J Solution Chem (2011) 40: 656–679
DOI 10.1007/s10953-011-9672-8

Hydration Structure of Cocaine and its Metabolites: A Molecular Dynamics Study

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Received: 6 May 2010 / Accepted: 30 August 2010 / Published online: 18 March 2011

Abstract We present molecular dynamics simulations of solvated cocaine and its metabolites in water, using both the Optimized Potentials for Liquid Simulations (OPLS) force field and the same force field but with Quantum Theory of Atoms In Molecules (QTAIM) atomic charges. We focus on the microscopic aspects of solvation, e.g. hydrogen bonds, and investigate influence of partial charges applied. Hydrophobicity or hydrophicility of these molecules were analyzed in terms of solute–solvent radial distribution functions and, for their most hydrophilic atoms, by spatial density functions. These hydration studies allowed us to classify these molecules according to their total coordination numbers, from the most hydrated metabolite to least hydrated, and this trend matches the degree of each metabolite is excreted in urine of patients with a high consumption of cocaine. Finally, we observed that QTAIM charges provide a more physically reasonable description of electrostatic environment of these solvated molecules than those of OPLS charges.

Keywords Cocaine · Cocaine metabolites · Hydration structure · OPLS · QTAIM charges

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1 Introduction

The (−)-cocaine (hereafter cocaine), a tropane alkaloid extracted from the leaves of the *Erythroxylum coca*, is one of the most widely used drugs of abuse. When sniffed, smoked, swallowed or applied to mucus membrane, cocaine is absorbed from all sites of exposure. It can also be taken intravenously. The onset of action of cocaine depends on its mode of intake, e.g., approximately 30 min for snorting or inhalation and 1–2 min for an intravenous injection. The elimination half-life of cocaine is approximately 40–60 min, except at very high doses [1]. It is metabolized by plasma and liver cholinesterases to water-soluble metabolites that are excreted in the urine [2].

Cocaine (COC) is rapidly metabolized in vivo by esterases with the concomitant hydrolysis of its ester groups, to produce ecgonine methyl ester (ECGME), ecgonine ethyl ester (ECGET) and benzoylecgonine (ECGBE) [3–12]. Cocaine is also biotransformed to norcocaine (NORCO) and to norecgonine methyl ester (NOREC) by a N-demethylation process [13]. However, when cocaine is consumed along with ethanol, a new metabolite is then produced by transesterification, cocaine ethyl ester (COCET) [14], which has a much longer plasma half-life (3.5 to 5.5 h) compared to cocaine.

Cocaine is one of the most reinforcing and addictive compounds ever studied [15, 16]. Its addiction is believed to result from the inhibition of dopamine uptake by the binding of cocaine to a specific recognition site located on the dopamine transporters. This inhibition results in elevated extracellular levels of dopamine that are believed to be responsible for the euphoria and addictive properties [17, 18]. This kind of neurotransmitter transporters and receptors can be dramatically disturbed by cocaine and its metabolites, in different degrees of binding/potency and selectivity, mainly ruled by the stereoselectivity [15]. These properties in turn can be strongly influenced by the type of substitution and hydration around the tropane ring. Thus, the study of the hydrophobicity of the tropane ring substituents of cocaine and its metabolites could be a valuable tool in interpreting the phenomenon in terms of the molecular mechanisms. In fact, research advances in this area are a prerequisite for understanding pharmaco/toxico-kinetics and pharmacogenetic variations [19].

The present work reports a detailed molecular dynamics (MD) simulation study of cocaine and its metabolites in aqueous solution, with the aim of describing their different solvation characteristics. Our results give useful insights for the classification of these substances based on their relative hydrophobicity. Moreover, we wish to determine how the values obtained for such properties are modified when the charge parameters of standard force fields are replaced by those derived from the Quantum Theory of Atoms in Molecules (QTAIM) [20]. Thus, we have carried out two sets of simulations, one using the charges of the OPLS potential [21] and another using the derived QTAIM charges obtained by separate density functional theory (DFT) calculations. We should remark here that the OPLS parameters have been optimized to fit experimental properties of liquids, such as density and heat of vaporization, in addition to fitting gas-phase properties determined by Quantum Mechanical (QM) calculations. Normally, QM−Mulliken charges are further optimized to fit experimental properties of the species [22]. In contrast, QTAIM charges are obtained by numerical integration of the molecular electron density, $\rho(\boldsymbol{r})$, within the atomic domain defined by a $\rho(\boldsymbol{r})$ isosurface and zero flux surface for the vector field of $\nabla\rho(\boldsymbol{r})$. Moreover, the integration of the density function within the same atomic domain provides the corresponding atomic properties (energy, dipole moment, informational entropy, etc.). It has been observed that the values of any of these atomic properties are nearly transferable within similar chemical environments. Thus, atoms make recognizable contributions to the total properties of a system, which can be reported as being additive and nearly transferable between molecules [23].

This paper is organized as follows: Sect. 2 details the computational methods used, whereas Sect. 3 presents the results and Sect. 4 summarizes the main conclusions of this work. To our knowledge, there is no previous work that investigates the microscopic and dynamics properties of cocaine and its metabolites in aqueous solution.

2 Computational Methods

All metabolites of cocaine share the 8-azabicyclo[3.2.1]octane-2-acid/alkyl-ester /carboxylate anion structure (Fig. 1). They only differ in the substitutuent groups at positions 2 and 3, and on the nitrogen, as shown in Table 1.

Under physiologic conditions (pH = 7.4), only a small amount of norcocaine or norecgonine methyl ester would be protonated at the amine group [24, 25] and benzoylecgonine would be a zwitterionic molecule [26, 27] while cocaine and the remaining metabolites mainly exist as the corresponding protonated amines. For this reason, the former species were considered in the base form and attributed an overall zero charge (Table 1).

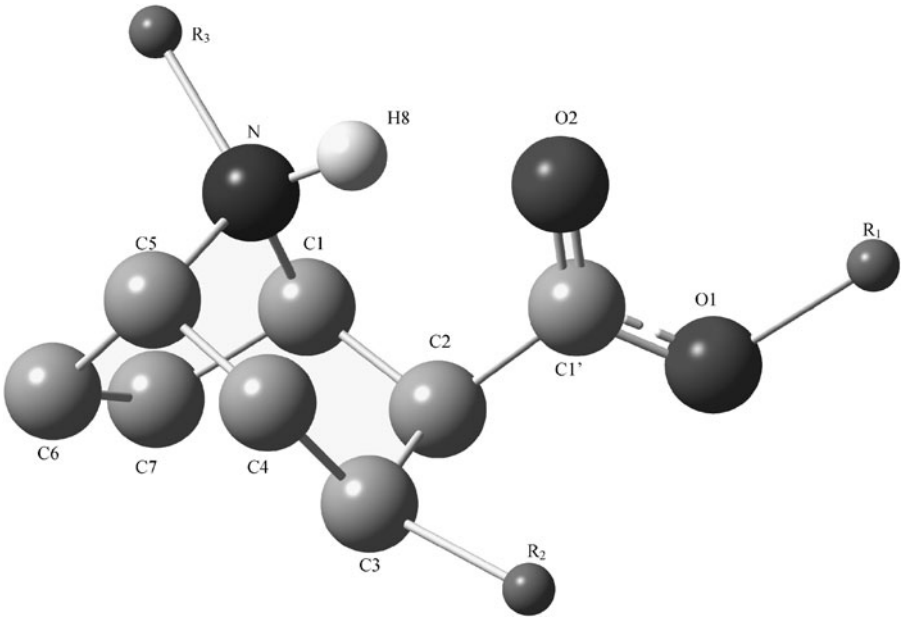
2.1 Quantum Mechanical Calculations

The optimized geometries of cocaine and its metabolites were obtained by DFT calculations with the standard 6-31G** basis set. B3LYP Hamiltonian with Becke exchange [28] and Lee–Yang–Parr [29] correlation functionals were employed as implemented in the GAUSSIAN03 package [30]. In addition, solvent effects have been considered by performing single-point energy calculations using the polarizable continuum model (PCM) of Tomasi and co-workers [31–33]. The default dielectric constant for water was used with the PCM procedure. Harmonic frequencies were computed at the fully-optimized geometries, allowing the assignment of stationary points as true minima [34].

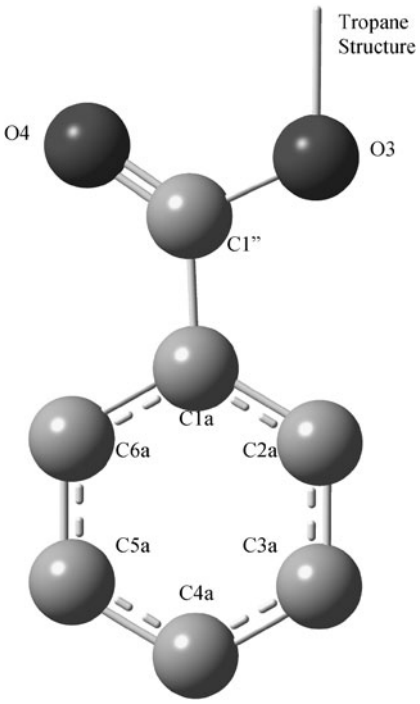
The electron densities of cocaine and all its metabolites were analyzed within the framework of QTAIM [20], using the AIMPAC [35] program, by resorting to single-point PCM energy calculations, along with the B3LYP/6-311++G(d, p) 6d basis set, on top of the optimized geometries described above. This analysis provided the atomic charges, which were then used in a set of our MD simulations.

2.2 Molecular Dynamics Simulations

The MD simulations were carried out using version 3.3 of the GROMACS software package [36–39]. The equations of motion were integrated with the Verlet leapfrog algorithm [40], using a time step of 2 fs, and all the bond lengths were constrained by applying the LINCS algorithm [41]. The temperature was kept fixed at 298.15 K using the Nose–Hoover (NH) thermostat [42, 43] and the pressure (p) was held constant at 1 bar by the Parrinello–Rahman (PR) coupling scheme [44]. The NH scheme resorted to a coupling constant of 1 ps; for the PR scheme a coupling constant of 1.0 ps and an isothermal compressibility of 4.5×10^{-5} bar^{-1} were used. Periodic boundary conditions were applied in all three Cartesian directions. The particle-mesh Ewald method [45, 46] was applied to deal with the long-range electrostatic forces, whereas the Lennard–Jones (L-J) interactions were handled using a twin range cut-off. Both the real-space part of the Ewald sum and the short-range L-J interactions, up to a distance of 1.0 nm, were calculated with the help of a neighbor list. In addition, a long-range dispersion correction was applied to both energy and pressure.



(a)



(b)

Fig. 1 The molecular structure and numbering for the 8-azabicyclo[3.2.1]octane-2-acid/alkyl-ester structure, which is shared by all metabolites of cocaine. Structure and numbering of the benzoyloxy substituent is also shown (hydrogens not shown except that of amine)

Table 1 Substituents on the 8-azabicyclo[3.2.1]octane-2-acid/alkyl-ester structure for cocaine and the diverse metabolites (with their acronyms) studied here

Molecule	Acronym	R_1	R_2	R_3	Overall charge
Cocaine	COC	Methyl	Benzoyloxy	Methyl	+1
Ethyl cocaine	COCET	Ethyl	Benzoyloxy	Methyl	+1
Ecgonine methyl ester	ECGME	Methyl	Hydroxyl	Methyl	+1
Ecgonine ethyl ester	ECGET	Ethyl	Hydroxyl	Methyl	+1
Benzoyloxy ecgonine	ECGBE	None	Benzoyloxy	Methyl	0[a]
Norcocaine	NORCO	Methyl	Benzoyloxy	None	0
Norecgonine methyl ester	NOREC	Methyl	Hydroxyl	None	0

[a]The total charge is zero, because it is a zwitterionic species which has both a positively charged and a negatively charged group

The force field used in our simulations included a harmonic angle-bending term, a torsional term of the Ryckaert–Bellemans form, a Lennard–Jones term for short-range dispersive and repulsive interactions, and a Coulomb term for point-charge electrostatics. In all simulation runs, water was modeled by the SPC/E potential [47]. Cocaine and its metabolites were modeled by the OPLS force field [21], save for the point charges: in the first set of runs, the standard OPLS charges were employed, while in the second set, we used the derived QTAIM charges. Solvation properties of these metabolites were calculated from simulations in the NPT ensemble of a single solute molecule dissolved in about 900 water molecules. After an energy minimization of at least 1000 steepest descent steps, the systems were equilibrated in the NPT ensemble until all observables fluctuated around their equilibrium values. Therefore, the equilibrium runs were performed for at least 1.0 ns. Finally, production runs of 10.0 ns were performed for data collection. In addition, we considered the influence of using two different types of charge definition, default OPLS charges and QTAIM ones, on the solvation behavior of the more electronegative atoms, as was outlined above. Notice that the internal and intermolecular interactions, e.g. the energy of the system due to the bonded and non bonded interactions, were not studied in detail in this paper. Also, radial distribution functions (RDFs) relative to the water atoms were determined for the most charged atoms of each metabolite in order to classify them in terms of their coordination numbers (N_{A-B}), computed taking into account the position of the first minimum of the RDFs. These RDFs are denoted as $gA-B(r)$, where A and B represent, respectively, the atoms of the species under study and of water.

3 Results and Discussion

In the following, we will discuss the differences between various properties calculated for different systems. We will focus first on the standard OPLS model, comparing the local solvation structure of cocaine and its metabolites by analyzing RDFs involving atoms of different substituent groups, and classifying these atoms according to their degree of hydration. After that, we will turn our attention to the models using QTAIM charges. We discuss the magnitude of the charges localized on each atom, as well as their influence on the RDFs, the coordination number of the most polarized atoms, and the total hydration of each metabolite. Finally we will analyze the 3D local structure around the most hydrophilic atoms by resorting to spatial density distribution functions.

3.1 Solvation Structure with the OPLS Model

3.1.1 Local Solvation Structure

Substituent on $\mathbf{R}_1$ Radial distribution functions for the carboxylic O2 relative to water hydrogen (HW) atoms (Fig. 2b) show a clear first-neighbor peak for all metabolites, which appears at nearly the same distance (0.2 nm) for both cationic and neutral metabolites. In the case of O1, however, peaks are only present for ECGBE (Fig. 2a), because in this metabolite the oxygen belongs to a carboxylic group, and hence is much more polar than that in the other metabolites where O1 is part of an ester group. Notice that the ECGBE peak for O1 is smaller than for O2, even though the OPLS charge on both atoms is the same. The difference is explained by a strong intramolecular hydrogen bond between O1 and H8. The relative distance between both oxygens and H8 is shown in Fig. 3, where it can be seen that O1

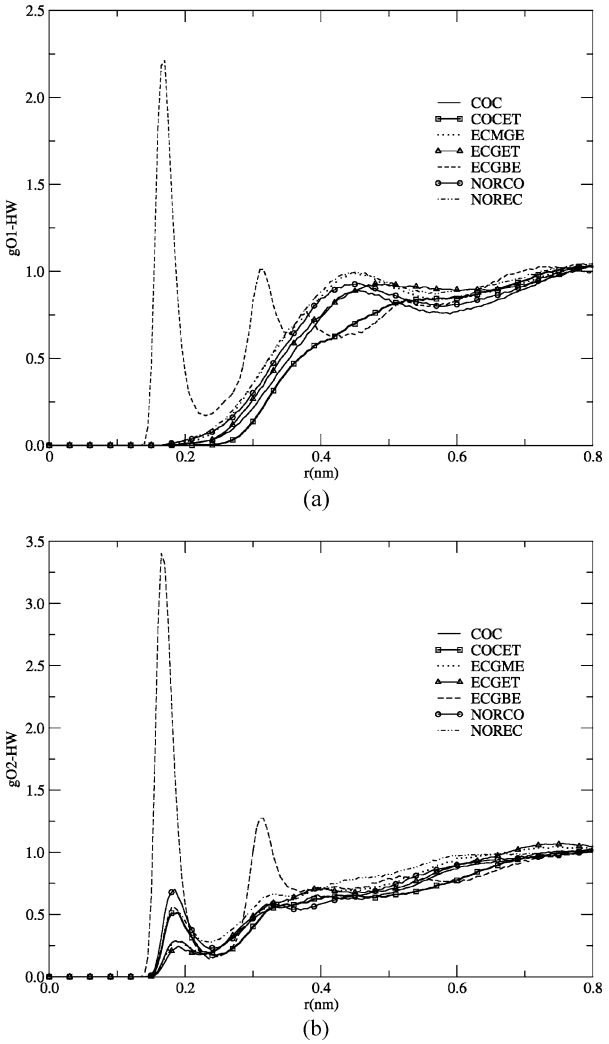


Fig. 2 The radial distribution functions between water and the most polar atoms of cocaine and its metabolites obtained with OPLS charges. Oxygens of the carboxylic group bonded to C2: (**a**) O1−HW and (**b**) O2−HW

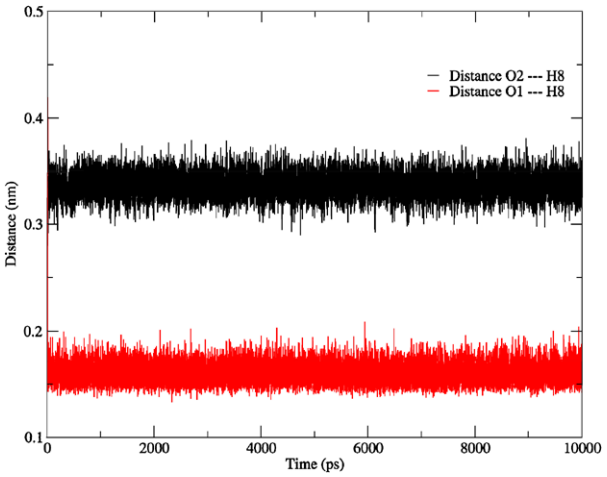


Fig. 3 The distances between the two carboxylate oxygens (O1, *bottom curve* and O2, *top curve*) and the amine hydrogen (H8) of ECGBE along the entire simulation run with QTAIM charges

forms a hydrogen bond during the entire simulation run, while O2 is further away from H8, and free to be hydrated by more water molecules.

The O2 peak height (Fig. 2b) increases with the number of carbons on R_1—the height of the peak of COCET is higher than that of COC and the same trend is observed for ECGET and ECGME. This is caused by the steric hindrance of the ethyl group, which reduces the hydration of O1 (Fig. 2a), strengthens the H8$\cdots$O1 intramolecular interaction (see Sect. 3.2.4 for a discussion of this effect) and favors the hydration of O2.

Substituent on R_2 The substituent on this oxygen differentiates cocaine and its metabolites into two groups, those with a hydroxyl substituent at the R_2 position (ECGME, ECGET and NOREC) and those with a benzoyl substituent at the same position (COC, COCET, ECGBE and NORCO). The RDF for O3 (Fig. 4a) reflects this classification clearly; the first group has well-defined peaks and the second one has not. This behavior is due to hydroxyl oxygens being more negatively charged than methoxyl oxygens in ester groups, which means that hydroxyl oxygens are significantly more hydrophilic. Indeed, the RDFs of Fig. 4a show evidence of strong O3$-$HW bonds for ECGME, ECGET and NOREC. Strong hydrogen bonds are also formed between the hydroxyl hydrogens of those metabolites and water oxygens (OW), as shown in Fig. 4b. For these metabolites, we also observe that the peaks for gH3$-$OW are higher than those for gO3$-$HW, revealing that hydrogen is more likely to be hydrated than oxygen in the hydroxyl group. In the case of the highly hydrophilic hydroxyl hydrogen (ECGME, ECGET and NOREC), gH3$-$OW exhibits a fairly well developed second neighbor hydrogen peak (Fig. 4b), which arises due to a proximity effect from the other hydrogen atoms of the H-bonded water molecules. RDFs related to O4 (Fig. 4c) calculated for all metabolites with the benzoyl substituent present peaks of ca. 0.5 height at 0.18 nm.

Substituent on R_3 In the case of the amine group, the RDFs of water hydrogens around the nitrogen (Fig. 5a) do not show a strong short-range peak for COC, COCET, ECGME, ECGET and ECGBE, due mostly to the low charge on the N atom in these cationic molecules, but also to the steric hindrance caused by the presence of the methyl group at the R_3 position. Also, ECGBE does not present any peak in the RDFs of the amine group due to the strong H8$\cdots$O1 interactions. On the contrary, there is a clear peak for NORCO and NOREC, which are charged-neutral and do not have a methyl substituent. This

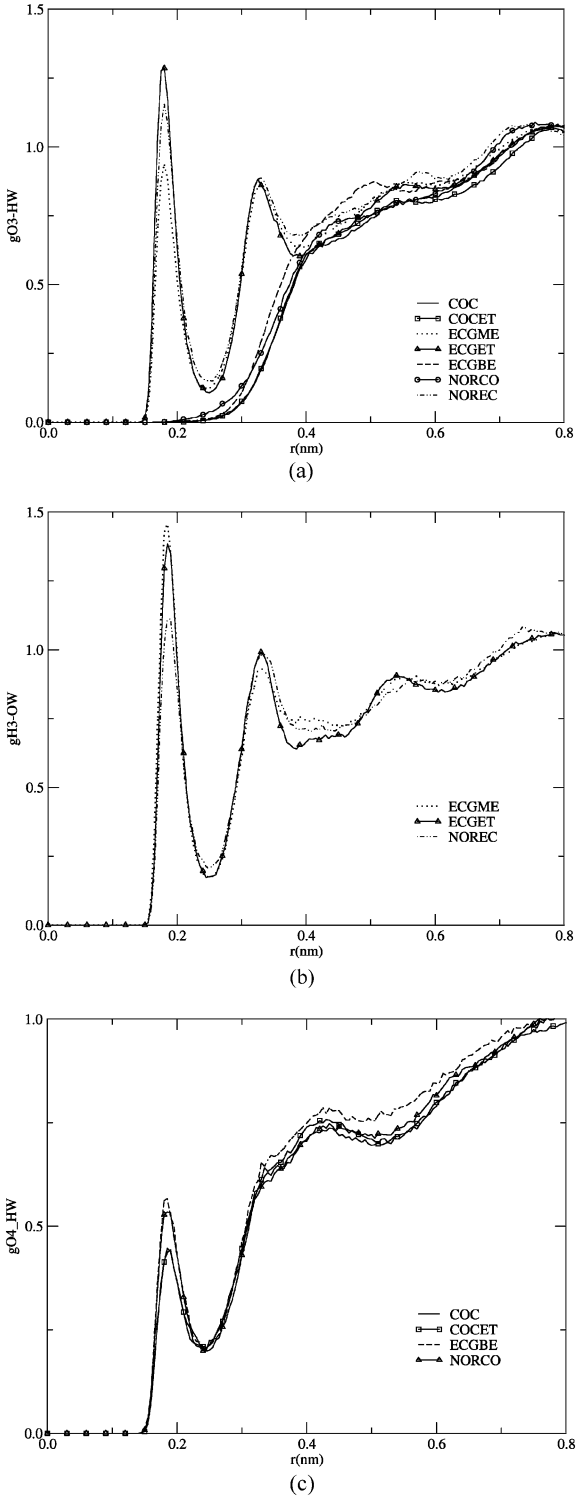


Fig. 4 The radial distribution functions between water and most polar atoms of cocaine and its metabolites with OPLS charges. Atoms of the benzoyl and hydroxyl groups: (**a**) O3−HW, (**b**) H3−OW and (**c**) O4−HW

peak is evidence of hydrogen bond formation between nitrogen and water hydrogens. The N−OW RDFs shown in Fig. 5b exhibit peaks with a height around unity at ca. 0.3 nm for all metabolites. However, these peaks have different origins: for the cationic metabolites, they reflect the strong H8−OW hydrogen bonds, whereas for the neutral metabolites, they reflect N−HW hydrogen bonds. The RDF between H8 and water oxygens (Fig. 5c) shows a strong peak at 0.2 nm for all cationic metabolites. This demonstrates the existence of H-bonds between these atoms. However, the two neutral metabolites (NORCO and NOREC) do not show these strong interactions, as can be observed by looking at gN−HW. This behavior means that in neutral metabolites the nitrogen is more likely to be hydrated by water molecules and in cationic metabolites the amine hydrogen is the preferred hydration site.

3.1.2 Classification of Atoms According to the Degree of Hydration

An analysis of the solute–solvent RDFs of cocaine and its metabolites indicates the existence of three main classes of atoms whose general behavior within each class is quite similar.

The first class contains very hydrophilic atoms: oxygens of the carboxylate anion (ECGBE, Fig. 2), amine hydrogen (COC, COCET, ECGME, ECGET, Fig. 5c) and hydroxyl hydrogen in the R_2 position (ECGME. ECGET, NOREC, Fig. 4b), which form strong hydrogen bonds. The very high first neighbor peaks in the RDFs involving atoms from this first class provide direct evidence of their high hydrophilicity and their strong interactions with water molecules. The gA−B(r) functions for most of these highly hydrophilic atoms also exhibit fairly well-developed second neighbor peaks that arise due to a proximity effect from the other hydrogen atoms in the H-bonded water molecules.

The second class consists of solute atoms that are surrounded by large groups, which exert a noticeable steric hindrance, but can still form hydrogen bonds with water. This class contains nitrogen (NORCO, NOREC, Fig. 5a) and oxygens of ester groups (O2 in all the metabolites except ECGBE (Fig. 2b) and O4 in COC, COCET, ECGBE and NORCO; Fig. 4c). The majority of these atoms are hydrophilic, as is evident from the existence of first neighbor peaks in gA−B(r) (e.g., Fig. 4a). However, a few members of this class appear to be hydrophobic because the first neighbor peak height in their gA−B(r) functions is only around 0.5 (Figs. 2b and 4c). This happens due to steric hindrances from hydrophobic groups that significantly limit the ability of water to form a hydrogen bond with these atoms. In general we observe that the atoms of the first class display peaks in the corresponding gA−B(r) functions, whose heights are more than double of those corresponding to the second class.

The third class comprises atoms that are incapable of forming hydrogen bonds with water but instead interact with the solvent through dispersion and electrostatic forces. i.e. N in COC, COCET, ECGME, ECGET and ECGBE (Fig. 5a), H8 in ECGBE, NORCO and NOREC (Fig. 5c), O1 in all metabolites except ECGBE (Fig. 2a), and O3 in COC, COCET, ECGBE and NORCO (Fig. 4a). Generally, the atoms of this class are mostly hydrophobic.

3.1.3 Substituent Effects

Another important observation emerging from inspection of the RDFs is how the degree of hydrophilicity of certain atoms is affected by the substituents in their neighborhood. The effect of each substituent group may be analyzed by comparing the RDFs of cocaine and its metabolites that vary in only one substituent. For example, an increase in the length of the alkyl chain at R_1 position causes an increase in hydrophilicity of the amine hydrogen. This is manifested by an increase in height of the H8−OW peak as we move from ECGME to ECGET (Fig. 5c).

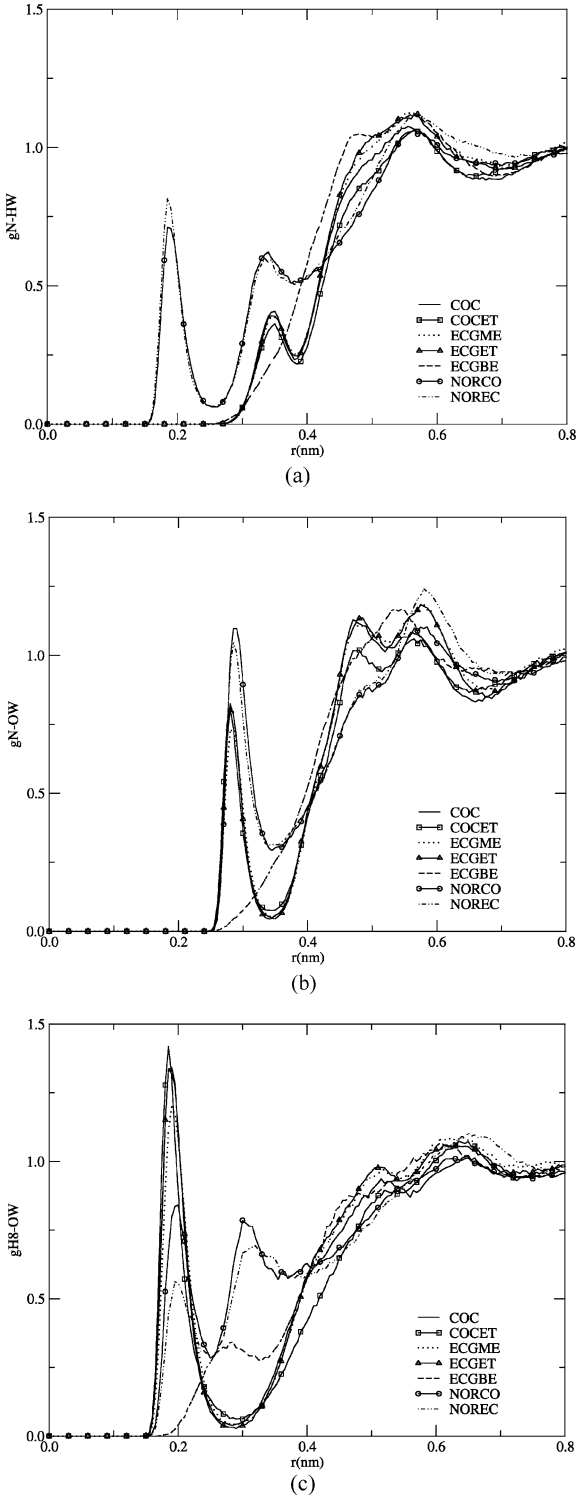


Fig. 5 The radial distribution functions between water and most polar atoms of cocaine and its metabolites with OPLS charges. Atoms of the amine group: (**a**) N−HW, (**b**) N−OW and (**c**) H8−OW

The presence of a benzoyl substituent instead of a hydroxyl substituent at the R_2 position causes, in general, a decrease in the hydrophilicity of the O2 atom (compare RDFs in Fig. 2b between COC and ECGME, COCET and ECGET, NORCO and NOREC). It also induces an increase in the hydrophilicity of the amine hydrogen, since the height of this peak (Fig. 5c) increases as we move from ECGET to COCET, from ECGME to COC, and from NOREC to NORCO. An opposite trend is observed for the nitrogen: it becomes more hydrophobic when a benzoyl substituent is present (compare RDFs for NORCO and NOREC in Fig. 5a).

Finally, the effect of the presence of a methyl group at the R_3 position can be assessed by comparing the distributions for COC and NORCO, and also those for ECGME and NOREC. In this case, we can observe that the methyl substituent induces a decrease in the hydrophilicity of the O2 atom (increase in the height of the peak in Fig. 2b from COC to NORCO and from ECGME to NOREC), of the O3 atom (compare curves for ECGME and NOREC in Fig. 4a) and of the O4 atom (compare curves for COC and NORCO in Fig. 4c).

Thus, the overall hydrophilicity of a given atom depends on a combination of local effects (the functional group to which that atom belongs) and the effects described above (substituents on neighboring groups). For example, the amine hydrogen becomes less hydrophilic when a methyl group is no longer connected to nitrogen (local effect), and its relative degree of hydrophilicity is also influenced by the type of substituent at the R_2 position (substituent effect). Similarly, the O3 becomes hydrophilic when it belongs to a hydroxyl rather than to an ester group, with the degree of hydrophilicity dictated by the nature of the R_2 and R_3 substituents. Also for amine hydrogen (Fig. 5c), the major effect is local (shifted peaks for NORCO and NOREC relative to the other metabolites) with relative peak heights controlled by the substituent effect of both R_1 and R_2.

3.2 Solvation Structure with QTAIM Charges

3.2.1 Charges

The QTAIM and OPLS charges differ in the conceptual way of defining them. The QTAIM charges, defined by a boundary condition associated to the topology of a molecular charge distribution, are obtained through partitioning the molecular system into a set of disjointed spatial regions, each region containing in general a single nucleus, as detailed above. On the other hand, the OPLS charges have been adjusted to fit thermodynamic properties, not to represent the charge density as well as possible. Herein, we will show that this leads to different QTAIM and OPLS charges for all the atoms of cocaine and its metabolites. These, in turn, are better understood if we differentiate and analyze separately the metabolites with an aminic methyl group and without it.

The QTAIM analysis of amine, carboxylic ester/acid/carboxylate anion and hydroxyl groups of cocaine and its metabolites was the subject of previous studies [27, 34], in which it was found that: (i) the positive charge of cocaine and its metabolites is shared among the amino hydrogen, the atoms of the methylamino group, and all of the hydrogens attached to the bicycle structure; (ii) the zwitterionic structure of benzoylecgonine, ECGBE, can be described by two partial charges of 0.63 au, the negative one shared by the oxygens of the carboxylate group, whereas the positive charge is distributed among all the hydrogens that bear the positive charge in cocaine; (iii) the hydrogen bond, N8–H8$\cdots$O2, is strengthened in metabolites without a benzoyloxy group and is also slightly strengthened as the size of the alkyl ester group, bonded to O1, increases. These properties are reflected in the variation of QTAIM charges among cocaine and its metabolites. In the positively charged metabolites, COC, COCET, ECGME and ECGET, the absolute values of the QTAIM charges of the

most polarized atoms slightly decrease when the number of carbons of O1-substituents is reduced (from ethyl to methyl). More evident is the trend related to the presence of a benzoyl group at O3 (COC and COCET), where the oxygen charges are more negative than without this substituent (ECGME and ECGET). In contrast, the absolute charge values are larger when there is no N-methyl group (NORCO and NOREC) than when it is present (COC and ECGME). The remarkable trends are the charges of O1, O2 and amine hydrogen in the zwitterionic metabolite ECGBE, which have the highest absolute values compared to the other metabolites.

It is important to point out the noticeable difference between QTAIM and OPLS charges for the most polarized atoms of cocaine and its metabolites. The QTAIM charge values of the oxygen, nitrogen, amine and hydroxyl hydrogen atoms are remarkably different from the OPLS charges for cocaine and its principal metabolites (Table 2), which can be expected as such atoms belong to the most charged groups, that is to say, the carboxylic, amine and hydroxyl groups. The differences between the values of these charged atoms are very small, being the majority of the OPLS charges values, closer to zero than their QTAIM counterparts. This lower degree of polarization can lead to weaker hydrogen bonds in force field simulations, as reported by other researchers [48], who have scaled all atomic charges to better reproduce the experimentally observed behavior. Furthermore, the nitrogen, oxygen, amine and hydroxyl hydrogen atoms have a higher absolute QTAIM charge value than their OPLS values, as is shown in Table 2. These charge differences will very likely influence the hydration properties of cocaine and its metabolites.

3.2.2 Local Solvation Structure

The most relevant differences in the RDFs obtained with the QTAIM charges, in comparison with the OPLS ones, are presented by polarized atoms belonging to the carboxyl, hydroxyl, and amine groups. The QTAIM RDFs of O1 present well defined RDFs (Fig. 6a) for charged and non-charged metabolites, but the corresponding RDF with OPLS charges do not present the same solvated behavior, except for ECGBE (Fig. 2a). In the case of O2, well defined RDFs were obtained with both models (Figs. 2b and 6b), but the peaks obtained with the QTAIM charges are almost 6 times higher than those obtained with OPLS (with the exception of ECGBE).

A similar effect is seen for O4, where the QTAIM peaks are almost 8 times higher than those obtained with OPLS (Figs. 4c and 7c). The RDF for O3 allows us to differentiate between metabolites with and without the benzoyl group—the height of RDF peaks for metabolites without this group are almost two times higher than those that possess this substituent (Fig. 7a), but in the OPLS simulations this last group does not present any peak (Fig. 4a). The polarized atoms of the amine group present the same qualitative behavior, with peak heights for QTAIM being slightly higher than OPLS (Figs. 5 and 8). Finally, it should also be mentioned that the main peak position shifts from around 0.20 nm for OPLS to 0.18 nm for QTAIM simulations. These local solvation effects are a consequence of the atomic charge values and it is important to understand how these values modify the hydration properties for the most polarized atoms in each metabolite.

3.2.3 Coordination Numbers

We can better judge the effects of the atomic charges on the most polarized atoms of these metabolites, by comparing their coordination numbers, obtained by integrating RDFs with

Table 2 QTAIM and OPLS atomic charges for the most polarized atoms of cocaine and its metabolites

	COC		COCET		ECGME		ECGET		ECGBE		NORCO		NOREC	
	QTAIM	OPLS	QTAIM	OPLS	QTAIM	OPLS	QTAIM	OPLS	QTAIM	OPLS	QTAIM	OPLS	QTAIM	OPLS
O1	−1.048	−0.330	−1.048	−0.330	−1.034	−0.330	−1.036	−0.330	−1.149	−0.800	−1.064	−0.330	−1.048	−0.330
O2	−1.150	−0.430	−1.153	−0.430	−1.137	−0.430	−1.137	−0.430	−1.174	−0.800	−1.157	−0.430	−1.163	−0.430
O3	−1.054	−0.330	−1.055	−0.330	−1.039	−0.683	−1.040	−0.683	−1.045	−0.330	−1.045	−0.330	−1.060	−0.683
O4	−1.156	−0.430	−1.155	−0.430	[a]	[a]	[a]	[a]	−1.138	−0.430	−1.163	−0.430	[a]	[a]
N	−0.949	−0.100	−0.952	−0.100	−0.951	−0.100	−0.953	−0.100	−0.979	−0.100	−0.990	−0.780	−0.985	−0.780
H8	0.467	0.290	0.468	0.290	0.469	0.290	0.473	0.290	0.526	0.290	0.402	0.380	0.382	0.290
H3	[a]	[a]	[a]	[a]	0.558	0.418	0.557	0.418	[a]	[a]	[a]	[a]	0.597	0.418

[a]Atom not present in this metabolite

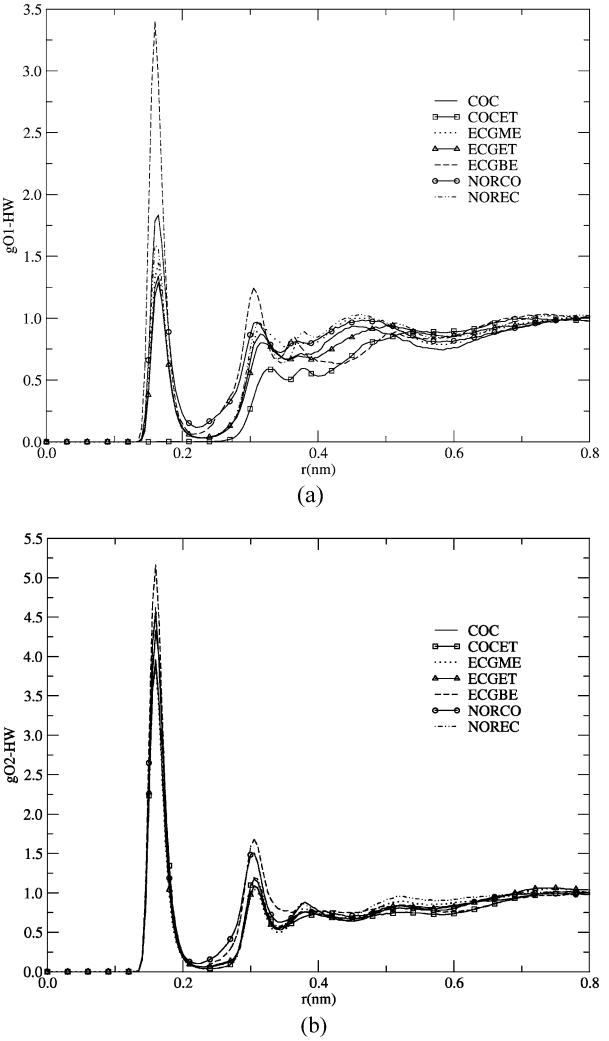
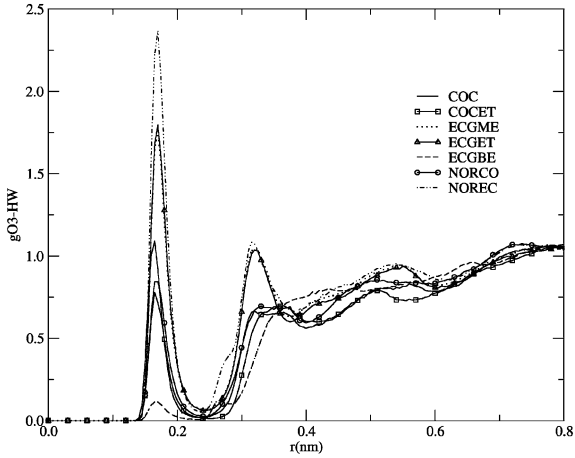


Fig. 6 The radial distribution functions between water and the most polar atoms of cocaine and its metabolites obtained with QTAIM-parameterized charges. Oxygens of the carboxylic group bonded to C2: (**a**) O1−HW and (**b**) O2−HW

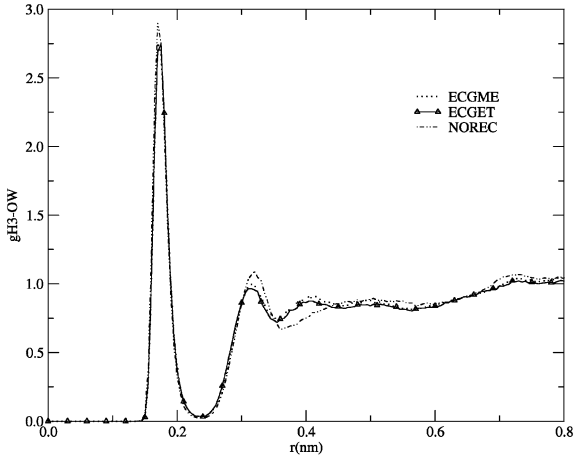
both OPLS and QTAIM charges. As seen in Table 3, such coordination numbers are significantly different, the former being much lower than the latter.

As commented above, using the OPLS charges, the hydrophilicity of the species at O2 and O4 can be classified according to the total molecular charge, i.e. the higher hydrophilicity is that of the neutral species. The orders are: ECGBE $\gg$ NORCO $>$ NOREC $>$ COCET $\gg$ COC $>$ ECGME $>$ ECGET for O2 and NORCO $>$ ECGBE $>$ COCET $>$ COC for O4. In the same vein, neutral metabolites display water molecules coordinated to N, while they hydrate H8 in cationic ones. For the hydroxyl group in ecgonine's metabolites, the hydrophilicity of its hydrogen decreases as a result of increasing the R_1 lateral chain. Finally, the carboxylic oxygen, O2, of ECGBE has the highest peak in its $g\mathrm{A{-}B}(r)$ and coordination number.

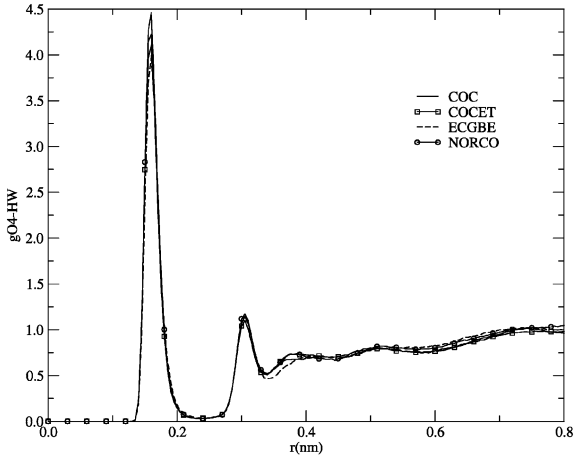
In the case of QTAIM charges, the coordination numbers of O1, O2, and O4 permit us to distinguish between neutral and positively charged species, with those atoms being more



(a)



(b)



(c)

Fig. 7 The radial distribution functions between water and the most polar atoms of cocaine and its metabolites obtained with QTAIM-parameterized charges. Atoms of the benzoyl and hydroxyl groups: (**a**) O3−HW, (**b**) H3−OW and (**c**) O4−HW

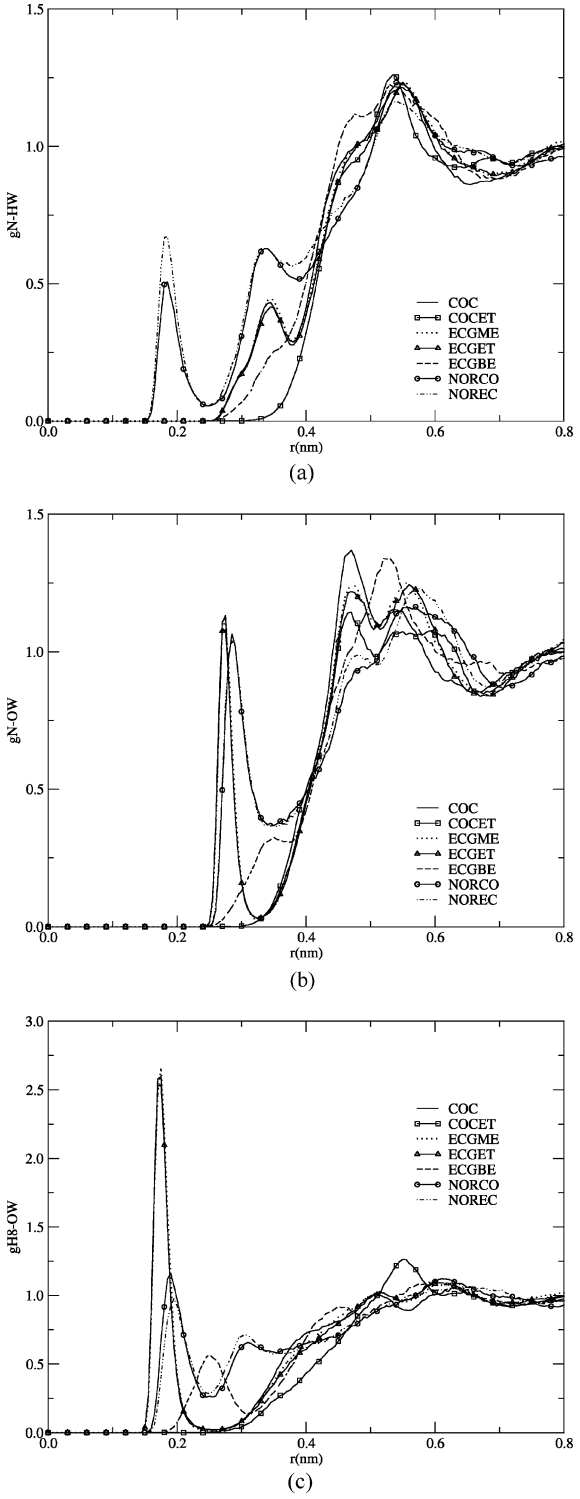


Fig. 8 The radial distribution functions between water and the most polar atoms of cocaine and its metabolites obtained with QTAIM-parameterized charges. Atoms of the amine group: (**a**) N−HW, (**b**) N−OW and (**c**) H8−OW

Table 3 Coordination numbers for the atoms of cocaine and its metabolites

Atom	COC	COCET	ECGME	ECGET	ECGBE	NORCO	NOREC
OPLS charges							
O1	[b]	[b]	[b]	[b]	0.992	[b]	[b]
O2	0.265	0.449	0.222	0.169	1.466	0.533	0.512
O3	[b]	[b]	0.598	0.781	[b]	[b]	0.797
O4	0.396	0.415	[a]	[a]	0.474	0.488	[a]
N	[b]	[b]	[b]	[b]	[b]	0.496	0.629
H8	1.012	1.009	0.979	0.995	[b]	0.790	0.515
H3	[a]	[a]	0.929	0.925	[a]	[a]	0.843
QTAIM charges							
O1	0.465	[b]	0.506	0.462	1.025	0.715	0.537
O2	1.205	1.360	1.184	1.198	1.560	1.422	1.399
O3	0.380	0.315	0.759	0.760	0.049	0.386	0.937
O4	1.259	1.203	[a]	[a]	1.271	1.323	[a]
N	[b]	[b]	[b]	[b]	[b]	0.328	0.398
H8	0.975	[b]	0.993	0.950	[b]	0.962	0.834
H3	[a]	[a]	0.995	0.988	[a]	[a]	0.994

[a]Atom not present in this metabolite

[b]No peak found for this atom in its corresponding $g\mathrm{A-B}(r)$

hydrated in the former species. Moreover, it is important to point out that the coordination numbers for O1 in COCET and ECGBE have the smallest and the largest values, respectively. This is due to a steric hindrance of the ethyl group at O1 in the former, and to the electronegative charges of the carboxylate oxygens in the latter. Also, the hydrophilicity of O2 is slightly decreased by the number of carbons of R_1. The hydrophilicity for O3 is classified according to the R_2 substituent, i.e. the coordination number for O3 is higher in metabolites without a benzoyl group, NOREC > ECGET > ECGME, than with it, NORCO > COC > COCET. For the amine group, water molecules prefer to hydrate N in NORCO and NOREC, and H8 in the other metabolites.

Figure 9 gives an overview of all coordination numbers per metabolite. It can be seen that the coordination numbers for the OPLS-charged atoms are almost half of the QTAIM values. The reason is that the magnitude of the QTAIM charges is much higher than the OPLS charges, which arises from their different theoretical definitions and methods of computation, as commented above. The total coordination number obtained by summing up the coordination numbers for each metabolite, obtained with the QTAIM charges, allows us to classify these molecules from the most solvated metabolite to the least solvated. The following order was gathered: NORCO > NOREC > ECGBE > ECGME > ECGET > COC > COCET. It is remarkable that this trend agrees with the order in which the principal metabolites are excreted in patients with a high consumption of cocaine [13, 49–51], and also gives an insight into the reason for the high half-life of COCET in plasma [14].

3.2.4 Hydrogen Bond Dynamics

In this section, we study the dynamic behavior of all the possible hydrogen bonds (HB) of cocaine and its metabolites. A hydrogen bond is defined by a geometrical criterion based

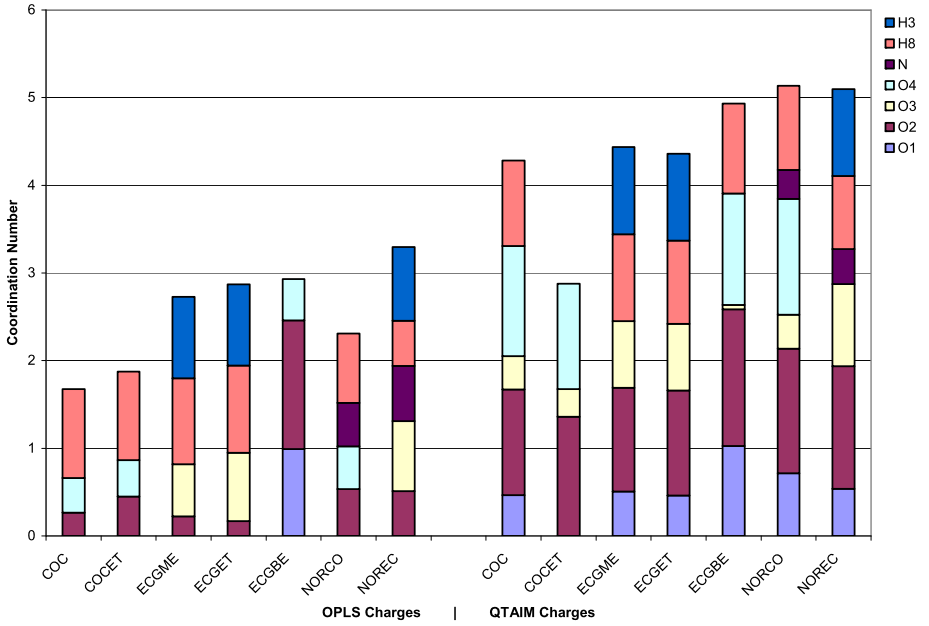


Fig. 9 (Color online) The coordination numbers (partial and total) of the most polarized atoms of cocaine and its metabolites: *gray* for O1, *green* for O2, *yellow* for O3, *light blue* for O4, *purple* for N, *orange* for H8, and *dark blue* for H3

on a maximum donor-acceptor distance (r) of 0.36 nm and a maximum donor-hydrogen-acceptor angle (θ) of 40 °. Its lifetime can be computed in two different ways, depending on the choice of what to regard as the duration of a hydrogen bond. The continuous HB definition states that this bond must exist continuously (i.e., without ever being broken), and we can compute a distribution of lifetimes $P(t)$ by making a histogram of the number of HBs that exist continuously from time 0 to t. An alternative definition is the interrupted HB, in which hydrogen bond dynamics are analyzed according to

$$-\frac{\mathrm{d}C_{\mathrm{HB}}(t)}{\mathrm{d}t} = kC_{\mathrm{HB}}(t) - k'N_{\mathrm{HB}}(t) \tag{1}$$

where C_{HB} is the intermittent hydrogen bond autocorrelation function, N_{HB} is the probability that a HB is broken at time zero but that the two fragments involved in the HB remain near each other, and k and k' are the forward and backward rate constants, respectively [52–61]. In our case, the HB lifetimes were calculated assuming the interrupted hydrogen bond definition. The functions C_{HB} and N_{HB} were calculated from the simulated trajectories and were fitted by least-squares analysis to Eq. 1, from which the two rate constants were extracted. The inverse of the corresponding forward rate constant is the average hydrogen bond lifetime [62–65].

The HB lifetimes were obtained from the QTAIM simulations, which have presented a more realistic description of the most relevant interactions, i.e. the H-bonds. The studied metabolites present two types of HB: intramolecular and intermolecular. The intramolecular HB is formed by N8H8···O1 of COCET and ECGBE, with lifetimes of 1358 ps and 880 ps, respectively. The lifetime of these bonds is justified by the steric hindrance of the ethyl group

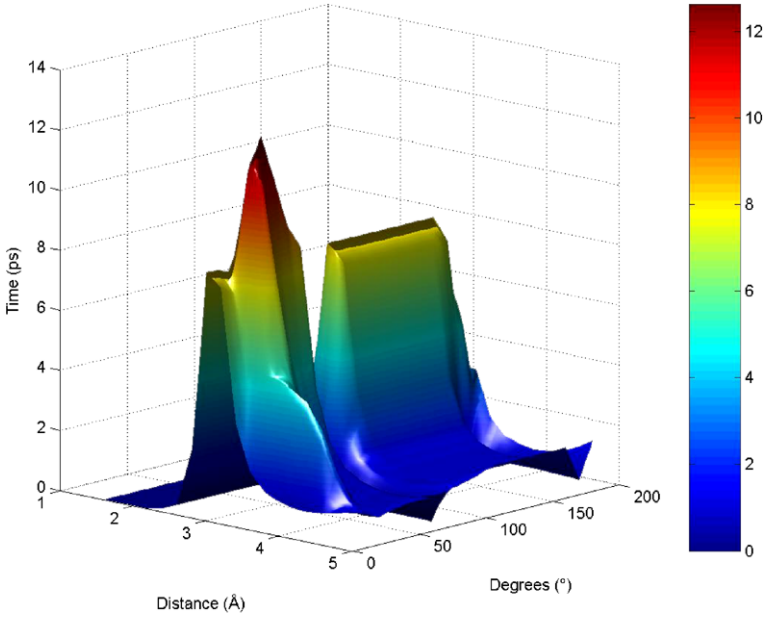


Fig. 10 The conformational surface of the most stable geometrical arrangements of the intermolecular hydrogen bond between O4 of NORCO and a water molecule

Table 4 Residence times, t_R (ps) of hydrogen bonding of the most polarized atoms of cocaine and its metabolites, with water molecules

Acronym	$t_{R\,[O1]}$	$t_{R\,[O2]}$	$t_{R\,[O3]}$	$t_{R\,[O4]}$	$t_{R\,[N]}$
COC	5.09	6.57	6.32	11.55	[b]
COCET	0.42	10.66	7.06	11.18	[b]
ECGME	4.85	6.73	3.27	[a]	[b]
ECGET	4.69	6.99	3.59	[a]	[b]
ECGBE	7.73	10.67	2.62	10.29	[b]
NORCO	2.54	5.63	4.42	12.62	1.63
NOREC	5.58	11.05	5.70	[a]	2.34

[a]Atom not present in this metabolite

[b]No peak found for this atom in its corresponding gA−B(r)

at the O1 in COCET impeding its hydration, and by the fact that O1 is more polarized in ECGBE than in COCET. This trend was also observed in the corresponding RDFs.

In order to understand the dynamical aspects of the intermolecular HB around the most polarized atoms, we calculated their most stable geometrical configurations (Fig. 10), as well as the average HB lifetimes (t_R) of the hydrogen bonds for cocaine and its metabolites. Only HBs with lifetimes of more than 0.5 ps were considered in this work. For instance, the most stable geometrical conformations of the interaction between water molecules and the O4 in NORCO, in terms of donor-acceptor distances and donor-hydrogen-acceptor angles, fall between 0.24 and 0.36 nm, and between 20.0° and 60.0°, respectively (Fig. 10, Table 4).

The HB lifetimes allow us to differentiate oxygens into two broad groups: carboxylic (O2, O4) and methoxylic/hydroxilic (O1, O3) groups. Atoms of the former group show longer HB lifetimes than the latter, which shows that carboxylic oxygens are more strongly

hydrated, in agreement with the analysis of the corresponding RDFs. Moreover, the values of HB lifetimes present similar trends to those obtained in analyzing the substituent effects on RDFs of the polarized atoms (O1, O2, O3, O4 and N). For example, the size of the alkyl chain at R_1 generally increases the HB lifetime of the oxygens, while the absence of the benzoyloxy group at R_2 reduces the HB lifetime of O2 and O3 in charged metabolites and increases them in non-charged ones. For the metabolites without a methyl group at R_3, the last trend is also observed for O1 and nitrogen.

3.2.5 Spatial Density Distribution Functions (SDF)

SDFs are accessible indirectly by neutron diffraction experiments [66, 67], using the method of empirical potential structure refinement (EPSR). Unfortunately, to the best of our knowledge there is no experimental SDF available for the metabolites of cocaine. Here, to get more direct detailed insights into the three-dimensional local structure around ECGBE, which appears to have the most hydrophilic atom, the hydrogen of the carboxylic group, among cocaine metabolites, its SDF [68] was calculated using the QTAIM charge parameters. Compared to RDFs, the SDFs span both the radial and angular coordinates of the interatomic separation vector, and describe the 3D density distribution of water molecules in a local coordinate system linked to the solute molecule or part of it. We show that this approach can thus be useful when studying systems where hydrogen bonds are an important mode of interaction. As a measure for short-range order in the water structure around the carboxylic group, we used the relative population of its solvation shell. This property was defined as the ensemble-averaged hydration-density maps, calculated from the number of times the oxygen of any water molecule falls within a 0.05 nm^3 grid volume, relative to the center of mass of the atom in the studied metabolite. Moreover, the electrostatic potential isomesh shows the highest probability regions for finding the preferable zones where water molecules are allowed to have a strong H-bond, such as that shown in Fig. 11. That is, around the oxygen, O2, of the carboxylic group, where it can be seen that the SDF has a well-defined cloud.

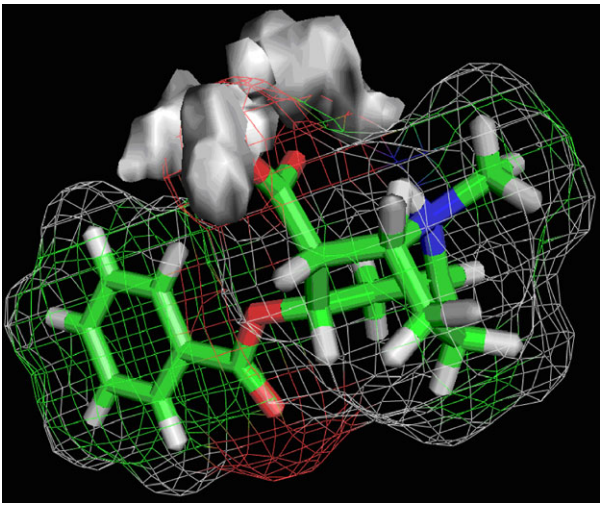


Fig. 11 The spatial density function for the hydrogen of the carboxylic group in ECGBE and its electrostatic isomesh obtained with QTAIM-parameters

4 Conclusions

In this work, we have presented a detailed and systematic study of the hydrated structures of cocaine and its metabolites by molecular dynamics simulations, based on the calculation of radial distribution functions hydrogen bond dynamics and coordination numbers, and spatial density functions. From these properties, we have proposed a classification of the hydrophobicity of the atoms of cocaine and its metabolites into three different classes: (i) charged atoms that form very strong interactions with water (oxygen atoms of the anionic carboxylate group in ECGBE, hydrogen in the amine group of cationic molecules, and hydroxyl atoms); (ii) atoms surrounded by large groups exerting noticeable steric hindrances, but that still allow H-bonding with water (i.e., carboxylic oxygen atoms O2 and O4, and nitrogen of the amine group in neutral molecules); (iii) atoms that only interact with the solvent through van der Waals and weak electrostatic forces, being denoted as hydrophobic (i.e., oxygens of ester groups, nitrogen and hydrogen of the amine group in uncharged molecules). It was shown that the degree of hydration of these atoms in each solute is determined by a combination of local effects, the type of group to which the atom belongs and the nature of the direct substituent and substituent effects, and the type and size of substituents on neighboring groups. A strong intermolecular bond, between the amine hydrogen and the O1, was identified in some metabolites, and this also significantly affects the respective degree of hydration.

Two sets of atomic charges were compared; the standard point charges of the OPLS force field and QTAIM atomic charges. It is observed that QTAIM charges are significantly higher in magnitude than their OPLS counterparts, and allow for a more realistic description of the most important hydrogen bond interactions. The differences between the two sets of charges are particularly significant for some of the most electronegative atoms. The total coordination numbers of each metabolite calculated using the QTAIM charges, allowed us to classify them in the following order of decreasing hydration: NORCO > NOREC > ECGBE > ECGME > ECGET > COC > COCET. This classification is in remarkable agreement with experiments evidence for the order of excretion in urine of these molecules in patients with a high consumption of cocaine, and might help explain the high observed half-life of COCET in plasma. We believe these studies, and those that will follow, will help us understand the pharmacological behavior of cocaine and its metabolites in humans.

Acknowledgements This work was supported by Fundação para a Ciência e a Tecnologia (FCT), POCTI/SAU–FCF/58330/2004. DR and MNDSC acknowledge FCT for research grants.

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