Solid-State Properties of Tobramycin

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Tobramycin (I) obtained from two different sources was subjected to powder X-ray diffractometry, thermal analyses, and Karl Fischer titrimetry. It was concluded to be tobramycin monohydrate ($C_{18}H_{37}N_5O_9 \cdot H_2O$). When heated in the differential scanning calorimeter (DSC), the dehydration of I resulted in the formation of metastable anhydrous tobramycin, which melted at 164°C. This was followed by the crystallization of the stable anhydrous tobramycin, which then melted at 217°C. The polymorphic transition was concluded to be monotropic and the calculated free energy difference between the metastable and the stable forms, at 25°C, was 348 cal · mol^{-1}. Both the heating rate in the DSC and the sample size had a significant influence on the enthalpy values of most of the thermal events. These observations were attributed to the presence of trace amounts of moisture in the sample. No detectable decomposition of I occurred when it was heated up to 224°C.

KEY WORDS: tobramycin; thermal analyses; polymorphism; solid-state.

INTRODUCTION

Tobramycin is an aminoglycoside antibiotic used almost exclusively in the treatment of gram-negative bacterial infections (1). It is commercially available as a crystalline free base (Sigma Chemical Company, St. Louis, MO) ($C_{18}H_{37}N_5O_9$) and it exists as a sulfate salt [$(C_{18}H_{37}N_5O_9)_2 \cdot 5H_2SO_4$] in the marketed parenteral dosage form (Nebicin, Eli Lilly and Company, Indianapolis, IN). The free base has been reported to occur as a monohydrate ($C_{18}H_{37}N_5O_9 \cdot H_2O$), a dihydrate ($C_{18}H_{37}N_5O_9 \cdot 2H_2O$), and a trihydrate ($C_{18}H_{37}N_5O_9 \cdot 3H_2O$) (2,3). This investigation deals with the characterization of the solid-state properties of tobramycin base. In addition, the thermal stability of tobramycin was studied using a high-pressure liquid chromatographic (HPLC) method (4).

MATERIALS AND METHODS

Tobramycin base (I) (Eli Lilly, Sigma), kanamycin B sulfate (hereafter referred to as kanamycin), tris(hydroxymethyl)aminomethane (Sigma), acetonitrile, chloroform, monobasic potassium phosphate (Mallinckrodt), and 2,4,6-trinitrobenzenesulfonic acid (Pierce) were used as received. A sample of the USP Tobramycin Reference Standard was also obtained. I and kanamycin were each stored in a tightly closed screw-capped container at 4°C in a chamber maintained at 0% relative humidity (RH). Unless otherwise mentioned, I obtained from Eli Lilly was used for the studies.

Thermal Analyses

A differential scanning calorimeter (Model 910, Du Pont) and a thermogravimetric analyzer (Model 951, Du Pont) were connected to a thermal analysis operating system (Thermal Analyst 2000, Du Pont). The differential scanning calorimeter (DSC) was calibrated with indium (Du Pont). Typically, about 3 mg of sample was weighed into an aluminum pan, and the pan crimped nonhermetically and heated in the DSC from 30 to 250°C under a stream of nitrogen. The heating rate was 10°C/min, unless otherwise stated. The DSC work was carried out under a variety of experimental conditions. The specific details are provided under Results and Discussion. The mechanical cooling accessory permitted programmed cooling of the samples. In all cases, the peak temperature, i.e., the point of maximum deviation from a horizontal baseline was calculated (5). For the thermogravimetric analysis (TGA), about 10 mg of sample was weighed into platinum pans and heated from 30 to 250°C, at 10°C/min, under a stream of nitrogen.

The thermomicroscopic studies were performed by placing about 0.1 mg of the sample on a glass slide and observing it while heating on a hot-stage microscope (Thermosystem FP800, Mettler).

Powder X-Ray Diffractometry

Samples were exposed to CuKα radiation (40 kV × 30 mA) in a wide-angle X-ray diffractometer (Model D500, Siemens). The instrument was operated in the step scan mode, in increments of 0.02° 2θ. The angular range was 5 to 25° 2θ and counts were accumulated for 1 sec at each step. The limited amount of sample available did not permit the use of a regular X-ray sample holder. Instead, double-stick tape (3M) was mounted on a glass holder, and the powder sprinkled on the tape and exposed to radiation. In the absence of sample, the tape mounted on the glass holder exhibited a broad amorphous halo between 5 and 25° 2θ.

Karl Fischer Titrimetry

The water content of I was determined using a Karl Fischer titrimeter (Model CA-05 Moisture Meter, Mitsubishi).

HPLC Analysis

The equipment consisted of a pump (Model LC-6A) programmed by a system controller (Model SCL-6A), an autoinjector (Model SIL-6A), an ultraviolet–visible spectrophotometric detector (Model SPD-6AV), and an integrator (Model C-R5A), all from Shimadzu, Japan. Following the derivatization of I and kanamycin (internal standard) with 2,4,6-trinitrobenzenesulfonic acid, the derivatives were extracted into chloroform, evaporated to dryness, reconstituted in acetonitrile, and injected onto the HPLC. The mobile phase was a mixture of acetonitrile and phosphate buffer (62:38, v/v) and the separation was carried out on a reversed-phase

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octyl column. The column effluent was monitored at 340 nm. The assay method was stability-indicating, and the full details of the assay procedure are described elsewhere (4).

RESULTS AND DISCUSSION

The powder X-ray diffraction pattern of I was identical to the powder pattern of the USP Tobramycin Reference Standard (Table I). The powder patterns of tobramycin base and tobramycin sulfate are not listed in the powder diffraction files of the International Centre for Diffraction Data (6).

The powder X-ray diffraction pattern of tobramycin base obtained from Sigma Chemical Company was superimposable on the powder pattern of the material from Eli Lilly. The results of thermal analyses and Karl Fischer titrimetry led to the conclusion that the materials from the two sources were identical.

Heating I up to 250°C in the DSC resulted in endotherms at 114, 164, and 217°C and an exotherm at 198°C (Fig. 1). Thermomicroscopy of I revealed three events. Melting was observed at about 163°C, crystallization at approximately 195°C, and melting again at 220°C. When I was subjected to TGA, a weight loss of 4.74 ± 0.05% (mean ± SD; $n = 4$) occurred between 30 and 120°C (Fig. 2a). The weight loss between 120 and 250°C was small (~0.2%). The water content of I, determined by Karl Fischer titrimetry, was 5.06 ± 0.04% (w/w; $n = 3$).

It was of interest to determine if any of the transitions observed on heating I in the DSC were reversed when the sample was cooled. Therefore, I was heated up to 140°C, so that the first endothermic event was complete, and cooled back to room temperature at 10°C/min. No exotherm was observed around 114°C, indicating that the thermal event was not readily reversed. This conclusion was confirmed by reheating this sample to 250°C, during which time the endotherm at 114°C did not occur. Fresh samples of I were heated to 180 and 209 and 224°C and cooled to room temperature at 10°C/min. In no case was any transition observed during the cooling of the sample. These studies led to the conclusion that none of the transitions observed on heating the sample were readily reversed on cooling.

Based on DSC and TGA, the endotherm at 114°C was attributed to a dehydration reaction. Since this temperature was above the boiling point of water, the dehydration would have been accompanied by vaporization of water. It was indicated earlier that tobramycin can exist in different states of hydration (2,3). The monohydrate, dihydrate, and trihydrate forms of tobramycin will contain, respectively, 3.7, 7.1, and 10.3% (w/w) water. Karl Fischer titrimetry indicated 5% (w/w) water content and this was supported by the TGA. Therefore I was likely to be tobramycin monohydrate containing some absorbed water. The hygroscopic nature of tobramycin is documented in the literature (7,8).

The derivative thermogravimetric curve supported the above conclusion. It showed maxima at approximately 70 and 105°C, suggesting that the weight loss during TGA occurred in two stages (Fig. 2b). From the nature of the derivative thermogravimetric curve, it could be concluded that there was overlapping of the two reactions (9). Though the first stage of the weight loss (up to about 85°C) was expected to be due to both desorption and dehydration, it was likely that the former reaction predominated. The second stage of the weight loss was expected to be predominated by the dehydration process. TGA was carried out in an open container under a stream of nitrogen, and because of the unrestricted nature of the sample, the dehydration was expected to occur over a broad temperature range (10).

According to the phase rule, a mixture of hydrates cannot exist in stable equilibrium over a range of vapor pressures at a constant temperature. However, since the existence of such mixtures has been reported in the literature (11,12), it was necessary to confirm that I was not a mixture of hydrates. I was stored at room temperature in chambers maintained at 0 and 9% RH for 1 month. In both of these samples, the water content determined by Karl Fischer titrimetry was close to 5% (w/w) and the weight loss observed

Table I. Powder X-Ray Diffraction Data of I and of I Heated to Various Temperatures

I		I heated to 140°C		I heated to 180°C		I heated to 209°C	
d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity (%)	d-spacing (Å)	Relative intensity (%)
15.07	27	14.72	46	15.63	36	15.77	10
10.35	48	10.27	50	7.69	49	7.69	18
8.87	49	8.89	50	7.13	100	7.08	89
8.14	36	8.11	37	5.94	27	5.90	29
7.48	33	7.49	36	5.50	29	5.50	29
6.17	53	6.19	54	4.99	69	4.98	84
4.97	57	4.98	54	4.72	27	4.72	25
4.79	100	4.82	100	4.64	18	4.64	20
4.69	47	4.67	50	4.28	80	4.27	100
4.56	35			3.95	27	3.95	31
4.39	56	4.39	55	3.78	31	3.77	27
4.24	36	4.23	42	3.59	24	3.60	31
4.08	54	4.09	45				
3.91	31	3.91	38				
3.79	53	3.78	48				

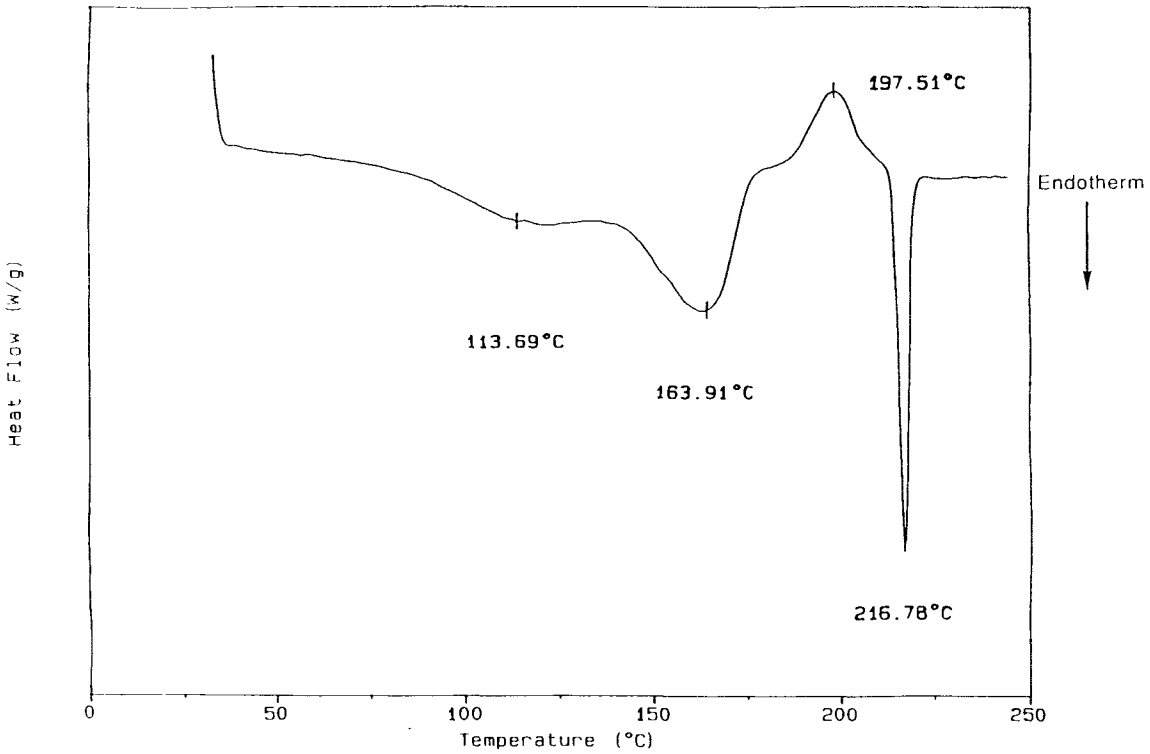

Fig. 1. Differential scanning calorimetric curve of tobramycin base (I). The sample weight was 3.10 mg.

in the TGA, between 30 and 120°C, was ~4.7%. Therefore, I stored at two relative humidities had about the same water content. This confirmed that I was not a mixture of hydrates.

Based on the direct evidence provided by thermomicroscopy, the endotherm at 164°C was attributed to melting of the solid and the exotherm at 198°C to crystallization of the melt. Therefore the anhydrous tobramycin formed on dehydration of I was likely to be a metastable form with a

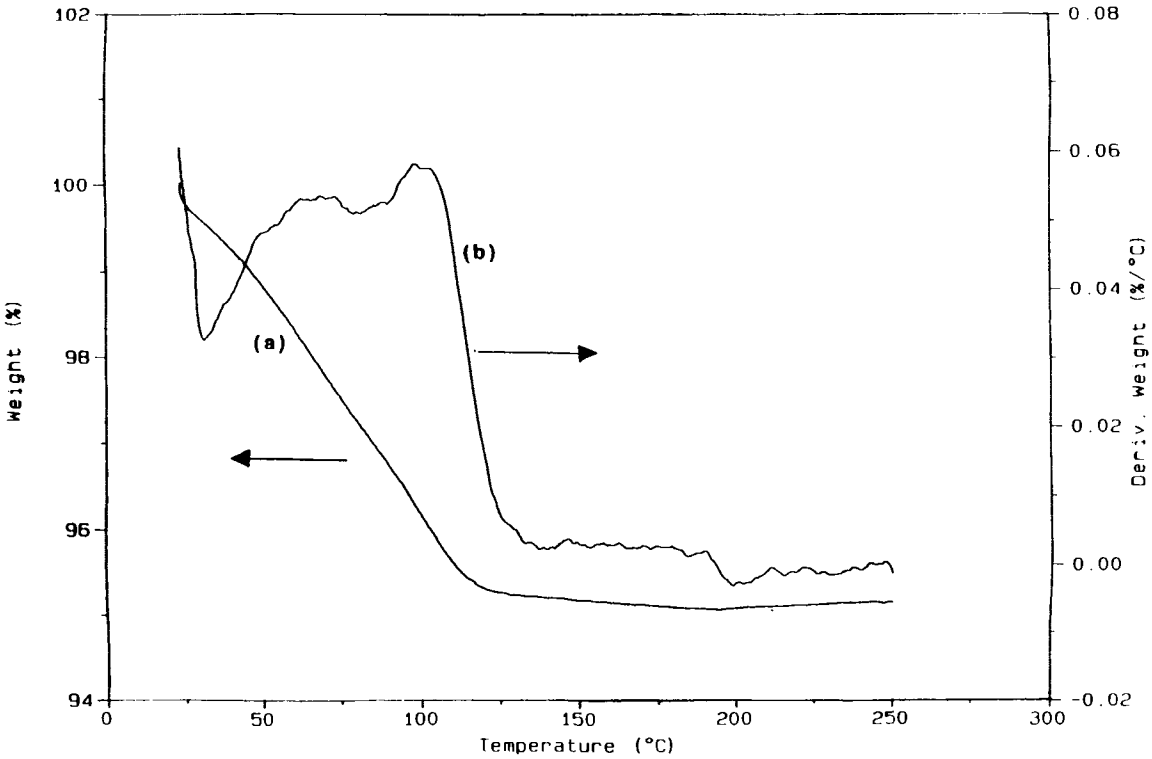

Fig. 2. Thermogravimetric (a) and derivative thermogravimetric (b) curves of I. The sample weight was 10.26 mg.

melting point of 164°C. The melting of the metastable anhydrous tobramycin was followed by the crystallization of the stable anhydrous tobramycin at 198°C. The stable anhydrous tobramycin melted at 217°C.

Powder X-ray diffractometry provided evidence supporting this conclusion. Samples of I were heated in the DSC to 140, 180, 209, and 224°C. In each case, the sample was allowed to cool back to room temperature under nitrogen purge, taken out, and immediately subjected to X-ray diffraction studies. The X-ray diffraction patterns of I and of I heated to 140°C were very similar (Table I). Based on crystal lattice studies, three cases have been distinguished following the dehydration of hydrates (13): (a) the crystal lattice of the residue is nearly identical to that of the original hydrate, (b) the residue is poorly crystalline, and (c) the residue recrystallizes with a different crystal lattice. The X-ray diffraction studies indicated that I belongs to the first case.

When the metastable anhydrous tobramycin was allowed to melt in the DSC and cooled at 10°C/min, the recrystallization of the metastable anhydrous tobramycin did not occur. Instead, partial crystallization of the stable anhydrous tobramycin seemed to have occurred. This conclusion was based on both DSC and X-ray results. When I was heated to 180°C, cooled, and reheated, the endotherm at 164°C due to the melting of the metastable form did not occur. In addition, the enthalpy of crystallization had a much lower value than the enthalpy of crystallization value obtained in samples of I heated directly from 30 to 250°C. This suggested that considerable crystallization of the stable anhydrous tobramycin had occurred between the first and the second heating. The powder X-ray diffraction patterns of I heated to 140°C was different from that of I heated to 180°C (Table I). However, I heated to 180°C and I heated to 209°C had identical powder patterns, indicating that the stable anhydrous tobramycin was formed in both these cases. I heated to 224°C exhibited a broad amorphous halo.

The first endotherm seen on heating I in the DSC was due to the dehydration and vaporization reaction. The events following the dehydration reaction lead to the conclusion that there were two polymorphic forms of anhydrous tobramycin in the temperature range studied. Polymorphic transitions can be of two types: enantiotropic and monotropic (14). The type of transition observed was monotropic for the following reasons: (a) no solid-solid transitions were observed in the experimental temperature range, and (b) none of the transitions observed were reversible.

Four rules were formulated by Burger and Ramberger (15), the use of which can aid in the identification of the type of polymorphic transition. The results from the DSC studies permitted the use of the heat of fusion and heat of transition rules. The heat of fusion rule states that "if the higher melting form has the lower heat of fusion, the two forms are usually enantiotropic, otherwise they are monotropic." The results in Table II show that, irrespective of sample size, the polymorphic form with the higher melting point (~217°C) had a higher enthalpy of fusion value than the polymorphic form with the lower melting point (~164°C). This supports our conclusion that the observed polymorphic system was monotropic. However, the results presented in Table II must be viewed with caution for two reasons. (a) Both the heating rate in the DSC and the sample size had a significant influence on the enthalpy values of most of the thermal events. This is discussed in detail later. (b) The melting of the metastable anhydrous tobramycin (peak at ~164°C) and the crystallization of the stable anhydrous tobramycin (peak at ~198°C) may have been occurring simultaneously. In such a circumstance, the observed enthalpy of fusion of metastable anhydrous tobramycin would have been lower than the actual value.

Table II. Effect of Sample Size on the Enthalpies of the Thermal Events (Mean ± SD; $n = 3$)

Sample weight (mg)	Enthalpy of fusion (J/G)[a]	Enthalpy of crystallization (J/G)[b]	Enthalpy of fusion (J/G)[c]
1.1 ± 0.01	41.2 ± 0.65	−60.6 ± 0.61	78.5 ± 1.05
2.1 ± 0.03	41.4 ± 0.49	−47.6 ± 0.35	74.5 ± 0.58
3.1 ± 0.02	38.5 ± 1.31	−42.8 ± 0.78	62.2 ± 1.54
5.1 ± 0.02	37.5 ± 0.42	−33.8 ± 0.65	45.5 ± 1.30

[a] Endotherm at 164°C (see Fig. 1).
[b] Exotherm at 198°C.
[c] Endotherm at 217°C.

The heat of transition rule states in part that "if an exothermal transition is observed at some temperature, it may be assumed that there is no transition point below it, i.e., the two forms are related monotropically or the transition temperature is higher." According to this rule, anhydrous tobramycin has no transition point (solid–solid) below 198°C. Above this temperature, the only event observed was the melting of the stable anhydrous tobramycin at ~217°C. Therefore based on this rule also the observed polymorphic transition was monotropic. Shafizadeh and Susott studied the thermal behavior of some phenyl glycosides, which are structurally quite similar to tobramycin, and observed monotropic polymorphic transition in some of these compounds (16).

In order to calculate the free energy difference between the two polymorphs, their solubilities were determined in both benzene and carbon tetrachloride. The metastable and stable forms of anhydrous tobramycin were obtained by heating I in the DSC up to 150 and 208°C, respectively. The samples were immediately transferred to screw-capped tubes containing the appropriate solvent and placed in a thermostated water bath maintained at 25°C. The water bath had a horizontal shaking arrangement. Preliminary studies indicated that no perceptible change in the solubility value occurred after 48 hr. After 48 hr, the slurry was filtered through a membrane filter (Millex-HV4, Millipore). A known volume of the filtrate was taken, the solvent evaporated to dryness, and the HPLC analysis carried out according to the procedure outlined earlier. Periodically, the solid in contact with the liquid was subjected to powder X-ray diffraction studies. In all cases, there was no detectable change in the powder X-ray diffraction pattern of the solid in contact with the liquid during the solubility studies. The solubility of the metastable anhydrous tobramycin in carbon tetrachloride and benzene was 0.930 ± 0.070 and 0.112 ± 0.010 μg/ml (mean ± SD; $n = 3$), respectively. The solubility of the stable anhydrous tobramycin in carbon tetrachloride and benzene was 0.520 ± 0.070 and 0.062 ± 0.006 μg/ml, respectively. The

Table III. Effect of Heating Rate on the Enthalpies of the Thermal Events (Mean ± SD; $n = 3$)

Heating rate (°C/min)	Sample weight (mg)	Enthalpy of fusion (J/G)[a]	Enthalpy of crystallization (J/G)[b]	Enthalpy of fusion (J/G)[c]
15	2.1 ± 0.01	37.2 ± 0.64	−11.7 ± 0.49	14.2 ± 0.62
10	2.1 ± 0.03	41.4 ± 0.49	−47.6 ± 0.35	74.5 ± 0.58
5	2.1 ± 0.06	50.1 ± 0.05	−57.9 ± 0.40	102.8 ± 0.40
2	2.1 ± 0.01	53.5 ± 0.90	−59.5 ± 0.45	117.5 ± 0.25

[a] Endotherm at 164°C (see Fig. 1).
[b] Exotherm at 198°C.
[c] Endotherm at 217°C.

solubility ratio, i.e., (solubility of the metastable form)/(solubility of the stable form), was found to be 1.81 in benzene and 1.79 in carbon tetrachloride. The solubility ratios in the two solvents were in excellent agreement. The free energy difference (ΔF_T) between the metastable and the stable forms of anhydrous tobramycin was calculated according to the formula

$$\Delta F_T = RT \ln \frac{\text{solubility of the metastable form}}{\text{solubility of the stable form}}$$

where T was the temperature at which the solubilities were determined and R was the gas constant. The above formula is an approximation, since the concentration has been substituted for activity (17). However, the error associated with this approximation was likely to be small because of the low solubilities of metastable and stable anhydrous tobramycin in these solvents. The ΔF_T based on the solubilities in benzene and carbon tetrachloride, at 25°C, was 351.3 and 344.8 cal/mol, respectively.

In an effort to optimize the DSC methodology, the runs were first carried out with sample weights ranging from 1 to 5 mg (Table II). In these experiments, a constant heating rate of 10°C/min was maintained. The enthalpy of crystallization and the enthalpy of fusion of stable anhydrous tobramycin appeared to be inversely related to the sample size (Table II).

We had earlier presented evidence suggesting that I contained both absorbed water and water of crystallization. Even after storage under 0% RH for several months, I contained ~5% (w/w) water. Therefore, it is possible that the dehydration of I in the DSC was not followed by the complete removal of the water. In other words, the dehydration reaction was accompanied by removal of most of the water but a trace amount of water was retained in the solid. TGA of I had indicated a weight loss of ~0.1% between 150 and 190°C (Fig. 2a). The weight loss between 150 and 190°C is clearly perceptible in the derivative thermogravimetric curve (Fig. 2b). It was determined to be ~0.002 %/°C. The presence of this water appears to have partially inhibited the crystallization of the stable anhydrous tobramycin (transition at 198°C, Fig. 1), thereby decreasing the enthalpy value. The incomplete crystallization of the stable anhydrous tobramycin affected the subsequent fusion reaction (transition at 217°C, Fig. 1) and this was reflected in the enthalpy values. It is believed that the observed enthalpy value was inversely related to the weight fraction of water retained in the solid. As the sample size in the DSC was increased, it was likely that the water liberated on dehydration had greater difficulty escaping from the pan. This would have increased the weight fraction of the retained water, resulting in the observed decrease in the enthalpies of the transitions. It must be realized that in all of these cases the amount of water retained after dehydration was likely to be less than 0.5% (w/w).

The optimization of the DSC methodology also required us to determine the effect of heating rate, if any, on the enthalpy values of the thermal transitions. Maintaining a

Table IV. Effect of Holding Time on the Enthalpies of the Thermal Events (Mean ± SD; $n = 3$)

Holding time (min)	Sample weight (mg)	Enthalpy of fusion (J/G)[a]	Enthalpy of crystallization (J/G)[b]	Enthalpy of fusion (J/G)[c]
0	3.1 ± 0.02	38.5 ± 1.31	−42.8 ± 0.78	62.2 ± 1.54
1	3.1 ± 0.13	43.1 ± 0.39	−48.8 ± 0.86	70.1 ± 0.30
3	3.1 ± 0.11	44.8 ± 0.84	−55.7 ± 0.61	82.5 ± 0.74
5	3.1 ± 0.04	48.0 ± 0.76	−58.6 ± 0.27	85.7 ± 1.00
10	3.2 ± 0.11	51.1 ± 0.62	−60.6 ± 0.20	90.1 ± 1.25
20	3.1 ± 0.06	57.4 ± 1.35	−68.0 ± 0.88	94.4 ± 0.40
30	3.1 ± 0.05	57.7 ± 1.41	−69.0 ± 0.26	94.4 ± 0.40
60	3.1 ± 0.06	57.0 ± 2.00	−67.8 ± 0.43	98.5 ± 1.57

[a] Endotherm at 164°C (see Fig. 1).
[b] Exotherm at 198°C.
[c] Endotherm at 217°C.

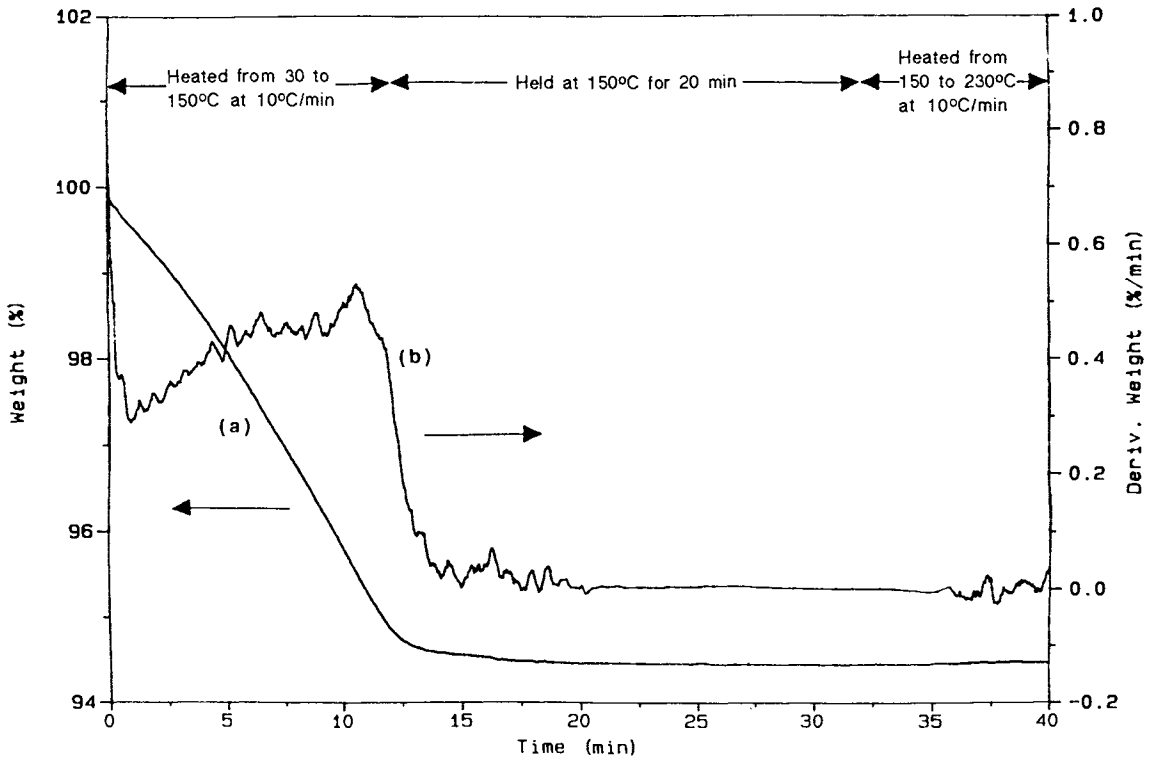

Fig. 3. Thermogravimetric (a) and derivative thermogravimetric (b) curves of I. The sample was heated from 30 to 150°C, then held at 150°C for 20 min, and the heating continued to 230°C. The regions where the sample was heated and the isothermal region are labeled. The sample weight was 10.29 mg.

constant sample size, the experiments were carried out at different heating rates. The enthalpies of the two melting transitions and the crystallization transition were affected by the heating rate (Table III). The slower the heating rate, the longer was the time taken by the sample to cover a given range of temperature. The longer the heating time, the more complete would have been the removal of the trace water in the solid. The observed enthalpy values support such a conclusion.

One possible approach to remove the presence of trace amounts of water will be to heat and maintain the sample at a high temperature. Earlier studies had indicated that the dehydration reaction was complete by about 130°C. Therefore, the sample was heated to 150°C in the DSC, then held at this temperature for varying time periods, and the heating continued (Table IV). As the holding time was increased up to 20 min, the enthalpy values of the observed transitions progressively changed. Holding at 150°C for longer periods of time caused no further changes in the enthalpies of the transitions. Up to a holding time of 20 min, progressive removal of the trace water in the solid may be occurring. Once all of the water was removed, any further increase in the holding time would have had no effect on the observed enthalpy values. TGA showed that when I was heated to 150°C, then held for 20 min, and the heating continued, no detectable weight loss was observed above 150°C (Fig. 3a). This is also evident from the derivative thermogravimetric curve (Fig. 3b). Since the heating rate was 10°C/min, and the sample was heated from 30°C, the temperature of 150°C was reached in 12 min. For the next 20 min, the sample was held at this temperature. It is clear from Fig. 3 that there was no weight loss after the sample had been held at 150°C for 10 min. However, the DSC work had suggested that the sample had to be held at 150°C for 20 min for complete removal of

Table V. Effect of Holding Time on the Enthalpies of the Thermal Events for Different Sample Sizes (Mean ± SD; n = 3)

Holding time (min)	Sample weight (mg)	Enthalpy of fusion (J/G)[a]	Enthalpy of crystallization (J/G)[b]	Enthalpy of fusion (J/G)[c]
20	3.1 ± 0.06	57.4 ± 1.35	−68.0 ± 0.88	94.4 ± 0.40
20	5.1 ± 0.01	57.1 ± 0.91	−68.2 ± 0.29	94.1 ± 0.20
20	7.1 ± 0.09	57.0 ± 0.32	−67.6 ± 0.42	94.4 ± 0.27

[a] Endotherm at 164°C (see Fig. 1).
[b] Exotherm at 198°C.
[c] Endotherm at 217°C.

Table VI. HPLC Analysis of I Heated to Various Temperatures[a] (Mean ± SD; $n = 3$)

Sample heated to (°C)	Nominal concn. (mg/L)	Measured concn. (mg/L)	Mean % nominal	CV (%)
150	7.8 ± 0.41	7.6 ± 0.12	97	6.0
185	8.2 ± 0.97	8.1 ± 0.77	99	2.9
210	9.5 ± 0.32	10.0 ± 0.69	105	3.6
224	8.1 ± 0.04	7.7 ± 0.18	95	1.9

[a] In each case, the sample was held for 5 min at the final temperature.

moisture (Table IV). In the DSC, the sample was heated in a crimped pan, while in the TGA, the sample was heated in an "open" pan. Therefore it is conceivable that the removal of water in the DSC requires heating for a longer time.

When the samples were heated to 150°C and held for 20 min at that temperature, sample size did not have any effect on the enthalpies of the two melting transitions and the crystallization transition (Table V). This suggested that complete removal of moisture, irrespective of sample size, was achieved by holding the sample at 150°C for 20 min.

It was necessary to ensure that none of the transitions observed in the DSC were due to sample decomposition. Therefore, I was heated in the DSC from room temperature up to 150°C and held at that temperature for 5 min. A weighed amount of the sample was immediately dissolved in Sorensen's phosphate buffer (pH 7.4), and after appropriate dilution the sample was analyzed by HPLC. The above experiment was repeated after heating the solid to 185, 210, and 224°C. The tobramycin content determined after heating I to various temperatures is given in Table VI. There was no appreciable sample decomposition even after heating I to 224°C. However, heating I to 224°C and holding at that temperature for 1 hr caused appreciable sample decomposition.

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