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5-Fluorouracil tautomers and their interactions with Ca^{++} ion – A DFT treatment

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Abstract

5-Fluorouracyl is an important cancer chemotropy agent widely used in medicine. In the present study, its 1,3- and 1,5-type proton tautomers and their interactions with calcium cation have been investigated within the restrictions of density functional theory at the level of B3LYP/6-311++G(d,p). All the structures considered are electronically stable, thermodynamically exothermic and have favorable Gibbs' free energy of formation values at the standard states. Various quantum chemical properties of them, including UV-VIS spectra, the HOMO and LUMO energies etc., have been obtained and discussed. The calculations have indicated that in the case of the composites some electron population transfer occurs from the tautomers to the cation, thus the initial formal charge of the calcium decreases in certain extent depending on the tautomeric structure.

1. Introduction

5-Flurouracil acts in several ways, but principally as a thymidylate synthase (TS) inhibitor. Interrupting the action of this enzyme and blocks the synthesis of the pyrimidine thymidylate (dTMP), which is a nucleotide required for DNA replication. 5-Fluorouracil also effectively blocks the replication of DNA viruses in the cell culture systems but it is relatively ineffective in *vivo* [1]. It is known that in body, 5-fluorouracil undergoes anabolic biotransformation to ribosyl and deoxyribosyl nucleotide metabolites [1]. One of these metabolites, 5-fluoro-2'-deoxyuridine-5'-phosphate (FdUMP), forms a covalently bound ternary complex with the enzyme thymidylate synthetase and its cofactor $N^{5,10}$-methylenetetrahydrofolate, a reaction critical for the synthesis of thymine nuleotides [1]. Thus, it results in inhibition of DNA synthesis through "thymineless death" [1].

It is a drug given as an injection to treat cancers of the breast, colon, rectum, stomach, and pancreas and as a cream to treat actinic keratosis (a skin condition that may become cancer) and certain types of basal cell skin cancer.

Following parenteral administration of 5-fluorouracil, there is rapid distribution of the drug and rapid elimination with an apparent terminal half-life of approximately 8 to 20 minutes. The rapid elimination is primarily due to swift catabolism of the liver [1]. However, 5-fluorouracil has "delayed toxicity" having gastrointestinal ulceration, bone marrow depression, etc., [1]. Drug interactions of 5-fluorouracil continue to be described with other antineoplastic drugs, as well as with other classes of agents [2].

Several assay methods are available to quantify 5-fluorouracil in serum, plasma and other biological fluids. Unfortunately, there is no evidence that plasma drug concentrations can predict antitumor effect or host cell toxicity. The recent development of clinically useful pharmacodynamic assays provides an attractive alternative to plasma drug concentrations, since these assays allow the detection of active metabolites of 5-fluorouracil in

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biopsied tumor or normal tissue. 5-Fluorouracil is poorly absorbed after oral administration, with erratic bioavailability [3].

Over the past decades, increased number and better understanding of the mechanism of action of 5-fluorouracil has led to the development of strategies that increase its anticancer activity. Despite these advances, drug resistance remains a significant limitation to the clinical use of 5-fluorouracil. Emerging technologies, such as DNA micro array profiling, have the potential to identify novel genes that are involved in mediating resistance to 5-fluorouracil. Such target genes might prove to be therapeutically valuable as new targets for chemotherapy, or as predictive biomarkers of response to 5-fluorouracil-based chemotherapy [3-8].

2. Method of Calculation

In the present study, all the initial optimizations of the structures leading to energy minima have been achieved first by employing MM2 method which is then followed by semi empirical PM3 self consistent fields molecular orbital method [9-11]. Afterwards, the structure optimizations have been achieved within the framework of Hartree-Fock and finally by using density functional theory (DFT) at the level of B3LYP/6-311++G(d,p) [12,13]. Note that the exchange term of B3LYP consists of hybrid Hartree-Fock and local spin density (LSD) exchange functions with Becke's gradient correlation to LSD exchange [14]. The correlation term of B3LYP consists of the Vosko, Wilk, Nusair (VWN3) local correlation functional [15] and Lee, Yang, Parr (LYP) correlation correction functional [16]. In the present study, the normal mode analysis for each structure yielded no imaginary frequencies for the $3N–6$ vibrational degrees of freedom, where N is the number of atoms in the system. This search has indicated that the structure of each molecule considered corresponds to at least a local minimum on the potential energy surface. Furthermore, all the bond lengths have been thoroughly searched in order to find out whether any bond cleavage occurred or not during the geometry optimization process. All these computations were performed by using SPARTAN 06 [17].

3. Results and Discussion

The 1,3- and 1,5-type proton tautomers of 5-fluorouracil are sequentially named as T1-T4 whereas their composites with calcium dication as T1+Ca^{+2}, T2+Ca^{+2}, etc. Figure 1 shows the optimized structures of 5-fluorouracil tautomers and their composites with Ca^{+2} ion. Note that T4 structure differs from the others because it possesses doubly occurred tautomerism and its ring has 6π-electrons. Note that T4 can also be considered as 1,3-tautomer of T3. Figure 1 also shows the direction of the dipole moment vectors. As seen in the figure, the positive end of the dipole of T1 originates somewhere around the nitrogen and the olefinic moiety. In the cases of T2 and T3, the head of the dipole moment vector aims at the final position of the tautomeric proton. Whereas, in T4 tautomer the positive end of the vector originates nearby the fluorine atom and ends to the OH moiety which is far away from the fluorine atom. It seems 1,3- or 1,5-tautomerism highly disturbs the electron distribution of the structures so that the direction of the dipole moment vector in each case depends on position of the tautomeric proton.

On the other hand, in the cases of composites the positive end of the dipole moment vector originates nearby the calcium cation and points to the OH moiety, except in T1+Ca^{+2} case, in which it aligns directly to the carbon-fluorine bond. Thus, the positive charge on the calcium atom is the most influential factor in dictating the direction of the vector.

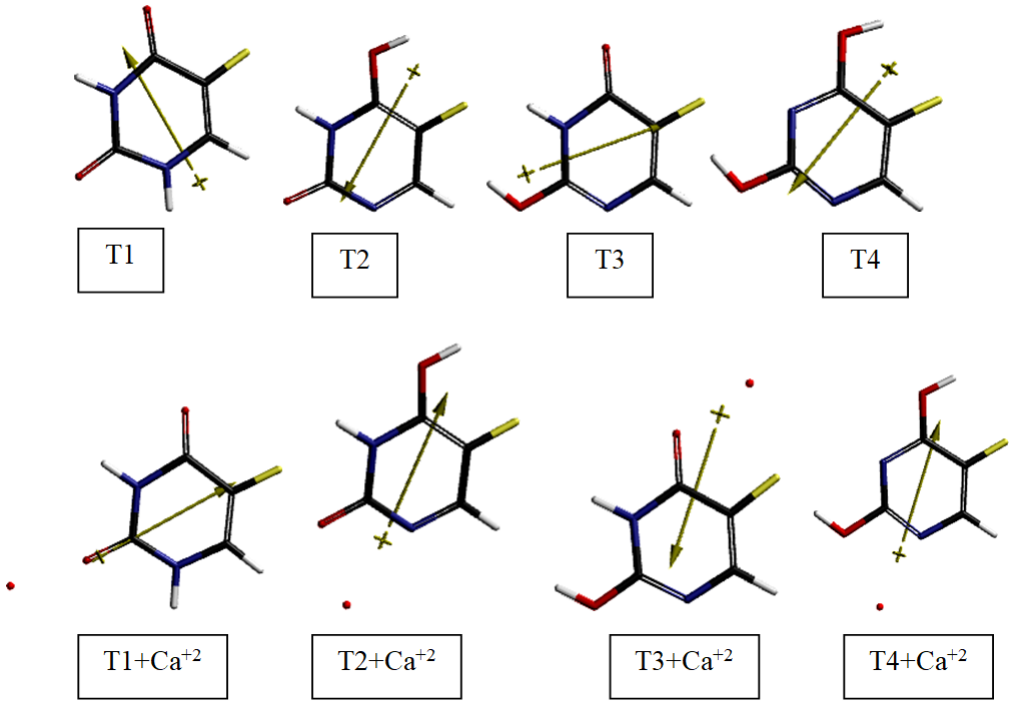


Figure 1. Optimized structures of 5-fluorouracil tautomers and their composites with Ca^{+2}.

Figures 2 and 3 respectively show the calculated bond lengths of 5-fluorouracil tautomers and their composites with Ca^{+2} ion. The tautomerism affects almost all the bond lengths even the C-F length (even though the fluorine atom is at the exocyclic position), implying that some changes occurs in the electron population in the C-F bond as the tautomerism proceeds.

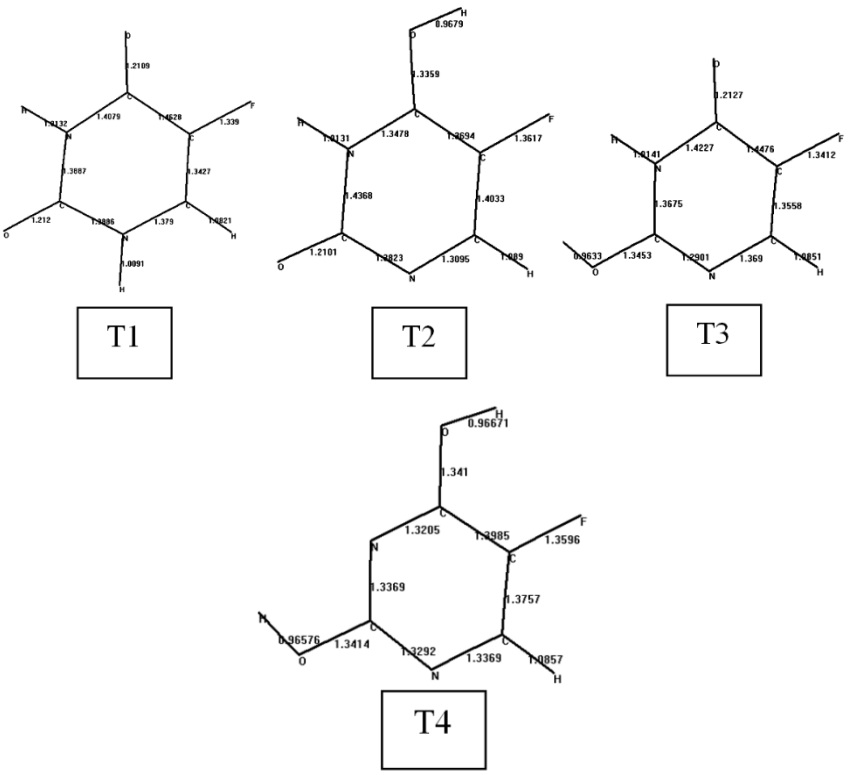


Figure 2. Calculated bond lengths (Å) of 5-fluorouracil tautomers.

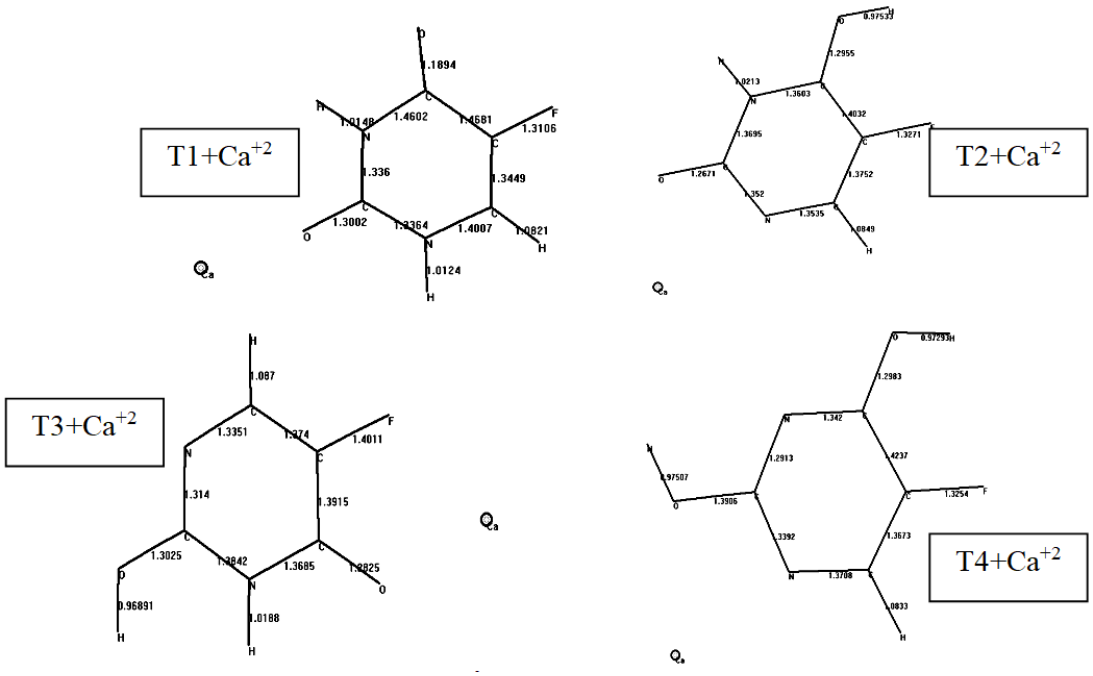
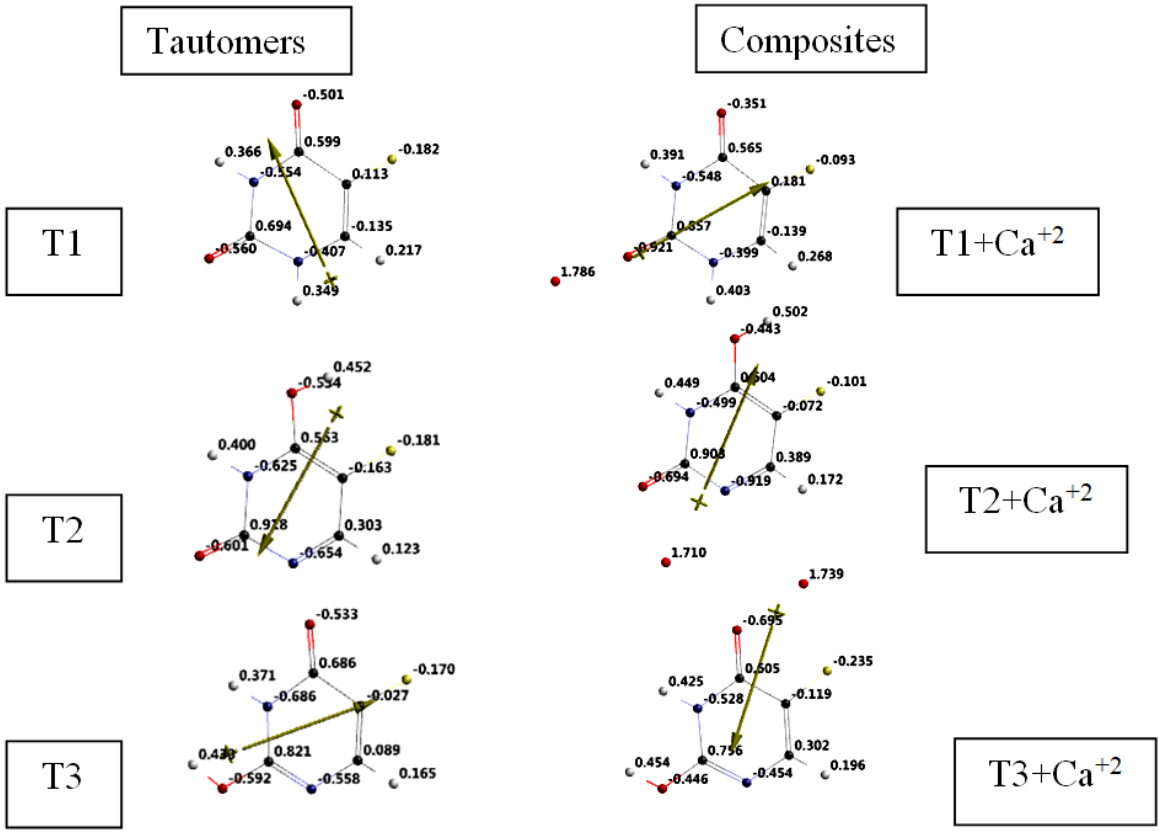


Figure 3. Calculated bond lengths (Å) of 5-fluorouracil composites considered.

Figure 4 displays the electrostatic potential (ESP) charges on the atoms of the tautomers and the composites considered. Note that the ESP charges are obtained by the program based on a numerical method that generates charges that reproduce the electrostatic potential field from the entire wavefunction [17].



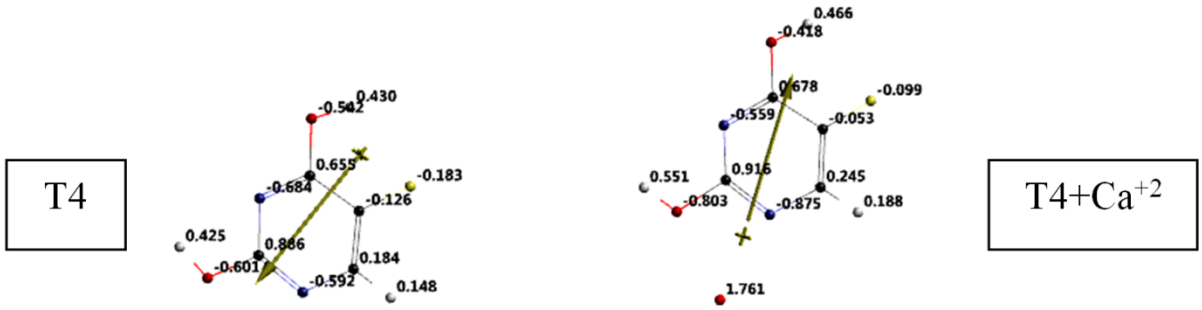


Figure 4. The ESP charges on the atoms of the tautomers and the composites considered.

As seen in the figure, the presence of the cation changes all the charges on the parent tautomers such that some are increased whereas some decreased. Note that the charge on the cation is no longer possesses its initial charge of +2 but also decreased as the tautomerism occurred, implying that some electron population has been transferred from organic component to the cation. The order of charge on the cation is T1+Ca^{+2}> T4+Ca^{+2}> T3+Ca^{+2}> T2+Ca^{+2}. Consequently, the ease or favorability of electron population transfer from the organic component to the cation should be T1> T4> T3> T2.

Figure 5 stands for the electrostatic potential maps of the tautomers considered where negative potential regions reside on red/reddish and positive ones on blue/bluish parts of the maps. As seen in the figure, in T1 through T3, the most negative regions are around the carbonyl oxygen atom(s) (in T2 it is partly on the doubly bounded nitrogen atom) whereas in T4 it is nearby one of the ring nitrogen atom.

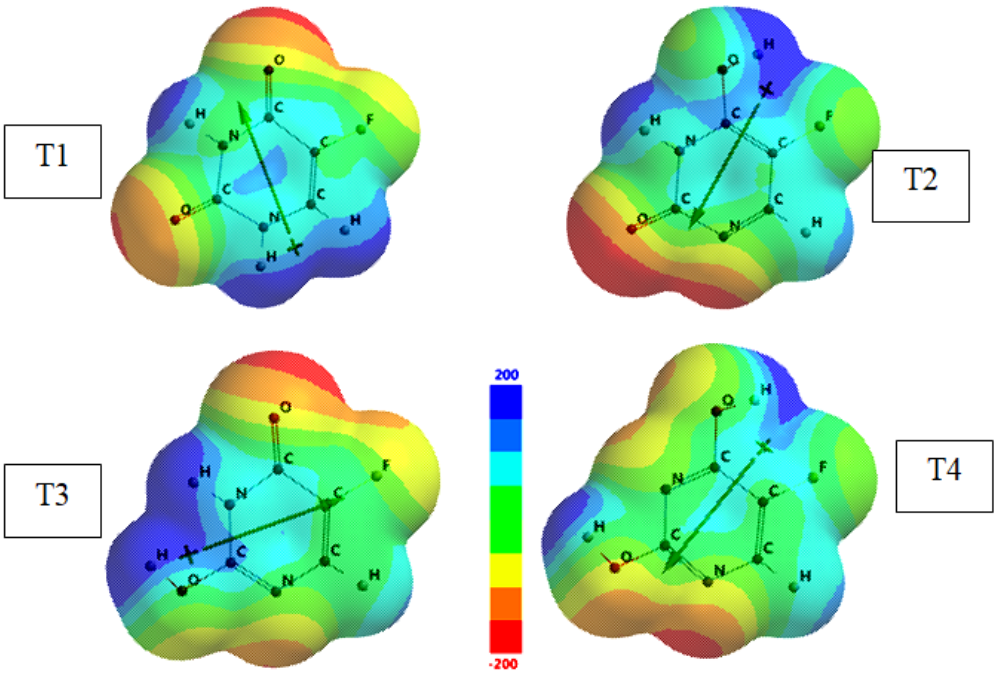


Figure 5. The electrostatic potential maps of the tautomers considered.

In the case of composites the cation so strongly influences the organic part that the electrostatic potential maps are all blue.

Tables 1 and 2 show some thermochemical properties of the tautomers and the composites considered, respectively where Hº and Gº stand for the standard heat of formation and the Gibbs free energy of formations. As seen in the tables, at the standard conditions all the tautomers as well as the composites possess exothermic heat of formation and favorable Gº values. The algebraic orders of Hº and Gº values of the tautomers are the same that is T1< T4< T3< T2. For the composites, the Hº and Gº values fallow the orders of $T2+Ca^{+2}$< $T3+Ca^{+2}$< $T1+Ca^{+2}$< $T4+Ca^{+2}$. All these orders should be dictated by the peculiarities of the sigma and π-skeletons engendered by the assembly of atoms present in the structures.

Table 1. Some thermo chemical properties of the tautomers considered.

Tautomers	Hº	Sº (J/molº)	Gº
T1	-1349821.253	342.72	-1349923.439
T2	-1349735.580	345.26	-1349838.522
T3	-1349748.831	348.14	-1349852.631
T4	-1349757.401	341.99	-1349859.366

Energies in kJ/mol.

Table 2. Some thermo chemical properties of the composites considered.

Composites	Hº	Sº (J/molº)	Gº
$T1+Ca^{+2}$	-3127431.489	374.99	-3127543.310
$T2+Ca^{+2}$	-3127496.759	371.38	-3127607.504
$T3+Ca^{+2}$	-3127431.778	374.55	-3127543.441
$T4+Ca^{+2}$	-3127414.239	373.20	-3127525.509

Energies in kJ/mol.

Tables 3 and 4 respectively show some energies of the tautomers and their composites considered, where E, ZPE and E_C stand for the total electronic energy, zero point vibrational energy and the corrected total electronic energy values, respectively. As seen in the tables, all the species considered are electronically stable. The stability order of the tautomers and the composites are T1> T4> T3 > T2 and $T2+Ca^{+2}$ > $T3+Ca^{+2}$ > $T1+Ca^{+2}$> $T4+Ca^{+2}$, respectively.

Table 3. Some energies of the tautomers considered.

Tautomers	E	ZPE	E_C
T1	-1350034.66	205.85	-1349828.81
T2	-1349946.44	202.88	-1349743.56
T3	-1349959.24	202.20	-1349757.04
T4	-1349970.03	205.07	-1349764.96

Energies in kJ/mol.

Table 4. Some energies of the composites considered.

Composites	E	ZPE	E_C
T1+Ca^{+2}	-3127636.02	210.19	-3127425.83
T2+Ca^{+2}	-3127701.18	210.39	-3127490.79
T3+Ca^{+2}	-3127633.39	206.84	-3127426.55
T4+Ca^{+2}	-3127617.00	208.32	-3127408.68

Energies in kJ/mol.

Table 5 lists the aqueous and solvation energies of the tautomers. The algebraic orders of Eaq and the solvation energy values are T1< T4< T3< T2 and T4< T2< T3< T1, respectively. These orders are dictated by the presence of various chemical functional descriptors and their variations as going from one tautomer to the other.

Table 5. The aqueous and solvation energies of the tautomers.

	T1	T2	T3	T4
Eaq	-1350072.66	-1350017.66	-1350029.55	-1350043.13
Solvation Energy	-38.000	-71.225	-70.310	-73.098

Energies in kJ/mol. Solvation model SM5.4/A

Tables 6 and 7 show some properties of the tautomers and their composites, respectively.

Table 6. Some properties of the tautomers.

	T1	T2	T3	T4
Dipole moment	4.21	5.87	3.90	2.28
Polarizability	48.68	48.80	48.70	48.53
Log P	-1.31	-1.08	-0.44	0.44
PSA (Å²)	51.649	52.369	52.840	53.376

Dipole moments in debye units. Polarizabilities in 10^{-30} m^3 units.

Molecules having no permanent dipole are capable of altering their shapes under the influence of an external field as a result of which they may acquire a dipole moment (induced dipole moment) [18].

In the present case, the dipole moments show some noticeable fluctuations from one tautomer to the other. Obviously, various structural, electronic and hydrogen bonding variations are responsible for it. The order of dipole moments is T4<T3< T1< T2. In the presence of the cation, the order turns out to be T3+Ca^{+2}< T2+Ca^{+2}< T4+Ca^{+2}< T1+Ca^{+2}. So, the cation perturbs the parent systems in different extents to establish new order of dipole moments in the case of composites.

Polarizability is to be an important factor in determining the nucleophilicity. The order of polarizabilities in the case of the tautomers is T4< T1< T3< T2 whereas it becomes T2+Ca^{+2}< T3+Ca^{+2}< T4+Ca^{+2}< T1+Ca^{+2} for the composites (Table 7).

The log P values follow the order of T1< T2< T3< T4. Note that a negative value for log P means the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when log P = 0 the compound is equally partitioned between the lipid and aqueous phases; whereas a positive value for log P denotes a higher concentration in the lipid phase (i.e., the compound is more lipophilic).

It is worth mentioning that the polar surface area (PSA) is defined as the amount of molecular surface area arising from polar atoms (N,O) together with their attached hydrogen atoms. Although these compounds are isomeric, their PSA values differ from each other meaning that the same kind of atoms might be influenced by electronic factors differently at different positions. The order of PSA values of the tautomers is T1< T2< T3< T4 whereas the order becomes T1+Ca^{+2}< T2+Ca^{+2}< T4+Ca^{+2}< T3+Ca^{+2} for the composites (Table 7).

Table 7. Some properties of the composites.

	T1+Ca^{+2}	T2+Ca^{+2}	T3+Ca^{+2}	T4+Ca^{+2}
Dipole moment	31.41	19.04	13.95	22.16
Polarizability	50.32	49.78	49.89	49.92
PSA (Å^2)	47.604	48.378	49.226	49.022

Dipole moments in debye units. Polarizabilities in 10^{-30} m^3 units.

Figure 5 shows the local ionization potential maps of the tautomers considered where conventionally red/reddish regions (if any exists) on the density surface indicate areas from which electron removal is relatively easy, meaning that they are subject to electrophilic attack. It is worth remembering that the local ionization potential map is a graph of the value of the local ionization potential on an isodensity surface corresponding to a van der Waals surface. In the case of composites, the perturbation is so great that the local ionization potential maps are all blue over the whole structures. Meaning that electron removal from the composites is highly hindered.

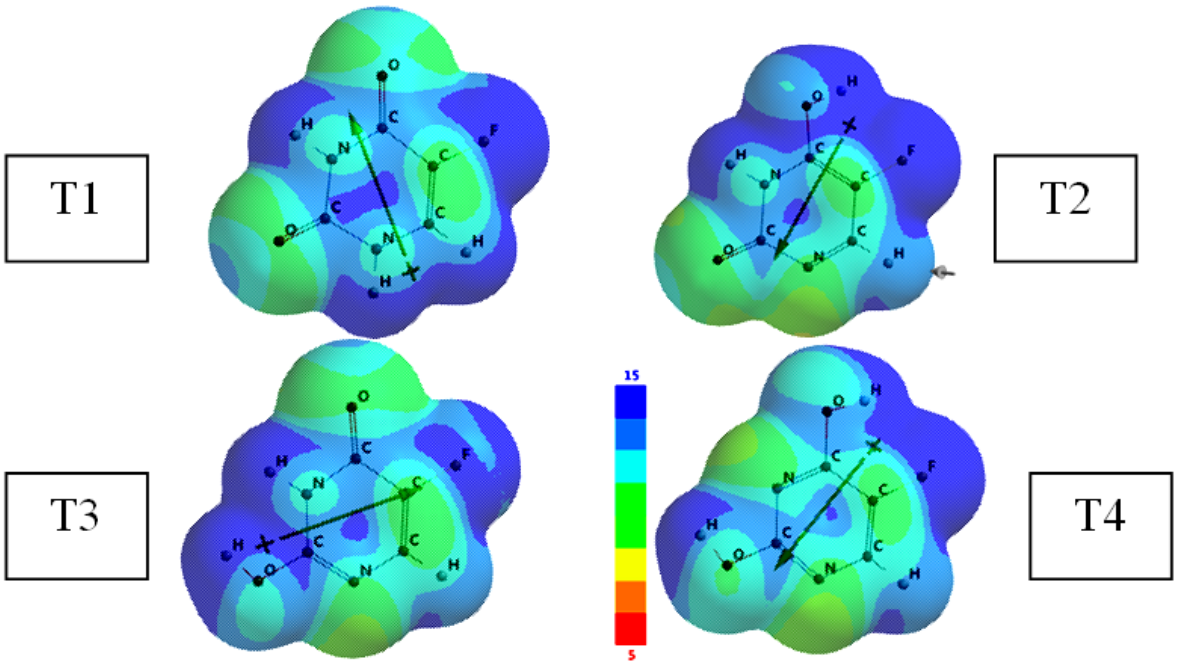


Figure 5. The local ionization potential maps of the tautomers considered.

Figure 6 shows the LUMO maps of the tautomers considered. Note that a LUMO map displays the absolute value of the LUMO on the electron density surface. The blue color (if any exists) stands for the maximum value of the LUMO and the red colored region, associates with the minimum value.

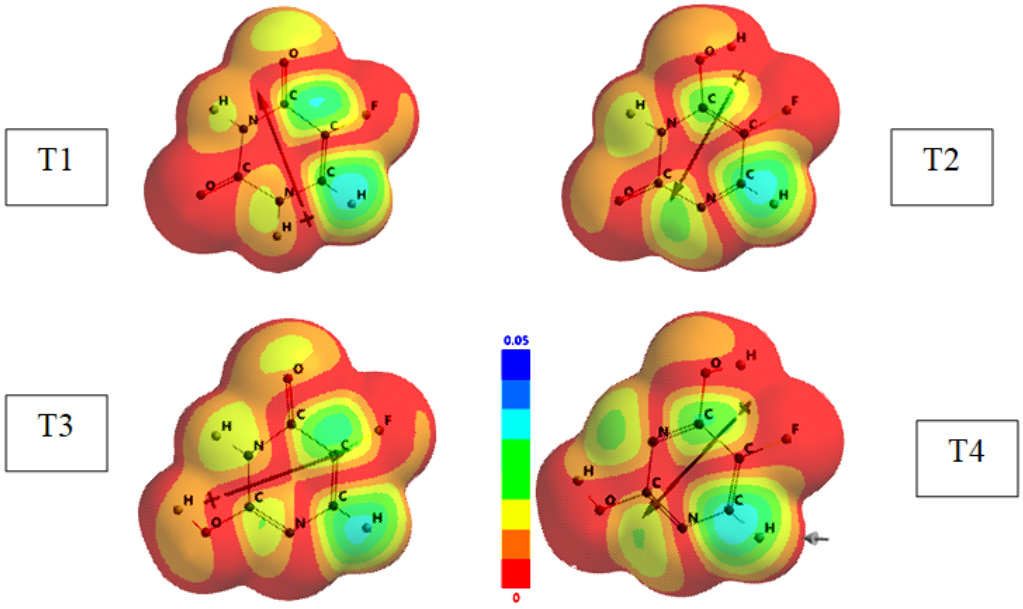


Figure 6. The LUMO maps of the tautomers considered.

Figure 7 displays the LUMO maps of the composites considered. It is note worthy that the LUMO map of $T2+Ca^{+2}$ is different from the others around the cation. That means the cation, in contrast to the other composites is no longer at the site having the maximum value of the LUMO. Actually, the cation in $T2+Ca^{+2}$ case has the lowest positive ESP charge (see Figure 4), meaning that greater value of electron population has been transferred to the cation compared to the other composites considered.

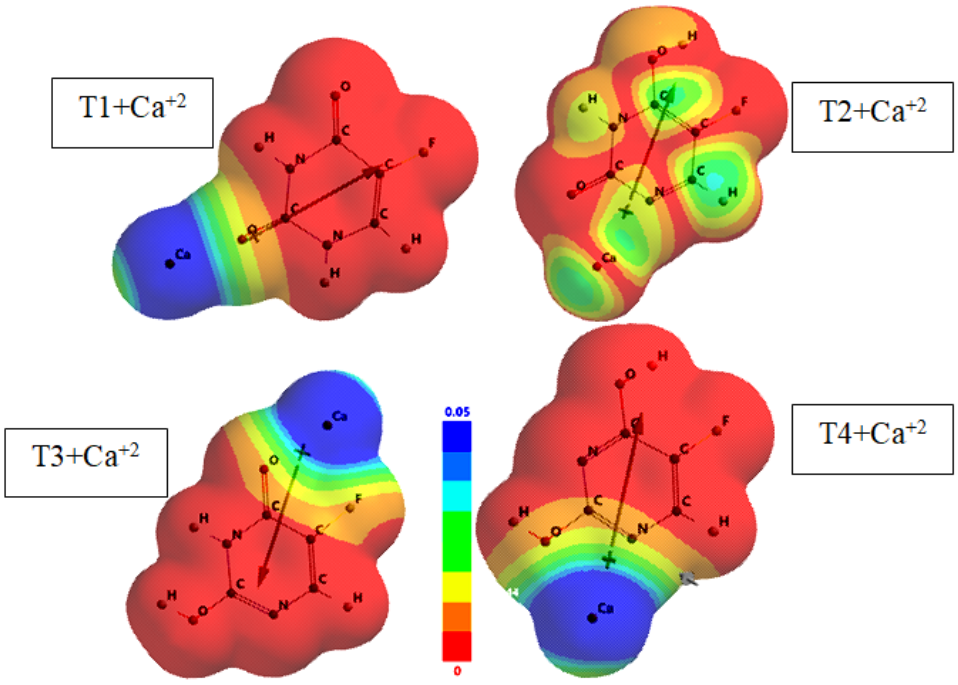


Figure 7. The LUMO maps of the composites considered.

Figure 8 and 9, respectively display some energy levels of 5-fluorouracil tautomers and their composites considered. The presence of calcium cation affects both the occupied and unoccupied energy levels of the tautomers considered, especially the HOMO and LUMO (the frontier molecular orbitals), energy levels of the parent tautomers.

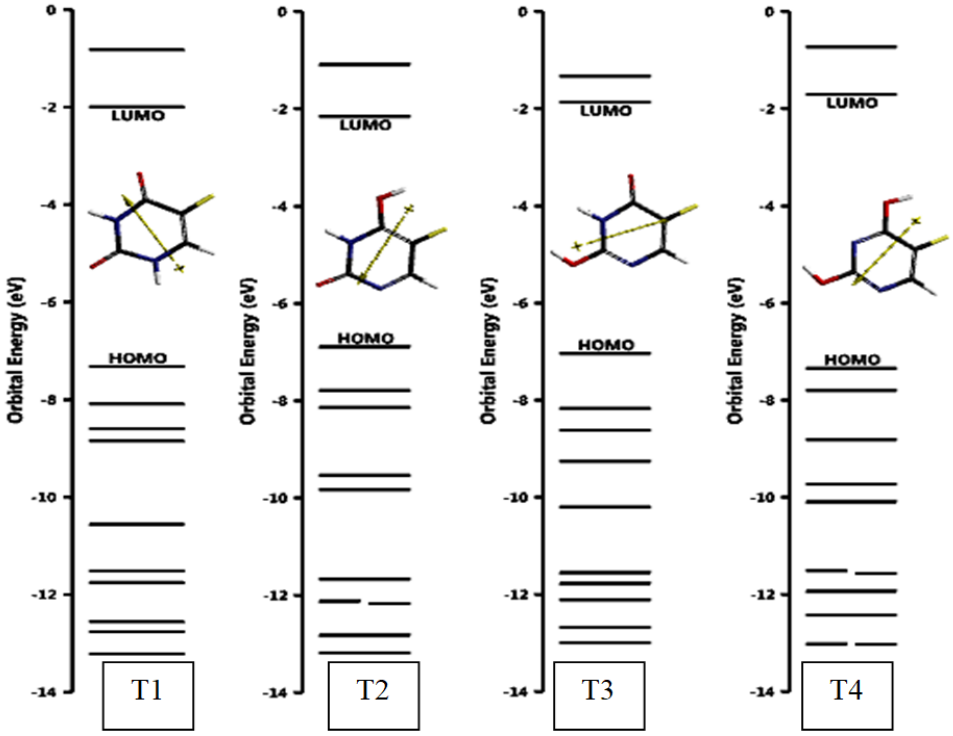


Figure 8. Some energy levels of 5-fluorouracil tautomers considered.

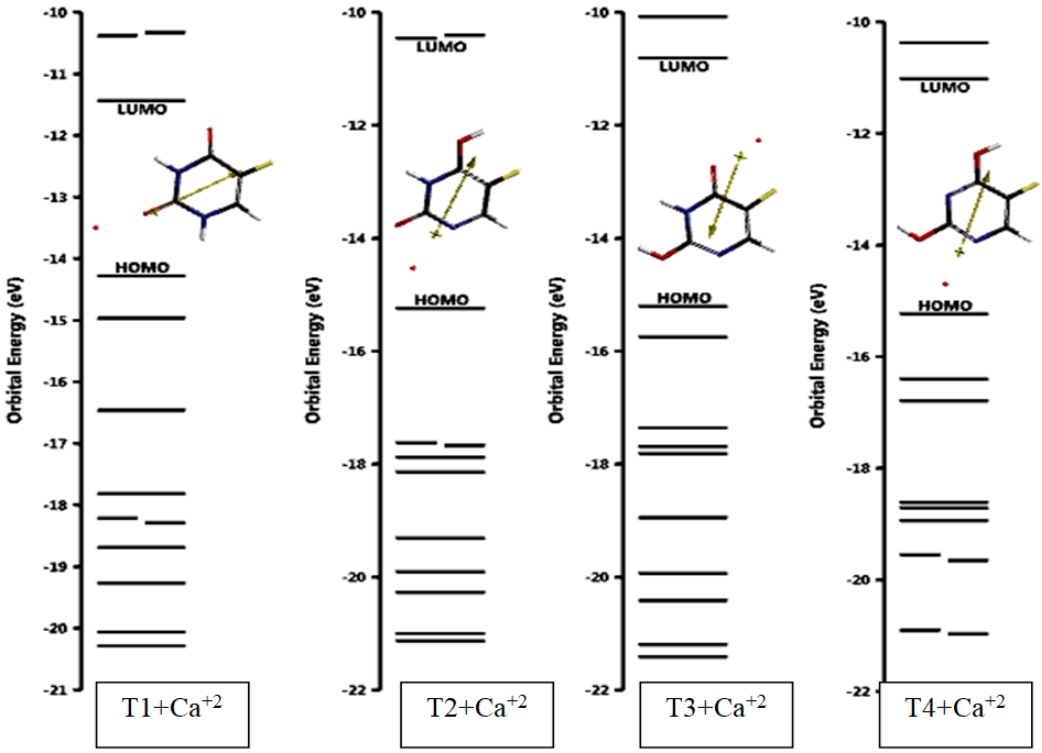


Figure 9. Some energy levels of the composites considered.

Table 8 lists the HOMO, LUMO energies and Δε values (interfrontier molecular orbital energy gap, $\Delta\varepsilon = \varepsilon_{LUMO} - \varepsilon_{HOMO}$) of the species considered. As seen in the table, the cation acting as if an electron attracting substituent on the organic component, lowers both the HOMO and LUMO energy levels at unequal extents [19].

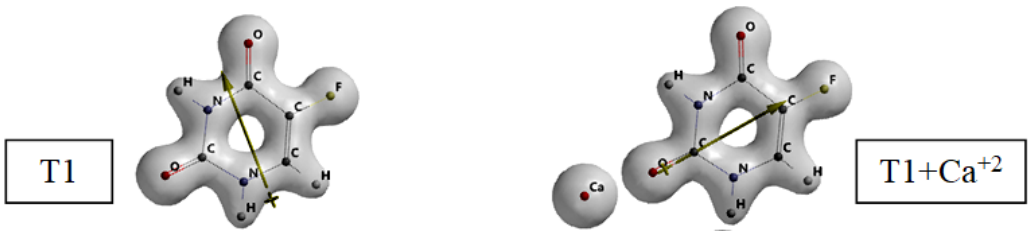
Table 8. The HOMO, LUMO energies and Δε values of the species considered.

Species	HOMO	LUMO	Δε
T1+Ca^{+2}	-1377.68	-1102.95	274.73
T1	-706.54	-192.30	514.24
T2+Ca^{+2}	-1470.85	-1009.11	461.74
T2	-664.94	-207.58	457.36
T3+Ca^{+2}	-1466.92	-1042.47	424.45
T3	-679.26	-179.72	499.54
T4+Ca^{+2}	-1469.75	-1062.89	406.86
T4	-708.14	-164.06	544.08

Energies in kJ/mol.

Algebraic order of the HOMO and LUMO energy levels for the parent tautomers are T4< T1<T3< T2 and T2< T1< T3< T4, respectively. In the case of the composites, the orders of the HOMO and LUMO energy levels become T2+Ca^{+2} < T4+Ca^{+2} < T3+Ca^{+2} < T1+Ca^{+2} and T1+Ca^{+2} < T4+Ca^{+2} < T3+Ca^{+2} < T2+Ca^{+2}, respectively. Consequently, the orders of Δε values for the parent tautomers and their composites are T2< T3< T1< T4 and T1+Ca^{+2} < T4+Ca^{+2} < T3+Ca^{+2} < T2+Ca^{+2}, respectively. Note that, in the case of composites the order of Δε values is the same as the order of LUMO energies. So the LUMO energies are more influential on the Δε values compared to the HOMO energies.

Figure 10 displays the bond densities of the tautomers and the composites considered. As seen in the figure, in the case of T1+Ca^{+2} the cation is in interaction with one of the carbonyl oxygen atoms, whereas in T2+Ca^{+2} it also interacts with the nitrogen atom in the vicinity of the carbonyl oxygen. In the case of T3+Ca^{+2}, the cation interacts with the carbonyl oxygen atom nearby the fluorine substituent, whereas in T4+Ca^{+2} composite the cation mutually interacts with the ring nitrogen atom and oxygen atom of hydroxyl group. In all the tautomers and the composites substituents are strongly bound the ring backbone. The cation in all the cases does not have any bond density with the organic component. Thus, the interaction between the components should be electrostatic type acting through the space.



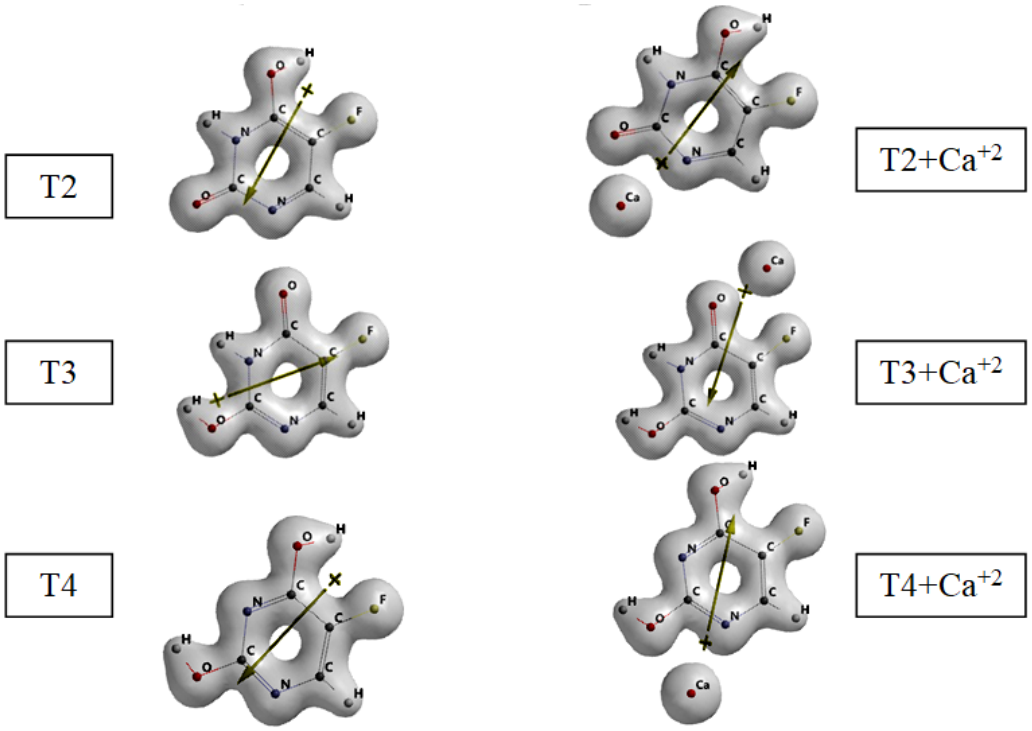


Figure 10. Bond densities of the tautomers and the composites considered.

Figure 11 displays the calculated UV-VIS spectra (time dependent density functional) of the tautomers and

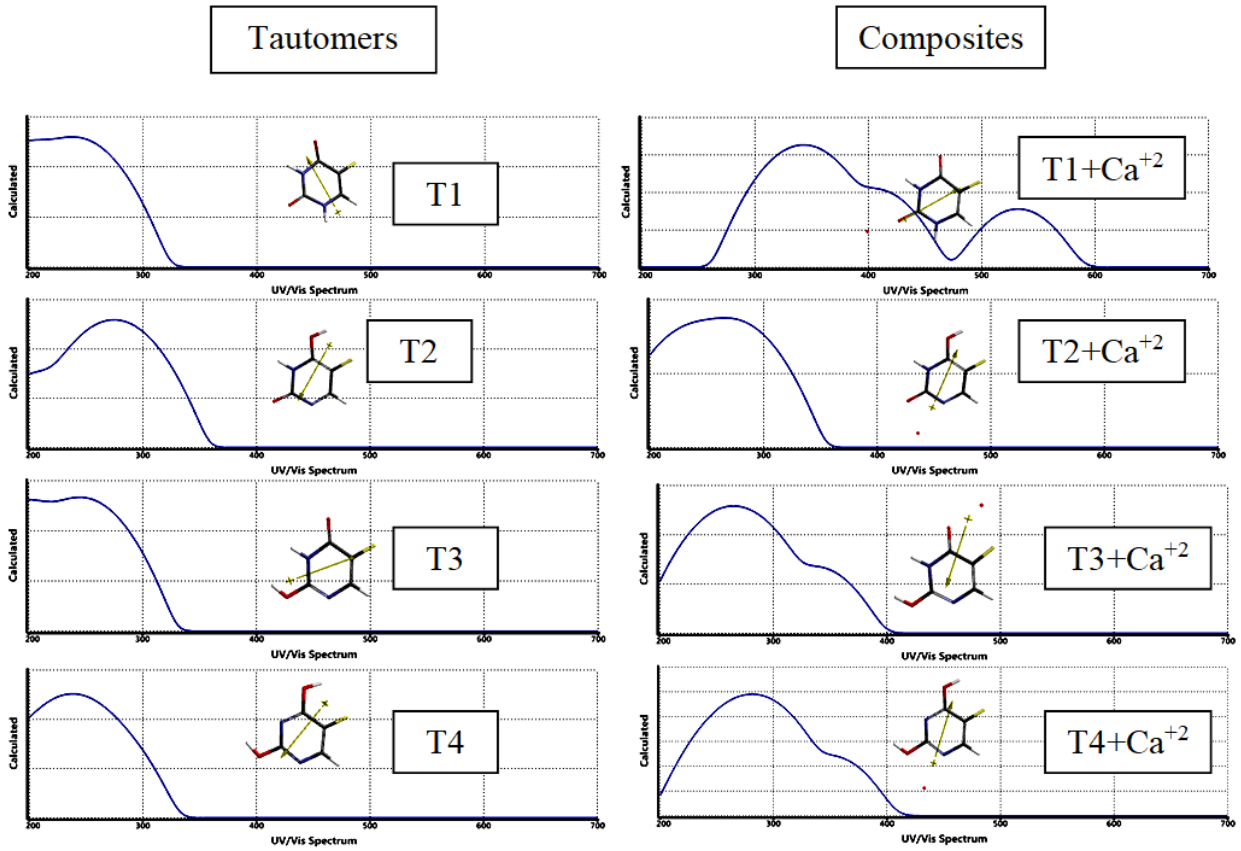


Figure 11. The calculated UV-VIS spectra of the tautomers and their composites.

their composites. As seen in the figure, the spectrums of the tautomers are confined to UV region of the spectrum only, having some shoulders which are not clearly discernable in the cases of T1 and T3. Tautomer T4 does not have any shoulder. It has 6π-electrons in a cyclic conjugation. Therefore it should have some aromatic character. The other tautomers have some short-range conjugation in their structures depending on the extend of the tautomerism which should be responsible for the emergence of weak shoulders. As for the composites, the presence of the cation in the case of T1+Ca^{+2} causes an effective bathochromic effect to the visible part of the spectrum having a couple of shoulders. Note that its organic component, T1, is the parent structure 5-fluorouracil. Whereas the other composites possess the organic components which have been involved in some tautomeric structures. As seen in Figure 11, T3+Ca^{+2} and T4+Ca^{+2} possess shoulders but still in the UV region. Therefore, the presence of the cation does not have any effective perturbational influence on organic components of those tautomers causing any appreciable narrowing of the interfrontier molecular orbitals of those composites to exhibit any bathochroming effect towards the visible region.

4. Conclusion

In the present computational study, within the restrictions of DFT study at the level of B3LYP/6-311++G(d,p), 1,3-proton tautomers of 5-fluorouracil and their interactions with the calcium cation have been considered. The present results indicate that in the vacuum conditions, all the structures (the agents and their composites) are characterized with exothermic heat of formation and favorable Gibbs free energy of formation values and they are electronically stable. The calculations revealed that although the cation does not cause any bond rupture on the organic partner of the composites, it affects the partner in various ways by accepting some electron population from it. Although, the calcium metabolism is governed by various factors, the calculations indicate that 5-fluorouracil does not have much direct perturbational effect at the initial stage of calcium metabolism. However, its indirect effects via some other controller on the calcium metabolism are open to future investigations.

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