Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation

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The thorough analysis of natural nanoparticles (NPs) and engineered NPs involves the sequence of detection, identification, quantification and, if possible, detailed characterization. In a complex or heterogeneous sample, each step of this sequence is an individual challenge, and, given suitable sample preparation, field-flow fractionation (FFF) is one of the most promising techniques to achieve relevant characterization.

The objective of this review is to present the current status of FFF as an analytical separation technique for the study of NPs in complex food and environmental samples. FFF has been applied for separation of various types of NP (e.g., organic macromolecules, and carbonaceous or inorganic NPs) in different types of media (e.g., natural waters, soil extracts or food samples).

FFF can be coupled to different types of detectors that offer additional information and specificity, and the determination of size-dependent properties typically inaccessible to other techniques. The separation conditions need to be carefully adapted to account for specific particle properties, so quantitative analysis of heterogeneous or complex samples is difficult as soon as matrix constituents in the samples require contradictory separation conditions. The potential of FFF analysis should always be evaluated bearing in mind the impact of the necessary sample preparation, the information that can be retrieved from the chosen detection systems and the influence of the chosen separation conditions on all types of NP in the sample. A holistic methodological approach is preferable to a technique-focused one.

Keywords: Analytical separation; Characterization; Complex sample; Environmental sample; Field-flow fractionation; Food sample; Method development; Nanoparticle; Particle separation; Particle-size analysis

Abbreviations: aF^4, Asymmetric flow-field-flow fractionation; DLS, Dynamic light scattering; EM, Electron microscopy; FFF, Field-flow fractionation; F^4, Flow-field-flow fractionation; HPLC, High-performance liquid chromatography; ICP-OES, Inductively coupled plasma-optical emission spectrometry; ICP-MS, Inductively coupled plasma-mass spectrometry; LC, Liquid chromatography; LS, Light scattering; MALLS, Multi-angle laser-light scattering; MS, Mass spectrometry; NP, Nanoparticle; SEC, Size-exclusion chromatography; SedFFF, Sedimentation-field-flow fractionation; sF^4, Symmetric flow-field-flow fractionation; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; UV-Vis, Ultraviolet-visible spectroscopy

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1. Introduction

There is a widespread consensus that new analytical methods are needed to quantify nanoparticles (NPs) in a wide variety of sample types [1], a task that appears currently to be difficult or even impossible, especially for impure, multi-component, heterogeneous and complex samples, which are obtained in the context of environmental, biological and food analysis.

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The analysis of NPs in a given sample has to address four main questions:

(1) Are NPs present in the sample (detection)?
(2) Which type of NPs are in the sample (i.e. what is their chemical identity) (identification)?
(3) How many NPs are in the sample (quantification)?
(4) What are the properties or the aggregation state and the surface chemistry of the NPs (characterization)?

Food and environmental samples are complex and heterogeneous matrices, which may contain natural NPs and/or engineered NPs (ENPs) and larger particles. Natural NPs and ENPs vary in composition, size and shape and can be polydispersely distributed in these matrices. In this article, we describe a collection of entities in a sample as polydisperse when they exhibit a broad range of properties, among which are size, shape and composition. One particular analytical challenge that arises in environmental and food samples is to distinguish quantitatively between ENPs and ubiquitous natural NPs of the same composition.

One approach to solve this problem of background NPs is to make use of an existing contrast between the NPs and the sample matrix or to find a solution to create one. To date, several analytical tools are available to obtain accurate results for fairly simple matrices. Nevertheless, they present limitations when dealing with complex samples.

For example, with detection by light-scattering (LS) techniques, the contrast stems from differences in the refractive index between the particles and the medium. LS is also sensitive to particle size and sometimes structure and shape. An overview of recent LS applications is available [2]. However, LS analysis is not substance specific, its size resolution is low and it offers no physical separation, so it may be impossible for LS analysis to distinguish ENPs from any other particulate entity. Besides, for dynamic light scattering (DLS), the polydispersity of the NPs in the sample limits its applicability [3].

With mass spectrometry (MS), the limitation stems from a potential difference between the composition of the particles and the sample matrix [4]. If the composition of the NPs is identical or similar to the background of the sample, then conventional MS measurement could not be used alone for NP detection [5].

Electron microscopy (EM) makes use of spatial resolution and differences in composition and structure of particles. EM is a highly valuable tool for identifying single particles but, without tediously counting thousands of particles, it fails to provide an accurate statistical representation of the whole sample [6].

However, combination with a hydrodynamic separation technique [e.g., field-flow fractionation (FFF)] offers the possibility of using all the potential of the three aforementioned techniques. By separating macromolecules and particles, FFF reduces sample polydispersity and complexity for each of the analytical devices, and ideally also adds particle-size information [7]. For the analytical separation of NPs, various methods can be applied {e.g., liquid chromatography (LC) [8], size-exclusion chromatography (SEC), hydrodynamic chromatography (HDC) [3] or FFF [4,5]}. Although much of an FFF system resembles a classic LC system (pumps, autosampler, detectors), FFF separates particles at low to medium pressures in an open channel without a stationary phase. Interactions of the NPs with the stationary phase or mechanical stress are therefore avoided. Due to its relatively gentle separation process, broad size range and versatility through different sub-techniques {e.g., sedimentation-FFF (SedFFF), flow-FFF (F^4) and thermal-FFF}, it is one of the most promising techniques for the analysis of NPs in complex samples [9,10].

Since FFF is prone to interferences by large particles or flocs (>1 μm), sample preparation is required for most samples. Indeed, a suitable sample for FFF separation is a stable dispersion of NPs in a liquid medium. The preparation methods depend on both the character of the matrix and the properties of the NPs, so development of suitable sample-preparation techniques plays a major role in FFF analysis.

The objective of this review is to present the use of FFF as a separation technique for the study of NPs in complex samples (e.g., food or environmental) and to discuss the principal challenges in method development. First, we present the general principles of FFF separation theory. We detail some examples of FFF applications for separation, and characterization of NPs in complex samples, giving special attention to sample-preparation methods, and the limitations and the optimization of the FFF methodology.

The information given substantiates the following needs:

(1) development of methods with respect to a target particle type and a certain matrix;
(2) standard or reference particles and matrices;
(3) reporting unified calibrated data to enable the intercomparison of results and interlaboratory tests.

Because of the analytical constraints mentioned, general ''one-for-all'' methods will have little success, given the broad range of particle properties that needs to be accounted for.

2. General principles and instrumentation for FFF

FFF is a flow-assisted hydrodynamic separation technique that permits physical separation of small quantities (injected masses in the ng–μg range) of macromolecules or particles. The rigors of FFF theory are described elsewhere [4,11–13]. Here, we describe only the general principles of particle-size fractionation by