AN ABSTRACT OF THE THESIS OF

Michelle R. Adlong for the degree of Honors Baccalaureate of Science in Environmental Engineering presented on June 1, 2010. Title: Quantification of C_{60} Particle Characteristics in Environmentally Relevant Aqueous Systems.

Abstract approved:

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The production of the fullerene C_{60} and its emerging applications in consumer products imply its ultimate release to the environment. Due to the molecule's relative novelty, the aqueous characteristics of C_{60} have not been fully assessed, though it may potentially harm human or ecological health. C_{60} is highly insoluble in water, and relatively little research has been performed on C_{60} aggregates that are released directly to natural waterways. In this research, multiple C_{60} suspensions were created via the suspended stirring method in natural Willamette River water, lab-made synthetic water, and deionized water. Analysis of these suspensions revealed that over a two month period, C_{60} particles in natural water are less stable than in synthetic water designed to mimic the natural water, and both are less stable than C_{60} particles that have been in suspension for a couple of years. Therefore, the behavior of C_{60} in real waterways with complex aqueous chemistry may be different than that predicted by lab experiments relying solely on synthetic water. This discrepancy should be considered when predicting the fate and bioavailability of C_{60} in natural waters.

Quantification of C_{60} Particle Characteristics in

Environmentally Relevant Aqueous Systems

by

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Michelle R. Adlong, Author

ACKNOWLEDGEMENTS

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DEDICATION

I dedicate this honors thesis to my family members, who have been a constant source of support. First, to my hard-working father Jeff, who has always encouraged me to push my boundaries and strive for my dreams. Second, to my politically active mother Jeannette, who has been a key influence on my desire to make the world a healthier, more equitable place. Third, to my passionate sister Justine, with whom I learned how to argue. Finally, to my cat Kenya, who passed away during the completion of this research. A loyal companion since before 5th grade, Kenya was a source of unconditional love, joy, and allergies, and he is deeply missed.

Quantification of C_{60} Particle Characteristics in Environmentally Relevant Aqueous Systems

1. BACKGROUND

1.1 Fullerene Discovery and Structure

Fullerenes remained undiscovered until 1985, when Robert Curl, Harold Kroto, and Richard Smalley unintentionally produced C_{60} while conducting astrophysics research at high temperatures.[1] The researchers identified the truncated icosahedral structure after inspiration from the geometry of the geodesic dome, a building designed by the architect R. Buckminster Fuller. This surname is the origin of the name "fullerene" as well as the nickname "buckyball." Although the researchers originally fabricated C_{60} in a lab, it also exists naturally at low concentrations in ash and other carbon sources that have been exposed to high temperatures.[2]

Fullerenes join graphite and diamond as the third allotrope of carbon. The carbon atoms in fullerenes form caged structures that resemble hollow spheres, ellipsoids, or tubes. C_{60} is the most commonly occurring fullerene, as well as the smallest possible perfectly spherical fullerene. C_{60} has 60 carbon atoms that are singly and doubly bonded in a pattern of 20 hexagons and 12 pentagons to form an enclosed molecule similar to a hollow soccer ball, as shown in Figure 1. The C_{60} molecule is extremely small; the diameter is approximately 7 Å, or 7 x 10^{-10} m.[3] Another common fullerene is C_{70}, while other bigger yet rarer fullerenes exist. Tubular fullerenes are commonly known as carbon "nanotubes." Finally, fullerene derivatives exist that have functional groups such as

alcohols attached.
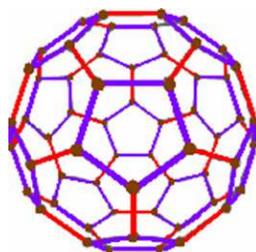
Figure 1: C_{60} structure.

1.2 Industrial Applications of Fullerenes

Though their existence has only been known for 25 years, fullerenes are already key components in a wide range of nanomaterials. Nanomaterials are man-made materials with a characteristic dimension smaller than 100 nm. Today, fullerene production on an industrial scale facilitates research and commercial use. Due to their unique cage-like structure, fullerenes offer promising new opportunities in the field of medicine. Like other nanoparticles, fullerenes have proven antibacterial properties, although the antibacterial mechanisms are not yet clear.[4] Fullerenes are currently being used in nanomaterials for medical imaging, drug delivery, cosmetics, and other products.[5]

The widespread production and use of C_{60} means that it is ultimately released to the environment. The physical and chemical characteristics of C_{60} and other fullerenes have not been completely assessed due to their relative novelty, however. C_{60} may potentially be harmful to the environment, biota, and humans. Many researchers are currently investigating the behavior of C_{60} in an attempt to determine their environmental

fate and toxicology.

One specific area of research—and the subject of this thesis—is the quantitative techniques used to characterize particulate C_{60}. These include the identification of C_{60} and the determination of particle size, zeta potential, and concentration. Essentially, this research aims to improve the methods of answering the questions, "What, how much, how big, and how stable?"

1.3 Theory

1.3.1 Particle Size

Hydrodynamic diameter is a measure for particle size, assuming that the particle is spherical. The hydrodynamic diameter is always slightly greater than the actual particle diameter, as it includes the double layer thickness associated with the particle as it moves through the surrounding fluid. Hydrodynamic diameter can be measured experimentally by dynamic light scattering that correlates light intensity with the diffusion coefficient and thus the diameter. Small particles undergo random movement called Brownian motion, which is proportional to the particle diffusion coefficient. The Stokes-Einstein equation relates the diffusion coefficient, D, to the inverse of the particle hydrodynamic diameter, d_p, as in Equation 1:

$$D = \frac{k_B T}{3\pi\mu d_p} \qquad [1]$$

where k_B is Boltzmann's constant, T is absolute temperature, and μ is dynamic viscosity. As hydrodynamic diameter increases, the diffusion coefficient decreases and the particle

motion slows.

The diffusion coefficient is measured by shining a laser through a particle sample at a known distance, and using a detector on the opposite side to record the intensity and angle of light passing through the sample. The decay time of the light signal fluctuations is an indication of particle size, as larger particles are expected to have longer signal decay times. The light signal patterns over time are put into an autocorrelation function, which calculates the diffusion coefficient. The diffusion coefficient is then used in combination with user-specified temperature and viscosity to calculate particle size from Equation 1.[6]

Dynamic light scattering is very effective at measuring monodisperse (uniform) particle size distributions. However, for wide-ranging distributions, the results will be more heavily weighted to the larger diameter particles. The instrument uses an intensity-weighted distribution to statistically determine the "average" particle effective diameter, d_{eff}, as shown in Equation 2:

$$d_{eff} = \frac{\Sigma N d_p^6}{\Sigma N d_p^5} \qquad [2]$$

where N is the number of particles of a particular diameter. Since d_{eff} is proportional to diameter to the sixth power, large particles, even if there are very few, will skew the output diameter reading to be bigger than the actual number-average or mass-average diameter.

Polydispersity is a unitless measure of the relative width of the particle size distribution, assuming a lognormal distribution. Polydispersity is less than 0.02 for monodisperse suspensions, 0.02 to 0.08 for narrow distributions, and greater than 0.08 for

polydisperse distributions. The polydispersity value is a useful indication of the variation of particle sizes within a suspension and the reliability of the effective diameter reading.

1.3.2 Zeta Potential

A particle's zeta potential is a good indication of its colloidal stability. Zeta potential is related to particle surface charge; it is the difference in electrical potential at the shear surface of the particle's electrical double layer and in the bulk fluid. Generally, particles with zeta potentials greater than 15 mV or less than -15 mV are considered stable. The high charge, either positive or negative, provides a repulsive force that prevents particles from further aggregation, stabilizing them. Figure 2 shows a diagram of zeta potential as it relates to surface charge.

(a)   (b)
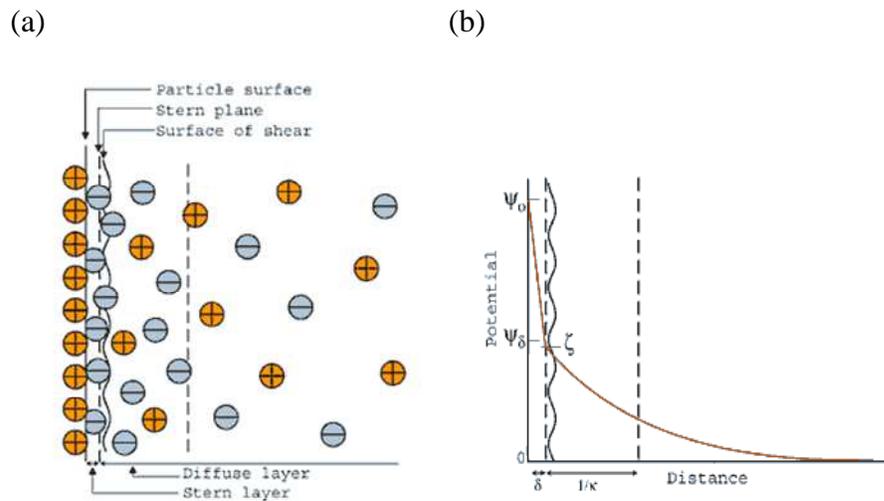
Figure 2: (a) Electrical double layer and its associated charges, and (b) zeta potential is measured at the shear surface.[7]

In practice, zeta potential, ζ, can be measured via electrophoretic mobility, *EPM*. The two are correlated as in Equation 3:

$$EPM = \zeta \frac{\varepsilon}{\mu} f(ka) \qquad [3]$$

where ε is the dielectric constant, μ is the fluid viscosity, and f(ka) is the order of unity. Electrophoretic mobility is determined by placing an electrode in a sample cuvette and exposing the sample to an electric field. Particles move in the presence of the field based on their EPM. As with DLS, the particle movement is measured by the scattering of light. The phase change of detected light is used to calculate the Doppler shift caused by the moving particles, which is then correlated to EPM.[8]

1.3.3 UV/VIS Absorbance

One method of measuring concentration of dissolved compounds is by light absorbance. Many organic compounds absorb or scatter light of a specific wavelength, either ultraviolet (UV) between 100-400 nm or visible (VIS) between 400-800 nm. The Beer-Lambert Law in Equation 4 describes the linear relationship between light absorbance, *A*, and the compound concentration, *C*:

$$A = alC \qquad [4]$$

where *a* is the extinction coefficient or molar absorptivity and *l* is the cell path length. A scan of the entire UV/VIS spectrum can reveal the wavelength of optimum absorbance for a particular compound. Different functional groups absorb light at different wavelengths, so a shift in peak wavelength may indicate a change in the chemistry of the compound. Although the UV/VIS technique is well established for dissolved compounds,

it is unclear whether it is also valid for particle suspensions.

1.3.4 Total Organic Carbon Analysis

One method of determining the total carbon concentration of an aqueous sample is by total organic carbon (TOC) combustion analysis. In this analysis, samples are acidified with hydrochloric acid and injected into a heated chamber with a platinum (Pt) catalyst. In the chamber the water is vaporized and the organic carbon undergoes catalytic combustion, being oxidized to carbon dioxide. The amount of CO_2 is then measured with an infrared detector, and the carbon concentration of the sample can be found. This TOC technique is useful because its method detection limit is 0.1 mg/L,[9] however, the process is also very sensitive to contamination. Additionally, the process takes several hours to complete, so samples from suspensions that settle gradually over time may not be representative.

2. OVERVIEW OF CURRENT RESEARCH & LITERATURE REVIEW

2.1 Methods of preparing aqueous C_{60} suspensions

C_{60} is extremely hydrophobic and is nearly insoluble in water after simply stirring. The molecule has been shown to have a solubility of approximately 8.0 ng/L, which is based on octanol-water partitioning coefficients and may be an overestimate. It also appears to have a high affinity for lipids, making its characterization and potential toxicity environmentally relevant.[10] Due to its low solubility in water, C_{60} tends to aggregate and form colloidal suspensions of particles. These particles are referred to as "nC_{60}" in this paper.

Characterizing particulates rather than soluble compounds presents challenges. The traditional method to overcome these is through preparation of C_{60} suspensions with organic solvents. Typically, suspensions are prepared by first dissolving dry, powdered C_{60} into an organic solvent such as toluene, tetrahydrofuran (THF), or acetone. Deionized (DI) water is added to the solution and stirred, and the C_{60} typically aggregates upon entering the aqueous phase. The organic solvent is then removed through extraction or distillation, leaving behind the nC_{60} particles and the water. The suspension may also be prepared through sonication of the mixture rather than stirring and distillation.[11]

A problem with this technique is that solvent residual may be associated with the fullerenes, altering its apparent behavior and toxicity. Extended stirring in water is an alternative preparation method that avoids the use of an organic solvent altogether. In this method, powdered or crystalline pure C_{60} is added to deionized water and stirred for long periods of time. Studies of dispersion kinetics have shown that it may take as little as two

weeks to several months to reach a stable dispersion by extended stirring in water.[12] Water-stirred suspensions of C_{60} are referred to here as aqu/nC_{60}.

Another method used to increase solubility is to derivatize the C_{60} with hydrophilic functional groups, but these fullerene derivatives may exhibit properties that differ from pure nC_{60}. For example, hydroxylated C_{60} is known as fullerol. Fullerol has been shown to produce reactive oxygen species such as superoxide under irradiation with ultraviolet or, to a lesser extent, visible light.[13] Another common functionalization is with a carboxyl (COOH) group. Further, carboxylic acids from natural organic matter, may also adsorb to C_{60} and act to stabilize it.

2.2 Effects of preparation method on particle size

The method by which a fullerene is introduced to the water has also been shown to affect the particle size. Methods which first dissolve C_{60} into an organic solvent typically result in particles with slightly smaller diameters than methods of extended stirring. Duncan et al. (2008) found that the mean hydrodynamic diameter of aqu/nC_{60} was 190 nm with a range of 20-600 nm, while the mean hydrodynamic diameter of a THF/nC_{60} particle was 220 nm with a range of 100-330 nm.[14] The polydispersity index (PDI) of the aqu/nC_{60} particles was a magnitude higher than the THF/nC_{60} particles, at 0.201 and 0.029, respectively. The PDI disparity indicates that the particle size range for aqu/nC_{60} was quite variable within the given range, while the THF/nC_{60} particles were more homogenous. The above results were reported after filtration of each suspension through a 0.45 μm pore-size syringe filter. However, filtration may have excluded large particles from consideration in the size analysis.

A previous study by Brant et al. (2006) concluded that there was no significant size difference between the two preparation methods. It reported a mean hydrodynamic diameter of 180 nm for aqu/nC$_{60}$ with a PDI of 0.146, similar to Duncan et al., but with a much wider range of 20-2000 nm.[15] It also reported a median diameter of 357 nm, which is nearly 100% greater than the mean diameter. The large difference between the median and mean diameters highlights the heterodisperse nature of the particles. It is difficult to compare particle sizes that are reported with different measures of central tendency. Most importantly, Duncan et al. found that filtration did indeed exclude some particles. Analysis of unfiltered samples identified aqu/nC$_{60}$ peaks at median values of 500 nm and 3 μm. However, it was not clear which analysis calculations were used to identify these peaks from the dynamic light scattering (DLS) data.

2.3 Effects of aqueous chemistry on particle size and stability

Environmental factors such as ultraviolet light (UV) exposure, natural organic matter (NOM), pH, and electrolytes may also alter the size and stability of nC$_{60}$ particles in water. These factors are important because they are typically present in surface waters in the natural environment. Most studies that try to simulate C$_{60}$ behavior in natural waters use lab-synthesized water with varying levels of NOM, pH, and electrolytes. Typically the particle size (via dynamic light scattering), concentration (via UV/VIS or total organic carbon analysis), and surface charge (via electrophoretic mobility) are measured to determine stability. The more negatively charged, smaller diameter, or highly concentrated suspensions are considered more stable.

Studies indicate that the presence of NOM stabilizes nC_{60} particles. Chen and Elimelech (2007) have shown that nC_{60} suspensions, in the presence of NaCl and humic acid, aggregate much more slowly, and ultimately have a smaller particle size, than those in the absence of humic acid.[16] They theorize that upon exposure to C_{60}, the NOM immediately adsorbs to the surface of the nC_{60} particle and causes steric repulsion, stabilizing it. However, it was also observed that in the presence of a high concentration of divalent salt such as 40 mM $MgCl_2$, the aggregation rate is higher than without humic acid. The authors suggest a different aggregation mechanism: that magnesium complexation occurs with the humic acid molecules, which then join C_{60} particles through bridging, forming larger overall particles. A TEM image of this aggregation effect is shown in Figure 3.
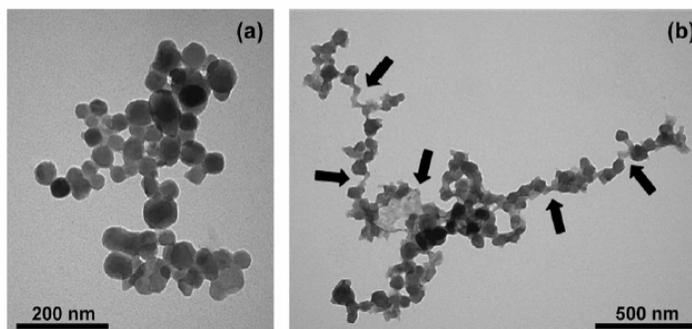
Figure 3: Chen and Elimelech's TEM images 3-4 hours after initiation of aggregation show (a) a fullerene aggregate in the presence of 1 mg/L TOC and 100 mM $MgCl_2$ at pH 7.5-8.5, and (b) a fullerene aggregate in the presence of 1 mg/L TOC and 40 mM $MgCl_2$ at pH 7.5-8.5.

The pH of the media also seems to affect the stability of the particles. In a study by Chang and Vikesland (2009), the electrophoretic mobility (EPM) of the more highly acidic suspensions are generally less negatively charged than the suspensions of higher

pH.[17] As EPM became increasingly negative, particle size also tended to decrease. Ma and Bouchard (2009) observed the same phenomenon; at lower pH, zeta potential was less negative and particle size was greater.[18] This may be because of the prevalence of positively-charged H^+ ions at low pH. Overall, higher stability was observed at higher environmentally relevant pH values.

Ionic strength also has a key effect on fullerene aggregation. In a separate study, Ma, Bouchard and Isaacson (2009) found that the fullerenes aggregated slowly at ionic strengths less than 50 mM NaCl, indicating a higher stability at lower ionic strengths.[19] Aggregation increased at ionic strengths between 50 mM and 300 mM NaCl, above which an increase in aggregation was no longer observed. They reported a critical coagulation coefficient of 260 mM NaCl. Although it was not specifically stated, the greater particle diameter at greater ionic strengths would be associated with a decrease in particle stability. As mentioned earlier, according to Chen and Elimelech (2007) the type of salt may also be important. At the same ionic strength, divalent cations such as Mg^{2+} or Ca^{2+} may cause a decrease in zeta potential compared to monovalent cations such as Na+.[16]

When water-stirred C_{60} is exposed to ultraviolet light, it tends to stabilize quickly, increase in suspended particle concentration, decrease in size, and possibly undergo chemical transformation. According to Li et al., the combination of UV light and NOM at a low ionic strength led to particle sizes below 5 nm and steadily increasing concentrations of nC_{60} in suspension, to upwards of 10 mg/L, after 3 days of stirring.[12] The particles also exhibited a more negative EPM, an indication of increased stability. In

comparison, solutions exposed to fluorescent lighting and darkness maintained low concentrations and relatively large particle sizes.

Based on UV/VIS spectra, the study even suggested that chemical transformation of the C_{60} was occurring, possibly due to the formation of photochemical oxidants as mentioned earlier. A study by Hou and Jafvert (2009) also concluded that photochemical transformation was occurring. In that study, nC_{60} in the presence and absence of NOM was exposed to sunlight, and concentrations of nC_{60} decreased.[20] However, the complete disappearance of a fraction of the total organic carbon, without explanation, makes the validity of the study questionable.

Already it has been seen that nC_{60} particle size and stability varies with many variables including level of exposure to ultraviolet light, ionic strength, pH, organic matter, and functional groups. Still, researchers have attempted to model nC_{60} particle characteristics in natural waters based on these parameters. How well can the experiments approximate natural water with the complex aqueous chemistry of, for example, the Willamette River? One nanoparticle study by Gao et al (2009) suggested that there are differences in the toxicology of nanoparticles in natural water, in this case the Suwannee River, when compared to nanoparticles in lab-made water.[21] Further investigation of the differences of particle characteristics in the natural water versus lab-made water would aid the scientific community to assess the behavior of nC_{60} in actual natural water.

3. C_{60} CHARACTERISTICS IN NATURAL WATERS

3.1 Goals

Many experiments with C_{60} have been performed to mimic environmentally relevant conditions. These experiments primarily involve adding salts and humic acid to double deionized (DDI) water in concentrations that are similar to that of natural waters. However, C_{60} behavior in natural water may be more complex than is observed in the "synthetic" water suspensions. The objective of this experiment was to determine how well the lab-made synthetic waters mimic C_{60} behavior in natural waters over extended periods of time. This was accomplished by extended mixing of C_{60} in actual Willamette River water as well as in synthetic water with the same characteristics as the river water. It was found that the two suspensions did behave differently, as reflected by significantly differing zeta potentials and visual appearance of the suspensions.

3.2 Sample Preparation

The natural water sample was taken from the inlet of the City of Corvallis's Taylor Water Treatment Plant. The natural water was analyzed for cations and anions, alkalinity, pH, and total organic carbon, and these results are summarized in Table 4 in Appendix A. A sample of "synthetic water" was prepared to match the natural water characteristics as closely as possible. DI water of resistance greater than 17 MΩ/cm was adjusted with a monovalent salt, NaCl, to an ionic strength of $8 \cdot 10^{-4}$ mol/L. Stock Aldrich humic acid that had previously been analyzed for total organic carbon (TOC) was added

to attain a TOC concentration of 1.2 mg/L. The synthetic water represents the media that most labs use to simulate natural water in their C_{60} experiments, while the Willamette River water represents actual natural water.

Both waters were then passed through a 0.22 μm vacuum filter, followed by a 20 nm syringe filter. The 0.22 μm filtration step was performed three times, while the 20 nm filtration step was performed once due to the highly time-consuming nature of such ultra filtration. The pH of the synthetic water was then adjusted to 6.3 to match that of the natural water. After preparation, each filtered media was stored in a 500 mL nalgene container in a 5°C refrigerator.

Samples of dry, powdered C_{60} of 99+% purity from MER Corporation were massed and added to 250 mL of each media to achieve a total theoretical suspension concentration of 100 mg/L. A stir bar was added, and the volumetric flasks were stoppered and placed on stir plates. The mixtures were continuously stirred for the duration of the eight-week experiment at room temperature and ambient lighting.

During weeks 1, 2, 3, 4, and 8, samples of approximately 30 mL were removed from each flask with pipettes. Samples were drawn about 2 cm below the water surface and then placed in small graduated cylinders or beakers and stored in a 4°C refrigerator until analysis was complete. Analysis typically took around 2 days, during which the samples were not being stirred. About 5 minutes prior to removing each sub-sample, the flasks were swirled around to resuspend any settled particles and draw a more uniform sample.

After sample removal, a series of filtration steps were performed, with sample analysis associated with each filtration step. Samples were first analyzed as whole,

unfiltered fractions. They were then were passed three times through a 1.0 µm glass microfiber (GMF) syringe filter, after which samples were analyzed again. Finally, they were passed through a 0.45 µm GMF syringe filter and analyzed a final time. The two filtration levels were decided after an earlier experiment with aqueous nC_{60}, which also filtered the samples at 0.20, 0.10, and 0.02 µm but found that the smaller filter sizes were unnecessary. A detailed experimental procedure and sampling schedule can be found in Appendix B.

3.3 Analysis Techniques

Samples were analyzed with multiple techniques. Zeta potential of the particles was measured via electrophoretic mobility with a BIC ZetaPALS Zeta Potential Analyzer using an aqueous (AQ) electrode. Testing of the standard with this electrode revealed that its readings were slightly high; measurement of a standard should read -54 mV with some room for error, though the electrode typically read -40 to -50 mV. Cuvettes were examined to ensure the absence of any air bubbles within the cuvette prior to sampling.

The particle hydrodynamic diameter was measured via dynamic light scattering (DLS) with the ZetaPALS particle sizing software. Samples of 50-200 µL C_{60} suspension were diluted into approximately 2.5 mL of filtered natural or synthetic water, depending on the origin of the sample. Prior to addition of the C_{60} samples, the background dilution water was analyzed by the DLS instrument to ensure no dust contamination.

Concentrations were measured via total organic carbon with a Shimadzu TOC autosampling combustion analyzer. Samples were tested at varying dilutions to ensure readings within calibration limits. Attempts were also made to measure concentration via

UV-VIS spectrum with an HP 8453 spectrophotometer. Prior to sampling, the instrument was blanked with the appropriate media. Quartz cuvettes were used and the samples were not diluted.

3.4 Results

3.4.1 Observations

When originally mixed, the C_{60} appeared extremely hydrophobic. The particles grouped together and swirled around like a soot-colored snow globe. After four to seven days, a brownish color predominated the suspension and the number of large clumps appeared to diminish. There were still particles, however, that clustered along the glass walls at the surface of the water. Other particles appeared to adhere to the surface of the magnetic stir bar. Over the next few weeks, there were fewer visibly noticeable black particles and the brown color of the suspension persisted. Although the main mixture was continuously stirred, when samples were taken each week, black particles tended to visibly settle to the bottom. Figure 4 shows the C_{60} suspensions after two weeks of stirring.

After approximately eight weeks, there were noticeable changes in the color and opacity of the synthetic water. It appeared to increase in opacity and become a richer, orange-brown. Particles seemed to remain suspended rather than settling out. In contrast, the Willamette River water did not exhibit such obvious changes. Its color persisted to be a weak gray-brown and particles continued to settle after periods of non-mixing.

Figure 5 shows the samples ready to be analyzed after eight weeks of stirring. It appeared that the Willamette River C_{60} settled out significantly more than the synthetic water C_{60}. The synthetic water also appeared to be more opaque and to have a more intense, rusty brown color. These visual observations indicate that after eight weeks, the synthetic water suspension was considerably more stable than the natural water suspension.

Figure 4: Continuously stirred solutions at two weeks in the volumetric flasks. C_{60} in Willamette River (left) and C_{60} in synthetic water (right) appear similar.
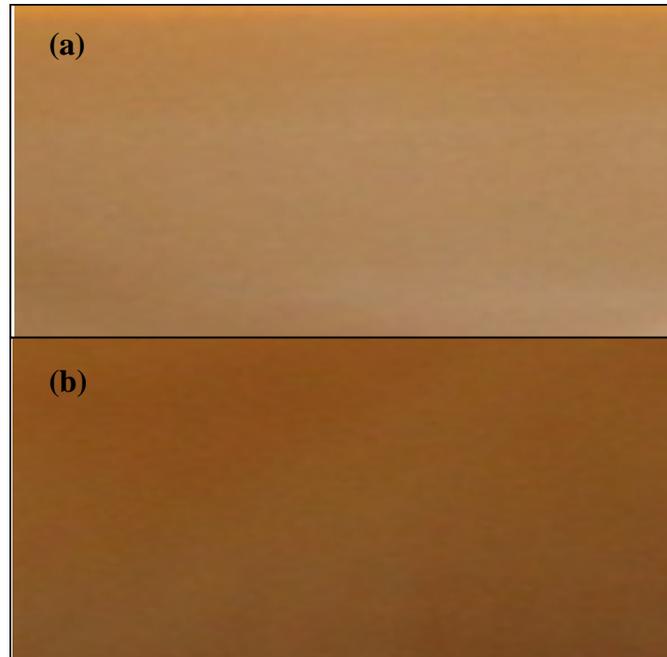
Figure 5: (a) Natural water and (b) synthetic water suspension samples at 8 weeks, after sitting unstirred for about 15 minutes.

3.4.2. Zeta Potential Results

Zeta potentials in this experiment were analyzed with a Brookhaven ZetaPALS (phase analysis light scattering) instrument. The zeta potential of the synthetic water C_{60} was consistently and significantly higher than the zeta potential of the C_{60} in natural water. The synthetic water zeta potential averaged -35 mV over the duration of the experiment with a standard deviation of 9 mV, while the natural water averaged -17 mV with a standard deviation of 3 mV. Additionally, the zeta potential of the synthetic water appeared to increase slightly over the time course of the experiment. However, the scatter in the data is too great to determine the significance of this trend. The zeta potential vs. time results are shown graphically in Figure 6.
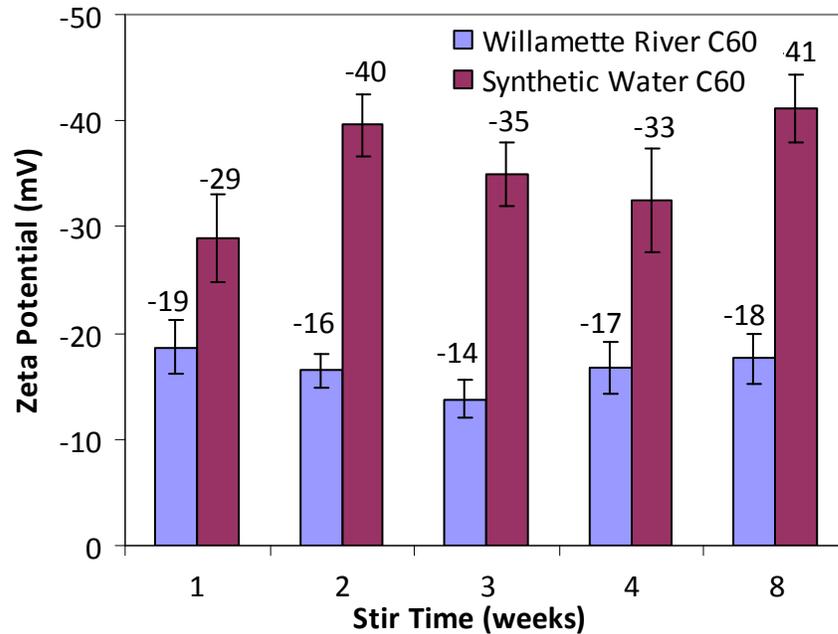
Figure 6: The average zeta potential versus time for the natural and synthetic waters. Error bars are one standard deviation above and below the means.

Conductivity of the suspensions was also recorded during the zeta potential measurements, and the conductivity was higher for the synthetic water than the natural water. The cause is likely differences in ionic strength, although the synthetic water solution was made with NaCl to match the ionic strength of the natural water. Figure 7 shows average zeta potentials for all filtration steps for the particular media and week number. The synthetic water had a more negative zeta potential as well as a higher conductivity. However, it has been observed by previous authors that a higher ionic strength generally causes a decrease in particle stability and a less negative zeta potential. Therefore, these results seem to be at odds with previous authors' findings.
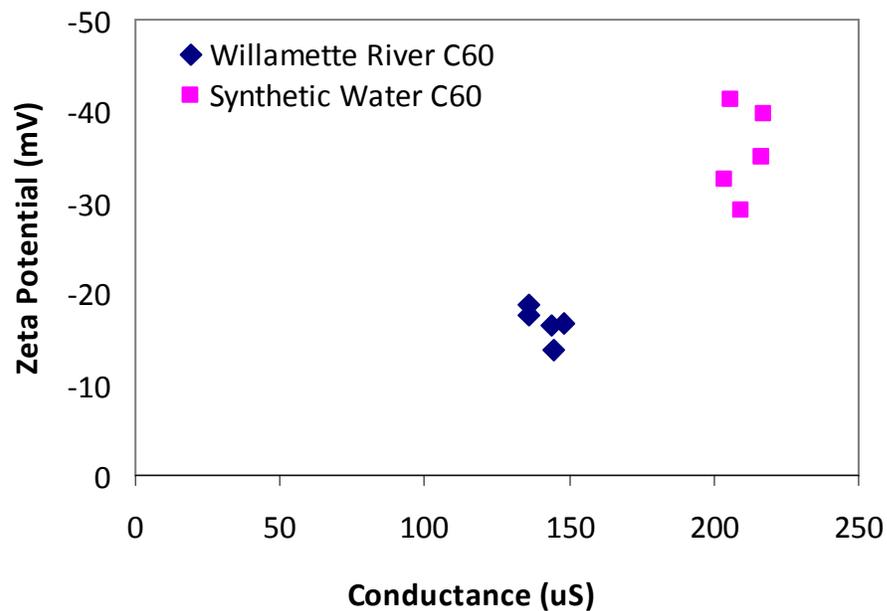
Figure 7: Average zeta potential vs. conductance over the 8-week experiment.

There may not necessarily be a direct cause-and-effect relationship between ionic strength and zeta potential here. Other factors, such as differences in the organic matter of the natural and synthetic water samples, may have caused the differences in zeta potential. Humic acid is a component of natural organic matter, and is present in soil and peat as the result of decaying plants. The composition of humic acid depends on the source geography, although in general it includes carboxylic and phenolic acid groups. Aldrich humic acid is a commercially sold humic acid from a specific source. In contrast, the natural organic matter in the Willamette River is more complex and variable, and may include different organic compounds. Therefore, the particle interactions with the NOM in the natural water may be different than with the Aldrich humic acid due to different humic acid composition.

Additionally, the molecular weight of the humic acids may also have an effect on particle interactions. The molecular weight of humic acid can vary from 2,000-500,000 g/mol.[22] According to a study by Johnson et al (2002), lower molecular weight components of humic acid adsorb to activated carbon preferably over higher molecular weight components.[23] If this is also true for nC_{60}, perhaps the Aldrich humic acid had a higher fraction of low-molecular weight compounds, while the NOM present in the Willamette River had longer chains of higher molecular weight.

3.4.3. Particle Size Results

Particle size measurements were taken with a Brookhaven ZetaPALS instrument with the BI-MAS dynamic light scattering (DLS) option. Prior to analysis, 50 μL of sample was placed into a plastic cuvette filled with filtered media. At least two samples were sized for each mixture and filtration step. The DLS instrument analyzed each sample for three runs at three minutes per run, equaling a total of nine minutes. The multiple runs resulted in a "combined" output reading for hydrodynamic diameter, which is statistically more accurate than the "mean" reading from each individual run. Other output data from the DLS instrument included the diameter standard error, the polydispersity, and the polydispersity standard error. The DLS instrument also has an option to use a "dust filter," which uses an algorithm based on standard deviation to exclude the particles of unusually large size from analysis. The dust filter was not used for nearly all of the runs.

As a whole, no significant particle size trends with time were observed. Although changes in individual particle size were likely occurring, the polydisperse, widely ranging particle sizes obscured the collection of meaningful data.

Table 1 summarizes the overall particle size results averaged over the entire experiment duration. nC_{60} particles in synthetic water appeared to be slightly smaller than the nC_{60} in natural water for each filtration step, although this difference may not be statistically significant. Particles were extremely heterodisperse, and the polydispersity at each filtration step was nearly the same for each media. The greatest polydispersity of 0.52-0.55 occurred after the 0.45 µm filtration step, and the least polydispersity of 0.35-0.36 occurred after the 1.0 µm filtration step.

Table 1: Average and range of particle sizes for both synthetic and natural waters over the entire duration of the experiment.

Media	Particle Hydrodynamic Diameter (nm)			Polydispersity
	Mean	Minimum	Maximum	Mean
Natural Water				
Unfiltered	20,000	1,120	44,200	0.43
1.0 µm filtered	1,097	660	1,589	0.36
0.45 µm filtered	360	9	640	0.55
Synthetic Water				
Unfiltered	6,500	1,040	23,900	0.43
1.0 µm filtered	1,000	360	1,820	0.35
0.45 µm filtered	200	0	400	0.52

As expected, each filtration step led to lower mean particle sizes. This is most likely due to size exclusion as a predictable result of the filtration process. However, there were still particles present that were greater than the rated filter size. This is most likely due to larger particles passing through the filter, but could possibly be due to aggregation after filtration. The average particle sizes for unfiltered particles were larger than expected, ranging from 1120-44200 nm in diameter. It is possible that dust may have contaminated the sample to cause such large diameters. However, when the dust filter was applied for comparison purposes, the results were not significantly different. Additionally, the instrument's size range is 2 nm to 3 µm, meaning that readings greater than 3 µm are not necessarily reliable or accurate. Figure 8, Figure 9, and Figure 10 show the data over the 8-week experiment for each filtration step and both medias. Trends with time were not observed for any of the filtration steps.

Unfiltered Average Particle Sizes
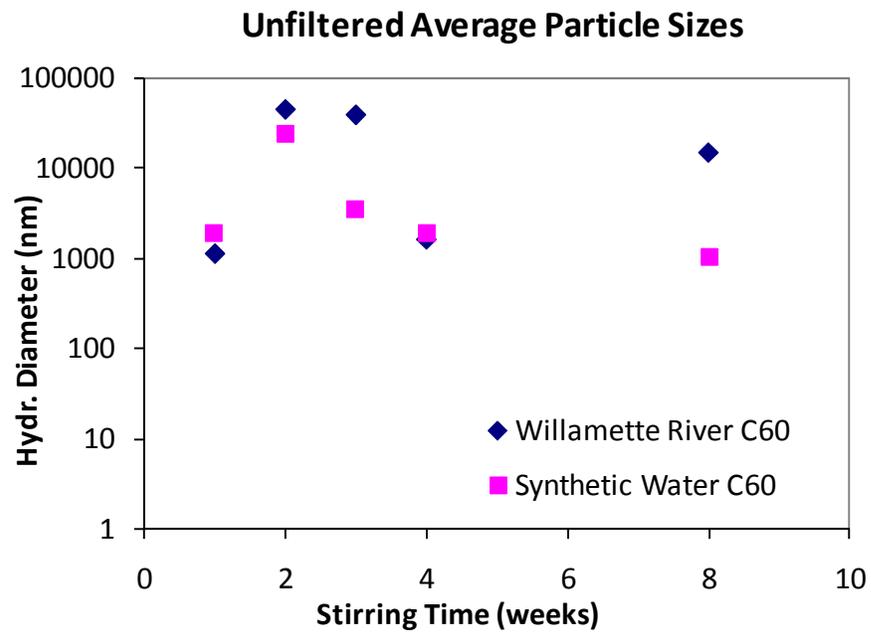
Figure 8: The average particle sizes for the unfiltered suspensions. The y-axis is logarithmic due to the wide range of results.

1.0 μm-Filtered Average Particle Sizes
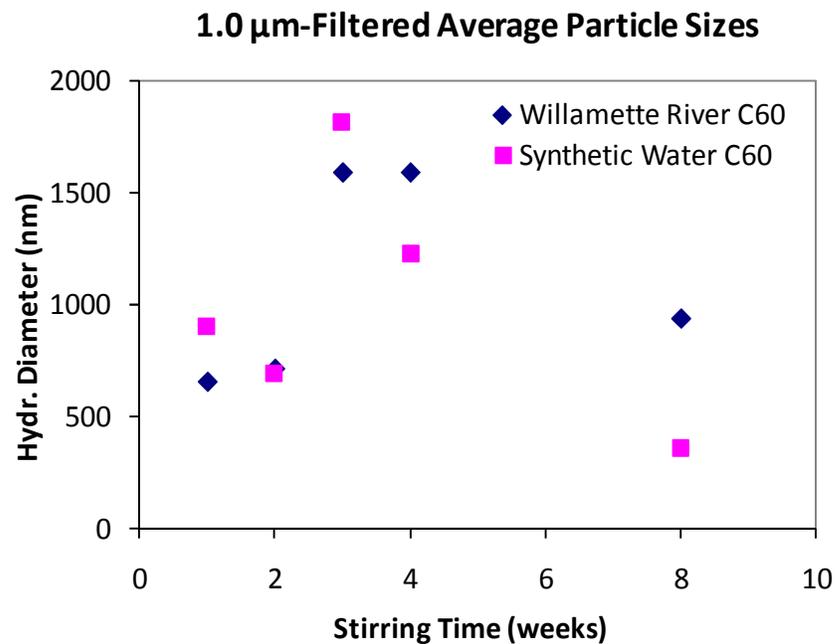
Figure 9: Average particle sizes for the 1.0 μm filtered suspensions, with a linear y-axis.

0.45 µm-Filtered Average Particle Sizes
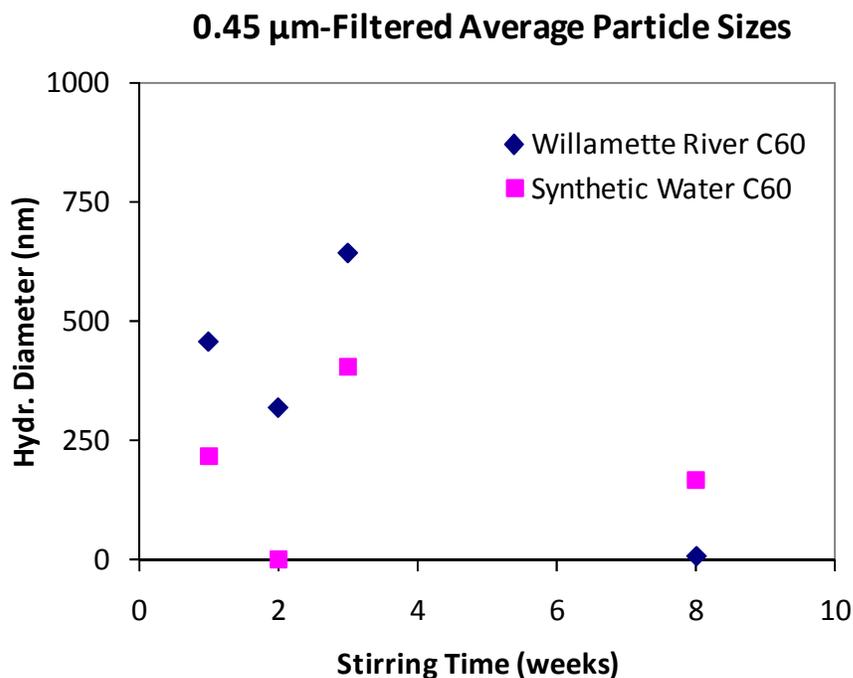
Figure 10: Average particle sizes for the 0.45 µm filtered suspensions.

Some of the extremely large particle diameters in the unfiltered samples could be due to dust particle contamination of the samples or instrumental correlation error as described previously. For the 1.0 and 0.45 µm-filtered samples, a few particles with a diameter greater than the filter size appear to have broken through the filter. However, this is possible due to the nature of the glass microfiber filters. Interestingly, at week eight the 0.45 µm-filtered samples resulted in particle diameters less than 200 nm for the synthetic water and nearly 0 nm for the natural water. There could be many explanations; there may have been only small particles left after filtration, or few to no particles less than 0.45 µm in the natural water case. Another possibility is that eight weeks of stirring

may have been enough to finally cause a decrease in particle size. However, in general data here is too scattered to make conclusions about the effects of time on particle size.

The particle size versus time results were inconclusive due to several potential factors. As described earlier, the samples may have been unintentionally contaminated with dust over the course of the experiment as samples were removed and the stirring flasks were exposed to air. Second, the polydispersity was greater than 0.2 in most cases indicating a heterodisperse suspension, and the standard deviations were also high for the unfiltered solutions. Therefore, a wide range of particle sizes were present. Third, the instrument heavily weights large particles over smaller particles, even though the ratio of the number of large to small particles may be quite small. This insensitivity to small particles when the suspension is heterodisperse may result in large diameter outputs unrepresentative of the majority of the suspension.

Finally, observations indicated that if the particle sizing was performed immediately after the cuvettes were inserted into the instrument, the results would be unusually high. Therefore, most trials were run 5 to 10 minutes after the cuvette was inserted, which tended to give more reasonable results; it is possible that this allows the extremely large outliers to settle out. However, the settling time was not standardized from the start of the experiment.

Overall, the most important finding from these particle size measurements is that filtration does indeed influence the particle size results. In many previous studies, a 0.45 µm filter was used prior to size analysis, and the resulting particle diameters were then found to be less than 0.45 µm. In contrast, this study examined the size of the whole unfiltered fraction of particles as well as filtered fractions, and found that the resulting

particle diameter depends on the size of the filter used. Therefore, at least for the first several weeks of stirring in aqueous suspensions, the particle size and range of nC_{60} may be much greater than indicated by previous studies.

3.4.5. TOC Results

After one week of stirring, samples were removed at all filtration steps and analyzed for total organic carbon with a Shimadzu TOC combustion analyzer at the Institute for Water & Watersheds Collaboratory. The instrument takes several hours to run, therefore most of the large particles would have settled out. As seen in Table 2 and Figure 11, less than 6% of the C_{60} was "dissolved" at this point in time after accounting for the background humic acid and natural organic matter. Predictably, this percentage decreased with each filtration step.

Table 2: TOC concentration and standard deviation after one week of stirring. Initial concentration of C_{60} added to the media was 100 mg/L. .

	Average TOC (mg/L)		
	Unfiltered	**1.0 μm filtered**	**0.45 μm filtered**
Willamette River Average	4.49 ± 1.48	0.91 ± 0.17	0.29 ± 0.03
Synthetic Water Average	3.3 ± 0.08	0.49 ± 0.09	0.44 ± 0.10

(a)

(b)
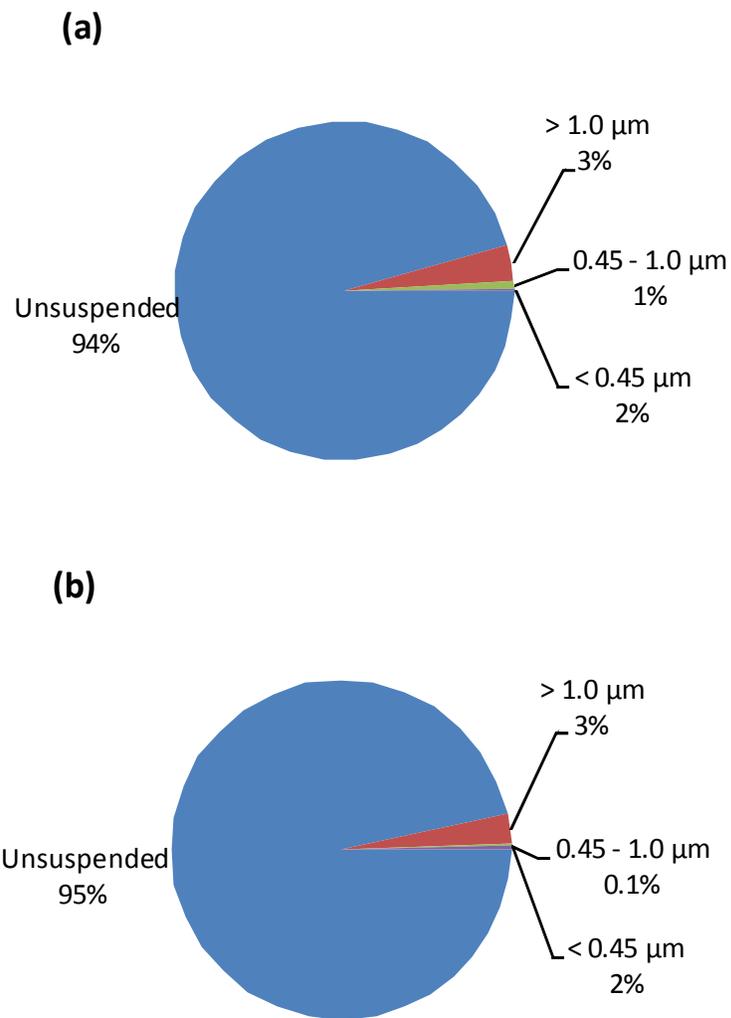
Figure 11: Pie chart of C_{60} concentration percentages after one week of stirring for various hydrodynamic diameters of (a) Willamette River C_{60} and (b) synthetic water C_{60}.

There is not enough information to draw conclusions about differences between the Willamette River and synthetic water dissolved C_{60} concentrations. In this data, it is assumed that *all* particles that pass through the filters are smaller than the pore sizes mentioned. However, in actuality there are most probably a small number of larger particles that were not filtered out. If there were more time, it would be valuable to

measure the TOC concentrations for the week 8 samples and compare them with the increased UV/VIS absorbance. As will be explained in the following section, the expected result would be that the TOC concentration increases, at least for the synthetic water, over the course of the eight weeks.

3.4.4. UV/VIS Results

An HP 8453 Spectrophotometer was used to measure the absorbance spectrum of each mixture at each filtration step. Samples were put in glass cuvettes and scanned over both the ultraviolet (UV) and visible (VIS) light spectrums, and the UV range of 300-400 nm appeared to be the range of interest. Nearly every sample had an absorbance peak around 366 ± 2 nm, with the more defined peaks occurring from the natural water samples.

Spectra of the unfiltered mixtures for this range are shown in Figure 12. Interestingly, the synthetic water C_{60}'s absorbance increased over time, suggesting a possible increase in unfiltered C_{60} concentration that was stable and did not settle out. This reading concurs with the physical appearance of the mixture and its richer, deeper brown color.
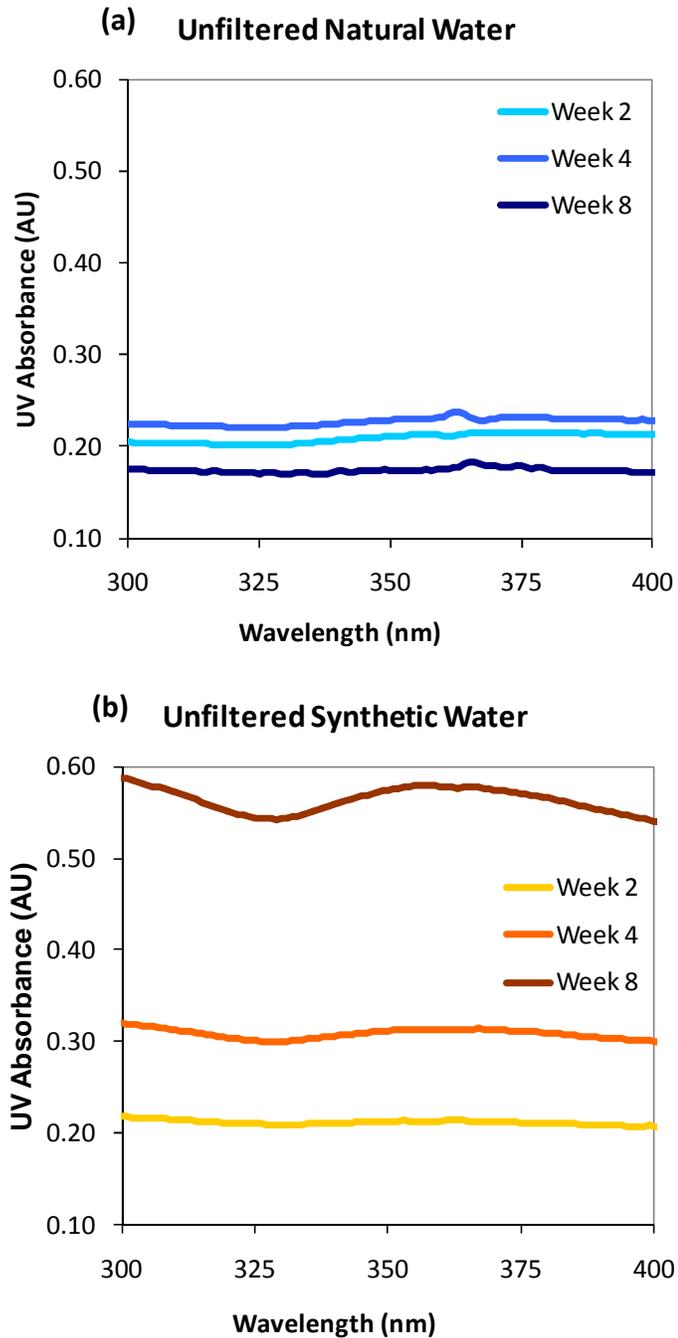
Figure 12: Absorbance from 300 – 400 nm of (a) unfiltered Willamette River and (b) unfiltered synthetic water C_{60} for two, four, and eight weeks.

The UV absorbance data is valuable because it is a simpler, quicker way to determine concentrations as compared to TOC combustion analysis. UV/VIS absorbance

is often used to find concentrations of soluble compounds. However, there have been

questions about whether UV/VIS can appropriately indicate concentrations of particles

rather than aqueous compounds. A comparison of results from two-week stirred nC_{60}

indicates that there is indeed a linear correlation between carbon concentration and

absorbance. This relationship is shown below in Figure 13. The square of the correlation

coefficient, r^2, is 0.83. Although the trend is significant, additional trials may be

performed to increase the precision of the correlation if UV/VIS is to be the sole

concentration determination method.
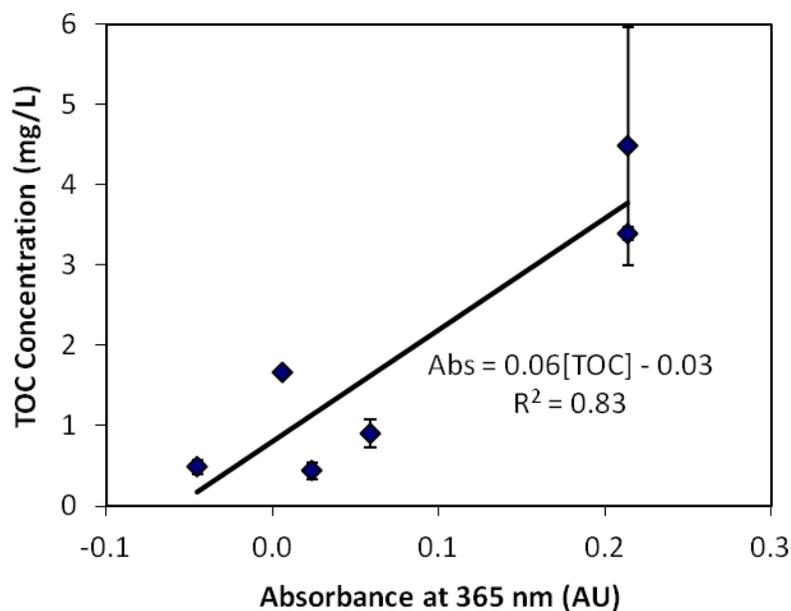
Figure 13: UV absorbance versus TOC concentration of fullerenes after two weeks of stirring. Both the natural and synthetic water media are shown; error bars represent one standard deviation above and below the mean.

Even if the UV/VIS is detecting dissolved C_{60} rather than the particles themselves, the overall carbon concentration can still be determined with this method. An assumption must be made that the dissolved concentration to particulate concentration ratio, although small, is constant throughout the duration of the experiment. Since TOC measures all carbon present, this ratio should be accounted for within the regression equation.

3.5 Overview of Findings and Suggestions for Future Work

Overall after eight weeks of stirring, the nC_{60} particles exhibited a higher stability in the synthetic water than in the natural Willamette River water. In particular, the synthetic water suspension had a higher zeta potential and visually appeared to be more stable. The differences between the two types of water may be due to error in experimental design. However, they also may be due to differences in the natural organic matter present. If this is the case, researchers should be aware that using a model humic acid, such as Aldrich or Suwannee, to mimic the natural organic matter a natural waterway may not accurately reflect the complex aqueous chemistry of that waterway. It is suggested that more experiments are performed with filtered natural water from local rivers and streams to better characterize fullerenes in the natural environment.

If this experiment were to be continued , it would be valuable to make a few changes. First, the ionic strength of the synthetic water should be double checked to make sure that it is indeed equivalent to that of the natural water. Second, the pH should be regulated throughout the course of the experiment. In this experiment the pH was initially set, but it was not altered during the course of the experiment because of the concern with

contamination of the entire stirred sample. A solution may be to add a pH buffer that is not expected to cause significant particle interaction. Finally, due to the potential photochemical reactions, it is suggested to stir a control sample in the dark.

It would also be useful to measure the zeta potential, particle size, UV/VIS absorbance, and possibly TOC of the suspensions for a longer time period. At this date (nine months later) the aqueous suspensions are still stirring, and could potentially be analyzed if there were more time. Visual observations suggest that the natural water is richer in color and settles more slowly than during the first eight weeks of stirring, meaning that over time it may have increased in stability. It would be interesting to see if zeta potential or any of the other characteristics validate this observation.

4. C_{60} PARTICLE SIZE AFTER TOLUENE AND SALT EXTRACTION

4.1 Background

One of the methods of quantifying the concentration of C_{60} is by liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS).[24] This method requires an organic solvent; it cannot measure C_{60} particle concentrations directly from the aqueous phase. However, it is possible to start with an aqueous suspension of fullerenes and perform a series of extractions with an organic solvent followed by LC/ESI-MS. First, the suspension is shaken with toluene, during which time the hydrophobic fraction of the C_{60} is believed to enter the toluene phase. After extraction of the toluene, salt is added to the remaining aqueous C_{60} followed by more toluene. Additional C_{60} then enters the toluene after being "salted out." The toluene is then extracted and the C_{60} concentration quantified by LC/ESI-MS.

4.2 Goals

The goal of this experiment is to determine the size of water-stirred particles that are associated with various steps of the LC/ESI-MS extractions. These results can then be coupled with concentration data in an attempt to characterize the nature of the fullerenes present in the aqueous suspension.

4.3 Sample Preparations & Procedures

4.3.1 Procedure for extractions of fullerenes in DDI water

In the first experiment, two simultaneous samples were prepared in double

deionized water. For each sample, approximately 20 mL of a stock fullerene suspension

was used that consisted of 100 mg/L C_{60} in DDI water that had been stirring for

approximately 2.5 years. This represented a well-stirred suspension with particle sizes

that were not expected to change with time. The initial C_{60} particle size was measured

with the Zeta PALS particle sizing instrument prior to performing any experiments. All

samples for the particle sizing were pipetted from approximately 2 cm below the surface

of the well-stirred suspension in order to pull a representative sample.

2.5 mL of toluene was then added to each aqueous sample and agitated on an

orbital shaker for 30 minutes, followed by 15 minutes of resting and extraction. This step

was repeated twice more for a total of three extractions with toluene. A sample from the

aqueous phase was then analyzed for particle size. Next, 2 mL of 1 M $Mg(ClO_4)_2$, the

salt, along with 2.5 mL of toluene was added to each aqueous sample. The samples were

agitated, rested, extracted, and repeated twice more as described above. Particle size was

again analyzed. All particle size measurements utilized aqueous C_{60} samples of 50-100

μL in a background media of approximately 3 mL DDI water; exact concentrations of the

C_{60} were not of concern. Data was collected for three runs at three minutes per run.

4.3.2 Procedure for extractions of fullerenes in humic acid & DDI water

In order to imitate more environmentally relevant conditions, a similar experiment

was performed for C_{60} in DDI water with humic acid added. Aldrich humic acid was

diluted to 40 mg/L and filtered three times through a 20 nm-pore size syringe filter. The stock well-stirred 100 mg/L C_{60} was also diluted with DDI, and the two were combined for final concentrations of 37.2 mg/L C_{60} and 13.6 mg/L AHA. The suspension was then stirred continuously for three days under ambient lighting conditions to allow the C_{60} to adjust to the presence of the humic acid. After the stirring period, the sample was divided into two simultaneous samples for analysis.

The basic extraction procedure was adjusted slightly to make the experiment more time-efficient. The series of three extractions between measurements was reduced to one extraction; 5 mL of toluene was added to the suspension, agitated for 30 minutes, and rested for 5 minutes. Prior to salt addition, the $Mg(ClO_4)_2$ was filtered three times through a 20 nm syringe filter to ensure no dust contamination. In the second extraction step, 1.5 mL of the 1 M $Mg(ClO_4)_2$ along with 5 mL toluene. Particle size measurements were taken as described in section 4.3.1.

4.3.3 Procedure for time-monitored fullerenes in salt/DDI water

In an attempt to further investigate salt's effects on C_{60} aggregation behavior, a separate experiment was run with monovalent (KCl) and divalent ($MgCl_2$) salts and without the toluene extractions. Each salt solution was prepared to ionic strengths of 0.01, and 0.1, and 1 mol/L. These solutions were used as the background in the cuvette during particle size analysis. The well-stirred stock C_{60} solution was diluted to 10 mg/L C_{60}. Immediately prior to analysis, 100 μL of the C_{60} was added to the salt media. Particle size was then measured every minute for 15 minutes after exposure to the salt.

4.4 Results

For both experiments, particle effective diameter remained relatively constant at about 370±19 nm for the first two extraction steps. This is consistent with previously observed median particle diameter of 357 nm, reported by Brant et al. However, the diameter increased to between 2 to 5 μm, with an equally large standard deviation, after the toluene/salt extraction. Figure 14 displays the results from both the experiment in deionized water as well as the experiment in humic acid.
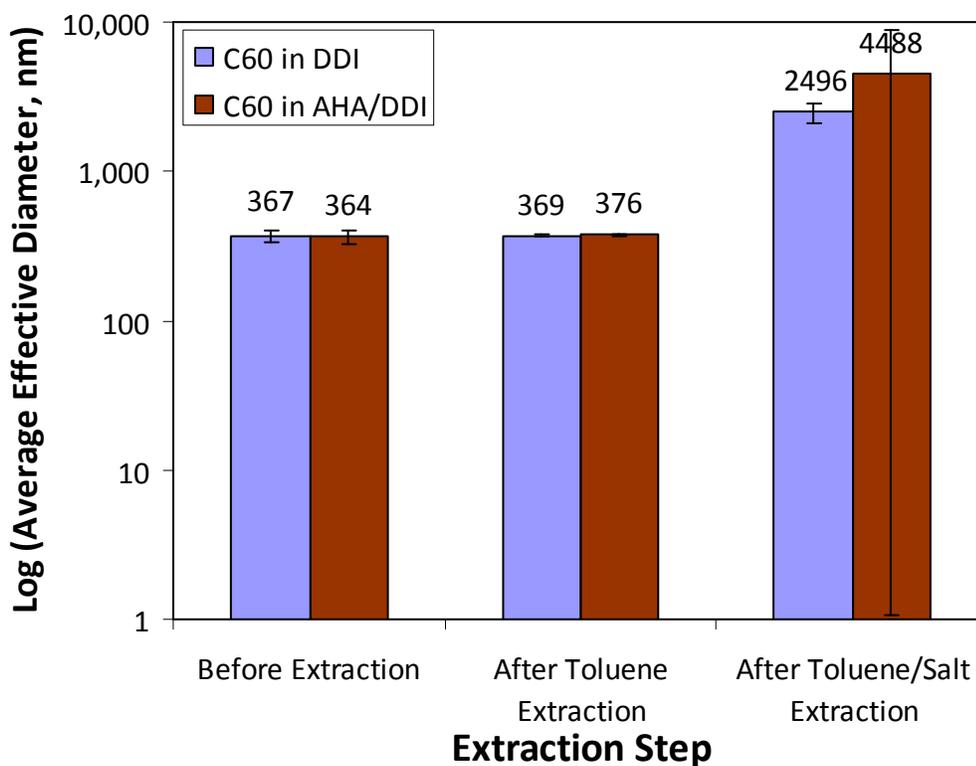
Figure 14: Particle size results of the extractions of C_{60} in the DDI water and in the Aldrich humic acid/DDI water. Data reported are the means of two samples. Error bars represent one standard deviation above and below the mean.

It was first supposed that the unexpectedly large and inconsistent particle diameter after the salt addition may have been due to contamination of the salt used rather than the actual particle diameter. The salt was filtered and blanked during the humic acid trial to ensure no particles such as dust entered the solution in this way. However, the same large diameter was observed in the humic acid experiment, indicating that the large diameter reading may have been due to aggregation of the particles.

This aggregation could have been due to the addition of the salt itself. The magnesium ions from the salt may act as a bridge between particles, effectively causing the diameter to increase. The increase in particle hydrodynamic diameter with increase in ionic strength has been observed by other authors as well, as described in section 2.3. Furthermore, there were no significant size difference between the particles in the DDI water and the Aldrich humic acid water. This indicates that the shortened extraction procedure does not significantly affect the particles removed, and that the salt addition affected the humic acid and DDI water to the same extent.

In an attempt to investigate why the hydrodynamic diameter increased so greatly during the third extraction step, water-stirred C_{60} was added to salt solutions and the particle size was measured over time. Both a monovalent salt, potassium chloride, as well as a divalent salt, magnesium chloride, were prepared at ionic strengths of 1, 0.1, and 0.01 mol/L. The expected result was that the diameter would increase quickly within a couple of minutes, displaying aggregation to hydrodynamic diameters greater than 1 µm due to the salt addition. This result was expected particularly for the magnesium chloride salt, and would have seemed to explain the behavior observed in the earlier experiment.

However, the actual result was that the hydrodynamic diameter remained relatively constant. Figure 15 shows the lack of trend in size with time, salt type, or ionic strength, while Table 3 summarizes the time-averaged results. The average hydrodynamic diameter for the $MgCl_2$ suspension was 420 ± 90 nm, with a polydispersity of 0.28 ± 0.06. The average hydrodynamic diameter for the KCl suspension was 400 ± 220 nm, with a polydispersity of 0.26 ± 0.06. These values represent heterodisperse systems. The high standard deviation for KCl is due to just a couple of points that were outside of the expected range, as can be seen in Figure 15b.
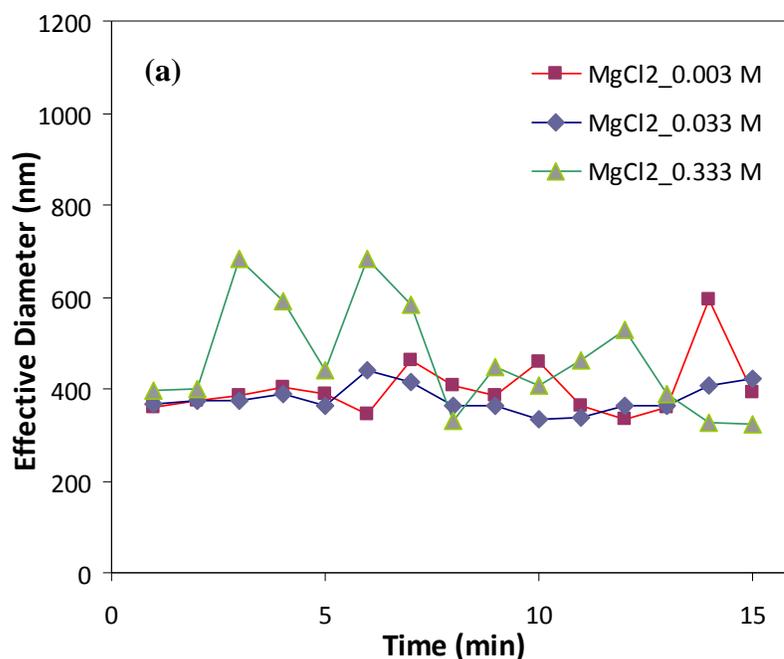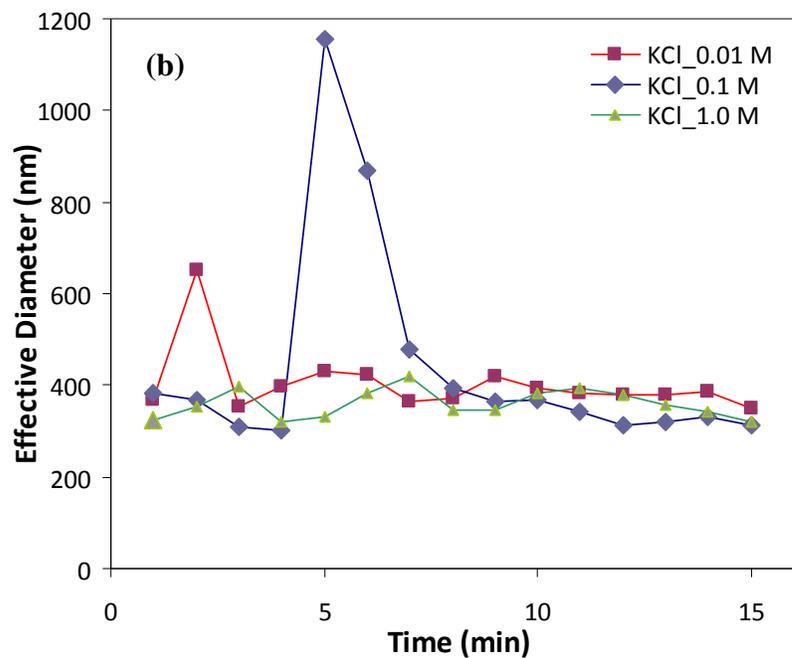
Figure 15: C_{60} particle size measured over 15 minutes after addition to (a) $MgCl_2$ salt and (b) KCl salt.

Table 3: Time-averaged values for particle mean hydrodynamic diameter and polydispersity index.

Ionic Strength	MgCl2			
(mol/L)	Mean Hydr. Diam. (nm)	Standard Dev. (nm)	Polydispersity	Polydisp. Standard Dev.
1	470	120	0.31	0.06
0.1	380	31	0.25	0.04
0.01	400	65	0.28	0.05
All	**420**	**90**	**0.28**	**0.06**
Ionic Strength	KCl			
(mol/L)	Mean Hydr. Diam. (nm)	Standard Dev. (nm)	Polydispersity	Polydisp. Standard Dev.
1	360	31	0.28	0.03
0.1	440	240	0.27	0.05
0.01	400	73	0.25	0.09
All	**400**	**220**	**0.26**	**0.06**

The diameter of 400-420 nm in the presence of salt is slightly greater than the diameter of 370 nm observed for the extended stirred C_{60} without salt added. This means that the salt may have had a small effect on the aggregate size. However, if this were truly the case then the particle size should have increased over time in Figure 15, unless it increased nearly instantaneously after the salt is added. The difference between the salt-exposed and non-salt suspensions is not great at 30-50 nm, and may just be a result of the different time of analysis.

A possible explanation for this unexpected lack of aggregation is that the C_{60} suspension added to the salt background was too dilute during this experiment. The total initial TOC concentration of the suspension was 10 mg/L, whereas in the previous extraction experiments the total initial TOC concentration was 100 mg/L. These values are based on the initial amount of C_{60} added to the water rather than actual TOC analysis. In both cases, the suspensions were further diluted by a factor of 30 as 100 µL of nC_{60} suspension was added to approximately 3 mL of salt solution. If the solution was indeed too dilute, there might not have been sufficient particle-particle interactions to cause aggregation and significantly increase the particle size.

Overall, the particles in the extended-stirred suspensions were heterodisperse. Both the size and standard deviation tended to be about twice as large as the particle sizes reported by Duncan et al. and Brant et al., which were mean diameters of 180-220 nm. Unlike the previous studies, the samples were not filtered prior to measurement, therefore no particles were excluded from analysis . They represent a direct sample of an aqu/nC_{60} sample that had been continuously stirred for about 2.5 years. The measured diameter of

400 nm is representative of a well-stirred aqueous suspension and the particle diameter would not be expected to change over time after such an extended stirring period.

The constant aggregate diameter measured for the extended-stirred aqueous nC_{60} was quite different than the extremely heterodisperse, inconsistent particle size measurements observed during the natural and synthetic water trials in Chapter 3. One possible explanation for the difference is time. For both cases, C_{60} was initially added to the water in the form of a solid powder. The chunks of C_{60} powder may take a very long time of stirring in aqueous suspension to establish a small, uniform particle size. Eight weeks of stirring time may not have been enough to establish suspensions that had a stable particle size.

4.5 Suggestions for Future Work

In many ways, the findings described above raise more questions than they answer. The data is the result of a merely preliminary investigation. With more time, further experiments could be run with water-stirred nC_{60} in the presence of salt, where the initial fullerene concentration is greater than 10 mg/L. Perhaps this would help to either explain the unexpected results that were achieved here, or to finally observe the aggregation that was originally expected.

In the long run it would be valuable to compare the particle size measurements to results from the LC/ESI-MS analysis. Perhaps this analysis could be extended to nC_{60} exposed to different salts and humic acids. The ultimate goal would be to quantify the particle size and concentration associated with various steps of the toluene/salt extraction procedure, giving an indication of the fraction of hydrophobic and hydrophilic nC_{60}. Such

analysis could help the research community to better predict the fate of fullerenes in the environment, and determine whether they are an environmental or human health risk.

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Table 4: Water quality of the Willamette River sample, including ions. Sample was taken from the untreated influent to the Taylor Water Treatment Plant in Corvallis, OR.

Characteristic	Concentration (mg/L)
Cations	
Ca^{2+}	4.4
Cu^{2+}	0
Fe^{2+}	0
K^+	0.7
Mg^{2+}	1.7
Na^+	4.9
Anions	
Cl^-	3.4
NO_2^-	0
NO_3^-	0.5
PO_4^{3-}	0.2
SO_4^{2-}	3.5
Alkalinity (as HCO_3^-)	20.2
Other	
TOC	1.2 mg C/L
pH	6.3

Procedure for the natural/synthetic water experiments.

Materials:

- DI water, 0.02 μm filtered
- Willamette River (WR) water, characterized for alkalinity, total carbonate, cations & anions, DOC, pH, 0. 02 μm filtered
- NaOH
- HCl
- Aldrich Humic Acid – 80 mg/L solution
- C_{60} powder, 99% purity from MER Corp, about 60 mg
- Glass microfiber syringe filters (1 μm, 0.45 μm, 0.02 μm pore size)
- 10 mL & 20 mL syringes
- 250 mL volumetric flasks, 2
- 500 mL volumetric flasks, 2
- Stir plates, 2
- Stir bars, 2
- Pipettes, electronic, 1000 μL, and 200 μL
- Glass buret
- Cuvettes, plastic and glass

Procedure:

Note: Prior to each use, all glassware and syringes should be rinsed with DI water and 20 nm-filtered water.

A. Preparation of Sample A, Willamette River water stirred C_{60}

1. Characterize WR water for alkalinity, cations and anions, and pH. Filter 500 mL WR water through a 0.02 μm syringe filter. Remove two 5 mL samples for DOC analysis.
2. Weigh 25 mg of C_{60} powder onto a weigh plate and transfer to a clean, dry 250 mL volumetric flask. Rinse the plate with filtered WR water into the flask. Fill the flask to 250 mL with filtered WR water.
3. Add stir bar and cap flask. Stir on stir plate indefinitely.

B. Preparation of Sample B, humic acid-adjusted water stirred C_{60}

1. Prepare stock solution of 100 mL 0.01 M NaCl: Weigh 58 mg dry NaCl, add to 100 mL volumetric flask, fill to 100 mL with DI water, cap, stir for 1 hr, and filter through 0.22 μm membrane.

2. To appx. 400 mL of DI water, add 10.27 mL of the 0.01 M NaCl stock solution and 7.50 mL of the 80 mg/L AHA stock. Fill to 500 mL with DI water. This should result in a 500 mL solution of 12 mg/L NaCl and 1.2 mg/L AHA.
3. Filter the solution of AHA-salt water through a 20 nm syringe filter. Remove two 5-mL samples for DOC analysis.
4. Test pH and add a couple drops of 0.01 M NaOH or HCl if needed to achieve same pH as WR water.
5. Weigh 25 mg of C_{60} powder onto a weigh plate and transfer to a clean, dry 250 mL volumetric flask. Rinse the plate with filtered AHA-salt water into the flask. Fill the flask to 250 mL with AHA-salt water.
6. Add stir bar and cap flask. Stir on stir plate indefinitely
7. Store remainder of AHA-salt water in a Nalgene container for later use.

C. Continuation of experiment and removal of samples

1. Allow each sample to stir, undisturbed, for seven days. On the seventh day, the first samples can be removed from each of the four flasks for particle analysis according to the following:
2. With a buret, remove 40 mL of sample from each of the two batches and place into small flasks. Do this while still stirring.
3. Set aside two samples of 100 µL from each batch into 40 mL glass vials that contain 5 mL DI water for TOC analysis. Set aside 2 mL into a cuvette for UV/VIS absorption.
4. Filter through a 1 µm syringe filter. Set aside two 500 µL samples into 40 mL glass vials that contain 5 mL 20 nm filtered water for TOC analysis.
5. Set aside 2 mL into a cuvette for UV/VIS absorption. When UV/VIS is run, transfer to a quartz cuvette.
6. Withdraw two 100 µL samples into cuvettes filled with the same medium (WR water, salt-adjusted water, humic acid-adjusted water, or filtered water). Analyze particle size with the Brookhaven instrument. Decrease volume of sample if it is too concentrated to accurately measure particle size.
7. Withdraw two 100 µL samples into cuvettes with 1.5 mL of the same medium. Analyze EPM & zeta potential with the Brookhaven instrument.
   a. *See what count rates are. If needed, use larger sample volumes.*
8. Filter through a 0.45 µm syringe filter. Set aside two 3 mL samples into a 40 mL glass vial for DOC analysis and 2 mL into a cuvette for UV/VIS absorption. Set aside 1 mL of sample for TEM analysis. Also repeat steps E.5 - E.7.
9. Every seven days for 42 days (six weeks), repeat this sampling and analysis procedure for each batch. This will result in 6 sample removals from each batch, or 12 sample removals total. However, only do DOC and TEM analysis on day 7 and day 42. On all other days, remove 10 mL (rather than 40 mL) of stock.

Table 5: Sampling schedule for synthetic and natural water nC$_{60}$ suspensions.

Day	Volume (mL) removed each step, per batch								
	UV/VIS	(Single samples)		**EPM**	(Double samples)		**Size**	(Double samples)	
	Pre-filtration	1 μm	0.45 μm	Pre-filtration	1 μm	0.45 μm	Pre-filtration	1 μm	0.45 μm
7	2	2	2	0	0.10	0.10	0	0.10	0.10
14	2	2	2	0	0.10	0.10	0	0.10	0.10
21	2	2	2	0	0.10	0.10	0	0.10	0.10
28	2	2	2	0	0.10	0.10	0	0.10	0.10
56	2	2	2	0	0.10	0.10	0	0.10	0.10
Day	**Volume (mL) removed each step, per batch**								
	TOC*	(Double samples)		**TEM**	(Double samples)		**Vol. Used**	**Stock removed**	**Start stock** / **End Stock**
	Pre-filtration	1 μm	0.45 μm	Pre-filtration	1 μm	0.45 μm	(mL)	(mL)	(mL) / (mL)
7	1.0	1.0	4.0	1	1	1	25	30	250 / 220
14	0	0	0	0	0	0	7	10	220 / 210
21	0	0	0	0	0	0	7	10	210 / 200
28	0	0	0	0	0	0	7	10	200 / 190
56	1.0	1.0	4.0	1	1	1	25	30	190 / 160