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SPECTROSCOPIC PROPERTIES OF CYTOSINE: A COMPUTATIONAL INVESTIGATION

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Abstract

IR, NMR and UV-Visible properties of cytosine (6-amino-1, 2-dihydropyrimidin-2-one, $C_4H_5N_3O$) have been investigated computationally using DFT/B3LYP/6-311+G(d,p) level of theory. The calculated of ^1H NMR chemical shift spectra of cytosine shows good agreement with experimental data. Analysis of the calculated vibrational spectra shows four distinct IR active mode of vibrations assigned as C7 = O stretching, symmetric -NH_2, free -N_9H and anti-symmetric -NH_2 vibration. It is noticeable that the IR spectrum obtained computationally in the work of cytosine agrees well with the experimental results. The electronic and optical properties were also calculated by time dependent-density functional theory (TD-DFT). A good agreement is obtained for the calculated optical absorption energy with the experimental value.

***Keywords*:** Cytosine, TD-DFT, IR, NMR, Electronic properties, Optical absorption

Introduction

Cytosine is a pyrimidine base that is a fundamental unit of nucleic acids. The deamination of cytosine alone is apparent and the nucleotide of cytosine is the prime mutagenic nucleotide in leukaemia and cancer. Cytosine encodes the genetic information of all organisms in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Szyc et al., 2010; Samoyloya et al., 2005; Canuel et al., 2005). It helps to stabilize the nucleic acid portion of these molecules. These nucleobases absorb UV light strongly and act as a primary chromophore of DNA (Pecourt et al., 2001; Kang et al., 2002; Gustavsson et al., 2010). Photostability of DNA bases is the essential criteria for the preservation of genetic information of DNA bases. As nucleobases absorb UV light strongly, the knowledge of the electronic properties and excited state lifetimes are of paramount importance for understanding the UV radiation induced DNA damage (Ullrich et al., 2004; Ho et al.,

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2011; Kwok et al., 2008; Lange et al., 2009). Numerous experimental and theoretical studies on DNA and DNA bases have been performed to understand structural, electronic properties and excited state lifetimes (Middleton et al., 2009; Ghane et al., 2012; Varsano et al., 2006; Sobolewski et al., 2002; Alauddin et al., 2016). All the calculations have been focused on the ground state, higher electronic singlet and triplet states of the neutral molecules.

In this work, we have performed a theoretical investigation of spectroscopic properties of cytosine with DFT/B3LYP level of theory and compared with the available experimental data. The optical properties have been calculated at the excited states using TD-DFT method.

Computational details

All the calculations have been performed using the hybrid approach of B3LYP as implemented in the quantum chemistry package G09 (Frisch et al., 2009). We have used 6-311+G(d,p) basis set in order to calculate the structure, IR, ^{1}H NMR and UV–Vis spectra of cytosine in the ground state at the B3LYP level of theory. The optimized structure of cytosine in the ground state was verified by calculating vibrational frequencies. There are several methods to calculate singlet excited states such as symmetry adapted cluster method/configuration interaction (SAC/CI) method (Honda et al., 2002), configuration interaction singles (CIS) method (Drougas et al., 2006) and time dependent density functional theory (TD-DFT) (Varsano et al., 2006) calculations. Among them, the TD-DFT is new approach for studying electronic excitations which gives more accurate results (Varsano et al., 2006). Therefore, excited state calculations have been done by TD-DFT method employing B3LYP function and 6-311+G(d,P) basis set to study electronic and optical properties of cytosine. To avoid complexity, we have calculated only six lowest excited states of cytosine.

Results and Discussion

Thermodynamic parameters

The optimized structure of cytosine by DFT/ B3LYP is shown in Fig.1. For optimizing minimum energy structure of cytosine, first we have done intrinsic reaction coordinate (IRC) calculation with HF/6-31G. This minimum energy structure was re-optimized using DFT/B3LYP/6-311+G(d,p) level of theory. The final geometry corresponds to true energy minima as revealed by the lack of imaginary

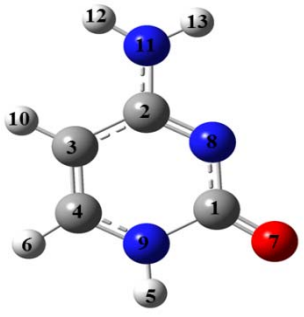

Fig. 1. Optimized Structure of Cytosine.

frequencies in the vibrational mode calculation.

Thermodynamical properties such as energy, specific heat capacity, entropy, enthalpy and dipole moment were studied theoretically. The total energy (SCF), zero-point vibrational energy (ZPVE), rotational constants, specific heat capacity (C_v) at constant volume, entropy (S) and dipole moment (μ) of cytosine are provided in the Table 1.

Table 1. Calculated thermodynamic parameters of cytosine.

Molecule	SCF* energy/ Hatree	Z PV** energy/ kcal $mole^{-1}$	Rotational constant /GHz	Specific heat(Cv)/cal mol^{-1} K^{-1}	Entropy (S)/cal mol^{-1} K^{-1}	Dipole moment μ/Debye
	-395.0530	61.1535	3.86647	23.922	79.281	6.8031
Cytosine			2.02027			
			1.32693			

*SCF = Self-Consistent Field and **ZPV = Zero Point Vibrational

Thermodynamics parameters can be changed depending upon the level of computational theory. To get more accurate results very high level computational calculations such as Møller-Plesset perturbation theory (MP2) is required.

^{1}H NMR spectral analysis

The NMR spectroscopy is one of the most powerful techniques for the structural analysis of organic compounds. It is well-established that the combined use of experimental NMR spectroscopic technique and computational simulation methods give a powerful gadget to interpret and predict the structure of organic compound, even for the structures of large biomolecules. To get theoretical ^{1}H NMR results of thymine, first the full geometry optimization is carried out with the B3LYP/6-311G+(d,p) in gas phase. After optimization, ^{1}H NMR calculations of the studied compounds is calculated by using the gauge-including atomic orbital (GIAO) method. The chemical shifts of the studied compounds are reported in ppm relative to tetramethylsilane (TMS) for ^{1}H NMR spectrum as presented in Table 2.

Table 2. Calculated ^{1}H NMR data of cytosine.

Molecule	Protons with number	Chemical Shift (Calc.)/ppm	Chemical Shift (Expt.)/ppm
cytosine	H(6)	7.00	7.486
	H(5)	6.48	-
	H(10)	5.05	5.98
	H(13)	4.45	2.98
	H(12)	3.78	2.97

H(12) and H(13) protons are highly shielded due to the lone pair of electrons on nitrogen atom. On the other hand, H(5) and H(6) protons are deshielded. the experimental ^{1}H NMR spectra of cytosine in DSS solvent are shown in Table 2 (Human, Metabolome Database, HMDB). The little difference of chemical shifts between theoretical and experimental results is due to the solvent effect.

Vibrational spectral analysis

The assignments of the vibrational frequencies are shown in Table 3. In the calculated spectrum of cytosine, four IR active mode of vibrations were found at 1700, 3420, 3445 and 3551 cm^{-1} which are assigned as C7=O stretching vibration, symmetric -NH_2 vibration, free -N_9H vibration and anti-symmetric -NH_2 vibration, respectively. Four IR absorption bands at 1710, 3400, 3465 and 3531 cm^{-1} were observed experimentally which are assigned as C7=O stretching vibration, symmetric -NH_2 vibration, free -N_9H vibration and anti-symmetric -NH_2 vibration, respectively (Alauddin et al., 2015). Calculated IR spectrum of C7=O stretching vibration, symmetric -NH_2 vibration, free -N_9H vibration and anti-symmetric -NH_2 vibration, respectively have well agreement with the experimental results.

Table 3. IR calculated and experimental frequencies of cytosine.

Molecule	IR calculated* frequencies/cm^{-1}	IR experimental frequencies/cm^{-1}	IR active mode of vibration
Cytosine	1700	1710	C7=O stretching vibration
	3420	3400	Symmetric -NH_2 vibration
	3445	3465	Free -N_9H vibration
	3551	3531	Anti-symmetric -NH_2 vibration

*Calculated values were corrected by multiplying the frequency factor, f = 0.950

Electronic absorption and optical properties

We have used TD-DFT method for the calculations of excited state properties of cytosine for excitation from the ground state to the higher electronic states. For this purpose, we have calculated six lowest singlet electronic states from the ground state of cytosine. The computed electronic values such as absorption wavelength (λ), excitation energies (E) and oscillator strengths (f) are tabulated in Table 4. The major transition with 0.0414 oscillator strength for cytosine molecule is at 267 nm (4.64 eV).

Table 4. Calculated wavelengths λ (nm), excitation energies E(eV) and oscillator strength (f) and major electronic transitions of cytosine.

Molecule	λ (nm)	E (eV)	f	Major transitions*
Cytosine	267	4.64	0.0414	H→L (96%)
	260	4.77	0.0010	H-3→L (14%) & H-1→L (85%)
	231	5.37	0.0043	H→L+1 (95%) & H→L+2 (3%)
	228	5.44	0.0893	H-2→L (87%), H-2→L+3 (3%) & H→L+3(7%)

*H = HOMO & L = LUMO

This transition occurs from HOMO to LUMO (96%) (n→π*). The second major transition occurs at 260 nm (4.77 eV) from HOMO-3 to LUMO (14%) and HOMO-1 to LUMO (85%). Another strong transition occurs at 228 nm (5.44 eV) from HOMO-2→LUMO (87%), HOMO-2→LUMO+3 (3%) & HOMO→LUMO+3 (7%). However, experimentally we could not scan smoothly from 200 nm to 100 nm region in UV-Visible spectrophotometer due to the absorption of solvent. The HOMO and LUMO that participate in the lowest electronic transitions of cytosine are presented in Fig. 2 as a Jablonski diagram.

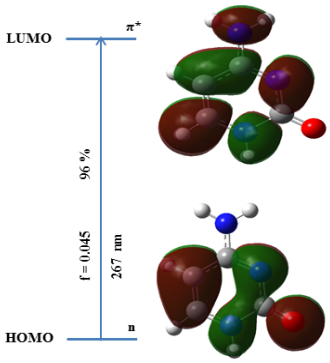

Fig. 2. Jablonski diagram and singlet excited-state transition from HOMO → LUMO of cytosine.

Theoretical and experimental UV-Visible absorption spectrum of cytosine are shown in Fig. 3. From the figure, it is very clear that cytosine is a colorless compound as it does not absorb any visible light. The experimental UV-Visible absorption spectrum of cytosine (*Photochem Cad*) is at 264 nm which shows that our calculated spectrum is in very good agreement with experiment. Little red shift from 264 nm to 267 nm is due to the solvent used to measure UV-Visible absorption spectrum of cytosine.

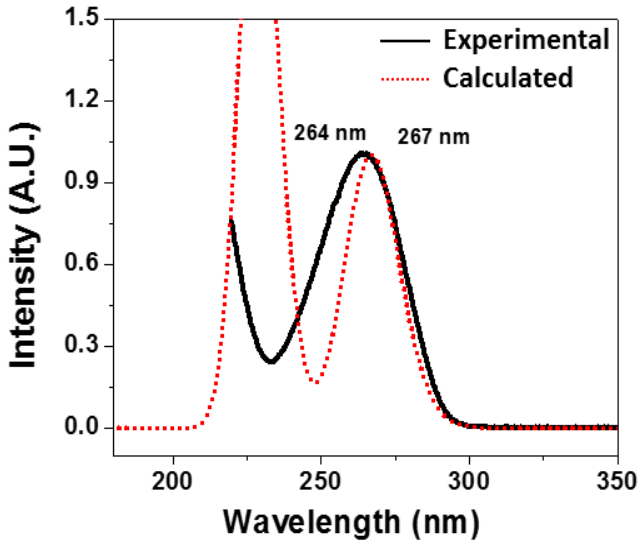

Fig. 3. Calculated and experimental UV-Visible absorption spectrum of cytosine.

The frontier molecular orbitals (FMO) play an important role in the optical and electronic properties. In the present study, the FMO energy gap is calculated as the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) as follows:

$HOMO_{energy}$ (B3LYP) = -6.65 eV,

$LUMO_{energy}$ (B3LYP) = -1.35 eV

HOMO–LUMO energy gap (B3LYP) = 5.30 eV

Therefore, the energy gap of FMO of cytosine is 5.30 eV.

Conclusion

The molecular geometry optimization, thermodynamic properties and spectroscopic properties of cytosine molecule were studied theoretically. The calculated ^{1}H NMR

chemical shift spectra are well accord with the experimental data. The calculated IR active mode of vibrations and their assignments are in well agreement with the available experimental data. The investigation of electronic properties shows that the HOMO-LUMO energy band gap of cytosine at B3LYP level is 5.30 eV. The calculation of optical properties at excited states shows that the major transition (from HOMO to LUMO (96%) ($n\rightarrow\pi^*$)) occurs at 267 nm (4.64 eV). Our theoretical UV-Visible absorption spectrum suggests that cytosine is a white compound as it does not absorb any visible light which is in good agreement with experiment.

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