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REGULAR ARTICLE

Solvent effects on the two lowest-lying singlet excited states of 5-fluorouracil

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Abstract Different solvation models based on the sequential-QM/MM methodology are used to investigate the two lowest n–π* and π–π* transitions of 5-fluorouracil in water and acetonitrile. Electronic polarization of the solute, use of discrete and explicit solvent models and different QM models ranging from semiempirical, time-dependent DFT, size-extensive CI and equation of motion are considered. The results show that DFT-based methods provide good results for the energy transitions, but fail to describe the relative energy shifts. Very good and equivalent shifts are obtained using CIS(D) and EOM-CCSD methods combined with the polarizable continuum model solute polarization and discrete solvent description. Our best results give the π–π* transition lower than the n–π* by 0.1 eV in water and, in acetonitrile, place these two states essentially as degenerate in the Franck–Condon region of the ground state. The relative position of these two excited states is of crucial importance for understanding the photophysics of 5-fluorouracil in solvent environment.

Keywords Solvent effects · 5-Fluorouracil · Absorption spectrum · Solvatochromic shifts

1 Introduction

Recent data of the World Health Organization show that cancer is now among the three main causes of death in the world [1]. One important possible treatment uses 5-fluorouracil (5FU, Fig. 1) because of its great efficiency in two cytotoxic effects: the RNA falsification and the inhibition of the TYMS enzyme [2]. This has increased the interest for more experimental and theoretical investigations on 5FU. Recent works have experimentally studied the absorption, emission and NMR spectra. Theoretical works have considered the isolated molecule or included the solvent effects using the continuum model [3–6]. In the aftermath of the work of Barone, Improta and coworkers [7], there is considerable interest in the photophysics of 5FU, and one central aspect is the location of the low-lying singlet excited states.

The two lowest-lying singlet excited states of 5FU are of n–π* and π–π* character, respectively. In the gas phase (isolated molecule) the n–π* should lie lower than the π–π* state. As it is normally expected, the solvent effect will blue shift the lowest n–π* and red shift the π–π*. Depending on the amount of the shifts, there may be a crossing and thus an inversion of the two states in solvent environment. Indeed, recent theoretical and experimental results suggest that there is such an inversion of the n–π* and π–π* transitions of the 5FU in solvent environment, compared to the gas phase [7]. Santoro et al. [7] used a cluster model composed of four water molecules within the polarizable continuum model (PCM) [8] to represent the solvent, to investigate 5FU in water and found evidences for the inversion. In a series of interesting theoretical and experimental studies, Improta and coworkers have analyzed the photophysics of 5FU and also the lifetime of the excited states [9]. The reversal of these two states in water seems clear, but in acetonitrile it is less certain. On the basis of the photochemistry studies, these two states should lie very close in the Franck–Condon region of the ground state, a condition that should be confirmed by high-level calculations.

Inversion of the excited states is known in the DNA bases and was pointed as one of the reasons for the low

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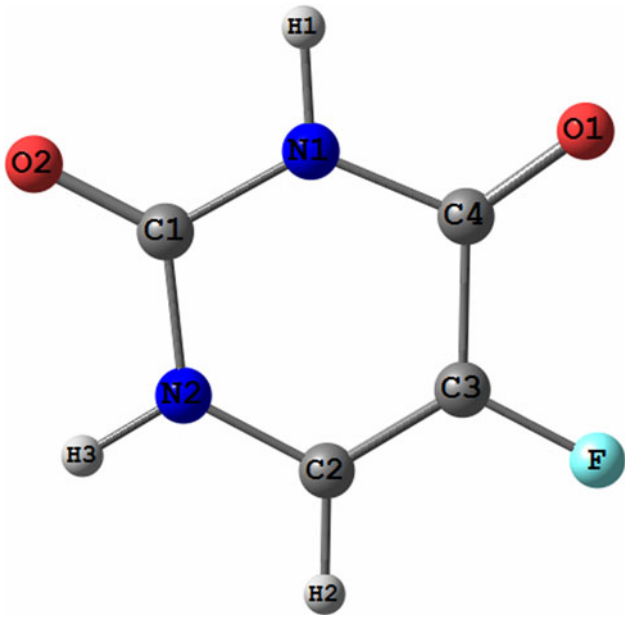

Fig. 1 5-Fluorouracil. Atomic labels are used in Table 1 and along the text

quantum yield presented by these molecules because this inversion would be related to coupling of the near excited states [10] and the existence of conical intersections [7]. Biologically, this is very desirable because the lifetime of the excited states decreases, becoming the order of a few tens or hundreds of femtoseconds [11, 12], drastically reducing the possibility that potentially dangerous photochemical reactions occur [13].

Experimentally, the typical low intensity of n–π* transitions makes it difficult to be observed, especially when it is located near or under the intense and broad π–π* transitions. The observed π–π* excitation in acetonitrile is located at 4.70 eV, whereas this same transition in water lies at 4.66 eV [3]. Thus, the redshift of the π–π* excitation in water is larger than in acetonitrile by 0.04 eV (i.e., only 300 cm^{-1}). The description of this very small shift may be considered a great challenge for the present solvation models. However, this difference is important for understanding the distinct photophysical behavior of 5FU in acetonitrile and water. Thus, in this work we analyze the performance of different solvation models and the possible inversion of the n–π* and π–π* lowest energy transition of the 5FU in solvent environment. We also consider the relative location of the π–π* in acetonitrile and water.

Solvent effects are considered using the sequential-QM/MM methodology [14], where Monte Carlo simulations are made to generate the liquid structure that will be submitted to the quantum mechanical (QM) calculations. Two central aspects are analyzed: (1) the electronic polarization of the solute due to its interaction with the solvent and (2) the corresponding QM model adopted for calculating the excitation energies. We consider two possibilities of including the solute polarization. First, the polarization is obtained using the PCM and, second, the iterative procedure that brings the solute into electrostatic equilibrium with the solvent [15]. Monte Carlo Metropolis simulations were performed to generate statistically uncorrelated configurations to represent the system, on which the QM calculations are performed. For the QM calculations, we select four distinct approaches. First, we consider different DFT models such as B3LYP [16], O3LYP [17], BHandHLYP and PBE0 [18] functionals. The excitation energies are obtained using the time-dependent DFT (TDDFT) approach. Second, we consider size-extensive CIS(D) configuration interaction models [19]. This is a singly excited CI calculation followed by a perturbative doubly excited calculation and has successfully been used in previous studies [20]. Thus, we also report CIS results, although this is normally expected to provide overestimated excitation energies. Third, we consider more sophisticated models such as the equation-of-motion coupled-cluster with single- and double-excitations (EOM-CCSD) [21]. Fourth, we also use the semiempirical QM intermediate neglect of differential overlap INDO/CIS model as this allows for consideration of a large number of explicit solvent molecules. The solvent was included using three approaches: (1) continuum model as obtained from PCM, (2) discrete model, where the solvent is treated as simple point charges, and (3) explicit model, where some explicit solvent molecules are used. In this latter case an electrostatic embedding surrounding the explicit solvent molecules is used to account for the long-range bulk effects.

This work thus focuses on the theoretical assessment of the relative location of the two lowest-lying excited states of 5FU in water and acetonitrile and the relative position of the observed π–π* states in these two solvents.

2 Computational details

The 5FU geometry was optimized both isolated and considering the PCM representation of the two solvents, water and acetonitrile. In all cases these optimizations were made using the B3LYP/aug-cc-pVDZ level, using Gaussian 03 [22]. Frequency calculations were performed only to verify that each geometry corresponds to true energy minimum.

The Monte Carlo simulations were made using the Dice Program [23], in the NVT ensemble, at 25 °C and 1 atm. The system was composed of a solute molecule surrounded by 500 solvent molecules in a cubic box. After a thermalization phase, a total of 10^8 MC steps, or 2×10^5 steps/molecule, were made in the simulation.

The site–site interaction was treated using Lennard-Jones (LJ) plus Coulomb potential. The 5FU LJ parameters were obtained from the all-atom optimized potentials for liquid simulation (OPLS-AA) force field [24]. The atomic charges were obtained using the CHELPG scheme [25] in the MP2/aug-cc-pVDZ level. The LJ potential for water was the SPC model [26] and, for acetonitrile, the model of Böhm and McDonald [27]. More details on the classical simulations can be found in our previous works [28–31].

To include the solute polarization effect, two methods were used: a computationally non-expensive PCM + MM/QM approach [15] and a more accurate, but expensive iterative procedure [32]. In the first, we obtain the solute geometry and the charges considering them surrounded by the solvent described by the PCM method, and the resulting atomic charges are directly used in the Coulomb part of the classical potential of the MC simulation. In the second procedure, we first determine the solute geometry and all the atomic charges for the isolate solute molecule and then perform a simulation using these values to generate an average solvent electrostatic configuration (ASEC) [33], which is used to calculate the dipole moment and a new set of charges. This new set is used in a next simulation, repeating the procedure until the convergence of the calculated dipole moment μ, within an accuracy of $\Delta\mu = 0.01$ D.

The absorption electronic transitions were calculated using the time-dependent DFT methods in the exchange–correlation parameterization adopted by B3LYP, O3LYP, BHandHLYP and PBE0. The reference states for the calculation of the excitation energies are obtained by using the Kohn–Sham approach. In addition, the CIS, CIS(D) and EOM-CCSD are used with Hartree–Fock as the reference state. In all cases we have used the 6-311++G(d,p) basis set. All these calculations were performed using Gaussian 03 and Gaussian 09 [34]. Because of the large computational demand, only a relatively small number of explicit solvent molecules can be used. For the use of a larger number of solvent molecules, the INDO semiempirical method in the spectroscopic parameterization (INDO/CIS) [35], as implemented in the ZINDO Program [36], was used.

In these calculations, three levels of approach were used to include the solvent: (1) *continuum*, using the default PCM available in Gaussian 03, which is a computationally inexpensive method, but with difficulties in treating specific interactions. It was noted before that the PCM results on 5FU do not depend on the choice of the cavity radii [7], so we did not consider analysis of the influence of the cavity radius in our study, although it may have some influence in the general case; (2) *discrete*, where the solvent molecules were represented only by point charges located in their atomic sites. The charges used are the same of the classical simulation. In this case instead of using the ensemble of configurations generated, it is possible to use only one average configuration (ASEC). This has similar computational cost than PCM, but only treats the electrostatic interactions between the solute and the solvent; (3) *explicit*, where some solvent molecules close to the solute are explicitly included and the remaining solvent molecules are treated as an electrostatic embedding generated by more than 200 molecules. In this third approach, the calculations were carried out over 100 statistically uncorrelated configurations (composed of the reference molecules, the explicit solvent molecules and the electrostatic embedding), and the values of the transition energies and oscillator strength (f) were obtained as an average. The use of a spectral convolution using the calculated transition energies and intensities will also be discussed. In this case the wave function is antisymmetric with respect to the electrons of the solute and the solvent molecules and allows for the exchange and van der Waals interactions.

3 Results and discussion

3.1 Geometry, charges and dipole moment

We start by discussing the geometry of 5FU. We have considered only the 5FU in the diketo form, which is known to be the most stable form of 5FU, being the only one found in nature [37]. The structures obtained for the isolated gas phase and PCM are very similar, as noted before [38], indicating that the solvent effect in the calculated geometry of 5FU is not very important. Using this structure, we have calculated the atomic charges (using the CHELPG scheme) and the dipole moments (Table 1).

The calculated dipole moment for the isolated molecule, corresponding to the in-vacuum situation, is 3.89 D, in reasonable agreement with a previous result of 4.20 D obtained using the same basis set and the B3PW91 functional [38]. Apparently, the experimental gas-phase dipole moment of 5FU has not been reported. These values are, however, not much different from the experimental value of uracil (3.87 D) [39]. This is simply because the charge in fluorine for 5FU is similar to the value obtained for the corresponding hydrogen atom in the case of uracil [38]. Table 1 summarizes the influence of the solute polarization on the dipole moment. In the case of water as the solvent, we obtain the dipole moments of 5FU as 5.92 D and 6.29 D in the PCM and iterative polarizations, respectively. Using the gas-phase geometry, the dipole moments considering the PCM polarization are calculated as 5.57 D and 5.52 D for 5FU in water and in acetonitrile, respectively. Relaxing the geometry, these corresponding numbers are 5.92 D and 5.85 D, respectively. In the iterative procedure, the calculated dipole moment in water increases to 6.29 D, whereas the value in acetonitrile decreases to 5.10 D. The dipole

Table 1 Atomic charges (e) and dipole moments (Debye) of 5-fluorouracil in gas phase, in water and in acetonitrile (parenthesis), calculated in the MP2/aug-cc-pVDZ level

Atom	Gas	PCM	PCM/PCM[a]	Iterative
N1	−0.6469	−0.6911 (−0.6910)	−0.7018 (−0.7029)	−0.7738 (−0.6882)
C1	0.7933	0.8741 (0.8726)	0.8886 (0.8874)	0.9881 (0.8404)
N2	−0.5238	−0.5603 (−0.5596)	−0.5613 (−0.5613)	−0.6215 (−0.5536)
C2	0.0077	0.0488 (0.0473)	0.0689 (0.0674)	0.1206 (0.0611)
C3	0.0349	0.0045 (0.0054)	−0.0110 (−0.0106)	−0.0404 (−0.0006)
C4	0.6965	0.7688 (0.7666)	0.7886 (0.7871)	0.8722 (0.7415)
H1	0.3672	0.4263 (0.4247)	0.4357 (0.4340)	0.4720 (0.4148)
O1	−0.5112	−0.6267 (−0.6229)	−0.6471 (−0.6424)	−0.7233 (−0.5848)
F	−0.1746	−0.2017 (−0.2006)	−0.2056 (−0.2042)	−0.1797 (−0.1949)
H2	0.1744	0.2134 (0.2125)	0.2110 (0.2100)	0.1964 (0.1909)
H3	0.3551	0.4233 (0.4218)	0.4292 (0.4274)	0.4587 (0.4096)
O2	−0.5728	−0.6796 (−0.6768)	−0.6952 (−0.6919)	−0.7695 (−0.6361)
μ	3.89	5.57 (5.52)	5.92 (5.85)	6.29 ± 0.03[b] (5.10 ± 0.02)[b,c]

[a] This means the geometry and the charges were obtained considering the solute involved in PCM

[b] These values were obtained adjusting an exponential plus a constant function to the data (see Fig. 2)

[c] Obtained value starting the iterative process using the gas-phase value. Using PCM as starting point gives the same value

moment of 5FU is expected to be larger in water than in acetonitrile. This feature is well reproduced by the iterative method that gives the dipole moment in water larger than in acetonitrile by 1.19 D. In the case of the PCM polarization, these values are very close, differing only in 0.07 D.

Figure 2 shows the calculated results using the iterative procedure. For water, the PCM results for the insolvent dipole moment represent ca. 90 % of the converged iterative value, as we have also seen in previous applications. However, in the case of acetonitrile, the PCM result is larger than the iterative value. As it can be seen, the iterative result is the same whether it starts from the isolated or the PCM results. However, it is unexpected that the PCM dipole moment value is so large for acetonitrile. In the next section we discuss how these different polarizations affect the transition energies.

3.2 Lowest electronic transitions of 5-fluorouracil in solvent environment

For reference, Table 2 shows the results obtained using the different theoretical models for the isolated molecule, corresponding to the case of a low-density gas. All theoretical models agree that the n–π* transition is lower than the π–π* transition. Next, Tables 3 and 4 summarize the results for the calculated transition energies in water and acetonitrile, respectively, using different QM methods. Only the two lowest transitions representing the n–π* and π–π* are presented. The n–π* transition is dominated by the HOMO-1(n) → LUMO(π) promotion, whereas the π–π* is dominated by the HOMO(π) → LUMO(π). The Kohn–Sham (KS) orbitals obtained in the B3LYP/6-311++G(d,p) level are shown in Fig. 3. They are very similar to that obtained with Hartree–Fock (HF). The delocalization of the orbitals when 5FU is in aqueous solution is seen, but it is less pronounced when the solvent is acetonitrile. This picture is obtained in both methodologies, KS and HF orbitals.

We will focus first on the π–π* transition of 5FU in water (Table 3), as it is experimentally observed. We first present the results obtained using the B3LYP functional and different treatments of the solvent, starting with PCM and improving until the explicit use of sixteen water molecules. Using the PCM polarization, the best agreement with experiment for the excitation energy using B3LYP is 4.79 eV. This value is an average over 100 configurations, where the solvent is represented by 16 explicit water molecules and an electrostatic embedding composed of the atomic charges of 238 water molecules. The value is equivalent to that of 4.80 eV obtained using the iterative polarization (shown in parenthesis) and slightly larger than the experimental result with a band maximum at 4.66 eV [3]. The PCM overestimates the transition energy, and the results improve by adding explicit water molecules. As noted before [40], the use of the electrostatic embedding is very important in accelerating the convergence with respect to the number of explicit solvent molecules used.

A single vertical excitation may not represent the absorption maximum observed. A more realistic way of obtaining the transition energies when dealing with a

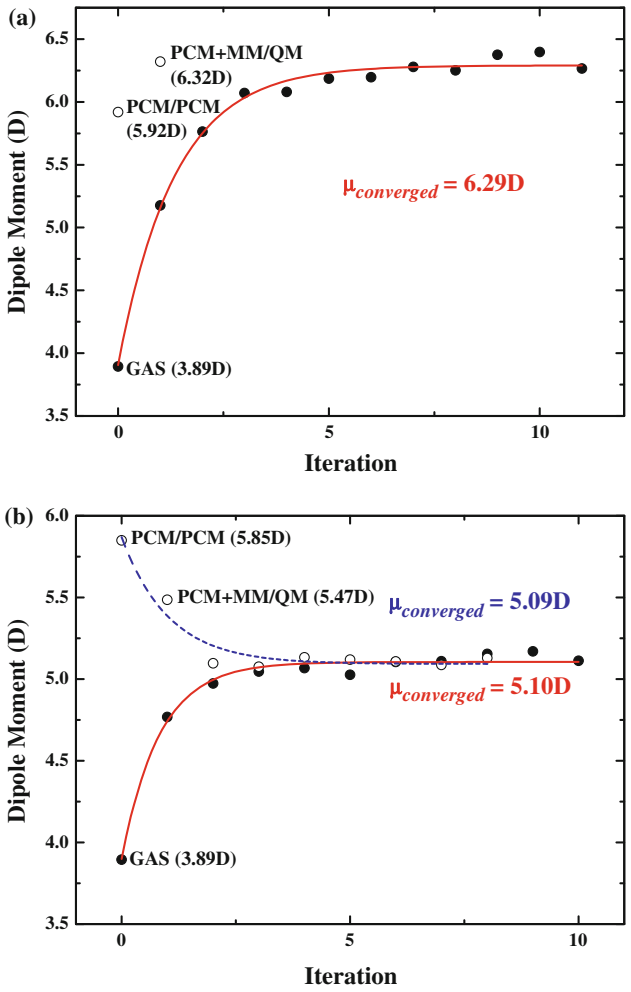

Fig. 2 Converged dipole moment of 5FU in **a** water and **b** acetonitrile, obtained using an iterative procedure. "GAS" indicates that the dipole moment was obtained using the optimized geometry of isolated 5FU; "PCM/PCM" indicates that dipole moment was calculated considering the solvent effect in this approach in a geometry optimized also in this approach; in "PCM + MM/QM," dipole moment is calculated using the ASEC generated after a Monte Carlo simulation which uses the values obtained in "PCM/PCM" as starting point. The converged dipole moment values were obtained by a fitting procedure

sample of configurations is by convoluting the results to obtain the spectral shape and include the inhomogeneous broadening due to the structural fluctuations, as we have done before [41]. However, in the present case, we obtained the same results. As we have an intense (π–π^*) band near the weak (n–π^*) transition, the Lorentzian convolution produced a broad π–π^* and a weak n–π^* bands, but with the two maxima corresponding to the average values.

The result of 4.42 eV, obtained using seven molecules (corresponding to the number of molecules in the microsolvation shell, using a minimum-distance distribution function) [42], underestimates the experimental value. Adding the electrostatic embedding, the result increases to 4.82 eV, close to the value that is converged with respect to the number of solvent molecules included. Figure 4 illustrates one of the configurations with 16 explicit water molecules in the electrostatic embedding composed of 238 water molecules. The result obtained with ASEC corresponds to the sole inclusion of the electrostatic interaction between 5FU and the solvent. Comparing this with the converged result obtained with explicit solvent indicates a decrease of 0.1 eV when using explicit solvent molecules. This value is relatively small, and the explicit use of solvent molecule will not be considered in the more expensive ab initio methods. As expected, CIS gives a value for the excitation energy that is too large. Using only the electrostatic interaction, the values obtained with CIS(D) and EOM-CCSD are equivalent and slightly larger than the experimental value. This indicates that the essential contributions of the double excitations are obtained in low-order perturbation theory. Assuming the correction of $\sim$0.1 eV for the contribution of the explicit solvent molecules still indicates that these values are overestimated by ca. 0.5 eV. The results for the n–π^* transition are also shown in Table 3, but the experimental location of this transition is uncertain [3]. It should be noted that except for the 5FU + 7 H_2O and INDO/CIS results, all theoretical models now agree that the π–π^* transition is lower than the n–π^* transition. The INDO/CIS calculation includes 150 explicit H_2O molecules, corresponding to including all solvent molecules within a distance of 8 Å from the center of mass of 5FU. In all cases, as expected, the n–π^* transition blue shifts compared to the isolated case, whereas the π–π^* transition red shifts. This is also the case in the INDO/CIS, where the two shifts have the correct sign, but quantitatively are not enough to promote the reversal of the two excited states. This failure has also been noted in the

Table 2 The two lowest electronic transitions of isolated 5-fluorouracil using the geometry optimized with the B3LYP/aug-cc-pVDZ level

QM method	n–π^*		π–π^*	
	E (eV)	f	E (eV)	f
B3LYP	4.67	0.000	4.88	0.125
O3LYP	4.33	0.000	4.69	0.096
PBE0	4.65	0.000	4.95	0.137
BHandHLYP	5.16	0.000	5.36	0.219
CIS	6.12	0.000	6.26	0.448
CIS(D)	4.82	0.000	5.36	0.448
EOM-CCSD	5.14	0.000	5.50	0.218
INDO/CIS	3.99	0.001	5.20	0.438

f is the calculated oscillator strength (dimensionless)

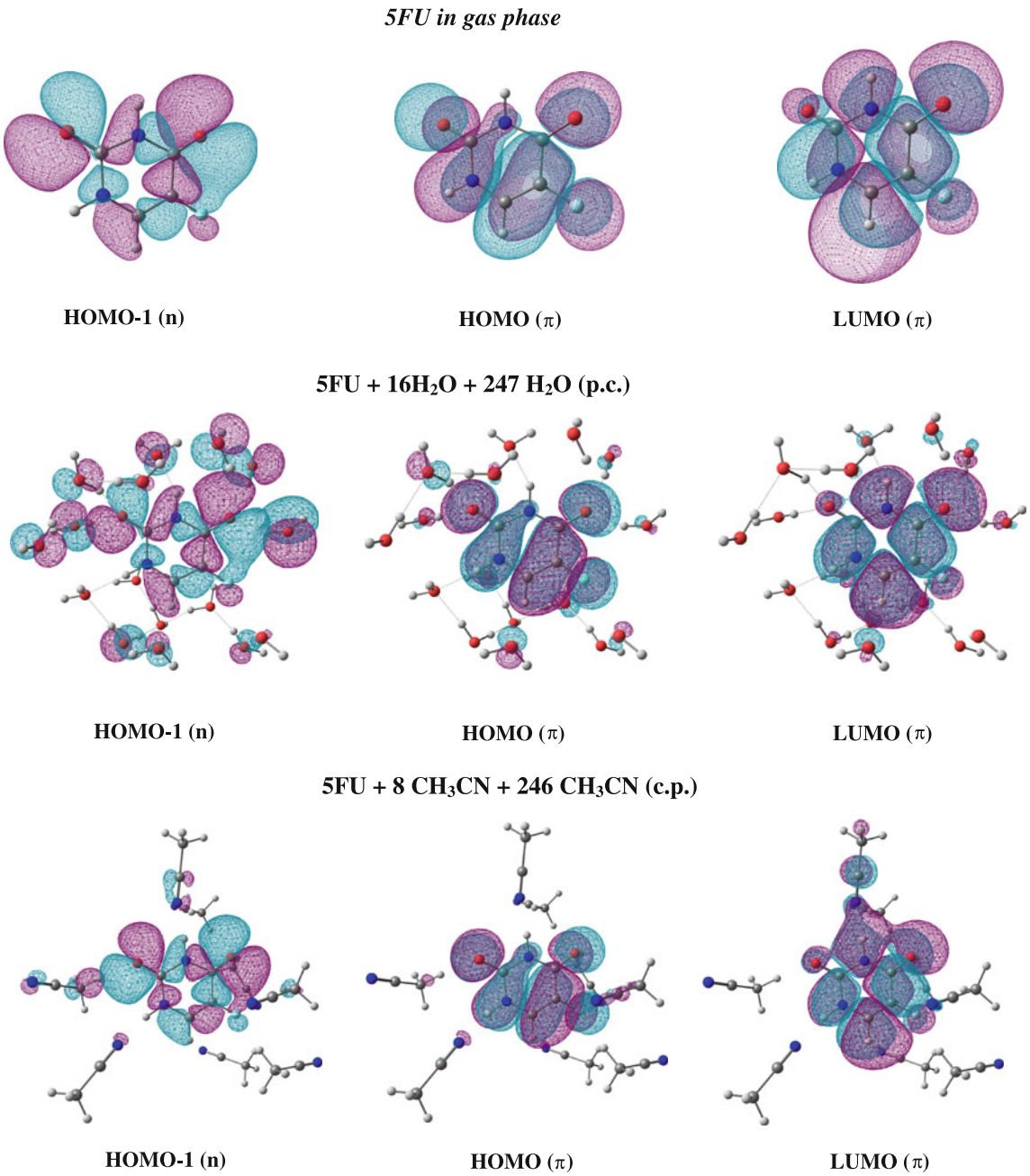

Fig. 3 The 5FU Kohn–Sham orbitals involved in the lowest transitions in gas phase (*top*) and a configuration in each solvent: water (*medium*) and acetonitrile (*bottom*). The B3LYP/6-311++G(d,p) level of calculation was used

case of uracil [43] and should be traced to the original parameterization.

Now, we analyze the case of acetonitrile as the solvent (Table 4). The theoretical results are obtained in a similar manner as in the case of water. Again, the explicit use of all solvent molecules within a distance of 8 Å is used in the INDO/CIS case. The experimental result locates the π–π* band maximum at 4.70 eV, separated from the same band in water by only 0.04 eV (corresponding to 300 cm^{-1} or 2 nm in the band maximum) [3]. As expected, the experimental redshift of the π–π* transition is larger in water than in acetonitrile, though the difference is very small. Qualitatively, one can note that some theoretical methods fail in this aspect. The PCM method is not able to distinguish the two solvents giving the π–π* transition of 5FU located at 4.81 eV for the two solvents. This has been noted before [7] and justified the use of some explicit solvent molecules. In general, all DFT-based methods also fail in giving the relative location of the π–π* transition of 5FU in water and acetonitrile. Interestingly, this is the case

Table 3 The two lowest electronic transitions of 5-fluorouracil in water

QM method	Solvent model	π–π*		n–π*	
		E (eV)	*f*	*E* (eV)	*f*
B3LYP	PCM	4.81	0.176	4.99	0.000
B3LYP	ASEC	4.88 (4.90)	0.139 (0.140)	5.10 (5.30)	0.000 (0.000)
B3LYP	5FU + 07 H_2O	4.42	0.034	4.40	0.003
B3LYP	5FU + 07 H_2O + 247 H_2O (p.c.)	4.82	0.164	5.07	0.003
B3LYP	5FU + 10 H_2O + 244 H_2O (p.c.)	4.81	0.164	5.07	0.003
B3LYP	5FU + 12 H_2O + 242 H_2O (p.c.)	4.80	0.161	5.05	0.004
B3LYP	5FU + 14 H_2O + 240 H_2O (p.c.)	4.79	0.156	5.05	0.004
B3LYP	5FU + 16 H_2O + 238 H_2O (p.c.)	4.79 (4.80)	0.154 (0.156)	5.04 (5.14)	0.004 (0.006)
PBE0	ASEC	4.99 (5.00)	0.151 (0.152)	5.22 (5.43)	0.000 (0.000)
BHandHLYP	ASEC	5.37 (5.38)	0.218 (0.215)	5.77 (5.96)	0.000 (0.000)
O3LYP	ASEC	4.76 (4.79)	0.117 (0.121)	4.90 (5.12)	0.000 (0.000)
CIS	ASEC	6.25 (6.28)	0.418 (0.409)	6.82 (6.96)	0.000 (0.000)
CIS(D)	ASEC	5.18 (5.19)	0.418 (0.409)	5.32 (5.55)	0.000 (0.000)
EOM-CCSD	ASEC	5.29 (5.30)	0.226 (0.220)	5.56 (5.76)	0.000 (0.000)
INDO/CIS	5FU + 150 H_2O	5.05	0.441	4.40	0.003
Exp. [3]		4.66	–	–	–

The results obtained using explicit solvent molecules are converged averages over 100 statistically uncorrelated configurations (the statistical error is less than the decimal presented). ASEC is an average electrostatic configuration. Results in parenthesis correspond to the iterative polarization. Except for the INDO/CIS, all results were obtained using the 6-311++G(d,p) basis set. *f* is the calculated oscillator strength (dimensionless)

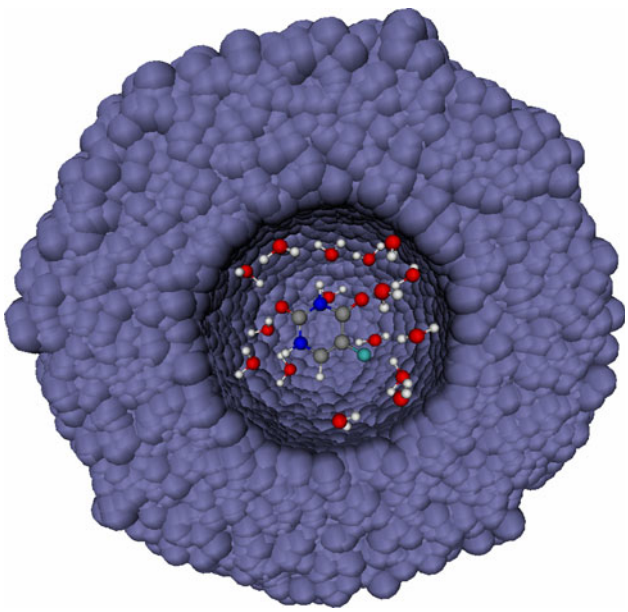

Fig. 4 One of the configurations used in the quantum mechanical calculations. The illustration shows 5FU and 16 explicit water molecules embedded in the electrostatic field of 238 remaining water molecules represented by simple point charges on the atomic positions

even when using the explicit solvent molecules of the first solvation shell in the electrostatic embedding of the remaining solvent molecules. Indeed, the B3LYP result of 4.77 eV for 5FU + 8CH_3CN + 246CH_3CN(pc) is lower in energy than the corresponding result for 5FU + 7 H_2O + 247 H_2O(pc) or even 5FU + 16 H_2O + 238 H_2O(pc). The INDO/CIS method with a large number of explicit solvent molecules correctly describes the qualitative positioning of the π–π* transition in water and acetonitrile. However, the magnitude of separation of this band in the two solvents is too large. As in the case of water, comparing the result for B3LYP using only the electrostatic solute–solvent interaction with the use of explicit solvent molecules gives an estimate of the contribution of the explicit molecules of ∼0.1 eV. Using only the electrostatic solute–solvent (ASEC), all CI-based and the EOM-CCSD methods correctly obtain the π–π* transition of 5FU in acetonitrile located higher in energy than in water. Table 5 summarizes the situation. The results indicate that within the approximations used here, common to several studies of solvent effects in spectroscopy, the CIS(D) and EOM-CCSD perform very well and give equivalent results. Although the individual transition energies are slightly larger than in experiment, the relative location of the π–π* transition is well described. Also, it can be noted that the PCM polarization used in the CIS(D) and EOM-CCSD gives the relative location with better precision, whereas the iterative polarization leads to an overestimation. There are different possibilities for this. One is that the iterative procedure may overpolarize the solute [44, 45]. Indeed, this has been analyzed before and may require the reconsideration of the LJ parameters for

Table 4 The two lowest electronic transitions of 5-fluorouracil in acetonitrile

The results obtained using explicit solvent molecules are converged averages over 100 statistically uncorrelated configurations (the statistical error is less than the decimal presented). ASEC is an average configuration. Results in parenthesis correspond to the iterative polarization. Except for INDO/CIS, the results were obtained using the 6-311++G(d,p) basis set. f is the calculated oscillator strength (dimensionless)

QM method	Solvent model	π–π*		*n*–π*	
		E (eV)	f	E (eV)	f
B3LYP	PCM	4.81	0.177	4.98	0.000
B3LYP	ASEC	4.84 (4.89)	0.128 (0.132)	4.83 (4.92)	0.001 (0.000)
B3LYP	5FU + 08 CH_3CN + 246 CH_3CN (p.c.)	4.77 (4.79)	0.152 (0.148)	4.84 (4.92)	0.003 (0.010)
PBE0	ASEC	4.95 (5.00)	0.139 (0.145)	4.95 (5.05)	0.002 (0.000)
BHandHLYP	ASEC	5.36 (5.40)	0.217 (0.217)	5.48 (5.55)	0.000 (0.000)
O3LYP	ASEC	4.71 (4.76)	0.106 (0.110)	4.62 (4.74)	0.000 (0.000)
CIS	ASEC	6.26 (6.30)	0.431 (0.428)	6.49 (6.51)	0.000 (0.000)
CIS(D)	ASEC	5.22 (5.29)	0.431 (0.428)	5.09 (5.21)	0.000 (0.000)
EOM-CCSD	ASEC	5.33 (5.38)	0.223 (0.218)	5.29 (5.38)	0.000 (0.004)
INDO/CIS	5FU + 63 CH_3CN	5.23	0.502	4.87	0.002
Exp. [3]		4.70	–	–	–

Table 5 Difference between the energy of 5FU π–π* transition in water and in acetonitrile

QM method	Solvent model	$E_{acet} - E_{water}$ (eV)
B3LYP	PCM	0.00
B3LYP	ASEC	−0.04 (−0.01)
B3LYP	Explicit + (p.c.)[a]	−0.02 (0.00)
PBE0	ASEC	−0.03 (−0.01)
BHandHLYP	ASEC	−0.01 (0.02)
O3LYP	ASEC	−0.05 (−0.03)
CIS	ASEC	0.00 (0.03)
CIS(D)	ASEC	0.04 (0.10)
EOM-CCSD	ASEC	0.04 (0.08)
INDO/CIS	Explicit[a]	0.19
Exp. [3]		0.04

Values in parenthesis mean that the solute was polarized using an iterative procedure

[a] Statistical error in this case is 0.01 eV

better results [44, 45]. Also, the present procedure considers only the polarization of the solute due to the solvent, assuming that the reversal is small. In the same vein, the geometry relaxation of the solute is assumed to lead to a negligible effect on the spectral transitions.

We now consider, in some more detail, the position of the unobserved *n*–π* transition in water and in acetonitrile. Theoretical considerations [7] along with consequences of the photophysical properties [9] indicate that the π–π* and *n*–π* transitions are reversed in water compared to the isolated condition. This is simple to understand on the basis of the redshift of the π–π* and blueshift of the *n*–π* transitions. Most theoretical models (Table 3) agree that in water the intense π–π* excited state of 5FU is lower than the dark *n*–π* state. The situation is less clear in acetonitrile. Experiments indicate that the fluorescence spectrum of 5FU in acetonitrile is much broader than in water [46] and the decay in acetonitrile is thus much faster. Experimental and theoretical works by Improta and coworkers [9] have shown that a decay channel is available for 5FU involving the dark *n*–π* state. One outcome of these investigations is that the π–π* and *n*–π* excited states are very close in energy in the Franck–Condon region and then they cross in the path for the minimum of the π–π* state. The present theoretical results reflect this aspect. Table 4 shows that some theoretical methods still obtain the *n*–π* located lower but close to the π–π* state in the Franck–Condon region of the ground state. This is the case for the CIS(D) and EOM-CCSD. The PBE0/ASEC gives the same result, and the BHandHLYP reverses, giving the π–π* state lower. The INDO/CIS even including 63 explicit acetonitrile solvent molecules also gives the *n*–π* state lower. Consideration of the results obtained with B3LYP with and without explicit solvent molecules indicates that the delocalization of the wave function over the solvent region is more important for the π–π* state than for the *n*–π*. Table 4 indicates, by comparing the results obtained with the B3LYP/ASEC and the B3LYP/5FU + 8CH_3CN + 246CH_3CN(pc), that this delocalization decreases the excitation energy of the π–π* state by ~0.1 eV, but is immaterial for the *n*–π* state. The non-electrostatic interactions thus play an important role in π–π* transition. Hence, we also calculated the transition energies of 5FU in acetonitrile using CIS(D)/6-311++G(d,p) and gradually increasing the number of explicit solvent molecules (Table 6). For this calculation we used only one representative configuration, since the CIS(D) method requires a very high computational demand. The results in Table 6 show that for the energy of the *n*–π* transition the electrostatic interactions are dominant. In contrast, the π–π* transition decreases in energy by about 0.11 eV, emphasizing the importance of considering the non-electrostatic interactions.

Table 6 Dependence of n–π* and π–π* transition energies (eV) with non-electrostatic interactions using CIS(D)/6-311++G(d,p)

Solvent model	n–π*	π–π*
5FU + 254 CH_3CN (p.c.)	5.10	5.22
5FU + 01 CH_3CN + 253 CH_3CN (p.c.)	5.08	5.23
5FU + 02 CH_3CN + 252 CH_3CN (p.c.)	5.10	5.17
5FU + 03 CH_3CN + 251 CH_3CN (p.c.)	5.12	5.14
5FU + 04 CH_3CN + 250 CH_3CN (p.c.)	5.13	5.11
5FU + 05 CH_3CN + 249 CH_3CN (p.c.)	5.11	5.13
5FU + 06 CH_3CN + 248 CH_3CN (p.c.)	5.12	5.11

Because of the very high computational demand, only one randomly chosen configuration was used

In summary, in solvent, the n–π* transition of 5FU molecule is displaced to the blue, while the π–π* transition undergoes a redshift. This shift is large enough to reverse the position of the transitions in water, but in acetonitrile it places them very close in energy, corroborating the discussion previously presented.

4 Conclusions

This work presents a theoretical study of the absorption spectra of the molecule 5-fluorouracil (5FU) in water and acetonitrile, using a sequential-quantum mechanics/molecular mechanics model that combines the Monte Carlo method and subsequent quantum mechanical calculations.

Both solvents are treated using more than one approach: continuous, discrete and explicit. The polarization was accounted by two procedures: using PCM and an iterative method. The quantum mechanical calculations were made with different methods: TD-DFT, CI, EOM and INDO.

Using TD-DFT, specifically the B3LYP functional, the value of the lowest π–π* transition obtained was very close to the experimental value, a difference of only 0.1 eV. However, the method fails calculating the transition in water as more energetic than in acetonitrile, when the opposite is experimentally measured. This occurs in all solvent approaches and persists even changing the functional or changing the polarization method.

The ab initio CIS(D) and EOM-CCSD methods slightly overestimate the transition energy, but present a very good description of the spectral shifts and the relative locations of the bright and dark states in the two solvents.

The semiempirical method INDO/CIS shows the correct trend, but too large values for the separation of the same π–π* state in the two solvents.

The CIS(D) gives equivalent results compared to EOM-CCSD, which gives the lowest π–π* and n–π* transitions very close in energy in the Franck–Condon region of the ground state, in agreement with the previous predictions. The non-electrostatic solute–solvent interactions are found to be not relevant to the n–π* state, but play an important role in the positioning of the π–π* transition, thus being of crucial importance for the relative location of these two levels and, hence, for the photophysical processes.

DFT is able to reproduce the position of the transitions in reasonable agreement with the experimental values. However, for describing the transition energy shifts, we find the CIS(D) and EOM-CCSD to perform better. With the exception of the INDO/CIS, all methods agree that there is a reversal of the 5FU lowest transitions in aqueous solution, if compared with gas phase, while in acetonitrile, they become very close in energy.

The sequential-QM/MM methodology within the PCM polarization combined with the CIS(D) approach is seen to provide a good description of the solvation effects in the low-lying excited states of 5-fluorouracil with the best ratio between performance and computational cost.

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