



Cu (II), Fe (II)-Adriamycin semiquinone complexes: computational investigation and organometallic synthesis

Rayene Kadri ^{a*}, Saad Bouchekioua ^{a,c}, Nour Hasni ^b, Mekki Kadri ^b

^a Pharmaceutical Sciences Research Center (CRSP), Constantine, Algeria.

^b Laboratoire de Chimie Physique (LCP), University 8 May 1945 Guelma.

^c Laboratory of Chemistry of Materials and Living Organisms: Activity & Reactivity (LCMVAR), University Batna 1, Algeria.

Abstract

Adriamycin semiquinone (ADRS) is an anticancer agent that has been successfully used in chemotherapy since 1963. However, this molecule exhibits a cardiotoxicity as a side effect, which is caused by the radical reactions in which the ADRS molecule is involved. It has also been reported that the presence of Fe (II) and Cu (II) ions catalyses the reaction of ADRS with free radicals. This finding prompted researchers to investigate the role of these ions in radical reactions and their interaction with ADRS. The goal of the current work is to perform a spectroscopic and thermodynamic study of Fe (II) and Cu (II) – ADRS interactions.

Distribution diagrams illustrating various chemical species were constructed, followed by the calculation of stability constants for both 1:1 and 1:2 complexes, along with the associated thermodynamic parameters. Solid-state complexes were synthesized in a methanol solution utilizing ultrasonic conditions at a frequency of 20 kHz, and these complexes were subsequently characterized. Additionally, the interactions between ions and ADRS were explored through molecular modelling studies employing DFT/B3LYP-6.311++G(d,p) and DFT/B3LYP-6.311++G(d,p) with Empirical Dispersion=GD3BJ, which incorporates Grimme dispersion effects. The results obtained from these studies were analysed and compared in terms of bond lengths, angles, and dihedral angles.

The best-performing exchange functional was used for subsequent calculations such as prediction of the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) then the corresponding including the energy gap, hardness, and electrophilicity, among others. Additionally, Natural Bond Orbital (NBO) and Quantum Theory of Atoms and Molecules (QTAIM) analysis were performed to clarify the intermolecular interactions occurring between ADRS molecules and metallic ions.

Keywords:

Adriamycin semiquinone, organometallic complexes, DFT, TD-DFT, NBO, QTAIM analysis.

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*Corresponding author

Email address: kadri.rayene94@gmail.com (Rayene Kadri)

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1. Introduction

Adriamycin semiquinone (**Fig. 1.**) has been an extensively used and efficacious chemotherapeutic drug for the treatment of different cancers, encompassing both solid tumours and haematological malignancies, since 1969[1]. However, cardiotoxicity, caused by the production of free radicals [2] and promotion of lipid peroxidation [3], limits its therapeutic applicability. Adriamycin semiquinone (ADRS) can promote the generation of reactive oxygen species (ROS) by redox cycling of its aglycones, especially in the presence of metal ions. As a result, various research has investigated the relationships between ADRS, Fe (II), and Cu (II) [4, 5]. Metal ions have a considerable impact on the metabolic characteristics of anthracyclines, indicating new opportunities for improving chemotherapeutic effectiveness while minimising toxicity [6]. The binding of metal ions has a significant impact on these medicines' redox characteristics [7, 8].

Iron serves a vital function as a redox centre, producing free radicals in the presence of dioxygen under reducing circumstances, potentially harming cellular components [9]. ADRS may be converted to its semiquinone form by biological reducing agents such as NADH and NADPH.

In the presence of transition metals [Cu (II) and Fe (II)], this reaction can result in the creation of hydrogen peroxide and superoxide when dioxygen takes electrons from the semiquinone [10]. Because transition metals like iron and copper may bind to biomolecules and dioxygen at the same time, frequently serving as a bridge between them, it is critical to maintain low concentrations of these metal ions. Consequently, it is essential to look into the properties and structure of metal–Adriamycin semiquinone complexes [11]. The production of Adriamycin semiquinone complexes with Fe (II) or Cu (II) has been reported in several investigations [12].

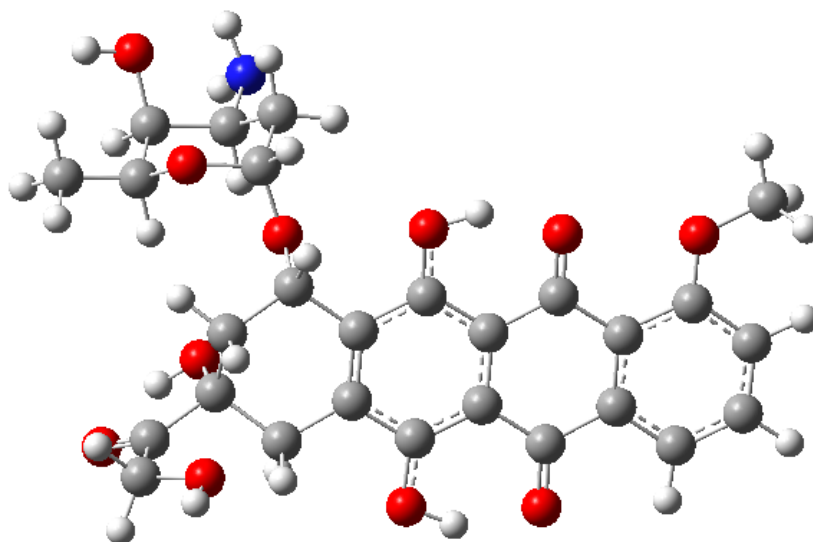


Fig.1. Adriamycin semiquinone structure.

Therefore, the goal of this work is to get some theoretical and experimental understanding of how the Fe(II) /ADRS) and Cu(II) /ADRS systems interact.

2. Experimental

2.1. Materials and equipment

(7*S*,9*S*) – 7 - [(2*R*,4*S*,5*S*,6*S*) -4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxy acetyl)-4-methoxy-8,10-dihydro-7*H*-tetracene-5,12-dione (ADRS), methanol, Copper (II) hexahydrated perchlorate [Cu (ClO₄)₂.6H₂O] and iron (II) hexahydrate perchlorate [Fe (ClO₄)₂.6H₂O] were obtained from Aldrich Chemical Co. and used unaltered.

The electronic absorption spectra were recorded in the range 400–220 nm using a Jasco UV-530 spectrophotometer equipped with a Jasco EHC-477S thermostat (± 0.1 C) using 1.0 cm matched quartz cells.

2.2. Preparation of the solid complexes

The equimolar concentrations of ADRS and either copper (II) hexahydrated perchlorate or iron (II) hexahydrated perchlorate, dissolved separately at room temperature in methanol, were used to isolate the solid complexes. At a frequency of 20 kHz, the two solutions were combined in an ultrasonic environment. The resultant precipitated solid compounds were first filtered out, then repeatedly cleaned with a methanol/dichloromethane combination to get rid of any leftover components, and lastly allowed to dry.

2.3. Computational details

Gaussian 09 software was used for all molecular modelling and computations. Using the Becke's three parameter hybrid technique with LYP correlation (B3LYP) /6.311++G(d,p) and B3LYPGD3BJ, which accounts for the Grimme dispersion under the same basis set, the geometries of the complexes were optimised at the Density Functional Theory (DFT) level. In order to prevent imaginary frequencies, frequency calculations were done after geometry optimisation. The CPCM solvation model was utilised to do calculations both in liquid and vacuum phases.

3. Results

3.1. Spectrophotometric study

The complex formation was investigated in methanol solution at 25°C and 0.1 M KCl ionic strength. *Hypspec*, a non-linear least-square fitting tool, was used to compute the stability constants and species distribution (Fig. 2.).

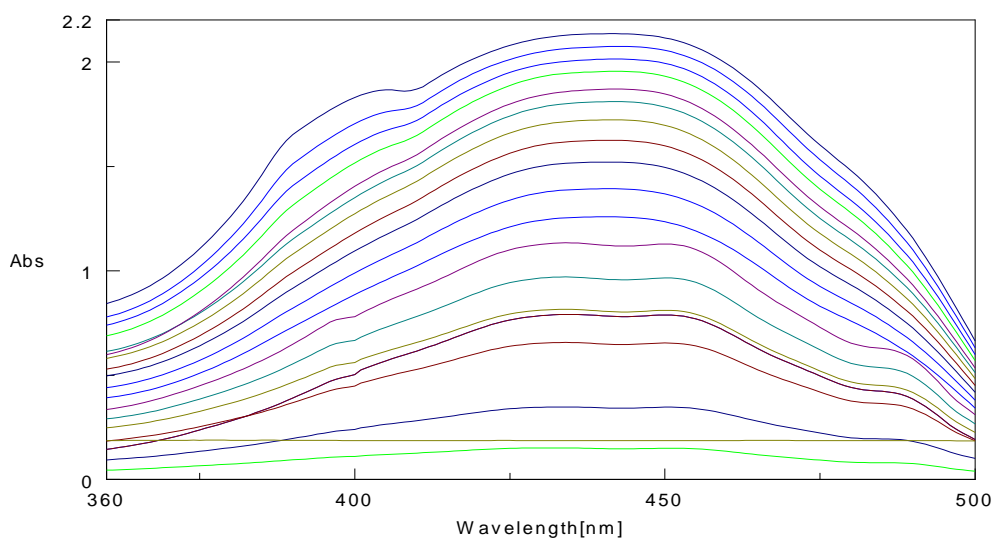


Fig.2. Absorption spectra of Fe (II)(4.10⁻⁴M) in methanol containing various concentrations of ADRS.

The stability constants calculated using a non-linear least-square fitting program were presented in **Table 1**.

Table 1. Stability constants of formed complexes in Fe (II)/ADRS and Cu (II)/ADRS systems.

Complex	$\log \beta_{11}$	β_{11}	$\log \beta_{12}$	β_{12}
Fe(II)/ADRS	3.4251±0.0257	2.66x10 ³	6.1448±0.024	1.39x10 ⁶
Cu (II)/ADRS	3.8022±1.655	6.3416x10 ³	9.812±0.22	6.48x10 ⁶

By determining the stability constants of the complexes in solution, it is possible to infer the species distribution diagrams (**Fig.3**), which show the relative abundance of each species based on the concentration of the ligand ADRS.

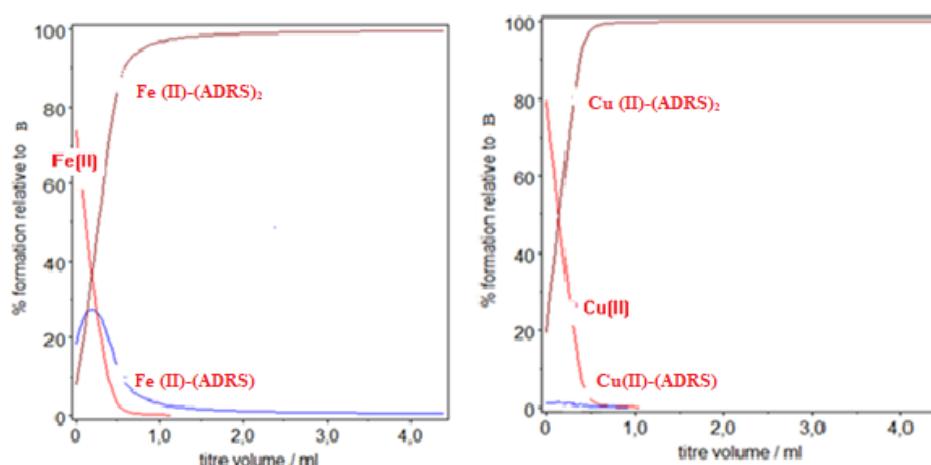


Fig.3. Distribution diagrams of species for Fe (II) –ADRS and Cu (II)-ADRS systems.

3.2. Thermodynamic parameters

Using the well-known van t Hoff plots, the thermodynamic standard quantities (ΔH^0 , ΔS^0 , ΔG^0) related to the complex's production were calculated, assuming constants ΔH^0 across the 20°–35°C temperature range. In all plots of $\ln \beta_{12} = f(1/T)$. The correlation coefficients were above 0.97. The enthalpies and entropies of complexation were determined (**Fig.4**) from the slopes and intercepts, respectively.

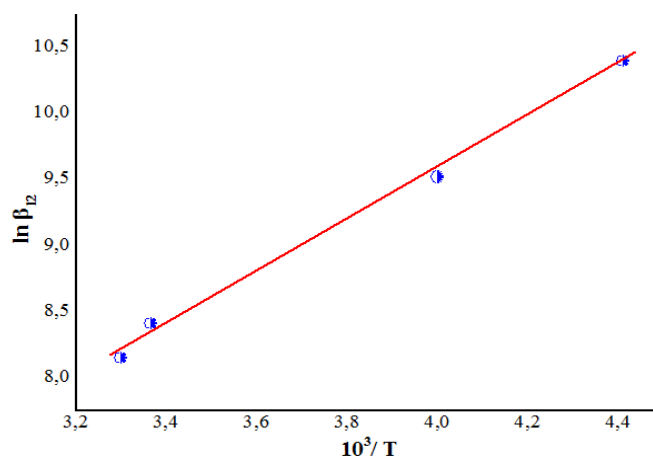


Fig.4. Determination of thermodynamic parameters.

The free enthalpy was computed from the following equation

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ} = -RT \ln \beta_{12}$$

Table 2. Thermodynamic parameters of the formed complexes: Fe (II)/ADRS and Cu (II)/ADRS systems.

Complexes	ΔH° (kJ/mol)	ΔS° (J/mol.K)	ΔG° (kJ/mol)
Fe (II)-(ADRS) ₂	-139	-396.59	-20
Cu (II)-(ADRS) ₂	-155	-280.12	-71

3.3. Molecular modelling study

3.3.1 Complexes optimization

The most stable compounds in solution, those with a 1:2 stoichiometry, were optimised using DFT using the same basis set, 6-311++G(d,p), but two distinct exchange functionals: B3LYP or B3LYP GD3BJ which takes into account the Grimme dispersion.

In order to estimate the performance of each exchange functional in DFT calculations, the predicted bond lengths in ADRS molecule at the given DFT level were plotted against the experimental ones, as reported in **Fig.5**. The correlation coefficient values R were 0.938 for B3LYP and 0.987 for B3LYPGD3BJ.

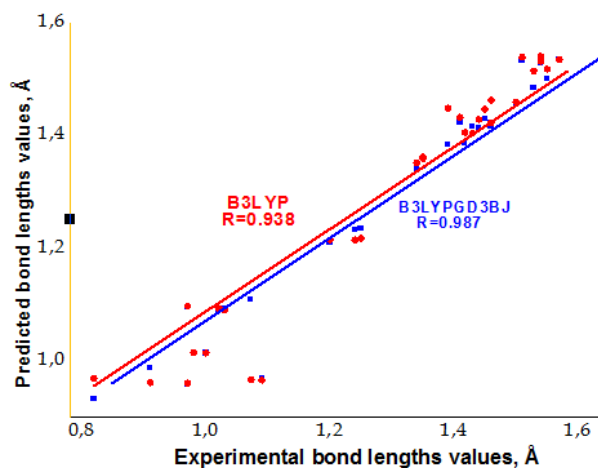


Fig.5. The performances of geometric parameter prediction using B3LYP and B3LYPGD3BJ.

The predicted structure of the Cu (II) complex obtained from the selected exchange functional (B3LYPGD3BJ) was depicted in **Fig. 6**. The metal ion was placed between two ADRS units.

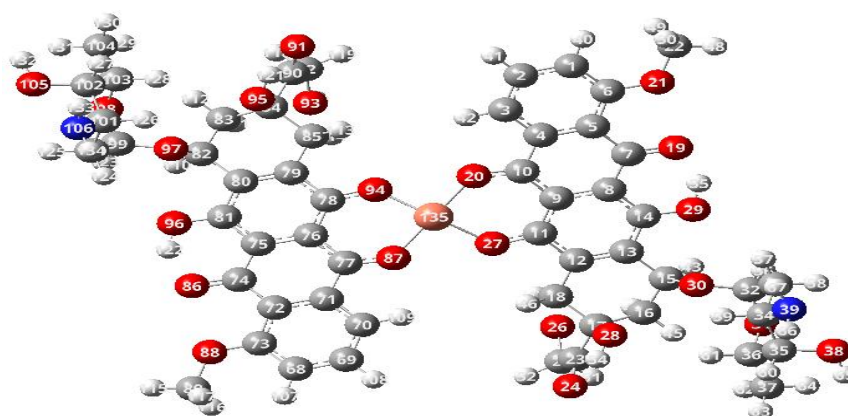


Fig.6. The optimized structure of Cu (II) ADRS₂ complex at B3LYPGD3BJ /6-31+(d,p) level With atomic numbering.

3.3.2. Frontier Molecular orbitals (FMO) and global reactivity indexes

The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), called frontier molecular orbitals (FMO) are the most important orbitals in a molecule (**Fig.7**).

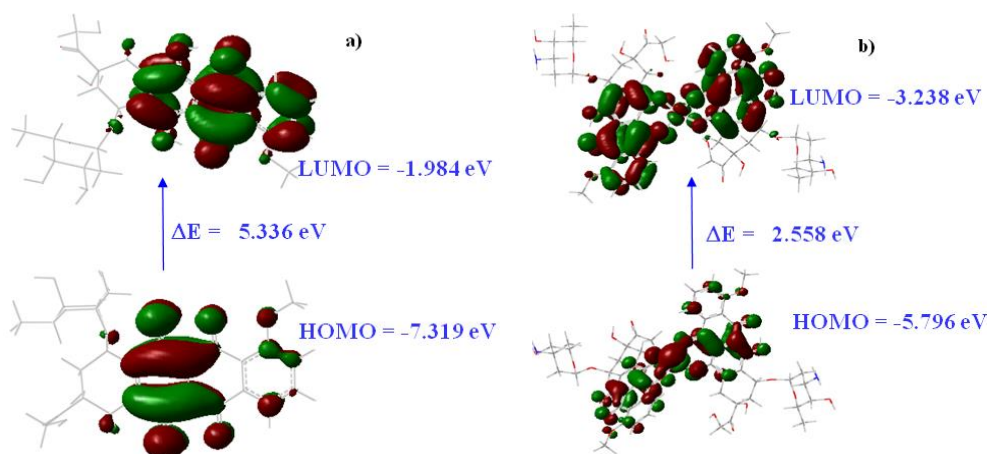


Fig.7. 3D plots of the HOMO and LUMO orbital of a) ARDS and b) copper (II) complex predicted at DFT/ B3LYPGD3BJ /6-31+(d,p) level.

We utilised the conceptual DFT [13] to identify certain ideas (indexes or reactivity descriptors) that can offer us a general sense about the reactivity or the stability of the system in order to emphasise the formation influence of the organometallic complexes, by comparing the values of the global organometallic complexes reactivity descriptors with those of ADRS alone one can conclude that the complexation improves the reactivity of the ligand and consequently the presence of the metal ions favor the involvement of ADRS in the radical reactions.

Table 3. Global reactivity of the formed complexes: ADRS - Fe (II) and ADRS -Cu (II) systems.

	ADRS	Cu(II)ADRS ₂		Fe(ADRS) ₂
		Spin α	Spin β	
LUMO, eV	-1.984	-3.238	-3.211	-3.156
HOMO, eV	-7.319	-5.796	-5.714	-5.415
A	1.984	3.238	3.211	3.156
I	7.319	5.796	5.714	5.415
ΔE	5.336	2.558	2.503	2.258
η	2.668	1.278	1.251	1.129
μ	-4.652	-4.517	-4.462	-4.286
ω	4.055	7.977	7.955	8.132
S	0.37	0.782	0.799	0.886

3.3.3 NBO analysis

The natural bond orbital analysis (NBO) approach [14, 15] is a straightforward way to study charge transfer or conjugative contact (interactions) in molecular systems. It has been widely utilised in the assessment of intra- and intermolecular interactions. To offer the contribution of atomic orbitals, NBO analysis was carried out. A set of occupied Lewis orbitals and a set of non-Lewis localised orbitals are used to interpret the electronic wave functions.

For Fe (III) - ADRS₂ and Cu (II)-ADRS₂ systems, the NBO results were reported in the following table (**Table 4**)

Table 4. NBO analysis studying the non-covalent interactions in Fe (II) - ADRS₂ and Cu (II)-ADRS₂ systems

	Donor	Acceptor	Energy (kcal/mol.)
Fe(II)-ADRS₂	LP(2) O 20	LP*(6) Fe 135	42.97
	LP(2) O 20	LP*(7) Fe 135	32.28
	LP(1) O 27	LP*(6) Fe 135	11.48
	LP(1) O 27	LP*(7) Fe 135	13.51
	LP(2) O 27	LP*(5) Fe 135	24.78
	LP(2) O 27	LP*(6) Fe 135	29.96
	LP(2) O 27	LP*(7) Fe 135	20.84
Cu(II)-ADRS₂	LP(1) O 20	LP*(6) Cu 135	5.44
	LP(2) O 20	LP*(6) Cu 135	15.96
	LP(1) O 27	LP*(6) Cu 135	5.05
	LP(2) O 27	LP*(6) Cu 135	18.95

The bonds are formed by electron transfer from the donor (oxygen) atoms of the ADRS molecule to the iron (II) or Cu (II) vacant orbitals. The energies of these transitions are strong; therefore, they stabilize the formed organometallic complexes.

3.3.4 Quantum Theory of Atoms in Molecules (QTAIM) analysis

The different intermolecular interactions stabilizing the studied metal complexes were explored with the topological analysis of the electron, within the conceptual framework of the quantum theory of atoms in molecules (QTAIM) [16, 17].

Among the topological properties of the electron density that were successfully used to analyze the bonding in the complexes, the electron density at bond critical point $\rho(r)$, the Laplacian of the electron density $\nabla^2\rho(r)$, the potential electron energy density ($V_{(r)}$), the local gradient kinetic energy density ($G_{(r)}$) and the total electron energy density ($H_{(r)}$), are of fundamental importance.

The presence of a critical point (CP) indicates a change in the topology of the density and, hence, a change in the molecular structure. As shown in **Fig.8**, following the interaction between the metallic ion and two ADRS molecules four bond critical points (BCPs) and two ring critical points (RCPs) were obtained. The corresponding topological data were reported in **Table 6**.

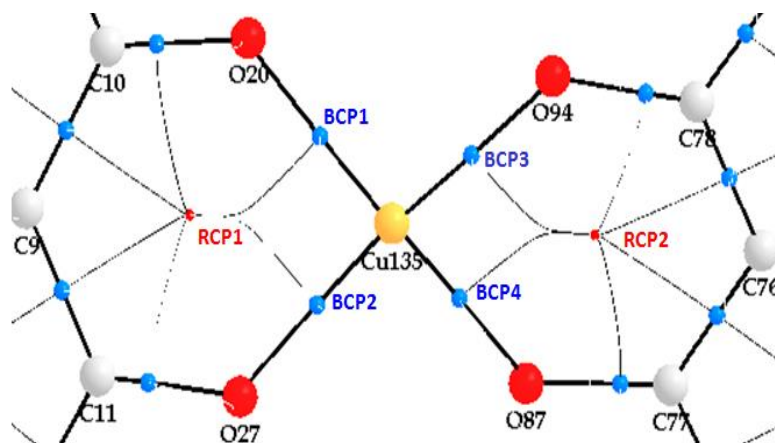


Fig.8 Observed critical points (BCPs and RCPs) following the formation of the Cu (II)-ADRS₂ complex.

In order to investigate the nature and the strength of these interactions, $\nabla^2\rho(r)$ and $H(r)$ were used as descriptors. For all bonds, the values of $\rho(r)$ were small ($\approx 10^{-2}$ a.u) and those of $\nabla^2\rho(r) > 0$ ranging from 0.778657 a.u to 0.875155 a.u for the iron(II) complex, and from 0.478381 a.u to 0.510266 a.u, for the copper(II) complex. These findings characterize the closed-shell interactions [51, 52] which were mostly dative ($H(r) < 0$) for the copper complex and weakly ionic ($H(r) > 0$) in the iron complex. Additionally, since $0.5 < -\frac{G(r)}{V(r)} < 1$, the interactions Cu (II) –O and Fe (II) have a dominant covalent character.

Table 5 Selected topological parameters of $\rho(r)$ function for intramolecular bonds in Cu (II) and Fe (II) complexes.

	BCP	$\rho(r)$	$\nabla^2\rho(r)$	$V(r)$	$G(r)$	$H(r)$	$-G(r)/V(r)$
Fe(II)-ADRS ₂	O20-Fe135	0.064544	0.778657	-0.176414	0.185539	0.009125	0.9517
	O27 -Fe135	0.067806	0.875155	-0.193361	0.206075	0.012714	0.8658
	O87 -Fe135	0.064545	0.778714	-0.176426	0.185552	0.009126	0.9517
	O94 -Fe135	0.067805	0.875047	-0.193340	0.206051	0.012711	0.9657
	RCP1	0.014245	0.072456	-0.14608	0.016361	-0.12972	-0.1120
	RCP2	0.014245	0.072453	-0.14607	0.016360	-0.12971	-0.1120
Cu(II)-ADRS ₂	O20-Cu135	0.089989	0.478381	-0.166153	0.142874	-0.023279	0.8599
	O27 - Cu135	0.094952	0.510213	-0.176380	0.151967	-0.024413	0.8616
	O87 - Cu135	0.089993	0.478420	-0.166164	0.142885	-0.023279	0.8599
	O94 - Cu135	0.094958	0.510266	-0.176396	0.151981	-0.024415	0.8616
	RCP1	0.016041	0.080497	-0.016347	0.018236	-0.12972	-0.1120
	RCP2	0.016041	0.080499	-0.016347	0.018235	-0.12971	-0.1120

Conclusion

Interaction of ADRS ligand Fe (II) and Cu (II) ions in ethanol at 25 °C were studied experimentally using UV-visible spectrophotometry. The data processing with a nonlinear least square fitting allowed the determination of stoichiometries, stability constants of the complexes and species distribution diagrams for each complex against ligand concentration.

DFT study was performed to obtain insights on Fe/ADRS and Cu (II)/ADRS systems. The calculations were firstly carried out at two levels: B3LYP and B3LYPGD3BJ under the same 6-311++G (d,p) basis set. The performance of each exchange functional was tested with respect to the prediction of structural parameters of ADRS. Subsequently, with the best functional thus obtained, calculations were performed on the most stable (1:2) complexes to obtain information on their structures and properties. The interactions nature within the metallic complexes were explored and studied with NBO and QTAIM analysis. Bonds were created by electron transfer from the donor (oxygen) atoms of the ADRS molecule to the empty orbitals of iron (II) or copper (II).

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