

Chapter 5

Ethionamide physical vapour deposition / sublimation products

5.1 Introduction

In this section the methods used to obtain these crystals will be explained and the characterisation of the properties of these crystals will be presented. The two methods utilised to obtain sublimation products were: cold glass finger and cover glass method.

A cold glass finger method was used in order to study the sublimation of ethionamide. During the sublimation process the crystals formed in higher yields on the hotter sides of the apparatus than on the cold finger itself and the crystals from the sides of the container were used for these analyses.

With this method a very small amount of crystals were formed at the bottom of the cold finger. The crystals were gathered and XRPD, DSC, TM, SEM and FTIR methods were used to determine the properties of the formed crystals. The abbreviation used for the crystals obtained by this method will be SV throughout this section (Different vacuum pumps were used and in the case of the stronger pump the abbreviation SV (HV) was used and in the case of the weaker vacuum pump SV (LV) was used).

Secondly, a cover glass method was employed to obtain sublimation crystals. The method as explained by Guillory (1999) was used. This method involves placing some of the ethionamide RM in a petri dish and covering it with an inverted watch glass. The petri dish is heated and the watch glass is observed for crystal growth. This method is referred to from this point onward as the cover glass method.

Nomenclature:

SV (HV) = Sublimation product high vacuum

SV (LV) = Sublimation product low vacuum

S = Sublimation product cover glass method

5.2 Cold finger method

5.2.1 X-ray powder diffractometry

Though the result obtained by using the cold glass finger method (Figure 5.1) does resemble the result obtained with the RM, there are some clearly visible differences implying that the crystal structure of the material has altered through this method.

The method for obtaining these crystals was repeated and another XRPD analysis was done to determine whether the results are reproducible (Figure 5.2).

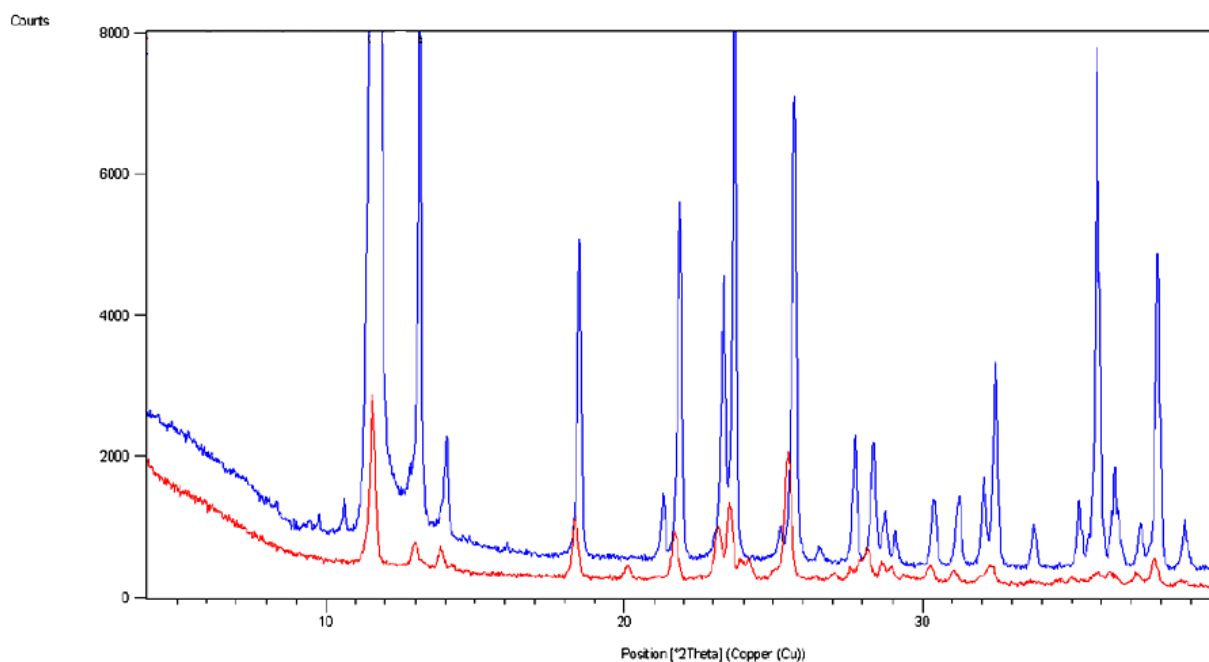


Figure 5.1 Overlay of XRPD results of ethionamide crystals gathered from the bottom of the cold finger (bottom) and of ethionamide RM (top).

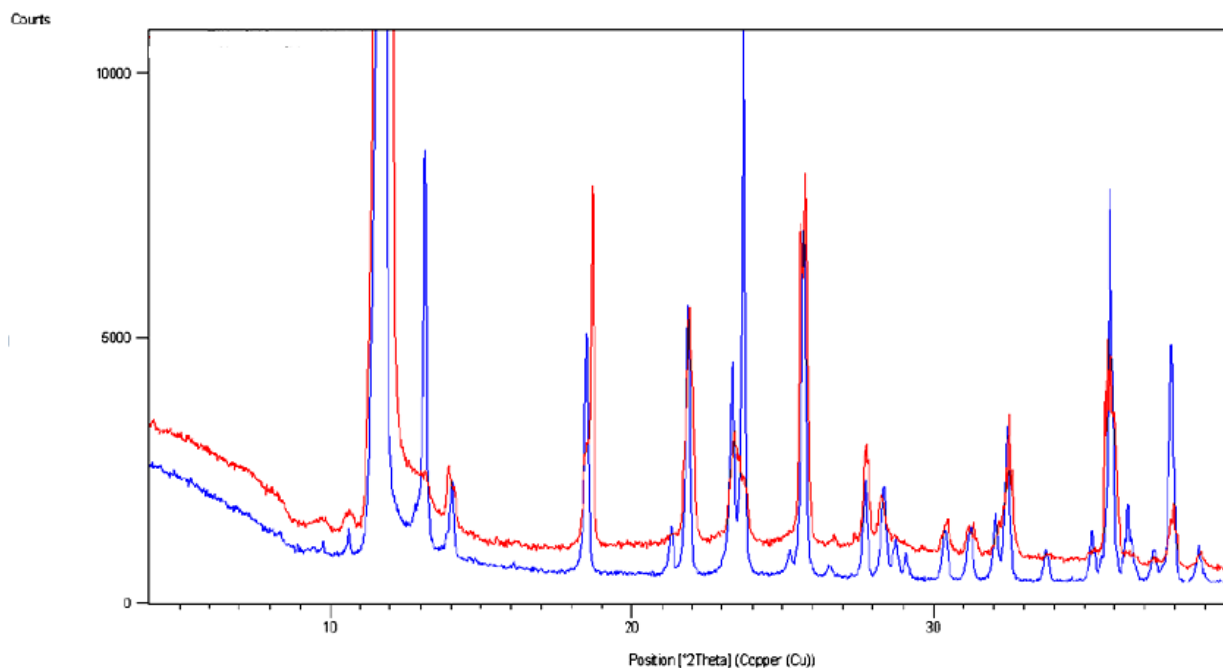


Figure 5.2 Overlay of the XRPD results of ethionamide crystals gathered from the bottom of the cold finger after a repeat (top) and of ethionamide RM (bottom).

In this diffractogram, the peaks appear to be more diffuse as in the case of the first experiment (Figure 5.1). Some peaks appear to be missing. Small amounts were gathered from the cold finger method, and it was, therefore, difficult to obtain a diffractogram with better quality peaks. Also, there was a possibility that the sublimation product could be less crystalline. The latter of these two options was the aim of this method and this possibility led to further analyses to determine whether this was the case or not.

Conclusion

The XRPD results showed that the crystals obtained from the cold glass finger method was possibly less crystalline. The intensity counts of the peaks were much lower in the newly formed crystals than the RM. This can be explained if one recalls that the intensities will be higher in crystalline materials where long-range order exists, because the additive refractive patterns cause constructive interference. If the same molecular coordinations causing the XRPD pattern are present, but the coordination is not repetitive throughout the entire lattice one will find that some, though less, constructive

interference occurs than in the case where the coordination is present throughout the lattice. It can also be as a result of less molecules existing in an individual plane. This will be as a result of lower packing density. This concept led to the presumption that the lower crystallinity would result in lower stability of the new form and perhaps result in better solubility. The yields obtained by employing this method were too low at this stage of the study to do a comparable solubility experiment.

5.2.2 Differential scanning calorimetry

This DSC trace only shows one endothermic event at a peak maximum of 162.78°C with a large change in heat of fusion (611.24 mJ). This is thought to be the melting point and is lower than the melting point of the RM ($\pm 164^\circ\text{C}$). Another difference is the single event where, in the case of the RM, there were two distinct endothermic events. From this it was suspected that a less stable monotropic form was created (a form with a lower melting point that does not transform to a more stable form) that corresponds with the conclusion made after the XRPD analysis.

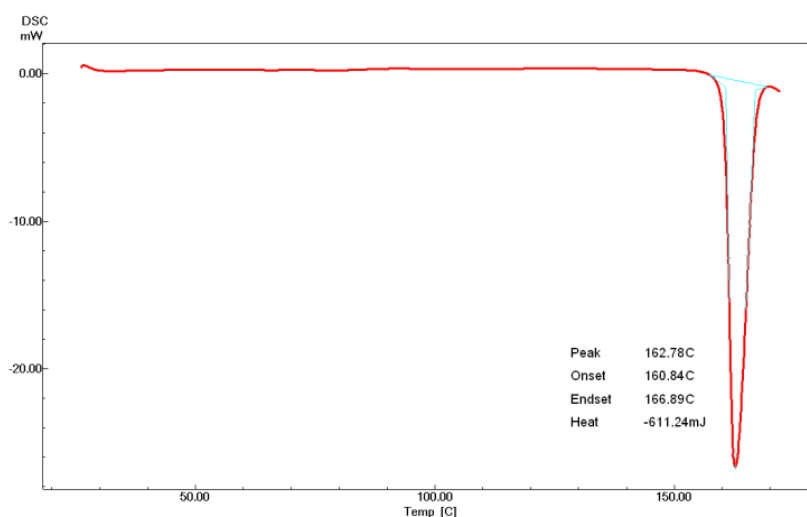


Figure 5.3 DSC trace of SV (LV) (heating rate of 10°C/minute).

Conclusion

It appears as if a different solid-state form than the ethionamide RM form has been prepared, though it is not clear whether there is a correlation between this form and the form produced by simply heating the RM to the point where it sublimates and undergoes a

phase transition. The possibility that degradation of ethionamide was induced during the sublimation process could be a cause for the difference observed in the melting point. However, this will be discussed further on in the chapter.

5.2.3 Scanning electron microscopy

Here one can observe how thin these crystals are. In Figure 5.4c one can see through the incredibly thin crystals, implying that at the intensity the SEM is set at, some electrons are able to pass through layers of sample and are reflected by the crystals beneath the ones at the top. One can also see that some of these thin crystals are not part of the larger crystals and that these layers formed independent crystals.

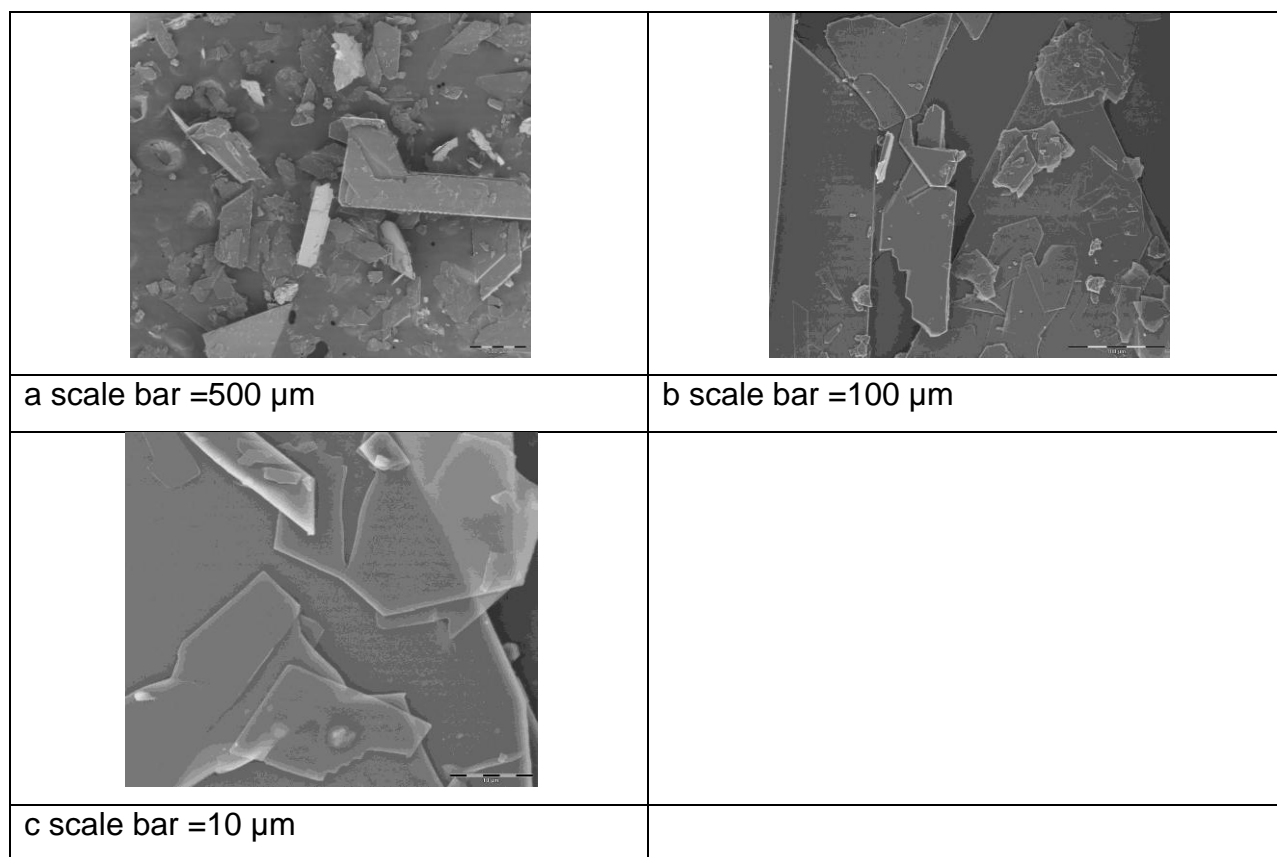


Figure 5.4 SEM micrographs of SV (LV) at different magnifications in order of lower to higher magnifications.

5.2.4 Thermal microscopy

Although it is not very clear from the micrographs (Figure 5.5), the same sublimation and subsequent recrystallisation as seen with the RM was observed again (Figures 3.13 and 3.14). From this it is possible to conclude that the SV (LV) crystals are not the same solid-state form as the sublimate formed when heating the RM under ambient pressure, otherwise there would be no transformation visible. The cooling to ambient temperatures of the crystals formed during sublimation, SV (LV), might also have caused a reversion back to the RM form. The assumption also arose that the SV (LV) crystals could be a mixture of more than one form.

It does, however, appear that the sublimation and subsequent recrystallisation occurs to a lesser extent in this case, though this was not measurable. The onset of melting appears to be near 140°C (Figure 5.5b) and at around 160°C the complete sample mass appears to have melted (Figure 5.5e). This can, however, be due to the dissipation of heat occurring with greater ease through these thin crystals.

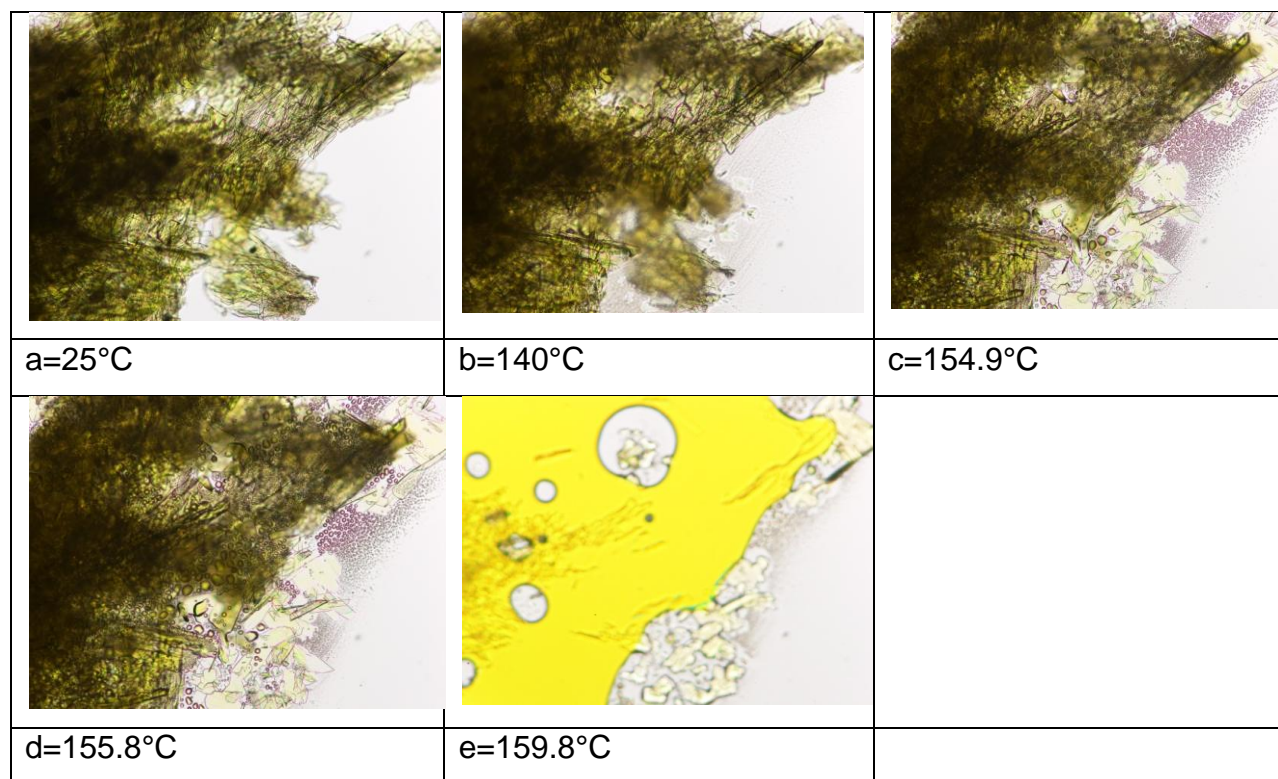


Figure 5.5 TM results for SV (LV)

Conclusion

From the results it was determined that the crystals obtained by SV (LV) were not a pure new form, because the phase transition seen with the RM was still present. Relating this to the results seen in Figures 5.1-5.2 it would appear that the less-crystalline attributes of the crystals obtained are only partly seen in the results. The assumption was made that the crystals present were possibly a mixture containing a form that might be less crystalline than the ethionamide RM and another form/s. This could be because the temperature at which the crystals formed was too high for the crystals to form as amorphous solid-state forms and that there was still enough molecular mobility at this temperature for the molecules to partially arrange in the stable form under the conditions present. The presumption was made that by altering the conditions at which the crystals were formed it would be possible to drive the formation of new crystals in favour of the less-crystalline form and reduce the presence of other forms or impurities.

When looking at the conditions at which the crystals formed it made sense that by lowering the ambient pressure while keeping the volume constant, it would be possible to have the crystals sublime at a lower temperature. This could then reduce the energy of the vapour molecules at the point at which it is adsorbed to the sides of the container and accordingly lowering the molecular mobility of these molecules. This could then result in a packing formation with less order, lower density and consequently lower stability and higher dissolution potential.

The method described in section 5.1 was then altered by using a vacuum pump with higher strength and is referred to as the SV (HV) method hereafter.

This method resulted in much larger yields of crystals and in the formation of significantly larger crystals. These crystals were then analysed by some of the methods used to analyse the SV (LV).

5.2.5 Thermal microscopy in combination with polarised microscopy

The same TM method was employed, but in this case the sample was viewed through crossed polarisers.

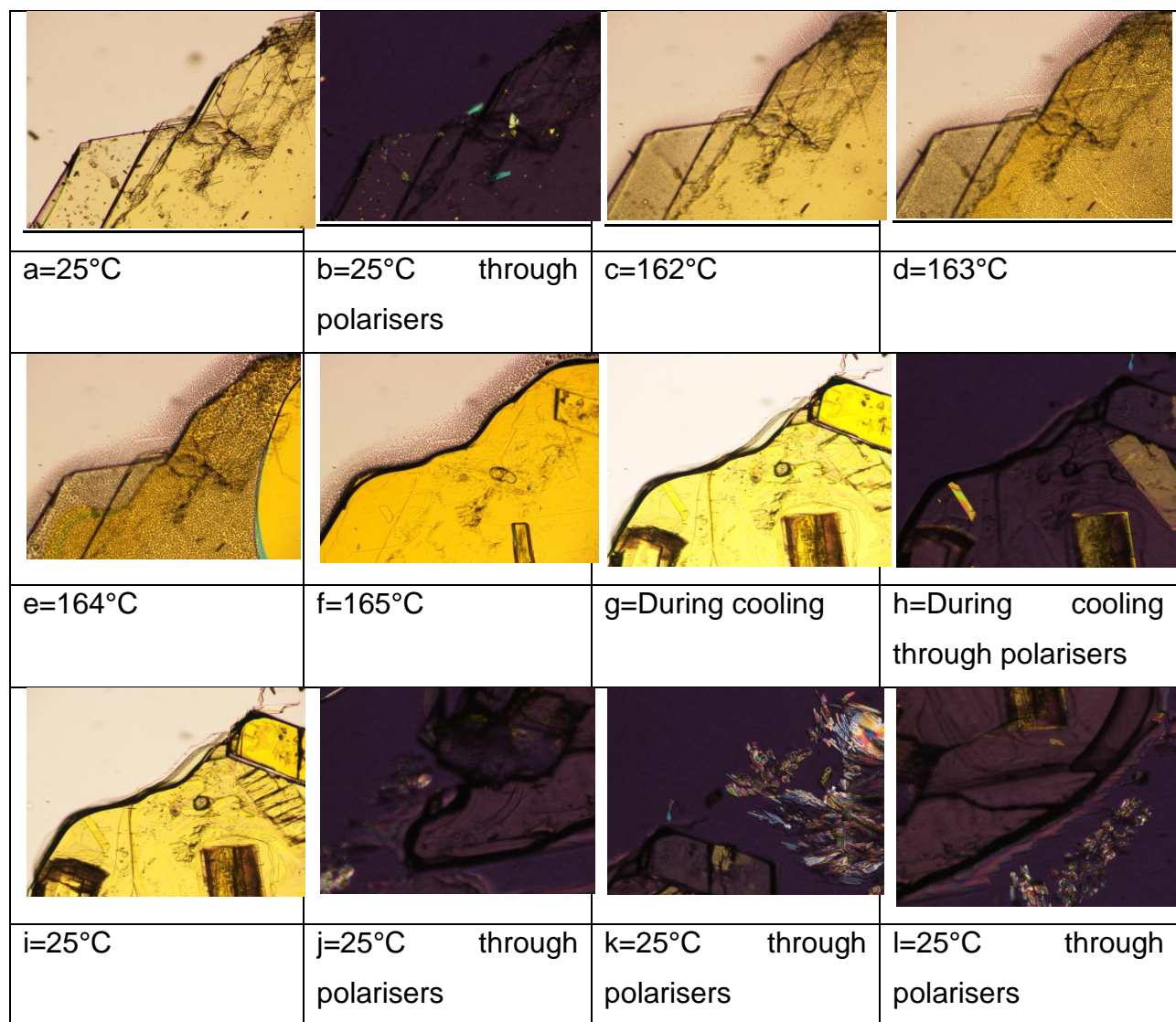


Figure 5.6 TM in combination with polarised microscopy of SV (HV).

In these images one can see the SV (HV) crystals during a heating and cooling run. The first image (Figure 5.6a) shows the sample at ambient conditions before starting the heating run. Figure 5.6b shows the same image as Figure 5.6a viewed through crossed polarisers. This could imply that the SV crystals are possibly isotropic and that they have an amorphous structure. In Figures 5.6c and d, although not very clear, one can see a small amount of droplets forming on the cover glass through condensation of vapour. The amount of condensated vapour appears to be lower in this case than for that seen with the RM. In Figure 5.6e melting started at approximately 164°C

corresponding to the melting point of the RM. The melted part flowed over the sample in view under the microscope, though the original sample in view has not completely melted yet at this stage (Figure 5.6f). The heating was stopped at 165°C when melting was complete, followed by cooling of the sample to ambient temperature. In this image one can see other crystals present within the molten phase. These were assumed to either be other solid-state forms present or that they are simply other crystals of the same solid-state orientated in different ways, aligned with the polarisers to not show extinction of the polarised light. Figures 5.6f and 5.6g show the sample while it was cooling down. Here one can see the molten phase returning to a solid state. During the cooling phase crystal growth was observed. Here one can see what appears to be the growth of different forms as the molecules in the liquid phase (molten part) arrange themselves in the molecular packing formation of the other crystals present. Some forming part of the crystals present and displaying the same colour through the crossed polarisers and others forming a separate phase that appears to have no colour (i.e. having the same colour as the background), possibly implying that the sample is isotropic, possibly representing an amorphous solid state. The solid state formed from recrystallisation of the melt shows crystallinity less clearly than before it was melted, though the tell-tale signs are still visible.

The fact that after melting and subsequent cooling the sample still shows no birefringence through the polarisers, can lead one to the conclusion that perhaps a glassy/amorphous form has been formed. However, the presence or absence of interference colours, when applying this method, is not conclusive for determining whether a solid is amorphous or not. The SV (HV) crystals obtained were viewed under the same microscope with crossed polarisers.

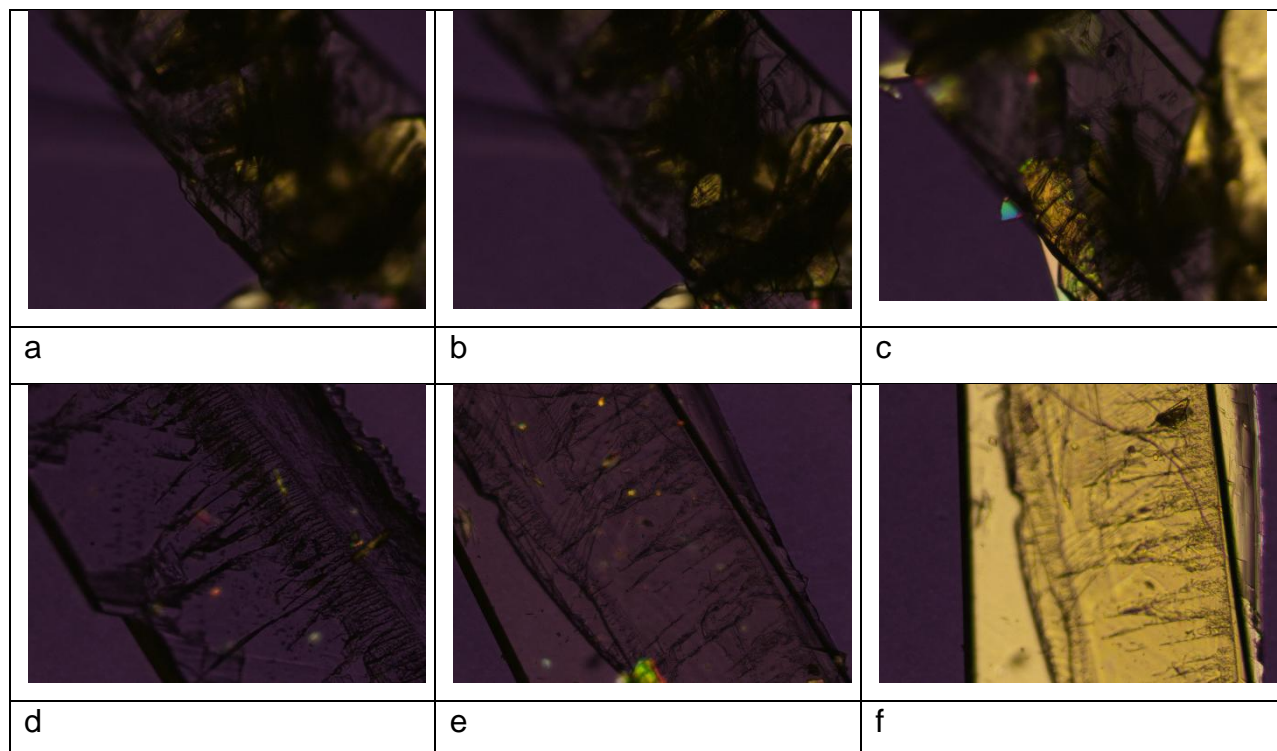


Figure 5.7 Different images of SV (HV) viewed under crossed polarisers. a-c (20x magnification). d-f depicts a single crystal being rotated (10x magnification).

The first three images show that though the crystals appear to have isotropic properties, as can be seen in the parts not showing any colours, there are anisotropic crystals present displaying birefringences resembling that seen in the RM through crossed polarisers. This can imply that all the crystals obtained by this method do not exist purely in a single state or that they exist in a single state, but some crystals are arranged in a way that their principle vibration directions are aligned with those of the polarising filters resulting in no colours being visible (Explained under extinction in section 2.3.3 and an example can be seen in Figure 5.8). The way to test this is fairly simple. One can rotate the crystals to see whether the interaction with light changes, resulting in the visualisation of colours. As these crystals were rotated the crystals displayed birefringence as can be seen in Figures 5.7d – 5.7f. This implies that the SV (HV) crystals are in fact quite crystalline in their molecular packing and have long-range order.

The following image shows an example of an extinction position and angle. In this example it is possible for one to see the parallel extinction positions where the crystals

are oriented in line with the crossed polarisers. This is why there are four extinction positions as the orientation is aligned with the polars at every 90° angle.



Figure 5.8 Extinction positions of acetylsalicylic acid after it has been melted and cooled. The sample recrystallised in a radial fashion and makes it possible for one to see the parallel extinction positions (seen as the dark crystals (Carlton, 2011)).

Conclusion

This result proves without a doubt that these crystals have a crystalline molecular packing order. This refutes the assumption that was made earlier that this form was a less-crystalline form. If one looks at the previous findings leading to the assumption that the SV crystals were less-crystalline with this knowledge in mind, it is possible to explain these results. On closer inspection, Figure 5.6f shows that the crystal was not fully melted. The melt recrystallised with the original sample crystal serving as a seed crystal. This led to the formation of a crystal with the same molecular packing as the original sample, arranged in the same directional formation as the original crystal that was not showing birefringence through the crossed polarisers. The crystals visible within the molten phase in Figure 5.6 were of the same solid-state form but simply orientated to interact with the light coming through the crossed polarisers.

This result did not fully explain the differences between the DSC results obtained with these crystals (Figures 5.3a and b) and with the RM (Figures 3.3, 3.4a and 3.4b)

though, but gave some more information on the transitions observed. Further analyses were needed for full clarification of the events seen in the DSC results.

5.2.6 Variable temperature X-ray diffractometry

VTX data were generated using a PANalytical EMPYREAN (PANalytical, Netherland) with CuK α -radiation at a minimum step size of 0.0001 $^{\circ}\theta$. Generator settings were 45 mA, 40 kV. An Anton Paar Cryo and humidity chamber (Anton Paar, Germany) was used for the heating of the samples. The samples were heated to a predetermined temperature where a 5 minute scan was done.

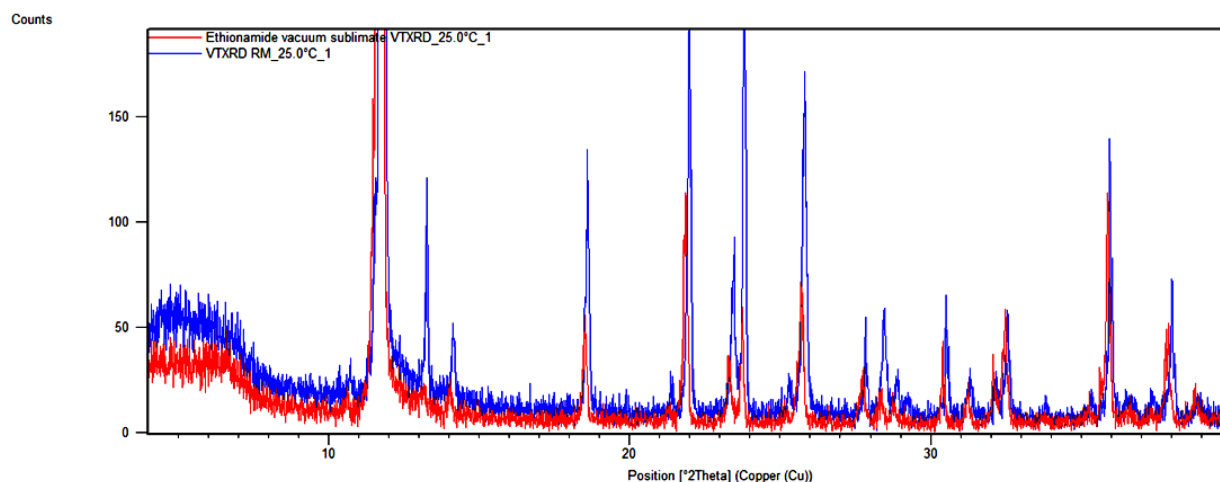


Figure 5.9 VTXRD of SV (HV) (bottom) and RM (top) at 25°C.

In this result one can see that there is a clear resemblance between the XRD result of the RM and that of the SV (HV) crystals. These results further disprove the assumption that was made earlier that the crystals are less crystalline than the RM. These XRD results are so alike that the assumption was made that the crystals obtained by this method possessed the same molecular packing as the RM crystals.

Conclusion

The results indicate that the crystalline molecular packing formations of the RM and the SV (HV) crystals are very similar if not identical.

This result still left the differences seen in the DSC results of these two forms unexplained. At this stage when relating the results seen in this section so far to the results seen in section 3.2.4, it can correspond with the assumption made that the RM is a mixture of more than one polymorphic form. This can be deduced from the results seen that the crystals formed by the SV (HV) method only have a single peak in the DSC results and much less sublimation and subsequent recrystallisation from the vapours was visible with the SV (HV) crystals. For this assumption to be verified more analyses needed to be done.

5.2.7 Fourier transform infrared (FTIR)

There were no clear differences visible in FTIR results and supports the idea that the SV (HV) crystals are of the same polymorphic form as the RM.

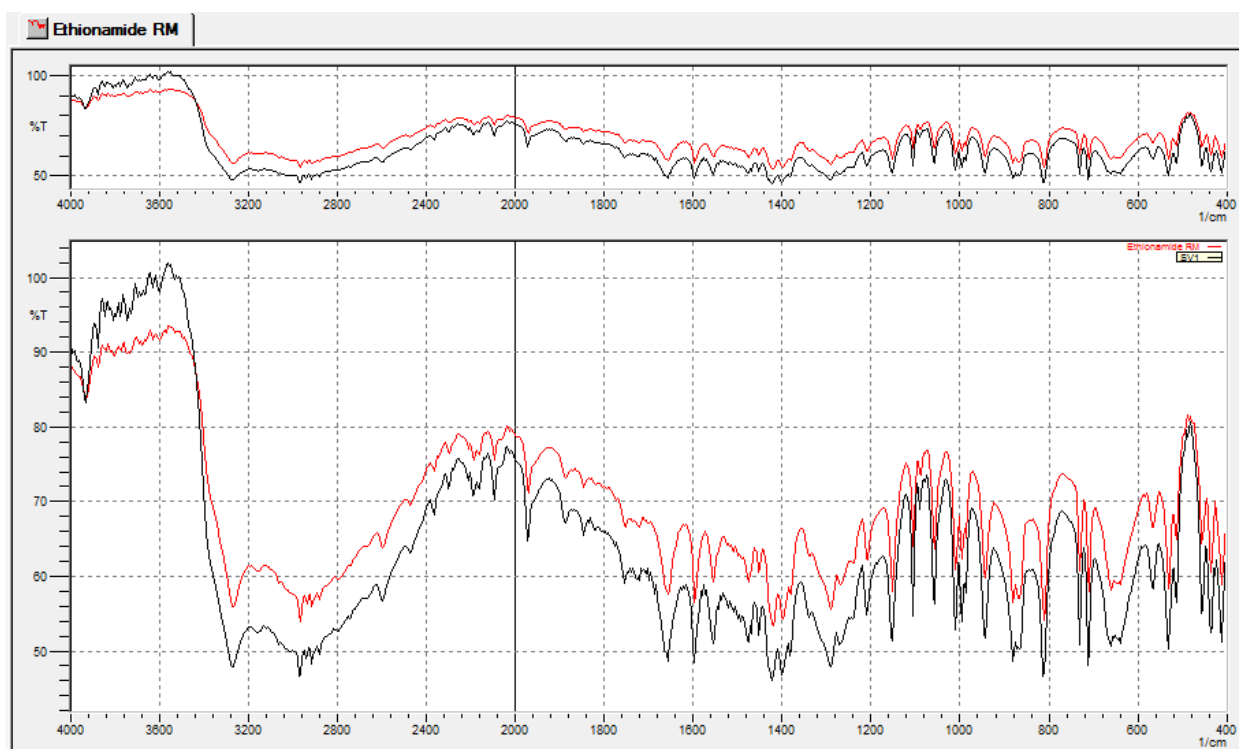


Figure 5.10 FTIR of SV (HV) and RM as an overlay.

Conclusion

Many observations hinting to the possibility of creating crystals by a physical vapour deposition method throughout this study were encountered and explained. The

subsequent alterations made to optimise the processes employed to obtain these crystals and alter the properties of these crystals were also described.

The resulting crystals displayed properties indicating that a crystalline form of the material was created that differed slightly from that of the RM and the sublimate formed when heating the RM at ambient pressure, though these forms were not fully characterised and differentiated between at this stage.

It also appears as though when under lower pressure (caused by stronger vacuum pump) the sublimate that recrystallises from the sublimation of the RM differs from the sublimate formed under higher pressure (caused by weaker vacuum pump). The difference caused is assumed to be as a result of the differences in temperature resulting from the differences in pressure (higher pressure should cause higher temperature as related from the ideal gas law (see section 1.5). The sublimate formed under lower pressure is assumed to be very similar to the RM, though the sublimate formed at higher pressure shows less resemblance to the RM. Though, this assumption remained to be verified at this stage.

5.3 The cover glass method

5.3.1 Introduction

At first a method was employed to see whether it was possible to create crystals from the vapours created by sublimation. The method as explained by Guillory (1999) was used. This method involves placing some of the ethionamide RM in a petri dish and covering it with an inverted watch glass. The petri dish is heated and the watch glass is observed for crystal growth. This method is referred to from this point onward as the cover glass method.

At first glance this method did not appear to result in the formation of crystals. Taking into consideration that the transition seen in the DSC results obtained with ethionamide RM was due to sublimation at ambient pressure and when seeing that the SV (LV) crystals were still forming new crystals upon sublimation, it was decided that a method should be formulated to isolate the crystals formed by sublimation at ambient pressure for identification. The yield of crystals obtained by heating the RM until sublimation

occurs utilising the TM, was too small. From the assumption that these crystals form by the recrystallisation from the vapour phase at around 162°C at ambient pressure, the decision was made to retry the cover glass method explained at the beginning of this section as this represented the exact conditions by which these crystals were forming on the cover glass in the TM.

When the RM is heated to the correct temperature (which is more difficult to obtain with the less accurate temperature of a heating magnetic stirrer (Velp[®] Scientifica - Italy) than with the TM) and left for a long enough time, the crystals do form. If the temperature is too high the crystals simply melt and decompose and if the temperature is too low, no sublimation will be induced. The crystals form amid the RM in the bottom petri dish. These crystals are difficult to separate from the raw material though and the possibility exists that one can simply gather a combination of both making the attempt to isolate the newly formed crystals redundant. The ideal is thus to gather the crystals solely from the surface of the cover glass to avoid this obstacle, even though the yield will be much lower. When the temperature of heating was optimised (the exact temperature was not measured, but the heating magnetic stirrer was set to a temperature at around 150°C), enough crystals were formed and gathered to do analyses on.

The abbreviation used for these crystals throughout the following section is S.

5.3.2 Differential scanning calorimetry

From the positions of the endothermic events (with peaks at 103.15°C and 113.29°C respectively) and what was presumed to be the much lower melting point endotherm (peak at 148°C compared to that of the RM at ±164°C) of this form, it was suspected that perhaps some degradation occurred.

To determine whether degradation occurred, the TLC method explained in section 3.2.12 was used. The resulting chromatogram did not show additional spots. After confirming that this was not a product of decomposition, the data obtained from the DSC result (Figure 5.11) was further interpreted. The thermogram appears to be a long continuous relaxation of enthalpy as the temperature is increased. This relaxation takes

place until there is a very small endothermic event at temperature $\pm 103^{\circ}\text{C}$ (with an enthalpy change of 1.02 mJ). The slope continues after this event and leads up to another endothermic event at $\pm 113^{\circ}\text{C}$ with a change in enthalpy of 12.44 mJ. The slope becomes steeper at $\pm 141^{\circ}\text{C}$ and forms a single broad endotherm. From the enthalpy change involved in this event it was assumed that this is where melting occurs, but required further analyses to know for certain whether this was the case.

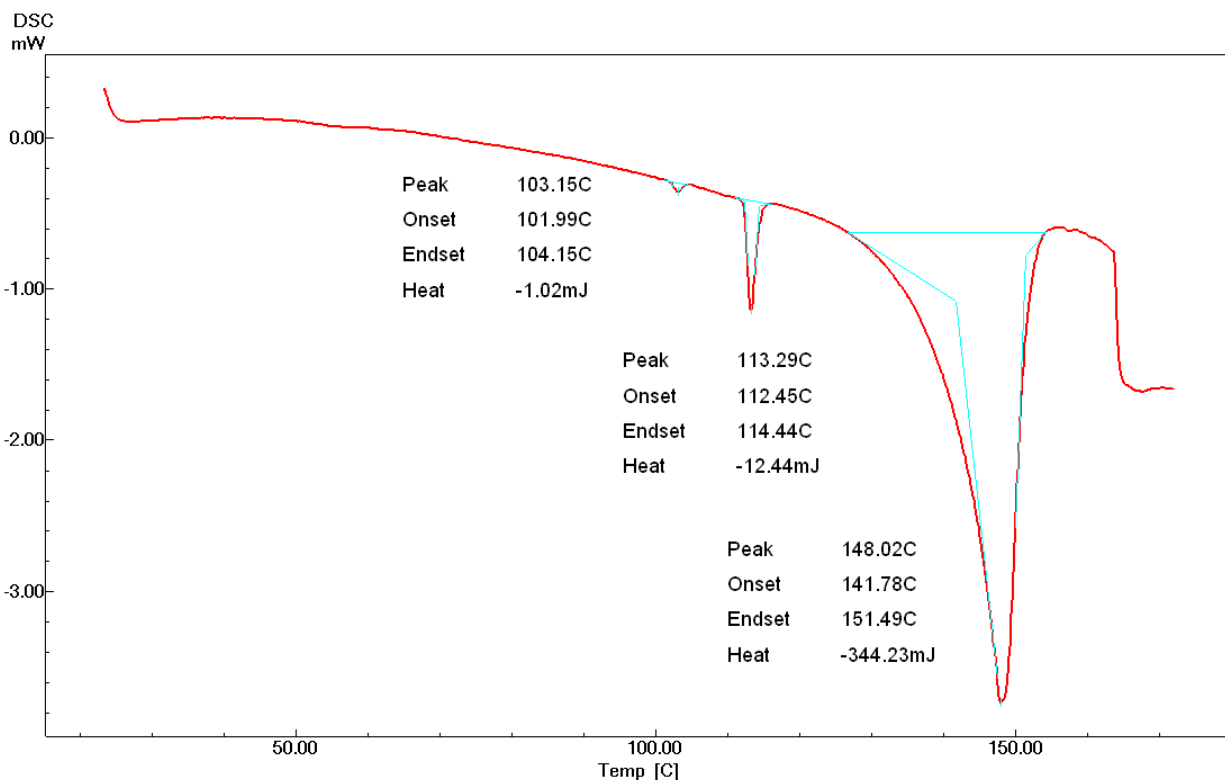


Figure 5.11 DSC of S (the crystals obtained by the cover glass method) (heating rate of $5^{\circ}\text{C}/\text{minute}$).

Figure 5.12 depicts an overlay of DSC thermograms obtained for ethionamide RM and ethionamide S. Clear thermal differences can be observed.

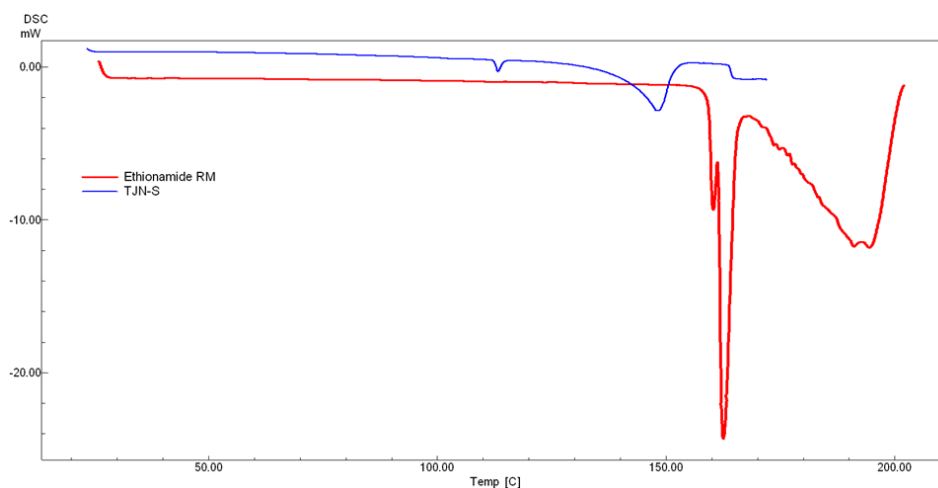


Figure 5.12 DSC overlay at 5°C/minute of RM and S.

Conclusion

The endothermic event at temperature 148°C seen in Figure 5.11 thought to be the melting point at this time in the study had to be elucidated with further analyses. The other events had very small changes in heat, but also remained to be verified.

A different heating rate was employed (2°C/minute) to see if the result seen in the previous DSC was reproducible and also to see if more information can be obtained on the events seen in the previous DSC by increasing the sensitivity of the analysis method.

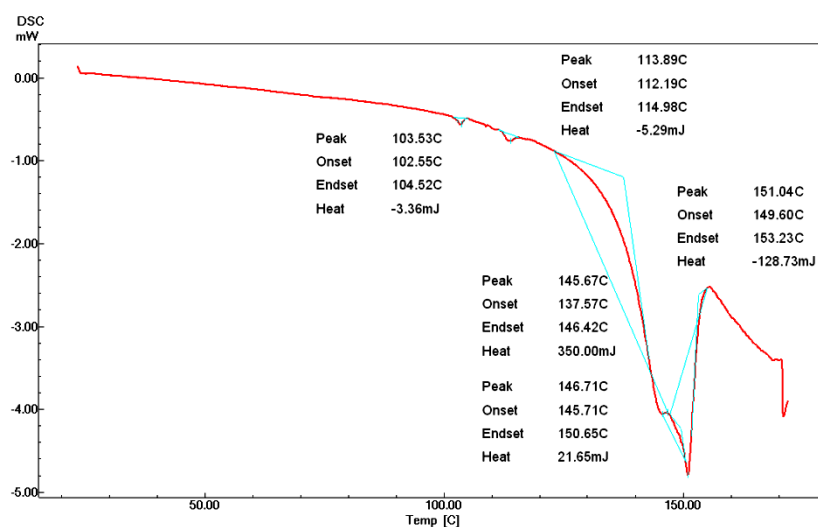


Figure 5.13 DSC of S at a heating rate of 2°C/minute.

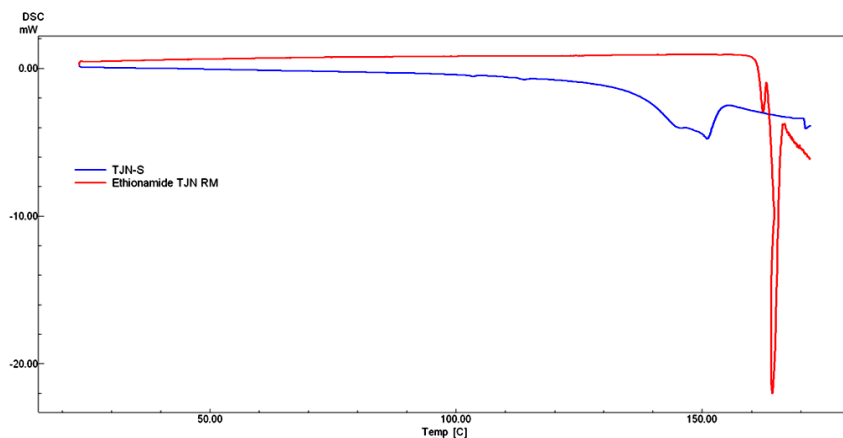


Figure 5.14 DSC overlay of S and RM at a heating rate of 2°C/minute.

From the results obtained by this experiment more information can be deduced from the event that was seen in Figure 5.11 at 148°C. At this heating rate it appears that this is not a single event, but appears to be an endothermic event with a peak at 145.6°C overlapping with a second endothermic event with a peak at 151°C.

When looking at Figure 5.14 the fact that there is more than one event occurring, becomes even more evident. The other endothermic events visible in Figure 5.11 are also visible in this result, though the heat changes involved are so small (3.36 and 5.29 mJ) that they seem insignificant, though can't be ignored.

Conclusion

From this information one could assume that this is not a single event and further investigation will be necessary to clarify these thermal events.

5.3.4 Thermal microscopy in combination with polarised light microscopy

The following figures show the results obtained with the TM in combination with the polarifilters of ethionamide RM.

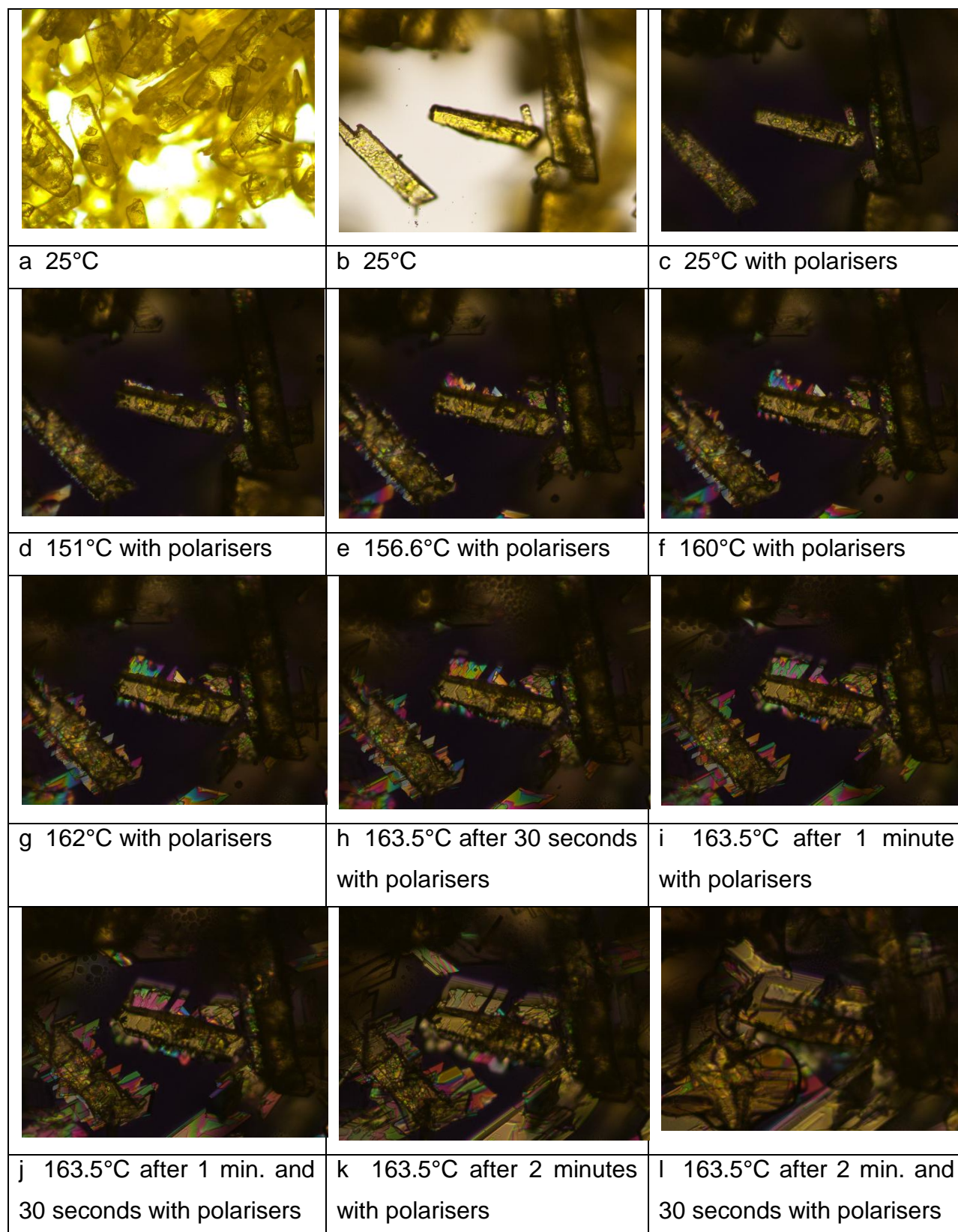


Figure 5.15 TM with polarised light microscopy of ethionamide RM.

Figure 5.15a shows the crystals without any filter at a high brightness. From this image one can see the actual colour of the crystals without the light being filtered. Figure 5.15b shows an image of the crystals that were used in this experiment at ambient conditions before the heating run was started without any filter and Figure 5.15c shows the exact same image where the crossed polarisers were used. In Figure 5.15c one can still see colours through the filters and this suggests that the material is anisotropic. The RM displays a single colour when viewed through the polarisers. This can be explained by the assumption that the birefringence is constant throughout the material; as a result of a uniform molecular coordination throughout the sample (the material is crystalline with a repeating long-range packing order throughout the crystal lattice).

The formation of new crystals is visible in Figure 5.15d. The colours that are seen in this image and of the subsequent images (Figures 5.15d – 5.15j) display a discontinuous change in the polarisation colours and can imply that a phase transition is taking place. As these crystals grow and increase in thickness the colours change as the colours seen are a product of the birefringence and the thickness of the material. This can be explained by the assumption that the newly formed crystals have a differing molecular packing formation than the RM, though it is still a crystalline solid-state form.

Conclusion

From the results obtained the presumption that the RM undergoes a phase transition at $\pm 162^\circ\text{C}$ as was suspected from the DSC results (Figure 3.3) is verified. From the evidence it can be seen that the phase transition starts at a lower temperature ($\pm 150^\circ\text{C}$) than the temperature seen in the DSC results (onset $\pm 160^\circ\text{C}$), but verifies that a transition takes place near this temperature. This change in onset temperature could imply that the transition reaction is not of a first-order and corresponds to the jump in enthalpy caused by an event such as sublimation.

In the following result the temperature was held at 150°C in an attempt to make the event seen in Figure 5.13 visible. The temperature was held again when the sample started to melt, this was at 168°C .

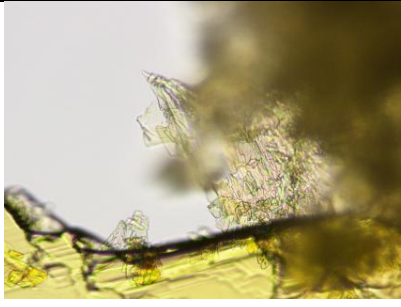
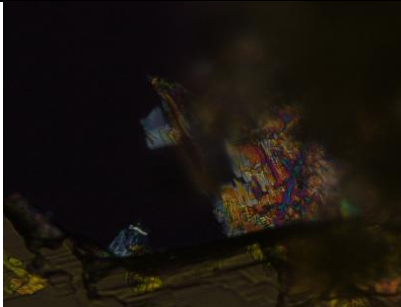

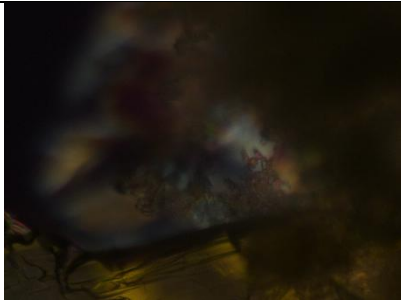
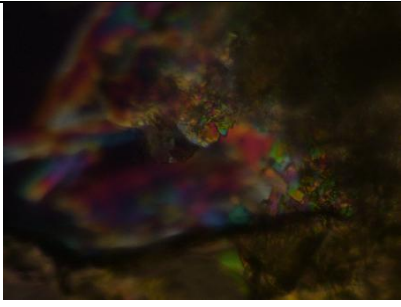
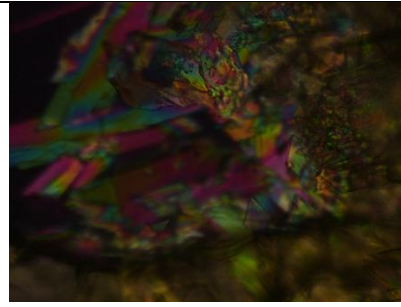
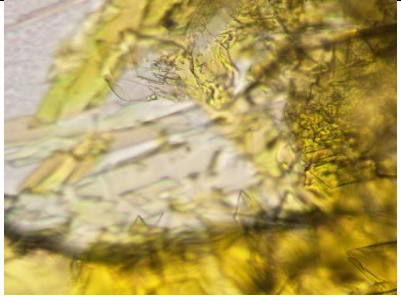
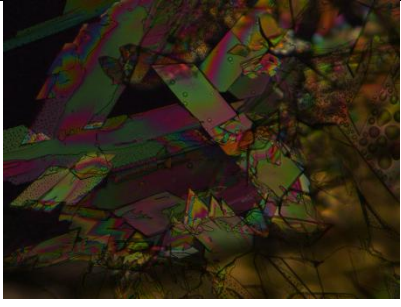
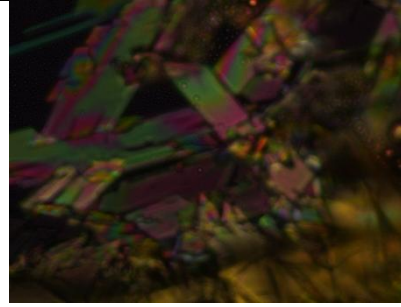
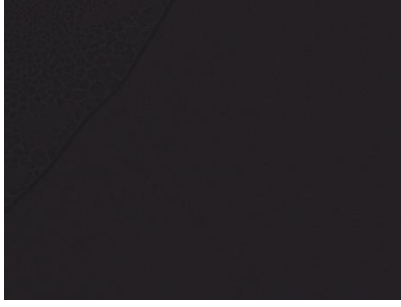
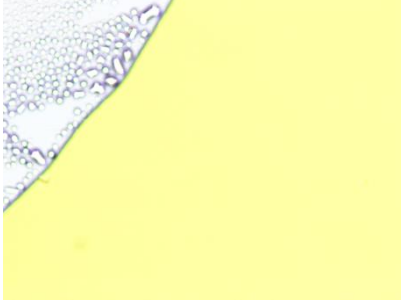
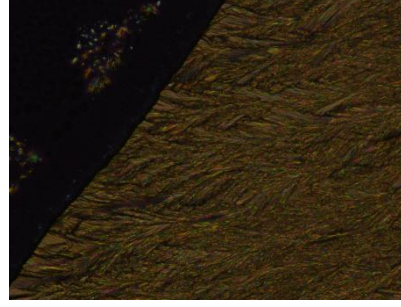
		
a 25°C	b 25°C with polarisers	c 148°C with polarisers
		
d 150°C with polarisers	e 150°C after 2 minutes with polarisers	f 150°C after 5 minutes with polarisers
		
g 150°C	h 165°C with polarisers	i 168°C with polarisers
		
j 170°C with polarisers	k 170°C	l 25°C with polarisers

Figure 5.16 TM with crossed polarisers of S.

Figure 5.16a is an image of the sample without light polarisers at ambient conditions. Figure 5.16b is the same image as Figure 5.16a seen through crossed polarisers. The image displays birefringence and implies that the material is anisotropic. The crystals look similar to those obtained in Figures 5.15d – 5.15i. This is a good outcome, because the aim of this preparation method was to isolate the crystals formed under those conditions. The problem in this case is that the whole sample mass does not consistently resemble these crystals and that there appears to be crystals present resembling the crystals formed by the cold glass finger method. This could imply that the method used in an attempt to isolate the sublimate formed at ambient pressure has led to the formation of a mixture of the sublimate acquired under lower pressure (under vacuum) and that obtained by means of sublimation at ambient pressure.

The assumed reason for the formation of the various forms was that the temperature differs with the two methods. This is because under vacuum the temperature at which sublimation and subsequent recrystallisation takes place is lower. This is explained by the ideal gas law. The formation of the one solid-state form appears to be favoured at lower pressure and the other at ambient pressure. The higher temperature at ambient pressure supposedly led to the formation of the sublimate seen in Figures 3.13 and 3.14.

From Figure 5.16c one can see that the crystals are starting to melt, corresponding to the temperature at which the peak of the large endothermic event can be seen in the DSC result for this material (Figure 5.13 $\pm 148^{\circ}\text{C}$). This implies that the event seen in Figure 5.13 is a process of melting. This could imply that this polymorphic form is monotropically related to the form it transforms into as melting takes place before a phase transition takes place. This result is, however, not a decisive verification of this as the other events seen in the DSC results Figures 5.11 and 5.13 have not been explained yet and it could be that these events represent phase transitions.

In Figure 5.16d it appears as if there is a partially molten phase that is starting to recrystallise when the temperature is held at 150°C . This relates to the exothermic event visible at $\pm 147^{\circ}\text{C}$ in Figure 5.13. This recrystallisation continues as the system is held at this temperature, as can be seen in Figures 5.16d – 5.16h. This correlates well

with the results seen in Figure 5.13 and shows that the endothermic peak and exothermic peaks (at $\pm 146^{\circ}\text{C}$ and $\pm 147^{\circ}\text{C}$ respectively) represent a process of melting and subsequent recrystallisation from the melt. The overlapping event seen in Figure 5.13 with an endothermic peak at $\pm 150^{\circ}\text{C}$ is not visible in this case. The heating run was held at 150°C but up to this temperature the heating rate was $10^{\circ}\text{C}/\text{minute}$. It is possible that the endothermic event seen at $\pm 148^{\circ}\text{C}$ in Figure 5.11 and at $\pm 151^{\circ}\text{C}$ in Figure 5.13 was skipped in this case. This could be because in this case the recrystallisation process seen in Figure 5.13 had time to continue to completion and resulted in the formation of a more stable form melting at a higher temperature. This theory works well with the temperature of the event being lower in the case where the sample was heated at $5^{\circ}\text{C}/\text{minute}$ than when it was heated at $2^{\circ}\text{C}/\text{minute}$. In the case where the sample was heated at a higher rate the recrystallisation was not visible and the sample melted at a lower temperature, because the recrystallisation to the more stable (higher melting point polymorph had not taken place). When the sample was heated at a lower rate the recrystallisation to the more stable form partially took place before the next melting event occurred and this caused the melting point to be slightly higher.

In Figures 5.16g and 5.16h one can clearly see that a new solid-state has formed by recrystallisation from the melt. In Figure 5.16i it seems as though the newly formed crystals along with the remainder of the original crystals start to melt at a temperature of $\pm 168^{\circ}\text{C}$ which is much higher than the melting point of the RM and could indicate that this is a more stable polymorphic form of ethionamide (with a higher melting point).

Conclusion

From the results obtained by this method it appears as though the S crystal melts when heated to near 150°C . It appears that the solid partly melts and starts to recrystallise from the melt. This can explain the endothermic and exothermic peaks seen in Figure 5.11 at $\pm 150^{\circ}\text{C}$. The results seen here indicate that it should be possible to separate the events that were overlapping in the DSC traces seen in Figures 5.11 and 5.14 as they are clearly separated in this result. It also appears that by separating these events it is possible to obtain a higher melting/more stable polymorphic form of ethionamide, though it could be that this is simply a product of decomposition recrystallising into a

more stable form, though this is highly unlikely. Further analyses are needed for clarification.

The events representing the peaks seen at $\pm 103^{\circ}\text{C}$ and $\pm 113^{\circ}\text{C}$ in Figures 5.11 and 5.14 were not visible in this experiment.

5.3.5 Differential scanning calorimetry

The events seen in the DSC results in Figures 5.11 and 5.14 were not visible in the TM results (Figure 5.16) and the idea that by increasing the heating rate to $10^{\circ}\text{C}/\text{minute}$ could alter the enthalpy involved in the events to make them more readily visible and give some more information on these events.

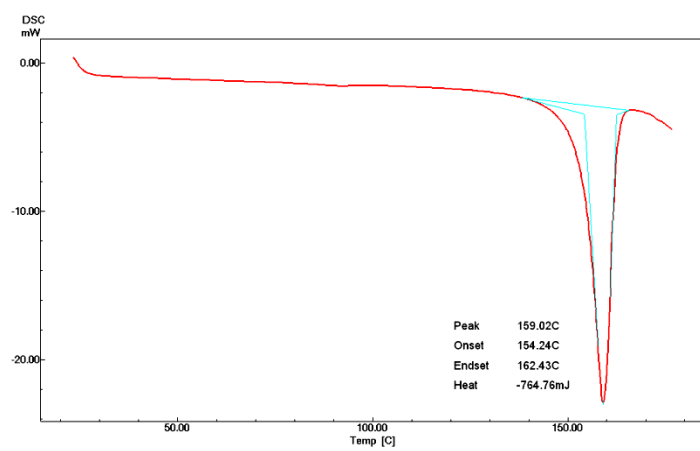


Figure 5.17 DSC of S at a heating rate of $10^{\circ}\text{C}/\text{minute}$.

Results

By heating the sample S at a rate of $10^{\circ}\text{C}/\text{minute}$ the events seen at temperatures $\pm 103^{\circ}\text{C}$ and $\pm 113^{\circ}\text{C}$ in Figures 5.11 and 5.14 are not visible. This could be that at this rate these events were skipped.

The sample continues with a single steady baseline to 154°C and an event takes place assumed to be melting of the sample with a change in enthalpy of 764.76 mJ (Figure 5.17). In this case the sample does not show the phase transitions seen in Figures 5.11 and 5.13 and melts at a temperature corresponding to that of the SV (HV) crystals. This could imply that the crystals represent the same solid-state form obtained by the cold

glass finger method as it was seen that the crystals obtained by the cover glass method are possibly a mixture. Because the events at $\pm 103^{\circ}\text{C}$ and $\pm 113^{\circ}\text{C}$ in Figures 5.11 and 5.13 are not visible and there is no extra peak, as was the case with the RM (Figures 3.3 and 3.4), it could also be assumed that the phase transitions seen at the other heating rates were skipped and that this is the melting point of this solid-state form when no transitions are undergone.

It was seen that by lowering the heating rate, the events seen in Figures 5.11 and 5.13 were more readily visible, and reducing the heating rate to $1^{\circ}\text{C}/\text{minute}$ was done in an attempt to gain more precise information on the events seen.

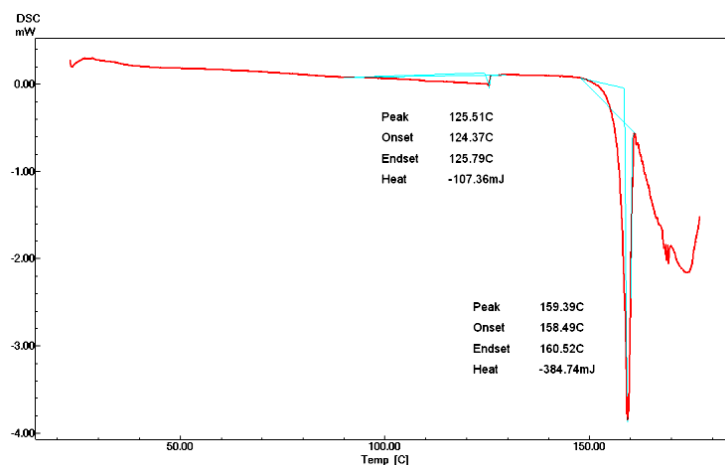


Figure 5.18 (a) DSC of S at $1^{\circ}\text{C}/\text{minute}$.

At a heating rate of $1^{\circ}\text{C}/\text{minute}$ the events seen at temperatures $\pm 103^{\circ}\text{C}$ and $\pm 113^{\circ}\text{C}$ in Figures 5.11 and 5.13 were also not visible. This could be because with the very small heat at a single point in time during this experiment, smaller events such as those mentioned will become too small to be observed. Giron (1995) explains that if the enthalpy involved in a solid to solid transition reaction is very low it may not be detected when using DSC with lower heating rates.

There is another endothermic event visible at $\pm 125^{\circ}\text{C}$ though and the eventual endothermic peak assumed to be the melting point can be seen at $\pm 159^{\circ}\text{C}$.

A DSC was done at which the temperature was held isothermally at $\pm 148^{\circ}\text{C}$ in order to separate the events seen in the DSC results as was visible in the TM results (Figure 5.16). The result can be seen in Figure 5.18b.

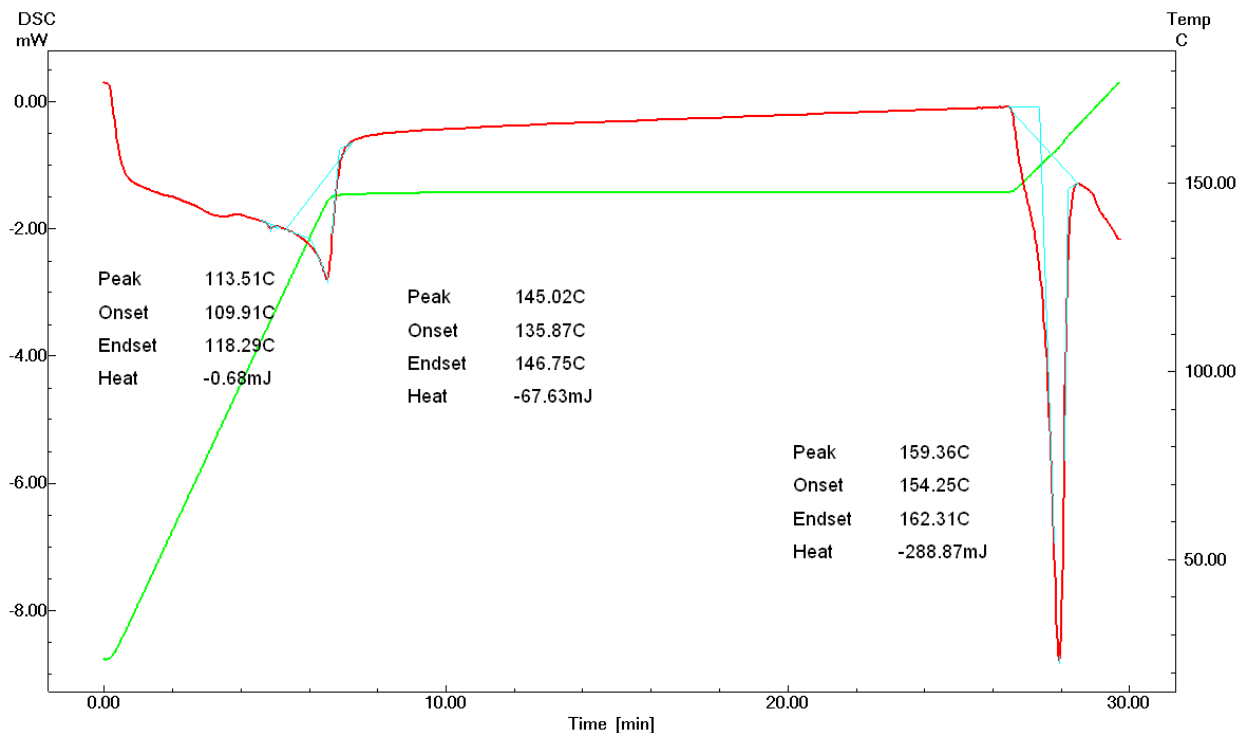


Figure 5.18 (b) DSC trace of S at a heating rate of $20^{\circ}\text{C}/\text{minute}$ up to 148°C and held at this temperature for 20 minutes and heated at a rate of $10^{\circ}\text{C}/\text{minute}$ to 175°C .

This result (Figure 5.18b) shows the relaxation of enthalpy that was visible in Figures 5.11 and 5.13. One of the events seen in these traces at 113°C is also visible in this case. This implies that the events are not skipped at higher heating rates. This could be because the sample used in this experiment was simply a higher percentage ratio of the S form than the sample used in the other DSC run (Figure 5.17) as the samples were taken from the same batch. After the exothermic transition, presumed to be recrystallisation of the melt, is done, the heat capacity is higher and represents a more stable form than before this transition. Though when considering the starting heat capacity the new heat capacity after the exothermic event appears to be lower. As the sample is held isothermally the heat capacity appears to return towards the higher heat capacity that was visible at the starting position. After the sample was kept at 148°C for

20 minutes the sample is heated at 10°C/minute up to a temperature of 175°C. Another large endothermic event can be seen at 159°C. This event appears to be a partial overlap of two events and this could imply that the exothermic transition to a more stable form was incomplete at the time where the heating was resumed.

5.3.6 Variable temperature X-ray diffractometry

This result shows a clear difference between the RM and the S crystals and implies that the molecular packing of the two crystals differs.

This means that the S crystals represent a different polymorphic form of ethionamide.

Only the VTXRD results of significance will be presented in this section.

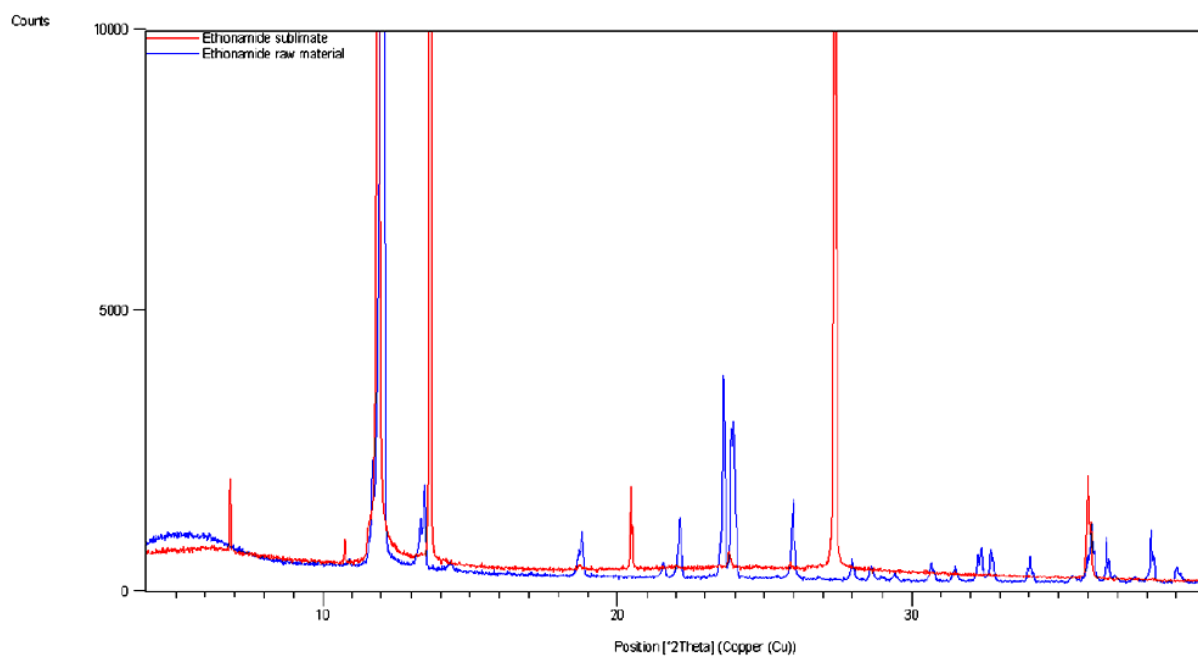


Figure 5.19 XRPD overlay of ethionamide S and ethionamide RM.

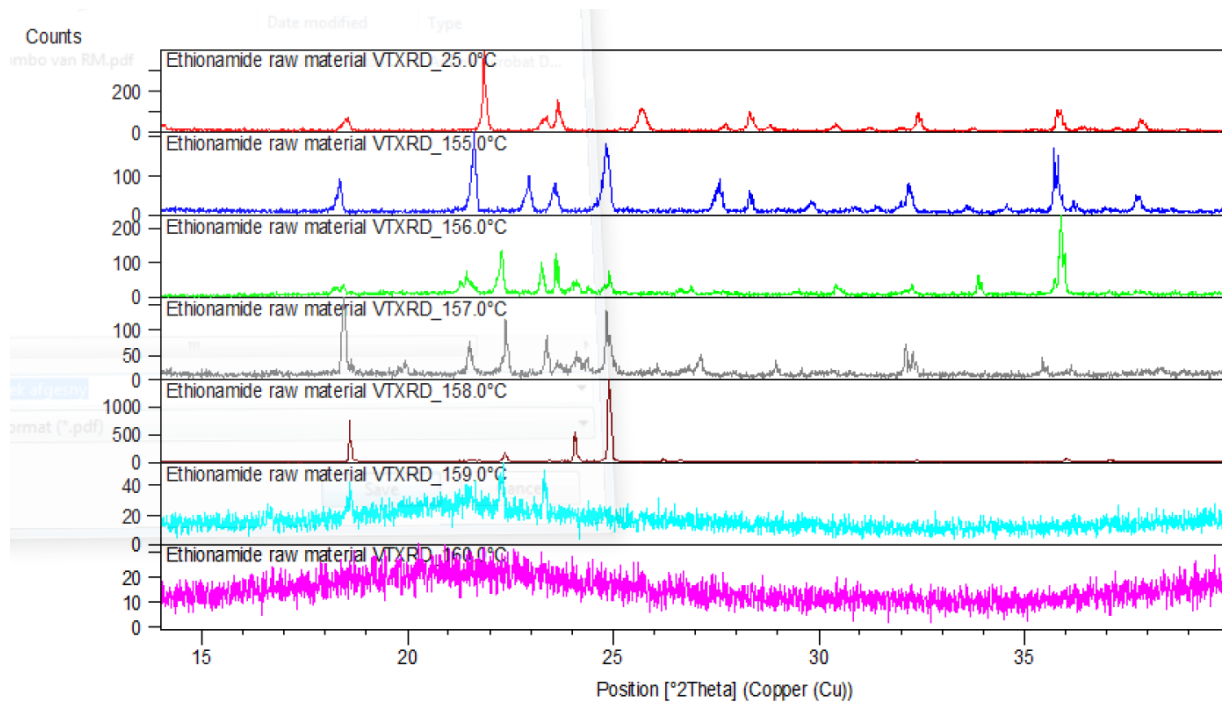
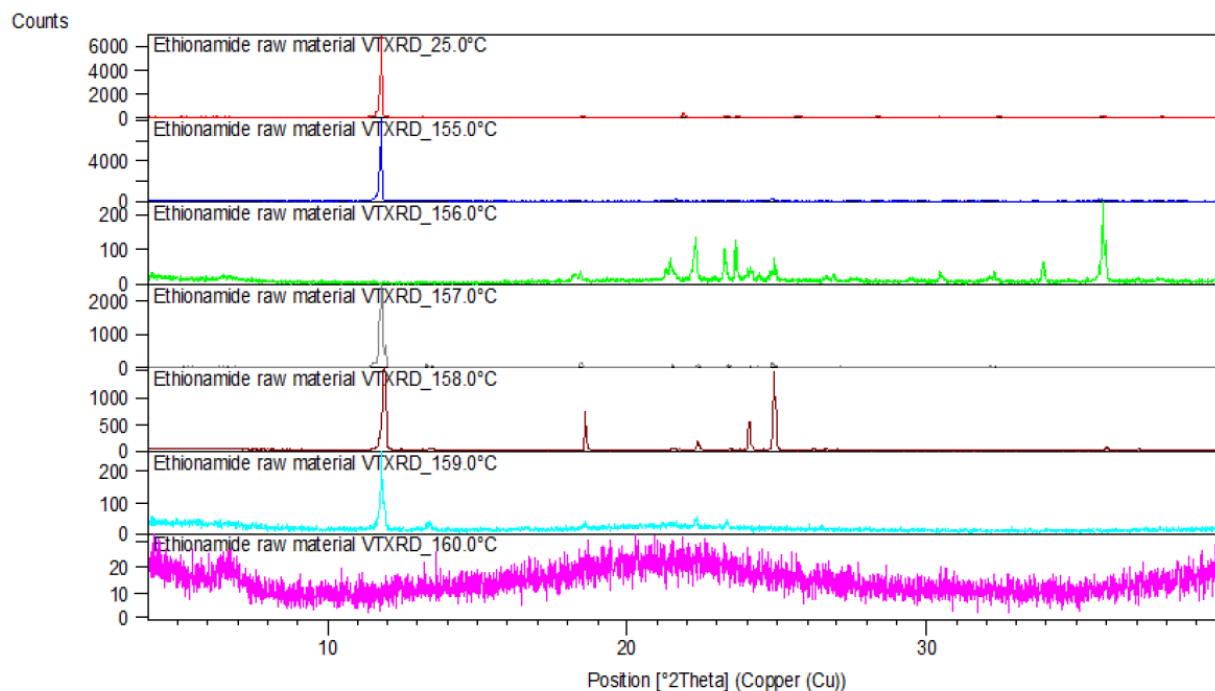


Figure 5.20 (top) VTXRD of ethionamide RM beginning at room temperature (25°C) up to 170°C. **(bottom)** VTXRD of ethionamide RM beginning at room temperature (25°C) up to 170°C, starting at position [°2Th.] of 14 to make the smaller peaks more readily visible.

The results obtained by this method give substantial information when relating the results seen with DSC and TM in combination with crossed polarisers.

When comparing the diffractogram at 25°C with that at 155°C in Figure 5.20 (top) it is very difficult to compare the peaks, but when looking at Figure 5.20 (bottom), the peaks are more readily visible and easier to compare. From Figure 5.20 (bottom) one can see some minor peak shifts at position [°2Th.] 25-26 and 29-33 when comparing the diffractograms at 25°C with 155°C. This can be due to small changes in the spacing of the molecules within the planes they find themselves in.

In Figure 5.20 (top), there appears to be a considerable difference between the results at 155°C and 156°C. The largest peak at position [°2Th.] 11-12 seems to have disappeared. This implies that something drastic has occurred in terms of the molecular coordination within this sample. When comparing these results from Figure 5.20 (bottom) the peaks show a resemblance in positions of some peaks, though appear to differ to a significant extent. This can imply that a transition of some sort has taken place or is busy taking place at this temperature.

When comparing the results at 157°C with that at 155°C and 156°C in Figure 5.20 (top) it appears that at 157°C the result looks fairly similar to that at 155°C but differs from the result at 156°C. When comparing the results in Figure 5.20 (bottom) though, the results at 157°C are similar in some peak positions and counts to that of 155°C and some are similar to the results at 156°C. This can imply that the transition that caused the differing results at 155°C and 156°C is partly reversible and causes a reversion to the molecular conformations that were present at 155°C when heated to 157°C. This idea will be in accord with the thought that at this temperature there are additional accessible dips in the energy landscape (see section 1.2.3) and the crystals can form with different molecular coordinations at this temperature, but can just as easily return to the former molecular coordination. When comparing this to the results seen in Figure 5.15 one will notice that in the TM results the transformation from the one solid-state to the other through sublimation and subsequent recrystallisation is incomplete at the temperature where the newly formed crystals and original crystals melt, but that the formation of the new crystals starts from about 150°C. The changes made visible from 150°C to 160°C

by means of TM with crossed polarisers relate to the VTXRD results in that a phase transformation is visible in both, though the change seen with VTXRD at 156°C is not visible. The change seen at 156°C was also not seen again when the method of analysis was repeated. Here the term disappearing polymorphs comes to mind.

The formation of a polymorph influences the environment in which it has formed in that the newly formed polymorph will be contending with the polymorphs that were present before it came into existence. A polymorph forming will usually be a result of a change of free energy to a lower level. Here one should consider Ostwald's law of stages. The polymorph that is formed will not necessarily be the most stable form but simply the form that is nearest the form that was initially present in terms of free energy. Under the specific conditions the polymorphic form might be the more stable form or simply very close in free energy, but by altering any aspect of the conditions present the highest stability might shift in favour of another form. Another aspect influencing which contending form will be the reigning one is that of the polymorphic forms present in the vicinity of the newly formed polymorphic form as these crystals can serve as seeds. A large number of variables in conditions can influence which form will dominate and in polymorphism the specific conditions favouring a certain polymorphic form is not always easy to determine (Bernstein, 2002).

An example of disappearing polymorphs is that of ritonavir (Bauer *et al.*, 2001).

This could be explained by referring to the concept of the energy landscape. The packing of the molecules in the specific coordination is not a definite result of heating a material to the temperature at which this formation is possible. It is merely a possible coordination at that temperature. This could explain why this was not seen with the TM or DSC results.

The changes seen when relating the VTXRD results at temperatures 157°C, 158°C, 159°C and 160°C with one another it shows that the diffraction pattern steadily becomes more diffuse as the sample material starts to melt. At higher temperatures than this the

pattern simply becomes more diffuse as can be seen in Figure 5.21.

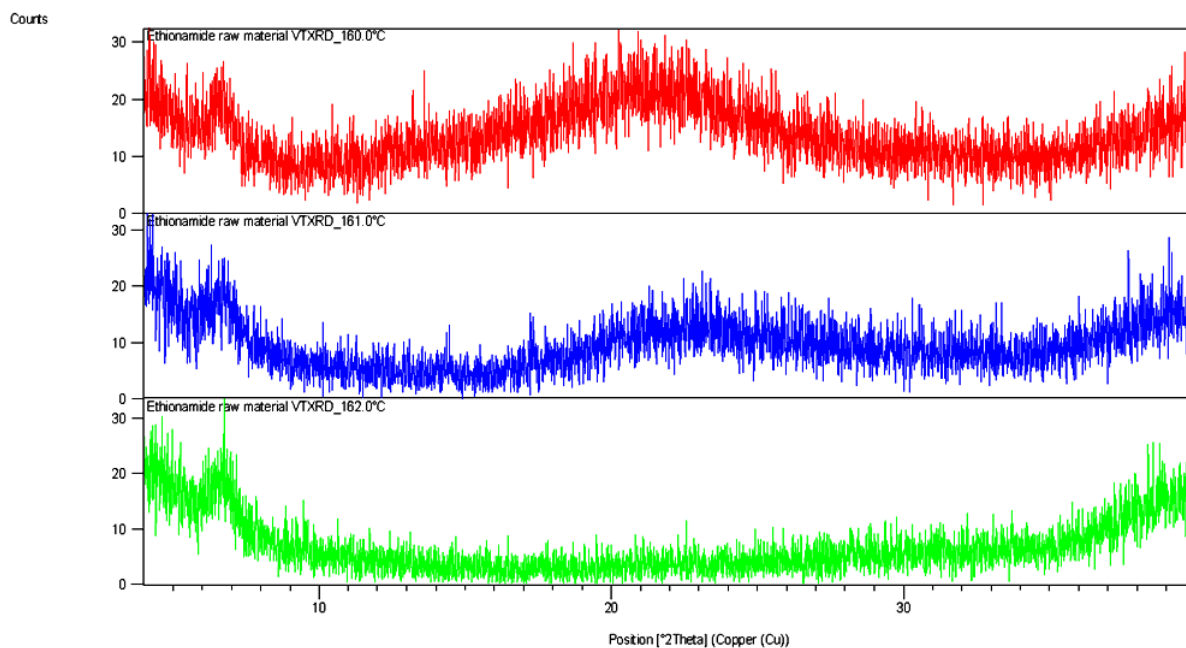


Figure 5.21 VT-XRD results of ethionamide heated near melting point.

Relating the results seen in Figures 5.20 (top and bottom) and Figure 5.21 to the DSC results seen in Figures 3.9a and 3.10 one will find that there was an event at around 158°C where the phase transition was visible and as was seen in the TM results in Figures 3.13 and 3.14 (which does not require melting of the sample). This is why in the VT-XRD results the diffractograms all have high counts (crystalline samples) and one cannot see melting in either results. The XRD results seen for the sublimate (Figure 5.19) do not resemble any of the diffractograms seen in Figures 5.20 (top and bottom) and Figure 5.21. This could be as a result of both forms that of the RM/SV crystals and the sublimate crystals being present at the same time and influencing the results as can be seen in the TM results (Figures 3.6 and 3.7) or as a result of the vapour escaping and not forming the sublimate in this case (this is unlikely as the sublimate forms in close proximity or as part of the original sample). Another explanation could be that there are simply more polymorphic forms created on the heating of the sample than were seen with any of the DSC results. Throughout this study it has been made clear that with this material the heating rates have a significant impact on the kinetics of

transitions occurring. The variability of the heating rates seen in these VTXR results are the most probable cause of the variable results seen and make the comparison between the methods near impossible.

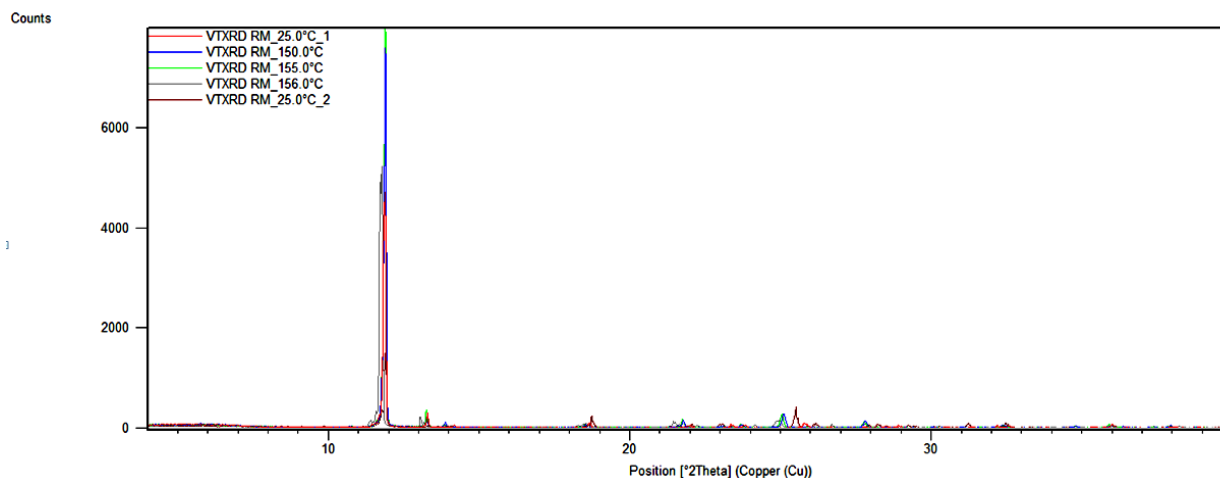


Figure 5.22a VTXR results of ethionamide RM heated up to 156°C and left to cool to 25°C.

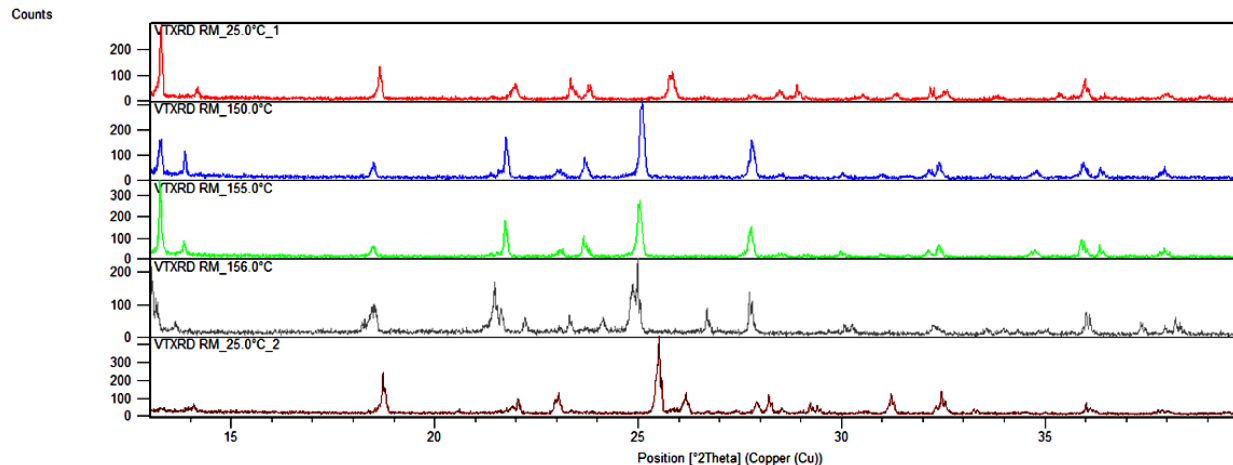


Figure 5.22b VTXR results of ethionamide RM heated up to 156°C and left to cool to 25°C, displaying the results starting at position [°2Th.] of 13 to make the smaller peaks more readily visible.

The results seen in Figures 5.22a and b show that some changes did take place in the molecular packing order of the sample and that after cooling back down to 25°C the resulting diffractogram (labeled VTXR RM_25.0°C_2) appeared similar to that of the

RM before being heated (labeled VTXRD RM_25.0°C) though does display some minor differences.

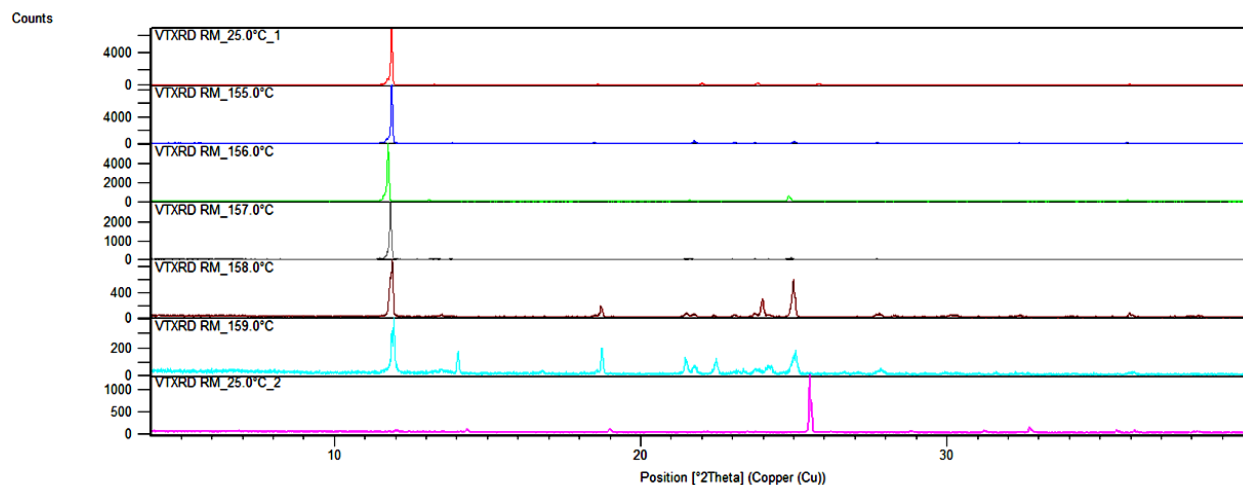


Figure 5.23a VTXRD results of ethionamide RM heated up to 159°C and left to cool to 25°C.

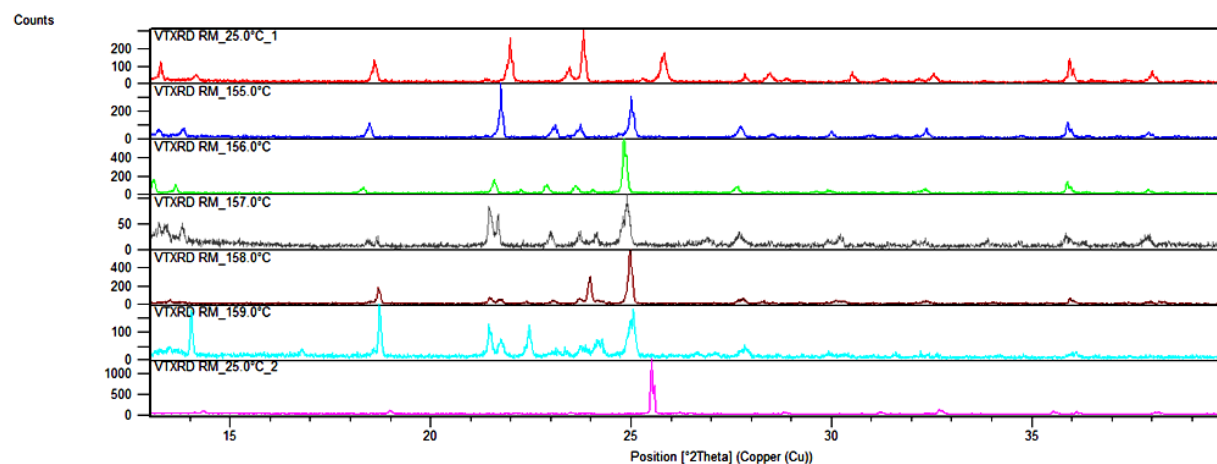


Figure 5.23b VTXRD results of ethionamide RM heated up to 159°C and left to cool to 25°C, displaying the results starting at position [°2Th.] of 13 to make the smaller peaks more readily visible.

From these results (Figure 5.23b) one can see a larger variation in molecular packing order of the sample as the sample is heated. From 156°C to 157°C there is a clearly visible change taking place and the change corresponds to Figures 5.20 (top and

bottom). The result seen at 25°C after the sample has cooled down appears to be very different when compared to the sample before heating though.

It is possible that a phase transition was undergone during the cooling of the sample to ambient temperature, relating well to the theory that the sublimate formed undergoes a transition upon cooling as was visible in Figure 3.10 though these results do not give this information and further analyses are required for clarification.

There are various VTXRD results to be presented here and none conform to that of the sublimate as shown in Figure 5.21. There were reasons stated why this could be, but none are very convincing. This result remains a mystery and makes it very difficult to relate these results with certainty to many of the results found throughout this study.

The possibilities thought of as an explanation for this was that the RM either has contaminants present or possibly the RM existed as a mixture of more than one polymorphic form. The idea was that the vacuum sublimation product was the pure RM form without contaminants present or existing as a single polymorphic solid-state form and that the sublimate formed at ambient pressure was another. (The sublimate formed at ambient pressure can possibly be a degradation product that was not detected with official test methods, like UV or TLC).

If the SV (HV) crystals are a fairly pure form, it would make it possible for one to view the single polymorphic solid-state form go through a heating run and witnessing the phase transitions without having contaminants or other polymorphic forms present influencing the results (as was assumed to be the case for the RM being a mixture of more than one polymorphic forms).

5.3.7 Results from various methods

The following results are of the SV (HV) analysed by VTXRD.

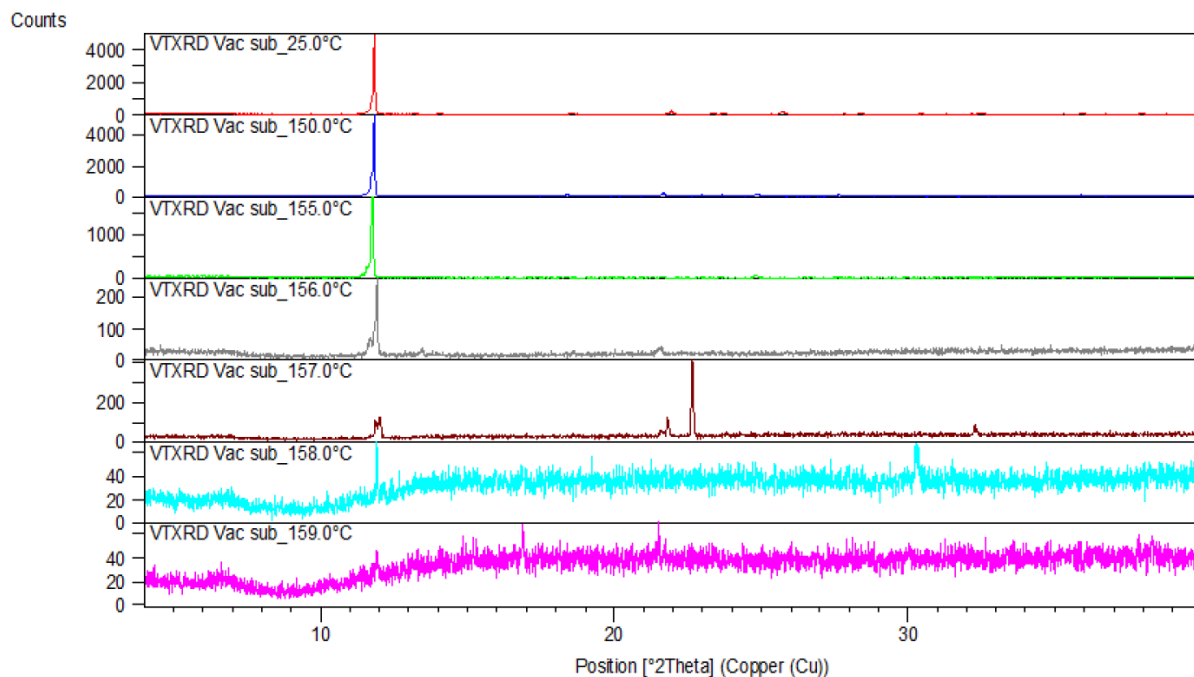


Figure 5.26a VTXRD of the SV crystals when heated from 25°C up to 170°C.

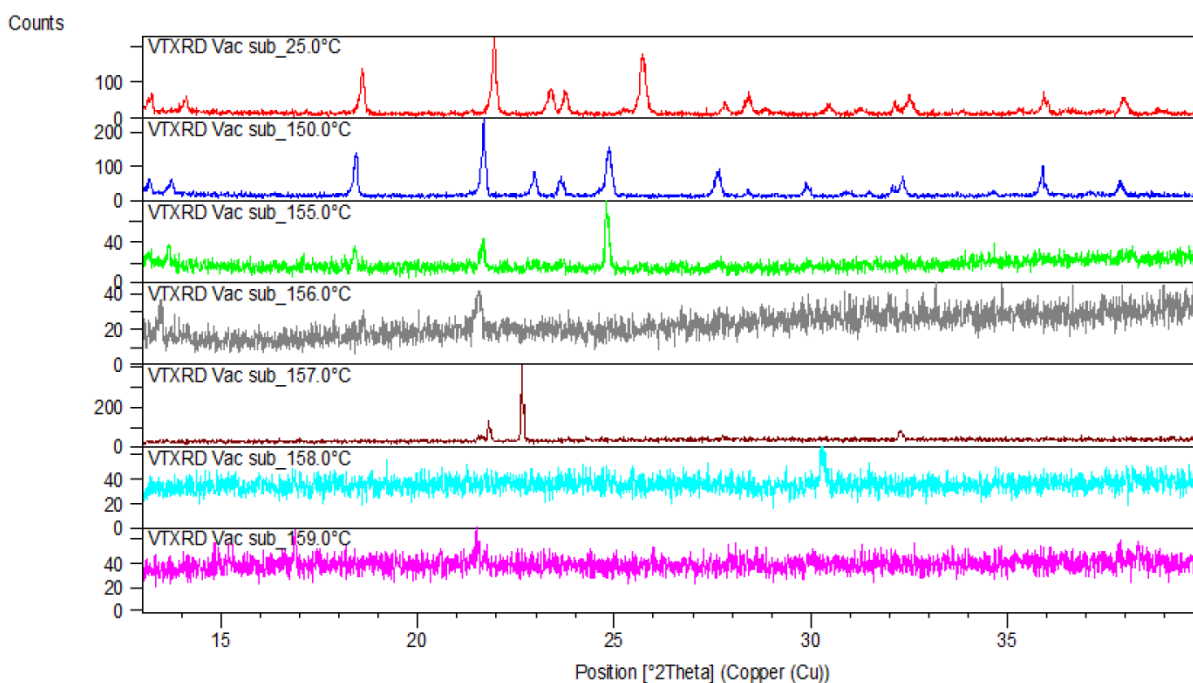


Figure 5.26b VTXRD of the SV crystals displaying the results starting at position [°2Th.] of 13 to make the smaller peaks more readily visible.

In this result it is possible to see a significant change at 157°C. In the DSC result of the SV crystals (Figure 5.4) there was only one large endothermic peak where in the DSC results for the RM there was more than one endothermic peak (Figures 3.3 - 3.4).

The results obtained for the RM and for the SV (HV) are fairly similar, though not precisely matching. This corresponds with the idea that the RM is a mixture of various polymorphic forms and the SV (HV) is a more pure form of a single solid state polymorphic form. This does, however, not confirm it and other methods could be employed to verify whether this is the case.

Mass spectrometry was done on S, SV (HV) and the RM for comparison in order to exclude the possibility of degradation. Also HPLC was implemented to check for degradation. Both methods prove to be negative for any degradation products. To recreate the conditions created in the VTXRD while the analyses were done, a TM with crossed polarisers was employed to represent the time intervals at the various temperatures used. The scans taken by the VTXRD take a few minutes at each temperature. For this reason the sample was held at the lowest temperature at which changes were visible (150°C) for a duration of 30 minutes. This was done to illustrate the effects caused by the temperature in the VTXRD over the time of the scans (Figure 5.28). As control the sample was heated at 10°C/minute up to a temperature where melting was visible (Figure 5.27).

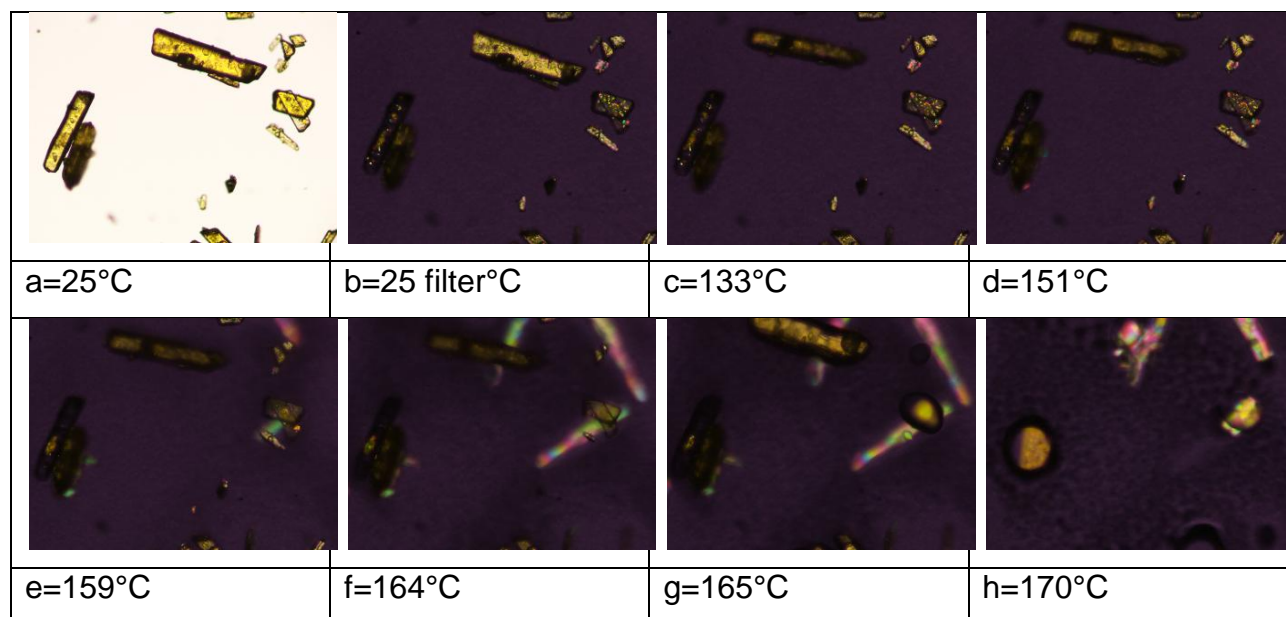


Figure 5.27 TM with crossed polarisers of ethionamide RM at a heating rate of 10°C/minute up to 170°C.

Figure 5.27a shows the sample at ambient conditions before the heating run was started. Figure 5.27b shows the sample before the heating run was started when viewed through the crossed polarisers. In Figure 5.27c the crystal in the top centre position appears to have partially melted, this is strange because this was at a temperature of 133°C, which is much lower than the melting point of this form. From Figures 5.27c - 5.28f the transformation from the original form to the sublimate is visible and the birefringence seen indicates a phase transition taking place. Figure 5.27g shows the original crystals melting, while the newly formed crystals appear not have melted. Figure 5.27h shows both forms melting. From this result (Figure 5.27) one can see that the phase transition is not completed at the stage when the sample starts to melt at 165°C. This represents the DSC traces when heating the sample at 10°C/minute and is meant to serve as the control for this experiment.

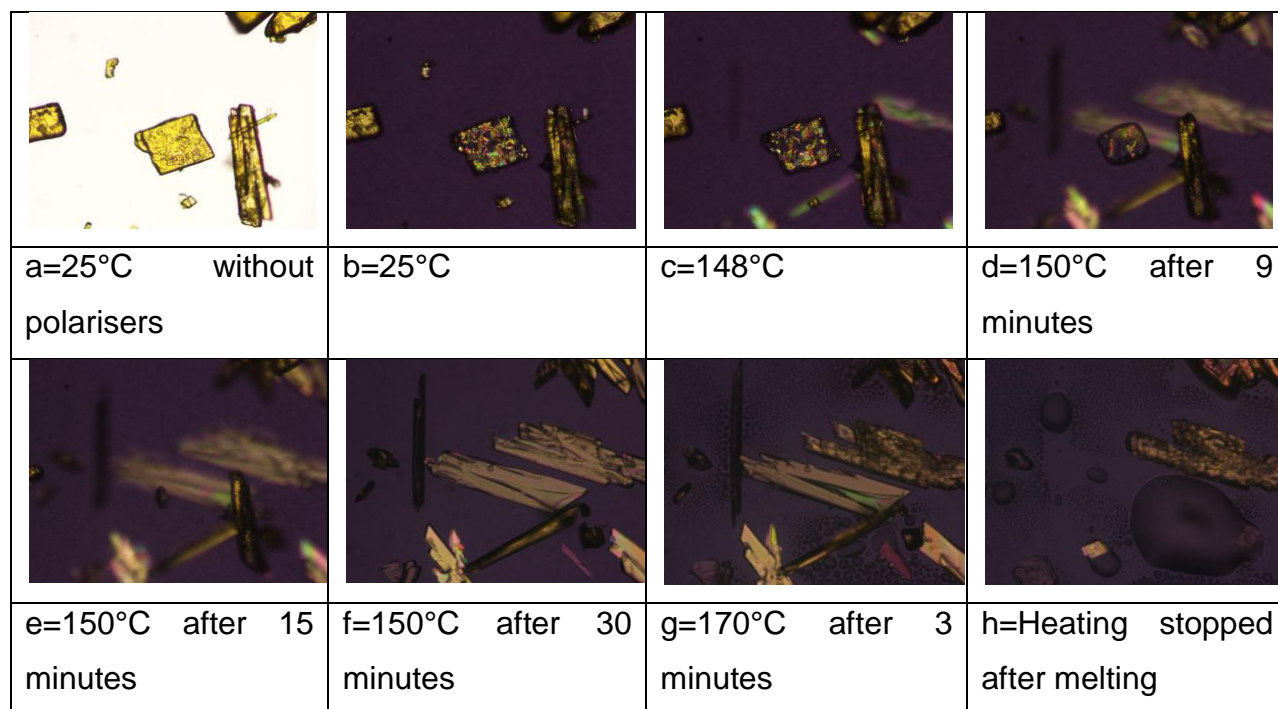


Figure 5.28 TM with crossed polarisers of ethionamide RM at a heating rate of 10°C/minute up to 150°C, held at 150°C for 30 minutes, resumed heating at 10°C/minute up to 170°C and held there until melting occurred.

Figures 5.28a and 5.28b show the sample at ambient conditions before the heating run was started without polarisers and with polarisers respectively. In Figures 5.28c – 5.28f the transition from the original form to the sublimate is visible. It appears as though the transition was completed after being kept at 150°C for 30 minutes. The heating run was resumed and the newly formed crystals appeared not to melt up to a temperature of 170°C. After being kept at 170°C some crystals appeared to have undergone decomposition and other crystals have melted (Figure 5.28h). The occurrence that the crystals formed melt at this high temperature, corresponds with the hypothesis that the S crystals formed without being cooled is a higher melting/more stable polymorph, though this was not seen in the DSC results. From this result (Figure 5.28) it is very clear that the VTXRD and DCS results cannot be compared, because in this instance complete transformation from the one form to the other occurred and in this case the sample melted at a much higher temperature (165°C and 170°C after held at this temperature for 3 minutes respectively).

Comparing the results seen in Figures 5.27 and 5.28 it irrefutably proves that the heating rate will influence the kinetics of transformations taking place with these crystals. The conditions created within the VTRXD were simulated and were shown to be clearly incomparable to the results seen with DSC, as the sample is held at these temperatures for variable and imprecise intervals of time.

Another factor possibly influencing the VTXRD and XRPD results was the milling of the sample in preparation for this method. The SV (HV) samples were milled and DSC traces of the resulting sample are shown in Figures 5.29a and 5.29b.

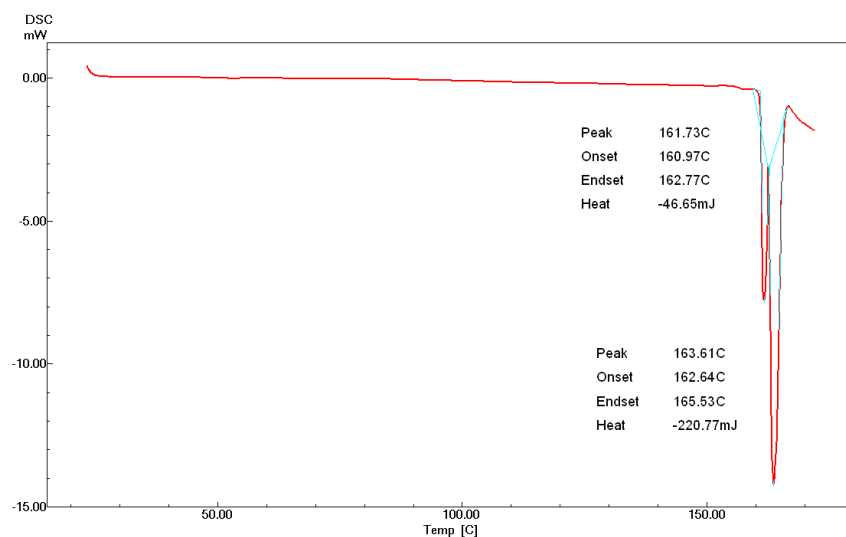


Figure 5.29 (a) DSC trace of SV (HV) after milling at a heating rate of 5°C/minute up to 175°C.

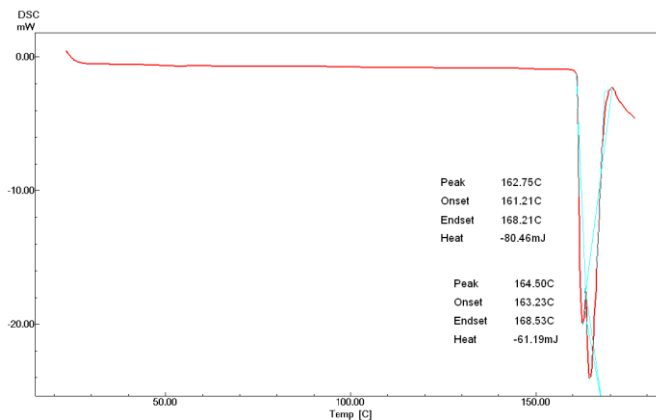


Figure 5.29 (b) DSC trace of SV (HV) after milling at a heating rate of 10°C/minute up to 175°C.

These results (Figures 5.29a and 5.29b) resemble the DSC traces of the RM (Figures 3.3, 3.4a and 3.4b). This could imply that milling may have an effect on the solid-state form of the sample. The preparation methods used for XRPD and FTIR require the samples to be milled and if it is the case that milling has an influence on the solid-state form present, it could explain why the XRPD and FTIR results of the RM and SV (HV) crystals are similar though the DSC differed in all the traces done.

5.3.8 Fourier transform infrared

There do not appear to be any differences. If the assumption is correct that the crystals isolated by the cover glass method partly consists of the SV crystals and that the RM is also a mixture of these two polymorphic forms it would make sense that the results should look similar. The milling required for the preparation of this method of analysis could also have had an influence on the results.

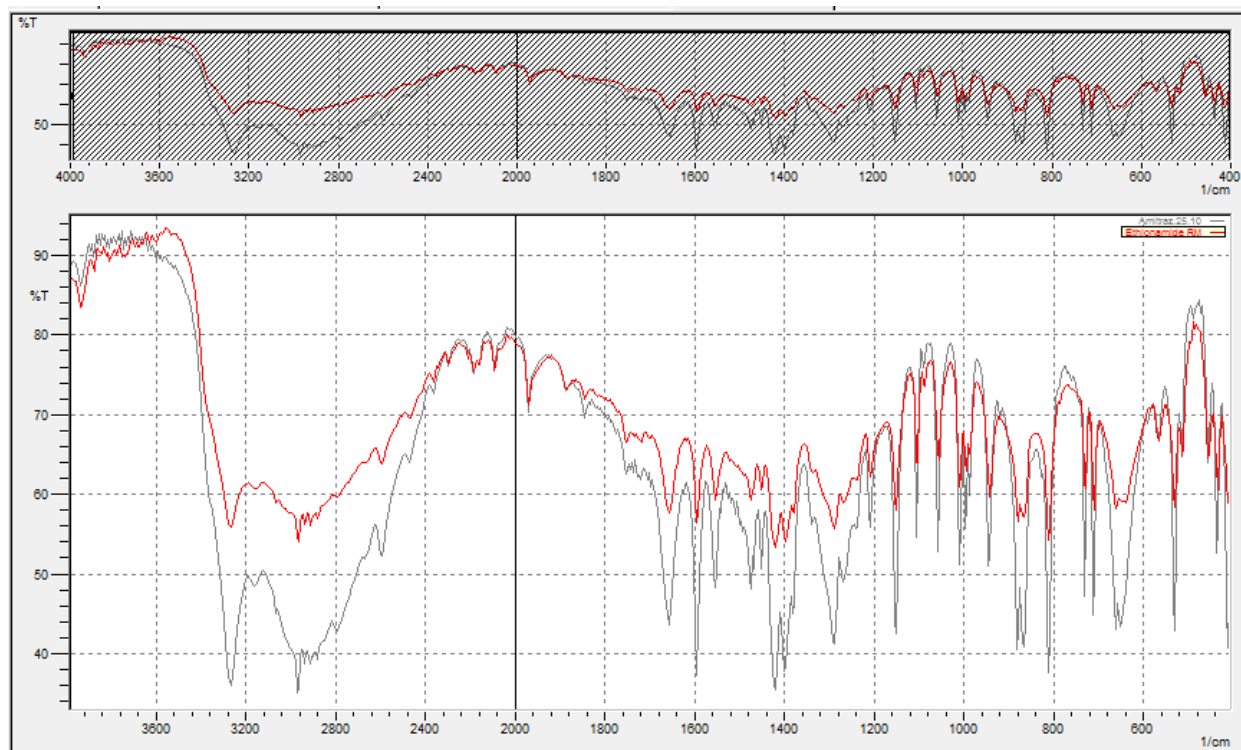


Figure 5.25 FTIR results of S and ethionamide RM.

Summary of results

The results obtained with VTXRD did not help clarify which forms were formed by which methods as the heating rates were not as carefully controlled as with the DSC. It has been visible throughout the results in Chapters 3 and 5 that the heating rate influences the kinetics of the events of the various solid-state forms analysed. The DSC traces obtained from the crystals formed by the methods in this chapter were reviewed and tabulated for ease of comparison (see Tables 5.1 – 5.5). The first table (Table 5.1) displays the results that had a single endothermic peak. The heating rates do not match

as the kinetics involved in the transitions of each form is influenced by the rate of heating.

Table 5.1: Summary of information from DSC traces with a single endothermic event.

Form / method	Heating rate (°C/minute)	Sample mass (mg)	Melting point (°C)	-ΔH of event (mJ)
RM	20	2.8	165.72	438.37
	20	2.2	165.34	415.70
S	10	4.4	159.02	764.76
	10	5.2	162.00	889.43
	10	2.0	160.00	355.24
	5	2.3	160.44	397.80
SV (LV)	10	2.3	160.20	357.43
	10	3.3	162.78	611.24
SV (HV)	5	4.0	161.41	605.00
	2	4.0	159.93	613.40
	5	4.0	161.41	572.43
	10	2.0	162.41	425.42

The variation in sample masses used as well as the differing heating rates make the comparison of the enthalpy values of the events close to being of no value, though in some cases these parameters were matching and these values can be used to compare future DSC results with.

When comparing the melting points, the only significant difference lies in the RM having a higher melting point than the other forms

The following tables (Tables 5.2 – 5.5) display more results, but in this case other events were visible or the heating rate was varied during a single run for comparison of the melting points and enthalpies involved with the various forms. The methods used to separate events or purposefully obtain a specific form are also listed.

Table 5.2: Summary of information from DSC traces of RM.

RM					
No	Heating rate (°C/minute)	Description	Sample mass (mg)	Melting point (°C)	-ΔH of event (mJ)
1	20	Figure C1.1 *	2.8	165.72	438.37
2	20	Figure 3.9b *	2.2	165.34	415.7
3	10	Figure 3.9a+	1.85	163.2	229.19
4	10	Figure 3.10+	2.5	164.64	432.69
5	2	Figure 3.3#	4.89	164.18	503.97
6	5	Figure 3.4a#	3.75	162.5	441.21
7	10	Figure 3.4b#	4.4	164.33	252.84

* There was only one endothermic event visible.

Two overlapping endothermic events were visible.

+ Variable heating rates for separation of events/ isolation of specific form.

It appears as though the crystals formed by sublimation and subsequent recrystallisation, undergo a transition of some sort when cooled, though the specifics of this transition are lacking at this point. The fact that an event is visible can lead to the assumption that perhaps the S crystals are the resultant product of the transition occurring during cooling. The resulting melting point after this transition (Table 5.2 no 4) is 164.64°C at a heating rate of 10°C/minute with a sample mass of 2.5 mg. This relates very well with the melting point of the RM. This can imply that the crystals formed by sublimation and subsequent recrystallisation of the RM undergo a phase transition back

to the RM when cooled corresponding to the conclusion made in section 3.4.1. If this assumption is true, the methods employed to obtain the S crystals should be redundant as the samples are eventually cooled to room temperature. This could be partially true, as there appears to be a mixture of various forms in the crystals obtained by this method, though perhaps the transition is incomplete and only partly reverts back to the RM form.

Table 5.3: Summary of information from DSC traces of S.

S					
No	Heating rate (°C/minute)	Description	Sample mass (mg)	Melting point (°C)	-ΔH of event (mJ)
1	5	Figure 5.11#	3.22	148.02	344
2	2	Figure 5.13#	4.5	145.67	350
3	10	Figure 5.17*	4.4	159.02	764.76
4	1	Figure 5.18a+	3.7	159.39	384.74
5	10	Figure C1.2 *	5.2	162	889.43
6	10	Figure C1.3 *	2	160	355.24
7	5	Figure C1.4 *	2.3	160.44	397.8
8	20	Figure 5.18b♦	2	145.02	67.6

* There was only one endothermic event visible.

More than one endothermic events were visible (at 103°C, 113°C and an overlapping event at 148°C).

+ An extra endothermic event at 125.

♦Variable temperature was used.

When heating S there are phase transitions visible in some, but not all of the cases and the melting points differ greatly. This is thought to be as a result of the sample being a mixture as was supposedly visible in Figure 5.16 and thus sometimes representing more of the one form and sometimes more of the other. This is true even when

analysing the same batch on different occasions. In the samples where the events are seen (Figures 5.11, 5.13 and 5.18b at 103°C and 113°C) the melting temperature is much lower.

The other results that do not have extra events visibly present in their DSC traces resemble that of the SV (LV) results, possibly implying that these forms are similar.

Table 5.4: Summary of information from DSC traces of SV (LV).

SV (LV)					
No	Heating rate (°C/minute)	Description	Sample mass (mg)	Melting point (°C)	-ΔH of event (mJ)
1	10	Figure C1.5*	2.3	160.21	357.43
2	10	Figure C1.6 #	2.3	158.04	510
3	10	Figure 5.3*	3.3	162.78	611.24
4	10	Figure C1.7 #	4	161.26	675.11
5	10	Figure C1.8 #	4.1	157.46	612.28

* There was only one endothermic event visible.

An extra endothermic event is visible at 111°C

The melting points differ greatly for the crystals obtained by this method, though there appears to be a resemblance between some of the traces in the events visible before the melting endotherm. The events are all seen at 111°C and appear to lower the melting point of the sample, implying that a transition might have occurred to a less stable form. The melting points were 157°C and 158°C in the case where these events were visible compared to 160°C and 162.78°C for the traces where the events did not occur. In Table 5.4 no 4 the event was visible and the melting point still corresponds to the higher melting points seen with this form. The enthalpy involved in the event is very small though (0.77mJ) and this event probably did not drastically alter the phase of the sample. The event seen at 111°C has not been clarified at this stage and remains a mystery. When relating the temperature of this event with that of S it appears to be

close, but the traces show clear differences and the patterns are distinct for each form. The SV (LV) events were all seen at 111°C and the events for S were all at 113°C - 114°C. The repetition of this result shows that this is not a coincidence and that there is a distinct pattern present.

Table 5.5: Summary of information from DSC traces of SV (HV).

SV (HV)					
No	Heating rate (°C/minute)	Description	Sample mass (mg)	Melting point (°C)	-ΔH of event (mJ)
1	5	Figure C1.9*	4	161.41	605
2	2	Figure C1.10*	4	159.93	613.4
3	10	Figure C1.11*	2	162.41	425.42

* There was only one endothermic event visible.

The melting points seen in Table 5.5 are lower than the melting point seen for the RM. In each case there was only one large endothermic peak, this does not resemble the results seen with the RM and resembles some cases of the SV (LV) results. The SV (LV) results where there were no other events visible are closer to the results of SV (HV) in melting points as well. This could imply that the lower vacuum creates a form partially resembling that of the SV (HV) and could be that the forms are partially present in both cases with varying ratios. The XRD results of this form and of the RM appear to be very similar (figure 5.9) and would imply that these are the same solid-state forms, though from the observation that milling alters the solid-state form, this correlation is made void. The explanation for the difference in the melting points or the fact that the RM shows two distinct endothermic events taking place, whereas the SV (HV) only shows a single event remains to be resolved. The variation in crystal sizes also does not explain this phenomenon as all the crystals obtained by the methods explained in Chapter 4 displayed two endothermic events in their DSC results and they had a range of varying crystal shapes and sizes. Clarification of this could be possible by making use

of single crystal X-ray diffractometry (SCXRD), though the crystal sizes obtained were not sufficient for using this method.

5.4 Final conclusion

The various results seen in this study pointed to at least two polymorphic forms being obtained by the various methods employed. The possibility of even more could not be ruled out, but isolating these forms was not successfully accomplished.

The fact that the RM undergoes sublimation upon heating proved to make the explanation of the enantiomeric/ monotropic relation of the various forms quite difficult.

It is speculated that the sublimate formed at ambient pressure is formed by an irreversible enantiotropic transition as the enthalpy involved in the transformation shows a jump in enthalpy caused by the transition and that the temperature at which this event takes place has a large range implying that the transition is possibly not of the first order. The RM is thought to be a mixture of polymorphic forms, with one of the forms being by far the largest fraction of the total mass, making analyses by FTIR and XRPD resemble the results of the most abundant form (the form found in the literature). The influence of milling in the preparation of these samples for the methods of analysis also showed a possibility of influencing these results. It appears as though the RM when heated under vacuum sublimates and forms a solid-state form that appears to be a more pure mass of this form. This can be related to the temperature involved in the sublimation and subsequent formation of the sublimate. In this study various pressures were used in the vapour deposition methods and the results showed that the lower the pressure of the environment, the higher the fraction of the SV (HV) polymorphic form obtained will be. At the lowest pressure used the sample was almost exclusively the SV (HV) polymorphic form and under ambient pressure the fraction of SV (HV) was still high, though a lot of the S form was visibly present.

The formation of the sublimate at lower pressures resulted in a higher melting point than when using the same method at a slightly higher pressure by making use of different strength vacuum pumps. Though the instability of the form created through sublimation at ambient pressures made the characterisation difficult. Analysing the effects of

various pressures on the solubilities of the products formed via sublimation and subsequent recrystallisation can also possibly lead to new finds.

Isolating the S form proved to be quite difficult and the yields obtained through the cover glass method were usually a mixture of the forms and this had an influence on the results obtained with the various analytical methods used. The possible phase transitions occurring during cooling might have further complicated this matter. This could be the reason why the S form has not been identified as a separate polymorphic form. It appears as though this form has the highest melting point of the two forms, though undergoes transitions when cooled and heated. The subsequent events seen upon the heating/cooling of the sublimate have not been thoroughly characterised and whether they are phase transitions and what the relation of the possible other forms might be to each other remains unanswered. Figures 5.16 and 5.29 also show forms appearing to be a more stable form (having a higher melting point) than the RM. By reproducing the conditions used in those methods it could be possible to isolate this form and it could then be characterised and checked whether it has a possibility of pharmaceutical application.

The various experiments done throughout this study gave lots of valuable information on the forms obtained, though there remains much to be clarified.

Doing a solubility study of these forms at various temperatures was not possible as the the yields obtained were too low. The information obtained by such an experiment can be of value when used in combination with the calorimetric information obtained throughout this study to determine stabilities of the various forms at different temperatures.

The patterns seen in melting points and events in DSC traces for the various forms mentioned in this chapter show reproducibility beyond chance or coincidence and further characterisation by means of VTXRD by more accurate heating rates could help characterise the forms and differentiate between them with more accuracy.

Making use of vacuum pumps with pressure gauges and variable suction capabilities could also help to clearly differentiate between the various forms obtained.

SCXRD is a possible method that could clearly differentiate between the forms obtained especially for SV (HV) as it is presumed that the preparation method for XRPD influences the solid-state form of the samples and this can be avoided with SCXRD.

The characterisation of the various forms is thus left incompleting, though with the information presented it should be possible to reproduce the forms obtained by the methods explained in this chapter and characterisation should be accomplished with greater ease if the information presented is taken into consideration.