J. Braz. Chem. Soc., Vol. 15, No. 6, 897-903, 2004.
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0103 - 5053 $6.00+0.00

Article

Solution Studies of Copper(II) Complexes as a Contribution to the Study of the Active Site of Galactose Oxidase

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Reportamos neste trabalho, a síntese, e a caracterização em solução dos compostos de coordenação de cobre(II) – [$Cu^{II}(H_2bbpeten)](NO_3)_2$, [$H_2$bbpeten = *N*-(2-hidroxibenzil)- *N*,*N*'-bis(2-piridilmetil)-*N*'-(2-hidroxietil)etilenodiamina]; [$Cu^{II}(H_3bpeten)](NO_3)_2$, [$H_3$bpten = *N*,*N*'-bis-(2-hidroxibenzil)-*N*-(2-piridilmetil)-*N*'-(2-hidroxietil)etilenodiamina]; [Cu^{II}(Hnbbpeten)]NO_3, [H_2nbbpeten, *N*-(5-nitro-2-hidroxibenzil)-*N*,*N*'-bis(2-piridilmetil)-*N*'-(2-hidroxietil)etilenodiamina] e [Cu^{II}(Hbnbpeten)], [H_3bnbpeten = *N*,*N*'-bis-(5-nitro-2-hidroxibenzil)-*N*-(2-piridilmetil)-*N*'-(2-hidroxietil)etilenodiamina] - com ligantes polidentados assimétricos N,O-doadores. Dois destes ligantes apresentam grupos - NO_2 em posição *para* aos grupos fenólicos. Estudos eletroquímicos foram realizados para os ligantes livres e para os complexos de cobre(II).

The synthesis and solution characterization of the coordination compounds – [$Cu^{II}(H_2bbpeten)](NO_3)_2$, [$H_2$bbpeten = *N*-(2-hydroxybenzyl)-*N*,*N*'-bis(2-pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine]; [$Cu^{II}(H_3bpeten)](NO_3)_2$, [$H_3$bpten = *N*,*N*'- bis- (2-hydroxybenzyl) -*N*-(2-pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine]; [Cu^{II}(Hnbbpeten)]NO_3, [H_2nbbpeten = *N*-(5-nitro-2-hydroxybenzyl)-*N*,*N*'-bis(2- pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine] and [Cu^{II}(Hbnbpeten)], [H_3bnbpeten = *N*,*N*'-bis-(5-nitro-2-hydroxybenzyl)-*N*-2-pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine] - are reported as copper(II) complexes containing alcoholic, pyridine and phenolic arms. Two of the ligands bear a NO_2 group at the *para* position of one or two of the phenolic moieties. Electrochemical studies have been performed for the proligands and for the copper complexes.

Keywords: Cu^{II} complexes, electrochemical properties, EPR, galactose oxidase

Introduction

Organic synthesis of unsymmetrical multidentate ligands that are necessary to enforce the desired coordination environments of the metal ions, has played an important role in the design of the active site of metalloprotein analogues.[1,2]

Copper in its various roles in biological systems displays different spectroscopic and chemical properties presumably because of the different ligand environments and coordination numbers.[3]

Galactose oxidase (Goase) is a mononuclear copper metalloenzyme which catalyses the oxidation of several primary alcohols to aldehydes. The geometry around the monomeric copper(II) center in galactose oxidase extracted from the fungus *Fusarium dendroides* at pH 4.5 can be described as square pyramidal, comprised of one tyrosyl oxygen atom, two histidine nitrogen atoms and one acetate oxygen atom in the plane and of a tyrosine oxygen atom occupying the apical position of the pyramid.[4, 5]

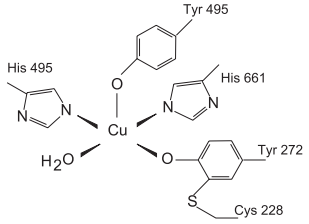

Figure 1. Design of the galactose oxidase active site, crystallografically characterized.

In the active and oxidized Goase form the enzyme contains coordinated Tyr-272. Unusual features of Tyr-272 are a thioether link in the *ortho*-position of the phenolate oxygen formed by the covalent binding to the

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S-atom of Cys-228. The major role of this thioether group is the lowering of the oxidation potential of the tyrosine, stabilizing the phenoxide oxidation product by several hundred millivolts.[6-9]

Thus the study of variously substituted proligands belonging to the same series seemed an essential prerequisite to the understanding and, consequently, the control of redox properties of the corresponding copper complexes.

We describe here solution studies of four copper(II) complexes derived from four unsymmetrical N,O-donor polyfunctional ligands, two of which bear no substituent at the phenol moieties (L1 and L2)[10-12] and two which bear the electron-withdrawing $-NO_2$ group at one or two of the phenol moieties (L3 and L4). These four compounds have been designed with the aim of studying the effect of $-NO_2$ group on the physicochemical properties of the copper complexes.

Experimental

Abbreviations

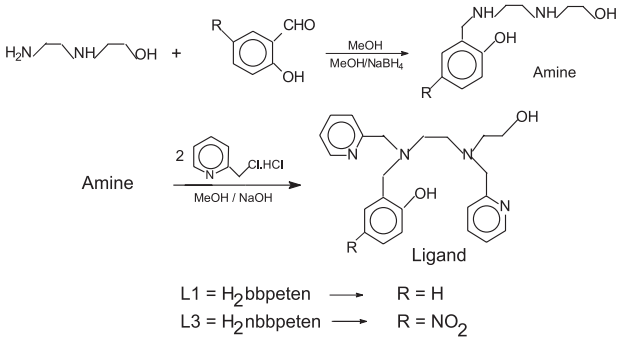
The following abbreviations are used throughout the text: H_2bbpeten, *N*-(2-hydroxybenzyl)-*N*,*N*'-bis(2-pyridylmethyl)-*N*'-(2-hydroxyethyl) ethane-1,2-diamine; H_3bpten, *N*,*N*'-bis-(2-hydroxybenzyl)-*N*-(2-pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine; H_2nbbpeten, *N*-(5-nitro-2-hydroxybenzyl)-*N*,*N*'-bis(2-pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine; H_3bnbpeten, *N*,*N*'-bis-(5-nitro-2-hydroxybenzyl)-*N*-(2-pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine; H_2beten, *N*-(2-hydroxybenzyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine; Hpeten, *N*-(2-pyridylmethyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine; H_2nbeten, *N*-(5-nitro-2-hydroxybenzyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine; H_3bnbeten, *N*,*N*'-bis-(5-nitro-2-hydroxybenzyl)-*N*'-(2-hydroxyethyl)ethane-1,2-diamine; Et_3N, triethylamine; $TBAPF_6$, tetrabutylammonium hexafluorophosphate; Fc^+/Fc, ferrocinium/ferrocene.

Materials

2-hydroxybenzyl alcohol (salicyl alcohol), 2-hydroxybenzaldehyde (salicylaldehyde), 2-pyridylcarboxyaldehyde, 2-bromomethylphenol, 2-hydroxy-5-nitrobenzaldehyde (5-nitrosalicylaldehyde), 2-picolyl chloride hydrochloride, *N*-(2-hydroxyethyl)ethylenediamine, 2-hydroxy-5-nitrobenzyl bromide (α-bromo-4-nitro-*o*-cresol), copper nitrate trihydrated and tetrabutylammonium hexafluorophosphate were obtained from Aldrich Chemical Co. For electrochemical and spectroscopic studies, high-purity solvents were used as received from Merck. High-purity nitrogen was used to deoxygenate solutions. All other chemicals and solvents were reagent grade.

Synthesis

*H_2bbpeten, (**L1**).*[10,12] This unsymmetric compound was obtained according to Scheme 1. The first step was a condensation reaction of *N*-(2-hydroxyethyl)ethylenediamine (2.60 g , 25 mmol) and salicylaldehyde (3.05 g , 25 mmol) followed by reduction with $NaBH_4$ (1.90 g, 50 mmol) in methanol producing N-(2-hydroxybenzyl)-N'-(2-hydroxyethyl)ethane-1,2-diamine (H_2beten). The final unsymmetric hexadentate proligand was synthesized by nucleophilic substitution of 2-(chloromethyl)pyridine hydrochloride (8.20 g , 50 mmol) previously neutralized with NaOH 4 mol L^{-1} with H_2beten (5.25 g , 25 mmol). Sodium hydroxide (4 mol L^{-1}, 5 mL) was added very slowly to the stirred and in a cool bath reaction.

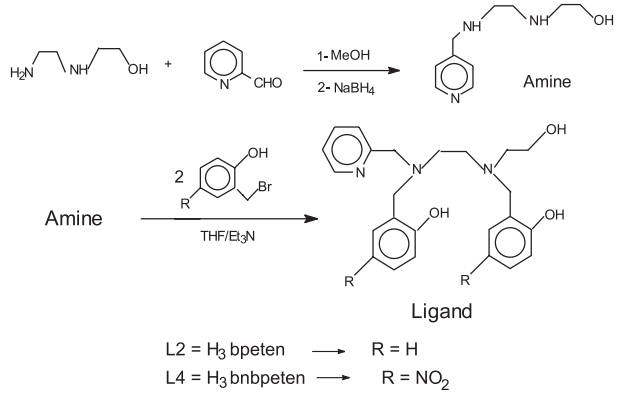


Scheme 1.

The proligand was extracted with eight 50 mL portions of $CHCl_3$, and the extracts were combined, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography employing ethanol-water (3:1) as eluent to afford 8.60 g (87.8%) of a yellow oil. IR ν_{max} /cm^{-1}: ν(O-H), 3250; ν(C=N, C=C), 1592, 1484, 1436; δ(O-H), 1368; ν(C-O), 1252. Anal. Calc. for $C_{23}H_{28}N_4O_2.H_2O$: C, 67.31; H, 7.32; N, 13.66. Found: C, 68.5; H, 7.5; N, 13.4%.

*H_3bpeten, (**L2**).*[11,12] This compound was obtained according to Scheme 2. The first step was a condensation reaction of N-(2-hydroxyethyl) ethylenediamine (2.60 g; 25 mmol) with 2-pyridinecarboxyaldehyde (2.68 g; 25 mmol), followed by reduction with $NaBH_4$ (1.90 g; 50 mmol) in methanol, producing N-(2-pyridylmethyl)-N'-(2-hydroxyethyl)ethane-1,2-diamine (Hpeten). The final unsymmetric hexadentate proligand was synthesized by

nucleophilic substitution of the 2-bromomethylphenol (3.74 g; 20 mmol), with Hpeten (1.95 g; 10 mmol) in 50mL of tetrahydrofuran under argon atmosphere. After the addition of Et_3N (22 mmol) and stirring for 24 h, a precipitate of Et_3NHBr formed, which was removed by filtration. After rotatory evaporation, a yellow oil was obtained. The proligand was extracted with eight 50 mL portions of $CHCl_3$, and the extracts were combined, washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. After concentration, the desired proligand was precipitated with acetone, filtered off, washed with propan-2-ol and dried under vacuum. The H_3bpeten was obtained as a pale yellow solid. Yield: 3.20 g (78.5%), mp=157 °C. Anal. Calc. for $C_{24}H_{29}N_3O_3.2C_3H_8O$**:** C, 68.24; H, 7.01; N, 7.97. Found: C, 68.2; H, 7.1; N, 7.5%. IR ν_{max}/cm^{-1}: ν(O-H), 3188; ν(C=N, C=C), 1592, 1488, 1456 and 1438; δ(O-H), 1368; ν(C-O), 1252.



Scheme 2.

*H_2nbbpeten, (**L3**).* This was also obtained according to Scheme 1, using 5-nitro-salicylaldehyde instead of salicylaldehyde. The H_2nbbpeten was obtained as a pale yellow oil. Yield: 4.08 g (99.6%). Anal. Calc. for $C_{23}H_{27}N_5O_4$**:** C, 63.14; H, 6.22; N, 16.01. Found: C, 63.2; H, 6.1; N, 15.9%. IR ν_{max} /cm^{-1}: ν(O-H), 3292; ν(C=N, C=C), 1617, 1589, 1512 and 1434; δ(O-H), 1335; ν(C-O), 1287; δ_{assim} (N=O)$_2$, 1589; δ_{sim} (N=O)$_2$, 1287 and δ_{C-N} (ArNO$_2$), 834.

*H_3bnbpeten, (**L4**).* This was also obtained according to Scheme 2, using 2-hydroxy-5-nitrobenzyl bromide instead the 2-bromomethylphenol. The H_3bnbpeten was obtained as a yellow solid. Yield: 3.18 g (91.4%), mp=208^{0}C. Anal. Calc. for $C_{24}H_{27}N_5O_7$**.:** C, 57.95; H, 5.43; N, 14.08%. Found: C, 58.2; H, 5.3; N, 14.6%. IR ν_{max}/cm^{-1}: ν(O-H), 3478; ν(C=N, C=C), 1.663, 1.597, 1.538 and 1.418; δ(O-H), 1343; ν(C-O), 1280; δ_{assim} (N=O)$_2$, 1597; δ_{sim} (N=O)$_2$, 1280 and δ_{C-N} (ArNO$_2$), 829.

*[Cu^{II}(H_2bbpeten)](NO$_3$)$_2$, (**1**), [Cu^{II}(H_3bpeten)](NO$_3$)$_2$, (**2**), [Cu^{II}(Hnbbpeten)]NO$_3$, (**3**) and [Cu^{II}(Hbnbpeten)], (**4**).* The coordination compounds were prepared by refluxing a methanolic solution of $Cu(NO_3)_2.3H_2O$ (0.24 g, 1.0 mmol) and the four proligands: H_2bbpeten – L1 (0.41 g, 1.0 mmol), H_3bpeten – L2 (0.53 g, 1.0 mmol), H_2nbbpeten – L3 (0.44 g, 1.0 mmol) and H_3bnbpeten – L4 (0.50 g, 1.0 mmol), with magnetic stirring for 1 h. All four reactions yield dark green precipitates, which were recrystallized in an acetonitrile-propan-2-ol (1:1) solution at room temperature. After a few days microcrystalline solids were filtered off, washed with propan-2-ol and dried with ether. The green crystals were unfortunately not suitable for X-ray diffraction measurements. Yield: (**1**) 0.32 g (55,2%), (**2**) 0.34 g (57.6%), (**3**) 0.38 g (56.6%) and (**4**) 0.30 g (53.6%). Anal. Calc. for $CuC_{23}H_{28}N_4O_2(NO_3)_2$ (**1**): C, 47.63; H, 4.83; N, 14.50%. Found: C, 47.8; H, 4.6; N, 14.3%. $CuC_{24}H_{29}N_3O_3(NO_3)_2$ (**2**): C, 48.44; H, 4.63; N, 11,77%. Found: C, 48.8; H, 4.8; N, 11.7%. $CuC_{23}H_{26}N_5O_4.NO_3$ (**3**): C, 49.15; H, 4.32; N, 14.96%. Found: C, 49.2; H, 4.8; N, 15.1%. $CuC_{24}H_{25}N_5O_7$ (**4**): C, 51.47; H, 4.64; N, 12.51%. Found: C, 50.9; H, 4.6; N, 12.6%.

Physical measurements

IR spectra were obtained on a FT-IR BOMEN - MICHELSON model MB spectrometer in KBr-disk or Nujol film. Elemental analyses were performed on a Perkin Elmer model 2400. EPR spectra were measured at 298 K and 77 K in a Bruker ESP 300E spectrometer. Molar Conductivity was measured in DMF (10^{-3} mol L^{-1}) at 298 ± 0.1 K with a Digimed CD-21. Visible and NIR spectra were recorded in DMF with a SHIMADZU model 2401 spectrometer. Cyclic voltammetry experiments were performed with a PAR 273 (Princeton Applied Research) in CH_3CN under argon at room temperature with 0.1 mol L^{-1} [TBA][PF_6] as the supporting electrolyte. Cyclic voltammograms were obtained by using a standard three-component system consisting of a carbon disk working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl reference electrode. The Fc$^+$/Fc couple[13] was used to monitor the reference electrode and was observed at +0.406 V *vs.* Ag/AgCl.

Results and Discussion

Syntheses

The H_2bbpeten, H_3bpeten, H_2nbbpeten and H_3bnbpeten characterization were established unambiguously by elemental analysis, and IR spectroscopy. They react in methanolic solution with $Cu(NO_3)_2.3H_2O$ to form the stable [Cu^{II}(H_2bbpeten)](NO$_3$)$_2$, (**1**), [Cu^{II}(H_2bpeten)](NO$_3$)$_2$, (**2**), [Cu^{II}(Hnbbpeten)]NO$_3$, (**3**) and [Cu^{II}(Hbnbpeten)], (**4**), which were isolated in good yields.

The IR spectra of **1**, **2**, **3** and **4** are similar to those of the proligands. They differ only in: (a) appearance of one well defined band at 3420, 3388, 3055 and 3416 cm^{-1}, respectively, attributed to the ν(O-H) stretching of uncoordinated primary alcohol; (b) the δ(O-H) in-plane bending of the phenol of the compounds **3** and **4**, at 1343 cm^{-1}, compared to those observed in the proligands, indicating that the phenol groups are deprotonated and coordinated; (c) row of bands at 1384 and 1268 cm^{-1} in the spectrum of **1**, **2**, and **3**, attributed to ν(N-O) of the NO_3^- counterion.

The molar conductivities of **1**, **2** and **3**, in DMF at 298 K are 154, 158 and 87 Ω^{-1} cm^2 mol^{-1}, respectively, which are consistent with 2:1, 2:1 and 1:1 electrolytes. The molar conductivity of **4** in DMF solution at 298K is insignificant which is consistent with it being a neutral coordination compound.[14]

Electronic absorption and electron paramagnetic resonance spectra

The electronic spectra of **1**, **2**, **3** and **4**, measured in DMF in the visible region are shown in Figure 2 and reveal the following transitions at λ_{max}/nm (ε/L mol^{-1} cm^{-1}): 426 (280) and 650 (111) for **1**; 424 (405) and 633 (114) for **2**; 387 (12,273), 473 (355) and 657 (111) for **3** and 390 (18,965) and 659 (117) for **4** that can be associated with metal-ligand coordination. Copper(II) ions can adopt square-planar, square-pyramidal, trigonal-bipyramidal, octahedral and tetrahedral geometries, which, except for the first, are generally distorted from the idealized structures. The d-d spectra shown by these coordination geometries are distinctive only in the case of the tetrahedral environment where the absorptions occur at much lower energies and generally show, in the distorted forms, well-separated absorption peaks; all the other geometries show closely spaced absorption manifolds.[3] The intense absorbance bands occurring at 387 and 390 nm for **3** and **4**, respectively, are assigned to $Cu^{II} \rightarrow O^-_{axial}$ and/or $O^-_{equatorial} \rightarrow Cu^{II}$ charge-transfer transitions. The bands in the ranges 659-633 (broad and distorted) and 424-473 nm are due to d-d transitions and suggest that the Cu^{II} atoms in the these complexes have similar distorted square-pyramidal geometries.[3,15-17]

The visible absorption spectrum of galactose oxidase is characterized by three absorption bands having molar extinction coefficients of the order of 1000 L mol^{-1} cm^{-1}. These occur at 444, 630 and 775 nm, the last two being broad and overlapping. The bands at 630 and 775 nm are assigned to d-d transitions and suggest that the copper(II) ion in the enzyme is in a somewhat distorted five-coordinated environment.[3,16] Thus, of the four copper (II) compounds described in this work, the $[Cu(H_3bpeten)](NO_3)_2$, (**2**) has chromophoric properties most similar to those found in the enzyme.

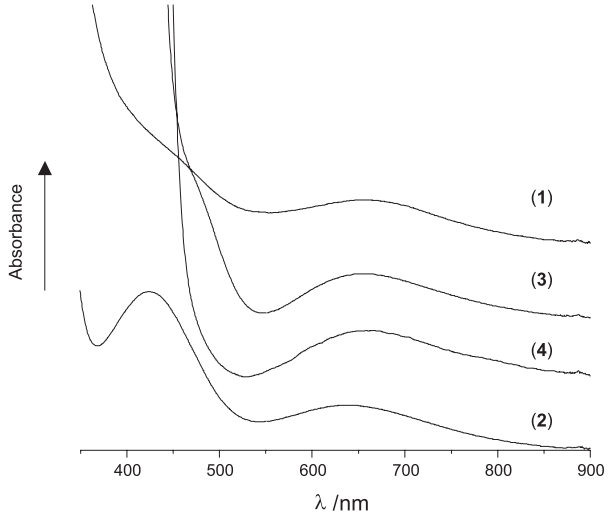


Figure 2. Electronic spectra of **1**, **2**, **3** and **4** in DMF, 10^{-3} mol L^{-1}.

Table 1. Electronic spectra data for the copper(II) compounds in DMF, and for GOase[16]

Compound	λ_{max} /nm (ε/mol^{-1} L cm^{-1})
$[Cu(H_2bbpeten)](NO_3)_2$, (**1**)	650(111) and 426(280)
$[Cu(H_3bpeten)](NO_3)_2$, (**2**)	633(114) and 424(405)
$[Cu(Hnbbpeten)]NO_3$, (**3**)	657(111); 473(355) and 387 (12,273)
[Cu(Hbnbpeten], (**4**)	665(73) and 390 (18,965)
Goase[16]	775(905); 630(1015) and 444(1155)

The X-band EPR spectrum of a frozen solution of **1**, **2**, **3** and **4**, in DMF are shown in Figure 3. The Hamiltonian parameters obtained from the spectra of these compounds and for Goase are resumed in Table 2.

The EPR spectra of the four copper(II) coordination compounds described here, indicate axial symmetry. For all four complexes, $g_{II} > g_{\perp} > 2$, suggesting distorted tetragonal, square-pyramidal or square-planar geometry. Moreover, the g_{II} and A_{II} values of $Cu[(H_2bbpeten)](NO_3)_2$, $[Cu(H_3bpeten)](NO_3)_2$, $[Cu(Hnbbpeten)]NO_3$ and [Cu(Hbnbpeten)] are found in the regions characteristic of CuN_4 and CuN_3O chromophores in the g_{II} *vs.* A_{II} diagram and near the value of galactose oxidase. Sagakushi and Addison[19] showed that the g_{II} / A_{II} ratio can be used as a convenient empirical index of tetrahedral distortion in CuN_4 units. This value ranges from *ca.* 105 to 135 cm for square-planar structure, and this quotient increases on the introduction of tetrahedral distortion to the chromophore.

Table 2. Parameters *g*, *A* (x 10^{-4} cm^{-1}) and g_{II} / A_{II} ratio for the compounds **1**, **2**, **3** and **4** and for GOase[18]

Compound	g_x	g_y	g_z	A_z	g_{II} / A_{II}
Goase[18]	2.058	2.048	2.273	176.5	129
Cu[(H_2bbpeten)](NO_3)$_2$, (**1**)	2.071	2.071	2.225	182.6	122
[Cu(H_2bpeten)](NO_3)$_2$, (**2**)	2.055	2.055	2.215	186.7	118
[Cu(Hnbbpeten)]NO_3, (**3**)	2,035	2,035	2.231	181.6	123
[Cu(Hbnbpeten)], (**4**)	2,040	2,040	2,241	178.6	125

Further, tetrahedral distortion of a square-planar chromofore is observed when any of biomimetic (N, O, S) donors reduces $A_{//}$ and increases $g_{//}$. Using that relationship and the results in Table 2, all the four compounds have a slightly tetrahedral distortion, that is, they seemed to be square-pyramidal. Probably the nitrogen atoms of **L1** and **L3** are in the equatorial plane of the respective compounds **1** and **3**. For the compounds **2** and **4** the equatorial ligand sets of the copper(II) ion are composed of three nitrogen atoms and one oxygen atom.

The increased $g_{//}$ values and decreased $A_{//}$ values in the compounds **2**, **1**, **3** and **4**, respectively, show that the ligand field strength decreases in these compounds in the same order. The complex [Cu(H_3bpeten)](NO_3)$_2$, (**2**) has a stronger ligand field than [Cu(H_2bbpeten)](NO_3)$_2$, [Cu(Hnbbpeten)]NO_3 and [Cu(Hbnbpeten)] ($A_{//}$ = 186.7 *vs.* 182.6, 181.6 and 178.6 x 10^{-4} cm^{-1} and $g_{//}$ = 2.215 *vs.* 2.225, 2.231 and 2.241) consistent with the more energetic d → d transition found in its electronic spectrum.

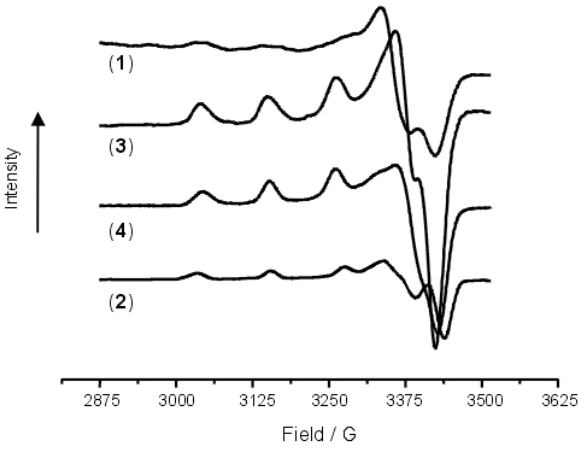


Figure 3. X-band EPR spectra of **1**, **2**, **3** and **4** in DMF at 77 K. The Hamiltonian parameters are: $g_{\perp}$ = 2.071, $g_{||}$ = 2.225 and $A_{||}$ = 182.6 x 10^{-4} cm^{-1} for (1); $g_{\perp}$ = 2.055, $g_{||}$ = 2.215 and $A_{||}$ = 186.7 x 10^{-4} cm^{-1} for 2; $g_{\perp}$ = 2.035, $g_{||}$ = 2.231 and $A_{||}$ = 181.6 x 10^{-4} cm^{-1} for 3; $g_{\perp}$ = 2.040, $g_{||}$ = 2,241 and $A_{||}$ = 178.6 x 10^{-4} cm^{-1} for **4**.

Electrochemistry

The redox properties of the four proligands and of the four copper(II) complexes were investigated by cyclic voltammetry in acetonitrile solution.

The electrochemical study of the four proligands focuses on the first one-electron oxidation of the phenolic units in view of studying the modulation of this property by the substituting groups. This may allow prediction of the ease of one-electron oxidation of the corresponding copper(II) complexes because the oxidation potential of the protonated free proligand is close to that of the corresponding copper(II) complex (protonation parallels metallation).[7] **L1**, **L2**, **L3** and **L4** exhibit an irreversible oxidation wave at E_{pa} = 0.79; 0.96; 0.91 and 1.20 V *vs.* Ag/AgCl (Table 3). These processes correspond to oxidation of the phenolic moieties leading to unstable radical cations, undoubtedly of the phenoxyl type. The E_{pa} values of the ligands are consistent with the electron-withdrawing character of the substituents on the phenolic arms. Compared with **L1**, the substitution by an electron-withdrawing group such as NO_2 (**L3**), increases E_{pa} by 0.12 V and compared with **L2**, the substitution by two electron-withdrawing group such as NO_2 (**L4**) increases E_{pa} by 0.24 V.

The cyclic voltammograms of **1**, **2**, **3** and **4**, in CH_3CN at scan rate of 100 mV s^{-1} are shown in Figures 4 and 5. In the four complexes, the ligands stabilize the copper(II) with respect to copper(I) state, since the reduction of the complexes occurs at low potentials (E_{pc} = -0.244; -0.700; -0.512 and -0.842 V for **1**, **2**, **3** and **4**, respectively). The cyclic voltammograms of **1** and **3** have a *quasi*-reversible redox couple at $E_{1/2}$ = -0.221 and -0.452 V *vs.* Ag/AgCl, (-0.626 and -0.857 V *vs.* Fc^+/Fc^0) respectively, which can be ascribed

Table 3. Anodic peak potential[a], E_{pa}, versus Ag/AgCl (Fc/Fc^+) for the first oxidation of the proligands **L1**, **L2**, **L3** and **L4**

Proligand	(*L1*), H_2*bbpeten*	(*L2*), H_3*bpeten*	(*L3*), H_2*nbbpeten*	(*L4*), H_3*bnbpeten*
E_{pa}/V[b]	0.79 (0.38)	0.96 (0.52)	0.91(0.50)	1.20 (0.76)

[a] v = 100 mV s^{-1}; [b] V *vs.* Ag/AgCl 10 mmol L^{-1} in CH_3CN + $TBAPF_6$ 0.1 mol L^{-1}.

to the $Cu^{2+} \leftrightarrows Cu^{+}$ redox couple. Compared with **1**, the presence of an electron-withdrawing group such as NO_2 in the coordination environment of **3** increases $E_{1/2}$ by 0.231 V. The cyclic voltammograms of **2** and **4** show a *ir*reversible reduction at E_{pc} = -0.700 and -0.842 V *vs.* Ag/AgCl, (-1.110 and -1.252 V *vs.* Fc^{+}/Fc^{0}) respectively, which can be ascribed to the $Cu^{2+} \rightarrow Cu^{+}$ process. The reduction process is irreversible for the two complexes and leads to a deposit of copper(0) on the electrode surface, as judged by the observation of a sharp oxidation peak during the reverse scan [E_{pa} = -0.242 and -0.278 V *vs.* Ag/AgCl (-0.652 and -0.688 V *vs.* Fc^{+}/Fc^{0}), respectively] with the typical features of a redissolution process.[9] However, compared with **2**, the substitution by two electron-withdrawing NO_2 group on the phenolic arms of the ligand in **4** also increases E_{pc} by 0.142 V. The most easily one-electron oxidizable proligand is H_2bbpeten – L1 that has one phenol moiety with no electron-donating or withdrawing group. The complex

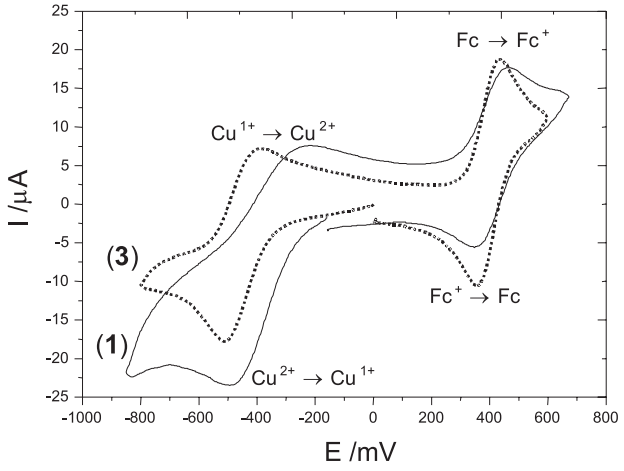


Figure 4. Cyclic voltammograms of **1** and **3** in CH_3CN (0.1 mol L^{-1} $TBAPF_6$), carbon working electrode, Ag/AgCl reference electrode and platinum wire electrode; scan rate 100 mV s^{-1}.

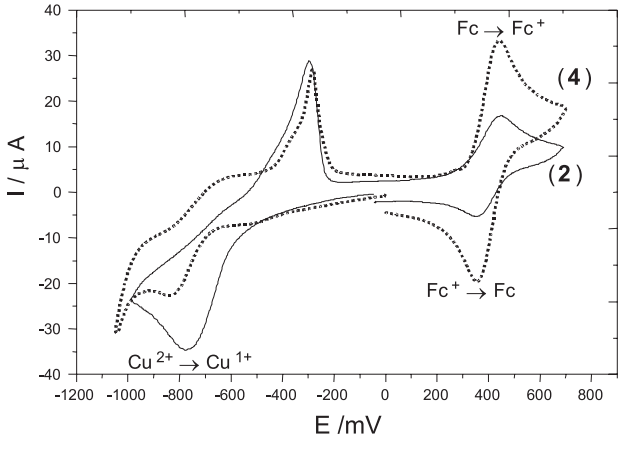


Figure 5. Cyclic voltammograms of **2** and **4** in CH_3CN (0.1 mol L^{-1} $TBAPF_6$), carbon working electrode, Ag/AgCl reference electrode and platinum wire electrode; scan rate 100 mV s^{-1}.

[$Cu(H_2bbpeten)](NO_3)_2$ **1** has a reduction potential more cathodically shifted than **2**, **3** and **4**.

Conclusions

Four Cu^{II} complexes containing four polyfunctional ligands, where two of which bear no substituent at the phenol moieties (**L1** and **L2**) and the other two bear an electron-withdrawing NO_2 group at one or two of the phenol moieties (**L3** and **L4**), have been prepared and characterized electrochemically and spectroscopically. The four compounds have absorption spectra bands in the ranges 665-636 (broad and distorted) and 422-463 nm due to d-d transitions. The EPR spectra showed that the four copper(II) coordination compounds have axial symmetry and a distorted square-pyramidal environment like the galactose oxidase. Electrochemical measurements gave E_{pa} values in agreement with the electron-withdrawing character of the substituents on the phenolic arms of the respective ligands. The substitution by $-NO_2$ stabilizes the copper(II) state by several hundred of millivolts. Electron-withdrawing substituent such as $-NO_2$ do not lower the oxidation potential of the phenol groups, just like the thioether linked in the *ortho*-position of the phenolate oxygen of the Tyr 272 of the enzyme Goase does. Further investigations of electron-donating substituents in the phenolic arms of the ligands are now in progress.

The major influence of the substitution of the phenolic moieties by electron-withdrawing substituent such as $-NO_2$ implies to us that it was not necessary to add base during the preparation of the copper complexes **3** and **4**, to obtain coordinated deprotonated phenol groups due the greater acidity of the respective ligands.

The results are consistent with the structures proposed for the copper(II) compounds presented in Figure 6.

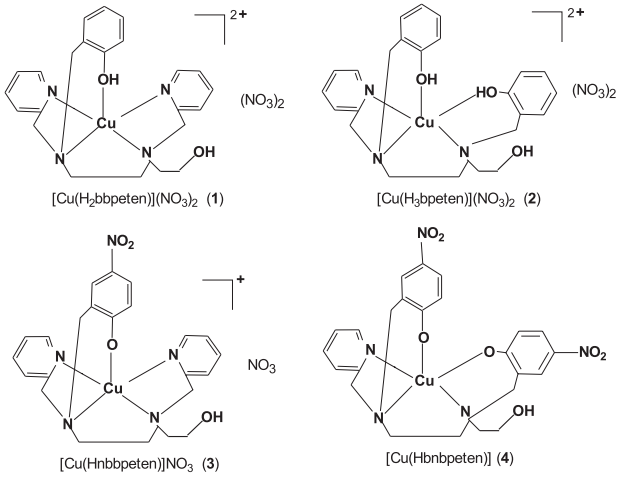


Figure 6. Proposed structures of **1**, **2**, **3** and **4**.

Acknowledgements

This work was supported by grants from Fundação Araucária, UFPR/TN and PADCT.

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Received: July 4, 2003
Published on the web: November 5, 2004