EXPLORATION AND CHARACTERIZATION OF NEW SYNTHESIS METHODS FOR C_{60} COLLOIDAL SUSPENSIONS IN WATER

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EXPLORATION AND CHARACTERIZATION OF NEW SYNTHESIS METHODS FOR C_{60} COLLOIDAL SUSPENSIONS IN WATER

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Abstract:

Buckminsterfullerene, C_{60}, has been used in the production of several commercial products from badminton racquets and lubricants for their mechanical properties to cosmetics and even dietary supplements for their "antioxidant" properties. Multi-ton production of C_{60} began in 2003 encouraging serious consideration of its fate in the environment in the case of an accidental release or improper disposal. Although C_{60} is practically insoluble in water, it readily forms stable aqueous colloidal suspensions (termed $n$$C_{60}$) through solvent exchange methods or long-term vigorous stirring in water. Two new solvent exchange methods for synthesizing $n$$C_{60}$ are presented. These methods combine key advantages of multiple existing synthesis methods including high yield, narrow particle size distribution, short synthesis time, and an absence of solvents such as tetrahydrofuran that have historically caused problems in laboratory synthesized aggregates. The resulting samples are attractive candidates for use in controlled environmental impact, biological, and toxicity studies. An improved method for quantifying residual solvents in $n$$C_{60}$ samples utilizing solid phase micro extraction gas chromatography mass spectrometry (SPME-GC-MS) is also discussed.

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CHAPTER I

INTRODUCTION

1.1 Background

The 1996 Nobel Prize in Chemistry was awarded to Richard E. Smalley, Robert F. Curl, and Sir Harold W. Kroto of Great Britain for the discovery (in 1985) of a new allotrope of carbon, C_{60}.[1] Named Buckminster Fullerene (or Buckyball) after the designer of the geodesic dome, this cluster of 60 carbon atoms is arranged in a hollow icosahedral structure resembling a soccer ball. The fullerenes were synthesized by condensing carbon vapor in an atmosphere of inert gas. C_{60} was first produced in bulk in 1990 by Krätschmer et al.[2] and is currently produced in multi-ton quantities.[3] As the production and use of nanomaterials such as fullerenes increases, so does the risk of environmental and human exposure. It is known that when exposed to visible or ultraviolet light C_{60} can convert oxygen from the triplet to the singlet state.[4] Singlet state oxygen can cause oxidative damage to biological systems[5] thereby creating cause for concern about potential health risks of C_{60}. Unfortunately, current chemical classifications have not caught up with the advancement of nanotechnology.

Although it is a hydrophobic molecule, chemical modifications have been made to C_{60} to render it water soluble. One example of such a modification is the functionalization of the C_{60} molecule with hydroxyl groups to produce a fullerol ($C_{60}(OH)_{20-24}$).[6, 7] Numerous techniques have been employed to stably disperse C_{60} in water including adding various surfactants or polymer solubilizers,[8] covalent derivatization (such as the fullerol above),[9] and colloid formation where no deliberate attempt to chemically modify the fullerene is made.[10-18] A method for producing a fine aqueous colloidal suspension of C_{60} without chemical modification was first proposed by Scrivens et al.[18] in order to monitor its uptake by human keratinocytes. A saturated solution of C_{60} in benzene was added to tetrahydrofuran. The resulting light purple-colored solution was added drop-wise to rapidly stirring acetone. Water was then slowly added to the solution forming a mustard yellow suspension. Subsequently, the organic solvents were removed by evaporation. This colloidal suspension of C_{60} in water has been termed in the literature as fullerene water system (FWS), nano-C_{60}, or just $n$$C_{60}$ (used herein).

These $n$$C_{60}$ colloidal aggregates can be formed either naturally by simply mixing C_{60} powder in water[17] or through solvent exchange methods such as Scrivens' work described above. Solvent exchange methods, in general, begin by dissolving C_{60} powder in an organic solvent such as toluene or tetrahydrofuran. The resulting solution is mixed with water followed by the evaporation of the organic solvent(s) either by rotary evaporation, boiling, or ultrasonication. When trying to address environmental health and safety (EHS) issues for fullerenes, the form of C_{60} that is most relevant is $n$$C_{60}$ since it seemingly forms naturally upon contact with water regardless of the original state of the C_{60}.

$n$$C_{60}$ has attracted significant interest for health and environmental impact studies and has become a touchstone material concerning guidelines for the handling and disposal of nanomaterials.[7, 16, 17, 19-36] These numerous studies on $n$$C_{60}$ highlight the limitations of the current guidelines for fullerenes, which are based on the properties of carbon black, because they demonstrate the availability of C_{60} to natural water systems whereas carbon black and other insoluble carbon powders are not readily available. In other words, the formation of water stable C_{60} aggregates allows for the transport of C_{60} through the

environment as opposed to the initial belief that C_{60} (a hydrophobic molecule) would simply stick to the soil and other organic matter. To make matters more complex, different $n$$C_{60}$ samples were initially thought to be largely similar and independent of production method but have later been determined to be quite sensitive to the method from which they were produced.[13] The diversity and complexity of $n$$C_{60}$ suspensions suggest that the level of characterization appropriate for biological and environmental impact studies will be adequately more sophisticated than similar studies on more traditional bulk materials such as carbon black.[16]

Since $n$$C_{60}$ is the most likely form of C_{60} to be transported through and thus effect the environment, it is necessary to understand the metabolic pathway of $n$$C_{60}$ within a living organism. Also, it is important to study the effects it will have on living cells and other life forms essential to different ecosystems such as bacteria. Synthesized $n$$C_{60}$ has been used in several projects to study its impact on biological or environmental systems. There has been much debate and controversy on the properties exhibited by $n$$C_{60}$ ranging across opposite extremes from causing oxidative damage[7, 29] to having antioxidant properties.[21]

The cytotoxicity of $n$$C_{60}$ was initially reported in 2004.[7, 29] These reports suggested that $n$$C_{60}$ demonstrates toxicity to several cell lines in culture through an oxidative damage mechanism. In response to these initial findings, numerous reports either confirmed[20, 26, 30, 35, 37] or contradicted[19, 21, 38, 39] the reported cytotoxicity. Following the initial confusion, more recent findings revealed that much of the oxidative damage initially observed was due to the presence of γ-butyrolactone, which is the main degradation product of tetrahydrofuran (an organic solvent often used in the preparation of $n$$C_{60}$). However, the extent of this contribution is still under debate.[22, 31] Elimination of γ-butyrolactone and other tetrahydrofuran degradation products by additional washing during $n$$C_{60}$ synthesis procedures that involve tetrahydrofuran decreased the toxic effect to *D. magna* and A549 lung cells significantly.[31]

No articles have been found that use $n$$C_{60}$ made via tetrahydrofuran (without intentional removal of tetrahydrofuran side products) that claim it is nontoxic. This shows support for the idea that the

degradation products of tetrahydrofuran do cause additional toxic effects. However, some studies report that nC$_{60}$ is toxic where tetrahydrofuran was not involved during the synthesis procedure.[20, 37] This evidence suggests that there is some concern for adverse effects from nC$_{60}$ suspensions other than the effects of tetrahydrofuran degradation products. There are at least three other possible sources for this observed toxicity: oxidation caused by nC$_{60}$ itself, C$_{60}$ photo-oxidation, or un-intended changes to the structure of C$_{60}$ upon the formation of the nC$_{60}$ colloid such as derivatization to make C$_{60}$O (making the C$_{60}$O responsible for the observed oxidation).[40] The ultimate goal of the work herein was to aid the quantification of sources of oxidation by nC$_{60}$ suspensions that can contribute to their reported toxicity as well as develop new methods for the preparation of nC$_{60}$ samples that are ideal for toxicity studies.

1.2 Synthesis

Since the synthesis of the first nC$_{60}$ suspensions by Scrivens, four commonly used synthesis methods (TTA, THF, SON, and AQU) have been developed and studied in detail as described by Brant.[13] It has been shown that the preparation method used to synthesize nC$_{60}$ can have a significant impact on the physical and chemical properties of the suspensions.[11, 13, 41-44] Variations of these four primary synthesis methods have been utilized in order to eliminate certain concerns that have arisen in studies on the toxicity or characterization of this material.[20, 28, 45-47] Each of the four main methods is described in detail below and summarized in **Table 1.1** followed by a brief discussion on some of the variations on these methods.

Method	Summary	Advantages	Disadvantages	References
TTA	Successive solvent exchange from toluene to tetrahydrofuran to acetone to water	Narrow and controllable size distributions, moderate yields	Use of tetrahydrofuran, low yields, long synthesis time	12, 13, 18, 48
THF	Dissolve C_{60} in tetrahydrofuran, mix rapidly with water, and evaporate tetrahydrofuran	Moderate yields, moderate size control	Use of tetrahydrofuran, which can interfere with environmental studies	11, 13, 49, 50
SON	Ultrasonicate toluene solution of C_{60} with water until toluene has evaporated	High yields	Poor size control, reactive conditions, long synthesis time	13, 51
AQU	Spontaneous C_{60} colloid formation in water upon vigorous mixing.	Most environmentally-relevant method (no organic solvents)	Low yield, poor size control, long synthesis times, poor reproducibility	11, 13, 14, 40

Table 1.1: Summary of the significant literature *n*C_{60} synthesis methods.

TTA

TTA is an abbreviation referring to the solvents used in this synthesis method: toluene, tetrahydrofuran, and acetone. The procedure for the TTA method[13] was taken almost directly from Scrivens[18] except for the replacement of benzene with toluene. Powdered C_{60} is first dissolved in toluene, tetrahydrofuran is added, and then acetone is added, followed by the slow addition of purified water. The mixture is generally stirred for a period of time between additions and before the removal of the organic solvents. The organic solvents are removed by evaporation. In order to ensure the removal of residual organic solvents, the suspension is repeatedly concentrated by evaporation then diluted.

Involving a gradual transition from a "good" solvent (toluene) to a "bad" solvent (water), this method offers the most controlled *n*C_{60} particle growth conditions and results in the narrowest range of particle sizes of any reported method (see **Tables 1.1** and **3.3**).[13, 18] Espinasse reported the average hydrodynamic radius for TTA suspensions to be 100 ± 3 nm.[42] Brant reported particle sizes ranging from 100-200 nm based on TEM imaging (this compared to 100-200 nm for SON and 20-500 nm for AQU suspensions in the same study).[13] However, the concentration of C_{60} in the resulting suspensions tends to

be low relative to the THF and SON methods. A summary comparison of the four methods is provided in **Table 3.3**.

THF

This method developed by Deguchi[15] is most commonly studied due to its simplicity and reproducibility. Purified water is slowly added to a solution of C_{60} in tetrahydrofuran (hence the abbreviation THF) with equal volumes. The rate of addition determines the resulting size of the nC_{60} particles, the average particle diameter ranging from ~75-350 nm.[16] The tetrahydrofuran can be removed completely by rotary evaporation following the repeated concentration and dilution procedure mentioned above. Although the particle size can more easily be controlled using this approach, the resulting suspensions are more polydisperse than TTA suspensions. Additionally, this approach, as well as the TTA method, is controversial due to concerns that tetrahydrofuran or its decomposition products can interfere with toxicity studies.[22]

SON

Andrievsky proposed the SON method in 1995.[10] A solution of C_{60} in toluene is mixed with an equal amount of purified water and put in an ultrasonic bath for several hours until the evaporation of toluene is complete. Additional aliquots of water can be added and subsequently removed by sonication to eliminate residual toluene. This method produces extremely high colloid concentrations suitable as stock solutions for controlled biological or environmental impact studies without further concentration steps.[10, 13] However, ultrasonication is a highly uncontrolled process; it is known to produce temperatures and pressures significant enough to induce a wide range of chemical reactions. As a result, the SON method often produces suspensions with the widest distribution of particle sizes of any synthesis method described here. Xie reported an average hydrodynamic radius of 56 ± 8 nm,[47] whereas Brant reported particle sizes between 100-200 nm based on TEM imaging.[13]

AQU

The most environmentally relevant method, AQU, can be formed by simply mixing C_{60} powder in nanopure water from a few days (~7) to months.[17] This approach avoids any use of controversial solvents like tetrahydrofuran and provides no extra energy that can facilitate unwanted reactions. The disadvantages to this method include the result of low colloid concentrations (similar to TTA), longer preparation time, and absolutely no control over particle size or polydispersity.

Variations on Synthesis Methods

As with any new material, determining the environmental impact or cytotoxicity of $n$$C_{60}$ is of great importance. Studies have shown $n$$C_{60}$ suspensions to be harmful to some biological related systems.[29, 30] However, there is much controversy regarding the mechanisms underlying the toxicity of $n$$C_{60}$. While Sayes et al.[7] claim that pure, underivatized, C_{60} itself exhibits oxidative properties and thus cytotoxicity, others argue that the presence of residual tetrahydrofuran intercalated into the $n$$C_{60}$ crystal lattice can cause reactive oxygen species (ROS)-mediated cytotoxicity.[19] In order to mediate these two claims, Dhawan[20] and Markovic[28] conducted studies using an $n$$C_{60}$ suspension prepared similarly to the THF synthesis method only replacing tetrahydrofuran as the starting solvent with ethanol. The advantages of this alteration of the THF method are that ethanol is known to be non-genotoxic at the concentrations used,[20, 52] has been shown to not form solvates within $n$$C_{60}$ crystals,[53] and does not usually contain any peroxides that can produce ROS such as those commonly present in tetrahydrofuran.

Much of the effort in studying $n$$C_{60}$ includes determining the fate of C_{60} if/when it is released into the environment through waste disposal or other means. By adding natural organic matter (NOM) to a solution of C_{60} during the synthesis of $n$$C_{60}$, researchers can observe and characterize its effects on particle formation and thus the effects $n$$C_{60}$ can have on the environment.[42, 47] Other research efforts, however, focus on learning about the reactivity of C_{60} in aqueous environments. Fortner et al. described

the effects of ozonating an aqueous suspension of fullerenes using extreme levels of ozonation.[46] These results can be compared to the reactivity of C_{60} with ozone in non-aqueous solutions.[45]

Of the four main nC_{60} synthesis methods, only two have been found to produce controllable particle sizes: the THF[16] and TTA[48] methods. Unfortunately, those are the two methods that involve the use of tetrahydrofuran, a solvent whose degradation products have been implicated in significant overestimates of the oxidative damage resulting from exposure to nC_{60}.[19, 22, 34] Although additional washing steps helped to significantly reduce the toxic effects of suspensions made using tetrahydrofuran,[31] avoiding the use of controversial solvents altogether would be beneficial. Therefore, if the community is going to explore any potential dependence on particle-size for the health or environmental impacts of nC_{60}, a new synthesis method that can control the resulting particle size but which does not use tetrahydrofuran is needed. This method should also produce stock suspensions with concentrations high enough to provide numerous diluted samples for performing controlled studies from which the results can be easily compared.

The THF method's control over particle size requires careful attention to solvent addition rates,[16] a parameter not often specified in reports nor routinely monitored and controlled. In contrast, the size control that has been achieved by the TTA method requires modifying the relative volumes of the solvents involved.[48] The gradual transition in solvent from a "good" solvent (toluene) to a "poor" solvent (water) achieved by using intermediate solvents is conceptually appealing because it allows the best potential for separating the colloidal particle nucleation step from the particle growth step. Therefore, in the work presented here, the principles underlying the TTA method have been used to motivate the development of new techniques that do not require the use of tetrahydrofuran and thus are not subject to concerns of oxidant contamination.[19, 21-23, 29, 31, 34, 35]

A further problem with the TTA method, and its parent method that began with benzene instead of toluene,[18] is that the solvents chosen (toluene/benzene, tetrahydrofuran, and acetone) have relative vapor-pressures that are in the opposite order to their fullerene solubility. Therefore as the solvents are evaporatively removed, the "poor" solvents are removed before the "good" solvents, leading to a gradual increase in solubility during evaporation rather than the desired gradual decrease. Thus, near the end of the standard TTA synthesis method, the C_{60} remains in the toluene layer above the aqueous layer. The difference in solubilities of C_{60} in these two immiscible layers is such that at that point, when the last of the toluene evaporates, the majority of the C_{60} precipitates as particles too large to be suspended in the water and thus decreases the yield of the procedure significantly.

The work reported here represents new approaches for producing aqueous $n$$C_{60}$ colloidal suspensions that retain the attractive gradual solvent-quality transition that successive solvent exchange promises but also address the shortcomings of the TTA method. Two solvent series are examined where both fullerene solubility and solvent vapor pressure decrease successively to controllably induce colloid seeding and growth and also allow the removal of solvents in the same order in which they were added. The most successful of these methods, termed HIPA, involves a transfer of the fullerenes from hexane to isopropyl alcohol (IPA) then to water. The second of these methods, termed HEA, replaces the IPA with ethanol. These approaches improve the yield of $n$$C_{60}$, avoid the use of controversial solvents such as tetrahydrofuran, and shorten the synthesis time. It is likely that this method, upon further study, will be able to control the resulting particle size similar to the TTA method.[48]

1.3 Characterization

The behavior of particles in suspension is a direct function of their size, structure, and chemical characteristics.[54] Differences in size, structure, and surface chemistry of nC$_{60}$ produced by the various synthesis procedures could have important implications for the interpretation of data from environmental transport and toxicity studies.[13] While little work has been done to fully characterize nC$_{60}$ samples from all four of the primary synthesis methods in a way that the results can be easily compared, individuals have analyzed particle size and structure and the concentration of nC$_{60}$ colloids before using them in their research. Some of the characterization techniques often used include zeta potential, UV-vis absorption, gas chromatography (GC), high performance liquid chromatography (HPLC), static/dynamic light scattering (SLS/DLS), transmission electron microscopy (TEM), infrared spectroscopy, and oxidation studies.

Zeta potential is used to measure the charge per unit surface area and electrophoretic mobility (EPM) on colloidally suspended particles. The zeta potential provides information on the stability of the colloid with respect to aggregation. A value of 25-30 mV or greater (positive or negative) indicates that the colloid is sufficiently charged to allow repulsion between adjacent, similarly charged particles. nC$_{60}$ suspensions produced from the various synthesis methods yield similar zeta potential values at pH=7: SON = -31 mV, TTA = -30 mV, THF = -50 mV, and Aqua = -30 mV (after at least two weeks of stirring).[13] SON and THF suspensions were reported as having an EPM ranging from -2.5×10^{-8} to -3.5×10^{-8} m^2/Vs.[47]

GC can be used to detect levels of residual solvents left in the suspensions from the synthesis procedure and more commonly identify degradation products from these residual solvents (namely tetrahydrofuran). Consistent with the results from the work presented herein and other work in the Ausman lab at OSU,[48] literature reports of GC performed by liquid-liquid extraction of trace solvents

typically yielded detection limits of < 1 ppm[15, 55, 56] whereas GC using solid phase micro extraction (SPME) yielded detection limits of < 1 ppb.[32, 34, 46] Deguchi was the first known author to address the amount of residual solvent left in the *n*C_{60} sample used in his study in 2001 and found no detectable amount of tetrahydrofuran to a limit of < 1 ppm.[15] Deguchi had sparged the suspension with nitrogen gas to eliminate traces of tetrahydrofuran but had not attempted to monitor or remove any degradation products from the tetrahydrofuran. In 2007, Fortner suggested a new method for removing residual tetrahydrofuran via a step-wise solvent exchange process using a membrane stirred-cell with a molecular weight cut off (MWCO) of 10,000.[46] Fortner found no residual tetrahydrofuran to a limit of < 1 ppb but also made no attempts to monitor its degradation products. During that same year, Henry reported a study comparing a THF/*n*C_{60} sample as-produced with a sample that had been sparged with nitrogen gas for 2.5 days to remove traces of tetrahydrofuran and its degradation products.[22] Henry could detect tetrahydrofuran after rotary evaporation in the as-produced sample but could detect none in the sample that had been sparged with nitrogen. However, in both the as-produced and sparged samples only the tetrahydrofuran degradation products γ-butyrolactone and tetrahydro-2-furanol were detected with an elevated level of γ-butyrolactone in the *n*C_{60} compared to a blank sample (no C_{60}). The amount of γ-butyrolactone also increased after a 72 hour wait period for the as-produced sample. Henry concluded that any residual tetrahydrofuran could not explain any observed toxicity (indicated as fish mortality) but the presence of the degradation products (specifically γ-butyrolactone) contributed to toxicity found in the *n*C_{60} samples. Later on, several studies were done showing the presence and effects of residual solvents (mostly tetrahydrofuran and its degradation products) on the toxicity of *n*C_{60} samples.[31, 34, 55, 56] Residual solvent levels were typically below detectable limits of sub ppm or ppb or otherwise in the lower ppm levels.

HPLC is used to detect low levels of fullerene functionalization when applied to organic solvent extracts of *n*C_{60}. Scrivens reported that a second peak was present in the HPLC chromatogram shortly after the main C_{60} peak.[15, 18] He attributed this peak to $C_{60}O$, which can be formed from the oxidation of

the suspended particles after prolonged exposure to air. In 2001, Weisman validated Scrivens' conclusion by synthesizing and characterizing $C_{60}O$ showing it to be in two possible forms: either as the [6,6]-closed epoxide or the [5,6]-open oxidoannulene.[57] However, Deguchi (as well as others) reported that no derivatization was detected in the THF/$n$$C_{60}$ suspensions prepared during his study.[15]

Infrared spectroscopy can determine the nature of any fullerene derivatives that may exist on the colloid surface.[46, 58] Based on FTIR reflectance, Andrievsky reported that suspensions produced by the SON method consisted of pristine, singly hydrated fullerene particles.[58] Using ATR-FTIR, Fortner observed hydroxyl groups on $n$$C_{60}$ particles that had reacted with ozone.[46] Fortner also described the presence of other oxygen moieties likely in the form of hemiketal arrangements. However, clear IR spectra can be difficult to obtain and interpret, especially when there may be water or intercalated solvents present in the samples and when the expected signals do not give strong absorptions.

UV-vis absorbance can be used to determine concentration and hydrophilicity/extraction efficiency.[13, 15, 18, 20, 41, 44, 47, 58] Scrivens first reported the difficulty of liquid-liquid extraction of colloidal C_{60} into toluene.[18] His solution was to filter out the fullerene material, dissolve it in toluene, and analyze it by UV-vis absorption spectroscopy. However, this approach is not entirely quantitative due to the unknown filtration removal efficiency and the imprecisions inherent in the solid transfer of the retentate. Andrievsky[58] and Sayes[7] independently attempted to determine the concentration of the colloids directly in water by UV-vis spectroscopy. Although $n$$C_{60}$ demonstrates a linear absorption,[41, 47, 58] colloidal fullerene aggregates are not well-suited to this approach because their particle sizes are often such that scattering will increase the light extinction over-and-above absorption. Furthermore, the suspension of the colloids often occurs with appreciable derivatization of the constituent fullerenes[40] resulting in potential changes to the absorption spectrum. Finally, the size distribution, nature, and extent of the derivatization are highly dependent on the colloidal synthesis method employed[13] so simple corrections to account for these problems are difficult to employ. While some groups have performed extractions of $n$$C_{60}$ into toluene via an oxidation reaction with potassium perchlorate,[44, 47] this process can also result in C_{60}

derivatization. Due to these concerns, we have adapted the extraction technique used by Deguchi[15] that allows the complete extraction of the fullerenes from their aqueous layer by salting out into a toluene layer. The concentration is then determined by measuring the absorbance of C_{60} in the organic layer and fitting the measured spectrum to a calibrated reference spectrum obtained from a standard solution of C_{60} in toluene according to Beer's law.

SLS/DLS are often used to measure the nC_{60} particle size, polydispersity, internal structure, and interparticle interactions.[13, 15, 20, 28, 41, 44, 46, 47] Some results of this work have been discussed in **Sections 1.2** and **3.3**. TEM is also used to measure the particle size, structure, and polydispersity[13, 15, 17, 20, 44, 46, 47, 58, 59] as well as to validate SLS/DLS measurements. From the TEM images, many reports have been made that nC_{60} consists of larger particles made up of aggregated smaller particles; these smaller particles have been shown to have a crystalline structure.[13, 16, 20, 41, 44, 47]

Some studies have indicated that nC_{60} shows oxidative behavior that may induce metabolic stress in living systems.[7, 30] nC_{60} has also been shown to cause cell death in some biological systems[7, 29, 30, 60] or to halt bacterial growth.[16] Part of the oxidative behavior can be attributed to the synthetic method employed, derivatization of the fullerene cage during synthesis, residual solvents in the nC_{60} suspensions, or peroxides present in utilized organic solvents such as tetrahydrofuran that can produce ROS. Oxidation studies have been performed to observe the production of ROS for cytotoxicity studies.[25, 28, 61] This characterization technique most specifically pertains to TTA and THF suspensions due to the use of tetrahydrofuran during the synthesis procedure. Other than monitoring ROS production, oxidation studies can also help in the quantification of other sources of oxidation by nC_{60} mentioned earlier such as C_{60} derivatization and photo-oxidation.

The physicochemical properties and ultimate fate of nC_{60}, and essentially our understanding of the fate of C_{60} itself, can vary significantly with the preparation method employed. For this reason, care must be taken when reporting and interpreting toxicology results. Thorough descriptions of the preparation procedures and a complete spectrum of characterization of the material used should be

reported. The current work in part has focused on developing and improving a method for the characterization of residual solvents by SPME-GC in nC$_{60}$ suspensions.

1.4 Stability

Andrievsky gave evidence that SON nC$_{60}$ suspensions exhibit high stability with no evident changes after having been stored in the absence of light and at low temperature for three months.[10] The suspensions were stable in the pH range 1-10, were unaffected by boiling, and were not easily extracted back into toluene.[10] Avdeev also reported SON suspensions as being stable for 3 months.[62] According to Brant, nC$_{60}$ suspensions (from all four methods) remain stable for several months at low ionic strengths and that an increase in ionic strength caused an increase in aggregation.[11, 12] Deguchi reported his THF suspensions as being stable for up to 9 months.[15] While many researchers such as these report how long nC$_{60}$ suspensions are stable and are stable under certain conditions, only speculations have been made regarding what causes the formation and stability of nC$_{60}$ colloids based on limited evidence.

Early reports discussed the presence of a charged surface on nC$_{60}$ but none could explain its source. This surface charge was attributed to either the formation of clathrate structures of nC$_{60}$ and water or hydration shells surrounding the nC$_{60}$ particles, both of which are explained by donor-acceptor and charge transfer interactions.[10, 15, 16, 58, 62-64] More recent studies have shown some evidence that the negative surface charge is due to hydroxyl groups either attached or adsorbed to the surface of the nC$_{60}$ particles.[65, 66]

However, Murdianti[67] demonstrated that the broad IR band near 1100 cm^{-1} described by Labille[66] as C-O stretching due to the presence of hydroxyl groups is more likely due to the C$_{60}$O epoxide. As pointed out by Murdianti, the small peak in the IR spectrum at 750-850 cm^{-1} shown (but unexplained) in

Labille's work is a characteristic transition of epoxides (symmetric and asymmetric stretches). Additional experiments by Murdianti using IR, UV-vis, HPLC, and synthesis of nC$_{60}$ using the AQU method under various conditions provide a strong argument supporting the model that the formation of the C$_{60}$O epoxide on the surface of nC$_{60}$ particles is responsible for the stabilization of AQU/nC$_{60}$ samples.[40, 67] The formation of the epoxide may also be responsible for the stabilization of nC$_{60}$ prepared by other methods such as the solvent exchange methods discussed above. However, HIPA/nC$_{60}$ samples often show no detectable amounts of C$_{60}$O and yet are still stable for several months. Therefore, there must be other sources of stabilization, one possibility being residual solvent molecules that associate with the C$_{60}$.[68]

Little is known of the reactivity of C$_{60}$ in water (nC$_{60}$ - after colloid formation); although, Fortner has described the reaction of nC$_{60}$ with ozone.[46] Fortner compared the results with similar reaction mechanisms proposed for organic phase reactions and described the formation of hemiketal arrangements on the nC$_{60}$ aggregates. According to Fortner, the aqueous-phase reaction occurs via the formation of a closed epoxide from the dissociation of a primary ozonide and loss of O$_2$; further hydrolysis results in the formation of the hemiketal functionalities.

1.5 Conclusions

Traditionally, the guidelines for the handling and disposal of nanomaterials are based on the properties of their bulk counterparts. Fullerenes have become a touchstone material for study comparing their chemicophysical properties to that of carbon black (its bulk counterpart). [C$_{60}$]fullerenes are hydrophobic molecules that are capable of forming stable colloidal aggregates in water through the formation of nC$_{60}$ particles, and it has been shown that the properties of these samples are dependent upon the synthesis method employed.[13] For these reasons, it is clear that full characterization of nC$_{60}$ samples

will not only help us understand and interpret the results from toxicity and environmental impact studies but will also be helpful in providing appropriate guidelines for the handling and disposal of [C_{60}]fullerenes and provide a framework for other nanomaterials.

There has been much speculation about what causes the stability of $n$$C_{60}$ aggregates in water. Many researchers have supported the model that the $n$$C_{60}$ particles are surrounded by a shell of hydration or arranged in a clathrate-like structure with water. However, it has recently been shown that the formation of $C_{60}O$ on the surface of C_{60} aggregates or clumps in water provides stable hydrophilic particles in AQU/$n$$C_{60}$ samples.[40, 67] $C_{60}O$ may also help to stabilize $n$$C_{60}$ samples produced by solvent exchange methods, but it is not always present in detectable amounts[15, 68] suggesting the role of other stabilizing factors such as residual solvent molecules.

Five primary methods for the synthesis of $n$$C_{60}$ are discussed in this work: four of these are found in the literature (TTA, THF, SON, and AQU) whereas the fifth (HIPA/HEA) is new to the literature. An ideal synthesis method would produce $n$$C_{60}$ suspensions with high yields; a high degree of particle size control with narrow size distributions; require a short synthesis time; avoid the use of harmful solvents that may interfere with toxicity, oxidation, and environmental impact studies; and be easily reproducible. While the SON method produces suspensions with high yields and the AQU method avoids the use of any organic solvents, the AQU method yields low concentrations of $n$$C_{60}$. Both methods provide little control over particle size, generally giving wide size distributions, and involve lengthy synthesis procedures (SON ~20+ hours and AQU ~7 days to months). Additionally, high energy sonication can induce unwanted side reactions. The TTA and THF methods have both been shown to control particle size with narrow and moderate size distributions, respectfully. However, the TTA method typically yields very low $n$$C_{60}$ concentrations and both methods involve the controversial solvent tetrahydrofuran that has been shown to confound toxicity studies.[19, 22, 34]

New approaches for producing aqueous $n$$C_{60}$ colloidal suspensions are presented that retain the attractive gradual solvent-quality transition that successive solvent exchange promises but that address the

shortcomings of the TTA method. Two solvent series are examined (HIPA and HEA) where both fullerene solubility and solvent vapor pressure decrease successively to controllably induce colloid seeding and growth while removing the solvents in the same order in which they were added. This approach improves the yield of nC$_{60}$, avoids the use of controversial solvents such as tetrahydrofuran, and shortens the synthesis time. It is likely that this method, upon further study, will be able to control the resulting particle size similar to the TTA and THF methods.[16, 48]

The ultimate goal of the work described here is to develop further methods for fully characterizing nC$_{60}$ suspensions, aid in the quantification of sources of oxidation by nC$_{60}$ suspensions that can contribute to their reported toxicity, as well as develop new methods for the preparation of nC$_{60}$ samples that are ideal for toxicity and environmental impact studies. Additionally, this work has focused on developing and improving methods for the characterization of residual solvents by SPME-GC. The HIPA/HEA method for synthesizing nC$_{60}$ samples has been developed to address concerns of other synthesis methods prominent in the literature. HIPA/nC$_{60}$ suspensions often produce particles with no detectable amounts of C$_{60}$O or other derivatives making them suitable for oxidation studies to help quantify any oxidation due to the nC$_{60}$ aggregate itself.

CHAPTER II

EXPERIMENTAL PROCEDURES

2.1 nC_{60} Sample Preparation

For HIPA and HEA samples, a stock solution was prepared by adding 3.4 - 3.6 mg powdered C_{60} (MER Corp., Tucson, AZ, MR6HP 99.9%) to 125 mL hexane isomers (SIGMA-ALDRICH, ≥ 99%) and stirring 1-2 hours. This solution was filtered through a Whatman® glass microfiber filter (934-AH, 42.5 mm Ø, 1.5 μm pore size) before use. The HEA and HIPA nC_{60} suspensions were prepared by adding 50 mL (at 1 L/min) of either ethyl alcohol (AAPER, ethyl alcohol USP, absolute 200 proof) or isopropyl alcohol (IPA) (PHARMCO-AAPER, HRGC/HPLC-trace grade) respectively to 50 mL of rapidly stirring C_{60} stock solution and mechanically mixing for 30 min. Approximately 50 mL of solvent (mostly hexanes) were removed by rotary evaporation (using a Heidolph® HB control rotavap) with an applied pressure of 150–200 mbar and temperature of 30 ^{o}C for approximately 20 minutes. To ensure the removal of all the hexanes, 20 mL of the alcohol were added to the reaction flask followed by the removal of an additional 10-20 mL of solvent achieved by reducing the pressure to 100 mbar. The resulting suspension was filtered through the Whatman® filter (after rinsing the filter with the alcohol).

Fifty milliliters of nanopure water (Millipore Direct-Q3 UV system, 18 MΩ.cm @ 25 ºC) were immediately added to the rapidly stirring suspension at 1 L/min. After mixing for ~1 min, the suspension was filtered again through the Whatman® filter. Approximately 50-60 mL of solvent (mostly alcohol) were removed by rotary evaporation over approximately a 30 minute time period by reducing the pressure to 65–50 mbar. To ensure complete removal of the alcohol, 20 mL of nanopure water were added followed by the removal of 20 mL of solvent. The removal of solvent, which was mostly water at this point, was achieved by decreasing the pressure to 45 mbar and increasing the temperature to 35–40 °C. This process was repeated twice. The suspension was concentrated to ~15 mL then filtered through a 0.45 μm MCE sterile filter (Fisherbrand).

For the synthesis of comparison *n*C_{60} samples, saturated solutions of C_{60} in ethyl alcohol and IPA were prepared by adding 3.5-4.8 mg C_{60} to 125 mL of the appropriate alcohol. These mixtures were stirred overnight then filtered with a new Whatman® glass filter (same as above). Fifty milliliters of nanopure water were added to 50 mL of the rapidly stirring C_{60}/alcohol solutions (separately). The alcohols were removed in the same fashion as described above, and the resulting suspensions were filtered through an MCE filter.

2.2 Concentration Determination

In our procedure modified from Deguchi *et al.*,[15] 1 mL of the *n*C_{60} suspension, 2 mL 10% (w/v) $NaNO_3$ (prepared with nanopure water), and 3 mL toluene (PHARMCO-AAPER, HRGC/HPLC-trace grade) were combined using a 1000 μL micropipette. The vial was sealed with a Teflon lined cap and suspended in a sonication bath (Fisher Scientific FS140H) for 10 minutes. The resulting mixture was set aside to rest for at least 2 hours or overnight in order to allow the layers to separate completely. The

aqueous and organic layers were analyzed separately by UV-visible spectroscopy (Varian Cary 5000 UV/vis/NIR) at λ = 200-800 nm using nanopure water, 1 mL nanopure water mixed with 2 mL 10% $NaNO_3$, or toluene for background subtractions as appropriate. Quartz, standard rectangular, 10 mm Starna cells (catalog # 1-Q-10) were used for the analysis. The concentration of C_{60} in the toluene layer was determined by fitting the measured spectrum to a calibrated reference spectrum obtained from a standard solution of C_{60} in toluene according to Beer's law.

Care was taken to avoid introducing experimental and instrumental errors into the absorption data. First, a single cuvette was used to take all absorption measurements and oriented in the same direction inside the instrument for each measurement (after cleaning the exterior with methanol and wiping with lens paper). The solution background was corrected by taking an absorption spectrum of toluene or nanopure water/$NaNO_3$ as appropriate (with the baseline correction) and manually (excel) subtracting this spectrum from the sample spectrum.

With the given extraction procedure, all cases showed that the aqueous layer's absorption spectrum matched that of 6.7% (w/v) $NaNO_3$ (1 mL nanopure water mixed with 2 mL 10% $NaNO_3$) (also taking into account that this solution contains a very small amount of toluene). No measurable absorbance at wavelengths characteristic for C_{60} (*e.g.*, λ = 347 nm) were detectable, thereby demonstrating complete fullerene extraction into toluene. The toluene layer's absorption spectrum matched that of standard solutions of C_{60} in toluene demonstrating that the synthesis and extraction procedures resulted in a toluene solution of primarily underivatized fullerenes. This was further confirmed by high-performance liquid chromatography (HPLC) analysis (**section 2.2**).

Studies are currently underway to validate the concentration determination calculations via UV-vis and HPLC. HPLC concentrations are based on a standard calibration curve that is consistent with UV-vis absorption. We are considering extraction by stirring (discussed in **section 2.2**) and analysis of C_{60} and $C_{60}O$ derivative peaks (and others if present) separately to gain a more accurate determination of the C_{60} content.

2.3 Derivative Analysis

Extraction of C_{60} and its derivatives from *n*C_{60} for HPLC analysis was performed by adding 2 mL of 10% (W/V) $NaNO_3$ and 1.5 mL toluene (HPLC Grade) to 6 mL of *n*C_{60} using a 1000 µL micropipette (the amount of *n*C_{60} can vary depending on its concentration). The mixture was stirred overnight and the toluene and aqueous layers were separated. The aqueous layer from the extraction was analyzed using UV-Vis spectrophotometer Varian Cary 5000 (or Cary 100) to verify that all C_{60} and its derivatives were extracted into toluene. The toluene layer was dried using anhydrous Na_2SO_4 and filtered through a 0.02 µm Whatman® Anotop™ filter (Fisher Scientific, Suwanee, GA).

The filtered toluene extracts were analyzed on an HPLC system from Varian consisting of a 210 ProStar Solvent Delivery Module, a Rheodyne® 7725i injection valve with an injection volume of 20 µL and a 355 ProStar Photo Diode Array detector with deuterium (UV) and quartz iodide (visible) lamp source, all operated through Galaxie™ Chromatography Workstation. UV spectra were collected at all retention times from 300 to 450 nm. The chromatograms presented in this work were recorded at 336 nm (unless otherwise stated), where the C_{60} spectrum has a local maximum. The column used was a Nacalai Tesque "Cosmosil™ Buckyprep", Waters type, 4.6 x 250 mm packed column protected by a NacalaiTesque "Cosmosil™ Buckyprep," Waters type, 4.6 x 10 mm guard column. HPLC Grade toluene was used as the mobile phase with a flow rate of 1 mL/min.

Since sonication conditions are harsh enough to induce chemical reactions in a number of systems[69] and is specifically known to cause reactions in fullerene systems,[10, 70, 71] we examined by HPLC *n*C_{60} extracted into toluene under both the sonication conditions described in **section 2.1** and under milder extended stirring conditions described above in this **section 2.2**. In both cases the primary derivative observed was the [6,6]-closed epoxide isomer of $C_{60}O$[40, 57] (see **section 3.3** for details). However, we found higher levels of derivatization in the stirred samples than we did in the sonicated samples

suggesting that the sonication-induced chemistry primarily resulted in a loss of oxygen from the fullerene oxide. We are continuing to investigate the details of this process, but for the purposes of the work in this section we concluded that extraction under stirring conditions left the fullerene derivatives more intact than did the alternative, and thus the stirred samples were more appropriate for HPLC analysis of fullerene derivatives. On the contrary, the conversion from the [6,6]-closed epoxide isomer of $C_{60}O$ to underivatized C_{60} facilitates the concentration determination, and thus extraction under sonication conditions was chosen as appropriate for concentration determination.

Some experimental concerns for running samples on the HPLC include avoiding cross contamination and making quantitative measurements. Toluene is used for the first injection each day to ensure there is no residual C_{60} in the syringe, injection port, or on the column. The injection port is left in the "inject" position for several seconds before returning it to the "load" position so that everything is flushed out of the injection loop. The syringe is rinsed many times with the sample before each injection and with toluene immediately afterwards. Also, the syringe is used to flush the injection port several times with toluene (in the "load" position) to ensure that the last of the previous sample has been removed. When loading a sample into the injection port, ~50 μL of sample is pushed through a 20 μL injection loop in order to flush out all the toluene in the loop. This step is particularly beneficial when determining C_{60} concentration by HPLC as an exact injection volume (without any dilution effects) is necessary to ensure quantitatively accurate results.

2.4 Trace Solvent Analysis

Previous studies have demonstrated that residual solvents within some *n*C_{60} samples can contribute to reported chemical, biological, and environmental behavior.[13, 16, 19, 20, 35, 44, 72] The samples in

the present work were analyzed for residual organic solvents using solid-phase microextraction (SPME) gas chromatography (GC). A divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco® #57348-U) was used for sampling followed either by gas chromatography (GC) analysis on a Hewlett Packard® (HP®) 5890 Series II GC system with an FID (fiber/solvent matching and reproducibility trials) or by GC-mass spectroscopy (GC/MS) on a Shimadzu 2010 with a QP2010S mass spectrometer (calibration curves and sample analysis).

For analysis on the HP® GC, a Restek® Corp. column was used containing an RTX-50 stationary phase with a length of 15 m, an inner diameter of 0.32 mm, and a DF (thickness of the coating) of 0.25 µm (cat # 10521). The injection temperature was set to 150 °C and the detection temperature set to 270 °C. The initial oven temperature was set to 40 °C with an oven equilibration time of 30 seconds. Thirty seconds after injection, the oven temperature was set to increase at a rate of 20.0 °C per minute until it reached 150 °C then held for three minutes. The total method/analysis time was 9.00 minutes. For all analyses on the HP® GC, the gas pressures from the tanks were 34 psi for helium (carrier gas), 50 psi for air (FID operation), and 30 psi for hydrogen (FID operation). The gas pressures through the instrument were set to 160 kPa (23.2 psi) for helium, 190 kPa (27.6 psi) for air, and 200 kPa (29.0 psi) for hydrogen.

For analysis on the Shimadzu GC/MS, a SHR5XLB Shimadzu column (cat # 220-94536-01) was used with a length of 30 m, an inner diameter of 0.25 mm, and a DF (thickness of the coating) of 0.25 µm (cat # 220-94536-01). The injection temperature was set to 250 °C. The initial oven temperature was set to 40 °C with a hold time of 1.0 minute. After one minute the oven temperature was set to increase at a rate of 20 °C per minute until it reached 150 °C then held for 3.00 minutes. The total method/analysis time was 9.50 minutes. The gas pressure from the helium tank (carrier gas) was 100 psi, the pressure through the instrument was set to 49.5 kPa (7.2 psi). The column flow was set to 1.00 mL/min with a total flow of 50.0 mL/min. The linear velocity was set to 36.1 cm/sec, the purge flow to 3.0 mL/min, and the split ratio to -1.0. The ion source and interface temperatures were both set to 260 °C. The solvent cut time

was set to 0.5 min. The detector voltage was set to "relative to tuning result" and the "set" value to 0 kV with a threshold of 1000.

All glassware used for gas chromatography analysis was cleaned and rinsed well with acetone at least one day before experimental data was taken and set out to dry overnight. All vials, magnetic stir bars, volumetric flasks, and stoppers for volumetric flasks were stored in the oven at ~90-100 °C overnight (or until the experiment was performed). The day the experiment was performed, the caps to the vials were placed in the oven for a few minutes. The vials and volumetric flasks were capped immediately upon removal from the oven to avoid the adsorption of any volatile chemicals in the air (notably acetone and toluene) on the interior walls of the containers as they cooled. For the HP® GC, the septum was changed daily before use, and the inlet liner was inspected for visible impurities such as pieces of the septa (the injection needle is blunt making the septum prone to leaks and degradation). The SPME fiber was conditioned in the injection port of a GC instrument according to the specifications of the manufacturer (for the gray fiber – DVB/CAR/PDMS, 270 °C for one hour). This thermally cleans the fiber before use by removing any un-desorbed analytes or other impurities which can cause extraneous peaks in the chromatogram. After conditioning, the fiber was allowed to cool inside a hood for at least ten minutes before exposing it to the first sample.

For constructing the calibration curves, the standard solutions were prepared while the fiber was conditioning. Micropipettes and volumetric flasks were used to make measurements and solutions. Care was taken when making solutions to give accurate concentrations. For example, while adding the solvents, virtually no liquid was allowed to accumulate on the walls of the volumetric flasks above the mark designating the specified volume of the flask. Where practical, the accuracy of the measurements was maximized by choosing measurement volumes that were around the middle or towards the higher end of the range specified on the micropipette. Each solution was well mixed and, especially in the case of hexanes, vortexed to give the most homogenous solution possible. Further, each solution was shaken well immediately before each aliquot was measured out; this should be particularly beneficial for hexanes

since they are practically insoluble in water. Solutions for the calibration curves were made ranging from one part per quadrillion (ppq) to fifty parts per million (ppm). Ranges for IPA and hexanes were slightly different based on their compatibility with the fiber (detection capabilities), baseline noise surrounding the chromatographic peak, and the amount of each solvent seen in HIPA samples.

HIPA samples and the standard solutions for the calibration curves were added in 2 mL aliquots to clean, cool 4 mL vials fitted with PTFE/silicone septum lined caps along with small cylindrical stir bars. A 2 mL volume was chosen in order to fully expose the fiber to a minimum volume of headspace without touching the liquid solution with the fiber. Touching the solution with the fiber would cause the injection of large amounts of water (the solvent) into the GC that could, in turn, cause a lot of background interference and could also destroy the column. Headspace sampling greatly improves sensitivity to target analytes both for the above reason and due to varying vapor pressures between the solvent and the different analytes.

Before exposing the fiber to the sample, the sample was agitated to release any air bubbles trapped around the stir bar, which can trap any volatile solvents that may be in the air, then the headspace of the vial was purged for 45 seconds with ultra high purity nitrogen gas (samples used for the fiber/solvent matching experiment were not purged with nitrogen). The sample was then allowed to stir for at least 5 minutes to allow equilibration between the solution and the headspace. The fiber was exposed to the headspace of the vial for 30 minutes while the solution was stirring then immediately injected into the GC/MS or (GC). The fiber was held in the injection port of the GC/MS for 30 seconds (at which time a burst of helium would clean out the injection port) then allowed to cool for at least 5 minutes before introducing it to the next sample. For the HP® GC (fiber/solvent matching), the fiber was kept in the injection port for ~5 min then removed and allowed to cool for at least 5 minutes.

2.5 Particle Size and Morphology Analysis

Particle sizes were determined by Dynamic Light Scattering (DLS) analysis, performed on a Malvern High Performance Particle Sizer, model HPP5001 using either a DTS0112 low volume disposable sizing cuvette or a DTS0012 disposable sizing cuvette. The manual measurement option was used when setting the instrument parameters each time a measurement was performed. The "type of measurement" was set to "size". The "material" was set to C_{60} with a refractive index = 2.200, absorption = 0.001, and "sample viscosity" set to "use dispersant viscosity as sample viscosity". The "dispersant" was set to "water" at 25.0 °C temperature, viscosity = 0.8872 cP, and refractive index = 1.330. The "temperature" was set to 25.0 °C and the "equilibration time" set to 4 minutes. Under the "result calculation settings" tab, the "multiple narrow modes" (as opposed to "general purpose") option was chosen and so was the "automatic" (# runs, run duration) setting for "measurement duration". The "multiple narrow modes" setting is appropriate for samples where each peak in the distribution is known to be narrow as is the case in the present work. Also under the "result calculation settings" tab under "advanced settings", the size range was set to "lower" equal to 0.100 d.nm and "upper" equal to 1.00e4 d.nm with the thresholds "lower" equal to 0.01 and "upper" equal to 0.01. Export options were set to export the "size distribution (intensity)" and the correlation report was exported separately after the measurement (only one type of data can be exported at a time).

Transmission Electron Microscopy (TEM) imaging was performed on a JEOL JEM-2100 Scanning TEM System using copper grids with ultra thin Carbon (< 3 nm) on carbon holey support film (product # 01824 from Tedpella). One drop of the sample was put on the dull side of the grid and allowed to dry while covered to prevent dust from settling on it. If the sample was very dilute, a second drop was added after the first dried completely. TEM was used to validate the accuracy of the DLS measurements of *n*C_{60} suspensions for particle size and polydispersity. TEM is also useful to see the general shape of the

particles and whether or not they are individual particles or aggregates of particles. However, it should be kept in mind that TEM images of dispersed particles dried onto a grid are often subject to clumping that occurs during the dry-down process. Thus, a common question in interpreting such TEM data is whether or not the particles shown are truly representative of the particles as seen in liquid suspension.

CHAPTER III

SYNTHESIZING AQUEOUS FULLERENE COLLOIDAL SUSPENSIONS BY NEW SOLVENT-EXCHANGE METHODS

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3.1 Introduction

There was a fundamental need to establish a method for producing nC$_{60}$ that will not interfere with toxicity and environmental studies as well as easily generate reproducible, concentrated colloids with the ability to control particle size. The recently published method presented here (the HIPA method) combines many advantages of other methods established in the literature (high yield, rapid and simple synthesis, narrow size distribution, lack of questionable solvents such as tetrahydrofuran, and the potential for controllable average particle size) while addressing some of the shortcomings of the other methods.

3.2 Method Development

The development of the HIPA and HEA methods for the synthesis of *n*C_{60} colloids was modeled from the TTA method. The gradual transition in solvent from a "good" solvent (toluene) to a "poor" solvent (water) that is allowed by using intermediate solvents is conceptually appealing because it allows the best potential for separating the colloidal particle nucleation step from the particle growth step. This approach results in narrow particle size distributions in the resulting colloidal suspensions and conceptually lends to the ability of particle size control. We therefore have used the principles underlying the TTA method to motivate our development of new techniques that do not require the use of tetrahydrofuran and thus are not subject to concerns over oxidant contamination.[19, 21-23, 29, 31, 34, 35]

A significant problem with the TTA method, and its parent method that began with benzene instead of toluene,[18] is that the solvents chosen (toluene/benzene, tetrahydrofuran, and acetone) have relative vapor pressures that are in the opposite order to their fullerene solubility as can be seen in **Table 3.1**. Therefore as the solvents are evaporatively removed, the "poor" solvents are removed before the "good" solvents leading to a gradual increase in solubility during evaporation rather than the desired gradual decrease. Thus, near the end of the standard TTA synthesis method, the C_{60} remains in the toluene layer above the aqueous layer. The difference in solubilities of C_{60} in these two immiscible layers is such that at the point when the last of the toluene evaporates, the majority of the C_{60} precipitates as particles too large to be suspended in the water thus decreasing the yield of the procedure significantly. In order to develop an improved method similar to the TTA method (gradually decreased C_{60} solubility that leads to narrow particle size distributions and the potential for particle size control), we need to find a series of solvents that decrease in C_{60} solubility and also decrease in vapor pressure to allow the solvents to be removed in the same order in which they are added while avoiding the use of tetrahydrofuran.

Solvent	C_{60} Solubility (10^3x Molarity)	BP (°C)	VP (mmHg @ 20 °C)
Benzene	1.22-2.58	80.0	74.6
Toluene	1.27-1.70	110.0-111.0	21.8
THF	0.05-0.8	65.0-67.0	143.0
Acetone	0.001	56.0	184.0
Water	1.6×10^{-10}	100.0	17.3

Table 3.1: C_{60} solubilities, boiling points, and vapor pressures for the solvents used in the TTA/*n*C_{60} synthesis method. C_{60} solubility values came from the book *Fullerenes* by Karl M. Kadish and Rodney S. Ruoff[73] and the boiling points and vapor pressures came from material safety data sheets (MSDS) from Sigma-Aldrich except water which came from the MSDS from sciencelab.com.

During the development of the HIPA/HEA methods, the first step was to compile a list of solvents in decreasing C_{60} solubility with their accompanying boiling point and vapor pressure values. A condensed version of this list is shown in **Table 3.2** to illustrate. The next step was to sort through this list and come up with a list of solvents that meet the above criteria of decreasing C_{60} solubility and decreasing vapor pressure (highlighted solvents in **Table 3.2**). The resulting list of solvents was somewhat limited including some halogenated hydrocarbons, hydrocarbons, and alcohols. The halogenated hydrocarbons were disregarded due to their known carcinogenicity. From the solvents that remained in the shortened list and the solvents on hand, n-hexane (or hexanes), isopropyl alcohol (IPA), ethyl alcohol (EA), and water seemed to be likely candidates for this new method.

Initial attempts for synthesizing *n*C_{60} using this new series of solvents involved imitating the TTA method by adding C_{60} to hexanes, filtering, adding IPA, adding water, then stirring for a few hours or overnight followed by rotary evaporation to remove the organic solvents. These attempts failed in that all of the C_{60} precipitated out of solution similar to what is seen during the evaporation of TTA samples.

Additionally, in the presence of hexanes most or all of the C_{60} would sometimes precipitate out upon the addition of water to the mixture even before the rotary evaporation step. Through trial and error, it was found that removing the hexanes from the C_{60}/hexanes/IPA mixture before adding water was necessary to produce *n*C_{60} samples with good concentrations. It is vital that all of the hexanes be removed before the addition of water which is achieved by a second addition of alcohol and further removal of solvent at lower pressures for the reasons given above.

Early during the method development process, it was noticed that if the stock solution consisting of C_{60} powder in hexanes was stirred too long (overnight or longer) it would turn yellow and the C_{60} that was initially dissolved had precipitate out again. Evaporation of the hexanes could be one reason for the precipitation of C_{60}, but this doesn't explain the yellow coloration of the solution. It was later found that hexanes react with the black rubber stopper that was used to seal the flask. The C_{60} solution does not change overnight when the flask is sealed with a silicon rubber septum. At the time the work presented here was performed the cause of this phenomena was unknown and thus it was decided that the stock solution should be prepared the same day the HIPA/HEA *n*C_{60} samples were to be made.

Solvent	C_{60} Solubility (10^3 x Molarity)	BP (°C)	VP (mmHg @ 20°C)
Dichloromethane	0.32-0.36	39.8-40.0	353.2
Chloroform	0.22-0.71	60.5-61.5	160.0
Heptane	0.067-0.42	98.4-99.0	40.0
Octane	0.028-0.42	125.0-127.0	11.0
n-Hexane	0.051-0.072	69.0	132.0
o-Cresol	0.02	191.0	0.3
1-Propanol	0.0057	97.0	14.5
Acrylonitrile	0.0055	77.0	86.0
2-Butanol	0.005	98.0	11.5
N-Pentane	0.004-0.01	35.0-36.0	434.3
Cyclopentane	0.003	50.0	275.0
Nitroethane	0.003	114.0-115.0	15.6
Isopropyl Alcohol	0.0029	81.0-83.0	32.4
Ethyl Alcohol	0.0011-0.0014	78.3	44.6
Methanol	0.000046	64.7	97.7
Water	1.6×10^{-10}	100.0	17.3

Table 3.2: C_{60} solubilities, boiling points, and vapor pressures for several of the solvents considered for the development of the HIPA/HEA/*n*C_{60} synthesis methods. C_{60} solubility values came from the book *Fullerenes* by Karl M. Kadish and Rodney S. Ruoff[73] and the boiling points and vapor pressures came from material safety data sheets (MSDS) from Sigma-Aldrich except water which came from the MSDS from sciencelab.com.

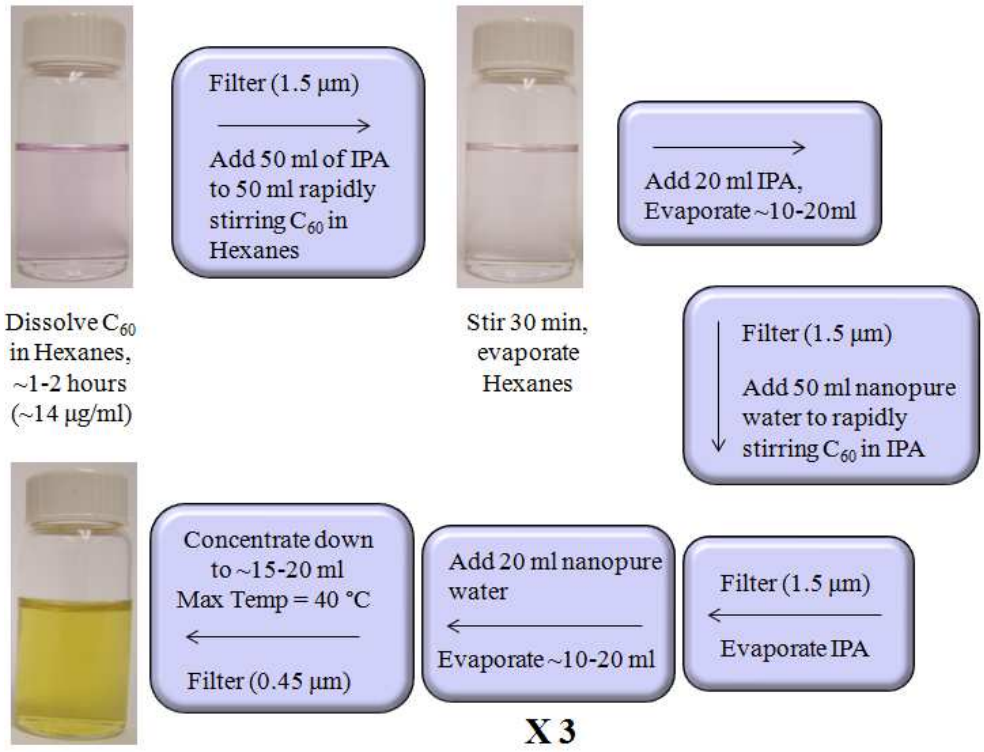

Figure 3.1: Schematic of HIPA nC$_{60}$ synthesis.

Figure 3.1 outlines the final synthesis procedure for HIPA/nC$_{60}$ samples which is also described in detail in **section 2.1** and takes ~4-5 hours from start to finish. HEA samples are made in the same way but with IPA replaced by ethyl alcohol. In short, C_{60} is dissolved in hexanes, IPA is added to this solution while it is stirring, the hexanes are removed by rotary evaporation, nanopure water is added, then IPA or ethyl alcohol is evaporated. The filtration steps indicated in **Figure 3.1** are included in order to remove any larger precipitates of C_{60} to avoid the rest of it from accumulating on them and crashing out of suspension. The maximum temperature indicated towards the end of the schematic in **Figure 3.1** is notable because it was thought that at temperatures above ~50 °C caused the C_{60} to crash out of solution. This concept will be discussed briefly in **section 5.2.2**.

3.3 nC_{60} Synthesis and Characterization

Four solvent-exchange synthesis methods for nC_{60} were examined in this study:

- **HIPA**: hexane → isopropyl alcohol → water
- **IPA:** isopropyl alcohol → water
- **HEA:** hexane → ethyl alcohol → water
- **EA:** ethyl alcohol → water

To evaluate the role of hexane in the HIPA and HEA samples, control samples were made by eliminating the hexane in the solvent exchange series. In these cases, saturated solutions of C_{60} in ethyl alcohol and IPA were used as starting points, leaving subsequent steps as described for the HIPA and HEA methods. Four replicates were prepared by each method and fully analyzed to ensure reproducibility; these results are reported in **Table 3.3** as the standard deviation. While the ethyl alcohol → water method is similar to a technique that has been previously reported,[20, 28] it has not been widely adopted in the community and thus is not included in the motivation for this work. Our inclusion of it here is primarily to evaluate the role of hexane in the HEA method.

Typical samples at each step for the HIPA method, the most successful method of those reported here, are shown in **Figure 3.1**. As is readily apparent from the dark coloration of **Figure 3.2 (C)**, the resulting concentration from this method is quite high. There are two main reasons why the intensity of the color of C_{60} in water is substantially more intense than in the parent organic solutions. First, the initial volume of C_{60} in hexanes was 50 mL, but as described above the final collected volume of nC_{60} in water was ~15 mL. This decrease in volume effectively concentrates the nC_{60} relative to the original stock solution, partially offsetting the decrease in concentration resulting from the loss of fullerene material during the filtration steps. Second, and more dramatically, the colloidal form of C_{60} has significantly higher extinction in the visible than does C_{60} in organic solvents because (a) the colloidal particles scatter

visible light and (b) the broad pseudo-solid-state C_{60} absorption band from 400-500 nm is significant.[16] This second explanation is demonstrated in **Figures 3.3** and **3.4** for typical HIPA and HEA suspensions. The resulting colloidal suspensions were analyzed by SPME-GC for hexane, IPA, and ethyl alcohol. All residual solvents were found to be below detectable limits (< 1 ppm using the SPME-GC method employed at the time these samples were analyzed: HP®-GC with FID). Improvements to the SPME-GC characterization method will be discussed in chapter four.

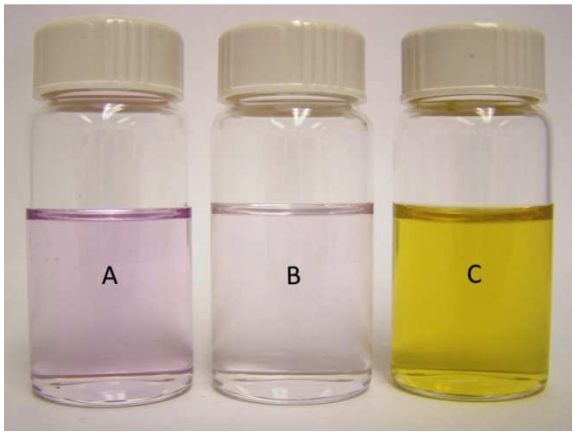

Figure 3.2: A) C_{60} in hexanes, B) C_{60} in IPA after removal of hexanes, C) nC_{60} in water, final suspension. Note that the higher optical density of the final suspension than the source suspensions is due to (a) the concentration effect resulting from starting with 50 mL of hexane solution, but concentrating the final sample to 15 mL, and (b) the greater optical extinction of nC_{60} than of organic solution of C_{60} in the visible region because of light scattering and to the broad pseudo-solid-state absorption band that is observed in such samples from ca. 400-500 nm.[16]

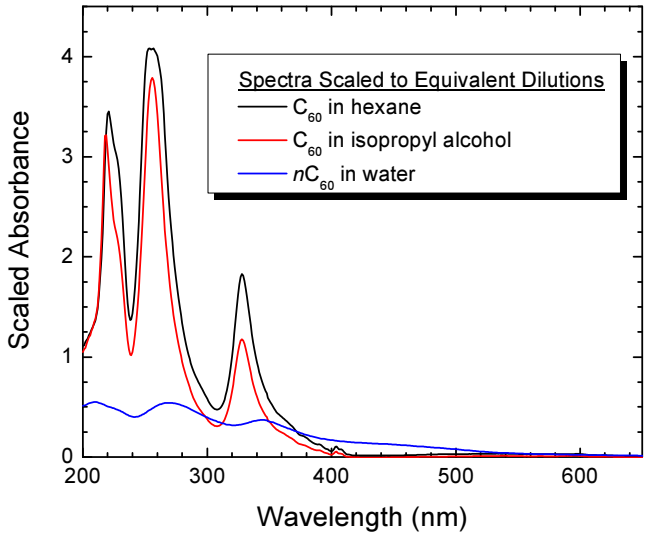

Figure 3.3: Absorption spectra for HIPA samples at various stages of synthesis scaled to equivalent dilutions, calculated as $A(\lambda)\times V_{sample}/V_{hexanes}$: original stock solution in hexanes, suspension in IPA after removal of hexanes, and suspension in water after removal of IPA. The original hexanes solution was 50 mL; the IPA suspension was 57 mL; the water suspension was 15 mL.

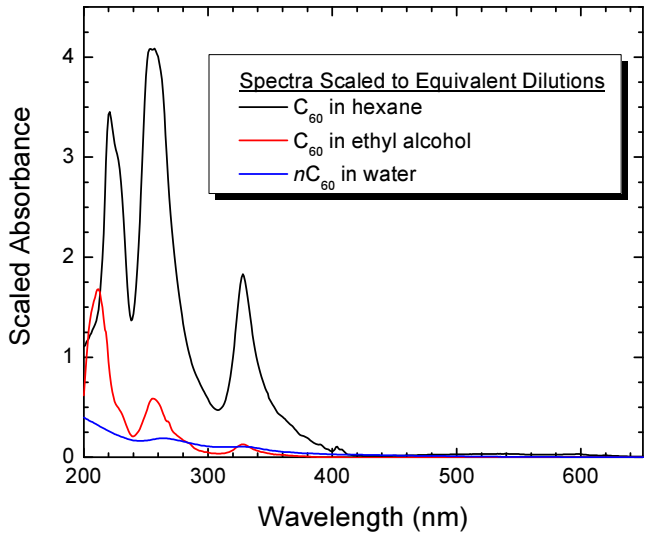

Figure 3.4: Absorption spectra for HEA samples at various stages of synthesis scaled to equivalent dilutions, calculated as $A(\lambda)\times V_{sample}/V_{hexane}$: original stock solution in hexanes, suspension in ethyl alcohol after removal of hexanes, and suspension in water after removal of ethyl alcohol. The original hexanes solution was 50 mL; the ethyl alcohol suspension was 52 mL; the water suspension was 17 mL.

The particle size distributions as determined by dynamic light scattering (DLS), the final concentrations, and percent yields for the four methods described here are given in **Table 3.3** along with comparison values for similar data available in the literature for the other four primary synthesis techniques. Representative transmission electron micrographs (TEM) of HIPA/nC$_{60}$ and HEA/nC$_{60}$ samples can be seen in **Figures 3.5** and **3.6** respectively. TEM images of dispersed particles dried onto a grid are often subject to clumping that occurs during the dry-down process. Thus, a common question in interpreting such TEM data is whether or not the particles shown are truly representative of the particles as seen in liquid suspension. **Figure 3.5** shows base particle sizes in the 30-70 nm range, but which are clumped together into larger aggregates with apparent radii that are more in line with the DLS data (133 nm mean particle diameter for HIPA – **Table 3.3**) than that of the 30-70 nm shown in the figure. We therefore conclude that the larger aggregates shown by TEM are likely more representative of the state of the particles in suspension than are the 30-70 nm sub-particles.

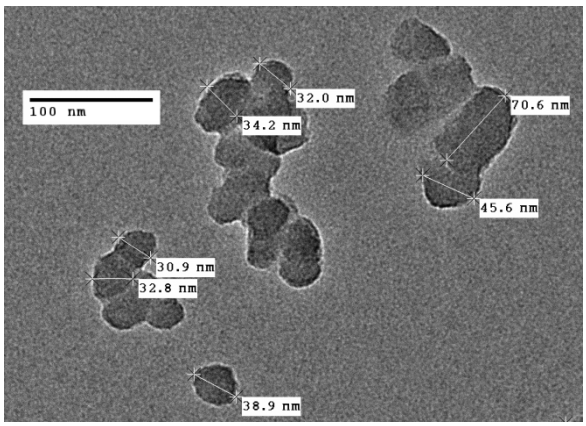

Figure 3.5: Representative TEM image for the HIPA method taken on a holey carbon grid.

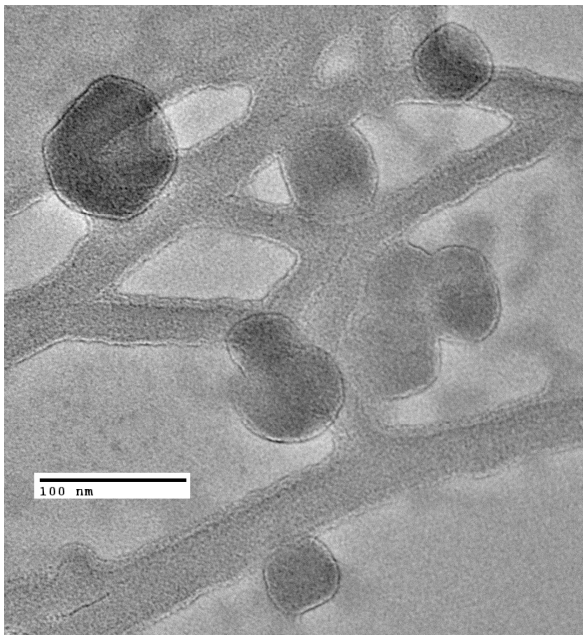

Figure 3.6: Representative TEM image for the HEA method taken on a holey carbon grid.

The four methods presented here exhibit a high degree of reproducibility in mean particle diameter across the four replicate samples as demonstrated by the reported standard deviations of the mean in **Table 3.3**. The particle sizes are uniformly smaller than are those produced by the AQU and SON methods. THF and TTA methods have both been demonstrated to provide a degree of particle size control over ranges that include the particle sizes produced by the methods introduced in this study. Similarly, as measured by the diameter distribution width and the polydispersity index (PDI), the level of polydispersity in the samples from this study is low compared to that reported for the AQU and SON methods and rivals that of the best THF and TTA samples reported.

While the EA method, similar to a technique that has been previously reported,[20, 28] produced the smallest colloidal particles of the methods investigated here, the HIPA method produced the most monodisperse samples. This claim is supported by the PDI and the peak width expressed as a percentage of the mean particle size.

	Mean particle diameter (nm)	Diameter distribution FWHM (nm)	Polydispersity Index (PDI)	[C_{60}] (ppm)	% yield
HIPA	133 ± 9	33 ± 13	0.09 ± 0.03	12 ± 3	14 ± 3
IPA	91 ± 8	24 ± 7	0.11 ± 0.02	4 ± 1	4.3 ± 0.4
HEA	88 ± 7	24 ± 8	0.12 ± 0.02	3.7 ± 0.6	4 ± 1
EA	77 ± 4 121.8 ± 0.8[20]	23 ± 5	0.15 ± 0.04	3.7 ± 0.4 4.2 ± 0.9[20] 6[28]	2.7 ± 0.3
AQU	180[11, 13] 200 – 340[17] 175.6 ± 1.2[20]	200 – 350[17]	0.146[13]	5[13] 0.22 ± 0.07[20] 0.41[40] 2.1[28]	0.08[40]
THF	160[11, 13] 62.8[15] 80 – 350[16]		0.090[13]	6[13] 3.6[15] 12[28]	
SON	160[13]		0.227[13]	5[10] 9[13]	
TTA	20 – 168[12] 170[13] 300[18] 76±5 – 202±8[48]	25 – 35[12] 50[18] 22±4 – 60±35[48]	0.283[13] 0.08±0.03 – 0.4±0.2[48]	3.5[13] 10 – 20[48]	2.7 – 20[48]

Table 3.3: Particle size distributions and yields for the four nC_{60} synthesis methods employed here and for the main literature methods. For the present study's data, the values are averages of four replicate samples ± the standard deviations. Particles sizes were stable for two months. References with a range of values refer to multiple samples produced by varying the core reported method. In all cases in the present study and in other reports of nC_{60} synthesis, the majority of the losses are in the form of solids removed by filtration.[1]

[1] A few literature data points have been excluded. Markovic *et al.28. Markovic, Z.; Todorovic-Markovic, B.; Kleut, D.; Nikolic, N.; Vranjes-Djuric; Misirkic, M.; Vucicevic, L.; Janjetovic, K.; Isakovic, A.; Harhaji, L.; Babic-Stojic, B.; Dramicanin, M.; Trajkovic, V., The Mechanism of Cell-damaging Reactive Oxygen Generation by Colloidal Fullerenes. Biomaterials* ***2007,*** *28, 5437-5448.*reported particle diameters (35.1 nm for EA/nC_{60}, 29.2 nm for AQU/nC_{60}, and 20.5 nm for THF/nC_{60}) that are out-of-line with those reported for the same techniques by other groups, and given that report's lack of TEM data to validate their DLS measurements, we find those results suspicious. Brant *et a.l. 12. Brant, J.; Lecoanet, H.; Wiesner, M. R., Aggregation and Deposition Characteristics of Fullerene Nanoparticles in Aqueous Systems. J. Nanopart. Res.* ***2005,*** *7 (4-5), 545-553.* reported post-synthesis concentrations of 0.21, 0.69, and 2.08 mM for the three samples studied. These values equal 100% yield from the starting 0.15, 0.5, and 1.5 mg/mL concentrations of C_{60} in toluene, so we suspect that the authors were reporting an assumed concentration rather than a measured one.

The final concentrations reported in **Table 3.3** are those acquired at the end of the published synthesis procedure. Many of the resulting samples have, in those studies and in other work, been successfully further concentrated by low-temperature evaporation of the remaining solvent, dialysis, or the use of centrifugal concentrators. Therefore, these reported concentrations should not be considered maximum concentrations for the reported technique. As compared to the literature reports of other techniques, the as-prepared HIPA method's concentrations rival or surpass those of most other reports.

Although the yields can be significantly increased (briefly discussed in **section 5.2.1**), concentrations as low as those in **Table 3.3** have been demonstrated for other lipophilic organic molecules to have significant ecological impacts in aqueous systems at 1-10 ppm.[74] However, having higher yields allows researchers doing biological, toxicological or environmental studies to test a wide range of concentrations as well as precisely control the relative concentrations of each solution (by dilution). The properties of these suspensions are very much affected by the synthesis method employed and also vary from sample to sample.[13] Having a single concentrated stock suspension provides the possibility to do a large variety of experiments while eliminating many variables that can come from using several different samples.

A dramatic difference can be seen in the synthesis yields comparing the HIPA and HEA methods to the IPA and EA methods. For ethyl alcohol, starting from a hexane solution roughly doubles the fraction of fullerene molecules that are converted to the colloidal form as compared to directly dissolving the fullerenes in alcohol. For IPA, the yield is nearly tripled by starting with a hexane solution. A review by Mchedlov-Petrossyan et al. gives strong evidence that low-level aggregation of C_{60} occurs in solvents that are considered to be excellent for fullerenes.[75] Thus, it is unlikely that the initial C_{60}/hexane stock solution is actually a true thermodynamic solution. This initial particle seeding in hexanes may be advantageous in producing colloids with superior narrow size distributions and increased yields. These increases in yield are consistent with DLS observations (not shown) in both the HIPA and HEA methods, which indicate that much of the growth occurs at the addition of alcohol and removal of hexanes. This process can also be seen visibly during the synthesis process and is the reason for the filtration step before

adding water (to prevent the C_{60} from crashing). The seeding of colloidal nC_{60} particles before the addition of water in these cases is further supported by the observation of higher concentrations of fullerenes in the alcohols when starting from a hexanes solution compared to the concentration of fullerenes dissolved directly in alcohol.

As a result of the particle seeding mentioned above, the synthesis procedure was designed in a way that the hexanes are removed very quickly to avoid over-aggregation in the alcohol and subsequent crashing out of the C_{60}. However, removing the hexanes too quickly can also cause crashing out of the C_{60} and thus result in a low yield in the final suspension. The removal of hexanes is typically carried out within ~35 min. The filtering steps before and after the addition of water should also be carried out fairly quickly for the same reasons.

3.4 Surface Chemistry and Colloid Stabilization

HPLC analysis demonstrated that the only fullerenes present in the colloid were underivatized C_{60} (peak at 8.24 min, 98.4% of integrated area), $C_{60}O$ (peak at 9.20 min, 1.34% of integrated area), and a very small amount of $C_{60}O_2$ (peak at 10.2 min, 0.003% of integrated area) (typical data shown in **Figure 3.7**). $C_{60}O$ is present as the [6,6]-closed epoxide isomer as opposed to the [5,6]-open oxidoannulene isomer. While the retention times of these two isomers overlap strongly on the Cosmosil Buckyprep column (which was designed specifically for fullerene analysis), the UV-vis spectrum extracted for the $C_{60}O$ peak in the HIPA/HEA/nC_{60} samples matched that of the epoxide isomer with a λ_{max} = 328 nm.[57] For the annulene isomer, the λ_{max} = 336 nm. Additionally, the annulene isomer is formed under relatively extreme conditions such as ozonation in the absence of light followed by UV irradiation (fluorescent desk lamp).[57] The synthesis of the annulene isomer was attempted in order to compare the UV/HPLC data. However, the synthesis was unsuccessful illustrating that the annulene is not easily

formed, particularly under normal laboratory conditions in which the appearance of the $C_{60}O$ in the nC_{60} samples described in this work is seen.

Since the extinction coefficients for $C_{60}O$ and $C_{60}O_2$ are lower than that for C_{60} at the reported wavelength of 336 nm, these reported percentages underestimate the oxide content of the samples. A similar analysis of the C_{60} starting material revealed that the original fullerene sample contained 0.2% $C_{60}O$, despite the 99.9% purity level of the starting material stated by the supplier. A study has recently been completed in the same lab as the research presented here that explains the importance of this data.[40] In particular, it has been demonstrated that the often-unrecognized, small amount of $C_{60}O$ that is present in nearly all solid fullerene samples is due to reaction with trace levels of atmospheric ozone. This work reveals that careful attention to the storage conditions of stock fullerene material is vital to keep $C_{60}O$ production to a minimum. Further, this study demonstrates that $C_{60}O$ is enriched in the resulting colloidal aggregates during nC_{60} preparation due to a preferential partitioning of this fullerene into the colloidal phase due to its greater hydrophilicity than that of pristine C_{60}. Finally, this study suggests that the stabilization of nC_{60} colloids in water is due in large part in many samples to the presence of these oxide molecules on the surfaces of the aggregate particles.

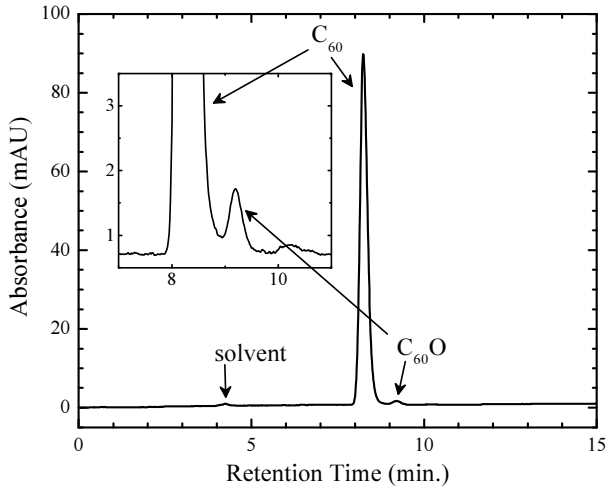

Figure 3.7: HPLC chromatogram for the initial HIPA method as described in the text.

The data presented in **Figure 3.7** supports the model that the oxide forms of the fullerenes, when present in small amounts in the original samples, preferentially partition into the colloidal aggregate phase: 0.2% $C_{60}O$ in the original sample with a 14% overall yield of colloid formation, corresponds to 1.4% $C_{60}O$ in the final product aggregates if all of the oxide successfully partitions, and this is discounting any increase in oxide concentration due to reaction with trace levels of atmospheric ozone during the course of the synthesis. However, unlike our studies on samples formed by simple stirring of C_{60} in water for extended periods, the presence of the oxide seems to not be required for the HIPA fullerene colloids to be stable in water. We performed a control study starting with freshly-received C_{60} that had been carefully stored under nitrogen and showed $C_{60}O$ concentrations below detectible limits. The resulting HIPA/*n*C_{60} samples had lower concentrations than was found from the original 0.2% $C_{60}O$ samples, but nonetheless produced measurable amounts of the colloid. We suspect that in these cases a sufficient amount of IPA remains, coating the fullerene aggregates to stabilize them in water.

To test this hypothesis, we performed a control experiment similar to the AQU/*n*C_{60} synthesis method where we purged a mixture of C_{60} powder and nanopure water, as well as the headspace, with nitrogen gas in a septum sealed flask. Separately, we also purged some IPA with nitrogen gas in a septum sealed flask. Next, using N_2 as a carrier gas, we exposed the headspace of the C_{60}/water mixture to IPA vapor for five minutes via the cannula technique. This mixture was then allowed to stir for 20 days at which time an aliquot was taken. The concentration of this aliquot was ~0.5 ppm which was lower than a normal AQU/*n*C_{60} sample (~0.83 ppm) that was prepared during the same time frame and also much lower than any HIPA/*n*C_{60} sample. However, this IPA/AQU/*n*C_{60} sample had a significantly higher C_{60} content than samples prepared under inert atmosphere stirred for the same amount of time (20 days = 0 ppm). This suggests that it is probable that IPA plays some role in the stabilization of HIPA/*n*C_{60} samples but may not be the full explanation.

Additional evidence for the role IPA can play in the stabilization of C_{60} in water can be seen in **Figure 3.8,** which shows the above mentioned mixtures of C_{60} in water under N_2/IPA and ambient atmosphere at different times after the initial setup. Image A shows the sample minutes after being setup;

the mixture is mostly clear since the C_{60} has not had sufficient time to interact with the water. Image B shows the same mixture after 2 hours of vigorous stirring; the mixture at this point is a muddy brown color showing that the C_{60} has begun to interact with the water, presumably due to the presence of IPA. Typical AQU samples under ambient atmosphere require a few days to a couple of weeks for the C_{60} to be incorporated into the water in this way, and it is not until this point that the nC_{60} colloidal particles begin to form. Further mixing allows the final concentration of nC_{60} in the sample to increase. Image C provides a comparison to image B showing an ambient AQU sample after 24 hours stirring; the mixture is still clear. These images give strong evidence that IPA can play a significant role in the formation and stabilization of nC_{60} colloidal particles in water. In a similar manner, other organic solvents may play a similar role in colloid formation in other solvent exchange synthesis methods.

Figure 3.8: A) C_{60} powder in water purged with nitrogen gas minutes after being exposed to IPA vapor, B) same mixture as in image A after 2 hours, C) control mixture of C_{60} powder in water under ambient atmosphere after 24 hours stirring.

3.5 Conclusions

We have synthesized aqueous colloidal aggregates of C_{60} by three new techniques (HIPA, HEA, and IPA) and by one previously-reported technique (EA).[20, 28] The HEA, IPA, and EA techniques produce colloids with sub-100 nm mean particle diameters with particle distribution widths of less than 25 nm

(PDI ≤ 0.15) with as-produced concentrations near 4 ppm. The HIPA technique produces colloids at significantly higher concentrations (12 ppm, an overall 14% yield) with only slightly larger particles (133 nm diameter) and a higher degree of monodispersity (PDI of 0.09). All of the techniques reported in this chapter produce particles that are smaller and significantly more monodisperse than the two main literature methods that do not involve the use of the controversial solvent tetrahydrofuran (AQU and SON) and yield comparable as-produced concentrations. From comparative work done in our lab, the THF, HIPA, and HEA methods only take 4 to 5 hours to carry out from beginning to end, while the SON method takes anywhere from 7 to 20 hours, TTA about 2 days, and AQU from weeks to months to prepare good quality samples that are free of residual solvents. These new methods provide the same simplicity, narrow size distributions, short synthesis time, and high concentrations of the THF and TTA methods but avoid the use of controversial solvents such as tetrahydrofuran.[19, 21-23, 29, 31, 34, 35] Further, the HIPA and HEA methods are designed on the same gradual solvent-exchange principles that have allowed the TTA method to produce colloidal samples of controlled particle size.[48] If future studies demonstrate that the HIPA or HEA methods can likewise produce controlled particle-size samples, they will be the first examples of such control over *n*C_{60} that doesn't require the use of tetrahydrofuran.

These advantages of high yield, rapid and simple synthesis, narrow size distribution, lack of questionable solvents, and the potential for controllable average particle size make HIPA and HEA attractive methods for producing *n*C_{60} suspensions to be used in controlled environmental impact, biological, and toxicity studies. The concentrations available as-produced by these methods, reported above to be to be in the 4-12 ppm range, are comparable or better than most of the alternate methods in the literature (**Table 3.3**). Even these low as-produced concentrations can have significant ecological impacts in aqueous systems for other lipophilic organic molecules (see, for example, Chung, *et al.*[74]). Follow-up work is underway to determine reasonable upper-limits on the concentrations possible in the *n*C_{60} systems.

Finally, we have demonstrated that the presence of a small amount of $C_{60}O$ in the original C_{60} material is preferentially partitioned into the resulting *n*C_{60} aggregates by the HIPA method, analogous to

the behavior found for the AQU method,[40] and is suspected to be required for the stabilization of samples produced by the THF and SON methods.[40] In the case of the HIPA synthesis method, however, the fullerene oxide seems to be helpful but may not be necessary for colloid formation, and we hypothesize that other available molecules such as IPA may form the necessary hydrophilic layer surrounding the final product.

CHAPTER IV

METHOD DEVELOPMENT FOR TRACE SOLVENT ANALYSIS OF *n*C_{60} SUSPENSIONS VIA SPME-GC-MS

4.1 Introduction

After developing the HIPA method, the main task of this work was to develop a method for quantifying any residual solvents from the synthesis procedure. Within this research group, a method existed that involved filtering an *n*C_{60} sample through a 0.02 μm sterile anotop filter to remove C_{60}, extracting any residual solvents from the water layer using dichloromethane, drying the dichloromethane layer with anhydrous Na_2SO_4, filtering out the Na_2SO_4, then injecting 0.5 μL of this solution into an HP® 5890 GC with an FID. Identification was difficult not only because of the large solvent peak but also because several extraneous peaks could be present in the chromatogram if an extremely pure solvent was not used for the extraction. Also, with using only an FID there was no way to distinguish between two different compounds with the same retention time (a problem inherent in the analytes of interest here). A GC with a mass spectrophotometer would later be used to aid in identification of residual solvents in *n*C_{60} samples.

This existing trace solvent analysis method, while sufficient for quantifying tetrahydrofuran, toluene, and possibly acetone with a detection limit around 1 ppm, could not be used for quantifying hexanes, isopropyl alcohol (IPA), or ethanol due to the huge solvent peak that overlaps with these analytes. Toluene can be used as the extraction solvent for these last three analytes since it has a retention time significantly later than all of them. However, this method was extremely labor intensive and time consuming without using two different extraction solvents for the different analytes. For these reasons, a more sensitive method that did not involve a solvent extraction was needed that could quantify all of the solvents used in the various synthesis methods. A method using SPME with reported detection limits from parts per billion (ppb) to parts per trillion (ppt) seemed to be the best candidate to solve these problems.

SPME was developed and first reported by Zhouyao Zhang who was working in Janusz Pawlisyn's research group in 1993 at the University of Waterloo.[76] SPME fibers currently available consist of a fused silica fiber coated with various polymers (the specific polymer coatings determine which analytes are best analyzed by the fiber). This coated fiber is housed inside a hollow needle. A specialized plunger assembly is used to push the fiber out of the needle so it can be exposed to the sample of interest, which can either be in the gas phase (often the headspace over a liquid-phase sample) or in the liquid phase. After being exposed to the sample, the fiber is retracted into the needle then the needle is inserted into the inlet of the GC (or HPLC) and the fiber is exposed again and heated to desorb the analytes (or in the case of HPLC simply exposed to the solvent flow). **Figure 4.1** below shows the fiber assembly and also depicts the sampling process used in this study (headspace sampling for GC).

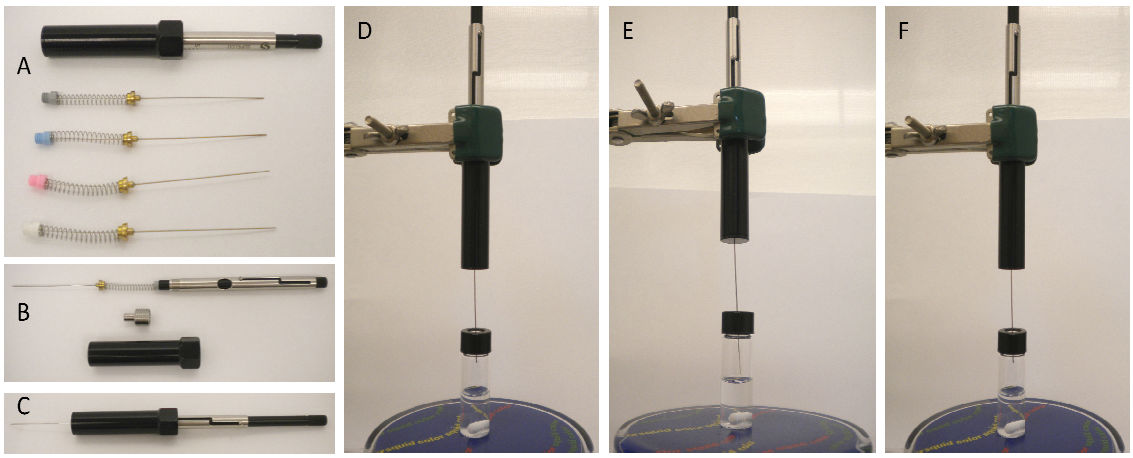

Figure 4.1: SPME fiber assembly and sampling process: A) manual injection fiber holder and color coded fibers that are coated with different types of polymers, B) assembly of the fiber into the fiber holder, C) fully assembled fiber and fiber holder, D) insert needle into vial, E) push down plunger and lock to expose fiber, F) unlock and pull up plunger to retract fiber into the needle then pull needle out of vial and insert into GC inlet. Re-expose fiber to load analytes onto column. Retract needle and remove from GC inlet.

A few advantages of SPME that make this technique desirable for trace solvent analysis include minimal sample preparation (single step extraction), the ability of headspace sampling that eliminates large solvent peaks in the chromatograms, and improved detection limits. Detection limits in the ppt range are possible with SPME due to decreased background signal from the solvent and the fact that the analytes are concentrated on the fiber and are rapidly delivered to the column. The analytes establish equilibria among the sample matrix, the headspace above the sample, and the polymer coating on the SPME fiber. The quantity of analyte extracted by the fiber is proportional to its concentration in the sample as long as equilibrium is reached (the equilibrium process can be sped up with the help of convection or agitation).[76, 77]

Before any quantification could be done on residual solvents in *n*C_{60} samples using SPME/GC, a suitable fiber needed to be found. Supelco® provides numerous fibers and guidelines describing the types of analytes for which they are best used. To limit the cost of this project, an assortment kit of four different fibers was selected, containing fibers that were said to be good for low molecular weight volatile or semi-volatile compounds. This kit contained fibers with the following polymer coatings: Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), Carboxen/Polydimethylsiloxane

(CAR/PDMS), Polydimethylsiloxane/Divinylbenzene (PDMS/DVB), and Polyacrylate (PA). The recommended use for each of these fibers is given in **Table 4.1**.

SPME Fiber	Recommended Use
DVB/CAR/PDMS	Trace Compound Analysis (MW = 40-275 g/mol)
CAR/PDMS	Gases and Low MW compounds (MW = 30-225 g/mol)
PDMS/DVB	Volatile Compounds, Amines, and Nitro-aromatic Compounds (MW = 50-300 g/mol)
PA	Polar Semi-volatiles (MW = 80-300 g/mol)

Table 4.4: SPME fibers tested in this study and the recommended analytes to be analyzed by each fiber.

4.2 Results and Discussion

4.2.1 SPME Fiber Selection for Trace Solvent Analysis

Based on the information in **Table 4.1**, we would expect the DVB/CAR/PDMS and CAR/PDMS fibers to show the greatest affinities for the residual solvents of interest: toluene, tetrahydrofuran, acetone, hexane, IPA, and ethanol. These solvents collectively have low molecular weights ranging from 46 to 92 g/mol and are considered volatile organic compounds.[78] **Table 4.2** and **Figure 4.2** contain some preliminary results showing the relative extraction efficiencies of these four SPME fibers with 20 ppm samples of the six solvents mentioned above. As predicted based on the information given by Supelco®, the DVB/CAR/PDMS and CAR/PDMS fibers show the highest responses for all six solvents. The PDMS/DVB and PA fibers gave significantly lower responses compared to the other two fibers and thus were excluded from consideration as suitable fibers.

	DVB/CAR/PDMS	CAR/PDMS	PDMS/DVB	PA
Toluene	1,010,000	720,000	475,000	51,000
THF	68,000	47,000	15,600	250
Hexanes	73,000	15,600	1,080	210
Acetone	53,000	86,000	21,500	380
IPA	9,300	3,800	1,170	190
EtOH	1,800	5,800	240	120
Water Blank	390	135	410	85

Table 4.5: Comparison of the response (counts) of the four available SPME fibers to the six solvents used in the most common methods for synthesizing nC$_{60}$. The values are the approximate peak heights at the retention time characteristic for each solvent. These results were obtained on an HP® 5890 series II GC system with an FID. All samples were 20 ppm in water.

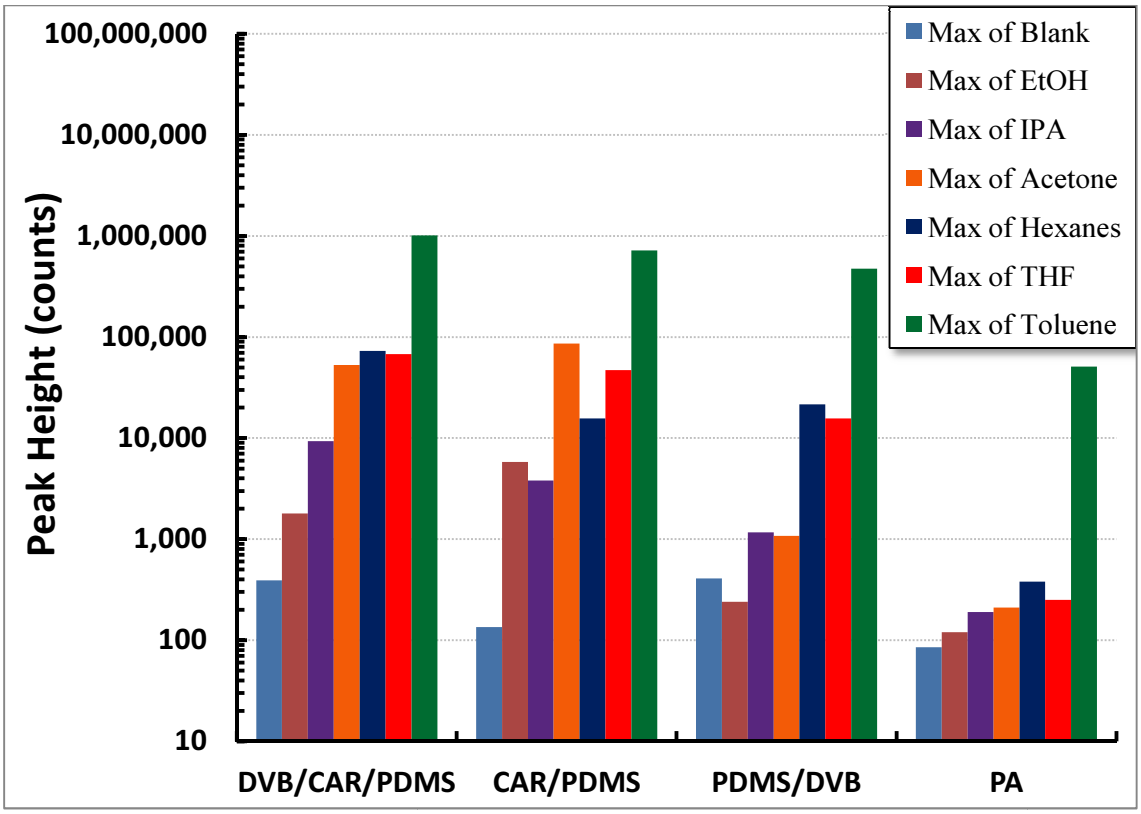

Figure 4.2: Comparison of the response of the four available SPME fibers to the six solvents used in the most common methods for synthesizing nC$_{60}$: a graphical depiction of the data in **Table 4.2**. The values are the approximate peak heights (counts) at the retention time characteristic for each solvent. These results were obtained on an HP® 5890 series II GC system with an FID. All samples were 20 ppm in water.

Although the DVB/CAR/PDMS and CAR/PDMS fibers show the highest peak intensities for different analytes, only one fiber should ideally be used for all six solvents due to the lengthy process of conditioning each fiber before use added to the limitation of having only one manual injection fiber holder. Using one fiber to quantify all of the residual solvents within a single sample is also beneficial with regards to reproducibility since only one extraction would be necessary for any certain sample. The DVB/CAR/PDMS fiber was chosen as the best fiber to use due to higher extraction efficiencies for the majority of the solvents of interest. Although acetone and ethanol were extracted more efficiently by the CAR/PDMS fiber, the DVB/CAR/PDMS fiber was able to extract these two solvents sufficiently. The reproducibility for the DVB/CAR/PDMS fiber with respect to hexane and IPA is discussed in **Section 4.2.3**.

4.2.2 SPME Method Development for Trace Solvent Analysis

All of the preliminary work described above concerning choosing the best SPME fiber to use was obtained using a GC with an FID. This method only provides identification based on retention time which gives rise to several problems. One concern was that four of the six solvents studied (acetone, hexane, IPA, and ethanol) had very similar retention times (0.55-0.60 min) which would make identification difficult in the quantification of residual solvents in some nC$_{60}$ samples that may contain more than one solvent (notably HIPA/nC$_{60}$ samples). Observations during the collection of this data led to an additional concern; acetone and toluene from the atmosphere can be present in the headspace of the sample vials which can interfere with the quantification of these solvents as well as other solvents with similar retention times to that of acetone. For example, in a HIPA sample, there would be no way to distinguish if a peak at 0.6 min was due to IPA or acetone from the atmosphere. Two ways to solve this problem were explored: purge the samples with N_2 gas and/or use a GC with a mass spectrometer both of which are discussed below. For reference, **Figures 4.3-4.14** show the chromatograms/retention times and mass spectra for standard solutions of four of the six solvents of interest (toluene, acetone, IPA, and hexanes).

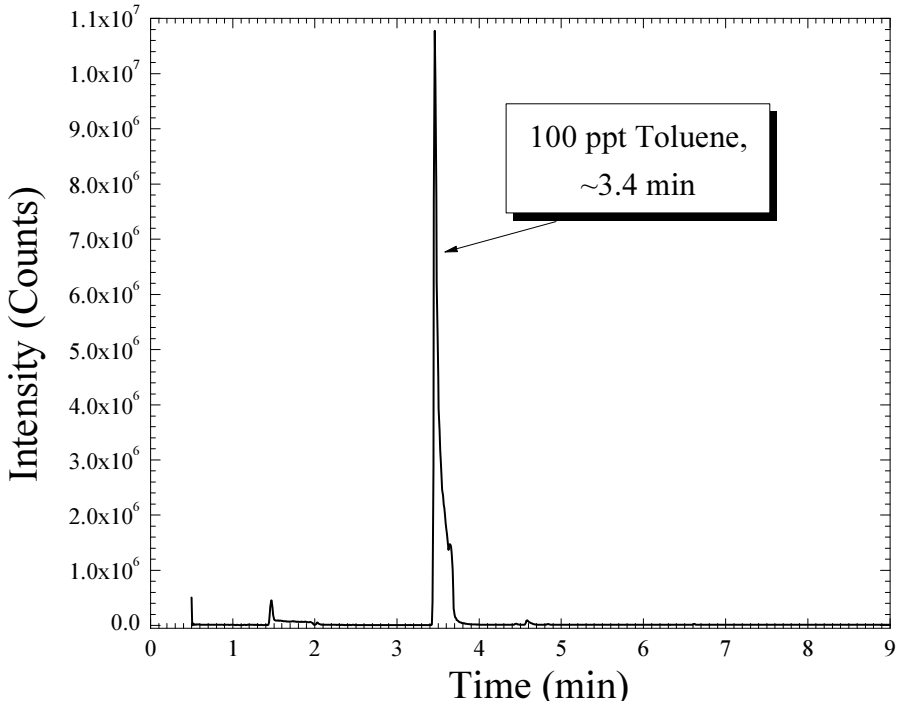

Figure 4.3: SPME-GC-MS chromatogram of standard solution of 100 ppt toluene in water. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The sample was allowed to equilibrate while stirring for 5 min before exposing the fiber.

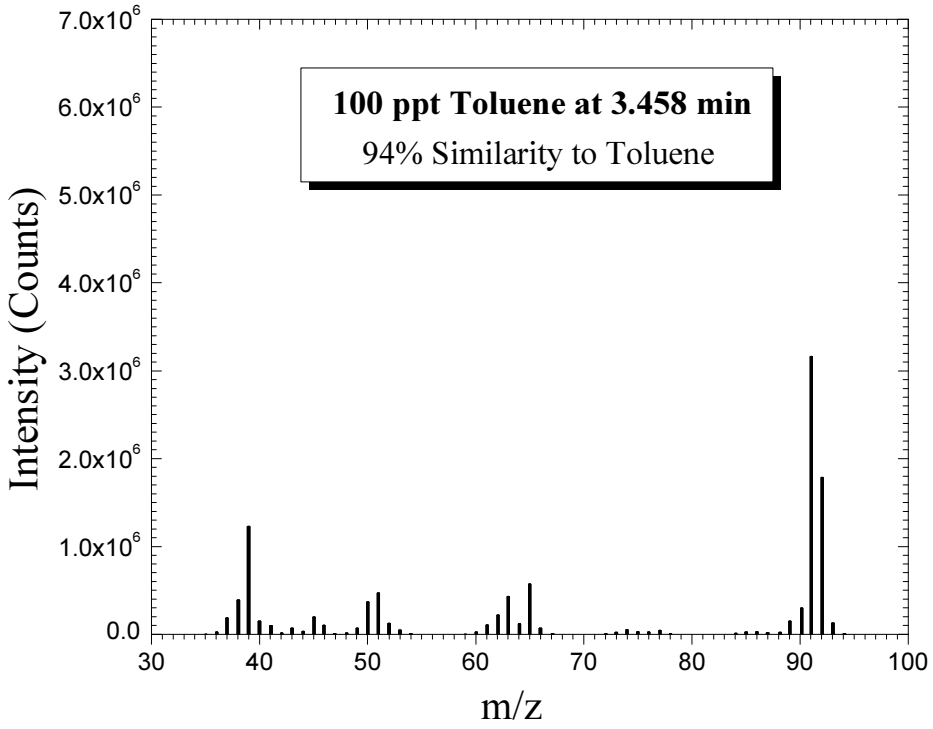

Figure 4.4: Mass spectrum for toluene extracted from the chromatogram (3.458 min peak) in **Figure 4.3** (SPME/GC-MS of 100 ppt toluene in water).

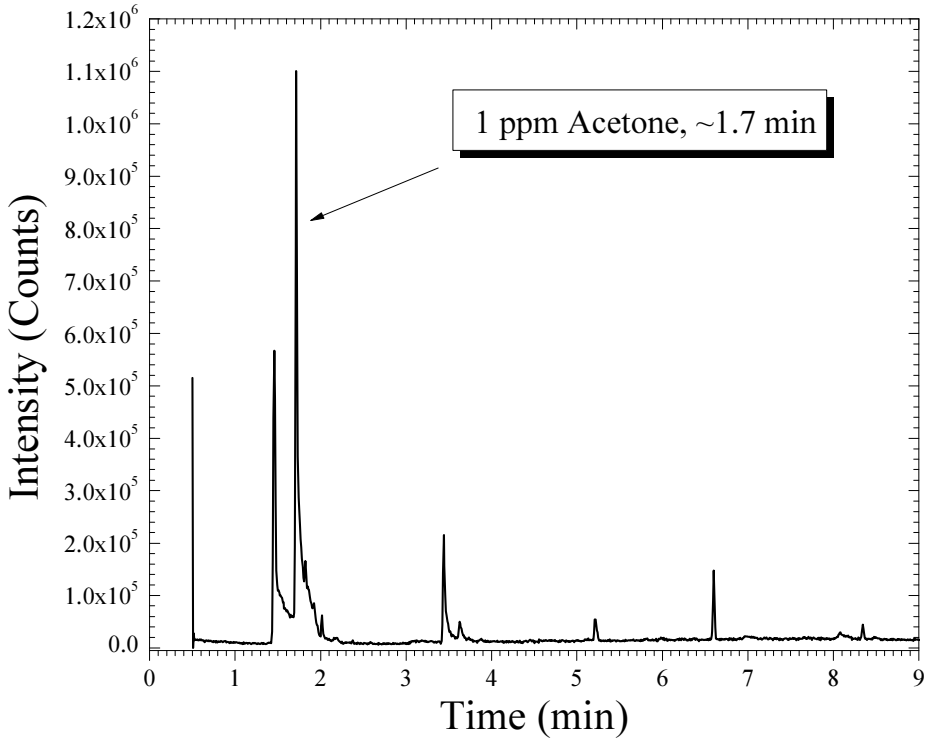

Figure 4.5: SPME-GC-MS chromatogram of standard solution of 1 ppm acetone in water. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The sample was allowed to equilibrate while stirring for 5 min before exposing the fiber.

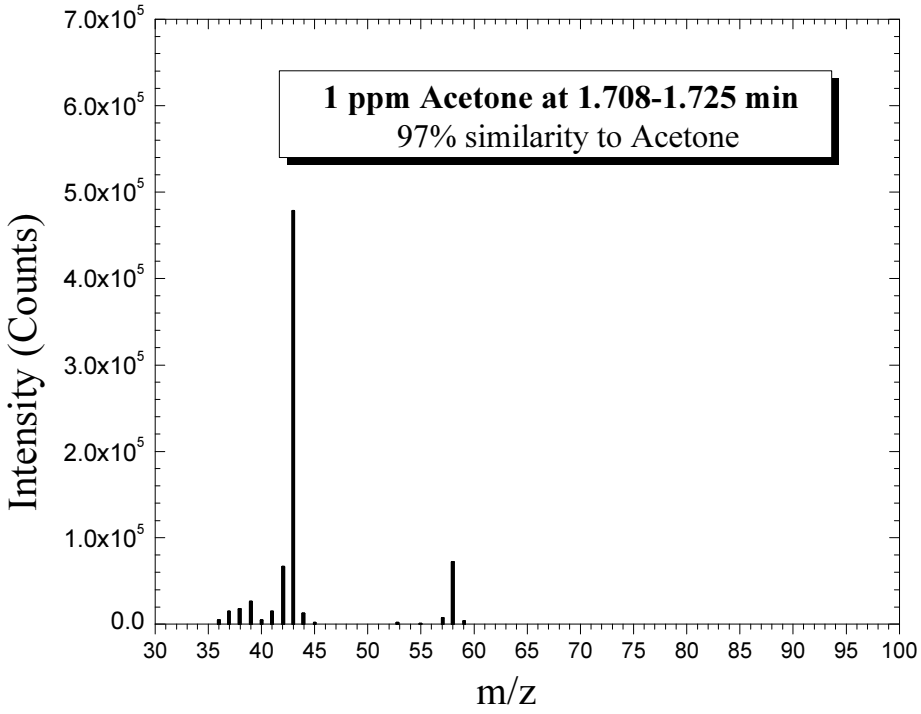

Figure 4.6: Averaged mass spectrum for acetone extracted from the chromatogram (1.708-1.725 min) in **Figure 4.5** (SPME/GC-MS of 1 ppm acetone in water).

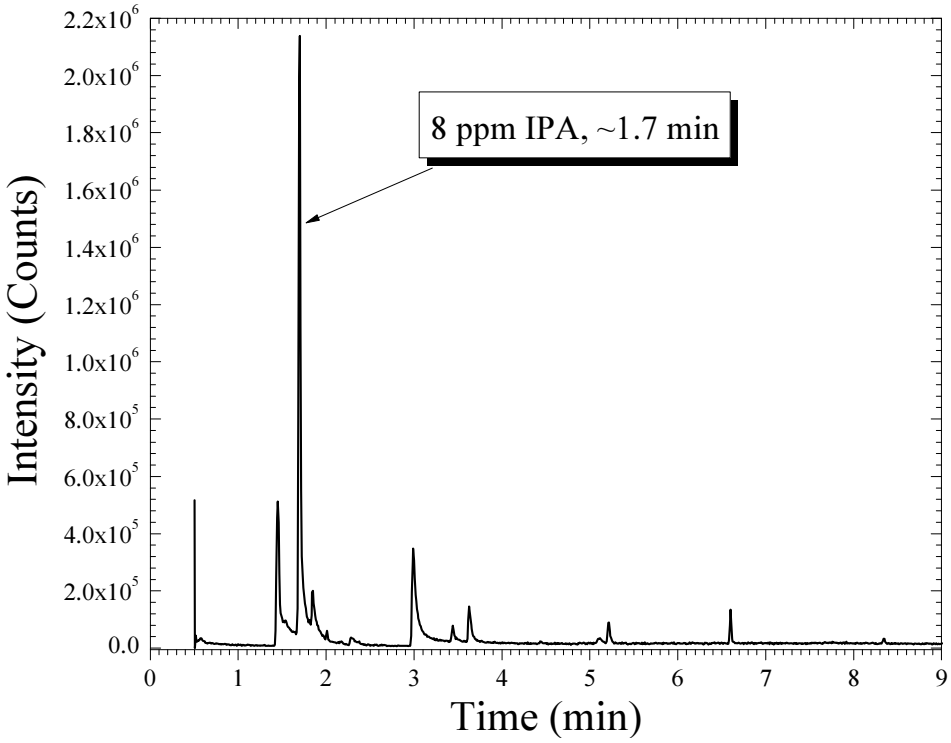

Figure 4.7: SPME-GC-MS chromatogram of standard solution of 8 ppm IPA in water. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The sample was allowed to equilibrate while stirring for 5 min before exposing the fiber.

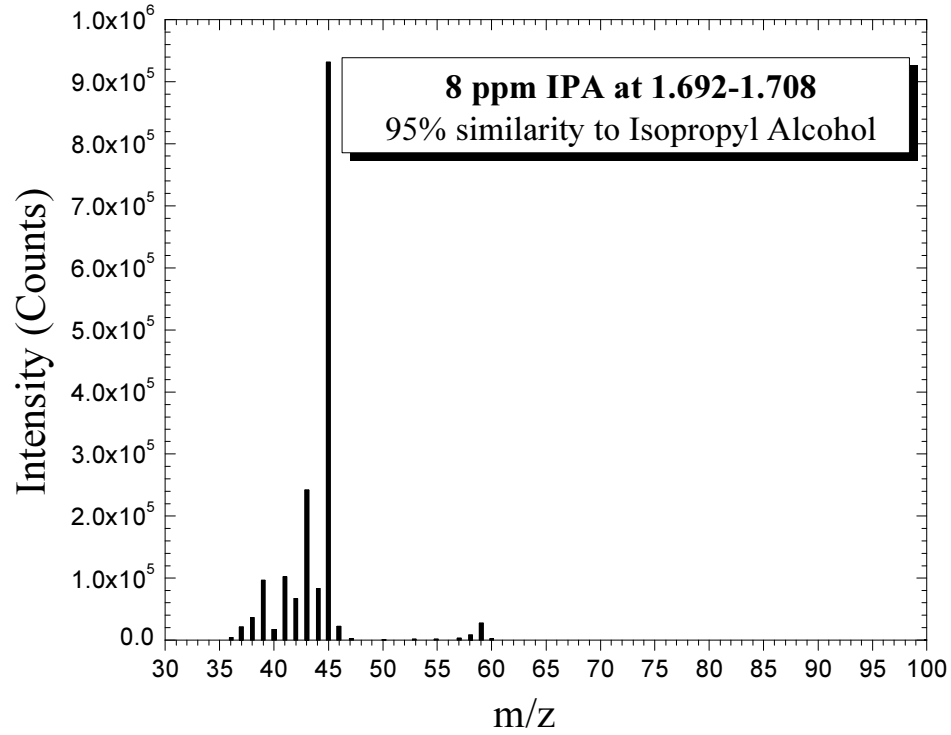

Figure 4.8: Averaged mass spectrum for IPA extracted from the chromatogram (1.692-1.708 min) in **Figure 4.7** (SPME/GC-MS of 8 ppm IPA in water).

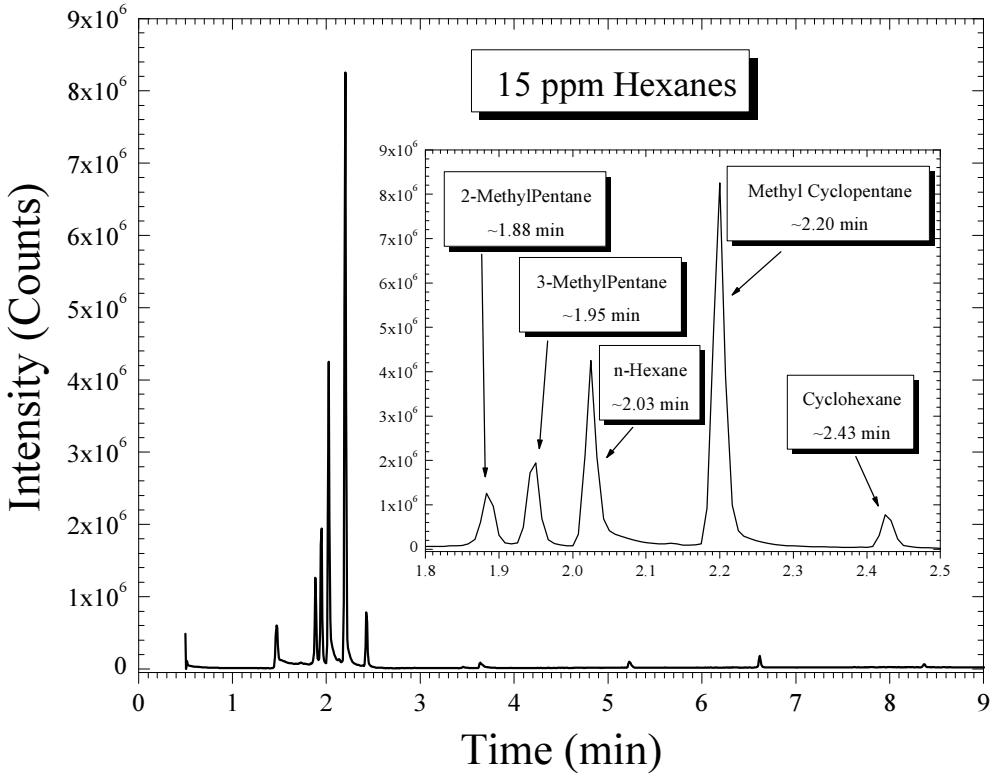

Figure 4.9: SPME-GC-MS chromatogram of standard solution of 15 ppm hexanes in water. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The sample was allowed to equilibrate while stirring for 5 min before exposing the fiber.

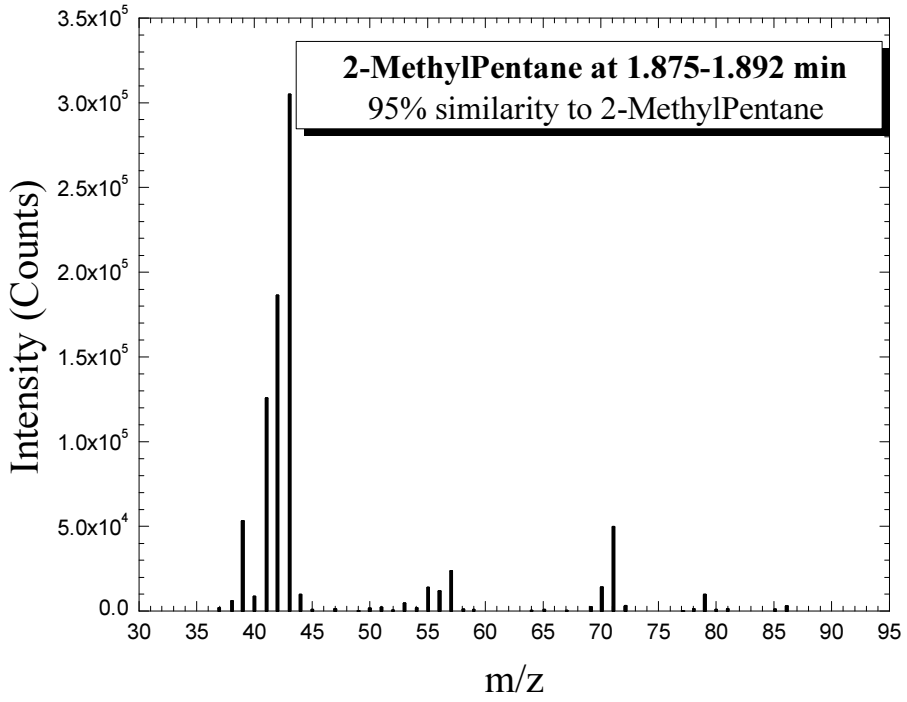

Figure 4.10: Averaged mass spectrum for 2-MethylPentane extracted from the chromatogram (1.875-1.892 min) in **Figure 4.9** (SPME/GC-MS of 15 ppm hexanes in water).

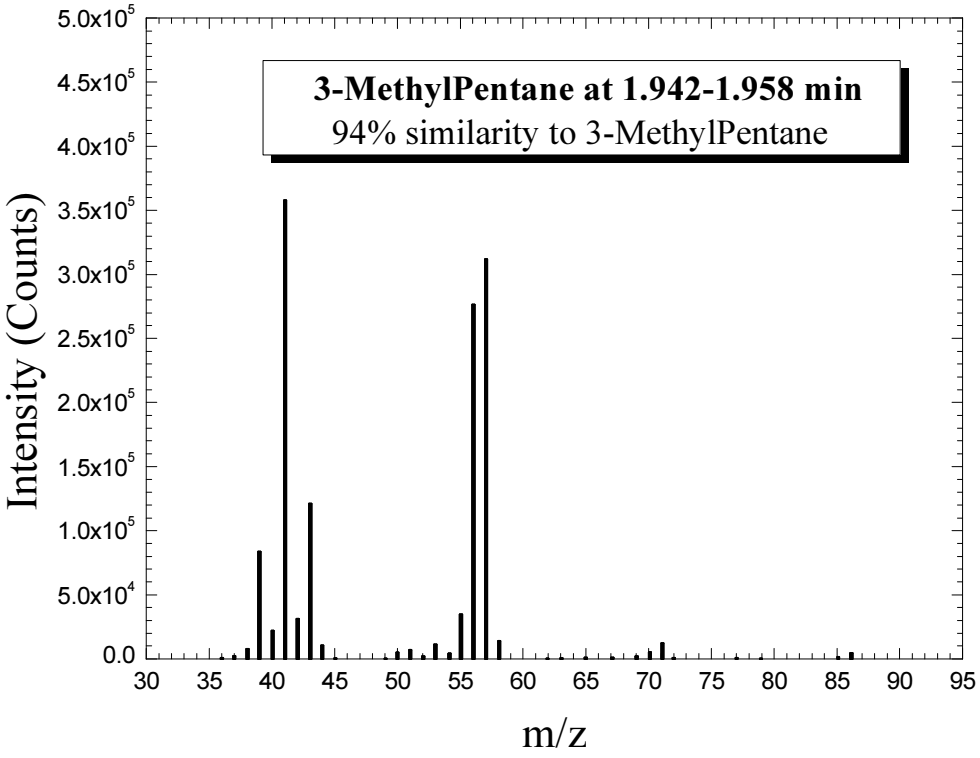

Figure 4.11: Averaged mass spectrum for 3-MethylPentane extracted from the chromatogram (1.942-1.958 min) in **Figure 4.9** (SPME/GC-MS of 15 ppm hexanes in water).

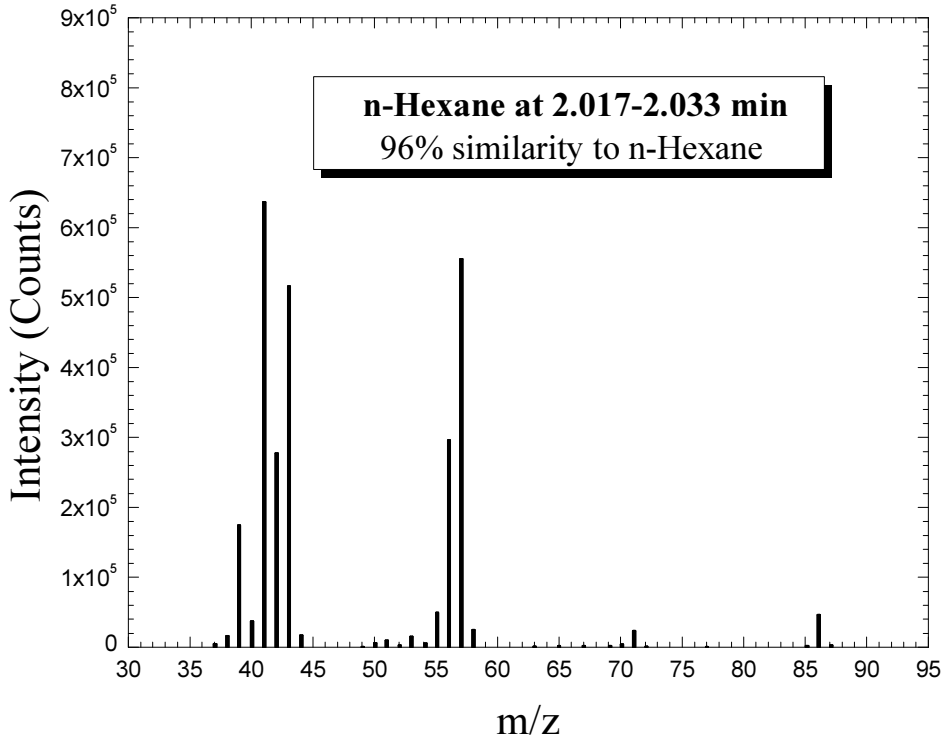

Figure 4.12: Averaged mass spectrum for n-Hexane extracted from the chromatogram (2.017-2.033 min) in **Figure 4.9** (SPME/GC-MS of 15 ppm hexanes in water).

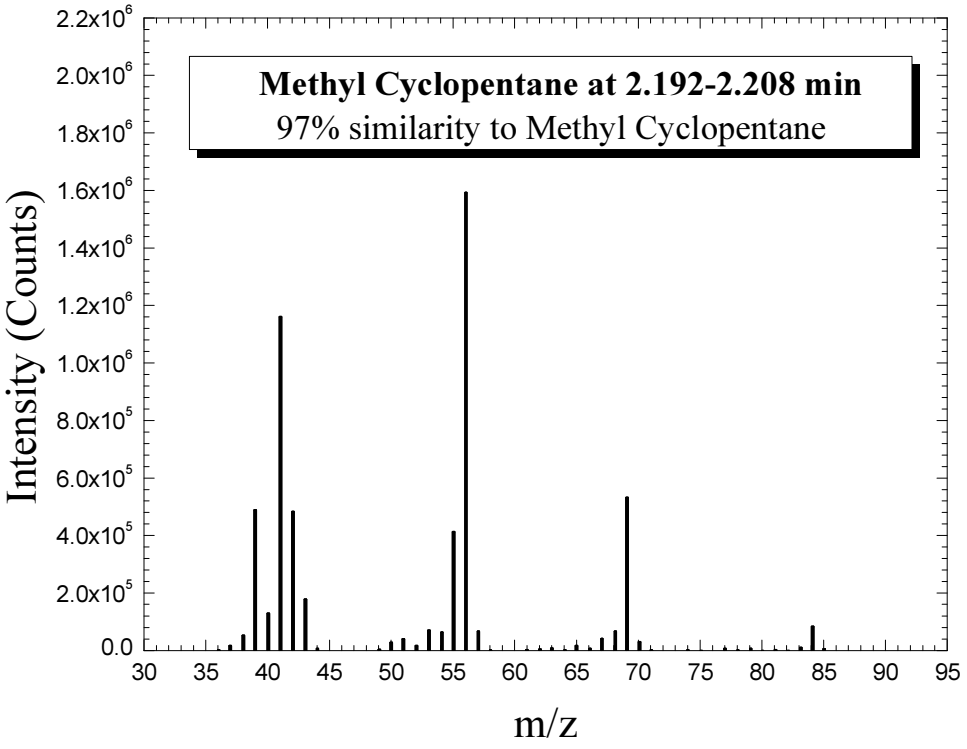

Figure 4.13: Averaged mass spectrum for Methyl Cyclopentane extracted from the chromatogram (2.192-2.208 min) in **Figure 4.9** (SPME/GC-MS of 15 ppm hexanes in water).

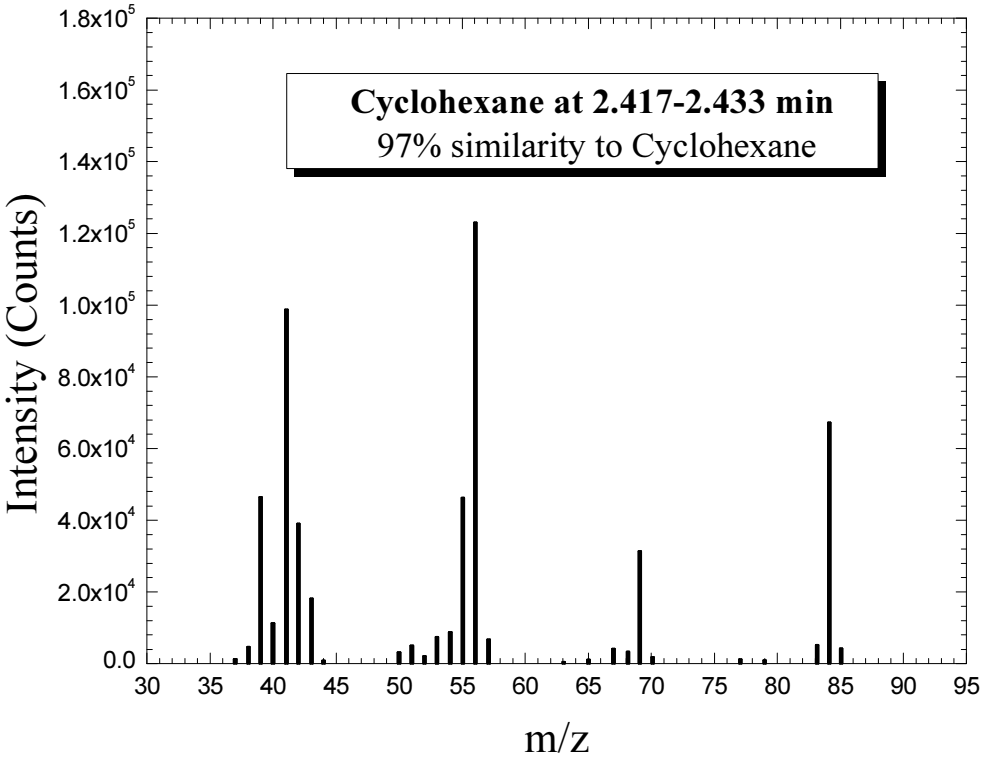

Figure 4.14: Averaged mass spectrum for Cyclohexane extracted from the chromatogram (2.417-2.433 min) in **Figure 4.9** (SPME/GC-MS of 15 ppm hexanes in water).

Figure 4.15 shows two aliquots from the same HIPA sample, one of which was analyzed by SPME/GC/MS using a DVB/CAR/PDMS fiber under ambient atmosphere. The second aliquot was analyzed in the same conditions except that the headspace was purged with N_2 gas before the sample was allowed to equilibrate and exposed to the SPME fiber. The biggest difference between the two chromatograms is the near disappearance of the toluene peak upon purging the headspace with N_2 gas. However, the peak at ~1.7 min (for acetone and IPA) did not change significantly as was expected. Purging the headspace was expected to cause a significant decrease in this peak's area/intensity, and the mass spec was expected to show mostly acetone for the ambient atmosphere aliquot and mostly IPA in the purged aliquot. **Figures 4.16-4.19** show the mass spectra for the acetone and toluene peaks in **Figure 4.15** in order to confirm their identities (compared to **Figures 4.3-4.14**).

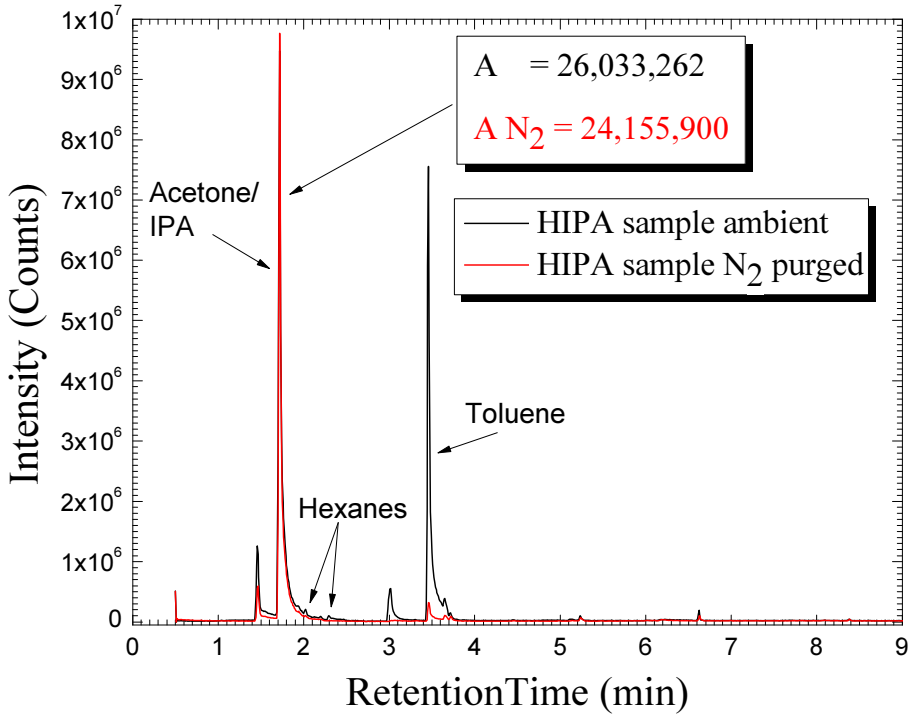

Figure 4.15: SPME-GC-MS chromatogram of a typical HIPA sample of nC_{60} in water. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The sample was allowed to equilibrate while stirring for 5 min before exposing the fiber. The fiber was exposed to two fresh aliquots of the HIPA sample: the first under ambient atmosphere (black) and the second under N_2 atmosphere (red).

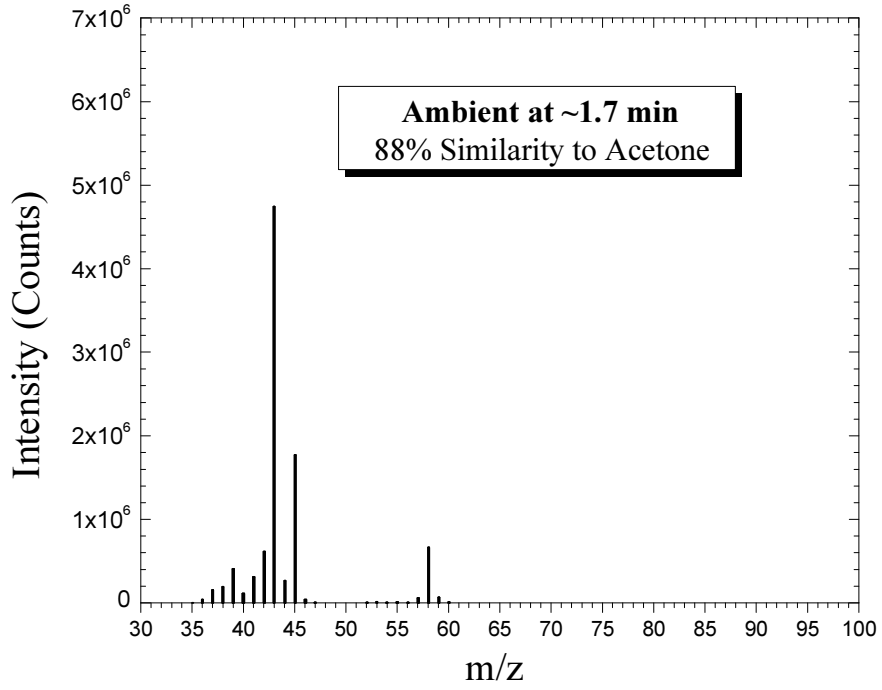

Figure 4.16: Mass spectrum for acetone/IPA extracted from the chromatogram (~1.7 min – Black trace) in **Figure 4.15** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where the headspace was under ambient atmosphere).

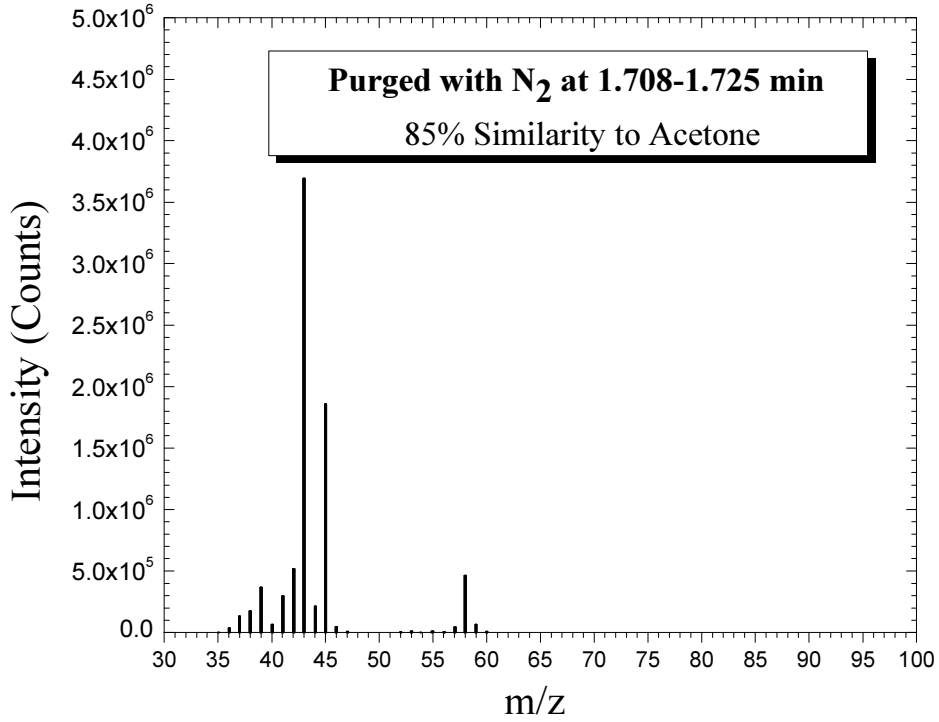

Figure 4.17: Averaged mass spectrum for acetone/IPA extracted from the chromatogram (1.708-1.725 min – Red trace) in **Figure 4.15** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where the headspace was purged with N_2 gas).

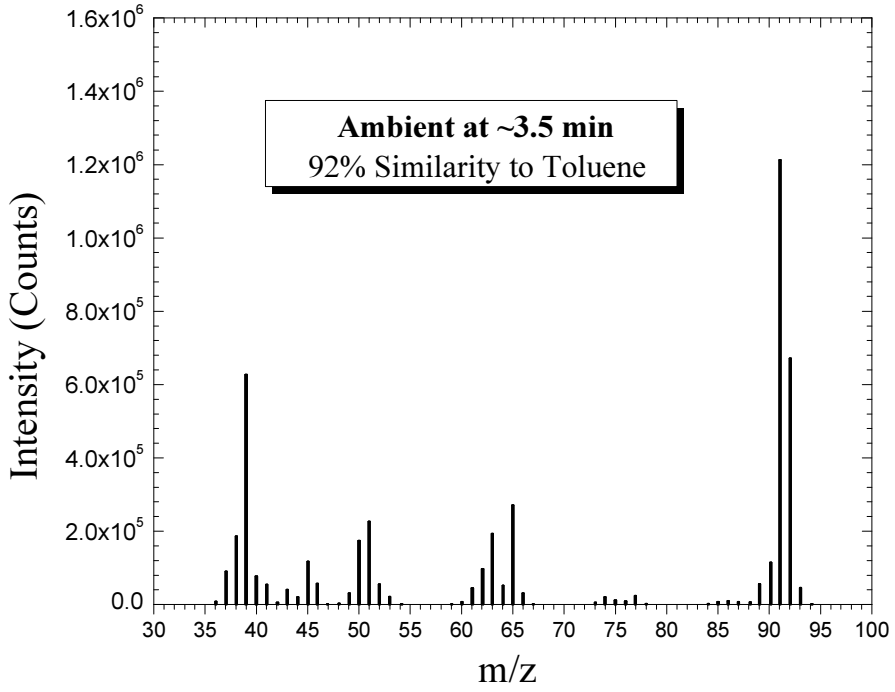

Figure 4.18: Mass spectrum for toluene extracted from the chromatogram (~3.5 min – Black trace) in **Figure 4.15** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where the headspace was under ambient atmosphere).

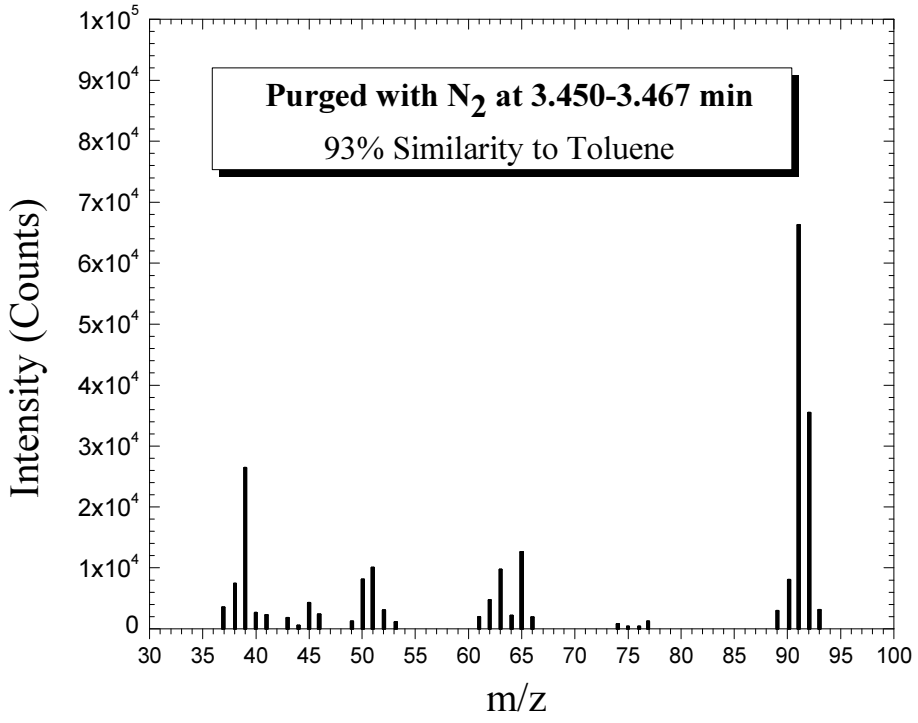

Figure 4.19: Averaged mass spectrum for toluene extracted from the chromatogram (3.450-3.467 min – Red trace) in **Figure 4.15** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where the headspace was purged with N_2 gas).

It was explained in **Section 2.4** that the glassware used in GC trace solvent analysis should be thoroughly cleaned, kept in the oven overnight, and capped (or closed to the atmosphere) upon removal from the oven until completely cooled in order to avoid solvent vapors from the atmosphere being adsorbed onto the interior walls of the containers and appearing in the chromatograms. An experiment shown in **Figure 4.20** demonstrates the reasoning behind this caution in sample preparation and explains why the ~1.7 min peak (acetone) in **Figure 4.15** did not significantly change. In **Figure 4.20**, two separate HIPA samples are represented: in red, the same HIPA sample shown in **Figure 4.15** that was purged with N_2 gas (prepared by normal procedures), and in black, a HIPA sample that was prepared with the same caution to avoid any solvent contamination from cleaning the glassware as described during GC sample preparation (also purged with N_2 gas). **Figures 4.15-4.22** confirm that careful sample preparation is necessary to accurately quantify residual solvents in $n$$C_{60}$ samples. Also, **Figures 4.23-4.28** show that the HIPA sample analyzed here does contain some residual hexanes and a significant amount of IPA.

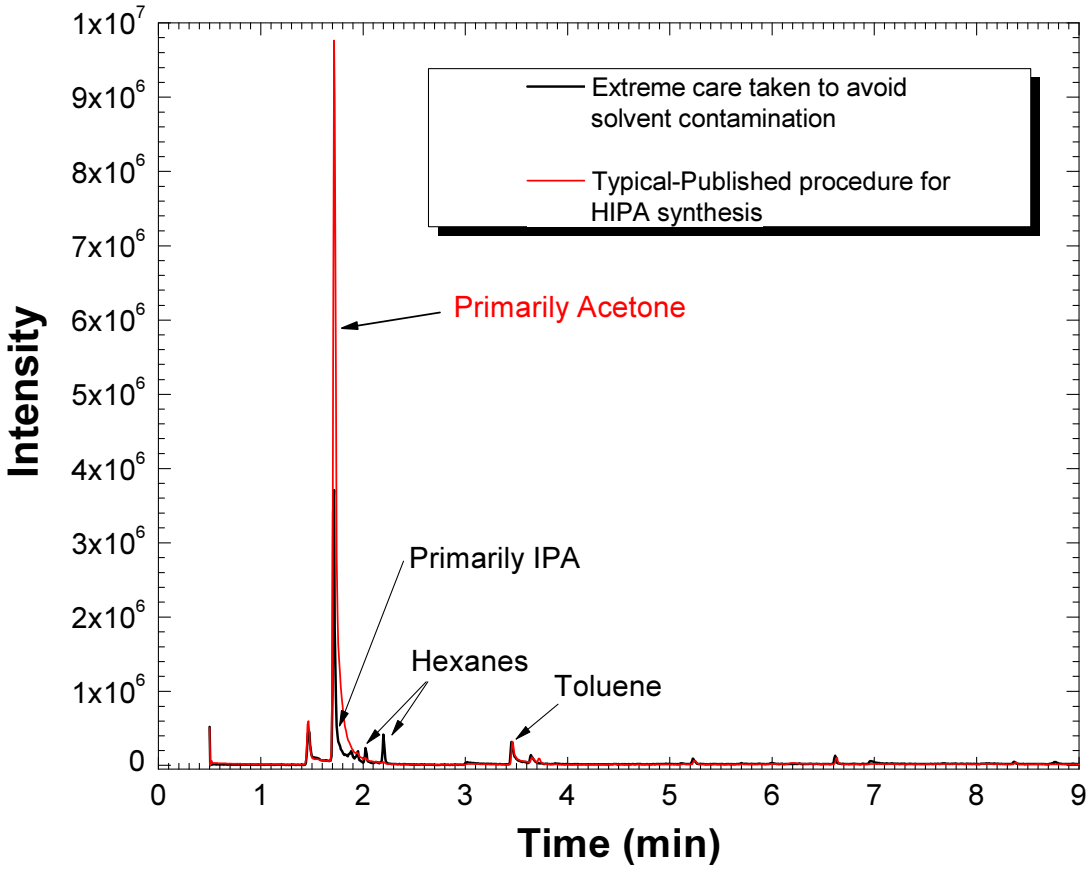

Figure 4.20: SPME-GC-MS chromatogram of a typical HIPA sample of nC$_{60}$ in water (Red) compared to a HIPA sample carefully prepared to avoid solvent contamination (black). A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The headspace above the sample was purged with N_2 then allowed to equilibrate while stirring for 5 min before exposing the fiber.

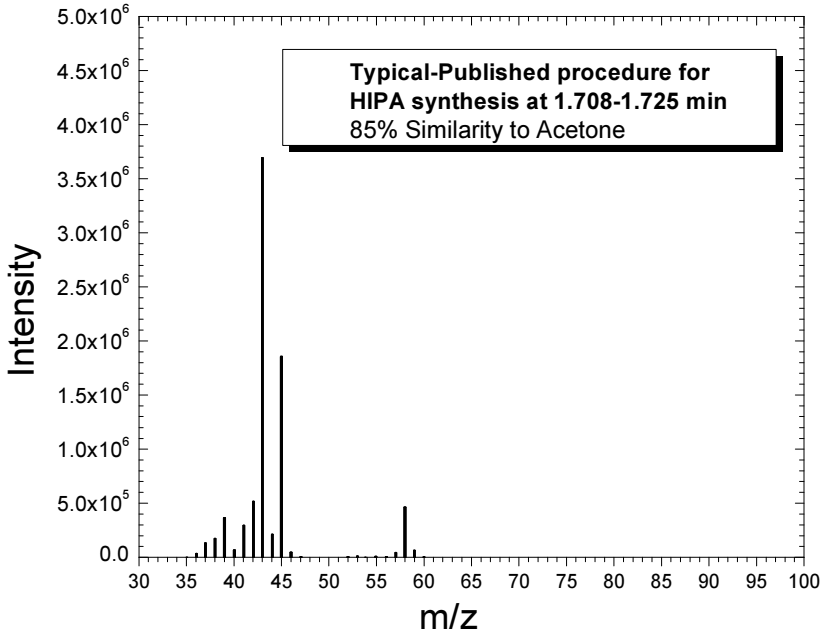

Figure 4.21: Averaged mass spectrum for acetone/IPA extracted from the chromatogram (1.708-1.725 min – Red trace) in **Figure 4.20** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where no special care was taken to avoid solvent contamination during synthesis and the headspace was purged with N_2 gas).

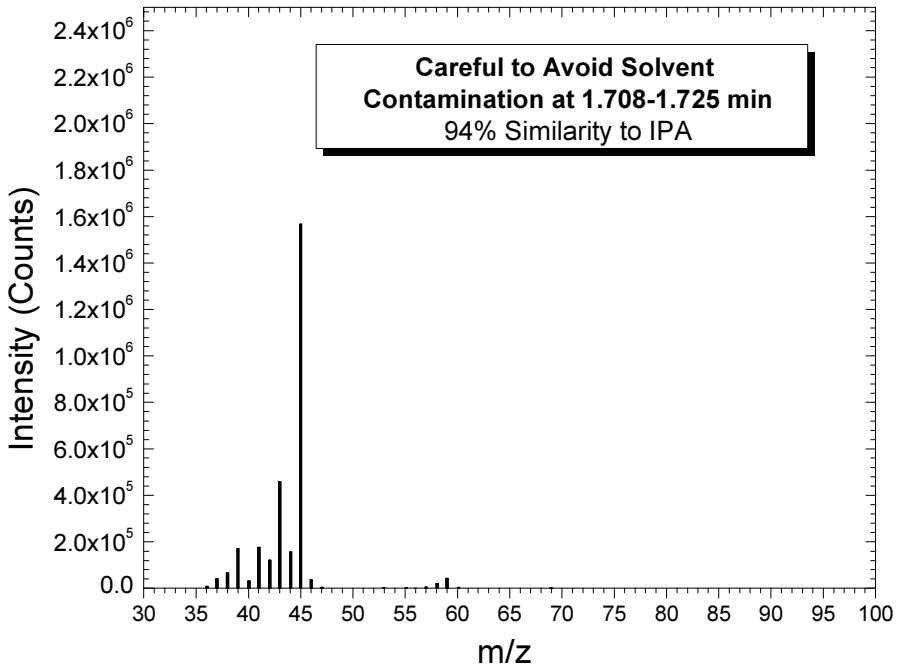

Figure 4.22: Averaged mass spectrum for IPA extracted from the chromatogram (1.708-1.725 min – Black trace) in **Figure 4.20** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where care was taken to avoid solvent contamination during synthesis and the headspace was purged with N_2 gas).

Figure 4.23 shows an expanded view of the same HIPA sample depicted in **Figure 4.20** that was prepared with care to avoid solvent contamination and purged with N_2 gas. The major identifiable components in the sample (IPA, n-hexane, methyl cyclopentane, and toluene) are labeled in the chromatogram. **Figures 4.22** and **4.24-4.28** show the mass spectra for these four main peaks as well as two other minor peaks (2-methylpentane and 3-methylpentane) that could not be identified by the similarity search function on the instrument software but match well with the respective mass spectra shown in **Figures 4.10** and **4.11**.

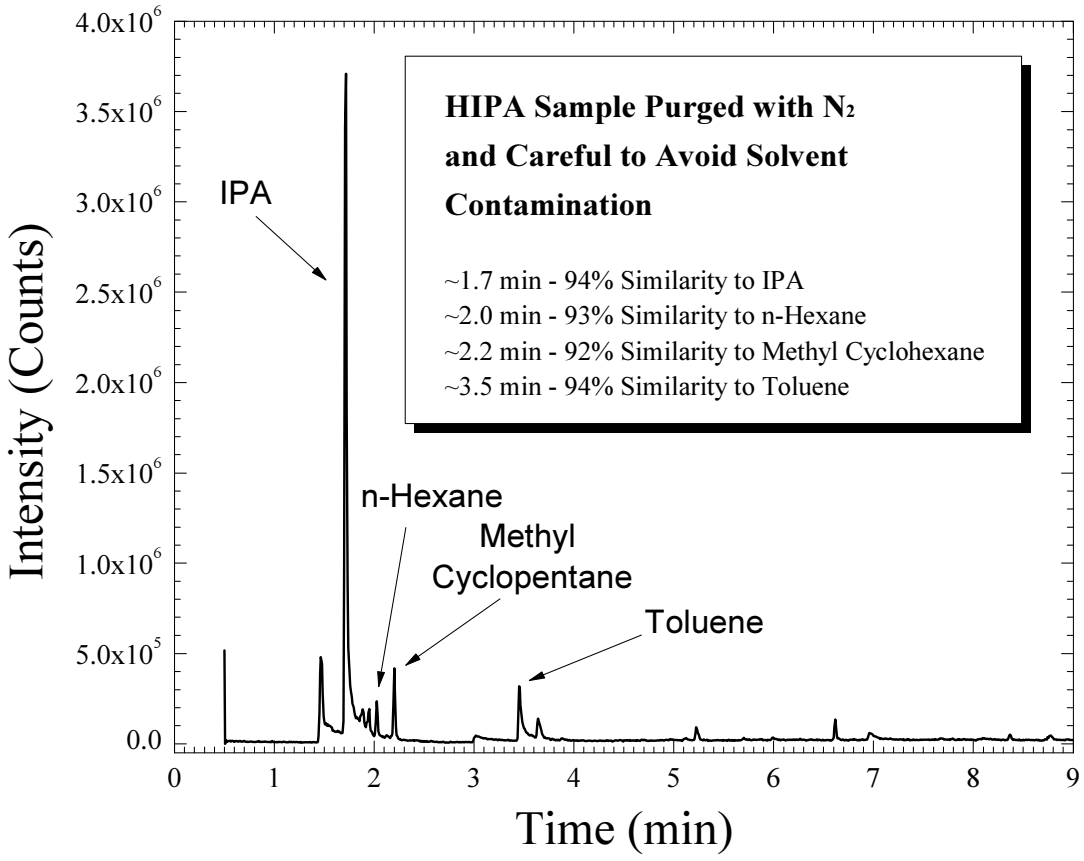

Figure 4.23: SPME-GC-MS chromatogram of a HIPA sample carefully prepared to avoid solvent contamination. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The headspace above the sample was purged with N_2 then allowed to equilibrate while stirring for 5 min before exposing the fiber.

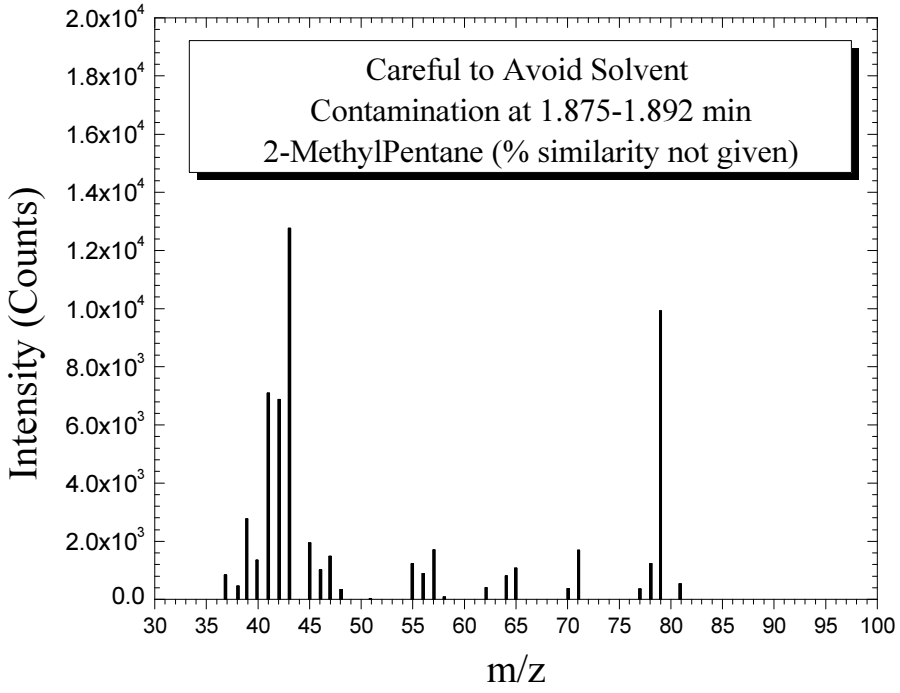

Figure 4.24: Averaged mass spectrum for 2-MethylPentane extracted from the chromatogram (1.875-1.892 min) in **Figure 4.23** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where care was taken to avoid solvent contamination during synthesis and the headspace was purged with N_2 gas).

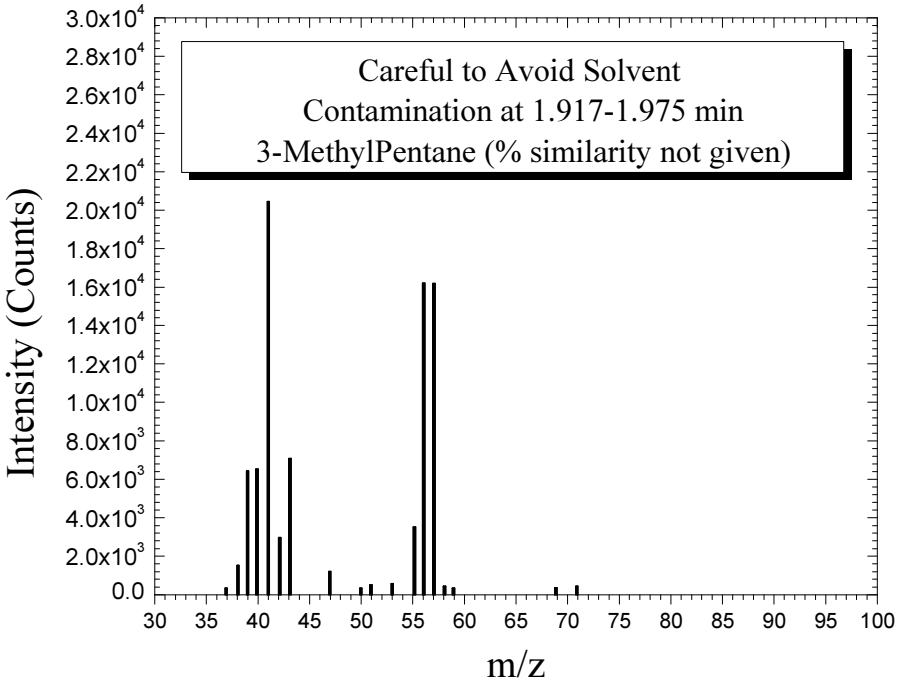

Figure 4.25: Averaged mass spectrum for 3-MethylPentane extracted from the chromatogram (1.917-1.975 min) in **Figure 4.23** (SPME/GC-MS of a typical HIPA/*n*C_{60} sample in water where care was taken to avoid solvent contamination during synthesis and the headspace was purged with N_2 gas).

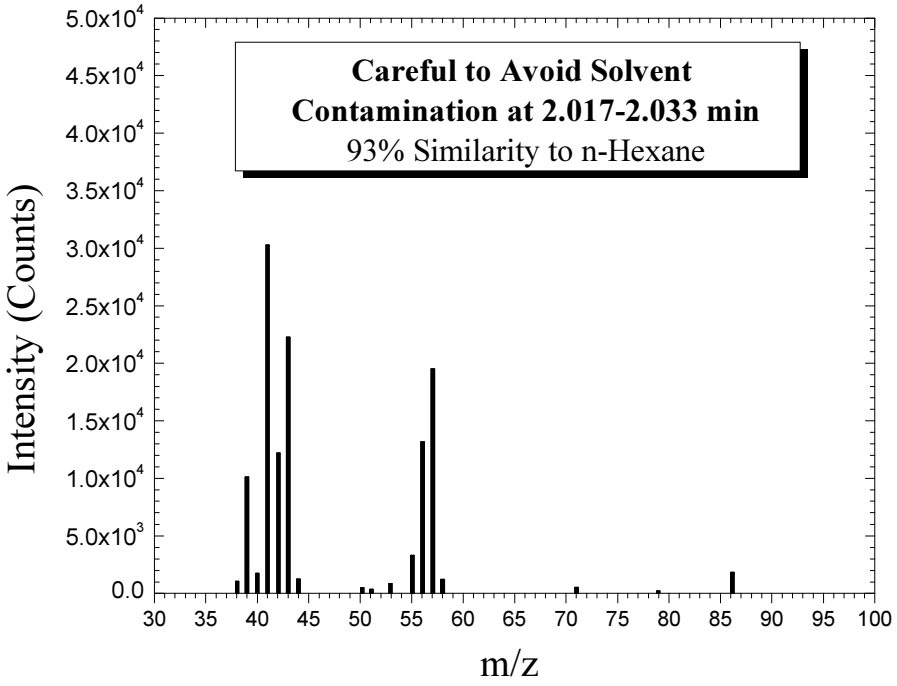

Figure 4.26: Averaged mass spectrum for n-Hexane extracted from the chromatogram (2.017-2.033 min) in **Figure 4.23** (SPME/GC-MS of a typical HIPA/nC$_{60}$ sample in water where care was taken to avoid solvent contamination during synthesis and the headspace was purged with N_2 gas).

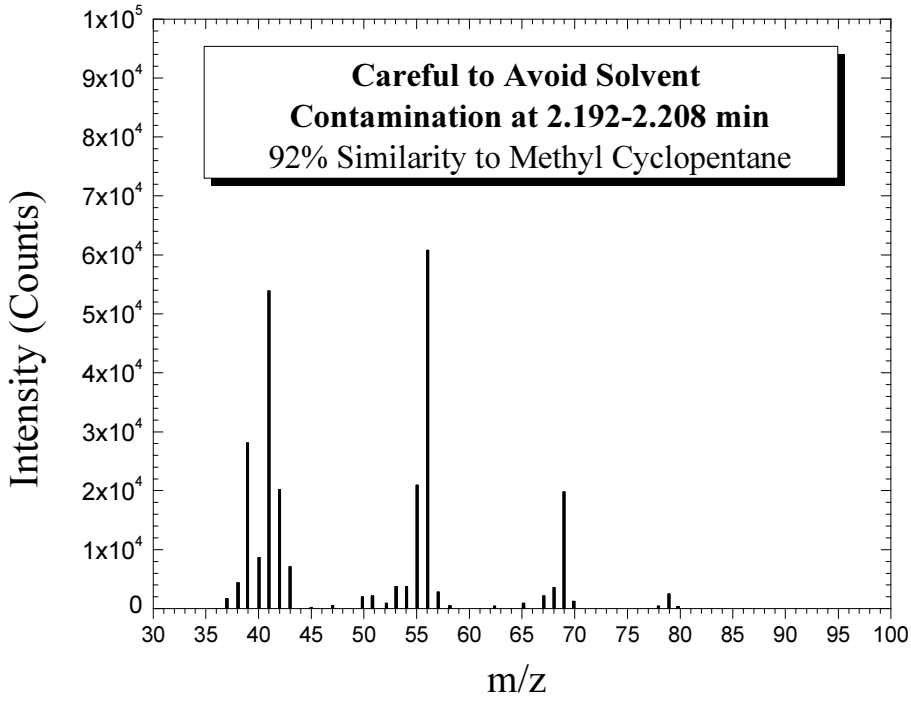

Figure 4.27: Averaged mass spectrum for Methyl Cyclopentane extracted from the chromatogram (2.192-2.208 min) in **Figure 4.23** (SPME/GC-MS of a typical HIPA/nC$_{60}$ sample in water where care was taken to avoid solvent contamination during synthesis and the headspace was purged with N_2 gas).

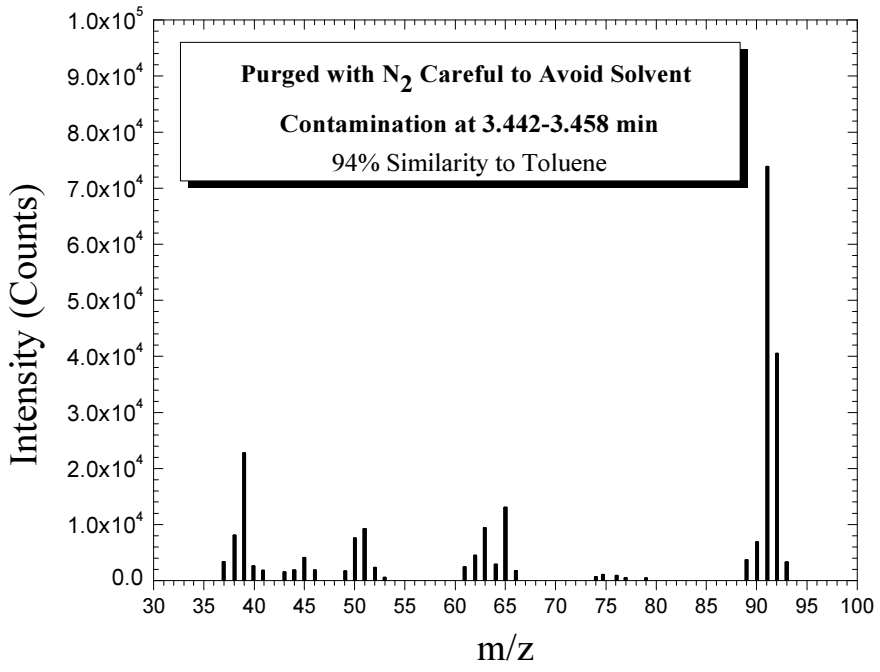

Figure 4.28: Averaged mass spectrum for toluene extracted from the chromatogram (3.442-3.458 min) in **Figure 4.23** (SPME/GC-MS of a typical HIPA/nC$_{60}$ sample in water where care was taken to avoid solvent contamination during synthesis and the headspace was purged with N_2 gas).

4.2.3 Quantification of Trace Solvents in HIPA/nC$_{60}$ Samples

Originally, it was thought that there would be no residual hexanes present in the final HIPA samples since hexanes are immiscible with water and have a vapor pressure that is significantly higher than that of water. However, **Figure 4.23** proves this hypothesis to be incorrect. Upon further research, it was found that hexanes do have a very small amount of solubility in water (9.5-18.3 ppm).[79-84] In order to quantify the amounts of hexanes and IPA present in HIPA samples, several standard solutions were prepared for these solvents in nanopure water. Calibration curves of concentration versus peak area were produced for both IPA and hexanes which are shown in **Figures 4.29** and **4.30** respectively. These plots show that the concentrations of IPA and hexanes in a typical HIPA/nC$_{60}$ sample are on the order of 17 ppm and < 1 ppm (based on the crude calibration plot in **Figure 4.30**) respectively.

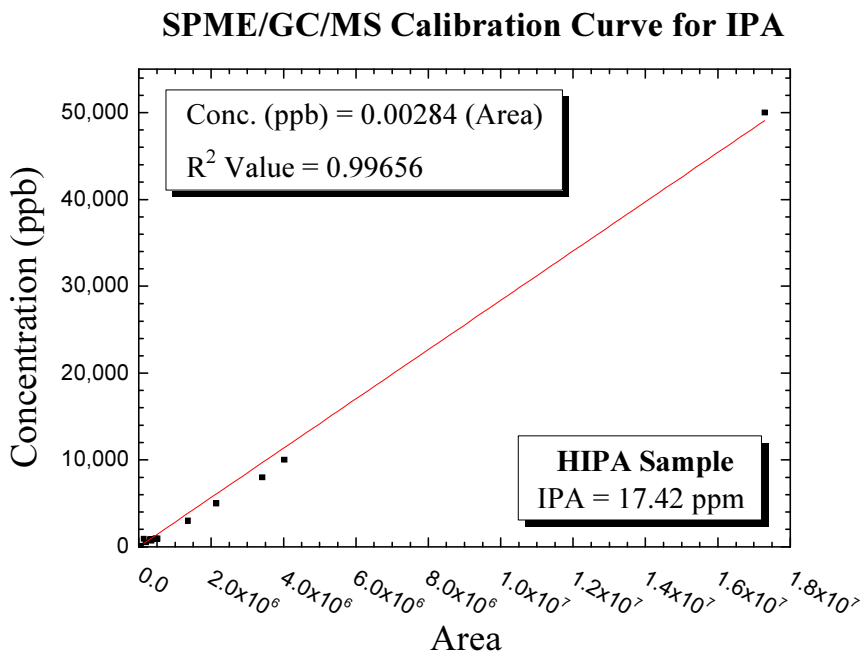

Figure 4.29: Calibration curve made from concentrations of IPA in water ranging from 25 ppb to 50 ppm. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The headspace above the sample was purged with N_2 then allowed to equilibrate while stirring for 5 min before exposing the fiber. The peak area in the chromatogram in **Figure 4.23** was applied to the given equation to calculate the concentration of IPA. Area is in counts/min.

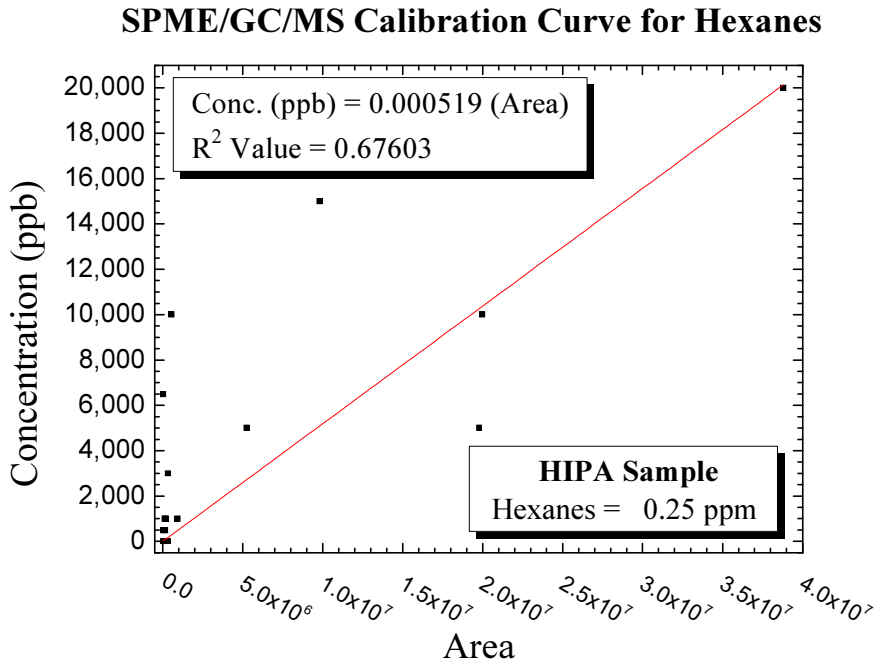

Figure 4.30: Calibration curve made from concentrations of hexanes in water ranging from 1 ppt to 20 ppm. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The headspace above the sample was purged with N_2 then allowed to equilibrate while stirring for 5 min before exposing the fiber. The peak area of the most abundant peak in the chromatogram in **Figure 4.23** (~2.2 min – Methyl Cyclopentane) was used to construct the calibration curve and applied to the given equation to calculate the concentration of hexanes. Area is in counts/min.

While the DVB/CAR/PDMS SPME fiber has been shown to efficiently extract the residual solvents in nC$_{60}$ samples (**Table 4.2** and **Figure 4.2**), **Figures 4.29** and particularly **4.30** suggest that there is a measure of irreproducibility. One major factor that helps explain some of the variability seen in **Figure 4.30** is given by the statement on the Supelco® website that "the 100 μm and 30 μm PDMS-coated fibers cannot be used with hexane." Although, no reason is given why this is the case.[85] The gray fiber used here is described as 50/30 μm DVB/CAR/PDMS. Although it is unclear in the description which coating layers the 50 μm and 30 μm are referring to, it is obvious from **Figures 4.29** and **4.30** that the quantification of hexanes is much less reliable than that of IPA.

There are numerous factors, other than fiber coating choice, which can affect the quantification of analytes by SPME: sample preparation, volume of headspace/sample, linear range for analyte/fiber combination, solubility of the analyte in the solvent used, temperature, stirring time/speed (if used), exposure time (fiber to sample), establishment of equilibrium, pH, and salt concentration. Many of these factors (volume, stirring, exposure time, equilibrium, pH, and salt concentration) should cause little variation in the results as these parameters were kept as consistent as possible and within the recommended limits.[77] Also, any variation in these factors would not explain the dramatic difference in linearity seen between IPA and hexanes in the calibration curves in **Figures 4.29** and **4.30**. Although there may have been fluctuations in the room temperature between some of the data points taken on different days, any effects from these fluctuations would be negligible as the same degree of variability was seen among samples taken from sample to sample within a single day. Thus, the most likely sources of variability in the hexanes samples would be 1) fiber choice, 2) sample preparation/solubility of analyte, and 3) linear range for the analyte/fiber combination.

The linear range for volatile analytes using SPME is typically around 1 ppb to 10 ppm but may vary some depending upon analyte polarity.[86] However, we are only interested in IPA concentrations in the ppm range that are present in HIPA samples. Including only the points within the range of 1-10 ppm in the calibration curve for IPA, provides a better linear regression fit to this data which can be seen in **Figure 4.31**. Using the equation from **Figure 4.31**, the concentration of IPA in the representative HIPA

sample is 14.7 ppm. In contrast, the data in the hexanes calibration curve does not show any linearity anywhere within the range shown in **Figure 4.30**. The fact that the range of data points in **Figure 4.30** is at or close to the actual solubility of hexanes in water, which is somewhere within the range of 9.5-18.3 ppm,[79-84] could explain a large part of the variability seen here. This solubility can affect the accuracy of the preparation of the "standard" solutions. Instead of being true solutions it is possible that each solution (at least the more concentrated solutions) could have a thin film of undissolved hexanes on the surface causing inaccurate dilutions for the entire series. This is especially true for the initial standard solution prepared at 20 ppm which is above the solubility for hexanes in water. However, starting with a standard solution of hexanes as low as 9 ppm (just below the reported solubility)[80, 81, 83] would require measuring 13.7 microliters of hexanes into 1 L of water which is not unreasonable but unfortunately was not considered during the collection of this data.

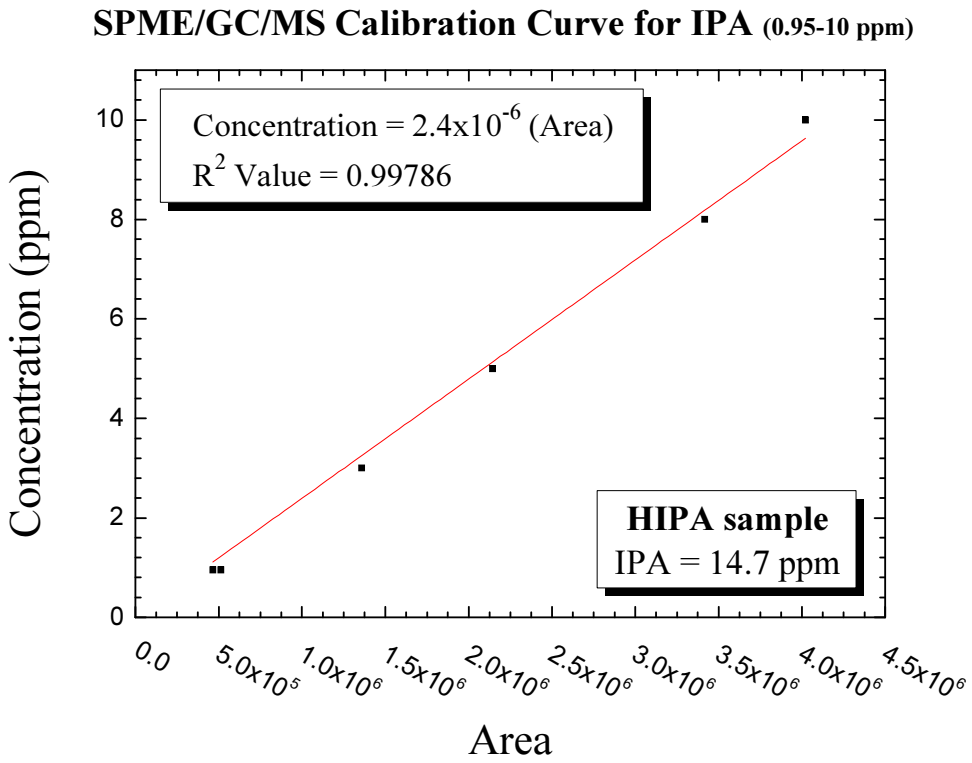

Figure 4.31: Calibration curve made from concentrations of IPA in water ranging from 0.95 ppm to 10 ppm. A DVB/CAR/PDMS SPME fiber was exposed for 30 min to the headspace of a 4 mL vial containing 2 mL of stirring sample. The headspace above the sample was purged with N_2 then allowed to equilibrate while stirring for 5 min before exposing the fiber. Area is in counts/min.

4.2.4 Significance of Trace Levels of Solvents in HIPA/nC_{60} Samples

Although the HIPA method was designed to avoid controversial solvents, the amounts of residual solvents would be useful information if these colloids were to be used in health and environmental safety studies. Quantifying the amount of residual solvents in HIPA/*n*C_{60} samples is vital in answering the question of whether or not residual IPA and hexanes will interfere with toxicity or oxidation studies for *n*C_{60} characterization. However, these solvents (IPA and hexanes in addition to ethanol) are extremely stable and are not known oxidants.

While hexanes are known to be toxic and dangerous to the environment, the levels present in HIPA/*n*C_{60} samples demonstrated here (~ < 1 ppm) are extremely unlikely to cause any negative effects to health and safety even if directly consumed or absorbed. No deaths have been reported in humans after exposure to n-hexane by any route.[79] Prolonged exposure (8-14 hours per day for six months to several years) of n-hexane to high concentrations (500 ppm to 2,500 ppm vapor) has caused significant toxic effects to the central nervous system (CNS) in workers.[79] Most of the workers recovered within six months to one year after removal from the contaminated environment without any lasting damage (more severe cases recovered within 1-2 years).[79]

The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) is 500 ppm for n-hexane in workplace air over an eight hour work period per day.[79] Additionally, hexane and solvents containing hexane are used to extract oils and protein from foods such as soy beans.[87] Some hexane can persist in the final food product created and has been measured to be as much as 10 ppm in soybean oil, 21 ppm in the resulting meal from soy bean processing, and 14 ppm in the grits from soy bean processing.[87] How much of this residual hexane ends up in the final packaged products on grocery store shelves has yet to be reported. The Food and Drug Administration (FDA) does not set a maximum level of hexane residue in soy foods and does not require that food manufacturers test for hexane residues.[87]

IPA would be expected to cause less harm than hexane if ingested as it gives similar effects to drinking alcohol (ethanol) and is sometimes ingested as a substitute for ethanol due to its low cost and availability. Although, ingestion of IPA is not recommended as it produces greater CNS depressant effects than ethanol at comparable concentrations (2-3 times more potent).[88-90] The OSHA PEL for IPA is 400 ppm total weight average (TWA) in workplace air over an eight hour period per day.[91] For comparison, the OSHA PEL for ethanol is 1000 ppm TWA.[92] According to a criteria document by the National Institute for Occupational Safety and Health, Kemal published results in 1927 showing that no acute effects were observed after the ingestion of 0.1-20.0 g (0.127-25.4 mL) of IPA by four healthy men in single quantities or in three repeated quantities of 5 g each at two hour intervals (in one case at three hour intervals).[93, 94] In 1969, Wills reported that daily ingestion of up to 6.4 mg/kg of IPA for six weeks produced no deleterious effects.[95] This amount would be equivalent to a 140 pound person ingesting ~ 0.5 mL of IPA daily which would relate to a significantly higher exposure than that from residual amounts in HIPA *n*C_{60} samples (~ 14-17 ppm).

4.3 Conclusions

A promising method has been found for quantifying the amount of residual solvents left in HIPA/*n*C_{60} samples using SPME-GC-MS techniques. The results shown here for IPA appear to be fairly accurate and reproducible; although, some work could be done to validate these results. There is yet much work to be done to improve the quantitation of hexanes. First and foremost, a sample of HIPA/*n*C_{60} should be made using pure n-hexane instead of hexanes. This would greatly reduce variability in the composition of the hexanes left in solution and make identification and quantitation much easier and more straightforward. Also, the CAR/PDMS SPME fiber may have shown slightly lower extraction efficiencies for most of the analytes of interest, but it is described as 85 μm CAR/PDMS suggesting that hexanes

should be more compatible with this fiber than the DVB/CAR/PDMS fiber used herein (30 and 100 μm PDMS-coated fibers are not compatible with hexane). Choosing a more compatible fiber, whether it be CAR/PDMS or a different fiber offered by Supelco®, would greatly improve the reproducibility of the quantification of hexanes. Additionally, there are several experimental procedures to improve or optimize that would increase reproducibility.

Another major contribution to reproducibility is error in the preparation of the standard solutions of hexanes in water. The initial solution should be less than the solubility limit (< 9.5 ppm for hexanes in water). The peak area for hexane in the HIPA samples should be a base target from which to build the calibration curve adding several samples with concentrations above and below this value. Other more subtle improvements to the experimental design include optimizing the extraction time, more closely monitoring the extraction time to keep it consistent, and either finding a method that keeps stirring/agitating the samples more consistent or refraining from stirring altogether (which might have negative effects in itself). Also, using an autosampler would greatly reduce the amount of variability in sampling in terms of consistency in extraction time, position of the fiber within the sample, and accuracy in retention time.

IPA and hexane should not interfere with oxidation and environmental studies for the characterization of *n*C_{60} samples (as does the more commonly used solvent tetrahydrofuran). However, if there is any concern about the toxicity of residual amounts of these particular solvents, IPA can be replaced with less toxic ethanol (using the same sample preparation procedure) and other solvents can be explored to replace hexane (provided that the vapor pressure is higher than that of ethanol/IPA).

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Summary of Current Work

Stable aqueous suspensions of C_{60}, nC_{60}, are complex systems that are poorly understood and need further study in order to describe their behavior. As with any new material, determining the environmental impact and cytotoxicity of nC_{60} is of great importance. Studies have shown nC_{60} suspensions to be harmful to some biological related systems.[29, 30] However, there is much controversy regarding the mechanisms underlying the toxicity of nC_{60}. Full characterization and a complete understanding of nC_{60} suspensions is necessary to interpret accurately the results of environmental impact and toxicity studies and can help solve the dispute among researchers that nC_{60} exhibits properties ranging from causing oxidative damage to acting as an anti-oxidant.

The behavior of particles in suspension is a direct function of their size, structure, and chemical characteristics.[54] Through different synthesis methods, it has been reported that the obtained suspensions have varied properties in terms of particle size, concentration, surface charge, surface chemistry, contaminant concentrations, synthesis reproducibility, and toxicity.[10, 13, 15, 17, 18, 68, 96, 97] Thus, differences in these physicochemical properties of nC_{60} produced by the various procedures could have important implications for the interpretation of data from environmental transport and toxicity studies and consequently contribute to our understanding of the ultimate fate of nC_{60} and that of its predecessor,

pristine C_{60}.[13] For this reason, the current work has focused on the development and improvement of methods for the characterization of residual solvents in $n$$C_{60}$ suspensions by SPME-GC and the development of a method for synthesizing $n$$C_{60}$ that will be ideal for use in toxicity studies as well as aid in the quantification of sources of oxidation by these suspensions that can contribute to their reported toxicity.

The work reported here represents new approaches for producing aqueous $n$$C_{60}$ colloidal suspensions that retain the attractive gradual solvent-quality transition that successive solvent exchange promises but that address the shortcomings of the TTA method. Two series of solvents were examined where both fullerene solubility and solvent vapor pressure decrease successively to controllably induce colloid seeding and growth while removing the solvents in the same order in which they were added. The most successful of these methods, termed HIPA, involves a transfer of the fullerenes from hexane to isopropyl alcohol (IPA) and then to water. The second of these methods, termed HEA, replaces the IPA with ethanol. These approaches improve the yield of $n$$C_{60}$, avoid the use of controversial solvents such as tetrahydrofuran, and shorten the synthesis time. It is likely that this method, upon further study, will be able to control the resulting particle size, similar to the TTA method.[48] Additionally, HIPA/$n$$C_{60}$ samples often contain no detectable amounts of $C_{60}O$ which is suspected to be a contributor to oxidative damage exhibited by $n$$C_{60}$ samples. The lack of the use of controversial solvents in conjunction with the comparison of $n$$C_{60}$ samples containing no detectable $C_{60}O$ with samples that have been intentionally enriched with $C_{60}O$ along with the combined advantages of other methods make the HIPA method ideal for producing $n$$C_{60}$ samples to be used in environmental, toxicity, and oxidation studies.

Improvements were made to an existing method for quantifying residual solvents in $n$$C_{60}$ samples, and new procedures were established for this determination. Previously, residual solvents were extracted into an organic solvent (dichloromethane) through a long, tedious process then injected into a GC with an FID detector. This method, while sufficient for quantifying acetone, toluene, and tetrahydrofuran to a limit of 1 ppm, was not suitable for quantifying hexanes, IPA, and ethanol due to an

overlap of these solvent peaks with the large dichloromethane solvent peak. A single procedure was needed to quantify all of the solvents used in the main nC$_{60}$ synthesis methods established in the literature being studied by this group (TTA, THF, SON, HIPA, and HEA – AQU does not use organic solvents). However, using the existing method, a suitable solvent could not be found to extract and quantify all of these solvents.

A method using SPME with reported detection limits from ppb to ppt was adopted in order to shorten the quantification procedure, lower detection limits, and eliminate the huge interfering solvent peak in the GC chromatogram. Eventually, the use of mass spectroscopy (MS) became available and was included in the procedure which made it possible to distinguish the solvents of interest from each other and from extraneous peaks due to solvent contamination among other sources. During the method development process for implementing SPME and MS, it was determined that it is necessary to thoroughly clean any glassware used, dry it overnight in an oven, then seal it immediately upon removal from the oven to avoid contamination of the samples to be analyzed. Also, it was found necessary to nitrogen purge the final prepared sample vials to be analyzed to avoid detecting traces of solvent in the air (particularly acetone and toluene). Although there is still much work to be done to improve the current method, it was determined that residual IPA in a typical nC$_{60}$ sample is on the order of 14-17 ppm and residual hexanes are on the order of less than 1 ppm. These levels are low enough that these particular solvents should not cause concern in or interfere with toxicity, environmental, and oxidation studies.

The inherent problem with the current SPME-GC-MS method for quantifying residual solvents in nC$_{60}$ samples is the lack of reproducibility with respect to hexanes as seen in **Figure 4.30**. A number of ways to improve this method have been identified that include using pure n-hexane to make HIPA-nC$_{60}$ samples (to avoid confusion and inconsistencies), finding an SPME fiber that works for all the solvents of interest and is more compatible with hexane, more accurately preparing standard solutions for the calibration curve, optimizing the sampling procedure (fiber exposure time, stirring/agitation, N_2 purge time, etc.), and using an autosampler (to reduce variability in sampling/injection). Although there is still much work left to be done to optimize the current method, much progress has been made from the

previous method. Results have shown reasonable reproducibility for IPA and it appears likely that the results for hexane can be greatly improved. Future directions in this work involve establishing calibration curves for the quantification of the remaining solvents used in the synthesis of nC$_{60}$ (ethanol, toluene, tetrahydrofuran, and acetone).

5.2 Future Directions

Several other tasks were accomplished during the timeframe of this work that have not been presented here and yet many other tasks remain to answer certain key questions in this research. These tasks, while in part are relevant to the present work, are largely part of other on-going projects within the same research group which encompass three main topics: (1) continuing efforts to fully characterize nC$_{60}$ suspensions as well as the possibility for controlling particle size during the synthesis of nC$_{60}$ suspensions (particularly for HIPA samples), (2) determining the source of stabilization for nC$_{60}$ produced by the various synthesis methods, and (3) quantifying the sources of oxidation by nC$_{60}$ suspensions that can contribute to their reported toxicity. These topics are addressed in summary below in order to outline future directions for this research.

5.2.1 Continued Method Development

While the next steps concerning the method development for trace solvent analysis have been discussed above, this section focuses on determining the best way to extract the C$_{60}$ in nC$_{60}$ suspensions back into toluene for either concentration or derivative analysis. Also discussed is further development of

the HIPA synthesis method in order to control the average size of the nC$_{60}$ particles in the resulting suspensions.

It was originally thought that if the HIPA method was modeled after the TTA method by gradually reducing the C$_{60}$ solubility, the resulting particle size could be controlled by particle seeding and subsequent growth. Since the TTA method has been shown to control particle size by altering the solvent volumes,[48] this experiment was tried initially with the HIPA method. However, instead of seeing differences in particle size with varying solvent volume ratios, a difference in the resulting suspension concentrations was observed. Preliminary data shows that the C$_{60}$ yield increases with an increase in the IPA/water ratio.

Not a lot of effort was devoted to particle size control efforts during the course of this study, but a few experiments in addition to varying solvent volumes were explored. As part of the initial HIPA method development, different solvent removal rates were tried with the result being that if the solvents were removed either too fast or too slow, most of the C$_{60}$ would crash out of the mixture giving a low yield in the final suspension. A very limited number of samples were synthesized varying the length of time that the C$_{60}$/hexane/IPA mixture was stirred (0 min, 30 min, and 3 days). This brief test did not show significant differences in the resulting particle size. However, some evidence has suggested that the length of time C$_{60}$ remains in the 'pure' IPA in the step between removing hexanes and adding water in some way does affect the particle size. Since the C$_{60}$ concentration in this step exceeds its solubility in IPA, the particles tend to aggregate over a relatively short amount of time (hence the filtration step before the addition of water). There may be some possibility of controlling particle size by further exploring the dynamics of this step in the HIPA synthesis procedure.

The THF method has been show to control particle size by varying the solvent addition rate.[16] Some attempts were made to replicate this with the HIPA method using different types of glassware to add the solvents (in an attempt to accurately control both the addition rate as well as the way in which the solvent was added from sample to sample). However, the results of this experiment were never known due to difficulties with instrumentation. Solvent addition has a high potential for controlling particle size

as this is when particle seeding and the subsequent growth begins. Controlling the speed and manner in which solvents are added to the system is potentially a critical step and should consist of much of the efforts in moving forward with this goal in further development of the HIPA synthesis method.

Probably the most promising effort in exploring particle size control (from the limited efforts described here) has been adding multiple aliquots of C_{60} dissolved in hexane (start with C_{60} in hexane, add IPA, remove most of the hexane, then add another aliquot of C_{60} in hexane). A second addition of C_{60} in hexane with the volume of the second varying did not result in any significant differences in particle size. Similarly the addition of second, third, and fourth aliquots did not make a significant difference. However, an increase in particle size was seen upon making a second addition equal in volume to the first while increasing the volume of the C_{60} solution in hexane (thus increasing the overall C_{60} concentration). These experiments suggest that a likely way to control $n$$C_{60}$ particle size via the HIPA synthesis method is to either vary the initial volume of the C_{60}/hexane solution adding a second equal volume aliquot of C_{60}/hexane or possibly to simply vary the initial C_{60} concentration in the first step of the published procedure.

As described here, there is much work left to be done in the area of particle size control research via the HIPA synthesis method. However, before this work is carried out, it would be wise to further refine the current method in order to achieve more reproducible sample to sample particle size. As mentioned above, controlling the solvent addition step is very important. Instead of manually pouring in the solvent, which can give varied results from sample to sample, a more consistent method of solvent addition should be utilized. For instance, a more evenly distributed misting of solvent could be introduced. Once the method is finalized, then many of the experiments described above should be revisited as some evidence has been seen that suggests the possibility of controlling $n$$C_{60}$ particle size.

Other method development tasks include determining the best way to extract C_{60} back into toluene for the characterization of $n$$C_{60}$ samples (notably concentration determination and derivative analysis). The working method of extraction consists of mixing an aliquot of $n$$C_{60}$, toluene, and an inorganic salt ($NaNO_3$); either sonicating or stirring this mixture to facilitate the extraction; then

separating out the toluene layer for analysis (when working with HPLC, the toluene layer is dried at this point with Na_2SO_4). Differences in the concentrations of C_{60} and its main derivative $C_{60}O$ have been seen when comparing sonication versus stirring as well as upon drying and not drying the toluene layer with Na_2SO_4. Thus, a series of experiments have been performed to sort out the best method of extraction for each type of characterization but thus far no conclusive data has been acquired and efforts in this area of research continue. Also, the benefits of using UV absorption versus an HPLC gravimetric calibration curve to calculate the concentration of C_{60} is being explored.

5.2.2 Stabilization of nC_{60} Suspensions

Strong evidence has been presented showing that $C_{60}O$ plays a vital role in the stabilization of AQU nC_{60} suspensions.[40] However, as discussed in **Section 3.4**, the HIPA method often produces samples that do not contain any detectable amounts of $C_{60}O$. A simple experiment (described in **Section 3.4**) showed evidence that residual IPA can play a small role in the formation and stability of nC_{60} particles in water although this is not the full explanation. Zeta-potential measurements would be useful in understanding the surface chemistry of the colloids as well as provide some indication of their stability. Surface charge and surface chemistry are also important factors in studying and understanding nC_{60}'s effect on the environment such as transport, fate, and toxicity. Unfortunately, an available instrument that can measure zeta-potential could not be found during the course of this research and the search continues to find such an instrument. In addition to the attempt of measuring the zeta-potential, efforts continue to explain the formation and stability of nC_{60} particles in water via the different solvent exchange synthesis methods.

Early evidence (unpublished) suggested that nC_{60} colloids may be unstable above 50 ˚C as the C_{60} had a tendency to crash out of suspension when heated during the synthesis procedure. The model proposed to explain this behavior was that above a certain temperature (50 ˚C) there is sufficient energy

in the system to allow $C_{60}O$ to combine with C_{60} to form $C_{120}O$ (a dimer). As part of an ongoing project to test this proposal, an effort was made to deliberately synthesize $C_{120}O$. This was achieved by following a published procedure[98] involving producing a $C_{60}O$ enriched solution, drying this solution down, then heating the solids in a vacuum oven at 200 ˚C for 1 hour. The HPLC chromatogram and UV spectrum of a solution of this $C_{120}O$ could then be compared to a solution of C_{60} made from a dried down nC_{60} suspension that had been heated above 50 ˚C for some time. While $C_{120}O$ is believed to have been successfully synthesized as part of the work presented here, the results of this experiment have yet to be determined.

Published reports typically state that the nC_{60} suspensions used in those studies were stable over a certain period of time or under certain conditions (such as varying pH or ionic strength) and often give zeta potential measurements, but there have been no known reports of a direct comparison of the relative stabilities of suspensions produced by the various synthesis methods. This kind of study might involve making reliable measurements of particle size (by DLS verified by TEM) and zeta potential for suspensions produced by all of the common synthesis methods periodically over several months and under various conditions (light, dark, ambient atmosphere, inert atmosphere, pH, ionic strength, temperature, etc.). These experiments should be repeated in order to verify reproducibility. Efforts to accomplish this task would be beneficial in better understanding nC_{60} suspensions, their behavior in controlled studies, and their potential impact on the environment. These experiments would also serve as a guideline for determining and comparing the stability of nC_{60} suspensions produced in other researcher's labs and by additional variations of synthesis methods.

5.2.3 Sources of Oxidation by nC_{60} Suspensions

Once the HIPA method has been fully developed (hopefully producing controllable particle sizes), well refined, and fully characterized, the resulting suspensions will be more useful in other

applications such as aiding in the quantification of sources of oxidation by nC$_{60}$. Preliminary oxidation studies using dihydrorhodamine-123 (DHR-123) as a probe molecule show that HIPA/nC$_{60}$ samples exhibit less oxidative behavior than THF/nC$_{60}$ samples and roughly the same amount as SON/nC$_{60}$ samples. An obvious explanation for this difference is that the presence of peroxides in residual tetrahydrofuran causes oxidation of the probe molecule. HIPA and SON samples (which do not use tetrahydrofuran during synthesis) do not contain any peroxides or other oxidative interferents. Another source of oxidation that has been proposed is the presence of C$_{60}$O. Early HIPA samples used in the preliminary oxidation studies mentioned above contained significant amounts of C$_{60}$O because they were made from an old stock of C$_{60}$ that was later found to contain 0.2 % C$_{60}$O. However, samples made with fresh C$_{60}$ that is free of C$_{60}$O often contain no measureable amounts of C$_{60}$O. These samples can be used in similar oxidation studies compared to C$_{60}$O enriched samples in order to determine if C$_{60}$O plays a role in any oxidative behavior exhibited by nC$_{60}$ samples.

REFERENCES

1. Kroto, H. W.; Heath, J. R.; O'Brian, S. C.; Curl, R. F.; Smalley, R. E., C60: Buckminsterfullerene. *Nature* **1985,** *318*, 162-163.
2. Kratschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R., Solid C(60): A New Form of Carbon. *Nature* **1990,** *347*, 354-358.
3. Murayama, H.; Tomonoh, S.; Alford, J. M.; Karpuk, M. E., Fullerene production in tons and more: From Science to Industry. *Fullerenes Nanotubes and Carbon Nanostructures* **2004,** *12*, 1-9.
4. Arbogast, J. W.; Darmanyan, A. P.; Foote, C. S.; Rubin, Y.; Diederich, F. N.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L., Photophysical Properties of C60. *The Journal of Physical Chemistry* **1991,** *95*, 11-12.
5. Kamat, J. P.; Devasagayam, T. P. A.; Priyadarsini, K. I.; Mohan, H.; Mittal, J. P., Oxidative Damage Induced by the Fullerene C60 on Photosensitization in Rat Liver Microsomes. *Chemico-Biological Interactions* **1998,** *114*, 145-159.
6. Chiang, L. Y.; Swirczewski, J. W.; Hsu, C. S.; Chowdhury, S. K.; Cameron, S.; Creegan, K., Multi-hydroxy Additions onto C60 Fullerene Molecules. *Journal of the Chemical Society-Chemical Communications* **1992,** (24), 1791-1793.
7. Sayes, C. M.; Fortner, J. D.; Guo, W.; Lyon, D. Y.; Boyd, A. M.; Tao, Y. J.; Sitharaman, B.; Wilson, L. J.; Hughes, J. B.; West, J. L.; Colvin, V. L., The differential cytotoxicity of water-soluble fullerenes. *Nano Letters* **2004,** *4* (10), 1881-1887.
8. Torres, V. M.; Posa, M.; Srdjenovic, B.; Simplicio, A. L., Solubilization of Fullerene C-60 in Micellar Solutions of Different Solubilizers. *Coll. Surf. B: Biointerfaces* **2011,** *82* (1), 46-53.
9. Bosi, S.; Da Ros, T.; Spalluto, G.; Prato, M., Fullerene Derivatives: an Attractive Tool for Biological Applications. *European Journal of Medicinal Chemistry* **2003,** *38*, 913-923.
10. Andrievsky, G.; Kosevich, M. V.; Vovk, O. M.; Shelkovsky, V. S.; Vashchenko, L. A., On the production of an aqueous colloidal solution of fullerenes. *J. Chem. Soc. Chem. Commun.* **1995,** *12*, 1281-1282.

11. Brant, J.; Lecoanet, H.; Hotze, M.; Wiesner, M., Comparison of Electrokinetic Properties of Colloidal Fullerenes (n-C60) Formed Using Two Procedures. *Environ. Sci. Technol.* **2005,** *39* (17), 6343-6351.
12. Brant, J.; Lecoanet, H.; Wiesner, M. R., Aggregation and Deposition Characteristics of Fullerene Nanoparticles in Aqueous Systems. *J. Nanopart. Res.* **2005,** *7* (4-5), 545-553.
13. Brant, J. A.; Labille, J.; Bottero, J. Y.; Wiesner, M. R., Characterizing the impact of preparation method on fullerene cluster structure and chemistry. *Langmuir* **2006,** *22* (8), 3878-3885.
14. Cheng, X.; Kan, A. T.; Tomson, M. B., Naphthalene Adsorption and Desorption from Aqueous C60 Fullerene. *j. Chem. Eng. Data* **2004,** *49* (3), 675-683.
15. Deguchi, S.; Alargove, r. G.; Tsujii, K., Stable dispersions of fullerenes, C_{60} and C_{70}, in water. Preparation and characterization. *Langmuir* **2001,** *17* (19), 6013-6017.
16. Fortner, J. D.; Lyon, D. Y.; Sayes, C. M.; Boyd, A. M.; Falkner, J. C.; Hotze, E. M.; Alemany, L. B.; Tao, Y. J.; Guo, W.; Ausman, K. D.; Colvin, V. L.; Hughes, J. B., C_{60} in water: nanocrystal formation and microbial response. *Environ. Sci. Technol.* **2005,** *39* (11), 4307-4316.
17. Labille, J.; Brant, J.; Villieras, F.; Pelletier, M.; Thill, A.; Masion, A.; Wiesner, M.; Rose, J.; Bottero, J. Y., Affinity of C-60 fullerenes with water. *Fullerenes Nanotubes and Carbon Nanostructures* **2006,** *14* (2-3), 307-314.
18. Scrivens, W. A.; Tour, J. M.; Creek, K. E.; Pirisi, L., Synthesis of ^{14}C labeled C_{60}, its suspension in water, and its uptake by human keratinocytes. *J. Am. Chem. Soc.* **1994,** *116* (10), 4517-4518.
19. Andrievsky, G.; Klochkov, V.; Derevyanchenko, L., Is the C_{60} fullerene molecule toxic? *Fullerenes, Nanotubes and Carbon Nanostructures* **2005,** *13* (4), 363-376.
20. Dhawan, A.; Taurozzi, J. S.; Pandey, A. K.; Shan, W.; Miller, S. M.; Hashsham, S. A.; Tarabara, V. V., Stable colloidal dispersions of C_{60} fullerenes in water: evidence for genotoxicity. *Environ. Sci. Technol.* **2006,** *40* (23), 7394-7401.
21. Gharbi, N.; Pressac, M.; Hadchouel, M.; Szwarc, H.; Wilson, S. R.; Moussa, F., [60]fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett* **2005,** *5* (12), 2578-85.
22. Henry, T. B.; Menn, F. M.; Fleming, J. T.; Wilgus, J.; Compton, R. N.; Sayler, G. S., Attributing effects of aqueous C_{60} nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression *Environ. Health Perspect.* **2007,** *115* (7), 1059-1065.
23. Isakovic, A.; Markovic, Z.; Nikolic, N.; Todorovic-Markovic, B.; Vranjes-Djuric, S.; Harhaji, L.; Raicevi, N.; Romcevic, N.; Vasiljevic-Radovic, D.; Dramicanin, M.; Trajkovic, V., Inactivation of nanocrystalline C-60 cytotoxicity by gamma-irradiation. *Biomaterials* **2006,** *27* (29), 5049-5058.

24. Isakovic, A.; Markovic, Z.; Todorovic-Markovic, B.; Nikolic, N.; Vranjes-Djuric, S.; Mirkovic, M.; Dramicanin, M.; Harhaji, L.; Raicevic, N.; Nikolic, Z.; Trajkovic, V., Distinct cytotoxic mechanisms of pristine versus hydroxylated fullerene. *Toxicological Sciences* **2006,** *91* (1), 173-183.

25. Lee, J.; Fortner, J. D.; Hughes, J. B.; Kim, J.-H., Photochemical Production of Reactive Oxygen Species by C60 in the Aqueous Phase During UV Irradiation. *Environ. Sci. Technol.* **2007,** *41*, 2529-2535.

26. Lyon, D. Y.; Alvarez, P. J. J., Fullerene Water Suspension (nC(60)) Exerts Antibacterial Effects via ROS-Independent Protein Oxidation. *Environ. Sci. Technol.* **2008,** *42* (21), 8127-8132.

27. Lyon, D. Y.; Brunet, L.; Hinkal, G. W.; Wiesner, M. R.; Alvarez, P. J. J., Antibacterial activity of fullerene water suspensions (nC(60)) is not due to ROS-mediated damage. *Nano Letters* **2008,** *8* (5), 1539-1543.

28. Markovic, Z.; Todorovic-Markovic, B.; Kleut, D.; Nikolic, N.; Vranjes-Djuric; Misirkic, M.; Vucicevic, L.; Janjetovic, K.; Isakovic, A.; Harhaji, L.; Babic-Stojic, B.; Dramicanin, M.; Trajkovic, V., The Mechanism of Cell-damaging Reactive Oxygen Generation by Colloidal Fullerenes. *Biomaterials* **2007,** *28*, 5437-5448.

29. Oberdorster, E., Manufactured nanomaterials (fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth bass. *Environ. Health Perspect.* **2004,** *112* (10), 1058-1062.

30. Sayes, C. M.; Gobin, A. M.; Ausman, K. D.; Mendez, J.; West, J. L.; Colvin, V. L., Nano-C_{60} cytotoxicity is due to lipid peroxidation. *Biomaterial* **2005,** *26*, 7587-7595.

31. Spohn, P.; Hirsch, C.; Hasler, F.; Bruinink, A.; Krug, H. F.; Wick, P., C60 fullerene: A powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays. *Environ. Pollut.* **2009,** (157), 1134-1139.

32. Tao, X. J.; Fortner, J. D.; Zhang, B.; He, Y. H.; Chen, Y. S.; Hughes, J. B., Effects of aqueous stable fullerene nanocrystals (nC(60)) on Daphnia magna: Evaluation of sub-lethal reproductive responses and accumulation. *Chemosphere* **2009,** *77* (11), 1482-1487.

33. Valant, J.; Drobne, D.; Sepcic, K.; Jemec, A.; Kogej, K.; Kostanjsek, R., Hazardous potential of manufactured nanoparticles identified by in vivo assay. *J. Hazard. Mater.* **2009,** *171* (1-3), 160-165.

34. Zhang, B.; Cho, M.; Fortner, J. D.; Lee, J.; Huang, C. H.; Hughes, J. B.; Kim, J. H., Delineating Oxidative Processes of Aqueous C-60 Preparations: Role of THF Peroxide. *Environ. Sci. Technol.* **2009,** *43* (1), 108-113.

35. Zhu, S. Q.; Oberdorster, E.; Haasch, M. L., Toxicity of an engineered nanoparticle (fullerene, C-60) in two aquatic species, Daphnia and fathead minnow. *Mar. Environ. Res.* **2006,** *62*, S5-S9.

36. Zogovic, N. S.; Nikolic, N. S.; Vranjes-Djuric, S. D.; Harhaji, L. M.; Vucicevic, L. M.; Janjetovic, K. D.; Misirkic, M. S.; Todorovic-Markovic, B. M.; Markovic, Z. M.; Milonjic, S. K.; Trajkovic, V. S., Opposite effects of nanocrystalline fullerene (C-60) on tumour cell growth in vitro and in vivo and a possible role of immunosupression in the cancer-promoting activity of C-60. *Biomaterials* **2009,** *30* (36), 6940-6946.

37. Zhu, X.; Zhu, L.; Lang, Y.; Chen, Y., Oxidative Stress and Growth Inhibition in the Freshwater Fish *Carassius auratus* Induced by Chronic Exposure to Sublethal Fullerene Aggregates. *Environmental Toxicology and Chemistry* **2008,** *27*, 1979-1985.

38. Levi, N.; Hantgan, R. R.; Lively, M. O.; Carroll, D. L.; Prasad, G. L., C60-Fullerenes: Detection of Intracellular Photoluminescence and Lack of Cytotoxic Effects. *Journal of Nanobiotechnology* **2006,** *4* (14).

39. Shinohara, N.; Matsumoto, T.; Gamo, M.; Miyauchi, A.; Endo, S.; Yonezawa, Y.; Nakanishi, J., Is Lipid Peroxidation Induced by the Aqueous Suspension of Fullerene C60 Nanoparticles in the Brains of *Cyprinus carpio*? *Environmental Science & Technology* **2009,** *43*, 948-953.

40. Murdianti, B. S.; Damron, J. T.; Hilburn, M. E.; Maples, R. D.; Hikkaduwa Koralege, R. S.; Kuriyavar, S. I.; Ausman, K. D., C60 Oxide as a Key Component of Aqueous C60 Colloidal Suspensions. *Envronmental Science & Technology* **2012,** *46*, 7446-7453.

41. Duncan, L. K.; Jinschek, J. R.; Vikesland, P. J., C60 Colloid Formation in Aqueous Systems: Effects of Preparation Method on Size, Structure, and Surface Charge. *Environmental Science & Technology* **2008,** *42*, 173-178.

42. Espinasse, B.; Hotze, E. M.; Weisner, M., Transport and Retention of Colloidal Aggregates of C60 in Porous Media: Effects of Organic Macromolecules, Ionic Composition, and Preparation Method. *Environmental Science & Technology* **2007,** *41*, 7396-7402.

43. Kulkarni, P. P.; Jafvert, C. T., Solubility of C60 in Solvent Mixtures. *Environmental Science & Technology* **2008,** *42*, 845-851.

44. Lyon, D. Y.; Adams, L. K.; Falkner, J. C.; Alvarezt, P. J., Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size. *Environ Sci Technol* **2006,** *40* (14), 4360-6.

45. Bulgakov, R. G.; Nevyadovskii, E. Y.; Belyaeva, A. S.; DGolikova, M. T.; Ushakova, Z. I.; Ponomareva, Y. G.; Dzhemilev, U. M.; Razumovskii, S. D.; Valyamova, F. G., Water-Soluble Polyketones and Esters as the Main Stable Products of Ozonolysis of Fullerene C60 Solutions. *Russian Chemical Bulletin, International Edition* **2004,** *53* (1), 148-159.

46. Fortner, J. D. K.; Doo-IL; Boyd, A. M.; Falkner, J. C.; Moran, S.; Colvin, V. L.; Hughes, J. B.; Kim, J. H., Reaction of Water-Stable C60 Aggregates with Ozone. *Environmental Science & Technology* **2007,** *41*, 7497-7502.

47. Xie, B.; Xu, Z.; Guo, W.; Li, Q., Impact of Natural Organic Matter on the Physicochemical Properties of Aqueous C60 Nanoparticles. *Environmental Science & Technology* **2008,** *42*, 2853-2859.

48. Maples, R. D.; Hilburn, M. E.; Murdianti, B. S.; Hikkaduwa Koralege, R. S.; Williams, J. S.; Kuriyavar, S. I.; Ausman, K. D., Optimized solvent-exchange synthesis method for C(60) colloidal dispersions. *J Colloid Interface Sci* **2012,** *370*, 27-31.

49. Deguchi, S.; Alargova, R. G.; Tsujii, K., Stable dispersions of fullerenes, C-60 and C-70, in water. Preparation and characterization. *Langmuir* **2001,** *17* (19), 6013-6017.

50. Fortner, J. D.; Lyon, D. Y.; Sayes, C. M.; Boyd, A. M.; Falkner, J. C.; Hotze, E. M.; Alemany, L. B.; Tao, Y. J.; Guo, W.; Ausman, K. D.; Colvin, V. L.; Hughes, J. B., C-60 in water: Nanocrystal formation and microbial response. *Environmental Science & Technology* **2005,** *39* (11), 4307-4316.

51. Andrievsky, G. V.; Kosevich, M. V.; Vovk, O. M.; Shelkovsky, V. S.; Vashchenko, L. A., On the Production of an Aqueous Colloidal Solution of Fullerenes. *Journal of the Chemical Society-Chemical Communications* **1995,** (12), 1281-1282.

52. Andersson, M.; Agurell, E.; Vaghef, H.; Bolcsfoldi, G.; Hellman, B., Extended-term Cultures of Human T-lymphocytes and the Comet Assay: A Useful Combination When Testing for Genotoxicity in Vitro? *Mutation Research* **2003,** *540*, 43-55.

53. Jafvert, C. T.; Kulkarni, P. P., Buckminsterfullerene's (C60) Octanol-Water Partition Coefficient (Kow) and Aqueous Solubility. *Environmental Science & Technology* **2008,** *42*, 5945-5950.

54. Weibel, A.; Bouchet, R.; Boulc'h, F.; Knauth, P., The Big Problem of Small Particles: A Comparison of Methods for Determination of Particle Size in Nanocrystalline Anatase Powders. *Chemistry of Materials* **2005,** *17*, 2378-2385.

55. Hadduck, A. N.; Hindagolla, V.; Contreras, A. E.; Li, Q.; Bakalinsky, A. T., Does Aqueous Fullerene Inhibit the Growth of *Saccharomyces cerevisiae* or *Escherichia coli*? *Applied and Environmental Microbiology* **2010,** *76* (24), 8239-8242.

56. Kovochich, M.; Espinasse, B.; Auffan, M.; Hotze, E. M.; Wessel, L.; Xia, T.; Nel, A. E.; Wiesner, M. R., Comparative Toxicity of C60 Aggregates Toward Mammalian Cells: Role of Tetrahydrofuran (THF) Decomposition. *Environmental Science & Technology* **2009,** *43*, 6378-6384.

57. Weisman, R. B.; Heymann, D.; Bachilo, S. M., Synthesis and Characterization of the "Missing" Oxide of C60: [5,6]-Open C60O. *J. Am. Chem. Soc.* **2001,** *123*, 9720-9721.

58. Andrievsky, G. V.; Klochkov, V. K.; Bordyuh, A. B.; Dovbeshko, G. I., Comparative Analysis of Two Aqueous-Colloidal Solutions of C60 Fullerene with Help of FTIR Reflectance and UV-Vis Spectroscopy. *Chem. Phys. Lett.* **2002,** *364*, 8-17.

59. Andrievsky, G. V.; Klochkov, V. K.; Karyakina, E. L.; Mchedlov-Petrossyan, N. O., Studies of Aqueous Colloidal Solutions of Fullerene C60 by Electron Microscopy. *Chem. Phys. Lett.* **1999,** *300*, 392-396.

60. Isaacson, C. W.; Usenko, C. Y.; Tanguay, R. L.; Field, J. A., Quantification of Fullerenes by LC/ESI-MS and Its Application to in Vivo Toxicity Assays. *Analytical Chemistry* **2007,** *79*, 9091-9097.

61. Zhao, B.; Bilski, P. J.; He, Y. H.; Feng, L.; Chignell, C. F., Photo-induced Reactive Oxygen Species Generation by Different Water-soluble Fullerenes (C60) and Their Cytotoxicity in Human Keratinocytes. *Photochemistry and Photobiology* **2008,** *84* (5), 1215-1223.

62. Avdeev, M. V.; Khokhryakov, A. A.; Tropin, T. V.; Andrievsky, G. V.; Klochkov, V. K.; Derevyanchenko, L. I.; Rosta, L.; Garamus, V. M.; Priezzhev, V. B.; Korobov, M. V.; Aksenov, V. L., Structural Features of Molecular-Colloidal Solutions of C60 Fullerenes in Water by Small-Angle Neutron Scattering. *Langmuir* **2004,** *20*, 4363-4368.

63. Ludwig, R.; Appelhagen, A., Calculation of Clathrate-Like Water Clusters Including H2O-Buckminsterfullerene. *Angewandte Chemie* **2005,** *44*, 811-815.

64. Scharff, P.; Risch, K.; Carta-Abelmann, L.; Dmytruk, I. M.; Bilyi, M. M.; Golub, O. A.; Khavryuchenko, A. V.; Buzaneva, E. V.; Aksenov, V. L.; Avdeev, M. V.; Prylutskyy, Y. I.; Durov, S. S., Structure of C60 Fullerene in Water: Spectroscopic Data. *Carbon* **2004,** *42*, 1203-1206.

65. Deguchi, S.; Mukai, S.; Yamazaki, T.; Tsudome, M.; Horikoshi, K., Nanoparticles of Fullerene C60 From Engineering of Antiquity. *Journal of Physical Chemistry* **2010,** *114*, 849-856.

66. Labille, J.; Masion, A.; Ziarelli, F.; Rose, J.; Brant, J.; Villieras, F.; Pelletier, M.; Borschneck, D.; Wiesner, M.; Bottero, J. Y., Hydration and Dispersion of C60 in Aqueous Systems: The Nature of Water-Fullerene Interactions. *Langmuir* **2009,** *25*, 11232-11235.

67. Murdianti, B. S. Stability of Nano-Engineered C60 Colloidal Suspensions in Water and Its Oxidative Behavior. Oklahoma State University, Stillwater, 2012.

68. Hilburn, M. E.; Murdianti, B. S.; Maples, R. D.; Williams, J. S.; Damron, J. T.; Kuriyavar, S. I.; Ausman, K. D., Synthesizing Aqueous Fullerene Colloidal Suspensions by New Solvent-Exchange Methods. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2012,** *401*, 48-53.

69. Cintas, P.; Luche, J.-L., Green Chemistry - The Sonochemical Approach. *Green Chemistry* **1999,** *1*, 115-125.

70. Hucho, C.; Kraus, M.; Maurer, D., Ultrasonic Investigations of C60/C70 - Compounds. *Physica B* **1994,** *194*, 415-416.

71. Mandrus, D.; Kele, M.; Hettich, R. L.; Guiochon, G.; Sales, B. C.; Boatner, L. A., Sonochemical Synthesis of C60H2. *Journal of Physical Chemistry B* **1997,** *101*, 123-128.

72. Oberdorster, E.; Zhu, S. Q.; Blickley, T. M.; McClellan-Green, P.; Haasch, M. L., Ecotoxicology of carbon-based engineered nanoparticles: Effects of fullerene (C-60) on aquatic organisms. *Carbon* **2006,** *44* (6), 1112-1120.

73. Kadish, K. M.; Ruoff, R. S., Fullerenes. John Wiley & Sons, Inc.: 2000.

74. Chung, N.; Alexander, M., Effect of Concentration on Sequestration and Bioavailability of Two Polycylic Aromatic Hydrocarbons. *Environ Sci Technol* **1999,** *33*, 3605-3608.

75. McHedlov-Petrossyan, N. O., Fullerenes in molecular liquids. Solutions in â€œgoodâ€ solvents: Another view. *Journal of Molecular Liquids* **2011,** *161* (1), 1-12.

76. Zhang, Z. a. P., Janusz, Headspace Solid-Phase Microextraction. *Analytical Chemistry* **1993,** *65*, 1843-1852.

77. Supelco. Solid Phase Microextraction: Theory and Optimization of Conditions *Bulletin 923* [Online], 1998. (accessed 10/13/2013).

78. EPA, U. Volatile Organic Compounds (VOCs) Technical Overview. http://www.epa.gov/iaq/voc2.html.

79. Harris, M. O.; Corcoran, J., Toxicological Profile for n-Hexane. DHHS, U. S., Ed. U.S. Government Printing Office: Atlanta, Georgia, 1999; pp 1-269.

80. McAliffe, C. J., Solubility in Water of C1-C9 Hydrocarbons. *Nature (London)* **1963,** *200*, 1092-1093.

81. McAuliffe, C., Solubility in Water of Paraffin, Cycloparaffin, Olefin, Acetylene, Cycloolefin, and Aromatic Hydrocarbons. *J. Phys. Chem.* **1966,** *70*, 1267-1275.

82. Nelson, H. D.; DeLigny, C. L., The Determination of the Solubilities of Some n-Alkanes in Water at Different Temperatures, by Means of Gas Chromatography. *Recueil des Travaux Chimiques des Pays-Bas* **1968,** *87*, 528-544.

83. Pereda, S.; Awan, J. A.; Mohammadi, A. H.; Valtz, A.; Coquelet, C.; Brignole, E. A.; Richon, D., Solubility of Hydrocarbons in Water: Experimental Measurements and Modeling Using a Group Contribution with Association Equation of State (GCA-EoS). *Fluid Phase Equilibria* **2009,** *275*, 52-59.

84. Polak, J.; Lu, B. C.-Y., Mutual Solubilities of Hydrocarbons and Water at 0 and 25 C. *Can. J. Chem.* **1973,** *51*, 4018-4023.

85. Supelco Selection Guide for Supelco SPME Fibers. http://www.sigmaaldrich.com/analytical-chromatography/sample-preparation/spme/selecting-spme-fiber.html.
86. Supelco SPME Fiber Performance Tips for Volatiles & Semi-Volatiles. http://www.sigmaaldrich.com/analytical-chromatography/sample-preparation/spme/performance-tips.html.
87. Vallaeys, C.; Kastel, M.; Fantle, W.; Christianson, L.; Hannah, M. *Behind the Bean: The Heroes and Charlatans of the Natural and Organic Soy Foods Industry*; The Conucopia Institute: Cornucopia, WI, 2009; pp 1-57.
88. Brown, R. *Tox Tidbits: Ingestion of Isopropanol*; Maryland Poison Center: 2009.
89. Church, A. S.; Witting, M. D., Laboratory Testing in Ethanol, Methanol, Ethylene Glycol, and Isopropanol Toxicities. *The Journal of Emergency Medicine* **1997,** *15* (5), 687-692.
90. Clark, J. D., Isopropyl Alcohol Intoxication. *Journal of Emergency Nursing* **2010,** *36* (1), 81-82.
91. OSHA Chemical Sampling Information: Isopropyl Alcohol. https://www.osha.gov/dts/chemicalsampling/data/CH_248400.html.
92. OSHA Chemical Sampling Information: Ethyl Acohol. https://www.osha.gov/dts/chemicalsampling/data/CH_239700.html.
93. Kemal, H., The Fate of Isopropyl in the Human Organism. *Biochemische Zeitschrift* **1927,** *187*, 461-466.
94. Valerino, D. M. *Criteria Document: Recommendation for an Occupational Exposure Standard for Isopropyl Alcohol*; National Institute for Occupational Safety and Health: 1976; pp 1-119.
95. Wills, J. H.; Jameson, E. M.; Coulston, F., Effects on Man of Daily Ingestion of Small Doses of Isoproplyl Alcohol. *Toxicology and Applied Pharmacology* **1969,** *15*, 560-565.
96. Chae, S. R.; Badireddy, A. R.; Farner Budarz, J.; Lin, S.; Xiao, Y.; Therezien, M.; Wiesner, M. R., Heterogeneities in Fullerene Nanoparticle Aggregates Affecting Reactivity, Bioactivity, and Transport. *Acs Nano* **2010,** *4*, 5010-5018.
97. Song, M.; Yuan, S.; Yin, J.; Wang, X.; Meng, Z.; Wang, H.; Jiang, G., Size-dependent Toxicity of Nano-C60 Aggregates: More Sensitive Indication by Apoptosis-related Bax Translocation in Cultured Human Cells. *Environmental Science & Technology* **2012,** *46*, 3457-3464.
98. Lebedkin, S.; Ballenweg, S.; Gross, J.; Taylor, R.; Ktatschmer, W., Synthesis of C120O: A New Dimeric [C60]Fullerene Derivative. *Tetrahedron Letters* **1995,** *36* (28), 4971-4974.

VITA

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