TOXIC-IONS CATALYZED HIDROLYSIS OF AMOXICILLIN: HPLC KINETIC STUDIES

Antonio Doadrio VILLAREJO*
José B. SOLELO*
Juan C. DOADRIO*
Regina ORENGA*
Antonio MAYORGA*

■ ABSTRACT: In this work, we have studied the kinetics of amoxicillin degradation catalyzed by toxic-ions, Cu(II), V(IV) and Ni(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 reversed phase HPLC and ion exchange HPLC, and the effects of pH, temperature and ion concentration in the hidrolysis reaction. The HPLC studies provide additional evidence for the reaction mechanism. The mechanisms of Cu(II) and V(IV) catalysis involve a ternary complex and Ni(II) shows no chelate mechanism.

■ KEYWORDS: Amoxicillin; HPLC; toxic-ions.

Introduction

It was previously suggested that the presence of Cu(II) in penicillin solutions pquelatos romotes the degradation of the penicillins studied to their corresponding penicilloic acid.

The transition metal interactions to penicillins[1-3,7] were studied previously by spectrophotometric and potentiometric methods.

In our work, the study of Cu(II)-amoxicillin and V(IV)-amoxicillin interactions by Reversed Phase Liquid Chromatography (RPHPLC) facilitates the separation of Cu(II) or V(IV) amoxicillin chelate, corresponding Cu(II) or V(IV) penicilloic acid chelate, free amoxicillin and degradated compounds of amoxicillin molecule; and in the other Ni(II)

* Departamento de Química Inorgánica y Bioinorgánica – Facultad de Farmacia – Universidad Complutense – 28040 – Madrid – Espanha.

interaction, the RPHPLC method provided the separation of amoxicillin and degradated products.

A great advantage of RPHPLC and Ion Exchange Liquid Chromatography (IEXHPLC) combined method, used in this work, 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)-amoxicillin and V(IV)-amoxicillin mechanisms.

Materials and methods

Chemicals and reagents. Amoxicillin trihydrate was supplied by Beecham Pharmaceutical Laboratories. 2-hydroxy-3-hydroxy-phenylpyrazine was prepared in our laboratory by Lebelle method.[5] Copper (II) sulfate solution was prepared from Merck analytical grade $CuSO_4$ anhydrous. Vanadium (IV) sulfate solution was prepared from Merck analytical grade $VOSO_4$ pentahydrate. Nickel (II) nitrate solution was prepared from Merck analytical grade $Ni(NO_3)_2$ hexahydrate. All other chemicals were HPLC grade.

Apparatus and instruments conditions. RPHPLC assays were performed with a Hewlett-Packard 1084B chromatograph equipped with a variable-wavelength UV detector and 10 µL loop injection automatic valve. The effluent was monitored at 236 nm. A Hewlett-Packard 3390 integrator was used to monitor the detector output. A Spherisorb ODS 5 µ (20 x 0.46 cm) reversed-phase column was maintained at 30°C. The mobile phase consisted of a 30% 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) and VO^{2+} concentrations used in the calibration plot were 1, 5, 10 and 20 ppm. In this assay the retention time of Cu(II) was 4.5 min and V(IV) was 8 min.

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

Buffer Solutions. For the general investigations we used the Sörensen buffer.[6] A constant ionic strength of 0.5 was maintained for each buffer by adding an appropriate amount of KCl. The solutions were freshly prepared and the pH were measured at 30°C by a Corning 140 pH meter and SC – glass electrodes.

Analytical procedure

Effect of pH. A series of experiments were performed at a pH 2, 3, 4 and 5. Amoxicillin was maintained constant at 1.19 mM, ionic strength at 0.5 and Cu(II), V(IV) and Ni(II) concentration at 1.19 mM.

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

Effect of temperature. The effect of temperature on the Cu(II), V(IV) and Ni(II) catalyzed degradation of amoxicillin was studied at 30, 40, 50, and 60°C ± 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. Amoxicillin was maintained constant at 1.19 mM, ionic strength at 0.5 and 40°C of temperature. The Cu(II), V(IV) and Ni(II) concentration were 11.9, 3.6, 2.4, 1.19 and 0.6 mM, in a 10:1, 3:1, 2:1, 1:1 and 0.5:1 molar relations metal:amoxicillin respectively.

Results and discussion

RPHPLC studies. In Figure 1 is shown a typical chromatogram of Ni(II) amoxicillin interaction and in the same way; in Figure 2 is shown a chromatogram of Cu(II) amoxicillin interaction. The chromatogram peaks were identified by UV – VIS spectra. The chromatogram of Ni(II) amoxicillin interaction shows four peaks (I-IV), at retention time indicated in Figure 1, with absorption maxima at 273 (peak I), 318 (peak II), 277 (peak III) and 364 nm (peak IV) respectively and were discussed in a previous work.[4]

The chromatogram of Cu(II) amoxicillin interaction shows in addition two new peaks at retention time of 3.7 min (peak V) and 6.1 min (peak VI) with absorption maxima at 273 nm (V) and 318 nm (VI) in UV range and 790 nm (V-VI) in visible zone. The last maximum is typical of Cu(II) chelates and the amoxicillin and Cu(II)-penicilloic band at 273 and 318 nm respectively has been reported.[3,4] The chromatogram of V(IV) amoxicillin interaction shows the same profile.

In other hand, the peak IV, due to 2-hydroxy-3-hydroxyphenylpyrazine (yellow fluorescent compound) is only registered at ultimate hydrolysis and this indicates that amoxicillin molecule follows two alternative degradation routes, penicilloic or penamaldate pathways. In the acid pH of our assays the only possible is the penamaldates route. We are discussed this route in a previous paper.[4]

On basis of HPLC results, in the Cu(II) or V(IV) amoxicillin reaction, metal ions interacts with amoxicillin through the formation of a catalytic five-membered chelate (peak V) in which the Cu(II) or V(IV) effect would consist on accelerating the hydrolysis of the amoxicillin by the increasing of the hydroxyl nucleofilic attack with formation of the corresponding Cu(II) or V(IV)-penicilloic acid chelate, in a non-equilibria reaction balance. In Figure 3 is shown this effect in the vanadium reaction.

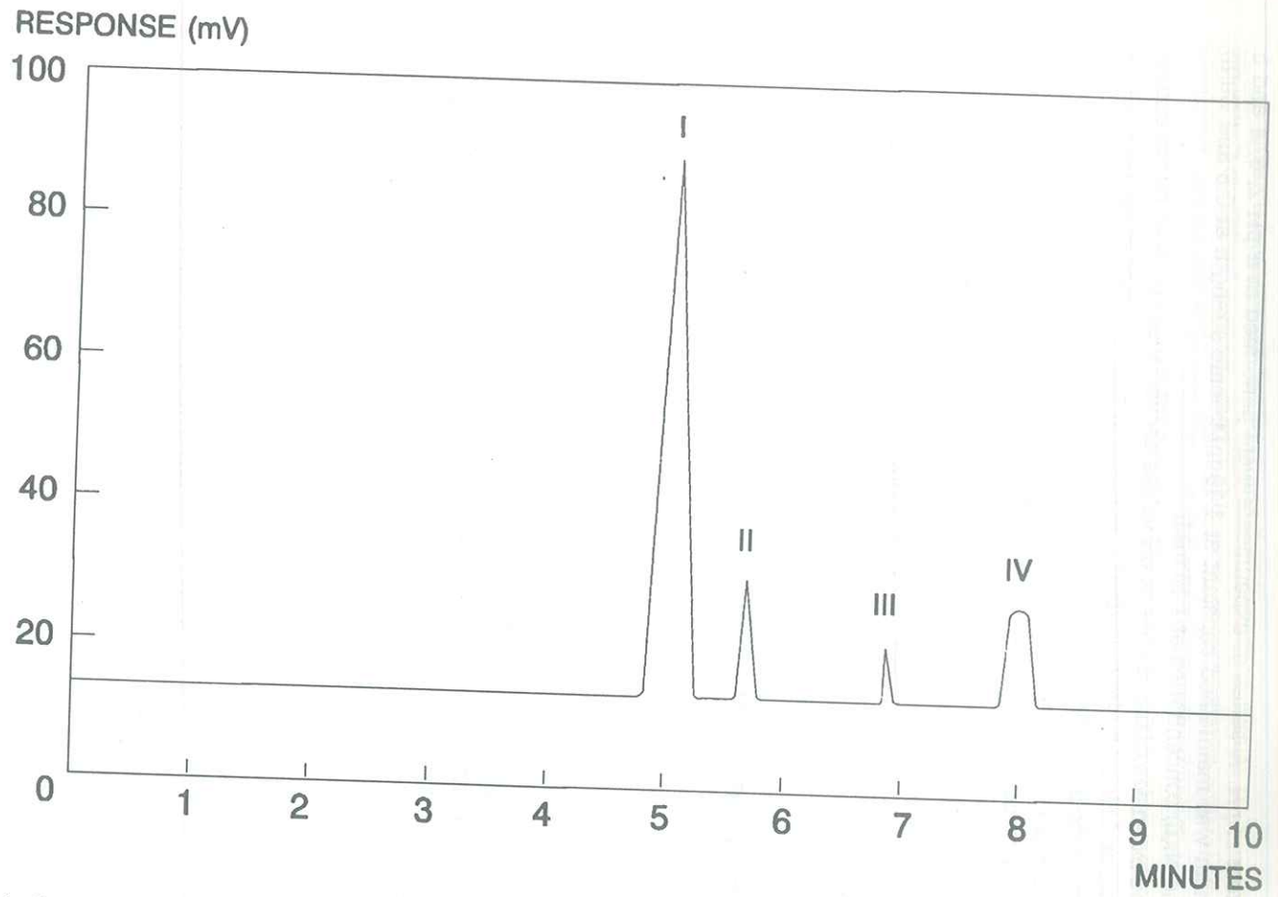

FIGURE 1 – Chromatogram obtained by RPHPLC of Ni(II)-amoxicillin interaction. Peak identification: I. amoxicillin (5 min); II. hydroxylpenicillenic acid (5.6 min); III. hydroxylpenamaldic acid (7 min); IV. 2-hydroxy-3-hydroxy phenylpyrazine (8 min).

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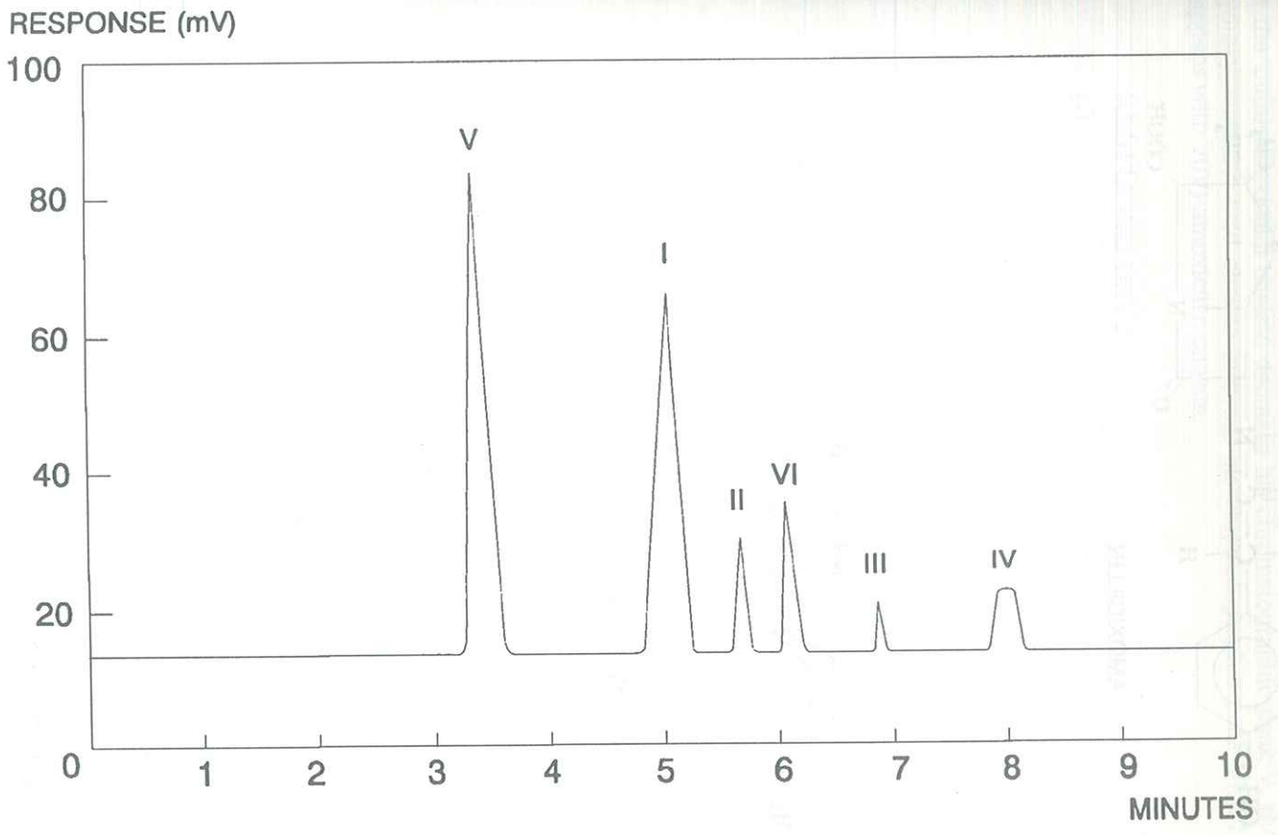
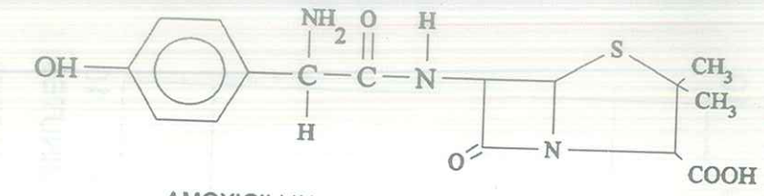
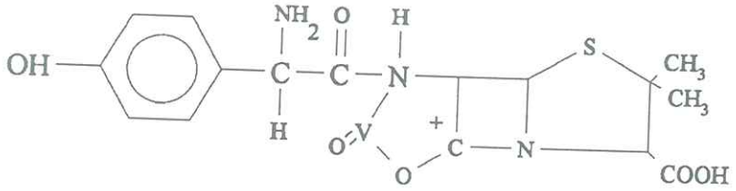


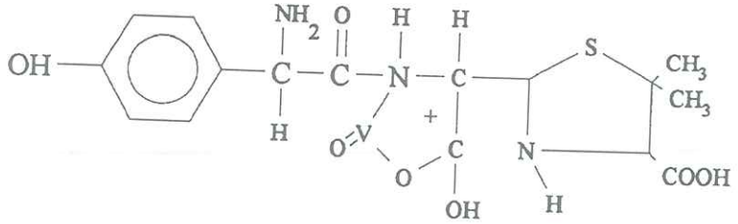
FIGURE 2 – Chromatogram obtained by RPHPLC of Cu(II)-amoxicillin interaction. Peak identification: I. amoxicillin (5 min); II. hydroxylpenicillenic acid (5.6 min); III. hydroxylpenamaldic acid (7 min); IV. 2-hydroxy-3-hydroxy phenylpyrazine (8 min); V. Cu(II)-amoxicillin chelate (3.7 min); VI. Cu(II)-hydroxylpenicilloic acid chelate (6.1 min).



AMOXICILLIN



AMOXICILLIN-V(IV)



PENICILLOIC-V(IV)

FIGURE 3 – VO^{2+}-amoxicillin interaction.

In Figure 4 is shown by plotting the peak area versus degradation time, where as the Cu(II)-amoxicillin chelate decrease his chromatographic peak V, the Cu(II)-hydroxypenicilloic acid chelate increase his chromatographic peak VI. The same happens with V(IV)-amoxicillin chelate.

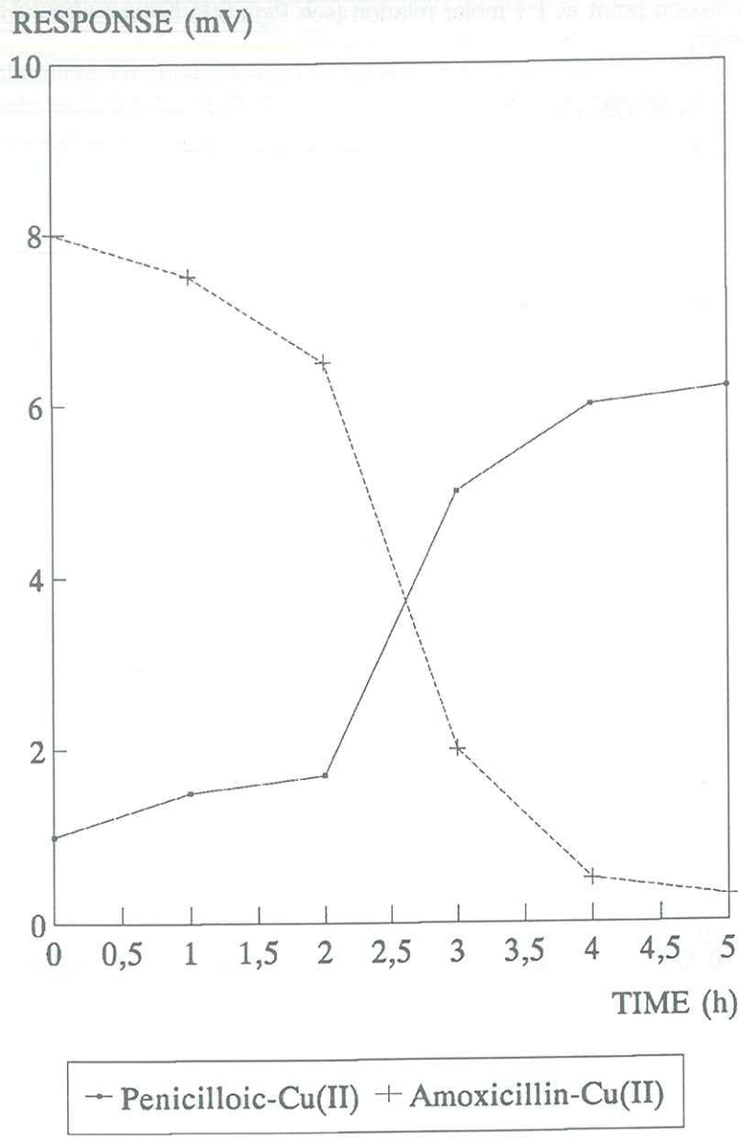


FIGURE 4 – Chromatographic response plot of Cu(II)-amoxicillin and hydroxypenicilloic chelate peaks versus degradation time at pH=3, T=40°C and 0.6 mM of Cu(II).

Metal ions-amoxicillin chelate

Chelate stoichiometry. By plotting the peak area of cupric-amoxicillin and vanadium-amoxicillin chelates (peak V) obtained by RPHPLC method versus Cu(II)/amoxicillin and V(IV)/amoxicillin molar relations (0.5:1 to 10:1) we have obtained an inflexion point at 1:1 molar relation (see Figure 5) in correlation to complex stoichiometry.

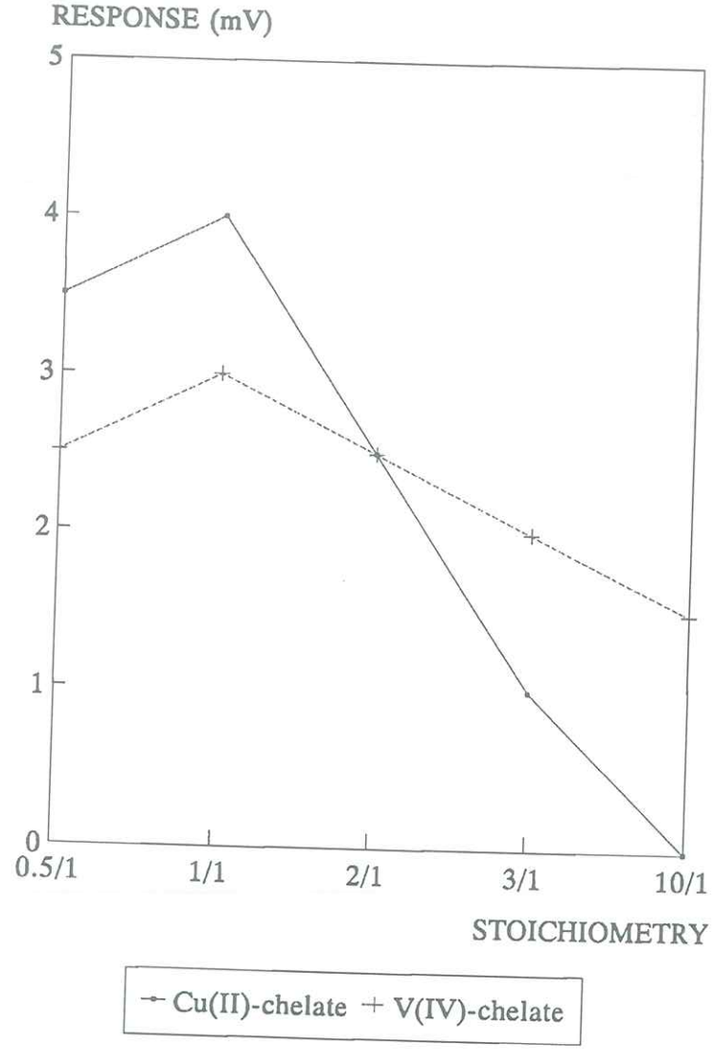


FIGURE 5 – Chromatographic response plots of Cu(II) (pH=5, T=30°C) and V(IV)-amoxicillin (pH=4, T=40°C) chelate peaks versus metal ions/amoxicillin molar relation.

Chelate kinetics constants. According to the observed in the RPHPLC method we have assumed in order to determinate the kinetics constants for the cupric-amoxicillin and vanadium-amoxicillin chelates, a degradation mechanism in which the cupric or vanadium chelate, by hydroxyl or H_2O molecule attack involved the cupric or vanadium-hydroxylpenicilloic acid chelate formation, with an equilibrium constant of chelate formation according to the proposed by Cressman et al.[1] in cupric-ampicillin chelate.

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

The K_e and k_1 constants are shown in Table I. The hydroxyl constant (k_1) values have a tendency to increase with the pH 1[1-3,7] according to the increase of the OH- attack. The K_e constant values have a similarly tendency to increase with the pH, according to a major chelate formation.

Table 1 – log k_1 and log K_e values at 30°C. ( ) values at 40°C.

[Me]=0.6 mM.

pH	log K_e		log k_1	
2	3.04(3.23)[a]	3.91(4.11)[b]	0.63[a]	0.61[b]
3	3.56(3.72)	4.17(4.36)	2.69	2.18
4	3.85(3.99)	4.21(4.38)	2.41	2.30

(a) Cu(II)-amoxicillin, (b) V(IV)-amoxicillin.

Thermodynamic constants. We have calculated the $\Delta G°$, $\Delta S°$ and $\Delta H°$ values that we shown in Table II. The calculated enthalpy change for the complexation between metal ions and amoxicillin is in the range 26.7-38.2 KJ/mol and decreasing with the pH; the change in free energy is in the range – 4.21 to – 5.83 KJ/mol and the entropy change is in the range + 105.8 to + 143.8 J/mol. These values for ΔS are to be expected for chelate formation.

Effect of pH. By plotting the logarithm of the observed rate constants (k_T) versus pH assays for the hydrolysis of cupric amoxicillin chelate, a minimum rate at a pH of 3 was observed (see Figure 6). Similar process were encountered in other Cu(II) concentration and temperatures. However in the V(IV) chelate, the kinetic values at pH 2 and pH 3 are in the same order. The degradation rate constants are of first-order, according to the best values of correlation coefficient.

Table 2 – Thermodinamic constants values at 30°C in KJ/mol

ΔS° in J/mol.

pH	ΔG°		ΔH°		ΔS°	
2	- 4.21[a]	- 5.39[b]	36.29[a]	38.21[b]	133.66[a]	143.89[b]
3	- 4.93	- 5.83	30.56	36.30	117.12	138.81
4	- 5.33	- 5.77	26.74	32.47	105.84	126.26

(a) Cu(II)-amoxicillin, (b) V(IV)-amoxicillin.

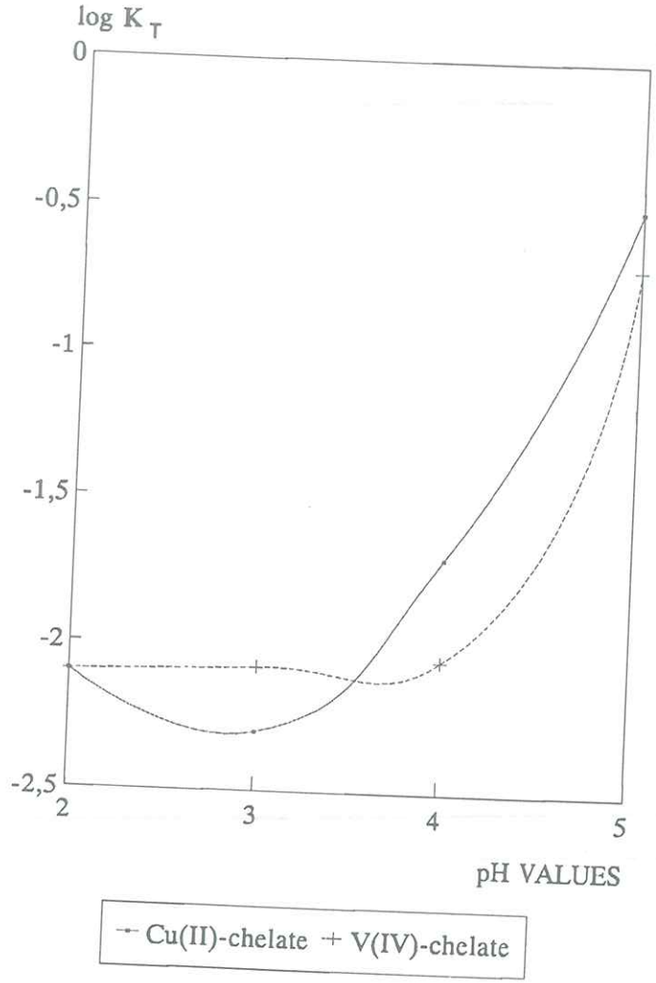


FIGURE 6 – log k_T plot of cupric-amoxicillin and vanadium-amoxicillin chelates versus pH values at 1:1 molar relation and T=30°C.

The pH-rate profile suggested that the rate in the hydrolysis reaction gave an order: 5 > 4 > 3 > 2 > 1, in Cu(II) chelate, and 5 > 4 > 3 > 2 > 1, in V(IV) chelate, which are inverses to pH-rate profile that we observed in the amoxicillin without metals addition.[4]

Effect of temperature. The temperature dependence of the hydrolytic reactions of cupric and vanadium chelates in buffers solutions was determined by measuring the first-order rate constants at various pH and Cu(II) and V(IV) concentrations and a constant ionic strength of 0.5. The corresponding Arrhenius-type plots are shown in Figure 7. The calculated heat of activation are in the range of 12.4-45.1 kcal/mol (see Table III).

Table 3 – Calculated heat of activation in kcal/mol

pH	0.5:1		1:1		2:1		3:1		10:1[1]	
2	29.5[a]	18.3[b]	31.4[a]	22.1[b]	30.3[a]	20.7[b]	27.5[a]	18.5[b]	33.1[a]	12.4[b]
3	17.2	16.2	20.4	20.3	21.0	21.0	22.1	16.5	42.61	3.5
4	35.2	20.1	32.7	25.7	35.4	18.5	37.4	22.3	45.1	14.6
5	37.4	19.7	27.8	18.4	31.4	14.7	17.6	21.0	13.9	17.5

(1) Molar relations, (a) Cu(II)-amoxicillin, (b) V(IV)-amoxicillin.

Effect of Cu(II) concentration. By plotting the chromatographic response of cupric chelate peak V versus time of hydrolytic reaction at a different molar relation of Cu(II)/amoxicillin, we have observed an increasing of chelate formation with Cu(II) concentration (see Figure 8). In molar relation upper at 10:1 not observed a major increment in the chelate formation. The same occur in the V(IV)-amoxicillin chelate.

Free amoxicillin. The free amoxicillin we have observed by a chromatographic peak (I) at retention time of 5 min (see Figure 2).

By plotting the logarithm of the observed rate constants versus the pH assays for the hydrolysis of free amoxicillin, in the Cu(II) or V(IV) interaction, a minimum rate at a pH of 2 was observed (see Figure 9) and the pH rate profile suggested that the rate in the hydrolysis reaction is: 5 > 4 > 3 > 2 > 1, which is inverse to pH rate profile that we have observed in the amoxicillin without metals addition.[4]

Furthermore, the observed rate constants of hydrolytic reaction of free amoxicillin with Cu(II) addition are more exalted that amoxicillin without Cu(II) or V(IV) addition.[4] This is due to a "super acid" catalysis of metal ion by the inductive effects of the positive charge.

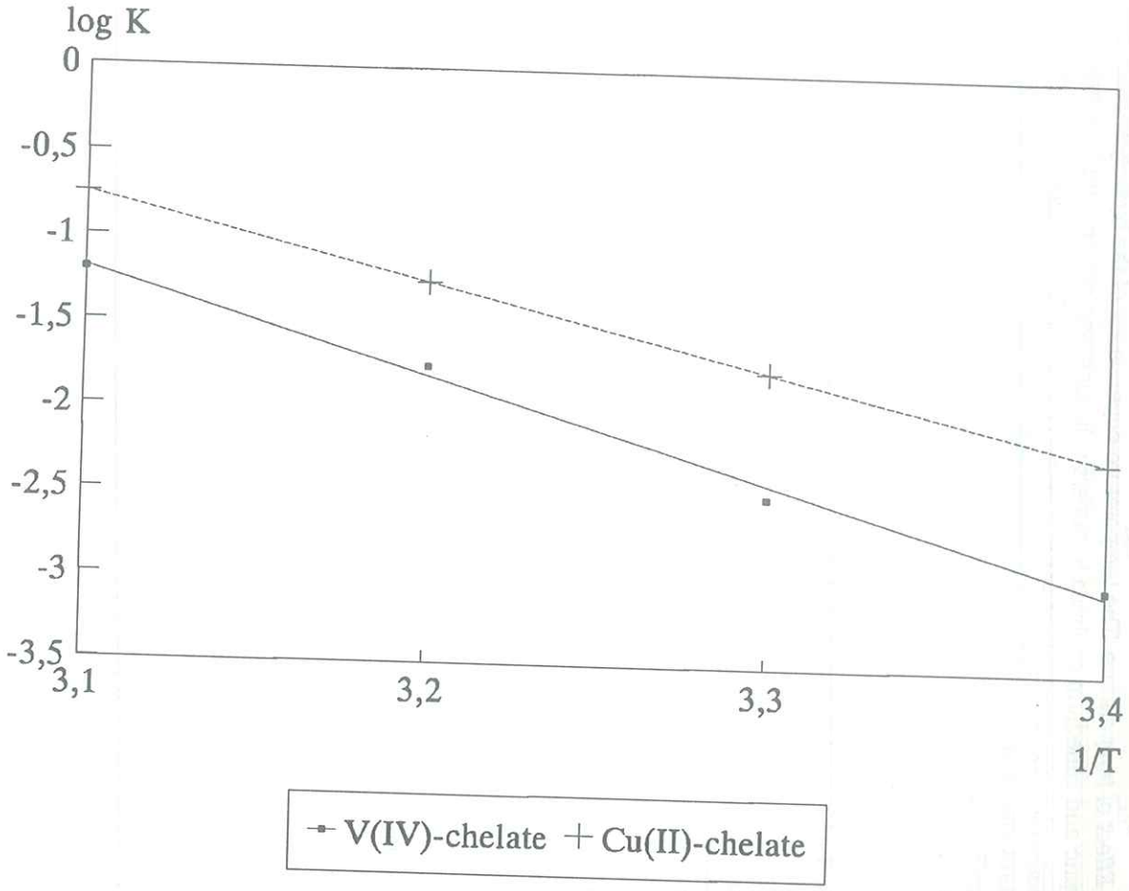


FIGURE 7 – Arrhenius-type plots of Cu(II)-amoxicillin (pH=2) and V(IV)-amoxicillin (pH=4) chelate at 1:1 molar relation.

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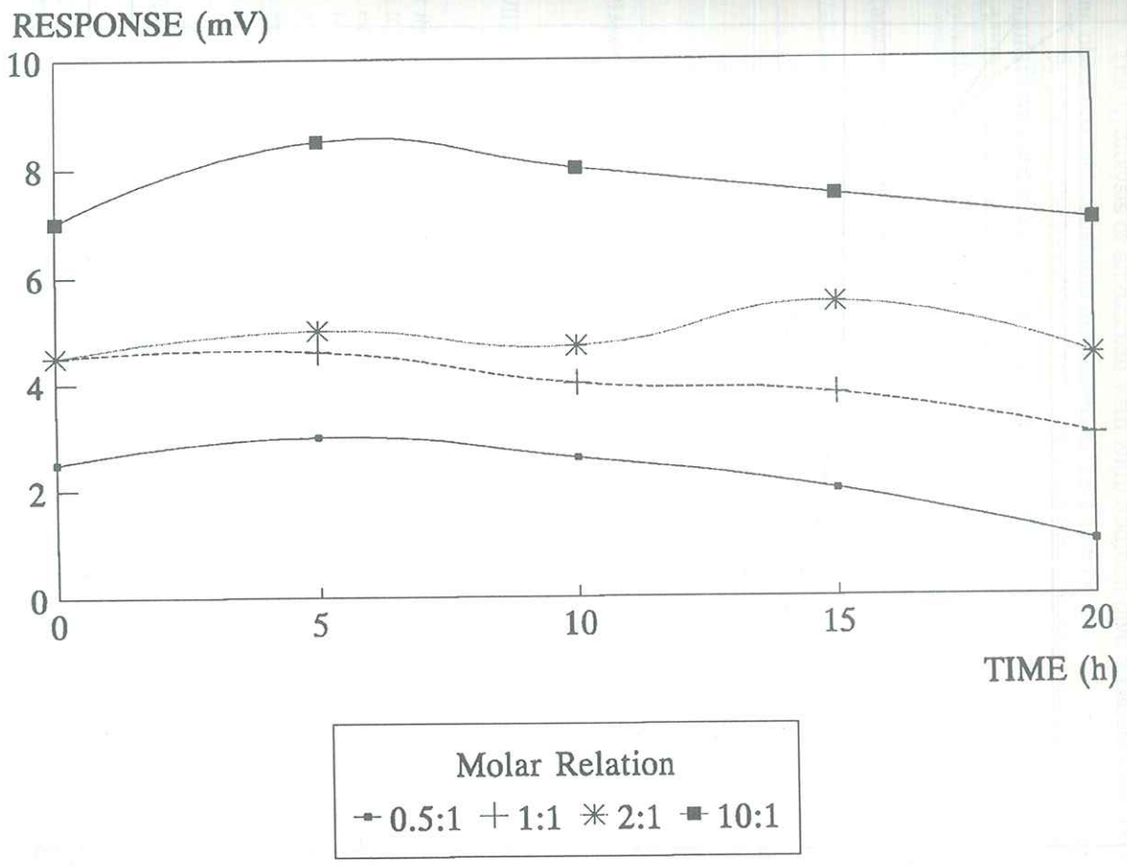

FIGURE 8 – Cupric-amoxicillin chelate chromatographic response (mV) plot versus time (h) at 40 °C and pH=2.

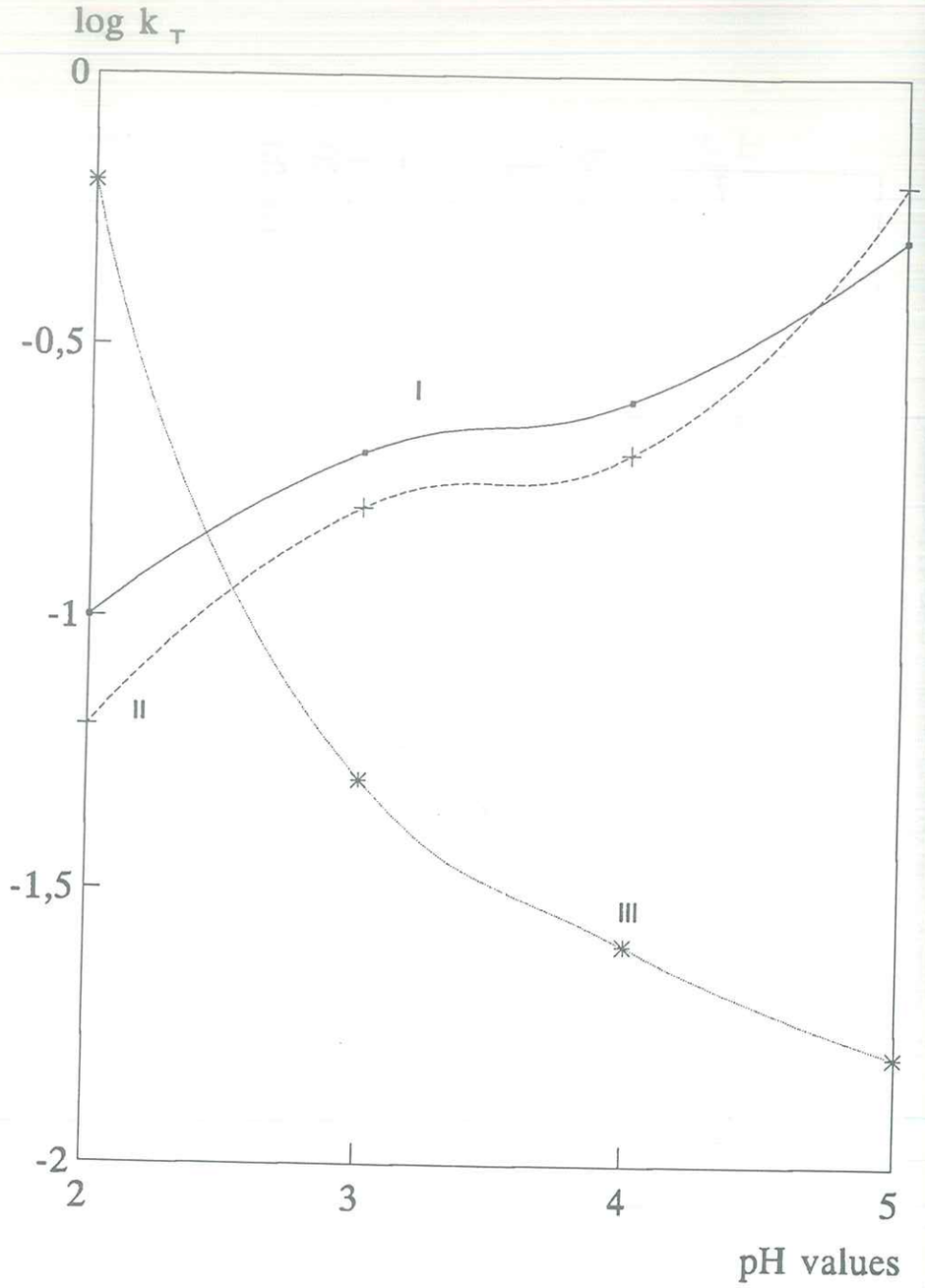

FIGURE 9 – log kT plots of free-amoxicillin versus pH in metals addition. I. Cu(II). II. V(IV). III. Ni(II).

Nickel (II) interaction

In the Ni(II) ion addition to amoxicillin we did not observe a chelate formation similarly to cupric-amoxicillin chelate.

The hydrolysis of amoxicillin with Ni(II) addition give a pseudo-first order and the observed rate constants are shown in the Table IV.

By plotting the logarithm of the observed rate constants versus pH assays (see Figure 9) we have obtained a pH rate profile similarly at the amoxicillin without metals addition[4] and the values of the rate observed constants suggested that Ni(II) showed no-activity in the hydrolytic reaction of amoxicillin.

Table 4 – Rate observed constants (h^{-1}) of pseudo first-order of hydrolitic reaction of amoxicillin in presence of Ni(II) at 30°C

pH	0.5:1	2:1	3:1	10:1*	
2	0.029	0.026	0.030	0.032	(0.028)
3	0.020	0.021	0.021	0.022	(0.020)
4	0.009	0.008	0.009	0.009	(0.009)
5	0.005	0.006	0.006	0.006	(0.005)

(*) Molar relation Ni(II)/amoxicillin ( ) values correspond with no metal interaction amoxicillin (7).

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- *RESUMO: Neste trabalho, estudamos a cinética da degradação da amoxicilina catalisada por íons tóxicos, Cu(II), V(IV) e Ni(II) em solução, em um intervalo de 30-60°C e força iônica constante de 0,5 em um intervalo de pH de 2 a 5, por HPLC em fase reversa e HPLC por troca iônica, e os efeitos do pH, temperatura e concentração iônica na reação de hidrólise. Os estudos de HPLC fornecem uma evidência adicional para o mecanismo da reação. Os mecanismos de catálise de Cu(II) e V(IV) envolvem um complexo ternário e o mecanismo para Ni(II) não apresenta a formação de quelatos.*

- *PALAVRAS-CHAVE: Amoxicilina; cromatografia líquida (HPLC); íons tóxicos.*

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