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Molecular structure and spectroscopic characterization of Metformin with experimental techniques and DFT quantum chemical calculations

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Abstract : Metformin is one of the important anti- diabetic drugs to treat non – insulin dependent diabetes mellitus. A complete vibrational assignment and analysis of the fundamental modes of Metformin was carried out using FT-IR, FT-Raman and Quantum Chemical studies. The observed vibrational data were compared with the wave numbers derived theoretically. Thermodynamic properties like entropy, heat capacity and enthalpy have been calculated for the molecule. HOMO, LUMO energy gap has been calculated, the intra-molecular contacts have been interpreted using natural bond orbital (NBO) and Natural Localized Molecular Orbital (NLMO) analysis. Finally the Mulliken Population Analysis on atomic charges of the title compound has been calculated. The B3LYP/6-311++G (d, p) based NMR calculation procedure was also done. The NLO properties of the molecule have also been studied. It was used to assign the 13C and 1 H NMR chemical shift of Metformin. The results of the calculations were applied to simulated spectra of the title compound which show excellent agreement with observed spectra.

Keywords: Molecular structure, spectroscopic characterization, Metformin, DFT quantum chemical calculations.

1. Introduction

Metformin is an anti-diabetic agent. It reduces blood glucose levels and improves insulin sensitivity. Its metabolic effects, including the inhibition of hepatic gluconeogenesis, are mediated at least in part by activation of the LKB1-AMPK (AMP-activated protein kinase) pathway. Metformin helps reduce LDL cholesterol and triglyceride levels and is not associated with weight gain, and is the only anti-diabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. Type 2 or NIDDM (Non Insulin Dependent Diabetes Mellitus) is a metabolic disorder that affects more than 150 million people world-wide [1]. The different classes of oral hypoglycemic agents used to treat NIDDM have complementary mechanism of action, and their use in combination often results in blood glucose reduction that are significantly greater than those that can be obtained with maximal dose of any single drug [2,3]. Metformin belongs to the class of biguanides and it is chemically known as 1, 1-dimethyl biguanide hydrochloride. It is a white hygroscopic crystalline powder with a bitter taste with a molecular formula C4H11N5 HCl. Metformin is an exciting drug having major impact on the treatment of type 2 diabetes that it increases insulin sensitivity by both decreasing hepatic glucose output and enhancing peripheral glucose uptake. Metformin reduces blood glucose levels primarily by suppressing glucose formation in the liver (hepaticgluconeogenesis). [4] More importantly, it activates an enzyme called AMPK (AMP-activated protein kinase) that plays an important role in insulin

signaling, systemic energy balance, and the metabolism of glucose and fats. [5] Activation of AMPK is one mechanism that may explain why diabetics prescribed Metformin have sharply lower cancer rates. For instance, in a controlled study at MD Anderson Cancer Center, the risk of pancreatic cancer was 62% lower in diabetics who had taken Metformin compared to those who had never taken it.

In one study lasting more than 10 years, patients who primarily received Metformin had a 39% reduction in the risk of heart attack and a 36% reduction of death from any cause. [6] The same study showed that Metformin did not cause weight gain in overweight patients, while patients prescribed sulfonylurea's gained more than 7 pounds, and those using insulin injections gained over 10 pounds. [7].

Recently several disputes are going on over the drug Metformin. The American medical establishment labored under an egregious misconception about the safety of Metformin. The reason was that drugs in the same class of Metformin (biguanides) can cause a potentially fatal condition called lactic acidosis, where the body becomes overly acidic in the presence of excess lactic acid. While other biguanide drugs were withdrawn because of lactic acidosis risk, it turned out that Metformin did not induce this same side effect in healthier people. [8] As long as one has sufficient kidney, liver, cardiac, and pulmonary function, any excess lactic acid caused by Metformin is safely removed by the kidneys. [9-11]. It turned out that only patients with severe kidney, liver, pulmonary, or cardiac impairment had to avoid Metformin because of lactic acidosis concerns, and even these worries were overblown. But ANALYSIS SHOWS METFORMIN DOES NOT CAUSE LACTIC ACIDOSIS. A Cochrane Systematic Review of over 300 trials evaluated the incidence of lactic acidosis among patients prescribed Metformin vs. non-Metformin anti-diabetes medications. Of 100,000 people, the incidence of lactic acidosis was 4.3 cases in the Metformin group and 5.4 cases in the non-Metformin group. The authors concluded that Metformin is not associated with an increased risk for lactic acidosis. [12]. Secondly Metformin is known to cause vitamin B12 deficiency which translates into higher levels of artery-clogging. So the present work on quantum chemical calculations on the drug may be helpful in overcoming the disputes over the same compound which is on headlines now.

In our present work, an attempt has been made to interpret the vibrational spectra of Metformin by applying abinitio and density functional theory calculations based on Hartree-Fock and Becke3-Lee-yang-Pars (B3LYP) level using 6-31 G (d.p) basis sets. Further the calculations of electronic excitations, particularly for valence-like transitions and oscillator strength of Metformin, were calculated employing the allowance e^- TD-DFT methods. In addition to these, bond orders and atomic charges calculated at the HF/6-31G (d, p), B3LYP, 6-31, G (d, p) levels. Experimentally observed spectral data of the little compound is found to be well compared with the data obtained by quantum chemical methods.

The aim of the present theoretical study is to investigate the structural, electronic and vibrational properties of Metformin. There are limited studies in the literature about the molecules considered in this work. The result of such theoretical work will aid in the elucidation of structure activity relationships of compounds in drugs before they can be safely evaluated and commercially developed as beneficial pharmaceuticals.

2. Experimental details

The title compound Metformin was obtained from Ranbaxy India ltd, a reputed pharmaceutical company, Pondicherry, India with more than 98% purity and was used as such without further purification to record FTIR and FTR spectra. The FTIR spectrum of the compound was recorded in the region4000-450 cm-1 in evacuation mode on Bruker IFS 66vspectrophotometer using Kbr pellet technique (solid phase)with 4.0 cm-1 resolution. The FT-Raman spectrum was recorded using 1064 nm line of Nd: YAG laser as excitation wavelength in the region 5000-10 cm-1 on Bruker IFS 66V spectrometer equipped with FRA 106 Raman module was used as an accessory. The FT-NMR measurements were carried out using NMR 500 BRUKER spectrometer. All these spectral measurements were carried out at Sophisticated Analytical Instrumentation Facility, IIT, Chennai, India, The UV-Vis spectral measurements were carried out at SAIF, SPU, AVADI, India.

3. Computational Details

The entire calculations conducted in the present work were performed at quantum chemical density functional calculations (DFT) using the Gaussian 03W package] [13]program employing the Becke's three-parameter hybrid functional [6][14] combined with Lee-Yang-Parr correlation[15]functional (B3LYP) method together with the 6-31G(d,p)basis set utilizing gradient geometry optimization. The DFT partitions the

electronic energy as E=T+EV+EJ+EXC, where ET, EV, and EJ are the electronic kinetic energy, the electron nuclear attraction and the electron-electron repulsion terms respectively. The electron correlation is taken into account in DFT via the exchange correlation term EXC, which includes the exchange energy arising from the antisymmetry of the quantum mechanical wave function and the dynamic correlation in the motion of individual electrons; it makes DFT dominant over the conventional HF procedure [16] Molecular geometries were fully optimized using the Berny optimization algorithm using redundant internal coordinates. The optimized structural parameters were used in the vibrational wave number calculation at the DFT levels to characterize all stationary points as mini ma.

Then vibration ally averaged nuclear positions of Metformin is used for harmonic vibrational frequency calculations resulting in IR and Raman frequencies together with intensities and Raman depolarization ratios. The DFT hybrid B3LYP functional also tends to overestimate the fundamental modes in comparison to the other DFT methods; therefore, scaling factors have to be used to obtain considerably better agreement with experimental data. Thus according to the work of Rauhut and Pulay (Rauhut *et al.,* 1995), a scaling factor of 0.963 has been uniformly applied to the B3LYP calculated wave numbers. Similarly, the vibrational modes studies through HF method were scaled by a value of 0.891. Finally, calculated normal mode vibrational frequencies, provide thermodynamic properties by way of statistical mechanics. Zero point vibrational energy was also calculated in the present work. By combining the results of the Gauss view program (Frisch *et al.,* 2000) with symmetry considerations, vibrational frequency assignments were made with high degree of accuracy. The transformation of force field, calculations of potential energy distribution (PED), IR and Raman intensities were done on a personal computer (PC) with a version V7.0-G77 of the MOLVIB program written by Tom Sundius (Sundius, 1991). There is always some ambiguity in defining internal coordination. However, the defined coordinate form complete set and matches quite well with the motions observed using the Gauss view program. To achieve a close agreement between observed and calculated frequencies, the least square fit refinement algorithm was used. For the plots of simulated IR and Raman spectrum, pure Lorentzian band shapes were used with a bandwidth of 10 cm^{-1}.

4. Results and Discussion

4.1 Molecular geometry

The optimized structure parameters of Metformin calculated by ab initio HF and DFT-B3LYP levels with the 6-31G (d, p) basis set are listed in the table 1 in accordance with the atom numbers given in Fig 1. From the theoretical values, we can find that most of the optimized bond angles are slightly larger than the experimental values, due to the theoretical calculations belong to isolated molecules in gaseous phase and the experimental results belong to molecules in solid state. Comparing bond angles and lengths of B3LYP with those of HF, as a whole the formers are on higher side than the latter and the HF calculated values correlates well compared with the experimental results. In spite of the differences, calculated geometric parameters represent a good approximation and they are the basis for calculating other parameters, such as vibrational frequencies and thermodynamic properties.

Table 1 Geometrical Parameters of Metformin calculated by DFT and HF methods

Bond Length	B3LYP	HF	Bond Angle	B3LYP	HF	Dihedral Angle	B3LYP	HF
R(1,2)	1.4115	1.4031	A(2,1,4)	112.814	112.6721	D(4,1,2,3)	128.6672	125.9146
R(1,4)	1.3984	1.3945	A(2,1,6)	118.9929	119.2522	D(4,1,2,10)	-35.5966	-29.6782
R(1,6)	1.2752	1.2515	A(4,1,6)	127.8927	127.8089	D(6,1,2,3)	-57.1337	-59.5593
R(2,3)	1.3967	1.388	A(1,2,3)	124.5821	124.2217	D(6,1,2,10)	138.6024	144.8479
R(2,10)	1.0088	0.9943	A(1,2,10)	117.0159	115.9473	D(2,1,4,8)	166.0763	164.3961
R(3,5)	1.4	1.3883	A(3,2,10)	116.626	115.5308	D(2,1,4,9)	-49.7003	-56.6685
R(3,7)	1.2749	1.2544	A(2,3,5)	110.747	110.6425	D(6,1,4,8)	-7.4916	-9.5569
R(4,8)	1.4543	1.4472	A(2,3,7)	121.3274	122.1127	D(6,1,4,9)	136.7318	129.3784
R(4,9)	1.4651	1.458	A(5,3,7)	127.7573	127.1075	D(2,1,6,13)	173.1217	175.3446
R(5,11)	1.0096	0.9953	A(1,4,8)	117.293	116.7704	D(4,1,6,13)	-13.6582	-11.0521
R(5,12)	1.0116	0.9967	A(1,4,9)	119.4337	117.4023	D(1,2,3,5)	158.4485	163.5109

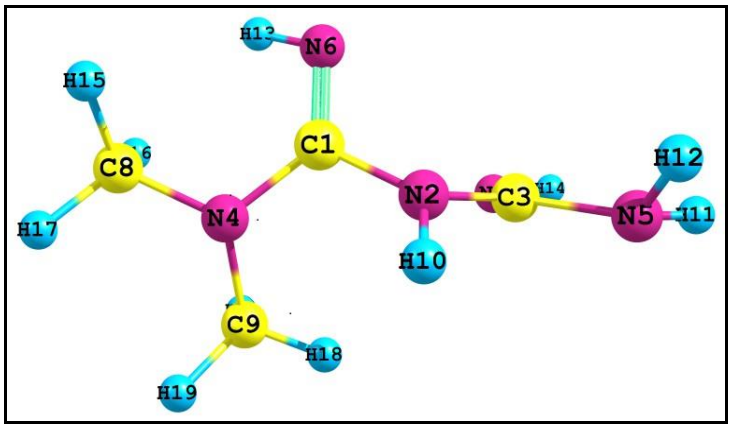


Fig.1 Atom numbering of Metformin molecule

4.2 Vibrational Assignments

The FTIR and FT Raman spectra of Metformin are shown in Fig 2 and 3. The observed and calculated frequencies using HF/6-31G (d, p), B3LYP/6-31 G (d, p) methods along with their relative intensities and assignments of Metformin are summarized in Table 2. The maximum number of values determined by HF/6-31G (d, p) method is well agreed with the experimental values and is also confirmed by the scale factors used to get the scaled frequencies. Therefore this method is considered to be more reliable and taken for discussion.

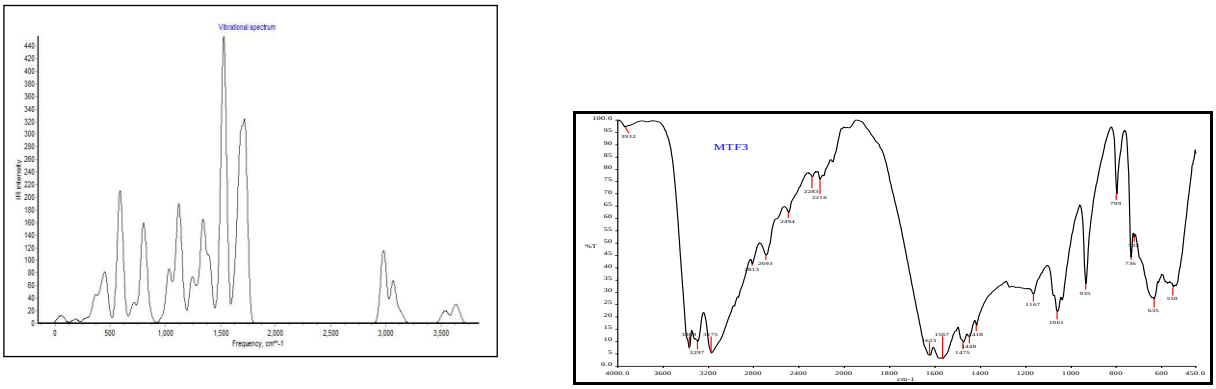


Fig.2 FT-IR Vibrational Spectra of Metformin (a) Theoretical (b) Experimental

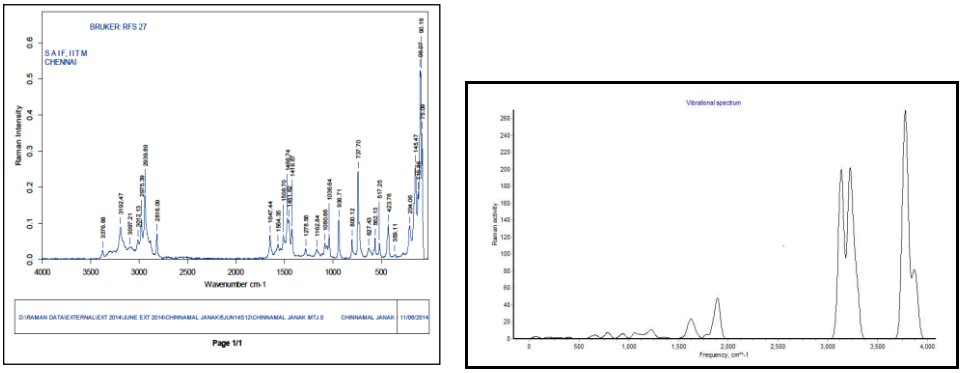


Fig.3 FT-Raman Spectra of Metformin (a) Experimental (b) Theoretical

Table 2 Observed and Theoretical Vibration (Selected) assignment of Metformin

DFT Method				HF Method				Experimental		
Frequencies	**Reduced Mass**	**Force Constant**	**IR Intensity**	**Frequencies**	**Reduced Mass**	**Force Constant**	**IR Intensity**	**FTIR**	**FTR**	**Assignments**
360.0024	1.4392	0.1099	34.6768	392.3493	1.5424	0.1399	14.2892		359.11	CNC bending
373.1719	1.8047	0.1481	7.6385	400.676	1.6696	0.1579	8.5428		423.76	NCN torsion
492.0606	3.3218	0.4739	7.0239	525.274	4.1928	0.6816	4.2659	517.02	517.25	NCN bending
586.4171	1.3328	0.27	165.7557	648.5294	1.4762	0.3658	135.214	614.35	627.13	NCN bending
793.7198	1.3321	0.4908	117.4485	894.6049	1.3551	0.639	141.9187	800.12	800.12	NH wagging
1030.0518	2.7002	1.688	80.4578	1112.4105	2.7776	2.0251	64.6995	935.5	936.71	NH wagging
1072.7451	1.4457	0.9802	23.529	1161.9134	1.4598	1.611	25.8906	1040.1	1036.64	CN stretching
1238.1723	2.9179	2.6356	67.1594	1349.6579	2.7468	2.948	74.1692	1245.21		
1284.9506	2.0665	2.0103	41.8037	1405.4173	1.9959	2.3228	31.5371		1278.56	NC stretching
1339.6114	1.9865	2.1004	158.804	1466.6319	1.9185	2.4313	185.7976	1342.26		
1485.9027	1.0495	1.3653	17.5483	1608.0821	1.049	1.5983	9.6418	1486.84	1466.74	CH3 asymmetric
1497.2045	1.0503	1.3872	15.8647	1621.8872	1.0506	1.6282	14.8917		1508.7	HNC bending
1531.5467	1.5988	2.2095	351.3457	1683.2087	2.1034	3.5112	491.4802	1534.78	1564.35	HNC bending
1630.4127	1.246	1.9515	69.0884	1777.6348	1.2602	2.3463	96.3549	1626.01	1647.44	HNH bending
2965.3883	1.0569	5.4757	57.5099	3120.4338	1.0561	6.0585	52.9019	2973.22	2975.39	CH stretching
2987.6351	1.053	5.5378	71.5008	3143.8916	1.053	6.132	68.6634		3012.13	CH stretching

C-N vibrations

Seshadri et al. [17] have observed the C-N stretching band at1305 cm $^{-1}$ in FTIR and 1307 cm^{-1} in FT Raman spectra of 7-chloro-3-methyl-2H-1, 2, 4-benzothiadiazine 1, 1-dioxide. Silverstein et al [18] assigned the C-N stretching absorption in the range 1382-1266 cm^{-1} for aromatic amines. In the present work, 1273.30, 1342.26 cm^{-1} in FTIR and 1278.56 cm^{-1} in FTR spectrum are assigned to C-N stretching vibrations. The theoretically computed values of C-N stretching vibrations also falls in the region 1284.95-1497.2095 cm^{-1} by both DFT and HF methods.

CH3 vibrations

T.Gnasambandan et al. [19] has assigned CH_3 asymmetric stretching vibrations for CBZ. He has assigned at 1213,1277 cm^{-1} in FTIR and FTR for CH_3 in plane bending vibrations and 1390,1366 cm^{-1} in FTIR spectrum for CH_3 out of plane bending vibrations. In our present work, 1211.78, 1245.21 cm^{-1} are assigned for CH_3 in plane bending vibrations and 1342.26 is assigned for CH_3 out of plane bending vibration. The computed values of CH_3 vibrations also fall in the region 1238, 1723-1339.61 cm^{-1} both DFT and HF methods.

CH Vibrations

Aromatic compounds commonly exhibit multiple weak bands in the region 3200–3000 cm^{-1} due to aromatic C-H stretching vibrations .[20-23] In our titled compound, the C-H symmetric stretching vibrations observed in FT-IR spectrum at 2938,2973, 3169,3178 cm^{-1}. Similarly in FT-Raman spectrum 975, 3012, 3097, 3192 cm^{-1} are assigned as C-H asymmetric stretching vibrations. The calculated values of CH vibrations also coincides with the experimental values in the region 3200-3000 cm^{-1} in both DFT and HF methods.

NH Vibrations

The NH stretching vibration gives rise to a weak band at 3500–3300 cm^{-1}. The band appear at 3414 cm^{-1} in the FTIR spectrum of 2-amino-4, 6-dimethoxy pyrimidine molecule was assigned to NH2 stretching vibration [24]. In our titled compound, 3393.11 cm^{-1} is assigned in FTIR for NH stretching vibration and 3376.88 cm^{-1} is assigned in FTR for NH vibrations. The stretching mode for NH vibrations calculated at the higher wave number 3506 cm^{-1} by DFT method.

4.3 Thermodynamic Properties

Several calculated thermodynamic parameters at room temperature are presented in Table 3. Scale factors have been recommended for an accurate prediction in determining the zero point vibrational energies

and the entropy S. The variation in the ZPVE seems to be insignificant. The total energies found to decrease with increase of the basis sets dimension. The changes in the total entropy of Metformin at room temperature at different basis sets are only nominal.

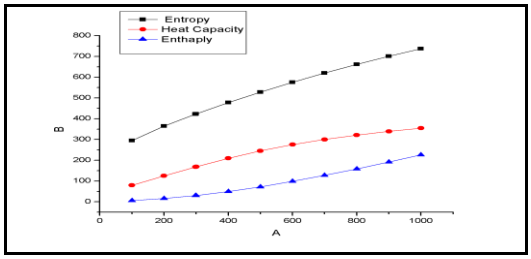


Fig 4. ThermoDynamics Properties of Metformin

Table 3 The calculated Thermodynamic parameters of Metformin

Position	Energy (Kcal/mol)		Heat Capacity (Cal/mol-kelvin)		Entropy (Cal/mol-kelvin)	
	DFT	HF	DFT	HF	DFT	HF
Translational	0.889	0.889	2.981	2.981	40.479	40.479
Rotational	0.889	0.889	2.981	2.981	29.170	29.106
Vibrational	111.719	119.483	32.199	29.537	31.233	28.272
Total	113.497	121.261	38.161	35.499	100.873	97.858
Rotational Constant(GHZ)	3.18875	.96118	0.16938	3.2447	0.9637	0.7500

Table 4

T (K)	S (J/mol.K)	Cp (J/mol.K)	ddH (kJ/mol)
100	294.75	79.54	5.56
200	364.14	125.3	15.84
298.2	422.16	167.98	30.24
300	423.2	168.77	30.55
400	477.47	209.76	49.51
500	528.23	245.52	72.32
600	575.72	275.4	98.42
700	620.09	300.26	127.24
800	661.59	321.2	158.34
900	700.48	339.07	191.38
1000	737.03	354.48	226.07

On the basis of vibrational analysis at B3LYP/cc-pVDZ level, the standard statistical thermodynamic functions: heat capacity ($C^0_{p,m}$),entropy (S^0_m), and enthalpy changes ($\Delta_H{}^0_m$) for the title compound were obtained from the theoretical harmonic frequencies and listed in Table 4. From Table 4, it can be observed that these thermodynamic functions are increasing with temperature ranging from 100 to 1000 K due to the fact that the molecular vibrational intensities increase with temperature [25,26]The correlation equations between heat capacities, entropies, enthalpy changes and temperature are fitted by quadratic formulas. The corresponding fitting factors (R^2) for these thermodynamic properties are 0.9998, 0.99972 and 0.9956 respectively. The standard deviation is very least in the calculation of enthalpy change. The corresponding fitting equations are as follows and the correlation graphics of those shows in Fig. 4.

$C^0_{p,m=}$ 26.35056 + 0.54287T- 2.15855E- 4T^2 (R^2= 0.99981)
$S^0_{m=}$ 232.50013 + 0.68241T- 1.80097E- 4T2 (R^2=0.99972)
$\Delta_H{}^0_m$ = -6.27853+ 0.08151t- 1.52812E- 4T2 (R^2=0.99956)

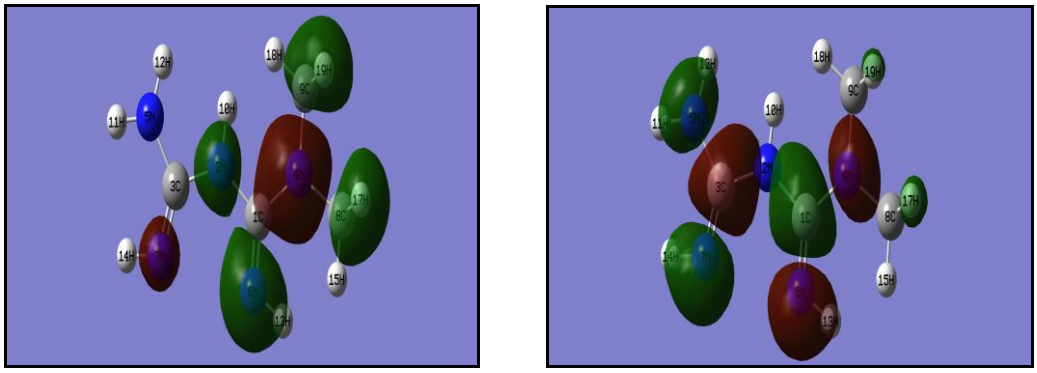
All the thermodynamic data supply helpful information for the study on the Metformin. They can be used to compute the other thermodynamic energies according to relationship of thermodynamic functions and estimate directions of chemical reactions according to the second law of thermodynamics. Notice: all thermodynamic calculations were done in gas phase and they could not be used in solution.

4.4 UV -Visible spectrum and electronic properties

Ultraviolet spectra analyses of Metformin have been investigated by theoretical calculation. The excitation energies are investigated for Metformin. The lowest singlet-singlet spin-allowed excited states are taken into account in order to investigate the properties of electronic absorption. The computed electronic features, such as absorption wavelength (k), excitation energies (E), oscillator strengths (f), and major contributions of the transition are tabulated in the table 5. Typically according to Frank–Condon principle, the maximum absorption peak (max) corresponds in a UV–Vis spectrum of vertical excitation. The computed absorption wavelength coincides well with the experimentally observed value in the reference work byL adikari et al [27].

4.5 HOMO -LUMO energies.

The energies of the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) are very important parameters for quantum chemistry. HOMO can be through the outermost orbital containing electrons tends to give these electrons such as an electron donor. On the other hand, LUMO can be through the innermost orbital containing free places to accept electron [28]. Owing to the interaction between HOMO and LUMO orbital of a structure, transition state transition of π^- - π^+ type is observed with regard to the molecular orbital theory [29, 30]. Therefore, while the energy of the HOMO is directly related to the ionization potential, LUMO energy is directly related to



HOMO **LUMO**

Fig5. 3D Plots of HOMO and LUMO

The electron affinity. Energy difference between HOMO and LUMO orbital is called as energy gap that is an important stability for structures [31]. The HOMO and LUMO energies of the drug under study are given in the table 6. The energy difference between HOMO and LUMO orbital's is called as energy gap and is calculated by the basis set as 5.1345568 eV. It is a critical parameter in determining molecular electrical transport properties Fig.5 shows the distributions and energy levels of HOMO and LUMO orbitals for the Metformin in gaseous phase. It is clear from Fig. 5 that the isodensity plots for the HOMO are well localized with the ring whereas; the orbital overlapping on the ring system of LUMO surface shows empty. Also, energies of HOMO and LUMO are used for the determination of global reactivity descriptors. It is important that Ionization potential (I), Electron affinity (A), Electrophilicity (ω), Chemical potential (μ), Electro negativity (χ), Hardness (η) and Softness (S) be put into a MO framework.

We focus on the HOMO and LUMO energies in order to determine the interesting molecular/atomic properties and chemical quantities. In simple molecular orbital theory approaches, the HOMO energy is related to the ionization potential (I) and the LUMO energy has been used to estimate the electron affinity (A) respectively by the following relations:

$I = -E_{HOMO}$ and $A = -E_{LUMO}$

The chemical potential of the molecule is (μ) = _ (I + A)/2.
The absolute hardness of the molecule is (η) = (I - A)/2.
The softness is the inverse of the hardness (S) = 1/ η.
The electro negativity of the molecule is (χ) = (I + A)/2.
The electrophilicity index of the molecule is (ω) = $\mu^2/2\ \eta$.
The quantum chemical parameters of the molecule are presented in Table 6.

4.6 Non linear optical properties

Table 5 Calculated absorption wavelength (λ), excitation energies (E), Oscillator strength (f)

λ (Cal. Nm)	E (eV)	F
278.91	4.4453	0.00071
265.98	4.664	0.0046
253.80	4.8852	0.0206

Nonlinear optical (NLO) effects arise from the interactions of electromagnetic fields in various media to produce new fields altered in phase, frequency, amplitude or other propagation characteristics from the incident fields [32]. The results of the calculated molecular polarizabilities at DFT/B3LYP/6-311++G (d, p) level on the basis of the finite-field approach are given in Table 5. By considering Table 3, the values of the second-order polarizability Or first hyperpolarizability (β), dipole moment (μ) and polarizability (α) of title molecule are reported in the atomic mass units (a.u) and electrostatic unit (esu). First hyperpolarizability is a third rank tensor that can be described by a 3 x 3x 3 matrix. The 27 components of the 3D matrix can be reduced to 10 components due to the Klein man symmetry [33]. It can be given in the lower tetrahedral format. It is obvious that the lower part of the 3 x 3x 3 matrices is a tetrahedral. The components of β are defined as the coefficients in the Taylor series expansion of the energy in the external electric field. When the external electric field is weak and homogeneous, this expansion becomes:

$$E = E^0 - \mu_\alpha E_\alpha - \tfrac{1}{2}\, \alpha_{\alpha\beta} F_\alpha F_\beta - 1/6\, \beta_{\alpha\beta\gamma} F \alpha F\beta F_\gamma + \ldots\ldots\ldots$$

Where E^0 is the energy of the unperturbed molecules, F_α is the field at the origin μ_α, $\alpha_{\alpha\beta}$ and $\beta_{\alpha\beta\gamma}$ are the components of dipole moment, polarizability and the first order polarizabilities respectively.

In Gaussian 09W output, the complete equations for calculating The magnitude of total static dipole moment (μ_{tot}), the mean polarizability (α_o), the anisotropy of the polarizability ($\Delta\alpha$) and the Mean first hyperpolarizability (β_0) by using the x, y, z components are defined as follows: [34, 35].

The mean polarizability is defined as,

$$\alpha_o = \alpha_{xx} + \alpha_{yy} + \alpha_{zz} \,/\, 3$$

The polarizability anisotropy invariant is

$$\Delta\alpha = 1/\sqrt{(2)}\ x\ \sqrt{((\alpha_{xx} + \alpha_{yy})^2 + (\alpha_{yy} + \alpha_{zz})^2 + (\alpha_{zz} + \alpha_{xx})^2 + 6\alpha^2_{xz} + 6\alpha^2_{xy} + 6\alpha^2_{yz})}$$

The components of the first hyperpolarizability can be calculated using the following equation. Using the x, y and z components Of β the magnitude of the first hyperpolarizability tensor can be

Calculated by:

$$\beta_0 = \sqrt{(\beta_x^2 + \beta_y^2 + \beta_z^2)}$$

The complete equation for calculating the magnitude of β_0 from

Gaussian 09W output is given as follows:

$\beta_0 = \sqrt{((\beta_{xxx} + \beta_{xyy} + \beta_{xzz})^2 + (\beta_{yyy} + \beta_{yzz} + \beta_{yzx})^2 + (\beta_{zzz} + \beta_{zxx} + \beta_{zxy})^2)}$

$\beta_x = \beta_{xxx} + \beta_{xyy} + \beta_{xzz}$

$\beta_y = \beta_{yyy} + \beta_{yzz} + \beta_{yxx}$

$\beta_z = \beta_{zzz} + \beta_{zxx} + \beta_{zyy}$

In Table 3, the calculated parameters described above and electronic dipole moment {μ_i (i = x, y, z) and total dipole moment μ_{tot}} for title compound were listed.

The total dipole moment can be calculated using the following Equation.

$\mu_{tot} = \sqrt{(\mu_x^2 + \mu_y^2 + \mu_z^2)}$

It is well known that the higher values of dipole moment, molecular polarizability and hyperpolarizability are important for more active NLO properties. The polarizabilities and hyperpolarizability are reported in atomic units (a.u), the calculated values have been converted into electrostatic units (esu) (for α; 1 a.u = 0.1482 $x10^{-24}$ esu, for β; 1 a.u = 8.6393$x10^{-33}$esu). The highest value of dipole moment is observed for component μ_z. In this direction, the value is equal to 0.00054 Debye. The lowest value of the dipolemomentof the titled compound isμ_y component (-2.4619). Urea is one of the prototypical molecules used in the study of the NLO properties of molecular systems. Therefore it was used frequently as a threshold value for comparative purposes. The total molecular dipole moment (μ_{tot}) and the first order hyperpolarizability are 2.4079 Debye and10.9998$x10^{-30}$ esu respectively and are depicted in Table 3. Total dipole moment of title molecule is nearly equal to urea and the first order hyperpolarizability of title molecule greater than that of urea (μ_{tot} and β_0 urea are 1.5256 Debye and 0.7803 $x10^{-30}$ esu [36] respectively, obtained by B3LYP/6-311++G (d, p) method. It is well known that the higher values of dipole moment, molecular polarizability and hyperpolarizability are important for more active NLO properties.

4.7 Atomic Charge Analysis

The calculation of effective atomic charges plays an important role in the application of quantum mechanical calculation to molecular systems. Our interest here is in the comparison of different methods (RHF and DFT) to describe the electron distribution in Metformin as broadly as possible, and to assess the sensitivity of the calculated charges to change the choice of quantum chemical method. The calculated natural atomic charge values from the natural population analysis (NPA) and Mulliken Population analysis (MPA) procedures using the RHF and DFT methods are listed in table6. It is worthy to mention that electro negative atoms C1, N2, N4, N5, N6, N7, C8 and C9 exhibit negative charge. The charge on H10 has the maximum magnitude of 0.37among the hydrogen atoms present in the molecule. It is to be noted that all the hydrogen atoms exhibit a net positive charge. The presence of large negative charge on C and N atom and net positive charge on H atom may suggest the formation of intramolecular interaction and intermolecular interaction through CH and NH bond in solid form.

Table 6 Molecular Properties of Metformin

HOMO energy (eV)	-5.99444
LUMO energy (eV)	-0.859886
Energy gap (eV)	5.134556804
Chemical Potential (μ)	-3.427163
Absolute hardness(η)	2.567277
Softness of the molecule (S)	0.389517765
Electro negativity of the molecule (χ)	3.427163
Electrophilicity index of the molecule (ω)	2.287529984

4.8 Molecular electrostatic potential surface

The molecular electrostatic potential surface (MESP) is a plot of electrostatic potential mapped on to the constant electron density surface. The MESP superimposed on top of the total energy density as a shell. Because of the usefulness feature to study reactivity given that an approaching electrophilic will be attracted to

negative regions. In majority of the MESP the maximum negative region which the preferred the site for electrophilic attack is indicated in as red color, while the maximum positive region which preferred the site for nucleophilic attack is symptoms indicated in blue color [37]. The computationally observed MESP surface map with the fitting point charges to the electrostatic potential for the title compound is shown in Fig 6. The purpose of finding the electrostatic potential is to find the reactive site of a molecule. These maps allow us to visualize variably charged regions of a molecule. Knowledge of the charge distributions can be used to determine how molecules interact with one another. The different values of the electrostatic potential at the surface are represented by different colors. Potential increases in the order red < yellow < green < light blue < blue. The color scheme for the MESP surface is red-electron rich, partially negative charge; yellow- slightly electron rich region; green-neutral; light blue-slightly electron deficient region and blue-electron deficient, partially positive charge respectively. Areas of low potential red are characterized by an abundance of electrons. Areas of high potential blue are characterized by the relative absence of electrons. Such mapped electrostatic potential surfaces have been plotted for Metformin in B3LYP/6-311++G (dip) method using the computer software Gauss view 5.0. The molecular electrostatic potential contour surface of Metformin is shown in the Fig. 6. The total electron density surface mapped with the molecular electrostatic potential of Metformin is shown in Fig. 7. The importance of MESP lies in the fact that it simultaneously displays molecular size, shape, positive as well as negative and neutral electrostatic potential regions in terms of color grading and is very useful in the research of molecular structure with its physicochemical property relationship. The color code of the map is between 0.0790 a.u. (red) to 0.1190 a.u. (blue) for the compound, where blue indicates the strongest attraction and red indicates the strongest repulsion[38,39] . The MESP map as shown in fig 6 shows the negative potential sites are on oxygen and nitrogen atoms as well as the positive potential Sites are around the hydrogen atoms.

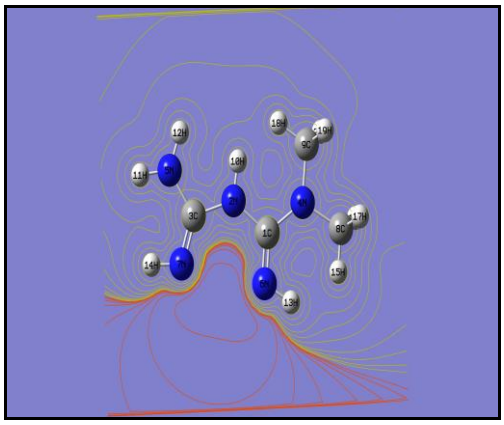


Fig 6. ESP of Metformin

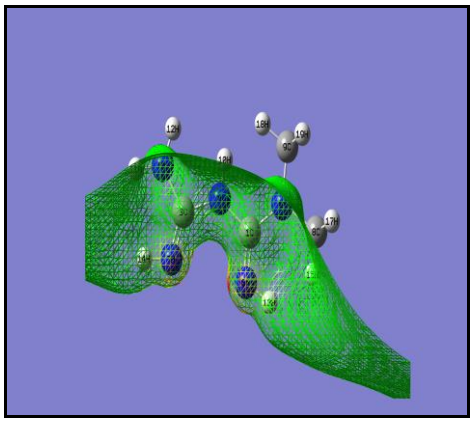


Fig 7. Electron Density Surface Mapped with ESP of Metformin

5. ^{13}C and ^{1}H – NMR Chemical Shift Assignment

The ^{13}C and ^{1}H- NMR simulated theoretically with the aid of ChemDra Ultra10.0 and experimentally observed spectra are as shown in Fig 8 & 9. Table 7 present the predicted chemical shift values of Metformin obtained by the DFT and HF methods, In general highly shielded electrons appear downfield and vice versa. The carbon atom C3appearing at very high chemical shift value (166.46) in DFT method and (161.03) in HF method is due to negative charges of two nitrogen atoms N2 and N4. Similarly C1appearing at higher chemical shift values (165.37) in DFT method and (160.48) in HF method is due to negative charges of three nitrogen atoms N2, N4, N6 respectively. The carbon atom C3 is electro positive and possess more positive charges than the other carbon atoms, and hence the Shielding is very small and appears up field. In both methods, the DFT calculated atomic charges revealed that the more electron rich atoms areC8, C9. They are highly shielded atoms and hence appear at downfield (lower chemical shift). The carbon atoms in the Benzene ring are deshielded than the carbon atoms in Aliphatic, so that the Benzene Carbon atoms in purine appear at higher chemical shift values than the aliphatic carbon atoms that were made by DFT methods. In this study, a good correlation between atomic charges and chemical shift was made.

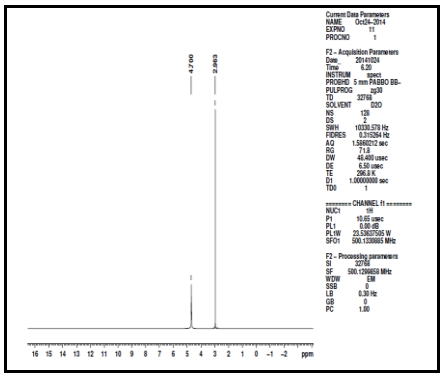


Fig 8. NMR Spectrum of Metformin (CShift)

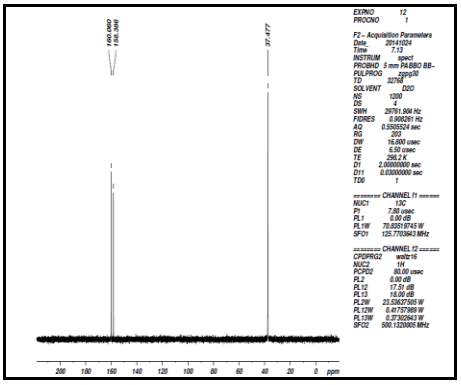


Fig 9. NMR Spectrum of Metformin (H Shift)

Table 7. Calculated α and β components – DFT method

parameters	DFT value	Esu x 10^{-34}	ß	DFT	Esu x 10^{-34}
α_{xx}	112.5621	16.6817	$ß_{xxx}$	76.5119	661.0092
α_{xy}	7.0472	1.044	$ß_{xxy}$	128.4952	1110.1085
α_{yy}	104.6978	15.5162	$ß_{xyy}$	1185.8524	10244.934
α_{xz}	0.0019	0.0028	$ß_{yyy}$	-220.0073	-1900.7090
α_{yz}	-0.0098	-0.0015	$ß_{xxz}$	-0.0842	-0.7274
α_{zz}	62.9180	9.3244	$ß_{xyz}$	-0.0319	-0.2756
α_0	93.3633		$ß_{yyz}$	0.3750	-3.2397
$\Delta\alpha$	230.6295		$ß_{xzz}$	-94.5003	-816.4164
μ_x	-0.3305		$ß_{yzz}$	-75.1067	-648.8693
μ_y	-2.4619		$ß_{zzz}$	-0.1145	-0.9892
μ_z	0.00054		$ß_0$	1273.2354	10999.866
μ_{tot}	2.4079				

Table 8 Mulliken and Natural atomic charges of Metformin

		MPA		NPA	
Sl.No	**Atom**	**HF**	**DFT**	**HF**	**DFT**
1	C	-0.2204	0.010918	0.78053	0.62601
2	N	-0.20956	-0.36922	-0.72693	-0.64833
3	C	0.199258	0.443703	0.74766	0.59598
4	N	-0.09947	-0.14558	-0.62836	-0.53753
5	N	-0.39686	-0.53702	-0.89009	-0.84951
6	N	-0.27643	-0.41142	-0.78043	-0.70808
7	N	-0.36696	-0.49969	-0.76405	-0.69395
8	C	-0.26791	-0.28751	-0.24796	-0.34206
9	C	-0.36152	-0.36872	-0.24094	-0.33713
10	H	0.302275	0.374504	0.3745	0.37341
11	H	0.292127	0.324288	0.39053	0.39017
12	H	0.216559	0.252635	0.37731	0.37544
13	H	0.19619	0.240724	0.32288	0.32258
14	H	0.190172	0.211231	0.31384	0.31603
15	H	0.12416	0.120717	0.17691	0.19811
16	H	0.151201	0.145682	0.15908	0.18424
17	H	0.151067	0.145525	0.15909	0.18425
18	H	0.061111	0.039171	0.14947	0.17567
19	H	0.157577	0.155134	0.16346	0.18732
20	H	0.15741	0.154929	0.16349	0.18735

Table 9 The calculated NMR Chemical Shift of Metformin

DFT Method			HF Method	
Atom Number	**Absolute Shielding**	**Chemical Shifts**	**Absolute Shielding**	**Chemical Shifts**
1	34.6081	165.3772	39.4996	160.4857
3	33.5216	166.4637	38.9459	161.0394
8	149.8638	50.1215	163.0257	36.9596
9	147.1878	52.7975	161.3298	38.6555
10	27.1873	5.41031	27.9461	4.6515
11	29.3148	3.2828	29.1787	3.4189
12	29.0941	3.5035	29.393	3.2046
13	25.9041	6.6935	26.2884	6.3092
14	26.2852	6.3124	26.7122	5.8854
15	28.424	4.1736	28.9043	3.6933
16	29.7492	2.8484	30.0058	2.5918
17	29.7494	2.8482	30.0061	2.5915
18	29.2927	3.3049	29.7752	2.8224
19	29.2575	3.3401	29.7342	2.8634
20	29.2577	3.3399	29.7345	2.8631

The Hydrogen atom H13 appearing at high chemical shift value (6.3092) in HF and (6.6935) in DFT method is due to negative charges of two atoms C1 and N6. Similarly H14 appearing at (6.3124) in DFT and (5.8854) in HF method is due to the negative charges of three nitrogen atoms N2, N4 and N7 respectively. The Hydrogen atom H10 is electropositive and possess more positive charge than the other hydrogen atoms and hence the shielding is very small and appears up field. In this study, a good correlation between atomic charges and chemical shift was made.

6. Conclusion

The geometry of Metformin was optimized with both DFT and HF methods using 6-31G (d, p) basis sets. The complete molecular structural parameters and thermodynamic Properties of the optimized geometry of the compound have been obtained from DFT calculations. The vibrational frequencies of the fundamental modes of the compounds have been precisely assigned and analyzed and the theoretical values were compared with the experimental vibrations. The bond order and atomic charges of the title molecule have been studied by DFT and HF methods. The energies of important molecules, absorption wavelength (λ_{max}), oscillator strength and excitation energies of the compound were also determined from TD-DFT method and compared with the experimental values. This study predicted that the molecular geometry, vibrational wave numbers and 13C and ^{1}H – NMR Chemical Shifts for Metformin could be successfully elucidated by the DFT and HF methods using Gaussian programs. The MESP map shows the negative potential sites are on oxygen, and nitrogen atoms as well as the positive potentialSites are around the hydrogen atoms. NLO properties of the compound also have been carried out. Thus, the present investigation provides complete vibrational assignments, structural informations and electronic properties of the compound which may be useful to upgrade the knowledge of Metformin.

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