A COMPUTATIONAL METHOD FOR PREDICTING THE SOLUBILITY OF DOXORUBICIN-PLLA-PEG

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ABSTRACT
Doxorubicin is a chemotherapeutic drug used as a cure for cancer. Being an anthracycline antibiotic, it is commonly used in treating a wide variety of cancers. In this report, the molecular structure, Binding Energy (BE), Dipole Moment (DM), Gibbs free energy of solvation ($\Delta G$ (solvation)) and some physicochemical properties of doxorubicin were conjugated chemically to the terminal end of a di-block copolymer which was composed of poly(L-lactic acid)(PLLA) and methoxy-poly(ethylene glycol) (mPEG) and were investigated using computational methods. In this report, computational calculation carried out on the acid–cleavable linkages, a hydrazone bond (complex A) and a cis-aconityl bond (complex B) were used for the conjugation of doxorubicin to the terminal end of poly(L-lactic acid) in a di-block copolymer of PLLA–PEG. Wherein Complex A and B being large molecules.

For large reactive systems, the calculation of energies can be simplified by treating the active part with a high-level quantum mechanical (QM) ab initio or density functional. One such method is the original "Our-own-N-layer Integrated molecular Orbital, Molecular Mechanics ONIOM" approach. We used this approach for optimization of DOX–PLLA-PEG complexes. Our results indicate that these complexes mentioned above can be used to improve anti cancer activity and water-solubility of Doxorubicin.

Keywords: Anti-cancer drugs, Doxorubicin, Ab initio calculation, di-block co-polymer, acid-cleavable linkages.

INTRODUCTION
Doxorubicin, an anthracycline ring antibiotic, is a widely used therapeutic drug in cancer cure1. The Doxorubicin scheme is seen as follows in Fig 1:

![Fig 1: Doxorubicin](image)

Further, although doxorubicin is used widely as an anticancer agent, the cytotoxicity that it shows to normal tissue and its inherent effect of multidrug resistance are major problems that have yet remained unsolved2-5. In order to increase the efficacy of this therapeutic nature of doxorubicin, several systems of drug delivery, based on liposomes, nanoparticles, polymer conjugates, and polymeric micelles, have been comprehensively researched. There have also been extensive studies on Polymer–drug conjugates using poly (N-(2-hydroxypropyl)methacrylamide) and are in the clinical trial stage6. Experimental work performed by other researchers has illustrated that the potential of biodegradable polymeric nanoparticles and solid lipid nanoparticles has also been identified and utilized as doxorubicin carriers7. The characteristics and uniqueness of structure of polymeric micelles have recently been the focus of much attention8-12. Composed of hydrophilic and hydrophobic polymer blocks, a di-block copolymer in aqueous solution can self-associate to form a micellar structure with a diameter of less than 100 nm. In our previous study12, however, an amide or ester linkage that was not readily cleaved under physiological conditions was used for conjugating doxorubicin to the terminal end of a PLGA chain. The conclusion showed that the released doxorubicin was composed of a mixture of doxorubicin species conjugated with water-soluble PLGA oligomers. In the present study, acid-cleavable linkages, a hydrazone bond and a cis-aconityl bond, were used for the conjugation of doxorubicin to the terminal end of poly (L-lactic acid) in a di-block copolymer of PLLA-PEG. Their acid cleavability makes the hydrazone linkage and the cis-aconityl bond instrumental in the conjugation of anticancer agents to monoclonal antibodies requiring a rapid cleavage of the drug under acidic conditions, as in the vicinity of tumor tissues or within endosomes13-15.

Tools are required for computational chemistry to study chemical reactions and processes. Computer software gives scientists insight into chemical processes. A well-defined structure needs to be generated before molecular properties can be calculated. A calculation often requires a structure that represents a minimum on a potential energy surface16. In view of the above, we optimized the complexes using Gaussian 03. The ONIOM approach was also carried out as the size of complexes was large.

The methods and basis sets for high and low level were set at B3LYP/6-311++G* and HF/6-31G*, respectively. The structure optimized was used as a starting point for subsequent calculations, such as, molecular energy, binding energy, dipole moment, $\Delta G$ (solvation), partition coefficient (logP), distance bound and angle bound17,18.

RESULTS AND DISCUSSION
The ketone group of doxorubicin was conjugated to the terminal hydroxyl moiety of PLLA segment in a PLLA-PEG di-block copolymer via a hydrazone linkage (complex A), as shown in fig. 2. This complex was synthesized by Eun Ah Lee and colleagues19. Similarly, the conjugation of doxorubicin to PLLA-PEG via a cis-aconityl bond is shown in fig. 3. This complex was also originally synthesized by Eun Ah Lee and colleagues20.

![Fig 2: Dox-plla-peg conjugate via a hydrazone linkage (complex a)](image)

$^{n=1\ m=2}$
The geometry structure of DOX-PLLA-PEG di-block copolymer was optimized at B3LYP/6-311+g** and HF/6-31g* level of theory via a hydrazone linkage (complex A) and then the Gibbs free energy of solvation (ΔG solvation) was calculated at B3LYP/6-31g* level of theory using Gaussian 03.15 Table 1 presents the geometrical parameters of this above mentioned complex around linking position (hydrazino group), see also Fig. 4. Some physicochemical properties of complex A, complex B and doxorubicin, such as, Refractivity, Polarizability, Log p, Hydration Energy, Binding Energies (BE), Gibbs Free Energy of Solvation (ΔG solvation) and Dipole Moment (DM) were obtained from optimal structure 16 as shown in Table 2.

The binding energy per molecule was computed using the formula (1):

$$\Delta E = E_{\text{complex}} - E_{\text{drug}} - E_{\text{carrier}}$$ (1)

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### Table 1: Geometrical Parameter Of Complex A Around Linking Position

<table>
<thead>
<tr>
<th>Geometrical Parameters of Complex A</th>
<th>H1-N2 (Å)</th>
<th>N2-N3 (Å)</th>
<th>N3-C4 (Å)</th>
<th>C4-C5 (Å)</th>
<th>H1-N2-N3 (°)</th>
<th>N3-C4-C5 (°)</th>
<th>N2-N3-C4 (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.016</td>
<td>1.358</td>
<td>1.278</td>
<td>1.513</td>
<td>119.573</td>
<td>122.750</td>
<td>119.973</td>
</tr>
</tbody>
</table>

### Table 2: Some Physicochemical Properties Of Complex A, Complex B And Doxorubicin

<table>
<thead>
<tr>
<th>Physicochemical Properties</th>
<th>Complex A</th>
<th>Complex B</th>
<th>Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractivity</td>
<td>202.46</td>
<td>242.73</td>
<td>135.50</td>
</tr>
<tr>
<td>Polarizability</td>
<td>78.33</td>
<td>95.01</td>
<td>52.00</td>
</tr>
<tr>
<td>Log p</td>
<td>-0.82</td>
<td>-2.91</td>
<td>0.110</td>
</tr>
<tr>
<td>Hydration energy</td>
<td>-29.710</td>
<td>-35.74</td>
<td>-24.03</td>
</tr>
<tr>
<td>Surface area (Å²)</td>
<td>1068.840</td>
<td>1335.25</td>
<td>729.45</td>
</tr>
<tr>
<td>ΔG solvation [kcal/mol]</td>
<td>-26.990</td>
<td>-33.47</td>
<td>-18.08</td>
</tr>
<tr>
<td>Dipole moment (Debye)</td>
<td>8.648</td>
<td>5.749</td>
<td>6.848</td>
</tr>
<tr>
<td>BE (ev/mol)</td>
<td>-1071.359</td>
<td>-0.3218</td>
<td>-0.218</td>
</tr>
</tbody>
</table>

* Data were calculated using Hyper Chem B software 21

The geometrical structure of DOX-PLLA-PEG di-block copolymer was optimized at B3LYP/6-311+g** and HF/6-31g* level of theory via a cis-aconityl bond linkage (complex B) and then the Gibbs free energy of solvation (ΔG solvation) was calculated at B3LYP/6-31g* level of theory using Gaussian 03.15 Table 3 presents the geometrical parameters of this complex mentioned above around linking position (amide group), see also Fig. 5.

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### Table 3: Geometrical Parameter Of Complex B Around Linking Position

<table>
<thead>
<tr>
<th>Geometrical Parameter Of Complex B</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2-O1 (Å)</td>
</tr>
<tr>
<td>C2-N3 (Å)</td>
</tr>
<tr>
<td>N3-H4 (Å)</td>
</tr>
<tr>
<td>C2-N3-H4 (*)</td>
</tr>
<tr>
<td>O1-C2-N3 (*)</td>
</tr>
</tbody>
</table>

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### CONCLUSION

The Density Functional Theory (DFT) and Hartree Fock (HF) calculation were applied to study some physicochemical properties of DOX-PLLA-PEG conjugate – via a hydrazone linkage (complex A), DOX-PLLA-PEG conjugate – via a cis-aconityl linkage (complex B) and Doxorubicin.

With regard to the calculations carried out, this significant conclusion was drawn that computational chemistry is closely consistent with experimental results. The experimental results show doxorubicin conjugated micelles with a cis-aconityl bond, doxorubicin was released much faster than doxorubicin conjugated micelles with a hydrazone linkage at pH 3. This fact can be verified through the Binding Energy (BE) obtained for complex A and complex B using formula (1). The value of the BE of these complexes mentioned in table 2, confirm that complex A is more stable than complex B. Thus, doxorubicin conjugated micelles with a cis-aconityl bond, doxorubicin was released much faster than doxorubicin conjugated micelles with a hydrazone linkage.

With regards to the calculation results, hydrophilicity of complex B is higher than that of complex A and Doxorubicin; this fact can be verified through the Gibbs free energy of solvation (ΔG solvation) obtained for these complexes using Gaussian 03. Therefore, complex B is more soluble than that complex A. Our results are clear proof that the complexes mentioned above can be used to improve the anti-cancer activity and the water-solubility of Doxorubicin.

### REFERENCES

1. Kushwaha PS, Mishra PC. Molecular electrostatic potential maps of the anti-cancer drugs daunomycin and adriamycin an ab initio theoretical study, J Mol Struct 2005; 699:149-156.


