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Article

VO^{2+} and Cu^{2+} Interactions with Ceftriaxone and Ceftizoxime. HPLC Kinetic Studies

Antonio L. Doadrio*, Antonio Mayorga and Regina Orenga

Departamento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain

Estudos do mecanismo, das constantes cinéticas e termodinâmicas das interações de VO^{2+} e Cu^{2+} com Ceftriaxone e Ceftizoxime, na faixa de pH entre 2 e 5 e nas temperaturas de 30 °,40 °, 50 ° e 60 ºC, usando HPLC em fase reversa e de troca iônica são descritos. Foram avaliados os efeitos de pH, temperatura e concentração dos íons na reação de hidrólise. Estudos de HPLC forneceram evidência satisfatória do mecanismo da reação. Mecanismos das reações dos complexes de Cu^{2+} e VO^{2+} envolvem um complexo 1:1.

Studies of the mechanism, kinetics and thermodynamic constants of VO^{2+} and Cu^{2+} Ceftriaxone and Ceftizoxime interactions at the pH range between pH 2 and 5 and at the temperatures of 30 °, 40 °, 50 ° and 60 ºC, using reversed phase HPLC and ion exchange HPLC, are reported. The effects of pH, temperature and ion concentration on the hydrolysis reaction have been evaluated. HPLC studies provided satisfactory evidence of the reaction mechanism. Mechanisms for Cu^{2+} and VO^{2+} induced reactions involve a complex of 1:1 stoichiometry.

Keywords: ceftriaxone, ceftizoxime, copper (II), vanadium (IV)

Introduction

Proper antibiotic characteristics make Ceftriaxone or Ceftizoxime to be among the third generation cephalosporins used in medicine. Our previous work on VO^{2+} and Cu^{2+} chelation with penicillins has already been reported.[1,2]

Several methods have been proposed for the determination of cephalosporin, such as spectrophotometric, potentiometric or electrophoretic methods[3-9] and, more recently, HPLC[10] and capillary electrophoresis.[11] In this work, Reversed Phase High Performance Liquid Chromatography (RP-HPLC), has been used to study Cu^{2+}-ceftriaxone and VO^{2+}-ceftriaxone and Cu^{2+}-VO^{2+}-ceftizoxime interactions, since this method permits the separation of Cu^{2+} and VO^{2+} cephalosporin chelates, the corresponding Cu^{2+} and VO^{2+} cephaloic acid chelates, free cephalosporin and degradated compounds of the cephalosporin molecule. The RP-HPLC method also provides evidence for the stoichiometry and the chelation mechanisms.

Thus, the goal of the present work consists in evaluating the influence of metal chelation in ceftizoxime or ceftriaxone degradation. Apparently only the VO^{2+} and Cu^{2+} ions cause this processes to take place.

* e-mail: aantoniov@telepolis.com

Experimental

Chemicals and reagents

Commercial sodium Ceftriaxone, with 99.80% purity, and commercial sodium Ceftizoxime, with 98.80% purity, tested by the standard methods of the USP[12] at a concentration of 0.5 mg mL^{-1}, and 7-ADCA (7-aminoceph-3-em-4-oic acid or 7-desacetoxy methyl aminocephalosporanic acid) were supplied by SKF Laboratories (Madrid, Spain).

The copper (II) sulphate solution was prepared from Merck analytical grade $CuSO_4$ pentahydrate. The vanadium (IV) sulphate solution was prepared from Merck analytical grade $VOSO_4$ pentahydrate. All other chemicals were HPLC grade and supplied by Micron Analítica S.A. (Madrid, Spain).

Apparatus and instrument conditions

RP-HPLC assays were performed with a liquid chromatographic system equipped with two Waters M6000A pumps (Waters, Milford, Massachusetts, USA), a variable-wavelength LDC SM 5000 diode array detector (supplied by Micron Analítica, Madrid, Spain) and a Kontron Auto Sampler 460 automatic injector (Kontron

Instruments, Münich, Germany) connected to a water-bath thermostat, controlled from a computer, through a RS232C connection. A Spherisorb ODS-18 reversed-phase column (10 μm, 25 x 0.46 cm), supplied by Micron Analítica S.A., was employed. The automatic injector, pumps and detector were controlled by software (D450 v.2.0) from Kontron Instruments. The mobile phase consisted of a 20% (ceftriaxone) or 30% (ceftizoxime) solution (v/v) of methanol in 0.01 mol L^{-1} KH_2PO_4. The flow rate was 0.8 mL min^{-1}. The effluent was monitored at 254 nm and the injection volume was 10 μL.

IEX-HPLC assays were performed with a liquid chromatographic system equipped with a Waters M-510 pump, a Waters 430 conductivity detector and a Waters U6K 20 μL loop injection valve. A Kontron Data Station with D450 software was used to monitor the detector output. An Ion-210 (Waters, Milford, Massachusetts, USA) cation column was used. The mobile phase consisted of a solution containing 10 mmol L^{-1}citric acid and 3.5 mmol L^{-1} ethylenediamine. The flow rate was 2 mL min^{-1}. The copper sulphate pentahydrate standard solution used in the calibration plot was supplied by Merck (Darmstadt, Germany) and the concentration used was 0.002 mmol L^{-1} of metallic ion.

Buffer Solutions

For the general investigations we used the Sörensen buffer (Table 1). A constant ionic strength of 0.5 was maintained for each buffer by adding an appropriate amount of KCl. All solutions were freshly prepared and the pH values were taken by a Methrom-Herisau E 520 pHmeter.

Table 1. Sörensen buffer solutions.

pH	Solution 1*	Solution 2 (0.1 mol L^{-1})
2	30.6 mL	69.4 mL HCl
3	40.3 mL	59.7 mL HCl
4	56.0 mL	44.0 mL HCl
5	96.4 mL	3.6 mL NaOH

* 21.04 g of citric acid dissolved in water plus 200 mL of 1 mol L^{-1} NaOH diluted with distilled water to 1L.

Analytical procedure

Effect of pH

The hydrolysis of the Cephalosporin-Metal complex was studied between pH 2 to pH 5 because, at pH values above 6, the hydroxo complexes formed precipitate and mask quantification by HPLC.

Effect of temperature

The effect of temperature was studied at 30º, 40º, 50º and 60ºC.

Effect of ion concentration

The influence of ion concentration was studied in the molar ratios of 0.5:1, 1:1, 2:1 and 3:1. The cephalosporin was kept constant at 1.26 mmol L^{-1}.

Results and Discussion

Chromatograms from Cu^{2+}-ceftriaxone interactions show three peaks, at retention times of 2.8 min (peak I), 4.3 min (peak II) and 4.8 min (peak III), indicated in Figure 1, with absorption maxima at 201, 240 and 280 nm (peak I), 197, 238 and 279 nm (peak II) and 308 nm (peak III), respectively. Peak II was not seen in chromatograms run at pH 4 and pH 5. Besides, peaks II and III do not appear at the initial time. The chromatogram peaks were directly identified by their UV spectra from the Diode Array Detector and were compared with the spectra of free ceftriaxone and with the 7-ADCA degradation product.

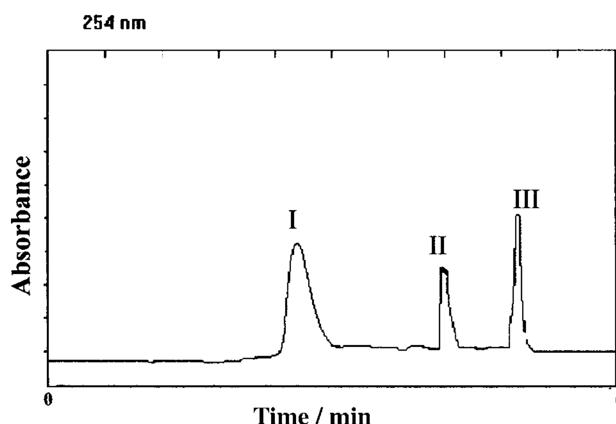


Figure 1. Chromatogram of Cu^{2+}-ceftriaxone interaction products at pH = 3 and T = 30 ºC after 60 min. Retention times: I = 2.8 min, II = 4.3 min, III = 4.8 min. Chromatographic conditions in the text. Detection at 254 nm.

Peak I is assigned to the Cu^{2+}-ceftriaxone complex, which has a similar UV-spectrum to uncomplexed ceftriaxone. Peak III is due to a Cu^{2+}-cephaloic complex, with typical absorption between 300-320 nm.[1,2] Finally, peak II, with retention time and absorption spectra similar to 7-ADCA, a product of ceftriaxone degradation, can be ascribed to a Cu-7-ADCA chelate.

Chromatograms of VO^{2+}-ceftriaxone interaction show the same profile, but the retention times are different (2.3 min, peak I; 4.0 min, peak II, and 4.7 min, peak III).

Peaks from free ceftriaxone (1.8 min)[13] are not present at all in any chromatogram.

The chromatogram from Cu^{2+}-ceftizoxime interactions shows four peaks, at retention times of 6.8 min (peak I), 8.1 min (peak II), 4.3 min (peak III) and 5.8 min (peak IV), as indicated in Figure 2, with absorption maxima at 201, 240 and 280 nm (peaks I and II), 197, 238 and 279 nm (peak III) and 308 nm (peak IV), respectively. Peak III is not present in the chromatogram at pH=5. Peaks III and IV do not appear at the initial time. The chromatographic peaks were also identified by comparing the UV spectra from the Diode Array Detector with the spectra of free ceftizoxime and with that of the 7-ADCA degradation product.

Peaks I and II are attributed to two isomers of a Cu^{2+}-ceftizoxime complex. Peak IV is due to a Cu^{2+}-cephaloic complex, with an absorption maximum between 300-320 nm. Finally, peak III, with retention time and absorption spectra similar to 7-ADCA, a product of ceftizoxime degradation, can be ascribed to a Cu-7-ADCA chelate.

The chromatogram of VO^{2+} ceftizoxime interaction shows the same profile, but the retention times of the peaks are different (5.8 min, peak I; 7.1 min, peak II, 3.9 min, peak III and 4.8 min, peak IV).

The two peaks attributed to free ceftizoxime (1.4 min and 1.6 min)[14] are not present in any of the chromatograms. Commercial ceftizoxime has two isomeric forms, *Z*-syn and *E*-anti, which are separated under our chromatographic conditions.

It is reasonable to think, on the basis of the HPLC results from the reactions of ceftriaxone with Cu^{2+} and VO^{2+}, that metal ions interact with the ceftriaxone through the formation of a five-member chelate (peak I). By this way, Cu^{2+} and VO^{2+} would accelerate the hydrolysis of ceftriaxone resulting in the formation of a cephaloic acid chelate (peak III). Additionally, Cu^{2+}-7-ADCA and VO^{2+}-7-ADCA complexes are formed (peak II) at pH between 2 and 3. The interaction mechanism for the Cu^{2+}-ceftriaxone complex is shown in Figure 3. Similar effects happen with the ceftizoxime interaction.

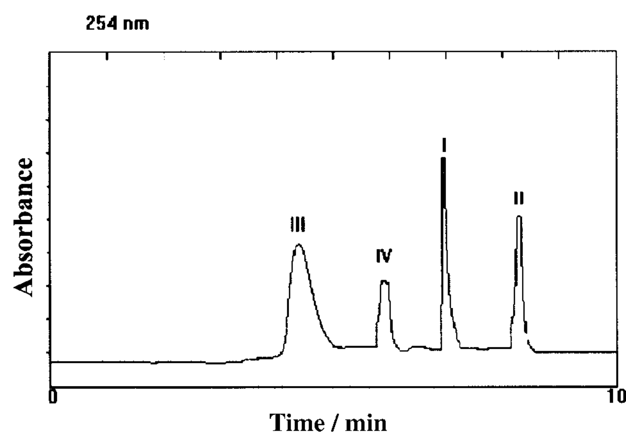


Figure 2. Chromatogram of Cu^{2+}-ceftizoxime interaction products at pH = 4 and T = 30 °C after 30 min. Retention times: I=6.8 min, II = 8.1 min, III = 4.3 min, IV = 5.8 min. Chromatographic conditions in the text. Detection at 254 nm.

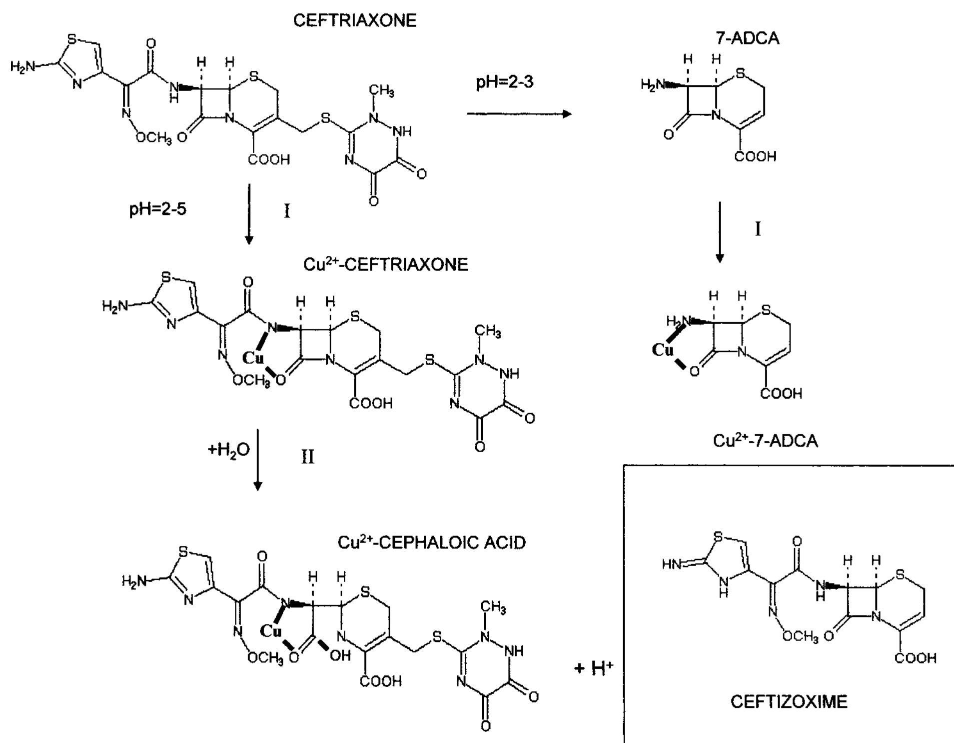


Figure 3. Cu^{2+}-ceftriaxone hydrolysis mechanism. I Chelate formation. II Complex hydrolysis.

Chelate stoichiometry

By plotting the peak area of Cu^{2+}-ceftriaxone and VO^{2+}-ceftriaxone chelates (peak I) obtained by the RP-HPLC method versus the Cu^{2+}/ceftriaxone and VO^{2+}/ceftriaxone molar ratios (0.5:1 to 3:1), at pH 2 to pH 5, an inflexion point was observed at a 1:1 molar ratio (see Figure 4) in correlation with the stoichiometry of the complex. Plots obtained from ceftizoxime are similar.

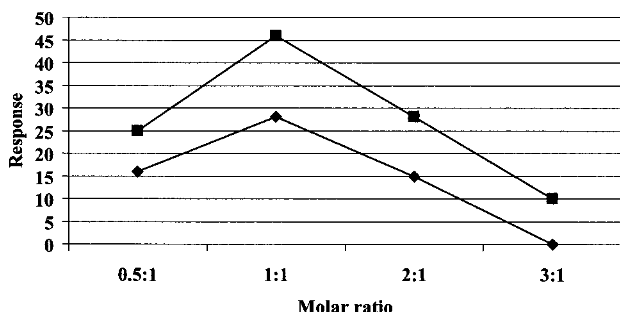


Figure 4. Determination of the stoichiometry of the Cu^{2+}-ceftriaxone and VO^{2+}-ceftriaxone complexes at T = 30 ºC and pH = 5. ◆ = Cu^{2+}. ■ = VO^{2+}.

Equilibrium reaction

Kinetic constant

According to results observed using the RP-HPLC method, in order to determine the kinetic constants for the cupric and oxovanadium ceftriaxone and ceftizoxime chelate formations, we assume that the following equilibrium reaction may occur:

$$Me^{2+} + \text{Cephalosporin} \rightleftarrows Me^{2+} - \text{Cephalosporin} \quad (1)$$

With an equilibrium constant:

$$K_e = \frac{K_1}{K_2} = \frac{[\text{Me-Cephalosp}]}{[\text{Cephalosp}]\,[Me^{2+}]} \quad (2)$$

Since the VO^{2+} ion was not detectable under our chromatographic conditions, we used VO^{2+} in excess, in order to consider, in the equilibrium constant, the unreacted VO^{2+} concentration as the initial analytical concentration.

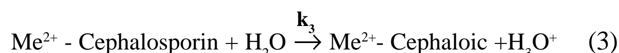
Values of log K_e at different values of pH are shown in Table 2.

Thermodynamic constants

Calculations of ΔG°, ΔS° and ΔH° values are also shown in Table 2. The calculated enthalpy change for the complexation between Cu^{2+} and VO^{2+} and ceftriaxone was found to be between of 24-38 kJ mol^{-1} (37-46 kJ mol^{-1} for ceftizoxime). The change in free energy was around –3.5 kJ mol^{-1} and the entropy change in the range of 85 to 130 J mol^{-1} (127-147 J mol^{-1} for ceftizoxime). These ΔSº values are to be expected for chelate formation.

Hydrolysis reaction

Now, we assume that the next reaction of the Cu^{2+} and VO^{2+} ceftriaxone or ceftizoxime complexes is a non-equilibrium hydrolysis reaction, as described in equation (3):



$$Me^{2+} - \text{Cephalosporin} + H_2O \xrightarrow{k_3} Me^{2+} \text{- Cephaloic} + H_3O^+ \quad (3)$$

We have evaluated the effects of pH, temperature and ion concentration on this hydrolysis reaction, through the

Table 2. Log K_e values as a function of pH at 30ºC and 40ºC, thermodynamic constant values in kJ mol^{-1} and ΔSº in J mol^{-1}, at 30 ºC.

pH	Log k_e				ΔGº		ΔHº		ΔSº	
	T = 30ºC		T = 40ºC							
	Cu^{2+}	VO^{2+}	Cu^{2+}	VO^{2+}	Cu^{2+}	VO^{2+}	Cu^{2+}	VO^{2+}	Cu^{2+}	VO^{2+}
2	2.56	2.52[1]	2.74	2.74	-3.50	-3.48	40.64	43.64	137.63	147.51
	2.42	2.44[2]	2.56	2.58	-3.30	-3.4	26.70	26.70	91.00	91.00
3	2.42	2.42[1]	2.63	2.64	-3.35	-3.35	41.32	41.32	139.73	139.74
	2.44	2.48[2]	2.64	2.65	-3.40	-3.4	38.20	32.40	130.00	110.00
4	2.42	2.42[1]	2.63	2.63	-3.35	-3.36	39.30	39.76	133.05	134.57
	2.44	2.48[2]	2.64	2.65	-3.40	-3.4	38.20	32.40	130.00	110.00
5	2.46	2.48[1]	2.66	2.68	-3.41	-3.44	37.65	38.30	127.66	129.83
	2.54	2.55[2]	2.68	2.68	-3.50	-3.5	26.70	24.80	91.00	85.00

[1]Ceftizoxime. [2]Ceftriaxone.

observed kinetic constants and the Arrhenius heats of activation.

Effect of pH

The logarithms of the observed kinetic constants versus pH, for the hydrolysis of the cupric-ceftriaxone chelate, show a minimum rate at pH 5 (Table 3). The series pH 2 > pH 3 > pH 4 > pH 5 indicates a better stability at pH 5. Similar processes were encountered for the VO^{2+}-ceftriaxone complex. If we compare the observed constants for the free ceftriaxone hydrolysis reactions with those of the cupric-ceftriaxone complex, at a 1:1 molar ratio, a situation of lesser stability is seen in the ceftriaxone values at pH 2 (k_3 of 0.584) and pH 3 (0.463) at 30 ºC,[15] in relation to the Cu^{2+}-ceftriaxone complex (0.082, pH 2 and 0.062, pH 3). These effects are less at pH 4 (0.162 vs. 0.065) and pH 5 (0.140 vs. 0.069). The same happens in the case of VO^{2+}-ceftriaxone. The degradation rate constants are of first-order, according to the best values of the correlation coefficient.[13]

However, the logarithms of the observed rate constants versus pH for the hydrolysis of the cupric-ceftizoxime chelate show a minimum rate at pH 2 (see Table 4), with an order of pH 2 < 3 < 4 < 5, which indicates a better stability at pH 2. Similar processes were encountered for the VO^{2+} -ceftizoxime complex. If we compare the observed constants for the free ceftizoxime hydrolysis reaction with those of the cupric-ceftizoxime complex, at a 1:1 molar ratio, a situation of lesser stability is seen in the ceftizoxime values at pH 2-3 (k of 0.172 and 0.091 at 30 ºC),[14] in relation to the Cu^{2+}-ceftizoxime complex (0.015 and 0.042). These values are similar at pH 4 (0.044, 0.043) and 5 (0.075, 0.072). The same is observed in the case of VO^{2+}-ceftizoxime. The degradation rate constants are also of first-order, according to the best values of the correlation coefficient.[14]

Effect of temperature

The temperature dependences of the hydrolytic reactions of the cupric and vanadium chelates in buffer solutions were determined by measuring the first-order rate constants at various pH and Cu^{2+} or VO^{2+} concentrations. Also, constant ionic strengths of 0.5, at 30º, 40º, 50º and 60 ºC, were considered useful for these assays. The calculated Arrhenius heats of activation are shown in Table 5.

Table 3. Logarithms of observed k values (h^{-1}) of the Cu^{2+}-ceftriaxone hydrolytic reaction.

Temperature	Molar ratios	pH= 2	3	4	5
30 ºC	0.5:1	-1.52	-1.70	-1.70	-1.80
	1:1	-1.10	-1.22	-1.22	-1.15
	2:1	-0.72	-0.77	-0.80	-0.85
	3:1	-0.55	-0.66	-0.72	-0.77
40 ºC	0.5:1	-1.22	-1.30	-1.40	-1.40
	1:1	-1.00	-1.10	-1.05	-1.15
	2:1	-0.66	-0.72	-0.74	-0.80
	3:1	-0.52	-0.62	-0.66	-0.70
50 ºC	0.5:1	-1.15	-1.22	-1.30	-1.30
	1:1	-0.92	-1.00	-1.05	-1.10
	2:1	-0.62	-0.68	-0.68	-0.72
	3:1	-0.49	-0.59	-0.62	-0.66
60 ºC	0.5:1	-1.05	-1.22	-1.22	-1.22
	1:1	-0.66	-1.00	-2.00	-1.05
	2:1	-0.47	-0.66	-0.66	-0.70
	3:1	-0.37	-0.57	-0.60	-0.64

Table 4. Logarithms of the observed k of the Cu^{2+}-ceftizoxime hydrolytic reaction.

Temperature	Molar ratios	pH= 2	3	4	5
30 ºC	0.5:1	-2.00	-1.41	-1.45	-1.18
	1:1	-1.82	-1.36	-1.38	-1.14
	2:1	-1.49	-1.27	-1.28	-1.02
	3:1	-1.30	-1.19	-1.20	-0.94
40 ºC	0.5:1	-1.54	-1.25	-1.26	-1.07
	1:1	-1.46	-1.20	-1.21	-1.04
	2:1	-1.35	-1.14	-1.15	-0.99
	3:1	-1.25	-1.08	-1.09	-0.87
50 ºC	0.5:1	-1.36	-1.15	-1.16	-1.00
	1:1	-1.31	-1.11	-1.12	-0.97
	2:1	-1.23	-1.06	-1.07	-0.94
	3:1	-1.15	-1.01	-1.01	-0.83
60 ºC	0.5:1	-0.95	-0.85	-0.86	-0.77
	1:1	-0.89	-0.84	-0.84	-0.76
	2:1	-0.86	-0.81	-0.81	-0.76
	3:1	-0.86	-0.78	-0.78	-0.66

Table 5. Calculated heats of activation in kJ mol^{-1}.

pH	0.5:1[1]		1:1		2:1		3:1	
2(I)	15.6[a]	15.2[b]	15.2 [a]	14.9 [b]	14.2 [a]	14.3 [b]	13.5 [a]	13.9 [b]
2(II)	13.3	13.5	13.4	14.3	12.9	13.2	12.6	12.4
3(I)	14.0	14.0	13.8	13.8	13.6	13.6	13.4	13.4
3(II)	13.6	13.9	12.8	12.7	12.5	12.5	12.3	12.4
4(I)	13.9	13.9	13.8	13.7	13.6	13.5	13.4	13.3
4(II)	13.8	13.6	12.6	12.8	12.5	12.5	12.4	12.5
5(I)	13.4	13.3	13.3	13.3	12.9	12.9	12.9	12.9
5(II)	14.0	14.0	12.4	12.9	12.6	12.5	12.5	12.5

[1]Molar ratios; (I) Ceftizoxime, (II) Ceftriaxone. [a]Cu^{2+}-chelate. [b]VO^{2+}-chelate.

Effect of Cu^{2+} and VO^{2+} concentrations

The k values observed versus different molar ratios of Cu^{2+}-ceftriaxone and VO^{2+}-ceftriaxone (or ceftizoxime) show an increase of the observed k values when the molar ratio increases (see Tables 3 and 4). This fact indicates less stability when the concentrations of Cu^{2+} or VO^{2+} increase.

Conclusions

Cu^{2+} and VO^{2+} complexation with ceftriaxone or ceftizoxime at pH between 2 and 5 results in the formation of a stable chelate that facilitates the nucleophilic attack of the H_2O molecule, with an equilibrium constant for equation 1 and a hydrolysis constant for equation 3. The effects of Cu^{2+} and VO^{2+} complexation in the ceftriaxone decomposition reaction are evident at pH between 2 and 5, because the rate constant is lower than that with uncomplexed ceftriaxone. With ceftizoxime these effects are evident at pH 2 and 3, but not at pH 4 and 5, where the observed constants are similar to uncomplexed ceftizoxime.

The 7-ADCA degradation product of ceftriaxone also gives rise to these reactions at pH 2 and pH 3 and the 7-ADCA degradation product of ceftizoxime also participates in similar reactions at pH between 2 to 4.

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