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The Prediction of Nanoscale Drug Molecular Structure and Acid Dissociation Constants of 5-Fluorouracil in Aqueous Solution Using DFT Methods

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Abstract

Background and Objective : In this work, dissociation of nano drug 5-Fluorouracil derivatives was studied theoretically. ***Methodology*** : For this purpose, Gibbs free energy values for neutral and deprotonated forms of 5-Fluorouracil were calculated at gas and aqueous phases by using density functional theory (DFT) method. Solvent effects are taken into account by means of polarizable continuum model (PCM). ***Result*** : It was shown that, theoretically calculated pK_a values are in good agreement with the existing experimental pK_a values, which are determined from capillary electrophoresis, potentiometric titration and UV–visible spectrophotometric measurements. ***Conclusion*** : In summary, cluster continuum method with implicit-explicit solvent molecules was used for calculation of pK_a values. Total energies and molecular parameters were obtained for 5-FUra nanoscale drug systems, at B3LYP/6-31G(d) level of theory for the anion, cation, and neutral species.

Keywords: Nanomedicine, 5-Fluorouracil, Acid dissociation constants, Computational Chemistry, DFT.

1. Introduction

The Controlled nanoscale drug delivery technology represents one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care. Nanoparticles hold tremendous potential as an effective drug delivery system. The nano drugs are target selective and specific towards tumors only resulting into better treatment [1].

The acid–base dissociation constant of substances) pK_a value) is a very important parameter in drug design, drug delivery and optimization. The degree of ionization strongly affects solubility, permeability, and drug disposition properties-absorption, distribution, metabolism and excretion [2]. One of the most important physicochemical properties of small molecules and macromolecules are the Dissociation constants for any weakly acidic or basic groups, generally expressed as the pK_a of each group [3].

The computational treatment and the underlying theoretical analysis of molecular properties have shown continuous growth in the degree of molecular complexity since the earliest efforts of chemist to employ computer calculations in advancing understanding. This will certainly continue right along with rapid increases in computing power as we have seen over the last decade. Computational investigation has reached a point where it is realistic to dis-play predictive simulations of complex biological molecules [4-6].

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In the field of industrial pharmacy, perhaps the most important physicochemical characteristic property of biologically active molecules is their acidity or basicity expressed by their pK_a values. Because most molecules have acidic and/or basic functionalities, relationships between dissociation constants and structure may prove useful in drug design studies and in explaining the biopharmaceutical properties of substances [6,7].

The theoretical prediction of pK_a values has received considerable attention and there have been many studies on this topic in recent years [8,9]. These studies are related with the use of different systems and different aspects of the computational methods used to determine acidity constants. Quantum chemical methods provide reliable pK_a values that allow a better understanding of the different factors on pKa values to be obtained, and are essential for interpretation of experimental values in various systems [6,10,11]. It was demonstrated that the calculation of pK_a values is possible by using simple ab initio methods. By using these methods it is possible to calculate pK_a values with an average error of less than 1 pK_a unit [12]. As the DFT calculation includes the effect of the electron correlation and can be calculated with a high accuracy, it needs only as much calculation time as the Hartree–Fock calculation, which is the cheapest ab initio calculation [13,14].

In general there are three methods known for calculation of pK_a: thermodynamic cycles, gas-phase free energy calculations and the change in free energy of solvation calculations. The methodology used in this work is Solvation free energy calculations.

The structure of 5-Fluorouracil (5-FUra) can be seen in Figure I.

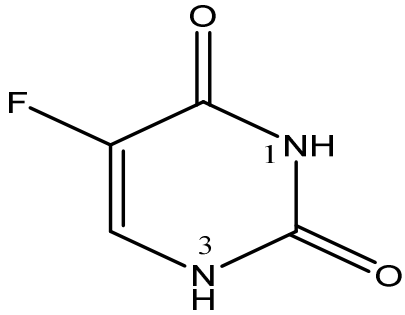


Figure I: Structure of 5-Fluorouracil

5-FUra was rationally developed in 1957 as a potential antitumor drug. Fluorouracil (5-FUra) is still considered the most active antineoplastic agent in the treatment of advanced colorectal cancer [15,16]. The chemotherapy agent 5-FUra, which has been used against cancer for about 40 years, acts in several ways, but principally as a thymidylate synthase inhibitor. Some of its principal uses are in colorectal cancer, and pancreatic cancer, in which it has been the established form of chemotherapy for decades. It is sometimes used in the treatment of inflammatory breast cancer, an especially aggressive form of breast cancer [17].

5- FUra is used in ophthalmic surgery, specifically to augment trabeculectomy in patients deemed to be at high risk for failure. 5- FUra acts as an anti-scarring agent in this regard, since excessive scarring at the trabeculectomy site is the main cause for failure of the surgery [18].

2. Methodology

Ab initio calculations of the 5- FUra nano molecule (Fig. II) were carried out with the Gaussian 09 computer code at density functional level of theory [19].

In order to evaluate the conformational behavior of these systems in solvent, Optimization calculations were performed in the presence of water. In this work cluster continuum method is used which uses implicit-explicit solvent molecules in calculations. Nanoscale drug structures were optimized at B3LYP/6–31G(d) level of theory without any geometrical constraint.

Bulk solvent effects were accounted by self-consistent reaction field (SCRF) approach based on the Polarized Continuum Model (PCM) method, which has been proven to be an effective tool to investigate on a variety of solution phase physicochemical properties.

Finally, we selected the solvation of the species by means of intermolecular hydrogen bonds (IHBs) that involve one molecule of the mentioned species and some molecules of water (see Table I and Fig. III) [20].

The tendency of a molecule to lose its hydrogen atom as an acidic proton is quantified as pK_a. Fully protonated 5- FUra have two acid groups. A first proton can be lost from N_1 Then second proton from N_3 group (see fig 1). The different models of molecule (zwitterions and unzwitterions) were investigated by the

Table I: Calculated total energy at B3LYP/6-31G(d) level of theory for cationic, neutral, and anionic species of 5- FUra at 298.15 K.

N	*Solvated species*	*$G°_{sol}$ (Hartree)*	*G^{o}_{sol}/molecule ($kJ{\cdot}mol^{-1}$)*
1	$H_2L^{2+}(H_2O)$	-591.3305	-371066
2	$HL^{+}(H_2O)_2$	-667.3514	-418769
1	$HL^{+}(H_2O)$	-590.9161	-370805
3	$L(H_2O)_3$	-743.3314	-466448
2	$(H_2O)_2$	-152.8798	-95933.5
2	$OH^{-}(H_2O)_2$	-228.84536	-143603
3	$OH^{-}(H_2O)_3$	-305.22555	-191532

N: total number of solvation water molecules
G°sol: total free energy in solution

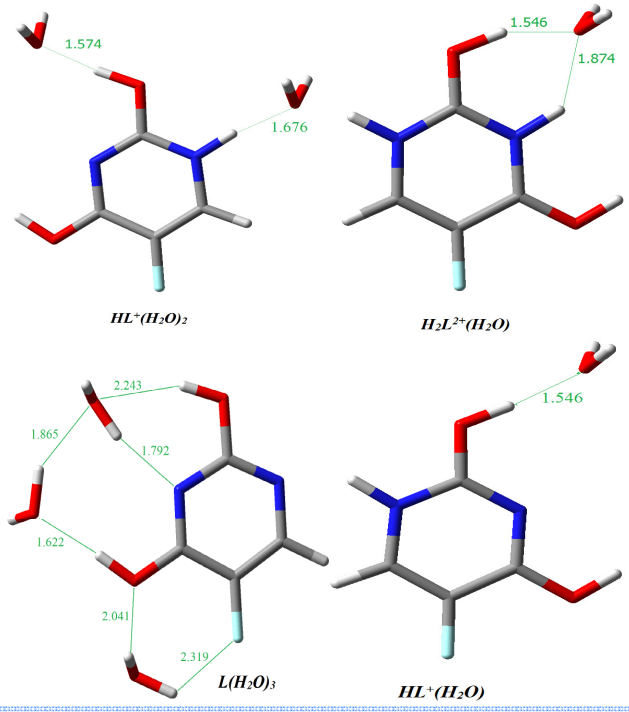


Figure II: Optimized molecular structures of nanoscale drug (5-FUra), in presence of one, two or three water molecules. Relevant hydrogen bond distances are reported in Angstrom.

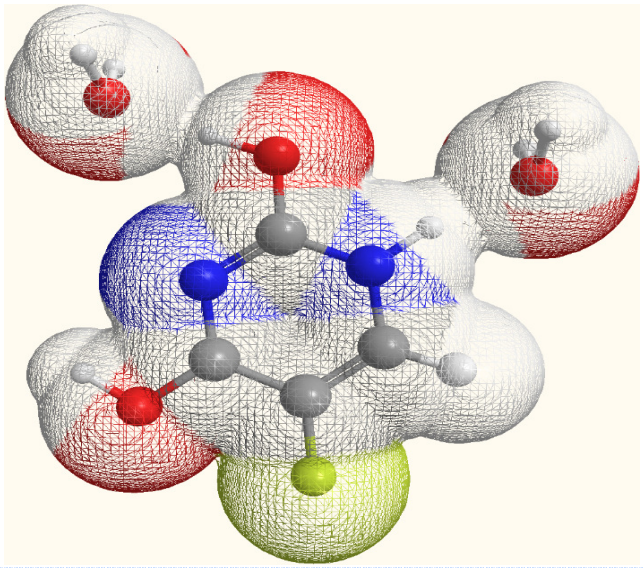


Figure III: Calculated molecular surface of (5-FUra) solvated with two water molecules $[HL^{+}(H_2O)_2]$ obtained at B3LYP/6-31G(d) level of theory.

G09 program. Different reactions including cationic, neutral, and anionic species were tested, but some of the reactions were not considered further because the estimated error in its acidic dissociation constants was unacceptable. The models finally chosen for the studied system and the calculated values of the acidic dissociation constants for this nanoscale drug is listed in

Table II: Calculated total energy at B3LYP/6-31G(d) level of theory for cationic, neutral, and anionic species of 5- FUra at 298.15 K. *a*: from Ref [24][24]

species	*selected equations*	*ΔG (Hartree) calculated*	*pKa (calculated) this work*	*pKa (experimental)*	**Relative deviations (RD) for pK_a**
5- FUra	Equation 1	-0.05539768	8.031	8.0[a]	0.003875
	Equation 2	-0.06957118	12.843	13.0[a]	0.012077

Table II.

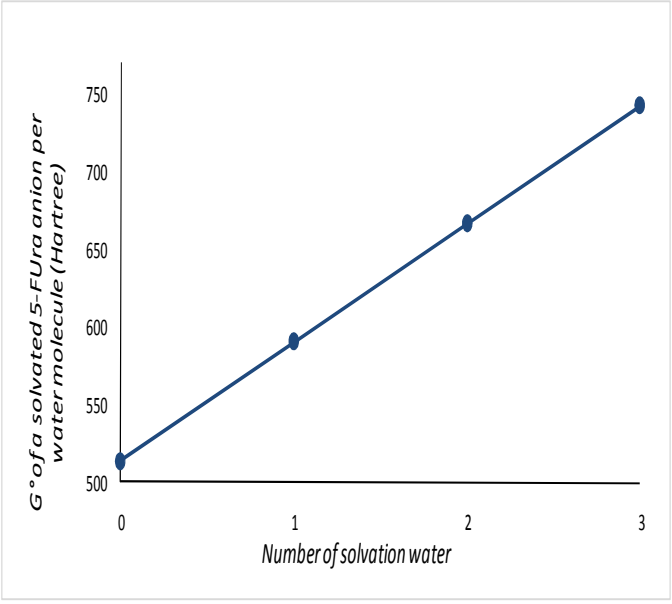
The acidic dissociation constants of this nano drug (5- FUra) have been determined using the potentiometric and spectrophotometric techniques. The method of determining acidic dissociation constants was previously described, and its values are used in this work [21]. These values are listed in Table II together with the calculated values using Cluster continuum method at the B3LYP/6-31G(d) level of theory.

The total energies of the single and solvated 5-FUra nanoscale drug species (cationic, neutral, and anionic) were calculated in water. Table I summarizes the variations of the total energy ($kJ \cdot mol^{-1}$) of the species per water molecule as a function of the total number of solvation water molecules. Figure IV, and Table I show the marked increase of the total energies of ions when the solvation decreases.

The data show that the water, exerting its hydrogen-bond-donor (HBD) capability, forms IHBs with the 5-FUra nano drug [22]. These hydrogen bonds have been classified as strong, moderate, and weak, according to their lengths, angles, and energies [23].

The calculated molecular volume and surface values, together with the corresponding values obtained at DFT- B3LYP/6-31G(d) level are summarized in Table III. The surface of ones calculated molecule $HL^+(H_2O)_2$ is also shown, as an example, in Figure III.

Figure IV: Plot of total energy (Hartree) of a solvated 5-FUra anion per water molecule against the total number of solvation water molecules.



3.1 First Ionization Constant of 5-FUra nanoscale drug:

It was selected that in alkaline solutions 5-FUra suffers a total neutralization as follows:

$$H_2L^{2+}(H_2O)+OH^-(H_2O)_2 \longrightarrow HL^+(H_2O)_2+2H_2O \quad ..(1)$$

In this reaction (eq.1), $H_2L^{2+}(H_2O)$ is the 5-FUra solvated with one water molecule, and $HL^+(H_2O)_2$ represents 5-FUra solvated with two water molecules. The above reaction (eq.1) was used to determine theoretically the value of the first ionization constant of 5-FUra nanoscale drug in water. Table II (section Equation 1) summarizes the optimized values of molecular physico-chemical properties of the $H_2L^{2+}(H_2O)$ cation (figure II), OH^- ion, and $HL^+(H_2O)_2$ cation molecule (figure II) obtained at B3LYP/6-31G(d) level of theory with cluster continuum method in water at 298.15 K.

3.2 Second Ionization Constant of 5-FUra nanoscale drug:

Here, it is selected that the neutral $HL^+(H_2O)_2$ suffers a reaction of partial neutralization as follows:

$$HL^+(H_2O)_2 + OH^-(H_2O)_3 \longrightarrow L(H_2O)_3 + 2H_2O \quad ....(2)$$

In the above reaction (eq.2), $L(H_2O)_3$ represents the 5-FUra neutral solvated with three water molecules. Table II (section Equation 2) summarizes the optimized values of molecular physico-chemical properties of the $HL^+(H_2O)_2$ cation molecule (Figure II), $OH^-(H_2O)_3$ ion, and $L(H_2O)_3$ neutral molecule (figure II) obtained at the B3LYP/6-31G(d).

The relative devations (RD) for pK_a can be calculated from the following equation [25]:

$$RD = \left| \frac{pKa_{(calculated)} - pKa_{(experimental)}}{pKa_{(experimental)}} \right| \quad .... (3)$$

Table III shows the computed values for surface and van der Waals volume of complex (solute-solvent) of 5-FUra species.

Conclusions

In summary, cluster continuum method with implicit-explicit solvent molecules was used for calculation of pK_a values. Total energies and molecular parameters were obtained for 5 -FUra nanoscale drug systems, at B3LYP/6-31G(d) level of theory for the anion, cation, and neutral species. For analyzing the solvent effects on all species involved in the selected ionization reaction, the polarized continuum model (PCM) of Tomasi et al. was used. The resulting values are shown in the Tables I and II.

According to table II computationally obtained pK_{a1} value (lost from N_1) is very close to the experimental pK_{a1} value and for pK_{a2} (lost from N_2) calculated value is relatively

Table III: Computed values for surface and van der Waals volume of nanoscale 5-FUra at B3LYP/6-31G(d) level of theory.

Solvated species	*Molecular Surface*	*Molecular volume* ($A^{\circ 3}$)
$H_2L^{2+}(H_2O)$	158.175	130.361
$HL^{+}(H_2O)_2$	191.207	149.560
$HL^{+}(H_2O)$	162.709	131.518
$L(H_2O)_3$	202.376	163.732

comparable with the experimentally determined pK_{a2} which obtained from reference 24. So one conclude that theoretically obtained pK_a values are in very good agreement with experimental data obtained from solution measurements.

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