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Standardization of microwave-assisted extraction procedures for characterizing non-labile metallic nanoparticles in environmental solid samples by means of single particle ICP-MS†

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Microwave-assisted extraction (MAE) treatments seem to be a promising sample preparation strategy to isolate nanomaterials (NMs) from environmental samples prior to single particle inductively coupled plasma mass (spICP-MS) determination. Nevertheless, because previous studies make use of domestic microwave ovens (DMOs), several shortcomings arise that compromise the analysis such as lack of control of experimental variables, limited operating conditions, and uncontrolled sample heating leading to irreproducibility issues. The goal of this work is to adapt previously developed MAE treatments for soil and air filters with the DMO apparatus to state-of-the-art scientific microwave ovens (SMOs) in order to standardize current analytical protocols for NM characterization. Results show that, by working with an SMO, non-labile metallic NMs (Pt-AuNPs) are quantitively extracted from soil and air filter samples unaltered in 10 min and 6 min, respectively, at 1200 W by using 10 mL of NaOH 0.1 M solution. The use of a SMO system allows improving accuracy (above 10% of the particle recovery), precision (above 5% of the RSD) and sample throughput (above 4-fold) when compared to the DMO ones. According to these findings, MAE seems to be a powerful strategy for routine analysis of non-labile NMs in environmental samples.

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1. Introduction

Single Particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS) has emerged as a leading technique for the analysis of nanomaterials in complex samples.[1–4] Though NMs could be directly determined in solid samples using non-conventional sample introduction systems (*e.g.*, laser ablation),[5–7] an extraction treatment is usually performed to isolate them[8–10] and reduce potential spectral and non-spectral interferences affecting NM determination.[11] To this end, several strategies have been successfully employed for NM isolation such as: (i) liquid extraction at ambient temperature;[12] (ii) ultrasound assisted extraction procedures;[13–16] and (iii) cloud point extraction.[17,18] However, they are not easy to apply for routine analysis due to the lack of robustness and accuracy as well as limited sample throughput.

Our research group has recently demonstrated the benefits of using microwave-assisted extraction (MAE) treatment for NM characterization in environmental samples. For instance, Au- and PtNPs are quantitatively recovered from soil samples by using 20 mL of a 0.1 M NaOH solution in an 800 W domestic microwave oven (DMO) for 6 min.[19] Similar findings were observed for air filters after a 4 minute treatment in the same MW oven using 40 mL of a 2.0% w/w NH_4OH solution.[20] These studies have shown that MAE offers significant advantages over other sample extraction strategies in terms of accuracy and sample throughput. Nevertheless, because NM extraction is performed using a domestic MW oven (DMO), some practical issues arise such as: (i) lack of control of experimental variables affecting sample heating (temperature, pressure, incident and reflected microwave power, *etc.*); (ii) a limited window of operating working conditions since extraction is performed at atmospheric pressure and, hence, temperature is limited to extraction solvent boiling point; (iii) uncontrolled sample heating can cause irreproducibility issues by negatively affecting extraction accuracy and precision due to solution splashes. Such issues can only be mitigated by using less efficient sample heating conditions, leading to limited sample throughput.[13,15,16] Though the number of samples that can be treated with a DMO (2 samples, three replicates each) are

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significantly higher than those afforded by other strategies (*e.g.*, ultrasound or cloud point extraction), it is still limited for routine analysis. *A priori*, all these shortcomings could be addressed by operating commercial scientific microwave oven (SMO) systems. To the best of the authors' knowledge, such apparatus has only been applied for sample digestion of organic food matrices to isolate metal oxide particles[21] but not for NM extraction. Therefore, the goal of this work is to evaluate the use of SMOs for extracting NMs from environmental samples (*i.e.*, soils and air filters) thus making feasible the development of standardized procedures for improving spICP-MS accuracy and precision as well as NM analytical metrology.

2. Materials and methods

2.1. Reagents

The solutions were prepared using ultrapure water from a Mili-Q-purification system (Millipore Inc, France). For preparing extraction media, sodium hydroxide was used (Sigma-Aldrich, Steiheim, Germany).

This work employed suspensions of different metallic NMs, namely: citrate-stabilized platinum nanoparticles (PtNPs) with a nominal diameter of 70 nm and a nominal concentration of 1.2×10^{10} mL^{-1} (NanoComposix, San Diego, USA); as well as citrate-stabilized gold nanoparticles (AuNPs) with nominal diameters of 50 and 150 nm and concentrations of 3.5×10^{10} and 3.6×10^{9} mL^{-1}, respectively (Cytodiagnostics, Burlington, Canada).

Finally, Pt and Au mono-elemental 1000 mg L^{-1} standards (Sigma-Aldrich, Steiheim, Germany) were used to prepare calibration curves with known mass concentrations for spICP-MS size measurements.

2.2. Samples

In this work, soil samples covering different matrix characteristics were employed, namely: (i) alkaline; (ii) acid; (iii) sandy; (iv) clayey; (v) ERM483 (JRC, Belgium); (vi) ERMCC141(JRC); and (vii) SL36 (IELAB, Alicante, Spain). A detailed description of soil physicochemical characteristics and sample preparation is given elsewhere.[19] Munktell Microquartz-fiber filters (50 mm diameter, 0.3 mm nominal pore size), from Thermo-Fisher Scientific (Waltham, USA) were employed to evaluate MAE of NMs from air filters.[20]

2.3. MAE extraction methodology

Nanomaterial extraction from soils and air filters was carried out using a high-pressure microwave assisted digestion system UltraClave, Milestone s.r.l. (Sorisole, Italy) which allows working with up to 40 samples simultaneously in PTFE vessels of 15 mL. In this work, the following variables were investigated: (i) MW power (800–1200 W); (ii) extractant concentration (0.01–1 M NaOH) and time (1–15 min). The microwave program was set up at an initial pressure of 30 bars for the extractions tested. To study the effect of the MW time on the NP extraction, MW power and target time were fixed at their maximum values (1200 W and 260 °C).

The extractant volume was fixed at 10 mL (maximum allowed volume per vessel) to soak air filters properly and facilitate supernatant–sample separation. For the same reason, when working with soil samples, the sample mass was also fixed at 0.2 g.

Extraction experiments were carried out following the procedure of previous studies; soil and air filter samples were spiked with a 0.2 g of 4×10^{6} mL^{-1} suspension of PtNPs and were allowed to stand overnight.[19,20] Once extraction methodologies were developed, the same procedure was replicated with AuNPs of different sizes.

For both soils and air filters, MAE operating conditions were optimized by means of Design of Experiments (DoE) using a Central Composite + Star model (Statgraphics® Centurion 16.1.11 32 bit software, Statpoint Technologies, USA). According to the general requirements for the competence of testing and calibration laboratories – ISO/IEC 17025:2017 standard, NM recoveries were considered quantitative within the range 80–120%.[22] After the MAE treatment, soil samples were left to settle, and the supernatant was separated from soil samples without any further treatment. In the case of air filters, a centrifugation step was required to remove microquartz fibers to avoid nebulizer blockage. Irrespective of the sample considered, extracts after the MAE treatment were diluted to 40 mL final volume to reach a concentration of 2×10^{4} particles per mL.

2.4 Instrumentation

A triple-quadrupole based 8900 ICP-MS from Agilent Technologies (Santa Clara, USA) was used throughout this work. Table 1 summarizes operating conditions. Calibration was carried out using the frequency method with a Pt suspension of known concentration 2×10^{4} mL^{-1}. This strategy was preferred over other approaches since it allows accurate transport efficiency assessment if robust plasma conditions are operated.[23] Data acquisition and treatment were conducted *via* instrument software (MassHunter version 4.5).

Table 1 ICP-MS operating conditions

	Single particle mode
Plasma forward power [W]	1550
Sampling depth [mm]	8
Argon flow rate [L min^{-1}]	
Plasma	15
Auxiliary	0.9
Nebulizer [Q_g]	1.00
Torch i.d. [mm]	1.0
Sample introduction system	
Nebulizer	MicroMist® nebulizer
Spray chamber	Scott double pass
Sample uptake rate [Q_l] [μL min^{-1}]	300
Dwell time [ms]	0.1
Measuring time [s]	60
Nuclides	^{197}Pt; ^{195}Au

3. Results and discussion

Microwave-based digestion and extraction procedures with SMO comprise different temperature-controlled heating steps. Normally, samples are initially heated following a temperature ramp for 5–10 min until a target temperature is reached. Next, the temperature is maintained for a given period and samples are left to cool down.[24–26] Additional steps might be added depending on sample characteristics. With this in view, an extraction procedure mimicking the experimental conditions previously employed with a DMO (*i.e.*, 20 mL of NaOH 0.1 M, 6 min, 800 W) was tested.[19] To this end, 0.2 g of PtNP spiked soils (0.2 mL of a 4×10^6 mL^{-1} suspension) was mixed with 10 mL of NaOH 0.1 M and poured into SMO quartz vessels (40 samples in total). Next, the sample–extractant mixture was heated using the following program: (i) target temperature: 100 °C; (ii) ramp time: 2 min; (iii) hold time: 4 min; (iv) MW power: 800 W. Irrespective of the soil considered (Appendix Table S1†), the mean diameter size of the NPs recovered was equivalent to the reference value obtained by means of TEM (69 ± 2 nm) thus confirming that this approach preserves PtNP size. However, PtNP recoveries were not quantitative (<25%) and they were significantly lower than those previously obtained with a DMO by a factor of 4. Similar findings were noticed when working with air filters. These results were totally unexpected considering SMO characteristics, but it should be considered that the total sample mass introduced into this system (40 samples × 10 mL; 400 mL) is higher than that introduced in the DMO (6 samples × 20 mL; 120 mL) and, hence, there is less energy per sample mass unit. On the other hand, when operating the above-mentioned program, the magnetron does not heat samples continuously but using on–off cycles to pursue the predefined temperature program. This means that samples are heated less efficiently with regard to the DMO which heats continuously using the highest power available. Because extraction conditions are not directly transferable between both types of devices, they should be specifically optimized for working with SMOs.

Table 2 Optimal working conditions for NM MAE from soil and air filter samples using an SMO

Extraction parameters	Soil	Air filters
MW power [W]	1200	
NaOH volume [mL]	10	
NaOH concentration [M]	0.1	
MW time [min]	10	6

3.1. Optimization of the extraction procedure for soils

Microwave-assisted extraction conditions for soils were optimized using DoE since it provides valuable information about the interactions of the different parameters potentially affecting NM recovery. As previously mentioned, the standard working procedure of SMO is not suitable for NM extraction since the sample is not continuously heated. Therefore, some modifications were implemented to enhance heating efficiency. First, the maximum power available was selected (1200 W) to increase energy per sample mass unit. On the other hand, the target temperature was prefixed at 260 °C (maximum target temperature available) in such a way that the magnetron is continuously heating the sample without any on/off cycle. This means that the MAE procedure comprises just a single step and, once it is finished, the samples are left to cool down for spICPMS analysis. Though the use of lower sample volumes than 10 mL could be advantageous for improving heating efficiency, the sample volume was kept at 10 mL since it was easier to separate the supernatant from soil particulate. Consequently, only NaOH concentration and extraction time were optimized using a central composite design (CCD). Both variables were investigated in five levels (NaOH concentration: 0, 0.01, 0.1 and 0.5 M; time: 1, 2.5, 7.5, 15 and 20 min) for a total of 9 experiments

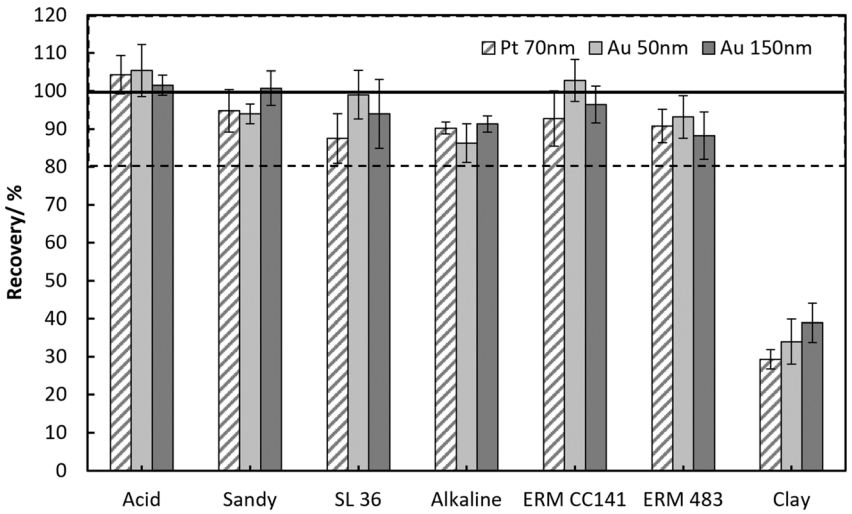

Fig. 1 Particle number recovery for the extractions of Pt and AuNPs in the soil samples using the SMO under optimum conditions. Error bars: standard deviation, $n = 5$. Dashed lines limit the quantitative recovery interval.

Table 3 Average NP diameter size obtained in soil samples using optimum extraction conditions with an SMO system. Results are expressed as the mean value ± s, $n = 5$

	PtNPs	AuNPs	
Reference	69 ± 2	52 ± 6	155 ± 12
Acid	69.4 ± 0.3	49.2 ± 0.5	146.1 ± 0.8
Sandy	68.5 ± 0.5	48.7 ± 0.3	145.4 ± 0.7
SL36	69.1 ± 0.5	49.3 ± 0.6	148.7 ± 1.0
Alkaline	68.2 ± 0.8	48.0 ± 0.4	146.2 ± 1.1
ERM CC141	68.5 ± 0.6	48.7 ± 0.4	145.1 ± 1.2
ERM 483	68.7 ± 0.5	48.3 ± 0.3	145.6 ± 0.9
Clayey	69.3 ± 0.4	48.4 ± 0.6	145.1 ± 0.9

(three replicates each) using the sandy soil sample (Appendix Table S2†). The ANOVA data analysis revealed that PtNP extraction was significantly dependent on time and the interaction between time and concentration (Appendix Fig. S1†). According to the CCD model, optimum experimental conditions for PtNP extraction from sandy soil were NaOH 0.1 M and 10 min (Table 2). Under these conditions, Pt and AuNPs from the different soils were re-evaluated obtaining quantitative recoveries for all of them except from the clay soil which shows recoveries below 30% (Fig. 1). The poor recoveries for the latter matrix were expected considering that NMs are efficiently retained by the soil clay fraction (*i.e.*, small pore size and electric attraction).[27–29] Irrespective of soil characteristics, the particle diameter size obtained (Table 3) was equivalent to that afforded by TEM thus confirming strategy robustness for non-labile NM extraction. Labile nanoparticles such as Ag and SeNPs were also tested using this approach but as expected from our previous work (Appendix Table S3†), recoveries were not quantitative at all since these species are prone to dissolution.[19] Consequently, MAE does not seem to be a suitable technique for characterizing this type of NMs.

Table 4 Particle number recovery and particle diameter size in air filters using optimum extraction conditions with an SMO system. Results are expressed as the mean value ± s, $n = 5$

	PtNPs	AuNPs	
Recovery [%]	94 ± 3	96 ± 4	96 ± 7
Reference size [nm]	69 ± 2	52 ± 6	155 ± 12
NP size [nm]	67.5 ± 1.2	48.4 ± 0.4	145.2 ± 0.7

3.2. Optimization of the extraction procedure for air filters

As it has been previously done with soil samples, SMO operating conditions were specifically optimized for extracting PtNPs from microquartz filter samples. Unlike our previous work, NH_4OH was replaced by NaOH for NM extraction to evaluate whether this extractant is potentially useful for multipurpose applications. Method optimization was carried out similar to that of soil samples but both NaOH concentration levels (0, 0.01, 0.05, 0.1 and 0.5 M) and MW time (1, 2, 3 and 4 min) were reduced considering previous findings with the DMO apparatus (Appendix Table S4†).[20] In this case, ANOVA data analysis revealed that PtNP extraction was significantly dependent on time, NaOH concentration and the interaction between both variables (Appendix Fig. S2†). According to the CCD model, NaOH 0.1 M and 6 min of MW time were the optimum conditions for PtNP extraction (Table 2). Interestingly, NaOH concentration is equivalent to that previously obtained for soils, but the length treatment is 4 min shorter, probably because air filters do not contain a complex matrix as soil samples. It is important to note that air filters were partially decomposed after the MW extraction treatment and microquartz fibers were present in the NM suspension. Therefore, in this particular case, a pseudo MW-based digestion was carried out instead of a pure extraction treatment as the air filters were mostly decomposed. When working with soil, however, sample preparation is closer to an extraction treatment given that soil (inorganic) particulate in the extracts after MAE is significant. Because microquartz fibers might cause irreversible nebulizer blockage, they should be eliminated prior to spICP-MS. To this end, aliquots of 10 mL of each sample were centrifuged for 10 min at 500 g to remove the filter fibres. No differences in particle number concentration and particle size distribution were noticed for the NM standards under these centrifugation conditions (Appendix Table S5†). When working with this approach, PtNP recoveries were again quantitative without any significant change in particle size. Similar findings were obtained on working with AuNPs and, at least, this strategy could be applied to NMs with a size up to 150 nm (Table 4).

3.3. Comparison of the MW apparatus for NM extraction

Results afforded by the SMO were compared with those previously obtained with a DMO (Table 5).[19,20] As expected, the former system is more advantageous for sample preparation than its domestic counterpart since it affords equal or higher NM recoveries, regardless of the sample considered. Recoveries

Table 5 Comparison of NM extraction performance between DMO and SMO systems

	DMO			SMO		
	Soil samples	Clay soil	Air filters	Soil samples	Clay soil	Air filters
Recovery [%]	71–96	9–11	96–107	86–105	29–39	94–96
RSD [%]	5–15	3–6	6–10	2–10	2–5	5–7
Extraction time [min]	6	6	4	10	10	6
Samples [run]	6	6	6	40	40	40

obtained in matrices homogeneously composed (air filter samples) were quantitative in equivalence with those obtained using the DMO (Appendix Fig. S3†). In the case of the samples with a complex matrix (soils), results show an enhancement of more than 10%, thus making all the recoveries within the quantitative range except from the clay soil (Appendix Fig. S4†). This enhancement of the recoveries can be explained because the SMO is a more energy-efficient process (*i.e.*, better heating and less sample losses). Second, precision was improved, since sample heating was enhanced, and sample losses were mitigated. This effect was more noticeable for soil samples than for air filters. Finally, although extraction time was increased (*i.e.*, from 4 to 6 min for air filters and from 6 to 10 min for soils), the number of samples extracted at the same time using an SMO was significantly higher (from 6 to 40 samples). In consequence, the sample throughput was increased 4-fold with regard to the DMO.

4. Conclusions

Results in this work confirm that MAE is a powerful sample preparation strategy to isolate non-labile metallic NMs from solid samples for their subsequent analysis by means of spICP-MS. When compared to DMOs, the use of SMOs offers significant advantages in terms of accuracy, precision, and sample throughput. In this regard, NaOH seems to be, apparently, a promising extraction agent for successfully isolating non-labile NMs from a wide range of samples. *A priori*, it is expected that this approach could also be applied to metal oxide and coated metallic NMs provided that both extractant and extraction conditions are properly selected. However, further research efforts are still required to apply MAE for the analysis of labile NMs (*e.g.* SeNPs and AgNPs). As it is possible to use commercial instrumentation to extract non-labile metallic NMs, *a priori*, standardization of analytical protocols to improve current spICP-MS metrology is feasible.

Conflicts of 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.

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