

CHAPTER 5

DIDANOSINE

5.1 BACKGROUND

Didanosine (2',3'-Dideoxyinosine, ddI) is a purine analogue and a highly potent nucleoside reverse transcriptase inhibitor (figure 5.1). The first phase I clinical trial data was published by Lambert *et al.* (1990:1333) in a dose-escalation study of didanosine in 17 patients with acquired immunodeficiency syndrome (AIDS) and 20 patients with AIDS-related complex. The rationale behind the trial was to find a suitable alternative to zidovudine, which was at that point the only antiviral drug used in the treatment of the human immunodeficiency virus (HIV). Zidovudine's adverse effects, particularly concerning bone marrow and lymphocyte toxicity in severely immunocompromised patients, made its use (back then as single-drug treatment) extremely undesirable. Zidovudine's efficacy also appeared to wane after about one year of treatment and zidovudine-resistant HIV strains had already been reported by Larder *et al.* (1989:1731).

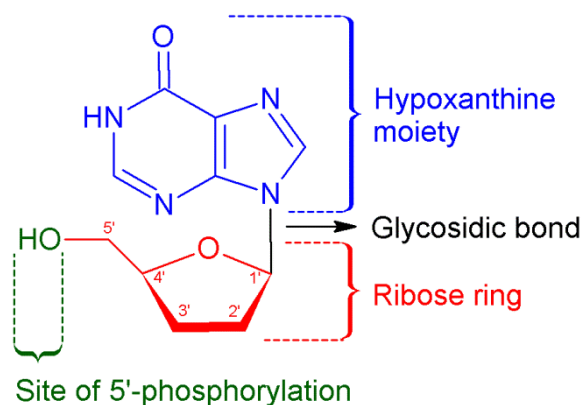


Figure 5.1: The chemical structure of 2',3'-dideoxyinosine (Safrin, 2004:813).

Didanosine was investigated as a possible alternative due to its high *in vitro* activity against HIV in both T cells (Mitsuya & Broder, 1986:1912) and monocytes (Perno *et al.*, 1989:933), and its low *in vitro* toxicity in human bone marrow progenitor cells (Bhalla *et al.*, 1989:569). In a preclinical animal study, El-hawari *et al.* (1989:625) had already proven didanosine to have a favourable toxicity profile, further increasing its appeal. Lambert *et al.* (1990:1336) found didanosine's adverse effects to be dose related, with the most prevalent being

hyperuricemia (in 10 patients), elevated liver enzymes, primarily serum aminotransferases (in 10 patients), peripheral neuropathy and pancreatitis (in 10 patients), all of whom had received ≥ 30 mg didanosine per kilogram per day.

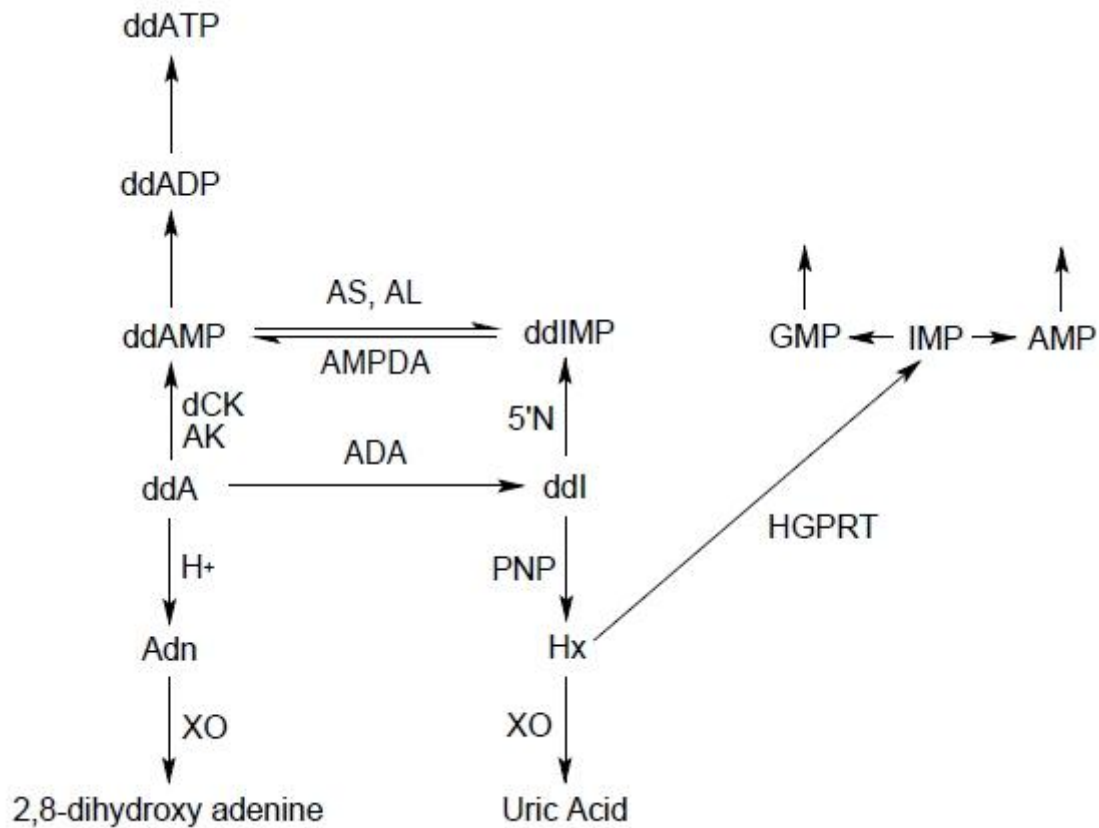


Figure 5.2: The metabolic pathways of ddI and ddA, adapted from Hartman *et al.* (1990:648). ADA, adenosine deaminase; Adn, adenine; AK, adenosine kinase; AMPDA, adenylate kinase; AL, adenylosuccinate lyase; AS, adenylosuccinate synthetase; dCK, deoxycytidine kinase; ddA, 2',3'-dideoxyadenosine; ddADP, 2',3'-dideoxyadenosine-5'-diphosphate; ddAMP, 2',3'-dideoxyadenosine-5'-monophosphate; ddATP, 2',3'-dideoxyadenosine-5'-triphosphate; ddl, didanosine; ddIMP, 2',3'-dideoxyinosine-5'-monophosphate; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; Hx, hypoxanthine; PNP, purine nucleoside phosphorylase; XO, xanthine oxidase.

These adverse effects seemed to disappear and liver function returned to normal with a lowering of the dose. Only the neuropathy and pancreatitis persisted, but at much lower frequency of only 2 patients, when the dose was lowered to ≤ 12 mg per kilogram per day.

The pharmacokinetics of didanosine (figure 5.2) was described in detail by Hartman *et al.* (1990:648). 2',3'-dideoxyadenosine (ddA) was another compound tested in their study, since it had an *in vitro* activity profile similar to that of didanosine and a very favourable therapeutic index. However, *in vivo* ddA was metabolised to didanosine so rapidly and quantitatively that it was undetectable in the plasma, even during continuous intravenous administration. Of particular interest here is the active metabolite, 2',3'-dideoxyadenosine-5'-triphosphate (ddATP), which acts at the level of viral reverse transcriptase as both a competitive inhibitor of physiologic nucleotides and a deoxyribonucleic acid chain terminator. From figure 5.1 it is clear that the activity of didanosine is totally dependent on the availability of the hydroxyl group on carbon 5'. Should the glycosidic bond between the ribose ring and the hypoxanthine moiety be broken, didanosine would lose all its antiviral activity, this will be discussed shortly.

Kahn *et al.* (1992:582) investigated the advantages of administering didanosine to patients who have become intolerant to zidovudine or in whom zidovudine treatment had failed. The results showed a significant decrease in AIDS-defining events and deaths among the patients who took didanosine after zidovudine efficacy began to diminish, when compared to patients who stayed on zidovudine. This was one of the first studies to show the benefit of changing from one antiretroviral (ARV) drug regime to the next in order to combat drug resistance and intolerance. Although didanosine was approved, in the fall of 1991, for treatment of patients in whom zidovudine treatment had failed, Kahn *et al.* (1992:586) argued that the administration of didanosine should not be limited to these patients.

Despite the early promise shown by didanosine, the rapid hydrolysis of its glycosidic bond in acidic pH was (and still is) a major cause for concern. With the nature of AIDS and the frequency of dosing, the most viable route of administration is the oral route. However, the rapid hydrolysis of didanosine raised serious questions about the efficiency and reproducibility of absorption after oral administration. Hartman *et al.* (1991:278) investigated the effects of different oral formulations on didanosine's bioavailability. When didanosine was given to fasting patients as an oral solution with antacid, the bioavailability was $41\% \pm 7\%$ (mean $\pm$ SEM), while buffered tablets gave a bioavailability of $25\% \pm 5\%$. However, when these tablets were given with supplemental antacid, the bioavailability increased to $36\% \pm 6\%$. They also tested two enteric-coated preparations which gave bioavailabilities of $36\% \pm 5\%$ and $26\% \pm 5\%$. Although the oral solution with antacid exhibited the highest bioavailability, it was also associated with serious gastrointestinal intolerance, making it less appealing considering patient compliance. A reconstituted mixture of didanosine, sucrose and buffer was also given to fasting patients and yielded a bioavailability of $29\% \pm 6\%$, but

when administered with food the bioavailability dropped to $17\% \pm 4\%$. Addition of ranitidine and ganciclovir to the formulation had no effect on the pharmacokinetics of didanosine.

The current formulations include buffered tablets (25 mg, 50 mg, 100 mg and 150 mg), enteric-coated capsules (250 mg and 400 mg) and a paediatric powder, all under the trade name, Videx® by BM Squibb. Aspen Pharmacare and Ranbaxy Be-Tabs also have generic formulations of the buffered tablets on the market. The current dose are 200 mg 12 hourly or 400 mg daily for patients weighing 60 kg or more, and 125 mg 12 hourly or 250 mg daily for patients weighing less than 60 kg. The tablets/capsules should be taken half an hour before, or 2 hours after a meal to minimise didanosine's deactivation (Rossiter, 2010:333). However, Safrin (2004:333) reports that, even if taken 2 hours after a meal, the modern formulations still only achieve a maximum bioavailability of 45 %, due to acid hydrolysis. Clearly this is a very difficult issue to address, with the best bioavailability achieved by a method that causes severe gastrointestinal intolerance and is extremely difficult to administer to babies.

A decade after the first clinical trial data was published, the first polymorphic studies on didanosine was done. The reason for this delay stems from the physical nature of didanosine crystals. Obtaining a crystal of adequate size for SXRD analysis is an extremely tedious task with didanosine, and will be discussed in the experimental section of this chapter. Bettini *et al.* (2010:1857) attempted to recrystallise didanosine from dimethylsulfoxide (DMSO), while using supercritical CO₂ as an antisolvent. The supercritical antisolvent process (SAS process) yielded didanosine needles with a particle size distribution of between 1.01 and 30.05 µm. Although these crystals were too small for SXRD analysis, modern solid-state nuclear magnetic resonance (SS NMR) spectroscopy techniques were used to investigate the different polymorphs obtained by the process. PXRD data identified one didanosine polymorph with a different diffraction pattern than commercial didanosine. This new polymorph was obtained from SAS-recrystallisation at a pressure of 200 bar. After various two-dimensional SS NMR analyses, the results for commercial didanosine suggested that there were two very similar molecules in an asymmetric unit, with the main difference between them being hydrogen bond strength. The same analysis for the polymorph obtained by SAS-recrystallisation at 200 bar gave hydrogen bond patterns which correlated with those of the commercial product, all the other hydrogen proximities were also identical. The fact that the main difference between the polymorphs lie in the strengths of the hydrogen bonds, suggests that the two differ in interaction lengths and intermolecular distances. This indicated that the two polymorphs possess similar structural conformations, but different molecular packing. In other words, since the interaction networks are similar, the two polymorphs differ mainly in unit cell volume and density. The polymorph obtained by SAS-recrystallisation proved to be thermodynamically metastable

and was more susceptible to external stresses than commercial didanosine. Upon being submitted to prolonged milling, commercial didanosine powder underwent a reduction in the degree of crystallinity after 2 hours, with no further decrease after that, while the SAS-recrystallised polymorph's degree of crystallinity continued to decrease, even after 4 hours of milling when there were almost no visible peaks left on the PXRD diffractogram (Bettini *et al.*, 2010:1868).

Later that same year, Martins *et al.* (2010:1885) published the results from their structural determination of recrystallised didanosine. Through solvothermal conditions, they had managed to prepare a didanosine crystal with dimensions of 0.15 x 0.10 x 0.02 mm³, falling just within the detection limits of the latest SXRD instruments, from isopropanol. They also found two conformers of didanosine in the unit cell, differing slightly from each other by rotation on the glycosidic bond axis. These crystals had the same melting point as commercial didanosine, as well as a similar PXRD diffraction pattern and FT-Raman spectra, indicating that the crystals were structurally similar. Therefore their article is not a polymorphism study, but rather a structural determination of didanosine crystals found to isostructural to commercial didanosine. The pressure generated from the solvothermal process introduced a certain degree of hydration into their crystals, which Martins *et al.* (2010:1889) claims is necessary for the formation of single crystal quality crystals. However, the slow onset of crystallisation brought on in the autoclave, and the subsequent effect that the BFDH Law (from section 2.5) has on the relative surface area of crystal faces was not taken into account. Furthermore, the exact amount of water loss was not reported. It was only mentioned that the water loss was continuous and its slow rate made TGA unreliable. However, no other methods of TGA were investigated, such as isothermal analysis or different heating rates. The reported heat of dehydration on a DSC trace was 0.39 J/g, at 98°C (Martins *et al.*, 2010:1889), and although this is very small, it should still have been possible to quantify with KF analysis. The alternative possibilities are however not investigated, such as the fact that continuous weight loss from the start of a TGA run is not associated with water entrapped in channels inside crystal, but rather with water loss from the surface of a material. The incorporation of water into the didanosine crystals are most probably due to the pressure under which the crystals were formed.

5.2 MATERIALS

Didanosine raw material (batch number B3-081102M) was purchased from Sri Sai Nikitha Pharma Pvt. Ltd., Hyderabad, India, as a fine white powder.

5.3 POLYMORPHISM STUDY

5.3.1 INITIAL POLYMORPH SCREENING

The first step in the study was the identification of solvents from which didanosine could be recrystallised and the subsequent investigation of the crystals thereby obtained for differences in polymorphism. For this initial screening, a fixed amount of didanosine (in this case 2 g), was placed in a beaker and solvent was added while stirring at 700 rpm. The mixture was heated to a temperature above the boiling point of the specific solvent. Upon reaching the boiling point, more solvent was added until all the didanosine was dissolved. As this point represents the point of supersaturation, a small amount of solvent was added and the mixture was removed from the heating mantle and allowed to cool under ambient temperature. The results of this initial screening are presented in table 5.1. Cases where more than 2000 ml of the specific solvent could not dissolve 2 g of didanosine are indicated as insoluble. These solvents were deemed impractical for recrystallising sufficient quantities of didanosine crystals needed for analytical tests. At first glance the solubility of didanosine seems to be dependent on the polarity of the solvent used, as well as the aqueous miscibility of the solvent, however there are several inconsistencies with this observation. Acetone, 1,4-dioxane and tetrahydrofuran (THF) are water miscible, yet didanosine was insoluble in all three. Furthermore, acetonitrile and dimethylformamide (DMF) share a similar dielectric profile (with a dielectric constant of 37.5 and 38 respectively), yet the solubility of didanosine is quite different in each of them. 1-Butanol is more polar than 2-butanol, however, didanosine dissolved more readily in 2-butanol and stayed in solution. Clearly there is more to didanosine's solubility in organic solvents than meets the eye. Didanosine is highly soluble in water and DMSO, so much so that even after 2 years no crystals were formed from supersaturated mixtures with these solvents. Based on the solubility of didanosine in water and the different alcohols, one could argue that the hydroxyl group plays a role in its solubility, however, this does not account for the high solubility in solvents without hydroxyl groups like DMSO, DMF and acetonitrile. Rather, didanosine's solubility might be the result of a combination of different chemical and physical properties of the solvent, for example electron negativity, polarity, surface tension, steric effects etc. Although didanosine's solubility in organic solvents is a very interesting topic, it is not the main theme of this study, therefore it was left at that and the crystals that were formed were analysed for polymorphic differences.

Table 5.1: The initial screening of didanosine for crystal forming solvents

Solvent	Recrystallisation information	
	ml solvent per 2 g didanosine	Crystals formed?
Acetone	Insoluble	No
Acetonitrile	1400	Yes
1-Butanol	1230	Yes
2-Butanol	600	No
Chloroform	Insoluble	No
Dichloromethane	Insoluble	No
Diethyl ether	Insoluble	No
Dimethylformamide	65	No
Dimethylsulfoxide	45	No
1,4-Dioxane	Insoluble	No
Ethanol	396	Yes
Ethyl Acetate	Insoluble	No
Methanol	275	Yes
1-Pentanol	Insoluble	No
2-Pentanol	Insoluble	No
Petroleum ether	Insoluble	No
1-Propanol	510	Yes
2-Propanol	915	Yes
Tetrahydrofuran	Insoluble	No
Toluene	Insoluble	No
Water	30	No

All of didanosine's recrystallisation products presented as extremely fine whiskers, with no visible morphologic difference between of them. Despite the lack of macroscopic differences in morphology, all of the samples obtained from recrystallisation were tested for polymorphic differences.

5.3.2 THERMAL ANALYSIS

To investigate the thermal behaviour of didanosine raw material and its recrystallisation products, DSC, hot stage microscopy and TGA were employed. All the DSC samples were prepared with pierced lids, to facilitate solvent evolution and minimise any increase in

pressure due to solvent evolution. All the DSC and TGA thermograms were obtained from heating rates of 10 K/min. All the hot stage micrographs presented in this study were taken at a magnification of 100 x and all the scanning electron microscope (SEM) micrographs at 5000 x.

5.3.2.1 DIDANOSINE RAW MATERIAL

Didanosine raw material was found to be an extremely fine white powder and, although it appeared to be dry, it was also analysed in pierced lid DSC crucibles for the sake of comparability with the recrystallisation products.

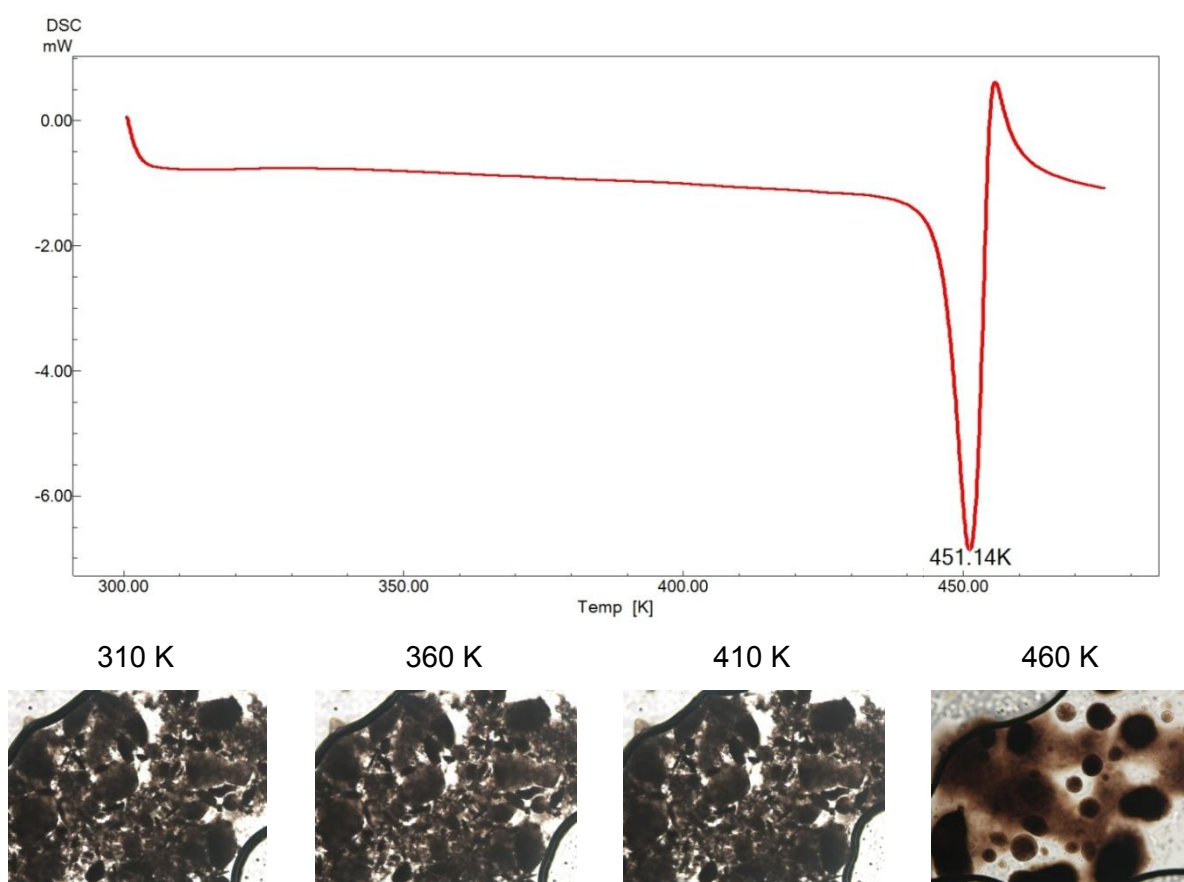


Figure 5.3: DSC thermogram and hot stage micrographs of didanosine raw material.

The DSC thermogram of the raw material (figure 5.3) shows no endothermic peaks prior to the melting endotherm, indicating that the material is dry and does not undergo any phase transitions. The melting endotherm rapidly changes to an exothermic peak, possibly due to oxidation (or some other method of decomposition) of the sample during melting. The hot stage micrographs also show no evolution of volatiles prior to or during melting, as well as a

clear change in colour and composition immediately after melting. The material changes to a charred-like substance during melting, and since charring is an oxidation process, it is detected as an exothermic event on a DSC. The melt immediately recrystallises into the decomposition product. This was investigated further by means of scanning electron microscopy (SEM) on a heating stage (figure 5.4).

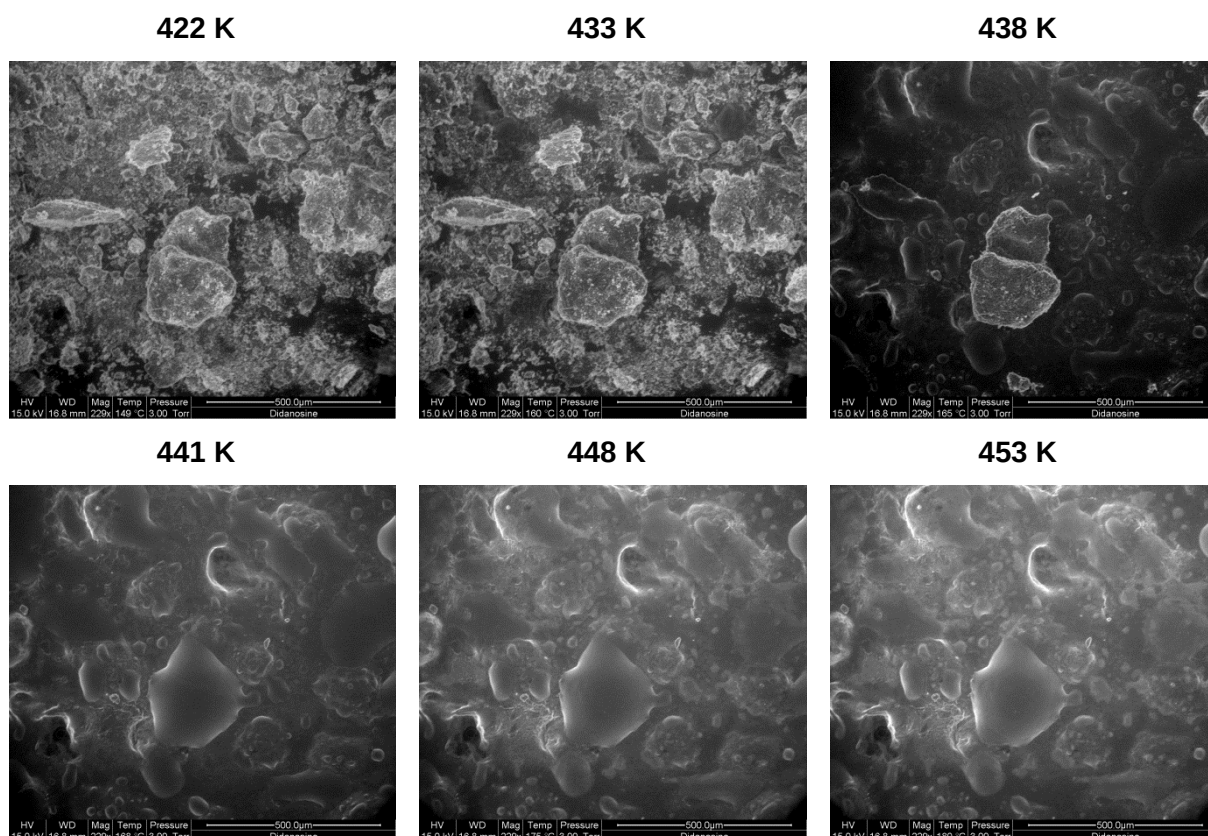


Figure 5.4: Hot stage SEM micrographs of didanosine raw material melting.

The hot stage SEM micrographs of didanosine raw material show the same trends as observed from the DSC thermogram and light microscopy. After the powder melts, the droplets recrystallise, as can be seen from the formation of stria on the large droplet and the increased resolution of the borders on the smaller droplets. It is worth noting that the intensity of the secondary electrons coming off the sample increases as the temperature is increased past the melting point. This phenomenon is usually associated with the emission of volatiles from the sample or decomposition. If the melt had begun to evaporate, the same increase in electron emission would be observed, however, the hot stage micrographs, SEM micrographs and DSC thermogram show the sample to recrystallise after melting and it is, therefore, a solid. Since no evolution was observed on the hot stage micrographs and

oxidation is redox reaction associated with electron loss, the increase in secondary electron emission from the sample after melting supports the previous findings which point to oxidation.

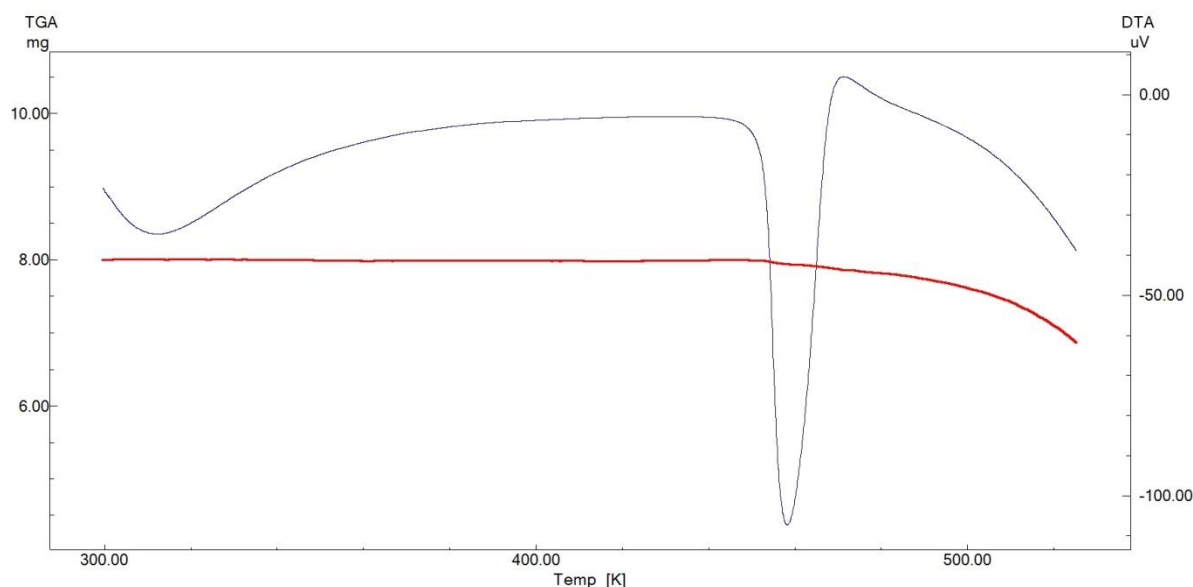


Figure 5.5: TGA of didanosine raw material.

To further investigate what happens after the recrystallisation exotherm on the DSC thermogram, TGA was employed. The differential thermal analysis (DTA) trace (blue in figure 5.5) is included in the background to show the coherence between the melting endotherm and the weight loss. The resulting TGA thermogram (figure 5.5) shows weight loss to coincide with the melting temperature, which increases progressively after the recrystallisation exotherm. This weight loss associated with an exothermic thermal event is consistent with charring, which was observed through hot stage microscopy. This offers further proof that didanosine decomposes upon melting, and that the mechanism of decomposition is oxidation. For this reason, the rest of the samples were analysed only up to 470 K.

5.3.2.2 DIDANOSINE CRYSTALS FROM ACETONITRILE

To investigate the fine crystals obtained from acetonitrile, a small amount was removed from the recrystallisation medium and placed on filter paper. The filter paper was then folded and the sample was blot dry. To test for dryness, a spatula was pressed onto the sample. If no crystals adhered to the spatula, it was considered dry and used for analysis.

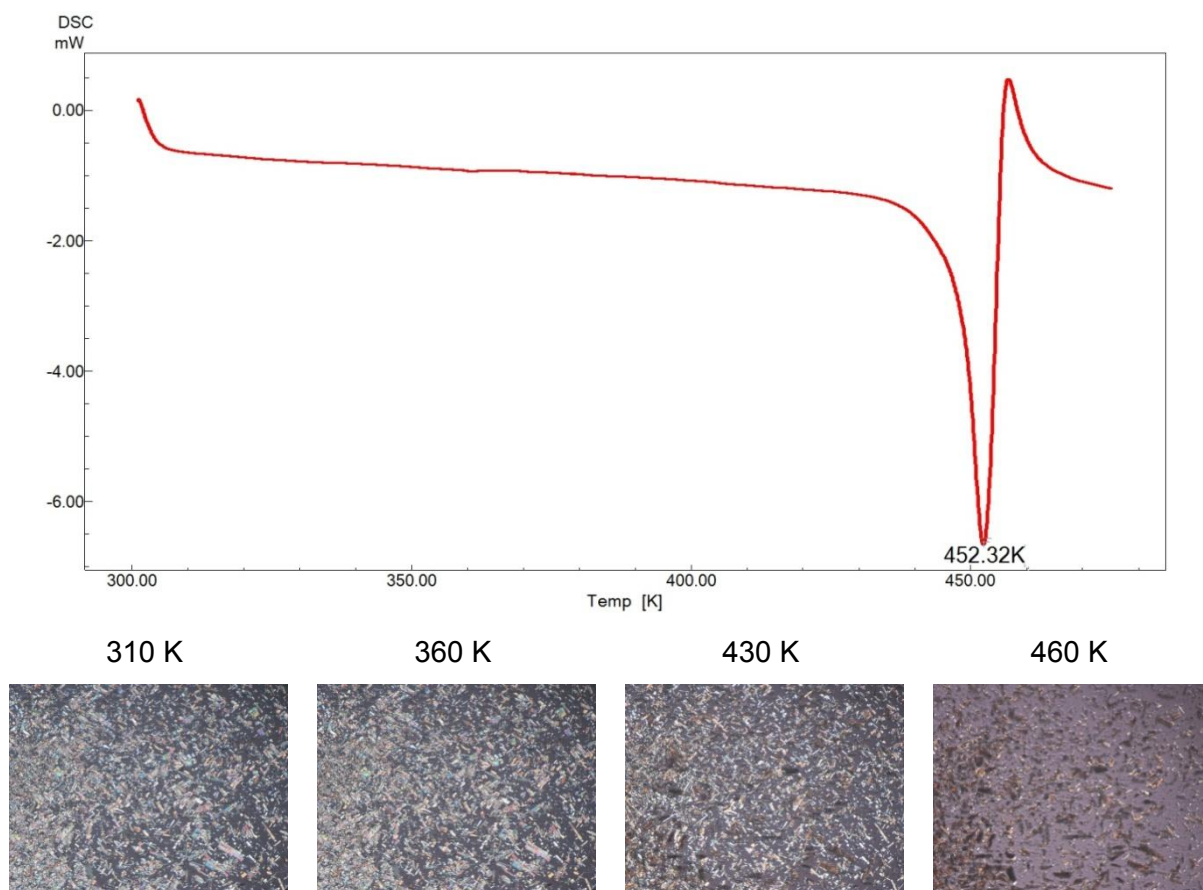


Figure 5.6: DSC thermogram and hot stage micrographs of didanosine recrystallised from acetonitrile.

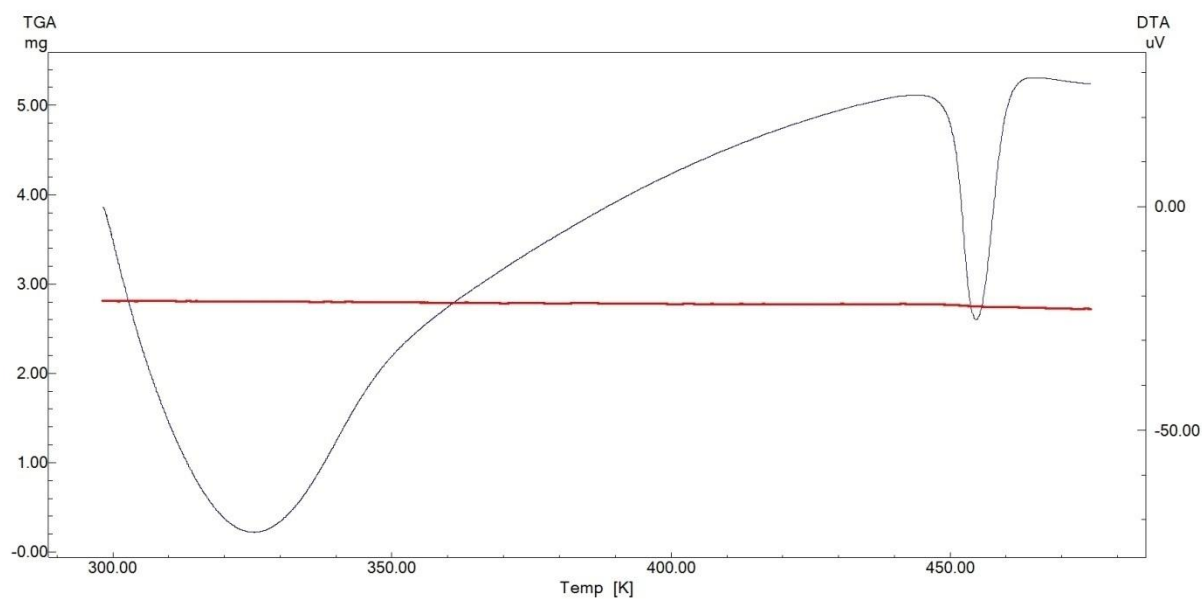


Figure 5.7: TGA of didanosine crystals obtained from acetonitrile.

The DSC thermogram of the didanosine crystals obtained from acetonitrile (figure 5.6) is similar to the one obtained from the raw material. No desolvation or phase transition endothermic peaks are present, and the melting point itself is only about 1 K higher than that of the raw material. The hot stage micrographs show the crystals to be small monolithic flakes. No solvent evolution during heating is observed and the crystals also exhibit the same decomposition and charring upon melting. TGA of the crystals from acetonitrile (figure 5.7) shows no weight loss, except during decomposition on melting. Except for the morphology of the crystals, the recrystallisation product from acetonitrile seems to be the same crystalline form as the raw material. This will be conclusively proven later in this chapter with PXRD analysis.

5.3.2.3 DIDANOSINE CRYSTALS FROM METHANOL

The crystals obtained from methanol were dried according to the method described for the crystals from acetonitrile.

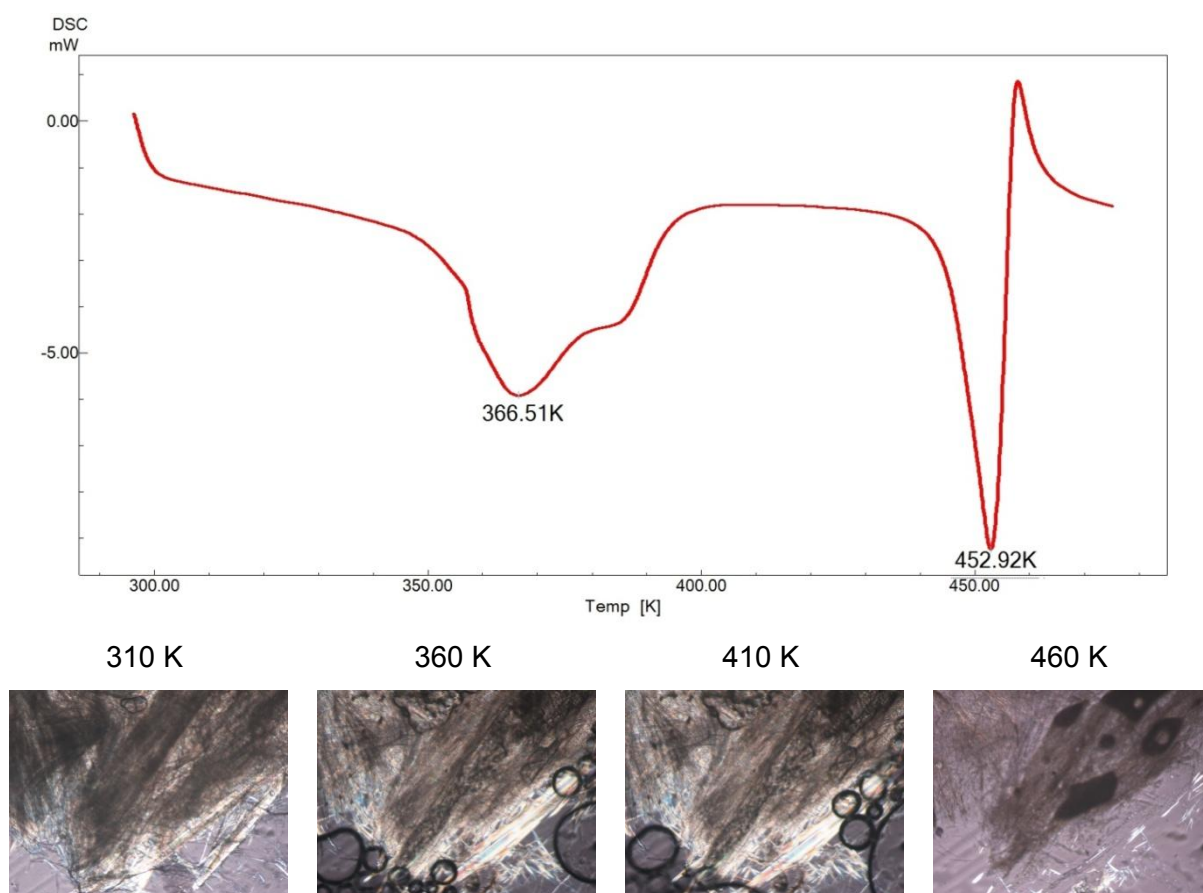


Figure 5.8: DSC thermogram and hot stage micrographs of didanosine recrystallised from methanol.

The DSC thermogram of the didanosine crystals obtained from methanol (figure 5.8) exhibits what appears to be a very broad desolvation endotherm prior to melting and decomposition. Interestingly, the base line of the thermogram after desolvation indicates that the system is at a lower energy state after desolvation. Furthermore, the slope of the thermogram slowly descends from the start of the run leading up to the desolvation peak. This indicates that the enthalpy of the system slowly increases leading up to desolvation, a thermal event that could be explained by the systematic loss of solvent from the surface of the crystals. Another indication of non-conventional solvent loss is the shape of the desolvation endotherm. The shape of this desolvation peak differs from the usual desolvation endotherms (which have a much steeper slope back to the base line) and the desolvation temperature is 28 K higher than the boiling point of methanol (338 K). It is therefore clear that some interaction between the solvent and the crystals is preventing total desolvation from occurring at a single temperature. The hot stage micrographs show pockets of solvent trapped inside the mesh network of the fibrous crystals. Upon heating the solvent volatiles are evolved from the mesh network and this evolution continues up to the point of melting, explaining the broad desolvation endotherm on the DSC thermogram. This phenomenon is not usually associated with solvent entrapped in a crystal. Indeed, solvent entrapped inside a crystal would also not be visible on a micrograph.

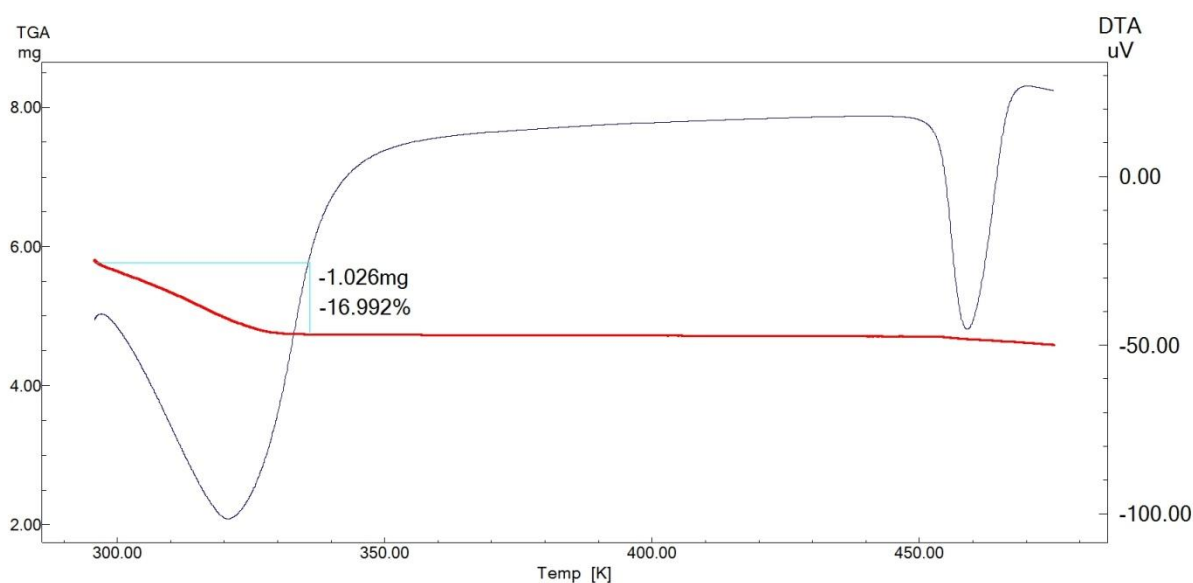


Figure 5.9: TGA of didanosine crystals obtained from methanol.

TGA of the didanosine crystals obtained from methanol (figure 5.9) shows a 17% weight loss on heating. The weight loss for a 1:1 stoichiometry is 11.95%, indicating a ratio of 1.5 methanol molecules for every didanosine molecule. The weight loss occurs from the start of

the heating run. This is very important, as it is indicative of weight loss from the surface of a substance, and not from within a crystalline structure. This observation corresponds with the thermal behaviour seen on the DSC thermogram and hot stage micrographs. Methanol seems to form a thin film on the didanosine whiskers, in much the same way as hygroscopic water forms a film around ground particles, while the mesh network of fibrous whiskers form a capillary system which retains solvent molecules. The formation of such a capillary network is nothing new, and is the basis of the Washburn method (Fell, 2002:65) for determining the contact angle of liquids on powders. The film of solvent (which is of course in a higher energy state than a solid) could cause the system to present itself in a higher energy state on a DSC and, after the solvent is removed from the system, and only the crystals remain, the system would seem to be in a lower energy state. This would explain the thermal behaviour on the DSC thermogram. The capillary solvent can be seen on the micrographs and the prolonged evolution of this loosely bound methanol along with the more tightly bound film around the crystals, explains the broad endothermic peak on the DSC.

What would happen then, if the crystals were to be dried completely? To investigate this, the didanosine crystals obtained from methanol were dried *in vacuo*, in the oven at high and at low temperatures and in the vacuum oven. To test for dryness, the samples were analysed on the TGA. The results were the same for each of the samples, regardless of the method of drying. Upon reaching total dryness, as was evident by no weight loss on the TGA, the entire mesh network of crystals lost its morphology and turned into powder.

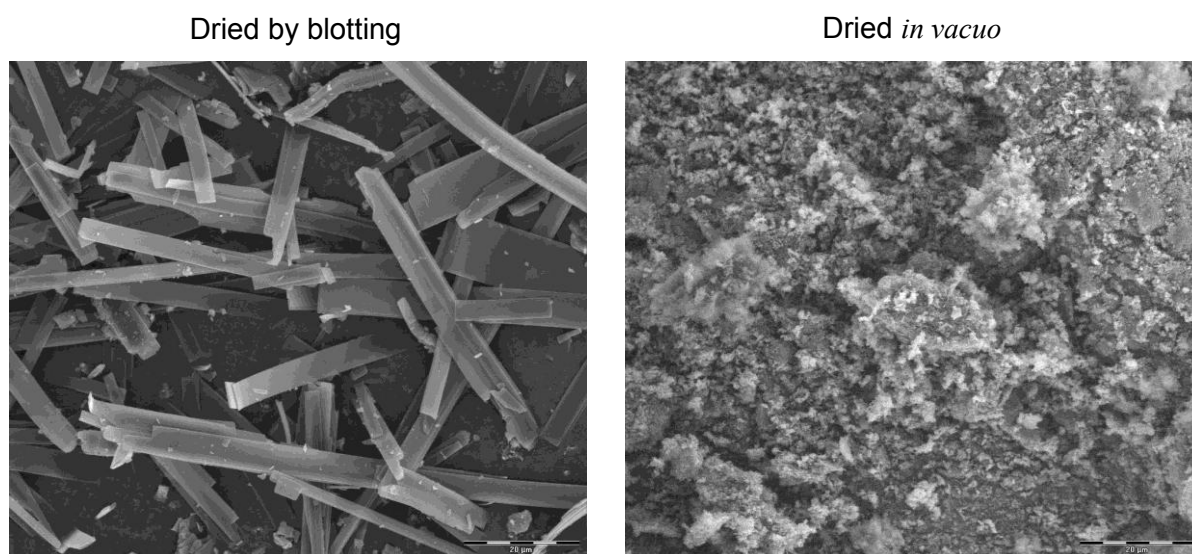


Figure 5.10: Didanosine crystals from methanol dried by blotting (as described in section 5.3.2.2) and dried *in vacuo*.

To best illustrate the change in morphology, SEM micrographs of the crystals from methanol before and after drying *in vacuo* are given in figure 5.10. This extremely fine powder that formed had the same macro- and microscopic appearance as didanosine raw material, and also exhibited the exact same thermal behaviour. Clearly, the presence of methanol around the crystals is needed for it to retain its morphology, in much the same way as hygroscopic and capillary water is needed for clay and pot ground to retain its structures. Some surface interaction between the didanosine and methanol molecules keeps the crystal structure intact.

It was mentioned in the background section of this chapter that Bettini *et al.* (2010:1868) found the didanosine polymorph prepared in their study to be thermodynamically metastable. Indeed, metastability was also observed for the crystals obtained from methanol (as well as all the other alcohols used in this study) when the recrystallisation product was left in the recrystallisation medium for a certain amount of time. Starting at around 2 weeks after the recrystallisation product was formed, small amounts of powder precipitated on top of the crystal network. After a month, no trace of the crystals was left and the bottom of the beaker was covered in a fine white powder, all the while whilst still under the recrystallisation medium. Thermal and microscopic analysis of the powder revealed it to exhibit the same behaviour as the raw material. SEM micrographs of the powder and the initial crystals are presented here in figure 5.11.

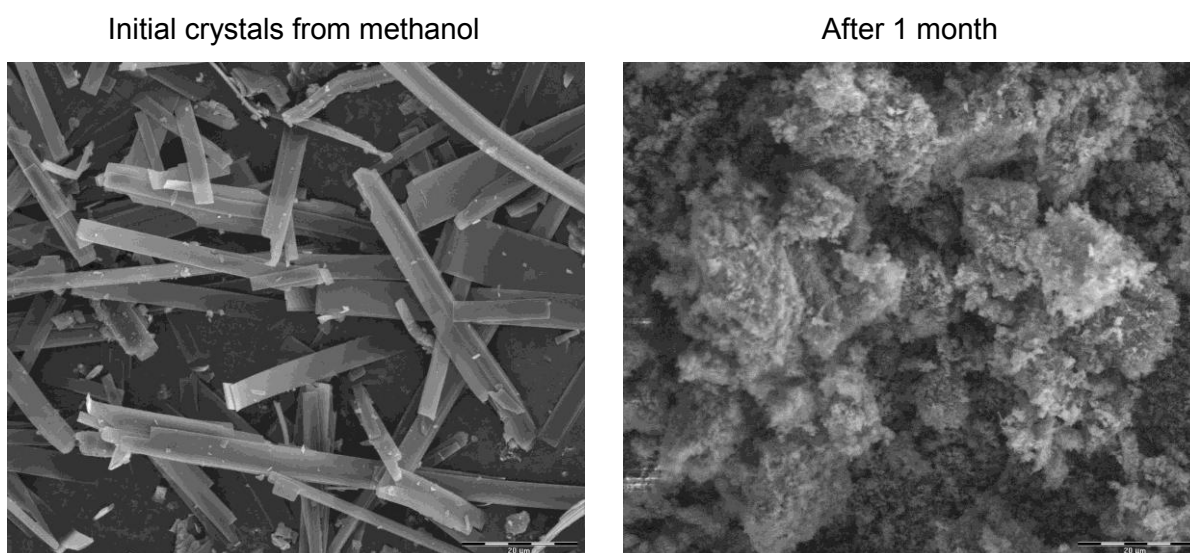


Figure 5.11: SEM micrographs of didanosine crystals obtained from methanol and the same crystals one month later.

Considering the observations thus far, there can be little doubt that the raw material is the most stable form of didanosine. Any stress added or alteration made to the recrystallisation products and it reverts back to the raw material, therefore special care was taken when handling and analysing the recrystallisation products.

5.3.2.4 DIDANOSINE CRYSTALS FROM ETHANOL

The crystals obtained from ethanol were dried according to the method described for the crystals from acetonitrile.

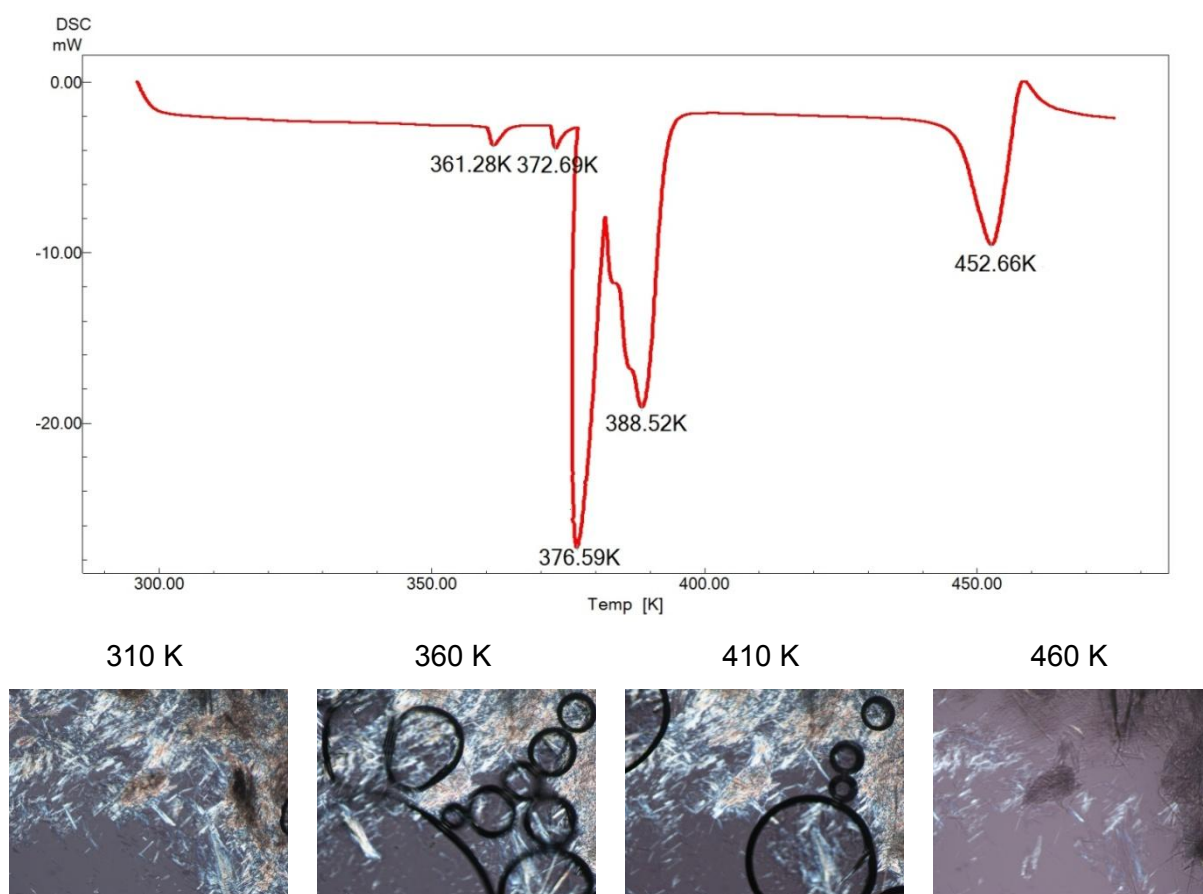


Figure 5.12: DSC thermogram and hot stage micrographs of didanosine recrystallised from ethanol.

The DSC thermogram (figure 5.12) again shows endothermic peaks at temperatures much higher than the boiling point of ethanol (351 K), indicating that the solvent is tightly bound to the crystals. Additionally, the peak at 376.59 K shows significant lag from the machine, indicating that the desolvation process was extremely rapid. Apart from the unusual desolvation endotherms, the thermogram exhibits the same unique thermal behaviour as

that of the crystals obtained from methanol, in the fact that the sample appears more liquid before desolvation and more crystalline afterwards. The hot stage micrographs show the same prolonged volatile evolution observed for the crystals from methanol, along with the characteristic charring upon melting. Considering the similarities between the thermal behaviours of the crystals from methanol and ethanol, one might conclude that the ethanol was also present on the surface of the crystals. This can be conclusively proven by TGA.

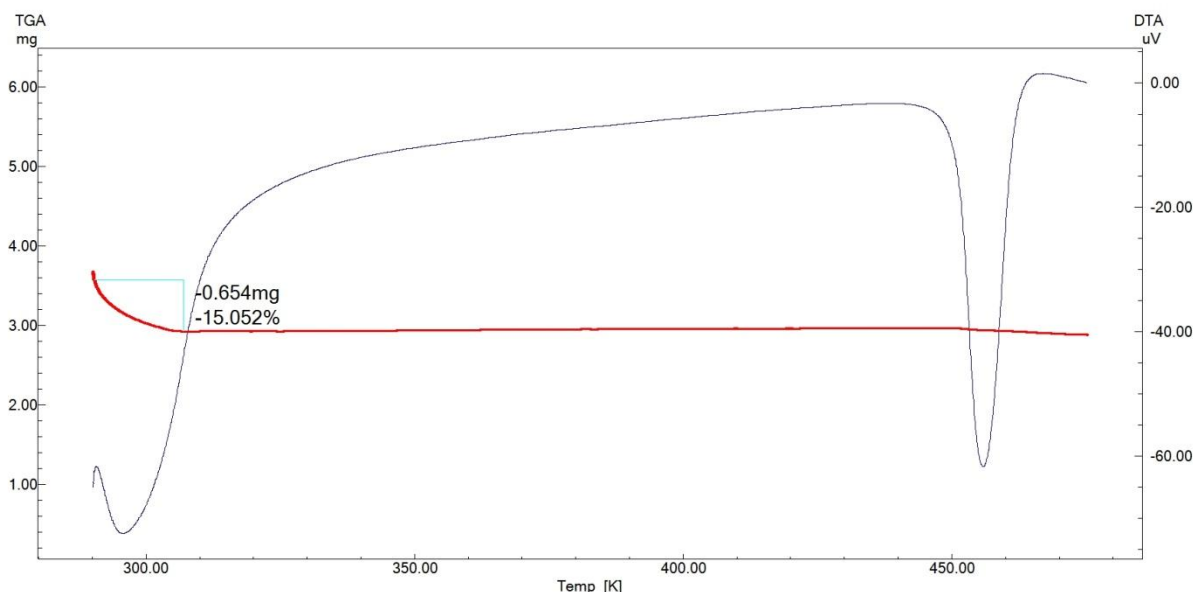


Figure 5.13: TGA of didanosine crystals from ethanol.

TGA of didanosine crystals from ethanol (figure 5.13) clearly shows weight loss occurring at the start of the heating run. Again, this is consistent with solvent loss from the surface of crystals (or any other material). At a stoichiometry of 16.32%, the non-stoichiometric weight loss of 15% can be attributed to the fact that the solvent is not entrapped within a crystalline structure, but rather adsorbed to the surface. Upon thorough drying, the crystals also lost their morphology and reverted back to the raw material. This phenomenon has now been observed for both the crystals obtained from methanol and ethanol, and it would be interesting to see how the crystals from the longer chain alcohols behave under the same drying conditions.

5.2.3.5 DIDANOSINE CRYSTALS FROM 1-PROPANOL

The crystals obtained from 1-propanol were dried according to the method described for the crystals from acetonitrile

The DSC thermogram (figure 5.14) shows a very sharp desolvation endotherm, with the same lag observed on the thermogram of the crystals from ethanol. The same trend described for the previous crystals also repeat here, where the system appears to be in a higher energy state before desolvation than afterwards. On the hot stage micrographs, evolution of volatiles can be seen from very early on in the analysis, as a direct result of the difficulty in drying these crystals. The solvent evolution also takes place over a prolonged period of time and the crystals also char upon melting.

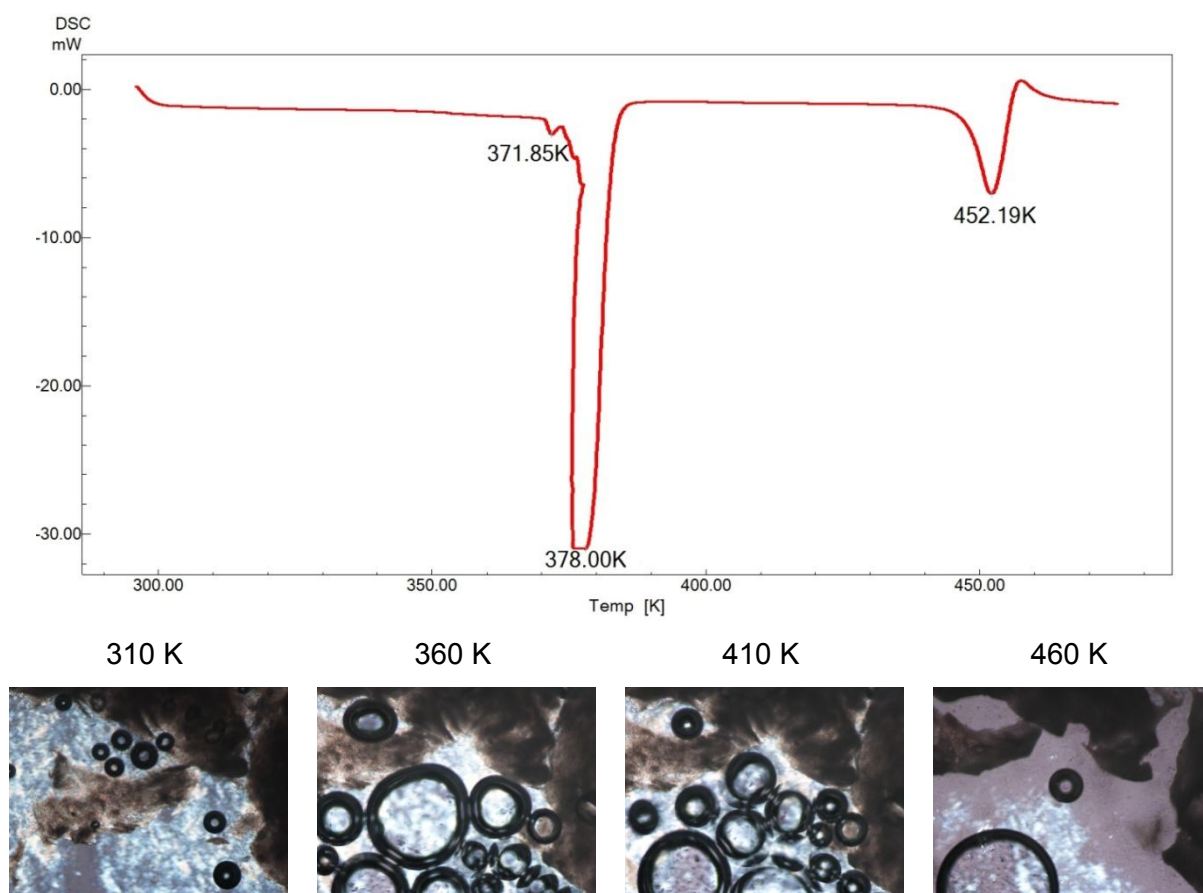


Figure 5.14: DSC thermogram and hot stage micrographs of didanosine recrystallised from 1-propanol.

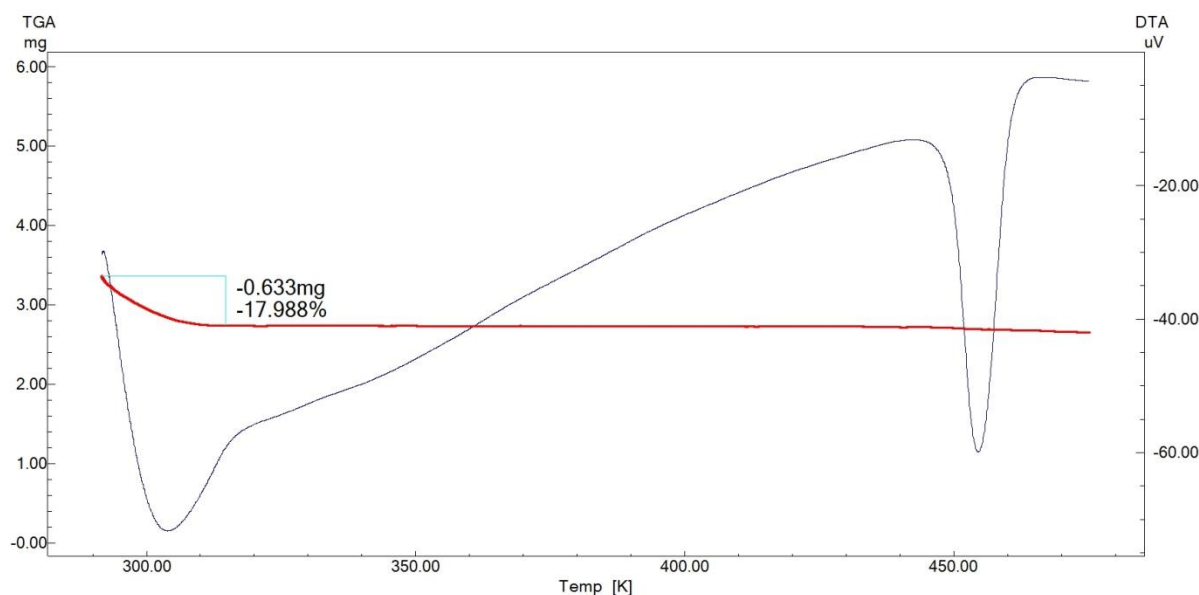


Figure 5.15: TGA of didanosine crystals from 1-propanol.

TGA of the crystals from 1-propanol (figure 5.15) exhibits the same characteristic weight loss from the surface that was observed for the previous recrystallisation products from alcohols. Similar to the crystals obtained from ethanol, the weight loss from these crystals is non-stoichiometric. The stoichiometric weight loss for a 1:1 solvent to crystal ratio is 20.28%, whilst the weight loss for these crystals is 17.99%.

5.2.3.6 DIDANOSINE CRYSTALS FROM 2-PROPANOL

The crystals obtained from 2-propanol were dried according to the method described for the crystals from acetonitrile. The DSC thermogram of didanosine crystals from 2-propanol (figure 5.16) exhibits the same instrument lag observed for the crystals from ethanol and 1-propanol, as well as the same base line shift after desolvation. The pockets of solvent can be seen on the micrographs and the same prolonged solvent evolution noted for the previous crystals obtained from alcohols can also be seen here.

TGA of the crystals from 2-propanol (figure 5.17) also exhibits the same weight loss which has now become synonymous with didanosine crystals obtained from alcohols. The weight loss of 41.17 % indicates the presence of two 2-propanol molecules for each didanosine molecule.

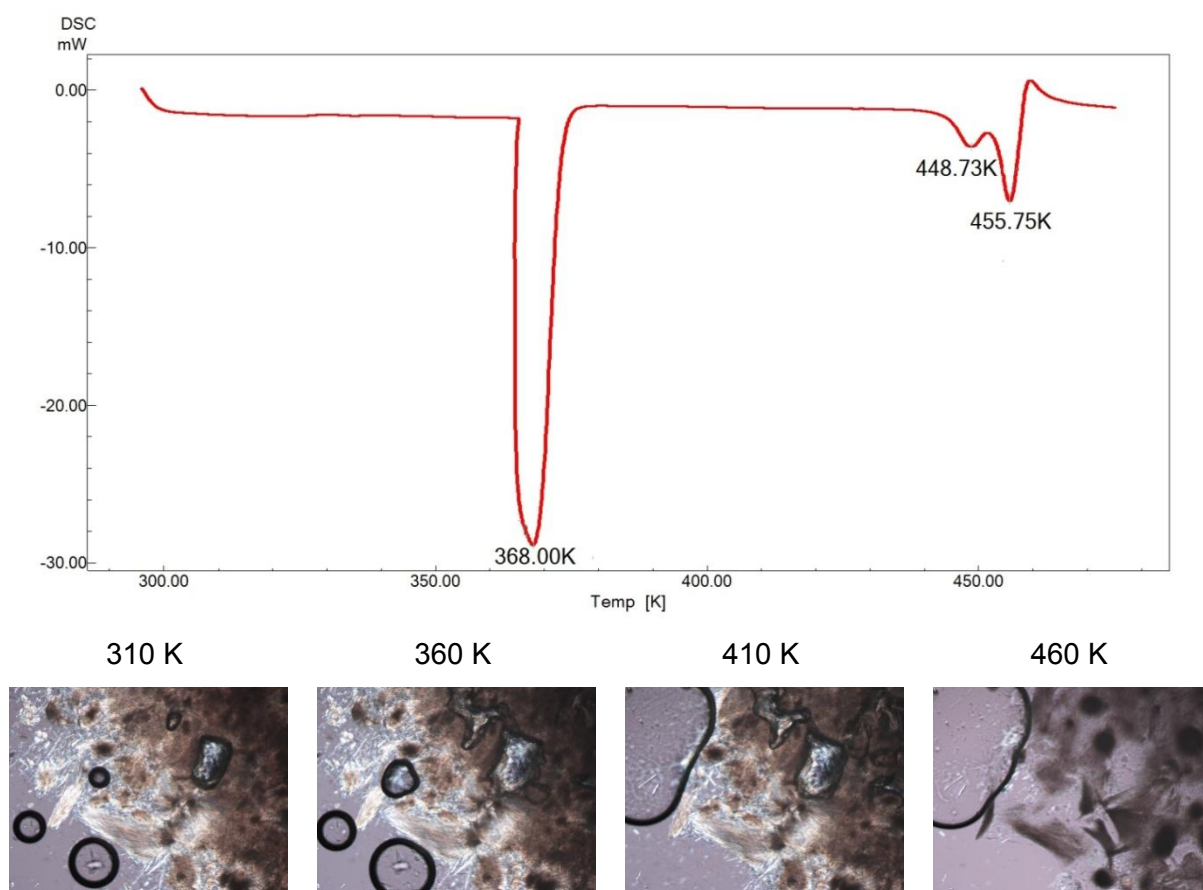


Figure 5.16: DSC thermogram and hot stage micrographs of didanosine recrystallised from 2-propanol.

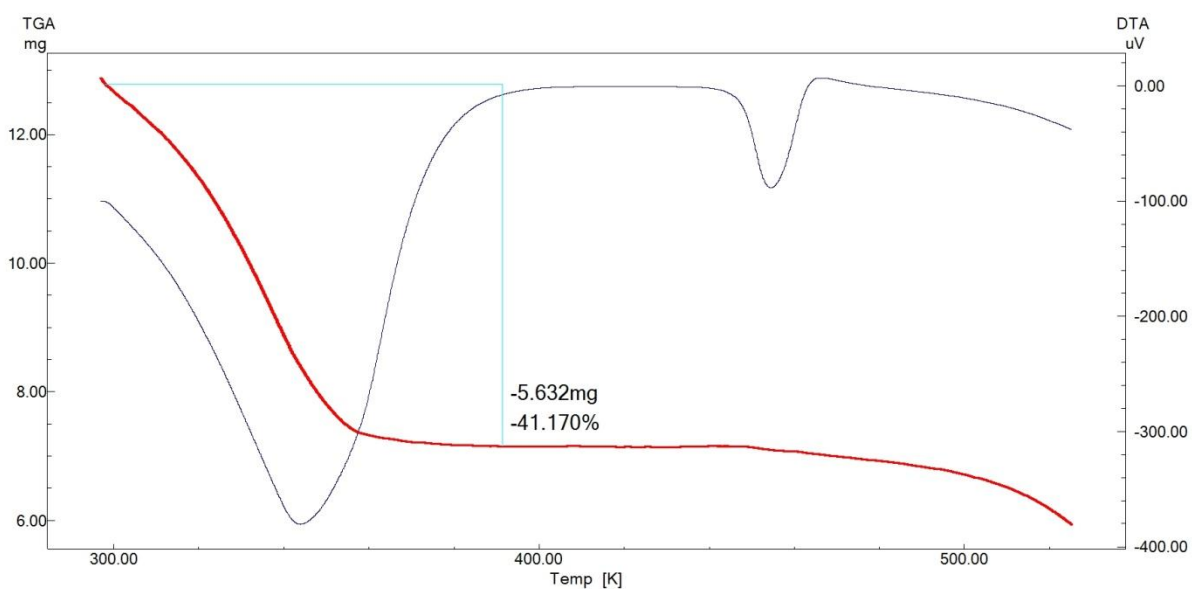


Figure 5.17: TGA of didanosine recrystallised from 2-propanol.

5.2.3.7 DIDANOSINE CRYSTALS FROM 1-BUTANOL

The crystals obtained from 1-butanol were dried according to the method described for the crystals from acetonitrile.

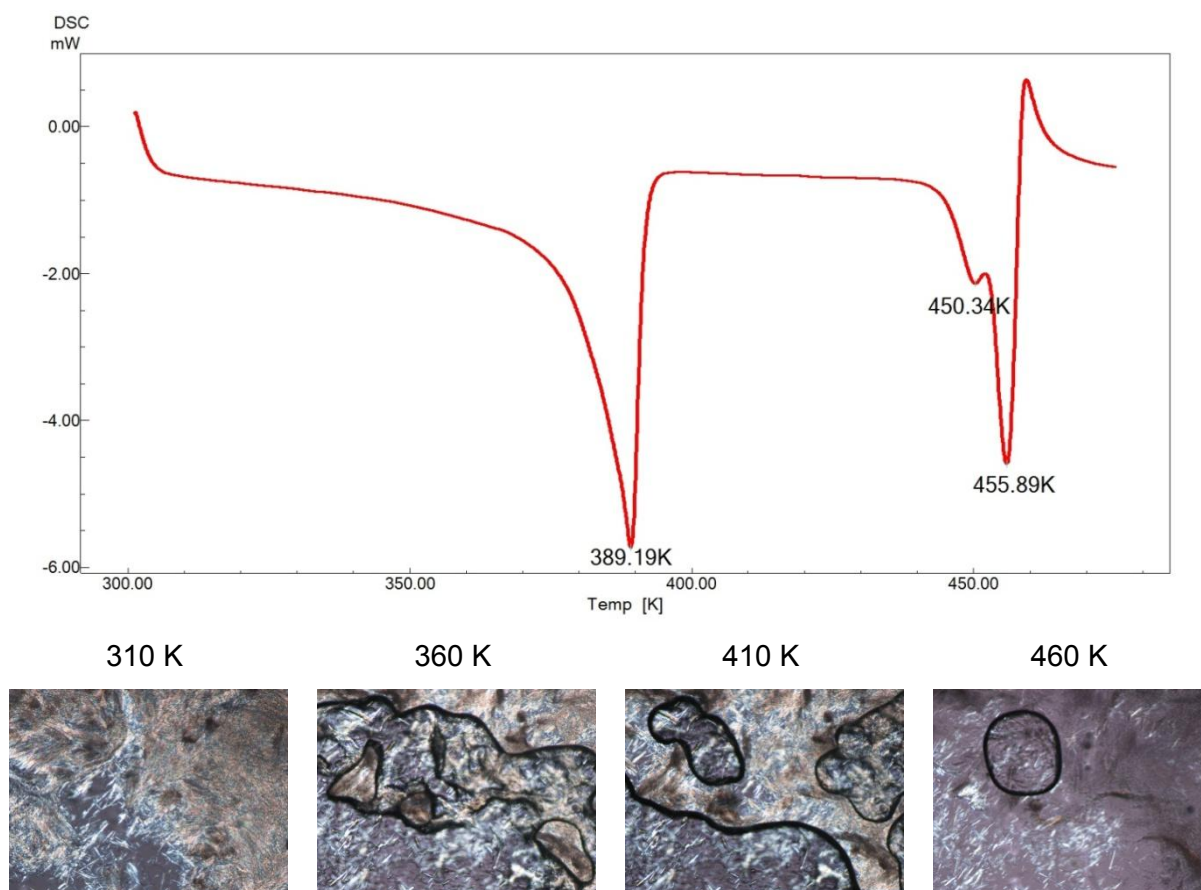


Figure 5.18: DSC thermogram of didanosine recrystallised from 1-butanol.

The DSC thermogram of didanosine recrystallised from 1-butanol (figure 5.18) exhibits a large desolvation endothermic peak, as well as the two melting endotherms observed for the recrystallisation product from 2-propanol. Similar to the crystals obtained from 2-propanol, the melting point of the crystals from 1-butanol is about 4 K higher than that of the raw material. Although this is usually indicative of a polymorphic difference between two crystals of the same substance, this will have to be proven with PXRD. The hot stage micrographs show pockets of solvent in the fibrous mesh network of crystals, with prolonged evolution of solvent volatiles. The intensity of the solvent evolution was much higher for these crystals than for its contemporaries from other alcohols. Indeed, weight loss on TGA of these crystals (figure 5.19) indicates two 1-butanol molecules for each didanosine molecule.

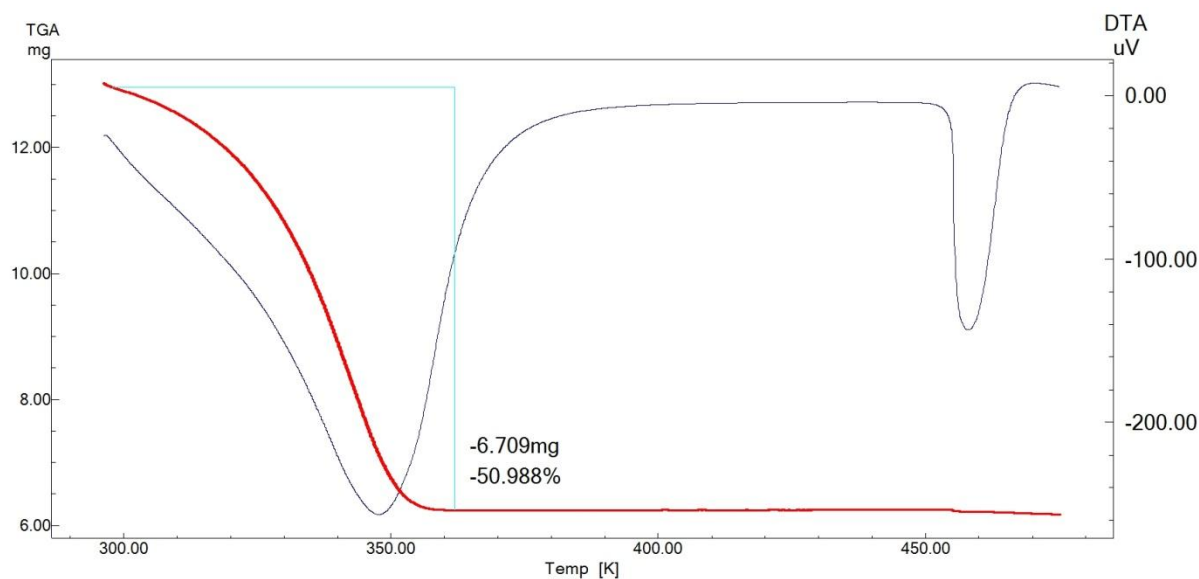


Figure 5.19: TGA of didanosine crystals from 1-butanol.

5.3.3 FTIR ANALYSIS

To investigate whether or not the inter- and intramolecular interactions between didanosine's functional groups differ from raw material to recrystallisation product, FTIR was employed.

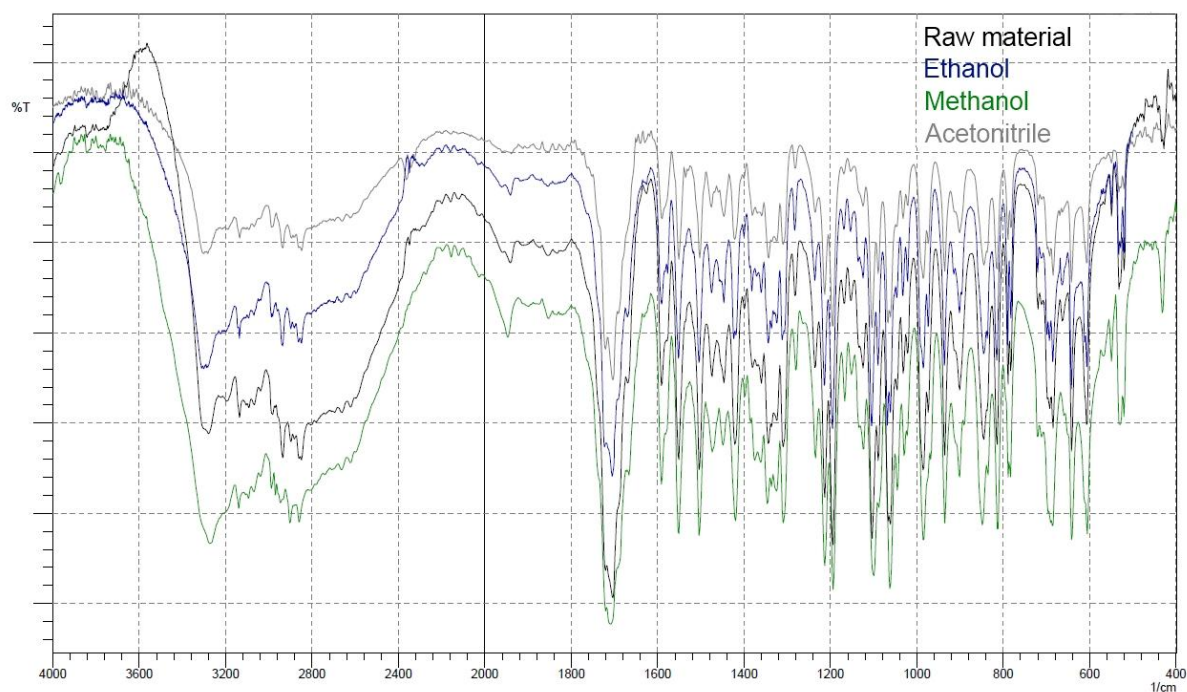


Figure 5.20: Overlay of the FTIR interferograms of didanosine raw material and its recrystallisation products.

The interferograms of the raw material and the recrystallisation products from acetonitrile, methanol and ethanol are presented in figure 5.20. The interferograms are identical, indicating that the molecules inside the unit cells of the raw material and the recrystallisation products have the same molecular interactions. This is not too surprising, especially when considering the fact that the melting points of all didanosine's recrystallisation products (with the exception of those from 2-propanol and 1-butanol) differed only slightly (± 2 K) from that of the raw material. This similarity in melting points is already an indication that the molecular interactions inside the crystals are the same, and the FTIR data is therefore in excellent coherence with the data from thermal analyses. The interferograms of the didanosine crystals obtained from longer chain alcohols are presented in figure 5.21.

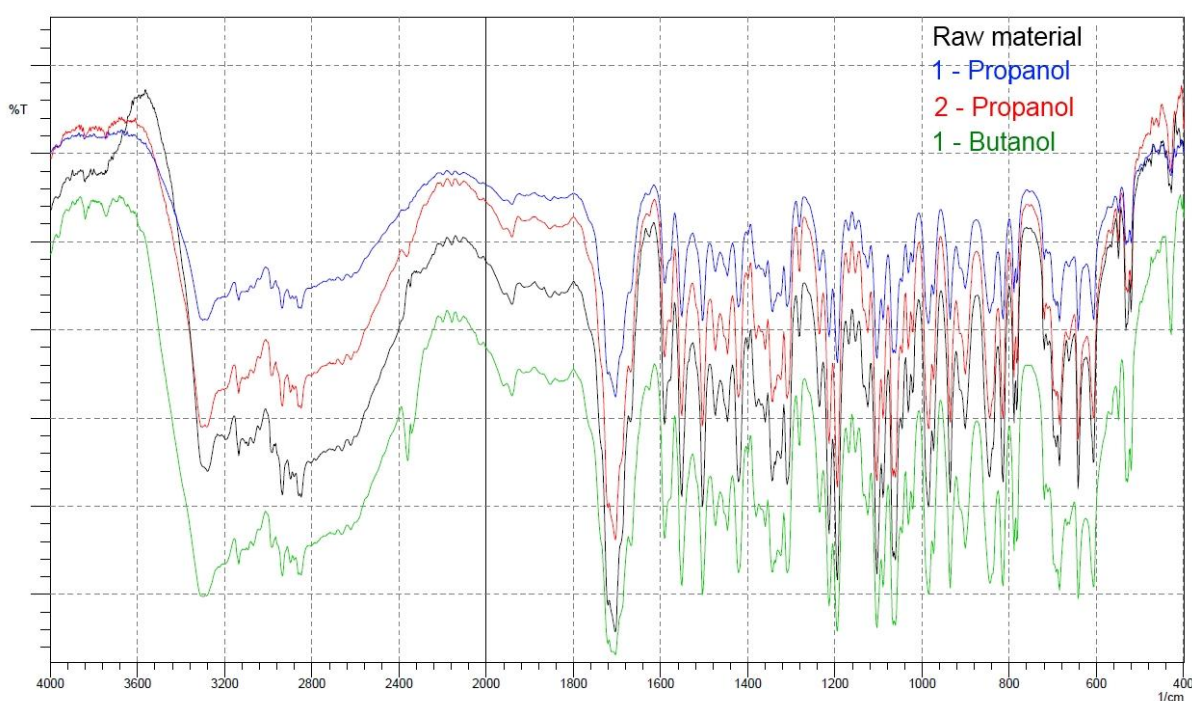


Figure 5.21: Overlay of the FTIR spectra of didanosine raw material and its recrystallisation products.

5.3.4 PXRD ANALYSIS

The final step in the polymorphism study, and possibly the most conclusive, involves comparison of the PXRD diffractograms of each recrystallisation product to that of the raw material. The positions of the peaks, according to Bragg's Law, is the result of x-ray diffraction from planes of a unit cell. Differences in the positions of the peaks are therefore indicative of differences in unit cell dimensions, and can be used as conclusive proof of

polymorphism. The diffractograms of didanosine raw material and its recrystallisation products are given here in figure 5.22.

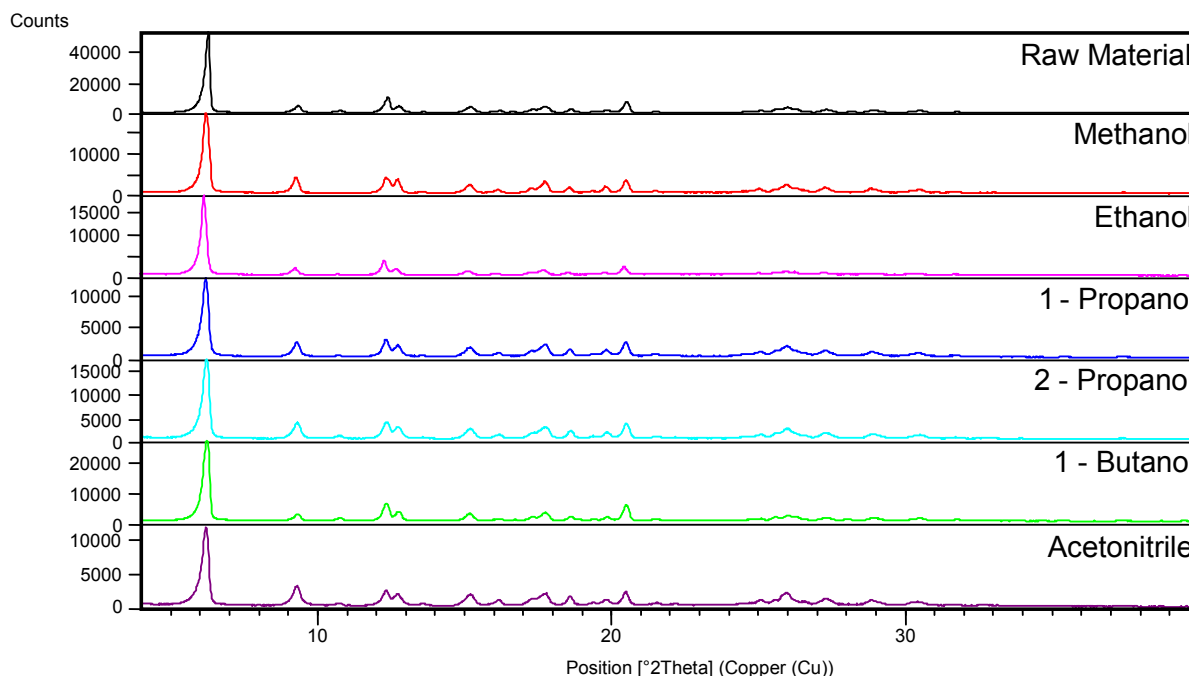


Figure 5.22: PXRD diffractograms of didanosine raw material and its recrystallisation products.

The diffractograms of all the samples tested appear to show peaks at the same positions. The exception being that of the crystals obtained from methanol, which lacks the small peak just below $11^\circ 2\theta$. This specific peak appears to be non-existent in the methanol crystals and slowly increase in intensity as the chain length of the alcohol used to recrystallise from increases. Because of the very high counts of the peak at $6^\circ 2\theta$, much of the smaller peaks are difficult to elucidate. To address this issue, an enlargement of the diffractograms between 10 and $20^\circ 2\theta$ is presented in figure 5.23.

The PXRD data clearly show that all the didanosine recrystallisation products accept those from methanol, have the same unit cell dimensions as the raw material. The absence of the peak at $10.7^\circ 2\theta$ indicates that a certain plane shared by the raw material and the recrystallisation products is absent in the methanol crystals. It was mentioned in chapter 4 that the intensity of the x-ray diffraction has nothing to do with Bragg's Law, but rather with the electron density of the atoms in the plane causing the diffraction. The fact that this peak is in general so small only means that there are few atoms occupying that specific plane. When viewing this fact alongside the increased intensity (counts) of the peak at $12.7^\circ 2\theta$, it is

reasonable to assume that the didanosine molecules in the unit cells of the crystals obtained from methanol are arranged in such a way that they reinforce the diffraction from the plane at $12.7^\circ 2\theta$, instead of that from the plane at $10.7^\circ 2\theta$.

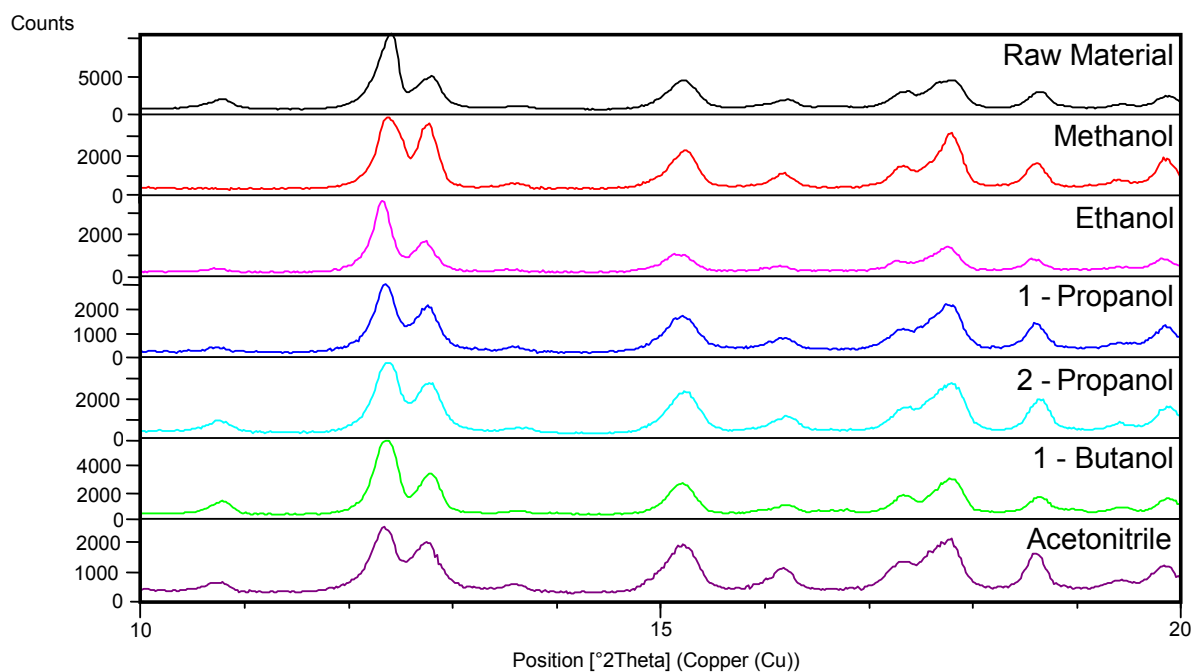


Figure 5.23: An enlargement of the PXRD diffractograms of didanosine raw material and its recrystallisation products between positions 10 and 20 at $^\circ 2\theta$.

Single-crystal x-ray diffraction (SXRD) would have been able to prove the difference in molecular arrangement between the molecules in the unit cells of the crystals and the raw material, however, neither of the recrystallisation products nor the raw material were of sufficient size for SXRD analysis. This extremely small size of any crystal obtained from didanosine is exactly the same problem encountered by both Bettini *et al.* (2010:1868) and Martins *et al.* (2010:1885). Despite numerous attempts to increase crystal size by altering the cooling rate, the pressure or the degree of supersaturation, no didanosine crystal of adequate size for SXRD could be prepared in our laboratories.

5.3.5 CONCLUSIONS FROM THE POLYMORPHISM STUDY

Despite the double melting peaks and higher melting points of the crystals obtained from 2-propanol and 1-butanol, PXRD conclusively shows these crystals not to be polymorphs of didanosine raw material. In fact, the only crystal obtained during the polymorphism study that exhibited different unit cell dimensions than that of the raw material, was the crystals

obtained from methanol, making these crystals the first reported didanosine polymorph in this study. To further investigate the ability methanol to yield new didanosine polymorphs, recrystallisation from different methanol concentrations was attempted.

5.4 METHANOL AND WATER MIXTURES

Since the crystals obtained from methanol were the only ones which displayed different unit cell dimensions to those of the raw material, it was decided to investigate these crystals further. A saturated solution of didanosine in water was prepared and methanol was added as anti-solvent. The crystals obtained from these mixtures were analysed for polymorphic differences according to the methods described in section 5.3.

5.4.1 THERMAL ANALYSIS

The mesh network of crystals obtained from each of the methanol/water mixtures were dried according to the method described for the crystals from acetonitrile in section 5.3.2.2.

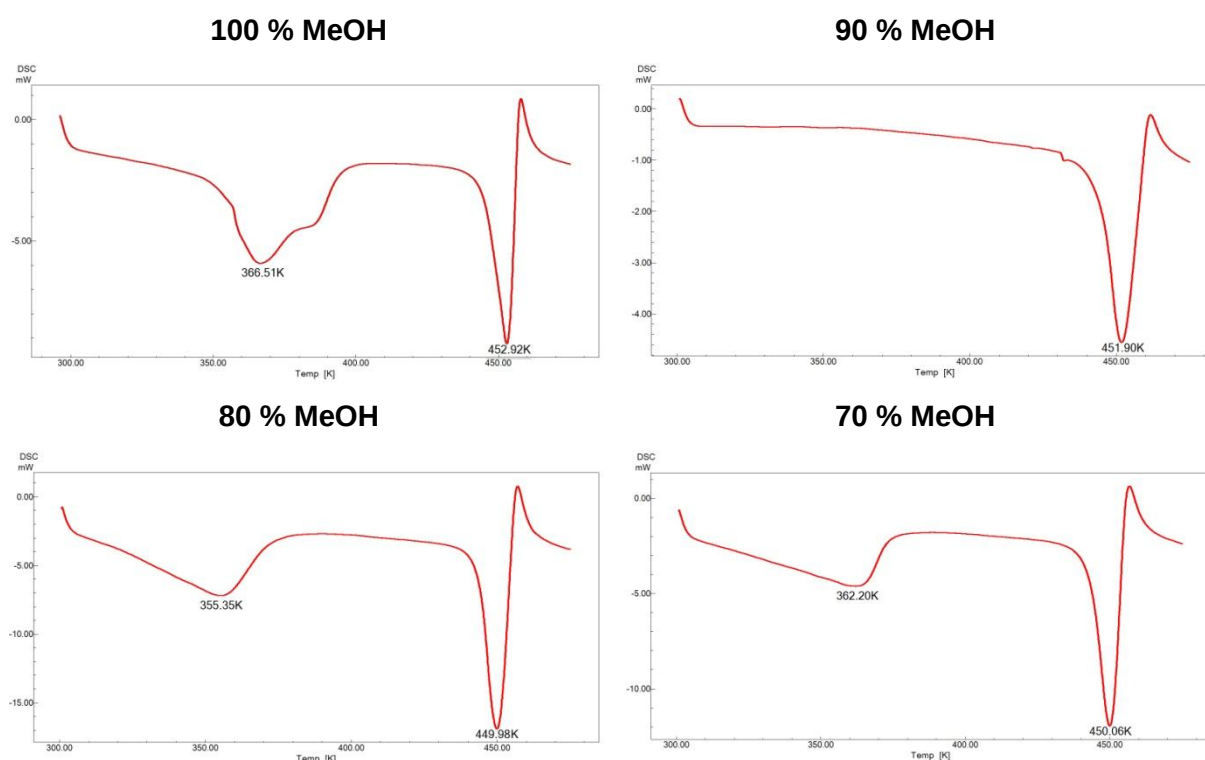


Figure 5.24: DSC thermograms of the didanosine crystals obtained from methanol and water mixtures.

The DSC thermograms of the first 4 samples are given in figure 5.24. With the exception of the crystals obtained from 90 % methanol, all the other samples display thermal behaviour consistent with that described earlier in this chapter for didanosine recrystallised from alcohols. The crystals obtained from 90 % methanol display no endothermic peaks prior to melting. This is quite curious, since it was dried according to the exact same method used for the rest of the samples, indicating the irreproducibility of drying these mesh crystals.

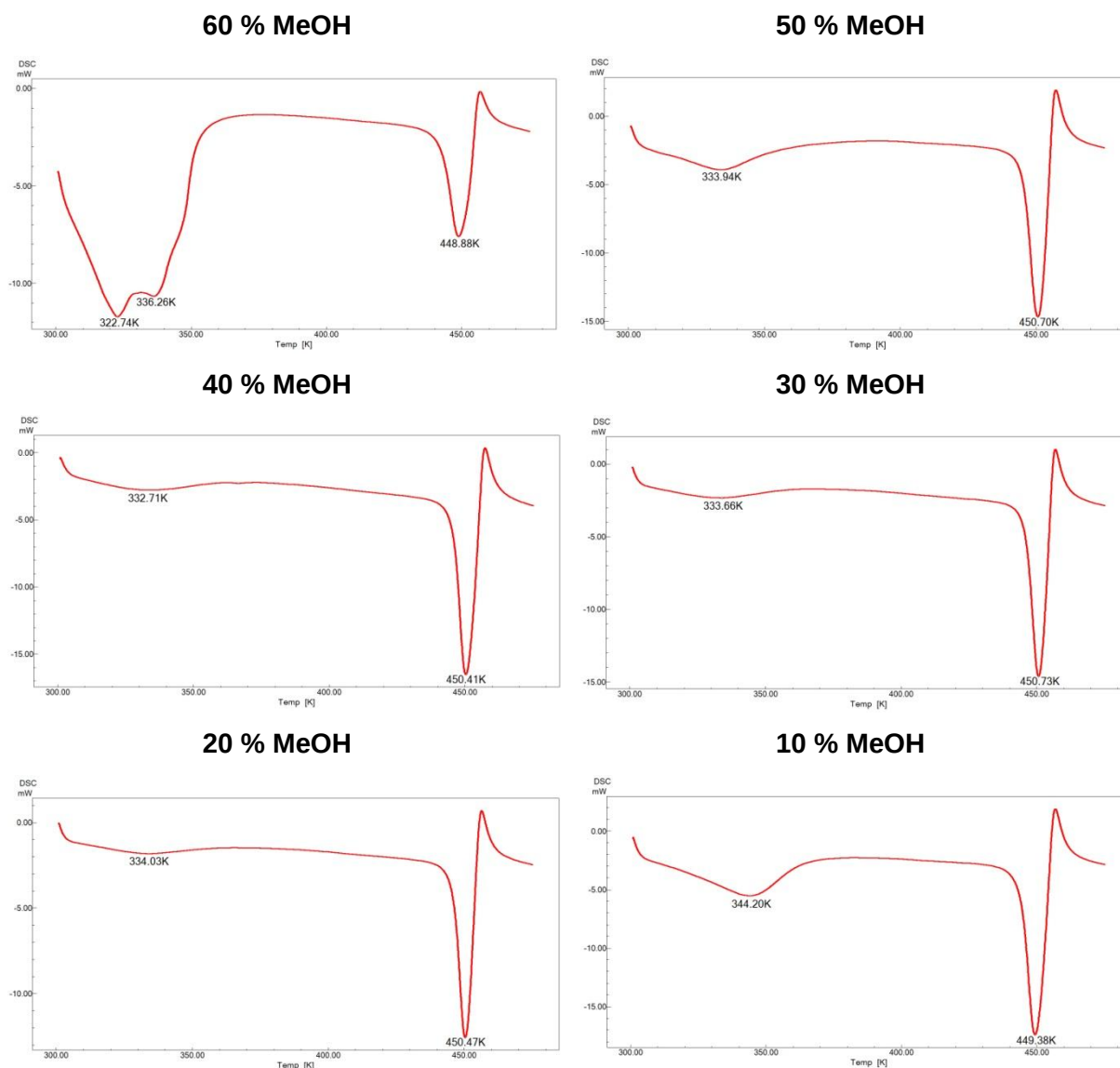


Figure 5.25: DSC thermograms of didanosine crystals obtained from methanol and water mixtures.

The didanosine crystals obtained from 50 % to 10 % methanol (figure 5.25) have similar thermal behaviour. The crystals obtained from 60 % methanol (figure 5.25) display a very

large endothermic event from the start of the heating run and also display the lowest melting point of all the samples analysed. However, this melting point is still only ± 2 K lower than that of the raw material and further analysis is needed to draw conclusions as to it being a new polymorph. To visually compare the endothermic events of each sample, hot stage microscopy was employed (figures 5.26 to 5.28).

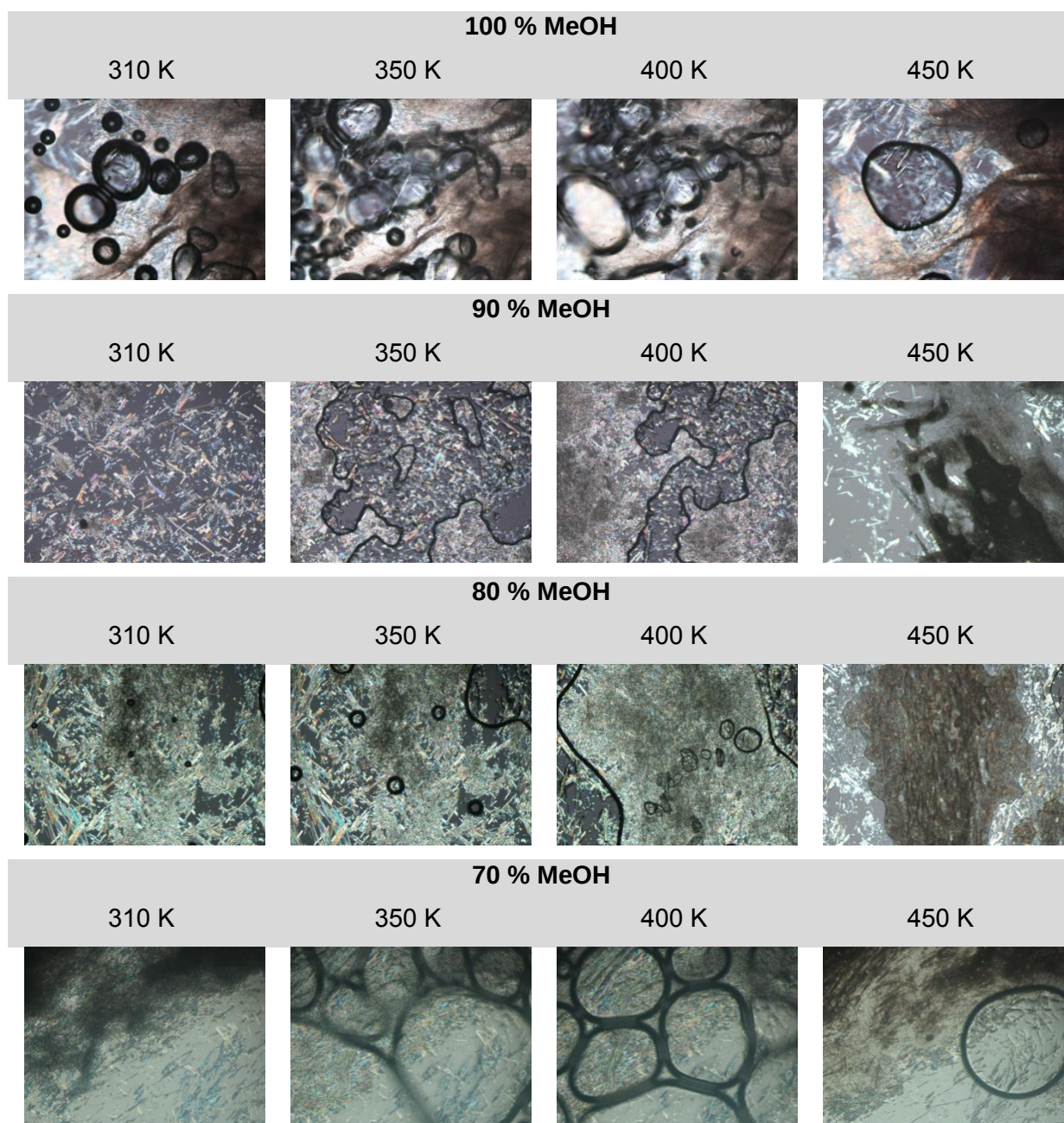


Figure 5.26: Hot stage micrographs of didanosine crystals from methanol and water mixtures.

The pockets of solvent which remain, even after drying, can be seen prior to their release. The same prolonged volatile evolution observed for the recrystallisation products from the alcohols can be seen for the products from the methanol/water mixtures, indicating that the solvent is also present on the surface of the crystals and in capillary networks and not inside the crystals.

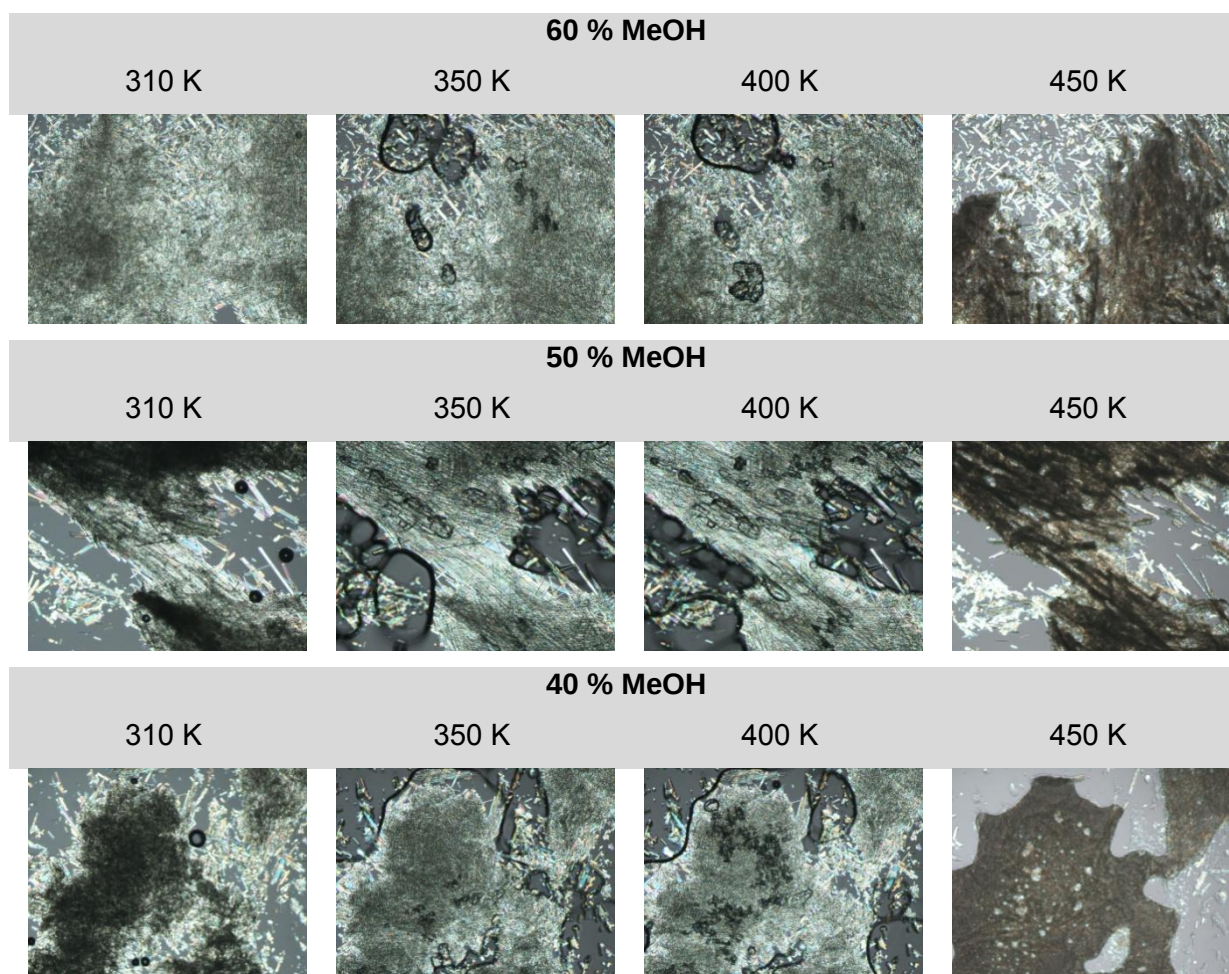


Figure 5.27: Hot stage micrographs of didanosine crystals from methanol and water mixtures.

Further evidence that these crystals are not solvates, but rather that the solvent is present in between the crystals, can be found in the random evolution points in the crystal mesh network while no evolution can be seen from the smaller crystals lying on their own. If these crystals were solvates the solvent evolution would have been homogeneous from the crystals network and would also have been visible from the individual crystals. This evolution of volatiles is more likely the result of solvent trapped in capillary networks forming random pathways throughout the mesh network of fibrous crystals. The prolonged release can be

explained again by the concept of hygroscopic water. It is a well known fact that plants cannot use hygroscopic water because it is bound too tightly to the ground particles. The solvent film coating the didanosine crystals can be bound so tightly that it only comes “loose” after absorbing enough energy. By this time, the solvent in the capillary networks is already escaping from the system and the film solvent slowly follows, giving rise to a prolonged time of volatile evolution from the system.

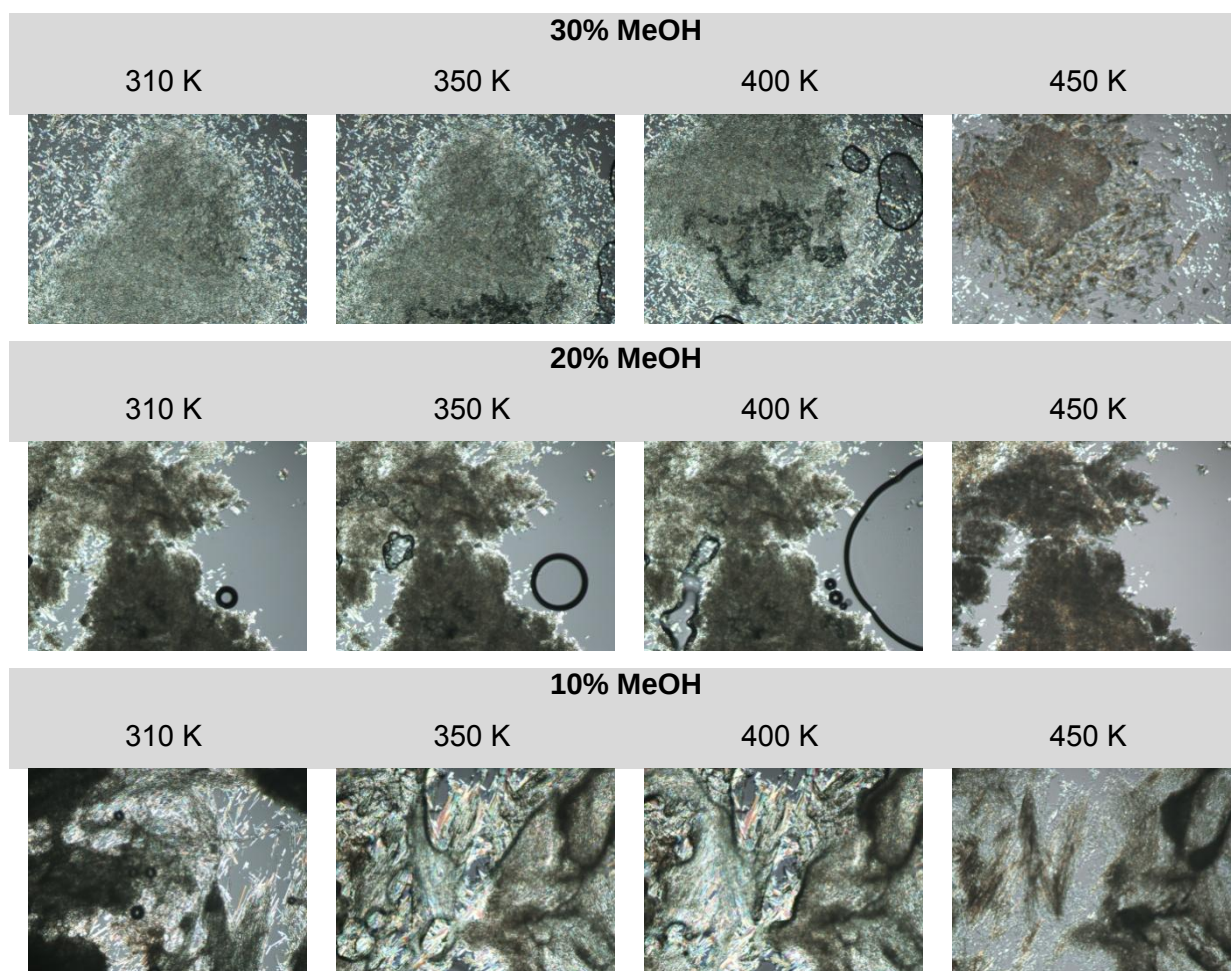


Figure 5.28: Hot stage micrographs of didanosine crystals from methanol and water mixtures.

Rather than presenting a figure containing 10 identical TGA's (the shortened version of which is presented in figure 5.29), the TGA results are given in table 5.2. Since these mixtures contained water, KF titration was also employed to determine which percentage of the total weight loss was due to water. All TGA's and KF titrations were run in triplicate. The large standard deviation values indicate the poor reproducibility of these analyses. Reproducible drying of didanosine crystals (even from the same batch) without changing the morphology was simply not possible.

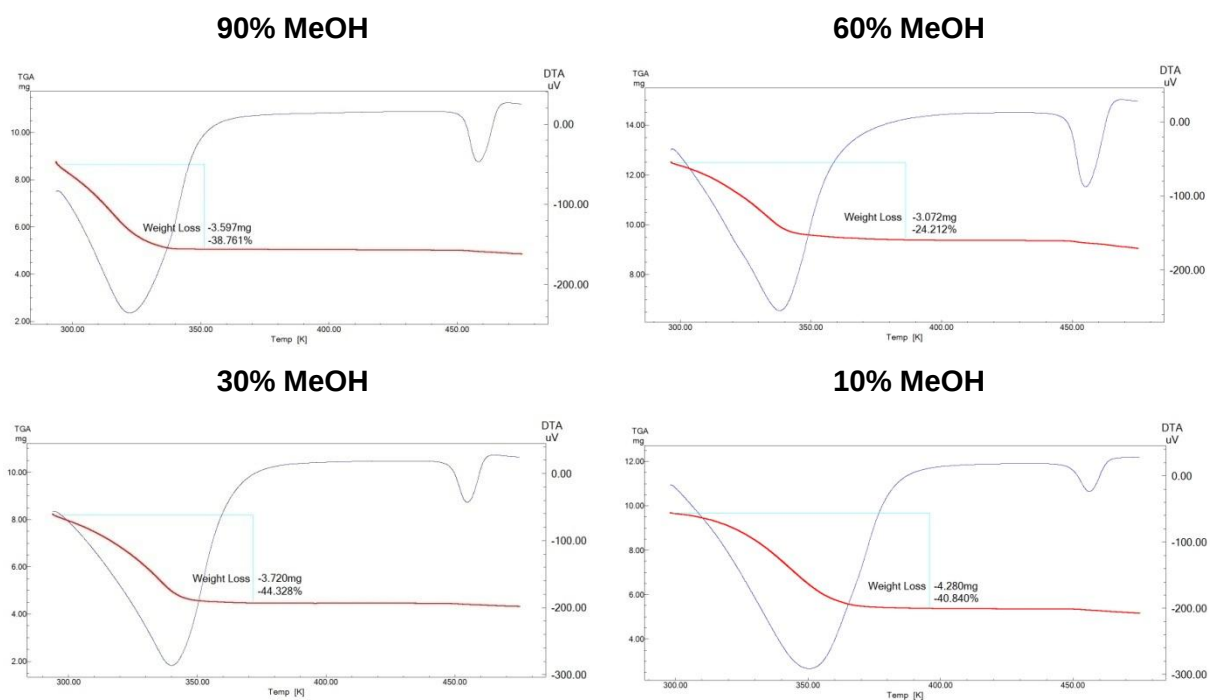


Figure 5.29: TGA of some of the recrystallisation products from methanol and water mixtures.

Despite these setbacks, there does seem to be a trend between the TGA weight loss and KF analysis. The weight loss was not the result of a single solvent, but rather a mixture of the two, the ratio of which is visible in the KF results.

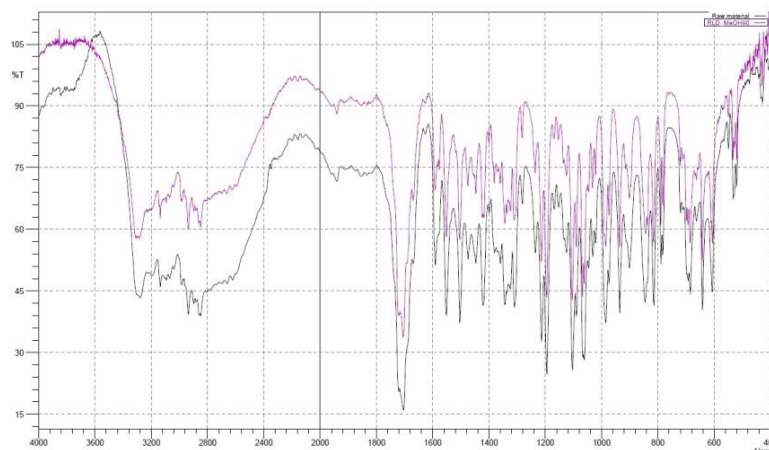
Table 5.2: Weight loss of didanosine crystals from methanol/water mixtures

Sample (% methanol)	Weight loss (%)	
	TGA	KF
100	18.4 ± 2.11	1.4 ± 0.2
90	33.2 ± 7.73	12.9 ± 7.42
80	19.0 ± 11.79	11.2 ± 2.72
70	15.4 ± 0.69	12.2 ± 7.87
60	28.5 ± 5.22	18.1 ± 2.4
50	31.1 ± 0.86	22.1 ± 7.55
40	43.7 ± 10.81	31.6 ± 11.82
30	36.1 ± 11.59	27.9 ± 10.94
20	33.5 ± 5.77	27.5 ± 6
10	40.2 ± 0.88	34.1 ± 11.6

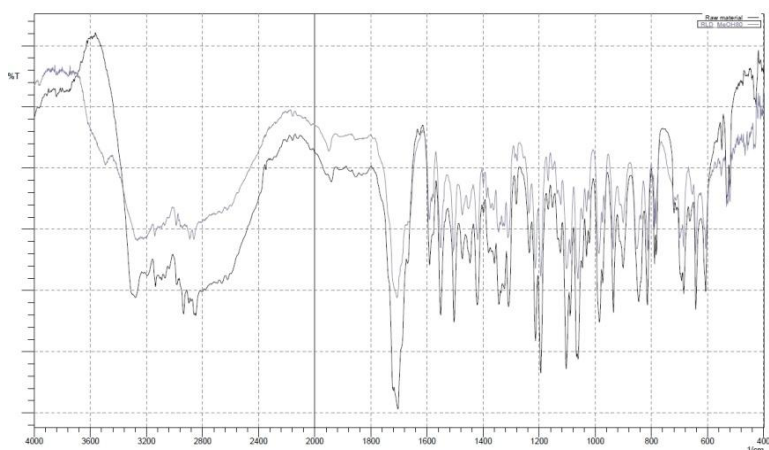
5.4.2 FTIR ANALYSIS

To investigate the solid-state molecular interactions between the didanosine molecules in the recrystallisation products from the methanol/water mixtures, FTIR was employed.

90% MeOH



80% MeOH



70% MeOH

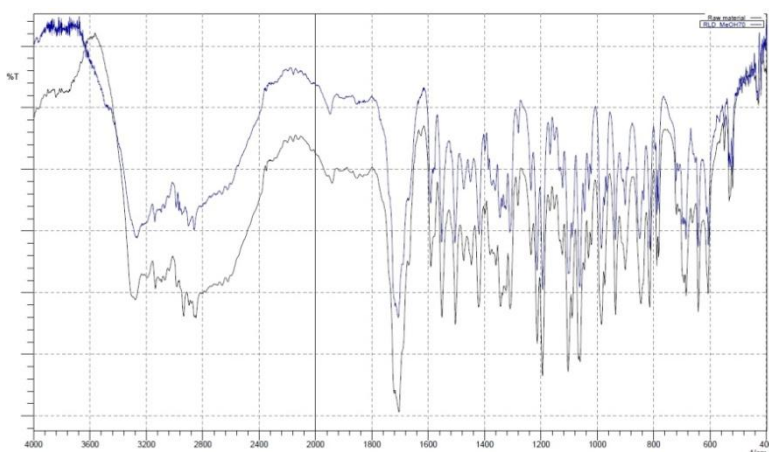
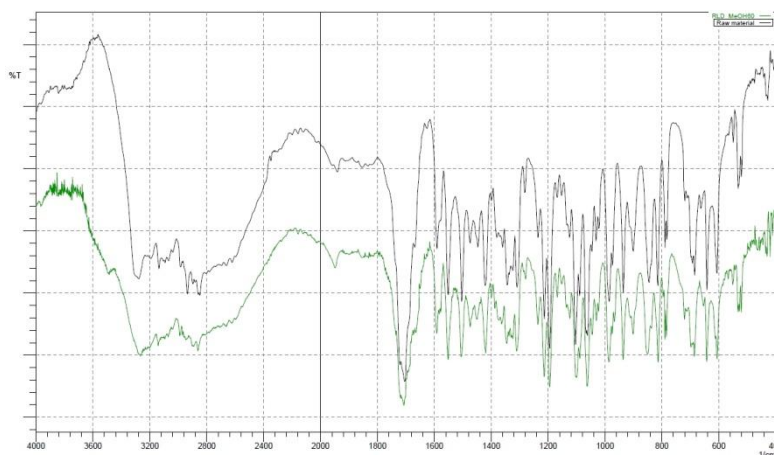


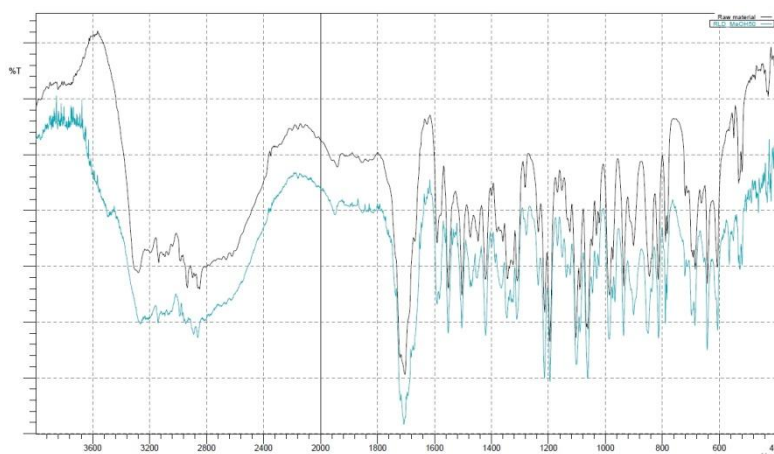
Figure 5.30: FTIR overlays of didanosine raw material (black interferogram) and its recrystallisation products from methanol and water mixtures.

The interferograms thereby obtained are presented here in figures 5.30 to 5.32.

60% MeOH



50% MeOH



40% MeOH

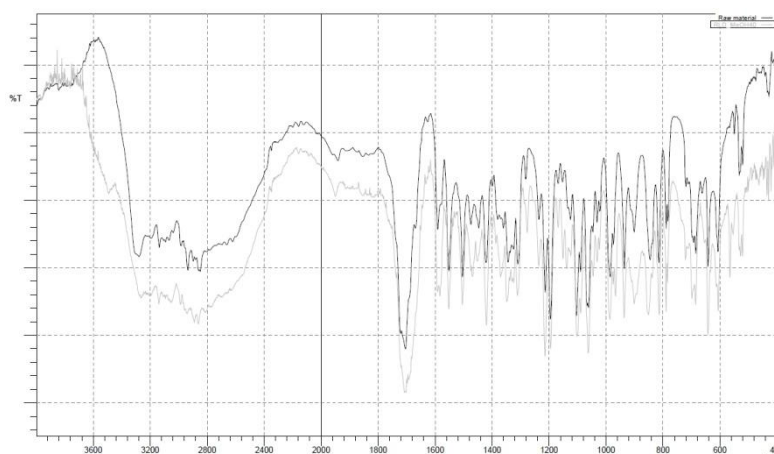
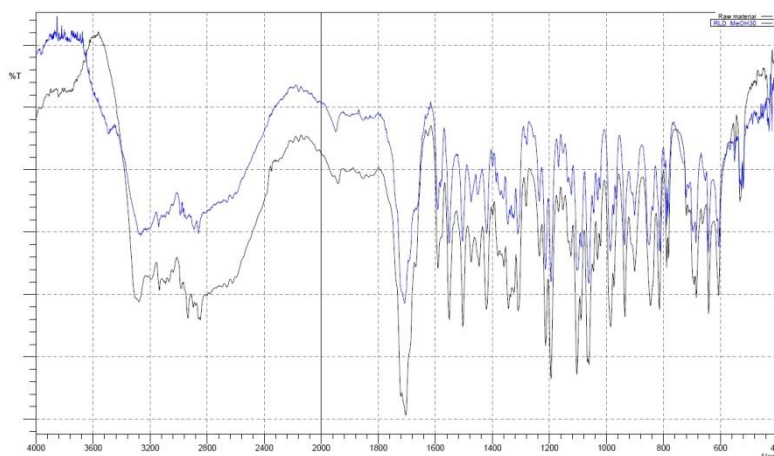


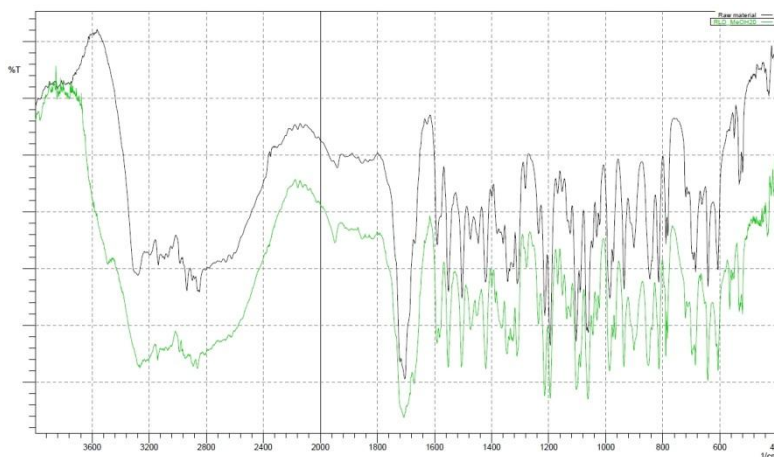
Figure 5.31: FTIR overlays of didanosine raw material (black interferogram) and its recrystallisation products from methanol and water mixtures.

With the exception of the broad weak peak between 3400 and 3600 cm⁻¹, the FTIR data correlates with those presented in section 5.3.3.

30% MeOH



20% MeOH



10% MeOH

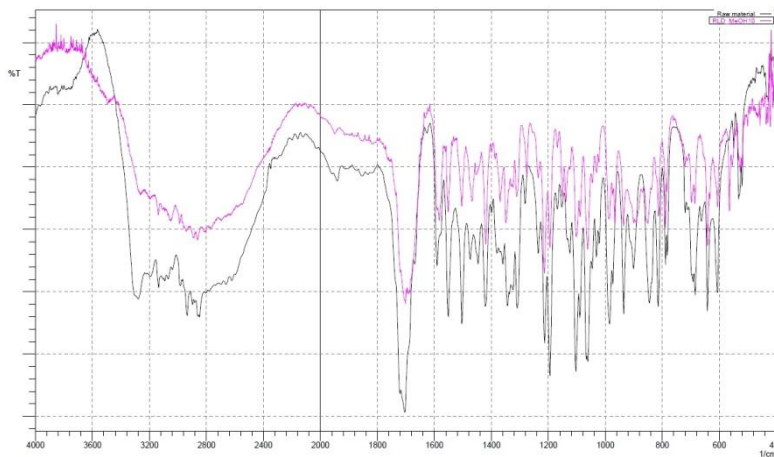


Figure 5.32: FTIR overlays of didanosine raw material (black interferogram) and its recrystallisation products from methanol and water mixtures.

The peak between 3400 and 3600 cm^{-1} is not present in the diffractograms of the crystals obtained from 100- and 90 % methanol and is consistent with the peak position and shape of a sample with a high concentration of alcohols (hydroxyl groups). Why the crystals obtained from the mixtures with the highest concentrations of methanol do not present this peak remains unanswered at this point. It is also possible that the peak between 3400 and 3600

cm^{-1} is the result of a change in the intermolecular interactions of amine groups. Although the absorption peaks of amine groups are usually very strong, secondary amine groups have been known to present as a weak band at various wave numbers above 3000 cm^{-1} . The hypoxanthine moiety of the didanosine molecule contains aromatic amine groups but resonance stabilisation of the aromatic hypoxanthine moiety, possibly due to electron pushing or pulling effects can change the absorption behaviour of these amine groups. The possibility of keto-enol tautomerism, as proposed by Bettini *et al.* (2010:1857), is also a possibility. Not only will it introduce more hydroxyl groups to the system, but it will also lead to resonance stabilisation of the hypoxanthine moiety. Then again, it could simply be water adsorbed to the surface of the crystals. SXRD would have proven invaluable in elucidating the origin of this peak, if only suitable crystals could be obtained. Any of these changes in molecular interactions can cause a change in molecular packing and, if a change in the molecular packing in the unit cell occurs, it will have an influence on the unit cell dimensions and a change in the unit cell's planes, which can be detected with PXRD.

5.4.3 PXRD ANALYSIS

To investigate the unit cell dimensions of the recrystallisation products from methanol and water mixtures, PXRD was employed.

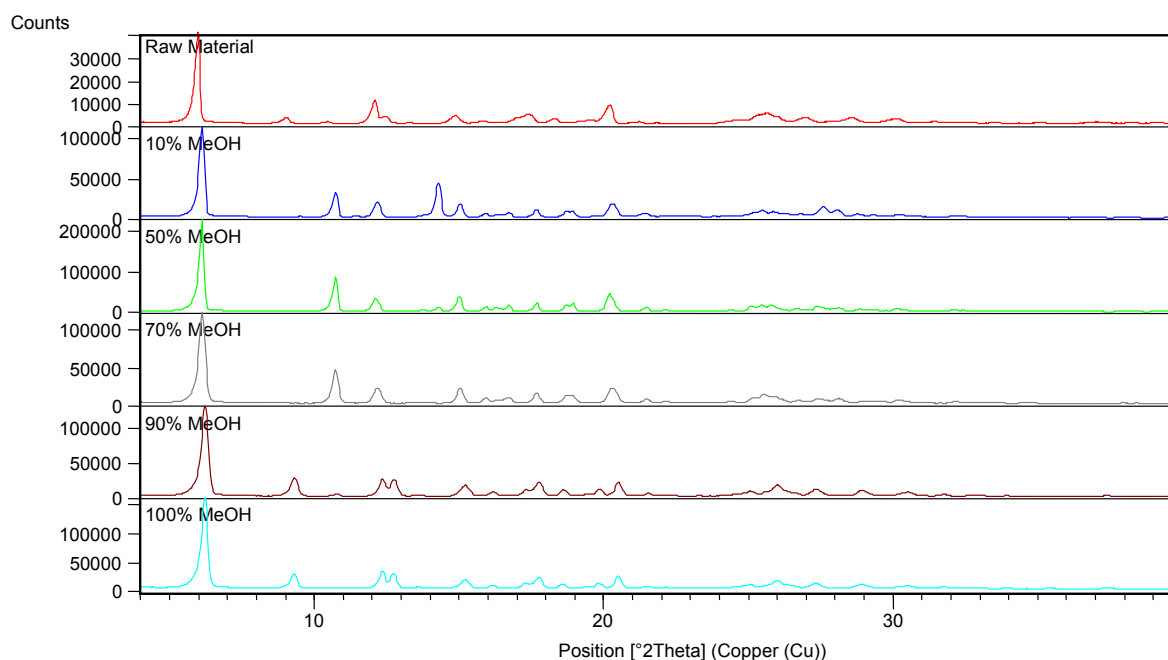


Figure 5.33: PXRD analysis of the differences in unit cell dimensions between didanosine raw material and its recrystallisation products from methanol/water mixtures.

Based on thermal and absorption behaviour, certain recrystallisation products were chosen for figure 5.33. The recrystallisation products from 100- and 90 % methanol did not exhibit the weak absorption peak between 3400 and 3600 cm^{-1} and were included in the overlay. The recrystallisation products from 80- and 70 % methanol shared similar thermal and absorption behaviour therefore the product from 70% methanol was included. From 60 % methanol onwards the recrystallisation products had similar absorption behaviour and from 50 % methanol they shared the same thermal behaviour and therefore the diffractogram of the product from 50 % methanol is included. The thermal and absorption behaviour of the product from 10 % methanol differed from the other products and therefore it was included in the overlay.

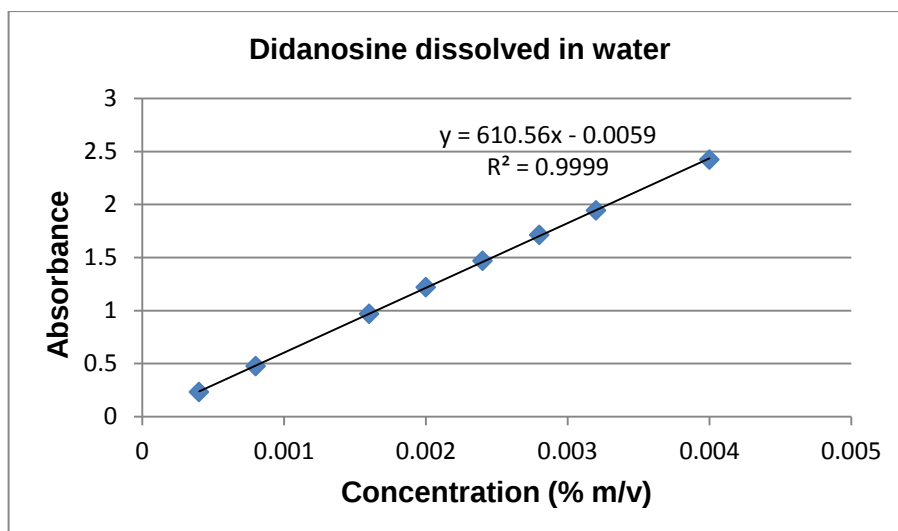
There is a clear difference in unit cell dimensions between the didanosine raw material and the recrystallisation products from the methanol/water mixtures. The peak at $10.7^\circ 2\theta$, which was the basis for identifying the recrystallisation product from methanol as a new polymorph, re-emerges in the products obtained from 90 % methanol onwards and the peak at $12.7^\circ 2\theta$ disappears. Furthermore, the peak at $9.3^\circ 2\theta$, which is present in the raw material and the recrystallisation products from 100- and 90 % methanol, is not present in the products from the more diluted methanol mixtures. A peak appears at $14.3^\circ 2\theta$ for the product from 50 % methanol and the more diluted mixtures, which is not present for the products from the more concentrated methanol mixtures. This peak's intensity is exceptionally high for the product from 10 % methanol, indicating a high electron density in that plane of the unit cell. Although the most conclusive method of proving differences in molecular packing between the recrystallisation products and the raw material would have been SXRD, the inability of didanosine to form crystals of adequate size means that PXRD will have to suffice.

The clear differences in unit cell dimensions conclusively prove that all of the recrystallisation products from methanol/water mixtures are polymorphs, not only of each other but most importantly, of the raw material. The PXRD patterns reported here in figure 5.33 differ from those reported by Bettini *et al.* (2010:1862), indicating that new didanosine polymorphs are presented in this study.

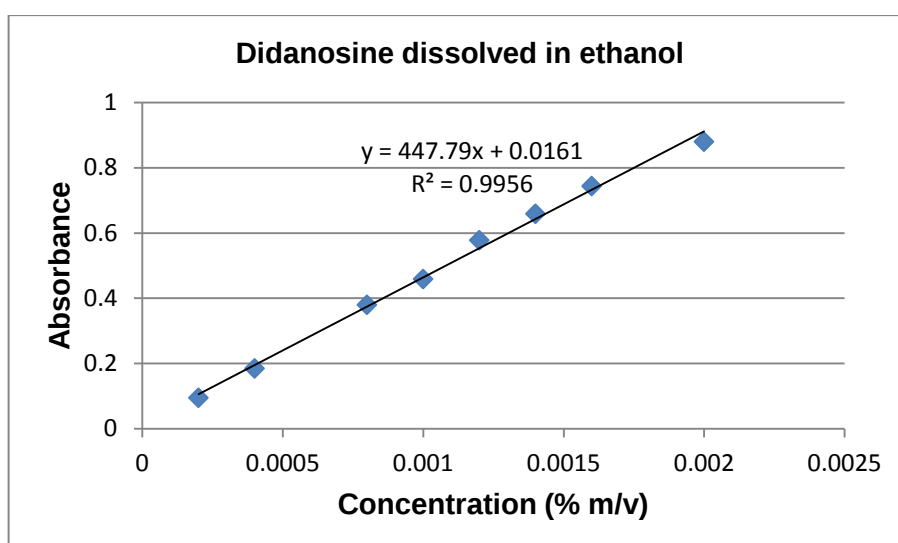
5.5. SOLUBILITY STUDY

To investigate the solubility of the novel polymorphs prepared in this study, 6 oversaturated solutions of the raw material and its recrystallisation products were prepared in double distilled water, 0.1 N HCl and ethanol. The mixtures were stirred overnight (for 24 hours) in a water bath kept at 310 K (37°C).

Water



Ethanol



0.1 N HCl

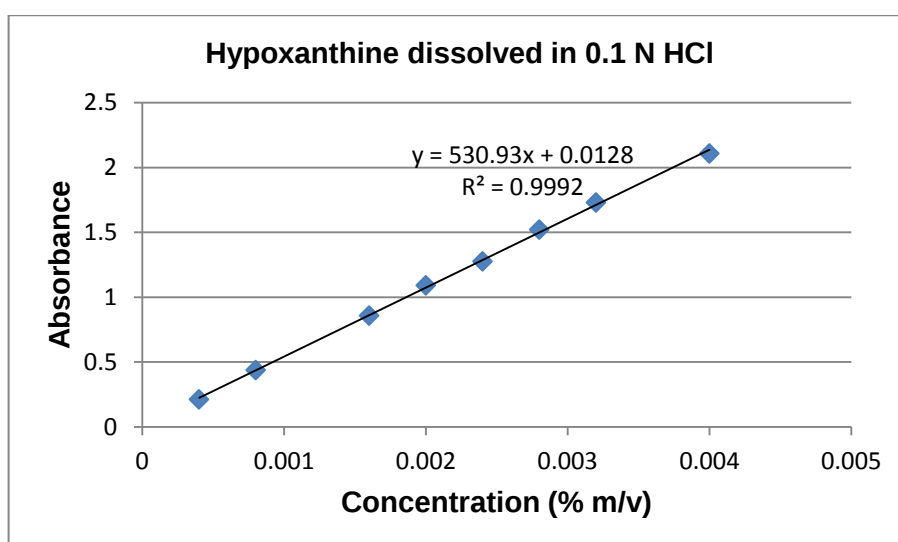


Figure 5.34: Standard curves for didanosine and hypoxanthine.

The time span of 24 hours was chosen as this represented the maximum amount of time needed for the sample to reach peak solubility.

Standard traces were drawn for didanosine in double distilled water and ethanol and for hypoxanthine in 0.1 N HCl, since the didanosine raw material and crystals will definitely have hydrolysed to hypoxanthine after 24 hours (figure 5.34). Both didanosine and hypoxanthine absorb UV radiation at 250 nm, in water and 0.1 N HCl respectively, while didanosine exhibits 2 absorbance peaks in ethanol, at 245- and 250 nm. The ratio of the absorbance peaks in ethanol is fixed and therefore, so too is the concentrations determined from them. It does not matter which peak is used, since both will yield the same concentration, but for this study the peak at 245 nm was used. The reason for working with this peak will become clear in the next section. Didanosine is only sparingly soluble in ethanol, and this lead to the standard trace from ethanol having a weak regression value when compared to that of the trace from water, in which didanosine is freely soluble. The solubility of didanosine raw material and its recrystallisation products from methanol and water mixtures are given in figure 5.35.

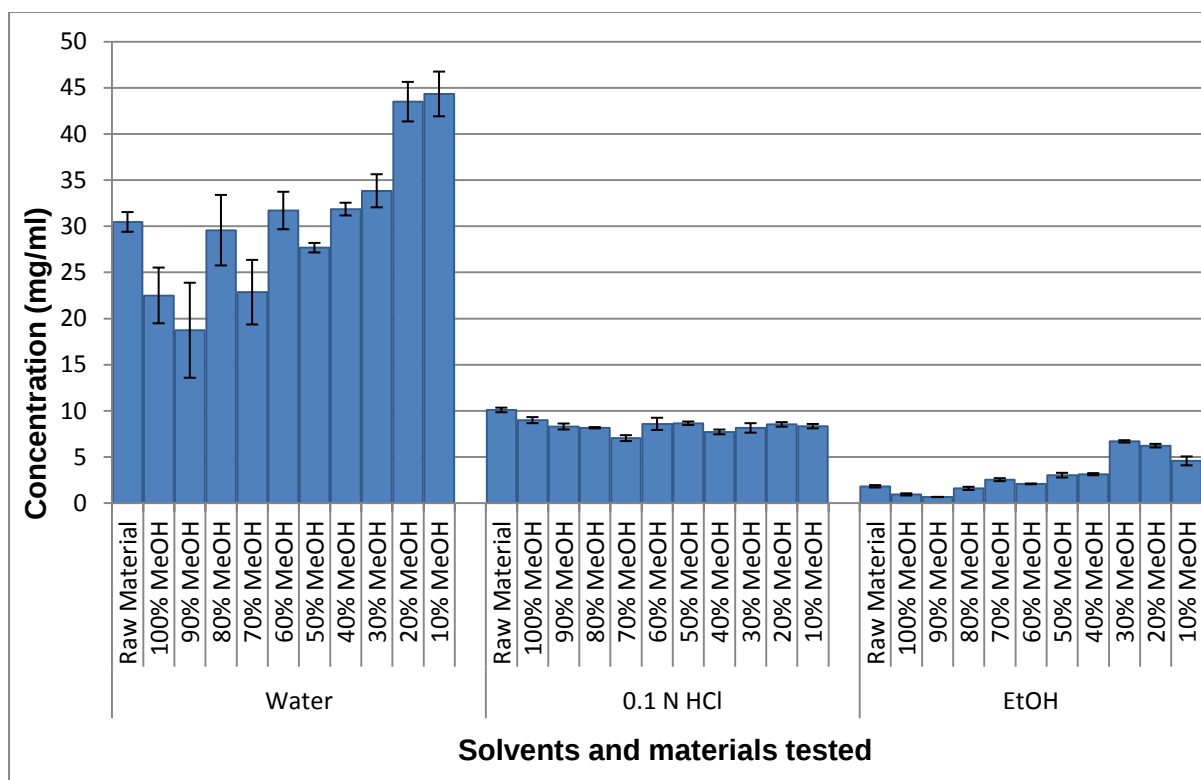


Figure 5.35: Solubility of didanosine raw material and its recrystallisation products from methanol and water mixtures.

There is no clear relation between the solubility and the medium from which the crystals were obtained. The fact that there is no clear variance between the concentrations obtained from 0.1 N HCl indicates that all the didanosine samples tested had completely hydrolysed to hypoxanthine, and that hypoxanthine itself is only moderately soluble in 0.1 N HCl. Although the solubility of the crystals obtained from 10- and 20 % methanol is greater than that of didanosine raw material, the raw material itself is so freely soluble in water that it is doubtful if this increase in solubility will have a noteworthy clinical effect. The increased solubility of the crystals from 30- to 10 % methanol in ethanol can have some pharmaceutical relevance, especially when deciding on a dosage form, however, the values remain quite small. The exact solubility values from which the histogram in figure 5.35 was drawn are presented in table 5.3.

Table 5.3: Solubility of didanosine raw material and its recrystallisation products from methanol and water mixtures

Sample	Solubility in solvent (mg/ml)		
	Water	0.1 N HCl	Ethanol
Raw Material	30.5 ± 1.07	10.1 ± 0.25	1.8 ± 0.13
100 % methanol	22.5 ± 3.02	9.0 ± 0.33	0.9 ± 0.12
90 % methanol	18.7 ± 5.15	8.3 ± 0.32	0.7 ± 0.01
80 % methanol	29.6 ± 3.82	8.2 ± 0.07	1.6 ± 0.17
70 % methanol	22.9 ± 3.50	7.0 ± 0.32	2.5 ± 0.16
60 % methanol	31.7 ± 2.03	8.6 ± 0.66	2.1 ± 0.04
50 % methanol	27.7 ± 0.52	8.7 ± 0.19	3.0 ± 0.25
40 % methanol	31.9 ± 0.69	7.7 ± 0.26	3.1 ± 0.11
30 % methanol	33.8 ± 1.79	8.1 ± 0.51	6.7 ± 0.13
20 % methanol	43.5 ± 2.14	8.5 ± 0.24	6.2 ± 0.19
10 % methanol	44.3 ± 2.42	8.3 ± 0.24	4.6 ± 0.48

The reported aqueous solubility of didanosine in water is 27.3 mg/ml at 298 K (25°C). Considering this, a solubility value of 30.5 ± 1.07 mg/ml for didanosine raw material at 310 K seems reasonable. Before making assumptions concerning the origins of the strange solubility behaviour of the recrystallisation products, it was decided to investigate the morphology of these crystals as a possible explanation.

5.6 MORPHOLOGY AND ACID LABILITY

To investigate the morphological differences between the didanosine crystals obtained from methanol/water mixtures, SEM was employed (figures 5.36 to 5.37).

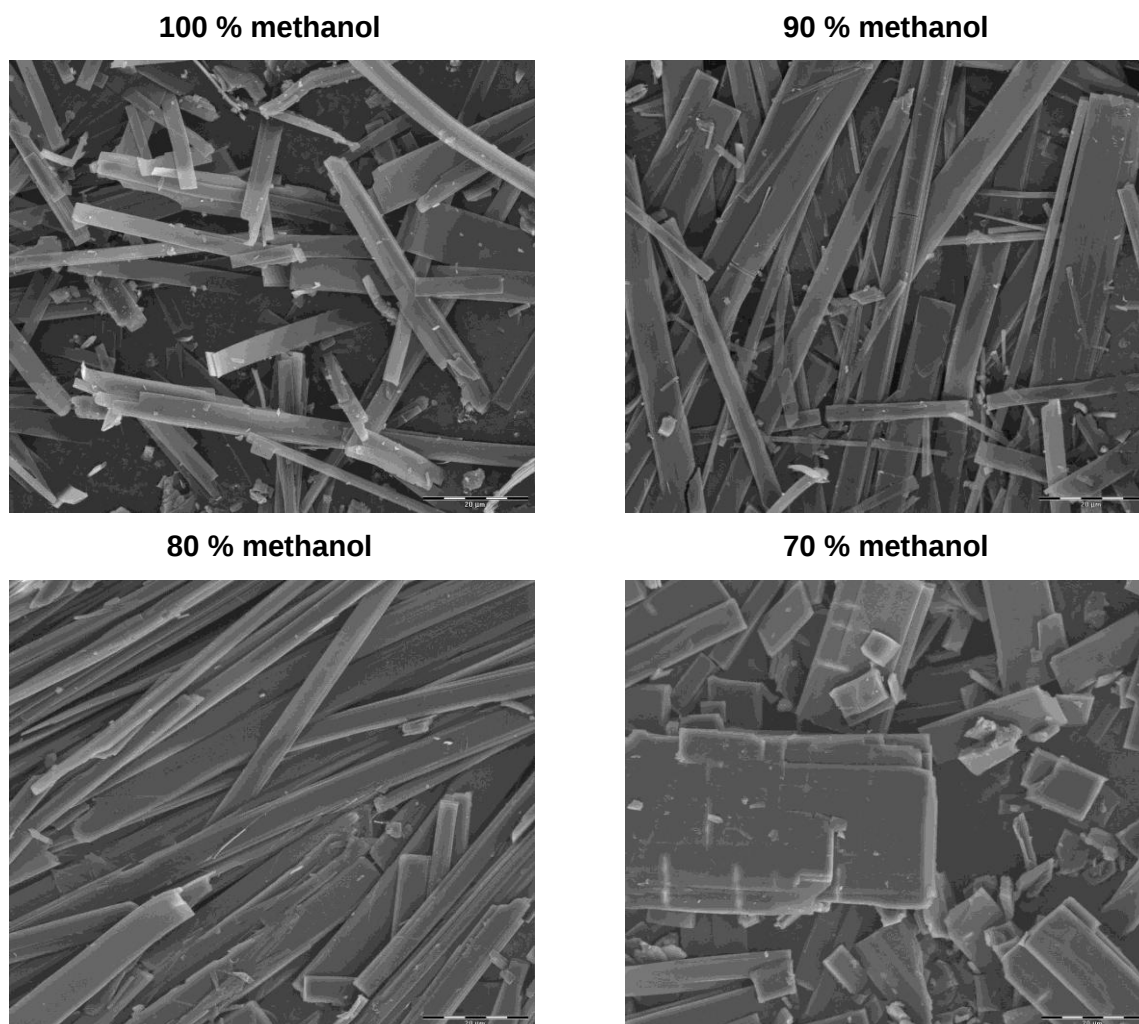


Figure 5.36: SEM micrographs of didanosine crystals from methanol and water mixtures.

The crystals obtained from methanol/water mixtures increase in thickness up to those obtained from 70 % methanol, after which they decrease in thickness until they reach the extremely fine morphology of the crystals obtained from 10 % methanol. The crystals from 70- and 60 % methanol took several days longer to form than the rest, and their morphology is consistent with the BDFH Law described in chapter 2 (section 2.5). The extra thickness of the crystals obtained from 70 % methanol increased its brittleness, while the thinner crystals were visibly more flexible. The enhanced solubility of the crystals from 10-, 20- and 30 %

methanol can be attributed to their morphology, where their thinner structure increases the effective surface area and subsequently their solubility.

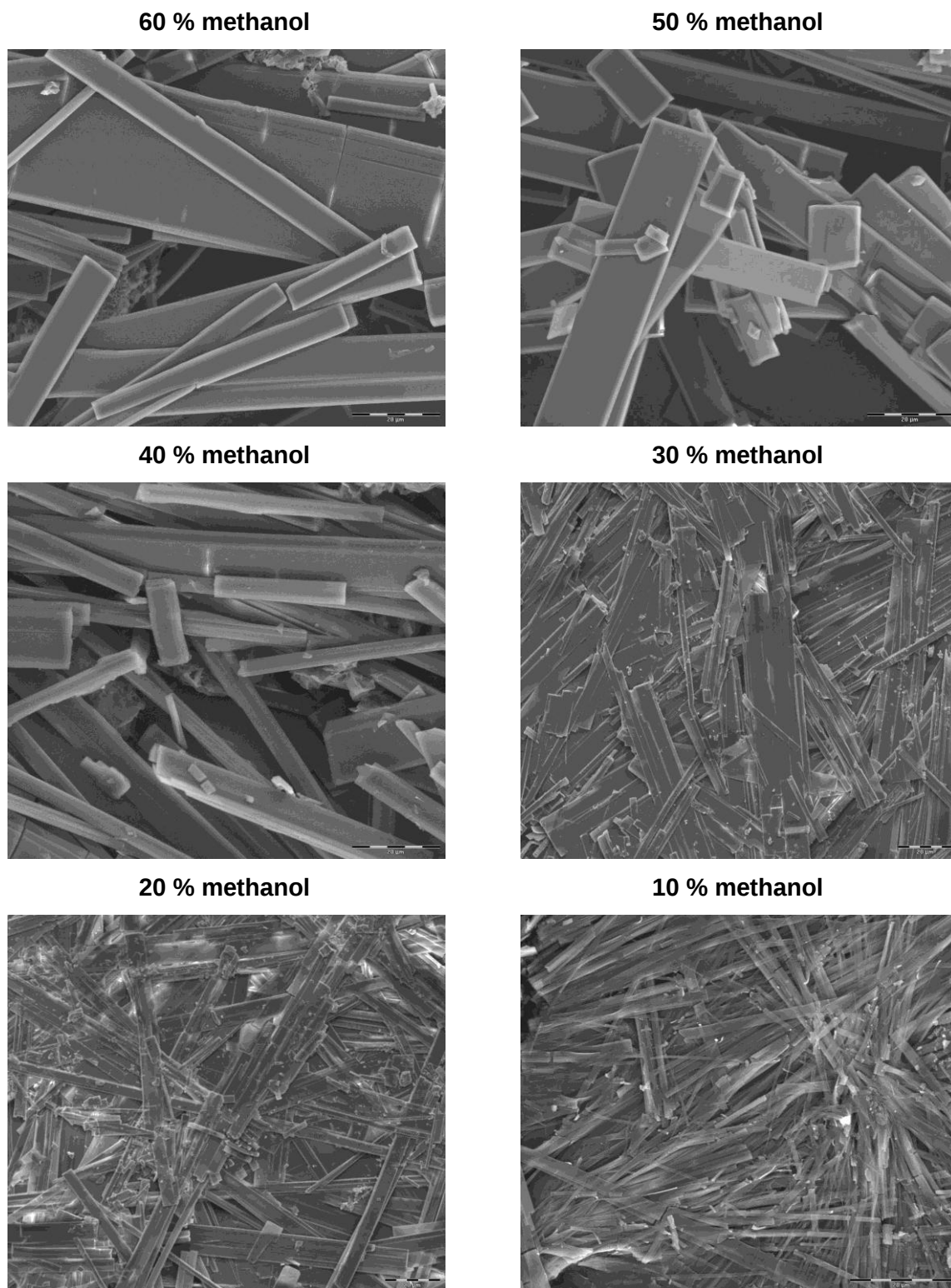


Figure 5.37: SEM micrographs of didanosine crystals from methanol and water mixtures.

An increase in effective surface area can also arise from the cracks that form in the thick, brittle crystals obtained from 60 % methanol. Stirring in the water bath can cause these crystals to break into many smaller pieces, thereby increasing its solubility. The crystals from 70 % methanol grew the slowest, inferring an increased stability in these crystals and explaining their weak solubility.

Concerning the acid lability of didanosine, an enteric coated preparation is already on the market. Since the contact angle (and hence the wettability) can also influence acid lability, it was decided to investigate the influence the morphology of the new didanosine polymorphs reported in study on its acid lability. It is reasonable to assume that the monolithic morphology of the polymorphs may offer protection against acid hydrolysis which the raw material cannot. Despite numerous attempts to determine the contact angle of the crystals prepared from methanol/water mixtures, all of the methods used failed. From the beginning of the study, the didanosine crystals obtained from alcohols were difficult to dry thoroughly and, upon reaching 100 % dryness the crystals lost their morphology and polymorphic character and reverted back to the raw material. The thin film of solvent adsorbed to the surface of the crystals as well as the capillary solvent made it impossible to punch tablets, make plates and even to use the Washburn method to determine the contact angle. Despite the inability to determine the contact angle, it was decided to proceed with the acid lability study, even if only as a proof of concept study.

To monitor acid hydrolysis in real time, UV spectrophotometry was employed. It was mentioned during the solubility study that didanosine exhibited 2 absorbance peaks in ethanol and, one of these peaks, the one at 245 nm, was unique to didanosine (figure 5.37). Hypoxanthine dissolved in ethanol does not present a peak at 245 nm. Clearly the presence of the ribose ring, and some subsequent change in π -electron distribution in the aromatic hypoxanthine moiety while in the presence of ethanol, is needed for didanosine to exhibit the peak at 245 nm. If hydrolysis has taken place, and didanosine has lost its ribose ring, the peak at 245 nm disappears. To determine the rate of hydrolysis in real time, the rate at which the peak at 245 nm disappeared was monitored. When no further decrease in absorbance was observed the reaction was considered complete.

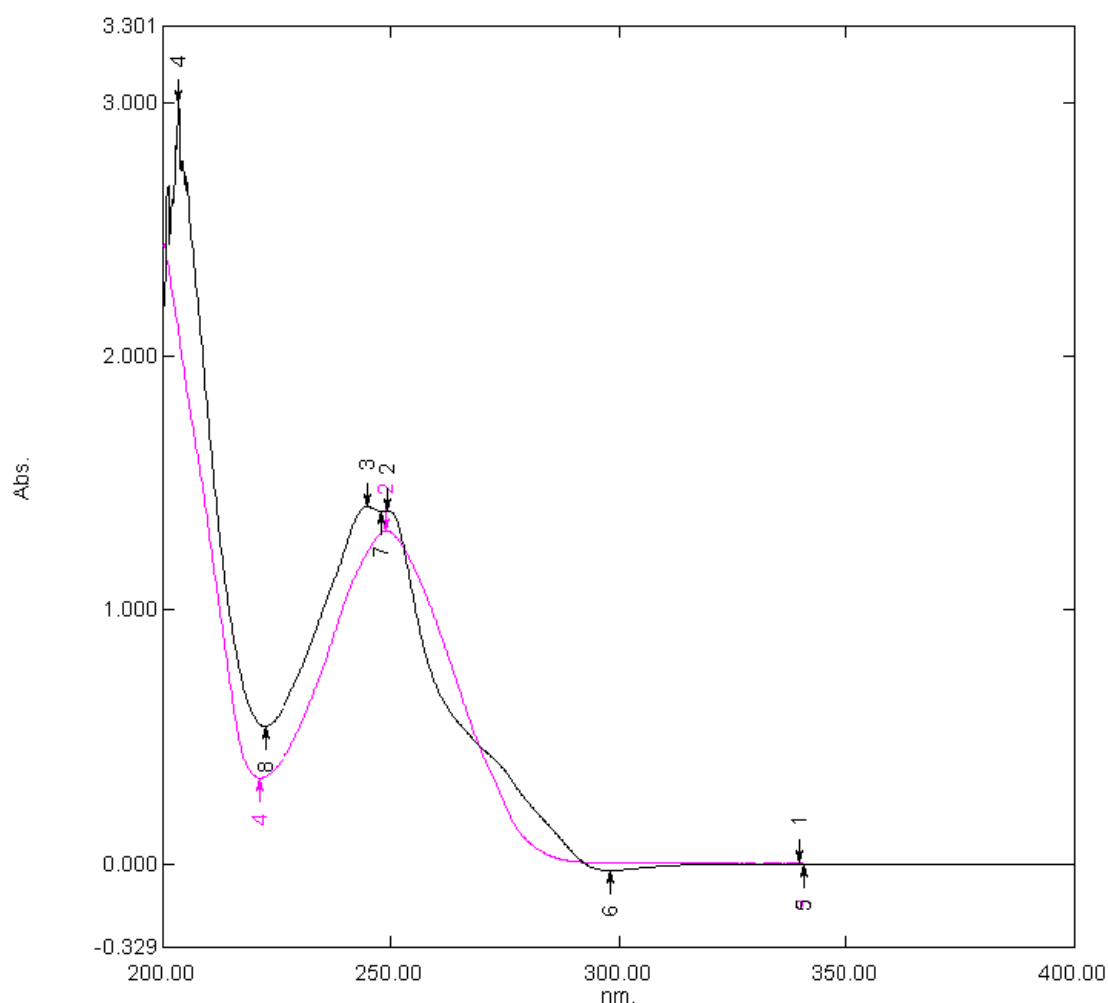
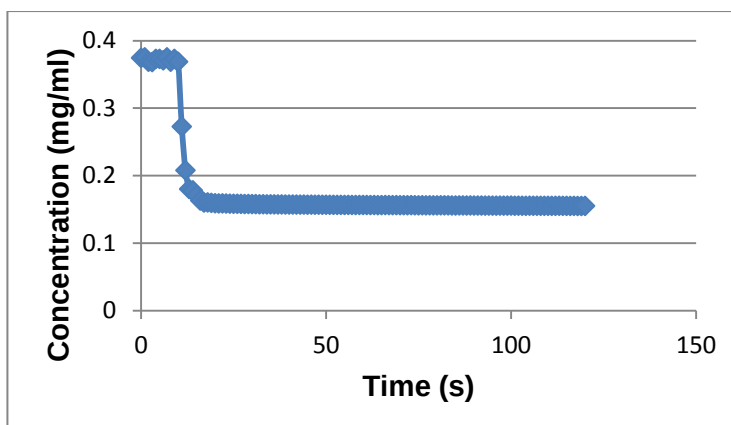


Figure 5.37: UV absorbance spectra of equimolar amounts of didanosine (black trace) and hypoxanthine (pink trace) in ethanol and 0.1 N HCl respectively.

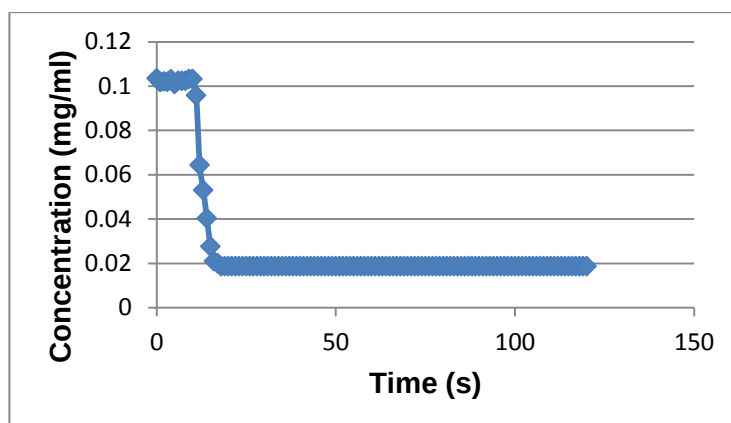
The analysis was carried out by dissolving didanosine raw material (as reference) and its recrystallisation products from methanol and water mixtures in 3 ml ethanol. The kinetics run was started on the UV-vis spectrophotometer, taking readings at 0.1 second intervals over a total run time of 120 seconds. After a 10 second lag time, 1 ml 0.1 N HCl was injected into the system and the decrease in absorbance at 245 nm was monitored.

After compensating for dilution and respective solubility, the concentration against time curves are presented in figure 5.38. The hydrolysis of didanosine was extremely rapid. Within 10 seconds of the 0.1 N HCl added no difference between the didanosine and hypoxanthine peaks could be detected and the hydrolysis was considered complete. Apart from small differences in concentration changes relative to time, there was no significant difference in the hydrolysis rate between the raw material and the recrystallisation products from 100-, 50- and 10 % methanol.

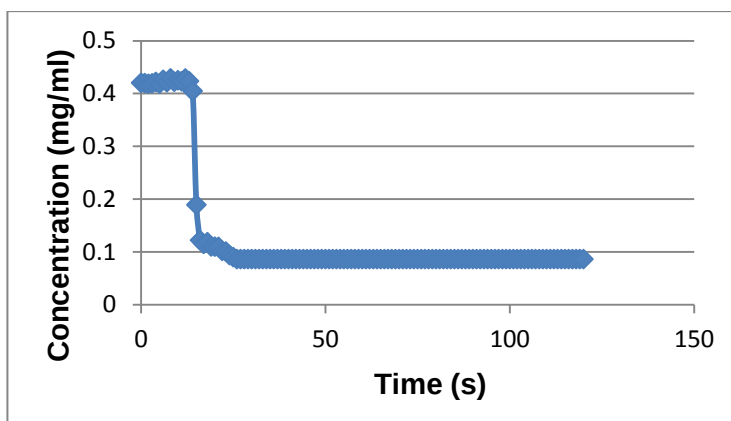
Raw Material



100 % MeOH



50 % MeOH



10 % MeOH

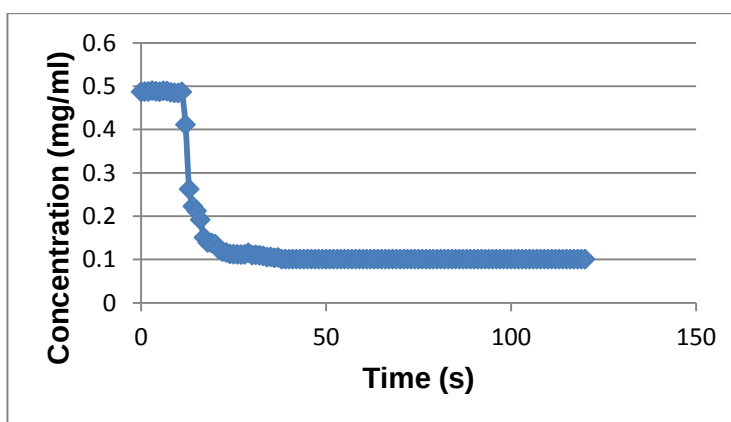


Figure 5.38: Real time hydrolysis study of didanosine and its recrystallisation products.

The morphologies of the crystals do not appear to have an influence on the acid lability of didanosine. The reason is the same as for the contact angle problem, namely the fact that the crystals are already wet. The thin film of solvent around the crystals eliminates the contact angle; any liquid coming into contact with the crystal is automatically dispersed across its surface.

5.7 CONCLUDING REMARKS

This study presents not only the first thorough screening of polymorphism in didanosine, but also the first detailed physicochemical analysis of didanosine. The data obtained from this study can be of significant use during formulation of didanosine preparations, as well as during *in vitro* and *in vivo* analyses. New polymorphs were obtained from methanol and dilutions thereof and the physicochemical properties of these novel polymorphs were reported.

Didanosine did not form a wide range of polymorphs, as seen from PXRD and SEM data, which can be attributed to the structural rigidity of the molecule. The hypoxanthine moiety is aromatic, and therefore flat, with the only point of rotation being the glycosidic bond (figure 5.1). This drastically limits the amount of conformations which the molecule can adopt and therefore the amount of energy optimised molecular coordinations on the energy landscape. The polymorphs (and even the other crystals from alcohols and acetonitrile) were metastable and displayed rapid and total transformation to the raw material state upon stress added to the system. Despite the preparation of new polymorphs, the inherent aqueous solubility of didanosine is so high, that the improvements shown by its polymorphs are of little significance. The morphologies of the polymorphs also did not offer significant protection against acid hydrolysis relative to the raw material.

The most interesting find was probably the adsorption of solvent to the surface of the crystals and the capillary solvent trapped in the mesh network of didanosine crystals. Although this solvent film coating the crystals made thermal analysis irreproducible, determination of the contact angle impossible and negated the effect of morphology on wettability, it is irrevocably needed for the polymorphs (and other didanosine crystals from alcohols) to maintain their structural integrity and thermal behaviour.

Acid hydrolysis of didanosine remains a concern, the rate of which was shown in this study to be far more rapid than initially believed. It is this researcher's opinion health practitioners should, as far as possible, dispense the enteric coated preparation.

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