Study of Nanoparticles in a Few Rivers in North East Italy using SP-ICP-MS

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Abstract

The use of nanoparticles (NP) has increased in these years. They are used in many fields, for example industrial and commercial sectors. The large employment may compromise the human and the ecosystem health, because the fate/damages/consequences of NP in the environment are still not known. SP-ICP-MS is a fast method and it can be used for a screening of environmental samples, it combines high sensitivity with fast analytical speed. This is a water surface study, few rivers in the North East of Italy are analysed. 3 litres of surface water are filtered with a vacuum pump with membrane by 0.22 μm pore. The filter is put into a test tube with 30 mL of ultrapure water and then sonicated for 20 minutes. The analyses are performed on a SP-ICP-MS NexION 350D Perkin Elmer using Syngistix Software. Transport efficiency and particle calibration are performed with standard gold nanoparticles of 30 nm, 60 nm and 90 nm. The detection limit (DL) is calculated from the analysis of 5 replicates of ultrapure water. The recovery test is made on water surface both spiked and unspiked and it appears to be between 61,8% and 97%. Considering the high dilution of NP in an environmental complex matrix, this study evaluates the efficiency of the concentration via filtration for SP-ICP-MS analysis of nanoparticles of ZnO, CeO_2, TiO_2, Ag. The results show that the analysis in SP-ICP-MS combined with the filtration enhance the detection sensitivity and the characterization of nanoparticles.

Keywords: SP-ICP-MS; Nanoparticles; Environmental pollution; River; Filtration

Introduction

This preliminary study puts the attention on propagation of nanoparticles (NP) into few rivers in Friuli Venezia Giulia (FVG)-Italy. The use of NP has been increasing in recent years. The scientific and engineering communities are driven heavily by government investments on nanotechnology applications. NP are used in industrial and commercial sectors (e.g. foods, pesticides, personal care products, pharmaceutical, and medicine). The large use may compromise the humans and the ecosystem health (EFSA 2016–2017) [1]. In order to understand the fate of NP in the environment, it is necessary to study all the characteristics that permit their correct identification. Six characteristics contribute to determine nanomaterials: size, shape, surface charge, surface porosity, composition and structure [2].

As suggested in EPA APM 32 [3], detecting, quantifying and characterizing the NP in environmental samples with traditional technique is difficult because they are present in a very low concentration and there are a lot interference coming from background natural colloid. The most used techniques to determine the NP and to achieve a reliable and quantitative determination of NP were hydrodynamic chromatography (HDC) and field flow fractionation (FFF) in combination with detectors such as multiple angle light scattering (MALS) and inductive coupled mass (ICP-MS). These methods require a complex preparation of the samples with the aim of removing interfering matrix and meet a low detection limit. To determine the real particle size number distribution, several methods are available: nanoparticle tracking analysis (NTA), differential mobility analyzers (DMA) and single particle inductive coupled mass spectrometry (SP-ICP-MS).

The use of FFF-ICP-MS permits to determine the size of NP. This method has more sensitivity than ICP-MS however the NP interactions with the porous membrane wall of the separation system can lead to peak tailing and even irreversible adsorption. In addition, the time of migration of NP is very high and it cannot permit to use this as routine method. Using an analytical approach as HDC permits to lower the resolution than FFF-ICP-MS, but the separation of the NP is more rapid. HDC-ICP-MS provide elemental information to size distribution, therefore the application for natural environmental media is limited. Imagines techniques as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are used for the characterizing of shape, structure, particle size and particle size distribution, agglomeration of NP; in this case the quantification is precluded.

SP-ICP-MS is by far the common tool used for NP analysis because of its trace element capability and extremely low detection limits is ideally suited to characterization of NP. The information obtained using this technique are concentration, particle size and particle size distribution, agglomeration and

composition. The shape of NP is assumed spherical. Traditional ICP-MS detects metal ions based on their mass to charge ratio. The analysis in single particles requires a different approach that measures dissolved elements with ICP-MS. Two parameters are very important: the dwell time (reading time) and the settling time (overhead and processing time). In a traditional ICP-MS multiple intensity are integrated with a long dwell time (0.03-0.1 s) to produce an overall metal concentration for the sample. In SP-ICP-MS each intensity reading is combined with a shorter dwell time (100 μs or less) and plotted individually. The principal assumption behind SP-ICP-MS is that each pulse represents a single particle event which depends on short dwell time, constant flow rate and a sufficient low particle concentration.

The better situation for analysis of nanoparticles is when the settling time is zero because every nanoparticle is detected. This parameter also permits a fast data acquisition with two benefits: to ensure an accurate particle counting and to eliminate the changes that particles miss or that only partial ion clouds from particles detect, as suggested in Chady [4]. Another important parameter in SP-ICP-MS is the transport efficiency. It is defined as the ratio of the amount of analyte entering the plasma and the amount of analyte aspirate. During each new analytical session, the transport efficiency must be determined for a good result [5].

This study puts the attention on the presence of NP in a few rivers in FVG and it describes the suitability of SP-ICP-MS for routine analysis, including adequate and simple sample preparation methods. This method researches nanoparticle of TiO_2, CeO_2, ZnO and Ag. To determine nanoparticles in the environment a very sensitive analytical technique is needed because the concentration of nanoparticles is very low in surface waters. Ten rivers surface water samples, downstream of productive settlements, are collected and analysed.

Methods

Reagents, standards and materials

Ionic Au calibration standards 1.25, 2.5, 5, 10 μg/L are used as ionic calibrations and made from 1000 mg/L. It is purchased from Ultra Scientific. Three sizes of Au (30-60-90 nm) with nanoparts carboxylic acid, purchased from Perkin Elmer, are used for the particles calibration and the transport efficiency determination. Nanopowder of TiO_2<100 nm particle size (BET), CeO_2<50 nm particle size (BET) ZnO <50 nm particle size (BET) and Ag dispersion 40 nm particle size 0.02 mg/mL in aqueous buffer contains sodium citrate as stabilizer.

All the nanoparticles standards are purchased from Sigma-Aldrich. Samples are filtered with a decontaminated vacuum pump with cellulose acetate membrane filters with a 0.22 μm pore size purchased from Millipore. HNO_3 suprapure (Panreac), H_2SO_4 hiperpure (Panreac) and H_2O_2 suprapure 30% (Merck) are needed for the digestion of the samples.

Instruments

Single Particle Inductively Coupled Plasma-mass Spectrometry (SP-ICP-MS) Perkin Elmer NexION 350D with Syngistix Nano Application Software ModuleVers.1.0 is used for this study. For the analysis of the NP dissolved concentration, Milestone Ultrawave and DigiPrep digestors (both with dedicated and decontaminated vessels) are used for the mineralization of the samples; to avoid the aggregation of the NP before the analysis, it's necessary the use of a sonicator.

Experimental protocol

Ten rivers (downstream of productive settlements) of different nature in FVG have been chosen for this study. Each sample is put into a polypropylene bottle and stored in the dark in a fridge at 4°C [6]. The samples of three rivers of different typologies have been selected: one river affected by industrial activities (A), one river exposed to an urban pollution (B) and one river in estuarine context (C). For the evaluation of the total metals content, river water is treated with two different mineralization methods.

To determine Ag and Zn total, the digestion is made according to UNI EN ISO 15587-2 [7], the mineralization is performed with an addition of 6.25 mL of nitric acid suprapure to 25 mL of sample water. The sample is mineralized in an open digestion system (DigiPrep) at 95°C for at least 3 hours. To extract Ti and Ce total from surface water a different treatment is required, as suggested in Poscher et al. [8]. 10 mL of H_2SO_4 are added to 5 mL of water, and the solution is mineralized via microwave, according to UNI EN 16173 [9]. After the digestion, few drops of hydrogen peroxide are added cautiously (to enhance the extraction of Ti and Ce) until the apparition of an orange-red colour. All extracts are brought to a volume of 50 mL with deionized water and analysed with ICP-MS NexION 350 D (**Table 1**).

Table 1 Range of concentrations obtained from samples analysis.

Constituents	Minimum	Maximum
Ti	3,7 μg/L	11.2 μg/L
Ce	0.2 μg/L	0.4 μg/L
Zn	0.12 μg/L	7.9 μg/L
Ag	0.64 μg/L	0.80 μg/L

Table 2 DLs estimated accoring to ISO/TS 19590: 2017.

Size detection limit (nm)	CeO_2	TiO_2	Ag	ZnO
	23.7	49.6	24.2	31.4

To evaluate the efficiency of the filtration in the NP research, three litres of water are filtered with a vacuum pump with cellulose acetate membrane filters with a 0.22 μm pore size to capture nanoparticles [10]. The filter is put into a vessel

of 50 mL then 30 mL of deionized water are added. Before determining and quantifying nanoparticles, the samples and standards are sonicated for 20 minutes. Particle size detection limit is performed according to ISO/TS 19590 [11]. The size detection limit is determined by three times the standard deviations (added to the mean size detected above the background) (**Table 2**). For accurate results, the sample flow rate should be determined (as in Syngistix Nano Application module) [12]. Ten millilitres of DW are weighted and aspirated through the nebulizer with a pump set 20 rpm for three minutes and the vial is weighted again. This operation is made for 5 times, then the flow rate is calculated on 5 readings average. The transport efficiency is calculated during the calibration. This operation is made every day and the average value of transport efficiency resulted between 4.85% and 7.96% (**Table 3**). Dissolved Au standards (1.25-2.5-5.0 and 10.0 µg/L) are prepared in 1% HCl acid. Au NP (30 60 and 90 nm) are used to determine transport efficiency and particle calibration **(Figure 1) (Table 4).**

Table 3 Example of parameters used in an analysis.

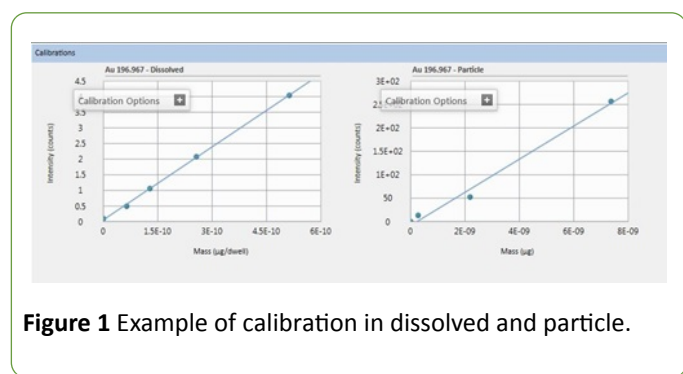| Mode	Gas Channel	Gas Flow (mL/min)	RPq	AFT (V)	Sample Flow Rate (mL/min)	Dwell Time (µs)	Scan Time (s)	TE (%)
STD	None	0	0,5	-	0,246	25	30	6,25

Figure 1 Example of calibration in dissolved and particle.

Even though nanoparticles have got a diameter between 1 and 100 nm, they form agglomerates and aggregates. For this reason, they don't pass through the pores of the filter.

Table 4 Calibration parameters.

Variables	Dissolved	Particle
Slope	7.782E+09	3.535E+10
Intercept	0.0578	-8.1727
R2	0.99938	0.98719

Table 5 comparison between filtered and unfiltered spiked samples.

Analyte	Sample	Data collected				% recovery			
		Mean Size (nm)	No. of Peaks	Part. Conc. (parts/mL)	Diss. Conc. (µg/L)	Mean Size (nm)	No. of Peaks	Part. Conc. (parts/mL)	Diss. Conc. (µg/L)
Ag 106.905	filtered	57,93	2780,00	462216,31	0,692	96,60	39,00	39,00	60,40
	unfiltered	59,98	7126,00	1184803,39	1,144				
Ce 139.905	filtered	64,07	2806,00	466539,20	0,868	88,80	41,50	41,50	55,90
	unfiltered	72,14	6758,00	1123617,92	1,554				
Ti 46.9518	filtered	92,29	4736,00	787430,38	0,953	81,50	49,80	49,80	36,00
	unfiltered	113,23	9510,00	1581178,82	2,649				
Zn 65.926	filtered	65,63	1764,00	293291,21	2,633	74,30	65,10	65,10	32,80
	unfiltered	88,31	2708,00	450245,24	8,024				

Table 6 Evaluation of the % recovery of total analytes.

Variables	Ti	Ce	Ti	Ce
Dilution	200 X	200 X	500 X	500 X
Theorical value (µg/L)	12000,00	16300,00	12000,00	16300,00
Experimental value (µg/L)	11216,31	11421,27	9949,49	15095,54
% of recovery	93,47	70,07	82,91	92,61

This is demonstrated with a recovery test. NP-spiked sample of river water (with the following concentration: Ag 2 µg/L, TiO$_2$ 20 µg/L, CeO$_2$ 40 µg/L and ZnO 200 µg/L) is divided in two quotes. The first is analysed directly and the second (3 L) is filtered. The filter is analysed in the same way of the previous experiments (**Table 5**). To evaluate more precisely the efficiency of recovery using the filtration, a spiked surface water (with a known concentration of TiO2 and CeO2 NP) is filtered and the total metal content on the filter is analysed (according to UNI EN ISO 17294-1:2007 and UNI EN ISO 17294-2:2016) [13,14] (**Table 6**).

Results

To characterize the presence of nanoparticles in water rivers is needed to concentrate them into a filter. This operation allows the reduction of the matrix interference and lowering of background signal as shown in **Table 6**. In particular there is an abatement in dissolved concentration of Zn up to 67.2%, which permit a reduction of mean size of 25.7%.

Figures 2 and 3 show the difference between filtered and unfiltered sample in the river C, in the case of TiO$_2$ NP.
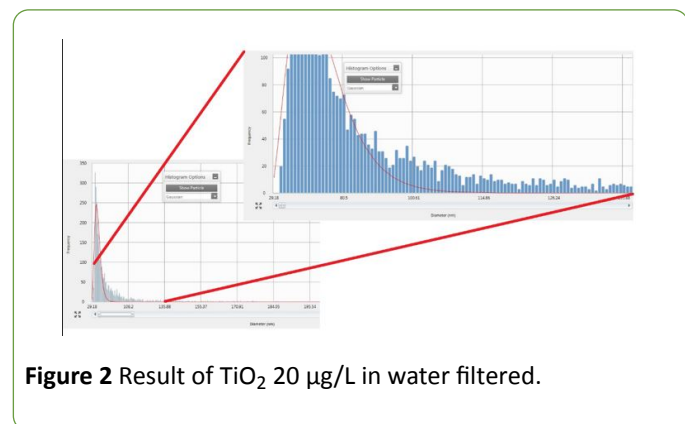

Figure 2 Result of TiO$_2$ 20 µg/L in water filtered.

The remarkable reduction of the background signal permits a lower detection limit of the particle size; therefore, the filtration allows to remove the interference of dissolved Ti. As a result, the signal is cleared than the unfiltered one. These images are from the SP-ICP-MS management software.

Table 7 Percentage of recovery for river A and B TiO2, CeO2 NP.

Rivers	Mean particle concentration of filtered samples	Mean particle concentration of unfiltered samples	% of recovery (filtered/not filtered parts/mL ratio)
River A (TiO2)	675151,6	864259,3	78.1
River B (TiO2)	225147,0	364500,9	61.8
River A (CeO2)	240908,4	248283,5	97

Table 7 shows the results of recovery tests for rivers A B in case of TiO2 and CeO2 NP.
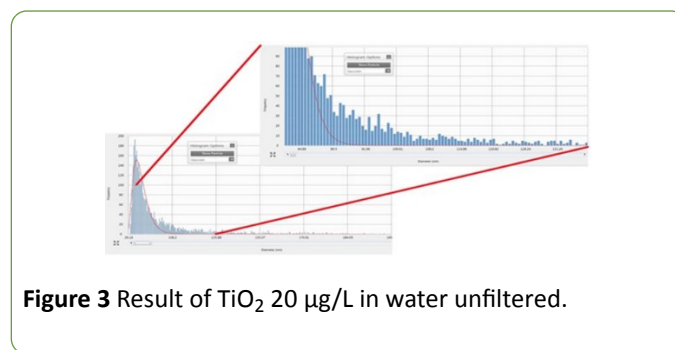

Figure 3 Result of TiO$_2$ 20 µg/L in water unfiltered.

Discussion

This research proved that SP-ICP-MS is a good technique to identify and characterize the nanoparticles in surface water.

The use of SP-ICP-MS presents some advantages: the high sensitivity of this method permitted to evaluate the presence of NP in a lower concentration (in order of µg/L or ng/L); the simple treatment of the surface waters reduce the cross contamination of sample and the transformation of nanoparticles in ions;

Furthermore, the filtration with a vacuum pump using a cellulose filter of 0.22 µm allows to lower the DL both concentration and size. This is possible because the filtration takes out the dissolved ions and depresses the signal of background with a good recovery of nanoparticles onto the filter; moreover, it's demonstrated the positive effect of the filtration in removing the matrix interferences, which could cause a significant false positive in the evaluation of NP.

Table 8 Comparison between experimental DL and results from Lee et al. in 2014.

DL particle size		
Lee et al. in 2014		
Ag	24.2	13
CeO$_2$	23.7	21
TiO$_2$	49.6	91.8

The size detection limit of this study is compare with the data obtained from Lee et al. [15]. The results showed in **Table 8**. They are similar for CeO$_2$ and Ag. In the case of TiO$_2$ there is a significant reduction of DL probably due to removal of matrix interferences.

Conclusion

The work of near future will be to compare this study with another analytical method as SEM or TEM.

The future development will put the attention on the bioaccumulation and biological effects of NP to evaluate the risk assessment perspective.

References

1. European Food Safety Authority (EFSA) (2016) Annual report of EFSA Scientific Network of Risk Assessment of Nanotechnologies in Food and Feed. pp. 1-14.

2. Salamon AW, Courtney P, Shuttler I (2010) Nanotechnology and engineered nanomaterials: A Primer. PerkinElmer FAQ Manuals 1-19.

3. United States Environmental Protection Agency (EPA) (2011) APM 32 screening methods for metal-containing nanoparticles in water.

4. Chady SKN (2014) Single particle inductively coupled plasma mass spectrometry: Understanding how and why. Perkin Elmer White Paper. pp. 1-5.

5. Pace HE, Rogers NJ, Jarolimek C, Coleman VA, Higgins CP, et al. (2011) Determining transport efficiency for the purpose of counting and sizing nanoparticles via single particle inductively coupled plasma-mass spectrometry. Anal Chem 15: 83.

6. Donovan AR, Shi H, Adams CD, Stephan C (2015) Monitoring cerium dioxide and zinc oxide nanoparticles through drinking water treatments using single particle ICP-MS. PerkinElmer Application Note 38-45.

7. UNI EN ISO 15587-2 (2002) Water quality-Digestion for the determination of selected elements in water-Part 2: Nitric acid Digestion 1-16.

8. Poscher A, Luidold S, Schnideritsch H, Antrekowitsch H (2016) Extraction of lanthanides from spent polishing agent. Rare Earths Industry 209-222.

9. UNI EN 16173 (2012) Sludge, treated bioiste and soil-Digestion of nitric acid soluble fractions of elements. UNI-Ente Nazionale Italiano di Unificazione database 1-11.

10. Gondikas AP, Von der Kammer F, Reed RB, Wagner S, Ranville JF, et al. (2014) Release of TiO2 nanoparticles from sunscreens into surface waters: A One-Year survey at the old danube recreational lake. Enviromental Sci Technol 5415-5422.

11. ISO/TS 19590 (2017) Nanotechnology–Size distribution and concentration of inorganic nanoparticles in aqueous media via SP-ICP-MS. ISO-International Organization for Standardization database 1-19.

12. Syngistix Nano Application module rev. 1.0 (2016) Single Particle- Inductively Coupled Plasma Mass Spectrometry (SP-ICPMS). PerkinElmer instrument manual 1-26.

13. UNI EN ISO 17294-1 (2007) Water quality: Application of inductively coupled plasma mass spectrometry (ICP-MS). Part 1: General guidelines. UNI - Ente Nazionale Italiano di Unificazione database. pp. 1-33.

14. UNI EN ISO 17294-2 (2016) Water quality: application of inductively coupled plasma mass spectrometry (ICP-MS). Part 2: Determination of selected elements including uranium isotopes. UNI - Ente Nazionale Italiano di Unificazione database. pp. 1-31.

15. Lee S, Bi X, Reed RB, Ranville JF, Herckes P, et al. (2014) Nanoparticle size detection limits by single particle ICP-MS for 40 elements. Environmental Sci Technology. pp. 1-31.