A theoretical study to understand the photophysical properties of a fluorescent compound (HINA)

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Abstract. The photophysical properties of fluorescent molecules are investigated theoretically using computational methods, including Hartree-Fock and density functional theory (HF/DFT) models. The observed UV-Visible spectra and related photophysical properties are interpreted using energy values from the ground-state geometry. From the optimized structure excited-state geometry is calculated for further analysis. The structure-property relationship of the fluorescent molecule is analyzed, and theoretical data will be validated against the experimental data. In our present study, the photophysical properties of 3-hydroxy-4-pyridine carboxaldehyde (HINA) are studied theoretically in the gas phase. Using DFT calculation with 6-311++G(d,p) basis set, the ground state geometry and potential energy surface are calculated. In addition, the Time-Dependent Density Functional Theory (TD-DFT) method is used to calculate the excited state geometry of the molecule, and finally, the computed results are compared with experimental data.

1 Introduction

Photochemistry is the study of chemical reactions, isomer formation, and changes in the physical behaviour of the reactants under UV-Visible light. Light can be used as a driving force for the chemical reaction. It reduces the reaction time and potentially improves the reaction efficiency. There are different types of photochemical reactions occurred [1]. Photochemistry is widely used in food processing, the decontamination of drinking water, the production of hydrogen fuel, and other fields. During the reaction in the presence of UV – Visible light, the reactant absorbs energy (a photon) and becomes excited. This absorbed energy is known as excitation energy. As a result, the molecule exists in its first excited electronic state. In this excited state, the molecule releases some amount of energy and relaxes from a higher to a lower excited vibrational state. This process is known as vibrational relaxation.

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The excited molecule returns to its ground electronic state and may release energy either in the form of heat or a photon. If it releases a photon, the process is called fluorescence (radiative process), and the energy is called emitted energy. This emission energy can be used to study the fluorescence property of the molecule [2].

The green fluorescent protein (GFP) is a naturally occurring molecule that emits green light when exposed to light in the UV-Visible range. GFP was first isolated from the jellyfish, *Aequorea victoria.* The sea pansy, known as *Renilla reniformis,* contains a GFP (Green Fluorescent Protein) [2,3]. Although the proteins from these organisms are not identical. They are thought to have the same chromophore, which is derived from specific amino acid residues within the primary amino acid sequence of GFP [3]. Structural differences arise from variations in the chromophore sequences. It has been proven that the cDNA encoding Aqua GFP can be successfully cloned, and proved that fluorescence occurs only in the presence of the Aquorea GFP protein. These findings indicate that GFP is a useful marker for tracking gene expression and protein location in both living and fixed tissues [3]. GFP found in *Aequorea Victoria*, has a sharp excitation peak at 395 nm and a small peak at 475 nm. The emission peak is observed at 509 nm, which lies in the lower green portion of the visible range.GFP remains stable over a wide pH range from mildly acidic (pH=5.6) to highly alkaline (pH=12) conditions.GFP is stable up to 65 °C. The Structural representation of GFP is shown in **Fig. 1**.It has a quantum yield of 0.79. Another type of GFP is found in the sea pansy that shows a sharp excitation peak at 498 nm. This kind of protein has various roles in biological processes, because it can form its chromophore without the need for external cofactors [4]. In cell microbiology, GFP has been widely used as a biosensor.It can be introduced into a variety of organisms through the transgenic techniques and control their progeny's genome. Most recently, GFP and similar fluorescent proteins are encoded by genes of approximately 730 base pairs.GFP can be used to track protein localization inside cells [2,4]. It is also used for monitoring the environmental toxicity levels and applied as a pollutant marker. GFP is widely used for imaging living cells. It is low-toxic in nature. Highly automated live-cell microscopy has been developed as an important application of GFP for studying living cells.

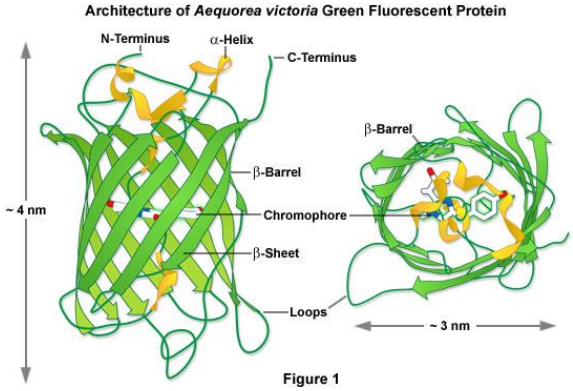

Fig.1 Structural representation of Green Fluorescent Protein(GFP) found in *Aequorea Victoria* [4].

In our present work, we selected 3-hydroxy-4-pyridine carboxaldehyde (HINA) shown in **Fig. 2a**, which is reported to be the smallest green-emitting fluorophore in water. It consists of 14 atoms. It shows the ratiometric emission response to biologically relevant pH changes [2]. HINA is also used in combination with metal complexes for fluorescence-based cysteine

detection in aqueous media and is readily taken up by cells. Detailed investigation of the photophysical properties of HINA reveals that, at 3.8<pH< 7.0, it exists in its neutral form and shows blue emission at a wavelength of 382 nm with a quantum yield 7%. In the alkaline medium, it shows an absorption peak within the range 325-385 nm [2]. With increasing alkalinity, the emission peak shifts toward the blue-green region, a phenomenon known as the hypochromic shift. At 525 nm, it shows green emission with a quantum yield of almost 15%. Unlike many hydroarenes, HINA does not show photoacidic behaviours [2]. Below pH 3.9, it becomes N-protonated and behaves as a fluorescent, with a low quantum yield 0.9%. At this stage, an emission band is observed at 395 nm. The absorption peak of the molecule shows a hypochromic shift in acidic conditions [2]. The emission lifetimes of HINA exist in three forms: cationic, neutral, and anionic, as shown in **Fig. 2b**. Three different emissive variants of HINA act as an emissive fluorescent dye [2].

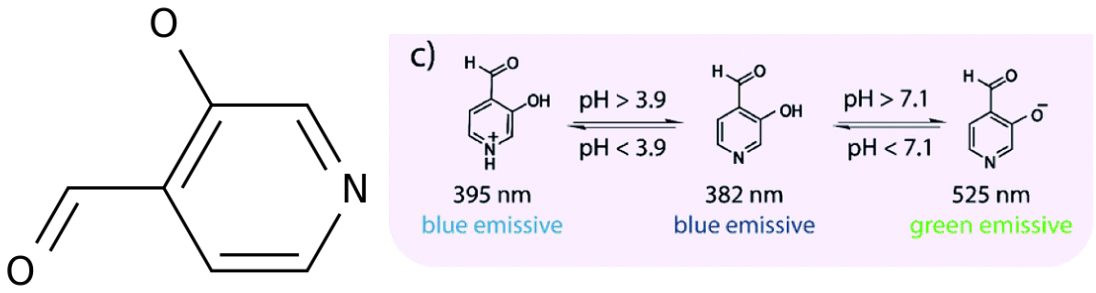

Fig.2 (a) Structural representation of {3-hydroxy-4-pyridine carboxaldehyde (HINA);(b) Different forms of HINA showing the fluorescence property [2].

In our present study, the photophysical properties of this molecule are studied theoretically in the gas phase.DFT calculations using [6-311++G(d, p)]basis set are performed. The ground state geometry and potential energy surface are optimized. The TD-DFT method is used to calculate the excited state geometry of the molecule and to correlate the obtained results with the experimental data.

2. Computational details

A theoretical model is an approach that uses a predetermined set of approximations to describe a system. Atomic orbitals, molecular orbitals, and energies are calculated using these approximations along with the computational algorithm [5]. Chemical phenomena can frequently be fully described mathematically using approximations, which are very useful concepts [5]. Avarge approximation are often used when quantitative results are required. Different approaches have been found to solve mathematical problems of the quantum system. Correlating experimental data with the theoretical data using different methods is essential [5]. The ab initio method is one of the most popular methods to solve different quantum chemical problems. It is mainly attempted to solve different mathematical models that depend on the Schrödinger wave equations. This method is widely used due of its accuracy in calculations [5]. DFT is a computational method widely used to solve problems relating to the quantum mechanical system. It is widely used in computational chemistry, physics, and material science to find out electric gradients in crystal systems [5]. By using this theory, one can analyse different fundamental and energetic parameters of systems such as electronic structure, thermodynamic energies, etc. As a powerful tool, DFT can be used to

study the intramolecular and intermolecular interactions of a system [6]. This theory has become highly popular over the last two decades due to its wide range of applications in various fields. The modern version of DFT defines self-consistent equations that must be solved for a set of orbitals whose electron density ρ(r) corresponds to that real system. In these equations, a vital contribution to the energy, the exchange-correlation (XC) energy, is expressed in terms of ρ (r). In modern physics, this theory is widely used as an alternative approach to solving Schrödinger equations [7]. Time-Dependent Density Functional Theory (TD-DFT) is a quantum chemical process to find out the different chemical, physical properties, and dynamics of many bodies system in the presence of time-dependent potentials, like magnetic or electric fields. The effects of these fields on molecules help to determine excitation energies, transition frequencies, and photo absorption spectra [7]. It is an extension of DFT. The TD-DFT method enables the theoretical calculation of excitation energies and allows comparison with ground state energies from DFT calculations [7,10].

3. Methodology

Gaussian is a very popular software, used in computational chemistry for solving different quantum problems and predicting different molecular properties. It provides a wide range of functionality of ab-initio code (HF, MPn, CI, CC, CBS, and MCSCF), density functional theory (DFT) functionals, and semi-empirical approaches. Gaussian also supports numerous basis sets, which are used together with exchange-correlation functionals to solve the Schrödinger equation. This software allows the use of the simplest input formats (ASCII) [7,9]. Using the software, molecular properties like IR, UV-Visible, and NMR spectra, bond angle, bond energy, and dihedral angle can be computed. The molecular structure was visualized using Gauss View software [8,9]. Structural representation of HINA was drawn using G09W software, which is depicted in **Fig.3**. Geometry optimization and frequency calculations were done by the exchange correlation function [B_3lyp] in the gas phase (without solvent). To get the lowest potential energy state (PES) of the molecule in its ground state geometry, this computation was performed. Frontier molecular orbitals (HOMO&LUMO) and IR spectra were obtained from the optimized structure. Vertical excitation energies and UV-Visible spectra were computed using TD-DFT at the same level of theory based on the Franck-Condon principle. To ensure the reliability of the results, additional TD-DFT calculations were performed using the Wb97xd/6-311++G(d,p) and, Cam-B3LYP /6-311++G(d,p) levels of theory for comparison.

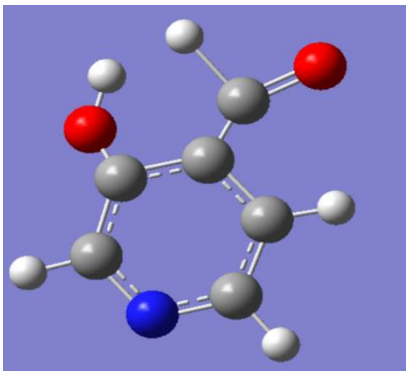

Fig.3 Structural representation of HINA drawn in Gauss view software.

4. Results & discussion

4.1 Structure analysis of ground state structure

Ground state geometry of (HINA) was optimized using Gaussian (G09W) software. The obtained structure was visualized using ChemCraft. All calculations were performed in the gas phase. Density Functional Theory (DFT) was employed to obtain the most stable molecular geometry. The exchange-correlation function (B3lyp) was used in combination with {6-311++G(d,p)} basis set for geometry optimization. **Fig. 4**(a) represents the optimized structure and (b) the most stable structure of HINA.

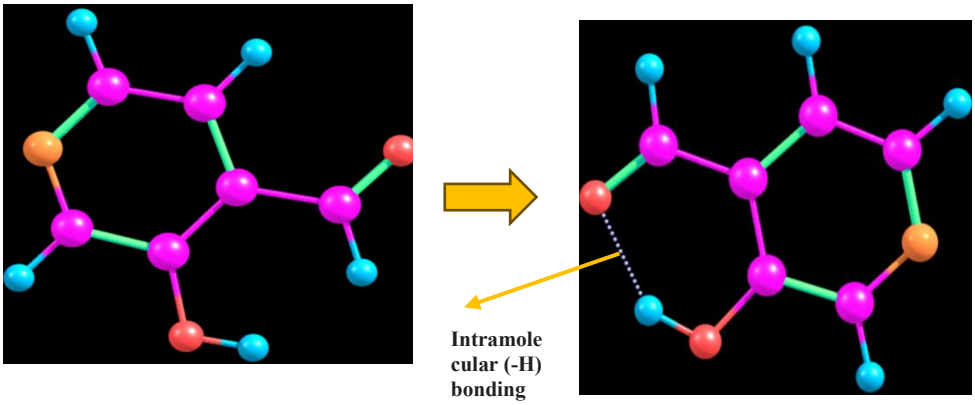

Fig. 4(a) Optimized structure of HINA and (b) most stable structure.

Frontier molecular orbitals (HOMO&LUMO) structures of HINA was analyzed using G09W software. **Fig. 5** depicts the obtained structure.

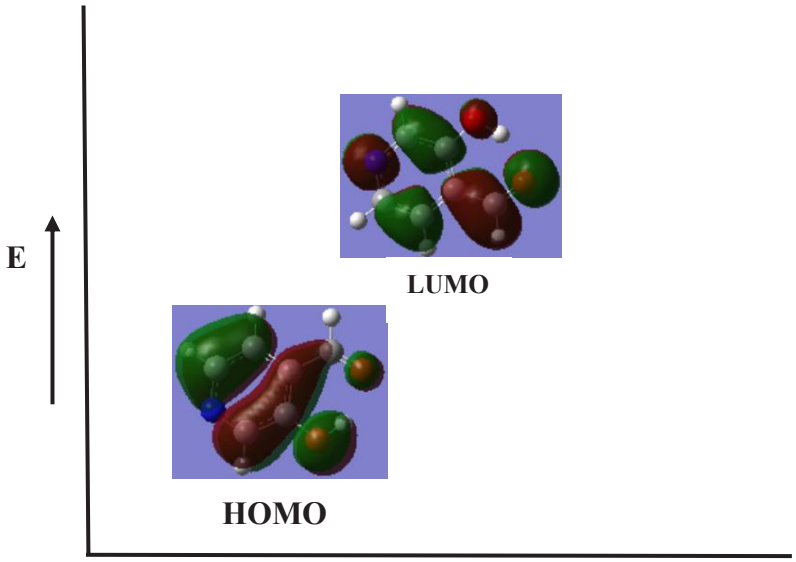

Fig.5 Structural representation of HOMO and LUMO of HINA (gr.state).

Using the same basis set and exchange correlation function, IR spectra was calculated for the most stable ground state geometry. **Fig.6** shows the result. **Table-1** represents a particular identification of the obtained spectra value.

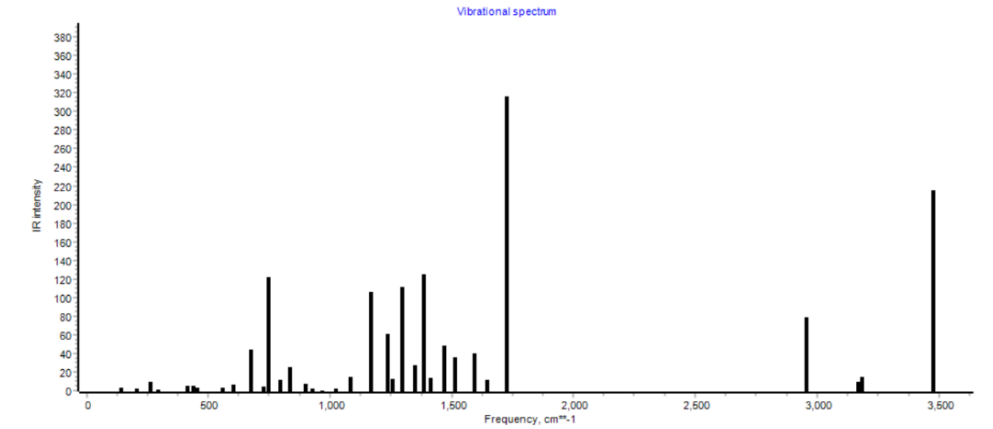

Fig. 6 Representation of IR intensities of HINA in its ground state geometry.

Table 1 . Particular identification of IR intensities of HINA in its ground state

Peak position (cm-1)	Peak intensity	Appearance	Functional group
789	Sharp	Strong	(C=C) bending alkene
897	Weak	Weak	(C=C) bending, alkene disubstituted
924.86	Medium	Medium	(C=C) bending, alkene
1080.42	Medium	Sharp	(C-O) stretching, primary aldehyde
1165.14	Medium	Sharp	(C=O) bending, aldehyde
1230.57	Strong	Sharp	(C-CO-C) stretching
1344.06	Weak	broad	(C-H) bending, alkene
1380.06	weak	broad	(C-H) bending, alkene
1410.22	Weak	Broad	(C-H) bending,
1465.58	Weak	Broad	(C-H) bending, (-Me) group present
1510.18	Weak	sharp	(C-H) bending, alkane

1641.85	Weak	Sharp	(C=C) stretching, conjugated alkene
1719.95	Strong	Sharp	(C=O) stretching, conjugated aldehyde
2953.18	Strong	Broad	(C-H) stretching, alkane
3163.49	Weak	small	(C-H) stretching, benzene group
3180.78	Weak	small	(C-H) stretching, benzene group
3475.11	Sharp	Broad	(O-H) stretching, alcohol present

4.2 Structure analysis of the excited state geometry

Excited state geometry of HINA was optimized using TD-DFT, the same basis set {6-311++G(d.p)}. Optimized and frequency calculations were performed to obtain the first excited state geometry, and the UV-Visible spectra were obtained. **Fig. 7** depicts the structure of HINA obtained using the B_3lyp function. **Fig. 7**(a) depicts structural formation and (b) UV-Visible spectra of HINA after applying B_3lyp.The maximum absorption wavelength was predicted at 329.42 nm.

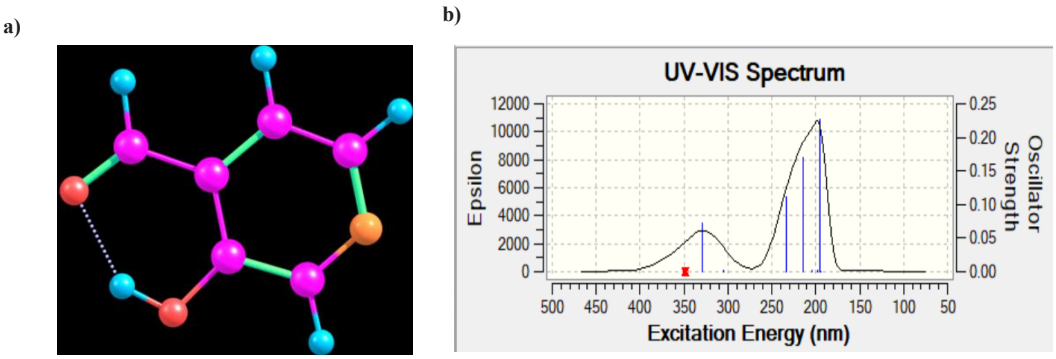

Fig.7 (a) Structural formation of HINA (b) UV-Visible spectra [B_3lyp].

Two additional exchange correlation functions Wb97xd and Cam-$b_3$lyp were applied to compare the structural difference. After completing the simulation, no significant structural variations were observed among all three methods. **Fig. 8** shows the structure of HINA obtained using the Cam$b_3$lyp function. **Fig. 8**(a) shows the optimized structure and (b) UV-

Visible spectra of HINA computed using Camb$_3$lyp. The maximum absorption wavelength was predicted at 309.25 nm.

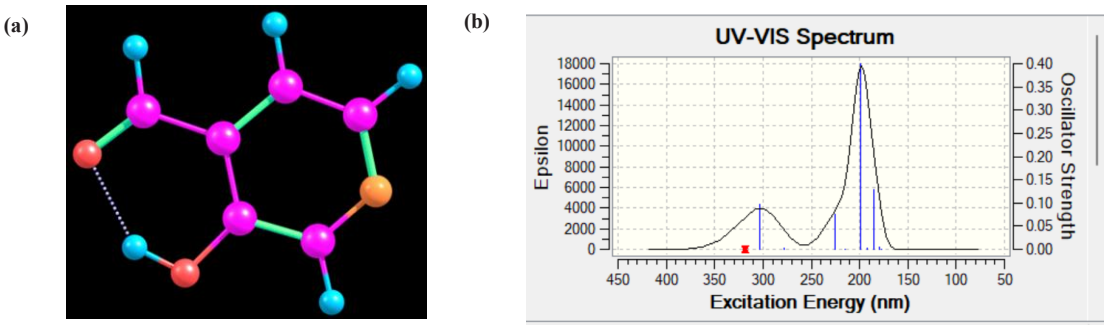

Fig.8 (a) Structural formation of HINA (b) UV-Visible spectra [Camb$_3$lyp].

Fig. 9 shows the structure of HINA obtained using the Wb97xd function. **Fig. 9** (a) depicts structural formation and (b) UV-Visible spectra of HINA computed using Wb97xd. The maximum absorption wavelength was observed at 303.05 nm.

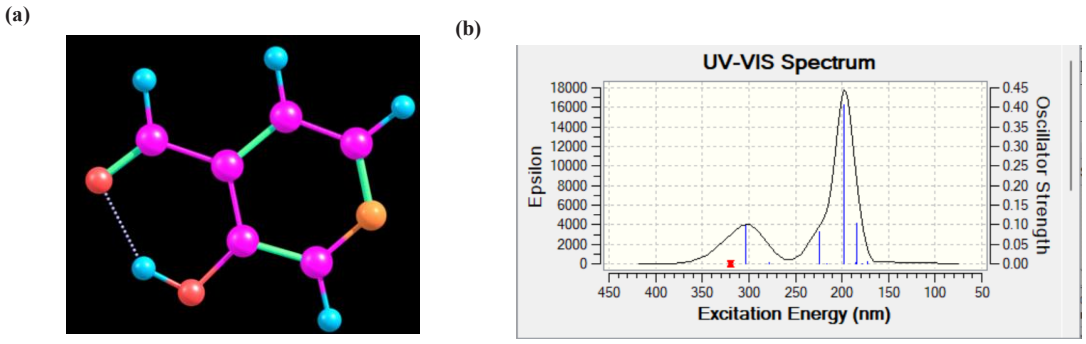

Fig.9 (a) Structural formation of HINA (b) UV-Visible spectra [Wb97xd].

Among the three functionals, the B3lyp method predicts a maximum absorption wavelength of 329.42 nm, which most closely matches the experimental value of 325 nm reported for neutral HINA. This close agreement suggests that the B3lyp functional offers the most accurate representation of the electronic structure and excited-state behavior of neutral HINA in this work. Finally, B3lyp is preferred as the best method for further analysis as it easily correlates with the experimental value.

5. Conclusion

In this study, the photophysical properties of HINA were studied using DFT and TD-DFT calculations in the gas phase. Geometry optimization and vibrational frequency analysis were performed using B3lyp /6-311++g(d,p) basis set. Performing this simulation, the most stable ground-state geometry was found. Excited -state geometry calculations were also performed using TD-DFT with B3lyp, Camb$_3$lyp, and Wb97xd to predict the absorption behaviour of

HINA. Among all three methods, B3lyp predicted the maximum absorption wavelength (329.42nm), which is in close agreement with the reported experimental value (325nm). This result demonstrates its superior reliability in describing the electronic and excited- state properties of neutral HINA. Overall, the computed results demonstrate good correlation with experimental data and provide a theoretical phenomenon for understanding the photophysical behaviour of this small green-emitting fluorophore.

Future Outlook

To investigate the structure of HINA in the solvent phase, DFT calculations will be performed using (B3lyp/Cam-b3lyp/Wb97xd functionals with 6-311++G (d, p) basis set in Gaussian 09 W, employing the Opt+freq keyword and an appropriate solvent model. After optimizing the ground state geometry, excited-state optimizations will be carried out using TD-DFT with the same functionals and basis set in the solvent phase. The emission wavelengths will be used to generate the fluorescence spectra, and the theoretical results will be compared with the previous experimental data.

Acknowledgement

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Conflict of Interest

No conflicts of interest have been declared.

Data Availability

All sources of data for this are available upon reasonable request.

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