

Investigation of the gas-phase hydrogen/deuterium exchange behavior of aromatic dicarboxylic acids in a quadrupole ion trap

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Abstract

Gas-phase hydrogen/deuterium (H/D) exchange reactions of four deprotonated aromatic dicarboxylic acids (phthalic acid, isophthalic acid, terephthalic acid and 2,6-naphthalic acid) with D₂O were performed in a quadrupole ion trap mass spectrometer. Experimental results showed significant differences in the rate and extent of exchange when the relative position of the carboxylic acid groups varied. Spontaneous and near complete exchange of one aromatic hydrogen atom occurred when the carboxylic acid groups were in the meta-position, whereas no additional exchange was observed for either the ortho- or para-isomers or for the structurally similar naphthalic acid. Computational investigations support the participation of several possible exchange mechanisms with the contribution of each relying heavily on the relative orientation of the acid moieties. A relay mechanism that bridges the deprotonation site and the labile hydrogen site appears to be responsible for the H/D exchange of not only the labile hydrogen atom of isophthalic acid, but also for the formation of a stable carbanion and corresponding subsequent exchange of one aromatic hydrogen atom. The impact of hydrogen bonding on the relay mechanism is demonstrated by the reaction of phthalic acid as the extent and rate of reaction are greatly retarded by the favorable interaction of the two carboxylic acid groups. Finally, a flip-flop mechanism is likely responsible for the exchange of both terephthalic acid and 2,6-naphthalic acid where the reactive sites are too remote for exchange via relay.

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1. Introduction

The hydrogen/deuterium (H/D) exchange reaction has been used extensively to investigate the availability, reactivity, and accessibility of active hydrogens and the conformations of molecules in the gas-phase. [1–31] In recent years considerable experimental effort, as well as extensive molecular modeling, has been invested to understand the various mechanisms associated with the reaction of a deuterated molecule with either cations or anions. Computations have typically probed the accessibility of labile hydrogen atoms and the ability of key functional groups to interact as these two variables assist in the interpretation of the locations of the exchanged sites and the kinetics of exchange.

Details about the mechanisms of H/D exchange continue to be uncovered and debated. Of particular interest to the present work are the relay and flip-flop H/D exchange mechanisms that were

first proposed in earlier studies involving protonated amino acids and glycine oligomers. [13,14] The relay mechanism requires the formation of a stable ion–molecule complex in which a deuterating reagent bridges the gap between a charge site and a labile hydrogen atom, thereby allowing indirect interaction of the two remote sites. In this case the charge site serves as the deuterium acceptor in the exchange process, while the labile hydrogen is transferred to the deuterating agent. In contrast, the flip-flop mechanism is independent of the charge site and involves the interaction of the deuterating reagent directly with the labile hydrogen atom. For this route, the ion–molecule complex forms a pseudo ring structure by the attraction of partially charged participants, and exchange occurs as one of the potential outcomes of its disassembly. The contribution of each mechanism is dependent on the identity of the deuterating reagent as it must be either capable of spanning the distance between the hydrogen donor and receptor sites [7,14,19,31] or have the proper electronic and steric configuration to allow direct interaction. [14] In addition, the gas-phase acidity of the reagent and basicity of the acceptor site have been cited as critical factors for successful exchange. [7,13,32]

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In the gas-phase H/D exchange process, the lifetime of the ion–molecule complex is critical, as results from numerous experiments have demonstrated that reactions proceed at varying rates. It is therefore not surprising that the bulk of the gas-phase H/D exchange studies have been performed using either a quadrupole ion trap (QIT) [1–12] or, more predominantly, a Fourier transform ion-cyclotron resonance (FTICR) mass spectrometer, [13–30] since these two techniques are well suited to trapping ions for variable periods to allow kinetic analysis. While the two methodologies have many fundamental similarities, they also have several inherent differences, including the temperatures of ions, the number of collisions that they undergo with the reagent or buffer gas, and the total reaction time, that may have an impact on the results of ion–molecule reaction experiments.

The gas-phase H/D exchange behavior of amino acids and peptides has been studied intensely. [22] In these studies, both the intramolecular interactions of the carboxylic acid group within the molecule and the intermolecular interactions with the exchange reagent has often been deemed crucial to the H/D exchange process and correspondingly to the gas-phase conformations of the compound. However, a recent study highlighted a potential difficulty in the interpretation of H/D exchange results of peptides as exchange of non-labile hydrogen atoms was reported for a series of deprotonated compounds, including 2-oxoglutaric acid, isophthalic acid and 2-methylisophthalic acid. [21] In these cases additional H/D exchange beyond the number of labile hydrogen atoms was observed when the initial product of the reaction obtained in the FTICR was also subjected to subsequent activation via SORI.

The present work focuses not only on the H/D exchange of isophthalic acid, one of the compounds for which abnormal exchange was reported, but also on the other isomeric phthalic acids and a structurally similar naphthalic acid. Of primary interest were the elucidation of the differences in the exchange behaviors of the isomers and the potential exchange at traditionally non-labile sites in a gas-phase environment. Previous theoretical studies have been performed to investigate the chemical behavior of particular aromatic dicarboxylic acids. [33–36] The computational analyses performed here assess the plausibility of the various reaction mechanisms as the location of the deprotonation site relative to the remaining labile hydrogen atom varied.

2. Experimental

2.1. ESI-QIT-MS

Gas-phase hydrogen–deuterium exchange reactions of four aromatic dicarboxylic acids (Fig. 1) with D₂O were conducted in a Hitachi 3DQ (Model: M-800 LC/3DQMS) quadrupole ion trap (QIT) mass spectrometer. All reagents were purchased from Sigma Aldrich (St. Louis, MO) and used without further purification. Stock standards were prepared at a concentration of 20–30 mM in 1:1 H₂O/methanol with the addition of <0.1% of concentrated ammonium hydroxide. Analytical standards at 100 μM were prepared from each stock via dilution with methanol.

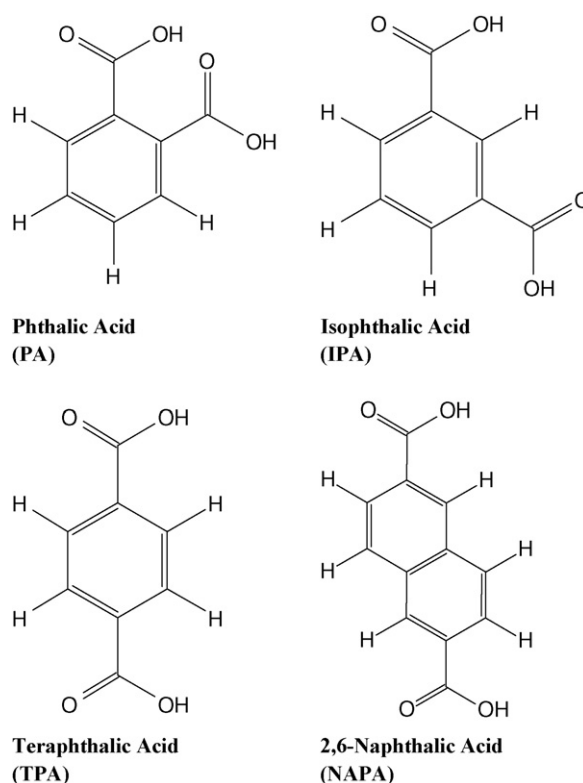


Fig. 1. Structures of the aromatic dicarboxylic acids.

Deprotonated dicarboxylic acids were introduced into the quadrupole ion trap via an electrospray ionization (ESI) source. The nitrogen sheath gas of the ESI was held at 2.2 kg/cm² and the helium buffer gas inside the trap was set to 3.0 kg/cm² yielding a helium pressure of approximately 1 mtorr. Dicarboxylic acid analytical standards were directly infused via a syringe pump at a rate of 15 μL/min with a makeup flow of 120 μL/min of HPLC grade methanol provided by a secondary pump. The adjustable temperature settings of the ESI source were set as follows: assist gas heater 200 °C, desolvator 200 °C and aperture 1 temperature of 190 °C. The ESI probe, drift, and focus voltages were set to 2.8 kV, 65 V, and 40 V, respectively. All analyses were performed in the negative ion mode.

2.2. H/D Exchange

All gas-phase H/D exchange reactions were conducted within the quadrupole ion trap. Deuterium oxide (D₂O) was used as the deuterating agent and introduced to the ion trap via a custom leak valve assembly as described previously. [10] Using this arrangement D₂O was admitted into the ion trap independent of the helium buffer gas. The typical pressure of the system including helium buffer gas was nominally 5.0 × 10⁻⁵ torr. The extent of exchange was observed by monitoring the relative abundance of the precursor ion and deuterated species while the total pressure of the system including D₂O was held near 1.5 × 10⁻⁴ torr, as measured by the ion gauge. Ions were accumulated for 25 ms with a subsequent mass selection and isolation time of 10 ms. Reaction times with D₂O were varied from 10 ms to 10 s. Each

compound was analyzed individually, and the experiments were repeated under similar conditions to assess reproducibility.

The kinetics of the H/D exchange reactions were modeled using the Kinfit program.[37] Prior to data fitting, all data were normalized and isotopic corrections to peak intensities were made based on the theoretical amount of ^{13}C represented in each peak. Peaks that were less than 5% of the intensity of the base peak of the spectrum were omitted from equation fitting.

2.3. Computational chemistry

All computations were performed in house using a combination of CAChe Worksystem Pro from Fujitsu and Gaussian 03W.[38] A comprehensive set of planar starting geometries for each compound was generated by systematically varying each rotational degree of freedom of the dicarboxylic acid anions. In the case of a carboxylate anion, the three degrees of freedom associated with the rotation of the deprotonated carboxylic acid, rotation of the intact carboxylic acid, and rotation of the remaining labile hydrogen atom relative to the plane created by the C–O bond in the carboxylic acid group produced eight possible starting structures for each compound. For terephthalic acid (TPA), rotational symmetry about the plane intersecting the two carboxylic acid moieties reduced this set to only four unique conformations.

In addition to the carboxylate anions, selected carbanion structures were also modeled for isophthalic acid (IPA) and terephthalic acid (TPA). In this case the four degrees of freedom associated with rotating either of the two carboxylic acid groups or either of the two labile hydrogen atoms produced 16 possible ion conformations. For IPA the symmetry created by placing the charge site between the carboxylic acid groups reduced this set to ten unique structures. Thus, a total of 18 IPA anions consisting of eight carboxylate ions and ten carbanions were modeled. For TPA the assignment of a negative charge to a particular carbon atom of the aromatic ring resulted in a lack of symmetry around the assigned charge site as one acid group lies adjacent to the charge while the other is two carbon atoms away. Therefore all 16 possible carbanion structures were unique for TPA and a total of 20 ions, four carboxylate ions and 16 carbanions, were modeled.

Starting structures were created in CAChe and subjected to a pre-optimization via molecular mechanics using the MM3 force field.[39] Resulting atomic coordinates were utilized as the starting structure for Density Function Theory (DFT) geometry optimizations using the B3LYP functional and the 6–31 + G(d, p) basis set.[40] Frequency calculations were also performed at the same level of theory to ensure that the optimized geometry was a local minimum of the potential energy surface and to derive a value for the zero point energy (ZPE) correction. Zero point energies were scaled using a factor of 0.9642 [41]. All final potential energies are reported at 0 K as the sum of the electronic energy and the scaled ZPE. In addition to potential energies, data for interatomic distances, bond angles, dihedrals and Mulliken partial charges were also assembled for each optimized conformation.

A subset of the low energy ion conformations were modeled as complexes with D_2O . In these calculations a potential ion–molecule complex was created by placing a single D_2O molecule at various starting positions around the optimized anion structure. The geometry of the entire system was then optimized at the same level of theory as the anion to assess their interaction. In addition, the energy and structures of several transition states were also modeled using the QST2 method in Gaussian at the same level of DFT theory used for all of the other calculations. The computational information was used to investigate the plausibility of the various mechanisms for each exchange reaction.

3. Results and discussion

3.1. ESI-QIT-MS

For each of the four dicarboxylic acids, the H/D exchange reactions with D_2O were monitored for up to 10 s, the relative rate constants were derived from the resulting kinetic plots, and then extensive DFT calculations were undertaken to model the anions of the four dicarboxylic acids and their complexes with D_2O . The kinetic results for the H/D exchange reaction of the deprotonated aromatic dicarboxylic acids with D_2O (acquired at a nominal pressure of 1.5×10^{-4} torr) are summarized in Table 1 and Fig. 2. As the reaction time was extended to the maximum of 10 s, a decrease in the abundance of the precursor ion, D(0), was observed with a corresponding increase in the abundance of the deuterated ions, D(*N*) where *N* signifies the number of incorporated deuteriums. The exchange reactions progressed at varying rates depending on the relative position of the carboxylic acid groups. The most rapid exchange was observed for isophthalic acid (IPA) where *k*₁ was roughly 500 times larger than *k*₁ for any other compound. The kinetic plots in Fig. 2 reveal that in the case of IPA, a majority of the precursor ion, D(0), was converted to a singly deuterated species, D(1), within 25 ms. In contrast, the formation of the D(1) product for the other acids occurred gradually over the 10 s reaction period, with the outcome of each reaction resulting in a distribution between D(0) and D(1) products. Interestingly, the exchange reaction for IPA proceeded beyond D(1) to a doubly deuterated species D(2), indicating exchange of an aromatic hydrogen. A majority of the D(1) ion was converted to D(2) within 1 s, and the rate associ-

Table 1
Relative rate constants for H/D exchange of dicarboxylic acids with D_2O

Molecule	<i>K</i> values ^a	
	<i>K</i> ₁	<i>K</i> ₂
Phthalic acid (PA)	0.031 ± 0.004	–
Isophthalic acid (IPA)	100.00 ± 5.98	4.70 ± 0.22
Terephthalic acid (TPA)	0.214 ± 0.006	–
2,6-Naphthalic acid (NAPA)	0.071 ± 0.004	–

^a Rate constant values as determined by kinetic fitting using Kinfit³³ after correcting for isotopic contribution and ignoring those corrected peaks that had a relative abundance of less than 5%. All rate constants are reported relative to isophthalic acid + D_2O and expressed as relative rate × 100.

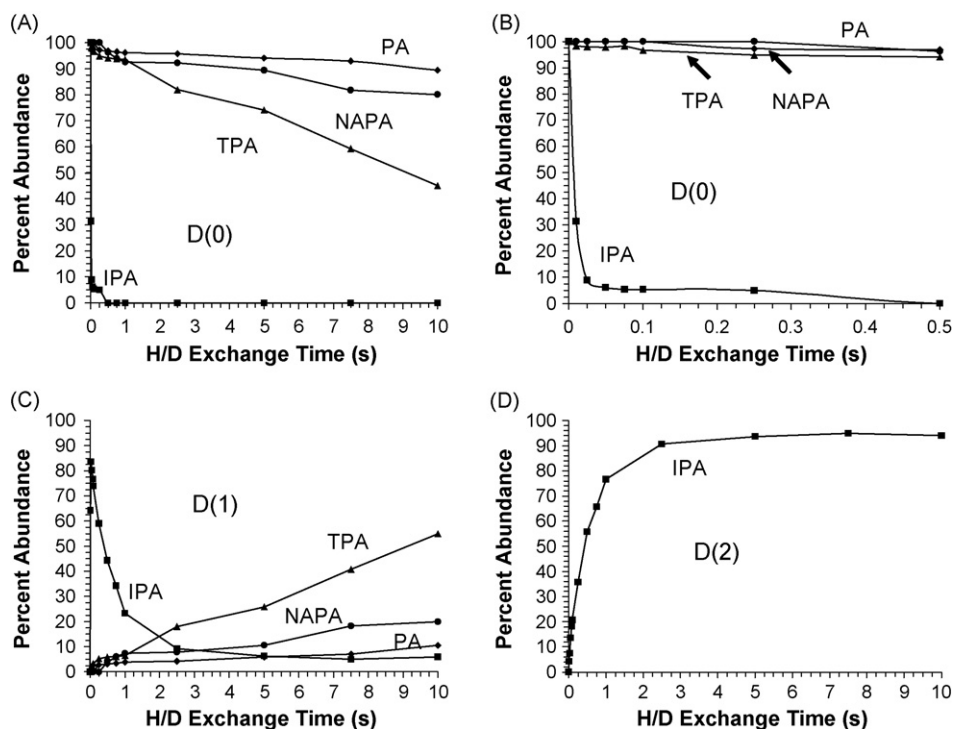


Fig. 2. Kinetic plots of the H/D exchange reaction of deprotonated phthalic acid isomers and 2,6-naphthalic acid with D_2O . Figure (A) depicts the depletion of the precursor ion D(0) over the full time scale from 0 to 10 s, while figure (B) highlights the depletion of D(0) over the first 500 ms of the exchange reaction. Figures (C) and (D) illustrate the formation of D(1) and D(2) over the 10 s reaction scale.

ated with this second reaction was over an order of magnitude larger than the exchange rates for the first deuteration of the other acids.

The mass spectra in Fig. 3 were taken after 10 s reaction time and represent a snapshot of the maximum extent of exchange for the reaction of each of the dicarboxylic acid ions with D_2O . Peaks are labeled with D(N) to represent the number of

deuterium atoms incorporated for each isomer and annotated for the inclusion of the ^{13}C isotopes. For example, the abundance of the ion of m/z 168 in Fig. 3B averaged 8.4% of the abundance of the ion of m/z 167 and could thus be attributed primarily to the ^{13}C isotope of the D(2) product (due to the presence of eight carbons in isophthalic acid), not a true D(3) product.

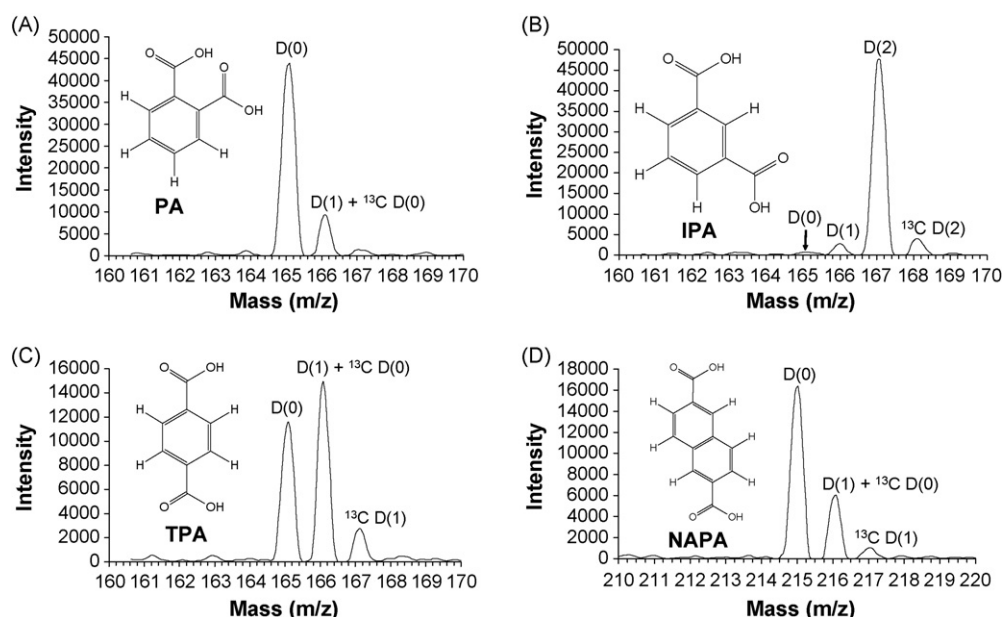


Fig. 3. H/D exchange spectra for deprotonated (A) phthalic acid; (B) isophthalic acid; (C) terephthalic acid; and (D) 2,6-naphthalic acid after 10 s exchange with D_2O .

For isophthalic acid (IPA) 94.5% of the total ion abundance in Fig. 3 is the D(2) product, signifying a nearly complete conversion while the balance of the ions, 5.5%, remained exclusively as D(1). The formation of D(2) reached a plateau near 2 s of reaction time and additional reaction time up to 10 s failed to produce any D(3) ions, thereby indicating that only one aromatic hydrogen atom is capable of undergoing exchange.

The distribution of the reaction products for the other acids is very different from IPA. Terephthalic acid (TPA), which has the two carboxylic acid groups para to each other, had a much larger extent of exchange than phthalic acid (PA), in which the two moieties were ortho. The TPA product distribution at 10 s reaction time averaged 52.4% D(1), while the corresponding distribution for PA averaged only 10.2% D(1). NAPA also underwent a single exchange, albeit at a slower rate than its phthalic counterpart, terephthalic, TPA.

Observation of the exchange of one deuterium atom for an aromatic hydrogen atom in isophthalic acid (IPA) has been reported previously in an FTICR instrument. [21]. Interestingly, it was reported that the D(1) species was the dominant exchange product and the presence of D(2) was observed only after increasing the internal energy of the ions by additional excitation via sustained off-resonance irradiation, SORI (~ 23.0 kcal/mol

estimated internal energy). Here the formation of D(2) in the IPA exchange reaction occurred both spontaneously and rapidly suggesting either that the higher number density of D₂O in the quadrupole ion trap relative to the FTICR instrument produces a larger number of collisions and thus more potential for complexation and subsequent reaction or that ions of elevated internal energy are present in the QIT.

3.2. H/D exchange of isophthalic acid (IPA)

Due to the unusual H/D exchange behavior of isophthalic acid relative to its ortho- and para-counterparts, molecular modeling of deprotonated isophthalic acid and its complexes with D₂O was performed to probe the potential mechanism of the hydrogen–deuterium exchange reactions. As discussed in the experimental section, multiple starting structures for the anions were generated for both carboxylate anions and carbanions by systematically varying the rotational degrees of freedom to derive coordinates for a set of 18 unique planar geometries. In most cases the location of the atoms in the optimized structures remained planar and relatively similar to the starting geometry. However, there were several instances where the simple planar rotation used to form the starting

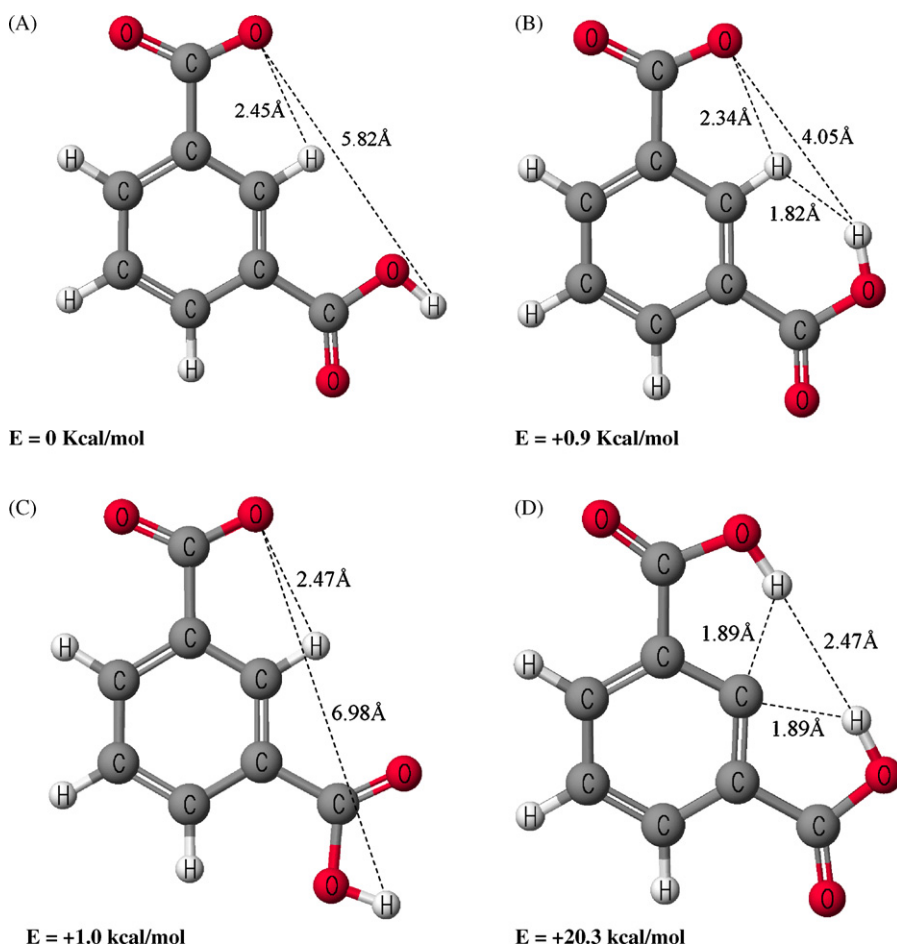


Fig. 4. Optimized structures of notable low energy isophthalic acid anions. Each structure was found to be a local minimum on the potential energy surface with figure (A) also being the global minimum. Figures are annotated with relative potential energies and interatomic distances that impact the H/D exchange reaction with D₂O.

structures resulted in unfavorable interactions between groups. The optimized geometries of these structures typically resulted in out-of-plane rotations and concomitant increases in potential energy. Furthermore, the assigned negative charge was consistently delocalized across the deprotonated carboxylic acid group making the conformations that differed simply by the rotational orientation of this group essentially indistinguishable.

Four of the most notable potential energy minima, along with their corresponding relative potential energies, of deprotonated isophthalic acid (IPA) are depicted in Fig. 4. The relative potential energies correlate with the differences in relative acidities of the four anion structures. The global energy minimum conformation (Fig. 4A) occurs when the labile hydrogen atom is rotated away from the deprotonation site, resulting in a distance of 5.82 Å between the assigned charge site and the exchangeable hydrogen. However, a simple rotation of the labile hydrogen atom through a transition state that lies approximately 11 kcal/mol above the global minimum results in another local minimum that lies only 0.9 kcal/mol above the global minimum (Fig. 4B). This conformation may be more suitable to hydrogen–deuterium exchange via the relay mechanism as it places the labile hydrogen atom approximately 4.0 Å from the assigned deprotonation site and also within 1.8 Å of the somewhat acidic aromatic ring hydrogen atom. For comparison, rotation of the intact carboxylic acid (Fig. 4C) results in a structure that is also fairly low in energy, but is suspected to be far less important for exchange via the relay mechanism since the distance between the labile hydrogen atom and the deprotonation site is nearly 7.0 Å. Finally, Fig. 4D depicts the most important carbanion structure. In this structure the two labile hydrogen atoms are both rotated inward toward the deprotonated aromatic ring and are symmetrically balanced at 1.89 Å from the assigned charge site. While the energy of the carbanion is higher than the global minimum by 20.3 kcal/mol, it would appear to be fairly suited to a relay type mechanism as all of the key atoms are in close proximity.

The mass spectra for the hydrogen–deuterium exchange of deprotonated isophthalic acid (IPA) show the formation of both D(1) and D(2) ions depending on the amount of time allotted for the reaction. It is assumed that the initial deprotonation occurs at one of the carboxylic acid moieties as these hydrogen atoms are more acidic than those of the aromatic ring. While it is certainly plausible that a carbanion structure can be formed by migration of a hydrogen atom from the aromatic ring to the carboxylate anion, none of the calculations performed here supported this path. Instead computational results suggested that the deprotonation site remained in a delocalized state on the carboxylate group regardless of its orientation and that significant electron density did not migrate to the potential carbanion charge center. Thus, it was assumed that the formation of the D(1) product was most likely to occur via reaction of a carboxylate-type anion structure, not a carbanion structure.

Complexes of D₂O with carboxylate anions amenable for H/D exchange via the relay mechanism were constructed to assess the plausibility of this exchange (Fig. 5). Here the D₂O molecule has oriented itself with the oxygen atom approximately 1.9 Å away from the remaining labile hydrogen, thereby increas-

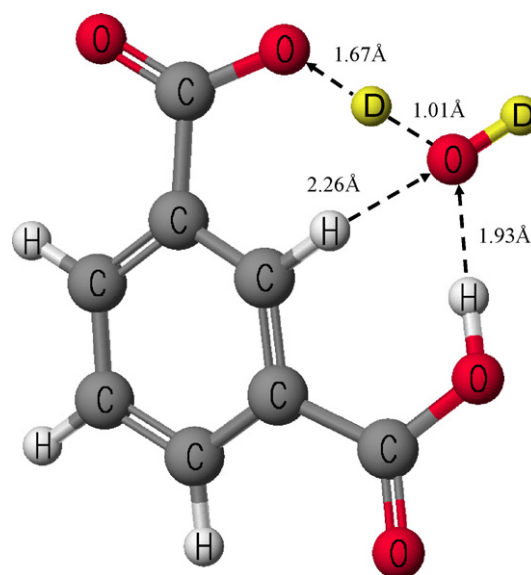


Fig. 5. Optimized structure of the carboxylate anion of isophthalic acid in the D(0) state complexed with a neutral D₂O molecule. Annotations of interatomic distances are included to support the plausibility of relay H/D exchange while arrowheads suggest possible exchange outcomes.

ing its interaction with the labile hydrogen and consequently participating in hydrogen bond formation with the carboxylate group. This orientation is enhanced by the presence of the relatively acidic aromatic hydrogen that also resides just 2.26 Å away.

Arrowheads in Fig. 5 show the possible movement of atoms within the complex. When the formerly deprotonated oxygen atom abstracts the deuterium atom from D₂O, the oxygen atom of the reagent may attract either the opposing carboxylic acid labile hydrogen or the relatively acidic aromatic hydrogen atom with either of the two paths resulting in the formation of the D(1) product. The first of these options is the classic relay mechanism in which labile hydrogen atoms are exchanged and the ion remains in the carboxylate configuration. The second path, however, results in the temporary transfer of the charge site to the aromatic ring thereby creating a carbanion in the D(1) state. Thermodynamics then dictates that D(1) ions formed via this second pathway must rearrange and form D(1) carboxylate ions with the deuterium located on the aromatic ring before exiting the complex. Based on the energy difference of the potential carboxylate and carbanion conformations given in Fig. 4 (~19 kcal/mol) and assuming similarity in the transitions along either exchange pathway of the complex, one could suspect the formation of the D(1) carboxylate product ion with the deuterium located on the carboxylic acid to be the favored exchange process.

Since a second exchange reaction involving D₂O and the two carboxylic acid groups can only result in the re-generation of the D(1) product, the formation of the D(2) product must proceed through the formation of a carbanion. Calculations suggested that the balanced carbanion shown in Fig. 4D is favored by between 10.7 and 36.3 kcal/mol over the other stable carbanions modeled. The full list of optimized carbanions and their calculated energies is included in the supporting information.

There are two possible paths for the subsequent formation of the D(2) product involving this carbanion form of the D(1) ion. On one hand this carbanion could simply become unbalanced if one of the carboxylic acid moieties rotated away from the charge site (see Fig. 4D, with one of the OH groups replaced by an OD group). This movement could force the migration of either a hydrogen or deuterium atom to the aromatic ring, depending on the carboxylic acid group that was involved in the rotation. The subsequent formation of the D(2) ion could then occur through the traditional relay mechanism between the two carboxylic acid groups, as described above. While this pathway does result in deuteration of the aromatic ring and the eventual formation of the D(2) product, computational investigation revealed that the transition state barrier associated with rotating the partially positive hydrogen/deuterium away from the charge site was high, requiring approximately 30 kcal/mol of additional energy.

An alternate path to D(2) also exists via the complexation and subsequent relay exchange of the stable carbanion in the D(1) state. The modeling of these complexes resulted in structures illustrated in Fig. 6. In the initial state (Fig. 6A), the interatomic distance between the oxygen and deuterium atom of D₂O is 0.95 Å, the typical D–O bond length. However, when the complex is optimized (Fig. 6B) this distance increases to 2.26 Å while the complimentary distance between the carbon and the deuterium atom is reduced from 1.5 Å to 1.04 Å, the typical C–D bond length. Most importantly, this process results in the formation of low energy D(2) carboxylate ions without passing through the high energy transition states associated with the first path.

In summary, the relay mechanism appears to be a favorable process for both the first and second H/D exchange of deprotonated IPA. The role of the ion–molecule complex appears to be of foremost importance as it allows the formation of both carboxylate and carbanions in the D(1) state. Various pathways from D(1) to D(2) are expected to proceed through a carbanion structure either by hydrogen migration or more likely by subsequent D₂O complexation and relay exchange. In either case, the

rapid and nearly complete exchange from D(0) to D(2) observed in the mass spectrum illustrates the efficiency of the process.

3.3. H/D exchange of phthalic acid (PA)

Molecular modeling was conducted on eight potential carboxylate anions of phthalic acid to elucidate the most energetically favorable arrangement of the ortho-oriented carboxylic acid groups. Unlike the results for IPA where optimized geometries were fairly consistent with the planar starting structure, optimization of the phthalic acid structures often resulted in not only significant movement of the atoms from their original position but also out of plane rotation. In addition, all of the starting conformations optimized to one of two minima, depending on both the rotational orientation of the intact carboxylic acid moiety and the initial location of the labile hydrogen atom. The global energy minimum (Fig. 7A) resulted from starting structures that placed the hydrogen atom both between the carboxylic acid groups and rotationally oriented toward the delocalized charge site. Here, the labile hydrogen atom bridges the two carboxylic acid groups orienting itself 1.89 Å from each oxygen atom in order to stabilize the deprotonated structure and dramatically reduce the potential energy. All of the other starting structures optimized to the structure in Fig. 7B in which the carboxylic acid group has rotated out of the plane to relieve the repulsive forces between the electron dense oxygen atoms. The potential energy of this second structure is 19.9 kcal/mol above the global minimum and results in a distance of 3.13 Å between the deprotonation site and the labile hydrogen atom. In both cases the interatomic distances between the deprotonation site and the labile hydrogen atom indicated that the relay mechanism may be suitable for H/D exchange with D₂O.

The mass spectra for the hydrogen-deuterium exchange of phthalic acid (PA) show the presence of both D(0) and D(1) ions, with the distribution being heavily weighted toward D(0). Furthermore, the rate of exchange for deprotonated PA was the lowest among all of the reactions studied. Thus, while inter-

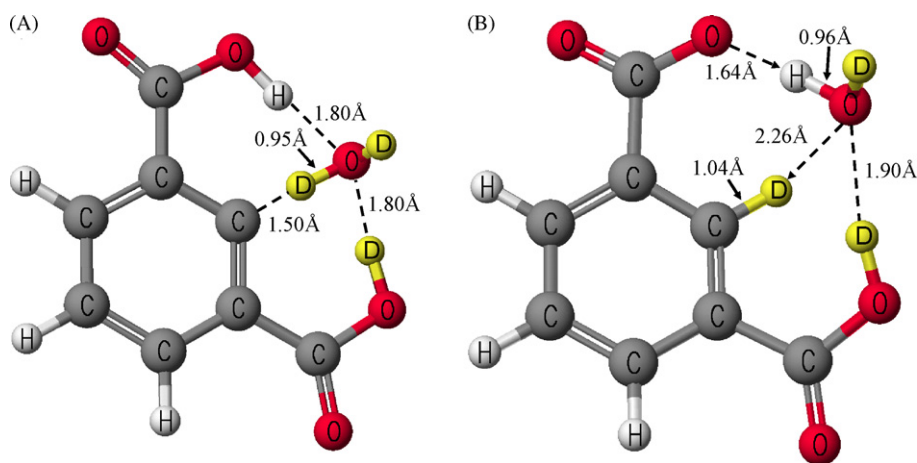


Fig. 6. Figure (A) depicts an example of isophthalic acid carbanion in the D(1) state symmetrically complexed with D₂O. Potential energy optimization of this complex produces the complex illustrated in figure (B). Here the carbanion has been deuterated as evidenced by the formation of the C–D bond (1.04 Å interatomic distance). The concerted deprotonation of the carboxylic acid and formation of the H–O–D product (0.96 Å interatomic distance) results in the formation of isophthalic acid carboxylate anions in the D(2) state.

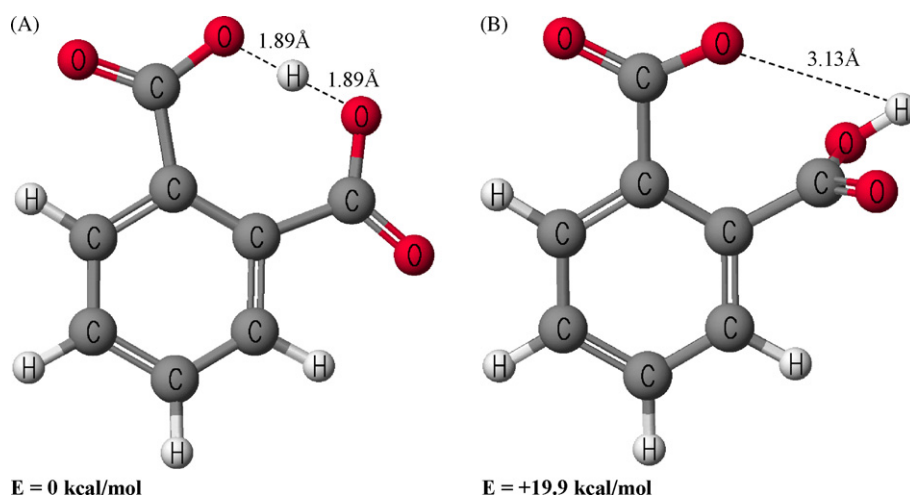


Fig. 7. Optimized structures of low energy phthalic acid anions. Each structure was found to be a local minimum on the potential energy surface with figure (A) also being the global minimum. Figures are annotated with relative potential energies and interatomic distances that impact the H/D exchange reaction with D_2O .

atomic distances may be favorable for exchange, energetics must remain unfavorable. Indeed the hydrogen bonding associated with the global energy minimum could be suspected to preclude the labile hydrogen from exchange. Hydrogen bonding has been implicated in the past as a reason for reduced H/D exchange of labile hydrogen atoms. [17–19] However, some D(1) product was formed as the reaction time was increased, and it can therefore be concluded that either the energetic restraint of breaking the hydrogen bonds was overcome or the exchange proceeded through a higher energy anion such as the one in Fig. 7B.

Complexes of the two phthalic acid anions with D_2O were modeled to assess their interaction (Fig. 8). For the global energy minimum anion (Fig. 8A), the interaction with D_2O typically resulted in a tightly bound complex where the labile hydrogen atom was approximately 1.2 Å from either of the carboxylic acid groups and about 2.8 Å from the oxygen atom of D_2O . The two deuterium atoms remained attracted to the oxygen atoms of the carboxylic acid groups and tended to orient themselves

between 2.0 and 2.4 Å away, resulting in a pseudo six-membered ring consisting of alternating oxygen and hydrogen/deuterium atoms. H/D exchange of this complex is unlikely because the very tightly bound labile hydrogen atom would have to overcome the attractive forces of both carboxylic acid groups to transfer to the deuterated reagent.

In contrast, the complex of the higher energy anion (Fig. 8B) resulted in complexes that were well suited to the relay mechanism as the deuterium of the oxygen atom was typically 1.7 Å from the deprotonation site, while the labile hydrogen atom was a similar 1.8 Å from the oxygen atom of the D_2O reagent and still remained nearly 2.9 Å from the charge site. Thus, the charge site may be somewhat attracted to the labile hydrogen atom, but the deuterium of D_2O is the most closely associated atom and hydrogen bonding of the labile hydrogen would not block the H/D exchange. In either case the reduced rate of hydrogen–deuterium exchange for phthalic acid relative to isophthalic acid can be attributed to energetic restraints, either those associated

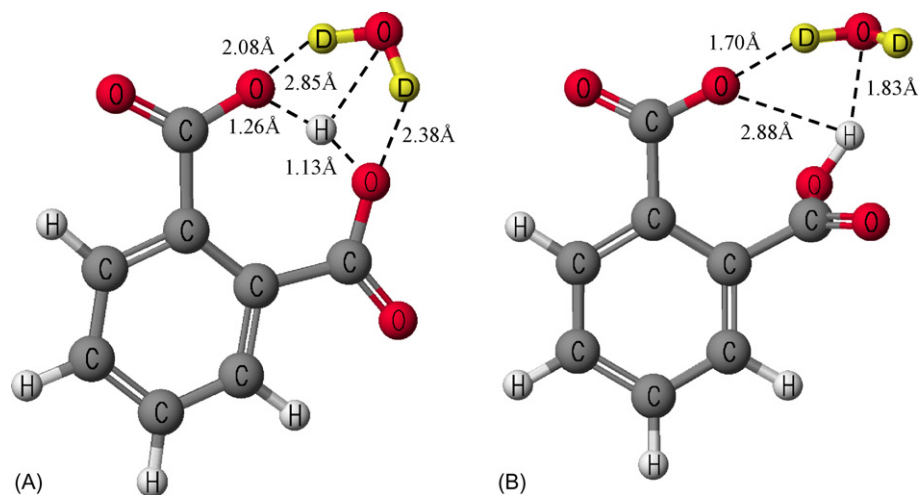


Fig. 8. Depictions of the energy optimized complexes of D_2O with phthalic acid carboxylate ions in the D(0) state. Figure (A) depicts the complex formed with the lower energy conformation while figure (B) correlates to the complex of the higher energy conformation. Annotations of interatomic distances in (A) suggest that the hydrogen atom remains bound to the carboxylate and is not exchanged while those in (B) demonstrate the plausibility of a relay H/D exchange of the higher energy conformation.

with overcoming the hydrogen bonding of the global energy minimum conformation or the lower probability of the creation of the higher energy deprotonated structure prior to the complexation.

3.4. H/D exchange of terephthalic acid (TPA) and 2,6-naphthalic acid (NAPA)

The mass spectra for the hydrogen–deuterium exchange of both terephthalic acid (PA) and 2,6-naphthalic acid (NAPA) show the presence of both D(0) and D(1) ions but no D(2) ions, with a maximum of 52.4% D(1) for TPA and 32.2% D(1) for NAPA. The exchange rate for both TPA and NAPA ions was greater than that for PA but much less than that of IPA, with the rate for TPA exchange being approximately three times that of NAPA.

Molecular modeling was conducted on four potential carboxylate anions and 16 potential carbanions of terephthalic acid (TPA) to determine their gas-phase structure and potential energies. Structures resulting from these calculations and the most energetically favorable anions of both TPA and NAPA are depicted in Fig. 9. Optimization of the carboxylate anions yielded low energy geometries that were for the most part planar and similar to the starting structures. In some cases, deviations in planarity were observed due to hydrogen–hydrogen repulsion, but overall they accounted for relatively little change in atomic position. Optimization of the carbanions led either to their collapse into carboxylate ions with the transfer of the labile hydrogen atom from the carboxylic acid to the aromatic ring or to structures with very high potential energy (>50 kcal/mol) compared to the global minimum. Most notably, the labile hydrogen atom is relatively distant from the deprotonation site

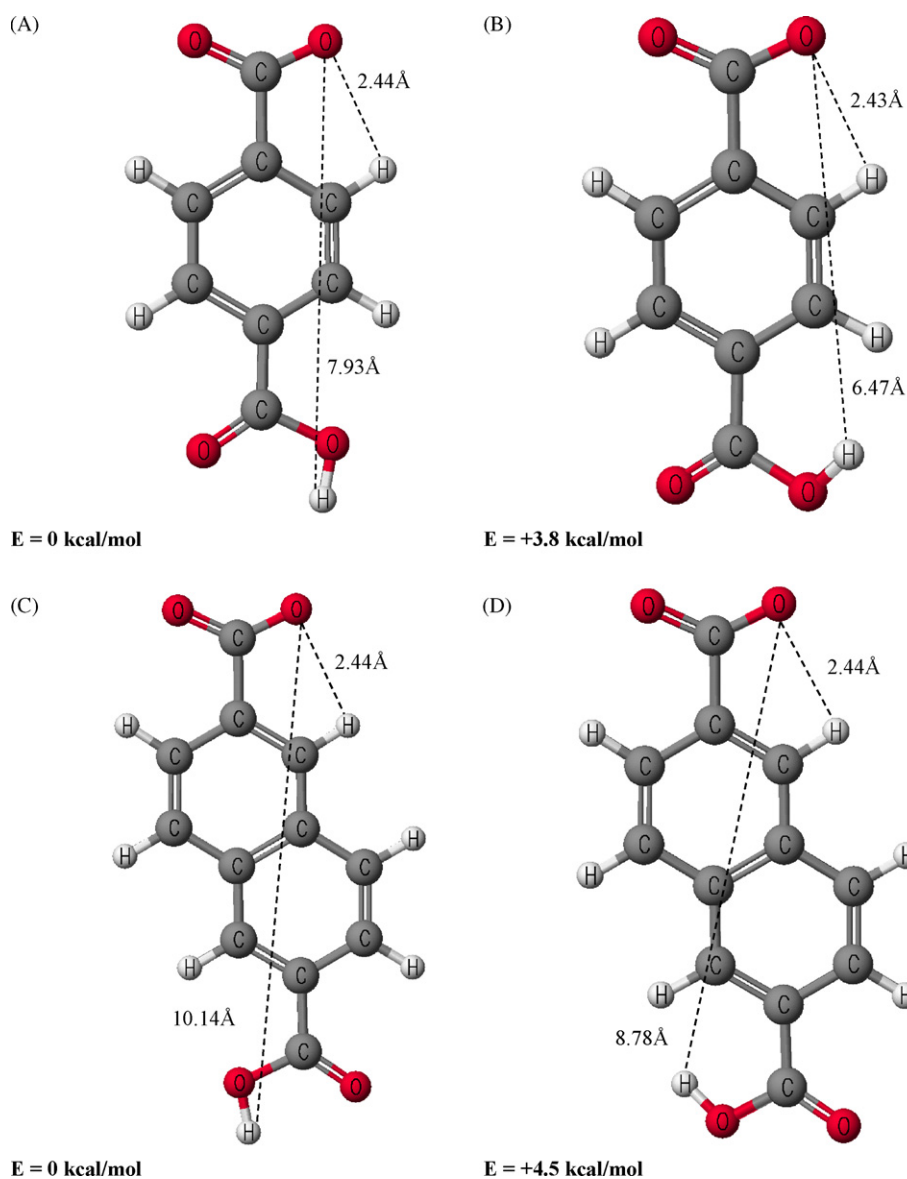


Fig. 9. Optimized structures of low energy terephthalic acid anions and 2,6-naphthalic acid ions. Each structure was found to be a local minimum on the potential energy surface with figures (A and C) also being the global minimum for each compound. Figures are annotated with relative potential energies and interatomic distances that impact the H/D exchange reaction with D_2O .

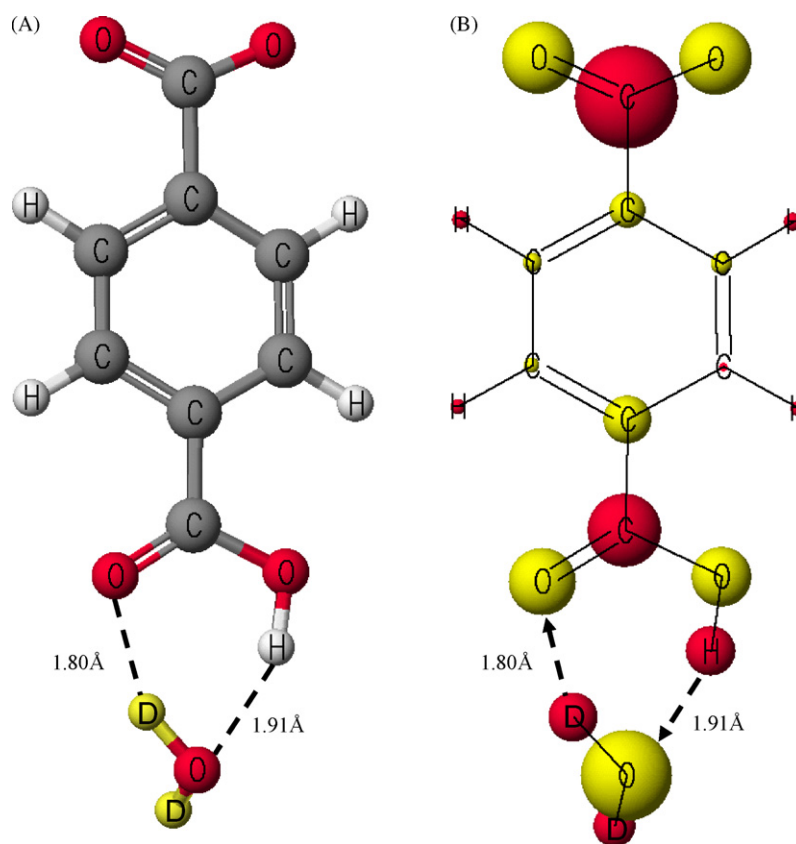


Fig. 10. The geometry optimized structure of the complexation of terephthalic acid carboxylate anion in the D(0) state with D₂O is depicted in figure (A). A charge transfer process for the exchange of hydrogen for deuterium is indicated by the arrowheads in figure (B). Here red spheres indicate positive partial charges while yellow spheres indicate negative partial charges with the magnitude of the partial charge at the atom being proportional to the size of the sphere (1e_u is depicted as 1 Å).

with distances exceeding 6 Å for TPA in the higher energy conformation (Fig. 9B) and nearly 8 Å in the global energy minimum (Fig. 9A). The results for NAPA showed even larger interatomic distances (Fig. 9C and D). Therefore, the carboxylate ions were highly favored over the carbanions for both the structures of TPA and NAPA, thus prohibiting exchange of the aromatic hydrogen atoms.

Unlike the other acids, the relay mechanism seems implausible for the H/D exchange of TPA and impossible for NAPA as the distance between the labile hydrogen atom and the deprotonation site is too great to be bridged by a D₂O molecule. Therefore, various complexes of the lowest energy conformations of TPA and NAPA anions with D₂O were modeled to investigate alternate exchange pathways. When the D₂O molecule was placed near the deprotonated charge site, an interaction between the deuterium and the delocalized charge site was observed, but no path for exchange could be elucidated as there was no acidic hydrogen in the vicinity of the oxygen atom of D₂O. However, when the D₂O molecule was placed in the vicinity of the other carboxylic moiety, simulating the ability of the reagent to access all points on the ion surface, an interaction was observed at the carboxylic acid. The D₂O neutral oriented itself such that one of the deuterium atoms was 1.80 Å from the doubly bonded oxygen atom of the carboxylic acid moiety, while the oxygen atom of the neutral was only 1.91 Å from the labile hydrogen atom thereby forming a pseudo six-membered ring (Fig. 10A).

When the complex is viewed from the standpoint of Mulliken partial atomic charge (Fig. 10B), a clear path to a flip-flop (charge transfer) exchange process is observed. Dissolution of the complex can result in either the regeneration of the initial structures or in the formation of the exchanged structure depending on which of the bonds of the pseudo six-membered ring are broken.

4. Conclusions

The exchange reactions of the aromatic dicarboxylic acids illustrate several interesting aspects of gas-phase H/D exchange. First, the relative positioning of the carboxylic acid groups has a dramatic impact on the extent and rate of the reactions. Mass spectral results show that the meta-arrangement of the groups is highly favorable as the reactions produce not only the exchange of the labile hydrogen atom but also exchange of the aromatic hydrogen atom located between the two groups well within 10 s of reaction time. The difference in the exchange rates of the various isomers is phenomenal.

Second, as the relative position of these moieties changes, the apparent contribution of various mechanisms also changes. For phthalic acid, the ortho-arrangement encourages a tightly bound global energy minimum conformation that either deters the hydrogen–deuterium exchange rate or inhibits it entirely. The limited exchange that is observed may proceed through a higher

energy deprotonated structure, thereby explaining the relatively slow formation of the deuterated product.

While the exchange of the ortho- and meta-isomers may be reasoned using a relay mechanism, the exchange of the para-compound cannot. Modeling results for both terephthalic acid and 2,6-naphthalic acid indicate that the distance between the labile hydrogen atom and the deprotonation site in the low energy conformations is too large to be bridged by a D₂O molecule. Therefore, the formation of deuterated products of these anions must occur via an alternate pathway such as the flip-flop (charge transfer) process.

Lastly, the mass spectral results suggest that the reaction environment of the ions stored in the quadrupole ion trap appears to be significantly different than those stored in the FT-ICR mass spectrometer. Specifically, reactions involving isophthalic acid that required additional excitation via SORI (~23 kcal/mol) proceeded spontaneously in the quadrupole ion trap. Apparently, the quadrupole ion trap produces conditions favorable for extensive H/D exchange, which can either be attributed to differences in ion internal energy or to the dramatic difference in number density of the exchange reagent. Computational results suggest that the additional exchange of an aromatic hydrogen atom of isophthalic acid can be correlated with the formation of a carbanion structure in the D(1) state that lies approximately 20 kcal/mol above the global energy minimum and remains stable within the complex after the first deuterium exchange. Continued investigation of the various exchange pathways of others sets of isomers should shed light on the intricacies of this non-traditional exchange process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2007.02.026.

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