Short communication

Asymmetrical flow field-flow fractionation hyphenated to Orbitrap high resolution mass spectrometry for the determination of (functionalised) aqueous fullerene aggregates

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ABSTRACT

In this short communication we report on the technical implementations of coupling an asymmetric flow field-flow fractionation (AF4) instrument to a high resolution mass spectrometer (Orbitrap) using an atmospheric photoionisation interface. This will allow for the first time online identification of different fullerenes in aqueous samples after their aggregates have been fractionated in the FFF channel. Quality parameters such as limits of detection (LODs), limits of quantification (LOQs) or linear range were evaluated and they were in the range of hundreds ng/L for LODs and LOQs and the detector response was linear in the range tested (up to ~20 µg/L). The low detection and quantification limits make this technique useful for future environmental or ecotoxicology studies in which low concentration levels are expected for fullerenes and common on-line detectors such as UV or MALS do not have enough sensitivity and selectivity.

1. Introduction

Interest in nanomaterial-related applications is growing due to their novel and unique characteristics compared to "normal scale" materials [1–3]. It can therefore be assumed that nanomaterials including nanoparticles (NP) are emitted into the environment [4]. To assess the environmental risks of NPs, the development of techniques to measure and characterise them in natural environments is a priority issue [5].

Field-flow fractionation (FFF) [6], especially the asymmetrical flow version (AF4), is one of the most promising particle separation techniques that can – especially in combination with different on-line detectors – be used for characterisation of NPs and colloids [7,8]. However, the lack of sensitivity of many detectors commonly used, such as UV or light scattering devices, limits its use under environmentally relevant conditions [7]. Inorganic NPs, such as gold and silver NPs, can be characterised and measured

at environmental concentrations by hyphenation of AF4 to an ICP-MS [9]. Carbon-based NPs, such as fullerenes [10] cannot be characterised using this combination. Several methods have been developed for the determination of concentrations of fullerenes in environmental matrices e.g., LC–UV [11], or LC–MS [12–14] using atmospheric pressure ionisation [15–17], but information about the size of their aggregates in water cannot be obtained as they need to be extracted from the aqueous phase. Information on the aggregate size is, however, crucial as the mobility and deposition of fullerenes in the aquatic environment strongly depends on this characteristic [18–20]. Therefore, up to now samples had to be analysed twice, once by using FFF to receive information on the size of the aggregates and then by using MS to determine the concentration and type of fullerene. Now it is possible to analyse each size fraction. Hence, one can see e.g., if compound A can only be found in size fractions < 50 nm and compound B in fractions > 50 nm. This was not possible with MALS or UV detectors.

It should be mentioned that fullerene clusters are destroyed during ionisation in the MS and size information cannot be obtained by use of APPI-MS alone. FFF is necessary.

To improve, shorten and ease the analysis of samples we suggest coupling AF4 to HRMS (accurate mass). In the present study AF4 was hyphenated with an Orbitrap-HRMS in order to combine

particle/aggregate separation with sensitive concentration detection of three different fullerenes. The description of the technical implementation will not only allow further development of the FFF method but might also open a door to the analysis of other organic particles and aggregates. To the best of our knowledge a combination of FFF, APPI and Orbitrap-HRMS has never been reported on before. The general feasibility of coupling FFF to a MS has been demonstrated before for ICP-MS [21–23] and ESI-MS/MS [24].

2. Experimental

2.1. Reagents and standards

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) and ultrapure water (resistivity > 18 MΩ) was obtained from a Milli-Q water purification system (Millipore, Amsterdam, The Netherlands). Milli-Q-water used as carrier liquid in the FFF was filtered through 0.1 μM membrane filters (Postnova Analytics GmbH, Landsberg, Germany) prior to entering the FFF channel.

The individual aqueous fullerenes suspensions (aqu/nC60) were prepared by extended stirring [25]. 10 mg of each compound was placed in a glass bottle containing 500 mL of Milli-Q water and they were stirred in the dark for more than one month at 25 °C. The exact concentration of the aqueous solution (filtered through 0.45 μm regenerated cellulose (RC) to remove larger particles) was determined by liquid–liquid extraction followed by LC-APPI-HRMS.

2.2. Asymmetrical flow field-flow fractionation

A Postnova AF2000 system (Postnova Analytics GmbH, Landsberg, Germany) was used. The AF4 module was coupled to a UV-detector (Shimadzu) and a Multi Angle Light Scattering detector (Postnova) The AF4 trapezoidal channel was 27.5 cm long from tip to tip, the height of the spacer was 250 μm and the permeable wall consisted of a 10 kDa RC membrane (Postnova). The carrier liquid was Milli-Q water and the injection volume was set to 100 μL using an autosampler device (Postnova). The fractionation conditions are summarised in Table 1.

2.3. APPI-LTQ-Orbitrap measurements

A hybrid LTQ Orbitrap (Thermo Electron) equipped with an atmospheric pressure photoionisation (APPI) interface (Thermo Electron) which uses a Syagen PhotoMate VUV Krypton lamp (20 eV) was employed for HRMS measurements. To optimise the MS conditions, the stock aqu/nC60 solution was infused in the source with a syringe pump using AF4 flow conditions. Toluene was used as a dopant and introduced in the auxiliary gas. The exact mass 720.00055 m/z $[C_{60}]^{\bullet-}$ corresponding to the molecular ion of C_{60} was monitored in full-scan in FTMS analyser at a resolution of 30,000 FWHM over a mass-range of 300–1300 Da. The optimal parameters are summarised in Table 1.

3. Results and discussion

3.1. AF4 optimisation

Information explaining the principle of FFF can be found elsewhere [6,26–28]. Generally, the optimisation of the different

Table 1
Selected parameters for AF4-APPI-LTQ-Orbitrap method.

AF4 conditions	
Membrane	Regenerated cellulose 10 kDa
Carrier liquid	Milli-Q water
Spacer thickness	250 μm
Detector flow	0.1 mL/min
Split flow	0.5 mL/min
Cross flow	1.2 mL/min (0–12 min); 1.2–0 mL/min (12–15 min, exp. 0.2); 0 mL/min (15–20 min)
Injection time	5 min
Injection volume	100 μL
Injection flow	0.2 mL/min
Focusing flow	1.2 mL/min
LTQ-Orbitrap conditions	
Interface	(−)APPI
Probe position	C, 0, 0.75 μm
Capillary temperature	350 °C
Vaporiser temperature	500 °C
Sheath gas	20 AU
Auxiliary gas	10 AU
Sweep gas	0 AU
Dopant	Toluene
Dopant flow	0.1 mL/min
Capillary voltage	−20 V
Tube lens	−200 V

parameters involved in AF4 aims at the separation of monodisperse components resulting in distinct signals for each size fraction. However, the size distribution of aqu/nC$_{60}$ solutions is polydisperse and consequently a broad signal in the fractogram is obtained [17]. Therefore, AF4 parameters were optimised to enhance the MS response but maintaining proper fractionation of fullerene aggregates which was assessed using MALS data obtained by analysing stock solutions of aqu/nC$_{60}$.

The carrier liquid is a limitation for coupling AF4 to MS because the latter should not be used with non-volatile electrolytes and surfactants commonly used in AF4 [29]. Ultrapure water was selected as carrier liquid because it is compatible with the MS interface and its use has been suggested before for fullerene analysis with AF4 [17].

The ratio between cross flow (F_c) and outlet flow (F_{out}) and their absolute values were optimised to separate the void peak from the analyte peak and to minimise the analysis time. Three different ratios were tested (1, 2 and 3) using a constant outlet flow of 0.8 mL/min. A ratio of 2 was selected as it results in good separation of the fullerene aggregates from the void peak, better size distribution than a ratio of 1 and less sample dilution than a ratio of 3. Different settings for F_c and F_{out} were used (all having a ratio of 2). F_{out} = 0.6 mL/min and F_c = 1.2 mL/min were selected as optimal flows. Afterwards, the focusing time was optimised until the peak area was remained constant. The optimum value was 5 min under the aforementioned AF4-conditions.

Taking into account that MS-ionisation performance with an APPI interface is highly affected by the flow rate (see Section 3.2) an interesting option to improve the analyte response is the use of a split pump to remove the upper layer of liquid at the end of the channel (slot outlet). Using this option, the resulting response measured in the MS shows an increase for the following reasons: first, under cross flow conditions the analytes are accumulated close to the membrane and the rest of the channel is void of analytes. Therefore, the upper layer of the carrier liquid is removed in the slot outlet and preconcentration in the detector flow is achieved. Secondly, an ionisation enhancement in MS is obtained when the detector flow is