Synthesis, Characterization, DFT calculation and Antimicrobial Activity of Co(II) and Cu(II) complexes with azo dye

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Abstract. In this study, the ligands based on azo dye of 3,6-diamino-10-methyl acridinium chloride (MAA) and its complexes of Co(II) and Cu(II) were synthesized and characterized successfully via mass, UV-Vis, and infrared spectroscopy and elemental analysis. The energies of HOMO and LUMO orbitals of the MAA ligand were calculated via density functional theory (DFT). The LUMO orbital of the MAA dye is appropriate to donate electron injection to the condition band of TiO₂. The band energy of MAA dye was 1.8 eV which encourages using it as a sensitizing dye in dye-sensitized solar cells. The MAA ligand exhibited interaction ability more than reacting material (3,6-diamino-10-methyl acridinium) according to results of click docking software. The MAA dye and its complexes exhibited attractive biological activity towards streptococcus and staph aureus.

Keywords: Azo dye, Biological Activity, DFT

Introduction
Hybrid nanomaterials based on organic or inorganic molecules are known to possess unique optical and electronic properties such as incorporating azo benzene molecules into a monolayer of gold nanoparticles. These properties were led to use them in different fields like photonics and optoelectronics. Photochromic materials like azo dyes and their complexes, showing a reversible transformation between two isomers upon the irradiation with appropriate light. This behavior leads to be using in many applications such as optical data storage, photochromic lenses, phototherapy, DNA scissors, electro-chemical writing, and multi-photon devices. Recently, Dye-sensitized solar cells, (DSSCs) have fascinated wide attention for clean energy applications and high efficiency. The dyes capture the solar energy then convert the photons to electrons which inject into nanoparticles for semiconductor such as titanium oxide (TiO₂).
The DSCs are very interesting because of easy fabrication and combing with low cost having a good conversion efficiency of sunlight reached 13% even upon low flux of sunlight\(^{9-13}\). The dye sensitizers are known to play essential role in DSSCs\(^{14}\). The first step in the DSSCs is photoexcitation which occurs in the dye sensitizer upon absorption the sunlight via the dye\(^{15}\). Therefore we were interested in azo dyes of 3,6-diamino-10-methyl acridinium to get lower energy level of LUMO orbital (low gap for photoexcitation) and large absorption with more utilization of visible light which leads to high efficiency. The acriflavine dye exhibited effects on bacterial growth of \textit{Klesienia} and \textit{K. Pneumonine}\(^{16}\). Azodye ligand composed from 4-Aminoantipyrine and 2, 4-dihydroxyacetophenone and its compounds of Co(II), Ni(II), Cu(II) and Zn(II) exhibited antibacterial activity\(^{3}\). The 3,6-diamino-10-methyl acridinium shows significant biological effects and promising spectroscopic properties therefore we were interested in synthesis and study of its azo dyes and its complexes.

**Experimental**

**Materials and apparatus**

The solvents used were reagent grade and used without further purification. 3,6- diamino-10-methyl acridinium chloride, P-anisidine, CoCl\(_2\).6H\(_2\)O and CuCl\(_2\). H\(_2\)O were purchased from BOC science, Sigma Aldrich and Merck. Infrared spectra were carried out by using Shimadzu, FTIR-8400S fourier transform. UV-Vis spectra were performance by spectrophotometer-1800 (Shimadzu). Mass spectroscopy was carried out by using a Hitachi-PerkinElmer model RMU-6L-Spectrometer. Elemental analysis (C, H and N) for the synthesized compounds was performed by using EU Elemental Analyzer.

**Synthesis of MAA ligand and its complexes**

(0.5 g, 1.9 mmol) of 3,6- diamino-10-methyl acridinium chloride and 5 mL of hydrochloric acid was mixed in 15 ml distillated water. Resulting solution was kept under cooling (0 °C). A solution of NaNO\(_2\) (0.26 g, 3.8mmol) in 10 ml water was added drop by drop in dark condition in 3,6- diamino-10-methyl acridinium chloride solution to form diazonium ion. The solution of p-anisidine was put under cooling (0 °C). The solution of diazonium ion was added slowly in the solution of p-anisidine (0.47 g, 3.8 mmol) in ethanol. The resulting mixture was stirred for one hour at 0-5°C. After one hour reaction mass was filtrated and washed with distilled water to afford dark brown precipitate no fluorescence in 75% yield. It is an important point that we wash the powder by little bit of ammonia to remove the acidity because the mass spectroscopy mentioned to find (M+H/+2). Anal. Calcd for MAA dye (C\(_{28}\)N\(_7\)H\(_{26}\)O\(_2\)Cl): C,63.69; H, 4.96; N, 18.57. Found: C, 63.81; H, 5.01; N, 18.72. FT-IR: (cm\(^{-1}\)) 3444 and 3381 for (N-H of amino group of amino of anisidine), 3182 (C-H aromatic), 2951 (C-H aliphatic), 1600 (C=N), 1556 (C=C), 1510 (N=N azo group), 1467 (C-H bending of methyl), 1184 and 1118 (C-O of methyl phenyl ether). 0.5 g, 0.94 mmol of MAA ligand was dissolved in 15 mL ethanol. 1.89mmol of salt (CoCl\(_2\).6H\(_2\)O or CuCl\(_2\).2H\(_2\)O) was dissolved in 15mL of ethanol. The solution of MAA ligand was added to solution of salt. The mixture of reaction was refluxed for 40 minutes in dark conditions. The solution was cold for 30 minutes. The solution was filtrated and dried. The yield of both complexes is 60-70%. Anal. Calcd for Co(II) complex (C\(_{28}\)N\(_7\)H\(_{26}\)O\(_2\)Cl\(_5\)Co): C,42.69; H, 3.33; N, 12.45. Found: C, 42.95; H, 3.82 ; N, 12.75 and the anal. Calcd for Cu(II) complex (C\(_{28}\)N\(_7\)H\(_{26}\)O\(_2\)Cl\(_5\)Cu\(_2\)): C,42.20; H, 3.29; N, 12.30. Found: C, 42.61; H, 3.64; N, 12.62.

**DFT calculations**

The optimization of the MAA ligand was done by Gaussian 09 package using the B3LYP function with basis set (6-31 G)\(^{17}\). The theoretical UV-visible spectrum of MAA ligand was done in DMF using TD-DFT with B3LYP function.
Biological Activity
Antibacterial activity of the MAA dye and its complexes Co(II) and Cu(II) was estimated against some Gram positive bacteria and Gram negative bacteria (Staph.aureus, Streptococci, E.coli and Klebsiella sip). We used Muler-Hinton agar and the ethanol as solvent. Crokporer was used to do three holes for each petri dish. Three concentrations (50, 75 and 100 mg/mL) were prepared for each synthesized compound in this study.

Result and discussion
The optimization of the MAA ligand was done by using B3LYP which depicts in figure 1.

Figure 1. Optimized structure of MAA ligand by B3LYP

The UV-Visible spectrum of the MAA dye was done in N,N-Dimethylformamide at 25 °C which is illustrated in Figure 2. The MAA dye exhibited three electronic transitions. The absorption bands at (275 nm) and (354 nm) are located in the UV region which are due to \( \pi \rightarrow \pi^* \). The third absorption band was at(460 nm, 20900 L.mol\(^{-1}\).cm\(^{-1}\)) which is due to n- \( \pi^* \).\(^{18}\)

Figure 2. Electronic spectrum of MAA ligand in DMF solvent at room temperature
The theoretical UV-Visible spectrum of the MAA dye was done in N,N-Dimethylformamide by using the TDDFT with B3LYP functional which depicts in Figure 3. The spectrum exhibited three bands having same absorption locates in experimental spectrum. That means that the theoretical spectrum accepts with experimental spectrum.

![Figure 3](image_url)

**Figure 3.** Electronic spectrum of MAA dye by DT-DFT using B3LYP function in DMF solvent

The complex Co(II) of MAA dye exhibited a new bands at 605 nm due to $^4A_2 \rightarrow ^4T_1 (F)$ and at 667 nm which is due to $^4A_2 \rightarrow ^4T_1 (P)$ transition. This visible transitions of Co(II) complex are due to a typical electronic transitions for tetrahedral of Co(II) complexes\(^9\). The complex Cu(II) exhibited a new band at 623 nm which is due to $^2B_2 \rightarrow ^2A_1$ transition. This visible transition of Cu(II) complex is consider a typical electronic transition for tetrahedral of Cu(II) complexes\(^10\). In addition, the bands of MAA dye ligand exhibited blue shift around 20 nm which reflects interaction the ligand with ions of Co(II) and Cu(II) and the data of infrared refer to the stretching of azo function of MAA ligand suffering red shift around 15 cm\(^{-1}\) in complexes and the amino group was suffered blue shift about 20 cm\(^{-1}\) in the complexes. Depending on these data and elemental analysis data of complexes, we can suggest the tetrahedral geometry for the synthesized complexes which depicts in Figure 4.

![Figure 4](image_url)

**Figure 4** Suggest structure for Co(II) and Cu(II) complexes with MAA ligand
Depending on the HOMO-LUMO energy levels of the MAA dye and the absorption of the dye in the visible region give a scientific insight on the performance of the dye in the DSSCs. The HOMO and LUMO of the dye, the conduction band edge level of the titanium oxide (IV) and the redox potential energy of Iodine/iodide electrolyte are shown in Figure 5. The LUMO potential of the dye locates above the conduction band edge level of the titanium oxide which is 4.00eV under vacuum. This state is favorable to injection the electron in the conduction band edge of titanium oxide from the dye in excited state. On the other hand, the potential of the HOMO of the dye is beneath the iodine/iodide redox potential which is 4.80 eV under vacuum. That means that, the dye has a fast regeneration which leads to avert the geminate charge recombination between oxidized dye and the photo-injected electrolyte in titanium oxide. It is great point that the energy gab between HOMO and LUMO of MAA dye is 1.8 eV less than many dyes. This means that the MAA dye is very sensitive for the light of sun.

![Energy levels of EHOMO and ELUMO of MAA dye, electrolyte (I/I3 -) and TiO2](image)

**Figure 5** Energy levels of EHOMO and ELUMO of MAA dye, electrolyte (I/I3-) and TiO2

The HOMO and LUMO orbitals of MAA dye supports the low band gab because the LUMO orbital of MAA dye spreads on all the dye (Figure 6) which refers to the stability of the exited state of dye.
Figure 6 HOMO and LUMO orbitals of MAA dye by B3LYP

Antimicrobial activity of MAA dye and its complexes Co(II) and Cu(II) were tested against *Staph. aureus*, *Streptococci*, *E.coli* and *Klebsiella* bacteria. The MAA dye exhibited high antibacterial activity against *Staph. aureus* (Figure 7). On the other hand, it has no antibacterial activity against other bacteria. The complexes of Co(II) and Cu(II) exhibited biological activity against *streptococcus* and *staph aureus* bacteria and they have no biological activity towards *E.coli* and *klebsiellaspp* bacteria.

Figure 7 The inhibitory effect of MAA dye on *Staph. Aureus*
Table 1 Antibacterial activity of MAA ligand and its complexes

| Bacteria                  | Concentration mg/mL | The diameter inhibition zone (cm) |
|---------------------------|---------------------|----------------------------------|
|                           | APP dye | Co(II) | Cu(II) |
| Streptococcus. gram-positive | 100     | -      | 1      | 1.5    |
| Staph.aureus (gram-positive) | 75      | -      | 1      | 1.5    |
| E.coli Gram-negative      | 100     | -      | -      | -      |
| Klebsiellaspp Gram-negative | 75      | -      | -      | -      |

We used 1-click docking to foretell the binging orientation of our ligand (MAA) on the tropinonereductase 1 protein and to know the valuation of the binding affinity. The MAA ligand exhibited high score of docking; it was 11 higher than acriflavin which the docking score of it was 8. It is noticeable that the protein surface shows that all the ligands were in the protein cavity which refers to a high binding affinity of MAA ligand to the target protein. It depicts in Figure 8.

![Figure 8](image_url)

**Figure 8** The protein surface for the MAA ligand in the cavity of tropinonereductase 1 protein

**Conclusion**

In conclusion we have reported synthesis of ligands based on azo dye of 3,6- diamino-10-methyl acridinium chloride (MAA) and its compounds with Co(II) and Cu(II) metal ions and their biological activity. The synthesized Dye (MAA ligand) was also analyzed for their DFT calculation study. Antimicrobial activity of synthesized ligand (MAA) and its complexes was also found to significant and
our finding suggested that present study is very useful from chemical as well as biological perspective. Our results may be useful for drug discovery programmer in near future.

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