Characterization of C$_{60}$ Nanoparticles in Aqueous Systems

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Abstract

The discovery that negatively charged aggregates of C$_{60}$ fullerene are stable in aqueous environments has elicited concerns regarding the potential environmental and health effects of these aggregates. Although many previous studies have used aggregates synthesized using intermediate organic solvents, this work primarily employed an aggregate production method that more closely emulates the fate of C$_{60}$ upon accidental release into the environment – extended mixing in water. The aggregates formed via this method (aqu/nC$_{60}$) differ from those produced using the more common solvent exchange methods. The aqu/nC$_{60}$ aggregates are heterogeneous in size (20 nm and larger) and shape (angular to round), negatively charged, and crystalline in structure – exhibiting a face centered cubic (fcc) system. Solution characteristics such as aqu/nC$_{60}$ aggregate size and concentration were found to be dependant upon preparation variables such as stirring time, initial C$_{60}$ concentration, and initial particle size.

Additional experiments indicate that aggregate charge, structure, and stability are highly dependant upon the identity of co-solutes (NaCl, CaCl$_2$, sodium citrate) and their concentrations. Citrate concentrations greater than 0.5 mM resulted in the formation of very small (< 20 nm) spherical aqu/nC$_{60}$ particles. At moderate citrate concentrations (~ 1 mM) a more negative surface charge was observed, which may be an indication of increased nC$_{60}$ stability. In contrast, high concentrations of monovalent and divalent electrolytes result in aggregation and sedimentation of nC$_{60}$ out of solution. Our research describes the effect that solution composition has on aggregate formation and stability, and suggests that C$_{60}$ fate and transport will be a function of solution composition.
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Table of Contents

ABSTRACT ...................................................................................................................... II
ACKNOWLEDGEMENTS ........................................................................................... III
TABLE OF CONTENTS ............................................................................................... IV
TABLE OF FIGURES .................................................................................................... VI
TABLE OF TABLES .................................................................................................... VII
I. INTRODUCTION ........................................................................................................ 1
   BACKGROUND .......................................................................................................... 1
   RESEARCH OBJECTIVES .......................................................................................... 2
   THESIS OUTLINE ....................................................................................................... 3
   REFERENCES ................................................................................................................ 4

II. LITERATURE REVIEW ........................................................................................... 5
   BACKGROUND ON THE C₆₀ MOLECULE: DISCOVERY, DESCRIPTION, AND USES ........ 5
   THE DISCOVERY OF WATER STABLE C₆₀ AGGREGATES .................................................. 6
   CHARACTERIZATION OF nC₆₀ .................................................................................... 8
   TOXICITY OF nC₆₀: FISH, BACTERIA, AND HUMAN CELLS ........................................... 11
   AGGREGATION, TRANSPORT, AND FATE IN THE ENVIRONMENT ................................ 14
   REFERENCES ................................................................................................................ 17
   TABLES AND FIGURES ............................................................................................ 19

III. EXPLORING C₆₀ COLLOID FORMATION IN AQUEOUS SYSTEMS:
    EFFECTS OF PREPARATION METHOD ON SIZE, STRUCTURE, AND
    SURFACE CHARGE ..................................................................................................... 26
   INTRODUCTION .......................................................................................................... 26
   MATERIALS AND METHODS ........................................................................................ 28
      nC₆₀ Suspension Preparation .................................................................................. 29
      Dynamic Light Scattering (DLS) ............................................................................ 30
      Electrophoretic mobility ....................................................................................... 31
      Transmission Electron Microscopy (TEM) ............................................................. 31
      UV-Visible Spectrophotometry .............................................................................. 31
   RESULTS AND DISCUSSION ...................................................................................... 32
      Comparisons of aggregate size between THF/nC₆₀ and aqu/nC₆₀ ............................. 32
      Aqu/nC₆₀ and THF/nC₆₀ crystallinity and shape ...................................................... 34
      Pulverization of C₆₀ ............................................................................................... 36
      Filtration vs. settling ............................................................................................. 37
      C₆₀ concentration ................................................................................................. 38
      Effects of pH and NaCl on aqu/nC₆₀ ...................................................................... 39
      Effect of NOM on aqu/nC₆₀ .................................................................................. 40
      Formation of nC₆₀ ................................................................................................. 41
   REFERENCES ................................................................................................................ 43
   TABLES AND FIGURES ............................................................................................ 45
IV. EFFECTS OF SODIUM CITRATE AND ELECTROLYTE CONCENTRATION ON THE FORMATION OF AQU/nC₆₀

INTRODUCTION .............................................................................................................. 52
MATERIALS AND METHODS ........................................................................................... 53
  nC₆₀ Suspension Preparation.................................................................................... 53
  Dynamic Light Scattering (DLS) .............................................................................. 54
  Electrophoretic mobility ........................................................................................... 54
  Transmission Electron Microscopy (TEM)............................................................... 54
RESULTS AND DISCUSSION............................................................................................. 55
  Effects of NaCl and CaCl₂ on aqu/nC₆₀.................................................................... 55
  Effect of sodium citrate on aggregate charge........................................................... 57
  Effect of sodium citrate on aggregate size and structure ......................................... 58
  Formation of aqu/nC₆₀.............................................................................................. 58
CONCLUSIONS................................................................................................................ 59
REFERENCES .................................................................................................................. 61
TABLES AND FIGURES ................................................................................................. 62

V. CONCLUSIONS ....................................................................................................... 67
CONCLUSIONS................................................................................................................ 67

APPENDIX A .................................................................................................................. 68
  DYNAMIC LIGHT SCATTERING (DLS) ANALYSIS ........................................................... 68
  REFERENCES .............................................................................................................. 70
  TABLES AND FIGURES ............................................................................................... 71

APPENDIX B .................................................................................................................. 76
  TABLES AND FIGURES ............................................................................................... 76
  TEM IMAGES .............................................................................................................. 79

VITA ................................................................................................................................. 96
Table of Figures

**Figure II - 1.** UV-VIS absorbance spectra of each type of $nC_{60}$................................. 24  
**Figure II - 2.** THF/$nC_{60}$ zeta potential as a function of pH. ........................................ 24  
**Figure II - 3.** Effect of the rate of water addition during the synthesis of nano-$C_{60}$........ 25

**Figure III - 1.** a) Intensity-weighed and b) volume-weighted particle size distributions for $nC_{60}$ solutions ........................................................................................................ 46  
**Figure III - 2.** a) Overview TEM image of THF/$nC_{60}$.................................................... 47  
**Figure III - 3.** Pulverized $aq/nC_{60}$ plotted as a function of stirring time ......................... 48  
**Figure III - 4.** Intensity-weighted size distributions for $aq/nC_{60}$ solutions....................... 49  
**Figure III - 5.** Electrophoretic mobilities of $aq/nC_{60}$ and THF/$nC_{60}$ solutions graphed as a function of pH. ................................................................. 50

**Figure III - 6.** Pulverized $C_{60}$ was added to NaCl solutions of 1-100 mM...................... 51

**Figure IV - 1.** Electrophoretic mobility and average hydrodynamic diameter ($Z_{ave}$) of $aq/nC_{60}$ solutions plotted as a function of NaCl and CaCl$_2$. ................. 62  
**Figure IV - 2.** HRTEM images of $aq/nC_{60}$ produced in a 10 mM NaCl solution.......... 63  
**Figure IV - 3.** Electrophoretic mobility and average hydrodynamic diameter ($Z_{ave}$) of $aq/nC_{60}$ solutions plotted as a function of sodium citrate ............................ 64  
**Figure IV - 4.** HRTEM images of $aq/nC_{60}$ produced in a 10 mM sodium citrate ...... 65  
**Figure IV - 5.** SEM images of pulverized $C_{60}$. ................................................................. 66

**Figure A - 1.** Linear relationship between relative concentration and UV-VIS absorbance at 357 nm........................................................................................................ 71  
**Figure A - 2.** SEM images of un-pulverized and pulverized $C_{60}$ at equivalent magnification. ........................................................................................................ 72  
**Figure A - 3.** $Z_{ave}$ and electrophoretic mobility of $aq/nC_{60}$ are shown as a function of NaCl (0.01-100 mM) added after preparation. .................................................. 73

**Figure B - 1.** Particle size distribution for the small spherical $aq/nC_{60}$ particles present in a 10 mM sodium citrate solution. .......................................................... 76
Table of Tables

Table II - 1. Summary of $nC_{60}$ results previously described in the literature .................. 19
Table III - 1. Hydrodynamic diameter ($Z_{ave}$), polydispersity index, intensity-weighted mean, and volume-weighted mean of $nC_{60}$ solutions ........................................ 45
Table A - 1. Summary of chapter III experiments. ............................................................ 74
Table B - 1. Summary of chapter IV experiments. ............................................................ 77
I. Introduction

Background

With nanomaterial production on the rise, concerns about the environmental and health impacts of their release into the environment have risen. Unless carefully regulated, it is inevitable that nanomaterials will eventually find their way into the air and water of our environment. The mobility and bioavailability of these materials will be dependent upon environmental factors as well as the characteristics of a particular nanomaterial.

This study focused on the fate of C₆₀ in the environment, specifically, in aqueous systems. This cage-like molecule, consisting of 60 carbon atoms bonded together to form a sphere, has caught the interest of many industries who would wish to harness its unique properties to enhance their products. Production of C₆₀ is on the rise, with some companies producing hundreds of tons each year. This increase in production raises environmental and health concerns about the release of C₆₀ into the environment (1). Despite the extremely low solubility of C₆₀, it is possible to produce high concentrations of stable C₆₀ aggregates in water (nC₆₀). Formation of these aggregates could dramatically increase the potential for the molecule to be transported and come into contact with aquatic organisms. The characteristics of these nC₆₀ aggregates are highly dependent upon the method used to produce them. Most nC₆₀ production methods require that C₆₀ be dispersed in a non-polar solvent prior to its addition to water. In a less commonly employed method, C₆₀ is added directly to water and stirred for extended periods. Although it is a topic of heated debate, several studies have found nC₆₀ to be toxic to bacteria and other organisms (2-4). Examples of the negative effects caused by
exposure to \( nC_{60} \) include oxidative stress in the brains of smallmouth bass and toxicity towards human liver carcinoma cells and dermal fibroblasts (5,6). Despite our knowledge that \( nC_{60} \) has the potential to be transported in aqueous environments, and despite studies showing negative health effects, a thorough study of the fate and transport of \( C_{60} \) released into the environment has not been conducted (5,6).

**Research Objectives**

A number of studies have been published on the toxicity, characterization, and behavior of \( nC_{60} \) in water. Variations in the methods used to produce \( nC_{60} \) and the types of experiments conducted leave us unable to predict the potential ramifications of \( C_{60} \) releases to the environment. Although much work remains to be done, this study informs us of many factors that may play a role in the fate of \( C_{60} \) in the environment. Experiments were conducted to explore the effects of preparation method variables on the formation and stability of \( nC_{60} \) in a variety of solutions. Dynamic light scattering (DLS), UV-Visible spectroscopy, and transmission electron microscopy (TEM) were employed to characterize the forms of \( nC_{60} \) produced. The following four objectives outline the main goals of this research:

1) To summarize what is currently known about the fate of \( C_{60} \) in the environment.

2) To characterize and differentiate between \( nC_{60} \) produced via a solvent exchange method (THF/\( nC_{60} \)) and that produced by a method that involves the direct addition of \( C_{60} \) to water (aqu/\( nC_{60} \)).

3) To determine the effect of stirring time, initial \( C_{60} \) concentration, initial \( C_{60} \) particle size, and filtration on the formation of aqu/\( nC_{60} \) aggregates.
4) To evaluate the effect of common water constituents (electrolytes, sodium citrate, and natural organic matter) on the formation of aqu/nC₆₀ aggregates.

**Thesis Outline**

This thesis contains a total of five chapters including this introductory chapter. Chapter II provides background information on C₆₀, describes several common preparation methods for nC₆₀, and discusses the results from studies on the mobility, toxicity, and aggregation of nC₆₀ produced via these methods. Chapter III compares aggregates produced via the THF/nC₆₀ and aqu/nC₆₀ methods. Chapter III also describes the affect of preparation variables (stirring time, initial C₆₀ concentration and size) on aggregate size, shape, concentration, and surface charge. Chapter IV discusses the affect of solution composition on the formation of aqu/nC₆₀ aggregates. In these experiments C₆₀ was added to solutions of NaCl, CaCl₂, and sodium citrate, and the affects of these solution constituents on particle size, surface charge, and stability were evaluated. The final chapter lists the key conclusions developed from this research.
References

II. Literature Review

Background on the C\textsubscript{60} molecule: Discovery, description, and uses

Kroto et al. (1) published the article “C\textsubscript{60}: Buckminsterfullerene” describing the discovery of C\textsubscript{60} and its production apparatus. To produce C\textsubscript{60}, carbon was vaporized from graphite into a high-density helium flow using a pulsing vaporization laser. Once vaporized, the carbon cooled and reacted to form clusters of carbon atoms (1). Through time-of-flight (TOF) mass spectrometry, it was determined that the clusters were composed of a variable number of carbon atoms, and that C\textsubscript{60} was the most stable and abundant size (1). The development of a new method as well as improvements in purification methods have made it possible to produce C\textsubscript{60} in larger quantities (2).

The C\textsubscript{60} molecule, nicknamed a “buckyball” after Buckminster Fuller’s geodesic dome, is a spherical cage-like molecule built purely of carbon (1). The 60 carbon atoms in C\textsubscript{60} take the structure of a truncated icosahedron with 12 pentagonal faces and 20 hexagonal faces (e.g., a soccer ball). Each carbon atom has two single bonds and one double bond to adjacent carbon atoms. Originally, this molecular structure was selected based on theory because it was the only one that would satisfy the valence of each carbon atom (1). However, the structure was verified to be correct using nuclear magnetic resonance (NMR) spectroscopy to confirm that the C\textsubscript{60} molecule has icosahedral point group symmetry (3).

Dravid et al. (4) published a paper on the crystal structure of C\textsubscript{60} as determined by imaging and diffraction. Although C\textsubscript{60} was originally interpreted to be hexagonal close packed (hcp) at room temperature (2), C\textsubscript{60} actually forms face centered cubic (fcc) crystals. Dravid et al. (4) determined that the symmetry of C\textsubscript{60} is consistent with that of
the m3m cubic point group and is thought to be in the Fm3m space group. Each unit cell contains 14 lattice sites occupied by a C$_{60}$ molecule (4 molecules per unit cell). The lattice constant of C$_{60}$ was found to be $a = 1.42$ nm with a 5% error. C$_{70}$, unlike C$_{60}$ was found to be hcp and to have lattice parameters of $a = 1.01$ nm and $c = 1.68$ nm with a 5% error (4).

Because of the unusual structure of C$_{60}$, the molecule has come to the attention of the biomedical, electronic, environmental, and optics industries (5). Despite the fact that only a few products containing C$_{60}$ have reached the shelf, there is growing interest in using the molecule for drug treatments, catalysts, and sensors (6-8). Production of C$_{60}$ has increased following the development of a mass production method in 1990 (2). Some companies currently aim to produce tens to hundreds of tons of C$_{60}$ per year (9). The increase in production has raised concerns about the health and safety of C$_{60}$.

**The discovery of water stable C$_{60}$ aggregates**

C$_{60}$ has an extremely low solubility in water, estimated through extrapolation to be $1.3 \times 10^{-11}$ ng/mL (10). However, many potential applications for C$_{60}$, especially in the biomedical industry, require a more hydrophilic molecule (11). Fortunately, the surface of C$_{60}$ can be derivatized, making it possible to tailor its solubility to a given application (12). One common example is the creation of a water soluble molecule via addition of hydroxyl groups to the C$_{60}$ cage, resulting in the fullerol molecule (C$_{60}$OH$_{20-24}$). Encapsulation in a surfactant can also be used to increase the solubility of C$_{60}$ (13).

To study un-functionalized C$_{60}$, several methods have been developed to suspend C$_{60}$ in water without chemical alteration of the molecule. Four of the most common
methods, BTA/nC₆₀, tol/nC₆₀, THF/nC₆₀, and aqu/nC₆₀, will be described in the following section using the notation developed by Brant et al. (14) of “initial solvent/colloidal species”.

The first method (BTA/nC₆₀) developed to produce stable suspensions of C₆₀ in water involved the use of benzene, tetrahydrofuran (THF), and acetone (15). The solution was synthesized by adding THF to a solution of benzene saturated with C₆₀. The resulting purple mixture was then mixed with acetone. Next, water was added resulting in the precipitation of yellow C₆₀ particles. The organic solvents were removed through distillation, leaving behind an nC₆₀ solution. The particles in the solution remained stable for more than 3 months, but it was possible to remove the majority of them with a 0.22 μm cellulose acetate filter. This method was modified by Brant et al. (16) who used toluene instead of benzene to make TTA/nC₆₀.

One year later, a second method (tol/nC₆₀) for producing highly stable aqueous suspensions of fullerene aggregates was published (17). The fullerene sample used to produce the colloidal solution contained both C₆₀ and C₇₀ at a ratio of 10:7. A solution of toluene, deionized water, and fullerene was sonicated for several hours, allowing all of the toluene to evaporate. Filtration of the solution through a 0.2 μm filter resulted in a brownish-orange suspension with a concentration of ≈ 5 μg/cm³. This fairly simple process produced a suspension of fullerene particles that remained stable over a pH range of 1 to 10 and was unaltered by boiling (17).

A paper by Deguchi et al. (18) described another method to produce stable suspensions of C₆₀ and C₇₀ in water via solvent exchange with THF (THF/nC₆₀). In this method, THF saturated with fullerene was injected into water inside a test tube. The THF
was then removed by purging the solution with nitrogen. The light magenta color of the THF-C\textsubscript{60} solution changed to yellow once injected into water (18).

The final method included in this review was developed by Cheng et al. (19) to study the adsorptive and desorptive behavior of naphthalene onto C\textsubscript{60} fullerene. This method differs significantly from previous methods because no intermediate solvent was used during the aggregate formation process. This method more closely emulates how nC\textsubscript{60} might form if C\textsubscript{60} powder were to be spilled in the environment, thus making this method more appropriate for studies investigating the toxicity or transport of C\textsubscript{60}. The Cheng et al. (19) study investigated C\textsubscript{60} large aggregates (20 to 50 \(\mu\)m), C\textsubscript{60} small aggregates (1 - 3 \(\mu\)m), a C\textsubscript{60} thin film, as well as crushed activated carbon. Because they are most relevant to this research, the production method for the C\textsubscript{60} small aggregates (aqu/nC\textsubscript{60}) will be described. C\textsubscript{60} was pulverized with a mortar and pestle and sieved with a 75 \(\mu\)m sieve prior to being added to a solution containing 0.01 M NaCl and 0.01 M NaN\textsubscript{3}. The solution was stirred for two days to produce a brownish suspension (19). Later variations of this method include the use of nanopure water instead of electrolyte solutions, and longer stirring periods.

Although many methods exist for the production of water stable suspensions of C\textsubscript{60}, the aggregates produced via the different methods have unique characteristics. As described in the following pages, the size and structure of the aggregates as well as their other properties vary dramatically depending on the preparation method employed.

**Characterization of nC\textsubscript{60}**

Despite the numerous publications that have investigated the UV-Visible absorbance spectra, surface charge, and aggregate size of nC\textsubscript{60}, it is difficult to compare
results from these studies because the aggregate characteristics are dependant upon the production method used. Details of many of these production methods, and the characteristics of the aggregates that form, are summarized in Table II-1.

**UV-VIS absorbance spectra.** Brant et al. (16) published a manuscript addressing the differences in $nC_{60}$ particles produced using four different production methods. Figure II-1 (16) shows the UV-VIS absorbance spectra for $nC_{60}$ produced via four different methods. The absorbance spectra for each of the $nC_{60}$ solutions differ from each other, and from the spectrum of $C_{60}$ in n-hexane (considered to be a true solution) (14). In general, the spectra for $nC_{60}$ show a broadening of the absorbance bands and a shifting of the peaks to higher wavelengths (red shift).

Andrievsky et al. (17) attributed the broad peaks to light scattering by particles in the tol/$nC_{60}$ solutions (also referred to as SON/$nC_{60}$), and hypothesized that the fullerene may be stabilized by the formation of clathrate-like networks of water molecules around the fullerene aggregates. A similar idea was presented by Deguchi et al. (18) for $nC_{60}$ produced using THF, who noted that the absorption peaks for $C_{60}$ in water show a broadening and a red shift compared to $C_{60}$ in THF. Similar to Andrievsky, Deguchi et al. (18) interpreted the shift in the absorption edge of $C_{60}$ in water as an indication of the formation of clathrate crystals that contain molecules of THF or water.

**Surface charge.** Each of the methods of production discussed thus far produce $nC_{60}$ aggregates with a negative surface charge. The negative charge causes an electrostatic repulsion between aggregates, thus helping to stabilize them in solution. The aggregates produced via the THF method were found to be stable over time and stayed suspended for at least 9 months (18). Data from THF/$nC_{60}$ solutions show that the
magnitude of the negative surface charge is strongly dependant upon pH and electrolyte concentration (Figure II-2) (14). An increase in the electrolyte concentration or a decrease in the pH lessens the negative charge on the particles and results in the loss of colloidal stability.

Comparison of zeta potential values obtained in various studies suggests that the surface charge has some dependence on the preparation method. Although many have reported that the aggregates possess a negative surface charge, no consensus exists within the literature on the origin of this charge. Deguchi et al. (18) attributed the formation of the negative surface charge to charge transfer reactions that take place when clathrate crystals are formed. Thus, this hypothesis requires that clathrate crystals form instead of pristine C₆₀ crystals. Alternatively, negative surface charge could result from hydroxyl ion absorption to the surface of the C₆₀ molecules (18). The negative charge may form in different ways that depend on the preparation method. For example, when solvents are used, charge could be transferred from these molecules to nC₆₀, while the charge developed in the aqu/nC₆₀ aggregates must be obtained via some other mechanism.

*Aggregate size.* As is true for UV-VIS absorbance and surface charge, aggregate size is dependant upon the preparation method. Dynamic light scattering measurements and TEM image analysis reveal a large assortment of nC₆₀ sizes and shapes that are dependant upon the preparation method: The tol/nC₆₀ method produces spherical particles, the TTA/nC₆₀, and the THF/nC₆₀ methods produce faceted particles, and the aqu/nC₆₀ method produces particles with irregular shapes (16). Although the preparation method has a dramatic effect on the morphology of nC₆₀ particles, the cause of these differences remains elusive. The mechanisms involved in the formation of different
types of aggregates need to be studied further. Figure II-3 shows the data from one such study conducted on THF/nC₆₀, which determined that aggregate size was dependant upon pH and the rate of water addition (20). While the THF/nC₆₀ method is fairly well understood, many questions remain about the formation of aggregates via other methods.

**Toxicity of nC₆₀: Fish, bacteria, and human cells**

$nC_{60}$ is likely the most environmentally relevant form of $C_{60}$ and for this reason a lot of effort has gone into investigating its toxicity. However, the results obtained thus far are mixed; some studies show positive or neutral effects (21), while others indicate negative health effects (11,22,23). Some of the discrepancies in the toxicity data can be attributed to differences in the form of $nC_{60}$ used or to the type of toxicity test conducted.

Oberdörster (22) studied the effects of $nC_{60}$ on juvenile largemouth bass using fullerene solutions prepared by the Center for Biological and Environmental Nanotechnology, Rice University. The $nC_{60}$ was made using a modified version of the THF/$nC_{60}$ procedure (22). Peroxidation of lipids and proteins can occur as a result of exposure to reactive oxygen species (ROS). Because $nC_{60}$ was thought to be capable of producing ROS, experiments were set up to evaluate the effect of THF/$nC_{60}$ on largemouth bass. When exposed to $nC_{60}$, the gill and liver of the fish showed a trend of decreased lipid peroxidation, while the brains showed an increase in lipid peroxidation. The cause of these opposing trends may be explained by two phenomena (22). The first is an increase in the activity of the repair enzymes in the gills and liver that protect against peroxidation. The second is a selective pathway allowing nanoparticles to be transported into the brain via the olfactory neuron, thus carrying redox-active fullerenes into this lipid rich zone. Interestingly, while increased lipid peroxidation was observed in
the brains of fish exposed to nC$_{60}$, there was no change in protein oxidation in the brain, gill, or liver, possibly due to partitioning of nC$_{60}$ into lipid-rich zones (22).

In research conducted by Sayes et al. (11), THF/nC$_{60}$ proved to be far more toxic to human skin and liver cells than the three more soluble C$_{60}$ derivatives tested: C$_3$, Na$^{2-3}$[C$_{60}$O$_{7-9}$(OH)$_{12-15}$]$^{(2-3)-}$, and C$_{60}$(OH)$_{24}$. As more of the surface of the C$_{60}$ molecule is derivatized the molecule becomes more water soluble and less toxic. The cause of cell death in this study was proposed to be oxidative damage to the lipid bilayer caused by superoxide anions produced by C$_{60}$ aggregates in solution (11).

Lyon et al. (23) conducted experiments to assess the toxicity of THF/nC$_{60}$ water solutions (nano-C$_{60}$) on bacterial cells. The gram positive bacterium *Bacillus subtilis* and the gram negative bacterium *Escherichia coli* were grown in a low salt medium before they were exposed to various concentrations of THF/nC$_{60}$. The low salt medium was used because higher salt levels led to aggregation and settling of C$_{60}$, which prevented any toxic effects. Fairly low concentrations of THF/nC$_{60}$ were found to inhibit growth (0.5 to 2 mg/L) and cause death (1.5 to 4 mg/L) of the bacteria. It was hypothesized that the toxicity was related to production of harmful reactive oxygen species (23). A derivative of C$_{60}$, fullerol, was found to have no effect on either type of bacteria. The authors’ hypothesized that the toxicity of C$_{60}$ in the environment will be highly dependant upon both the extent to which it has been derivatized and the concentration of other constituents in the water such as salts and proteins.

Some other researchers believe the THF present in THF/nC$_{60}$ aggregates may be the actual cause of the toxicity observed in these studies, and debate exists regarding the validity of using THF/nC$_{60}$ aggregates in toxicity studies (21). Each of the previously
described studies used aggregates produced via the THF/nC₆₀ method. Andrievsky et al. (21) reported that solvent exchange methods produce aggregates that may contain up to 10% (w/w) of organic impurities such as THF. THF is known to have a narcotizing effect that may be responsible for the negative effects observed in the toxicity studies described above (21).

Some light was shed on the debate over nC₆₀ toxicity by a 2006 publication investigating the antibacterial activity of nC₆₀ produced via the four methods (THF/nC₆₀, tol/nC₆₀, aqu/nC₆₀, and PVP/C₆₀), the latter of which uses poly(vinylpyrrolidone) to encapsulate C₆₀ (8). To study the effect of aggregate size on toxicity, the solutions were centrifuged to separate small particles from large ones. Each of the suspensions was found to have toxic effects on the gram-positive bacterium Bacillus subtilis, and the smallest aggregates of THF/nC₆₀ and aqu/nC₆₀ proved to be far more toxic than the larger aggregates. This size dependant toxicity was also found from batch to batch for THF/nC₆₀, where batches with smaller particles were more toxic than those with larger particles. At the 95% confidence level, THF/nC₆₀ was found to be one order of magnitude more toxic than tol/nC₆₀, aqu/nC₆₀, and PVP/C₆₀ (8). In a control study, no negative effects were observed for bacteria exposed to THF without nC₆₀ present. However, this control does not rule out the possibility that THF and nC₆₀ together are far more toxic than nC₆₀ alone. The increased toxicity of THF/nC₆₀ may result from locally high concentrations of THF caused by contact of THF/nC₆₀ with the organisms (21).

Results from the Lyon et al. (8) study suggest nC₆₀ is highly toxic to bacteria when produced with THF and, to a lesser degree, with the three other methods, including aqu/nC₆₀. Toxic effects were observed in experiments conducted both in the dark and
under anaerobic conditions where ROS are not produced (8). Although ROS may be responsible for antibacterial activity in some situations, these results show it cannot be the only mechanism of \( nC_{60} \) toxicity. Accordingly, the toxicity of \( nC_{60} \) released into the environment is going to be highly dependant upon the characteristics of the aggregates including aggregate size, surface charge, and the pathway by which \( C_{60} \) is introduced into the environment.

**Aggregation, transport, and fate in the environment**

The mobility and toxicity of \( C_{60} \) released to the environment will depend on the colloidal stability of the aggregates that form. Aggregation of \( nC_{60} \) into larger clusters will result in sedimentation and this will reduce the ability of the material to be transported or come into contact with aquatic organisms. The aggregation kinetics of \( tol/nC_{60} \) were studied using sodium and calcium cations (24). For both salts, aggregation was reaction-limited (slow) at low salt concentrations and diffusion-limited (fast) at high salt concentrations. Below the critical coagulation concentration (CCC), aggregation was reaction-limited because the surface charge of the particles was great enough to create an energy barrier to aggregation. However, as the electrolyte concentration increases the energy barrier decreases. The CCC occurs when the energy barrier is minimal and the aggregation kinetics are only limited by diffusion. The aggregation behavior of \( tol/nC_{60} \) could be well characterized by Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory, which describes the balance of attractive and repulsive forces between particles (25).

\( Tol/nC_{60} \) deposition to a collector surface was measured using a quartz crystal microbalance. At a concentration of either 30 mM NaCl or 0.6 mM CaCl\(_2\) the attachment
efficiencies for the fullerene particles approach 1, indicating the electrostatic repulsive forces were significantly decreased thus allowing maximum deposition. Further experiments indicated that particles were not released if the NaCl concentration was dropped to 1 mM, where deposition was not favored. However, raising the pH to 12.3, via addition of NaOH, resulted in particle release. The results from these experiments show that the aggregation and deposition behavior of tol/$nC_{60}$ is in accordance with DLVO theory.

The transport of TTA/$nC_{60}$ under a variety of electrolyte concentrations was investigated using columns packed with spherical glass collector beads (26). Electrolyte concentrations as low as 0.001 M resulted in aggregation and sedimentation of TTA/$nC_{60}$, indicating that aggregate stability is related to repulsive electrostatic forces. The percentage of TTA/$nC_{60}$ retained in the columns increased from 29% for 0.001 M NaCl to 67% for 0.1 M NaCl (26). The enhancement of aggregation and deposition of TTA/$nC_{60}$ caused by the presence of salt is likely to reduce the ability of TTA/$nC_{60}$ to be transported in porous media. However, it will be necessary to study many other environmental factors before coming to any conclusions about the mobility of $nC_{60}$. The colloidal stability of $nC_{60}$ may be increased by some constituents found in natural waters. Natural organic matter (NOM), for example, has been shown to stabilize carbon nanotubes and $nC_{60}$ (27,28).

It is too early to make conclusions about the risks associated with the release of $C_{60}$ into the environment. If $C_{60}$ is spilled into the environment, the aggregates formed will probably be more similar to aqu/$nC_{60}$ than to aggregates produced via one of the solvent exchange methods. Fewer studies have been conducted on this form of $nC_{60}$. We
know that many characteristics of these aggregates are dependant upon the preparation method. Data from experiments conducted using aggregates produced via solvent exchange should not be considered representative of those that would be acquired with aqu/nC₆₀ suspensions. Investigations on the formation and behavior of aqu/nC₆₀ in various aqueous solutions are essential to help determine the fate of C₆₀ upon release. The stability of nC₆₀ is strongly dependent upon the water composition, and thus the toxicity, stability, and transport of nC₆₀ may change from one aqueous environment to another.
References


(10) Heymann, D. Solubility of fullerenes C\textsubscript{60} and C\textsubscript{70} in seven normal alcohols and their deduced solubility in water. *Fullerene Science and Technology* 1996, 4, 509-515.


### Tables and Figures

#### Table II-1. Summary of \( n C_{60} \) results previously described in the literature.

<table>
<thead>
<tr>
<th>Paper</th>
<th>Prep Method &amp; Details</th>
<th>DLS</th>
<th>TEM / SEM</th>
<th>Zeta (mV)</th>
<th>UV-Vis</th>
<th>Other Experiments</th>
</tr>
</thead>
</table>
| **Synthesis of \( ^{14}C \)-labeled \( C_{60} \), its suspension in water and its Uptake by human keratinocytes** *J. Am. Chem. Soc.* (1994) Scrivens, Tour, Creek, Pirisi | Basis for TTA/\( n C_{60} \) method \( C_{60} \) synthesized based on Krätschmer –Huffman method  
- Add 100 µL of \( C_{60} \) (0.15 mg) saturated benzene to 10 mL THF.  
- Add this solution drop by drop to 100 mL of acetone (stirring rapidly)  
- Add 150 mL water slowly  
- Evaporate off solvent and 50 mL water leaving 100 mL of solution | SEM  
- 300 nm avg. size  
- 95% in range of 250 - 350 nm  
- roughly spherical | 227, 280, 360 Broadened peaks | \( ^{14}C \)-Labeled \( C_{60} \) was taken up by human keratinocytes  
The \( C_{60} \) became cell associated and did not leave the cells during washing or during an 11 hour period when in contact with new \( C_{60} \) free media. |
- A solution of 0.2 mg/cm\(^2\) fullerene (ratio of 10:7, \( C_{60} \) and \( C_{70} \)) in toluene was added to water  
- Solution was subjected to sonication until the toluene evaporated  
- Filtered though 0.22 µm filter | Broadened peaks – not shown or identified | | Stable for pH 1-10  
Stable for at least 3 months  
Plasma desorption mass spectroscopy |
| **Stable dispersions of fullerenes, \( C_{60} \) and \( C_{70} \), in water. Preparation and characterization** *Langmuir* (2001) Deguchi, Alargova, Tsuji | First THF method 1  
- \( C_{60} \) added to 20 cm\(^3\) THF and stirred overnight under an argon atmosphere to produce a saturated solution (excess removed with 0.45 µm filter)  
- 2 mL of this solution was injected into a test tube containing 2 mL water  
- The solution was then purged with nitrogen to remove the THF  
- Water was added to replace any that evaporated and the solution was filtered | HRTEM  
Crystals are polycrystalline  
| TEM  
Size in good agreement with DLS | 218, 256, 339 Broader and less intense than the peaks in THF or \( n \)-hexane of 213, 257, 330  
- New broad absorption between 420 and 520 nm  
- Blue shift in absorption edge indicates clathrate crystals  
- Red shift of 5 nm when 1% NaCl is added | No change in DLS size or UV-Vis spectra after storage in the dark for 1 mo.  
1% NaCl addition after synthesis caused a red shift (see UV) and precipitation of yellow sediment  
Concentration of the solution does not increase aggregate size |
| **Naphthalene adsorption and desorption from aqueous \( C_{60} \) fullerene** *Journal of Chemical & Engineering Data* (2004) Cheng, Kan, Tomson | “\( C_{60} \) small aggregates” >99.5% Aldrich  
- \( C_{60} \) stirred in 30 mL electrolyte solution for 2 days at 1000 rpm | SEM  
Aggregates observed in the size range of 1-3 µm | When dissolved in toluene the sample showed characteristic peak at 408 nm and no peak for the \( C_{60} \) oxide at 424 nm. | Also investigated “\( C_{60} \) large aggregates”  
Adsorption of naphthalene onto “\( C_{60} \) small aggregates” was high while adsorption to the large aggregates was not. |
<table>
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<th>Paper</th>
<th>Prep Method &amp; Details</th>
<th>DLS</th>
<th>TEM / SEM</th>
<th>Zeta (mV)</th>
<th>UV-Vis</th>
<th>Other Experiments</th>
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</thead>
<tbody>
<tr>
<td>Laboratory assessment of the mobility of nanomaterials in porous media <em>E</em> &amp; <em>T</em> (2004) Lecoanet, Bottero, Wiesner</td>
<td>TTA/nC₆₀  • termed n-C₆₀ in this paper  • Added 100 μL of C₆₀ (0.15 mg) saturated toluene to 10 mL THF and 100 mL acetone  • Added 150 mL of water slowly  • Evaporated off solvent</td>
<td>• Uniform size of 168 ± 33 nm</td>
<td>Electro-photoretic mobility = -1.99 UNITS?</td>
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<td>• A total of 8 nanomaterials were tested to evaluate their transport behavior through porous media</td>
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<tr>
<td>Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems <em>E</em> &amp; <em>T</em> (2005) Brant, Lecoanet, Wiesner</td>
<td>TTA/nC₆₀  • Three different size fractions prepared by changing the initial concentration of C₆₀ in toluene (0.15, 0.5 &amp; 1.5 mg/ml)  • From lowest conc. to highest 18.7, 135, and 168 nm  • Small increase in size after two months of sitting</td>
<td>• faceted particles hexagonal or pentagonal in cross-section</td>
<td>NaCl (mM) 0.01 -51 0.1 -52 1 -50 10 -33 100 -25</td>
<td></td>
<td>• Addition of salt caused aggregation forming (m-(n-C₆₀)) that settled out of solution as a brown precipitate  • For NaCl 0.01, 0.1 &amp; 1 M the particle size increased to 298, 680 and 897* nm respectively</td>
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<tr>
<td>Study of C₆₀ transport in porous media and the effect of sorbed C₆₀ on naphthalene transport Journal of Materials Research (2005) Cheng, Kan &amp; Tomson</td>
<td>Nano-C₆₀  • mixed a C₆₀/tetrahydrofuran (THF) solution with water  • removed THF</td>
<td>• faceted particles  • particle size ~100 nm</td>
<td>Concentration of samples from breakthrough curve were determined by UV abs. at 446 nm</td>
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<td>transport in a soil column</td>
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<tr>
<td>C₆₀ in water: Nanocrystal formation and microbial response <em>E</em> &amp; <em>T</em> (2005) Fortner, Lyon, Sayes, Boyd, Falkner, Hotze, Alemany, Tao, Guo, Ausman, Colvin, Hughes</td>
<td>THF/nC₆₀ - method 2  (Solutions sparged with N₂ to remove oxygen)  • Stirred 100 mL C₆₀ in 4 L THF for 24 hrs  • Filtered through 0.22 μm nylon membrane  • Added 250 mL water to 250 mL of THF-C₆₀ solution at a variety of rates (1000 to 10 mL/min)  • Starting with 1 L evaporated off 550 mL, diluted with 100 mL water, evaporated to 450 mL, and added 100 mL water. Evaporated to 500 mL and filtered.</td>
<td>• DLS to study particle size over time after IS was increased  • starting particle size 100 nm</td>
<td>CRYO-TEM-  • Size and shape dependant on rate of water addition &amp; pH  • pH increases led to decreased sizes  • small particles are circular and larger ones are rectangular or triangular in cross-section  • SAED shows crystallinity - indexing most consistent w/ simple hexagonal unit cell</td>
<td>• peak for C₆₀ between 330 - 350 nm and adsorption from 400-500 nm characteristic of solid-state, crystalline C₆₀  • Blue shift in 330-350 peak as water addition rate is increased and as pH is increased</td>
<td>Antibacterial effect of C₆₀ nanocrystals on <em>E coli</em> and <em>B subtilis</em>  • nC₆₀ concentration of 0.4 mg/L resulted in no growth in MD media  • Stability of solutions was tested by increasing the IS to concentrations ranging from 0.001 to 0.7 M  • Particle size stayed constant at the starting particle size of around 100 nm for 0.001 and 0.01 M IS, while 0.05 M caused increase and fluctuation in size</td>
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<td>Paper</td>
<td>Prep Method &amp; Details</td>
<td>DLS</td>
<td>TEM / SEM</td>
<td>Zeta (mV)</td>
<td>UV-Vis</td>
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<td>THF/nC₆₀ - method 2</td>
<td>• 160 nm mean particle diameter</td>
<td>Salt = 0.001 M Na₂SO₄ -50 KCl -50 NaCl -50 Na₂SO₄ -13 KCl -18 NaCl -25</td>
<td>• Peaks more narrow than aqu/nC₆₀</td>
<td>• CaCl₂ causes charge reversal at 0.0001 M and BaCl₂ has local max and min</td>
<td>• Zeta increases from ~-63 mV at pH 10 to ~-7 mV at pH 1.3</td>
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<tr>
<td>aqu/nC₆₀ - method 1</td>
<td>• Added 80 mg C₆₀ to 100 mL water • Stirred at 500 rpm for about 2 weeks • Filtered (0.45 μm)</td>
<td>Salt = 0.001 M NaCl -34</td>
<td>• Red shifted &amp; broader • peaks at 200, 260, 349 nm • larger band from 430-530 nm</td>
<td>• Conductometric and Potentiometric titrations</td>
<td>• Conductometric and Potentiometric titrations • Concentration = 5 mg/L</td>
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<tr>
<td>Characterizing the impact of preparation method on fullerene cluster structure and chemistry Langmuir (2006) Brant, Labille, Bottero, Wiesner</td>
<td>TTA/nC₆₀</td>
<td>• 237 nm median • PDI = 0.283 • Intensity wt. peak at 2.2 μm • No. wt. peaks 43.8 and 15.7</td>
<td>NaCl (mM) 0.01 -31 0.1 -33 1 -30 10 -10 100 -4</td>
<td>Spectra varies depending on the preparation method</td>
<td>• Hydrophobicity • Rg (nm) = 57.22 • Concentration = 3.5 mg/L • SLS 170±20 nm</td>
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<tr>
<td>SON/nC₆₀ - method 1</td>
<td>• mixed 5 mL toluene-C₆₀ (1 mg/mL) with 20 mL water ~3 hrs • evaporated toluene and filtered (0.45 μm)</td>
<td>• 10-86 nm with less well defined particles in the 5-10 nm range NaCl (mM) 0.1 -33 1 -33 10 -25 100 -16</td>
<td>Spectra shown in graph - differs for each prep method</td>
<td>• Hydrophobicity • Rg (nm) = 57.87 • Concentration = 9 mg/L • Spherical clusters • SLS 160±20 nm</td>
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<td>THF/nC₆₀ - method 1</td>
<td>• 271 nm median • PDI = 0.090 • Intensity wt. peak at 1.4μm • Unfiltered peaks 300 nm &amp; 5 μm</td>
<td>• Spherical clusters ≤50 nm and elongated aggregates ~200 nm in diameter NaCl (mM) 0.01 -51 0.1 -52 1 -50 10 -34 100 -26</td>
<td>Spectra shown in graph - differs for each prep method</td>
<td>• Hydrophobicity • Rg (nm) = 70.47 • SLS 160±20 nm</td>
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<td>aqu/nC₆₀ - method 1</td>
<td>• 357 nm median • PDI = 0.146 • Unfiltered peaks 300 nm &amp; 5 μm</td>
<td>• 20-500 nm and often in aggregate form NaCl (mM) 0.01 -32 0.1 -34 1 -34 10 -25 100 -17</td>
<td>Spectra shown in graph - differs for each prep method</td>
<td>• Hydrophobicity • Rg (nm) = 80.10 • SLS 180±20 nm • Random shaped particles</td>
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<td>Paper</td>
<td>Prep Method &amp; Details</td>
<td>DLS</td>
<td>TEM / SEM</td>
<td>Zeta</td>
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<tr>
<td>Antibacterial activity of fullerene water suspensions: Effect of preparation method and particle size ES&amp;T (2006) Lyon, Adams, Falkner, Alvarez</td>
<td>THF/nC&lt;sub&gt;60&lt;/sub&gt; - method 2 • 50 mL of C&lt;sub&gt;60&lt;/sub&gt; - Toluene solution (1 g/L) was added to 500 mL of water &amp; then sonicated • Filtered (0.45 µm then 0.22 µm)</td>
<td>Mean = 74.6 nm • Small = 39.1 nm • Large = 97.4 nm</td>
<td>TEM 50 - 150 nm</td>
<td></td>
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<td>• Measured minimal inhibitory concentration (MIC) for E. Coli and B. subtilis</td>
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<td>SON/nC&lt;sub&gt;60&lt;/sub&gt; - method 2 • 50 mL of C&lt;sub&gt;60&lt;/sub&gt; - Toluene solution (1 g/L) was added to 500 mL of water &amp; then sonicated • Filtered (0.45 µm then 0.22 µm)</td>
<td>Mean = ~2 nm* • Small = ~2 nm* • Large = ~2 nm* • * below DLS detection limit</td>
<td>TEM 10 - 25 nm</td>
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<td>• centrifuge - nugget = “large” supernatant = “small”</td>
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<td>Aq/nC&lt;sub&gt;60&lt;/sub&gt; • 1 g of C&lt;sub&gt;60&lt;/sub&gt; stirred in 1 L of water at 40 °C for 2-4 weeks • Filtered (0.45 µm then 0.22 µm)</td>
<td>Mean = 74.9 nm • Small = 2 nm* • Large = 142.3</td>
<td>TEM 30 - 100 nm</td>
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<td>PVP/C&lt;sub&gt;60&lt;/sub&gt; • PVP in chloroform was added to C&lt;sub&gt;60&lt;/sub&gt; in toluene and stirred and heated (45 °C) overnight until solvents evaporated • Re-suspended in water and filtered (0.45 µm then 0.22 µm)</td>
<td>Mean, small and large = 2 nm* • * below DLS detection limit</td>
<td>TEM 10 - 25 nm</td>
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<td>Stable Colloidal Dispersions of C&lt;sub&gt;60&lt;/sub&gt; Fullerenses in Water: Evidence for Genotoxicity ES&amp;T (2006) Dhawan, Taurozzi, Pandey, Shan, Miller, Hashsham, Tarabara</td>
<td>aqu/nC&lt;sub&gt;60&lt;/sub&gt; – Method 2 • 14 days and (b) for 11 months with 10 mM NaN&lt;sub&gt;3&lt;/sub&gt; and 0, 10, or 100 mM NaCl - no filtration step</td>
<td>14 days • 178.6 nm &amp; 865 nm 11 mo. • 10 mM NaN&lt;sub&gt;3&lt;/sub&gt; = 211.8 nm • 10 mM NaN&lt;sub&gt;3&lt;/sub&gt; &amp; NaCl = 211.1 nm • 10 mM NaCl = 211.3 nm</td>
<td>TEM • diffraction patterns show that particles are crystalline • Small particles down to 20 nm observed</td>
<td>14 days - pH 5.6 • 13.5 mV 11 mo. - pH 5.6 • 10 mM NaN&lt;sub&gt;3&lt;/sub&gt; = -44.5 mV • 10 mM NaN&lt;sub&gt;3&lt;/sub&gt; &amp; NaCl = -39.9 mV • 10 mM NaCl = -36.6 mV</td>
<td>UV-Vis to measure conc. • Concentration dependant DNA damage • Particles mostly spherical or hexagonally shaped</td>
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<td>EthOH/nC&lt;sub&gt;60&lt;/sub&gt; • added 0.8 mg of powdered C&lt;sub&gt;60&lt;/sub&gt; to 800 mL of ethanol • multistage heating, sonication, rotary evaporation • filtered through 0.45 µm filter</td>
<td>121.8 nm &amp; 304 nm diameters (smaller sized peak dominant)</td>
<td>• Small particles down to 10 nm</td>
<td>-31.6 mV UV-Vis to measure conc. • same as above • particles mostly spherical or hexagonally shaped</td>
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<td>Paper</td>
<td>Prep Method &amp; Details</td>
<td>DLS</td>
<td>TEM / SEM</td>
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<tr>
<td>Affinity of C\textsubscript{60} fullerenes with water</td>
<td>(WSC-C\textsubscript{60}) Water stable Clusters (similar to aq/nC\textsubscript{60})</td>
<td>50-600 nm</td>
<td>20-500 nm</td>
<td>Small are round Large are angular</td>
<td></td>
<td>• SLS- Rg = 80nm; Fractal dimension = 2.37 • Wettability- C\textsubscript{60} no; WSC- C\textsubscript{60} = wettible • Water adsorption- stld-C\textsubscript{60} P/P\textsubscript{o}=0.7; C\textsubscript{60} P/P\textsubscript{o}=0.2</td>
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<td>Fullerenes, Nanotubes, and Carbon Nanostructures (2006)</td>
<td>Stired 14 d</td>
<td>Filtered</td>
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<td>Settled 24h</td>
<td>Up to 1.5 μm</td>
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<td>Filtered with 0.45 μm</td>
<td>Unfiltered</td>
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<tr>
<td>Aggregation and Deposition Kinetics</td>
<td>Toluene and ethanol method</td>
<td>Hydrodynamic radius = 55.7 nm</td>
<td>TEM</td>
<td>EPM</td>
<td></td>
<td>• aggregation of C\textsubscript{60} as a function of added NaCl or CaCl\textsubscript{2} • Deposition and release of C\textsubscript{60} nanoparticles using a quartz crystal microbalance</td>
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<tr>
<td>of Fullerene (C\textsubscript{60}) Nanoparticles Langmuir (2006)</td>
<td>36 mg C\textsubscript{60} dissolved in 30 mL toluene</td>
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<td>Number-average diameter is 59.2 nm with SD = 22.7 nm</td>
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<td></td>
<td>5 mL of this solution was added to 50 mL water &amp; 1.5 mL ethanol</td>
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<td>sonicated for more than 3 hours to allow for the evaporation of toluene and ethanol (adding water as needed)</td>
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<tr>
<td></td>
<td>used 0.45 μm then 0.2 μm membrane filters</td>
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<tr>
<td>Facile Generation of Fullerene Nanoparticles by Hand-Grinding Advanced Materials (2006)</td>
<td>Hand-ground C\textsubscript{60} with SDS</td>
<td>235 nm</td>
<td>SEM</td>
<td></td>
<td></td>
<td>• hand-ground nanoparticles do not inhibit growth of E. coli at a concentration of 5 μg/mL • sonication breaks apart agglomerates but does not reduce basic particle size • \textsuperscript{13}C NMR spectrum shows that C\textsubscript{60} is underivatized (single resonance at 145.86 ppm)</td>
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<td>7 mg hand-ground C\textsubscript{60} was mixed with 5 mg 40 mM sodium dodecylsulfate (SDS) in water to form a turbid dispersion</td>
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<td>Followed by ultrasonic treatment</td>
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<td>34% dispersion efficiency</td>
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<td></td>
<td>ground C\textsubscript{60} added to pure water and sonicated or stirred for 1 day</td>
<td>229 nm</td>
<td>-39 mV</td>
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<td>17% dispersion efficiency</td>
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</table>
Figure II-1. UV-VIS absorbance spectra of each type of $nC_{60}$ in water (pH = 5.6, T = 20$^\circ$C). Inset shows spectra for $C_{60}$ in n-hexane. Figure from Brant et al. (2006) reproduced with permission from *Langmuir*. 2006, 22, 3787-3885. Copyright 2007 American Chemical Society.

Figure II-2. THF/$n$-C$_{60}$ zeta potential as a function of pH using sodium chloride as a background electrolyte (pH = 7, $T = 25$°C, $n = 10$). Figure from Brant et al. (2005) reproduced in part with permission from *Environ. Sci. Technol.* 2005, 39, 6343-6351. Copyright 2007 American Chemical Society.
Figure II-3. Effect of the rate of water addition during the synthesis of nano-C$_{60}$. (a) DLS average particle size (nm) value as a function of the rate of water addition during the formation process. Representative dried TEM images of these nano-C$_{60}$ suspensions: (i) Prepared at 1000 mL/min (ii) 500 mL/min (iii) 250 mL/min (iv) 63 mL/min. Figure from Fortner et al. (2005) reproduced with permission from *Environ. Sci. Technol.* 2005, 39, 4307-4316. Copyright 2007 American Chemical Society.
III. Exploring C$_{60}$ Colloid Formation in Aqueous Systems: Effects of Preparation Method on Size, Structure, and Surface Charge

Introduction

Following the discovery of C$_{60}$ more than 20 years ago, there has been great interest in the use of C$_{60}$ in biomedical, electronic, and other applications (1-3). Recent improvements in production methods have brought C$_{60}$ costs down. The drop in prices has lead to an increase in production, with some companies hoping to produce 300 tons per year (4). With increased production, concerns about the environmental and health effects of C$_{60}$ that is either spilled or released into the environment have grown.

Andrievsky et al.(5) determined that C$_{60}$, although virtually insoluble in water (6,7), can form stable colloidal solutions containing high concentrations of C$_{60}$ aggregates (nC$_{60}$). These aggregates, which consist of numerous C$_{60}$ molecules, possess a negative surface charge that acts to stabilize them. There is concern that these stable C$_{60}$ aggregates may be transported in surface and groundwater systems and come into contact with receptor organisms. In addition, environmental pollutants, such as naphthalene, may sorb to nC$_{60}$ (8). The mobility and bioavailability of the sorbed contaminants may increase as a result of the presence of nC$_{60}$ in the system if the contaminated aggregates remain stable. Additionally, the presence of nC$_{60}$ itself remains a concern, as discussed below.

Although it is a topic of vigorous debate (9), studies have suggested that nC$_{60}$ exhibits toxicity towards fish, human skin and liver cells, and gram-positive as well as gram-negative bacteria (10-14). However, in most of these studies, nC$_{60}$ was produced by adding fullerene to a non-polar solvent such as toluene, tetrahydrofuran (THF), or
some combination of solvents and then transferring the C$_{60}$ into water (5,15-18). The use of nC$_{60}$ produced via solvent exchange methods in toxicity studies is a point of controversy as there is debate about the amount of solvent retained within a given aggregate. It has been suggested that solvent molecules, such as THF, remain bound within nC$_{60}$ aggregates and may contribute up to 10% of the weight of a given aggregate (9). This bound THF may contribute to the toxic effects of the nC$_{60}$ solutions (9). A study by Lyon at al. (7), comparing the toxicity of aggregates produced using four different preparation methods shows THF/nC$_{60}$ (here we use the nomenclature of Brant et al. (19) with the initial solvent indicated) to be about one order of magnitude more toxic than aggregates produced via other methods. A control experiment revealed that THF by itself was not toxic at the concentration present in the THF/nC$_{60}$ solutions. However, this control did not take into account the possibility that THF and nC$_{60}$ may have a synergistic toxic effect. One explanation for the increased toxicity of THF/nC$_{60}$ is that these aggregates come into contact with the organism and create local concentrations of THF high enough to cause cell damage (9). Another possibility is that THF/nC$_{60}$ may enhance the movement of THF into cells.

Several studies have investigated nC$_{60}$ formed via direct addition of C$_{60}$ to water (aqu/nC$_{60}$) (8,17-21) including one that compared the toxicity of three different forms of nC$_{60}$ (7). Lyon et al. (7) indicated that aqu/nC$_{60}$ was toxic to bacteria at a concentration less than 1 mg/L, and that THF/nC$_{60}$ was about one order of magnitude more toxic than either aqu/nC$_{60}$ or the other forms of nC$_{60}$ tested. C$_{60}$ released into the environment will enter natural systems of air, water, and soil where it may form aqu/nC$_{60}$ without being
dispersed in an organic solvent. To evaluate the health and environmental risks of aqu/nC₆₀ we need to understand its formation and stability in natural systems.

In this study we confirm that differences exist between the nC₆₀ particles formed via mixing in water (aqu/nC₆₀) and via a solvent exchange method (THF/nC₆₀). Several studies have previously published particle sizes for aqu/nC₆₀ and THF/nC₆₀ suspensions (7,17-19,21); however, a comprehensive comparison of particle size distributions including analysis of particles smaller than 100 nm has not been reported for these methods. Size distributions developed from our DLS data for aqu/nC₆₀ and THF/nC₆₀ indicate the presence of particles as small as 20 nm. These small particles are also visible in TEM images, which we have used to determine aggregate crystallinity and shape. Additionally, we conducted a thorough investigation of aqu/nC₆₀ to determine the size distribution, surface charge, and crystallinity of the aggregates that form using this method. Solution variables such as salt concentration and stirring time were tested to establish a base of information from which we will be better able to understand the formation of aqu/nC₆₀ and its eventual fate in the environment.

Materials and Methods

All experiments were conducted using 99.9% sublimed C₆₀ (Sigma Aldrich). Experiments were conducted using ACS grade sodium chloride (Sigma Aldrich) and aquatic natural organic matter (GT-NOM) provided by Baohua Gu of ORNL. All water used was obtained from a NANOpure ultrapure water system and filtered through a 0.22 μm Millipore steritop filter. In preliminary experiments it was determined that C₆₀/nC₆₀ readily adsorbs to plastic and PTFE surfaces, thus all nC₆₀ solutions were prepared and
stored in glass containers that had been washed with aqua regia and rinsed thoroughly before use. The C₆₀ powder and nC₆₀ solution were stored in the dark.

**nC₆₀ Suspension Preparation.** C₆₀ fullerene nanoparticle suspensions (nC₆₀) were produced using two basic methods, aqu/nC₆₀ and THF/nC₆₀, previously described in the literature (17). The first method, extended mixing of fullerene powder in water (aqu/nC₆₀) was selected because it may most closely represent aggregate formation in nature. As described below, we modified the procedure to test the effect of several variables on nC₆₀ formation. The second method, THF/nC₆₀, was chosen because it is a popular method for preparation of fullerene suspensions and several studies have suggested that the particles produced via this technique are toxic (7,12-14). In this method, C₆₀ is added to THF then transferred to water via solvent exchange.

**aqu/nC₆₀** - The general method for production of aqu/nC₆₀ suspensions is based on that from Cheng et al. (8). In this method, C₆₀ was added to nanopure water at a concentration of 800 mg/L. A magnetic stir-bar was added and the solution was stirred in the dark for two weeks. The general procedure was modified to test the effect of the following variables on aggregate development: 1) Initial C₆₀ particle size. C₆₀ was either used as received or was pulverized to a very fine powder. C₆₀ pulverization was achieved with a Fritsch pulversette 0 ball mill at amplitude 0.5 mm for 20 minutes. The pulverized material was then sieved through a 63 μm metal sieve. 2) Filtered vs. settled C₆₀. Samples were either filtered with a 0.45 μm cellulosic filter (Osmonics Inc.) or settled for one hour. 3) Initial C₆₀ concentration. The mass of C₆₀ initially added to nanopure water was varied between 80 and 800 mg/L. These concentrations represent the mass of C₆₀ added and not the final concentrations of the solutions, as not all of the
$C_{60}$ becomes stabilized in the water. 4) Effect of NaCl and NOM. $C_{60}$ was added to solutions containing either 1-100 mM NaCl or 1-10 mg/L GT-NOM to evaluate the effect of these constituents on particle size and surface charge.

*THF/n$C_{60}$ - The second preparation method, THF/n$C_{60}$, incorporates modifications (17) of the original procedure developed by Deguchi et al. (16). $C_{60}$ powder was added at a concentration of 25 mg/L to a previously unopened bottle of tetrahydrofuran (THF; Fisher Scientific). This solution was purged with argon to remove dissolved oxygen both before and after $C_{60}$ addition. The bottle was then resealed and left to stir overnight on a magnetic stirrer. After 24 hours, the solution was filtered through a 0.2 μm nylon filter (GE Osmonics) resulting in a THF/$C_{60}$ solution. Next, 250 mL of filtered nanopure water was added at a rate of 1 L/min to 250 mL of THF/$C_{60}$. A rotary evaporator was then used to remove and collect 300 mL of THF and water. To remove residual THF, 100 mL of water was added to the solution and 100 mL was evaporated off. The addition of 100 mL of water was repeated and 50 mL of water was evaporated off leaving 250 mL of THF/n$C_{60}$ solution. As a final step, the yellow solution was filtered through a 0.45 μm cellulosic filter (Osmonics Inc.).

**Dynamic Light Scattering (DLS).** Particle measurements were made using either a Malvern Zetasizer 3000HS equipped with a helium/neon laser ($\gamma = 633$ nm) and a 10 mm sample cell or a Malvern Nano ZS equipped with a helium/neon laser ($\gamma = 633$ nm) and a folded capillary cell. Samples were sonicated for 15 seconds prior to measurement. A minimum of three measurements were made on each sample to ensure accuracy and repeatability. The refractive index of $C_{60}$ was set at 2.2 (17) and
temperature was held at 25 °C by the instrument. Details regarding the DLS measurements may be found in Appendix A.

**Electrophoretic mobility.** Electrophoretic mobility was measured using a Malvern Nano ZS equipped with a helium/neon laser (\( \lambda = 633 \) nm) and disposable folded capillary cells. Temperature was maintained at 25 °C by the instrument and at least three measurements, each consisting of numerous sub runs, were made on each sample.

**Transmission Electron Microscopy (TEM).** TEM samples were imaged with a JEOL 100 CX-II TEM or a Zeiss 10CA TEM operated at 100 kV or 60 kV, respectively. Samples were prepared by placing one drop of solution on a carbon/formvar coated 200-mesh copper grid and allowing the sample to dry in a desiccator at room temperature. A FEI Titan scanning/transmission electron microscope (S/TEM) was operated at 200 kV to obtain high-resolution transmission electron microscope (HRTEM) images of a limited subset of samples. Ultrathin carbon grids with a holey carbon support were selected for samples investigated with the HRTEM to allow greater visibility and detection of the crystallinity and size of small \( n \)C\(_{60} \) aggregates. A representative selection of particle images was obtained from multiple sites on each grid.

**UV-Visible Spectrophotometry.** A Cary 5000 UV-VIS-NIR spectrophotometer was used to measure solution absorbance over a spectral range of 200-800 nm. The absorbance of C\(_{60} \) is dependant upon the dispersing solvent and the preparation method. C\(_{60} \) in \( n \)-hexane forms a true solution that was used as a reference with characteristic peaks at 210, 256, 328, and 404 nm (17). Peaks for aqu/\( n \)C\(_{60} \) occur at 222, 278, and 357 nm and a plateau extends from 420 nm to about 520 nm. The peak at 357 nm was used to determine the \( n \)C\(_{60} \) concentration of the suspensions because C\(_{60} \) has strong absorbance at
this wavelength and it is far from the absorbing range of NaCl (below 245 nm)(16). A calibration curve for aqu/nC_{60} was obtained by diluting a series of aqu/nC_{60} solutions and then measuring the absorbance of each of these samples at 357 nm (Appendix A, Figure A - 1). The absorbance of a given aqu/nC_{60} solution was plotted against its actual concentration, as determined by weight analysis, to develop this calibration curve. Weight measurements were obtained by drying an aqu/nC_{60} sample in the oven for 3 days and then measuring sample mass. Using this approach, the molar absorptivity of nC_{60} at 357 nm was found to be 35,000 M^{-1}cm^{-1}.

Results and Discussion

Comparisons of aggregate size between THF/nC_{60} and aqu/nC_{60}. Average hydrodynamic diameters (Z_{ave}) and standard deviations for the THF/nC_{60} and aqu/nC_{60} solutions (Table III-1) were calculated by averaging Z_{ave} values for replicate solutions. As indicated in Table III-1, small standard deviations illustrate a high level of agreement between replicates. Average aggregate sizes for the 400 mg/L aqu/nC_{60}, the 800 mg/L aqu/nC_{60}, and the THF/nC_{60} solutions were similar, 186, 171, and 219 nm, respectively. In contrast, the polydispersity index (PDI) values varied significantly and were 0.201, 0.139, and 0.029, respectively, for these solutions. The PDI values for the two aqu/nC_{60} solutions are almost one order of magnitude greater than the PDI value for the THF/nC_{60} solutions, indicating that the aqu/nC_{60} solutions are far more heterodisperse than THF/nC_{60}.

The CONTIN algorithm was used to develop aggregate size distributions over the range 20-2000 nm. As expected from the small PDI value for THF/nC_{60}, both the intensity-weighted and the volume-weighted size distributions for this solution were
narrow and monomodal (Figure III-1). The $Z_{ave}$ value of 219 nm is consistent with the mean aggregate sizes of 224 and 233 nm for the intensity-weighted and the volume-weighted distributions, respectively (Table III-1). Differing significantly from the results for THF/$nC_60$, both aqu/$nC_60$ solutions had broad intensity-distributions as suggested by the large PDI values. Although the heterogeneity of the aqu/$nC_60$ solutions is evident from the broad peaks in the intensity-weighted distributions, a conversion to a volume-weighted distribution allows the aggregates to be represented based upon the percentage of the total aggregate volume. This conversion, based on Mie theory, allows for better visualization of small particles in the midst of larger ones (see Appendix A).

The aqu/$nC_60$ volume-weighted size distributions reveal the presence of aggregates ranging in size from $\leq 20$ nm to 600 nm. The size distributions for the 400 mg/L and 800 mg/L aqu/$nC_60$ solutions were very similar to one another, each containing three peaks, including one smaller than 50 nm. This latter peak has not been previously reported in DLS measurements of aqu/$nC_60$ suspensions. This group of small particles may prove to be the most environmentally significant, as a recent study has shown small particles of $nC_60$ to be more toxic than their larger counterparts (7). The mean values for the peaks were 27, 122, and 280 nm for the 400 mg/L solutions and 26, 151, and 290 nm for the 800 mg/L solutions (Figure III-1). Because these solutions are heterodisperse, the $Z_{ave}$ values do not match any one peak in the aqu/$nC_60$ the distributions, rather, the $Z_{ave}$ values represent an average of all peaks. In heterodisperse systems, $Z_{ave}$ can be a useful metric for assessing trends in average particle size; however, the volume-weighted distributions provide much more information about the aggregates present in complicated samples such as aqu/$nC_60$.  

33
Our results differ from a previous study by Brant et al. (17), which reported almost identical intensity distributions for aqu/nC_{60} and THF/nC_{60} that consisted of aggregates ranging in size from 50 to 2000 nm. Our intensity distributions for aqu/nC_{60} contained particles ranging in size from 20 to 600 nm, while the particles in THF/nC_{60} ranged from 100 to 330 nm. Also, we have identified a new peak in the aqu/nC_{60} solutions containing particles smaller than 50 nm in the volume-weighted size distributions. The disparity in aggregate sizes between the studies may be a result of actual differences in the solutions produced, or the use of a different algorithm or size range in the development of the size distributions.

Both TEM and HRTEM were used to further characterize the size of aqu/nC_{60} and THF/nC_{60} aggregates. Aggregate sizes for aqu/nC_{60} and THF/nC_{60} as observed in the HRTEM and TEM micrographs were consistent with the DLS measurements (Figure III-2a-b). Analysis of aqu/nC_{60} indicates the presence of both large and very small particles, down to 20 nm in diameter. Although isolated particles of various sizes were found, the majority of the particles exist as aggregates. It is not known how much of this aggregation occurred during sample drying, but the DLS data suggests that aggregates are also present in solution. Nonetheless, some particle aggregation may have occurred during sample drying.

**Aqu/nC_{60} and THF/nC_{60} crystallinity and shape.** The crystallinity and shape of THF/nC_{60} and aqu/nC_{60} particles were studied using HRTEM. The majority of particles exhibited lattice fringes, which indicate that the particles are crystalline (Figure III-2c-f). The fringes are created by the crystal structure, and were observed for both small (< 30 nm) and large particles. Fast Fourier transformations (FFT) of the lattice
images of multiple THF/nC_{60} and aqu/nC_{60} particles were analyzed to determine crystal structure (Figure III-2c-d). FFT patterns matching those of the face centered cubic (FCC) [112], [011], and [-111] orientations were identified in both the aqu/nC_{60} and the THF/nC_{60} samples. Although the pattern of the FCC [-111] orientation also matches the hexagonal HCP [0001] orientation, we were able to confirm that the crystal class was FCC by identifying two other patterns, FCC [112] and FCC [-111], that do not exist in the HCP class. Our classification differs from that previously published by Fortner et al. (12) and Sayes et al. (11), who identified THF/nC_{60} as HCP; however, the FCC crystal structure of our particles is consistent with that of C_{60} in bulk crystalline form (22), which has been misidentified as HCP in the past (23).

The shapes of the THF/nC_{60} particles were different from those found in the aqu/nC_{60} sample. The THF/nC_{60} solution contained both spherical and faceted particles, while almost no irregular particle shapes were detected. In contrast, the majority of the particles in the aqu/nC_{60} sample were irregular in shape. Some of the aqu/nC_{60} particles were faceted, but they did not exhibit the symmetry and regularity seen in the THF/nC_{60} particles. The differences in particle shape between THF/nC_{60} and aqu/nC_{60} are relics of their formation. THF/nC_{60} particles precipitate when water is added to a solution of C_{60} dissolved in THF and their size and shape is dependent upon the rate of water addition, as described by Fortner et al. (12). The process of aqu/nC_{60} formation has not been established; however, considering the irregularity in particle size and shape and the low solubility of C_{60} in water (1.3 \times 10^{-11} \text{ ng/mL}) (6), it is likely that many of the particles formed through a weathering process where larger particles broke down into smaller particles over time.
**Pulverization of C\textsubscript{60}.** Initially, aqu/nC\textsubscript{60} solutions were made using un-pulverized C\textsubscript{60}. The material was received from the manufacturer as a heterodisperse black crystalline powder containing particles of dimension as large as several mm in length (Appendix A, Figure A-2). As a result of this heterodispersity, replicate solutions with the same nominal initial C\textsubscript{60} concentration made with un-pulverized C\textsubscript{60} often varied greatly in their final concentration of nC\textsubscript{60} (as determined by visual observation of the suspension color). Despite this variation in concentration, the average hydrodynamic diameters of the aggregates formed were remarkably similar (see Table III-1).

C\textsubscript{60} powder was pulverized prior to its addition to water to minimize inconsistency in the final nC\textsubscript{60} concentration. Pulverization of the material resulted in a visible increase in the speed and extent to which C\textsubscript{60} became stabilized in solution and made it possible to reach higher concentrations of nC\textsubscript{60} more rapidly. Additionally, by reducing variations in the initial C\textsubscript{60} size, the solution concentration could be reproduced more readily. A pulverized C\textsubscript{60} solution was filtered so that aggregate size could be compared with solutions prepared with un-pulverized C\textsubscript{60}. For solutions with the same initial C\textsubscript{60} concentration (800 mg/L), the average hydrodynamic diameters of the pulverized and un-pulverized solutions were very similar, being 163 nm and 171 nm, respectively. Based upon this DLS data and TEM images indicating similar aggregate shape and size (results not shown), it seems that pulverization of C\textsubscript{60} speeds up the process of nC\textsubscript{60} formation without dramatically altering the characteristics of the aggregates that ultimately form.

Following pulverization, the concentration of nC\textsubscript{60} in solution, as determined by UV-Vis spectroscopy at 357 nm, increases over time starting at about 6 mg/L after 1 day,
and reaching 75 mg/L after 14 days (Figure III-3). The continual increase in concentration over a two week period indicates that time will be an important factor in assessing the concentration of C₆₀ that enters an aqueous system. A spill of C₆₀ would most likely not immediately create high concentrations of nC₆₀, instead particulate C₆₀ may continually release low concentrations of nC₆₀ over an extended period.

**Filtration vs. settling.** The filtration of nC₆₀ solutions during preparation facilitates the use of methods such as DLS and TEM by removing large particles that can be problematic during size measurement or TEM grid preparation. However, this type of filtration is not a natural process and may result in an inaccurate representation of solutions produced by the gradual weathering of C₆₀ particles released to the environment. This experiment was designed to compare how aggregates in filtered solutions differ from those that were allowed to settle for one hour. Two samples from the same aqu/nC₆₀ - pulverized solution were taken. One sample was filtered through a 0.45 μm filter (Osmonics Inc.) and the other was allowed to settle for one hour. The hydrodynamic diameter, PDI, and electrophoretic mobility were 163 nm, 0.113, and \(-3.22 \times 10^{-8}\) m²/Vs, respectively for the filtered solution and 392 nm, 0.447, and \(-3.12 \times 10^{-8}\) m²/Vs, respectively, for the settled solution. Filtration of the sample reduced the polydispersity and hydrodynamic diameter of the solution because the larger particles are removed leaving behind more uniformly sized small particles. Nonetheless, the filtered and settled solutions have very similar electrophoretic mobilities, suggesting that filtration does not affect the surface properties of the aggregates. The filtration process simply removes large particles from the solution, while a good number of particles larger than 450 nm remain suspended in the settled solutions. Because filtration removes the
larger aggregates which have been shown to have decreased toxicity relative to the small aggregates (7), the simple act of filtration serves to pre-concentrate smaller aggregates and may lead to overestimates in the toxicity of a given material.

**C$_{60}$ concentration.** Solutions of aqu/nC$_{60}$ made by adding 2, 5, 10, and 20 mg of pulverized C$_{60}$ to 25 mL of nanopure water (80, 200, 400, and 800 mg/L) were produced to study the effect of the initial C$_{60}$ concentration on nC$_{60}$ formation. Increasing the amount of pulverized C$_{60}$ powder added to nanopure water resulted in an increase in the final nC$_{60}$ concentration after 14 days of stirring from 0.4 mg/L for the 80 mg/L solution to 84 mg/L for the 800 mg/L solution, as measured after 1 hour of settling (Figure III-4). The results show no indication of reaching a plateau in nC$_{60}$ concentration, which would be indicative of a maximum stable concentration of nC$_{60}$, instead, the nC$_{60}$ concentration increases as a function of the initial amount of C$_{60}$ added, indicating that equilibrium has not been reached. Even after two weeks of stirring, the concentration of nC$_{60}$ formed remains far below the concentration of C$_{60}$ added with a maximum of $\approx 10\%$ nC$_{60}$ production efficiency.

The hydrodynamic diameters of the aggregates present in the 80, 200, 400, and 800 mg/L solutions, measured with the Nano ZS, were 298, 277, 383, and 350, respectively. Intensity-weighted size distributions indicate that the aggregates become larger and more heterodisperse as the C$_{60}$ concentration is increased (Figure III-4). This trend of increasing size was most likely not observed in the experiment comparing 400 and 800 mg/L aqu/nC$_{60}$ because filtration of those samples reduced the polydispersity and selectively removed the larger aggregates from the solutions.
Effects of pH and NaCl on aqu/nC₆₀. The stability and electrokinetic properties of filtered aqu/nC₆₀ solutions were investigated through adjustments in solution pH and electrolyte concentration. The aqu/nC₆₀ particles had an electrophoretic mobility of zero at a pH of about 2 and became more negatively charged with an increase in pH (Figure III-5). Our findings with aqu/nC₆₀ differ slightly from those of Brant et al. (19) for THF/nC₆₀, which was found to have a point of zero charge closer to pH = 1, and a more linear trend of decreasing charge with increasing pH. Differences in the surface charging properties of aqu/nC₆₀ and THF/nC₆₀ may be an indication of compositional differences between the two types of aggregates. The presence of THF within the structure of the THF/nC₆₀ aggregates has been suggested to affect the charging of these particles (9).

In another experiment, NaCl was added to five samples from a filtered solution of aqu/nC₆₀ (at unaltered solution pH = 5.5), to attain samples with concentrations of 0.01, 0.1, 1, 10, and 100 mM NaCl. The electrophoretic mobility was fairly stable at NaCl concentrations ≤ 1 mM NaCl. However, at higher NaCl concentrations of 10 and 100 mM, the electrophoretic mobility became increasingly less negative, indicating compression of the electrical double layer of the aggregates by the increasing concentration of electrolytes in solution (Appendix A, Figure A-3). The trend in electrophoretic mobility as a function of NaCl added is similar to that observed by Brant et al. for a suspension produced using a similar method (mix method). However, our solutions generally had a slightly more negative electrophoretic mobility. As might be expected from the decrease in electrophoretic mobility, concentrations of NaCl > 1 mM caused aggregation and an increase in the hydrodynamic diameters from around 160 nm
for the 1 mM NaCl solution to 209 nm for the 10 mM NaCl solution and 254 nm for the 100 mM NaCl solution.

Another experiment was conducted to evaluate the differences in aggregate charge and formation when \( C_{60} \) powder was added to a solution of NaCl instead of nanopure water. In this experiment, pulverized \( C_{60} \) was added to electrolyte solutions ranging in concentration from 1 to 100 mM NaCl. Similar trends occurred in these solutions, where electrophoretic mobility became less negative as a function of increasing NaCl concentration (Figure III–6a). Likewise, the hydrodynamic diameter increased with increasing NaCl concentration (Figure III–6b). The trend was very similar to that of the \( \text{aq/nC}_{60} \) solutions where NaCl was added after the two weeks of stirring, indicating that increasing concentrations of NaCl result in compression of the electrical double layer and aggregation of \( \text{aq/nC}_{60} \).

**Effect of NOM on \( \text{aq/nC}_{60} \).** Multi-walled carbon nanotubes (CNT), which ordinarily settle out of solution, have been shown to form stable suspensions in the presence of NOM (24). To test the effect of NOM on the formation and stabilization of \( nC_{60} \), an experiment was conducted where un-pulverized \( C_{60} \) (800 mg/L) was added to solutions containing 1 or 10 mg/L GT-NOM. The solutions were stirred for two weeks and then filtered. The \( Z_{\text{ave}} \), PDI, and electrophoretic mobility were 134 nm, 0.140, and \(-2.07 \times 10^{-8} \text{ m}^2/\text{Vs}\), respectively for the 1 mg/L NOM sample and 144 nm, 0.119, and \(-1.83 \times 10^{-8} \text{ m}^2/\text{Vs}\), respectively for the 10 mg/L NOM sample. The average particle sizes for the 1 mg/L and the 10 mg/L NOM solutions were 37 nm and 27 nm smaller, respectively, than the \( Z_{\text{ave}} \) for the 800 mg/L \( \text{aq/nC}_{60} \) solution made in nanopure water. This decrease in particle size could be a result of NOM acting to stabilize small \( nC_{60} \).
particles. Additionally, we see a slight decrease in the PDI values for aqu/nC₆₀ in the presence of NOM indicating that aggregates of a more uniform size may be forming. However, the increase in stability was not a result of electrostatic stabilization, as the electrophoretic mobility of the NOM aqu/nC₆₀ solutions was less negative than aqu/nC₆₀ produced in pure water. The decrease in size and increase in stability is more likely a result of steric stabilization of the aqu/nC₆₀ aggregates by NOM. In other research, humic acid acted as a stabilizing agent, which inhibited the aggregation of nC₆₀ (produced via a solvent exchange method)(25).

**Formation of nC₆₀.** The aggregates in THF/nC₆₀ and aqu/nC₆₀ solutions may be forming via a top-down process, as larger aggregates are broken down into smaller and smaller aggregates or by a bottom-up process, where individual C₆₀ molecules or small clusters (26) of molecules are ordering themselves to form new crystals. In THF/nC₆₀ solutions the aggregates are formed during the step in which water is added to the THF-C₆₀ solution. This is a bottom-up process that results in the formation of crystals with regular sizes and shapes (12). In the case of aqu/nC₆₀ it is possible that both mechanisms take place simultaneously. Some of the smaller, more spherical particles may form from either advanced weathering, while the irregular shapes and angular edges observed in many of the larger particles are more consistent with a top-down process, such as weathering of the bulk material over time during the stirring process. Experiments to examine the mechanism of aqu/nC₆₀ aggregate formation are described in the next chapter.

In this investigation we have described numerous differences between aqu/nC₆₀ and THF/nC₆₀ aggregates. Unfiltered aqu/nC₆₀ solutions are highly heterodisperse
containing aggregates ranging in size from 20 nm to several microns in diameter. Unlike THF/nC\textsubscript{60} aggregates, the aqu/nC\textsubscript{60} aggregates are irregular in shape. These characteristics as well as the specific surface charging characteristics, stability in solution and evolution over time are distinctive attributes of the aqu/nC\textsubscript{60} aggregates making them quite different from nC\textsubscript{60} produced via other methods. Because the characteristics of nC\textsubscript{60} aggregates are dependant upon the preparation method, results from studies using aggregates produced via solvent exchange methods may not be comparable with those obtained with aqu/nC\textsubscript{60}. Recent research has informed us that certain aggregate characteristics, such as particle size and production method, have an impact on their toxicity (7). Therefore, in investigations of the fate, transport, and toxicity of nC\textsubscript{60}, the production method should be carefully selected to reflect a predicted pathway for C\textsubscript{60} to enter the environment.
References


Tables and Figures

**Table III-1** Hydrodynamic diameter ($Z_{\text{ave}}$), polydispersity index, intensity-weighted mean and volume-weighted mean of $nC_{60}$ solutions prepared via different methods. $n$ represents the number of replicate solutions.

<table>
<thead>
<tr>
<th>Method</th>
<th>$n$</th>
<th>Hydrodynamic diameter / $Z_{\text{ave}}$ (nm)</th>
<th>Polydispersity index (PDI)</th>
<th>Intensity-weighted mean (nm)</th>
<th>Volume-weighted mean (nm)</th>
</tr>
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<tr>
<td>aqu/nC$_{60}$ Co = 400 mg/L</td>
<td>4</td>
<td>186 ± 12.6</td>
<td>0.201</td>
<td>Peak 1 = 30</td>
<td>Peak 1 = 27</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Peak 2 = 122</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Peak 3 = 280</td>
<td>Peak 3 = 280</td>
</tr>
<tr>
<td>aqu/nC$_{60}$ Co = 800 mg/L</td>
<td>3</td>
<td>171 ± 0.2</td>
<td>0.139</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>THF/nC$_{60}$</td>
<td>2</td>
<td>219 ± 2.2</td>
<td>0.029</td>
<td>224</td>
<td>233</td>
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</tbody>
</table>
Figure III-1. a) Intensity-weighed and b) volume-weighted particle size distributions for $nC_{60}$ solutions labeled according to preparation method or initial $C_0$ concentration ($C_0$) for aqu/$nC_{60}$ solutions. Each distribution is the average of five measurements from two or more solutions. (CONTIN method with size range 20-2000 nm used for data analysis). Mie theory was used to convert data from intensity to volume using a refractive index of $n_{C_{60}} = 2.2$. 
Figure III-2. a) Overview TEM image of THF/nC$_{60}$. All other images are HRTEM images acquired at 200 kV with a FEI Titan scanning/transmission electron microscope (S/TEM). (b) Overview of aqu/nC$_{60}$ showing heterogeneity in particle size and shape. (c) Image of a THF/nC$_{60}$ face centered cubic (FCC) crystal in [011] zone axis with inset FFT. (d) Lattice image of an aqu/nC$_{60}$ FCC crystal in [112] orientation with inset FFT. (e) Two crystal defects in the central aqu/nC$_{60}$ nanoparticle. (f) Visible lattice fringes reveal the crystalline nature of the small aqu/nC$_{60}$ particles.
Figure III-3. The concentration of a pulverized aqu/$n\text{C}_60$ solution was measured over the 14 day stirring period and is plotted as a function of stirring time. Inset are the UV-VIS absorbance spectra of the pulverized aqu/$n\text{C}_60$ samples taken on four different days during the stirring period. (Initial $\text{C}_60 = 800 \text{ mg/L}$)
Figure III-4. Intensity-weighted size distributions for aqu/$nC_{60}$ solutions with initial $C_{60}$ concentrations of 80, 160, 400, 800 mg/L. Inset is a plot of initial $C_{60}$ concentration vs. final $nC_{60}$ concentration after stirring 14 days.
Figure III-5. Electrophoretic mobilities of aqu/nC₆₀ and THF/nC₆₀ solutions graphed as a function of pH.
Figure III-6. Pulverized C$_{60}$ was added to NaCl solutions of 1-100 mM. (a) The electrophoretic mobility of each aqu/nC$_{60}$ solution is plotted as a function of NaCl concentration. (b) The $Z_{\text{ave}}$ of each aqu/nC$_{60}$ solution is plotted as a function of NaCl concentration. PDI in nm (the standard deviation of the Gaussian distribution) is shown by the error bars.
IV. Effects of sodium citrate and electrolyte concentration on the formation of aqu/nC$_{60}$

Introduction

Numerous methods have been developed for the production of a water stable form of C$_{60}$ known as “nC$_{60}$” (1-5). Each production method produces a unique aggregate that contains multiple C$_{60}$ molecules. Differences between aggregates result from variations in the techniques employed and the solvents used to make these aggregates. A recent investigation on the effect of four different types of nC$_{60}$ on bacteria indicates that the level of nC$_{60}$ toxicity is related to both the aggregate size and the method used to produce the aggregates (6). In addition to these factors, the composition of the water affects the stability of nC$_{60}$ in solution. In accordance with DLVO theory, the addition of electrolytes to nC$_{60}$ solutions leads to aggregation and sedimentation of particles (7,8). However, it has been shown that the stability of nC$_{60}$ (produced via a solvent exchange method) can be enhanced by the presence of natural organic matter (NOM) in the water (9). Given this information, it is apparent that the fate of C$_{60}$ in the environment requires assessment of many factors, such as its release pathway, and the concentrations of constituents, such as electrolytes or NOM, in the water that C$_{60}$ comes into contact with.

In this study, we characterized the effect of water composition on the formation of aqu/nC$_{60}$, a form of nC$_{60}$ produced through simple addition of C$_{60}$ to water. This method of nC$_{60}$ production was selected because it represents a pathway by which C$_{60}$ is likely to enter the environment. We stirred C$_{60}$ in solutions containing various concentrations of NaCl, CaCl$_2$, and sodium citrate and monitored these solutions over time to evaluate increases or decreases in aggregate size, stability, and structure. Additionally, we used
this information to understand the mechanisms responsible for the formation of very small particles (< 100 nm) that exist in aqu/nC$_{60}$ solutions. These small particles may prove to be highly significant in the overall evaluation of the dangers associated with release of C$_{60}$ because they are more toxic than the larger particles (6), and they may possess the ability to cross biological membranes that larger particles are unable to breach.

**Materials and Methods**

All experiments were conducted using 99.9% sublimed C$_{60}$ (Sigma Aldrich), which was stored in the dark. Experiments were conducted using ACS grade sodium chloride, calcium chloride, and sodium citrate (Sigma Aldrich). All water used in these experiments was obtained from a NANOpure ultrapure water system and filtered through a 0.22 μm Millipore steritop filter. All glassware and lab equipment in contact with nC$_{60}$ solutions was washed with aqua regia and rinsed thoroughly before use.

The **nC$_{60}$ Suspension Preparation**. C$_{60}$ fullerene nanoparticle suspensions (nC$_{60}$) were produced via a method modified from Cheng et al. (4). In this method, C$_{60}$ was pulverized to reduce irregularity in the initial particle size. Pulverization was achieved using a Fritsch pulverisette 0 ball mill at amplitude 0.5 mm for 20 minutes. The resulting fine powder was sieved thorough a 63 μm sieve. C$_{60}$ was added (800 mg/L) to 25 mL solutions of NaCl, CaCl$_2$, sodium citrate, or nanopure water. The solutions were stirred for two weeks with a magnetic stirrer and a 1 inch PTFE stir bar. Stirring was stopped for 1 hour prior to measurements to allow the larger particles to settle so that accurate DLS measurements could be made. Samples were removed from just below the surface of the solutions and diluted as necessary.
**Dynamic Light Scattering (DLS).** Particle measurements were made using a Malvern Nano ZS equipped with a helium/neon laser (λ = 633 nm) and a folded capillary cell. Samples were sonicated for 15 seconds prior to measurement. A minimum of three measurements were made on each sample to ensure accuracy and repeatability. The refractive index of C₆₀ was set at 2.2 (2) and temperature was held at 25 °C by the instrument.

**Electrophoretic mobility.** Electrophoretic mobility was measured using a Malvern Nano ZS equipped with a helium/neon laser (λ = 633 nm) and disposable folded capillary cells. Temperature was maintained at 25 °C by the instrument and at least three measurements, each consisting of numerous sub runs, were made on each sample.

**Transmission Electron Microscopy (TEM).** TEM samples were imaged with a JEOL 100 CX-II TEM or a Zeiss 10CA TEM operated at 100 kV or 60 kV, respectively. Samples were prepared by placing one drop of solution on a carbon/formvar coated 200-mesh copper grid and allowing the sample to dry in a desiccator at room temperature. A FEI Titan scanning/transmission electron microscope (S/TEM) was operated at 200 kV to obtain high-resolution transmission electron microscope (HRTEM) images of a limited subset of samples. Ultrathin carbon grids with a holey carbon support were selected for samples investigated with the HRTEM to allow greater visibility and detection of the crystallinity and size of the small carbon nanoparticles. A representative collection of particle images was obtained from multiple sites on each grid. Determination of particle size distributions from the collected micrographs was completed using the Psion software package. A minimum of 200 particles were counted to determine particle size.
Results and Discussion

Effects of NaCl and CaCl₂ on aqu/nC₆₀. Pulverized C₆₀ was added to solutions containing 1-100 mM NaCl or 1-10 mM CaCl₂. Three control aqu/nC₆₀ solutions prepared in nanopure water had an average electrophoretic mobility of \(-3.7 \pm 0.1 \times 10^{-8}\) m\(^{-2}\)/Vs. Upon comparing the control solutions to those mixed in the presence of NaCl, we see that at low NaCl concentrations (1 and 10 mM), the aggregates had a more negative electrophoretic mobility than those with no salt (Figure IV-1). The enhanced negative charge of the aqu/nC₆₀ aggregates produced in 1 and 10 mM NaCl may indicate adsorption of co-ions (Cl\(^-\)) to the surface of the aqu/nC₆₀ aggregates, thus increasing the absolute value of the negative charge. Additional experiments need to be conducted to determine if specific adsorption is taking place or if the co-ions are simply approaching the surface of the aqu/nC₆₀ aggregates. A similar phenomena was observed for the addition of KCl, NaNO₃, or Na₂SO₄, to solutions of polystyrene latex particles (10). However, this trend was not observed in an experiment where NaCl was added to solutions of THF/nC₆₀ (another form of nC₆₀ produced via solvent exchange) (11). The disparity in charging properties of aqu/nC₆₀ and THF/nC₆₀ aggregates may be a result of differences in these two types of aggregates (as discussed in Chapter III), or may be a result of producing aqu/nC₆₀ in a solution of NaCl, resulting in the formation of an aggregate with a different surface charge that those produced in nanopure water and later exposed to NaCl solutions. At higher NaCl concentrations, the electrophoretic mobilities become less negative increasing to \(-2.5 \times 10^{-8}\) m\(^{-2}\)/Vs at 100 mM NaCl. At higher NaCl concentration the ionic strength of the solution is increased and the counterions in...
solution compress the electrical double layer surrounding the aggregates, thus reducing
the magnitude of the negative charge.

In experiments conducted on THF/\textit{n}C\textsubscript{60} by Brant et al. (11), charge reversal was
observed in a THF/\textit{n}C\textsubscript{60} solution to which 0.1 mM CaCl\textsubscript{2} was added. At higher CaCl\textsubscript{2}
concentrations, the aggregates regained their negative charge. Brant et al. (11) proposed
that the reversal in charge was caused by the adsorption of Ca\textsuperscript{2+} to the surface of the
aggregates. In our experiments, all aqu/\textit{n}C\textsubscript{60} solutions produced in CaCl\textsubscript{2} had negative
electrophoretic mobilities; however, they were much less negative than aqu/\textit{n}C\textsubscript{60}
aggregates produced in NaCl solutions. The decreased magnitude of the negative charge
on the aggregates produced in CaCl\textsubscript{2} solutions is likely caused by increased electrical
double layer compression by divalent Ca\textsuperscript{2+}, and by the adsorption of Ca\textsuperscript{2+} to the surface
of aqu/\textit{n}C\textsubscript{60} aggregates, which counteracts some of the negative surface charge.

The hydrodynamic diameters for the three control solutions ranged from 312 to
372 nm. Interestingly, low NaCl concentrations resulted in a slight decrease in aggregate
size to 263 nm. The smaller aggregate size and the more negative electrophoretic
mobilities of the 1 mM NaCl solutions may indicate that aqu/\textit{n}C\textsubscript{60} stability is enhanced at
this low NaCl concentration. However, as would be expected from the electrophoretic
mobility data, higher electrolyte concentrations of 10 mM NaCl and 1 mM CaCl\textsubscript{2} resulted
in an increase in the average hydrodynamic diameter of aqu/\textit{n}C\textsubscript{60} (Figure IV-1). In these
solutions with higher electrolyte concentrations, the measured increase in hydrodynamic
diameter was accompanied by sedimentation of particles (as observed visually) indicting
the destabilization of the aqu/\textit{n}C\textsubscript{60} aggregates.
HRTEM micrographs of aqu/\(n\)C\(_{60}\) from a 10 mM NaCl solution show extensive aggregation of primary particles (Figure IV-2a). The particles are heterogeneous in size and shape, exhibiting jagged edges and a wide range of particle sizes. Lattice fringes of overlapping crystallites indicate that the primary particles are crystalline in nature (Figure IV-2b). Fast Fourier transformations (FFT) of lattice images are consistent with the face centered cubic (fcc) crystal class observed previously (see Chapter III).

**Effect of sodium citrate on aggregate charge.** The presence of sodium citrate over the concentration range of 0.01-100 mM had a complex effect on the electrophoretic mobility of the aqu/\(n\)C\(_{60}\) aggregates. As shown in Figure IV-3, the electrophoretic mobility becomes increasingly negative when the sodium citrate concentration was increased from 0.01 to 1 mM. The solutions with the most negative electrophoretic mobilities were the 1 mM sodium citrate solutions that had an average electrophoretic mobility of about \(-5 \times 10^{-8}\) m\(^2\)/Vs. However, increases in citrate above 1 mM resulted in the opposite effect, with the electrophoretic mobility becoming less negative as the citrate concentration was increased from 1 to 100 mM. The competing trends in the electrophoretic mobility data for the citrate aqu/\(n\)C\(_{60}\) solutions may be dictated by two different phenomena. The presence of small amounts of citrate may enhance the negative charge on the aqu/\(n\)C\(_{60}\) aggregates thus resulting in more negative electrophoretic mobilities and, possibly, an increase in particle stability. However, at higher concentrations the build up of electrolytes in solution compresses the electrical double layer surrounding the aggregates. Compression of the electrical double layer results in the electrophoretic mobilities becoming less negative, as illustrated with both NaCl and CaCl\(_2\).
**Effect of sodium citrate on aggregate size and structure.** Dynamic light scattering data from aqu/nC$_{60}$ solutions with 12 different sodium citrate concentrations indicate that the aggregate size is stable at 360 ± 31 nm over a citrate range from 0 to 1 mM. Higher sodium citrate concentrations from 2.5 mM to 100 mM show a trend of increasing average hydrodynamic diameter with increasing sodium citrate concentration.

Although they were not detected by DLS measurements, very small (< 20 nm) spherical aqu/nC$_{60}$ particles were identified in TEM images obtained for solutions containing > 0.5 mM sodium citrate (Figure IV-4). The average diameter of these small aqu/nC$_{60}$ particles was 12.8 ± 2.8 nm (Appendix B, Figure B1). These particles were often in an aggregated form, occasionally surrounding a larger particle as shown in figure IV-5b. Because these small particles were not detected in the DLS measurements it is highly likely that the particles are aggregated to some degree in solution; however, additional aggregation may have occurred during the sample drying process.

The structure of the aqu/nC$_{60}$ particles found in the citrate solutions differs dramatically from that of the aggregates found in the NaCl or nanopure solutions, which contain a heterogeneous assortment of sizes and shapes. These differences may indicate that the uniform, spherical particles present in the citrate aqu/nC$_{60}$ solutions may be forming via a different process. The decrease in primary particle size may also indicate that citrate is acting to stabilize C$_{60}$ or aqu/nC$_{60}$ in solution, enabling formation of these very small particles.

**Formation of aqu/nC$_{60}$.** As shown in SEM images of pulverized C$_{60}$ (Figure IV-5), nanoparticles of C$_{60}$ are present on the surface of the particles prior to their addition to water, however, the majority of the material, by mass, is larger in size. Nanoparticles
found in the suspensions may have detached from the surface of larger particles, formed as a result of the stirring process, or have been produced by a combination of these processes. Another possibility is that individual C_{60} molecules or small clusters (12) of C_{60} molecules may have ordered themselves to form nanoparticles. In the case of aqu/nC_{60} produced in nanopure water or solutions of NaCl or CaCl_{2}, the aggregates are highly irregular, and often exhibit morphologies similar to that of the pulverized C_{60} particles (Figure IV-5). Conversely, the aqu/nC_{60} produced in citrate solutions contained particles that were very regular in size and shape. Nanocrystals of nC_{60} with regular sizes and shapes have been formed via solvent exchange methods where re-crystallization occurs as water is added to a solution of C_{60} dissolved in a non-polar solvent (13). The regular, spherical nature of the aqu/nC_{60} particles formed in citrate solutions may be a clue that these particles form through re-crystallization, as their structure is more similar to nC_{60} particles formed by re-crystallization during the solvent exchange process. In the case of aqu/nC_{60}, the smaller, more spherical particles may have formed from either advanced weathering or re-crystallization, while the irregular shapes and angular edges observed in many of the particles are more consistent with weathering of the bulk material. It is likely that the presence of citrate enhances the ability of C_{60} to initially disassemble, and eventually form the more regular particles observed in the citrate solutions.

**Conclusions**

Diversity in aggregate charge, size, structure, and stability has proven to be highly dependant upon the composition of the solution to which C_{60} powder is introduced. The fate and transport of aqu/nC_{60} in natural systems, and its potential to be taken up by
organisms will be dependant upon the propensity of aqu/nC$_6$0 to aggregate or remain stable in solution. A recent study has shown that the presence of NOM increases the stability of nC$_6$0 (produced via solvent exchange) in the presence of electrolytes and decreases the speed and extent to which aggregation occurs (9). In the aforementioned study, as well as in our sodium citrate experiments, the aqu/nC$_6$0 aggregates became more negatively charged in the presence of NOM or citrate, respectively. Therefore the stabilization of these particles may result from enhanced electrostatic repulsion between aggregates. However, in another study, the addition of humic acid and fulvic acid to solutions of nC$_6$0, caused a decrease in particle size that could not be attributed to electrostatic stabilization (14). Our understanding of the stability of nC$_6$0 in natural systems and the mechanisms responsible for its stabilization is far from complete. In natural systems a combination of electrostatic and steric stabilization mechanisms are likely to play a role in the particle stabilization, while high electrolyte concentrations will encourage aggregation.
References


Figure IV-1. Electrophoretic mobility and average hydrodynamic diameter ($Z_{\text{ave}}$) of aqu/$n$C$_{60}$ solutions plotted as a function of NaCl and CaCl$_2$ concentration at the unaltered pH of 5.5.
Figure IV-2. HRTEM images of aqu/$nC_{60}$ acquired at 200 kV with a FEI Titan scanning/transmission electron microscope (S/TEM). a) Irregular size and shape of aqu/$nC_{60}$ aggregates produced in a 10 mM NaCl solution. b) Overlapping lattice fringes indicate the crystallinity of aqu/$nC_{60}$ particles produced in a 10 mM NaCl solution.
Figure IV-3. Electrophoretic mobility and average hydrodynamic diameter ($Z_{ave}$) of aqu/$n$C$_{60}$ solutions plotted as a function of sodium citrate concentration (0.01 to 100 mM).
Figure IV-4. HRTEM images of aqu/$nC_{60}$ acquired at 200 kV with a FEI Titan scanning/transmission electron microscope (S/TEM.  a) Small, spherical aqu/$nC_{60}$ particles produce in a 10 mM sodium citrate solution.  b) Larger triangular particle surrounded by numerous spherical particles in a 10 mM sodium citrate solution.
Figure IV-5. SEM images of pulverized C$_{60}$ showing the irregularity in particle size and shape. In the bottom image it is possible to see many particles with dimensions $<100$ nm that are attached to the surface of a larger C$_{60}$ particle.
V. Conclusions

Conclusions

The primary conclusions obtained during the course of this research project are as follows:

- THF/nC\textsubscript{60} and aqu/nC\textsubscript{60} aggregates differ from one another in size, polydispersity, shape, and surface charge. These differences may play an important role in their toxicity and behavior in natural systems. And thus conclusions from studies conducted with one form of nC\textsubscript{60} may not be valid for nC\textsubscript{60} produced via other methods.

- Both THF/nC\textsubscript{60} and aqu/nC\textsubscript{60} particles exhibited face centered cubic (fcc) crystal structure. Our classification of THF/nC\textsubscript{60} as fcc differs from that of recent publications, but is consistent with the well established structure of bulk C\textsubscript{60}.

- The addition of monovalent and divalent electrolytes resulted in enhanced aqu/nC\textsubscript{60} aggregation and sedimentation, as would be expected from DLVO theory.

- The presence of NOM resulted in a decrease in aggregate size, however, the electrophoretic mobilities of these aggregates were less negative that those produced in nanopure water. Therefore, this enhanced stabilization is most likely a result of steric not electrostatic stabilization.

- Sodium citrate concentrations > 0.5 mM resulted in the formation of very small (12.8 ± 2.8 nm) spherical particles. At low concentrations (0.01 – 1 mM) sodium citrate also had the affect of making the electrophoretic mobility values more negative. The increase in negative surface charge indicates that electrostatic forces may be responsible for the stabilization of aqu/nC\textsubscript{60} in the presence of sodium citrate.
Appendix A

Dynamic Light Scattering (DLS) Analysis

DLS data was used to determine the hydrodynamic diameter of particles in the aqu/nC$_{60}$ and THF/nC$_{60}$ suspensions. DLS is based on the principle that measured particles move according to Brownian motion and the speed, or translational diffusion coefficient, of the particles is inversely proportional to their hydrodynamic diameter as defined by the Stokes-Einstein equation:

$$d(H) = \frac{kT}{3\pi\eta D}$$  \hspace{1cm} (1)

A correlogram for each suspension was developed through temporal correlation of changes in the intensity of light scattered by particles moving in the suspension. The correlogram can be fit with the cumulants algorithm, which is a single exponential function, to determine a single particle size ($Z_{ave}$) and a polydispersity index (PDI). The PDI is a measure of the standard deviation of the Gaussian distribution generated about $Z_{ave}$ and is described by the following:

$$PDI = \left(\frac{\sigma}{Z_{ave}}\right)^2$$ \hspace{1cm} (2)

In polydisperse suspensions, additional information about the distribution of particles within the suspension can be obtained by fitting the correlogram with one of several more complex algorithms that allow for multiple exponential decays and the development of a non-Gaussian distribution. Size distributions for THF/nC$_{60}$ and aqu/nC$_{60}$ were developed using the CONTIN algorithm (1). The CONTIN algorithm uses the principal of parsimony to calculate 12 possible size distributions, ordered based upon their ability to fit the data. CONTIN was selected because of its success in characterizing samples with complex particle size distributions (2). To improve the
resolution of the final CONTIN solution a size range of 20 to 2000 nm was chosen. This range was selected to be large enough to include all particle size peaks, while remaining small enough to give sufficient resolution of particle size peaks.

Intensity-weighted distributions are the direct output of the CONTIN analysis and can be converted to volume-weighted distributions via Mie theory (3). Volume-weighted distributions may detect the presence of small particles that exist in heterogeneous solutions, but are often obscured by the presence of large particles in intensity distributions. In the limited set of experiments where volume-weighted distributions were obtained, each distribution was generated by averaging the data from replicate solutions. Each solution was filtered with a 0.45 μm filter (Osmonics Inc.) so that the size distribution of the smaller particles in solution could be developed.
References


Figure A-1. Relative concentration (from samples diluted from a maximum concentration at 100%) vs. UV-VIS absorbance at 357 nm. The trend line is a linear regression developed from the data.
Figure A-2. SEM images show un-pulverized (left) and pulverized (right) C₆₀ at equivalent magnifications.
Figure A-3. $Z_{\text{ave}}$ and electrophoretic mobility of aqu/$nC_60$ are shown as a function of NaCl (0.01-100 mM). NaCl was added to aqu/$nC_60$ solution prepared in nanopure water.
### Table A-1. Summary of chapter III experiments.

<table>
<thead>
<tr>
<th>Prep Method &amp; Details</th>
<th>Solution Composition</th>
<th>Zave</th>
<th>PDI</th>
<th>Intensity waited peaks (nm)</th>
<th>Volume waited peaks (nm)</th>
<th>TEM &amp; HRTEM</th>
<th>pH</th>
<th>Electrophoretic Mobility $(10^8 \text{ m}^2/\text{Vs})$</th>
<th>UV-Vis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aqu/nC$_{60}$ - Un-Pulverized Filtered (0.45μm)</strong></td>
<td>A - (0.4 mg/mL)</td>
<td>186 ± 12.6</td>
<td>0.201</td>
<td>Peaks: 1 = 29 2 = 221</td>
<td>Peaks: 1 = 28 2 = 123 3 = 281</td>
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<td></td>
<td>• Added C$_{60}$ to nanopure water in a 60 mL glass</td>
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<td></td>
<td>• Stirred for 2 weeks at 500 rpm</td>
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<tr>
<td></td>
<td>• Filtered (0.45 μm)</td>
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<tr>
<td></td>
<td>B - (0.8 mg/mL)</td>
<td>171 ± 0.2</td>
<td>0.139</td>
<td>Peaks: 1 = 194</td>
<td>Peaks: 1 = 26 2 = 150 3 = 289</td>
<td>B1 &amp; B4 Small and large particles and aggregates of particles - some v. small and light</td>
<td>-</td>
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<td></td>
<td>• 4 replicates</td>
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<td></td>
<td>• 40 mg in 50 mL of filtered nanopure water</td>
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<td></td>
<td>THF/nC$_{60}$ Filtered (0.45μm)</td>
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<tr>
<td></td>
<td>• Transferred from THF to nanopure water and filtered</td>
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<td></td>
<td></td>
<td>Average of two 250 mL samples</td>
<td>219 ± 2.2</td>
<td>0.029</td>
<td>Peaks: 1 = 224</td>
<td>Peaks: 1 = 233</td>
<td>Faceted particles of similar size and shape</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><strong>Aqu/nC$_{60}$ - Pulverized Settled</strong></td>
<td>2 mg C$_{60}$ in 25 mL nanopure *1</td>
<td>298</td>
<td>0.442</td>
<td>-</td>
<td>-</td>
<td>5.5</td>
<td>-4.23</td>
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<tr>
<td></td>
<td>• Added pulverized C$_{60}$ to nanopure water in a 40 mL glass bottle</td>
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<td>• Stirred for 2 weeks at 500 rpm</td>
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<td>• Let settle (1 hr)</td>
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<td>* marks Experiment number</td>
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<td></td>
<td>5 mg C$_{60}$ in 25 mL nanopure *1</td>
<td>277</td>
<td>0.326</td>
<td>-</td>
<td>-</td>
<td>5.5</td>
<td>-3.63</td>
<td>-</td>
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<td>10 mg C$_{60}$ in 25 mL nanopure *2</td>
<td>383</td>
<td>0.438</td>
<td>-</td>
<td>-</td>
<td>Large size distribution of irregularly shaped particles</td>
<td>5.5</td>
<td>-3.66</td>
<td>-</td>
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<td>20 mg C$_{60}$ in 25 mL nanopure *2</td>
<td>350</td>
<td>0.401</td>
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<td>-</td>
<td>Large size distribution of irregularly shaped particles</td>
<td>5.5</td>
<td>-3.66</td>
<td>-</td>
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<tr>
<td></td>
<td>20 mg C$_{60}$ in 25 mL nanopure *1</td>
<td>372</td>
<td>0.420</td>
<td>-</td>
<td>-</td>
<td>Large size distribution of irregularly shaped particles</td>
<td>5.5</td>
<td>-3.76</td>
<td>-</td>
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<tr>
<td></td>
<td>20 mg C$_{60}$ in 25 mL nanopure *1</td>
<td>312</td>
<td>0.320</td>
<td>-</td>
<td>-</td>
<td>5.5</td>
<td>-3.79</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Aqu/nC$_{60}$ - Pulverized Settled or Filtered</strong></td>
<td>Settled 1 hour 20 mg C$_{60}$ in 25 mL nanopure *3</td>
<td>392 ± 19.3</td>
<td>0.447</td>
<td>-</td>
<td>-</td>
<td>-3.12 ± 0.0313</td>
<td>-</td>
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<td>• Added pulverized C$_{60}$ to nanopure water in a 40 mL glass bottle</td>
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<tr>
<td></td>
<td>• Stirred for 2 weeks at 500 rpm</td>
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<td>* marks Experiment number</td>
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<td></td>
<td>Filtered (0.45μm) 20 mg C$_{60}$ in 25 mL nanopure *3</td>
<td>163 ± 0.658</td>
<td>0.113</td>
<td>-</td>
<td>-</td>
<td>-3.22 ± 0.0163</td>
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<td><strong>Aqu/nC$_{60}$ - Un-Pulverized Filtered</strong></td>
<td>40 mg in 50 mL stirred two weeks and filtered</td>
<td>155</td>
<td>0.133</td>
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<td>-</td>
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<td>• (prepared for pH titration)</td>
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<td>Prep Method &amp; Details</td>
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<td>PDI</td>
<td>Intensity waited peaks (nm)</td>
<td>Volume waited peaks (nm)</td>
<td>TEM &amp; HRTEM</td>
<td>pH</td>
<td>Electrophoretic Mobility ($10^{-8}$ m$^{-2}$/V·s)</td>
<td>UV-Vis</td>
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<td>• Added C$_{60}$ to NOM solutions in 60 mL glass bottles</td>
<td>• Stirred for 2 weeks at 500 rpm</td>
<td>• Filtered (0.45 μm)</td>
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<td>B Samples 10 mM NOM</td>
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<td>Aqu/nC$_{60}$ - Un-Pulverized Effect of NaCl addition after (9-01-06)</td>
<td>0 mM NaCl*</td>
<td>161 ± 2.55</td>
<td>0.198</td>
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<td>-</td>
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<td>-3.09$^*$</td>
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<td>• Added C$_{60}$ to nanopure water in a 60 mL glass bottle</td>
<td>• Stirred for 2 weeks at 500 rpm</td>
<td>• Filtered (0.45 μm)</td>
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<tr>
<td>• Mixed Aqu/nC$_{60}$ with salt solution (1:1) to produce the concentrations listed*</td>
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<tr>
<td>0.01 mM NaCl*</td>
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<tr>
<td>0.1 mM NaCl*</td>
<td>159 ± 1.61</td>
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<tr>
<td>1 mM NaCl*</td>
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<tr>
<td>10 mM NaCl*</td>
<td>209 ± 22.3</td>
<td>0.317</td>
<td>-</td>
<td>-</td>
<td>-2.55$^*$</td>
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<tr>
<td>100 mM NaCl*</td>
<td>254 ± 12.2</td>
<td>0.283</td>
<td>-</td>
<td>-</td>
<td>-1.25$^*$</td>
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<tr>
<td>Aqu/nC$_{60}$ - Pulverized Settled</td>
<td>1 mM NaCl-a *1</td>
<td>283</td>
<td>0.396</td>
<td>266</td>
<td>-</td>
<td>Irregular particles forming chains of aggregates</td>
<td>5.5</td>
<td>-4.65</td>
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<tr>
<td>• Added pulverized C$_{60}$ to nanopure water in 40 mL glass bottles</td>
<td>• Stirred for 2 weeks at 500 rpm</td>
<td>• Settled (1 hr)</td>
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<tr>
<td>1 mM NaCl-b *1</td>
<td>242</td>
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<td>257</td>
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<td>-</td>
<td>5.5</td>
<td>-4.43</td>
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<tr>
<td>10 mM NaCl-a *1</td>
<td>514</td>
<td>0.475</td>
<td>486</td>
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<td>Dominated by irregular particles but some aggregate chains</td>
<td>5.5</td>
<td>-4.17</td>
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<tr>
<td>10 mM NaCl *2</td>
<td>699</td>
<td>0.572</td>
<td>524</td>
<td>-</td>
<td>HRTEM - aggregates of irregular particles as small as 10 nm</td>
<td>5.5</td>
<td>-4.34</td>
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<tr>
<td>30 mM NaCl *2</td>
<td>648</td>
<td>0.553</td>
<td>521</td>
<td>-</td>
<td>Dominated by irregular particles but some aggregate chains</td>
<td>5.5</td>
<td>-3.36</td>
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<tr>
<td>70 mM NaCl *2</td>
<td>820</td>
<td>0.558</td>
<td>659</td>
<td>-</td>
<td>Large particles and chains of smaller particles</td>
<td>5.5</td>
<td>-2.53</td>
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<td>100 mM NaCl-a *1</td>
<td>941</td>
<td>0.495</td>
<td>808</td>
<td>-</td>
<td>Irregular particles and very small 10 nm and 50 nm round particles</td>
<td>5.5</td>
<td>-2.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM NaCl-b *1</td>
<td>795</td>
<td>0.606</td>
<td>577</td>
<td>-</td>
<td>-</td>
<td>5.6</td>
<td>-2.45</td>
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Appendix B

Tables and Figures

Figure B-1. Particle size distribution for the small spherical aqu/$nC_{60}$ particles present in a 10 mM sodium citrate solution. Average diameter is 12.8 ± nm. Determination of particle size from TEM micrographs was achieved by measuring >200 particles using Psion software.
Table B-1. Summary of chapter IV experiments.

<table>
<thead>
<tr>
<th>Prep Method &amp; Details</th>
<th>* Solution Composition</th>
<th>Z_{ave}</th>
<th>PDI</th>
<th>Intensity waited peaks</th>
<th>TEM &amp; HRTEM</th>
<th>pH</th>
<th>Electro-phoretic mobility ($10^{-8} \text{ m}^2/\text{V}\cdot\text{s}$)</th>
<th>UV-VIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqu/Nanopure C_{60} - Nanopure Pulverized - settled</td>
<td>20 mg C_{60} in 25 mL nanopure *2</td>
<td>350</td>
<td>0.401</td>
<td>-</td>
<td>Large size distribution of irregular shaped particles</td>
<td>5.5</td>
<td>-3.66</td>
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<td>20 mg C_{60} in 25 mL nanopure *1</td>
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<td>0.420</td>
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<td>Large size distribution of irregular shaped particles</td>
<td>5.5</td>
<td>-3.76</td>
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<td></td>
<td>20 mg C_{60} in 25 mL nanopure *1</td>
<td>312</td>
<td>0.320</td>
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<td>5.5</td>
<td>-3.79</td>
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<td>Aqu/Nanopure C_{60} - Nanopure Pulverized - settled</td>
<td>Aqu/NaCl - Nanopure Pulverized - settled</td>
<td>1 mM NaCl-a *1</td>
<td>283</td>
<td>0.396</td>
<td>Irregular particles forming chains of aggregates</td>
<td>5.5</td>
<td>-4.65</td>
<td></td>
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<td></td>
<td>1 mM NaCl-b *1</td>
<td>242</td>
<td>0.294</td>
<td>-</td>
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<td>5.5</td>
<td>-4.43</td>
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<tr>
<td></td>
<td>10 mM NaCl-a *1</td>
<td>514</td>
<td>0.475</td>
<td>-</td>
<td>Dominated by irregular particles but some aggregate chains</td>
<td>5.5</td>
<td>4.17</td>
<td></td>
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<tr>
<td></td>
<td>10 mM NaCl *2</td>
<td>699</td>
<td>0.572</td>
<td>-</td>
<td></td>
<td>5.5</td>
<td>-4.34</td>
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<td>30 mM NaCl *2</td>
<td>648</td>
<td>0.553</td>
<td>-</td>
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<td>5.5</td>
<td>-3.36</td>
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<td>70 mM NaCl *2</td>
<td>820</td>
<td>0.558</td>
<td>-</td>
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<td>5.5</td>
<td>-2.53</td>
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<tr>
<td></td>
<td>100 mM NaCl-a *1</td>
<td>941</td>
<td>0.495</td>
<td>-</td>
<td>Irregular particles and v. small 10 nm and 50 nm round particles</td>
<td>5.5</td>
<td>-2.51</td>
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<tr>
<td></td>
<td>100 mM NaCl-b *1</td>
<td>795</td>
<td>0.606</td>
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<td>5.6</td>
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<td>Aqu/Nanopure C_{60} - CaCl_2 Pulverized - settled</td>
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<td>494</td>
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<td>5.5</td>
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<td>5 mM CaCl_2 *2</td>
<td>638</td>
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<td>Dominated by larger particles and aggregates of large particles</td>
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<td>10 mM CaCl_2 *2</td>
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<td>-</td>
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<td>5.5</td>
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<td>Prep Method &amp; Details</td>
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<td>Z&lt;sub&gt;ave&lt;/sub&gt;</td>
<td>PDI</td>
<td>Intensity waited peaks</td>
<td>TEM &amp; HRTEM</td>
<td>pH</td>
<td>Electro-phoretic mobility (10&lt;sup&gt;4&lt;/sup&gt; m&lt;sup&gt;2&lt;/sup&gt;/Vs)</td>
<td>UV-VIS</td>
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<td>Aqu/C&lt;sub&gt;c60&lt;/sub&gt; - Citrate Pulverized - settled</td>
<td>0.01 mM Citrate *2</td>
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<td>0.394</td>
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<td>1 mM Citrate-a *1</td>
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<td>1 mM Citrate-b *1</td>
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<td>2.5 mM Citrate *3</td>
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<td>Irregular particles and round particles 25 nm and smaller</td>
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<td>5 mM Citrate *3</td>
<td>614</td>
<td>0.608</td>
<td>Combination of (1) irregular shaped particles over a large size range and (2) 10 nm or 50 nm diameter spheres HRTEM - 10 mM Citrate which shows 10 nm single crystals &amp; polycrystalline 50 nm crystals in addition to a few single crystal 2-D squares and triangles</td>
<td>7-8</td>
<td>-4.22</td>
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<td>10 mM Citrate-a *1</td>
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<td>-1.95</td>
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* marks Experiment number

- Added pulverized C<sub>c60</sub> to Citrate solutions
- Stirred for 2 weeks at 500 rpm
- Settled (1 hr)
TEM images

TEM - aqu/nC$_{60}$ (concentration 400 mg/L)

TEM - THF/nC$_{60}$
HR-TEM - aqu/nC₆₀ (concentration 800 mg/L)
HR-TEM - aqu/nC$_{60}$ (concentration 800 mg/L)
HR-TEM - aqu/nC₆₀ (concentration 800 mg/L)
HR-TEM - aqu/nC$_{60}$ (concentration 800 mg/L)
HR-TEM - aqu/nC$_{60}$ (concentration 800 mg/L)
HR-TEM - aqu/nC$_{60}$ (concentration 800 mg/L)
HR-TEM - THF/nC$_{60}$
HR-TEM - THF/nC$_{60}$
TEM - aqu/nC$_{60}$ NaCl
HR-TEM - aqu/nC₆₀ NaCl
HR-TEM - aqu/nC$_{60}$ NaCl
TEM - aqu/nC₆₀ Sodium citrate
HR-TEM - aqu/nC₆₀ Sodium Citrate
HR-TEM - aqu/nC<sub>60</sub> Sodium Citrate
HR-TEM - aqu/nC$_{60}$ Sodium Citrate
VITA

Laura K. Duncan was born in Denver, Colorado on March 19, 1982 to Mack and Julie Duncan.

She attended Virginia Tech from the fall of 2000 to 2004 where she earned a B.S. in Geosciences. In 2005, she began her graduate studies in Environmental Engineering at Virginia Tech. Her M.S. ENE degree was completed in spring 2007.