



Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: [www.elsevier.com/locate/chroma](http://www.elsevier.com/locate/chroma)



## Characterization of aggregates of surface modified fullerenes by asymmetrical flow field-flow fractionation with multi-angle light scattering detection

Alina Astefanei<sup>a,\*</sup>, Wim Th. Kok<sup>b</sup>, Patrick Bäuerlein<sup>c</sup>, Oscar Núñez<sup>a,d</sup>,  
Maria Teresa Galceran<sup>a</sup>, Pim de Voogt<sup>e</sup>, Peter J. Schoenmakers<sup>b</sup>

<sup>a</sup> Analytical Chemistry Department, University of Barcelona, Martí i Franquès 1-11, E-08028 Barcelona, Spain

<sup>b</sup> Analytical Chemistry Department-HIMS, University of Amsterdam, PO Box 94157, 1090 GD Amsterdam, The Netherlands

<sup>c</sup> KWR Watercycle Research Institute, Groningehaven 7, PO Box 1072, 3430 BB Nieuwegein, The Netherlands

<sup>d</sup> Serra Hunter Fellow, Generalitat de Catalunya, Spain

<sup>e</sup> IBED, University of Amsterdam, PO Box 94248, 1090 GE Amsterdam, The Netherlands

### ARTICLE INFO

#### Article history:

Received 8 May 2015

Received in revised form 30 June 2015

Accepted 1 July 2015

Available online xxx

#### Keywords:

Asymmetrical flow field-flow fractionation

Multi-angle light scattering

Fullerene aggregates

### ABSTRACT

Fullerenes are carbon nanoparticles with widespread biomedical, commercial and industrial applications. Attributes such as their tendency to aggregate and aggregate size and shape impact their ability to be transported into and through the environment and living tissues. Knowledge of these properties is therefore valuable for their human and environmental risk assessment as well as to control their synthesis and manufacture. In this work, asymmetrical flow-field flow fractionation (AF4) coupled to multi-angle light scattering (MALS) was used for the first time to study the size distribution of surface modified fullerenes with both polyhydroxyl and carboxyl functional groups in aqueous solutions having different pH (6.5–11) and ionic strength values (0–200 mM) of environmental relevance. Fractionation key parameters such as flow rates, flow programming, and membrane material were optimized for the selected fullerenes. The aggregation of the compounds studied appeared to be indifferent to changes in solution pH, but was affected by changes in the ionic strength. Polyhydroxy-fullerenes were found to be present mostly as 4 nm aggregates in water without added salt, but showed more aggregation at high ionic strength, with an up to 10-fold increase in their mean hydrodynamic radii (200 mM), due to a decrease in the electrostatic repulsion between the nanoparticles. Carboxy-fullerenes showed a much stronger aggregation degree in water (50–100 nm). Their average size and recoveries decreased with the increase in the salt concentration. This behavior can be due to enhanced adsorption of the large particles to the membrane at high ionic strength, because of their higher hydrophobicity and much larger particle sizes compared to polyhydroxy-fullerenes. The method performance was evaluated by calculating the run-to-run precision of the retention time (hydrodynamic radii), and the obtained RSD values were lower than 1%. MALS measurements showed aggregate sizes that were in good agreement with the AF4 data. A comparison of the scattering radii from the MALS with the hydrodynamic radii obtained from the retention times in AF4 indicated that the aggregate shapes are far from spherical. TEM images of the fullerenes in the dry state also showed branched and irregular clusters.

© 2015 Published by Elsevier B.V.

### 1. Introduction

Fullerenes, the third carbon allotrope discovered by Kroto et al. in 1985 [1], are hollow-sphere nanoparticles composed entirely of carbon. Due to their unique physical and chemical properties they find widespread application in diverse fields, such as

in photovoltaics [2], cosmetics [3], and biomedicine [4]. Nowadays, fullerenes functionalized with polar groups are produced in higher quantities than native fullerenes due to their increasing number of biomedical applications [5]. For instance, polyhydroxy-fullerenes have attracted attention for their good water solubility and biological compatibility [6], and have been demonstrated to be radical scavengers against superoxide anions and hydroxyl radicals [7,8]. Among these compounds, fullerol (C<sub>60</sub>(OH)<sub>24</sub>) holds a special place, being investigated for clinical application as drug carrier, tumor inhibitor [9] and mitochondrial protective antioxidant [10].

\* Corresponding author. Tel.: +34 93 402 1286; fax: +34 93 402 1233.  
E-mail address: [astefanei.alina@gmail.com](mailto:astefanei.alina@gmail.com) (A. Astefanei).

Moreover, it is being considered as starting material for synthesis of fullerene containing polymers [11] and as coating for solid-phase microextraction [12]. Carboxyl  $C_{60}$ -derivatives have potential use for photodynamic therapy [13,14] and as inhibitors of the HIV-1 protease [15].

The anticipated market growth of surface modified fullerenes, in combination with the risk of direct human exposure *via* several applications, has led to concerns about their potential to cause adverse effects on the environment and human health. However, there is a significant lack of knowledge on fullerene exposure, as well as of data on their inherent properties and toxicity. Fullerenes aggregate in aqueous media leading to the formation of structures with various shapes and sizes. It was recently found that the size and shape of the nanoparticles formed dictate their *in vitro* toxicity [16–18] as well as their mobility, fate, bioavailability and toxicity in the environment [19,20]. However, the lack of adequate methods for their characterization and analysis in environmental samples is currently a bottleneck in this field. Currently, the prediction of the fate and behavior of fullerenes is mostly focused on pristine compounds and is being based on laboratory experiments. A direct comparison of the data is not possible because of variations in the experimental conditions which are not consistent among studies. The data gained from the existing studies is not nearly enough to create a detailed prediction model of the behavior and fate of these nanoparticles in the environment, but represent a good starting point for their risk assessment. Therefore, it is crucial to develop reliable methods for the characterization of fullerene aggregates in terms of size distribution and shape, at variant key environmental parameters (e.g., pH, ionic strength natural organic matter) especially for the water soluble compounds for which there is a lack of studies.

The most commonly used techniques for particle sizing are microscopy, e.g., transmission electron microscopy (TEM) or scanning electron microscopy (SEM), optical spectroscopy (UV–vis) and light scattering techniques [21]. Nonetheless, with microscopic methods the integrity of the particles and aggregates is not always guaranteed, and light-scattering methods (e.g., static (MALS) and dynamic light scattering (DLS)) give average size values and are less suited to obtain information on the particle size distributions [22]. Therefore, these methods alone are often not conclusive when applied to nanoparticles and they must be combined with separation techniques for more accurate sizing or aggregation determination [23].

FIFFF is a separation technique introduced by J. Calvin Giddings in the late seventies [24] and has become the most commonly employed mode of FFF. This is a versatile analytical tool for the separation and characterization of macromolecules and particles over a wide size range. Asymmetrical flow-field flow fractionation (AF4), including its hollow fiber format (HF5), have been the completely dominating FIFFF techniques generally used during the last decade [25]. The principles of both AF4 and FIFFF have been reviewed elsewhere [26–29]. Briefly, the fractionation principle is based on applying a perpendicular field (cross-flow) to main parabolic flow in an open flat channel. The retention of sample components of different sizes can be controlled by tuning the cross-flow rates and the separation is based on differences in the diffusion coefficients of eluting particles. The particles with larger diffusion coefficients or smaller sizes diffuse faster to upper layers inside the channel and, therefore, reach to the detector faster than the bigger ones. This is the most widely used mechanism for analytes smaller than  $1\ \mu\text{m}$  referred to as normal-mode separation mechanism [30]. When the particle size exceeds approximately  $1\ \mu\text{m}$ , the steric/hyperlayer mode prevails, and the elution order is reversed in that larger particles elute before smaller particles [31,32]. The relation between the retention time and diffusion coefficient helps in calculating hydrodynamic radii using standard AF4 theory [28,29]. Recently, AF4 was

reported as a method for the size measurement of some fullerene aggregates [16,33–36]. Because of its versatility, this technique is used in a wide range of research and quality control applications, including nanotechnology, molecular biology and environmental analysis. Moreover, when combined with online light-scattering detection, AF4 is a powerful tool for the determination of the size distribution of particles. For instance, Kato et al. [16] studied the size distribution of aqueous  $C_{60}$  and  $C_{70}$  fullerene in a cell culture medium for *in vitro* toxicity assessment by AF4 coupled to MALS, and reported values of  $256 \pm 90\ \text{nm}$  ( $C_{60}$ ) and  $257 \pm 90\ \text{nm}$  ( $C_{70}$ ) for their diameters. Isaacson and Bouchard [33] used AF4 coupled to dynamic light scattering (DLS) for the characterization of  $C_{60}$  fullerene aggregates in deionized water and reported hydrodynamic diameters between 80 and 260 nm. Recently, Herrero et al. [35] described a method for the fractionation and online identification of  $C_{60}$  and two hydrophobic  $C_{60}$ -derivatives by coupling AF4 to high resolution mass spectrometry and to MALS. The authors reported very similar size distribution for the three fullerenes, with particle radii of gyration ( $r_G$ ) ranging between 20 and 80 nm. Regarding water soluble functionalized fullerenes, there are limited studies on their size distribution and aggregation behavior. AF4 with offline atomic force microscopy (AFM) was proposed for the characterization of  $C_{60}(\text{OH})_{24}$  [34] as a function of pH and ionic strength. The authors found that fullerol present aggregate sizes of only few nanometers in size ( $\approx 2\ \text{nm}$ ) at basic pH and low ionic strength. Fullerol aggregate size increased with the salt concentration from 1.8 nm at zero ionic strength up to 6.7 nm at 0.1 M NaCl, but was not affected by the pH of the solutions. These results disagree with a previous study [37] reporting sizes on the order of 100 nm for this compound as found by dynamic light scattering and TEM. This could be due to the different methodology used for the size measurements of the particles since imaging in air induces aggregation due to partial drying of the sample before analysis.

In the present paper we describe the development and optimization of a separation method for the characterization of four surface modified fullerenes (polyhydroxy- and carboxy-derivatives) that find increasing biomedical application, in aqueous solutions by AF4 on-line coupled to UV and MALS detectors. The effect of the fractionation parameters such as carrier liquid composition, membrane material, cross flow and focus flow rate, focusing time and flow programming were evaluated. Additionally, TEM was employed to visualize the morphology and aggregate structures of the compounds studied. This research provides relevant information regarding the effect of the aqueous solution chemistry on the aggregate sizes and shapes of surface modified fullerenes.

## 2. Experimental

### 2.1. Chemicals and solutions

Fullerol ( $C_{60}(\text{OH})_{24}$ ) was purchased from Materials & Electrochemical Research M.E.R. Corporation (Tucson, Arizona, USA). Polyhydroxy small gap fullerene, hydrated ( $C_{120}(\text{OH})_{30}$ ), (1,2-methanofullerene  $C_{60}$ )-61-carboxylic acid ( $C_{60}\text{CHCOOH}$ ) and  $C_{60}$ -pyrrolidine tris acid ( $C_{60}$ -pyrr tris acid) were purchased from Sigma–Aldrich (Steinheim, Germany). The chemical structures and abbreviations of these compounds are given in Fig. 1.

Bovine serum albumin (BSA, molecular weight  $\approx 66\ \text{kDa}$ ) was purchased from Sigma–Aldrich (Steinheim, Germany). NaCl and phosphate buffered saline (PBS) were purchased from Merck (Darmstadt, Germany).

Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA) and filtered using a  $0.22\ \mu\text{m}$  nylon filter integrated into the Milli-Q system.

Download English Version:

<https://daneshyari.com/en/article/7611668>

Download Persian Version:

<https://daneshyari.com/article/7611668>

[Daneshyari.com](https://daneshyari.com)