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# DFT ANALYSIS ON CHARGE DISTRIBUTION, ELECTRONIC ABSORPTION SPECTRA, NON-LINEAR OPTICAL PROPERTY AND MOLECULAR DOCKING STUDY OF (R) – EPINEPHRINE (ADRENALINE)

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**Abstract**

The electronic properties of the title compound were examined using charge distribution, Natural Bond Orbital Analysis (NBO), UV absorption spectra and Frontier molecular orbital analysis. Experimental UV absorption spectrum (200-400 nm) of the title compound was recorded in solid phase and ethanol solution. Theoretical UV-spectra was computed using time dependent self-consistent field (TD-SCF) theory at B3LYP/6-311++G (d, p) level in gas and solvent phases. Population analysis, Frontier Molecular Orbital Analysis, Nonlinear optical properties were calculated using the same functional and basis set. NBO analysis was done using B3PW91 functional and 6-311++G (d, p) basis set. Molecular docking study shows that the title compound might exhibit inhibitory activity against bacterial species of Klebsiella.

## INTRODUCTION

The title compound has a catechol nucleus (benzene with two hydroxyl side groups) connected to an amine side chain [1] and its IUPAC name is 4-[(1R)-1-hydroxy-2-(methyl amino) ethyl] benzene-1, 2-diol. Epinephrine diverts blood to tissues under stress and hence, it is essential for maintaining cardiovascular homeostasis [2]. When a person is subjected to threat, a signal is sent to brain, which in turn sends nerve impulses to the adrenal gland present in the kidneys. The medulla of the adrenal gland secretes the R-isomer of Epinephrine [3]. Thus, Epinephrine is involved in fight or flight response in human beings.

Owing to its wide important biological actions, several studies have been reported [4-12] from time to time. Yuanzhi et al.[4] have performed DFT computations using B3LYP/6-31G(d) and B3PW91/6-31G (d) methods to study the molecular structure, solvation energies, sum of electronic and thermal free energies and the prominent vibrational frequencies of epinephrine. Gunasekaran et al. [5] have discussed important vibrational frequencies of epinephrine using experimental values of FT-IR and FT-Raman. Omar B. Ibrahim et al. [6] have discussed the interaction of epinephrine with heavy elements. The thermal behavior of epinephrine was discussed by Gilbert Bannach et al. [9]. Ilhami Gulcin [10] has studied the

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antioxidant activity of L-adrenaline. Andersen [12] has studied the crystal structure of epinephrine using XRD techniques.

Though various spectroscopic studies have been done, the electronic properties, Non-linear optical properties and molecular docking studies have not been explored so far on (R)-Epinephrine. Hence, the probing was extended by rigorous computation using DFT methods in combination with a larger basis set. The experimental and theoretical results support each other and the calculations provide an insight into the electronic and molecular properties.

## EXPERIMENTAL DETAILS

The compound under investigation namely (R)-Epinephrine was purchased in the powder form from Sigma-Aldrich Chemicals, Chennai with 98% purity. The UV-Vis spectra were recorded in solid and ethanol solution in the range 200-800 nm, with the scanning interval of 0.2 nm, using Shimadzu UV-250 spectrometer.

## COMPUTATIONAL DETAILS

All the quantum chemical computations, of the compound (R)-Epinephrine was carried out with the Gaussian 09W program [13] and visualized through Gauss view program [14]. The charge distribution was computed through Mulliken population analysis, Natural charge and Electrostatic potential charge using B3LYP functional and 6-311++G (d, p) basis set. The electronic absorption spectra for optimized molecule were calculated using time dependent self-consistent field (TD-SCF) theoryat B3LYP/6-311++G (d, p) level in gas and solvent phases. It obtains the wave functions of Molecular Orbitals that oscillate between ground state and the first excited states.The number of excited states and the type of transition to be calculated, can be selected, for example TD=Nstates=3, TD=singlet. Natural Bond Orbital Analysis was done using B3PW91 functional and 6-311++G (d, p) basis set. Parameters such as dipole moment (μ), polarizability (α), anisotropy (Δα) and the first order hyper polarizability (β) are determined to study the non-linear optical property of (R)-Epinephrine using B3LYP functional with 6-311++G(d,p) basis set [15]. The β components of Gaussian output are reported in atomic units and therefore the calculated values are converted into e.s.u units (for α: 1 a.u. = 0.1482 X $10^{-24}$ e.s.u, for β; 1 a.u. = 8.6393 X $10^{-33}$ e.s.u).

## RESULTS AND DISCUSSION

### *Mulliken Population (MP), Natural Atomic Charge (NAC) and Electro Static Potential (ESP) Analysis*

The molecular structure of (R)-Epinephrine is displayed in the Fig. 1. The charges on the atoms of the title compound are calculated by MP, NAC and ESP using B3LYP method with

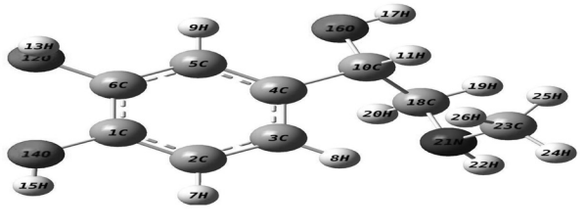


**Fig: 1. Molecular structure of (R)-Epinephrine**

6-311++G (d, p) basis set. On analyzing the conjugation, due to the presence of two electron releasing OH groups, attached directly to the ring, the negative charge can be delocalized by resonance to three different carbons of the aromatic ring. The lone pair delocalization increases the electron density at the two ortho positions and one para position, making them more reactive than pure benzene. With respect to C1, C2 and C6 are ortho positions, C3 and C5 are meta positions and C4 is para position. With respect to C6, C1 and C5 are ortho positions, C2 and C4 are meta positions and C3 is para position. Here, C2, C3, C4 and C5 are electronegative by NAC and ESP predictions. MP shows electro-positivity on C4, because at this carbon atom, the aliphatic chain is attached. C10 is electropositive through NAC and ESP primarily due to the fact that, it is attached to OH group. It is electronegative through MP. C18 attached to nitrogen, is electronegative through all the methods. All the hydrogen atoms have a net positive charge. H13, H15, H17 are found to be more electropositive because they are attached to oxygen. Oxygen and nitrogen are found to be electronegative through all the methods and hence,they can interact with the positive part of the receptor. Both the atoms are able to share the free electron pair to form a bond with a proton and other electrophilic atoms. Epinephrine is a base due to presence of lone pair on nitrogen. On protonation, the product obtained is a conjugated acid [16]. The

protonated amine acts as a cationic center and is responsible for binding to many drug targets including receptors and enzymes. This is confirmed through molecular docking.

***Natural Bond Orbital (NBO) analysis***

NBO is used to investigate the intra and inter-molecular interactions of the title compound using second-order perturbation theory. This was done at B3PW91/6-311++G (d, p) level of theory. There is a charge transfer from O14 LP (2) to $\pi^*_{C1-C2}$ antibond and O12 LP (2) → π*C5-C6 antibond. On observing the bonding of the natural hybrid orbital of lone pair electrons, we understand that, n1 (O12) which has a higher occupation number of 1.97847 has a considerable p character (54.81%). n2 (O12) which has a lower occupation number of 1.87460 has a p character of (99.94%). n1 (O14) has a higher occupation number of 1.97962 has a p character of 55.66%. n2 (O14) which has a lower occupation number of 1.89855 has a p character of 99.93%. Hence, we infer that a very close to pure p-type lone pair orbital participates in the electron donation to π* (C-C) orbital for n2 (O12) to π*(C-C), n2 (O14) to *(C-C).

We observe that σ to σ* transition occur from various bonds within our molecule, σ (C1-C2), σ (C1-C6), σ (C10-O16), σ (N21-H22) with antibonding σ* (C2-C3), σ* (C1-C2), σ* (C3-C4), σ*(C18-H20) and the corresponding contribution energies are 3.19, 4.23, 1.97, 1.04 Kcal/mol respectively. The highly probable transitions in this molecule are C1-C2 to C3-C4 (π - π*, 17.82 Kcal/mol), C1-C2 to C5-C6 (π - π*, 17.99 Kcal/mol), C3-C4 to C1-C2 (π - π*, 20.75 Kcal/mol), C3-C4 to C5-C6 (π - π*, 19.49 Kcal/mol), C5-C6 to C3-C4 (π - π*, 21.07 Kcal/mol), C5-C6 to C1-C2 (π - π*, 20.87 Kcal/mol).Two other transitions which involve lone pair O14 to C1-C2 (n- π*, 24.14 Kcal/mol), O12 to C5-C6 (n- π *, 27.25 Kcal/mol), also have high stabilization energies. Hence their transitions are significant in this molecule. They are observed in UV spectrum. O16 is attached to a sigma bond and hence does not exhibit n- π* transition. Hyper conjugation, that is σ to π* transitions, also occur between, C10-H11 to C3-C4 of the benzene ring, with a stabilization energy of 1.09 Kcal/mol.

***Frontier Molecular Orbitals (FMO) and ultraviolet-visible spectral analysis***

HOMO stands for Highest Occupied Molecular Orbital and LUMO stands for Lowest Unoccupied Molecular Orbital. They are collectively called as Frontier Molecular Orbitals and play an important role in UV-Vis spectra [17]. The energy difference between HOMO and LUMO is related to the kinetic stability, chemical reactivity, optical polarizability and chemical hardness/softness of the molecule [18, 19].

The calculated energies of HOMO are 6.00 eV and 6.08 eV in gas and ethanol solution respectively. Similarly, the LUMO energies are 0.54 eV and 0.56 eV. The values thus obtained, are compared to the benzene ring. The energies of HUMO-1 is observed to be 6.33eV and LUMO+1 is found to be 0.56 eV. Their difference is 5.81 eV. The energy gap in epinephrine ($E_{HOMO}$ - $E_{LUMO}$) is 5.46 eV. It has been reduced by 1.15 eV, on comparing with pure benzene ring. Due to the effect of substituents, the electronic energy levels have moved closer together. The dipole moment of benzene is almost zero, whereas, by addition of the substitution, dipole moment of the present molecule is increased to 3.89 and 6.86 Debye in gas phase and ethanol phase respectively, which shows that the charge flow occur from negative to positive direction. They also indicate that the title molecule has strong intermolecular interactions. The values of electronegativity, chemical hardness, softness and electrophilicity index are 3.27 eV, 2.73 eV, 0.18 eV and 1.96 eV, respectively, in gas phase, for the title molecule.

On the basis of HOMO & LUMO, TD-SCF has been used to find out the excitation energies, oscillator strength (f), absorption wavelength (λ) of the prominent or most probable transitions in the present molecule, in gas phase and ethanol solution. The experimental values and calculated values along with spectral assignments are given in Table 1. The major contribution is observed at 268.89 nm absorption wavelength for HOMO→LUMO transition with 89% using Gauss Sum Program[20]. In Epinephrine, two OH groups are attached to benzene ring. Substituents that carry nonbonding electrons, shifts the absorption bands to longer wavelength. This is bathochromic shift or red shift [21]. Also, when two functional groups are ortho to each other, the magnitude of the observed shift is approximately equal to the sum of the shifts

caused by individual groups [21]. In pure benzene, the allowed transition is at 180 nm and in phenol, the absorbance is at 270 nm [21, 22]. In epinephrine, the experimental absorption bands are viewed at 382 nm, 288 nm and 250 nm, in solid phase.They are assigned n→π π*, n→π π* and π π→ππ* transitions according to [23].

In ethanol solution, the computed absorption bands are found at 260.14 nm, 250.88 nm, 232.12 nm, 229.63 nm, 224.61 nm and 222.74 nm with excitation energies 4.7661 eV, 4.9419 eV, 5.3414 eV, 5.3994 eV, 5.5200 eV and 5.5663 respectively. The experimentally observed band is viewed at 281 nm for HOMO→LUMO transition with 82%. It is assigned n→π* transition according to [23]. Both the computational and experimental spectrum show that there is a decrease in intensity of absorption and a considerable shift in the absorption wavelengths. The wavelengths are reduced when compared to the gas phase. Thus, it may be concluded that the solvents not only change the observable peaks in the UV-Vis spectrum, but also the wavelengths of absorptions.

**Table 1: Experimental and Theoretical Electronic Absorption Spectra of R-Epinephrine using Td-Scf/ B3lyp/6-311++G (D, P) Method**

| Experimental Value | | | Theoretical Value | | | Major Contribution | Assignment |
|---|---|---|---|---|---|---|---|
| **ƛ (nm)** | E (eV) | (f) | **ƛ (nm)** | E (eV) | (f) | | |
| ETHANOL SOLUTION | | | ETHANOL SOLUTION | | | | |
| 281 | 4.3979 | - | 260.14 | 4.7661 | 0.0710 | H→L (82%) | ***n***→π* |
| - | - | - | 250.88 | 4.9419 | 0.0022 | H → L+1 (64%) | ***n***→π* |
| - | - | - | 232.12 | 5.3414 | 0.0020 | H-1 → L (93%) | ***n***→π* |
| | | | 229.63 | 5.3994 | 0.1030 | H→L+2 (45%) | ***n***→π* |
| | | | 224.61 | 5.5200 | 0.0054 | H→ L+3 (82%) | ***n***→π* |
| | | | 222.74 | 5.5663 | 0.0116 | H-1→L+1 (65%) | ***n***→π* |
| SOLID PHASE | | | GAS PHASE | | | | |
| 382 | 3.2351 | - | 268.69 | 4.6144 | 0.0020 | H→L (89%) | ***n***→π* |
| 288 | 4.2910 | - | 260.42 | 4.7610 | 0.0528 | H→L+1 (75%) | ***n***→π* |
| 250 | 4.932 | - | 237.94 | 5.2107 | 0.0037 | H-1 → L +1 (77%) | ***n***→π* |
| | | | 235.37 | 5.2677 | 0.0083 | H→L+3 (23%) | ***n***→π* |
| | | | 234.07 | 5.2969 | 0.0209 | H→L+3 (38%) | ***n***→π* |
| | | | 228.97 | 5.4148 | 0.0430 | H→L+2 (41%) | ***n***→π* |

***Molecular Docking Study***

Prediction of activity spectra for biologically active substances (PASS) [24] is an online tool which predicts different types of activities based on the structure of a compound. PASS analysis of (R)-Epinephrine predicts amongst other activities, Klebsiella pneumoniae inhibitor activity. Klebsiella pneumoniae is a Gram-negative, non-motile, rod-shaped bacterium which cause destructive changes to human lungs via inflammation and haemorrhage with cell death. Bronchitis and bronchopneumonia is triggered due to proliferation of K.pneumoniae [25-29].

The 3D crystal structure of Klebsiella pneumoniae was obtained from Protein Data Bank (PDB ID:1N9B). 1N9B has a good resolution 0.9 Å and attached co-crystallized inhibitors were used to identify the

active site. Molecular docking is an efficient tool to get an insight into ligand-receptor interactions. All molecular docking calculations were performed on Auto Dock-Vina software [30] and visualized through Discovery studio software 4.0 [31]. The Auto Dock Tools (ADT) graphical user interface was used to calculate Kollmann charges for the protein and to add polar hydrogen. Water molecules and co-crystallized ligands were removed. The ligand was prepared for docking by minimizing its energy at B3LYP/6-311++G (d, p) level of theory. Partial charges were calculated by Gasteiger method. Torsion and rotatable bond were defined. The active site of the enzyme was defined to include residues of the active site within the grid size of 32 Å, 34 Å and 34 Å.

Lamarckian Genetic Algorithm (LGA) available in Auto Dock-Vina was employed for docking. The docking protocol was tested by removing co-crystallized inhibitor from the protein and then docking it at the same site. To evaluate the quality of docking results, the common way is to calculate the Root Mean Square Deviation (RMSD) between the docked pose and the known crystal structure confirmation. RMSD values up to 2 Å are considered reliable for docking protocol [32]. The ligand binds at the active sites of the protein by conventional hydrogen bonding, carbon hydrogen bonding and Pi-Alkyl interaction which are depicted in dotted lines.ASP 104, ASN 132, ASN 170 of amino acid form hydrogen bonds with three –OH groups and NH group respectively. The inhibitor forms a stable complex with 1N9B as it is obvious from the ligand-receptor interactions. Binding free energy (ΔG in kcal/mol) of -5 to -5.5 as predicted by Autodock-Vina suggests good binding affinity. It is evident that the hydroxyl group and the amino group are crucial for binding. These results draw us to the conclusion that the compound might exhibit inhibitory activity against Klebsiella and may act as anti-bacterial agent. However, biological tests need to be done to validate the computational predictions. Fig.2 shows the protein 1N9B with docked ligand embedded in the active site.

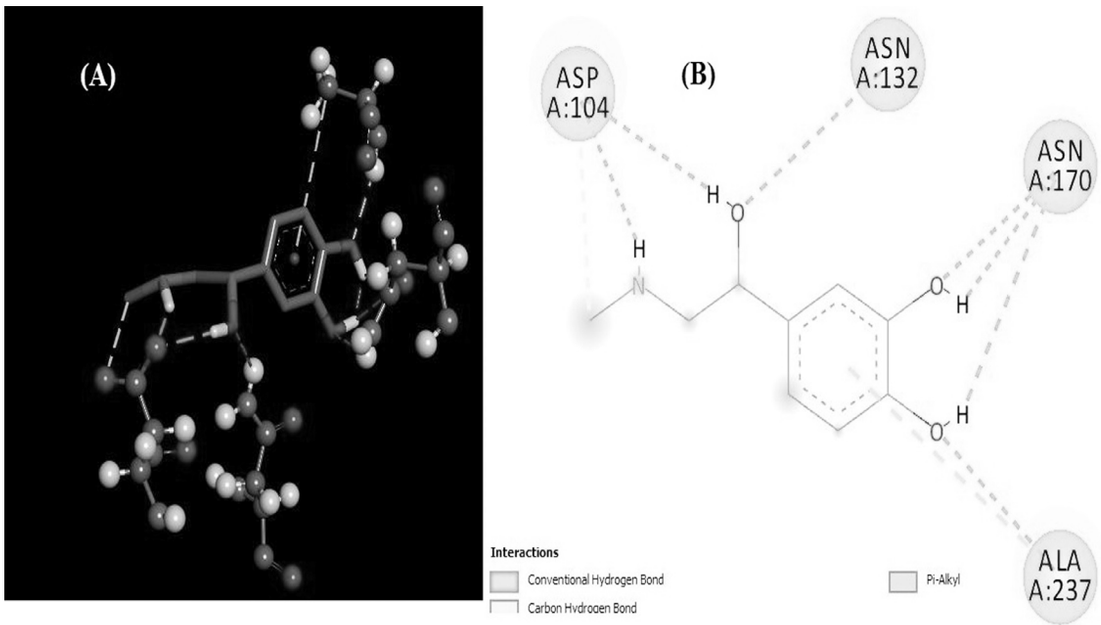


**Fig 15.2: Molecular Docking of (R)-Epinephrine with Klebsiella Pneumoniae (Pdb Id: 1n9b) is Depicted in (A). the Various Types of Interactions between the Receptor and Ligand are Depicted in (B)**

***Non-linear Optical effect (NLO)***

The non-linear optic properties (NLO) of the title compound was studied by calculating the dipole moment, polarizability, anisotropy and first order polarizability using B3LYP method with 6-311++G(d,p) basis set. Nonlinear optics explains non-linear response of properties such as frequency, polarization, phase or path of incident light. In general, compounds with electron releasing substituents are promising for NLO applications [33]. DFT has been extensively used as an effective method to investigate the organic NLO materials. The calculated values of the dipole moment is observed to be 3.69 Debye. The highest value of

dipole moment is observed for component $\mu_z$, which is equal to 0.34 Debye and the lowest value of the dipole moment of the molecule for the component $\mu_y$ is -3 Debye. The calculated average polarizability and anisotropy is -10.8341 X $10^{-24}$e.s.uand 3.464 X $10^{-24}$e.s.u respectively. Here, negative polarizability indicates that the molecule attains dipole moment opposite to the applied electric field. The hyper polarizability (β) is one of the fundamental important factors in NLO system and the computed value is 371.896 X $10^{-33}$e.s.u. The values of dipole moment and hyper polarizability for urea are (1.37 D and 372 X $10^{-33}$e.s.u) [34].

## CONCLUSION

Atoms associated with electronegative elements exhibit greater electropositvity. For example, in the charge analysis, C1, C6 and C10 which are associated with oxygen atom exhibit higher positivity. The hydrogen atoms H13, H15, H17 attached to oxygen are found to be more electropositive than the hydrogens associated with the ring. Similarly, H22 attached to nitrogen also exhibits higher positivity compared to hydrogens in the aliphatic chain. This indicates that the molecule has greater probability of interacting with a receptor.In electronic transitions $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions have high stabilization energies. These transitions observed in NBO are correlated with UV spectrum. Molecular docking was applied to explore the binding mechanism and docking score of the title compound. The results imply that (R)-Epinephrine and its derivatives can exhibit inhibitory activity against Klebsiella species of bacteria. This can be further validated through lab studies for its proper function. The NLO computations made in relation with hyperpolarizability, polarizability and dipole moment show that the molecule is a promising candidate for NLO material.

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