International Journal of Pharmaceutics, 73 (1991) 33–36
© 1991 Elsevier Science Publishers B.V. 0378-5173/91/$03.50
ADONIS 037851739100240X

IJP 02432

Stability testing of pharmaceuticals by isothermal heat conduction calorimetry: Ampicillin in aqueous solution *

Reza Oliyai and Siegfried Lindenbaum

Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045 (U.S.A.)

(Received 28 August 1990)
(Modified version received 2 January 1991)
(Accepted 26 February 1991)

Key words: Ampicillin; Stability testing; Calorimetry; Microcalorimetry

Summary

The utility of isothermal heat conduction calorimetry for the study of stability of pharmaceuticals in solution is demonstrated in this study. The rate of heat evolution is measured as a function of the concentration of ampicillin, pH and the temperature. The pseudo first-order reaction rate constants, k, for the hydrolysis reaction are calculated from the variation of the heat evolution with time. The pH-rate profile for this reaction, as determined from the calorimetric data, is shown to correlate well with the literature data determined by other standard analytical methodology. The molal enthalpy of reaction was also calculated as function of pH and temperature.

Introduction

In order to establish stability and shelf life for a drug substance it is frequently necessary to invoke accelerated stability testing. The rate of decomposition at room temperature may be too slow to assess accurately the rate of disappearance of the drug substance or the appearance of a decomposition product. It is often useful, in these cases, to study the rate of decomposition at elevated temperatures, and to assume that the rate of decomposition at the storage temperature may be extrapolated from the high temperature data using the Arrhenius relationship. This relationship assumes that the activation energy for the decomposition reaction is independent of temperature and that the reaction pathway and mechanism do not change. When these conditions are met, accelerated stability testing is a valid approach to the estimation of slow reaction rate at low temperatures. However, these assumptions are not always valid, and long-term stability tests under normal storage conditions are generally required in order to validate the preliminary estimate of stability or projected shelf life — a process that can take months or even years to acquire sufficient data. It is desired to have an analytical methodology in which the decomposition rate can be rapidly and accurately estimated at room temperature. Since most chemical reactions are associated with production or consumption of heat, measurement of

Correspondence: S. Lindenbaum, Pharmaceutical Chemistry Dept, The University of Kansas, Lawrence, KS 66045, U.S.A.

* Presented in part at the 4th annual meeting of the American Association of Pharmaceutical Scientists, Atlanta, October 22–26, 1989.

the rate of heat produced or consumed by the sample can be used to evaluate the kinetics of reaction. Introduction of high-sensitivity isothermal heat conduction calorimeters enables one to detect heat production as low as 0.05 μW/g.

A number of studies have been published in which the utility of heat flow calorimetry for the study of stability of drugs in the solid state and in solution (Angberg et al., 1988; Hansen et al., 1989; Pikal et al., 1989) has been documented.

The theoretical basis for the use of heat evolution data to obtain reaction rates has been published (Angberg et al., 1988; Hansen et. al., 1989; Pikal and Dellerman, 1989). Briefly, assuming first order degradation we may write:

$$\ln[A]/[A_0] = -k(t - t_0) \quad (1)$$

where [A] is the concentration of starting material at time t, $[A_0]$ is the initial concentration at time t_0 and k is the first-order rate constant. If the rate of heat evolution q (μW/g) is proportional to the concentration of remaining reactant

$$\ln \alpha[A]/\alpha[A_0] = \ln q/q_0$$

where α is the proportionality constant, then the first-order rate equation may be rewritten:

$$\ln q/q_0 = -k(t - t_0) \quad (2)$$

Plotting $\ln q$ vs t yields a straight line as t approaches zero (initial rate approximation), and the slope is k, the first-order rate constant. Applying the same assumptions also yields the enthalpy of the reaction. Since $\Delta H = -\int q \, dt$, and $q = q_0 e^{-kt} \, dt$, $\Delta H = -\int q_0 e^{-kt} \, dt$ which may be integrated from $t = 0$ to $t = \infty$ to yield:

$$\Delta H = -q_0/k \quad (3)$$

The mechanism of ampicillin decomposition has been investigated (Hou and Poole, 1969). Ampicillin is susceptible to β-lactam hydrolysis. Its pH-rate profile reveals specific acid- and specific base-catalyzed hydrolysis. General acid and general base catalysis are known to occur in citrate and phosphate buffer systems. The rate of degradation increases substantially with increasing initial drug concentration due to dimerization. The purpose of this paper is to show the applicability of isothermal heat conduction calorimetry in estimating the solution degradation rate constants of ampicillin as a function of pH and temperature by comparing these results with the rate constants obtained under similar conditions using other standard analytical methods.

Materials and Methods

Calorimetry: The system used in these studies was the Thermometric Thermal Activity Monitor, TAM (Thermometric AB, Spjutvägen SA, S-17561 Järfälla, Sweden, LKB 2277). The unit may be equipped to accommodate four independent differential heat flow microcalorimeters. Calibrations were performed electrically every time upon changing the temperature and sensitivity of the instrument. The glass sample containers were loaded with 2 ml of buffer solution containing around 2 mg/ml of ampicillin in the sample site and 2 ml of buffer in the reference site. The sample and reference were immediately lowered to a thermal equilibration position in the calorimeter and allowed to equilibrate with the calorimeter for about 15 min. The sample and reference were then slowly lowered into the measuring zone of the calorimeter.

Temperature equilibrium was generally achieved within 30–60 min, leaving the signal representing the thermal activity of the sample. The output of thermal activity was read digitally and with strip chart recorders. The rate of heat evolution from the sample was recorded as a function of time. All studies were performed at least in duplicate. The rate of decomposition of ampicillin was also investigated as a function of temperature. Citric acid-dibasic phosphate buffer (pH 2–8) was used. A constant ionic strength of 0.5 was maintained for each buffer used by adding an appropriate amount of KCl. The pH electrode was calibrated at the temperature of the sample using standard buffer solutions. The pH values of ampicillin solutions were measured before and after every run to ensure consistency of pH within

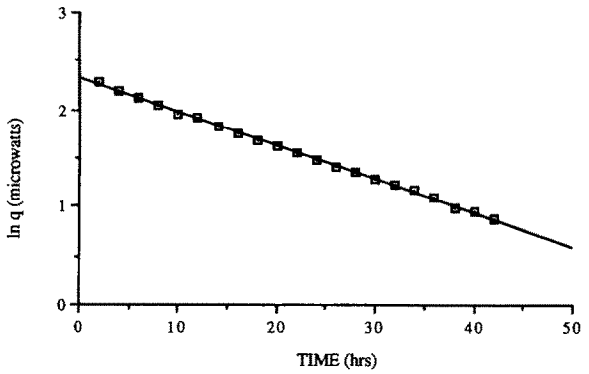

Fig. 1. Plot of ln q vs time for decomposition of ampicillin at pH 8.0 (37°C).

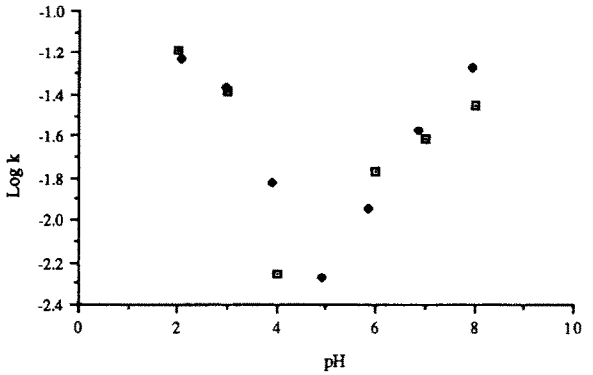

Fig. 2. pH-rate profile of ampicillin degradation in citric acid phosphate buffer at 37°C. (⊡) TAM, (◆) Hou and Poole (1969).

the experimental error (± 0.03 pH unit) throughout the run.

Ampicillin sodium salt was obtained from Sigma. The sample was used as received. All other chemicals were reagent grade. Water was purified by reverse osmosis followed by ion exchange and finally distilled from a quartz system.

Results and Discussion

Calorimetric data demonstrate that at constant temperature, pH, and ionic strength, the degradation of ampicillin for at least three half-lives follows pseudo first-order kinetics with respect to the substrate. Fig. 1 is a typical graph obtained upon plotting the natural logarithm of rate of heat evolved (q) from the sample vs time.

The degradation rate constant was calculated from the slope of the line. Table 1 compares the rate constants obtained at 37°C for various pH values, using isothermal heat conduction calorimetry, with the literature values. These results are fairly consistent and are in good agreement at both ends of the pH-rate profile where the decomposition rate is fast (except pH 8.0), but greater differences are observed around pH 4–6. An explanation for this is that the rate constants around pH 4–6 are very small, and the heat evolved from the sample is low, reaching the limit of sensitivity of the instrument. Fig. 2 shows the pH-rate profile obtained by microcalorimetry data compared to standard analytical methodology.

The activation energy for ampicillin hydrolysis was calculated from the temperature dependence of the degradation rate constants. The results presented in Table 2 clearly show that these values are in agreement (except pH 8.0) with literature

TABLE 1

Observed rate constants of degradation of ampicillin at 37°C using microcalorimetric and iodometric methods

Microcalorimetry		Iodometric [a]	
pH	$k_{obs}(\times 10^2)\,(h^{-1})$	pH	$k_{obs}(\times 10^2)\,(h^{-1})$
2.0	6.5 ± 0.1	2.05	5.9
3.0	5.1 ± 0.3	2.96	5.9
4.0	0.7 ± 0.02	3.91	1.5
6.0	1.6 ± 0.07	5.86	1.1
7.0	2.8 ± 0.04	6.85	2.7
8.0	3.8 ± 0.3	7.94	5.4

[a] Hou and Poole (1969).

TABLE 2

Activation energies of ampicillin hydrolysis obtained using microcalorimetry and iodometric methods

pH	E_a/R (K)
Microcalorimetric data	
2.0	8.6 ± 0.4
3.0	8.5 ± 0.5
8.0	8.6 ± 1.0
Iodometric data [a]	
1.35	8.3
4.93	9.2
9.78	11.0

[a] Hou and Poole (1969).

TABLE 3

Heat conduction calorimetric data for ampicillin decomposition in aqueous buffer solutions

pH	q_0 (J s^{-1} mol^{-1})	$k_{obs}(\times 10^2)$ (h^{-1})	ΔH (kJ/mol)
25°C			
2.0	0.66 ± 0.01	2.30 ± 0.08	103 ± 4
3.0	0.42 ± 0.01	1.80 ± 0.03	84 ± 3
7.0	0.20 ± 0.01	0.74 ± 0.03	97 ± 5
8.0	0.36 ± 0.00	1.20 ± 0.02	108 ± 2
37°C			
2.0	1.53 ± 0.04	6.5 ± 0.1	85 ± 4
3.0	1.43 ± 0.01	5.1 ± 0.3	101 ± 2
7.0	0.75 ± 0.04	2.8 ± 0.04	96 ± 1
8.0	1.10 ± 0.02	3.8 ± 0.3	104 ± 4
50°C			
2.0	5.86 ± 0.31	21.8 ± 0.6	97 ± 6
3.0	2.99 ± 0.12	14.7 ± 0.5	73 ± 4
7.0	1.22 ± 0.01	5.0 ± 0.1	88 ± 2
8.0	2.05 ± 0.05	11.7 ± 0.3	63 ± 2

data within the experimental error. These experiments demonstrate that high sensitivity heat conduction calorimetry should be considered as an alternative method for the determination of degradation kinetics of drug substances.

The molal enthalpy change (ΔH) of ampicillin hydrolysis at a given temperature was calculated using Eqn 3 with reproducibility of ± 10%. Table 3 lists the calorimetric data and shows the pH and temperature dependence of the molal enthalpy of ampicillin hydrolysis. This quantity was calculated at both ends of the pH rate-profile. However, at pH values where degradation is slow, larger experimental variation was observed. Although the mechanism of ampicillin degradation is pH dependent, the molal enthalpy change of reaction was found to vary only slightly with pH in the regions examined. It is postulated that the heat of reaction results from β-lactam cleavage. At both ends of the pH-rate profile, the degradation pathway involves β-lactam cleavage which is the thermochemical limiting step, regardless of the different pathways which may lead to the β-lactam cleavage. The heat of reaction remains fairly constant over the temperature range 298–323 K, and ΔC_p for the reaction is small.

References

Angberg, M., Nyström, M. and Castensson, S., Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. I. Precision and accuracy for static experiments in glass vials. *Acta Pharm. Suec.*, 25 (1988) 307–320.

Hansen, L.D., Lewis, E.A., Eatough, D.J., Bergstrom, R.G. and Degraft-Johnson, D., Kinetics of drug decomposition by heat conduction calorimetry. *Pharm. Res.*, 6 (1989) 20–27.

Hou, J.P. and Poole, J.W., Kinetics and mechanism of degradation of ampicillin in solution. *J. Pharm. Sci.*, 58 (1969) 447–454.

Pikal, M.J., Results of the LKB 2277 Calorimeter stability Testing of Pharmaceuticals, LKB Application Note 335, LKB-Produkter AB, Bromma, Sweden, 1983.

Pikal, M.J. and Dellerman, K.M., Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25°C: cephalosporins in the solid and aqueous solution states. *Int. J. Pharm.*, 50 (1989) 233–252.