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Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 65–73
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Rapid estimation of kinetic parameters for thermal decomposition of penicillins by modulated thermogravimetric analysis

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Received 5 March 2003; received in revised form 15 December 2003; accepted 6 January 2004

Abstract

Modulated thermogravimetic analysis (MTGA) is evaluated for the rapid estimation of thermal stability using several penicillin antibiotics as model compounds. The MTGA technique utilizes an oscillatory temperature program to obtain Arrhenius kinetic parameters through a mass loss during thermal degradation. To evaluate the reliability of this technique, activation energies (E_a), log pre-exponential factor ($\log Z$), and log first order rate constants ($\log k$) obtained by MTGA for the thermal decomposition of ampicillin anhydrous, ampicillin trihydrate, ampicillin sodium salt, and penicillin G potassium salt are compared to existing literature values. The $\log k$ values estimated by MTGA agreed well with literature values when the weight loss observed by MTGA was shown to be due to the first decomposition step of the compound. The E_a and $\log Z$ values determined by MTGA did not consistently agree with literature values as these parameters increased with decreasing heating rate (β). The increase in E_a and $\log Z$ values with decreasing β seemed to offset each other to some extent to yield a relatively consistent $\log k$ estimate regardless of β.

Keywords: Modulated thermogravimetric analysis; Decomposition kinetics; Activation energy; Thermal stability; Penicillins

1. Introduction

Thermal stability is an important physical parameter in the design and selection of novel drug candidates. Indeed, thermal instability can pose major problems in the synthesis, processing, formulation, and shelf-life of new drug molecules. This can lead to significant delays in development and regulatory approval. For these reasons, it is necessary to obtain information on the thermal stability of promising drug candidates as early as possible in the discovery process. A conventional stability assessment involving holding the compound in an oven at elevated temperature and assaying periodically by chromatographic methods is employed early on in the drug development process. However, this process requires a substantial amount of time and sample and is usually carried out only after the drug molecule has been selected for development. More

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doi:10.1016/j.jpba.2004.01.007

rapid techniques such as microcalorimetry can provide some stability information, however this technique also requires a substantial amount of sample that is often not available early in the discovery of a new drug.

In 1998, a new technique called modulated thermogravimetric analysis (MTGA) was introduced [1,2]. The method is based on the work of Flynn and Wall [3] and is analogous to the modulated differential scanning calorimetry (MDSC) concept patented by Reading et al. [4]. In MTGA, a sinusoidal temperature modulation is superimposed on the underlying heating rate to obtain the Arrhenius kinetic parameters of activation energy (E_a) and log pre-exponential factor ($\log Z$) through a mass loss during thermal degradation. The MTGA method would be ideal for rapid assessment of thermal stability of drug candidates since the run times are relatively short (2–3 h) and sample consumption is relatively low (15–30 mg). However, the reliability of the MTGA method is not well established. The E_a and $\log Z$ values for decomposition of polymers and inorganic molecules obtained using MTGA have been compared with those obtained by non-isothermal and isothermal thermogravimetric methods [1,5,6]. However, kinetic parameters estimated by MTGA have not been compared to those obtained by more conventional stability assessments. Furthermore, the applicability of MTGA for the estimation of the thermal stability of pharmaceutical molecules has not been assessed.

In this work, we have determined the E_a and $\log Z$ values obtained by MTGA for the thermal decomposition of ampicillin anhydrous, ampicillin trihydrate, ampicillin sodium salt, and penicillin G potassium salt. To gain a more reliable evaluation of the MTGA method, the results are compared to those obtained several years ago by Pawelczyk et al. using the more traditional approach of holding the compounds at elevated temperatures and assaying periodically by iodometric titration [7,8]. Log first order rate constants ($\log k$) for the thermal decomposition of the penicillins are also estimated and compared.

2. Experimental

2.1. Materials

Ampicillin anhydrous, ampicillin sodium salt, ampicillin trihydrate, and penicillin G potassium salt were obtained from Sigma (St. Louis, MO). All compounds were of the highest available quality and were used as received.

2.2. Methods

2.2.1. MTGA

MTGA experiments were performed using a TA Instruments (New Castle, DE) TGA 2950 equipped with Windows NT Thermal Advantage and Universal Analysis 2000 software.

The sample and balance were continuously purged with N_2 at 60 and 40 ml/min, respectively.

Sample size was 25 ± 2 mg. The temperature was modulated at a programmed amplitude of $\pm 5\,^{\circ}C$ for all experiments, however the observed amplitude was about $\pm 3.6\,^{\circ}C$, as reported previously [5]. The modulation period was 200 s for all experiments. Each compound was run at four different heating rates (β) in the range of 0.3–2.0 °C/min.

2.2.2. HPLC

HPLC experiments were performed on an Agilent Technologies (Palo Alto, CA) HPLC 1100 equipped with photodiode array detector and ChemStation for LC 3D software. Ampicillin forms were assayed using a 250 mm × 4.6 mm Phenomenex (Torrance, CA) Luna C18 column with 5 μm particle size. The detection wavelength was 260 nm. The mobile phase consisted of water–acetonitrile–KH_2PO_4 (1.0 M)–acetic acid (1.0 M) (909:80:10:1, v/v/v/v) and was pumped at a flow rate of 1.5 ml/min. Typical sample size was about 1 mg/ml and injection volume was 20 μl. Potassium penicillin G was assayed on a 150 mm × 4.6 mm Zorbax (Aston, PA) SB-C18 column with 5 μm particle size. The detection wavelength was 220 nm. The mobile phase consisted of KH_2PO_4 (0.01 M)–methanol (60:40 v/v) and was pumped at a flow rate of 1.0 ml/min. Typical sample size was about 0.1 mg/ml and injection volume was 10 μl.

3. Theory

Reaction kinetics can be expressed by the rate equation, which describes the relationship between the rate of reaction, time, and amount of material. The rate of

reaction as a function of temperature can be described by the Arrhenius equation. The rate equation and Arrhenius equation are frequently combined into a single equation of the following form [1]:

$$\frac{d\alpha}{dt} = Z[f(\alpha)]\, e^{-E_a/RT} \quad (1)$$

where α is the fraction reacted, $d\alpha/dt$ is the rate of reaction, Z is the pre-exponential factor, $f(\alpha)$ is the kinetic expression, R is the universal gas constant and T is the temperature.

In MTGA experiments, a sinusoidal temperature modulation is superimposed on the underlying heating rate. The E_a can be obtained from oscillatory response in the rate of weight loss via the following equation [1,2]:

$$E_a = \frac{R(T^2 - A^2)L}{2A} \quad (2)$$

where A is the temperature amplitude, L is the ratio of the natural log of the maximum and minimum rate of weight loss taken at adjacent half cycles of the sine wave, and T is the average temperature. The values for T, A, and L are obtained by deconvolution of the oscillatory rate of weight loss using real-time discrete Fourier transform (DFT) [1,2].

The calculation of E_a does not assume any kinetic model, i.e., it is "model free". However, a kinetic model must be assumed to calculate $\log Z$. Assuming first order kinetics, $\log Z$ can be obtained from the value of E_a via the following equation [1,2]:

$$\log Z = \log\left[\frac{d\alpha}{1-\alpha}\right] + \frac{E_a}{2.303RT} \quad (3)$$

For an initial approximation of $\log Z$, the assumption of first-order kinetics is reasonable given that many decompositions in solid dosage forms approach first-order kinetics [9].

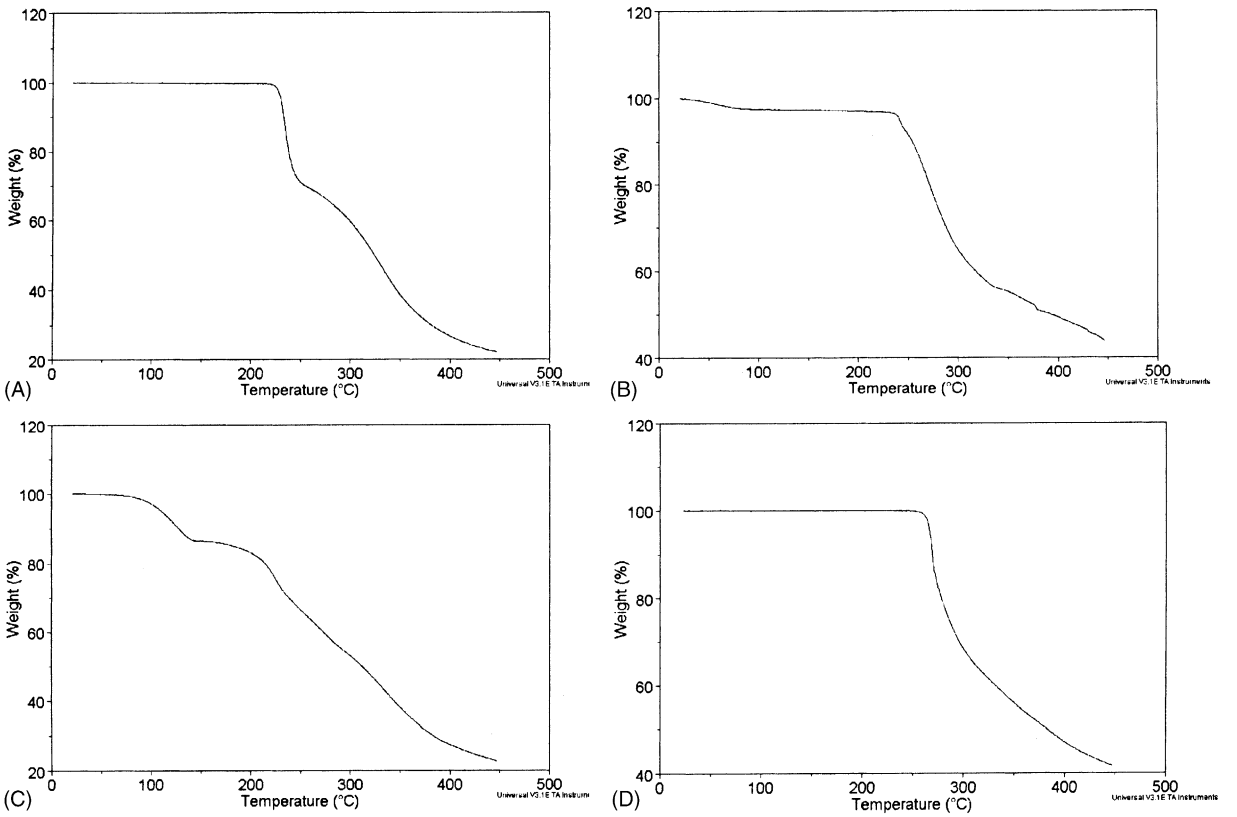

Fig. 1. TGA scouting runs of (A) ampicillin anhydrous, (B) ampicillin sodium salt, (C) ampicillin trihydrate and (D) penicillin G potassium salt at $\beta = 10\,^{\circ}C/min$.

4. Results and discussion

Non-isothermal TGA runs were initially carried out on ampicillin anhydrous, ampicillin trihydrate, ampicillin sodium salt, and penicillin G potassium salt to compare their decomposition profiles and decomposition onset temperatures. The non-isothermal TGA runs are shown in Fig. 1. The compounds were then analyzed by MTGA using an initial temperature that was 30–40 °C lower than the decomposition onset point to shorten the analysis times. Fig. 2 contains the MTGA thermogram for ampicillin anhydrous at $\beta = 0.7$ °C/min. Each compound was run at four different β because previous studies have shown that E_a values tend to increase with decreasing β [5]. E_a and $\log Z$ versus α plots were then generated for each of the MTGA runs. As an example, the E_a and $\log Z$ versus α plot for the thermal decomposition of anhydrous ampicillin is shown in Fig. 3. At the beginning of the decomposition step, the E_a and $\log Z$ values are unrealistically high as the extent of conversion is low and very few modulation cycles have been completed. As the decomposition proceeds, the E_a and $\log Z$ values plateau and become more consistent. Rather than arbitrarily choosing any point in the decomposition step, we took an average and standard deviation (S.D.) of E_a and $\log Z$ over the $\alpha = 0.9$–0.5 range, as this range of α values is most relevant for shelf-life and half-life predictions.

Table 1 compares the E_a and $\log Z$ values obtained by MTGA to various literature values obtained by holding the compounds at elevated temperatures and periodically determining % decomposed [7,8]. The number of modulation cycles contained in Table 1 was calculated using the following equation:

$$\text{cycles} = \frac{T_{1/2}}{\beta p} \tag{4}$$

where $T_{1/2}$ is the peak width at half height of the derivative of the modulated weight % plot (°C), β is the heating rate (°C/min), p is the modulation period (min).

For all compounds, the E_a and $\log Z$ values obtained by MTGA increased with decreasing β as reported

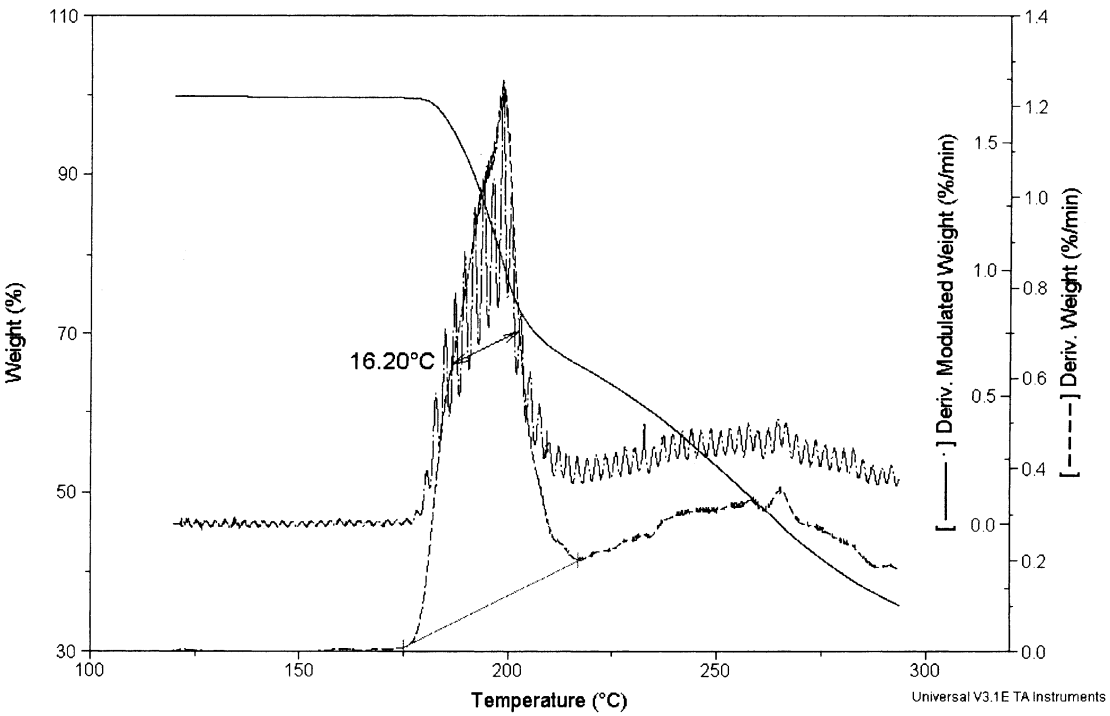

Fig. 2. MTGA of anhydrous ampicillin at $\beta = 0.7$ °C/min.

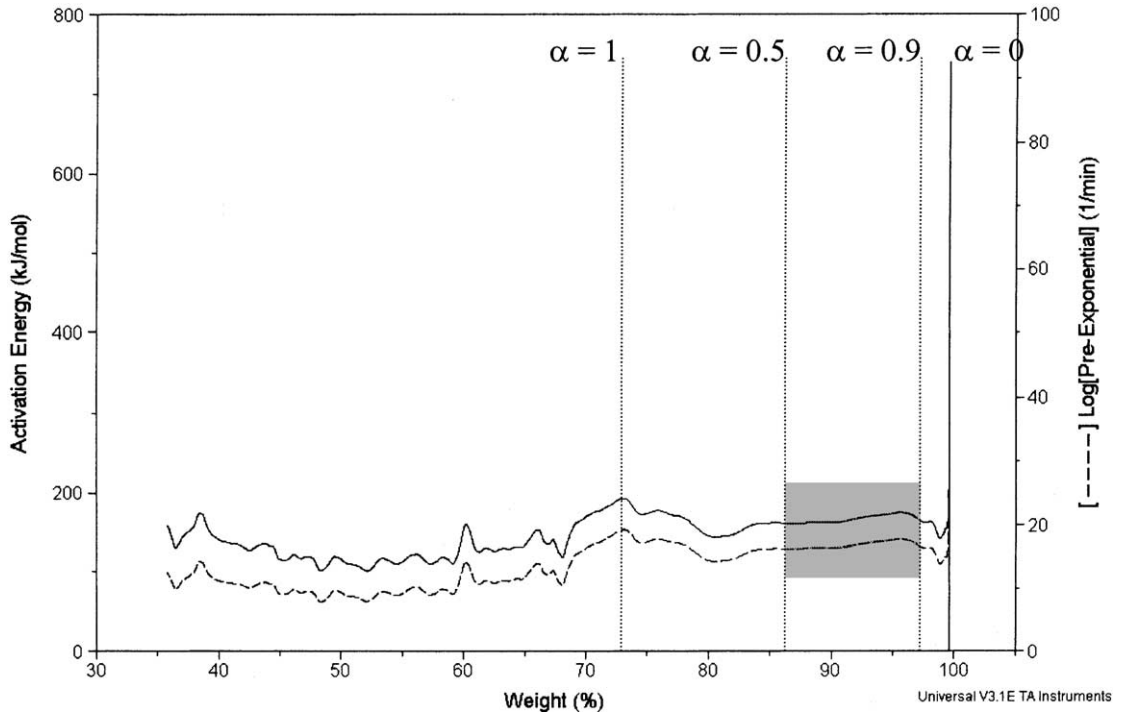

Fig. 3. E_a and $\log Z$ as a function of α for the thermal decomposition of anhydrous ampicillin at $\beta = 0.7\,^{\circ}$C/min.

previously [5]. This trend continued even when the β used gave more than the recommended number of five modulation cycles over the decomposition range [1]. The E_a and $\log Z$ values obtained by MTGA agreed reasonably well with literature values for some determinations but agreement was not as good for others. The level of agreement also depended on the β that was employed. The agreement for ampicillin anhydrous was relatively good at the slowest β of 0.3 °C/min. The opposite trend was observed for the potassium salt of penicillin G as the fastest β of 1.0 °C/min gave E_a and $\log Z$ values most consistent with the literature. The $\log Z$ values obtained by MTGA for the sodium salt of ampicillin agreed well with literature values for the slowest β of 0.3 °C/min, however the E_a estimates agreed best at the fastest β of 1.0 °C/min. As shown in Fig. 4, the MTGA of ampicillin trihydrate contained two weight loss steps. The first step was due to dehydration of the compound while the second step was due to decomposition. The E_a and $\log Z$ values obtained by MTGA at the fastest β of 2.0 °C/min for the dehydration (step 1) of ampicillin trihydrate were more consistent with literature values than the values for the second weight loss step.

Next we evaluated the reliability of the MTGA method for estimation of $\log k$. We compared the $\log k$ values reported in the literature using conventional stability assessments [7,8] to those calculated via the Arrhenius equation using the E_a and $\log Z$ values determined by MTGA. Table 2 compares the literature $\log k$ values for degradation of the penicillins to those estimated by MTGA. For all compounds, the $\log k$ values obtained by MTGA varied slightly as a function of β. However, the increase in E_a and $\log Z$ values with decreasing β seemed to offset each other to some extent to yield a relatively consistent $\log k$ estimate regardless of β. The $\log k$ values estimated by MTGA were in good agreement with the literature values for ampicillin anhydrous. The $\log k$ estimates for ampicillin anhydrous were within 1 log unit of the literature for all β values, however the $\log k$ values determined at the fastest β of 2 °C/min were in closest agreement. Similar results were observed for the potassium salt of penicillin G as the

Table 1
Comparison of literature and MTGA values of E_a and $\log Z$ for degradation of beta-lactam antibiotics

Compound	β (°C/min)	Cycles	MTGA		Literature	
			E_a (kJ/mol)	$\log Z$ (min^{-1})	E_a (kJ/mol)	$\log Z$ (min^{-1})
Ampicillin anhydrous	2.0	2.4	157.6 ± 7.0	15.8 ± 0.7	187.1	20.0
	1.0	4.7	170.7 ± 3.8	17.3 ± 0.4		
	0.7	6.9	167.2 ± 4.7	16.8 ± 0.5		
	0.3	16.8	182.7 ± 7.6	18.5 ± 0.9		
Ampicillin sodium salt	1.0	1.9	149.7 ± 4.1	13.7 ± 0.4	143.9	18.9
	0.7	3.1	162.5 ± 6.6	15.1 ± 0.7		
	0.5	4.6	188.0 ± 4.0	18.1 ± 0.4		
	0.3	8.1	200.9 ± 5.0	19.6 ± 0.5		
Ampicillin trihydrate (step 1)	2.0	3.2	102.6 ± 10.6	13.1 ± 1.6	112.5	13.2
	1.5	4.3	108.1 ± 12.0	14.0 ± 1.9		
	1.0	5.8	121.1 ± 15.0	16.1 ± 2.4		
	0.7	6.5	163.1 ± 20.8	22.2 ± 3.2		
Ampicillin trihydrate (step 2)	2.0	3.0	181.9 ± 4.8	18.9 ± 0.7		
	1.5	4.0	186.9 ± 3.1	19.4 ± 0.4		
	1.0	6.5	200.0 ± 5.5	21.0 ± 0.6		
	0.7	9.6	215.4 ± 6.1	22.8 ± 0.7		
Penicillin G potassium salt	1.0	2.0	92.4 ± 17.8	8.0 ± 1.8	84.5	7.3
	0.7	2.9	106.1 ± 6.5	9.4 ± 0.5		
	0.5	4.0	107.5 ± 5.4	9.5 ± 0.5		
	0.3	6.8	123.4 ± 8.2	11.2 ± 0.7		

$\log k$ values estimated by MTGA were in good agreement with literature values. Again, the best agreement was observed for the fastest β of 1.0 °C/min, even though this β gave the smallest number of modulation cycles.

The $\log k$ values estimated by MTGA did not agree well with literature values for the decomposition of ampicillin sodium. The $\log k$ values determined by MTGA were >7 log units higher than the literature values, which would result in a gross overestimate of stability for ampicillin sodium by MTGA. Reasonable estimates of $\log k$ are obtained by MTGA for ampicillin trihydrate when E_a and $\log Z$ values for the first weight loss (dehydration) step are used. However, gross overestimates of $\log k$ are obtained when the kinetic parameters for the second weight loss step are used.

We hypothesized that the $\log k$ values obtained for ampicillin sodium and ampicillin trihydrate (step 2) were higher than the literature values because the primary mechanism of decomposition for these compounds occurs before any weight loss is observed by MTGA. To test this hypothesis, we reanalyzed the compounds by MTGA only this time we stopped the analysis just before each weight loss step and analyzed by HPLC. Ampicillin trihydrate was also analyzed just after the dehydration step on the MTGA to see if decomposition may have occurred simultaneously with the dehydration step. Table 3 contains the remaining percentage of the antibiotics just before mass loss on the MTGA as determined by HPLC. No degradation was observed for ampicillin anhydrous and penicillin G potassium salt just prior to the weight loss on the MTGA. This indicates that the weight loss observed by MTGA is due to the decomposition of the parent molecules leading to more realistic $\log k$ estimates for the thermal decomposition of these compounds. Ampicillin sodium salt had degraded >30% before any weight loss was observed by MTGA suggesting that the primary decomposition step did not involve a loss of mass for this compound. Ampicillin trihydrate had degraded >20% during the dehydration process and had completely degraded prior to the second weight loss step on the MTGA. Fig. 5 contains the HPLC chromatograms of ampicillin trihydrate before and after dehydration. The chromatogram after dehydration

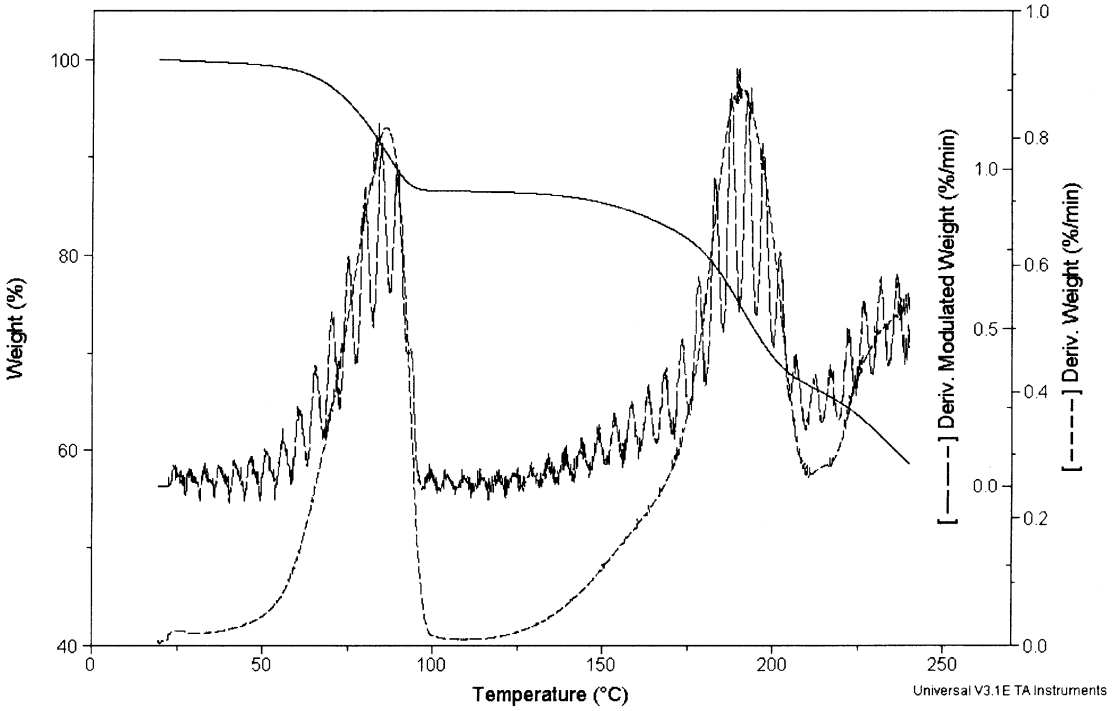

Fig. 4. MTGA of ampicillin trihydrate at $\beta = 1.5\,^{\circ}$C/min.

contains the degradation product, confirming that decomposition does occur simultaneously during the dehydration step. These observations indicate that the $\log k$ values estimated by MTGA were higher than the literature values for ampicillin sodium salt and ampicillin trihydrate because significant decomposition of these compounds occurs before any weight loss is observed by MTGA. Therefore, MTGA is not a suitable method for investigating the decomposition kinetics of these compounds.

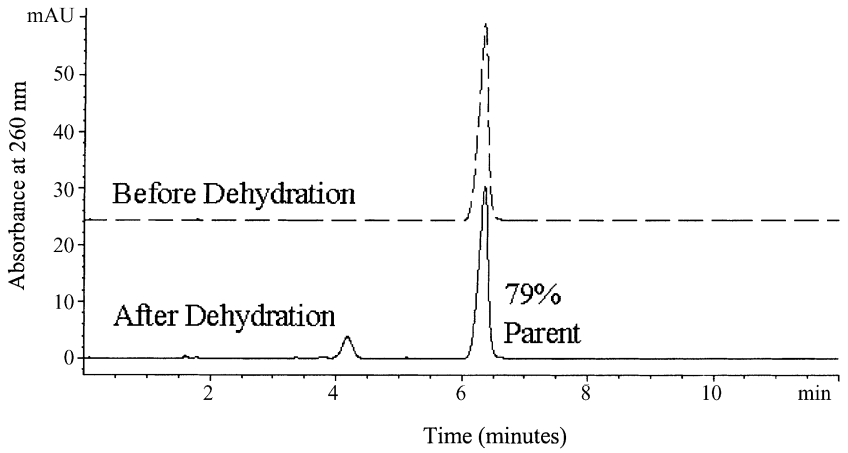

Fig. 5. HPLC chromatograms of ampicillin trihydrate before and after dehydration by MTGA.

Table 2
Comparison of literature and MTGA values of $\log k$ for degradation of beta-lactam antibiotics

Compound	Literature temperature (°C)	Literature $\log k$ (min^{-1})	MTGA $\log k$ (min^{-1})			
			$\beta = 0.3$	$\beta = 0.7$	$\beta = 1.0$	$\beta = 2.0$
Ampicillin anhydrous	102	−6.1	−6.9	−6.5	−6.5	−6.1
	115	−5.3	−6.1	−5.7	−5.7	−5.4
	140	−3.7	−4.6	−4.3	−4.3	−4.1
			$\beta = 0.3$	$\beta = 0.5$	$\beta = 0.7$	$\beta = 1.0$
Ampicillin sodium salt	60	−3.7	−11.9	−11.4	−10.3	−9.7
	80	−2.3	−10.1	−9.7	−8.9	−8.4
	100	−1.3	−8.5	−8.3	−7.6	−7.2
			$\beta = 0.7$	$\beta = 1.0$	$\beta = 1.5$	$\beta = 2.0$
Ampicillin trihydrate (step 1)	80	−3.4	−1.9	−1.8	−2.0	−2.1
	96	−2.8	−0.8	−1.0	−1.3	−1.5
	100	−2.6	−0.6	−0.8	−1.2	−1.3
	110	−2.1	0.0	−0.4	−0.8	−0.9
Ampicillin trihydrate (step 2)	80	−3.4	−9.0	−8.6	−8.2	−8.0
	96	−2.8	−7.7	−7.3	−7.0	−6.9
	100	−2.6	−7.3	−7.0	−6.7	−6.6
	110	−2.1	−6.6	−6.3	−6.0	−5.9
			$\beta = 0.3$	$\beta = 0.5$	$\beta = 0.7$	$\beta = 1.0$
Penicillin G potassium salt	110	−4.3	−5.6	−5.1	−5.1	−4.6
	120	−3.8	−5.2	−4.7	−4.7	−4.3
	140	−3.4	−4.4	−4.0	−4.0	−3.7

Table 3
Remaining percentage of beta-lactam antibiotics at different points on MTGA as determined by HPLC

Compound	Point on MTGA	Remaining %
Ampicillin anhydrous	Before decomposition	99.2
Ampicillin sodium salt	Before decomposition	69.6
Ampicillin trihydrate	After step 1 (dehydration)	78.6
	Before step 2	0.0
Penicillin G potassium salt	Before decomposition	100.1

5. Conclusions

Several important conclusions regarding the applicability of MTGA for rapid estimation of thermal stability may be drawn from the results reported here. Most importantly, the observed weight loss must be shown to be due to the primary decomposition step of the compound in order to obtain realistic estimates of thermal stability. The favorable results for ampicillin anhydrous and penicillin G potassium salt indicate that MTGA may serve as a useful tool for rapid estimation of thermal stability when the weight loss observed by MTGA is shown to be due to the first decomposition step of the compound. We have shown that the compound of interest can be analyzed just prior to the decomposition onset temperature in order to confirm that the weight loss observed on the MTGA is due to decomposition of the parent molecule. The unfavorable results for ampicillin sodium salt and ampicillin trihydrate suggest that MTGA may overestimate the stability of compounds in which the primary decomposition step does not involve a weight loss. It should also be noted that the compounds studied here did not melt before decomposition. Therefore, if the compounds under investigation melt before decomposing on the MTGA, the results are indicative of liquid-state rather than solid-state stability. Other factors such as particle size, amorphous content, and exposed surface area, were not investigated here but may also impact the MTGA stability estimate.

The data reported here indicate that E_a values alone cannot be used to estimate thermal stability as E_a and $\log Z$ increase with decreasing β. The E_a results must be combined with the observed $\log Z$ values to estimate $\log k$. The $\log k$ estimate remains relatively consistent regardless of β, which leads to a more reliable estimate of thermal stability. The current MTGA theory may need to be revised in order to account for these observations.

Acknowledgements

The authors would like to acknowledge Howard Ando, Suresh Babu, Roger Blaine, Anthony Blackburn, Ben Collman, Laila Kott, Rita Lodaya, and Jay Sisco for their insights and support.

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