METAL-IONS CATALYZED HYDROLYSIS OF AMPICILLIN: RPHPLC-IEXHPLC KINETIC STUDIES

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- ABSTRACTS: In this work, we have studied the kinetics of Ampicillin degradation catalyzed by Cu(II), Ni(II), Fe(II) and Zn(II) in solution at a range of 30-60°C and constant ionic strength of 0.5 over a pH range of 2-5 by RPHPLC-IEXHPLC, and the effects of pH, temperature and ion concentration in the hydrolysis reaction. The RPHPLC-IEXHPLC studies provides additional evidence for the reaction mechanism. The mechanism of Cu(II) catalysis involves a ternary complex and Fe(II), Ni(II) and Zn(II) shows no hydrolysis mechanism.

- KEYWORDS: Ampicillin; kinetics constants; reversed-phase liquid chromatography (RPHPLC); ion-exchange liquid chromatography (IEXHPLC).

Introduction

The transition metal interactions to penicillins[1-5], were studied previously by spectrophotometric and potentiometric methods.

In our work, the study of Cu(II)-ampicillin interaction by reversed-phase liquid chromatography (RPHPLC), method which we proposed, facilitates the separation of Cu(II)-ampicillin, corresponding Cu(II)-penicilloic acid chelate, free ampicillin and degradated compounds of ampicillin molecule; and in the others Ni(II), Fe(II) and Zn(II) interactions, the RPHPLC method provided the separation of ampicillin and degradated products.

It is not possible in spectrophotometric and potenciometric methods proposed in other works.[1,5]

On the other hand, a great advantage of our reversed-phase liquid chromatography and ion-exchange liquid chromatography (RPHPLC-IEXHPLC) combined method

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is that kinetics constants may be calculated without any other requirement that registered peak information.

On the other hand, the RPHPLC method provides evidence for the stoichiometry and the Cu(II)-ampicillin mechanism.

Experimental section

Chemical and reagents

Ampicillin trihydrate was supplied by Beecham Pharmaceutical Laboratories. Cu(II) sulfate solution was prepared from Merck analytical grade $CuSO_4$ anhydrous. Ni(II) nitrate solution was prepared from Merck analytical grade $NiSO_4$ hexahydrate. Fe(II) and Zn(II) sulfate solutions was prepared from Merck analytical grade $FeSO_4$ heptahydrate and $ZnSO_4$ heptahydrate, respectively. These stock-solutions were standardized by complexometric methods. All other chemicals were HPLC grade.

Apparatus and instruments conditions

RPHPLC assays were performed with a Waters Associated chromatograph equipped with a variable-wavelenght UV detector and 10μL loop injection valve.

The effluent was monitored at 254 nm. A Kontron Station Data with D450 software was used to monitor the detector output. A Hypersil ODS 5μ (10x0.46 cm) reversed-phase column was maintained at 30ºC. The mobile phase consisted of a 50% solution (V/V) of methanol in 0.01M PO_4H_2K. The pH of the final solution was adjusted to 2.5 with phosphoric acid. The flow rate was 1 mL/min.

IEXHPLC assays were performed with a liquid chromatograph system equipped with a Waters M6000A pump, a Waters 430 conductivity detector and a Waters U6K 20 μL loop injection valve. A Kontron Station Data with D450 software was used to monitor the detector output. A Ion-210 (Waters Associated) cation column was used. The mobile phase consisted of a 10 mM citric acid and 3.5 mM EDA solution. The flow rate was 2 mL/min. The Cu(II) concentrations used in the calibration plot are 1, 5, 10 and 20 ppm. In this assay the retention time of Cu(II) is 4.5 min.

Absorption spectra was obtained with a Beckman 5240 double-beam spectrophotometer with a 0.1 cm cell.

Buffer Solutions

For the general investigations was used the Sörensen buffer.[6] A constant ionic strength of 0.5M was maintained for each buffer by adding an appropriate amount of

KCl. The solutions were freshly prepared and the pH were measured at 30ºC by a Corning 140 pHmeter and SC-glass electrodes.

Analytical procedure

Effect of pH

A series of experiments were performed at pH 2, 3, 4 and 5. Ampicillin was maintained constant at 0.02 mM, ionic strength at 0.5M and metal-ions concentration at 0.12 mM.

The extension of the studies to pH values above 6 was hindered by the metals hydroxo formation and the reaction proceeded too rapidly to be followed by the instruments.

Effect of temperature

The effect of temperature on metal-ions catalyzed degradation of ampicillin was studied at 30, 40, 50 and 60ºC±0.05. The reaction was studied at pH 2, 3, 4 and 5.

Effect of ion concentration

A series of experiments were performed at pH 2 to 5. Ampicillin was maintained constant at 0.02 mM, ionic strength at 0.5M and 40ºC of temperature. The metal-ions concentration are 0.006, 0.012, 0.024 and 0.036 mM, in a 0.5:1, 1:1, 2:1 and 3:1 molar relations metal:ampicillin, respectively.

Results and discussion

RPHPLC Studies

In Figure 1 it is shown a chromatogram of Ni(II)-ampicillin interaction and in the same way, in Figure 2 it is shown a chromatogram of Cu(II)-ampicillin interaction. The chromatograms of Zn(II) and Fe(II) metal-ion interaction are similary to Ni(II) interaction. The chromatogram peaks were identified by UV-VIS spectra. The chromatogram of Ni(II)-ampicillin interaction shows three peaks (I-III), at retention time indicated in Figure 1, with absorption maxima at 354 (peak I), 322 (peak II) and 260 (peak III) nm, due to penillic acid, penicillenic acid and ampicillin molecules respectively and were discussed in a previously work.[7] The chromatogram of Cu(II) ampicillin interaction shows in addition two new peaks at retention time of 5 min (peak V) and 3 min (peak IV), with absorption maxima at 260 nm (V) and 310 nm (IV) in UV range and 600 nm (IV-V) in visible zone. The last maximum is typical of Cu(II) chelates and the ampicillin and Cu(II)-penicilloic band at 260 and 310 nm respectively has been reported.[4, 7]

In the Cu(II) ampicillin reaction, the Cu(II) ion interacts with ampicillin through the formation of a catalytic five-membered chelate (peak IV) in which the Cu(II) effect would consist on accelerating the hydrolysis of the ampicillin by the increasing of the hydroxyl nucleofilic attack with formation of the Cu(II)-penicilloic acid chelate, in a non-equilibria reaction balance.

In Figure 3 it is shown, by plotting the peak area (mV) *versus* degradation time, that when the Cu(II)-ampicillin chelate decrease chromatographic peak IV the Cu(II)-penicilloic acid chelate increase its chromatographic peak V.

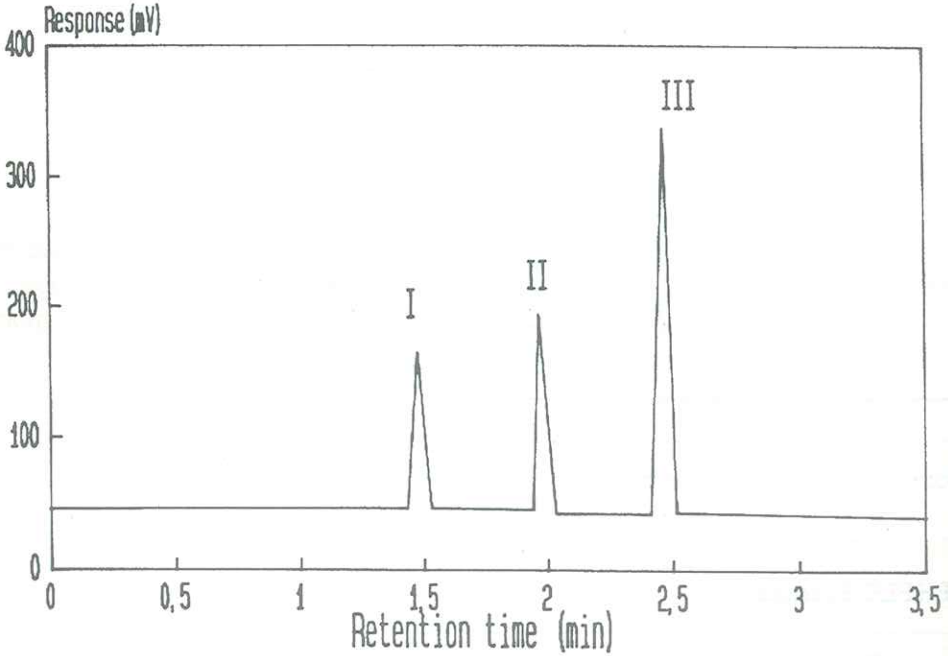

FIGURE 1 – Chromatogram obtained by RPHPLC of Ni (II)-ampicillin interaction. Peak identification: I. Penillic acid (1.6 min). II. Penicillenic acid (2.0 min). III. Ampicillin (2.5 min).

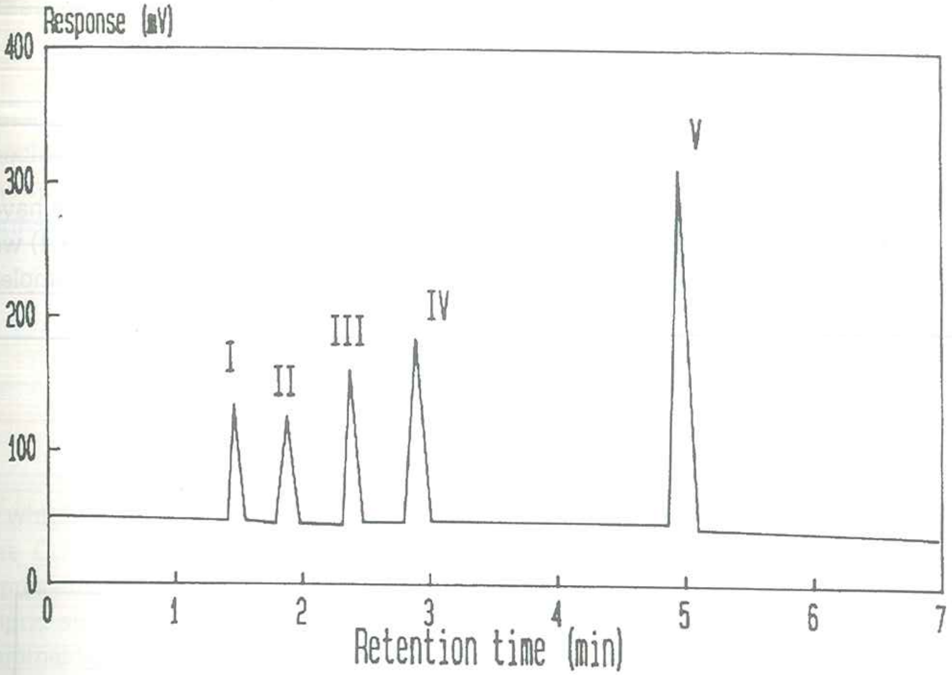

FIGURE 2 – Chromatogram obtained by RPHPLC of Cu(II)-ampicillin interaction. Peak identification: I. Penillic acid (1.6 min). II. Penicillenic acid (2.0 min). III. Ampicillin (2.5 min). IV. Cu(II)-penicilloic acid chelate (3 min). V. Cu(II)-ampicillin acid chelate (5 min).

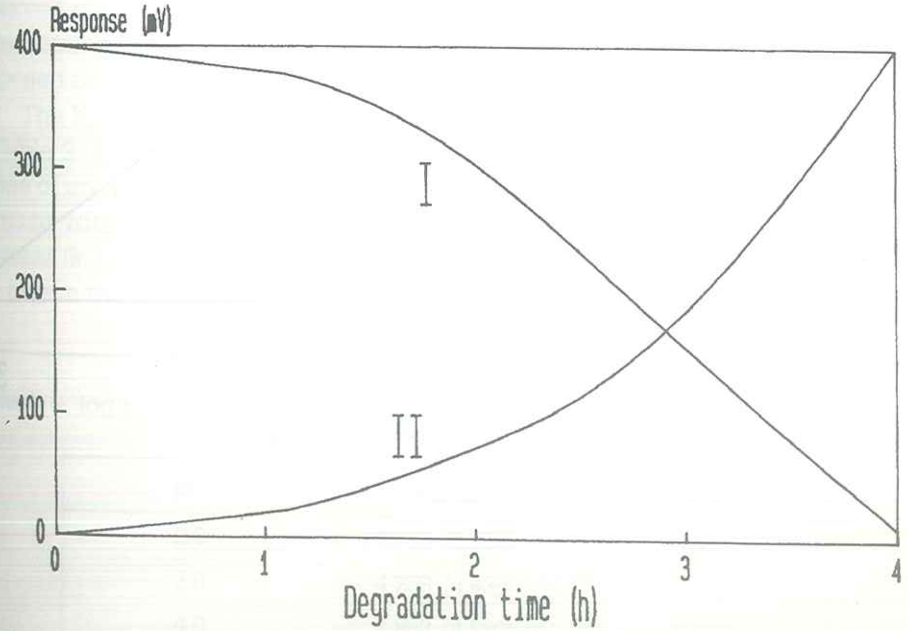

FIGURE 3 – Chromatographic response (mV) plot of I. Cu(II)-ampicillin chelate and II. Cu(II) penicilloic chelate peaks *versus* degradation time (pH=2, T=30°C and 1:1 molar relation).

Cupric-ampicillin chelate

Chelate stoichiometry

By plotting the peak area(mV) of cupric-ampicillin chelate (IV) which we have obtained by RPHPLC method *versus* Cu(II)/ampicillin molar relation (0.5:1 to 3:1) we have obtained a inflexion point at 1:1 molar relation (Figure 4) in correlation to complex stoichiometry.

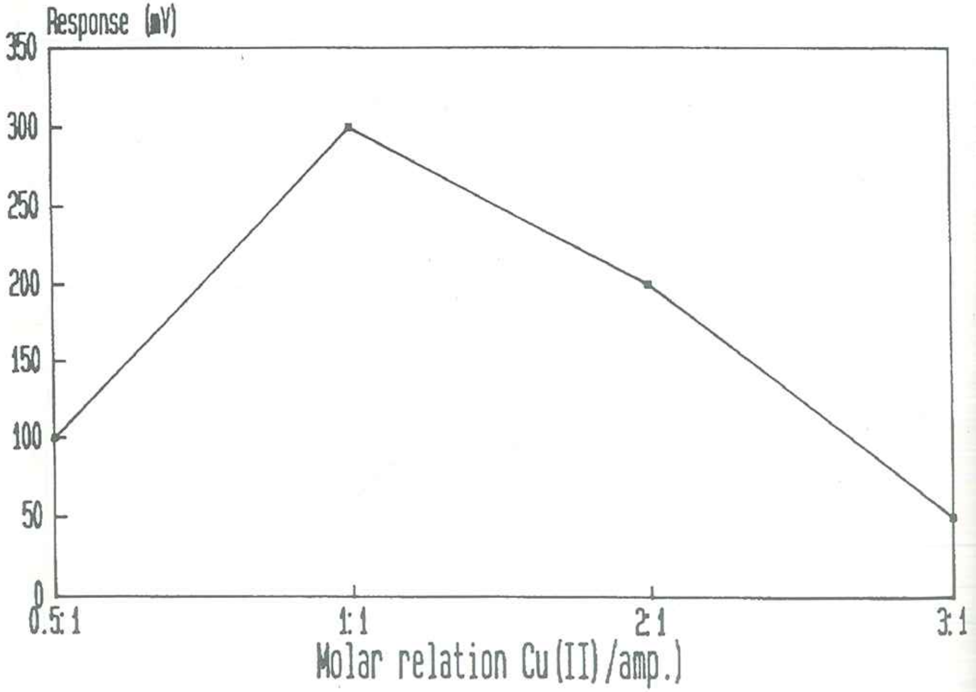

FIGURE 4 – Chromatographic response (mV) plot of Cu(II)-ampicillin chelate peak *versus* Cu(II)/amp. molar relation (pH=2 and 30°C).

Chelate kinetics constants

According to the observed in the RPHPLC method we have assumed in order to determinate the kinetics constants for the cupric-ampicillin chelate, a degradation mechanism in which the cupric chelate, by hydroxyl or H_2O molecule attack, involved the cupric-penicilloic acid chelate formation, with an equilibrium constant of chelate formation:

$$Ke = \frac{[Cu - Amp.]}{[Amp.]\,[Cu(II)]}$$

in which [Amp.] is the free concentration of ampicillin, [Cu(II)] is the concentration of free Cu(II) that we have determinate by IEXHPLC method, and the [Cu-Amp.] represents the chelate concentration calculated by RPHPLC method *versus* a pure cupric-ampicillin chelate that we have obtained by adequate fraction collection of chromatographic peak IV and hinder injection in the chromatographic system. This reaction scheme is according to the scheme proposed by Cressman.[1]

Furthermore, two degradation rate constant, k_1 and k_2 of hydroxyl or H_2O attack are present, with a total degradation rate constant $k_T = k_1 + k_2$, in which the k_T is the observed rate constant at different temperatures and pH of our RPHPLC assays. We have obtained the k_1 hydroxyl ion attacks constant following the scheme equation proposed by Tomida.[8]

The K_e and k_1 constants are shown in Table 1, and the K_T values are summarized in Table 2. The degradation rate constants are of first-order, according to the best values of correlation coefficient. The hydroxyl constant (k_1) values has a tendency to increase with the pH according to the increase of the OH^- attack. The total rate constant (k_T) values has a similary tendency to increase with the pH, according to a less rate in the hydrolysis reaction.

Table 1 – log k_1 and log K_e values at 30°C and 0.036 mM ion concentration

pH	log K_e	log k_1
2.0	3.780 (3.989)	0.501
3.0	4.258 (4.439)	0.903
4.0	4.863 (4.980)	1.605

( ) values at 40°C

Table 2 – $k_T(h^{-1})$ observed rate constants values at different molar relations Cu(II)/amp. in the cupric-ampicillin chelate hydrolysis

T°C	pH	0.5:1	1:1	2:1	3:1
30	2	0.009	0.007	0.006	0.005
	3	0.006	0.004	0.003	0.003
	4	0.043	0.056	0.110	0.137
	5	0.120	0.126	0.160	0.260
40	2	0.021	0.020	0.016	0.014
	3	0.020	0.016	0.011	0.010
	4	0.049	0.089	0.118	0.140
	5	0.140	0.130	0.190	0.280
50	2	0.058	0.047	0.038	0.036
	3	0.056	0.043	0.037	0.031
	4	0.090	0.140	0.150	0.170
	5	0.160	0.200	0.220	0.301
60	2	0.160	0.130	0.110	0.100
	3	0.141	0.130	0.110	0.101
	4	0.190	0.220	0.250	0.280
	5	0.290	0.330	0.470	0.670

Thermodynamic quantities

We have calculated the ΔG°, ΔS° and ΔH° are values that we shown in Table 3. The calculated enthalpy change for the complexation between cupric ion and ampicillin is in the range 21.73-35.94 kJ/mol and increasing with the pH (2-4); the change in free energy was in the range -21.73 to -28.0 kJ/mol and the entropy change are in the range +164.27 to +191.86 J/mol. These values for Δ S are to be expected for chelate formation.

Table 3 – Thermodynamic constants values at 30°C in kJ/mol. ΔS° in J/mol

pH	ΔG°	ΔH°	ΔS°
2	–21.904	36.24	191.862
3	–24.494	32.645	188.977
4	–28.131	21.736	164.566

Effect of pH

By plotting the logarithm of the observed rate constants (k_T) *versus* pH assays for the hydrolysis of cupric-ampicillin chelate at 30°C and 0.012 mM Cu(II) concentration, a minimum rate at a pH of 3 was observed (Figure 5). Similar process were encountered in other Cu(II) concentration and temperatures.

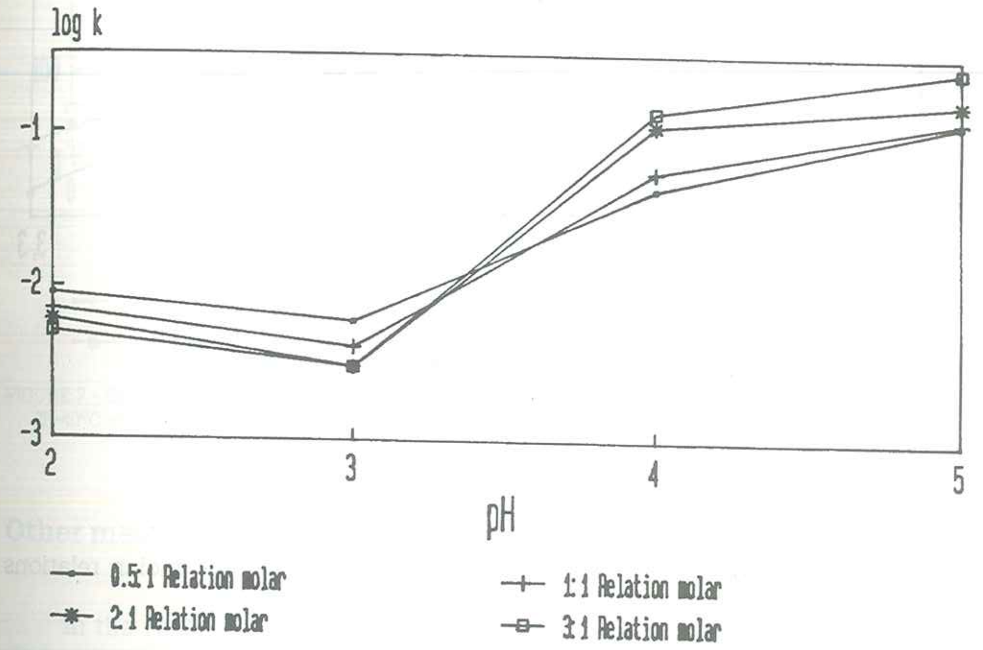

FIGURE 5 – Log k_T plot of Cupric-ampicillin chelate hydrolytic reaction *versus* pH values at 30°C.

The pH-rate profile suggested that the rate in the hydrolysis reaction gave an order: 5>4>2>3>, which is inverse to pH-rate profile that we observed in the ampicillin without metals addition.[7]

Effect of temperature

The temperature dependence of the hydrolytic reactions of cupric chelate in buffers solution was determined by measuring the first-order rate constants at various pH and Cu(II) concentration and a constant ionic strength of 0.5. The corresponding Arrhenius-type plots are shown in Figure 6. The calculated heat of activation are in the range of 19.64-94.88 kJ/mol (Table 4).

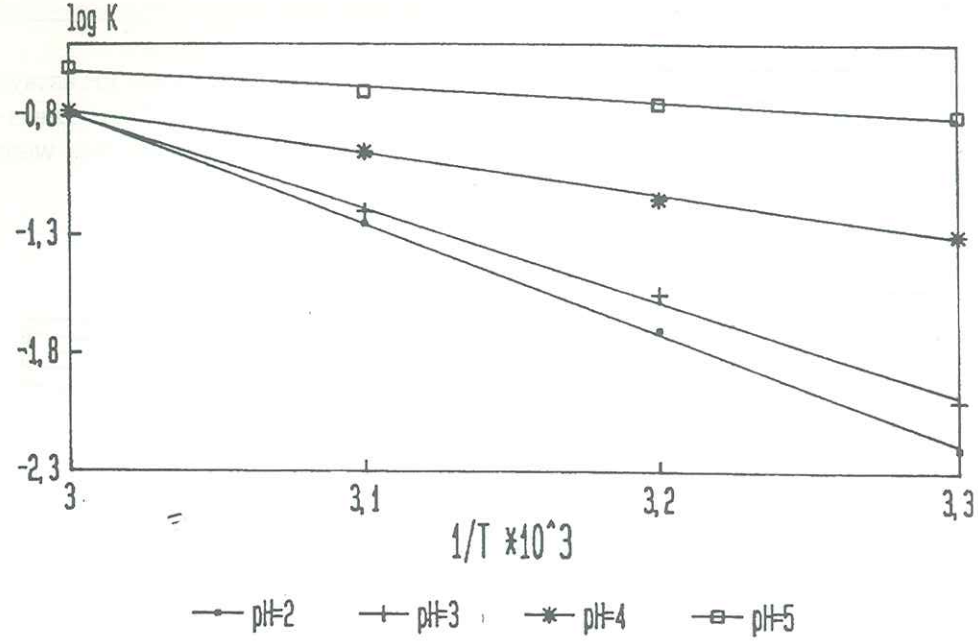

FIGURE 6 – Arrhenius-type plots of Cu(II)-ampicillin chelate (0.5:1 molar relation).

Table 4 – Calculated heat of activation in kJ/mol at different molar relations Cu(II)/amp.

pH	0.5:1	1:1	2:1	3:1
2	79.00	79.839	81.510	80.250
3	89.034	96.140	94.886	94.886
4	42.218	37.620	22.572	19.646
5	22.900	28.00	27.170	27.170

Effect of Cu(II) concentration

By plotting the chromatographic response of cupric chelate peak IV *versus* time of hydrolytic reaction at different molar relation of Cu(II)/amp. we have observed an increase of chelate formation with Cu(II) concentration (Figure 7). In molar relation upper at 3:1 it was not observed a major increment in the chelate formation.

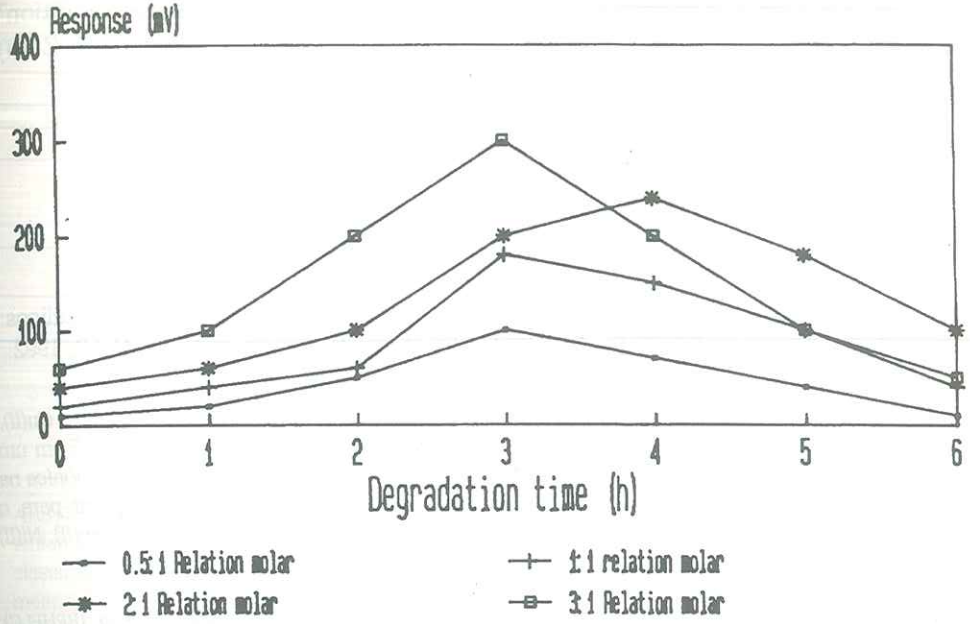

FIGURE 7 – Cupric-ampicillin chelate chromatographic response (mV) plot *versus* time (h) at different molar relation (T=40°C and pH=2).

Other metal-ions interactions

In the Ni(II), Fe(II) or Zn(II) ions addition to ampicillin it was not observed a chelate formation similary to cupric-ampicillin chelate.

The hydrolysis of ampicillin with Ni(II), Fe(II) or Zn(II) addition gives a pseudo first-order and the observed rate constants in the Ni(II) addition we have shown in the Table 5. Similary k_T values we have obtained in the Fe(II) and Zn(II) interaction.

Table 5 – Rate observed constants (h^{-1}) of pseudo first-order of hydrolitc reaction of ampicillin in presence of Ni(II) at 30°C

pH	0.5:1	1:1	2:1	3:1*
2	0.025	0.025	0.030	0.032
3	0.016	0.019	0.021	0.018
4	0.013	0.009	0.015	0.014
5	0.014	0.013	0.011	0.009

* Molar relation Ni(II)/amp.

This observed rate constants are similary to ampicillin without metals addition[7] and the values of rate observed constants suggested that Ni(II), Fe(II) and Zn(II) showed no-activity in the hydrolytic reaction of ampicillin.

DOADRIO-VILLAREJO, A. L. et al. Hidrólise da ampicilina catalizada por íons metálicos: estudos cinéticos por RPHPLC e IEXHPLC. *Ecl. Quím.*, São Paulo, v. 17, p. 41-52, 1992.

- *RESUMO: Neste trabalho, estudamos a cinética da degradação da ampicilina, catalizada por Cu(II), Ni(II), Fe(II) e Zn(II) em solução, em um intervalo de 30-60°C e força iônica constante de 0,5 em um intervalo de pH de 2-5 por RPHPLC-IEXHPLC, e os efeitos do pH, temperatura e concentração iônica na reação de hidrólise. Os estudos de RPHPLC-IEXHPLC fornecem uma evidência adicional para o mecanismo de reação. O mecanismo da catálise de Cu(II) envolve um complexo ternário; Fe(II), Ni(II) e Zn(II) não apresentam mecanismo de hidrólise.*

- *UNITERMOS: Ampicilina; constantes cinéticas; cromatografia líquida em fase reversa (RPHPLC); cromatografia líquida por troca iônica (IEXHPLC).*

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