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Solid phase extraction materials as a key for improving the accuracy of silver nanoparticle characterization with single-particle inductively coupled plasma mass spectrometry in natural waters through dissolved silver removal

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A B S T R A C T

The accurate characterization of silver nanoparticles (Ag NPs) in environmental samples is crucial for understanding the potential impact of these materials on the environment and human health. Single-particle inductively coupled plasma mass spectrometry (spICP-MS) is one of the few techniques available for quantifying NPs at environmentally relevant concentrations. However, the spICP-MS detection of Ag NPs can be interfered with coexisting dissolved analyte causing high background signals, which mask NP signals leading to a significant bias in NP characterization. In this paper, a simple sample pre-treatment procedure for efficient removal of dissolved silver in natural waters is proposed using solid phase extraction (SPE) materials, allowing more accurate characterization of NPs in environmental water matrices. The applicability of eight commercially available SPE materials was evaluated based on their effects on sample Ag NP properties and dissolved silver extraction efficiency in environmental waters, which were shown to depend on sample matrix. The SPE materials found most efficient in dissolved silver extraction were further studied for improving the characterization of 30 nm Ag NPs in colorless and dark-colored waters. Whereas dissolved silver concentrations of ≤ 1 µg kg^{-1} were shown to lead to a significant bias in NP characterization (up to +58% increase in NP size and − 90% decrease in particle concentration), pre-treatment of the samples with SPE materials 'SiliaMetS Thiol' and 'Purolite C115HMR' minimized the effect of dissolved silver interference. As a result, highly more accurate NP sizing (28 ± 2 nm) and relatively stable particle concentration was obtained for 30 nm Ag NPs in environmental waters.

1. Introduction

Over the past decades, the human population has witnessed a widespread beneficiation of engineered nanomaterials in various fields and consumer products. Among metallic nanoparticles (NPs), silver NPs are one of the most frequently exploited due to their exceptional antimicrobial, anti-angiogenic and optical properties. Ag NPs are in fact widely utilized in textiles, water purification systems, health care products, cosmetics, and in medical diagnostics just to name a few [1–3]. The increased production and use of nano-enhanced products inevitably lead to their growing release into the environment, which has raised serious concerns about their potential adverse effects on the environment and human health [4–10]. Despite the vast amount of previous research focused on the matter, knowledge gaps in the risk

assessment of NPs still exist due to a number of factors confounding the issue. In addition to the properties of the NPs themselves (such as size, shape, concentration, surface functionalization, etc.), the environmental behavior and toxicity of NPs are also affected by environmental conditions, such as pH, temperature, and other elements and compounds present [11–20]. Clearly, understanding the potential impact of NPs on the environment and human health requires careful characterization of these materials at environmentally relevant concentrations.

Single-particle inductively coupled plasma mass spectrometry (spICP-MS) is widely recognized as a powerful tool in the characterization of engineered NPs. As a highly sensitive technique, spICP-MS enables the simultaneous determination of NP size, size distribution, particle number concentration, and ionic (dissolved) content at realistic environmental concentrations [21–25]. In spICP-MS, a sufficiently

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dilute NP suspension is nebulized into the plasma, resulting in vaporization, atomization, and ionization of an analyte element. Whereas the ionization of the dissolved form of the element produces a steady-state signal, individual NPs generate a burst of ions producing a transient signal, i.e. "a spike". The discrimination between the signal originating either from the analyte in the dissolved or NP form is based on the separation of the pulse intensities originating from individual NPs over a certain threshold limit, which typically is determined using an iterative 'mean + 3σ' computation [21,24,26–29]. However, distinguishing the NP events from the background signal is not a trivial task, especially in the presence of a high level of dissolved analyte. In these cases, the high background signal might overlap the NP signals resulting in a calculated threshold value being larger than some or possibly all NP signals. As a result of only a partial detection of the NP size distribution, the obtained NP number and size information will be biased (overestimation of the NP size and underestimation of the particle concentration). The situation is particularly challenging for samples containing high concentrations of dissolved analyte and/or small NPs, e.g., for environmental samples [30–32]. For accurate characterization of the NPs, minimizing the dissolved analyte concentration in NP samples is crucial.

Differentiation of the NP events from the continuous background signal can be improved by careful selection of the data acquisition parameters used, especially in the presence of a high level of dissolved analyte. As compared to millisecond dwell times frequently used [25], faster data acquisition with dwell times in the microsecond range (e.g. ≤ 100 μs) allows smaller NPs to be detected [24,29,33]. Mathematical methods can be further used to improve the accuracy of NP event identification [34,35]. For instance, Mozhayeva et al. [34] developed a data deconvolution procedure using Poisson statistics for improved identification of NP events using microsecond time resolved signals. The dissolved analyte content in the sample can also be reduced physically, thus eliminating the need for mathematical discrimination techniques. A simple way to reduce dissolved (background) signal at spICP-MS is by sample dilution [30,36]. However, considering environmental samples with low NP concentrations [5,30,32,37,38], sampling times might need to be extended to ensure the capture of a sufficient number of NP events for reliable NP characterization. Over the past years, a variety of approaches for discrimination of metallic NPs and their corresponding ionic counterparts have been developed including e.g. filtration and centrifugation methods [39,40], cloud point extraction [18,41–43], chromatographic separation [44–46], field flow fractioning methods [47,48] and magnetic solid phase extraction [49–53]. Even though effective, these methods suffer from some pitfalls including e.g. time-consuming sample pre-treatment stages, the need for careful optimization of separation conditions, and long analysis times. In addition, not all aforementioned methods are directly compatible with spICP-MS or changes in the original sample properties might also occur during complex sample treatment processes (e.g. changes in NP size or concentration), which should be avoided.

The applicability of solid phase extraction (SPE) on the separation of metal ions and their corresponding NPs in various matrices has been demonstrated in numerous papers over the past years [31,39,54–57,59,60]. In this technique, the analyte either in NP or dissolved form can be selectively extracted from the sample solution by careful selection of the SPE material and possible surface functionalization of the NPs. Alternatively, both forms of the analyte can simultaneously be extracted and the separation of the two is achieved by differential desorption [54]. The first on-line coupling of spICP-MS with an ion-exchange column for the characterization of Ag NPs was presented by Hadioui et al. [31]. The dissolved signal was efficiently decreased using cationic Chelex 100 resin, allowing more accurate characterization of the NPs. Since then, Chelex 100 resin has been applied for the removal of dissolved analyte in the characterization of Ag, La$_2$O$_3$, and Zn NPs in various matrices [55,60–62]. However, lengthy washing and regeneration steps (up to 60 min) needed to ensure the proper functioning of the resin and to minimize carry-over between

samples [31,62] cause a significant increase in the analysis time, especially in on-line systems.

Despite the previous studies focused on the matter, more research is still urgently needed on the applicability of SPE materials on dissolved silver removal from real environmental waters before spICP-MS analysis, which is currently still rare. As such, the aim of this work was to develop a simple sample pre-treatment procedure for spICP-MS allowing more accurate NP characterization by dissolved silver signal removal using SPE materials. The effect of eight commercial SPE materials (Chelex 100, Lewatit MonoPlus TP 214, Purolite C115HMR, SiliaBond Tosic Acid, SiliaMetS (SM) DEAM, SM DMT, SM Thiol and SM Triamine) on silver in both dissolved or NP form is thoroughly investigated in several water matrices (spring, lake, river, brook and ultrapure (UP) water and synthetic water sample (SWS)). The effect of competing elements, matrix components, and humic substances on the dissolved silver extraction efficiency of the SPE materials is investigated. Finally, the most suitable SPE materials were selected for efficient dissolved silver removal in different environmental water matrices, and their benefit on improving NP characterization is demonstrated.

2. Experimental

2.1. Materials

UP water (18.2 MΩ•cm) obtained from PURELAB Ultra water purification system (ELGA LabWater, Buckinghamshire, UK) was used for the preparation of aqueous solutions and sample dilutions. Single-element standard stock solutions of silver, yttrium, and ruthenium (1000 mg L^{-1}, pure grade) and multi-element standard solutions (29-Element and 12-Element Solution, 10 mg L^{-1}) used for calibration purposes and preparation of Ag sample solutions were obtained from PerkinElmer (MA, USA). Citrate-stabilized Ag NPs with nominal sizes of 30, 40, 50, and 80 nm (NanoComposix, San Diego, CA, USA) were used for the preparation of NP dispersions. The certified values of the standard particles used in the experiments were further verified in our laboratory (see Chapter 2.3). A dispersion containing ultra-uniform 50 nm PEG-Carboxyl-stabilized Au NPs specially designed for spICP-MS calibration was obtained from PerkinElmer and used for transport efficiency determination.

Sodium thiosulfate (STS, purum p.a., $\geq 98.0\%$) and thiourea (Reagent plus, $>99.0\%$) were obtained from Sigma-Aldrich (Saint Louis, MO, USA) and used for adjusting solution matrix and elimination of the memory effects of silver in spICP-MS and ICP-MS (standard mode) measurements, respectively. High-purity nitric (HNO$_3$) and hydrochloric (HCl) acids (Analpure, Analytika spol. s r.o., Prague, Chech Republic) were used for adjusting the sample matrix for inductively coupled plasma optical emission spectrometry (ICP-OES)-measurements and for acid digestion of the commercial NP standards. Nitric acid (puriss p.a., $\geq 65\%$) and NaOH ($\geq 97\%$) were obtained from Sigma-Aldrich and used for the pre-treatment of the SPE materials.

SWS used in some experiments was prepared from stock solutions (1000 mg L^{-1}) of the individual elements. The composition of the SWS (0.5 mg L^{-1} of Al, 10 mg L^{-1} of Ca and Na, 1.5 mg L^{-1} of Fe, 2 mg L^{-1} of K and Mg, 4 mg L^{-1} of S, 72 mg L^{-1} NO$_3^-$ and 5 mg L^{-1} Cl$^-$) was designed to represent the elements and their levels commonly found in natural waters. Al(NO$_3$)$_3$•9H$_2$O (ACS Grade), KCl ($\geq 99\%$) and Mg(NO$_3$)$_2$•6H$_2$O (98.0–102.0%) was obtained from J.T.Baker (NJ, USA), FeCl$_3$ •6H$_2$O (99.0–102.0%) was obtained from VWR International, NaNO$_3$ ($\geq 99.5\%$) was obtained from Sigma-Aldrich, Ca(NO$_3$)$_2$•4H$_2$O (99.0–103.0%) was obtained from Merck (NJ, USA) and 1000 mg L^{-1} solution of S (in H$_2$O) was obtained from PerkinElmer. Only UP water was used for the preparation of the stock solutions to maintain the pH of the SWS as close to the environmental waters as possible. Humic acid (HA) solution was used as humic substances' source in selected experiments and prepared by dissolving 1 g of HA (virgin, Carl ROTH, Karlsruhe, Germany) in 1000 ml of UP water under constant stirring for 24 h. The undissolved

fraction of the material was removed by filtration (Whatman grade 41) and the filtrate was stored in the dark until use in HDPE-container. The soluble fraction of the material was determined by weighting the un-dissolved material and found to be approximately 20%.

2.2. spICP-MS measurements

All spICP-MS measurements were performed on a NexION350 D ICP-MS (PerkinElmer) operating in a single particle mode equipped with an ESI 4DX autosampler (Elemental Scientific, NE, USA). General instrumental parameters are shown in Table 1. At least duplicate readings were recorded of all samples to monitor any sample introduction errors. A commercial NexION Setup Solution (1 µg L^{-1} Be, Ce, Fe, In, Li, Mg, Pb, and U in 1% HNO$_3$, Pure Plus, PerkinElmer) was used to adjust the equipment according to instrument software's tuning protocols and to check the overall performance (i.e., maximum sensitivity with CeO$^+$/Ce$^+$-level < 2.5% and Ce^{++}/Ce <3%). All data processing was performed using the Syngistix Nano Application Module (v. 2.5), which automatically separates the NP events from the background (dissolved) signal using a threshold value determined using an iterative 'mean + 3σ' computation [63].

Transport efficiency was determined on daily basis at least in triplicate using the particle frequency method described by Pace et al. [28]. A dispersion containing 50 nm PEG-Carboxyl-stabilized Au NPs (PerkinElmer) diluted to a particle concentration of approximately 10^5 particles g^{-1} with UP water was used for its stability. The sample uptake rate was measured daily at least in duplicate by weighing the water uptake after 3 min aspiration, and its value was regularly checked during measurements.

Dissolved silver calibration standards ranging from 0.1 µg kg^{-1} to 2 µg kg^{-1} were prepared from a standard stock solution of silver (1000 mg L^{-1}). All calibration standards were diluted with a 0.5 mM solution of STS to ensure the solution stability of silver. Particle calibration was performed using citrate-stabilized Ag NPs with nominal sizes of 40, 50, and 80 nm (NanoComposix) after dilution to a particle concentration of approximately 10^5 particles g^{-1} with UP water. Before spICP-MS-measurements, an appropriate amount of 1000 mM STS solution was added to adjust the matrix of the NP standard solutions to 0.5 mM STS. The purpose of this was to match the matrix of the calibration standards of dissolved and NP silver, thus minimizing any matrix differences between the two.

All solutions used in the experiments were diluted gravimetrically fresh on a daily basis before the experiments. 15 and 50 ml polypropylene (PP) centrifuge tubes and high-density polyethylene (HDPE) bottles (Abdos Life Science, VWR International, and Fisher Scientific) were used for the preparation of the samples. The matrix of all sample solutions of silver was adjusted to 0.5 mM STS before measurements to ensure the chemical stability of dissolved silver and to minimize any matrix interferences. To minimize the effect of time on the sample

properties, all samples were measured within 2 h from their initial preparation.

2.3. Verification of the Ag NP standards

The certified values of the commercial Ag NPs used in the experiments were verified in our laboratory (Table S1), as at times these values have been shown to deviate from the information provided by the manufacturer [64–67]. The mass fraction of silver (mg L^{-1}) of each Ag NP dispersion was quantified in duplicate by ICP-MS (NexION350 D ICP-MS, standard mode) using ruthenium (^{102}Ru) as an internal standard. 0.25 g of each sample was accurately weighed into new 15 ml PP tubes followed by the addition of 0.4 ml of aqua regia (Analpure). For quality control purposes, an accurately weighed amount of yttrium (approximately 7.5 µg) was added, and the samples were allowed to digest at room temperature for several days. Once dissolved, samples were brought to 10 ml using 0.1% (m/v) thiourea solution (Sigma-Aldrich) and further diluted 50-fold using a solution composed of 0.1% thiourea (*w/v*), 2.4% HCl (*v/v*) and 0.5% HNO$_3$ (v/v). The recovery of yttrium was monitored during analysis and used to compensate for any deviations originating from the sample preparation procedure (recoveries of 92–98% were attained in all experiments).

The diameters of the commercial Ag NPs were determined with transmission electron microscopy (TEM, JEM-1400HC, JEOL, MA, USA). For the calculation of average particle diameters and to ensure the statistical significance of the results, a minimum of 250 particles were considered. The in-lab verified values of the commercial Ag NPs were found to differ <10% from the values provided by the manufacturer (Table S1) and were used throughout the study.

2.4. Environmental water samples

Several environmental water samples (lake, river, brook, and two spring waters) were collected from various locations situated in Southern Ostrobothnia and Central Finland and stored in HDPE-containers in the refrigerator at 6–8 °C until use. Samples containing any solid material were filtrated before use (Whatman grade 41) to minimize the risk of blockage of the sample introduction system. The elemental composition of all the water samples used in the experiments (environmental water samples, UP water, SWS, and HA solution) were characterized in the laboratory and is presented in Table S2 (Appendix). Silver was measured with spICP-MS as described in Chapter 2.2, while ICP-OES (PerkinElmer) was used for the characterization of other minor and major elements. Other physicochemical parameters including e.g. chemical oxygen demand (COD$_{Mn}$) and dissolved organic carbon (DOC) used to characterize the humic substances' content of the water samples are presented in Table S3 (Appendix).

2.5. SPE materials

Various SPE materials having the potential to extract dissolved silver from water samples were obtained from commercial vendors (Tables 2 and S4). All SPE materials used in the experiments were pre-treated in the laboratory before use to remove any possible residual impurities and the finest particles, which could cause blockage of the sample introduction system. 3–4 g of each material was weighted into new 50 ml PP centrifuge tubes (VWR International) followed by the addition of 30 ml of 1 mM nitric acid. The tubes were shaken for 10 min (800 osc./min) using a flask shaker (Stuart SF1 Flask Shaker, Cole-Parmer, IL, USA), left to stand for 5–10 min and the supernatant liquid was removed by decanting. The residue was thoroughly rinsed with UP water until a neutral pH was achieved. To avoid any pH changes of the sample solutions caused by ion-exchange processes, some of the materials were further transformed to their sodium (Na$^+$) form (Table 2) using 30 ml of 1 mM NaOH following the same procedure as above. The excess water was then removed by vacuum filtration. All SPE materials except for

Table 1
General instrumental parameters used in spICP-MS measurements.

Parameter	Value
ICP RF-power (W)	1600
Nebulizer gas flow (L min^{-1})	0.93–0.95
Plasma gas flow rate (L min^{-1})	18
Auxiliary gas flow rate (L min^{-1})	1.2
Spray chamber	Baffled Cyclonic, Glass (cooled to 2 °C)
Nebulizer	PFA-ST MicroFlow Concentric
Injector	1.8 mm i.d. Sapphire
Sampling depth (mm)	11
Dwell time	100 µs
Sampling time (s)	30
Readings/sample	≥2
Transport efficiency (%)	6.1–8.7
Sample uptake rate (g min^{-1})	0.265–0.314
Isotope monitored	^{107}Ag$^+$

Table 2

SPE materials used in the experiments.

SPE material (Abbreviation)	Manufacturer	Particle size (µm)	Functional group	Conversion to Na$^+$ form
Chelex 100	Bio-Rad	300–1180 (wet)	Iminodiacetate ion	Yes
Lewatit MonoPlus TP 214 (TP 214)	Lanxess	500–600	Thiourea	–
Purolite C115HMR (C115HMR)	Purolite	<150	Carboxylic Acid	Yes
SiliaBond Tosic Acid (SB TA)	SiliCycle	40–63	Tosic Acid	Yes
SiliaMetS DEAM (SM DEAM)	SiliCycle	40–63	N-alkyldiethanolamine	Yes
SiliaMetS DMT (SM DMT)	SiliCycle	40–63	Dimercaptotriazine (DMT)	Yes
SiliaMetS Thiol (SM Thiol)	SiliCycle	40–63	Thiol	Yes
SiliaMetS Triamine (SM Tri)	SiliCycle	40–63	Triamine	–

Chelex 100 were further oven-dried at 50 °C overnight. All materials were stored in tightly-sealed containers until use.

2.6. Separation of the solid SPE materials

To investigate the effect of centrifugation speed and duration on sample Ag NP properties, dispersions containing 50 nm Ag NPs (10^5 particles g^{-1}) were prepared both in UP and lake water and treated with SPE materials 'C115HMR' and 'SM DEAM' as described in Chapter 2.7. The two SPE materials were chosen for their different properties (Tables 2 and S4), which might affect the separation process. Samples were then centrifuged at 1500, 2500, or 3500 rpm for 3, 5, or 10 min using Heraeus Labofuge 400 centrifuge (Thermo Scientific, MA, USA).

2.7. Extraction experiments using SPE materials

The ability of the different SPE materials (Tables 2 and S4) to remove dissolved silver and their possible effect on NP properties (particle size or -concentration) was estimated. Dispersions containing either 1 µg kg^{-1} of dissolved silver or 10^5 particles g^{-1} of 50 nm Ag NPs were prepared in brook, lake, spring (1), or UP water. The dispersions were then divided on 15 ml centrifuge tubes (10 ± 1 ml each, $n = 2$) containing 45 ± 5 mg of SPE materials, which were weighted in the tubes beforehand. 2 sample tubes were used as reference samples and thus did not contain any SPE material. Sample tubes were protected from light with aluminum foils and manually shaken for 5 min to ensure efficient mixing. Samples were then centrifuged at 2500 rpm for 3 min using Heraeus Labofuge 400 centrifuge (Thermo Scientific). 6 ml of liquid samples were carefully separated from the surface of the tubes and an appropriate amount of 100 mM STS solution was added to adjust the matrix background to 0.5 mM STS. The silver content (dissolved and NP silver) of the samples was measured with spICP-MS.

The effect of different SPE materials on dissolved or NP silver was evaluated by comparing the obtained results of the samples to those of the reference samples according to Eqs. (1) and (2), where EE-% is the material's dissolved silver extraction efficiency (%), C_{Ag} is the dissolved silver concentration (µg kg^{-1}) and C_p is the particle concentration (particles g^{-1}):

$$EE - \% = \frac{C_{Ag\ (Ref.)} - C_{Ag\ (Sample)}}{C_{Ag\ (Ref.)}} \bullet 100\% \qquad (1)$$

$$C_p\ recovery\ (\%) = \frac{C_{p\ (Sample)}}{C_{p\ (Ref.)}} \bullet 100\% \qquad (2)$$

In addition, the possible effect of SPE materials on Ag NP size or particle size distribution was evaluated by comparing the obtained mean particle size and the size distribution histograms of the samples to those obtained for reference samples.

2.8. Interference and selectivity experiments

The SPE materials' ability to extract silver in the presence of humic substances and various competing elements (Al, Ca, Cl$^-$, Fe, K, Mg, Na, NO$_3^-$ and S) was evaluated. Solutions containing 1 µg kg^{-1} dissolved

silver were prepared in spring (2) and river water, SWS, and in HA solution with different physicochemical properties (Tables S2 and S3). All samples were then treated as described in Chapter 2.7 and the concentration of dissolved silver was analyzed with spICP-MS. After extracting the sample for spICP-MS measurements, 3 ml of the residual sample was further extracted for ICP-OES to determine the concentration of the extracted competing elements (Al, Ca, Fe, K, Mg, Na, and S). Following this, 0.25 ml of high-purity HNO$_3$ was added, and the volume was finally brought up to 5 ml with UP water for ICP-OES analysis.

3. Results

3.1. Dissolved silver interference

As discussed before, elevated concentrations of dissolved analyte can severely affect the accuracy of NP characterization by masking NP signals. The situation is particularly challenging for environmental samples, where dissolved silver usually coexists with Ag NPs due to Ag NP dissolution [20]. As the intensity of observed NP pulses is directly proportional to the NP size, the high background signal interferes foremost with the detection of small NPs as a result of increased size detection limit [31,36].

To study the effect of dissolved silver interference on NP detection, dispersions containing 30, 40, 50, and 80 nm Ag NPs in UP water were spiked with increasing concentrations of dissolved silver (0–1 µg kg^{-1}, Fig. 1). Whereas dissolved silver concentration up to 1 µg kg^{-1} was not found to affect the detection of 50 and 80 nm Ag NPs, characterization of the 30 and 40 nm Ag NPs was clearly interfered by dissolved silver concentrations of ≥0.1 µg kg^{-1}. For these NPs, increasing dissolved concentration leads to an overestimation of the particle size and a significant underestimation in particle concentration. As expected, elevated concentrations of dissolved silver affected foremost the detection of the smaller 30 nm Ag NPs, and at dissolved silver concentrations >0.5 µg kg^{-1} the majority (>80%) of the 30 nm Ag NP signals were in fact masked by the dissolved signal and thus were undetected. This is because the increased dissolved background signal masked the pulses of the smaller NPs, leading to significant bias in particle sizing (up to +80%) and concentration determination (up to −93%).

As the Ag NPs in environmental samples are usually 20 nm in size or less [68], the dissolved signal should be reduced as much as possible for accurate NP characterization. As seen in Fig. 1, for accurate characterization of ≤30 nm Ag NPs, the dissolved silver concentration should be decreased to a concentration below 0.1 µg kg^{-1}.

3.2. Separation of the solid materials

SPE materials can be highly effective in improving the accuracy of NP detection by removing dissolved analyte in NP dispersions. Even though these materials are often utilized in in-lab built columns connected on-line with spICP-MS [31,61,62], laborious washing and regeneration steps (16–60 min depending on the author [31,62]) needed to ensure the proper functioning of the SPE materials and to minimize the risk of carryover between samples often increase total sample analysis time. As such, in this paper, SPE materials were utilized as an
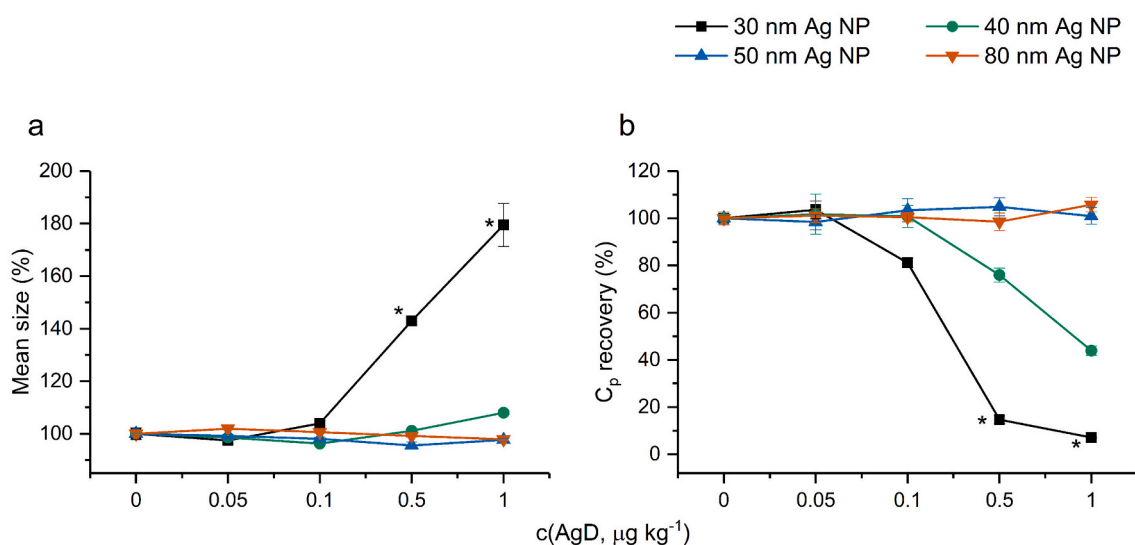
Fig. 1. The effect of dissolved silver concentration (0–1 µg kg^{-1}) on the accuracy of Ag NP characterization. Obtained Ag NP mean size (a) and particle concentration (b) as function of dissolved silver (AgD) concentration, given as percentual (%) values as compared to the values obtained at a dissolved silver concentration of 0 µg kg^{-1}. Results are given as mean ± 1 standard deviation (1 s) of three replicate samples ($n = 3$). The obtained NP size detection limits were 10, 16, 17, 26 and 30 nm at dissolved silver concentrations of 0, 0.05, 0.1, 0.5 and 1.0 µg kg^{-1}, respectively. The data points for 30 nm Ag NP at AgD concentrations of 0.5 and 1.0 µg kg^{-1} are highlighted with an asterisk, as an insufficient number of NPs (⟨100) were captured for statistically significant characterization.

off-line sample pre-treatment procedure to maximize sample throughput in spICP-MS.

For the small particle size of the solid SPE materials (Table 2), sufficient separation of the sample solution and solid materials should be achieved to prevent the risk of blockage of the sample introduction system. However, the original sample NP properties (size and concentration) should be maintained in the process. Even though centrifugation is an efficient method for the separation of the solid materials, using high centrifugation speeds and durations has in some cases shown to lead Ag NP losses [59,69–72]. As such, the effect of centrifugation conditions on sample properties was investigated.

Firstly, the effect of centrifugation duration on Ag NP recoveries was evaluated. 50 nm Ag NP dispersions prepared in UP and lake water were centrifugated for 3, 5, and 10 min at 3500 rpm (Fig. S1a and b in Appendix). Even though the Ag NP recoveries are generally observed to decrease slightly as the centrifugation duration is increased, no significant differences are observed in the Ag NP concentration in either matrix. In addition, sufficient separation of the solid SPE materials was achieved even at the shortest centrifugation duration of 3 min. As such, 3 min was chosen as the optimal centrifugation duration to minimize sample preparation time. Once the centrifugation duration of 3 min was set, the effect of centrifugation speed (1500, 2500, and 3500 rpm) on 50 nm Ag NP concentration was further studied (Fig. S1c and d in Appendix). Increasing centrifugation speed was not found to significantly affect the Ag NP concentration or separation of the SPE materials in either matrix. As such, a centrifugation speed of 2500 rpm was chosen.

A closer investigation of Fig. S1 reveals some interesting differences in the recoveries of Ag NPs between samples and matrices treated with SPE materials. Significantly (up to 40%) lower Ag NPs recoveries are obtained for samples treated with SPE material 'SM DEAM' in lake water matrix (Fig. S1b and d). Similar behavior was not observed in UP water, indicating that the SPE material itself is not responsible for the loss of the Ag NPs. It seems SPE material 'SM DEAM' interacts with sample Ag NPs to some extent leading to NP losses, however, the process is matrix dependent. The effect of different SPE materials on Ag NPs in different matrices is discussed in more detail in Chapter 3.3.1.

Neither the centrifugation speed nor duration was found to significantly affect the recovery of Ag NPs in either of the matrices studied. This is somewhat in contrast with the findings of other authors, who reported a significant reduction of the NP concentration with increasing

centrifugation speed [59,69,71,72]. However, in these studies either significantly longer centrifugation times (up to 20 min) or different sample matrices were used, which could explain the differences in the findings. In addition, differences in the equipment used for centrifugation (e.g. the centrifuge itself and the size of centrifugation tubes used) can affect the results, making the direct comparison of the results challenging. As such, it seems advisable to study the effect of these variables on the sample properties in the early stages of the method development to minimize the risk of NP losses.

Compared with on-line systems, the optimized off-line procedure presented here reduces the required analysis time considerably, as laborious washing and regeneration steps needed between samples [31,39,61,62] are omitted. Up to 12 samples can be pre-treated with the proposed off-line procedure in less than 10 min (<1 min/sample), whereas 16–60 min (depending on the author [31,62]) was required for the regeneration of the resin between samples in on-line systems. In addition, the risk of carryover is minimized by washing the sample introduction system between every sample.

3.3. Extraction experiments of the SPE materials

The effect of the commercially available SPE materials used in the experiments (Table 2) on sample properties was evaluated. Considering spICP-MS analyses, the aim is to identify suitable SPE materials capable of efficient removal of dissolved silver, while maintaining the original Ag NP properties of the sample. As such, the SPE materials were evaluated based on two factors. Firstly, the effect of these materials on Ag NP properties (particle size or -concentration) and secondly, the extraction efficiency of dissolved silver.

3.3.1. Effect of SPE materials on Ag NPs properties

As the purpose of this study is to improve the accuracy of NP detection, maintaining the samples' original NP properties is of utmost importance. As such, the effect of the different SPE materials on Ag NP concentration and size was carefully investigated. To evaluate the possible effect of SPE materials on NP properties, the results obtained for samples treated with SPE materials were compared to those obtained for reference samples, which did not contain any SPE materials. The obtained results for the Ag NP concentration and size in various natural waters are presented in Fig. 2a and b, respectively, and in Table S5
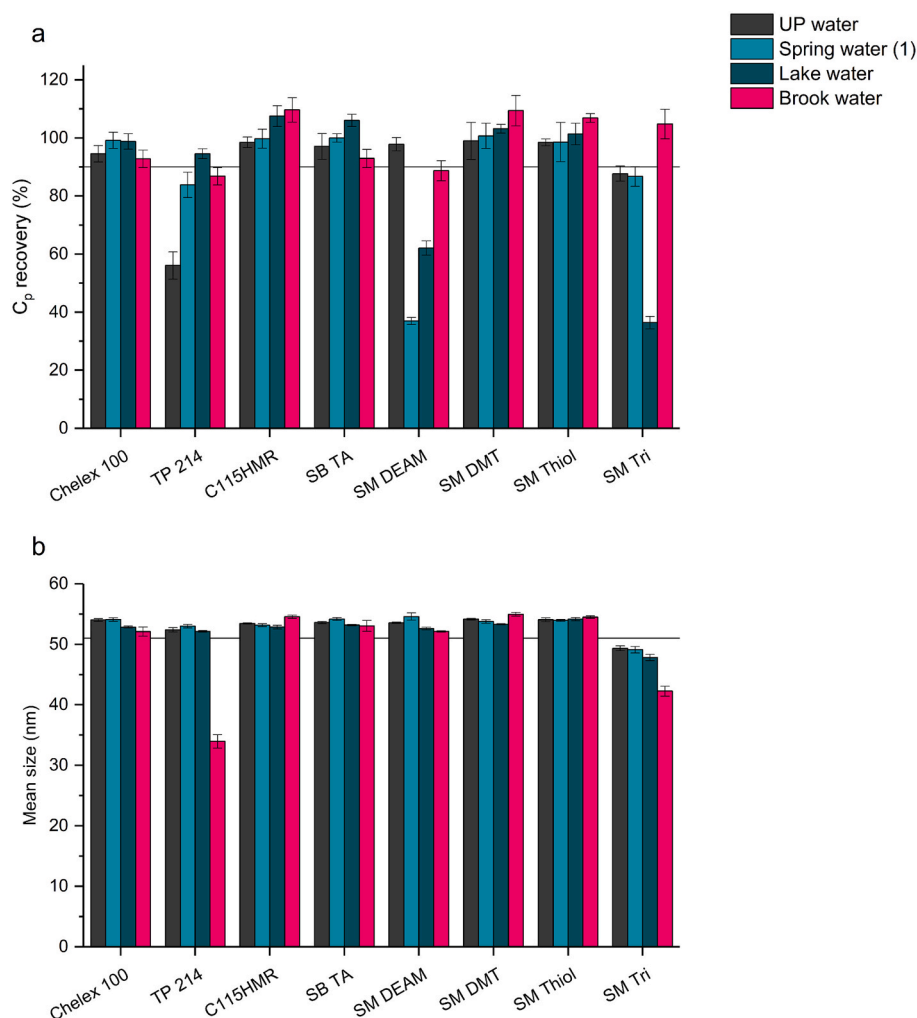
Fig. 2. The effect of SPE materials on 50 nm Ag NPs a) particle recovery (C_p recovery (%)) and b) mean size in different matrices. Results are given as mean $\pm$ 1 standard deviation of two replicate samples with 4 readings. The black reference lines given in Figs. 2 a and b represent 90% C_p recovery and the mean size of the 50 nm Ag NPs (51 nm as obtained with TEM), respectively.

(Appendix). The obtained size distribution histograms are presented in Figs. S2–S5 (Appendix).

Most of the SPE materials were not found to cause significant changes in Ag NP concentration or size (Fig. 2a and b). In addition, similar size distribution histograms were in general obtained for samples analyzed as such and after treatment with SPE materials (Figs. S2–S5 in Appendix). As such, most of the materials were not found to significantly interact with sample Ag NPs in any of the matrices studied, making them potential candidates for dissolved silver removal in NP dispersions. However, significant (up to 64%) loss of Ag NPs is observed in samples treated with SPE materials 'TP 214', 'SM DEAM' and 'SM Tri' (Fig. 2a). The effect of these materials, however, is found matrix-dependent; in general, losses are more severe in spring (1) and lake water (Tables S2 and S3 in Appendix). A significant reduction in particle size was also observed for samples containing SPE materials 'TP 214' and 'SM Tri' (Fig. 2b), indicating particle dissolution. As the observation was made for the latter in all the matrices studied, it can be expected, that the material itself is causing changes in particle size. The observed changes in particle size and concentration for the SPE materials 'TP 214', 'SM DEAM' and 'SM Tri' can also be clearly seen in the size distribution histograms (Figs. S2–S5 in Appendix).

As the observed changes in particle size or concentration are clear indicators of the material's interaction with the sample Ag NPs, materials 'TP 214', 'SM Tri', and 'SM DEAM' cannot be considered for

dissolved silver removal in NP dispersions. However, SPE materials 'Chelex 100', 'SB TA', 'C115HMR', 'SM Thiol' and 'SM DMT' were able to maintain the original sample Ag NP properties in all the matrices studied. As such, these materials can be considered as viable alternatives for dissolved silver removal and the extraction efficiency of these materials is evaluated in the following chapter.

3.3.2. Effect of SPE materials on dissolved silver

As presented in Fig. 1 and shown before [31], even low concentrations of dissolved silver can severely interfere with the detection of Ag NPs, leading to inaccurate characterization of these materials. Efficient removal of the dissolved silver is thus required for accurate sizing and counting of the NPs. In addition, the possible effect of the sample matrix should be considered, as it is expected to affect the extraction efficiency of SPE materials [55,59,61,73].

After identification of the SPE materials capable of maintaining the original sample NP properties (Chapter 3.3.1), their ability to extract dissolved silver was evaluated in various matrices (UP, spring (1 and 2), lake, river, and brook water, SWS and HA solution). The obtained results for colorless (with dissolved organic carbon (DOC) <5 mg L^{-1}) and dark-colored (DOC >5 mg L^{-1}) waters are presented in Fig. 3a and b, respectively and in Table S6 (Appendix).

As the obtained results in colorless and dark-colored waters are compared (Fig. 3a and b), a significant difference in the dissolved silver
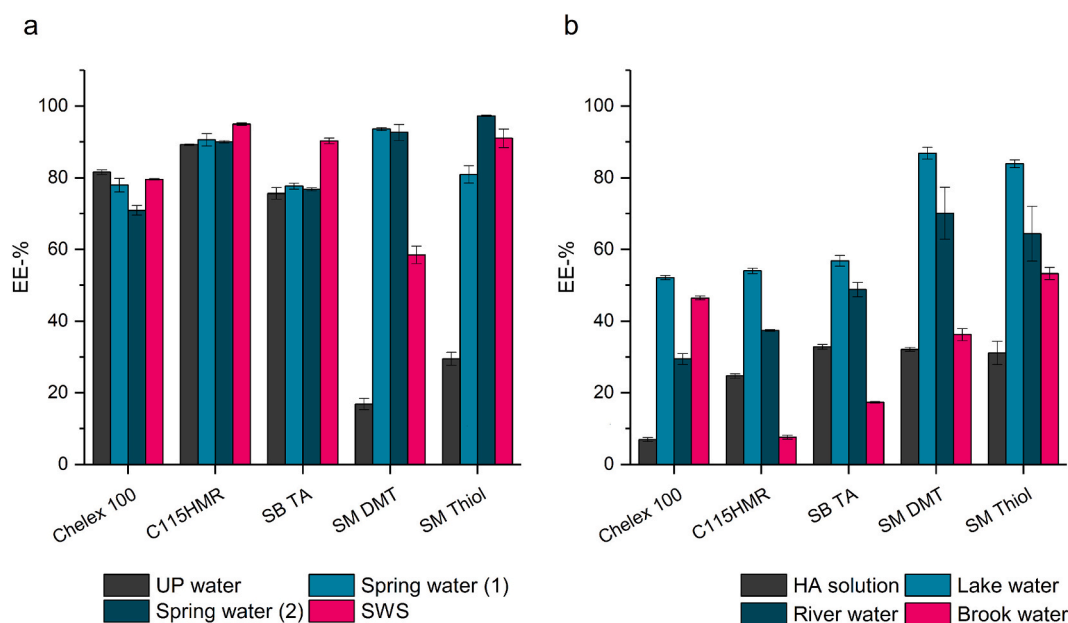
Fig. 3. The different SPE materials' dissolved silver extraction efficiency (EE-%) a) in colorless waters (DOC <5 mg L^{-1}) and b) in dark-colored waters (DOC >5 mg L^{-1}). Results are given as mean $\pm$ 1 standard deviation (1 s) of two replicate samples with 4 readings.

extraction efficiency is observed. In colorless waters, clearly higher extraction efficiencies (up to 97%) are obtained virtually unaffected by the SPE materials used. Cation exchange materials 'C115HMR', 'SB TA', and 'Chelex 100' with carboxylic or sulfonate groups proved most efficient in dissolved silver removal. The highest extraction efficiencies (89–95%) were obtained with a weak cation exchange resin 'C115HMR', followed by 'SB TA' and 'Chelex 100' with highly similar efficiencies (71–90%).

In dark-colored waters with higher amounts of humic substances (DOC >5 mg L^{-1}), however, the ability of the different SPE materials to remove dissolved silver is clearly decreased (Fig. 3b). The latter is true especially for the ion-exchange resins 'C115HMR', 'SB TA' and 'Chelex 100', which extraction efficiencies fell below 60% in dark-colored waters. A possible explanation for this observation is the presence of humic substances in these waters (Table S3, Appendix), which are known to affect the efficiency of SPE extraction [55,61]. As seen in Fig. 3b, poor dissolved extraction efficiencies ($<40\%$) were obtained in HA solution independent of the SPE material used, which might be due to competitive binding of silver to humic substances' active sites (e.g. sulphur and carboxyl groups) [74]. The effect of humic substances can be more clearly seen in Fig. S6, where the SPE materials' dissolved silver EE-% in different waters is presented with their corresponding DOC-values indicating the humic substances' content in these waters. As can be seen, the dissolved silver extraction is found most efficient in colorless waters with DOC-values <5 mg L^{-1}, but generally lower efficiencies are obtained in waters with DOC-values >5 mg L^{-1}.

Comparing the obtained results in colorless and dark-colored waters (Fig. 3a and b and S6), two SPE materials stand out. In contrast to the other SPE materials, clearly lower extraction efficiencies are obtained for thiol-containing materials 'SM DMT' and 'SM Thiol' in UP water as compared to environmental waters. This is somewhat unexpected since UP water contains no competing elements or compounds that could interfere with the extraction process. Considering that UP water lacks buffering capacity, changes in solution pH during the extraction process might affect the extraction efficiency. As compared with the reference sample, a significantly higher pH value was indeed measured for the sample treated with SPE material 'SM DMT' (9.9 vs. 5.4), which could explain the observed results. In addition, the extraction efficiency of these materials might depend on specific compounds or elements present. When the extraction efficiency of these materials is presented with

the increasing concentration of dissolved anions (Cl$^-$ and SO$_4^{2-}$) present (Fig. S7 in Appendix), some correlation can be observed. The presence of these anions may be favouring the dissolved silver extraction efficiency of SPE materials 'SM DMT' and 'SM Thiol', for instance by affecting the chemical state of silver.

As the purpose of this paper is to develop a sample pre-treatment procedure for dissolved silver removal in environmental waters, it is important to evaluate the possible interfering effect of matrix components on the extraction process. Since other elements present in environmental waters might decrease the extraction efficiency of dissolved silver by competing with the SPE materials' active sites, the effect of competing elements on the dissolved silver extraction efficiency was investigated in more detail. The results for extraction of dissolved silver and other competing elements are presented in the supplementary material (Tables S7–S9 for SWS, spring (2), and river water, respectively). Due to the conversion of SPE materials into their sodium (Na$^+$) form, higher concentrations of sodium were measured for samples treated with SPE materials as compared to the reference samples, and thus results for the extraction of sodium were omitted. As all the SPE materials were found to extract competing elements to varying extents, none of the SPE materials were found selective towards silver. As such, the application of SPE materials on improving NP detection by dissolved signal removal can potentially be extended for other elements as well, as has already been demonstrated for Zn and La$_2$O$_3$ [55,60,61].

Because of the high molecular loading capacity of SPE materials (Table S4, Appendix) and the low elemental concentrations commonly found in natural waters, the presence of competing elements was not found to affect the materials' dissolved silver extraction efficiency. As seen in Fig. 3a and b, similar extraction efficiencies were obtained in waters containing various competing elements as in UP water, indicating that the extraction of other elements does not negatively affect the extraction of silver in the concentration range studied. Interestingly, the presence of humic substances was not found to affect the SPE materials' extraction efficiency of the competing elements (Tables S7–S9 in Appendix), especially in the case of ion-exchange resins, as was noticed for silver. This confirms that the observed decrease in dissolved silver extraction efficiency in dark waters is due to humic substances' interaction with silver ions, thereby reducing the interaction with the SPE materials.

3.4. Selection of the SPE materials for dissolved silver extraction in NP dispersions

The suitability of the SPE materials on dissolved silver removal in Ag NP dispersions was evaluated based on their effects on NP properties and dissolved silver extraction efficiency. As maintaining the original sample Ag NP properties is of utmost importance for accurate NP characterization, any SPE materials found to cause changes in the original sample NP properties were excluded ('SM Tri', 'SM DEAM' and 'TP 214', see Chapter 3.3.1). The remaining five SPE materials were assessed based on their ability to extract dissolved silver in colorless (DOC <5 mg L^{-1}) and dark-colored waters (DOC >5 mg L^{-1}), for which the numerical results are given in Table S6 (in Appendix) and summarized in Table 3.

Based on the obtained results, SPE materials 'C115HMR', 'Chelex100' and 'SB TA' were found generally most suitable for dissolved silver removal. These materials were found to extract dissolved silver efficiently especially in colorless waters and importantly, were not found to interact with the Ag NPs in any of the matrices studied. However, in dark-colored waters, these materials' extraction efficiency decreased significantly probably because of the interfering effect of humic substances. In dark-colored waters with higher amounts of humic substances (DOC >5 mg L^{-1}), SPE materials 'SM Thiol' and 'SM DMT' were found more efficient and should be preferred. As such, care should be taken in choosing SPE materials for dissolved silver removal, as the sample matrix greatly affects the efficiency of the SPE materials.

Finally, SPE materials 'C115HMR' and 'SM Thiol' were chosen as the final materials for dissolved silver removal in colorless and dark-colored environmental waters, respectively. Even though 'C115HMR' was found more efficient in colorless waters, tests were performed also with 'Chelex 100' for comparative purposes due to its frequent use in previous studies [31,62].

3.5. Analysis of environmental samples

To demonstrate the effect of the chosen SPE materials on improving the accuracy of NP characterization in environmental waters, dispersions containing 30 nm Ag NPs and 0–1 µg kg^{-1} of dissolved silver were prepared in colorless waters with COD <5 mg L^{-1} (UP and spring (2) water), and dark-colored water with COD >5 mg L^{-1} (river water). Samples were then analyzed as such and after treatment with the chosen SPE materials as described in Chapter 2.7. The results are displayed in Fig. 4a–c.

As can be seen in Fig. 4a–c, when samples are analyzed as such without treatment with the SPE materials, the coexisting dissolved concentration causes a significant interference on the detection of the 30 nm Ag NPs in all matrices. With increasing dissolved silver concentration, more and more NPs are masked by the higher background signal, causing a significant increase in particle mean size (up to +58%, from 26 to 29 nm to 42–43 nm) and a decrease in particle concentration (up to −90%). By pre-treating the samples with SPE materials before spICP-MS-measurements, however, a significant portion of the dissolved silver can be removed, allowing highly more accurate characterization

of the 30 nm Ag NPs over the entire concentration range studied. It should be noted, that significantly lower (up to 40%) particle concentration is obtained in UP water for samples analyzed as such (i.e., for the reference sample) as compared to the values obtained for samples treated with SPE materials, even in the presence of low background signal (no dissolved silver added, Fig. 4a). The adsorption of Ag NPs onto solid surfaces has been previously reported [75] and shown to depend both on the surface of the Ag NPs and the sample container materials. As such, it seems possible, that the lower recoveries obtained in UP water for the reference sample might result from the interaction of Ag NPs with e.g. sample introduction system. The addition of SPE materials might reduce the adsorption of Ag NPs onto solid surfaces by e.g. affecting the physicochemical properties of the sample matrix or particle surface, thereby explaining the observed results.

In clear waters (UP- and spring water (2) with DOC <5 mg L^{-1}, Fig. 4a and b), the dissolved silver interference can be minimized by treating the samples with ion-exchange materials 'Chelex 100' or 'C115HMR'. The efficient extraction of dissolved silver (up to 95%) improves the detection of the 30 nm Ag NPs, allowing more accurate sizing and particle concentration determination over the entire concentration range studied. Even though 'Chelex 100' is more often used in previous publications [31,62], 'C115HMR' proved more efficient in dissolved silver removal, as noticed already in Chapter 3.3.2. As a result, highly accurate values are obtained for the particle mean size (28 ± 2 nm) even in samples containing relatively high amounts (up to 1 µg kg^{-1}) of dissolved silver, whereas a slight increase is observed for samples treated with 'Chelex 100' (from 29/27 nm to 32/33 nm). At the same time, relatively stable particle concentration values are also obtained for samples treated with 'C115HMR' over the entire concentration range studied.

In river water with higher amounts of humic substances (DOC 11 mg L^{-1}), the detection of the 30 nm NPs can be significantly improved by pre-treating the samples with SPE material 'SM Thiol' (Fig. 4c). As a result, accurate readings are obtained for particle mean size (28 ± 2 nm) and particle concentration ($<21\%$ different) over the entire concentration range studied. For the ion-exchange materials 'Chelex 100' and 'C115HMR', the extraction efficiency is significantly decreased in the river water matrix rich with humic substances, resulting in only a slight improvement in NP detection. This demonstrates the better suitability of 'SM Thiol' for dissolved silver removal in dark-colored waters as compared to 'Chelex 100' and 'C115HMR', which were found more efficient in colorless waters.

4. Conclusions

Accurate characterization of Ag NPs in environmental samples can be severely interfered by high background (dissolved) signal, leading to inaccurate characterization of these materials. As the smallest NPs are most susceptible to the interfering effect of dissolved silver, for accurate characterization of ≤ 30 nm NPs, dissolved silver concentration should be decreased at least to a concentration below 0.1 µg L^{-1}. In this paper, a simple sample pre-treatment procedure for more accurate NP characterization with spICP-MS is presented using solid phase extraction (SPE) materials for dissolved silver removal in environmental waters. The developed off-line procedure maximizes sample throughput in spICP-MS with 12 samples treated in less than 10 min, as lengthy (up to 60 min) washing and regeneration steps necessary on commonly employed on-line systems are eliminated. The effect of centrifugation conditions (speed and duration) used for the separation of the solid SPE materials on the NP properties were investigated to minimize NP losses. Using the optimal conditions (2500 rpm for 3 min), the applicability of eight commercially available SPE materials was thoroughly evaluated based on their dissolved silver extraction efficiency and ability to maintain original sample Ag NP properties in various water matrices. Though many of the SPE materials were not found to interact with the sample Ag NPs, SPE materials 'SiliaMets (SM) Triamine', 'SM DEAM' and 'Lewatit
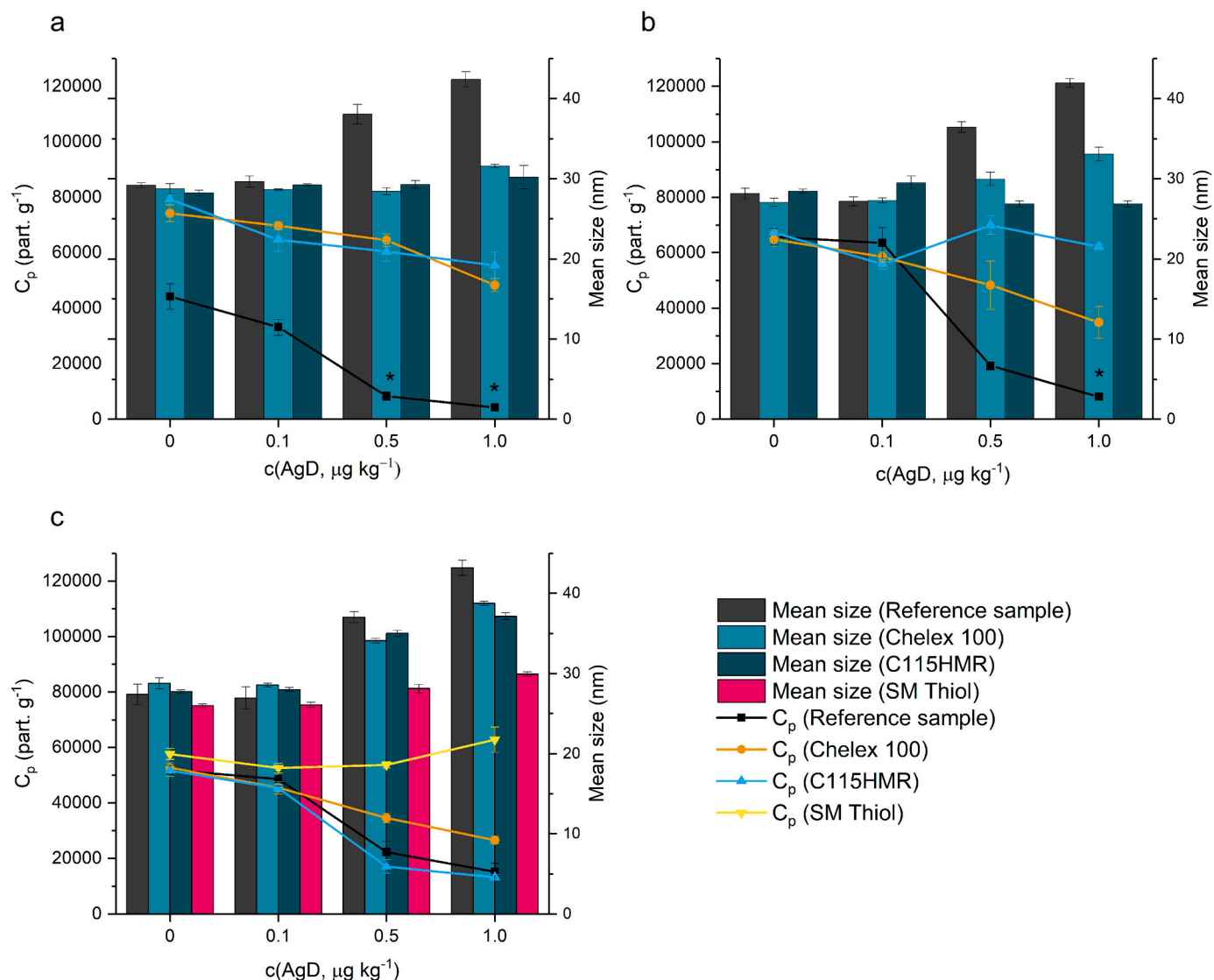
Table 3
Effect of the selected SPE materials on sample properties.

SPE Material	C_p recovery (%)[1]	EE-%(AgD), colorless waters[2]	EE-%(AgD), dark-colored waters[2]
Chelex 100	Excellent	Good	Poor
C115HMR	Excellent	Excellent	Poor
SB TA	Excellent	Good	Poor
SM DMT	Excellent	Moderate	Moderate
SM Thiol	Excellent	Moderate	Moderate

[1] The effect of SPE materials on C_p recovery was estimated as follows: $\geq 90\%$ in all matrices: Excellent, $<90\%$ in any matrix: Poor.

[2] Dissolved silver extraction efficiency (EE-%(AgD)) estimated as follows: $>85\%$ Excellent, 75–85% Good, 55–75% Moderate and $<55\%$ Poor.

Fig. 4. The obtained results for 30 nm Ag NP dispersions spiked with increasing concentrations (0–1 µg kg^{-1}) of dissolved silver (AgD) in a) UP water, b) spring water (2) and c) river water. All samples were analyzed as such (i.e., reference sample) and after treatment with the chosen SPE materials ('Chelex 100'; 'C115HMR' and 'SM Thiol') in triplicate. The obtained results for particle concentration (C$_p$, given in part. g^{-1}) are represented with lines and mean size with bars. The error bars represent one standard deviation (1 s) of three replicate samples ($n = 3$). The data points for 30 nm Ag NP at AgD concentrations of 0.5 and 1.0 µg kg^{-1} are highlighted with an asterisk, as an insufficient number of NPs (<100) were captured for statistically significant characterization.

MonoPlus TP 214' were found to cause Ag NP dissolution or losses and as such were found unsuitable for dissolved silver removal in NP dispersions. The remaining SPE materials capable of maintaining original NP properties ('Chelex 100', 'Purolite C115HMR' ('C115HMR'), 'Silia-Bond Tosic acid' ('SB TA'), 'SM Thiol' and 'SM DMT') were assessed based on their dissolved silver extraction efficiency in several water matrices. In addition, the effect of various competing elements commonly found in natural waters (Al, Ca, Na, Fe, K, Mg, S, NO$_3^-$ and Cl$^-$) and humic substances on the extraction efficiency were evaluated. Even though competing elements were not found to affect the dissolved silver extraction efficiency due to the high molecular capacity of the SPE materials used, the presence of humic substances was shown to significantly decrease the dissolved silver extraction efficiency, especially with ion-exchange materials 'Chelex 100', 'C115HMR' and 'SB TA'. This is probably due to the competitive binding of silver through humic substances' active sites (e.g. sulphur and carboxyl groups).

Three SPE materials found most efficient in dissolved silver extraction ('C115HMR', 'Chelex 100' and 'SM Thiol') were chosen and their effect on improving the accuracy of Ag NP characterization was demonstrated in ultrapure, spring, and river water with different physicochemical properties. Whereas measurement of the samples with coexisting dissolved silver (up to 1 µg kg^{-1}) as such without treatment with SPE materials resulted in significant bias in 30 nm Ag NP sizing (up to +58% increase) and counting (up to −90% decrease), pre-treatment of the samples with SPE materials 'C115HMR' and 'SM Thiol' minimized the effect of dissolved silver interference in colorless and dark-colored waters, respectively. As a result, highly more accurate readings were obtained for NP size (28 ± 2 nm) with relatively stable particle concentration values (<21% different).

CRediT authorship contribution statement

Virva Kinnunen: Conceptualization, Validation, Formal analysis, Investigation, Visualization, Writing – original draft. **Siiri Perämäki:** Supervision, Writing – original draft. **Rose Matilainen:** Project administration, Supervision, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sab.2022.106431.

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