Aggregation and Coagulation of C60 Fullerene as Affected by Natural Organic Matter and Ionic Strength

Hamid Mashayekhi
hamid@umass.edu

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AGGREGATION AND COAGULATION OF C_{60} FULLERENE AS AFFECTED BY NATURAL ORGANIC MATTER AND IONIC STRENGTH

A Dissertation Presented

by

HAMID MASHAYEKHI

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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Plant and Soil Sciences Program
AGGREGATION AND COAGULATION OF C$_{60}$ FULLERENE AS AFFECTED BY NATURAL ORGANIC MATTER AND IONIC STRENGTH

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HAMID MASHAYEKHI

Approved as to style and content by:

________________________________
Dr. Baoshan Xing, Chair

________________________________
Dr. Dula Amarasiriwardena, Member

________________________________
Dr. Stephen Simkins, Member

________________________________
Dr. Richard Vachet, Member

Dr. Wesley Autio, Director
Stockbridge School of Agriculture
DEDICATION

I dedicate this work to my wife Lourdes who always encouraged me and provided all her intellectual and professional help in my research. And to my children Sam and Susana, so that they always remember to accomplish their goals and dreams.

I dedicate this dissertation to my brother Ali who always believed in me, encouraged, and supported me in my research as well as my life.

I dedicate this work to my mother and sisters for their love and emotional support, and to the living memory of my father who loved science and believed in it.
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ABSTRACT

AGGREGATION AND COAGULATION OF C₆₀ FULLERENE AS AFFECTED BY NATURAL ORGANIC MATTER AND IONIC STRENGTH

SEPTEMBER 2016

HAMID MASHAYEKHI, B.A., ISFAHAN UNIVERSITY OF TECHNOLOGY

M.A., TARBIAT MODARES UNIVERSITY

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Baoshan Xing

With widespread production and use of C₆₀ fullerene nanoparticles, their release to the environment and natural waters is inevitable. The colloidal nature of C₆₀ fullerene in the aquatic environment significantly influences its behavior in the environment including its transport, bioavailability and toxicity to different organisms. Natural organic matter (NOM) is a ubiquitous and reactive material in aquatic environments with significant structural heterogeneity. NOM is known to interact with other particles present in waters, to modify their surface structure and thus to affect their environmental behavior. Therefore, the effect of NOM molecules on the colloidal behavior of fullerene particles needs to be studied.

A major part of NOM consists of humic acids (HA). HAs have pronounced effects on the aggregation of C₆₀ fullerene in water. To obtain structurally different HAs, they
were sequentially extracted from compost. The structure of extracted HAs became increasingly aliphatic and hydrophobic with increasing number of extractions. Molecular weights of HAs and their affinity for adsorbing simple nonpolar molecules from water also increased with increasing number of extractions.

The effect of structurally different HAs, as well as fulvic acid (FA) and tannic acid (TA) on the aggregation behavior of C_{60} fullerene suspension (nC_{60}) was investigated. Aggregation was started by addition of varying amounts of Ca^{2+} to the nC_{60} with 2 mg/L of each type of NOM. The absolute value of the zeta potential |\zeta| of pure nC_{60} increased after the addition of any type of NOM. Addition of Ca^{2+} to the (nC_{60} + NOM) system decreased |\zeta| of fullerene almost uniformly for all types of NOM. The nC_{60} critical coagulation concentration (CCC) was equal to 14.5, 6.5, 5.4, and 3.7 mM Ca^{2+} for HA7, HA1, FA, and TA respectively. A nearly constant |\zeta| for (nC_{60} +NOM) system across a wide range of Ca^{2+} concentrations, suggested steric stability of the aggregates rather than electrostatic stability. Then the feasibility of nC_{60} removal from water sources with commonly used coagulants was investigated, as there is little information available on the transformation and removal efficiency of C_{60} during the process of water treatment. Results showed that C_{60} can be removed with 80% efficiency at an optimum Al^{3+} dose. Divalent anions, such as CO_3^{2-}, SO_4^{2-}, HPO_4^{2-} and humic acid (HA) could act as a bridge to connect C_{60}-Al(OH)_{n\text{-(m/δ)}} particles together and induce the formation of flocs, therefore, the addition of these anions enhanced C_{60} removal significantly. Other cations such as Na^+ and Ca^{2+} increased the ionic strength of aqueous solution which enhanced the
function of pressing electric double layer and induced the $C_{60}^{m-n}\text{Al}(OH)_{n}^{(m-n)^{+}}$ particles to further aggregate.

This study highlighted the role of omnipresent, very reactive and structurally diverse HAs on the transportation, aggregation, precipitation and fate of $C_{60}$ fullerenes, when they enter the natural waters. The interaction between fullerene nanoparticles with NOM and different cations and anions in the environment, is an essential factor in understanding fate and behavior of these particles in the environment.
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CHAPTER 1

GENERAL INTRODUCTION

1.1. Overview

Nanotechnology is the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications (National Nanotechnology Initiative, 2010). When the size of ordinary materials such as carbon or silicon is reduced to nanoscale, they show novel and unpredictable characteristics such as extraordinary strength, chemical reactivity, electrical conductivity, or other properties that the same material does not possess in the micro or macro scales (Aitken et al., 2004). When material dimensions reach the nanometer scale, quantum mechanical and thermodynamic properties that are insignificant for large, predominate, everyday materials, cause these nanomaterials to display new and interesting properties.

Nanotechnology holds revolutionary scientific and engineering promise that may impact the infrastructure of consumables. But just like any other technology, nanotechnology can be like a double-edged sword. Environmental and health concerns regarding nanotechnology have been discussed during the past decade. The small size and unique physical and chemical properties of some nanoparticles, for instance, makes them valuable in medicine, but the same features might make them active in unusual ways in the environment and might allow them to react with living organisms.

Nanomaterials are a diverse class of small-scale (< 100 nm in at least one dimension) substances formed by molecular-level engineering to achieve unique mechanical, optical, electrical, and magnetic properties. Nanomaterials are expected to improve virtually all
types of products (Tsuji et al., 2006).

Generally speaking nanomaterials can be organized into four types (AZ Nanotechnology, 2010):

- **Carbon Based Materials.** These materials are composed of carbon atoms. They may take the form of hollow spheres, ellipsoids (such as fullerenes), or tubes (such as carbon nanotubes). These particles have many potential applications, including improved films and coatings, stronger and lighter materials, and applications in electronics.

- **Metal Based Materials.** They include quantum dots, metal nanoparticles like gold, silver or iron, as well as bimetallic nanoparticles, and metal oxides, such as titanium dioxide. They have a vast application range in different industries, biomedicine, environmental remediation, and cosmetics.

- **Dendrimers.** These nanomaterials are nanosized polymers built from branched units. The surface of a dendrimer has numerous chain ends, which can be tailored to perform specific chemical functions.

- **Composites.** These are the combination of nanoparticles with other nanoparticles or with larger, bulk-type materials.

**1.2. \( C_{60} \) Fullerene**

Fullerenes were discovered in 1985 by Harold Kroto and Richard Smalley (1985). These products have even-numbered closed-cage structures consisting of carbon atoms. \( C_{60} \) was one of the first molecules discovered in this family of spheroidal carbon clusters with the carbon number ranging from 20 to 240. The discoverer of \( C_{60} \) named it “buckminsterfullerene” and the entire family as “fullerenes” after the American architect
R. Buckminster Fuller who made geodesic domes (Fig 1.1). A general chemical formula of $C_{20+2H}$ has been proposed for them, in which $H$ stands for the number of hexagonal phases. For example the soccer-ball structure of $C_{60}$ is made of 20 hexagonal carbon rings interlocked together with 12 pentagonal carbon rings. In the structure of $C_{60}$ there is no five-membered rings fused directly together, while the six-membered rings are fused to the alternating six- and five- membered rings. The structure of $C_{60}$ is highly aromatic having 60 $\sigma$ and 30 $\pi$ electrons, giving it 12,500 possible resonance structures (Taylor, 1995).

The first method of production of fullerenes used laser vaporization of carbon (graphite) in an inert atmosphere of helium (~100 torr). This method however produces only minor amounts of fullerenes. The soot from graphite vaporization contains only few percent of fullerene ($C_{60}$ and $C_{70}$). Soxhlet extraction is used to solubilize fullerene molecules from the soot in benzene or other nonpolar solvents. Benzene then is evaporated to get pure fullerene. Another method used to separate $C_{60}$ from the soot is heating it in an inert atmosphere or vacuum to 400° C and subliming $C_{60}$. Separation of $C_{60}$ from $C_{70}$ or higher fullerenes is done by chromatographic techniques (Kratschmer, 1995). Today a simple AC or DC arc technique is used to produce fullerenes commercially from graphite, which has a reasonable yield of around 40%.

Fullerenes have many promising biomedical and biological applications such as cytoprotection, DNA photocleavage, enzyme inhibition, diagnostic imaging, and antimicrobial and anticancer therapy (Isakovic et al., 2006). $C_{60}$ is used in fuel cells, drug delivery, imaging agents in medicine, and cosmetics (Xin and Bouchard, 2008).
1.3. Environmental concerns for C\textsubscript{60} fullerene

As the production and use of fullerene continue growing and expanding, it is easy to predict that fullerenes will be introduced into the environment during their production, transportation, use, or by disposing or discharging of consumer products containing fullerenes. There are several concerns about the diverse effects of released fullerenes into the environment, including their toxicity and their potential for acting as a carrier and accumulator of xenobiotic compounds. For instance, it has been shown that the toxicity of phenanthrene and pentachlorophenol (PCP) in the presence of C\textsubscript{60} suspensions was altered (Baun et al., 2008). Fullerene aggregates showed high sorption for phenanthrene and PCP as well. Phenanthrene sorbed to the C\textsubscript{60}-aggregates contributed to the overall toxicity to algae and daphnids, and consequently an increase in toxicity was observed for phenanthrene after addition of C\textsubscript{60} in aqueous solution. In the case of PCP toxicity, however, addition of fullerene aggregates to the system reduced overall toxicity of PCP, because absorbed PCP was deactivated and could not be desorbed inside the organism. Atrazine toxicity to test organisms did not show any changes in toxicity in the presence or absence of fullerene aggregates (Baun et al., 2008). Thus, diverse interactions of fullerenes with xenobiotic compounds should also be considered during an overall assessment of environmental risks of released fullerenes into the environment. In another study Cheng (2004) showed that dispersing fullerene in water increased its solid-water distribution coefficient (K\textsubscript{d}) for naphthalene sorption by several orders of magnitude. This fact significantly affects transport, distribution and even the biological interactions of naphthalene, a model organic pollutant, in the presence of fullerene aggregates in water.
From the toxicity point of view, even though still subject to debate (Gharbi et al., 2005) several reports have shown toxicity of fullerene to various microorganisms (Fortner et al., 2005; Lyon et al., 2006; Dhawan et al., 2006; Lyon and Alvarez, 2008), aquatic organisms (Oberdorster et al., 2006; Zhu et al., 2006; Usenko et al., 2008), and animal/human cell lines (Sayes et al., 2004; Sayes et al., 2005; Fiorito et al., 2006). Reactive oxygen species (ROS) are believed to be responsible for eukaryotic cell membrane disruption and eukaryotic lipid peroxidation in the presence of C\textsubscript{60} fullerene with consequent toxicity. It is not clear if ROS are produced by C\textsubscript{60} aggregates or by the eukaryotic cells’ immune response to the fullerene (Lyon et al., 2006). Lipid peroxidation and the subsequent damage to the cell membrane were reported to be the mechanism of fullerene C\textsubscript{60} toxicity in human cell lines (Sayes et al., 2005).

There is a debate whether the observed toxicity of fullerene might be caused by traces of the organic solvents used during preparation. However, research has shown that fullerene suspensions prepared without the use of any organic solvent and only by mixing fullerene powder with water, also show signs of toxicity (Lyon et al., 2006). Some toxicity studies on fish and invertebrates suggest that C\textsubscript{60} fullerene is toxic in the ppm range, but the reported LC\textsubscript{50} values are largely dependent on the preparation method of the fullerene suspension. However, dispersed C\textsubscript{60} fullerenes are more toxic than the non-dispersed C\textsubscript{60} (Lovern and Klaper, 2006; Zhu et al., 2006). Lyon et al. (2006) reported that small fullerene aggregates were significantly more toxic to bacteria than larger aggregates; but there was no clear linear relationship between aggregate size and observed toxicity. Nevertheless, aggregate size of fullerene appears to affect its toxicity and makes aggregation an important factor in predicting the toxic effects of fullerene.
1.4. $C_{60}$ fullerene in water

$C_{60}$ is essentially insoluble in water and other polar and H-bonding solvents such as acetone, tetrahydrofuran, acetonitrile, nitromethane, methanol and ethanol. It is sparingly soluble in alkanes like hexane and its solubility increases with number of carbons in the solvent. The solubility of $C_{60}$ in chloroalkanes is generally higher than that of alkanes. Fullerenes have rigid, well-defined geometries and their shape does not change as a result of interaction with solvents. The solubility of $C_{60}$ fullerene in toluene is 2.8 mg/mL, 1.7 mg/mL in benzene and 36 mg/mL in dimethylnaphthalene (Ruoff et al., 1993).

A $C_{60}$ molecule could be considered as a colloidal particle itself, considering that its diameter is just 1 nm. Traditionally, 1 nm (or 10 Å) is considered as the lower range for colloidal particles (Andrievsky et al., 2002). Colloidal particles are defined as any organic or inorganic particles large enough to show supramolecular structure and properties (such as conformational changes for organic materials and electrical surface fields for inorganic materials), but small enough not to sediment quickly. Particles having a size range between 1 nm to 1 \(\mu\)m usually exhibit the above characteristics of colloidal particles (Buffle et al., 1998).

Andrievsky et al., (1995) were one of the first research groups to report the formation of a stable aqueous colloidal suspension of $C_{60}$ and $C_{70}$ fullerenes in the absence of any additives (such as surfactants). They solubilized $C_{60}$ in toluene (200 mg/L) and then mixed it with deionized (DI) water and sonicated the 2-phase mixture until full evaporation of toluene was achieved. The resulting yellowish solution was passed through a 0.2 \(\mu\)m filter and was slightly opalescence which indicates a colloidal
suspension with particles smaller than 0.2 µm. The suspension was unaffected by boiling and was stable for several months at neutral pH. The mass spectrum of the suspension showed the presence of C\textsubscript{60} and C\textsubscript{70} in the same ratio as the original fullerene mixture, and there were no indications of fullerene derivatives formation. Scharff et al. (2004) used this technique to prepare water-soluble fullerenes to use in biological studies, and reported that maximum possible fullerene concentration in water was 1.4 mg/ml. Another method that has been used in making water-soluble fullerenes is to functionalize them. The first generation of water-soluble fullerenes was made by adding 18 hydroxyl groups on the C\textsubscript{60} molecule in 1992. This poly-hydroxylated C\textsubscript{60} is also called fullerenol-I. Compounds such as polyalkyl-sulfonated C\textsubscript{60} with 4-6 sodium butyl sulfonate moieties (FC\textsubscript{4}S\textsuperscript{1}) are considered as the second generation of water soluble fullerenes (Chen et al., 1998).

Besides these solvent exchange mechanisms, stirring a mixture of fullerene and distilled water alone for long periods of time (usually two weeks) also leads to the production of a stable fullerene water suspension (FWS) or nC\textsubscript{60} (Cheng et al., 2004). This process could be the most probable route for the formation of fullerene suspension in natural waters, if a spill of fullerene powder into the environment takes place. Jakubczyk et al. (2004) reported that the colloidal C\textsubscript{60} in water prepared by sonicating fullerene powders in water did not extract back easily and in an appreciable quantity to a nonpolar solvent such as toluene or benzene. This indicates that the process involved in the creation of C\textsubscript{60} water colloids alters the properties of fullerene. It has been reported that the surface of fullerene colloids in water is negatively charged (Deguchi et al., 2001; 

\textsuperscript{1} C\textsubscript{60}((CH\textsubscript{2})\textsubscript{4}SO\textsubscript{3}Na)\textsubscript{4-6}
Fortner et al., 2005). This fact explains why the fullerene colloidal aggregates cannot be extracted back into a nonpolar solvent. The source of the negative surface charge of fullerene is not clearly understood, however there are several theories in the literature that will be reviewed and discussed later in this work.

The fate and transport of fullerene nanoparticles in natural aquatic systems determines their bioavailability to living organisms. Since the mobility of the nanoparticles in aquatic systems is significantly dependent on their ability to remain suspended, studying the aggregation behavior of nanoparticles in natural waters is important for the prediction of their biological activities or for their removal from water. Aggregation and stability of colloidal suspensions including fullerene suspension in general follows the classical DLVO theory of colloid stability.

1.5. The DLVO Theory

The DLVO theory is named after Derjaguin Landau, Verwey and Overbeek and is the theory describing colloid stability. This theory is based on the quantitative representation of the electrical double layer repulsive and van der Waals attractive forces between adjacent colloid particles. DLVO theory suggests that the stability of a colloidal system is determined by the sum of these van der Waals attractive ($V_A$) and electrical double layer repulsive ($V_R$) forces that exist between particles as they approach each other due to the Brownian motion they are undergoing. This theory proposes that an energy barrier resulting from the repulsive force prevents two particles approaching from each other and sticking together.

These interactions are expressed in terms of potential energy as a function of distance between two particles. The repulsive energy ($V_R$) between two particles
decreases exponentially with increasing distance between two particles. At very short distances between particles the attraction energy \((V_A)\) is significantly higher than the repulsive energy; therefore flocculation is favored and results in the instability of a colloidal system. At longer separating distances, the counterions of an electrical double layer of the interacting particles repel each other thus the repulsive energy overcomes the attractive force. In this case the stability of the colloidal system is maximum. In classical DLVO potential energy curves features like energy barrier, primary and secondary minima are characteristics (Figure 1.2). DLVO theory does not include the effects of particle shape, charge heterogeneity, and surface roughness, which may also influence the collision efficiency (Elimelech et al., 1995).

1.6. Aggregation behavior of pure fullerene suspension in water

Chen and Elimelech (2006) reported the intensity-weighted hydrodynamic radius of a freshly synthesized fullerene by sonication technique was measured to be 55.7 nm (±1.8 nm) based on 20 Dynamic Light Scattering (DLS) measurements. This size was constant for 2 months after the synthesis of colloidal suspension and after 6 months the aggregate radius increased to 58.2 nm. Fortner et al. (2005) evaluated the stability of fullerene suspension created by solvent exchange method in solutions with ionic strengths in the range 0.7–0.001 M. Their results showed that ionic strengths more than 0.05 M increased particle size and ionic strengths of 0.1 and 0.7 M are high enough to precipitate the fullerene particles out of solution after 72 and 48 h, respectively. However, in ionic strengths lower than 0.05 M, a considerable fraction of particles remained stable at \(C_{60}\) concentrations up to 100 ppm for at least 15 weeks. Thus, fullerene suspensions in water are strongly sensitive to ionic strength, which is an indication of the electrostatic
stability of fullerene suspension. However, in real conditions of natural waters, the presence of other natural colloids and macromolecules could affect or mask the electrostatic stability effect. This raises the need to study the aggregation behavior in more complicated and realistic solutions which are closer to natural waters.

The negative surface charge of the fullerene aggregates becomes less negative as the electrolyte concentrations increase (i.e., at higher ionic strength), and divalent cations are more effective than monovalent cations in decreasing surface charge of fullerene suspensions. In the presence of NaCl and CaCl$_2$ electrolytes, an increase in the electrolyte concentrations resulted in a monotonic increase in the rate of aggregation until their respective critical coagulation concentrations (CCC). At concentrations above the CCC, aggregation occurs at the same maximum rate in both electrolytes, confirming diffusion-controlled aggregation kinetics (Chen and Elimelech, 2006).

1.7. Source of negative surface charge on fullerene aggregates

Fullerene suspensions, regardless of the method of their production, possess a relatively high negative charge (~ -60 mV). This is a very interesting phenomenon, because the surface charge of colloidal suspensions usually arises from the ionization of functional groups on the surface of particles, differential loss of ions from a crystalline structure, or through adsorption of cations or anions (Ma and Bouchard, 2009). However, the negative charge of a fullerene water suspension does not fall into any of those categories. NMR and titration experiments do not support the existence of any functional groups on the fullerene molecules in water, but show rather than that the fullerene molecule is in pristine condition. Different theories are proposed to justify the presence of negative charge in the fullerene suspension that take account of fullerenes’ electronic
properties. Recalling that fullerene molecule is an electron acceptor, having an electron affinity of 2.65 eV (measured in gas phase) makes it reactive with nucleophilic species. One possibility is that during the interaction of $C_{60}$ with water molecules (while stirring or sonicating) some kind of donor-acceptor reaction takes place and the observed charge is due to the adsorption of hydroxyl ions on the surface of fullerene (Marinova et al., 1996). Based on this theory the free electron pair of oxygen in water molecule becomes more polarized in the vicinity of electron deficient fullerenes. As a result water molecules will align themselves around the fullerene with their oxygen side towards the particle. Then hydroxyl ions in water will adsorb to the hydrogen atoms of water layer surrounding the fullerene particle with strong dipole or H-bonding and give fullerene its negative charge in water (Deguchi et al., 2001; Marinova et al., 1996). However, a comparable zeta potential has been observed for dispersions of fullerene in organic polar solvents. Thus, this theory could not be the sole reason for the observed negative charge in $C_{60}$ molecules in water. Brant et al. (2005), however, disagreed with this hypothesis, reasoning that hydroxyl ion adsorption was not the major contributor to surface charge, because of unchanged pH after formation of colloidal $C_{60}$ and no observed decrease in $\zeta$ potential at higher pHs.

Andrievsky et al. (1995) proposed a theory based on the geometry of fullerene molecule and water structure for the stability of fullerene water suspension. Based on their theory, $C_{60}$ molecules enter into cavities of water structure due to the ultrasound treatment which causes formation of clathrate-like layers of water around fullerene molecules (inclusion complex). At the same time electronic properties of fullerene may lead to donor-acceptor and charge transfer interactions, which cause intermolecular
Chen and Elimelech (2009) examined a few of theories on the origin of electric charge of fullerenes:

1.7.1. Preferential adsorption of anions

Preferential adsorption of anions cannot be the source of negative surface charge of fullerene particles. They measured electrophoretic mobility of fullerene suspension in the presence of Cl\(^-\), I\(^-\), and SO\(_4^{2-}\) anions paired with K\(^+\) cation but did not see any significant change in the surface charge of fullerenes. Thus, adsorption of anions is not the source of surface charge. This is in line with the work of Brant et al. (2005) which ruled out anion adsorption as a source of negative charge for fullerene particles.

1.7.2. Oxidation of the surface of fullerene when it is exposed to water

XPS data showed that the oxygen content of fullerene particles which were in contact with water for long periods of time (40 days) was significantly higher than that of pristine fullerene (3.7% O as compared to 1.7% in pristine fullerene). Thus, deprotonation of these oxygen-containing surface functional groups could be a reason for negative surface charge of fullerene. However, there is evidence that even if such oxygenated groups are present on fullerene surface, their deprotonation could not explain the negative charge of a fullerene suspension, because, a fullerene suspension is negatively charged even at pHs as low as 2 (Brant et al., 2005). But at such low pH, almost all oxygen-containing acidic functional groups should be protonated and could not be contributing to the negative charge of particle. Another reason is that potentiometric titration of a fullerene suspension does not show any significant change with the
potentiometric titration curve of water, i.e. the presence of functional groups cannot be confirmed via titration (probably due to their low abundance).

1.7.3. Higher pH increases stability of fullerene suspension

The $\zeta$ potential of fullerene suspensions (regardless of their method of production) becomes increasingly negative as pH increases. The increase in the surface charge of the nanoparticles could be partly due to more surface functional groups being deprotonated as the pH of the solution is increased. However, the fact that at a pH as low as 2, fullerene suspension is still negatively charged, shows that functional groups could not be the only source of observed negative charge. It is also possible that a preferential adsorption of hydroxyl ions over hydronium ions contributes to the negative surface charge of these fullerene nanoparticles (Ma and Bouchard, 2009).

More recent works on the source and origin of negative charge of $C_{60}$ molecule in water using molecular dynamics simulation models suggest the importance of charge polarization transfer processes. Based on these simulation models, the $C_{60}$ molecules are stabilized in water by a net negative charge produced by networks of charge polarization interactions in large fullerene aggregates. Based on these findings single $C_{60}$ molecules or even small aggregates of few $C_{60}$s would not be stable in the aqueous phase and aggregate into larger particles. This is in line with observations from many experimental studies that $C_{60}$ aggregates in water have a size of around 100 nm (Mashayekhi et al., 2012). Results of simulations like this show that networks of hydrogen bonded water molecules are formed around the $C_{60}$. The hydrogen bonding interaction is more favorable than the $C_{60}$-water interaction. This provides the primary thermodynamic driving force to form $nC_{60}$ in the aqueous phase (Choi et al., 2015).
1.8. Effect of NOM on fullerene aggregation

Aggregation of fullerene nanoparticles in water as discussed earlier, follows the classical DLVO theory of colloids stability. Based on this theory, aggregate stability is related to the equilibrium between attractive (Van der Waals) forces and repulsive forces (the electrostatic double layer) between aggregates. So here we consider how NOM could alter the surface charge and the size of fullerene aggregates.

The ubiquitous natural organic matter (NOM) in the environment is quite reactive and will interact with fullerene molecules. A major part of NOM consists of humic acid (HA) and fulvic acid (FA); plant, animal and bacterial residues are transformed via chemical, biological and physical processes into the more stable form of humic substances. A few studies have focused on the effect of NOM on the physicochemical properties of fullerene suspensions in water. For example, an increase in the solubilization of fullerene molecules in water has been reported in the presence of 100 mg/L fulvic or humic acids. The increase in fullerene solubility was due to surface interaction of fullerene with fulvic or humic acids but the structure of fullerene was not altered. Humic acid was more effective than fulvic acid in increasing fullerene solubility, which was attributed to its lower charge density and larger hydrophobic structure (Terashima et al., 2007). Xie et al. (2008) reported a decrease in the particle size of fullerene water suspension (FWS) prepared with different methods after addition of fulvic acids, which could be due to disaggregation of fullerene after the surface sorption of NOMS. Espinasse et al. (2007) studied the effect of alginate and tannic acid on the aggregation of fullerene in porous media and varying concentrations of NaCl. They observed that tannic acid enhanced stability of FWS, while the long chain alginate led to
production of bigger aggregates indicated by increasing attachment efficiency. They also reported increasing surface charge of FWS with increasing tannic acid or alginate concentrations, indicating sorption of these macromolecules on the surface of fullerene aggregates. Interaction of NOMs with colloidal particles including $C_{60}$ fullerene can affect their stability and mobility in soils and sediments. It has been shown that proteins, humic, fulvic and tannic acids could inhibit aggregation of colloidal particles through steric stabilization and/or increasing the electrical charge of particles which leads to suspension stability (Tsai et al., 2004, Chen and Elimelech, 2007). A decrease in antibacterial activity of FWS was reported after its interaction with humic acid, probably due to modification of fullerene surface chemistry (Li et al., 2008). However, the size of fullerene aggregates decreased in the presence of humic acids in the same study. Duncan et al. (2008) stated that addition of NOM to FWS increased aggregate stability most probably due to steric stabilization, because the electrophoretic mobility of FWS did not change after addition of NOM in their studies. Thus NOM can greatly affect the environmental fate and behavior of $C_{60}$ fullerene by influencing its aggregate size, aggregate stability and by affecting its toxicity and biological interactions.

At the same time, we have to consider that NOM is highly heterogeneous in terms of composition and structure. NOM consists mainly of plant residues, microbial tissues, and humic substances (Stevenson, 1994). Structural properties of NOM varying by their age, origin, and source greatly influence their physicochemical properties such as sorption properties, their functional groups and surface charge, solubility and other properties (Kang et al., 2003). It has been shown that humic acids (HA), sequentially extracted from a single soil or compost sample could vary greatly in their properties and
structure (Kang and Xing, 2005). For instance aliphaticity and molecular weight of HAs increases with increasing sequential extraction while the polarity of HA decreases. Xing (2001) also showed that aromaticity of HA increased with increasing depth of soil even in a single soil profile. Structural and polarity differences in HAs caused the changes in their sorption characteristics for organic compounds in terms of sorption capacity and isotherm nonlinearity. For example, Grathwohl (1990) showed that organic matter extracted from unweathered shales had enhanced sorption capacity for nonpolar organic compounds compared to the organic matter present in younger soils. His results also displayed a decrease in sorption capacity with increasing ratio of oxygen-containing functional groups in the natural organic substances. Also, fractionation of HA has been observed during their adsorption by clay minerals which is due to heterogeneous nature of HA as a molecular mixture (Wang and Xing, 2005).

Thus, HA has been shown to be extremely heterogeneous and the effect of structural characteristics on the sorption properties has been established quite well in the literature. Therefore, when structural characteristics of HA could affect their sorption properties for simple organic compounds, their interactions and affinities with fullerene molecule can be expected to be affected as well. This could lead to different capabilities of HAs to stabilize fullerenes (or any other particle) in water. Different HAs could stabilize or destabilize a colloidal suspension through neutralizing, or enhancing their surface charge or by means of steric stabilization. However, to our knowledge only a few studies have focused on the effect of physicochemical properties of NOM on the stability and aggregation behavior of fullerene, and our understanding about the mechanism of aggregate stabilization in the presence of NOM is very limited (Ghosh et al., 2009). Thus
the main goal of this proposed work is to investigate how structural characteristics of NOM could affect aggregation behavior of fullerene in water.

Considering the complex nature and unknown structure of HA, researchers have always been trying to use simpler organic materials to mimic HAs or NOM. For example Chang and Vikesland (2009) studied the effect of some carboxylic acids on the aggregation behavior of fullerene suspensions. They used acetate, tartrate and citrate sodium salts to investigate changes in fullerene aggregates shape, size and surface charge. They concluded that surface charge of fullerene aggregates did change significantly in the presence of these carboxylic ions. However shape and size of fullerene aggregates was different in the presence of different acids which suggest interaction of these carboxylic ions with the fullerene aggregates. The effect other carboxylic acids with longer C chain or benzene rings has not been studied on the aggregation fullerene suspension.

As mentioned before polysaccharides are other portion of NOM besides humic materials which could affect aggregation of fullerenes. There are some reports on the effect of alginate as a natural polysaccharide on the colloidal behavior of nanoparticles including fullerenes. Alginites are naturally occurring polysaccharides found in the marine environments which are produced by brown algae species, like Laminaria hyperborea, Ascophyllum nodosum, and Macrocystis pyrifera. Alginate is an unbranched block copolymer that contains 1,4-linked β-D-mannuronic acid and α-L-guluronic acid residues. There are three different block types in alginate: mannuronic acid blocks (M), guluronic acid blocks (G), and the alternating M and G blocks (Chen et al., 2006). The conformation of adsorbed alginate on the surface of hematite nanoparticles was found to be dependent on the ionic strength of the background solution. The adsorbed alginate
polyelectrolytes extend into bulk solution due to repulsive forces between negatively charged functional groups in the polymers at low ionic strengths. The introduction of electrolytes results in electrostatic shielding (NaCl) and charge neutralization (CaCl$_2$) of alginate functional groups. Sodium and magnesium could affect the stability of hematite suspension through a reduction of surface charge, but calcium caused enhanced aggregation with alginate. Possible mechanisms of enhanced aggregation with calcium are an apparent increase in the collision radii of alginate-coated hematite nanoparticles through alginate gel network formation from the particle surface. In addition, cross-linking between unadsorbed (suspended) alginate macromolecules may form bridges between hematite-alginate gel clusters (Chen et al., 2006). Chen and Elimelech (2008) studied deposition of fullerene aggregates on alginate coated silica surfaces. In their study deposition of fullerene on the silica coated by alginate was higher than on surfaces coated with HA. They suggested that alginate macromolecules are larger than humic acid, and that the alginate layer on the silica is much rougher than the humic acid layer. This irregular surface of alginate may allow for the fullerene nanoparticles to be trapped within the depressions of the alginate layer. In the presence of calcium ions the deposition kinetics onto the alginate-coated surfaces are relatively high, probably due to alginate forming complexes with Ca$^{2+}$ which leads to charge neutralization of the alginate layer. Alginate also undergoes gelation in the presence of Ca$^{2+}$, which results in the modification of the physical properties and surface morphology of the alginate layer (Chen and Elimelech, 2008).

Thus structural properties of NOM have enormous and even contradictory effects on the stability or aggregation of C$_{60}$ fullerene in water. NOMs with longer carbon chains
who can bridge multivalent cations would destabilize and coagulate nC$_{60}$ colloidal system. On the other hand, less charged NOMs who do not interact with multivalent cations tend to make nC$_{60}$ system more stable.

1.9. Removal of C$_{60}$ fullerenes from water sources

When and if municipal water sources are contaminated with fullerene particles, it is important to have strategies for their removal. Wastewater discharge is possibly one of the most likely routes that nC$_{60}$ might be released to the aqueous environment (Wiesner et al., 2006). Farré et al. (2010) reported the occurrence of nC$_{60}$ in the effluents of 14 out of 22 wastewater treatment plants (WWTPs) in Spain and concentrations of nC$_{60}$ in the µg/L range were found in the effluents of some WWTPs located in highly populated and industrialized areas. Only limited studies have addressed the removal of nC$_{60}$ from water and wastewater treatment processes (Wang et al., 2013). The nC$_{60}$ can be effectively removed by the conventional coagulation flocculation–sedimentation process that is used for water and wastewater treatment plants.

Jar tests are usually performed to evaluate the removal of nC$_{60}$ by a conventional water treatment processes. These processes usually consist of employing a coagulant (such as alum, hydrated aluminum sulfate), flocculation of fullerenes followed by their sedimentation. The sedimented materials are then removed from the water through simple filtration techniques. Water quality parameters such as pH, alkalinity, and ionic composition, as well as NOM usually affect the removal efficiency of nC$_{60}$. The other important parameter in efficiency of nC$_{60}$ removal is the dosage or the concentration of alum (Huang and Kim, 2009; Wang et al., 2013). Electrolytes are considered as an important factor affecting the stability of nC$_{60}$ as well as other nanoparticles (Chen et al.,
Both monovalent and divalent salts could destabilize fullerene $C_{60}$ nanoparticles. However, humic acid can enhance the stability of $C_{60}$ by imparting negative charge on it and also through steric repulsion (Chen et al., 2007; Mashayekhi et al., 2012).

Thus, in this research we also evaluate the coagulation and removal efficiency of n$C_{60}$ as affected by different types of ions, and humic acid as a representative of dissolved organic materials.

1.10. Objectives

Fullerenes will go through aggregation and also interact with NOM as soon as they enter natural waters. The interactions of NOM with $C_{60}$ fullerenes alter their surface properties and subsequently their aggregation behavior and other environmental behaviors. Considering the heterogeneous nature of NOM, our knowledge about the effects of the physicochemical characteristics of HA on aggregation behavior of fullerene is very limited. Thus, the overall goal of this work is to study the effect of physicochemical characteristics of HAs (a model NOM) on the aggregation and subsequently on the environmental behavior of $C_{60}$ fullerene in water. The specific objectives of this proposed work are:

1- To extract HA as model NOM from soil and obtain HAs with structural differences via sequential extraction. To study the sorptive behaviors of extracted humic materials for polycyclic aromatic hydrocarbons (PAHs) in order to get more indirect information about the structure of humic materials, as well as the mechanisms of interactions with other organic molecules.
2- To study the effect of structurally different HAs on the aggregation of fullerene suspension.

3- To study the effect of different ions, the solution ionic strength, and HA on the flocculation and removal efficiency of C$_{60}$ fullerenes.

Humic acids as a major component of NOM are very reactive and diverse in their structure, thus need to characterize to better understand their effect on fullerene particles. Aggregation and coagulation of C$_{60}$ are major factors in predicating transport and availability of C$_{60}$ in the environment as well as their removal from water sources. Thus accomplishing this research work will enable us to better understand the fate of C$_{60}$ nanoparticles in water under different aquatic conditions and to evaluate their removal efficiency from (potentially) contaminated water sources.
Figure 1.1. $C_{60}$ Buckminsterfullerene molecule showing 5 and 6 member carbon rings (Kroto et al., 1991).

Figure 1.2. The DLVO theory typical energy diagrams. In the equations, $A$ is Hamaker constant, $H$ is the distance between particles, $\varepsilon$ is the permittivity of water, $\kappa$ is the inverse of Debye length, and $\xi$ is zeta potential.
CHAPTER 2

PHENANTHRENE SORPTION BY COMPOST HUMIC ACIDS

2.1. Abstract

Nonlinear sorption isotherms have been observed for sorption of polycyclic aromatic hydrocarbons (PAHs) to humic materials, but the exact sorption mechanism is not clearly understood. This study investigated sorption of PAHs by compost humic materials. Humic acids (HAs) were progressively extracted from two compost samples by 0.1 M sodium pyrophosphate solution. Sorption of phenanthrene by the extracted HAs was studied with a batch equilibration technique. Competitive sorption between pyrene and phenanthrene in the HAs was also examined. Elemental analysis and solid-state $^{13}$C NMR were used to characterize HAs. All sorption isotherms were nonlinear although these samples contained little black carbons (BC) if any. Isotherm linearity increased with increasing number of extractions. Isotherm nonlinearity was negatively related to HA aliphaticity. Addition of pyrene to the phenanthrene-HA system increased isotherm linearity. Competition between phenanthrene and pyrene sorption was more pronounced in the first fraction of HAs with a higher content of aromatic moieties. This study showed that humic materials alone could lead to non-ideal sorption for PAHs.

2.2. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced and enter the environment via different human activities such as combustion of fossil fuel, forest fires, and direct inputs of mineral oils. From the human health prospective PAHs have drawn
considerable research interest because some of them are potent carcinogens. In addition, PAHs have a high tendency to bioaccumulate, and thus these compounds are among the most intensively investigated organic pollutants and are usually considered as model compounds in environmental studies (Schwarzenbach et al., 2003).

Soil and sediment organic matter (SOM) is the predominant sorbent of PAHs in soils and sediment (Chiou, 1989; Huang and Weber, 1997; Schwarzenbach et al., 2003). Sorption of PAHs by SOM can reduce the degradation of PAHs as well as the rate with which bioaccumulation occurs. The strong association between PAHs and organic matter often limits passive remedial methods, such as bioremediation. A quantitative understanding of sorption process is necessary to evaluating the hazards these pollutants pose and to planning any effective remediation process (Accardi-Dey and Gshwend, 2002; Salloum et al., 2002; Schwarzenbach et al., 2003).

Humic acid (HA), the base soluble and acid insoluble portion of SOM is usually employed as a model sorbent for SOM, considering the complex structure of the latter. Sorption of PAHs to HA has been the subject of many investigations, but the exact sorption mechanism is still not clear. Solid-phase dissolution (partitioning) is usually considered as the sorption mechanism. Based on this model, sorption of PAHs to SOM is concentration independent which yields linear sorption isotherms (Gunasekara and Xing, 2003). However nonlinear sorption isotherms and other evidences such as competitive sorption, and hysteresis have been reported indicating that partitioning cannot be the only mechanism involved in the sorption of PAHs to SOM (Xing, 2001a).

Sorption nonlinearity has been attributed to the presence of small amounts of high surface area carbonaceous materials (HSCMs) such as black carbons (BC) in soils and
sediments (Accardi-Dey and Gshwend, 2002; Kleineidam et al., 1999). BC is defined as combustion-produced black particulate carbon having a graphitic microstructure (Glaser et al., 1998). BC in the environment occurs in numerous forms, ranging from partially charred plant materials to soot particles, forming from the gas phase during combustion and pyrolysis (Nguyen et al., 2004). Upon their formation these particles accumulate in the environment, due to their resistance to decomposition. It has been shown that surface sorption (adsorption) of PAHs to BC can be exceptionally strong and highly nonlinear: sorption coefficients decrease significantly with increasing concentrations (Cornelissen and Gustafsson, 2004). Accardi-Dey and Gshwend, 2003 hypothesized that PAH sorption in soils and sediments must be considered as a two-component mechanism: absorption into SOM, and adsorption onto BC. As a result, sorption of PAHs by soils would be nonlinear.

BC will cause isotherm nonlinearity if present in soil. However nonlinear sorption isotherms have been reported for sorption of PAHs by other sorbents, rather than SOM. For example, Gunasekara and Xing (2003) observed nonlinear isotherms for sorption of naphthalene by lignin, a natural biopolymer. Salloum et al. (2002) and Chefetz et al. (2000) reported nonlinear isotherms for sorption of PAHs by plant cuticle materials. Nonlinear sorption isotherms have also been observed for sorption of PAHs to PVC, a synthetic polymer (Xing and Pignatello, 1997). Lignin and plant cuticle materials are precursors of SOM and sorption of PAHs to synthetic polymers may be analogous to that of SOM. Since these systems are free of BC and nonlinear isotherms are still observable, we raise the question: does sorption nonlinearity occurs by SOM in the absence of BC? Thus the objective of this study was to examine the sorption behavior of BC-free humic
materials extracted from compost samples of different sources. Compost humic materials were chosen in this study, because compost contains humic materials that have been created by the natural processes similar to those occurring in soils. In addition, composting process can be controlled to minimize introduction of BC and to produce BC-free compost.

2.2. Materials and Methods

2.2.1. Humic acids

Humic acids were obtained from two compost samples which here are referred as compost 1 and compost 2. Compost 1 was produced mainly from food wastes, horse manure, and horse bedding materials (straw) by the Intermediate Processing Facility, University of Massachusetts. Leaves of deciduous trees, and garden waste were the ingredients of the compost 2 sample, prepared by a private producer in Amherst, Massachusetts. Both compost samples were one year old. In the composting process no soil was added to the compost materials. Because soils may contain BC in much elevated levels than plant materials, eliminating soils from composting process can lead to production of BC-free compost.

HA was progressively extracted from the compost samples by using 0.1 M sodium pyrophosphate (Na$_4$P$_2$O$_7$) solution (Ding et al., 2002; Xing et al., 2005). Briefly 80 g of compost along with 800 ml of the Na$_4$P$_2$O$_7$ solution were placed in a one-liter polyethylene bottle under N$_2$ atmosphere and shaken overnight. A total of 28 and 24 consecutive extractions were performed for compost 1 and compost 2, respectively. The extraction procedure was stopped when HA was no longer extractable from the solid
residue (humin). Different numbers of consecutive HA extracts were pooled together in order to get enough HA for characterization and sorption experiments. As a result, seven HA fractions (F-1 to F-7) were obtained for compost 1, and six HA fractions (F-1 to F-6) for compost 2. All HA fractions were demineralized with a mixture of 0.3 M HF and 0.1 M HCl, freeze-dried, and ground. The detailed extraction procedure is described elsewhere (Heymann et al., 2005).

2.2.2. NMR studies

Solid State Cross-Polarization Magic-Angle-Spinning with Total Sideband Suppression (CPMAS-TOSS) $^{13}$C-NMR spectra were obtained using a Bruker Avance DSX-300 spectrometer operated at $^{13}$C frequency of 75 MHz. HA samples were packed in a 7 mm Zirconia rotor with Kel-f cap. Spin rate was 5 KHz, contact time 2 ms, and the number of scans between 5000 and 7000. Within the NMR spectra carbon atoms were assigned to aliphatic C (0-110 ppm) and aromatic C (110-165 ppm).

2.2.3. Sorption and competitive sorption studies

Ring-UL-$^{14}$C and unlabeled phenanthrene was purchased from Sigma-Aldrich Chemical Company. Phenanthrene sorption by the extracted HA fractions was studied using a batch equilibration technique (Xing, 2001a) in 8 ml screw-cap vials with aluminum foil layered between the solution and silica septa at 22 ± 1 °C. Initial phenanthrene concentration ranged from 0.006 to 0.8 µg/mL in all sorption experiments. The background solution was 0.01 M CaCl$_2$ with 200 µg/mL NaN$_3$ as biocide. The ratio of sorbate solution to sorbent was adjusted in a way to get 30 - 80% sorption of added sorbate. Each isotherm consisted of 10 concentration points in duplicate. Vials containing
HA suspension were shaken on a rotary shaker for five days, and then centrifuged at 1000 × g for 30 minutes. One mL of the supernatant was sampled for liquid scintillation counting (Beckman-Coulter LS 6500). Sorbed phenanthrene was determined by mass balance calculations because phenanthrene sorption to the vials was not significant.

Competitive sorption studies were performed by using non-labeled pyrene as the competing sorbate and phenanthrene as the main sorbate. Pyrene concentration was kept close to its water solubility (0.16 µg/ml) in all sorption systems. Other details of competitive sorption studies were the same as the regular sorption studies as above.

Sorption and competitive sorption data were fitted to the linear form of the Freundlich equation:

\[ \log q = \log K_f + N \log C_e \]

where \( q \) (µg/g) is the sorbed concentration, \( C_e \) (µg/mL) is the equilibrium solution concentration, \( K_f \) (µg/g)(µg/mL)^N is the sorption coefficient, and \( N \) (unitless) is the index of isotherm nonlinearity. \( N \) values equal to one indicate linear sorption isotherms.

2.2.3. Elemental analysis and BC content

Elemental composition of HA fractions was determined by an Exeter 11 Analytical 240XA Elemental Analyzer in a micro-analytical lab at the University of Massachusetts. Ash content was determined by heating samples at 750 °C for four hours in a muffle furnace (Xing, 2001b). BC content of the compost samples was estimated with a chemical oxidation method (Song et al., 2002). Briefly, compost was first demineralized with a 2:1 mixture of 22 M HF and 6 M HCl at 60 °C for 20 hours. Then the residue was oxidized in a solution of 0.1 M K_2Cr_2O_7 and 2 M H_2SO_4 for 60 hours at 55 ± 1 °C. BC content of the sample was estimated based on its weight loss during the
oxidation process.

2.3. Results and Discussion

Black carbon (BC) of the two compost samples was not detectable with the dichromate oxidation method (Song et al., 2002), indicating that all the organic matter of the two samples was not resistant to the dichromate oxidation. We preferred using this method to the alternative dry oxidation methods to avoid charring of non-BC fractions of SOM (Nguyen et al., 2004), a positive error in BC estimation. Skjemstad and Taylor, 1999 however reported that chemical oxidation by dichromate can oxidize BC as well as SOM in the soil. Presently, BC determination methods for soils and sediments are generally a subject of debate and it is recommended using them for semi-quantitative purposes (Nguyen et al., 2004; Skjemstad and Taylor, 1999). Therefore, the compost samples in this study were chosen from known producers who had not added soil to the composting materials. Thus, these samples were expected to have no or negligible BC content, regardless of the laboratory estimation method.

Elemental composition, ash content, and atomic ratios of H/C and O/C for each HA fraction are listed in Table 2.1. Chemical heterogeneity is apparent among the different fractions of HAs. As a result of progressive extraction, carbon content of HAs increased from 46 to 51% in compost 1, and from 50 to 52% in compost 2. Hydrogen content of the extracted HAs also increased while oxygen content decreased from 43 to 37% for compost 1 HAs and from 42 to 39% for compost 2 HAs. Similar heterogeneity in the chemical characteristics of HAs progressively extracted from a forest soil sample (Kang et al., 2003) and a Mollisol soil (Xing et al., 2005) was reported. From the first to last HA fraction, atomic O/C ratio decreased in both compost samples whereas H/C
increased. This trend is more pronounced in compost 1 HAs, however similar changes are observed for compost 2 HAs. Higher H/C values indicate a more aliphatic structure and high O/C values imply a more polar structure. Hence the structure of HAs gradually became more aliphatic and less polar with progressive extraction.

CPMAS-TOSS $^{13}$C-NMR spectra of the first two and the last two HA fractions from each compost sample are shown in Figure 2.1, and the percentage of the integrated peak areas in aliphatic (0-110 ppm) and aromatic (110-160 ppm) regions are shown in Table 2.2. A gradual change in the characteristics of HA fractions during the process of extraction is evident. Percentage of aromatic moieties decreased from the first to last fraction of HA, and at the same time percentage of aliphatic moieties increased. This trend is again clear in compost 1 HAs, but a similar overall trend can be found for compost 2 HAs. As a result the structure of HA becomes more aliphatic by continuing the sequential extraction procedure, consistent with elemental analysis (Table 2.1). This observation is in agreement with the results of Kang et al. (2003) and Li et al. (2003).

Sorption of phenanthrene by all HA fractions exhibited nonlinear isotherms. The nonlinearity index ($N$) ranged from 0.86 to 0.93 for the HAs extracted from compost 1 and from 0.92 to 0.95 for the HAs of compost 2 (Figure 2.2). Sorption isotherms became more linear ($N$ approached one) by increasing the number of HA extractions in both compost samples. Apparent solid to water distribution coefficient ($K_d = q / C_e$) was calculated at equilibrium concentration range of 0.006 to 0.8 µg/mL of phenanthrene for all HA fractions (Figure 2.3). If partitioning was the only sorption mechanism, $K_d$ had to be constant throughout the whole concentration range (linear relationship). Negative relationship between $K_d$ and $C_e$ indicates that surface sorption mechanisms (adsorption)
are involved in addition to partitioning. Isotherm linearity and HA aliphaticity appear positively related as shown in Figure 2.4 with some variability for compost 2 HAs. Similar behavior was reported by Xing, 2001 that isotherm nonlinearity increased with aromaticity of HAs extracted from different depths of the same soil.

Competitive sorption isotherms for the first and last HA fractions from compost 1, are shown in Figure 2.5. The sorption isotherm of the two HAs became more linear when pyrene was added in the system as the competing sorbate. This is in agreement with the results of Gunasekara et al., 2003. The $N$ value increased from 0.86 to 0.90 for F-1, and from 0.92 to 0.97 for F-7. The displacement of phenanthrene by pyrene is more evident at the lower concentrations of phenanthrene. A possible mechanism for this observation is that because of the limited number of high energy sites for a given HA, at low phenanthrene concentrations a higher percentage of phenanthrene would be displaced by pyrene in comparison with that of higher phenanthrene concentrations (Gunasekara et al., 2003). As a result, a large proportion of phenanthrene molecules were removed by pyrene from HAs at lower phenanthrene concentrations than higher concentrations, leading to a larger deviation between phenanthrene isotherms with and without pyrene at lower phenanthrene concentrations (Figure 2.5). When comparing competitive sorption between F-1 and F-7 HAs, the degree of competition between phenanthrene and pyrene was greater for F-1 HA than F-7 HA (Figure 2.5). This result indicates that the percentage of nonlinear domains of F-1 HA was more than that of F-7 HA, consistent with the increase of $N$ values with progressive extraction (or increasing aliphaticity) (Table 2.2, Figures 2.2 and 2.4). Combining the data of this study with the literature results (Kang and Xing, 2005; Gunasekara and Xing, 2003; Gunasekara et al. 2003; Xing, 2001b), it may be
concluded that aromatic moieties of HAs are more responsible for nonlinear sorption while aliphatic domains more for linear sorption.

2.4. Conclusions

Sorption of phenanthrene by compost extracted HAs exhibited non-ideal sorption characteristics, such as non-linear isotherms and competition between sorbates. Thus partitioning is not the only involved mechanism in the sorption process. This study also showed that SOM alone could cause sorption isotherm nonlinearity. Nonlinearity and competitive sorption were negatively related to the aliphaticity of HAs. Structure of sequentially extracted HAs became more aliphatic with continuing extraction. Competition between sorbates was more pronounced in the first HA fraction with higher aromaticity.
Table 2.1. Elemental composition and atomic ratios of HA fractions extracted from compost samples.

<table>
<thead>
<tr>
<th>Source</th>
<th>HA Fraction</th>
<th>C %</th>
<th>H %</th>
<th>N %</th>
<th>O %</th>
<th>Ash %</th>
<th>H/C&lt;sup&gt;a&lt;/sup&gt;</th>
<th>O/C&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost 1</td>
<td>F-1</td>
<td>46.3</td>
<td>4.1</td>
<td>5.2</td>
<td>43.2</td>
<td>1.3</td>
<td>1.05</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>F-2</td>
<td>50.8</td>
<td>4.5</td>
<td>5.6</td>
<td>38.3</td>
<td>0.8</td>
<td>1.06</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>F-3</td>
<td>51.4</td>
<td>5.0</td>
<td>5.8</td>
<td>37.3</td>
<td>0.5</td>
<td>1.17</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>F-4</td>
<td>52.4</td>
<td>5.5</td>
<td>5.9</td>
<td>35.6</td>
<td>0.7</td>
<td>1.25</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>F-5</td>
<td>53.2</td>
<td>6.0</td>
<td>6.1</td>
<td>34.1</td>
<td>0.6</td>
<td>1.34</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>F-6</td>
<td>52.4</td>
<td>6.0</td>
<td>5.5</td>
<td>35.7</td>
<td>0.9</td>
<td>1.28</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>F-7</td>
<td>50.7</td>
<td>6.0</td>
<td>5.4</td>
<td>36.8</td>
<td>1.2</td>
<td>1.41</td>
<td>0.54</td>
</tr>
<tr>
<td>Compost 2</td>
<td>F-1</td>
<td>50.0</td>
<td>4.8</td>
<td>2.9</td>
<td>42.2</td>
<td>0.3</td>
<td>1.15</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>F-2</td>
<td>50.4</td>
<td>5.1</td>
<td>3.3</td>
<td>40.5</td>
<td>0.7</td>
<td>1.20</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>F-3</td>
<td>50.6</td>
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<td>4.1</td>
<td>38.7</td>
<td>1.1</td>
<td>1.31</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>F-4</td>
<td>51.6</td>
<td>5.7</td>
<td>4.3</td>
<td>38.1</td>
<td>0.3</td>
<td>1.32</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>F-5</td>
<td>52.0</td>
<td>5.4</td>
<td>3.8</td>
<td>38.3</td>
<td>0.5</td>
<td>1.25</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>F-6</td>
<td>52.0</td>
<td>5.4</td>
<td>3.2</td>
<td>38.8</td>
<td>0.6</td>
<td>1.24</td>
<td>0.56</td>
</tr>
</tbody>
</table>

<sup>a</sup>H/C: Atomic ratio of hydrogen to carbon
<sup>b</sup>O/C: Atomic ratio of oxygen to carbon

Table 2.2. Integration of solid-state <sup>13</sup>C-NMR spectra in aliphatic (0-110 ppm) and aromatic (110-160 ppm) regions (%).

<table>
<thead>
<tr>
<th>Source</th>
<th>HA Fraction</th>
<th>Compost 1</th>
<th>Compost 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-1</td>
<td>F-2</td>
<td>F-6</td>
</tr>
<tr>
<td>Aliphatic C %</td>
<td>33.1</td>
<td>36.2</td>
<td>47.7</td>
</tr>
<tr>
<td>Aromatic C %</td>
<td>40.3</td>
<td>38.5</td>
<td>34.1</td>
</tr>
<tr>
<td>Aliphaticity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45</td>
<td>0.48</td>
<td>0.58</td>
</tr>
</tbody>
</table>

<sup>a</sup>Aliphaticity is the ratio of aliphatic C % to the total aromatic plus aliphatic C %.
Figure 2.1. Solid-state $^{13}\text{C}$-NMR spectra for HA fractions extracted from compost 1 (a) and compost 2 (b).
Figure 2.2. Index of nonlinearity \((N)\) increased with the number of HA extractions in compost 1 and compost 2.
Figure 2.3. Apparent solid to water distribution coefficient ($K_d$) as a function of equilibrium concentration for 3 HA fractions extracted from compost 1 (a) and compost 2 (b).
Figure 2.4. Relationship between isotherm linearity and H/C atomic ratio in compost 1 (a) and compost 2 (b) HAs.
**Figure 2.5.** Competitive sorption between phenanthrene and pyrene using the first (a) and last (b) HA fraction of compost 1. \( N \) is the index of nonlinearity.
CHAPTER 3

EFFECT OF NATURAL ORGANIC MATTER ON AGGREGATION BEHAVIOR OF C\textsubscript{60} FULLERENE IN WATER

3.1. Abstract

The stability of C\textsubscript{60} fullerene particles in water affects their mobility, bioavailability and toxicity to organisms. Natural organic matters (NOM) have pronounced effects on the aggregation behavior of C\textsubscript{60} fullerene. This study was to examine the effects of NOM structural properties on the aggregation behavior of fullerene water suspension (FWS). Fulvic acid (FA), tannic acid (TA), and two structurally different humic acids (HA1 and HA7) were studied. HA1 and HA7 were sequentially extracted HAs, where HA7 was more hydrophobic than HA1 and had a higher molecular weight. Aggregation was induced by addition of varying amounts of Ca\textsuperscript{2+} to the FWS with 2 mg/L of each NOM. The absolute value of zeta potential |ζ| of pure FWS increased after addition of any type of NOM. Addition of Ca\textsuperscript{2+} to the FWS+NOM system decreased |ζ| of fullerene almost uniformly for all types of NOM. FWS critical coagulation concentration (CCC) was equal to 14.5, 6.5, 5.4, and 3.7 mM Ca\textsuperscript{2+} for HA7, HA1, FA, and TA, respectively. The order of increasing CCCs was positively correlated to the NOMs molecular weight and negatively to their polarity. A nearly constant ζ for FWS+NOM system at a wide range of Ca\textsuperscript{2+} concentrations, suggested the steric stability rather than electrostatic one. This study highlighted the role of NOM in the fate of manufactured nanoparticles in the environment and linked the structural properties of NOM to their interaction with manufactured nanoparticles.
3.2. Introduction

C$_{60}$ fullerene molecule has been subject of numerous research and attention since its discovery, because of its different potential applications. Consumer products containing fullerene such as lubricants and badminton racquets are already in the market (Franco et al., 2007). Thus it is easy to foresee the inevitability of fullerene introduction to the environment during their production, transportation, usage, and disposal. There are several concerns about the diverse effects of released fullerenes into the environment, including their toxicity and acting as a carrier and accumulator of xenobiotic compounds (Baun et al., 2008). Several reports have shown toxicity of fullerene to various organisms and human or animal cells (Sayes et al., 2004; Oberdorster et al., 2006; Lyon and Alvarez, 2008; Usenko et al., 2008).

Though C$_{60}$ fullerene molecule is extremely nonpolar and virtually insoluble in water (Ruoff et al., 1993), it forms stable suspensions in water at low ionic strengths (Deguchi et al., 2001). Solvent exchange and extended mixing in water are different methods used in laboratory for preparing fullerene water suspension (FWS) (Cheng et al., 2004). Several studies have shown that fullerene particles in water, regardless of their production routes carry negative surface charges with yet unclear origin, leading to suspension stability (Brant et al., 2005). Pure FWS behavior perfectly follows the classic Dejaguin-Landau-Verwey-Overbeek (DLVO) theory (Chen and Elimelech, 2009).

Natural organic matter (NOM) in water greatly affects colloidal stability, and thus the mobility and bioavailability of fullerene. However, different degrees or types of surface modifications of fullerenes induced by NOM can be perceived, depending upon spatial and temporal variations of NOM (Xie et al., 2008). A few studies have been
focused on the effect of NOM on the physicochemical properties of FWS. An increase in the concentration of fullerene molecules in water was reported in the presence of 100 mg/L FA or HA (Terashima and Nagao, 2007). Xie et al. (2008) reported a decrease in the particle size of fullerene after addition of FA, which could be due to disaggregation of fullerene aggregates after the surface sorption of NOM. Espinasse et al. (2007) reported that tannic acid increased stability of FWS in the presence of Na⁺. Chen and Elimelech (2007) showed that HA could effectively increase stability of FWS.

However NOM is known to be composed of materials with a wide range of structural properties and molecular weights. Structural properties of NOMs varying by their age, origin, and source greatly influence their physicochemical properties such as sorption, their dominant functional groups, surface charge, solubility, and other properties (Kang et al., 2002). This could lead to different capabilities of HAs to stabilize fullerenes in water. In a recent study, Ghosh et al. (2010) showed that surface adsorbed, long chain humic acid molecules could provide steric stability to Al₂O₃ nanoparticles, while polar short chain HAs enhanced aggregation of Al₂O₃ nanoparticles. To our knowledge only a few studies have been focused on the effect of physicochemical properties of NOM on the stability and aggregation behavior of C₆₀ fullerene in water.

The objective of this work was therefore, to study the effect of different NOM structural properties on the FWS aggregation behavior, by comparing FWS stability in the presence of a series of NOMs with a wide range of structural characteristics. We utilized four different NOMs: Two structurally different humic acids sequentially extracted from an organic soil, tannic acid and Suwannee River fulvic acid. Different concentrations of calcium chloride were used to induce aggregation of fullerene in the
presence of each of NOM. Early stage aggregation kinetics of FWS was studied using dynamic light scattering (DLS) technique in the presence of each NOM. Then several structural characteristics of NOM such as molecular weight and polarity were related to the stability of FWS with NOM.

3.3. Materials and Methods

3.3.1. Preparation of FWS and NOM solutions

$C_{60}$ fullerene powders were purchased from MER Corp. (Tuscan, AZ). Tannic acid was purchased from Fisher Scientific and Suwannee River Fulvic acid Standard I from International Humic Substances Society (IHSS). Humic acids (HAs) were sequentially extracted using alkali solution from an organic soil in Amherst, MA in our previous research and were fully characterized (Wang and Xing, 2007; Ghosh et al., 2008). HA1 was the first sequential extract while HA7 was the 7th progressive extract. Characterization experiments showed that HA7 was more aliphatic with a relatively high carbohydrate content compared to HA1 (Ghosh et al., 2008). Fullerene powders were dissolved in HPLC grade toluene (2 mg/mL), then 2 mL of the purple color toluene solution was added to 150 mL of Milli-Q water and sonicated with a sonicator probe at 100 W (Fisher Scientific) for several hours until fullerene was transferred to water and all toluene was evaporated. The amber colored suspension of fullerene in water (FWS) was filtered through a 0.2 µm cellulose filter, its pH was adjusted to 7 using 0.1 M NaOH or HCl solutions, and stored at room temperature in the dark. Solutions of HAs, FA and TA were made at 250 mg/L and filtered through a 0.2 µm sterile nylon filter after adjusting
their pH to 7 and stored refrigerated in sterile tubes to avoid potential microbial interference.

3.3.2. Molecular weight measurement of NOMs

High performance size exclusion chromatography (HPSEC) was used to determine and compare the relative molecular weights of NOMs. The detailed procedure was described by Kang and Xing (2008). A TSKGEL column (Tosoh Bioscience, G3000PWXL, 7.8 mm × 30 cm, and particle size 6 µm) and TSK-GEL guard column (Tosoh Bioscience, 6.0 mm × 4 cm, and particle size 12 µm) were used with a Perkin-Elmer 200 LC diode array detector at 254 nm. Sodium polystyrene sulfonate (PSS) was used as standard molecular weight ranging from 1.8 to 35 kDa and acetone as the lowest molecular weight index. All NOMs samples and standards were injected at a concentration of 250 mg/L. The mobile phase was 0.1 M NaCl buffered with 2 mM phosphate at pH 7 and flow rate was 0.5 mL/min.

3.3.3. Sorption of NOMs by C₆₀ fullerene

A batch sorption experiment was designed to estimate sorption of each NOM by fullerene in powder form. Ten mg of fullerene powders were transferred to 8 mL screw cap vials with Teflon liners (in duplicate) then 8 mL of 5 mg/L solution of each NOM in 200 mg/L sodium azide at pH 7 was added. The vials were shaken for 4 days to achieve equilibrium then centrifuged at 1000 ×g and the supernatant was analyzed by a Total Organic Carbon Analyzer (TOC-L Shimadzu Corp., Japan) to determine initial and equilibrium concentrations of NOMs. The amount of sorbed NOM was calculated based
on mass-balance with reference to blank vials (without fullerene). The adsorption coefficient ($K_d$) was calculated for each NOM, based on the following equation:

$$K_d = \frac{q}{C_e}$$

where $q$ ($\mu$g/g) is the concentration of sorbed NOM on the solid phase and $C_e$ ($\mu$g/mL) is the NOM concentration at equilibrium in the solution.

### 3.3.4. Transmission electron microscopy (TEM) and Atomic Force Microscopy (AFM) imaging

Formvar coated 400 mesh cupper grids (Electron Microscopy Sciences, Hatfield, PA) were placed on the top of a drop of 5 mg/L FWS with or without different NOMs, and allowed the adsorption of particles on the formvar coating for 15 minutes. We chose to place grid on the top of suspension droplet to minimize possible precipitation of bigger aggregates on the grid. Then the grids were gently rinsed with Milli-Q water and allowed to dry in a laminar flow hood to avoid airborne contaminations. A JEOL 100CX TEM (JOEL Ltd, Tokyo, Japan) at 100 kV was used to capture the images. Two samples from each treatment were prepared and at least 3 images at different magnifications were captured by the microscope.

For the AFM studies, fullerene particles with or without NOMs were adsorbed on the surface of Ca saturated freshly cleaved mica and images were captured in tapping mode. A Dimension 3100 AFM instrument (Digital Instruments) in tapping mode was used to capture height and phase AFM images. The cleaved mica surface was saturated with Ca to increase adsorption of negatively charged fullerene by immersing mica in 1 M CaCl$_2$ solution for 30 minutes followed by washing with Milli-Q water to remove free
CaCl$_2$. Then Ca saturated mica was vertically placed in FWS with different NOMs and allowed adsorption for 30 minutes. After gently washing to remove excess particles, mica samples were dried under a laminar flow hood to avoid dust contamination. Two slides in each treatment were prepared and at least three images (5 µm×5 µm) per slide were captured by the AFM instrument.

3.3.5. Zeta potential measurements

Zeta potential (ζ) of FWS was measured using a ZetaSizer Nano ZS (Malvern Instruments, Bedford, MA) equipped with a folded capillary cell at 25°C. This instrument employs a He-Ne laser at 633 nm and collects back-scattering data at 173°. Electrophoretic mobility was measured by a combination of laser Doppler velocimetry and phase analysis light scattering technique. Electrophoretic mobility measurements were converted to ζ using Smoluchowski’s equation. Predetermined amounts of 1 M CaCl$_2$ solution were added to the fullerene suspension to get desired ionic strengths ranging from 2 to 10 mM. Concentration of all NOMs in the fullerene suspension was kept at 2 mg/L. The ζ of pure HAs were measured at a concentration of 250 mg/L while that of TA and FA measured at 1000 mg/L. We had to use the high concentration of 1000 mg/L for TA and FA to get enough intensity of the back-scattered light from these smaller NOMs.

3.3.6. Aggregation kinetics studies

Growth rate of fullerene aggregates was also measured using the ZetaSizer Nano ZS. Three mL FWS was added to a polystyrene measurement cell and the intensity average aggregate size was recorded at 15 s intervals for 16 min at 25° C. Predetermined
amounts of NOM stock solutions were added to the FWS to get a final concentration of 2 mg NOM/L which is in range of typical concentration range of NOM in most surface waters. Different concentration levels of Ca\(^{2+}\) were obtained by adding predetermined amounts of 1 or 2 M CaCl\(_2\) solutions into the cell containing FWS and mixing it with the FWS just prior to conducting measurements. Final concentration of Ca\(^{2+}\) was corrected considering the final total volume of solution in the cell. Collected data were analyzed and plotted against the measurement time. At a fixed angle scattering experiment setup, the initial rate of fullerene particles aggregation can be expressed as (Chen et al., 2007):

\[
\left[ \frac{da_h(t)}{dt} \right]_{t \to 0} \propto k_{11} N_0
\]

where \(a_h(t)\) is the hydrodynamic radius of fullerene particles at time \(t\), \(N_0\) the initial number of particles (suspension number concentration) and \(k_{11}\) the initial aggregation rate constant. The left side of this equation is the slope of fullerene aggregate size vs. time which can be estimated using the collected DLS data. Increasing Ca\(^{2+}\) concentration increased aggregation rate (reaction controlled mechanism, slow) until a limit where increasing Ca\(^{2+}\) concentration did not further affect the rate of aggregation (diffusion controlled mechanism, fast). The ratio of \(k_{11}\) in any desired calcium concentration to \(k_{11}\) in the diffusion controlled regime is defined as attachment efficiency (\(\alpha\)) or inverse stability ratio (\(1/W\)). Attachment efficiencies (\(\alpha\)) of FWS at different calcium concentrations and constant \(N_0\)'s were calculated to quantify kinetics of fullerene aggregation using the following equation (Li et al., 2008):
3.4. Results and Discussion

3.4.1. Characteristics of FWS and NOM

FWS had a transparent yellow color and was stable for several weeks during and after the experiments. The concentration of C_{60} fullerene in water was 4.6 mg/L having a UV absorbance of 0.42 AU at 245 nm. The suspension polydispersity measured by intensity-weighed DLS measurements was 0.16. Ninety eight percent (98%) of particles had hydrodynamic diameters ranging from 59 to 295 nm and the Z-average (mean diameter based on the intensity of scattered light) particle diameter was 96.8 nm, which was used to track and report particle size changes in the kinetics studies. The remaining 2% of particles were fullerene aggregates bigger than 1 µm in diameter (Fig. 3.1). TEM studies showed fullerene aggregates having a “crystalline polynomial structure” with rounded edges. Average size of particles in TEM images was about 89 nm which was measured along the longest dimension of aggregates. Zeta potential of fullerene particles in the suspension was -47 mV at pH 7 and 25° C. This high ζ of fullerene particles in water as also reported in several previous studies (Deguchi et al., 2001; Brant et al., 2005; Chen and Elimelech, 2009), was the reason for high stability of suspension.

The Zeta potentials of pure TA, FA, HA1, and HA7 were -40, -37, -33, and -35 mV, respectively. At pH 7 all carboxylic and a fraction of phenolic functional groups of

\[
\alpha = \frac{1}{W} = \frac{K_{11}}{(K_{11})_{(fast)}} = \frac{1}{N_0} \left( \frac{da_h(t)}{dt} \right)_{t \to 0} = \left( \frac{da_h(t)}{dt} \right)_{t \to 0, fast}
\]
humics as well as a large portion of TA phenolic groups were deprotonated, which gave the high negative charge of these NOM molecules. Molecular weight (MW) of HAs was compared using their GPC chromatograms at pH 7 in Figure 3.2. In general FA had the lowest MW while HA7 had the highest. FA had an average molecular weight of 1.2 kDa and the average molecular weight of HA1 was 2.9 kDa. In size exclusion chromatogram of HA7, two peaks at 13.7 and 5.1 kDa were identified revealing the presence of relatively high molecular weight moieties along with other smaller fractions. Molecular weight of Suwannee River FA was reported between 800 to 1200 Da with HPLC method, which is similar to our results (Chin et al., 1994). They also reported molecular weight of Aldrich HA equal to 3200 Da which is in range with HA1 in our study. We believe the larger MW fraction of HA7 consisted of long chain nonpolar moieties, which were identified and reported in our earlier work (Ghosh et al., 2008). Molecular weight of TA was reported equal to 1.7 kDa.

3.4.2. Effect of NOM on FWS zeta potential

The $\zeta$ of FWS in the presence or absence of different NOM at varying Ca$^{2+}$ concentrations is presented in Figure 3.3-a. Without Ca$^{2+}$, addition of any type of NOM increased the $|\zeta|$ of fullerene particles. At pH 7, most of the polar functional groups of NOM were deprotonated; hence NOM adsorption on the surface of fullerene aggregates increased their $|\zeta|$. The fact that NOMs increased $|\zeta|$ of FWS even though their $|\zeta|$ were similar or lower than that of pure FWS, indicates that their sorption mechanism on the fullerene particles was not electrostatic. NOM sorption was probably due to hydrophobic or $\pi$-$\pi$ interactions, thus it had an additive effect in increasing dissociated functional groups on the surface of NOM coated fullerene aggregates leading to increased overall $|\zeta|$. 
for the FWS+NOM systems. TA increased the $|\zeta|$ of fullerene aggregates as much as other types of NOM, indicating deprotonation of most phenolic groups of TA at pH 7. Adsorption of TA on the surface of fullerene was due to the attraction by the abundant $\pi$ electrons in the aromatic structure of TA and fullerene (Lin and Xing, 2008).

Increasing Ca$^{2+}$ concentration decreased the $|\zeta|$ of FWS due to the electrostatic effect and decreasing the thickness of electric double layer around the particles. Calcium ions also could interact with the functional groups of surface adsorbed NOMs and screen their charge which ultimately causes a reduction in $|\zeta|$. At concentrations of Ca$^{2+}$ higher than 3 mM, $\zeta$ of FWS with FA, HA1 and TA was almost identical with that of the pure FWS + Ca$^{2+}$ system. However the $|\zeta|$ of FWS with HA7 was always slightly higher than the rest of NOMs throughout the whole concentration range of Ca$^{2+}$. This could be explained by the fact that although HA7 had a lower carboxylic acidity (2.5 me/g) compared to HA1 (4.3 me/g) or FA (5.6 me/g), it had a significantly higher percentage of long chain polysaccharides (Wang and Xing, 2007). Carboxylic groups attached to these long C chains, have higher pKa values (Green, 1981), thus their interaction with Ca$^{2+}$ would not be as favorable when compared with carboxylic groups attached to aromatic Cs or other oxygen containing C groups. Thus the addition of Ca$^{2+}$ had a lesser effect on decreasing $|\zeta|$ of FWS with HA7. For any Ca$^{2+}$ concentration, HA7 increased FWS $|\zeta|$ slightly more than other tested NOMs. This could be due to a stronger hydrophobic interaction of long chain HA7 fraction with fullerene aggregates, which led to more sorption of HA7 on the fullerene aggregates. Decrease in fullerene $|\zeta|$ with TA due to Ca$^{2+}$ charge screening was slightly higher than other NOMs in this study. This may be due to readily binding of Ca$^{2+}$ with the $\pi$ electrons in the structure of TA (Mecozzi et al., 1996). Also TA could act as a...
chelating agent for Ca\(^{2+}\) and the presence of Ca\(^{2+}\) caused a higher reduction in its surface charge (Smith, 1975).

3.4.3. FWS aggregation in the absence of NOM

Pure FWS was stable and aggregation rate was very low, so increasing concentrations of Ca\(^{2+}\) were used to initiate particle aggregation. Divalent calcium ions effectively increased the aggregate size immediately after their addition to the stable FWS. Figure 3.3-b shows increasing aggregation rate of FWS by increasing Ca\(^{2+}\) concentration from 0 to 50 mM during 900 s measured by DLS. Calcium ions could effectively decrease the extent of diffuse double layer around fullerene aggregates, reduced the energy barrier and caused rapid aggregation starting at concentrations as low as 1 mM of Ca\(^{2+}\). Aggregation rate of fullerene particles increased linearly with increasing Ca\(^{2+}\) concentrations up to 5.4 mM. At Ca\(^{2+}\) concentrations higher than 5.4 mM, the rate of fullerene aggregation did not further respond to the increased Ca\(^{2+}\) concentration and reached a maximum rate which is controlled only by the diffusion rate of fullerene aggregates in water. At such concentrations energy barrier between particles is completely eliminated due to the charge screening by high Ca\(^{2+}\) concentrations, thus the only limiting factor in aggregation is the rate of aggregate diffusion in the electrolyte (Chen and Elimelech, 2007). This is clearly shown in Figure 3.3-c where two distinct regions were identified: a reaction controlled region (slow) where \(\alpha\) increases with increasing Ca\(^{2+}\) concentration up to a maximum of 1, and a diffusion controlled region (fast) where increasing Ca\(^{2+}\) concentration did not further increase \(\alpha\). The intersection of these two regions, i.e. critical coagulation concentration (CCC) was calculated equal to 5.4 mM Ca\(^{2+}\). Chen and Elimelech (2006) with the same technique calculated CCC equal
to 4.8 mM Ca$^{2+}$ for a fullerene suspension at pH 5.2. One difference between our work and that of Chen and Elimelech (2006) is that they performed aggregation studies at a fullerene concentration about 20 times lower than ours, however the CCC and the general observed aggregation behavior of pure fullerene system was quite similar. This suggested that even at concentrations as high as 5 mg/L fullerene, particle-particle interactions of FWS were not considerable and the system follows the classic DLVO theory of aggregation.

### 3.4.4. FWS aggregation in the presence of different NOMs

Effect of different NOMs (at 2 mg/L) on the aggregation rate of FWS at 4 mM Ca$^{2+}$ is compared in Figure 3.4. Pure FWS started aggregating at this Ca concentration, but as discussed earlier it was still in the reaction controlled regime. Addition of FA, HA1, and HA7 decreased aggregation rate with order of FA<HA1<HA7. TA on the other hand enhanced FWS aggregation, even higher than that of the pure FWS. To take a more quantitative approach in comparing the effect of different NOMs on the stability of FWS, $\alpha$ was calculated for a range of Ca$^{2+}$ concentrations in the presence of each NOM (Fig. 3.5). Highest CCC was shown for HA7 equal to 14.5 mM CaCl$_2$. HA1, FA, and TA had CCCs at 6.4, 5.4, and 3.7 mM Ca$^{2+}$, respectively.

Each NOM affected FWS stability differently, which could be due to their effect on the $\zeta$ of fullerene particles or through steric repulsion. Because $\zeta$ of FWS in the presence of different NOMs and Ca$^{2+}$ did not significantly change (Fig. 3.3-a), an electrostatic effect could not explain the observed stability differences of FWS with various NOMs. The increased FWS stability with FA, HA1, and HA7 was mainly due to the steric effect: The adsorbed layer of NOMs (organic coating) on the surface of
fullerene aggregates did not allow aggregates to get close enough to form bigger aggregates, thus aggregation stability increased. This is in line with the study by Duncan et al. (2008) which showed addition of NOM to FWS increased aggregate stability due to steric stabilization. Chen and Elimelech (2007) reported similar increase of CCC for NaCl and MgCl$_2$ in the presence of HA. However, in their study, addition of HA to the fullerene suspension with CaCl$_2$ caused enhanced aggregation, i.e. $\alpha$ values greater than 1. They believed that the enhanced aggregation with CaCl$_2$ was due to bridging of free HA molecules with Ca$^{2+}$ that caused enhanced aggregation, which was not observed in our study, most likely because the concentration ratio of fullerene to HA was much higher in our study leading to less abundant free HA molecules in the mixture.

In the sorption study $K_d$ was 0.113, 0.140, 0.167, and 0.184 mL/mg for TA, FA, HA1, and HA7, respectively. Sorption experiment was performed on fullerene powder which consists of big aggregates and crystals of fullerene, thus sorption by fullerene powder would be much lower than that of FWS, where fullerene particles are much smaller. However, these data could give us a basis on which to compare sorption of different NOMs by fullerene. The trend of increasing $K_d$s is in line with increasing stability of FWS by different NOMs, suggesting higher sorption of NOMs on the fullerene particles could lead to a thicker NOM layer thus providing greater steric stability.

The thickness of NOM layer on the surface of fullerene particles which controls effectiveness of steric repulsion depends on the nature of interactions between fullerene and NOM and also physicochemical properties of NOM. The main structural differences between these NOMs were their molecular weight and polarity. As shown in Figure 3.2,
HA7 had the highest molecular weight among the NOMs used in this study and the presence of long chain hydrophobic moieties in this fraction of HA was previously reported (Ghosh et al., 2008). The long chain hydrophobic nature of HA7 not only caused its strong attachment to the surface of hydrophobic fullerene aggregates, but also prevented the coated fullerene aggregates from getting close to each other. Terashima and Nagao (2007) also showed that hydrophobic structural features of NOM could be a factor determining the degree of fullerene dispersion and stability in water. Effectiveness of NOM in C$_{60}$ stability decreased with decreasing molecular weight of NOMs: HA7 > HA1 > FA (Fig. 3.7-c). FA and HA1 not only contained less hydrophobic moieties which impeded their sorption on the surface of fullerene aggregate (compared to that of HA7), but also their smaller size provided a thinner coating on the surface of fullerene aggregates which was not as effective in providing steric stability (Figs. 3.7-a and 3.7-b).

Polarity of NOMs negatively affected their effectiveness in increasing FWS colloidal stability (Fig. 3.7-c). Polarity was calculated as the atomic ratio of oxygen and nitrogen divided by that of carbon. More polar NOMs (FA and HA1) had a lower ability to stabilize FWS. Considering the hydrophobic nature of fullerenes, polar NOMs have lower tendencies in adsorbing on the surface of fullerene particles, as a result the layer of organic coating would be thinner. Terashima and Nagano (2007) reported that HA was more effective than FA in increasing fullerene solubility, due to its lower charge density and larger hydrophobic structure. Polarity and molecular weight of NOM are often inversely proportional, because higher molecular weight NOMs usually contain long chain molecules with fewer functional groups, which lead to lower polarity.
TA behavior in aggregation stability of FWS was quite different from that of the humified NOMs. TA with a central carbohydrate (glucose) and 10 galloyl groups, had a relatively low molecular weight (1.7 kDa) and its structure was more spherical than that of HA or FA. Sorption of TA on the surface of fullerene aggregates was probably due to $\pi-\pi$ interaction with the aromatic structure of fullerene. However because of the low molecular weight and shape of TA, the thickness of the organic layer coated on the surface of fullerene aggregates would be smaller than that of the humified NOMs in this study. This reduced the effectiveness of steric stabilization. On the other hand as mentioned before, the surface charge of fullerene aggregates in the presence of TA was not significantly different from other types of NOM (Fig. 3.3-a). But CCC of pure FWS was reduced from 5.4 to 5.1 mM CaCl$_2$ in the presence of TA suggesting that TA has a lesser effect on the stability of FWS. Two possible reasons could be proposed for this observation. First there was a possibility of $\pi$-Ca$^{2+}$ interaction between Ca$^{2+}$ in the solution with the aromatic structure of adsorbed TA molecules on the surface of fullerene (Fig. 3.6-a). This is actually an electrostatic interaction of positively charged calcium cations with the negatively charged benzene ring surface (Keiluweit and Kleber, 2009). Another possibility could be the chelation or bridging of surface adsorbed and free TA molecules via Ca$^{2+}$ (Fig. 3.6-b). Chelating properties of TA is well studied in literature especially with iron ions, and Smith (1974) has also reported Ca$^{2+}$ chelation with TA. Chelation of TA molecules could form a network that traps fullerene aggregates and cause enhanced aggregation. We also observed spherical structures of TA+CaCl$_2$ in TEM studies which suggests the possibility of chelation of Ca$^{2+}$ by TA molecules (Fig. 3.6-c). TEM images of fullerene aggregates from different conditions are shown in Figure 3.8.
Pure fullerene in the absence of Ca\textsuperscript{2+} had minimum aggregation. Addition of Ca\textsuperscript{2+} (2 mM) showed enhanced aggregation with any type of NOM. However fullerene in the presence of HA7 displayed the lowest degree of aggregation compared to the other types of NOMs. Similar observations were made in the AFM height images (Fig. 3.9). Huge aggregates with heights well above 150 nm were observed on the surface of mica with Ca\textsuperscript{2+} and TA (Fig. 3.9-b), while minimum aggregation with heights lower than 20 nm observed with HA7 and Ca\textsuperscript{2+} (Fig. 3.9-c).

3.5. Conclusions

In summary, different NOM characteristics could significantly affect stability and size of fullerene particles in water and potentially their transport and bioavailability. More hydrophobic NOMs showed a higher affinity in adsorbing to the surface of fullerene aggregates. Sorption of NOMs on the surface of fullerene aggregates could affect their surface charge, but steric stability seems to be the dominant mechanism for increasing stability of fullerene aggregates. Cation bridging in long chain molecules of NOM leads to colloidal instability and precipitation of nanoparticles from water. Because different NOMs are expected in different environments, thus NOMs cannot be treated the same when discussing their impact on the environmental behavior and toxicity of fullerene and other nanoparticles. Considering the abundance of NOMs and their vastly different properties in natural waters, they can effectively alter the aggregation state of released fullerene in the environment. This study showed that structural characteristics of different NOMs could promote or inhibit aggregation of fullerene particles, which needs to be evaluated for the fate and toxicity of fullerene.
Figure 3.1. Intensity based size distribution of pure FWS obtained from DLS data. Z-average particle diameter was 96.9 nm. 2% of particles had diameters greater than 1000 nm.

Figure 3.2. Size exclusion chromatograms of FA, HA1 and HA7 used in the study. HA7 has a very wide range of molecular sizes and two peaks were observed in the chromatogram showing the presence of two types of molecular size range.
Figure 3.3. Effect of different types of NOM on the \( \zeta \) of FWS measured at varying concentrations of \( \text{CaCl}_2 \) at pH 7 and 25°C (a). Concentration of FWS was 5 mg/L and NOMs concentration was kept at 2 mg/L. All NOMs decreased \( \zeta \) of fullerene particles (to more negative values) in the absence of \( \text{Ca}^{2+} \); however increasing \( \text{Ca}^{2+} \) concentration increased the \( \zeta \). Aggregation rate of pure FWS as a function of \( \text{Ca}^{2+} \) concentrations (b). Increasing \( \text{Ca}^{2+} \) concentration increased FWS aggregation rate at concentrations as low as 1.5 mM \( \text{Ca}^{2+} \). Aggregation rate of FWS continued to increase by increasing concentrations of \( \text{Ca}^{2+} \) up to 5.7 mM \( \text{Ca}^{2+} \), after which increasing \( \text{Ca}^{2+} \) concentration had no effect on the aggregation rate. Attachment efficiencies (\( \alpha \)) of FWS as a function of \( \text{Ca}^{2+} \) concentration (c), in this graph two distinct aggregation regimes can be observed: reaction controlled regime, where \( \alpha \) increased linearly with increasing \( \text{Ca}^{2+} \), and diffusion controlled regime, where rate of aggregation was controlled by diffusion rate of FWS aggregates in the solution. The intercept between two regimes is CCC which was determined equal to 5.4 mM \( \text{Ca}^{2+} \) for the pure FWS system.
Figure 3.4. Effect of different NOMs on the aggregation rate of FWS in the presence of 4 mM Ca$^{2+}$. Concentration of all NOMs was 2 mg/L. Aggregation rate of pure FWS was higher than its aggregation rate in the presence of HAs and FA, but TA increased aggregation rate. The aggregation rate was significantly reduced in the presence of bigger molecular weight, more hydrophobic NOMs.

Figure 3.5. Attachment efficiencies ($\alpha$) for different fullerene-NOM systems as a function of Ca$^{2+}$ concentration. Reaction and diffusion controlled regimes were distinguishable for all NOMs. Highest calculated CCC was 14.5 mM Ca$^{2+}$ for HA7 followed by 6.4 mM for HA1, 5.4 mM for FA, and 3.7 mM for TA.
Figure 3.6. $\pi$-Ca interaction with aromatic structure of surface adsorbed TA molecules (a). Chelation of free or adsorbed TA by Ca$^{2+}$ causing entrapment of fullerene aggregates leading to enhanced aggregation (b). TEM image of TA showing spherical structure of adsorbed TA on a formvar coated TEM grid in the presence of CaCl$_2$, confirming the possibility of chelation. Center of each structure is a CaCl$_2$ crystal (c).
Figure 3.7. Schematic diagram showing sorption of FA or HA1 on the surface of fullerene particles (a). The thickness of NOM is not enough to provide effective steric stabilization for the suspension at CaCl\textsubscript{2} concentrations as low as 5.4 mM. On the other hand HA7 with higher molecular weight created a thicker coating layer on the surface of fullerene particles which could effectively provide steric stability for the suspension at CaCl\textsubscript{2} concentrations up to 14.5 mM (b). Relationship between NOM molecular weights with the CCC and polarity index of NOMs (c). Most colloidal stability represented by the highest CCC was observed with HA7 (highest molecular weight of 13.7 kDa). CCC decreased with increasing polarity of humified NOMs. TA did not follow the same trend as the humics. Relationship of CCC with K\textsubscript{d} (d). CCC increased with increasing K\textsubscript{d}s supporting the idea that more sorbed NOM on the surface of aggregates, provides more aggregation stability through steric effect.
Figure 3.8. TEM images of fullerene aggregates precipitated from water suspension. Pure fullerene without Ca\(^{2+}\) (a), and with Ca\(^{2+}\) (b). Enhanced aggregation of fullerene in the presence of 2 mg/L TA and Ca\(^{2+}\) (c), compared to less aggregation in the presence of 2 mg/L HA1 and Ca\(^{2+}\) (d). Concentration of Ca\(^{2+}\) in each case was 2 mM.

Figure 3.9. AFM height images of fullerene particles adsorbed on the surface of freshly cleaved, Ca saturated mica with different NOMs. Low aggregation of pure FWS in the absence of Ca\(^{2+}\) (a). Enhanced aggregation of fullerene particles in the presence of Ca\(^{2+}\) and TA (b), and minimum aggregation and smallest fullerene aggregates in the presence of HA7 and Ca\(^{2+}\) (c). Concentration of Ca\(^{2+}\) in each case was 2 mM.
CHAPTER 4

INFLUENCE OF IONS ON THE COAGULATION AND REMOVAL OF C\textsubscript{60} FULLERENES FROM WATER

4.1. Abstract

With C\textsubscript{60} widely used, these nanoparticles will be discharged into natural waters and produce pollution. Drinking water is a pathway for human exposure to nanoparticles, but little attention has been made on the transformation and removal efficiency of C\textsubscript{60} in the process of water treatment. Therefore, C\textsubscript{60}, as one kind of nanoparticulated pollutant, was selected to investigate its coagulation removal efficiency in the presence of different ions. The effects of these ions and the mechanism of C\textsubscript{60} removal in coagulation were evaluated. Results showed that C\textsubscript{60} could be removed with 80% efficiency at optimum Al\textsuperscript{3+} dose. Divalent anions, such as CO\textsubscript{3}\textsuperscript{2-}, SO\textsubscript{4}\textsuperscript{2-}, HPO\textsubscript{4}\textsuperscript{2-}, and humic acid (HA) could act as a bridge to connect C\textsubscript{60}-Al (OH)\textsubscript{n}(m-δ)+ particles together and induced the formation of flocs. Therefore, the addition of these anions enhanced C\textsubscript{60} removal significantly. However, monovalent anions, such as OH\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-} and SDS, could not bridge C\textsubscript{60}-Al\textsuperscript{n+} particles together and did not enhance C\textsubscript{60} removal. Addition of Na\textsuperscript{+} and Ca\textsuperscript{2+} increased the ionic strength of aqueous solutions which compressed the electric double layer and induced the C\textsubscript{60}-Al (OH)\textsubscript{n}(m-δ)+ particles to aggregate. However the cations were not the most important factor in flocs formation. This work highlighted the presence of multivalent anions and cations in raw water as a crucial factor in C\textsubscript{60} removal.

4.2. Introduction

With increasing commercial interest in its unique chemical and physical
properties, $C_{60}$ has been widely used in different products such as new high-strength materials, electronics, optics, biomedicines and catalysts (Jensen et al., 1996; Innocenzi and Brusatin, 2001; Sherigara et al., 2003; Tenne and Seifert, 2009). As the use of fullerene $C_{60}$ continues to grow rapidly, it is inevitable that fullerene particles will enter natural aquatic systems. Therefore, it is important to understand the fate and transformation of these nanoparticles in the environment, because of their potentially severe ecological impacts. Furthermore, the possibility of nanoparticles entering the drinking water system is related to their behavior in natural waters. Electrolytes are considered an important factor affecting the stability of $C_{60}$ as well as other nanoparticles (Chen et al, 2006; Chen et al, 2007; Zhang et al, 2009). Both monovalent and divalent salts could destabilize fullerene $C_{60}$ nanoparticles. However, humic acid can enhance the stability of $C_{60}$ suspension by imparting negative charge on the particles and also through steric repulsion (Chen et al, 2007; Mashayekhi et al, 2012).

Exposure to $C_{60}$ suspensions has been reported to cause oxidative damage to human cell membranes, DNA damage and human cell death, partially due to the production of reactive oxygen species and their uptake by cells (Sayes et al., 2004; Dhawan et al., 2006; Isakovic et al., 2006). Drinking water is a direct way for humans to ingest nanoparticles. The risk of exposure for humans to nanoparticles in drinking water is affected by the efficiency of water treatment processes. These processes usually include coagulation, flocculation, sedimentation and filtration. Coagulation is an effective technique for removing colloidal particles and dissolved organic matter (DOM) from surface water sources. It was reported that the particle size of colloids and molecular weight of DOM affected their coagulation removal efficiency (Volk et al, 2003).
Therefore, nanoparticles with their tiny size were assumed to have a different removal efficiency and mechanism than colloidal particles in the process of coagulation. However, the fate and transformation of commercial nanoparticles during the water treatment processes is not completely understood. Zhang et al, 2008 reported that 20-60% of commercial metal oxides such as TiO$_2$, NiO, Fe$_2$O$_3$, ZnO could be removed in the process of coagulation followed by sedimentation, but the size of these commercial metal oxide nanoparticles was actually bigger than 100 nm. Hoon et al, 2009 examined the fate of C$_{60}$ nanoparticles in the process of conventional water treatment and found that aggregated C$_{60}$ could be effectively removed in the operation units, while the efficiency of C$_{60}$ removal depended on various parameters, such as pH, alkalinity, organic materials and coagulant dosage. Although much attention has been paid to nanoparticle removal from drinking water, there is still a lot unknown. Such as evaluating the exact removal mechanism of nanoparticles, determining different parameters affecting the nanoparticles removal in the process of water treatment, and understanding the byproducts of nanoparticles and water treatment chemicals and so on.

Thus, this research was done to evaluate the coagulation removal efficiency of C$_{60}$ as affected by different types of ions, and humic acid as a representative of dissolved organic materials. The mechanisms of C$_{60}$ removal affected by these parameters were also discussed.

4.3. Materials and Methods

4.3.1. Preparation of C$_{60}$ water solutions and HA solution

C$_{60}$ powders were purchased from MER Corp. (Tuscan, AZ). About 50 mg of
99.9% pure C\textsubscript{60} powders were dissolved in 25 mL of HPLC-grade toluene (Sigma-Aldrich, St. Louis, MO) by shaking for more than 12 hours, resulting in a clear dark purple mixture. The mixture was gently introduced into a solution of 750 mL of deionized water with pH 10 (NaOH added). Then the mixture was sonicated with a sonicating probe (450 Sonifier, Branson Ultrasonics Corporation, Danbury, CT) in a fume hood for 1-1.5 h to allow complete evaporation of toluene and transfer of fullerene into water. The final solution was filtered using a vacuum filter, first through a glass fiber pre-filter followed by a 0.20 µm nylon membrane filter (Fisher Scientific). The resulting clear amber color fullerene suspension was stored in the dark at 4 °C. The total carbon content (TC) of the filtered fullerene suspension was measured equal to 16.035 mg/L, using a TOC-L CSH (Shimadzu, Kyoto, Japan) instrument.

Commercial Aldrich HAs were purified according to a previous report (Pan et al., 2006). Briefly, a solution containing 0.1 M NaOH and 0.1 M Na\textsubscript{4}P\textsubscript{2}O\textsubscript{7} was mixed with Aldrich HA (50:1, v:w) to extract the HA. The supernatants were filtered and collected, and the HAs were precipitated with 6 M HCl. The precipitated HAs were washed using distilled water until a chloride test using AgNO\textsubscript{3} was negative for chloride, freeze-dried and ground to 500 µm particles. An aliquot of the purified HAs was dissolved in deionized water, and the mixture was adjusted to pH 12 with 0.5 M NaOH. After 10 h stirring, the solution was filtered through a 0.20 µm cellulose acetate membrane filter, and the supernatant was collected. The concentration of HA in the filtrate was quantified by TOC, and the HA solution was stored at 4 °C for other experiments.

**4.3.2. Jar test procedure**

Jar tests were performed with 50 mL of C\textsubscript{60} stock solution transferred to 200 mL
deionized water in 400 mL beakers using a Jar Mixer (Phipps & Bird. INC Richmond. VA). One milliliter NaHCO$_3$ (12.5g/L) was used to buffer the water sample and keep the pH value in the range of 8.5-9.0. One milliliter of NaCl (1M) was added to the solution to adjust ionic strength unless otherwise stated. After adding a predetermined amount of alum (Al$_2$(SO$_4$_3)·18H$_2$O) (Aldrich, Milwaukee, WI) from 0.01M stock, the solution in the jar was mixed at a paddle speed of 120 rpm for 5 min (coagulation), followed by 30 min slow mixing at 40 rpm (flocculation). After allowing 1 h of quiescent settling (sedimentation), the supernatant was removed for further analyses.

When investigating the effect of Ca$^{2+}$ on C$_{60}$ removal, 1 mL NaCl (1M) was replaced with an equal valance of Ca$^{2+}$. Similarly, HCO$_3^-$ was replaced with other anions, such as HPO$_4^{2-}$, SO$_4^{2-}$, NO$_3^-$, HA, Sodium Dodecyl Sulfate (SDS), when investigating the effect of anions on C$_{60}$ removal.

4.3.3. Analytical methods

The size of C$_{60}$ was analyzed by dynamic light scattering (DLS) using a Zetasizer ZS90 (Malvern Instruments, Bedford, MA). Zeta potential was analyzed by a Zeta 90 Plus Zeta Potential Analyzer (Brookhaven Instruments Co., Holtsville, NY). The concentration of C$_{60}$ in organic free water was determined by UV absorption at 268 nm using an Agilent 8453 UV–Vis Spectroscopy System (Palo Alto, CA). All samples were run in triplicate.

4.4. Results and discussion

4.4.1. Characterization of C$_{60}$ in different electrolytes

Zeta potential and particle size (PS) of C$_{60}$ were measured in the presence of
different ions. The results are shown in Table 2.1. Determination was carried out after the process of jar test in the absence of coagulant. Zeta potential of C₆₀ particles in the deionized water was negative which is similar with the data from previous research that showed fullerene nanoparticles have a negative electrophoretic mobility (Andrievsky et al, 1995; Chen et al, 2007). The addition of cations caused zeta potential to increase in the following order: Ca²⁺ > Na⁺, while the addition of anions (with Na⁺ as a common counter-ion) decreased zeta potential as the order of²: HCO₃⁻ < HPO₄²⁻ < A⁻ < SDS. The results revealed that cations neutralized the negative charge on the surface of C₆₀, while the anions added to the negative surface charge. The hydrodynamic diameter of C₆₀ measured by intensity-weighed DLS was varied from 57.9 to 148.6 nm. The mean diameter based on the intensity of scattered light was 97.0 nm. The concentration of cations in this work was well below the reported (Chen and Elimelech, 2006) critical coagulation concentration (CCC) for Na⁺ and Ca²⁺ (120 mM and 4.8 mM, respectively).

4.4.2. Effect of coagulant dose on C₆₀ removal and Zeta potential

Aluminum sulfate was used as coagulant and its dose effect was investigated on C₆₀ removal efficiency as well as the change of the zeta potential. The results are presented in Fig. 4.1. In all cases, C₆₀ removal first increased and then decreased with increasing dose of Al₂(SO₄)₃. The maximum C₆₀ removal (82%) was observed at a dose of 0.02 mM Al₂(SO₄)₃ when no other cations were presented. Overdose of coagulant caused the C₆₀ removal to decrease. The presence of Na⁺ or Ca²⁺ widened the range of coagulant dose to obtain the effective C₆₀ removal. More than 80% of C₆₀ removal was

² A⁻ represents negatively charged humic acid ions, and SDS here stands for dodecyl sulfate anions.
obtained in a range of coagulant from 0.02 to 0.05 mM for Na$^+$ and 0.02 to 0.06 mM coagulant for Ca$^{2+}$. The decrease of C$_{60}$ removal due to overdose of coagulant, was attributed to the accumulation of hydrolyzed species of Al$^{3+}$ on the outer surface of C$_{60}$ double layer. This phenomenon caused reversal of C$_{60}$ charge (from negative to positive) and re-stabilized it. The Al$^{3+}$ ions were hydrolyzed upon their addition to the C$_{60}$ aqueous solution. This can be confirmed by a decrease in the pH of C$_{60}$ suspension upon addition of Al$^{3+}$ to it. It was reported that the chemical species of hydrolyzed Al(III) can be categorized into three types: monomeric species (Al$^{3+}$, Al(OH)$^{2+}$, Al(OH)$_2^+$, Al(OH)$_3$, Al(OH)$_4$), medium polymer species (Al$_2$(OH)$_2^{4+}$, Al$_{13}$O$_4$(OH)$_{24}^{7+}$) and species of sol or gel (Malgorzata, 2005). Although the hydrolyzed Al$^{3+}$ species were not quantified in this work, it is almost likely that the hydrated Al$^{3+}$ which is simply shown as Al(OH)$_{n}^{m+}$ carried positive charge, because it caused the zeta potential of C$_{60}$ shifted from negative to positive (Fig 4.1b). The positive Al(OH)$_{n}^{m+}$ ions tend to be adsorbed by negative C$_{60}$ because of electrostatic attraction which caused the charge reversal of C$_{60}$, then the re-stabilization took place. In the presence of Na$^+$ or Ca$^{2+}$, charge reversal was occurred at a lower concentration of Al$_2$(SO$_4$)$_3$ because these cations screened some part of negative charge carried by C$_{60}$ (Table 4.1). However, the aggregation of C$_{60}$ particles observed even though they carried positive charge. The results revealed that the addition of Na$^+$ and Ca$^{2+}$ could enhance the aggregation of C$_{60}$ particles, but the effect of Na$^+$ and Ca$^{2+}$ on C$_{60}$ removal needs to be further investigated.

4.4.3. Effect of Na$^+$ and Ca$^{2+}$ on C$_{60}$ removal

The effect of Na$^+$ and Ca$^{2+}$ dose on C$_{60}$ removal was investigated by fixing the dose of Al$_2$(SO$_4$)$_3$ at 0.04 mM, and varying Ca$^{2+}$ or Na$^+$ concentrations. The results are
shown in Fig 4.2. It was observed that C₆₀ removal first increased and then reached an equilibrium at concentration of 1.0 mM Ca²⁺ or 2.0 mM Na⁺ respectively (Fig 4.2a). No decrease in C₆₀ removal was observed over these two doses of Na⁺ or Ca²⁺, even when the dose of Na⁺ was more than 40 mM (data not shown). The results revealed that the addition of Na⁺ and Ca²⁺ did not cause the re-stabilization of C₆₀, but enhanced their aggregation. The effect of Na⁺ and Ca²⁺ on C₆₀ removal can be explained by the function of compressing electric double layer. According to the DLVO theory, C₆₀ adsorbed Al(OH)ₙᵐ⁺ to form C₆₀-Al(OH)ₙ(ₘ₋δ⁺) particles which carried positive charges. In order to keep the electric charge at equilibrium, the exterior of C₆₀-Al(OH)ₙ(ₘ₋δ⁺) particles is balanced by opposite charged ions and formed electrical double layer. The presence of Na⁺ and Ca²⁺ enhanced the ionic strength of aqueous solution and caused the electric double layer to shrink. Therefore, the colloidal particle was destabilized and formed flocs. Furthermore, a lower concentration of Ca²⁺ than Na⁺ was needed to cause aggregation of C₆₀, which is due to a stronger potential of divalent Ca²⁺ for compressing the electric double layer.

4.4.4. Effect of pH on C₆₀ removal

As stated in the jar test procedure, HCO₃⁻ was used to buffer the aqueous solutions and adjusting the ionic strength. In order to make a comparison, the pH was adjusted by NaOH and HCl in the absence of HCO₃⁻. The effect of pH on C₆₀ removal is shown in Fig 4.3. No flocs were formed and fewer C₆₀ were removed in the absence of HCO₃⁻ in a pH range from 5 to 10. In the presence of HCO₃⁻, obvious flocs were formed and C₆₀ removal could reach to 80% at a pH near 9, while much lower C₆₀ removal was obtained at other pH values. Three classic mechanisms have been used to describe NOM
removal in the process of coagulation by alum. These mechanisms were: charge neutralization, entrapment and adsorption. The optimum pH for NOM removal was reported to be in the range of 5-7 (Yang et al., 2010, Kabsch-Korbutowicz, 2005). In this pH range, Al$^{3+}$ hydrolyzes to form higher positively charge species, which could neutralize the negatively charged NOM and make them destabilized (Edzwald and Tobiason, 1999). The pH range for C$_{60}$ removal was different from NOM removal in the process of coagulation by alum. No C$_{60}$ flocs were seen in the pH range of 5-10 in the absence of HCO$_3^-$.

Charge neutralization of C$_{60}$ took place by the hydrolyzed Al (OH)$_n^{m+}$. While it did not cause destabilization of C$_{60}$, but caused its re-stabilization. The reaction can be shown as follow:

$$C_{60}^{δ-}(s) + Al(OH)_n^{m+}(s) \rightarrow [C_{60}-Al(OH)_n]^{(m-δ)^+}(s)$$

Negatively charged C$_{60}$ adsorbed Al(OH)$_n^{m+}$ and its charge reversed to positive values which hindered further aggregation. Therefore, re-stabilization of C$_{60}$ occurred and negligible C$_{60}$ removal was observed in the pH range of 5-10 in the absence of HCO$_3^-$. However, flocs were seen at pH 9 in the presence of HCO$_3^-$. As HCO$_3^-$ dissociated and formed CO$_3^{2-}$ at pH 9, it was assumed CO$_3^{2-}$ acted as a bridge to connect two positive C$_{60}$-Al(OH)$_n^{(m-δ)^+}$ particles together, but the assumption should be further confirmed.

### 4.4.5. Effect of anions on C$_{60}$ coagulation removal

In order to verify the bridging function of CO$_3^{2-}$, effect of other divalent anions was also compared in C$_{60}$ removal and the results are shown in Fig 4.4. As anticipated, more flocs were formed and higher C$_{60}$ removal was observed in the presence of multivalent anions, such as HPO$_4^{2-}$ and SO$_4^{2-}$, while negligible C$_{60}$ removal was observed with a monovalent anion, NO$_3^-$. The results confirmed the speculation on bridging role of
multivalent anions for $\text{C}_{60}\text{-Al(OH)}_n^{(m-\delta)+}$ particles.

To further understand the behavior of ions involved in the process of coagulation, a schematic model was prepared to describe this mechanism (Fig 4.6). The $\text{Al}^{3+}$ hydrolyzed as it was added to the $\text{C}_{60}$ aqueous solution and formed hydrated $\text{Al(OH)}_n^{m+}$ which carried positive charge. Due to electrostatic attraction, $\text{Al(OH)}_n^{m+}$ tends to be adsorbed by $\text{C}_{60}$ and formed $\text{C}_{60}\text{-Al(OH)}_n^{(m-\delta)+}$ particles which carried positive charge (Fig 4.1-b). The $\text{C}_{60}\text{-Al(OH)}_n^{(m-\delta)+}$ particle could not aggregate further because of electrostatic repulsion, therefore re-stabilization occurred (Fig 4.1-a). Multivalent anions, such as $\text{CO}_3^{2-}$, $\text{SO}_4^{2-}$ and $\text{HPO}_4^{2-}$, can act as a bridge to connect two positive $\text{C}_{60}\text{-Al(OH)}_n^{(m-\delta)+}$ particles together and enhance the formation of flocs, while the monovalent anions cannot serve as a bridge to cause the $\text{C}_{60}\text{-Al(OH)}_n^{(m-\delta)+}$ particles to aggregate. The presence of $\text{Na}^+$ or $\text{Ca}^{2+}$ increased the ionic strength and stimulated the particles to aggregate by compressing the electric double layer. However, they were not required to cause floc formation, because aggregation was also observed in the absence of $\text{Na}^+$ or $\text{Ca}^{2+}$ (Fig 4.4, No Cations).

4.4.6. Theoretical analysis on stability of nanoparticles

The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory is a well-known theory to describe stability of particles in water by analyzing the interaction of forces between particles. In terms of classical DLVO theory, two major forces determine the stability of particles: Van Der Waals (VDW) attraction (FVDW) and electrical double layer (EDL) repulsion (FEDL). The net interaction between two particles whether it is repulsive or attractive depends on the sum of these two forces which is expressed as follow (Hiemenz and Rajagopalan, 1997):
\[ \Phi_{\text{Total}} = \frac{4\pi \varepsilon r^2 \zeta^2}{H + 2r} e^{(-\kappa H)} - \frac{A}{6} \left[ \frac{2r^2}{H^2 + 4rH} + \frac{2r^2}{H^2 + 4rH + 4r^2} + \ln \left( \frac{H^2 + 4Hr + 4r^2}{H^2 + 4Hr} \right) \right] \]

where, \( r \) is the radius of a particle (m); \( \zeta \) is the Zeta potential (V); \( \varepsilon \) is the permittivity of water \( (6.95 \times 10^{-12} \text{ C}^2/\text{J}) \); \( H \) is the distance between two particles (m); \( k \) is the inverse of the Debye Length \( (\text{m}^{-1}) \); \( A \) is the Hamaker constant of fullerene particles in water \( (6.7 \times 10^{-21} \text{ J}) \). Hamaker constant of C\(_{60}\) nanoparticles was obtained from Chen and Elimelech, 2006.

Fig 4.5 shows DLVO calculation results for C\(_{60}\) nanoparticles in the absence or presence of Ca\(^{2+}\). Zeta potentials of C\(_{60}\) nanoparticles were obtained from Fig 4.1b. The ionic strengths of the suspensions were determined from the electrolyte concentration.

The results revealed that the repulsive energy was higher than the attractive energy, which means the aggregation of C\(_{60}\) nanoparticles could not occur. However, the aggregation of C\(_{60}\) nanoparticles did occur and higher C\(_{60}\) removal was obtained in the presence of HCO\(_3^-\). This calculation further revealed that the aggregation of C\(_{60}\) in the process of coagulation did not follow the DLVO theory. It indirectly confirmed that the bridging process took place by multivalent anions on C\(_{60}\)-Al(OH)\(_n\)\(^{(m-\delta)}\) particles.

4.4.7. Effect of HA on C\(_{60}\) removal

It was reported that the presence of humic acid (HA) could stabilize C\(_{60}\) particles by imparting negative charge and steric hindrance (Chen and Elimelech, 2007; Mashayekhi et al., 2012). Furthermore, the presence of HA can cause more alum to be consumed resulting in the stabilization of colloids in the process of coagulation.
Therefore, the effect of HA on C_{60} removal was investigated and results are shown in Fig 4.7.

In the presence of HA, the trend of removal curves was similar with the cases without HA (Fig 4.1), but more alum was consumed in the absence of Na\(^{+}\) and Ca\(^{2+}\). More alum consumption was also observed for colloid coagulation in the presence of HA (Shin et al., 2008) which was attributed to the extra negative charge imparted on the particles by HA and free HA being in aqueous solution. More flocs were observed (Fig 4.8) and more than 80% of C_{60} was removed in the absence of multivalent anion. Therefore, it was assumed that a bridge function occurred between HA and C_{60}-Al(OH)\(_n\)(m-δ\(^{+}\)) particles, because HA could provide more than two negative functional group per molecule. To testify this inference, the effect of sodium dodecyl sulfate (SDS) which can not provide two negative charges per molecule, was also investigated on the C_{60} removal. As anticipated, no flocs were observed and a lower C_{60} removal was obtained in the presence of SDS. The results revealed that the bridging function could be induced not only by multivalent anions, but also by negatively charged molecules, which can provide more than two negative charges per molecule.

4.5. Conclusions

C_{60} can be removed with more than 80% efficiency at the optimum Al\(^{3+}\) dose, while overdose of Al\(^{3+}\) can cause the re-stabilization of C_{60}. It is inconvenient for operation to control the dose of alum in actual water treatment process. Fortunately the addition of Na\(^{+}\) and Ca\(^{2+}\) widened the range of coagulant concentration providing optimum C_{60} removal efficiency. Multivalent anions, such as CO\(_3^{2-}\), SO\(_4^{2-}\), HPO\(_4^{2-}\), and HA could act as a bridge to link C_{60}-Al(OH)\(_n\)(m-δ\(^{+}\)) particles together and cause them to
aggregate and to be removed in the process of coagulation. Anions which could not serve as a bridge, such as NO$_3^-$, OH$^-$, SDS, had negligible effect on flocs formation and C$_{60}$ removal.
Table 4.1. Characterization of C$_{60}$ in different aqueous solutions.

<table>
<thead>
<tr>
<th>C$_{60}$</th>
<th>Blank</th>
<th>Na$^+$</th>
<th>Ca$^{2+}$</th>
<th>HCO$_3^-$</th>
<th>HPO$_4^{2-}$</th>
<th>A$^-$ (TOC)</th>
<th>SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 mM</td>
<td>1 mM</td>
<td>0.59 mM</td>
<td>0.4 mM</td>
<td>1.9 mg/L</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>PS (nm)</td>
<td>97.0</td>
<td>97.9</td>
<td>98.8</td>
<td>98.1</td>
<td>97.1</td>
<td>98.4</td>
<td>94.7</td>
</tr>
</tbody>
</table>

A$^-$: Humic acid ion; SDS: Sodium dodecyl sulfate

Figure 4.1. Effect of Al$_2$(SO$_4$)$_3$ dose on C$_{60}$ removal (a) and Zeta potential of C$_{60}$ colloidal solution (b).
Figure 4.2. $C_{60}$ removal% (a) and Zeta potential (b) of $C_{60}$ colloidal solution as affected by dose of Na$^+$ and Ca$^{2+}$.

Figure 4.3. Effect of pH on $C_{60}$ removal and the Zeta potential.
Al₂(SO₄)₃: 0.02 mM; NaCl: 4 mM; CaCl₂: 1 mM

**Figure 4.4.** Effect of different anions on C₆₀ coagulation and removal.

**Figure 4.5.** Theoretical net energy between C₆₀ particles calculated based on the DLVO equation, versus separation distance.
Figure 4.6. A schematic model for $C_{60}$ aggregation in the process of coagulation by alum.

Figure 4.7. Effect of HA on $C_{60}$ removal efficiency. Concentration of HA (TOC) was 1.9 mg/L; Ca$^{2+}$: 1 mM, and Na$^+$: 4 mM.
Figure 4.8. Pictures of C$_{60}$ aqueous solutions in jar test experiments coagulated with Al$^{3+}$ in the presence of different ions. Concentration of ions in the solution was as follow: $c$(Na$_2$CO$_3$) = 0.59 mM, $c$(Na$_2$HPO$_4$) = 0.4 mM; $c$(NaHSO$_4$) = $c$(CaCl$_2$) = 1 mM; $c$(NaCl) = $c$(NaNO$_3$) = 4 mM; $c$(SDS) = 0.5 mM, and HA concentration (TOC based) was 1.9 mg/L.
CHAPTER 5

CONCLUSIONS AND ENVIRONMENTAL IMPLICATIONS

The general goal of this work was to investigate the fate and transport of $C_{60}$ fullerene nanoparticle in the water. Considering the ubiquitous presence of NOM in natural waters, humic materials as their representative were separately studied and characterized. Aggregation and coagulation of all natural or manmade particles is one of the most important factors in determining their fate, mobility and availability in the aquatic environments. Thus, the interaction of $C_{60}$ fullerene with NOM and in the presence of commonly found salts in the environment in regard to aggregation and coagulation of fullerene was studied. Based on these, three major projects were defined and studied in my academic research work:

(1) Humic materials were extensively extracted from composites and characterized. Then their sorptive properties for PAHs were studied.

(2) A water suspension of $C_{60}$ fullerene was made, characterized, and its aggregation behavior was studied at different ionic strengths and in the presence of the structurally different humic materials and tannic acid as representatives of NOM.

(3) The efficiency of removal of $C_{60}$ from water or waste water in the presence of different ions and NOM was evaluated, in a scenario modeling a water source that is actually contaminated with $C_{60}$ fullerene.

In the first project, humic acids (HAs) were progressively extracted from two compost samples by 0.1 M sodium pyrophosphate solution. Seven different HAs were extracted through progressive extraction from the composites. The structural differences
and sportive characteristics of each fraction for polycyclic organic hydrocarbons (PAHs) were scrutinized. This was important because NOM is known to be very heterogeneous mixture of compounds, and their structural characteristics greatly affect their behavior. Structure of sequentially extracted HAs was showed to become more aliphatic and less polar with the successive extraction. The more aliphatic HA fractions showed more affinity to adsorb simple nonpolar organic materials from water. Structural and polarity differences in HAs caused changes in their sorption characteristics for organic compounds in terms of sorption capacity and isotherm nonlinearity. In the following projects we will see these structural differences among the HAs could affect their interaction with fullerenes as well as fullerene aggregation.

In the second project, aggregation kinetics of fullerene were investigated as affected by ionic strength and different NOMs. It was concluded that pure fullerenes colloidal suspension was quite instable and susceptible to aggregation with increasing ionic strength of solution. Charge screening by sodium or calcium ions was the reason for aggregation in a pure nC₆₀ colloidal suspension. Addition of NOM to the system greatly improved nC₆₀ colloidal stability. We observed that NOMs with larger molecular weights were more effective in stabilizing the fullerene suspension. Steric stability was concluded to be the reason for increasing suspension stability. Even though sorption of NOMs on the surface of fullerene aggregates could affect their surface charge, but steric stability seemed to be the dominant mechanism for increasing stability of fullerene aggregates. Divalent cation bridging in long chain molecules of NOM led to colloidal instability and precipitation of associated nanoparticles from water. This study showed that the structural characteristics of different NOMs could promote or inhibit aggregation of fullerene
particles, which needs to be evaluated for its consequences on the fate and toxicity of fullerene.

In the third project, the efficiency of removal of $C_{60}$ from water was evaluated. A classical jar study was employed in this project. Alum was used as a classical and common coagulant. The optimum dose of alum was evaluated. The results showed that $C_{60}$ could be removed with efficiency of more than 80% at the optimum $Al^{3+}$ dose, however overdose of $Al^{3+}$ could cause re-stabilization of $C_{60}$. Multivalent anions, such as $CO_3^{2-}$, $SO_4^{2-}$, $HPO_4^{2-}$, and HA could act as bridges to link $C_{60}$-$Al(OH)_{n}^{(m-δ)+}$ particles together and enhanced coagulation. Monovalent anions such as $NO_3^-$, $OH^-$, and SDS which do not act as bridge, had negligible effect on floc formation and on $C_{60}$ removal. Addition of $Na^+$ and $Ca^{2+}$ enhanced the aggregation of $C_{60}$-$Al(OH)_{n}^{(m-δ)+}$ particles, while they were not absolutely required to cause floc formation.

If this line of work and research is to continue further, natural waters collected from streams, lakes or drinking water reservoirs should be used to evaluate removal efficiency and aggregation behavior of $C_{60}$ fullerenes. Natural waters have a much more complex composition of both inorganic and organic dissolved materials, thus the behavior and stability of $C_{60}$ fullerene in those needs to scrutinize in more details.

In this work we only studied alum as a coagulant, because it is a classic and popular coagulant. However, there are many problems associated with the use of alum as a coagulant in water treatment plants. Because all the produced sludge from water treatment plants contains considerable percentage of aluminum salts and complexes, and their safe disposal and treatment is a huge environmental challenge.
Ferric-based coagulants can also be used as coagulants to replace alum, considering environmental issues associated with aluminum. Ferric ions are especially very efficient in removing medium sized NOM from water, however they need more buffering salts added to the water. PAC (polyaluminum chloride) is a poly-hydrolized aluminum chloride with a higher ratio of highly charged species such as Al$^{13+}$, which has a higher efficiency in removing NOM from water. PFS (polyferric sulfate) is pre-hydrolyzed iron sulfate with higher ratios of highly charged iron species, which provides better performance compare to the regular ferric sulfate (Ma et al., 2016). Other coagulant alternatives to alum are organic coagulants, which are mostly used to treat waters with high amounts of dissolved NOM.

Polyamines are a major group of organic coagulants which are commercially used. These organic compounds have two or more primary amine groups. Polyamines are liquid, cationic polymers of differing molecular weights which work effectively as primary coagulants and charge neutralization agents. Melamine formaldehydes and tannins are also organic polymers which are used instead of alum or other inorganic coagulants. Even though these organic coagulants are more expensive than inorganic coagulants, especially than alum, their efficiency and lower cost of their “environmental friendly” removal make their use appealing in certain conditions. To the best of our knowledge, there is no study on the efficiency of these products in removing nanoparticles and nC$_{60}$ in particular from water. This would be an interesting follow up to this line research for future work.

This study in general emphasizes the role of omnipresent, very reactive and structurally diverse humic materials on the transport, aggregation, precipitation and fate
of C$_{60}$ fullerenes, when they enter natural waters. Cations commonly found in fresh waters such as sodium and calcium were also the subject of investigation in this work. The interaction between fullerene nanoparticles, NOM, and different cations and anions in the environment is the essential factor in understanding fate and behavior of these particles in the environment. Having this type knowledge and insight enables us to better predict the fate of released nanoparticles in water. In water treatment plants knowing these interactions in part is essential to the management of plants to reach maximum cleanup efficiency.
REFERENCES


