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Detection and sizing of nanoplastics by AF4-FFF-MALS-UV: the case of polystyrene nanoparticles

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BACKGROUND

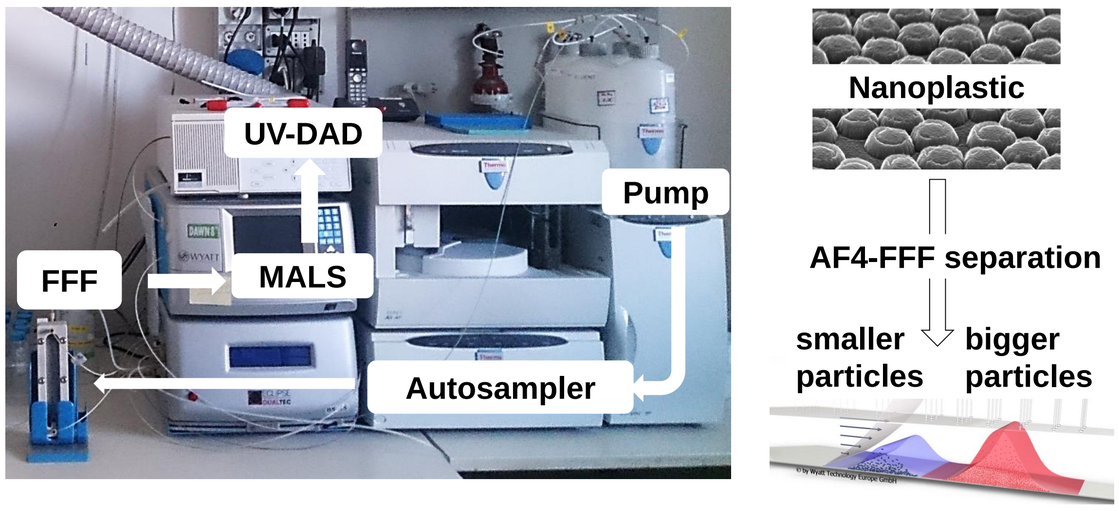
Nowadays Microplastic (MP) can be found in almost all environmental habitats, and degradation and fragmentation lead to smaller particles. Recently the actual fragmentation of Polystyrene (PS) into Nanoplastic (NP) was proven experimentally. However, identification methods are still under development, consequently information on quantity, quality and fate of especially small NPs are limited.

AIM

We tested the suitability of asymmetric flow field-flow fractionation (AF4-FFF) on-line coupled with two detectors, the multi-angle light scattering (MALS) and the UV diode array detector (DAD) to simultaneously detect PSNPs and collect information about the size (**Figure 1**).

In AF4-FFF the particles flow along the separation channel with smaller particles that move faster and are eluted at lower retention times (Rt) before larger slower moving particles. The MALS then measures the root mean square radius (Rg) of particles in solution, by detecting how they scatter light. The UV-DAD detector is used to perform a semiquantitative analysis by measuring peak absorbance of particles.

Figure 1. AF-FFF-MALS-UV for PSNPs determination



MATERIALS AND METHODS

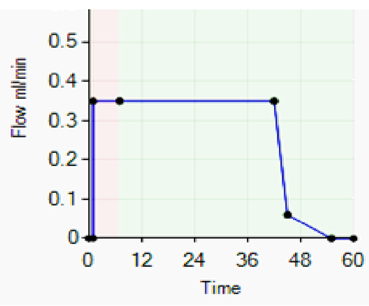
The selected nanoplastics were PSNPs (Thermo Scientific) with certified size of 20±2 nm, 60±4 nm, 100±3 nm and 203±5 nm (1% solid suspension, each). A mixture of all the four dimensions of PSNPs was prepared by dilution with ultrapure deionized water and gentle mixing and was used as test standard for a polydisperse nanoplastic system.

For AF4-FFF separation, a volume of 25 µl of the mixture of PSNPs was injected into a short separation channel (SC) with 10 kDa Regenerated Cellulose (RC) membrane and a 350 µm height spacer.
After a focus step of 6 minutes, a gradient elution in a total time of 48 min (**Table 1**) was applied. Mobile phase was the 0.2% SDS (sodium dodecyl sulfate) and detector flow (Vd) and focus flow (Vx) rates were set at 0.65 ml/min and 0.35 ml/min, respectively.

In MALS analysis the 90° scattering angle was used to determine the size, while in UV-DAD analysis the maximum peak absorbance was registered at 215 nm.

Table 1. AF4-FFF-MALS-UV parameters for PSNPs mixture

AF4-FFF Separation	**Eclipse Dualtec (Wyatt)**		
MALS Detection	**Dawn Heleos II (Wyatt), 8 angles**		
UV-DAD detection	**Thermo Scientific Dionex**		
Separation channel	**SC 10 kD RC, 350 µm height**		
Injection volume	**25 µL**		
Carrier	**SDS 0.2%**		
Detector flow (V_d)	**0.65 mL/min**		
Focus flow (V_x)	**0.35 mL/min**		
Elution program	**Duration (min)**	**Description**	**Cross-flow (mL/min)**
	1	**Elution**	**0.0**
	6	**Focus+Inject**	**0.0**
	35	**Elution**	**0.35-0.35**
	3	**Elution**	**0.35-0.06**
	10	**Elution**	**0.06-0.0**
	5	**Elution+Inject**	**0.0**



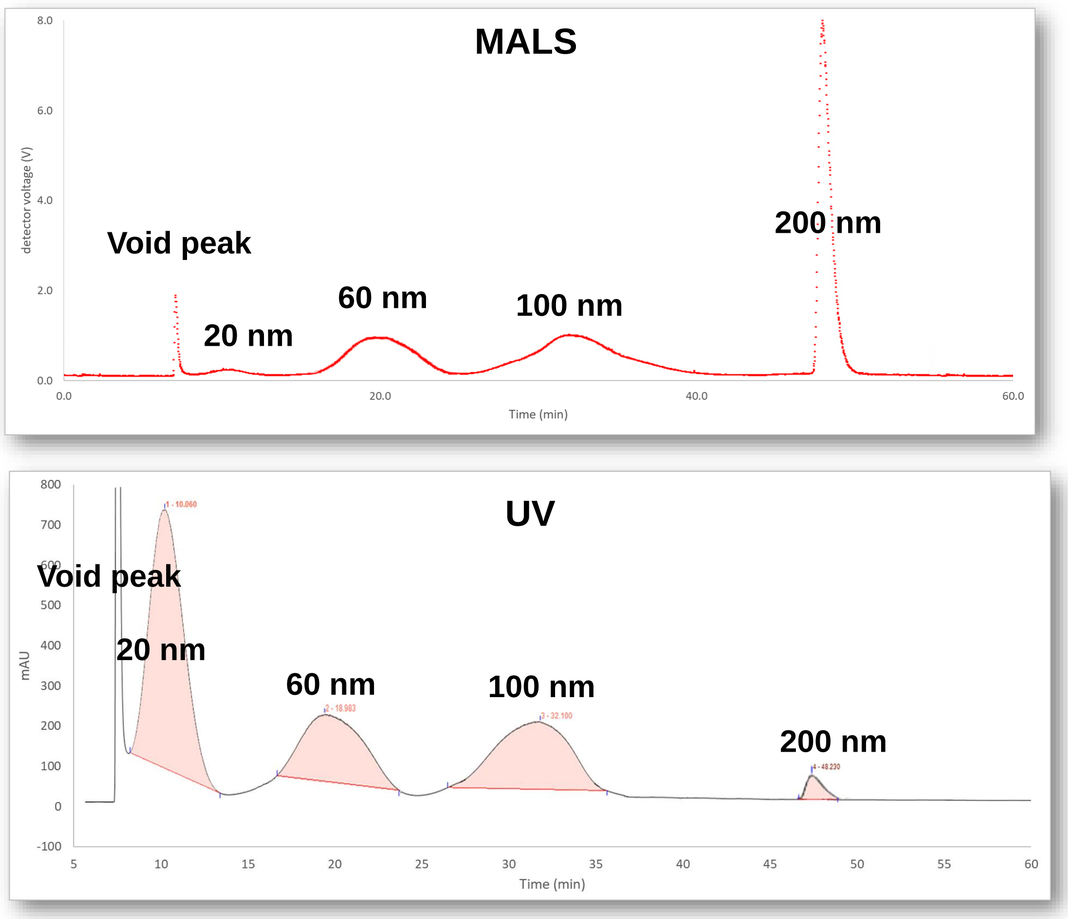
RESULTS

The coupling of AF4-FFF with MALS and UV-DAD gave the fractograms reported in **Figure 2** for the mixture of PSNPs 20 nm, 60 nm, 100 nm and 200 nm. Accuracy, repeatability and limits of detection (LoDs) were evaluated (n=5 injectons) and reported in **Table 2**.

Table 2. AF4-FFF-MALS-UV validation results

Size	**PS 20 nm**	**PS 60 nm**	**PS 100 nm**	**PS 200 nm**
Concentration (µg/ml)	**1000**	**400**	**200**	**50**
Rt (min)	**10.6±1.1**	**20.1±1.2**	**32.8±1.9**	**48.2±2.1**
Repeatability on Rt (RSD%)	**10.4**	**5.9**	**5.8**	**4.4**
Found size (nm)	**nd**	**54±5**	**95±5**	**220±10**
Accuracy on size (%)	**nd**	**90**	**95**	**110**
Repeability on size (RSD%)	**nd**	**9.2**	**5.3**	**4.5**
Concentration LoDs (µg/ml)	**24.7**	**27.4**	**29.1**	**28.5**

Figure 2. AF4-FFF-MALS-UV fractograms for PSNPs mixture



DISCUSSION

The fractograms reported in **Figure 2** for the mixture of PSNPs showed an overlay of MALS and UV-DAD signals.
The different particles eluted at different Rt with 20 nm particles eluting at 10 min and 200 nm particles at 48 min, with good repeatability (<11%) over five injections (**Table 2)**. A good resolution between the void peak and the first particle peak, and between the different particle peaks was observed.

Smaller particles (20 nm) produced an UV signal more intense that the larger ones. On the contrary, MALS signal was much more intense and accurate for bigger particles (200 nm).

CONCLUSIONS

The AF4-FFF-MALS-UV method here developed could detect nanoplastic of PS sized between 20 nm and 200 nm with high resolution between nanoplastic fractions, good repeatibility between measurements and quantification at relatively low concentration (ca. 25 µg/ml or 0.6 µg injected).

FUTURE WORK

The pyrolysis coupled to Gas Chromatography Mass Spectrometry (GC-MS) will be applied to unequivocally identify the type of plastic.
The method will be also exploited for other types of nanoplastics as Polyethylene (PE).