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Synthesis, characterization and DNA binding Studies of hydrazine-based ligands and their corresponding copper (II) complexes

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Abstract

Hydrazone Schiff base ligands were synthesized using benzhydrazide, nicotinoyl hydrazide and 3,5-dibromo salicylaldehyde and two new hydrazine-based copper (II) complexes were also synthesized by reacting Cu $(CH_3COO)_2$ with *N'*– (3,5-dibromo-2-hydroxy benzylidene) benzo hydrazide (H_2L^1). The synthesized complexes were characterized by elemental analyses, IR and UV spectral studies. Based on these studies, the metal center was found to be in a square planar geometry. The binding mechanism of the synthesized ligands ($[H_2L^1]$, $[H_2L^2]$) and metal complexes ($[CuL^1]_2$, $[CuL^1Py]$) with CT-DNA have been investigated by electronic absorption titration and luminescence spectra. The binding constants from UV-visible absorption studies were found to be 5.1277×10^4, 3.7500×10^4, 5.2031×10^4, 1.4817×10^4. The results showed that both the ligands and their corresponding metal complexes interact with DNA by intercalative mode.

Keywords: Metal complexes, DNA binding, Florescence spectroscopy, Hydrazone.

1. Introduction

Hydrazones are class of ligands which contain -NH-N=HC- group in their molecule(1). They are well attracted and have exceptional importance in the field of research because of their chelating capacity and structural pliability. Diverse studies on acyl hydrazones were published and here we are incorporating more contributions to the field of hydrazines and their biological or medicinal applications(2). Due to the presence of C=O in acyl hydrazones they show various biological activities like, anti-microbial, anti-cancerous, and anti-viral(3). Generally, the acyl hydrazones have ability to coordinate through carbonyl oxygen and azomethane nitrogen to metal ions by the reaction with the transition metal salts(4). The coordination of ligands using ONO donor atoms with metal complexes are found to have excellent biological activity(5). Cu (II) and Zn (II) ions are less toxic and have shown good involvement in biological applications which act as structural and catalytical co-factor(6)(7). These complexes have high selectivity towards oxygen and nitrogen donor atoms. For constructing efficient anti cancerous drugs and developing diagnostic agents, it is necessary to consider the binding ability of metal complexes with DNA(8). Generally, more stable and inert complexes with active metal centers are responsible for the better binding with DNA. Groove binding, intercalation, and electrostatic effects are the different modes of interactions occurs in the metal complexes with DNA(9)(10). One of the important modes of binding is intercalation which helps for cellular degradation. Depending upon the change in planarity of the complexes the ability for intercalation also varies(11). The degree of binding depends on the geometry of the metal complexes and the donor atom of the ligand(12). Copper and zinc complexes are known to be the suitable candidates in the field of coordination chemistry with greater pharmacological importance(7).

In search for DNA binding studies of acyl hydrazones and corresponding transition metal complexes, we synthesized two complexes of Cu (II) using H_2L^1 ligand. Here, we discovered the DNA binding ability of four complexes by UV and fluorescence spectroscopy with CT DNA and predicted the mode of binding contrasted with the past outcomes through literature.

2. Review of Literature

In the past decades, the cancer and the infectious diseases has increased drastically which lead the

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researchers to put forward their effort to invent novel anti-cancer drugs, which paved a way to synthesize Schiff base ligands due to their stability and structural flexibility(13). Hydrazone ligands became the backbone of the coordination chemistry due to the presence of carbon – nitrogen bonding and various applications in the present era(3). The coordinated hydrazone ligands undergo nucleophilic reactions which resulted in the synthesize of nanoscale molecular clusters, which opens an another area for research(14). Aryl group substituted Schiff bases are the best candidates among hydrazone derivatives, due to their ability to form different molecular structures(2). According to the literature survey, the complexes which contain zinc and copper metal ions at the center, are the complexes which shows more stability and flexibility as compared to the other transition elements(15).

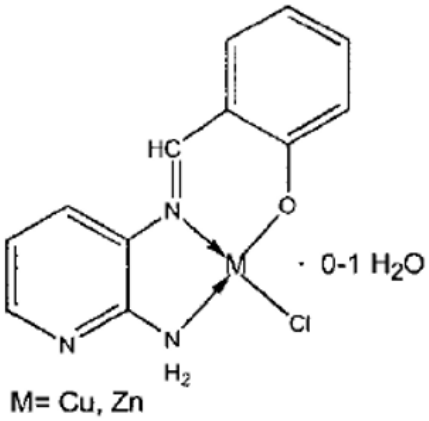


Fig 1. Structure of Schiff base complexes

3. Materials and Methods

3.1. Materials

The compounds Cu $(OAc)_2$ (Sigma-Aldrich), 3,5-Dibromo salicylaldehyde (Merck), Benzhydrazide (Sigma Aldrich) and Nicotinic Hydrazide (Sigma Aldrich) were used as received. Dimethyl Sulfoxide, ethanol and methanol were the solvents used for all the experiments. Tris- hydrochloride and Calf-Thymus DNA (CT-DNA) for DNA binding studies were purchased from Sigma.

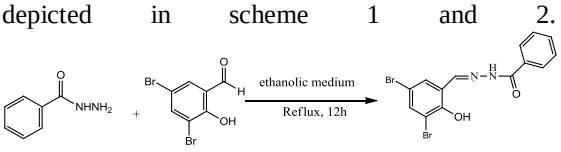
3.2. Physical and spectral measurements

The elemental analyses for the ligands and complexes were performed on a Vario ELIII CHNS analyzer at SAIF, Cochin University of Science and Technology, Kochi, India. Infrared spectra were recorded on PerkinElmer Spectrum Version 10.02.00 using KBr pellets in the range of 4000-400 cm^{-1} at Marine Biotechnology, Cochin University of Science and Technology, Kochi, India. Electronic spectra were recorded with UV-1800, Shimadzu UV Spectrophotometer, Christ University, Bangalore, India. Fluorescence spectra were made with RF-5301 pc, Shimadzu, Spectrophotometer Christ University, Bangalore, India.

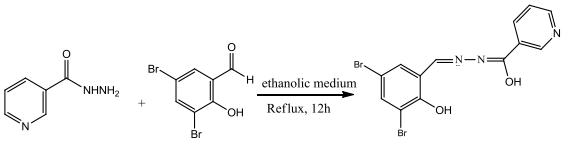
Fig 2. Schematic representation of synthesis of ligand H_2L^1

3.3. Synthesis of ligands

The ligands H_2L^1 and H_2L^2 were synthesized by reported method and the reaction for the above is depicted in scheme 1 and 2.



H_2L^1



H_2L^2

Fig 3. Schematic representation of synthesis of ligand H_2L^2

To the solution of H_2L^1 (1.5 mmol) in methanol, a methanolic solution of Cu $(CH_3COO)_2$ (1.5 mmol) was added with constant stirring followed by reflux and continued for 5 hours. The green compound formed was separated, filtered and washed with ethyl alcohol. And drying was done at room temperature.

3.4.2. $[CuL^1Py]$

A solution of H_2L^1 (1 mmol) in methanol, and the methanolic solutions of Cu $(CH_3COO)_2$ (1 mmol) and Pyridine (1 mmol) were mixed and refluxed for 5 hours with constant stirring. Green solid attained was separated, filtered and washed with ethyl alcohol. Drying was done at room temperature.

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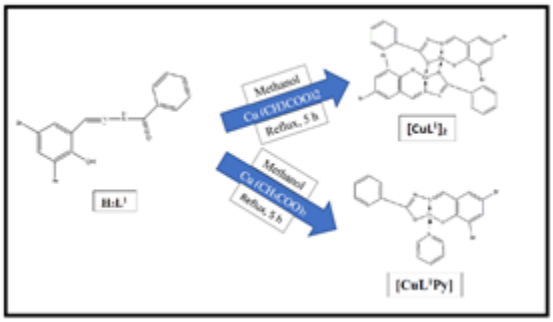

Fig.4. Schematic representation of metal hydrazone complexes of H_2L^1

3.5. DNA binding studies

At room temperature, DNA binding studies of metal complexes were carried out. The CT DNA samples were prepared by dissolving in Tris Buffer solution (25mM of NaCl and 3.8mM of Tris-HCl) with pH 7.2. The prepared DNA solution was systematically added to the metal hydrazone complexes, and observed the absorption spectra. Stock solutions were refrigerated and used within four days. To each of the metal complex solutions 63 µl of DNA solution was added to carry out the DNA absorption titration. The spectra observed were at the ratio of 260 nm to 280 nm of 1.89, which demonstrates CT DNA was adequately in protein free frame. To determine the absorbance of DNA only, identical amount of DNA was added to the compound and the reference solution. The intrinsic binding constant values of metal hydrazone complexes bound to DNA were calculated using equation (1),

$$[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA] / (\varepsilon_b - \varepsilon_f) + 1/ K_b \times (\varepsilon_b - \varepsilon_f) \quad (1)$$

Where, [DNA] stands for the concentration of DNA, ε_a is the extinction coefficient obtained by calculating A(observed)/ [complex], $\varepsilon_{f\ is}$ the extinction coefficient for the free metal complex and finally, ε_b represents the extinction coefficient for fully bound complex. The $1/ [\varepsilon_a - \varepsilon_f]$ and $1/K_b [\varepsilon_b - \varepsilon_f]$ were obtained by plotting the slope and intercept of linear fit of $[DNA] / (\varepsilon_a - \varepsilon_f)$ against [DNA](16).

3.6. Fluorescence studies

This is an effective method for finding the efficiency of metal complex-binding to DNA. The all fluorescence spectra were recorded by the successive addition of DNA (63 µl) to the fixed amount of metal hydrazone complex solutions. The excitation wavelength and the range of emission wavelength of the all four Zn (II) complexes were recorded at 280 nm and 285-800 nm respectively.

4. Results and Discussion

4.1. Properties of the complexes

The analytical data obtained for ligands and the corresponding metal complexes which explained in the experimental are in accord with the expected values and we confirmed the formulae $[CuL^1]_2$ and $[CuL^1Py]$ proposed for the new metal hydrazone complexes. DMSO is a common solvent for complexes.

4.2. Elemental analysis

The first preliminary confirmation of ligands and complexes were done by analyzing the CHN data obtained. All the complexes obtained have solid color and the percentage yield of each of the complexes are given in the following table. Both the complexes showed solubility in DMSO and DMF. The CHN data so obtained was leading to the confirmation of all the metal complexes synthesized.

Table 1. Elemental Analysis Data

Compound	Yield (%)	Color	**C** Calc. (Theo.)	**H** Calc. (Theo.)	**N** Calc. (Theo.)
$[CuL^1]$	69.26	Dark green	36.60 (36.59)	1.79(1.75)	6.13(6.10)
$[CuL^1Py]$	62.00	Darkgreen	42.39(42.36)	2.48(2.43)	7.85(7.80)

4.3. Infrared spectroscopy

The comparison of Infrared spectra of the hydrazone ligands and their respective metal complexes were held in the region of 4000–200 cm−1. The ligands H_2L^1 and H_2L^2 were showing the characteristic absorption bands of ν(N–H), ν(O-H), ν(C=O), ν(C=N) and ν(N–N) vibrations, at 3055/3054, 3220/3453, 1655/1657, 1604/1602, and 1103/1101 cm−1 respectively. The absorption bands of ν(N–H), ν(O–H) and ν(C=O) vibrations of the hydrazone ligands were inattentive in the corresponding complexes because enolization and deprotonation took place advance to coordination. The other scientific evidence to confirm the new complexes were identified by the presence of new absorption bands in the range of 1598–1504 cm−1 and 1358–1372 cm−1 that corresponds to newly introduced ν(C=N) and ν(C–O) stretching

vibrations, respectively. The ν(C=N) stretching vibration bands obtained were showing shift to frequencies having longer wavelengths, and ν(N–N) stretching vibrations showed a positive shift of about 41–51 cm−1 after the comparison with, the corresponding ligands. These two important features implied that the nitrogen atom corresponding to the azomethine group were bonded or coordinated to the metal (Cu) in the complexes. So, the hydrazone ligands appear as dibasic tridentate (ONO) chelating ligand in all the complexes.

Table 2. The important IR frequencies (cm^{-1}) of ligands and complexes

Compound	ν(C=N)	ν(C=O)	ν(N–N)	ν(N–H)	ν(N–H)
H_2L^1 **-1**	1604	1655	1103	3055	3220
H_2L^2 **-2**	1602	1657	1101	3054	3453
$[CuL^1]_2$ **(3)**	1611	____	1164	___	___
$[CuL^1Py]$ **(4)**	1608	____	1146	___	___

4.4. Electronic Spectroscopy

The UV spectra for ligands and the corresponding metal complexes were recorded by dissolving in DMSO, and the values are shown in table 3. The intensive bands present in the range of 400 nm- 250 nm corresponds to the n-π* and π- π* transitions of benzene, carbonyl and imine groups existing in them. The UV-Visible spectra of new copper complexes displayed two to three bands in the range 260- 410 nm. The bands appeared in the region of 260-270 nm have been assigned to intra ligand transitions and those around 390- 410 nm are assigned to LMCT transitions. The bands at 270 and 268 nm in ligand molecules are shifted to the range of 260- 272 nm and 402-405 nm indicating the formation of the complexes.

Table 3. Electronic spectral assignments (nm) of ligands and metal complexes

Compound	**n→π*/ π→π***	**LMCT**	**d-d transitions**
H_2L^1 **(1)**	270	399	____
H_2L^2 **(2)**	268	403	____
$[CuL^1]_2$ **(3)**	269	402	____
$[CuL^1Py]$ **(4)**	272	405	____

4.5. DNA binding studies

The interaction of DNA with the ligands and newly formed complexes were investigated using the absorption spectra. Normally, the binding of intercalative molecules with DNA is confirmed by red shift and hypochromism. The binding constant (K_b) values for ligands, ($[H_2L^1]$ and $[H_2L^2]$), and complexes, ($[CuL^1]_2$ and $[CuL^1Py]$) with CT DNA, were found and shown in table 4. The following graphs show the binding ability of ligands and complexes to CT-DNA.

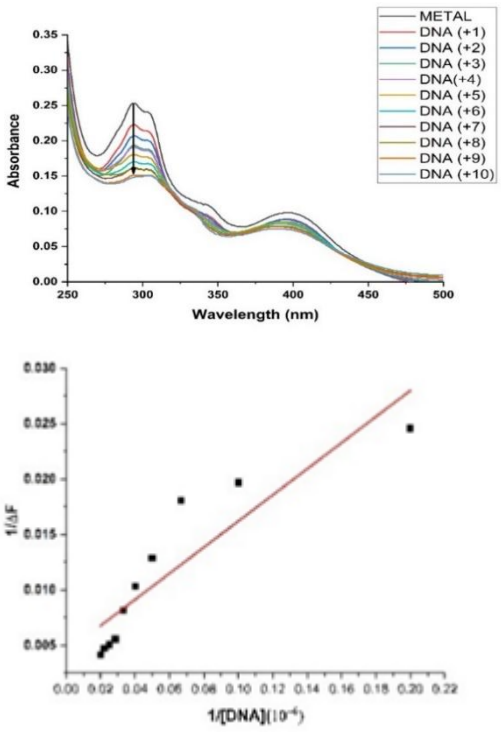

Fig 5. UV-Visible Spectrum of H_2L^1 and its linear fitting curve

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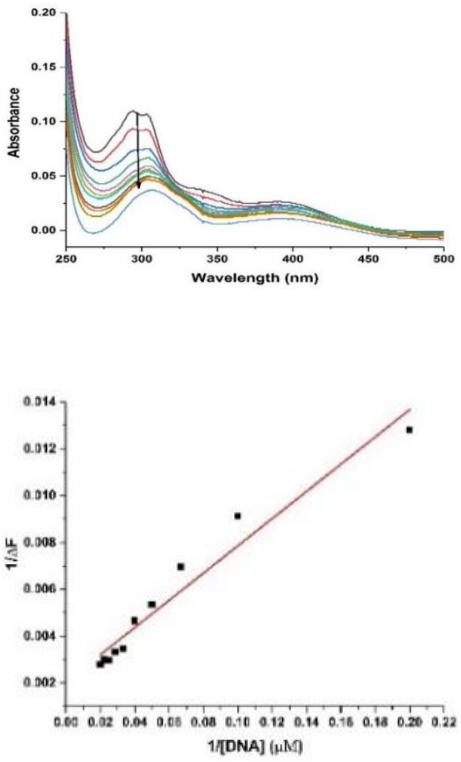

Fig 6. UV-Visible Spectrum of H_2L^2 and its linear fitting curve

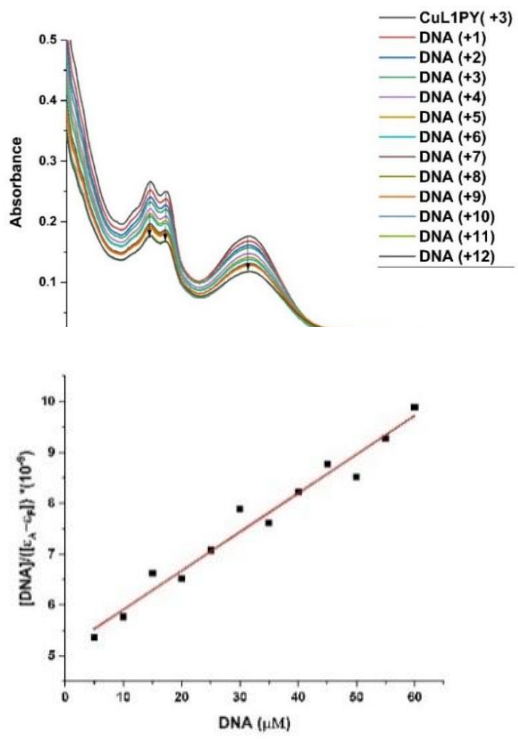

Fig 7. UV absorption spectrum for [CuL^1Py] with CT DNA and its linear fitting curve

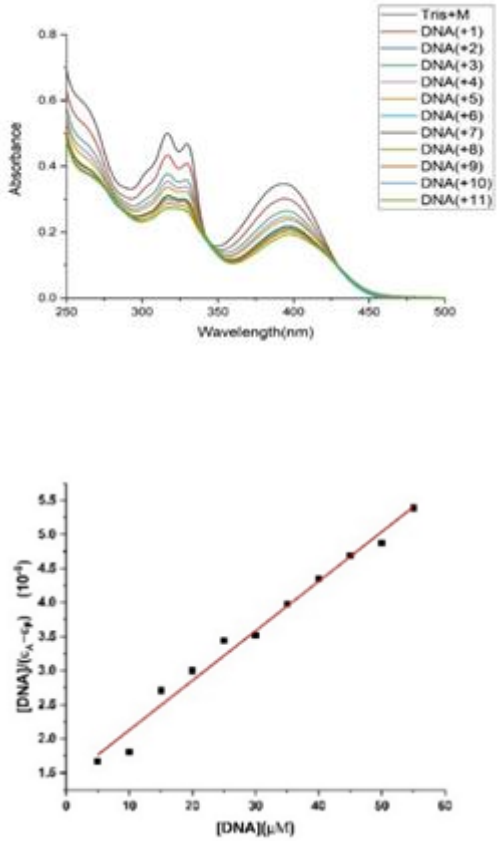

Fig 8. UV absorption spectrum for [CuL^1]$_2$ with CT DNA and its linear fitting curve

The UV absorption spectra of metal complexes show two peaks in the region of 300-400 nm, characteristic of intra ligand and metal to ligand charge transfer transitions. The absorption spectra of complexes in the presence of increasing amounts of CT-DNA have been recorded (figures 7 and 8). By the gradual addition of DNA, the complexes showed pronounced hypochromism with a slight red shift of 2-4 nm in both the bands. These results suggest that, title complexes intercalate into DNA base pairs, because as the intercalate action progresses concentration of the free metal complexes decreases which in turn decreases the absorption. In order to evaluate the binding affinity of the complexes with DNA, the intrinsic binding constant values were calculated using the following equation through a plot of [DNA]/ (ε_a - ε_f) versus [DNA].

$$[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA] / (\varepsilon_b - \varepsilon_f) + 1/ K_b \times (\varepsilon_b - \varepsilon_f)$$

The Kb values obtained for newly synthesized copper complexes are found to be very similar to that of other known mono nuclear or binuclear copper complexes(17). These spectral characteristics support the binding of both the

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complexes to CT-DNA by intercalation into the base pairs of DNA. $[CuL^1]_2$ shows more binding affinity than $[CuL^1Py]$ which is consistent with extended aromatic surface of binuclear metal complexes.

Table 4. Binding constant values of ligands and metal complexes.

Compound		K_b Constant
$[H_2L^1]$	[1]	5.1277×10^4
$[H_2L^2]$	[2]	3.7500×10^4
$[CuL^1]_2$	[3]	5.2031×10^4
$[CuL^1Py]$	[4]	1.4817×10^4

4.6. Fluorescence study

The emission spectra of the synthesized compounds in presence of DNA allow an investigation of the interaction both the ligands and the complexes to DNA. Both the ligands and complexes show luminescence in Tris-buffer at room temperature around 560 nm. The luminescence of the complexes and the ligands is enhanced appreciably with DNA binding, and this luminescence enhancement implies that all these compounds can strongly interact with DNA. The fluorescence emission spectra for the complexes are shown in figures (9-12). It is evident that all the metal complexes and ligands have shown an enhancement in the emission intensity. The enhancement in spectra can also be due to protection of imine nitrogen by the attack from H_2O molecule.

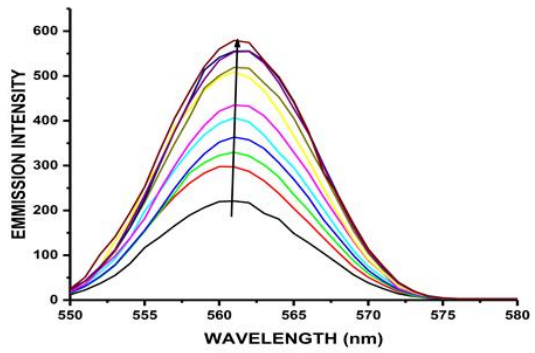

Fig 9. Fluorescence spectrum of ligand H_2L^1

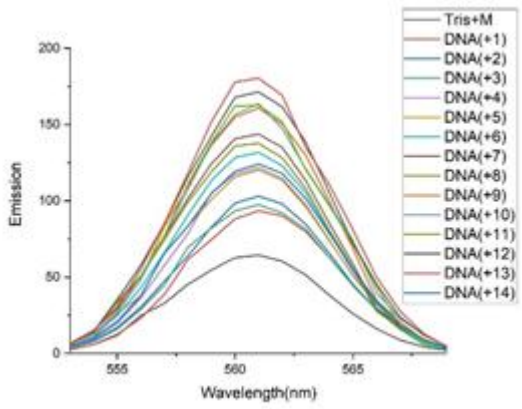

Fig 10. Fluorescence spectrum of ligand H_2L^2

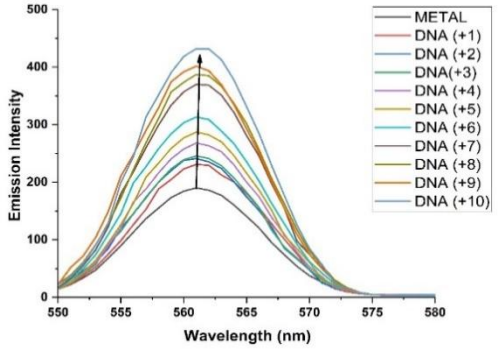

Fig 11. Fluorescence spectrum of $[CuL^1Py]$

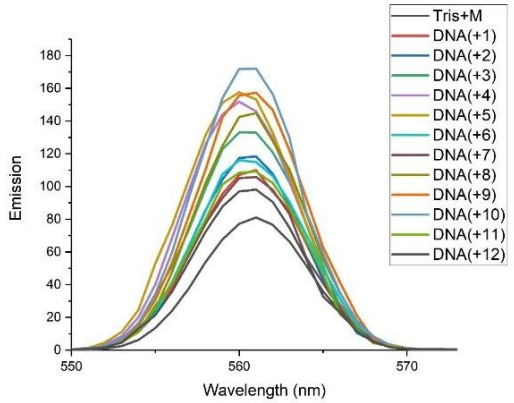

Fig 12. Fluorescence spectrum of $[CuL^1]_2$

5. Conclusion

The characterization of ligands and copper complexes were carried out using, C, H, N analysis, infra-red and ultra-violet visible spectroscopy. According to DNA binding studies, both the ligands and metal complexes shown binding ability to bind with DNA. When DNA

added gradually, the decreased absorption spectra (hypochromism) shown for both the ligands and complexes, led to the confirmation that they show binding ability with DNA through intercalation. Further studies on DNA binding studies is done by fluorescence spectroscopy. From the fluorescence studies, by the successive addition of DNA in to the corresponding ligands and metal complexes showed enhancement in the spectra, which confirms the complexes and ligands have fluorescence property. Based on the results, the novel copper metal complexes have ability to bind with the DNA and are luminescent. These complexes can be used in many bio- medical applications.

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