IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 13, Issue 1 Ser. I (January. 2020), PP 45-51
www.iosrjournals.org

UV-Visible absorption spectroscopy and Z-scan analysis along with corresponding molecular electronic structure analysis at DFT level for L-Tyrosine.

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Abstract:*Aqueous solutions of L-tyrosine prepared with a wide range of concentrations from 3.0 μM to 1.5 mMwere examined by UV-Visible spectroscopy and Z-scan analysis along with quantum mechanical calculation for its molecular structure for possible cause of linear and nonlinear optical activities. L-tyrosine has been found to absorb light at three regions around 193 nm, 224 nm and 275 nm in aqueous solution. These absorptions exhibit ^1L_a and^1L_btransitions in the side chain containing phenol ring structure. The aqueous solution of L-tyrosine also exhibits third order optical nonlinearity with negative refractive index of 3.18×10^{-8} m^2/W in the thermal regime as found by Z-scan technique. The HOMO-LUMO structure of L-tyrosine in solvated form calculated at DFT level using CAM-B3LYP parameters and aug-ccPVTZ /ccPVQZ basis sets give results consistent with observation of UV-Vis spectroscopy.*

Key Word: *UV-Visible Spectroscopy, amino acids, aromatic chromophores, nonlinear refraction, z-scan, DFT*

Date of Submission: 17-01-2020 Date of Acceptance: 04-02-2020

I. Introduction

UV-visible spectroscopy is one of the oldest modern technique used for electronic structure determination of compounds either in condensed phase or gaseous phase. This technique is based upon absorption of electromagnetic photons by atoms or molecules. As molecular orbitals are formed by overlapping of the adjacent atomic orbitals and further complicated by vibrational and rotational energy levels, the absorption spectra of molecules are not narrow as is in the case of elemental atomic spectrum, but rather consists of broad absorption bands whose peak positions and shapes are determined by structural features of absorbing material in question and its surrounding environment. Among the biomolecules, proteins and nucleic acids absorb light in the near UV and visible region of 150 - 800 nm. Except these others mostly absorb in the deep UV which are not experimentally suitable.

It has been found that the UV absorption in proteins comes mainly from the absorptions by the constituent aromatic amino acids L-tyrosine, L-tryptophan, L-phenylalanine [1-9] and also from cysteine disulfide bond[10,11]. Simple peptide structure containing only one type of the aromatic amino acid exhibits absorption pattern much like that of the constituent aromatic amino acid. Tryptophan absorbs most strongly having absorption peak at 280 nm with extinction coefficient of 5600 $L\ mol^{-1}cm^{-1}$and tyrosine at 275 nm with extinction coefficient 1400 $L\ mol^{-1}cm^{-1}$[12]. However, in case of human serum albumin (HSA) the contribution of L-tyrosine is expected to be more weighty compared to tryptophan as it is much more abundant (18:1) in the HSA structure[13]. Again its side chain chromophore phenol is more polar than the other aromatic chromophores and hence its absorbance is strongly influenced by the polar surrounding as it is exposed to the solvent[14]. Investigations on absorption by aromatic amino acids have been reported as early as 1883 which wereincomplete[1-5]. Katherinetal. in 1935 made extensive study on tyrosine, tryptophan and phenylalaninewhich produced somewhat different results than before[15]. Earlier absorption studies were made above 220 nm which was later extended up to 177 nm[3,16,17].

The absorption band position and intensity for the conjugated π-bonded systems depend strongly on the micro environment of the chromophores and the conformations of the respective molecules. It has been found that the absorption bands of tyrosine and tryptophan are redshifted by *1-3 nm*when incorporated in protein structure[6,7,9]. In particular cases, the absorption patterns have been directly correlated to its structural features. As a part of optical study of HSA we need to look at the different linear and nonlinear optical responses of tyrosine as well as the others.During absorption of electromagnetic photon a range of incidents take place in sequence like electronic excitation followed by charge redistribution, reorganization of dipole moment,

alteration of bond strengths, ensuing of vibration and finally response of the environment in the form of solvent solute relaxation resulting in lowering of overall energy of the system[18]. All these occurring in a wide time scale of 10^{-15}s to 10^{-8} s and are related to the change in internal geometry, the extent of which determines the shape of the absorption bands[19].

The tyrosyl side chain having aromatic ring structure makes it not only UV absorbing but also a candidate for nonlinear optically active material as organic compounds containing conjugated-π bond have been found to exhibit third order nonlinearity [20,21]. The formalism developed by Sheikh -Bahaeet al. is a suitable method to look for third order nonlinearity in solution [22,23].

On the other hand, molecular orbital theory has been successfully utilized to explore the molecular structure of compounds and elucidating its spectral features [24-26]. With the advent of quantum mechanical (QM) approach the theoretical calculations of molecular structure and the effect of conformations and surrounding environment on its electronic properties have become a useful tool [27-32]. Abinitio procedure (MP2/6-31G*) [33,34] and semi empirical studies [34] have been performed to look at the ground state and excited state configuration. The hybrid form of Density functional theory (DFT) with B3LYP formalism has been established as an accurate QM chemical method for analysis of coordination compounds and biomolecules [35,36]. The correctness of these calculations depended on the level of sophistication utilized.

In section 2 we develop our method of experiment and in section 3 we give the result of the experiment and thereof finally in section 4 we conclude.

II. Experimental methods

2.1. UV-Vis Spectroscopy

Shimadzu UV-1800 spectrophotometer was used for collecting the light absorption data in the range of 190*nm* to 400 *nm*. Aqueous solutions of reagent grade (≥98%) L-tyrosine supplied by Sigma Aldrich were prepared at concentration 1.5 mM at 25 ^{0}C and then diluted to different concentrations up to 3.0 μM. The sample cell was 2.75 *mL* quartz cuvette with 1.0 *cm* path length and wall thickness 1.0 *mm*. The spectrophotometer uses D_2lamp as UV source and Tungsten as visible light source. A medium scan speed with sampling interval 0.5 *nm* and 4 repetition have been used. Spectroscopic scans were repeated with freshly prepared solutions at different specific concentrations for reliability test of the data. The obtained data was presented in graphical form using UV-Probe^{TM} that comes with the spectrophotometer. The absorbance at three wavelengths namely 275 nm, 224 nm and 193 nm were plotted separately as a function of concentrations and the extinction coefficients were calculated.

2.2. Z-Scan

A standard Z-scan setup as shown in Fig. 1 at the *'Nonlinear Optics'* lab has been used for the Nonlinear Optical (NLO) study. The experimental setupconsisted of a 665 *nm* laser beam from a LED source adjusted for power by Neutral Density (ND) filter and the beam profile corrected byF_1 - F_2lens system and then focused by F_3onto the sample holder. The power meter A (Newport 918D-SL-0D3) measures the incident power on the sample and the meter B measures the power transmitted through the aperture. As the sample is translated along the beam axis past the focus of $F_3$the power meters record the energy of the incident beam and the transmitted beam every 10 *mS* for time 7 *s* over a length of 5.5 *cm*. The beam waist is determined using beam profiler (Spiricon SP620U). The sample cuvette provides path length of3.0 *mm* and the beam waist at the focus was 85 μM.A range of incident power on the sample incrementally varied from 9.2 *mW* to 89.5 *mW* have been used.

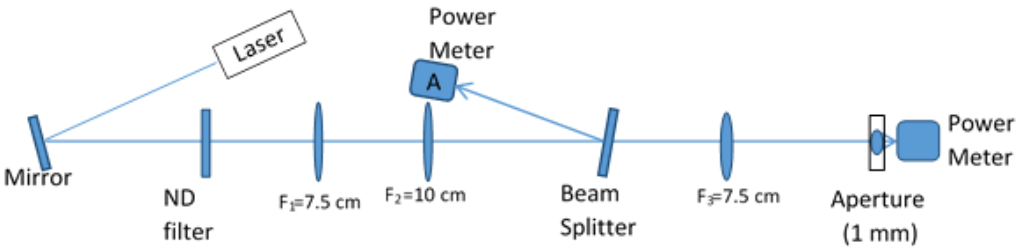

Fig.1 Close aperture Z-scan setup

2.3 Computational Details

Initial molecular geometry of L-tyrosine was taken from PubChem [37] which was optimized and processed with GaussView5 [38]. This structure was then further optimized at MM level followed by semi-empirical and finally at quantum mechanical DFT level with 6-31G basis set using computational chemistry program Gaussian 09 [39]. Then the minimum energy of the system at ground state was determined using cc-PVDZ and cc-PVTZ basis set developed by Dunning et al. [40,41]. Finally the excitation energy and absorption spectra was calculated at TDDFT level using Coulomb Attenuating Method (CAM) in Becke's three Parameter hybrid functional combined with Lee Yang and Parr (B3LYP) functional [42]and aug-cc-PVTZ and cc-PVQZ basis sets [43] with IEFPCM [44] as solvation model. The results of simulation were compared with experimental values for excitation energies.

III. Results

3.1 Results of UV-Vis spectroscopy

Three absorption peaks have been observed in the UV region of the spectrum. At concentration greater than 0.5 *mM* only one peak at 274.7 *nm* with a shoulder at 282 *nm* is distinguishable and the absorbance below 240 *nm* rose steeply to a continuous end absorption band (Fig.2). However, as concentration is decreased, two more absorption peaks became discernible. The second absorption peak appears at 224 *nm* at concentration below 0.5 *mM* and remains there till 7.5 μM. At further lower concentration it shifts toward 221 *nm.*

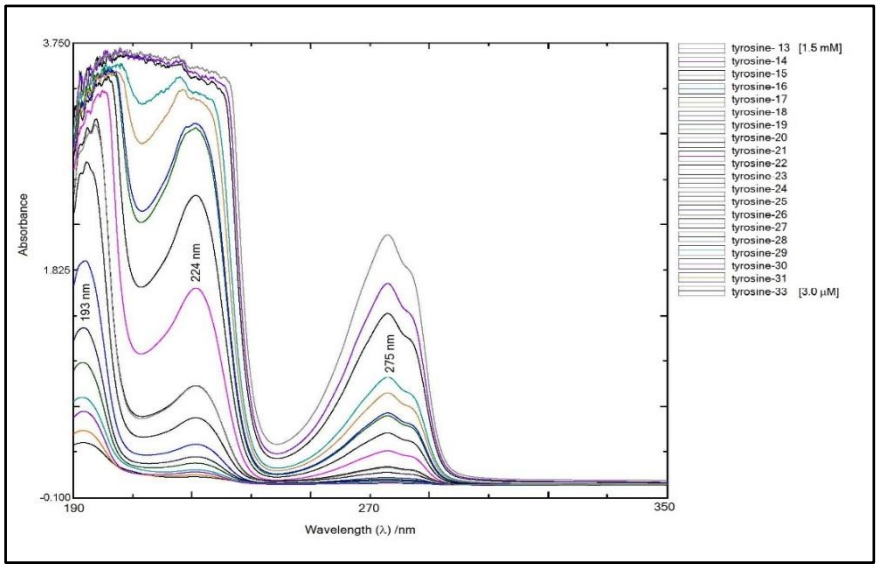

Fig. 2UV absorption spectrum of L-tyrosine at concentrations 1.5 mMto3.0 μM.

Another peak at 193 nm appears at concentration 0.05 *mM*as a shoulder to an absorption band around 196 *nm* and with decreasing concentration it gradually shifts toward shorter wavelength around 192 *nm*. These three absorption bands correspond to excitation energies of 4.51 eV, 5.54 eV and 6.40 eV respectively. Similarly the shoulder at 282 *nm* can be identified as a separate band with excitation energy 4.40 eV. The absorptions bands at 275 nm and 224 *nm* are due to singlet π to $\pi^{\star}$ transition from S_0 to S_1 state and from S_0 to S_2 state respectively [45,46]. These two transitions have been identified as ^1L_a and ^1L_b respectively as per Platt's convention[47]. The asymmetric structure of the phenol ring favours the ^1L_b transition as has been observed in case of p-cresol[11]. The absorption at 193*nm* is due to transition from S_0 to S_3 in analogy with benzene transition at 184 *nm* [48]. In the case of all the absorption bands the positions of the absorption peaks changed slightly with concentration. This is because of the change in the pH of the solution. The position of the absorption bands in aromatic amino acids depend on the pH and are found to be red shifted with increase in alkalinity [45]. As the response of aromatic chromophore of tyrosine to UV radiation changes substantially with environment [11], the features of UV-Vis Spectroscopy can be used to study protein structure.

Fig.3 to Fig.5 shows the variations of absorbance at the wavelengths 275 nm, 224 nm and 193 nm respectively. Absorbance at these three peak positions do not vary with concentration in the same manner and are found to follow Beer-Lambert law at different concentration ranges. The graphs are plotted at their respective linear regions. For the shortest of the wavelength corresponding to the absorption peak at 193 *nm*is linear in the lowest concentration range of 3.0 μM to 30 μM. The absorbance at 224 nm is linear in the range of 3.0 μM to 75 μM, whereas the

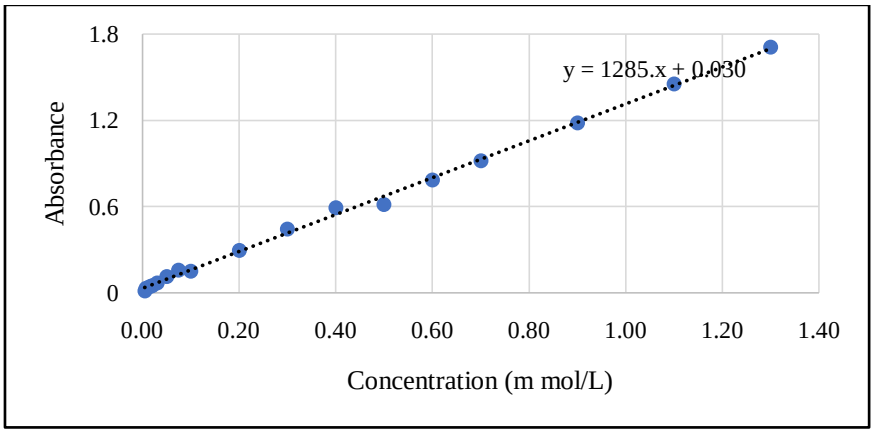

Fig.3 Absorbance at 275 nm at concentrations 5.0 $mM\ to$ 1.3 mM

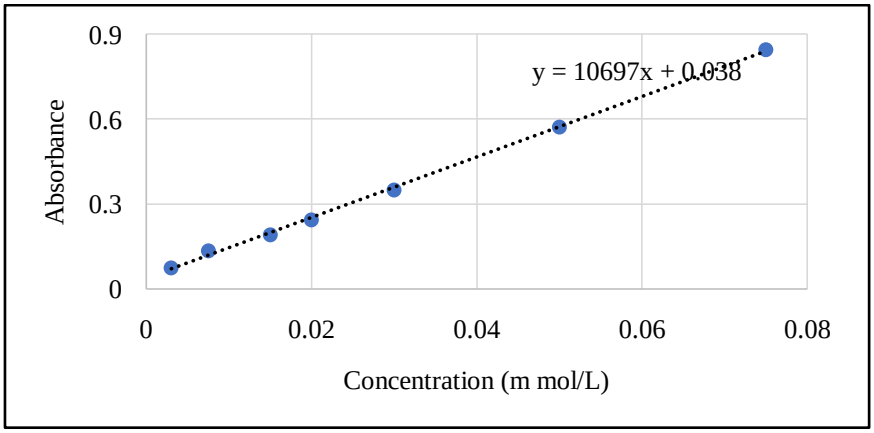

Fig.4 UV absorbance at 224 nm at concentrations 3.0 $\mu M\ to$ 75 μM

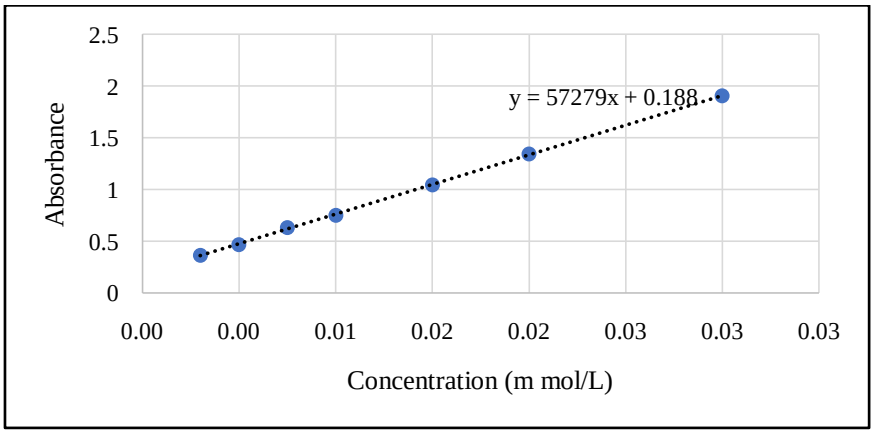

Fig. 5 UV absorbance at 193 nm at concentrations 3.0 $\mu M\ to$ 30 μM

absorbancein the longest wavelength, 275nm, varies linearly at the widest range of 5.0 μM to 1.3 mM. Hence the graphs are plotted only for their respective linear regime. The slope of these graphs indicates the extinction coefficients at these wavelengths to be1285 $L\ mol^{-1}cm^{-1}$, 10679 $L\ mol^{-1}cm^{-1}$ and 57279 $L\ mol^{-1}cm^{-1}$respectively. These values differ from the values cited by Schmid [49]. The possible reason for this discrepancy is the pH of the solution and the concentration at which these values are determined.

3.2 Results of Z-Scan Analysis

Z-scan patterns of L-tyrosine at different powers are all similar with a peak followed by valley exhibiting its negative nonlinear refractive property. TheT_{pv}'s obtained from the normalized transmittance curve increased with the increase of incident power(Fig.6). The Fig.7 shows the concurrence between the experimental

data and theoretical curve fitting at 65 mW incident power. The resulting on axis phase shift ($\Delta\Phi_0$) determined from theT_{pv} following the equation

$$\Delta\Phi_0 = T_{pv}/\{0.406(1-S)^{.25}\}$$

is found to vary linearly with the power (Fig.8), the slope of which gives a nonlinear refractive index of 1.74 × 10^{-8} m^2/W. The value is very large to be of electronic origin and hence must be due to thermal lensing effect. The possible source of this nonlinearity is the presence of conjugated π-bond in the structure.

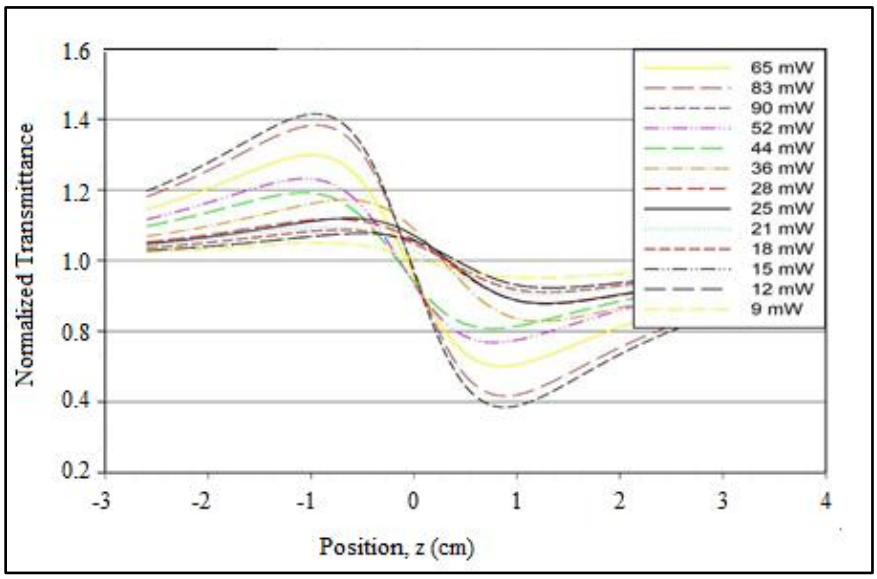

Fig.6 Normalized transmittance through aperture as function of sample position (Theoretical fit)

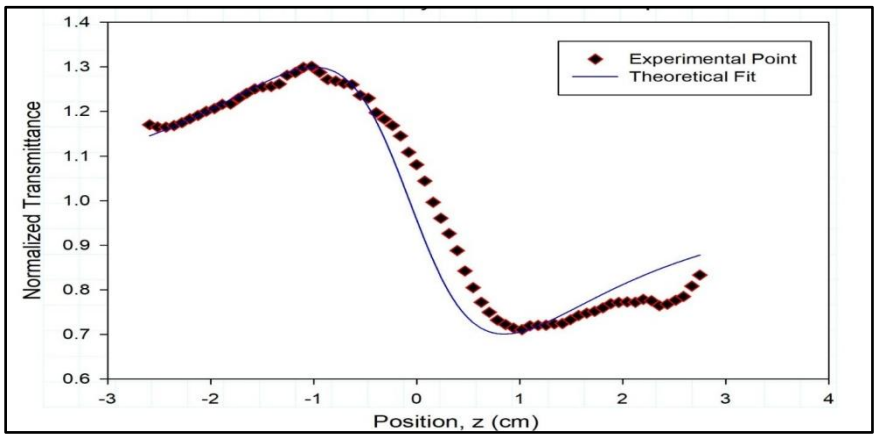

Fig. 7 Experimental data and theoretical fit at 65 mW incident power

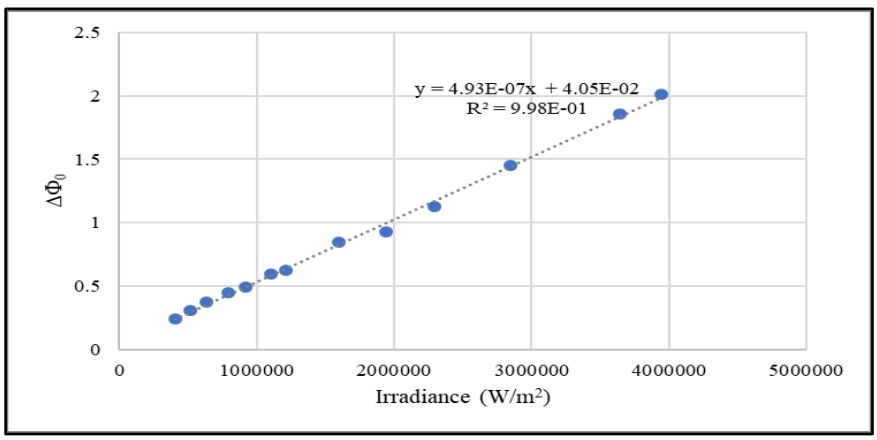

Fig. 8 Variation of on axis phase-shift with irradiance

3.2 Results of Theoretical Analysis

The HOMO-LUMO gaps giving excitation energies using cc-PVTZ basis set and CC-PVQZ along with the experimental values obtained from UV-VIS spectra are summarized in table no 1. The UV-Vis absorption peaks are at 275*nm*, 224*nm* and 193 *nm* respectively whereas the corresponding values obtained from calculation using cc-PVTZ basis set are 259*nm*, 224*nm* and 197.6 *nm* and with cc-PVQZ the absorption peaks are at 260*nm*, 225 *nm*and 198 *nm* respectively . The calculated dipole moment of L-tyrosine using these two basis sets are 13.16 D and 13.46 D respectively which are very close to the reported value of 13.85 D.

Table no 1 : Comparison of experimental results and theoretical calculations of UV absorption bands

Experimental observation			Cc-PVTZ		Cc-PVQZ	
Wavelength	Excitation energy	Extinction coefficient	Excitation Energy	Oscillator strength	Excitation Energy	Oscillator strength
275	4.51	1285	4.79	0.0342	4.77	0.0325
224	5.54	10857	5.54	0.0898	5.51	0.0852
193	6.42	57279	6.28	0.2083	6.26	0.3548

IV Conclusion

As tyrosine's peaks position and absorbance depend strongly on the environmental factors, its UV absorption analysis can be used to study protein structure. The theoretical calculation at DFT level with polar valence basis sets gives HOMO-LUMO structure from which the calculated excitation energies of two absorption bands of L-tyrosine at 193 nm and 224 nm are almost same and the other at 275 nm is within 6 % of the experimental value. However the thermal effect of nonlinear response is not calculable with this approach.

Acknowledgement

We acknowledge the support of UGC of Bangladesh for their funding through the HEQEP. We also acknowledge the support of the High-Performance Computing group of the Department of Physics, Shahjalal University of Science and Technology, Bangladesh.

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Sarfuddin Ahmed Tarek, et al. "UV-Visible absorption spectroscopy and Z-scan analysis along with corresponding molecular electronic structure analysis at DFT level for L-Tyrosine." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 13(1), (2020): pp45-51.