ELSEVIER

Carbohydrate Research 315 (1999) 319–329

CARBOHYDRATE RESEARCH

Kinetics of the degradative oxidation of sugar-type ligands catalyzed by copper(II) ions

Renata Trinquinato Ródio, Eleonice Maria Pereira, Marina Franco Maggi Tavares, Ana Maria da Costa Ferreira *

Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo, PO Box 26077, São Paulo 05599-970, SP, Brazil

Received 24 September 1998; accepted 19 January 1999

Abstract

The autoxidation of gluconate (Gluc) and glucuronate (GlcA) catalyzed by copper(II) ions was studied in alkaline medium (pH 13), at 30.0 ± 0.1 °C, by monitoring the oxygen uptake and the oxidative degradation of the ligands. Different complexes of copper with gluconate were obtained, and their reactivities were compared. The compound $[Cu(Gluc)_2]$ is commercially available, while the compound Na[Cu(Gluc)(OH)] was isolated at pH 11.5. A third complex, the dimeric $Na_2[Cu_2(Gluc)_2(OH)_2]$, was obtained at pH 5.0. The most active complex was Na[Cu(Gluc)(OH)], showing a first-order dependence on both complex and gluconate concentrations. A saturation effect was observed when a large excess of gluconate was added to the reaction solution. Induction periods of ca. 90 min were also observed in the presence of excess gluconate. A second-order rate constant of $k = (1.44 \pm 0.09) \times 10^{-3}$ mol^{-1} dm^3 s^{-1} was determined, based on manometric measurements of the consumed oxygen. Comparative studies revealed a faster oxidation of glucuronate in the presence of copper(II) ions, with $k = (2.5 \pm 0.3)$ mol^{-1} dm^3 s^{-1}. Free radicals were detected as reactive intermediates in these catalyzed oxidations by EPR spectroscopy, using DMPO or POBN as spin traps. Evidence of the degradative oxidation of both ligands was obtained by the formation of formate, glycolate and carbonate ions, resulting from carbon–carbon bond cleavage, monitored by capillary electrophoresis.

Keywords: Copper(II)–gluconate complexes; Aldonic and uronic acids; Degradative oxidation; Kinetics; Capillary electrophoresis

1. Introduction

Selective oxidation reactions are usually catalyzed by metal ions having more than one oxidation state, which are easily accessible with common oxidizing agents. Most of the increasing interest in these reactions comes from the search for new catalysts, which aim at the preparation of more valuable oxidized products [1], but there is also an interest in elucidating mechanisms and catalytic cycles in the metabolism of various biological substrates [2–4].

Particularly, copper and iron ions have been involved in a number of oxidative processes, frequently with the generation of oxygen-reactive species [5,6]. Among these species, hydroxyl radicals are thought to be capable of causing damage to cellular membranes as well as imparting structural modifications in many

Abbreviations: Gluc, gluconate, $C_6H_{11}O_7^-$ or $C_6H_{10}O_7^{2-}$; GlcA, glucuronate, $C_6H_8O_8^-$; CTAB, cetyltrimethylammomium bromide; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; POBN, α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone.

* Corresponding author. Fax: +55-11-815-5579.

E-mail address: amdcferr@quim.iq.usp.br (A.M. da Costa Ferreira)

0008-6215/99/$ - see front matter
PII: S0008-6215(99)00026-9

important biomolecules, such as DNA, proteins and enzymes [7]. More recently, simple carbohydrates, such as glucose, have been recognized as important agents of oxidative stress, although the relative oxidative potencies of other biologically relevant sugars have not been adequately studied [8]. In the presence of copper ions, some of these substrates are potent pro-oxidants, and some can have antioxidant properties.

Earlier studies on the oxidation of reducing sugars by copper(II) ions showed that the process occurred in alkaline medium, exhibiting a first-order dependence on the concentration of the alkali and the carbohydrate, but was independent of copper concentration for all the sugars studied [9]. An enediol–Cu(II) intermediate was postulated to be formed, which in a subsequent step gave the final products, including cuprous oxide.

Gluconate ions, usually obtained by enzymatic oxidation of glucose, occur as metabolic intermediates in plants, animals and microorganisms. They are known to form coordination compounds with several metallic ions, especially iron, copper and manganese, and their efficiency as sequestering agents is markedly enhanced in alkaline medium [10,11]. Different binding modes have been described in solid and in aqueous solution, depending on the coordinated metal ion [12]. With copper, different stable compounds were observed with interesting structural properties determined by spectroscopic methods. Binuclear species coordinated by the carboxylate and deprotonated alcoholic hydroxy groups were prepared in alkaline solutions [13].

Glucuronate ions, derived from a uronic acid, play an important role in the biosynthesis of polysaccharides and in the metabolism of carbohydrates [14] that undergo Amadori rearrangements in the so-called Maillard reactions, leading to brown products in food and in some biological systems [15]. They are also intermediates in the synthesis of ascorbic acid [16], and similarly to gluconate, they can form coordination compounds with many metal ions [17,18].

We have been investigating oxidative processes that use molecular oxygen or hydrogen peroxide as the oxidizing agent and are catalyzed by transition metal ions coordinated to different ligands [19–21], including biomolecules [22]. In most of these reactions, oxygen free radicals are formed, which lead to damage of a coordinated substrate. In this work, the autoxidation, catalyzed by copper(II) ions, of the polyhydroxylated ligands, gluconate and glucuronate, is described with the aim of studying their antioxidant and/or pro-oxidant properties. The formation of formate, glycolate and carbonate ions was verified. These components probably formed by chain steps involving free radicals that were responsible for the oxidative cleavage of the coordinated ligands. These reactive intermediates were detected in the studied systems by spin-trapping EPR, while different anions were monitored by capillary electrophoresis.

2. Results and discussion

The autoxidation of gluconate ions in the presence of copper(II) ions was observed in very alkaline solutions (at pH 13). Different complexes copper–gluconate were then obtained as described in Section 3, and their reactivities were compared. The results of elemental analyses obtained for the two copper(II)–gluconate compounds prepared at different pHs, as well as for the commercially obtained compound, $[Cu(Gluc)_2]$, are shown in Table 1. The dimeric $Na_2[Cu_2(Gluc)_2(OH)_2]$ species, where Gluc is a dianion, was found to be too insoluble, even for catalytic studies. The commercial compound exhibits a d–d band at 782 nm, with $\varepsilon = 26.3$ mol^{-1} dm^3 cm^{-1} at pH 4, while the monomeric species Na[Cu(Gluc)(OH)] shows a similar band at 650 nm ($\varepsilon = 63.8$ mol^{-1} dm^3 cm^{-1}) at pH 9. An analogous band at 662 nm was observed in saturated solutions of the dimeric compound at pH 7.

Different binding modes have been suggested for carbohydrate complexes, depending on the central metal ion [26,27]. The most favorable arrangement for complex formation is the axial–equatorial–axial sequence of hydroxyl groups, although the 1,3,5-triaxial arrangement has also been described, and evidence for the formation of tri- and tetra-

Table 1
Elemental analyses and spectral parameters of copper(II)–gluconate complexes

Compounds[a]	% C Exp. (calcd)	% H Exp. (calcd)	λ_{max}, nm (ε, mol^{-1} dm^3 cm^{-1})
$[Cu^{II}(Gluc)_2]$	30.77 (31.76)	4.85 (4.89)	782 (26.3) at pH 4.0
$Na[Cu^{II}(Gluc)OH]\cdot H_2O$	22.95 (22.83)	4.18 (4.15)	650 (63.8) at pH 9.0
$Na_2[Cu^{II}_2(Gluc)_2(OH)_2]\cdot H_2O$	21.91 (23.50)	3.46 (3.94)	662[b] at pH 7.0

[a] Gluc, gluconate ion.
[b] In saturated aqueous solution.

dentate complexes was observed with suitable polyols [27b] or functionalized carbohydrates [26]. D-Glucuronate complexes seem to prefer the α-pyranose form in the $^4C_1$ conformation. In acidic solutions, weak copper complexes show coordination only at the carboxylate group, but above pH 5 many bidentate complexation sites are allowable [28]. Based on differential line broadening analysis of ^{13}C NMR spectra, additional binding of copper ion to the endocyclic oxygen O-5 of the sugar ring or to O-3 and O-1 in a $^1C_4$ conformation was suggested [17a]. However, at pH > 5 stronger species are formed exhibiting the $[Cu_2(OH)_2]$ moiety [26,27]. Those dimeric species $[Cu(OH)_2Cu]^{2+}$ are believed to catalyze the mutarotation of the glucuronate, enabling subsequent ring opening. At higher pH the complexation at O-3 increases in importance and causes breakdown of the complex, a phenomenon attributed to its redox behavior [17a]. On the other hand, gluconate ions prefer an open structure in which the copper ions are coordinated by the carboxylate and a hydroxyl group [11,13], although in solution these undergo equilibration with the corresponding γ- and δ-lactones [10].

Kinetics studies.—In Fig. 1, manometric curves relative to the complexes Na[Cu(Gluc)(OH)] and $[Cu(Gluc)_2]$ are compared. The former were shown to be the most catalytically active species that involved gluconate as a ligand.

Further kinetic studies indicated a first-order dependence on the complex concentration when using the compound Na[Cu(Gluc)(OH)] as catalyst in the presence of an excess of the ligand, as shown in Fig. 2(A). The influence of the gluconate concentration on the initial reaction rate was then verified by adding different amounts of excess sodium D-gluconate to the reacting solution (see Fig. 2(B)). A first-order dependence was also observed, followed by a saturation effect with $[gluc] > 8 \times 10^{-3}$ mol dm^{-3}. Based on these results, a second-order rate constant was determined, $k = (1.44 \pm 0.09) \times 10^{-3}$ mol^{-1} dm^3 s^{-1}. This oxidation reaction was only observed in very alkaline solutions that exhibit induction periods of ca. 90 min in the presence of excess D-gluconate. At pH < 12 the reaction rate was negligible. These results are indicative of a dimerization equilibrium where the monomer is the catalytically most active species. Dimeric complexes of iron(III)–gluconate have been characterized by X-ray absorption studies (EXAFS and XANES), wherein the metal ions are coordinated by one deprotonated alcoholic and one carboxylic oxygen atom and bridged by two hydroxide ions [29]. Similar complexes are formed with copper(II) ions [13].

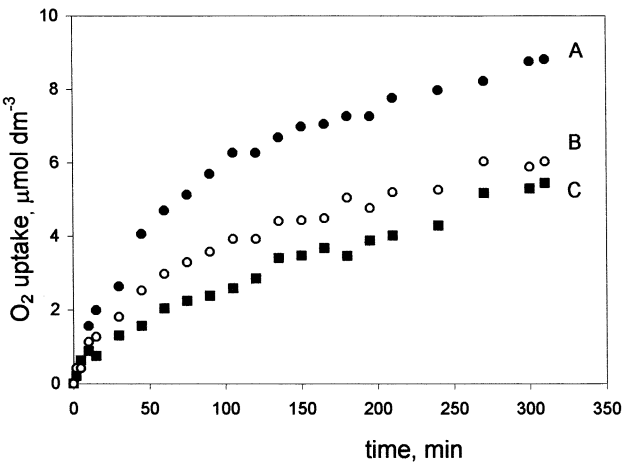


Fig. 1. Oxygen uptake curves of copper(II)–gluconate complexes at 30.0 ± 0.1 °C, pH 13, and $I = 0.10$ mol dm^{-3} KNO_3. (A) $Na[Cu(Gluc)(OH)] = 4.04 \times 10^{-3}$ mol dm^{-3}; (B) $Na[Cu(Gluc)(OH)] = 2.02 \times 10^{-3}$ mol dm^{-3}; (C) $[Cu(Gluc)_2] = 4.00 \times 10^{-3}$ mol dm^{-3}.

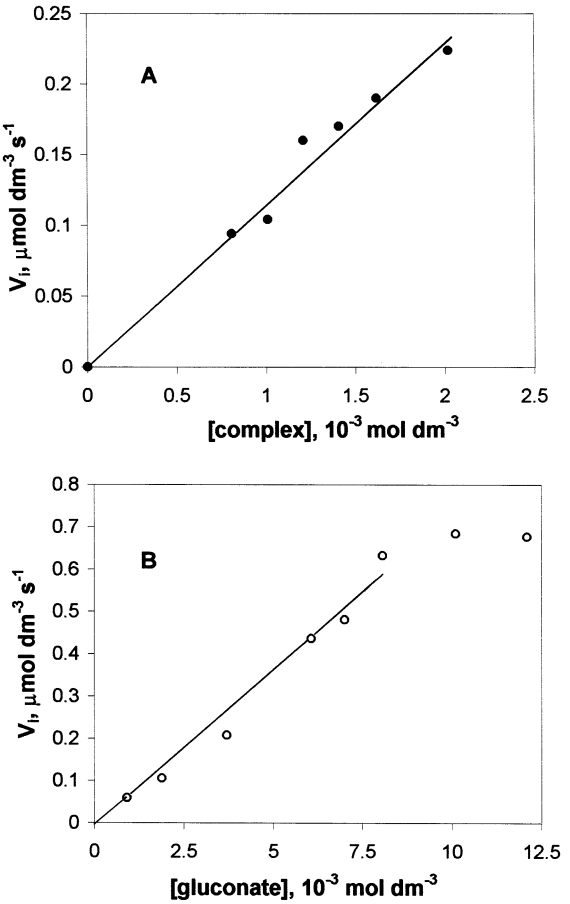


Fig. 2. Dependence of the initial oxidation rate of gluconate on: (A) the concentration of the catalyst, [Cu(Gluc)(OH)$^-$], in the presence of excess ligand; $[Gluc]_T = 10.1 \times 10^{-2}$ mol dm^{-3}; (B) the gluconate concentration; [Cu(Gluc)(OH)$^-$] = 4.04×10^{-3} mol dm^{-3}. At 30.0 ± 0.1 °C, pH 13 and $I = 0.10$ mol dm^{-3} KNO_3.

The isolation of analogous copper(II)–glucuronate complexes under the same experimental conditions was not successful since the ligand was very rapidly oxidized in alkaline medium (pH > 7) with concomitant reduction of the copper ion [17a]. Therefore, in this case comparative kinetic studies were performed by adding copper(II) ions (as nitrate) to excess sodium D-glucuronate in aqueous solution. The corresponding kinetic curves showed faster reaction rates, exhibiting a first-order dependence on both glucuronate and copper(II) concentrations, as shown in Fig. 3. In contrast to the previous results with gluconate, an appreciable non-catalyzed step was verified, with $V_0 = 2.73 \times 10^{-7}$ mol dm^{-3} s^{-1} (Fig. 3(A)), and no saturation effect on substrate concentration was observed (Fig. 3(B)). The determined value for the corresponding second-order rate constant was $k = (2.5 \pm 0.3)$ mol^{-1} dm^3 s^{-1}.

Additional experimental data obtained by varying the temperature led to the Arrhenius curves shown in Fig. 4. Both ligands provided very similar values for the activation energy, 4.82 kcal mol^{-1} or 1.15 kJ mol^{-1}, for glucuronate, and 5.17 kcal mol^{-1} or 1.24 kJ mol^{-1}, for gluconate, indicating little dependence of the rate constants on the temperature, in the range 25–45 °C.

Reactive intermediates.—By using spin-trap EPR spectroscopy, it was possible to detect carbon- and oxygen-centered free radicals as reactive intermediates in the catalyzed oxidation of gluconate and glucuronate ions. Fig. 5 shows the spectra of the adducts observed during the oxidation of gluconate in the presence of the spin-trap DMPO. At the very beginning of the reaction ($t = 2$ min), the char-

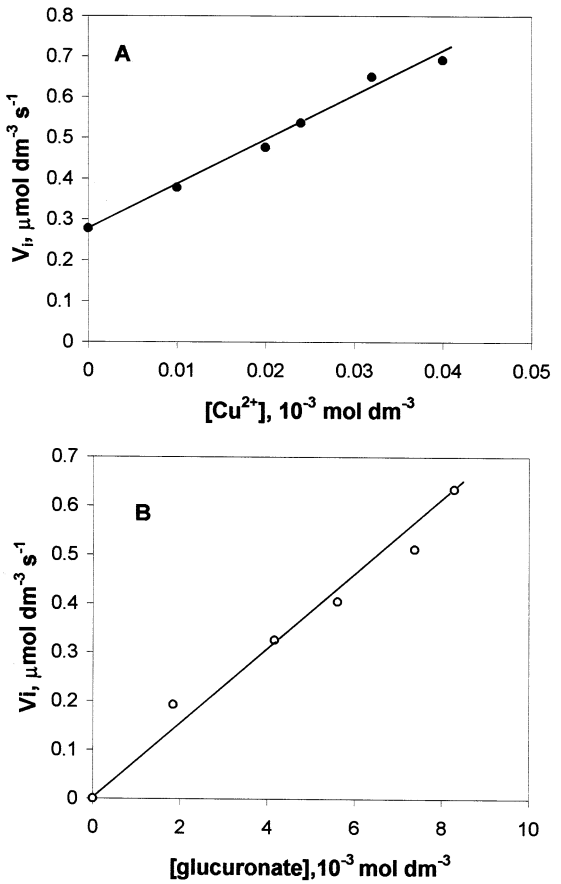


Fig. 3. Dependence of the initial rate of the oxidation of glucuronate on: (A) the copper(II) ion concentration; [glucuronate] = 6.00×10^{-3} mol dm^{-3}, and (B) the glucuronate concentration; $[Cu(II)]_T = 2.40 \times 10^{-5}$ mol dm^{-3}. At 30.0 ± 0.1 °C, pH 13 and $I = 0.10$ mol dm^{-3} KNO_3.

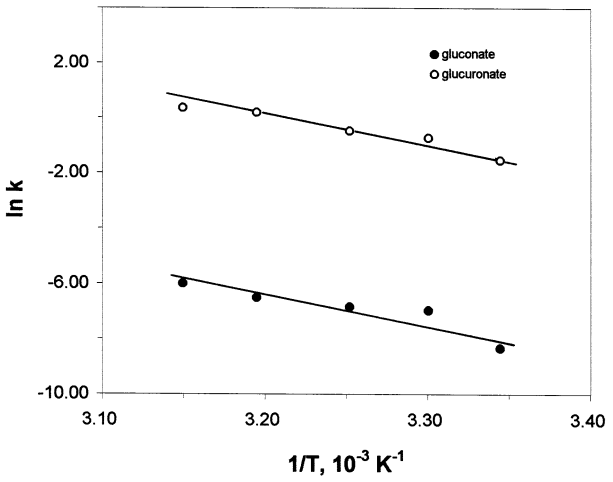


Fig. 4. Influence of the temperature on the copper(II)-catalyzed oxidation of (●) gluconate ($[Cu(Gluc)OH^-] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[gluconate]_T = 10.2 \times 10^{-2}$ mol dm^{-3}); and (○) glucuronate ($[Cu^{2+}] = 2.40 \times 10^{-5}$ mol dm^{-3}; $[glucuronate] = 6.46 \times 10^{-3}$ mol dm^{-3}). Reaction at pH 13 and $I = 0.10$ mol dm^{-3} KNO_3.

acteristic spectrum of the adduct DMPO-OH was identified, with $a_N = a_H = 15.47$ G. Then, with further development of the reaction, the

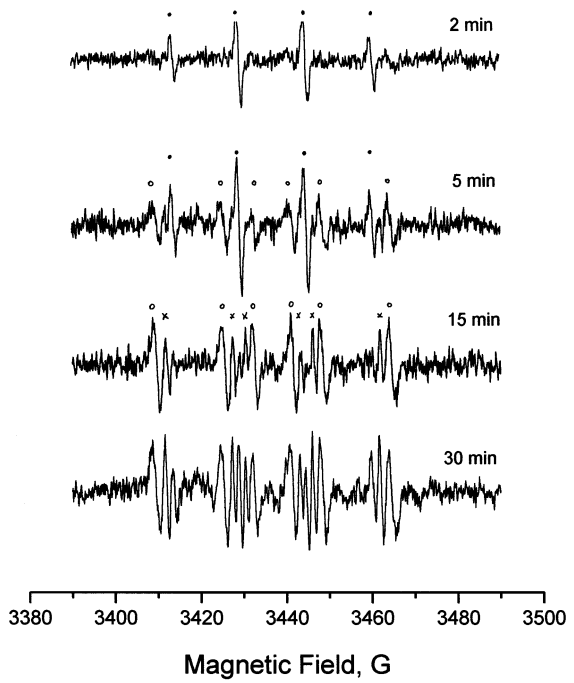


Fig. 5. EPR spectra of the adducts formed in the presence of DMPO (0.25 mol dm^{-3}) during the catalyzed oxidation of gluconate, at 25 °C. $[Cu(Gluc)OH^-] = 4.43 \times 10^{-3}$ mol dm^{-3}. (●) DMPO-OH, with $a_N = a_H = 15.47$ G; (○) DMPO-R, with $a_N = 15.96$ and $a_H = 23.13$ G; and (×) DMPO-CO_2, with $a_N = 15.70$ and $a_H = 18.77$ G. Spectra registered with modulation amplitude = 1 G; receiver gain = 2.00×10^6; 16 scans.

increasing signals of two other adducts were observed. First, the broad lines of a carbon-centered radical ($a_N = 15.96$; $a_H = 23.13$ G) were detected at $t = 5$ min, followed by the formation of another adduct, with thinner lines ($a_N = 15.70$; $a_H = 18.77$ G) at $t = 15$ min. The first adduct is probably due to radicals derived from the original ligand after suffering hydrogen atom abstractions. By comparison, the hyperfine constants of this adduct were found to be very similar to that of hydroxylated radicals, such as $^{\bullet}CH_2OH$ ($a_N = 16.00$; $a_H = 22.70$ G) [30]; the glycerol radical ($a_N = 15.9$; $a_H = 22.4$ G) [31], or sorbitol radical ($a_N = 15.90$; $a_H = 22.50$ G) [32]. The second adduct, observed after 15 min of reaction, was attributed to the formation of $CO_2^{\bullet -}$ radical anions that showed corresponding adduct hyperfine constants $a_N = 15.60$ and $a_H = 18.70$ G [33]. Therefore, the degradation of the ligand that leads to C–C bond cleavage occurs only after some time of reaction. After 30 min of reaction, the three adducts were simultaneously observed (Fig. 5).

When POBN was used as scavenger, two radical species were observed (data not shown): one adduct exhibiting six lines, with $a_N = 15.78$ and $a_H = 2.20$ G, which can be identified as the POBN-OH adduct ($a_N = 15.60$; $a_H = 2.60$ G) [34], and another one showing only three lines, with $a_N = 17.25$ G. This latter species is probably formed by the degradative oxidation of the scavenger itself.

In the case of glucuronate, the oxidation also leads to oxygen- and carbon-centered radicals similar to those observed in gluconate oxidation. As shown in Fig. 6, after 2 min of reaction, three adducts with DMPO were already formed: (●) DMPO-OH, with $a_N = a_H = 15.59$ G; (○) a derivative of a carbon-centered radical, with $a_N = 16.04$ and $a_H = 23.38$ G; and (×) DMPO-CO_2, with $a_N = 15.70$ and $a_H = 18.77$ G. After 15 min of reaction, this latter adduct was shown to predominate in solution.

Products of reaction.—In order to verify which products are formed in these oxidations, reacting solutions were monitored by capillary electrophoresis as described in Section 3. By comparison with standard solutions of inorganic and organic anions, carbonate

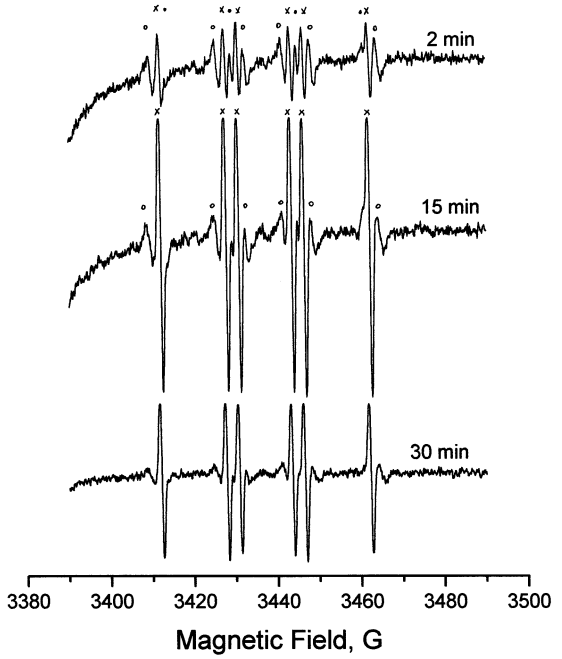


Fig. 6. EPR spectra of the adducts formed in the presence of DMPO (0.25 mol dm^{-3}) during the catalyzed oxidation of glucuronate, at 25 °C. $[Cu^{2+}] = 1.00 \times 10^{-4}$ mol dm^{-3}; [glucuronate] $= 5.75 \times 10^{-3}$ mol dm^{-3}. (●) DMPO-OH, with $a_N = a_H = 15.59$ G; (○) DMPO-R, with $a_N = 16.04$ and $a_H = 23.38$ G; and (×) DMPO-CO_2, with $a_N = 15.70$ and $a_H = 18.77$ G. Spectra registered with modulation amplitude = 1 G; receiver gain $= 2.00 \times 10^6$; 16 scans.

ions were identified as the main anionic product formed in the autoxidation of gluconate, as well as that of glucuronate. Those ions showed a migration time of 2.16–2.32 min, under the experimental conditions, while the initial gluconate ions exhibit a migration time of 2.75–2.88 min, as observed in Fig. 6. No evidence for substantial formation of other anionic species was obtained in the case of gluconate complex during 2 h of reaction. Control experiments were done and showed neglecting absorption of CO_2 by the alkaline reacting solution under the experimental conditions. Oxalate ions that co-eluted with inorganic ions, such as nitrate or hydroxide (peak 1 in the control electropherogram), proved impossible to detect unequivocally.

When the oxidation of glucuronate in the presence of copper(II) ions was studied, evidence for considerable formation of formate and glycolate, in addition to carbonate anions, was obtained. The respective electropherograms are also shown in Fig. 7. Glucuronate and gluconate ions exhibit very similar migration times under the same experimental conditions (peak 6 in the control electropherogram). Fig. 8 shows the relative amounts of the products obtained in this catalyzed oxidation, with the predominance of

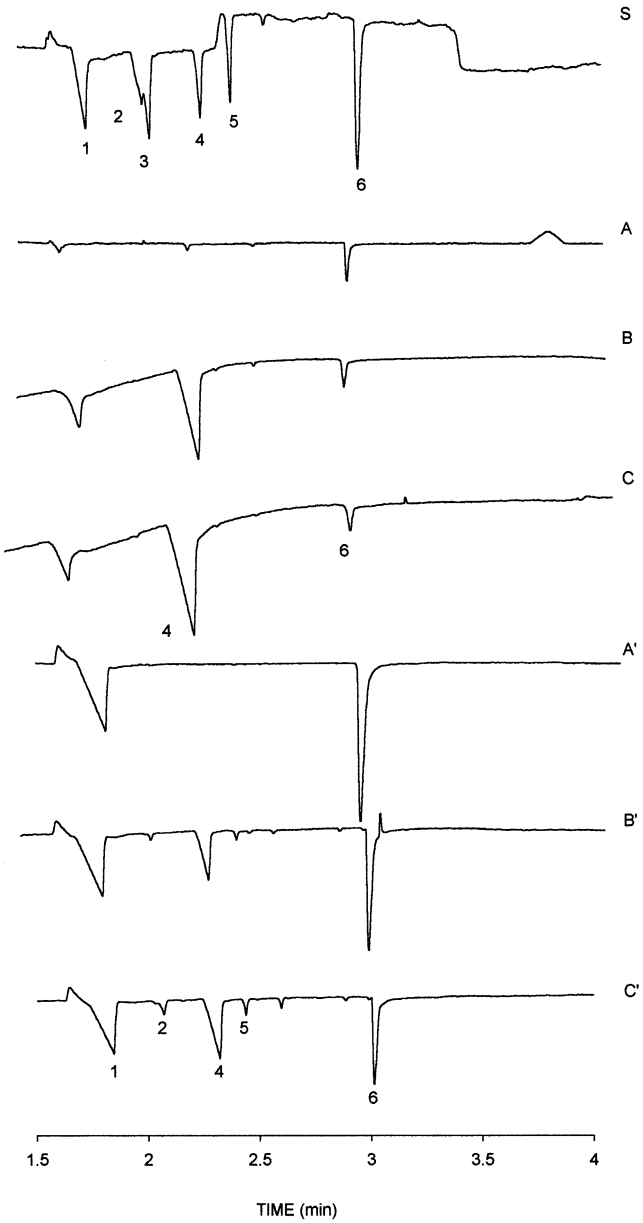


Fig. 7. Electropherograms obtained during the autoxidation of the complex Na[Cu(Gluc)(OH), total concentration = 1.15×10^{-3} mol dm^{-3}, at (A) initial time, (B) after 30 min, (C) after 60 min; and the autoxidation of glucuronate ions, in the presence of copper(II) ions (added as $Cu(NO_3)_2$), at (A′) initial time; (B′) after 60 min; (C′) after 120 min. [Cu(II)] = 5.10×10^{-3} mol dm^{-3}; $[glucuronate]_T = 5.78 \times 10^{-3}$ mol dm^{-3}. (S) Control electropherogram (1.00×10^{-3} mol dm^{-3}): 1, oxalate; 2, formate; 3, tartrate; 4, carbonate; 5, glycolate; 6, gluconate or glucuronate. Reactions at 30.0 ± 0.1 °C, and pH 13. All samples were diluted 100-fold in water, immediately before analysis.

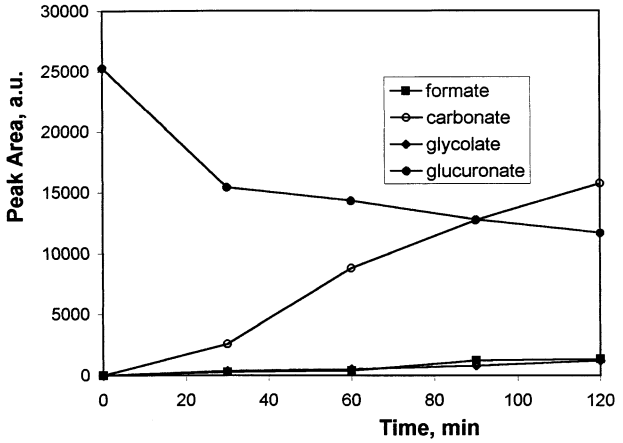


Fig. 8. Comparative curves of product formation during the degradative oxidation of glucuronate ions catalyzed by copper(II). Experimental conditions as in Fig. 6.

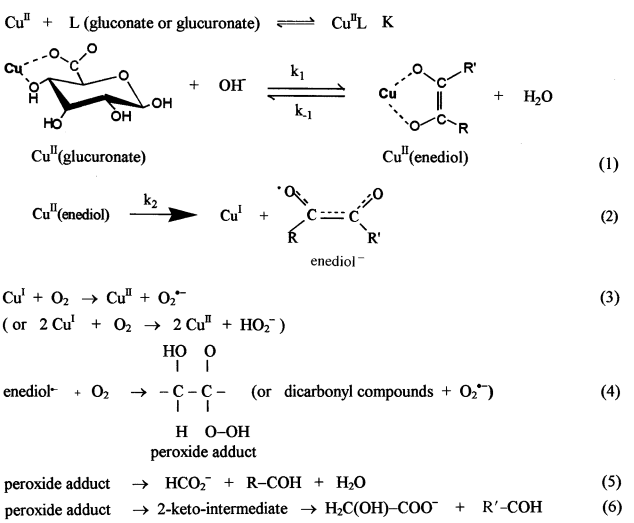
carbonate ions, which is in agreement with the reported EPR results.

Additionally, the precipitation of cuprous oxide as a fine orange powder was observed after many hours of reaction in the oxidation of both ligands. In these reactions, as well as that which has been observed in monosaccharide autoxidation [35], the oxygen molecule is reduced predominantly to hydrogen peroxide, which by interaction with the copper ions gives hydroxyl radicals that are responsible for the oxidative degradation of the ligands.

Mechanism of reaction.—Hydroxylated substrates as antioxidant compounds can act as scavengers of oxygen free radicals. Mannitol, ethanol and sorbitol, all of which react very quickly with hydroxyl radicals, are examples of such substrates and have been used for this purpose in biological studies [36]. On the other hand, non-reducing carbohydrates can react with an alkaline solution of hydrogen peroxide and iron(II) ions known as Fenton's reagent. Aldonic acids, such as D-gluconic and D-xylonic acid, gave formate, oxalate and carbonate ions, which were identified by conventional chemical methods [37]. Studies of disaccharides reacting with the same reagent under aerobic physiological conditions indicated that the reaction of the hydroxyl radicals generated with sugars is initiated by hydrogen abstraction, followed by oxidative C–C cleavage [38]. In addition, DNA damage caused by oxidants with free radical character, such as metal complexes and oxygen metabolites, is also initiated by the abstraction of a hydrogen atom from ribose or deoxyribose to produce a carbon-based sugar radical that can rearrange and lead to the scission of the nucleic acid strand. If unrepaired, this type of lesion may contribute to mutagenesis, carcinogenesis, aging, inherited disease or cell death [39].

Our results showed that, when reacting with molecular oxygen in the presence of copper ions and in very alkaline medium, gluconate and glucuronate ions also generated free radical species that were responsible for the degradative oxidation of both anions. While gluconate was mainly converted into carbonate ions, glucuronate was rapidly degraded to formate and glycolate ions, in addition to carbonate ions.

These experimental data support a mechanism analogous to that suggested for the oxidation of simple monosaccharides shown in Scheme 1. In very alkaline medium glucuronate ions can undergo tautomeric change, forming initially an enediol [36] that is stabilized by coordination to the copper(II) ions. In our studies the oxidation process is first order, dependent on both copper(II) and substrate concentrations. Conversely, in the oxidation of hexoses by copper(II) ions [9], the enediol formation was considered as the rate-determining step for all the sugars studied, and the kinetic law thus determined was independent of copper concentration. However, in these studies the catalyst concentrations used



Scheme 1.

were higher (>2.5 mmol dm^{-3}). As previously postulated in the mechanism for the oxidation of alduronic anions by alkaline hydrogen peroxide [40], a further nucleophilic addition of the dioxygen molecule or the hydroperoxide anion to the enolized form of the ligand can also be proposed.

In the presence of molecular oxygen as the oxidant, an intramolecular redox reaction can occur with reduction of the copper(II) ion and oxidation of the hydroxylated ligand to an enediol radical anion, which is similar to that proposed in the glucose autoxidation [41], or more recently in the copper(II)-catalyzed autoxidation of δ-aminolevulinic acid [42]. Subsequently, the radical anion reacts by faster steps with the dioxygen species to give peroxide derivatives, which are degraded stepwise to formate and carbon dioxide. The reoxidation of the metal ion occurs with reduction of dioxygen to the superoxide radical or hydrogen peroxide. In the case of glucuronate ligands, the production of glycolate ions can be indicative of the intermediary formation of a 2-ketose [35].

According to Scheme 1, the rate of oxygen uptake is given by the expression below, considering the intramolecular redox reaction, preceded by the enolization step, as the rate-determining step:

$$-\mathrm{d}[O_2]/\mathrm{d}t = k_2[\mathrm{Cu(enediol)}][O_2] = k_2K_1[OH^-][O_2]K[Cu^{2+}][\mathrm{ligand}]$$

or

$$-\mathrm{d}[O_2]/\mathrm{d}t = k[Cu^{2+}][\mathrm{ligand}]$$

where [ligand] = total gluconate or glucuronate concentration, and k is the determined second-order rate constant: 1.44×10^{-3} mol^{-1} dm^3 s^{-1} (for Gluc), and 2.5 mol^{-1} dm^3 s^{-1} (for GlcA).

The verified small dependence of both rate constants on the temperature, which exhibit very similar activation energies (1.2 kJ mol^{-1}), reinforced the proposed mechanism. Further deprotonation of the ligands, which is carried out only at very high pH and favored in the presence of copper ions, is essential for the initiation of the process.

With gluconate, however, the enolization of the ligand demands two oxidation equivalents.

$$LCu^{II} + H_2O_2 + 2OH^- \rightarrow LCu^{I} + O_2^{\bullet -} + 2H_2O \quad (7)$$

$$LCu^{I} + H_2O_2 \rightarrow LCu^{II} + {}^{\bullet}OH + OH^- \quad (8)$$

$$LCu^{II} + O_2^{\bullet -} \rightarrow LCu^{I} + O_2 \quad (9)$$

$$^{\bullet}OH + HL \rightarrow L^{\bullet} + H_2O \quad (10)$$

$$^{\bullet}OH + HCO_2^- \rightarrow CO_2^{\bullet -} + H_2O \quad (11)$$

Scheme 2.

In this case, a concerted mechanism can be proposed in which one electron from the ligand is transferred to the copper(II) ion, and simultaneously another one to the oxygen molecule:

$$[Cu^{II}(\mathrm{gluconate})OH]^- + O_2 \rightarrow Cu^{I} + O_2^{\bullet -} + \mathrm{enediol} + H_2O \quad k_i \text{ (initiation)}$$

$$Cu^{II} + \mathrm{enediol} \rightleftharpoons Cu^{II}(\mathrm{enediol})$$

This previous initiation step could explain the slower rate of the reaction compared with glucuronate, as well as the observed induction period. Once the enediol complex is formed, the other steps are analogous to those in the glucuronate oxidation.

The detected oxygen reactive intermediates are produced by the interaction of the copper ions with the formed hydrogen peroxide, generating hydroxyl radicals in a Fenton-like scheme of reactions (Scheme 2). Carbon-centered radicals are formed from H abstraction of the ligands by the hydroxyl radicals, while $CO_2^{\bullet -}$ radicals can be formed by the attack of these radicals on the formate ions that are produced. Both enediol–oxy radicals and superoxide anions were not observed by spin-trapping, since this technique of detection shows relatively low sensitivities for these radicals [23b].

Faster oxidation of glucuronate compared with gluconate can be indicative of an intramolecular redox reaction (step 2 in Scheme 1) thermodynamically more favorable in the case of the copper(II)–glucuronate complex. The catalysis of the glucuronate mutarotation by copper ions makes easier the pyranose ring opening, and the subsequent degradation of the complexes in alkaline solution, as previously reported [17a]. The intermediary Cu(II)–enediol species can be less stabilized in

this case. Additionally, in the subsequent redox steps with hydrogen peroxide (Scheme 2), the reduction of copper(II) can be easier when the ligand is glucuronate, a reducing sugar that undergoes Fehling-type oxidation. In the catalytic cycle the reaction rate is significantly influenced by the relative stabilization of the oxidized form compared with the reduced one.

In conclusion, our comparative kinetic results revealed that glucuronate ions, which exhibited rate constants at least 10^3 faster in the presence of copper(II), are much more susceptible to autoxidation than gluconate. Also, those ions were oxidized to different products by concomitant steps, including glycolate ions, which comes from a keto-intermediate (step 6 in Scheme 1). This type of compound, in particular, can cause protein damage by interaction with amino groups. In contrast, gluconate ions were predominantly degraded to carbonate ions, which are less harmful in oxidative steps. Therefore, these results suggest that the pro-oxidant properties of glucuronate and gluconate ions are responsible for the dioxygen reduction observed in the presence of cupric ions. For both ligands the process is more remarkable than that of simple monosaccharides. For comparison, the value determined for D-glucose oxidation was $k = 2.1 \times 10^{-6}$ mol^{-1} dm^3 s^{-1}, at 35 °C [9].

Recent studies showed that copper-catalyzed oxidation reactions can play a major role in promoting in vitro the formation of advanced glycation end products [43] from long half-life proteins, such as collagen, a process that is implicated in the pathogenesis of diabetes mellitus.

3. Experimental

Materials.—The sodium salts of the D-gluconic acid and D-glucuronic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Sigma (St Louis, MO), respectively. The reagent DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide, from Aldrich) was previously purified by either distillation or filtration on charcoal as recommended [23]. Other chemicals used were analytical grade from various sources. All the solutions were prepared with nanopure water from a Barnsted apparatus.

The copper complexes with the ligand gluconate (Gluc), a monomer $Na[Cu^{II}(Gluc)(OH)]$, and a dimer $Na_2[Cu^{II}_2(Gluc)_2(OH)_2]$, were prepared in aqueous solution at pH 11.5 and 5.0, respectively, according to previously reported procedures in the literature [13], with minor modifications. Stoichiometric amounts (4 mmol) of $Cu(ClO_4)_2 \cdot 6H_2O$ and sodium D-gluconate (from Aldrich) were dissolved in a minimum amount of water (15 mL), and the pH of the solution, monitored with a pH meter, was carefully adjusted by addition of sodium hydroxide solution. Then, ethanol up to 80% (v/v) was added under stirring to the final solution, which led to the precipitation of fine blue crystals of the corresponding product. The crystals were collected by filtration under reduced pressure and dried in a desiccator under vacuum for 2 days. After that time period, these products were purified by recrystallization, adjusting again the correct final pH in each case. The final products were then collected and dried over phosphorus pentoxide (P_4O_{10}). The commercial complex $[Cu^{II}(gluconate)_2]$ was purchased from Aldrich and used as received. The results of elemental analyses for these compounds are shown in Table 1.

Methods.—Electronic spectra were recorded in a Beckman DU-70 spectrophotometer (Fullerton, CA) equipped with a temperature-controlled cell compartment. Elemental analyses were performed at the Núcleo de Instrumentação de Análises Químicas do IQ-USP using a Perkin–Elmer model 2400 CHN instrument (Norwalk, CT).

Kinetic measurements of the oxygen uptake were performed by manometric technique in a Warburg apparatus from B. Braun (Melsungen, Germany), model V-85, at 30.0 ± 0.1 °C, adjusted to pH 13 by addition of NaOH solution, and with an ionic strength of 0.10 mol dm^{-3} KNO_3.

The final products obtained in the oxidation of gluconate, or glucuronate ions, were analyzed in a capillary electrophoresis system [24,25], model 270-HT (Perkin–Elmer, Applied Biosystem Div., Foster City, CA), at

−30 kV and 25 °C, in a 50 μm × 72 cm capillary, with hydrodynamic injection of 5 inchHg and 2.5 s, using indirect photometric detection at 254 nm. The instrument was equipped with a data acquisition and treatment software (Turbochrom™, PE–Nelson, Norwalk, CT). Different compounds were tested as electrolytes, including phthalate, benzoate and chromate, but the best results for organic anions were obtained when a solution of 3,5-dinitrobenzoic acid (5×10^{-3} mol dm^{-3}) containing the surfactant agent CTAB (cetyltrimethylammomium bromide, 1×10^{-4} mol dm^{-3}) was used at pH 6.0. Samples of the oxidized solution were collected at different times and diluted 100-fold immediately before analysis.

EPR spectra were obtained using a Bruker EMX instrument with DMPO or POBN as the spin trap in standard flat quartz cells. Different samples were collected at different times of reaction, performed at 25 °C in the presence of 0.25 mol dm^{-3} spin trap, and their spectra were immediately recorded.

Acknowledgements

Financial support from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CAPES (Conselho para Aperfeiçoamento do Pessoal de Nível Superior), and fellowships from CNPq/PIBIC (Conselho Nacional de Desenvolvimento Científico e Tecnológico/Programa de Iniciação Científica) are gratefully acknowledged.

References

[1] R.A. Sheldon, J.K. Kochi (Eds.), Oxidations with molecular oxygen, in *Metal-Catalyzed Oxidations of Organic Compounds*, Academic Press, New York, 1981, Chapter 2, pp. 18–32.
[2] K.D. Karlin, Z. Tyeklar, A.D. Zuberbühler, Formation, structure, and reactivity of copper dioxygen complexes, in J. Reedjik (Ed.), *Bioinorganic Catalysis*, Marcel Dekker, New York, 1993, Chapter 9, pp. 261–315.
[3] E. Spodine, J. Manzur, *Coord. Chem. Rev.*, 119 (1992) 171–198.
[4] N. Kitajima, Y. Moro-oka, *Chem. Rev.*, 94 (1994) 737–757.
[5] S.M. Lynch, B. Frei, *J. Lipid Res.*, 34 (1993) 1745–1753.
[6] S. Kawakishi, Y. Okawa, K. Uchida, *J. Agric. Food Chem.*, 38 (1990) 13–17.
[7] E.R. Stadtman, *Free Radic. Biol. Med.*, 9 (1990) 315–325.
[8] K.R. Wehmeier, A.D. Mooradian, *Free Radic. Biol. Med.*, 17 (1994) 83–86.
[9] S.V. Singh, O.C. Saxena, M.P. Singh, *J. Am. Chem. Soc.*, 92 (1970) 537–541.
[10] D.T. Sawyer, *Chem. Rev.*, 64 (1964) 633–643.
[11] (a) R.L. Pecsok, R.S. Juvet Jr., *J. Am. Chem. Soc.*, 77 (1955) 202–206. (b) K. Blomqvist, E.R. Still, *Anal. Chem.*, 57 (1985) 749–752.
[12] (a) H.A. Tajmir-Riahi, *Can. J. Chem.*, 67 (1989) 651–654. (b) G.D. Bailey, W.R. Carper, *J. Inorg. Biochem.*, 52 (1993) 99–108.
[13] L. Nagy, L. Zsikla, K. Burger, A. Rockenbauer, J.T. Kiss, *J. Crystallogr. Spectr. Res.*, 19 (1989) 911–918.
[14] G.J. Dutton (Ed.), *Glucuronic Acid: Free and Combined Chemistry, Biochemistry, Pharmacology, and Medicine*, Academic Press, New York, 1966.
[15] (a) F. Ledl, E. Schleicher, *Angew. Chem., Int. Ed. Engl.*, 102 (1990) 565–594. (b) M. Namiki, *Adv. Food Res.*, 32 (1988) 115–184.
[16] G. Banhegyi, L. Braun, M. Csala, F. Puskas, J. Mandl, *Free Radic. Biol. Med.*, 23 (1997) 793–803.
[17] (a) I.B. Cook, R.J. Magee, R. Payne, B. Ternai, *Aust. J. Chem.*, 39 (1986) 1307–1314. (b) S. Stojkovski, R.J. Magee, J. Liesegang, *Aust. J. Chem.*, 39 (1986) 1205–1212.
[18] (a) H.A. Tajmir-Riahi, *Bull. Chem. Soc. Jpn.*, 62 (1989) 1281–1286. (b) S.B. Etcheverry, P.A.M. Williams, E.J. Baran, *J. Inorg. Biochem.*, 63 (1996) 285–289.
[19] (a) A.M. da C. Ferreira, H.E. Toma, *J. Coord. Chem.*, 18 (1988) 351–359. (b) A.M. da C. Ferreira, L.L.O. Duarte, *J. Coord. Chem.*, 24 (1991) 339–350.
[20] (a) C. Rocha, A.M. da C. Ferreira, *Int. J. Chem. Kin.*, 26 (1994) 1121–1134; (b) C. Rocha, A.M. da C. Ferreira, *J. Braz. Chem. Soc.*, 6 (1995) 229–234.
[21] M.L.P. Santos, A. Faljoni-Alário, A.S. Mangrich, A.M. da, C. Ferreira, *J. Inorg. Biochem.*, 71 (1998) 71–78.
[22] (a) M.C. de A. Ribeiro, O. Augusto, A.M. da C. Ferreira, *J. Chem. Soc., Dalton Trans.* (1995) 3759–3766. (b) M.C. de A. Ribeiro, O. Augusto, A.M. da C. Ferreira, *J. Inorg. Biochem.*, 65 (1997) 15–23.
[23] (a) G.R. Buettner, L.W. Oberley, *Biochem. Biophys. Res. Commun.*, 83 (1978) 69–74. (b) E. Filkenstein, G.M. Rosen, E. Rauckman, *Arch. Biochem. Biophys.*, 200 (1980) 1–16.
[24] Y. Xu, *Anal. Chem.*, 67 (1995) 463R–473R.
[25] J. Romano, P. Jandik, W.R. Jones, P.E. Jackson, *J. Chromatogr.*, 546 (1991) 411–421.
[26] D.M. Whitfield, S. Stojkowski, B. Sarkar, *Coord. Chem. Rev.*, 122 (1993) 171–225.
[27] (a) S.J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 1–43. (b) S.J. Angyal, *Carbohydr. Res.*, 200 (1990) 181–188.
[28] R. Aruga, *Bull. Chem. Soc. Jpn.*, 54 (1981) 1233–1235.
[29] L. Nagy, H. Ohtaki, T. Yamaguchi, M. Nomura, *Inorg. Chim. Acta*, 159 (1989) 201–207.
[30] F.P. Sargent, E.M. Gardy, *Can. J. Chem.*, 54 (1976) 275–279.
[31] J. Rashba-Step, E. Step, N.J. Turro, A.I. Cederbaum, *Biochemistry*, 33 (1994) 9504–9510.
[32] H.D. Grimes, K.K. Perkins, W.F. Boss, *Plant Physiol.*, 72 (1983) 1016–1020.
[33] P. Riesz, D. Berdhal, C.L. Christman, *Environ. Health Perspect.*, 64 (1985) 233–252.
[34] E.G. Janzen, Y.Y. Wang, R.V. Shetty, *J. Am. Chem. Soc.*, 100 (1978) 2923–2925.

[35] P. Thornalley, S. Wolf, J. Crabbe, A. Stern, *Biochim. Biophys. Acta*, 797 (1984) 276–287.
[36] (a) P. Neta, L.M. Dorfman, *Adv. Chem. Ser.*, 81 (1968) 222–230. (b) C. Walling, *Acc. Chem. Res.*, 8 (1975) 125–131. (c) G. Czapski, A. Samuni, D. Meisel, *J. Phys. Chem.*, 75 (1971) 3271–3280.
[37] (a) H.S. Isbell, H.L. Frush, E.W. Parks, *Carbohydr. Res.*, 51 (1976) C5–C9. (b) H.S. Isbell, *Carbohydr. Res.*, 49 (1976) C1–C4.
[38] K. Uchida, S. Kawakishi, *Carbohydr. Res.*, 173 (1988) 89–99.
[39] W.K. Pogozelski, T.D. Tullius, *Chem. Rev.*, 98 (1998) 1089–1107.
[40] H.S. Isbell, H.L. Frush, Z. Orhanovic, *Carbohydr. Res.*, 36 (1974) 283–291.
[41] S.P. Wolff, R.T. Dean, *Biochem. J.*, 245 (1987) 243–250.
[42] Y. Hiraku, S. Kawanishi, *Cancer Res.*, 56 (1996) 1786–1793.
[43] G.B. Sajithlal, P. Chithra, G. Chandrakasan, *Free Radical Biol. Med.*, 25 (1998) 265–269.