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Studies of the Interaction of the Salicylideneserinatecopper(II) Complex with β-Cyclodextrin: Characterization and Reactivity of the Inclusion Compound

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Abstract. The compound formed by the copper-Schiff base complex salicylideneserinatecopper(II), [Cu(sal-ser)(H_2O)], interacting with β-cyclodextrin was prepared, and characterized in the solid state by infrared, UV-visible and EPR spectroscopies, X-ray diffraction, and thermoanalytical techniques. The catalytic activity of this compound, [Cu(sal-ser)CD], in the decomposition of hydrogen peroxide, and in the dismutation of superoxide radicals was also verified, in comparison with the reactivity of the free complex, in aqueous solution. In both cases, a decreasing in the reaction rate was observed for the CD-containing compound. The results of structural characterization, in addition to the substantial differences observed in the catalytic activities of the compounds, are indicative of partial insertion of the copper complex in the cavity of the oligosaccharide.

Key words: copper(II)-Schiff base complex, β-cyclodextrin inclusion compound, catalase activity, superoxide dismutase mimic

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of D-glucopyranose units linked by an $\alpha(1 \rightarrow 4)$ glycoside linkage. The commonly available cyclodextrins are those with 6, 7 and 8 glucose units, known as α, β, and γ-CD, respectively [1, 2]. The most important property of CDs is their ability to encapsulate a wide variety of molecules of appropriate size into their hydrophobic cavity, with the formation of the so called inclusion compounds [3–5]. This inclusion can modify

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the physicochemical properties of the guest molecules, increasing the interest for this type of compounds in research, as well as in industrial applications. CDs can impart water solubility to otherwise insoluble compounds [6]; they can also modulate reactions, protecting reactive substrates by inclusion, or stabilizing light- or oxygen-sensitive molecules.

Metal complexes included in CDs can be an excellent model of metalloenzymes, and consequently there is much interest in their use as catalyst, both in enzymatic and nonenzymatic reactions [7]. Recently, many studies have been reported on the effect of this encapsulation on the rates, products, and yields of different reactions [8–11]. Most of these studies described the coordination of the metal ions by modified CDs, which usually is very useful for analytical applications [12]. Particularly, copper complexes have been extensively investigated as models for biomolecules involved in oxidative processes [13–15].

In the present work, studies on the interaction of a slightly soluble copper-Schiff base complex, the salicylideneserinatecopper(II), [Cu(sal-ser)(H_2O)], with β-cyclodextrin are reported. Evidence for the inclusion of the complex into the cyclodextrin cavity was then investigated by usual structural techniques, including IR, UV-Vis and EPR spectroscopies, X-ray diffraction, and thermoanalytical methods. Comparative kinetic studies of the reactivity of the original copper complex, and the corresponding inclusion compound, towards hydrogen peroxide and superoxide radicals were also performed. The obtained results suggested an effective intercalation of the complex into the oligosaccharide.

1. Experimental

1.1. MATERIALS AND METHODS

The salicylideneserinatecopper(II) complex was prepared according to methods previously described in the literature [16, 17]. Salicylaldehyde (Aldrich, 0.24 mL) dissolved in 10 mL ethanol was added to an aqueous solution of L-serine (Sigma, 0.42 g in 2.5 mL H_2O), under constant stirring, and the resulting solution was refluxed for 4 hours, at 45–50 °C. The subsequent slow addition of an aqueous solution of copper(II)perchlorate hexahydrate (Aldrich, 1.9 g in 5 mL H_2O) led to the formation of a green solution, from which dark green crystals were obtained by filtration, after cooling. This solid was washed with cool water and ethanol, and dried in a desiccator under vacuum. The elemental analyses, performed at the Microanalytical Section of the University of São Paulo, are consistent with [Cu $C_{10}H_9O_4N$ (H_2O)]. The fourth coordination site of the metal is occupied by an aqua ligand. Calcd. (%): C, 41.59; H, 3.81; N, 4.85; Cu, 22.0; Found (%): C, 39.75; H, 3.67; N, 4.71; Cu, 20.5. The copper assay was performed spectrophotometrically with EDTA [18], and by atomic absorption spectroscopy.

The corresponding inclusion compound in β-cyclodextrin (Aldrich) was prepared in a 1 : 1 molar ratio by co-precipitation or freeze-drying methods [5, 19]. In the former, the salicylideneserinatecopper(II) complex previously obtained was

dissolved in ethanol, and added to an aqueous solution of β-CD, at room temperature, under constant stirring. The reaction mixture was kept at 40–50 °C for 15 hours, until the total volume was reduced to 10 mL. The light green solid obtained was then removed by filtration, and dried in vacuum, at room temperature. Results of copper analyses by atomic absorption spectroscopy indicated the ratio [1Cu : 1(β-CD)]. Calcd.: 4.56% Cu; Found: 3.80% Cu.

Physical mixtures of β-cyclodextrin and the salicylideneserinatecopper(II) complex were prepared by mixing both solids, carefully homogenized in a mortar for 15 minutes.

Hydrogen peroxide, 35% weight and free from stabilizers, was kindly supplied by Peroxidos do Brasil Ltda. All the solutions of peroxide were prepared by dilution of this reagent with deionized water, and analysed spectrophotometrically by the vanadate method, based on the formation of a vanadium (V)-peroxo complex [20], with maximum absorption at 454 nm (ϵ = 360 mol^{-1} dm^3 cm^{-1}).

Copper, zinc-superoxide dismutase (Cu_2Zn_2 SOD), xanthine, and xanthine oxidase were purchased from Sigma. The SOD activity was monitored by the inhibition of the reduction of nitroblue tetrazolium (NBT^{2+}, from Aldrich) with superoxide radicals, generated by the system xanthine/xanthine oxidase [21, 22]. The formation of formazan was monitored at 560 nm during 10 minutes, for different concentrations of the copper complexes.

1.2. INSTRUMENTATION

X-ray powder diffraction patterns were obtained in a Rigaku difractometer, fitted with a LiF monochromator, and employing Cu K_α radiation as source (15 kV, 15 mA). The TG/DTG curves were registered using a TA-4000 Mettler equipment, and the DSC curves using a Shimadzu DCS-50 instrument. All these curves were obtained under a nitrogen atmosphere, at a heating rate of 10 °C/min.

IR spectra were registered in a MATTSUM 3500-FTIR spectrometer, using KBr pellets, in the range 4000–500 cm^{-1}. UV-Vis spectra were obtained using a Beckman DU-70 instrument, using quartz cells of 1.000 cm optical length. Copper analyses were performed using a Spectroflame Modula instrument (from Spectro Co.), using 1.2 kW power, at 324.754 nm. The limit of detection is 0.002 μg/mL, and the sample was introduced at 1.5 mL/min.

EPR measurements of the pure solid copper(II) compounds were performed using a Bruker ESP 300E instrument, operating at X-band, at room temperature and at 77 K, using Wilmad suprasil tubes. EPR spectra of solutions of the complexes in acetonitrile were registered using a Bruker E 200-SR instrument, at room temperature, in standard flat cells.

The catalase activity of the compounds, in aqueous solution, was monitored by manometric measurements of the oxygen evolved, using a Warburg apparatus from B.Braun, model V-85, at constant temperature of (30.0 $\pm$ 0.1) °C, and pH = (10.8 $\pm$ 0.3), adjusted with concentrated NaOH solution.

2. Results and Discussion

Elemental analysis of the isolated (salicylideneserinate)copper(II)-cyclodextrin compound showed a 1 : 1 ratio of copper to β-cyclodextrin, as determined by atomic absorption spectroscopy (vide Experimental section). It was also observed that this compound is more soluble in water than the original copper complex.

The UV/Visible spectrum of the free (salicylideneserinate)copper(II) complex in pure N, N'-dimethylformamide (DMF) showed bands at 270 nm (ε = 10.6 $\times$ 10^3 mol^{-1} Lm^3 cm^{-1}), 368 nm (5.09 $\times$ 10^3 $mol^{-1}Lm^3$ cm^{-1}), and 634 nm (120 mol^{-1} Lm^3 cm^{-1}), attributed to ligand transitions, and a d-d band, respectively. In a DMF-aqueous solution (1 : 10, v/v), the d–d band shifted to 662 nm (ε = 99.4 mol^{-1} Lm^3 cm^{-1}), while the other bands appeared at 266 and 358 nm, exhibiting also a decrease in the molar absorptivity values ($\sim$10%).

On the other hand, the isolated compound with β-cyclodextrin, in saturated aqueous solution ($\sim 1 \times 10^{-3}$M), showed bands at 256 nm (ϵ = 5.4 $\times$ 10^3 mol^{-1} Lm^3 cm^{-1}), 358 nm (4.6 $\times$ 10^3 mol^{-1} Lm^3 cm^{-1}), and 656 nm (87.7 mol^{-1} Lm^3 cm^{-1}). When dissolved in DMF-aqueous solution (1 : 10, v/v), small variations in the electronic spectrum relatively to that of the free complex in the same solvent were verified. Bands with maxima at 268 nm (4.20 $\times$ 10^3 mol^{-1} Lm^3 cm^{-1}), 362 nm (3.81 $\times$ 10^3 mol^{-1} Lm^3 cm^{-1}), and 654 nm (88.2 mol^{-1} Lm^3 cm^{-1}) were observed in this case.

The XRD patterns of the compound β-cyclodextrin-(salicylideneserinate) copper(II) complex, obtained by freeze-drying and co-precipitation methods, are shown in Figure 1, compared with that of the physical mixture of the reagents. The compound prepared by co-precipitation showed a more crystalline pattern than the analogous compound obtained by the freeze-drying method. This can be due to the rapid decrease of the temperature, by immersion in liquid nitrogen, performed in the last method, which can lead to a less crystalline product [23]. The pattern verified with the physical mixture does not seem to be a sum of the XRD patterns of the free components. In this case an amorphous halo was observed, which can be ascribed to the coexistence of the non-included crystalline components and the inclusion compound [23, 24]. This observation was reinforced by the partial solubility in water of the physical mixture. Even in the solid mixture, some interaction between the copper(II) complex and the β-cyclodextrin can probably occur by hydrogen bond formation between the hydroxyl groups in the guest, and the O(6)H groups in the host [19]. It can also be a consequence of the higher release of water molecules inside and outside of the host, after the freeze-drying process. This evidence was verified by comparison of the TG curves of the inclusion complexes obtained by both methods. A loss of mass around 4% was observed with the product prepared by the freeze-drying method, while a 10% loss was obtained with the co-precipitation product, in the range of temperature 30–110°C.

The DSC curves of β-cyclodextrin, the (salicylideneserinate)copper(II) complex, the freeze-drying inclusion compound, and the physical mixture of the

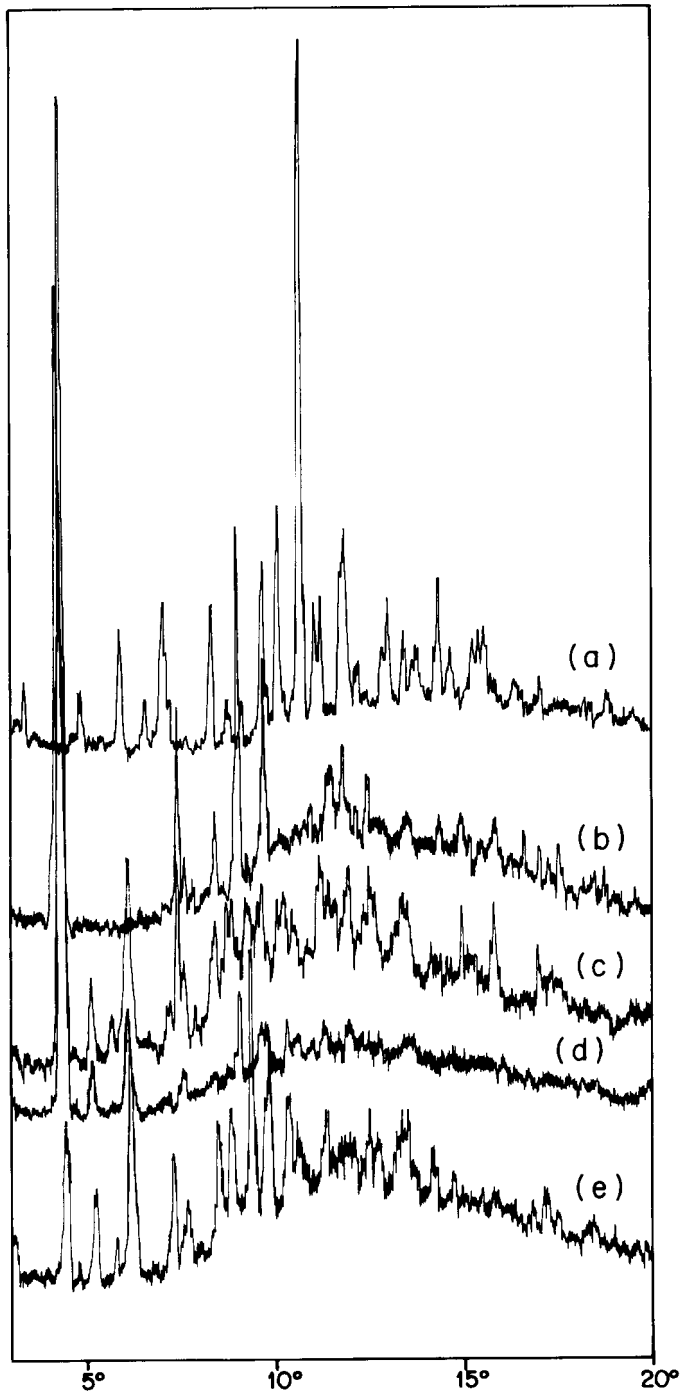

Figure 1. X-ray diffraction pattern of (a) β-cyclodextrin, (b) salicylideneserinate copper(II) complex, (c) corresponding 1 : 1 inclusion compound (co-precipitation method), (d) freeze-drying inclusion compound, and (e) physical mixture of the complex and β-cyclodextrin.

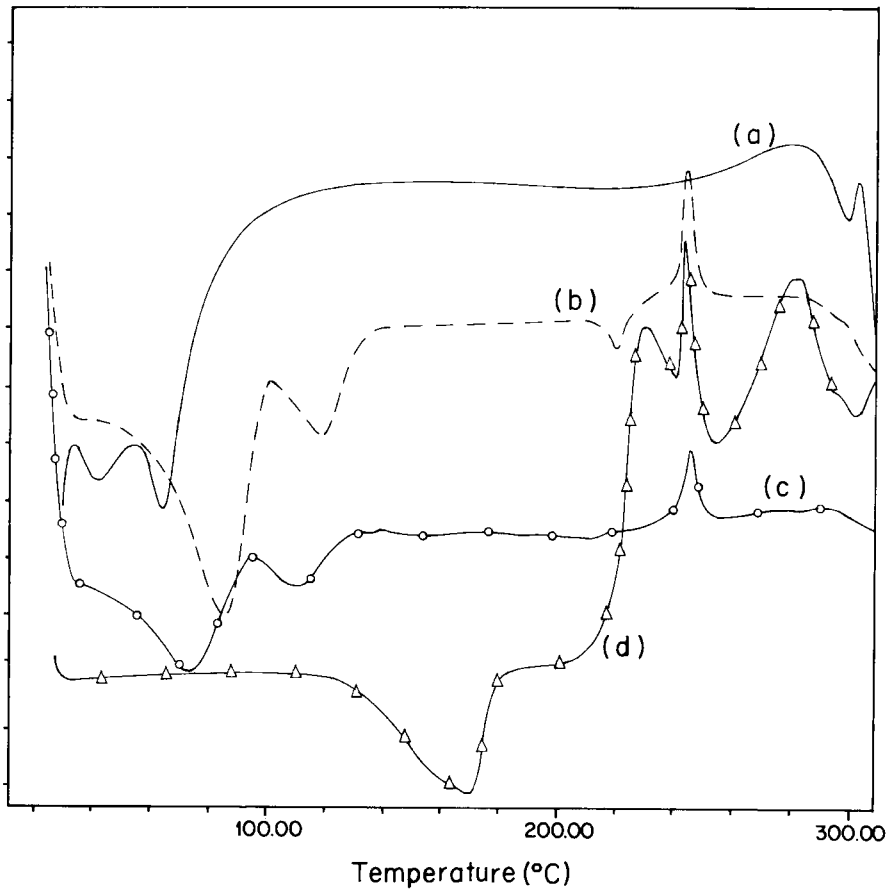

Figure 2. DSC curves of (a) β-cyclodextrin, (b) 1 : 1 inclusion compound of the complex in β-cyclodextrin (co-precipitation method), (c) physical mixture of the salicylideneserinatecopper(II) complex and β-cyclodextrin, and (d) free salicylideneserinatecopper (II) complex.

components, all in the solid state, are compared in Figure 2. The co-precipitation inclusion compound showed a DSC curve very similar to that registered for the freeze-drying inclusion compound (curve b, Figure 2). The β-cyclodextrin and the inclusion compound curves exhibited endothermic events at temperatures lower than 100 °C, corresponding probably to the release of water molecules in the host compound. For the physical mixture (curve c) this loss of water is much less substantial. On the contrary, the free complex (curve d) lost the coordinated water molecule only around 160 °C. Additionally, the physical mixture and the inclusion compound exhibited an exothermic peak around 240 °C, also observed in the guest, but that is not present in the analogous curve for the host compound. On the other hand, it can be observed in the DSC curve of the inclusion compound another

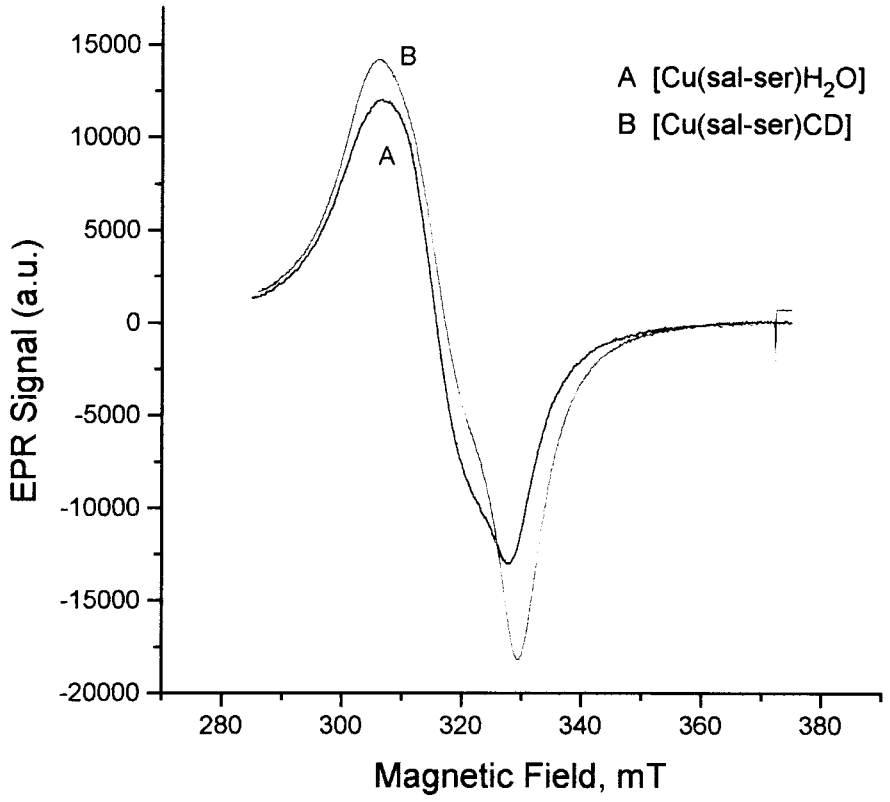

Figure 3. EPR spectra of the solid complexes, [Cu^{II}(sal-ser)H_2O] and the corresponding 1 : 1 inclusion compound in β-cyclodextrin, [Cu^{II}(sal-ser)CD], at 77 K. Frequency = 9.445 GHz, power = 2.00 mW, gain = 1.00 $\times$ 10^3, modulation amplitude = 0.1138 mT, modulation frequency = 100 kHz.

endothermic event around 220 °C. These results are indicative of soft host : guest interactions, occurring slightly in the physical mixture.

EPR spectra of the studied copper(II) complex and of the corresponding inclusion compound, prepared by the co-precipitation method, were registered at 77 K, as shown in Figure 3. In both cases, three g-values were estimated as for pure copper complexes where the exchange interaction is larger than the electron-copper hyperfine interaction, and hyperfine splitting is averaged into single exchange-narrowed lines. The g-values are in according to EPR spectra of pure copper complexes of ligands with N and O as coordinating atoms [25]. The g-values for the free complex are lower and less spread, g_1= 2.056, g_2 = 2.137 and g_3 = 2.204, than those for the inclusion compound: g_1 = 2.058, g_2 = 2.144 and g_3 = 2.214. This indicates that when included in the cavity of the β-cyclodextrin, in the solid state, the copper ion is submitted to a more distorted and weaker ligand field than that observed in the original complex.

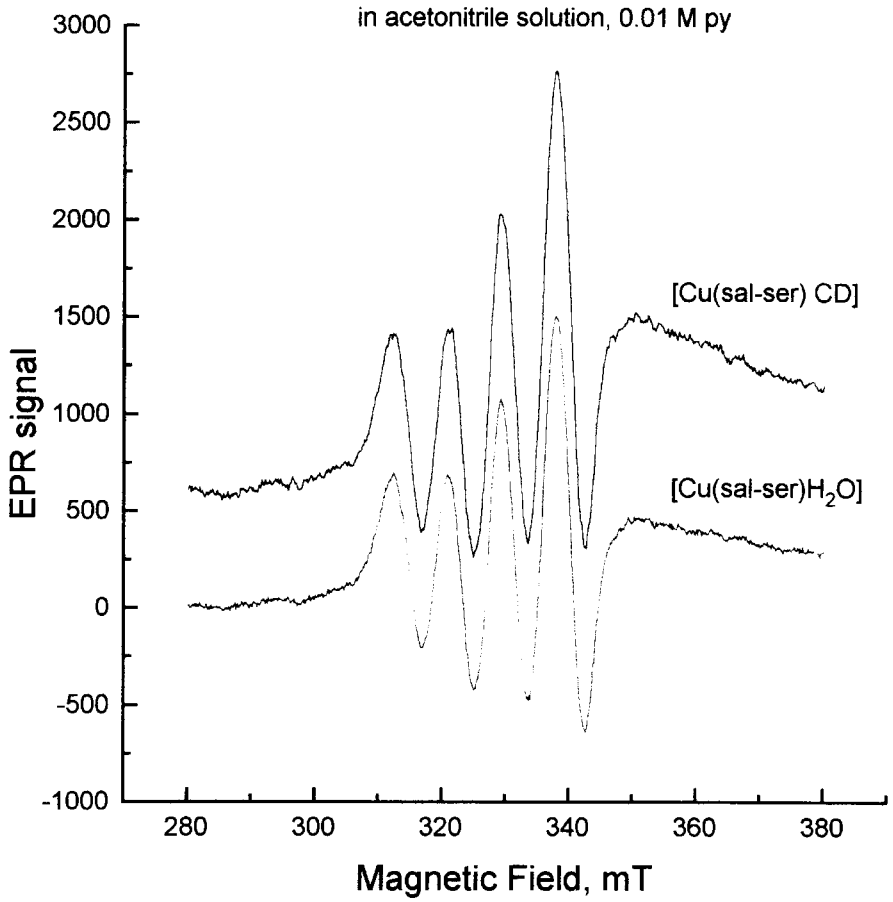

Figure 4. EPR spectra of the complex [Cu^{II}(sal-ser)H_2O], and of the corresponding 1:1 inclusion compound in β-cyclodextrin, [Cu^{II}(sal-ser)CD], dissolved in acetonitrile containing 0.01 M pyridine. Frequency = 9.690 GHz, power = 20.0 mW, gain = 1.00×10^5 (free complex) and 2.00×10^5 (inclusion compound), modulation amplitude = 1.25 mT, modulation frequency = 100 kHz.

For the free complex dissolved in *N*-methylformamide, a three-line pattern of superhyperfine splitting due to one ^{14}N atom bound to copper has been previously observed [26], with determined parameters $g_{iso} = 2.129$ ($A_{iso} = 73 \times 10^{-4}$ cm^{-1}) and $g_{\|} = 2.272$ ($A_{\|} = 184 \times 10^{-4}$ cm^{-1}), at room temperature. The coordinated solvent molecule occupying the fourth coordinating position does not seem to be coplanar with the chelate ring composed by the carboxylate oxygen, the imine nitrogen, and the phenolic oxygen.

We obtained similar results, for the free complex and for the inclusion compound, using acetonitrile as solvent. In addition, both the free complex and the inclusion compound gave identical EPR spectra in saturated acetonitrile solution, in the presence of 0.01 M pyridine, at room temperature (Figure 4), suggesting that

the coordination of pyridine in the fourth equatorial position leads to a transfer of the coordinating solvent to the axial position, in a pentacoordinated species [26], and also causes an exchange of the included complex by molecules of pyridine or the solvent in the β-cyclodextrin cavity.

The reactivity of the free complex and of the corresponding inclusion compound in β-cyclodextrin was then compared, in relation to the reagent hydrogen peroxide. Many copper complexes have shown catalase activity [27, 28], although native catalase enzymes are not copper dependent. More recently, the interaction of copper compounds with hydrogen peroxide has been related to oxidative damage of biomolecules [29, 30]. Manometric curves of the oxygen release during the catalytic decomposition of the peroxide were obtained, in aqueous solution, in the presence of both complexes. A first-order dependence of the initial rate on the concentration of copper compounds was determined, as shown in Figure 5, with rate constants $k_{obs} = 2.78 \times 10^{-2}$ and 1.64×10^{-2} s^{-1}, for the free complex and the inclusion compound, respectively. The influence of the hydrogen peroxide concentration was also verified, indicating a first-order dependence, followed by a saturation effect (vide Figure 6). The determined second-order rate constants, considering only the initial and linear part of the curves in Figure 6, were: k = 72.3, and 28.1 mol^{-1} dm^3 s^{-1}, for the free complex and the inclusion compound, respectively. The observed saturation effect is indicative of the coordination of the peroxide molecule to the copper center [28], in a pre-equilibrium to the rate determining step.

Accordingly, a decrease in the rate constant of circa 60% was noticed when the compound with β-CD was used, suggesting an effective insertion of the catalyst in the hydrophobic cavity of the β-cyclodextrin, and/or the formation of a lidlike association compound [3]. The potential ability of β-cyclodextrin to act as a free radical scavenger can partially explain this effect, since reactions of hydrogen peroxide in the presence of copper ions usually involve such reactive species [31], and hydroxylated substrates has been described as good scavengers of hydroxyl radicals in metal-catalyzed reactions [32]. However, the comparative results were sufficiently dissimilar to indicate specific interactions between the host and the guest molecules.

The catalytic activity of the studied complexes in the dismutation of superoxide radicals were also investigated, as many copper complexes with related ligands have been described as good SOD mimics [33]. In Figure 7, curves of the % of inhibition of the reduction of the nitroblue tetrazolium reagent, verified in the presence of the studied complexes, are compared with that of the native Cu_2Zn_2 SOD enzyme. Similarly to that observed in the catalase activity, a lower activity was also verified in the case of the inclusion compound in relation to the free complex.

The corresponding IC_{50} values obtained were 1.63×10^{-6} mol dm^{-3}, and 4.54×10^{-6} mol dm^{-3} for the free complex and the inclusion compound, respectively. For the native Cu_2Zn_2 SOD enzyme the determined IC_{50} value was 0.0059×10^{-6} mol dm^{-3}, which is in good agreement with reported values in the literature [34].

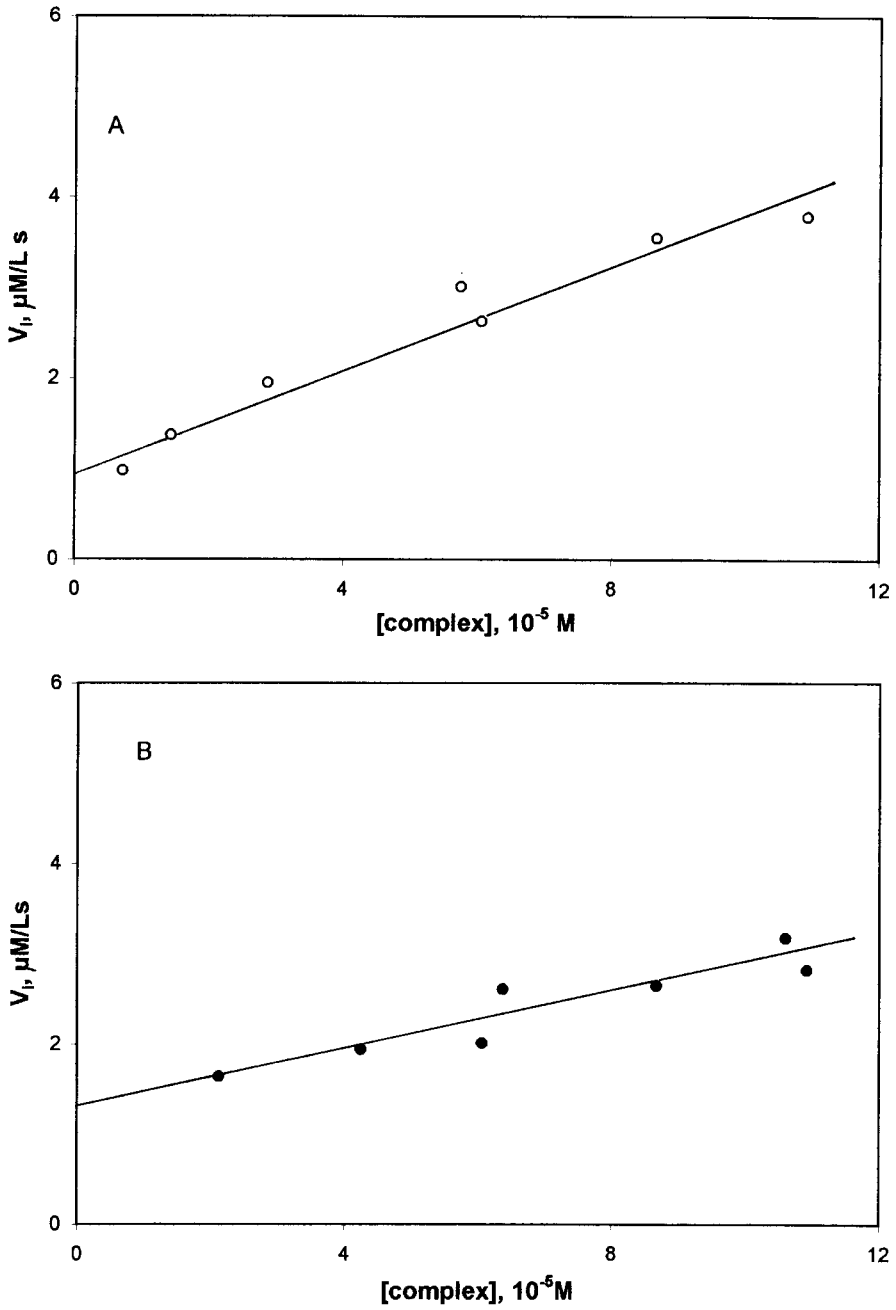

Figure 5. Dependence of the initial rate of the catalyzed hydrogen peroxide decomposition, in aqueous solution, with the catalyst concentration. (A) free complex, $[Cu^{II}(sal\text{-}ser)H_2O]$; (B) 1 : 1 inclusion compound in β-cyclodextrin. $[H_2O_2] = 18.2 \times 10^{-3}$ mol dm^{-3}, T = (30.0 ± 0.1)°C, and pH = 10.8.

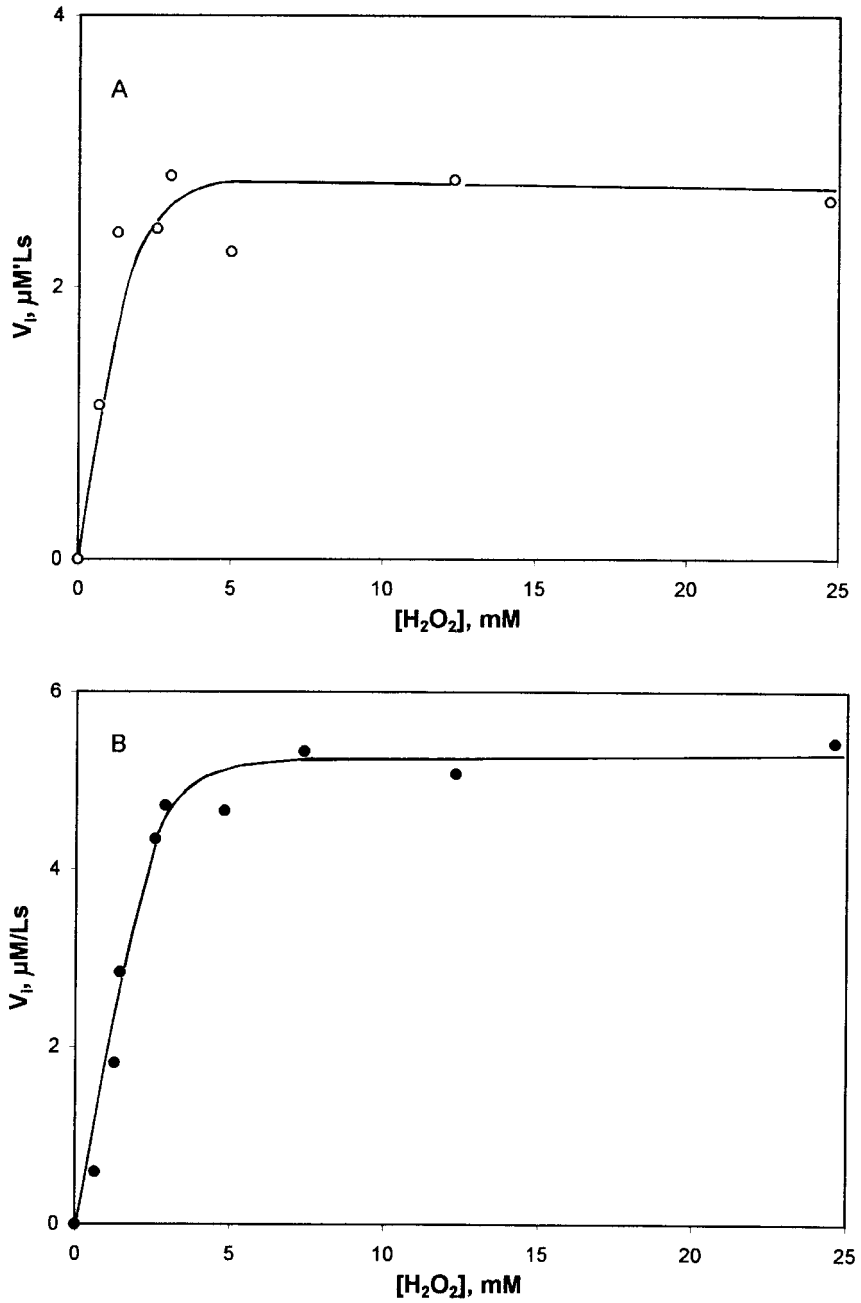

Figure 6. Influence of the hydrogen peroxide concentration on the initial rate of the catalyzed peroxide decomposition, in aqueous solution. Reaction at (30.0 ± 0.1)°C, pH = 10.8, with catalyst: (A) free complex, $[Cu^{II}(sal\text{-}ser)H_2O] = 12.6 \times 10^{-6}$ mol dm^{-3}; (B) 1 : 1 inclusion compound in β-cyclodextrin, $[Cu(sal\text{-}ser)CD] = 60.9 \times 10^{-6}$ mol dm^{-3}.

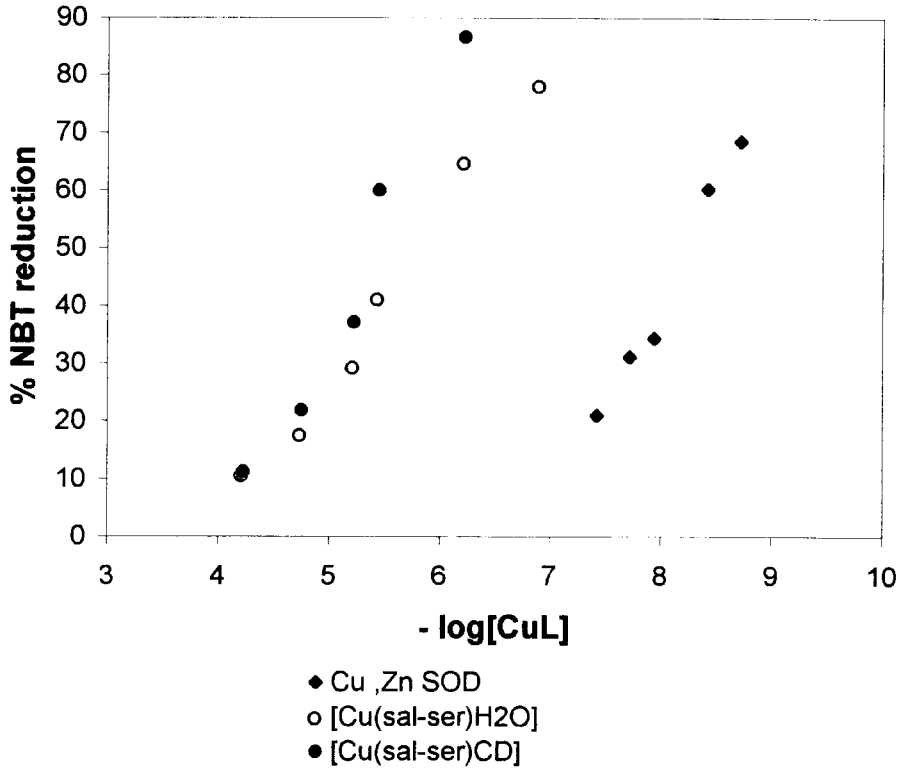

Figure 7. Curves of the percentage of inhibition of the nitroblue tetrazolium reduction, in aqueous solution, in the presence of copper complexes. Reaction at (25.0 ± 0.5)°C, and pH = 7.4 (phosphate buffer).

Recently, copper(II) complexes of difunctionalized β-cyclodextrins were prepared [35], with IC_{50} values in the range (0.12–0.30) $\times$ 10^{-6} mol dm^{-3}. Again, the differences verified in the catalytic parameters are sufficiently high to suggest the formation of an inclusion compound between the cooper complex, and the β-CD.

Therefore, the structural results, as well as the data of reactivity studies, are indicative of a partial insertion of the copper complex into the hydrophobic cavity of the β-cyclodextrin. The inhibitory effect observed both in the SOD and in the catalase activities can mainly be attributed to the fact that the reactive copper center is less available to the substrate, when included in the host structure. In both reactions the reactive oxygen species must interact with the copper center in the rate determining step [28, 36].

In addition, our results showed that the β-cyclodextrin framework provides a more tetrahedral geometry around the copper ion. Usually, the SOD activity is favored in a slightly distorted square-pyramidal environment [34], very similar to that of the native Cu_2Zn_2SOD.

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