Analysis of fullerenes and functionalized fullerenes in water samples by liquid chromatography coupled to high resolution mass spectrometry

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Dit rapport is selectief verspreid onder medewerkers van BTO-participanten en is verder (nog) niet openbaar.

Summary

Engineered nanoparticles are manufactured in rapidly increasing quantities nowadays. Engineered nanoparticles (ENP) are manufactured in rapidly increasing quantities nowadays. Fullerenes, which belong to class of organic nanoparticles, are applied at an increasing rate in commercial applications such as for example personal care products and organic photovoltaic cells (solar cells), and in medical applications, such as imaging probes and drug carriers. Due to this and their unique properties, there is a growing concern about the human health and environmental effects of fullerenes.

One of the main challenges in the environmental risk assessment of fullerenes is to develop analytical methods that can detect and quantify fullerene in natural environmental samples at low concentrations. In the present paper we report on the development of a highly specific and robust analytical method for the quantitative determination of C_{60} and C_{70} as well as six functionalized fullerene derivatives, namely, [6,6]-Phenyl-C_{61}-butyric acid methyl ester ([60]PCBM), [6,6]-Phenyl-C_{61}-butyric acid methyl ester ([60]PCBB), [6,6]-Phenyl-C_{61}-butyric acid octyl ester, [6,6]-Bis-Phenyl-C_{61}-butyric acid methyl ester [60]bisPCBM,[6,6]-Thienyl-C_{61}-butyric acid methyl ester [60]ThCBM, and [6,6]-Phenyl C_{71} butyric acid methyl ester [70]PCBM in different aqueous matrices. The selection of these fullerenes was based on the application of these materials in consumer's products. For example C_{60} and C_{70} are common constituents in cosmetics, while [60]PCBM is the most important fullerene derivative *n*-type semiconductor used in organic solar cells. Moreover, the different compounds were chosen to obtain a selection of fullerenes with a wide range of different physiochemical characteristics to study the behavior and fate in the environment of the different compounds.

The analytical method was based on extraction of the fullerenes from the aqueous phase using solid phase extraction (SPE), with subsequent analysis on a LC-Orbitrap MS system. Solid phase extraction was optimized by varying different conditions in order to improve recovery of the suite of fullerenes. Different SPE column materials were tested, namely C18, C18e, C8 and CN, and recoveries appeared to be the highest at the C18 material. The recoveries were improved by adding NaCl to the water sample during extraction. Very low LOD values were obtained for all compounds with the optimized method, ranging from 0.17 ng/L for [70]PCBM to 0.28 ng/L for C_{60}, and subsequent LOQ values of 0.57 ng/L to 0.91 ng/L. Recoveries for the fullerenes were found to be on average 120% in ultra pure and drinking water samples. The recoveries appeared to be lower, but still acceptable (e.g. >78%), in surface water samples, which could be explained by the higher concentration humic acids present in surface water samples.

So far there are no papers describing a comparable analytical technique that is suitable for the analysis of such a large suite of fullerenes at a very low, environmental relevant, detection level. The use of high resolution accurate mass spectrometry as applied in this method creates the possibility to restrospectively look for the presence of other functionalized fullerenes in environmental samples. The developed approach is promising and can be applied for (1) environmental monitoring, (2) a more in-depth study of environmental fate and transformation products and (3) studying the water treatment efficiency of C_{60}, C_{70} and the various functionalized fullerenes.

Inhoud

1 Introduction

Nanomaterials are defined as particles with at least one dimension smaller than 100 nm, and include nanofilms, nanotubes and nanoparticles, with one, two and three dimensions smaller than 100 nm respectively. Nanomaterials exhibit a very large surface-area-to-volume ratio and the percentage of atoms at the particle surface is high [1, 2]. Therefore they exhibit exceptional physical and chemical properties, which can be totally different compared to their respective bulk material. Due to their, unique properties, like high strength, thermal stability, low permeability, high catalytic activity, and high conductivity, these materials have great potential for employment in, e.g. medicines, solar panels or personal care products. Engineered nanoparticles (ENP) are manufactured in rapidly increasing quantities nowadays. Since a few decades nano science and –technology play an important role in industry. It is estimated that by 2015 about 7 million people will be employed in this sector and the projected revenue will be about $1 trillion [3].

ENP can be either of inorganic or organic nature. The carbon-based fullerenes belong to the latter category [4]. Fullerenes are spherical molecules containing aromatic moieties [5] and can be exclusively comprised of carbon, such as C_{60} and C_{70}, or carry additional functional groups [6, 7]. Especially the purely-carbon based apolar fullerenes exhibit a very low solubility in water (e.g $C_{60} < 10^{-9}$ mg/L) [8, 9]. However, fullerenes have the ability to form stable aggregate clusters with nanoscale dimensions upon contact with water [10]. The so called nano-C_{60} clusters or nC_{60} are crystalline and their formation can be undone. Hence, the C_{60} molecules can be retrieved [10]. Aqueous nC_{60} suspensions can be prepared via different techniques, like solvent exchange, sonication and extensive stirring [11-13].

Fullerenes are applied at an increasing rate in commercial applications such as example personal care products [14] and organic photovoltaic cells (solar cells) [15], and in medical applications, such as imaging probes and drug carriers [16, 17]. Due to this and their unique properties, there is a growing concern about the human health and environmental effects of fullerenes. Waste water discharge, manufacturing factories, storm-water runoff, erosion from composites and wet deposition from the atmosphere are likely routes of release of fullerenes into the aqueous environment [18]. In addition, it should be noted that fullerenes are formed in natural processes including volcanic eruptions and forest fires and can be produced unintentionally by human activity (e.g. soot and diesel exhaust).

One of the main challenges in the environmental risk assessment of fullerenes is to develop analytical methods that can detect and quantify fullerene in natural environmental samples at low concentrations. Different analytical techniques have been described to detect fullerenes in environmental samples (see reference [19, 20] for a comprehensive review). Extraction of fullerenes from the environmental matrices are carried out mostly using liquid liquid extraction (LLE) [21, 22] or solid phase extraction (SPE) [21-23]. Recently, novel techniques like ultrasound-assisted dispersive liquid-liquid micro extraction have been applied successfully for the extraction of fullerenes from aqueous matrices [24]. For most studies high performance liquid chromatography for the separation of fullerenes is employed, but recently also other separation techniques such as asymmetric flow field flow fractionation [25] and non-aqueous capillary electrophoresis [26] have been used. UV detection [21, 22] and mass spectrometry [23, 24, 27-29] are typically used for detecting and quantifying fullerenes. Different ionization techniques, like electrospray ionization (ESI), atmospheric pressure ionization (APCI) and atmospheric pressure photo ionization (APPI) have been applied to ionize fullerenes [23, 27, 30, 31].

Gottschalk et al. [32] calculated that environmental concentrations of fullerenes can range from 0.003 ng/L for European surface waters to 5.2 ng/L for European sewage treatment effluents. Some recent studies have reported the presence of fullerenes in environmental water samples. For example Farré et al. detected C_{60} and C_{70} and C_{60}-methylfulleropyrrolidine fullerenes in the suspended organic material of Spanish wastewater effluents in concentration up to the µg/L range [29]. Chen et al. reported the measurement of C_{60} in surface water and in industrial effluents up to concentrations of 98 ng/L and 130 ng/L respectively [24]. Núñez et al. detected fullerenes at locations near landing strips of an airport and a racetrack in the low ng/L range in the suspended matter of surface water samples [27]. In their study,

C_{84} was present in higher concentration than C_{60} and C_{70}. In a study of Dutch surface water samples, Van Wezel et al. did not observe C_{60} to be present. although sensitive analytical methods (LOQ of 5 ng/L) were applied [23].

Most environmental studies focus on C_{60} [23, 33] or higher fullerenes [27]. Only a few groups report on analytical methods that can detect functionalized fullerenes in environmental samples [22, 29, 34]. In this paper we report on the development of a highly specific and robust analytical method for the quantitative determination of C_{60} and C_{70} as well as six functionalized fullerenes namely, [6,6]-Phenyl-C_{61}-butyric acid methyl ester ([60]PCBM), [6,6]-Phenyl-C_{61}-butyric acid methyl ester ([60]PCBB), [6,6]-Phenyl-C_{61}-butyric acid octyl ester, [6,6]-Bis-Phenyl-C_{61}-butyric acid methyl ester [60]bisPCBM,[6,6]-Thienyl-C_{61}-butyric acid methyl ester [60]ThCBM, and [6,6]-Phenyl-C_{71}-butyric acid methyl ester [70]PCBM in different aqueous matrices. The selection of these fullerenes was based on the application of these materials in consumer's products. For example C_{60} and C_{70} are common constituents in cosmetics (REF), while [60]PCBM [35] is the most important fullerene derivative *n*-type semiconductor used in organic solar cells. Moreover, the different compounds were chosen to obtain a selection of fullerenes with a wide range of different physiochemical characteristics to study the behavior and fate in the environment of the different compound. For example, the effect of the functionalized groups compared with the bare fullerene (e.g. (1) C_{60} versus [60]PCBM versus [60]bisPCBM, and (2) C_{70} versus [70]PCBM), the length of the alkyl chain in [60]PCBM, [60]PCBB and [60]PCBO. The analytical method was based on extraction of the fullerenes from the aqueous phase using solid phase extraction, with subsequent analysis on a LC-Orbitrap MS system. So far there are no papers describing a comparable analytical technique that is suitable for the analysis of such a large panel of fullerenes at a very low, environmentally relevant, detection level (i.e.. ng/L) as we report in this study.

2 Materials and Methods

2.1 Reagents and chemicals

C_{60} fullerene (purity >99.9%) was obtained from MER Corporation. (Tuscon, Arizona, United States). C_{70} fullerene (purity >99%), [6,6]-Phenyl-C_{61}-butyric acid butyl ester ([60]PCBB) (purity >97%) and [6,6]-Phenyl-C_{61}-butyric acid octyl ester ([60]PCMO) (purity >99%) were purchased from Sigma-Aldrich (Steinheim, Germany). [6,6]-Phenyl-C_{61}-butyric acid methyl ester ([60]PCBM) (purity >99), [6,6]-Bis-Phenyl-C_{61}-butyric acid methyl ester [60]bisPCBM (purity >99.5%), [6,6]-Thienyl-C_{61}-butyric acid methyl ester [60]ThCBM (purity >99%) and [6,6]-Phenyl-C_{71}-butyric acid methyl ester [70]PCBM (purity >99%) were purchased from Solenne B.V. (Groningen the Netherlands). Toluene, methanol, acetonitrile, and acetone (all analytical grade or higher), NaCl and see sand (100 µm) were purchased from Mallinckrodt Baker B.V. (Deventer, the Netherlands). Humic acid (HA) was obtained form Sigma-Aldrich (Steinheim, Germany). 6 mL polypropylene columns filled with 1000 mg C18 or 1000 mg CN material were obtained from Mallinckrodt Baker B.V. (Deventer, the Netherlands), and 6 mL polypropylene columns filled with 1000 mg C18e , and 1000 mg C8 material were obtained from Phenomenex (Utrecht, The Netherlands).

2.2 Water samples

Ultrapure water was obtained by purifying demineralised water in a Milli-Q-system (Millipore, Bedford, MA). Tap water was obtained from the town of Nieuwegein (The Netherlands). Surface water samples were taken from the Lekkanaal at Nieuwegein (which is hydrologically connected to the River Rhine) in a stainless steel container that had previously been thoroughly washed and rinsed. These surface water samples were stored at 4^0C in the dark for a maximum time of 1 week.

2.3 Preparation of the standards in toluene

Stock solutions of the individual fullerenes were prepared at a concentration of ~500 mg/L in toluene. The solutions were placed in the dark overnight on a rotary shaker to achieve complete dissolution of the fullerenes. Next, a mixture stock solution was prepared by diluting aliquots from the individual stock solutions to a level of 40 mg/l in toluene.

2.4 Preparation of aqueous fullerene suspension

3 mL ethanol and 30 mL ultra pure water were added to a 40 mL vial (ultraclean EPA vial), and the vial was placed in an ultrasonic bath to degas for a minimum of 15 min. The ultrasonic bath was kept at a constant temperature of 34°C by means of a cooling circuit. Next, 500µL from the combined fullerenes stock solution (40 mg/L toluene) was infused (at a flow rate of 25 µL/min) near the bottom of the vial under continuous sonification, which resulted in a white cloudy emulsion. After 4 h of sonification the toluene was evaporated and the resulting solution was yellow and clear. This procedure was repeated 15 times and the solutions were pooled and filtrated through a glass fiber filter (Whatman GFC, 47 mm). The filtrate was collected and the volume was adjusted to 500 mL ultra pure water. The resulting aqueous fullerene suspension was used to spike the different water samples in this study.

2.5 Liquid liquid extraction

Liquid liquid extraction (LLE) was used (1) to determine the concentration of the individual fullerenes in the aqueous fullerene suspension and (2) as reference method for recovery determination (set at 100%) to compare it with recoveries obtained with SPE. LLE was performed by adding 4 mL of the aqueous suspension, 4 mL of toluene and 4 mL of ultra pure water with 20% (w/v) NaCl to a vial. This solution was shaken at 200 rpm during 2 h to allow extraction of the fullerenes into the toluene phase.

2.6 Solid phase extraction of water samples

Various parameters were optimized and studied during this study: (1) Different SPE column materials, namely C18, C18e, C8 and CN were tested, by extracting 1L drinking water (n=2), fullerenes were spiked at 200 ng/L and in this experiment no salts were added. (2) NaCl was spiked to ultrapure water (concentration range: 0, 1, 2, 5, 10, 20, 30, 50, 70, 100 mM) and recoveries for all fullerenes (spiked at 30 ng/L) were determined. (3) The filtration column (e.g. a 6 mL polypropylene column with a 20 µm frit filled with 4 grams of see sand), which was used in order to prevent the SPE material from clogging, was extracted with toluene (n=4, 1L ultrapure water, addition of 50mM NaCl, spiking of 40 ng/L fullerenes aqueous suspension). The 20 µm frit and the sand were extracted individually using toluene. (4) Humic acid was spiked to ultrapure water (0, 1, 2, 5, 7, 10, 20, 30 mg/L) and recoveries for all fullerenes (spiked at 40 ng/L) were determined.

The final optimized SPE protocol is as follows: sample preparation pre-concentration was carried out on a 6 mL polypropylene column filled with 1000 mg of C18 material (J.T. Baker, Deventer, The Netherlands). For surface water samples, the SPE column was preceded by a 6 mL polypropylene column with a 20 µm frit) filled with 4 g of see sand in order to prevent the C18 material from clogging. The extraction column and the filtration column were preconditioned by first rinsing with 6 mL of toluene, followed by 12 mL of methanol and finally with 6 mL of ultra pure water. 3 g of NaCl was added to the water sample (1L) (0.05 M). After passing the water sample (1L) through the sand filter column and SPE column, both columns were dried under vacuum overnight and subsequently eluted with 3 x 6 mL of toluene. The eluate was evaporated to less than 1 mL by using an automated blow down apparatus (Barkey, Germany) and transferred to an amber glass vial. The volume of the extract was adjusted to 1 mL with toluene by weight.

2.7 LC MS UV high resolution mass spectrometry measurements

A hybrid LTQ Orbitrap mass spectrometer (Thermo Electron Bremen Germany) provided with an ESI interface was interfaced to a Surveyor HPLC system (Thermo Electron Bremen Germany) for the chromatographic separation. Mobile phases consisted of toluene and acetonitrile at a flow rate of 200 µl/min. The separation was performed on a custom made, Cosmosil ® Buckyprep column (column dimensions were 2.0mm ID x 250mm with 5µm particles, from Nacalai Tesque, Japan) column, which is a pyrenylpropyl group bonded silica based column specifically designed for fullerene separation. In addition, a Buckyprep precolumn was used (4.6mmID x 10 mm 5µm, Cosmosil Japan). 25 µl of the sample was injected. The following elution conditions were used: the first 10 min the composition consisted of toluene/acetonitrile (75/25 v/v), then the concentration toluene was increased to 100% in 1 min. Isocratic elution at 100% toluene was continued for 20 min. Post-column addition of methanol was performed to improve ionization efficiency in ESI by means of a peek high pressure mixing-tee at a flow rate of 100 µl/min.

Analysis was performed using negative electrospray ionization. Instrument settings were optimized by infusing a C_{60} solution (5 mg/L in 60/40 (v/v) toluene/methanol) at a flow rate of 10 µL/min. The capillary used was a metal needle maintained at a temperature of 400°C. The sheath, sweep and auxiliary gas were set to arbitrary units of respectively 30, 10, 10. A source voltage of 3 kV and a capillary voltage of -80 V were used. The tube lens was set to -200 V. Full scan high accuracy mass spectra were acquired in the range of 300-2000 m/z with the resolution set at 30,000 (FWHM). For confirmation purposes accurate MS/MS spectra were recorded at a resolution of 7,500 at normalized collision energy (NCE) of 40.

2.8 Quantification

External calibration curves were analyzed using standards in toluene ranging from 1 µg/L to 64 µg/L. Quantification was based on the sum of the peak areas of the accurate masses of the fullerene compound and all its related adducts as described by van Wezel et al. [23].

2.9 Validation

The LOD is determined in conformity with the guideline NEN 7777. The limit of detection (LOD) of the whole method was determined by spiking fullerenes in ultrapure water at a concentration of 1 ng/L (n=7). The LOD is defined by using the standard deviation of the repeatability and a taking into account a confidence interval of 99% with one side probability (table 1). The limit of quantitation (LOQ) for each compound was determined by using the LOD multiplied by 3. The linearity of the whole method was determined by spiking ultrapure water at 10 different levels ranging form 1ng/L to 40 ng/L and linearity was determined by ANOVA analysis.

3 Results and Discussion

3.1 LC MS method

We report for the first time an analytical method that is applicable for the determination of the fullerenes C_{60} and C_{70} and 6 functionalized fullerenes in a single run. Representative ion chromatograms of all components are shown in Figure 1. All compounds eluted within 30 min. Although not all components are base-line separated, the hyphenation of the LC system to a high resolution Orbitrap mass spectrometer allows unambiguous identification of all components by their accurate mass and their fragments.

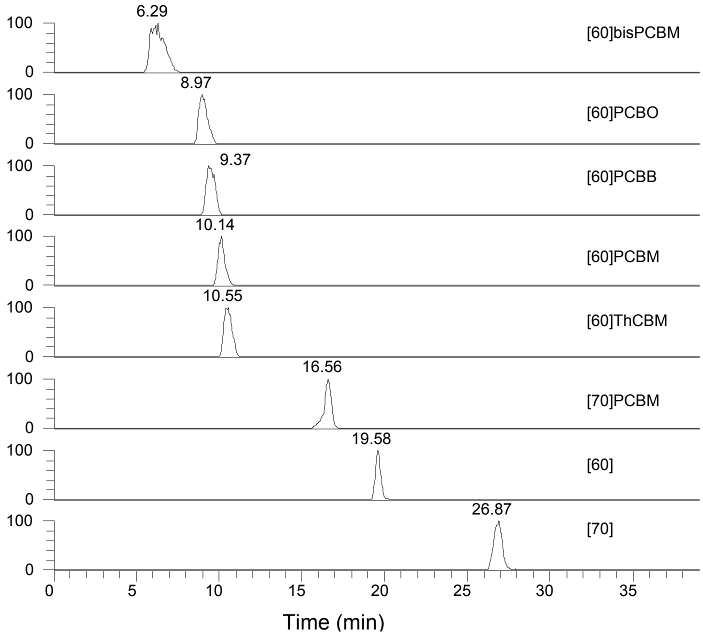


Figure 1: Extracted ion chromatograms of C_{60}, C_{70} and the 6 functionalized fullerenes.

Firstly, Figure 1 shows that all functionalized fullerenes elute before C60 and C70. Secondly, larger fullerenes elute later than the smaller ones, e.g. C60 elutes before C70 and [60]PCBM elutes earlier than [70]PCBM. This can be explained by the smaller surface area available on the smaller fullerene for binding to the Buckyprep column. Also the functional groups reduce the surface area able to interact with the column. Further, it can be seen that the length of the alkyl chain in [60]PCBO, [60]PCBB, and [60]PCBM is inversely correlated with the retention time, e.g. the longer the alkyl chain the earlier the elution of the component. This latter observation matches the elution order principle of normal phase chromatography.

The peak width in the chromatogram of [60]bisPCBM is broader (e.g. ~1 minute) compared to with the other fullerenes compounds (e.g. 20-40 seconds). A plausible explanation of this peak broadening is that bis[60]PCBM consists of more than one structural isomer [36]. When the percentage of acetonitrile in the mobile phase was increased to 35% or higher, separation of the different isoforms was observed (data not shown).

3.2 MS detection

In table 1 the mass spectrometric results are presented. For every fullerene compound the accurate masses of the radical parent ion and the fragmentation ions are presented. The accurate mass was used to determine the elemental composition of the parent ion and the fragments ions.

All analyzed fullerenes take up an electron and were detected as singly charged radical molecular ion. Upon using an ESI-interface complex adduct formation of C_{60} and C_{70} takes place, which also occurred for the functionalized fullerenes, albeit to a lesser extent. For all analyzed fullerenes in this study, next to the molecular ion also oxidized adduct ions, methanol adduct ions, and oxidized toluene adduct were detected in the ESI mass spectrum. Adduct formation of fullerenes during ESI is a known phenomenon, which has been described by van Wezel et al. [23] and others [31]. When atmospheric pressure photo ionization (APPI) is used as ionization, adduct formation on C_{60} occurs to a much lesser extent compared with ESI [27], which can result in higher sensitivity of the method.

C_{60} and C_{70} do not show fragmentation ions in the MS2 spectrum, similar to what has been reported in various other studies. C_{60} and C_{70} are known to be very stable during mass spectral fragmentation and cage fragmentation does not occur. MS/MS of the mono-functionalized fullerenes resulted in a product ion with the highest intensity at m/z 720.0005 and m/z 840.0005 for the C_{60} and C_{70} functionalized fullerenes, respectively. These fragment ions correspond to the “bare fullerene”, e.g. the loss of the functionalized group.

For all the mono-functionalized fullerenes, next to the bare fullerene, additional fragmentation products can be seen in the MS2 spectrum. For fullerenes which contained a butyric acid alkyl ester moiety, e.g. [60]PCBM, [60]PCBB, [60]PCBO, [60]bisPCBM, [60]ThCBM and [70]PCBM the loss of the propyl acid alkyl ester group (e.g. loss of $(CH_2)_2COO(CH_2)_xH$) was observed. Fragmentation occurred at the same place in these compounds, namely at the C-C bond adjacent to the quarternary carbon atom in the functionalized group. For fullerenes with a larger alkyl moiety in their ester-group namely, [60]PCBB and [60]PCBO, an additional fragment ion occurred at m/z 896.0835. This fragment ion is a result of the cleavage of the carboxylate ester and corresponds to the $[C_{60}+C_6H_5C(CH_2)_3COOH]^{-\bullet}$ ion.

The fullerene with two functionalized groups, namely [60]bisPCBM showed a different fragmentation mass spectrum compared with the mono-functionalized fullerenes. The most intense MS2 ion is at m/z 910.1001, which corresponds to the loss of one of the PCBM groups ($[M-C_{12}H_{14}O_2]^{-\bullet}$). Also a product ion at m/z 720.0005 was seen, but it was less intense in comparison with this fragment ion in the MS2 spectra of the mono functionalized fullerene derivatives. The ion at m/z 720.0005 corresponded to the loss of the two PCBM groups. A third product ion is seen at m/z 1013.1547 which corresponds to $[C_{60}+C_6H_5CCH_2+C_6H_5C(CH_2)_3COOCH_3]^{-\bullet}$, which can be explained by the loss of one propyl acid methyl ester group.

3.3 SPE optimalisation

Since low concentrations of fullerenes are expected in environmental water samples, sample preparation is needed to concentrate the fullerenes to obtain the desired sensitivity. In this research we have build further on our previous work [23], in which we applied solid phase extraction with C18 cartridges. Recoveries for C_{60} of 32±2% in tap water, and 26±4% for surface water samples were obtained, which in the present study we wanted to improve.

Solid phase extraction was optimized by varying different conditions in order to improve the recovery of the suite of fullerenes. First, different solid phase extraction materials (Figure 2A), were tested and recoveries for all fullerenes appeared to be lowest on the C8 material. Recoveries were highest for the fullerenes, except for C_{60}, on C18 SPE material. Based on these results C18 SPE material was chosen for further optimization experiments.

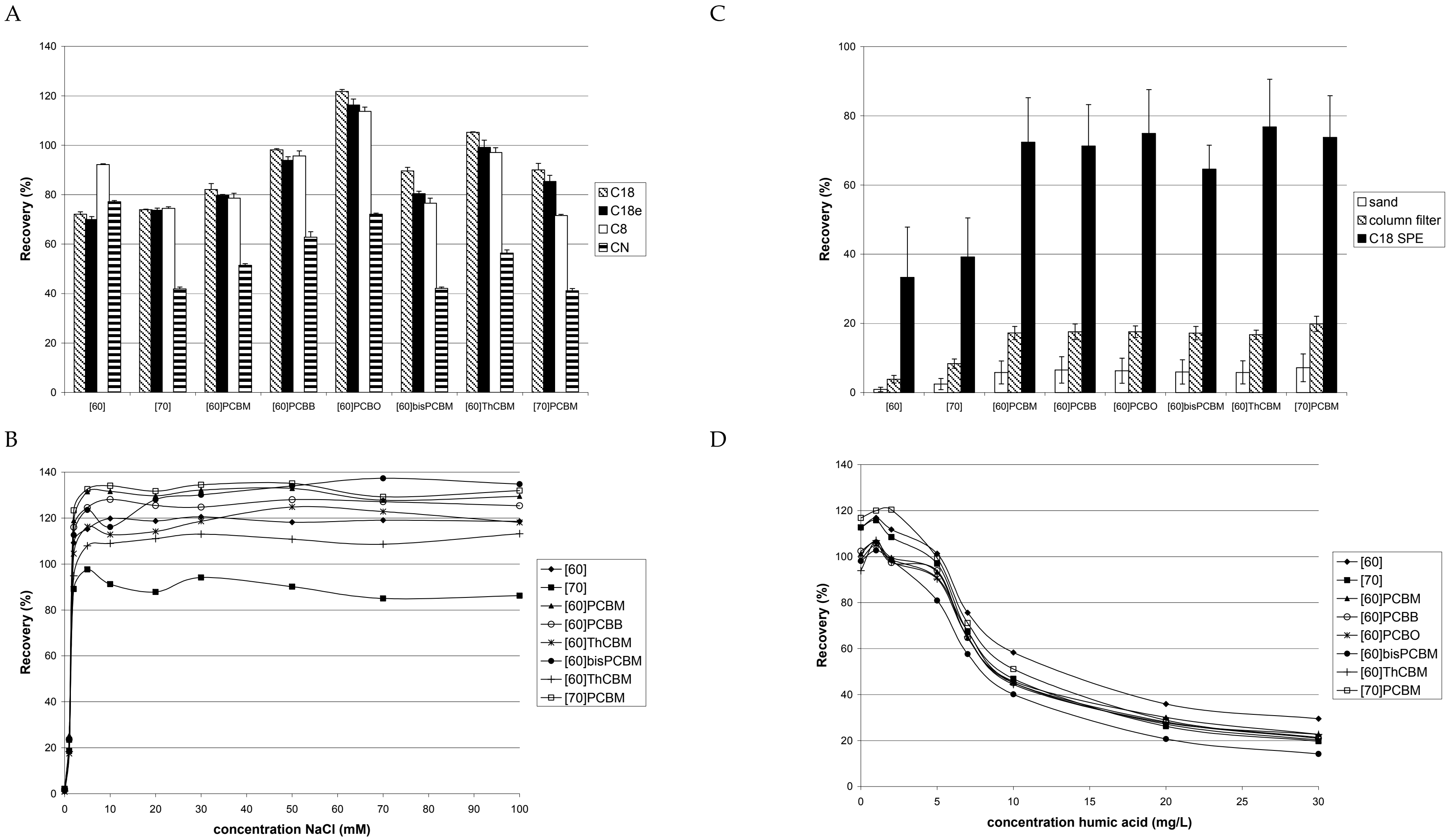
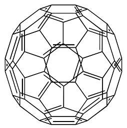
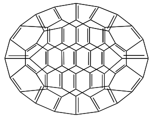
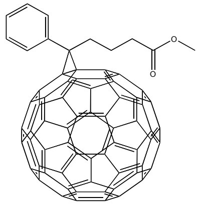
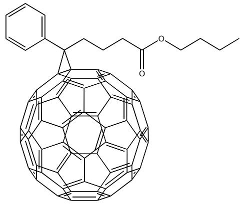
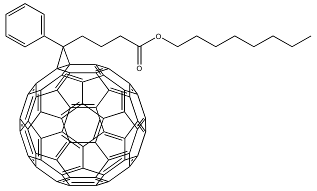
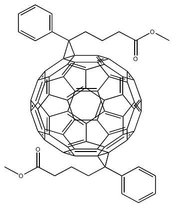
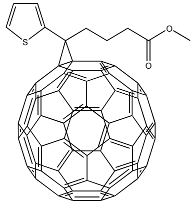
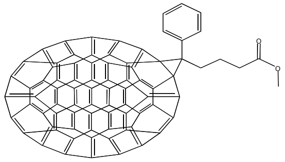


Figure 2: Results of the experiments carried out for optimalization of SPE of fullerenes and functionalized fullerenes a) Recoveries obtained with different solid phase materials (n=2, addition of fullerenes 200 ng/L to drinking, b) Effect of the salt concentration on the recovery (n=1 , addition of fullerenes at 40 ng/L to ultrapure water) , c) Separate extraction of the sand, column frit, and C18 SPE column (n=4, addition of fullerenes 40 ng/L to ultrapure water) and d) Effect of humic acid on the recovery (n=1, addition of fullerenes at 40 ng/L to ultrapure water). Error bars in a) and c) represent standard deviations.

Table 1: Mass spectrometric results of C_{60}, C_{70} and the 6 functionalized fullerenes.

Component	Abbreviation	Bruto formula	Cas-nr	Structure	mass $[M]^{-\bullet}$	Accurate mass MS2	Fragment ions	Loss
C_{60}	[60]	C_{60}	99685-96-8		720.0005	720.0005	$[C_{60}]^{-\bullet}$	-
C_{70}	[70]	C_{70}	115383-22-7		840.0005	840.0005	$[C_{70}]^{-\bullet}$	-
[6,6]-Phenyl-C_{61}-butyric acid methyl ester	[60]PCBM	$C_{72}H_{14}O_2$	160848-21-5		910.0999	720.0005	$[C_{60}]^{-\bullet}$	$-C_6H_5C(CH_2)_3COOCH_3$
						823.0553	$[C_{60}+C_6H_5CCH_2]^{-\bullet}$	$-(CH_2)_2COOCH_3$
[6,6]-Phenyl-C_{61}-butyric acid methyl ester	[60]PCBB	$C_{75}H_{20}O_2$	571177-66-7		952.1469	720.0005	$[C_{60}]^{-\bullet}$	$-C_6H_5C(CH_2)_3COO(CH_2)_3CH_3$
						896.0835	$[C_{60}+C_6H_5C(CH_2)_3COOH]^{-\bullet}$	$-(CH_2)_3CH_3$
						823.0553	$[C_{60}+C_6H_5CCH_2]^{-\bullet}$	$-(CH_2)_2COO(CH_2)_3CH_3$
[6,6]-Phenyl-C_{61}-butyric acid octyl ester	[60]PCBO	$C_{79}H_{28}O_2$	571177-68-9		1008.2095	720.0005	$[C_{60}]^{-\bullet}$	$-C_6H_5C(CH_2)_3COO(CH_2)_7CH_3$
						896.0835	$[C_{60}+C_6H_5C(CH_2)_3COOH]^{-\bullet}$	$-(CH_2)_7CH_3$
						823.0553	$[C_{60}+C_6H_5CCH_2]^{-\bullet}$	$-(CH_2)_2COO(CH_2)_7CH_3$

[6,6]-Bis-Phenyl-C_{61}-butyric acid methyl ester	[60]BisPCBM	$C_{72}H_{14}O_2$	n/a		1100.1993	910.0999 720.0005 1013.1547	$[C_{60}+C_6H_5C(CH_2)_3COOCH_3]^{-\bullet}$ $[C_{60}]^{-\bullet}$ $[C_{60}+C_6H_5CCH_2+C_6H_5C(CH_2)_3COOCH_3]^{-\bullet}$	$-C_6H_5C(CH_2)_3COOCH_3$ -2 x $(C_6H_5C(CH_2)_3COOCH_3)$ $-(CH_2)_2COOCH_3$
[6,6]-Thienyl-C_{61}-butyric acid methyl ester	[60]ThCBM	$C_{70}H_{12}O_2S$	925673-03-6		916.0563	720.0005 829.0117	$[C_{60}]^{-\bullet}$ $[C_{60}+C_4H_3SCCH_2]^{-\bullet}$	$-C_4H_3SC(CH_2)_3COOCH_3$ $-(CH_2)_2COOCH_3$
[6,6]-Phenyl C_{71} butyric acid methyl ester	[70]PCBM	$C_{82}H_{14}O_2$	609771-63-3		1030.0999	840.0005 943.0553	$[C_{70}]^{-\bullet}$ $[C_{70}+(C_6H_5CCH_2)]^{-\bullet}$	$-C_6H_5C(CH_2)_3COOCH_3$ $-(CH_2)_2COOCH_3$

Moreover, the influence of the addition of salt to the aqueous fullerene suspension on the recovery was studied. At increasing NaCl concentrations the recoveries for all fullerene compounds increased and reached a plateau at a concentration of about 10 mM (Figure 2B). 50mM NaCL was chosen in the final optimized protocol to ensure an excess of salt during SPE to obtain optimal recoveries. This phenomenon has been described before. Xiao et al studied the influence of salts on the recovery of C_{60} and C_{70} in LLE and SPE sample preparation [37]. Their results indicated that the addition of $MgCl_2$ during extraction facilitated the partitioning of C_{60} and C_{70} into the organic phase and consequently the recovery was increased. This was explained by the fact that the zeta-potential increased dramatically, as a consequence of the salt added. Others [22] have proposed that the electric double layer of the C_{60} aggregates is destabilized by the addition of salts, which subsequently results in facilitated extraction of C_{60} into the toluene phase.

For surface water samples, a filtration step using sea sand was used to remove particles that might cause clogging of the SPE column. After the filtration step, fullerenes were extracted using the optimized SPE method. It was observed that recoveries were lower for the filtered samples compared with non-filtered samples (Figure 2C). An elution with toluene of the different components of the sand filter showed that the fullerenes absorbed to the sand and to the column frit (Figure 2C). The average recovery for the different fullerenes was 20% for column frit and 5% for the sand. In the final optimized protocol it was decided to elute the sand filter and the SPE column simultaneously, i.e. on top of each other. Analysis by means of Field Flow Fractionation (FFF) in combination with Multi Angle Light Scattering (MALS) showed that the size distribution of nC_{60} before and after sand filtration coincides (data not shown).

3.4 Method performance

The method performance of the whole analytical method was determined in ultrapure water. The linearity of the whole analytical method was determined for a concentration range of 1-40 ng/L, and r^2 values of more than 0.99 were obtained for all analyzed fullerenes (Table 2). LOD were determined by spiking fullerenes to ultra pure water at a concentration of 1 ng/L (n=7) and very low LOD values were obtained for all compounds, ranging from 0.17 ng/L for [70]PCBM to 0.28 ng/L for C_{60}, and subsequent LOQ values of 0.57 ng/L to 0.91 ng/L, which is about a ten-fold improvement compared with previous work [23].

Table 2: Whole method performance characteristics for all analyzed fullerenes in ultra pure water.

Compound	LOD (ng/L)	LOQ (ng/L)	Linearity - r^2 (1-40 ng/L)
[60]	0.28	0.91	0.9960
[70]	0.21	0.69	0.9992
[60]PCBM	0.16	0.53	0.9991
[60]PCBB	0.22	0.74	0.9988
[60]PCBO	0.24	0.79	0.9985
[60]bisPCBM	0.17	0.57	0.9984
[60]ThCBM	0.19	0.65	0.9993
[70]PCBM	0.17	0.57	0.9986

Next, recoveries for all components were determined in ultrapure water, drinking water and surface water (Table 3). Relative standard deviations (RSD, e.g. repeatability) were <7% for all components in all matrices. Recoveries in ultrapure water and in drinking water were high varying from 103% for C_{60} till 133% for [70]PCBM. Recoveries in surface water samples were in general lower than the recoveries obtained in ultrapure water and drinking water, and ranged from 75% for C_{60} and C_{70} till 89% for [60]PCBM. The recovery for [60]bisPCBM in surface water was much lower, e.g. 49% compared with the other compounds, which might be explained by the fact there is less surface of the fullerene available for binding to the SPE material compared with mono-functionalized or non-functionalized fullerenes.

Table 3: Recoveries in % for all analyzed fullerenes in ultrapure water (UPW), drinking water (DW) and surface water (SW) at 40 ng/L spiking level for all compounds. Values in parenthesis correspond to relative standard deviation (RSD).

Compound	Recovery (%) UPW (n=8)	Recovery (%) DW (n=7)	Recovery (%) SW (n=4)
[60]	103 (5)	118 (3)	75 (4)
[70]	114 (5)	119 (3)	75 (4)
[60]PCBM	127 (6)	119 (3)	89 (4)
[60]PCBB	131 (6)	117 (2)	83 (3)
[60]PCBO	129 (6)	121 (4)	87 (4)
[60]bisPCBM	116 (4)	114 (4)	49 (7)
[60]ThCBM	126 (5)	116 (5)	86 (4)
[70]PCBM	122 (4)	133 (5)	80 (5)

The recoveries were determined using liquid liquid extraction (LLE) as a reference method (e.g. set at 100%).

The most likely explanation for the lower recoveries in surface water compared with the other matrices is that matrix suppression occurs. The concentration of humic acids in surface waters is higher compared with ultrapure and drinking water. To confirm this hypothesis, humic acid was spiked to ultra pure water and recoveries for all fullerenes were determined (Figure 2D). From this figure it can be seen that when the concentration of humic acid increased, the recovery of all fullerene compounds decreased. Xiao et. al. [37] observed the same effect for C_{60} and C_{70} with increasing concentrations of humic acid as well as fulvic acid. In the present study we also see the effect on the recoveries of the functionalized fullerenes.

4 Conclusion

In the present paper we report on the development of a highly specific and robust analytical method for the quantitative determination of C_{60} and C_{70} as well as six functionalized fullerene derivatives in different aqueous matrices. So far there are no papers describing a comparable analytical technique that is suitable for the analysis of such a large suite of fullerenes at a very low, environmental relevant, detection level (e.g. LOD = 0.3 ng/L and LOQ = 1 ng/L). The use of high resolution accurate mass spectrometry as applied in this method creates the possibility to restrospectively look for the presence of other functionalized fullerenes in environmental samples. The developed approach is promising and can be applied for (1) environmental monitoring, (2) a more in-depth study of environmental fate and transformation products and (3) studying the water treatment efficiency of C_{60}, C_{70} and the various functionalized fullerenes.

5 References

1. Rogers, N. J.; Franklin, N. M.; Apte, S. C.; Batley, G. E.; Angel, B. M.; Lead, J. R.; Baalousha, M., Physico-chemical behaviour and algal toxicity of nanoparticulate CeO_2 in freshwater. *Environ. Chem.* **2010,** *7*, (1), 50-60.

2. Poole, C. P.; Owens, F. J., *Introduction to Nanotechnology* **2003**.

3. Roco, M. C., Broader Societal Issues of Nanotechnology. *Journal of Nanoparticle Research* **2003,** *5*, (3-4), 181-189.

4. Kroto, H. W.; Heath, J. R.; O'Brien, S. C.; Curl, R. F.; Smalley, R. E., C_{60}: Buckminsterfullerene. *Nature* **1985,** *318*, (6042), 162-163.

5. Bühl, M.; Hirsch, A., Spherical Aromaticity of Fullerenes. *Chem. Rev.* **2001,** *101*, (5), 1153-1184.

6. Zhang, W.; Isaacson, C. W.; Rattanaudompol, U. s.; Powell, T. B.; Bouchard, D., Fullerene nanoparticles exhibit greater retention in freshwater sediment than in model porous media. *Water Res.* **2012,** *46*, (9), 2992-3004.

7. Gao, J.; Wang, Y.; Folta, K. M.; Krishna, V.; Bai, W.; Indeglia, P.; Georgieva, A.; Nakamura, H.; Koopman, B.; Moudgil, B., Polyhydroxy Fullerenes (Fullerols or Fullerenols): Beneficial Effects on Growth and Lifespan in Diverse Biological Models. *PLoS ONE* **2011,** *6*, (5), e19976.

8. Jafvert, C. T.; Kulkarni, P. P., Buckminsterfullerene's (C_{60}) octanol-water partition coefficient (Kow) and aqueous solubility. *Environmental science & technology* **2008,** *42*, (16), 5945-50.

9. Heymann, D., Solubility of fullerenes C_{60} and C_{70} in seven normal alcohols and their deduced solubility in water. *Fullerene Science and Technology* **1996,** *4*, (3), 509-515.

10. 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.

11. Ma, X.; Bouchard, D., Formation of aqueous suspensions of fullerenes. *Environmental Science and Technology* **2009,** *43*, (2), 330-336.

12. Hyung, H.; Kim, J.-H., Dispersion of C60 in natural water and removal by conventional drinking water treatment processes. *Water Research* **2009,** *43*, (9), 2463-2470.

13. 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 C60 colloidal dispersions. *Journal of Colloid and Interface Science* **2012,** *370*, (1), 27-31.

14. Lens, M., Recent progresses in application of fullerenes in cosmetics. *Recent Pat Biotechnol* **2011,** *5*, (2), 67-73.

15. Thompson, B. C.; Fréchet, J. M. J., Polymer-fullerene composite solar cells. *Angewandte Chemie - International Edition* **2008,** *47*, (1), 58-77.

16. Zhang, Y.; Bai, Y.; Yan, B., Functionalized carbon nanotubes for potential medicinal applications. *Drug discovery today* **2010,** *15*, (11-12), 428-35.

17. Bakry, R.; Vallant, R. M.; Najam-ul-Haq, M.; Rainer, M.; Szabo, Z.; Huck, C. W.; Bonn, G. K., Medicinal applications of fullerenes. *International journal of nanomedicine* **2007,** *2*, (4), 639-49.

18. Wiesner, M. R.; Lowry, G. V.; Alvarez, P.; Dionysiou, D.; Biswas, P., Assessing the risks of manufactured nanomaterials. *Environmental science & technology* **2006,** *40*, (14), 4336-45.

19. Isaacson, C. W.; Kleber, M.; Field, J. A., Quantitative analysis of fullerene nanomaterials in environmental systems: A critical review. *Environmental Science and Technology* **2009,** *43*, (17), 6463-6474.

20. Pycke, B. F. G.; Halden, R. U.; Benn, T. M.; Westerhoff, P.; Herckes, P., Strategies for quantifying C_{60} fullerenes in environmental and biological samples and implications for studies in environmental health and ecotoxicology. *TrAC - Trends in Analytical Chemistry* **2011,** *30*, (1), 44-57.

21. Wang, C.; Shang, C.; Westerhoff, P., Quantification of fullerene aggregate nC_{60} in wastewater by high-performance liquid chromatography with UV-vis spectroscopic and mass spectrometric detection. *Chemospere* **2010,** *80*, 334-339.

22. Bouchard, D.; Ma, X., Extraction and high-performance liquid chromatographic analysis of C_{60}, C_{70}, and [6,6]-phenyl C_{61}-butyric acid methyl ester in synthetic and natural waters. *Journal of Chromatography A* **2008,** *1203*, (2), 153-159.

23. van Wezel, A. P.; Morinière, V.; Emke, E.; ter Laak, T.; Hogenboom, A. C., Quantifying summed fullerene nC_{60} and related transformation products in water using LC LTQ Orbitrap MS and application to environmental samples. *Environment International* **2011,** *37*, 1063-1067.

24. Chen, H. C.; Ding, W. H., Determination of aqueous fullerene aggregates in water by ultrasound-assisted dispersive liquid-liquid microextraction with liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry. *Journal of Chromatography A* **2012,** *1223*, 15-23.

25. Isaacson, C. W.; Bouchard, D., Asymmetric flow field flow fractionation of aqueous C_{60} nanoparticles with size determination by dynamic light scattering and quantification by liquid chromatography atmospheric pressure photo-ionization mass spectrometry. *Journal of Chromatography A* **2010,** *1217*, (9), 1506-1512.

26. Astefanei, A.; Núñez, O.; Galceran, M. T., Non-aqueous capillary electrophoresis separation of fullerenes and C_{60} fullerene derivatives. *Analytical and bioanalytical chemistry* **2012,** *404*, (2), 307-13.

27. Núñez, O.; Gallart-Ayala, H.; Martins, C. P.; Moyano, E.; Galceran, M. T., Atmospheric pressure photoionization mass spectrometry of fullerenes. *Anal Chem* **2012,** *84*, (12), 5316-26.

28. Fellows, M. D.; O'Donovan, M. R., Cytotoxicity in cultured mammalian cells is a function of the method used to estimate it. *Mutagenesis* **2007,** *22*, (4), 275-280.

29. Farré, M.; Pérez, S.; Gajda-Schrantz, K.; Osorio, V.; Kantiani, L.; Ginebreda, A.; Barceló, D., First determination of C_{60} and C_{70} fullerenes and N-methylfulleropyrrolidine C_{60} on the suspended material of wastewater effluents by liquid chromatography hybrid quadrupole linear ion trap tandem mass spectrometry. *Journal of Hydrology* **2010,** *383*, (1-2), 44-51.

30. Kawano, S.-i.; Murata, H.; Mikami, H.; Mukaibatake, K.; Waki, H., Method optimization for analysis of fullerenes by liquid chromatography/atmospheric pressure photoionization mass spectrometry. *Rapid Communications in Mass Spectrometry* **2006,** *20*, (18), 2783-2785.

31. Li, L.; Huhtala, S.; Sillanpää, M.; Sainio, P., Liquid chromatography-mass spectrometry for C_{60} fullerene analysis: optimisation and comparison of three ionisation techniques. *Analytical and bioanalytical chemistry* **2012,** *403*, (7), 1931-8.

32. Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B., Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions. *Environmental science & technology* **2009,** *43*, (24), 9216-9222.

33. Chen, Z.; Westerhoff, P.; Herckes, P., Quantification of C_{60} fullerene concentrations in water. *Environmental Toxicology and Chemistry* **2008,** *27*, (9), 1852-1859.

34. Sanchis, J.; Berrojalbiz, N.; Caballero, G.; Dachs, J.; Farré, M.; Barcelo, D., Occurrence of aerosol-bound fullerenes in the Mediterranean Sea atmosphere. *Environmental science & technology* **2012,** *46*, (3), 1335-43.

35. Hummelen, J. C.; Knight, B. W.; LePeq, F.; Wudl, F.; Yao, J.; Wilkins, C. L., Preparation and Characterization of Fulleroid and Methanofullerene Derivatives. *The Journal of Organic Chemistry* **1995,** *60*, (3), 532-538.

36. Bouwer, R. K. M.; Hummelen, J. C., The Use of Tethered Addends to Decrease the Number of Isomers of Bisadduct Analogues of PCBM. *Chemistry – A European Journal* **2010,** *16*, (37), 11250-11253.

37. Xiao, Y.; Chae, S. R.; Wiesner, M. R., Quantification of fullerene (C_{60}) in aqueous samples and use of C_{70} as surrogate standard. *Chemical Engineering Journal* **2011,** *170*, (2-3), 555-561.