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Structural, Electronic, Topological and Vibrational Properties of Isomers of Thymidine - A Study Combining Spectroscopic Data with DFT Calculations



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ABSTRACT

In this work, the structural, electronic, topological and vibrational properties of six Cis and Trans isomers of Thymidine (THY) in gas and aqueous solution phases were studied combining the hybrid B3LYP/6-31G* method with the Polarized Continuum Model (PCM) model and the available experimental infrared, Raman, ¹H-NMR, ¹³C-NMR and UV spectra. Hence, theoretical molecular structures of two Cis isomers and one Trans isomers of THY were determined in the two media for first time. The population analyses suggest the presence in solution of a Trans isomer and of two Cis isomers in accordance with the experimental structures reported by X-ray diffraction by different authors. The study of the charges show clearly the differences between the Cis and Trans isomers where the charges on the O atoms belonging to the ribose rings in all the structures, on the N atoms belonging to the glycosidic bond and on the two chiral C atoms show the higher modifications. The Molecular Electrostatic Potential (MEP) surface mapped evidence clearly that the group's acceptors of H bonds in all the isomers are the two C=O groups of the pyrimidine rings while the OH groups of the ribose rings and the NH group of the pyrimidine rings are donors of H bonds showing their surfaces colorations red and blue in those sites, respectively. Natural Bond Orbital (NBO) and Quantum Atoms in Molecules (QAIM) calculations clearly reveal the high stabilities of those three isomers of THY in both media. The frontier orbitals show that in gas phase the decreasing of the gap values are different than those in solution. The vibrational analyses show the presence of different isomers in both media and confirm that the different positions of the bands are characteristic of their different structures. Here, the ¹H-NMR, ¹³C-NMR and UV spectra present a reasonable concordance with the corresponding experimental ones. Finally, the spectroscopic studies support the presence of more of an isomer of THY in solution.



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1. INTRODUCTION

The structural and vibrational properties of different isomers expected for the nucleosides emtricitabine (FTC), idoxuridine (IDU) and trifluridine (TFT), as a consequence of their two asymmetric C atoms present in the ribose rings are of great interest due to their antiviral properties [1-6]. In this work, we have compared and analyzed the different *Cis* and *Trans* configurations of the antiviral thymidine (THY) agent because in the recent study reported by Márquez and Brandán only the structural and vibrational properties corresponding to three *Trans* conformations were presented [3]. In this case, the study of the *Cis* isomers of thymidine was also considered because a *Cis* isomer in the nucleoside emtricitabine has a high antiviral activity while the other *Cis* one exhibit low activity [7]. Hence, to know exactly the Structure Activity Relationship (SAR) of this important antiviral agent it is necessary to know the existent relations among the atomic charges, molecular electrostatic potentials, stabilization energies, frontier orbitals and their *Cis* (*2R,5S*) and (*2S,5R*) structures. Particularly, in these nucleosides is of interest to understand why especially the *Cis* configuration present, for example in emtricitabine, a high biological activity despite that it is not the more stable in both media. In this study, we observed that the *Cis* (*2R,5S*) isomers for THY is the most stable than the other configurations. Besides, the topological properties were also compared in order to analyze the characteristics and nature of the intra-molecular interactions. Here, a series of interesting descriptors were also compared among them in order to know the influence of the electronic density on the two different pyrimidines and ribose rings present in all the different structures. These properties were calculated for THY using the optimized structures with the hybrid B3LYP/6-31G* method. It is very important to clarify that the comparisons of the properties were performed in gas and aqueous solution phases because we observed in the above studies and, in this work in particular, that the populations of the isomers of those species notably changes with the media considered. The Self Consistent Reaction Field (SCRF) calculations and the (PCM) were employed to study all the species in aqueous solution [8,9] while the solvation model [10] was used to compute the solvation energies using the same level of theory. The volume contractions or expansions expressed as the difference between the values in solution and those obtained in the gas phase were calculated with the Moldraw program [11]. The energy gap values for all the species and the calculations of some descriptors were performed with the frontier orbitals and with equations reported in the literature [12-18]. Here, the descriptor properties were calculated using NBO and QAIM [19,20] calculations together with the charges derived from Merz-

Kollman [21]. On the other hand, the assignments of the new *Cis* isomers were performed taking into account the available infrared and Raman spectra [22], the internal coordinates and the force fields determined at the same level of theory by using the Scaled Quantum Mechanics Force Field (SQMFF) and the Molvib program [23,24]. In this work, the predicted $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra for all the isomers of THY were compared for first time with those available experimental in DMSO- d_6 and D_2O [25,26] while the predicted UV-visible using the B3LYP/6-31G* method were compared here with those reported in the literature for THY in aqueous solution [27].

2. COMPUTATIONAL DETAILS

Here, the structural and vibrational properties of six isomers of THY were compared. In particular, the properties for the C4, C5 and C6 isomers were only studied here because the corresponding to the C1, C2 and C3 isomers were already reported [3], as was before mentioned. The *GaussView* program was used to model those three structures of THY while for their optimizations was employed the Gaussian program [28,29]. The hybrid B3LYP/6-31G* method was used in all the calculations in gas and aqueous solution phases [30,31]. The structures of the three *Trans* isomers of THY are presented in **Figure 1** together with the atoms labelling while in **Figure 2** are presented the other three isomers. Two charge's types were considered in this study, on one hand, the atomic natural population (NPA) computed from the NBO calculations [19] and, on the other hand, the MK charges which were derived from Merz-Kollman calculations [21]. The bond orders were also computed together with the molecular electrostatic potentials while the behaviours of each isomer in the different media and their reactivities were predicted by using the HOMO-LUMO calculations and useful equations reported for some descriptors by different authors [12-18]. The predictions of the theoretical $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra for all the isomers of THY were presented for first time in this work and, they were carry out using the self-consistent perturbation theory of diamagnetism with the gauge-invariant molecular orbital theory (GIAO) method [32]. Later, these spectra were compared with the available experimental in DMSO- d_6 and D_2O [33,34] showing reasonable concordance. On the other hand, the electronic UV-visible spectra for all the isomers of THY in aqueous solution were also predicted for first time using the B3LYP/6-31G* method and, then, they were compared with the available experimental for thymidine in aqueous solution [27] evidenced among them a very good correlation.

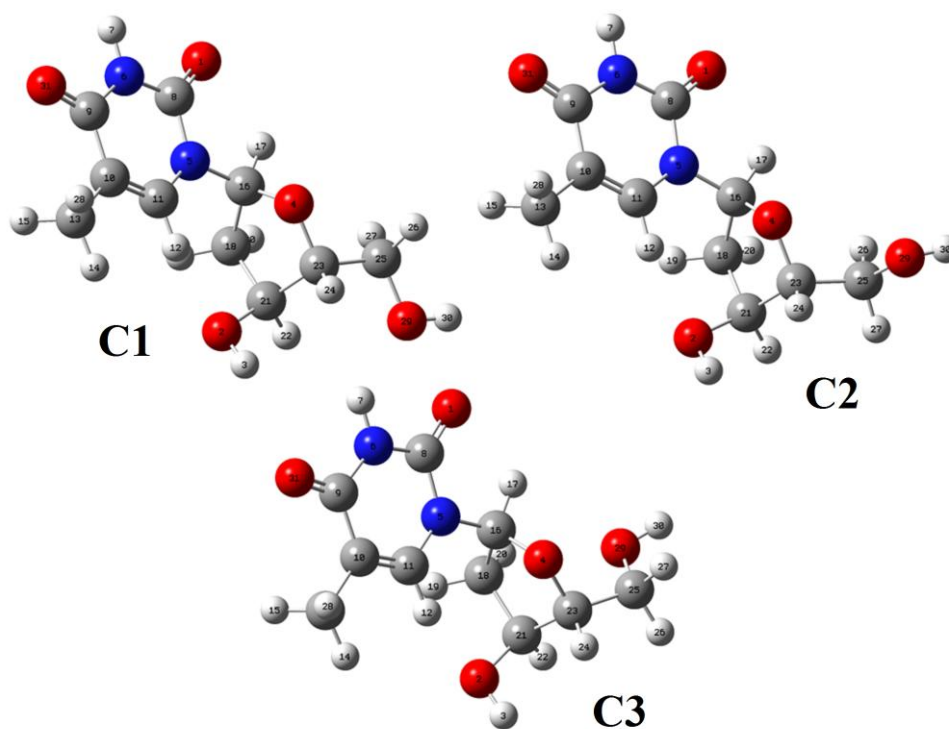


Figure 1. Theoretical molecular structures and atoms labelling for the *trans* C1, C2 and C3 isomers of thymidine.

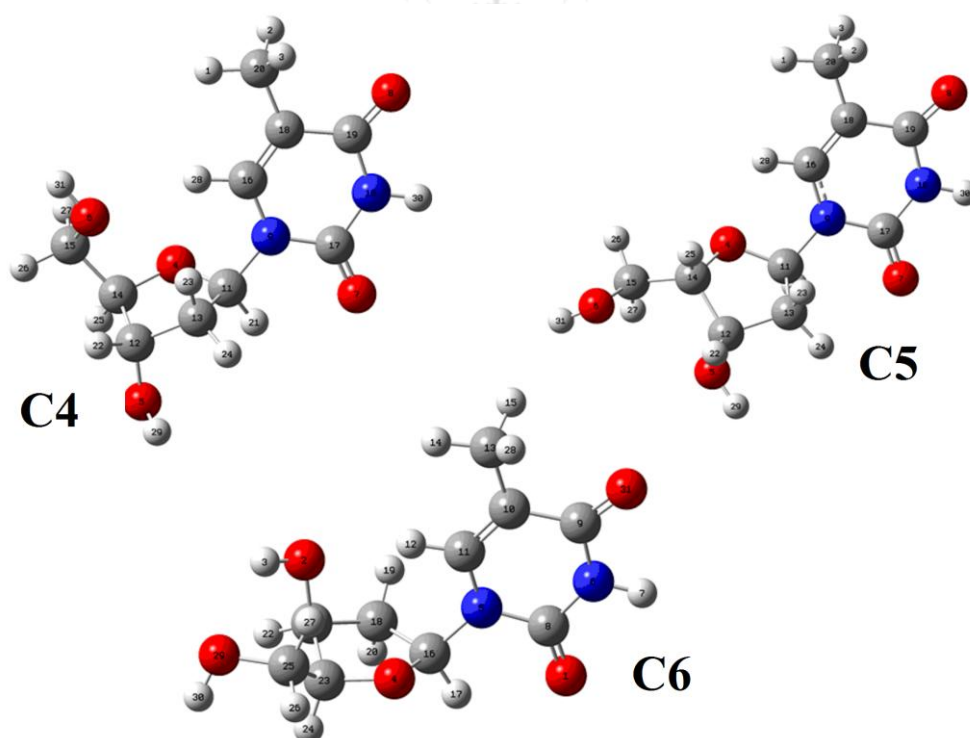


Figure 2. Theoretical molecular structures and atoms labelling for the *Cis* C4 and C6 and, *trans* C5 isomers of thymidine.

The force fields for the isomers C4, C5 and C6 were calculated at the same level of theory that for C1, C2 and C3 with the SQMFF methodology and the Molvib program [23,24] while the internal coordinates were those similar to the previously reported [3]. This way, the complete assignments of the new *Cis* and *Trans* isomers were performed using the available infrared and Raman spectra [22]. Finally, the force constants of all the isomers were compared among them evidenced an excellent correlation.

3. RESULTS AND DISCUSSION

3.1. Optimization geometries

The total and relative energies, dipole moment values and populations for all the isomers of THY in both media are presented in **Table 1**.

Table 1. Total (*E*) and relative (ΔE) energies and dipole moment (μ) for all conformers of thymidine

Name	Isomers	E (Hartree)	ΔE (kJ/mol)	μ (D)	Population %
Gas phase ^a					
C1	<i>Trans</i>	-875.1242	11.01	6.73	0.92
C2	<i>Trans</i>	-875.1195	23.34	7.39	0.02
C3	<i>Trans</i>	-875.1246	9.97	7.75	1.85
C4	<i>Cis</i>	-875.1255	7.61	6.81	4.63
C5	<i>Trans</i>	-875.1215	18.10	4.95	0.06
C6	<i>Cis</i>	-875.1284	0.00	6.91	92.52
Aqueous solution ^a					
C1	<i>Trans</i>	-875.1567	4.98	9.02	5.81
C2	<i>Trans</i>	-875.1566	5.24	11.07	5.36
C3	<i>Trans</i>	-875.1576	2.62	11.06	15.62
C4	<i>Cis</i>	-875.1581	1.31	9.29	26.34
C5	<i>Trans</i>	-875.1558	7.34	7.12	2.23
C6	<i>Cis</i>	-875.1586	0.00	9.18	44.64

^aThis work The results clearly show that the *Cis* C6 isomer is the most stable in both media exhibiting the biggest populations but the C4 isomer also shows higher populations in the two media, as observed in Table 1. **Figure 3** shows the variations in the population of the different isomers in both media. The graphic shows clearly that C4 and C6 present the higher values in the two media. On the other hand, **Table 2** shows the molar volume variations that experiment each isomer, expressed as a difference between the values in solution and those in

gas phase, as a function of the different isomeric structures while the variations of the dipole moment values in both media are represented in **Figure 4** at B3LYP/6-31G* level of theory. Note that the behaviours of the dipole moments in both media are similar for all the isomers showing the C2 and C3 isomers the higher values while the low values are observed for C5. Figure 3 shows that the C3 isomer presents the higher volume values in both media which could easily explain the higher dipole moment values that present the C2 and C3 isomers in the two media, as observed in Tables 1 and 2.

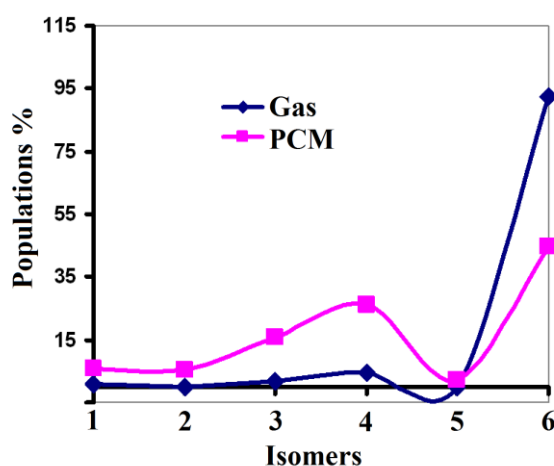


Figure 3. Variation of the populations in function of the isomers of thymidine in both media at the B3LYP/6-31G* level of theory.

Table 2. Molecular volume for the stable conformations of thymidine by the B3LYP/6-31G* method

Isomers	Molar Volume (Å ³)		#ΔV= V _{AS} - V _G (Å ³)
	GAS	PCM/SMD	
C1	236.7	236.0	-0.7
C2	242.6	236.9	-5.7
C3	257.3	257.3	0.0
C4	238.8	239.8	1.0
C5	240.5	238.4	-2.1
C6	237.0	238.3	1.3

#See text

On the other hand, **Figure 5** shows that the C2 and C5 isomers present the higher variations in the molar volumes probably because these isomers present lower populations in the two media and, as a consequence, they can undergo higher expansions in these media.

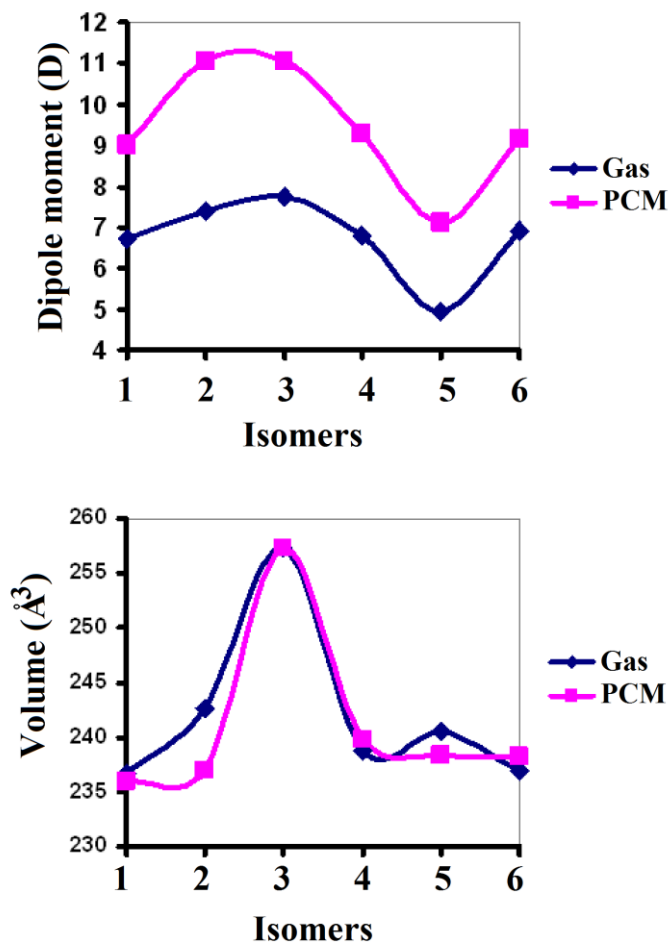


Figure 4. Variations observed in the dipole moment (upper) and volume (bottom) values in the different isomers in both media at B3LYP/6-31G* level of theory.

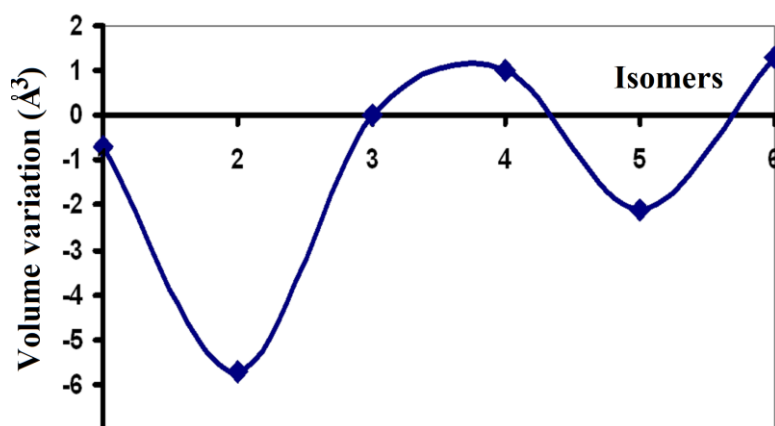


Figure 5. Variations observed in the volume values in the different isomers in both media at B3LYP/6-31G* level of theory.

The solvation energy values for the six isomers of THY calculated at B3LYP/6-31G* level of theory are summarized in

Table 3 while the representation of these values can be seen in **Figure 6**.

Table 3. Calculated Solvation energies (ΔG) for the six isomers of thymidine

PCM/B3LYP/6-31G*			
Species	ΔG (kJ/mol)		
	$\Delta G_u^{\#}$	ΔG_{ne}	ΔG_c
C1	-85.24	29.68	-114.92
C2	-97.31	29.72	-127.03
C3	-86.56	29.59	-116.15
C4	-85.51	29.51	-115.02
C5	-89.97	29.18	-119.15
C6	-79.21	28.84	-108.05

$$\Delta G_c = \Delta G_{\text{uncorrected}}^{\#} - \Delta G_{\text{Total non-electrostatic}}$$

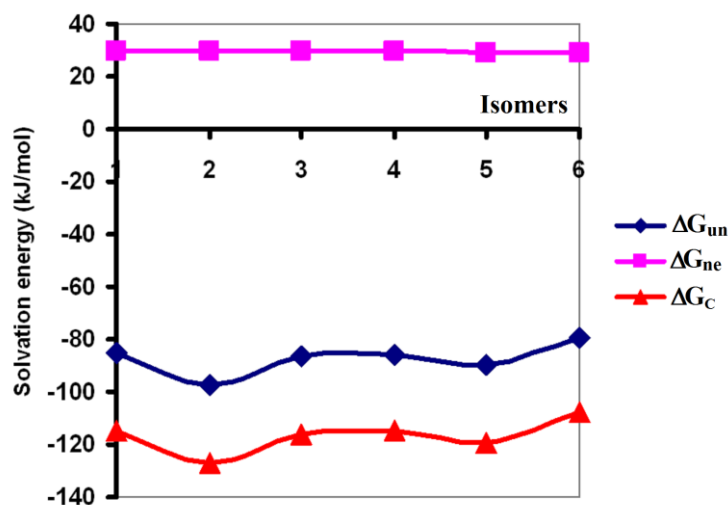


Figure 6. The uncorrected and corrected solvation energy values calculated at B3LYP/6-31G* level of theory in function of the six isomers of THY together with the corresponding total nonelectrostatic terms due to the cavitation, dispersion and repulsion energies.

The graphics clearly evidence the diminishing of the corrected values, in relation to those uncorrected, when the nonelectrostatic terms are included. Notice that the C2 and C5 isomers have the lowest values than the other ones. Hence, probably the higher variations in the molar

volumes due to their low populations can in part justify the lower solvation energies of these two isomers in solution.

For the six isomers of THY, the Root-Mean-Square Deviation (RMSD) were used to compare the calculated geometrical parameters with the corresponding experimental ones reported by Görbitz *et al.* [33] for α -thymidine at low temperatures. The results for the six isomers in gas phase can be seen in **Table 4**. Obviously, the variations observed in those parameters can be, on one hand, attributed to the temperature because the calculations were computed at 298 K while the experimental values are reported at 105 K and, on other hand, to the packing forces observed in the solid phase. In general, we observed a good concordance between the calculated and experimental bond lengths and angles values for all the *Cis* and *Trans* isomers of THY and only significant differences are observed in the dihedral angles, having the C2 isomer the lowest RMSD value. Analyzing the glycosidic N5-C16 bonds of all the *Cis* and *Trans* isomers, we observed that the calculated values, in general, are in conformity with the experimental value of 1.478Å [33] and only in the C5 isomer the B3LYP/6-31G* calculations overestimated this value, as observed in Table 4. When the O4-C23-C25-O29 torsion angle for the new isomers considered in this work were carefully analyzed we observed that in C4 that dihedral angle has a value of -68.9 °, similar to that observed in C3 of -69.5°, for which, the structures of both isomers correspond to that B structure reported by Görbitz *et al.* [33]. Note that these two last isomers have respectively *Trans* and *Cis* structures. On the other hand, in the previous work reported for the three *Trans* isomers by Márquez and Brandán [3], was observed that the structure of the C2 isomer is similar to the A structure reported at low temperatures by Görbitz *et al.* [33] while in this work we have observed for C6 a positive O4-C23-C25-O29 torsion angle but with a value of 161.6 ° different from that experimental value of 59.34 (9)° reported for the A structure. Experimentally, the *Cis* structures for THY were reported by both Young *et al.* and Hübschle *et al.* [34,35], of which the C4 isomer corresponds to the *Cis* structure reported by those first authors. On the other hand, the *Cis* and *Trans* structures were also predicted at low temperatures by Ivanov *et al.* [36]. Besides, in that experimental structure of THY at low temperatures [36], it was possible to observe dimeric species linked by intermolecular bonds and intra-molecular H bonds between atoms belonging to the two pyrimidine and ribose rings with O4-C16-N5-C8 torsion angles of 124°.

Table 4. Comparison of calculated geometrical parameters for the six isomers of thymidine in gas phase

Parameter	B3LYP/6-31G** ^a						Exp ^b
	C1	C2	C3	C4	C5	C6	
Bond lengths (Å)							
N5-C8	1.399	1.399	1.399	1.397	1.391	1.400	1.380
N5-C11	1.386	1.386	1.385	1.385	1.380	1.387	1.388
N5-C16	1.463	1.462	1.463	1.463	1.483	1.462	1.478
N6-C8	1.383	1.383	1.384	1.384	1.382	1.383	1.381
N6-C9	1.406	1.406	1.406	1.406	1.408	1.406	1.392
C9-C10	1.462	1.462	1.462	1.462	1.461	1.462	1.454
C10-C11	1.352	1.352	1.352	1.352	1.354	1.352	1.361
C8-O1	1.221	1.221	1.221	1.221	1.223	1.221	1.237
C9-O31	1.222	1.222	1.223	1.222	1.222	1.222	1.237
O4-C16	1.429	1.426	1.429	1.424	1.413	1.429	1.436
O4-C23	1.428	1.427	1.425	1.435	1.444	1.427	1.452
C16-C18	1.548	1.551	1.549	1.535	1.538	1.548	1.546
C18-C21	1.531	1.530	1.530	1.530	1.532	1.531	1.535
C21-C23	1.538	1.540	1.543	1.543	1.533	1.544	1.537
C23-C25	1.529	1.528	1.526	1.521	1.520	1.532	1.523
RMSD	0.012	0.012	0.012	0.011	0.011	0.012	
Bond angles (°)							
N5-C8-N6	113.4	113.3	113.3	113.3	113.7	113.4	115.1
C8-N6-C9	128.7	128.7	128.7	128.6	128.3	128.7	126.7
N6-C9-C10	113.8	113.8	113.8	113.8	113.8	113.8	115.5
C9-C10-C11	118.5	118.5	118.4	118.5	118.5	118.5	117.9
N5-C11-C10	123.8	123.7	123.8	123.7	123.6	123.8	123.1
N5-C16-C18	115.2	115.1	114.9	114.6	113.4	115.1	114.2
N5-C16-O4	108.9	108.5	108.2	108.5	109.4	109.0	107.0
O4-C16-C18	106.4	106.4	106.6	105.4	106.4	106.3	114.2
C16-C18-C21	105.1	104.7	105.0	102.3	101.9	105.5	104.7
O4-C23-C21	106.1	105.1	105.4	106.6	105.3	106.3	104.2
C23-C25-O29	106.9	107.6	109.1	109.2	106.3	111.7	109.4
RMSD	2.8	2.7	2.6	3.0	2.9	2.8	
Dihedral angle (°)							
C11-N5-C16-	59.6	63.0	59.2	-68.8	-104.1	56.4	61.6
C8-N5-C16-C18	-125.6	-121.4	-126.2	112.7	76.0	-129.0	-117.0
C8-N5-C16-O4	114.8	119.3	114.6	-129.6	-165.2	111.4	124.8
C11-N5-C16-O4	-59.8	-56.1	-59.8	48.7	14.5	-63.0	-56.3
O4-C23-C25-	174.4	67.0	-69.5	-68.9	-170.2	161.6	89.7
C21-C23-C25-	49.9	-174.1	-66.8	51.2	71.3	43.9	
O4-C23-C21-O2	-85.1	-81.4	-83.6	-96.0	-82.5	-86.8	-79.7
RMSD	35.1	9.7	65.3	168.8	192.1	30.6	

^aThis work

^bFrom Ref [33]

Here, the B3LYP/6-31G* calculations predicted the O4-C16-N5-C8 torsion angles values for the C1, C2, C3 and C6 isomers between 119.3 and 111.4° while for the C4 and C5 isomers those torsion angles exhibit values of -129.6 and -165.2°, respectively. For C2, C4 and C5, the calculations predicted the O4-C23 bond longer than the O4-C16 bond in accordance with the observed values in Table 4 while for C1, C3 and C6 contrary results are predicted. The population analysis suggests that the C6 isomer is the only expected in both media while the

C3, C4 and C6 isomers could be present no more than in aqueous solution. This way, these three isomers could be present in both media.

3.2. Charges, molecular electrostatic potentials, bond orders studies

The study of the atomic charges in all the isomers of THY is of great structural importance taking into account the presence of N and O atoms with lone pairs, of -OH groups and of the two chiral C atoms in their structures that obviously have influence on their antiviral properties, as was observed before for the antiviral emtricitabine, IDU and TFT agents. Hence, the MK and NPA charges [19,218] for the six isomers of THY were studied in both media at B3LYP/6-31G* level of theory. The results of both charges can be seen in **Tables 5** and **6**; respectively while in **Figures 7** and **8** are presented the variations of the MK charges for all the O, N and C atoms of the different isomers in gas phase. First, the complete analysis of the MK charges on the five O atoms of the different isomers show clearly that the charges on the O4 atoms belonging to the ribose rings in all the structures present the higher variations, especially in the C5 isomer while the charges on the O1 and O31 atoms belonging to the C=O bonds practically no present variations. Other very important results are that the MK charges on the O2 and O29 atoms belonging to the -OH groups also present slightly variations in the different isomers being more notable in C5. On the other hand, when the MK charges on the two N atoms are analyzed in **Figure 7**, we observed that only the charges on the N5 belonging to the glycosidic bond present higher variations, having the C6 isomer the lowest value than the other ones while the charges on the N6 atoms remain practically constant in all the isomers.

Table 5. Atomic MK charges for the six isomers of thymidine in gas and aqueous solution phases

Atoms	Gas phase						Aqueous solution					
	MK' charges						MK' charges					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
1 O	-	-	-	-0.541	-0.544	-	-0.550	-0.555	-0.554	-0.548	-	-
	0.545	0.550	0.549			0.548					0.544	0.551
2 O	-	-	-	-0.620	-0.619	-	-0.598	-0.561	-0.587	-0.612	-	-
	0.600	0.566	0.595			0.647					0.619	0.637
3 H	0.378	0.370	0.386	0.416	0.422	0.426	0.376	0.371	0.382	0.413	0.422	0.427
4 O	-	-	-	-0.401	-0.548	-	-0.433	-0.322	-0.346	-0.397	-	-
	0.452	0.334	0.353			0.389					0.548	0.362
5 N	-	-	-	-0.169	-0.157	-	-0.126	-0.179	-0.179	-0.156	-	-
	0.148	0.175	0.226			0.295					0.157	0.261
6 N	-	-	-	-0.633	-0.621	-	-0.655	-0.636	-0.643	-0.611	-	-
	0.672	0.658	0.658			0.643					0.621	0.629
7 H	0.380	0.375	0.371	0.371	0.373	0.369	0.381	0.374	0.374	0.369	0.373	0.371
8 C	0.685	0.695	0.709	0.659	0.621	0.717	0.675	0.685	0.691	0.653	0.621	0.696
9 C	0.638	0.636	0.636	0.622	0.606	0.630	0.622	0.619	0.617	0.600	0.606	0.622
10 C	-	-	-	-0.015	-0.010	-	-0.012	-0.016	-0.005	0.000	-	-
	0.029	0.025	0.020			0.007					0.009	0.009
11 C	-	-	-	-0.151	-0.174	-	-0.137	-0.104	-0.124	-0.146	-	-
	0.125	0.112	0.103			0.116					0.174	0.118
12 H	0.194	0.191	0.202	0.216	0.199	0.233	0.193	0.184	0.200	0.206	0.199	0.225
13 C	-	-	-	-0.369	-0.390	-	-0.411	-0.425	-0.416	-0.382	-	-
	0.403	0.418	0.423			0.460					0.390	0.449
14 H	0.123	0.125	0.124	0.100	0.114	0.133	0.124	0.125	0.122	0.104	0.115	0.129
15 H	0.127	0.131	0.131	0.124	0.130	0.142	0.129	0.133	0.129	0.128	0.130	0.141
16 C	0.309	0.196	0.301	0.311	0.560	0.419	0.259	0.191	0.236	0.278	0.560	0.401
17 H	0.082	0.121	0.093	0.092	0.003	0.062	0.091	0.125	0.115	0.104	0.003	0.067
18 C	-	-	-	-0.312	-0.333	-	-0.459	-0.327	-0.346	-0.309	-	-
	0.498	0.322	0.363			0.422					0.332	0.436
19 H	0.182	0.156	0.157	0.110	0.077	0.160	0.177	0.161	0.157	0.115	0.076	0.169
20 H	0.151	0.100	0.128	0.107	0.113	0.120	0.144	0.106	0.129	0.110	0.112	0.127
21 C	0.334	0.248	0.251	0.167	0.159	0.413	0.344	0.233	0.234	0.156	0.159	0.409
22 H	0.028	0.023	0.057	0.063	0.044	-	0.016	0.024	0.056	0.068	0.044	-
						0.022						0.009
23 C	0.261	0.068	0.036	0.223	0.381	-	0.210	0.084	0.084	0.217	0.381	-
						0.053						0.165
24 H	0.050	0.109	0.078	0.073	0.013	0.075	0.071	0.103	0.069	0.075	0.013	0.116
25 C	0.070	0.163	0.129	0.011	0.093	0.110	0.102	0.124	0.084	0.039	0.094	0.162
26 H	0.055	0.031	0.000	0.044	0.039	0.069	0.052	0.039	0.011	0.036	0.039	0.060
27 H	0.029	-	0.055	0.070	0.055	0.096	0.024	0.001	0.063	0.064	0.055	0.090
		0.013										
28 H	0.129	0.134	0.133	0.118	0.123	0.145	0.132	0.137	0.132	0.122	0.123	0.144
29 O	-	-	-	-0.594	-0.648	-	-0.652	-0.577	-0.578	-0.600	-	-
	0.660	0.587	0.579			0.617					0.647	0.616
30 H	0.437	0.402	0.410	0.423	0.427	0.409	0.428	0.400	0.414	0.423	0.427	0.402
31 O	-	-	-	-0.515	-0.509	-	-0.517	-0.518	-0.519	-0.518	-	-
	0.511	0.512	0.515			0.510					0.509	0.518

Table 6. NPA charges for the six isomers of thymidine in gas and aqueous solution phases

Atoms	Gas phase						Aqueous solution					
	NPA						NPA					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
1 O	-	-	-	-	-	-	-	-	-	-	-	-
	0.638	0.638	0.637	0.636	0.643	0.636	0.645	0.644	0.643	0.642	0.643	0.643
2 O	-	-	-	-	-	-	-	-	-	-	-	-
	0.763	0.758	0.761	0.756	0.762	0.776	0.762	0.757	0.759	0.756	0.762	0.770
3 H	0.482	0.480	0.477	0.476	0.481	0.500	0.482	0.480	0.476	0.476	0.481	0.494
4 O	-	-	-	-	-	-	-	-	-	-	-	-
	0.586	0.577	0.587	0.594	0.596	0.590	0.584	0.575	0.586	0.594	0.596	0.588
5 N	-	-	-	-	-	-	-	-	-	-	-	-
	0.479	0.477	0.478	0.474	0.475	0.479	0.474	0.474	0.473	0.470	0.475	0.473
6 N	-	-	-	-	-	-	-	-	-	-	-	-
	0.667	0.667	0.668	0.668	0.666	0.667	0.659	0.659	0.659	0.659	0.666	0.659
7 H	0.447	0.447	0.446	0.447	0.447	0.447	0.450	0.450	0.450	0.450	0.447	0.451
8 C	0.835	0.835	0.835	0.834	0.829	0.835	0.831	0.831	0.830	0.830	0.829	0.831
9 C	0.664	0.664	0.664	0.664	0.661	0.664	0.656	0.656	0.656	0.655	0.661	0.656
10 C	-	-	-	-	-	-	-	-	-	-	-	-
	0.177	0.178	0.179	0.180	0.182	0.176	0.174	0.175	0.176	0.177	0.182	0.173
11 C	0.036	0.038	0.038	0.037	0.036	0.032	0.037	0.040	0.039	0.039	0.036	0.035
12 H	0.261	0.260	0.259	0.259	0.258	0.260	0.260	0.258	0.258	0.258	0.258	0.259
13 C	-	-	-	-	-	-	-	-	-	-	-	-
	0.682	0.682	0.682	0.681	0.682	0.683	0.682	0.681	0.681	0.681	0.682	0.682
14 H	0.235	0.234	0.234	0.231	0.233	0.236	0.234	0.233	0.234	0.231	0.233	0.235
15 H	0.251	0.251	0.250	0.251	0.252	0.251	0.251	0.251	0.251	0.252	0.252	0.251
16 C	0.264	0.264	0.266	0.275	0.287	0.269	0.266	0.267	0.269	0.275	0.287	0.271
17 H	0.265	0.263	0.269	0.263	0.252	0.265	0.264	0.265	0.270	0.264	0.252	0.263
18 C	-	-	-	-	-	-	-	-	-	-	-	-
	0.524	0.522	0.520	0.523	0.522	0.521	0.525	0.523	0.521	0.522	0.522	0.522
19 H	0.272	0.271	0.263	0.259	0.248	0.271	0.273	0.273	0.265	0.258	0.248	0.271
20 H	0.257	0.255	0.274	0.250	0.266	0.258	0.257	0.257	0.272	0.251	0.266	0.259
21 C	0.068	0.071	0.071	0.073	0.072	0.069	0.068	0.070	0.071	0.072	0.072	0.070
22 H	0.238	0.223	0.228	0.226	0.234	0.224	0.236	0.223	0.227	0.227	0.234	0.227
23 C	0.045	0.038	0.039	0.047	0.054	0.035	0.043	0.037	0.036	0.044	0.054	0.032
24 H	0.239	0.244	0.237	0.253	0.229	0.211	0.241	0.244	0.238	0.254	0.229	0.210
25 C	-	-	-	-	-	-	-	-	-	-	-	-
	0.120	0.118	0.120	0.117	0.111	0.120	0.121	0.120	0.120	0.117	0.111	0.117
26 H	0.215	0.198	0.198	0.206	0.206	0.218	0.217	0.198	0.198	0.207	0.206	0.218
27 H	0.193	0.196	0.217	0.217	0.221	0.235	0.194	0.198	0.217	0.217	0.221	0.230
28 H	0.251	0.251	0.250	0.250	0.251	0.251	0.251	0.252	0.251	0.252	0.251	0.252
29 O	-	-	-	-	-	-	-	-	-	-	-	-
	0.762	0.743	0.768	0.772	0.759	0.764	0.761	0.740	0.764	0.770	0.759	0.758
30 H	0.482	0.478	0.486	0.485	0.481	0.480	0.481	0.476	0.484	0.485	0.481	0.475
31 O	-	-	-	-	-	-	-	-	-	-	-	-
	0.600	0.600	0.603	0.602	0.602	0.599	0.607	0.608	0.610	0.609	0.602	0.606

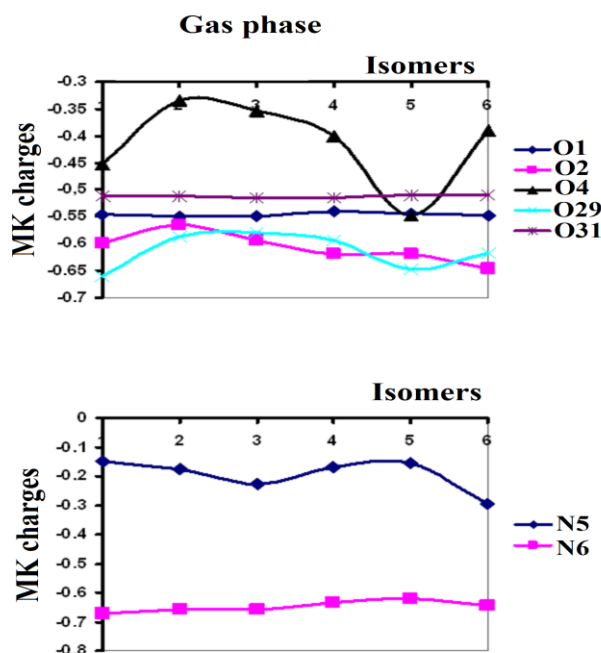


Figure 7. The MK charge values on the O (upper) and N (bottom) atoms corresponding to the six isomers of THY calculated in gas phase at B3LYP/6-31G* level of theory in function of their structures.

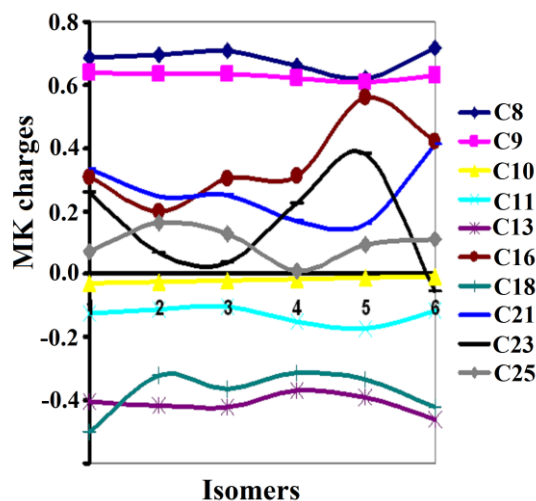


Figure 8. The MK charge values on the C atoms corresponding to the six isomers of THY calculated in gas phase at B3LYP/6-31G* level of theory in function of their structures.

Analyzing the MK charges on the C atoms, according to the **Figure 8**, we observed that the charges on the C16 and C23 atoms show the higher modifications, as expected because they

are the chiral atoms where C5 exhibit the higher charges comparing C16 with C23, respectively. **Figure 8** shows that the MK charges on the C21 atoms also undergo notable variations in the six isomers being important in C3 and C5. The analysis of the MK charges on the H atoms show strong variations on the H17 atoms belonging to the chiral C16 atoms of all the isomers showing C5 the lowest value than the other ones and the higher variations are observed on the H atoms belonging to the CH₂ and C-H groups while the H atoms belonging to the pyrimidine rings show few variations. The higher MK charge values in all the isomers are observed on the H3, H7 and H30, as expected because they belong to the NH and OH groups. In aqueous solutions similar variations in the MK charges on those atoms are observed, for these reasons, the graphics are not presented here.

In relation to the NPA charges, there are not significant variations of these charges on all the O, N, C and H atoms of the six isomers. For instance, only the most negative values are observed on the O2 and O29 atoms, as expected because these atoms belong to the OH groups. The NPA charges on the two N atoms remain practically constant in all the isomers having the lower values the N6 atoms of the N-H groups. All the NPA charges on the C atoms show the same values in all the isomers even those chiral atoms, then, this charge type do not produce variations in the different structures. In all the isomers, the most negative values are observed on the C13 atoms belonging to the CH₃ groups while the most positive values on the C8 and C9 atoms that belong to the C=O groups. This way, the MK charges on the O, N, C and H atoms evidence different behaviors of the six structures when compared with those NPA and, they suggest their importance to understand the properties of the *Cis* and *Trans* isomers in both media.

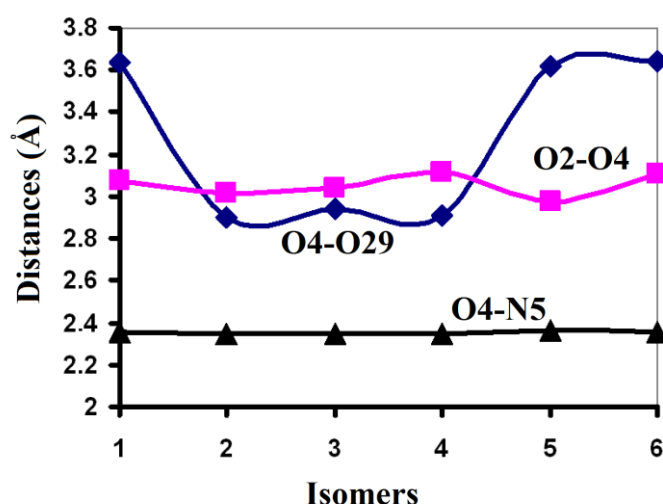
In this chapter, we have also performed the study of the separations between the most electronegative N and O atoms because these parameters are very important to know the stabilities of these isomers. Thus, in **Table 7** are summarized the calculated values in both media while in Figure 9 are represented the variations in function of the six isomers in gas phase. Figure 9 shows clearly that the O4-N5 distances are independent of the *Cis* or *Trans* conformations because they remain nearly constant in all the isomers while the O4-O29 distances exhibit the higher values in C1, C5 and C6 and the lower values in C2, C3 and C4. This way, in C1, C5 and C6 are expected lower repulsions between those atoms indicating that these isomers are most stable while, for the same reasons, lower stabilities can be

expected for C2, C3 and C4. In aqueous solution, the same variations in those distances are observed; hence, the corresponding graphic was not presented here.

Another property studied for the six isomers of THY were the molecular electrostatic potentials because they are essential to describe the reaction sites with potential biological electrophiles and nucleophiles. Thus, in Table 8 are presented the Molecular Electrostatic Potential (MEP) for the six isomers of thymidine in both media using the B3LYP/6-31G* level of theory. In **Figure 10**, the MEP values for all the O atoms of the six isomers in gas phase are represented as a function of the different structures where we observed that the O1 and O31 atoms belonging to the C=O groups of the pyrimidine rings remain practically constant in all the isomers and they have the most negative values.

Table 7. Distances values between the more electronegative atoms for the stable isomers of thymidine

B3LYP/6-31G* method ^a							
Gas phase							
Distances	C1	C2	C3	C4	C5	C6	Exp ^b
O4-O29	3.635	2.897	2.941	2.909	3.617	3.645	
O2-O4	3.074	3.018	3.043	3.114	2.976	3.104	2.976
O4-N5	2.355	2.346	2.344	2.344	2.365	2.355	2.343
Aqueous solution							
O4-O29	3.649	2.829	2.913	2.941	3.617	3.668	
O2-O4	3.069	3.069	3.083	3.128	2.975	3.082	2.976
O4-N5	2.352	2.349	2.347	2.348	2.365	2.351	2.343



^aThis work

Figure 9. Distances between the more electronegative atoms for the stable isomers of thymidine in gas phase at B3LYP/6-31G* level of theory.

Table 8. Molecular electrostatic potential (in a.u.) for the six isomers of thymidine

Gas phase							Aqueous solution					
Atoms	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
1 O	-	-	-	-	-	-	-	-	-	-	-	-
	22.343	22.344	22.347	22.344	22.339	22.342	22.346	22.348	22.351	22.347	22.339	22.345
2 O	-	-	-	-	-	-	-	-	-	-	-	-
	22.290	22.285	22.291	22.301	22.297	22.297	22.288	22.283	22.289	22.300	22.297	22.289
3 H	-0.972	-0.967	-0.973	-0.981	-0.977	-0.981	-0.970	-0.965	-0.971	-0.981	-0.977	-0.974
4 O	-	-	-	-	-	-	-	-	-	-	-	-
	22.295	22.298	22.301	22.300	22.287	22.294	22.293	22.297	22.299	22.299	22.287	22.291
5 N	-	-	-	-	-	-	-	-	-	-	-	-
	18.285	18.286	18.290	18.285	18.280	18.284	18.283	18.284	18.288	18.282	18.280	18.281
6 N	-	-	-	-	-	-	-	-	-	-	-	-
	18.303	18.303	18.306	18.303	18.299	18.302	18.301	18.303	18.305	18.301	18.299	18.300
7 H	-1.000	-1.001	-1.004	-1.000	-0.996	-0.999	-0.998	-0.999	-1.001	-0.998	-0.996	-0.996
8 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.593	14.593	14.597	14.594	14.590	14.592	14.593	14.595	14.598	14.594	14.590	14.592
9 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.634	14.634	14.637	14.634	14.631	14.633	14.635	14.636	14.638	14.635	14.631	14.634
10 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.728	14.729	14.731	14.728	14.724	14.727	14.727	14.729	14.731	14.726	14.724	14.726
11 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.687	14.687	14.689	14.685	14.678	14.686	14.685	14.686	14.688	14.683	14.678	14.683
12 H	-1.080	-1.081	-1.083	-1.079	-1.073	-1.079	-1.079	-1.080	-1.082	-1.078	-1.073	-1.077
13 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.740	14.740	14.742	14.737	14.736	14.739	14.739	14.740	14.741	14.737	14.736	14.738
14 H	-1.118	-1.118	-1.120	-1.115	-1.114	-1.117	-1.117	-1.118	-1.119	-1.115	-1.114	-1.117
15 H	-1.122	-1.122	-1.124	-1.120	-1.118	-1.121	-1.121	-1.122	-1.123	-1.119	-1.119	-1.120
16 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.635	14.635	14.644	14.640	14.629	14.634	14.632	14.634	14.641	14.638	14.629	14.631
17 H	-1.102	-1.103	-1.113	-1.110	-1.104	-1.102	-1.100	-1.102	-1.112	-1.109	-1.104	-1.099
18 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.718	14.716	14.729	14.726	14.722	14.717	14.715	14.713	14.725	14.724	14.722	14.714
19 H	-1.092	-1.090	-1.102	-1.100	-1.094	-1.091	-1.088	-1.087	-1.099	-1.098	-1.094	-1.087
20 H	-1.093	-1.090	-1.107	-1.095	-1.095	-1.092	-1.090	-1.088	-1.104	-1.093	-1.095	-1.088
21 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.666	14.658	14.666	14.668	14.668	14.664	14.663	14.655	14.663	14.666	14.668	14.660
22 H	-1.102	-1.092	-1.102	-1.105	-1.103	-1.100	-1.100	-1.089	-1.100	-1.103	-1.103	-1.096
23 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.673	14.670	14.671	14.673	14.673	14.669	14.669	14.668	14.668	14.671	14.673	14.667
24 H	-1.099	-1.095	-1.092	-1.105	-1.106	-1.099	-1.094	-1.092	-1.088	-1.102	-1.106	-1.094
25 C	-	-	-	-	-	-	-	-	-	-	-	-
	14.674	14.671	14.671	14.670	14.684	14.669	14.672	14.670	14.670	14.669	14.684	14.669
26 H	-1.105	-1.099	-1.101	-1.098	-1.119	-1.097	-1.105	-1.098	-1.096	-1.097	-1.119	-1.100
27 H	-1.103	-1.102	-1.100	-1.101	-1.117	-1.100	-1.098	-1.097	-1.101	-1.099	-1.117	-1.098
28 H	-1.122	-1.122	-1.124	-1.120	-1.118	-1.121	-1.121	-1.122	-1.123	-1.119	-1.118	-1.120
29 O	-	-	-	-	-	-	-	-	-	-	-	-
	22.293	22.302	22.292	22.285	22.306	22.276	22.293	22.303	22.292	22.285	22.306	22.282
30 H	-0.974	-0.983	-0.973	-0.967	-0.987	-0.961	-0.974	-0.984	-0.973	-0.968	-0.987	-0.966
31 O	-	-	-	-	-	-	-	-	-	-	-	-
	22.350	22.351	22.353	22.350	22.348	22.349	22.354	22.355	22.357	22.354	22.348	22.353

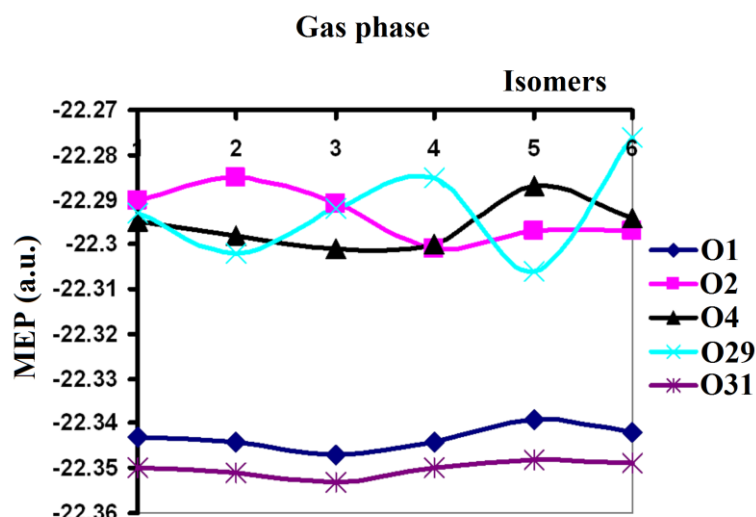


Figure 10. Molecular electrostatic potentials (MEP) for the stable isomers of thymidine in gas phase at B3LYP/6-31G* level of theory.

On the other hand, in this graphic we observed that the MEP values for the O2, O4 and O29 atoms have the less negative values than the other ones and, also, show significant differences in all the isomers indicating clearly that the variations of this property is a consequence of two chiral atoms present in their structures. If we analyzed the surface mapped on the C6 structure in gas phase in **Figure 11** different colorations are observed.

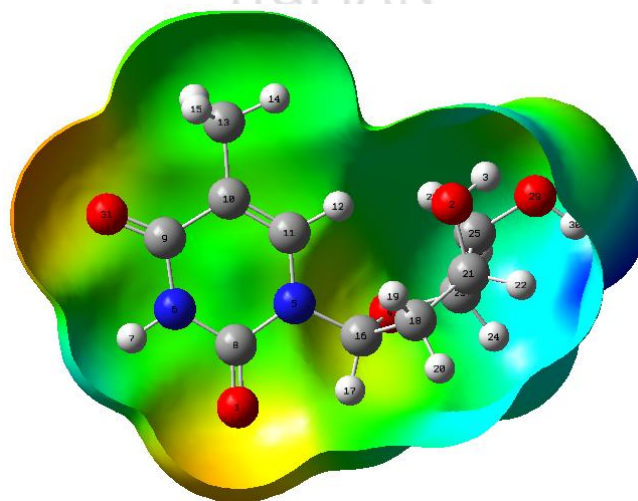


Figure 11. Calculated electrostatic potential surface on the molecular surface of C6 structure of thymidine in gas phase. Colour ranges, in au: from red -0.077 to blue + 0.077. B3LYP functional and 6-31G* basis set. Isodensity value of 0.005.

The strong red colors are observed on the O1 and O31 atoms whose MEP values are the most negative values and, moreover, weak red colors on the O4 atoms are also observed. These regions are acceptor of H bonds, are nucleophilic regions that reacting with potential biological electrophiles. On the other hand, the strong blue colors are observed on the H atoms belonging to the N-H and OH groups, as expected because these regions are donor of H bonds that obviously reacting with potential biological nucleophiles. This study, evidence clearly that the two C=O groups of the pyrimidine rings in all the isomers are principally acceptors of H bonds while the OH groups of the ribose rings and the NH group of the pyrimidine rings are donors of H bonds.

The bond orders expressed as Wiberg indexes were also studied for all the isomers of THY. The values that experiment the different isomers in both media in function of their structures are summarized in **Table 9**.



Table 9. Wiberg indexes for the six isomers of thymidine

Gas phase		Aqueous solution										
Atoms	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
1 O	1.984	1.984	1.985	1.985	1.974	1.986	1.969	1.969	1.970	1.971	1.974	1.971
2 O	1.791	1.796	1.793	1.789	1.787	1.784	1.791	1.795	1.792	1.786	1.787	1.787
3 H	0.769	0.771	0.774	0.775	0.770	0.752	0.769	0.771	0.775	0.776	0.770	0.758
4 O	2.006	2.016	2.006	1.996	1.995	2.001	2.004	2.014	2.005	1.992	1.996	2.000
5 N	3.382	3.383	3.385	3.386	3.391	3.381	3.388	3.389	3.390	3.391	3.391	3.387
6 N	3.236	3.236	3.235	3.235	3.238	3.236	3.243	3.243	3.243	3.243	3.238	3.243
7 H	0.804	0.804	0.805	0.804	0.804	0.804	0.801	0.801	0.802	0.801	0.804	0.801
8 C	3.871	3.870	3.870	3.871	3.872	3.871	3.873	3.873	3.873	3.873	3.872	3.873
9 C	3.895	3.895	3.895	3.895	3.896	3.895	3.899	3.899	3.899	3.899	3.896	3.899
10 C	3.992	3.992	3.992	3.991	3.991	3.992	3.992	3.992	3.991	3.991	3.991	3.992
11 C	3.877	3.878	3.879	3.878	3.881	3.877	3.877	3.878	3.880	3.879	3.881	3.879
12 H	0.936	0.937	0.937	0.938	0.936	0.937	0.936	0.937	0.938	0.938	0.936	0.937
13 C	3.833	3.833	3.834	3.835	3.833	3.833	3.833	3.833	3.834	3.834	3.833	3.832
14 H	0.946	0.946	0.947	0.948	0.947	0.946	0.946	0.947	0.947	0.948	0.947	0.946
15 H	0.939	0.939	0.939	0.939	0.938	0.939	0.939	0.939	0.939	0.939	0.938	0.939
16 C	3.799	3.801	3.794	3.795	3.801	3.794	3.799	3.800	3.791	3.795	3.801	3.795
17 H	0.934	0.935	0.932	0.936	0.941	0.934	0.935	0.934	0.931	0.935	0.941	0.935
18 C	3.883	3.884	3.879	3.890	3.887	3.883	3.882	3.883	3.879	3.891	3.887	3.882
19 H	0.928	0.928	0.933	0.935	0.941	0.929	0.927	0.927	0.932	0.936	0.941	0.928
20 H	0.935	0.936	0.927	0.940	0.931	0.935	0.935	0.935	0.927	0.939	0.931	0.934
21 C	3.854	3.861	3.858	3.863	3.860	3.870	3.856	3.863	3.860	3.864	3.860	3.867
22 H	0.947	0.953	0.951	0.952	0.948	0.954	0.948	0.953	0.951	0.951	0.948	0.952
23 C	3.859	3.864	3.869	3.855	3.849	3.862	3.858	3.865	3.869	3.856	3.849	3.863
24 H	0.947	0.944	0.947	0.939	0.952	0.961	0.946	0.944	0.946	0.939	0.952	0.961
25 C	3.813	3.828	3.812	3.805	3.804	3.795	3.814	3.830	3.815	3.807	3.804	3.800
26 H	0.957	0.964	0.965	0.961	0.961	0.955	0.956	0.964	0.965	0.961	0.961	0.955
27 H	0.966	0.965	0.956	0.956	0.955	0.949	0.965	0.964	0.956	0.956	0.955	0.951
28 H	0.939	0.939	0.939	0.939	0.939	0.939	0.939	0.939	0.939	0.939	0.939	0.938
29 O	1.777	1.795	1.776	1.776	1.776	1.786	1.775	1.792	1.775	1.774	1.776	1.784
30 H	0.769	0.773	0.765	0.766	0.770	0.771	0.770	0.775	0.767	0.766	0.770	0.776
31 O	2.007	2.007	2.005	2.005	2.006	2.008	1.990	1.990	1.988	1.989	2.006	1.991

As expected, the higher values are observed in the O1, O4 and O31 atoms belonging to the C=O groups and to the ribose rings while the lower values are observed in the O2 and O29 atoms belonging to the OH groups because they are the most labile atoms. In relation to the N atoms, the higher bond order values are observed in the N5 atoms belonging to the NH groups because they are most labile while the higher values are observed in the N6 atoms belonging to the glycosidic bonds. In all the isomers the values are practically constant and only a slightly variation are observed in the values of both atoms in the C5 isomer. Analyzing the values for the C atoms, we observed that the C10 atoms in all the isomers present the higher values because these have double bond character while the lowest values are observed in the C16 atoms of the glycosidic bonds. All the values are practically constant in the

different isomers and only slightly modifications are observed in the C25 atoms of all the conformations. In reference to the H atoms, the lowest values are observed in the H3, H7 and H30 atoms because these atoms are labile due to that are linked to the N and O atoms and because they have a higher ability to form H bonds, in relation to the other ones.

3.3. NBO and QAIM studies

The stabilities of all the isomers of THY were studied using NBO and QAIM calculations [19,20] in both media and at B3LYP/6-31G* level of theory. The main delocalization energy values for the six isomers of thymidine in both phases are presented in **Table 10** while in **Figure 12** are represented their variations in function of the different isomers in gas phase.

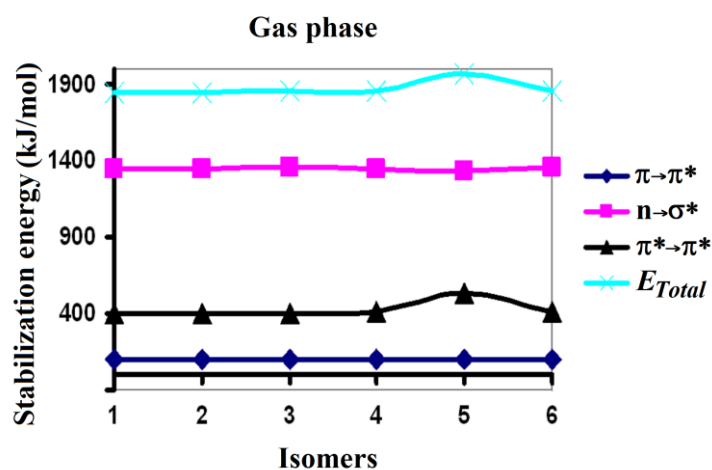


Figure 12. Main stabilization energies for the stable isomers of thymidine in gas phase at B3LYP/6-31G* level of theory.

Table 10. Main delocalization energy (in kJ/mol) for the six isomers of thymidine

B3LYP/6-31G* method												
Delocalization	Gas phase						Aqueous solution					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
$\pi C10-C11 \rightarrow \pi^* C9-$	98.23	98.23	98.44	102.16	97.73	97.56	102.49	102.62	102.58	102.16	97.73	101.82
$\Delta ET_{\pi \rightarrow \pi^*}$	98.23	98.23	98.44	102.16	97.73	97.56	102.49	102.62	102.58	102.16	97.73	101.82
$LP(2)O1 \rightarrow \sigma^* N5-C8$	114.99	114.99	115.33	109.93	113.57	115.53	109.47	109.31	109.39	109.93	113.53	109.81
$LP(2)O1 \rightarrow \sigma^* N6-C8$	106.76	106.84	107.43	102.45	106.42	107.00	102.28	102.37	102.49	102.45	106.42	102.45
$LP(2)O2 \rightarrow \sigma^* C21-H22$	25.08	27.46	25.71	22.24	22.36	26.54	26.17	26.42	25.54	22.24	22.36	23.20
$LP(1)N5 \rightarrow \sigma^* O1-C8$	241.39	241.35	242.73	256.94	252.14	241.64	255.23	254.27	256.98	256.94	252.18	254.35
$LP(1)N5 \rightarrow \sigma^* O4-C16$	39.71	35.95	39.21	27.13	2.55	42.47	38.08	34.36	37.03	27.13	2.55	39.54
$LP(1)N5 \rightarrow \sigma^* C10-C11$	150.86	151.19	152.61	155.04	159.47	150.40	152.61	153.15	154.62	155.04	159.47	152.36
$LP(1)N6 \rightarrow \sigma^* O1-C8$	257.44	256.82	255.23	263.63	264.72	258.91	262.92	260.75	261.54	263.63	264.72	263.46
$LP(1)N6 \rightarrow \sigma^* C9-O31$	211.55	211.55	213.18	222.83	210.34	211.55	222.12	222.63	223.42	222.83	210.34	222.21
$LP(2)O31 \rightarrow \sigma^* N6-C9$	122.52	122.52	122.01	113.36	123.27	122.51	113.44	113.32	113.19	113.36	123.27	113.44
$LP(2)O31 \rightarrow \sigma^* C9-C10$	77.79	77.83	77.71	73.48	77.83	77.91	73.36	73.36	73.40	73.48	77.83	73.44
$\Delta ET_{n \rightarrow \sigma^*}$	1348.09	1346.5	1351.15	1347.03	1332.67	1354.46	1355.68	1349.94	1357.63	1347.03	1332.67	1354.26
$\pi^* C9-O31 \rightarrow \pi^* C10-$	397.68	398.94	401.50	409.30	533.12	406.13	384.35	387.57	391.33	409.30	533.16	394.26
ΔET_{Total}	1844.00	1843.67	1851.09	1858.49	1963.52	1858.15	1842.52	1840.13	1851.51	1858.49	1963.56	1850.34

^aThis work

Note that there are three $\Delta ET_{\pi \rightarrow \pi^*}$, $\Delta ET_{n \rightarrow \sigma^*}$ and $\Delta ET_{\pi^* \rightarrow \pi^*}$ charge transfers that are related to the C10=C11 bonds, to the lone pairs corresponding to the O1, O2, O31, N5 and N6 atoms and, to the C9=O31 bonds, being the most significant the $\Delta ET_{n \rightarrow \sigma^*}$ contributions. All these delocalization energies contribute to the total energy of each isomer and, where we can see that in both media the total energy values favors to the C5 isomer being the stability order in both media the following: C5 > C4 > C6 > C3 > C1 > C2. The high values observed in the $\Delta ET_{n \rightarrow \sigma^*}$ charge transfers probably suggest that in all the isomers the pyrimidine rings are the

most stable than the ribose rings and, where the C6 isomer has the high value in gas phase while C3, in solution, as observed in **Table 10**.

In the previous study of the *Trans* C1, C2 and C3 conformations of THY [3] were observed different intra-molecular interactions in the three structures which are related to the H bonds formation and to the stabilities of their structures. Thus, C3 present three H bonds interactions, as observed in **Table 11** while, both C1 and C2, present only two interactions, this way, C3 is the most stable conformer than the other ones.

Table 11. An Analysis of the Bond Critical points (BCP) for the C1, C2 and C3 isomers of thymidine

B3LYP/6-31G* ^a							
Gas phase							
Parameter (a.u.)	C1		C2		C3		
	O2---H12	O1---H17	O2---H12	O1---H17	O2---H12	O1---H17	O29---H20
$\rho(r_c)$	0.0164	0.0204	0.0149	0.0201	0.0144	0.0194	0.0101
$\nabla^2\rho(r_c)$	0.0489	0.0801	0.0454	0.0793	0.0440	0.0780	0.0416
λ_1	-0.0189	-0.0220	-0.0168	-0.0216	-0.0162	-0.0206	-0.0078
λ_2	-0.0184	-0.0127	-0.0165	-0.0121	-0.0158	-0.0103	-0.0047
λ_3	0.0864	0.1149	0.0787	0.1130	0.076	0.1089	0.0541
$ \lambda_1 /\lambda_3$	0.2188	0.1915	0.2135	0.1912	0.2132	0.1892	0.1442
Distances (Å)	2.243	2.199	2.293	2.208	2.303	2.229	2.551
Aqueous solution							
Parameter (a.u.)	C1		C2		C3		
	O2---H12	O1---H17	O2---H12	O1---H17	O2---H12	O1---H17	O29---H20
$\rho(r_c)$	0.0161	0.0188	0.0150	0.0187	0.0133	0.0185	0.0082
$\nabla^2\rho(r_c)$	0.0483	0.0752	0.0458	0.0749	0.0411	0.0750	0.0344
λ_1	-0.0185	-0.0197	-0.0170	-0.0196	-0.0146	-0.0192	-0.0052
λ_2	-0.0181	-0.0096	-0.0166	-0.0095	-0.0143	-0.0086	-0.0023
λ_3	0.0849	0.1046	0.0794	0.1041	0.0700	0.1029	0.0417
$ \lambda_1 /\lambda_3$	-0.2179	-0.1883	-0.2141	-0.1883	-0.2086	-0.1866	-0.1247
Distances (Å)	2.252	2.247	2.287	2.249	2.344	2.256	2.680

^aFrom Ref [3]

Later, it is very important to analyze the topological properties for the C4, C5 and C6 isomers in the bond critical points (BCPs) in order to investigate their stabilities in both media. Thus, for those three isomers the electron density distribution, $\rho(r)$, the values of the Laplacian, $\nabla^2\rho(r)$, the eigenvalues (λ_1 , λ_2 , λ_3) of the Hessian matrix and, the λ_1/λ_3 ratio were also computed by using the AIM2000 program [20]. These parameters describe the characteristic of the interactions, hence, the interaction is covalent when $\lambda_1/\lambda_3 > 1$, $\nabla^2\rho(r) < 0$ and

moreover has high values of $\rho(r)$ and $\nabla^2\rho(r)$. On the contrary, the interaction is ionic, highly polar covalent or of hydrogen bonds when $\lambda_1/\lambda_3 < 1$ and $\nabla^2\rho(r) > 0$ [37]. The calculated parameters for the C4, C5 and C6 isomers of THY are presented in **Table 12** where we observed that both *Cis* C4 and C6 isomers show three H bonds interactions in the two media while in C5 only an interaction in each medium is observed.

Table 12. An Analysis of the Bond Critical points (BCP) for the C4, C5 and C6 isomers of thymidine

B3LYP/6-31G*						
C4						
Parameter (a.u.)	Gas phase			Aqueous solution		
	O1---H17	O29---H12	O29---H19	O1---H17	O29---H12	O29---H19
$\rho(r_c)$	0.0186	0.0109	0.0084	0.0180	0.0085	0.0072
$\nabla^2\rho(r_c)$	0.0772	0.0346	0.0334	0.0760	0.0288	0.0293
λ_1	-0.0192	-0.0118	-0.0066	-0.0183	-0.0088	-0.0054
λ_2	-0.0076	-0.0115	-0.0045	-0.0061	-0.0086	-0.0030
λ_3	0.1039	0.0579	0.0445	0.0999	0.0461	0.0377
$ \lambda_1 /\lambda_3$	0.1848	0.2038	0.1483	0.1832	0.1909	0.1432
Distances (Å)	2.258	2.438	2.605	2.278	2.551	2.678
C5						
Parameter (a.u.)	Gas phase		Aqueous solution			
	O4---H12		O4---H12			
$\rho(r_c)$	0.0186		0.0186			
$\nabla^2\rho(r_c)$	0.0744		0.0744			
λ_1	-0.0196		-0.0196			
λ_2	-0.0113		-0.0113			
λ_3	0.1054		0.1054			
$ \lambda_1 /\lambda_3$	0.1860		0.1860			
Distances (Å)	2.255		2.255			
C6						
Parameter (a.u.)	Gas phase			Aqueous solution		
	O1---H17	O2---H12	O29---H3	O1---H17	O2---H12	O29---H3
$\rho(r_c)$	0.0201	0.0165	0.0196	0.0186	0.0144	0.0095
$\nabla^2\rho(r_c)$	0.0795	0.0494	0.067	0.0750	0.0445	0.0422
λ_1	-0.0216	-0.0190	-0.0240	-0.0195	-0.0162	-0.0064
λ_2	-0.0120	-0.0185	-0.0203	-0.0091	-0.0158	-0.0007
λ_3	0.1131	0.0869	0.1111	0.1037	0.0765	0.0495
$ \lambda_1 /\lambda_3$	0.1910	0.2186	0.2160	0.1880	0.2118	0.1293
Distances (Å)	2.208	2.237	2.119	2.252	2.294	2.472

Comparing the O--H bonds distances in all the isomers we observed that in aqueous solution these distances undergo increase as a consequence of the hydration of each isomer with water molecules. Besides, these interactions are very different in the six isomers, for example, in C3 the interactions are O2---H12, O1---H17 and O29---H20 while the three observed in C4 are O1---H17, O29---H12 and O29---H20 and, finally the three observed in C6 are the O1---H17, O2---H12 and O29---H3 interactions. Analyzing the values of the $\rho(r)$ and $\nabla^2\rho(r)$ properties for the common interaction, these are the O1---H17 interactions observed in all the isomers, with exception of C5, we can see of **Figure 13** that the behaviours of their density values are

similar in both media, having C4 the lower values in both media. On the other hand, the behaviours of the Laplacian values of those isomers in gas phase are different from those observed in aqueous solution, thus while C4 shows the low value in gas phase, in solution present the higher value.

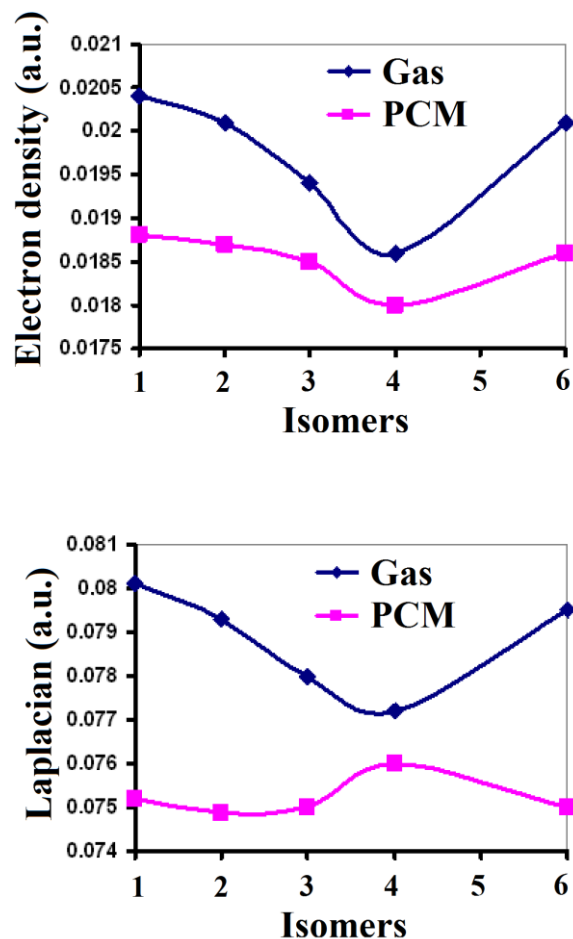


Figure 13. Electron density distribution, $\rho(r)$ and the Laplacian, $\nabla^2\rho(r)$ values for the stable isomers of thymidine in gas phase at B3LYP/6-31G* level of theory.

Probably, the low density values that present the O1---H17 interactions in C3, C4 and C6 in aqueous solution probably can in part justify their higher populations, as observed in Table 1. Therefore, this study clearly reveals that the C3, C4 and C6 isomers are the most stable isomers of THY in both media.

3.4. HOMO-LUMO and descriptors

The calculations of the frontier orbitals in molecules with great biological importance such as in the thymidine isomers these parameters are necessary to predict their reactivities and behaviours in different media, as reported by many authors [12-18]. Thus, in this work, we have computed those orbitals and some interesting descriptors for C4, C5 and C6 because for C1, C2 and C3 they were already determined in a previous work [3]. To comparison, in Table 13 are summarized the results for all the isomers in both media while in **Figure 14** are represented the calculated electronegativity (χ), global electrophilicity index (ω) and global nucleophilicity index (E) for these six isomers in both phase at the same of theory.

Table 13. Calculated HOMO and LUMO orbitals, energy band gap, chemical potential (μ), electronegativity (χ), global hardness (η), global softness (S), global electrophilicity (ω) and nucleophilic (E) indexes for the six isomers of thymidine at B3LYP/6-31G* level of theory

Gas phase						
Orbitals	C1 ^b	C2 ^b	C3 ^b	C4 ^a	C5 ^a	C6 ^a
HOMO	-6.2014	-6.1878	-6.1061	-6.1658	-6.2027	-6.2332
LUMO	-0.7184	-0.7020	-0.6313	-0.7134	-0.7762	-0.7606
GAP	-5.4830	-5.4858	-5.4748	-5.4524	-5.4265	-5.4726
Descriptors (eV)						
χ	-2.7415	-2.7429	-2.7374	-2.7262	-2.7133	-2.7363
μ	-3.4599	-3.4449	-3.3687	-3.4396	-3.4895	-3.4969
η	2.7415	2.7429	2.7374	2.7262	2.7133	2.7363
S	0.1824	0.1823	0.1826	0.1834	0.1843	0.1827
ω	2.1833	2.1633	2.0728	2.1698	2.2439	2.2345
E	-9.4853	-9.4490	-9.2215	-9.3770	-9.4678	-9.5686
Aqueous solution ^a						
Orbitals	C1 ^b	C2 ^b	C3 ^b	C4 ^a	C5 ^a	C6 ^a
HOMO	-6.2201	-6.1758	-6.1128	-6.1919	-6.2027	-6.2634
LUMO	-0.7734	-0.7297	-0.6800	-0.7758	-0.7760	-0.8206
GAP	-5.4467	-5.4461	-5.4328	-5.4161	-5.4267	-5.4428
Descriptors (eV)						
χ	-2.7233	-2.7230	-2.7164	-2.7081	-2.7134	-2.7214
μ	-3.4967	-3.4527	-3.3964	-3.4839	-3.4894	-3.5420
η	2.7233	2.7230	2.7164	2.7081	2.7134	2.7214
S	0.1836	0.1836	0.1841	0.1846	0.1843	0.1837
ω	2.2449	2.1890	2.1233	2.2410	2.2436	2.3050
E	-9.5229	-9.4020	-9.2260	-9.4344	-9.4678	-9.6392

^aThis work, ^bFrom Ref [3]

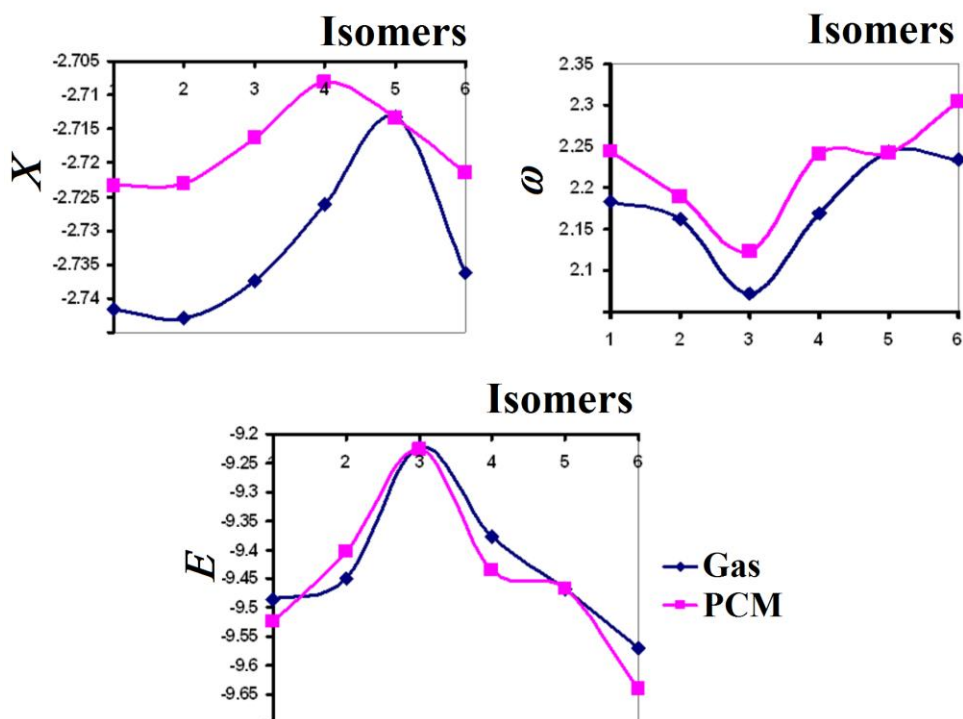


Figure 14. Calculated electronegativity (χ), global electrophilicity index (ω) and global nucleophilicity index (E) for the six isomers of thymidine in both phase at B3LYP/6-31G* level of theory.

The equations to calculate the descriptors were presented in other above papers [4-6] and, for this reason, they were not described here. The exhaustive analysis of the separation between both frontier orbitals for each isomer, these are the gap values in gas phase are different from those observed in aqueous solution. Thus, in gas phase the decreasing of this separation follow the order: C2 > C1 > C3 > C6 > C4 > C5 while in solution change to: C1 > C2 > C6 > C3 > C5 > C4. Note that the gap values of all the isomers decrease in solution which implies that they are more reactive in this medium independently of the *Cis* or *Trans* conformation that present the isomer. This way, the C2 isomer in gas phase is the most stable and the less reactive while C5 is the most reactive and the less stable than the other ones. On the other hand, in solution the most reactive and the less stable is C4 while C1 is the most stable and the less reactive in water. These results show that both *Cis* isomers are most reactive in gas phase while in solution C4 is most reactive than C6. The behaviors of the calculated electronegativity (χ), global electrophilicity index (ω) and global nucleophilicity index (E) descriptors for the six isomers of thymidine in gas phase are different from in aqueous solution, as observed in Figure 14. This way, the χ descriptors present higher negative values

in gas phase while all the values decrease in solution, having C5 the lower value in gas phase and C4 in solution. These results are in complete concordance with their higher reactivities in each medium. Analyzing the ω indexes we observed that C3 has the lower values in both media while the higher values are observed for C1 and C6 in the two media. Note that C4 and C5 have practically the same ω values in solution but different in gas phase. The nucleophilicity values show that the lower values are observed for C3 in both media while C1 and C6 present the most negative values in those media. In solution, we also observed that C4 and C5 have practically the same E indexes. This study shows clearly that the three χ , ω and E indexes are factors very important to predict the reactivity of an isomer of thymidine in a medium determined, thus, C4 and C5 are the most reactive in solution due to that both isomers have practically the same ω and E values but low χ values.

3.5. Vibrational study

For this analysis, only the structures of the C3, C4 and C6 isomers with higher populations have been considered, in accordance with the values observed in **Table 1**. Structurally, these isomers were optimized with C_1 symmetries and they have 87 normal vibration modes where all the modes are active in both infrared and Raman spectra. The calculations of their force fields at B3LYP/6-31G* level of approximation were performed in both media by using the SQMFF methodology, the internal normal coordinates already reported for C1, C2 and C3 in a previous work [3] and the Molvib program [23,24]. Later, the assignments of the available infrared and Raman spectra [22,38] were carrying out taking into account only the Potential Energy Distribution (PED) contributions $\geq 10\%$. Comparisons among the available experimental spectra and the corresponding predicted by calculations for the three isomers in different regions can be seen from **Figure 15** to **18** while the calculated and observed positions of the bands together with their tentative assignments are shown in **Table 14**. It is necessary to clarify that the isomers C3, C4 and C6 were observed experimentally by those three authors [33-35] and predicted by Ivanov *et al.* [36]. For these reasons, all the comparisons presented in those figures show the presence of various isomers of THY, thus, between 3100 and 2900 cm^{-1} there are very good concordance among the experimental spectrum and those corresponding to the C4 and C6 isomers while in the 1900-1300 cm^{-1} region the intensities of the bands show the probable presence of the three isomers and maybe of some dimeric species, as was experimentally reported for the *Cis* structure at low temperatures by Görbitz *et al.* [33] and by Young *et al.* [34].

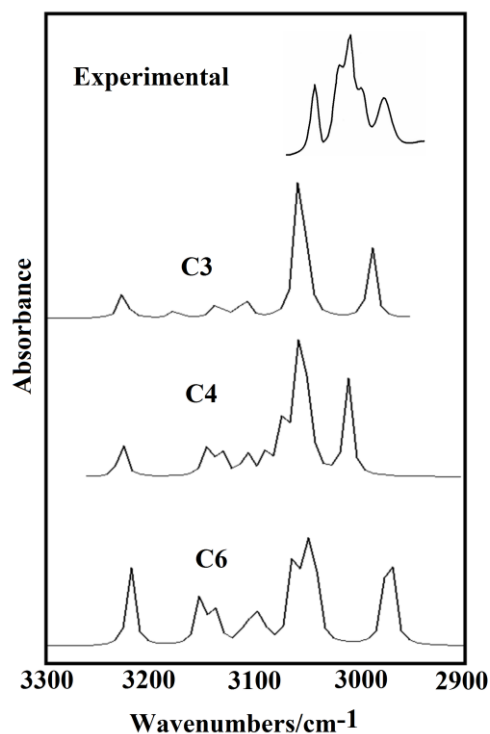


Figure 15. Comparison between the experimental Infrared spectra of thymidine in the 3300-2900 cm^{-1} region from Ref [22] with the corresponding theoretical for the C3, C4 and C6 isomers in gas phase.

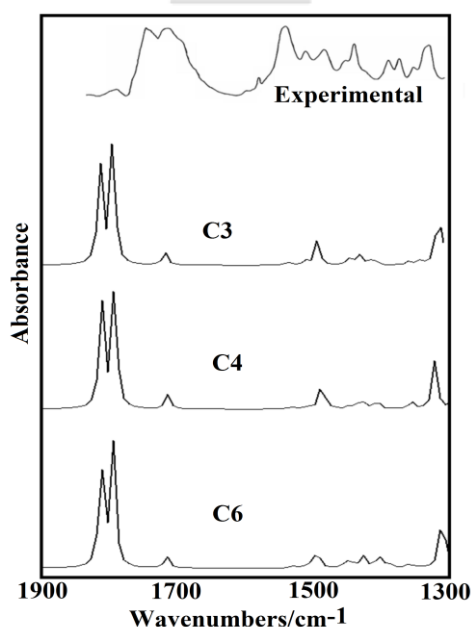


Figure 16. Comparison between the experimental Infrared spectra of thymidine in the 1900-1300 cm^{-1} region from Ref [22] with the corresponding theoretical for the C3, C4 and C6 isomers in gas phase.

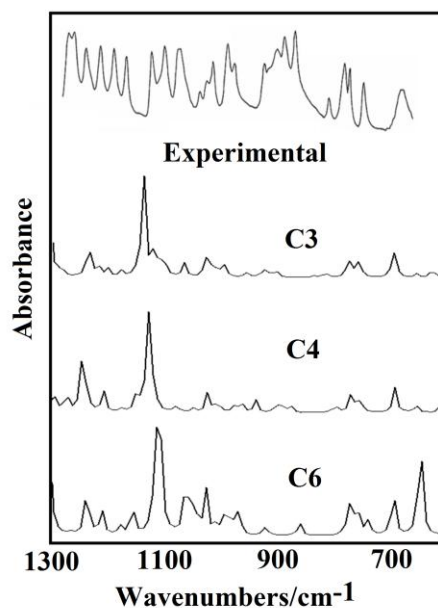


Figure 17. Comparison between the experimental Infrared spectra of thymidine in the 1300-600 cm⁻¹ region from Ref [22] with the corresponding theoretical for the C3, C4 and C6 isomers in gas phase.

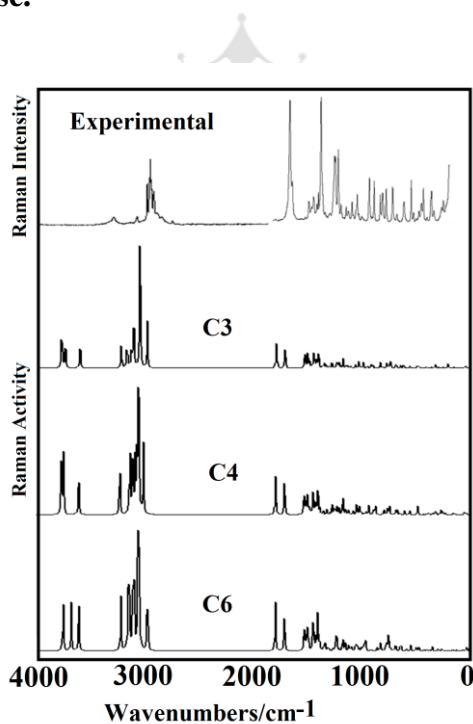


Figure 18. Comparison between the experimental Raman spectra of thymidine in the 4000-10 cm⁻¹ region from Ref [38] with the corresponding theoretical for the C3, C4 and C6 isomers in gas phase.

Table 14. Observed and calculated wavenumbers (cm⁻¹) and assignments for the isomers of thymidine in gas phase and aqueous solution

Experimental*	C3*				C4*				C6*				
	Gas phase		Aqueous solution		Gas phase		Aqueous solution		Gas phase		Aqueous solution		
Ir	Raman*	SQM*	Assignment	SQM*	Assignment	SQM*	Assignment	SQM*	Assignment	SQM*	Assignment	SQM*	Assignment
3641*													
3620*		3616	νO29-H30	3589	νO29-H30	3606	νO29-H30	3586	νO29-H30	3591	νO29-H30	3555	νO29-H30
3482*		3585	νO2-H3	3571	νO2-H3	3588	νO2-H3	3576	νO2-H3	3520	νO2-H3	3554	νO2-H3
3428*	3306(10)	3455	νN6-H7	3422	νN6-H7	3454	νN6-H7	3431	νN6-H7	3454	νN6-H7	3425	νN6-H7
3130*	3157(1)	3089	νC11-H12	3100	νC11-H12	3092	νC11-H12	3109	νC11-H12	3084	νC11-H12	3097	νC11-H12
3075*	3092(9)	3042	νCH2(C18)	3044	νC16-H17	3015	νC16-H17	3025	νCH2(C18)	3022	νCH2(C18)	3030	νC16-H17
2994	2996(50)	3030	νC16-H17	3034	νCH2(C18)	3002	νCH2	3016	νCH2	3017	νCH2(C25)	3019	νCH2(C18)
2977	2975(54)	3003	νCH2	3014	νCH2	2999	νCH2(C18)	3012	νC16-H17	3013	νC16-H17	3007	νCH2(C25)
2970	2967(63)	2985	νCH2(C18)	2989	νCH2(C18)	2978	νCH2	2976	νCH2	3005	νCH2	3006	νCH2
		2977	νCH2	2976	νCH2	2959	νCH2(C18)	2972	νCH2(C18)	2978	νCH2	2976	νCH2(C18)
2964	2956(55)	2930	νC21-H22	2969	νC23-H24	2942	νC23-H24	2955	νC23-H24	2965	νCH2(C18)	2976	νCH2
2934	2935(40)	2927	νCH2(C25)	2954	νC21-H22	2928	νCH2(C25)	2954	νC21-H22	2935	νCH2(C25)	2953	νC21-H22
		2926	νCH2	2938	νCH2(C25)	2926	νCH2	2946	νCH2(C25)	2927	νCH2	2930	νCH2(C25)
2902	2905(14)	2920	νC23-H24	2927	νCH2	2920	νC21-H22	2927	νCH2	2918	νC21-H22	2925	νCH2
	2861(10)	2861	νCH2(C25)	2901	νCH2(C25)	2884	νCH2(C25)	2908	νCH2(C25)	2848	νC23-H24	2884	νC23-H24
1748	1810(2)	1744	νC8-O1			1744	νC8-O1			1745	νC8-O1	1703	νC8-O1
1700	1690(15)	1725	νC9-O31	1666	νC8-O1	1724	νC9-O31	1666	νC8-O1	1727	νC9-O31	1675	νC9-O31
1664	1665(89)	1648	νC10-C11	1643	νC10-C11	1646	νC10-C11	1642	νC10-C11	1648	νC10-C11		
	1643(40)			1621	νC9-O31			1620	νC9-O31			1636	νC10-C11
	1627(5)												
1513	1516(2)	1472	δCH2(C25)	1464	δCH2(C25)	1473	δCH2(C25)	1465	δCH2(C25)	1467	δCH2(C25)	1473	δCH2(C25)
1478	1483(20)	1459	δaCH2	1449	νC9-C10	1459	δaCH2	1446	νN5-C11	1459	δaCH2	1464	δaCH2 νC9-C10
1456	1459(15)	1441	δCH2(C18)	1435	δaCH2	1442	δCH2(C18)	1441	wagCH2(C25)	1443	δCH2(C18) ρCH(C16)	1452	δaCH2
1435	1437(23)	1436	δaCH2	1432	wagCH2(C25)	1436	νN5-C8	1431	δaCH2	1436	δaCH2	1442	δCH2(C18)
1411	1414(8)	1434	νN5-C8	1427	δCH2(C18)	1435	δaCH2	1426	δCH2(C18)	1436	δCH2(C18)	1437	δaCH2
1402	1404(13)	1428	wagCH2(C25)	1423	δaCH2	1431	wagCH2(C25)	1418	δaCH2	1406	wagCH2(C25)	1409	wagCH2(C25)

ν, stretching; δ, scissoring; wag, wagging or out- of plane deformation; ρ, rocking; τ, torsion, twist, twisting; a, antisymmetric; s, symmetric ; ip, in-phase; op, out-of-phase; R, ring; pyrimidine ring, (A6); sugar ring, (A5), ^aThis work, ^bFrom Ref [3], ^cFrom Ref [22], ^dFrom scaled quantum mechanics force field B3LYP/6-31G*, ^eFrom scaled quantum mechanics force field PCM/B3LYP/6-31G*, [#]From Ref [36].

In general, we observed that those vibration modes related to the chiral C16 atoms of the three isomers are predicted at higher wavenumbers than the corresponding to the chiral C23 atoms, thus, the C16-H17 stretching modes are predicted between 3044 and 3012 cm^{-1} while the C23-H24 stretching modes between 2964 and 2861 cm^{-1} . A similar behaviour is observed in the rocking modes related to those two C atoms. The assignments only of some important groups are discussed and presented below.

3.5. 1. Assignments

3.5.1.1. OH groups. In this work, the two OH stretching modes belonging to the ribose ring can be assigned to the IR bands observed between 3641 and 3428 cm^{-1} while the band at 3428 cm^{-1} is assigned to the N6-H7 stretching modes of all the isomers of thymidine, as in the previous work [3]. The IR bands at 1288, 1197 and 1173 cm^{-1} were assigned to the OH in-plane deformation modes of the C1, C2 and C3 isomers [3], hence, in C4 and C6, one of them can be assigned between 1319 and 1277 cm^{-1} and the remain to those two latter bands, as observed in **Table 14**. The corresponding out-of-plane deformation or torsion modes can be assigned between 474 and 180 cm^{-1} , in according to the reported paper [3].

3.5.1.2. CH₃ groups. The antisymmetric and symmetric stretching modes of these groups for the all the isomers of thymidine can be assigned to the IR and Raman bands between 2994 and 2902 cm^{-1} while the corresponding antisymmetric and symmetric deformation modes are assigned to the set of bands between 1478 and 1366 cm^{-1} . Note that the intense Raman band at 1366 cm^{-1} is easily assigned to the symmetric deformation modes. The groups of IR and Raman bands between 1052 and 1000 cm^{-1} are assigned to the rocking modes, as was previously reported [3] while the Raman bands observed between 175 and 100 cm^{-1} are assigned to the expected twisting modes, as reported for C1, C2 and C3 [3].

3.5.1.3. CH₂ groups. The two expected stretching modes were predicted between 3042 and 2861 cm^{-1} ; accordingly, they are associated to the IR bands observed in the 3075-2902 cm^{-1} region. As predicted by calculations, the scissoring or deformation modes can be assigned between 1513 and 1435 cm^{-1} , the wagging modes between 1456 and 1292 cm^{-1} and the rocking modes between 1288 and 1173 cm^{-1} in similar form as was assigned for C1, C2 and C3 in the previous work [3]. The IR and Raman bands between 870 and 663 cm^{-1} are assigned to the twisting modes, as predicted by the SQM calculations and, as observed in Table 14.

3.5.1.4. Skeletal groups. The two C=O stretching modes expected for all the isomers of THY are predicted by SQM calculations in the 1744-1620 cm^{-1} region, thus, they are assigned to higher wavenumbers than the C=C stretching modes. Only for the C3 and C4 isomers in aqueous solution, the C10=C11 modes are predicted at higher wavenumbers than the C9=O31 stretching modes, as observed in Table 14. In relation to the C-N stretching modes, we observed that in all the isomers, the modes related to the pyrimidine rings appear at higher wavenumbers than those corresponding to the ribose rings, especially, the N5-C16 stretching modes related to the chiral C16 atoms. This way, these modes can be assigned to the Raman bands at 1102 cm^{-1} , with exception of C5 in solution, which is assigned to the Raman band at 898 cm^{-1} because in this isomer that mode is predicted at 890 cm^{-1} . Analyzing the C-O stretching of the ribose rings, we observed that the C23-O4 stretching modes are predicted at higher wavenumbers than the C16-O4 stretching modes, as expected because the calculations predicted the O4-C23 bond longer than the O4-C16 bond, as can be seen in **Table 4**. The vibration deformation and torsion modes of both rings are predicted for C3, C4 and C6 approximately in the same regions and, in some cases, they are observed coupled with other modes, as observed in Table 4. The remaining deformation, rocking and torsion modes were also assigned according to the Scaled Quantum Mechanical (SQM) calculations, as indicated in Table 4.

3.6. Force fields

The force constants for the C1, C2 and C3 isomers of thymidine in gas and aqueous solution phases were previously determined [3] using their corresponding scaled force fields at B3LYP/6-31G* level of theory and the Molvib program [24]. Here, we have calculated those force constants for the C4, C5 and C6 isomers expressed in valence internal coordinates using the above procedure. The results for all the isomers of THY can be seen in **Table 15**. First, we observed a decreasing in some force constant values of all the isomers in solution, in relation to the values in gas phase, with exception of those calculated for C5 because their values remain without change in solution. This fact can be explained due to that the optimized coordinates practically remain constant in both media and, their properties are not modified in solution, as was previously studied. In this work, we observed that the $f(O-H)$, $f(\nu N-H)$, $f(\nu C=O)$ and $f(\nu C-O)$ force constants values for C4 and C6 also decrease in solution as a consequence of the hydration in these groups in form similar to the previously observed for C1, C2 and C3. However, the $f(\nu C-N)$, $f(\nu C-C)_{A6}$ and $f(\nu C-C)_{A5}$ force constants increase

in solution probably are related with the shortening of their bonds in solution, with exception of C6 where a slightly decreasing in the $f(\nu C-N)$ is observed in solution. Thus, these variations can be attributed to the calculated geometrical parameters in the different media.

Table 15. Scaled force constants for the six stable isomers of thymidine in gas and aqueous solution phases.

B3LYP/6-31G* ^a						
Gas phase						
Force	C1 ^b	C2 ^b	C3 ^b	C4	C5	C6
$f(\nu O-H)$	7.24	7.23	7.25	7.24	7.28	7.07
$f(\nu N-H)$	6.62	6.62	6.62	6.62	6.62	6.62
$f(\nu C-H)_{A6}$	5.23	5.26	5.22	5.24	5.34	5.20
$f(\nu C-H)_{A5}$	4.85	4.80	4.83	4.81	4.76	4.72
$f(\nu C=C)$	8.17	8.17	8.17	8.15	8.07	8.18
$f(\nu C=O)$	11.62	11.62	11.63	11.62	11.56	11.64
$f(\nu C-O)_{A5}$	4.47	4.47	4.48	4.48	4.51	4.43
$f(\nu C-O)_{OH}$	4.91	4.98	4.88	4.88	4.97	4.88
$f(\nu C-N)$	5.38	5.39	5.38	5.40	5.37	5.38
$f(\nu C-C)_{A6}$	4.88	4.87	4.88	4.87	4.87	4.87
$f(\nu C-C)_{A5}$	3.87	3.84	3.86	3.88	3.90	3.93
$f(\delta H-C-H)$	0.78	0.77	0.77	0.77	0.77	0.77
$f(\delta C-O-H)$	0.71	0.71	0.70	0.70	0.70	0.74
Aqueous solution						
$f(\nu O-H)$	7.17	7.16	7.17	7.17	7.28	7.07
$f(\nu N-H)$	6.52	6.51	6.49	6.53	6.62	6.51
$f(\nu C-H)_{A6}$	5.22	5.23	5.25	5.29	5.34	5.25
$f(\nu C-H)_{A5}$	5.08	5.08	5.08	4.87	4.76	5.03
$f(\nu C=C)$	8.07	8.05	8.09	8.07	8.07	8.10
$f(\nu C=O)$	10.49	10.50	10.50	10.50	11.56	10.78
$f(\nu C-O)_{A5}$	4.29	4.24	4.26	4.21	4.51	4.29
$f(\nu C-O)_{OH}$	4.76	4.75	4.74	4.74	4.97	4.81
$f(\nu C-N)$	5.44	5.44	5.45	5.48	5.37	5.23
$f(\nu C-C)_{A6}$	5.13	5.12	5.12	5.11	4.87	5.14
$f(\nu C-C)_{A5}$	3.93	3.91	3.91	3.95	3.90	3.94
$f(\delta H-C-H)$	0.76	0.76	0.76	0.76	0.77	0.77
$f(\delta C-O-H)$	0.72	0.71	0.73	0.73	0.70	0.74

ν , stretching; δ angle deformation.

Units in $\text{mdyn } \text{Å}^{-1}$ for stretching and $\text{mdyn } \text{Å rad}^{-2}$ for angle deformations

^aThis work, ^bFrom Ref [3]

3.7. NMR study

In this analysis, we only have considered the C3, C4 and C6 isomers because these are the conformations with higher populations in aqueous solution and, also, because they were experimentally observed by different authors [33-36]. Thus, the predicted ¹H-NMR and ¹³C-

NMR spectra for those three isomers of THY in aqueous solution and their corresponding chemical shifts were computed by using the GIAO method [32] at the B3LYP/6-31G* level of theory. These results for the hydrogen and C atoms were compared with the experimental data taken from the literature for thymidine in DMSO-*d*₆ and in D₂O [39,40] by means of RMSD values which can be seen in **Tables 16** and **17**, respectively.

Table 16. Calculated hydrogen chemical shifts (δ , in ppm) for the C3, C4 and C6 isomers of thymidine in aqueous solution

B3LYP/6-31G*Method ^a				Experimental
Atoms	C3	C4	C6	DMSO- <i>d</i> ₆ ^d
3-H	0.98	0.65	2.19	5.25
7-H	6.69	6.68	6.70	11.3
12-H	8.05	7.85	8.03	7.71
14-H	1.69	1.57	1.73	1.78
15-H	2.43	2.42	2.42	1.78
17-H	6.95	6.93	6.62	6.18
19-H	2.33	2.44	2.44	2.08
20-H	3.28	2.09	2.75	2.08
22-H	4.94	5.00	4.68	4.26
24-H	4.25	4.52	3.98	3.78
26-H	3.96	4.23	4.39	3.55
27-H	4.27	4.44	4.35	3.60
28-H	2.42	2.41	2.42	1.78
30-H	0.69	0.81	0.47	5.04
RMSD^b	2.12	2.14	1.97	

^aThis work/GIAO method Ref. to TMS.

^bFrom Ref [39]

Table 17. Calculated hydrogen chemical shifts (δ , in ppm) for the C3, C4 and C6 isomers of thymidine in aqueous solution

B3LYP/6-31G* Method ^a				Experimental
Atoms	C3	C4	C6	D ₂ O ^b
8-C	152.16	151.73	152.11	152.42
9-C	163.99	163.88	163.91	167.17
10-C	117.33	116.90	118.01	112.17
11-C	143.16	141.27	142.56	138.28
13-C	26.22	26.38	26.11	12.34
16-C	96.45	95.46	94.71	85.88
18-C	51.69	54.14	49.38	39.36
21-C	85.36	84.86	82.16	71.26
23-C	100.17	95.24	92.90	87.34
25-C	75.84	75.10	71.66	62.01
RMSD^b	10.35	9.84	8.21	

^aThis work/GIAO method Ref. to TMS

^bFrom Ref [40]

The analysis of the calculated chemical shifts for all the H atoms of the C3, C4 and C6 isomers shows in general a very good correlation with the experimental values, with exception of the H3, H7 and H30 atoms, due to that probably these atoms belonging to the N6-H7, O2-H3 and O29-H30 groups could be involved in the H bonds formation in solution, as was before observed by MEP and AIM calculations. Note that the C6 isomer presents a better correlation than the other ones which maybe could explain their presence in solution. When the calculated chemical shifts for all the C atoms are analyzed we observed that for the C atoms belonging to the pyrimidine rings there are a better correlation than those corresponding to the ribose rings. Hence, this observation could be explained by the presence of various isomers in solution. The representation of the calculated chemical shifts for the H and C atoms in function of the corresponding experimental ones taken from Refs [39,40] are shown in **Figure 19**. Both graphics show clearly the differences observed between the theoretical and experimental chemical shifts showing apparently the C atoms a better concordance.

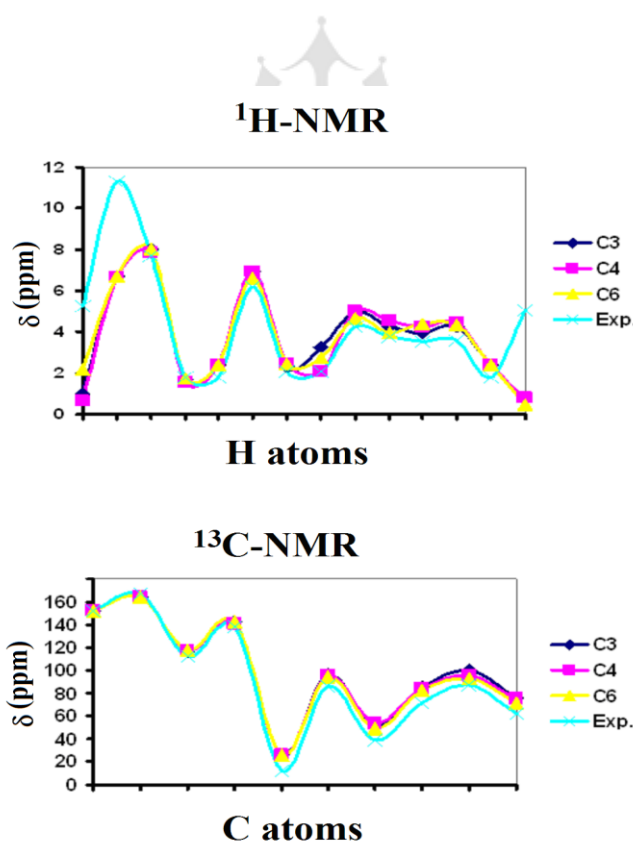


Figure 19. The calculated chemical shifts for the C3, C4 and C6 isomers of thymidine in aqueous solution at B3LYP/6-31G* level of theory compared with the corresponding experimental ones taken from Refs [39,40].

The differences observed could be attributed to the solvent, because the calculations were performed in aqueous solution instead DMSO- d_6 and in D_2O , to the 6-31G* basis set used instead 6-311++G** basis set, to the presence of various isomers in solution and, to the H bonds formation that probably shift the experimental peaks toward higher or lower fields, in relation to the theoretical predicted by calculations.

3.8. Electronic spectra

For the C3, C4 and C6 isomers of thymidine in aqueous solution the electronic spectra were also predicted by using the Time Dependent Density Functional Theory (TD-DFT) calculations with the B3LYP/6-31G* method and the Gaussian program [29]. The positions of the observed bands in the experimental spectrum of thymidine in water, taken from Ref [27], and those calculated for the three isomers can be seen in **Table 18** together with their corresponding assignments. Experimentally only two bands are observed due to that the spectrum was recorded in the 200-350 nm region. On the other hand, **Figure 20** shows the comparisons between that experimental electronic spectra of THY in water with the predicted for the C3, C4 and C6 isomers in aqueous solution at B3LYP/6-31G* level of calculation. In general, there is a very good concordance between those spectra in solution. The assignment was performed with the aid of the NBO calculations because of the $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$ or $n \rightarrow \pi^*$ and $\pi^* \rightarrow \pi^*$ charge transfers are expected for the thymidine isomers in aqueous solution. Thus, the most intense bands can be related to the $n \rightarrow \sigma^*$ or $n \rightarrow \pi^*$ charge transfers which are predicted with higher energy values while the other two could be assigned to the $\pi \rightarrow \pi^*$ and $\pi^* \rightarrow \pi^*$ charge transfers, as indicated in **Table 18**.

Table 18. TD-DFT calculated visible absorption wavelengths (nm) for the C3, C4 and C6 isomers of thymidine in aqueous solution compared with the corresponding experimental one in the same medium.

B3LYP method ^a				
Experimental	Thymidine ^a			Assignment ^a
Water ^b	C3	C4	C6	
	127.77 s	128.44 s	122.93 sh	$n \rightarrow \sigma^*$ O1, O2, O3I
	140.81 s	139.06 s	140.94 s	
	165.13 vs	165.14 vs	163.79 vs	$n \rightarrow \pi^*$ N5, N6
211	202.96 m	197.25 m	203.13 m	$\pi^* \rightarrow \pi^*$ C9=O3I
268	250.36 m	251.12 m	249.79 m	$\pi \rightarrow \pi^*$ C=C

^aThis work, ^bRef. [27]

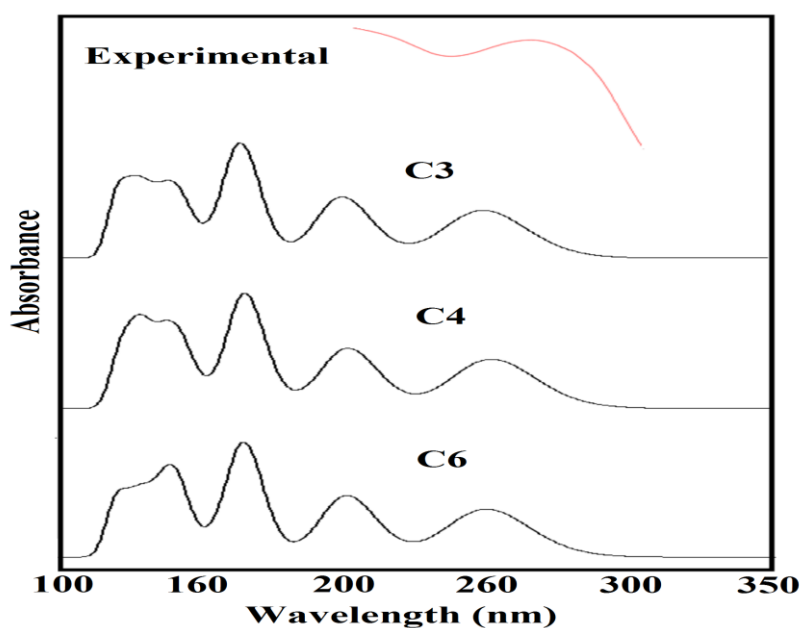


Figure 20. Comparisons between the experimental electronic spectra of thymidine in water with the corresponding predicted for the C3, C4 and C6 isomers in aqueous solution at B3LYP/6-31G* level of calculation.

Obviously, we observed that each isomer cannot be completely differentiated with this study because the three isomers show theoretically five bands in the 200-350 nm region. Note that C6 presents a shoulder at lower wavelength instead of good defined bands as in C3 and C4. This fact could be related to the $LP(1)N5 \rightarrow \sigma^*O1-C8$ and $LP(1)N5 \rightarrow \sigma^*C10-C11$ charge

transfers observed in this isomer with lowest values than the values corresponding to C3 and C4, as shown **Table 18**.

4. CONCLUSIONS

In this study, the theoretical molecular structures of two *Cis* isomers of THY, named C4 and C6 and, of a *Trans* isomers named C5 were determined in gas phase and in aqueous solution combining the hybrid B3LYP/6-31G* method with the PCM model. Here, the comparisons of their structural and vibrational properties were presented together with the corresponding to other three *Trans* isomers previously published. The population analyses suggest the presence in solution of a *Trans* isomer C3 and of two *Cis* isomers C4 and C6 in accordance with the experimental structures reported by X-ray diffraction by different authors. On the contrary, in gas phase, only the presence of the C6 isomer is expected. On the other hand, the higher populations observed in the *Cis* C4 and C6 isomers in solution could explain their volume expansions while C3 do not undergo variation in solution. The study of the charges shows that the MK charges have higher variations in both media and clearly these show the differences between the *Cis* and *Trans* isomers while the NPA charges remaining practically constant in all the isomers. The MK charges on the O4 atoms belonging to the ribose rings in all the structures, on the N5 atoms belonging to the glycosidic bond and on the chiral C16 and C23 atoms show the higher modifications. Besides, the higher MK charge values in all the isomers are observed on the H3, H7 and H30 belonging to the NH and OH groups. The MEP surface mapped evidence clearly that the acceptors of H bonds are the two C=O groups of the pyrimidine rings in all the isomers while the OH groups of the ribose rings and the -NH group of the pyrimidine rings are donors of H bonds showing their surfaces colorations red and blue in those sites, respectively. The NBO study suggests that in all the isomers, the pyrimidine rings are the most stable than the ribose rings and, where the C6 isomer has the high value in gas phase while C3, in solution. The QAIM analysis clearly reveals the high stabilities of the C3, C4 and C6 isomers of THY in both media. The HOMO-LUMO study show that in gas phase the decreasing of the gap values follow the tendency: $C2 > C1 > C3 > C6 > C4 > C5$ while in solution change to: $C1 > C2 > C6 > C3 > C5 > C4$. These results show that both *Cis* isomers are most reactive in gas phase while in solution C4 is most reactive than C6. The evaluation of the descriptors evidence that the three χ , ω and E indexes are factors very important to predict the reactivity of an isomer of thymidine in a medium determined, thus, C4 and C5 are the most reactive in solution due to that both isomers have

practically the same ω and E values but low χ values. The vibrational analyses show the presence of different isomers in both media and confirm that the different positions of the bands are characteristic of the different structures that they present. Here, the complete assignments for C4 and C6 in both media are presented, their force fields and the corresponding force constants at the same level of theory. Finally, the $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and U-Violet spectra were predicted for the three expected structures of THY in aqueous solution showing a reasonable concordance with the corresponding experimental ones. Here, the spectroscopic studies support the presence of more of isomer of THY in solution.

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