Chapter 3

Ethionamide raw material

3.1 Introduction

In the following chapters the specifications of the materials and methods used in this study are described along with the results obtained through these methods. The principles of these methods were explained in Chapter 2, but now the focus is on the specifications of these methods and the results obtained. The results are displayed and described in the following format:

Firstly, the ethionamide is characterised as the raw material (RM) form. From the results obtained, comparisons can be made between the other experimental forms obtained throughout this study (Chapter 3).

Secondly, the experimental forms obtained through recrystallisation from solvents are characterised and the resulting data on the properties of these forms are compared to the properties of the raw material (Chapter 4).

Thirdly, the properties of the experimental forms obtained through recrystallisation from vapour through sublimation recrystallisation/ physical vapour deposition methods are compared to that of the raw material (Chapter 5).

In trying to create new solid-state forms or polymorphs having different molecular coordinations, as was depicted by the energy landscape in the first chapter (section 1.2.3), with useful properties, it is usually quite difficult or even seemingly impossible to predict the outcomes of the experiments. A range of methods were used in an attempt to acquire new useful polymorphs and/or solid-state forms. The thought processes behind the methods used and how they changed throughout this study will be explained in this section.

3.2 Ethionamide raw material

3.2.1 Nomenclature

Chemical name

2-ethylpyridine-4-carbothioamide; 2-ethyl-4-thiopyridylamide; ethionamide; 2-ethylisonicotine thioamide; 2-ethyl-thioisonicotinamide; 2-ethylisonicotinthioamide; 2-ethylthioisonicotinamide

Non-proprietary name

Ethionamide

Proprietary names

Trecator® (Wyeth); Nisotin; Trescatyl (M&B); Aetina; Ethimide; Iridocin (Bayer); Tio-Mid

CAS registry

536-33-4

3.2.2 Formulae

Empirical formula

$C_8H_{10}N_2S$

Structural formula

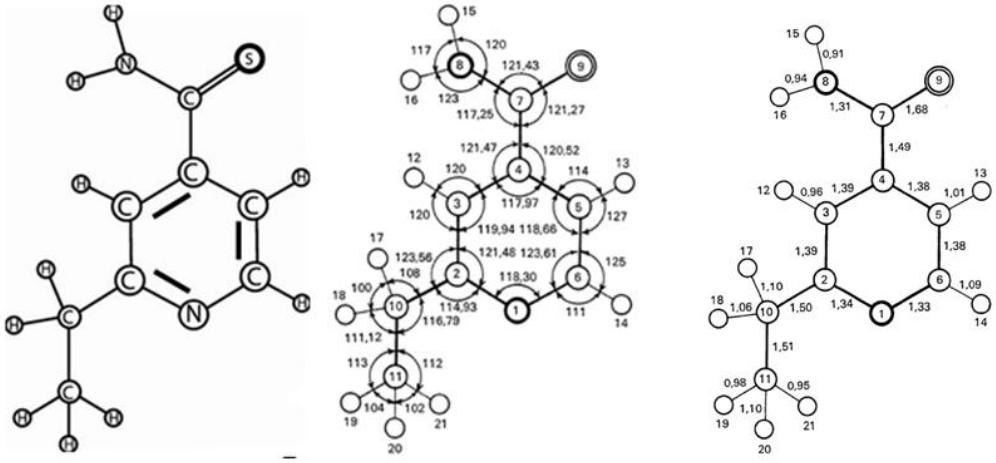


Figure 3.1 Structural formulae (a) ball and stick model, (b) showing interatomic angles and (c) showing interatomic distances in Å (Adapted from Alléaume *et al.*, 1973).

3.2.3 Molecular weight

166.24 g/mol

3.2.4 Appearance and colour

Ethionamide is a yellow, crystalline powder with a sulfide odour (USP, 2012).

3.2.5 Formulation and optimal human dosage

Supplied in 250 mg tablets, recommended dose 750 mg orally [FDA label]. The marketed sugar-coated tablets have been replaced by film-coated tablets. In order to obtain a plasma concentration above the MIC the lowest possible dose is 500 mg (Zhu *et al.*, 2002).

3.3 Physical properties

3.3.1 Solubility

It is practically insoluble in water and ether, but soluble in methanol and ethanol. It has a partition coefficient (octanol/water) Log P value of 0.3699 (USP, 2012).

Very sparingly soluble in water or ether. Sparingly soluble in methanol, ethanol or propylene glycol. Soluble in hot acetone or dichloroethane. Freely soluble in pyridine (O'Neil *et al*., 2001).

3.3.2 Polarity

Log P 0.705 (DrugBank, 2012).

3.3.3 Melting point

164-165ºC (O'Neil *et al*., 2001).

3.4 Characterisation of ethionamide raw material

3.4.1 Differential scanning calorimetry (DSC)

Method

DSC thermograms were recorded by making use of a Shimadzu DSC-60A differential scanning calorimeter with TA60 Version 2.11 software (Shimadzu, Kyoto, Japan). Dry nitrogen was used as purge gas at a rate of 35 ml/minute. Approximately 2-10 mg of a sample was weighed and placed in an aluminium crucible. The lid of the crucible was pierced and the crucible was sealed. The samples were heated at a rate of 10°C/minute (the heating rates used varied from 2 to 10°C/minute depending on what the purpose of the experiment was (see following section) and the resulting thermograms were recorded.

In the case of heat flux DSC, which is the method that was used in this study, the reference consisting of an empty crucible and the sample are heated in the same furnace and the dissimilarity in temperature is measured by sensors (as was explained in Chapter 2). The dissimilarity in temperature is converted to differential power by the software of the apparatus. This can then be used to compute the temperature at which as well as the energy required for a thermal event to take place (Brown, 2001). Interpretation of these results can lead to the identification of boiling points, sublimation, vaporisation, crystallisation and other phase transitions of the sample analysed.

As was mentioned earlier, there are dissimilarities in the temperature between the sample and the reference due to the heat needed to melt the sample, while the reference stays effectively isothermal. Because of this, greater resolution should be expected as a result when lower heating rates are utilised and greater sensitivity should result from higher heating rates (Reading & Craig, 2007).

Because the DSC measures the flow of energy, a higher heating rate will increase the amount of energy flowing in a given time period. This will show up as bigger peaks on the plot and can thus be used to find transitions or events that are otherwise hard to detect. Resolution on the other hand will decrease with higher heating rates because of thermal gradients across a sample and for sharper resolution a slower rate is needed (Saunders & Gabbott, 2011).

The kinetics of events also need to be taken into consideration, as a heating rate that is too high might skip certain events that take longer to complete (Saunders & Gabbott, 2011). It is, therefore, important to find the correct balance between resolution and sensitivity for each experiment to optimize results. For this reason different heating rates were used in this study.

In trying to find the middle ground heating rate according to Lever (2007), 10°C/minute is a sensible starting point for DSC studies. This is true for samples having small variances in transition temperatures such as in polymorphs.

In Figure 3.2 one can see examples of some events that are detectable by means of DSC.

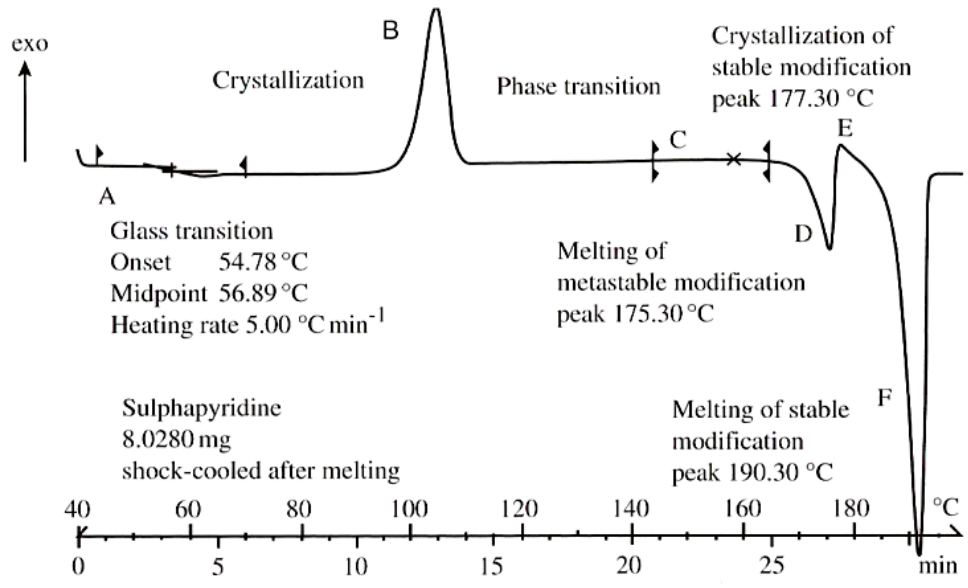


Figure 3.2 An example of a DSC of sulphapyridine that has been prepared by quench cooling the melt to illustrate some events that are possible to detect by means of DSC (Bernstein, 2002).

Results

The following DSC results were obtained by various heating rates (2-, 5- and 10°C/minute) of ethionamide RM and illustrate the variation in resolution and sensitivity resulting from the change. This was done to characterise the RM by acquiring knowledge of temperatures at which events take place as well as the changes in heat involved for these events. Another reason for these analyses was to obtain results that can serve as a reference standard for comparison for all the other experimental forms created.

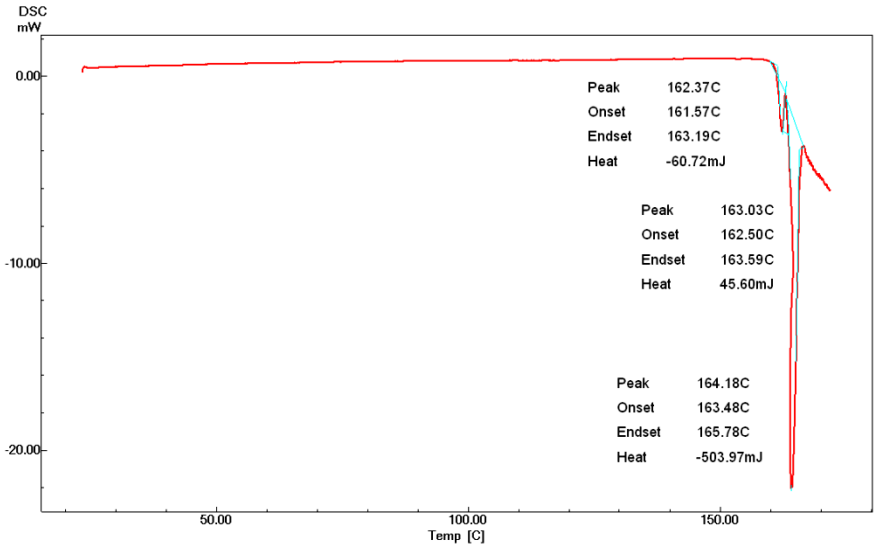


Figure 3.3 DSC trace of ethionamide RM at a heating rate of 2°C/minute.

In Figure 3.3 the first endotherm is quite small (heat change of 60.72 mJ) as can be explained by noting that at a lower heating rate less energy is flowing in a certain time period and thus a smaller amount of energy, only enough to cause the event, will be detected. The temperatures of this event were as follows: Onset temperature of 161.57°C, peak at 162.37°C and endset at 163.19°C. When comparing these temperatures with those obtained at the other heating rates one will find that the temperatures are similar to that of the results obtained at the heating rate of 10°C/minute (Figure 3.4). This can imply that this is a first order phase transition because it is not influenced by the rate of heating.

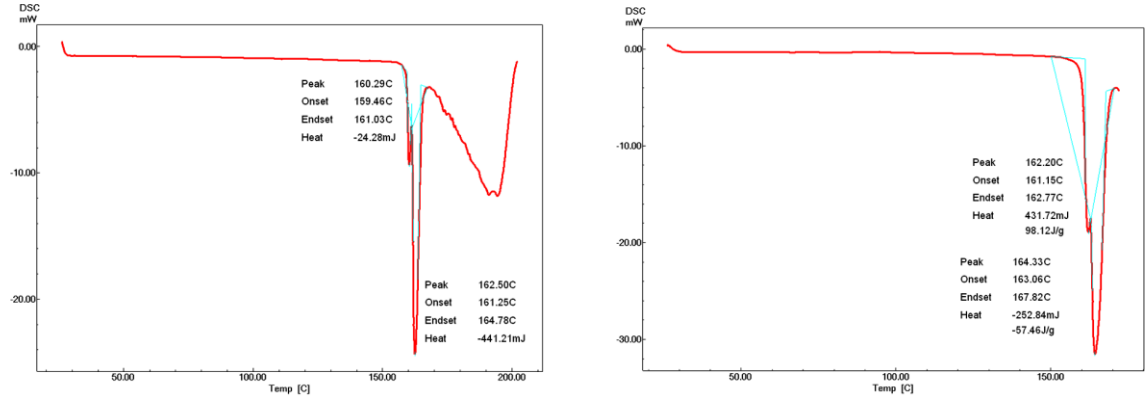
During the first endothermic event the return of the trace to the baseline is interrupted by a second endothermic event. These overlapping endothermic events cause what appears to be an exothermic peak at 163.03°C. It is possible that the first event is just the sample melting, but that would make the second endothermic event an odd occurrence, that could perhaps be explained as being decomposition. If, however, the overlapping event is interrupting recrystallisation of the melt (from the first endothermic event), it would be an exothermic event being interrupted by the melting of the partially recrystallised form and is a more reasonable explanation. Whether the first endothermic event seen represents melting, remains to be seen. The event could also be a solid-

solid phase transition. The point of overlap occurs below the original baseline, possibly implying that the heat capacity of the resulting form has changed to a lower value, which is highly unlikely, or that the event was not completed when the following event took place (i.e. different events overlapped). The various heating rates clearly show the differences in sensitivity and resolution mentioned earlier as can be seen in the variation in heats of the first endothermic and exothermic events when comparing Figures 3.3 and 3.4a and b. In Figure 3.4b one can see a larger change in heat at the first endothermic peak (431.72 mJ) than in Figure 3.3 (60.72 mJ), but the peak formed by the overlapping events is much further from the original baseline in Figure 3.4b than in Figure 3.3. This may be because before the event is complete, the next event starts. There is, therefore, not enough time for the event to be completed and is an example of the effect of a higher heating rate on resolution and sensitivity.

When relating this result to that of the DSC of sulphapyridine (Figure 3.2) one can see the difference being that in the case of sulphapyridine, the metastable form melts and recrystallises into a more stable modification where the baseline surpasses the original baseline during recrystallisation from the melt. In the case of ethionamide, whether the sample has melted is yet to be determined, though this seems a likely explanation of the event. The exothermic peak could be a recrystallisation from the melt though in this case the transition might not be to a more stable form as the recrystallised form has possibly shifted to a lower baseline level, though this is not likely. It appears as though while recrystallisation from the melt was taking place, an overlapping event, possibly melting of the newly formed/forming crystals occurred. This could be that the less stable polymorphic form melted and recrystallised into the more stable form or underwent a solid-state transition to a more stable form, but the melting point of the lower melting (i.e. less stable) form was reached before the recrystallisation to the more stable form was completed.

During this event the interruption appears to be at around 163°C where another endothermic event occurs. This event causes a substantial change in heat and can lead one to assume with some certainty that this is the melting point and takes place at the temperature listed as the melting temperature found in literature of 164 -165°C (O'Neil *et al*., 2001).

The temperatures of this event are as follow: The onset was at 163.48°C, the peak was at 164.18°C and the endset was at 165.78°C. The temperatures related to this event are not identical to that of those obtained at the other heating rates but are close enough to be considered the same event. During this endothermic event as the heat returns to the baseline as a result of the return of the temperature of the sample to that of the reference pan, another endothermic event can be seen. This is assumed to be decomposition and/or evaporation of the sample and occurs at a temperature above 165°C while the sample is still supposedly melted.



a **b**

Figure 3.4a and b DSC of ethionamide RM at a heating rate of 5°C/minute (a) (in this case the sample was heated up to a temperature of 200°C) and DSC of ethionamide RM at a heating rate of 10°C/minute (b).

The following is a DSC thermogram where ethionamide was heated at a rate of 5°C/minute up to a temperature of 170°C and cooled down to ambient and heated again up to 170°C. This was done to see whether the events seen in the normal DSC run are reversible. This experiment was done without the first endothermic peak in mind and was essentially flawed in principle for that reason, but the results were useful nonetheless as will be explained later in Chapter 4.

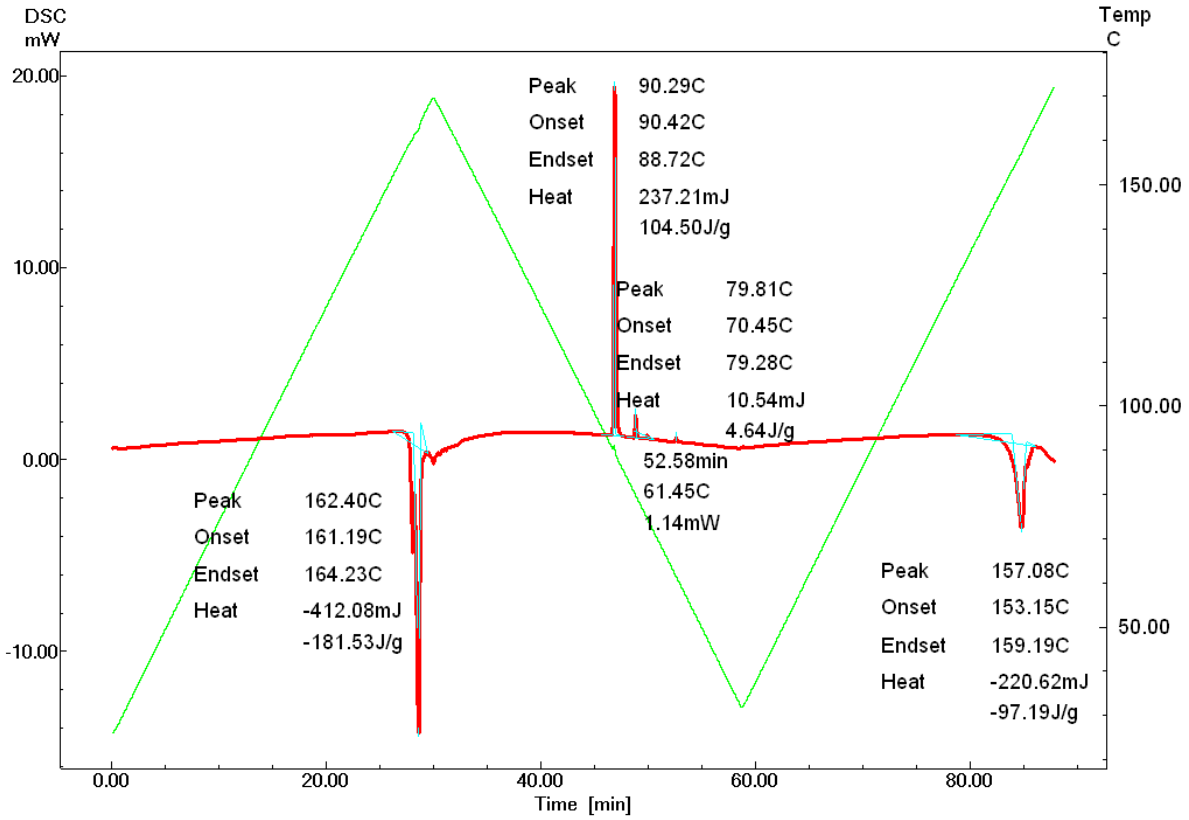


Figure 3.5 DSC of ethionamide RM at a heating rate of 5°C/minute up to a temperature of 170°C, cooled to 30°C and heated again to 170°C at 5°C/minute.

This result (Figure 3.5) shows that after being heated to 170°C the crystal has melted and recrystallises while being cooled into a form that has a single melting endotherm at 157°C. When looking at the second endothermic event during the first heating run one can see what is suspected to be decomposition or evaporation taking place just as the event assumed to be the melting event is ending. The cooling run starts during this occurrence though and complete decomposition or evaporation does not seem to have occurred. This is all just speculation and further analyses needed to be done to verify what was seen in the DSC results.

Looking at the results obtained thus far it is possible to make some assumptions as to what occurred when comparing the results to cases found in literature. Here some speculation was done on the enantiotropic or monotropic properties (see section 1.3.3) witnessed so far.

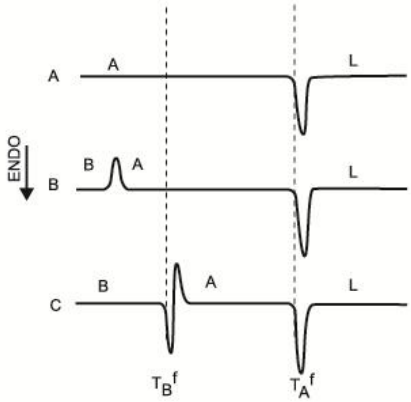


Figure 3.6 Theoretical DSC curves representing monotropically related polymorphs: A, the thermodynamically high melting form A melts; B, the low melting form undergoes an exothermic transition into A; C, B melts and A crystallizes from the melt, then A melts. Where T_0 is the temperature of the transition A to B; T_A^f is the melting temperature of A and T_B^f is the melting temperature of B (Adapted from Giron, 1995).

Figure 3.6 is in relation with Figure 1.13(b) as it represents monotropic relationships between polymorphs. When relating the events seen in Figure 3.3 with Figure 3.6 the event resembles C in this figure. This relates to the same events that were shown in Figures 3.2 D, E and F and would be true if the solid melts before recrystallising into the new form. Further analyses will confirm whether or not this is the case.

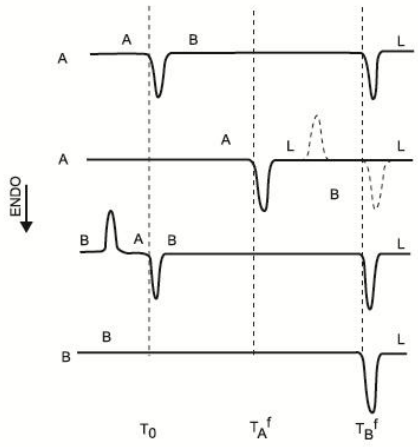


Figure 3.7 Theoretical DSC curves representing enantiotropically related polymorphs: A, endothermic solid-solid transition into B, then B melts or A melts and eventually B crystallises from the melt. B, is at room temperature and a spontaneous exothermic transition into A occurs or B melts. Where T_0 is the temperature of the transition A to B; T_A^f is the melting temperature of A; T_B^f is the melting temperature of B (Adapted from Giron, 1995).

Figure 3.7 is in relation with Figure 1.13(a) as it represents enantiotropic relationships between polymorphs. When relating the events seen in Figure 3.3 with Figure 3.7 the event resembles A in this figure, corresponding to a solid-solid transition before melting takes place. Further analyses will confirm whether or not this is the case.

Giron (1995) states that the resolution of two peaks can be heavily influenced by the heating rate (this has been clearly demonstrated thus far), but the kinetics of the reaction are also heavily influenced by the heating rate. It is stated that if the heating rate is too high a recrystallisation reaction from the molten lower melting point form to the more stable form will not be completed and only a single melting point (that of the lower melting form) will be visible. Temazepam is an example of a substance that shows this trait and the effects can be seen in Figure 3.8. If, however, decomposition takes place while the sample is melting a slower heating rate cannot be used.

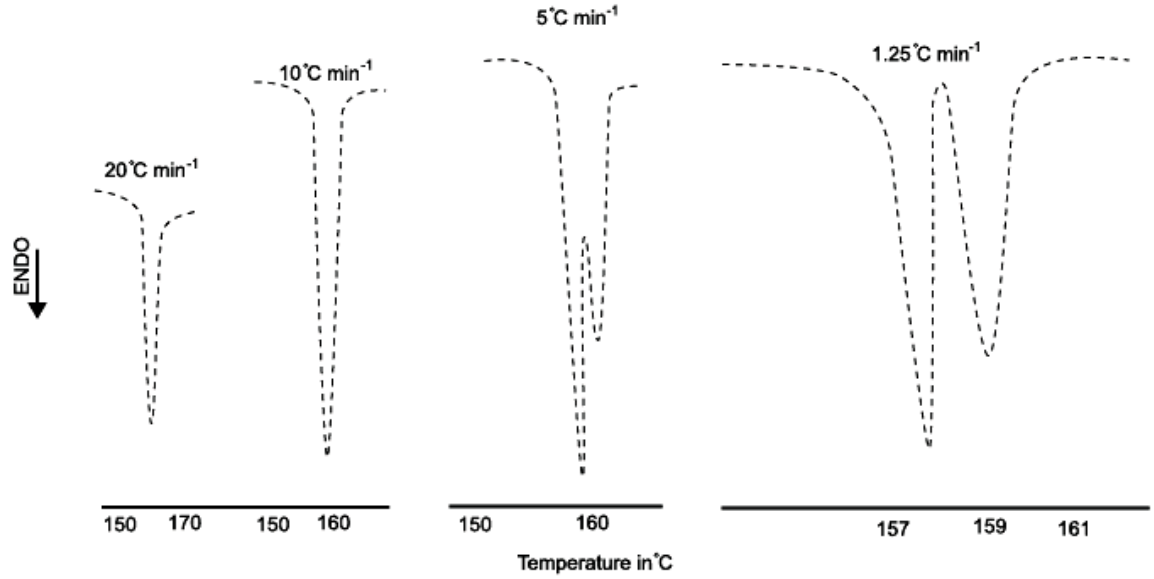


Figure 3.8 The influence of the heating rate on a DSC scan for temazepam (Adapted from Giron, 1995).

One can make use of the melting enthalpy rule presented by Burger (1982) to determine whether polymorphs are monotropically or enantiotropically related. This rule states that if the enthalpy change for the higher melting form is higher, the polymorphs are monotropically related and if it has the lower change in enthalpy of the two, they are enantiotropically related. In order for one to use this rule, one has to separate the events seen in a DSC run when there are two melting peaks. The author used a method of tempering (holding the sample isothermally) the sample at the correct heat until the transformation to the higher melting polymorph was completed. This made it possible to check the enthalpies involved in melting for each form.

An attempt was made to separate the events seen in the previous DSC results (Figures 3.3-3.5) by tempering the sample near the temperature where the first endothermic and exothermic events were seen at 162°C. At first this method proved to be fruitless and was only retried later on in this study. At the later stage the results seen through the other methods helped in perfecting the variables to obtain this result.

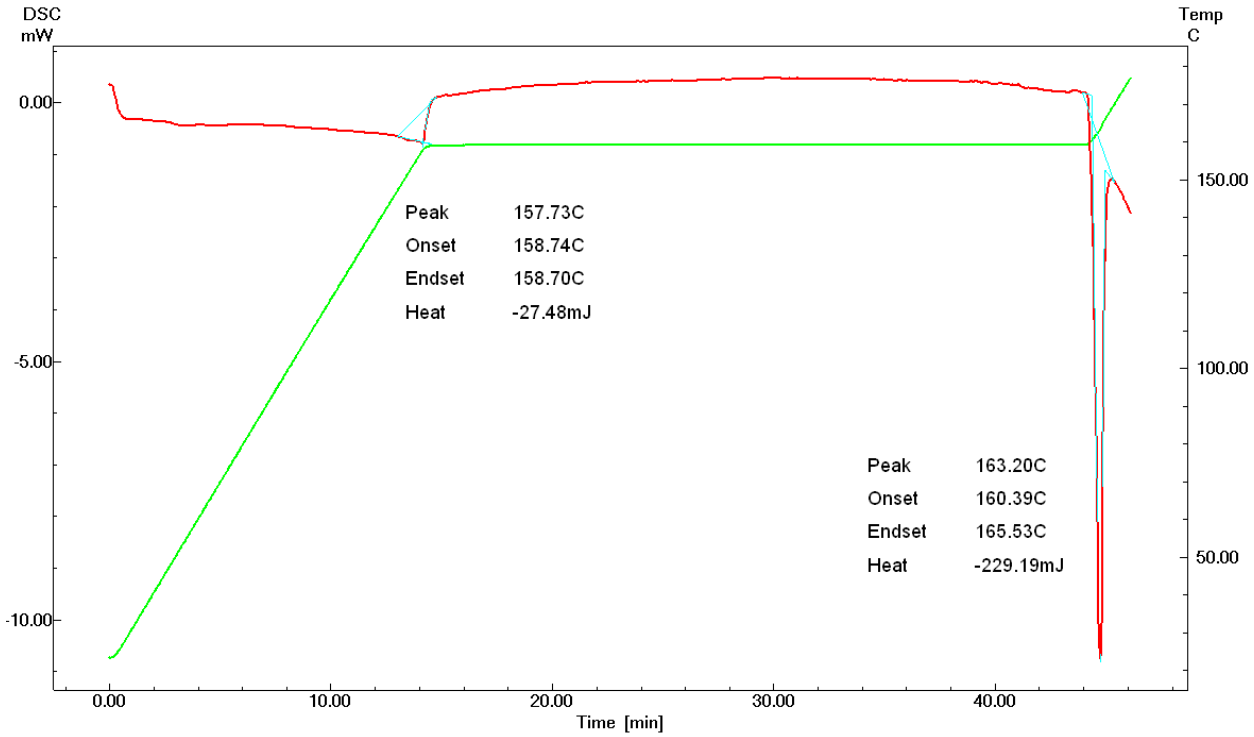


Figure 3.9 (a) DSC of ethionamide RM at a heating rate of 10°C/minute up to 162°C, held at 162°C for 30 minutes and continued heating at a rate of 10°C/minute to 175°C.

This DSC was done to separate the events seen in the previous DSC results to make it possible to interpret each event individually and evaluate the enthalpies involved.

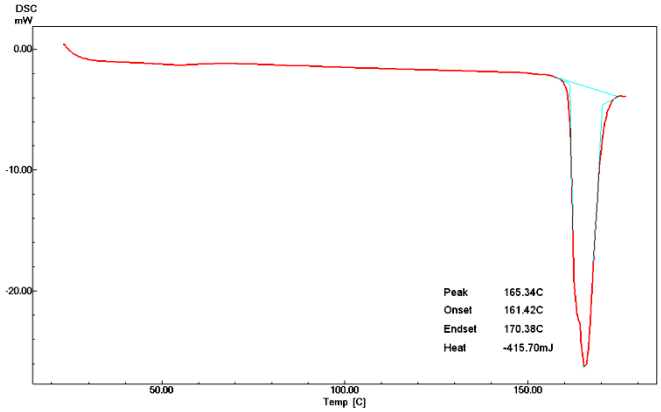


Figure 3.9 (b) DSC of ethionamide RM at a heating rate of 20°C/minute up to 170°C.

If one relates the results in Figures 3.9.a and 3.9.b to the melting enthalpy rule these forms would be monotropically related if the first endothermic event turned out to be a melting point. Later in this study the first endothermic peak was proven to be the

sublimation of the RM and thus makes the enthalpy rule not valid in this case. At this point of the study this was not yet determined.

Explaining the events seen by making use of the heat of transition rule it is possible to envision the two forms as enantiotropically related. Lohani and Grant (2006) state that this rule, though fairly reliable can, however, be invalid in some cases as a result of polymorphic conformation. The rule assumes that the free energy isobars do not cross more than once and that the enthalpy isobars of the various polymorphic forms do not cross. The latter of these assumptions can be made void if considerable differences exist between the molecular conformations of the polymorphs in question.

The event seen is assumed to be a first-order reaction, because the temperature at which it takes place does not seem to be affected by the heating rate used. Kawakami and Ida (2005) state that the enthalpy-temperature curves of polymorphs (see section 1.3.3.1) do not cross if the change in enthalpy is positive and the reaction is a first order reaction. If, however, the transition is of the second-order, the enthalpy temperature curves of polymorphs can cross. This is the reason why enantiotropic transitions are grouped into two types, i.e kinetically reversible and irreversible transitions. An enthalpy jump associated with a transition can cause a kinetically irreversible transition.

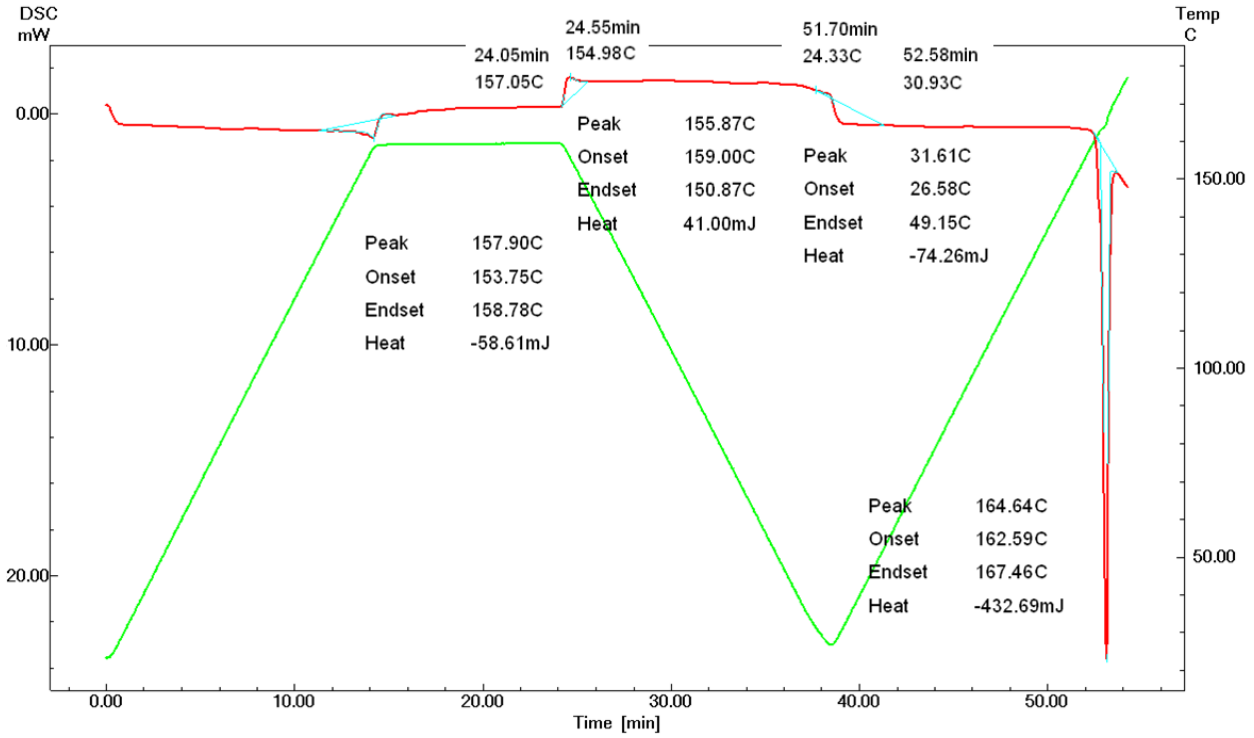


Figure 3.10 DSC result of ethionamide RM where the temperature was increased up to 162°C at a heating rate of 10°C/minute, held at 162°C for 10 minutes, cooled down to 25°C at a rate of 10°C/minute and then heated to 175°C.

This was done to see whether the event seen in Figures 3.3-3.5 at ±162°C was reversible. The result shows the endothermic event seen at around 162°C in Figures 3.3-3.5 taking place, though in this case the temperature at which it takes place is slightly lower (with a peak at 157.9°C). The endothermic event transitions into an exothermic event seen just above this temperature (at around 158°C). This event looks very much like the event seen in the other DSC results (Figures 3.3-3.5) though the event is not interrupted by an overlapping event in this case. As the temperature is held at 162°C one can see that the baseline has shifted to a higher point and this can imply that perhaps the original sample has melted and recrystallised into a more stable form, as was represented in the DSC result in Figures 3.2 D and E, or that a solid-state transition took place from a less to a more stable form. The baseline stays constant at this temperature. When the sample starts to cool down at around 26 minutes after being held at 162°C for 10 minutes, it appears as though another endothermic event is visible.

This could be that the sample has melted and partially recrystallised at 162°C and then further recrystallised when the cooling started or that the recrystallised form underwent another phase transition to yet another different form. The baseline has shifted to a higher position representing a higher heat capacity. The most probable explanation would be that the machine caused the difference in baseline by dropping the heat input by a miniscule amount to keep the sample at an isothermal state. This would then cause a drop in enthalpy for the sample and cause an exothermic shift in baseline. This shift is then made more visible when the sample is actively cooled and can be seen in the baseline shift between 24 and 38 minutes. The possibility also still exists that decomposition took place after the first endothermic event and afterwards the decomposition products recrystallised into more stable forms.

There appears to be another event causing a shift in the baseline when the second heating run is happening and occurs at around 30°C. This event results in the baseline returning to around the same height as it was when heating the RM before any events took place. This could be a phase transition to the polymorphic form that was present when the heating run was started (i.e. the solid-state form of the RM). The temperature related to this event appears strange though, because it did not take place while cooling, but while being heated. The temperature was low though and this event could be a transition from a third most stable polymorphic form (of the three seen in this DSC result) to the least stable polymorphic form (seen in this DSC result). This could be because the transition needed more time to take place. The other possibility could also be true i.e. that the sample was simply in a state of being partly recrystallised and the slight lowering of temperature caused the completion of this recrystallisation event. If this proves to be the case the event seen at around 38 minutes can be the reverse of the original transition and the sample has transitioned from the more stable second form back to the first less stable form.

Both ideas are speculative and require further analyses for clarification.

The baseline stays constant from here until an endothermic event with a very large change in enthalpy (432.69 mJ), assumed to be the melting endotherm takes place at

around164°C corresponding to the melting point found for ethionamide in literature of 164 -165ºC (Merck Index, 1996).

Giron (1995) explains that a DSC scan showing two “melting points” can imply monotropy or enantiotropy as was explained in the previous section, but can also be due to the sample being pure or being a mixture.

Whether these DSC results are the usual results seen when analysing ethionamide remains to be verified as the purity of the RM has not been tested at this stage of the study. The possibility that impurities or various polymorphic forms are present in the RM obtained from DB Fine Chemicals, South Africa, exists and this could influence the results obtained. An example of impurities in the sample causing altered results mentioned by Giron (1995) is that of β-hydroxy-propylteophylline. In this example the sample was heated to a temperature slightly higher than its melting point and then cooled. A second heating run was done and in this instance the sample (a metastable form) melted and underwent another recrystallisation to a higher melting form representing a third separate polymorphic form. What makes this case interesting is the fact that the method was not reproducible when using a pure sample.

DSC analysis - Conclusion

The aim of the DSC as seen in Figure 3.9a was to prepare the higher melting form to analyse the melting point of this event without interference from the melting of the lower melting form and subsequent recrystallisation to the higher melting form. The problem here is that it has not yet been determined whether the “lower melting form” melts before transitioning into the more stable (higher melting) polymorph. This was not, however, the result obtained through this experiment and left more questions than answers.

The aim of the DSC run, as seen in Figure 3.10, was to determine whether the first event seen in the previous DSC was a reversible transition. The method used will not prove with certainty that an event is kinetically irreversible, but can possibly show whether a transition is reversible. In this case the result did not give a clear answer to

the question posed and more investigation was necessary. The information could prove to be of importance when related to results found in other methods.

From the results obtained through the DSC measurements, one can find much useful information, but when used as a standalone method the information is incomplete for identifying events with certainty. As the name implies this method only measures the changes in heat, but does not fully explain what causes the changes. By relating the results of these experiments to that of other analytical methods it can clarify exactly what occurred in the events seen by this method.

3.4.2 Thermogravimetric analysis (TGA)

Method

Thermogravimetric analysis was done by making use of a Shimadzu DTG-60 differential thermogravimetric analyser with TA60 Version 2.11 software (Shimadzu, Kyoto, Japan). Approximately 2-10 mg of each sample was placed in an aluminium pan, left uncovered and then heated from 25°C to 200°C at a heating rate of 10°C/minute. Nitrogen was used as purge gas with a flow rate of 35 ml/minute. (The first TGA was done up to 200°C, but after noticing that decomposition takes place before this temperature the samples were only heated up to 170°C during further analyses).

Galwey and Craig (2007) explain that one will reach higher precision when larger sample masses are used in these analyses, because the results give values such as percentage weight loss. This larger sample mass can, however, cause uncertainties in kinetic transformations and increases the chance of reversible reactions influencing results and can cause variations of temperature within samples. The influence of reaction enthalpy by means of self-cooling or self-heating will increase with the use of larger sample masses. This problem can be reduced by spreading the sample in the pan without creating big sample masses that are closely compacted.

For this reason Galwey and Craig (2007) suggest using the smallest practicable sample masses. This will reduce thermal inertia as well as the time it will take the sample to reach the furnace temperature at the beginning of a TGA run and result in reduced thermal lag.

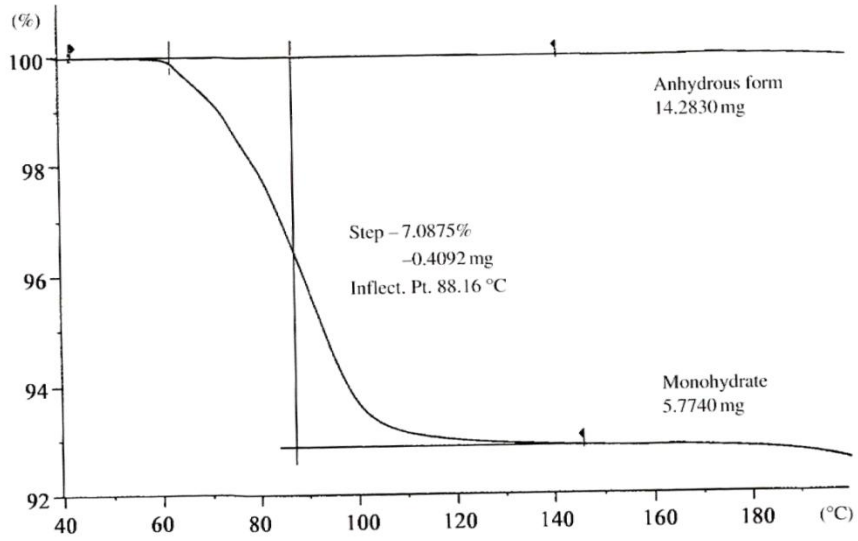


Figure 3.11 An example of a TGA for the anhydrous and monohydrate forms of glucose (Bernstein, 2002).

Figure 3.11 is a clear example of a TGA showing weight loss resulting from the loss of solvent (in this case water), compared to no weight loss in the case of the anhydrous form.

Results

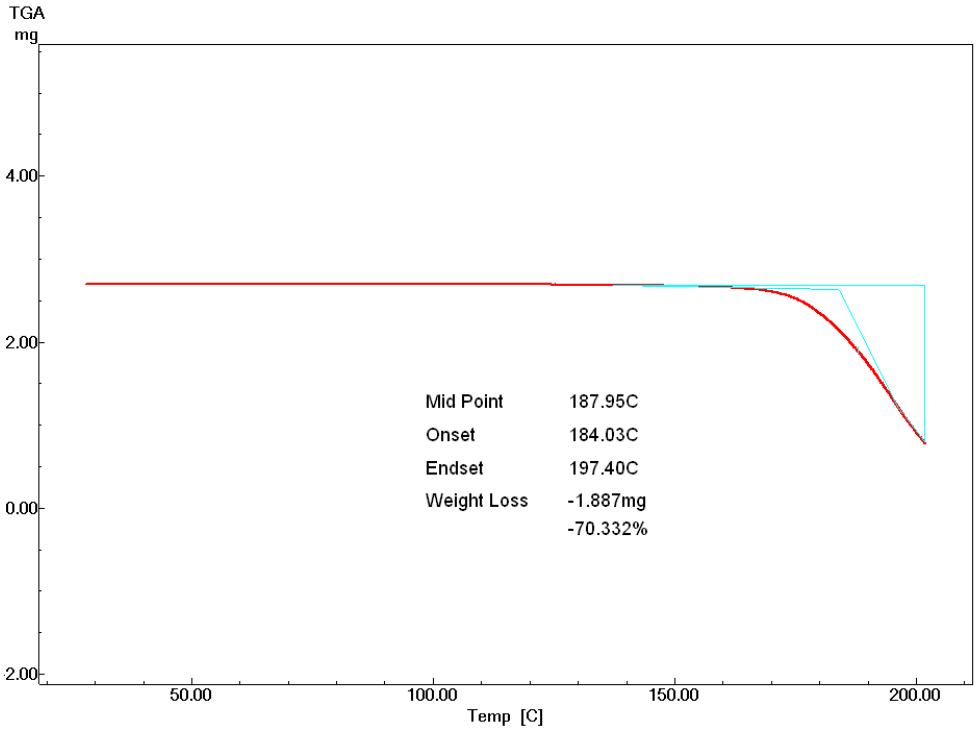


Figure 3.12 TGA of ethionamide RM done at a heating rate of 10°C/minute to a temperature of 200°C.

From Figure 3.12 one can see that the baseline stays constant past the temperature assumed to be the melting temperature (±164°C) from the DSC results. This can imply that though decomposition might be taking place, no weight loss is taking place up to a temperature of 184.03°C (the onset of the weight loss from the TGA). After the analysis was completed, a fluffy yellow powdery substance was found on the vents of the analyser. This led to the assumption that sublimation/vaporisation of ethionamide occurred followed by the subsequent recrystallisation of ethionamide in the vents of the analyser.

TGA analysis - conclusion

Though there are many important hints to be found from TGA results, at this stage of the study the TGA results had not given too much valuable information. The result can serve as a reference for comparison of other forms obtained though. The results found

here can be used in conjunction with that of other methods to explain the results with more certainty.

The substantial weight loss of 70% shows that the material most probably evaporated. The supposed formation of crystals by means of coincidental vapour deposition led to the hypotheses that it might be possible to form crystals purposefully by a physical vapour deposition method. This led to the methods employed for acquisition of new solid-state forms employed in section chapter 5.

3.4.3 Thermal microscopy (TM)

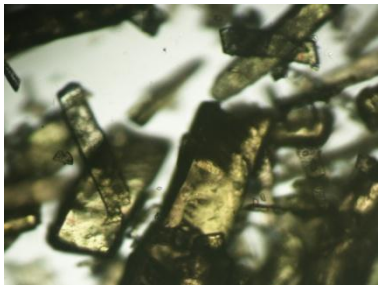
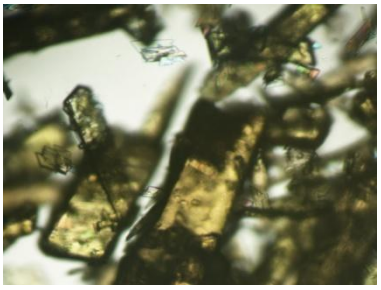
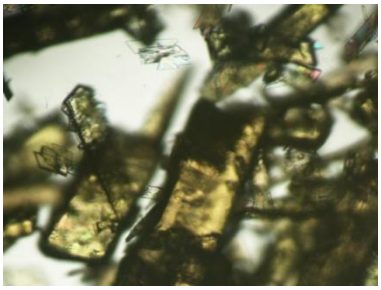
Two different microscopes were used in this study on separate occasions and it is specified which method was used in each instance.

Method 1

A very small amount of sample was placed on a microscope slide and a cover slide was used to cover it. This was then placed in a Nikon Eclipse E400 thermal microscope (Nikon, Tokyo, Japan). The sample was then heated by means of a Leitz 350 heating unit (Leitz, Wetzlar, Germany) and the temperature monitored by making use of a Metratherm 1200d thermostat (Metratherm, Nuremberg, Germany). Photos were taken of occurrences of interest by means of a Nikon DS-Fi1 digital camera that was affixed to the microscope.

Melting point determination can be somewhat troublesome when using TM for this purpose. This is because all the crystals that are visible in the analyst's field of view will not necessarily melt at the same time. One can make use of various methods to resolve this problem. One option is to use the temperature at which the last crystal in view melts. Another option is to try and reverse the melting process by cooling the sample directly after it melts and determining the midpoint between melting and recrystallisation (Carlton, 2003). The latter of these two options will not be possible if phase transitions occur shortly after melting and this method will not be used in this dissertation for this reason.

Results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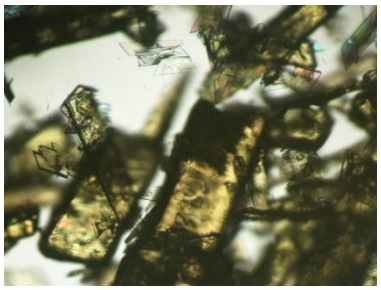
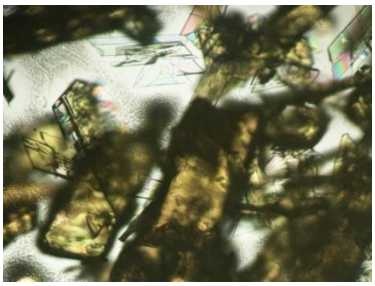
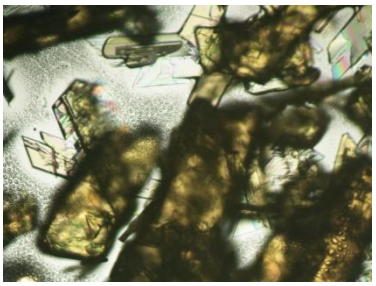
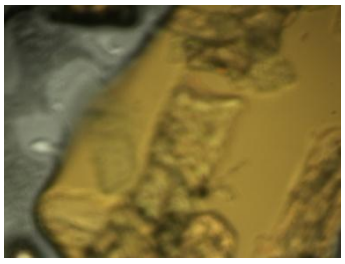
		
d=150°C	e=161.4°C	f=163°C
		
g=164.1°C	h=167°C	

Figure 3.13 TM results for ethionamide RM. As the sample is heated one can see the formation of new crystals starting at around 150°C. These crystals form on top of the RM crystals present but also in the vicinity of these crystals in rather close proximity. These crystals were thought to be possible degradation products, crystals formed through a solid-state reaction or possible crystals formed by recrystallisation from the vapours formed through sublimation.

At 161°C small droplets in the vicinity of the crystals that appear to be on the cover glass are observed. These droplets can be explained by the assumption that sublimation took place and the droplets were formed by condensation corresponding to the finding of the crystals in the vents of the TGA mentioned in the previous section.

The exact onset temperature of melting is hard to pinpoint from these results as it happened gradually. Melting started at 161°C, at 164°C the sample was almost completely melted but one can still see solid structures amid the melt. The sample was completely melted by 167°C.

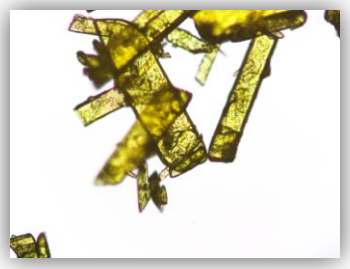
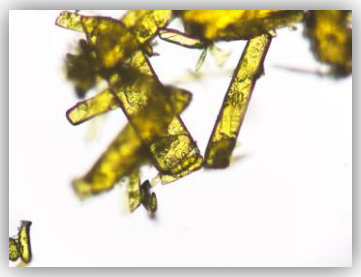
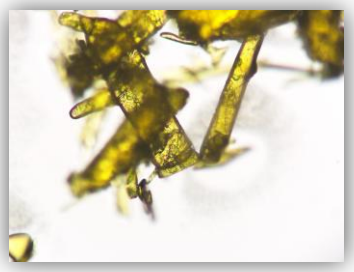
Conclusion

From the finding of the droplets on the coverglass, the assumption that recrystallisation from vapours occurred, was strengthened. The assumption that the second endotherm in the DSC results was the melting point, was confirmed as the temperature in the DSC results (±164°C) correspond to the visual occurrence of melting in the result found here. It was seen that the supposed phase transition represented by the first endothermic and exothermic peaks in the DSC results were not recrystallisation from the melt, but was either a solid-state transition or recrystallisation from vapour formed by sublimation. The latter of these two was the supposed explanation as the new crystals formed in isolation as well. The crystals formed cannot be classified with certainty from these results and required further analyses for clarity.

Method 2

The specifications for the second thermal microscope used are as follows: the heating stage was a Linkam Scientific Instruments, THSM600 (Surrey, UK), the camera control unit was a Nikon Eclipse 50i and the camera head was a DS-Fi1 (Nikon, Tokyo, Japan). The imaging software used to capture all photomicrographs was NIS Elements, F package, version 3.22

The heating rate used was 5°C/minute and the sample was heated up to a temperature of 164°C where the temperature was held until the crystals that were seen in the previous TM experiment (Figure 3.13) were visible. The heating was continued at 5°C/minute to a temperature of 167°C until the the crystals were fully melted.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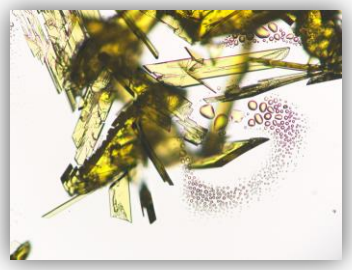
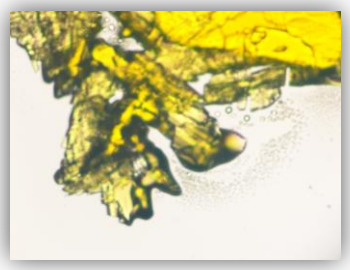
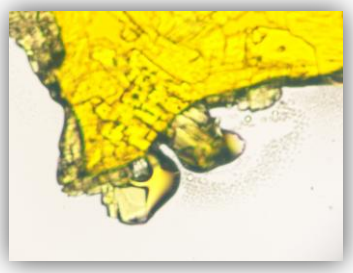
		
d=164°C	e=164°C	f=167°C
		
g=167°C	h=167°C	i=167°C

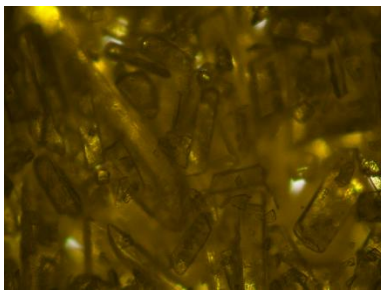
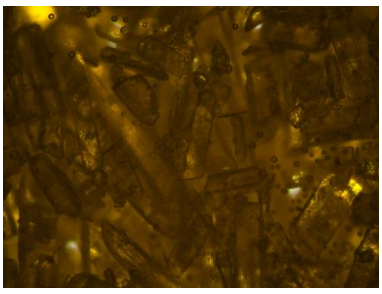
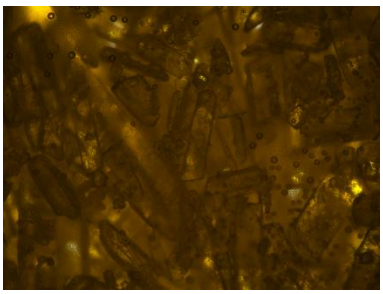
Figure 3.14 TM results of ethionamide RM.

At 25°C one can see the crystals at ambient temperature at the start of the heating run. From 158°C to 164°C the formation of new crystals in the vicinity of the crystals that were present at 25°C can be observed. This corresponds to the results found with the first TM experiment (Figure 3.13). At a temperature of 164°C (Figure 3.14b) one can

see the newly formed crystals with clarity as in this case the focus was shifted to them. Also, at 164°C (photo d) one can also see the formation of droplets on the coverglass in the vicinity of the crystals. This strengthens the assumption that sublimation occurred and the droplets were formed as a result of condensation. At 164°C (photo e) it is possible to see an interesting occurrence that was not observed with the previous TM experiment. At this temperature, the droplets disappear. When viewing this event in real-time it appears as if these droplets disappear and that the newly formed crystals appear instantaneously. An explanation for this is the possibility that the droplets recrystallise at a quick rate and form part of the old crystals or form the new crystals that can be seen from temperatures 158 to 164°C. The temperature was held at 164°C to show that the original form present at 25°C continues to transform from the one solid-state to the other at a temperature lower than the temperature where complete melting takes place.

At temperature 167°C one can see that the onset of melting has occurred, though it appears that the original form starts to melt first and flows over the newly formed crystals after which melting of both forms continues to completion at this temperature.

In the following experiment the sample was heated at a rate of 10°C/minute up to a temperature of 157°C and held there for 25 minutes. This was done in order for the RM to sublime and recrystallise into the crystals seen in Figures 3.13 and 3.14. After holding the sample at this temperature and seeing that the new crystals had formed, the heating run was continued at 10°C/minute up to a point where melting was observed. This was done to determine the melting point of the newly formed crystals.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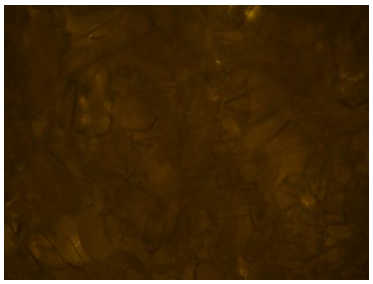	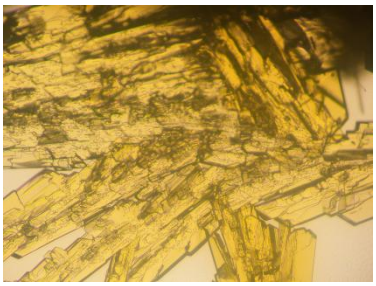	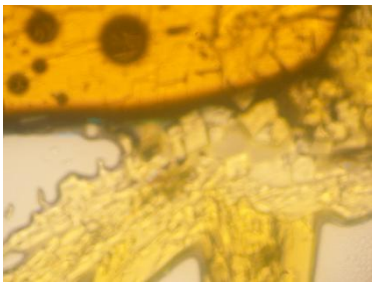
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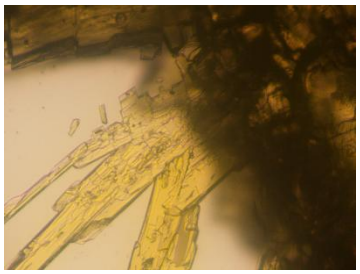		
g=25°C		

Figure 3.15 TM of ethionamide RM.

At temperature 150°C droplets forming on the cover glass are observed. Photo c is the initial image of the sample held at 157°C, and 157°C (photo d) is at the same temperature after being held here for 25 minutes. Here the newly formed crystals are evident, though the crowding of the crystals makes it difficult to see whether the original crystals are still present. At temperature 157°C (photo e) the focus was shifted to an edge of the solid mass that has formed to make the crystals more visible. In this image one can see the newly formed crystals quite clearly. The heating run was resumed at a rate of 10°C/minute. At temperature 168°C, a melt formed. Photo g (temperature = 25°C) shows the sample after cooling back to ambient temperature in which one can see the crystals formed by sublimation and subsequent recrystallisation not having fully melted.

Conclusion

These results also clearly show that the sample transforms into new crystals. From the finding that the small droplets appear to recrystallise into the new form and that this new

form is formed onto the crystals present or in close proximity to these crystals, it is possible to explain the results obtained with TGA. The onset of weight loss seen in the TGA results (Figure 3.12) starting at 184.03°C can be explained by the formation of the new crystals. The original crystals sublime at temperatures (onset ±150 to 160°C), far below the detected weight loss seen in the TGA results. The reason for this is that the vapours from the sample in the pan recrystallise into the new crystals that form in the pan and there is no net weight loss. This means that the weight loss from this event cannot be seen in the TGA result, but it does not mean that no sublimation occurred.

If one compares the temperature at which the sublimation takes place as seen in the TM results (onset ±160°C) to that of the first endothermic event in the DSC results the event appears to be identified as the phase transition through sublimation and subsequent recrystallisation as seen in the TM results. The results obtained here can also be used in conjunction with results obtained through other analytical methods.

Carlton (2011) mentioned a similar example as that seen here where polymorphic Form II of caffeine sublimes and subsequently crystallises to Form I at around 150°C and melts at 236°C. The higher melting polymorph in this case was not stable at ambient conditions and entails that the various forms are enantiotropically related and it was said that the transition temperature was near the melting temperature. This scenario looks very much like the one encountered here, though this reference source was not of much use in explaining the events seen, since the author sidestepped the topic, as this was not the focus of that work. The author writes: “It is somewhat difficult for me to wrap my mind around the complication of sublimation”. These words also held true during this study and the entire Chapter 4 was focused on this issue.

3.4.4 Light microscopy (stereomicroscopy)

Images were viewed under an Optiphot® stereomicroscope (Nikon, Tokyo, Japan).

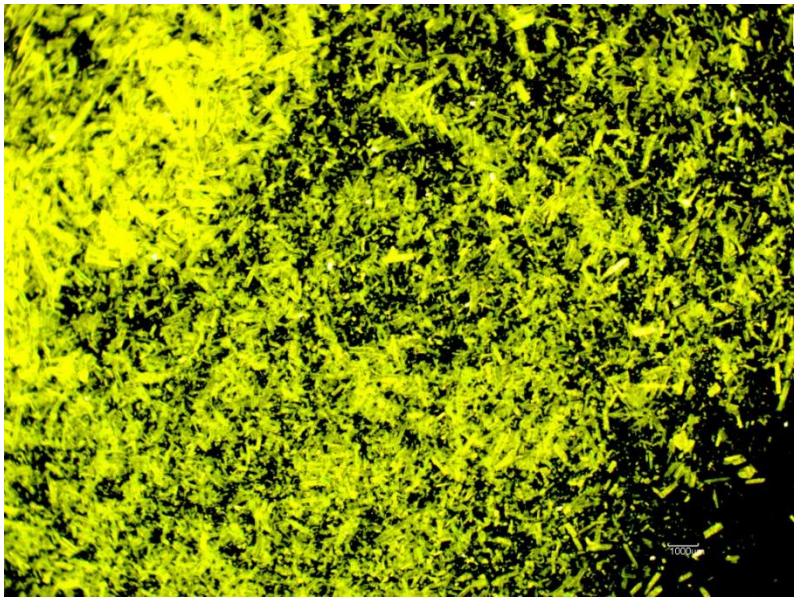

Figure 3.16 Photo image of ethionamide RM as seen under a stereomicroscope.

This image shows the RM powder and can serve as a reference for comparison of other solid-state forms of ethionamide obtained in the future.

3.4.5 Scanning electron microscope (SEM)

Method

Scanning electron microscopy was done by making use of an FEI Quanta 200 ESEM (FEI, Hillsboro, USA). The ESEM was operated under high and low vacuum modes at 10 kV and samples coated with Au/Pd to a thickness of 20 nm.

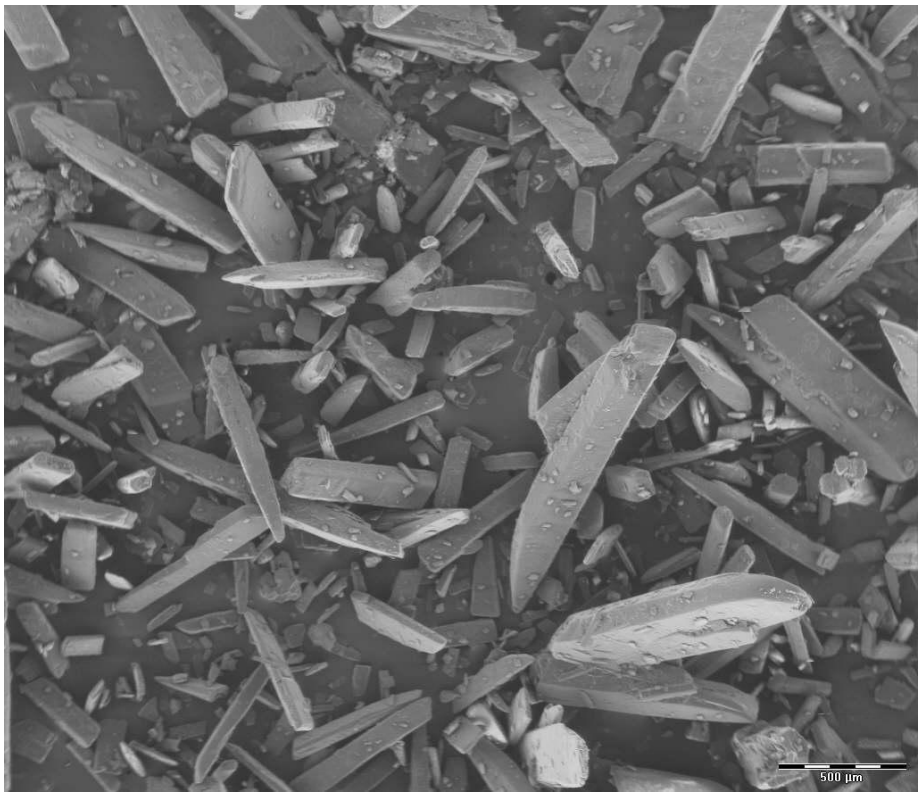

Figure 3.17 SEM image of ethionamide RM.

This image shows what the ethionamide RM looks like under a SEM and can be used as a reference for comparison of solid-state forms obtained in the future.

3.4.6 Ultra-violet spectroscopy (UV)

Method

The identification method used to check the RM as well as serving as a method to compare experimental forms obtained to the RM was as follows. 10 mg of the raw material was dissolved in 100 ml of methanol. 10 ml of the resulting solution was then diluted to 100 ml with the same solvent. The sample is analysed by UV spectral analysis with a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The UV absorbance spectrum should show a maximum absorption at 290 nm with a specific absorbance of 380 to 440 (BP, 2011).

3.4.7 Fourier transform infrared spectroscopy (FTIR)

Method

A Shimadzu IR Prestige-21 (Shimadzu, Kyoto, Japan) was used for the recording of infrared spectra within the range of 400-4000 cm^{-1}. The sample was dispersed in a matrix of the background which was potassium bromide. The spectra were recorded in a reflectance cell by means of DRIFT (diffuse reflectance infrared Fourier transform) spectroscopy.

To minimise the possibility of polymorphic transformations or solvent loss occurring, DRIFT spectroscopy is used. In this method the sample is dispersed in a powdered alkali halide (KBr in this case). This does not require such aggressive grinding as that used for mull preparations and does not require the pressure that is used to form pellets (Bernstein, 2002).

Results

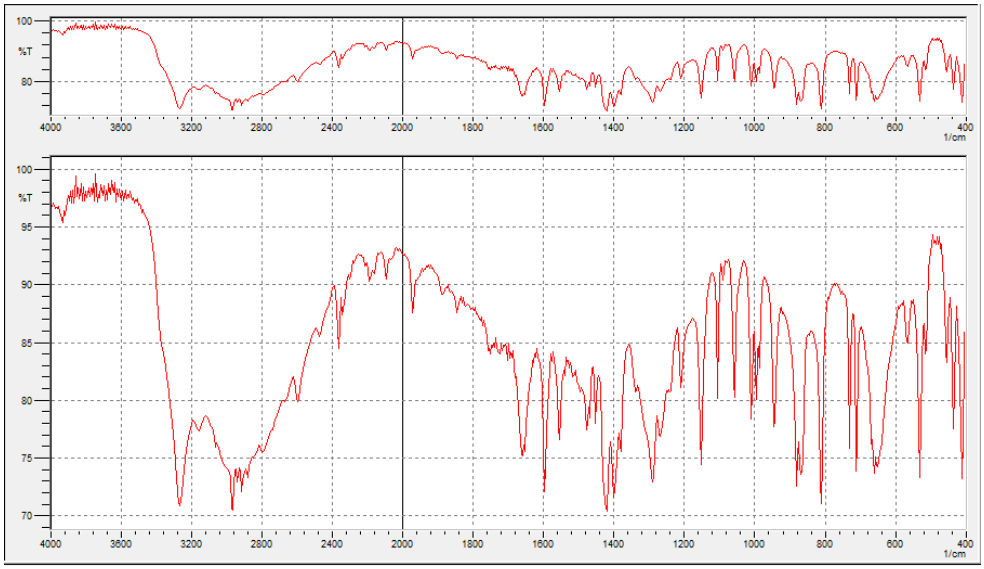

Figure 3.18 FTIR spectrum of ethionamide RM.

3.4.8 X-ray powder diffraction (XRPD)

Method

Sample readings were recorded on a Philips X'Pert-Pro (Philips, Almelo, Netherlands). With measurement conditions of: target, Cu; voltage, 40 kV; current, 45 mA; divergence slit, 0.9570 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/minute with a step size of 0.0170° and a step time of 5.8142 seconds. Prepared samples were packed in an aluminium sample holder. Eva© software (version 10.0, revison 1) was used to determine the peak positions and intensities from the diffractograms and store them in a format that is functional.

Results

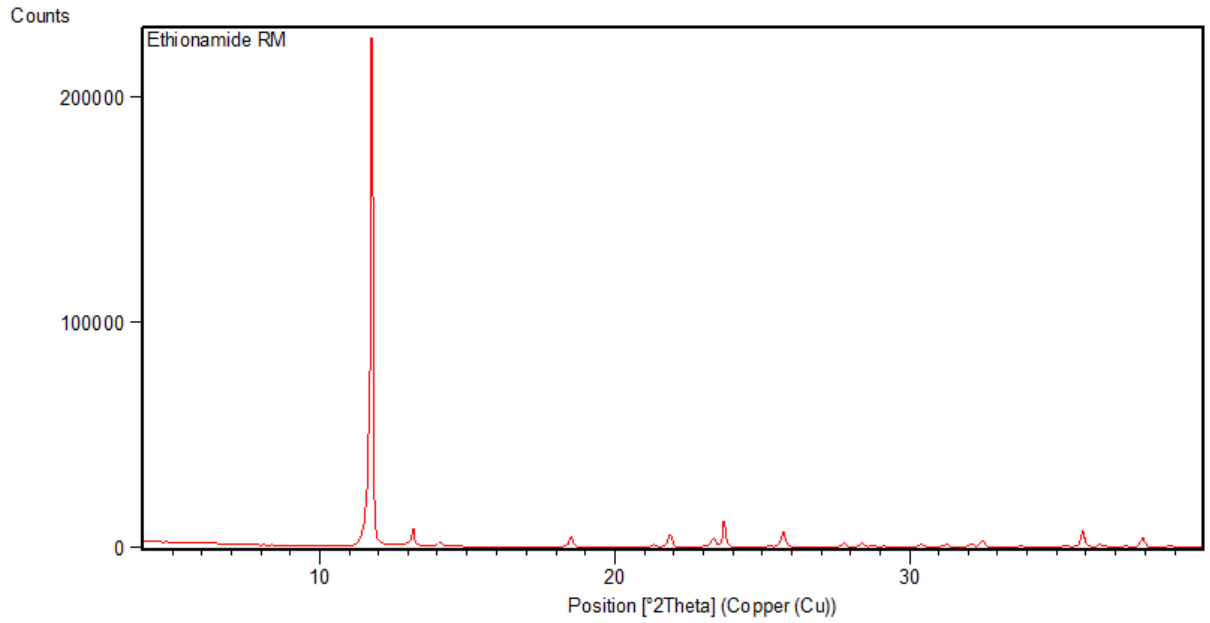

Figure 3.19 XRPD of ethionamide RM.

Conclusion

The XRPD shows that this sample is a highly crystalline material, with intensity counts far above 20000. This result can serve as a reference to which other results can be compared.

3.4.9 Thin layer chromatography (TLC)

Method

A TLC Silica gel 60 F254 plate was placed in a mobile phase consisting of methanol and chloroform in the ratio 1:9. To create the test solution, 0.2 g of the sample to be tested is dissolved in 10 ml of acetone. Two reference solutions were prepared by diluting 0.5 ml of the test solution to 100 ml with acetone for the first and by diluting 0.2 ml of the test solution to 100 ml with acetone for the second. The reference solutions are applied to the plate at different positions in 10 µl applications. The solvent front is left to move a path length of 15 cm. It is then left to dry and is examined afterwards by ultraviolet light at 254 nm.

The sample being tested should not form a spot on the chromatogram with a higher intensity than that of the spot formed by the first reference solution (0.5%) at any other point than the principle spot. It should not have more than one such spot with a higher intensity than that obtained with the second reference solution (0.2%) (BP, 2011).

3.5 Conclusion

In this section the specifications of many of the analytical methods used throughout this study were explained. In the following sections only the alterations made to these methods are explained.

A significant amount of information on the characteristics of the RM was gained by these analytical methods and this information makes it possible for one to formulate ways to manipulate the RM to alter its properties. The data obtained can also serve as a reference for the results obtained when analysing the potential new forms created via the various methods explained in the following sections. At this stage of the study there was also much information on the various supposed forms seen by these methods that was not explained with certainty, as the sublimation aspect complicates things and a straight forward explanation found in an everyday textbook proved difficult to obtain. The following sections were also aimed at clarifying what was still unexplained in this matter.