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The Influence of Potassium Clavulanate on the Rate of Amoxicillin Sodium Degradation in Phosphate and Acetate Buffers in the Liquid State

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The stability of aqueous admixtures of amoxicillin sodium and potassium clavulanate was studied in the liquid state at selected pH values. Potassium clavulanate was found to catalyze the rate of degradation of amoxicillin sodium under the conditions of this study. In phosphate buffer (at pH 7.0) both amoxicillin sodium and potassium clavulanate showed first-order degradation when stored separately. However, when combined the rate of amoxicillin degradation increased and t_{90} values for amoxicillin decreased from 69.6 min for amoxicillin alone to 10.8 min for amoxicillin in the combination at 55°C. A kinetic model was developed that explained the catalytic behavior of potassium clavulanate and phosphate buffer. In acetate buffer the rate of degradation of amoxicillin sodium followed first-order kinetics, but the catalytic effect of clavulanate caused curvature in the rate plots at higher temperatures and clavulanate concentrations. This catalytic effect was less than that occurred in phosphate buffer (where the t_{90} value of amoxicillin decreased from 137.3 min for amoxicillin alone to 52.5 min for amoxicillin in combination at 55°C). First-order bi-exponential decay occurred with amoxicillin degradation, which explained this change in rate.

Keywords kinetics; stability; amoxicillin clavulanate combination; catalysis; liquid state

INTRODUCTION

Amoxicillin is D(−)-α-amino-*p*-hydroxybenzyl penicillin with a broad spectrum of antibacterial activity. A combination formulation with clavulanate was subsequently marketed to extend the spectrum to include β-lactamase producing organisms. This combination is still widely used as an antibiotic of choice for wide range of clinical conditions (Klein, 2003; Martinez, Inglada, Ochoa, & Villagrasa, 2007; Salvo, Polimeni, Moretti, Conforti, & Leone, 2007).

Several workers have evaluated the stability of aminopenicillins (Ashwin, Lynn, & Taskins, 1987; Bundgaard, 1977; Tsuji, Nakashima, Hamano, & Yamana, 1978; Zia, Shalchian, & Borhanian, 1977) in solution. In dilute aqueous solution, the degradation rate of amoxicillin follows first-order kinetics over a wide pH range (Doadrio & Sotelo, 1988; Moll & Esperester, 1984; Tsuji et al., 1978; Zia et al., 1977) with a minimum rate occurring at approximately pH 6. General acid–base catalysis was also evident in citrate and phosphate buffers (Moll & Esperester, 1984; Tsuji et al., 1978; Zia et al., 1977).

At higher concentrations ($\geq 6 \times 10^{-2}$ mol/dm^3) and in the pH range 8.6–10 the rate of degradation followed higher order kinetics. This arose from the occurrence of a simultaneous dimerization (Bundgaard, 1977) reaction. This occurs via nucleophilic attack of the side chain amino group on the β-lactam group of another molecule and is concentration dependent. The hydrolytic degradation of clavulanate has also been investigated in aqueous solutions (Finn, Harris, Hunt, & Zomaya, 1984; Haginaka, Yasuda, Uno, & Nakagawa, 1983, 1985). These reactions lead to a range of degradation products including an amino ketone and pyrazines. The reaction has been reported as pseudo first-order with a minimum at pH 6.0. The reactions are reportedly (Haginaka, Nakawa, & Uno, 1981) catalyzed by acetate, phosphate, and borate.

To date, the combination of amoxicillin and clavulanate has been marketed in solid state dosage forms and powder suspensions but not as a parenteral dosage form.

There are advantages in some clinical conditions in enabling the combination to be administered parenterally.

Amoxicillin in aqueous solution can exist in up to five different ionic species. In this study, we selected three pH values (2.0, 4.6, and 7.0) which included the ionic species in formulation

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conditions relevant to the stability of the combination. Thus in the hydrochloric acid system, the predominant (about 80%) species would be the cationic form with minor quantity (about 19%) zwitterion. The cationic form of amoxicillin present in the hydrochloric acid system would be relevant clinically in studies of gastrointestinal fluid. Similarly in acetate buffer (pH 4.6) the predominant ionic form (about 99%) of amoxicillin was the zwitterion species, with very small quantities of the anion (about 0.1%) and the remainder the cationic form. Because the pH of maximum stability of amoxicillin happens to be at the region around the isoelectic point, the zwitterionic form of amoxicillin present in the acetate system is considered to be an important species in formulation stability. The zwitterionic species has also been suggested (Hou & Poole, 1969) to be responsible for the broad antimicrobial activity of the amphoteric penicillins such as amoxicillin which makes it a clinically relevant species. Likewise studies in phosphate buffer (pH 7.0) involved the presence of (about 22%) anionic form, another relevant species to formulation stability. The phosphate system provided a neutral medium relevant to parenteral formulations.

The percentage quantities stated were obtained from Bundgaard (1977) who reported pK_a of the carboxyl group (2.63) and the α-amino group (7.55) at 23°C. Additionally, the phenolic hydroxide has a pK_a of 9.6.

This study was the initial part of the study which evaluated the stability of solutions of amoxicillin sodium in combination with potassium clavulanate at selected pH values and temperatures. The stability of these solutions under frozen states has been reported elsewhere (Vahdat & Sunderland, 2007). This paper mainly focusses on interesting and novel findings that resulted from the influence of the potassium clavulanate on the rate of amoxicillin degradation in the phosphate and acetate buffer systems.

MATERIALS AND METHODS

Amoxicillin sodium was kindly provided by SmithKline Beecham Pharmaceuticals, Australia and potassium clavulanate was a gift from SmithKline Beecham Pharmaceuticals, UK. Buffers were prepared from AR reagents and water was deionized by passing through the apparatus (Permutit, Australia) that had a specific conductivity of less than 5.5×10^{-6}/ohm/cm.

Experiments in the buffer systems were carried out at four temperatures (35.0, 42.0, 49.0, and 55.0°C) at pH 7.00 ± 0.05 (1.0×10^{-1} mol/dm^3 phosphate buffer) and pH 4.60 ± 0.05 (2.2×10^{-1} mol/dm^3 acetate buffer). In the hydrochloric acid system (1.24×10^{-2} mol/dm^3 hydrochloric acid), however, the reactions were monitored at lower temperatures (14, 20, 27, and 35°C) and the pH of the solutions were adjusted to 2.00 ± 0.05. All pH measurements were carried out at 25°C. All the runs were performed at a constant ionic strength (μ = 0.5) using sodium chloride.

The phosphate buffer consisted of 6.3×10^{-2} mol/dm^3 of disodium phosphate and 3.6×10^{-2} mol/dm^3 of potassium dihydrogen phosphate. The acetate buffer contained 1.3×10^{-1} mol/dm^3 sodium acetate and 9.3×10^{-2} mol/dm^3 acetic acid.

Admixtures were prepared to a theoretical concentration of 1.29×10^{-3} and 1.05×10^{-3} mol/dm^3 for amoxicillin and clavulanate, respectively. The experiments for the catalytic effects of clavulanate were carried out at four concentrations of potassium clavulanate (5.3×10^{-4}, 1.05×10^{-3}, 2.1×10^{-3} and 3.15×10^{-3} mol/dm^3) and constant amoxicillin sodium (1.29×10^{-3} mol/dm^3) concentration.

The reactants were monitored by a stability indicating HPLC assay method developed from a modified USP method for amoxicillin (USP, 1992). The HPLC system consisted of a reverse phase HPLC column, C_{18} (Altima 5μ, 25 cm × 4.5 mm, NSW, Australia), a C_{18} Guard (Alltech), a Varian Vista 5500 HPLC pump (Victoria, Australia), Varian UV detector with the integrator connected to a Delta chromatography digital system and a 20 μL injector (Rheodyne, Contati, CA, USA). The mobile phase was a mixture of aqueous phosphate buffer and methanol (95:5) adjusted to pH 4.4 ± 0.1. The detection wavelength was set at 228 nm and the flow rate was 1.5 mL/min.

The stability indicating nature of the assay method was determined by inducing degradation of the drug compounds in water, acid (2×10^{-2} mol/dm^3 HCl), and alkali (1×10^{-2} mol/dm^3 NaOH) at room and elevated temperatures (60°C) and restoring the analytical response by addition of the drugs to the almost completely degraded samples. The stability indicating nature of the method was also verified by using a photodiode array detector (Waters 991 Photo diode array UV spectrophotometer, Milford, MA, USA, connected to a 3396 Hewlett Packard integrator, Avondale, PA, USA) to identify peak purity. The wavelength 228 nm was found to give a high response for both drugs when used in combination under these experimental conditions. The peak height rather than the peak area was concluded to give more consistent results in the presence of degradation products (Vahdat, 2000; Vahdat & Sunderland, 2007).

The validity of the method was ascertained by the method described previously (Vahdat & Sunderland, 2007). Standard solutions of the drug combination in water were prepared over the concentration range from 6.45×10^{-5} to 2.58×10^{-3} mol/dm^3 (amoxicillin sodium) and from 4.2×10^{-5} to 1.68×10^{-3} mol/dm^3 (potassium clavulanate), where the linearity $r > .999$ was established for both compounds. The precision of the method was found by calculating the coefficient of variation (n = 6) which was found to be 0.66% and 0.3% for amoxicillin (1.29×10^{-3} mol/dm^3) and clavulanate (1.05×10^{-3} mol/dm^3), respectively. A set of six replicates from each sample set were analyzed at the start of each experimental set. The replicate runs showed high reproducibility with relative standard deviation (RSD) under all experimental conditions and was estimated to be <1%.

Solutions of clavulanate, amoxicillin, and their combinations were prepared from double strength solutions of the buffer and hydrochloric acid media in volumetric flasks (Vahdat & Sunderland, 2007). The flask was then placed in a thermostat water bath (Grant, Cambridge, UK) (±0.1°C) at the required

temperatures for 5 min for equilibration. Simultaneously in another volumetric flask, double strength solutions of the drug samples were prepared in water and placed in the water bath for the same period. The two temperature-equilibrated solutions were then mixed and shaken well. Immediately, a 2 mL aliquot of the final sample solution was removed from the flask brought to room temperature and an aliquot was injected into the HPLC column. The time when the first sample was injected was denoted as time zero. Subsequently, samples were drawn at specified time intervals for analysis until 2–4 half-lives of the reaction were complete. The initial and final pH of the runs were recorded. No significant change in pH was observed under all conditions of this study.

The amount of reactants remaining with time (expressed in percentage) were calculated by dividing the height of each individual peak obtained at each sampling time to the one obtained at time zero, which was designated as 100%. All data were fitted by a least squares program.

RESULTS AND DISCUSSION

The stability indicating nature of the assay was validated in water, alkali, and acid at 60°C. Under these conditions, the reactants were essentially completely decomposed to give base-line chromatographs. These could be quantitatively restored by spiking with known concentrations of amoxicillin and clavulanate. The overall recovery values obtained from these experimental runs fell within the range 100 ± 4% which was considered to be within the experimental error limits. The homogeneity of these peaks was confirmed using a photodiode array detector.

The hydrolytic degradations of amoxicillin sodium and potassium clavulanate were examined separately and in combination in both hydrochloric acid media and each of the acetate (pH 4.6) and phosphate (pH 7.0) buffer systems. Typical first-order plots shown in Figure 1A and B demonstrated the linearity over 2–4 half lives ($t_{1/2}$) of the reaction.

However, the degradation rate of amoxicillin in combination with clavulanate in acetate and phosphate buffers was nonlinear (Figure 2A and B), leading to the conclusion that simple first-order kinetics were not followed. Greater nonlinearity occurred at higher clavulanate concentrations. These data were found to follow a bi-exponential first-order model where amoxicillin underwent two first-order reactions: k_1, which was catalyzed by clavulanate; and k_2, the underlying hydrolytic degradation evident after clavulanate, the more labile reactant, had completely degraded.

This model described the overall rate of amoxicillin degradation as bi-exponential model and C, the overall concentration of amoxicillin expressed by the following relationship:

$$C = A\,k_1^{-kt} + B\,k_2^{-kt}. \qquad (1)$$

The k_1 and k_2 values were determined from the nonlinear regression fitting using the above bi-exponential equation.

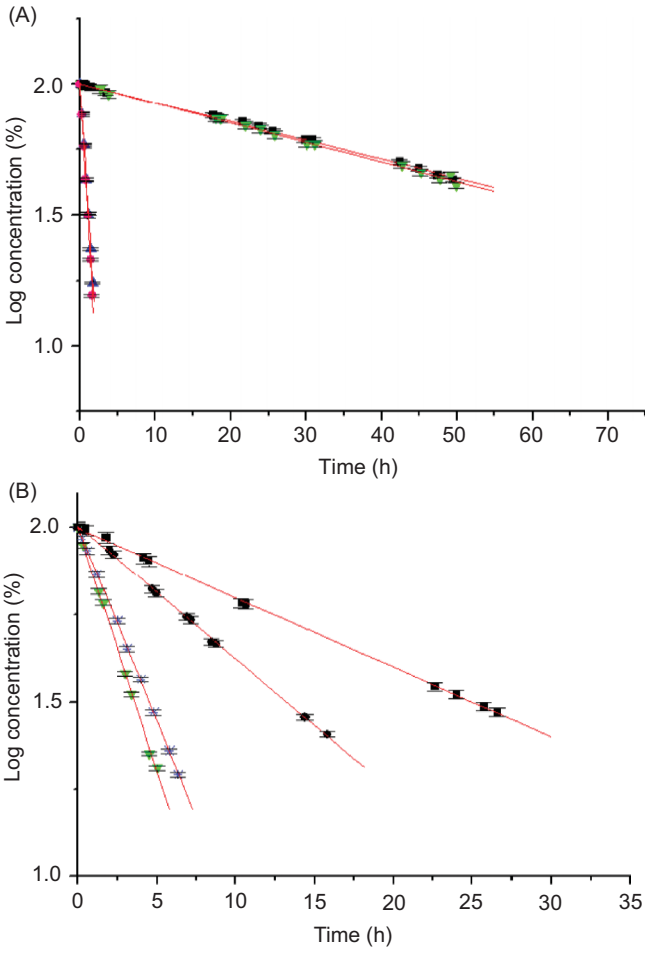

FIGURE 1. Typical first-order plots for amoxicillin and clavulanate at constant ionic strength (μ = 0.5). (A) In hydrochloric acid, pH 2.0 at 27ºC: ■, amoxicillin in combination with clavulanate; ▲, amoxicillin; ▲, clavulanate in combination with amoxicillin; ●, clavulanate. R^2 of all the plots were between .9981 and .9998 and (B) in acetate and phosphate buffer at 55ºC: ●, amoxicillin in phosphate buffer pH 7.00; ▲, clavulanate in combination with amoxicillin in phosphate buffer pH 7.0; *, clavulanate in combination with amoxicillin in acetate buffer pH 4.6; ■, amoxicillin in acetate buffer pH 4.6. R^2 of all the plots were between .9994 and .9999.

The data in Table 1 summarizes the rate constants obtained at pH values of 2.0, 4.6, and 7.0 at several selected temperatures. In the hydrochloric acid system (Table 1A), there was no significant change between the first-order rate constant values of amoxicillin in individual and combination runs. This demonstrated that at this pH any effect of clavulanate on the rate of degradation of amoxicillin in combination was undetectable. The overall rate constant for clavulanate is much greater than amoxicillin, about 60-fold, indicating that clavulanate was much more susceptible to acid hydrolysis than amoxicillin. The summarized data in Table 1A also shows that the observed rate constants for the degradation of clavulanate in individual runs were generally slightly faster than that of the combination. Although this effect was small, it may be related to the slight

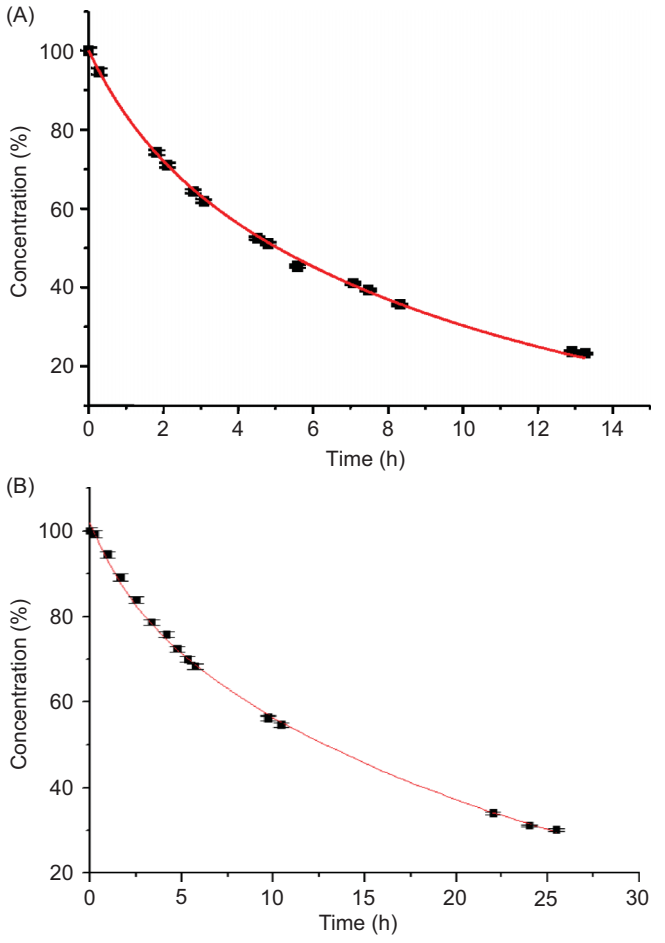

FIGURE 2. First-order bi-exponential plot of amoxicillin in combination with clavulanate at constant ionic strength ($\mu = 0.5$) and 55ºC. (A) Phosphate buffer pH 7.0: $chi^2 = 0.56$ and (B) acetate buffer pH 4.6 where the concentration of clavulanate (3.15×10^{-3} mol/dm^3) is three times greater than (A): $chi^2 = 1.01$.

pH change (0.1 units) observed between the two runs. This was because of the basic nature of amoxicillin solution, which when added to the hydrochloric system tended to increase the pH of the system slightly. There was no significant change in pH observed during the course of each experimental run. The maximum pH change observed was 0.07 pH unit.

It was notable that in the buffer systems (Table 1B) the catalytic rate constants of amoxicillin (k_1) in the combination were markedly greater than that of uncatalyzed rate constant (k_2) values. These data were in acceptable agreement with those evaluated in the absence of clavulanate (Table 1B) and were all statistically significant ($p < .01$ Student's t-test) as shown in Table 1C. This suggested the possibility of a catalytic effect of clavulanate on amoxicillin in the buffer media. In contrast, amoxicillin did not appear to have any effect on the rate of clavulanate decomposition in the combination runs, since there was little change between the rate constant values of clavulante in combination or alone. No catalytic effect was observed on the rate of clavulanate degradation when in combination with amoxicillin. This would tend to eliminate a specific reaction of clavulanate with amoxicillin and support a catalytic effect of clavulanate on amoxicillin degradation. It is notable that this effect was greater in phosphate than in acetate buffers. No significant change in pH occurred under all conditions used in this study. Since the pK_a for clavulanate is 2.7, it would be largely in the ionized form in both buffers suggesting that the ionized clavulanic acid is the responsible species for this catalytic effect.

In addition, it is evident (Table 2A) that the initial concentration of clavulanate has a marked overall effect on the catalytic rate constant for the degradation rate of amoxicillin. However, the concentration of clavulanate had no effect on the rate constant for its own degradation (Table 2B).

Buffer Effects

The effect of buffer species on the rate of degradation of amoxicillin and clavulanate is shown in Figure 3. Extrapolation of these plots to zero-buffer concentration, at the intercepts, provides the values of the non-buffer-catalyzed degradation rate constants (k_{ph}) that correspond to each reaction system (Table 3).

Studies in Phosphate Buffer

The influence of phosphate buffer on the catalytic effect of clavulanate was also investigated. It was worth noting that when the concentration of phosphate was doubled at pH 7.0 there were marked increases (Table 4) in the rate of clavulanate catalysis. Although the catalytic effects of phosphate buffer on the rates of amoxicillin and clavulanate degradations have been documented (Haginaka, et al., 1981; Tsuji, et al., 1978; Zia et al., 1977), there were no reports of clavulanate catalysis. The data from this study are shown in Figure 3 and Table 4.

Studies in Acetate Buffer

The data in Table 2 and Figure 3 show the influence of acetate buffer species on the rate of degradation of amoxicillin. The catalytic effect of clavulanate in acetate was less prominent than in phosphate. This made it difficult to estimate k_1 values particularly at lower concentrations and temperatures, which resulted in considerable errors arising under these conditions. However, the intercept value in Figure 4B is 4.3×10^{-3}/h which is close to the rate constant for amoxicillin in the absence of clavulanate in acetate. This reinforced the evidence of catalysis of amoxicillin by the ionized form of clavulanate in this buffer system.

A kinetic model was developed to explain the overall behavior of the several factors responsible for the catalysis of amoxicillin in the presence of clavulanate in phosphate buffer.

$$k_1 = k_{Amx} + k_{Clav}, \qquad (2)$$

TABLE 1
First-order k_{obs} Values of Amoxicillin and Clavulanate Individually and in Combination at Constant ($\mu = 0.5$) Ionic Strength

(A) In Hydrochloric Acid System

pH	T (°C)	AMOX (h^{-1})	CLAV (h^{-1})	CLAV–COMB (h^{-1})	AMOX–COMB (h^{-1})
2.0	14	4.40×10^{-3}	2.22×10^{-1}	2.07×10^{-1}	4.28×10^{-3}
	20	8.18×10^{-3}	5.03×10^{-1}	4.44×10^{-1}	7.99×10^{-3}
	27	1.72×10^{-2}	1.12×10^{0}	1.02×10^{0}	1.66×10^{-2}
	35	3.44×10^{-2}	2.80×10^{0}	2.67×10^{0}	3.46×10^{-2}

(B) In Acetate and Phosphate Buffer System

pH	T (°C)	AMOX (h^{-1})	CLAV (h^{-1})	CLAV–COMB (h^{-1})	AMOX–COMB (k_1 h^{-1})	AMOX–COMB (k_2 h^{-1})
4.6	35	8.06×10^{-3}	3.59×10^{-2}	3.59×10^{-2}	1.91×10^{-2}	7.99×10^{-3}
	42	1.46×10^{-2}	7.24×10^{-2}	7.29×10^{-2}	5.85×10^{-2}	1.27×10^{-2}
	49	2.37×10^{-2}	1.44×10^{-1}	1.36×10^{-1}	1.02×10^{-1}	2.45×10^{-2}
	55	4.59×10^{-2}	2.08×10^{-1}	2.00×10^{-1}	1.20×10^{-1}	4.75×10^{-2}
7.0	35	1.54×10^{-2}	6.50×10^{-2}	6.03×10^{-2}	1.83×10^{-1}	1.56×10^{-2}
	42	2.91×10^{-2}	1.14×10^{-1}	1.17×10^{-1}	2.28×10^{-1}	3.02×10^{-2}
	49	5.51×10^{-2}	2.17×10^{-1}	2.05×10^{-1}	4.06×10^{-1}	5.70×10^{-2}
	55	9.06×10^{-2}	3.36×10^{-1}	3.29×10^{-1}	5.83×10^{-1}	9.40×10^{-2}

Data presented as average values from two independent experimental run with error <1%. pH 2.0 is in 1.24×10^{-2} mol/dm^3 hydrochloric acid; pH 4.6 is in 2.2×10^{-1} mol/dm^3 acetate buffer; pH 7.0 is in 1.0×10^{-1} mol/dm^3 phosphate buffer. Amoxicillin sodium initial concentration was 1.29×10^{-3} mol/dm^3 in buffers and 9.03×10^{-4} mol/dm^3 in hydrochloric acid system. Initial concentration of potassium clavulanate was 1.05×10^{-3} mol/dm^3 in buffers and 7.38×10^{-4} mol/dm^3 in hydrochloric acid system. CLAV, potassium clavulanate; CLAV–COMB, potassium clavulanate in combination with amoxicillin; AMOX, amoxicillin sodium; k_1, catalyzed first-order rate constant of amoxicillin; k_2, uncatalyzed first-order rate constant of amoxicillin.

(C) Student's t-test for k_1 and k_2

pH	T(°C)	t_{cal}	d.f.	t_{tab} (0.01)
4.6	35	13.8	16	2.921
	42	51.9	16	2.921
	49	10.5	12	3.055
	55	13.5	10	3.169
7.0	35	4.36	16	2.921
	42	5.5	12	3.055
	49	4.42	10	3.169
	55	25.5	6	3.707

where

$$k_{Clav} = \left[k_{cvc} + k_{phccv} \frac{[phos]}{[clav]} \right] [clav] \quad (3)$$

and

$$k_{Amx} = k_0 + k_H[H^+] + k_{hyd}[OH^-] + k_{ph}[phos], \quad (4)$$

where k_1 is the catalytic first-order rate constant for amoxicillin (in combination) because of degradation at the initial stage; k_{Amx} the rate of degradation of amoxicillin because of hydrolysis of amoxicillin; k_{Clav} the rate of degradation of amoxicillin because of the presence of clavulanate; k_0 the uncatalyzed reaction rate constant; k_H the second-order rate constant for acid catalysis of amoxicillin degradation; k_{hyd} the second-order rate constant for base catalysis of amoxicillin degradation; k_{ph} the second-order rate constant for phosphate catalysis of amoxicillin degradation; k_{cvc} the second-order rate constant for clavulanate catalysis of amoxicillin degradation; k_{phccv} the second-order rate constant for phosphate catalysis of clavulanate catalysis of amoxicillin degradation;

TABLE 2
(A) Catalytic Effect of Clavulanate on the Rate of Degradation of Amoxicillin: First-Order Rate Constants of Amoxicillin at Constant Amoxicillin Initial Concentration, $\mu = 0.5$ and 55°C and (B) Effect of Clavulanate Initial Concentration on the Rate of Clavulanate Degradation: First-Order Rate Constant Values of Clavulanate at Constant Amoxicillin Initial Concentration, $\mu = 0.5$ and 55°C

pH	CLAV (mol/dm^3)	k_1 (h^{-1})	*SE* (h^{-1})	k_2 (h^{-1})	*SE* (h^{-1})
4.6	5.3×10^{-4}	1.44×10^{-1}	2.07×10^{-3}	3.9×10^{-2}	4.43×10^{-4}
	1.05×10^{-3}	1.20×10^{-1}	1.19×10^{-3}	4.8×10^{-2}	3.69×10^{-4}
	2.10×10^{-3}	2.87×10^{-1}	2.52×10^{-3}	4.9×10^{-2}	5.05×10^{-4}
	3.15×10^{-3}	4.21×10^{-1}	4.67×10^{-3}	4.6×10^{-2}	5.14×10^{-4}
7.0	5.3×10^{-4}	5.02×10^{-1}	4.90×10^{-3}	9.4×10^{-2}	3.20×10^{-4}
	1.05×10^{-3}	5.83×10^{-1}	5.89×10^{-3}	9.4×10^{-2}	6.30×10^{-4}
	2.10×10^{-3}	7.99×10^{-1}	6.94×10^{-3}	9.2×10^{-2}	9.20×10^{-4}
	3.15×10^{-3}	8.88×10^{-1}	9.77×10^{-3}	1.03×10^{-1}	4.64×10^{-4}

pH	CLAV (mol/dm^3)	CLAV (h^{-1})	*SE* (h^{-1})	CLAV–COMB (h^{-1})	*SE* (h^{-1})
4.6	5.3×10^{-4}	2.20×10^{-1}	5.5×10^{-5}	2.12×10^{-1}	1.02×10^{-3}
	1.05×10^{-3}	2.08×10^{-1}	3.33×10^{-5}	2.00×10^{-1}	1.54×10^{-4}
	2.10×10^{-3}	2.18×10^{-1}	5.67×10^{-4}	2.19×10^{-1}	9.86×10^{-5}
	3.15×10^{-3}	1.95×10^{-1}	6.44×10^{-4}	1.88×10^{-1}	3.37×10^{-4}
7.0	5.3×10^{-4}	3.60×10^{-1}	3.13×10^{-4}	3.30×10^{-1}	9.79×10^{-5}
	1.05×10^{-3}	3.36×10^{-1}	2.18×10^{-3}	3.29×10^{-1}	3.13×10^{-4}
	2.10×10^{-3}	3.39×10^{-1}	2.22×10^{-4}	3.23×10^{-1}	5.36×10^{-4}
	3.15×10^{-3}	3.30×10^{-1}	1.46×10^{-4}	3.34×10^{-1}	2.30×10^{-3}

Amoxicillin initial concentration, (1.29×10^{-3} mol/dm^3); SE, standard errors.

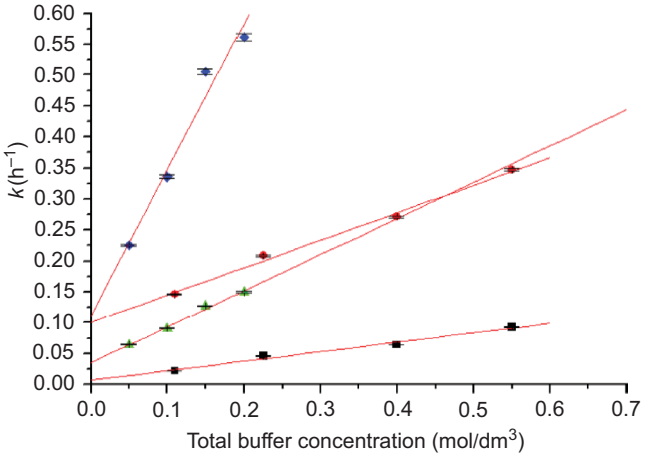

FIGURE 3. Effect of buffer on rate of degradation of amoxicillin and clavulanate at constant ionic strength ($\mu = 0.5$) and 55ºC. ■, amoxicillin in acetate buffer pH 4.6; ●, clavulanate in acetate buffer pH 4.6; ▲, amoxicillin in phosphate buffer pH 7.0; ◆, clavulanate in phosphate buffer pH 7.0.

TABLE 3
k_{ph} (h^{-1})/(mol/dm^3) Values of Amoxicillin Sodium and Potassium Clavulanate at 55°C and $\mu = 0.5$

pH	AMOX k_{ph} (h^{-1})	CLAV k_{ph} (h^{-1})
4.6	0.007	0.101
7.0	0.035	0.113

pH 4.6, 0.22 mol/dm^3 acetate buffer; pH 7.0, 0.10 mol/dm^3 phosphate buffer.

[phos] the concentration of phosphate buffer; and [clav] the concentration of clavulanate.

k_0 was found from the work of Bundgaard (1977), k_{ph} of the phosphate buffer was taken from Table 3, and k_{hyd} was taken as 1.15×10^{-3}/h/(mol/dm^3). The value of k_{ph} was obtained from the slope of the plot of the phosphate buffer effect (Figure 3) and k_{phccv} was estimated to be 2.87/h^{-1}/(mol/dm^3) which was obtained from the slope of the plots of phosphate buffer concentration versus k_1 values. The value of k_{cvc} was found to be 1.75×10^2/h/(mol/dm^3) which was obtained from the slope of the plot of concentration of clavulanate versus k_1 (calculated for zero phosphate buffer effect) values (Figure 4A).

The above model, Eq. 2, was verified by incorporating into equations Eqs. 3 and 4 various rate constant values predicted from the experimental results. Thus, the k_1 values obtained from the model [equation (2)] was compared with the k_1 values obtained directly from the experimental runs (Table 4). The results indicated a satisfactory similarity between the two data sets as shown in Figure 4A which implied that equation (2)

TABLE 4
Comparison Between Catalyzed Rate Constant (k_1) Values of Amoxicillin Obtained from the Experimental Runs Versus Calculated Rates from Model Equation (2)

CLAV (mol/dm^3)	Experimental k_1 (h^{-1})		Model k_1 (h^{-1})	
	1.0×10^{-1a}	2.0×10^{-1a}	1.0×10^{-1a}	2.0×10^{-1a}
5.3×10^{-4}	5.02×10^{-1}	7.85×10^{-1}	4.74×10^{-1}	7.61×10^{-1}
1.05×10^{-3}	5.83×10^{-1}	8.63×10^{-1}	5.66×10^{-1}	8.53×10^{-1}
2.10×10^{-3}	7.99×10^{-1}	1.10×10^{0}	7.50×10^{-1}	1.03×10^{0}
3.15×10^{-3}	8.88×10^{-1}	1.23×10^{0}	9.34×10^{-1}	1.22×10^{0}

CLAV, clavulanate concentration.
[a]Concentration of phosphate buffer (pH 7.0) in mol/dm^3.

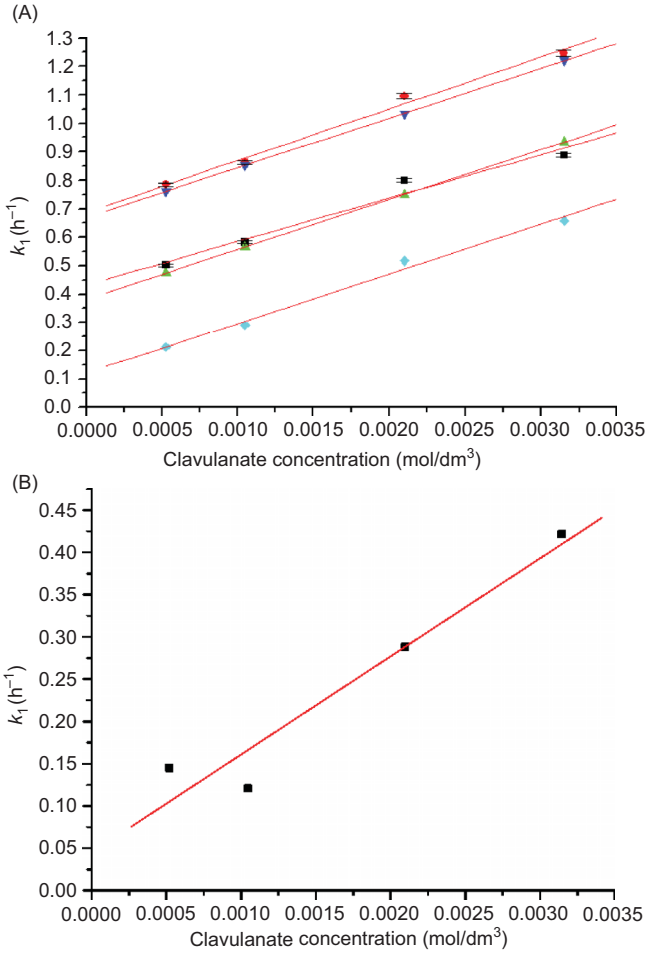

FIGURE 4. Effect of clavulanate concentration on the initial rate of amoxicillin at constant ionic strength μ = 0.5 and temperature 55°C. (A) In phosphate buffer pH 7.0: ■, experimental runs in 0.1 mol/dm^3 phosphate buffer; ●, experimental runs in 0.2 mol/dm^3 phosphate buffer; ▲, calculated value from Eq. 2 for the runs in 0.1 mol/dm^3 phosphate buffer; ▼, calculated value from Eq. 2 for the runs in 0.2 mol/dm^3 phosphate buffer; ◆, calculated value for zero phosphate effect by subtracting the phosphate buffer catalytic effect (k_{phccv}) and (B) in acetate buffer pH 4.6: the k_1 values are the average of two independent experimental runs.

TABLE 5
Activation Energy (E_a) Values at Constant Ionic Strength (μ = 0.5)

pH	Activation Energy (E_a) kJ/mol[1]			
	AMOX	CLAV	AMOX–COMB	CLAV–COMB
2.0	72.49	88.12	73.45	88.19
4.6	71.21	75.12	77.17(k_1) 74.83(k_2)	72.66
7.0	74.80	69.82	50.67 (k_1) 75.51(k_2)	71.01

CLAV, potassium clavulanate; CLAV–COMB, potassium clavulanate in combination with amoxicillin; AMOX, amoxicillin sodium; AMOX–COMB, amoxicillin sodium in combination with clavulanate. pH 2.0, 1.24×10^{-2} mol/dm^3 hydrochloric acid; pH 4.60, 0.22 mol/dm^3 acetate buffer; pH 7.0, 0.10 mol/dm^3 phosphate buffer.

could be a suitable model for clavulanate-catalyzed rate constant of amoxicillin in combination.

Temperature Dependency on Reaction Rates

The temperature dependence of amoxicillin sodium and potassium clavulanate alone and in combination were studied under these experimental conditions. The Arrhenius plots were obtained by the normal procedure of plotting log k versus (1/T) and the apparent energies of activation were calculated (Table 5 and Figure 5). The data obtained were in close agreement with literature values obtained for amoxicillin (Zia et al., 1977) and for clavulanic acid (Haginaka et al., 1981). The lower value for k_1 for amoxicillin in the combination in phosphate buffer may be indicative of the catalyzed reaction.

CONCLUSIONS

This study has identified that significant catalysis of amoxicillin occurred in the selected acetate and phosphate buffers. Clavulanate is an additional catalytic species and

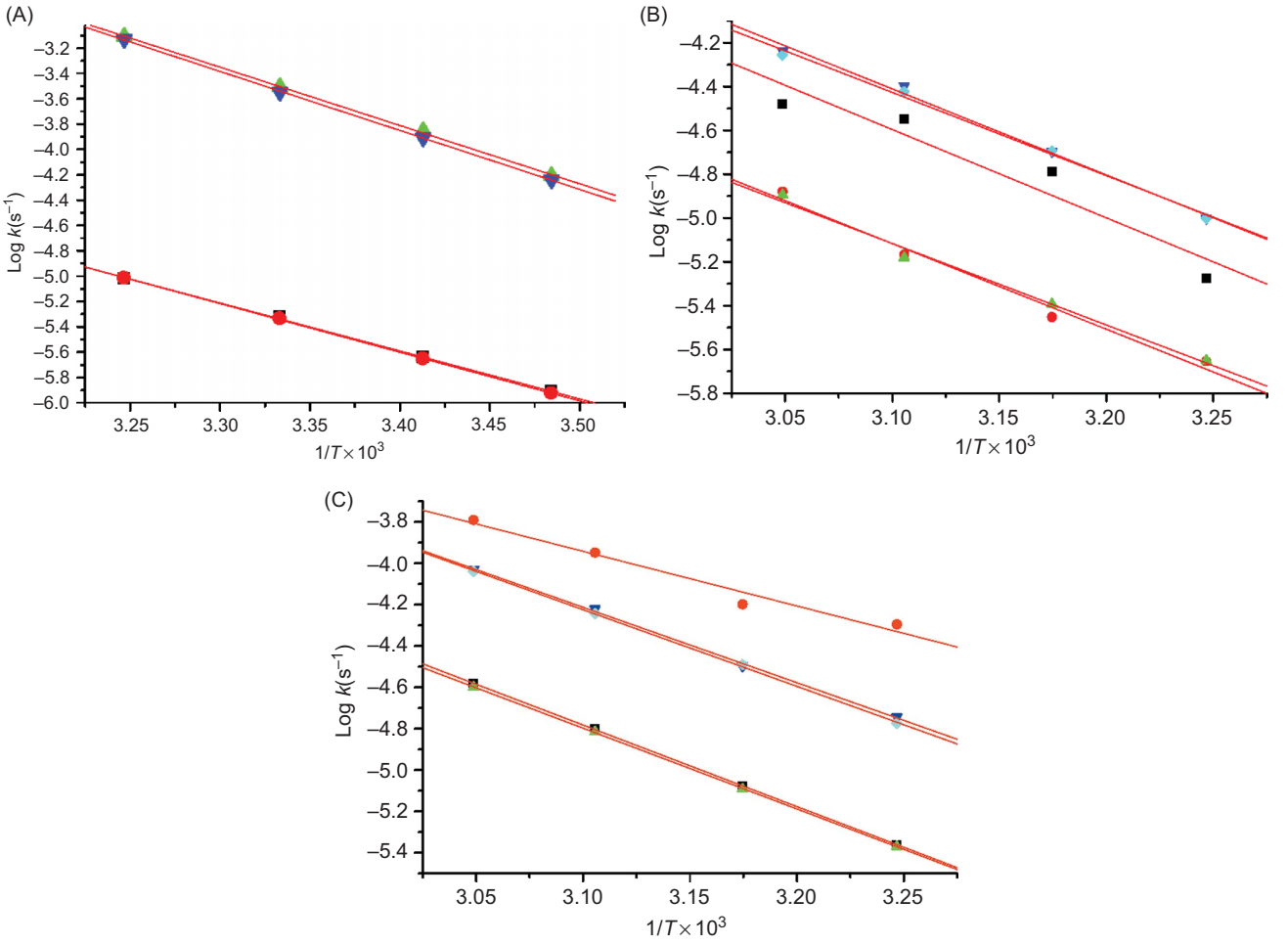

FIGURE 5. Arrhenius plots of amoxicillin sodium and potassium clavulanate in separate solution and in combination at constant ionic strength $\mu = 0.5$. (A) In hydrochloric acid system pH 2.0: ■, amoxicillin; ●, amoxicillin in combination with clavulanate; ▲, clavulanate; ▼$_c$, Clavulanate in combination with amoxicillin; (B) in acetate buffer pH 4.6: ■, k_1 of amoxicillin in combination with clavulanate; ●, k_2 of amoxicillin in combination with clavulanate; ▲, amoxicillin; ▼, clavulanate; ◆, clavulanate in combination with amoxicillin; and (c) in phosphate buffer pH 7.0: ■, k_2 of amoxicillin in combination with clavulanate; ●, k_1 of amoxicillin in combination with clavulanate; ▲, amoxicillin; ▼, clavulanate; ◆, clavulanate in combination with amoxicillin.

Note: All the data are the average of two independent runs with error <1%.

therefore destabilizes amoxicillin in the combination. This causes a rapid initial loss of amoxicillin. For example at 35°C, the shelf-life of amoxicillin is 5.5 h in acetate buffer (pH 4.6) and 0.57 h in phosphate buffer (pH 7.0). Both of these systems are unsuitable for the preparation of this combination for administration.

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