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Kinetic studies of the degradation of an aminopenicillin antibiotic (amoxicillin trihydrate) in aqueous solution using heat conduction microcalorimetry

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Abstract

Recent developments in isothermal microcalorimetry allow the direct determination of kinetic and thermodynamic parameters for slow reactions from studies conducted at appropriate temperatures and under designated environmental control. The degradation kinetics of amoxicillin trihydrate has been investigated as a function of pH (1–10) and temperature (303.15–318.15 K) at 0.5 M ionic strength using heat conduction microcalorimetry. Equations were developed incorporating calorimetric accessible data, rate constants and change in enthalpy, which showed that the degradation of amoxicillin trihydrate in aqueous solution followed pseudo-first-order kinetics under our experimental conditions. The enthalpy of degradation reaction was found to be exothermic in nature. The values of the rate constant k for individual steps were determined from the values of the overall rate constants at different pH. Energy of activation of overall reaction as a function of pH and for individual rate constants was determined. The log k-pH profiles indicated specific-acid and specific-base catalysis and there were inflection points near pH 3 and pH 7 corresponding to the pK_{a1} and pK_{a2} values. Quantitatively, there was good correlation between calorimetric determined half-life (t1/2) and the literature value in the acidic region determined by other methods at 310.15 K. The presence of a β-lactam ring and of an α-amino group in the C-6 side chain played a critical role in the degradation of amoxicillin trihydrate and the zwitterionic form of the drug was found to be more stable.

Introduction

The biological activity of aminopenicillins is closely related to the particular grouping of atoms and specific features of their molecular structures (Indelicato & Wilham 1974; Indelicato et al 1974). During formulation and storage these chemical structures are susceptible to instability or other undesired reactions leading to loss in activity. Shelf-life stability of drugs and pharmaceuticals needs to be determined as speedily and accurately as possible. The phenomenon of drug instability has been found to be thermodynamic in nature accompanied by absorption or evolution of heat. The technique of calorimetry has much potential in the prediction of long-term stability and compatibility data for pharmaceutical materials (Pikal & Dellerman 1989; Willson et al 1995; Beezer et al 1999). Stability can refer to both chemical and physical stability of a pure drug or its formulation. Microcalorimetry has been shown, in some instances, to give more information as to the mechanism, kinetics and thermodynamics of a degradation reaction than either of the conventional techniques can provide (Willson et al 1995; Liang et al 2002). The heat effects produced due to degradation are influenced only by the progress of the reaction and are unaffected by static conditions existing in the solution. The high sensitivity heat conduction microcalorimetry measures the rate of heat evolved from a sample and can be studied as function of concentration, pH and temperature. In recent years, isothermal microcalorimetry has evolved as an alternative technique to HPLC for drug stability studies (Phipps & Mackin 2000). In this investigation, the heat conduction microcalorimeter has been used to study the degradation kinetics of amoxicillin trihydrate, with particular emphasis placed on determining the total change in enthalpy associated with the degradation processes. Sufficient data have been collected to determine rate constants as a function of temperature and over the pH range 1–10.

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Amoxicillin, an aminopenicillin, is extensively used alone and in combinations (Bertoni et al 1996; Hirschl & Rotter 1996; Erah et al 1997; Shah et al 1999) because of its stability in acid, low toxicity, efficient absorption and low minimum inhibitory concentration against a wide range of bacterial infections when given orally. It is now available for intramuscular and intravenous injection. The acid stability of aminopenicillin over other types of penicillin has been attributed to the incorporation of an electron withdrawing substituent (NH_2) on its side chain (Kessler et al 1983). It also influences the stability of amoxicillin under neutral and alkaline conditions (Van Krimpen et al 1987; Robinson-Fuentes et al 1997). A study by Valvo et al (1997) detected amoxicillin piperazine 2,5-dione and penicilloic acid as degradation products formed by the intramolecular attack of the amino group on the β-lactam ring in amoxicillin sodium commercial injectable preparations.

HPLC, a standard method for stability analysis, has been used to study stability of aminopenicillins in solution and in the solid state (Cook et al 1982; Concannon et al 1986; McDonald et al 1989; Mendez et al 1989; Hsu & Hsu 1992; Charles & Chulavatnatol 1993; Robinson-Fuentes et al 1997). Comparative stability studies of amoxicillin trihydrate and amoxicillin sodium, performed using an HPLC technique in solid state (at different temperatures), and an iodometric method for determination of amoxicillin (at different relative humidity and temperatures) have shown that amoxicillin sodium degrades faster than amoxicillin trihydrate (Mendez et al 1989; Plotkowiak & Nogowska 1989). The main advantage of using calorimetry for stability studies compared with HPLC techniques is that it requires less time, as the latter involves sample preparation/extraction and method development. Other methods employed, such as the conventional UV technique, have limited application to study the stability of amoxicillin as degradation products have absorbance maxima overlapping with the parent drug (Tokumura & Machida 2001).

Although there have been several reports on the stability of amoxicillin and its sodium salt in aqueous solution, very few have studied amoxicillin trihydrate, which is mainly used in oral dosage form. A literature survey indicated that amoxicillin trihydrate was much more susceptible to hydrolysis, but that the degradation reaction indicating the enthalpy of reaction over a wide pH range had not been studied. Also, no systematic study on the determination of individual rate constants and their activation parameters for the degradation reaction had been made.

Materials and Methods

Materials

Amoxicillin trihydrate (99.9% pure) was procured as a gift sample from Osaka Pharmaceutical Ltd., India, and was used without further purification. The drug was sieved and the fraction with the particle size 300–350 μm was used throughout the study.

Buffers

Phosphate buffers were prepared using AR grade mono-, di- and tri-sodium salts of phosphoric acid according to given procedures (Christian 1986). A buffer of pH 1 was prepared using 0.08 M hydrochloric acid. The ionic strength of all buffers was 0.5 M, adjusted using potassium chloride. The solutions were freshly prepared and the pH values were measured using a pH meter and a SC-glass electrode (Elico, India) standardized with standard buffer solutions of pH 4.0, 7.0 and 9.2. No attempt was made to exclude oxygen from the solution.

Solution calorimeter

A heat flux microcalorimeter (model-C-80, Setaram, France) was used to study the degradation process of amoxicillin trihydrate in aqueous solutions. In accordance with the Calvet principle, two experimental vessels (reference and sample) were placed in a calorimetric block. The temperature control by the thermostat of the calorimeter was within ± 0.001 K. The performance of the calorimeter was tested by measuring the enthalpy of a solution of potassium chloride in triple-distilled water (Balk & Benson 1959). The precision of any individual measurement was better than 0.02 kJ mol^{-1} for three consecutive experiments and agreed with the standard value within $\pm$ 0.03 kJ mol^{-1}. The amoxicillin trihydrate was weighed in the lower container of the calorimetric vessel itself using a single pan Mettler balance with an accuracy of $\pm$ 0.01 mg. Therefore, the maximum error in concentration ($4.77 \pm 0.0103 \times 10^{-3}$ M and $7.15 \pm 0.0157 \times 10^{-3}$ M) for lower and higher concentrations due to their experimental uncertainties was ± 0.0022, 0.0021 of their molarities.

Degradation of amoxicillin trihydrate was followed by loading the reference cell of the calorimeter with 5-mL buffer of the desired pH. The sample cell was filled with the desired buffer (5.00 mL) and an accurately weighed amount (10.00 mg or 15.00 mg) of drug, which was separated from the liquid by a displaceable lid. After stabilization, the calorimetric block containing the vessels was rotated through 180° several times, which displaced the lid between the drug and solution leading to their mixing (Jain et al 2000). The signal was automatically recorded on the strip chart recorder. The degradation reaction of drug was followed for a few hours at extreme pH values and for two to four days at other pH values in the pH range 1–10. The deviation of the sample signal from the baseline is the rate of heat produced by the sample and is proportional to the reaction rate under a particular set of experimental conditions such as concentration, pH and temperature.

Statistical methods

The rate constants obtained from the experiments over pH range 1–10 and different temperatures were compared statistically. One-way analysis of variance followed by multiple comparison with Tukey's test was performed. The experiments were performed for two concentrations of amoxicillin trihydrate (4.77×10^{-3} and 7.15×10^{-3} M) in triplicate over

the pH range 1–10 and temperatures 303.15, 310.15 and 318.15 K.

Results and Discussion

Enthalpy of degradation

Heat evolution as a function of time was studied for degradation of amoxicillin trihydrate at different initial concentrations in the temperature range 318.15–338.15 K and in aqueous solution (pH range 1–10). The calorimetric data of heat evolution vs time were plotted for the degradation of amoxicillin trihydrate (7.15×10^{-3} M and pH 1) at 318.15 K (Figure 1). It was assumed that total heat produced (q_0) for complete degradation was proportional to the initial concentration of drug ($q_0 = c_0 V \Delta_r H^0$) for first-order reaction (Beezer et al 1999). Here, c_0 is the initial concentration of the drug, V is the volume of the solution taken in the calorimeter and $\Delta_r H^0$ is the enthalpy of the degradation reaction. The heat evolved up to time t,

$$q_t = (c_0 - c)V\Delta_r H^0 = q_0 - cV\Delta_r H^0 \quad (1)$$

where c is the concentration of the drug at time, t.

For this first-order reaction it can be shown that

$$dq_t/dt = k(q_0 - q_t) \quad (2)$$

From the calorimetric data we know q_t and for determining q_0 we follow the following procedure. It is known that dq_t/dt is a function of time and we denote it by ϕ and determine the values of ϕ at two different times t_1 and t_2.

We have at $t = t_1$, $\phi_1 = k\,(q_0 - q_{t1})$ and at $t = t_2$, $\phi_2 = k\,(q_0 - q_{t2})$. Therefore, we get:

$$q_0 = q_{t1} + (q_{t1} - q_{t2})/[(\phi_2/\phi_1) - 1] \quad (3)$$

where, q_0 is the total heat evolved for complete degradation reaction starting with V L solution and initial concentration (c_0), q_{t1} is the heat evolved up to time t_1, q_{t2} is the heat evolution up to time t_2, ϕ_1 is the rate of heat evolution at t_1, and ϕ_2 is the rate of heat evolution at t_2.

We have also:

$$\Delta_r H^0 = q_0/c_0 V \quad (4)$$

The q_0 is estimated using equation 3. Table 1 gives the value of heat evolved at various intervals for 5 mL solution with initial concentration equal to 4.77×10^{-3} M and at pH 2 (temperature 318.15 K). The value for q_0 under these conditions has been calculated to be 2.53 J and $\Delta_r H^0 = -106.13$ kJ mol^{-1}. It may be noted that q_0 is proportional to initial concentration where $q_0 - q_t$ is propor-

Table 1 Heat evolved at various time intervals for 4.77×10^{-3} M amoxicillin trihydrate at pH 2 and 318.15 K.

t (min^{-1})	$10^2 \bullet q_t$ (J)	$\ln(q_0 - q_t)$
10	2.492 ± 0.0029	0.918
20	4.981 ± 0.0029	0.908
30	7.444 ± 0.0029	0.898
40	9.884 ± 0.0029	0.888
60	14.690 ± 0.0029	0.868
80	19.400 ± 0.0029	0.848
100	24.020 ± 0.0029	0.828
120	28.546 ± 0.0029	0.808
140	32.982 ± 0.0029	0.788
160	37.333 ± 0.0029	0.768
200	45.775 ± 0.0029	0.730
250	55.865 ± 0.0029	0.790
300	65.463 ± 0.0029	0.629
400	83.281 ± 0.0029	0.529
500	99.408 ± 0.0029	0.429

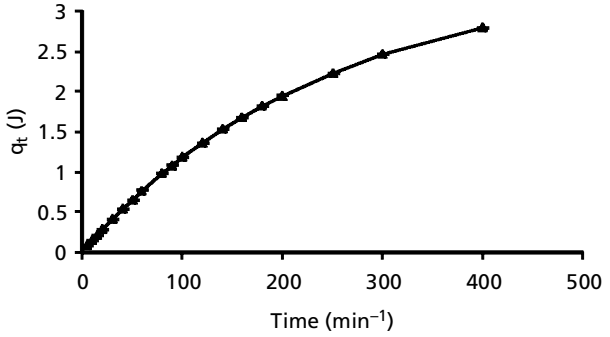

Figure 1 Calorimetric data of heat evolution (q_t) vs time for degradation of amoxicillin trihydrate (7.15×10^{-3} M and pH 1) at 318.15 K.

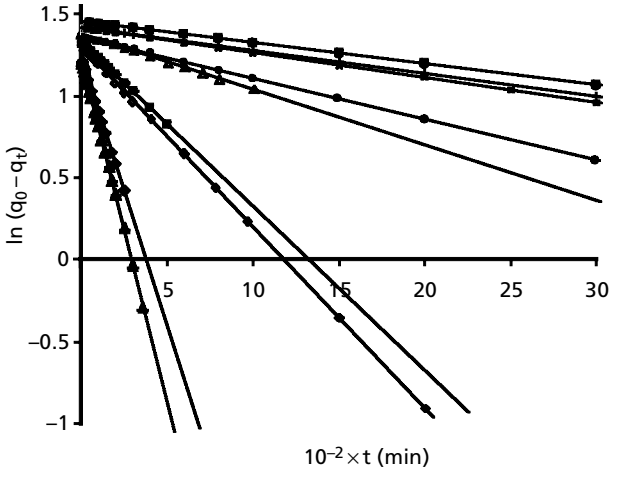

Figure 2 First-order plots for the degradation of amoxicillin trihydrate (7.15×10^{-3} M) over pH range 1–10 (pH 1.0 (▲), 2.0 (■), 3.0 (△), 4.0 (×), 5.0 (□), 6.0 (●), 7.0 (+), 8.0 (○), 9.0 (◇) and 10.0 (◆)) at 310.15 K.

Table 2 Reaction parameters of degradation of amoxicillin trihydrate over the pH range 1–10.

pH	10^3 • Amoxicillin trihydrate concn (M)	$\Delta_r H^0$ (kJ mol^{-1})	$10^{-1} \times q_0$ (J)	$10^3 \times k$ (h^{-1}) [1]Cal.	Experimental values	$^2t_{1/2}$ (h)
303.15K						
1	4.77 ± 0.010	−107.38 ± 0.12	25.59 ± 0.01	81.14 ± 0.14	81.19 ± 0.01	8.51 ± 0.003
	7.15 ± 0.016	−107.95 ± 0.09	38.59 ± 0.02		81.61 ± 0.03	
2	4.77 ± 0.010	−114.12 ± 0.13	27.20 ± 0.02	18.77 ± 0.04	18.80 ± 0.03	36.86 ± 0.058
	7.15 ± 0.016	−114.11 ± 0.09	40.80 ± 0.02		18.80 ± 0.03	
3	4.77 ± 0.010	−121.21 ± 0.14	28.90 ± 0.02	6.12 ± 0.03	6.13 ± 0.01	113.14 ± 0.184
	7.15 ± 0.016	−121.38 ± 0.12	43.40 ± 0.03		6.12 ± 0.02	
4	4.77 ± 0.010	−125.41 ± 0.14	29.89 ± 0.02	2.62 ± 0.01	2.56 ± 0.01	270.17 ± 2.10
	7.15 ± 0.016	−125.03 ± 0.11	44.71 ± 0.03		2.57 ± 0.02	
5	4.77 ± 0.010	−129.61 ± 0.14	30.90 ± 0.01	2.05 ± 0.01	1.99 ± 0.02	349.12 ± 3.52
	7.15 ± 0.016	−130.05 ± 0.12	46.50 ± 0.03		1.98 ± 0.01	
6	4.77 ± 0.010	−129.20 ± 0.14	30.81 ± 0.02	2.15 ± 0.01	2.14 ± 0.01	322.32 ± 2.25
	7.15 ± 0.016	−130.05 ± 0.11	46.50 ± 0.03		2.16 ± 0.02	
7	4.77 ± 0.010	−125.84 ± 0.13	30.00 ± 0.01	2.41 ± 0.01	2.32 ± 0.01	300.00 ± 2.58
	7.15 ± 0.016	−126.16 ± 0.11	45.10 ± 0.03		2.30 ± 0.02	
8	4.77 ± 0.010	−119.12 ± 0.13	28.40 ± 0.02	4.84 ± 0.01	4.66 ± 0.02	149.51 ± 0.64
	7.15 ± 0.016	−118.90 ± 0.11	42.50 ± 0.03		4.61 ± 0.02	
9	4.77 ± 0.010	−112.83 ± 0.12	26.90 ± 0.01	23.22 ± 0.02	23.12 ± 0.03	29.98 ± 0.039
	7.15 ± 0.016	−113.00 ± 0.09	40.40 ± 0.02		23.11 ± 0.03	
10	4.77 ± 0.010	−106.54 ± 0.11	25.40 ± 0.01	72.40 ± 0.44	79.95 ± 0.02	8.68 ± 0.003
	7.15 ± 0.016	−107.14 ± 0.10	38.30 ± 0.03		79.78 ± 0.03	
310.15K						
1	4.77 ± 0.010	−102.78 ± 0.11	24.51 ± 0.01	139.81 ± 0.25	139.80 ± 0.05	4.95 ± 0.002
	7.15 ± 0.016	−102.64 ± 0.10	36.70 ± 0.03		140.01 ± 0.06	5.2*
2	4.77 ± 0.010	−109.52 ± 0.12	26.11 ± 0.01	32.72 ± 0.07	32.87 ± 0.06	21.05 ± 0.038
	7.15 ± 0.016	−109.90 ± 0.09	39.30 ± 0.02		32.98 ± 0.03	19*
3	4.77 ± 0.010	−116.20 ± 0.13	27.71 ± 0.01	10.55 ± 0.02	10.87 ± 0.04	63.58 ± 0.349
	7.15 ± 0.016	−115.75 ± 0.10	41.39 ± 0.03		10.93 ± 0.02	
4	4.77 ± 0.010	−112.04 ± 0.14	28.62 ± 0.01	4.83 ± 0.01	4.70 ± 0.01	147.29 ± 0.626
	7.15 ± 0.016	−119.72 ± 0.12	42.81 ± 0.03		4.71 ± 0.02	176.9*
5	4.77 ± 0.010	−125.03 ± 0.14	29.80 ± 0.02	3.89 ± 0.01	3.74 ± 0.02	183.58 ± 0.981
	7.15 ± 0.016	−124.73 ± 0.10	44.60 ± 0.02		3.81 ± 0.01	176.6*
6	4.77 ± 0.010	−125.93 ± 0.14	30.02 ± 0.02	3.89 ± 0.01	3.87 ± 0.01	178.38 ± 0.953
	7.15 ± 0.016	−124.98 ± 0.12	44.69 ± 0.03		3.90 ± 0.01	166.1*
7	4.77 ± 0.010	−120.82 ± 0.13	28.80 ± 0.01	4.37 ± 0.01	4.28 ± 0.01	162.10 ± 0.378
	7.15 ± 0.016	−121.10 ± 0.12	43.31 ± 0.03		4.27 ± 0.01	153.1*
8	4.77 ± 0.010	−114.54 ± 0.12	27.30 ± 0.01	8.34 ± 0.02	7.92 ± 0.02	87.39 ± 0.220
	7.15 ± 0.016	−113.81 ± 0.12	40.70 ± 0.03		7.94 ± 0.01	86.9*
9	4.77 ± 0.010	−108.26 ± 0.12	25.81 ± 0.01	38.75 ± 0.03	38.60 ± 0.03	17.91 ± 0.014
	7.15 ± 0.016	−107.98 ± 0.10	38.60 ± 0.02		38.79 ± 0.03	
10	4.77 ± 0.010	−101.52 ± 0.11	24.20 ± 0.01	120.36 ± 0.81	135.31 ± 0.05	5.11 ± 0.002
	7.15 ± 0.016	−102.08 ± 0.10	36.52 ± 0.03		135.80 ± 0.05	
318.15K						
1	4.77 ± 0.010	−96.06 ± 0.10	22.90 ± 0.01	250.89 ± 0.39	251.05 ± 0.07	2.76 ± 0.001
	7.15 ± 0.016	−95.98 ± 0.09	34.32 ± 0.02		252.00 ± 0.06	
2	4.77 ± 0.010	−106.22 ± 0.12	25.32 ± 0.02	59.86 ± 0.11	59.90 ± 0.05	11.55 ± 0.002
	7.15 ± 0.016	−105.71 ± 0.09	37.80 ± 0.02		60.10 ± 0.05	
3	4.77 ± 0.010	−111.22 ± 0.12	26.51 ± 0.01	20.42 ± 0.04	20.40 ± 0.05	34.02 ± 0.028
	7.15 ± 0.016	−111.30 ± 0.09	39.80 ± 0.02		20.34 ± 0.05	

Table 2 (Cont.)

pH	10^3 • Amoxicillin trihydrate concn (M)	Δ_rH^0 (kJ mol^{-1})	$10^{-1} \times q_0$ (J)	$10^3 \times k$ (h^{-1}) [1]Cal.	Experimental values	[2]t1/2 (h)
4	4.77 ± 0.010	−114.97 ± 0.13	27.41 ± 0.02	9.10 ± 0.02	9.10 ± 0.04	76.15 ± 0.334
	7.15 ± 0.016	−115.27 ± 0.09	41.22 ± 0.02		9.10 ± 0.04	
5	4.77 ± 0.010	−120.05 ± 0.14	28.62 ± 0.02	7.67 ± 0.02	7.72 ± 0.02	89.52 ± 0.232
	7.15 ± 0.016	−120.01 ± 0.10	42.91 ± 0.02		7.71 ± 0.02	
6	4.77 ± 0.010	−119.62 ± 0.13	28.51 ± 0.01	7.81 ± 0.02	7.84 ± 0.02	88.45 ± 0.225
	7.15 ± 0.016	−119.71 ± 0.10	42.80 ± 0.02		7.83 ± 0.02	
7	4.77 ± 0.010	−115.00 ± 0.12	27.41 ± 0.01	8.74 ± 0.02	8.53 ± 0.03	81.62 ± 0.287
	7.15 ± 0.016	−115.78 ± 0.10	41.40 ± 0.02		8.45 ± 0.03	
8	4.77 ± 0.010	−108.62 ± 0.13	25.89 ± 0.02	15.62 ± 0.02	15.00 ± 0.03	46.26 ± 0.092
	7.15 ± 0.016	−108.77 ± 0.09	38.89 ± 0.02		14.96 ± 0.03	
9	4.77 ± 0.010	−102.82 ± 0.11	24.51 ± 0.01	66.53 ± 0.04	66.67 ± 0.05	10.42 ± 0.007
	7.15 ± 0.016	−102.93 ± 0.08	36.81 ± 0.02		66.38 ± 0.05	
10	4.77 ± 0.010	−94.39 ± 0.10	22.50 ± 0.01	203.29 ± 0.10	194.39 ± 0.05	3.53 ± 0.001
	7.15 ± 0.016	−95.12 ± 0.08	34.02 ± 0.02		198.06 ± 0.05	

[1]Values of rate constants calculated from equation 6. *Literature values for amoxicillin (Erah et al 1997). [2]$t_{1/2} = 0.693\,k^{-1}$.

tional to concentration at time t. For the first-order reaction the rate constant is given by the equation

$$\ln q_0 - \ln(q_0 - q_t) = kt$$
$$\ln(q_0 - q_t) + kt - \ln q_0 = 0 \qquad (5)$$

The plots between $\ln(q_0 - q_t)$ against t were a straight line at various concentrations and pH (Figure 2). The values of k calculated from the slope of the straight line plot at different concentration, pH and temperature are given in Table 2. The values of the rate constants for two concentrations (4.77×10^{-3} and 7.15×10^{-3} M) were almost constant at all pH and temperature, confirming that it followed pseudo-first-order kinetics. The enthalpies of reaction (Δ_rH^0) calculated from the (q_0) total heat evolved at various concentrations and pH values are given in Table 2. It can be seen that the values of Δ_rH^0 obtained with two different initial concentrations were in good agreement. However, these depended on the pH of the reaction and passed through a broad minimum at pH 5–6. The Δ_rH^0 did not vary much as the temperature was increased, indicating the completion of the reaction and absence of equilibrium (Beezer et al 2001). However, the slight decrease in Δ_rH^0 with increasing temperature at all pH values was due to different heat capacities of the products at different temperatures. This also showed that Δ_rC^0p for the reaction was positive (0.77, 0.55, 0.68, 0.72, 0.66, 0.66, 0.71, 0.69, 0.67 and 0.80 kJ mol^{-1}K^{-1} over the pH range 1–10) and was expected for the degradation process proposed here.

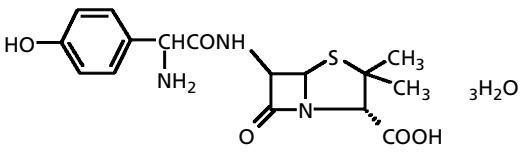

Figure 3 The structure of amoxicillin trihydrate.

pH-rate profile of amoxicillin trihydrate (Figure 3)

In the pH range studied, the amphoteric antibiotic amoxicillin (fH) exists in four different ionic forms: as a cation (fH_2^+), a zwitterion ($fH^{\pm}$), an anion (f^-) and a dianion (f^{2-}), the apparent pK_a values of fH_2^+, $fH^{\pm}$, f^- being 2.63, 7.16 and 9.55, respectively (Tsuji et al 1978). The pH rate profile for the degradation of amoxicillin showed three regions:

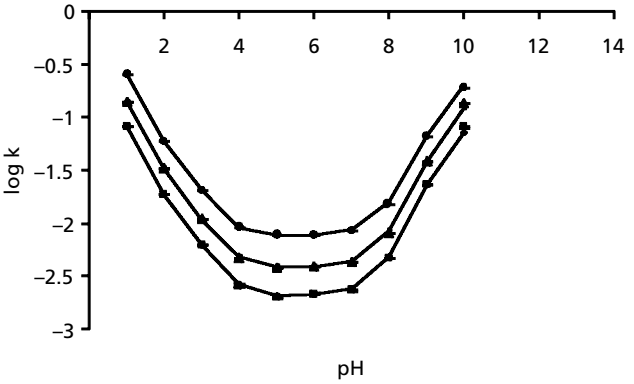

Figure 4 Plot of log k–pH profile in the temperature range of 303.15–318.15 K. Solid lines represent the calculated values from equation 6 and points are the experimental values (303.15 K (■), 310.15 K (▲) and 318.15 K (✶)).

Table 3 Fraction of various species of amoxicillin trihydrate in pH range 1–10.

pH	Cationic species (fH_2^+)	Zwitterionic species (fH)	Anionic species (f^-)	Di-anionic species (f^{2-})
1	0.97709	0.022905	–	–
2	0.81009	0.18990	0.00001	–
3	0.28519	0.66855	0.04626	–
4	0.04088	0.9584	0.00067	–
5	0.00421	0.9889	0.00684	–
6	0.000398	0.9349	0.06468	0.00002
7	0.00002	0.59038	0.40844	0.00116
8	–	0.12325	0.85260	0.02403
9	–	0.01115	0.77143	0.21742
10	–	0.000378	0.26178	0.73783

hydrogen ion catalysed, hydroxide ion catalysed and a plateau (Figure 4). The shape of the experimental curves without strong inflection was characteristic of the reaction with hydrogen ion and hydroxide ion catalysis. The pH dependent rate constants can be explained by various equations but the best rate expression has to be found out to take into account the fraction of various species calculated from the corresponding pK_a values and also the individual rate constants for these species. Our analysis of the results showed that the following equation explained adequately the kinetics of the reaction at all pH values.

$$k_{pH} = k_H\,[H^+]\,fH_2^+ + k_1\,fH_2^+ + k_2\,fH^{\pm} + k_3\,f^- + k_{OH}\,[OH]\,f^- \quad (6)$$

where, k_H is a second-order rate constant for hydrogen ion catalysed reaction of species fH_2^+, k_{OH} is a second-order rate constant for hydroxide ion catalysed reaction of species f^-, and k_1, k_2, and k_3 are first-order rate constants for hydrolytic degradation of fH_2^+, $fH^{\pm}$, and f^-, respectively.

Other equations including the reaction of f^{2-} were tried but equation 6 reproduced the entire pH profile satisfactorily with standard deviation ± 0.0025 at 303.15 K.The various fractions for different species calculated from the ionization constants taken from the literature are given in Table 3. The rate constant k_H, k_1 and k_2 were calculated by simultaneously solving the equations for overall rate constant at pH 1, 2 and 3. Other rate constants (k_3 and k_{OH}) were calculated from data at higher pH. The calculated rate constants are listed in Table 4. These calculated second- and first-order rate constants were used to construct the pH rate profile. There was good agreement between the experimental data and calculated values. The calorimetrically determined enthalpy of reaction and rate constants gave an estimation of the β-lactam ring opening as the main degradation pathway of amoxicillin trihydrate degradation. This kinetic analysis of the degradation of amoxicillin trihydrate using the calorimetric technique supported previous observations that the β-lactam ring of amoxicillin was more susceptible to hydrolytic degradation when the pH deviated significantly from the isoelectric point (pH 4.8) (Tsuji et al 1978; Erah et al 1995), and the zwitterionic form of the drug was the most stable species ($k_2 = 3.84 \times 10^{-3}\,h^{-1}$ at 310.15K) (Mendez et al 1989).

Amoxicillin was observed to degrade most rapidly under acidic conditions, and at pH 1 the observed half-life of amoxicillin trihydrate (4.95 h) was in agreement with Erah et al (1997) (5.2 h). In the acidic pH region amoxicillin degraded to give amoxicillin penamaldic acid and amoxicillin penicilloic acid as the main degradation products (Hou & Poole 1969; Longridge & Timms 1971; Blaha et al 1976; Tsuji et al 1978). However, at pH 1 penamaldic acid may be the only degradation product (Longridge & Timms 1971; Blaha et al 1976). Therefore, in this study the value of the rate constant at pH 1 ($k = 139.90 \times 10^{-3}\,h^{-1}$) corresponded to the formation of penamaldic acid from the cationic species (fH_2^+). As the pH increased the formation of penamaldic acid goes on decreasing and the penillic acid formation increased. This may be the reason that $\Delta_r H^0$ changed with pH. In the neutral and basic pH region amoxicillin penicilloic acid and 2-(6′-phenylpiperazine-2′,5′-dione 3′-yl)5,5′-dimethylthiazolidone-4-carboxylic acid were the major products facilitated by intramolecular attack of the amino group to the reactive β-lactam moiety (De Pourcq et al 1985; Mendez et al 1989; Robinson-Fuentes et al 1997; Valvo et al 1997). At pH 6 amoxicillin present as the zwitterionic form degraded to give amoxicillin penicilloic acid

Table 4 Individual rate constants defined in equation 6 for the degradation of amoxicillin trihydrate at ionic strength 0.5 and various temperatures.

Temperature (K)	k_H ($M^{-1}\,h^{-1}$)	$10^2 \bullet k_1$ (h^{-1})	$10^3 \bullet k_2$ (h^{-1})	$10^3 \bullet k_3$ (h^{-1})	$10^{-2} \bullet k_{OH}$ ($M^{-1}\,h^{-1}$)
303.15	0.670 ± 0.001	1.60 ± 0.004	2.05 ± 0.006	2.68 ± 0.012	27.42 ± 0.060
310.15	1.152 ± 0.002	2.80 ± 0.006	3.84 ± 0.010	4.68 ± 0.010	45.51 ± 0.110
318.15	2.051 ± 0.003	5.16 ± 0.010	7.67 ± 0.020	9.55 ± 0.015	76.70 ± 0.140

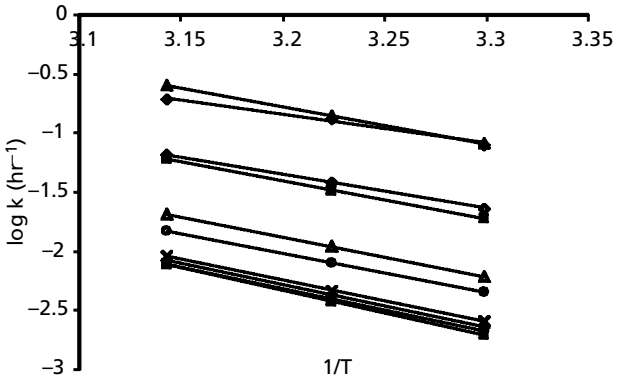

Figure 5 The Arrhenius plot for degradation of amoxicillin trihydrate (pH 1.0 (▲), 2.0 (■), 3.0 (△), 4.0 (×), 5.0 (□), 6.0 (●), 7.0 (+), 8.0 (○), 9.0 (◇) and 10.0 (◆)).

($k = 3.89 \times 10^{-3}\,h^{-1}$). As the pH approached the basic region the amount of cyclic derivative piperazine-2,5-dione decreased. The amount of amoxicillin penicilloic acid formed in the neutral region decreased also (Longridge & Timms 1971) to give amoxicillin penilloic acid above pH 12 (Robinson-Fuentes et al 1997). The primary amino group played the significant role in the initial rupture of the β-lactam ring responsible for the overall degradation regardless of the pathway by which the degradation proceeded.

Figure 4 shows that at pH values near pK_{a1} there was a decrease in k_{pH} within the pH range 2–4, indicating some influence of the dissociation equilibria of the 4-carboxyl acid group on the degradation rate. However, at pH values near pK_{a2}, there was a small increase in k_{pH} with the pH range 6–8, indicating a microscopic effect of the dissociation equilibria of the side chain amino group on the degradation rate. At pH > 8, the observed rate of the degradation increased rapidly and uniformly with increasing pH. This showed that the hydroxide ion catalysed reactions of anionic amoxicillin took place in this pH region and accounted exclusively for the total amoxicillin trihydrate degradation. The hydrogen ion catalysed degradation was negligible compared with the water catalysed reaction above pK_{a1} and above pK_{a2} the hydroxide catalysed degradation predominated. The calculated values of k_H and k_{OH} indicated that the degradation of amoxicillin trihydrate was much faster in basic solution due to the susceptibility of the β-lactam ring to nucleophilic attack by hydroxide ion (Hou & Pool 1969).

Effect of temperature

The temperature dependence of the degradation of amoxicillin trihydrate was studied in buffered solution over the range pH 1–10 using heat conduction microcalorimetry. The plot of log k vs 1/T was found to be linear (Figure 5) and energy of activation (E_a) was determined from the slope along with pre-exponential factor (log A) (Table 5). The correlation coefficient ($r = 0.9998$) for the Arrhenius plot at all pH was nearly unity. Also, the energy of activation and pre-exponential factor were calculated for each individual rate constant along with other activation parameters such as free energy ($\Delta G^{\#}$), entropy ($\Delta S^{\#}$) and enthalpy ($\Delta H^{\#}$) (Table 6).

In aqueous solution at acidic pH amoxicillin trihydrate degraded mainly via acid catalysed reaction and to some extent water catalysed β-lactam opening reaction with activation energy of 59.79 kJ mol^{-1}. In aqueous solution at pH 5–7 amoxicillin trihydrate degraded mainly via the intramolecular reaction initiated by the attack of the amino group on the β-lactam moiety. However, at pH 5 the predominant species was the zwitterionic form, which degraded with the highest value of activation energy (70.56 kJ mol^{-1}), indicating it was the more stable form of the drug. Above pH 8, the hydroxide ion-assisted degradation proceeded exclusively, with some possible contribution from the intramolecular catalysed reaction and the activation energy for the base catalytic constant (k_{OH}) was calculated to be 54.98 kJ mol^{-1}. The smaller value of activation energy for the base catalytic constant may be due to the susceptibility of the β-lactam ring to hydroxide ion attack. The negative values of entropy of activation (ΔS^0) for all individual rate constants indicated that activated complex involved the formation of associated species involving the drug molecule, water molecule in neutral region, H_3O^+ or $H_3O_2^-$ in acid/base catalysed reaction, respectively. Furthermore, larger values for negative entropy of activation for water catalysed degradation of zwitterionic species in neutral pH region indicated

Table 5 Activation parameters for the degradation reaction of amoxicillin trihydrate over the pH range 1–10.

pH	E_a (kJ mol^{-1})	log A h^{-1}	$\Delta G^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (J mol^{-1} K^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})
1	60.31	9.30	103.42 ± 0.005	−135.46 ± 0.016	60.304 ± 0.263
2	62.06	8.97	107.21 ± 0.005	−141.89 ± 0.014	62.049 ± 0.022
3	64.25	8.86	110.07 ± 0.004	−143.99 ± 0.012	64.239 ± 0.022
4	67.71	9.07	112.20 ± 0.000	−139.82 ± 0.000	67.698 ± 0.005
5	72.59	9.80	112.64 ± 0.002	−125.83 ± 0.006	72.586 ± 0.015
6	69.18	9.25	112.60 ± 0.002	−136.42 ± 0.006	69.174 ± 0.037
7	69.61	9.35	112.38 ± 0.013	−134.42 ± 0.039	69.597 ± 0.018
8	63.79	8.65	110.88 ± 0.004	−147.98 ± 0.011	63.782 ± 0.018
9	55.93	8.00	106.94 ± 0.006	−160.30 ± 0.018	55.919 ± 0.082
10	46.80	6.98	104.08 ± 0.025	−180.00 ± 0.079	46.789 ± 0.722

Table 6 Arrhenius parameters for individual rate constants for the degradation reaction of amoxicillin trihydrate.

Rate constants	E_a (kJ mol^{-1})	log A h^{-1}	$\Delta G^{\#}$ (kJ mol^{-1})	$^{1}\Delta S^{\#}$ (J mol^{-1} K^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})
k_H	59.79 ± 0.01	10.13 ± 0.00	95.95 ± 0.004	−119.25[1] ± 0.04	59.78 ± 0.01
k_1	62.61 ± 0.03	8.99 ± 0.01	105.37 ± 0.006	−141.00 ± 0.13	62.60 ± 0.03
k_2	70.56 ± 0.02	9.47 ± 0.01	110.55 ± 0.007	−131.86 ± 0.08	70.55 ± 0.02
k_3	68.02 ± 0.13	9.14 ± 0.02	109.87 ± 0.011	−140.24 ± 0.42	68.01 ± 0.13
k_{OH}	54.98 ± 0.02	12.91 ± 0.01	74.98 ± 0.013	−65.98[1] ± 0.08	54.97 ± 0.02

[1]The standard state for entropy of activation 1 mol L^{-1}

the formation of cyclic piperazine-2′,5′-dione derivative with a more ordered transition state.

Statistical analysis

To compare the degradation of drug at pH range 1–10, one way analysis of variance followed by multiple comparison with Tukey's test were performed on rate constants obtained in triplicate experimentally. The difference in the mean values of rate constants among the treatment groups (pH) were greater then would be expected by chance and there was a statistically significant difference ($P < 0.001$). The values of rate constants with different initial concentration agreed with each other with least square deviations ±0.31%, 0.40% and 0.37% at 303.15, 310.15 and 318.15 K, respectively. The values of $\Delta_r H^0$ with different initial concentration agreed with each other with least square deviations ± 0.225%, 0.231% and 0.195% in the temperature range of 303.15–318.15 K, respectively. The pH dependence of the rate constant was given by equation 6 in terms of various species of amoxicillin trihydrate. The calculated values of rate constant from the equation agree with standard deviation ± 0.0025, 0.016 and 0.0024 in the temperature range of 303.15–318.15 K, respectively.

Conclusion

This study demonstrated the application of isothermal heat conduction calorimetry to determine the degradation rate, activation parameters and enthalpy change of the degradation of amoxicillin trihydrate. The technique did not involve any sample treatment and the decomposition reaction could be studied directly; the amount of heat produced was proportional to the rupture of the β-lactam ring. This calorimetric study has revealed that amoxicillin trihydrate (zwitterionic form) was most stable at pH 5–7 with the half-life being 183.58 h at pH 5 (310.15 K).

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