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# SYNTHESIS, CHARACTERIZATION, DFT CALCULATIONS AND BIOLOGICAL EVALUATION OF AZO DYE LIGAND CONTAINING 1,3-DIMETHYLXANTHINE AND ITS Co(II), Cu(II) AND Zn(II) COMPLEXES

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**ABSTRACT**. The azo dye of 1,3-dimethylxanthine, namely 8-[(4-acetyl phenyl azo)]-1,3-dimethylxanthine (APAX), was prepared in two steps. The first step included the formation of the diazonium salt by reacting 4-aminoacetophenone with sodium nitrite $NaNO_2$ under acidic conditions and completing the diazotization process while maintaining a low temperature below 5 °C. The resulting diazonium salt was reacted with the 1,3-dimethylxanthine compound and obtained on the azo dye, which was characterized by UV-Vis, FT-IR, $^1$H-NMR spectroscopies and elemental analysis. DFT calculations were performed to obtain optimized geometry of the ligand. These calculations were performed using three functionals and 6-311++G (d,p) basis and allow to determinate bond length, bond angles, dihedral and HOMO and LUMO energies. The APAX ligand exhibited considerable color changes when it mixed with solution of cobalt, copper and zinc ions, therefore, it is considered a probe to detect these ions. Metallic complexes of APAX ligand were prepared by interacting aqueous solutions of the metallic salts of Co(II), Cu(II) and Zn(II) with APAX ligand under a molar ratio equal to 2:1 (APAX ligand : metal). The APAX dye and the complexes showed exciting biological activity against *E. coli*, *Staph. aureus* and *Aspergillus*. The APAX dye was pH sensitive and exhibited interesting photochromic behavior.

**KEY WORDS**: 1,3-Dimethylxanthine, Dye, Complexes, pH sensitive, Probe, Bacteria, DFT calculations

## INTRODUCTION

Azo dyes have received considerable attention due to their importance in numerous disciplines such as medicine, clinics, biochemistry, industry and the environment. Azo dyes have a good ability to coordinate with the metal ions, therefore this type of dye considers as an efficient sensor to detect the metal ions besides the simple process of their preparation, low cost, and sensitivity to pH changes [1, 2].

The heterocyclic azo dyes get wide attention in the textile industry and extensive applications in medicine like photodynamic therapy, antiviral, antifungal, and antioxidant properties due to the presence of N, O, S atoms in their structures [3, 4].

Azo dyes are color compounds which mean that the agitation process of electrons needs light in the visible area or the red area; therefore, there are an attraction to use as non-linear and photo-electronic, essentially in optical information storage [5] and in photodynamic treatment as photosensitizer [6, 7]. Azo dyes and their complexes are also suitable for two-photon excitation (TPE) in photodynamic therapy (PDT) [8].

The 1,3-dimethyl xanthine is one of the most essential compounds used for therapeutic purposes such as bronchitis, asthma, chronic obstructive pulmonary disease and emphysema among respiratory diseases all over the world due to its low cost and availability on large scale steroids or sodium cromoglycate [9, 10].

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The 1,3-dimethel xanthine compounds are among the important compounds used in various fields of life, especially medicine and pharmacology, where they have proven biological efficacy against microbes (antimicrobial) and anti-cancer, the most important of which are breast and skin cancer [11–13], which is most prevalent in developing countries. As the activity of a compound depends on its geometry's stability, it is very important to determinate the most stable conformation of a compound using molecular modelling. Indeed, several works have been done to study the geometry of ligands and their complexes [14, 15]. Therefore, we prepared and characterized azo dye of 1,3-dimethylxanthine and its complexes of Co(II), Cu(II), and Zn(II) and study their biological activity and study spectral properties of prepared dye. Also, we studied the geometry of the ligand using DFT calculations to obtain its optimized geometry, structural parameters and HOMO and LUMO energies.

## EXPERIMENTAL

The UV-Vis spectra were done by Spectrometer Shimadzu UV1800 Spectrophotometer and the data of mole ratio were obtained by Spectrophotometer-J7310, the NMR was made by BAMX400 MHZ Spectrophotometer, the melting point was done by Stuart Scientific SMP3 Melting Point Apparatus. The data of elemental analysis were obtained from EURO EA 300 C.H.N. Element analysis2012. Data on magnetic susceptibilities were acquired from MSB-Auto Magnetic Susceptibility Balance. Samples of APAX ligand and its complexes were prepared for examination by infrared spectroscopy using FTIR-8400 Shimadzu in potassium bromide in the range of 4000 to 400 $cm^{-1}$.

The solvents used in this research have a high degree of purity. 1,3-Dimethylxanthine was used from Sigma Aldrich, 4-aminoacetophenone from Scharlau, cobalt(II) chloride hexahydrate and sodium hydroxide from Fluka, copper(II) chloride dihydrate from Merck, zinc(II) chloride and sodium nitrite from B.D.H.

### *Preparation of ligand 8-[(4-acetyl phenyl azo)]-1,3-dimethylxanthine (APAX)*

The ligand (APAX) was prepared by dissolving 0.5 g (0.0036 mol) of the 4-aminoacetophenone in 10 mL of distilled water then was mixed with 3 mL of hydrochloric acid. The mixture was cooled to 0 °C and then was mixed with 15 mL aqueous solution of 0.24 g (0.0036 mol) sodium nitrite. The mixture solution was left to settle for twenty minutes in order to complete forming diazonium salt. Then the diazonium salt solution was added to the aqueous solution of the coupling compound resulting from dissolving (0.64 g, 0.0036 mol) of 1,3-dimethylxanthine in 30 mL of an aqueous solution having 0.4 g (0.01 mol) of sodium hydroxide. After completing this addition process, the solution is left under constant stirring and cooling for 1 hour; after that, the solution was left for the next day. Then the solution was acidified with dilute hydrochloric acid to form a dark brown precipitate. Then the precipitate was filtered and washed with distilled water several times. Then it was recrystallized by ethanol. The dark brown precipitate formed was placed in an oven for several hours at the temperature 50 °C.

The ratio of the ligand to metal [L: M] was determined by the mole ratio method (fixing the amount of the metallic salts and changing the amount of the ligand. The size of the salt solution was fixed at 1 mL and $10^{-4}$ M in some volumetric flasks with variable volumes of APAX ligand solution (0.25 mL to 6.5 mL, with a step of 0.25 mL). We added in the every case a suitable volume of distilled water to complete the volume of solution in the volumetric flasks to 10 mL.

### *Preparation of metallic complexes for APAX ligand*

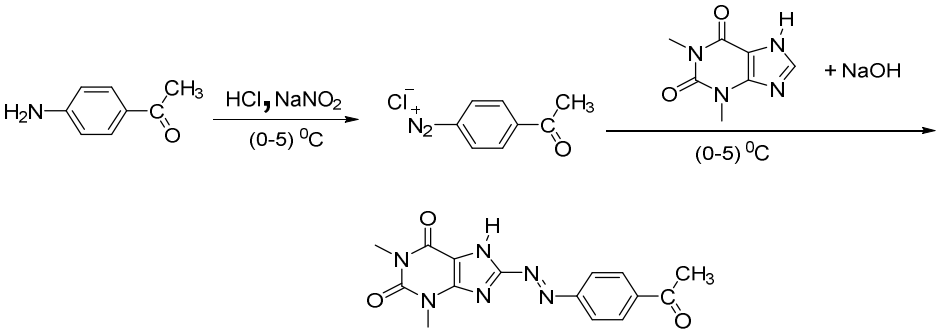
The metal complexes were prepared in a mole ratio (2:1) L: M by dissolving 0.1 g, 0.0003 mol of APAX ligand in 10 mL of ethanol, then the heated resulting solution was added gradually to 5

mL aqueous solution of having 0.00015 mol of metal chloride solutions of cobalt, copper and zinc. The resulting solution was refluxed at 60 °C for 50 min. After that, a residue of complexes of these metals was observed. The precipitate was filtered and was washed with distilled water several times, then was washed with a little of ethanol.

The culture media was prepared according to the manufacturer's instructions by 38 g of (Mueller-Hinton agar) was added to 1000 mL of distilled water in a conical flask. The mixture was heated until perfect dissolving of powder. Then the medium was sterilized within a period of 15 min under 120 °C and pressure (15 pounds/inch). Then poured into dishes called Petri dishes 10 mL and then the temperature of the dish was lowered to room temperature to solidify. Preparation solutions of APAX ligand and its metallic complexes under study were 250 ppm. The bacteria were spread in the dishes on the surface of the culture media (Muller-Hinton agar) and were made three holes of 6 mm in diameter using an alcohol-sterilized cork drill with a space left between one hole and another to avoid overlapping of the damping areas between them. In these holes were added 0.1 mL of every compound using a fine pipette for that and then placed in an incubator for 24 hours at 37 °C. We measured the inhibition zone of these compounds with a millimetre ruler.

## RESULTS AND DISCUSSION

The azo ligand of 1,3-dimethylxanthine was prepared by preparing the diazonium salt from the reaction of 4-aminoacetophenone in an acidic medium with sodium nitrite, then reacting the diazonium salt with the 1,3-dimethylxanthine in basic aqueous solution to form the dye. The yield was 79% and dark brown color powder and a melting point is 172 °C as shown in Scheme 1.




Scheme 1. The preparation steps of 8-[(4-acetyl phenyl azo)]-1,3-dimethylxanthine.

The $^1$H NMR spectrum of the APAX ligand showed two bands in the aromatic area, both are doublet, with a multiplicity equal to two protons. They have same coupling constant of 6.8 Hz, which confirms that they have the same environment. These signals are due to protons of the phenyl ring. It also showed a single signal at 14.06 ppm belonging to the N-H proton of 1,3-dimethylxanthine. As well as three single signals in the aliphatic region, every one presents three protons, as shown in Figure 1. Overall, the dye showed six signals with a number of 14 protons in agreement with the number of protons of the ligand (14.06 (s, H), 8.25 (d, 2H, J = 6.8 Hz), 8.06 (d, 2H, J = 6.8 Hz), 3.49 (s, 3H), 3.31 (s, 3H), 3.25 (s, 3H), 2.60 (s, for water of solvent), 2.49 (s, for the DMSO solvent)).

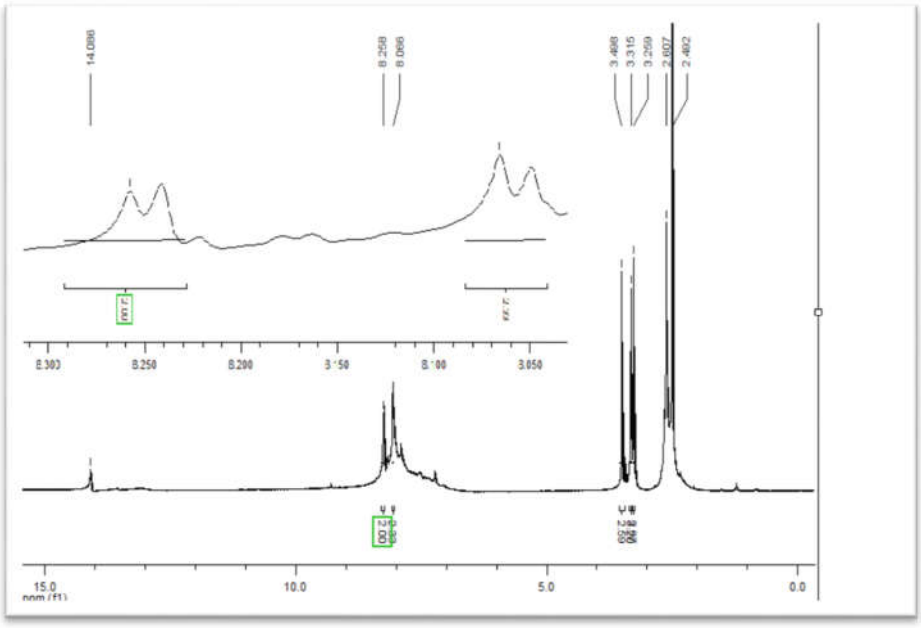



Figure 1. $^{1}$H NMR spectrum of the APAX ligand in dimethyl sulfoxide solvent *d6* at room temperature.

The color of the neutral form of APAX dye is orange in an aqueous solution and the colors of APAX salts in acidic condition at pH = 2 is red, while in the basic condition at pH = 10 is violet. This feature of different colors in different values of pH serves that APAX dye is used as acid-base indicator [16]. Color changes were monitored by the changes in UV-Vis spectra under different values of pH and it was observed that there are essential differences between the spectra. The colorimetric behavior of APAX dye is in agreement with UV-Vis spectra under different values of pH.

The APAX dye can exist in two forms called cis and trans, which can stimulate under irradiation or heating. Under the light of 359 nm in an aqueous solution at room temperature, the spectrum of APAX in trans isomer goes down. After 5 min of irradiation, there was no change. Important point, when we removed source of irradiation, the spectrum backed to apply on the initial state before irradiating. That means that, the azo dye of APAX exhibits real photoisomerization behavior [17, 18].

The mole ratio method is an essential spectroscopic method to determine the ratio of ligand to metal (L : M) in the composition of metal complexes. The mole ratio of APAX ligand to Co, Cu and Zn divalent is 2:1, as shown in Figure 2. On the other hand, the addition of APAX ligand to the metal ions of cobalt, copper and zinc exhibited significant changes in colors in aqueous solutions. That indicates to easy coordinating APAX ligand to these metal ions with high selectivity and sensitivity for selective metal ions in this study. Metal sensors of copper, zinc and cobalt are important topic right now, because these metal ions are engaged in a wide range of biological and environmental processes.

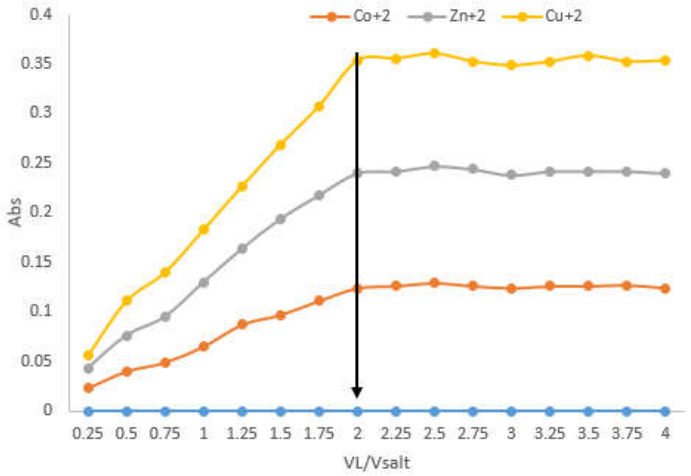



Figure 2. Curves of the molar ratio of the Co(II), Cu(II) and Zn(II) ions with APAX ligand.

Molar conductivity is a technique used to arrive at the ionic formulas for metallic complexes based on the direct proportionality between the degree of electrical conductivity and the charged components. From the results listed in Table 1, the molar conductivity values were low for these complexes in DMF solvent at $10^{-3}$ M and under laboratory temperature, which indicates the absence of ionic character in these complexes.

Analysis ratio of carbon, hydrogen and nitrogen in the studied compounds was adopted for the purpose of supporting the validity of mixing ratios of metal ions with the ligand. It was found that there is a remarkable convergence between the theoretically determined results and the obtained results in the practice, which are included in Table 1. On the other hand, the physical properties of these complexes (melting point, color and yield), are also listed in Table 1.

Table 1. The elemental analysis of APAX ligand and its complexes and their physical properties

| Compound | Molar conductivity $S.Cm^2.mol^{-1}$ | Found (calc.) % | | | Color | Yield% | Mp °C |
|---|---|---|---|---|---|---|---|
| | | C | H | N | | | |
| APAX Ligand | - | 55.04 (55.21) | 4.21 (4.32) | 25.56 (25.75) | Dark brown | 79 | 172 |
| $[Cu(APAX)_2Cl_2]$ | 5 | 45.67 45.78 | 3.48 (3.59) | 21.30 (21.36) | Dark red | 71 | 230 |
| $[Co(APAX)_2Cl_2]$ | 10 | 45.91 (46.05) | 3.31 (3.61) | 21.25 (21.48) | Brown | 73 | 199 |
| $[Zn(APAX)_2Cl_2]$ | 10 | 45.45 (45.67) | 3.47 (3.58) | 21.29 (21.31) | Pale red | 65 | 153 |

Infrared spectroscopy is one of the vital techniques to characterize the active groups in the ligands and complexes, as well as observing and following up on the difference between before the interaction of the ligands with the metal ion and after the formation of the complex. The changes indicate coordinating of these groups to metal ions in the complexes.

The infrared spectrum of the APAX ligand showed a number of characterized peaks for the active groups in the APAX ligand, which are listed in Table 2, including the NH group at 3419 $cm^{-1}$, the CH aromatic group at 3005 $cm^{-1}$, the CH aliphatic group at 2918 and 2850 $cm^{-1}$. In addition, the carbonyl group appeared at 1697 $cm^{-1}$. A peak at 1647 $cm^{-1}$ is due to the absorption of C=N group, as well as a peak at 1552 $cm^{-1}$ due to the absorption of C=C group. As well as the absorption of azo group at 1529 $cm^{-1}$. The infrared spectra of the APAX ligand showed some differences, like decreasing the frequencies of the C=N group and azo group in the complexes relative to what is in the ligand, which indicates the coordination of these groups with the metal ions in the complexes [19].

Table 2. The frequencies ($cm^{-1}$) of the important groups of APAX ligand and its complexes.

| Compound | NH | CH aromatic | CH aliphatic | C=O | C=N | C=C | N=N |
|---|---|---|---|---|---|---|---|
| APAX Ligand | 3419 | 3005 | 2918, 2850 | 1987 | 1647 | 1552 | 1529 |
| Co complex | 3524 | 3001 | 2918 | 1683 | 1600 | 1538 | 1508 |
| Cu complex | 3480 | 3003 | 2955 | 1705, 1689 | 1600 | 1556 | 1477 |
| Zn complex | 3485 | 3003 | 2922, 2852 | 1707 | 1600 | 1556 | 1477 |

*Electronic spectra of the APAX ligand and its complexes*

The spectrum of the APAX ligand showed bands at 240, 279 and 341 nm that belong to the electronic transition of $\pi \rightarrow \pi^*$ and a band at 460 nm belongs to the $n \rightarrow \pi^*$ electronic transition [20]. The cobalt complex of the APAX ligand showed a band at 324 nm due to the $\pi \rightarrow \pi^*$ of APAX ligand. As well as three d-d-type electronic absorption bands, a transition at 450 nm due to the $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ electronic transition, the second transition was at 714 nm due to the $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ electronic transition, and the third d-d electronic transition is $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ at 901 nm. The value of the magnetic sensitivity was 4.8 B.M. This is in agreement with the octahedral complexes of cobalt [21].

The spectrum of the copper complex showed a band at 333 nm belonging to the electron transition of the APAX ligand and three bands belonging to the metal transition. The first transition at 456 nm belonging to the $^2B_{1g} \rightarrow {}^2E_g$ (dxz, dyz$\rightarrow dx^2\text{-}y^2$) and a band at 600 nm belonging to the electron transition $^2B_{1g} \rightarrow {}^2B_{2g}$ (dxy $\rightarrow dx^2\text{-}y^2$) and the third transition at 872 nm due to the electronic transition $^2B_{1g} \rightarrow {}^2E_g$ ($dz^2 \rightarrow dx^2\text{-}y^2$). These transitions are consistent with the transitions of the octahedral copper complexes and this is supported by the magnetic sensitivity of the copper complex, which is equal to 1.96 B.M. The spectrum of the zinc complex of APAX ligand showed four bands at 233 nm, 276 nm, 354 nm and 471 nm, like the ligand spectrum with a red shift in the last band, which confirms the occurrence of coordination between APAX ligand and the zinc ion.

*Antimicrobial activities*

Azo dyes, especially those with heterocyclic aromatic rings, have shown anti-bacterial activity against many bacteria and fungi. Therefore, the efficacy of APAX dye of 1,3-dimethylxanthine and its complexes against two types of staph bacteria was tested against *S. aureus*, which is gram-positive, and against *E. coli*, which is gram-negative. These compounds were also tested against the human pathogenic fungus *Aspergillus niger*.

The APAX ligand and its chelating complexes were able to inhibit the growth of *E. coli* and *S. aureus* bacteria. The APAX ligand showed a high inhibition rate against both types of bacteria, the inhibition rate was 2 cm against *E. coli* and 2.8 cm against *S. aureus*. The cobalt complex showed good inhibition against *S. aureus* bacteria, which was 1.3 cm, while against *E. coli* showed a medium inhibition rate of 0.5 cm. The copper complex did not show any inhibitory activity

against both types of bacteria. The zinc complex showed varying inhibition, it showed a medium inhibition about of 0.1 cm against *E. coli* bacteria, and inhibition rate was high against and *S. aureus* bacteria up to 0.9 cm.

Table 3. The effect of APAX ligand and its complex on bacteria (inhibition zone, cm) in aqueous solution.

| Compound | *E. coli* (Gram-negative) | *S. aureus* (Gram-positive) | *Aspergillus* |
|---|---|---|---|
| APAX ligand | 2 | 2.8 | 3 |
| Cu complex | 0 | 0 | 1.9 |
| Co complex | 0.5 | 1.3 | 1.2 |
| Zn complex | 0.1 | 0.9 | 1.7 |

*Antifungal activity*

To evaluate antifungal properties of APAX dye and its complexes against the pathogenic fungus of *Aspergillus*. We used the minimum concentration of these substances, which was 50 mg/mL. Where all compounds showed a high efficacy inhibition between 3 to 1.2 cm. The APAX ligand showed a high inhibition rate in the range of 3 cm, while the complexes showed a good inhibition rate of 1.9 to 1.2 cm. These results are included in Table 3.

*Theoretical study of the APAX ligand*

The geometry of the studied ligand has been optimized with 6-311++ G (d,p) basis. We used three functionals; RCAM-B3LYP, RB3PW91 and RB3LYP; which respectively give the following energies -1055.412060 Hartree, -1055.475111 Hartree and -1055.893870 Hartree. The obtained energies with the three functionals agree and do not differ dramatically. However, the data show that RB3LYP gives the lowest energy and the most stable geometry of the APAX ligand is represented in Figure 3.

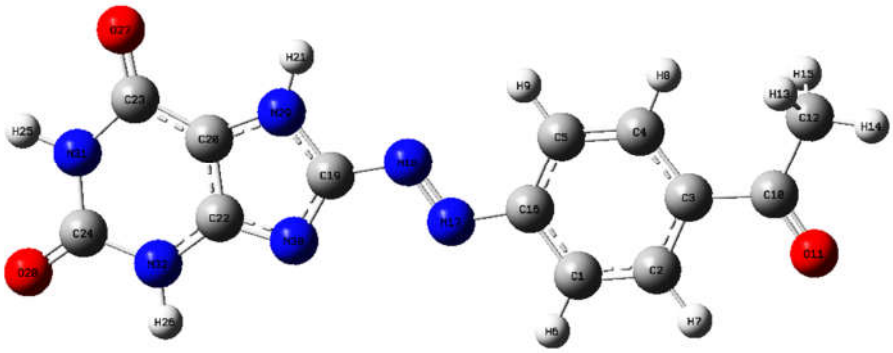


Figure 3. optimized geometry of APAX, 6-311++ G(d,p) / RB3LYP.

We reported structural parameters of the ligand (bond lengths, bond angles and dihedral) in Table 4.

Table 4. Bond lengths (Å), bond angles (degree), dihedral (degree) in the APAX ligand, determined by DFT calculations.

| Atoms | Bond lengths (Å) | Atoms | Bond angles (degree) | Atoms | Dihedral (degree) |
|---|---|---|---|---|---|
| N17=N18 | 1.25929 | C1-C16-N17 | 115.07072 | C19-N17=N18-C16 | -179.96909 |
| C10=O11 | 1.21579 | C5-C16-N17 | 125.01336 | N29-C19-N18=N17 | -179.98637 |
| C23=O27 | 1.21715 | C16-N17=N18 | 115.18145 | N30-C19-N18=N17 | 0.01414 |
| C24=O28 | 1.21149 | N17=N18-C19 | 114.91282 | | |
| C20-C22 | 1.38872 | C19-N29-H21 | 125.93723 | | |
| C20-N29 | 1.36891 | C19-N30-C22 | 104.04207 | | |
| C20-C23 | 1.43910 | C22-N32-C24 | 121.71283 | | |
| C23-N31 | 1.40948 | N32-C24-O28 | 123.13856 | | |
| N31-C24 | 1.40086 | N31-C23-O27 | 122.32034 | | |
| C24-N32 | 1.38910 | C20-C23-O27 | 127.58286 | | |
| N32-C22 | 1.37320 | C19-N29-C20 | 106.84300 | | |
| C22-N30 | 1.34546 | C3-C10-O11 | 120.23268 | | |
| N30-C19 | 1.33641 | C12-C10-O11 | 120.81977 | | |
| C19-N29 | 1.36579 | | | | |
| C19-N18 | 1.38298 | | | | |
| N17-C15 | 1.40996 | | | | |
| C5-C15 | 1.40453 | | | | |
| C5-C4 | 1.38553 | | | | |
| C4-C3 | 1.40578 | | | | |
| C3-C2 | 1.40097 | | | | |
| C2-C1 | 1.38634 | | | | |

For a more detailed study on the geometry of the APAX ligand, frequency calculations were carried out (Table 5). The comparison of these results with those of the experimental study shows some difference in values. This is probably due to the behavior of the molecule in the real environment. Indeed, several approximations are made during the theoretical study.

Table 5. APAX frequencies calculated, 6-311++/G(d,p) RB3LYP.

| Functional groups | Frequencies |
|---|---|
| N-H out-of-plane deformation | 625.93 |
| N=N deformation | 929.48 |
| C-N stretching | 1055.37 |
| C-N cyclic stretching | 1340.05 |
| C-C stretching | 1385.80 |
| C=C cyclic | 1483.15 - 1640.73 |
| C=O stretching | 1748.53, 1761.78, 1793.34 |
| $CH_3$ symmetric stretching | 3033.30 |
| $CH_3$ asymmetric stretching | 3090.56 - 3145.28 |
| C-H cycle | 3189.17 - 3312.17 |
| N-H stretching | 3599.13, 3626.77, 3633.95 |

Frontier molecular orbitals (FMOs) of the studied complex were carried out with RB3LYP functional. HOMO ((highest occupied molecular orbital) whose electrons participate in nucleophilic attacks, LUMO (lowest unoccupied molecular orbital), which are electrophilic sites and the gap (HOMO-LUMO) energies are represented in Figure 4. The frontal orbital gap allows

characterizing the chemical reactivity [22] and the kinetic stability of the molecule [23]. The value of the gap shows a high reactivity of the studied ligand. This is in good agreement with the experimental results. Indeed, the APAX ligand was able to form stable complexes with metal ions Co(II), Cu(II) and Zn(II).

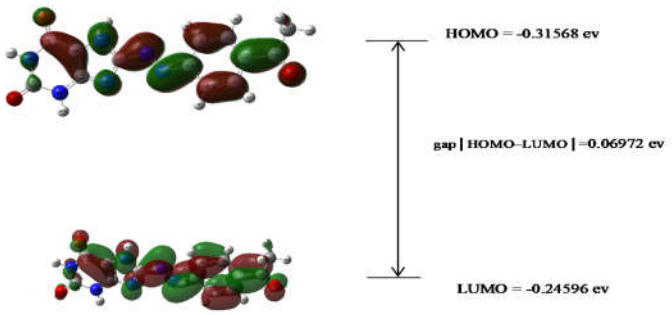



Figure 4. Frontier molecular orbitals of APAX ligand.

## CONCLUSION

The APAX ligand namely, 8-[(4-acetyl phenyl azo)]-1,3-dimethylxanthine was synthesized in good yield in two steps: preparing diazonium salt of 4-aminoacetophenoe, then coupling diazonium salt with 1,3-dimethylxanthine. The colorimetric studies exhibited ability of the APAX ligand to detect of Co, Cu and Zn ions. The APAX ligand could be used as an indicator. The stoichiometry of APAX ligand to metal ions was 2:1, getting by the molar ratio method. The complexes of APAX were synthesized and characterized by analytical methods. The behaviour of the APAX ligand is bidentate in all its complexes coordinated through the nitrogen atoms of imine in xanthine and nitrogen of the azo group. APAX complexes are octahedral. The APAX ligand and the complexes have appreciable activity against bacteria and fungi and the APAX ligand exhibited more potent than other complexes. The optimized geometry of the APAX ligand was performed using RB3LYP functional and 6-311++ G (d,p) basis. Bond lengths, band angles, dihedral, frequencies and frontier molecular orbitals were calculated. Theoretical study of the ligand confirms the experimental data. We plan in the future to study the structure of the complexes isolated in this work by molecular modelling which can provide more precisions on the complexes geometry.

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