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Kinetics of degradation of cefaclor: I. Effects of temperature, phosphate buffer, pH and copper(II) ion on the rate of degradation

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Abstract

Acidic, neutral and alkaline degradation of cefaclor was followed by ultraviolet spectroscopy, high-pressure liquid chromatography and iodometric method. Degradation of the drug follows first-order kinetics under constant pH and temperature conditions. The pH-rate profile was like that of other cephalosporins with an essentially similar structure. Phosphate buffer catalysis was observed. The copper(II)-ion catalyzed hydrolysis of cefaclor was investigated and no significant effect was found. The temperature effects on the reaction rate were followed and from Arrenius plots of the rates of hydrolysis, different activation energies were calculated.

Keywords: Cefaclor; Degradation kinetics; pH-rate profile; Temperature effect; Phosphate buffer effect; Copper(II) ion effect

1. Introduction

Penicillins and cephalosporins represent the most important class of drugs against infections caused by bacteria. The biologically active principle of these antibiotics is the β-lactam ring, the reactivity and selectivity of which can be decisively modified by substituents (Sweet and Dahl, 1970; Flynn, 1972; Boyd et al., 1975). The effect of the 3-substituents and the influence of the group in the 7-position on the reactivity of the β-lactam ring of cephalosporins have been investigated in detail by several authors (Hermann, 1973; Indelicato and Wilham, 1974; Boyd et al. 1980; Schanck et al., 1983).

Penicillins and cephalosporins have been subjected to facile cleavage of their β-lactam bonds in aqueous solution (Hou and Poole, 1971). The neutral and basic degradation of cephalosporins possessing an α-amino group in their C-7 side chain is facilitated by the intramolecular attack of the side chain amino group upon the β-lactam carbonyls (Indelicato et al., 1974; Bungaard, 1976;

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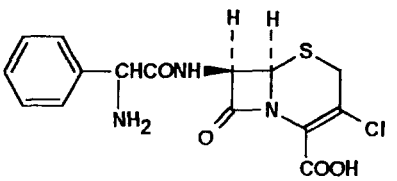

Fig. 1. Chemical structure of cefaclor.

Yamana and Tsuji, 1976; Tsuji et al., 1981). The acidic degradation of penicillins and cephalosporins has been studied and specific acid catalysis is observed (Hou and Poole, 1971; Yamana and Tsuji, 1976).

Some attention has been paid to the comparative chemical stability of cefaclor (Indelicato et al., 1977; Boyd, 1983; Matsukuma et al., 1989; Blaszczak et al., 1990; Pasini and Indelicato, 1992). Since, to our knowledge, there has been no systematic study of the degradation kinetics of cefaclor, we describe here the kinetics of degradation of cefaclor and the influence of different factors such as pH, temperature, phosphate buffer and copper(II) ion on the rate of cefaclor hydrolysis.

Cefaclor is a semisynthetic cephalosporin antibiotic for oral administration. The chemical structure of cefaclor is similar to that of cephalexin. Substitution of a chloro group for the methyl group of cephalexin produced a compound with an extended antibacterial spectrum of activity (Indelicato et al., 1977). The chemical structure of cefaclor, shown in Fig. 1, has two ionizable groups with ionization constants pK_{a_1} = 1.5 and pK_{a_2} = 7.17 for the dissociation of carboxylic acid and the α-amino group, respectively (Lorenz, 1980).

2. Experimental

2.1. Materials

Cefaclor monohydrate (953 μg/mg) was obtained from production lots (Eli Lilly and Company) and used without further purification. All other chemicals used were reagent grade.

2.2. Kinetic procedures

All kinetic experiments were carried out at 30°C except for the Arrhenius studies. The reaction solution pH was maintained at the desired value by the use of pH-stat or phosphate buffers (pH 5.98–7.40, respectively). The buffer solutions used were monobasic-dibasic sodium phosphates. The pH values of the reaction solutions were measured at the experimental temperature initially and at the end of the experiment on the pH-meter, standardized with standard buffer solutions at the same temperature. No significant changes in pH were observed. The ionic strength of all solutions (pH stat and buffer) was adjusted at 0.5 with sodium chloride.

An accurately weighed amount of cefaclor was dissolved in distilled water or in appropriate buffer solution preheated at a desired temperature to produce a final concentration of 5×10^{-5} M. The reactions were conducted in a constant temperature water bath at 30 ± 0.1°C. Samples were taken at appropriate time points and assayed immediately.

Solutions which were used for exploring the influence of copper(II) ion on the hydrolysis of cefaclor were prepared in water that had been distillated after deionization. The purity of the water was checked on a conductivity meter and atomic absorption spectrophotometer. All water used contained less than 0.01 ppm total solids, expressed as sodium chloride.

A series of different kinetic runs were carried out at pH 4.08, 5.00 and 6.00, maintained by a pH stat. The concentration of cefaclor was held constant at 5×10^{-5} M and Cu(II) at 5×10^{-6} M. The extension of the study to pH values above 6.00 was hindered by copper hydroxide formation. Another series of experiments were performed at pH 6.00 and the rate of degradation of cefaclor was measured in the presence of different concentrations of copper(II) ion in the range from 1.25×10^{-6} to 1×10^{-5} M. All experiments were performed at 30°C and ionic strength μ = 0.5.

The kinetic parameters describing the degradation of cefaclor were determined by a combination of appropriate analytical methods.

2.3. Analytical procedures

2.3.1. Spectrophotometric method

The rates of hydrolysis were determined by following the loss of the characteristic UV absorbance at 264 nm due to the β-lactam system on a recording HP 8452 diode array spectrophotometer Hewlett Packard, having a thermostated cell compartment. Disappearance of this absorption band is characteristic of β-lactam opening, either chemically or enzymatically (Flynn, 1972). As a result, the UV spectrophotometric method is acceptable for good estimation of the β-lactam opening rate of the cephalosporins.

2.3.2. HPLC method

The degradation rate of cefaclor was followed by measuring the remaining drug concentration by a reversed-phase high-pressure liquid chromatographic (HPLC) method.

The liquid chromatograph (Varian system) was equipped with 9010 Pump, variable wavelength detector (9050 UV/Vis) set at 264 nm and reverse-phase 4 × 250 mm LiChrospher column as a stationary phase. The mobile phase was glacial acetic acid-acetonitrile-water (2 : 15 : 83). The samples were eluted at a flow rate of 1.2 ml/min, at room temperature. 20 μl of degradation solution (1×10^{-3} M total antibiotic) was injected at suitable intervals using a Marathon autosampler with 20 μl loop. Peak areas were measured with a Varian 4400 integrator. A good linear relationship between the peak area and the concentration of intact cefaclor was obtained and the concentrations of cefaclor were calculated from the calibration curves.

2.3.3. Iodometric method

The working solutions with initial cefaclor concentration of 5×10^{-3} M were analyzed according to the method for iodometric determination of cephalosporins (Yamana and Tsuji, 1976) and the concentration of intact cefaclor in solution containing degradation products was determined.

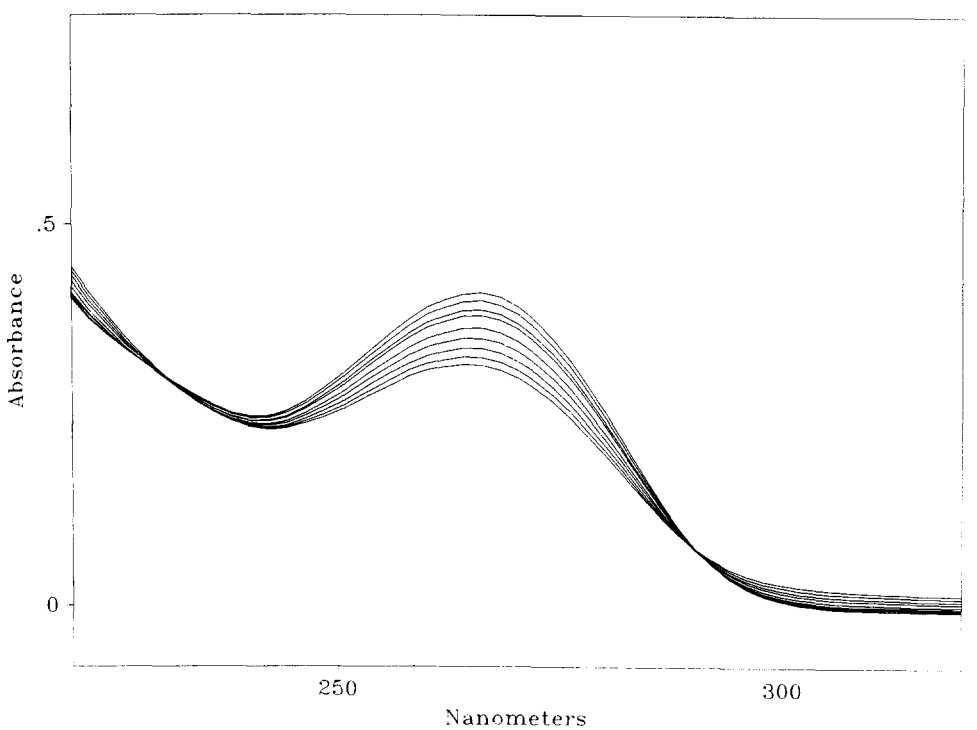

Fig. 2. Spectral changes for the degradation of 5×10^{-5} M cefaclor at pH 9.00, 30°C and $\mu = 0.5$ with time in min. The first five spectra were scanned after 15 min and the rest after every 30 min.

3. Results and discussion

3.1. Determination of rate constants by UV spectroscopy, HPLC method and iodometry

The degradation kinetics of cefaclor in acidic, neutral and alkaline solution was followed in detail mainly by UV spectroscopy whereas these reactions were examined by HPLC and the iodometric method only at two different pH values.

The rates were investigated by monitoring the UV absorbance at 264 nm. UV spectral changes of cefaclor as a function of time at pH 9.00, maintained by the use of a pH stat, are shown in Fig. 2.

During the kinetic runs cefaclor lost its characteristic UV absorbance at 264 nm. The values of the pseudo-first-order rate constants, k_{obs}, were obtained from the slopes of linear relationship between plots of $\ln(A_0-A_\infty)$ at λ_{max} vs time (Eq. 1), where A_t, A_0 and A_∞ are the absorbance at the time t, zero and infinity, respectively.

$$\ln(A_t-A_\infty) = \ln(A_0-A_\infty)-k_{obs}\cdot t \qquad (1)$$

The degradation of cephalosporins and penicillins is influenced by different factors like pH, temperature, metal ions, buffer concentration, ionic strength, etc. (Yamana and Tsuji, 1976; Gensmantel et al., 1980; Tsuji et al., 1981; Pasini and Indelicato, 1992).

The course of the hydrolysis and the nature of the degradation products are influenced by the pH of the solution (Cohen et al., 1973; Dinner, 1977). Fig. 3 and 4 shows typical first-order plots for the degradation of cefaclor at various pH values, followed by UV spectroscopy.

Degradation kinetics were also followed by HPLC and iodometry. The logarithmic plots of the percent residual cefaclor vs time were linear, and the pseudo-first-order rate constants were obtained from the slopes. Fig. 5 shows changes in the high-pressure liquid chromatogram during cefaclor degradation at pH 8.00 (maintained by using the pH stat), 30°C, and ionic strength 0.5, with time.

The rate constants determined by UV spectroscopy, HPLC method and iodometry are given in Table 1.

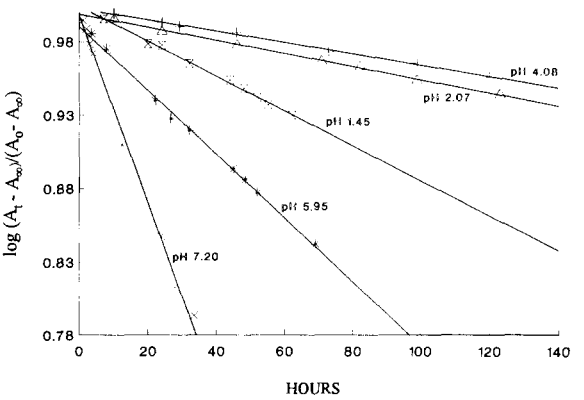

Fig. 3. Apparent first-order plots followed spectrophotometrically for the degradation of cefaclor at pH 1.45 (⧖), 2.07 (△), 4.08 (+), 5.95 (∗) and 7.20 (×), 30°C and $\mu = 0.5$.

The values of the rate constants are in relatively good agreement (Table 1). The data appear to be consistent with those of Indelicato et al., (1977) and Blaszczak et al. (1990).

Pseudo-first-order rate constants for four different initial concentrations of 2.5, 5, 7.5 and 10×10^{-5} M were determined by UV spectroscopy. The values were identical to the overall degradation of cefaclor and it was found that they were independent of the initial drug concentration.

In the kinetic studies, the catalytic effect of phosphate buffer on the rate of cefaclor degradation was determined by UV spectroscopy at constant pH, temperature (30°C), ionic strength (μ =

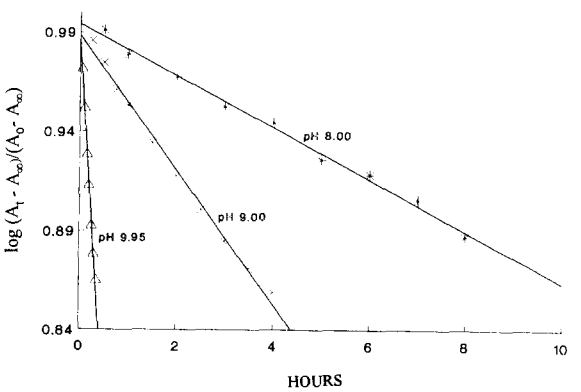

Fig. 4. Apparent first-order plots followed spectrophotometrically for the degradation of cefaclor at pH 8.00 (∗), 9.00 (×) and 9.95 (△), 30°C and $\mu = 0.5$.

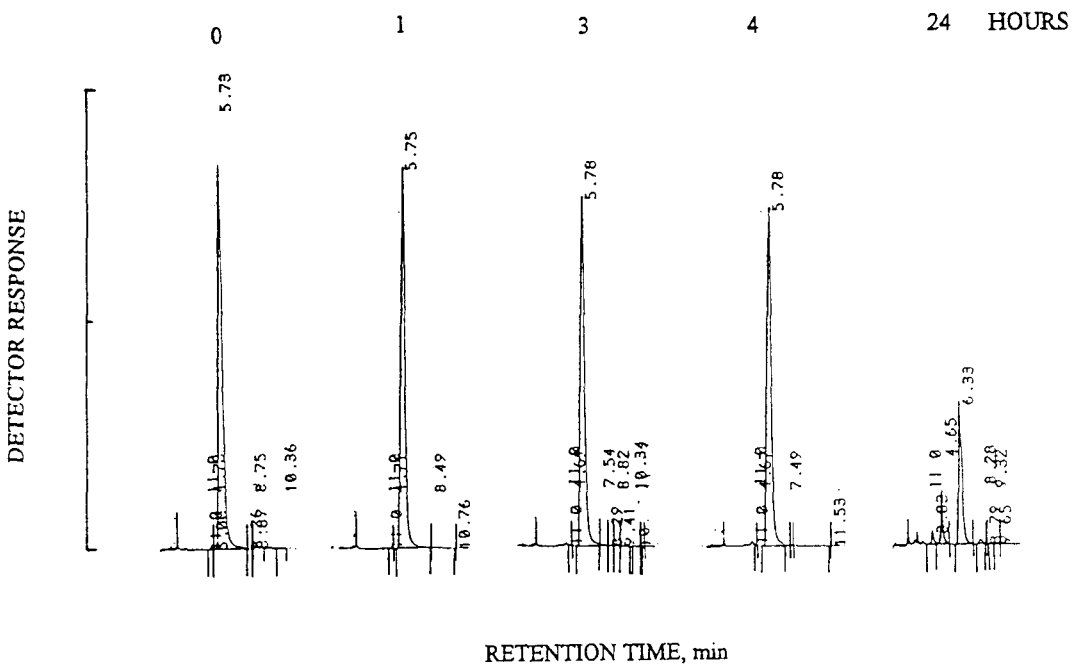

Fig. 5. HPCL changes for the degradation of cefaclor at pH 8.00, 30°C and $\mu = 0.5$ with time in h.

0.5) and concentration of cefaclor (5×10^{-5} M). Only the total buffer concentration was varied. The catalytic effect of phosphate buffer was determined between pH 5.90 and 7.40. Plots for phosphate buffer catalytic effects on the rate constants of cefaclor are shown in Fig. 6.

The results indicate that the degradation of cefaclor at constant pH in the presence of excess buffer followed pseudo-first-order kinetics and the rate constants increased linearly with increase in the buffer concentration. Extrapolation of the curves obtained for each pH values (Fig. 6) to zero buffer concentration provides, as intercepts, the values of the pseudo-first-order rate constants, k_{pH}, for the nonbuffer catalyzed reactions.

Table 1
Rate constants of degradation of cefaclor determined by UV spectroscopy, at 30°C and at different pH values

pH [a]	k_{obs} ($\times 10^2$) (h^{-1}) [b]
1.45	0.026
2.07	0.104
4.08	0.086
5.95	0.491
7.20	1.461
8.00	3.209; 3.479 [c]; 4.790 [d]
9.00	7.782; 6.300 [c]; 9.670 [d]
9.95	82.498

[a] pH values were maintained by a pH stat.
[b] Rate constants were obtained from Eq. 1.
[c] Determined by HPLC.
[d] Determined by iodometry.

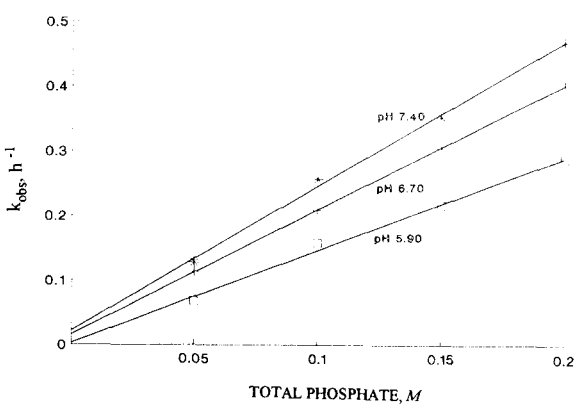

Fig. 6. Plots of the pseudo-first-order rate constants vs total buffer concentration for cefaclor degradation at various pH values, 30°C and $\mu = 0.5$.

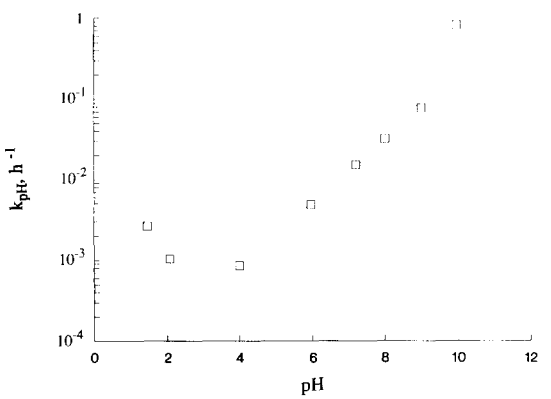

Fig. 7. Log k_{pH}-pH profile for the degradation of cefaclor in aqueous solution at 30°C, $\mu = 0.5$, where k_{pH} is the apparent first-order rate constants for the degradation in nonbuffer solution.

3.2. pH-rate profile

Log k_{pH} vs pH for degradation of cefaclor at 30°C and $\mu = 0.5$ is plotted in Fig. 7.

The rate constants, k_{pH}, used in construction of the graph were determined by UV spectroscopy in aqueous solution at various pH values, maintained by the pH stat. The pH-stability profile of cefaclor was found to be similar to that of the other correspondingly substituted 7-aminocephalosporanic acids. The log k_{pH}-pH profile is U-shaped and shows maximum stability at the isoelectric point of cefaclor.

Hydrolysis of cephalosporins in acidic media is thought to be the result of specific H_3O^+ ion catalyzed reaction and it has been found that there was no significant influence of the side chain on the acidic rate of hydrolysis of cephalosporins (Yamana et al., 1974; Dinner, 1977). Fig. 7 shows that cefaclor undergoes specific acid-catalyzed degradation below pH 2.00. The increase in k_{pH} for cefaclor with decreasing pH below 2.00 results in a negative slope which indicates specific H_3O^+ ion catalysis.

It has been suggested (Indelicato et al., 1977) that the increase in reactivity of cefaclor over drugs without an α-aminophenylacetyl side group is probably due to intramolecular nucleophilic attack at the β-lactam ring. Three different mechanisms, for participation of the neighboring α-amino group in facilitation of β-lactam opening of aminocephalosporins, have been cited and it was established that drug hydrolysis proceeded dominantly at neutral and basic pH by spontaneous aminolysis, rather than by water catalyzed reaction.

In this case, the rate constants of cefaclor increased with pH values above 4.50. At pH values near pK_{a_2}, there was a sigmoid dependence of k_{pH} on pH. This inflection indicates that the dissociation equilibria of the α-amino group ($pK_{a_2} = 7.17$) influenced the degradation rates. The results obtained in the present study and earlier findings (Yamana and Tsuji, 1976; Indelicato et al., 1977) for other cephalosporins supported the idea that the pH-rate profile in neutral and basic pH could be explained mainly by intramolecular nucleophilic attack at β-lactam.

It was also observed that no decrease occurred in the degradation rates in aqueous dioxane at pH 8.00, indicating no significant participation of water in this reaction (Yamana and Tsuji, 1976).

The effect of solvent on the degradation rate of cefaclor is shown in Table 2.

There are small increases in the degradation rates of cefaclor in H_2O-dioxane mixtures at pH 8.00 which indicate no significant participation of water in the reaction.

In spite of the approximation of MO theory and the lack of data for solvation effects, CNDO/2 calculations for 7-NH_2-3-cephem model compounds indicate that the 3-chloro compound formed a slightly more stable transition complex than a similar one with a CH_3 group. These results would be consistent with differ-

Table 2
Effect of water-dioxane mixtures on the degradation rate of cefaclor at pH 8.00, 30°C and $\mu = 0.5$

Solvent composition	k_{obs} (h^{-1}) [a]
Water	0.032
10% dioxane-water	0.033
25% dioxane-water	0.037
50% dioxane-water	0.084

[a] Rate constants were determined spectrophotometrically. pH value was maintained by a pH stat.

ences in the solution stability and relative reactivity for cefaclor and other cephalosporins (Boyd, et al., 1980; Pasini and Indelicato, 1992).

3.3. Dependence of rate on temperature

The temperature dependency of rate constants was followed from 30 to 60°C at pH 7.20 and 9.95. Arrhenius plots for the observed first-order rate constants of cefaclor are shown in Fig. 8.

The apparent activation energies calculated in the absence of phosphate buffer from the slope of the lines were 25.95 and 17.08 kcal/mol at pH 7.20 and 9.95, respectively.

3.4. Influence of cupric ion on the rate constants

The influence of copper(II) ion on the hydrolysis of cefaclor was determined by UV spectroscopy. The relevant data for the influence of copper(II) ion on the degradations of cefaclor are listed in Table 3.

In the presence of copper(II) ion a small increase in degradation rate was observed, indicating no significant catalytic effect of copper(II) ion on the hydrolysis of cefaclor. The ratio between the degradation rate constants of cefaclor in aqueous solution in the presence and absence of copper(II) ion at pH 4.08 and 6.00 were determined to be 1.25 and 1.49, respectively (Table 3).

In contrast to the penicillins and some cephalosporins (Gensmantel et al., 1978; Gensmantel et al., 1980) where it was found that in the presence of copper(II) ion there is an enormous increase in the rate of drug decomposition, the rate enhancement of hydrolysis of cefaclor in the presence of copper(II) ion practically is not observed. These results are consistent with the assumption that the metal ion probably is not involved in the stabilization of the tetrahedral transition complex.

The possibility arises that interaction between Cu(II) ion and cefaclor takes place through the side chain or carboxyl group in a similar mode to cephalexine (Moratal et al., 1989).

Our results indicate that, although the kinetics of degradation of drug could be complicated by the presence of chloride ions, there is no significant influence of copper(II) ion on the rate of drug decomposition (Gensmantel et al., 1980).

Table 3
Kinetics of degradation of cefaclor in the presence of copper(II) ion at 30°C and $\mu = 0.5$ (initial drug concentration was held constant at 5×10^{-5} M)

pH	[Cu(II)] (M)	k_{obs} ($\times10^{2}$) (h^{-1}) [a]
4.08	5×10^{-6}	0.107
5.00	5×10^{-6}	0.210
	1×10^{-5}	0.920
	5×10^{-6}	0.733
6.00	2.5×10^{-6}	0.761
	1.66×10^{-6}	0.715
	1.25×10^{-6}	0.680

[a] Determined by UV spectroscopy.

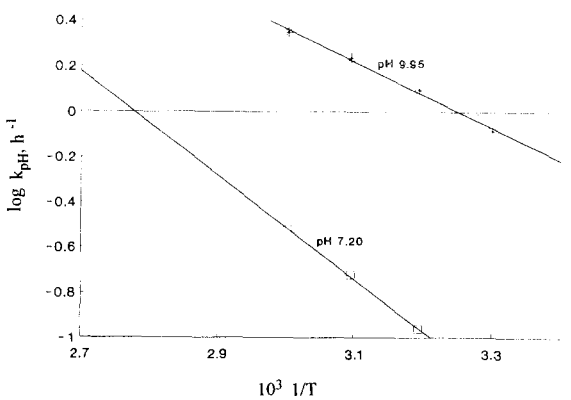

Fig. 8. Arrhenius plots for the apparent first-order rate constants, k_{pH}, for cefaclor at pH 7.20 and 9.95 and $\mu = 0.5$.

4. Conclusion

The present study shows that degradation of cefaclor is observed to follow first-order kinetics. The pH-rate profile is U-shaped and is similar with the other cephalosporins. A specific acid catalysis of degradation of cefaclor is proposed. In neutral and basic pH mainly intramolecular nucleophilic attack is observed. An appreciable phosphate buffer effect on the degradation of cefaclor is found. No significant influence of copper(II) ion on the degradation reaction is observed. Studies are in progress to examine the

interaction between the metal(II) ions and cefaclor moiety.

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