COMPUTATIONAL MODEL OF ADSORPTION FOR PARALYTIC SHELLFISH TOXINS (PSTs) ON GRAPHENE SURFACE.

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Abstract

Paralytic Shellfish Toxins (PSTs) are polar analytes, most of them with positive charges resulting in a charge-induced dipole at the graphene surface when they approach to it. Graphene is a novel material with great potentials to be used as sorbent due to its ultrahigh surface area. Herein, we perform the simulation about the retention mechanism of PSTs on the graphene through Merck Molecular Force Field (MMFF94) minimizations. The overall retention on graphene is a combination of two mechanisms:

- Adsorption: The strength of analyte interactions with graphene is largely dependent on the molecular area in contact with the graphene surface, and also on the type and positioning of the functional groups in relation to the graphene surface at the points of contact.

- Charge induced interactions of a polar analyte with the polarizable surface of graphene: when the polar group with apermanent dipole approaches the surface, an induced dipole is formed, increasing the attraction between the analyte and graphene surface.

Computational results were compared with those obtained after elution using a HPLC-Hypercarb column: they showed a good correlation pattern where it was seen that the theoretical model exhibited the potential of graphene as an excellent sorbent material for saxitoxin and analogues.

Hypercarb model, elution order: (shorter retention time) dcSTX < NEO < STX < GTX5 < GTX3 < C2 < GTX2 < C1 (longer retention time).

Merck Molecular Force Field (MMFF94) model, interaction energy values order: (minor complex energy) dcSTX < STX < NEO < GTX5 < GTX3 < GTX2 < C2 < C1 (mayor complex energy)

Introduction

Paralytic shellfish toxins (PSTs) are a wide group of natural neurotoxic alkaloid that are produced mainly by marine dinoflagellates belonging to genera: *Alexandrium*, *Pyrodinium* and *Gymnodinium*; and freshwater cyanobacteria of the genera: *Anabaena*, *Cylindrospermopsis*, *Aphanizomenon*, *Planktothrix* and *Lygbya* [1].

PSTs contain two guanidinium groups, which are responsible for their high polarity. They have similar structures, sharing a common tetrahydropurine skeleton but differ widely in toxicity. Saxitoxin (STX) was the first PSTs identified and isolated [2] and it is the most toxic. To date, more than 57 STX analogues are known [1, 3] and they can be divided into three families based on structural differences in their functional groups: the carbamate family includes saxitoxin (STX), neosaxitoxin (NEO) and the gonyautoxins (GTX1-4); the decarbamoyl family includes decarbamoyl-gonyautoxins (dcGTX1-dcGTX4) and decarbamoyl-saxitoxins (dcSTX, dcNEO); the N-sulfocarbamoyl toxins include GTX5, GTX6 (also called B1 and B2, respectively) and C toxins (C1-4). In recent years, a novel group were identified and named GC toxins (GC1-6) [4]. PSTs used in this work are shown in *Figure 1*

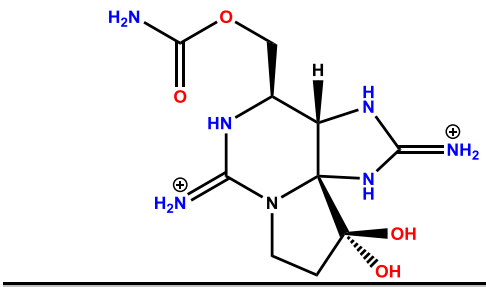
PSTs are responsible for the Paralytic Shellfish Poisoning syndrome (PSP), a potentially lethal clinical syndrome and one of the most dangerous and widespread in the world. The most common route of intoxication is through the ingestion of contaminated seafood, mainly bivalve mollusks but they have been reported in other vectors [5]. Due to structural differences, each toxin has a slightly different affinity to the binding site of voltage-gated sodium channels, which correlates to differential toxicity [6].

Graphene, a single-atom-thick carbon nanosheet, is a novel carbon material with great potentials to be used as sorbent due to its ultrahigh surface area.

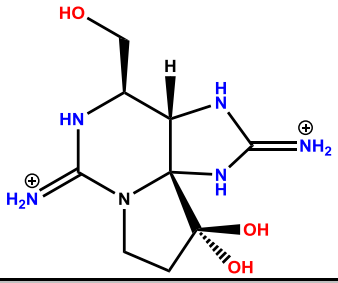
Due to their adsorption capacity graphene and family are promising nanomaterials in wastewater treatment [7]. Graphene oxide could be used for removal of algal toxins: Microcystin-LR and Microcystin-RR [8]. Graphene oxide showed a very high adsorption capacity when compared to that commercially available activated carbon. The graphene oxide performance allowed to reuse it as an adsorbent following 10 cycles of adsorption/desorption with not significant loss in its adsorption capacity.

Regarding marine toxins, *Shen et al*. [9] have employed solid phase extraction materials (SPE) with graphene as sorbent, due to its ultrahigh surface area, to extract marine lipophilic toxins in shellfish muscle, such as yessotoxins, okadaic acid and dinophysistoxin-1. Graphene shows high extaction efficiency as SPE sorbent since it is more effective than other commonly used sorbents namely C18, Strata-X ad HLB.

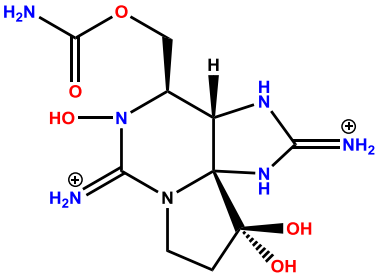
Recently, we developed a liquid-chromatography method with fluorescence detection to analyze PSTs and a variation of it to analyze TTX alongside PSTs. It was based on the use of a porous graphitized column with good properties related to separation performance and robustness. This method contributes to improve the analytical methodology for separation and quantitation of PSTs and reveals a starting point to graphene applications for this toxin family.



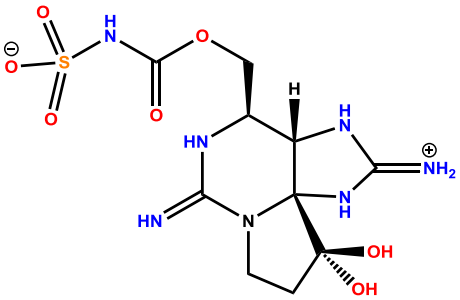
Saxitoxin (STX)



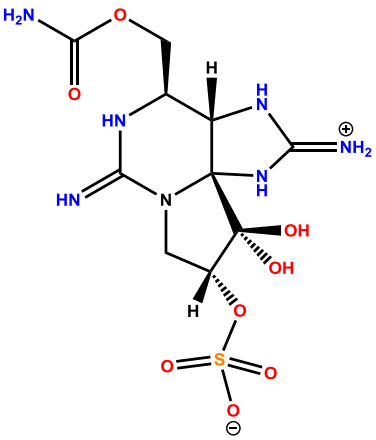
Decarbamoylsaxitoxin (dcSTX)



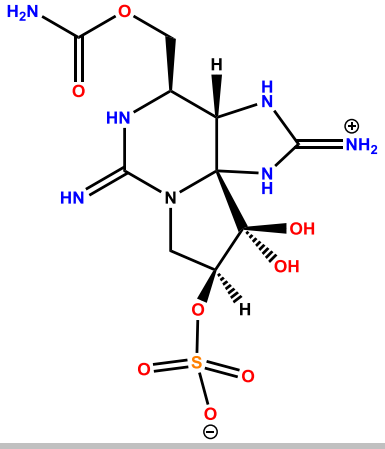
Neosaxitoxin



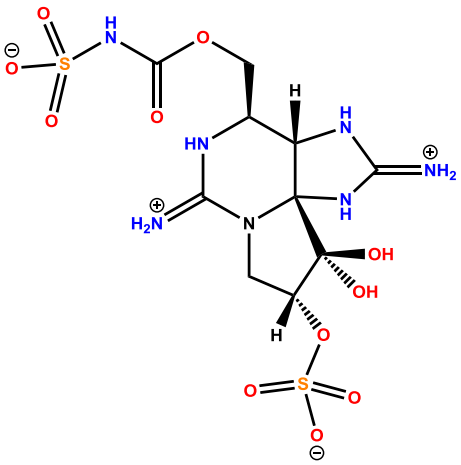
Gonyautoxin-5



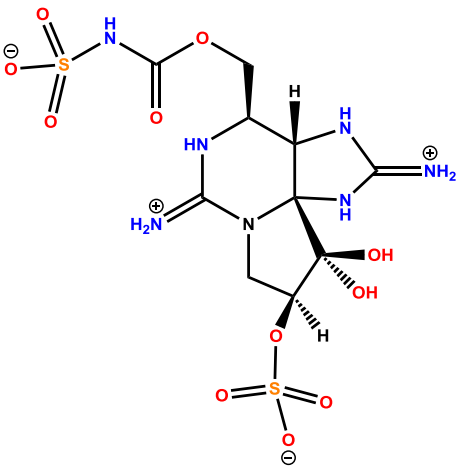
Gonyautoxin-2 (GTX2)



Gonyautoxin-3 (GTX3)



N-sulfocarbamoyl-gonyautoxin-2 (C1)



N-sulfocarbamoyl-gonyautoxin-3 (C2)

Figure 1.- Structures of PSTs used in this work. The most prevalent PSTs can be divided into three groups based on structural differences in their functional groups, namely the carbamoyl (STX, GTX2, GTX3), decarbamoyl (dcSTX) and sulfocarbamoyl (GTX5, C1, C2) derivatives.

The selectivity of the Hypercarb packing differs from the selectivity of silica and polymeric phases. The Hypercarb material excels at the separation of highly polar compounds with closely related structures. Its retention mechanism is completely different from conventional C18 columns. According to overall retention on Hypercarb, adsorption on porous graphitized column is a combination of two mechanisms:

1) **Adsorption**: The strength of analyte interactions with Hypercarb columns is largely dependent on the molecular area in contact with the graphite surface, and also on the type and positioning of the functional groups in relation to the graphite surface at the points of contact. The strength of the interaction depends upon the size and orientation of the molecular area that is able to come in contact with the flat graphite surface. Planar molecules will show more retention than rigid molecules with a 3-dimensional spatial arrangement.

2) **Charge induced interactions of a polar analyte with the polarizable surface of graphite**: As the polar group with a permanent dipole approaches the surface, an induced dipole is formed, increasing the attraction between the analyte and graphite surface. These charges should not be confused with the ionic charge of the molecule, such as a basic compound ionized in acidic pH conditions. The charge-induced dipole mechanism is strictly due to the interaction of the electrostatic charge of the polar molecule with the graphite surface. The results of intermolecular force field simulations are critically dependent on the initial spatial conformation of the system. Some different starting geometries were generated and then optimized.

To obtain information about the retention mechanism of PSPs on graphene surface Merck Molecular Force Field (MMFF94) minimizations were performed. The difference in molecular interaction energy values can be used as a relative retention capacity of graphene surface.

Objectives.

The purpose of this molecular simulation was to understand the non-binding mechanism of PSTs over a graphene surface, to evaluate the practical use of graphene to trap compounds keeping their initial structures and to predict the order of elution and develop a new strategy for computing the experimental binding energy of a PST molecule to an extended graphene surface.

Metodology

To help the understanding of the mechanism of supramolecular interaction of PSTs with graphene surface, computational simulation of the single molecules and final supramolecular complexes was performed using the Merck Molecular Force Field 94 (MMFF94) implemented in ChemDraw Bio3D 11. The procedure involves the following steps:

(1) The 2D structures of all the compounds were constructed in builder module of ChemDraw software. The 2D structures of the compounds were transferred to Chem3D with different charges as structure function.

(2) The initial structures using Merck Molecular Force Field 94 (MMFF94) in ChemBio3D 12 were minimized. Geometry optimizations for all molecules were computed using convergence

criteria for root-mean-square for the gradient of the potential energy surface of 0.001 kcal/mol, being E_T the energy of the toxin at the minimum and E_G the energy of graphene sheet at the minimum.

(3) Then, the toxin was placed at the center of the graphene sheet (352 carbon atoms, $C_{352}H_{52}$) separated by approximately 2 Amstrongs and the energy / geometry of the supramolecular complex optimized, without fixing the position of the graphene atoms in space, to obtain E_{TG} (potential energy of the complex in vacuum at the minimum).

(4) The calculations were made in an Intel Dual Core 2.6 GHz computer with 4 Gb of RAM.

Computational analysis of liquid chromatography retention was carried out taking into account the following approximations:

1. Minimizations were performed without solvents and dynamic flow in the calculations (vacuum is the media of minimizations).

2. Graphene sheet (single extended graphitic layer) was implemented to simulate graphite surface. In some cases the graphene sheet is curved in front of a rigid structure of graphite; 0.51 kcal/mol was the difference between the maximum curvature energy value and the minimum planar conformation energy of grapheme that can be used as the minimum value to evaluate the absorption energy .

3. Counter-anions were associated to PSTs: chloride and trifluoroacetate. While the former has only been used as counterion as a function of the net charge, the latter was implemented in simulation to increase or decrease TFA concentration, so that one or two molecules of TFA were added irrespectively to the net molecular charge.

4. To keep the procedure as general as possible, no particular attention was paid to the initial position of the toxin on the surface. PSTs molecules were centered over grapheme sheet so that no hydrogen bonding was allowed to retain molecules at the edge of the model phase.

In order to define a useful combined computational/chromatographic empirical approach for the high-performance liquid chromatography (HPLC) method development of PSTs over Hypercarb, porous graphite carbon, stationary phase and 8 PSTs with different chemical structures were used as test probes.

The binding energy of single toxins (with and without counter anions) adsorbed on a large poly-aromatic hydrocarbon mimicking graphene substrate was determined by subtracting the energy of the toxin and the graphene model from that of the supramolecular complex.

$$E_{ABS} = E_{TG} - (E_T + E_G)$$

Results.

Toxin absorption over graphene surface were evaluated without counter anions, taking into account only the real conformation for di-zwitterionic forms C1 and C2 with one TFA molecule, and for Gonyautoxins; with two TFA molecules, the normal situation for doubly charged dcSTX, Neo and STX. Chloride counter anion was explored for real conformations. The corresponding energy values are shown in Table 1, and the 3D molecular configurations of some of the minimum energy conformations of the PSP-graphene system are shown in *Figure 2.*

When the PSTs are adsorbed on the graphene then they move away from the surface gradually and the graphene deplection moves to planar configuration. They completely deposits on the surface at around 2.9 Amstrongs. The adsorption by beta face is lower than by alpha face in most of the cases. The employment of TFA as counter-ion at different stages modify this observation in two ways:

1. it reduces the energy difference between both approximations or,

2. it changes the interaction order. This is because the TFA interacts with the guanidinium residues and the charges will be screened in order to remain available for the interaction with the surface, therefore cation - π interaction is reduced. Experimental HPLC reflects that the higher TFA concentration the higher retention time in contrast with molecular calculations, although PSTs interaction with graphene surface is deeper when using TFA as counter anion than chloride.

Optimized energy has been made completely independent of graphene curvature. It is conceivable that the non-bonding interactions in PGC may not be very sensitive to the layer curvature. To include graphene curvature deformation in the nonbonding interactions, we used the adaptive 0.51 kcal/mol difference to predict a relative retention order. Graphene layers smaller (160 carbon atoms, $C_{160}H_{34}$) and bigger ($C_{432}H_{52}$) than ours were tentatively tested. The former toxin molecules were oriented to the edges, while the latter gave results at a much higher computational cost and a more pronounced curvature in grapheme in order to comparison with Hypercarb rigid model.

To evaluate the steric influence of the lateral alkyl substituent, relative energies of 3 different conformers were compared: dcSTX (hydroxyl), STX (carbamate), GTX5 (N-sulfocarbamate). Data are summarised in *Table 1*: dcSTX showed less absorption energy, -16.55 kcal / mol, with an alpha preferred orientation, dcSTX and STX energy changes indicated that the adsorption of these PSTs is mainly governed by the interaction between side-chain and the graphene surface. Delocalized electronic pair in carbamate moiety in STX (and Neo) contributed to have higher adsorption than hydroxyl group in dcSTX toxin. Gonyautoxin-5, rendered higher interaction than STX, while GTX5, with an extra sulphate group in this side-chain, rendered the highest value of this set, -24.97 kcal / mol, also with alpha orientation.

Table 1.- Energy values of PSTs supramolecular complexes with graphene sheet, without counter-anion and with TFA and chloride. Values for alpha and beta approx. were computed. The reference structures should be shown so that the energy terms of interest are highlighted. Values are given in kcal/mol.

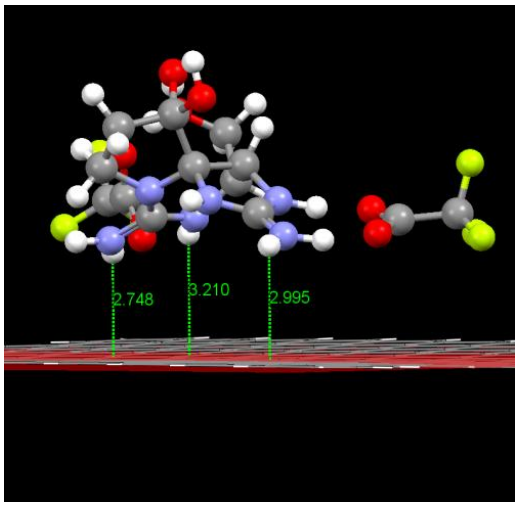
	No TFA		1 x TFA		2 x TFA		n x Cl	
	ΔE (alpha)	ΔE (beta)	ΔE (alpha)	ΔE (beta)	ΔE (alpha)	ΔE (beta)	ΔE (alpha)	ΔE (beta)
dcSTX	-27,13	-23,34	-22,75	-25,02	**-16,55**	-16,21	-11,36	-11,80
NEO	-27,42	-30,07	-23,47	-24,70	-19,44	**-21,55**	-16,99	-13,88
STX	-31,77	-29,96	-23,07	-23,94	-15,55	**-20,88**	-16,41	-14,03
GTX5	-32,35	-28,65	**-24,97**	-24,06	-22,67	-21,51	-22,68	-21,96
GTX3	-32,29	-32,54	**-26,54**	-25,02	-17,12	-21,09	-13.19	-26.11
GTX2	-38,80	-30,29	**-30,76**	-28,48	-20,03	-21,55	-24,44	-24,51
C2	**-33,14**	-31,47	-27,19	-26,35	-22,97	-23,80		
C1	**-36,98**	-30,90	-26,36	-22,20	-25,04	-23,45		

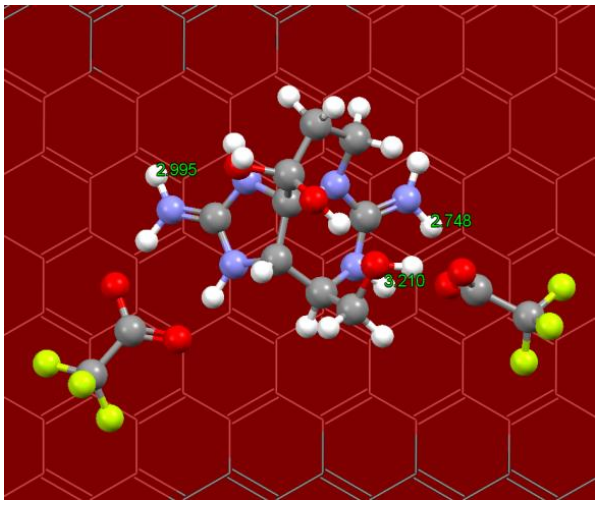
To evaluate the hydroxyl substituent in N1, relative energies of STX and Neo were compared. In this case both values were close with minimum energy beta orientation geometry, being 21.55 kcal / mol for Neo and -20.88 kcal / mol for STX. N1-OH group does not offer an extra stabilization or destabilization of supramolecular interaction versus STX.

And to evaluate sulphate group in 11-C position, GTX2 vs GTX3 and C1 vs C2 were checked. In both epimeric pairs sulphate group in beta position gives as a result less absorption energy. As a general rule, the mostly preferred of supramolecular complex is the orientation of positively charged guanidinium groups to graphene surface. This interaction could be seen an example of non-covalent bonding between a monopole (cation) and a quadrupole (π system), a Cation – π interation.

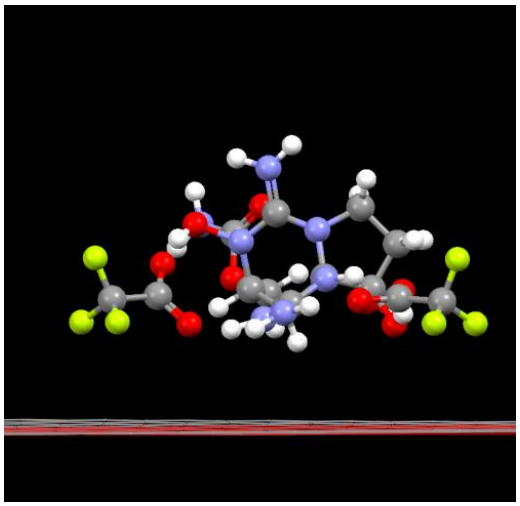
Experimental and molecular calculation reflect that C1 molecule shows the highest retention time and absorption energy, respectively. C1 optimized configuration shows an almost extended coplanar assembly with graphene layer with sulphate groups vicinity to π surface. These sulphate groups result in anion - π interation. Moreover, it should be noted that PGC packing has been probed to possess an anion-exchange capacity [11].

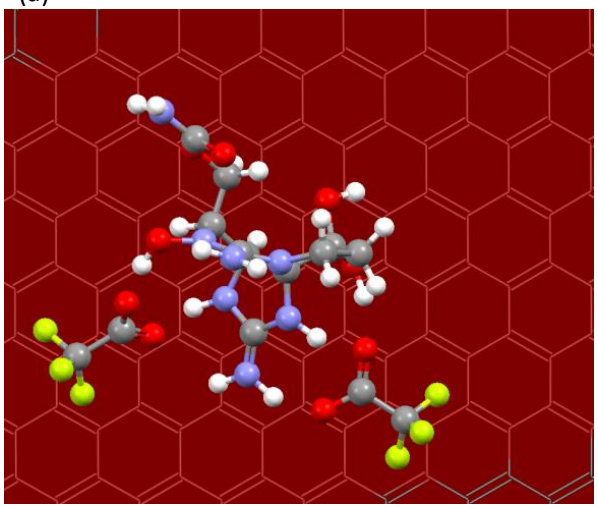
Therefore, C1 maybe presents a synergic effect of spatial charge distribution. The favourable cation – π pairs contributes to attract a negative charge and viceversa, the local charge distribution of the π system should be reversed. The anion–π interactions observed here are favored by dipole moment induced by guanidinium functional groups on local graphene surface, which enhances their electron-poor character. In addition to the π interaction, the sulphate groups are close to the aromatic surface.





(a)





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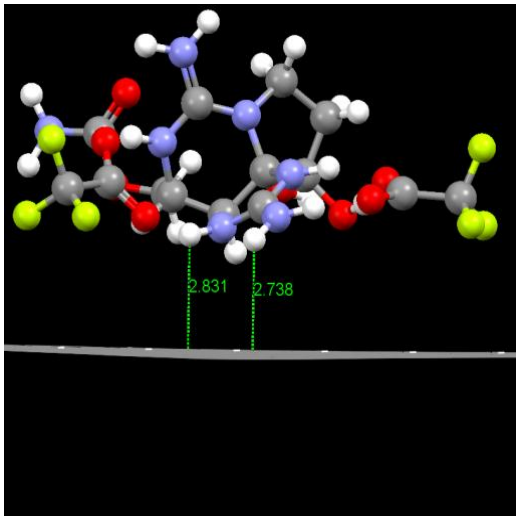
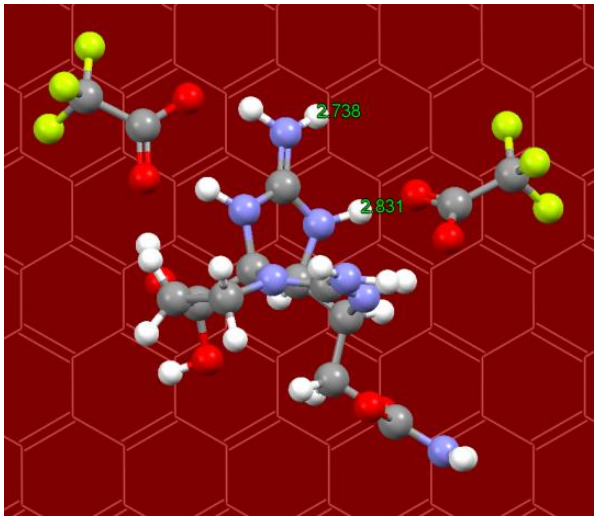
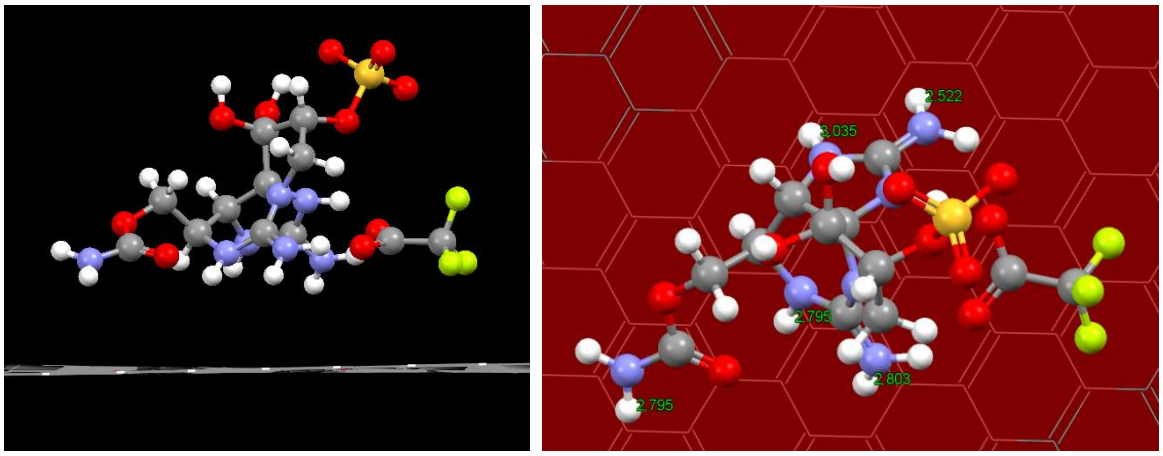




(c)

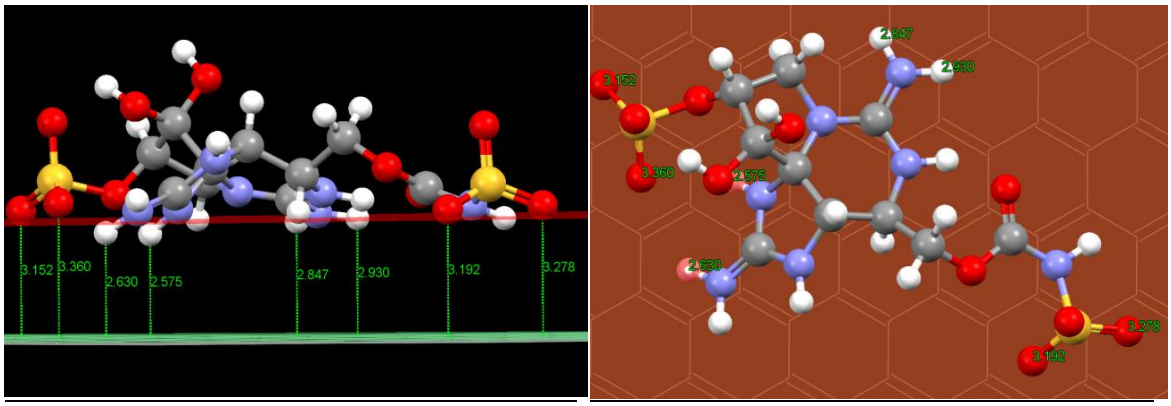


(d)

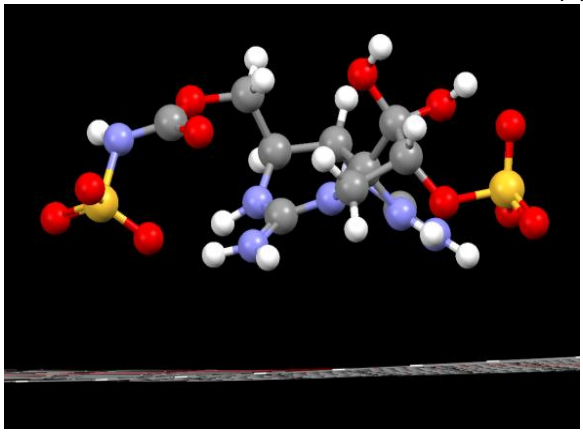
Figure 2.- 3D model of minimum energy of supramolecular complex of graphene layer with: (a) dcSTX with alpha orientation saw through lateral and top views. (b) Neo with beta orientation. (c) STX with beta orientation and (d) GTX2 through alpha orientation.

C1 minimum structure 3D model depicts a quasi-planar interaction between guanidinium and sulphate groups with graphene layer, an average plane of van der Waals contact atoms of gunidinium and sulphate moieties depicts a < 1º angle with graphene plane. It must be noted that guanidinium contact with surface is closer, approximately 2.6 amstrongs, than sulphate groups, around 3.2 amstrongs. Top view of this interaction shows that mostly ion – π interaction takes place when the cation is centered directly over the π-system and is in direct van der Waals contact with it, *Figure 3*.

Two additional supramolecular complexes of C1 with graphene with defferent sulphate spatial arrangements were computed, see *Figure 3*. When sulphate group of alkyl chain is spatial close to guanidium group the absorption energy increases 1.48 kcal / mol due to auto-screened charges. Moreover, if sulphate group of 11-C is far away from π surface the increment turns into 4.09 kcal / mol which shows the stabilization due to sulphate – π interaction. In GTX2 case, *Figure 2d*, TFA avoids sulphate group approximation to graphene layer, while without TFA GTX2 renders the most favourable complex.



(a)



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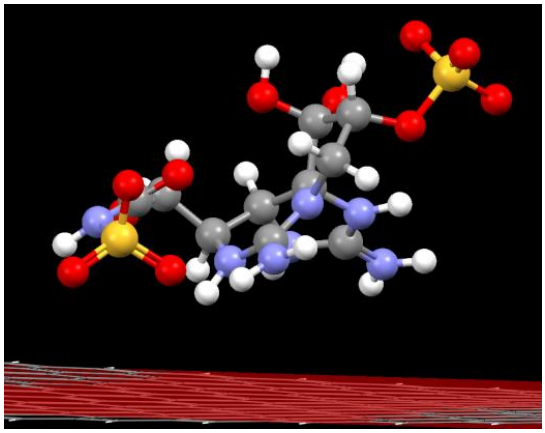

(c)

Figure 3.- Conformation of minimum energy for supramolecular interaction of C1 with graphene layer (a) and tentative minimization of C1 with sulphate side chain close to guanidinium group (b) and with sulphate of 11-C position far away from graphene layer (c).

Chromatographic elution order according to MMFF94 minimization for molecules PSTs under evaluation is: dcSTX – STX – Neo – GTX5 – GTX3 – GTX2 – C2 – C1 vs experimental HPLC model: dcSTX – STX – Neo – GTX5 – GTX3 – C2 – GTX2 – C1. Both profiles do not match fully but are very close. Chromatographic conditions have a strong influence on the adsorption of the PSTs with the stationary phase through non-binding intermolecular interactions. Experimental HPLC conditions use a mixture of water and acetonitrile plus TFA as the mobile phase and chromatographic analysis of PSTs provided the retention effect on graphite and the differentiation of analytes based on their fit to the graphite surface.

GTX2 and C2 computational order does not match well the chromatographic data. C2 minimum energy model reveals a sulphate group of 11-C position far away of graphene layer just in case of GTX2 but this latter with a TFA molecule intermediate of sulphate and surface. A suggestion is that TFA molecule could be situated between beta-sulphate of C2 and the surface diminished energy of C2 interaction or an average configuration with and without TFA conformation; this maybe depicts a real interaction.

For STX and Neo, complexes energy differ less than 1 kcal / mol so elution order can be greatly affected with slight chromatographic modifications, therefore higher energy differences need more important changes in experimental conditions.

Conclusions.

From a panoramic view of chromatographic data and computed molecular properties, several collective structure-chromatographic behavior relationships emerged:

(i) molecular shapes and steric assembly strongly influence enantio separation.
(ii) electron donating groups strengthen interactions.
(iii) side chain influences the recognition mechanism.

Therefore, graphene and family are promising materials for PSTs adsorption.

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