

CHAPTER 4

POLYMORPHISM OF AZITHROMYCIN

4.1 Introduction

Polymorphism is a phenomenon that relates to the existence of two or more crystal forms of a single compound in its solid state (Cui, 2007:9; Yu *et al.*, 1998:118). The pharmaceutical industry is dominated by solid state materials, whether API's or excipients (Cui, 2007:5). As discussed in Section 1.3.1, the solid phase can be characterised as crystalline or non-crystalline, depending on the packing of molecules in the crystal lattice (Cui, 2007:6; Stephenson *et al.*, 2001:67; Yu *et al.*, 1998:118). Active characterisation of the different crystal forms of an API is essential, since the existence of prepared forms cannot be predicted and neither can their physical and chemical properties (Florence & Attwood, 2009:18; Raw *et al.*, 2004:400; Singhal & Curatolo, 2004:336; Wu *et al.*, 2010:4).

Crystalline hydrates account for almost a third of all active pharmaceutical solids (Vippagunta *et al.*, 2001:15). Azithromycin (AZM) has three known solid states, of which azithromycin dihydrate (AZM-DH) is the most stable form, whereas the other two forms are the monohydrate and the anhydrate.

This chapter describes the recrystallisation of AZM-DH from various organic solvents, to determine the unique characteristics of the resulting crystal forms obtained, if any. Several similar, patented, recrystallisation studies, aimed at improving the pharmaceutical properties of AZM-DH are discussed in literature by Centellas *et al.* (2006:4), Khan *et al.* (2002:2), Li and Trask (2006:35), Rengaraju (2002:2) and Suh *et al.* (2007:10). During this study, only a few solvents were hence chosen for performing recrystallisations with. The preparation of an anhydrous form of AZM, according to the conventional dehydration process was attempted, as well as through a method that comprised dissolving of the API in a solvent, followed by desolvation through rotary evaporation. The preparation of a stable, anhydrous form of AZM, that would display better solubility properties, due to its higher free energy, was sought during this study.

4.2 Recrystallisation method

The organic solvents being utilised during recrystallisation included acetone, acetonitrile, 1-butanol, dichloromethane, diethyl ether, 1,4-dioxane, ethyl acetate and methanol. Saturated solutions for recrystallisation of AZM-DH were prepared by dissolving 2 g of the API in each chosen solvent. Since AZM-DH is very soluble in all of the said solvents, the amount of solvent needed to prepare each solution was approximately 10 mL. AZM-DH powder samples were weighed into small glass beakers, the solvents added and heated to just below the boiling point of each individual solvent, while continuously stirring, until reaching the saturation point. Each solution was then allowed to cool to ambient temperature, during which time the solutions each reached a point of supersaturation due to solvent evaporation and started to form crystals upon precipitation. Prior to analysis, the crystals were carefully removed from the solution and cautiously dried, to prevent the induction of desolvation of the solvates (Byrn *et al.*, 1999:16; Hilfiker *et al.*, 2006:289).

The crystals obtained from the recrystallisations were analysed, using the instruments, as described in Section 2.2. These analyses only served as screening tests and hence not all of the methods, as described in Chapter 2, were applied during this recrystallisation study.

4.3 Results

The results obtained from these screening analyses confirmed the reported findings, among which are registered patents, regarding the general tendency of AZM to either form the monohydrate or the dihydrate when recrystallised from solvents (Li & Trask, 2006:2). Suh *et al.* (2007:10) describe a process of preparing an azithromycin clathrate by using acetone, 1,2-propyleneglycol and water. Centellas *et al.* (2006:4) describe different processes through which stable monohydrates can be obtained through each process. These processes relate to the addition of a boronated hydride, where after it is hydrolysed, methylated, and ultimately azithromycin is isolated from the compound. Khan *et al.* (2002:2) describe the preparation of anhydrous azithromycin by using different types of solvents (isopropanol, chloroform and water) and ultimately drying the solutions to obtain the anhydrous forms of azithromycin. Rengaraju (2002:2) describes the process of preparing a non-hygroscopic dihydrate by converting the monohydrate into the stable dihydrate.

4.4 Discussion

Since investigation of the occurrence of polymorphism in APIs is such an important component of product development and in the improvement of their pharmaceutical properties, polymorphic studies on AZM were performed to serve as a means of screening a few recrystallisations from different organic solvents. The outcomes from this study demonstrated, similarly to those described in the patents being referred to (Section 4.3), that AZM tended to form the stable dihydrate, although at times it produced the hygroscopic monohydrate (Centellas *et al.*, 2006:4; Khan *et al.*, 2002:2; Li & Trask, 2006:35; Rengaraju, 2002:2; Suh *et al.*, 2007:10). The abovementioned findings encouraged further attempts to successfully prepare an anhydrous form of AZM during this study.

4.5 Anhydrous azithromycin prepared from azithromycin dihydrate

Different methods were used in an effort to prepare an anhydrous form of AZM, including the application of dry heat to initiate dehydration, by using the DSC. The drying process was monitored and finally isopropanol was chosen as solvent for preparing the anhydrate. The stability of anhydrous AZM required careful consideration, due to its tendency to convert into the stable dihydrate.

4.5.1 Anhydrous azithromycin prepared *via* dry heat

AZM-DH was placed in a conventional oven that was pre-heated at 100°C. The sample was allowed to dry for 60 minutes (at 100°C) in order to determine whether that would be sufficient for dehydration to occur. The sample was removed from the oven and placed in a dried desiccator (ambient temperature) to avoid exposure to atmospheric moisture, which could result in the transformation into its stable dihydrate. Samples for analysis were collected fast and immediately prior to each type of analysis. Exposure time and time in the desiccator was kept to the minimum to reduce risk of phase transformation. The results from the DSC and TGA are illustrated in Figures 4.1 and 4.2, respectively.

As seen in Figure 4.1, the DSC trace (green) of the AZM sample (prepared in the oven at 100°C for 60 minutes) exhibited three endotherms, the first at 48.89°C, the second at 80.28°C and the third at 118.58°C. The first two endotherms represented the dehydration of the water molecules from the crystal structure. The presence of these dehydration

endotherms meant that the prepared AZM sample (prior to analysis) was hydrated to an extent, thus indicating that 60 minutes was inadequate for complete dehydration; and hence not effective for the preparation of the anhydrous AZM *via* dehydration. The endotherm occurring at 118.58°C was indicative of the melting point of the sample. The DSC trace was similar to that of AZM-DH (red in Figure 4.1), although the first endotherm (48.89°C) occurred at a lower temperature than the first dehydration endotherm of AZM-DH (76.35°C). The dried AZM seemed to exhibit two resolved dehydration endotherms that are observed over two distinct temperature intervals. For AZM-DH that was not the case. The endotherms are broader; they overlap and are not distinct. The overall area of the dehydration region seems greater for AZM-DH. This means that the drying process probably caused some but not complete dehydration.

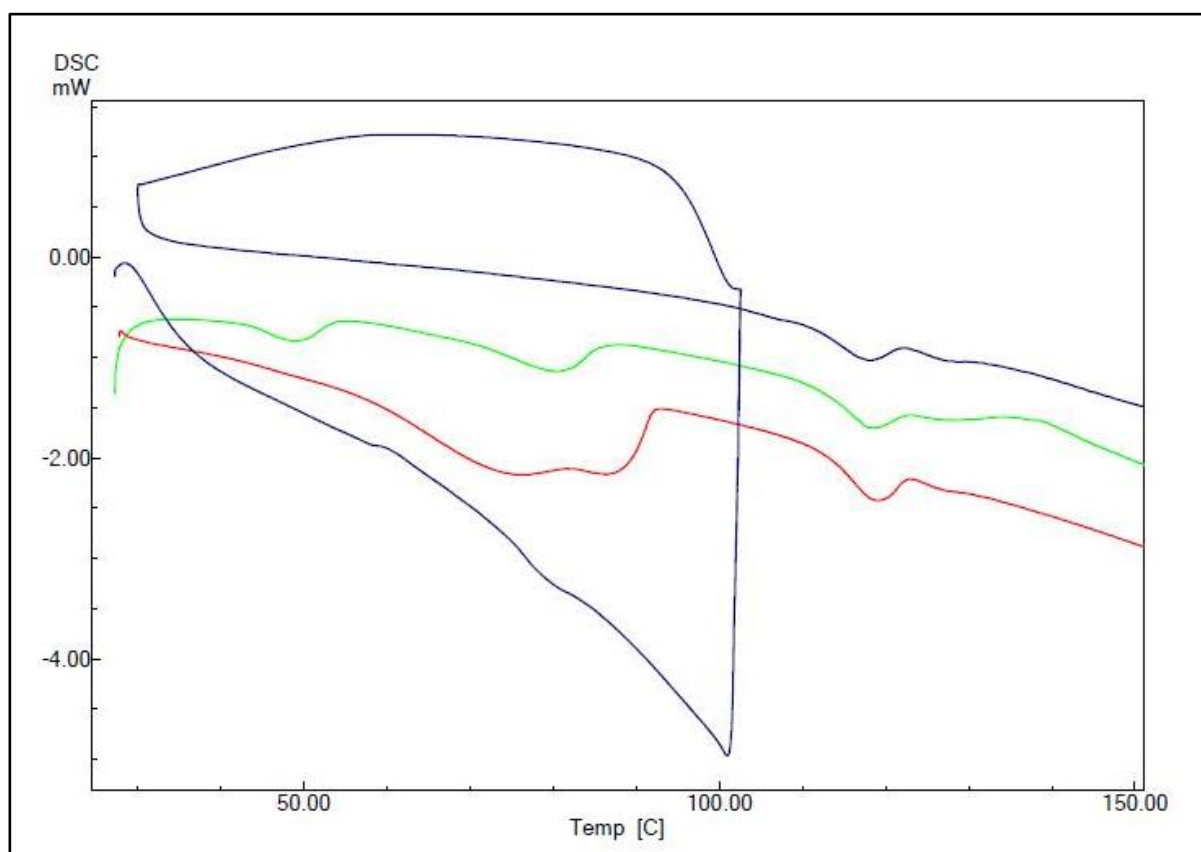


Figure 4.1 Overlay of DSC traces of AZM-DH (red) and AZM (green), dried in an oven for 60 minutes at 100°C, and of anhydrous AZM (magenta), dried in a DSC prior to analysis.

The TGA of the AZM sample showed a 2.79 % weight loss (Figure 4.2). The KFT analysis showed the presence of 3.91 % (n = 2) of water in the samples. The theoretical weight

loss for a dihydrate is 4.59 %, whereas that of a monohydrate is 2.3 % (USP, 2010). The TGA result (2.79%) thus corresponded with the weight loss of a monohydrate, whereas the KFT (3.91%) was indicative of the presence of more than just a monohydrate, i.e. that the monohydrate had possibly transformed into the stable dihydrate, which meant that a mixture of the monohydrate and dihydrate may have been present in the sample. This could have explained why the DSC trace (Figure 4.1) showed an endotherm at 48.89°C, in that one of the water molecules may not have yet been tightly bound and hence dehydrated at this low temperature.

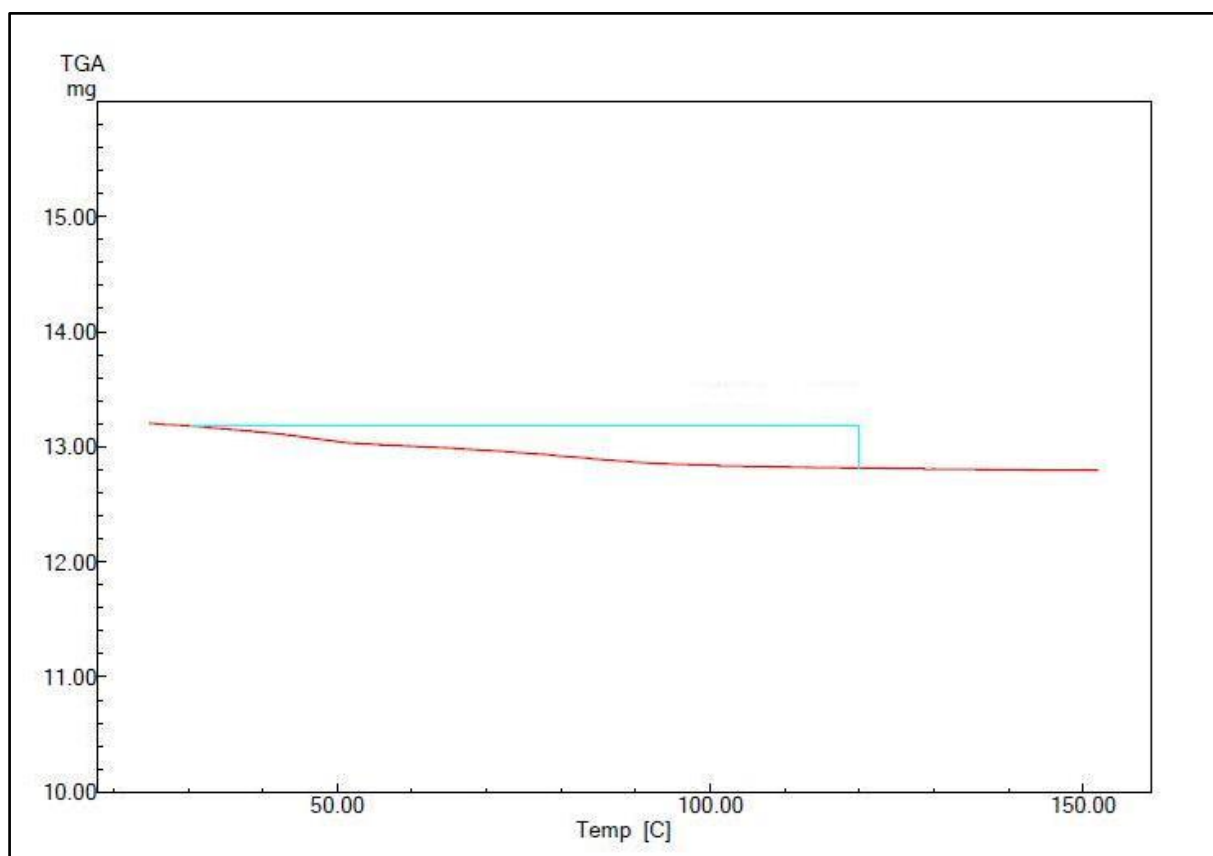


Figure 4.2 TGA thermogram indicating a 2.79 % weight loss for AZM, dried in an oven for 60 minutes at 100°C prior to analysis.

The FTIR spectrum of the dried AZM sample (green) is shown in Figure 4.3. This spectrum illustrates the two typical sharp peaks in the OH-stretching region (3600-3000 cm^{-1}), characteristic of the two water molecules present in the stable form of AZM-DH. This spectrum was identical to that of AZM-DH (red in Figure 4.3).

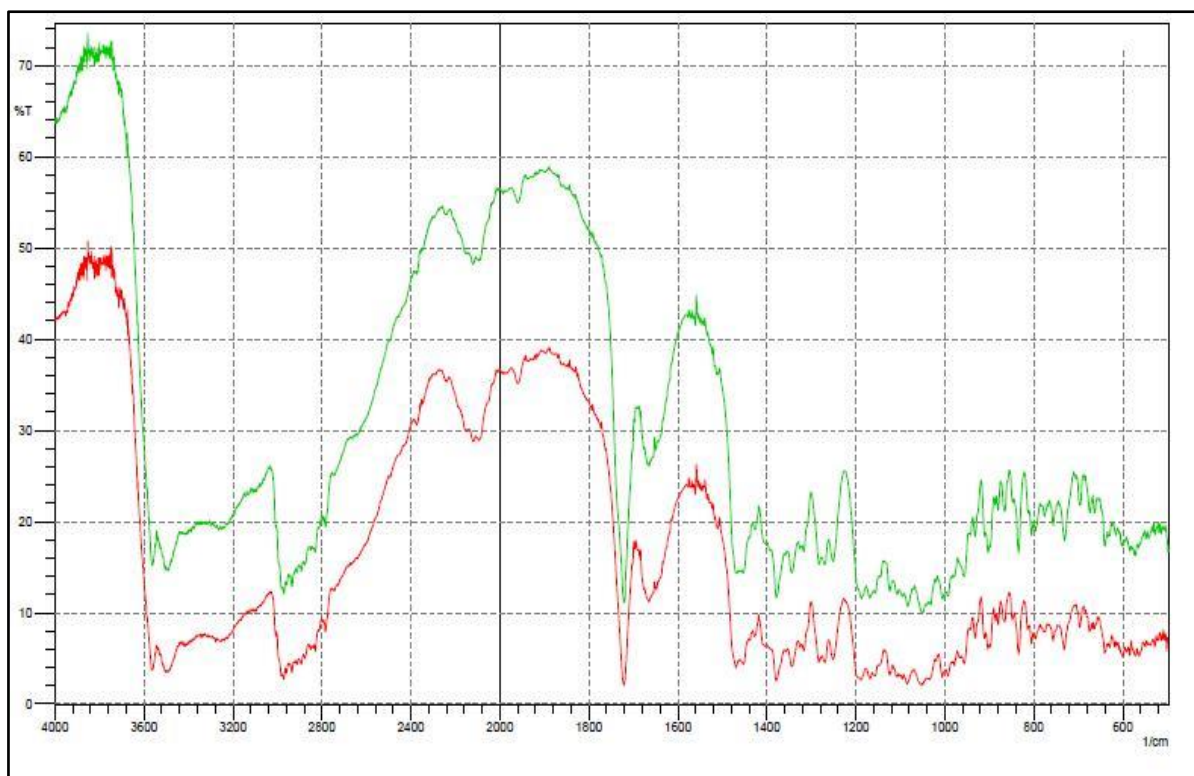


Figure 4.3 FTIR spectra of AZM-DH (red), and AZM (green), dried in an oven for 60 minutes at 100°C prior to analysis.

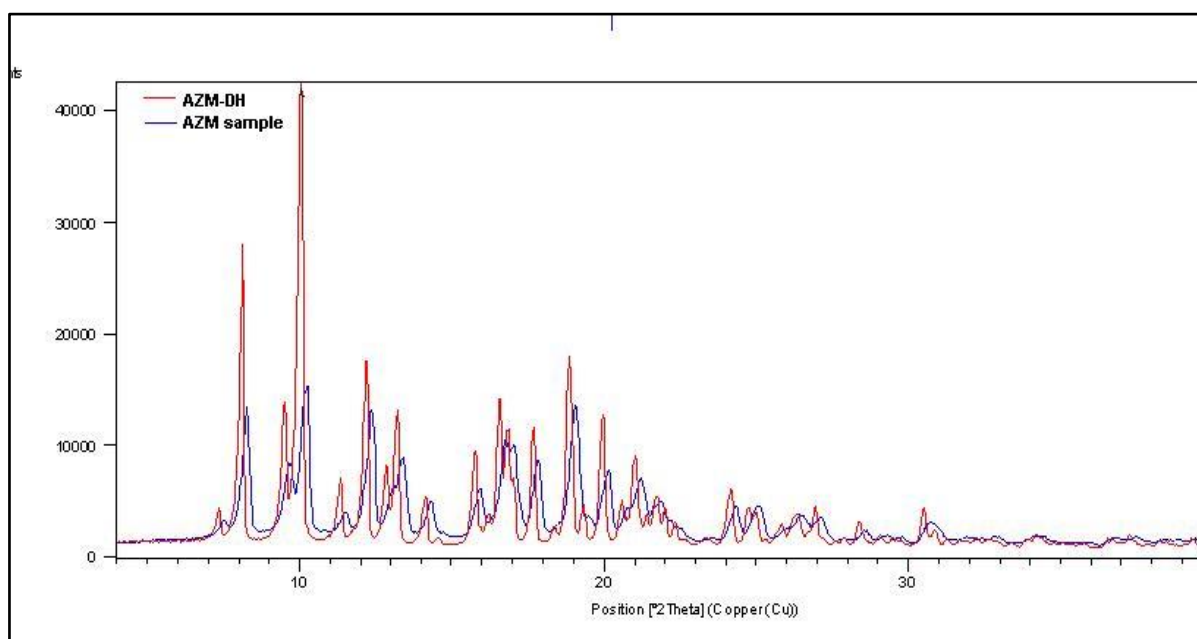


Figure 4.4 XRPD patterns of AZM (blue) and AZM-DH (red), dried in an oven for 60 minutes at 100°C prior to analysis.

The XRPD pattern displayed in Figure 4.4 illustrates the crystallinity of the dried AZM sample (blue). The peaks were similar (but not completely identical, due to the differences

in peak intensities and the shifting of the XRPD pattern to the right, $0.5 - 1^\circ 2\theta$) to the XRPD pattern of AZM-DH (red in Figure 4.4), thus meaning that the AZM sample was still in a hydrated state and that the 60 minutes of drying in the oven was insufficient for complete dehydration to occur. This, together with the DSC and TGA results, may have indicated the presence of both the monohydrate and the dihydrate in the sample. All of the results obtained with TGA, FTIR, DSC and KFT hence confirmed that the 60 minutes of drying in the oven was insufficient for complete dehydration, but furthermore that the resulting AZM sample could be a mixture of the monohydrate and the dihydrate, which meant that the transformation from the monohydrate into the dihydrate was still in progress.

4.5.2 Anhydrous azithromycin dried using the DSC and TGA apparatuses

The successful preparation of an anhydrate was further explored by employing two other methods, using DSC and TGA (Gandhi *et al.*, 2002:176). A small amount of AZM-DH was placed in individual aluminium pans, where after they were heated to 100°C in both apparatuses. The temperatures were each held at 100°C for 60 minutes (similar to the method described in Section 4.5.1), where after they were reduced. The samples were then analysed to determine the nature of the individual samples after exposure to controlled heating.

The DSC trace (magenta in Figure 4.1) showed only a melting endotherm at 118.03°C . The two dehydration endotherms that are usually present on DSC traces of AZM-DH, were absent, thus indicating that exposure to 100°C for 60 minutes in a DSC was sufficient to dehydrate AZM-DH into its anhydrous form, AZM. Contrary to the preparation and analysis of the anhydrous AZM, as described in Section 4.5.1, it was concluded that exposure to heat in the DSC furnace was more effective for dehydrating the AZM-DH sample. The area of exposure in the furnace is much smaller and concentrated than the conventional oven. The better dehydration can be attributed to these aforementioned reasons. A time of 30 minutes at 102°C is reported by Gandhi *et al.* (2002:180) as being adequate for dehydration to occur in the TGA furnace. From the DSC data being generated during this study, it was proven that for an anhydrous form of AZM to be prepared *via* dry heat in an oven, the exposure time of AZM to a temperature of 100°C should be longer than one hour.

The TGA trace of the anhydrous AZM after drying in the TGA is shown in Figure 4.5. The percentage weight loss achieved during this analysis was 0.89 %, which correlated with the USP guidelines for an anhydrous solid (USP, 2010).

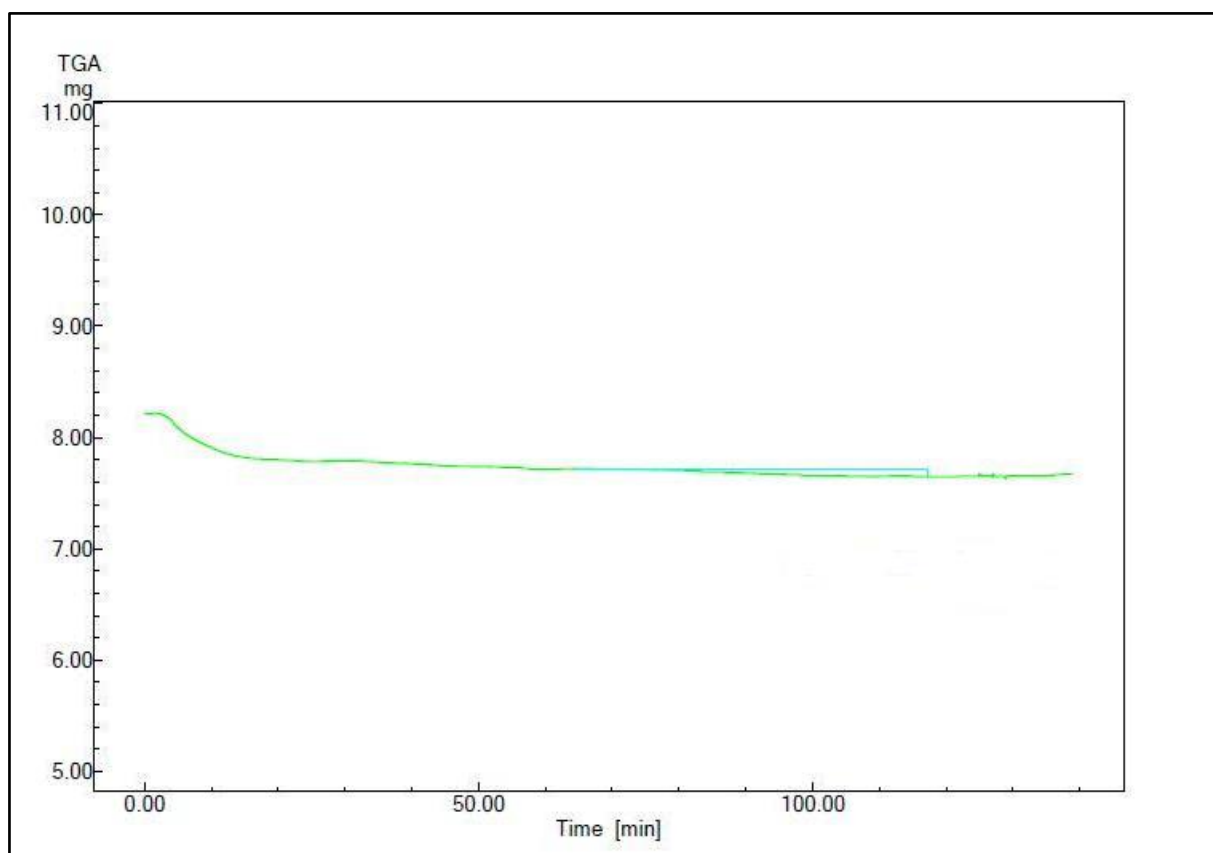


Figure 4.5 TGA thermogram indicating a 0.89 % weight loss for anhydrous AZM, dried in the furnace of a TGA prior to analysis.

4.5.3 Anhydrous azithromycin prepared with isopropanol

The principles of the patented method for preparing an anhydrous form of AZM-DH, as described by Khan *et al.* (2002:8), was followed in another attempt to prepare an anhydrous form of AZM. AZM-DH powder (5 g) was accurately weighed and dissolved in 30 mL isopropanol. The solution was stirred while slowly heating it to just below the boiling point of isopropanol (82°C), where after the remaining solution was dried under vacuum, by using a rotary evaporator. The resulting dried residue was placed in a desiccator to preserve it from external moisture prior to analysis. It was noted that the dried residue immediately became sticky upon breaking of the vacuum.

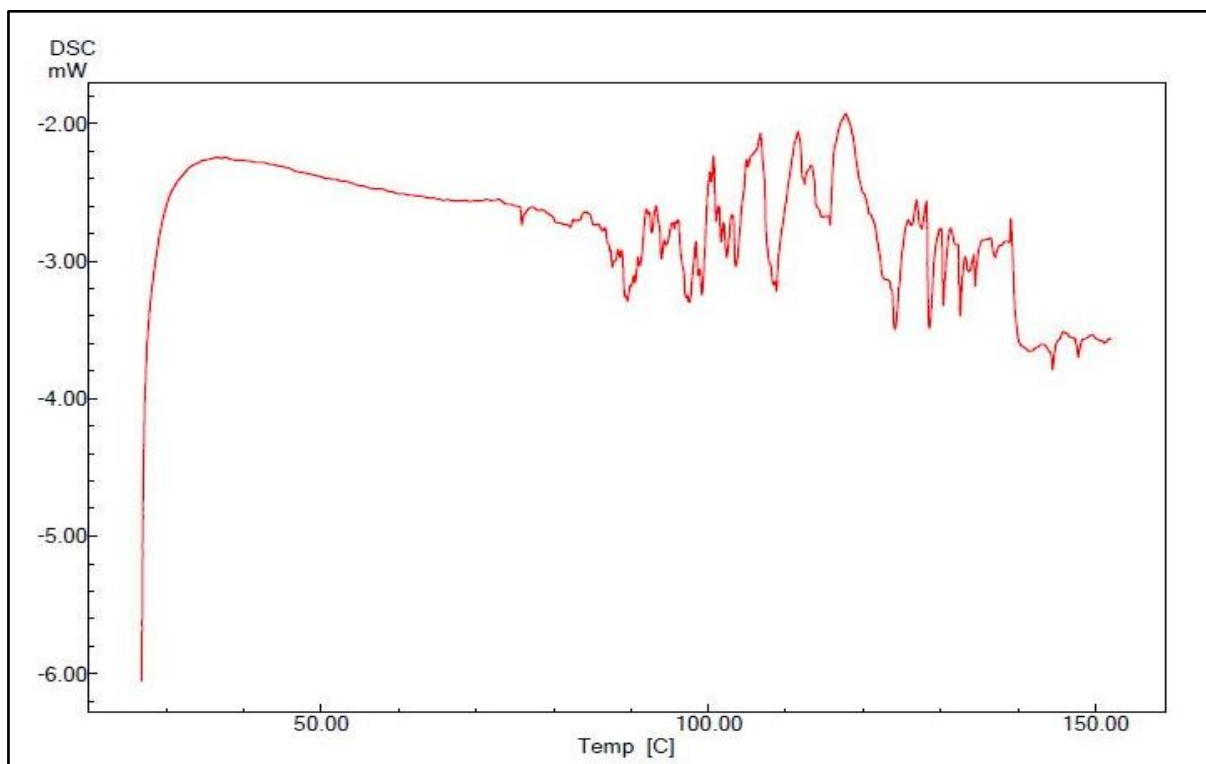


Figure 4.6 DSC trace of anhydrous AZM, prepared with isopropanol.

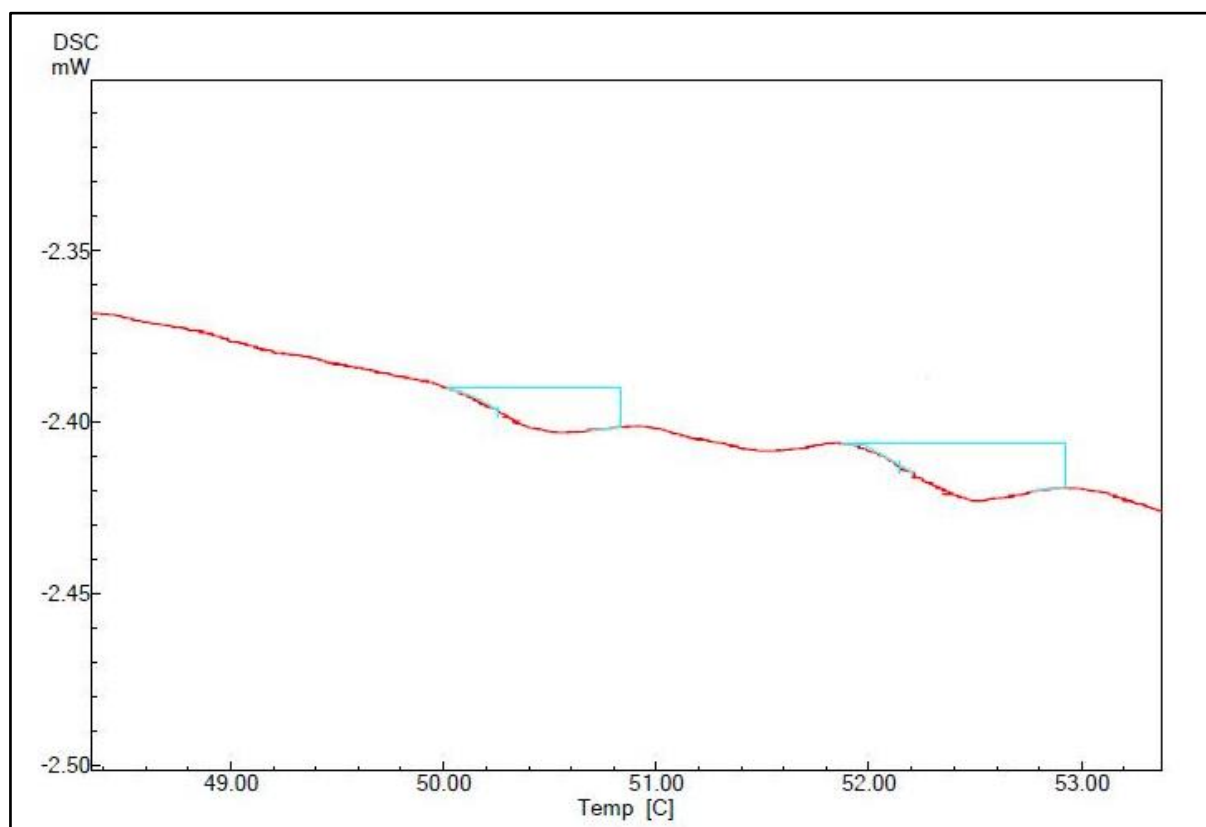


Figure 4.7 Enhanced DSC trace ranging between 49°C and 53°C of anhydrous AZM, prepared with isopropanol.

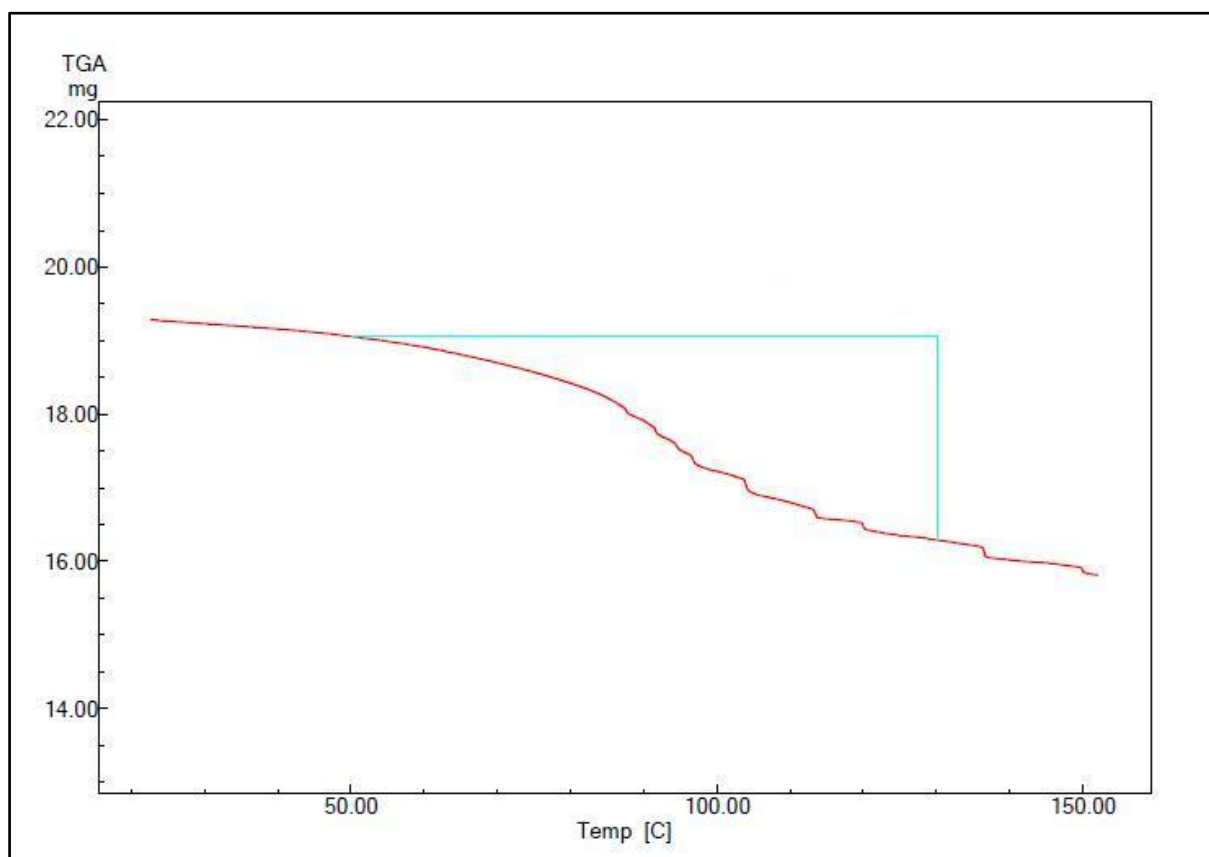


Figure 4.8 TGA thermogram indicating a weight loss of 14.39 % for anhydrous AZM, prepared with isopropanol.

The DSC trace being illustrated in Figure 4.6 shows a mixture of endothermic and exothermic events (starting at approximately 80°C), without showing any typical or definitive melting endotherm. These multiple events may have been as a result of the desolvation of isopropanol, since the boiling point of isopropanol is 82°C. The enhanced DSC trace, as represented by Figure 4.7, shows two endotherms (50.26°C and 52.14°C) indicative of transformation, which, according to the relevant HSM finding at 52°C (Figure 4.11), represents the glass transition. The crystallisation (HSM as illustrated by Figure 4.11) and glass transition imply that the said anhydrous form was amorphous prior to the phase transformation, starting at 62°C.

The weight loss being obtained with the TGA was 14.39 % (Figure 4.8). The theoretical weight loss of a solvate containing isopropanol is 7.11 %. This meant that there were probably 2 isopropanol molecules attached to the AZM structure, hence being amorphous with the inclusion of solvent molecules. To determine the water content of this amorphous AZM, the sample was analysed with KFT, which proved that the dried form of AZM was in fact anhydrous. An average water content of 0.64 % ± 0.04 % was recorded during the

analysis, which, according to KFT analysis and USP (2010) specifications, confirmed that the dried AZM was anhydrous. This meant that the weight loss being obtained with TG analysis was not caused by dehydration, but was as a result of the endothermic transitions (crystallisation and desolvation), occurring with the increase in temperature (Reutzel-Edens, 2011:219).

The FTIR spectrum of the anhydrous AZM that was prepared with isopropanol is shown in Figure 4.9. Compared to the spectrum of AZM-DH (Figure 4.9), it shows no sharp peaks in the OH-stretching region, thus indicating a lack of water molecules in the sample. The broadened peaks furthermore indicate that this AZM sample was amorphous, as peak broadening typically occurs in amorphous solids. The FTIR spectrum of the dried anhydrous AZM resulted in a similar spectrum to that of anhydrous AZM, as described in literature (Khan *et al.*, 2002:2). Isopropanol use was thus suitable for preparing an anhydrous form of AZM.

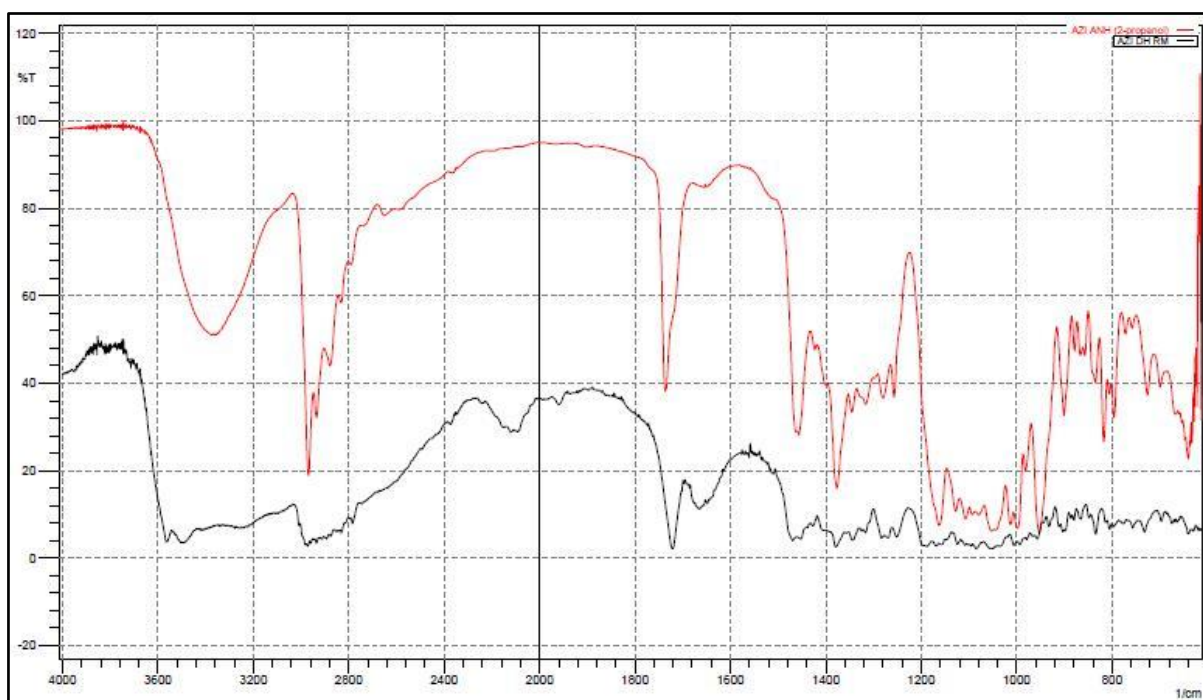


Figure 4.9 FTIR spectra of anhydrous AZM, prepared with isopropanol (red) and of AZM-DH (black).

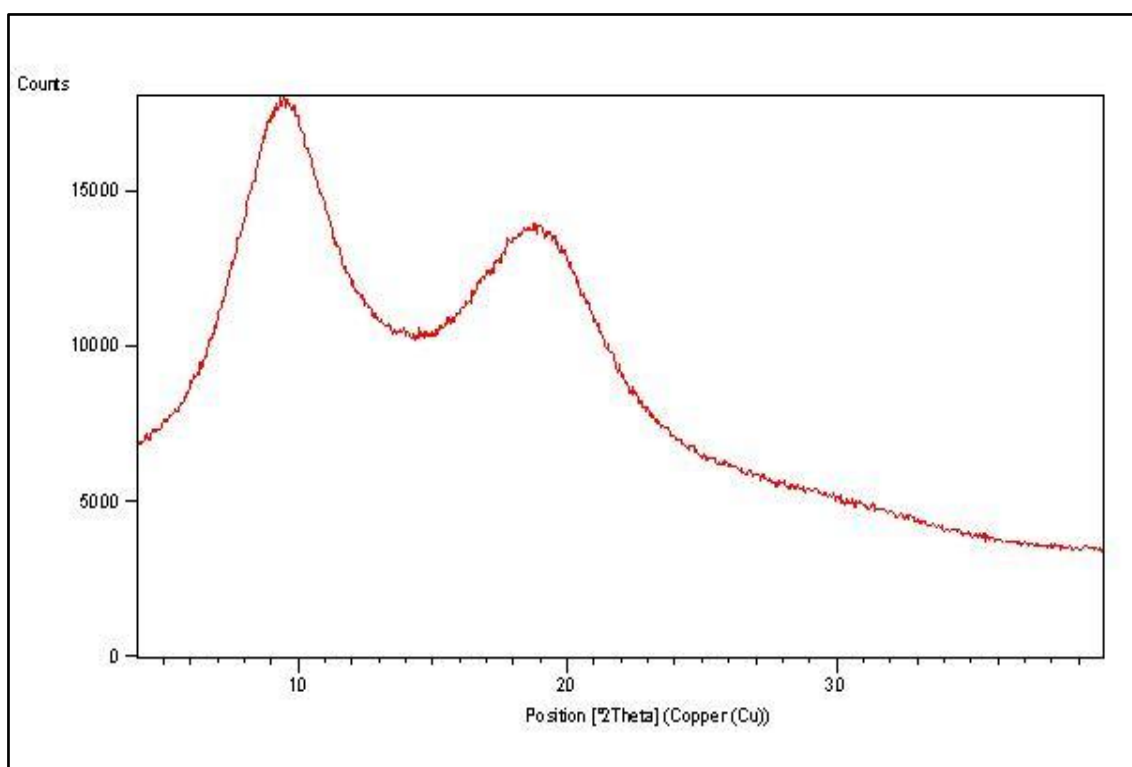


Figure 4.10 XRPD pattern of anhydrous AZM, illustrating the halo pattern typical of amorphous solids.

With regards to the XRPD pattern being shown in Figure 4.10, the halo pattern depicts the amorphous nature of the dried anhydrous AZM, since amorphous solids typically display a halo pattern when analysed with XRPD (Suga, 2011:1).

This amorphous form, however, proved unstable, as it recrystallised with an increase in temperature. The amorphous solid became crystalline at a relatively low temperature (62°C), after exceeding the glass transition temperature of 52°C (Figure 4.11c-d). This was evident from the analysis being performed with HSM (Figure 4.11c-d). The amorphous AZM seemed glass-like at room temperature (Figure 4.11a). The transition from an amorphous AZM solid to a liquid occurred at 52°C (Figure 4.11b), where after crystallisation commenced at 62°C (Figure 4.11c). Crystal growth increased with an increase in temperature (Figure 4.11d). At 101°C, the crystals started to melt very slowly until all of the crystals were melted at 142°C, thus indicating a broad melting range of the crystallised form.

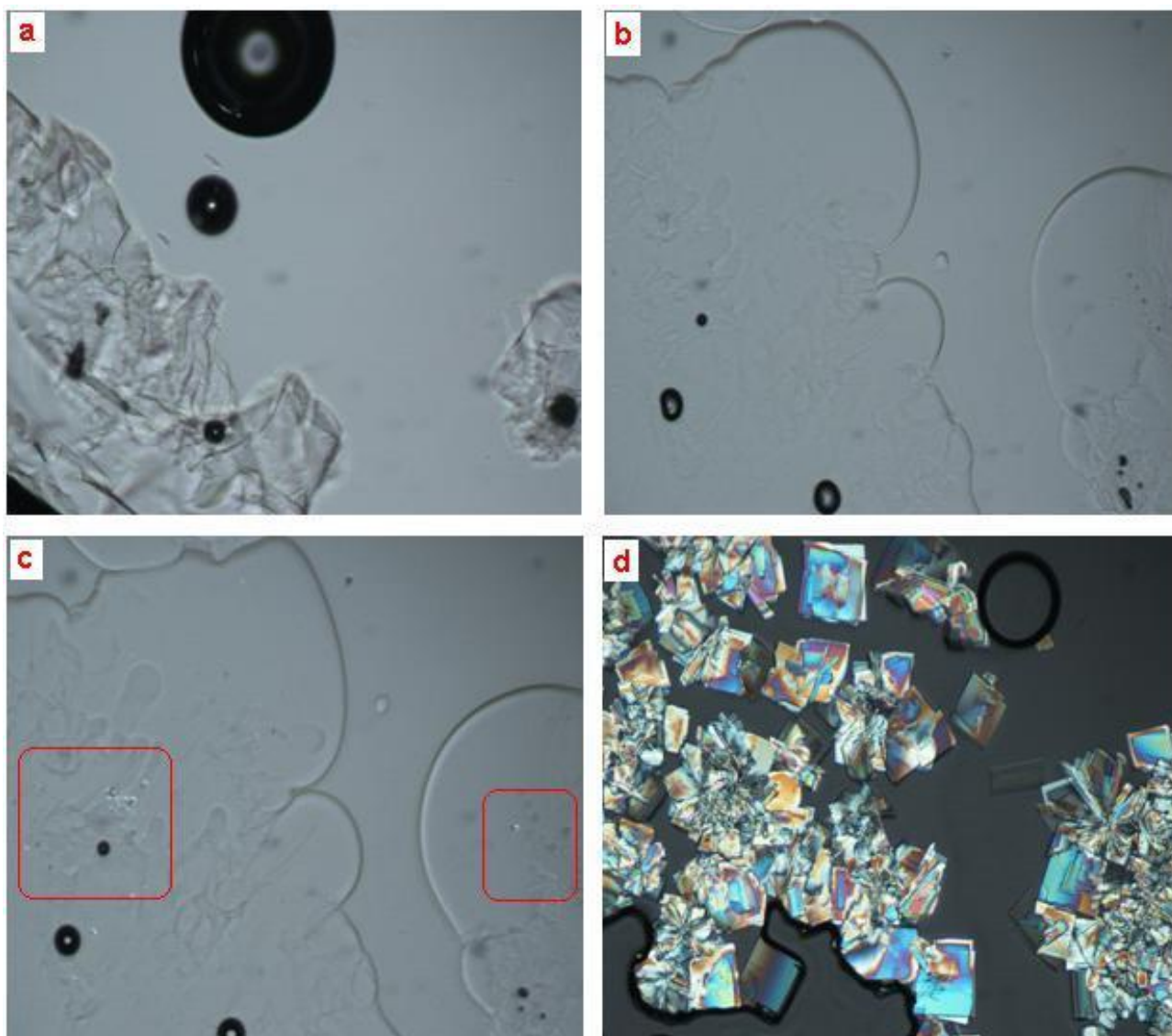


Figure 4.11 HSM images (taken at increasing temperatures) of anhydrous AZM, prepared with isopropanol. (a) The anhydrous AZM morphologically presents as an amorphous glass at room temperature, (b) The transition from solid to liquid occurs at 52°C, (c) Crystals start to form from the liquid at 62°C as indicated by the red squares, (d) Crystals resulting from the continuous growth at increasing temperatures up to 101°C, the point at which the crystals start to melt.

4.6 Discussion

Attempts made during this study to prepare an anhydrous form of AZM were successful. Since the dihydrate represents the stable form of AZM, it was considered that an anhydrous form could be unstable, because of its higher potential free energy, therefore

posing the risk of a tendency to transform into the stable dihydrate (Gandhi *et al.*, 2002:181). The dehydration method of drying AZM-DH in a conventional oven, at 100°C for 60 minutes, seemed unsuccessful, as complete dehydration did not occur. When those same conditions were applied using the DSC and TGA, it was concluded that drying at 100°C for 60 minutes was sufficient to accomplish complete dehydration. Gandhi *et al.* (2002:181) confirmed the instability and hygroscopicity of anhydrous AZM by storing anhydrous AZM (prepared through drying in an oven) at ambient temperature, during which the anhydrous form indeed converted into the dihydrate. The anhydrous AZM being prepared through isopropanol evaporation resulted in an anhydrous, amorphous AZM, which proved to be unstable at increasing temperatures, as it crystallised at 62°C, with the glass transition occurring at 52°C. This form proved unstable which means that it would require storage at a maximum of 2°C, according to the principle for storing amorphous forms ($T_g - 50^\circ\text{C}$) (Kratochvíl, 2011:138). This further emphasised the difficulty in preparing a stable, anhydrous, or even an amorphous form of AZM.

4.7 Conclusion

The studies conducted by Centellas *et al.* (2006:4), Khan *et al.* (2002:2), Li and Trask (2006:35), Rengaraju (2002:2) and Suh *et al.* (2007:10) revealed that recrystallisation of AZM-DH from various organic solvents most often resulted in the stable dihydrate, or an unstable monohydrate. According to reported studies and patents, AZM being recrystallised from alcohols tended to form monohydrated solvates. Polymorphism of AZM had been widely studied, due to its worldwide use as a leading antimicrobial, hence making the need to improve the pharmaceutical properties of AZM a matter of significance to the pharmaceutical industry. Amorphous glasses are usually very unstable (Cui, 2007:11). The anhydrous AZM being prepared with isopropanol evaporation also proved to be amorphous and presented as a glass. It was unstable at increasing temperatures and ultimately crystallised, because of its instability and inherent high levels of free energy. This finding resulted in pursuing the preparation of a glassy form of AZM, as discussed in Chapter 5.

4.8 References

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