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Spectroscopic (NMR, UV, FT-IR and FT-Raman) analysis and theoretical investigation of nicotinamide N-oxide with density functional theory

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ABSTRACT

The spectroscopic properties of the nicotinamide N-oxide (abbreviated as NANO, $C_6H_6N_2O_2$) were examined by FT-IR, FT-Raman, NMR and UV techniques. FT-IR and FT-Raman spectra in solid state were observed in the region 4000–400 cm⁻¹ and 3500–50 cm⁻¹, respectively. The ¹H and ¹³C NMR spectra were recorded in DMSO. The UV absorption spectrum of the compound that dissolved in water was recorded in the range of 200–800 nm. The structural and spectroscopic data of the molecule in the ground state were calculated by using Density Functional Theory (DFT) employing B3LYP methods with the 6–311++G(d,p) basis set. The geometry of the molecule was fully optimized, vibrational spectra were calculated and fundamental vibrations were assigned on the basis of the total energy distribution (TED) of the vibrational modes, calculated with scaled quantum mechanics (SQM) method and PQS program. The optimized structure of compound was interpreted and compared with the reported experimental values. The observed vibrational wavenumbers, absorption wavelengths and chemical shifts were compared with calculated vibrational geometry and calculated spectroscopic data show a good agreement with the experimental results.

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1. Introduction

Nicotinamide (also known as 3-pyridinecarboxylic acid amide and vitamin B3) and its derivatives have been subjected to many types of scientific studies due to its importance of biological activity [1–3]. For example, nicotinamide prevents immune suppression caused by UV-A and UV-B radiations. It is essential for many aspects of health and biological systems, including energy metabolism, growth and development, hormone synthesis and healthy skin, genetics, digestive tract, blood cells, brain and nervous system [4–10].

Extensive experimental and theoretical investigations have been focused on elucidating the structure and normal vibrations of nicotinamide and its derivatives. Takeshima et al. [11] determined the gas phase molecular structure of nicotinamide by using electron diffraction combined with MP2 calculations. Wright and King [12] have determined the crystal structure of nicotinamide by X-ray method. Miwa et al. [13] determined the crystal structure of nicotinamide by X-ray and neutron diffraction methods. The amide rotational barriers of nicotinamide and picolinamide have been studied using NMR and ab initio methods by Olsen et al. [2]. The molecular structure and vibrational spectra of zinc (II) halide complexes of nicotinamide [14] were investigated by computational vibrational study and scaled quantum mechanical (SQM) analysis.

The solid state FT-IR and micro-Raman spectra of nicotinamide have been recorded in the range of 650–4000 cm⁻¹ and 100–1200 cm⁻¹, respectively [15]. FT-IR spectrum of nicotinamide has been reported in the range 400–4000 cm⁻¹ and vibrational assignments have been done for all observed IR bands [16]. Akalin and Akyuz [17] calculated the optimized geometry and vibrational fundamentals of nicotinamide employing DFT method and the FT-IR experimental technique. The molecular structure and force field of nicotinamide δ_0 and δ_2 have been studied using IR spectra, ab initio and DFT calculations [18]. Borba et al. [19] have investigated the aggregation of nicotinamide using matrix-isolation, supersonic jet and solid state.

Nicotinamide N-oxide has been recognized as an excretory product of nicotinamide in the mouse and the hog [20]. The biological reduction of various N-oxide compounds has also been reported although very little is known about the enzyme, or enzymes, involved [21]. The enzymatic reduction of nicotinamide was investigated by Murray et al. [1]. Biological effects of nicotinamide N-oxide and nicotinic acid were researched by Fukuwatari et al. [22]. Kumar et al. [23] have present FT-IR spectra of nicotinamide and its N-oxide has been recorded and analyzed in the range 400–4000 cm⁻¹.

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Literature survey reveals that to the best of authors' knowledge no literature has been yet available on the structural and to fully determine the molecular structure with FT-IR, FT-Raman, ¹H and ¹³C NMR, UV-Vis spectra. Therefore, the aim of this study is to fully determine the molecular structure, vibrational modes and wavenumbers, isotropic chemical shifts and absorption bands of nicotinamide N-oxide experimentally and theoretically. For computations, we have carried out DFT calculations with the combined Becke's three-parameter exchange functional in combination with the Lee, Yang and Parr correlation functional (B3LYP) [24]. All calculations have been studied trans form and cis form of NANO. These calculations are valuable for providing insight into the vibrational spectrum and the geometric structure, vibrational energies, chemical shifts, absorption wavelengths, excitation energies and electric dipole moment of NANO. Detailed interpretations of the vibrational spectra of our compound have been made based on the calculated total energy distribution (TED).

2. Experimental

The compound NANO in solid state was purchased from Sigma Aldrich Company with a stated purity of 98%. The FT-IR spectrum of NANO was recorded between 4000 and 400 cm⁻¹ on a Perkin-Elmer FT-IR System Spectrum BX spectrometer, which was calibrated using KBr disc technique. The spectrum was recorded at room temperature, with a scanning speed of 10 cm⁻¹ min⁻¹ and the spectral resolution of 4.0 cm⁻¹. FT-Raman spectrum of the sample was recorded on a Bruker RFS 100/S FT-Raman instrument using 1064 nm excitation from an Nd:YAG laser. The detector is a liquid nitrogen cooled Ge detector. Five hundred scans were accumulated at 4 cm⁻¹ resolution using a laser power of 100 mW. ¹H and ¹³C NMR spectra were performed in Varian Infinity Plus spectrometer at 300 K. The compound was dissolved in dimethyl sulfoxide (DMSO). Chemical shifts were reported in ppm relative to tetramethylsilane (TMS) for ¹H and ¹³C NMR spectra. ¹H and ¹³C NMR spectra were obtained at a base frequency of 75 MHz for ¹³C and 400 MHz for ¹H nuclei. The ultraviolet absorption spectra of NANO are examined in the range 200-800 nm using Shimadzu UV-2101 PC, UV-VIS recording Spectrometer. The UV pattern is taken from a 10⁻⁵ molar solution of NANO, solved in water. Data are analyzed by UV PC personal spectroscopy software, version 3.91.

3. Quantum chemical computations

The first task for the computational work was to determine the optimized geometry of the compound. The hybrid B3LYP [25,26] method based on Becke's three parameter functional of DFT and 6-311++G(d,p) basis set level were chosen. Optimized structural parameters were used in the vibrational frequency, isotropic chemical shift and calculations of electronic properties. However, the frequency values computed at these levels contain known systematic errors [27]. Therefore, it is customary to scale down the calculated harmonic frequencies in order to improve the agreement with the experiment. In our study, we have followed two different scaling factors, i.e. 0.983 up to 1700 cm⁻¹ and 0.958 for greater than 1700 cm⁻¹ [28]. Analytic frequency calculations at the optimized geometry were done to confirm the optimized structures to be an energy minimum and to obtain the theoretical vibrational spectra. The stability of the optimized geometries was confirmed by frequency calculations, which give positive values for all the obtained frequencies. The total energy distribution (TED) was calculated by using the scaled quantum mechanics (SQM) method and PQS program [29,30] and the fundamental vibrational modes were characterized by their TED. The ¹H and ¹³C NMR chemical shifts of the compound were calculated using the gauge-invariant atomic orbital (GIAO) method. The GIAO approach allows the computation of absolute chemical shielding due to the electronic environment of the individual nuclei and this method, that one of the most common approaches for calculating nuclear magnetic shielding tensors, is often more accurate than those calculated with other approaches for the same basis set size. The time dependent DFT (TD-DFT) [24,31,32] is proved to be a powerful and effective computational tool for the study of ground and excited state properties by comparison to the available experimental data. Hence, we used TD-B3LYP to obtain wavelengths λ_{max} and compare with the experimental UV absorption spectra of NANO.

3.1. Prediction of Raman intensities

The Raman activities (SRa) calculated with Gaussian 03 program [24] converted to relative Raman intensities (IRa) using the following relationship derived from the intensity theory of Raman scattering [33,34]:

$$I_{i} = \frac{f(v_{0} - v_{i})^{4}S_{i}}{v_{i}[1 - \exp(-hcv_{i}/kT)]}$$

where v_0 is the laser exciting wavenumber in cm⁻¹ (in this work, we have used the excitation wavenumber $v_0 = 9398.5 \text{ cm}^{-1}$, which corresponds to the wavelength of 1064 nm of a Nd:YAG laser), v_i the vibrational wavenumber of the *i*th normal mode (cm⁻¹), while S_i is the Raman scattering activity of the normal mode v_i . f (is a constant equal to 10^{-12}) is a suitably chosen common normalization factor for all peak intensities. h, k, c and T are Planck and Boltzmann constants, speed of light and temperature in Kelvin, respectively. The simulation of calculated FT-Raman spectra was plotted using pure Lorentizian band shape with a bandwidth of Full Width and Half Maximum (FWHM) of 10 cm^{-1} .

4. Results and discussion

The calculated geometric parameters of NANO have been seen to be equal in size for *trans* and *cis*. Calculated energies and energy difference for two conformers of title molecule, determined by DFT/B3LYP/6-311++G(d,p) are presented in Table 1. The formation of hydrogen bonding between an amide group cause the structure of the conformers *trans* and *cis*. Using the energy of the lowest energy (*trans*) as reference point, the relative energy of the other conformers was as: $\Delta E = E_{\text{trans}} - E_{\text{cis}}$. From DFT calculations, the *trans* conformation was predicted to be 0.8723 kcal/mol smaller than *cis* conformer.

4.1. Geometrical structures

The optimized structures of two conformers of NANO are shown in Fig. 1 with numbering of the atoms. Table 2 compares the calculated bond lengths and angles for NANO with those of experimentally available from X-ray data for nicotinamide [12]. From the theoretical values we can find that most of the optimized bond lengths are slightly larger than the experimental values at the calculated values using B3LYP level, due to that the theoretical calculations belong to nicotinamide and isolated molecule in gaseous phase and the experimental results belong to molecule in solid state. The molecule of NANO, which have two substituents such as the oxygen atom and amide group ($CONH_2$), attached to a planar pyridine ring. Amide group and pyridine ring have nonplanar structures, therefore C1 point group symmetry is used for computation.

Several authors [35,36] have explained the changes in frequency or bond lengths of the C–H bond on substitution due to a change in the charge distribution on the carbon atom of the ring. Title

Та	bl	e 1

Calcı	ılated	l energies and	l energies	difference	for two con	formers	(trans & cis) of NANO	by DFT	/B3LYP/0	6-311+	+G(d,p)	
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Conformers	Energy		Energy difference	Dipole moment	
	Hartree	kcal/mol	Hartree	kcal/mol	Debye
Trans	-492.29830941	-308921.8660	0.0000	0.0000	6.9054
	-492.29091934	-308920.9937	0.0014	0.8723	2.4041

^a Energies between the most stable *trans* conformer and *cis* conformer.

molecule bond lengths for amide group have shown positive correlation for experimental values and literature. Worth mentioning differences are C–N bond lengths both pyridine ring and amide group. These lengths calculated bigger than experimental values. This enhancement could be due to the presence of the oxygen atom near the pyridine and amide group. In the ring part optimized geometry of the molecule shows very good agreement with experiment. For example, the CC bond lengths of the pyridine ring of nicotinamide were observed in the range of 1.384–1.388 Å [12] which calculated in the range 1.381–1.397 Å for *trans* structure (see Table 2). The optimized NH (amino group) bond length were calculated as 1.009 Å and 1.007 Å by DFT with 6-311++G(d,p) method for two structures. By comparing these values with experimental value of 1.000 and 1.015 Å for nicotinamide, it is observed that B3LYP estimates good.

The structural parameters are found to be similar for two structures (*trans* and *cis*) which are in good agreement with experimental values [12]. We can also enounce most of the bond angles and distances are almost the same for two conformers but we have assessed energy and general correlation *trans* structure could be the most stable structure.

The asymmetry of the pyridine ring is also evident from the negative and positive deviations from the normal value of 120° . This clearly shows that because of electronegative oxygen atom (O11), the C1–N6–C5 angle is the smallest value (118.0 and 118.6 for exp. and cal., respectively) in the pyridine ring. Also, for C4–C5–H10 bond angle is bigger than experimental values, this can be due to the presence of oxygen atom attached at pyridine ring in our title molecule. Therefore some bond angles near the oxygen atom are different experimental values. Also the optimized values of NANO are in very good agreement with the literature [12–19].

4.2. Vibrational spectra

The molecule of NANO consists of 16 atoms, so it has 42 normal vibrational modes. On the assumption of Cs symmetry the



Fig. 1. The theoretical geometric structures of NANO molecule for (a) *trans* and (b) *cis.*

numbers of vibration modes of the 42 fundamental vibrations will be distributed as 29A' + 13A''. The vibrations of the A' species are in plane and those of the A'' species are out of plane. The experimental wavenumbers tabulated in Table 3 are given together with the calculated wavenumbers for *trans* and *cis* of studied molecule. The resulting vibrational frequencies for the optimized geometries and the proposed vibrational assignments are given in Table 3. Modes are numbered from biggest to smallest frequency within each fundamental wavenumbers, ν . In the last column is given a

Table 2

Experimental and optimized bond lengths (Å) and bond angles (°) by using B3LYP/6-311++G(d,p).

Parameters		Cis	Trans
	X-ray ^a	B3LYP	B3LYP
Bond lengths (Å)			
C1-C2	1.384	1.386	1.386
C1-N6	1.341	1.374	1.371
C1-H8	1.074	1.080	1.080
C2-C3	1.388	1.398	1.397
C2-C12	1.492	1.508	1.508
C3-C4	1.385	1.394	1.391
C3-H7	1.092	1.082	1.081
C4–C5	1.384	1.379	1.381
C4-H9	1.082	1.083	1.083
C5-N6	1.338	1.368	1.371
C5-H10	1.077	1.080	1.080
N6-011	_	1.274	1.277
C12-013	1.230	1.217	1.218
C12-N14	1.337	1.369	1.367
N14-H15	1 015	1 009	1 009
N14-H16	1.000	1.007	1.007
Bond Angles (°)			
C2-C1-N6	119.5	121.6	121.7
C2-C1-H8	_	123.3	125.2
N6-C1-H8	_	115.1	113.1
(1-(2-(3	_	119.9	119.8
C1 - C2 - C12	1179	116.3	121.9
$C_{3}^{-}C_{2}^{-}C_{12}^{-}$	124.8	123.7	118 3
$C_2 - C_3 - C_4$	123.1	118.0	118.2
C2-C3-H7	118.4	121.5	119.5
C4-C3-H7	122.0	120.4	122.3
C3-C4-C5	118 3	120.6	120.7
C3-C4-H9	121 5	121.0	121.0
C5-C4-H9	120.2	118.4	118.4
C4-C5-N6	123.1	121.2	121.1
C4-C5-H10	120.4	125.0	125.0
N6-C5-H10	_	113.8	113.9
C1-N6-C5	118.0	118.6	118.6
C1-N6-O11	-	120.9	120.5
C5-N6-011	_	120.5	120.0
$C_{2}-C_{12}-O_{13}$	1197	121.7	121.1
C2-C12-N14	117.8	115.7	116.3
013-C12-N14	_	122.6	122.6
C12-N14-H15	118.5	116.6	116.7
C12-N14-H16	121.3	121.4	121.9
H15-N14-H16	_	117.6	1177
Selected dihedral angles	(°)		
C3-C2-C12-O13	· ´ _	24.5	-23.5
C2-C12-N14-H16	_	18.7	-17.7
013-C12-N14-H15	_	-6.3	5.3
013-C12-N14-H16	_	-162.3	163.1

^a X-ray data from Ref [12].

Table 3

 $Comparison of the calculated harmonic wavenumbers and experimental (FT-IR and FT-Raman) wavenumbers (cm^{-1}) using by B3LYP method 6-311++G(d,p) basis set for two conformers (trans & cis) of NANO.$

Modes no.	. Experimental		Trans structure		Cis structure		TED (≥10%)
	FT-IR	FT-Raman	Unscaled freq.	Scaled freq. ^a	Unscaled freq.	Scaled freq. ^a	
ν_1	3526		3711	3555	3710	3555	νNH ₂ (100) asym.
ν_2	3432		3586	3436	3585	3434	νNH ₂ (100) sym.
ν_3	3182		3241	3105	3250	3114	νCH_{ring} (96)
ν_4			3234	3098	3242	3106	νCH_{ring} (99)
ν_5		3091	3221	3086	3205	3070	νCH_{ring} (99)
ν_6		3065	3194	3060	3190	3056	νCH_{ring} (98)
V7	1684	1719	1756	1682	1758	1684	$\nu C = 0$ (78)
ν_8		1642	1645	1617	1647	1619	$\nu CC (56) + \beta CNH (10)$
Vg		1609	1624	1596	1620	1593	$\rho \text{NH}_2(57) + \beta \text{C} - \text{NH}_2(30)$
ν_{10}	1568	1594	1588	1561	1583	1556	$\nu CC (48) + \nu CN (15) + \beta CCH (14)$
V ₁₁		1509	1512	1487	1510	1484	$\nu CC(32) + \beta CCH(30) + \beta CNH(16)$
V12	1436	1442	1460	1435	1462	1437	ν CC (24) + β CNH (21) + β CCH (17)
V13			1369	1345	1364	1341	vCH (27) + v CC (20) + v NO (12)
V14	1304		1334	1312	1331	1308	β CCH (48) + ν NO (19) + β CNH (16)
V15			1298	1276	1304	1282	$\nu NO(34) + \beta CCH(26)$
V16	1236	1231	1253	1232	1257	1236	$\nu CN(55) + \nu CC(35)$
V17		1141	1183	1163	1186	1166	β CCH (76) + ν CC (14)
V19	1118	1118	1135	1116	1128	1108	$\nu CC(25) + \nu CN(10) + \beta CNH(23) + \beta CCH(13)$
V10			1107	1089	1114	1095	$\nu CC(33) + \beta CCH(33)$
Vao		1031	1087	1068	1088 1070		$\nu CN(27) + \beta CNH(26) + \beta CNC(18)$
V21	1022	1001	1025	1008	1024	1007	$\nu CC (25) + \nu CN (17) + \beta CCC (19) + \beta CCH (18)$
V21	1022		983	966	966 950		τ CHCH (59) + τ CCCH (19) + τ CNCH (10)
V22	938	936	958	941	955	939	$vC = NH_2(35) + vC = CONH(14) + vNO(11)$
V23	550	550	907	891	890	875	$\tau CCCH (46) + \tau CHCH (26) + \tau COCH (12)$
V24			870	855	885	870	$\tau CCCH (54) + \tau CHNO (17)$
V25			810	796	800	787	$\tau CCCH(32) + \tau CNCH(22) + \tau CNCO(15)$
V26	748	746	764	751	760	747	$\tau CCCN (30) + \tau CNCH (16) + \tau CCCH (12) + \tau CCCO (10)$
V27	740	740	745	732	7/3	730	$\mu CC(22) + \mu CN(16) + \beta CCC(13) + \beta CCN(10)$
1/28	142	653	678	666	674	663	$\tau CCCN (31) + \tau CCCH (27) + \tau CCCC (15) + \tau CNCH (15)$
1/29	648	033	640	630	628	617	BCCO(23) + BCNO(21) + BCCC(10)
V30	040		590	580	594	584	$\tau CONH(29) + \tau CCNH(22)$
V31	554	557	560	551	567	558	$BCCC(18) + BCNC(17) + \tau CNO(13) + BCCN(10)$
V32	554	337	548	520	546	527	$\beta CCC(18) + \beta CCC(17) + \beta CCC(13) + \beta CCN(10)$ $\beta CNO(22) + \beta CCN(15) + \beta CCC(14) + \tau CCNH(12)$
V33			505	106	502	405	$\beta CNO(23) + \beta CCN(13) + \beta CCCC(14) + \beta CCCNI(13)$
V34			303	490	JUJ 4EE	495	$\beta CNO(21) + i CCON(15) + i CCCC(15)$
V35	40.4		444	450	455	447	$\beta C(N) (19) + \ell C(C(N) (15) + \ell C(C(C) (10))$
V36	404	277	403	390	405	398	$p_{\text{CCN}}(22) + t_{\text{CCCN}}(10) + p_{\text{CCC}}(10)$
V37		3//	300	300	303	330	VCC(30) + pCON(13) + pCCC(11)
v_{38}			353	347	352	346	$\tau_{\rm CONH}(40) + \tau_{\rm CCNH}(37)$
v_{39}			213	210	211	207	$\tau \text{LCNU}(37) + \tau \text{LCNU}(20) + \tau \text{LCNH}(13)$
v_{40}			186	183	185	182	βCC_{ring} of CONH (93)
V41		144	147	144	150	147	$\tau \text{LCCN}(34) + \tau \text{CCCC}(19) + \tau \text{CCCH}(16)$
V42		69	56	55	55	54	τ CCCN (49) + τ CCCO (46)

TED: total energy distribution; ν stretching; β bending; ρ scissoring; τ torsion.

^a Wavenumbers in the ranges from 4000 to 1700 cm⁻¹ and lower than 1700 cm⁻¹ are scaled with 0.958 and 0.983 for B3LYP/6-311++G(d,p) basis set, respectively [28].

detailed description of the normal modes based on the total energy distribution (TED).

4.2.1. NH₂ vibrations

Calculations were made for free molecule in vacuum, while experiments were performed for solid samples, so there are disagreements between calculated and observed vibrational wavenumbers. All frequencies are calculated, however some of them are not observed in the FT-IR and FT-Raman spectra.

Fig. 2 presents the experimental and calculated Infrared and Raman spectra. The calculated IR and Raman spectra where the calculated intensity is plotted against the harmonic vibrational frequencies were drawn for comparison.

In Table 3 there is great mixing of the ring vibrational modes and between the ring and substituent modes. The descriptions of the modes are very complex because of the low symmetry of the studied molecule. Especially, bending modes and torsion modes are the most difficult to assign due to mixing with the ring modes. But there are some strong frequencies useful to characterize in the IR and Raman spectra. There are stretching vibrational modes (NH, CH, CO, CC and CN), bending modes (CNH, CCH, CCC, CCN, CNO) and torsion modes for title molecule.

The title molecule is a type of amino pyridine which is comprised of carbonyl and amino groups. The molecule under investigation possesses only one NH₂ group and hence expects one symmetric and one asymmetric N-H stretching vibrations in NH₂ group. The asymmetric stretching for the NH₂, CH₂ and CH₃ has magnitude higher than the symmetric stretching. In all the primary aromatic amines the N-H stretching frequency occurs in the region 3300–3500 cm⁻¹ [37]. In this region, the bands are not affected appreciably by the nature of the substituent. N-H asymmetric and symmetric stretching vibrations were assigned at 3526 and 3432 cm⁻¹, respectively in NH₂ group. These vibrations were calculated at 3555 and 3436 cm⁻¹ for *trans* form of title molecule. As expected these two modes are pure stretching modes as it is evident from TED column, they are almost contributing 100%. These observations agree well with the earlier reports [38]. For NH₂ scissoring modes are suggested in the region $1590-1650 \text{ cm}^{-1}$ [37-41]. The NH₂ scissoring mode is observed at 1609 cm⁻¹ in the FT-Raman and calculated 1596 cm⁻¹ using B3LYP method. The computed NH₂ scissoring vibration is in excellent agreement with the recorded



Fig. 2. Calculated and experimental infrared and Raman spectra of NANO molecule.

spectral data observed by Kumar et al. [23]. The C-NH₂ stretching mode were observed at 938 cm⁻¹ in FT-IR (936 cm⁻¹ in FT-Raman), calculated 941 and 939 cm⁻¹ for *trans* and *cis*, respectively.

4.2.2. C-H vibrations

The heteroaromatic structure shows the presence of the C–H stretching vibrations in the 3000–3100 cm⁻¹ range which is the characteristic region for the ready identification of C–H stretching vibrations [42]. In this region, the bands are not affected appreciably by the nature of the substituents. Accordingly, in the present study, the four adjacent hydrogen atoms left around the ring the NANO give rise four C–H stretching modes ($\nu_3 - \nu_6$), four C–H in plane bending (ν_{14} , ν_{15} , ν_{17} , ν_{19}) and four C–H out-of plane bending (ν_{22} , $\nu_{24} - \nu_{26}$) vibrations which corresponds to stretching modes of C5–H, C1–H, C3–H and C4–H units. The vibrations ($\nu_3 - \nu_6$) were assigned to as an aromatic C–H stretching in this region.

In aromatic compounds, the C–H in plane bending frequencies appear in the range of $1000-1300 \text{ cm}^{-1}$ and the C-H out-of-plane bending vibration in the range 750–1000 cm⁻¹ [43–48]. According to TED, bending modes which were assigned BCCH were calculated in the range 1089–1312 cm⁻¹ with in very good agreement experimental values. The C-H in plane modes were assigned in FT-IR and FT-Raman spectra at 1304 and 1141 cm⁻¹, respectively. The C–H out-of-plane bends are assigned from 751 to 907 cm⁻¹. Both the in-plane and out-of-plane bending vibrations are described as mixed modes. This band seems to be diagnostic for the pyridine ring since a similar band of medium intensity has been observed at 1250 cm⁻¹ in the FT-IR spectrum of 5-bromo-2-nitropyridine [27]. The ring stretching frequencies of the pyridine molecule were observed in the range 990–1585 cm⁻¹ by Wilmshurst and Bernstein [49]. Kumar et al. [23] have predicted this band in the range $1055-1630 \text{ cm}^{-1}$ for the nicotinamide molecule. Similarly, Gambi and Ghersetti [50] observed the six ring stretching frequencies in the range $1015-1605 \text{ cm}^{-1}$ for pyridine N-oxide and calculated in the range of 955-1645 cm⁻¹ for NANO.

4.2.3. C=O vibrations

The interaction of carbonyl group with amide group did not produce such a drastic and characteristic changes in the frequency of C=O stretch as did by interaction of N-H stretch. The carbon-oxygen double bond is formed by $p_{\pi}-p_{\pi}$ between carbon and oxygen. Because of the different electro-negativities of carbon and oxygen atoms, the bonding electrons are not equally distributed between the two atoms. The lone pair of electrons on oxygen also determines the nature of the carbonyl group. The position of the C=O stretching vibration is very sensitive to various factors such as the physical state, electronic effects by substituents, ring strains [51]. Normally carbonyl group vibrations occur in the region 1850–1600 cm⁻¹ [52]. The C=O stretching vibrations of xanthine are observed at 1693, 1672 cm^{-1} in FT-IR and 1689 cm^{-1} in the FT-Raman spectrum [53]. The C=O stretching mode was calculated as a strong IR and medium Raman intensities at 1755 cm⁻¹ for NA and its N-oxide by Kumar et al. [23]. In the present work, the ν (C=O) mode is observed at 1684 cm⁻¹ with very strong IR intensity in FT-IR and 1719 cm⁻¹ with FT-Raman. That vibration was calculated at 1682 cm⁻¹ which is in very good agreement with experimental value and above the other authors' works [23,51-53].

4.2.4. C-C vibrations

The ring stretching vibrations are very much important in the spectrum of the aromatic rings. The ring carbon–carbon stretching vibrations appear in the region $1400–1650 \text{ cm}^{-1}$ in benzene derivates [44–48]. In general, the bands are of variable intensity and are observed at 1625–1590, 1590–1575, 1540–1470, 1465–1430

and 1380–1280 cm⁻¹ from the frequency ranges given by Varsanyi [48] for the five bands in this region. It follows from Table 3, the C–C aromatic stretching mode, known as semicircle stretching modes calculated at 1437–1619 cm⁻¹ (extensive region) is in excellent agreement with experimental values. The most intense modes of these v_8 , $v_{10}-v_{12}$, showed excellent agreement with experimental data. This is also supported by the literature data [54–60]. The CC bending modes assigned β CCC, β CCN and β CCO using by TED in Table 3 and numbered v_{28} , v_{30} , v_{32} and v_{33} have weak intensity (ca. 15–20%) and as mixed modes.

The ring-breathing mode at 848 cm^{-1} coincides satisfactorily with the very strong FT-Raman band at 861 cm^{-1} [55]. We can also see in Table 3 vCC (-CONH₂) stretching modes assigned according to TED calculations as modes v_{23} and v_{37} . These modes were observed at 936–377 cm⁻¹ FT-Raman and 938 cm⁻¹ FT-IR and calculated 941 and 360 cm⁻¹ respectively.

4.2.5. C-N vibrations

The identification of C-N vibrations is a very difficult task, since the mixing of several bands is possible in this region. Silverstein et al. [44] assigned C-N stretching absorption in the region 1382–1266 cm⁻¹ for aromatic amines. In benzamide the band observed at 1368 cm⁻¹ is assigned due to C–N stretching [61]. In benzotriazole, the C-N stretching bands are found to be present at 1382 and 1307 cm⁻¹. Sundaraganesan et al. [56] observed at 1485 and 1395 cm⁻¹ in FT-IR spectrum have been assigned to C=N, C-N stretching vibrations, respectively. The theoretically computed values of C=N and C-N stretching vibrations also fall in the region 1464–1254 cm⁻¹ [56]. Kumar et al. [23] have calculated the frequency for the CN stretching mode lies in the region 1350–1370 cm⁻¹ for both NA and its N-oxide with strong IR intensity. In our present work, C=N and C-N stretching vibrations are observed at 742-1568 cm⁻¹ in FT-IR and at 936-1594 cm⁻¹ in FT-Raman spectra, respectively. This band has been calculated at 732–1561 cm⁻¹ by using B3LYP method in very good agreement with experimental values. It is worth mentioning that, v_{16} and v_{23} modes the highest contribute for this vibration at about 1231 cm^{-1} .

4.2.6. N–O vibrations

The most characteristic bands in the spectra of nitro compounds are due to NO₂ stretching vibrations, which are the most useful group wavenumbers, not only because of their spectral position but also for their strong intensity [62]. According to TED, N–O stretching mode is observed at 938 and 936 cm⁻¹ FT-IR and FT-Raman (ν_{23} mode), respectively. It should be emphasized that the wavenumbers calculated by the B3LYP/6-311++G(d,p) method for the modes ν_{23} (941 cm⁻¹) and ν_{15} (1276 cm⁻¹) with a major contribution (34%) from the N–O stretching vibration. N–O stretching mode is not observed in our study (ν_{15}), Kumar et al. [23] have observed at 1279 cm⁻¹ IR spectrum. β CNO modes are assigned wide range in the fingerprint region at 350–650 cm⁻¹ shown in last column of Table 3.

Empirical assignments of vibrational modes for peaks in the fingerprint region are difficult. In the wavenumber region of 600–1660 cm⁻¹, the spectrum observed in the experiments closely resembles the calculated spectrum, except for differences in details. These wavenumbers in the same range are in reasonable agreement with experimental results for nicotinamide N-oxide. Torsion modes, other not mentioned vibrations and the detailed assignment of the remaining bands in the vibrational spectra of NANO are shown in Table 3.

4.3. NMR spectra and calculations

The experimental ¹H and ¹³C NMR spectra of NANO are shown in Fig. 3a and b. The recorded and calculated ¹H and ¹³C

Table 4

Experimental and theoretical, ¹H and ¹³C NMR isotropic chemical shifts (with respect to TMS) of NANO by DFT (B3LYP/6-311++G(d,p)) method.

Atoms	Carbon		Atoms	Hydrogen		
	Exp.	B3LYP		Exp.	B3LYP	
C(1)	138.42	144.29	H(7)	7.733	7.761	
C(2)	134.17	139.05	H(8)	8.570	8.484	
C(3)	124.98	129.02	H(9)	7.493	7.549	
C(4)	127.07	131.46	H(10)	8.319	8.359	
C(5)	141.40	148.97	H(15)	4.102	5.139	
C(12)	164.77	170.27	H(16)	4.102	5.672	

chemical shifts in the dimethylsulfoxide (DMSO) solvent are gathered in Table 4. The atom positions were numbered according to Fig. 1. The isotropic chemical shifts are frequently used as an aid in identification of reactive ionic species. It is recognized that accurate predictions of molecular geometries are essential for reliable calculations of magnetic properties. Therefore, full geometry optimization of present compound was performed at the gradient corrected DFT/B3LYP/6-311++G(d,p) method. The isotropic shielding values were used to calculate the isotropic chemical shifts δ with respect to tetramethylsilane (TMS), $\delta^{\text{TMS}}(\delta_{\text{iso}}^X = \sigma_{\text{iso}}^{\text{TMS}} - \sigma_{\text{iso}}^X)$. The values of $\sigma_{\text{iso}}^{\text{TMS}}$ are 182.46 ppm and 31.88 ppm for C and H NMR spectra, respectively.

It is clear from Table 4 that the agreement with experimental data is good, even the trends in relative values are well reproduced. As in Fig. 1, the studied molecule shows six different carbon atoms. The range of the ¹³C NMR chemical shifts for a typical organic molecule is usually >100 ppm [63-65] and the accuracy ensures reliable interpretation of spectroscopic parameters. In the present paper, the ¹³C NMR chemical shifts for the title molecule are >100 ppm, as they would be expected. The largest differentiation in ¹³C chemical shifts is observed amounting to about 8 ppm. The pyridine ring include nitrogen atom which shows electronegative property therefore the chemical shift values of C1 (138.42 ppm) and C5 (141.40 ppm) are bigger than C2 (134.17 ppm), C3 (124.98 ppm) and C4 (127.07 ppm). They have been calculated 144.29 and 148.97 ppm for C1 and C5, and 139.05, 129.02 and 131.46 ppm for C2, C3 and C4 respectively (in Table 4). Similarly, other carbon peak (which bonded oxygen and nitrogen atoms, O=C-NH₂) is observed at 164.77 ppm and calculated at 170.27. That is too high, because of electronegative oxygen and nitrogen atoms.

The studied molecule has four hydrogen atoms in the ring, two hydrogen atoms attached to the nitrogen atom of amino group. Signals for protons were observed at 7.493–8.570 ppm for ring hydrogen atoms. But the chemical shifts obtained and calculated for the hydrogen atoms of amino groups are quite low. Two signals result from NH₂ group (H15 and H16) are observed combined one peak as a coupling (4.102 ppm) which calculated 5.139 and 5.672 ppm.

4.4. UV spectrum and electronic properties

Ultraviolet spectra analysis of NANO have been investigated by theoretical calculation. The UV spectrum of NANO is shown in Fig. 4, was measured in water solution and the density of states (DOS) with HOMO and LUMO diagrams for NANO can be seen in Fig. 5. Calculations of the molecular orbital geometry show that the visible absorption maxima of this molecule correspond to the electron transition between frontier orbitals such as translation from HOMO to LUMO. The HOMO energy characterizes the ability of electron giving, the LUMO characterizes the ability of electron accepting, and the gap between HOMO and LUMO characterizes the molecular chemical stability [66]. The energy gap between the



Fig. 3. (a) ¹³C NMR spectrum of NANO. (b) ¹H NMR spectrum of NANO.

HOMOs and LUMOs is a critical parameter in determining molecular electrical transport properties because it is a measure of electron conductivity. These results are illustrated in Fig. 6. Surfaces for the frontier orbitals were drawn to understand the bonding scheme of present compound. We examine the two important molecular orbitals (MO) for NANO: the highest occupied MOs and the lowest unoccupied MOs which we denote HOMO and LUMO, respectively. According to Fig. 6, the HOMO of free NANO presents a charge density localized mainly on the pyridine ring and N=O double bond and LUMO is characterized by a charge distribution on all pyridine ring, C=O double bond and NH₂ group. Absorption maxima (λ_{max} ; nm) for lower-lying singlet states of the molecule have been calculated by DFT/B3LYP method. The calculated visible absorption maxima of λ which are functions of the electron availability were presented in Table 5. As can be seen from the Table 5, the calculated absorption maxima values have been found to be 236.51, 223.32 nm for in water solution at DFT/B3LYP/6-311++G(d,p) method. The spectrum shows two broad bands, a long wavelength band covering the 280–240 nm region and a short wavelength one covering the 240–200 nm region. Each of these bands corresponds to a number of overlapping transitions. These absorption maxima values are observed 260.42 and 217.67 nm those show agreement in theoretical values. The deviation between experiment and theory may be resulted from solvent effects. Solvent make the chemical

Table 5

Experimental and calculated (*cis*/6-311++(d,p)) wavelengths λ (nm), excitation energies (eV), oscillator strengths (*f*), in water solution.

Exp.	Calculated			Major contribution (\geq 10%)
$\lambda \left(nm ight)$	λ (nm)	<i>E</i> (eV)	f	
217.67 260.42	223.3 236.5	5.55361 5.24668	0.0068 0.2189	$\begin{array}{l} H \rightarrow L + 2 \ (64\%) \\ H - 1 \rightarrow L + 2 \ (24\%), \ H \rightarrow L + 4 \ (47\%) \end{array}$

H, HOMO; L, LUMO.



Fig. 4. The theoretical and experimental UV spectra of NANO.

environment of molecule in the simulation become very complex. The maximum absorption wavelength corresponds to the electronic transition from the highest occupied molecular orbital HOMO to lowest unoccupied molecular orbital LUMO + 2 with 64% contribution. All the observed transitions are π - π * ones. For nicotinamide absorption wavelengths were observed 262.0 and 214.0 nm [67]. All assignments agree well with the values available in the literature and experimental values.



Fig. 5. The density of states (DOS) with HOMO and LUMO diagrams of NANO.



Fig. 6. The frontier molecular orbitals of NANO by using B3LYP.

5. Conclusions

In this study, the spectroscopic properties of title compound were examined by NMR. UV. FT-IR and FT-Raman techniques. Ouantum chemical calculations help us to identify the structural and symmetry properties of the molecule. A comparison of the result of experimental and theoretical study gave us a full description of the geometry and vibrational properties of title molecule. The chemical shifts were compared with experimental data, showing a very good agreement both for ¹³C and ¹H. ¹H calculations gave a slightly better coefficient and lower standard error than for ¹³C chemical shifts although two chemical shifts are not observed in the ¹H spectra. The electronic properties were also calculated and experimental electronic spectrum was recorded (200-800 nm) with help of UV-Vis spectrometer. The calculated geometric parameters and vibrational frequencies obtained with DFT method are in well agreement with the experimental values obtained for the investigated molecule. In summary, a very complete characterization of studied compound was given in this paper. It has been shown; moreover, hybrid density functional calculations are able to supply a variety of very reliable molecular properties.

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