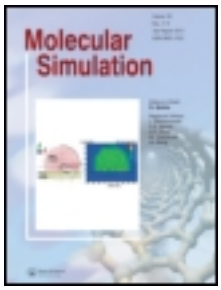

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Interaction between epoxidised estradiol and fullerene (C_{60}): possible anticancer activity

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Interaction between epoxidised estradiol and fullerene (C_{60}): possible anticancer activity

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In this article, the antioxidant activity of fullerene is theoretically studied by applying the density functional theory (DFT) method in terms of its protective effects against the derivatives of estrone that constitute species known to exhibit carcinogenic activity. Several reactions involving fullerene C_{60} in different possible reactive centres of estradiol and epoxidised estradiol were studied. Surprisingly, the ring that supports the epoxide group is able to react with fullerene by means of a $2 + 2$ cycloaddition, forming a very stable compound. This new compound has the potential to avoid known reactions between the epoxidised molecule and DNA fragments causing the mutagenic process of breast cancer. Therefore, fullerene C_{60} represents the possibility of a new agent for combating this disease.

Keywords: mutagenic process; breast cancer; fullerene complexes; DFT calculations; estradiol

1. Introduction

Unadulterated fullerenes and their water-soluble derivatives are revealed as potent free radical scavengers, making this class of compounds an attractive tool for regulating free radical processes and thus reducing the severity of oxidative stress in biological systems [1, 2].

C_{60} readily accepts up to six electrons [3], because of its low lying and triple degenerate LUMOs [4, 5]. The electron affinity of C_{60} can be explained qualitatively by considering its numerous pyracyclene units, as these are able to convert from an unstable $4n$ π-system to a stable aromatic $4n + 2\pi$ system, upon receiving two electrons [6]. Moreover, the tendency for pyramidalisation to occur has promoted interest in the electronic transfer capabilities of fullerenes in biological systems [7].

Until now, it has been demonstrated that due to its structure, the hydrated form of chemically unmodified C_{60} fullerene can act simultaneously as an antiradical, antioxidant and radio-protective agent [3]. The structure consists of 60 carbon atoms connected by sp^2 bonds that determine its pseudo-aromatic structure, due to delocalisation of π-electrons over its carbon core. This makes possible for C_{60} to readily react with oxygen free radicals [3].

From the time of the first studies defining antioxidant abilities of C_{60}, it was supposed that an extended electron-conjugation system only determines the high reactivity of fullerene molecules towards reactive oxygen species. Recently, fullerene has been considered to be a novel 'structural' antioxidant and characterised as a 'radical sponge' [8].

Based on results indicating that unadulterated C_{60} is non-toxic (in its hydrated form) with any side effects known, as well as providing ample radio-protective effects in low doses, C_{60} may be considered as a novel anti-oxidising agent, which substantially diminishes the harmful effects of ionising radiation [3].

Several reports present the solubility of fullerene in various solvents [9–13]. It has also been shown that the solubility of C_{60} in polar solvents is quite low [9, 12]; however, a study on ^{14}C labelled as C_{60} reported that it was possible to form a suspension of C_{60} that remained stable for long periods in water and could be delivered to cells [14, 15].

Due to their ability to modulate cell death, buckminsterfullerene (C_{60}) and its water-soluble derivatives, synthesised by attaching various functional groups (—OH, —COOH, —NH2 and others) to the fullerene cage, represent promising candidates for both cyto-protection and anticancer therapy [16].

Likewise, because of its nano-sized carbon cage, fullerene can be easily modified when juxtaposed with the most suitable chemical group with the purpose of developing new compounds manifesting desired biomedical functions. As a result, biomedical applications have been proposed and likewise attracted much attention since the discovery of C_{60} [17]. For instance, in 1993 and subsequent years, C_{60} derivatives were found to inhibit HIV protease [18, 19]. Besides this, in 1997 poly(ethylene glycol) (PEG)-modified C_{60} was suggested as a possible agent for photodynamic tumour therapy [20, 21]. This was because conjugated C60-PEG showed higher accumulation in tumour tissue and minimised skin cancer among mice exposed to visible light.

Another example refers to the cytotoxicity of polyethylene. In certain animal studies, glycol-modified

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C_{60} and multihydroxylatedmetalofullerene $[Gd@C_{82}(OH)_{22}]n$ were successfully used to target tumour tissue, causing tumour regression without discernible damage to healthy tissues [22, 23].

In the case of menopausal women, whose ovaries have ceased producing estrogene, the peripheral conversion of androgens to estrogene by aromatisation becomes the main source for the endogenous estrogene pool [24]. It has been established that estradiol, the most important precursor of estrone, is one of the main risk factors for the genesis and evolution of breast tumours [25, 26]. The reason for this is due to the fact that estradiol can easily generate the corresponding anion, thus producing epoxide, whereas estrone is clearly impeded [27]. In this context, fullerene can work as an electron scavenger to prohibit carcinogenic activity on the part of the estradiol.

Therefore, the main goal of this article is to study the reaction between C_{60} and epoxidised estradiol using theoretical methods, where epoxide is exposed to the fullerene with the expectation that the fullerene will catch the electrons of the epoxide radical, thus reducing the molecule to a species of estrogene which is non-harmful to DNA units. This experiment should reveal the anti-cancerigenous activity of the fullerene under ideal conditions.

2. Model and methodology

The structures were optimised by applying the BPW91 [28, 29] method included in the Gaussian 03 package [30]. The nature of the hydrogen bonds between the biomolecules, estradiol and its derivatives and the DNA fragments was approximated by applying the framework of the modified DFT MPWB95 [31] included in the Gaussian09 package [32], with the basis set 6-31G**. Reaction profile was calculated using the QST2 method [33, 34] with the same basis set at the B3LYP level of theory and verified by identifying a single imaginary frequency. This level of theory and basis set has been already proven to be a relatively low computational cost yet high accuracy method of calculation [35, 36]. The calculation of frequencies was carried out at BPW91/6-31G** level and applied in order to calculate the enthalpy values needed for the thermochemical analysis. No basis set superposition error correction was applied to the results, as it was proven that high-level, single-point energy calculations correlate more accurately with experiments where no correction has taken place [35, 37]. The dynamic processes between the epoxides and fragments of DNA were studied, applying the first principle *ab initio* atom-centred density matrix propagation (ADMP) molecular dynamics model [38, 39], with 1000 steps in trajectory and a step size with the dynamics of 0.1 fs. The temperature and pressure conditions were taken as 310 K and 1 atm, to simulate the normal conditions of the human body as closely as possible. Once again the method used was the DFT MPWB95 with 6-31G** basis set, as this functional provided an adequate representation of weak interactions. Besides, a similar calculation by means of molecular dynamics taking into account a water environment was also carried out at the same level of theory and applying the polarisable continuum model [40].

3. Results and discussion

The antioxidant nature of fullerene has been widely examined [41]. The source of this phenomenon comes from the pronounced symmetry of C_{60} (point group I_h) which leads to a known arrangement of the frontier orbitals with a fivefold degenerate set for the HOMO and a threefold degenerate set for the LUMO [42]. This scheme promotes electronic attraction to the LUMO, so that C_{60} becomes an electron-withdrawing species.

Previously, the potential of the fullerene in terms of promoting antioxidant activity for medical purposes was revealed, as it acts simultaneously as an antiradical, antioxidant and radio-protective agent [1]. It is well known for acting as an electron scavenger, reducing the severity of oxidative stress in biological systems [6, 7]. This is also the case for the (C_{60}) fullerene and its water-soluble derivatives which offer important alternatives for cyto-protection and anticancer therapy [16], as well as manifesting inhibitory activity against HIV enzymes [42–48] and portraying bactericidal [49–51] and antiviral properties [52, 53]. It is also important to emphasise that other studies have found that the covalent binding of fullerene C_{60} to pharmacologically important compounds may lead to the design of a new generation of biologically active compounds, where the fullerene moiety will serve as a transport component for the delivery of active substances to damaged cells, due to the ability of carbon clusters to efficiently penetrate cell membranes [54]. Thus, it is important to emphasise the diversity of possible medical applications for this compound.

The main incentive for this study is to continue previous research on the part of our group, who investigated the nature of cancerigenous activity of derivatives of estrone [27], where the greatest activity was attributed to estradiol. Indeed, the species which manifests cancerigenous behaviour is the epoxide of estradiol [55], formed by an enzymatic reaction and able to make a direct attack on the DNA chain [56]. Therefore, the original idea was to take advantage of the antioxidant activity of C_{60} and search for possible reaction centres on the epoxide of the estradiol molecule. The interesting point here is that a very strong centre of this kind is found on the ring of the molecule which supports the epoxide group;

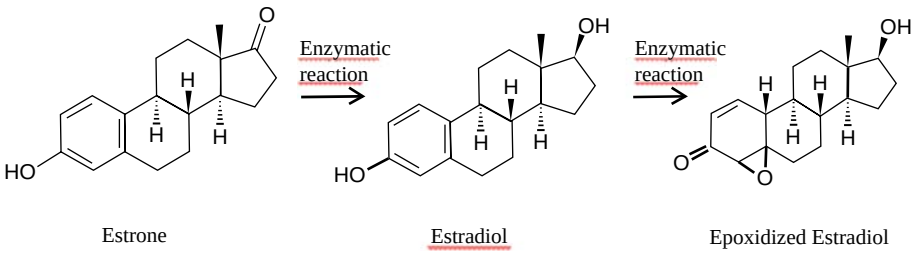

Figure 1. (Colour online) Scheme of the reaction pathway from estrone to epoxidised estradiol. Notice that the epoxidised estradiol loses the aromatic structure displayed by the estrone.

however, this is the double bond, and thus a feature deserving particular explanation.

The pathway of reactions involved in the transformation of estrone to epoxide of estradiol is shown in Figure 1; it is very similar to those reactions associated with the carcinogenicity of polyaromatic hydrocarbons [57]. Evidently, the most interesting feature in this entire scheme is that we initiate with an aromatic molecule that is preserved in the transformation from estrone to estradiol, but which loses its aromatic character when it becomes an epoxide, however, one of the original double bonds still remains in the last stage.

The original idea of this study was to analyse the interaction between the fullerene and epoxidised estradiol. Due to the antioxidant properties of the fullerene, it was expected to react with the epoxide of the estradiol precluding the interaction between epoxidised estradiol and a fragment of DNA [58]. This reaction, recognised as the initiation of the mutagenic process in breast cancer, is presented in Figure 2.

There were several attempts to carry out the simulation of interaction between the fullerene and epoxidised estradiol, first an input in which the fullerene molecule and the estrogene were put in such a position that the oxygen atom coming from the epoxide ring and a double bond of a six members ring of the fullerene were

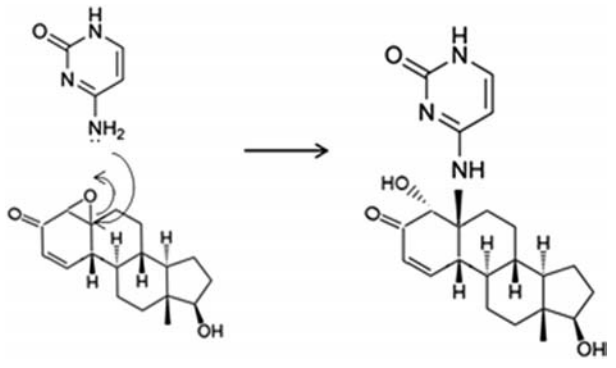

Figure 2. Scheme showing the interaction between estradiol and a DNA fragment. This reaction represents the initiation of the mutagenic process in breast cancer.

positioned to a ~ 1.4 Å bond length, but there was no reaction, other different inputs were proved searching for some kind of redox reaction (including all the oxygen atoms), however, in any case it was found the beginning of a chemical process, but curiously in all cases the fullerene molecule move to a position near the double bond of the estradiol group, therefore, the next attempt was to put both molecules in a position in which two double bonds, one of the fullerene and the other from the estradiol can interact, then the simulation shows a very clear chemical process and surprisingly this was a $[2 + 2]$ cycloaddition (see Figure 3).

Figure 3 depicts the cycloaddition between the species involved.

It is a known fact from the Woodward–Hoffmann rules [59] that a thermal $[2 + 2]$ cycloaddition is a forbidden reaction because of the lack of accessibility of adequate orbitals for the electronic exchange, however in the case of fullerenes, the cited abundance of reactive places in the LUMO as well as in the HOMO promotes this process; therefore, there are several examples showing this kind of reaction with fullerenes [60–62].

In the present case, referring to the reaction shown in Figure 3, the cycloaddition follows the next pathway: a pair of electrons from the fivefold degenerate set of the fullerene HOMO reaches the LUMO of the epoxidised estradiol, this electronic pair belongs to the double bond that is breaking, resulting in two new bonds that constitute a four member ring forming a cycloadditioned complex. The corresponding molecular orbital interaction diagram is shown in Figure 4, as well as the LUMO of the epoxidised estradiol and the HOMO of the fullerene.

The study of this reaction is divided into four parts: first, taking a thermochemical approach, when the reaction shown in Figure 3 is analysed on the basis of the change in enthalpy. Secondly, a statically quantum approach, when the possible formation of hydrogen bonds and other electronic interactions are assessed; this part was carried out, applying the Gaussian 03 package and the MPWB95 functional. Thirdly, the approach of the reaction profile was traced by means of a QST2 subroutine included in the same Gaussian 03 software and finally the dynamic process corresponding to the cycloaddition reaction was

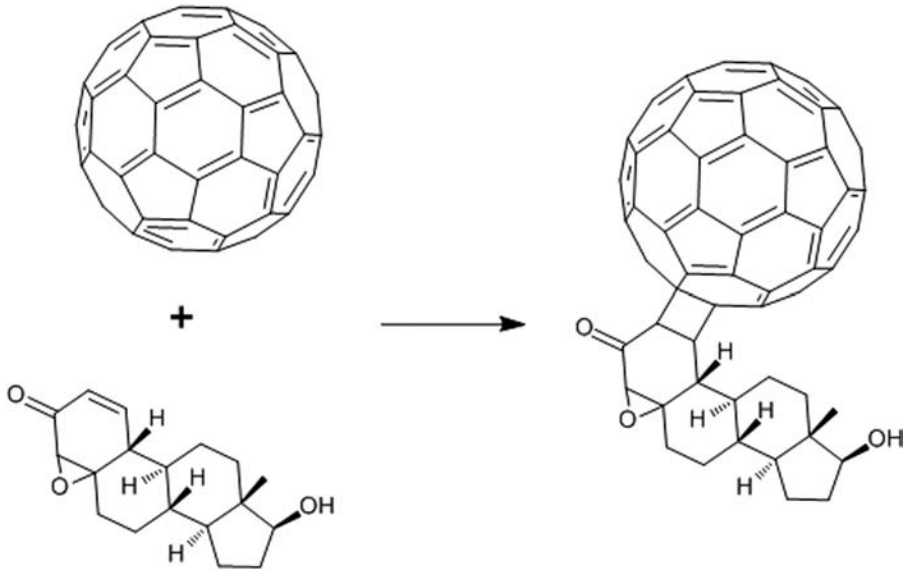

Figure 3. Cycloaddition [2 + 2] between the epoxidised estradiol and the fullerene. This new cycloadditioned complex cannot react with any other DNA fragment; therefore, the mutagenic process is precluded.

assessed, applying *ab initio* molecular dynamics (ADMP) [63] and using Gaussian 09.

Thermochemical analysis ($\Delta H_{\text{react}} = \Sigma\Delta H_{\text{P}} - \Sigma\Delta H_{\text{R}}$) indicates that the process of cycloaddition comprises a mild exothermic reaction because the enthalpy of the reaction is -59.11 kcal/mol, therefore, its occurrence at room temperature is predicted to take place without any difficulty.

The next study involves the possible weak interactions between fullerene and several potential reactive centres of the epoxide of estradiol; centres that may be the carbonyl group, the epoxide group or even the hydroxyl group. It is important to mention that the ADMP simulations were proved in the various reactive sites of the molecules involved, indicating weak interactions that can be classified as hydrogen bonds, van der Waals interactions or even Heitler–London contacts. These results revealed interesting aspects related to the chemistry of this reaction. However, this type of study requires the application of more accurate and sophisticated methods, therefore it is only cited here; to be developed and presented in a future paper.

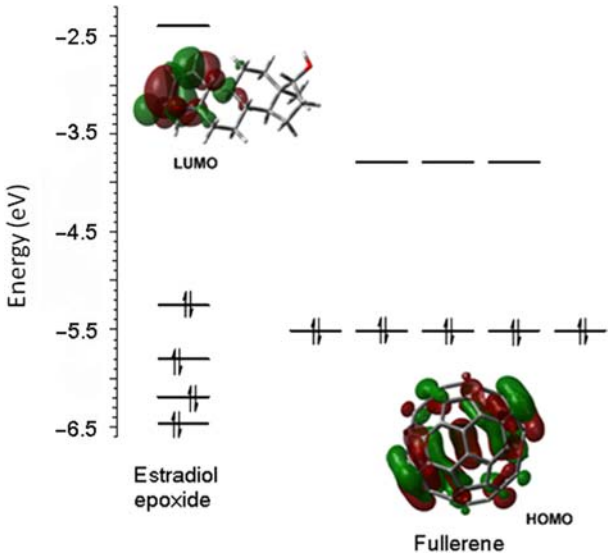

Figure 4. (Colour online) Molecular orbital interaction diagram (B3LYP/6-31G(d,p)) with the HOMO and LUMO of the fullerene and the epoxidised estradiol, respectively.

The reaction's profile was achieved by the QST2 subroutine included in the Gaussian09 package; the optimised structures were used as an initial approximation without any suggestion of transition state. The reaction's profile is shown in Figure 5, giving an energy barrier of 12.55 kcal/mol. This value can be compared with published results in two forms: first, processes carried out into live beings have been kinetically studied and their corresponding energy barriers have been reported, it is possible to cite the work by Otiepka [64] in which they found a barrier of $\sim 15-20$ kcal/mol for a process of cleavage of the active site of cytosine. Other example is those of the work by Sordo [65] for a water-assisted aminolysis of β-lactamas in which the barrier is 31 kcal/mol. Other interesting comparison is with other works in which a cycloaddition to a fullerene is considered, good examples are those from the work by Martín et al. [66] in which several barriers were calculated from different theoretical levels and yield results from 15 to 32 kcal/mol. Besides, the cycloaddition mechanism has

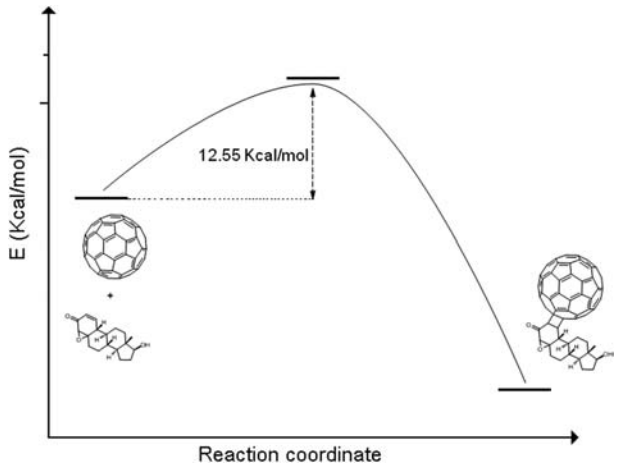

Figure 5. Reaction profile for cycloaddition of fullerene with epoxidised estradiol.

been studied in different systems [67] and the profile of that work is very similar to the one shown here and even the energy barrier found in that case is ~ 10 kcal/mol being very similar to the one reported here.

Assuming that the interaction between a DNA fragment and the epoxidised estradiol is responsible for the initiation of a mutagenic process and assuming that the presence of fullerene could prohibit this interaction with a DNA fragment; two different reactions were analysed by applying dynamic simulations. First, a normal reaction between the epoxide of estradiol and a fragment of DNA was implemented. Second, an analogous procedure was designed to detect the possible reaction of the cycloadditioned compound between the fullerene and the epoxidised estradiol.

The chosen fragments of DNA represent the interaction between a guanine and a cytosine base with hydrogen bonds (see Figure 2). It has been suggested that the reaction that produces a mutagenic process on the DNA, as a result of the attack by the epoxidised estradiol takes place on segments that contain these two unities by forming a covalent binding with the DNA. This causes gene mutations generated by a miss repair in the replication process which leads to DNA damage and tumour initiation in breast cancer [56].

The results from this section should be analysed very carefully. The dynamic processes are very sensitive to the position suggested in the initial guess, in the present case there were many different positions where this process was carried out, but in all cases the tests were undertaken under the same conditions for both systems, i.e. in both cases, the free epoxide of the estradiol and the cycloadditioned compound reacted with the cytosine–guanine complex (cyt–gua). Following the suggestions of Cavallieri's group [56], one of the most important tests simulated the attack of the epoxidised molecules on the proposed DNA fragment. The results are very interesting and will be described separately.

Figure 6 shows the total energy versus time in trajectory at 310 K and atmospheric pressure of the

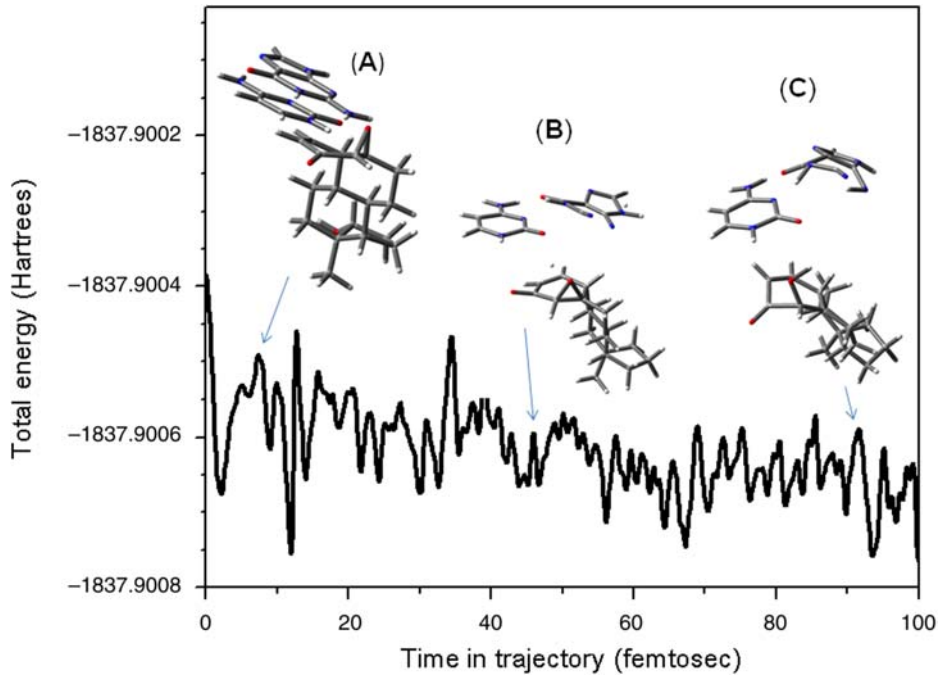

Figure 6. (Colour online) Total energy versus time for the interactions involved between epoxidised estradiol and a DNA fragment. Structural changes in the DNA fragment as a result of interaction with epoxidised estradiol (A, B and C) are also shown.

interactions involved between the epoxidised estradiol and a DNA fragment. Structure (A) corresponds to the start of the reaction, whereas in structure (B) it is apparent that both units are separated and the hydrogen bonds are broken. Subsequently, both hydrogen atoms derived from the nitrogen atom were separated. In structure (C), the epoxide bridge is open. Both molecules suffer tensions and distortions mainly on the DNA bases; and then ionised species are shown as products. Obviously this is not a stable product, but it is evident that a very energetic and spontaneous reaction takes place causing the loss of the cyt–gua complex due to the rupture of the hydrogen bonds, resulting in the adduct and leaving an apurinic site on the DNA.

In the first case, the natural process would be the attack of the epoxidised estradiol on the DNA fragment; it has been suggested [58] that the main focus for this attack is the terminal nitrogen atom as this is the chosen site of the guanine unit. This reaction is very destructive and causes severe damage to the cyt–gua complex.

Previously it was shown [56] that this is the first stage of a procedure involving the preferential insertion of Ade in the opposite DNA strand, leading to a G → T transversion at the site of the adduct and resulting in the mutagenic process.

The second case is very different; in this instance the analysed system was the new cycloadditioned molecule interacting with the DNA fragment. The molecular dynamic did not manifest strong evidence of a real reaction.

Figure 7 shows total energy versus time at 310 K and atmospheric pressure relating to the interactions involved between the cycloadditioned complex and a DNA fragment. Initial position to start the dynamic is (A), intermediate state is (B), where there are weak hydrogen atom separations, but these return to their original position, besides there are discernible but not significant distortions on the cyt–gua complex. End state for the dynamics is (C) showing that the hydrogen bonds remain throughout the entire process. Thus, it can be concluded that there is no reaction at all.

It was assumed that these molecules are in the gas phase and that no interactions with any other chemical substances take place. It is apparent that a study surmising a solvent environment would be more informative, however, the gas phase condition is established for one reason, the original idea was to study the reaction where the epoxide of estradiol becomes oxidised, a procedure which can be observed without the presence of the solvent. Therefore, the cycloaddition reaction was a surprise. However the presence of the solvent is essential. The simulation of the same kind of reaction in a solvent environment was achieved. The corresponding calculation considers the same input of the last description but now a water environment is introduced considering the polarisable continuum model by Truhlar and co-workers [40]. In this case, it is important to consider the physical properties

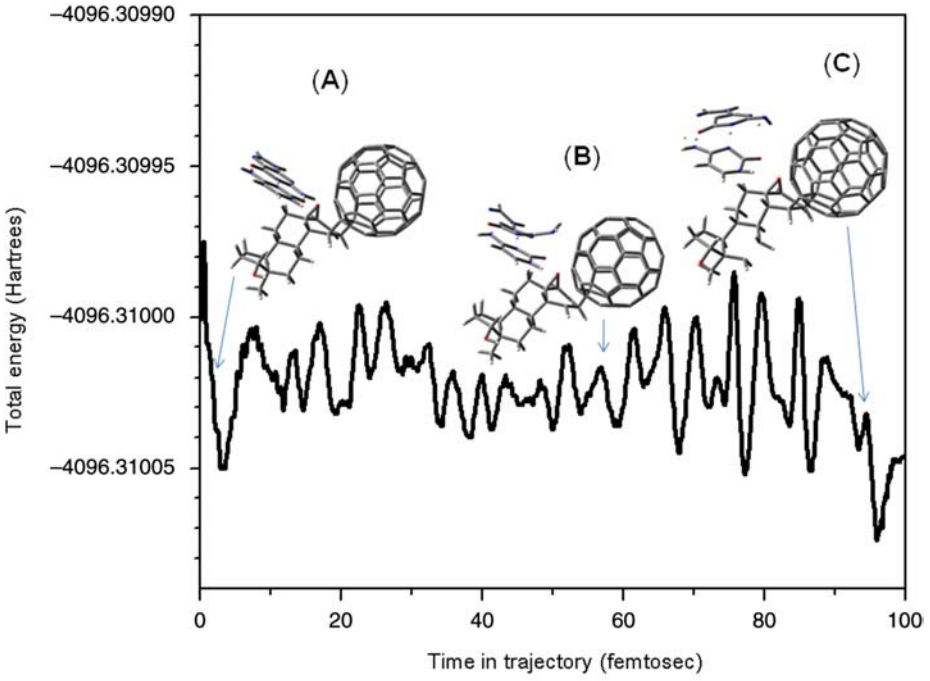

Figure 7. (Colour online) Total energy versus Time for the interaction between cycloadditioned complex and a DNA fragment. It is also showed the structural changes in the DNA fragment as a result of interaction with cycloadditioned complex (A, B and C).

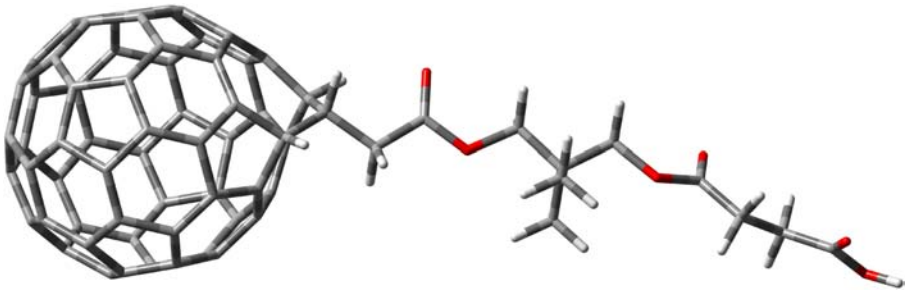

Figure 8. (Colour online) Fullerene water-soluble derivative.

of fullerene, this molecule is not soluble in water or other polar solvents, then a simulation in which a reaction between fullerene and an active molecule (as the epoxide of estradiol) is carried out in water must consider a reasonable reactive; therefore, a soluble derivative of fullerene is introduced (see Figure 8) [66].

The result is astonishing because the initial hypothesis about the destruction of the epoxidised estradiol is reached for this case, the process starts with a normal cycloaddition as it was established in the other simulation but continue with a strong vibration on the substituent groups joint to estradiol (the epoxide bridge included) and the separation of a methyl group, several hydrogen atoms and the breaking of the epoxide group. Curiously the fullerene and its anchorage have little changes and keep practically their original configuration; Figure 9 shows the final result of this process. The energy changes are summarised in the plot shown in Figure 9, considering that the product of the reaction is a broken species the plot shows an approximated stable process. This result must be handled with care because, it is possible that this reaction can occur but in general the estrogene molecules are liposoluble and maybe these two species do not appear in the same environment, a more detailed study is necessary and our group is working already in this problems. The important point is that in the gas phase there is a reaction which was characterised as a cycloaddition which can deactivate the estrogene molecule in its carcinogenic effect; on the other hand, in water media there is also a reaction and again deactivate the same estrogene and now by its destruction.

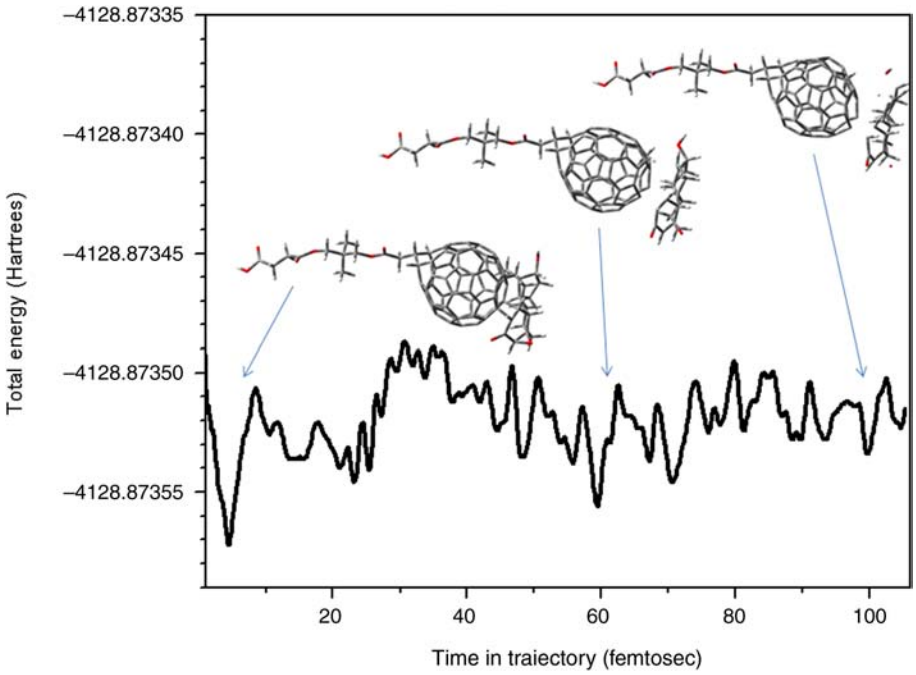

Figure 9. (Colour online) Total energy versus time for the interaction between fullerene water-soluble derivative and DNA fragment. It is also shown the structural changes in the DNA fragment as a result of interaction with cycloadditioned complex.

4. Conclusions

The fullerene C_{60} is able to interact with the epoxide of estradiol forming a very stable cycloadditioned compound. This reaction is simulated as it would take place in the human body at 310 K (which is the normal body temperature). The fullerene displayed a strong influence in possible collateral/mutagenic reactions of the derivative of estradiol by forming a cycloadditioned compound. This compound effectively inhibits the reaction between the epoxidised estradiol and fragments of DNA, avoiding the mutagenic reaction between the free epoxide and the DNA chain which leads to cancer. It is thus possible to propose pharmaceutical derivatives based on fullerene in order to prevent and avoid the development of cancer.

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