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Some thermochemical and structural properties of penicillin V benzathine tetrahydrate [1]

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Abstract

The thermochemical and structural properties of penicillin V benzathine tetrahydrate were studied in the temperature interval from 20 to 200°C. It has been found that, on increasing the temperature, the penicillin undergoes two phase transitions before reaching the melting point. The first is accompanied by complete dehydration and the corresponding structural changes are small, whereas the penicillin's crystal lattice before and after the second transition, as determined by X-ray diffraction, differs significantly. The overall enthalpy change is $250\,J\,g^{-1}$. Both transitions are reversible; the penicillin can be successively heated and cooled an indefinite number of times, retaining its chemical and structural properties, provided that the temperature does not exceed 100°C. From differential scanning calorimetry data one cannot resolve the kinetic parameters of the two phase transitions; the overall process is second order and the activation energy is $207\,kJ\,mol^{-1}$. Carroll–Freeman analysis of the thermogravimetric data indicates that the reaction order of the dehydration is 0.6 and the activation energy is $111\,kJ\,mol^{-1}$. All these parameters were obtained at a $1.25°C\,min^{-1}$ heating rate. The enthalpy of fusion of the anhydrous penicillin is $45\,kJ\,mol^{-1}$. The seeming inconsistency of the X-ray data with the DSC data is found to be related to the very pronounced effect of heating rate on the kinetics, and is explained in detail.

INTRODUCTION

The thermochemical and structural properties of penicillins are important from both the industrial and the therapeutic point of view. Storage conditions, heat shock sterilization and heat treatment after radiosterilization [1] to reduce the concentration of free radicals are all commercially important. In the literature, detailed studies devoted to the thermochemical properties of penicillins can be found although dealing more with the

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degradation kinetics [2, 3] than with the properties at elevated temperatures before degradation. On the other hand, structural properties such as degree of crystallinity and crystal size distribution are important, since the concentration of antibiotics in the blood is strongly influenced by them. In addition to this the kinetics of phase transitions accompanied by dehydration is an interesting subject from the point of view of general solid state chemistry.

The present work deals with the thermochemical and structural properties of penicillin V benzathine tetrahydrate in the interval 20–200°C.

EXPERIMENTAL

Penicillin V benzathine tetrahydrate [4] was kindly supplied by ICN Galenika, Belgrade, Yugoslavia. For heat capacity studies, a Perkin-Elmer DSC-2 instrument was used. The thermogravimetric curves and their first differentials were obtained using a Perkin-Elmer TGS-2 instrument. The specimen was purged with argon. Ultraviolet (UV) spectroscopy studies were carried out using a Perkin-Elmer Lambda 5 spectrometer. Infrared (IR) spectroscopy studies were performed using a Beckman IR 4220 spectrometer. X-ray diffraction data were obtained with a Philips APD 1700 automated powder diffractometer with a PW 1820 vertical goniometer, graphite monochromator and Cu Kα radiation. Samples were continuously scanned from 3° to 40° 2θ with a sample interval of 0.050° and a rate of 0.020° s^{-1}. The measurements at elevated tetmperatures up to 120°C were performed by heating the sample holder within a TTK temperature attachment produced by Anton Paar.

RESULTS AND DISCUSSION

Figure 1 shows the combined results of thermogravimetric (TG) and differential scanning calorimetry (DSC) measurements, both obtained at 1.25°C min^{-1} heating rate. The reasons for choosing this particular heating rate will be explained in the last paragraph of this section. Several regions of weight change can be observed on the TG curve. Starting from 20°C, the specimen loses weight on heating to 70°C. The weight then remains constant, and above 100°C decreases with an inflection point at about 115°C. The weight loss in the first stage is 6.6%, corresponding to 3.7 mol of water per mol of penicillin. This is 0.3 mol less than it should be for the tetrahydrate compound, owing to the presence of some amorphous phase. It should be noted that the water content varies by 10% from batch to batch, depending on the synthetic procedure adopted, and can be increased by recrystallization from methanol. In the DSC curve, the endothermic peak at 60°C is attributed to the dehydration process, and the exothermic

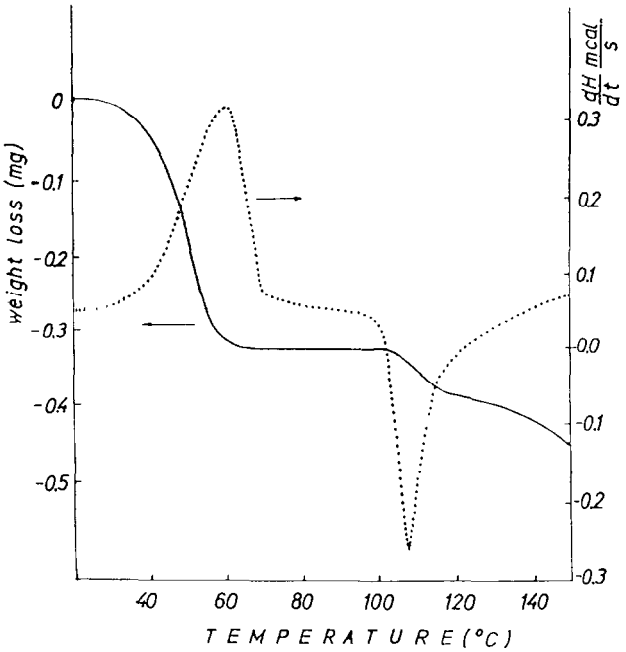

Fig. 1. TG (solid line) and DSC (dotted line) curves of the penicillin. TG: 4.98 mg, heating rate 1.2°C min^{-1}, purged with nitrogen. DSC: 3.24 mg, heating rate 1.25°C min^{-1}, sensitivity 0.5 mcal s^{-1}.

peak at 108°C is attributed to chemical reactions which result in microbiological deactivation of the penicillin. Integration of the peaks gave 3.8 kJ per mol of water for the enthalpy of dehydration and −110 kJ per mol of the dehydrated penicillin for the enthalpy of the subsequent reaction. From analysis of the peak asymmetry, carried out by a procedure suggested by Kissinger [5], the dehydration reaction was found to be second order, and the corresponding activation energy from an Arrhenius plot is equal to 207 kJ mol^{-1}.

However, analysis of the TG data over the interval 20–70°C gave results which differed significantly from those obtained by DSC. The kinetic analysis using the Freeman–Carroll method [6], gave a reaction order of 0.6 and an activation energy of 111 kJ mol^{-1}. In order to find the reason for such a large discrepancy, the results obtained by the two methods are compared in Fig. 2, where the percentage weight loss in the range 20–75°C is plotted against the percentage of the heat taken in by the sample in the same range. It is pertinent to note that at 57°C almost all the crystalline water is lost, although only 50% of the heat (corresponding to the area under the peak) is consumed. This somewhat surprising result is not an artefact of the different heating rates, since both TG and DSC experiments were performed under the same conditions, but indicates that there are two

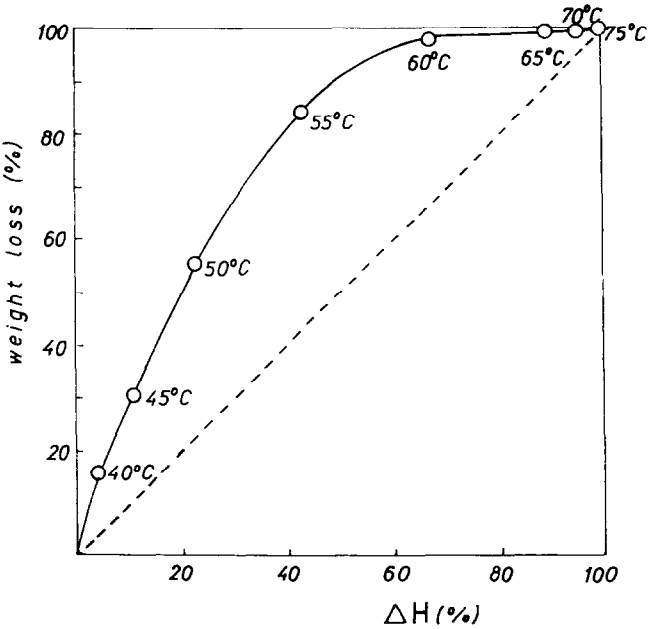

Fig. 2. Percentage weight loss vs. percentage heat taken in by the sample from 20 to 75°C. See text for details.

(or more) endothermic processes. The result could be explained if dehydration is followed by melting of the penicillin, which is also endothermic. The hypothesis is also supported by analysis of the chemical reaction which occurs between 100 and 150°C. Figure 3 shows the UV spectra of the penicillin before and after heating in vacuo to 120°C for 5 min. The thermolysis product contains the chromophore which is shown in the inset. The strong shift towards higher wavelengths indicates that its formation must be a multistep chemical reaction which requires significant molecular rearrangement and is not very likely to be performed in the solid state owing to restricted motion. The IR spectrum of the thermolysis product also indicates that there has been a chemical reaction with elementary steps, such as the opening of the β-lactam ring, for example, which require a significant molecular rearrangement.

For further investigation, the X-ray diffraction patterns of the penicillin at various temperatures were taken and are presented in Fig. 4, along with the corresponding d spacings and peak intensities in Table 1. The X-ray diffraction pattern taken at 60°C, i.e. after loss of 98% of the crystalline water indicates that the penicillin remains crystalline. The changes in the crystalline structure on dehydration are relatively small. This is to be expected, because the volume of a water molecule is small compared with that of the penicillin, and thus the removal of water does not have to be followed by a large change in the position of the penicillin molecules. The

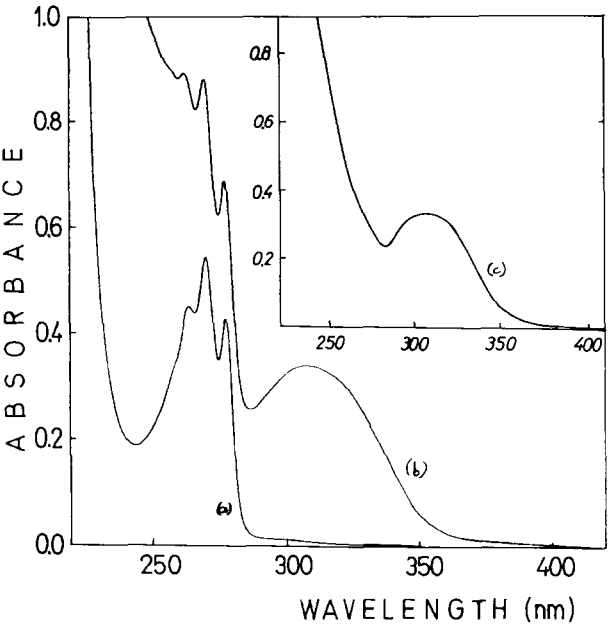

Fig. 3. UV spectra of the penicillin samples dissolved in methanol: (a) the untreated sample (200 mg l^{-1}); (b) after heating to 120°C for 5 min in vacuo (187 mg l^{-1}); (c) difference spectrum.

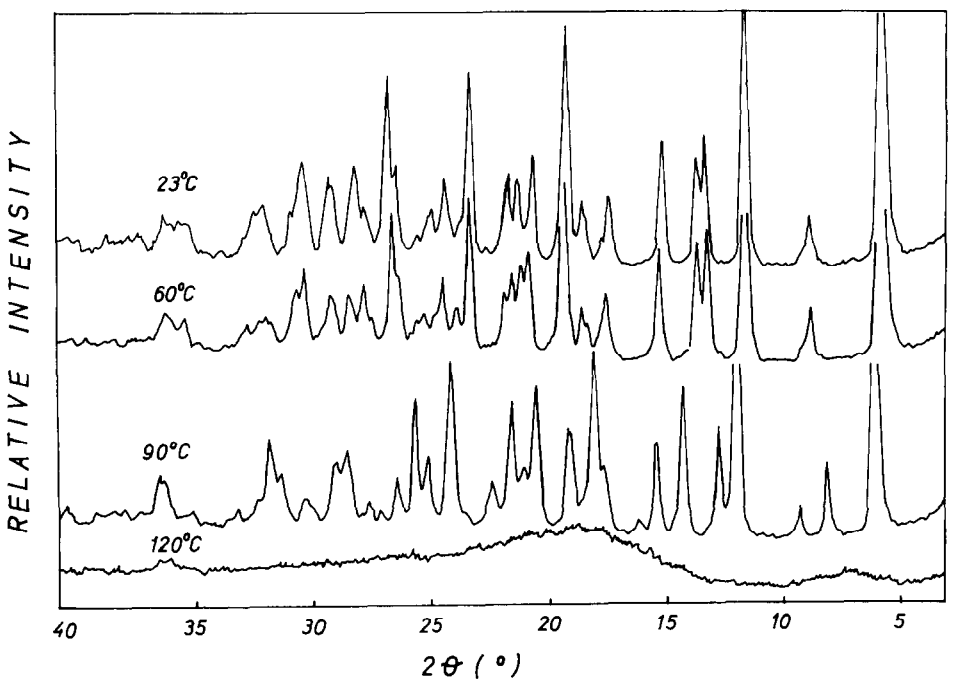

Fig. 4. Wide angle X-ray scattering of the penicillin at the indicated temperatures. The structureless pattern taken at room temperature after heating in a vacuum oven at 120°C for 5 min.

TABLE 1

X-ray parameters of penicillin V benzathine measured at the indicated temperatures

23°C		60°C		90°C	
d spacing (nm)	I/I_{max} (%)	*d* spacing (nm)	I/I_{max} (%)	*d* spacing (nm)	I/I_{max} (%)
1.525	100.0	1.526	100	1.475	100
0.999	11.7	1.002	13.4	1.107	21.7
0.763	46.0	0.762	55.8	0.964	11.7
0.655	34.1	0.659	38.4	0.739	97.1
0.583	18.8	0.579	21.0	0.623	41.6
0.508	14.4	0.506	16.0	0.576	26.9
0.457	36.5	0.457	43.1	0.495	67.5
0.427	17.5	0.422	32.5	0.464	40.5
0.406	20.1	0.409	23.5	0.433	49.2
0.379	31.9	0.379	27.9	0.413	43.2
0.360	14.5	0.361	12.7	0.369	58.8
0.333	34.6	0.334	26.9	0.349	41.6
0.316	21.1	0.319	11.0	0.309	35.1
0.305	17.6	0.305	11.0	0.295	8.16
0.292	22.4	0.292	21.2	0.282	33.9
0.277	15.4	0.278	9.40	0.247	21.8
0.253	10.2	0.273	6.00		
0.250	10.8	0.250	10.8		
0.238	5.50				

change of enthalpy on elimination of crystalline water is relatively small; approximately 2.5 per gram of water, which is slightly higher than the specific heat of vaporization of water, and the heat change is mainly associated with the water being transferred to the gas phase. As the energy used for the lattice rearrangement is very small, the corresponding changes of structure are expected to be small as well.

The pattern X-ray shows that the penicillin is a crystalline solid at 90°C. This result indicates that the initial hypothesis, that the dehydration is followed by melting, is wrong. The large difference between the patterns taken at 60 and 90°C clearly shows that the penicillin has undergone a phase transition after dehydration. This phase transition is not associated with the loss of water into the gas phase, and all the energy is used in this rearrangement. A greater change in structure may then be expected.

This also means that re-hydration on lowering the temperature is exothermic and therefore should be fast. Indeed, the isothermical rehydration measurement presented in Fig. 5 shows this to be the case. The sample was first slowly heated to 95°C to lose water, and then the furnace was pulled down, exposing the sample to room temperature. As the heat capacities of the sample and the sample pan are small, they will cool quickly

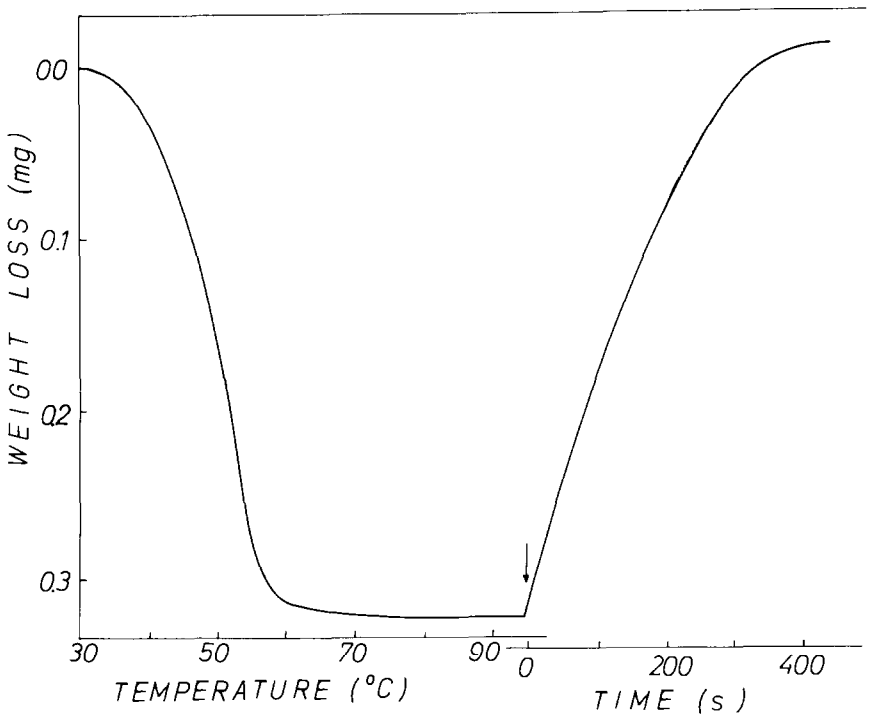

Fig. 5. TG dehydration of the penicillin on heating, followed by isothermal re-hydration at 22°C, relative humidity = 65%. See text for details.

to ambient temperature. On removing source of heat, the weight increase was followed as a function of time. It was found that the original weight of the sample was reached in approximately 5 min. It is pertinent to note that, no matter how many times dehydration and subsequent hydration were repeated, it did not affect the chemical and structural properties of the penicillin, provided the temperature did not exceed 100°C and heating was carried out in oxygen-free conditions. This result is also significant from the analytical point of view, in that precautions should be taken when determining the degree of hydration solely by measuring the weight loss. For example, if only 1 min has passed between taking the sample out of the oven and weighing the result obtained will be 25% smaller than the actual value. This estimate is applicable for the experimental conditions used in Fig. 5, but differences in the relative humidity and ambient temperature at which the weight measurements were carried out would certainly affect this error.

The DSC analysis as depicted in Fig. 1 shows no change from 80°C to the point where the chemical reaction starts, indicating that there is no additional energy consumption as would occur in any melting process. On the other hand, the X-ray analyses of the products undoubtedly shows that

the penicillin is a crystalline solid at 90°C. It is difficult to reconcile this with a chemical reaction which requires considerable molecular rearrangement. Accordingly, the influence of heating rate on the kinetics of the solid-state reaction was investigated. The results of the DSC experiment are presented in Fig. 6. One can observe that at moderate to high heating rates another endothermic process appears just before the exothermic one corresponding to the chemical reaction. The area under the endothermic curve also increases with heating rate. Bearing this result in mind, the seeming inconsistency between DSC and X-ray data can be explained as follows. Upon slow heating, when the sample temperature reaches 99°C, the chemical reaction starts on the surface of the crystals, because that is where the molecules have sufficient mobility. Being exothermic, the reaction provides energy for further melting of the crystal surface, thus enabling the chemical reaction to continue, and this goes on until the product of the first reaction step is in the liquid state. Thus there is not endothermic peak produced on melting when the rate of heating is low compared with that of the chemical reaction. When the heating rate and the rate of chemical reaction are of the same order of magnitude, then the energy necessary for melting is taken both from the calorimeter's heater and from the chemical reaction heat. From Fig. 1 one can calculate that, in the first 2 min of the reaction, less than 1% of the specimen has reacted. This means that at high

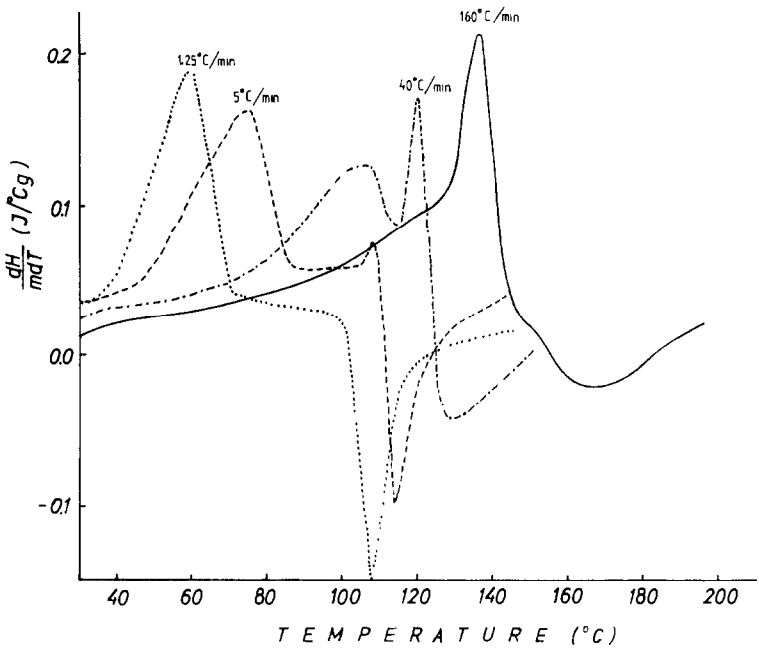

Fig. 6. Effect of heating rate on DSC of the penicillin. All curves are normalized with respect to the calorimeter sensitivities and sample weights.

heating rates (above 100°C min^{-1}, when the temperature is elevated from 100 to 150°C in less than half a minute) the penicillin may melt completely before the chemical reaction has started. Indeed, at a rate of 160°C min^{-1} the inflection between the endothermic and the exothermic peak suggests that melting is finished whereas the chemical reaction has not yet started (or more precisely, the amount of the specimen which has reacted is negligible). Integration over the peak gives a value of 298 J g^{-1}. The enthalpy change of 250 J g^{-1} (which is the corresponding value obtained at low heating rates) subtracted from 298 J g^{-1} gives 48 J g^{-1}, which is the enthalpy of function of the penicillin. The enthalpy of the reaction should be increased by this amount to give 155 kJ mol^{-1}.

Another test of this proposed scheme was carried out by analysing the kinetic parameters obtained from both the TG and DSC data at various heating rates (Table 2). In order to determine at which heating rate the experimental conditions can be considered reversible from a thermodynamic point of view, the temperatures of the peak maxima are plotted against the heating rates. The procedure was first proposed by Barrall and Rogers [7], although without a solid theoretical base. A linear dependence fitted the experimental results well and on extrapolation to zero heating rate the endothermic peak maximum is placed at 58°C, which is 2°C lower than the corresponding value obtained at the lowest heating rate of 1.25°C min^{-1} (Fig. 7). This means that the heating rate will need further lowering if reversible conditions are to be achieved. Unfortunately, because of the high instrumental sensitivity required (0.2 $mcals^{-1}$ s), which leads to a noisy baseline, we could not perform the DSC experiment below 1.25°C min^{-1}. Also, the analysis of the DSC data is meaningful only at heating rates equal to or lower than 5°C min^{-1}, since at higher heating rates there is a strong distortion of the peak, which corresponds to two solid state phase transitions due to overlap with the peaks corresponding to the penicillin melting and degrading. Although overlapping of the process at

TABLE 2

Kinetic parameters obtained at various temperatures

Heating rate (°C min^{-1})	Activation energy (kJ mol^{-1})		Reaction order		Linear regression coefficient	
	TGA	DSC	TGA	DSC	TGA	DSC
1.25	111	207	0.60	2.0	0.998	0.987
2.50	101	105	0.65	2.0	0.999	0.982
5.00	94	96	0.70	2.3	0.999	0.997
10.0	61		0.15		0.983	
20.0	55		0.19		0.987	

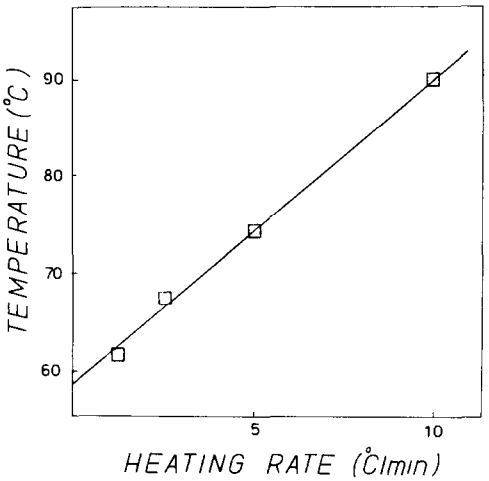

Fig. 7. Effect of heating rate on the temperature of the endothermic peak maxima.

moderate and higher heating rates does not occur with the TG curves themselves, the kinetic analysis shows, that on increasing the heating rate from 5 to 10°C min^{-1}, the slight decrease of the activation energy is no longer observed; instead there is an abrupt change. At low heating rates, the Carroll–Freeman plot is a straight line with the linear regression coefficient very close to unity. The activation energy is within the experimental error equal to that calculated from the TG data. However, at a heating rate of 10°C min^{-1}, the linear regression coefficient is significantly less than unity, and the plot bends downwards on increasing the temperature, which indicates complex kinetics. Further increase of the heating rate causes even sharper deviation from linearity because of greater overlap of the various processes, and this is in agreement with results from other experimental techniques.

Parameters obtained from the DSC data also depend strongly on the heating rate at which the experiment is performed. At a heating rate of 1.25°C min^{-1}, the Arrhenius plot can be reasonably well approximated by a straight line, although there is a noticeable upwards curvature on increasing the temperature. At a heating rate of 2.5°C min^{-1} the plot shows the same trend but less pronounced than in the previous case, resulting in an increase of the linear regression coefficient. The activation energy calculated is half that of the previous case. At a heating rate of 5°C min^{-1} the Arrhenius plot is a straight line and the linear regression coefficient is very close to unity.

Such a discrepancy in the results is a consequence of the existence of several elementary steps in the phase transitions that the penicillin undergoes on heating from room temperature to a temperature at which degradation occurs. In the first phase transition a dehydration takes place (which can formally be divided into two steps; breaking of hydrogen bonds and diffusion of water through the crystal lattice) as well as lattice

rearrangements. In the second phase transition there is a lattice reordering that consists of at least one step. Each of these elementary steps has its own activation energy, the values of which may differ considerably; because of such a complex mechanism, one has to be careful in determining which of the kinetic parameters are correct and which are artefacts due to the effect of the heating rate. Of all the processes that occur on heating, TG experiments can detect only those which are accompanied by a change in weight. In this case these are dehydration and degradation. In the heating rate range 1.25–5°C min^{-1}, all the crystalline water was removed at temperatures lower than 80°C; this means that there is no overlap of the dehydration process with melting and degradation, which occur above 100°C. Consequently, the kinetic parameters calculated from the TG data obtained under these conditions refer only to dehydration. As the calculated value of the activation energy changes very little in that interval, and as the experimental conditions at a heating rate of 1.25°C min^{-1} do not differ very much from the reversible ones, it can be considered that the calculated value of the dehydration activation energy is very close to the actual value.

At a heating rate of 5°C min^{-1}, 90% of the crystalline water is lost at 69°C, whereas only 50% of the heat of the process is taken in the sample. At 75°C there is less than 1% of the crystalline water left and 70% of the heat is consumed. The endothermic peak ends at 90°C. This means that the kinetic parameters calculated from the DSC data obtained under these conditions refer to the processes of dehydration and lattice rearrangement, and that they are not affected by melting and degradation. The Arrhenius plot is a straight line over the entire interval and the activation energy is equal to that calculated from the TG data, which refers to the dehydration only. This can only be rationalized if the value of the dehydration activation energy is very similar to that corresponding to the lattice rearrangement. In this case, it is not at first sight clear why, at a heating rate of 1.25 °C min^{-1}, the Arrhenius plot is bent upwards and the calculated activation energy is twice the previous value. The most plausible explanation is that, along with a change of heating rate, there is a change of the temperature interval in which the measurements are performed. At higher heating rates the reactions will occur at higher temperatures. If any reaction from a set of consecutive reactions has a high activation energy (in this case it is a lattice rearrangement in the second phase transition), at higher temperatures it will occur at a much higher rate and will no longer be a rate determining step, thus affecting the Arrhenius plot.

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