

Sulfite Induced Autoxidation of Cu(II)/Tetra/ Penta and Hexaglycine Complexes. Spectrophotometric and Rotating-ring-disk Glassy Carbon Electrode Studies and Analytical Potentialities

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A oxidação de complexos de Cu(II) com tetra, penta e hexaglicina, em solução aquosa de tampão borato, pelo oxigênio dissolvido é fortemente acelerada por sulfito. A formação de complexos de Cu(III) com máximos de absorvância em 250 nm ($\epsilon = 9000 \text{ mol}^{-1} \text{ L cm}^{-1}$) e 365 nm ($\epsilon = 7120 \text{ mol}^{-1} \text{ L cm}^{-1}$) foi também caracterizada usando-se voltametria com eletrodo rotativo disco-anel, na qual componentes anódicos e catódicos foram observados em voltamogramas registrados em solução contendo Cu(II). Voltamogramas, obtidos com várias velocidades de rotação, mostraram que a espécie de Cu(III) gerada eletroquimicamente não é estável em toda a janela de tempo do experimento, e em solução contendo tetraglicina a corrente limite é controlada pela cinética de um equilíbrio envolvendo espécies de Cu(II). O valor calculado da constante de decomposição de primeira ordem foi $4,37 \times 10^{-3} \text{ s}^{-1}$. Experimentos eletroquímicos realizados em solução de Cu(II) após a adição de quantidades relativamente pequenas de sulfito demonstraram que a espécie de Cu(III), formada na reação química, é a mesma que foi coletada no eletrodo anel quando Cu(II) é oxidado no eletrodo disco. A concentração dos complexos de Cu(III) é proporcional à quantidade de sulfito adicionada e os resultados indicaram a possibilidade de desenvolvimento de um método analítico indireto para sulfito, com detecção espectrofotométrica ou amperométrica do produto quimicamente gerado.

The oxidation of Cu(II) complexes with tetra, penta and hexaglycine in borate buffer aqueous solution, by dissolved oxygen is strongly accelerated by sulfite. The formation of Cu(III) complexes with maximum absorbances at 250 nm ($\epsilon = 9000 \text{ mol}^{-1} \text{ L cm}^{-1}$) and 365 nm ($\epsilon = 7120 \text{ mol}^{-1} \text{ L cm}^{-1}$) was also characterized by using rotating ring-disk voltammetry, whose anodic and cathodic components were observed in voltammograms recorded in solutions containing Cu(II). Voltammograms, obtained at various rotation speeds, showed that the Cu(III) species electrochemically generated is not stable over the entire time window of the experiment and in solutions containing tetraglycine the overall limiting current is controlled by the kinetics of an equilibrium involving Cu(II) species. The calculated first order rate constant of the decomposition was $4.37 \times 10^{-3} \text{ s}^{-1}$. Electrochemical experiments carried out in Cu(II) solutions after the addition of relatively small amounts of sulfite demonstrated that the Cu(III) species formed in the chemical reaction is the same as the one collected at the ring electrode when Cu(II) is oxidized at the disk electrode in ring-disk voltammetry. The concentration of Cu(III) complexes is proportional to the amount of added sulfite and the results indicated that indirect analytical methods for sulfite may be developed by means of spectrophotometric or amperometric detection of the chemically generated product.

Keywords: copper (III), glycines, sulfite, catalysis, autoxidation, rotating ring disc electrode

Introduction

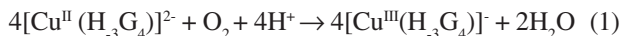
The present article is a comparative study of the sulfite induced oxidation of Cu(II) complexes with tetra, penta and hexaglycine by dissolved oxygen. The symbols G_n (G_4 , G_5 and G_6) are used here as general terms for

tetraglycine, pentaglycine and hexaglycine respectively. $(H_{-x}G_n)^{-(x+1)}$ refers to a peptide ligand with x deprotonated nitrogens coordinated to the copper ion. The degree of protonation of the copper peptides complexes depends on the medium acidity. The representations $Cu^{II}G_n$ and $Cu^{III}G_n$ refer to all complexes species present in solution.

The autoxidation of $Cu^{II}G_4$ and the decomposition of $Cu^{III}/G_4/G_5$ in the absence of sulfite were previously

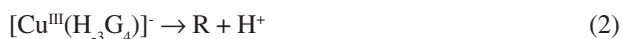
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studied by Margerum's research group.^{1,2} The reaction of O₂ with [Cu^{II}(H₃G₄)]²⁻, represented by the general reaction (equation 1), is very slow at room temperature and pH 7-10. This reaction is thermodynamically favorable (E°_{Cu^{III}G₄} = 0.63 V and E°_{O₂/H₂O} = 0.815 V vs NHE).



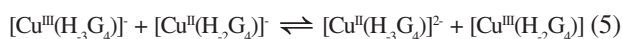
This reaction is catalyzed by initial traces of [Cu^{III}(H₃G₄)]⁻ (10⁻⁷ mol L⁻¹), with an induction period which becomes smaller by addition of strong oxidants as [Cu^{III}(H₃G₄)]⁻ (electrochemically generated). The Cu^{III}G₄ is moderately stable in neutral solution, with a half-life of 5.5 h at 25 °C.¹

The rate of subsequent decomposition of [Cu^{III}(H₃G₄)]⁻ is dependent on the pH and the oxygen concentration. The main species in solution (pH 7-10) prior to decomposition is [Cu^{III}(H₃G₄)]⁻. The pK_a value of [Cu^{III}(H₃G₄)]H and [Cu^{III}(H₃G₄)]⁻ are 4.2 and 12.1 (Table 1), respectively.² After the completion of the oxygenation and the decomposition of [Cu^{III}(H₃G₄)]⁻, new species were detected in the solution as suggested in the following mechanism (pH 7-9).¹



R is a reactive intermediate (either a carbon-centered free radical or a Cu(I) complex), RO₂ is either a peroxy radical or a copper(III) peroxide and G₄DHP is a dehydropeptide which hydrolyzes to give glycylglycinamide and glyoxyglycine.¹

Further studies² of the redox decomposition of Cu^{III}G₄ at pH 6-8 showed that the species [Cu^{II}(H₂G₄)]⁻ is responsible for the catalysis of the decomposition of [Cu^{III}(H₃G₄)]⁻ (equations 5 and 6):



The [Cu^{III}(H₂G₄)] is expected to have a higher reduction potential than [Cu^{III}(H₃G₄)]⁻ because one of the peptidic nitrogen is not coordinated and a faster redox decomposition reaction occurs. Kirschenbaum and Meyerstein³ also concluded the relative instability of neutral [Cu^{III}(H₂G₄)] compared to [Cu^{III}(H₃G₄)]⁻ species.

Studies related to Cu^{III}G₅ complex in basic medium (pH 11.6), in experimental condition where [Cu^{III}(H₄G₅)]²⁻ is the predominant species, showed that the initial

products, are dehydropeptides which hydrolyze to form amides and corresponding carbonyl species.^{4,5}

Margerum *et al.*^{1,2,4,6} studied the oxidation of Cu^{II}G₄ complexes by oxygen in the presence and absence of sulfite, these data could not clearly explain the induction period and the autocatalytic behaviour. The present work brings more information about the reaction of Cu^{II}G₄ with oxygen accelerated by sulfite. The studies were carried out in excess of Cu(II), G₄ and oxygen compared two sulfite concentration, such as a first order formation of Cu^{III}G₄ could be evaluated. Besides we also investigated the Cu^{II}G₅ and Cu^{II}G₆ complexes.

We also did complementary study about the decomposition of Cu^{III}G₄, electrochemically generated, by using the rotating ring-disk electrode (RRDE). The great advantage of the RRDE is that it can be used to analyse short-live species (unstable intermediate or product). The used RRDE technique is more sensitive and precise for kinetic studies than the one used by Margerum group,^{2,4} which consisted of a flow system, not adequate for very short-live product as further discussed.

In the present study, Cu(III) formation was followed when sulfite was added to air saturated solutions of Cu^{II}G₄, G₅ and G₆ complexes in borate buffer in aqueous medium. These new comparative studies, using spectrophotometric and electrochemical measurements bring a new contribution to better understanding of the mechanism and development of a new alternative analytical method.

Experimental

Reagents

All reagents used were of analytical grade (Merck or Sigma). The water used to prepare the solutions was purified with a Milli-Q Plus Water system (Millipore).

Stock solutions of sulfite (2.00x10⁻² mol L⁻¹) were daily prepared by dissolving Na₂S₂O₅ salt in water previously purged with nitrogen. To prepare diluted solutions of sulfite, small volumes of the stock solutions were properly added to air saturated water.

Cu(II) perchlorate stock solution (0.2 mol L⁻¹) was prepared from the direct reaction of Cu(II) carbonate with perchloric acid followed by standardization with EDTA by a conventional procedure.⁷ This solution also contained 2.0x10⁻³ mol L⁻¹ Ni(II), 6.6x10⁻⁶ mol L⁻¹ Mn(II) and 4.2x10⁻⁵ mol L⁻¹ Fe(III) ions, as impurities (determined by ICP OES technique).

Cu(II)/peptide complex solutions, Cu^{II}G_n, were freshly prepared by dissolving an appropriate amount of the peptide in 20.0 mL of borate buffer solution followed by

the addition of 0.2 mL of Cu(II) perchlorate. The final pH (7, 8, 9 and 10) was adjusted with 1.0 mol L⁻¹ NaOH or 1.0 mol L⁻¹ HCl solutions. Most of the working solutions (solution A) were: 2.0x10⁻³ mol L⁻¹ Cu^{II}G_n (with 25 % (5.0x10⁻⁴ mol L⁻¹) of peptide in excess over Cu^{II}G_n) in 0.02 mol L⁻¹ borate buffer solution. This working solution contains 2x10⁻⁵ mol L⁻¹ Ni(II). Ni(II) at this level concentration increases the reaction rate and the maximum absorbance at 365 nm.

The borate medium was chosen since the Cu(II) and Cu(III) complexes are stable in this medium. In phosphate buffers the Cu(III) formation is less effective as the anion may displace the peptide ligand.

The final concentration in air saturated solutions after mixing the reactants, with or without sulfite, are indicated in all figures.

Spectrophotometric measurements

Freshly prepared solutions were mixed prior to the experiments. An equal volume (1.0 mL) of sulfite solution was mixed with Cu^{II}G_n solution in borate buffer (1mL, solution A) in a Tandem spectrophotometric cell (optical path length = 0.875 cm).

The UV/VIS spectra were recorded on a HP8453 spectrophotometer, which was also used for kinetic measurements. Stopped flow data were acquired with a Pro-K.2000 Stopped-Flow Mixing Accessory (Applied Photophysics).

In all experiments, air saturated solutions were employed for which the oxygen concentration can be considered 2.8x10⁻⁴ mol L⁻¹.⁸ All UV/VIS spectra were recorded using water as a blank solution, since only the product, Cu^{III}G_n, absorbs in 365 nm.

A pHmeter Metrohm model 713 with a glass electrode (filled with saturated NaCl) was used in the pH measurements. The temperature was kept at 25.0 ± 0.1 °C.

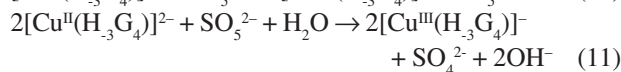
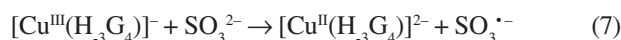
Electrochemical measurements

Rotating ring-disk electrochemical experiments were carried out using an analytical rotator (AFMSRX) connected to an AFCBP1 bipotentiostat (Pine Instrument Company), recording current potential curves typically at a 50 mV s⁻¹ potential scan rate with a data acquisition software made available by the manufacturer (ASWCV2, PineChem). The glassy carbon / glassy carbon ring-disk electrodes (AFMT29) had the following dimensions: disk radius = 0.5613 cm, inner radius of the ring = 0.6248 cm and outer radius of the ring = 0.7925 cm. The electrodes were polished using 0.3 μm alumina before using. A

platinum wire and a Ag/AgCl (saturated NaCl) were used as counter and reference electrodes respectively. During rotating ring-disk electrode (RRDE) experiments, the disk electrode potential was scanned between the limits 0 and 0.6 V, the ring being maintained at 0.1 V to collect the material generated at the disk. 0.1 mol L⁻¹ KNO₃ in borate buffer was used as supporting electrolyte.

Results and Discussion

The Cu^{II}G₄ complex is rapidly oxidized to Cu(III) in the presence of dissolved oxygen, Ni^{II}G₄ (traces) and sulfite, with the simultaneous formation of sulfate. The Cu(III) complex can be followed at 365 nm (ε = 7120 mol⁻¹ L cm⁻¹). Anast and Margerum⁶ proposed an autocatalytic mechanism, where the rate constant depends on the initial Cu(III) concentration. This species reacts with SO₃²⁻ to form the SO₃^{•-} radical (equation 7) and the further reaction with O₂ gives SO₅^{•-} (equation 8). The initiation in the absence of Cu(III) may be due to the disproportionation of Cu(II) to Cu(I) and Cu(III), or Ni(III) and formation of peroxomonosulfate by the reaction (equation 9).⁶ The peroxomonosulfate can then oxidize Cu(II) to Cu(III) (equations 10 and 11).



Surprisingly SO₃²⁻, a reducing agent, induces the autoxidation of the metal ion. It follows that a critical balance between the oxygen and sulfite concentration will control the overall reaction, S(IV) will be oxidized to S(VI) and oxygen is consumed.⁹⁻¹³

Spectrophotometric studies of the sulfite induced autoxidation of Cu^{II}G_n

The absorbance changes at 365 nm of an air saturated solution of (1.0x10⁻³ mol L⁻¹) Cu^{II}G₄ in the absence of sulfite (Figure 1A) indicate that the Cu(III) formation due to the spontaneous oxidation by dissolved oxygen (equation 1) is slow with an induction period of about 10000 s. The length of the induction period depends on the acidity and peptide, for instance at pH 7 (data not reported in the present work), the induction period is longer for G₅ and G₆ complexes, about 5.5 h, while for the G₄ it is about 3.5 h.

In order to evaluate the first fast reaction step, some

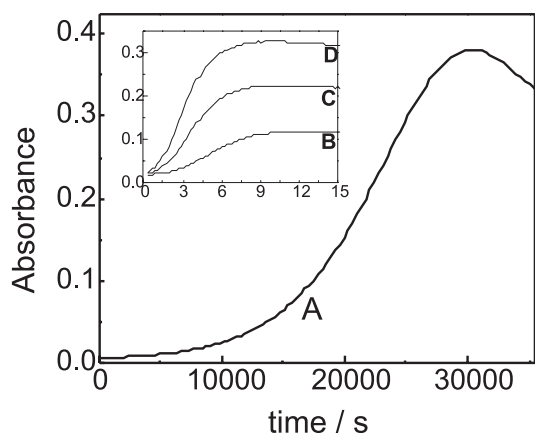


Figure 1. Absorbance changes at 365 nm after addition of sulfite to a $\text{Cu}^{\text{II}}\text{G}_4$ solution. Conditions: $[\text{Cu}^{\text{II}}\text{G}_4] = 1.0 \times 10^{-3} \text{ mol L}^{-1}$; $[\text{G}_4] = 0.25 \times 10^{-3} \text{ mol L}^{-1}$; 0.01 mol L^{-1} borate buffer (pH 9.0). $[\text{SO}_3^{2-}]_i$: (A) = Zero, (B) = 1×10^{-5} , (C) = 2×10^{-5} and (D) = $3 \times 10^{-5} \text{ mol L}^{-1}$.

kinetic measurements were performed on a stopped-flow instrument equipped with an online data acquisition system (Figure 1B, C and D).

Figure 1B, C and D shows the absorbance changes at 365 nm after addition of sulfite to a $\text{Cu}^{\text{II}}\text{G}_4$ solution in borate buffer (pH 9). At the indicated experimental conditions, two maximum peaks appear, one at 365 nm ($\text{Cu}^{\text{III}}\text{G}_4$ complex) and other around 250 nm ($\text{Cu}^{\text{III}}\text{G}_4$ and $\text{Cu}^{\text{II}}\text{G}_4$ complexes). The same spectrum profile was observed for G_5 and G_6 complexes.

At 365 nm only $\text{Cu}^{\text{III}}\text{G}_n$ species absorb^{1, 2, 6} and the absorbance before and after sulfite addition is higher, when compared to the one at 250 nm, and better for analytical purposes.

Figure 1B, C and D shows that in the presence of sulfite and oxygen the $\text{Cu}^{\text{III}}\text{G}_4$ formation is accelerated by sulfite, the induction period still exists (around 3 s) and depends on the initial $\text{Cu}^{\text{III}}\text{G}_4$ concentration (around $10^{-7} \text{ mol L}^{-1}$, due to the spontaneous oxidation with O_2 (equation 1)) and sulfite. This induction period is an evidence of autocatalytic behavior.

Figure 1B, C and D (data at the first 15 seconds) and Figure 2 (data at longer time) shows that one fast reaction occurs at the beginning, with $\text{Cu}(\text{III})$ formation, oxygen consumption and simultaneous oxidation of $\text{S}(\text{IV})$ to $\text{S}(\text{VI})$ (equations 7-11). In some cases, after the fast first step, this process is followed by a slow absorbance increase (the oxidation of $\text{Cu}^{\text{II}}\text{G}_n$ with oxygen still remaining in solution, equation 1, *i.e.*, Figure 2B) and further decrease due to its decomposition (equations 2-4). The formation and decomposition depends on the oxygen concentration and acidity.

$\text{Cu}(\text{III})$ formation, calculated neglecting the induction

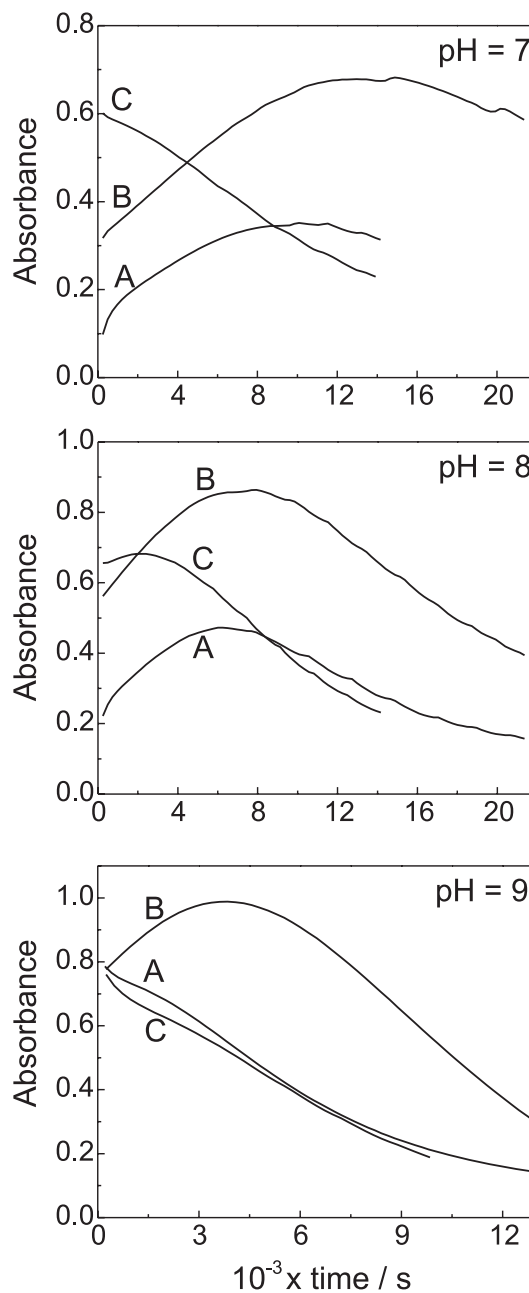


Figure 2. Absorbance changes at 365 nm after addition of $[\text{SO}_3^{2-}] = 8.0 \times 10^{-5} \text{ mol L}^{-1}$ to a $\text{Cu}(\text{II})$ complex solution. $[\text{Cu}^{\text{II}}\text{G}_n] = 1.0 \times 10^{-3} \text{ mol L}^{-1}$; $[\text{G}_n] = 0.25 \times 10^{-3} \text{ mol L}^{-1}$; 0.01 mol L^{-1} borate medium; (A) $[\text{Cu}^{\text{II}}\text{G}_4]$; (B) $[\text{Cu}^{\text{II}}\text{G}_5]$ and (C) $[\text{Cu}^{\text{II}}\text{G}_6]$.

period, can be obtained by the slope of $\ln(\text{Absorbance})_t$ vs. time, for several initial sulfite concentrations, $[\text{SO}_3^{2-}]_i$. The slope increases with $[\text{SO}_3^{2-}]_i$ (Figure 3). According to Coichev and van Eldik⁹ and Atkins¹⁴ the later part the kinetics trace exhibits the maximum rate of $\text{Cu}(\text{III})$ formation. The curves show an induction period and autocatalytic behaviour.

The dependence of $[\text{SO}_3^{2-}]_i$ was studied over a limited

concentration range ($[\text{SO}_3^{2-}]_i = 1.0\text{--}8.0 \times 10^{-5} \text{ mol L}^{-1}$) in air saturated solutions ($[\text{O}_2]_i = 2.8 \times 10^{-4} \text{ mol L}^{-1}$), where $[\text{O}_2]_i$ was in excess compared to $[\text{SO}_3^{2-}]_i$. The slope (Figure 3) is linearly correlated with $[\text{SO}_3^{2-}]_i$ in the cited range.

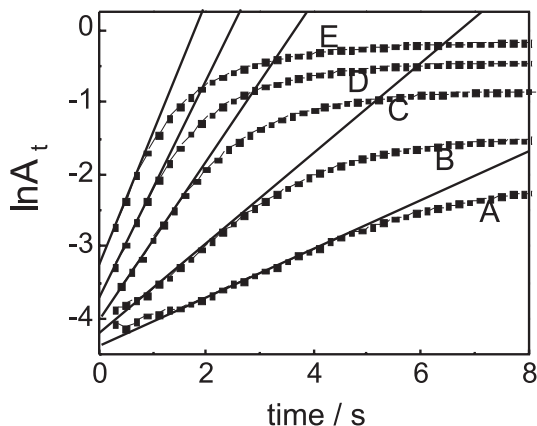


Figure 3. Formation of $\text{Cu}^{\text{III}}\text{G}_4$ as function of $[\text{SO}_3^{2-}]_i$. Conditions: $[\text{Cu}^{\text{II}}\text{G}_4] = 1.0 \times 10^{-3} \text{ mol L}^{-1}$; $[\text{G}_4] = 0.25 \times 10^{-3} \text{ mol L}^{-1}$; 0.01 mol L^{-1} borate buffer (pH 9.0). Ionic strength = 0.1 mol L^{-1} (NaClO_4). $T = 25.0 \text{ }^\circ\text{C}$. Calculated slope (s^{-1}) for each $[\text{SO}_3^{2-}]_i$ (mol L^{-1}): (A) 0.30 ± 0.01 , 1.0×10^{-5} ; (B) 0.57 ± 0.01 , 2.0×10^{-5} ; (C) 1.03 ± 0.02 , 4.0×10^{-5} ; (D) 1.45 ± 0.03 , 6.0×10^{-5} ; and (E) 1.83 ± 0.01 , 6.0×10^{-5} .

Some conclusions can be done regarding to the pH dependence shown in the absorbance vs. time profile (Figure 2), which is a result of Cu(III) formation induced by SO_3^{2-} in the presence of oxygen (equations 7-11) and its further decomposition (equations 2, 4, 5 and 6). The pH dependence might be due to the $[\text{Cu}^{\text{II}}(\text{H}_{-x}\text{G}_n)]^{(1-x)}$ species present in the solution, as a result of variable degree of protonation of the Cu(II) peptides complexes.¹⁵ Besides, shift in the $\text{HSO}_3^-/\text{SO}_3^{2-}$ equilibrium ($\text{pK}_a = 6.3^{16}$) will lead to an increase in the redox rate constant at $\text{pH} > 6$. According to Anast and Margerum,⁶ the maximum net generation of $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_4)]^-$ was found at pH 8, which also corresponds to the maximum rate of formation of $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_4)]^-$, the rate constant value decreasing at both higher and lower pH values.

As can be seen by the pK_a values listed in Table 1, at pH range 7 to 10 the Cu(III) complex formed is present only as the triply deprotonated peptide complex $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_n)]^-$ ($\text{pK}_a_{[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_n)]^-} = 11.4 - 12.1$).

The $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_n)]^{2-}$ must be more reactive than $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_n)]^-$. The pK_a values of $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_n)]^-$ follow the order: $\text{pK}_a_{[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_6)]^-} < \text{pK}_a_{[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_5)]^-} < \text{pK}_a_{[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_4)]^-}$, such as, at pH 9-10 the $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_5)]^{2-}$ and $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_4)]^{2-}$ are the predominant species in solution and the ratio $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_4)]^- : [\text{Cu}^{\text{II}}(\text{H}_3\text{G}_4)]^{2-}$ is about 1:1 at pH 9. This is in agreement with the higher maximum absorbance values obtained at pH 9 (Figure 2). As reported in previous studies, the

decomposition of $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_4)]^-$, the predominant Cu(III) species at pH 7-9, is catalyzed by $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_4)]^-$ ($\text{pK}_a = 9.14^{17}$) (equations 5 and 6),² mainly at lower pH.

Figure 4 shows the effectiveness of Cu(III) complexes formation as the sulfite concentration increases. The maximum generation of Cu(III) peptide is attained at pH 9.

The relative concentrations of SO_3^{2-} and O_2 determine the Cu(III) formation (equations 7 and 9). At low O_2 concentration, sulfite reduces Cu(III) with the formation of $\text{SO}_3^{\cdot-}$ (equation 7) with no further Cu(II) oxidation (equations 10 and 11).

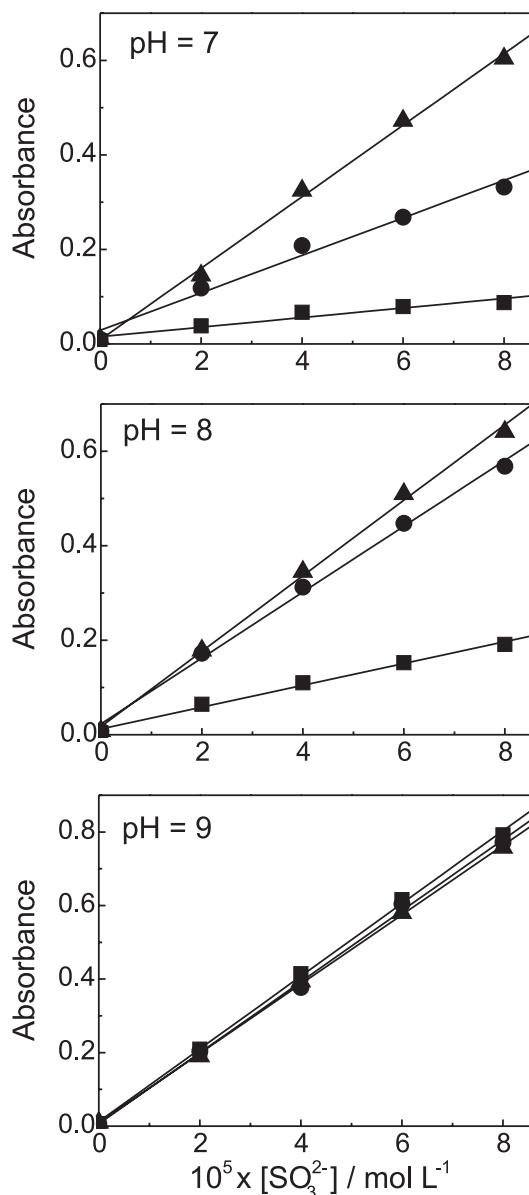


Figure 4. Absorbance at 365 nm (after 190 s of reaction) as a function of added sulfite to a Cu(II) complex solution. $[\text{Cu}^{\text{II}}\text{G}_n] = 1.0 \times 10^{-3} \text{ mol L}^{-1}$, $[\text{G}_n] = 0.25 \times 10^{-3} \text{ mol L}^{-1}$; 0.01 mol L^{-1} borate medium; ■ $[\text{Cu}^{\text{III}}\text{G}_4]$; ● $[\text{Cu}^{\text{III}}\text{G}_5]$; ▲ $[\text{Cu}^{\text{III}}\text{G}_6]$.

For analytical purposes, it is interesting to note that absorbance values at 365 nm are proportional to the initial SO_3^{2-} concentration (Figure 4). A flow injection procedure has already been developed for kinetic studies by measuring absorbance values at 365 nm originated from the formation of $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_4)]$.¹⁸ These kinetic studies showed the catalytic effect of some transition metal ions on the oxidation of S(IV), which are likely to exist in environmental samples.¹⁸ The influence of formaldehyde on the kinetics of the S(IV) oxidation process was also addressed. The well-known property of this aldehyde in the stabilization of S(IV) in non complexant medium containing metallic ions being confirmed.⁸ The pseudo-first-order rate constants of sulfite consumption were determined in the presence of formaldehyde, and lower values were found at higher formaldehyde concentrations ($1.0 \times 10^{-3} \text{ mol L}^{-1}$).¹⁸

Electrochemical studies of the oxidation of $\text{Cu}^{\text{II}}\text{G}_4$ in the absence of sulfite

Some rotating ring-disk (RRD) voltammetry studies were carried out to characterize the formation of $\text{Cu}^{\text{III}}\text{G}_4$. Figure 5 shows a typical steady state voltammogram, recorded at the disk, for a $5.0 \times 10^{-4} \text{ mol L}^{-1} \text{ Cu}^{\text{II}}\text{G}_4$ solution in borate medium (pH 10) also containing $0.1 \text{ mol L}^{-1} \text{ KNO}_3$, at $\omega = 900 \text{ rpm}$; the corresponding signal at the ring (maintained at 0.1 V) is also presented. At this pH $\text{Cu}^{\text{II}}\text{G}_4$ complex in the solution is present partially as $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_4)]$ and predominantly as $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_4)]^{2-}$, some $\text{Cu}(\text{OH})_2$ precipitates, which does not interfere in the electrodes reactions. The $\text{Cu}^{\text{III}}\text{G}_4$ generated complex is present as $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_4)]$. The following equations are responsible for the anodic and cathodic processes respectively:



Since the half-wave potentials for both processes (oxidation and reduction) are very similar, it may be

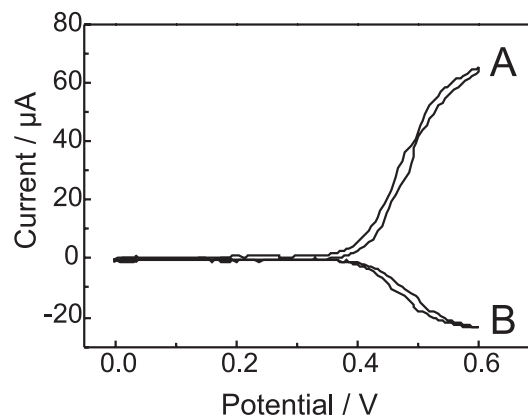


Figure 5. Current responses in RRDE experiments. Solution: $[\text{Cu}^{\text{II}}\text{G}_4] = 5.0 \times 10^{-4} \text{ mol L}^{-1}$, in 0.05 mol L^{-1} borate buffer (pH 10) and $0.1 \text{ mol L}^{-1} \text{ KNO}_3$. (A) voltammogram recorded at the disk (scan rate = 50 mV s^{-1}), (B) at the ring ($E = 0.1 \text{ V}$). Rotation speed = 900 rpm . Potential vs. Ag/AgCl (saturated NaCl) electrode.

concluded that the electrochemical process related to the couple Cu(II)/Cu(III) in a medium containing G_4 is reversible. The half-wave potential found 0.66 V vs. NHE (Figure 5), for all these peptides it is in agreement with the literature (see Table 1).

Figure 6 shows the results of RRDE experiments performed at various rotation speed values in the $100 - 4900 \text{ rpm}$ range. The current signals obtained at relatively high rotation speeds ($> 900 \text{ rpm}$) led to $I_{\text{ring}}/I_{\text{disk}}$ values (N_k collection efficiency) around 0.37, similar results being obtained by using an electroactive species characterized by fast electron transfer with no further chemical steps ($\text{Fe}(\text{CN})_6^{4-}$)²¹ as a probe. Therefore, in the $\text{Cu}^{\text{II/III}}\text{G}_4$ system no chemical transformation involving the electrogenerated $\text{Cu}^{\text{III}}\text{G}_4$ is noticeable at this time window (rotation speed $> 900 \text{ rpm}$), suggesting that the $\text{Cu}^{\text{III}}\text{G}_4$ decomposition could not be monitored at such short time. However, proportionally lower collection efficiency values were obtained at slower rotation rates, *i.e.*, 0.26 at 100 rpm , demonstrating the possibility of a following chemical step involving the Cu(III) complex formed at the disk, which decomposition, according to the literature,^{1,2} is favoured

Table 1. Some Cu(II)/(III) complexes characteristics

Copper-peptide	$\text{Cu}^{\text{III}}\text{G}_n$ ϵ ($\text{mol}^{-1} \text{ L cm}^{-1}$) 365 nm (ref. 20)	pK_a $[\text{Cu}^{\text{II}}(\text{H}_1\text{G}_n)]^a$ (ref. 17)	pK_a $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_n)]^b$	pK_a $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_n)]^c$ (ref. 5)	E (V) vs. NHE $\text{Cu}^{\text{III/II}}(\text{H}_3\text{G}_n)^d$ (ref. 20)
G_4	7120 ± 200	6.8	9.14 (ref. 17)	12.1 ± 0.2	0.63
G_5	7670 ± 300	6.9	8.04 (ref. 19)	11.6 ± 0.1	0.66
G_6	7580 ± 300	---	---	11.4 ± 0.2	0.67

^{a, b} ionization constant for the loss of coordinated peptide hydrogen from $[\text{Cu}^{\text{II}}(\text{H}_1\text{G}_n)]$ and $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_n)]$ complexes, respectively, $25 \text{ }^\circ\text{C}$, ($\mu = 0.1 \text{ mol L}^{-1}$, NaClO_4); ^c for amine hydrogen from $[\text{Cu}^{\text{III}}(\text{H}_3\text{G}_n)]$ complexes, ($25 \text{ }^\circ\text{C}$, $\mu = 1.0 \text{ mol L}^{-1}$, NaClO_4); ^d Potencial Redox: cyclic voltammetric-carbon paste working electrode 100 mV s^{-1} (scan rate).

at higher pH values (also in agreement with the data in Figure 2) and relatively high Cu(III) concentration.

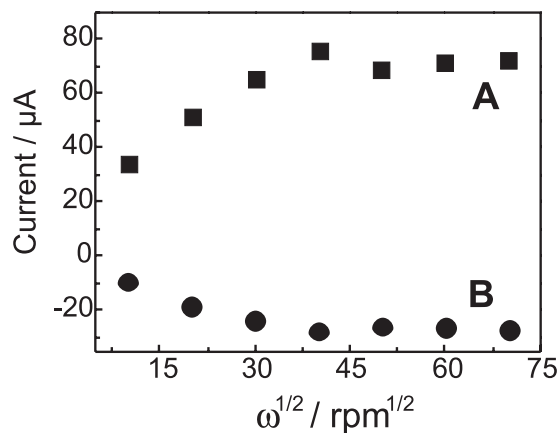


Figure 6. Dependence of the limiting currents on the square root of the rotation rate in RRDE experiments. Solution: $[\text{Cu}^{\text{II}}\text{G}_4] = 5.0 \times 10^{-4} \text{ mol L}^{-1}$ in 0.05 mol L^{-1} borate buffer (pH 10) and $0.1 \text{ mol L}^{-1} \text{KNO}_3$. (A) anodic current measured at the disc, (B) cathodic current measured at the ring ($E = 0.1 \text{ V}$). Collection efficiency values N_k as function of rotation rate to calculate the first order constant. Slope = $k = 4.37 \times 10^{-3} \text{ s}^{-1}$

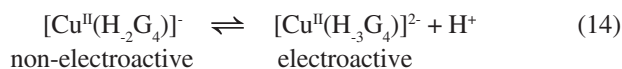
The RRDE experiments performed at various rotation speeds have a particular application in mechanisms study of electrochemical processes happening on two electrodes, it is possible the detection of unstable products (such as $\text{Cu}^{\text{III}}\text{G}_4$ complexes) formed on the disk electrode. The advantage of these electrodes is the transport enhancement of the electroactive species to the ring electrode, leading to higher currents and therefore, to a higher sensibility and reproducibility.^{22, 23}

According to the literature,²⁴ for cases where the intermediate undergoes a first-order homogeneous chemical reaction a plot of N_k as a function of ω^{-1} leads to a straight line whose slope contains the information on the first order kinetic constant (k). From the data shown in Figure 6, k was found to be $4.37 \times 10^{-3} \text{ s}^{-1}$ at 25°C , ionic strength 0.12 mol L^{-1} and pH 10 (borate buffer), from which $t_{1/2}$ was calculated as 159 seconds. The observed first-order rate constant reported in the literature² at pH 10.07 (0.05 mol L^{-1} carbonate buffer, 25°C), $1.12 \times 10^{-3} \text{ s}^{-1}$, is smaller. It can be explained by the different buffer composition and also depends on the concentration of oxygen and Cu(II) complexes (equations 3 and 5), which has being found to be responsible for the catalysis of the decomposition of Cu(III).^{2, 3} Besides the studies of Margerum *et al.*^{2, 4} were carried out in a flow system, which consisted of an electrochemical flow cell, used to generate $\text{Cu}^{\text{III}}\text{G}_4$, followed by spectrophotometric measurements of Cu(III) decomposition. With such experimental design is difficult to

evaluate the life time of short-live product ($\text{Cu}^{\text{III}}\text{G}_4$).

In fact the RRDE experiments allowed the determination of first-order constant of $\text{Cu}^{\text{III}}\text{G}_4$ decomposition, which would not be possible by spectrophotometric measurements. For instance Figure 2A (pH 9) shows the absorbance decrease due to $\text{Cu}^{\text{III}}\text{G}_4$ decomposition. In this case $\text{Cu}^{\text{III}}\text{G}_4$ was chemically generated by the reaction of $\text{Cu}^{\text{II}}\text{G}_4$ with oxygen accelerated by sulfite, with simultaneous consumption of oxygen and sulfite (equation 1). The kinetics of the $\text{Cu}^{\text{III}}\text{G}_4$ decomposition (equations 2-4) proved to be quite complex, and depends on $\text{Cu}^{\text{II}}\text{G}_4$ and oxygen concentration. At the conditions in Figure 2A (pH 9) the oxygen concentration decreases during the global process of Cu(III) formation (equations 7-9) and decomposition (equations 2-4), and it will influence on the rate decomposition. Figure 2 also shows that the kinetic profile depends on the ligand (G_n).

Also from Figure 6, it can be concluded that the I vs $\omega^{1/2}$ dependence does not correspond to a diffusion-controlled process since a clear deviation from a straight line is noticed at more intense hydrodynamic conditions.^{25, 26} This observation may be associated with the existence of an equilibrium between different $\text{Cu}^{\text{II}}\text{G}_4$ species in solution which controls the overall electrode process. As is further discussed, from the data in Figure 7, $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_4)]^{2-}$ is the electroactive species in solution, such as the acid-base equilibrium (equation 14) is displaced by the fast oxidation of the electroactive species. Hence, for relatively long time windows the depletion of the electroactive species by the electrochemical oxidation process may be compensated by the conversion of the non-electroactive species near to the electrode surface. At high rotation speeds this replenishment is not fast enough and the current at the disc is pure kinetically controlled and independent of the rate of mass transport. This conclusion is in agreement with the electrochemical studies reported in literature on the electrooxidation of copper (II) G_4 and G_5 complexes.²⁷



The voltammograms obtained at the 7-10 pH range (Figure 7) clearly show the importance of the acid-base equilibrium of the $[\text{Cu}^{\text{II}}(\text{H}_x\text{G}_n)]^{(1-x)-}$ species in solution. The current increases with the pH, that is, at higher pH the $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_4)]^{2-}$ species concentration also increases, such as it can be concluded that $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_4)]^{2-}$ is the electroactive species in solution. This evidence was also obtained from Figure 6. The conclusion that $[\text{Cu}^{\text{II}}(\text{H}_3\text{G}_n)]^{2-}$ must be more reactive than $[\text{Cu}^{\text{II}}(\text{H}_2\text{G}_n)]^-$ was already discussed from the spectrophotometric results presented in Figure 2.

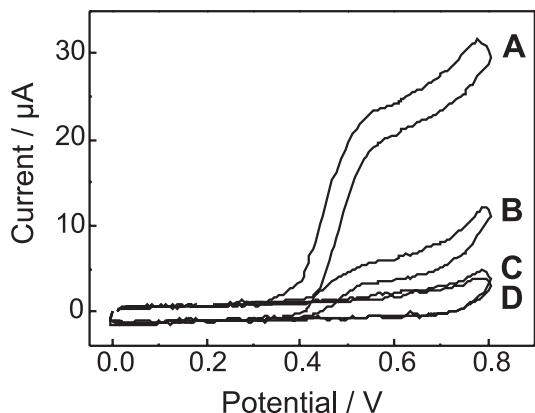


Figure 7. Voltammograms of air saturated solutions containing $2.0 \times 10^{-4} \text{ mol L}^{-1} \text{ Cu}^{\text{II}}\text{G}_4$, in 0.05 mol L^{-1} borate medium (pH 10) and $0.1 \text{ mol L}^{-1} \text{ KNO}_3$. (A) pH 10; (B) pH 9; (C) pH 8 and (D) pH 7. Working electrode = disk, rotation speed = 1000 rpm, scan rate = 50 mV s^{-1} . Potential vs. Ag/AgCl (saturated NaCl) electrode.

Electrochemical studies of the sulfite induced autoxidation of $\text{Cu}^{\text{II}}\text{G}_4$

The formation of a $\text{Cu}^{\text{III}}\text{G}_4$ complex when sulfite is added to a $\text{Cu}^{\text{II}}\text{G}_4$ solution under aerobic conditions was investigated electrochemically as shows Figure 8, where voltammograms were recorded at the rotating disc electrode ($\omega = 900 \text{ rpm}$). After the addition of sulfite a voltammogram with an anodic and a cathodic component is obtained, confirming that both $\text{Cu}^{\text{II}}\text{G}_4$ and $\text{Cu}^{\text{III}}\text{G}_4$ complexes exist in solution. A comparison of the voltammograms presented in Figures 5 and 8 suggests that the $\text{Cu}(\text{III})$ complex formed as a consequence of the induced sulfite oxidation is the same species electrochemically generated from $\text{Cu}^{\text{II}}\text{G}_4$.

Preliminary spectrophotometric studies have shown that there is a linear dependence between the $\text{Cu}^{\text{III}}\text{G}_4$

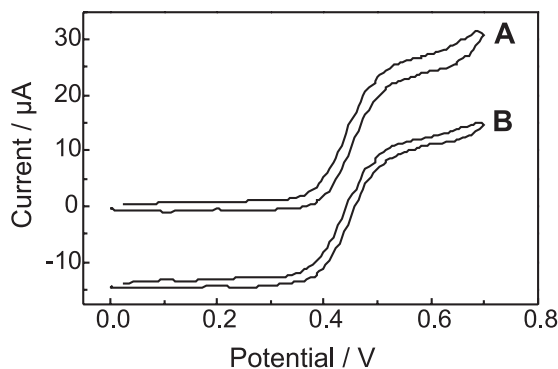


Figure 8. Voltammograms of an air saturated solution containing $2.0 \times 10^{-4} \text{ mol L}^{-1} \text{ Cu}^{\text{II}}\text{G}_4$, in 0.05 mol L^{-1} borate buffer (pH 10) and $0.1 \text{ mol L}^{-1} \text{ KNO}_3$, before (A) and after (B) addition of $2.0 \times 10^{-4} \text{ mol L}^{-1}$ sulfite (final concentration in solution). Working electrode = disk, rotation speed = 900 rpm, scan rate = 50 mV s^{-1} . Potential vs. Ag/AgCl (saturated NaCl) electrode.

concentration and the amount of sulfite added to the solution when experiments are performed in conditions of excess of both $\text{Cu}^{\text{II}}\text{G}_4$ and oxygen in respect to sulfite.¹⁸ From an analytical point of view this relationship could be useful for the development of an indirect method for sulfite. At the experimental condition reported in the Figure 4 (pH 9) the estimated detection limit was $2.0 \times 10^{-6} \text{ mol L}^{-1}$. By flow injection analysis this limit was $7.0 \times 10^{-6} \text{ mol L}^{-1}$.¹⁸

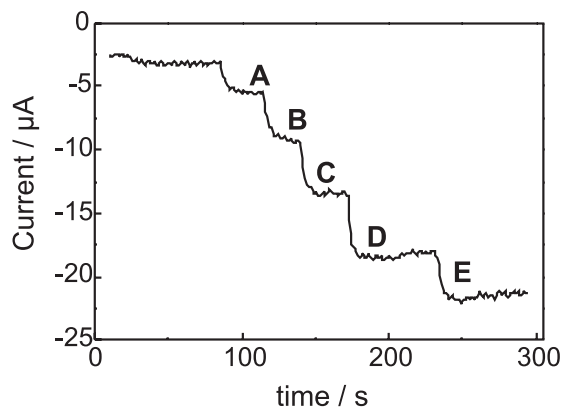


Figure 9. Current signals measured at the disk polarized at 0.1 V for a $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ Cu}^{\text{II}}\text{G}_4$ solution, in 0.05 mol L^{-1} borate buffer (pH 10) and $0.1 \text{ mol L}^{-1} \text{ KNO}_3$, during consecutive additions of sulfite to give the following final concentrations: (A) 6.0×10^{-5} , (B) 1.1×10^{-4} , (C) 1.8×10^{-4} , (D) 2.7×10^{-4} and (E) $3.7 \times 10^{-4} \text{ mol L}^{-1}$. RRDE at 900 rpm.

Owing to the advantages of the amperometric detection as its excellent signal to noise ratio²⁶ (since the capacitive current is virtually zero at constant potential), some experiments were performed at $E = 0.1 \text{ V}$, where the $\text{Cu}^{\text{III}}\text{G}_4$ species is electroactive (Figure 8). Accordingly, Figure 9 shows the results for successive injections of sulfite solution to an aerated solution containing $\text{Cu}^{\text{II}}\text{G}_4$. The fast increase in the cathodic current after addition of relatively low amounts of sulfite to the working solution indicates the feasibility of an indirect method for determination of sulfite with electrochemical detection of the $\text{Cu}^{\text{III}}\text{G}_4$ compound chemically generated. The proposed indirect approach is particularly relevant because the direct electrooxidation of sulfite at bare electrode surfaces has been reported to be irreversible.²⁸ Hence, drastic pre-treatment procedures combined with very positive potentials^{29, 30} or the modification of the electrode surface by incorporating catalytic layers^{31, 32} have been suggested to enhance the sensitivity. In this way, owing to the good results noticed in Figure 9 the proposed method is being fitted for a flow injection configuration and the preliminary results are very promising.

Interesting mechanistic studies⁹ and development of alternative analytical methods^{10-13, 33} based on metal ion catalyzed reactions, for determination of S(IV) in food, environmental samples and degraded hexafluoride have been already reported by our group.

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