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Article

The Kinetics and Mechanism of the Reaction of ZINCON, *o*-[1-(2-hydroxy-5-sulfophenyl)-3-phenyl-5-formazane] Benzoic Acid, with Zn^{2+}, Cu^{2+} and [Zn^{2+} + Cu^{2+}] Equimolar Mixtures

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Estudaram-se a cinética e o mecanismo das reações entre ZINCON e Zn^{2+}, Cu^{2+} e misturas equimolares de Zn^{2+} + Cu^{2+}. As reações foram realizadas em soluções aquosas (pH = 9,0) e monitoradas pelo método espectrofotométrico, em 610 nm. As velocidades das reações foram determinadas pela técnica de fluxo interrompido. O modelo mecanístico proposto para explicar as reações considera pontes OH^- e/ou H_2O envolvendo espécies poliméricas de Zn^{2+} e Cu^{2+} na solução aquosa. Em resumo, a reação entre os cátions metálicos e ZINCON pode ser descrita ocorrendo em duas etapas: i) uma reação inicial muito rápida entre pontes OH^-/H_2O ou moléculas de água da esfera de solvatação dos cátions e os átomos de oxigênio do ligante; ii) a ligação dos cátions ao nitrogênio do ligante, formando efetivamente o complexo ZINCON-cátion.

The kinetics and mechanism of the reactions between ZINCON and Zn^{2+}, Cu^{2+} and Zn^{2+} + Cu^{2+} equimolar mixtures were studied. The reactions were performed in aqueous solution (pH = 9.0) and monitored spectrophotometrically at 610 nm. The rates of the reactions were determined using the stopped-flow technique. The mechanistic model proposed to explain these reactions assumes OH^- and/or H_2O bridges involving polymeric species of Zn^{2+} and Cu^{2+} in the aqueous solution. The reaction between the metal cations and ZINCON can be described as occurring in two general steps: i) an initial very fast reaction between the OH^-/H_2O bridges or solvation water molecules of the cations and the oxygen atoms of the ligand; and ii) the binding of the cations to the nitrogen of the ligand, forming the ZINCON-cation complex.

Keywords: *ZINCON complexes, complex formation kinetics, stopped-flow kinetics*

Introduction

ZINCON, o-[1-(2-hydroxy-5-sulfophenyl)-3-phenyl-5-formazane] benzoic acid (Fig. 1a) is an analytical absorptiometric reagent utilized since 1952 when Yoe and Rush described it for zinc determination[1,2]. This ligand has been used for the determination of zinc, copper and other metals such as cobalt, cadmium, mercury and nickel, by such different techniques as titration (indicator), spectrophotometry, and by kinetic methods[3]. Figure 1b represents the most accepted structure for the ZINCON 1:1 metallic cation complexes[4].

The sequence shows the ionization scheme of ZINCON in aqueous solution. The carboxyl hydrogen is lost at a pH of about 4 ($pK_1 = 4.0$), lower pH values can decompose the ligand. Diprotoned and monoprotoned species are formed between pH 5.9 and 10.0, $pK_2 = 7.8$. The deprotoned form occurs in strongly alkaline media, $pK_3 = 15.0$[4].

$$H_3L^- \underset{pK_1}{\rightleftharpoons} H_2L^{2-} \underset{pK_2}{\rightleftharpoons} HL^{3-} \underset{pK_3}{\rightleftharpoons} L^{4-}$$

Although the reactions of the formation of the ZINCON complexes have been widely applied in analytical chemistry, their kinetics and mechanism, as far as we know, have been not studied in detail. The formation of the zinc-ZINCON complex was assumed to be so fast that it could not be studied even by the stopped-flow technique[5].

Usually, kinetic-mechanistic studies of the complexation of metal transition cations have been performed in conditions where only one cation reacts with the ligand. Nevertheless, in nature, for example, in living organisms,

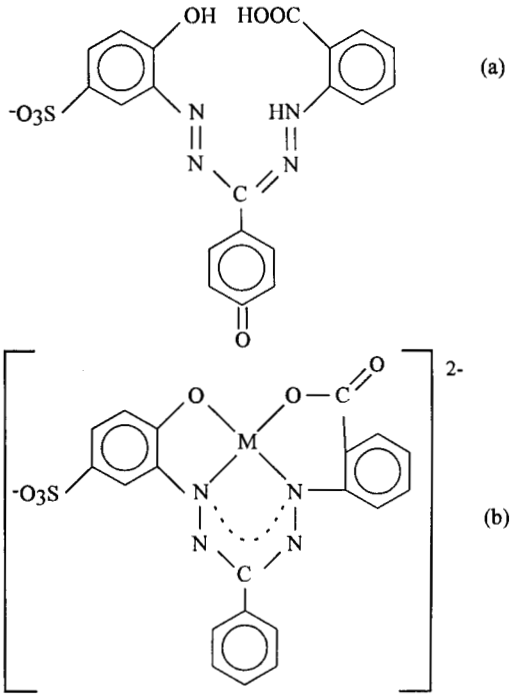

Figure 1. (a) Structure of ZINCON; (b) proposed structure of a divalent metallic cation ZINCON complex [4].

complexation reactions occur under conditions where cations and ligands are not alone but are in the presence of other species. Therefore, resulting reactions are a consequence of the competition between several equilibria. For example, it is known that the zinc level in the liver regulates the copper level in this organ[6]. If it is supposed that zinc and copper absorption in the organism is a consequence of complexation reactions, a reaction mechanism could be determined for this phenomenon.

Zinc is an essential micro-nutrient for all forms of life and it has a great variety of biochemical functions: catalytic, regulatory (inhibitors) and structural. In addition, there is a great variety of zinc metalloenzymes. Copper is also an essential micro-nutrient for many organisms. Some proteins are copper complexes, such as ceruloplasmin. Unlike zinc, copper can take part in biochemical redox processes[3,6-8]. Some diseases, such as anemia, are caused by copper deficiency. Zinc deficiency causes so many disorders that supplements have been used for treating diseases ranging from acne to AIDS. Some symptoms of AIDS resemble those of serious zinc deficiency, and the beneficial effects of zinc supplements in two hospitalized AIDS patients have been reported[8].

In order to investigate the relationship between copper and zinc in complexation reactions, the ZINCON ligand was chosen as a model reagent since it reacts with both under the same conditions. The kinetics of the reaction of ZINCON with Cu^{2+} and Zn^{2+}, including equimolar mixtures of these cations were studied, followed by a spectroscopic study of the aqueous cation solutions. Some unexpected facts were observed, since the equimolar mixtures react as a unique species. The measured spectroscopic effects were weak, but quite reproducible, making possible to proposal a plausible mechanism. A summation of some different weak effects seems to assume a strong influence on the reaction paths in ZINCON complexes formation.

Experimental

Materials

All reagents were of analytical grade and solutions were prepared with water obtained from a glass distiller. KCl was used to maintain the ionic strength constant (1.00 M) in all working solutions. A $(1.530 \pm 0.001) \times 10^{-2}$ M stock solution of zinc chloride was prepared by dissolving metallic zinc in 6.0 M HCl. Working Zn^{2+} solutions were prepared by adequate dilutions. A $(1.557 \pm 0.005)10^{-2}$ M stock solution of copper sulfate was prepared by dissolving $CuSO_4.5H_2O$ in distilled water and standardizing by the iodometric method[9]. Working Cu^{2+} solutions were prepared by adequate dilutions with distilled water. Working solutions of $Zn^{2+} + Cu^{2+}$ equimolar mixtures were prepared by diluting mixtures of adequate volumes of the above stock solutions with distilled water. Buffer solutions (pH 9.0) were obtained by mixing 50.0 mL of 0.10 M H_3BO_3 prepared in a 1.00 M KCl solution with 21.4 mL of 0.10 M NaOH solution[10]. The buffer was used only in ZINCON solutions. ZINCON solutions were prepared daily by directly weighing ZINCON monosodium salt and dissolving in the buffer solution (pH 9.0). For the near infrared and mid infrared spectra, aqueous solutions of $ZnSO_4$, $CuSO_4$ and the $ZnSO_4 + CuSO_4$ equimolar mixture were prepared by directly weighing the salts and standardizing[9].

Spectra

The visible electronic spectra were determined with a Carl Zeiss DMR-21 spectrophotometer and a 1.00 cm path quartz cell. The solutions were obtained by mixing cation solutions with the ZINCON buffered solution in equal volumes in adequate concentrations to give a 3.03×10^{-5} M zinc complex and a 3.08×10^{-5} M copper complex as the final concentration. The near infrared spectra were obtained with a Cary 2300 spectrophotometer and 0.10 cm path quartz cell. The mid infrared spectra were obtained with a Nicolet 520-FTIR, with a resolution of 4 cm^{-1}, 512 scans, using a SpectraTech micro CIRCLE™ cell with a ZnSe ATR crystal. Distilled water was used for reference.

Kinetics

The reactions were monitored spectrophotometrically at 610 nm, at 25.00 ± 0.03 °C, using a stopped-flow apparatus built in our laboratory[11,12]. In order to study the influence of metal and ligand concentration on the reaction,

two series of experiments were performed for each metal and for the equimolar mixture of zinc and copper. Series **A** was to study the influence of the metal concentration on the reaction rate, where the concentration of the metal varied from 3.80 x 10^{-6} to 1.55 x 10^{-5} M, and the ZINCON concentration was fixed at 5.16 x 10^{-5} M. Series **B** was to study the influence of ZINCON concentration on the reaction rate, where the concentration of ZINCON varied from 9.30 x 10^{-6} to 3.06 x 10^{-4} M, and the metal concentration was fixed at 1.50 x 10^{-5} M.

The results presented here are the average of at least five individual determinations obtained in immediate succession.

The reaction rate constants were determined considering the variation of absorbance over about 60% of the total reaction, *i.e.*, from approximately 20% up to 80%. The experimental readings were done with the aid of a microcomputer and electronically saved. Rates were calculated using a program developed especially for this work, which includes unweighted average smoothing to improve its performance[3].

The stopped-flow experiments were done with the ZINCON solutions buffered at pH = 9.0 and an ionic strength of 1.00 M. The Cu^{2+} and Zn^{2+} solutions were prepared only with distilled water and enough KCl to obtain an ionic strength = 1.00 M; buffering was not done in this case in order to avoid the formation of hydroxides and hydroxymetaborates[13,14] of the cations of the metals. In fact, the experiments have been performed in the absence of precipitation. Despite the fact that borate can undergo complexation with Cu^{2+} and Zn^{2+}, borate buffer was chosen as the least troublesome. The formation of hydroxo and/or borate aquo complexes of Cu^{2+} and Zn^{2+} cannot be actually ignored. However, a general mechanism can be proposed for the studied reactions, where the eventual participation of borate may or may not be considered without changes in the "core" of the mechanism. For instance, borates could be assumed to be bonded to cations complexed with ZINCON, substituting water molecules or OH^-, acting as intermolecular bridges or not. This would not change the proposed mechanism as a whole.

Results and Discussion

The visible electronic spectra of the aqueous solutions of ZINCON complexes with Zn^{2+}, Cu^{2+} and the Zn^{2+} + Cu^{2+} equimolar mixture can be observed in Fig. 2. At 610 nm, the Zn^{2+}-ZINCON and Cu^{2+}-ZINCON complexes have equal absorptivity coefficients, so this wavelength was chosen for monitoring the reaction. Figure 2 clearly shows that the spectrum of the equimolar mixture Zn^{2+} + Cu^{2+} (b) does not correspond to the mathematical sum (d) of the spectra of the separate solutions of the Zn^{2+}-ZINCON (a) and Cu^{2+}-ZINCON (c) complexes, since at the maximum absorbance wavelength the absorption of the mixture is

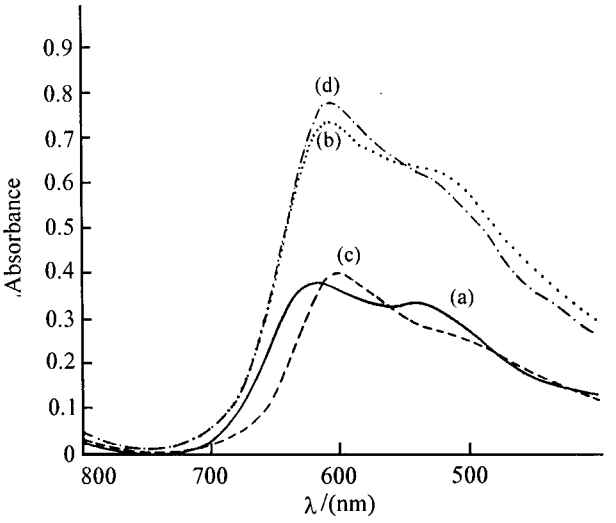

Figure 2. Electronic visible spectra of aqueous solutions, pH = 9.0, with distilled water as reference. (a) [ZnL] = 3.03 x 10^{-5} M, (b) [ZnL] = 3.03 x 10^{-5} M + [CuL] = 3.08 x 10^{-5} M (mixture), (c) [CuL] = 3.08 x 10^{-5} M, and (d) mathematical sum of a + c.

smaller than that calculated from the sum of the individual solution spectra. This is a quite reproducible observation within the performance of the instrument used. From about 560 nm, an inversion in the absorbance intensities is observed, indicating that free ligand is present in solution (for ZINCON solutions at pH = 9.0, λ_{max} = 470 nm). This observation suggests that polynuclear complexes $M_nZINCON_m$ (M is a bivalent metallic cation, Zn^{2+} or Cu^{2+}, and n > m) are formed at least when these ions are in a mixture.

The different observed stabilities of these ZINCON complexes in aqueous solution at pH = 9.0 at room temperature (*ca.* 25 °C) reinforces this idea. The copper complex is quite stable, and no significant discoloration was observed over a period of 10 days. On the other hand, the Zn^{2+}-ZINCON complex became colorless in about two days. The equimolar "mixture" zinc+copper-ZINCON complex was also quite stable, with just a little discoloration observed over 10 days.

In Figs. 3 and 4, the correlation between the rates and the concentration of metals and ligand can be observed. The **symbols** correspond to the **experimental values**, and the **lines** represent the **calculated** rates obtained from the proposed mechanism. Tables 1A and 1B show the experimental and calculated reaction rates that were obtained on the basis of the proposed mechanism.

Both in the Tables and in the plots, a very good agreement is observed between the experimental and calculated rates.

Figure 3 shows the variation of the reaction rate, for the solutions of zinc (a), copper (c), and for the mixture zinc + copper (b), as a function of the metal ion concentration. The 5.16 x 10^{-5} M concentration of ZINCON was maintained constant and the metal ion concentration varied from

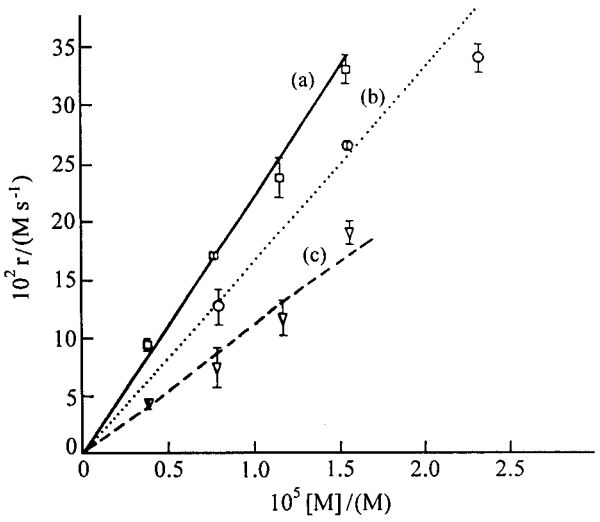

Figure 3. Plot of the reaction rates for the series **A** experiments at 25.00 ± 0.03 °C. Considering: [L] = 5.16 x 10^{-5} M constant and [$M(H_2O)_f$] is variable: (a) Zn^{2+}, (b) Zn^{2+}+Cu^{2+} equimolar mixture, (c) Cu^{2+}. The plotted lines were calculated according to the proposed mechanism. The error bars are ± σ.

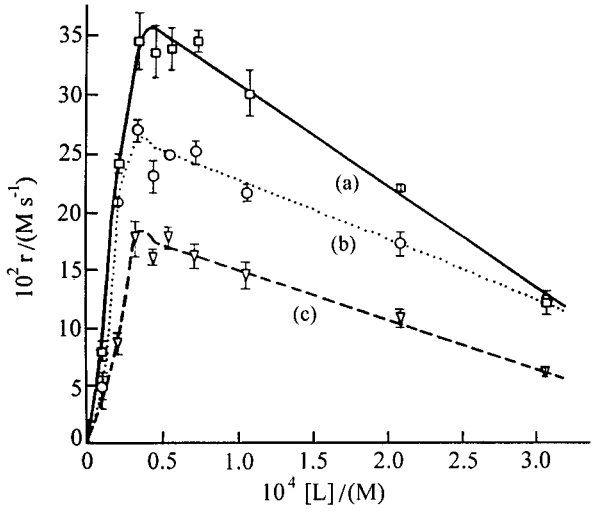

Figure 4. Plot of the reaction rates for the series **B** experiments at 25.00 ± 0.03 °C. Considering: [L] is variable and [$M(H_2O)_f$] is constant. (a) [Zn^{2+}] = 1.52 x 10^{-5} M, (b) [Zn^{2+}] = 7.76 x 10^{-6} M + [Cu^{2+}] = 7.78 x 10^{-6} M (mixture), (c) [Cu^{2+}] = 1.55 x 10^{-5} M. The plotted lines were calculated according to the proposed mechanism. The error bars are ± σ.

4.00 x 10^{-6} to 2.00 x 10^{-5} M. Within these metal concentration limits, in the three cases (Zn^{2+} (a), Zn^{2+} + Cu^{2+} (b), and Cu^{2+} (c) solutions) an increase in the rate is observed with increasing metal ion concentration.

In Fig. 4 the dependence of the reaction rate for the solutions of zinc (a), the zinc + copper mixture (b), and copper (c), as a function of the ZINCON concentration is shown. The metal ion concentration was maintained constant, [Zn^{2+}] = 1.52 x 10^{-5} M, [Cu^{2+}] = 1.55 x 10^{-5} M, and

Table 1A. Reaction rates for ZINCON complex formation, at 25.00 ± 0.03 °C, pH = 9.0, with [ZINCON] = 5.16 x 10^{-5} M solution. Series A experiments.

i) for Zn^{2+} complex

[Zn^{2+}]/(10^{-5} M)	$r_{exp} \pm \sigma$	r_{calc}
	(10^{-4} M x s^{-1})	(10^{-4} M x s^{-1})
0.38	9.6 ± 0.5	8.7
0.76	17.2 ± 0.4	17.4
1.14	24.1 ± 1.7	26.1
1.52	33.4 ± 1.2	34.8

ii) for Zn^{2+}+ Cu^{2+} complex

[Zn^{2+}]	[Cu^{2+}]	[Me^{2+}]$_{total}$	$r_{exp} \pm \sigma$	r_{calc}
(10^{-5} M)	(10^{-5} M)	(10^{-5} M)	(10^{-4} M x s^{-1})	(10^{-4} M x s^{-1})
0.38	0.39	0.77	12.8 ± 1.5	12.2
0.76	0.78	1.54	26.9 ± 0.3	25.1
1.14	1.16	2.30	34.5 ± 1.2	38.7

iii) for Cu^{2+} complex

[Cu^{2+}]	$r_{exp} \pm \sigma$	r_{calc}
(10^{-5} M)	(10^{-4} M x s^{-1})	(10^{-4} M x s^{-1})
0.39	4.4 ± 0.3	4.0
0.78	7.6 ± 1.7	8.2
1.16	11.9 ± 1.5	12.5
1.55	19.3 ± 1.0	17.0

the mixture ([Zn^{2+}] + [Cu^{2+}]) = 1.54 x 10^{-5} M. The ZINCON concentration varied from 9.30 x 10^{-6} to 3.06 x 10^{-4} M. It should be noted that in the three cases the reaction rate reaches a maximum at a ligand concentration of about 3 x 10^{-5} M, closely corresponding to a 1:2 molar proportion of metal:ZINCON. After these maxima, the rates decrease with increasing ligand concentration. It can be clearly noted that the reaction of the zinc + copper mixture does not simply correspond to the behavior of a mixture, but is intrinsic to a "new" species reacting at intermediate rates. In the part of the curve where the rate increases, the mixture curve resembles that of zinc. In contrast, the part of the curve of the decreasing rate follows that of copper. These observations extend the idea suggested above by the visible spectra that these complexes are probably polymeric in solution.

Polynuclear aquo-hydroxo copper and zinc complexes are reported in the literature[7,15]. In complexation reactions

Table 1B. Reaction rates for ZINCON complex formation, at 25.00 ± 0.03 °C, with [Zn^{2+}]= 1.52 x 10^{-5} M, mixture: [Cu^{2+}]= 7.78 x 10^{-6} M + [Zn^{2+}] = 7.6 x 10^{-6} M, [Cu^{2+}] = 1.55 x 10^{-5} M solutions at pH = 9.0. Series B experiments.

[ZINCON] (10^{-5} M)	Zn^{2+}		Zn^{2+} + Cu^{2+}		Cu^{2+}	
	$r_{exp} \pm \sigma$ (10^{-4} M x s^{-1})	r_{calc} (10^{-4} M x s^{-1})	$r_{exp} \pm \sigma$ (10^{-4} M x s^{-1})	r_{calc} (10^{-4} M x s^{-1})	$r_{exp} \pm \sigma$ (10^{-4} M x s^{-1})	r_{calc} (10^{-4} M x s^{-1})
0.92	8.1 ± 0.9	8.8	4.9 ± 1.0	4.9	4.6 ± 1.6	4.6
1.85	24.2 ± 0.8	25.4	20.9 ± 0.2	19.6	8.8 ± 0.9	9.0
3.05	34.6 ± 2.4	36.6	27.0 ± 0.9	26.1	17.7 ± 1.5	17.8
4.15	33.7 ± 2.2	35.7	23.1 ± 1.4	25.6	16.1 ± 0.6	17.4
5.25	34.5 ± 2.3	34.7	24.9 ± 0.5	25.1	17.8 ± 0.9	17.0
6.90	34.6 ± 0.9	33.2	25.2 ± 0.9	24.2	16.2 ± 1.0	16.3
10.30	30.2 ± 1.9	30.2	21.7 ± 0.7	22.6	14.6 ± 1.1	14.9
20.65	22.3 ± 0.4	21.1	17.5 ± 1.0	17.5	11.2 ± 0.7	10.7
30.60	12.7 ± 1.0	12.3	12.9 ± 0.7	12.6	6.7 ± 0.2	6.6

of ZINCON with zinc and copper, such aquo-hydroxo complexes can be assumed to play an important role. In this case, some water molecules and/or OH^- bonded to the cations must be replaced by the ligand. If one considers the borate also bonded to the cations, its replacement by the ligand could also occur. This fact, however, will not change the path of the mechanism.

In order to test the hypothesis of the existence of polynuclear aquo-hydroxo complexes in the studied solutions, near infrared spectra of aqueous solutions of Zn^{2+} and Cu^{2+}, and of equimolar mixtures of Zn^{2+} + Cu^{2+} were obtained, and are shown in Fig. 5. In this wavelength range, overtones and/or combination bands due to the vibration of water molecules and/or OH^- ions surrounding the ions of the added salt appear. These bands are very weak and they are placed on a tail of the corresponding stronger band of the solvent (water). As in the case of Fig. 2, the spectrum of the Zn^{2+} + Cu^{2+} mixture (b) does not correspond to the sum of the individual spectra of the solutions of Zn^{2+} (a) and Cu^{2+} (c). On the other hand, observing the "band shape" that reflects the contribution of the individual components of the bands, it can be noted that the mixture "band shape" is a little different. These small spectral differences, despite the operational difficulties, are quite reproducible, within the instrumental precision.

When the spectra of solutions with 99.8% D_2O were carried out, these bands were displaced to other wavelengths out of the range of the spectrophotometer used. This drastic effect of D_2O on the spectra can be considered to be a strong evidence for the fact that the observed bands are OH^- and/or water vibrations.

According to Herzberg[16] and Nakamoto[17], there are several combinations of water vibrational frequencies that could correspond to this band in the near infrared range. Some combinations of these may be: i) the first overtone (2 ν_1) of the symmetric HOH stretching; ii) the first overtone (2 ν_3) of the asymmetric HOH stretching; iii) a perturbation that corresponds to $\nu_1+\nu_3$; iv) a combination ν_2 + ν_3.

The infrared spectra of the cations solutions, from 3200 to 2600 cm^{-1}, are shown in Fig. 6. The maximum absorptions are *ca.* 3100 cm^{-1} (Zn^{2+} solution), 3070 cm^{-1} (Zn^{2+}+ Cu^{2+} solution), and 3050 cm^{-1} (Cu^{2+} solution). Considering the experimental precision, the maximum absorbance wavenumber of the mixture can be considered to be the mean value of the wavenumbers corresponding to the solutions of the individual salts of copper and zinc. Certainly this could simply be attributed to the instrumental coalescence of the two isolated peaks. However, considering the resolution of the infrared spectrophotometer used, it is quite reasonable to suppose that this indicates a mean arrangement in the water molecules and/or OH^- bonded to cations. This supposition is in agreement with the other results obtained in this work, for example, that of the stability of the ZINCON complex with Zn^{2+}, Cu^{2+} and the Zn^{2+} + Cu^{2+} mixtures, and their kinetics, reinforcing the idea that in solutions where there are both Cu^{2+} and Zn^{2+}, "mixed" copper-zinc polymeric aquo-hydroxo species are formed.

The first overtones of these vibrations are at *ca.* 1620 - 1640 nm, corresponding to the right shoulder of the obtained spectra in the near-infrared (*ca.* 1640 nm) range. More detailed work on this specific subject has been developed[18].

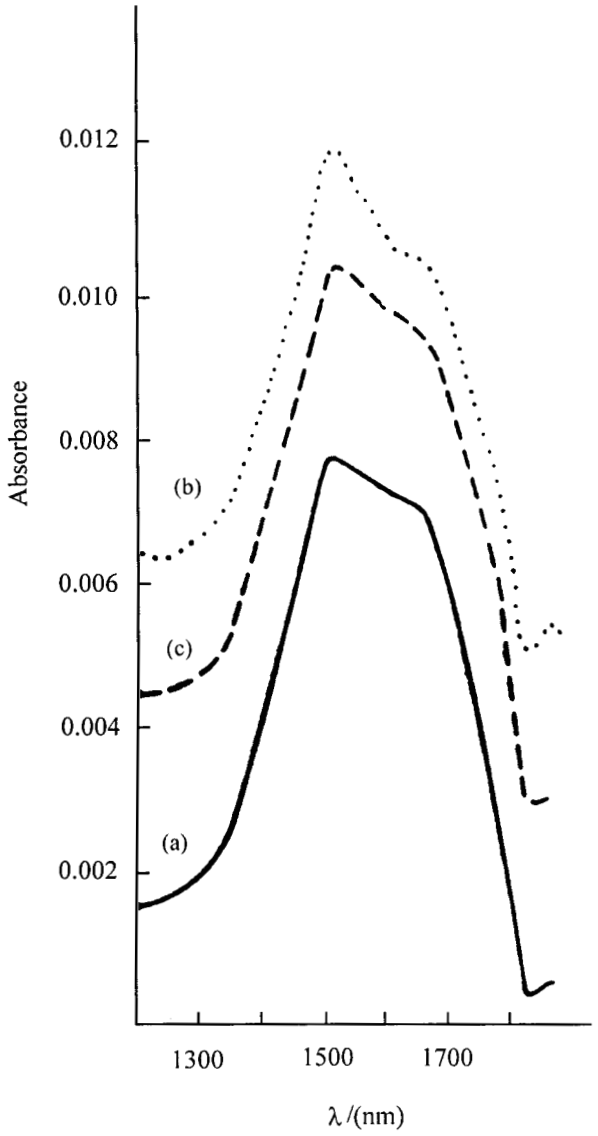

Figure 5. Near-infrared spectra of aqueous solutions using distilled water for reference. (a) $[ZnSO_4] = 5.00 \times 10^{-2}$ M, (b) $[ZnSO_4] = 2.50 \times 10^{-2}$ M + $[CuSO_4] = 2.50 \times 10^{-2}$ M (mixture), (c) $[CuSO_4] = 5.00 \times 10^{-2}$ M.

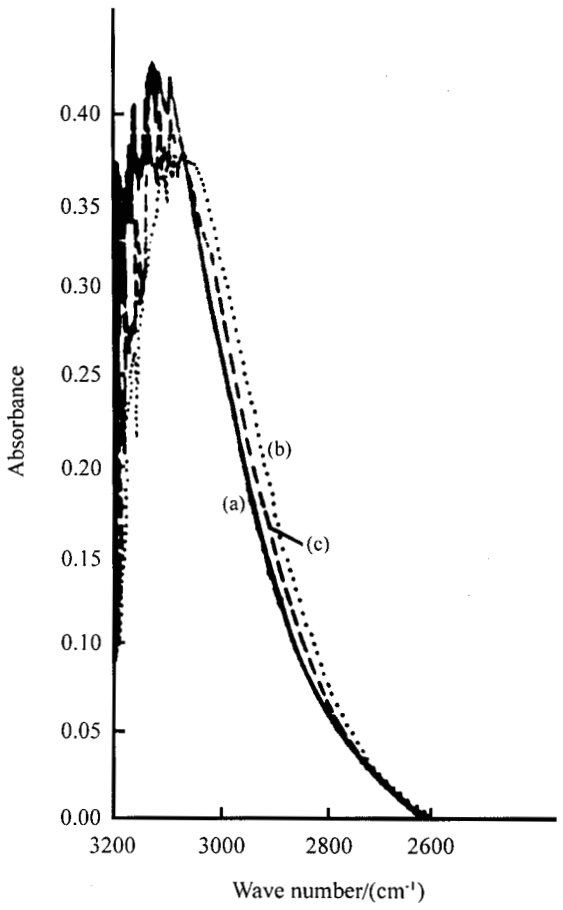

Figure 6. Infrared spectra of aqueous solutions using distilled water for reference. (a) $[ZnSO_4] = 1.0$ M, (b) $[ZnSO_4] = 0.50$ M + $[CuSO_4] = 0.50$ M (mixture), (c) $[CuSO_4] = 1.0$ M.

On the basis of the kinetic and spectrophotometric results obtained in this work, a plausible mechanism of this reaction can be proposed (Fig. 7). The following representations are used: r_i = reaction rates; k_i = rate constants; $\mathbf{M(H_2O)_f}$ represents the metal cation and its solvation sphere; **L** is ZINCON; and **ML** is the **simplified** representation of the complex formed, *i.e.*, the spectroscopically monitored species. **CM** and **CL** are the initial concentrations of the metal solutions and ZINCON, respectively. To simplify the scheme, water molecules of hydration and OH^- (and / or borate) bound to the metals are not shown in the formed complexes, **ML**. It must be noted that in the "intermediates" $\mathbf{M(H_2O)_xL_2}$ and $\mathbf{M(H_2O)_yL_3}$, where x = y or $x \neq y$, where the ligand **L** is supposed to be bound to the metallic cation through H_2O and/or OH^- (and/or borate) bridges, water molecules are represented to emphasize this circumstance. The water molecules are also represented in the metallic cations $\mathbf{M(H_2O)_f}$, where f = x or $f \neq x$. $\mathbf{M(H_2O)_f}$, **ML**, $\mathbf{M(H_2O)_xL_2}$ and $\mathbf{M(H_2O)_yL_3}$ may be polynuclear species, and **the representations used are simplifications**.

As the working pH is 9.0, based on the ZINCON pKa values (see above), it may be assumed that the reacting species is the monoprotoned one.

In the proposed mechanism, the initial interactions among the metallic cations and the ligand occur through H_2O and/or OH^- bridges from the solvation sphere of all of the reagent species. It is supposed to be a very fast step, represented by r_1. The binding of the cations to the nitrogen of the ligand, with the releasing of solvation water molecules, to form the ZINCON-cation complex, involves all the steps described in Fig. 7.

Figures 3 and 4 show the **experimental** (symbols) and the **calculated** (lines) rates from the proposed mechanism. An excellent fitting is observed.

Table 2. Kinetic constants for ZINCON complex formation, at 25.00 ± 0.03 °C.

Kinetic constants	Zn^{2+}	equimolar mixture Zn^{2+}+ Cu^{2+}	Cu^{2+}
$k_1 / 10^{13}$ $M^{-2} s^{-1}$	6.80	3.73	3.50
$k_{-1} / 10^4$ s^{-1}	9.95	-	1.46
$k_2 / 10^4$ s^{-1}	2.40	1.70	1.15
$k_3 / 10^7$ $M^{-1} s^{-1}$	5.50	3.19	2.62

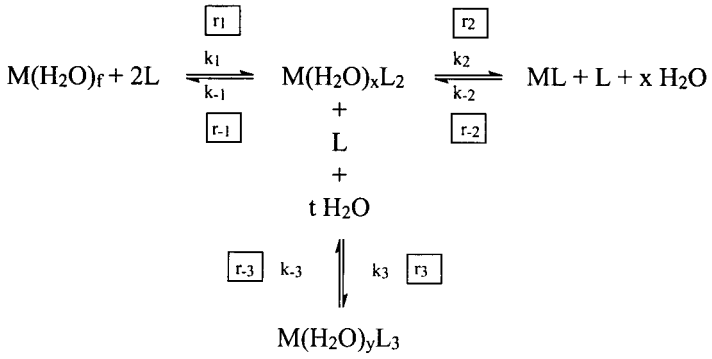

Figure 7. Mechanism of the reaction in aqueous solutions of Cu^{2+}, Zn^{2+} and Cu^{2+} + Zn^{2+} equimolar mixtures with ZINCON. To simplify the scheme, only the more important water molecules of the solvation sphere are represented. $M(H_2O)_f$, $M(H_2O)_xL_2$ and $M(H_2O)_yL_3$ could be polynuclear species and the representations used are simplifications.

The following procedure was used to obtain experimental rate constants and calculated rates (Tables 1A and 1B, Figs. 3 and 4).

In the first part of the rates curves, as a function of the ligand concentration (Fig. 4), *i.e.*, when CM ≥ CL, the reaction rate r_1 can be assumed to be higher than r_{-1} and lower than r_2 , and it is therefore the determining reaction rate:

$$r = r_1 = k_1 \times CM \times CL^2 \quad (1)$$

k_1 can be calculated because CM and CL are assumed to be the initial concentrations of the reagents.

With the increase of the ligand concentration, r_1 increases enough to very rapidly form [ML_2] in sufficient quantities to observe r_{-1} decelerating the total reaction:

$$r = r_1 - r_{-1} = k_1 \times CM \times CL^2 - k_{-1} \times [M(H_2O)_xL_2] \quad (2)$$

At these concentrations, a rate is calculated from Eq. 1. The difference between this result and the experimental rate gives a value for (k_{-1} x [$M(H_2O)_xL_2$]). To obtain k_{-1} , an estimate for [$M(H_2O)_xL_2$] is necessary. Supposing a rough value for [$M(H_2O)_f$], where a concentration of the complexed metal is subtracted from CM, an effective [L] can be calculated from Eq. 1, using the experimental rate. After some successive approximations, the best [$M(H_2O)_xL_2$] value is obtained and results an estimate for k_{-1}.

At about 2 x CM = CL, $r_1 - r_{-1} \cong r_2$ and r_3 is still very small. Therefore, from this maximum, it is expected that the reaction rate was determined by r_2, ($r \approx r_2$) giving constant values. Nevertheless a decrease in the observed rate is verified as a function of the increase in the ligand concentration. This can be understood in terms of the negative contribution of the rate r_3.

$$r_3 = k_3 \times [M(H_2O)_xL_2] \times [L] \quad (3)$$

r_3 increases when the free ligand concentration, [L] = CL - 2 x CM, increases and r decreases.

$$r = r_2 - r_3$$

$$\text{or } r = k_2 \times [M(H_2O)_xL_2] - k_3 \times [M(H_2O)_xL_2] \times [L] \quad (4)$$

In this situation, [$M(H_2O)_xL_2$] = CM and the total reaction rate can be calculated by Eq. 5:

$$r = CM \times (k_2 - (k_3 \times (CL - 2 \times [M(H_2O)_f])) \quad (5)$$

k_{-2} and k_{-3} are considered to be very small, and therefore r_{-2} and r_{-3} are negligible.

At the maximum of the curve, the rate can be expressed as:

$$r = k_2 \times [M(H_2O)_xL_2] \quad (6)$$

k_2 can be obtained by a first approximation, assuming that [$M(H_2O)_xL_2$] = CM as the ligand concentration is enough to complex all metal, and the equilibrium constant is considered to be sufficiently high.

The calculated rates using the rate constants were compared with the experimental rates. After successive approximations, the best values for the reaction rates and

kinetic constants k_1, k_{-1}, k_{-2}, and k_{-3} (Tables 1A, 1B and 2) were obtained.

In Figs. 3 and 4, the experimental (symbols) and calculated (lines) reaction rates are shown. Tables 1A and 1B show the calculated rate constant values. Since several approximations were done for the calculations, the calculated rate constants are not exact. This is of secondary importance, since the main interest of this work is to understand the mechanism and not to find the exact values of the constants. In this sense, as can be seen in the curves of Figs. 3 and 4 and in Tables 1A and 1B, a good agreement is observed between the experimental and calculated rates within the experimental error, indicating that the proposed mechanistic approach is consistent.

As shown above, solvation of the species must play a decisive role in this kind of mechanism, and the first step of the reaction should be mainly controlled by the interaction of the ligand with H_2O and/or OH^- bound to the cations, through the oxygens of the ligand (Fig. 1a), as if they were "claws of a crab", to form the species $M(H_2O)_xL_2$ or $M(H_2O)_yL_3$, when a third ligand is competing. The formation of ML species from ML_2, with the liberation of a ligand and water solvation molecule(s), is related to cation bonding to the ZINCON nitrogens, as if it were the "mouth" of the "crab" (Fig. 1b).

Conclusions

It is interesting that this behavior of zinc and copper towards ZINCON seems to be similar to these cations in the human organism, where the two show interrelated behavior. For example, an excess of zinc affects the metabolism of copper and *vice-versa*[6], as in Wilson's disease[8].

It is very interesting to observe that this mechanism shows a certain "rate buffering effect", in function of the increase or decrease of the concentrations of the reacting species, maintaining the rate values within certain limits. This can be clearly seen in Figs. 3 and 4 where the experimental and calculated results can also be compared. On the other hand, the fact that the mixtures of zinc + copper react as a "single" species is in good agreement with the observed interrelation of these cations in biological reactions.

On the basis of this mechanism studied as a model, it is reasonable to suppose that similar processes could occur in the metabolic reactions of such metals. For instance, the Zn/Cu/Mn interactions seem to be manifestations of a more general tendency towards biochemical **competition/antagonism** between various M^{2+} species, including nutrients, such as Ca^{2+} and Mn^{2+}, and toxins such as Pb^{2+}, Hg^{2+} and Cd^{2+}. Ratios of nutrients and toxins are probably more relevant to biochemical and physiological disorders than the tissue levels of single factors, though these multidimensional relationships have not yet been extensively studied[8].

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