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Size and concentration determination of (functionalised) fullerenes in surface and sewage water matrices using field flow fractionation coupled to an online accurate mass spectrometer: Method development and validation

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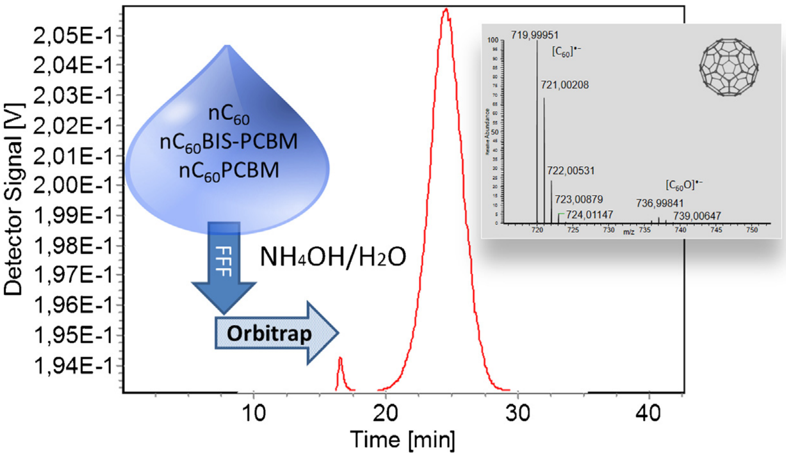
HIGHLIGHTS

- A 0.05(v/v)% NH_4OH solution was used for three different fullerene suspensions.
- LOD of the Orbitrap HRMS is about 0.1 $\mu g\,L^{-1}$.
- The water matrix (e.g. sewage water) does effect the size of the aggregates.

GRAPHICAL ABSTRACT



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ABSTRACT

In order to assess the environmental risks of a compound it is imperative to have suitable and reliable techniques for its determination in environmental matrices. In this paper, we focused on a method development for the recently introduced online coupling of a field flow fractionation (FFF) system to an Orbitrap-HRMS, that allows the simultaneous size and concentration determination of different aqueous fullerene aggregates and their concentrations in different size fractions. A 0.05% NH_4OH solution in water was identified as the best carrier liquid for the analysis of the three different aqueous fullerene suspensions (C_{60} [60], [6,6]-phenyl-C_{61} butyric acid methyl ester ([60]PCBM) and [6,6]-(bis)phenyl-C_{61} butyric acid methyl ester ([60]bisPCBM)). The multi-angle light scattering (MALS) data received after employing the ammonia solution was consistent with both the theory and calibration using well defined Au and latex particles. The LODs obtained using Orbitrap HRMS detection were 0.1 $\mu g\,L^{-1}$ for an injection volume of 100 μL which are significantly better than the LODs obtained by using UV (20 $\mu g\,L^{-1}$) and

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MALS detectors (5 μg L^{-1}). However, these LODs can be further improved as in theory there is no limit to the amount of sample that can be injected into the FFF. Environmental samples (river and sewage water) were spiked with fullerenes and the fractograms obtained for these samples revealed that the matrix does affect the size of fullerene aggregates. Information on the size distribution can be useful for the risk assessment of these particles.

1. Introduction

Nanoparticles (NPs) are by definition 1–100 nm in size in at least one dimension. They display unique chemical and physical properties such as large surface/area ratio, thermal stability, low permeability and high conductivity [1–3]. Therefore, nanoparticles have been applied in personal care products, drug delivery systems [4] and solar cells [5]. The estimated revenue for nanotechnology and nanomaterials in consumer products in 2015 is around five trillion dollars [6]. This growing interest in nanotechnology applications and products led to consider NPs as environmental contaminants [7]. Meanwhile, several papers report on the presence of various NPs in the environment [8–12].

NPs can be subdivided into inorganic and organic compounds. The latter category includes the carbon-based fullerenes. These nanoparticles are produced in amounts of 1500 t per year [13]. Therefore, the OECD placed them on the list of environmentally relevant nanomaterials [14].

Due to an anticipated rising production level, fullerenes can find their way into the environment via various routes and can be expected in the air, water, soil and sediment [15,16]. For surface water [16,17] industrial effluent [17] and sewage water [18] this has already been shown. In rivers and industrial effluent concentrations up to 130 ng L^{-1} were found. In sewage the concentrations were often significantly above 1 μg L^{-1}. In these studies fullerenes were analysed after extraction from the aqueous matrix. This allows the determination of the total concentration. Unfortunately, this approach does not enable the determination of the fullerene concentrations in different size fractions nor does one get information on the size of the aggregates in water. However, the tendency of fullerenes to form stable nanometer-sized clusters is an important characteristic. Without this ability their solubility would be less than 10^{-11} mg L^{-1} in water [19,20]. The moment these aggregates are formed the solubility is several orders of magnitude better (up to mg L^{-1}). As size dependent toxicity has been demonstrated for other nanoparticles [21], knowledge about the size of the aggregates is crucial for risk assessment.

To enable measurement of the aggregates or to determine in which size fraction they accumulate, samples need to be analysed directly without any pre-treatment that could result in their disintegration. A technique that allows this is flow field fractionation (FFF) in combination with light scattering techniques, such as multi angle light scattering (MALS).

During the last years, FFF techniques (especially asymmetrical flow field-flow fractionation (AF4)) have been applied in different fields [8,22–26]. FFF is a size separation technique similar to size-exclusion chromatography (SEC) but without the use of a packed column and with a wider size range than SEC. The fractionation principle of FFF is based on applying an external perpendicular field to a main parabolic flow in an open flat channel (a detailed explanation of FFF and AF4 principles is given in several papers and books [12,25,27–32]). The elution time is related to the diffusion coefficient of the particle which in turn can be linked to their radius (Stokes–Einstein equation). As FFF alone does not yield any information on the particles (except for the size calculated from the elution time, Supporting information Eq. (E3)), a detector is needed. Most commonly UV, ICP-MS, ICP-OES or MALS detectors are used. However, for measuring organic compounds at environmentally relevant concentrations these detectors are not suitable as they either cannot detect organic particles (ICP) or they are not sensitive enough (UV, MALS) [33–35]. Neither of these detectors is able to characterise the fullerenes to boot.

Isaacson and Bouchard proposed the use of AF4 with offline LC–MS analysis for the size distribution determination of aqueous fullerene aggregates (aqu/nC_{60}) at environmental concentrations but it has not been done yet [36]. In an earlier short communication, we describe the technical setup needed to allow the analysis of fullerenes in environmental samples with an AF4 coupled to an Orbitrap MS with an APPI [37]. The coupling of AF4 to an HRMS has never been reported before. The high flow rates needed for AF4, as compared with liquid chromatography, makes online coupling of AF4 to MS difficult and therefore we used a low detector flow rate, taking advantage of a split pump that allows to divert analyte-free solution away from the detector. As far as we know only one other paper reported on online MS detection to an FFF. However, these authors used hollow-fibre FFF (HF5) for the analysis of proteins [26]. The coupling of HF5 to MS is more feasible than other FFF techniques because of the lower flow rates required for the fractionation (below to 400 μL min^{-1}).

In the present paper, we report on the optimisation of the AF4 method to analyse fullerenes in environmental samples and will make use of the earlier mentioned online-coupling of the Orbitrap HRMS to the AF4 [37]. Fullerenes aggregates were separated in the AF4 channel and subsequently measured by MS using an atmospheric pressure photo ionisation interface (APPI), as this proved to be the most suitable technique [38,39]. Size determination is achieved by (I) measuring at a concentration high enough for the MALS detector and (II) by using calibration compounds (Au nanoparticle and latex particles). We propose the use of a different eluent than MilliQ-water, as we can demonstrate that in this eluent the fullerenes aggregate size does not coincide with the theory nor does it overlap with the size of other nanoparticles, that were used for calibration purposes.

2. Materials and methods

2.1. Reagents and chemicals

C_{60} (purity > 99.9%) was purchased from Materials and Electrochemical Research Corporation (Tucson, AZ, USA). [6,6]-phenyl-C_{61} butyric acid methyl ester ([60]PCBM) (purity > 99%) and [6,6]-(bis) phenyl-C_{61} butyric acid methyl ester ([60]bisPCBM) (purity > 99.5%) were purchased from Solenne B.V. (Groningen, The Netherlands). Toluene (ultraresidue analyse grade) and anisole were obtained from J.T. Baker (Boom, Meppel, The Netherlands), NaCl from Sigma–Aldrich (Steinhem, Germany) and ultrapure water (resistivity > 18 MΩ cm^{-1}) was obtained from a Milli-Q water purification system (Millipore, Amsterdam, The Netherlands). The individual stock solutions of fullerenes (500 mg L^{-1}) and a mixture stock solution (40 mg L^{-1}) were prepared in toluene and used for quantification purposes by LC-HRMS method. The individual aqueous fullerenes suspensions were prepared by extended stirring [37]. Around 10 mg of each compound was weighted and placed in a glass bottle containing