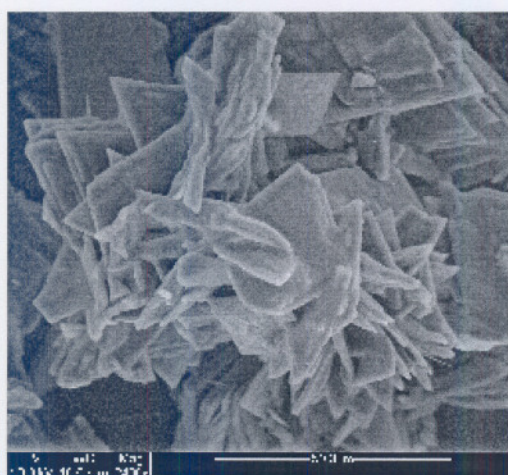


Figure 5.9 SEM photomicrograph of EP03D.

SEM photomicrographs of EP03D revealed that this form exhibited a mixture of plate-like and shapeless crystals with a tendency to agglomerate.

5.2.1.6 Dissolution studies

Powder dissolution studies were performed on both EP03W and EP03D using a sieve fraction (106-250 μm) of the crystals of each of these two forms. The results obtained from these dissolutions are tabulated in table 5.7 and the dissolution profiles of the two samples are displayed in figure 5.10.

Table 5.7 Dissolution results of EP03W and EP03D in 0.01 N HCl at $37\pm0.2^\circ\text{C}$

Dissolution vessel	% Dissolved					
	7.5 minutes		15 minutes		30 minutes	
	EP03W	EP03D	EP03W	EP03D	EP03W	EP03D
1	85.9	100.8	88.5	100.5	91.3	101.1
2	95.0	97.4	95.7	98.8	95.6	100.8
3	93.8	100.9	94.1	97.9	95.1	102.7
4	89.6	97.8	92.1	99.3	92.0	102.0
5	90.7	99.0	92.7	100.7	93.5	101.7
Average	91.0$\pm$3.6	99.0$\pm$1.6	93.0$\pm$2.7	99.0$\pm$1.2	94.0$\pm$1.9	102.0$\pm$0.8

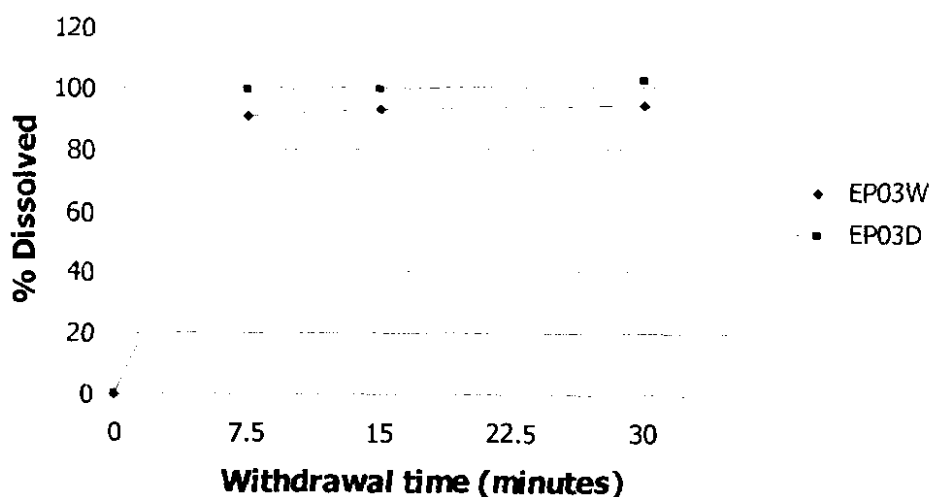


Figure 5.10 Dissolution profiles of EP03W and EP03D in 0.01 N HCl at $37\pm0.2^\circ\text{C}$.

According to the guidelines of the Medicines Control Council, for the calculation of the f_2 value only one measurement should be considered after 85% dissolution of both samples have occurred (Medicines Control Council, 8:2003). If $>85\%$ dissolution occurred for both samples, the dissolution profiles of the two samples

may be regarded similar. Thus the f_2 value (similarity factor) need not be calculated to determine the similarity between these two dissolution profiles. However, for the sake of completeness the f_2 value (similarity factor) was calculated and included here. An f_2 value of >50 indicates that the dissolution profiles are similar and the value decreases with an increase in differences between the profiles. EP03D was used as reference in this calculation. The f_2 value of EP03W was calculated as 56.36, which indicated that the profiles of EP03W and EP03D were similar. However, Q (% gatifloxacin dissolved at time (t)) at 30 minutes indicated that EP03D dissolved completely whereas only 94.0% of EP03W dissolved.

The dissolution profiles of EP03W and EP03D were also compared to that of the sesquihydrate raw material. The dissolution profile of the sesquihydrate form was used as reference in the calculations. The results are shown in table 5.8.

Table 5.8 f_2 values for EP03W and EP03D using the sesquihydrate raw material as reference

Crystal form	f_2 value
EP03W	53.57
EP03D	84.95

The similarity factor (f_2 value) of EP03W and EP03D indicated that these two forms displayed dissolution profiles similar to that of the sesquihydrate form. EP03D displayed a better dissolution profile fit to the sesquihydrate form as the higher f_2 value indicated.

5.2.1.7 Solubility studies

The solubility values (in mg/ml) of EP03W and EP03D are summarised in table 5.9. Figure 5.11 displays a graphic comparison of the solubility profiles of these two forms. EP03D revealed a higher solubility in all the media, with the greatest solubility in acidic media. The solubility of EP03W ranged in the following order: 0.01 N HCl > acetate buffer > phosphate buffer > MilliQ water. The solubility of EP03D ranged in the order: 0.01 N HCl > acetate buffer > MilliQ water > phosphate buffer.

Table 5.9 Solubility values (in mg/ml) of EP03W and EP03D in various media at $37\pm 2^{\circ}\text{C}$

Medium	pH	EP03W		EP03D	
		Solubility (mg/ml)	%CV	Solubility (mg/ml)	%CV
0.01 N HCl	2.20	4.23	2.11	5.17	2.69
Acetate buffer	4.50	4.19	1.88	4.95	1.23
MilliQ Water	5.50	2.36	2.51	3.09	3.09
Phosphate buffer	6.80	2.51	2.90	3.03	0.81

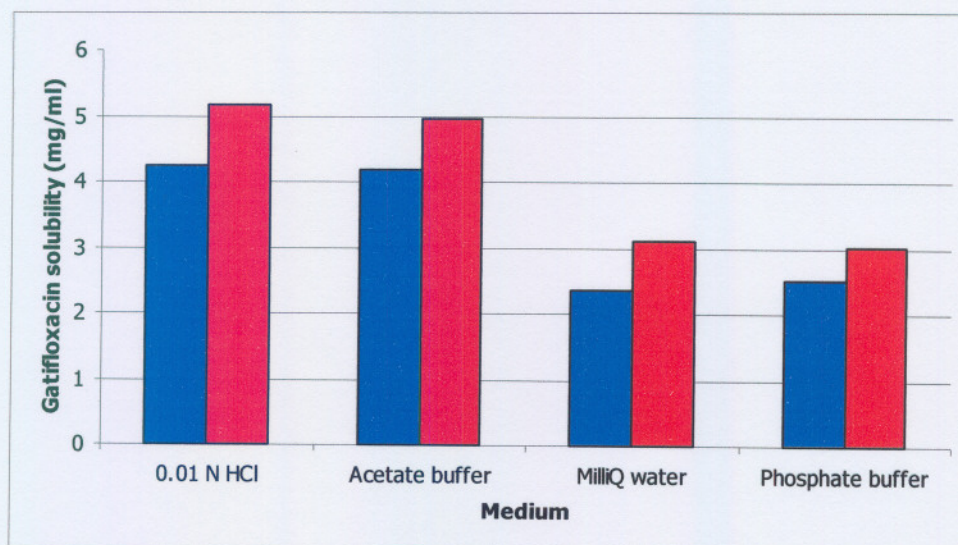


Figure 5.11 Solubility profiles of EP03W (blue) and EP03D (red) in various media at $37\pm 2^{\circ}\text{C}$.

5.2.1.8 Flowability

The flowability of the samples was investigated using the method described in chapter 3. The results obtained for EP03W and EP03D are tabulated in table 5.10 and 5.11.

Table 5.10 Initial density (D_0) and final density (D_f) measurements of EP03W and EP03D

Sample	D_0 (g/ml)		D_f (g/ml)	
	EP03W	EP03D	EP03W	EP03D
1	0.17	0.17	0.27	0.26
2	0.16	0.18	0.26	0.27
3	0.18	0.17	0.28	0.26
Average	0.17±0.01	0.17±0.01	0.27±0.01	0.26±0.01

Table 5.11 Hausner ratio and Carr's index of EP03W and EP03D

Sample	Hausner ratio		Carr's index	
	EP03W	EP03D	EP03W	EP03D
1	1.55	1.50	35.42	33.33
2	1.60	1.50	37.50	33.33
3	1.53	1.52	34.78	34.17
Average	1.56±0.04	1.51±0.01	35.90±1.4	33.61±0.5

The Hausner ratios and Carr's index of the samples were evaluated according to the criteria given in chapter 3. A Hausner ratio of approximately 1.2 is indicative of samples with relatively good flow, while samples with poor flow usually have a Hausner ratio of approximately 1.6 or higher. Table 3.2 summarises the interpretation of Carr's index. According to table 3.2 a sample with good flow properties has a Carr's index value of 12-16.

EP03W and EP03D both had Hausner ratios of approximately 1.5-1.6, which indicated that both samples had poor flow. EP03W had a Carr's index of 35.90, which is indicative of samples with very poor flow, and EP03D had a Carr's index of 33.61, indicating that this sample exhibited poor flow. Although the differences between these two samples were minimal, EP03D exhibited slightly better flow properties compared to EP03W.

5.2.2 EP04W and EP04D

The crystal form EP04W was prepared using iso-propanol as solvent for recrystallisation. The recrystallisation method described in section 5.1 was used to obtain this crystal form. EP04D was obtained by drying EP04W in an Ecotherm drying oven (Labotec, South Africa) at 130°C for 12 hours.

5.2.2.1 X-ray powder diffraction (XRPD) and variable temperature x-ray powder diffraction (VT-XRPD)

The x-ray powder diffraction patterns of EP04W (figure 5.12) displayed some similarities to that of EP03W (figure 5.13). However, some minor changes were observed including peaks with low relative intensities in the area of $7-10^{\circ}2\theta$ in the diffractogram of EP03W, which were absent in the diffractogram of EP04W. The diffractogram of EP03W also displayed peaks at $13.5^{\circ}2\theta$, $19.9^{\circ}2\theta$, $21.8^{\circ}2\theta$ and $23.2^{\circ}2\theta$ that were absent or not clearly distinguishable in the diffractogram of EP04W. The resolution of the diffractogram of EP03W was slightly better than that of EP04W.

EP04W further displayed similar thermal behaviour to that of EP03W with changes occurring in the VT-XRPD pattern at 95°C (figure 5.11). These changes included the appearance of peaks at $19.4^{\circ}2\theta$, $20.3^{\circ}2\theta$, $23.5^{\circ}2\theta$, $25.7^{\circ}2\theta$, and $28.3^{\circ}2\theta$. Further changes occurred at 100°C, including the disappearance of the peaks at $6.3^{\circ}2\theta$, $11.2^{\circ}2\theta$, and $16.5^{\circ}2\theta$. Other changes that occurred at 100°C include the appearance of peaks at $7.7^{\circ}2\theta$, $13.5^{\circ}2\theta$, $14.0^{\circ}2\theta$, and the peaks at $16.3^{\circ}2\theta$ and $16.9^{\circ}2\theta$. These changes occurred due to the desolvation of the sample. The diffractogram remained relatively unchanged from 100-170°C, with no significant changes occurring between these temperatures. At 180°C a characteristic melting XRPD pattern with no peaks was obtained. Figure 5.12 shows the variable temperature x-ray powder diffractograms of EP04W and figure 5.13 is an overlay of EP03W and EP04W at 25°C, indicating similarities between these two forms.

The x-ray powder diffractogram of EP04W contained some of the characteristic peaks of form J described in the patents. These peaks were present at the following $^{\circ}2\theta$ -positions: $11.4^{\circ}2\theta$, $13.9^{\circ}2\theta$, and $16.4^{\circ}2\theta$. The characteristic peak at $6.7^{\circ}2\theta$ in

the diffractogram of form J was absent from the diffractogram of EP04W (form J is described in section 2.4.1).

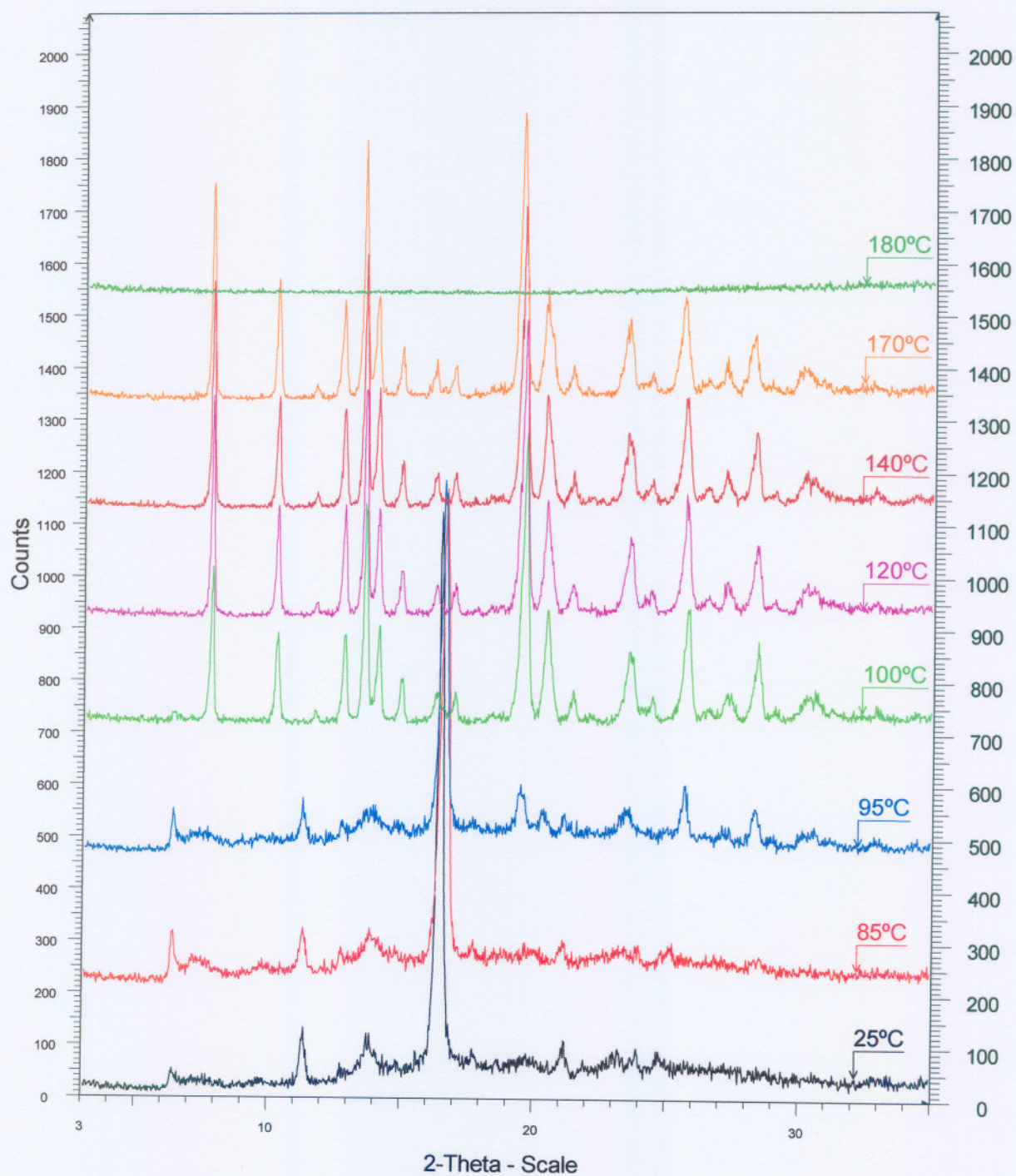


Figure 5.12 Variable temperature x-ray powder diffractograms of EP04W.

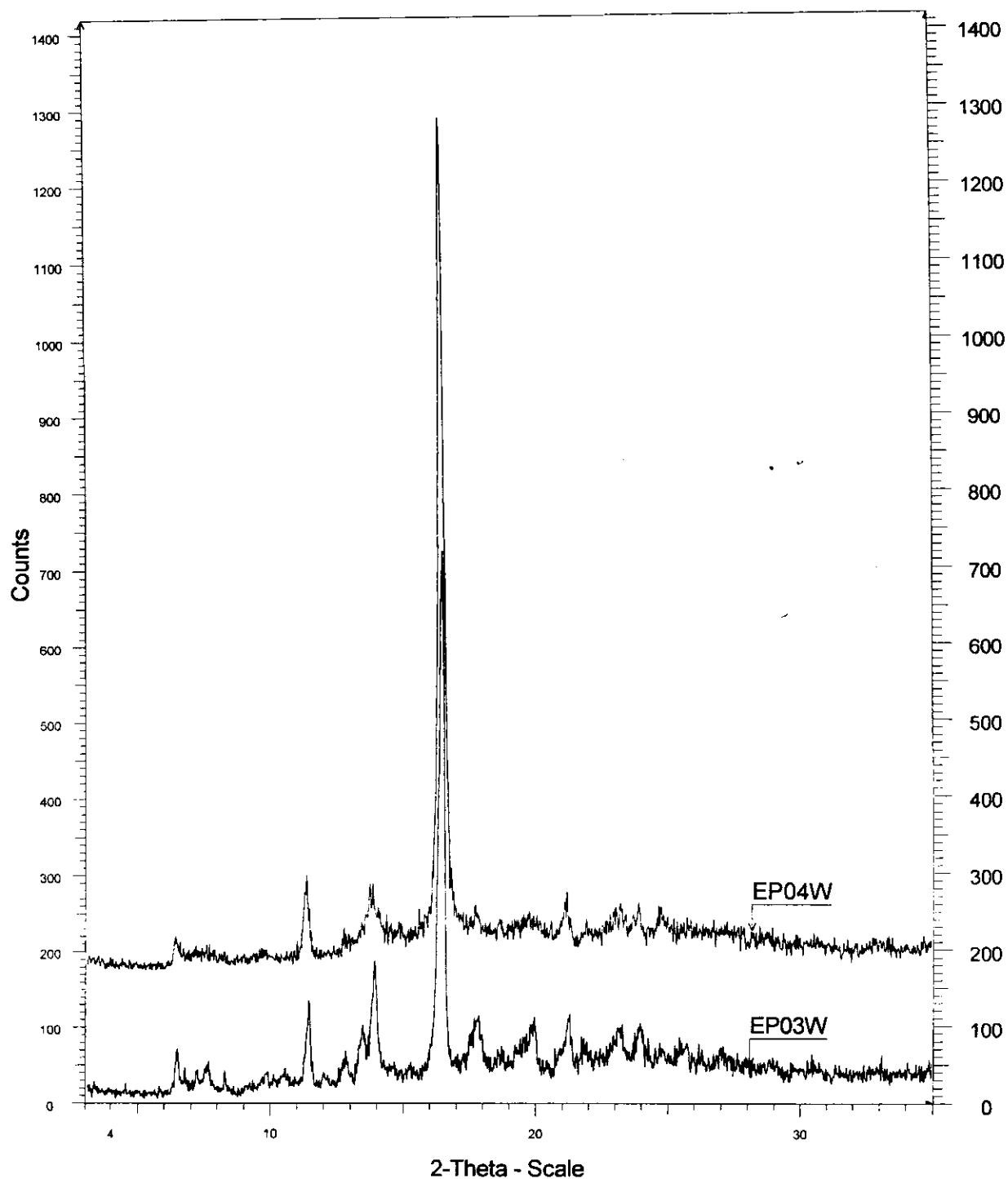


Figure 5.13 Overlay of the x-ray powder diffractograms of EP03W and EP04W at 25°C.

The XRPD pattern of EP04D was comparable to the diffractogram of EP04W obtained above 100°C, indicating that the desolvated crystal form remained stable when

exposed to elevated temperatures (130°C) for a prolonged time period (12 hours). Figure 5.14 displays the XRPD pattern of form EP04D at 25°C.

The x-ray powder diffractogram of form EP04D was comparable to that of the diffractogram of form EP03D. Form omega and form I (anhydrous forms described in the patents), showed strong resemblances in their XRPD patterns. The x-ray powder diffractogram of form EP04D, like form EP03D, resembled the diffractogram of form omega (form omega and form I are described in sections 2.4.2 and 2.4.3 respectively).

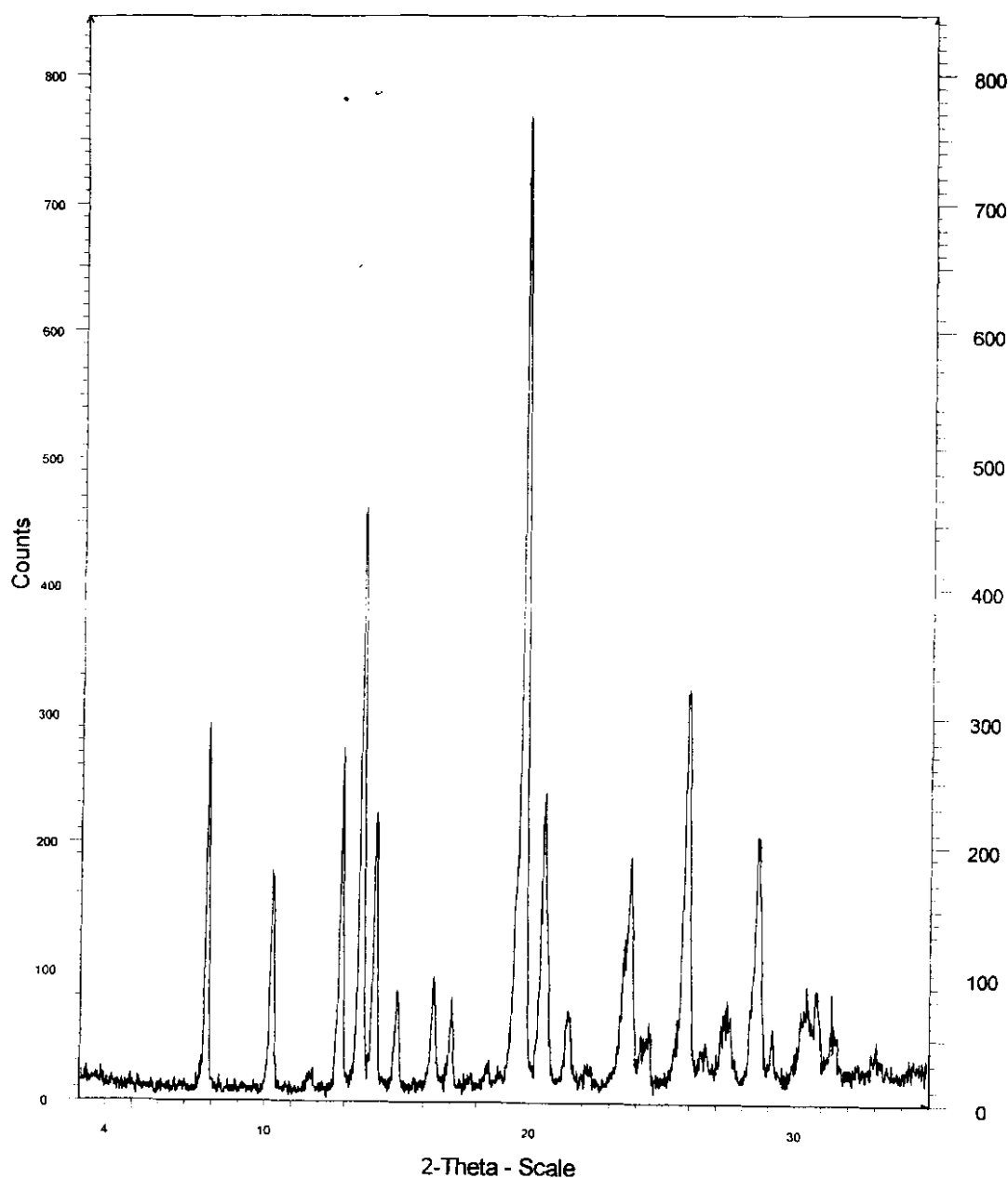


Figure 5.14 X-ray powder diffractogram of form EP04D at 25°C.

5.2.2.2 Infrared spectroscopy (IR)

Table 5.12 shows the main infrared absorptions of EP04W and EP04D and the differences between the DRIFT-IR spectra of these two forms are highlighted in the table. The most significant differences between the DRIFT-IR spectra of EP04W and EP04D were observed in the region 2400-3750 cm^{-1} .

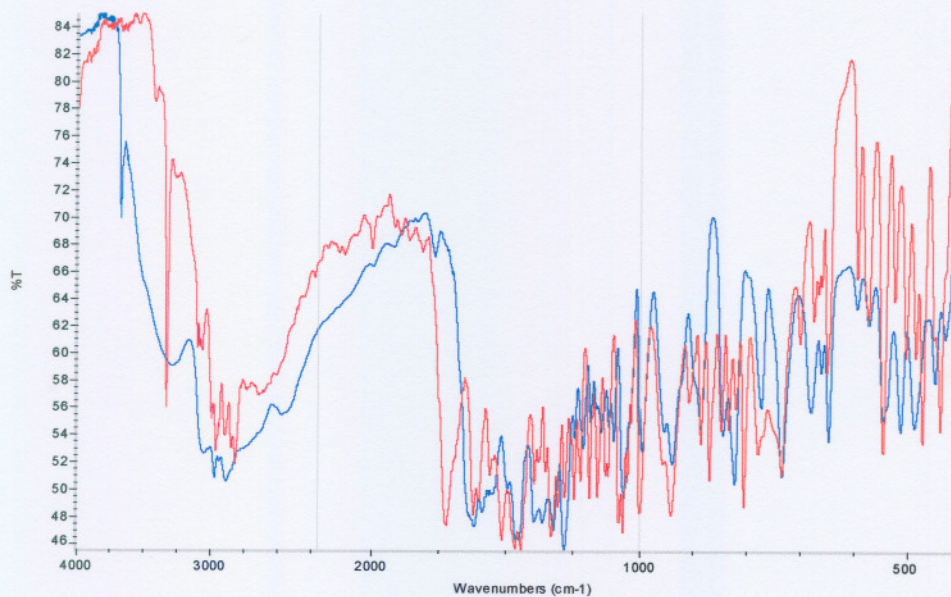
Table 5.12 Main absorptions in the DRIFT-IR spectra of EP04W and EP04D

Main absorptions	Wavelength (cm^{-1})	
	EP04W	EP04D
1	3676	-
2	-	3329
3	3284	-
4	-	3098
5	-	3062
6	2967	-
7	-	2960
8	-	2891
9	2883	-
10	-	2812
11	-	1999
12	1764	-
13	-	1719
14	1615	1618
15	-	1556
16	-	1509
17	1448	-
18	-	1439
19	-	1391
20	1359	-
21	-	1349
22	-	1327
23	1319	-
24	-	1302
25	1278	1277
26	1241	1242
27	-	1218
28	1208	-
29	1181	1185
30	-	1154
31	1143	-
32	1114	1115
33	1094	-
34	-	1075
35	1055	1059
36	-	1043
37	-	1029
38	996	999
39	940	942
40	-	909

Table 5.12 (continued)

Main absorptions	Wavelength (cm ⁻¹)	
	EP04W	EP04D
41	886	886
42	-	870
43	845	848
44	-	834
45	823	821
46	-	805
47	-	779
48	773	-
49	734	735
50	-	701
51	680	675
52	661	666
53	647	650
54	594	594
55	573	573
56	545	545
57	-	524
58	513	-
59	-	503
60	487	485
61	-	472
62	448	-
63	-	438
64	430	-

An overlay of the DRIFT-IR spectra of EP04W and EP04D is shown in figure 5.15.

**Figure 5.15** Overlay of the DRIFT-IR spectra of EP04W (blue) and EP04D (red).

The IR spectra of EP04W and EP03W were comparable and the IR spectrum of EP04D was comparable to the IR spectrum of EP03D. Figure 5.16 displays an overlay of the DRIFT-IR spectra of EP03W and EP04W.

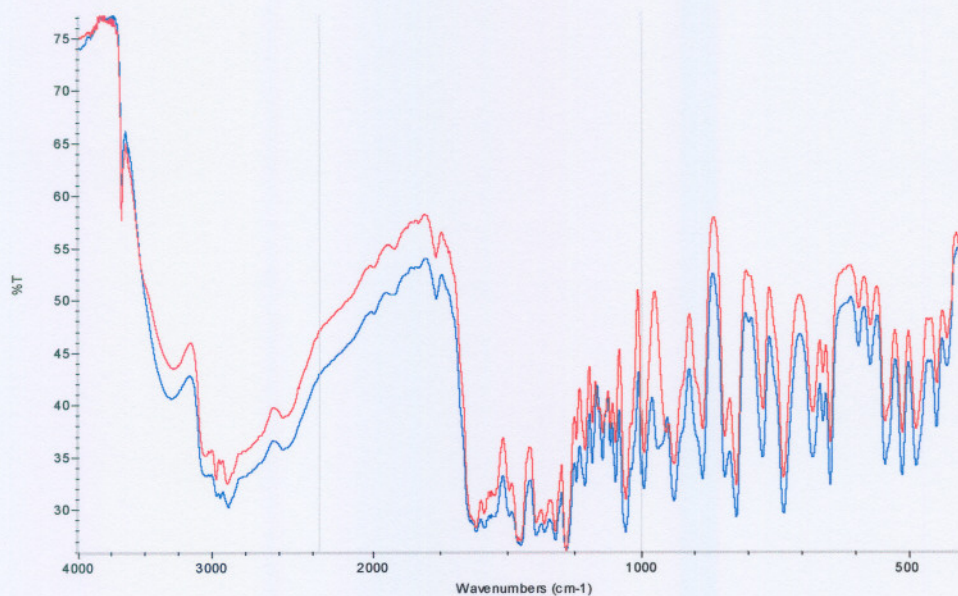


Figure 5.16 Overlay of the DRIFT-IR spectra of EP03W (blue) and EP04W (red).

5.2.2.3 Differential scanning calorimetry (DSC)

The DSC thermogram of EP04W is shown in figure 5.17.

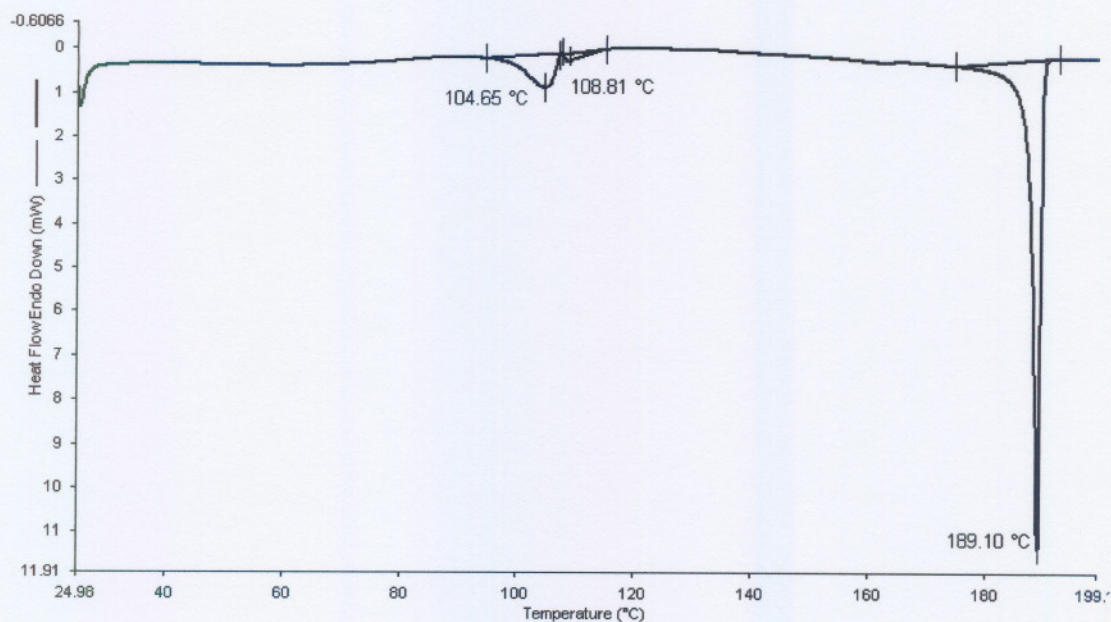


Figure 5.17 DSC thermogram of EP04W.

The thermogram of EP04W displayed three endotherms. The endotherms at 104.65°C and 108.81°C indicated desolvation (confirmed by TGA in section 5.2.2.4) and melting of a fraction of the sample (confirmed by HSM in section 5.2.2.5) respectively. The desolvation endotherm (104.65°C) was due to the overlapping desolvation and dehydration of the crystals, seeing that the boiling points of isopropanol and water are 82.50° and 100.00°C respectively. The third endotherm present at 189.10°C signified the melting of the sample.

The melting endotherm observed at 108.81°C in the thermogram was due to the melting of a fraction of the crystals upon dehydration. The melted fraction solidified and finally melted together with the rest of the crystals at the melting point of the dehydrated (anhydrous) form (189.10°C), as indicated in the HSM photomicrographs (section 5.2.2.5).

A similar behaviour was described for citric acid hydrate by Kuhnert-Brandstätter (1971:16) where some of the crystals dehydrated and became turbid, while another fraction of the crystals melted as the hydrate. The melted fraction solidified and melted together with the rest of the crystals at the final melting point of the desolvated (anhydrous) form.

Figure 5.18 displays the DSC thermogram of EP04D. The drift in the baseline of this thermogram could be attributed to the fact that the instrument used for analysis was a power-compensation DSC.

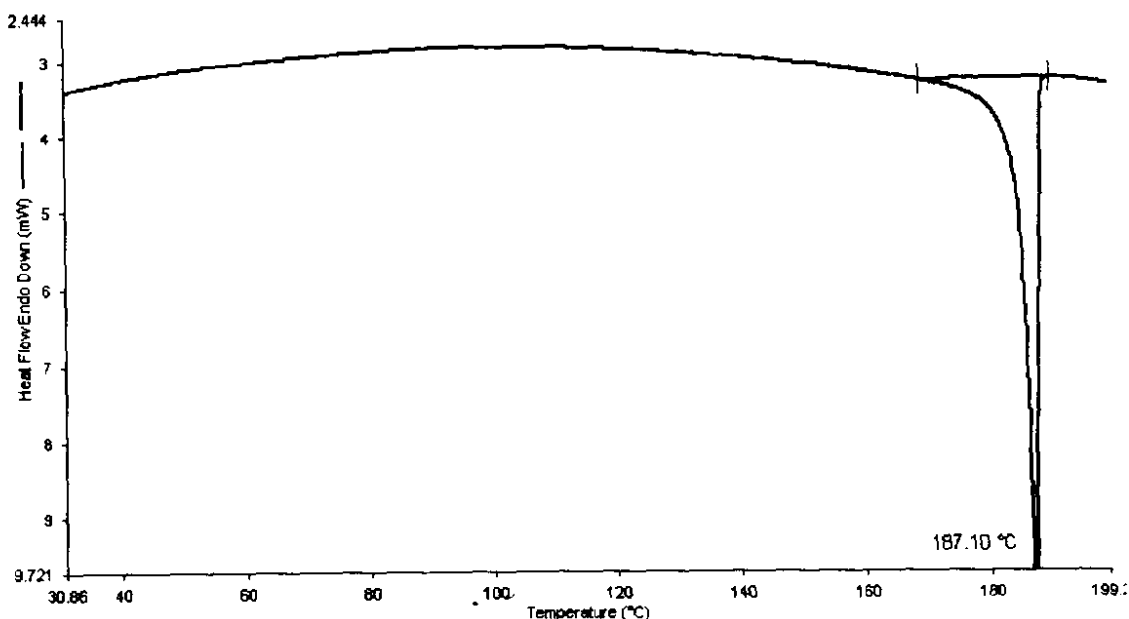


Figure 5.18 DSC thermogram of EP04D.

EP04D displayed a thermogram with only one event, a melting endotherm present at 187.10°C.

5.2.2.4 Thermogravimetric analysis (TGA) & Karl Fischer titration (KF)

The TGA and KF results of EP04W and EP04D are tabulated in table 5.13 and an overlay of the TGA thermograms of these two forms is displayed in figure 5.19.

Thermogravimetric analysis revealed a total % weight loss of 11.52% for EP04W. Karl Fischer titration confirmed that approximately 3.62% of this weight loss was due to crystal water. This correlated well with the theoretical water content of a monohydrate, which is 4.58%, and therefore this form could possibly be a monohydrate. However, iso-propanol was also included into the crystal lattice in an amount of 7.90% (as illustrated by TGA). The amount of iso-propanol included into the crystal could be compared to that of a hemi-solvate. EP04W could therefore possibly be classified as a hydrated solvate.

EP04D revealed no significant weight loss as determined by TGA, and KF confirmed that no significant amount of moisture was present in the sample.

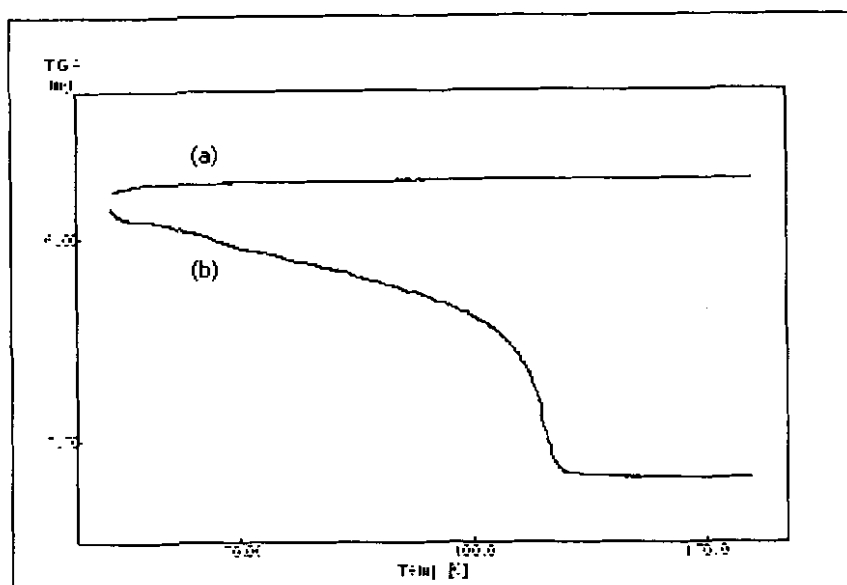


Figure 5.19 TGA thermogram of (a) EP04D and (b) EP04W.

Table 5.13 TGA and KF results of EP04W and EP04D

Crystal form	% Weight loss (TGA)	% Moisture content (KF)
EP04W	11.52	3.62
EP04D	0.35	0.19

5.2.2.5 Microscopic analysis

Hot-stage microscopy (HSM) was performed on EP04W and EP04D to investigate the thermal behaviour of these two forms. The thermal microscopic observations of EP04W are summarised in table 5.14.

Table 5.14 Summary of HSM observations of EP04W in the temperature range of 25-188°C

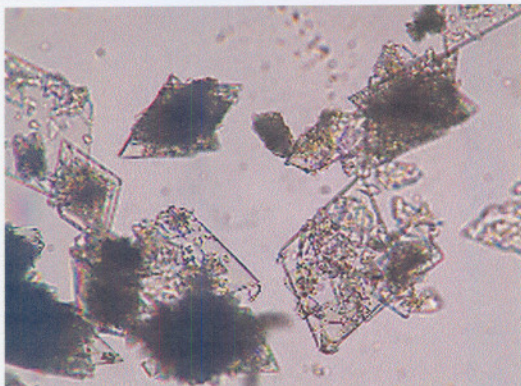
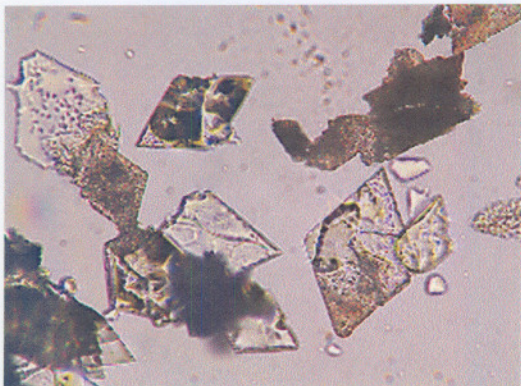
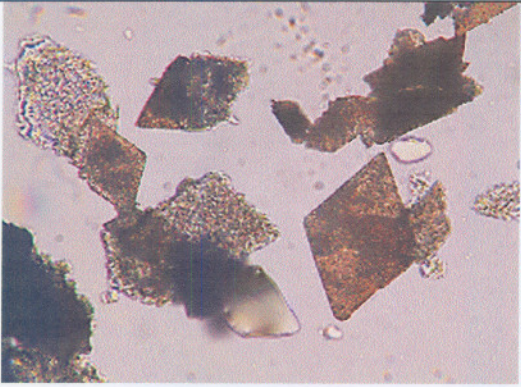
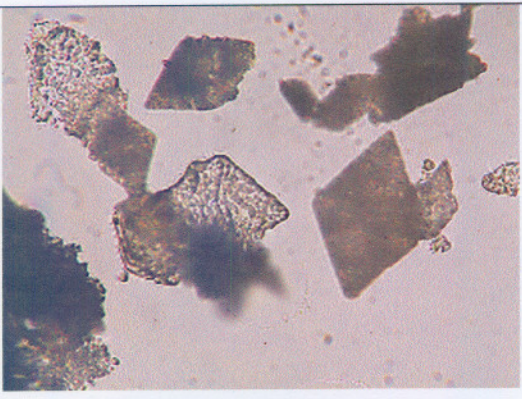
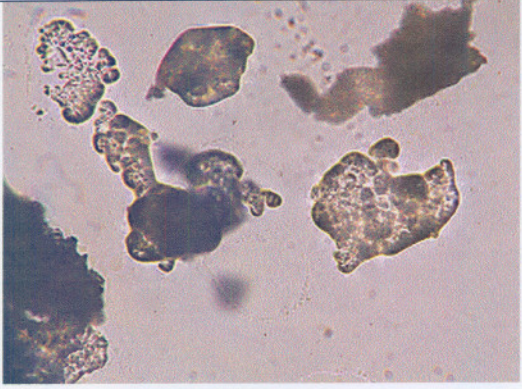
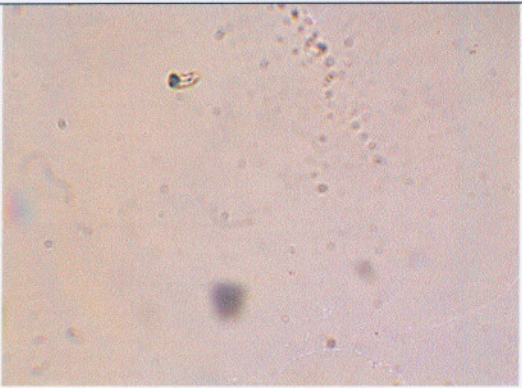
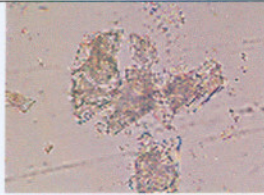
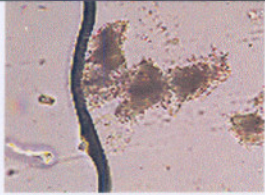
Temperature (°C)	Photomicrograph	Remarks
25		EP04W crystals at room temperature.
111		Melting of a fraction of the crystals occurred at 111°C during dehydration/desolvation; neighbouring crystals darkened during dehydration/desolvation without melting.
123		Solidification of the melted fraction occurred and darkening of the neighbouring crystals was visible.

Table 5.14 (continued)

Temperature (°C)	Photomicrograph	Remarks
181		Melting of all crystals started at 181°C.
185		Melting continued at 185°C.
188		Melting completed at 188°C.

Darkening of the crystals and bubble formation (due to gas evolution) indicated desolvation while the crystals were submerged in silicon oil. Table 5.15 displays the HSM photomicrographs of the darkening and bubble formation observed when crystals of EP04W were heated while submerged in silicon oil.

Table 5.15 HSM observations of EP04W when submerged in silicon oil

25°C	96°C	106°C
		
EP04W crystals at room temperature.	Darkening of the crystals and bubble formation occurred.	Darkening of the crystals and bubble formation was observed.

The thermal microscopic observations confirmed that the endotherms at 104.65°C and 108.81°C in the DSC thermogram were due to desolvation and melting respectively. The melting of a fraction of the crystals was followed by the solidification of the same melted fraction, which was not observed in the DSC thermogram signifying that the energy released during this solidification process might have been below the detection limit of the instrument. The temperature for the final melting of the crystals corresponded with the final melting endotherm observed in the DSC thermogram.

Table 5.16 displays a summary of the HSM observations of EP04D. The crystals of EP04D displayed no significant changes in form or colour upon heating. The only event observed by thermal microscopy was melting of the sample at 183-187°C.

Table 5.16 Summary of HSM observations of EP04D in the temperature range 25-187°C.

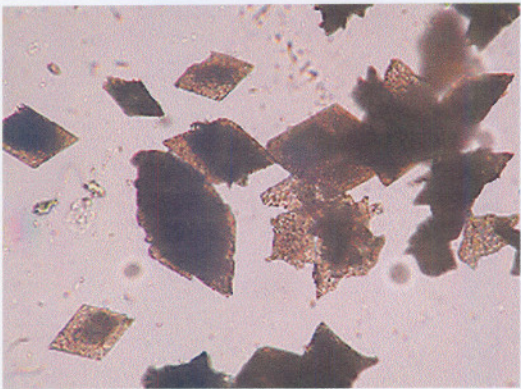
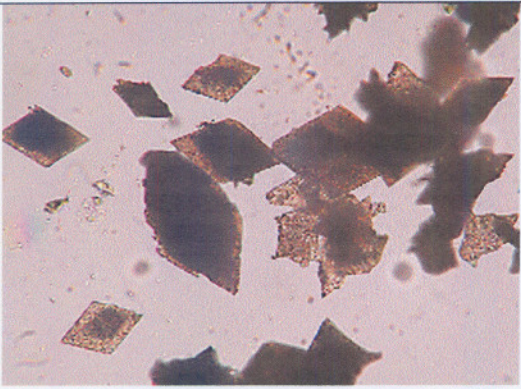
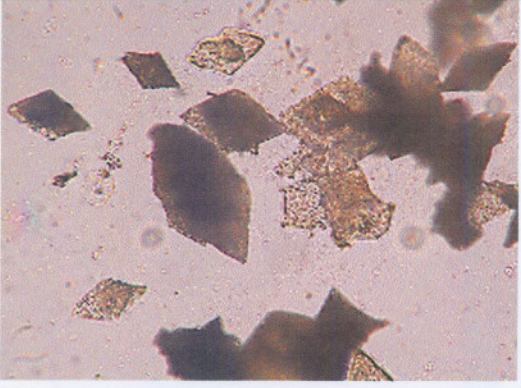
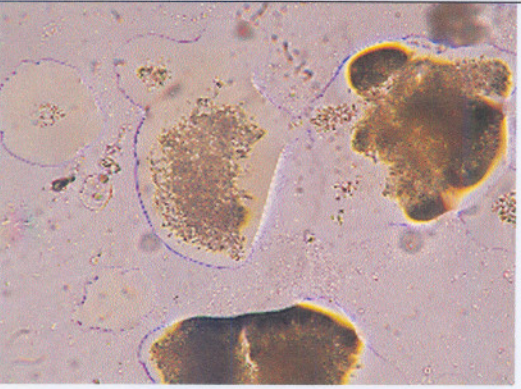
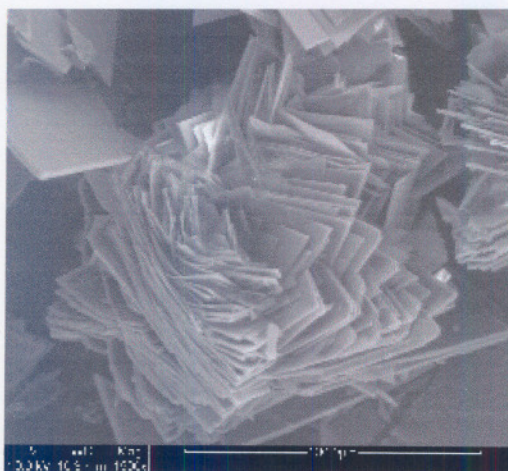
Temperature (°C)	Photomicrograph	Remarks
25		EP04D crystals at room temperature.
100		No change in the appearance of the crystals.
183		Melting of the crystals started at 183°C.
185		Melting continued at 185°C.

Table 5.16 (continued)

Temperature (°C)	Photomicrograph	Remarks
187		Melting completed.

Scanning electron microscopy (SEM) was used to compare the morphology of the crystals of EP04W and EP04D. Figure 5.20 shows a SEM photomicrograph of EP04W. This form displayed plate-like habit and the crystals tended to agglomerate. The crystals of EP04D were plate-like and tended to agglomerate in the same way as EP04W. A SEM photomicrograph of EP04D is shown in figure 5.21.

**Figure 5.20** SEM photomicrograph of EP04W.

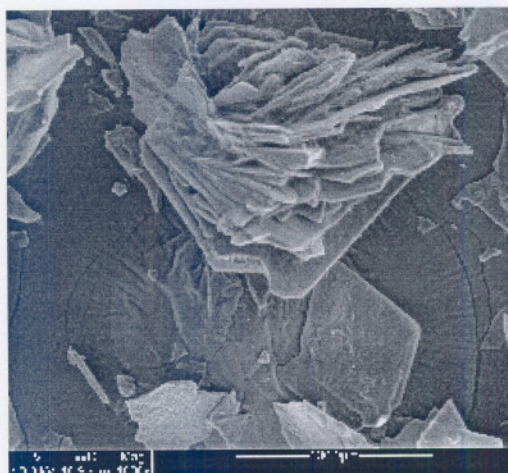


Figure 5.21 SEM photomicrograph of EP04D.

The morphology of the crystals of EP04W and EP04D were similar to that of EP03W.

5.2.2.6 Dissolution studies

Powder dissolution studies on EP04W and EP04D were performed using sieved fractions (106-250 μm) of the crystals to ensure particle size uniformity. The dissolution results of EP04W and EP04D are tabulated in table 5.17 and the dissolution profiles of these two forms are displayed in figure 5.22.

92.0% of the sample of EP04W was dissolved after 7.5 minutes, while the sample of EP04D was 99.0% dissolved at this time point. The final withdrawal was made at 30 minutes where 99.0% of EP04W and 102.0% of EP04D was dissolved.

The guidelines of the Medicines Control Council states that for the calculation of the f_2 value only one measurement should be considered after 85% dissolution of both samples have occurred (Medicines Control Council, 8:2003). The dissolution profiles of the two samples may be regarded similar if >85% dissolution occurred for both samples. Thus the f_2 value (similarity factor) need not be calculated to determine the similarity between the dissolution profiles of EP04W and EP04D. However, for the sake of completeness the f_2 value (similarity factor) of EP04W was calculated and included here. The dissolution profile of EP04D was used as reference in the calculation of the f_2 value of EP04W.

The f_2 value of EP04W was calculated as 63.57. An f_2 value of greater than 50 indicates that the profiles could be considered to be similar. Thus the differences between the dissolution profiles of EP04W and EP04D might be considered to be insignificant seeing that the similarity factor indicated that these two profiles were similar.

Table 5.17 Dissolution results of EP04W and EP04D in 0.01 N HCl at $37\pm0.2^\circ\text{C}$

Dissolution vessel	% Dissolved					
	7.5 minutes		15 minutes		30 minutes	
	EP04W	EP04D	EP04W	EP04D	EP04W	EP04D
1	96.8	100.8	98.9	101.0	101.7	101.1
2	85.1	97.7	89.4	98.8	96.0	100.8
3	87.3	100.9	90.3	101.7	96.9	102.5
4	99.4	97.7	100.7	99.3	99.5	101.7
5	93.0	98.9	96.0	99.5	100.6	101.8
Average	92.0 $\pm$ 6.1	99.0 $\pm$ 1.6	95.0 $\pm$ 5.1	100.0 $\pm$ 1.2	99.0 $\pm$ 2.4	102.0 $\pm$ 0.7

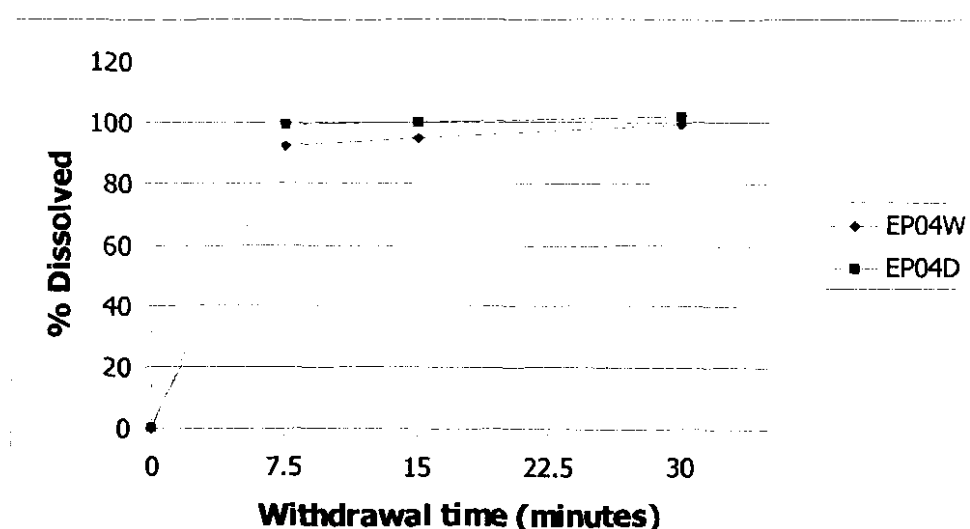


Figure 5.22 Dissolution profiles of EP04W and EP04D in 0.01 N HCl at $37\pm0.2^\circ\text{C}$.

To compare the dissolution profiles of EP04W and EP04D with that of the sesquihydrate, the similarity factors (f_2 values) of these two forms were calculated using the dissolution profile of the sesquihydrate as reference. The f_2 values of EP04W and EP04D (compared to the sesquihydrate form) are shown in table 5.18.

Table 5.18 f_2 values for EP04W and EP04D using the sesquihydrate as reference

Crystal form	f_2 value
EP04W	59.59
EP04D	88.07

The f_2 values of EP04W and EP04D indicated that the dissolution profiles of these two forms might be considered to be similar to the dissolution profile of the gatifloxacin sesquihydrate. EP04D displayed a better dissolution profile fit to the sesquihydrate form as the higher f_2 value indicates.

5.2.2.7 Solubility studies

Solubility studies were performed on both EP04W and EP04D according to the method described in chapter 3. The results obtained from these studies are tabulated in table 5.19. The solubility profiles of EP04W and EP04D are displayed in figure 5.23. The solubility of EP04W ranged in the following order: 0.01 N HCl > acetate buffer > phosphate buffer > MilliQ water. The order in which the solubility of EP04D ranged was: 0.01 N HCl > acetate buffer > MilliQ water > phosphate buffer.

Table 5.19 Solubility values (in mg/ml) of EP04W and EP04D in various media at 37±2°C

Medium	pH	EP04W		EP04D	
		Solubility (mg/ml)	%CV	Solubility (mg/ml)	%CV
0.01 N HCl	2.20	4.30	0.95	5.16	3.17
Acetate buffer	4.50	4.10	2.53	4.97	0.48
MilliQ Water	5.50	2.42	3.66	3.08	0.45
Phosphate buffer	6.80	2.50	1.32	3.03	1.15

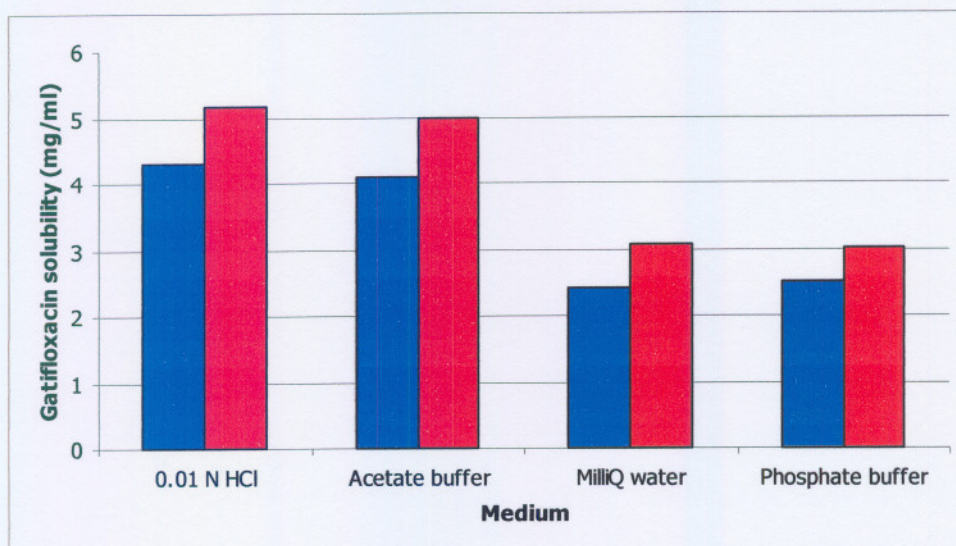


Figure 5.23 Solubility profiles of EP04W (blue) and EP04D (red) in various media at $37 \pm 2^\circ\text{C}$.

5.2.2.8 Flowability

The Hausner ratio and Carr's index were calculated for both EP04W and EP04D to compare the flowability of these forms. The results of these calculations are displayed in tables 5.20 and 5.21.

Table 5.20 Initial density (D_0) and final density (D_f) measurements of EP04W and EP04D

Sample	D_0 (g/ml)		D_f (g/ml)	
	EP04W	EP04D	EP04W	EP04D
1	0.14	0.17	0.20	0.26
2	0.14	0.18	0.21	0.26
3	0.14	0.17	0.20	0.26
Average	0.14 ± 0.00	0.17 ± 0.01	0.20 ± 0.01	0.26 ± 0.00

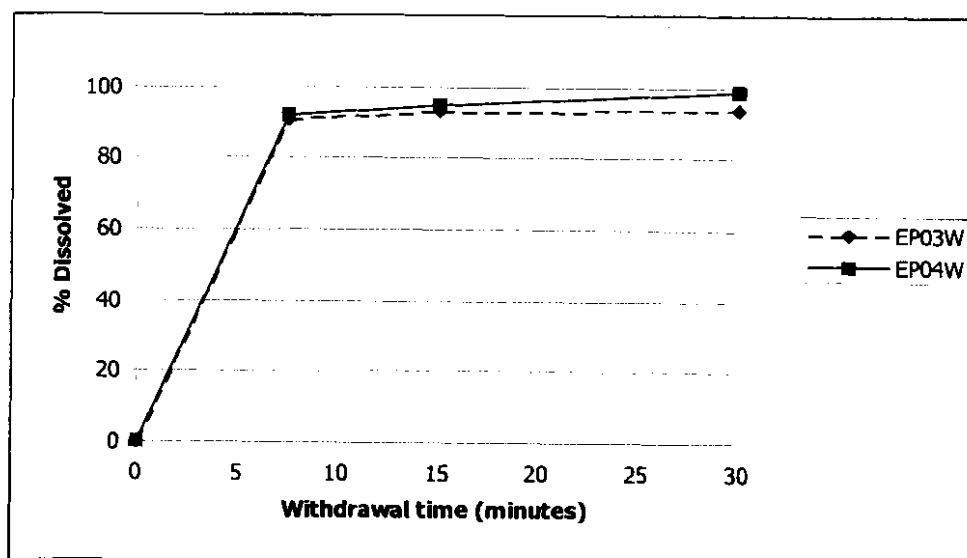
Table 5.21 Hausner ratio and Carr's index of EP04W and EP04D

Sample	Hausner ratio		Carr's index	
	EP04W	EP04D	EP04W	EP04D
1	1.40	1.51	28.57	33.90
2	1.45	1.50	30.99	33.33
3	1.40	1.53	28.57	34.48
Average	1.42±0.03	1.51±0.02	29.38±1.40	33.90±0.58

A powder with relatively good flowability has a Hausner ratio of 1.2, while the powders with poor flowability have Hausner ratio's of >1.6. Carr's index of the samples was interpreted using table 3.2. According to table 3.2 both forms had poor flowability. The Hausner ratio and Carr's index of EP04W indicated that this form had slightly better flowability than EP04D.

5.3 Comparison of the dissolution & solubility results of EP03 and EP04

The dissolution profiles of the two solvated crystal forms obtained in this chapter (EP03W and EP04W), are displayed in figure 5.24. The dissolution profiles of EP03W and EP04W displayed only slight differences. Q (% dissolved) after 30 minutes was 94.0% for EP03W and 99.0% for EP04W.

**Figure 5.24** Dissolution profiles of EP03W and EP04W in 0.01 N HCl at 37±0.2°C.

The dissolution profiles of EP03D and EP04D are displayed in figure 5.25. The dissolution profiles of these two forms were similar and Q after 30 minutes was 102.0% for both EP03D and EP04D.

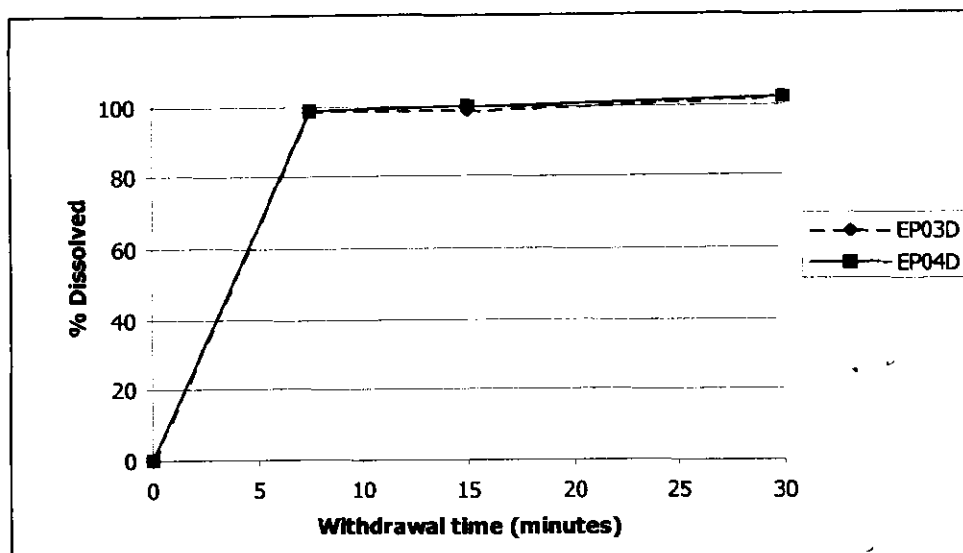


Figure 5.25 Dissolution profiles of EP03D and EP04D in 0.01 N HCl at 37±0.2°C.

The solubility values of EP03W and EP04W are compared in table 5.22 and figure 5.26 displays the solubility profiles of these two forms. Only slight differences were observed in the solubility profiles of EP03W and EP04W, which indicated that the inclusion of the different solvents into the crystal lattice did not significantly influence the solubility profile of this crystal form.

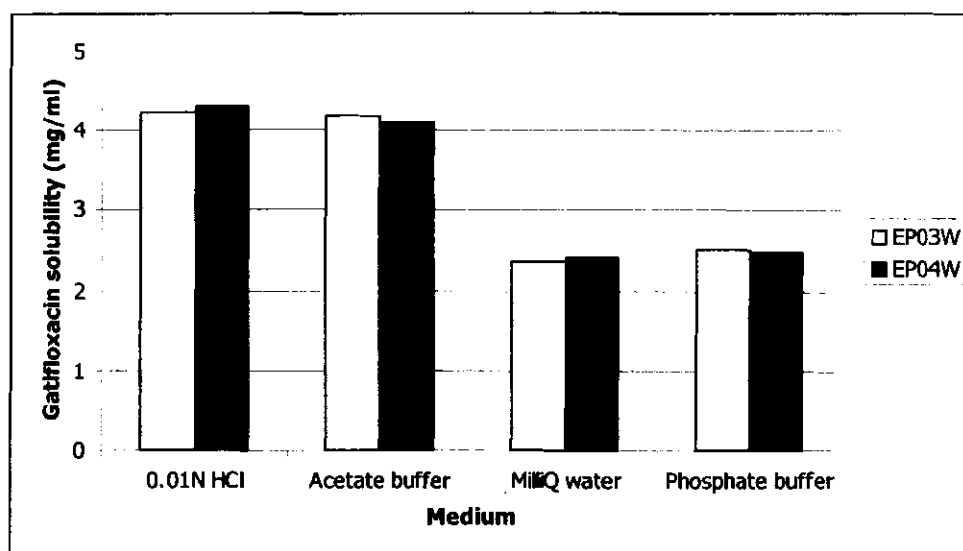


Figure 5.26 Solubility profiles of EP03W and EP04W in various media at 37±2°C.

Table 5.22 Solubility values (in mg/ml) of EP03W and EP04W in various media at 37±2°C

Medium	EP03W		EP04W	
	Solubility (mg/ml)	% CV	Solubility (mg/ml)	% CV
0.01 N HCl	4.23	2.11	4.30	0.95
Acetate buffer	4.19	1.88	4.10	2.53
MilliQ water	2.36	2.51	2.42	3.66
Phosphate buffer	2.57	2.90	2.50	1.32

The solubility profiles of EP03D and EP04D can be considered similar, as indicated in table 5.23 and figure 5.27. The solubility of both forms ranged in the order: 0.01 N HCl > acetate buffer > MilliQ water > phosphate buffer.

Table 5.23 Solubility values (in mg/ml) of EP03D and EP04D in various media at 37±2°C

Medium	pH	EP03D		EP04D	
		Solubility (mg/ml)	% CV	Solubility (mg/ml)	% CV
0.01 N HCl	2.20	5.17	2.69	5.16	3.17
Acetate buffer	4.50	4.95	1.23	4.97	0.48
MilliQ water	5.50	3.09	3.09	3.08	0.45
Phosphate buffer	6.80	3.03	0.81	3.03	1.15

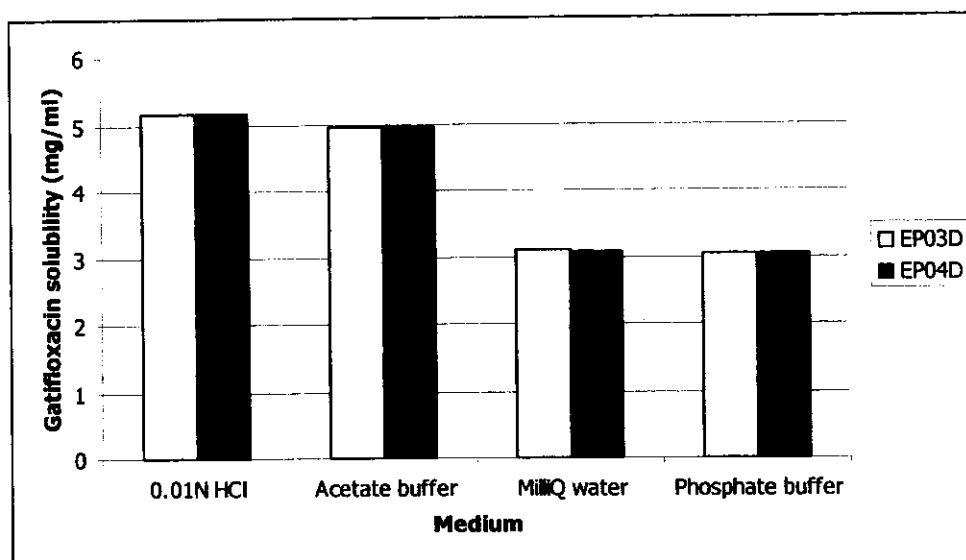


Figure 5.27 Solubility profiles of EP03D and EP04D in various media at 37±2°C.

Conclusion

The aim of this chapter was to characterise the crystal forms obtained from recrystallisation with the propanolic alcohols, and to determine the influence of solvent inclusion on the physico-chemical properties of these forms.

Crystal form EP03W was obtained from recrystallisation with n-propanol and was classified as a hydrated solvate (½ water : ½ n-propanol : 1 gatifloxacin). EP03D (an anhydrous crystal form) was obtained from the drying of EP03W at 105°C for 12 hours.

The dissolution profiles of EP03W and EP03D can be considered to be similar as indicated by the f_2 value. Comparison of the flow properties of EP03W and EP03D indicated that EP03W exhibited very poor flowability and EP03D displayed poor flow properties.

Recrystallisation from iso-propanol yielded form EP04W, a hydrated solvate (1 water : ½ iso-propanol : 1 gatifloxacin). The crystal form EP04D was obtained from the drying of EP04W at 130°C for 12 hours. This form was classified as an anhydrous crystal form.

The dissolution profiles of EP04W and EP04D were compared using the similarity factor (f_2 value) and was found to be similar. EP04W and EP04D both displayed poor flow properties. However, the Hausner ratio and Carr's index of EP04W indicated that this form had better flowability than EP04D.

Crystal forms EP03W and EP04W both had crystal structures with the solvent of recrystallisation and water incorporated into the crystal lattice. The formation of this type of crystal structure with more than one solvent included into the crystal lattice is not often found in pharmaceutical solids. Another API that displays this behaviour is triamterene as described by Dahl *et al.* (1989:598-606).

The crystal forms obtained from n-propanol and iso-propanol (EP03W and EP04W) resembled form J described in the patents. As illustrated in this chapter the inclusion of different ratios of water and different solvents into the crystal lattice of this form did not result in significant changes in the crystal structure. The XRPD patterns and IR spectra of the solvated crystal forms were comparable (figure 5.13 and figure 5.16). However, other physico-chemical properties (such as dissolution and flow properties) of these crystal forms differed due to the different ratios of water and different solvents incorporated into the crystals.

The XRPD patterns of the anhydrous forms obtained in this chapter resembled that of form omega described in the literature. The XRPD pattern was the only description of form omega available in the literature. The physico-chemical properties of EP03D and EP04D were comparable.

The physico-chemical properties i.e.: XRPD, IR, DSC, etc. of the solvated and desolvated crystal forms differed significantly. However, the dissolution properties of these crystal forms were not profoundly influenced by the absence of solvent.

The influence of solvent inclusion on crystal forms obtained from various solvents will be investigated in the next chapter.

CHAPTER 6

Solvent inclusion properties: crystals recrystallised from diverse solvents

Introduction

Several active pharmaceutical ingredients (API's) exhibit polymorphism and pseudopolymorphism. The different crystal packings of the polymorphs and pseudopolymorphs usually lead to significant differences in the physico-chemical properties of the API, such as melting point, solubility, dissolution rate, infrared spectra and XRPD patterns (Chapter 1).

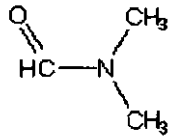
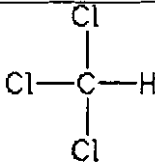
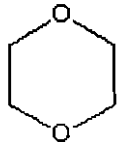
An understanding of the solid state properties of API's are necessary to ensure that the safest, most efficient crystal form is used in product development. Although most solvated API's that are used for the manufacturing of pharmaceutical products are hydrates, it is still important to fully characterise and study the solvates obtained from organic solvents. Solvates are occasionally used in their desolvated form due to improved dissolution profiles or they may be used in the purification process of the API (Byrn *et al.*, 1999:236).

The aim of this chapter was to characterise the crystal forms obtained from recrystallisation with diverse solvents, and to determine the influence of solvent inclusion on the physico-chemical properties of these forms. The methods described in chapter 3 were used in the characterisation of these crystal forms.

6.1 Preparation method

The crystal forms were recrystallised with the solvents listed in table 6.1. Small amounts of gatifloxacin sesquihydrate powder were added to the solvent, under constant stirring at the boiling temperature of the solvent (see table 6.1 for boiling temperatures), until saturated solutions were obtained. The saturated solutions were covered with Parafilm® and allowed to crystallise at ambient conditions.

Table 6.1 Diverse solvents used in recrystallisations

Solvent	Molecular weight (g/mol)	Boiling point at room temperature (°C)	Chemical structure	Manufacturer
DMF (Dimethylformamide)	73.09	153.0		Merck, South Africa
Chloroform	119.38	61.5		Saarchem, South Africa
Dioxane	88.10	101.1		Merck, South Africa

The desolvated crystals were prepared by drying the solvated crystal forms obtained from recrystallisation in an Ecotherm drying oven (Labotec, South Africa) for 12 hours at a temperature ranging between 100-110°C, depending on the desolvation temperature of the crystal form.

6.2 Characterisation of crystal forms

6.2.1 EP05W & EP05D

EP05W was recrystallised from dimethylformamide (DMF) using the method described in section 6.1. EP05D was obtained by drying EP05W (as described in section 6.1) at a temperature of 100°C.

6.2.1.1 X-ray powder diffraction (XRPD) and variable temperature x-ray powder diffraction (VT-XRPD)

The variable temperature x-ray powder diffractograms of EP05W are displayed in figure 6.1. The VT-XRPD patterns of EP05W displayed no significant changes in the temperature range 25-45°C. Noticeable changes were observed in the diffractogram at 55°C. These changes included the disappearance of the peaks at $7.7^{\circ}2\theta$, $8.7^{\circ}2\theta$, $9.7^{\circ}2\theta$ and $10.4^{\circ}2\theta$. A peak appeared at $10.6^{\circ}2\theta$. Other noticeable changes included the disappearance of the peaks at $12.4^{\circ}2\theta$, $14.1^{\circ}2\theta$, and $15.3^{\circ}2\theta$. Peaks that appeared at 55°C that were not present in the initial (25°C) diffractogram included the peaks at $14.4^{\circ}2\theta$ and $17.2^{\circ}2\theta$. The peaks at $17.6^{\circ}2\theta$, $20.8^{\circ}2\theta$, the peaks in the area of $25-27^{\circ}2\theta$ and the peak at $30.1^{\circ}2\theta$ also disappeared at 55°C. The diffractogram obtained at 55°C showed an overall decrease in the relative intensities of all peaks.

Slight differences were observed between 55-65°C, which included the appearance of a peak at $7.1^{\circ}2\theta$. The changes observed at 55 and 65°C could possibly be attributed to the desolvation of the DMF-solvate. The XRPD remained relatively unchanged from 65-85°C, with major changes occurring at 95°C. Some significant changes included the appearance of peaks at $19.6^{\circ}2\theta$, $23.6^{\circ}2\theta$, $25.8^{\circ}2\theta$ and $28.5^{\circ}2\theta$. An overall increase in the relative intensities of the peaks was observed.

Significant changes were also observed at 100°C. The peak at $6.4^{\circ}2\theta$ displayed a significant decrease in intensity, while the peak at $7.7^{\circ}2\theta$ increased significantly in intensity. The overlapping peaks in the area $16-17^{\circ}2\theta$ clearly divided into 2 visible peaks ($16.3^{\circ}2\theta$ and $17.0^{\circ}2\theta$). The changes that occurred at 95-100°C could be attributed to the change in crystal structure caused by dehydration of the hydrated fraction. The increase in overall relative intensity of the peaks in the diffractogram indicated that dehydration led to a more crystalline crystal structure. Only slight changes were observed between 100-110°C such as the disappearance of the peak at $6.4^{\circ}2\theta$.

No significant changes were observed between 110-170°C. The diffractogram obtained at 180°C was characteristic of the melting process.

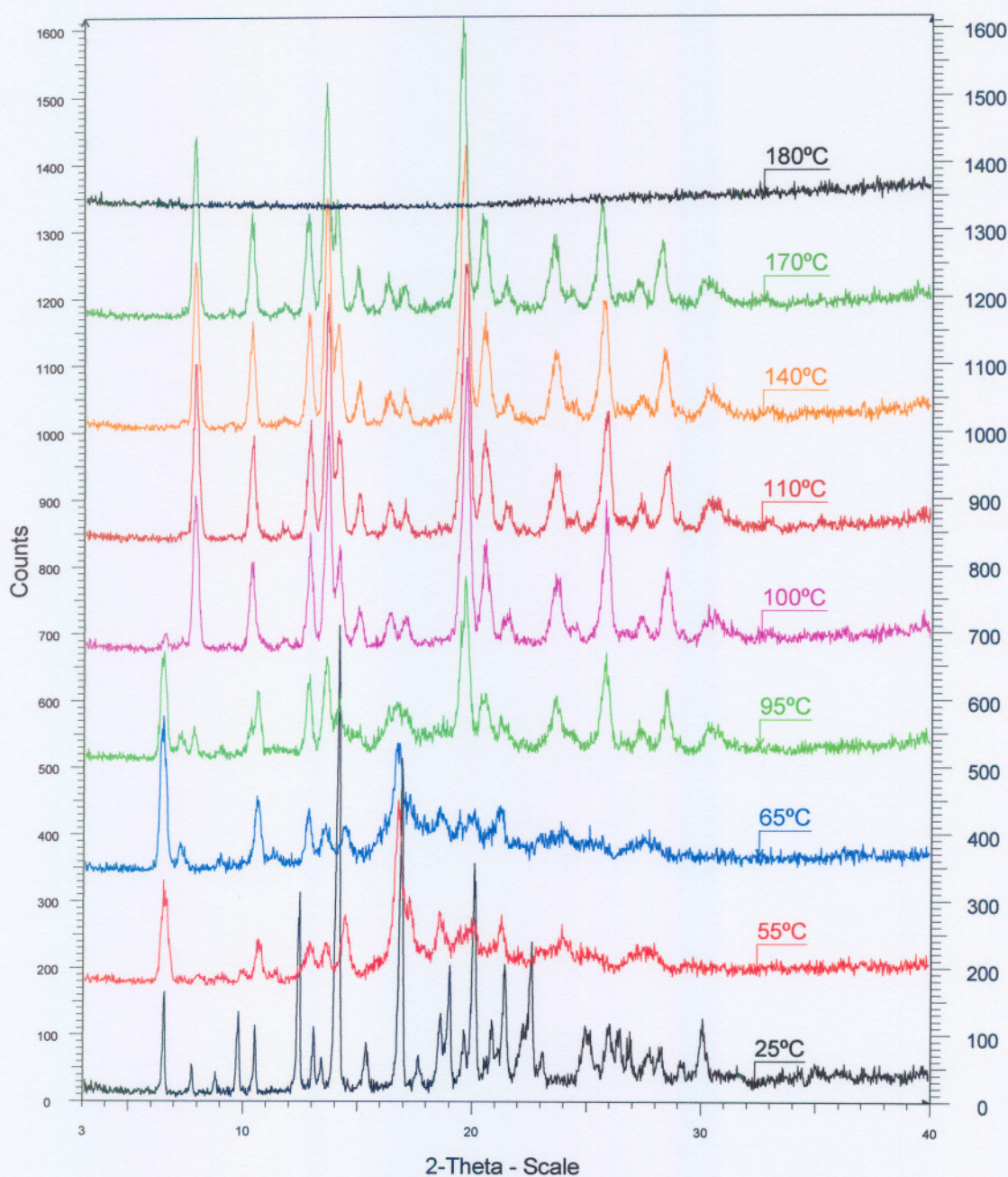


Figure 6.1 Variable temperature x-ray powder diffractograms of EP05W.

EP05W was found to possibly be a mixture of two pseudopolymorphic forms, i.e.: a sesquihydrate and a DMF solvate. No DMF solvates have currently been described in the literature. The XRPD pattern of EP05W displayed some peaks that correlated with that of the sesquihydrated forms described in the literature. Forms H1, H4 and H5 are sesquihydrate forms described in the literature (see sections 2.4.5-2.4.7 for a

discussion of these forms). The sesquihydrate form of which the most characteristic peaks were present in the diffractogram of EP05W was form H4. Characteristic peaks of this form that were present in the diffractogram of EP05W included the peaks at $7.7^{\circ}2\theta$, $9.7^{\circ}2\theta$, $13.0^{\circ}2\theta$, $13.4^{\circ}2\theta$, $20.1^{\circ}2\theta$, $20.8^{\circ}2\theta$ and $26.0^{\circ}2\theta$ ($\pm 0.1^{\circ}2\theta$).

The VTXRPD patterns of EP05D remained relatively unchanged through the entire temperature study (25-170°C) and no significant changes were observed. Figure 6.2 shows the variable temperature x-ray powder diffractograms of EP05D.

EP05D was compared to the anhydrous crystal forms described in the literature and revealed similarities to form I as well as form omega (see section 2.4.2-2.4.3 for a discussion of these two forms). EP05D displayed peaks at $10.1^{\circ}2\theta$, $14.0^{\circ}2\theta$, $14.8^{\circ}2\theta$, $16.2^{\circ}2\theta$ and $16.9^{\circ}2\theta$ ($\pm 0.1^{\circ}2\theta$). These peaks are characteristic peaks in the diffractograms of both form I and form omega. The peaks at $19.6^{\circ}2\theta$, $23.6^{\circ}2\theta$ and $24.3^{\circ}2\theta$ ($\pm 0.1^{\circ}2\theta$) were comparable to that of form I and form omega.

The diffractogram of EP05D correlated well with the diffractogram obtained for EP05W at 110-170°C, which indicated that prolonged exposure of EP05D to elevated temperature did not cause significant changes in the anhydrous crystal structure. An overlay of these diffractograms is shown in figure 6.3.

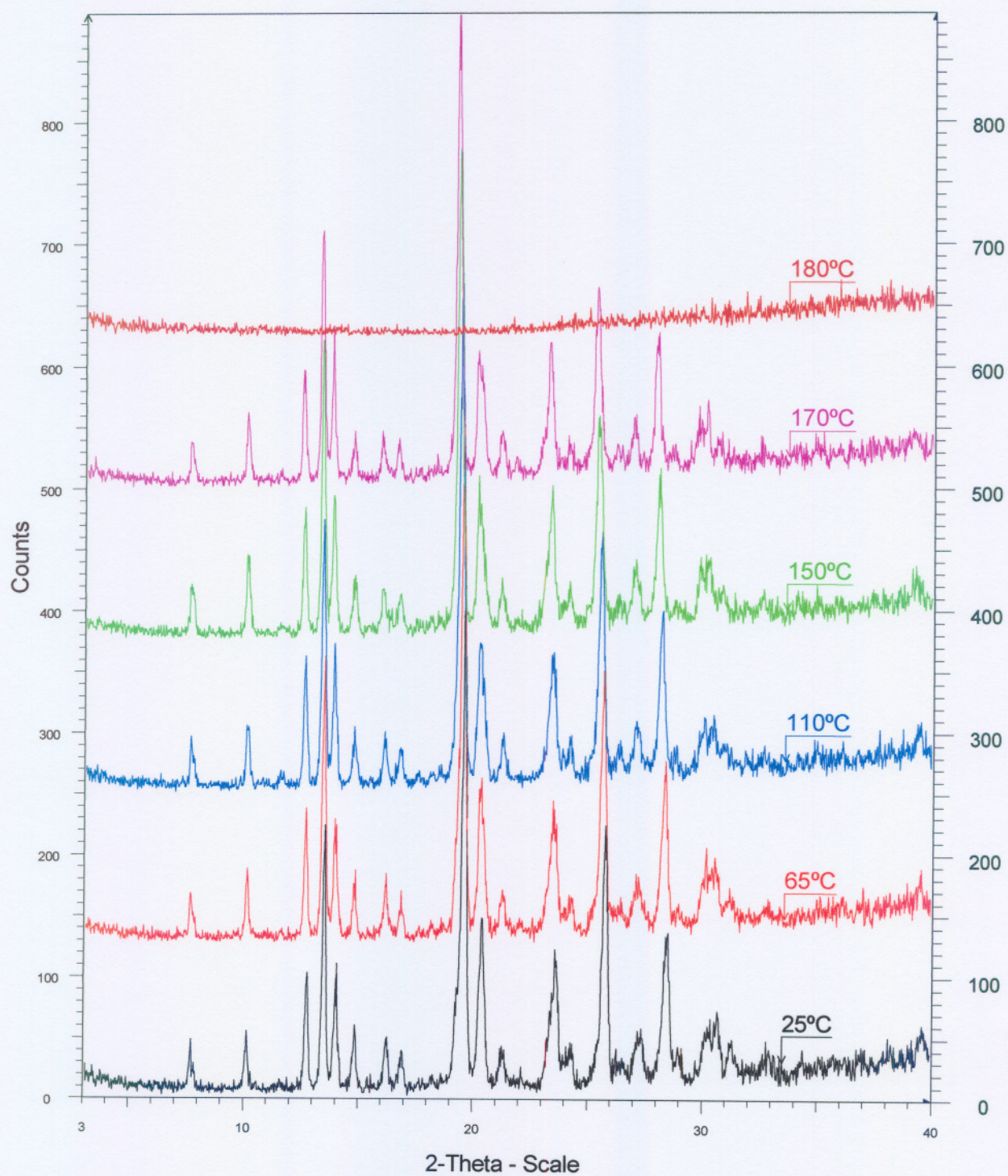


Figure 6.2 Variable temperature x-ray powder diffractograms of EP05D.

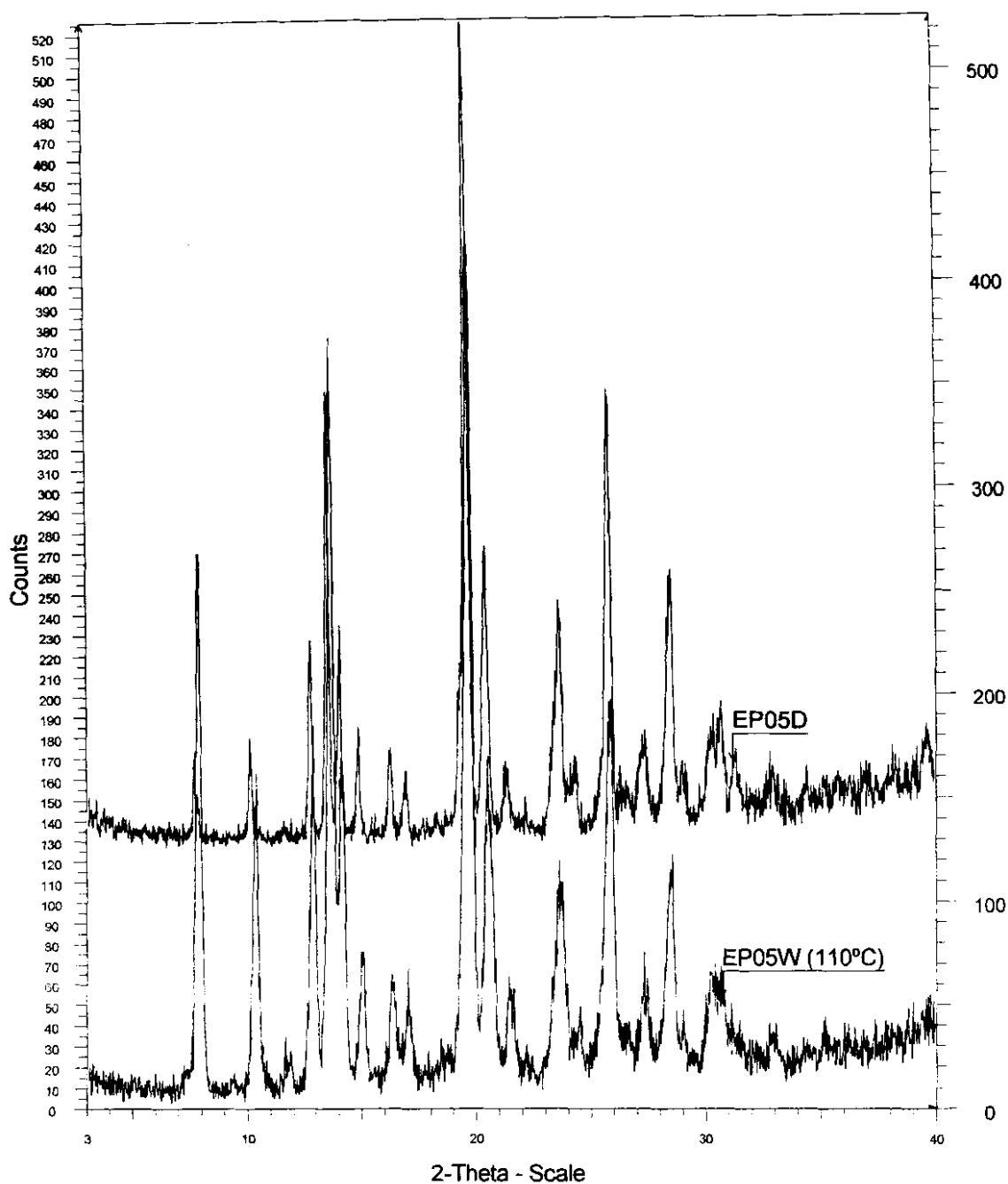


Figure 6.3 Overlay of the x-ray powder diffractograms of EP05W (at 110°C) and EP05D (at 25°C).

6.2.1.2 Infrared Spectroscopy (IR)

Table 6.2 lists the main absorptions in the IR spectra of EP05W and EP05D. An overlay of the DRITF-IR spectra of these two forms is shown in figure 6.4. Significant differences were observed between the spectra of EP05W and EP05D. The

differences in the peak positions between EP05W and EP05D are highlighted in table 6.2.

Table 6.2 Main absorptions in the DRIFT-IR spectra of EP05W and EP05D

Main absorptions	Wavelength (cm ⁻¹)	
	EP05W	EP05D
1	3676	-
2	3650	-
3	3630	-
4	3568	-
5	-	3420
6	3325	3329
7	-	3098
8	-	3062
9	-	2992
10	-	2960
11	2939	-
12	-	2891
13	2846	-
14	-	2812
15	-	2637
16	2471	-
17	2363	-
18	-	1999
19	1715	1719
20	1702	-
21	1671	-
22	-	1617
23	1578	-
24	1560	1556
25	1541	-
26	1508	1509
27	1458	1459
28	1390	1391
29	-	1376
30	-	1349
31	1324	1326
32	-	1302
33	1279	1277
34	-	1242
35	-	1218
36	1211	-
37	1182	1185
38	-	1154
39	1146	-
40	-	1115
41	1094	-
42	-	1075
43	1068	-
44	-	1059
45	-	1043
46	-	1030
47	999	999
48	961	-

Table 6.2 (continued)

Main absorptions	Wavelength (cm ⁻¹)	
	EP05W	EP05D
49	940	941
50	-	909
51	885	886
52	-	870
53	-	848
54	842	-
55	-	834
56	822	821
57	-	805
58	-	779
59	770	-
60	736	735
61	-	701
62	-	675
63	662	667
64	649	650
65	-	594
66	-	574
67	544	545
68	-	524
69	514	-
70	-	503
71	490	485
72	-	472
73	451	451
74	442	438
75	430	-
76	409	-

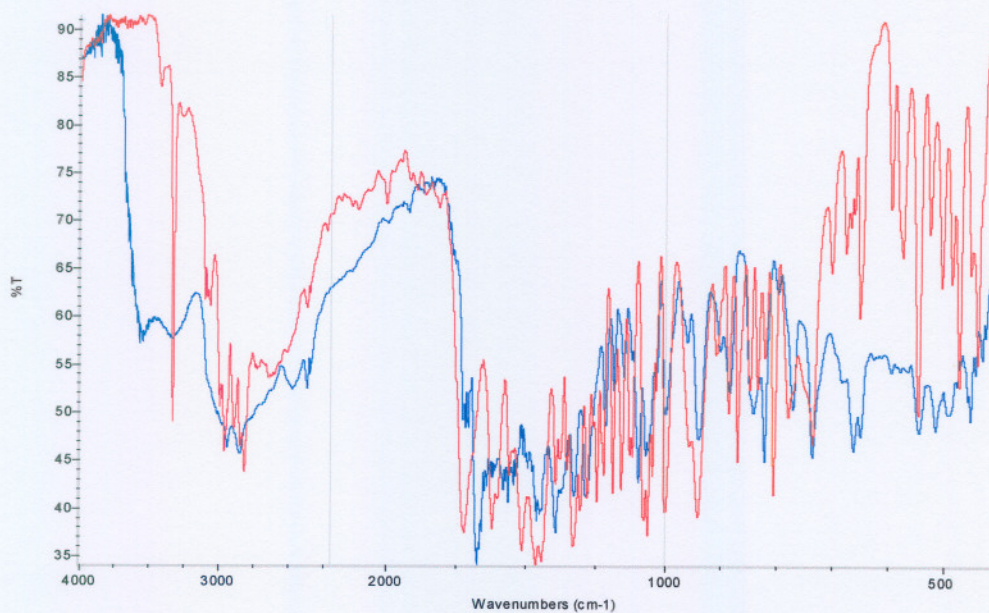


Figure 6.4 Overlay of the DRIFT-IR spectra of EP05W (blue) and EP05D (red).

6.2.1.3 Differential scanning calorimetry (DSC)

Figure 6.5 displays the DSC thermogram of EP05W.

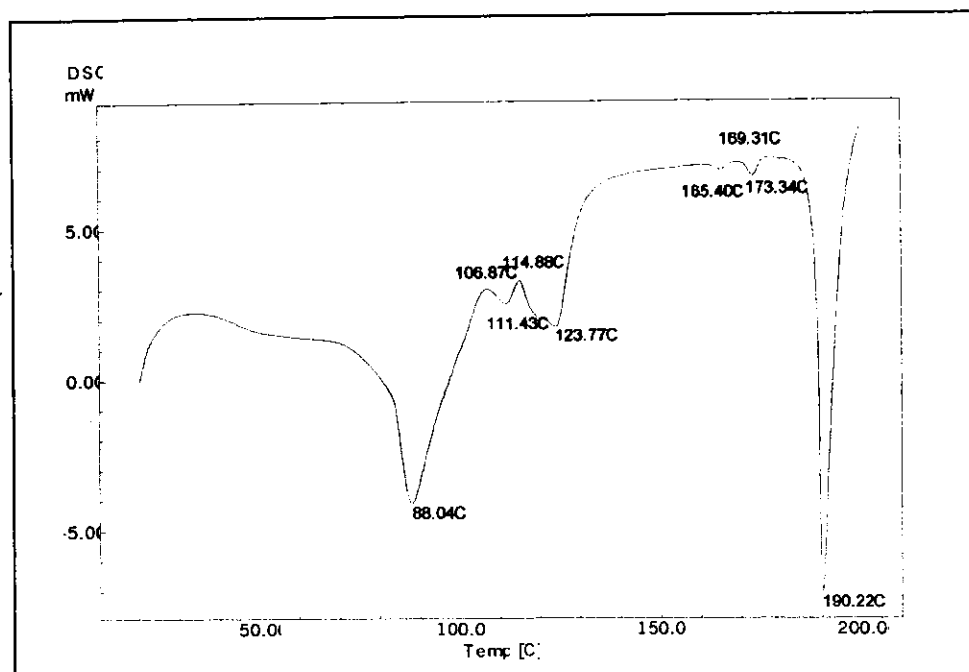


Figure 6.5 DSC thermogram of EP05W.

The DSC thermogram of EP05W displayed several endotherms that were interpreted with the help of HSM, TGA and KF. This sample consisted of a probable mixture of two forms, i.e.: a sesquihydrate and a DMF solvate. The HSM photomicrographs clearly showed the presence of two types of crystals (section 6.2.1.5).

To explain the thermal events in the DSC thermogram the two crystal forms present in the mixture will be discussed separately. The events caused by the DMF solvate will be discussed first, followed by a discussion of the events caused by the sesquihydrate fraction in the mixture.

The first endotherm at 88.04°C (figure 6.5) indicated the desolvation of the DMF solvate. This corresponded with the first step of the TGA (figure 6.8), which indicated a % weight loss of 16.60% in this temperature range (section 6.2.1.4). This weight loss correlated well with the theoretical weight loss of a mono-solvate (DMF solvate

1:1), which is 16.30%. Evaluation of the sample with HSM revealed movement of a fraction of the crystals at 90°C and bubble formation at 103°C, which indicated the desolvation of the crystals. The desolvation of the DMF solvate crystals was at a temperature much lower than expected seeing that the boiling point of DMF is at 153.0°C. Byrn *et al.* (1999:234) stated that the ease with which a solvate loses its solvent varies widely. Some solvates lose their solvents at room temperature while others retain their solvents even above the boiling temperature of the solvent.

The endotherm observed in the DSC thermogram (figure 6.5) at 111.43°C was due to the melting of a fraction of the crystals (the desolvated crystals of the DMF solvate). The melted fraction immediately recrystallised from the melt at 114.88°C (recrystallisation exotherm), which was clearly visible in the HSM photomicrographs (see section 6.2.1.5). This recrystallised fraction remained unchanged and melted at 173.34°C causing an endotherm at this temperature (HSM confirmed the melting of the recrystallised fraction at this temperature).

The endotherm present at 111.43°C was also caused by the dehydration of the sesquihydrated fraction in the mixture. The second step present in the TGA thermogram of EP05W (figure 6.8) confirmed the dehydration of the sesquihydrated fraction (section 6.2.1.4). The total weight loss for this step was 8.05% and KF analysis revealed a moisture content of 7.29% in the sample. This correlation between the KF data and the second weight loss step of TGA indicated that the other crystal form present in the mixture was possibly a sesquihydrate. HSM photomicrographs (section 6.2.1.5) revealed bubble formation at 112°C when the sample was submerged in silicon oil and this corresponded with the dehydration temperature indicated in the DSC thermogram. The endotherms at 111.43°C and 119.80-123.77°C could be attributed to the vaporisation of the water released from the crystal upon dehydration. The TGA confirmed that no weight loss occurred at 119.80-123.77°C, thus the endotherm could not be attributed to further desolvation or dehydration, but rather the vaporisation of the water from dehydration.

Literature described that dehydration and vaporisation could display overlapping or separate endotherms in a DSC thermogram. This separation of the endotherms is usually caused by elevated pressure in the sample pan. An example of an API that displayed this behaviour is carbamazepine (see figure 6.6). At ambient pressure two

overlapping endotherms were observed in the temperature range of 85-100°C. This overlapping of the dehydration and vaporisation endotherms usually occurs if the dehydration reaction is above or near the boiling point of water. Elevated pressure caused the dehydration and vaporisation endotherms to separate. At pressures above 200 psi continuous vaporisation caused a noisy baseline at higher temperatures (Han & Suryanarayanan, 1997:213).

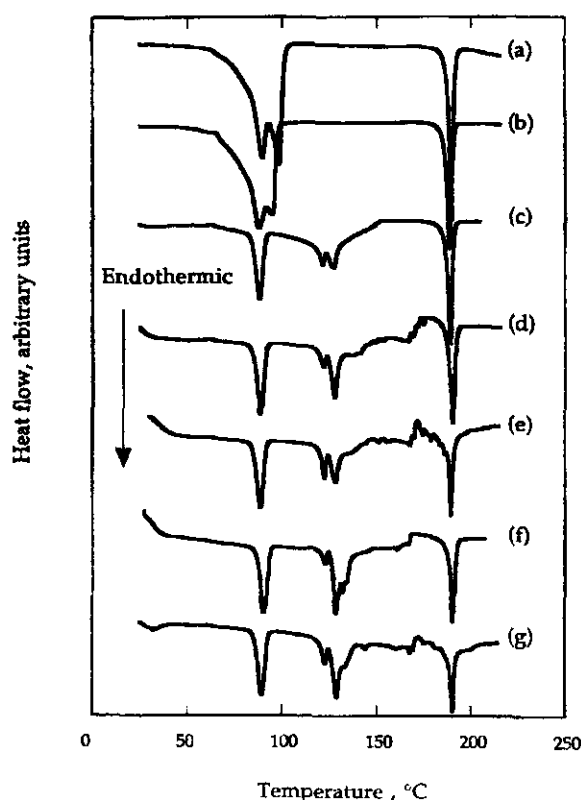


Figure 6.6 DSC thermograms of carbamazepine at different pressures: (a) at ambient pressure in a conventional DSC pan, (b) at ambient pressure in a pressure DSC pan, (c) 100 psi, (d) 200 psi, (e) 300 psi, (f) 400 psi, and (g) 600 psi (Han & Suryanarayanan, 1997:213).

A small endotherm was observed at 165.40°C (figure 6.5), which is characteristic of the sesquihydrate form of gatifloxacin (see section 4.2.3). This endotherm probably indicated melting of the anhydrous form in the mixture (sesquihydrate after dehydration), immediately followed by a very small recrystallisation exotherm. There was no prominent recrystallisation exotherm, which could be due to the fact that the energy released during recrystallisation was low and not detected by the DSC apparatus. The final endotherm in the thermogram (at 190.22°C) was due to the

melting of the form that recrystallised from the melt at 165.40°C. The melting at approximately 165°C immediately followed by recrystallisation and final melting at 189-190°C is characteristic of the sesquihydrate crystal form after dehydration (see section 4.2.3).

Figure 6.7 shows the DSC thermogram of EP05D. This thermogram exhibited only one peak, an endotherm at 189.46°C, caused by the melting of the sample. No desolvation or dehydration peaks were present in the thermogram, which indicated that this was an anhydrous crystal form.

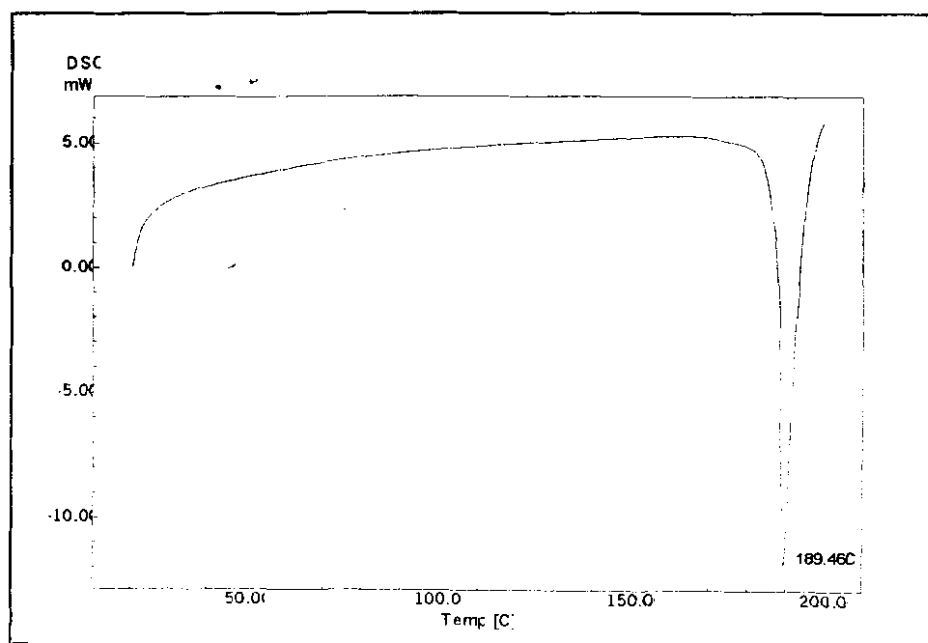


Figure 6.7 DSC thermogram of EP05D.

6.2.1.4 Thermogravimetric analysis (TGA) & Karl Fischer titration (KF)

The TGA thermogram of EP05W is shown in figure 6.8.

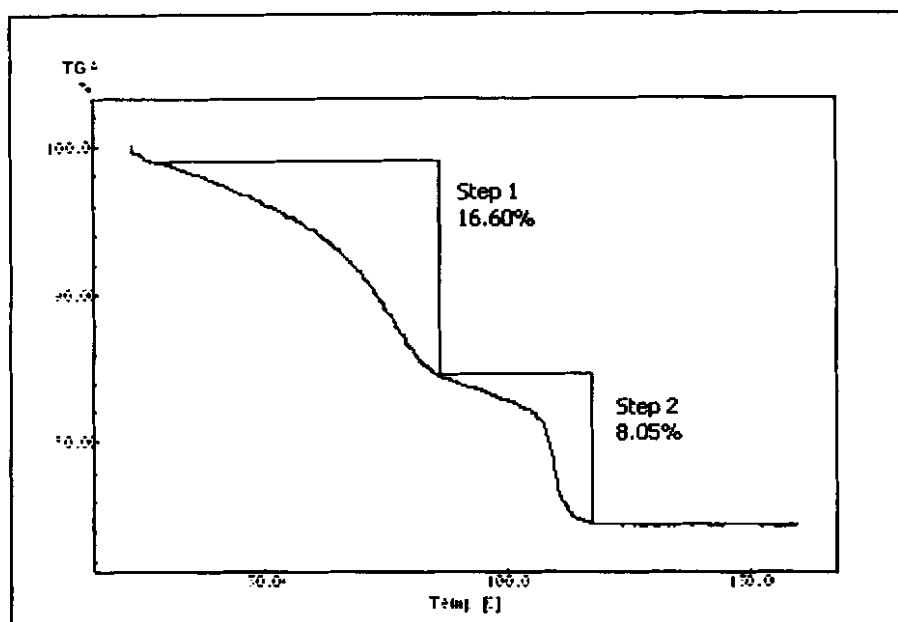


Figure 6.8 TGA thermogram of EP05W indicating the two weight loss steps.

The thermogram of EP05W showed that the weight loss of this form occurred in two steps. The first step was caused by the desolvation of the DMF solvate (1:1) fraction and the second step was due to the dehydration of the sesquihydrate fraction of the crystals. Table 6.3 displays the % weight loss of EP05W with each step in the TGA thermogram.

Table 6.3 TGA results of EP05W indicating the % weight loss of each step

% Weight loss (TGA)	EP05W
Step 1	16.60
Step 2	8.05
Total	24.65

Table 6.4 displays the total % weight loss of EP05W and EP05D (determined by TGA) and the moisture content of these two forms (determined by KF).

Table 6.4 TGA and KF results of EP05W and EP05D

	% Weight loss (TGA)	% Moisture content (KF)
EP05W	24.65	7.29
EP05D	0.22	0.46

TGA and KF of EP05D showed that this form had no significant amount of solvent included into the crystal lattice and could therefore be classified as an anhydrous crystal form (table 6.4). The TGA thermogram of EP05D is shown in figure 6.9.

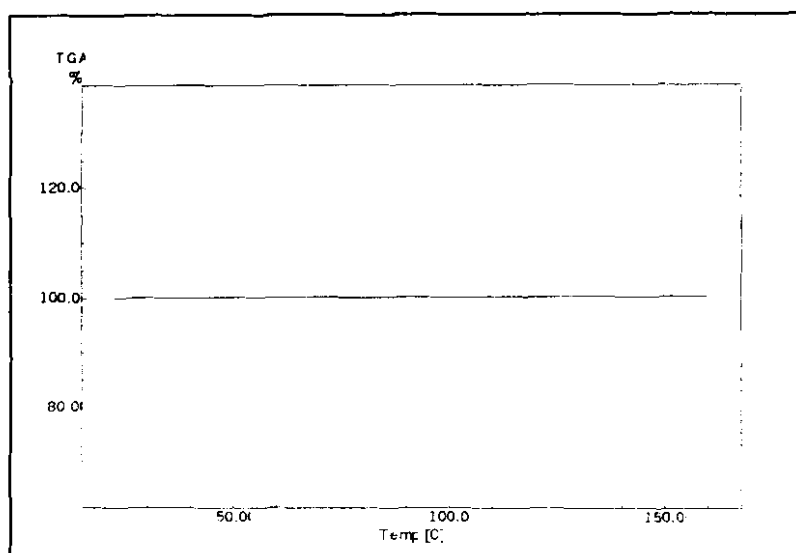


Figure 6.9 TGA thermogram of EP05D.

6.2.1.5 Microscopic analysis

Hot-stage microscopy (HSM) was performed on EP05W and EP05D to investigate the thermal behaviour of these forms. A summary of the HSM observations of EP05W is shown in table 6.5.

Table 6.5 Summary of HSM observations of EP05W in the temperature range of 25-187°C

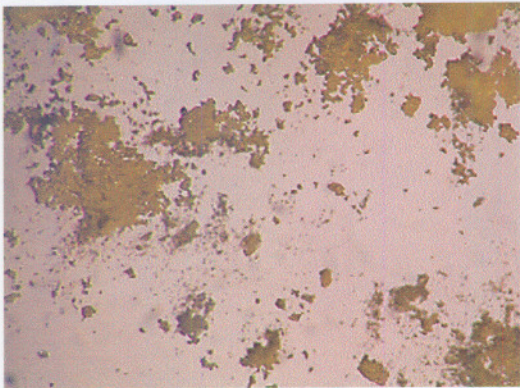
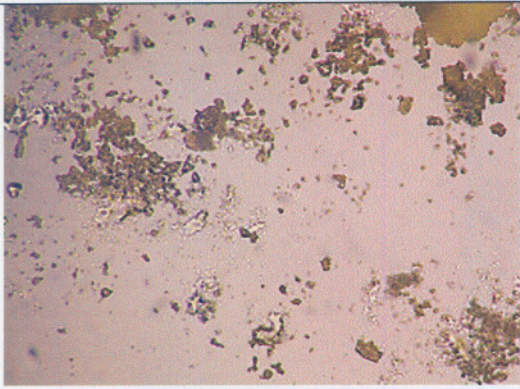
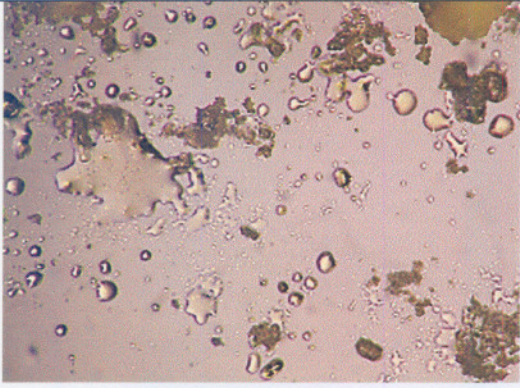
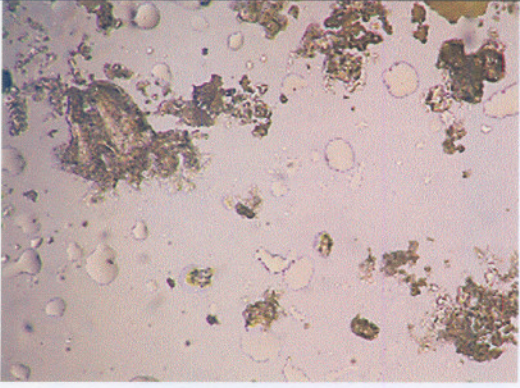
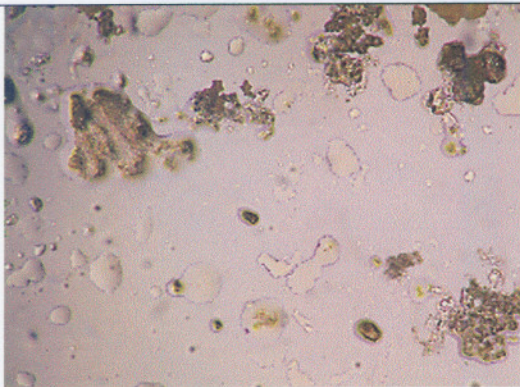
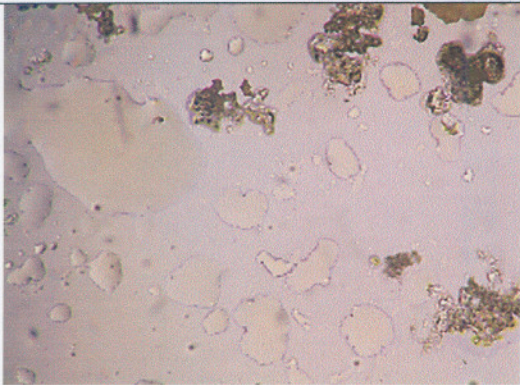
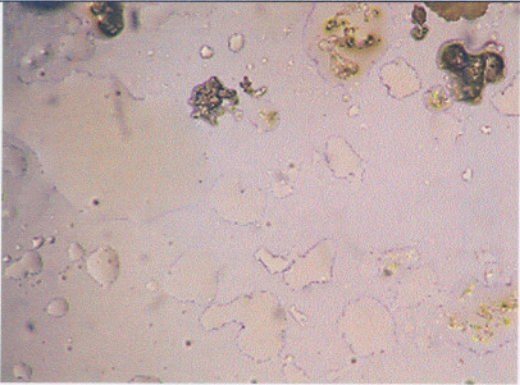
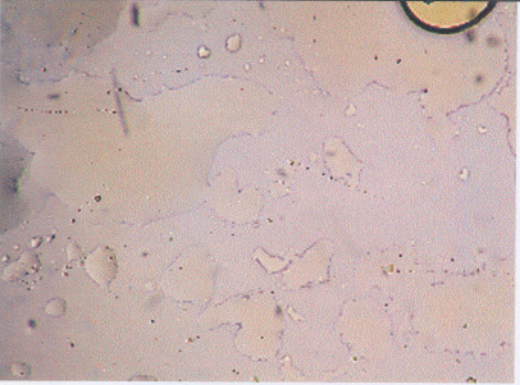
Temperature (°C)	Photomicrograph	Remarks
25		EP05W crystals at room temperature.
111		Melting of one fraction of the crystals started.
115		Recrystallisation occurred from the melted fraction.
120		Recrystallisation product.

Table 6.5 (continued)

Temperature (°C)	Photomicrograph	Remarks
170		Melting of the recrystallised product started.
180		Melting of recrystallised product completed.
184		Melting of the second fraction of crystals started.
187		Melting completed.

The melting and recrystallisation of the hydrated fraction at approximately 165°C was not clearly visible in the photomicrographs most likely due to the fact that only a small fraction of the hydrate was present in the sample.

The crystals of EP05W became turbid and bubble formation occurred when heated (submerged in silicon oil), which indicated that desolvation and dehydration of the crystals occurred. Table 6.6 displays the darkening and bubble formation of EP05W when submerged in silicon oil.

Table 6.6 HSM observations of EP05W when submerged in silicon oil

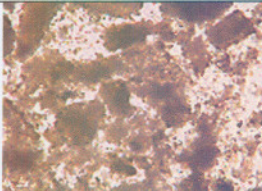
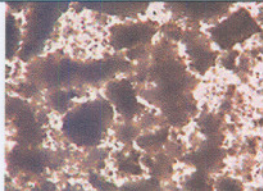
25°C	90°C	108°C
		
EP05W crystals at room temperature.	Darkening of the crystals indicating desolvation of one fraction of the crystals.	Bubble formation indicating dehydration of the second fraction of crystals.

Table 6.7 lists the important events that occurred with HSM of EP05D.

Table 6.7 Summary of HSM observations of EP05D in the temperature range of 25-189°C

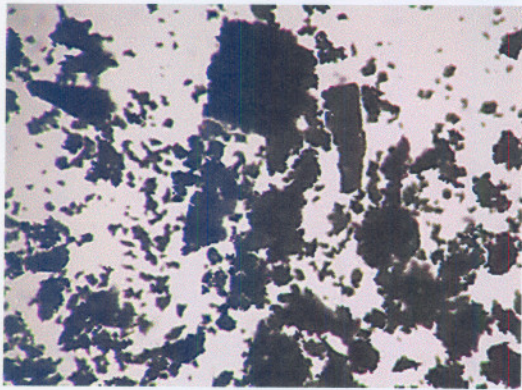
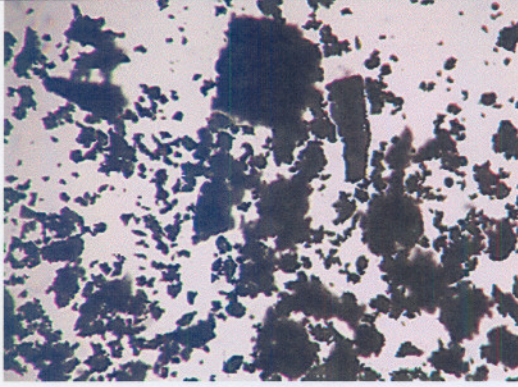
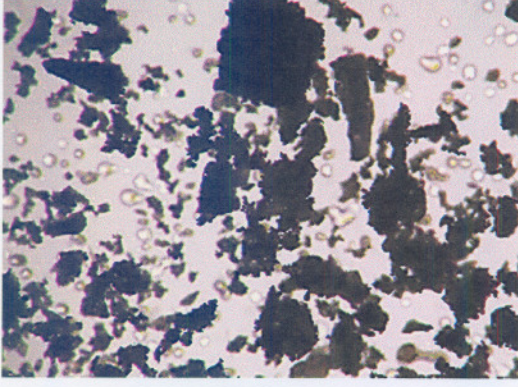
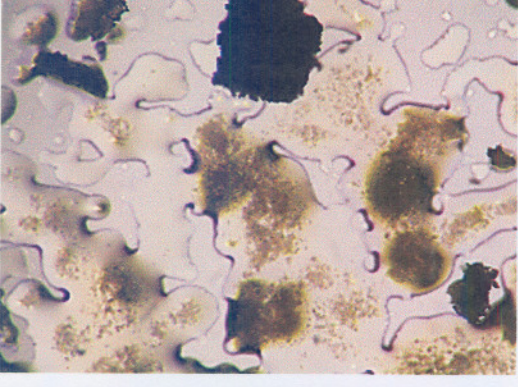
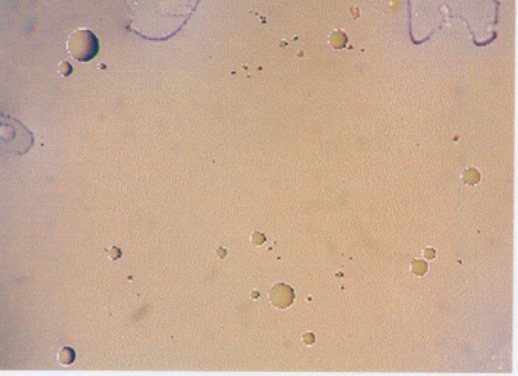
Temperature (°C)	Photomicrograph	Remarks
25		EP05D crystals at room temperature.

Table 6.7 (continued)

Temperature (°C)	Photomicrograph	Remarks
100		No change was observed.
184		Melting of the crystals started.
185		Melting continued.
189		Melting completed.

The dark colour of the crystals of EP05D indicated that these crystals were already desolvated. The HSM photomicrographs of EP05D confirmed the melting of the crystals at a temperature of approximately 189°C.

Scanning electron microscopy (SEM) analysis was performed on EP05W and EP05D to study the morphology of the crystals. Figure 6.10 and figure 6.11 shows the photomicrographs obtained for these two forms.

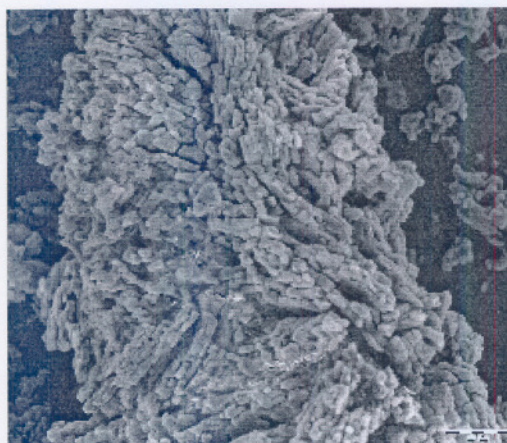


Figure 6.10 SEM photomicrograph of EP05W.



Figure 6.11 SEM photomicrograph of EP05D.

No significant differences were observed between the morphology of the crystals of EP05W and EP05D. The crystals of both forms were shapeless and showed a tendency to agglomerate.

6.2.1.6 Dissolution studies

Powder dissolution studies were performed on sieved fractions (106-250 μm) of the crystals of EP05W and EP05D to ensure particle size uniformity. The dissolution results of EP05W and EP05D are summarised in table 6.8 and a graphic comparison of the dissolution profiles of these two forms is displayed in figure 6.12. The average % dissolved for EP05W after 7.5 minutes was 99.0%, and this remained the same for the entire dissolution. At the time point 7.5 minutes, EP05D was 90.0% dissolved and a final value at time point 30 minutes was 98.0%. Both forms complied with an in-house criteria set for the dissolution of gatifloxacin (more than 75% of the sample should be dissolved after 30 minutes).

Table 6.8 Dissolution results of EP05W and EP05D in 0.01 N HCl at $37\pm0.2^\circ\text{C}$

Dissolution vessel	% Dissolved					
	7.5 minutes		15 minutes		30 minutes	
	EP05W	EP05D	EP05W	EP05D	EP05W	EP05D
1	101.8	102.1	101.6	102.7	101.3	102.3
2	101.6	89.3	101.2	92.9	101.4	96.0
3	100.7	83.5	101.1	88.1	100.8	94.2
4	90.9	97.6	90.9	98.8	91.5	100.2
Average	99 $\pm$ 5.3	93 $\pm$ 8.3	99 $\pm$ 5.2	96 $\pm$ 6.4	99 $\pm$ 4.8	98 $\pm$ 3.7

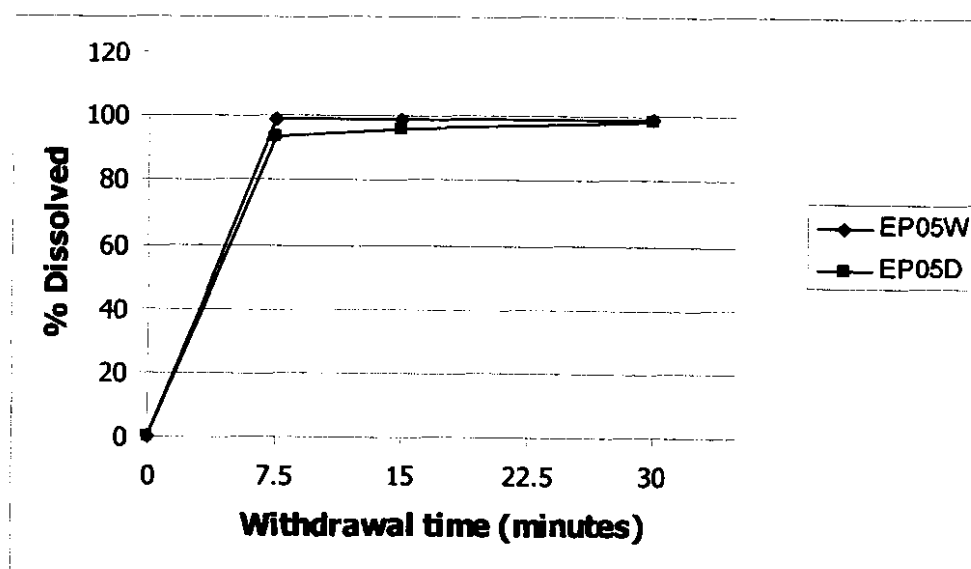


Figure 6.12 Dissolution profiles of EP05W and EP05D in 0.01 N HCl at $37\pm0.2^\circ\text{C}$.

A comparison of the dissolution profiles of EP05W and EP05D was made using the f_2 value (similarity factor described by Moore and Flanner (1996:65)). The dissolution profile of EP05D was used as reference in the calculation of the f_2 value of EP05W. According to the guidelines of the Medicines Control Council, for the calculation of the f_2 value only one measurement should be considered after 85% dissolution of both samples have occurred (Medicines Control Council, 8:2003). The dissolution profiles of the two samples may be regarded similar if >85% dissolution occurred for both samples. Thus the f_2 value (similarity factor) need not be calculated to determine the similarity between the dissolution profiles of these two forms. However, for the sake of completeness the f_2 value (similarity factor) of EP05W was calculated and included here.

The f_2 value of EP05W was calculated as 59.59. Seeing that this value is greater than 50, the two dissolution profiles could be considered to be similar. The dissolution profiles of EP05W and EP05D were also compared to the dissolution profile of the sesquihydrate. The similarity factors (f_2 values) of EP05W and EP05D were calculated using the dissolution profile of the sesquihydrate as reference in the calculation. The f_2 values of EP05W and EP05D are shown in table 6.9.

Table 6.9 f_2 values for EP04W and EP04D using the dissolution profile of the sesquihydrate as reference

Crystal form	f_2 values
EP05W	82.53
EP05D	61.82

According to Moore and Flanner (1996:65) an f_2 value of >50 indicates similarity between the dissolution curves. The dissimilarities increase as the f_2 value decreases. The comparison of EP05W and EP05D with the sesquihydrate raw material revealed that these two forms displayed similar dissolution profiles to that of the sesquihydrate, seeing that both forms had values of >50.

6.2.1.7 Solubility studies

The solubility values of EP05W and EP05D are listed in table 6.10. The solubility of both EP05W and EP05D ranged in the order: 0.01 N HCl > acetate buffer > MilliQ

water > phosphate buffer. Both EP05W and EP05D displayed significantly better solubility in the 0.01 N HCl and acetate buffer than in the MilliQ water and phosphate buffer.

Table 6.10 Solubility values (in mg/ml) of EP05W and EP05D in various media at $37\pm 2^{\circ}\text{C}$

Medium	pH	EP05W		EP05D	
		Solubility (mg/ml)	%CV	Solubility (mg/ml)	%CV
0.01 N HCl	2.12	3.64	0.21	5.02	0.48
Acetate buffer	4.52	3.54	0.85	5.00	0.24
MilliQ Water	5.65	2.13	2.43	3.09	1.96
Phosphate buffer	6.81	2.12	0.42	2.90	2.71

A graphic comparison of the solubility profiles of EP05W and EP05D is displayed in figure 6.13.

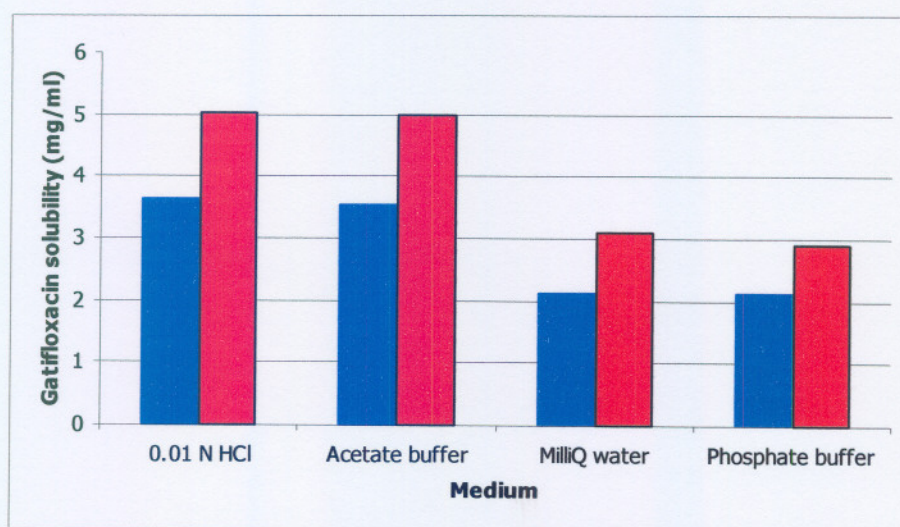


Figure 6.13 Solubility profiles of EP05W (blue) and EP05D (red) in various media at $37\pm 2^{\circ}\text{C}$

6.2.1.8 Flowability

The flow properties of EP05W and EP05D were investigated using the method described in chapter 3. Tables 6.11 and 6.12 list the results of the flowability study for EP05W and EP05D.

Table 6.11 Initial density (D_0) and final density (D_f) measurements of EP05W and EP05D

Sample	D_0 (g/ml)		D_f (g/ml)	
	EP05W	EP05D	EP05W	EP05D
1	0.29	0.22	0.45	0.41
2	0.28	0.23	0.47	0.41
3	0.27	0.23	0.47	0.41
Average	0.28±0.01	0.23±0.01	0.46±0.01	0.41±0.00

Table 6.12 Hausner ratio and Carr's index of EP05W and EP05D

Sample	Hausner ratio		Carr's index	
	EP05W	EP05D	EP05W	EP05D
1	1.57	1.83	36.11	45.24
2	1.65	1.75	39.47	42.86
3	1.77	1.75	43.59	42.86
Average	1.66±0.10	1.78±0.05	39.72±3.75	43.65±1.37

A Hausner ratio of 1.2 is indicative of good flowability and a ratio of >1.6 indicates that the sample exhibits poor flowability. The Hausner ratio for EP05W and EP05D was 1.66 and 1.78 respectively. The Hausner ratios of both forms indicated that these forms exhibited poor flowability. Carr's index was interpreted using table 3.2. According to table 3.2 powders with a Carr's index of 35-38 have very poor flowability, while powders with a Carr's index of >40 exhibits extremely poor flowability. EP05W had a Carr's index of 39.72 and EP05D a Carr's index of 43.65, which indicated that EP05W had very poor flow and EP05D displayed extremely poor flowability.

6.2.2 EP06W & EP06D

Chloroform was used as solvent for the recrystallisation of EP06W. This form was recrystallised by means of the method described in section 6.1. EP06D was obtained from drying EP06W in an Ecotherm drying oven (Labotec, South Africa) at 110°C for 12 hours.

6.2.2.1 X-ray powder diffraction (XRPD) and variable temperature x-ray powder diffraction (VT-XRPD)

Figure 6.14 displays an overlay of the VT-XRPD patterns of EP06W. At first glance the variable temperature x-ray powder diffractograms of EP06W revealed no major changes at increased temperatures. Closer inspection of the diffractograms revealed the following slight changes: the two peaks at $8.9^{\circ}2\theta$ and $9.5^{\circ}2\theta$ as well as the peaks at $12.9^{\circ}2\theta$ and $13.9^{\circ}2\theta$ shifted towards each other and started to overlap with an increase in temperature. At 45°C a peak appeared at $16.1^{\circ}2\theta$ that increased in intensity with an increase in temperature. The peak in the area of $18.5\text{--}20.0^{\circ}2\theta$ showed a sharp increase in intensity. The peak at $24.3^{\circ}2\theta$ slowly shifted to the right (towards $35.0^{\circ}2\theta$) and displayed an increase in peak shape and intensity. The peaks in the area $25\text{--}28^{\circ}2\theta$ became more pronounced with an increase in relative intensity, the peaks in this area also shifted slightly to the right of the diffractogram (towards $35.0^{\circ}2\theta$).

The most remarkable change in the XRPD patterns (figure 6.14) was an increase in the intensities of all peaks. Desolvation of the crystals caused the formation of a more crystalline crystal structure (as indicated by the increase in total counts). The removal of the relatively bulky solvent (chloroform) from the crystal lattice led to the formation of a more densely packed crystal structure that was more crystalline than the solvated crystal form.

At 160°C a slight decrease in the total counts was observed, followed by a melting XRPD pattern at 170°C .

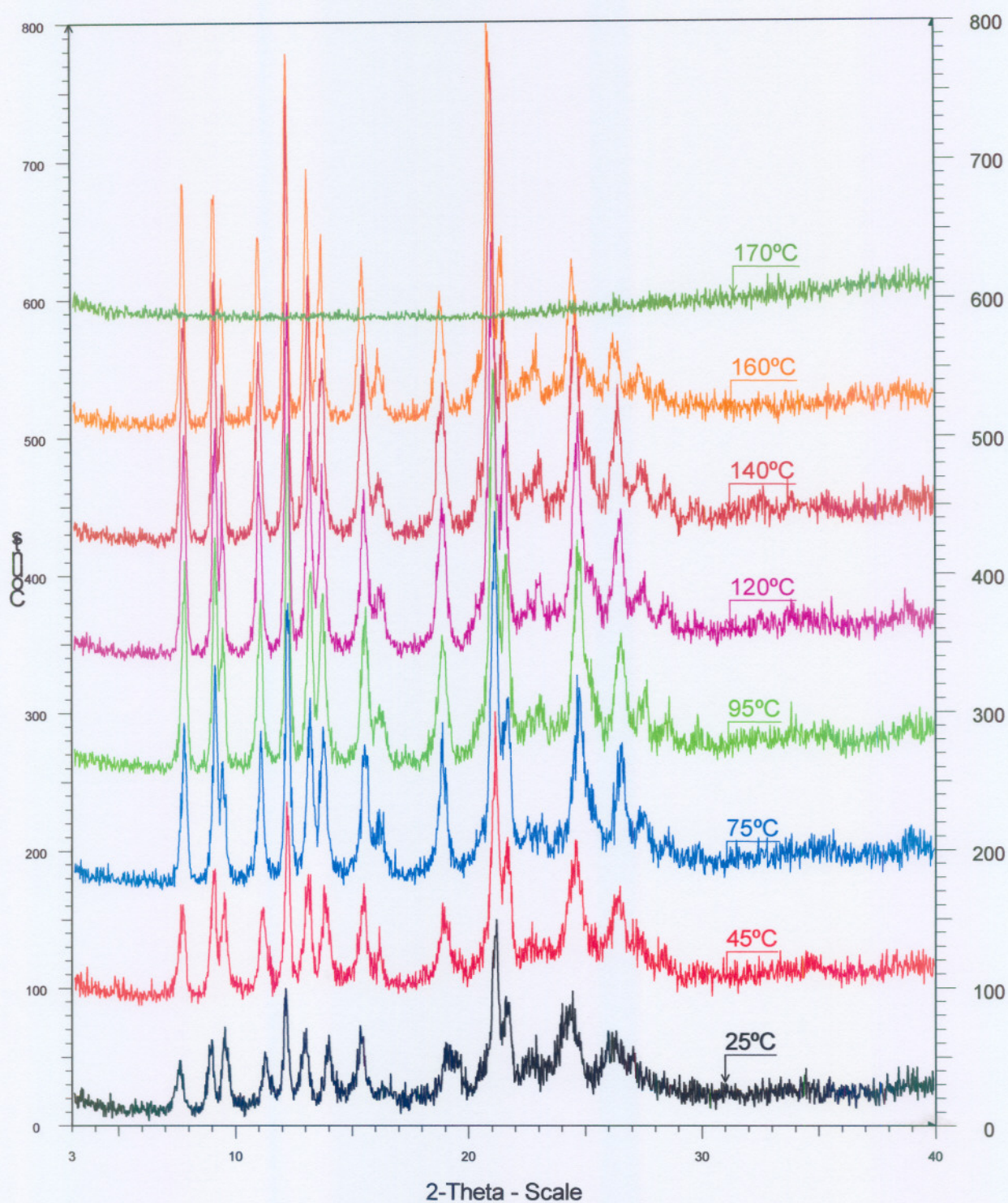


Figure 6.14 Variable temperature x-ray powder diffractograms of EP06W.

EP06W was classified as a chloroform solvate (1:1). Currently no chloroform solvates have been described in the literature. Comparison of EP06W with the available forms

in the literature indicated that EP06W displayed resemblances to the XRPD pattern of the crystalline form T1 recrystallised from acetonitrile (see section 2.4.4 for a discussion of form T1). A general overview of the diffractograms of form T1 and EP06W revealed similarities between these two forms with the exception of the absence of the peak at $26.0^{\circ}2\theta$ in the diffractogram of EP06W. EP06W was classified as a new pseudopolymorph of gatifloxacin, seeing that this form was a chloroform solvate and currently no chloroform solvates have been reported in the literature.

The variable temperature XRPD patterns of EP06D are shown in figure 6.15. No major differences were observed between the VT-XRPD patterns of EP06D. Slight changes such as the appearance of a peak at $26.4^{\circ}2\theta$ was observed in the XRPD pattern at 45°C . Peaks with weak intensities such as the peaks at $8.9^{\circ}2\theta$ and $9.3^{\circ}2\theta$ and the peak at $20.9^{\circ}2\theta$ disappeared in the temperature range of $140\text{--}160^{\circ}\text{C}$ which signified the onset of the melting process of the sample. At 170°C a characteristic melting XRPD pattern was observed.

EP06D was found to be an anhydrous crystal form. Comparison of EP06D with the anhydrous forms described in the available literature indicated similarities between this form and the anhydrous form omega and form I (see sections 2.4.2-2.4.3 for a discussion of these forms). The diffractograms of form omega and form I displayed similarities and most of the characteristic peaks of both these forms were present in the diffractogram of EP06D. Characteristic peaks of these forms that were absent in the diffractogram of EP06D included the following peaks at: $27.6^{\circ}2\theta$, $28.7^{\circ}2\theta$ and $30.5^{\circ}2\theta$.

The initial diffractogram of EP06D (at 25°C) differed from that of the desolvated EP06W. Major differences included the appearance of peaks at $10.1^{\circ}2\theta$, $12.8^{\circ}2\theta$, $14.0^{\circ}2\theta$ and $14.9^{\circ}2\theta$ on the diffractogram of EP06D. Peaks that appeared in the diffractogram of the desolvated EP06W (at 120°C) and not in the diffractogram of EP06D included the peaks at $13.1^{\circ}2\theta$, $15.4^{\circ}2\theta$, $18.8^{\circ}2\theta$ and $24.7^{\circ}2\theta$. The peaks that appeared in the diffractogram of EP06D at $19.6^{\circ}2\theta$, $20.4^{\circ}2\theta$ and $25.8^{\circ}2\theta$ was absent in the diffractogram of the desolvated EP06W. Figure 6.16 reveals an overlay of the desolvated EP06W (at 120°C) and EP06D (at 25°C).

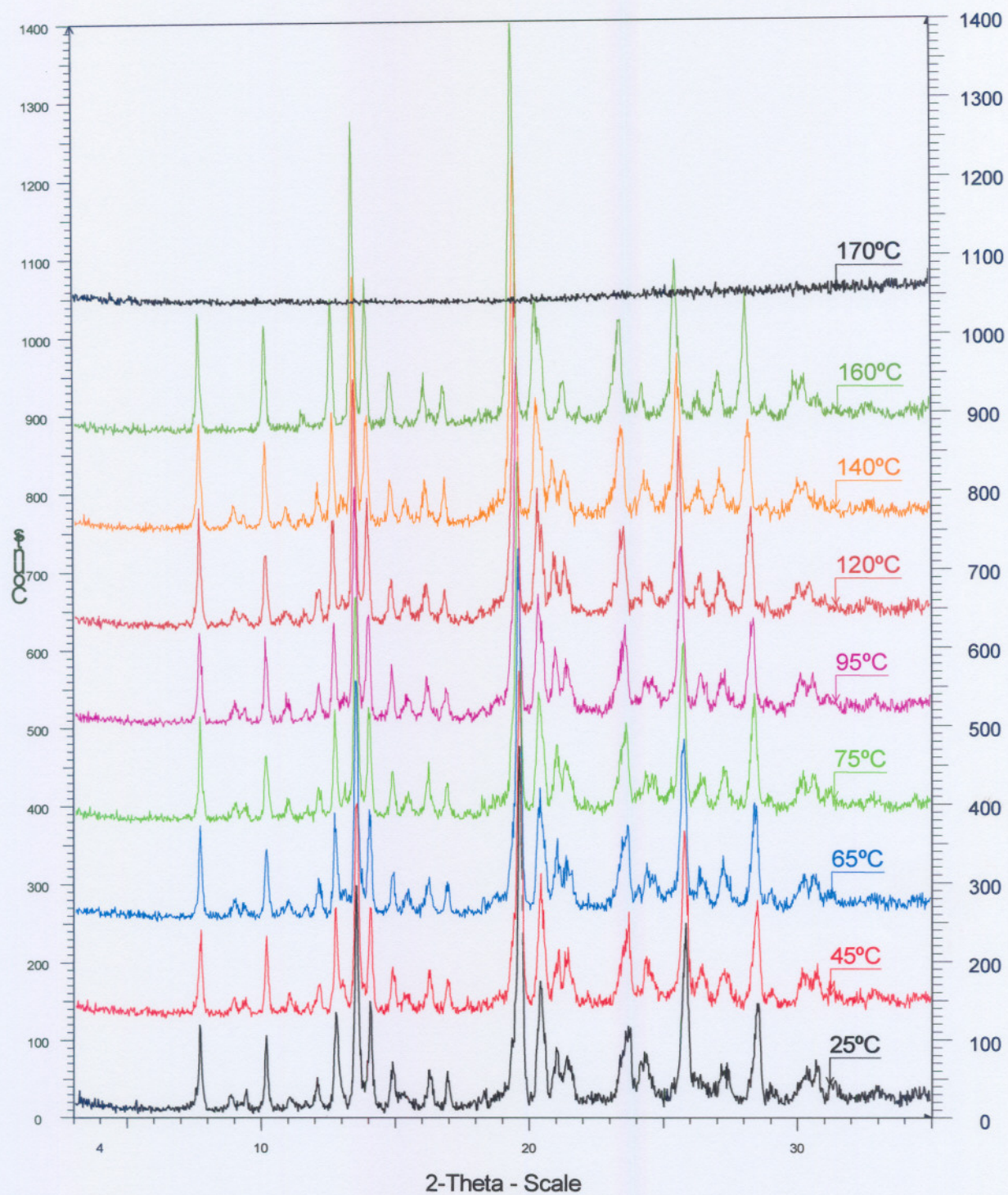


Figure 6.15 Variable temperature x-ray powder diffractograms of EP06D.

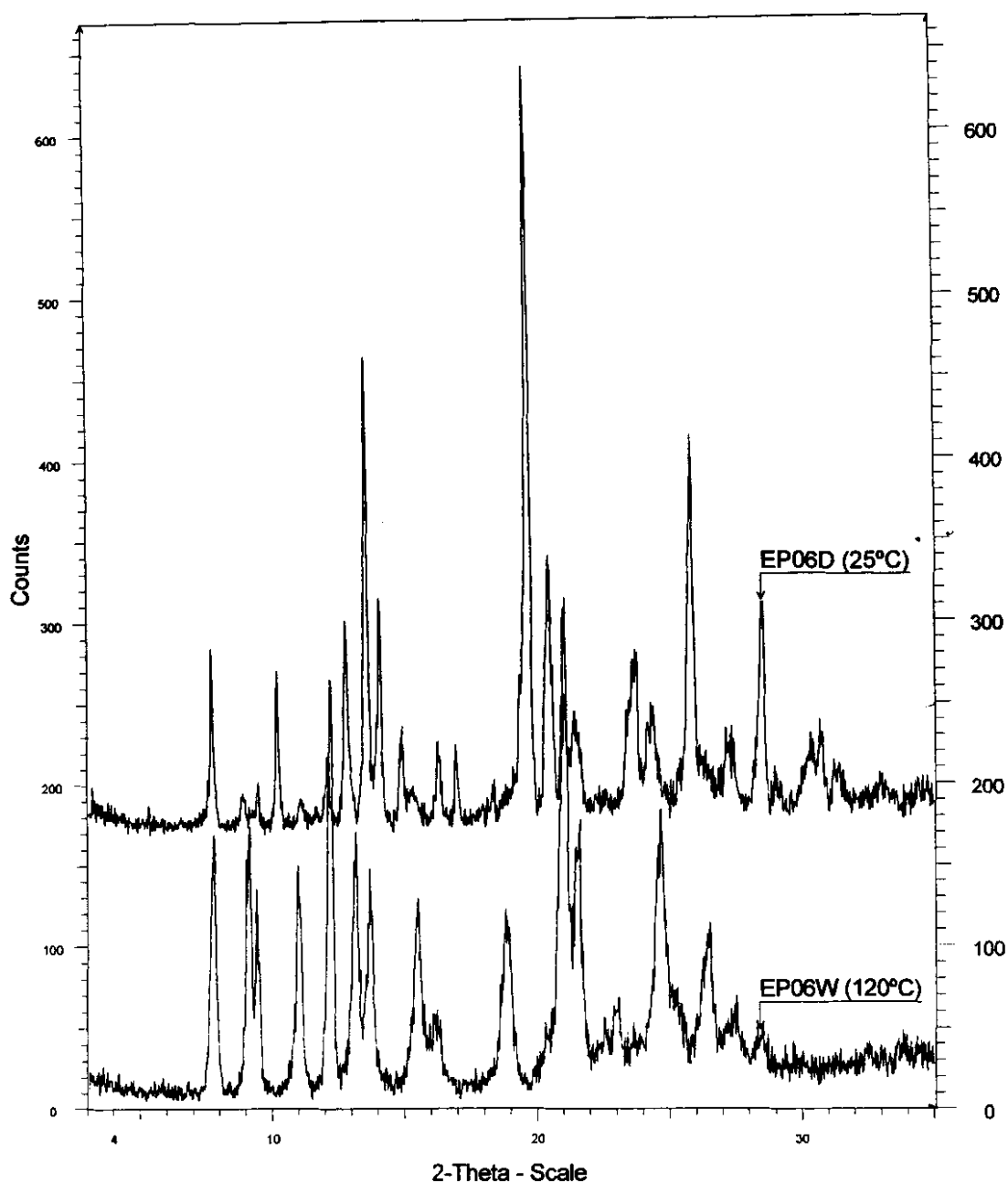


Figure 6.16 Overlay of the x-ray powder diffractograms of EP06W (at 120°C) and EP06D (at 25°C).

6.2.2.2 Infrared spectroscopy (IR)

Table 6.13 lists the main absorptions in the IR spectra of EP06W and EP06D. All the differences between the DRIFT-IR spectra of these two forms are highlighted in the mentioned table. An overlay of the spectra of these two forms is shown in figure 6.17.

Table 6.13 Main absorptions in the DRIFT-IR spectra of EP06W and EP06D

Main absorptions	Wavelength (cm ⁻¹)	
	EP06W	EP06D
1	3412	-
2	-	3328
3	2986	-
4	-	2944
5	2837	-
6	-	2812
7	2448	-
8	1727	-
9	-	1721
10	1647	1647
11	-	1616
12	1542	1541
13	1447	1447
14	1397	1395
15	-	1350
16	1318	1319
17	1277	1277
18	-	1242
19	1214	1217
20	-	1184
21	1176	-
22	-	1153
23	1144	-
24	1115	1115
25	1095	-
26	1070	-
27	1055	1058
28	1037	-
29	1002	1000
30	941	941
31	914	-
32	887	887
33	-	870
34	845	847
35	-	834
36	821	821
37	-	805
38	-	782
39	776	-
40	735	735
41	-	700
42	687	686
43	595	594
44	571	573
45	549	546
46	-	523
47	517	-
48	-	503
49	486	485
50	-	472
51	-	438
52	403	-

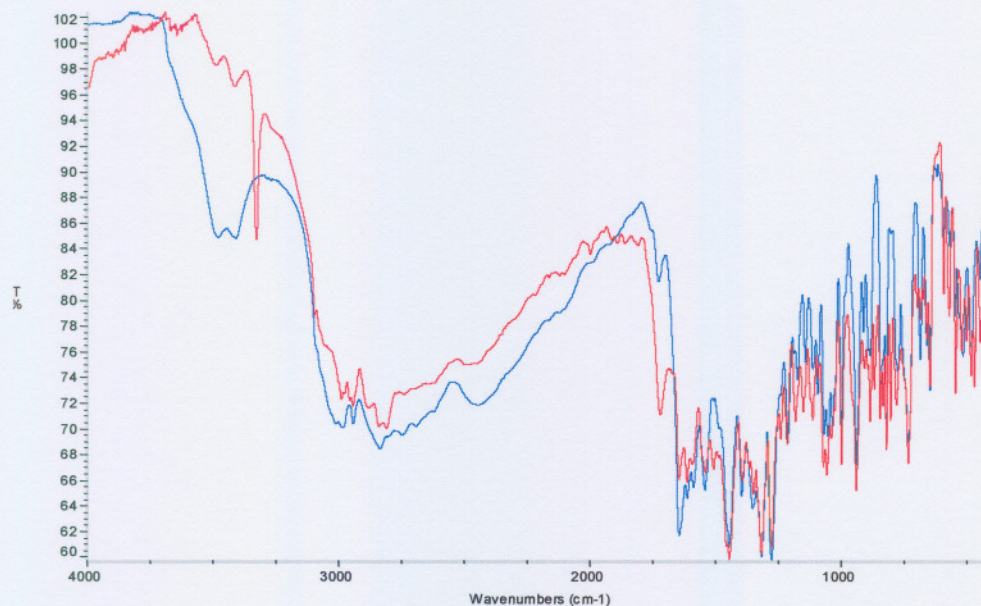


Figure 6.17 Overlay of the DRIFT-IR spectra of EP06W (blue) and EP06D (red).

6.2.2.3 Differential scanning calorimetry (DSC)

The DSC thermogram of EP06W is displayed in figure 6.18. The endotherm at 88.79°C was representative of desolvation. An endotherm present at 166.05°C was due to the melting of the crystals. This melting endotherm was followed by a recrystallisation exotherm at 169.56°C (these events were confirmed by thermal microscopy in section 5.2.2.5). The final melting endotherm of the recrystallised product was observed at 188.64°C.

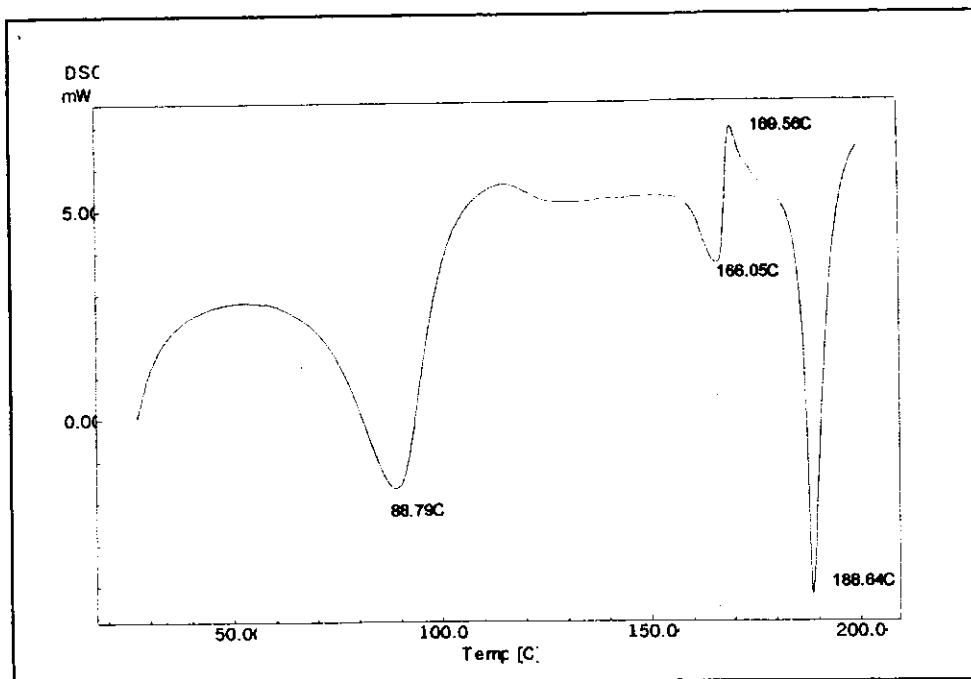


Figure 6.18 DSC thermogram of EP06W.

Figure 6.19 displays the DSC thermogram of EP06D. An endotherm was present at 187.32°C, which indicated the melting of the sample.

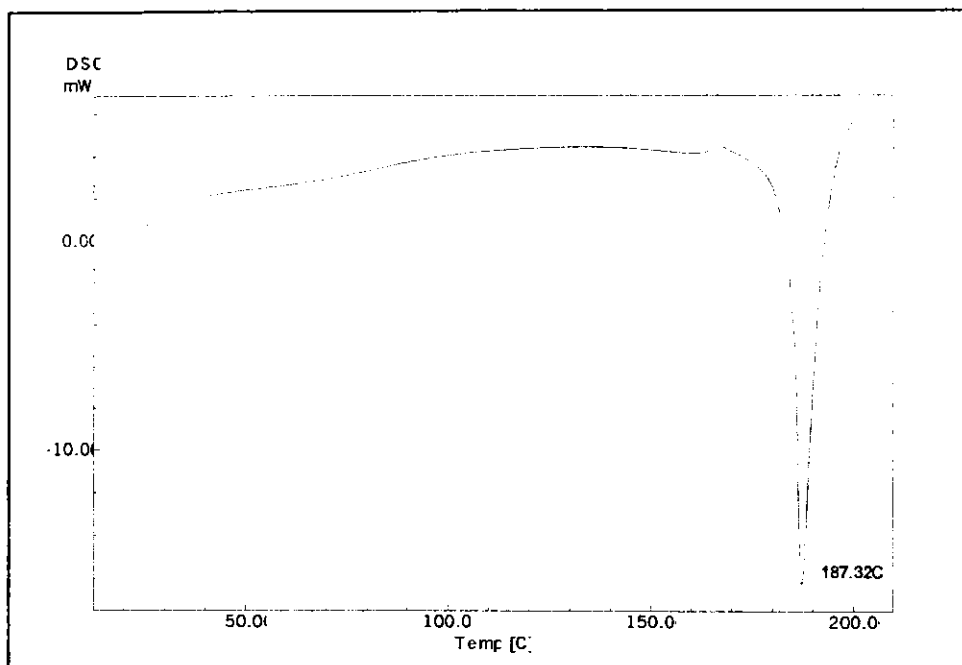


Figure 6.19 DSC thermogram of EP06D.

6.2.2.4 Thermogravimetric analysis (TGA) & Karl Fischer titration (KF)

The TGA thermograms of EP06W and EP06D are displayed in figure 6.20. The TGA thermogram of EP06W displayed two steps of weight loss. The first step showed a weight loss of 28.44% with an onset temperature at 46.31°C and an endset temperature at 68.10°C. This correlated with the boiling point of chloroform, which is 61.5°C. The weight loss of 28.44% was comparable with the theoretical weight loss of a chloroform solvate (1:1), which is 24.13%. The slight difference between the observed weight loss and the theoretical weight loss of a chloroform solvate (1:1) could possibly be due to the presence of some surface solvent on the sample.

The second step in the TGA curve of EP06W displayed a weight loss of 1.78%, which correlated with the KF moisture determination of 1.63%. No dehydration endotherm was observed in the DSC thermogram (figure 6.18) and hot-stage microscopy did not reveal any bubble formation that could indicate dehydration. It can be derived that this form is a chloroform solvate (1:1) and that the second step of weight loss was due to the loss of surface moisture. Table 6.14 displays the % weight loss of EP06W with each step in the TGA thermogram.

Table 6.14 TGA results of EP06W indicating the % weight loss of each step

% Weight loss (TGA)	EP05W
Step 1	28.44
Step 2	1.78
Total	30.22

The TGA and KF results of EP06W and EP06D are tabulated in table 6.15.

Table 6.15 TGA and KF results of EP06W and EP06D

	% Weight loss (TGA)	% Moisture content (KF)
EP06W	30.22	1.63
EP06D	0.11	0.89

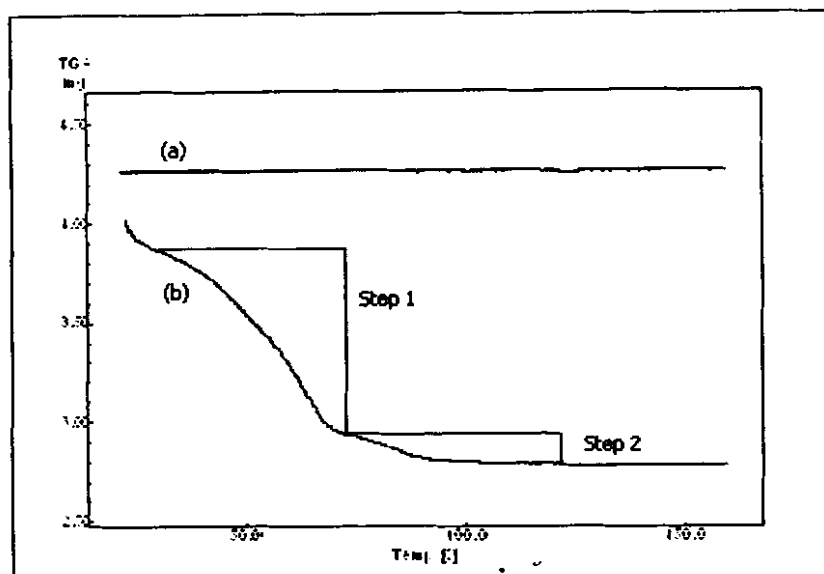


Figure 6.20 TGA thermograms of EP06D (a) and EP06W, indicating the two weight loss steps (b).

TGA revealed no significant weight loss for EP06D and KF confirmed that the moisture content of the sample was ommissible (table 6.15). EP06D can be classified as an anhydrous crystal form.

6.2.2.5 Microscopic analysis

Hot-stage microscopy (HSM) was performed on samples of EP06W and EP06D to study the thermal behaviour of these two crystal forms. Table 6.16 and table 6.17 summarises the HSM observations of these two crystal forms.

Table 6.16 Summary of HSM observations of EP06W in the temperature range of 25-188°C when submerged in silicon oil

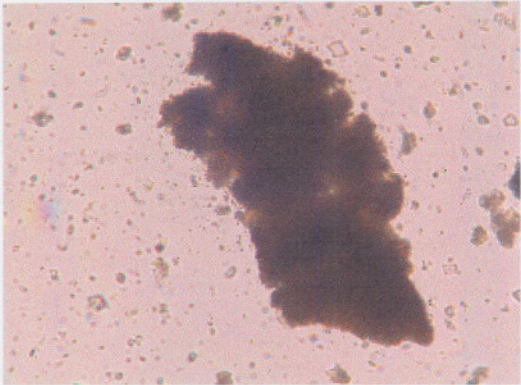
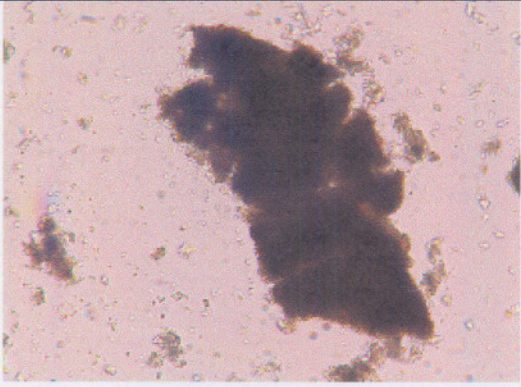
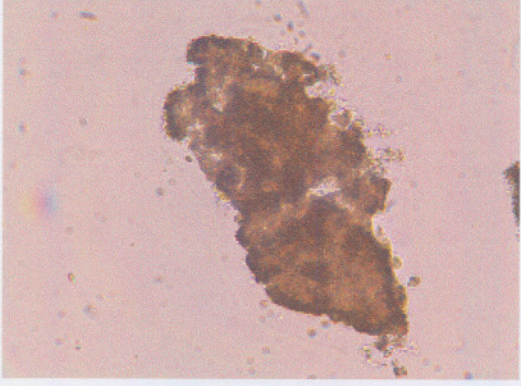
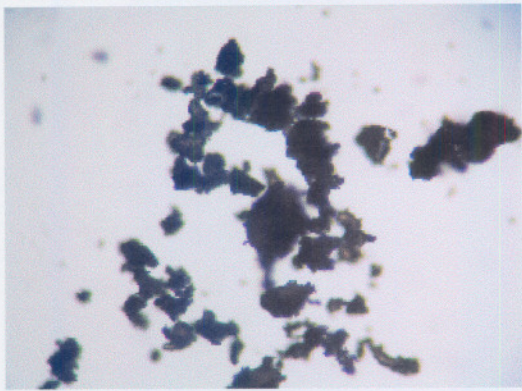
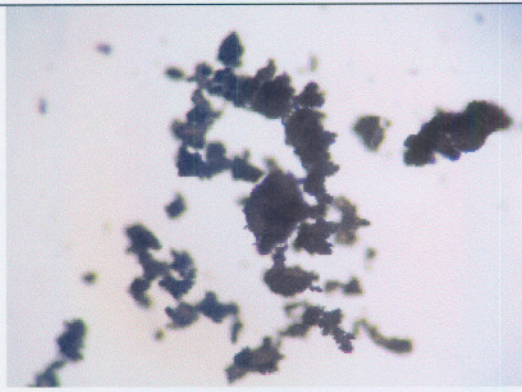
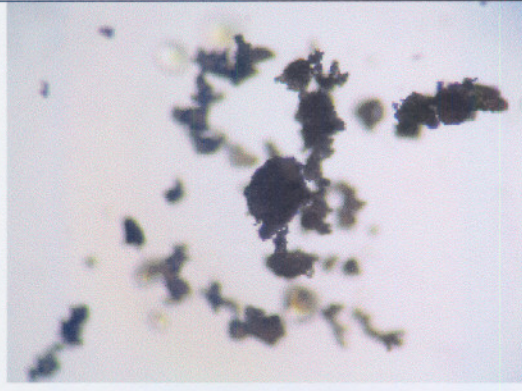
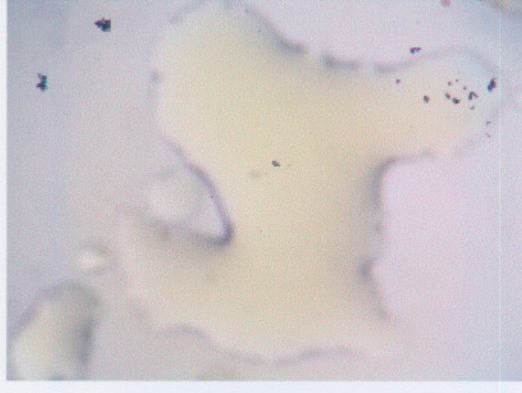
Temperature (°C)	Photomicrograph	Remarks
25		Crystals of EP06W at room temperature.
90		Slight darkening of the crystals occurred in the temperature range 70-100°C, which suggested desolvation.
167		Melting of the crystals occurred at 164°C.
169		Recrystallisation from the melt occurred at 169°C.

Table 6.16 (continued)

Temperature (°C)	Photomicrograph	Remarks
170		Recrystallisation product.
181		Melting of the recrystallised product occurred in the temperature range of 179-188°C.
188		Melting of the recrystallised product was completed at 188°C.

No bubble formation was observed with heating of EP06W when submerged in silicon oil and only a slight darkening of the crystals indicated desolvation.

Table 6.17 Summary of HSM observations of EP06D in the temperature range of 25-187°C

Temperature (°C)	Photomicrograph	Remarks
25		EP06D crystals at room temperature.
132		No change in the crystals was observed.
184		Melting of the crystals started.
187		Melting completed.

The HSM photomicrographs of EP06D confirmed the final melting point of the crystal form at 184-187°C.

Scanning electron microscopy (SEM) was utilised to investigate the morphology of EP06W and EP06D. Figures 6.21 and 6.22 displays SEM photomicrographs of EP06W and EP06D.



Figure 6.21 SEM photomicrograph of EP06W

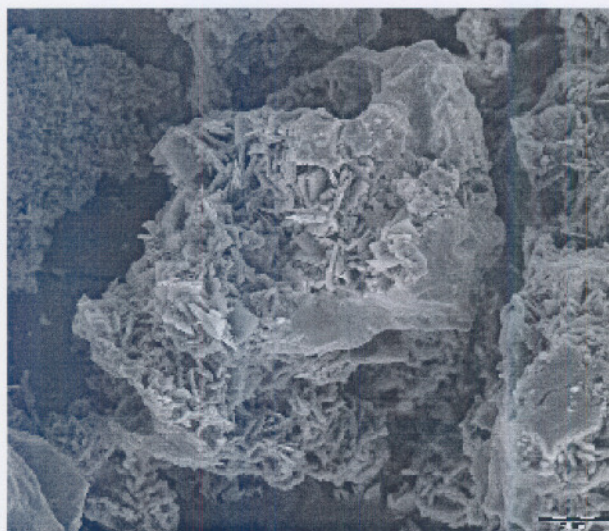


Figure 6.22 SEM photomicrograph of EP06D

EP06W displayed small plate-like crystals and the crystals of EP06D were shapeless. The crystals of both forms displayed a tendency to agglomerate.

6.2.2.6 Dissolution studies

Powder dissolution studies were performed on sieved fractions (106-250 μm) of the crystals to ensure particle size uniformity. The dissolution results of EP06W and EP06D are summarised in table 6.18 and an overlay of the dissolution profiles of these two forms is shown in figure 6.23. 83.0% of EP06W was dissolved after 7.5 minutes. This value remained unchanged for the duration of the dissolution study. This correlated with the solubility results obtained for EP06W in 0.01 N HCl, which indicated that only 80.8% of the initial mass of crystals used during the solubility study dissolved in the mentioned medium (see section 6.2.2.7). After 7.5 minutes 97.0% of EP06D was dissolved and after 30 minutes 100.0% of the sample of EP06D was dissolved. Both forms conformed to the in-house criteria for the dissolution of gatifloxacin (more than 75% of the sample should be dissolved within 30 minutes).

Table 6.18 Dissolution results of EP06W and EP06D in 0.01 N HCl at $37\pm0.2^\circ\text{C}$

Dissolution vessel	% Dissolved					
	7.5 minutes		15 minutes		30 minutes	
	EP06W	EP06D	EP06W	EP06D	EP06W	EP06D
1	86.8	92.0	86.6	94.9	87.1	96.9
2	86.6	96.1	86.3	98.9	86.4	100.4
3	83.7	96.0	84.8	99.2	84.4	100.8
4	78.4	99.8	78.6	100.9	78.6	101.3
5	81.4	101.1	81.2	101.1	80.9	101.5
Average	83.0 $\pm$ 3.6	97.0 $\pm$ 3.6	83.0 $\pm$ 3.5	99.0 $\pm$ 2.5	83.0 $\pm$ 3.6	100.0 $\pm$ 1.9

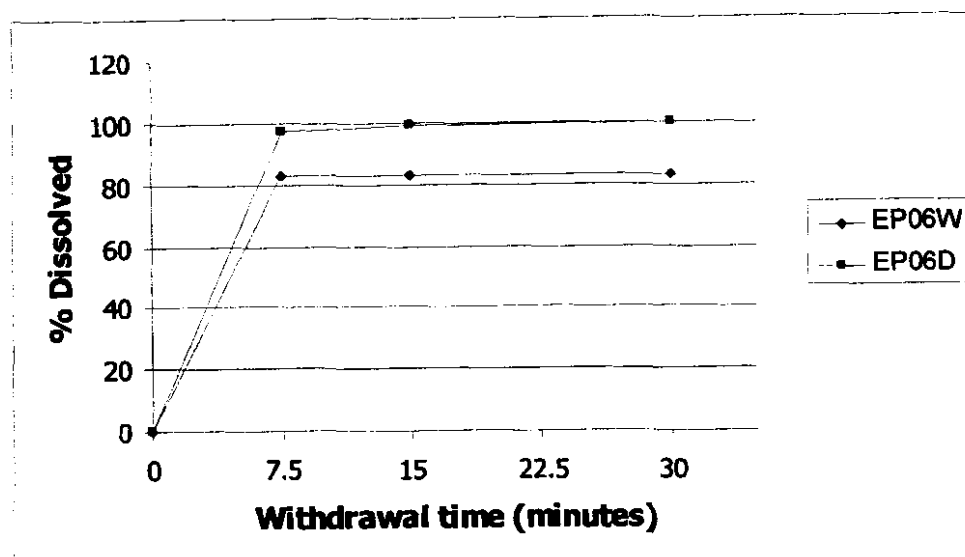


Figure 6.23 Dissolution profiles of EP06W and EP06D in 0.01 N HCl at 37±0.2°C.

The similarities between the dissolution profiles of EP06W and EP06D were determined using the equation described by Moore and Flanner (1996:65). An f_2 value of >50 indicates that the dissolution curves are similar. The f_2 value of EP06W was calculated using the dissolution profile of EP06D as reference.

The f_2 value of EP06W was calculated as 40.14, which indicated that the dissolution profiles of these two forms were not similar and displayed significant differences.

The dissolution profiles of EP06W and EP06D were compared to that of the sesquihydrate by calculating the f_2 values (similarity factor) of these forms using the dissolution profile of the sesquihydrate as reference. The f_2 values calculated for EP06W and EP06D are shown in table 6.19.

Table 6.19 f_2 values of EP06W and EP06D using the dissolution profile of the sesquihydrate form as reference

Crystal form	f_2 value
EP06W	37.20
EP06D	77.42

The f_2 value of EP06W was calculated as 37.20, which signified that the dissolution profile of EP06W and that of the sesquihydrate differed considerably and could not be considered to be similar. The f_2 value for EP06D was calculated as 77.42, which

indicated that the dissolution profiles of EP06D and the sesquihydrate could be considered to be similar.

6.2.2.7 Solubility studies

The solubility of EP06W and EP06D was determined in four media using the method described in chapter 3. The results for this study are tabulated in table 6.20 and figure 6.24 is a graphic comparison of the solubility profiles of these two forms. The solubility of EP06W ranged in the order: 0.01 N HCl > acetate buffer > phosphate buffer > MilliQ water. EP06D was more soluble than EP06W in all the media. The solubility of EP06D ranged in the following order: 0.01 N HCl > acetate buffer > MilliQ water > phosphate buffer.

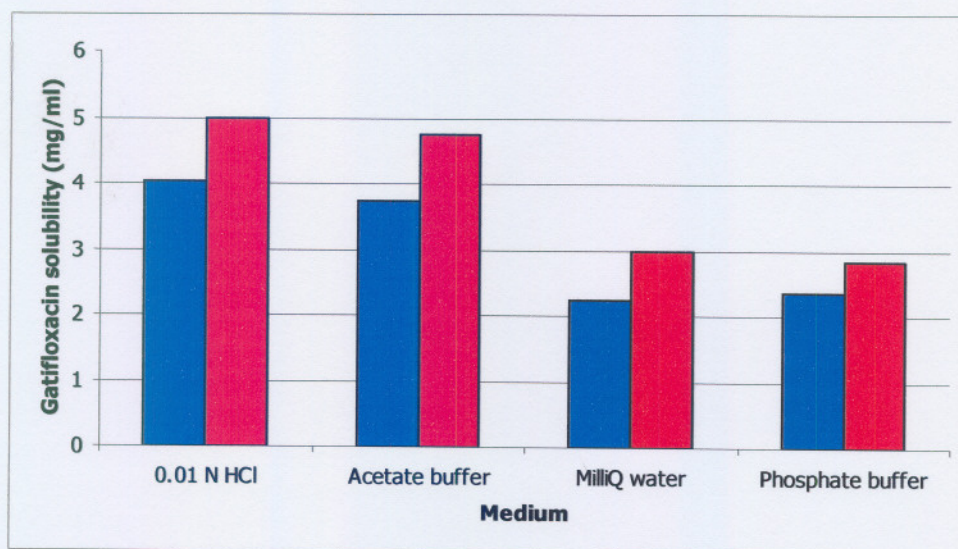


Figure 6.24 Solubility profiles of EP06W (blue) and EP06D (red) in various media at $37\pm 2^{\circ}\text{C}$.

Table 6.20 Solubility values (in mg/ml) of EP06W and EP06D in various media at $37\pm 2^{\circ}\text{C}$

Medium	pH	EP06W		EP06D	
		Solubility (mg/ml)	%CV	Solubility (mg/ml)	%CV
0.01 N HCl	2.25	4.04	3.00	4.99	2.25
Acetate buffer	4.53	3.73	3.21	4.74	1.70
MilliQ Water	5.57	2.24	2.04	2.99	0.49
Phosphate buffer	6.80	2.36	4.26	2.84	2.97

6.2.2.8 Flowability

The flowability of both EP06W and EP06D was evaluated by calculating the Hausner ratio and Carr's index for these forms. The results obtained in this study are listed in tables 6.21 and 6.22.

Table 6.21 Initial density (D_0) and final density (D_f) measurements of EP06W and EP06D

Sample	D_0 (g/ml)		D_f (g/ml)	
	EP06W	EP06D	EP06W	EP06D
1	0.28	0.25	0.42	0.32
2	0.27	0.25	0.40	0.30
3	0.29	0.25	0.40	0.32
Average	0.28 ± 0.01	0.25 ± 0.0	0.41 ± 0.01	0.31 ± 0.01

Table 6.22 Hausner ratio and Carr's index of EP06W and EP06D

Sample	Hausner ratio		Carr's index	
	EP06W	EP06D	EP06W	EP06D
1	1.48	1.27	32.43	21.43
2	1.46	1.23	31.58	18.60
3	1.38	1.31	27.78	23.81
Average	1.44±0.05	1.27±0.04	30.60±2.48	21.28±2.61

Both forms had a Hausner ratio of less than 1.6, indicating no significant difference in the flowability of these two forms. A Hausner ratio of approximately 1.2 is indicative of good flowability and above 1.6 indicates that the powder is less free-flowing. The data for Carr's index was interpreted using table 3.2. EP06D had a Carr's index value of 21.28, which indicated that this form exhibited fair flowability and EP06W exhibited poor flowability with a Carr's index value of 30.60.

The inclusion of a solvent into the crystal lattice of an API could change the crystal structure, which might influence the crystal habit. A change in crystal habit could lead to altered flowability of the sample as Rasenack and Muller (2002:45-57) illustrated for ibuprofen and paracetamol. The poor flowability of these API's was improved by a change in crystal habit.

The difference in the flowability of EP06W and EP06D could therefore be attributed to a possible difference in the morphology of the crystals of these two forms (see section 6.2.2.5 for SEM photomicrographs).

6.2.3 EP08W & EP08D

EP08W was recrystallised using dioxane. EP08D was prepared by drying EP08W in an Ecotherm drying oven (Labotec, South Africa) at a temperature of 110°C for 12 hours.

6.2.3.1 X-ray powder diffraction (XRPD) and variable temperature x-ray powder diffraction (VT-XRPD)

Figure 6.25 displays the VT-XRPD patterns of EP08W in the temperature range of 25-180°C.

EP08W displayed a relatively amorphous XRPD pattern at 25°C with two prominent diffraction peaks at $13.5^{\circ}2\theta$ and $16.5^{\circ}2\theta$ respectively. Slight changes were observed in the diffractogram of EP08W at 35°C. The peak at $16.5^{\circ}2\theta$ displayed an increase in total counts at this temperature. At 45°C the XRPD pattern displayed minor changes, including an increase in intensity of the peak at $11.2^{\circ}2\theta$ and a visible decrease in intensity of the peak at $13.5^{\circ}2\theta$. The diffractogram of EP08W remained relatively unchanged in the temperature range 55-65°C. The XRPD pattern obtained at 75°C displayed subtle changes such as the appearance of a peak at $25.1^{\circ}2\theta$. Major changes were observed in the diffractogram at 85°C. A more crystalline XRPD pattern was obtained at 85°C with an increase in the total counts of most of the peaks. Some of the most significant changes that were observed in the diffractogram included the appearance of peaks at the following diffraction angles: $9.2^{\circ}2\theta$, $14.5^{\circ}2\theta$, $17.2^{\circ}2\theta$, $22.9^{\circ}2\theta$ and $28.0^{\circ}2\theta$. The dramatic change in the diffraction patterns was due to desolvation of the sample. Removal of the solvent from the crystal lattice resulted in a more crystalline crystal structure.

The diffractogram remained relatively unchanged during the temperature range 95-160°C. A XRPD pattern with only a few noticeable peaks was observed at 170°C, which indicated that melting of the sample had commenced. The diffractogram obtained at 180°C was a characteristic melting XRPD pattern.

Table 6.23 lists the main peak angles ($^{\circ}2\theta$) and relative intensity values (I/I_0) in the diffractogram of EP08W at 25°C, 75°C, 85°C, 95°C and 150°C. The most prominent changes in the diffractograms of EP08W at these temperatures can be seen in the mentioned table.

Table 6.23 The main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I₀) in the diffractogram of EP08W at 25°C, 75°C, 85°C, 95°C and 150°C

25°C		75°C		85°C		95°C		150°C	
($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)
6.3	10.0	6.3	5.5	6.3	10.4	-	-	-	-
6.7	11.7	-	-	-	-	-	-	-	-
-	-	7.1	4.0	-	-	-	-	-	-
-	-	7.4	4.6	7.5	11.5	7.5	10.6	7.5	12.0
8.3	10.7	8.2	4.4	-	-	-	-	-	-
-	-	-	-	9.2	64.4	9.2	90.1	9.2	97.7
10.4	21.0	-	-	-	-	-	-	-	-
11.3	19.2	11.1	11.7	11.5	55.2	11.5	76.8	11.4	80.4
12.6	19.6	12.6	7.8	-	-	12.5	6.3	-	-
13.5	86.6	-	-	13.8	73.3	13.8	70.5	13.8	79.7
-	-	-	-	14.5	36.1	14.5	33.3	14.5	35.3
-	-	-	-	-	-	15.2	22.2	15.1	24.9
15.6	41.6	-	-	15.4	35.4	15.4	33.3	15.4	37.3
16.5	100.0	16.6	100.0	16.5	80.4	16.5	83.3	16.4	82.9
-	-	-	-	17.2	71.8	17.2	68.8	17.1	72.4
17.9	52.6	-	-	-	-	-	-	-	-
18.6	35.1	18.5	11.5	18.5	23.2	18.5	18.1	18.5	9.4
-	-	-	-	19.7	9.7	-	-	19.4	6.9
20.3	46.4	-	-	-	-	-	-	20.4	14.5
20.9	49.8	20.9	10.8	21.0	74.6	21.0	71.3	20.9	72.1
-	-	21.6	9.0	21.5	96.7	21.5	93.2	21.4	96.3
-	-	-	-	22.9	21.6	22.9	21.7	22.9	27.4
-	-	-	-	23.1	26.7	23.1	27.5	-	-
24.0	37.1	24.0	8.2	-	-	23.8	11.4	23.9	10.8
-	-	25.1	11.8	25.1	100.0	25.1	100.0	24.9	100.0
-	-	-	-	26.7	12.2	26.7	14.0	26.6	9.9
-	-	-	-	28.0	35.4	27.9	36.5	27.8	35.7
-	-	-	-	28.5	24.4	28.5	23.2	28.3	22.4
-	-	-	-	29.3	19.8	29.3	18.8	29.1	18.0
-	-	-	-	31.1	11.5	-	-	31.0	9.7
-	-	-	-	32.6	17.3	32.6	17.9	32.5	16.4
-	-	-	-	-	-	-	-	34.0	7.4

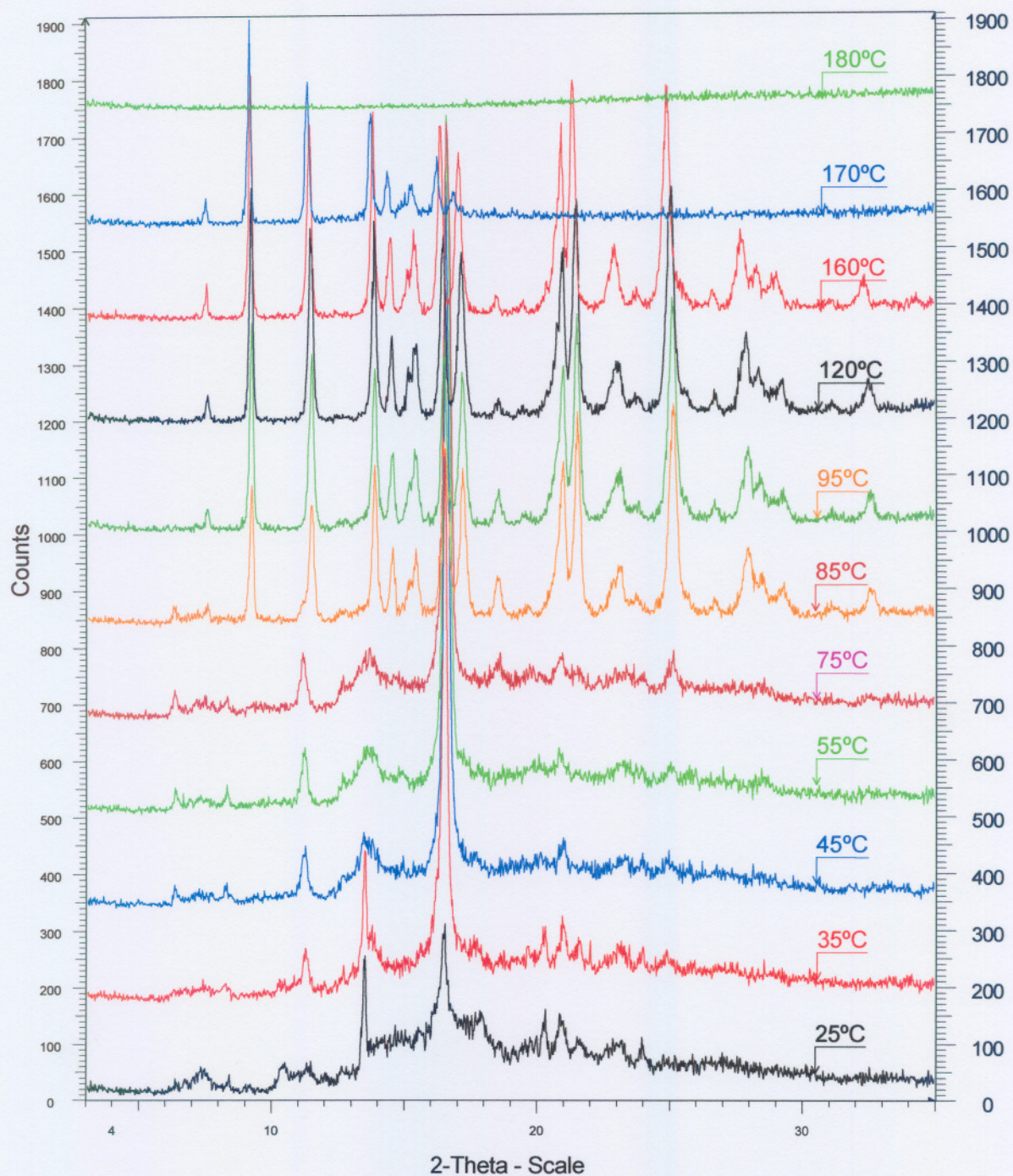


Figure 6.25 Variable temperature x-ray powder diffractograms of EP08W.

The XRPD pattern of EP08W displayed some of the peaks that were characteristic to form J described in the literature (see section 2.4.1 for a discussion of form J). The peak at 13.8 that is characteristic of form J was however, not present in the diffractogram of EP08W.

The XRPD patterns of EP08D in the temperature range of 25-180°C are shown in figure 6.26. The x-ray powder diffractogram of EP08D remained relatively unchanged for the entire temperature range of 25-160°C. At 170°C the sample started to melt, which was apparent from the relatively amorphous diffractogram with only a few peaks visible. At 180°C the sample was completely melted as indicated by the diffractogram obtained at this temperature.

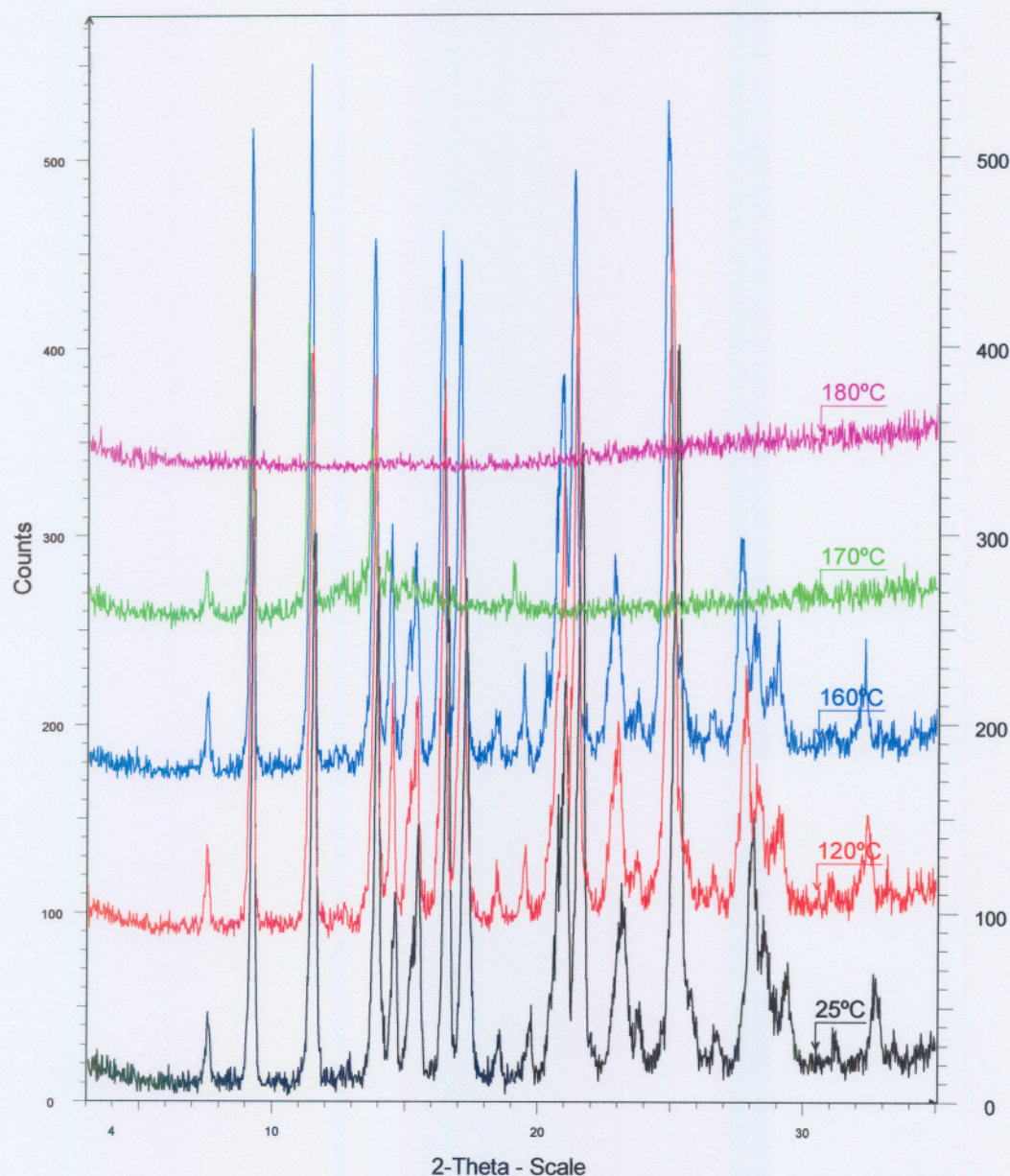


Figure 6.26 Variable temperature x-ray powder diffractograms of EP08D.

The x-ray powder diffractogram of EP08D was comparable to the diffractogram of EP08W obtained at temperatures above 95°C, as illustrated in figure 6.27. This indicated that the desolvated crystal form remained relatively unchanged when exposed to 120°C for a prolonged time period (12 hours).

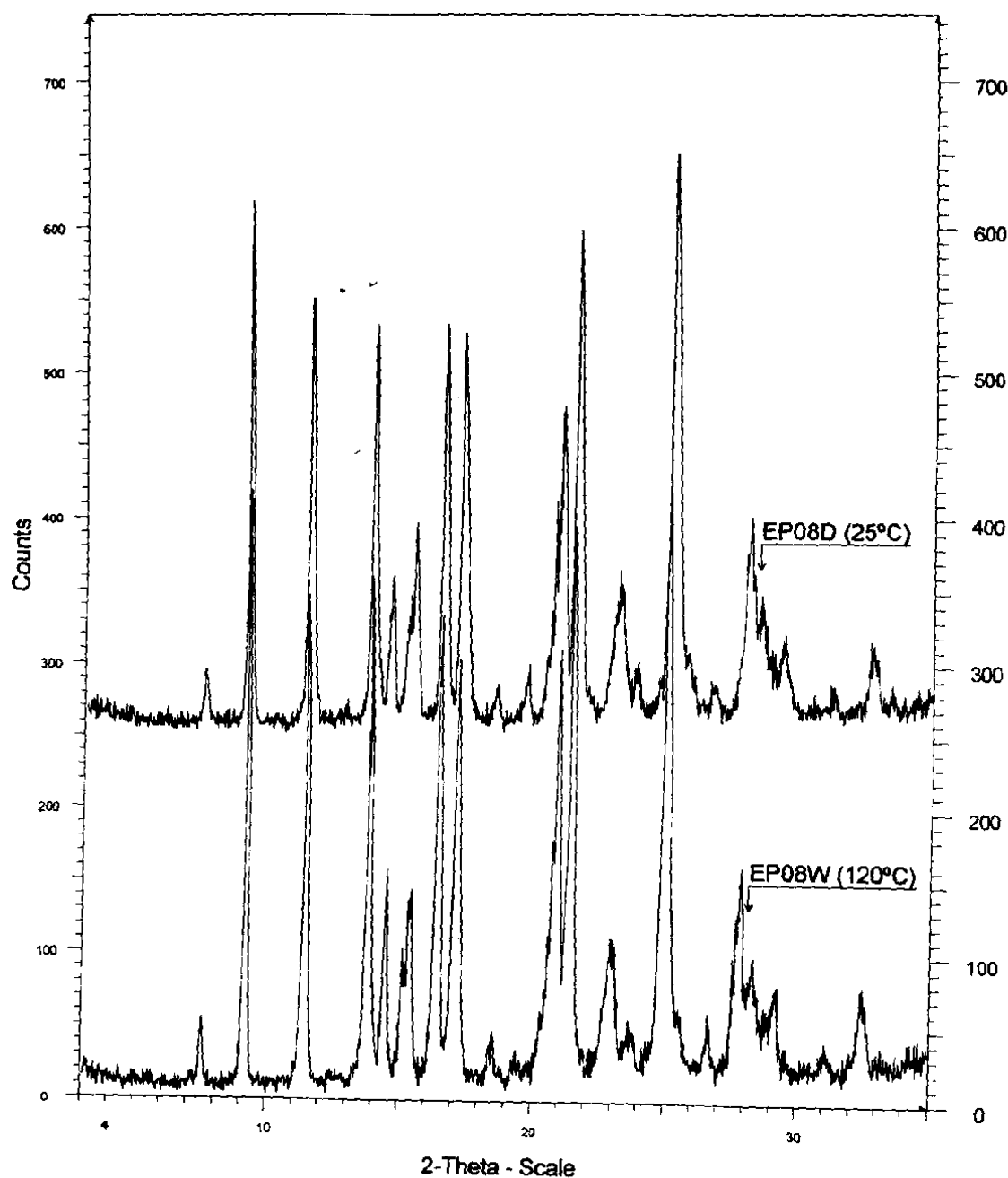


Figure 6.27 Overlay of the x-ray powder diffractograms of EP08W (at 120°C) and EP08D (at 25°C).

EP08D was compared to the crystal forms described in the literature and did not show any resemblances to any of these forms. EP08D could therefore be classified as a new anhydrous crystal form.

6.2.3.2 Infrared Spectroscopy (IR)

The main absorptions in the IR spectra of EP08W and EP08D are listed in table 6.24 and an overlay of the DRIFT-IR spectra of these two forms is shown in figure 6.28. All the differences between the spectra of the two forms are indicated in table 6.24. The DRIFT-IR spectra of these two forms differed significantly and only a few overlapping absorptions were found.

Table 6.24 Main absorptions in the DRIFT-IR spectra of EP08W and EP08D

Main absorptions	Wavelength (cm ⁻¹)	
	EP08W	EP08D
1	3672	-
2	-	3438
3	-	3276
4	-	3084
5	-	2990
6	-	2959
7	-	2927
8	2862	-
9	-	2835
10	-	2633
11	-	2108
12	-	1906
13	-	1850
14	-	1733
15	1614	1619
16	1578	-
17	-	1515
18	1448	1443
19	-	1375
20	1358	-
21	-	1331
22	1321	-
23	-	1299
24	1277	1279
25	-	1239
26	1209	1213
27	1181	1185
28	-	1153
29	1120	-
30	-	1109
31	1094	-
32	-	1078
33	1055	1054

Table 6.24 (continued)

Main absorptions	Wavelength (cm ⁻¹)	
	EP08W	EP08D
34	-	1031
35	-	1007
36	997	993
37	-	958
38	939	-
39	-	910
40	888	888
41	872	874
42	-	830
43	822	-
44	-	804
45	773	770
46	735	731
47	-	704
48	681	-
49	-	673
50	-	663
51	648	649
52	614	-
53	-	590
54	572	569
55	-	550
56	544	-
57	514	513
58	-	497
59	485	485
60	-	474
61	451	-
62	-	440
63	-	402

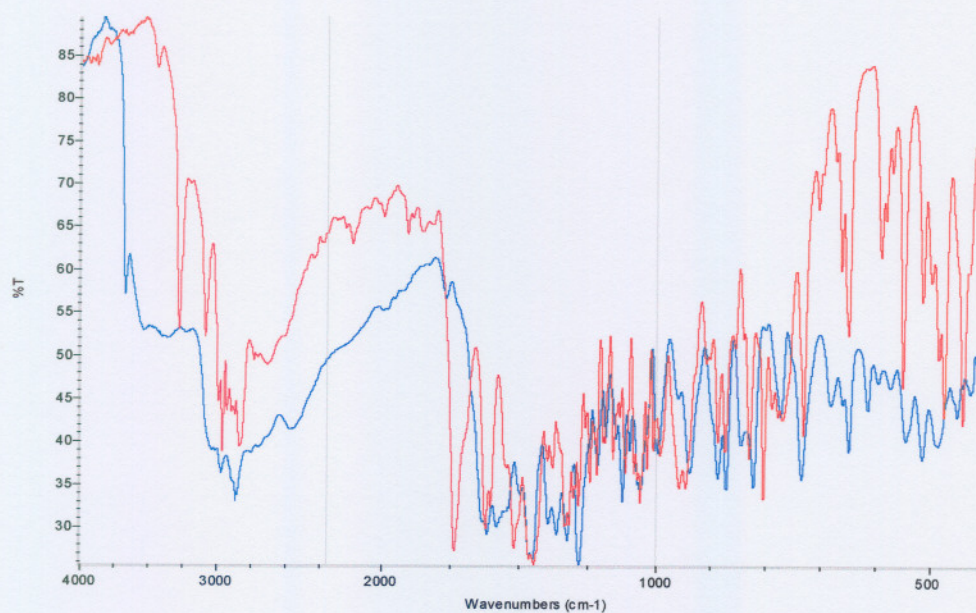


Figure 6.28 Overlay of the DRIFT-IR spectra of EP08W (blue) and EP08D (red).

6.2.3.3 Differential scanning calorimetry (DSC)

Figure 6.29 displays the DSC thermograms of EP08W before and after desolvation. The sample (EP08W) was heated to 90°C, cooled down to 25°C and heated again to 200°C to obtain the thermogram for the desolvated EP08W.

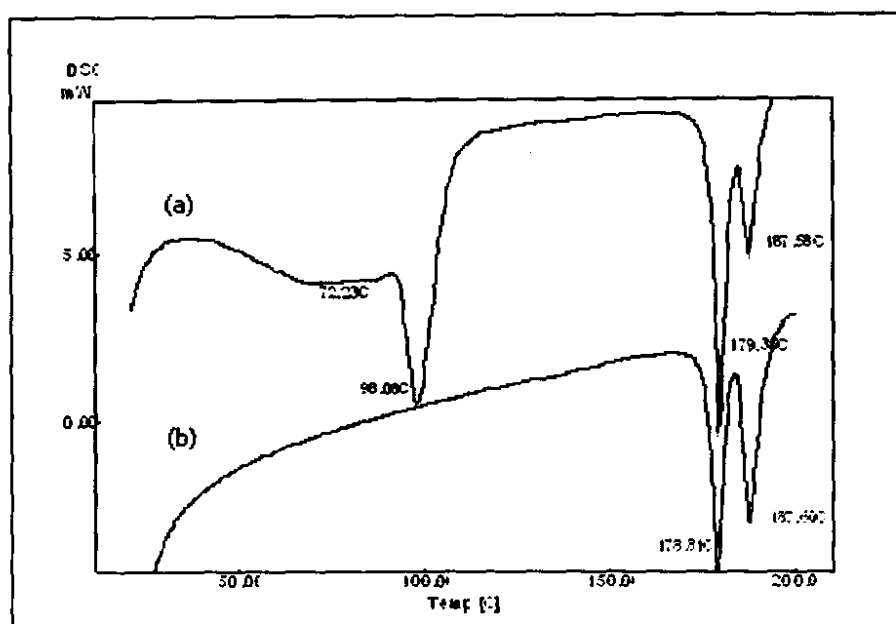


Figure 6.29 DSC thermograms of EP08W before (a) and after desolvation (b).

The DSC thermogram of EP08W displayed a broad endotherm at 72.23°C, which was possibly due to the loss of surface solvent. The endotherm at 98.08°C was due to the overlapping desolvation and dehydration of the sample (water and dioxane have boiling points of 100.0°C and 101.1°C respectively). The endotherm at 98.08°C was also due to the melting of a small fraction of the hydrate that occurred upon dehydration. Melting of a small fraction of the crystals was observed in this temperature range and it correlated with the temperature at which darkening of the crystals and bubble formation (which is indicative of dehydration) was observed with HSM (see HSM photomicrographs in section 6.2.3.5). The melted fraction solidified at 120°C. A similar behaviour has been described by Kuhnert-Brandstätter (1971:16) that in some cases a fraction of the hydrated crystals dehydrate and become turbid while the neighbouring crystals melt as the hydrate. The melted crystals solidify and melt together with the other (dehydrated) crystals at the melting point of the dehydrated (anhydrous) form (Kuhnert-Brandstätter, 1971:16).

The endotherm at 179.35°C was due to the melting of all the crystals. This was immediately followed by recrystallisation from the melt. The recrystallised product melted at 187.58°C. These events were confirmed with HSM (section 6.2.3.5).

Figure 6.30 displays the DSC thermogram of EP08D. The thermogram of EP08D correlated well with the thermogram obtained for EP08W after desolvation (figure 6.29). The thermogram of EP08D displayed no desolvation endotherms, which indicated that this was an anhydrous crystal form. The endotherm observed at 179.42°C was due to the melting of the crystals, which was followed by recrystallisation at 183.90°C. The final endotherm at 187.97°C indicated the melting of the recrystallised form. All the events observed in the thermogram were confirmed with HSM (section 6.2.3.5).

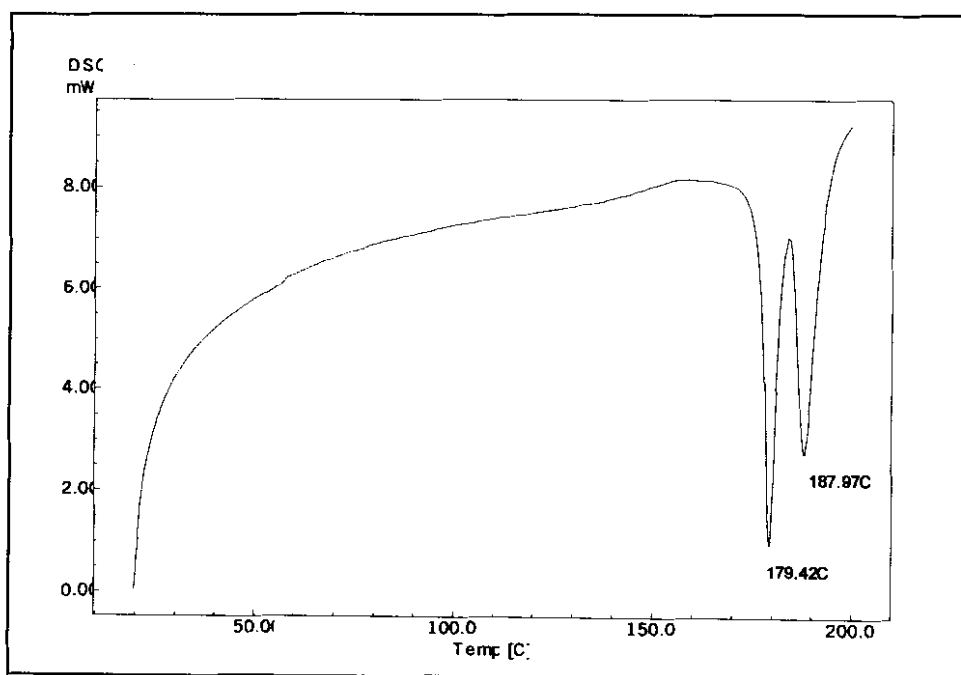


Figure 6.30 DSC thermogram of EP08D.

6.2.3.4 Thermogravimetric analysis (TGA) & Karl Fischer titration (KF)

The TGA and KF results of EP08W and EP08D are tabulated in table 6.25.

Table 6.25 TGA and KF results of EP08W and EP08D

	% Weight loss (TGA)	% Moisture content (KF)
EP08W	18.34	5.13
EP08D	0.11	0.30

The TGA thermogram of EP08W is displayed in figure 6.31. The TGA thermogram revealed a three-step weight loss for EP08W. The % weight loss of each step is shown in table 6.26.

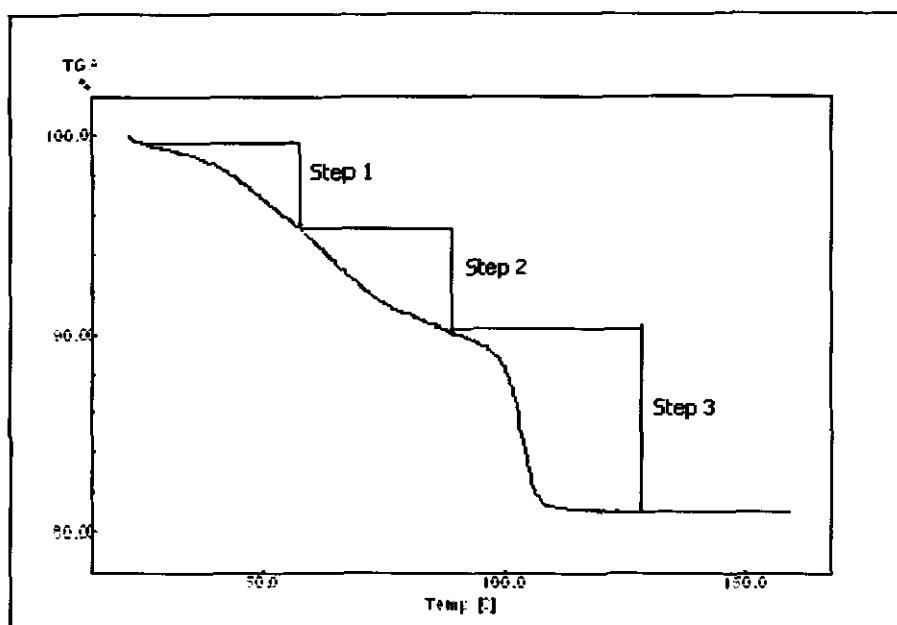


Figure 6.31 TGA thermogram of EP08W showing the three steps of weight loss.

Table 6.26 TGA results of EP08W indicating the % weight loss of each step

% Weight loss (TGA)	EP08W
Step 1	4.24
Step 2	5.08
Step 3	9.02
Total	18.34

The first weight loss step in the TGA thermogram can be attributed to the loss of surface solvent. The second step displayed a weight loss of 5.08%, which correlated with the % moisture content of the sample as determined by KF analysis. This value could be compared to the theoretical value of a monohydrate, which is 4.58%. The third step in the TGA thermogram was due to the desolvation of the sample. A weight loss of 9.02% was observed in this step, which was comparable to the theoretical weight loss of a hemisolvate of dioxane (9.50%). EP08W could be classified as a possible hemi-dioxane-monohydrate.

The TGA thermogram of EP08D is displayed in figure 6.32. TGA revealed that no significant weight loss occurred and KF analysis displayed no significant moisture content for the sample (table 6.25).

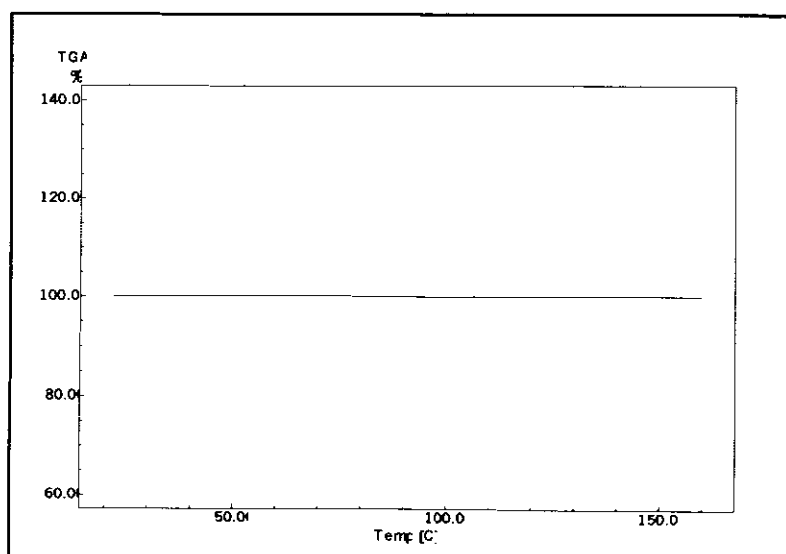


Figure 6.32 TGA thermogram of EP08D.

6.2.3.5 Microscopic analysis

Hot stage microscopy (HSM) was performed on EP08W and EP08D to study the thermal behaviour of these crystal forms. A summary of the observations with HSM of EP08W is shown in table 6.27.

Table 6.27 Summary of HSM observations of EP08W in the temperature range of 25-192°C

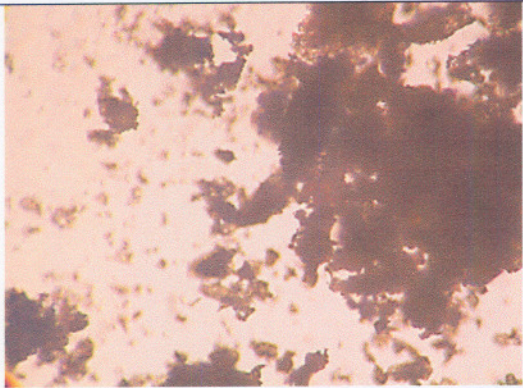
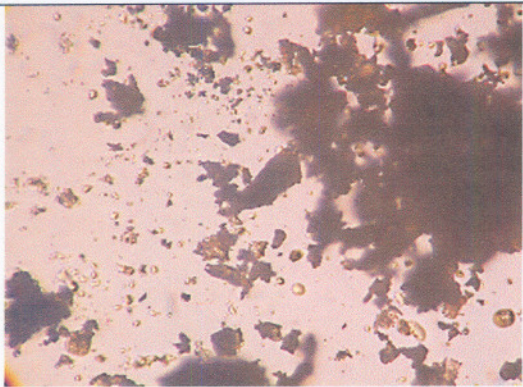
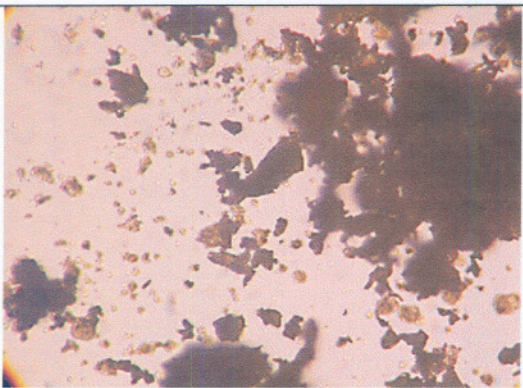
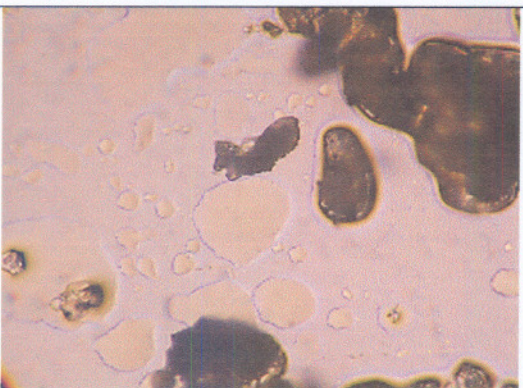
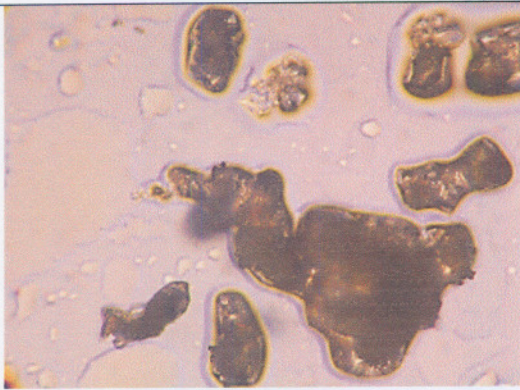
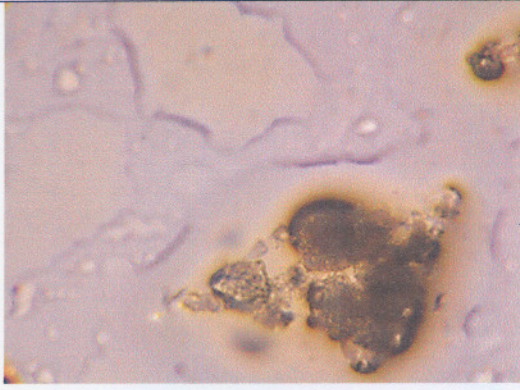
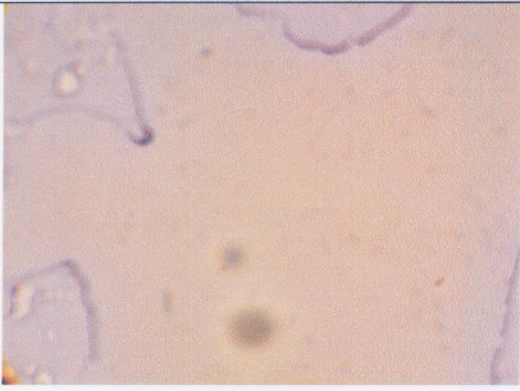
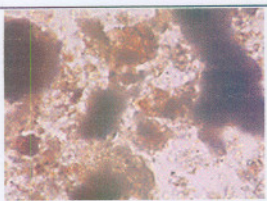
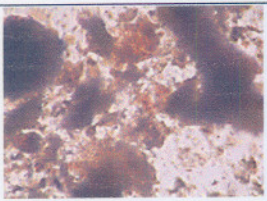
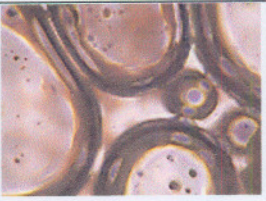
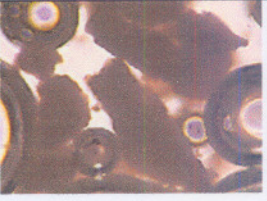
Temperature (°C)	Photomicrograph	Remarks
25		EP08W crystals at 25°C.
105		Melting of a fraction of the crystals upon dehydration.
120		Solidification of the melted fraction.
182		Melting of all the crystals was observed between 170-185°C.

Table 6.27 (continued)

Temperature (°C)	Photomicrograph	Remarks
185		Recrystallisation occurred from the melt.
190		Melting of the recrystallised form.
192		Melting completed.

The crystals became turbid and bubble formation due to gas evolution was observed when the crystals were submerged in silicon oil, which indicated desolvation/dehydration of the sample. Table 6.28 displays the HSM photomicrographs of the darkening and bubble formation observed for EP08W when submerged in silicon oil.

Table 6.28 HSM observations of EP08W when submerged in silicon oil

25°C	92°C	103°C	116°C
			
EP08W crystals at room temperature.	Darkening of the crystals.	Bubble formation.	Bubble formation.

The events that were observed with HSM for EP08D are summarised in table 6.29.

Table 6.29 Summary of HSM observations of EP08D in the temperature range 25-192°C

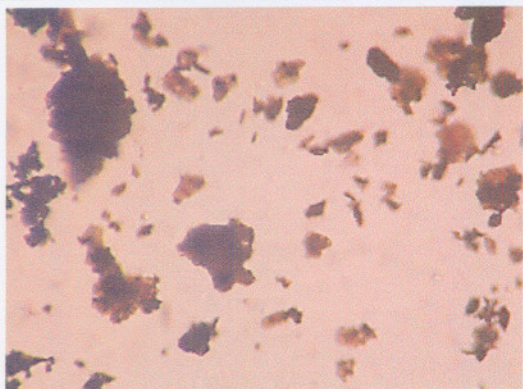
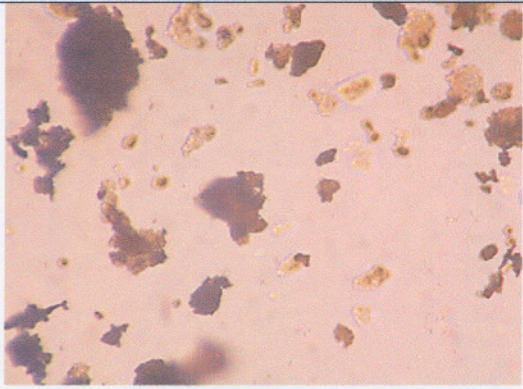
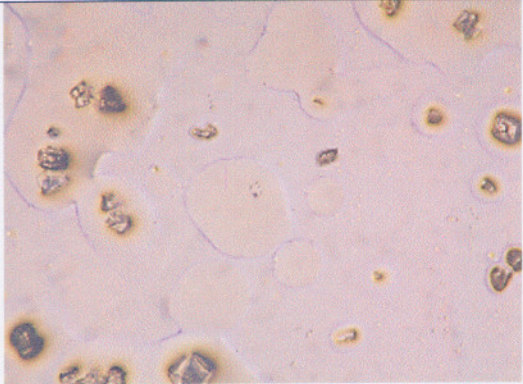
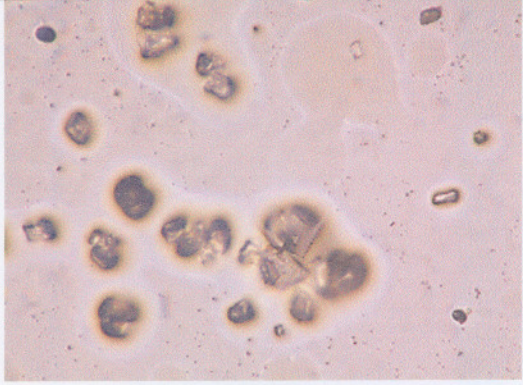
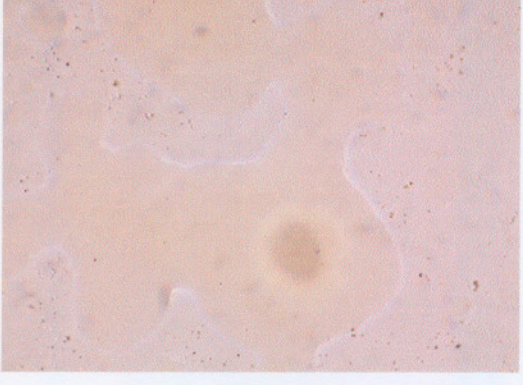
Temperature (°C)	Photomicrograph	Remarks
25		EP08D crystals at 25°C.
179		Melting of all the crystals started.

Table 6.29 (continued)

Temperature (°C)	Photomicrograph	Remarks
183		Melting completed and recrystallisation from the melt occurred at 183°C.
186		Recrystallisation product.
189		Melting of the recrystallised product started at 189°C.
192		Melting completed.

The morphology of EP08W and EP08D was studied by means of scanning electron microscopy (SEM). The SEM photomicrographs of EP08W and EP08D are shown in figures 6.33 and 6.34.

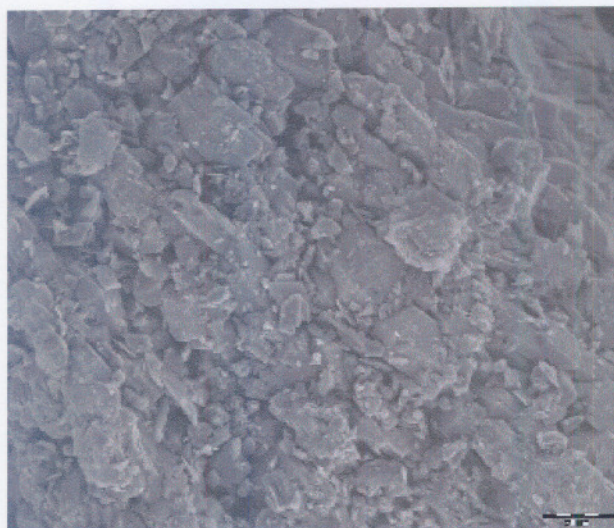


Figure 6.33 SEM photomicrograph of EP08W.



Figure 6.34 SEM photomicrograph of EP08D.

The morphology of the crystals of EP08W and EP08D was similar. Both EP08W and EP08D had shapeless crystals that showed a tendency to agglomerate.

6.2.3.6 Dissolution studies

Powder dissolution studies were performed on EP08W and EP08D and the results are tabulated in table 6.30. The samples were sieved and the fraction between 250-106 μm was used to ensure particle size uniformity. Figure 6.35 displays the dissolution profiles of EP08W and EP08D. The % dissolved ranged between 87.0% at 7.5 minutes to 95.0% at 30 minutes for EP08W. 91.0% of the sample of EP08D was dissolved after 7.5 minutes and the sample was fully dissolved (100.0%) after 30 minutes. Both EP08W and EP08D conformed to the in-house criteria set for the dissolution of gatifloxacin, which is that more than 75% of the sample should be dissolved after 30 minutes.

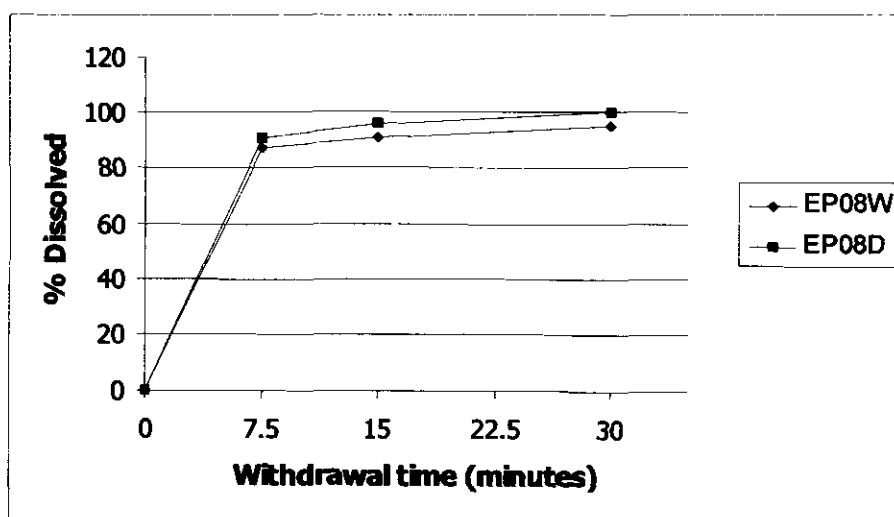


Figure 6.35 Dissolution profiles of EP08W and EP08D in 0.01 N HCl at $37\pm 0.2^\circ\text{C}$.

Table 6.30 Dissolution results of EP08W and EP08D in 0.01 N HCl at $37\pm 0.2^\circ\text{C}$

Dissolution vessel	% Dissolved					
	7.5 minutes		15 minutes		30 minutes	
	EP08W	EP08D	EP08W	EP08D	EP08W	EP08D
1	84.5	92.4	88.9	95.8	93.1	100.1
2	88.9	91.5	92.7	96.2	97.0	100.8
3	88.8	93.7	91.9	97.0	96.0	101.8
4	80.3	89.9	85.6	94.2	92.3	99.8
5	92.1	89.7	94.5	95.3	97.7	99.7
Average	87.0$\pm$4.6	91.0$\pm$1.7	91.0$\pm$3.5	96.0$\pm$1.0	95.0$\pm$2.4	100.0$\pm$0.9

The dissolution profiles of EP08W and EP08D were compared using the similarity factor (f_2 value) described by Moore and Flanner (1996:65). This calculation determines whether two dissolution profiles could be regarded as similar or if the differences between the two profiles are significant enough to regard the profiles as dissimilar. An f_2 value above 50 indicates that the two profiles are similar. The guidelines of the Medicines Control Council states that for the calculation of the f_2 value only one measurement should be considered after 85% dissolution of both samples have occurred (Medicines Control Council, 8:2003). If >85% dissolution occurred for both samples, the dissolution profiles may be regarded similar. Thus the f_2 value (similarity factor) need not be calculated to determine the similarity between the dissolution profiles of EP08W and EP08D. However, for the sake of completeness the f_2 value (similarity factor) of EP08W was calculated and included here. The dissolution profile of EP08D was used as reference profile in the calculation.

The f_2 value calculated for EP08W was 65.96, which indicated that these two profiles might be considered to be similar.

The dissolution profiles of EP08W and EP08D were compared to that of the sesquihydrate form by calculating the f_2 value for both of these forms. The dissolution profile of the sesquihydrate form was used as reference. The results are shown in table 6.31.

Table 6.31 f_2 values of EP08W and EP08D using the dissolution profile of the sesquihydrate as reference

Crystal form	f_2 value
EP08W	48.80
EP08D	59.16

EP08W had an f_2 value of 48.80, which indicated that the dissolution profile of this form and that of the sesquihydrate was not similar.

The f_2 value of EP08D was 59.16, which indicated that the dissolution profile of this form and that of the sesquihydrate could be regarded as being similar.

6.2.3.7 Solubility studies

The solubility of EP08W and EP08D in the four specified media was determined using the method described in chapter 3. EP08D was more soluble in all the media. The solubility for both forms ranged in the following order: 0.01 N HCl > acetate buffer > MilliQ water > phosphate buffer. The solubility of EP08W did not display a noticeable difference between the MilliQ water and phosphate buffer. The solubility values of EP08W and EP08D are listed in table 6.32 and a graphic comparison of the solubility of these two forms is shown in figure 6.36.

Table 6.32 Solubility values (in mg/ml) of EP08W and EP08D in various media at 37±2°C

Medium	pH	EP08W		EP08D	
		Solubility (mg/ml)	%CV	Solubility (mg/ml)	%CV
0.01 N HCl	2.25	3.86	7.33	4.98	1.10
Acetate buffer	4.52	3.58	7.26	4.85	0.86
MilliQ Water	5.62	2.25	3.59	2.97	0.97
Phosphate buffer	6.77	2.24	3.58	2.93	1.89

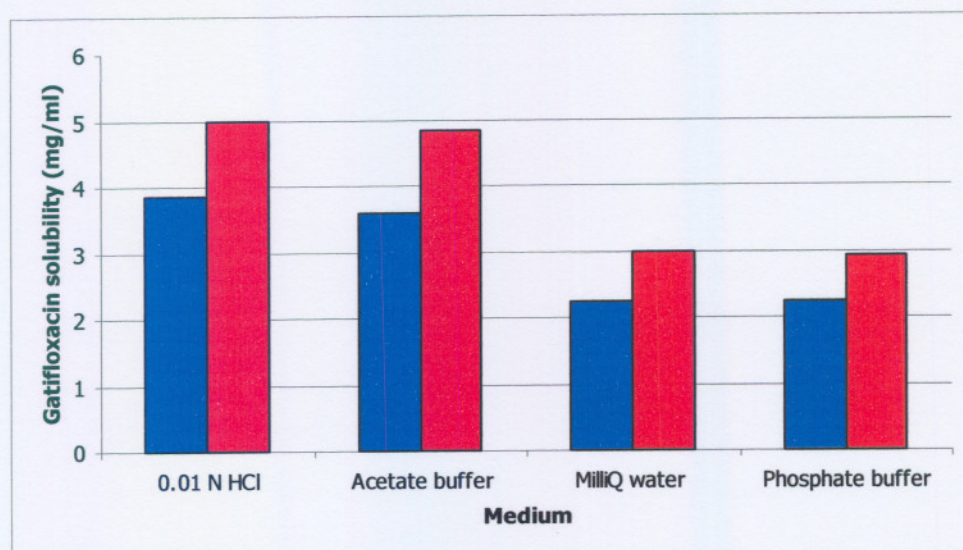


Figure 6.36 Solubility profiles of EP08W (blue) and EP08D (red) in various media at $37\pm 2^\circ\text{C}$.

6.2.3.8 Flowability

The flowability of EP08W and EP08D was investigated using the method described in chapter 3. Table 6.33 shows the calculated initial density (poured density) and final density (tapped density) values of both forms and table 6.34 displays the Hausner ratios and Carr's index of EP08W and EP08D.

Table 6.33 Initial density (D_0) and final density (D_f) measurements of EP08W and EP08D

Sample	D_0 (g/ml)		D_f (g/ml)	
	EP08W	EP08D	EP08W	EP08D
1	0.30	0.29	0.53	0.50
2	0.33	0.32	0.56	0.53
3	0.33	0.30	0.53	0.48
Average	0.32 ± 0.02	0.31 ± 0.02	0.54 ± 0.02	0.50 ± 0.03

Table 6.34 Hausner ratio and Carr's index of EP08W and EP08D

Sample	Hausner ratio		Carr's index	
	EP08W	EP08D	EP08W	EP08D
1	1.74	1.75	42.42	42.86
2	1.72	1.63	41.94	38.71
3	1.58	1.57	36.67	36.36
Average	1.68±0.09	1.65±0.09	40.34±3.19	39.31±3.29

A Hausner ratio above 1.6 indicates that the powder is more cohesive and less free-flowing. Both EP08W and EP08D had Hausner ratios above 1.6 and could therefore be seen as powders with poor flowability. The interpretation of Carr's index was done according to table 3.2. The Carr's index values of these two forms indicated that EP08W exhibited an extremely poor flowability and EP08D exhibited a poor flowability. The values for these two forms did not differ significantly and it could be assumed that solvent inclusion in this specific crystal does not cause a noticeable change in powder flowability.

6.3 Comparison of the dissolution & solubility results of EP05, EP06 and EP08

The dissolution profiles of EP05W, EP06W and EP08W are displayed in figure 6.37. Significant differences were observed between the dissolution profiles of these three forms, indicating that different solvent inclusions influenced the dissolution properties of gatifloxacin crystal forms significantly.

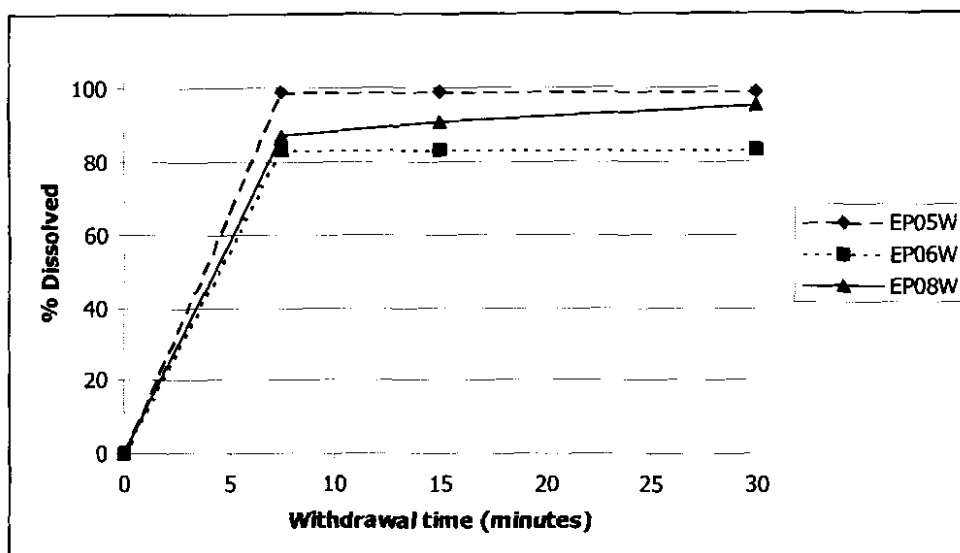


Figure 6.37 Dissolution profiles of EP05W, EP06W and EP08W in 0.01 N HCl at $37\pm0.2^{\circ}\text{C}$.

The dissolution profiles of EP05D, EP06D and EP08D are displayed in figure 6.38. The differences between the dissolution profiles were more subtle compared to those observed for the solvated crystal forms. All the desolvated crystal forms (EP05D, EP06D and EP08D) displayed comparable % dissolved (Q) at 30 minutes: 98.0%, 100.0% and 100.0% for EP05D, EP06D and EP08D respectively.

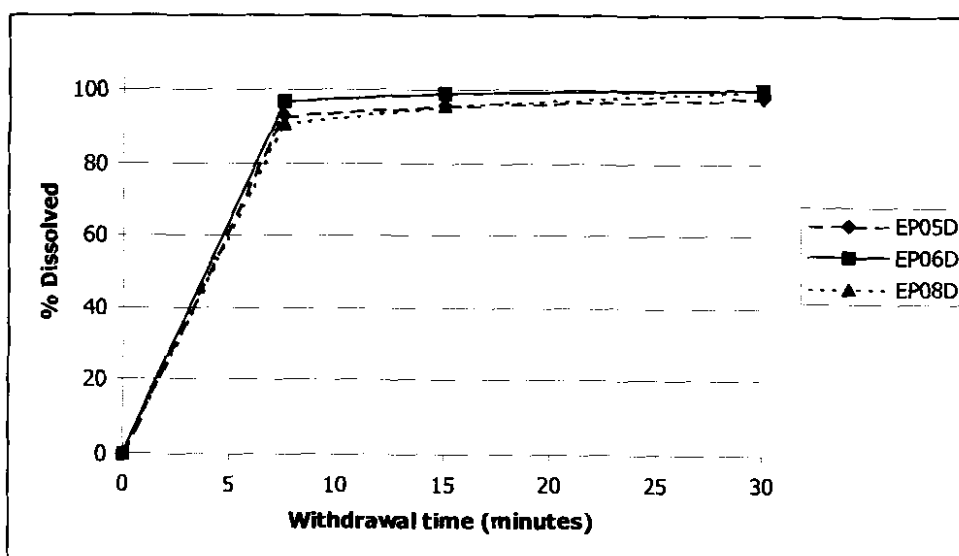


Figure 6.38 Dissolution profiles of EP05D, EP06D and EP08D in 0.01 N HCl at $37\pm0.2^{\circ}\text{C}$.

The solubility results of EP05W, EP06W and EP08W are tabulated in table 6.35 and the solubility profiles are displayed in figure 6.39.

Table 6.35 Solubility values (in mg/ml) of EP05W, EP06W and EP08W in various media at 37±2°C

Medium	EP05W		EP06W		EP08W	
	Solubility (mg/ml)	% CV	Solubility (mg/ml)	% CV	Solubility (mg/ml)	% CV
0.01 N HCl	3.64	0.21	4.04	3.00	3.86	7.33
Acetate buffer	3.54	0.85	3.73	3.21	3.58	7.26
MilliQ water	2.13	2.43	2.24	2.04	2.25	3.59
Phosphate buffer	2.12	0.42	2.36	4.26	2.24	3.58

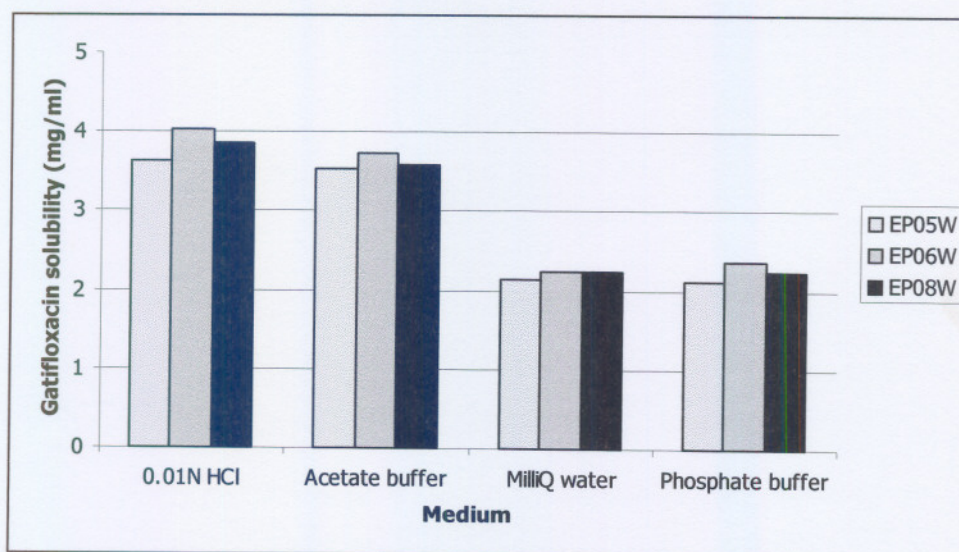


Figure 6.39 Solubility profiles of EP05W, EP06W and EP08W in various media at 37±2°C.

The solubility values of EP05D, EP06D and EP08D are tabulated in table 6.36. Figure 6.40 displays the solubility profiles of these three forms. The solubility profiles of

EP05D, EP06D and EP08D displayed only slight differences. The solubility of all three forms ranged in the order: 0.01 N HCl > acetate buffer > MilliQ water > phosphate buffer.

Table 6.36 Solubility values (in mg/ml) of EP05D, EP06D and EP08D in various media at $37\pm 2^\circ\text{C}$

Medium	EP05D		EP06D		EP08D	
	Solubility (mg/ml)	% CV	Solubility (mg/ml)	% CV	Solubility (mg/ml)	% CV
0.01 N HCl	5.02	0.48	4.99	2.25	4.98	1.10
Acetate buffer	5.00	0.24	4.74	1.70	4.85	0.86
MilliQ water	3.09	1.96	2.99	0.49	2.97	0.97
Phosphate buffer	2.90	2.71	2.84	2.97	2.93	1.89

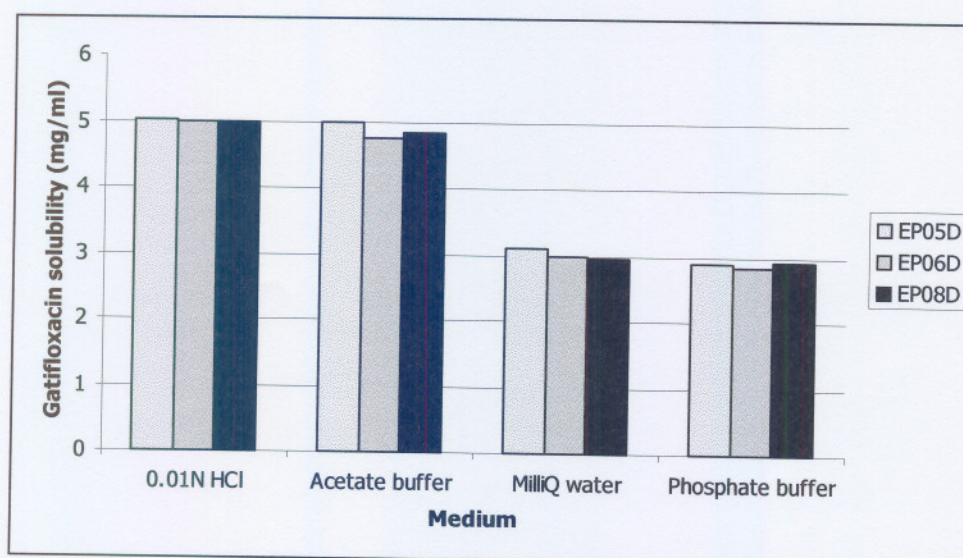


Figure 6.40 Solubility profiles of EP05D, EP06D and EP08D in various media at $37\pm 2^\circ\text{C}$.

Conclusion

The aim of this chapter was to characterise the crystal forms obtained from recrystallisation with DMF, chloroform and dioxane. The influence of solvent inclusion on the physico-chemical properties of these forms was also investigated.

EP05W was obtained from recrystallisation with DMF and was found to be a mixture of two forms, i.e. a sesquihydrate and a DMF solvate (1:1). Currently no DMF solvates have been documented in the literature.

EP05D was an anhydrous form prepared by drying EP05W. The XRPD pattern of EP05D showed some resemblances to that of form omega and form I described in the literature (section 2.4.2-2.4.3).

The dissolution profiles of EP05W and EP05D were similar and Q after 30 minutes was 99% for EP05W and 98% for EP05D. Comparison of these two forms to the sesquihydrate form revealed that EP05W displayed a better dissolution profile fit to the sesquihydrate than EP05D. Both EP05W and EP05D had Hausner ratios above 1.6, which indicated that these forms had poor flowability and Carr's index indicated that EP05W exhibited slightly better flow properties than EP05D.

Solvent inclusion did not have a profound influence on the dissolution or flow properties of these forms seeing that the dissolution profiles of these forms were similar and both forms displayed poor flowability; however other physico-chemical properties differed significantly.

EP06W was obtained from recrystallisation with chloroform and was classified as a chloroform solvate (1:1). Currently no chloroform solvates have been documented in the available literature and EP06W could therefore be classified as a new pseudopolymorph of gatifloxacin. The XRPD pattern of EP06W displayed some of the characteristic peaks of form T1 described in the patents (section 2.4.4).

EP06D was classified as an anhydrous crystal form, which was obtained from drying EP06W. The XRPD pattern of EP06D displayed similarities to that of form omega and form I described in the literature (section 2.4.2-2.4.3).

Significant differences were observed between the dissolution profiles of EP06W and EP06D, which indicated that solvent inclusion played a role in the dissolution properties of this crystal form. Q at 30 minutes was 83% and 100% for EP06W and EP06D respectively. The dissolution profile of EP06W differed significantly from that of the sesquihydrate form, while the dissolution profiles of EP06D and the sesquihydrate were comparable. Differences were observed between the flowability of EP06W and EP06D. EP06D exhibited fair flowability and EP06W displayed poor flowability.

The desolvation of the chloroform solvate resulted in a crystal with better dissolution and flow properties compared to that of the solvated crystal form.

EP08W was recrystallised from dioxane and was found to be a hemi-dioxane-monohydrate. This form displayed some of the characteristic peaks of form J described in section 2.4.1. No hemi-dioxane-monohydrates have been documented in the available literature and therefore EP08W could be classified as a new pseudopolymorphic form.

EP08D was obtained by drying EP08W and was classified as a new anhydrous crystal form. The dissolution profiles of EP08W and EP08D differed slightly. Q after 30 minutes was found to be 95% and 100% for EP08W and EP08D respectively. The similarity factor (f_2 value) for these two forms indicated that the differences between the dissolution profiles of these forms might be considered to be insignificant. Both EP08W and EP08D displayed poor flowability. Solvent inclusion did not influence the dissolution or flow properties of this crystal form significantly.

Overall the desolvated forms of the prepared crystal forms displayed better solubility properties than that of the solvated hydrated forms.

From this chapter it became clear that solvent inclusion influenced some of the physico-chemical properties of the crystal. Seeing that minor changes in the physico-chemical properties of pharmaceutical solids may lead to problems during the manufacturing process, it is important to select a crystal form (solvated or desolvated) with the most desired physico-chemical profile.

CHAPTER 7

Characterisation of a gatifloxacin commercial product

Introduction

The crystal form chosen for the development of a pharmaceutical solid dosage form is of importance seeing that it may influence the manufacturing process, stability and efficacy of the dosage form. The most suitable and stable crystal form should be identified during preformulation studies to avoid possible polymorphic phase transitions during the manufacturing or upon storage of the product.

Gatifloxacin is a broad-spectrum fluoroquinolone antibiotic used in the treatment of a range of eye, skin, respiratory- and urinary tract infections (see section 2.2.1). Gatifloxacin is normally administered as tablets, injections or eye drops depending on the indication. The sesquihydrate form of gatifloxacin is currently the preferred crystal form used in commercial products, possibly due to its thermodynamic stability.

Internationally available gatifloxacin products include ZYMAR[®] (gatifloxacin ophthalmic solution 0.3%) manufactured by Allergan; Gatiflox[®] (gatifloxacin 400 mg tablets) manufactured by Incepta Pharmaceuticals Ltd. (figure 7.1); and Gatiquin[®] (200/400 mg tablets, IV infusion, eye drops and eye ointment) manufactured by CIPLA.



Figure 7.1 Gatiflox[®] (400 mg gatifloxacin) tablet packaging (Incepta Pharmaceuticals Ltd., 2005).

The only commercially available gatifloxacin product in South Africa is Tequin® (200 mg and 400 mg tablets, and injections) manufactured by Bristol-Myers Squibb (figure 7.2).



Figure 7.2 Tequin® (400 mg gatifloxacin) packaging.

Various studies in the literature indicated that manufacturing processes influenced the polymorphic form used during manufacturing, thus the polymorph present in the final product is not the same as the crystal modification used initially.

The aim of this chapter was to use a set of analytical techniques (i.e. XRPD, IR spectroscopy, DSC and TGA) in an attempt to compare the crystal form present in the commercial product (Tequin®) with the sesquihydrate crystal form.

7.1 Materials and methods

7.1.1 Materials

Tequin® 400 mg gatifloxacin tablets (Bristol-Myers Squibb) were obtained from a local pharmacy. The batch number and expiry date of the tablets were as follows:

Batch number	: 427241
Expiry date	: April 2006

The Tequin® tablets were white to off-white in colour, almond shaped, biconvex and film coated. The letters "BMS" were debossed on one side of the tablets and "TEQUIN" and "400" on the other side.

7.1.2 Methods

The tablets were powdered and analysed by means of XRPD, IR spectroscopy, DSC and TGA analysis in an attempt to possibly identify the crystal form present in the tablets. These techniques are discussed in chapter 3.

7.2 Results and discussion

7.2.1 X-ray powder diffraction (XRPD) and variable temperature x-ray powder diffraction (VT-XRPD)

The XRPD pattern obtained for the powdered Tequin® tablets is shown in figure 7.3.

The diffractogram of the powdered Tequin® tablets was compared to that of the sesquihydrate raw material. Table 7.1 lists the main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I_0) of the powdered Tequin® tablets and that of the sesquihydrate crystal form.

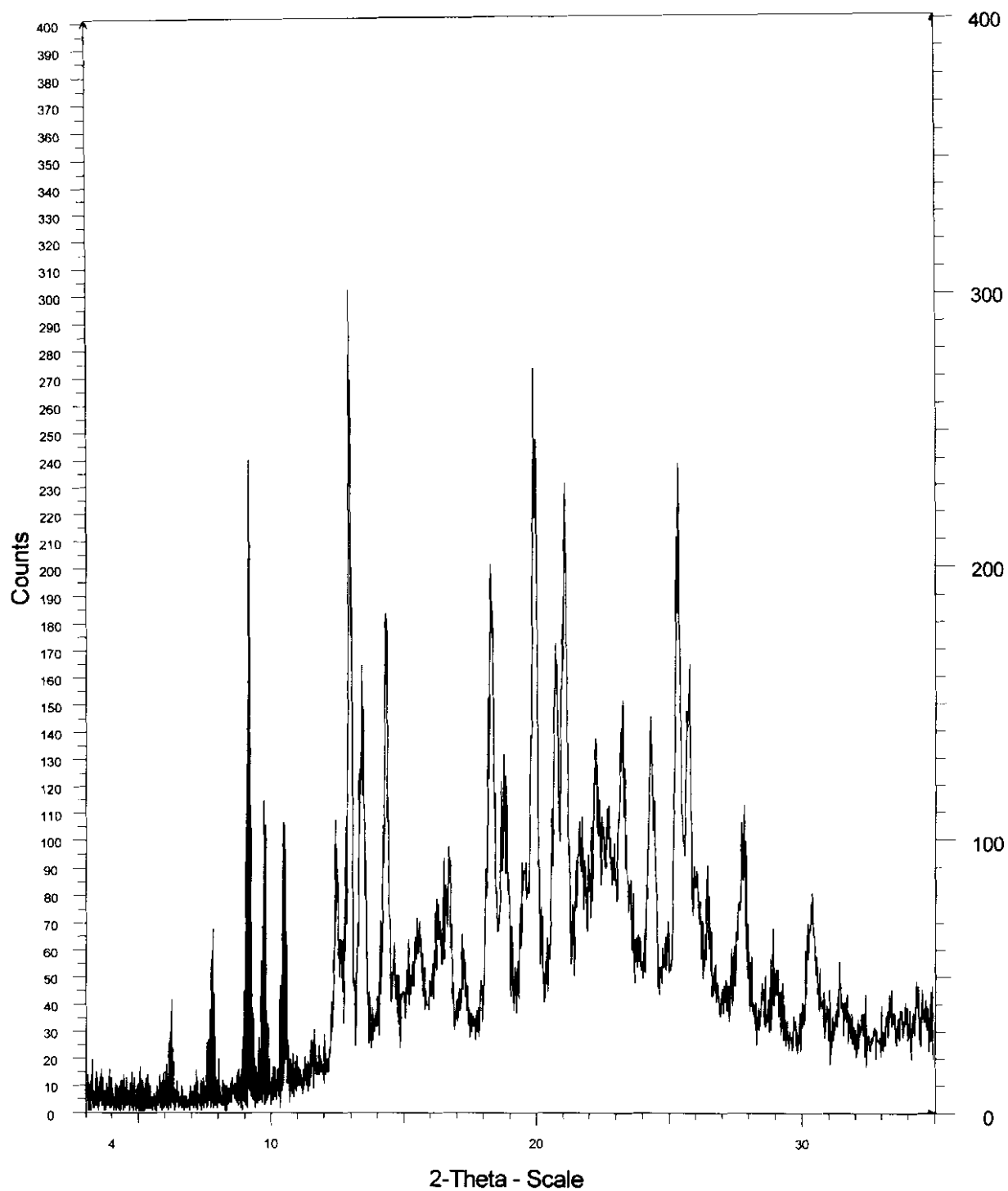


Figure 7.3 X-ray powder diffractogram of powdered Tequin® tablets.

Table 7.1 Main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I₀) of the powdered Tequin[®] tablets and gatifloxacin sesquihydrate raw material

Main peaks	Tequin [®] tablets		Sesquihydrate	
	($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)
1	6.2	13.6	6.3	5.8
2	7.7	22.2	7.8	10.0
3	9.1	79.5	9.1	100.0
4	9.7	37.7	9.7	22.6
5	10.4	35.1	10.5	16.0
6	11.5	9.9	11.4	14.6
7	12.4	35.4	12.3	12.7
8	12.9	100.0	13.0	33.8
9	13.4	54.3	13.5	18.1
10	14.3	60.6	14.3	14.8
11	-	-	14.8	6.9
12	15.5	23.2	15.6	5.3
13	16.3	25.2	16.3	6.6
14	16.7	32.1	16.7	5.5
15	17.2	19.9	-	-
16	-	-	17.8	5.2
17	18.3	66.6	18.3	56.5
18	18.8	43.4	18.9	14.3
19	19.6	29.1	19.6	23.2
20	19.9	81.8	20.0	47.4
21	-	-	20.2	18.5
22	20.7	57.0	20.8	21.2
23	21.1	76.5	21.1	37.0
24	21.7	35.1	21.8	15.2
25	22.2	45.4	22.2	7.2
26	-	-	22.9	6.6
27	23.2	50.0	23.3	15.1
28	24.3	48.0	24.3	22.7
29	25.3	78.8	25.4	22.9
30	25.7	48.7	25.8	18.1
31	26.0	29.1	26.1	13.0
32	26.5	29.8	26.5	7.9
33	27.8	37.1	27.8	8.8
34	28.6	15.6	-	-
35	-	-	29.0	7.0
36	-	-	29.3	9.4
37	30.4	26.5	30.4	15.4
38	31.5	18.2	-	-
39	-	-	32.3	6.3
40	34.4	15.9	-	-

The peaks between 3-15 $^{\circ}2\theta$ in the diffractogram of the powdered Tequin[®] tablets were comparable to that of the sesquihydrate form, except for the peak at 14.8 $^{\circ}2\theta$ that was present in the XRPD pattern of the sesquihydrate form and not clearly visible in the

diffractogram of the powdered Tequin® tablets (these peaks are highlighted in table 7.1). The XRPD peaks between 15-35°2 θ showed resemblances, but due to the presence of various amorphous excipients (seen by the drift in the XRPD pattern baseline and halo-shape thereof) in the tablets the peaks were not easily distinguishable and some of the peaks of the sesquihydrate could not clearly be identified in the diffractogram of the powdered Tequin® tablets.

7.2.2 Infrared spectroscopy (IR)

An overlay of the DRIFT-IR spectra of the powdered Tequin® tablets and the sesquihydrate raw material is shown in figure 7.4 and the main IR absorptions are listed in table 7.2.

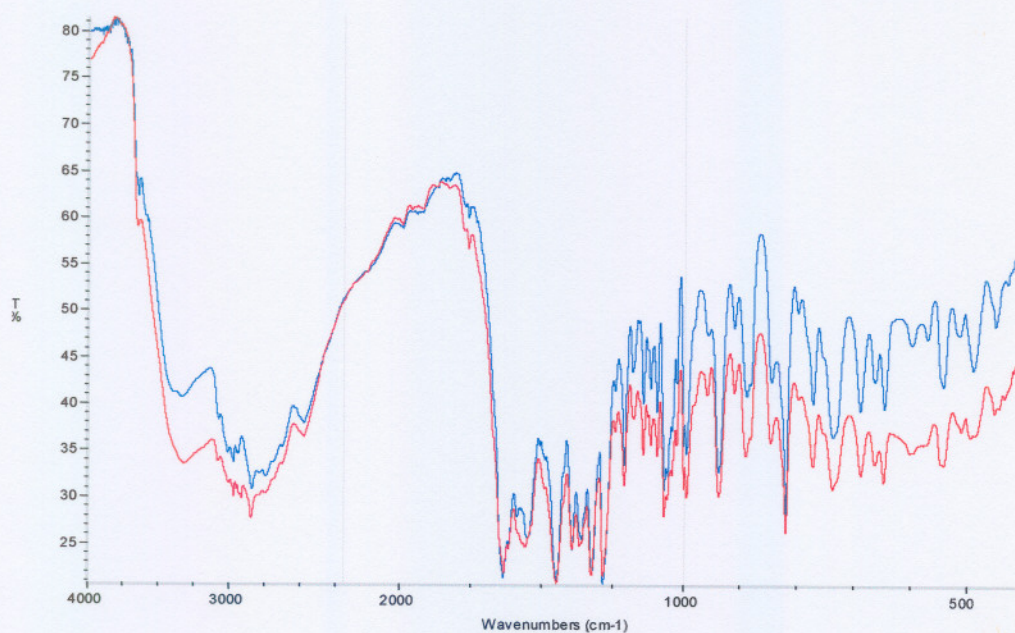


Figure 7.4 Overlay of the DRIFT-IR spectra of powdered Tequin® tablets (red) and sesquihydrate raw material (blue).

Table 7.2 Main absorptions in the DRIFT-IR spectra of the powdered Tequin® tablets and the sesquihydrate raw material

Main absorptions	Wavenumber (cm ⁻¹)	
	Tequin®	Sesquihydrate
1	3651	3649
2	-	3340
3	3326	-
4	3078	3078
5	-	3013
6	2974	2975
7	2954	-
8	-	2940
9	2917	-
10	2847	2842
11	-	2746
12	2477	2476
13	1994	1993
14	1922	-
15	1830	1830
16	1760	1760
17	1633	1635
18	-	1587
19	1560	-
20	-	1551
21	-	1507
22	1448	1448
23	1393	1394
24	1367	-
25	-	1359
26	1325	1322
27	1283	1283
28	1243	1243
29	1209	1209
30	1177	1179
31	1143	1143
32	1118	1118
33	1094	1095
34	1070	1070
35	1057	1057
36	1042	1041
37	1025	1025
38	997	997
39	961	960
40	940	940
41	912	912
42	892	890
43	884	883
44	848	847
45	821	822
46	-	800

Table 7.2 (continued)

Main absorptions	Wavenumber (cm⁻¹)	
	Tequin®	Sesquihydrate
47	774	773
48	738	738
49	689	689
50	664	664
51	648	647
52	602	599
53	-	571
54	542	542
55	511	514
56	495	490
57	452	450
58	434	429
59	412	-
60	401	405

Overall the spectra of the tablets and that of the raw material seemed to be similar. Small differences were observed which was possibly due to the presence of tablet excipients in the Tequin® tablets.

7.2.3 Differential scanning calorimetry (DSC)

The DSC thermogram of the powdered Tequin® tablets is shown in figure 7.5. A broad endotherm was observed at 50.95°C followed by a sharp endotherm at 91.23°C. These endotherms could possibly be identified as the dehydration of the sesquihydrate form used in Tequin® tablets. Melting of the sample caused an endotherm at 160.17°C, which was followed by a recrystallisation exotherm at 166.58°C. The final event in the DSC thermogram was the melting of the recrystallised sample at 184.96°C.

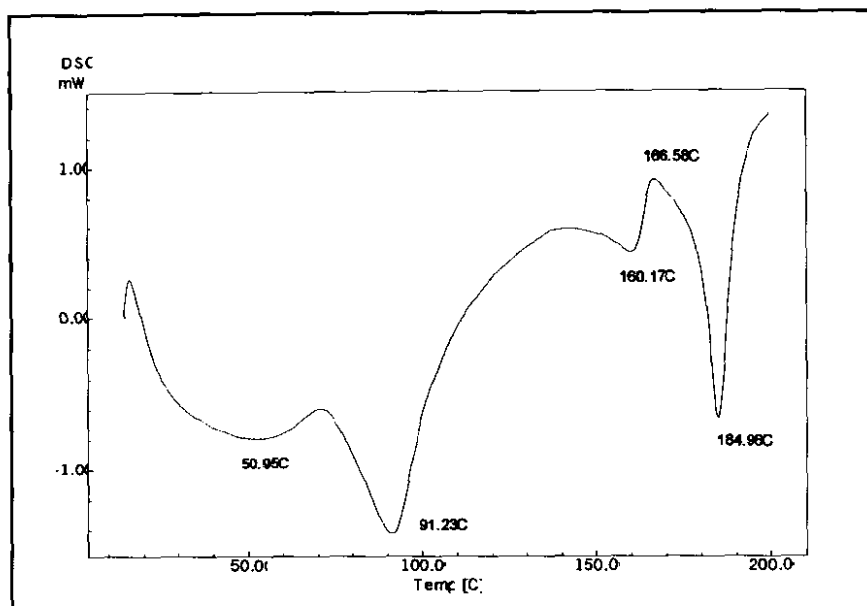


Figure 7.5 DSC thermogram of the powdered Tequin® tablets.

The DSC thermogram of the powdered tablets was comparable to that of the sesquihydrate. All the events observed in the thermogram occurred at lower temperatures in the Tequin® tablets. This could be due to the presence of tablet excipients. Figure 7.6 displays an overlay of the DSC thermograms of the powdered Tequin® tablets and the sesquihydrate raw material.

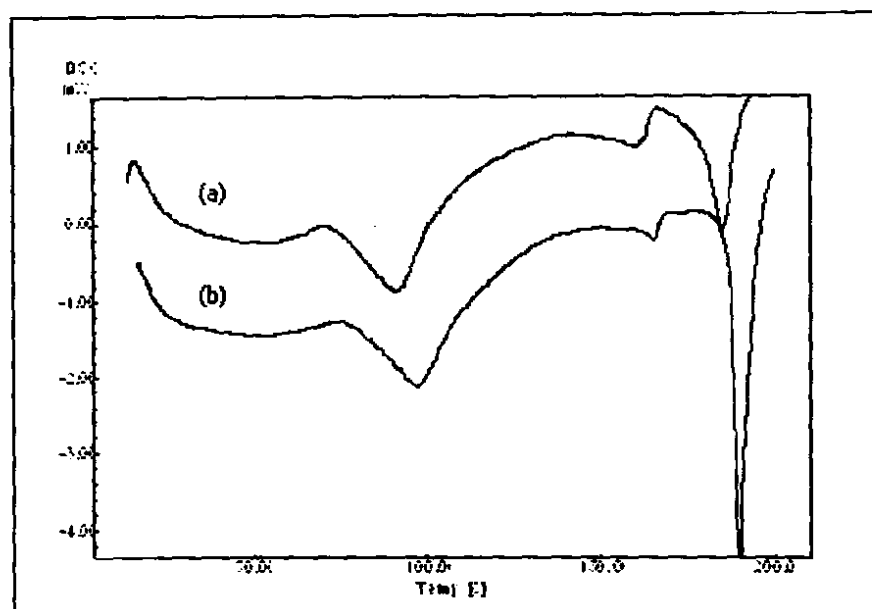


Figure 7.6 Overlay of the DSC thermograms of the powdered Tequin® tablets (a) and the sesquihydrate crystal form (b).

7.2.4 Thermogravimetric analysis (TGA)

The TGA thermogram of the powdered Tequin® tablets is shown in figure 7.7. TGA of the powdered Tequin® tablets revealed a weight loss of approximately 5.30%. This value was comparable to the theoretical weight loss of a sesquihydrate, which is 6.87%. The difference between the experimental and theoretical values was possibly due to minimal dehydration that could have occurred during the manufacturing process. Drying processes in the manufacturing of the tablets could have caused the sesquihydrate crystal form to lose some water.

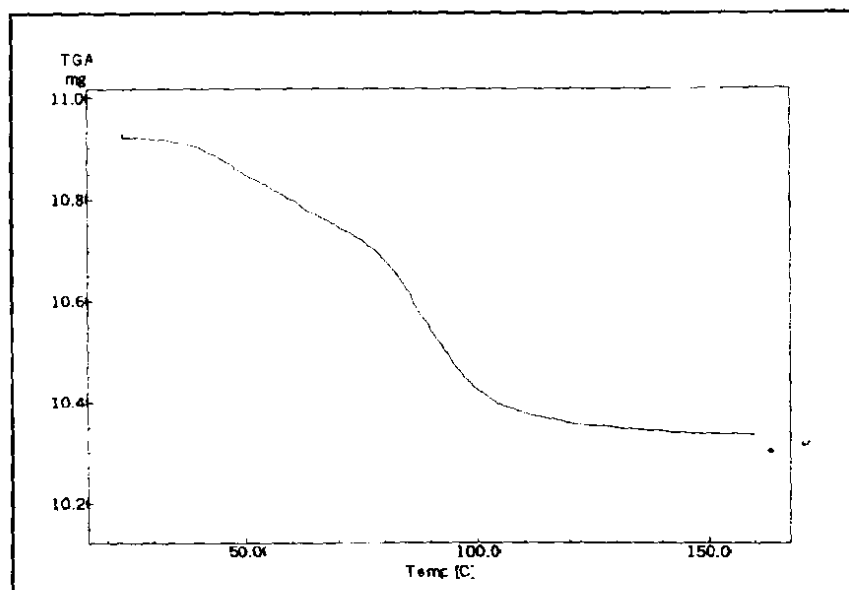


Figure 7.7 TGA thermogram of the powdered Tequin® tablets.

Conclusion

The aim of this chapter was to compare the crystal form present in the commercially available product (Tequin®) to the sesquihydrate crystal form by means of characterisation techniques such as XRPD, IR spectroscopy, DSC and TGA.

The XRPD pattern of the powdered Tequin® tablets showed strong resemblances to that of the sesquihydrate raw material. The x-ray powder diffractogram of the Tequin® tablet powder was less crystalline compared to that of the raw material possibly due to the presence of amorphous tablet excipients. The presence of tablet excipients complicated the identification of the crystal form (sesquihydrate) present in the 15-35°2θ region of the XRPD pattern.

The IR spectra and DSC thermogram of the tablet powder showed resemblances to that of the sesquihydrate crystal form.

The physico-chemical properties of the gatifloxacin crystal form present in the Tequin® tablets were found to be comparable with that of the sesquihydrate crystal form.

It can thus be concluded that the manufacturing process did not significantly influence the crystal form used initially in the manufacturing process.

From this chapter it became clear that the sesquihydrate crystal form seemed to remain stable during the manufacturing process. Prospective studies will investigate the stability of the newly identified crystal forms discussed in chapters 5 and 6.

CHAPTER 8

Solvent inclusion properties of crystal forms recrystallised from absolute methanol and binary mixtures of methanol and water

Introduction

The aim of this chapter was to investigate the solvent inclusion properties and thermodynamic behaviour of gatifloxacin crystal forms recrystallised from absolute methanol and binary mixtures of methanol and water. Another aim of this chapter was to investigate the thermal induced phase transitions of form F using VT-XRPD.

Form F is a pseudopolymorphic form of gatifloxacin, which is obtained from recrystallisation with a mixture of methanol and water (90:10) (Niddam-Hildesheim *et al.*, 2004:7). This crystal form is characterised by XRPD peaks at $8.0^{\circ}2\theta$, $14.2^{\circ}2\theta$, $18.7^{\circ}2\theta$, $21.8^{\circ}2\theta$ and $23.0^{\circ}2\theta$ ($\pm 0.2^{\circ}2\theta$). Figure 8.1 displays the typical x-ray powder diffractogram of form F.

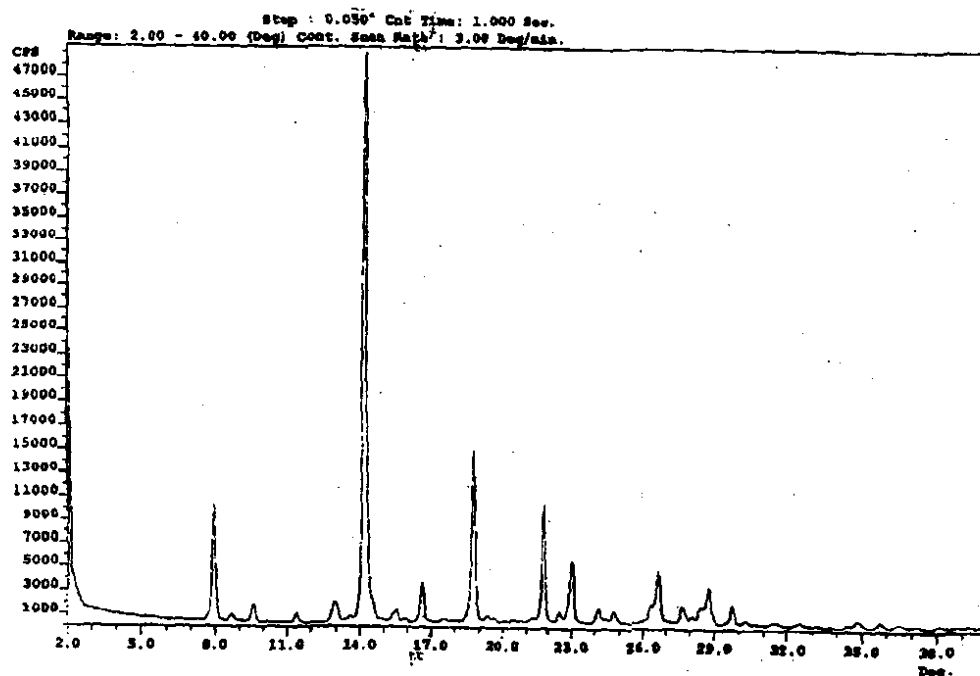


Figure 8.1 X-ray powder diffractogram of form F (Niddam-Hildesheim *et al.*, 2004:4).

Niddam-Hildesheim *et al.* (2004:7) described a method for the preparation of form F which included the following steps: prepare a heated solution of gatifloxacin in a mixture of methanol and water (90:10); cool the solution to ambient temperature; and isolate the suspended form F crystals.

TGA results of form F provided in the literature indicated a total weight loss of 14.78%, which was observed in two steps: 9.38% and 5.4% respectively (Niddam-Hildesheim *et al.*, 2004:4). However, no KF results were supplied in the literature and no absolute classification of this form was thus provided. The TGA and DSC thermograms of form F are displayed in figures 8.2 and 8.3.

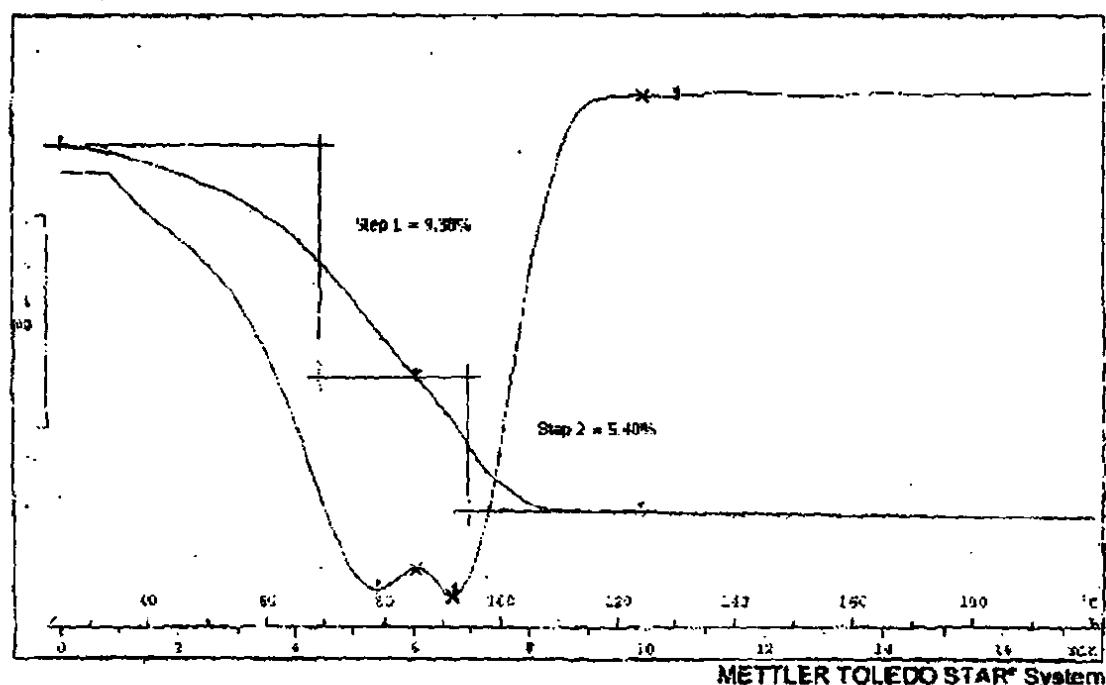


Figure 8.2 TGA thermogram of form F (Niddam-Hildesheim *et al.*, 2004:4).

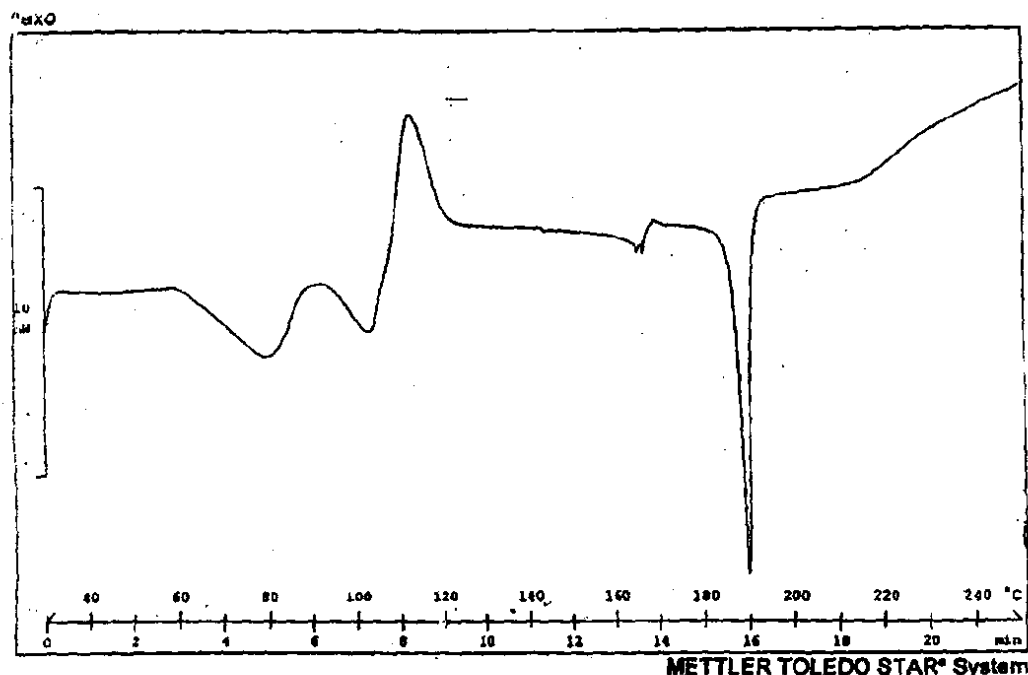


Figure 8.3 DSC thermogram of form F (Niddam-Hildesheim *et al.*, 2004:4).

Niddam-Hildesheim *et al.* (2004:7) described another pseudopolymorphic form of gatifloxacin, form G, which was characterised by XRPD peaks at $17.2^{\circ}2\theta$ and $17.6^{\circ}2\theta$ ($\pm 0.2^{\circ}2\theta$). The typical x-ray powder diffractogram of form G is displayed in figure 8.4.

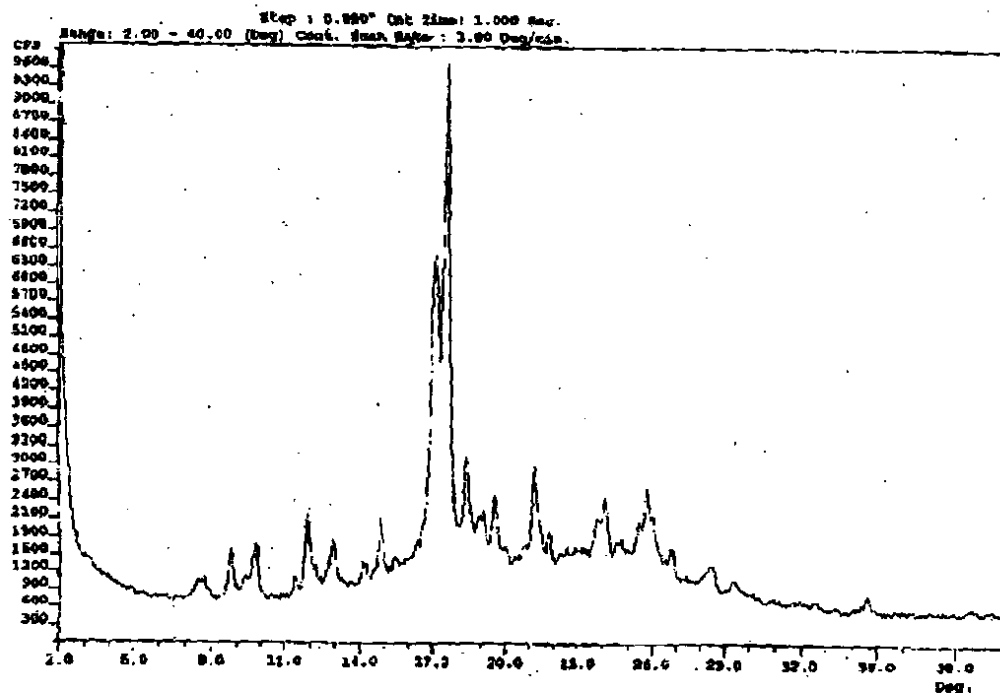


Figure 8.4 X-ray powder diffractogram of form G (Niddam-Hildesheim *et al.*, 2004:4).

Niddam-Hildesheim *et al.* (2004:7) stated that the drying of form F at approximately 50°C and atmospheric pressure for at least 20 hours resulted in the formation of form G.

The IR spectrum of form G is displayed in figure 8.5. Form G displays characteristic absorptions at 1614 cm^{-1} and 1267 cm^{-1} (Niddam-Hildesheim *et al.*, 2004:7).

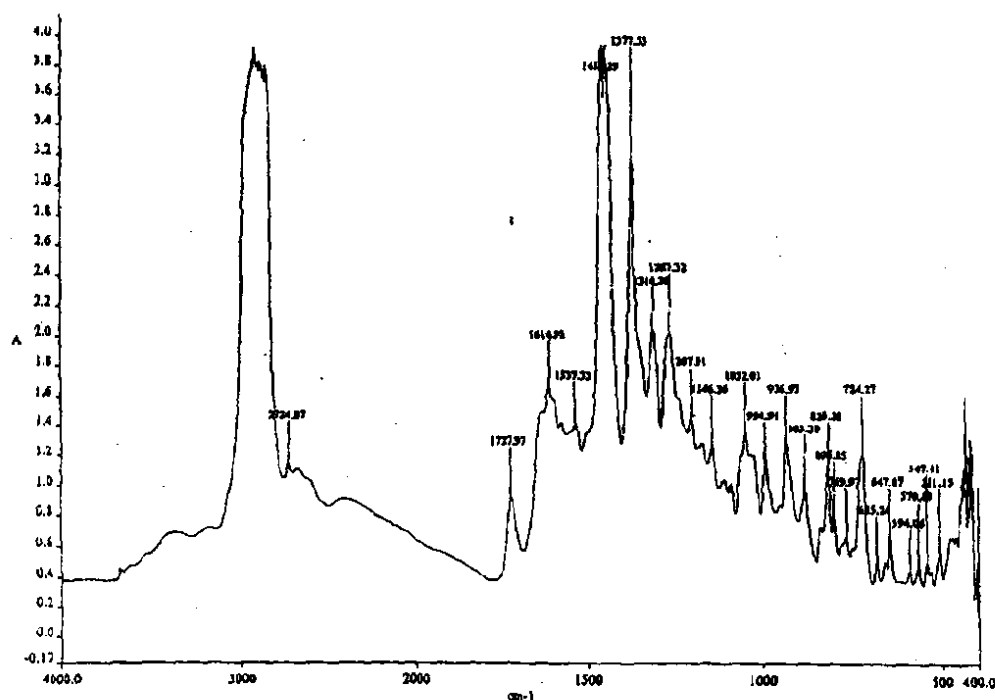


Figure 8.5 IR spectrum of form G (Niddam-Hildesheim *et al.*, 2004:4).

TGA results of form G indicated that this crystal form was a solvate. A weight loss of 5.70% was observed with TGA (Niddam-Hildesheim *et al.*, 2004:7). The literature did not provide any KF results or an absolute classification of form G. The TGA and DSC thermograms of form G are displayed in figures 8.6 and 8.7 respectively.

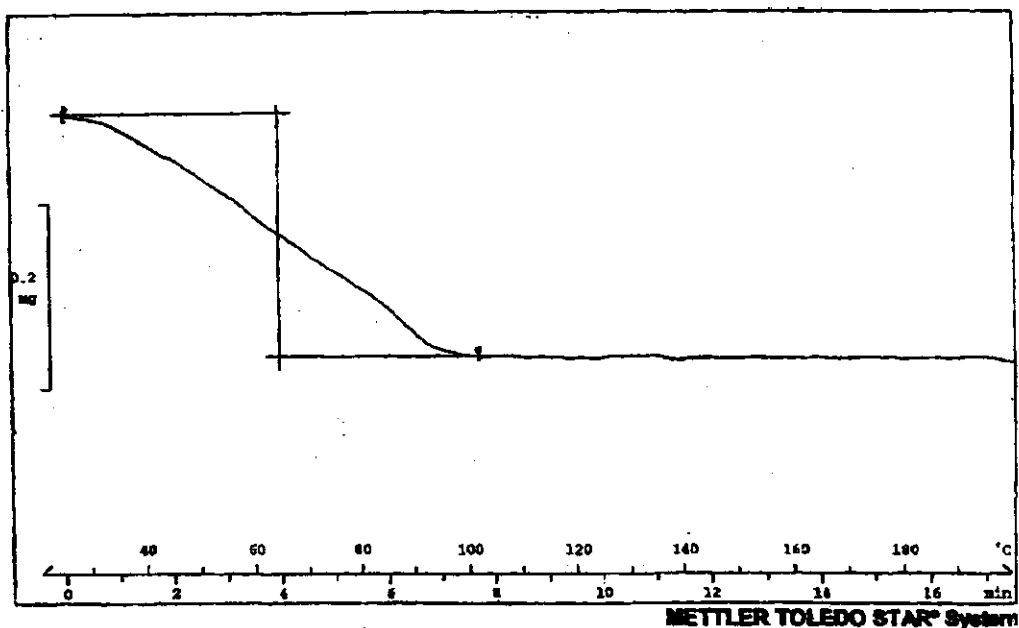


Figure 8.6 TGA thermogram of form G (Niddam-Hildesheim *et al.*, 2004:4).

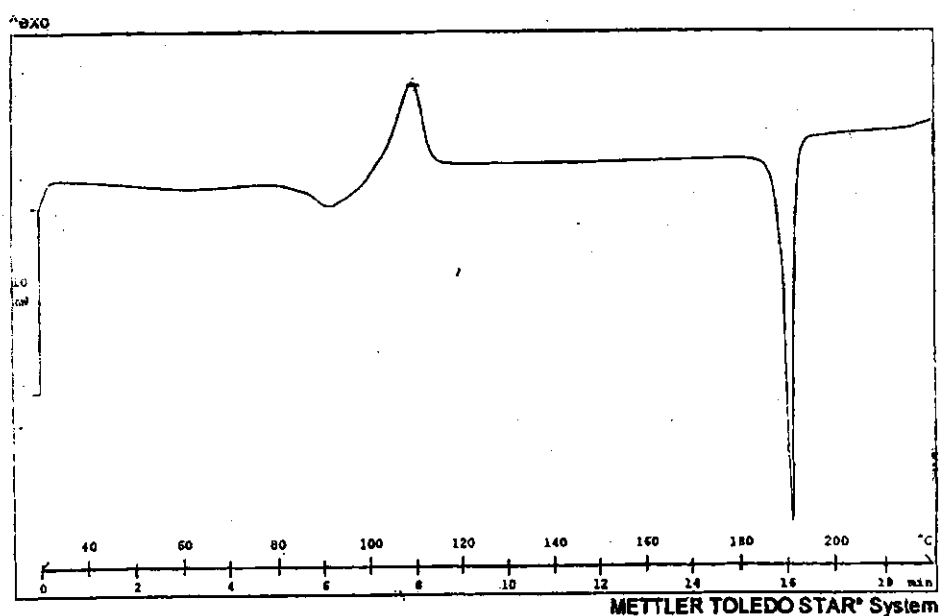


Figure 8.7 DSC thermogram of form G (Niddam-Hildesheim *et al.*, 2004:4).

It was also documented that the drying of form G at 120°C yields either form omega (anhydrous) or form T2RP (hemihydrate) (Niddam-Hildesheim *et al.*, 2004:8-9). The XRPD pattern of form T2RP is displayed in figure 8.8. Various x-ray powder diffractograms of form omega were disclosed in the literature, only two of these are displayed in figure 8.9 and 8.10.

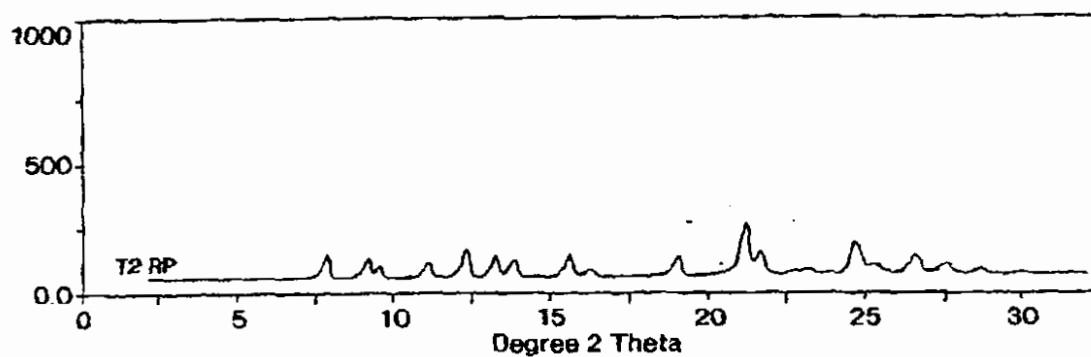


Figure 8.8 X-ray powder diffractogram of form T2RP (adapted from Raghavan *et al.*, 2002:1).

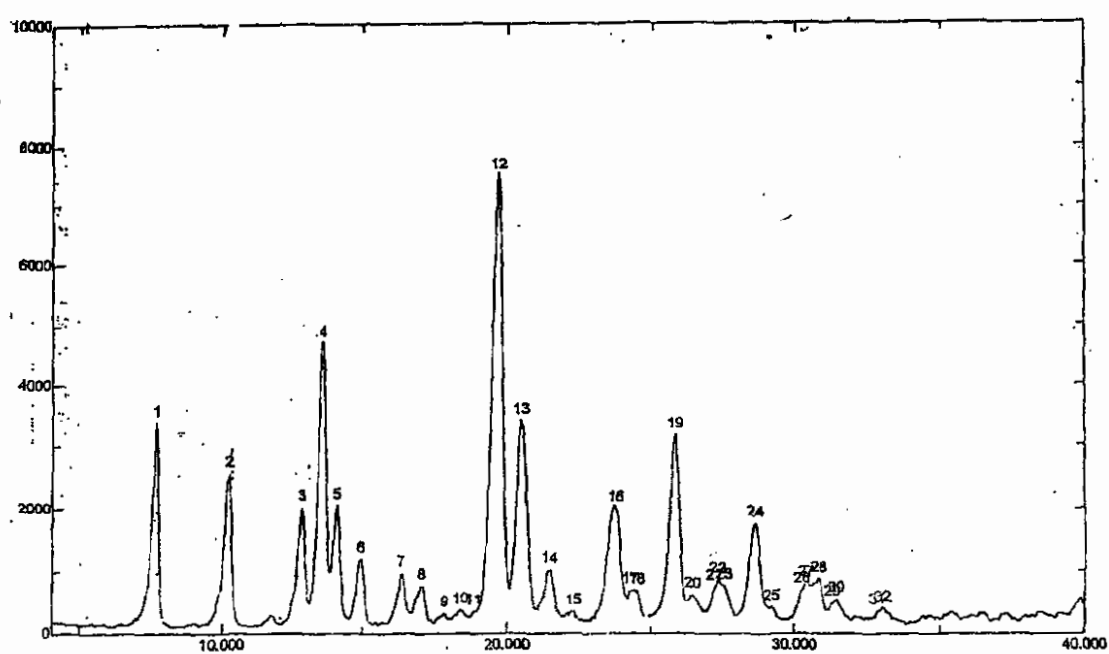


Figure 8.9 X-ray powder diffractogram 1 of form omega (Vakil *et al.*, 2004:4).

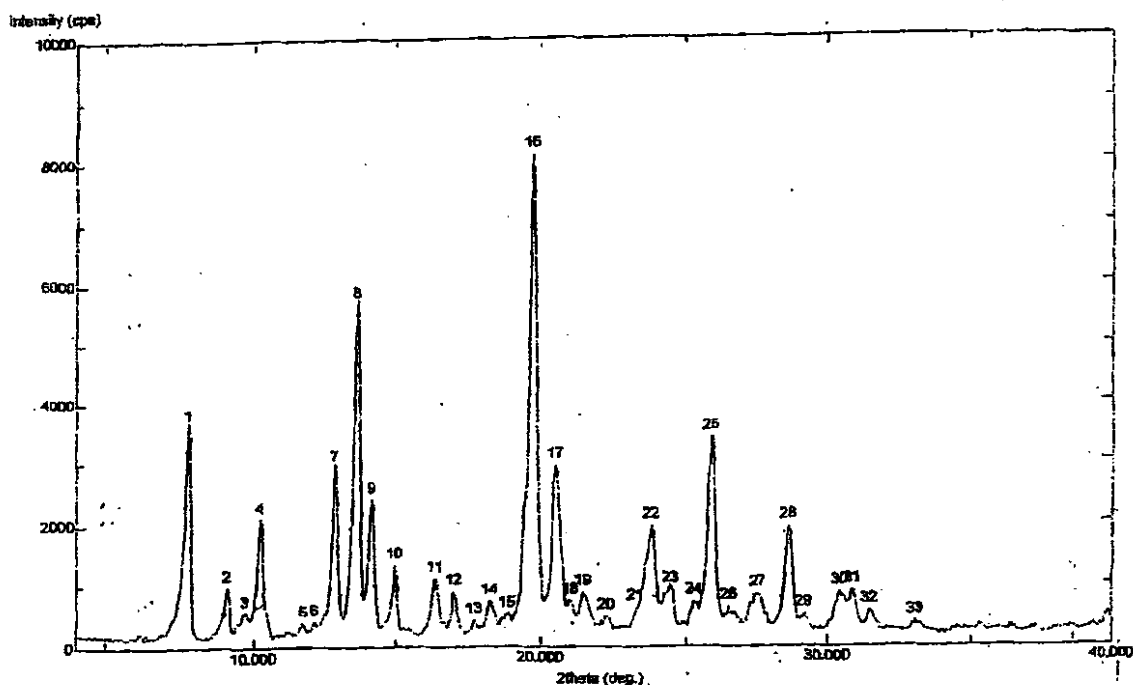


Figure 8.10 X-ray powder diffractogram 2 of form omega (Vakil *et al.*, 2004:4).

8.1 Materials and methods

8.1.1 Materials

Gatifloxacin sesquihydrate raw material was obtained from Dr. Reddy's Laboratories Ltd., India. The details of the raw material used during this study are as follows:

Batch number : GS002C05
 Manufacturing date : March 2005
 Expiry date : February 2010

Table 8.1 summarises the analytical results obtained prior to the release and distribution of the raw material by the supplier (certificate of analysis, or CoA).

Table 8.1 Certificate of analysis of gatifloxacin sesquihydrate raw material (Dr. Reddy's Laboratories Ltd., India, Certificate of analysis)

Test	Specification	Result
Description of the raw material	White to pale yellow coloured crystalline powder	Complies
Water content	6.00-9.00%	7.00%
Total impurities as determined by HPLC	≤1.00%	0.24%
Assay by HPLC	98.00-102.00%	100.30%
Residue on ignition	≤0.20%	0.09%
Heavy metals	≤20 ppm	Complies

The absolute methanol (boiling point 64.70°C) used in the recrystallisations was obtained from Saarchem, South Africa. Purified water was used in the recrystallisation with binary mixtures of methanol and water.

8.1.2 Methods

The crystal forms investigated in this study were recrystallised using the following method. Gatifloxacin sesquihydrate powder was added to small volumes of the recrystallisation solvent, whilst the solution was heated to the boiling point of the solvent under constant stirring by means of a magnetic stirrer until a saturated solution was obtained. The saturated solutions were prepared in glass beakers and covered with Parafilm® containing small holes to allow solvent evaporation at ambient conditions.

The various solvents used and the recrystallisation products obtained are listed in table 8.2.

Table 8.2 Solvents used and recrystallisation products obtained in this study

Solvent	Recrystallisation product
Absolute methanol	Product A
Methanol: water (75:25)	Product B
Methanol: water (50:50)	Product C

The crystals of each product were removed from the mother solvent and dried on filterpaper for approximately 30 minutes to ensure that the samples were free from any surface solvent, prior to the analysis. The following analytical techniques were used in this study: XRPD, VT-XRPD, DRIFTS, TGA, KF, and DSC. These techniques are described in chapter 3.

8.2 Results and discussion

8.2.1 Recrystallisation product A

The crystal form obtained from recrystallisation with absolute methanol is referred to as product A. The XRPD pattern for product A is displayed in figure 8.11. This crystal form displayed an XRPD pattern comparable to that of form F, with the exception of some small peaks that are absent in the diffractogram of form F, such as the peaks at $7.3^{\circ}2\theta$ and $21.1^{\circ}2\theta$.

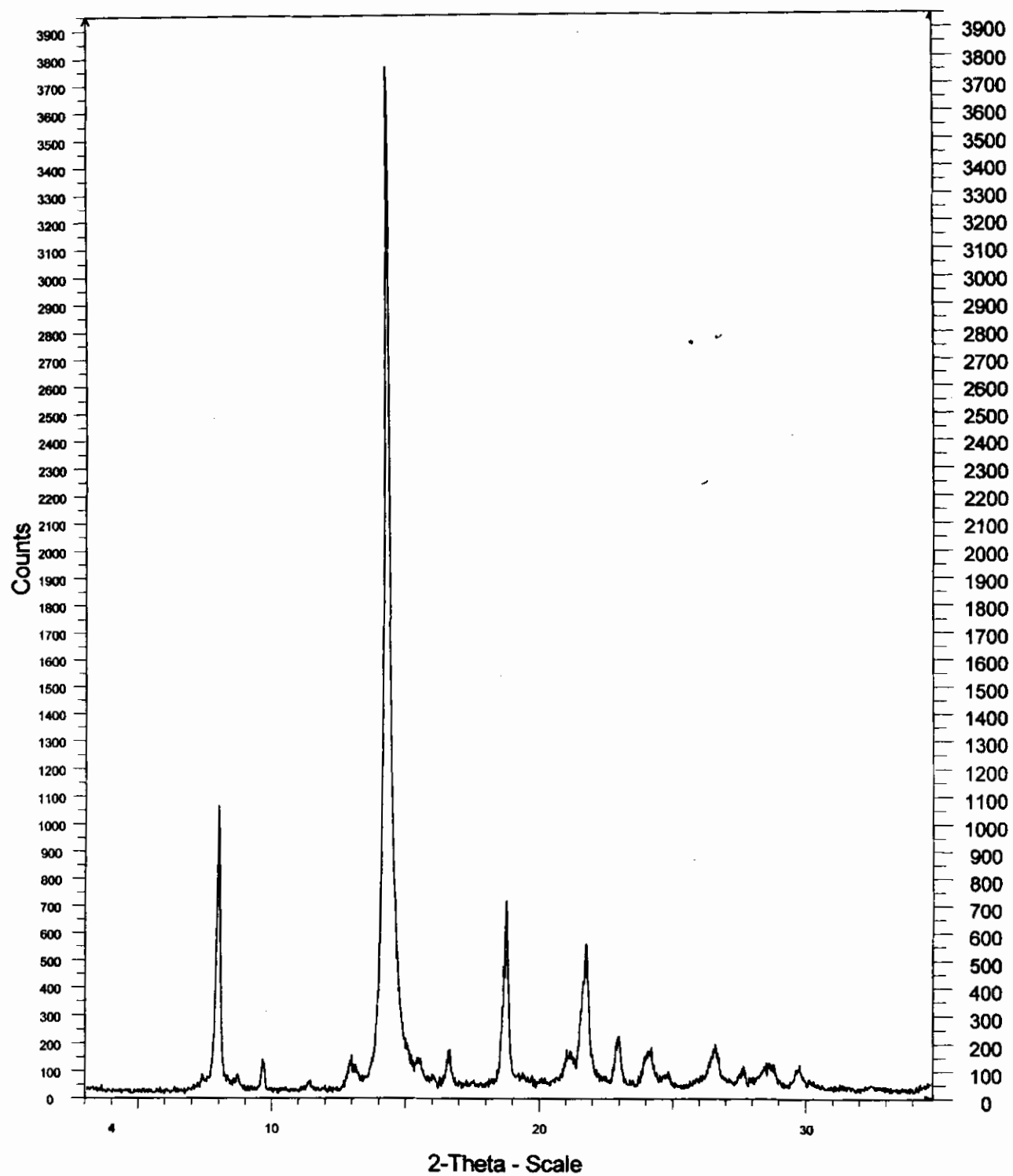


Figure 8.11 X-ray powder diffractogram of product A.

The DRIFT-IR spectrum of product A is displayed in figure 8.12.

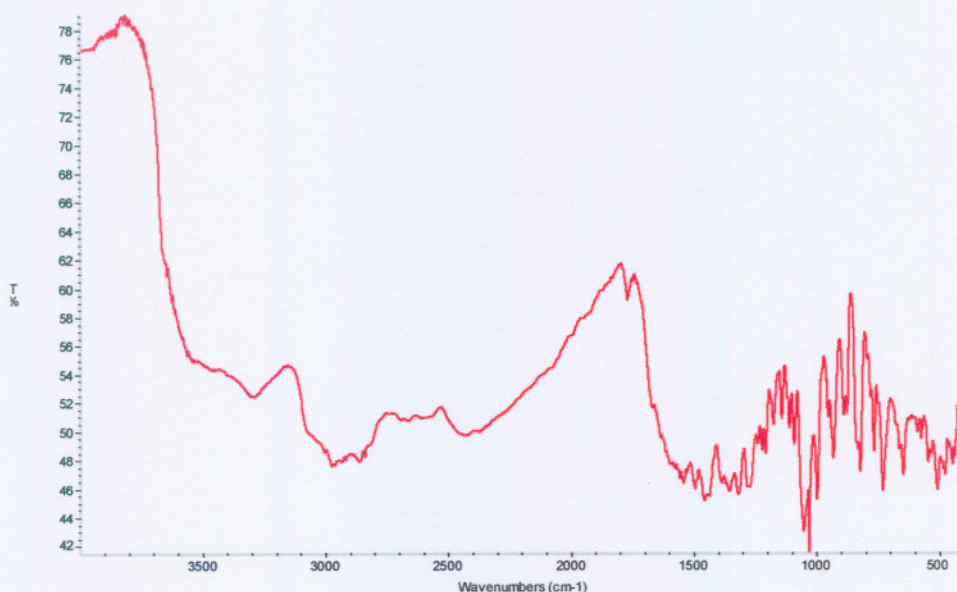


Figure 8.12 DRIFT-IR spectrum of product A.

TGA results of product A revealed that this was a solvated crystal form. The TGA thermogram of product A is displayed in figure 8.13.

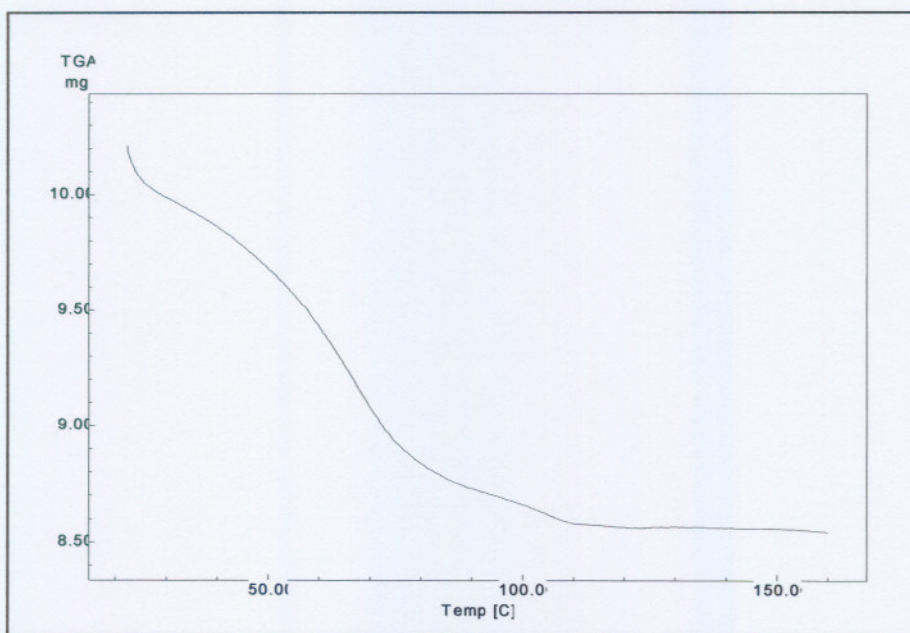


Figure 8.13 TGA thermogram of product A.

The TGA and KF results of product A are tabulated in table 8.3.

Table 8.3 TGA and KF results of product A

	Product A
% Weight loss (TGA)	14.51%
Moisture content (KF)	8.30%

The % weight loss observed with TGA was 14.51% and Karl Fischer titrations revealed a moisture content of 8.30% for product A. This KF value is comparable with the theoretical water content of a dihydrate, which is 9.15%. The rest of the weight loss observed with TGA might have been due to loss of methanol incorporated into the crystal lattice. This weight loss was 6.21%, which is comparable with the theoretical weight loss (7.86%) of a methanol solvate (1:1). Product A could therefore be classified as a possible hydrated solvate (2 water : 1 methanol : 1 gatifloxacin) or a mixture of hydrated and solvated forms.

The DSC thermogram of product A is displayed in figure 8.14.

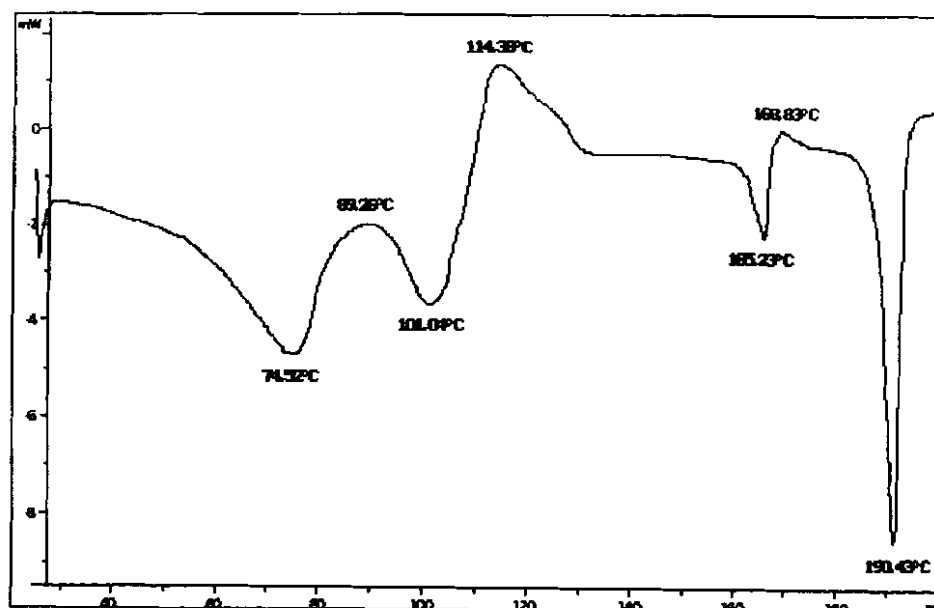


Figure 8.14 DSC thermogram of product A.

Product A was exposed to ambient conditions for one hour after which it was analysed again. The XRPD pattern of this sample (referred to as product A₂) was comparable to that of form F described in the literature, and is displayed in figure 8.15. Figure 8.16 displays an overlay of product A and product A₂.

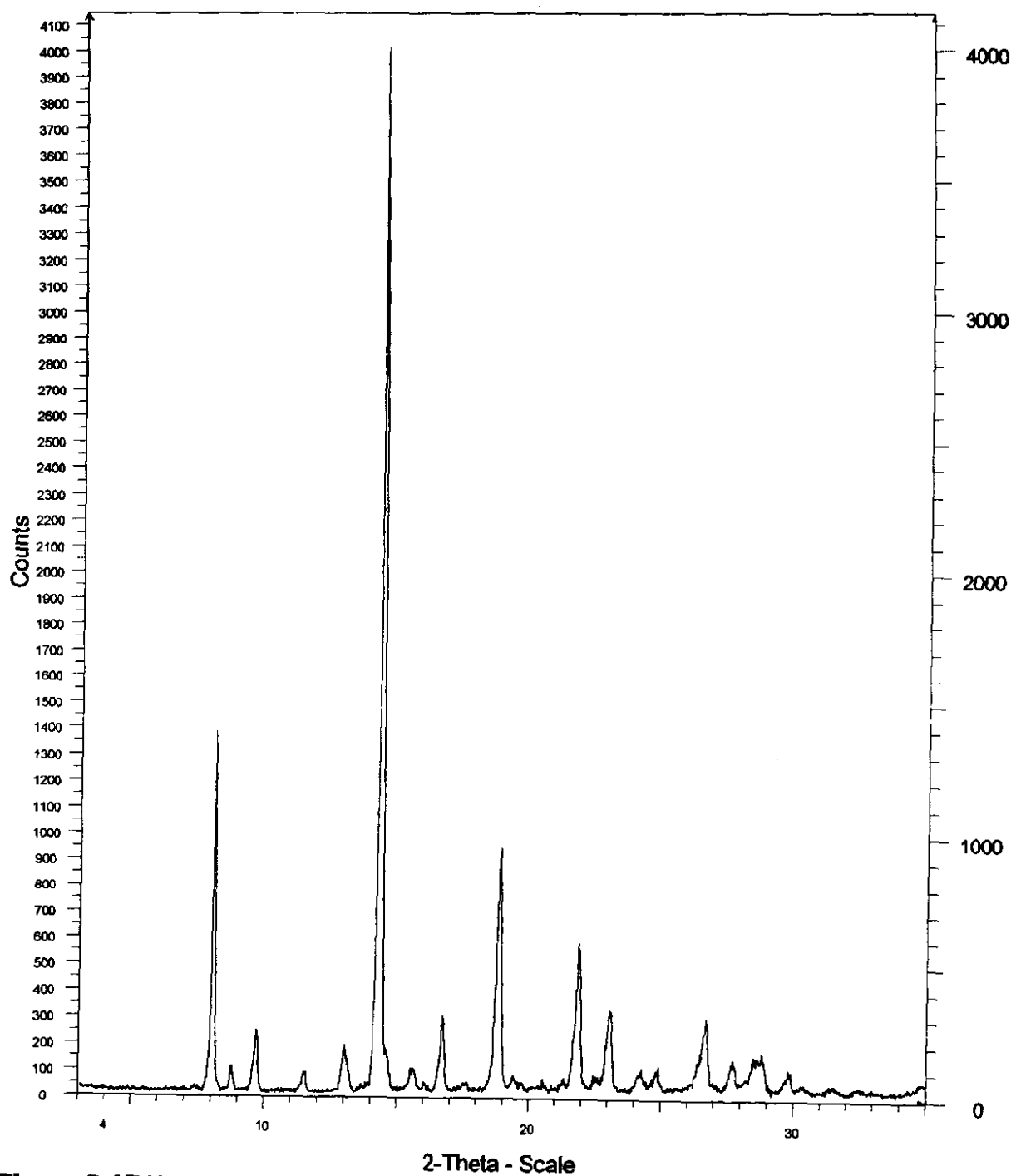


Figure 8.15 X-ray powder diffractogram of product A₂.

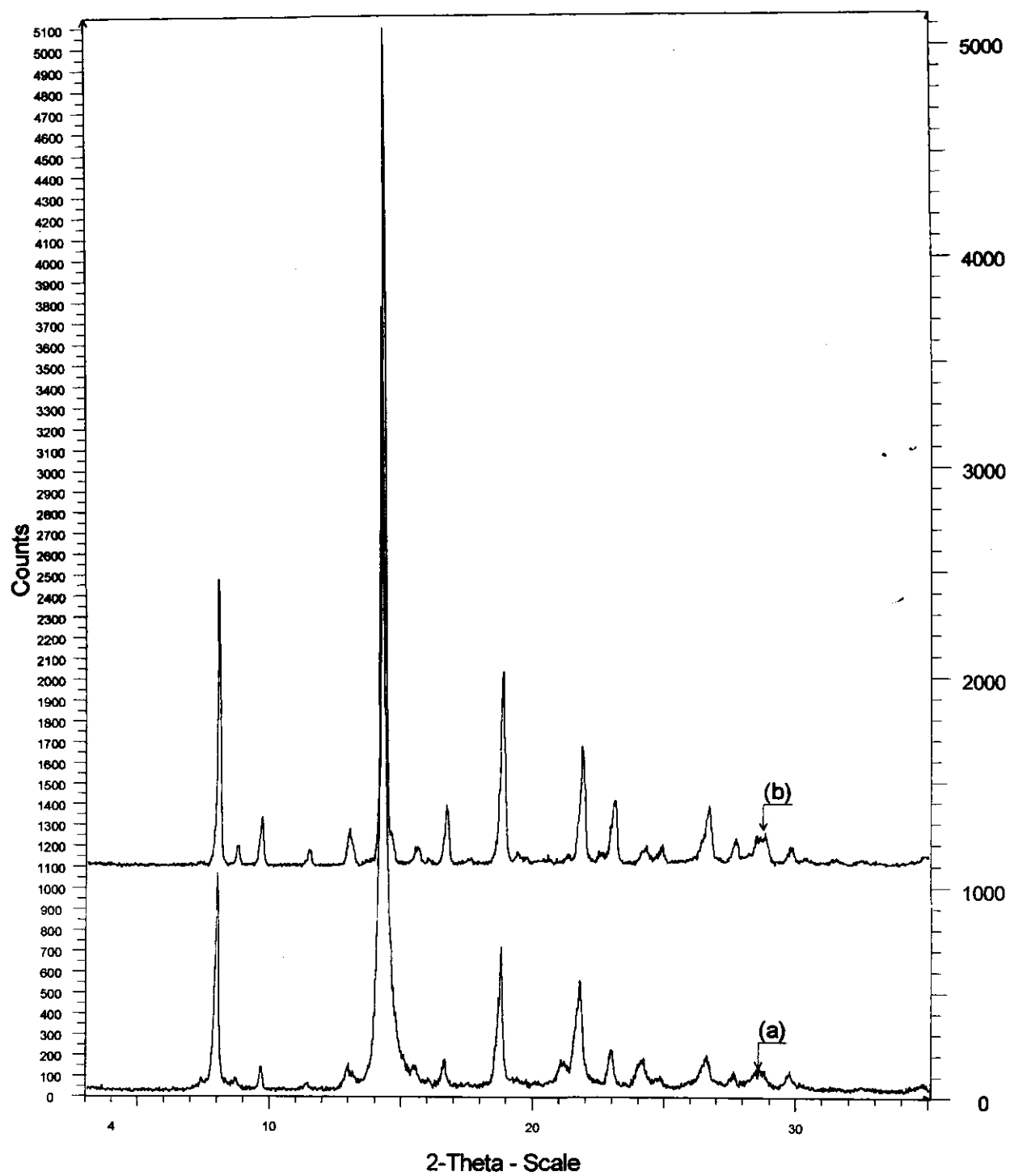


Figure 8.16 Overlay of the x-ray powder diffractograms of (a) product A and (b) product A₂.

The DRIFT-IR spectrum of product A₂ is displayed in figure 8.17 and figure 8.18 displays an overlay of the DRIFT-IR spectra of product A and product A₂.

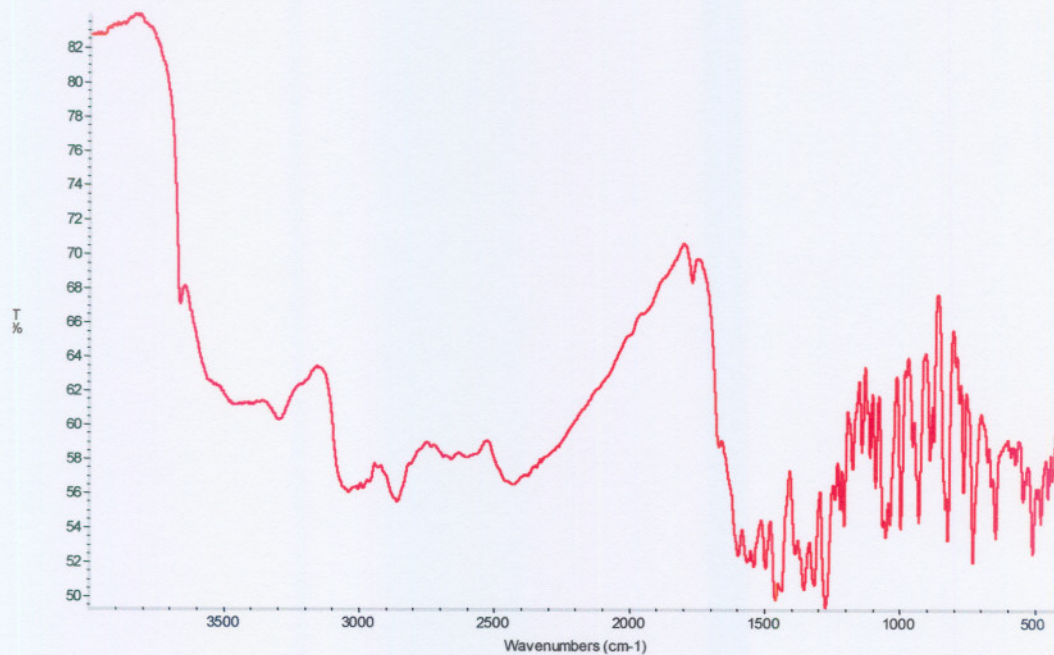


Figure 8.17 DRIFT-IR spectrum of product A₂.

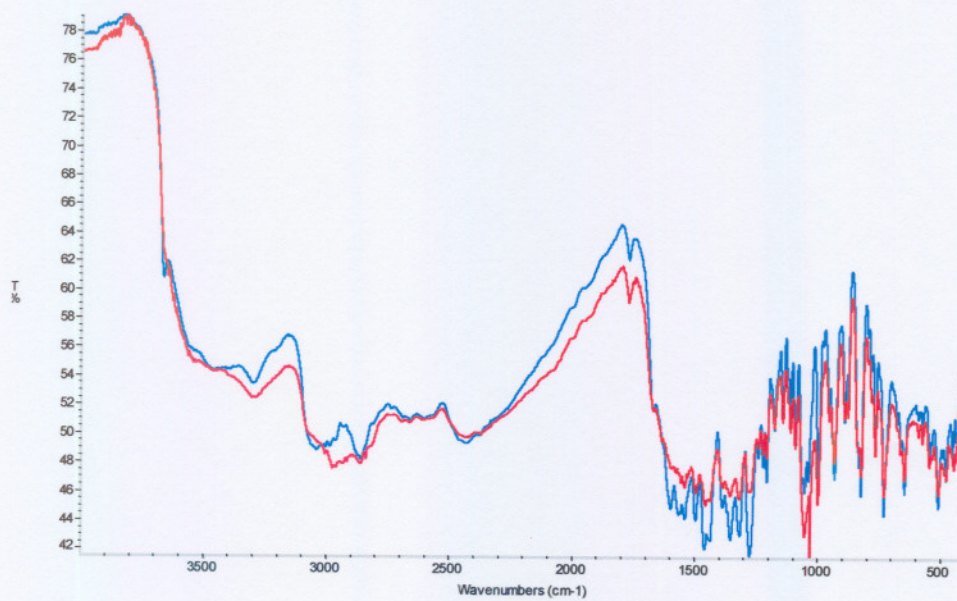


Figure 8.18 Overlay of the DRIFT-IR spectra of product A (red) and product A₂ (blue).

The TGA and DSC thermograms of form F presented in the literature suggested that form F is a solvated crystal form. This study confirmed that form F is indeed a solvated crystal form. Table 8.4 displays the TGA and KF results of product A₂ (form F).

Table 8.4 TGA and KF results of product A₂

	Product A ₂
% Weight loss (TGA)	12.58%
Moisture content . (KF)	13.02%

TGA results indicated a total % weight loss of 12.58% and KF titrations displayed a moisture content of 13.02% for this sample, which is comparable to the theoretical moisture content of a trihydrate (13.74%). These corresponding results thus indicated that the solvent incorporated into the crystal lattice was water. Product A₂ may thus be classified as a trihydrate. A possible reason for this uptake of water could be that the crystals of product A was highly higroscopic (product A displayed a water content comparable to that of a dihydrate). The TGA and DSC thermograms of this crystal form are displayed in figures 8.19 and 8.20 respectively.

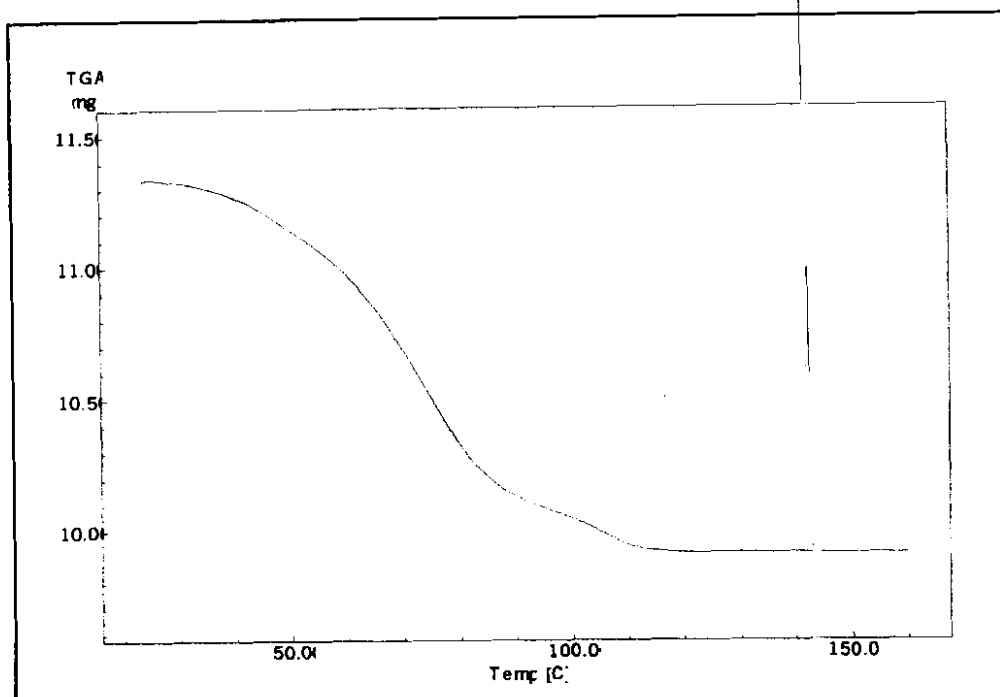


Figure 8.19 TGA thermogram of product A₂.

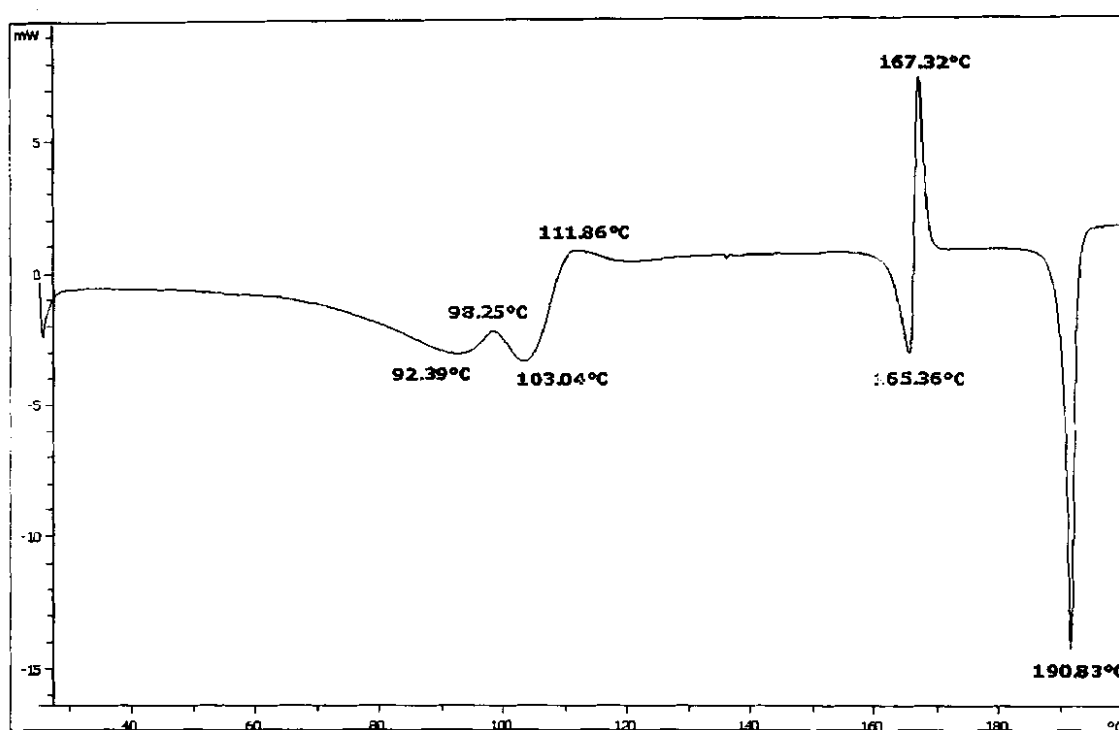


Figure 8.20 DSC thermogram of product A₂.

8.2.2 Recrystallisation product B

Product B was recrystallised from a methanol:water mixture (75:25) and displayed an XRPD pattern comparable to that of form F. The X-ray powder diffractogram of this form is displayed in figure 8.21. The XRPD pattern of product B was comparable

to that of product A₂. Figure 8.22 displays an overlay of the XRPD patterns of product A₂ and product B.

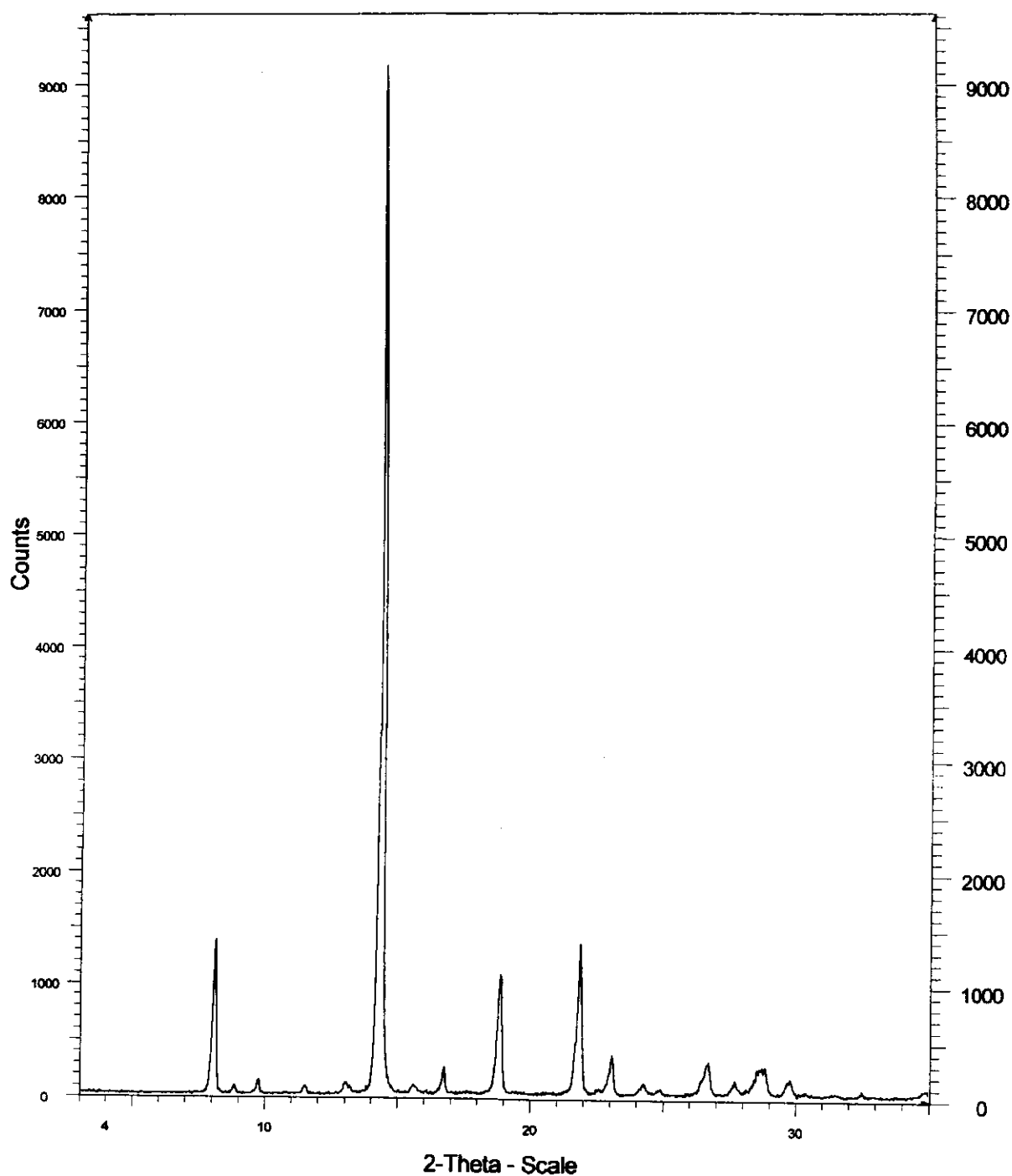


Figure 8.21 X-ray powder diffractogram of product B.

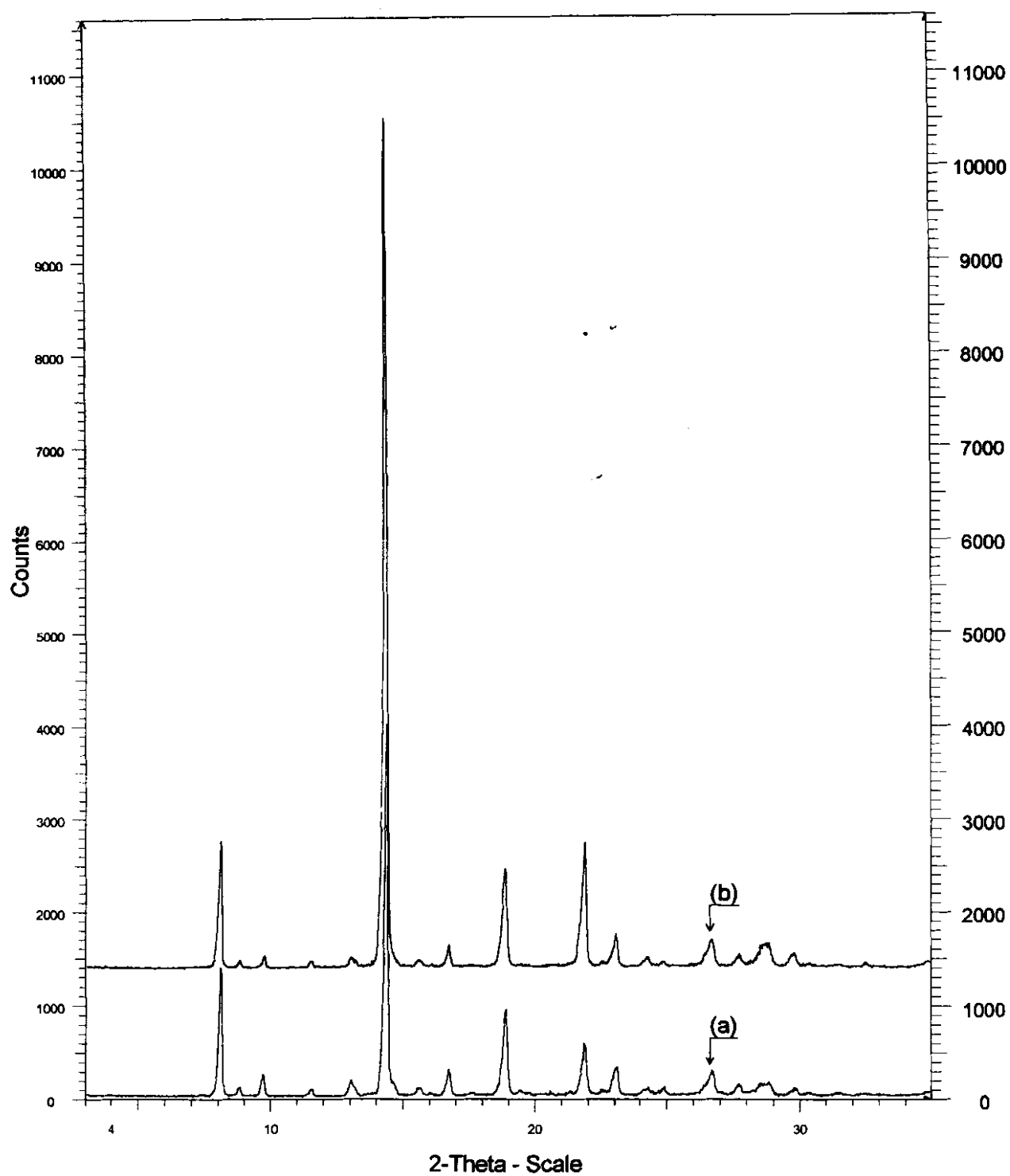


Figure 8.22 Overlay of the x-ray powder diffractograms of (a) product A₂ and (b) product B.

The DRIFT-IR spectrum of product B is displayed in figure 8.23. Figure 8.24 displays an overlay of the DRIFT-IR spectra of product A₂ and product B.

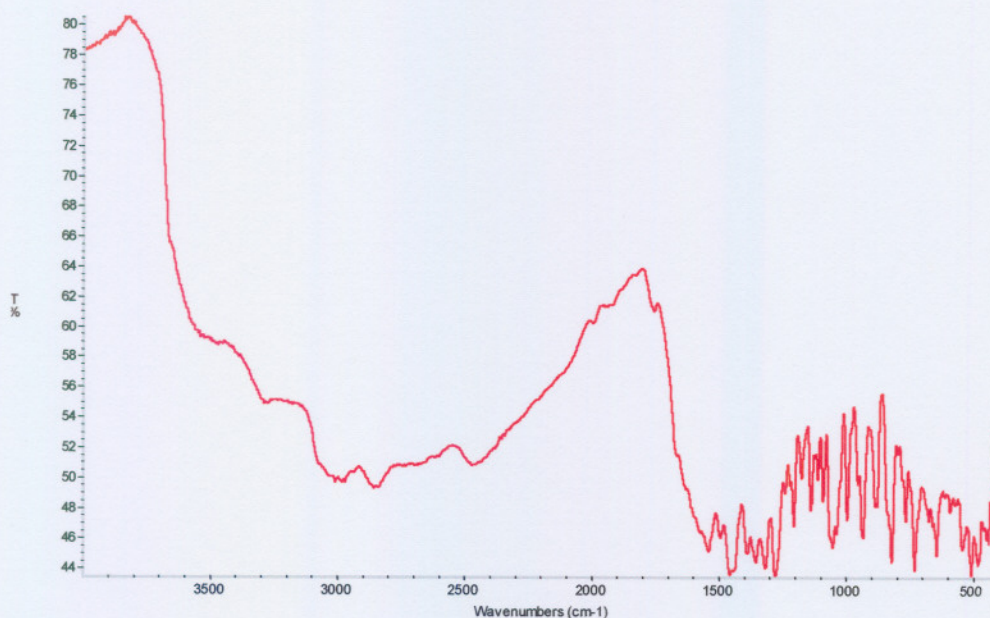


Figure 8.23 DRIFT-IR spectrum of product B.

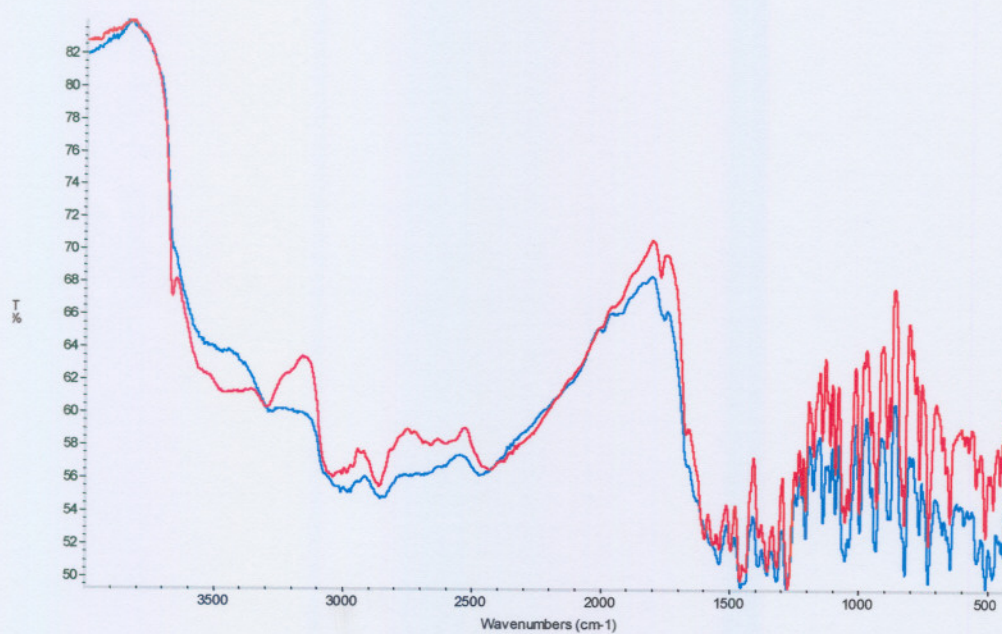


Figure 8.24 Overlay of the DRIFT-IR spectra of product A₂ (red) and product B (blue).

The TGA and KF results for product B are summarised in table 8.5. TGA revealed a total weight loss of 13.09 % and the total moisture content of the sample (as determined by KF) was 13.38 %. This correlated with the theoretical moisture content of a trihydrate, which is 13.74%. The correlation between the KF and TGA results indicated that water was the only solvent incorporated into the crystal lattice of this form and product B may therefore be classified as a trihydrate.

Table 8.5 TGA and KF results for product B

	Product B
% Weight loss (TGA)	13.09 %
Moisture content (KF)	13.38 %

The TGA thermogram of product B is displayed in figure 8.25.

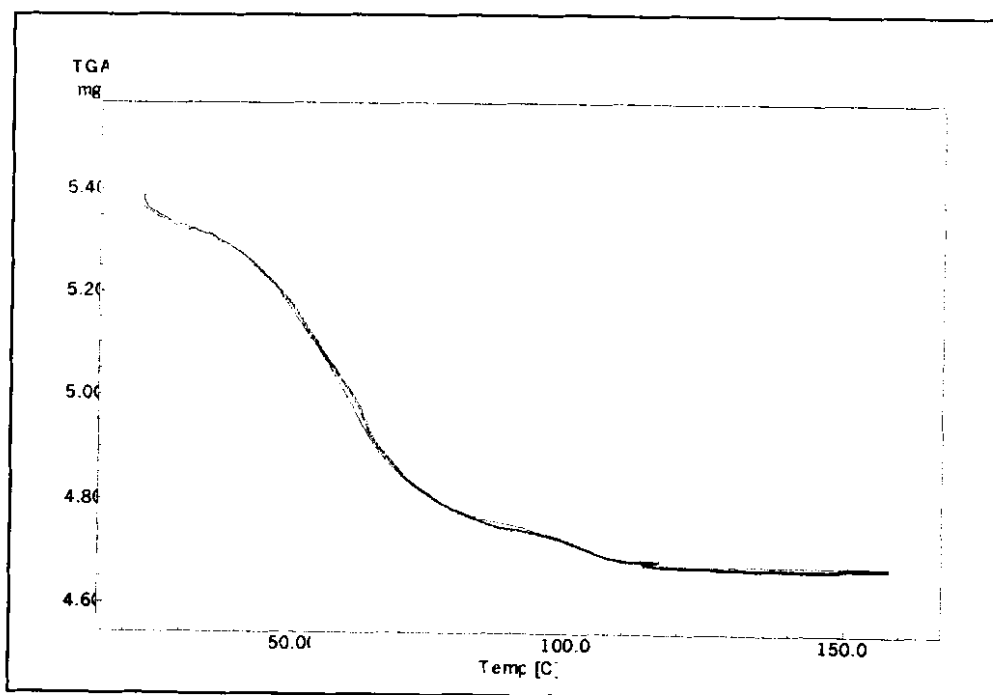


Figure 8.25 TGA thermogram of product B.

The DSC thermogram of product B is shown in figure 8.26. Minor differences were observed between the DSC thermograms of product A₂ and product B. The differences between the two thermograms are mostly present in the region of the desolvation endotherms and up to 120°C. Possible reasons for the slight differences observed in the thermograms of these two forms include the following: Slight differences in the piercing of the sample pan lid or the crimping of the sample pan that could have led to differences in the dehydration environment thus influencing the dehydration process.

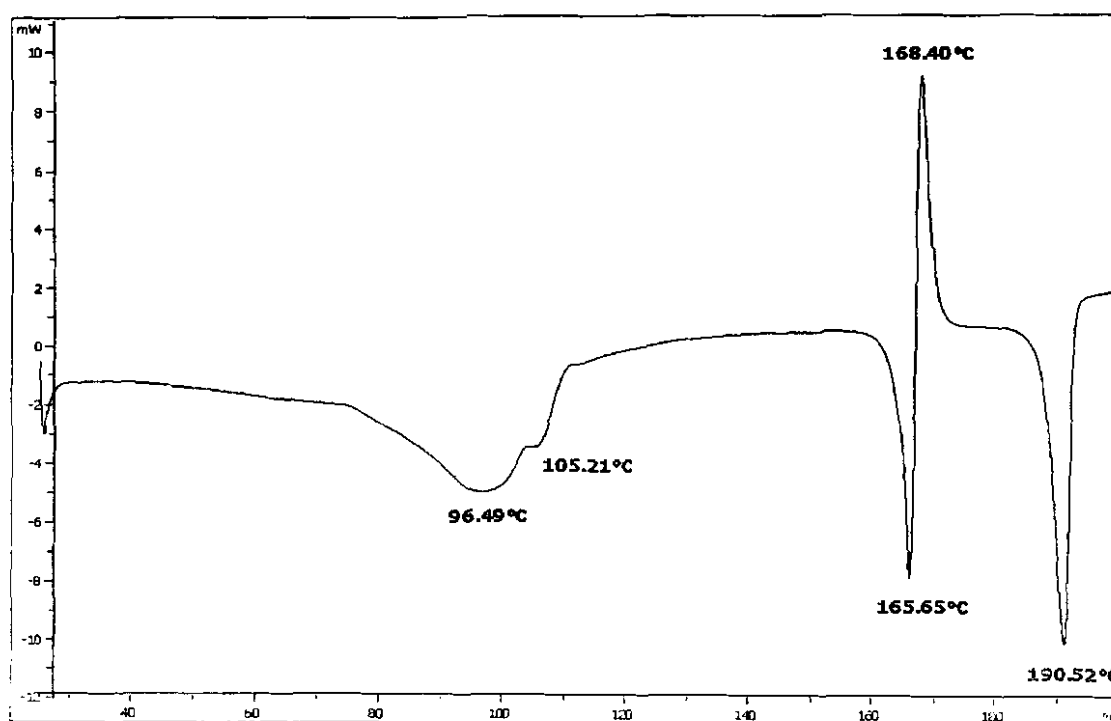


Figure 8.26 DSC thermogram of product B.

8.2.3 Recrystallisation product C

Figure 8.27 displays the x-ray powder diffractogram of product C, which was recrystallised from a binary methanol:water mixture (50:50). The XRPD pattern of product C differed from that of product A₂ and product B. The most noticeable differences were in the area between 3.0-10.0°2θ, the peak at 19.9°2θ, and the area between 24.0-26.0°2θ.

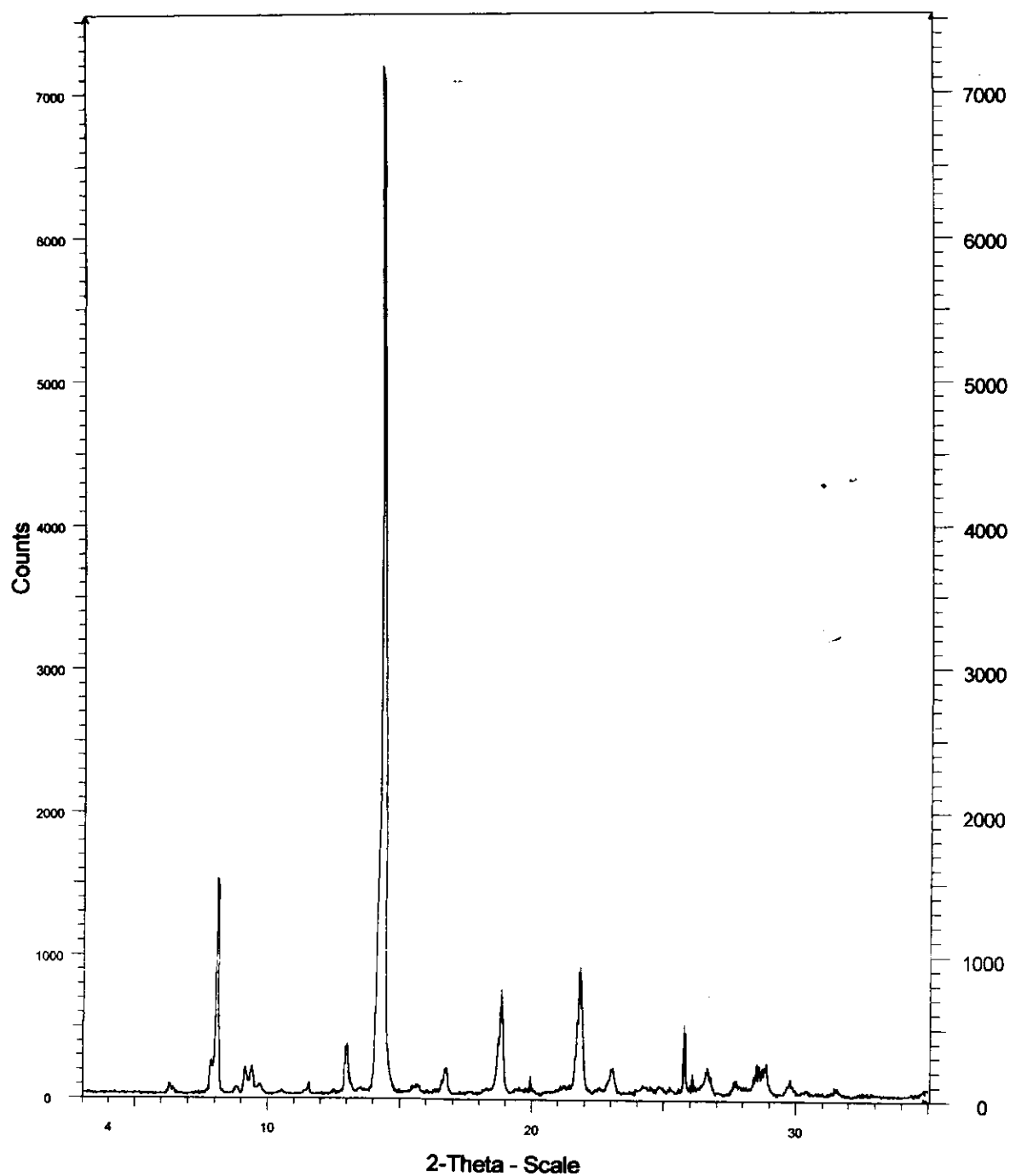


Figure 8.27 X-ray powder diffractogram of product C.

The DRIFT-IR spectrum of product C is displayed in figure 8.28.

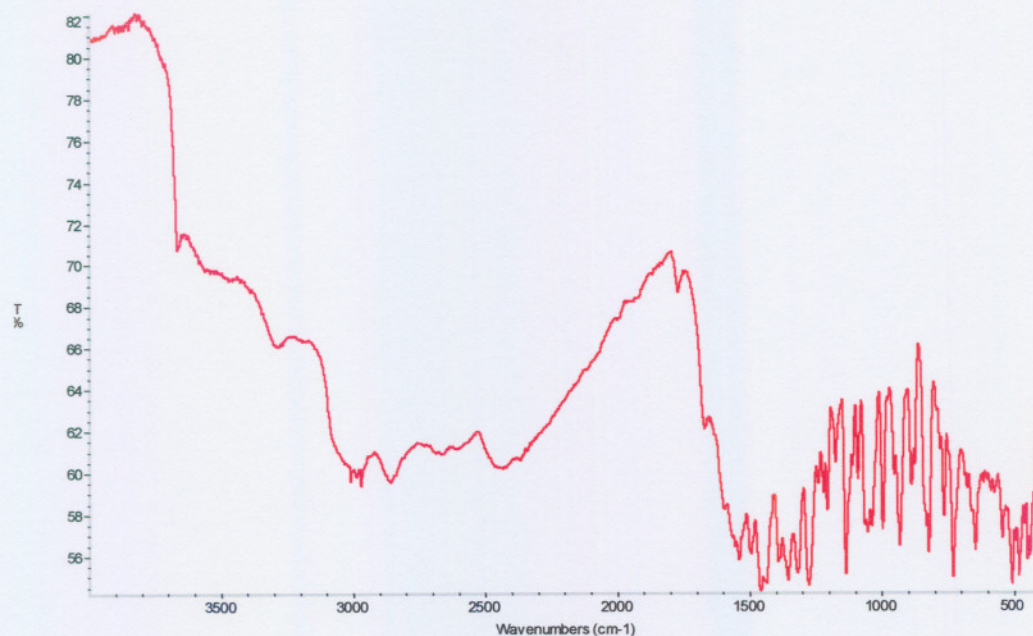


Figure 8.28 DRIFT-IR spectrum of product C.

The TGA and KF results for this crystal form are displayed in table 8.6.

Table 8.6 TGA and KF results of product C

	Product C
% Weight loss (TGA)	13.12%
Moisture content (KF)	13.07%

Thermogravimetric analysis of the sample revealed a total weight loss of 13.12% and the moisture content of the sample was determined as 13.07%. This value was found to be comparable to the theoretical moisture content of a trihydrate (13.74%), and this form may therefore also be classified as a trihydrate. The TGA thermogram of this crystal form is displayed in figure 8.29.

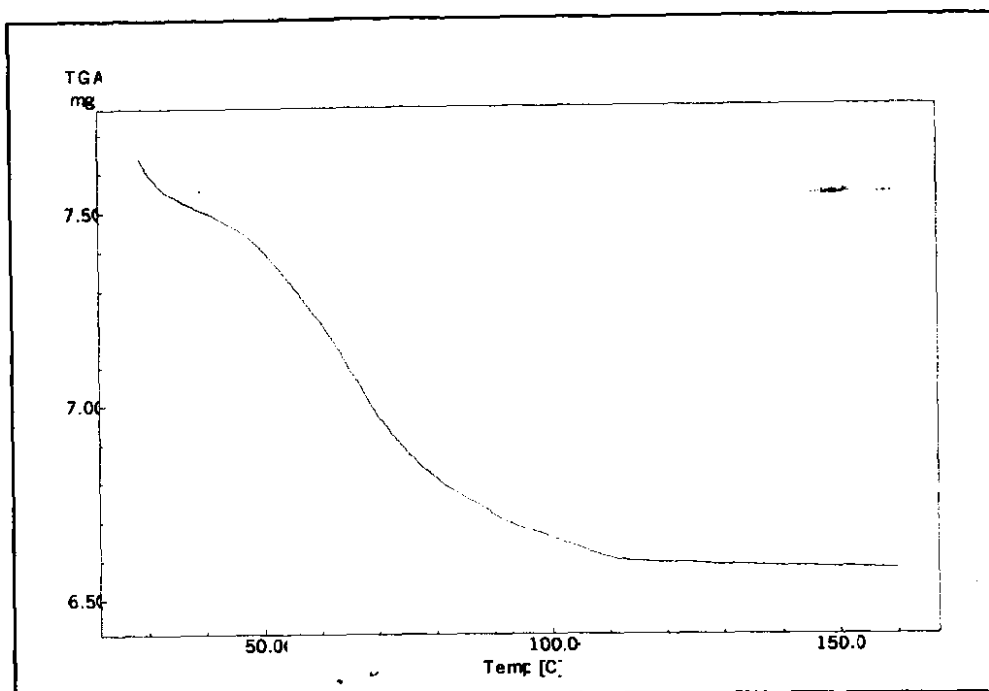


Figure 8.29 TGA thermogram of product C.

The DSC thermogram of product C is displayed in figure 8.30. Differences in the dehydration endotherms and up to 120°C were observed when compared to the thermogram of form F.

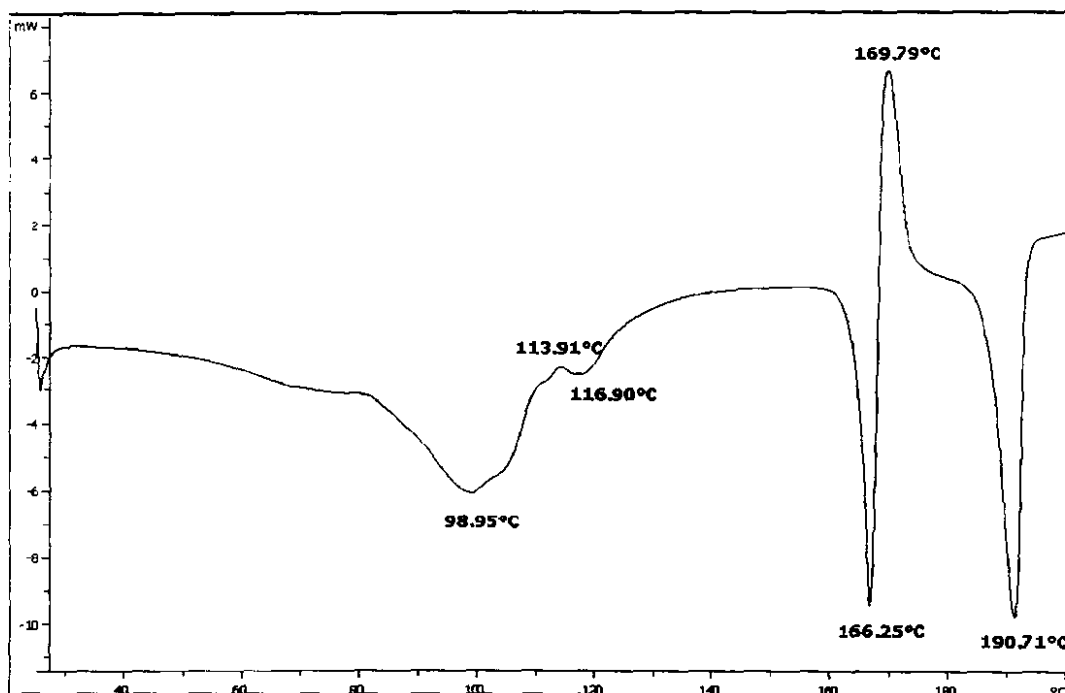


Figure 8.30 DSC thermogram of product C.

8.2.4 Comparison of the crystal forms obtained from the various solvents

Table 8.7 displays the main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I₀) of the various crystal forms obtained in this study.

Table 8.7 The main XRPD peak angles ($^{\circ}2\theta$) and relative intensity values (I/I₀) of the various recrystallisation products investigated in this study

Main peaks	Product A		Transformed product A		Product B		Product C	
	($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)	($^{\circ}2\theta$)	(I/I ₀)
1	3.2	0.9	-	-	-	-	3.2	0.4
2	-	-	-	-	-	-	3.3	0.3
3	-	-	-	-	-	-	6.2	1.2
4	7.3	2.1	7.3	0.8	-	-	-	-
5	7.9	28.1	-	-	-	-	7.8	3.5
6	-	-	8.0	34.6	8.0	15.5	8.0	21.2
7	8.7	2.1	8.7	2.7	8.8	1.0	8.8	0.9
8	-	-	-	-	-	-	9.1	2.8
9	-	-	-	-	-	-	9.3	2.9
10	9.6	3.4	9.6	6.1	9.7	1.6	9.6	1.2
11	-	-	-	-	-	-	10.5	0.5
12	-	-	-	-	10.7	0.3	-	-
13	11.4	1.5	11.5	2.2	11.5	1.0	11.5	1.3
14	-	-	-	-	-	-	12.5	0.6
15	12.9	3.6	13.0	4.7	13.0	1.3	13.0	5.1
16	-	-	-	-	-	-	13.4	0.9
17	-	-	13.6	1.0	-	-	-	-
18	-	-	13.7	0.8	-	-	-	-
19	14.2	100.0	14.3	100.0	14.3	100.0	14.3	100.0
20	15.5	3.8	15.6	2.5	15.6	1.1	15.6	1.3
21	16.0	2.1	16.0	1.2	-	-	-	-
22	16.6	4.4	16.7	7.5	16.7	2.9	16.7	2.9
23	17.5	1.6	17.6	1.3	-	-	17.6	0.6
24	-	-	-	-	-	-	18.2	0.9
25	18.8	18.9	18.8	23.6	18.8	12.1	18.8	10.5
26	-	-	19.4	2.0	-	-	19.4	0.8
27	-	-	-	-	-	-	19.9	2.1
28	-	-	20.6	1.6	-	-	20.6	0.7
29	-	-	-	-	-	-	20.7	0.7
30	21.1	3.8	-	-	-	-	-	-
31	-	-	21.3	1.8	-	-	-	-

Table 8.7 (continued)

Main peaks	Product A		Transformed product A		Product B		Product C	
	(°2 θ)	(I/I ₀)	(°2 θ)	(I/I ₀)	(°2 θ)	(I/I ₀)	(°2 θ)	(I/I ₀)
32	21.6	8.2	-	-	-	-	-	-
33	21.8	14.7	21.9	14.6	21.9	15.3	21.8	12.6
34	-	-	22.6	1.9	22.6	0.9	22.6	1.1
35	23.0	5.8	23.1	8.2	23.1	4.2	23.0	2.9
36	24.2	4.5	24.3	2.7	24.3	1.5	24.2	1.2
37	24.8	2.4	24.9	2.4	24.9	0.9	24.8	1.2
38	-	-	-	-	-	-	25.2	1.1
39	-	-	-	-	-	-	25.8	7.1
40	-	-	-	-	-	-	26.1	2.4
41	26.6	5.1	26.7	7.5	26.7	3.6	26.7	3.0
42	27.7	3.0	27.7	3.7	27.7	1.8	27.7	1.8
43	-	-	28.6	3.9	-	-	28.6	2.3
44	28.8	3.2	28.8	4.3	28.8	3.1	28.9	3.1
45	29.8	2.6	29.9	2.7	29.8	2.0	29.8	2.0
46	-	-	30.4	1.3	-	-	30.4	0.9
47	-	-	31.6	1.3	31.5	0.6	31.5	1.1
48	-	-	-	-	32.5	0.9	-	-
49	34.4	1.0	-	-	-	-	-	-
50	34.8	1.6	35.0	1.5	34.9	1.0	34.9	1.0

An overlay of the XRPD patterns of the various crystal forms is displayed in figure 8.31.

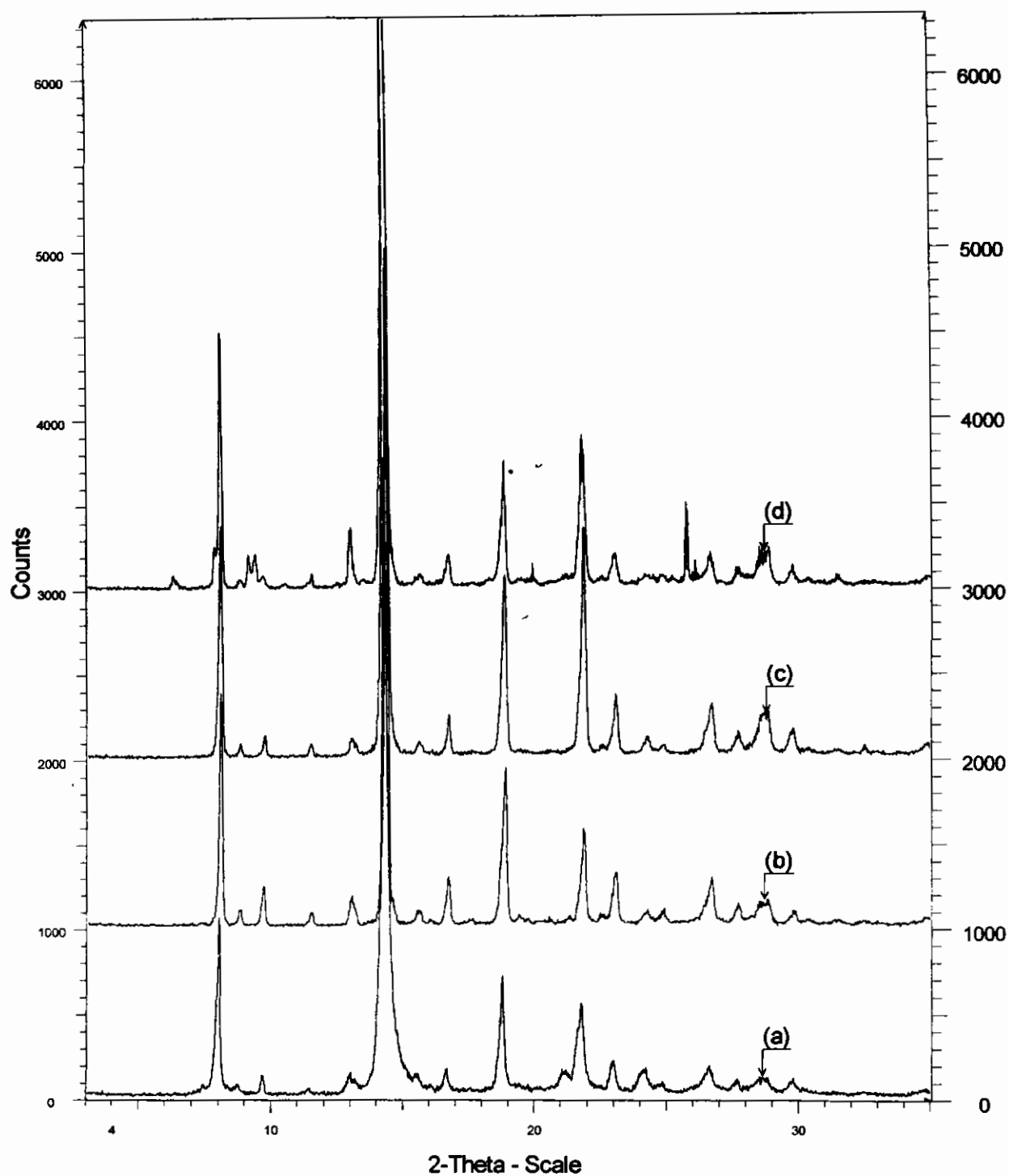


Figure 8.31 Overlay of the x-ray powder diffractograms of (a) product A, (b) product A₂, (c) product B, and (d) product C.

Table 8.8 displays the main absorptions in the DRIFT-IR spectra of the various products in this study.

Table 8.8 Main absorptions in the DRIFT-IR spectra of the various recrystallisation products obtained in this study

Main absorptions	Wavenumber (cm ⁻¹)			
	Product A	Product A ₂	Product B	Product C
1	-	3304	-	-
2	3293	-	-	-
3	-	3046	-	-
4	2981	-	-	2975
5	-	2864	2861	2863
6	2439	2436	-	2438
7	1773	1772	-	1773
8	-	1601	-	-
9	1545	1544	1545	1544
10	1499	1501	-	1500
11	1460	1463	1460	1460
12	-	-	-	1396
13	-	1358	1358	1358
14	1322	1321	1322	1323
15	1284	1278	1282	1279
16	1226	1225	-	1225
17	1210	1210	1210	1210
18	1179	1179	1180	1179
19	1146	1146	1143	1140
20	1113	1113	1114	-
21	1093	1093	1095	1094
22	1056	1056	1056	1056
23	-	1040	-	-
24	1033	-	-	-
25	1002	1001	1000	1001
26	-	958	-	-
27	934	933	937	935
28	892	892	-	891
29	-	878	882	-
30	826	827	825	826
31	770	769	770	770
32	732	733	733	733
33	650	650	648	650
34	577	576	-	-

Table 8.8 (continued)

Main absorptions	Wavenumber (cm ⁻¹)			
	Product A	Product A ₂	Product B	Product C
35	548	548	546	547
36	512	513	512	510
37	-	482	486	486
38	450	456	-	452
39	-	441	444	-
40	-	-	432	-
41	420	423	-	-
42	-	-	407	405

8.2.5 Solid state phase transformations of form F

8.2.5.1 Effect of recrystallisation period (harvesting time) on form F stability

Gatifloxacin was recrystallised in duplicate using absolute methanol as recrystallisation solvent. The first batch of crystals (referred to as product A) was harvested after 5 days and revealed an XRPD pattern comparable to that of form F (results discussed in section 8.2.1).

The second batch of crystals was harvested after 30 days. These crystals (referred to as product A₃) displayed an XRPD pattern (figure 8.32), which at first glance seemed to be comparable to that of form F. The TGA and KF results of product A₃ are summarised in table 8.9 and figure 8.33 displays the TGA thermogram of product A₃.

The TGA and KF values of product A₃ were slightly lower compared to the water content of form F prepared (products A₂ and B). A closer inspection of the x-ray powder diffractogram (of product A₃) revealed that peaks at 17.2 and 17.6°2θ (characteristic of form G) had started to emerge. Thus it can be assumed that product A₃ consisted of a mixture of form F and form G (form F >>> form G). This could elucidate the lower water content of this sample, seeing that form G displayed lower TGA and KF values compared to form F.

As discussed in the introduction to this chapter, form F can be transformed to form G. Thus from the results obtained it can be assumed that the aging of crystals (recrystallised from absolute methanol) could influence the crystal modification obtained for example: 5 days after recrystallisation pure form F crystals were obtained, 30 days after recrystallisation a small fraction of form F crystals transformed into form G, producing a mixture of forms F and G.

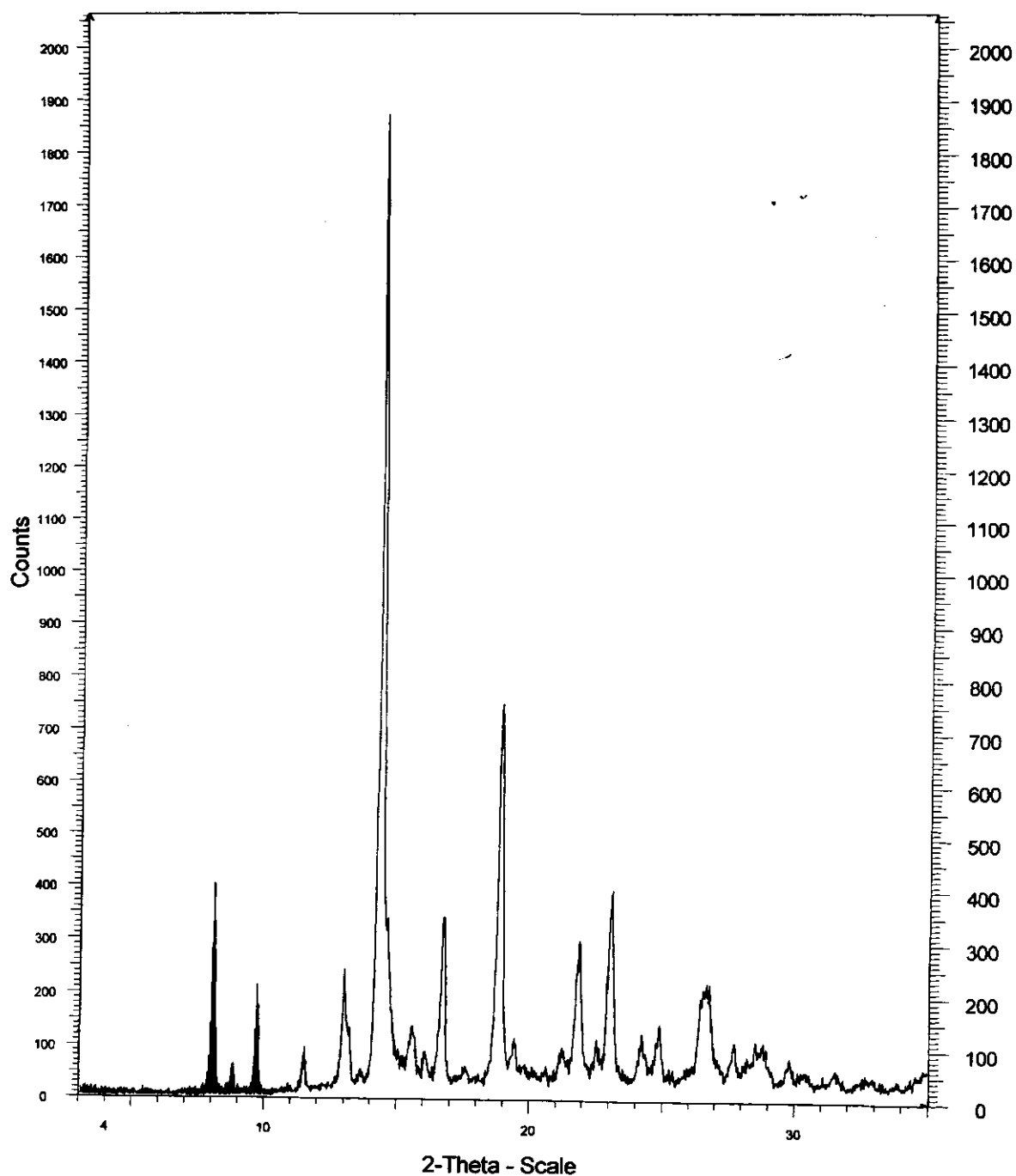


Figure 8.32 X-ray powder diffractogram of product A₃.

Table 8.9 TGA and KF results of product A₃

	Product A ₃
% Weight loss (TGA)	11.53%
Moisture content (KF)	10.65%

The TGA and KF results of product A₃ indicated that this was a hydrated crystal form.

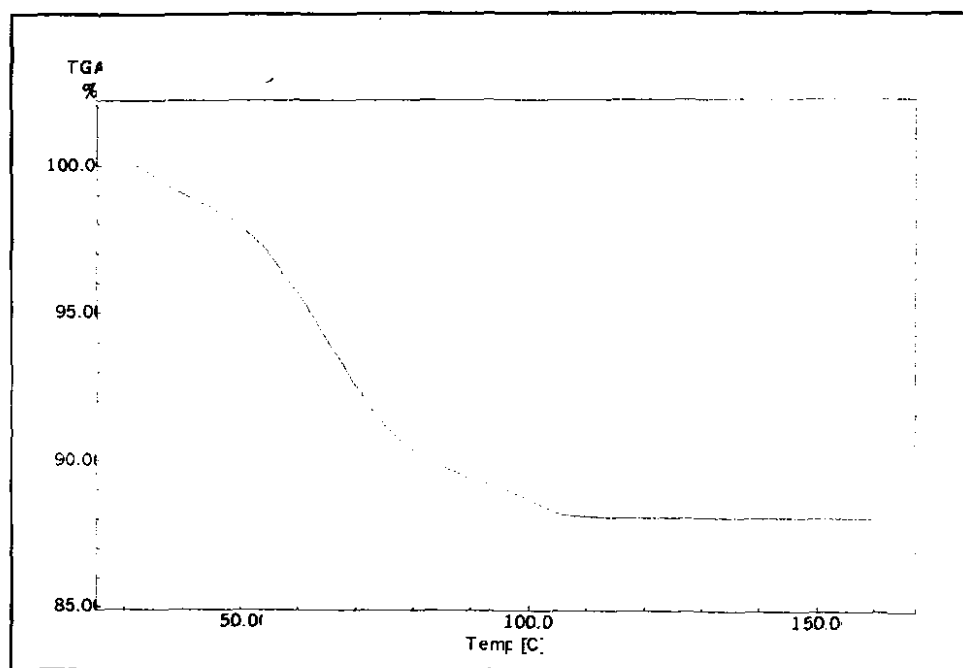


Figure 8.33 TGA thermogram of product A₃.

Product A₃ was exposed to ambient conditions for one hour and analysed. This sample is referred to as product A₃₀. The x-ray powder diffractogram obtained suggested product A₃₀ to be a mixture of form F and form G (figure 8.34). Most of the characteristic peaks of form F (such as the peaks at 7.9°2 θ , 14.2°2 θ , 18.8°2 θ , and 21.8°2 θ) were present in the diffractogram, and the intensities of the characteristic peaks of form G (17.2°2 θ and 17.6°2 θ) increased significantly,

suggesting that the form G fraction increased in the mixture (figure 8.35). Figure 8.35 displays an overlay of the XRPD patterns of product A₃ (mixture: form F>>>form G), product A_{3D} (mixture: form F & G) and product A₄ (form G) (please note that product A₄ will be discussed later in this section).

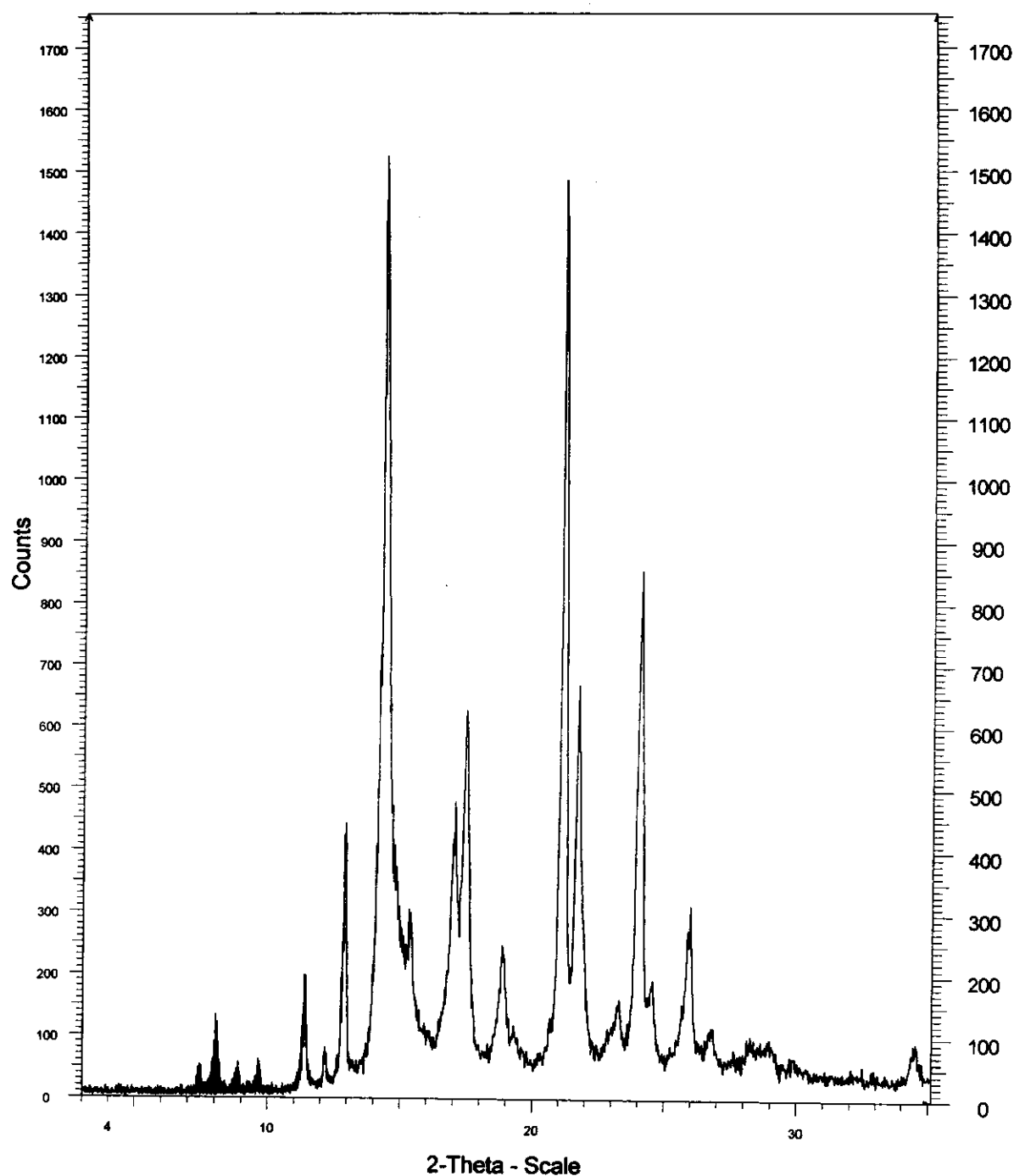


Figure 8.34 X-ray powder diffractogram of product A_{3D}.

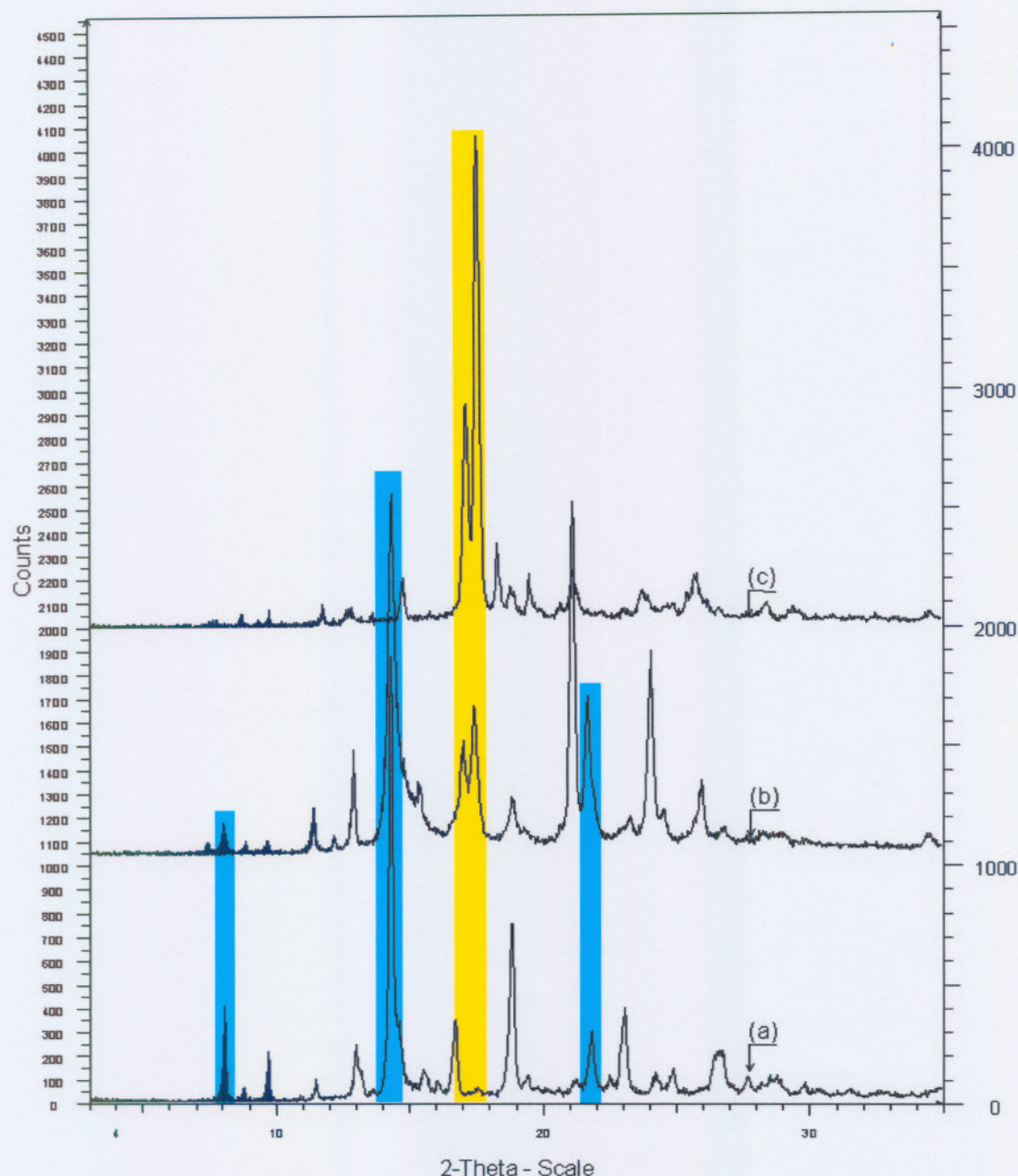


Figure 8.35 Overlay of the x-ray powder diffractograms of product A_3 (a), product A_{3D} (b) and product A_4 (c). The characteristic peaks of form F present in the diffractogram are highlighted in blue and the characteristic peaks of form G present in the diffractogram are highlighted in yellow.

The TGA and KF results of product A_3 , product A_{3D} and product A_4 are tabulated in table 8.10. The TGA thermogram of product A_{3D} is shown in figure 8.36.

Table 8.10 TGA and KF results of product A₃, product A_{3D}, and product A₄

	Product A ₃	Product A _{3D}	Product A ₄
% Weight loss (TGA)	11.53%	6.88%	3.17%
Moisture content (KF)	10.65%	6.82%	3.03%

Product A_{3D} displayed a weight loss of 6.88% (TGA), which correlated with the moisture content (KF) of the sample (6.82%). Table 8.10 revealed that the transition of form F to form G was accompanied by dehydration as the decrease in moisture content from product A₃ to product A₄ indicated.

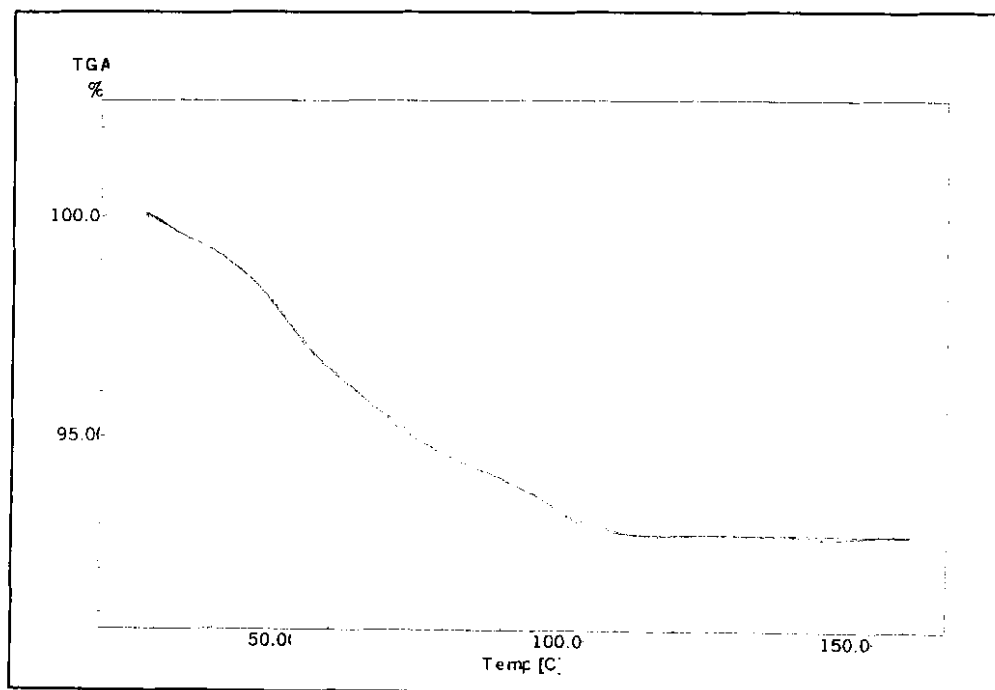


Figure 8.36 TGA thermogram of product A_{3D}.

Another fraction of the sample of product A₃ was dried at 70°C for 4 hours and the XRPD pattern of the dried product was found to be comparable to that of form G. The typical x-ray powder diffractogram of form G, obtained in this study (referred to as product A₄) is shown in figure 8.37.

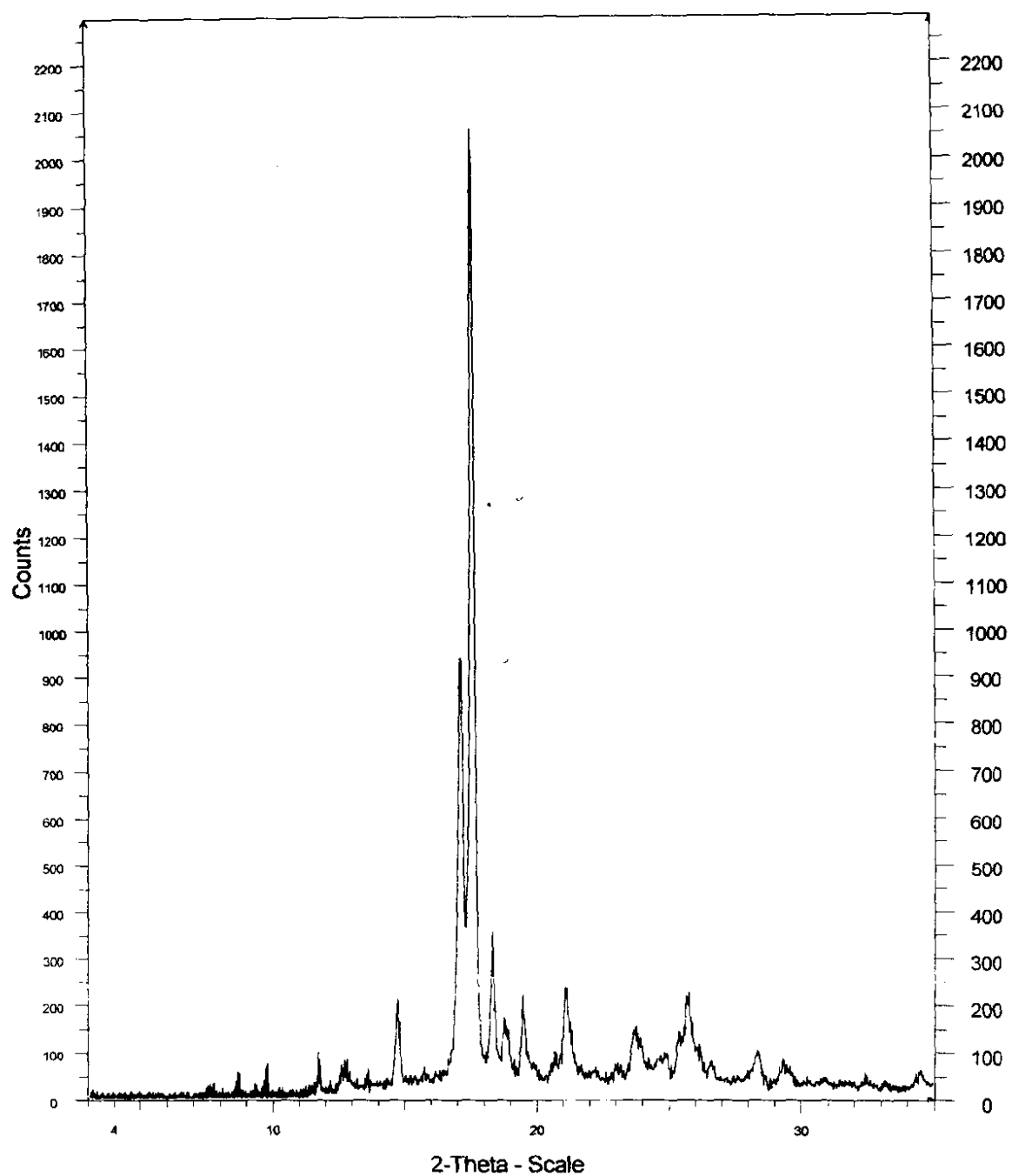


Figure 8.37 X-ray powder diffractogram of product A₄.

The TGA and KF results of product A₄ are displayed in table 8.10.

TGA results in the available literature indicated that form G was a solvated crystal form (a weight loss of 5.70% was displayed in the TGA thermogram). However, no KF results of form G was provided and no absolute classification was made. The TGA results of product A₄ revealed a total weight loss of 3.17%, which differed from the 5.70% documented in the literature. KF analysis revealed a moisture content of 3.03% for this crystal form. The comparable TGA and KF values indicated that water was incorporated into the crystal lattice. The TGA and KF results were found to be comparable to the theoretical % weight loss observed for a hemihydrate, thus product A₄ could be classified as a hemihydrate. The TGA thermogram of product A₄ is displayed in figure 8.38.

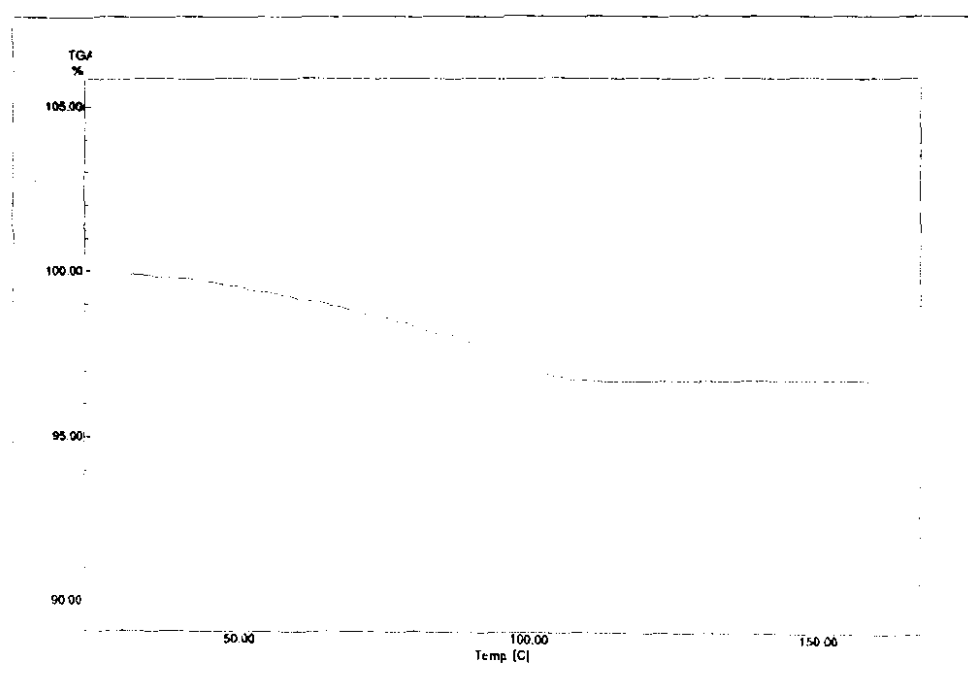


Figure 8.38 TGA thermogram of product A₄.

8.2.6 Thermal induced phase transformations of gatifloxacin form F

In the previous section it was observed that when a mixture of forms F and G was dried at 70°C for 4 hours, the fraction of form F transformed to form G. The thermal induced phase transformations of pure form F obtained were investigated using VT-XRPD. The variable temperature x-ray powder diffractograms are displayed in figure 8.39(a) and (b).

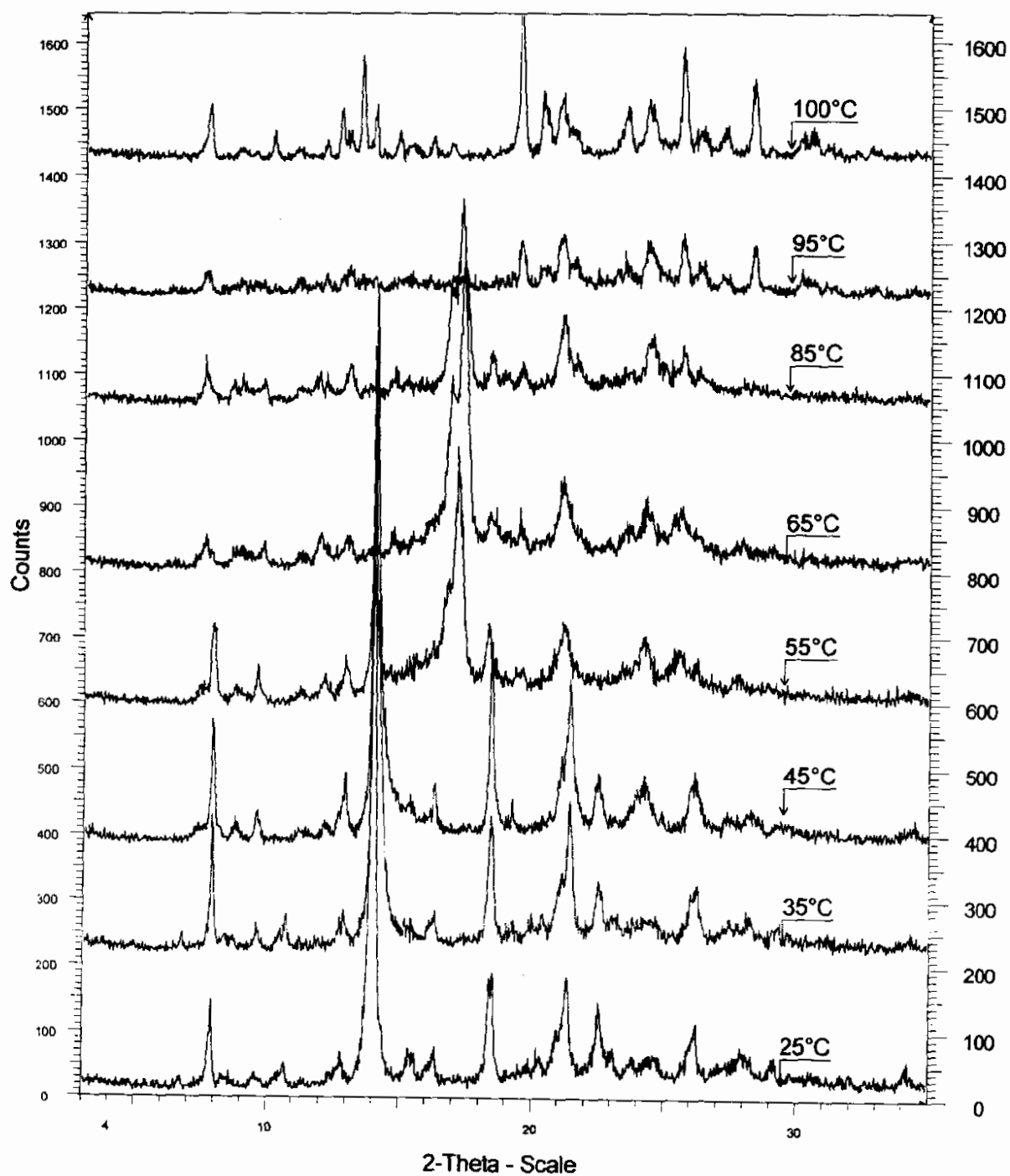


Figure 8.39(a) Variable temperature x-ray powder diffractograms of the recrystallisation product from absolute methanol (form F) in the temperature range 25-100°C.

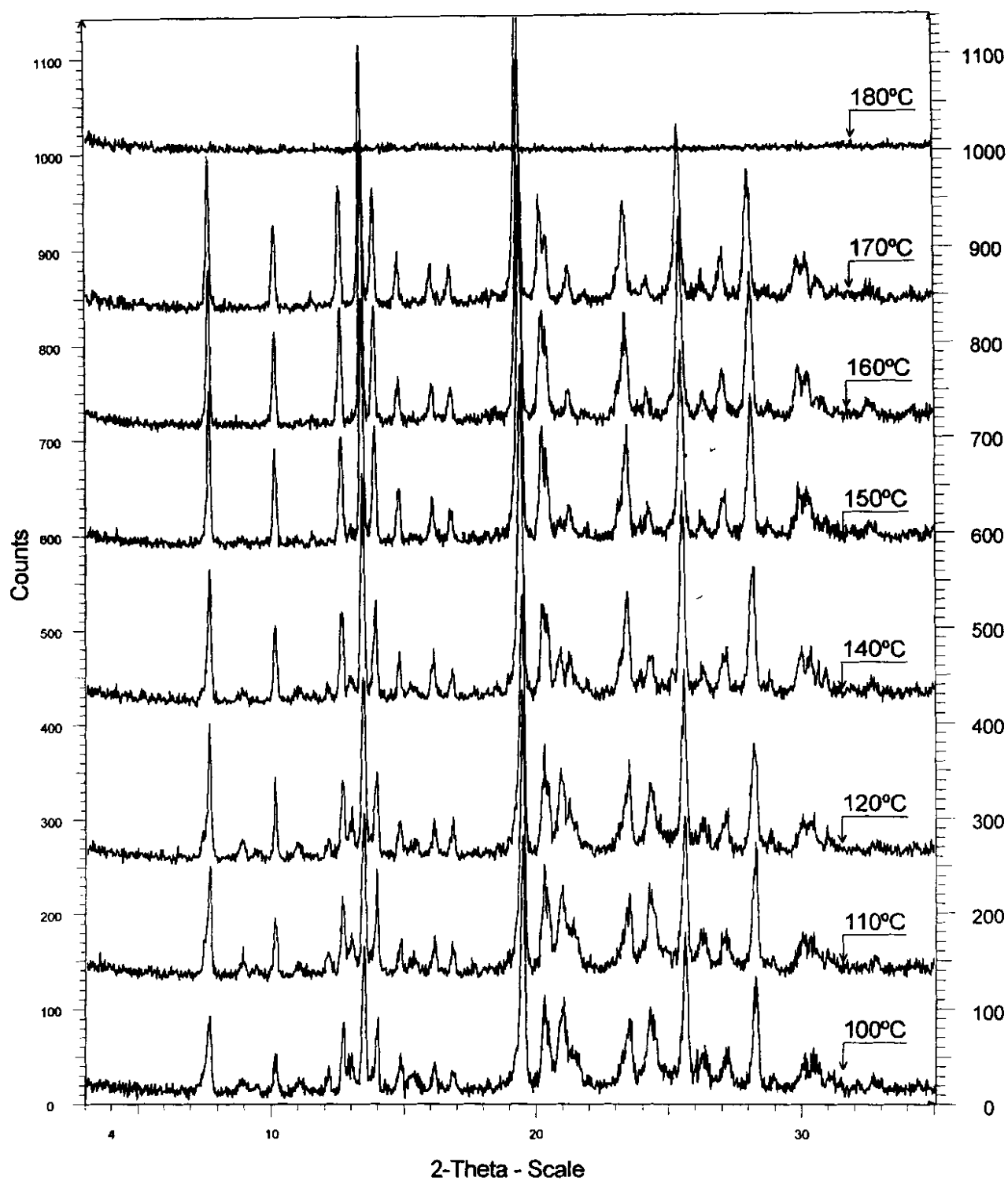


Figure 8.39(b) Variable temperature x-ray powder diffractograms of the recrystallisation product from absolute methanol (form F) in the temperature range 100-180°C.

An increase in crystallinity of the sample was observed between 25-45°C in the XRPD patterns. The x-ray powder diffractogram obtained at 45°C showed the greatest resemblances to that of form F.

The XRPD pattern changed significantly between 45-65°C. The XRPD pattern obtained at 65°C was comparable to that of form G. This correlated with the literature, which stated that heating of form F results in the transition to form G (as stated in the introduction of this chapter).

Further changes were observed in the diffractogram between 65-95°C. The XRPD pattern of form T2RP disclosed in the literature (figure 8.8) has a poor resolution and no peaklist was available (no other physico-chemical data on this form was discussed in the literature). The XRPD pattern obtained at 95°C is comparable to that of form T2RP. These results were corresponding to the literature, which stated that drying of form G resulted in form T2RP (as discussed in the introduction to this chapter).

The x-ray powder diffractograms of the sample changed noticeably between 95-100°C and only slight changes were observed in the temperature range of 100-170°C. The diffractogram obtained at 100°C was comparable to that of form omega (figure 8.10). The XRPD patterns obtained at 160°C and 170°C were comparable to that of form omega in figure 8.9.

The final diffractogram in this study (at 180°C) displayed no peaks, which suggested that the sample had melted.

This VT-XRPD study clearly indicated the thermodynamic transition of form F to form G and of form G to form T2RP and finally form omega (figure 8.40). Various intermediate phases were obtained between these forms.

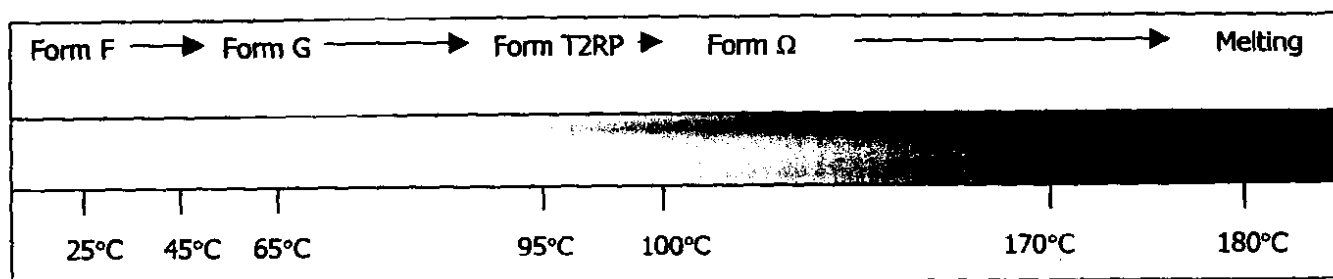


Figure 8.40 Thermodynamic transition of form F.

Conclusion

Various recrystallisation products were obtained from absolute methanol and binary mixtures of methanol and water. Three different crystal forms were obtained, namely a crystal form comparable to form F with the exception of peaks present at $7.3^\circ 2\theta$ and $21.1^\circ 2\theta$ (figure 8.11)(product A), form F (product A₂ and B) and a trihydrate crystal form (product C). The results obtained for product A, B and C indicated that the crystal forms obtained from absolute methanol and binary mixtures of methanol and water favoured the incorporation of water into the crystal lattice rather than methanol.

No absolute classification of form F was provided in the available literature. This study made it possible to classify form F as a trihydrated pseudopolymorph of gatifloxacin. Alternative preparation methods of form F have also been identified and discussed in this chapter.

A second trihydrated form (product C) was also prepared in this study, which differed from form F (indicating polymorphic pseudopolymorphism). It is therefore important to distinguish between possible trihydrated crystal forms by means of XRPD, as TGA and KF data does not reveal significant differences.

The influence of harvesting time after recrystallisation on form F was investigated. The results obtained indicated that longer harvesting times resulted in a mixture of form F and G, while shorter harvesting times produced pure form F. These results revealed that solid state phase transformations are possible whilst the crystals are submerged in the recrystallisation solvent.

As stated in the literature, form F transforms to form G, and form G to form T2RP or omega. A VT-XRPD study was performed to investigate these thermal induced phase transformations of form F. This study revealed the transition of form F to G, and clarified that form G first transformed into form T2RP, and that form T2RP then transformed into form omega.

CHAPTER 9

Summary and Conclusion

Gatifloxacin is a fluoroquinolone antibacterial agent with broad spectrum of activity against various gram-positive and gram-negative organisms as well as some atypical organisms (Schaeffer, 2002:49S). Gatifloxacin exhibits an improved pharmacokinetic profile over earlier fluoroquinolones and is often preferred over the preceding fluoroquinolone agents (Schaeffer, 2002:52S).

Gatifloxacin displays polymorphism and pseudopolymorphism. Polymorphism occurs when the solid crystallises into different crystal packing arrangements with the same elemental composition. Pseudopolymorphism is a phenomenon where one or more solvents are incorporated into the crystal lattice of the solid in either stoichiometric or non-stoichiometric amounts. Due to the tendency of gatifloxacin to crystallise into various crystal forms, polymorphic studies on this drug have become extremely important.

This study investigated the solid state and solvent inclusion properties of gatifloxacin (figure 9.1). It was investigated whether gatifloxacin displayed solvent inclusion and how this influenced the physico-chemical properties of the various crystal forms.

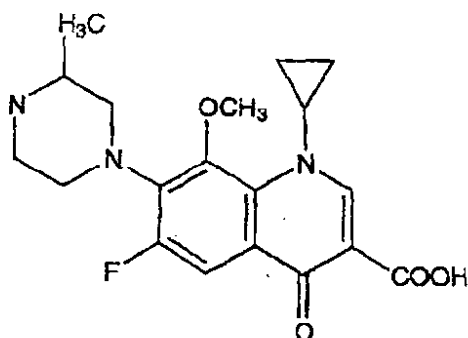


Figure 9.1 Chemical structure of gatifloxacin.

A literature overview revealed various patents that are currently available claiming intellectual property of the various crystal forms of gatifloxacin. Various hydrates, solvates and anhydrous forms of gatifloxacin are described in the literature.

However, insufficient physico-chemical data describing these various crystal forms of gatifloxacin are provided in the patents and one of the aims of this study was to comprehensively characterise some of these crystal forms.

A schematic overview of this study is displayed in figure 9.2. A thorough literature overview of polymorphism and pseudopolymorphism (solvent inclusion) was performed in chapter 1. The mechanism of solvent inclusion, the influence thereof on the physico-chemical properties of the pharmaceutical solid and the stability of the various crystal forms were investigated in chapter 1.

Chapter 2 focused on the pharmaceutical and pharmacological properties as well as the various patent claims of gatifloxacin crystal forms. These properties along with choosing the best crystal form for manufacturing ensures that the final product is safe, effective and stable.

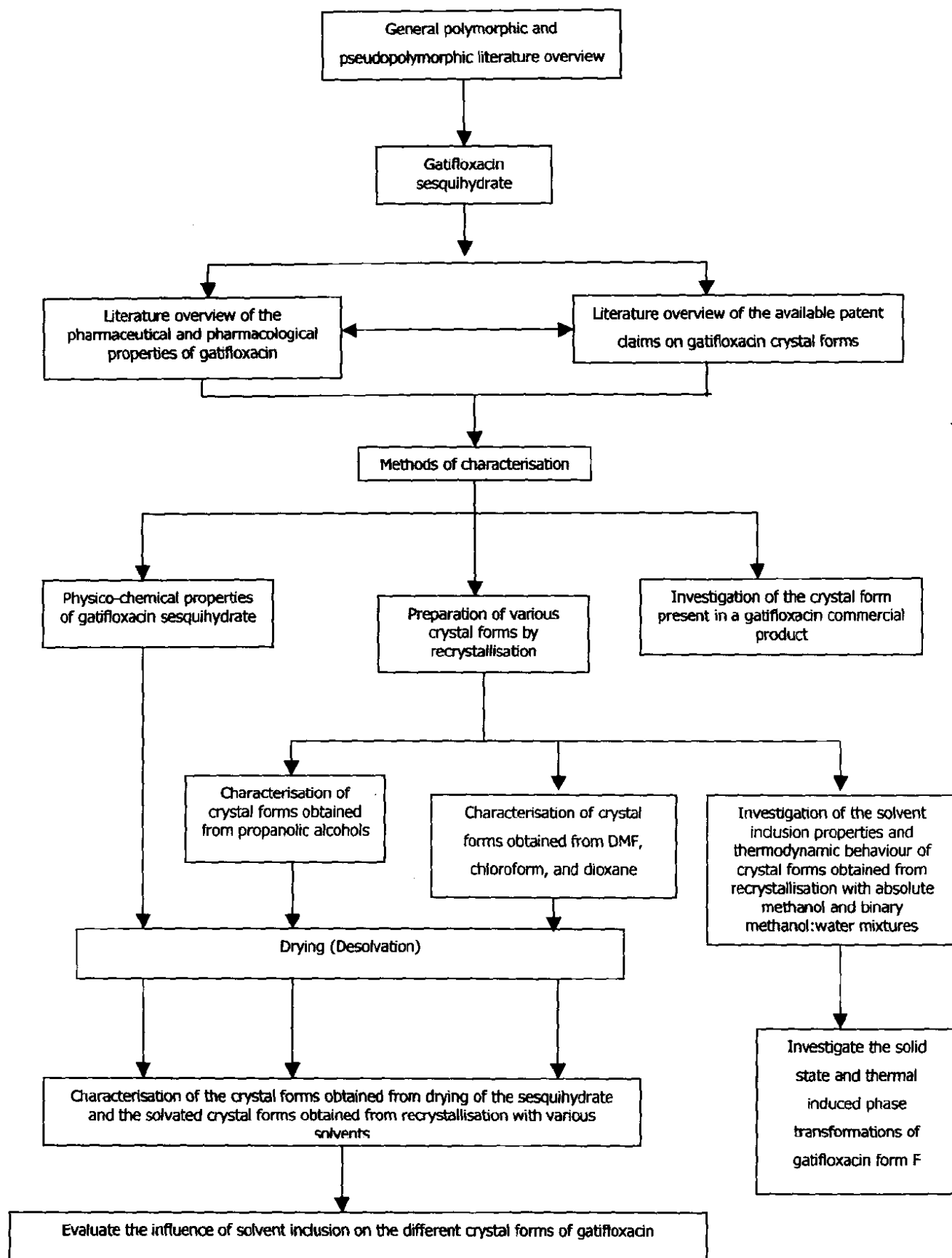


Figure 9.2 Schematic overview of this study.

Chapter 3 describes the analytical techniques used in this study. These techniques (such as XRPD, IR, DSC, TGA, KF, powder dissolution studies, HSM etc.) were used to determine the physico-chemical properties of the raw material (gatifloxacin sesquihydrate), its anhydrous form, and the various crystal forms prepared in this study and the desolvation products thereof.

The influence of solvent inclusion on the physico-chemical properties of the various crystal forms was investigated. The physico-chemical properties of these crystal forms and their dried products (i.e. desolvated forms) were investigated and compared. In all the studies it was found that solvent inclusion influenced some of the physico-chemical properties.

In chapter 4 the influence of the hydration of the crystal on the physico-chemical properties of gatifloxacin was investigated. This was achieved by comparing the physico-chemical properties of the sesquihydrate with that of the anhydrous form. Significant differences were observed in most of the physico-chemical properties of these crystal forms.

The crystal forms recrystallised from the propanolic alcohols were investigated in chapter 5 and the influence of solvent inclusion on the properties of these forms were evaluated in this chapter. Recrystallisation from n-propanol yielded a hydrated solvate ($\frac{1}{2}$ water : $\frac{1}{2}$ n-propanol : 1 gatifloxacin) referred to as EP03W, and recrystallisation with iso-propanol yielded a hydrated solvate (1 water : $\frac{1}{2}$ iso-propanol : 1 gatifloxacin) referred to as EP04W. The XRPD patterns of both these crystal forms were comparable to that of form J described in the literature. The inclusion of different ratios of water and different solvents into the crystal lattice of this form did not result in significant changes in the crystal structure thereof.

Drying of EP03W and EP04W both resulted in an anhydrous crystal form with an XRPD pattern comparable to that of form omega described in the literature. From this chapter it became clear that the absence of solvent did not have a profound effect on the dissolution properties of these crystal forms, seeing that the dissolution behaviour of the solvated and anhydrous forms did not differ significantly. However,

other physico-chemical properties (XRPD, DSC, TGA, KF, etc.) of these crystal forms differed.

In chapter 6 the physico-chemical properties of the crystal forms obtained from recrystallisation with DMF, chloroform, and dioxane were investigated. The impact of the solvent incorporation on the physico-chemical properties of these crystal modifications was investigated.

Recrystallisation with DMF resulted in EP05W, i.e. a mixture of a sesquihydrate form and a DMF (1:1) solvate. Drying of EP05W resulted in an anhydrous crystal form (EP05D). The XRPD pattern of EP05D displayed resemblances to that of forms omega and I described in the literature. Solvent inclusion did not have a major influence on the dissolution or flow properties of these crystal forms.

The crystal form obtained from recrystallisation with chloroform (EP06W) was classified as a chloroform solvate (1:1). This was a new pseudopolymorphic form seeing that no chloroform solvates have been documented presently in the available literature. Some resemblances were found between the XRPD pattern of EP06W and that of form T1 (a crystal form recrystallised from acetonitrile as described in the literature), which could indicate possible iso-structuralism between these two crystal forms.

Drying of EP06W resulted in an anhydrous crystal form (EP06D) with an XRPD pattern comparable to that of forms omega and I described in the literature. Comparison of the physico-chemical properties of the solvated and anhydrous crystal forms (EP06W and EP06D) revealed significant differences. The anhydrous form displayed improved dissolution, solubility and flow properties compared to the solvated crystal form.

Recrystallisation with dioxane yielded a hemi-dioxane-monohydrate, referred to as EP08W. Currently no hemi-dioxane-monohydrates have been documented in the literature and therefore this could be classified as a new pseudopolymorphic form. The XRPD pattern of EP08W displayed some of the characteristic peaks of form J, indicating the possible iso-structuralism between these two forms.

EP08D was obtained by drying EP08W. This crystal form was found to be a new anhydrous form. Differences were observed in the XRPD, DSC, IR and other physico-chemical properties of EP08W and EP08D, however solvent inclusion (dioxane and water) did not cause significant differences in the dissolution and flow properties of these two forms (i.e. EP08W and EP08D).

Chapter 7 focused on the stability of the crystal form (i.e. sesquihydrate) present in a commercial product. The physico-chemical properties (XRPD, IR, DSC, TGA, etc.) of the crystal form present in the commercial product (Tequin® tablets) were found to be comparable with that of the sesquihydrate crystal form, thus signifying that the manufacturing process did not induce any polymorphic transformations of the crystal form (i.e. sesquihydrate) used initially in the manufacturing process.

The solvent inclusion properties and thermodynamic behaviour of gatifloxacin crystal forms recrystallised from absolute methanol and methanol:water mixtures were investigated in chapter 8. Recrystallisation from absolute methanol resulted in product A, which displayed an XRPD pattern that was comparable to that of form F with the exception of a few peaks. This form was classified as a hydrated solvate (2 water : 1 methanol : 1 gatifloxacin) or a mixture of hydrated and solvated forms. An exact classification will only be possible with the assistance of single crystal crystallography.

Product A₂ was obtained by exposing product A to ambient conditions for approximately one hour. Product A₂ displayed an XRPD pattern that was comparable to that of form F and was classified as a trihydrate.

Recrystallisation from a methanol: water mixture (75:25) resulted in product B, which was classified as a trihydrate. Product B displayed an XRPD pattern that was comparable to that of form F and product A₂. Product C was obtained by recrystallisation with a methanol: water mixture (50:50). This form displayed an XRPD pattern, which differed from that of products A, A₂ and B. Product C was found to be a trihydrate, suggesting a polymorphic pseudopolymorphic behaviour of gatifloxacin.

Recrystallisation from absolute methanol and methanol:water mixtures resulted in crystal forms that favoured the incorporation of water into the crystal lattice rather than methanol, as the physico-chemical properties of product A, B and C indicated.

Chapter 8 also indicated that form F was a metastable crystal form. The harvesting time of the crystals after recrystallisation influences the final crystal form obtained. A longer harvesting time (1 month) resulted in a mixture of form F and form G, and the pure form F was obtained after a short harvesting time (5 days). An VT-XRPD study was performed in this chapter to investigate the thermal induced phase transitions of form F. This study captured the transition of form F to form G, followed by a transition to form T2RP and finally a transition to form omega. The results obtained in this study thus revealed that water present in the recrystallisation solvent may influence the recrystallisation product.

This study could be deemed successful seeing that the set aims and objectives (as stated in aims and objectives) were pursued and achieved.

As George B. Shaw (2006) once said: "*Science...never solves a problem without creating ten more*". The questions and problems posed at the beginning of this study were answered, but not without raising new questions that created an opportunity for future research.

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Finally, I would like to thank my heavenly Father for giving me this opportunity and providing me with the determination and endurance to successfully finish this study.

ANNEXURE 1

**Poster presented at the 26th Annual conference of
the Academy of Pharmaceutical Sciences, Port
Elizabeth, 2005.**

Physico-chemical properties of the commercially used Gatifloxacin sesquihydrate and the dehydration product

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PURPOSE

To provide physico-chemical data of the dehydration product and the sesquihydrate form of gatifloxacin and to compare the solid-state properties of these two forms.

BACKGROUND

Gatifloxacin is a fluoroquinolone antibacterial agent. It is bactericidal and acts by inhibiting DNA gyrase, interfering with the reproduction of the bacterial DNA. Gatifloxacin is active against various gram-positive organisms, including *S. pneumoniae*. It also has some activity against gram-negative organisms. Gatifloxacin is frequently used in lower respiratory tract and urinary tract infections⁽¹⁾.

METHODS

The anhydrous form was prepared from the sesquihydrate form: the sesquihydrate was dried for 4 hours in a vacuum oven at 140°C and 15 mbar. The two forms were analysed using XRPD, TGA, KF, FT-IR, DSC, TM and SEM photos. A solubility study was also conducted to compare the solubility of the two mentioned forms. Four different media were used in the solubility study, i.e.: Milli-Q water, 0.01 M HCl, acetate buffer pH 4.5 and phosphate buffer pH 6.8.

RESULTS

The physico-chemical properties of the sesquihydrate and its dehydration product differed significantly. The TGA and KF values of the sesquihydrate and anhydrous forms are compared in Table 1. The sesquihydrate showed an average moisture content of 6.338% and 6.094% using KF and TGA respectively, indicating the presence of 1.5 H₂O : 1 gatifloxacin molecules. The average moisture content for the anhydrous form was 1.359% and 0.847% for KF and TGA analysis respectively. These values are comparable to the theoretical moisture content for these two forms.

The solubility of the two forms was compared in the abovementioned media. The anhydrous form was found to be more soluble in all the media. However, the difference in solubility between the two forms, was especially noticeable in water (Fig 1).

The XRPD patterns (Fig 2), and the IR spectra (Fig 3) of the two forms can be utilized to distinguish between these forms. These characterisation methods display significant differences for these two forms.

The two forms also showed differences in their DSC thermograms. A dehydration endotherm at 97.21°C was present in the thermogram of the sesquihydrated form. TGA analysis confirmed that this was a dehydration peak (with a dehydration onset and endset temperature of 74.27°C & 102.67°C respectively). As expected, the anhydrous form displayed no peaks in this temperature range and TGA confirmed that no dehydration occurred (Fig 4).

The morphology and thermal behaviour of the two forms were also investigated using SEM (Fig 5) and TM photos. The TM microphotographs of the sesquihydrate sample revealed bubbles (at approximately 98°C), due to dehydration, when emerged in silicon oil. The anhydrous form showed no activity at 98°C. The SEM showed differences in the morphology of the two forms.

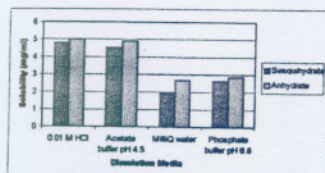


Fig 1. Solubility data of gatifloxacin sesquihydrate and its dehydration product.

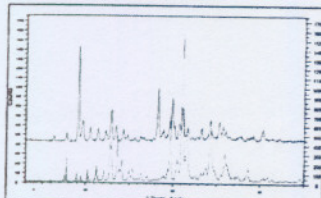


Fig 2. XRPD patterns of gatifloxacin sesquihydrate (blue) and gatifloxacin anhydrate (red).

Table 1. Comparison of the TGA and KF values of the sesquihydrate and anhydrous forms of gatifloxacin

Form	KF (%)	TG (%)	Theoretical moisture content (%)
Sesquihydrate	6.338	6.094	6.869
Anhydrous	1.359	0.847	0.0

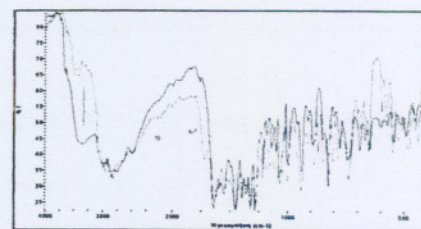


Fig 3. FT-IR spectra of gatifloxacin sesquihydrate (blue) and gatifloxacin anhydrate (red).

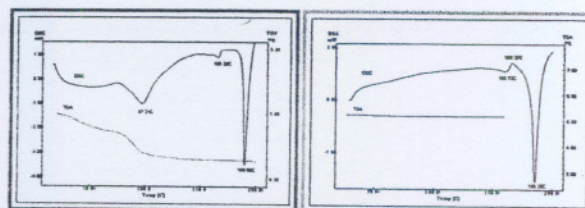


Fig 4. DSC thermograms for the sesquihydrate (blue) and anhydrous (red) forms of gatifloxacin.

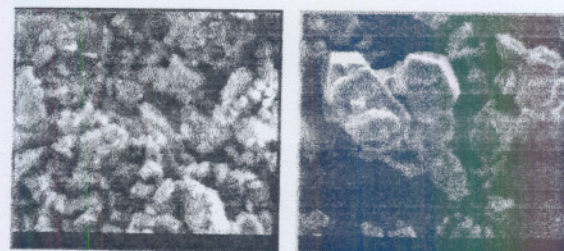


Fig 5. SEM photos of gatifloxacin sesquihydrate (blue) and its dehydration product (red).

CONCLUSION

Supplementary data on the sesquihydrated form and its dehydration product is presented. As expected, there were significant differences in the physico-chemical properties of the two forms. XRPD, FT-IR, DSC and TGA were identified as methods to distinguish between these two forms. A noticeable difference in the solubility of the two forms in water was found.

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ANNEXURE 2

**Article in the process of being submitted for
publication**

Solvent inclusion properties of crystal forms recrystallised from absolute methanol and binary mixtures of methanol and water

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Potchefstroom Campus, South-Africa

Abstract

The internal structure of API's is determined by the orientation and configuration of the molecular units and may thus be classified as: amorphous or crystalline. Crystalline solids may be classified as polymorphs or pseudopolymorphs. Solvent molecules can be included into the various crystal forms. This is called *pseudopolymorphism* [1]. If the solvent that is included into the crystal lattice is water, this crystal form is called a hydrate. If a solvent (other than water) is included, it is called a solvate [2].

The aim of this study was to investigate the solvent inclusion properties and thermodynamic behaviour of gatifloxacin crystal forms recrystallised from absolute methanol and binary mixtures of methanol and water and to investigate the thermal induced phase transitions of form F using VT-XRPD.

Keywords: Solvate, Gatifloxacin, Solvent inclusion, VT-XRPD, Trihydrate, Pseudopolymorph

1. Introduction

Gatifloxacin, 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid, is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent with activity against a wide range of gram-negative and gram-positive organisms [3]. A number of patents are currently available claiming intellectual property of various crystal forms of gatifloxacin.

Form F is a pseudopolymorphic form of gatifloxacin, which is obtained from recrystallisation with a mixture of methanol and water (90:10) [4]. This crystal form is

* Corresponding author.

characterised by XRPD peaks at $8.0^{\circ}2\theta$, $14.2^{\circ}2\theta$, $18.7^{\circ}2\theta$, $21.8^{\circ}2\theta$ and $23.0^{\circ}2\theta$ ($\pm 0.2^{\circ}2\theta$).

Figure 1 displays the typical x-ray powder diffractogram of form F.

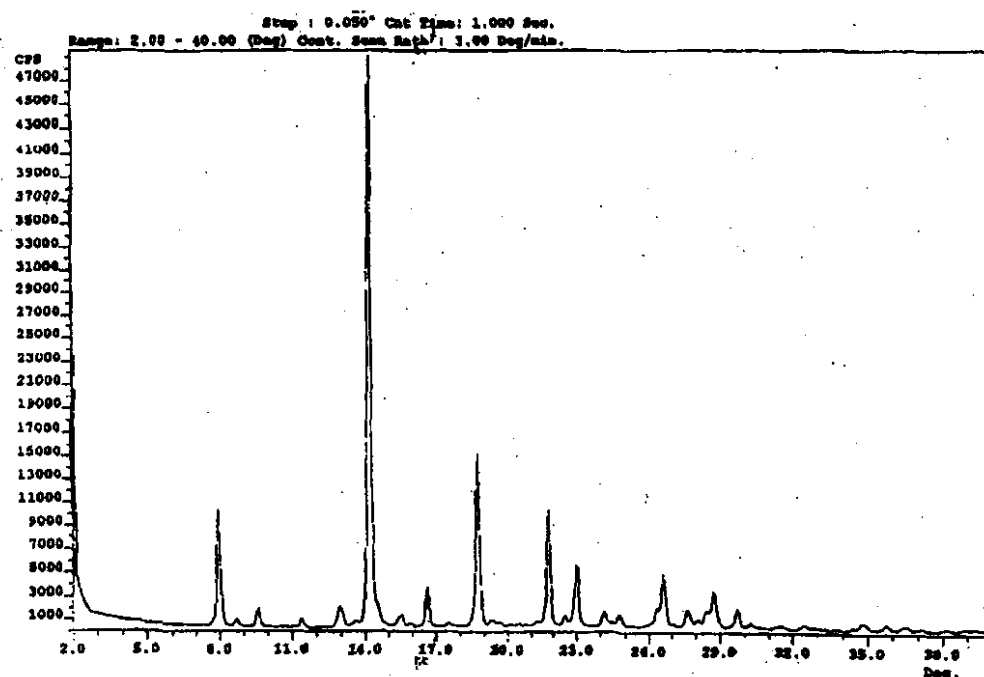


Figure 1. X-ray powder diffractogram of form F [4].

TGA results of form F provided in the literature indicated a total weight loss of 14.78%, which was observed in two steps: 9.38% and 5.4% respectively [4]. However, no KF results were supplied in the literature and no absolute classification of this form was thus provided.

Niddam-Hildesheim *et al.* [4] described another pseudopolymorphic form of gatifloxacin, form G, which was characterised by XRPD peaks at $17.2^{\circ}2\theta$ and $17.6^{\circ}2\theta$ ($\pm 0.2^{\circ}2\theta$). The typical x-ray powder diffractogram of form G is displayed in figure 2.

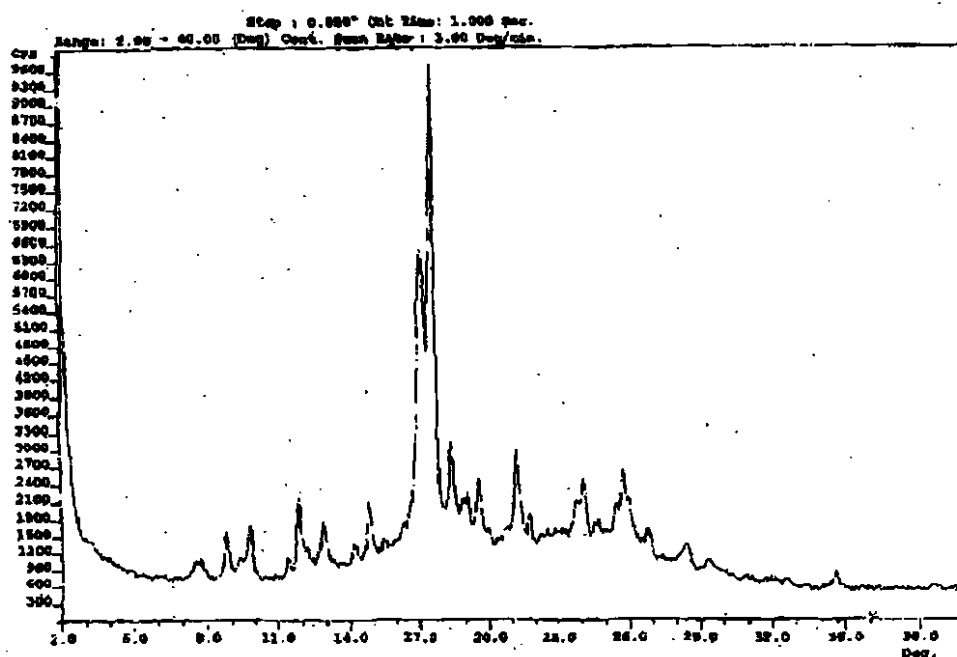


Figure 2. X-ray powder diffractogram of form G [4].

Niddam-Hildesheim *et al.* [4] stated that the drying of form F at approximately 50°C and atmospheric pressure for at least 20 hours resulted in the formation of form G.

TGA results of form G indicated that this crystal form was a solvate. A weight loss of 5.70% was observed with TGA [4]. The literature did not provide any KF results or an absolute classification of form G.

It was also documented that the drying of form G at 120°C yields either form omega (anhydrous) or form T2RP (hemihydrate) [4]. The XRPD pattern of form T2RP is displayed in figure 3. Various x-ray powder diffractograms of form omega were disclosed in the literature, only two of these are displayed in figure 4 and 5.

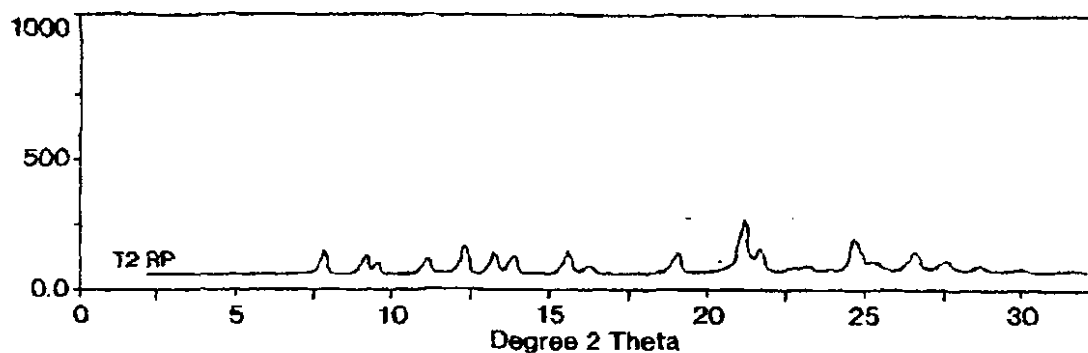


Figure 3. X-ray powder diffractogram of form T2RP (adapted from [5]).

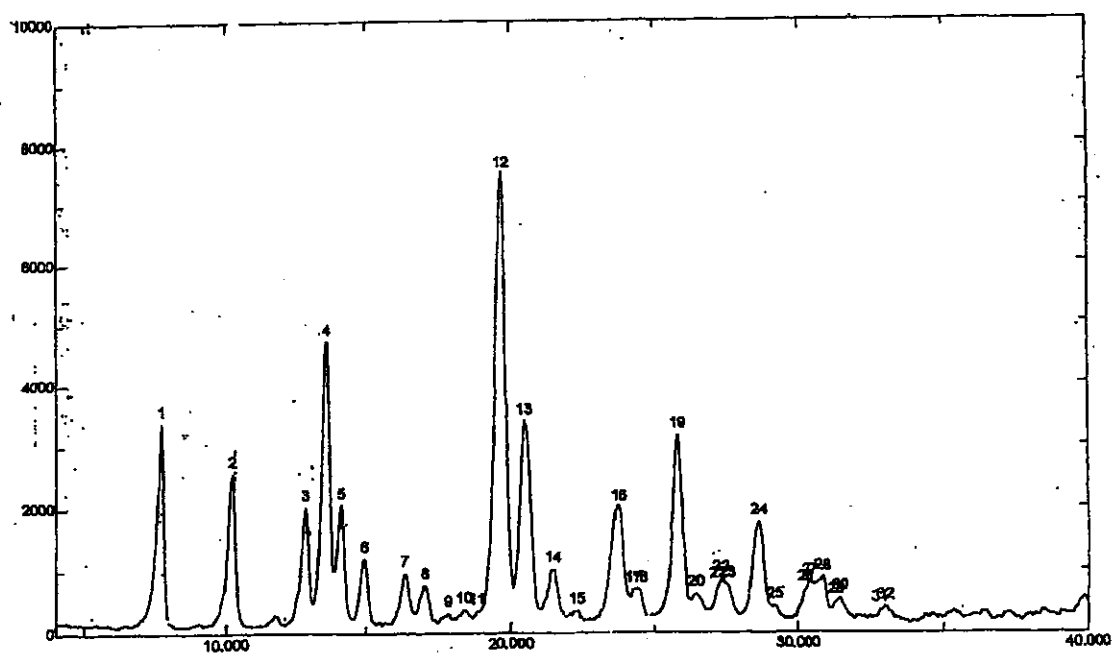


Figure 4. X-ray powder diffractogram 1 of form omega [6].

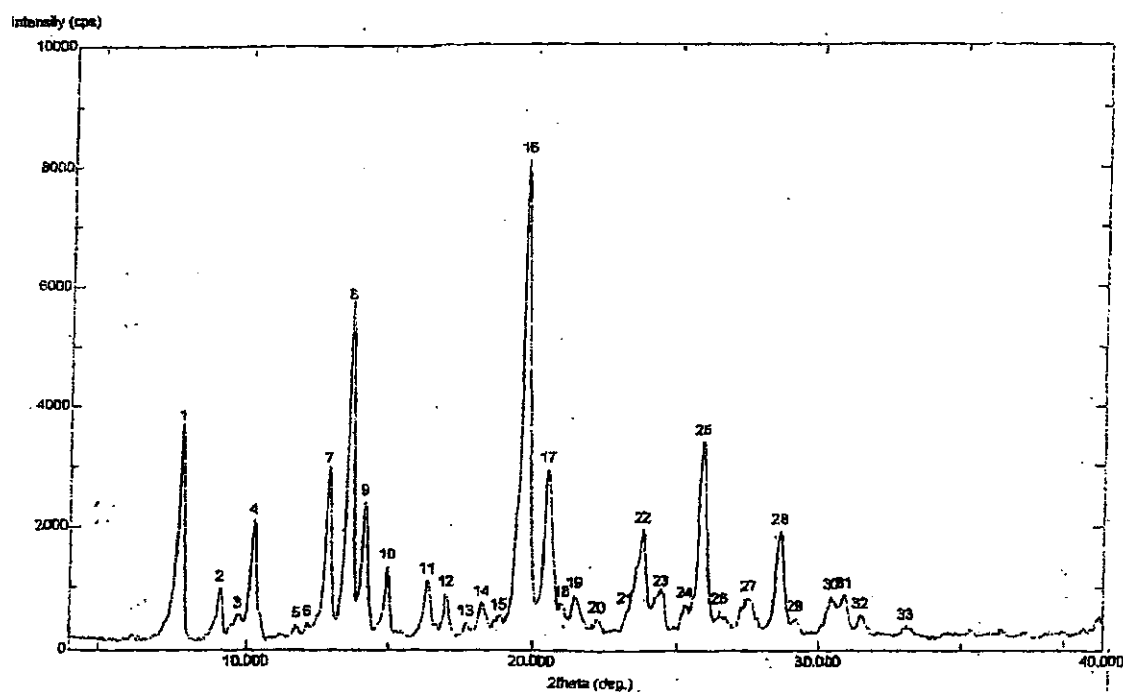


Figure 5. X-ray powder diffractogram 2 of form omega [6].

2. Experimental

2.1 Materials

Gatifloxacin sesquihydrate raw material was obtained from Dr. Reddy's Laboratories Ltd., India. The details of the raw material used during this study are as follows:

Batch number : GS002C05
Manufacturing date : March 2005
Expiry date : February 2010

The absolute methanol (boiling point 64.70°C) used in the recrystallisations was obtained from Saarchem, South Africa. Purified water was used in the recrystallisation with binary mixtures of methanol and water.

2.2 Recrystallisation method

The crystal forms investigated in this study were recrystallised using the following method. Gatifloxacin sesquihydrate powder was added to small volumes of the recrystallisation solvent, whilst the solution was heated to the boiling point of the solvent under constant stirring by means of a magnetic stirrer until a saturated solution was obtained. The saturated solutions were prepared in glass beakers and covered with Parafilm[®] containing small holes to allow solvent evaporation at ambient conditions.

The various solvents used and the recrystallisation products obtained are listed in table 1.

Table 1. Solvents used and recrystallisation products obtained in this study

Solvent	Recrystallisation product
Absolute methanol	Product A
Methanol: water (75:25)	Product B
Methanol: water (50:50)	Product C

The crystals of each product were removed from the mother solvent and dried on filter paper for approximately 30 minutes to ensure that the samples were free from any surface solvent, prior to the analysis. The following analytical techniques were used in this study: XRPD, VT-XRPD, DRIFTS, TGA, KF, and DSC.

2.3 Analytical Techniques and instrumentation

2.3.1 Differential scanning calorimetry (DSC)

A Mettler Toledo DSC (Mettler Toledo, Switzerland) instrument was used in this study. This is a heat-flux DSC instrument. Samples weighing 2-4 mg were heated in aluminium sample pans with pierced lids. Samples were heated from 25-200°C at a heating rate of 10°C/min, under a nitrogen purge with a flow of 30 ml/min.

2.3.2 Thermogravimetric analysis (TGA)

A Shimadzu TGA-50 instrument (Shimadzu, Kyoto, Japan) was used. The sample weight varied between 5-10 mg each. Samples were weighed in an open platinum cell and the changes in mass upon heating were recorded. The samples were heated at a heating rate of 10°C/min under a nitrogen purge of 35 ml/min. Samples were heated to a maximum temperature of 160°C.

The experimental weight loss, as determined by the TGA, was compared with the theoretical weight loss.

2.3.3. X-ray powder diffraction (XRPD)

The x-ray powder diffraction data for gatifloxacin crystal forms was obtained using a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; antiscatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size, 0.025°; step time, 1.0 sec.). An aluminium sample holder was used, and approximately 200 mg of powdered sample was loaded into the sample holder. Care was taken not to introduce a preferential orientation of the crystals.

XRPD analyses at ambient conditions and at increased temperatures, variable temperature x-ray powder diffraction (VT-XRPD), were performed on the crystals. The same conditions were used for both types of x-ray diffraction. For the VT-XRPD an Anton Paar TTK 450, low temperature camera (Anton Paar, Austria), was attached to the Bruker D8 Advance diffractometer (Bruker, Germany). A heating rate of 10°C/cycle was used during determinations and all samples were heated to a maximum temperature of 180°C.

2.3.4 Infrared spectroscopy (IR)

A Nicolet Nexus 470-FT-IR spectrometer (Nicolet instrument corporation, Maddison, Wisconsin, USA) was used to record the IR-spectra, over a range of 400-4000 cm⁻¹. The DRIFTS method was used. KBr was used as background. The samples were dispersed in KBr and the IR-spectra measured in a reflectance cell.

2.3.5 Karl Fischer titration

The moisture content of the samples was determined with a Metrohm 701 KF Titrino autotitrator (Metrohm, Switzerland). Calibration of the instrument was performed using a predetermined mass of water and approximately 250 mg of sodium tartrate dihydrate dibasic powder. Approximately 50 mg of each sample was used for the moisture determination. The experiment was performed in duplicate for each crystal form. The percentage of water (m/m) within the sample was then calculated.

3. Results and discussion

3.1 Recrystallisation product A

The crystal form obtained from recrystallisation with absolute methanol is referred to as product A. The XRPD pattern for product A is displayed in figure 6. This crystal form displayed an XRPD pattern comparable to that of form F, with the exception of some small peaks that are absent in the diffractogram of form F, such as the peaks at $7.3^{\circ}2\theta$ and $21.1^{\circ}2\theta$.

TGA results of product A revealed that this was a solvated crystal form. The TGA and KF results of product A are tabulated in table 2.

Table 2. TGA and KF results of product A

	Product A
% Weight loss (TGA)	14.51%
Moisture content (KF)	8.30%

The % weight loss observed with TGA was 14.51% and Karl Fischer titrations revealed a moisture content of 8.30% for product A. This KF value is comparable with the theoretical water content of a dihydrate, which is 9.15%. The rest of the weight loss observed with TGA might have been due to loss of methanol incorporated into the crystal lattice. This weight loss was 6.21%, which is comparable with the theoretical weight loss (7.86%) of a

methanol solvate (1:1). Product A could therefore be classified as a possible hydrated solvate (2 water : 1 methanol : 1 gatifloxacin) or a mixture of hydrated and solvated forms.

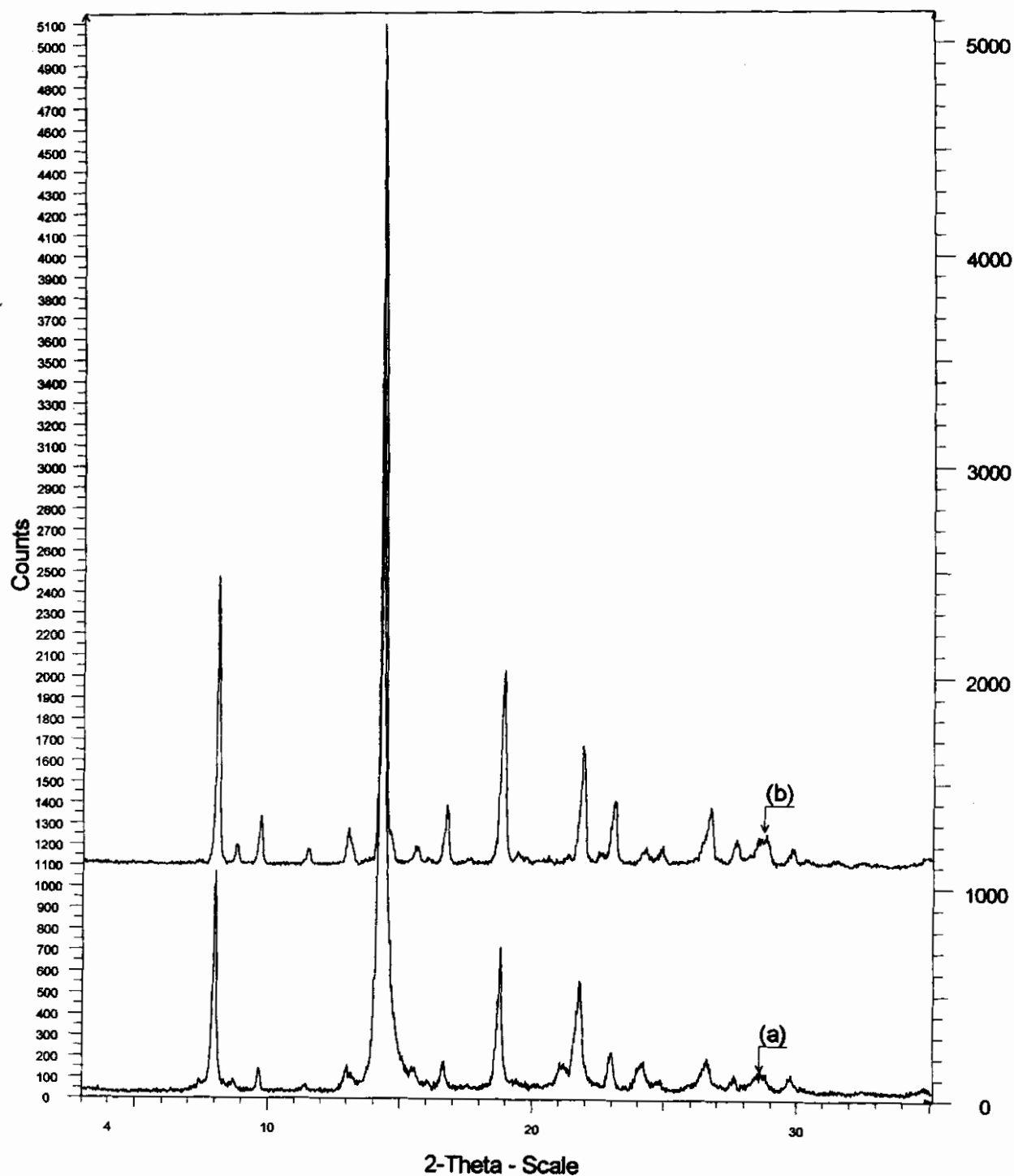
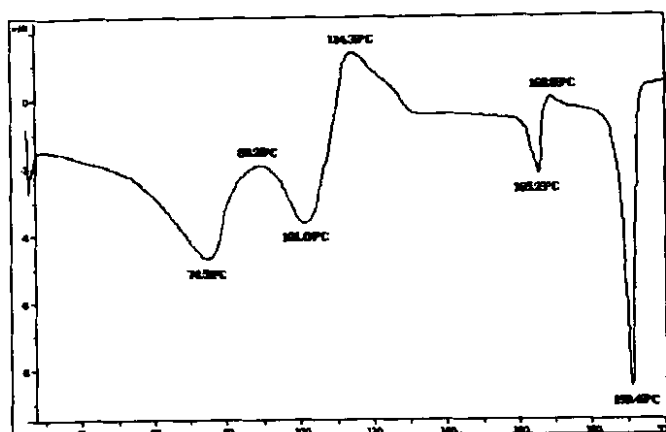
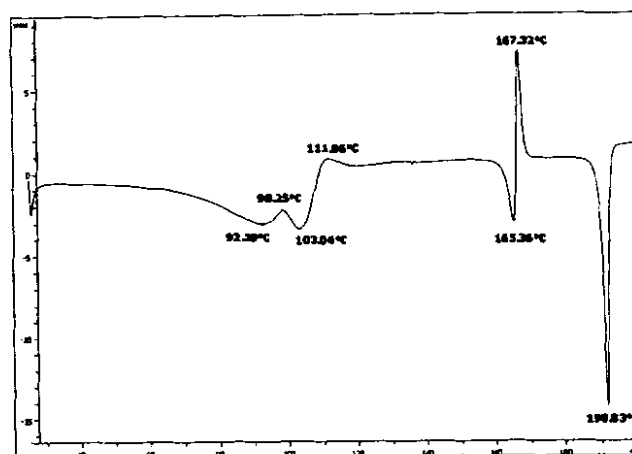


Figure 6. Overlay of the x-ray powder diffractograms of (a) product A and (b) product A₂.

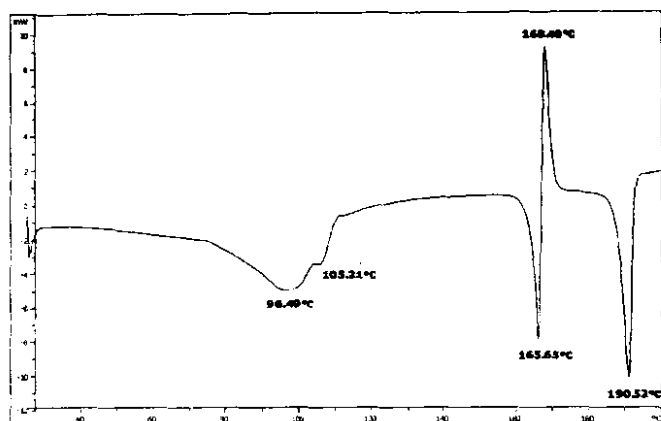
The DSC thermogram of product A is displayed in figure 7.



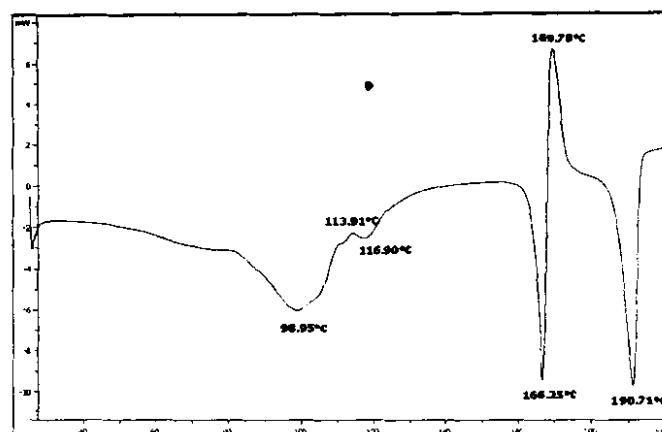
(A)



(B)



(C)



(D)

Figure 7. DSC thermogram of : (A) product A, (B) product A₂, (C) product B, (D) product C.

Product A was exposed to ambient conditions for one hour after which it was analysed again. The XRPD pattern of this sample (referred to as product A₂) was comparable to that of form F described in the literature, and is displayed in figure 6.

The DRIFT-IR spectrum of product A and A₂ is displayed in figure 8.

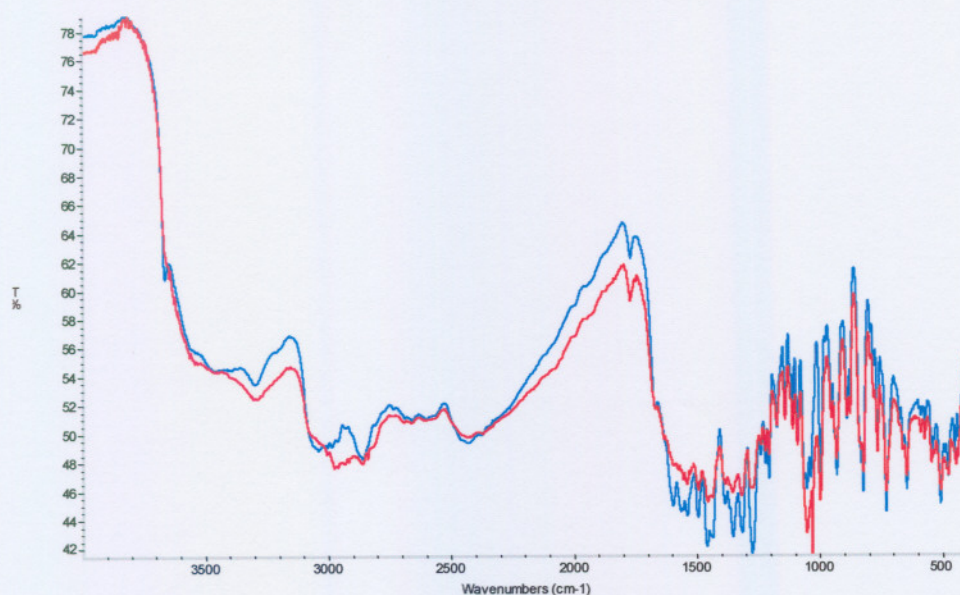


Figure 8. Overlay of the DRIFT-IR spectra of product A (red) and product A₂ (blue).

The TGA and DSC thermograms of form F presented in the literature suggested that form F is a solvated crystal form. This study confirmed that form F is indeed a solvated crystal form. Table 3 displays the TGA and KF results of product A₂ (form F).

Table 3. TGA and KF results of product A₂

	Product A ₂
% Weight loss (TGA)	12.58%
Moisture content (KF)	13.02%

TGA results indicated a total % weight loss of 12.58% and KF titrations displayed a moisture content of 13.02% for this sample, which is comparable to the theoretical moisture content of a trihydrate (13.74%). These corresponding results thus indicated that the solvent incorporated into the crystal lattice was water. Product A₂ may thus be classified as a trihydrate. A possible reason for this uptake of water could be that the crystals of product A was highly higroscopic (product A displayed a water content comparable to that of a dihydrate). The DSC thermogram of this crystal form is displayed in figure 7(b).

3.2 Recrystallisation product B

Product B was recrystallised from a methanol:water mixture (75:25) and displayed an XRPD pattern comparable to that of form F. The XRPD pattern of product B was comparable to that of product A₂.

The DRIFT-IR spectrum of product B is displayed in figure 9.

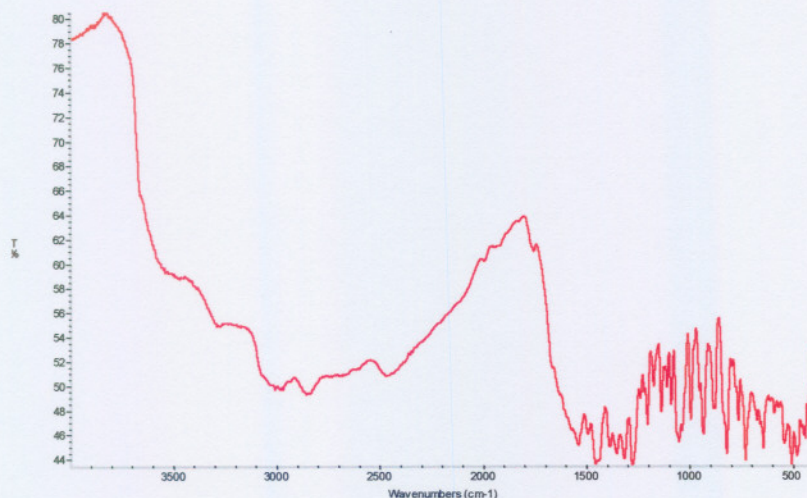


Figure 9. DRIFT-IR spectrum of product B.

The TGA and KF results for product B are summarised in table 4. TGA revealed a total weight loss of 13.09 % and the total moisture content of the sample (as determined by KF) was 13.38 %. This correlated with the theoretical moisture content of a trihydrate, which is 13.74%. The correlation between the KF and TGA results indicated that water was the only solvent incorporated into the crystal lattice of this form and product B may therefore be classified as a trihydrate.

Table 4. TGA and KF results for product B

	Product B
% Weight loss (TGA)	13.09 %
Moisture content (KF)	13.38 %

The DSC thermogram of product B is shown in figure 7(c). Minor differences were observed between the DSC thermograms of product A₂ and product B. The differences between the two thermograms are mostly present in the region of the desolvation endotherms and up to 120°C. Possible reasons for the slight differences observed in the thermograms of these two forms include the following: Slight differences in the piercing of the sample pan lid or the crimping of the sample pan that could have led to differences in the dehydration environment thus influencing the dehydration process.

3.3 Recrystallisation product C

Figure 10 displays the x-ray powder diffractogram of product C, which was recrystallised from a binary methanol:water mixture (50:50). The XRPD pattern of product C differed from that of product A₂ and product B. The most noticeable differences were in the area between 3.0-10.0°2θ, the peak at 19.9°2θ, and the area between 24.0-26.0°2θ.

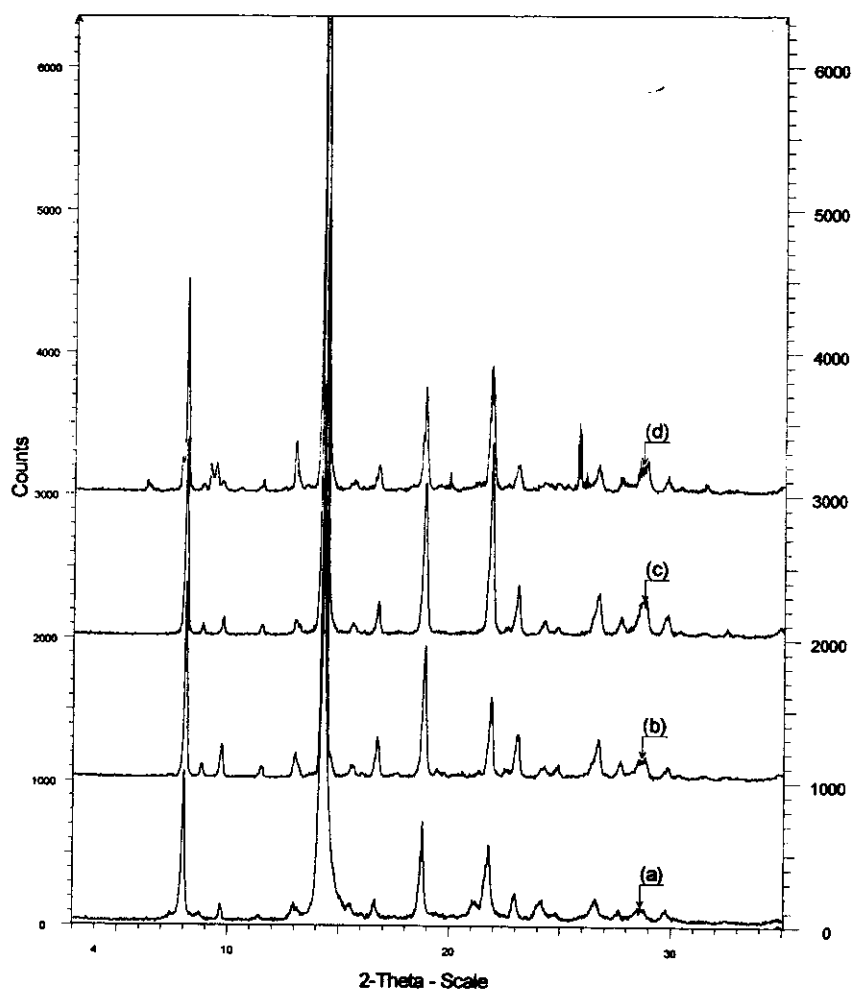


Figure 10. Overlay of the x-ray powder diffractograms of (a) product A, (b) product A₂, (c) product B, and (d) product C.

The TGA and KF results for this crystal form are displayed in table 5.

Table 5. TGA and KF results of product C

	Product C
% Weight loss (TGA)	13.12%
Moisture content (KF)	13.07%

Thermogravimetric analysis of the sample revealed a total weight loss of 13.12% and the moisture content of the sample was determined as 13.07%. This value was found to be comparable to the theoretical moisture content of a trihydrate (13.74%), and this form may therefore also be classified as a trihydrate.

The DSC thermogram of product C is displayed in figure 7(d). Differences in the dehydration endotherms and up to 120°C were observed when compared to the thermogram of form F.

3.4 Thermal induced phase transformations of gatifloxacin form F

The thermal induced phase transformations of pure form F obtained were investigated using VT-XRPD. The variable temperature x-ray powder diffractograms are displayed in figure 11.

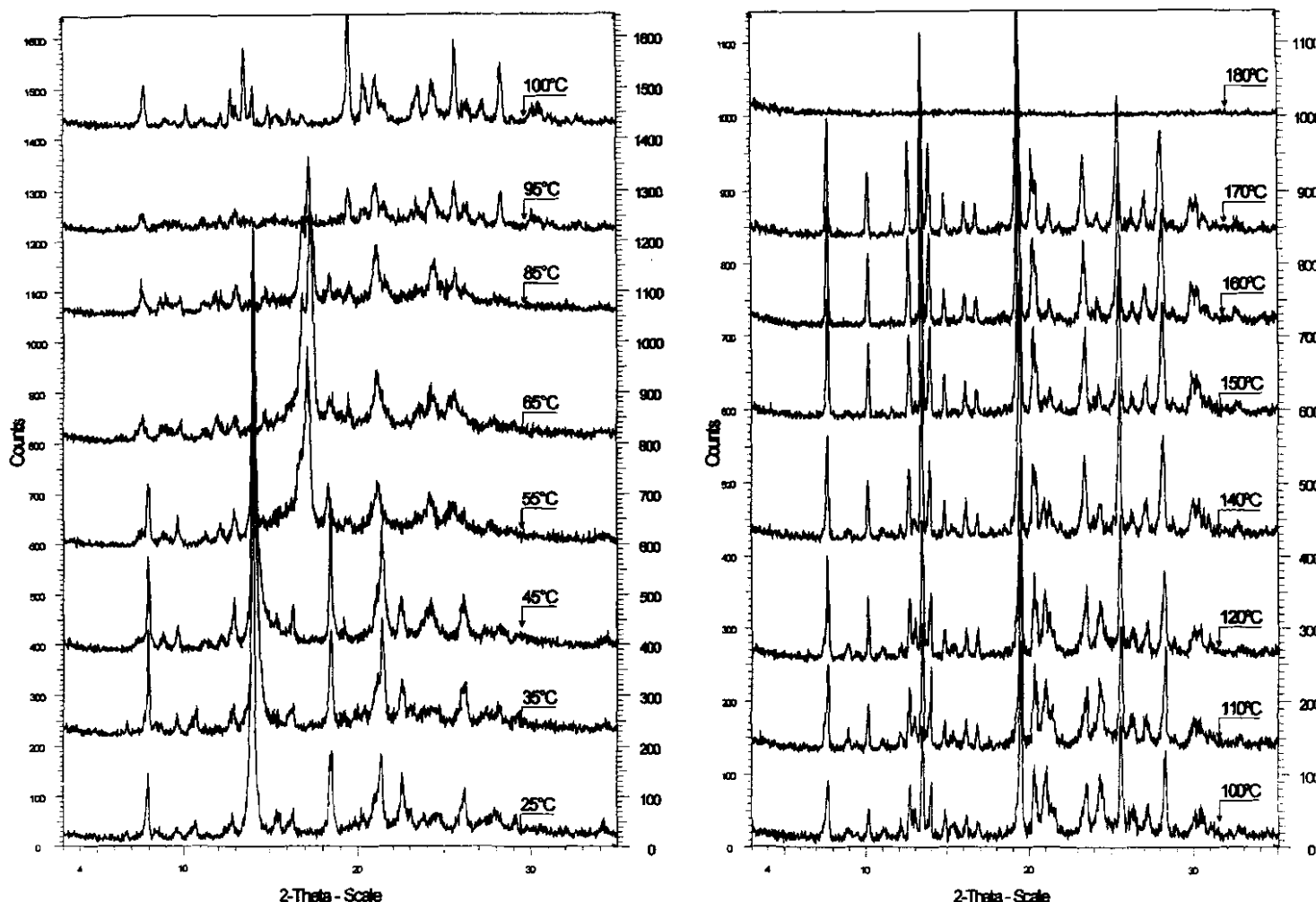


Figure 11. Variable temperature x-ray powder diffractograms of the recrystallisation product from absolute methanol (form F) in the temperature range 25-180°C.

An increase in crystallinity of the sample was observed between 25-45°C in the XRPD patterns. The x-ray powder diffractogram obtained at 45°C showed the greatest resemblances to that of form F.

The XRPD pattern changed significantly between 45-65°C. The XRPD pattern obtained at 65°C was comparable to that of form G. This correlated with the literature, which stated that heating of form F results in the transition to form G.

Further changes were observed in the diffractogram between 65-95°C. The XRPD pattern of form T2RP disclosed in the literature (figure 3) has a poor resolution and no peaklist was available (no other physico-chemical data on this form was discussed in the literature). The

XRPD pattern obtained at 95°C is comparable to that of form T2RP. These results were corresponding to the literature, which stated that drying of form G resulted in form T2RP.

The x-ray powder diffractograms of the sample changed noticeably between 95-100°C and only slight changes were observed in the temperature range of 100-170°C. The diffractogram obtained at 100°C was comparable to that of form omega (figure 5). The XRPD patterns obtained at 160°C and 170°C were comparable to that of form omega in figure 4.

The final diffractogram in this study (at 180°C) displayed no peaks, which suggested that the sample had melted.

This VT-XRPD study clearly indicated the thermodynamic transition of form F to form G and of form G to form T2RP and finally form omega (figure 12). Various intermediate phases were obtained between these forms.

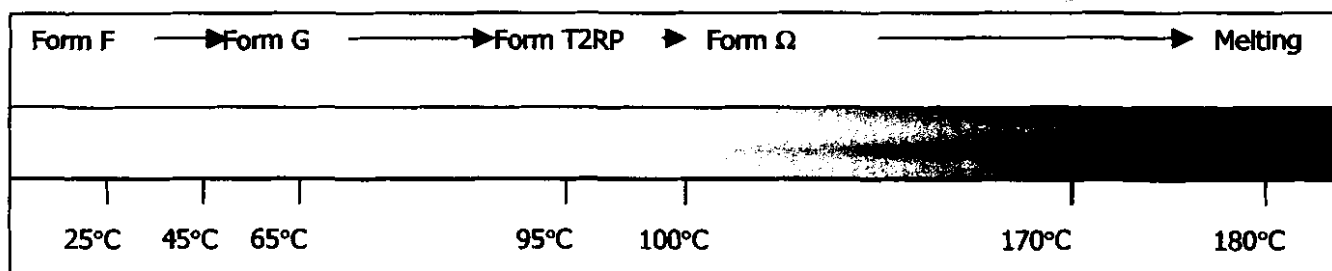


Figure 12. Thermodynamic transition of form F.

4. Conclusion

Various recrystallisation products were obtained from absolute methanol and binary mixtures of methanol and water. Three different crystal forms were obtained, namely a crystal form comparable to form F with the exception of peaks present at $7.3^{\circ}2\theta$ and $21.1^{\circ}2\theta$ (product A), form F (product A₂ and B) and a trihydrate crystal form (product C). The results obtained for product A, B and C indicated that the crystal forms obtained from absolute methanol and binary mixtures of methanol and water favoured the incorporation of water into the crystal lattice rather than methanol.

No absolute classification of form F was provided in the available literature. This study made it possible to classify form F as a trihydrated pseudopolymorph of gatifloxacin.

Alternative preparation methods of form F have also been identified and discussed in this article.

A second trihydrated form (product C) was also prepared in this study, which differed from form F (indicating polymorphic pseudopolymorphism). It is therefore important to distinguish between possible trihydrated crystal forms by means of XRPD, as TGA and KF data does not reveal significant differences.

As stated in the literature, form F transforms to form G, and form G to form T2RP or omega. A VT-XRPD study was performed to investigate these thermal induced phase transformations of form F. This study revealed the transition of form F to G, and clarified that form G first transformed into form T2RP, and that form T2RP then transformed into form omega.

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