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What is the hydrophobic interaction contribution to the stabilisation of micro-hydrated complexes of trimethylamine oxide (TMAO)? A joint DFT-D, QTAIM and MESP study

Imene Derbali,¹ Emilie-Laure Zins,¹ Mohammad Esmail Alikhani¹

1-Sorbonne Université, CNRS, De la Molécule aux Nano-Objets: Réactivité, Interactions Spectroscopies, MONARIS, 75005, Paris France

Abstract

Micro-hydrated trimethylamine oxide (TMAO) has been investigated using a range-separated-hybrid functional including empirical dispersion correction. Electrophilic and nucleophilic sites on TMAO and water clusters have been identified using the molecular electrostatic potential (MESP). The nature of the chemical bonding in the different isomers of the micro-hydrated complexes has been investigated with the topological analysis of the electron density (QTAIM) method. For complexes containing one to four water molecules, the strongest intermolecular interactions consist in hydrogen bonding between the oxygen atom of the TMAO and hydrogen atoms of water molecules. From five water molecules, interactions between water molecules become the main source of stabilization of the most stable isomer. From four stationary points corresponding to the 1:1 (TMAO:H₂O) complex, we determined the minimum distances between water molecules and central TMAO allowing the latter molecule to be encapsulated within a water clathrate-type cage. Optimization of TMAO encapsulated within two water cages (5¹² and 5¹²6²) suggests that only in the case of the 5¹² 6² water cage the insertion of TMAO, the preservation of the hydrogen bonding between water molecules is energetically favorable. The interaction energy between one inserted TMAO and the 5¹² 6² water cage was calculated to be around 150 kJ/mol with respect to the ground state of two partners. This result suggest that a thorough investigation of mono-hydrated complexes may be particularly relevant to identify the most suitable water cage for encapsulating a given solute.

Introduction

The precise characterization of water-solute interactions is crucial for the understanding of many phenomena, such as hydration of atmospheric molecules, biological processes, clathrate formation and exchange processes of the host molecule. In the case of methane clathrates, it is known that the methane host molecule can be exchanged by injecting CO₂.

From an environmental, energetic and ecological point of view, understanding how this exchange takes place is very important. If the exchange of host molecules in clathrates could be understood and controlled, there would also be other possible applications: storage of H₂, transport and reaction in confined environments, to name but a few.

The concept of hydrophobic interactions was introduced around the 1960s to describe the solvation of hydrophobic organic molecules.^{1,2} It seems that a global consensus is emerging on how water molecules arrange themselves around a small hydrophobic solute:^{3,4,5,6} the water molecules would be organized in such a way to maintain as much as possible the existing hydrogen bonding network between water molecules alone. An "exclusion volume" would be formed around small hydrophobic solutes, due to the impossibility of a water molecule to approach the solute without breaking the network of hydrogen bonds between water molecules. The hydration of amphiphilic molecules is more complex, and it has been shown that the hydrophilic part of solutes considerably slows down the dynamic processes of liquid water. It is known that, on the scale of an aqueous solution, amphiphilic molecules, with a hydrophilic part and a hydrophobic part, can modify the local structure and dynamics of water molecules.

These phenomena have been particularly studied in the case of the TMAO molecule.

The hydrophilic part is centered in the vicinity of the oxygen atom.⁷ In aqueous solution, the oxygen atom interacts with three water molecules via interactions of the hydrogen bond type. The hydrophobic part consists of methyls groups. Various advanced studies of ab initio molecular dynamics show, in solution, the existence of three types of water molecules, depending on their position:

- the water molecules with a maximum distance between the oxygen of the TMAO and one of the hydrogen of these water molecules of 2.1 Å are called "hydrophilic water molecules" by D. Marx et al.^{8,9} Three water molecules meet this requirement, and together with the TMAO molecule, they constitute the "supermolecular solvation complex" in the spectral decomposition model introduced by D. Marx et al. for the theoretical analysis of the THz spectra of zwitterionic molecules by AIMD (ab initio molecular dynamic) simulation,¹⁰
- water molecules located beyond this limit, and whose oxygen atom is located within 4.4 Å of one of the carbon atoms of the TMAO are called "hydrophobic water molecules",
- the other water molecules are considered to be bulk water molecules.

These studies provided a precise description of the overall behavior of water molecules around the TMAO in solution: AIMD calculations suggest that methyl groups would not be involved in hydrogen bond interactions. However, the experimental and theoretical molecular-scale study of other organic solutes showed that a water molecule could act as

an acceptor of hydrogen bonds in weak secondary interactions with methyls groups. To our knowledge, at the molecular level, only one theoretical study of TMAO:(H₂O)_n hydrated complexes involving an increasing number of water molecules up to about ten, has been carried out. This work was carried out by Hammer et al. in 2011, with DFT calculations at the B3LYP/aug-cc-pVTZ level.¹¹ The results obtained suggest that methyls could act as hydrogen bond donors, particularly in complexes containing 5 and 7 water molecules, but the formation of such hydrogen bondings was not demonstrated to date in these complexes.

In order to provide a better understanding of water-solute interactions, we looked for the TMAO:(H₂O)_n isomers for $1 \leq n \leq 24$. We characterized the nature of water-water and water-solute interactions in the complexes studied until a hydration sphere was formed around the solute. We were particularly interested in the characterization of interactions:

- between a water cluster and the hydrophilic part of the TMAO,
- between a water cluster and the hydrophobic part of the TMAO,
- between a clathrate-type water cage and the TMAO.

Method

Intermolecular interactions such as Burgi-Dunitz hydrogen bonds as well as interactions with a strong dispersive contribution have been demonstrated in the micro-hydrated complexes around small, weakly polar organic solutes. Even in the case of a small solute S, the number of isomers for an S:(H₂O)_n complex is quickly increasing when n increases, and it is not always easy to identify the most stable isomer using DFT or ab initio calculations based on the chemist's simple intuition. Politzer and Murray have shown that all these interactions come from electrostatic effects in the sense of Hellman-Feynmann's theorem.^{12,13,14} This is the reason why the study of the molecular electrostatic potential (MESP) of isolated partners makes it possible to propose preferred directions of approach, before geometry optimizations. Gadre et al. introduced the topology of the MESP function to study the formation of hydrated or other complexes. Another approach, simpler and directly based on the physical nature of interactions between molecules at long distances, has been proposed: it consists in studying the MESP of isolated partners and seeking all associations to maximize interactions between complementary sites. The initial structures thus obtained are then optimized. Which "isolated partners" to choose for the study of an S:(H₂O)_n complex? Different choices are possible: S and (H₂O)_n, S:(H₂O) and (H₂O)_{n-1}, as well as S:(H₂O)_x and (H₂O)_y, with x+y = n. All these starting points should be considered when looking for the TMAO:(H₂O)_n most stable isomers.

To further understand the formation of hydrogen bondings and other interactions, the topological analysis of the Electron Localization Function (ELF)^{15,16} of isolated water and TMAO molecules is particularly relevant, since it allows the identification of valence basins.

Protonated basins can be localized and characterized, as well as electron pairs in keeping with the Lewis structures.^{17,18} Furthermore, after geometry optimization, all the hydrated complexes were further studied by means of the QTAIM¹⁹ approach in order to analyze both qualitatively and quantitatively the intermolecular interactions involved in the complexes. We were particularly interested in quantifying the water-water interaction and the water-solute interactions in order to shed some light on the origins of the stability of the complexes.

All the calculations were carried out with the Gaussian09 software.²⁰ Dispersion-corrected density functionals within the density functional theory (DFT-D) are methods of choice for quantum chemical calculations on large systems including long-range, non-covalent interactions such as hydrogen bondings and electrostatic interactions. For the present study, we used the LC- ω PBE functional^{21,22,23} in combination with the 6-311++G(d,p) triple ζ Pople basis set. The Grimme empirical dispersion correction with Becke–Johnson damping function (GD3BJ)²⁴ was applied (using the "EmpiricalDispersion=GD3BJ" keyword). Furthermore, to deliver more accurate results, two additional keywords were used for the optimization calculations: scf=Tight Int=UltraFine. All the geometry optimization have been supplemented by frequency calculation to ascertain the nature of the stationary point (transition state versus local minimum).

The zero point energy (ZPE) correction was taken into account in all the energy calculations. Four energy differences were calculated for all the TMAO:(H₂O)_n isomers (Table 1):

- the binding energy corrected for the ZPE, D0, was calculated as the difference between the energy of the TMAO:(H₂O)_n complex ($E_{\text{TMAO:(H}_2\text{O})_n}$) and the ones of isolated TMAO ($E_{\text{TMAO}}^{\text{isolated}}$) and (H₂O)_n partners ($E_{(\text{H}_2\text{O})_n}^{\text{most stable}}$) in their most stable geometries,
- the interaction energy, I.E., was calculated as the difference between $E_{\text{TMAO:(H}_2\text{O})_n}$ and the sum of $E_{\text{TMAO}}^{\text{isolated}}$ with n times the energy of an isolated water molecule ($nE_{(\text{H}_2\text{O})}^{\text{isolated}}$),
- $\Delta(E)$ was calculated as the difference between the energy of the TMAO:(H₂O)_n and the energy of the most stable TMAO:(H₂O)_n isomer,
- $\Delta(G)$ calculated as the difference between the free energy of the TMAO:(H₂O)_n and the free energy of the most stable TMAO:(H₂O)_n isomer.

Table 1: Energy differences that were used throughout this work to characterize the stabilization of the isomers studied.

$D0 = E_{\text{TMAO:(H}_2\text{O})_n} - [E_{\text{TMAO}}^{\text{isolated}} + E_{(\text{H}_2\text{O})_n}^{\text{most stable}}]$	$\Delta(E) = E_{\text{TMAO:(H}_2\text{O})_n} - E_{\text{TMAO:(H}_2\text{O})_n}^{\text{most stable}}$
$\text{I. E.} = E_{\text{TMAO:(H}_2\text{O})_n} - [E_{\text{TMAO}}^{\text{isolated}} + nE_{(\text{H}_2\text{O})}^{\text{isolated}}]$	$\Delta(G) = G_{\text{TMAO:(H}_2\text{O})_n} - G_{\text{TMAO:(H}_2\text{O})_n}^{\text{most stable}}$

The Gaussian09 software was also used to generate the .wfn files further used for the topological analyses. From these .wfn files:

- the Topmod²⁵ software was used for the topological analysis of the ELF function,
- the Aimall software was used for the QTAIM analysis as well as for the MESP analysis.²⁶

The structures of the water cages used for the study of the complexes TMAO:(H₂O)₂₀ and TMAO:(H₂O)₂₄ were taken from the literature.^{27,28}

The main QTAIM topological features of the species herein discussed are presented in Supporting Information S1-S10, and the Cartesian Coordinates of all the complexes are given in Supporting Information S11.

Characterization of isolated TMAO and H₂O partners

The topological analysis of the TMAO leads to the identification of three particular regions (Figure 1):

- a highly nucleophilic zone located near the oxygen atom
- an electrophilic zone, diametrically opposed to this nucleophilic region. It is an electrophilic region located on the axis of a σ -bond, which corresponds to the definition of a σ -hole,
- three weakly electrophilic zones, in the median plane of the molecule, close to the hydrogen atoms of the methyl groups.

Figure 1: Molecular electrostatic potentials and the topological analysis of the ELF function of the TMAO and water molecules.

Furthermore, the topology of the ELF function leads to the identification of three monosynaptic basins for the oxygen atom (Figure 1). For sake of comparison, the topological analysis of the isolated water molecule is also presented in Figure 1.

In the literature, one hydrophilic and one hydrophobic regions are often considered for the TMAO molecule. However, for consistency with the MESP analysis, it is interesting to distinguish three different regions (Scheme 1):

- a hydrophilic region in the vicinity of the oxygen atom; this corresponds to the nucleophile region characterized from the MESP analysis,
- a hydrophobic region that is diametrically opposite to the hydrophilic region; this region will be called the "opposite hydrophobic region"; this corresponds to the σ -hole identified from the MEP analysis,
- a hydrophobic region between the two above-mentioned regions, that will be called the "vicinal hydrophobic region"; this corresponds to the weak electrophile regions identified from the MESP analysis.

Scheme 1: Identification of three different regions on the surface of the TMAO molecule

Identification and characterization of TMAO:(H₂O) isomers

From the topological analysis, three different directions of approaches of a water molecule relative to the TMAO may be proposed to maximize interactions between complementary sites (Figure 2):

- an approach allowing an interaction between a protonated basin of the water molecule (an electrophilic site) and a monosynaptic basin (a nucleophilic zone) of the TMAO. This approach also makes it possible to consider the formation of a secondary interaction between the nucleophilic zone of the water molecule and the weakly nucleophilic zone of the TMAO (Figure 2A);
- an approach in which the electrophilic zone of the water molecule interacts with the most nucleophilic part of the TMAO (Figure 2B);
- an approach in which the electrophilic zone of the water molecule interacts with a weaker nucleophilic zone of the TMAO, in the median plane of the solute (Figure 2C).

Figure 2: Three different direction of approaches of a water molecule relative to the TMAO molecule leading to the maximization of interactions between complementary sites.

After geometry optimization and frequency calculations, four structures are obtained for the mono-hydrated complex. Two structures correspond to minima on the potential energy surface: they will be denoted as S1 and S2 (Table 2). The two other structures correspond to transition states: they will be denoted as TS1 and TS2 (Figure 3). The S1 isomer corresponds to the global minimum, and lies 38.6 kJ.mol^{-1} below the S2 isomer. The binding energies for the S1 and S2 isomers are -61.4 and $-13.0 \text{ kJ.mol}^{-1}$, respectively. The QTAIM analysis of the S1 and S2 isomers demonstrates that, in both cases, three water-solute interactions are simultaneously formed (Table 2).

Table 2: Structure, energetic and QTAIM topological features of the TMAO:(H₂O) isomers identified at the LC- ω PBE / 6-311++G(d,p) level of theory. The small green spheres correspond to Bond Critical Points (BCP) and the black lines to Bond Paths (BP), as defined in the QTAIM theory. $\rho(r)$ and $\nabla^2\rho(r)$ correspond to the electron density and its Laplacian at the BCP, respectively (the values are given in a.u.).

S1				S2			
	BCP	$\rho(r)$	$\nabla^2\rho(r)$		BCP	$\rho(r)$	$\nabla^2\rho(r)$
	N=O...HOH	0.049	0.14		2 × H ₂ O...HC	0.008	0.03
	2 × H ₂ O...HC	0.010	0.04		H ₂ O...HC	0.005	0.02
$\Delta(E) = 0.0 \text{ kJ.mol}^{-1}$ $\Delta(G) = 0.0 \text{ kJ.mol}^{-1}$ D0 = $-51.6 \text{ kJ.mol}^{-1}$				$\Delta(E) = 38.6 \text{ kJ.mol}^{-1}$ $\Delta(G) = 29.4 \text{ kJ.mol}^{-1}$ D0 = $-13.0 \text{ kJ.mol}^{-1}$			

In the S1 isomer, the main interactions consists in an hydrogen bonding between a hydrogen atom of the water molecule and the oxygen atom of the TMAO, in full agreement with structures already proposed in the literature. Simultaneously, two other weak hydrogen bondings involving hydrogen atoms of the "vicinal hydrophobic region" of the TMAO and the oxygen atom of the water molecule, are also identified. Interactions with the hydrogen atoms of the TMAO cause a significant deformation of the main hydrogen bond. Indeed, according to the IUPAC report, in the case of an undisturbed hydrogen bond, the OHO angle should be close to 180° .²⁹ However, in the S1 complex, the OHO angle is equal to 162.7° . For secondary interactions, both OHC angles are equal to 129.8° .

In the S2 isomer, the water molecule interacts with the TMAO via the "opposite hydrophobic region". Three weak hydrogen bondings involving a hydrogen atom of each methyl group are identified from the QTAIM analysis. This isomer is significantly less stable than the S1 isomer. However, the negative binding energy shows that the formation of this isomer is energetically possible.

Figure 3: Identification of the global minimum, a local minimum, and two transition states on the potential energy surface of the TMAO:(H₂O) isomers at the LC- ω PBE / 6-311++G(d,p) level of theory

In the structures corresponding to transition states, the water molecule is solely interacting with the "vicinal hydrophobic region". The TS1 and TS2 species lie 45.6 and 42.9 kJ.mol⁻¹ above the global minimum, respectively, and they both correspond to a switch of the water molecule between the S1 and S2 isomers (Figure 3).

Identification and characterization of TMAO:(H₂O)₂₋₉ isomers

As soon as the dihydrated species is considered, many questions arise, and several isomers can be considered:

1. Does the micro-hydration occur with segregation of water molecules as observed with some other organic solutes? The term "water segregation" refers to a water cluster interacting with the solute. This expression being regularly used in the literature, we will employ it in the following of the manuscript.
2. Can the hydrogen bonding between the water molecules observed in the water dimer be preserved in the interaction with the TMAO?
3. Can interactions between water molecules and the hydrophobic part of the TMAO stabilize the TMAO complex:(H₂O)₂?

Several starting points should be used to generate the initial structures for the TMAO:(H₂O) complex:

- the TMAO molecule with the water dimer. The analysis of the MESP maps suggests two main directions for the approach of these two partners (Figure 4): the electrophilic site and the nucleophilic site of the water dimer may interact with the hydrophilic (nucleophilic) region of the TMAO and the "vicinal hydrophobic (weak electrophilic) region", respectively. Alternatively, the water dimer may interact with the TMAO via a the "opposite hydrophobic (electrophilic) region",
- the TMAO:(H₂O) complex with an additional water molecule. This corresponds to two different starting points: the S1 isomer and a water molecule, or the S2 isomer with an additional water molecule.

Figure 4: Proposal of two possible preferred approach direction for the water dimer relative to the TMAO molecule, as well as of two possible preferred approach direction for a water dimer relative to the TMAO:(H₂O) isomers, based on the molecular electrostatic potential (MESP) analysis. The guess structures thus obtained were used as starting points for the identification of TMAO:(H₂O)₂ isomers prior to geometry optimizations.

After geometry optimizations, four isomers were obtained, all of them corresponding to minima on the PES (Table3):

- In the most stable isomer, the two water molecules interact separately and symmetrically with the TMAO. The two main interactions correspond to hydrogen bondings. As in the case of the S1 isomer of the TMAO:(H₂O) complex, the OHO angles are smaller than those of a conventional hydrogen bond, and are 161.5°. Each water molecule is also involved in two secondary interactions of the hydrogen bond type with hydrogen atoms of methyls groups, and the OHC angles are 130.2° and 131.3°. The complexation energy is -80.7 kJ.mol⁻¹ for this isomer
- The D2 isomer is almost isoenergetic with D1, with $\Delta(E) = 4.2 \text{ kJ.mol}^{-1}$ and $\Delta(G) = 3.3 \text{ kJ.mol}^{-1}$. In this isomer, the water dimer interacts with the TMAO molecule. The

electron density at the BCP ($\rho(r)$) corresponding to the hydrogen bond between the two water molecules is 0.040. In the isolated water dimer, the value is 0.024. The significant increase of $\rho(r)$ in the complex suggests a cooperative effect due to the formation of three additional interactions with TMAO: a main interaction of the hydrogen bond type with the oxygen atom of TMAO, and two secondary interactions with two hydrogen atoms of the methyls groups. For the interaction between the oxygen atom of the TMAO and the water molecule, the higher value of $\rho(r)$ in the D2 complex (0.058) compared to the S1 and D1 complexes (0.049 and 0.043, respectively) again suggests a cooperative effect. The complexation energy is $-76.5 \text{ kJ.mol}^{-1}$ for this isomer.

- The D3 isomer lies significantly higher in energy, with $\Delta(E) = 30.7 \text{ kJ.mol}^{-1}$ and $\Delta(G) = 23.1 \text{ kJ.mol}^{-1}$. In this isomer, one water molecule occupies a position close to the one of the water molecule in the S1 isomer, while the other water molecule occupies a position close to the one of the water molecule in the S2 isomer. The QTAIM analysis reveals that each water molecule is involved in three non-covalent interactions with the TMAO molecule. The complexation energy is $-50.0 \text{ kJ.mol}^{-1}$ for this isomer.
- In the D4 isomer, the water dimer is in interaction with the TMAO molecule via the "opposite hydrophobic region".

Table 3: Structure, energetic and QTAIM topological features of the TMAO:(H₂O) isomers identified at the LC- ω PBE / 6-311++G(d,p) level of theory. The small green spheres correspond to Bond Critical Points (BCP) and the black lines to Bond Paths (BP), as defined in the QTAIM theory. $\rho(r)$ and $\nabla^2\rho(r)$ correspond to the electron density and its Laplacian at the BCP, respectively (the values are given in a.u.).

D1		
	BCP	$\rho(r)$	$\nabla^2\rho(r)$
	2 × N=O...HOH	0.043	0.13
	2 × C-H...OH ₂	0.011	0.04
	2 × C-H...OH ₂	0.0094	0.03
	$\Delta(E) = 0.0 \text{ kJ.mol}^{-1}$ $\Delta(G) = 0.0 \text{ kJ.mol}^{-1}$ D0 = $-80.7 \text{ kJ.mol}^{-1}$
D2		
	BCP	$\rho(r)$	$\nabla^2\rho(r)$
	N=O...HOH	0.058	0.15
	Hw...Ow	0.040	0.13
	C-H...OH ₂	0.011	0.04
	C-H...OH ₂	0.011	0.04
	$\Delta(E) = 4.2 \text{ kJ.mol}^{-1}$ $\Delta(G) = 3.3 \text{ kJ.mol}^{-1}$ D0 = $-76.5 \text{ kJ.mol}^{-1}$

D3		
	BCP	$\rho(r)$	$\nabla^2\rho(r)$
	N=O...HOH (S1)	0.050	0.13
	2 $\times$ C-H...OH ₂ (S1)	0.010	0.04
	2 $\times$ C-H...OH ₂ (S2)	0.008	0.03
	C-H...OH ₂ (S2)	0.005	0.02
$\Delta(E) = 30.7 \text{ kJ.mol}^{-1}$ $\Delta(G) = 23.1 \text{ kJ.mol}^{-1}$ D0 = -50.0 kJ.mol ⁻¹
D4		
	BCP	$\rho(r)$	$\nabla^2\rho(r)$
	Hw...Ow	0.026	0.11
	2 $\times$ C-H...OH ₂ (S2)	0.009	0.03
	C-H...OH ₂ (S2)	0.007	0.02
	C-H...OH ₂	0.003	0.01
$\Delta(E) = 60.1 \text{ kJ.mol}^{-1}$ $\Delta(G) = 46.0 \text{ kJ.mol}^{-1}$ D0 = -20.6 kJ.mol ⁻¹			

Table 4: Comparison of the geometric and topological features of the hydrogen bonding between water molecules in the water dimer and in the D2 and D4 isomers.

Complex \ Feature	$d_{\text{Hw...Ow}}$ (Å)	$\rho(r)$	$O - \widehat{H} \dots O$ (°)
(H ₂ O) ₂	1.93	0.024	175.9
D2	1.73	0.040	162.7
D4	1.88	0.026	172.0

Data reported in the Table 4 demonstrate that the water dimer is not dramatically affected by the interaction with the "opposite hydrophobic region" of the TMAO: the intermolecular distance between water molecules, $\rho(r)$ and the $O - \widehat{H} \dots O$ angle are close to the ones observed in the isolated water dimer. This is in strong contrast with what is observed when both water molecules interact with the hydrophilic and the "vicinal hydrophobic region" : in the D2 isomer, the $d_{\text{Hw...Ow}}$ distance is significantly shorter (by 0.20 Å) than that of the dimer water, suggesting a strengthening of the water/water interaction. The concomitant increase of $\rho(r)$ in the D2 isomer relative to the (H₂O)₂ complex confirms the strengthening of the water-water interaction. In addition, one of the water molecules of the D2 isomer occupies a similar position to that of the water molecule of the S1 isomer, near the oxygen atom of the TMAO, and it is interesting to compare the characteristics of these interactions (Table 5). The distance $d_{\text{Hw...OTMAO}}$ is slightly shorter (by 0.09 Å) in the D2 isomer than in the S1 isomer. In addition, for this interaction, $\rho(r)$ is significantly higher in the D2 (0.058) isomer than in the S1 (0.049) isomer. The interaction of the water cluster with the hydrophilic part of the TMAO therefore has a cooperative effect, resulting in enhanced water-water and water-solute interactions. This is not the case for the interaction of the water cluster with the hydrophobic part of the TMAO solute (Table 5).

Table 5: Comparison of the geometric and topological features of the hydrogen bonding between water molecules and the solute in the S1 and D2 isomers, as well as in the S2 and D4 isomers.

Complex \ Feature	Interacting atoms, $d_{\text{water} \cdots \text{TMAO}}$ (Å), $\rho(r)$			$O_w - \widehat{H_w} \cdots O_{\text{TMAO}}$ (°)
S1	$H_{\text{water}} \cdots O_{\text{TMAO}}$	1.69	0.049	162.6
D2	$H_{\text{water}} \cdots O_{\text{TMAO}}$	1.60	0.058	176.4
S2	$O_{\text{water}} \cdots H_{\text{TMAO}}$	2×2.55 2.75	2×0.008 0.005	
D4	$O_{\text{water}} \cdots H_{\text{TMAO}}$	2×2.48 2.59 3.12	2×0.009 0.007 0.003	

A similar approach was used to identify and characterize the TMAO:(H₂O)_n complexes, $3 \leq n \leq 9$. Finally, 45 stable isomers were found. The QTAIM features of the intermolecular interactions as well as the energetic features of the complexes are presented in Supporting Information S1 - S9.

Table 6 shows several structures for complexes containing 3 to 9 water molecules:

- the most stable complexes herein obtained,
- complexes involving the highest fraction of hydrophilic water-solute interactions,
- complexes involving the highest fraction of hydrophobic water-solute interactions.

Fully consistent with earlier studies presented in the literature,⁸⁻¹¹ the most stable isomer for the TMAO:(H₂O)₃ complex consists of three water molecules each forming an hydrogen bonding with the oxygen atom of the TMAO. It is worth noting that no interaction between the water molecules exists in the most stable isomer. This is consistent with what is also obtained for the complex containing two water molecules. However, it is interesting to note that TMAO does not lead to segregation of water molecules, unlike other solutes.³⁰⁻³⁷

The fact that the most stable TMAO:(H₂O)₂ and TMAO:(H₂O)₃ isomers consist of isolated water molecules interacting with the solute rather than slightly perturbed water dimer and water trimer can easily be understood by considering the interaction energies between the TMAO and a single water molecule on the one hand, and between water molecules in the water dimer and water trimer on the other hand. Indeed, the interaction energy between a single water molecule and TMAO is equal to -61.4 kJ.mol⁻¹ (Table 2), whereas the interaction energy in the water dimer and trimer are calculated to be -16.1 and -53.8 kJ.mol⁻¹, respectively at the same level of theory (Supporting Information). A quantitative analysis of the MESP features of isolated partners also shed some light on their electrophilic and nucleophilic strengths (Figure 5): the oxygen atom of the TMAO molecule is a stronger nucleophile than the oxygen atom of the water molecule. As a consequence, the TMAO molecule is a better hydrogen-bond acceptor than the water molecule.

Figure 5: MESP features of the TMAO and water molecules.

In the case of TMAO:(H₂O)₄ complex, five isomers very close in energy, with $\Delta E \leq 5.1 \text{ kJ.mol}^{-1}$ were identified (See Supporting information S3). The most stable isomer found at the chosen level of theory consists of two water dimer, both simultaneously acting as hydrogen-bond donor with the oxygen atom of the TMAO solute, and as double hydrogen-bond acceptor with two hydrogen atoms of methyl groups (See Supporting information S3 for the QTAIM features of the intermolecular interactions). Isomers involving higher fraction of hydrophilic water-solute interactions or higher fraction of hydrophobic water-solute interactions lies 13.5 kJ.mol^{-1} and 21.6 kJ.mol^{-1} above the global minimum, respectively.

In the case of TMAO:(H₂O)_{n=5 to 9} complexes, at least *n* water-water interactions are identified in the most stable isomers on the basis of the QTAIM analysis. For *n*= 7-9, among the most stable structures identified in the present study the ones involving the highest fraction of hydrophilic water-solute interactions also involve the highest fraction of hydrophobic water-solute interactions.

Table 6: Structures of some of the most interesting TMAO:(H₂O)₄₋₉ complexes. See Supporting Information for energetic and topological features, as well as for the structures of other isomers. Additional features are presented in Supporting Information.

TMAO:(H ₂ O) _n n= Complexes	3	4	5	6
Most stable	<p>T1: D0 = -82.4 kJ.mol⁻¹</p>	<p>Q1: D0 = -73.6 kJ.mol⁻¹</p>	<p>Pent1: D0 = -80.6 kJ.mol⁻¹</p>	<p>Hex1: D0 = -97.8 kJ.mol⁻¹</p>
involving the highest fraction of hydrophilic water-solute interactions	<p>T1: D0 = -82.4 kJ.mol⁻¹</p>	<p>Q6: D0 = -66.4 kJ.mol⁻¹</p>	<p>Pent4: D0 = -67.7 kJ.mol⁻¹</p>	<p>Hex6: D0 = -56.8 kJ.mol⁻¹</p>
involving the highest fraction of hydrophobic water-solute interactions	<p>T1: D0 = -82.4 kJ.mol⁻¹</p>	<p>Q8: D0 = -52.1 kJ.mol⁻¹</p>	<p>Pent2: D0 = -73.8 kJ.mol⁻¹</p>	<p>Hex8: D0 = -28.0 kJ.mol⁻¹</p>

TMAO:(H ₂ O) _n , n= Complexes	7	8	9
Most stable	Hept1: D0 = -93.3 kJ.mol ⁻¹	Oct1: D0 = -63.9 kJ.mol ⁻¹	Nona1: D0 = -85.1 kJ.mol ⁻¹
involving the highest fraction of hydrophilic water-solute interactions and also the highest fraction of hydrophobic water-solute interactions	Hept3: D0 = -53.2 kJ.mol ⁻¹	Oct3: D0 = -53.5 kJ.mol ⁻¹	Nona2: D0 = -67.9 kJ.mol ⁻¹

Encapsulation

From five water molecules, some isomers can be seen as the onset of encapsulation. It is therefore very interesting to look for the structure of a water cage allowing the encapsulation of the TMAO solute. For this study, two water cages were considered: 5^{12} and $5^{12}6^2$ water cages constituting elementary assemblies of some water clathrates.²⁷ The energy of the encapsulation ($E_{\text{encapsulation}}$) was calculated as the difference between the sum of the energies of the isolated water cage and the TMAO on the one hand, and the energy of the $\text{TMAO} @ (\text{H}_2\text{O})_{20,24}$, on the other hand. The D0 was calculated from the most stable $(\text{H}_2\text{O})_{20,24}$ isomers.³⁸ The most stable structures and energetics are presented in Table 7, and the topological analysis as well as additional isomers are presented in Supporting Information.

Table 7: Structure and energetics $\text{TMAO} @ (\text{H}_2\text{O})_{20,24}$ of the complexes. Additional features are presented in Supporting Information.

$\text{TMAO} @ (\text{H}_2\text{O})_{20}$	$\text{TMAO} @ (\text{H}_2\text{O})_{24}$

$D0 = -53.1 \text{ kJ.mol}^{-1}$ $E_{\text{encapsulation}} = -101.3 \text{ kJ.mol}^{-1}$	$D0 = -70.9 \text{ kJ.mol}^{-1}$ $E_{\text{encapsulation}} = -150.5 \text{ kJ.mol}^{-1}$

The presence of TMAO inside the $(\text{H}_2\text{O})_{20}$ water cage causes a breakdown of the hydrogen bonding network between the water molecules. However, very strong interactions, characterized by high values of electronic densities at BCP (0.069 and 0.040) are established between the oxygen atom of the TMAO and two adjacent water molecules. Nineteen water-solute interactions are identified within the QTAIM framework, as well as twenty-seven water-water interactions (as a comparison, thirty-five hydrogen bondings were identified in the most stable $(\text{H}_2\text{O})_{20}$ isomer³⁹). Finally, the complex is characterized by a very high encapsulation energy ($E_{\text{encapsulation}} = -101.3 \text{ kJ.mol}^{-1}$). The value of D0 calculated from the most stable $(\text{H}_2\text{O})_{20}$ isomer (edge-sharing) is also strongly negative ($D0 = -53.1 \text{ kJ.mol}^{-1}$). This shows that the breakdown of the network of hydrogen bonds between water molecules for encapsulation of amphiphilic solute is energetically favorable. Another isomer without breakage of the hydrogen bonding network was obtained, but the size of the cavity was notably bigger than the of the empty cage. This results in a slight destabilization of the structure ($D0 = -38.3 \text{ kJ.mol}^{-1}$, Structure 1_20_2 in Supporting Information)

The insertion of the TMAO into the $(\text{H}_2\text{O})_{24}$ cage does not lead to any breakage of the hydrogen bond between the water molecules. The QTAIM analysis identifies three very strong interactions between water molecules and the oxygen atom of the TMAO ($\rho(r) = 0.052$). Eighteen other water-solute interactions involving the hydrogen atoms of the TMAO are also identified, as well as thirty-six interactions between water molecules (See Supporting Information). The encapsulation energy is even higher than in the case of the $\text{TMAO} @ (\text{H}_2\text{O})_{20}$ complex, with $E_{\text{encapsulation}} = -150.5 \text{ kJ.mol}^{-1}$. Furthermore, comparing to the most stable $(\text{H}_2\text{O})_{24}$ isomer, the binding energy for the $\text{TMAO} @ (\text{H}_2\text{O})_{24}$ complex is particularly favorable to the encapsulation, with $D_0 = -70.9 \text{ kJ.mol}^{-1}$.

Discussion

Fifty one $\text{TMAO}:(\text{H}_2\text{O})_n$ complexes were finally investigated. To better understand the origin of the stability of the various isomers identified, we sought to determine the relative influence of intermolecular water-water, water-solute interactions with the hydrophilic part of the TMAO, and water-solute interactions with the hydrophobic part of the TMAO. To this end, all intermolecular BCPs were considered, and it was assumed that the sum of electron density at these intermolecular BCPs was a good indicator of the strength of interactions. To validate this assumption, the sum of $\Sigma\rho$ at intermolecular BCPs was plotted as a function of the interaction energy (Figure 6A and Supporting Information S10). The linear relationship observed confirms that intermolecular BCPs may be used as quantitative indicators of the strength of the interactions.

In the most stable isomers (Figure 6B), hydrophilic interactions dominate: for complexes containing up to 4 water molecules, water-soluble interactions with the hydrophilic part of the TMAO are dominant. For complexes containing more than five water molecules, water-water interactions become dominant in the most stable isomers. They are maximum for the $\text{TMAO}:(\text{H}_2\text{O})_{20}$ complexes, where they represent more than 80% of intermolecular interactions. It should be noted that in all these most stable isomers, there is a significant fraction of water-solute interaction with the hydrophobic part of the TMAO. For small complexes, we obtained isomers for which the water-solute interaction fraction with the hydrophobic part of the TMAO was significant. These isomers are never the most stable, but they could play a role in certain chemical processes, or be observed experimentally under certain conditions. Figure 6C shows how the intermolecular interactions are distributed in these complexes. As the size of the complexes increases, the fraction of these interactions decreases, particularly in favor of water-water interactions. This may be explained by the fact that most of these isomers involve a water cluster interacts with the solute that have been obtained, with little disruption of the hydrogen bonding network between the water molecules. Figure 6D shows the distribution of interactions in isomers for which hydrophilic water-solute interactions are the highest. For complexes containing up to three water

molecules, these are the most stable isomers, and the fraction of these interactions is more than 60%. From the pentahydrated complex, the fraction of these hydrophilic water-solute interactions decreases, with a concomitant increase of water-water interactions.

Figure 6: Characterization of the intermolecular interactions in the TMAO:(H₂O)_n complexes, 1 ≤ n ≤ 24. More data are given in Supporting Information S10.

Thus, all three types of intermolecular interactions are important in these complexes. Not surprisingly, the isomers that maximize hydrophilic water-water or water-solute interactions are the most stable. However, in all the most stable isomers obtained, there is also a fraction (at least 10%) of hydrophobic interactions, even in the largest complexes.

Finally, we also sought to determine whether it was possible to predict the minimum cage for encapsulation of TMAO from the study of the smallest isomers, and more precisely the isomers of the monohydrate complex. Hydration of hydrophobic solutions is generally considered to be governed by the existence of an exclusion volume around the solute, into

which water molecules cannot penetrate. The study of the distance between the water molecules and the solute in the S1, S2 isomers as well as in the two transition states TS1 and TS2 could therefore provide information on the minimum size of a water cage allowing complete encapsulation of the solute.

Figure 7A shows all the monohydrate isomers (minimum and TS) superimposed, taking into account the symmetry of the solute. The distances between oxygen atoms of the water molecules and the nitrogen atom have been reported: they are 3.16 Å for S1, 3.76 Å for S2, 3.94 Å for TS2 and 4.61 Å for TS1. The diameter of the (H₂O)₂₀ water cage (Figure 7B) is 7.6 Å. The cavity of the (H₂O)₂₄ water cage (Figure 7C) has a rectangular parallelepiped shape of 7.6 x 6.0 x 9.1 size. An "exclusion volume" for water molecules around the TMAO can be defined, and superimposed on the two water cages (Figure 7D and 7E). The volume fits in the (H₂O)₂₄ cage but not in the (H₂O)₂₀ cage. It may thus be hypothesized, from the study of the geometric features of the TMAO:(H₂O) isomers, that the (H₂O)₂₀ cage is too small to encapsulate the TMAO, unlike the (H₂O)₂₄ cage. This indeed corresponds to what is obtained after geometry optimizations of the TMAO@(H₂O)_{20,24} species: the encapsulation of the TMAO in the (H₂O)₂₀ cage leads to the rupture of the hydrogen bonding network between water molecules and the opening of the cage. On the other hand, the hydrogen bonding network between water molecules is maintained in the TMAO@(H₂O)₂₄ complex.

Figure 7: Comparison of water-solute distances in isomers S1, S2, TS1 and TS2 with the sizes of the water cages (H₂O)₂₀ and (H₂O)₂₄ to understand the minimal size of the water cage enabling the encapsulation of the solute (See text for more details).

Conclusion

The MESP analysis of isolated solutes makes it possible to predict and explain the structures of the most favorable TMAO:(H₂O)_n isomers. The oxygen atom of the TMAO is a particularly nucleophilic site, particularly suitable for acting as an hydrogen bond acceptor. In addition, the QTAIM analysis of hydrated complexes reveals that water molecules acting as hydrogen bond donors to the oxygen atom of the TMAO may be involved in other hydrogen bond interactions as hydrogen bond acceptors. A homodromic network of hydrogen bonds is thus formed,⁴⁰ which is known to generally stabilize the complexes.⁴¹ The most stable isomer for the TMAO:(H₂O)₃ isomer was already identified in the literature: it is composed of three water molecules solely interacting with the TMAO. Such a structure significantly differ to "water segregation" ³⁰⁻³⁷ reported in the literature for the micro-hydration of several organic solutes. The presence of a stronger nucleophilic site on the TMAO than the oxygen atom of the water molecule may explain that the most stable isomer of the TMAO:(H₂O)₃ complex does not involve "water segregation". As the number of water molecule in the complex increases, water-water interactions are also formed in the most stable isomers identified in the present study. The QTAIM analysis of the intermolecular interactions suggests that, from n=6, water-water interactions overtake water-solute interactions in strength. However, unlike what is observed with some other solutes, the most stable isomers of TMAO:(H₂O)₆₋₉ complexes does not consist of the most stable (H₂O)₆₋₉ isomers slightly deformed and interacting with the solute. Instead, the arrangement of water molecules in the most stable TMAO:(H₂O)_n isomers is notably different than the ones in the isolated (H₂O)_n clusters.

The most stable isomer for the TMAO:(H₂O)₅ complex can be considered as the beginning of encapsulation (Figure 8): if we assimilate, as a first approximation, the TMAO molecule to a sphere centered on the nitrogen atom, about a fourth of this sphere is covered by an incomplete water layer. It is interesting to further notice that the beginnings of this encapsulation follow a spheroid arrangement of water molecules, with a diameter close to 9 Å in the equatorial axis (Figure 8).

Figure 8: The most stable isomer of the TMAO:(H₂O)₅ complex can be seen as the beginning of the encapsulation of the solute.

These characteristics are in line with those of the water cage which allows the encapsulation of the TMAO without breaking water-water interactions: the solute can be encapsulated within a (H₂O)₂₄ cage. This 5¹²6² water cage has a spheroid shape with a diameter of 9.1 Å in the equatorial axis and 6.0 Å in the axial axis. Conversely, when the TMAO molecule is inserted inside the smaller 5¹² water cage, some hydrogen bondings between water molecules are broken.

Thus, some small TMAO:(H₂O)_n complexes share some geometric features with the smaller water cage allowing a total encapsulation of the solute. This geometric similarity paves the way for a question: would it be possible to predict the structure of a water cage that can encapsulate the solute from complexes as small as TMAO:(H₂O)? The preliminary study presented here suggests that an extrapolation from the structure of the different forms (minima and TS) involving a single water molecule around the solute could provide initial clues as to the minimum approach distance of a water molecule, and therefore the minimum size of a water box that would be able to host the solute.

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