

Carbon Nanoparticles in Aquatic Environments: Fate of Fullerenes (C₆₀) in Freshwaters and their Effects on Organism

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Abstract

Carbon nanoparticles, such as fullerenes, are one of the most promising groups of nanoparticles. Their versatile properties give them potential in various applications, for example in electronics, optics, medicine, and cosmetics. These applications include widely produced and sold consumer products. The relatively short life-cycle of these products can result in their quick disposal as waste, or even provides fullerenes direct access to waste waters. In addition, it is unrealistic to assume that fullerenes could be produced without any releases.

Keyword:-Nanoparticles, fullerenes, C₆₀, Graphite, Diamond.

1. INTRODUCTION

Nanotechnology has produced plenty of new applications since the middle of the 1990's, and its growth is continuous. The success of nanotechnology is based on nano-size particles (NPs) and structures which provide new, unique properties with several practical possibilities in technology, medicine, and consumer products. Increasing manufacturing and use of NPs give raise to a risk that they reach to the environment. The situation is similar to that of other chemicals: there is no reason to

assume that the manufacturing and use of NPs can or will be a perfectly closed system without any discharges: thus, the potential ecological risks of NPs need to be understood. Nanoproducts are already on the market – more than 1300 products exist today – but the knowledge of the environmental fate and effects of nanoparticles is still defective. Concern about the environmental effects of NPs has increased in the 2000s, and today nanoecotoxicology is already its own discipline.

1.1 Fullerenes

Fullerene (C₆₀) is an allotrope of carbon, consisting of 60 carbon atoms (figure 1a). Unlike other forms of carbon: graphite, grapheme, and diamonds, which are repeating atomic cage structures (figure 1b-d), and amorphous carbon without regular structure (figure 1), fullerenes have a molecular shape with hexagons and pentagons of carbon atoms. Fullerene structured molecules exist from the smallest known one, C₂₈ built by four hexagons and 12 pentagons to those built up with even hundreds of carbon atoms. Universally the structure of fullerene is described as C_{20+2m} meaning 12 pentagons and m hexagons. The most stable fullerenes are C₆₀ and C₇₀ because of energetic reasons (Zhang et al. 1992). In this thesis the word “fullerenes” means C₆₀ explicitly. This molecule consists of 20 hexagons and 12 pentagons and has diameter of 0.72 nm (figure 2a).

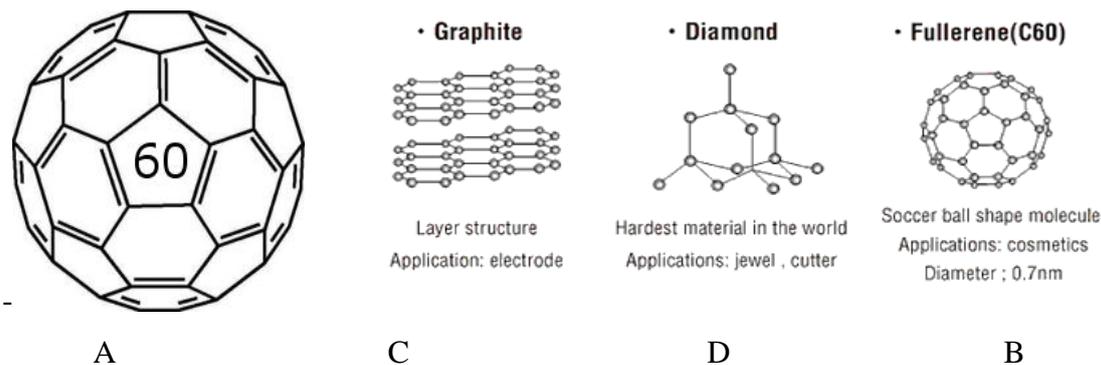


Figure 1:-Structures of carbon allotropes. A) Fullerene-C₆₀ (Di Venetra), B) graphite (benbest.com), C) graphene, a layer of graphite (metrolic.com), D) diamond (uwgb.edu), and E)

The electronic structure of fullerene molecule is stable because all the electrons are placed in bonding molecular orbitals. Four valence electrons of each carbon atom are bonded into the molecule cage, allowing only weak van der Waals interactions between molecules. All carbon atoms in the fullerene molecule are equivalent, having sp² hybridization. However, two different lengths of bonds occur: the length between two six rings is 1.38Å, and between six and five rings 1.45Å.

1.1.1 Unique shape: A promise of new possibilities

Since their discovery in 1985 by Curl, Heath, O'Brian, Kroto, and Smalley (Kroto et al. (1985)) fullerenes have interested and inspired researchers and inventors. For example, some of the first ideas included using "Buckyballs" as solid lubricants rolling between surfaces. Still more are under development because nano-size and molecular structure do provide fullerenes with unique properties different from those exhibited by other forms of carbon. Fullerenes have proven to have such potential that the scientists who found it were awarded a Nobel Prize in 1996.

In visible scale fullerenes appear as black powder. Some of their chemical and physical properties compared to other forms of carbon are presented in table 1. Unlike other carbon allotropes, fullerenes are soluble in nonpolar solvents, modifying colored solutions

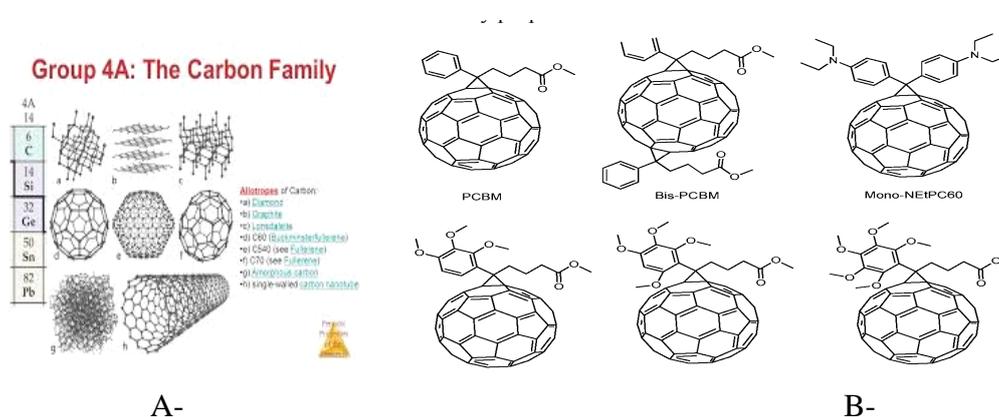


Figure 2: Examples of derivatized C60. A) Halogenated fullerenes. B) PCBM-fullerenes, the *most commonly used in semiconductor*.

1.1.2 Fullerenes in aquatic environments: Do good properties turn bad?

As for any other chemical, safe production and utilization in commercial applications can be achieved by knowing fullerenes' potential to cause harmful effects in the environment, and creating relevant legislation. Some properties of fullerenes are raising concern about their environmental effects. Firstly, they belong to nanoparticles, a group of new materials which urgently need attention in terms of risk assessment and legislation. In addition, some properties of fullerenes complicate the assessment of their fate and targets in the environment.

1.1.2.1 Water solubility

Fullerenes have a dualistic character in water solubility, which makes them very interesting and challenging especially in aquatic ecotoxicology. Traditionally the fate of pollutants in aquatic environments is assessed on the basis of their water solubility

using the octanol-water distribution coefficient $\log K_{ow}$. Poorly water soluble pollutants are expected to end up to bottom sediments and to lipids of organisms in aquatic environments. For example, water solubility for an organic pollutant, PCB77, is $5.5-7.5 \times 10^{-4}$ mg/L and $\log K_{ow}$ is 6.6 giving a strong affinity to organic carbon in sediments to PCB77 (Sabljić et al. 1993). Also fullerenes are insoluble to water and other polaric solvents: water solubility is less than 10^{-9} mg/L (Ruoff et al. 1993), and $\log K_{ow}$ is measured to be 6.67 (Jafvert and Kulkarni 2008). Along these values fullerenes should express an orientation away from water. However, fullerenes have a characteristic of modifying water-stable colloidal clusters of several single molecules when they are stirred in water. These clusters, or agglomerates, are named nano-C60 or nC60 for short, and they can be formed either in a laboratory by vigorous stirring, or in the environment due to natural processes like water flow and mixing.

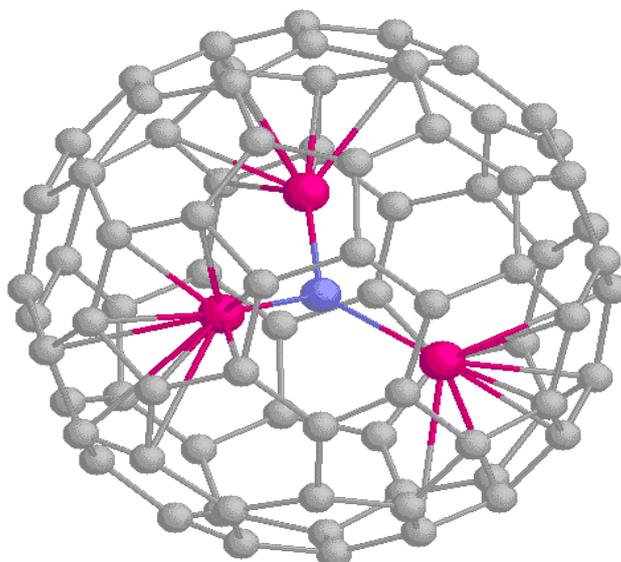


Figure 3: A model of fullerene molecule surrounded by water molecules. Adopted from Scharff et al. 2004.

2. AIMS OF THE STUDY

The aim of this study was to assess the fate of fullerenes in natural freshwaters and their effects on aquatic organisms. Understanding the fate is critical ground for risk assessment. Thus one aim was to map, where fullerenes end up if they are released to natural waters. This study proved that fullerenes can reach the sediment (paper I), which gave reason to investigate fullerenes' distribution between water and sediment (paper II) and effects on a benthic organism (paper IV). A part of fullerenes were found in water phase (paper I) directing the other aims of the study to effects on aquatic organism (paper III) and seeking explanations for varying water stability (paper I).

3. MATERIALS AND METHODS

3.1 Chemicals

Carbon nanoparticle, fullerene (C60), was 98 % purity from Sigma Aldrich. Purity percentage seemed that C60 could contain 2 % impurities, thus C60 was checked by plasma emission spectrometer and thermal gravimetric analysis. This determination indicated only minor occurrence of other elements (Waissi-Leinonen et al. 2012).

3.2 Test Organisms

Experiments concerning the fate of fullerenes revealed that they can be found both in water and sediment, thus, test organisms were chosen to represent both of these phases. Chosen species, an aquatic organism water flea *Daphnia magna* and a sedimentliving black worm *Lumbriculus variegatus* are both well-known and commonly used test organisms in aquatic ecotoxicolog.

3.2.1. Aquatic test organism, water flea *Daphnia magna*

Water flea *Daphnia magna* (figure) is a freshwater crustacean. It lives in temperate and cold climates and is a part of the freshwater food chain. *Daphnia* filter large volumes of water and water-suspended particles which make it a significant target of water suspended xenobiotics. It is also relatively easy to maintain in laboratory.

3.2.2. Benthic test organism, black worm *Lumbriculus variegatus*

Oligochaetes are an ubiquitous species that include both terrestrial and aquatic worms, a total more than 3000 species (Hickman et al 1997). Most aquatic oligochaetes, nearly 1700 are freshwater species (Martin 2008). Generally, aquatic oligochaetes are benthic species living on the bottom or burrowing in the sediment.

3.3. Experimental Matrixes

Both artificial and natural matrixes were used in experiments. An artificial matrix made it possible to assess the fate and effects of fullerenes themselves, representing a simple system without interactions with natural substances, such as dissolved natural organic matter. This is important as the first step to understanding fullerenes' fate and effects.

3.3.1 Waters

Artificial freshwater (AFW) was used as a basis for fullerene exposures on *D. magna*, and as a control for natural waters as well. This water corresponded "typical Finnish freshwater" with its pH and [Ca²⁺+Mg²⁺]-hardness (pH~6.8, 0.5 mM), and it was prepared by diluting salts (CaCl₂, MgSO₄, NaHCO₃, and KCl) to milliQ-water. Thus,

AFW was very simple compared to natural waters. They represented relatively wide range of DNOM concentration, and, as this study revealed, also a different composition of DNOM. Waters were filtered through 0.45 μm membrane before experiments to consider that all the material was dissolved, and they were stored in the dark at +4°C prior use. More detailed description for natural waters is provided in paper I.

3.3.2. Sediments

Experimental sediments were collected from Lake Kuorinka, Lake Höytiäinen, and Lake Mekrijärvi, thus they represented same origins than used natural waters, except for Lake Kontiolampi. Natural sediment was also used as experimental environment in exposure of *L. variegatus*. All sediments were sieved to grain size less than 1 mm, and they were stored in the dark at +4°C prior experiments.

3.4 Equipment

Because nanoparticles express both chemical and particulate character, different techniques compared to traditional chemicals are necessary to characterize the exposure caused by them. This means that physical dimensions of nanoparticles need to be determined in addition to their chemical composition, properties and concentration. Size and shape are critical for many properties and effects of nanoparticles.

3.4.1. Light and electron microscopy

Microscopy techniques were used for qualitatively identifying fullerenes inside test organisms. Light microscopy (Leica CME, Buffalo, NY, USA) with magnifications 40, 100, and 400x were used to reveal and locate fullerene masses inside and on surfaces of the test organisms. Additionally, estimated intake rate of fullerenes in *D. magna* was assessed by light microscopy to choose relevant sampling times. Nevertheless, resolution of light microscopy is restricted to 0.2 μm , thus transmission electron microscopy (TEM) was needed to observe individual fullerene agglomerates. Transmission electron microscopy was used to characterize fullerene agglomerates in suspensions and natural particles in test waters and sediments by determining particle sizes and shapes. In addition to TEM, 3D shapes of the particles in fullerene suspensions were visualized by scanning electron microscopy (SEM). Electron microscopy offered also more detailed information of location of fullerenes inside the gut and on the surfaces of test organisms and their effects on cells as well.

3.4.2 Spectroscopy

Fullerenes absorb ultraviolet (UV) and visible light (VIS) in their molecular structure, which offers a possibility to quantify fullerenes in solvents. The spectrum of

fullerenes in toluene consists a specific and strong absorption peak at 335 nm, another weaker peak at 407 nm, and a weak absorption band between 415 and 680 nm (Bensasson et al. 1994).

3.4.3 ¹³C-CPMAS-NMR and HPSEC

Semi-quantitative determination of chemical characteristics of DNOM in natural waters (excluding Lake Kuorinka) was done by ¹³C solid-state CPMAS NMR (Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance). Generally in NMR, analyzed atom, as ¹³C here, gives signals to special part of the measured magnetic field depending on their chemical environment, i.e. functional groups adjacent to the analyzed atom. The position of these signals on the x-axis of the spectrum is called the chemical shift and is presented as ppm values. Thus, presence of special functional groups, such as carbonyl, carboxylic, aromatic, and aliphatic, in the sample can be visualized. Measurement of peak areas offers a percentage estimation of these groups. The measurements were done by CPMAS technique, which offers better resolution than NMR itself. This technique requires sufficient organic carbon content, thus, Lake Kuorinka was excluded because of its very low carbon content.

3.5 Experiments

3.5.1. Fullerene suspensions and their characterization

At the beginning of this research, several methods for preparing fullerene suspensions were reported in literature. Some methods were based on solvent exchange using organic solvents like tetrahydrofuran or ethanol as a vehicle to transfer fullerenes to water (Deguchi et al. 2001, Fortner et al. 2005).

3.5.2. Method development

Methods developed throughout the experiments. Methods were needed for quantifying fullerenes in environmental samples: organisms, natural waters, sediments, and the fecal pellets of *L. variegatus*. Also, the suitability of traditional methods for testing toxicity and bioaccumulation-depuration kinetics of nanoparticles was assessed, and the relevancy of risk assessment parameters LC50 and BCF was be schussed.

3.5.3. Fate of fullerenes in four natural freshwaters

Their size, shape and even composition if other substances are present during Formation. In real environmental conditions DNOM and as discussed earlier, fullerene agglomerates produced with different methods vary in other natural substances are present and may affect agglomeration process.

3.5.4. Behavior of fullerenes in freshwater- sediment systems

Sediments have been modeled to be a sink of fullerenes (Gottschalk et al 2009). Nevertheless, the sediment is not a final sink for many traditional chemicals (Baker, 1991), and it cannot be assumed that it is for fullerenes either.

3.5.5. Effects of fullerenes in an aquatic species, *Daphnia magna*: Accumulation and depuration kinetics

Several preliminary tests were done before accumulation and depuration experiments. Fullerene agglomerates formed by the stirring method described earlier may vary in their size and shape. It was assumed that the acute toxicity of fullerenes would be rather low but because properties of agglomerates can affect toxicity, a set of toxicity tests was done. Other goals were to have a common picture of how fullerenes are placed in daphnids, examine extraction of fullerenes from organisms, and to learn how to handle daphnids as well. Initial questions and preceding work are presented.

3.5.6. Adverse effects of fullerenes on a benthic organism, *Lumbriculus variegatus*

In a preliminary study, a group of worms were exposed to nC60 water suspension without sediment at concentrations 0, 10, and 20 mg/L for two weeks. The aim of the preliminary test was to ensure if worms are able to survival in these fullerene concentrations, and if fullerenes could be observed by light and electron microscopy in their bodies and how fullerenes are placed in these organisms.

3.5.7 Work safety and waste handling

Work safety and environment protection must be taken account in all chemical work. Safety issues are included in the thesis, because a new kind of material was used - neither instructions for work safety and waste handling were available from the supplier. Generally, small size causing high surface to mass ratio, and surface reactivity of nanoparticles are characteristics which may cause risk in the workplace,

4. RESULTS AND DISCUSSION

4.1 Method Development

The quantification of fullerenes was based on their selective extraction to toluene from different matrices. Blank samples of the same matrices did not show UV-VIS absorptions at 335 nm (figures 20 – 23). Generally, extractions of environmental samples yielded high recoveries between 85–115 % (table 7). The method development for daphnids raised the yield dramatically compared to the first attempts: the first daphnia extractions produced only 0.8 µg/mg organism wet weight, which was roughly 20% of the fullerene contents extracted according to the final method. The only matrix where extraction was not successful was the *L. variegatus* samples.

This failed because of the small amount of the gut content remaining in worms after depuration, which was verified with TEM and light microscopy observations.

4.2 Different Water Chemistry Lead Different Fates

Water stabilities varied among different water sources. A fraction of suspended fullerenes settled rapidly during the first few days in all tested waters but differences in water stable fullerene concentrations between them were substantial.

5 CONCLUDING REMARKS

5.1 Role of Water and Sediment Chemistry

Fullerenes are very stable molecules. With increasing use stability allows them to potentially accumulate in aquatic environments which may raise the environmental concentrations of fullerenes. Fullerenes' fate in aquatic environments depends on water chemistry.

5.2 Trophic Transferring and Cycling By Organisms

Fullerenes were effectively ingested by water fleas. Those characters which led enhanced agglomeration were connected to more effective intake by water fleas. Effective intake makes trophic transferring possible and can lead to adverse impacts in other species.

5.4 Needs For Future Research

**Bioturbation was observed in one kind of sediment in these studies, and should be studied in other types of sediments, too. Bioturbation process may be different in another kind of sediment due to differences between sediment fullerene interactions, especially in sediments with high re-suspending potential.

**Intake and bioavailability in different kind of sediments: Does more effective intake and bioavailability occur if fullerenes are less strongly adhered to sediment particles?

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