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Experimental and Theoretical Study of Tetracaine-Hydrochloride β-Cyclodextrin Complexation

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Abstract

The complexation of the drug Tetracaine-hydrochloride (TC,HCl) with β-cyclodextrin (β-CD) was studied by means of UV-Visible Spectrophotometry and positive electrospray ionization mass spectrometry ESI+/MS and 1D ^{1}H-NMR. The experimental results confirm that an inclusion complex was formed between the drug and β-CD and that the cationic end of the guest molecule is inserted into β-CD cavity. Quantum semi-empirical calculations were performed to determine the best inclusion pathway between the cationic Tetracaine and the β-CD. Potential energy scan showed that the most stable inclusion complex is obtained when the guest molecule is inserted with its ammonium group into the cavity of β-CD from its wider rim in accordance to experimental results.

1. Introduction

In the two last decades, intensive researches were focused into biological and pharmaceutical compounds thanks to the fast advanced of physico-chemical techniques and the large-scale use of quantum computational calculations and drug design methods [1]. These permit the investigation of more disease-target molecules and the exploration of mechanism which govern the interaction between therapeutic molecules and their target site. Among all pharmaceutical compounds, the local anesthetic family is one of the most used for its therapeutic effects as analgesic, amnesic and hypnosis [2]. In fact, these compounds are known to be very efficient agents once applied for preventing pain surgery of elderly patient and eyes disease [3]. Unfortunately, as the majority of drug compounds most of the anesthetic molecules are poor water-soluble compounds and needs to be modified as chloride salts or transported as complexes to become more soluble in aqueous solutions and enable to reach their bioactive locations [4]. Their transport can be performed with the aid of macrocyclic carrier molecules as cyclodextrin, sulfonatocalixarene, and cucurbituril types [5]. They are employed as discrete supra-molecular drug delivery systems complementing existing supra-molecular drug formulation strategies based on polymers, hydrogels, liposomes, and related micro-heterogeneous systems used as carriers [6]. They must be better water soluble molecules then drugs able to carry the whole drug molecule as a complex.

For this purpose, one of the intensively used therapeutic molecules carrier is β-Cyclodextrin (β-CD) which is a hollow torus-shaped cyclic oligosaccharide formed from the α-1,4 linkages of seven glucopyranose units. This special architecture permits to other fitting molecules to be caged in the interior of its cavity. This process is known as Guest-Host inclusion phenomena and is governed partly by interactions as electrostatic interaction, Van der Waals interaction, hydrogen bonding and charge–transfer interaction and in the other part by hydrophobic forces [7-9]. Indeed, because of its chemical composition and its structural configuration, β-Cyclodextrin has a hydrophilic exterior rims constituted of hydroxyl moieties and hence is relatively a good water soluble compound as well as the majority of oligosaccharides compound but oppositely its interior is of hydrophobic nature. This peculiarity makes such molecule behaving like an attractive pole for other poor water soluble molecules as drugs which through inclusion process look for decreasing their surface contact with water molecules, to minimize their free enthalpy and thus enhance their aqueous solubility.

In practice, it is interesting to specify which ends of the guest molecule enters the β-Cyclodextrin cavity and weather the formed complex still stable with respect of changes in physico-chemical and thermodynamic parameters of the surrounding media and further in vivo conditions. To achieve this, one has to use the suitable method compatible with the physical and chemical specificities of the studied system. In fact, characterization methods for the host-guest phenomena can be folded into two categories; the first ones are exclusively used to evidence the formation of the complex and are handled as detectors of any modification of a chosen complexation dependent parameter; Colligative and mass spectrometry methods are the best candidates [10]. The second ones are more sensitive and bring out information about the manner the inclusion phenomena take place and can explore the formed complex behavior and its stability. They enable to depict the inclusion complex in space; one and two dimension nuclear magnetic resonance 1D and 2D-NMR spectroscopy constitute the best choices [11].

But as mentioned above computational methods are the other alternative to get information about the conformation of the formed complex and furthermore its energy. Many computational methods are used to investigate the complexation and their calculation level choice is dictated by the study objective. The advanced Ab initio methods, such as Hartree–Fock (HF) and density functional theory (DFT), are the best ones to explore the electronic aspect of the inclusion phenomena and permit comparison with experimental results obtained from NMR or other spectroscopic techniques. However, they are time consuming for β-CD complexes once compared to other quantum semi-empirical methods, such as AM1 (Austin Model 1) and PM3 (Parameterized Model 3) which are preferred and are widely used in such theoretical investigation [12–13]. Among these latter methods PM3 seems to be the most appropriate in the conformational study of supra-molecular systems, such as β-CD inclusion compounds and provides better performance compared to the other cited semi-empirical method for molecular geometry optimization, due to its improved description of hydrogen bonds [12–13].

In this study, electrospray ionization mass spectrometry (ESI- MS), UV-Visible Spectrophotometry and proton NMR were used to verify the formation of β-Cylodextrin/tetracaine-hydrochloride complex in aqueous solution. The guest molecule, Tetracaine hydrochloride (TC,HCl) is a potent local anesthetic, primarily used for topical anesthesia and spinal block with unfortunately much adverse effects as slight edema [14]. A lot of experimental works show that the association of such drug with Cyclodextrin leads to better therapeutically effects with the decrease of many adverse effects [15, 16]. Several theoretical works using both Ab-initio and semi-empirical quantum-mechanical calculations were used to characterize free neutral Tetracaine (TC) or its cationic charged form (TCH^+) in gas and water phases but relatively few works are published for β-CD/TCH+ inclusion complex [17-20]. So in this work, theoretical investigation was further performed to examine the insertion pathways and to determine the configuration of the most stable inclusion complex of β-CD/$TCH^{+\cdot}$ This information can be useful since the charged form of the tetracaine is identified as the bioactive form.

2. Experimental

2.1 Chemicals and reagents

Both Tetracaine hydrochloride (PubChem CID: 8695) and β-CD (PubChem CID: 444041) were purchased from Sigma-Aldrich (Germany) and used without further treatment. Double distilled water was used throughout the experiments. The aqueous solutions were prepared at ambient temperature and were subject to sonication for 4 hours before use. Figure 1 shows the chemical structures of both Tetracaine hydrochloride and β-CD.

2.2 *Mass spectrometry measurements*

A complex of Tetracaine hydrochloride with β-CD was prepared by the addition of an equimolar portion of the Tetracaine hydrochloride to an aliquot of β-CD in aqueous solution. Then the whole solution was shaken to mix it effectively before being introduced to the mass spectrometer. All ESI–MS experiments were performed on a Waters-Micromass (LCQ-DUO Finningan). The following settings were used: electrospray ionization (ESI) in positive mode. The dry gas (nitrogen) flow rate was set at 0,6liter/min and the dry heater operated at 150°C. The capillary voltage was set at 5000 V and the collision energy was varied from 30 to 35eV. MS data were recorded in full scan mode in the range 200 to 2,000 m /z). The assignments of the constituents of the ions are based on the exact masses and isotopic profiles.

2.3 *UV-Visible spectrophotometry measurements*

The UV-Vis spectra experiments were recorded with a double beam UV-Vis spectrophotometer (Specord 200 plus) at 25 °C. Data acquisition of UV-Vis spectra was performed with software (Winspec) supported by the manufacturer and converted to ASCII format for their analysis for the wavelength range 190 to 380nm.

2.4 *[1]HNMR Experiments*

Quantitative proton ^{1}H NMR spectroscopy analyses were performed using a Bruker spectrometer operating at 200.13 MHz for proton. Deuterium water (D_2O) was used to dissolve equi-molar quantities of protonated tetracaine (TCH+) and β-CD. The proton data were acquired using a 1.3664 s for acquisition time, a 2997.60 Hz sweep width and no recycle delay. The proton spectra were referenced to tetramethylsilane (TMS).

2.5 Computational Details

All calculations were performed with the Gaussian 03 software package [21]. The two structures, protonated tetracaine (TCH+) and β-CD, were firstly optimized separately by PM3 method in gas phase to get the starting optimized geometries needed for the oriented docking experiments. For all the starting structures frequency calculations were carried out to confirm the completeness of optimization and no negative eigenvalues were found for the optimized structures.

Figure 1: Chemical structures of β-CD and Tetracaine Hydrochloride TCH^+,Cl^-.

For the docking process into the β-CD cavity, several possible orientations of the guest molecule were investigated in order to examine the possible inclusion pathways and to detect the possible global minimum. In the first one, the N-butyl group (-HN-C_4H_9) of the drug tetracaine points toward the primary face of β-CD and was called the ‘‘A Orientation’’ and in the second one, it points toward the β-CD secondary face and was called the ‘‘B Orientation’’ as shown on figure 2.

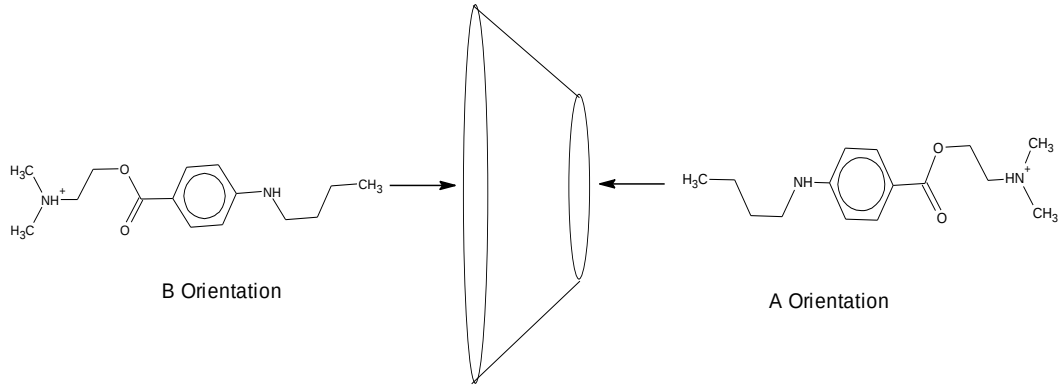

Figure 2: A and B Orientations for the proposed structures of TCH^+ and β-CD (torus-shaped)

A third orientation was considered with the ammonium group -(N+(CH_3)$_2$) pointing toward the secondary face of β-CD and was called the ‘‘C Orientation’’ as seen on figure 3. The orientation of the ammonium end toward the primary face of β-CD was discarded because the bulky ammonium group hinders the inclusion process from this narrower rim.

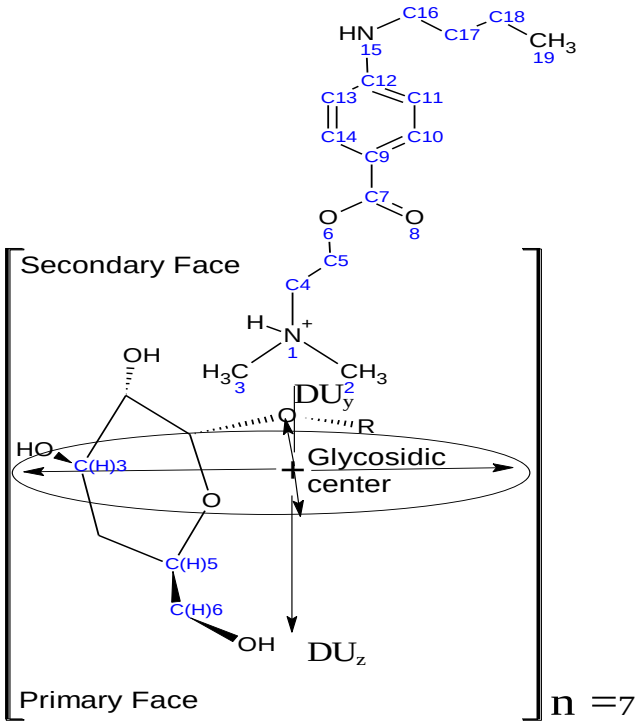

Figure 3: Starting orientation of the inclusion process C, (DUy-DUz) is the approaching distance between the two dummy atoms

The controlled insertion coordinate (Z) of the inclusion process is the distance between two dummy atoms, one located at the center of mass of the tertiary amine group (ammonium) or at the last carbon atoms of the N-butyl group in the protonated tetracaine and the second one is located right below the center of the glycosidic oxygen atoms of the β-CD as illustrated in figure 3. Dummy atoms DUz and β-CD atoms are kept frozen at their initial position throughout the calculation.

3. Results and discussion

The UV-Visible spectra of the drug in aqueous solution were collected at different concentrations at 25°C as reported on Figure 4. It can be observed that the spectra show three peaks centered at wavelength 195, 227 and 310 nm, with absorbance values (A) increasing with drug concentration, obeying to a typical Lambert-Beer behavior (no shown herein). The assignment of these peaks was detailed by Alcolea and al. [17] by means of different semi empirical methods and they correspond to π-π* and n-π* electronic transitions of the para-aminobenzoate fragment of the drug.

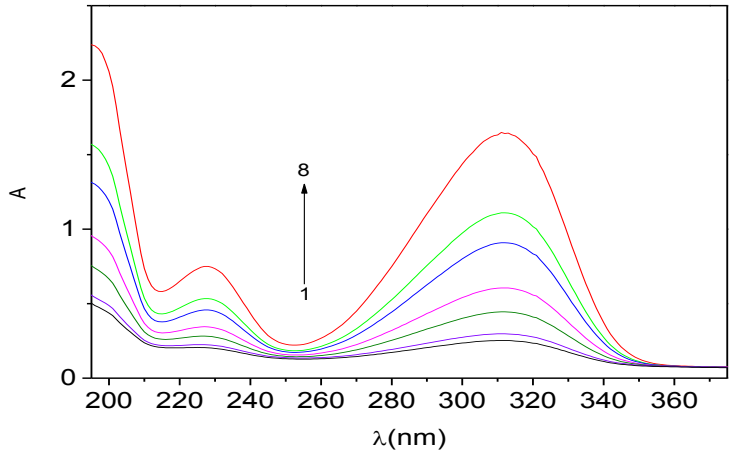

Figure 4: UV-Visible spectra of aqueous solution of tetracaine hydrochloride for different concentrations at 25°C (1: 9.2 10^{-6} M, 2 : 5.52 10^{-6} M, 3: 3.43 10^{-6}M, 4 : 1.48 10^{-5}M, 5: 2.48 10^{-5} M, 6: 3.1 10^{-5} M, 7: 5 10^{-5}M)

When a cyclodextrin solution is added to the tetracaine aqueous solution, the CD and the drug interact to form an inclusion complex CD/Drug as shown on the following equilibrium

$$CD + Drug \rightleftharpoons CD/Drug$$

As a result of inclusion, a complex is formed and the included portion of the guest molecule is surrounded by the hydrophobic microenvironment of the cavity. This induces noticeable variation on some physicochemical parameters depending on the extent of the difference between CD cavity and the outer medium in terms of polarity. Thus, since CD don't absorb in the near UV-Visible domain, the detection of such process can be proved by comparing the UV-Visible spectra of the drug before and after the addition of cyclodextrin.

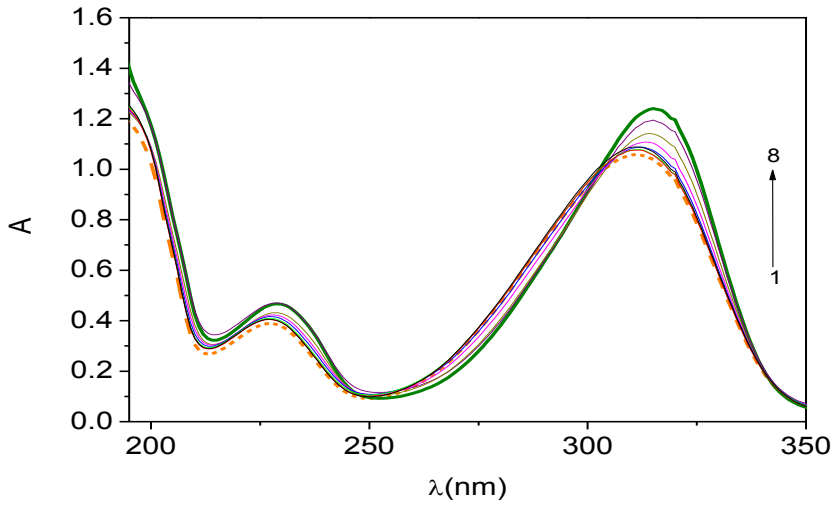

Figure 5: UV spectra of aqueous solutions of tetracaine hydrochloride at a constant concentration of 3 10^{-5} M in the absence (- - - -) and in the presence of different *β*-CD concentrations, at 25 °C. [β-CD]= (1) : 7.5 10^{-6}M, (2) : 2.25 10^{-5}M, (3) : 4.5 10^{-5}M, (4) : 3.16 10^{-4}M, (5) : 7.9 10^{-4}M, (6) : 1.99 10^{-3}M, (7) : 4.99 10^{-3}M, (8) : 10^{-2}M

The features of the spectra obtained after the addition of cyclodextrin are slightly different in terms of wavelength shift and the occurrence of isosbestic points on UV-Visible spectra of the mixture. On the figure 5, we remark the clear shifts of the three peaks already detected in the spectrum of the pure drug and the existence of several isosbestic points indicating the presence of a CD/drug complex are present. These differences prove the existence of a balance between the free molecules of both CD and drug with their corresponding complex CD/drug.

The inclusion process can also be directly detected by ESI spectrometry which shows real potential for the detection of molecular mass of any compounds especially the polar easy-ionisable ones. In this last decade the use of such technique becomes a routine process to the identification of natural molecules in biology field. Indeed, this technique is generally non-destructive and through nebulisation procedure one is able to inject all molecular species to generate positive or negative molecular-ions and hence deduce the molecular mass of the compound. Also, the molecular peaks obtained on the spectra express the stability of the detected formed-ions, so it's appropriate to reveal if other type of inclusions are encountered in the MS experiment conditions for the case of Cyclodextrin.

The positive ESI mass spectrum of β-CD and TCH^+ mixture in water is shown on figure 6 where the most abundant peak at 265 m/z corresponds to free TCH^+ ion, while the peak at 1399 m/z can be attributed to the 1:1 complex CD/TCH^+. The β-CD/sodium adduct peak is observed at 1157 m/z and the one noted at 1661 m/z corresponds probably to [β-CD–$(TCH^+)_2$] complex which directly suggest the existence of 1:2 complex between β-CD and two TCH^+ molecules.

To confirm the complexation and explore the structure of the formed complex quantitative proton NMR experiments are performed and the corresponding ^{1}HNMR spectra studied in this section. To facilitate the assignment of the signals on the HNMR spectra, all the carbon atoms of the molecule of TC, HCl were numbered as shown on the figure 7 as well as the internal protons of the βCD (figure 8). The description of the

inclusion of the TC, HCl in the βCD is simply established on the observation of the variations of the chemical shift δ of the various protons from each molecule between their isolated state and in the complex [11].

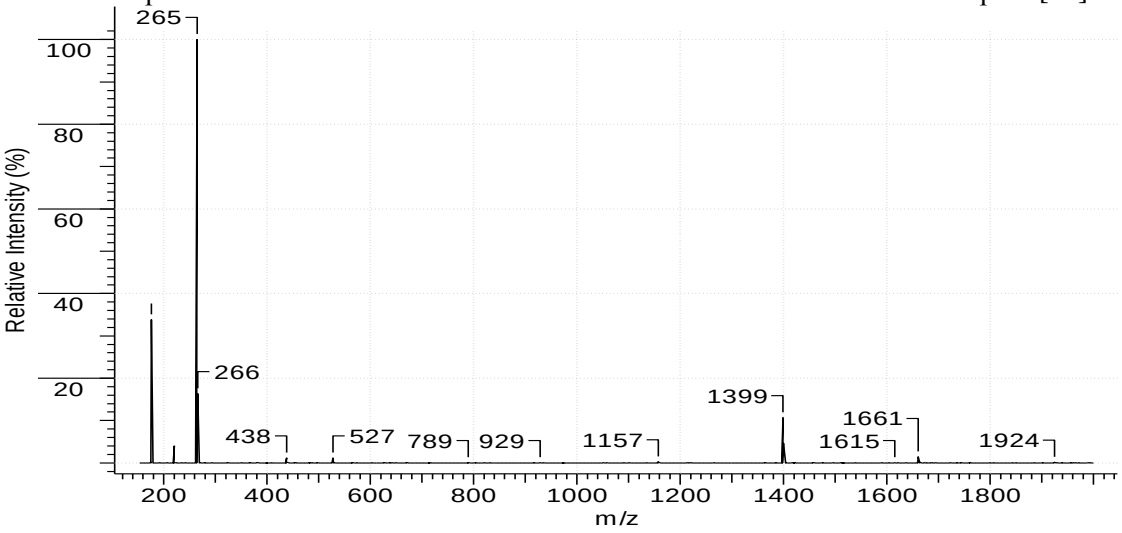

Figure 6: ESI+/MS spectrum of β-CD–TCH^+ inclusion complex in water

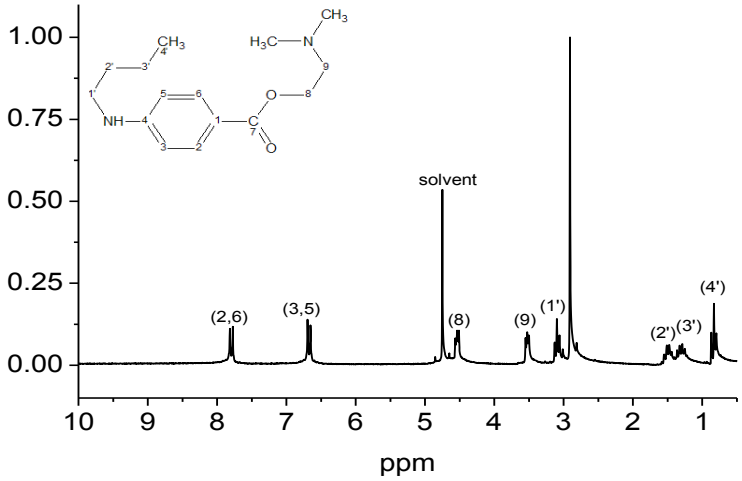

Figure 7: Spectrum ^{1}HNMR of the TTAC, HCl in D_2O (1mM)

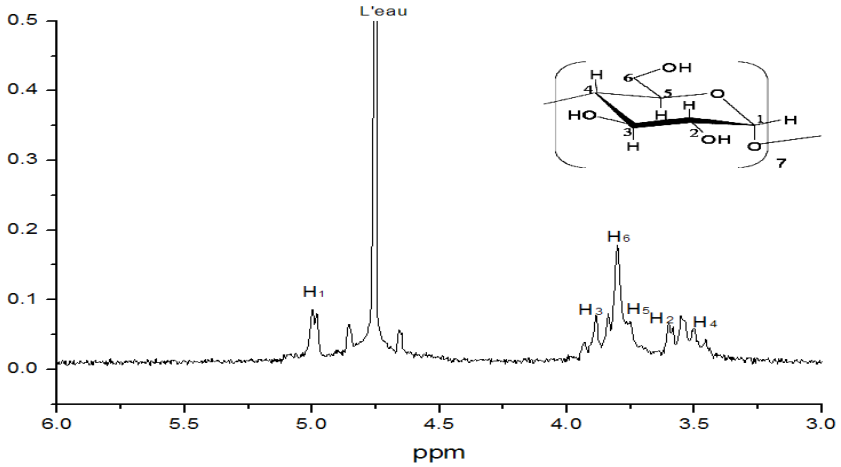

Figure 8: ^{1}H NMR Spectrum of the βCD in D_2 O (1mM)

H NMR Spectra of the βCD, in deuterated water and in the presence of the TC, HCl, are visible on figure 9. The analysis of these spectra shows that δ varies of about 0.06 ppm for H-3 and H-5 protons, both located inside the cavity of the βCD (see third column of table 1). In the same manner, the comparative analysis of H NMR spectrum of TC, HCl in the presence of βCD (figure. 10) shows that a variation of δ of 0.03 ppm is observed for N-methyl groups, and for (2,6), (3,5) and (8,9) protons. These values are comparable with those found in the literature for such system by Liu et al. [8] and Van Santvliet et al. [11]. This weak variation of δ could indicate that the inclusion complex has a weak constant of association.

Table 1: Chemical shifts δ of ^{1}H NMR spectrum of TC, HCl and variations of the chemical shifts Δδ due to the complexation with the βCD.

Protons	δ(ppm)	Δδ(ppm)
2.6	7.81 -7.76	0.03
3.5	6.69-6.64	0.03
8	3.09	0.03
9	4.53	0.03
N-methyl	3.54	0.03
1’	3.09	0.01
2’	1.51	0.01
3’	1.28	0.01
4’	0.83	0.01

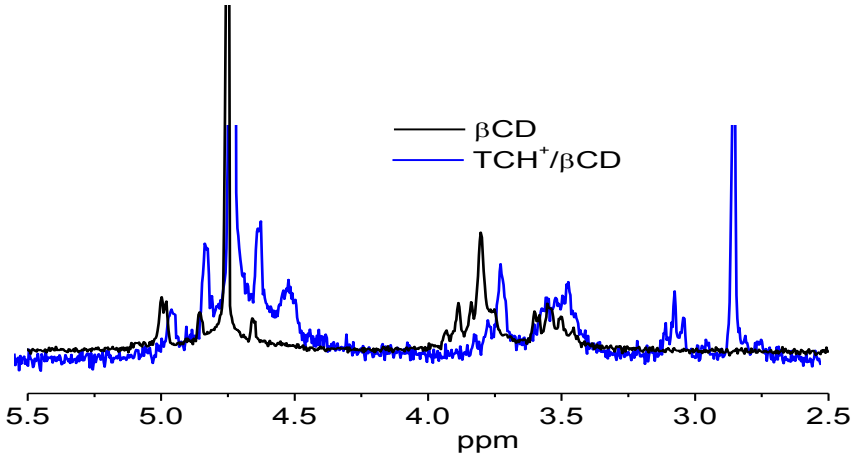

Figure 9: ^{1}H NMR Spectra of the isolated βCD and in the mixture with TCH^{+} in D_2O.

These results state clearly that the complexation takes place and that the ammonium group is the molecular end which is sensitive to the inclusion process because all its protons chemical shifts are disturbed. This conclusion is unexpected and interesting owing to the fact that the presence of the positive charge on the nitrogen atom of the dimethylaminoethyl is expected to render this group more water solvable, less hydrophobic and must be consequently the non-encapsulating end of tetracaine. This implies that inclusion within the βCD is not exclusively governed by the hydrophobic nature of the invited molecule but that other parameters have contributed.

To gain more insight in these results theoretical calculations are performed because quantum calculation may bring out information about charges, hydrophobicity of the guest molecule and the eventual interaction between the different protons. With the optimized protonated tetracaine in hand one can initiate the theoretical inclusion experiments. To achieve this, the amino-butyl end of the drug was inserted separately into the primary face of the cyclodextrin or into its secondary one. The potential energy profiles of the insertion processes named A, B show a minimum for each pathway as shown on figure 11. We note from the total energy values that the formed complex obtained when the inclusion of the $NH(C_4H_9)$ group penetrates the β-CD from the secondary face (B orientation) is more stable than one obtained through the A orientation. For the B orientation which occurs through the wider rim, the interaction is stabilized when the benzene begins its insertion into the second rim and that the first carbon atom of the butyl nitrogen group begins surpassing the primary rim level from the opposite side.

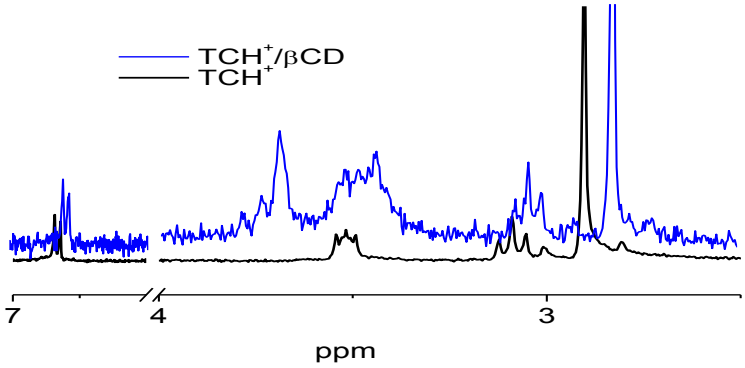

Figure 10: ^{1}H NMR Spectra of isolated TCH+ and in the mixture with βCD in D_2O. [4, 6 ppm] domain was voluntarily omitted to show the variation of the δ as well as possible.

For the C orientation, a better insertion profile is observed since only one minimum is observed and a more stable complex is obtained. The insertion process seems to be related to the bulkiness of the tertiary ammonium nitrogen group. The stability of the complex obtained with the C orientation agrees with the NMR results which suggest that the ammonium group is preferentially inserted into the CD cavity.

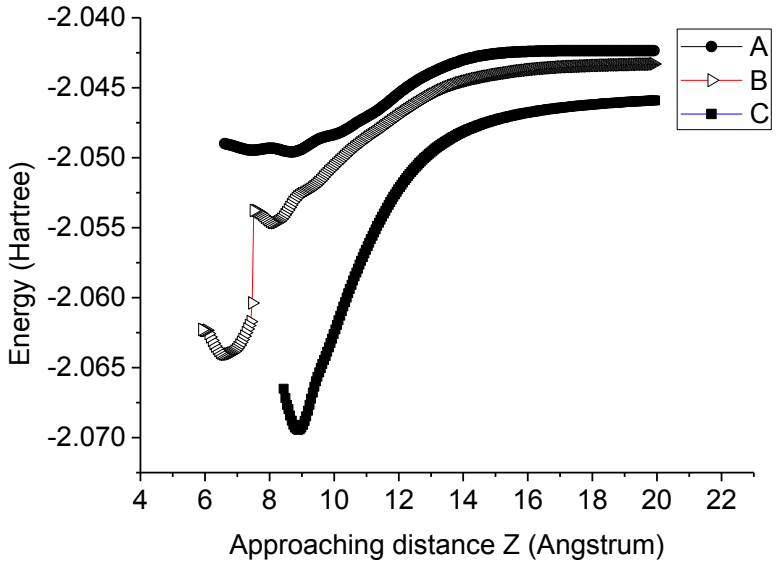

Figure 11: The potential energy profile of the inclusion for the different orientations A, B and C

These insertion profiles are obtained under rigid conditions for CD molecule since the host molecule is kept frozen during the insertion process. So the C minimum complex doesn't reflect the real minimum obtained under free conditions. So it is imperative to relax this complex structure to obtain more energetically stable one. To achieve this, the C minimum complex is allowed to relax with all structural parameters of the host and guest molecules set free. The final corresponding calculation parameters are reported on table 2. From table 2, it can be noted that the complex has higher stability than the isolated molecules indicating that the β-CD/TCH+ association is thermodynamically favorable.

The complexation energy for the inclusion process is evaluated by using the equation (1)

$$\Delta E_{Complexation} = E_{complex} - \left(E_{\beta-CD} - E_{Drug}\right) \quad (1)$$

Where $E_{complex}$, $E_{\beta\text{-}CD}$ and E_{Drug} are respectively: the total energy of the complex, the free optimized β-CD and the free optimized drug. The deformation energy of the guest or the host molecule can be obtained by the following expressions:

$$E_{déformation}\ (Guest) = E_{sp}[G]^{opt} - E\ \ [G]^{opt} \quad (2)$$

$$E_{deformation}\ (Host) = E_{sp}[H]^{opt} - E[H]^{opt} \quad (3)$$

Where $E_{deformation}$ stands for the deformation energy, $E_{sp}[\ ]^{opt}$ is the single point energy in the optimized complex, and $E[\ ]^{opt}$ is the energy of the optimized geometry.

Table 2: Calculated energies at the minimum of insertion for the relaxed C complex using PM3. 1Hartree = 627.51 Kcal/mol.

Starting point		**C minima**					
$E_{\beta\text{-CD}}$ (Hartree)	E_{TCH^+} (Hartree)	$E_{complex}$ (Hartree)	$E_{\beta\text{-CD}}$ (Hartree)	E_{TCH^+} (Hartree)	$\Delta E_{complexation}$ (kcal/mol)	$\Delta E_{def\ (TCH^+)}$ (kcal/mol)	$\Delta E_{def\ (\beta\text{-CD})}$ (kcal/mol)
-2.31408	0.11964	-2.23365	-2.31236	0.12358	-24.60	-2.47	1.07

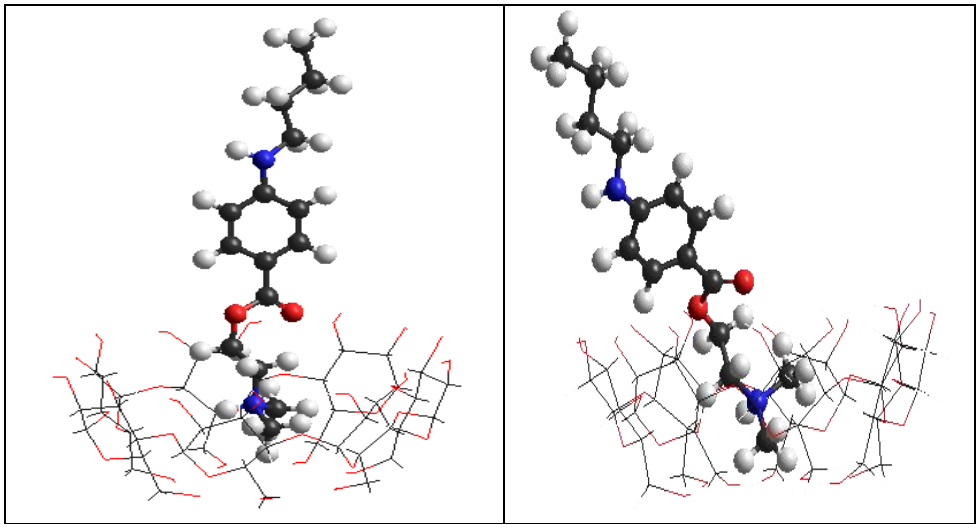

Figure 12: Rigid and Relaxed complexes obtained for C orientation

The analysis of the deformation energies variation shows that both the molecules (guest and host) are modified during the relaxation-optimisation process and that the known belt-like structure observed for the initial β-CD molecule is destroyed when the guest front end enters the secondary β-CD rim. On figure 12 the rigid and the relaxed structures of the obtained stable complexes are visible and it is obvious to note that under rigid conditions only the ammonium group insertion governs the stability of the rigid complex.

However, when the relaxed conditions are allowed a deeper insertion reaching the opposite rim is observed with a significant deformation of the guest molecule. This underlines that under these conditions the process is simultanously sensitive to the size of the group penetrating the cavity and to the interaction of the benzo ester chemical functions of the tetracaine with the hydroxyl moities of the β-CD. These results are in good agreement with those found by Bernardi et al. [19] who performed, by using density functional calculations and molecular dynamics (MD), simulations to investigate the structure and pharmacological action of neutral and charged tetracaine among other anesthetic molecules in water medium. The radial distribution function was used to study solvent effects in different regions of the molecule and to estimate the degree of hydrophobicity for every region of the molecule especially the protonated nitrogen. It was found that chlorine ion forms hydrogen bonds with the proton attached to the ammonium nitrogen, yielding to a neutral-like molecule, which could, in principle, increase the hydrophobicity of this fragment. The MD simulation result contributes to the experimental results obtained herein because the guest molecule gathers in the same time an unsuspected hydrophobic character, a well size-fitted bulky end with the Cyclodextrin cavity and finally Vander Walls type interactions.

Conclusion

Experimental and theoretical studies were carried out to investigate the complexation of β-CD with tetracaine hydrochloride in aqueous solution. In fact, the results obtained by UV-Visible Spectrophotometry and Electrospray mass spectrometry indicate clearly that a complex is formed between β-CD and the cationic form of tetracaine. Furthermore, NMR experiment suggests that the ammonium group of the guest molecule is inserted into the cavity. The PM3 method was applied to explore energetically the possible pathways of the insertion process. The results obtained from this analysis suggest that the insertion of the ammonium group into the secondary face of β-CD produces the most stable complex under rigid inclusion. The complexation energy scan for the inclusion process shows that the host–guest association is thermodynamically favorable and that size-fitting effect associated to hydrophobic and Vander waals interaction are the dominant parameters during the relaxed complexation.

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