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Stability kinetics of piperacillin in aqueous solutions

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Summary

The degradation process of piperacillin in acidic, neutral and alkaline solutions was followed by both high-pressure liquid chromatographic and spectrophotometric assays. Pseudo-first-order rate constants were determined in a variety of buffer solutions. The overall pH–rate profile was determined at 35°C and an ionic strength of 0.5. β-Lactam moiety degradation occurred in acidic media to produce the hydrolysis products. In alkaline solutions, the piperazinyl ring of piperacillin was hydrolyzed about 20 times faster than the β-lactam moiety.

Introduction

Penicillin have been shown to be ready to undergo cleavage of the β-lactam moiety in aqueous solutions (Schwartz et al., 1969; Hou et al., 1971; Yamana et al., 1974).

The compound, sodium-6-{2-[(4-ethyl-2,3-dioxo-1-piperazinyl)-formamido]-2-phenylacetamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3,2,0] heptane-2-carboxylate, is a new antibiotic with a broad spectrum of antibacterial activity. Its general name is piperacillin. Interest in the kinetics of piperacillin degradation arose from previous studies (Saikawa et al., 1977) demonstrating an easy cleavage of the piperazinyl ring compared to the β-lactam moiety in pH 9.0 buffer solution, unlike the easier cleavage of β-lactam in acidic media.

The purpose of the present study was to investigate the hydrolytic behavior of piperacillin over a wide pH range and to compare the stability of the piperazinyl ring with that of β-lactam moieties in aqueous solutions.

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Materials and Methods

Chemicals

Piperacillin was supplied by Sankyo, Japan. All other chemicals were of the highest grade available and were used without further purification except imidazole which was purified as described in the literature (Bundgaard et al., 1972).

Kinetic procedures

The degradation of piperacillin was studied at 35 ± 0.1°C, unless otherwise stated. The pH of the reacting solution of piperacillin (5×10^{-3} M) was maintained at the desired value with a pH-stat (Radiometer TTT-60 and ABU-12) or appropriate buffer systems. Where the half-life was more than 1 day, the solution was sealed in 2 ml ampoules. At appropriate intervals, samples were removed, cooled and assayed for intact piperacillin and/or products. The buffer solutions employed were hydrochloric acid–potassium chloride, acetic acid–sodium acetate, hydrochloric acid–monobasic phosphate, mono–dibasic phosphate, and sodium carbonate–sodium bicarbonate. The ionic strength of each solution was adjusted to 0.5 by the addition of potassium chloride.

Determination of piperacillin and its degradation products

Liquid chromatography. High-pressure liquid chromatography (Model LC-1. Shimadzu) was used to analyze intact piperacillin and its degradation products. The liquid chromatograph was equipped with a UV detector set at 210 nm and a stainless steel column (4.6 mm × 25 cm), prepacked with octadecylsilane chemically bonded on totally porous silica gel (ZORPAX-ODS, Du pont). Reversed-phase chromatography was employed with 40% methanol–0.05 M ammonium carbonate as a mobile phase. Samples were eluted at 50 kg/cm^2 at room temperature, resulting in a flow rate of 0.6 ml/min. Five μl of degradation solution (5×10^{-3} M total penicillin) was injected. The peak heights were measured, and concentrations were calculated from the calibration curves (obtained daily).

Spectrophotometric method. The remaining β-lactam of penicillin was assayed by the method of Bundgaard (1972). To 1 ml of sample in a test tube was added 5 ml of the imidazole reagent. After allowing the mixture to stand in a water bath at 60°C for 20 min, the tube was cooled to room temperature. The absorption in a 1 cm cell of the mixture was measured at 325 nm.

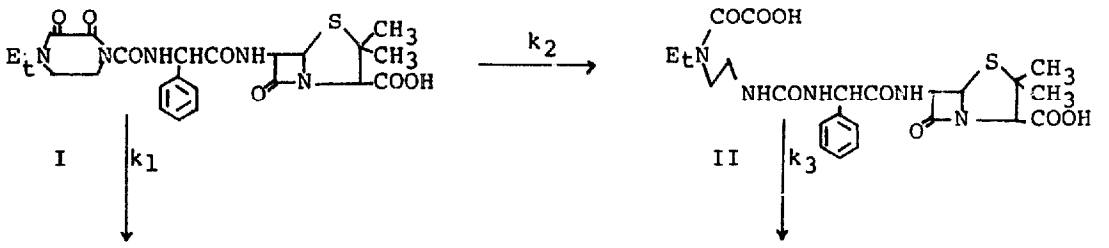
Preparation of the degradation product

The degradation intermediate, II, was prepared by the method of Takano et al. (1977). Equimolar piperacillin sodium and NaOH solution were mixed. After stirring for 10 min at 0–5°C, the solution pH was adjusted to 2.0 with HCl and the resultant solution was extracted with ethyl acetate. This ethyl acetate solution saturated with NaCl was filtered, washed with saturated NaCl aqueous solution, and dried over sodium sulfate. After evaporation, a white crystal (II) was obtained. The NMR and IR spectra of this crystal were in good agreement with the data given by Takano et al. (1977): 3.28 (broad singlet, CH_2); IR (KBr), 1770 cm^{-1} (β-lactam). HPLC of the crystal revealed a single peak.

The first-order rate constants estimated by using crystallized II were in good agreement with the rate constants determined by using the stock solution, which was obtained freshly by the degradation of piperacillin at pH 10.0 and 35°C for 30 min and neutralized by the addition of HCl and stored at 0°C. Since it was difficult to prepare a large quantity of pure crystalline II, the above stock solutions were sometimes used for estimation of the rates of degradation of II. These stock solutions contained II at about 82%, its degradation products at less than 18% and piperacillin at less than 1.0%.

Results and Discussion

The kinetics of degradation of piperacillin were investigated at various pH values by HPLC and spectrophotometric assays. Typical chromatograms of acidic (pH 3.0) and alkaline (pH 8.0) reaction mixtures are illustrated in Fig. 1. The peak designated II is the degradation product in Scheme 1 and that designated I is piperacillin itself. Scheme 1 shows the degradation pathway of piperacillin proposed by Saikawa et al. (1977).



Scheme 1

By solving the differential equation for Scheme 1, the rate expression for the molar fraction of each species may be written as:

$$[I]/[I_0] = e^{-(k_1+k_2)t} \tag{1}$$

$$[II]/[I_0] = \frac{k_2}{k_3-(k_1+k_2)} \cdot (e^{-(k_1+k_2)t} - e^{-k_3 t}) \tag{2}$$

By plotting the logarithm of $[I]/[I_0]$ against time, the apparent first-order rate constants, k_{obs}, for the overall degradation of I could be obtained from the slopes, i.e.:

$$k_{obs} = k_1 + k_2 \tag{3}$$

Fig. 2 shows the typical time course of the molar fractions of I and II during the degradation of I in alkaline solution of pH 9.5. In this experiment, compounds I and II were determined by HPLC and the molar fraction of the remaining β-lactam was

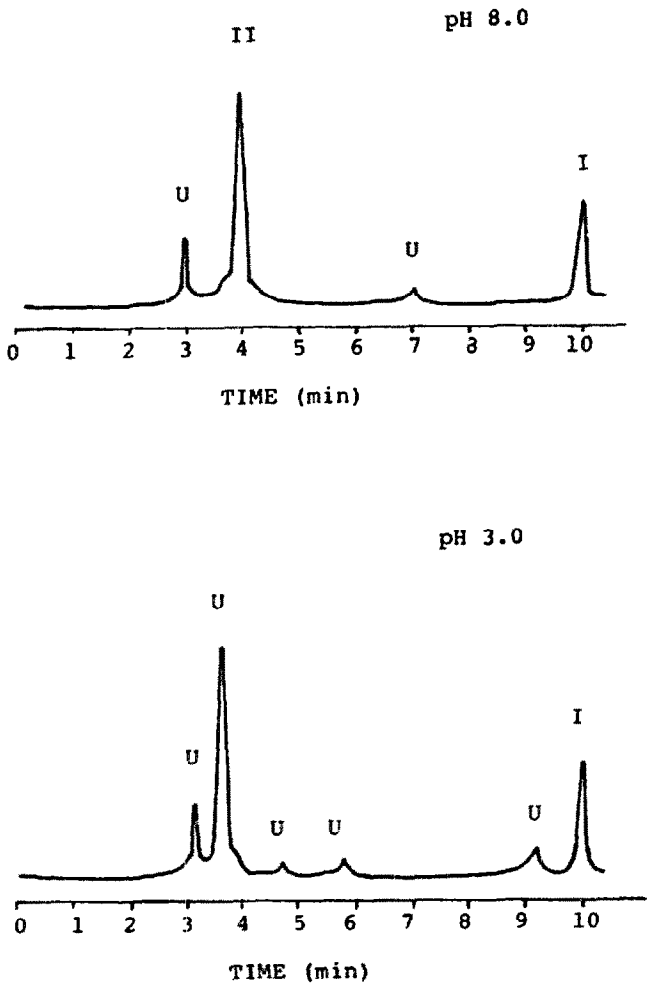


Fig. 1. High-pressure liquid chromatograms of 50% degraded piperacillin at 35°C and an ionic strength of 0.5. Key: I, piperacillin; II, degradation intermediate; U, unknown.

estimated by spectrophotometric assay. To evaluate k_3, the degradation of II was followed under the same conditions by spectrophotometric assay (Fig. 2).

As a result of the degradation of I and II in Fig. 2, $k_1 = k_3$ was confirmed, since the time course of the sum of the molar fractions of I and II as determined by spectrophotometric assay showed first-order kinetics and its value at $t = 0$ was assumed to be 1.0. Eqn. 4 could therefore be obtained from Eqn. 2.

$$[II]/[I_0] = e^{-k_3 t}(1 - e^{-k_2 t}) \quad (4)$$

As shown in Fig. 2, the experimental values for I and II closely approximated to the calculated line obtained by using Eqns. 1 and 4, suggesting that the degradation pathway of I in alkaline solution was actually followed as in Scheme 1. Thus, the value of k_1 determined by spectrophotometric assay was employed.

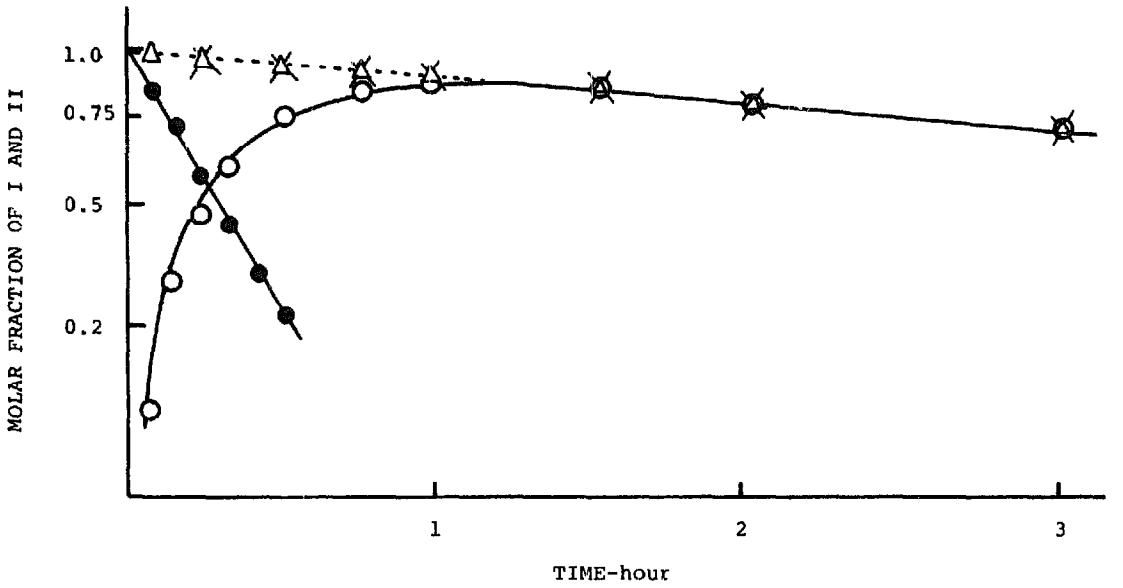


Fig. 2. Typical time course for piperacillin (I, ●) and intermediate (II, ○) determined by the HPLC method, and the sum of I and II (△) determined by the spectrophotometric method during the degradation of piperacillin at pH 9.5 [a], 35°C and an ionic strength of 0.5. The dotted line represents the time-course of II obtained by the spectrophotometric method (×) during the degradation of II. The solid lines were generated by using Eqns. 1 and 4 based on the assumption of $k_1 = k_3$ ($k_1 = k_3 = 0.12\ h^{-1}$ and $k_2 = 2.7\ h^{-1}$).

[a] The pH of the solution was maintained with a pH-stat.

In acidic media below pH 3.5, k_{obs} appeared to be almost identical with k_1 and the contribution of k_2 to k_{obs} was negligible, since II was scarcely detected by HPLC during the degradation of I (see Fig. 1) and the rate constants, k_{obs}, of total degradation of I evaluated by the HPLC method were in good agreement with the first-order rate constant evaluated by spectrophotometric assay of the same sample (see Fig. 5 below).

Fig. 3 shows typical first-order plots for the total loss of piperacillin at various pH values.

At constant pH and in the presence of excess buffer, the rate constants, k_{obs}, for the total loss of piperacillin were affected by general acid–base catalysis of the buffer components. Typical plots for the catalytic effect of various buffers on k_{obs} are given in Fig. 4, yielding a reasonably straight line at a constant pH level in all cases. Extrapolation of such plots to zero buffer concentration provides, as intercepts, the values of the pseudo-first-order rate constants, $(k_{obs})_{pH}$ or $(k_1)_{pH}$, corresponding to the non-buffer-catalyzed degradation of I. These values were also determined with the pH-stat.

Fig. 5 shows log $(k_{obs})_{pH}$–and $(k_1)_{pH}$–pH profiles for piperacillin degradation at 35°C and an ionic strength of 0.5.

For the degradation of the β-lactam of piperacillin, $(k_1)_{pH}$ obeys the general rate

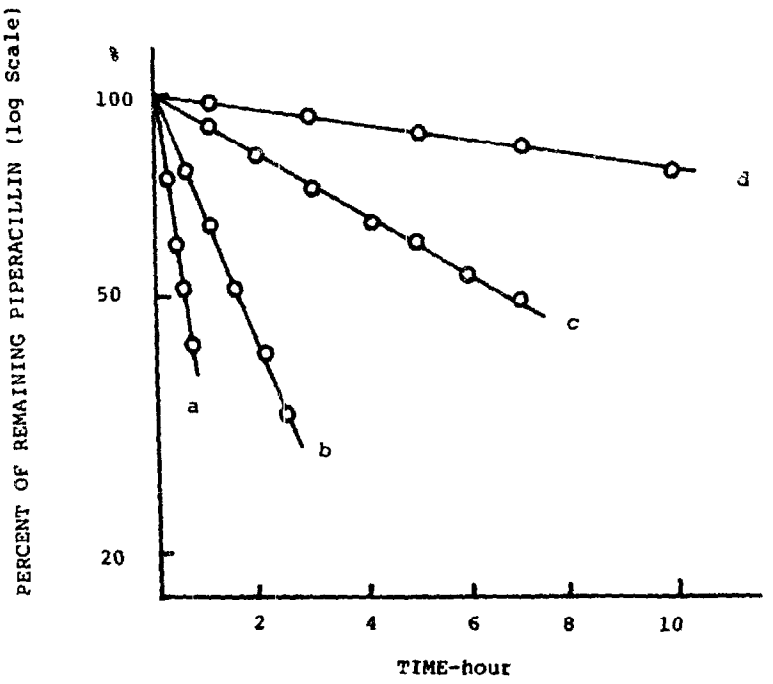


Fig. 3. Apparent first-order plots followed by HPLC for the degradation of piperacillin at various pH values, 35°C and an ionic strength of 0.5. Key: a, pH 9.3 (pH-stat); b, pH 1.6 (hydrochloric acid–monobasic phosphate); c, pH 2.4 (hydrochloric acid–monobasic phosphate); d, pH 7.0 (mono–dibasic phosphate).

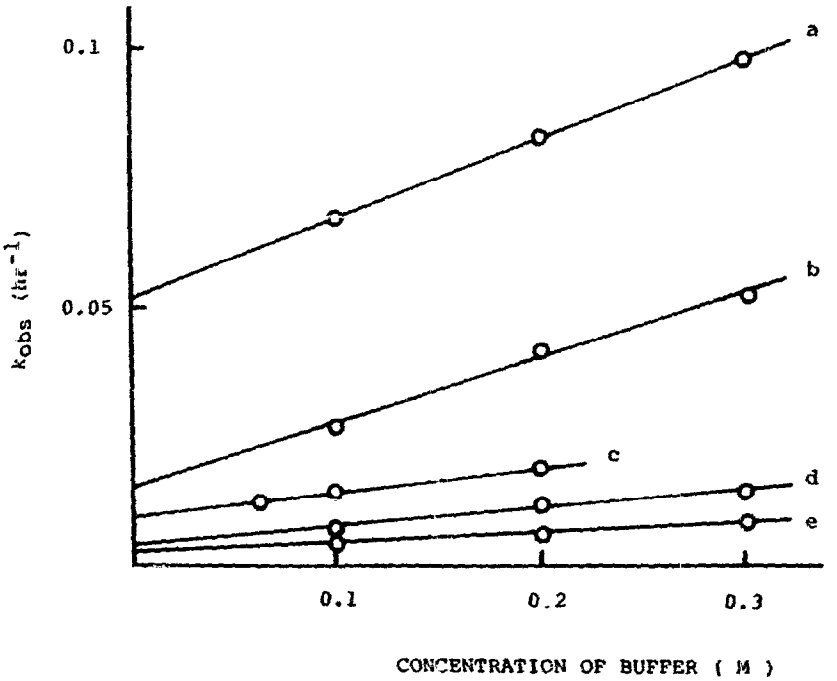


Fig. 4. Plots of the pseudo-first-order rate constants, k_{obs}, vs the total buffer concentration for the degradation of piperacillin at 35°C and an ionic strength of 0.5. Key: a, pH 2.4 (hydrochloric acid–monobasic phosphate); b, pH 3.0 (hydrochloric acid–monobasic phosphate); c, pH 7.0 (mono-dibasic phosphate); d, pH 4.0 (acetic acid–sodium acetate); e, pH 6.0 (mono–dibasic phosphate).

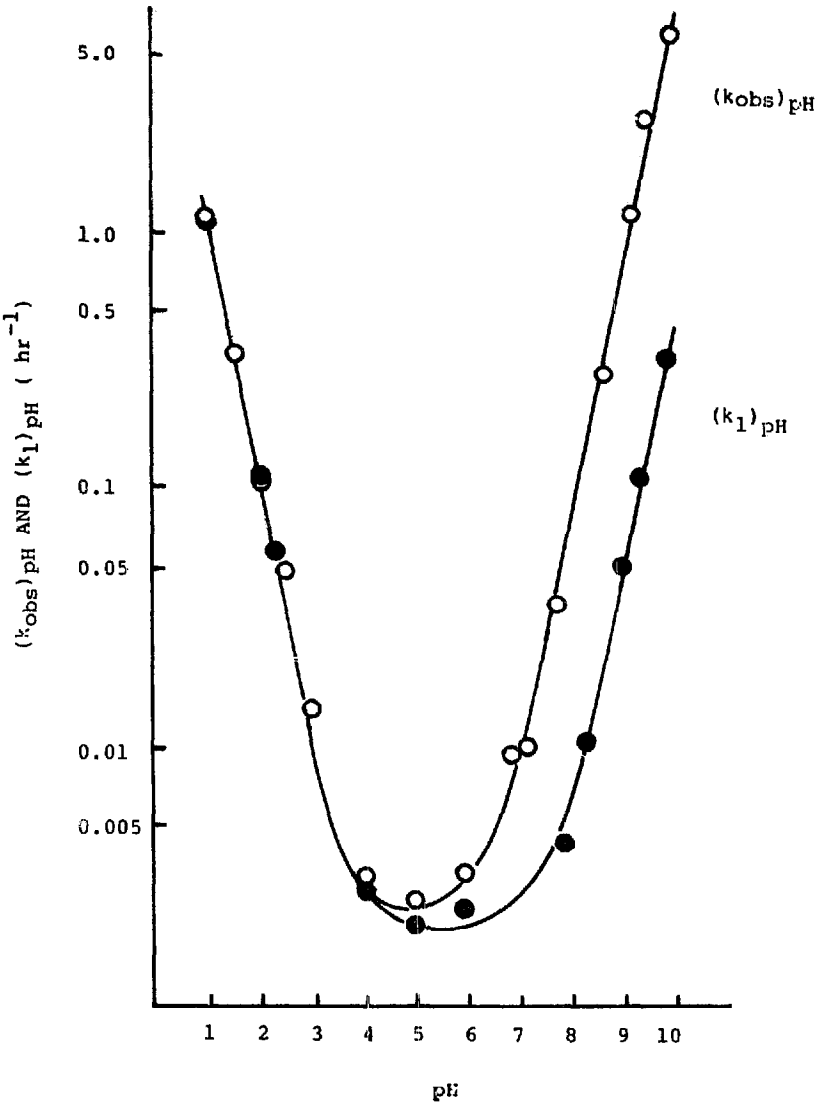


Fig. 5. $\log(k_{obs})_{pH}$– and $(k_1)_{pH}$–pH profiles for the degradation of piperacillin at 35°C and an ionic strength of 0.5. Key: O, determined by the HPLC method; ●, determined by the spectrophotometric method.

law (Yamana et al., 1974, 1977).

$$(k_1)_{pH} = [(k_1)_H a_H + (k_1)_0] \frac{a_H}{K_a + a_H} + \left[(k'_1)_0 + (k'_1)_{OH} \frac{K_w}{a_H}\right] \frac{K_a}{K_a + a_H} \quad (5)$$

where $(k_1)_H$ and $(k'_1)_{OH}$ are the second-order rate constants for the hydrogen-ion- and hydroxide-ion-catalyzed degradation, $(k_1)_0$ and $(k'_1)_0$ are the first-order rate constants for spontaneous and/or water-catalyzed degradation, and a_H is the activity of the hydrogen ion as measured with a pH meter. The value for the autoprotolysis constant of water, K_w, at 35°C is 2.09×10^{-14} (Harned, 1933). The

pK_a value was determined potentiometrically at 35°C and an ionic strength of 0.5 to be 2.9.

At pH < 4, $(k_{obs})_{pH}$ was almost identical with $(k_1)_{pH}$, indicating that piperazinyl ring cleavage was negligible in this pH region. Below pH 8, $(k_{obs})_{pH}$ was about 20 times larger than $(k_1)_{pH}$. This suggests that piperazinyl ring cleavage rather than β-lactam cleavage proceeds predominantly in the alkaline region.

For the alkaline cleavage of the piperazinyl ring of piperacillin, $(k_2)_{pH}$ could be written as:

$$(k_2)_{pH} = (k'_2)_{OH} \frac{K_w}{a_H} \frac{K_a}{K_a + a_H} \tag{6}$$

where $(k'_2)_{OH}$ represents the hydroxide-ion-catalyzed degradation rate of the piperazinyl ring of piperacillin.

The calculated curve for $(k_{obs})_{pH}$ was generated from Eqn. 7 corresponding to the sum of Eqn. 5 and 6, by the use of appropriate parameters:

$$(k_{obs})_{pH} = [(k_1)_H a_H + (k_1)_0] \frac{a_H}{K_a + a_H} + \left[(k'_1)_0 + [(k'_1)_{OH} + (k'_2)_{OH}] \frac{K_w}{a_H}\right] \frac{K_a}{K_a + a_H} \tag{7}$$

The rate constants which produced the best fits to the observed rate–pH profiles by using a digital computer (FACOM M 160, Data Processing Center, Kanazawa University) are given in Table I.

Dependence of rate on temperature

Good Arrhenius-type plots were obtained for the observed pseudo-first-order rate constants, $(k_{obs})_{pH}$, at 4 different pH values (Fig. 6). The activation energy, E_a, calculated from the slope of the lines, was 16.5 kcal/mol at pH 3.0, 12.3 kcal/mol at pH 5.0, 12.6 kcal/mol at pH 6.0 and 20.5 kcal/mol at pH 7.0.

Based on the present kinetic studies, piperacillin injection for general use appears to be adequately stable, because piperacillin dissolved in distilled water for injection gave pH 6.0 and the 10% loss time was predicted to be 40 h at 35°C and 115 h at 20°C.

TABLE I

CALCULATED VALUES OF RATE CONSTANTS FOR THE DEGRADATION OF PIPERACILLIN ($pK_a = 2.9$)

$(k_1)_H$ ($M^{-1}h^{-1}$)	$(k_1)_0$ (h^{-1})	$(k_1)_0$ (h^{-1})	$(k_1)_{OH}$ ($M^{-1}h^{-1}$)	$(k_2)_{OH}$ ($M^{-1}h^{-1}$)
1.1×10	1.3×10^{-2}	2.1×10^{-3}	2.0×10^{3}	3.5×10^{4}

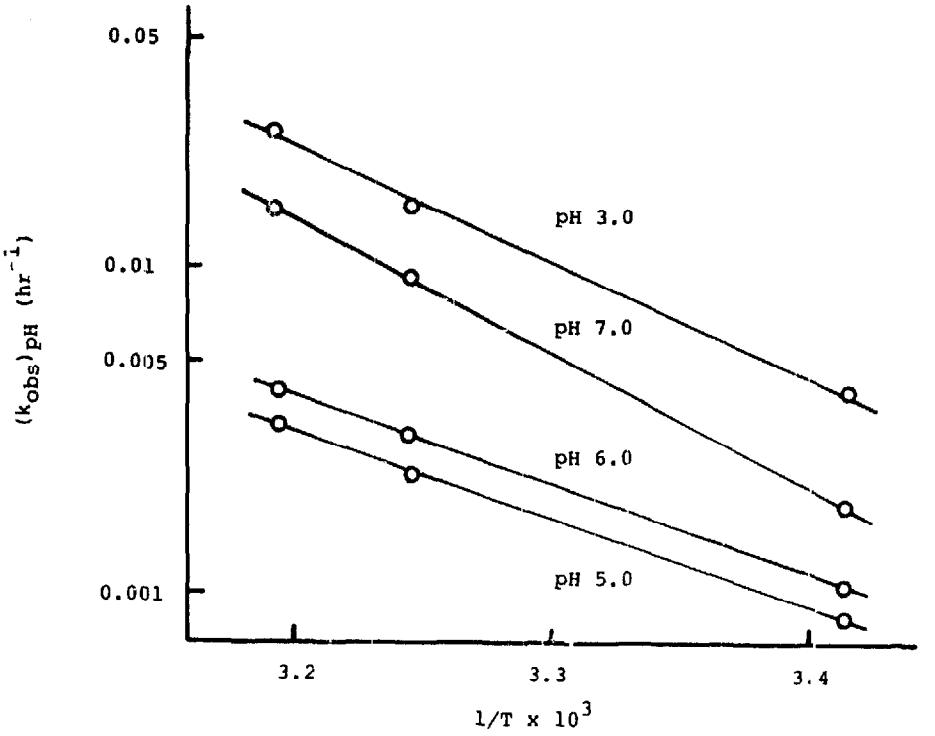


Fig. 6. Arrhenius type plots of $(k_{obs})_{pH}$ for the degradation of piperacillin in four different pH solutions at an ionic strength of 0.5.

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