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Kinetic studies of carbohydrate oxidation catalyzed by novel isatin–Schiff base copper(II) complexes

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Abstract

With the aim of elucidating the mechanism of oxidative processes initiated by copper(II)–carbohydrate interactions, new isatin–Schiff base copper(II) complexes, [Cu(*isaen*)H_2O]$ClO_4 \cdot 2H_2O$ (**1**), and [Cu(*isaepy*)$_2$]$(ClO_4)_2 \cdot 2H_2O$ (**2**), where *isaen* = *N*-[(3-indolin-2-one)]-1,3-ethylenediamine and *isaepy* = *N*-[(3-indolin-2-one)]-2-aminoethyl pyridine were isolated, and characterized by elemental analyses, UV–vis, FTIR, EPR, and molar conductivity measurements. These imine ligands are capable of modifying selectively the environment of the copper(II) ion in a pH controlled process, through keto–enolic equilibria similar to those occurring with carbohydrates. Therefore, the obtained complexes had their catalytic activity in the oxidation of common carbohydrates (glucose, fructose and galactose) by molecular oxygen, compared to that of an analogous complex [Cu(*isapn*)]$(ClO_4)_2$ (**3**), previously reported. The determined rate law, from kinetic curves of oxygen consumption, showed a pseudo-first-order dependence both on the catalyst and substrate concentrations, followed by a saturation effect, for all the compounds studied. Further, the pH profile indicated that reaction occurs significantly only in very alkaline medium (pH $\geq$ 10), and some influence of ionic strength (controlled by carbonate buffer) was also verified. The participation of very reactive intermediates in the oxidative degradation of the substrates was monitored by EPR spin trapping, while final products were identified by capillary electrophoresis. An extensive mechanism is proposed, explaining new kinetic studies as well as earlier data.

Keywords: Copper(II) complexes; Isatin; Catalysis; Carbohydrate oxidation; Reactive oxygen species

1. Introduction

Carbohydrate oxidation by molecular oxygen has been studied for many decades [1–3], since some of the oxidized products formed, gluconic acid for instance, have a significant economic impact [4]. Most of these carbohydrate oxidation reactions are performed in mild conditions, in the presence of supported palladium and platinum catalysts, which are environmentally less dangerous, and that can replace stoichiometric oxidations with mineral oxidizing agents, in more severe conditions [5].

On the other hand, common pentose and hexose oxidations are implicated in many diseases, such as diabetes mellitus, cataracts, arteriosclerosis, Alzheimer, and aging in general, leading to protein glycation [6,7], where different intermediates can cause severe injury. Enolization of Amadori compounds (1-amino-1-deoxy-2-ketose derivatives) usually formed in this process is thought to play an important role, contributing to the generation of oxygen free radicals, and their subsequent oxidative damage to proteins [8]. Particularly, copper(II) ions were verified to markedly increase the rate of those oxidative processes [9–11].

Many earlier studies indicated that the initial formation of a carbohydrate enediol, favored in alkaline medium, is essential for its further oxidation in the presence of metal ions [12–14]. However, most of those studies were carried out in experimental conditions very drastic, using copper coordinated to weak ligands (aqua, phosphate, tartrate, citrate), and

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at very high concentrations (10^{-4} to 10^{-3} mol dm^{-3}), when the metal is simultaneously precipitated as copper(I) oxide [12–14].

Therefore, systematic studies that selectively change the structural and electronic features in copper complexes, and modulate their effect on catalytic properties could help to clarify the reactivity of those metal centers toward carbohydrate oxidation.

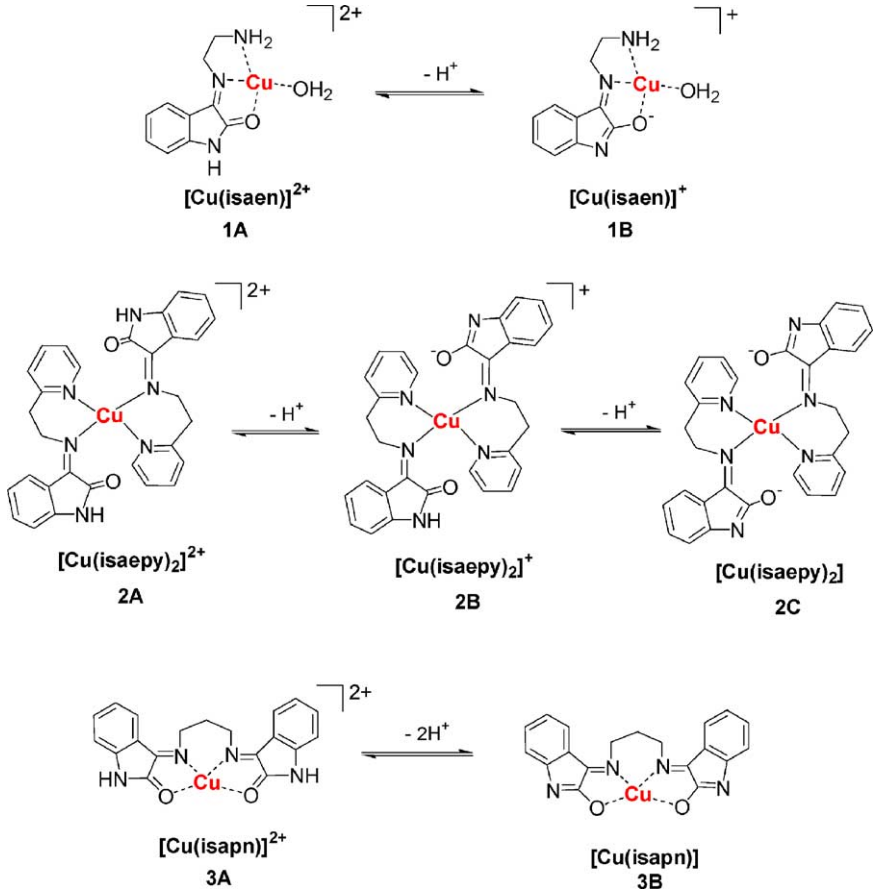
With the aim of elucidating the mechanism of oxidative processes initiated by copper(II)–carbohydrate interactions, we are studying some copper(II) complexes with different ligands, capable of modifying the environment of the copper ion, and influencing its reactivity.

In a previous work, we have studied the oxidative degradation of uronates derived from glucose, catalyzed by gluconate–copper(II) complexes, and occurring in alkaline medium by a mechanism propagated by an enediol radical [15]. More recently, we focused on some copper(II) complexes with ligands derived from isatin, an endogenous indole that exhibit a keto–enolic equilibrium similar to that showed by sugars (shown in Scheme 1), and that are also able of generating reactive oxygen species. This internal equilibrium can modulate the reactivity of the metal center related to it, facilitating its interaction with carbohydrate substrates.

2. Experimental

2.1. Materials

Carbohydrate substrates used in the kinetic studies, D(+)glucose (99%), and D(−)fructose (99%), were obtained from Merck, and D(+)galactose (99%) from Sigma Chemical Co. Isatin (98%), 1,3-diaminopropane, ethylenediamine, 2-aminoethyl pyridine and catalase (from bovine liver) were purchased from Aldrich, and Cu,Zn SOD from bovine erythrocytes was from Sigma Chemical Co. DMPO (2,2′-dimethyl-pyrroline-*N*-oxide, from Aldrich) was previously purified as recommended [16]. DBNBS (3,5-dibromo-4-nitrosobenzene-sulfonate) was prepared from 3,5-dibromosulphanilic acid and glacial acetic acid, by procedure already described in the literature [17]. The standards xylonic acid, formic acid, glyceric acid, glycolic acid and cetyltrimethyl ammonium chloride (CTAB), used in capillary electrophoresis experiments were purchased from Aldrich (Milwaukee, WI, USA). The reagent 3,5-dinitrobenzoic acid (3,5-DNB) was purchased from Merck (Darrmstadt, Germany). Standard stock solutions were prepared at 1000 mg/L and stored in freezer until analysis. Electrolyte stock solutions of 3,5-DNB at 20 mmol dm^{-3} concentration and CTAB at 10 mmol dm^{-3} concentration were prepared. The



Scheme 1. Structure of complexes studied, and equilibria involving the corresponding keto–enolic tautomers.

working electrolyte was comprised of 10 mmol dm^{-3} 3,5-DNB and 0.2 mmol dm^{-3} CTAB, adjusted to pH 3.7 with a 0.1 mol dm^{-3} HCl solution. All other reagents were of analytical grade, purchased from different sources, and the solvents were of chromatographic purity. Deionized water from a Milli-Q (Millipore, Bedford, MA, USA) or a Barnstead D 4700 apparatus was used in the preparation of all solutions.

2.2. Syntheses of the copper(II) complexes

Caution: Although the prepared species were shown to be very stable, perchlorate salts of metal complexes with organic ligands are potentially explosive, and should be very carefully handled, only in small amounts.

Copper(II) isatin–imine and diimine complexes were synthesized by condensation reaction of the amine ligands ethylenediamine (*en*), or 2-aminoethyl pyridine (*epy*) with isatin (*isa*), followed by coordination to copper(II) ions, added as perchorate salt, according to general procedure in the literature [18], with suitable modifications. The analogous complex (compound **3**, in Scheme 1) obtained from 1,3-diiminepropane (*pn*) and isatin has been recently investigated [19].

2.2.1. *[Cu(isaen)(H_2O)]ClO_4·2H_2O* (**1**)

Isatin (5 mmol) were dissolved in ethanol (0.050 dm^{-3}) and the solution pH adjusted to 5.5 with concentrated chloridric acid. Freshly distilled ethylenodiamine (5 mmol) was slowly added to this solution, maintained under stirring for 4 hours. The reaction was monitored by TLC using the mixture EtOAc/CH_2Cl_2 (20%, v/v) as eluent. After this period, yellow crystals formed were filtered off, and washed with cooled ethanol and ethyl ether. This compound was then dried in vacuum for 1 hour, protected from light. Yield: 78% (738 mg, 3.9 mmol). Afterwards, the ligand prepared was dissolved in ethanol (0.020 dm^{-3}), and the pH was adjusted to 7.5–8 with sodium acetate solution. Copper(II) perchlorate hexahydrated (3.9 mmol) dissolved in water (0.005 dm^{-3}) was slowly added, under constant stirring. Immediately, a fine brown precipitate was formed, and kept under stirring for more 2 hours. The solution was then cooled, and the precipitate was filtered, and washed with cooled ethanol and ethyl ether. The final copper(II) complex was dried in vacuum, and recrystallized from ethanol/ethyl ether solution. Yield: 90% (1.29 g, 3.5 mmol). Found: C, 30.40; H, 3.95; N, 10.89%. Calc. for $C_{10}H_{12}N_3O_2$ $Cu(ClO_4)$·2H_2O: C, 29.64; H, 3.97; N, 10.47%. Λ_M = 107.8 S cm^2 mol^{-1} in water solution, and 55.4 S cm^2 mol^{-1} in methanolic solution (both at 298 K). IR (cm^{-1}, KBr): 3433 m, ν(OH); 3318 and 3272 l, ν(NH); 2954–2894 w, ($C_{sp^2}H$) and ν(C=C); 1709 s, ν(CO); 1590 s, ν(C=N); 1618 s, ν(C–N); 1574 s, ν(N–H); 1449 m, ν(C–O); 1088 and 629 s, ν(Cl–O). MS (ESI+): *m/z* = 293.03 [MW = 293.81, in acetonitrile; $C_{10}H_{11}N_3O_2$ Cu·CH_3CN]; 295.03 [isotopic pattern ($Cu^{63/65}$) monocation]; 269.03 [MW = 269.77, in CH_3OH: $C_{10}H_{12}N_3OCu$); 252.01 [MW = 252.03, in CH_3CN, fragment $C_{10}H_{11}N_3OCu$]; 236.96 (MW = 235.73, in CH_3CN, fragment $C_{10}H_8N_2OCu$]; 224.01 [MW = 224.75, in CH_3CN, fragment $C_9H_{11}N_3Cu$].

2.2.2. *[Cu(isaepy)$_2$]$(ClO_4)_2$·2H_2O* (**2**)

This compound was analogously prepared, by the same procedure described for compound **1**. In this case, the corresponding ligand was obtained from isatin (5 mmol) and 2-aminoethylpyridine (5 mmol) with 74% yield (930 mg, 3.7 mmol), from which brown crystals were collected, after metallation under constant stirring, during 12 hours, in ethanolic solution, at pH 7.5–8. This precipitate formed was filtered off, washed with cooled ethanol and ethyl ether, and dried in vacuum for 6 hours. Yield: 86% (2.44 g, 3.2 mmol). Found: C, 45.69; H, 3.52; N, 10.08%. Calc. for $C_{30}H_{26}N_6O_2Cu(ClO_4)_2$·2$H_2O$: C, 44.98; H, 3.76; N, 10.45%. Λ_M = 110.0 S cm^2 mol^{-1} in water solution, and 56.0 S cm^2 mol^{-1} in methanolic solution (both at 298 K). IR (cm^{-1}, KBr): 3438 m, ν(OH); 3354 w, ν(NH); 3081–2995 w, ν($C_{sp^2}H$) and ν(C=C); 1725 s, ν(CO); 1614 s, ν(C=N); 1574 s, ν(N–H); 1444 m, ν(C–O); 1094 and 624 s, ν(Cl–O). MS (ESI+): *m/z* = 565.12 [MW = 565.15, in CH_3CN, fragment $C_{30}H_{26}N_6O_2Cu$]; 563.12 [isotopic pattern ($Cu^{63/65}$) monocation]; 312.02 [MW = 312.81, in CH_3CN, fragment $C_{15}H_{11}N_3OCu$].

2.3. Physical measurements

Elemental analyses were performed at the Central Analítica of our Institution, using a Perkin–Elmer 2400 CHN Elemental Analyser. The mass spectrometric measurements were performed using a high resolution hydrid quadrupole (*Q*) and orthogonal time-of-flight (Tof) mass spectrometer (QqTof from Micromass, UK) operating in the positive ion electrospray ionization mode. The nebulizer temperature was 200 °C, and the cone voltage was 40 V. Infrared spectra of the complexes obtained were recorded in a BOMEM 3.0 instrument, in the range 4000–400 cm^{-1}, using KBr pellets. Electronic spectra were registered in a Beckman DU-70 spectrophotometer, or an Olis modernized-Aminco DW 2000 instrument, with termostated cell compartment. EPR spectra were recorded in a Bruker EMX instrument, operating at X-band frequency, using standard Wilmad quartz tubes, at 77 K. DPPH (α,α′-diphenyl-β-picrylhydrazyl) was used as frequency calibrant (g = 2.0036) with samples in frozen methanol/water (4:1, v/v) solution, at 77 K. Usual conditions used in these measurements were 2.00×10^4 gain, and 15 G modulation amplitude. The pH of the solutions was monitored in a Digimed DMPH-2 instrument, coupled to a combined pH electrode, from Ingold or Radiometer. Appropriate buffer solutions were used to calibrate the instrument. Conductivity experiments with the complexes studied (in 1 mmol dm^{-3} aqueous solution) were carried out in a Digimed DM-31 instrument, using a 10.0 mmol dm^{-3} KCl solution as standard (specific conductivity = 1412.0 μS cm^{-1} for aqueous solution or 0.141 μS cm^{-1} for organic solutions, both at 298 K) [20]. All capillary electrophoresis experiments

were conducted in a P/ACE 5510 system, from Beckman Coulter Instruments (Fullerton, CA, USA), equipped with a diode array detector set at 254 nm, and a temperature control device set at 25 °C. The data acquisition and treatment software was supplied by the manufacturer (Beckman P/ACE System Gold® Software). Fused-silica capillaries with dimensions of 57 cm total length (50 cm effective length), 75 μm i.d. and 365 o.d. were used. Samples were injected hydrodynamically, at 0.5 psi pressure during 3 s. The system was operated under constant voltage conditions and reverse polarity (−25 kV). Working electrolyte solutions were prepared fresh daily and membrane filtered prior to use. At the beginning of the day, the capillary was conditioned by pressure flushes of 1 mol dm^{-3} NaOH solution (5 min), deionized water (5 min) and electrolyte solution (10 min) followed by a electrokinetic flush of electrolyte solution (−25 kV during 10 min). In between runs, the capillary was just replenished with fresh electrolyte solution (2 min flush).

2.4. Kinetic studies

The catalyzed oxidation of D(+)glucose, D(−)fructose and D(+)galactose was performed under pseudo-first-order conditions. Kinetic measurements were carried out in a GILSON oxygraph apparatus (Medical Electronics Inc. USA), monitoring the oxygen consumption during the catalytic oxidation of carbohydrates. A Clark platinum electrode was used as O_2 probe, with internal reference Ag/AgCl, and YSI membrane (from Yellow Spring Instruments Co.), using saturated KCl solution between the electrode and the membrane. A standard cell was utilized in these experiments, with total volume of 1.8 mL, closed by a capillary cap with no oxygen exchange through the atmosphere, and wrapped in a thermostat bath at (298.0 ± 0.1) K. Sodium dithionite was used to calibrate the oxygraph. The solubility of oxygen was taken as 244 μmol dm^{-3} O_2 dissolved in pure water, at 298 K [21]. The experiments were performed in alkaline medium, using 250 mmol dm^{-3} carbonate buffer (Na_2CO_3/NaOH), at pH (12.0 ± 0.2), previously treated with Chelex resin to eliminate any metal traces. The stock solution of catalysts was 1.0 mmol dm^{-3} in carbonate buffer, and adequate volumes were added to oxygraph cell, to make the suitable final concentration of catalyst in the range 10^{-6} to 10^{-4} mol dm^{-3}. The stock solutions of carbohydrates were usually 0.10 mol dm^{-3} in water, and a constant volume of 18 μL was added to oxygraph cell, to give the final concentration of 1.00 mmol dm^{-3}. In the experiments with varied concentrations of the carbohydrate, a 2.0×10^{-2} mol dm^{-3} stock solution was used, adding adequate volumes to obtain final concentrations from 10^{-3} to 10^{-2} mol dm^{-3}. When pH values were varied, NaOH solutions were used to adjust the pH in the range 8.0 to 12.0. All the measurements were done at least in triplicate and deviations in values of rate constants were ≤5% as indicated, estimated by repeated experiments.

3. Results and discussion

3.1. Characterization of the catalysts prepared

New isatin–Schiff base copper(II) complexes, [Cu(*isaen*)(H_2O)]$ClO_4 \cdot 2H_2O$ (**1**) and [Cu(*isaepy*)$_2$]$(ClO_4)_2 \cdot 2H_2O$ (**2**) (shown in Schemes 1 and 2), were isolated from ethanol solution, and characterized by elemental analyses, conductivity measurements, UV–vis, IR and EPR spectroscopy. Their spectroscopic properties were then compared to those of compound **3**, [Cu(*isapn*)]$(ClO_4)_2$, previously described [19], in order to correlate structural features and catalytic activity in the oxidation of carbohydrates.

3.1.1. Infrared spectra

The spectra of [Cu(*isaen*)(H_2O)]$ClO_4 \cdot 2H_2O$ (**1**) and [Cu(*isaepy*)$_2$]$(ClO_4)_2 \cdot 2H_2O$ (**2**), registered in KBr pellets, showed the expected characteristic bands of imine and diimine compounds, as indicated in experimental section. The bands observed at 3318 and 3272 cm^{-1} for **1**, and 3354 cm^{-1} for **2** were attributed to ν(N–H), and those around 3081–2954 cm^{-1}, for both complexes, to $\nu(C_{sp^2}–H)$ and $\nu(C_{sp^3}–H)$, while the corresponding bending were verified in the 990–750 cm^{-1} range. The characteristic ν(C=N) was found at 1590 cm^{-1} for **1**, and 1614 cm^{-1} for **2**, being observed at 1591 cm^{-1} for **3**. A strong band found around 1700 cm^{-1} is characteristic of ν(C=O) from five-member lactam groups, and that around 1590–1570 cm^{-1} of ν(N–H). A characteristic broad band in the ν(O–H) range was observed, indicating the presence of water molecules, or that some amount of the enol-form of these complexes was also present. Additional bands observed around 1090 and 625 cm^{-1} are characteristic of non-coordinated perchlorate ions. Based on these spectroscopic data and the elemental analysis results, complex **1** was isolated predominantly in the enol-form (structure **1B**, in Scheme 1), while for complex **2** the keto-form was prevalent (structure **2A**, in Scheme 1), in the solid state.

3.1.2. Conductivity measurements

The conductivity measurements of the studied complexes in deionized water or methanol, at 298 K, as indicated in experimental section, are consistent with a 1:1 electrolyte, for both the compounds **1** and **2** [20]. The pH measured in 1 mmol dm^{-3} aqueous solution was 5.6 and 5.9–6.0, respectively, for compounds **1** and **2**, indicating the occurrence of equilibria very dependent on protonation. Here a tautomeric form different from that isolated in solid state is predominant, and ascribable probably to structures **1B** and **2B**, respectively, for **1** and **2**, in Scheme 1. Enolic equilibrium of this type, also observed with the free indole, depends strongly on the pH of the solution.

3.1.3. Electronic and EPR spectra

Electronic spectra of [Cu(*isaen*)(H_2O)]$ClO_4 \cdot 2H_2O$ (**1**) and [Cu(*isaepy*)$_2$]$(ClO_4)_2 \cdot 2H_2O$ (**2**) were carried out in

Table 1
EPR parameters for the complexes prepared in frozen methanol/water (4:1, v/v) solution, at 77 K

Compound	EPR parameters				
	$g_{\perp}$	$g_{\|\|}$	$A_{\perp}$ (G)	$A_{\|\|}$ (10^{-4} cm^{-1})	$g_{\|\|}/A_{\|\|}$ (cm)
[Cu(*isaen*)H_2O]ClO_4 (**1**)					
pH ≈ 5	2.059	2.278	28.5	225	102
pH 11	2.057	2.234		192	116
pH < 3	2.083	2.421		131	185
[Cu(*isaepy*)$_2$]$(ClO_4)_2$ (**2**)					
pH ≈ 5	2.082	2.503	15.8	132	188
pH 11	2.054	2.236		186	120
pH < 3	2.082	2.425		130	187
[Cu(*isapn*)]$(ClO_4)_2$ (**3**)[a]					
pH ≈ 5	2.091	2.301	14.2	126	183
pH 11	2.065	2.240		189	118
pH < 3	2.086	2.420		130	186

[a] Ref. [19].

aqueous solution, and showed characteristic bands expected for this type of copper(II) complexes [22]. The corresponding maximum wavelengths were observed at 207 and 242 nm (ε = 9.1 and 8.9 × 10^3 mol^{-1} dm^3 cm^{-1}) for **1**, and at 206 and 247 nm (ε = 1.3 and 5.6 × 10^3 mol^{-1} dm^3 cm^{-1}) for **2**; corresponding internal ligand transitions (n → π, or n → π*) detected at 298 nm (ε = 2.2 × 10^3 mol^{-1} dm^3 cm^{-1}), and 456 nm, (ε = 536 mol^{-1} dm^3 cm^{-1}), for compound **1**, and at 406 nm (ε = 1.2 × 10^3 mol^{-1} dm^3 cm^{-1}), for **2**, were attributed to LMCT transitions (π → dπ). The characteristic d–d band for **1** appears at 675 nm (ε = 188 mol^{-1} dm^3 cm^{-1}), and it was not observed for compound **2**, mostly because of its very low solubility in water. In a previous related work [23], some metal complexes with isatin-3-hexamethyleneiminylthiosemicarbazone ligand were reported, showing very similar data, typically with intra-ligand bands at 261, 289 and 361 nm, LMCT bands at 400 and 448 nm, and the d → d band around 601 nm. For our former prepared complex **3**, [Cu(*isapn*)]$(ClO_4)_2$, the corresponding maximum wavelengths were observed, respectively, at 242 nm; 303 and 402 nm, with a shoulder at 434 nm, while the d–d band appears at 688 nm, in DMF solution [19].

EPR spectra of complexes **1** and **2** in frozen methanol:water (4:1, v/v) solutions showed a characteristic profile of an axial environment around the copper(II) center, with a $g_{||} > g_{\perp}$ (as shown in Table 1).

The empirical ratio $g_{||}/A_{||}$ is frequently used to evaluate tetrahedral distortions in tetragonal structures of copper(II) compounds, where the ratio $g_{||}/A_{||}$ close to 100 cm indicates a roughly square-planar or tetragonal structure around the copper(II) ion, and values from 170 to 250 cm are indicative of a distorted tetrahedral symmetry around the copper(II) ion [24]. Based on these data, complex **1** exhibited a more tetragonal symmetry with the unpaired electron in the dx^2–y^2 orbital, while complex **2** showed geometry more closely to a distorted tetrahedron. In compounds of this type, with a ligand derived from isatin, changes in the ratio $g_{||}/A_{||}$ with the pH suggest substantial modification in the environment of copper(II) center, as a consequence of the keto–enolic equilibrium presented in the ligand structure (Scheme 1), as already observed with compound [Cu(*isapn*)]$(ClO_4)_2$ (**3**). In this case, the ratio $g_{||}/A_{||}$ changes from 188 cm in acid pH to 118 cm in basic pH, indicating a geometry change from a distorted tetrahedron to a more square-planar or tetragonal structure around the copper(II) ion [24]. Similar data were obtained for the new complexes studied (see Table 1). If the EPR parameters at pH 5 and 11 are compared, more significant changes were observed for complex **2** than for complex **1**. Therefore, depending on the pH one of the species predominates in solution, that is, the keto-form at more acidic pH, and the enol-form in very alkaline medium. From these data, it was also verified that at pH < 3 all the studied complexes seem to be decomposed to a common species, exhibiting the same EPR parameters.

Table 2
Rate constants observed in glucose, fructose and galactose oxidation by molecular oxygen, catalyzed by the complexes studied

Compound	k_{obs} (10^{-2} s^{-1})		
	Glucose	Fructose	Galactose
[Cu(*isaen*)H_2O]ClO_4 (**1**)	1.91	13.7	1.81
[Cu(*isaepy*)$_2$]$(ClO_4)_2$ (**2**)	2.72	2.75	1.41
[Cu(*isapn*)]$(ClO_4)_2$ (**3**)	1.66	12.4	0.52

Reaction conditions: [Substrate] = 1.11 × 10^{-3} mol dm^{-3}, and [OH^-] = (2.2 ± 0.8) × 10^{-2} mol dm^{-3}, corresponding to pH (12.0 ± 0.2), at (25.0 ± 0.2) °C.

3.2. Kinetic studies

Kinetic experiments monitoring the oxygen consumption in the oxidation of fructose, glucose or galactose showed that the studied complexes [Cu(*isaen*)(H_2O)]ClO_4 (**1**) and [Cu(*isaepy*)$_2$]$(ClO_4)_2$·$2H_2O$ (**2**) can act as a catalyst in this reaction. In previous studies, the catalytic activity of a similar complex, also exhibiting an isatin–Schiff base ligand, [Cu(*isapn*)]$(ClO_4)_2$ (**3**) has been already observed [19]. Reactions were carried out in aqueous solution, at pH 12.0 controlled by carbonate buffer (250 mmol dm^{-3}), at (25.0 ± 0.2) °C. Experimental curves of O_2 consumption versus time were shown in Fig. 1, for glucose, fructose, and galactose as substrates, in the presence of all the studied complexes. These curves indicated that fructose was the most reactive substrate, and that major differences in the catalytic activity were observed in the case of galactose. A first-order dependence of the initial reaction rate with the catalyst concentration was observed for all substrates, followed by a saturation effect at [catalysts] > 2.0 × 10^{-5} mol dm^{-3} or >1.0 × 10^{-4} mol dm^{-3}, respectively, to fructose and galactose oxidation.

Based on these data the corresponding observed rate constant for these catalysts was calculated, as shown in Table 2.

The variation of the initial rate with concentrations of substrate and OH^- was also verified, for the fructose oxida-

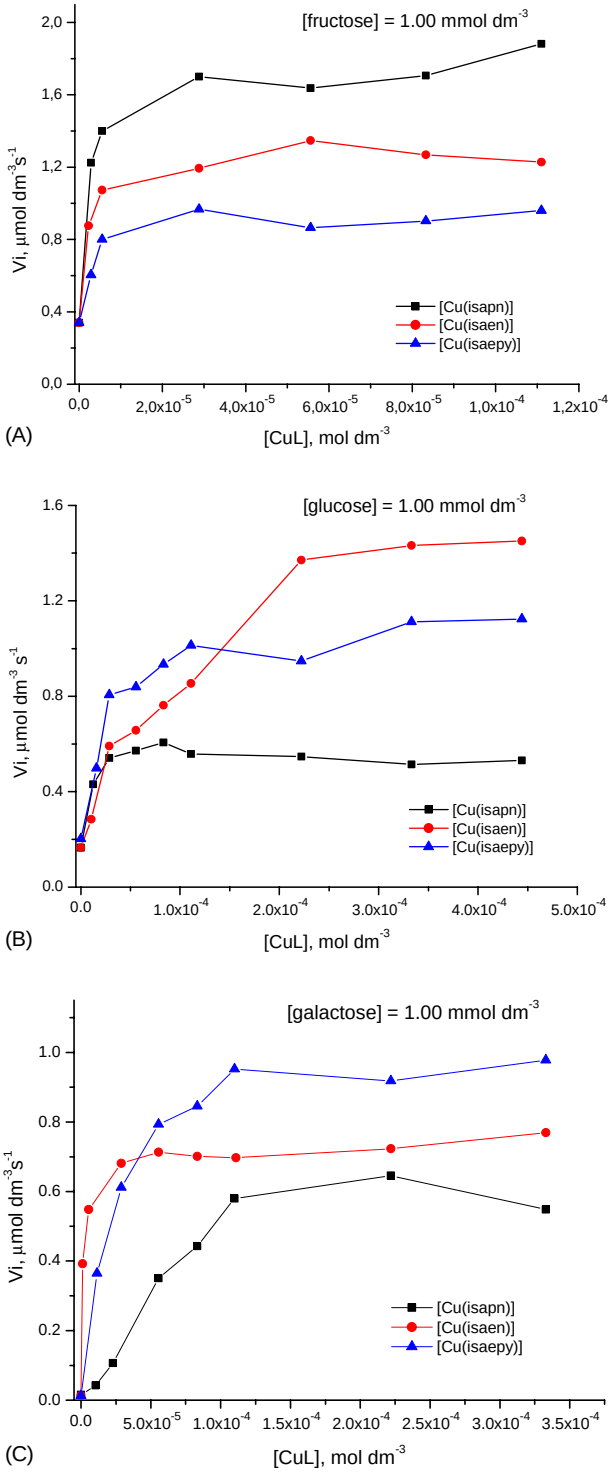


Fig. 1. Oxygen consumption in carbohydrate oxidation catalyzed by different isatin–Schiff base copper(II) complexes. Influence of catalyst concentration on initial rate. [Hexose] = 1.00 mmol dm^{-3}. (A) Fructose, (B) glucose, and (C) galactose. Reactions at $T = (25.0 \pm 0.1)\,^{\circ}$C, and pH 12.0, in carbonate buffer (250 mmol dm^{-3}).

tion catalyzed by [Cu(*isaen*)(H_2O)]$ClO_4 \cdot 2H_2O$ (**1**), which seems to be the most reactive compound. Similarly to what was observed for the catalyst dependence, the kinetic curves exhibited also a first-order dependence on substrate con-

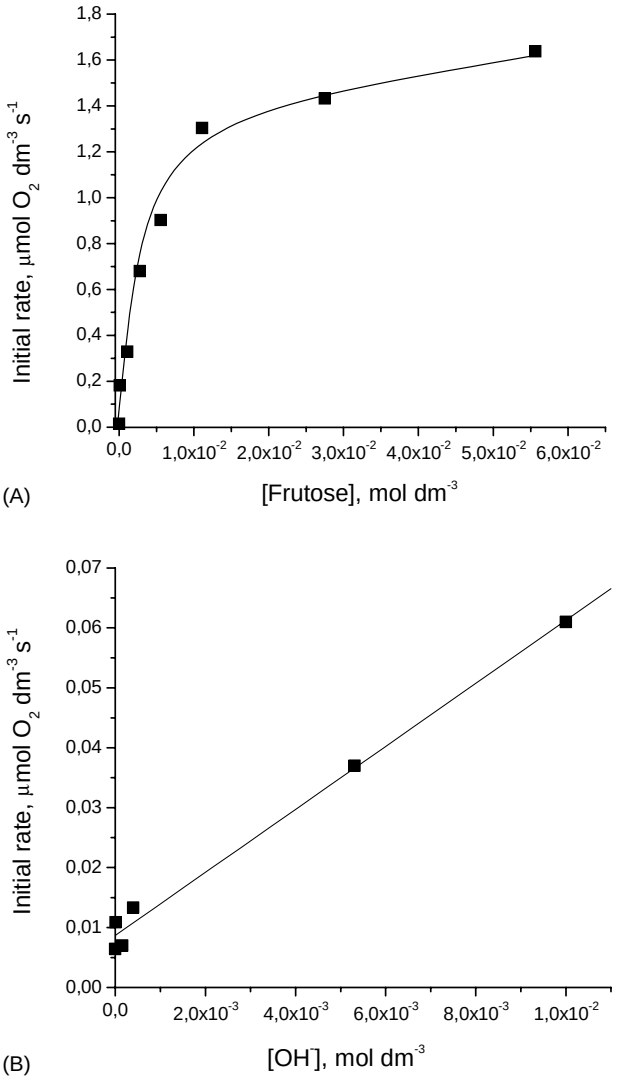


Fig. 2. (A) Dependence of initial rate on fructose concentration, in the presence of catalyst **1**, [Cu(*isaen*)H_2O](ClO_4), 0.110 mmol dm^{-3}. Reaction at $T = (25.0 \pm 0.1)\,^{\circ}$C, and pH 12.0, in carbonate buffer (250 mmol dm^{-3}). (B) Dependence on [OH^-] concentration. Reaction at $T = (25.0 \pm 0.1)\,^{\circ}$C, [Cu(*isaen*)($H_2O$)]($ClO_4$), 0.110 mmol dm^{-3}, and [Fructose] = 1.00 mmol dm^{-3}.

centration followed by a saturation effect, as shown in Fig. 2A.

On kinetic experiments at different pH values, it was observed a first-order dependence of initial rate with [OH^-] concentration (shown in Fig. 2B), with $V_i = k_2 + k_3$ [OH^-], where $k_2 = (8.70 \pm 0.05) \times 10^{-9}$ mol dm^{-3} s^{-1}, and $k_3 = (5.26 \pm 0.05) \times 10^{-6}$ s^{-1}. Additionally, these data showed a non-catalyzed step, with $V_o = (0.65 \pm 0.03) \times 10^{-6}$ mol dm^{-3} s^{-1}, attributed to the base-catalyzed enolization of the substrate. This value is consistent with earlier literature data ($k_s = 5.65 \times 10^{-5}$ mol dm^{-3} min^{-1} = 0.94×10^{-6} mol dm^{-3} s^{-1}) for the enolization step, in the D-fructose oxidation [12].

Table 3
Kinetic parameters for the fructose oxidation (1.11×10^{-3} mol dm^{-}3) catalyzed by complexes 1 or 3

Compound	Kinetic parameters		
	k_0	k_1	k_2
[Cu(*isaen*)(H_2O)]ClO_4 (**1**)	1.31×10^{-5}	2.65×10^{-2}	6.01×10^{3}
[Cu(*isapn*)](ClO_4)$_2$ (**3**)	1.17×10^{-5}	2.99×10^{-2}	5.17×10^{3}

Reaction conditions: [OH^-] = $(2.2 \pm 0.8) \times 10^{-2}$ mol dm^{-3}, corresponding to pH (12.0 ± 0.2), at (25.0 ± 0.2) °C.

According to these data, the experimental global rate law determined was:

$$V_i = \{k_0 + (k_1 + k_2[\text{cat}])[OH^-]\}[\text{fructose}]$$

where $k_0 = 1.3 \times 10^{-5}\,s^{-1}$ (neglected), $k_1 = 2.65 \times 10^{-2}\,mol^{-1}\,dm^3\,s^{-1}$, and $k_2 = 6.01 \times 10^{3}\,mol^{-2}\,dm^6\,s^{-1}$. An analogous rate law has been obtained for the reaction catalyzed by complex **3**, [Cu(*isapn*)]$^{2+}$ [19], and particularly, the determined values for constants k_0 and k_1 were very close, as shown in Table 3. Control experiments with copper perchlorate, or with a similar diimine–copper(II) complex not exhibiting a keto–enol equilibrium, showed that their activity are much lower than those of isatin-derivatives [19].

In contrast, the constants k_2 differ for both complexes. The value calculated for complex [Cu(*isaen*)(H_2O)]$^+$ (**1**) was a little higher, $(6.01 \pm 0.24) \times 10^{3}\,mol^{-2}\,dm^6\,s^{-1}$, than that observed for complex **3**, $(5.17 \pm 0.21) \times 10^{3}\,mol^{-2}\,dm^6\,s^{-1}$, previously studied. Therefore, both processes the base-catalyzed carbohydrate enolization, as well as the metal-catalyzed enediol formation are rate-determining steps, in the range of concentrations used.

The initial rate showed also to be influenced by variations in carbonate buffer concentration, as shown in Fig. 3, indicating a significant dependence on the ionic strength. However, these results can also be indicative of carbonate ion participation on the oxidation process, since carbonate radical anion $CO_3^{\bullet -}$ is a strong oxidant with $E^\circ = 1.78$ V at pH 7.0 [25], and has been reported as a product of oxidation of carbonate anions by hydrogen peroxide, catalyzed by the Cu,Zn SOD enzyme [26].

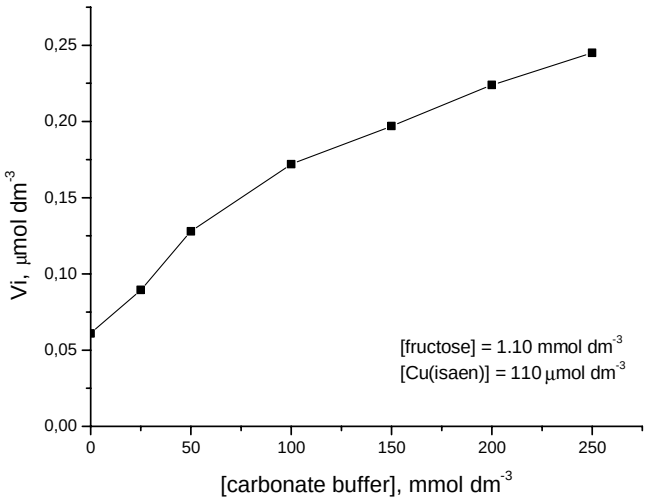


Fig. 3. Influence of carbonate buffer concentration in the initial rate of fructose oxidation. Reaction at T = (25.0 ± 0.1) °C, pH 12.0, [Cu(*isaen*)H_2O](ClO_4), 0.110 mmol dm^{-3}, and [Fructose] = 1.00 mmol dm^{-3}.

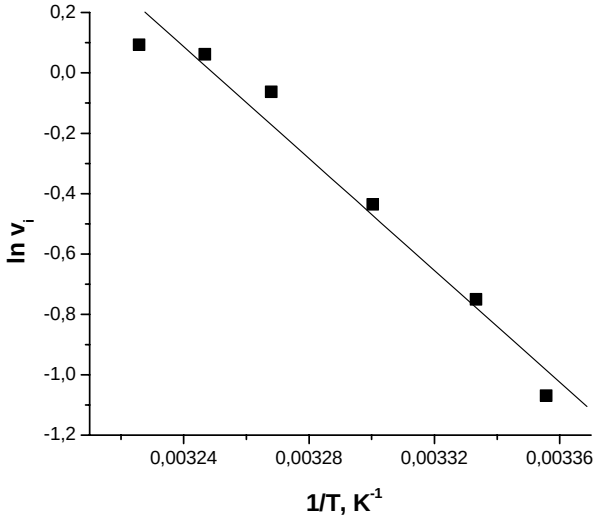


Fig. 4. Arrhenius curve, ln V_i (initial rate of oxygen consumption) versus 1/T (1/K), using catalyst **1**, [Cu(*isaen*)H_2O](ClO_4) = 0.11 mmol dm^{-3}, and [Fructose] = 1.11 mmol dm^{-3}. Reaction at pH 12.0, in carbonate buffer (250 mmol dm^{-3}). Used: R = 8.3144 J mol^{-1} K^{-1}.

In addition, it was noticed a typical Arrhenius behavior on the fructose oxidation catalyzed by **1**, [Cu(*isaen*)(H_2O)]$^+$ (see Fig. 4). From the curve ln V_i (initial rate) versus 1/T, it

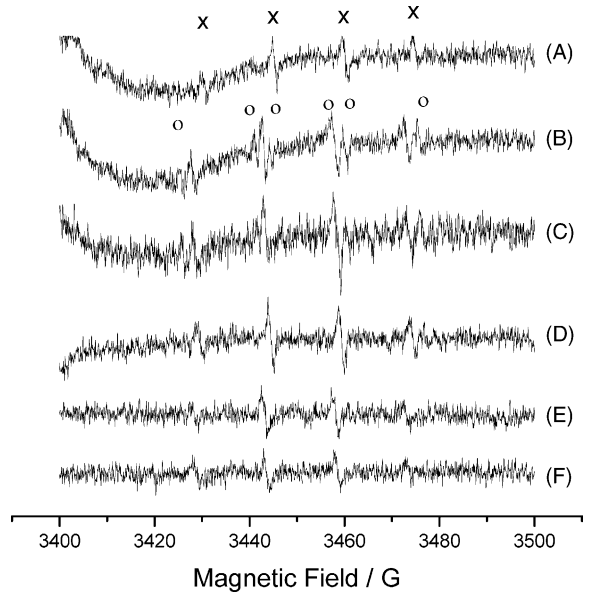


Fig. 5. Spin trapping EPR experiments, using DMPO (50 mmol dm^{-3}) as scavenger. Spectra recorded after 5 min of reaction, at (25.0 ± 0.1) °C and pH 12.0, in phosphate buffer (25 mmol dm^{-3}). [Substrate] = 1.00 mmol dm^{-3}. [Cu(*isaen*)H_2O]$^+$ = 100 µmol dm^{-3}: (A) fructose, (B) galactose, (C) glucose; [Cu(*isaepy*)]$^{2+}$ = 100 µmol dm^{-3}: (D) fructose, (E) galactose, (F) glucose. Conditions: modulation amplitude = 1 G; Gain = 2.00×10^5; eight scans.

was possible to estimate the activation energy of this process as 1.1 kJ mol^{-1}. This is a very low value, corresponding most likely to the enolization of the substrate. Very similar value has been obtained in previous studies of uronate oxidation, catalyzed by copper(II)–gluconate complex [15].

3.2.1. Detection of intermediates

In order to detect intermediary species in these catalyzed oxidations of carbohydrates, EPR spin trapping experiments were performed using DMPO as scavenger of free radicals. Although the formation of oxygen free radicals in processes catalyzed by copper ions is a controversial subject [27], in the presence of DMPO formation of DMPO–OH adduct was observed, in the fructose oxidation catalyzed by both complexes, [Cu(*isaen*)]$^{+}$ (**1**) and [Cu(*isaepy*)$_2$]$^{2+}$ (**2**), at pH 12.0 in phosphate buffer 25 mmol dm^{-3}, with typical parameters $A_N = A_H = 14.9$ G (see Fig. 5). As already observed in the fructose oxidation catalyzed by complex **3**, [Cu(*isapn*)]$^{2+}$ [19], no evidence of others adducts were detected. However, in the case of substrates galactose and glucose, a further adduct with A_N = 15.8 and A_H = 18.8 was also observed, for the reaction catalyzed by complex **1** [Cu(*isaen*)(H$_2$O)]$^{+}$, and it was ascribed to the formation of $CO_2^{\bullet -}$ radical anion, with very similar reported hyperfine constants [28]. In the case of complex **2** [Cu(*isaepy*)$_2$]$^{2+}$, this adduct was not observed, due probably to the low reactivity of this complex compared to **1**. Those results are expected since glucose and galactose can form the corresponding aldonates, in addition to formiate [29] that could explain the formation of $CO_2^{\bullet -}$ radical.

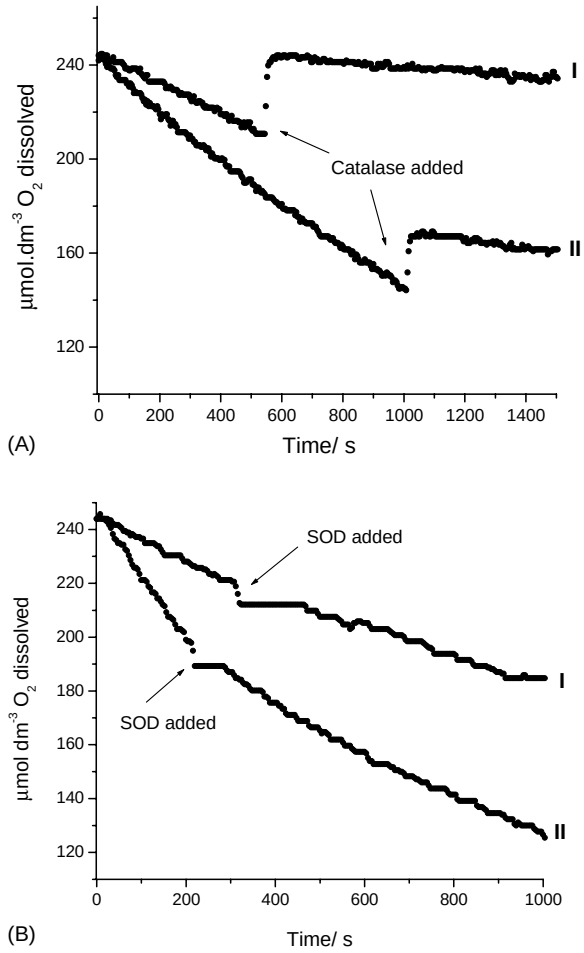


Fig. 6. (A) Addition of catalase (18,000 u) during kinetic run of fructose oxidation (1.11 mmol dm^{-3}), catalyzed by **1**, [Cu(*isaen*)H$_2$O](ClO$_4$) (0.11 mmol dm^{-3}), and **2**, [Cu(*isaepy*)$_2$](ClO$_4$)$_2$ (0.11 mmol dm^{-3}). (B) Addition of Cu,Zn Superoxide Dismutase (SOD, 15,000 u) during kinetic run of fructose oxidation (1.11 mmol dm^{-3}), catalyzed by **1**, [Cu(*isaen*)H$_2$O](ClO$_4$) (0.11 mmol dm^{-3}), and **2**, [Cu(*isaepy*)$_2$](ClO$_4$)$_2$ (0.11 mmol dm^{-3}). Both reactions (A and B) were performed at T = (25.0 ± 0.1) °C, and pH 12.0, adjusted with NaOH solution.

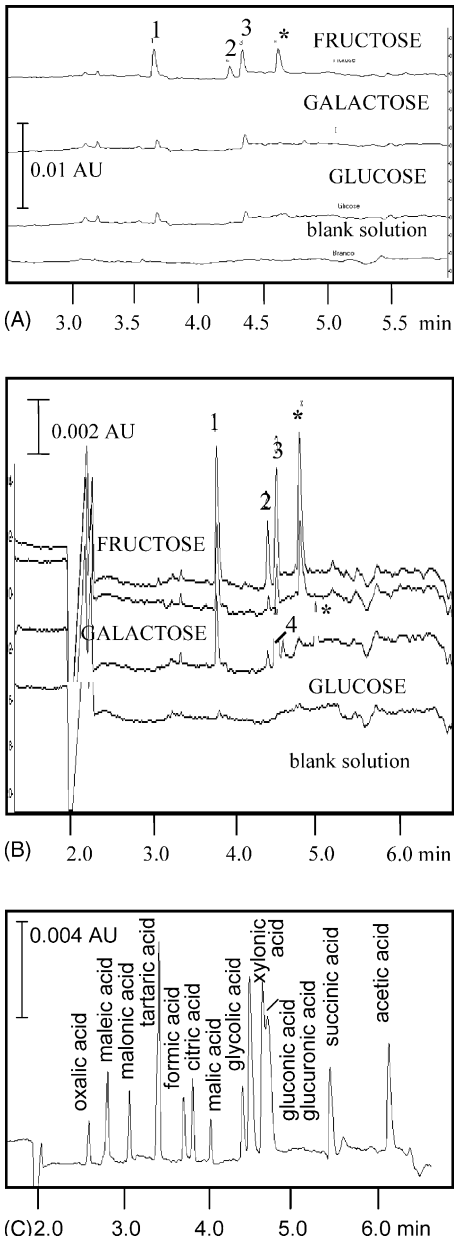


Fig. 7. Products detected by capillary electrophoresis in the hexose oxidation catalyzed by the complexes studied. (A) [Cu(*isapn*)](ClO$_4$)$_2$ (**3**); (B) [Cu(*isaen*)H$_2$O](ClO$_4$) (**1**). (C) is a standard mixture. Peak legend: (1) formic acid; (2) glyceric acid; (3) glycolic acid; (4) xylonic acid; (*) non-identified.

In further experiments, detection of carbon centered radicals in these carbohydrate oxidations by DBNBS adduct formation was not verified, with the studied systems. DBNBS was shown to be oxidized at the high pH value of reaction, possibly to a nitrosyl radical, showing a characteristic spectrum with three lines, with A_N = 9 G (not shown) [30]. At lower pH (<10) these oxidations were very slow, and radical formation was undetectable.

Superoxide radicals were qualitatively identified by addition of Cu,Zn SOD, causing a temporary suppression of the fructose oxidation, followed by a changing in the reaction rate, as shown in Fig. 6A, for both catalysts **1** and **2**. Analogous experiments permitted to verify the formation of hydrogen peroxide by addition of catalase during the kinetic run (Fig. 5B). Both intermediates superoxide radical anion and hydrogen peroxide probably react afterwards with the copper species, being dismutated or decomposed, respectively.

3.2.2. Detection of products

By capillary electrophoresis, it was possible to detect the main products formed in the oxidation of the carbohydrates, fructose, glucose, and galactose [31,32]. As shown in Fig. 7, the carboxylate anions formiate and glycolate were detected in the oxidation of all substrates, catalyzed by complexes **1** or **3**. Additionally, in the catalysis by **1**, glycerate was also verified in the oxidation of all substrates, while xylonate was detected only during the galactose oxidation. These products were identified by comparison with standard samples, using spiking techniques; theoretical mobility calculations were also made. In analogous studies, but in acidic medium (pH 4.0–4.8) and high temperature (110 °C), galactonic acid was detected as final product, by paper chromatography, in the galactose oxidation catalyzed by copper(II) ions [33].

These results indicated a degradative oxidation of all the substrates, expected in a process that occurs with the participation of very reactive free radicals.

3.2.3. Mechanism proposed

The experimental rate law indicated that enolization of the substrate and the coordinated ligand is essential for the process occur. A mechanism based on the steps shown in Scheme 2 is proposed.

Assuming that Eq. (2) refers to a rapidly achieved keto–enol equilibrium involving the catalyst, with the prevailing enol-form at very high pH as verified by spectroscopic techniques, and that Eqs. (1) and (3) are rate-determining

$$\text{Fructose} + OH^- \rightleftarrows \text{Fru-enediol} + H_2O \qquad K_1 = k_1/k_{-1} \qquad (1)$$

$$Cu^{II}L + OH^- \rightleftarrows Cu^{II}L_{enol}{}^{*} + H_2O \qquad K_2 \qquad (2)$$

$$\text{Fru-enediol} + Cu^{II}L_{enol}{}^{*} \rightarrow [(\text{Fru-enediol})Cu^{II}L_{enol}] \qquad k_2 \qquad (3)$$

$$[(\text{Fru-enediol})Cu^{II}L_{enol}] \rightarrow Cu^{I}L_{enol} + \text{Fru-enediol}^{\bullet} \qquad \text{fast} \qquad (4)$$

Catalyst Regeneration:

$$Cu^{I}L_{enol} + O_2 \rightarrow O_2^{\bullet-} + Cu^{II}L_{enol} \qquad (5)$$

$$H_2O_2 + Cu^{I}L_{enol} \rightarrow Cu^{II}L_{enol} + OH^{\bullet} + OH^- \qquad (6)$$

$$O_2^{\bullet-} + Cu^{I}L_{enol} + H_2O \rightarrow Cu^{II}L_{enol} + HO_2^- + OH^- \qquad (7)$$

Reaction Propagation:

$$OH^{\bullet} + \text{Fru-enediol} \rightarrow \text{Fru-enediol}^{\bullet} + H_2O \qquad (8)$$

$$OH^{\bullet} + HCOO^- \rightarrow CO_2^{\bullet-} + H_2O \qquad (9)$$

$$Cu^{II}L_{enol} + H_2O_2 \rightarrow Cu^{I}L_{enol} + HO_2^{\bullet} \qquad (10)$$

Reaction termination:

$$\text{Fru-enediol}^{\bullet} + O_2 \rightarrow \text{products} + H_2O_2 \qquad (11)$$

$^{*}Cu^{II}L_{enol}$ is the enol form of the complex (stable in alkaline medium).

Scheme 2. Proposed mechanism for the carbohydrate oxidation catalyzed by isatin–Schiff base copper(II) complexes.

steps, the rate reaction is given by:

$$-\frac{d[O_2]}{dt} = k[I][O_2]$$

where *I* is the intermediary species involving interaction between the substrate and the catalyst, [(Fru-enediol)$Cu^{II}L_{enol}$], which is rapidly decomposed into the corresponding reduced $Cu^{I}L$ species and Fru-enediol$^{\bullet}$ radical, by internal electron transfer. At steady-state conditions d[I]/dt = 0, and

$$k_2[\text{Fru-enediol}][Cu^{II}L_{enol}] = k[I][O_2]$$

Then,

$$\frac{-d[O_2]}{dt} = k_2[\text{Fru-enediol}][Cu^{II}L_{enol}]$$

Also, from steps (1) and (3):

$$\frac{d[\text{Fru-enediol}]}{dt} = k_1[\text{Fructose}][OH^-] - k_{-1}[\text{Fru-enediol}] + k_2[\text{Fru-enediol}][Cu^{II}L_{enol}] = 0$$

Therefore,

$$[\text{Fru-enediol}] = \frac{k_1[\text{Fructose}][OH^-]}{k_{-1} + k_2[Cu^{II}L_{enol}]}$$

Substituting this equation into the reaction rate expression:

$$-\frac{d[O_2]}{dt} = \frac{k_2[Cu^{II}L_{enol}]k_1[\text{Fructose}][OH^-]}{k_{-1} + k_2[Cu^{II}L_{enol}]}$$

This expression is analogous to that of earlier studies [12]. If $[Cu^{II}L_{enol}] \gg k_{-1}/k_2$, this expression becomes: $V_i = k_1[\text{Fructose}][OH^-]$, indicating that the process is independent of copper complex concentration.

However, when $[Cu^{II}L_{enol}] \ll k_{-1}/k_2$ the equation assumes the form:

$$-\frac{d[O_2]}{dt} = \left(\frac{k_1}{k_{-1}}\right) k_2[Cu^{II}L_{enol}][\text{Fructose}][OH^-]$$

According to this catalytic cycle (Scheme 2), and considering the wide range of catalyst concentration used in our studies, the global expression for initial reaction rate is consistent with the experimental rate law previously determined, $V_i = \{(k_1 + k_2[\text{cat}])[OH^-]\}[\text{fructose}]$, since in the used conditions (carbonate buffer, pH 12.3) $[Cu^{II}L_{enol}] \approx [CuL]_T$, that is, the total concentration of the catalyst. The process is autocatalytic, being propagated by reactive oxygen species formed at the beginning of the reaction.

4. Conclusions

Earlier studies on carbohydrate oxidation, in alkaline aqueous solution, catalyzed by copper ions were usually carried out at very high concentration of metal ion, in conditions where simultaneously copper(I) oxide precipitated [12–14].

Using different isatin–Schiff base ligands, capable of stabilizing copper ions in both oxidation states in very alkaline solutions, kinetic studies on copper-catalyzed oxidation of common hexoses were performed. Based on these results, an initial first-order dependence of reaction rate on catalyst concentration was observed, followed by a saturation effect. Under zero-order dependence on catalyst, a first-order dependence on substrate for all complexes studied was also verified, consistent with earlier data in the literature. Our kinetic studies also showed that the reaction occurs significantly only in very alkaline solutions, when both complexes and substrates suffer enolization.

Steps in the proposed mechanism, under pseudo-first-order conditions, combine intramolecular electron transfer with reduction of the copper ion by the coordinated reducing substrate, leading to substrate–enediol radical species, responsible for the initiation of the process. In this scheme, the dependence of reaction rate on the copper catalyst is detected only at very low concentrations, since afterwards the reaction is autocatalytic, occurring through diverse radical species and causing oxidative degradation of the substrates. This scheme is more comprehensive than those previously reported in the literature, avoiding the catalyst leaking as insoluble oxide.

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