Chapter 2

Methods of characterisation

2.1 Introduction

With polymorphism the physical or chemical property of the various modifications of a material differs. This being the reason for the multitude of analytical methods available for detection of different polymorphic or solid-state forms (Bernstein, 2002). It is, however, possible for different polymorphic forms to have physico-chemical properties that do not differ. As was stated by Borka (1991), it is possible for various polymorphic forms to have matching melting points. A good example is that of trimorphic D-mannitol (Burger *et al*., 2000), having a melting point of 166.5°C for Form I and 166°C for From II. This example is used to show that it is not always possible to distinguish between these forms by a single method and it is thus important to use different methods (preferably complimenting the other methods used) in order to detect correctly, differentiate between and classify polymorphs.

Molecular energies can be categorised into translational, electronic, rotational and vibrational energy. In order to assess these energies one should make use of the region of the electromagnetic spectrum (see Figure 2.1) that is related to the various energies involved. For electronic transitions one would use the ultraviolet and visible regions of the electromagnetic spectrum, for rotational transitions one would use the microwave region and for vibrational energy one would use the mid-infrared region of the spectrum (Anderton, 2003).

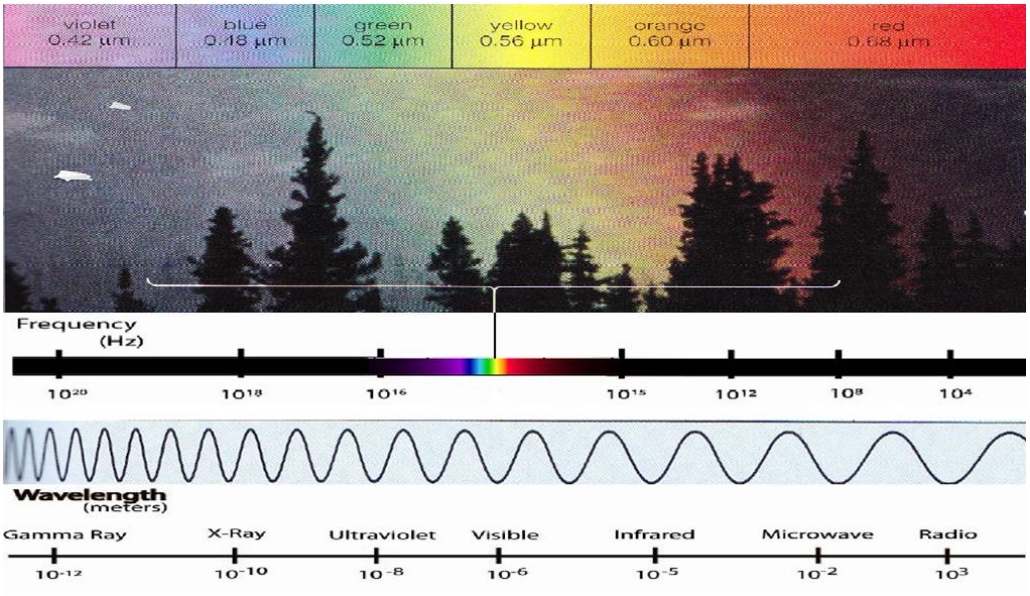

Figure 2.1 The electromagnetic spectrum (adapted from Ingraham & Ingraham, 2004).

The electromagnetic spectrum is grouped into the following wavelengths (listed here from shorter to longer wavelengths i.e. higher to lower frequencies): gamma rays, X-rays, ultraviolet radiation, visible radiation, infrared radiation, microwave radiation, short radio waves and long radio waves (Carlton, 2011).

Based on elementary quantum theory, it is possible to relate energy transitions taking place within atoms and molecules with the specific value of radiation absorbed by the particular atom/molecule. The analytical methods mentioned in this chapter make use of this principle in that they portray the atomic/molecular information by creating spectra from values of the interactions between electromagnetic energy and the atoms/molecules being analysed (Martin, 1993).

For each of the regions of the electromagnetic spectrum different equipment and/or methods can be used. The various methods and equipment and how they can be used to acquire information on the properties of materials will be explained in the following section. The methods show that by making use of a range of analytical procedures one can acquire much information on the molecular properties of the solids formed. The various methods used in this study should enable one to identify clearly and differentiate between different forms created.

2.2 Thermal analysis

Thermal analysis can be used as a quantitative measure to classify polymorphs by obtaining information on the relative stabilities of the various polymorphic modifications. Thermal analysis can also be used to acquire information on phase changes taking place as well as the energies associated with it and the monotropic or enantiotropic nature of these changes (Bernstein, 2002).

2.2.1 Differential Scanning Calorimetry (DSC)

Laye (2002) explains that the principle of DSC is based on the information of thermal changes taking place, because of a loss or gain in heat during the controlled heating and cooling of a sample while compared to an inert reference.

The term differential implies that in order to measure heat changes, a sample and a reference are needed. This makes the twin-type design a distinguishing feature for this method (see Figure 2.2) (Griesser & Stowell, 2003).

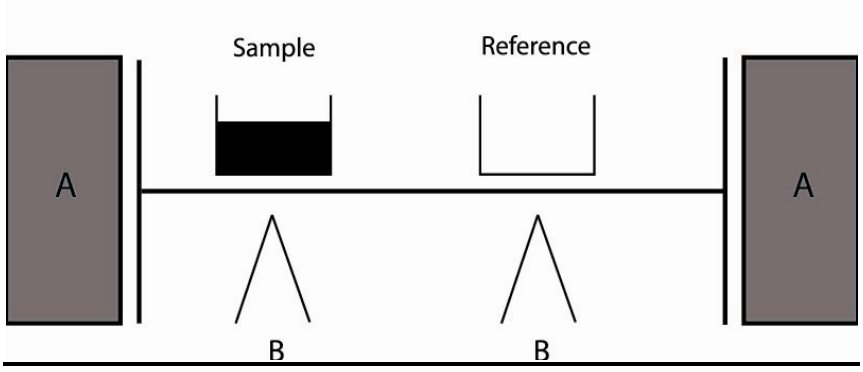

Figure 2.2 Schematic representation of a heat flux DSC, where A is the furnace and B is the thermocouple (Adapted from Reading & Craig, 2007).

The DSC is set up in a way that the dissimilarity in temperature between the sample and the reference is calculated directly by means of detecting the voltage produced by a corresponding back-to-back placement of thermocouples (Reading & Craig, 2007).

The flow of heat from the furnace can then be calculated via the following equation:

$$\frac{dQ}{dt} = \frac{\Delta T}{R}$$

(Equation 2.1)

where Q is heat, t is time, ΔT is the temperature difference between the furnace and the crucible and R is the thermal resistance of the heat path between the furnace and the crucible (Reading & Craig, 2007).

In the process of melting a sample, heat is used to break bonds between the molecules of the solid substance and this creates a lower rate of temperature increase between the sample and the reference pan (Reading & Craig, 2007).

Saunders and Gabbott (2011) state that when a single crystal is melted the combination of liquid and unmelted solid will stay at the melting point until all of the solid has melted.

The reason for this is that it takes a finite amount of time for the heat to penetrate the sample and overcome the latent heat of fusion. In other words heat energy is used to melt the sample in the sample pan, where in the reference pan it is used solely to increase the temperature. After the bonds are broken and the sample is in liquid form, the sample will return to the programmed temperature (Reading & Craig, 2007).

When melting is complete the temperature in the sample pan will catch up with the temperature in the reference (Saunders & Gabbott, 2011). This difference in energy taken up is seen on the DSC curve and used to detect events such as the melting point of the sample (Reading & Craig, 2007).

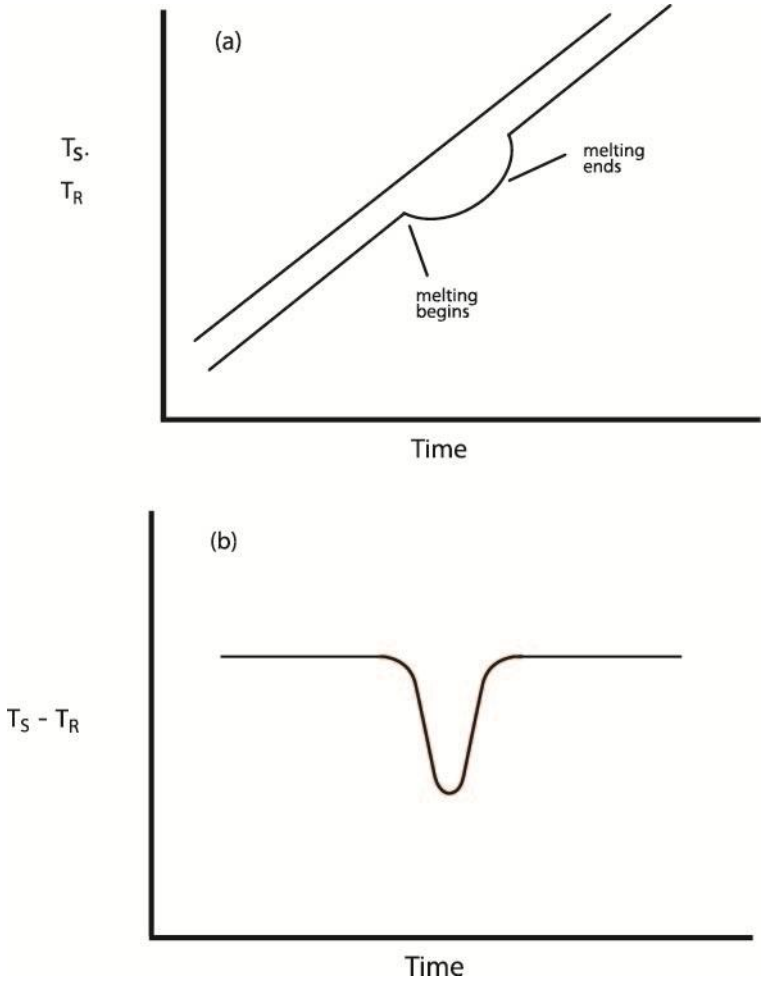

Figure 2.3 Representation of a common DSC response of (a) sample and reference temperature (T_S and T_R) over time and (b) the differential of the temperatures (T_S - T_R) over time (Adapted from Reading & Craig, 2007).

When a DSC is run without any event/transition such as, for instance, melting of the sample taking place, heat flow can be portrayed by means of the following equation:

$$\frac{dQ}{dt} = \frac{dQ}{dT} \cdot \frac{dT}{dt} \qquad \text{(Equation 2.2)}$$

where dQ/dt is the heat flow, dQ/dT is the heat capacity, and dT/dt is the heating rate (This equation was used in Chapter 1 to explain heat capacity, though in Chapter 1 heat capacity was represented by C_p) (Lever, 2007).

So putting this equation into words, it would look like this:

Heat flow = Heat capacity × Heating rate

When an event/transition does take place, however, it could be seen as an adjustment of the heat capacity of the sample or another term, i.e. kinetic event f(T,t), could be added to the equation making it appear as follows:

$$\frac{dQ}{dt} = \frac{dQ}{dT} \cdot \frac{dT}{dt} + f(T,t) \qquad \text{(Equation 2.3)}$$

or

Heat flow = Heat capacity . Heating rate + Kinetic events

When the resistance increases in order for the event/transition to take place in the sample, the temperature dissimilarity between the sample and the reference will also increase. This makes the heat flow increase to a similar extent and is thus controlled by it and is portrayed in the following equation:

$$\frac{dQ}{dt} = \frac{T_R - T_S}{R} \qquad \text{(Equation 2.4)}$$

where T_R and T_S are the reference and the sample temperatures, respectively, and R is the thermal resistance between reference and sample (Reading & Craig, 2007).

The onset of the melting peak, which is really the temperature at which one will find the most rapid heat change and not the actual thermodynamic melting point, is usually termed the melting point (Craig, 2006).

Evaluating the melting point transition:

To determine the extrapolated onset temperature a line can be extrapolated from the slope of the leading edge to the x-axis. The extrapolated onset temperature is the mark where this line crosses the x-axis (This process is done by making use of the software of the apparatus) (Saunders & Gabbott, 2011).

Melting point determination for identification of samples:

Every API has a unique melting point, and polymorphs as described earlier have unique melting points. This makes it possible to identify various polymorphic forms by means of melting point analysis. (As was mentioned earlier, there are possible exceptions to this principle and this should be used as a general guideline from which complimenting analyses can be used to verify whether it is the case) (Saunders & Gabbott, 2011).

In short a DSC can be used to identify different polymorphic or crystalline forms by means of melting point, the behaviour of transformation of these forms can be analysed and the interrelationship between various forms can be studied (Craig, 2006).

This method is thus useful for showing a possibility of new polymorphic forms and to study kinetics of transformations, but to confirm the presence of new forms and to determine their molecular structures other methods will be needed to accompany this method. In this case spectroscopic techniques will be applied for this purpose (Craig, 2006).

2.2.2 Thermogravimetric analysis (TGA)

Brown (2001) explains that a sample is heated or cooled while being weighed at the same time with an electronic microbalance coupled with a furnace and temperature programmer. The temperature, mass and time data are then captured by means of a computer.

Heal (2002) describes this form of analysis as the change in mass of a substance being subjected to a controlled temperature program when measured as a function of temperature.

When studying reaction kinetics in solid samples, as is the case in this study, it is assumed that reactions occur in the solid phase or the aforementioned reaction zone when looking at the rate data. This, however, in TGA is often not the case. Galwey and Craig (2007) explain that the physical changes taking place, like for instance melting is not observed by the TGA as no weight loss occurs. It is, therefore, important to find out in which state a reaction occurs to explain the reaction taking place satisfactorily (Galwey & Craig, 2007).

The weight loss observed in a TGA when studying a solid is usually from solvents or water vapour escaping from the solid, the melt evaporating or the solid going from solid to gas/vapour by means of sublimation (Galwey & Craig, 2007).

This does not give one very much information on all the kinetic transformations taking place in a sample, though when used in combination with DSC more clarity can be found on such matters. This method does, however, provide a substantial amount of information on weight loss of a sample and the temperatures at which this

occurs. This makes it possible for the analyst to detect solvates or hydrates and determine whether the solvent or water is lost at the regular boiling point or perhaps at a higher temperature. This might indicate that the solvent or water is more tightly bound to the solid at a molecular level and does not just fill a cavity (Galwey & Craig, 2007).

Craig (2006) states that TGA plays a key role in the verification of the stoichiometry of solvates and can possibly be used to identify the type of binding that is to be found in samples being analysed.

Numerous solids do not conduct heat well and when held in a vacuum, the differences between the temperatures inside the solid where the reaction is taking place, i.e. the aforementioned reaction zone may be quite different from the controlled reaction environment and thus the recorded temperature in a TGA run (Galwey & Craig, 2007).

This method is used to create a partial pressure of zero and the temperature can be altered and by this means the stability of solvates can be tested (Galwey & Craig, 2007).

2.3 Microscopy

2.3.1 Thermal microscopy (TM)

This is a method that makes use of visual observations to detect polymorphs/solvatism, identify boiling/melting points or phase changes. Thermal microscopy is best used in conjunction with other thermal analysis methods.

One can make use of thermal microscopy to determine whether or not evaporating solvents or water is released from a solid when heated by placing a drop of oil on the sample being investigated. The escaping gases will be observable as gas bubbles in the oil escaping from the solid. The temperature at which this takes place can be compared to the boiling point of the solvent in question to determine if it is in fact the solvent escaping or simply air bubbles or perhaps gases formed by decomposition. Results from other methods such as TGA can be compared to these results as well in order to verify the loss of solvent (Vitez & Newman, 2007).

2.3.2 Light microscopy

This method involves the use of visual imagery (visible light falls in the wavelength region of 400 to 700 nm (Carlton, 2011)) to analyse crystal habits and by means of measuring the birefringence and refractive indices of samples, various polymorphs could also be detected.

A refractive index of a material can be defined as the ratio of the speed of light propagating through a vacuum in comparison to the speed of light propagating through the particular material or stated as an equation:

$$n = \frac{v_{vacuum}}{v_{medium}} \qquad \text{(Equation 2.5)}$$

Where n is the refractive index and *v* is the speed of light (Carlton, 2011).

Light microscopy makes use of a microscope that magnifies the image of a sample to an extent where fine details that are impossible to see with the naked eye are made visible. This method sometimes requires specific sample preparation methods, altering the image contrast by various methods or by processing the image after the analysis has been done with software designed for this purpose (Nichols *et al.*, 2011).

Advantages of optical crystallography is that it provides information on some of the solid-state properties that other methods neglect and, therefore, makes it a good method to use in combination with other techniques (Carlton, 2011).

<u>The microscope as analytical tool:</u>

When studying crystals with non-polarised light one is unable to link known vibrational directions with optical properties and thus can only see the average value of properties such as colour or refractive index (Nichols *et al.*, 2011).

This is because non-polarised waves of light oscillate in various directions as is depicted in Figure 2.4. To do a more selective study of optical properties of a crystal one can make use of a polariser to filter the light into a single plane. By doing this one limits the directions in which the waves oscillate. This is the principle of polarised light microscopy (Carlton, 2011).

2.3.3 Polarised light microscopy

Differences in the optical properties of various polymorphs such as the refractive index, colour, extinction angle and optical dispersion can be seen because of the dissimilarities in their crystal structures. It is, therefore, possible to detect various polymorphs by means of analysing these properties. This can be done by making use of crossed polarisers in a method called polarised light microscopy (Nichols *et al.*, 2011).

This method makes use of the interaction between polarised light and the long-range alignment of molecules in crystals to make a distinction in birefringence visible (Nichols *et al.*, 2011).

The crossed polarisers mentioned earlier are additional components that can be added to regular light microscopes. They are plane polarizing filters called the polariser and the analyser and are attached to the microscope below and above the specimen respectively. When these pieces are angled in a way that the privileged vibration directions are at right angles to each other they are referred to as crossed polarisers (see Figure 2.4) (Nichols *et al.*, 2011).

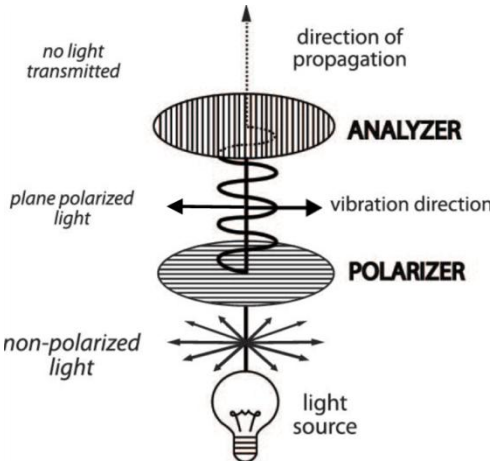

Figure 2.4 Representation of polarised light microscopy where the effects of polarisation on non-polarised light caused by a polariser and an analyser is shown (Adapted from Nichols *et al.*, 2011).

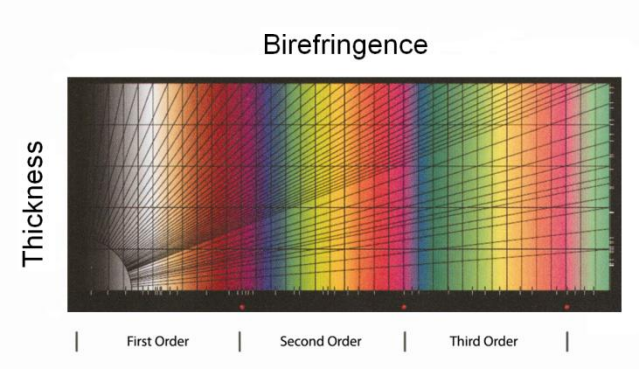

Figure 2.5 Michel-Levy colour chart (Adapted from Carlton, 2011).

Phase changes can be detected by visualising changes in polarisation colours that are of a discontinuous nature during a heating run (Bernstein, 2002).

Isotropic and anisotropic materials:

The terms isotropic and anisotropic for crystalline materials refers to the influence of directional aspects of the composition of these materials on their physical, chemical and optical properties. Where in anisotropic materials the direction influences these properties and in isotropic materials it does not (Nichols *et al.*, 2011).

Anisotropic materials will show colours, termed interference/polarisation colours, when observed through crossed polarisers. This is because the plane polarised white light that enters the anisotropic material is resolved into two mutually perpendicular rays with different refractive indices as a result of the vibration directions they are directed in. For this reason they are called doubly refractive. The difference in refractive indices causes one of the rays to move through the material at a slower rate. When these two rays rejoin at the other end of the material into a single plane at the analyser, they will differ by a few nanometers because of this delay (Nichols *et al.*, 2011).

The delay caused will be unique to a specific material as it results from the interactions of light with the functional groups within the molecules of the material. This delay of the speed of light moving through the sample can be calculated mathematically by the following equation:

$$r = 1{,}000t \times B \qquad \text{(Equation 2.6)}$$

Where r is the delay in speed of light measured in nm, t is the thickness of the sample in µm and B is the birefringence of the sample (Carlton, 2011).

This will result in the alteration of the colours that are seen by the analyst and the total delay as shown in equation 2.6, will be determined by the thickness of the sample analysed as well as its birefringence (this relationship can be seen in the Michel-Levy chart (Figure 2.5)). The term birefringence can be defined as the numerical difference between the highest and lowest refractive indices (Nichols *et al.*, 2011).

This will then result in the observation of a specific colour for a specific combination of particle thickness and birefringence. This occurrence can be explained by making use of the progression sequence of colours that is portrayed in the Michel-Levy chart (Figure 2.5) (Carlton, 2011).

If the principle vibration directions of the crystal being analysed are orientated to line up with those of the two polarising filters, one will not see any colours. This occurrence is termed extinction (Nichols *et al.*, 2011).

The delay of the rays created by the differing refractive indices in anisotropic materials does not occur in isotropic crystals because the light travels through them at the same rate in all directions without any interference (Carlton, 2011). One can explain this by recalling that they possess a single direction of vibration and no difference in refractive index. This means that the colours formed by the constructive or destructive convergence of the rays will not be present and the polarisation colours formed in the case of anisotropic materials will not be seen. This is the case for amorphous solids and cubic crystals. This means that they will not have double refraction, birefringence and will not display interference colours when studied through crossed polarisers. It is, however, possible for isotropic solids to display weak interference colours when a partial ordering of molecules is present (Nichols *et al.*, 2011).

Other factors than crystallinity can influence interference colours and for this reason this method, like most methods, should not be used in isolation for definitive results (Nichols *et al.*, 2011).

“The observation of the melting process under polarized light microscope, accompanied with a relatively inexpensive hot-stage is a very powerful method of studying polymorphism” (Borka, 1991).

2.3.4 Scanning electron microscope (SEM)

Scanning electron microscopy is used for the superior magnification that it provides when compared to regular optical microscopy. This enhanced magnification can lead to better understanding of dissimilarities in the properties of polymorphs and also characterisation of these polymorphs (Bernstein, 2002).

Where light microscopy usually goes up to about a 1000 times magnification, by making use of this method one can reach a magnification of up to 250 000 times. A difference between the two methods though is that SEM images only display the surface features where with a light microscope one can also see the internal features of the sample analysed if light is able to pass through it (Nichols *et al.*, 2011).

A SEM works by emitting a beam of electrons from a cathode situated in what is known as an electron gun (Nichols *et al.*, 2011).

In light-microscopy, lenses are used to refract and focus light in order to illuminate the sample being analysed to obtain an image. When using a SEM, however, instead of lenses, variable-power electromagnets are used. These electromagnets do not form an image of the sample being analysed, but rather focus the electron beam emitted by the electron gun to illuminate a single part of the surface of the specimen (Nichols *et al.*, 2011).

This beam is deflected through scan coils that cause the beam to scan across the surface of the sample. The deflection of the electron beam is synchronized with the viewing monitor by a scan generator. When viewing the sample through the monitor one can adjust the brightness of an image by means of intensifying the electron beam emitted (Nichols *et al.*, 2011).

X-rays and electrons that are emitted when the primary scanning electron beam strikes the sample are detected by various detectors that are situated a few millimeters away. It is possible to move the sample around in various ways to alter the image obtained and to acquire different magnifications one can adjust the area scanned (Nichols *et al.*, 2011).

2.4 Spectroscopy

These methods are primarily used to identify the short-range arrangement of molecules (i.e. the electronic environment resulting from molecular functions) in

solids and are thus very useful when used in combination with X-ray diffractometry, because it is used for the identification of long-range order in solids (Griesser & Stowell, 2003).

2.4.1 Fourier-transform infrared spectroscopy (FTIR)

Infrared (IR) waves are used in this method, more specifically waves having a wavelength in the range of 400 and 4000 cm^{-1}, which falls under the mid-infrared region. These are the wavelengths corresponding to vibrational energies and are what is necessary to cause a frequency shift related to the specific vibrational modes of covalent bonds in molecules as was mentioned earlier. The spectra created by this method are created by the measurement of the absorption of the IR radiation that is related to these vibrational modes of molecules (O'Neil & Edwards, 2011).

The results are then used for characterisation primarily for molecular properties of solid materials instead of solid state properties. Differences in spectra can then be related to environmental or conformational factors or used to identify polymorphs (Bernstein, 2002).

FTIR spectra show the number of absorptions and their associated wavenumbers as well as their intensity and sharpness. This is obtained from analysing the transmission, absorption, scattering and reflection of IR radiation (O'Neil & Edwards, 2011).

The various vibrational modes detected by FTIR can be categorised into changes in bond length and changes in bond angles. Two possible modes of stretching vibrations exist that fall under bond length changing vibrations and four of vibrational bending that fall under bond angle changing vibrations (O'Neil & Edwards, 2011).

To determine the amount of vibrational modes also referred to as fundamental modes of a drug molecule, one can make use of the equation M=(3N-6) where M represents the number of vibrational modes and N represents the amount of atoms in the molecule. Whether or not a vibrational mode will be detected is dependent on the atomic masses of the atoms involved and the bond strength of the specific bond (O'Neil & Edwards, 2011).

For a vibrational mode to absorb IR radiation it is required that a change in dipole moment of the molecule will come to be when absorbing the IR energy. The higher

the change in dipole moment the higher the intensity of the absorption band will be in proportion to the square of change in dipole moment. This means that polar bonds such as O-H or N-H bonds will usually be displayed with high intensities in IR spectra (Anderton, 2003).

One can assume that most bonds will exist in the ground state at ambient temperature and will therefore need to absorb the energy from the fundamental IR frequency to reach the fundamental mode. One can therefore identify a bond and consequently identify functional groups by linking it to a fundamental frequency (O'Neil & Edwards, 2011).

This means that one can find information on which functional groups are at hand and on the characteristics of the forces involved in their bonds by means of analysing the positions of the bands in the spectra (Anderton, 2003).

An advantage of FTIR methods over other IR methods is that it gives accurate locations of absorption bands. This makes it possible to compare results more effectively, as various polymorphs can have features that are very alike, especially when viewed as graphs only (Bernstein, 2002).

Vibrational spectroscopy of polymorphs, hydrates and solvates:

As was explained in the section on crystal habits/morphology (section 1.4.1.1), it is possible for crystals to have different morphologies though not differ in terms of crystal structure (Anderton, 2003).

This is, however, not the case when one considers polymorphs, hydrates and solvates. In the case of these forms one should be able to detect differences in the micro-environments of the functional groups involved. This is because the alterations in conformation and crystal packing (that is by definition what differentiates the various polymorphic forms) can alter the steric forces and/or inter or intra-molecular bonding between or within molecules. If one considers the explanation in the previous sections of how the spectra are formed from the various frequencies involved it will make sense that these forms could show differences in spectra for some or in some cases, even all the vibrational modes involved. In the case of solvates and hydrates one will definitely detect differences in spectra as they will contain different functional groups (Anderton, 2003).

The symmetry of the molecular arrangement of solids can be altered by the molecules changing into different solid-state forms. This can influence the intensities of peaks or in some cases result in band splitting. It is also possible for unit cells to become asymmetric and include more than one molecule in its boundaries. This will also cause band splitting. This will often be the case for molecular arrangements lacking repeating periodic order through its structure such as amorphous materials. Chemical information of these amorphous forms can still be acquired by this method. Each solid-state form will have a unique IR spectrum and for this reason one can use this method to distinguish between different forms. This unique spectrum is known as a spectral fingerprint. One can determine which structural differences are to be found between the various solid-state forms by analysing these spectra (Anderton, 2003).

2.4.2 Ultraviolet spectroscopy

Will be discussed in pararagraph 2.7.

2.5 X-ray powder diffraction (XRPD)

When using X-ray diffractometers, radiation with wavelengths near that of a bond length are used to identify the structures of various materials such as ionic compounds, molecular compounds and complex assemblies. In the case of organic molecules as it was for this study, the X-ray source is a hot cathode tube with a copper anode that is able to obtain a resolution of 0.75Å (Griesser & Stowell, 2003).

The principle of this method is that the X-rays produced by this source are diffracted off of the electrons that surround atoms in the sample being analysed (Griesser & Stowell, 2003).

Because of the wave nature of X-ray radiation it is possible for it to be diffracted from crystal planes in a way that each atom serves as a centre from which scattering takes place (Mattox, 2010).

Since the X-rays are diffracted from the atomic electrons the positions of atoms with few electrons are more difficult to determine with accuracy and makes the positions of hydrogen atoms and particularly acidic hydrogen atoms very difficult to determine accurately. The positions of atoms with high electron densities, such as bromine or

iodine, on the other hand will be easier to determine with accuracy because of their greater abilities to diffract X-rays (Griesser & Stowell, 2003). This ability to diffract x-rays is termed the atomic scattering factor (Gilmore, 2011).

With this method, as is suggested by the name, one analyses powders by means of x-ray diffractometry. This is done by making use of what is known as the Bragg equation (Equation 2.7) to determine the arrangement of molecules within a crystal. The Bragg equation uses the angle (θ) at which the monochromatic x-ray beam from the source mentioned earlier is diffracted from the sample and relates it to the interplanar spacing (*d*) within the sample. In order to obtain a diffractive pattern one needs to meet the requirements stated in the Bragg equation.

This equation is represented by:

$$n\lambda = 2d\,\sin\theta \qquad \text{(Equation 2.7)}$$

where λ is the wavelength of the X-ray beam and *n* is any positive integer (Griesser & Stowell, 2003). These terms are depicted in Figure 2.6.

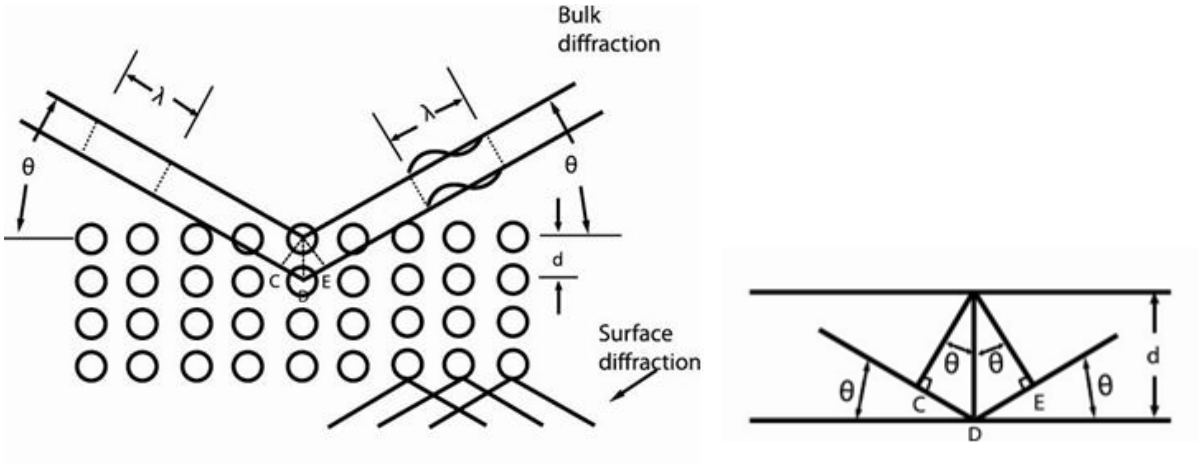

Figure 2.6 Left: Depiction of X-rays as they are diffracted by the sample being analysed; Right: Angles of diffraction causing interference where d is the distance between planes λ is the wavelength and θ is the angle of refraction (Adapted from Mattox, 2010).

It is possible that diffracted X-rays can cause constructive interference when being diffracted from the bulk or the surface of the solid being analysed. The reason for this, as displayed in Figure 2.6, is that when the divergence of the pathlength is

equal to the integral number of wavelengths the interference will be additive. This can be written as Equation 2.7 (where n is a positive integer) (Mattox, 2010).

The diffraction pattern of x-rays will differ when the direction from which the probing x-rays come from is changed. The intensity of the signal is dependent upon the population of the plane being analysed. This difference is depicted in Figure 2.7. Here one can see that the plane population and the spacing between these planes will be consequential of the direction of the probing x-rays (Mattox, 2010).

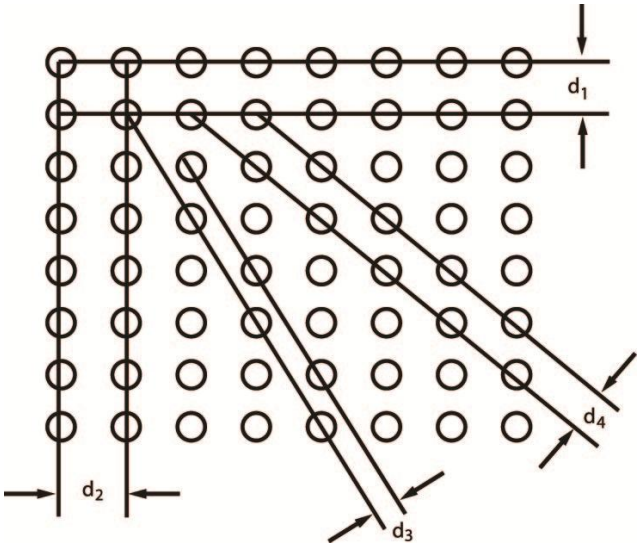

Figure 2.7 Representation of the plane population and spacing between planes for a 2D lattice (Adapted from Mattox, 2010).

For this reason the sample being analysed is rotated for the diffraction pattern to be a representative of the whole and not be of only a specific directional diffraction. This can, however, be a problem if the sample being analysed has what is known as a preferred orientation. This can occur for instance if the sample has a plate-like or needle-like shape (Griesser & Stowell, 2003).

In the first instance, as described by Griesser and Stowell (2003), one can picture the crystals as a deck of cards having been flung in the air. Some of the cards will fall face up and some will fall face down though very few, if any, will land on its edge. In the second instance, as described by Gilmore (2011), the needle shaped crystals will tend to align themselves in a way that they are all in line with one another.

This causes the diffraction pattern to be representative of only the “preferred” orientation of a single crystal face and not of the whole. A limited amount of grinding can be used to reduce this problem (Gilmore, 2011).

The specific positions and intensities of diffraction peaks are thus a depiction of the molecular arrangement of the sample. The intensities measured are then used as data for comparison and identification of molecular arrangements and is usually measured as °2θ (degrees two-theta). The range most pharmaceutical compounds diffract x-rays in is between 4 and 40°2θ when the x-rays are obtained from a copper source (λ=1.5405 Å) (Griesser & Stowell, 2003). This method can therefore be used as a means of identifying various crystal forms of an API, as each form will have a specific diffraction pattern. This also makes it possible to determine whether polymorphs or solvates were formed (Griesser & Stowell, 2003).

XRPD in analysing amorphous solid-states and polymorphic forms:

According to Byrn *et al.* (1999), XRPD is a useful means of differentiating between forms of APIs with divergent internal structures and can be performed on powdered samples. This alleviates the problem of not having large single crystals in certain instances. This method can be used to identify definitively and characterise polymorphs (Bernstein, 2002).

Because of the lack of long-range order in amorphous substances, an XRPD will show a broad, featureless diffraction pattern with a low intensity that is known as an amorphous halo. Some features might be evident though, because of short-range order in certain amorphous substances (Griesser & Stowell, 2003).

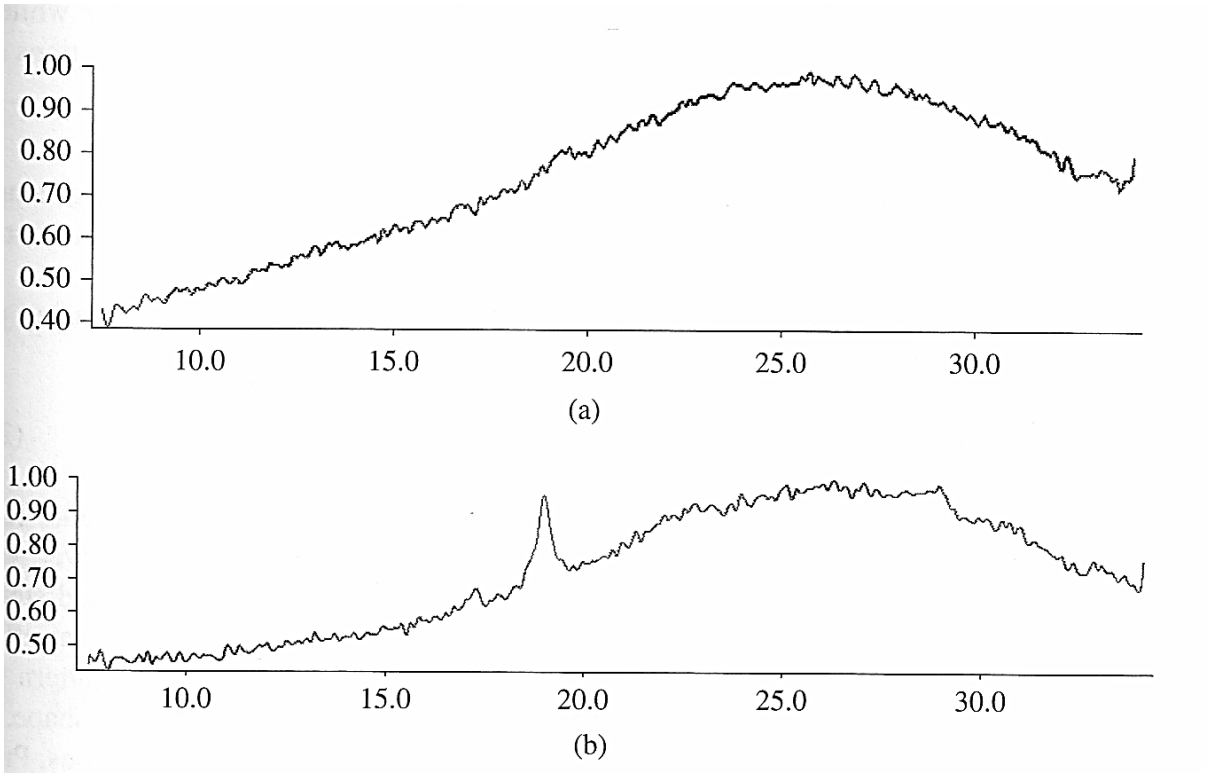

Figure 2.8 XRPD patterns for amorphous samples. (a) Amorphous substance without any large peaks showing what is known as a “halo” pattern. (b) Amorphous substance with some crystalline content (Gilmore, 2011).

If one starts off with a crystalline material and gradually reduces the size of the particles being analysed in steps, taking X-ray images of each step along the way, one will find that the intensities of the resulting images will gradually diminish until it becomes diffuse. This will occur when the sizes of the particles being analysed become less than 10^{-5} cm. If the sizes are reduced even further, the lines of the images will continue to become more and more diffuse, until the limit is reached at a size of about 10^{-8} cm. At this scale one will reach atomic dimensions and no diffraction patterns will exist as there is no longer any crystal structure, seeing as though no atoms or molecules are bound to form an ordered array. This absence of diffraction when examined through X-ray is also present in amorphous materials, as was discussed earlier. This means that amorphous forms will only have short-range order or otherwise termed crystal sizes of 10^{-5} Å or less (Ymén, 2011).

2.6 Chromatographic analysis

2.6.1 Thin layer chromatography (TLC)

This method is classified as a flat-bed chromatographic method. The principle of this method is that one can differentiate between materials by detecting and measuring differences in the distances moved by the material in a system consisting of two separate phases, of which one is static and the other mobile and justly termed the static phase and the mobile phase respectively. In this case the static phase is a solid adsorbent porous material (known as the chromatogram) and the mobile phase a liquid (Gasparič & Churáček, 1978).

The variation in adsorption between various substances is caused by the variation in affinities of the substances being analysed with the mobile and static phases.

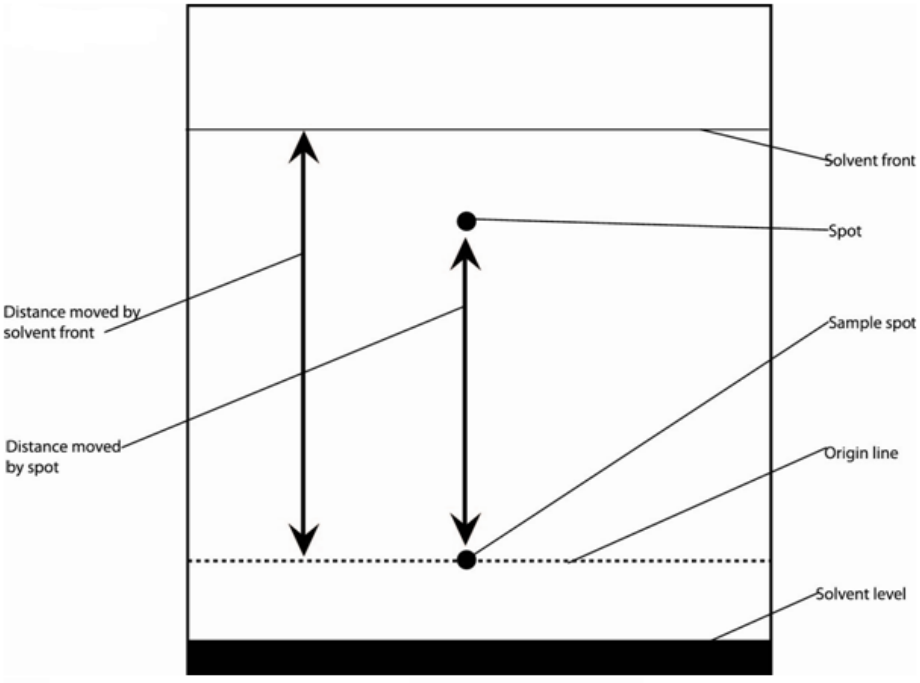

Figure 2.9 Depiction of a standard TLC plate (Adapted from Hamilton & Hamilton, 1987).

The results of this method can be seen as spots on the chromatogram when making use of chemical, biological or physical detection methods. If the correct method of detection, mobile phase and static phase is used, each material will move a specific distance that is unique to that material and can be used as a means of differentiating between materials (Gasparič & Churáček, 1978).

The value used to describe the variation in distance moved by various materials on the chromatogram is the R_f and is determined by the following equation:

$$R_f = \frac{Distance\ moved\ by\ spot}{Distance\ moved\ by\ solvent\ front}$$

(Equation 2.8)

The various physical forces and chemical properties governing the distances moved by the various materials in the system are the electrostatic interactions that are found between ions, dipoles (or between ions and dipoles), π-interactions, van der Waals forces, etc. resulting in variation in affinity for the different phases of the system and altering the adsorption (Gasparič & Churáček, 1978).

One can make use of a static phase that has a fluorescent indicator for making detection by means of ultraviolet light possible or assist in it (Gasparič & Churáček, 1978).

The wavelengths of visible light range from 400 to 700 nm. Coloured substances absorb some of the polychromatic (white) light and one sees the radiation that is not absorbed but is that is reflected by the substance. Substances that do not display visible colours absorb radiation with wavelengths below that of visible light in the range of 200 to 400 nm known as the UV range. These substances can be seen by irradiating the TLC plate with UV light if the plate/chromatogram being used is treated with a layer of a fluorescent indicator. The UV light (of the right wavelength) will be absorbed by the substance while the rest of the UV light will be reflected by the fluorescent plate and the substance will thus show up as a dark spot on the fluorescent surroundings of the chromatogram (Jork *et al.*, 1990).

Adamovics and Escbach (1997) states that this method offers more flexibility in terms of choice of solvents than other methods for instance HPLC.

2.7.1 Ultraviolet spectroscopy (UV)

Beer's law states that the amount of light absorbed at a fixed wavelength can be related to the concentration of the sample and the distance the radiation has to pass through the sample by making use of a constant known as absorptivity (Martin, 1993). Absorptivity is described by the following equation:

$$A = abc$$

(Equation 2.9)

Where A is the amount of light absorbed, a is the absorptivity in units of liter g^{-1} cm^{-1}, b is the length of the path of radiation passing through the sample measured in cm, c is the concentration of the absorbing substance in g/liter (Martin, 1993).

Organic molecules are known to absorb light when they are in liquid form or are in solution. This takes place at specific wavelengths for the reason that the light absorbed has a specific type of electronic transition coupled to it. This can be used to identify samples, determine rates of reactions or calculate concentrations in solubility studies (Martin, 1993).