Fullerene nanoparticles in soil: Analysis, occurrence and fate

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Chapter 7

Synthesis
Fullerenes are considered to be novel environmental contaminants and the characterization of their occurrence and fate in the environment is fundamental for the general assessment of their possible effects on humans and the environment. At the start of the present work, several studies claimed the lack of analytical methodologies able to fulfil these goals and in one of the first critical reviews on the topic, Isaacson et al. (2009) suggested that “Sensitive and mass-selective detection, such as that offered by mass spectrometry when combined with optimized extraction procedures, offers the greatest potential”. In this work, such methodologies were developed to address two specific scientific issues, the analysis of fullerenes in environmental samples and the study of the presence and fate of these chemicals in soil.

7.1 Development of analytical methods for the analysis of fullerenes in the terrestrial environment

The work achieved in this thesis contributed to overcome some limitations in the soil analysis of fullerenes and particularly (I) the development of routine extraction procedures (Chapter 2), (II) the analysis of mixtures of pristine and functionalized structures (Chapter 3), (III) the determination of these nanoparticles at environmentally relevant concentrations (Chapters 4 and 5) and (IV) the analysis of transformation products (Chapters 5 and 6). These are discussed in the following synthesis.

With regard to the extraction methodology, Chapters 2 and 3 show that the combination of ultrasonication and shaking extraction is a robust and reproducible technique to recover fullerenes from soil independent of the particle size distribution (texture) of the soil under investigation. Furthermore it has the advantages of being applicable to large batches of samples with relatively low costs in terms of materials and energy. It was observed that functionalized fullerenes (Chapters 2 and 3) and unknown fulleroid structures (Chapters 4 and 5) can be recovered to a similar extent of the pristine \( C_{60} \), making this procedure suitable for the study of both engineered and naturally occurring nanoparticles as well as transformation
products that may result in the environment. A current drawback of this technique is that it likely alters the conditions of the fullerenes in the soil, i.e. may not be representative of their natural state (homo- or hetero-aggregated).

Non-aqueous reverse phase (ultra) high performance liquid chromatography can provide sub-optimal separation of several fullerenes and functionalized fullerenes species (Chapters 3 and 4) and, in combination with selective detection methods such as high resolution mass spectrometry (Chapter 4), can provide the unambiguous determination of the fullerenes, even in complex mixtures where co-extractants and similar structures are present. In particular, the experiments reported in this thesis highlighted the performance of novel stationary phases such as core-shell biphenyl (Chapter 4) and pyrenylpropyl silica (Chapter 3). Although the retention mechanism has not been completely elucidated yet, the main retention is likely provided by pi-pi interactions between the aromatic rings of both the fullerenes and the functional groups of the stationary phases. This indicates that such materials can be applied for the analysis of most of the fullerenes and related structures, but also that some functionalized species such as the poly-hydroxylated fullerols may not be retained. A direct comparison of the column’s performances is not possible due to the differences in the overall analytical setup they were employed in. The chromatographic study reported in Chapter 3 was optimized for the separation of more structures, for which pyrenylpropyl silica is more suitable than biphenyl by virtue of the larger surface available for the interaction with the fullerenes. However, the higher retention is achieved at the expense of longer analysis time and this is not always desirable, especially in routine analysis. Thus, further methods development, aimed to monitoring studies, employed the core-shell biphenyl stationary phase that can provide much shorter analysis with complete resolution of the pristine structures only (Chapter 4), which are the most abundant in the environment (Chapter 5). Chapter 4 and 6 also show how methodologies employing this stationary phase can be optimized with regard to the separation of co-extractants and functionalized structures, respectively.

Eventually, the work in this thesis showed that, when coupled to high performance liquid chromatography, both UV and high resolution mass
spectrometry detection can be successfully applied in the analysis of fullerenes. However, for environmental studies these techniques must necessarily address different goals. In particular, UV detection lacks the specificity and sensitivity required for the analysis of the low concentrations present in real soil samples. Nevertheless, since both fullerenes and functionalized structures generally display similar absorptivity behaviour (Chapter 3), UV detection can still be a valuable tool for the identification of unknown species, i.e. products of transformation during incubations and ecotoxicological studies, and in general in experiments where higher concentrations are applied (Chapter 6). Mass spectrometry can fulfil the requirements of specificity and sensitivity necessary for an accurate determination in soil matrices and is therefore the detection method of choice. In particular, most experiments carried out in this thesis rely on a high resolution instrument, the quadruple time-of-flight (Q-TOF) mass spectrometer. Chapters 4 and 5 show how Q-TOF-based methodologies can be applied for the investigation of fullerenes in soil and sediment matrices and in environmental surveys addressing these chemicals. Furthermore, software-based data collection and analysis represent a valuable tool for the search and identification of non-target species in the samples (Chapter 6).

Ionization of the fullerenes (singly-charged molecular ions) was obtained with a heated electrospray ionization (H-ESI) interface, operating in negative mode. This is a hard ionization technique in comparison with standard ESI and particularly fits the analysis of fullerenes due to the resistance of these chemicals to high temperatures. As shown in Chapter 4, the Ion-Booster ESI (IB-ESI) interface applied in this study presents several advantages in comparison with existing methodologies and specifically: (I) a higher ionization efficiency and (II) a lower tendency to create adducts than standard ESI, and (III) the production of an isotopic pattern distribution better matching the theoretical pattern in comparison with other techniques. This latter point is particularly important in the analysis of fullerenes. Indeed, one of the peculiarities in their mass spectrometric analysis is the difficulty (impossibility in this work) to obtain fragmentation of the closed-cage structure and, although exohedralic species (e.g. [60]PCBM) can undergo fragmentation of the functional group from the cage, this limitation is especially relevant in the analysis of pristine species.
Thus, correct and highly resolved isotopic clusters in combination with the accurate mass provided by the Q-TOF can compensate these drawbacks and provide identification criteria needed for the unambiguous determination (Chapters 4 and 5). Nonetheless, the lack of fragmentation allows analysis at high collision energies with the advantage of a “background clean-up” in MS2, i.e. the removal of co-extractants from the samples. As shown in the illustration of MS1 and MS2 spectra in Chapter 4, this result in extremely clean mass spectra and is especially useful during the analysis of complex matrices. A further advantage of this approach is that in-source fragmentation of functionalized fullerenes allows identification of fullerenes that would be otherwise not detected by target analysis e.g. the C_{60}-like products found in environmental samples (Chapter 5) and incubation studies (Chapter 6). Such “defunctionalization” strategy is similar to that suggested by Pycke et al., (2012) and can represent a valid tool for future analysis of heterogeneous mixtures as well as colloidal nC_{60} structures, were fullerenes congeners may represent the majority of the species in the samples. In this context the main drawback of the IB-ESI Q-TOF methodology hereby proposed is the impossibility to prevent the defunctionalization, since any attempt to prevent the fragmentation (i.e. with “softer” analysis) was inconclusive. Thus, further studies should address such limitations with the optimization of the current methods or its combination with alternative techniques. Eventually, although optimized for the study of soil samples, the methodologies developed in this study should in principle be applicable to the analysis of fullerenes extracted from other media (e.g. biological matrices).

Eventually, it must be noted that the current methodologies/strategies present several limitations that future studies should address. One is represented by the lack of proper internal standards for quantitative purposes. Although standard addition methods (Chapters 4 and 5) and the use of other fullerenes as internal standard (Chapter 6) can allow a proper quantitation in certain experimental conditions, there is a need for high purity, isotopically labelled, materials that are currently unavailable on the market at reasonable prices. A second limitation, especially in the analysis of complex matrices, is the lack of optimized clean-up procedures that can improve the quality of the extracts and allow a better identification of the fullerenes. In conclusion, further enhancements of the current strategies
for the characterization of fullerenes in soil, and generally in environmental matrices, will necessarily involve the combination, and possibly hyphenation, of more analytical techniques. Indeed, characterization of fullerenes’ behaviour in the environment necessarily relies on the study of the interaction with natural components that will determine transport, accumulation and the general fate of these nanoparticles. Methods such as those hereby proposed provide qualitative and quantitative data about the presence of fullerenes but cannot completely describe these interaction. Thus, imaging techniques (e.g. transmission electron microscopy, Goel et al., 2004) and novel methodologies such as the recent coupling of field flow fractionation (FFF) with HRMS (Herrero et al., 2014) could be employed in order to characterize these processes with regard to properties such as the composition, size, size distribution and morphology.

7.2 Occurrence and fate in the environment

Some of the main research questions that formed the basis of this project were: “To which extent are fullerenes present in the soil compartment?” and “what is the contribution of engineered nanomaterials to their overall concentration?”. The answers were mostly hindered by the lack of monitoring studies that allow estimation and modelling of the environmental concentrations on the basis of empirical data.

The environmental survey reported in Chapter 5 shows that fullerenes are widely present in the environment but at relatively low concentrations. In particular, pristine structures occur in the soil in the part per billion range and, although a precise assessment of their presence will need further work, similar studies carried out in the last two years support these observations. These findings are also in line with the amounts predicted in soils due to use and production of engineered nanoparticles (Gotthschalk et al., 2009). However, although the presence of manufactured materials cannot be excluded a priori, the occurrence of fullerenes in the Dutch soils investigated in this thesis was likely due to incidental sources, i.e. due to combustion processes of carbonaceous materials such as coal and fuel. Thus, the overall occurrence in the terrestrial environment may nowadays be of anthropogenic origin but related to incidental sources more than the
production and application of ENMs. This hypothesis is also supported by a recent modelling study (Tiwari et al., 2016), which suggests that the global emission of incidental $C_{60}$ may be several orders of magnitude higher than that of manufactured $C_{60}$. However, no methodologies are available yet that can clarify the origin of the nanoparticles detected in the environment and, although some species could be linked to a source (e.g. [60]PCBM in photovoltaics application), this is not valid for pristine structures (e.g. $C_{60}$, $C_{70}$) that can result from both natural and anthropogenic activities as well as resulting from transformations of precursors and other species in the environment. In Chapter 4 it is proposed that an assessment of the ratio at which different species occur in the environment may be helpful in clarifying their origin, but this hypothesis is hindered by the lack of similar environmental surveys and the fact that most studies are focused on the determination of $C_{60}$ only. Thus, the best strategy for the unequivocal identification of fullerenes’ origins may be the characterization of source-related (or process-related) products, such as the unknown $C_{60}$-like structure presented in Chapter 5 that, although not characterized yet, may represent a marker for nanoparticles production during incinerators activity.

The incubation studies in Chapter 6 show that, upon release in the environment, $C_{60}$ will interact with the ultraviolet portion of the sunlight irradiation and that it will be degraded as a consequence. However, this process will take place at a relatively slow rate and will likely affect only the fullerenes that are released in the atmosphere and those that deposit on the top-soil (i.e. those that are directly exposed to the light). Once mixed into soil, other biotic and abiotic phenomena will likely determine their fate to a larger extent. In particular, biotic factors could determine faster decay rates similar to those observed for $C_{60}$ incubated into sandy soil in this study. The study in Chapter 6 also suggests that, upon release in the environment, fullerenes in the “solid” state will be degraded by abiotic factors and that the transformation pathways will be similar to those already observed during ozonation and irradiation of the colloidal structures. However, it is not possible to clarify yet whether biotic factors will determine similar pathways and if they will proceed until mineralization of the fullerenes. The figure below shows a putative pathway of degradation of $C_{60}$ in the environment.
Fig. 7.1. Possible degradation pathway of C$_{60}$ upon release in the environment. Light and oxygen related reactions on the closed cage structure (1) can lead to the functionalization of the fullerenes (2) with formation of oxidized species (3). Destabilization of the fullerenes can determine carbon removal and opening of the cage (4) that could in turn trigger the fragmentation of the cage (5) with the production of transformation products or mineralization (6).

In general, reactions on the cage, enhanced by interactions with light and oxygen species, are expected to involve the carbon(s) in the junctions between two hexagonal rings (Diederich, 1997). This can lead to the oxidation of the fullerenes with consequent production of oxidized functionalized structures that can in turn determine a destabilization of the fullerenes and their progressive fragmentation. Such hypothesis is supported by the detection reported in Chapter 6 of fulleroid traces that display carbon loss and several degrees of oxidation, hydroxylation and methoxylation. These species are more polar than the pristine fullerenes and will likely exhibit enhanced transport in soil. In order to characterize the life-cycle of fullerenes in the environment, further studies will need to focus on such oxidative pathway and the interaction of the transformation products with environmental components such as atmosphere particulates and organic matter.
7.3 Concluding remark

The studies reported in this thesis provide tools and pioneering data about the occurrence and fate of fullerenes in soil. Hopefully, these will be helpful for future studies and will enhance the assessment, regulation and the general understanding of these nanoparticles in the environment. A further ambition of this work is that of being helpful for environmental studies focussed on other carbon-based nanomaterials, such as graphene and carbon nanotubes, whose assessment is at least as difficult and whose production and application is emerging as well.

Nonetheless, future perspectives about fullerenes occurrence and fate are very uncertain. To date, the majority of these nanoparticles in the environment is likely due to incidental sources (both natural and anthropogenic), but the production of engineered nanomaterials will play a major role in determining both the amount and the number of species that will enter the environment. This is particularly relevant for the colloidal nC\textsubscript{60}, whose behaviour and fate are expected to differ from the “solid” structures and that could easily find industrial application in the near future due to the impressive properties that they display and the relative ease and low costs of production. In addition, while C\textsubscript{60} by-product release may decrease in the future, due to current and future policies aimed to limit the global emissions and the use of coal and fossil fuels (e.g. Kyoto protocol), engineered nanomaterials will likely find increasing applications and uses. In this context is at least interesting to note that sustainable energy technologies, such as photovoltaics, could strongly rely on the application of engineered fullerenes. Eventually, an increasing contribution of the manufactured fullerenes to the global load could present new scenarios where their presence is enhanced in a wider variety of ecosystems. For instance, while incidental species are necessarily dispersed onto soil by the atmosphere, resulting in a more homogeneous distribution in the environment, manufactured nanomaterials will likely present hotspots of accumulation close to production, use and dumping sites. In general, future studies focussed on the characterization of fullerenes in the environment will necessarily need to characterize (I) such hotspots of accumulation, (II) the origin of fullerenes found in the environment, with particular regard to
the ENMs and (III) to identify the totality of the fulleroid species that may result from nanotechnology as well as from natural processes.