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Synthesis and evaluation of structural and functional biomimetic models of oxidase-type metalloenzymes based on polynuclear compounds with copper (II) and manganese (II) ions

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Abstract:

Biomimetic compounds are an alternative for to the limited action and fragile nature of enzymes. This work deals with the synthesis, characterization and evaluation of catalytic activity of two new biomimetic models for the active centers of *ascorbate oxidase* and *catalase*. $[\text{Cu}_3(\text{S},\text{S}(+)\text{cpse})_3(\text{H}_2\text{O})_3][\text{Cu}_3(\text{R},\text{R}(-)\text{cpse})_3(\text{H}_2\text{O})_3] \cdot 17\text{H}_2\text{O}$ (model for *ascorbate oxidase*, **1**), and $[\text{Mn}_2(\text{S},\text{S}(+)\text{Hcpse})_4(\text{NaClO}_4)_2(\text{NaOH})(\text{CH}_4\text{O})]_n \cdot [(\text{C}_2\text{H}_6\text{O})_2]_n \cdot [(\text{CH}_4\text{O})_2]_n$ (model for *catalase*, **2**) were prepared through the synchronic method (yields > 78%). The compound **1** has electronic and optical characteristics for racemic compound. The magnetic properties and electrochemical behavior evidence electronic transfer between metal centers. Meanwhile, the compound **2** showed polymeric properties in solid state and dimeric behavior in solution. Compound **1** was able to effectively catalyze the oxidation of ascorbic acid to dehydroascorbic acid (65.6% and 78.24% for racemic and enantiomeric pure compounds) showing structural and functional similarity to the natural enzyme. Besides, Compound **2** catalyzed the decomposition of hydrogen peroxide toward oxygen and water molecules (45%), evidencing that the prepared complex mimics the action of *catalases*. These two biomimetic models are relationship between them for the structural ligands, the coordination form to metal center and the catalytic activity as *oxidase*. This research shows the relationship with the design, evaluation, and comprehension of fundamentals aspects for the biomimetic models of active center of metalloenzymes that have importance for biological and industrial processes.

Keywords: Ascorbate oxidase; Biomimetics; Catalase, Coordination compounds; Peroxidase.

1. Introduction

Enzymes are biocatalysts highly studied in recent decades given their wide functional range. These can catalyze numerous reactions that include fundamental reactions, e.g., the formation of C-C bonds [1], [2]. The catalytic properties of enzymes are achieved thanks to the synergistic work among multiple factors: microenvironment, affinity, reactivity, and selectivity for substrates and high recognition abilities [3]. The enzymes can be used in the industry, but they are somewhat limited by its fragile nature, low thermal stability and high activity with organic solvents and metal ions. [4-5]. Then, to overcome the drawbacks in practical applications and preserve the functional properties of an enzyme, biomimetic catalysts have been proposed [6], [7]. Among the many catalysts that have been synthesized for multiple applications are found functional binuclear biomimetic catalysts containing manganese. This kind of catalysts is used in water oxidation taking advantage lowest oxidation state of the manganese [8]. Furthermore, biomimetic oxidases (belonging to the oxidoreductive class) are able to catalyze oxidation-reduction reactions using dioxygen as an electron acceptor, leading to the formation of water or hydrogen peroxide as a by-product [9], [10].

On the other hand, natural enzymes such as *laccase* and *ascorbate oxidase* (AO) are part of multicopper oxidases and blue multilayer oxidases [11]. Generally, these enzymes have four copper atoms in their active center, which participate in the decline of oxygen and the production of water [12], and contributes to the degradation of dangerous organic pollutants such as phenols, chlorophenols and pharmaceutical products [13]. This biocatalytic behavior is extremely useful for application in various biotechnological processes, e.g., the detoxification of effluents, mainly from the pulp and paper, textile, and petrochemical industries. Moreover, *laccases* and *AO* are also used as catalysts in the pharmaceutical industry for development anticancer drugs [12], [14].

AO has been extensively investigated, and this is one of the first enzymes whose structure and active center were elucidated [10]. This metalloenzyme acts on L-ascorbic acid (AA), the most abundant antioxidant in the plant tissues (at millimolar “mmol L⁻¹” levels). At the cellular level, AA is found in the cytosol, chloroplasts, vacuoles, mitochondria, and apoplasts. The AA concentration is especially high in the cytosol and chloroplasts; ranging between 10 and 20 mM, respectively. The catalytic action of AO on AA and O₂ produces 2-Dehydroascorbate (DHA) and H₂O molecules. DHA is then transported to the cytosol through the plasma membrane by a specific carrier transferring between the oxidized form and the reduced form, ensuring a continuous flow of energy reduction to the cell wall, **scheme 1**.

Scheme 1. Reaction catalyzed by *Ascorbate Oxidase* enzyme (AO).

As above-mentioned AO is a multi-copper oxidase, related to laccase and ceruloplasmin. AO has diverse domains. For instance, the X-ray structure of AO from zucchini shows that the monomer of 552 residues is built up by three domains arranged sequentially on the polypeptide chain. The folding of all three domains is of a β -barrel type. Despite, each monomer has three disulfide bridges. It contains a mononuclear type 1 copper site in domain 3 and a trinuclear type 2/type 3 copper site between domains 1 and 3. The mononuclear copper site has the four canonical type 1 copper ligands (His, Cys, His, Met) also found in plastocyanin and azurin. The trinuclear copper site has eight histidine ligands (symmetrically supplied by domains 1 and 3) and two oxygen ligands. The type 3 copper pair is bridged by an OH^- or O_2^- . The remaining copper has two histidine ligands and an OH^- or H_2O ligand. The average copper–copper distance in oxidized AO is 3.74 Å. The shortest distance between the type 1 copper center and the trinuclear copper center is 12.2 Å. In the fully reduced form, the trinuclear site undergoes strong changes. The bridging oxygen ligand is released and the copper–copper distances are reported with a range of 3.7 Å to 5.1 Å for the copper pair, and to around of 4.2 Å for the third copper atom. In the peroxide form, the peroxide is bound end-on to one copper atom pair while two azide molecules are bound in the same copper atom. Spectroscopic and kinetic studies reveal that the entry site for electrons from the reducing substrate is the mononuclear copper and the dioxygen binding site is the trinuclear copper species. The enzyme acts in a ‘two - site ping - pong bi - bi’ mechanism.

Regarding enzymes containing manganese, we can mention that these catalysts are widely used in the transformation of compounds (e.g., peroxidases that use hydrogen peroxide to catalyze reactions [15]). Many peroxidases have been identified in fungal species [16]. Indeed, manganese peroxidase is the most common lignin-modifying peroxidase, and this is produced by almost all wood-colonizing basidiomycetes that cause white rot. This enzyme requires hydrogen peroxide as a co-substrate oxidizing Mn^{2+} ion present in wood and soil to Mn^{3+} ion (Equation (1)), which is stabilized by fungal chelates such as oxalic acid [17]. Manganese peroxidase is unique among plant and fungal, and it is dependent on hydrogen peroxide to oxidize/transform a wide variety of phenolic substrates, including lignin itself [18]. In general, the main component of the active center of catalases is manganese, as a metallic center. This can present different oxidation states depending on the activity, bound anionic ligands and charge compensation with other atoms of the same nature (iron or manganese itself). An acid medium favors the reduction of Mn^{3+} ion to Mn^{2+} ion ($E^\circ = 1.559 \text{ V}$, Equation (2)), which enhances its oxidative capacity against substrates such as hydrogen peroxide or organic molecules such as lignin [8].

Since eighties of past century, complexes of manganese have shown to be relevant in biological systems [19]. Currently, binuclear complexes of Mn^{2+} - Mn^{2+} have been synthesized for the construction of biomimetic sensors for the determination of phenolic compounds. These complexes show mimic efficacy to the active site of *Manganese peroxidase* [19], specifically in the bind to nitrogen and oxygen donor atoms [20]. In the context of preparation and evaluation of multi-nuclear catalysts, the design, synthesis and testing of two new biomimetic models of *AO* and *catalase* are reported in this work. For the *AO* biomimetic model is presented a trinuclear copper compound as structural and functional model of its active center.

The first compound, $[\text{Cu}_3(\text{S,S}(+)\text{cpse})_3(\text{H}_2\text{O})_3][\text{Cu}_3(\text{R,R}(-)\text{cpse})_3(\text{H}_2\text{O})_3] \cdot 17\text{H}_2\text{O}$ (**1**), has chiral aminoalcohols forming a racemic unit in the crystalline cell. This compound also contains a trinuclear arrangement conformed for three copper atoms such as in the *AO* center active. This was characterized through electronic analyses, magnetism measurements and electrochemical techniques, and the catalytic activity on ascorbic acid as substrate was evaluated and followed using HPLC- UV. The other synthesized compound was a binuclear manganese (II) compound, $[\text{Mn}_2(\text{S,S}(+)\text{Hcpse})_4(\text{NaClO}_4)_2(\text{NaOH})(\text{CH}_4\text{O})]_n \cdot [(\text{C}_2\text{H}_6\text{O})_2]_n \cdot [(\text{CH}_4\text{O})_2]_n$ (**2**). This last complex presented similar structural and geometric environment in relationship to catalases. Electronic and electrochemical properties of the compound (**2**) were evaluated in dissolution and solid state using electronic paramagnetic resonance (EPR), magnetic susceptibility at variable temperature and X-ray photoelectron spectroscopy (XPS). In solution was reported an electronic transfer of the system to the medium stabilizing oxidation states Mn^{3+} , which facilitates its catalytic activity. This change was determined electrochemically, by comparison to the oxidation of ferrocenecarboxaldehyde. Finally, taking advantage of its dimeric unit in solution, the catalytic oxidation of H_2O_2 (as substrate) to O_2 was evaluated, using spectrophotometry. It is important to remark that this research contributed with the design, evaluation and comprehension of fundamentals aspects for two biomimetic models of active center of metalloenzymes relevant for biological and industrial processes.

2. Materials and Methods

2.1. Materials

$\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ (Merck), and methanol (J.T. Baker) were used without further purification. The synthesis of ligands N-[2-hydroxy-1(R)-methyl-2(R)-phenylethyl]-N-methylglycine (R,R(-)Hcpse and N-[2-hydroxy-1(S)-methyl-2(S)-phenylethyl]-N-methylglycine (S,S(+)Hcpse), was carried out as described previously in reference [28].

$\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ (Merck), NaClO_4 (J.T. Baker), NaOH (J.T. Baker), and methanol (J.T. Baker) were used without further purification. The tests of peroxidase and catalase activities used DPPH (2,2-diphenyl-1-picrylhydrazyl) and peroxide hydrogen 30% (Aldrich), respectively.

Ascorbic acid (Merck). The isomer $[\text{Cu}_3(\text{R,R}(-)\text{cpse})_3(\text{H}_2\text{O})_3] \cdot 17\text{H}_2\text{O}$ was synthesized following the guidelines reported by [8].

2.2. Synthesis of the biomimetic compounds

For the synthesis of compound $[\text{Cu}_3(\text{S,S}(+)\text{cpse})_3(\text{H}_2\text{O})_3][\text{Cu}_3(\text{R,R}(-)\text{cpse})_3(\text{H}_2\text{O})_3] \cdot 17\text{H}_2\text{O}$ (**1**), mononuclear compounds $[\text{Cu}(\text{R,R}(-)\text{Hcpse})_2] \cdot 2\text{H}_2\text{O}$ (339 mg, 0.65 mmol) reported previously [28] and $[\text{Cu}(\text{S,S}(+)\text{Hcpse})_2] \cdot 2\text{H}_2\text{O}$ (343 mg, 0.66 mmol) dissolved in methanol (20 mL), plus 4 equivalents of

solid NaOH (104 mg) and $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ (264 mg, 1.2 mmol) were mixed. After a period of four weeks, blue crystals suitable for X-ray studies were obtained, yield 78.7%. Anal. Calcd. For $2(\text{C}_{36}\text{H}_{51}\text{N}_3\text{O}_{12}\text{Cu}_3) \cdot 17(\text{H}_2\text{O})$: C%, 36.1; H%, 6.87; N%, 3.52. Found: C%, 35.59; H%, 7.05; N%, 3.45. IR (KBr cm^{-1}): 1615 ($\nu_{\text{asCOO-}}$), 1385 ($\nu_{\text{sCOO-}}$), $\Delta\nu=230\text{cm}^{-1}$.

The synthesis of compound $[\text{Mn}_2(\text{S,S}(+)\text{Hcpse})_4(\text{NaClO}_4)_2(\text{NaOH})(\text{CH}_4\text{O})]_n \cdot [(\text{C}_2\text{H}_6\text{O})_2]_n \cdot [(\text{CH}_4\text{O})_2]_n$ (**2**) and its enantiomer involved a solution of $\text{S,S}(+)\text{H}_2\text{cpse}$ (479 mg, 2.14 mmol) in methanol (15 mL), added to $\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ (265 mg, 0.108 mmol). This mixture was stirred for 15 min. Then, NaClO_4 (13 mg, 0.109 mmol), in basic pH adjusted with NaOH solid, was added to the mixture. This last was stirred for 15 min. After two weeks, brown needles suitable for X-ray diffraction analysis were obtained. Yield 84% (261 mg). The compound showed to be hygroscopic. Anal. Calcd for $\text{Mn}_2\text{C}_{54}\text{H}_{91}\text{Cl}_2\text{N}_4\text{Na}_3\text{O}_{28}$: C%, 42.90; H%, 6.21; N%, 3.71. Exp.: C%, 43.15 %; H% 5.77; N% 3.84. IR (KBr cm^{-1}): 1569 ($\nu_{\text{as COO-}}$) and 1443 ($\nu_{\text{s COO-}}$) $\Delta\nu=126\text{ cm}^{-1}$. MS: m/z $[\text{M}]^+$, 99(87), 305(57), 146(38), 575(32), 875(30), 1179(13), 1098(12).

2.3. Physical measurements

Infrared spectra in the range 4000-400 cm^{-1} were recorded on a Nicolet FT-IR 740 spectrophotometer using KBr pellets. Elemental analyses were performed on a Fisons EA 1108 elemental analyser. Electronic spectra (diffuse reflectance) were measured on a Cary 5E Uv-Vis-NIR spectrophotometer over the range 250 - 2500 nm (40000 - 5000 cm^{-1}). Magnetic susceptibility measurements were carried out with a pendulum-type magnetometer (MANICS DSM8) equipped with an Oxford CF 1200 S helium continuous-flow cryostat working in the temperature range 300 - 4 K in a magnetic field of 3 Oe. Diamagnetic corrections were estimated using Pascal constants. Powder diffraction was recorded on SIEMENS D500, graphite-monochromated $\text{Cu-K}\alpha$ ($\lambda=1.5406\text{ \AA}$) radiation at 293 K, CT: 0.6 s, SS: 0.020 dg, WL= 1.5406 $\text{\AA}$. The chemical analysis was obtained using X-ray photoelectron spectroscopy (XPS). This was performed using a VG Microtech ESCA2000 Multilab UHV system, with an Al $\text{K}\alpha$ x-ray source ($h\nu = 1486.6\text{ eV}$), and a CLAM4 MCD analyzer. XPS spectrum was obtained at 55° from the normal surface in the constant pass analyzer energy mode (CAE), $E_0 = 50$ and 20 eV for survey and high-resolution narrow scan. Peak positions were referenced to the background silver 3d5/2 core level at 368.20 eV, having a FWHM of 1.00 eV, Au 4f7/2 in 84.00 eV and C 1s hydrocarbon groups in 285.00 eV central peak position. The XPS spectra were fitted with the program SDP v 4.1. The XPS error is based considering a detection limit estimated to be 0.1% in mass and uncertain propagation. For deconvolution analysis the uncertain was estimated at 5% (ie $\pm 0.05\text{ eV}$).

2.4. Electrochemical analyses

Cyclic voltammetry (CV) experiments for the copper-based compound (**1**, biomimetic model for the AO) were conducted with a CH Electrochemical Analyzer (CH Instruments, Inc.) in dry, degassed methanol with tetrabutylammonium hexafluorophosphate (TBAPF₆, 0.1 M) as a supporting electrolyte. Differential pulse voltammetry (DPV) studies were conducted to confirm the presence of all redox waves (pulse amplitude of 0.020 V, pulse width of 0.050 V, a sample with of 0.020 V, and a pulse period of 0.100 sec). Final concentrations of all compounds were 1 mM, with a final volume of 5 mL, and all samples were degassed for 5 min with N_2 before each experiment. CV experiments were conducted with a scan rate of 100 mV/s, and the samples were cycled between -2.0 V and 2.0 V using a glassy carbon working electrode, a Pt counter electrode, and a Ag/AgCl (+0.197 V vs. NHE5) reference electrode.

Electrochemical analyses for the manganese-based compound (**2**, biomimetic as model for catalase) were carried out in a conventional three-electrode cell, with a glassy carbon disc ($\phi = 3\text{ mm}$),

a platinum mesh and a saturated calomel electrode (SCE) as working, auxiliary and reference electrodes, respectively. The supporting electrolyte was n-tetrabutylammonium hexafluorophosphate (n-Bu₄NPF₆) 0.1 M. The reference electrode was connected to the working solution by means of a salt bridge containing the same supporting electrolyte. Distilled acetonitrile was employed as solvent. All the experiments were carried out under an argon atmosphere and at room temperature. Cyclic voltammetry was carried out at a scan rate of 0.1 Vs⁻¹, unless otherwise stated. All potentials are reported versus the Saturated Calomel Electrode.

2.5. X-ray crystallographic study

For [Cu₃(S,S(+)-cpse)₃(H₂O)₃][Cu₃(R,R(-)-cpse)₃(H₂O)₃].17H₂O (**1**); all X-ray diffraction data were measured using standard procedures on a Nonius Kappa instrument with CCD area detector using graphite-monochromated Mo-K α radiation at 293 K. Intensities were measured using ω + φ scans. The structure was solved using direct methods, using SHELX-97 and the refinement (based on F² of all data) was performed by full-matrix least-squares techniques with Crystals 12.84. All non-hydrogen atoms were refined anisotropically and all hydrogen atoms were placed geometrically and allowed to ride on their respective atoms. For [Mn₂(S,S(+)-Hcpse)₄(NaClO₄)₂(NaOH)(CH₄O)]_n·[(C₂H₆O)₂]_n·[(CH₄O)₂]_n (**2**), X-ray diffraction data were measured using on an Agilent Xcalibur Atlas Gemini diffractometer using graphite-monochromated Mo-K α radiation and ω scans at 130 K. Analytical numeric absorption correction using a multifaceted crystal was applied.

The structure was solved using direct methods, using SHELXS-2012 and the refinement (based on F² of all data) was performed by full-matrix least-squares techniques with SHELXL-2014.²⁴ The perchlorate anions, two phenyl rings (C21→C26 and C27→C32), coordinated and un-coordinated solvent molecules, exhibit orientational and/or statistical disorder which was modeled (mainly) over two positions. All disordered atoms (except the solvent methanol molecule C49-O22 which turn to be NPD and was refined isotropically) were refined with restraints (SIMU and DELU) on anisotropic displacement parameters and the bond geometries of the disordered groups were constrained or restrained to be similar with the AFIX or SADI commands of SHELXL. In addition, the contribution of a smeared un-coordinated solvent was "squeezed" using the PLATON program, **Table 1**.

Table 1. Crystal data and structure refinement for compound (**1**) and (**2**)

Chemical formula	2(C ₃₆ H ₅₁ N ₃ O ₁₂ Cu ₃) · 17(H ₂ O) (1)	C ₅₁ H ₇₉ Cl ₂ Mn ₂ N ₄ Na ₃ O ₂₅ (2)
Formula weight (g mol ⁻¹)	2123.17	1397.93
Crystal size (mm)	0.20 x 0.15 x 0.15	
Crystal color	Blue	dark purple
Crystal system	Trigonal	Monoclinic
Space group	R-3c	C2
Unit cell dimensions		
a (Å)	15.5811 (2)	21.5749(8)
b (Å)	15.5811 (2)	18.8865(5)
c (Å)	72.7841 (9)	17.6692(6)
α (°)	90.0	90.0
β (°)	90.0	113.624(4)
γ (°)	120.0	90.0
V (Å ³)	15302.5 (2)	6589.4(4)
Z	6	4

D _{calc} (g/cm ³)	1.382	1.409
F(000)	6671.905	2920
Temp (K)	293 (2)	130
θ range (°)	1.87-27.489	3.3855- 29.4984
Index range	-21 ≤ h ≤ 21	-27 ≤ h ≤ 24
	-21 ≤ k ≤ 21	-25 ≤ k ≤ 24
	-50 ≤ l ≤ 50	-24 ≤ l ≤ 21
Reflections measured	44628	27019
Independent reflections	3897	15140
Reflections	3146	9048
R _{int}	0.0679	0.0660
R	0.0466	0.0621
R _w	0.1346	0.1363
S	1.04	0.928
Δρ Maximum (e/ Å ³)	0.78	-----
Δρ Minimum (e/ Å ³)	-0.43	-----
Flack parameter	-----	-----

$$R_{\text{int}} = \sum |F_o^2 - \langle F_o^2 \rangle| / \sum F_o^2, R_1 = \sum \|F_o\| - |F_c| / \sum |F_o|, wR_2 = \left[\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2 \right]^{1/2}$$

2.6. Catalytic activities for biomimetic models

Ascorbate oxidase biomimetic model: The catalytic activity of compound **1** was evaluated by reactions at different concentrations of substrate (Ascorbic acid, AA) in HPLC vials (1.5 mL) using 10 ppm of the catalyst. The reaction was monitored by a high-performance chromatograph (UHPLC-ULTIMATE 3000, Thermo Scientific), equipped with a diode array detector (UV-VIS), a Hi-plex-H® column, using sulfuric acid (0.005 M) as mobile phase at 0.6 mL / min, and an injection volume of 20 µL. The detection was performed at different wavelengths of (220, 240, 270 and 300 nm) because these wavelengths allow to detect both AA (as substrate), and DHA (as final product). The concentration of the catalyst agent (CT) was 10 mg/Kg, it was evaluated in different concentrations of substrate to determine its effectiveness (300, 600, 900, 1200, 1500 mg / Kg), likewise it was possible to determine the catalytic activity of the compound (**1**) previously synthesized and the RR isomer synthesized by Ávila et al [28].

Catalase biomimetic model: The catalytic activity of compound **2** was measured by adding 1 mL of the catalyst (68 ppm) to the reactor body with 2 mL H₂O₂ (68 ppm), using the reactor described below. The reaction was carried out during 30 min, generating oxygen in small bubbles that are deposited in a gas collector. The reaction system consisted of a vessel (cylinder of glass, 10 mL capacity), with a cork cap (diameter 17 mm and height 10 mm) adapted to the mouth of the cylinder, an injection system (a 10 mL N₂ intravenous injection set, which was used to supply the substrate into the vessel and initiate the reaction), a gas transport system (a 15 mm long, 3 mm internal diameter intravenous catheter hose embedded within the cork at the mouth of the reactor vessel), a collection vessel (a 10 mL capacity cylinder held by tweezers in a universal support filled with water inside a gas trap), a gas trap (this was configured with a crystallizer of 15 mm in diameter and 8 mm deep filled with water where the collecting glass was submerged), and a security system to avoid diffusion of the oxygen produced through the cork (a septum stopper with a diameter of 12 mm and a thickness of 3 mm was placed on the outside of the vessel).

3. Results

3.1. Crystal structure for the ascorbate oxidase biomimetic model.

Compound **1** was obtained from a mixture of corresponding enantiomerically pure mononuclear copper(II) compounds $[\text{Cu}(\text{R,R(-)Hcpse})_2] \cdot 2\text{H}_2\text{O}$ and $[\text{Cu}(\text{S,S(+Hcpse})_2] \cdot 2\text{H}_2\text{O}$. The IR spectra of compound **1** shows that the ν_{as} and ν_{s} stretching modes of the carboxylic group at 1615 cm^{-1} and 1385 cm^{-1} , are shifted to lower frequencies in its coordination compound **1**, with an $[\Delta\nu(\text{cm}^{-1})]$ coordination modes at ca. 230 cm^{-1} . This shift is indicative of a monodentate coordination mode. No electronic transitions were observed in the CD spectrum of the racemic compound **1**, as expected.

The compound **1** presented a reflectance spectrum with an electronic transition centered at 14636 cm^{-1} (683 nm), indicative of a pentacoordinated square based geometry. The X-ray structure of this compound presents two enantiomer units $[\text{Cu}_3(\text{S,S(+Hcpse})_3(\text{H}_2\text{O})_3][\text{Cu}_3(\text{R,R(-)Hcpse})_3(\text{H}_2\text{O})_3] \cdot 17\text{H}_2\text{O}$ (**1**) in a 1:1 relationship with 17 water molecules in the crystalline center-symmetric group ($R\text{-}3\text{c}$), **Figure 1**, forming a racemic compound. The ligands, $\text{R,R(-)H}_2\text{cpse}$ or $\text{S,S(+H}_2\text{cpse}$, are coordinated to the metallic atom, and a water molecule in the apical position. Selected bond angles for compound (**1**) are listed in **Table 2**. Between units of trinuclear compounds there are two sets of 17 water molecules, with a cubic arrangement in the center; where in three of its edges three pentagons are formed, as depicted in **Figure 2**. This is an unusual arrangement. Each unit enantiomer presents a corresponding water molecule arrangement, **Figure 3a**. The packing in the supramolecular arrangements on c axes shows interesting cavities from of water molecules, **Figure 3b**.

Figure 2. In cubic center an arrangement for water molecules, compound 1.

Figure 3. Molecular arrangements. a). $[\text{Cu}_3(\text{S,S}(+)\text{cpse})_3(\text{H}_2\text{O})_3][\text{Cu}_3(\text{R,R}(-)\text{cpse})_3(\text{H}_2\text{O})_3] \cdot 17\text{H}_2\text{O}$ (1)
b). Packing in the unit cell (c axes).

Table 2. Selected angles for the compound 1.

Atoms	Angles (°)
O5-Cu1-N1	82.4(1)
N1-Cu1-O3	84.5(1)
O4-Cu1-O4A	166.7(1)
O5-Cu1-O4	97.0°(1)
N1-Cu1-O4	106.1(1)

π stacking interactions for each isomer, give to place to supramolecules with Δ and Λ isomerism within the same structure, **Figure 4**. This unique arrangement shown as the water molecules are trapped in the blades, which leads to stabilization of heptadimeric polygons. The distances between the centroids corresponding to aromatic rings, it shows moderate interaction enough for to allow this conformation. These structures are important in biological systems, in where water molecules are outside sphere coordination. Likewise, the binding of water molecules towards metal, allow coordination labile positions that facilitate biological functions, such as the case of metalloenzymes. Additionally, these mimetic models facilitate understanding in biological systems with similar helically arrangements such as the structure of DNA.

Figure 4. Arrangement of water molecules through of Δ and Λ isomer

3.2. Crystal structure for the *catalase* biomimetic model.

The molecular structure of (2) compound features an infinite 1D coordination polymer constructed from monomeric $\text{Mn}(\text{S,S}(+)\text{Hcpse})$ units and polymeric $\{(\text{NaClO}_4)_2(\text{NaOH})\}_n$ chains. The geometry around both Mn atoms is slightly distorted octahedral and is formed by two monodeprotonated, bis-chelating, triconnected N,O,O'' -N-[2-hydroxy-1(*S*)-methyl-2(*S*)-phenylethyl]-N-methylglycinate (*S,S*(+) H_2cpse) ligands that adopt a facial arrangement, in which the equatorial plane is formed by the nitrogen atoms N1(N3) and N2(N4), and the O5(O7) and O6(O8) oxygen atoms from the carboxylic groups while the apical position are occupied by the hydroxyls groups O1(O3) and O2(O4) for Mn1 and Mn2 atoms respectively, **Figure 5**; selected bond lengths and bond angles are reported previously [36].

Figure 5. Ortep representation for the compound 2.

Each of the four crystallographically unique Na^+ ions [two (Na1 and Na4) in special positions and two (Na2 and Na3) in general positions] is -excluding the minor components of disorder- effectively surrounded by six O atoms (from carboxylate, hydroxyl, perchlorate and methanol groups). The shortest $\text{Na}^+ \dots \text{Na}^+$ distance is 3.339(3) Å, while the Na–O distances range from 2.320(5) Å ($\text{Na4}-\text{O11}$) to 3.048(6) Å ($\text{Na3}-\text{O7}$). The connection of the $\text{Mn}[\text{S},\text{S}(+)\text{Hcpse}]_2$ units with $\{(\text{NaClO}_4)_2(\text{NaOH})\}_n$ chains is realized through the $\text{O7} \rightarrow \text{O12}$ μ_2 -carboxylate oxygen atoms, simultaneously bridging the $\text{Na1} \rightarrow \text{Na4}$ atoms and contributing to their distorted octahedral geometry. The octahedral geometry is completed by: unique and symmetry-equivalent $\text{O13}(\text{Cl1}$ perchlorate group) for Na1 ; hydroxy O21 and methanol coordinated solvent O22 , for Na2 and the second perchlorate $\text{O17}(\text{Cl2})$, for Na3 and Na4 , resulting in the generation of repeated —Na—O—Na—O— core with up-down alternate of $\text{Mn}[\text{S},\text{S}(+)\text{Hcpse}]_2$ and ClO_4 . Due to disorder, the second perchlorate group displays two μ_2 -coordination modes between Na3 and Na4 ; μ_2 -monodentate ($\text{Cl2} \rightarrow \text{O20}$) and μ_2 -bidentate ($\text{Cl2B} \rightarrow \text{O20B}$).

1D-chains run along the vector $[001]$ with the phenyl and methyl groups as pendants toward the outside surface of the endless chain generating a lipophilic surface. Pairs of chains running in opposite directions are weakly connected by $\text{C}-\text{H} \dots \text{O}(\text{perchlorate})$ and $\text{C}-\text{H} \dots \pi$ hydrogen bonds (**Figure 6** and **Table 6**). Adjacent pairs of phenyl groups together with the methyl groups create cavities (A and B in **Figure 6**) which are filled with solvent molecules and hydration water, those sitting in the cavity “A” could be located and refined with disorder, while for those sitting in the cavity B the electron density was found to be too sparse to model it and was “squeezed” crystal using the SQUEEZE routine of PLATON program, **Figure 6**.

Figure 6. Packing structure of the compound 2.

Table 3. Hydrogen Bonds for the compound 2.

D-H...A	d(D-H)	D(D...A)	<(DHA)
O1- H1A...O23		1.78	166.3
O3- H3...O22 ^I		1.84	178.5
O21- H21...O20	0.85	2.34	122.7
O21- H21...O22C	0.85	2.14	139.6
O23-H23C...O21	0.84	2.26	127.6
O24-H24C...O7 ^I	0.85	2.66	179.4
O25-H25C...O24 ^I	0.85	1.97	179.6
O25-H25D...O10 ^I	0.85	1.89	179.6
C6- H6...O15 ^I		1.00	136.1
C17- H17C...O19 ^{II}	0.98	2.65	167.4
C19- H19B...O14 ^{III}	0.98	2.61	164.7
C26- H26...O18 ^{II}	0.98	2.54	165.7
C45- H45B...O20 ^{II}	2.46	3.42	166.1
C37- H37...Cg1 ^{III}	0.98	2.84	145
C48- H48A...Cg2 ^{III}	0.98	2.83	128

Symmetry transformations used to generate equivalent atoms: ^I-x+1,y,-z+1, ^{II}-x+1/2,y+1/2,-z, ^{III}-x+1/2,y-1/2,-z+1 . Cg1: C21→C26; Cg2: C27→C32

3.3. Electrochemical studies for ascorbate oxidase biomimetic model.

Electrochemical studies were performed on three chiral H₂cpse ligands, the four mononuclear Cu²⁺ complexes, and the two trinuclear Cu²⁺ complexes listed in Table 4. All experiments were conducted in degassed methanol due to the limited solubility of both the ligands and their copper complexes in other solvents. Cyclic voltammograms of (+) S,S-H₂cpse and (+) R,R-H₂cpse exhibit a single irreversible oxidation wave, with E_pa values of 0.926, 0.927 V, respectively.

Table 4. Electrochemical potentials (versus NHE) from cyclic voltammetry studies of the tested complexes in methanol.

Compound	E_{pa} (V)	E_{pc} (V)	ΔE (V)	$E_{1/2}$ (V)
(+) <i>S,S</i> -H ₂ cpse	0.926	—	—	—
(-) <i>R,R</i> -H ₂ cpse	0.927	—	—	—
Cu(<i>S,S</i> (+)-Hcpse) ₂	0.075 ^a , 0.264 ^b , 0.926	-1.115 ^a , -0.454 ^b	1.040 ^a , 0.718 ^b	-0.520 ^a , -0.095 ^b
Cu(<i>R,R</i> (-)-Hcpse) ₂	0.075 ^a , 0.178 ^b	-1.124 ^a , -0.461 ^b	1.049 ^a , 0.639 ^b	-0.524 ^a , -0.142 ^b
Cu ₃ (<i>S,S</i> (+)-cpse) ₃	0.145 ^a , 0.414 ^b	-1.399 ^a , -0.982 ^a , -0.430 ^b	1.254 ^a , 0.837 ^a , 0.844 ^b	-0.627 ^a , -0.415 ^a , -0.008 ^b
Cu ₃ (<i>R,R</i> (-)-cpse) ₃	0.193 ^a	-1.321 ^a , -0.958 ^a , -0.445 ^b	1.514 ^a , 1.151 ^a	-0.564 ^a , -0.383 ^a

^aCu⁺⁰ potential. ^bCu^{2+/+} potential.

The cpse ligands in the mononuclear Cu²⁺ complexes are chemically similar, but their CuII complexes are different. The tridentate cpse ligands form octahedral Cu²⁺ complexes, each ligand coordinating through two oxygen atoms and the central nitrogen atom, **Figure 7a**. Oxidation waves for the ligand are only observed for the mononuclear Cu(*S,S*(-)-Hcpse)₂ complex; there is no change for the ligand potential in the *S,S* complex. The quasi-reversible Cu^{2+/+} redox couples for Cu(*S,S*(+)-Hcpse)₂ and Cu(*R,R*(-)-Hcpse)₂ are similar ($E_{1/2}$ of -0.520 and -0.524 V, respectively; **Figure 7 b**; however, the quasi-reversible Cu⁺⁰ couples vary by 0.047 V ($E_{1/2}$ values of -0.095 and 0.142 V, respectively).

Niklas et al. [39] reported only the Cu^{2+/+} redox couple for chiral and achiral derivatives of [bis(picoly)amino]acylglycine ethyl ester and [bis(picoly)amino]acylphenylalanine methyl ester complexes of Cu²⁺, complexes with square pyramidal or distorted octahedral geometry and N₃O coordination of the amino acid ligands; additional ligands such as acetonitrile, oxygen, and chloride complete the coordination spheres. These studies were conducted under similar conditions (in methanol, with 0.1 M tetrabutylammonium hexafluorophosphate as the supporting electrolyte), but with a smaller electrochemical window of -0.4 to 0.2 V versus NHE. No electrochemical data were reported for the uncoordinated ligands, and no observed redox waves in the copper complex voltammograms were assigned to either ligand. The Cu^{2+/+} $E_{1/2}$ values reported for these amino acid Cu²⁺ complexes are between -0.16 V and -0.10 V, relatively similar to the $E_{1/2}$ values determined for the Cu^{2+/+} couples of Cu(*S,S*(+)-Hcpse)₂ and Cu(*R,R*(-)-Hcpse)₂ ($E_{1/2}$ values of -0.142 and -0.095 V, respectively). However, the significant shift observed for the Cu^{2+/+} redox couple (between -0.104 V and 0.044 V) $E_{1/2}$ value compared to the amino acid complexes of Niklas et al. is likely attributed to the presence of the borderline nitrogen donor pyridines in the amino acid complexes that stabilize Cu²⁺ substantially more than soft Cu⁺.

Similar electrochemical studies were conducted on the trinuclear copper complexes [Cu₃(*S,S*(+)-cpse)₃] and [Cu₃(*R,R*(-)-cpse)₃], **Figure 7c**, in which the H₂cpse ligands are doubly deprotonated. No ligand-based oxidative waves are observed in the voltammograms of these trinuclear complexes. The voltammogram of [Cu₃(*S,S*(+)-cpse)₃] exhibits three reductive waves but only two oxidative waves. The two reductive waves at -1.399 and -0.952 V are attributed to the reduction of Cu⁺ to Cu⁰ and are similar in shape to the Cu⁺⁰ reductive wave observed for the mononuclear copper complexes (Figure 7). The third reductive wave at 0.430 V is attributed to the reduction of Cu²⁺ to Cu⁺. The oxidative wave at 0.145 V is attributed to the oxidation of Cu⁰ to Cu⁺, and the wave at 0.414 V, is attributed to the oxidation of Cu⁺ to Cu²⁺; however, the Cu^{0/+} oxidative wave is broader than expected, suggesting that all three Cu²⁺ centers are electrochemically similar but not equivalent.

Two reductive waves are observed in the voltammogram of [Cu₃(*R,R*(-)-cpse)₃]; whereas [Cu₃(*R,R*(-)-cpse)₃] exhibits a single broad oxidative wave observed at 0.193 V, caused by the

overlapping of the Cu^+ stripping and Cu^{2+} oxidation waves. Comparison of the $\text{Cu}^{+/0}$ $E_{1/2}$ values of $\text{Cu}(\text{S},\text{S}(+)\text{Hcpse})_2$ and $\text{Cu}(\text{R},\text{R}(-)\text{Hcpse})_2$ to the trinuclear complexes with similar chirality reveal shifts of -0.127 and -0.040 V, respectively. Previous studies with a trinuclear Cu^{2+} complex with tetradentate aromatic ligands (three nitrogen and one oxygen donor atoms), showed three separate oxidative waves for the $\text{Cu}^{2+/+}$ redox couple but they did not report any redox activity for the $\text{Cu}^{+/0}$ couple or identify any ligand-based waves. These studies with the trinuclear cpse complexes indicate that the three Cu^{2+} centers are not equivalent and that the overall effect of ligand chirality on the redox activity of the Cu^{2+} centers is significant. For the racemic mixture, the authors observed the same properties for each redox center separately, relative to each trinuclear independently. This is because structurally the two enantiomers coexist in the crystalline structure. Also, the distance between trinuclear centers estimated by π -stacking interaction is greater than 5 Å.

Figure 7. Results for the electrochemical analyses for compound **1**. a). Cyclic voltammograms vs. NHE for the ligands (+)S,S-H₂cpse (1 mM), b). (-)R,R-H₂cpse (1 mM), c). [Cu(S,S(+)-Hcpse)₂] (1 mM) d). [Cu(R,R(-)-Hcpse)₂] (1 mM), e). [Cu₃(S,S(+)-cpse)₃] (1 mM), and f). [Cu₃(R,R(-)-cpse)₃] (1 mM) in methanol with 0.1 M tetrabutylammonium hexafluorophosphate as the supporting electrolyte.

3.4. Electrochemical studies for *catalase* biomimetic model.

Cyclic voltammetry experiments were also carried out to analyze the electrochemical behavior of compound **2**. The results are dependent on the starting direction of the CV. When the scan started in the negative potential direction, no peaks were observed. In contrast, when starting the scan in the positive potential direction, an oxidation peak was observed ($E_{pIa} = 0.61$ V), which can be attributed to oxidation of the metal center. In the inverse scan, three reduction peaks were observed ($E_{pIc} = 0.47$ V, $E_{pIIc} = 0.06$ V and $E_{pIIIc} = -0.6$ V), where peak **Ic** is related to the reduction of the oxidation products of peak **Ia** back to the original species. Due to the current intensity in peak **Ic** is considerably smaller than that corresponding to peak **Ia**, the redox wave **Ia-Ic** corresponds to at least one electron transfer followed by a chemical reaction, where the chemical step could be the result of either a bond breaking or bond formation. On the other hand, peaks **IIc** and **IIIc** should correspond to the reduction of some sort of intermediates produced in the chemical reaction, since they do not appear when the CV is started in the negative direction. No other electrochemical signals were observed when the cyclic voltammetry was extended in the whole potential window of the system (from -2.5 to 2.0 V).

In order to estimate the number of electrons transferred during the oxidation process, a comparison of the electrochemical behavior of compound **2** with a one-electron transfer system, such as ferrocenecarboxaldehyde (Fc-CHO), was done. The current peak due to oxidation of compound **2** (20.5 μ A) has the same order of magnitude as the oxidation current peak of Fc-CHO (29.3 μ A), which implies that compound **2** undergoes a one-electron oxidation as well. The small difference (~ 9 μ A) between those two anodic currents could be due to a lower diffusion coefficient of compound **2**, because this is a larger molecule, and to the fact that this complex did not dissolve completely in the used solvent. The peak-to-peak separation ($\Delta E_p = E_{pIa} - E_{pIc}$) at 0.1 Vs^{-1} is 140 mV, which may indicate that the electron transfer process is slow. In order to corroborate this idea, an analysis of the electrochemical behavior with respect to the scan rate was carried out.

Figure 8. Linear dependence of oxidation peak current (I_{pa}) on scan rate (v).

Before any mechanistic analysis, it is necessary to investigate whether the electroactive species diffuse from solution or adsorb on the electrode surface [36]. For this reason, the variation of anodic peak current with respect to the scan rate (Log I_{pa} -Log v plot) was constructed, **figure 8**. The slope of this plot was 0.52, which indicates that the electron transfer is controlled by diffusion. Consequently, no species are adsorbed during the potential scan. It can be observed that as the scan rate is increased, the oxidation potential of peak **Ia** becomes more positive, while the reduction peak **Ic** slightly shifts to less positive values. Thus, the higher the scan rate, the larger E_p , in agreement with a slow electron transfer rate. The sluggishness of the electron transfer step is supported by the

slope of Epa-Logv plot ($\partial E_{pa}/\partial \log v = 0.066$ V). Thus, when this value was used in a ($\partial E_{p}/\partial \log v$) vs $\log k_s$ working curve, where k_s is the heterogeneous electron transfer constant, it was observed that the value of k_s for the system under study is lower than 10^{-1} cm $\cdot$ s $^{-1}$. Additionally, the fact that the current for peaks **IIc** and **IIIc** decreases with the increasing scan rate supports the idea that these signals correspond to the reduction of intermediates produced in the chemical reaction that follows the electron transfer step. An interesting feature that can be deduced from the electrochemical study. This is the fact that since the starting molecule possesses two redox centers, the only presence of one oxidation peak implies that this species bears a single oxidation state, and it may be due to the dissociation in solution of the compound **2** into two metallic complexes.

3.5. Magnetic properties for ascorbate oxidase biomimetic model.

The trinuclear copper (II) compound (**1**) obtained from the racemic mixture from the mononuclear compounds presented hugely different magnetic properties concerning the pure enantiomers. The curve $\chi_M T$ vs. T (K) for the compound **1** shows a typical behavior of an antiferromagnetic coupling between T = 92 K to T = 30 K. Compound **1** exhibits a minimum around 30 K, $\chi_M T = 0.48$ emu mol $^{-1}$ K. Furthermore, there is an abrupt increase as the temperature decreases, reaching a peak of $\chi_M T = 0.44$ emu mol $^{-1}$ K to T = 6.5 K, a decreasing $\chi_M T$ at T = 6.5 K is due to saturation effects [28], and strong antiferromagnetic coupling at T = 298 K. For this system, a mixture of oxidation states for Cu1-Cu2-Cu3 dependent on thermal activation is suggested. Three possible states: quartet $|3/2, 1\rangle$ and two doublets, $|1/2, 1\rangle$. For the range 92 K < T < 92 K is considered a state: $|1/2, 1\rangle$ 92 K and $|3/2, 1\rangle$, see **figure 9a**. External fields between 1 - 5 T, for pure enantiomer compounds and racemic compound was measured. There are structural differences between those three compounds. The racemic mixture presents a double water molecule that their respective pure enantiomers and more directional intermolecular interactions. We suggest that the amount of water outside the coordination sphere will significantly influence the paramagnetic-antiferromagnetic transition, **figure 9b**.

Figure 9. Magnetism analyses for compound **1**. **a).** Temperature dependence of $\chi_M T$ vs. T for compound (**1**), **b).** Magnetization at variable magnetic field for trinuclear compounds, T= 4K.

3.6. Magnetic properties for catalase biomimetic model.

The plots $\chi_M T$ vs. T(K) and $1/\chi_M$ vs. T(K) for the compound **2** show a typical behavior of an antiferromagnetic coupling at 3 KOe. $\chi_M T$ versus T(K). A decreasing in $\chi_M T$ as the temperature is lowered was observed. The value of $\chi_M T$ at T = 300K is 10.64 cm 3 K mol $^{-1}$, which corresponds for two manganese (II) ions (S=5/2), is consistent with a dimer (It decreases to T=2K, $\chi_M T = 3.80$ cm 3 mol $^{-1}$ K). The inverse molar susceptibility suggests an antiferromagnetic behavior, considering the parameters obtained by fitting the Curie-Weiss plot, between 300- 2K; $\theta = -16.58$ K, C = 0.0917. The corresponding molar magnetic susceptibility is represented by using the Heisenberg model (H= -2J S_1 · S_2 , $S_1=S_2=5/2$), and Equation 3.

$$\chi_M = \frac{2N\beta^2 g^2}{3KT} x \frac{e^{2x} + 5e^{6x} + 30e^{20x} + 55e^{30x}}{1 + 3e^{2x} + 5e^{6x} + 7e^{12x} + 9e^{20x} + 11e^{30x}} \quad (3)$$

Where, N, g, β , x are the Avogadro number, g factor, Bohr magneton and J/KT. The parameters giving the best fit were obtained using a nonlinear regression analysis, which are: $g = 2.58$, $2J = 20.88 \text{ cm}^{-1}$; coefficients with 95% confidence bounds, indicating a very good data fit and moderate antiferromagnetic ordering. The X-band EPR spectra for a polycrystalline sample of the compound **2** in solid state, consists of an isotropic featureless signal centered at $g \sim 2.0$, with typical signals for the axial geometry around the manganese (II) ions. However, the EPR spectra in solution at 2.03 mM, $T = 110 \text{ K}$; showed the non-Kramers doublet near 1500 G. This behavior is related with manganese (III), with four unpaired electrons resulting in a $5E_g$ ground state, and a Jahn-Teller distortion. Consequently, is indicating that there is a dissociation of the binuclear unit in solution and a subsequent stabilization of manganese (III) species [36].

3.7. Assessment of the activity for the biomimetic model of ascorbate oxidase

The structural similarities between the active center of the AO enzyme and the structural model presented in this work provides an initial idea of the catalytic properties of the compound **1**. Both AO and compound **1** have a Cu^{2+} trinuclear center at similar distances (in the order of $3.5 \text{ \AA}$). These substances present electro-donating atoms such as nitrogen and oxygen in amino and carboxyl groups, respectively. Also, AO and compound **1** have optical activities associated to ligands with possible biological influences. For this reason, the degradation of ascorbic acid was carried as biomimetic model for the AO. The degradation of L- ascorbic acid and consequently the formation of dehydroascorbic acid by action of the biomimetic model (compound **1**) is presented in **Figure 10**. There is a directly proportional relationship between the decreases in the substrate concentration with respect to the increase in the concentration of the trinuclear compound (**1**), as illustrated in **Figure 10a**.

Figure 10. Chromatograms. a) Degradation of AA b) Increase of formed DHA.

It should be indicated that ascorbic acid (AA) degradation was carried out at neutral pH, since no significant changes were observed in the degradation process compared to the process at basic pH. From the FT-IR spectra, can be noted that the band corresponding to the oxidation of secondary alcohols (around 1600 cm^{-1}) disappears as a function of the formation of dehydroascorbate products. This behavior was also correlated with complete oxidation of AA via hydrogen peroxide (**Figure 11**).

Figure 11. IR spectra for ascorbic acid degradation.

The AA oxidation was followed through HPLC-UV, detecting the degradation of the substrate, and the formation of dehydroascorbate as degradation product (as presented in **Figure 10**). For the racemic mixture of the biomimetic compound, the ascorbic acid (at concentrations between 300 ppm and 1200 ppm) degradation followed a linear trend (**Figure 12a**). However, the comparison between the racemic mixture and the pure trinuclear compound at 900 ppm showed an effect similar for this concentration. The behavior to other concentrations suggested that the racemic system has two trinuclear compound units communicated through intermolecular hydrogen bonding interactions. These interactions allow molecule by molecule to increase the efficiency of the catalyst. Otherwise, it happens with the enantiomerically pure trinuclear compound, which only has three Cu^{2+} ions per center. Another interesting factor occurred at 900 ppm of acid ascorbic, in where this behavior is inverted (**Figure 12a**). For the degradation rate the same behavior is observed (**Figure 12b**, the trinuclear compound pure presented higher degradation rate). It is proposed that the racemic compound is affected with successive oxidations of the substrate. The intermolecular interactions are weakened, and due to the oxidation, opening of the ring for each trinuclear system could occur (**Figure 1**). On the other hand, the formation for dehydroascorbic (**Figure 12c**) showed a lineal response for the trinuclear pure compound. It is proposed that there is an important effect of intermolecular interactions in the catalysis of the model compound. Also, the spatial orientation of the binder gives to rise a better interaction between substrate and catalyst [37].

Figure 12. Oxidation of ascorbic acid by the compound **1**. **a)** Ascorbic acid degradation for biomimetic racemic compound and trinuclear compound, **b)** Rate of degradation for Ascorbic acid and **c)** Dehydroascorbic acid produced with the biomimetic model of *ascorbate oxidase*

3.8. Assessment of the activity for the biomimetic model of *catalase*

The oxidizing activity of the compound **2** can be represented by Equation 4. Thus, the catalytic action of the biomimetic complex based on manganese was tested through the hydrogen peroxide oxidation to oxygen. In the **Figure 13a** is shown the $O_{2(g)}$ production, as an indicator of the degree of the reaction. There is a linear trend ($R=0.92$) to produce O_2 with the increasing of the substrate. It is suggested that the slight deviation from linearity is due to the formation of dimeric species in solution. In these units, there is an electronic transfer between the metallic centers, generating the oxidation of Mn^{2+} to Mn^{3+} , as observed in the electrochemical measurements and magnetic properties. This charge transfer would generate a decreasing of the catalytic sites available for oxidation of the hydrogen peroxide. Furthermore, the manganese (III) ion presents an absorption in the visible region due to electronic transition in octahedral geometry ($t_{2g}^3 e_g \rightarrow t_{2g}^2 e_g^2$) [36]. In contrast, the manganese (II) has no transitions in high spin configuration for this geometry, which shows the structural differences between solid and solution state. This behavior has allowed us the identification of the catalytic activity via oxidation of hydrogen peroxide, through visible spectroscopy (**Figure 13b** shows absorbance evolution for the catalytic reaction at 5min). The results indicate that the compound **2** is a good biomimetic oxidase model not only structural but also functional.

Figure 13. Oxidation of hydrogen peroxide by the compound **2**. **a)** O_{2(g)} production, as an indicator of the degree of the oxidation reaction, **b)** Monitoring the oxidation reaction through visible spectroscopy at different times.

4. Conclusions

Two new biomimetic compounds (**1** and **2**), as models of active centers of *ascorbate oxidase* and *catalase*, were synthesized successfully. The compound **1** presented antiferromagnetic coupling between trinuclear systems, optical properties of a racemate compound and similar structure to center active metalloenzyme *Ascorbate oxidase*. In turn, compound **2** had an antiferromagnetic coupling between metal ions, optical properties such enantiomer pure and polymeric structure with asymmetric unit like active center of the metalloenzyme *catalase*. The *ascorbate oxidase* model (**1**) is associated with stereoselective processes and this study discriminates activity in relation to pure enantiomers and racemic mixtures, for concentrations different of 900 ppm. The trinuclear compound with an enantiomer pure presented major oxidation of ascorbic acid toward dehydroascorbic acid. Indeed, it is suggested that the racemic compound is affected by successive oxidations of the substrate, and an opening of the ring for each trinuclear system could occur. For *catalase* biomimetic model (**2**) presented high catalytic activity to the oxidation of hydrogen peroxide to oxygen and water. This synthesized compound exhibited electronic transfer between its metal centers in aqueous medium, which decreased the catalytic activity. Then, it is necessary to improve the compound **2** structure in solution with respect to the solid state. The results from the present research suggest that, in future, these biomimetic models could be applied to industrial systems where substrates require oxidation with compound that involves environmentally friendly metal centers, such as manganese and copper. Also, we should mention that although catalysis of the models has been proven effectively, different studies on the reuse cycles are necessary in further works, as well as analyses of the toxicology of aqueous solution after the catalytic action of **1** and **2**.

Supplementary Materials: CCDC data 893740 contains the supplementary crystallographic data for [Cu₃(S,S(+)-cpse)₃(H₂O)₃][Cu₃(R,R(-)-cpse)₃(H₂O)₃]·17H₂O (**1**). CCDC data 2071434 contains the supplementary crystallographic data for [Mn₂(S,S(+)-Hcpse)₄(NaClO₄)₂(NaOH)(CH₄O)]_n·[(C₂H₆O)₂]_n·[(CH₄O)₂]_n. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html>, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk

Author Contributions: D.G., J.A., H.S., N.B., E.S., R.T., R.A.T and Y.A.T.: They participated in the analysis of the images and contributed to the writing; all authors participated in the corrections. D.G., J.A., H.S., N.B., E.S., R.T., and Y.A.T: Conceptualization, methodology and investigation; writing—original draft preparation, writing—review and editing, R.A.T, D.G., J.A., H.S., N.B., E.S., R.T, and Y.A.T.

Funding: The authors gratefully acknowledge the Universidad Santiago de Cali DGI project 939- 621 120- 1867.

Acknowledgments: The authors gratefully acknowledge the USC, UNAM and UDEA.

Conflicts of Interest: The authors declare no conflict of interest.

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