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Method for extraction of nanoscale plastic debris from soil†

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Sample preparation for extraction of nanoscale plastic debris (NPD, size < 1 μm) from environmental samples is a critical step to prepare NPD for further identification and quantification. Developing a NPD extraction method from soil matrices is particularly challenging due to the complexity of solid matrices. In the present study, we built upon the lessons learned from method development for extraction of microplastics and nanomaterials from environmental samples to develop a sample preparation method for extraction of NPD from soil matrices. The evaluation criteria for the extraction method are size distribution, particle number recovery, and particle mass recovery. Since there is no validated method available to trace and quantify the mass of NPD in complex matrices, we applied polystyrene particles doped with europium (Eu-PS NPs). Standard LUFA soil and field soil were spiked and mixed for 24 h with 1 mg of Eu-PS NPs and the particles were extracted from the matrices of the soils. The extraction method did not significantly influence the size distribution of the particles and the extraction agents did not degrade the Eu-PS NPs. Mass balance calculation suggested recoveries of 82 and 77% of the added Eu-PS NPs in LUFA soil and field soil, respectively. The number recoveries of the particles were 81 and 85% for LUFA soil and field soil, respectively. This method can be further optimized and used as the first building block to develop a generic sample preparation method for the extraction of NPD from soil samples. By combining this developed and verified extraction method with identification and quantification techniques, a fit-for-purpose workflow can be developed to quantify and subsequently understand the fate of NPD in soil.

Introduction

Plastics in the environment are exposed to various environmental factors, *e.g.* UV-radiation, varying temperatures, and the activities of micro-organisms.[1] This can lead to the formation of nanoscale plastic debris (NPD, size < 1 μm) in the environment. Nanoscale polymeric materials are also intentionally produced to be used in some consumer products.[2] These materials often end up in sewage systems, which act as a source of NPD pollution of the environment.[3] Soils in urban and agricultural areas are expected to contain NPD[4] as a result of *e.g.* transport from landfills and sewage sludge application for agricultural purposes and atmospheric deposition.[5–7] While the number of studies on microplastics and NPD in marine ecosystems is increasing, there is a lack of knowledge on the occurrence and distribution of NPD in soil ecosystems.[8]

Soil is a complex matrix that consists of different components *e.g.*, clay mineral components, iron oxides, aluminum oxides, carbonate matrices, soil organic matter (OM), *etc.* Currently, it is challenging to trace, quantify and characterize NPD in complex matrices of soil.[8,9] Due to the nanoscale size of NPD and their similarity in chemical composition to biomolecules and biogenic polymers present in the soil, most of the available analytical techniques cannot directly analyze NPD in soil matrices.[10] For example, Fourier-transform infrared spectroscopy and Raman spectroscopy are widely used to identify the properties of microplastics. These techniques, however, cannot identify microplastics and NPD in soil matrices.[11] Pyrolysis gas chromatography-mass spectrometry (Py-GC/MS)[12–15] and the combination of thermogravimetric analysis and solid-phase extraction with thermal desorption (TED)-GC/MS[13,16–18] offer chemical structural information about polymers in complex matrices by determining their thermal degradation products. In theory, both techniques allow for *in situ* (without the need for particle extraction) identification of NPD.[18] In practice, however, the presence of high concentrations of

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biological molecules, such as cellulose and lignin, interfere with the identification of NPD of interest. This is particularly the case when the concentration of the NPD in the sample is low as expected in soils.[10]

An alternative procedure to direct measurement techniques is to, first, extract NPD from the soil matrix and bring the NPD in a state that is measurable by the existing analytical techniques. Several protocols for the extraction of microplastics from environmental samples were presented in recent years.[4,10,19] Nevertheless, only very few extraction methods were specifically focused on NPD extraction from soil.

For example, floating methods using distilled water[20] or high-density solutions of NaCl (density ~1.2 kg L^{-1}),[21] NaI (density ~1.6–1.8 kg L^{-1})[22,23] and $ZnCl_2$ (density ~1.6–1.8 kg L^{-1})[24] are usually applied to extract microplastics from the sediment and sandy matrices,[11,25] benefiting from density differences. However, these extraction methods are not suitable when high OM content is present in the solid matrix.[11] Digestion agents *e.g.* hydrogen peroxide (H_2O_2),[8] enzymes[26] Fenton's reagent[27] and nitric acid (HNO_3)[26] have been used to remove OM and extract microplastics from samples. It was, however, reported that some of the digestive agents such as HNO_3 and H_2O_2 can degrade plastics and these solvents may, thus, influence the NPD extraction.[28] Alkaline and enzymatic digestion[26] have been recommended as suitable digestive agents. The influence of these agents on NPD and the efficiency of NPD extraction from soil using these agents remain unknown. The inherent features of small size and high surface energy make NPD prone to aggregation with soil particles, causing difficulties in the extraction of NPD from soils.[8] All challenges combined have so far resulted in the absence of fit-for-purpose analytical methods to characterize and quantify NPD in complex matrices of soils.

The overall aim of this study is to develop a straightforward method for the extraction of NPD from soil matrices. Two research questions were formulated to address the aim: (1) to what extent do the extraction agents influence the physicochemical properties of the NPD? (2) what is the extraction efficiency of NPDs from the soil matrix? Polystyrene nanoparticles (PS NPs) doped with a rare metal (Europium) are used to mimic the NPD because metal-doped polymeric NPs can be used as a model of NPD to circumvent the existing analytical challenges associated with NPD tracing, characterization and quantification in complex matrices.[29] We are aware that, in the environment, NPD are not doped with rare metals. However, doping PS NPs with Eu (Eu-PS NPs) and analyzing the Eu allows us to evaluate how the extraction method may influence the physicochemical properties of NPD and whether the extraction step can efficiently isolate the NPD from soil matrices. Particle size distribution, particle number recovery, and particle mass recovery were used as criteria for developing the extraction method. Developing an analytical method for the identification of NPD in soils is out of the scope of this study. The presented sample preparation method can however be combined with different characterization techniques, *e.g.*, identification technique, to provide the intended information about NPD in soil.

Experimental

Materials

All chemicals used were of analytical grade and purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands) or Merck (Darmstadt, Germany) unless otherwise mentioned. We used Eu-PS NPs as a model of NPD because Eu can be used as a proxy for tracing and quantification of the polymeric particles in complex matrices.[30] The application of metal-doped PS NPs allows the usage of well-established techniques, such as inductively coupled plasma mass spectrometry (ICP-MS) for analyzing the Eu-PS NPs. Spherical Eu-PS NP of 640 nm (PDI = 0.08) and Eu^{3+} content of <0.2% were purchased from Micro-Particles (GmbH, Berlin, Germany). The density of the Eu-PS NPs was 1.05 g cm^{-3}. Standard LUFA soil was purchased from LUFA Speyer (Speyer, Germany). The physicochemical parameters of the used soil are reported in Table S1 (ESI†). The water used in the present study was deionized by reverse osmosis and purified by a Millipore MilliQ (MQ) system. Soil samples were collected from a clean area in the city of Leiden, The Netherlands (52°10′16.1″N 4°26′57.8″E). The physicochemical parameters of the used soils are reported in Table S1 (ESI†).

Characterization and quantification of NPD

Transmission Electron Microscopy (TEM, Jeol JEM-1400 Plus) operating at 80 kV accelerating voltage was used to characterize the Eu-PS NPs in terms of particle shape and size. The hydrodynamic size and the ζ-potential of the Eu-PS NPs in MQ water were measured using dynamic light scattering (DLS) and laser Doppler electrophoresis by operating a Zetasizer Ultra (Malvern Panalytical, The Netherlands). Nanoparticle Tracking Analysis (NTA, NanoSight's NS200, Malvern, The Netherlands) was used to measure the number of particles in the samples following a method reported previously.[31]

To measure the size distribution of the particles in the samples, asymmetric flow field flow fractionation (AF4, Eclipse™ AF4, Wyatt Technology Europe GmbH, Dernbach, Germany) coupled on-line to multi-angle light scattering (MALS, DAWN® EOS™, Wyatt Technology Europe GmbH, Dernbach, Germany) was used (see S2, ESI†) following a method reported previously.[31] The carrier solutions [0.001% sodium dodecyl sulfate (SDS)] used for AF4 analyses were filtered before use (Anodisc <0.1 μm filter, Whatman, Maidstone, UK). The agglomeration rate of the Eu-PS NPs over time was determined by measuring the hydrodynamic size of the particles over time using the Zetasizer Ultra. A PerkinElmer NexION 2000 ICP-MS operating was used to measure the quantity of Eu in the samples. The conditional set up of the ICP-MS is given in S3, ESI.†

Spiking of the soils with Eu-PS NPs

The field soil and the LUFA standard soil were dried for 24 hours at 40 °C. From a 1 g L^{-1} stock dispersion of Eu-PS NP, an aliquot was spiked in the field and standard soils to obtain a concentration of 1 mg Eu-PS NPs per kg soil, which is equal to 6.9×10^9 particles per kg soil. The spiking procedure was performed by dropping the particle dispersion in the soil and mixing the soil for 10 min in a metal bowl. The spiked soils were left for 24 hours at room temperature. To confirm that the Eu-PS NPs were homogeneously distributed in the soil matrices, six samples (with three replicates) from the field and the standard LUFA soil were taken. Samples were digested using *aqua regia* (S3, ESI†) and the concentration of Eu was measured by ICP-MS.

Six replicates of (50 g) field and standard soils spiked with Eu-PS NPs and control samples (50 g) standard soil without Eu-PS NPs were placed into a 250 mL Erlenmeyer flask. Control soils without Eu-PS NPs included a LUFA soil blank and a field soil blank were used to test for the background concentration of Eu.

Four-step extraction of the NPD from the soil

After 24 hours of interaction between soil and Eu-PS NPs, the samples (6 replicates of each treatment and the controls) were used to extract the particles from the soil in four steps.

Step 1. This step was performed to wash the unbound Eu-PS NPs, the Eu-PS NPs loosely attached to the soil matrix and dissolved OM-coated Eu-PS NPs (coated with small molecules but free and unbound to soil surfaces) from the matrix of the soil. Accordingly, 5 g of each soil sample was mixed using a metallic spoon for 5 minutes and dispersed in 5 mL of MQ water. After 30 minutes, 5 mL ammonium sulphate solution [$(NH_4)_2SO_4$, adjusted to pH 7 and a density of 1.11 g cm^{-3}] was added to reach a final concentration of 1.8 M. The suspensions were mixed for 30 minutes (the 30 minutes was the optimal time, see the S4, ESI†). The $(NH_4)_2SO_4$ solution offers a higher density to the medium than the density of the Eu-PS NPs (1.04 g mc^{-3}). Thus, upon centrifugation (using Sorvall RC5Bplus centrifuge, Fiberlite F21-8 at 2800×*g* for 10 min) (see the S4, ESI†) the NPs were separated from the soil particles due to density differentiation. The supernatants were separated, and the particle number and the concentration of Eu were measured in the supernatants using NTA and ICP-MS, respectively.

Step 2. To separate the loosely attached NPs (attached to some positively charged surfaces in the soil) from the soil matrix and from other positively charged surfaces which could not be done using MQ water, the pellets resulting from step 1 were dispersed in a 5 mL SDS solution (1%) for 1 hour. The SDS offers a highly negative charge to the soil surfaces and the NPs, which leads to particle repulsion in the soil, and facilitates the dispersion of NPs in the aqueous phase. After 1 hour, 5 mL solution of $(NH_4)_2SO_4$ was added to reach the final concentration of 1.8 M. The suspensions were left for 30 minutes at room temperature followed by centrifugation at 2800×*g* for 10 minutes. The supernatants were carefully removed, and the particle number and the concentration of Eu were measured in the supernatant.

Step 3. To isolate Eu-PS NPs that were not washed off by MQ water and SDS solution, the pellets resulting from step 2 were treated with 5 mL of 5% tetramethylammonium hydroxide (TMAH) to digest the OM and to dissolve the oxyhydroxides in the soil.[32] It was reported that diluted (5%) concentrations of TMAH do not influence some polymeric particles such as PS.[33] After 1 hour, 5 mL of $(NH_4)_2SO_4$ solution was added to the suspension, the pH was adjusted to 7 using HCl (1 M) and it was left for 30 minutes. The suspensions were centrifuged at 2800×*g* for 10 minutes and the supernatants were removed. The particle number and the concentration of Eu were determined in the supernatants.

Step 4. The pellets resulting from step 3 were digested using *aqua regia* (see the S5, ESI†). The suspensions were centrifuged, the supernatants were removed and the total concentration of Eu in the supernatants was measured to obtain the total concentration of the retained Eu-PS NPs in the soil.

Influence of the extraction steps on the Eu-PS NPs

To assure that the Eu-PS NPs extraction procedures did not influence the physicochemical properties of the particles, the performance of the method was evaluated. Accordingly, the Eu-PS NPs were dispersed in MQ water and the physicochemical properties of the particles *e.g.*, particle size distribution, particle number and the Eu total mass, were determined. The particles in the MQ water dispersion were extracted step by step according to the extraction method described above (steps 1–3). To evaluate the influence of the extraction agents on the stability of the NPs against agglomeration, the particle size distribution was measured again after each step and compared with the size distribution of the particles before extraction. To calculate the particle and mass recovery after each extraction step, the particle number and the Eu total mass were measured and compared to the particle number and the Eu total mass obtained before particle extraction. The size distribution of the NPs before and after extraction was measured using AF4-MALS (S2, ESI†). The particle number was measured using NTA and the total mass of Eu was measured using ICP-MS.

Data analysis

The IBM SPSS Statistics 25 software was used for statistical analysis of the results. The normality and homogeneity of variances were checked using Kolmogorov–Smirnov and Levene tests, respectively. Significant differences between particle sizes and particle number recoveries of samples treated with different agents were analyzed by one-way ANOVA followed by Dunnett's test. The variation in the Eu mass recoveries was also determined using ANOVA. The difference between the values of ζ-potential of the particles before and after the extraction was measured using a *t*-test. The differences were significant at $p < 0.05$. Astra software (software version 5.3.4.20, Wyatt Technology Europe GmbH, Dernbach, Germany) was used to obtain the light scattering signal. Plotting of the fractograms was carried out in the software OrginLab 9.1. The Eu mass recovery (Formula S1†) and the particle number recovery (Formula S2†) calculation are described in S6 (ESI†).

Results and discussion

Characterization of the Eu-PS NPs in MQ water

The Eu-PS NPs dispersed in MQ water were thoroughly characterized regarding physicochemical parameters before performing the particle extraction experiment. TEM images showed that the particles are spherical in shape and that they

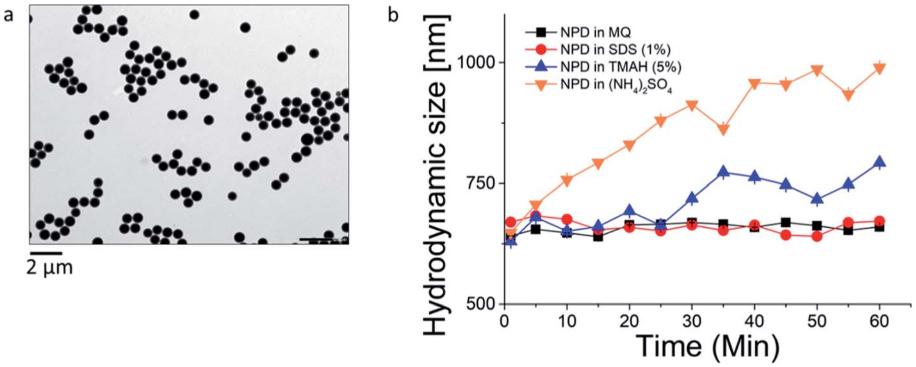

Fig. 1 (a) A TEM image of the Eu-PS NPs in MQ water. (b) The measured hydrodynamic size of the particles in different media during 1 hour exposure time (mean ± SD).

did not agglomerate upon dispersion in MQ water (Fig. 1a). No Eu release from the Eu-PS NPs was measured when the particles were dispersed in MQ water as measured by ICP-MS (S7, ESI†). The hydrodynamic size of the particles was 640 ± 20 nm (mean ± standard deviation) and the ζ-potential was −38 ± 2 mV (Table 1). The highly negative ζ-potential may lead to particle repulsion and this can be the reason for the stability of the particles, as reported previously.[34] The hydrodynamic size of the Eu-PS NPs in MQ water did not increase over 1 hour as measured by DLS (Fig. 1b). This confirmed that the Eu-PS NPs were stable against agglomeration in MQ water during the exposure.

Evaluation of the particle extraction steps on the Eu-PS NPs

Different particle extraction agents were used in this study, including $(NH_4)_2SO_4$ (1.8 M), SDS (1%) and TMAH (5%). It is possible that these media influence the physicochemical properties of the Eu-PS NPs and, consequently, lead to degradation and/or agglomeration of the particles as reported for their microplastic counterparts.[27] The hydrodynamic size and ζ-potential of the Eu-PS NPs in these media were measured immediately after sonication (Table 1). The value of the ζ-potential in MQ water was −38 ± 2 mV and it remained highly negative in SDS (−40 ± 2 mV) and in TMAH (−39 ± 3 mV) solutions (Table 1). However, in $(NH_4)_2SO_4$ solution the absolute value of the ζ-potential decreased significantly and reached the value of −17 ± 3 mV compared to the value in MQ water. Lessons learned from nanomaterial studies showed that in order to reach colloidal stability, the ζ-potential should be at least at ∼−30 mV.[35] The ζ-potential of Eu-PS NPs in the SDS and TMAH solution was highly negative. Particle repulsion could, thus, be the reason for the stability against agglomeration.[31] However, in the $(NH_4)_2SO_4$ solution, the particles may undergo agglomeration. This was tested by measuring the agglomeration profile of the Eu-PS NPs in different media, where the hydrodynamic size of the particles was determined for 1 hour (Fig. 1b). The particles doubled in size in the $(NH_4)_2SO_4$ solution after 1 hour of mixing. Ammonium sulphate dissolves in water to form monovalent cations of NH_4^+ and divalent anions of SO_4^{2-}. Although both ions may influence the double layer of the particles and alter the stability of the Eu-PS NPs,[36] SO_4^{2-} as a divalent ion is reported to be more efficient in screening the surface charge than monovalent ions.[37] Moreover, specific adsorption of SO_4^{2-} regardless of the surface charge of the particles[38,39] may eliminate the influence of NH_4^+ on the surface charge of the Eu-PS NPs. The DLVO theory explains that a higher electrolyte concentration (*i.e.*, NH_4^+) could compress the particles' electrical double layer through charge screening effects. This leads to a fast particle aggregation[40] as also observed in this study. The agglomeration profile of the particles allowed us to optimize the time that is required to keep the particles stable in each specific dispersant without causing significant increases in the size of the particles due to agglomeration. The TMAH percentage was optimized as we observed that 10% TMAH could lead to Eu-PS NPs degradation, while 5% TMAH had no influence on the particles (see Fig. S3, ESI†). This finding is in agreement with our previous study.[31] However, we must

Table 1 Physicochemical properties of Eu-PS NPs, the particle number recovery of Eu-PS NPs treated with different extraction agents, and the mass recovery of Eu[a]

Extraction agents	Hydrodynamic size [nm]	ζ-potential [mV]	Particle number recovery [%]	Total Eu mass recovery [%]
MQ water	640 ± 20 nm	−38 ± 2[b]	96 ± 0.5[b]	103 ± 0.8
$(NH_4)_2SO_4$	647 ± 32 nm	−17 ± 3[a]	88 ± 1.7[a]	97 ± 0.6
SDS (1%)	669 ± 16 nm	−40 ± 2[b]	94 ± 1.5[b]	98 ± 1
TMAH (5%)	630 ± 28 nm	−39 ± 3[b]	95 ± 0.7[b]	96 ± 0.4

[a] a and b show the significant differences between the treatments ($p < 0.05$), the data are the mean and standard deviation of 10 measurements.

emphasize that this concentration of TMAH has been tested for particle of 640 nm size. It is possible that 5% TMAH has a more pronounced effects on particles of smaller size because by decreasing size, the surface area to volume ratio of particles increase, exposing more atoms of the particles to TMAH.

The particle number recovery and the mass recovery of Eu were calculated for the Eu-PS NPs in MQ water by comparing the particle number and the Eu mass of the Eu-PS NPs before and after particle extraction. The calculated recoveries (Table 1) were 96% and 103% for particle number and Eu mass as measured by NTA and ICP-MS, respectively. The size distribution of the particles in MQ water was measured using AF4-MALS and compared to the size distribution of the same particles after performing the particle extraction method from MQ water (Fig. 2). The Eu-PS NPs in MQ water eluted in between 15-30 minutes with a peak at around 22 minutes (black line). The obtained recoveries for the Eu-PS NPs in the SDS solution were higher than 90% (Table 1) and the size distribution of Eu-PS NPs in SDS (red line) was similar to the size distribution of the particles in MQ water. This indicates that as expected, the solution of 1% SDS did not significantly influence the size distribution of the particles.[41] In the TMAH solution (blue line), the particle number and the Eu recoveries were higher than 90% (Table 1). The size distribution of the particles shifted slightly towards a smaller particle size with a peak eluting at around 20 minutes. This earlier elution compared to the Eu-PS NPs in MQ water could not be due to the influence of TMAH on the particles by degrading the surface of the Eu-PS NPs. It is likely to be related to the influence of the TMAH on the eluent itself, *e.g.*, changing the viscosity or pH. The recovery obtained for particle number in $(NH_4)_2SO_4$ decreased to 88% compared to the recovery of the Eu-PS NPs in MQ water (Table 1). However, the mass recovery of the Eu (97%) was similar to the recovery of particles in MQ water. The fractogram obtained for Eu-PS NPs in $(NH_4)_2SO_4$ showed that the particles eluted at the same time as the particles in MQ water. However, the intensity was lower for Eu-PS NPs in $(NH_4)_2SO_4$ solution. The higher release peak at the end of the $(NH_4)_2SO_4$ fractogram suggests that some of the particles might be agglomerated and retained in the channel. They thus eluted when the crossflow stopped as a release peak at the end of the fractogram.

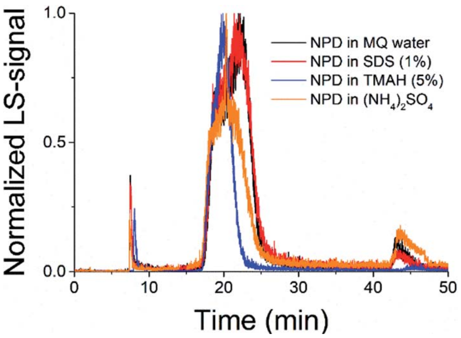

Fig. 2 The AF4-MALS fractograms obtained for Eu-PS NPs dispersed in MQ water (black line), SDS solution (red line), TMAH solution (blue line) and $(NH_4)_2SO_4$ (orange line).

Extraction of Eu-PS NPs from the soil matrix

By estimating the possible influences of the extraction agents on the physicochemical properties of the Eu-PS NPs as a model of NPD, the extraction method was used to isolate the NPD mimics (Eu-PS NPs) from the spiked standard LUFA soil and from field soils. Although the concentration of Eu in soil in The Netherlands reported to be 0.2–0.8 mg kg^{-1},[42] we could not detect the concentration of Eu in the soil samples using ICP-MS. This could be attributed to the mild digestion method that could not bring the Eu of the soil into the solution phase.

Before particle extraction, we made sure that the Eu-PS NPs were homogeneously distributed in the spiked soils. The concentration of the Eu in the 6 samples of each soil, which were randomly selected from 6 spots in each spiked soil, was measured. There was no significant difference between the concentrations of Eu in the 6 samples (see S9, ESI†). Then, we extracted the Eu-PS NPs from the spiked soil according to the method developed in this study to systematically assess to what extent the method is capable of isolating NPD from the soil matrix. This method was developed for PS particles and may be optimized for the extraction of other types of NPD from soils. The physicochemical properties of the particles such as size and shape must be also considered while developing an extraction method, as they might be influenced by the method. We emphasize that the method is used as a sample preparation method to extract and prepare NPD in soils for further analysis *e.g.*, identification and quantification. This implies that the method is a complementary method and may not be used alone without a suitable identification technique because in the natural condition the soil may be contaminated with different types of NPD and that they are not doped with metals.

Extraction using MQ water

Some of the spiked Eu-PS NPs could be extracted from the soil using MQ water. We assumed that these particles were freely present in the soil matrix and *e.g.*, not bound to the soil surfaces and/or stabilized with dissolved OM in the soil dispersion. No significant differences were obtained between the particles extracted from LUFA soil and field soil regarding particle number recovery and EU mass recovery.

The hydrodynamic size of the extracted Eu-PS NPs from the soils (Table 2) was comparable with the hydrodynamic size (slightly increased) of the pristine Eu-PS NPs in MQ water. However, the absolute value of the ζ-potential of particles in both soils (LUFA soil and field soil) decreased in comparison to the ζ-potential of Eu-PS NPs in MQ water. This is not surprising as soils contain many mono- and divalent electrolytes that can compress the double layer and screen the particle charges.[43,44] This could lead to a lower absolute value of the ζ-potential. Understanding the colloidal state of NPD, *e.g.*, by measuring aggregation rate and ζ-potential of NPD, is of paramount importance in developing methods for extraction of NPD from a soil matrix because it can influence the behavior of NPD and the interaction of NPD with the soil surfaces. Consequently, the colloidal state of NPD can have a direct influence on the NPD extraction.

Table 2 Physicochemical properties of Eu-PS NPs, the mass recovery of Eu, and the particle number recovery of Eu-PS NPs extracted from the standard and field soil

	Hydrodynamic size [nm]		ζ-potential [mV]		Particle number recovery [%]		Total Eu mass recovery [%]	
Steps	Standard soil	Field soil	Standard soil	Field soil	Standard soil	Field soil	Standard soil	Field soil
Step 1 (MQ water)	756 ± 74	682 ± 42	−23 ± 2	−20 ± 0.5	7 ± 2	4 ± 0.5	4 ± 1	5 ± 1
Step 2 (SDS)	710 ± 61	694 ± 57	−31 ± 1	−27 ± 2	51 ± 5	43 ± 3	45 ± 5	37.5 ± 3
Step 3 (TMAH)	854 ± 118	740 ± 86	−18 ± 1	−16 ± 0.5	23 ± 2	38 ± 1.5	18.5 ± 4	22.5 ± 2.5
Step 4 (residual in the soil)	—	—	—	—	—	—	15 ± 4	12 ± 2
Total					81	85 ± 9	82.5	77 ± 11.5

The recovery of Eu mass and particle number of the extracted Eu-PS NPs from the soil using MQ water was between 4% and 7%, respectively. This confirms that after entering soils, NPD immediately attach to the constituents of the soil solid matrix.[45–47]

Extraction using SDS solution

By using an SDS solution, a higher percentage of the spiked Eu-PS NPs was extracted from the soil matrices in comparison to using MQ water (Table 2). One explanation of this finding is that the formation of negatively charged surfaces in the soils upon contact with SDS solutions[48] gives rise to an electrical double layer. This negative charge layer decreases the attachment efficiency of the particles and the negatively charged surfaces in the soil. This increases the mobility of NPD in soil matrices.[49] Moreover, SDS is amphipathic in nature, which allows it to attach to the hydrophobic surface of NPD. This increases the dispersion of NPD in water and removes NPD from the soil matrix. The particle number and the EU mass recoveries of the NPD extracted from LUFA soil were significantly higher than the corresponding values measured for field soil. This could be due to differences in soil texture. We measured some of the physicochemical properties of the soil (see Table S1†) and observed that, for instance, the amount of OM as measured by total C in the field soil was higher than in the LUFA soil. This indicated that the method development for NPD extraction may not be directly applicable for different types of soil and some soil-specific optimization might be required to increase the recoveries.

The hydrodynamic size and the ζ-potential of the extracted Eu-PS NPs using SDS (1%) solution from both soils were comparable to the corresponding values obtained for Eu-PS NPs in MQ water (Table 2). This showed that there was no significant influence of SDS solution on the hydrodynamic size and ζ-potential of the particles. The particle number and Eu mass recoveries of the Eu-PS NPs extracted by SDS solution were <43% and <37%, respectively.

Extraction using TMAH solution

The TMAH solution was used to dissolve the OM and oxyhydroxide fractions of the soil and to isolate the Eu-PS NPs that were bound to these fractions. The particle number and Eu mass recoveries were <23% and <18.5%, respectively. The variation obtained between particle number and Eu mass recoveries could be due to the heterogeneous distribution of Eu in the particles and also could be attributed to the techniques used for measuring each property, NTA for particle number and ICP-MS for Eu mass. It is also likely that the particle losses during sample preparation for each technique is different, resulting in different recoveries. This supports our hypothesis that a fraction of the particles could be adsorbed to OM and/or strongly attached to oxyhydroxide colloids, which were isolated upon application of the TMAH solution. The recoveries obtained for particle number and Eu mass upon application of TMAH were both higher and lower than after application of MQ water and SDS solution, respectively. The recoveries calculated for the Eu-PS NPs extracted from the field soil using TMAH were higher than the recoveries obtained following particles extracted from the LUFA soil. This was expected as we measured a higher amount of OM in field soil compared to the standard soil.

Extraction of Eu-PS NPs from the soil matrix using TMAH slightly increased the hydrodynamic size and decreased the absolute value of the ζ-potential of the particles (Table 2). This decrease in the absolute value of the ζ-potential could be due to the presence of a high concentration of ions in the soil, which facilitated particle agglomeration and slightly increased the hydrodynamic size.[50]

Unrecovered Eu-PS NPs

Our data showed that the extraction method could extract 67.5% (step 1 + step 2 + step 3) of the Eu-PS NPs from the standard soil, around 15% was retained in the soil matrix (Table 2). Some explanation could be put forward. For example, 1 hour exposure to TMAH was not enough to break down all OM and oxyhydroxides, as needed to isolate all attached Eu-PS NPs. It is also likely that particles are attached to or complexed with other fractions of the soil that could not be extracted using our extraction method and an additional extraction solvent might be helpful in this respect. The results also showed that we could only recover 82.5% of the total added Eu-PS NPs into the soil. This means that around 17.5% of the particles was lost during the sample preparation. This could be due to the sample handling and the attachment of the particles to the tubing or to the glassware used, which results in the loss of the Eu-PS NPs.

Previous methods mostly developed for the separation of microplastics from the soil and other environmental compartments. The presented sample preparation method in this study is the first step toward developing a generic method for the extraction of NPD from the soil. The method considers the nanoscale physicochemical properties of the NPD, such as small size and the tendency of the particles for aggregation and agglomeration. The method also for the first time considers the presence of the NPD in different fractions of the soil matrix, *e.g.* coated with small molecules, attached to some positively charged surfaces in the soil, attached to OM and oxyhydroxides in the soil, and optimized to extract the NPD from each fraction sequentially.

Conclusion and future perspective

We emphasize that the aim of this study was to develop a sample preparation method to extract NPD from soil matrices. Eu-PS NPs were used as a model of NPD to facilitate tracing the particles and evaluating the extraction method to obtain a method with minimum artefacts and high particle number and mass recoveries. The results showed promising prospects to optimize the method for NPD of different sizes, morphologies, and chemistry. This method can be used as a sample preparation method to bring the NPD to a state that makes them measurable by some identification and quantification techniques. This method is one of the starting points for developing a sample preparation method that can extract intact NPDs of different types and sizes from the field soil. We suggest that future studies be carried out to optimize the parameters such as the concentration of the digestive agents and time of digestion. Our proposed method was shown to have a minimal impact on the physicochemical properties of NPD such as size distribution. Application of $(NH_4)_2SO_4$ for density differentiation decreased the absolute value of the ζ-potential and led to particle agglomeration over time. Future studies may optimize the concentration of $(NH_4)_2SO_4$ for each type of NPD to prevent any influence on the ζ-potential of NPD. The developed method may not cover all types of plastic and might for example not be applicable to NPD with a high density such as polyvinyl chloride or polymers with a higher sensitivity to TMAH. Nevertheless, the presented method in this study can be a suited starting point for developing a generic sample preparation method for the extraction of many types of NPD from soil matrices and can provide a workflow for future method development. In conclusion, NPD occur in different fractions of soil matrices and the method development must consider the texture of the soil.

Conflicts of interest

There are no conflicts to declare.

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