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A computationally derived structural model of doxorubicin interacting with oligomeric polyalkylcyanoacrylate in nanoparticles

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Abstract

We report a molecular simulation study of doxorubicin interacting within a frame of *n*-butyl polycyanoacrylate, one of the most commonly encountered polymers in the production of nanoparticles. Emphasis is put on the tetrameric, hexameric and octameric oligomers (PACA's). Log *P* was calculated for all interacting species. Molecular dynamics along with energy minimization processes (molecular mechanics MM2, semi-empirical quantum mechanics PM3) were employed to probe the conformational behavior of doxorubicin and polyalkylcyanoacrylate both as isolated species and interacting with each other. A docked structure of protonated doxorubicin with two octamers of *n*-butyl polycyanoacrylate is described. Among the main stability factors of the assembly was the charge–dipole interaction representing a stabilizing contribution of −33 kcal/mol. The mechanism of aggregation and desegregation (doxorubicin release) can be summarized as follows: oligomeric PACA's are lipophilic entities that scavenge amphiphilic doxorubicin already during the polymerization process by extraction of the protonated species from the aqueous environment to the increasingly lipophilic phase of the growing PACA's. The establishment of hydrogen bonds between the ammonium N–H function and the cyano groups is noteworthy. The cohesion in PACA nanoparticle comes therefore from a blend of dipole–charge interaction, H bonds, and hydrophobic forces,

Keywords: Doxorubicin; Nanoparticles; Polyalkylcyanoacrylate; Molecular mechanics; Quantum mechanics; Lipophilicity; Log *P*

1. Introduction

A wide range of antineoplastic agents with remarkable antitumor activity have been found to be effective in combating different types of cancers. However, to achieve complete eradication of tumors, antineoplastic agents have to be administered systemically in high doses, and almost all drugs effective in eradicating cancer cells cause damage to other healthy tissues and organs. This is due to the non-specific uptake of these agents by healthy organs such as the kidney, liver, bone marrow, and heart.

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The adverse side effects include severe immune suppression, myelosuppression, nephrotoxicity, and cardiotoxicity [1,2]. An alternative approach to the systemic delivery of antineoplastic agents is their association with polymers in order to control drug release and biodistribution [3]. Conjugation or mixing of drugs with polymers may thus reduce their side effects, increase their specificity, prolong their activity, and improve their resistance to degradation in body fluids [4].

Polymeric nanoparticles have received increasing attention as targeted delivery systems of antitumor drugs because they were found to be able to concentrate efficiently in certain types of tumors. This was observed with doxorubicin, a potent anticancer agent [5], loaded onto polyalkylcyanoacrylate (PACA) nanoparticles, which was found to cure experimental hepatic metastases much more efficiently than the free drug [6]. Interestingly, PACA nanoparticles loaded with doxorubicin also allowed multidrug resistance to be overcome in vitro [7]. This effect was due to both the adsorption of the nanoparticles onto the cell surface and to the increased doxorubicin diffusion into cells following accumulation of a lipophilic (ion pair) complex at the surface of plasma membrane [8,9].

In an effort to shed light on the nature of the PACA–doxorubicin interaction, we have undertaken a molecular modeling study of doxorubicin (as protonated species, Fig. 1) either alone (PM3) or in a simulated water environment (PM3 COSMO). Doxorubicin was subsequently included in a frame of PACA to see the types of interaction which are created under such conditions. Thus, this paper represents a first study applying molecular modeling in the field of drug delivery, initially as an exploratory tool to get insight into the cohesion forces operating in the drug–polymeric matrix relationship, and subsequently as a forecast tool in the design of new drug delivery systems.

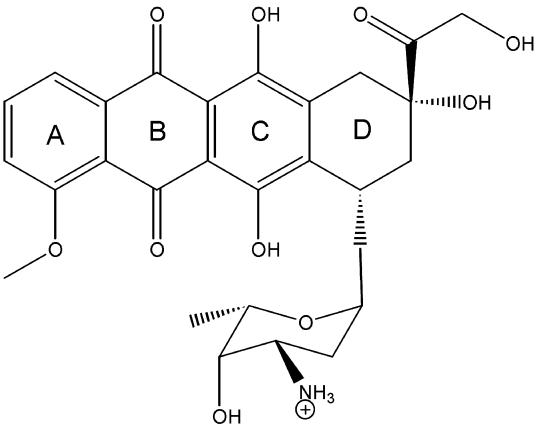


Fig. 1. Structure of doxorubicin in its protonated form.

2. Experimental

To explore the conformational behavior of the protonated doxorubicin and the various oligomeric polyalkylcyanoacrylates, a molecular dynamics trajectory for each species was generated at 600 K and extended over 1000 ps by steps of 1 fs. Conformers sampled every 1 ps were then energy minimized using first the Allinger's MM2 method. Minimization of energy was performed using the Block-Diagonal Newton–Raphson method until the gradient achieved was lower than 0.01 kcal/Å mol. In this way, low energy conformers were obtained. In order to assess the energy content of the various low energy conformers obtained above, semi-empirical quantum mechanics calculations were undertaken using the PM3 method [10] and, when useful with COSMO water solvation parameters as implemented in MOPAC 97, the latest version supported by Fujitsu Corporation. The COSMO method (conductor-like screening model) is useful for determining the stability of various species in a solvent environment, and particularly water. Theoretical calculations were carried out at the restricted Hartree–Fock level (RHF) using the PM3 semi-empirical SCF-MO methods, as implemented in the CS MOPAC package found in CS Chem3DPro 7.0.0 (CambridgeSoft Corporation, Cambridge, MA, USA). For water simulation, a relative permittivity of 78.4 was employed with up to 60 surface segments per atom for the COSMO model being used to construct a solvent accessible surface area based on van der Waals radii. The solute charge distribution was evaluated from an atom centered model including charges, dipole and quadrupole moments. All structures were optimized to a gradient $f<0.1$, using the eigenvector following method. The keyword LMIN=−100 was used to assist convergence. The general sequence of applica-

tions followed here is similar to previous studies [11,12].

Calculated log *P* (Clog*P*) values were obtained using ACD/logP (Version 1.0, ACD, Toronto, Ontario, Canada).

3. Results and discussion

3.1. Molecular modeling studies of doxorubicin

Conformational features of the tricyclic naphtoquinone (cycle A, B, C in Fig. 1) residue present in doxorubicin has been examined previously via ab initio calculations [13]. The results of that study suggested a planar and internally hydrogen-bonded structure in its preferred conformations. The optimized structural features were essentially the same as those found in the crystal. Molecular electrostatic potential (MEP) calculations using ab initio SCF methods were performed to visualize their three-dimensional topography and indicated that two factors—(i) the depth, extent, and relative location of negative potential around hydroxyl and quinonoid oxygens; and (ii) a gradual loss of negative potential over the molecular plane due to the presence and orientation of the hydroxyl groups in the phenolic part of the molecules—were crucial for recognition interaction at their biological target. This study later on confirmed by Monteagudo et al. [14] also emphasized the importance of solvation for the conformational behavior of this molecule.

To explore the conformational behavior of doxorubicin, a molecular dynamics trajectory of the protonated form of doxorubicin was generated at 600 K and extended over 450 ps in steps of 1 fs. Conformers sampled every 1 ps were then energy minimized using the PM3 method. In this way, low energy conformers were obtained. While a different relative orientation of the aminosugar moieties was encountered among low energy-conformers, they all were indeed characterized by hydrogen bonds established between the phenols of the C-ring with the carbonyls of the B-ring as previously reported by Bhattacharjee et al. [13], and Monteagudo et al. [14].

In support of these theoretical calculations comes the fact that there is a considerable difference between the calculated (−1.18) and measured (+1.27) log *P* value for doxorubicin [15]. This Δlog *P* of 2.35 is typical of a so-called ‘cameleon’ molecule, i.e. a molecule undergoing a hydrophobic collapse when transferred from a polar to an apolar medium [16,17]. As anticipated, when the sampled conformers obtained from the above 1000-ps molecular dynamics were recalculated using the PM3 COSMO method—which simulates an aqueous surrounding—the internally H-bonded structures were no longer favored, and this suggests that the molecule exposes polar moieties toward the solvent in order to establish H bonds with water.

Another important aspect of the doxorubicin molecule is that it is protonated at physiological pH on the N(3) of the aminosugar moiety and this formally positive group has clearly a significant influence on the molecular recognition for doxorubicin binding. Calculation of the charges (either by Mulliken or Wang–Ford’s theory) or molecular electrostatic potentials revealed that the net positive charge on the ammonium nitrogen irradiates on the neighboring atoms of the aminosugar moiety.

3.2. Molecular modeling studies of PBCA

The same approach of molecular dynamics (typically a 1000-ps trajectory) and energy minimization of the conformers sampled every 10 ps (as described in the previous section) was used to probe the conformational behavior of PACA. The tetramer (PBCA4), hexamer (PBCA6), and octamer (PBCA8) of *n*-butyl polycyanoacrylate were chosen for the study as they are representative of the main population of the oligomers formed during the anionic polymerization process [18]. Low energy conformers were obtained by energy minimization using the PM3 method for PBCA4 and PBCA6 while, due to the higher number of atoms present in PBCA8, the less computationally intensive molecular mechanics MM2 method was used.

It should be noted that PACA’s are chiral objects with asymmetric centers positioned at all quaternary carbons. All calculations were performed on the diastereoisomers possessing identical relative configuration at quaternary carbons since preliminary work indicated that the ‘stereochemically homogeneous’ diastereoismers were energetically favored over the alternate relative configuration diastereo-

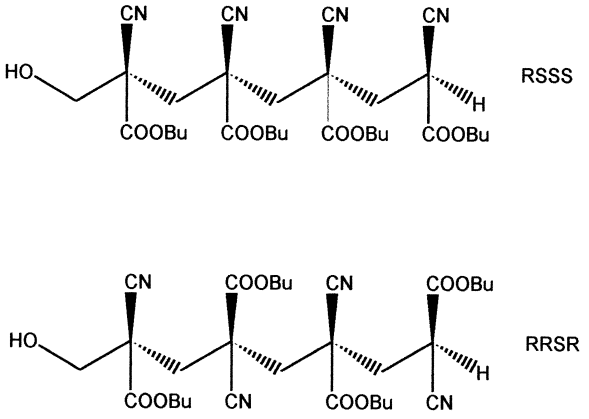


Fig. 2. Absolute configuration of the two diastereoisomeric species of the tetrameric *n*-butyl cyanoacrylate oligomers. The terminology *R* or *S* refers to the convention of Cahn–Ingold–Prelog.

isomers (i.e. (*RSSS*)-PBCA4 vs. (*RRSR*)-PBCA4, for example, see Fig. 2). For the tetramer, the ΔHf difference value amounted to 3.5 kcal/mol in favor of (*RSSS*)-PBCA4. Further work should be devoted to the stereotacticity of these oligomers, particularly those with complex long ester side-chains such as PEGylated PACA's [19–21].

Low energy conformers within the range of the molecular sizes covered by this study tend to adopt a helical conformation with a radial display of the cyano and ester moieties around the carbon–carbon backbone (Fig. 3). The segmental motional freedom of the carbon–carbon backbone appears rather limited and most of the kinetic energy is dissipated via the vibration motion of the *n*-butyl ester side-chains. The poor mobility of the carbon–carbon backbone noted here is in agreement with the signal broadening of the methylenes observed in ^{1}H-NMR resulting from the short T_2 (spin–spin relaxation). The signal of the methylene linking the quaternary carbons in oligomeric PACA was found to a line shape of ~20 Hz while in the monomer the corresponding singlet had a natural peak broadness of 1.25 Hz [22].

3.3. Molecular modeling studies of doxorubicin in interaction with PACA

A model of the interaction of doxorubicin–PACA was obtained by inserting protonated doxorubicin in its lowest energy conformation found above in a sandwich between two PBCA8 oligomers, also positioned in their lowest energy conformation. Molecular dynamics were then performed using leapfrog algorithms as implemented in Discover (Discover 2.7 package, Biosym Technologies Inc., San Diego, CA). Dynamics were equilibrated for 6 ps with time steps of 1 fs and then continued for 330 ps with time steps of 1 fs at 350 K. The resulting structures sampled every 1 ps were energy minimized. In another similar experiment, the trajectory was extended to 850 ps and conformers were sampled every 10 ps. This protocol was found to be an effective method for exploring conformational space in a complex close to its lowest energy structure. The assembly was found to be rather stable over the time with no particular trend to dissociate although the potential energy content of the assembly was higher than that of the sum of the individual entities due to some local bad contacts mainly between side-chain alkyl groups of PACA and doxorubicin. The main stabilizing factor of the complex comes from the charge–dipole interaction representing a contribution of −33 kcal/mol. Closer inspection showed that the nitrile groups from PBCA8 were the main actors in this scenario, e.g. two of them being in close interaction with the ammonium moiety present in the protonated aminosugar moiety from doxorubicin (Fig. 4). To a lesser extend, ester carbonyls from PBCA8 interact with hydroxyl groups from the aminosugar. It should be noted that the naphtoquinone tricyclic system (A, B, and C rings, see Fig. 1) was not internally hydrogen-bonded when included in the PBCA8 sandwich, which reflects the strong solvating properties of PACA's.

Due to the strong interaction between the protonated aminosugar ammonium and the cyano groups of the PACA, the formal net positive charge on the ammonium nitrogen was 'diluted' on the neighboring cyano nitrogens resulting in negative values of the Mulliken's charges (−0.26) or molecular electrostatic potentials (−0.56).

Another stabilizing factor likely to account for this remarkable aggregation property originates from hydrophobic forces. Indeed, the lipophilicity of the PACA increases with the molecular weight. Log *P* for PBCA4, for example, is 6.17 and this figure

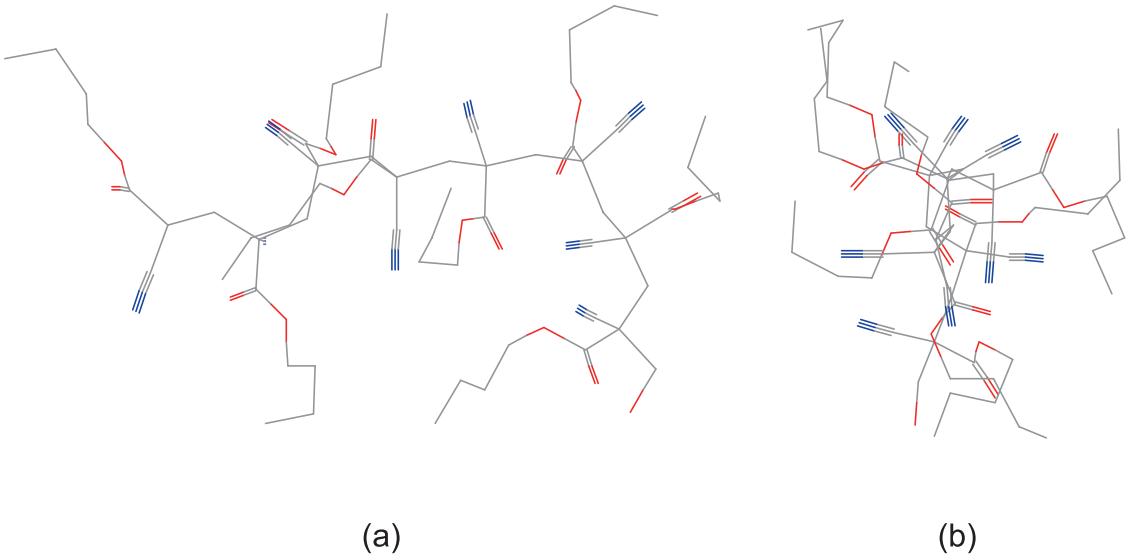


Fig. 3. Low-energy conformer of *n*-butyl hexacyanoacrylate [(a) cross view and (b) enfilade view] showing the radial disposition of cyano and ester carbonyl groups.

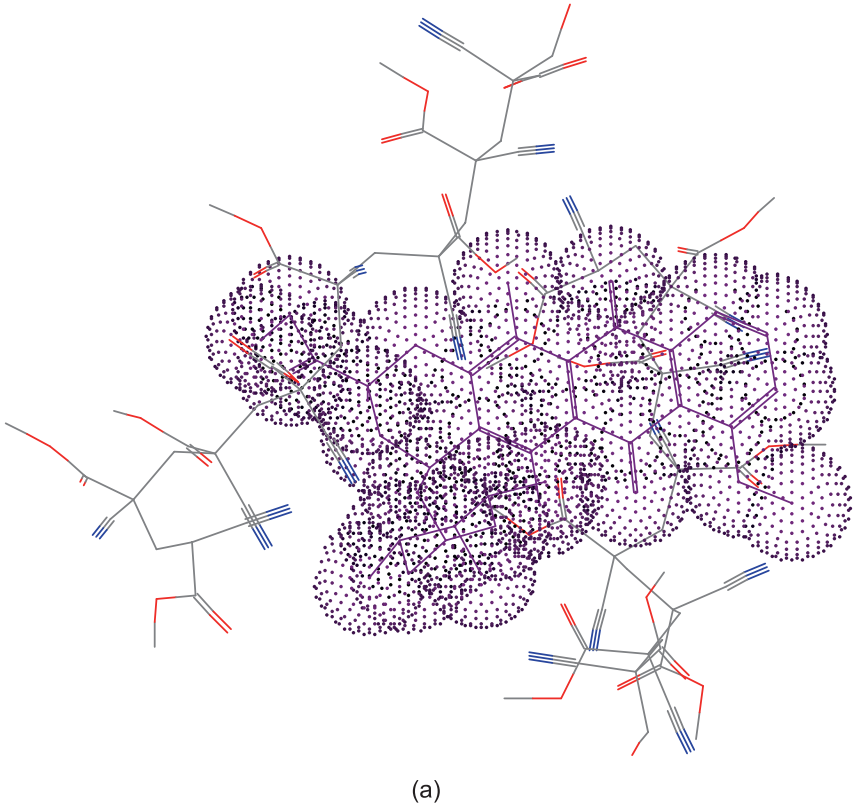
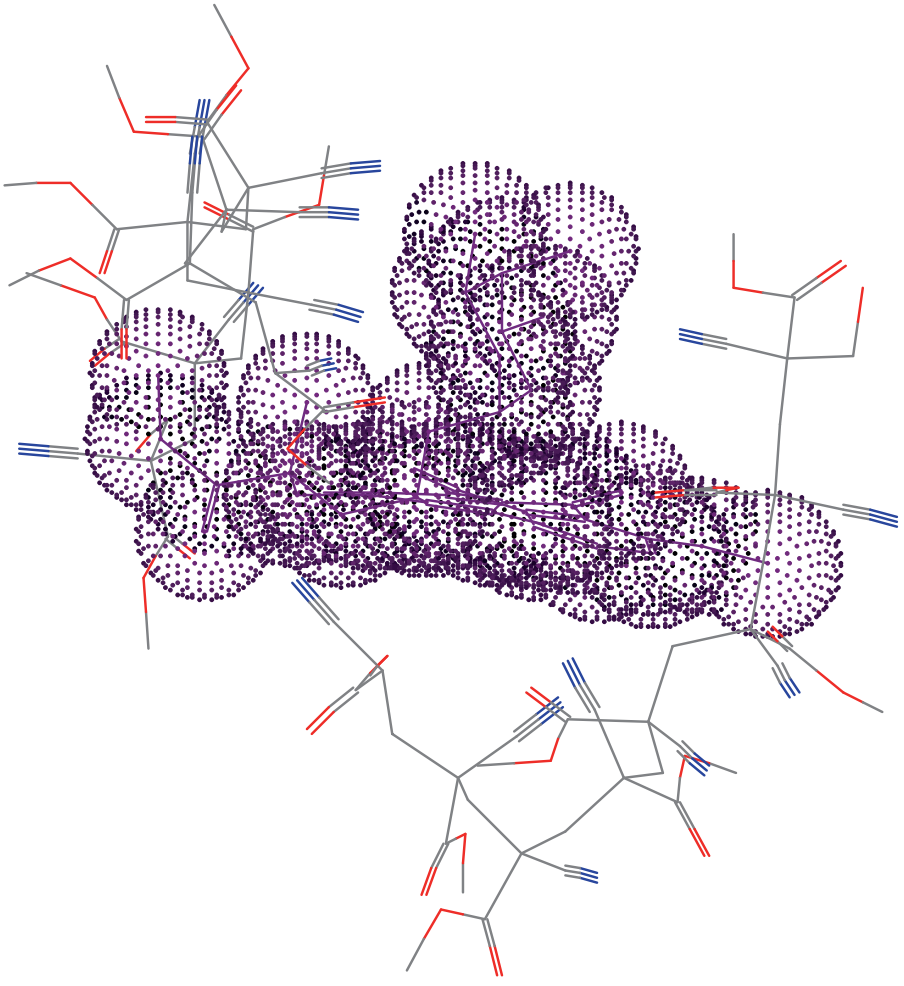


Fig. 4. Structure of a sandwich of protonated doxorubicin and two octamers of PBCA after thermal equilibration for 245 ps at 310 K and MM2 energy minimization [(a) and (b) cross view rotated by 90° within the *xz* plane].



(b)

Fig. 4. (*continued*)

increases by an increment of 1.76 for each addition of a *n*-butyl cyanoacrylate unit (Table 1).

4. Conclusion

As a result of this study, the following picture emerges: oligomeric PACA's which are highly lipophilic entities scavenge amphiphilic doxorubicin already during the polymerization process by extraction of the protonated species from the aqueous environment to an increasingly lipophilic phase embodied by the growing PACA's. In particular, the establishment of hydrogen bonds between the ammonium N–H function and the cyano groups is noteworthy. The cohesion in this assembly comes therefore from a blend of dipole–charge interaction, H bonds, and hydrophobic forces, a situation reminiscent of the interaction of drugs with proteins.

The mechanism by which nanoparticles reverse multidrug resistance (MDR) of P388 cells to doxorubicin has been studied previously [7–9]. As the

Table 1
Physico–chemical parameters of PACA

Species (*n*, *R*)	Formula	MW	Clog*P*	Δ*Hf*
1, *n*-C_4H_9	$C_8H_{13}NO_3$	171.2	0.9	−112.44
2, *n*-C_4H_9	$C_{16}H_{24}N_2O_5$	324.4	2.66	−193.81
4, *n*-C_4H_9	$C_{32}H_{46}N_4O_9$	630.7	6.17	−280.52
6, *n*-C_4H_9	$C_{48}H_{68}N_6O_{13}$	937.1	9.67	−385.72
8, CH_3	$C_{40}H_{42}N_8O_{17}$	907.4	–	−439.70

reversion of MDR by the nanoparticles cannot be explained by an endocytic pathway, the explanation of this phenomenon probably resides in the fact that nanoparticles adsorb onto the cell membrane, resulting in a high drug gradient close to the cell. Upon hydrolytic erosion of the nanoparticles involving mainly the hydrolysis of ester groups to carboxylate, the assembly of doxorubicin–PACA tends to loose cohesion as the hydrophobic forces decline due to water intrusion and increasing contribution of repulsive forces between anionic carboxylates. This creates a high local gradient of doxorubicin at the cell membrane surface permitting MDR efflux to be overwhelmed. The present molecular modeling studies are in agreement with this interpretation [7–9].

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