

FINAL PROJECT REPORT FOR THE SHELL CENTER FOR SUSTAINABILITY

MONITORING ENGINEERED NANOPARTICLES IN THE ENVIRONMENT

W.C. Hockaday¹, Y.-S. Hwang², Q. Li², C.A. Masiello¹

¹ *Department of Earth Science, Rice University, wch2@rice.edu, masiello@rice.edu*

² *Department of Civil and Environmental Engineering, Rice University, Qilin.li@rice.edu*

[1] Summary

The environmental and health implications of nanotechnology are of great concern as the escalating production of nanoscale materials will inevitably result in the introduction of nanoparticles into the environment. In this study, we used aqueous fullerene C₆₀ as a model system for the development of mass spectrometric methods capable of quantifying carbonaceous nanoparticles in water.

Key Findings:

- Using atmospheric pressure photo-ionization (APPI) mass spectrometry, we can identify and quantify C₆₀ fullerenes and their chemical-derivatives in solutions that simulate natural aquatic environments (i.e. waters containing environmentally-relevant levels of mineral and organic matter).
- The majority of the aqueous C₆₀ fullerenes are suspended in the form of colloidal nanoscale aggregates (nC₆₀), where they can be dispersed to part-per-million levels in water. APPI mass spectrometry detects a very small fraction of the total dispersed C₆₀, corresponding to the parts-per-trillion level. We hypothesize that the photo-ionizable fraction of aqueous C₆₀ corresponds to that which exists in the molecular form, rather than the nanoparticle form. If substantiated, ours would be the first direct measurements of molecular C₆₀ in water.
- Aqueous C₆₀ solution chemistry and preparation procedures influence the chemical and physical speciation of C₆₀. In APPI mass spectra this is most evident through differences in ionization efficiency of C₆₀ and the fullerol C₆₀(OH)_x:fullerene C₆₀ ratio.
- The presence of natural organic matter (NOM) substantially enhanced the instrumental response to aqueous C₆₀. This “NOM effect” could be attributed to:
 - the ability of NOM to act as a solvent or surfactant in the aqueous phase, and thereby stabilizing C₆₀ in a solvated (NOM-associated) form as opposed to nanoparticle-associated form (nC₆₀),
 - and/or an enhancement in ionization efficiency due to the action of NOM as a proton transfer reagent in the gas phase during ionization in mass spectrometer.

- The direct addition of fullerene to water containing environmentally realistic NOM levels (20 mg / L) increased the dispersible nC₆₀ concentration by a factor of 5 and the dissolved molecular C₆₀ concentration increased by a factor of 40 relative to NOM-free water.
- The current design of the APPI ion source is prone to “carryover” between consecutive samples, requiring a time-consuming cleaning procedure between sample analyses, limiting its potential application to high throughput environmental monitoring applications.

These findings demonstrate that APPI mass spectrometry can be a powerful tool for quantifying fullerenes and identifying derivatives formed under environmental conditions. Natural organic matter appears to be an important factor controlling the apparent aqueous solubility of molecular fullerenes and fullerene derivatives, and therefore may affect the chemical and biological availability fullerenes. Our future work will focus on using APPI mass spectrometry to study environmental factors (e.g., natural organic matter concentration and UV exposure) that control the quantity and chemical transformation of molecular fullerenes in aquatic systems.

[2] Background

Early research on carbonaceous nanoscale particles (fullerenes and nanotubes) demonstrates the potential for significant ecological risk. Exposure to part-per-million aqueous concentrations of fullerene can cause morbidity in microorganisms and oxidative, reproductive, and metabolic stress in crustaceans, copepods, and fishes (2-4).

We selected aqueous C₆₀ fullerene as a model system for method development because:

- current commercial production of fullerenes has exceeded 1500 tons yr⁻¹ (5) making them relevant as a potential environmental contaminant (6),
- there is a rapidly growing body of literature on their chemical and environmental behavior,
- fullerenes can be conveniently purchased in highly purified molecular form (>99%), as opposed to nanotubes which are complex polydisperse mixtures of nanoparticles, and
- the fullerene structure is fundamental to other engineered carbon nanoparticles, such as nanotubes, and therefore may provide some general insight to the environmental behavior of engineered nanocarbons.

Fullerene C₆₀ is an extremely hydrophobic molecule, and although the aqueous solubility can not be directly measured by any currently-available technique, the calculated C₆₀ solubility in pure water is 1.8×10^{-20} to 1.1×10^{-11} moles/L⁻¹ (7, 8). However, fullerenes can be transported in aqueous systems at concentrations significantly higher than aqueous solubility by forming water-stable aggregates that typically range in size from 5 - 500 nm (9-11). These nanometer sized aggregates of C₆₀ are known as nC₆₀. Most natural waters also contain part-per-million levels of NOM which consists of an array of organic components that behave as powerful solvents, sorbents, and chelating agents. Therefore, we hypothesized that NOM may participate in physical and chemical interactions with fullerenes and other engineered carbon nanoparticles that could further increase their concentration in aquatic ecosystems.

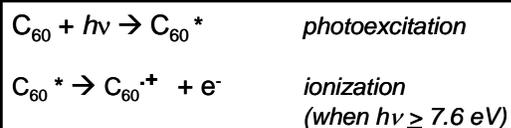
To identify release points, assess fate, and monitor exposure, we need reliable methods for detecting engineered nanoparticles the environment. However, both extraction and spectroscopic identification of carbonaceous nanoparticles such as fullerenes from geomeia has proven to be highly problematical (12-14). Poor extractability might be explained by physical sorption or chemical bonding to natural organic matter ($\log K_{OC} = 7.7$), which also inhibits physical (e.g. chromatographic) separation of fullerene from NOM (15, 16). Finally, the spectroscopic identification of fullerene in natural samples is complicated by shared spectroscopic features between fullerene and NOM. Therefore, the goal of this project is to develop methods that can detect fullerenes in the presence of natural organic matter.

[3] Approach

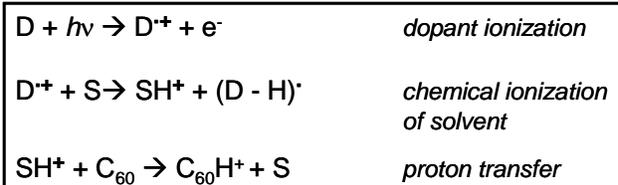
Our approach to identifying carbonaceous nanoparticles in environmental samples is based upon a unique molecular property of fullerenes - *zero mass defect*. The carbon atom has a mass of 12.0000 Dalton (Da). The decimal portion of the mass is termed the mass defect. Thus, structures comprised of pure carbon have *zero mass defect*. For instance, the molecular weight of C₆₀ is 720.0000 Da. Natural organic molecules in the environment are hydrogen- and nitrogen-rich (H = 1.0078 Da, N = 14.0031 Da), and therefore, have positive mass defects. However, determining the mass defect of a molecule or mixture of molecules is not trivial, as it requires a very accurate mass measurement of the type that can only be made in the gas phase by state-of-the-art mass spectrometers. Furthermore, once in the gas phase the molecule must be made into an ion (with positive or negative electrical charge) in a manner that does not alter the chemical structure. These are substantial challenges for nonvolatile substances such as fullerenes, especially underivatized fullerenes without electronegative atom to stabilize electrical charge.

The gas phase ionization of fullerenes is made possible by a recently-developed technique known as atmospheric pressure photoionization (17). The photoionization of C₆₀ fullerene, depicted in **Scheme 1**, occurs when C₆₀ absorbs UV photons ($h\nu$) promote electrons to the excited state (C₆₀^{*}) followed by the loss of an electron when the energy of the absorbed photon exceeds the ionization energy of 7.6 electron volts (eV) [Yoo, R.K.; Ruscic, B.; Berkowitz, J., Vacuum ultraviolet photoionization mass spectrometric study of C₆₀, J. Chem. Phys., 1992, 96, 911]. Photoionization of C₆₀ forms a stable radical cation (C₆₀^{•+}) that is readily detected by mass spectrometry. A chemical ionization mechanism can also be induced within the APPI source by introducing a photosensitizer and proton transfer reagent, also known as a “dopant”. Suitable

Scheme 1: Direct photoionization



Scheme 2: Dopant / Chemical ionization



[modified from *Rafaelli & Saba, 2003*]

dopants are organic molecules with a large UV light absorption cross section and low ionization energy (<10 eV) low proton affinity. As illustrated in **Scheme 2**, chemical ionization is achieved when photon energy, $h\nu$, absorbed by the dopant (D) results in the loss of a proton (H⁺) which is then transferred by the solvent (S) to the fullerene, yielding C₆₀^{H+}. Ideally, the solvent molecule should have a low proton affinity to ensure a high proton transfer from the ionized dopant to the C₆₀. In this study we use toluene to serve the dual purposes of dopant and solvent.

To examine the utility of APPI-MS for identifying and quantifying fullerene C₆₀ in aqueous samples we identified 3 critical tasks:

Task 1: Develop a method to measure C₆₀ via photoionization mass spectrometry within the Rice Shared Equipment Authority Mass Spectrometry lab. This includes optimizing instrument parameters and determining detection limits;

Task 2: Measure standards of C₆₀ in a natural organic matter (NOM) matrix prepared to simulate 2 pathways by which ECNs could enter aquatic systems: a) solid spillage followed by dispersion with natural organic material (NOM); and b) phase transfer from a heavy organic solvent into water and partitioning of C₆₀ into dissolved NOM;

Task 3: Assure sample quantitation by quantifying all chemical derivatives of C₆₀ in the presence of NOM by using a higher-resolution mass spectrometer (FTICR MS) located at Old Dominion University (Norfolk, VA).

[4] Methods

C₆₀ Sample preparation

Sublimed C₆₀ fullerene powder (99.9%) was obtained from Materials Electronics Research Corporation (Tucson, AZ). Suwannee River humic acid II (SRHA) was purchased from International Humic Substances Society (IHSS, Atlanta, GA).

For this study we prepared 3 types of samples.

Dissolved C₆₀ in toluene solvent. Fullerene powder was dissolved in HPLC grade toluene (Fisher scientific) to make a stock solution concentration of 30 mg C₆₀/L. The solution was transferred to a solvent washed amber glass bottle and stored in the dark.

nC₆₀ + NOM suspension by mechanical stirring.

Approximately 100 mg “as received” C₆₀ fullerene powder was added to one liter of aqueous NaCl (0.1mM) with SRHA (20mg/L) and stirred for 3 weeks in the dark using a magnetic stir bar. The suspension was filtered through Millex-AP membrane filter with pore size of 2 μm to remove larger C₆₀ particles. Samples were stored in the dark at room temperature until APPI-MS analysis.

nC₆₀ suspension by ultrasonic dispersion. Before preparation, C₆₀ fullerene powders were pulverized to a very fine power using an agate mortar and pestle for 10min. 150 mg of pulverized C₆₀ was added to 200ml of deionized water in a 500ml glass beaker. The mixture was then sonicated using the Vibra-Cell from Sonic (VCX 500) operated at 20% intensity for 30min, approximately 26 W. Then, the resulting brown-yellowish suspension was filtered through Millex-AP membrane filter with pore size of 2 μm to remove larger C₆₀ particles. Several batches were synthesized and combined and stored in the dark at 4°C.

APPI-MS analysis

Liquid samples were analyzed using a Bruker (Billerica, MA, USA) MicroTOF time-of-flight mass spectrometer (MS) at Rice University that is equipped with the atmospheric pressure photo-ionization ion source, depicted in **Figure 1**, from Syagen Technologies (Tustin, CA, USA). The APPI source consists of a nebulizer/vaporizer operated at a gas pressure of 20 psi and 300 °C and a UV lamp (krypton) which emits photons at 10 eV.

Samples were diluted until the total C₆₀ concentration was in the 1 – 50 nanomolar (10⁻⁹ moles L⁻¹) concentration range and directly infused to the ion source at a flow rate of 10 - 20 μL min⁻¹ using a syringe pump. Data were acquired over a range of 50 to 1500 m/z at a sampling frequency of 1Hz. Data used for quantitative measurements of ion abundance were summed over a minimum 60 seconds (i.e. 60 spectral acquisitions).

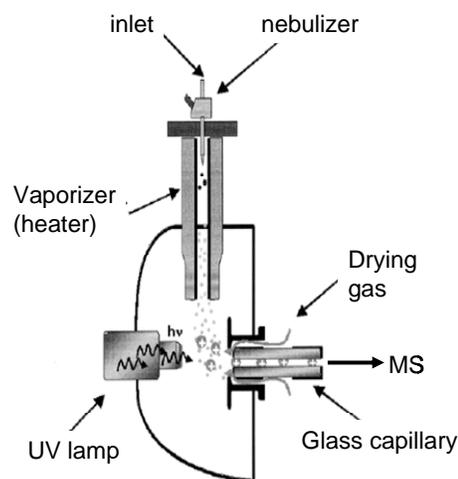


Figure 1. Diagram of the Syagen APPI ion source [modified from Hanold et al., (1)].

[5] Results

Task 1: Mass spectral method development

C₆₀ in toluene solutions. The APPI MS analysis of pure C₆₀ dissolved in toluene yields both the radical cation (C₆₀⁺) and the protonated fullerene (C₆₀^{H+}) likely produced by ionization processes shown in **Scheme 1** and **Scheme 2**. The APPI MS instrument was optimized for fullerene detection by systematically varying the recommended operating conditions of the ion source, ion optics, and mass analyzer until the maximum-possible signal intensity was obtained at 720 m/z. Under the optimized conditions the Bruker MicroTOF APPI-MS at Rice University routinely detects 1nM

concentration C₆₀ and has a detection limit of ~ 2 × 10⁻¹³ g C₆₀. This is a promising prospect for the detection of low levels of fullerene in environmental samples. Furthermore, **Figure 2** shows that the magnitude of the signal intensity is linearly related to C₆₀ concentration (in the nanomolar concentration range) which is critical to quantitative measurements of C₆₀ in environmental samples.

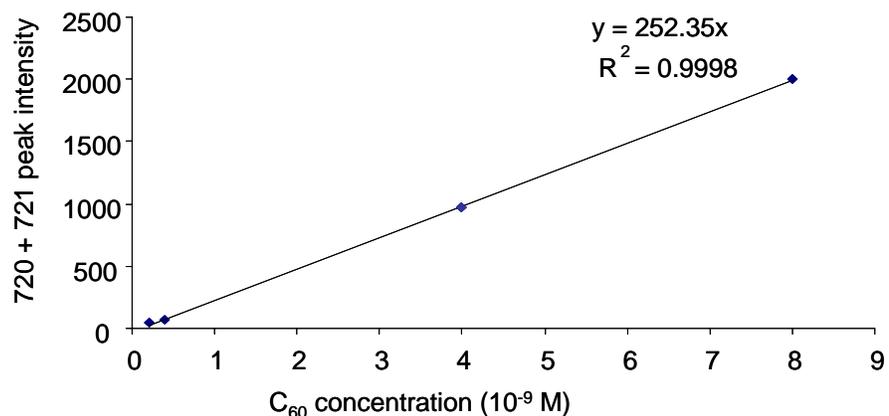


Figure 2. Summed intensity of the signals at 720 m/z (photo-ions) and 721 m/z (chemical ions) as a function of C₆₀ concentration in toluene solution.

Task 2: The effect of natural organic matter on aqueous C₆₀ fullerene

Aqueous nC₆₀. Aqueous nC₆₀ nanoparticles were prepared in two ways: (1) direct dispersion by mechanical stirring, and (2) direct dispersion by ultrasound. The total dispersed C₆₀ concentration was determined by measuring the total dissolved carbon concentration. With mechanical stirring it was possible to disperse nC₆₀ to a maximum concentration of 1.5 μM (1.5 × 10⁻⁶ moles C₆₀/ L). Substantially higher nC₆₀ concentrations, 5.3 μM, can be produced rapidly (30 min) using ultrasonic dispersion (sonication). APPI-MS offers insight to the differences in the dispersible concentration of C₆₀ achieved by mixing versus ultrasound. **Figure 3** shows that oxidized fullerene species are present in aqueous nC₆₀ samples created by both sonication and stirring methods. However, sonication either generates or facilitates dissolution of greater relative quantities of oxidized fullerene. The peak at 737 m/z represents C₆₀ plus one covalently bound oxygen atom plus one hydrogen atom (720 + 16 + 1 = 737). Similarly, the peak at 755 m/z represents C₆₀(OH)₂. Each fullerene species produces a parent peak followed by a series of peaks each separated by 1 mass unit in the mass spectrum. The series of peaks arise from ¹³C

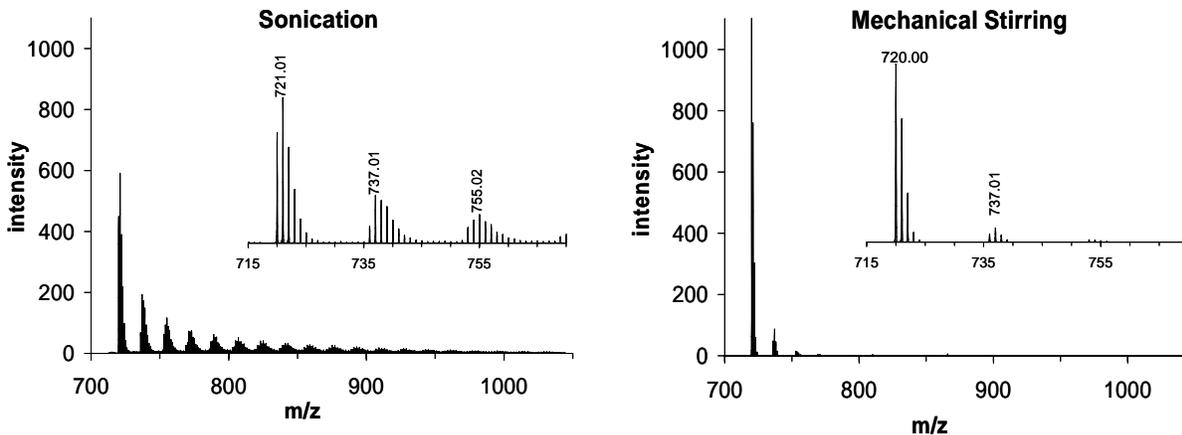


Figure 3. APPI MS spectra of aqueous nC₆₀ suspensions generated by 30 minutes of sonication (left panel) and 3 weeks of mechanical stirring (right panel). Peak intensities are scaled to remove differences in dissolved concentration. Insets are expansions of the 715 – 770 m/z region.

isotopologues of the parent ion in which one ¹²C atom is replaced by a ¹³C atom. Thus by simply counting the number of parent peaks separated by 17 mass units and their relative intensities we can determine the number of hydroxyl (OH) groups that have been added to fullerenes, and their relative abundances. The sonicated sample contains C₆₀(OH)₁₋₆ and a number of more complex oxidized fullerenes. For instance we also detect the epoxides (C₆₀O_x). **Figure 3** also demonstrates that the fullerol:fullerene ratio (C₆₀(OH)₁₋₆:C₆₀) is substantially greater in the sonicated sample (0.75) than in the mechanically stirred sample (0.1). The greater number and relative abundance of oxidized fullerenes in the sonicated sample likely contribute to the higher dispersed concentration of fullerene.

Aqueous nC₆₀ in the presence of NOM. Using mechanical stirring, it was possible to disperse nC₆₀ to a maximum concentration of 7.5 μM in water containing 20 mg/L Suwanee River NOM.

Compared to the 1.5 μM concentrations achieved in NOM-free water, this is a factor of 5 enhancement in the aqueous solubility of C₆₀. **Figure 4** shows that the dominant ionization mechanism in the presence of NOM is chemical ionization (yielding the protonated [C₆₀]^{H+} ion signal at 721 m/z) in contrast to C₆₀ dissolved in toluene where the dominant ionization mechanism is photo-ionization (yielding the [C₆₀]⁺ photo-ion signal at 720 m/z). This indicates that NOM serves as an effective proton transfer reagent in the photoionization process. Consequently, the presence of

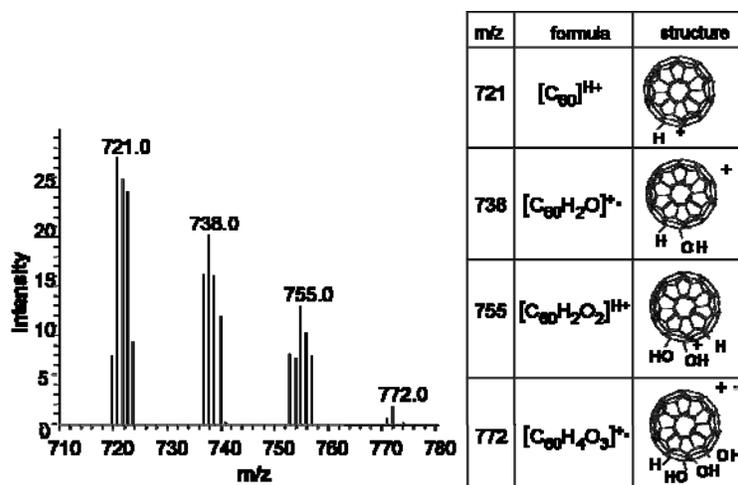


Figure 4. APPI mass spectral detection of nC₆₀ [5.4 mg/L] mixed with Suwanee River NOM [20 mg/L] by mechanical stirring (left). Several chemical derivatives of C₆₀ are also detected. The proposed molecular formulas and possible chemical structures are shown on the right.

NOM also enhances the ionization efficiency of fullerene C_{60} . The ionization efficiency of C_{60} is approximately doubled in the presence of 20mg/L NOM relative to NOM-free water. This may be an indication that the physical speciation of fullerene C_{60} is altered by aquatic NOM, specifically, the movement of fullerenes outside of the aggregated nanoparticle (nC_{60}) and into an association with the organic molecules that comprise NOM. It is also important to note that the apparent fullerol:fullerene ratio of 1.2 in **Figure 4** is substantially higher than the value of 0.1 for the corresponding nC_{60} sample made without NOM (**Figure 3**, right panel). Applying the calibration in **Figure 2** to the peak intensities of C_{60} and $C_{60}(OH)_{1-3}$, we estimate that the dissolved fullerene concentration is $\sim 5 \times 10^{-10}$ moles/L ($\sim 0.36 \mu\text{g/L}$). Thus the ionizable molecular fullerene concentration is only a small fraction (0.01%) of the total dispersed C_{60} concentration of 5.4 mg/L. Nevertheless, this is 45 times higher than the aqueous solubility in NOM-free water (1.1×10^{-11} moles/L (7)), and therefore may represent an ecologically significant increase in exposure for sensitive organisms.

Problems with APPI-MS of aqueous fullerenes. Despite the inherently high sensitivity of the APPI-MS for dissolved molecular fullerenes, the apparently low relative abundance of molecular fullerene in aqueous solutions is problematical. Since a very small fraction (<1%) of the total C_{60} is ionized by APPI, relatively high total C_{60} concentrations are required to achieve an adequate signal. Thus, the majority of the C_{60} which exists in the form of nC_{60} nanoparticles, does not ionize and instead accumulates inside the ion source (e.g. on the walls of the vaporizer). This creates problems for subsequent sample analysis. Our experience has been that solvents, dopants, or molecules in NOM that act as proton transfer reagents very effectively desorb and ionize the fullerene residues deposited during previous sample injections. It does not appear to be possible to circumvent this problem without cooling and thoroughly cleaning the APPI source between each sample. This is not a substantial issue for mechanistic laboratory studies or environmental case studies, but it suggests that commercially available APPI sources are not well-suited to routine environmental monitoring.

Task 3: Ultrahigh-resolution mass spectrometry of aqueous C_{60} fullerenes

High resolution MS analysis was originally intended to ensure that all C_{60} derivatives could be accounted for in a situation where NOM peaks are overlapping with chemical derivatives of C_{60} . However, we have delayed this task because we find that C_{60} and its derivatives photoionize more efficiently than the molecules present in NOM and therefore, interfering peaks from NOM are not a significant problem (see **Figure 5**, left panel). Nevertheless, it is interesting to note that with APPI it is possible to “turn on” the ionization of molecules present in the NOM by activating the chemical ionization mechanism (scheme 2) through the addition of toluene dopant (see **Figure 5**, right panel). In this case, APPI provides important chemical information about the NOM in addition to the C_{60} and its chemical derivatives. This important discovery may prove very useful in future studies because it would allow us to investigate the aqueous solubility of C_{60} in the presence of different types of NOM (i.e. from different aquatic ecosystems). Thus searching for correlations between NOM chemical composition and C_{60} solubility, it may be possible to determine which types of molecules in aquatic NOM interacting with, and facilitate the dissolution and transport of C_{60} . Similar investigations of coupled NOM and C_{60} chemistries are possible for photochemical or microbial decomposition experiments. These types of

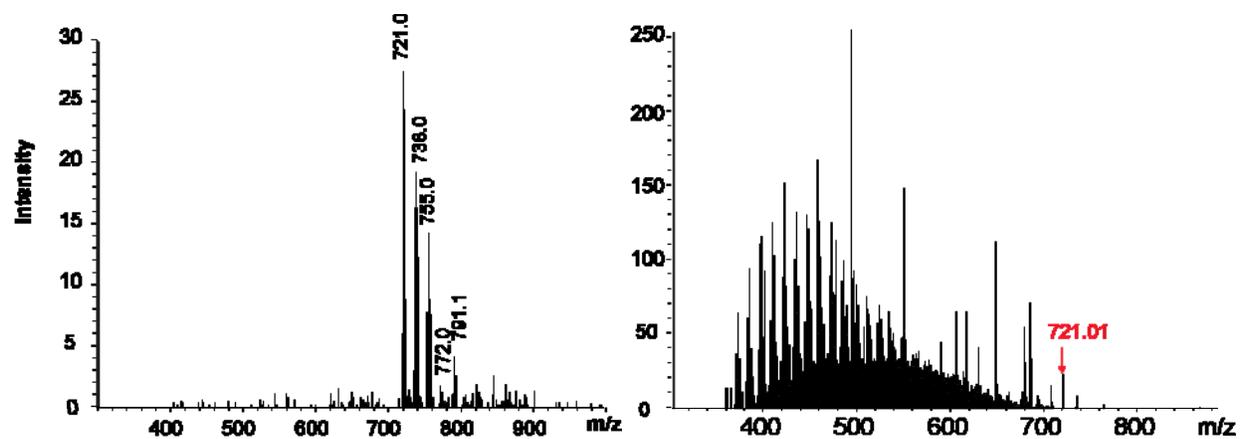


Figure 5. APPI-MS spectra of aqueous nC₆₀ mixed with Suwannee River NOM (left panel) and with addition of toluene dopant during sample injection (right panel). Toluene dopant facilitates chemical ionization of C₆₀ and molecules in the NOM (scheme 2) that are not amenable to direct photoionization (scheme 1). Note differences in intensity scales.

investigations would benefit from more sophisticated mass spectrometry, such as the Fourier transform ion cyclotron resonance (FT-ICR) MS originally proposed in Task 3.

[6] Discussion: Qualitative Aspects of APPI-MS Performance

The chemistry of the solution has a substantial influence on the appearance of the APPI-MS spectra of C₆₀ fullerenes. Presumably, this is because the solvents alter the physical speciation of the fullerenes themselves. **Table 1** summarizes the effects of the solution preparation upon the C₆₀ ionization mechanism and relative ionization efficiency. We suggest that the approximate

Table 1. C₆₀ ionization mechanisms and their relative efficiencies in various solution states

C ₆₀ solution state	Photo-ionization efficiency	Chemical ionization efficiency
Dissolved molecular C ₆₀ (organic solvent, toluene)	very high	high
Aqueous nC ₆₀ aggregates	low	TBD
Aqueous nC ₆₀ + NOM	moderate	moderate - high

Relative chemical- and photo-ionization efficiency is determined from the intensity of 721 and 720 m/z signals, respectively.
TBD = to be determined

doubling of C₆₀ ionization efficiency in the presence of NOM is evidence of the movement of fullerenes outside of the aggregated nanoparticle (nC₆₀) and into an association with the NOM in the same way that C₆₀ can be solvated and dispersed by commercial surfactants. This interpretation agrees with observations of decreasing nC₆₀ particle size upon addition of NOM

(16). However, the data shown in this report are not definitive evidence of C₆₀-NOM association in water because it remains unclear whether detachment of molecular C₆₀ from the nC₆₀ aggregate occurs in the solution phase (via dissolution into the NOM) or in the gas phase during nebulization in the ion source. Future work should determine the relative role of aqueous versus gas phase C₆₀-NOM interactions (see section 8).

With respect to fullerenes, APPI is a “soft ionization” process in the sense that the C₆₀ cage is not fragmented and functional groups (e.g., hydroxyl groups shown in **Figure 3**) are not eliminated during ionization. These are significant results because other ion sources such as electrospray and laser desorption are known to cause fragmentation problems and artifacts (e.g., 18, 19). Although ionization efficiency is low, the ability of APPI to ionize fullerenes in aqueous nC₆₀ nanoparticles is surprising because nC₆₀ nanoparticles are held together by electron donor-acceptor interactions (9) and the APPI source is free of electromagnetic fields that might disrupt the electronic interactions between fullerenes. We can not explain this phenomenon except to hypothesize that photoexcitation (see **Scheme 1**) and subsequent ionization of fullerenes on the surface of nC₆₀ aggregates may cause electrostatic repulsion between the positively charged [C₆₀]⁺ ion and the net – negatively charged aggregate to sufficiently disrupt a detectable quantity of C₆₀ molecules from the aggregate.

The poor ionization efficiency nC₆₀ nanoparticles could represent a shortcoming of APPI-MS in applications to environmental samples. Fortunately, this can be overcome by disrupting the nC₆₀ aggregates by increasing the ionic strength of the solution aqueous solution and then extracting the fullerene into an organic solvent such as toluene (20) for analysis by mass spectrometry (21).

[7] Discussion: Environmental Implications / Speculative Synthesis

While the formation of nC_{60} nanoparticles in water is a widely observed phenomenon, **Figures 3, 4, and 5** represent the first *in-situ* detection of molecular C_{60} and its derivatives in water. These novel APPI-MS data reveal important differences in abundance and chemical speciation of fullerenes as a result of aqueous solution composition. Especially noteworthy is the enhanced ionization of C_{60} and its oxidized forms in the presence of natural organic matter. **Figure 6** is a preliminary interpretation of the physical and chemical processes responsible for the gross differences of C_{60} solubility and ionization efficiency (**Table 1**) in presence of NOM.

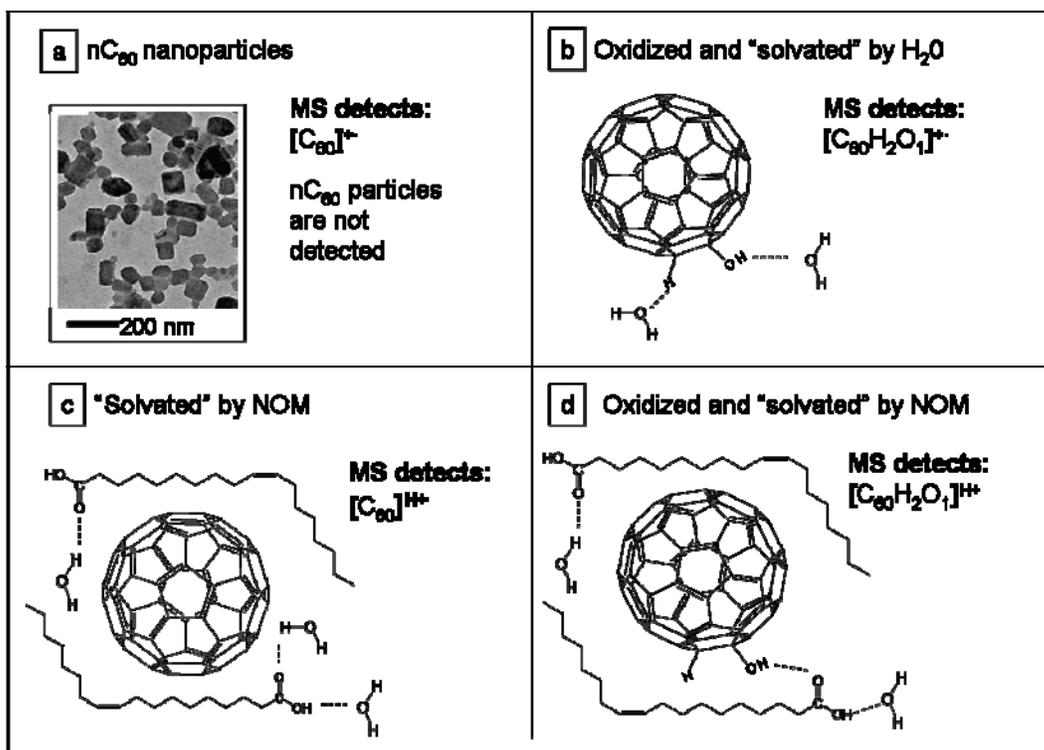


Figure 6. Schematic representation of 4 potential scenarios for aqueous C_{60} speciation based upon APPI-MS data (a) nanoparticle form, (b) oxidized molecular C_{60} dissolved by water molecules (c) native fullerene C_{60} dissolved by nonpolar regions of NOM, and (d) oxidized C_{60} dissolved by polar regions of NOM. Captions within each panel describe the MS signals detected for each scenario.

Our data suggest that NOM plays a role in stabilizing aqueous C_{60} outside of the nC_{60} aggregate, and therefore, may facilitate transport of molecular C_{60} and its oxidation products in aquatic ecosystems. This finding has major implications for the reactivity, bioavailability, and ultimate fate of engineered nanoparticles in the environment. For instance existence of molecular C_{60} fullerene and fullerene oxides in the aqueous phase may help explain discrepancies in fullerene toxicity which can not be explained by the production of reactive oxygen species (6)

[8] Future work

The primary focus of our future work on this topic will be on the fraction of C_{60} which exist in molecular form at low aqueous concentrations. *We believe that the ability to detect molecular fullerenes in the aquatic environment is an important advancement in our ability to assess the environmental implications of engineered nanoparticles.* Molecular C_{60} is probably a more biologically-available form of fullerene than the nanoparticulate form because its small size (0.7 nm) allows transport across biological membranes whereas particles are too large (50 – 200 nm) for direct uptake. However the behavior and chemical fate of molecular C_{60} is unknown because it has been undetectable until now. Therefore, the immediate future APPI mass spectroscopic analyses will be performed on the molecular fraction of aqueous C_{60} that has been separated from the nanoparticle forms by an ultrafiltration process. This will eliminate the contribution of ions from the nanoparticle C_{60} and simply interpretation of mass spectral data.

The NOM Effect on C_{60} Speciation. Interpretation of the results presented above on nC_{60} nanoparticles would benefit from a more rigorous investigation of ion source behavior. Therefore, future work will determine whether the “NOM effect” on C_{60} peak intensity is due enhanced dissolution of the C_{60} (**Figure 6c**) or simply an enhancement in the chemical ionization mechanism (Scheme 2). This requires the introduction of a well-known proton transfer reagent (e.g. toluene) to the nebulizer gas stream in the APPI source to determine whether the gas phase disruption of C_{60} aggregates is a viable mechanism for the enhanced chemical ionization we observed with NOM. If signal enhancement does not occur upon introduction of proton transfer reagent in the gas phase, then the enhancement by NOM can be attributed to dissolution of nC_{60} by NOM in aqueous phase. If signal enhancement from gas-phase dopants does occur then we have not disproved the aqueous phase dissolution mechanism, but it should be possible to apportion the relative amount of signal enhancement which occurs in the gaseous versus aqueous phases. Furthermore, we will have improved the ionization efficiency of nC_{60} – a valuable prospect for future experiments.

We will also briefly explore APPI ionization with the mass analyzer set to detect negatively charged ions. This may provide additional insight to the physical and chemical speciation of aqueous fullerenes. The formation of negatively charged ions occurs by an ionization mechanism known as electron capture (22). C_{60} fullerenes are excellent electron acceptors, and therefore, the capture of electrons from the photoionization of dopants may be another means of improving ionization efficiency of nC_{60} fullerenes.

Effect of UV irradiation on C₆₀ concentration and speciation

We prepared aqueous nC₆₀ by mechanical stirring and exposed it to UV light for 7 days. Using the method of Brant et al., (20), we dispersed the nanoparticles with MgClO₄ and extracted them into toluene for analysis by APPI-MS. As shown in **Figure 7**, the concentration of C₆₀ decreased by 97% relative to the dark control. We also found that the oxidized C₆₀ derivatives present in the aqueous sample (**Figure 3**, right panel) are not extracted into the toluene phase, and presumably remain in the aqueous phase. Therefore, future APPI-MS analyses will focus on determining the identity of C₆₀ photodegradation products in the aqueous phase.

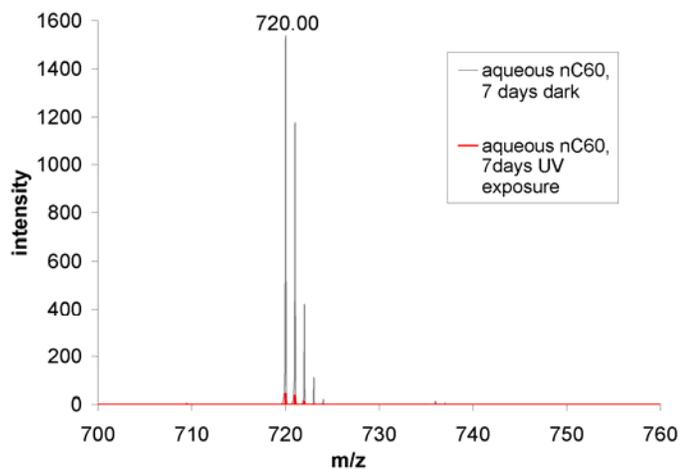


Figure 7. Exposure to UV light for 7 days caused the concentration of toluene-extractable C₆₀ (red spectrum) to decrease by one order of magnitude relative to the dark control (grey spectrum).

[9] References

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