Selective quantification of nanoplastics in environmental matrices by asymmetric flow field – flow fractionation with total organic carbon detection

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Selective quantification of nanoplastics in environmental matrices by asymmetric flow field – flow fractionation with total organic carbon detection

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Abstract: Nanoplastics are of rapidly emerging concern as ubiquitous environmental pollutants. However, fate and transport assessments are currently hindered by a need for new analytical methods that can selectively quantify nanoplastics in environmental matrices. This study presents the first proof of principle to hyphenate asymmetric flow field-flow fractionation (AF4) with total organic carbon (TOC) detection for nanoplastics analysis, as evaluated on mixtures of multimodal polystyrene nanoplastics in the presence of dissolved organic matter and clay colloids. The AF4-TOC method enables more robust, size-resolved quantification of nanoplastics over other AF4 detection modes, including UV-Vis, refractive index, and fluorescence tagging. This method development can fill a critical gap in analytical methodology for environmental nanoplastics research.

Nanoplastics (which have been defined as having dimensions from 1 nm up to either 100 nm or 1000 nm)[1] and microplastics (< 5 mm) have received increasing concern in recent years because of their risks, persistence, and prevalence in the environment.[2-7] In contrast to engineered metal and metal oxide nanoparticles or larger microplastics, nanoplastics have been less widely studied, and effective separation, detection and quantification methods are still under development for nanoplastics in environmental samples.[8-10] Commonly reported separation methods for plastic particles include filtration, flotation, or centrifugation. For detection, Nile Red staining has been reported as a simple method to enable semi-quantitative detection by fluorescence microscopy.[11-13] State-of-the-art detection methods include infrared and Raman microscopy and pyrolysis gas chromatography – mass spectrometry (Py-GC-MS). Each of these methods has limitations for characterization or quantification of plastic particles, particularly in the nanoscale range.[8-10] For example, batch separation methods produce only discrete size fractions and are better suited to isolate large particles.[8] Imaging analysis is limited by size resolution, and the data cannot easily be converted to mass concentrations. Py-GC-MS can provide selective identification and quantitative analysis with detection limits < 1 µg,[10, 14] but is a sample-destructive process that does not distinguish particles by size. A major analytical gap hence remains in developing methods capable of quantifying size-resolved mass concentrations of nanoplastics, particularly in complex mixtures with a broad range of particle sizes and interfering environmental species.

Interest is emerging in the potential applications of asymmetric flow field – flow fractionation (AF4) for nanoplastics analysis.[8-10] AF4 is a size separation method that covers the nanometer to micron range and can be "hyphenated" with online detectors for nanoparticle separation, detection, and characterization. AF4 has previously been coupled with inductively coupled plasma – mass spectrometry (ICP-MS) for selective detection of engineered metal and metal oxide nanoparticles in environmental matrices,[15-17] but nanoplastics must typically be labelled or associated with metals to be observed by ICP-MS.[18, 19] Other common AF4 detectors such as UV-vis and light scattering have been used for nanoplastics analysis[20] but have limited capability to selectivity distinguish nanoplastics from other matrix constituents (e.g., inorganic colloids or biological matrices[21]) or show disproportionate detector response to different size particles, making absolute quantification of nanoplastics mass concentrations highly challenging. A recent advance coupling AF4 with optical tweezers and Raman spectroscopy excels in identifying nanoplastics but also shows variable sensitivity with particle size.[22]

The objective of this study is to develop and demonstrate proof of principle for hyphenation of AF4 with total organic carbon (TOC) detection to achieve efficient separation and selective, unambiguous quantification of nanoplastics having multimodal size distributions and in complex mixtures representing natural waters. To our knowledge, this study represents the first demonstration of AF4-TOC for nanoplastics analysis, either alone or in environmental matrices (here, with clay colloids and humic acid), and only the second reported coupling of AF4 with TOC detection, with prior analysis focusing on dissolved polymers or organic matter,[23] as opposed to particles that may present greater challenges. The AF4-TOC method

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Electronic Supplementary Information (ESI) available: Sample preparation and instrumentation details, and additional nanoplastics evaluations and AF4 results. See DOI: 10.1039/x0xx00000x is simultaneously compared to three other AF4 detection modes (UV-vis, differential refractive index (dRI), and also for the first time, fluorescence detection (FLD) on Nile Red stained nanoplastics), along with light scattering for size determination, to establish AF4-TOC as a more robust detection mode for nanoplastics analysis.

Sample preparation and instrumentation are fully described in the Electronic Supplementary Information (ESI). In brief, stock suspensions of polystyrene (PS) nanoplastics (nominal 10 g L^{-1} with 1 g L^{-1} Tween 20 surfactant) in four diameters (nominal 50 nm, 100 nm, 200 nm, and 500 nm) were purchased from Phosphorex (Hopkinton, MA, USA). PS was selected because it is available in well-defined, narrow size ranges, which can be used to prepare multimodal mixtures of known concentrations to validate the new AF4-TOC method for simultaneous size separation and mass quantification. Batch sizes and zeta potentials were measured (ESI Figure S1), as well as functional groups by infrared spectroscopy (ESI Figure S2). For AF4 analysis, the PS nanoplastics were evaluated as multimodal mixtures of the four sizes except where otherwise noted. For calibration analyses, four mixture concentrations (containing 5 mg L^{-1}, 10 mg L^{-1}, 15 mg L^{-1}, and 20 mg L^{-1} of each PS size) were evaluated. Kaolin clay (ASP 600 hydrous aluminum silicate, BASF Corporation, Charlotte, NC, USA) and Elliott soil humic acid (ESHA Standard V, International Humic Substances Society (IHSS), St. Paul, MN, USA) were used to represent background constituents in natural waters. The nanoplastics (mixtures of 20 mg L^{-1} of each PS size) were mixed with clay and ESHA at 235 mg L^{-1} and 10 mg L^{-1} as carbon, respectively, for AF4 analysis. For AF4-FLD evaluation, Nile Red dye was added to the samples at 10 mg L^{-1} to fluorescently label the PS nanoplastics, both alone and in the presence of clay and ESHA.

The AF4 system was comprised of a high performance liquid chromatography system (1290 Infinity binary pump, degasser, and autosampler, Agilent Technologies, Santa Clara, CA, USA), AF4 module (Eclipse AF4, Wyatt Technology, Santa Barbara, CA, USA), and several online detectors: UV-Vis diode array and fluorescence detectors (Agilent); dRI, multi-angle light scattering (MALS), and dynamic light scattering (DLS) detectors (Wyatt); and a TOC detector (Sievers M9 SEC, Suez Water Technologies, Trevose, PA, USA). The AF4 short channel was equipped with a 250 μm, wide spacer and a 10 kDa regenerated cellulose ultrafiltration membrane. The mobile phase was 0.15 mM Na_2SO_4 and sample injection volume was 100 μL. The detector flow was 0.5 mL min^{-1}, injection flow was 0.2 mL min^{-1}, and focus flow was 2.0 mL min^{-1}. The cross flow was optimized as 0.7 mL min^{-1} for separation of the nanoplastics (ESI Figure S3). For online TOC analysis, the eluting sample flow is acidified with phosphoric acid for inorganic carbon removal and oxidized to CO_2 by ammonium persulfate with UV activation for detection. The AF4 separation method and optimization, as well as the detector settings and AF4-TOC data integration, are described in the ESI.

The overarching goals of the method development herein are to provide proof of principle for hyphenated AF4-TOC as a reliable method to quantify nanoplastics mass concentrations, regardless of particle size, and to selectively detect nanoplastics in complex environmental matrices. For quantitative analysis, accurate mass concentrations of the PS stocks were first determined by gravimetric analysis, followed by evaluation of the nanoplastics by TOC analysis, either in batch mode or as an online detector with AF4 (ESI Figure S4). The batch TOC analysis showed increasing oxidation efficiency with PS nanoplastics size. Accounting for oxidation efficiency, the online TOC analysis was validated to show nearly 100% recovery for quantification of the individual 50 nm, 100 nm, and 200 nm PS nanoplastics injected through the AF4 system without cross flow. Declining recovery of the 500 nm nanoplastics from 81% to 49% over triplicate injections was attributed to instrument fouling.

The online TOC analyzer was then qualitatively evaluated for its suitability to distinguish multimodal particle mixtures as separated by AF4 (with cross flow applied). Chromatograms for the UV and TOC detectors are shown in Figure 1, and light scattering and dRI detectors in ESI Figure S5. Nearly baseline resolution was achieved in the AF4 separation between the 50 nm, 100 nm, and 200 nm particles, as observed by UV detection (Figure 1a). Lower resolution was observed in the online TOC analysis and can be attributed to high dispersion in the relatively large flow volume through the TOC detector (≈ 6 min delay time) (ESI Figure S6), but peak separation was still clearly observed (Figure 1b). Particle sizes for the 50, 100, and 200 nm nanoplastics determined by online MALS and DLS were slightly smaller than batch DLS sizes without separation, which are more heavily weighted toward larger particles that scatter light more efficiently. Reliable sizes could not be acquired on the 500 nm particles, similar to prior reports and potentially attributable to the more significant error introduced by advective motion with the detector flow relative to the slower diffusion of the larger particles.[24]

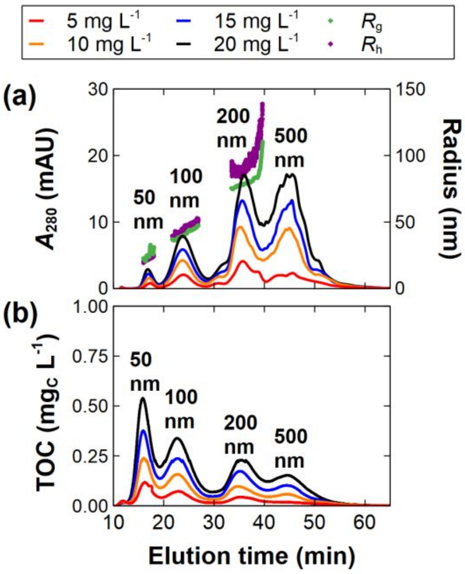

Figure 1. AF4-UV (a) and AF4-TOC (b) chromatograms of the nanoplastics mixtures at four concentrations, with radius of gyration (R_g) from MALS and hydrodynamic radius (R_h) from DLS (a). Detector signals were aligned based on delay volume between the detectors.

An alternative approach was also investigated to label the PS nanoplastics with Nile Red for detection by AF4-FLD. Nile Red has previously been reported to stain microplastics[11-13] and hence could hypothetically be utilized to detect nanoplastics in AF4 analysis. Interestingly, the resulting AF4-FLD chromatogram (Figure 2a) shows that the fluorescence response is not consistent across all four nanoplastics sizes, with particularly low uptake for 200 nm PS; results were verified on individually stained nanoplastics of each size. The variable uptake may stem from differences in functional groups on the nanoplastics (ESI Figure S2). Such inconsistencies in dye uptake can diminish the robustness of the Nile Red staining approach to detect all nanoplastics, even those comprised of the same polymer.

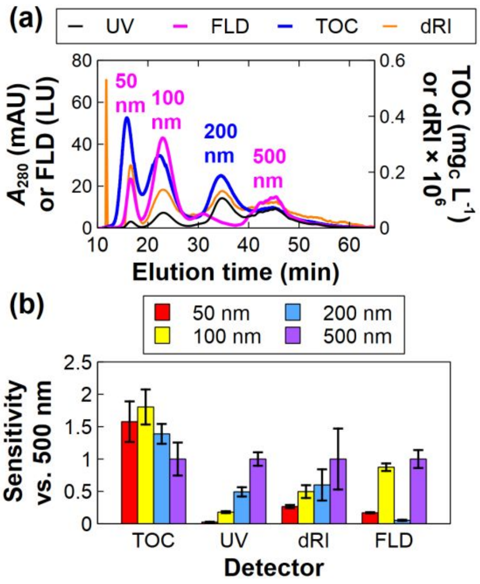

Figure 2. AF4 chromatograms of PS nanoplastics after staining with Nile Red (a), and sensitivity of each online detector to each of the four PS nanoplastic sizes, relative to the detector peak area for the 500 nm particles (b). Error bars represent standard deviations for triplicate AF4 runs for FLD and six replicate runs for TOC, UV, and dRI (with and without Nile Red).

The AF4 detectors were then compared quantitatively. Ideally, quantification of the nanoplastics mass concentration would be independent of other properties such as particle size, which may not be known a priori on any given sample. Although all detectors in Figure 1 show a linear peak area response to each individual size of nanoplastics (ESI Figure S7), the sensitivity or slope of the response varies with particle size. Considering the successful demonstration of the online TOC quantification when injecting nanoplastics without AF4 crossflow, lower TOC responses observed during AF4 separation are taken to represent losses to the membrane, a common issue in AF4 separation, with lower recovery observed for large particles that reside closer to the membrane. Still, the limit of quantitation based on signal to baseline noise was estimated to be < 0.3 μg injected mass for all particle sizes (ESI Figure S8 and Table S2). Furthermore, the TOC sensitivity is relatively consistent across size, i.e., within a factor of 2 (Figure 2b), even with the variable recovery. In contrast, UV and dRI show more highly variable sensitivities across different sizes of particles. In particular, the UV sensitivity decreases drastically as particle size decreases (by a factor of ≈ 40, comparing the 500 nm and 50 nm nanoplastics) because light scattering, which is highly size dependent, contributes significantly to the overall extinction coefficient of the particles. The dRI detection is similarly influenced by light scattering.[25] As discussed previously, the FLD detection for the Nile Red stained nanoplastics shows not only a highly variable response with particle size, but also anomalously low dye uptake by the 200 nm PS. These results imply that determining the mass concentration of nanoplastics comprising a range of particle sizes would be challenging when utilizing either UV, dRI, or fluorescence staining, whereas TOC detection provides a more robust measurement with lesser degree of error.

Finally, the capability of AF4-TOC to enable selective detection of nanoplastics in environmental matrices containing inorganic colloids (here, 235 mg L^{-1} of kaolin clay, particle diameter ≈ 300 to 400 nm) and dissolved organic matter (10 mg$_C$ L^{-1} of ESHA) was evaluated. Quantifying organic nanoplastics separately from natural organic matter is challenging without pre-separation or a selective detector; for example, bulk TOC analysis would not be suitable to quantify nanoplastics suspended with dissolved organic matter. Here, AF4 serves to provide effective separation of nanoplastics from smaller ESHA species (eluting as a void peak at the beginning of the AF4 elution stage in Figure 3).

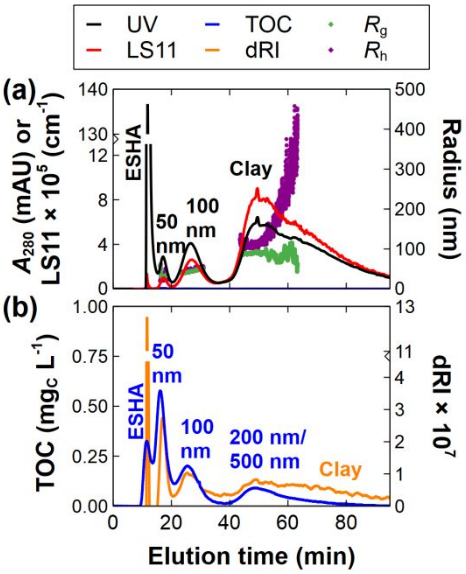

Figure 3. AF4 chromatograms of the PS nanoplastics mixture with clay and ESHA by UV and LS (Rayleigh ratio at detector 11, 90°) (a) and TOC and dRI detection (b). R_g and R_h are also presented in (a).

However, the AF4 separation alone cannot resolve colloidal species of the same size range as the nanoplastics that will coelute. Most AF4 detection modes are not capable to selectively distinguish nanoplastics in the presence of other coeluting colloids. For example, batch DLS analysis on unseparated mixtures of clay and nanoplastics reflects primarily the size of the clay colloids (ESI Figure S1a). In such cases, AF4-TOC is expected to be highly advantageous if oxidation of the TOC in the nanoplastics is maintained with minimal interfering signal from the inorganic colloids. Although zeta potential analyses suggest that some ESHA adsorbs onto the clay (imparting a more negative zeta potential, ESI Figure S1b), AF4 analyses on mixtures of ESHA and clay (without nanoplastics) show that clay colloids detected by UV produced minimal signal in the TOC detector (ESI Figure S9) and hence should not contribute significantly to the TOC measurement. In mixtures with the PS nanoplastics, it is not feasible to identify the presence of the larger nanoplastics (200 nm and 500 nm) by UV, LS, or dRI analysis because of the interference from the coeluting clay in those detectors (Figure 3). On the other hand, the TOC detector maintains sensitivity to the nanoplastics without interference from the clay, even in cases where overloading of clay resulted in worsened coelution over the particles (ESI Figure S10). The primary limitation is that instrument fouling by the clay results in further peak broadening and overlap of the 200 nm and 500 nm peaks (see ESI Figure S9 comparing the individual sizes of nanoplastics injected in the complex matrix). Nile Red staining was also tested in the complex mixture. The AF4-FLD chromatogram in ESI Figure S11 shows that the Nile Red selectively labels the PS nanoplastics without staining the clay or ESHA, but the issue of poor dye uptake by the 200 nm nanoplastics again precludes identification of those nanoplastics in the samples by the Nile Red approach. In summary, the selectivity and robustness of the TOC detection mode can be exceptionally useful in combination with size separation by AF4 to detect and quantify nanoplastics in environmental waters.

In conclusion, this study demonstrates the first proof of principle for hyphenating AF4 with TOC detection for the separation and size-resolved quantification of nanoplastics mass concentrations in complex and polydisperse mixtures. Advantages over other online AF4 detectors include a more uniform sensitivity across nanoplastics sizes, and especially selective detection in the presence of inorganic colloids. This method can fill an important analytical gap for the broader nanoplastics research community to evaluate mass concentrations of nanoplastics. Future development of the AF4-TOC method is suggested to further optimize the AF4 separation for improved recovery, e.g., by membrane or mobile phase modification,[26] and to evaluate a broader variety of nanoplastics, such as polyethylene (PE) and polyvinyl chloride (PVC), that are commonly observed in environmental samples.

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