



Elemental Concentration in Atmospheric Particulate Matter: Estimation of Nanoparticle Contribution

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ABSTRACT

Atmospheric nanoparticles (NPs) are often contained in aggregates or included in larger particles. We show that some of these structures can be crushed in water media by the application of ultrasounds, leading to a suspension of insoluble NPs. The contribution of these NPs to the total elemental concentration is evaluated as the difference between the inductively coupled plasma (ICP-MS) analysis before and after the elution of the suspension from an ion exchange cartridge. Total elemental content in PM can be therefore fractionated into three contributions - soluble species, solid NPs released from larger structures, insoluble particles - that may likely have different health and environmental effects.

The method was applied to both Certified Material NIST 1649a and size-segregated atmospheric PM samples collected by a 13-stage impactor. The results indicate that alkaline and alkaline earth metals are found in the suspension only as water-soluble species, also when they are contained in the fine fraction of PM. Instead, a significant fraction of most elements typically emitted from combustion sources (Pb, Sb, Sn, Cd, V and As) is present in fine PM as insoluble nanoparticles that are easily dispersed in water under ultrasound application.

Keywords: Nanoparticles; Atmospheric PM; Elemental concentration; Aggregates; Size distribution.

INTRODUCTION

Several epidemiological studies suggest that lung and cardiovascular diseases due to the exposure to atmospheric particulate matter (PM) are mainly associated with the inhalation of small particles, able to penetrate deeply into the respiratory tract (Dockery and Pope, 1994; Hagdnagy *et al.*, 1998; Oberdörster *et al.*, 2005a). Among these, particular attention has been recently paid to particles smaller than 100 nm in diameter (ultrafine particles, or nanoparticles, NPs). From the point of view of toxicology, NPs behave differently from larger particles because of their peculiar morphology, chemical composition and surface area (Oberdörster *et al.*, 2005a, b). Their minute size and their very high exposed surface and mobility, in fact, make NPs very reactive towards their environment and able to penetrate living cells. Moreover, several studies have shown that NPs are able to adsorb and carry heavy metals and other carcinogenic compounds (Oberdörster *et al.*, 2001; Xia *et al.*, 2004; Terzano *et al.*, 2010).

In the scientific literature, much attention has been paid up to now to engineered NPs (Luther, 2004; Salata, 2004; Bystrzejewska-Piotrowska, 2010), which, however, constitute a small minority of environmental nanomaterials. NPs released into the atmosphere, instead, come mostly from natural and anthropogenic combustion processes, such as volcanic eruptions, forest fires, cars exhausts, industry, waste incinerators and biomass burning for domestic heating (Buzea *et al.*, 2007; Nowack and Bucheli, 2007, Kumar *et al.*, 2013).

Due to their high surface area, all atmospheric NPs easily aggregate and form large clusters, (Hays *et al.*, 2009). When in contact with biological systems, however, clusters may disaggregate again releasing free NPs able to translocate across the respiratory epithelium and enter the lymphatic and the circulatory system. For this reason, the quantitative relevance of aggregated NP contribution to PM becomes of great importance in air quality studies.

The numerical relevance of nanoparticles and their contribution to atmospheric PM is usually largely underestimated (Manigrasso *et al.*, 2013). The measurement of atmospheric NP concentration, in fact, is generally carried out by using instruments that select incoming particles on the basis of their physical characteristics (optical diameter, aerodynamic diameter, electric mobility). As clusters exhibit a different physical behavior with respect to single NPs,

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these technique fatally neglect aggregated NPs, as they physically behave as a single, larger particle (generally between 0.1 and 1 μm). Currently, the only techniques able to directly show NPs and give qualitative information about their aggregation state are the Scanning Electron Microscopy (SEM; Wittmaack, 2004) and Transmission Electron Microscopy (TEM; Murr and Soto, 2005), which, however, cannot yield quantitative results and are hardly applicable to intensive atmospheric monitoring studies.

Up to now, most studies addressing the environmental impact of atmospheric NPs concerned diesel exhausts, which are considered as a primary source of NPs in urban areas (Kittelson, 1998). It has been shown that diesel exhausts emit both inorganic and carbonaceous particles in the size range of 5–20 nm, and also that these NPs quickly join to form aggregates (Kittelson, 1998; Murr *et al.*, 2006; Matti Maricq, 2007). Only a very limited number of studies address other NP sources releasing mainly inorganic particles (e.g., urban solid waste incinerators), whose environmental and health effects are still largely unknown (Buonanno *et al.*, 2011; Cernuschi *et al.*, 2012). In this contest, one of the main issues concerns the release of NPs rich in toxic elements. It is known, in fact, that the bio-availability - and danger - of elements in PM depends on their solubility in water (Mukhtar and Limbeck, 2013). However, insoluble particles of very small size may be even more dangerous than soluble species, given their ability to physically interact with cells (Cho *et al.*, 2012).

In this paper we report the optimization of a method able to fractionate the total elemental concentration in atmospheric PM into three contributes: the water-soluble fraction, the insoluble non-nanometric fraction and the fraction due to insoluble NPs, both individual and contained into larger clusters. The application of the method to the reference material NIST1649a (urban dust) and to size-segregated real atmospheric PM samples is also reported.

Environmental studies will benefit from the availability of a method able to evaluate the contribution of insoluble NP contained in aggregates to the elemental concentration in atmospheric PM; the effects of this fraction could be considerably different from those caused by the same elements in soluble forms or contained in coarser particles.

EXPERIMENTAL

Materials

All the chemical and reagents were of analytical grade. Water was produced by a deionizer Elga LabWater Purelab Plus. Acid digestion was carried out by using HNO_3 65% Suprapur (Merck, KGaA, Darmstadt - Germany) and H_2O_2 30% Suprapur (Merck KGaA, Darmstadt - Germany). A micro-analytical balance (Sartorius M5P-F, Sartorius Mechatronics, Italy, sensitivity 0.001 mg) was used to weight solid samples. Reference material NIST 1649a was purchased from National Institute of Standard and Technology (USA).

Physical and Morphological Features of Reference Material NIST1649a

Specific surface area of NIST1649a (10 mg) was measured by the BET method (Brunauer-Emmett-Teller), using a Nova 2200e Surface Area and Pore Size Analyzer (Quantachrome Instruments, Boynton Beach, FL-USA). Density was measured on the same material by a helium stereopycnometer (Quantachrome Instruments, Boynton Beach, FL-USA). In both cases, 10 replicates were carried out during each test. These measurements were aimed to evaluate the presence of NPs in the certified material.

Tridimensional images of the NPs were obtained by a high resolution scanning electron microscope (HRSEM; LEO 130, LEO Electron Microscopy, Oberkochen - Germany) operating at 20 kV; for sample preparation a small drop (50 μL) of the suspension was placed in the center of a graphite stub and dried. Individual particle analysis was performed using a scanning electron microscope (Philips XL30 ESEM, FEI Company, OR-USA) combined with energy dispersive spectrometer (EDAX Inc. Mahwah, NJ-USA); in this case, sample were prepared on aluminium stubs (Subst Foil 47 mm, MSP Corporation, MI-USA), so as to allow the analysis of carbonaceous NPs.

For measuring the size distribution of the certified material, 10 mg of NIST1649a were dispersed in deionized water; final concentration was 1.0 mg/mL. The suspension was sonicated (Cole Parmer 8890, Vernon Hills, IL-USA) for different time durations in the range 1–17 hours, by using a tailor-made rotating device able to uniform ultrasound (US) exposure. Then the suspension was centrifuged at 6000 rpm for 15 min (refrigerated centrifuge PK130R, ALC, Milan - Italy) to separate insoluble coarser particles (defined as *residual fraction* in the text) from the NP suspension (*suspended fraction*). The residual fraction was then re-suspended in the same initial water volume (10 mL).

Aliquots of 1.0 mL of the two suspensions were analyzed by Dynamic Light Scattering (DLS, Schurtenberger and Newman M, 1993; Finsy, 1994) and by Zetasizer (Nano Series, Malvern Instruments, Worcestershire - UK). The analyses were carried out at 25°C; thermal equilibrium time was 2 minutes; 14 replicates were carried out for DLS tests, 20 replicates for the Zetasizer. These two techniques allow the determination of the hydrodynamic diameter of the particle population and the measurement of the zeta potential, respectively. Although the hydrodynamic diameter cannot be directly correlated with the aerodynamic diameter and the particle shape, this parameter supplies useful indication about the aggregation state of the NPs (Baalousha, 2009; French *et al.*, 2009; Jiang *et al.*, 2009). The measurement of the zeta potential provide information about the dispersion stability: when the absolute value of the zeta potential is high (> 30 mV) the degree of repulsion among NPs is also high and the dispersion will resist aggregation; when it is low, attraction exceeds repulsion and the dispersion will flocculate.

Collection of Field Samples

Field PM samples were collected during a period of 15 days (spring 2010) at a sampling site located in the area of the Sapienza University of Rome, inside a parking site close to the Department of Chemistry and at about 50 m

from the nearest urban road. We used a 13-stage low-pressure impactor (DLPI, DEKATI Ltd., Tampere, Finland). The instrument operates at the flow rate of 10 L/min and at the pressure of 100 mbar under the last impactor stage. The nominal values for the equivalent aerodynamic 50% cut-off diameters of the stages are: 10, 6.8, 4.4, 2.5, 1.6, 1.0, 0.65, 0.40, 0.26, 0.17, 0.108, 0.060, and 0.030 μm . PTFE membranes, 25 mm diameter, (ALBET, Barcelona, Spain) were used as substrates on the impactor collection plates.

Elemental Analysis

All samples (NIST 1649a, ca. 3 mg, and field PM samples) were dissolved in 3 mL deionized water, sonicated for 3 hours and then filtered by using 0.22 μm Millipore filters (cellulose nitrate membranes, Millipore Corporation, MA-USA). Comparison tests between filtration at 0.22 μm and centrifugation (used to separate the suspended and residual fractions before DLS and SEM measurements) were performed to ensure the equivalence of the two separation systems. The differences in the elemental concentrations in the suspended fraction (6 replicates; NIST 1649a) were below the repeatability of the analytical method (about 5%).

The suspended fraction was divided into two aliquots: the first one was diluted (1:10) and directly analyzed by ICP-MS (Varian 820, Mulgrave, Australia). The second aliquot was injected by a 500 μL loop into a ion chromatograph (mod. 761 Compact, Metrohm, Origgio, Va-Italy) equipped with a pre-column only (Metrosep A SUPP 4/5 Guard/4.0, Metrohm). The sample was eluted with deionized water; the eluted solution was monitored for the total ionic content by a conductimetric detector, then collected into vials and analyzed by ICP-MS.

The residual fraction was acid digested, using a 4:2 $\text{HNO}_3/\text{H}_2\text{O}_2$ mixture (Canepari et al., 2006a, b; Canepari et al., 2010b); the digestion was microwave-assisted (Milestone Ethos Touch Control equipped with HPR 1000/6S rotor, Sorisole, Bg, Italy). The acid solutions were filtered and analyzed by ICP-MS.

The operating conditions for ICP-MS were as follows. Nebulizer: cross-flow Micromist (Varian, Mulgrave, Australia); RF forward power: 1,400 W; plasma Ar flow rate: 18.0 L/min; auxiliary Ar flow rate: 1.80 L/min; nebulizer Ar flow rate: 1.00 L/min; number of replicates: 3. Matrix-matched standard solutions were used for calibration and Y and Sc were used as internal standards. The collisional reaction interface (CRI) was used to reduce polyatomic interferences of ^{75}As and ^{51}V .

The analyzed elements were: As, Ba, Cd, K, Li, Mg, Mn, Na, Pb, Rb, Sb, Sn, Sr, Tl, V.

OPTIMIZATION OF THE EXPERIMENTAL CONDITIONS

Dispersion of NPs

Presence of NP Aggregates

In order to optimize the experimental conditions for an efficient dispersion of NPs contained in larger clusters and for their separation from larger particles, it was necessary to use a number of techniques requiring a high amount of

homogeneous material. For this reason, we began our study by using a certified material (NIST 1649a). Although this material, certified for its elemental and polycyclic aromatic hydrocarbon content, is not completely similar to field atmospheric PM samples in terms of size distribution and chemical composition, anyway it is constituted by urban dust and can be assumed to contain the main type of atmospheric particles (NIST, 2001).

Firstly, the presence of NP aggregates in NIST 1649a was evaluated by measuring its specific surface area and density. The specific area of the sample was $3.18 \pm 0.34 \text{ m}^2/\text{g}$, a sufficiently high value to hypothesize the presence of NPs; the average density of the sample was $2.51 \pm 0.11 \text{ g/cm}^3$. From these two data we roughly estimated the value of the average equivalent diameter: $r = 0.38 \pm 0.12 \mu\text{m}$. The analytical certificate of the NIST 1649a, however, reports an average radius of about 5 μm , much higher than our experimental result. This difference may be due to the different measurement techniques: the certified size distribution of the NIST 1649a was obtained by means of laser scattering, which measures the particles in aqueous suspensions and considers the aggregates as a single large particles. The technique we used, instead, is based on the penetration of gases inside the material and is much more adequate to consider interstices and to account for the surface of NPs in aggregate form. The presence of NP aggregates in NIST 1649a is also confirmed by the microphotographs obtained by HRSEM (Fig. 1).

Effect of pH and Ionic Strength

Some other preliminary trials carried out on NIST 1649a were addressed to select the best conditions for an efficient NP dispersion. As inorganic NPs are mainly composed of oxides and are thus hydrophilic, we used water as dispersion medium. By using DSL, we evaluated the effect of pH, ionic strength and duration of US application on the efficiency of NP dispersion and on the stability of the obtained suspended fraction.

At different pH values in the range 1–12, the number size distribution of the NIST 1649a dispersion was centered at $60 \pm 10 \text{ nm}$, independently of the pH value (3-h US application). The stability of the dispersions, instead, was dependent on pH: when the solution was acid the value of the zeta potential was close to zero, while at $\text{pH} \geq 6$ we obtained z potentials values between -24 mV and -28 mV . The stability of the suspension was further evaluated by studying the variations in the size distribution after fixed time intervals (0, 1 hour, 2 hours, 3 hours, 1 day, 2 days, 3 days). At spontaneous pH (pH 6.6) the size distribution remained unchanged up to at least 2 hours, then a partial re-aggregation of NPs was observed; at higher pH values ($\text{pH} \geq 10$) re-aggregation phenomena were observed after the first 24 h. However, since at alkaline pH many of the elements that are contained in PM as soluble species might form hydroxides, we preferred to keep the dispersion at spontaneous pH and to complete the experiments within two hours.

The influence of the ionic strength was evaluated by adding increasing NaCl amounts to the dispersions, up to reach a

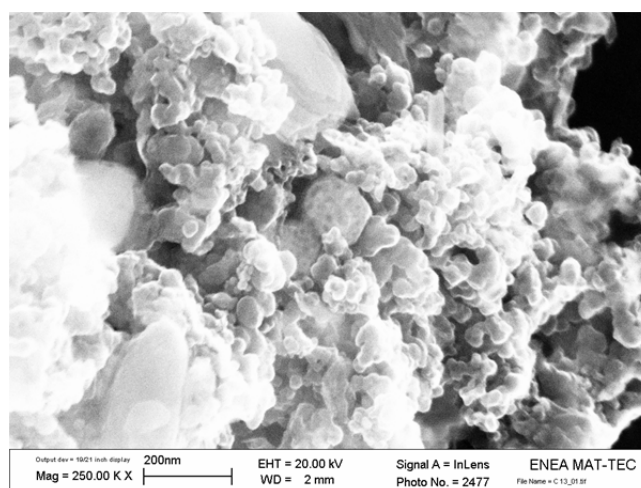


Fig. 1. Microphotographs of the NIST 1649a obtained by High-Resolution SEM.

concentration of 1 g/L (3-h US application). As far as the ionic strength increased, we observed an increase of the hydrodynamic dimensions of the particles up to about 200 nm; besides, the addition of NaCl did not improve the stability of the suspensions (the z potential of the 1 g/L solution was -24 ± 2 mV; re-aggregation occurred after 2 h).

Effect of US Supply Duration

To verify the influence of US supply duration on NP disaggregation, we carried out tests at different application times. Fig. 2 (upper panel) shows the size distribution of the suspended fraction when the US supply duration varied from 0 to 3 hours. The data show that the size of the suspended particles decreased when increasing the US supply duration, suggesting the occurrence of a steady disaggregation. After 3 hours, we obtained particles almost completely below 100 nm in hydrodynamic diameter. No further decrease in the particle dimensions was observed when increasing the exposure time over 3 hours.

We also checked the effect of US supply duration on the

size-distribution of the re-suspended residual fraction. When increasing US exposure time, the size distribution of the residual fraction moved towards lower hydrodynamic diameters, showing that US was able to crush particles (Fig. 2, lower panel). After three hours, the hydrodynamic diameter of the particles in the residual fraction was higher than 150 nm, well separated from the size distribution of the suspension. It is worth noting that the coarsest particles (above 1–2 μm) may undergo sedimentation during the measurement period and thus they were not included in the size distribution. Fig. 3 (upper panel) shows a HRSEM microphotograph of the suspended fraction after 3-h US exposure. There are some isolated NPs and some small aggregates on a background of soluble salts that were deposited following solvent evaporation. EDS analysis (supplementary material) showed that this background was mainly constituted by sulfates, while particles were mainly made of metal oxides. In the residual fraction, instead (Fig. 3, lower panel), there are many more and much more mixed particles, with a prevalence of carbon and soil particles.

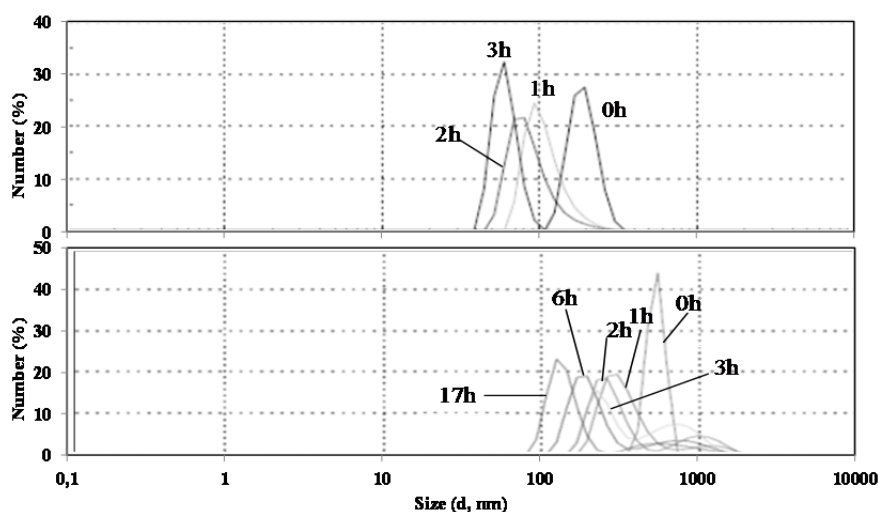


Fig. 2. Hydrodynamic size distribution of the suspended fraction (upper panel) and of the residual fraction (lower panel) as a function of US application time (average of 14 replicate measurement).

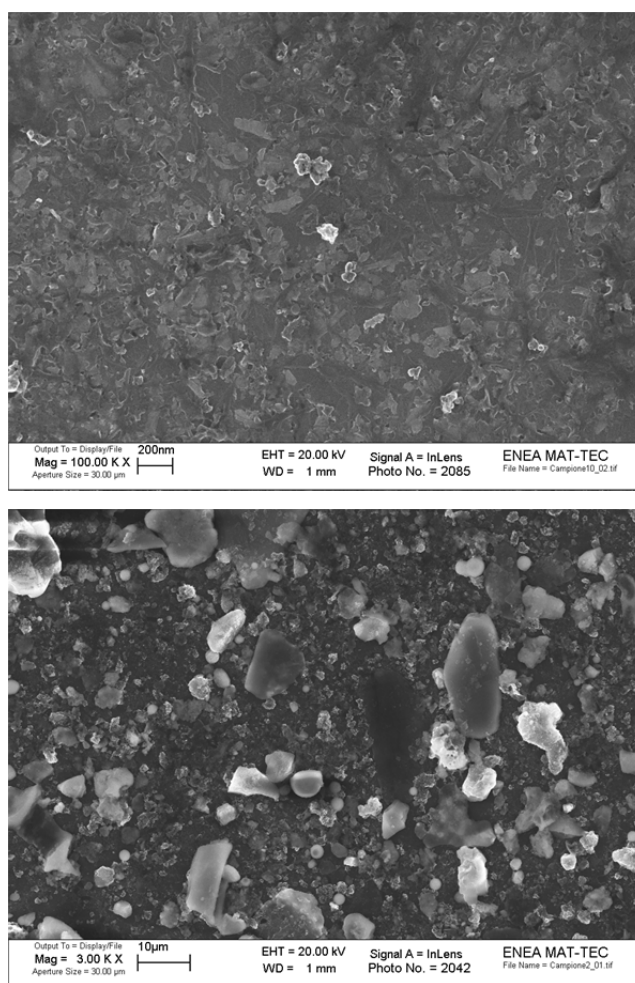


Fig. 3. High-Resolution SEM microphotograph of the suspended fraction (upper panel) and of the residual fraction (lower panel) after 3-h US exposure.

These results were confirmed by studying the effect of US exposure time on the concentration of elements. As an example, Fig. 4 reports the results obtained for Vanadium. The suspended and the residual fractions are mirror images: for exposure times up to three hours, the amount of Vanadium in the suspended fraction increased while it decreased in the residual fraction. These results indicated that by increasing US exposure time, a part of the particles containing vanadium moved from the residual to the suspended fraction, a behavior that is in agreement with the release of NPs from larger particles. The same pattern was shown also by As, Sb, Cd and Pb, while US exposure time had no detectable effect on the concentration of all the other elements.

As a result of this optimization phase, in all the subsequent experiments dust was suspended in deionized water at spontaneous pH and US were supplied for 3 hours.

Separation of Suspended NPs from Soluble Species

Since a fraction of the elemental concentration in PM is generally in a water-soluble chemical form (Heal *et al.*, 2005; Birmili *et al.*, 2006; Canepari *et al.*, 2010b), it is necessary to physically separate suspended solid NPs from

the soluble fraction. To this aim, we referred to previous results obtained by Canepari *et al.* (2010a). In that paper we had shown that solid NPs suspended in a solution are able to pass through the ICP nebulizer but are retained by a chromatographic column. The solid NP contribution to the elemental concentration in the suspended fraction can be thus estimated by measuring the elemental concentration by ICP-MS before and after elution of the suspension from a chromatographic column.

To apply this method one must assure that the elution of the soluble species from the column is quantitative. As there are no reference materials to check the fulfillment of this requirement, we collected the eluted fractions every 2 mL and determined the concentration of all the elements of interest.

For all these elements, the concentration in the eluted solution matched the background after the first three fractions (total volume: 6 mL; flow rate: 1.2 mL/min), as shown for Sb, V, Mg and Na in Fig. 5. Furthermore, to exclude that soluble species are retained into the chromatographic pre-column, we injected and eluted (with 6 mL of deionized water) the suspended fraction five times. Then we eluted the pre-column with different mobile phases (ethylendiaminetetraacetic acid, ammonium bicarbonate,

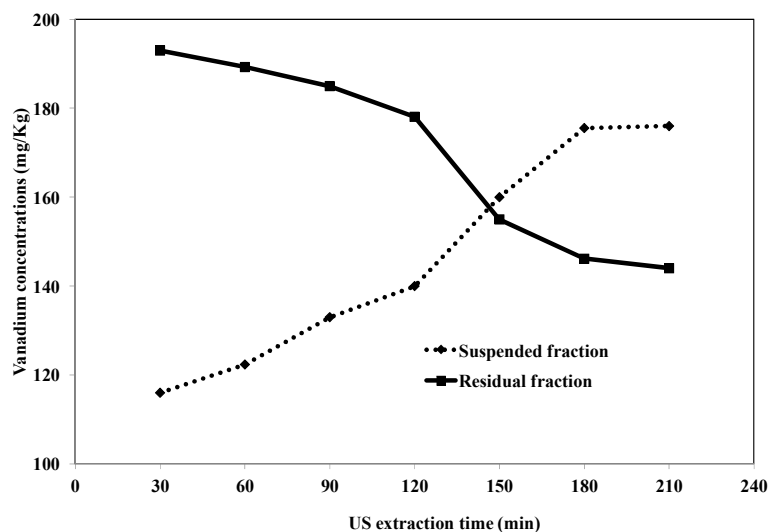


Fig. 4. Variation of Vanadium concentration in the suspended and residual fraction as a function of US application time.

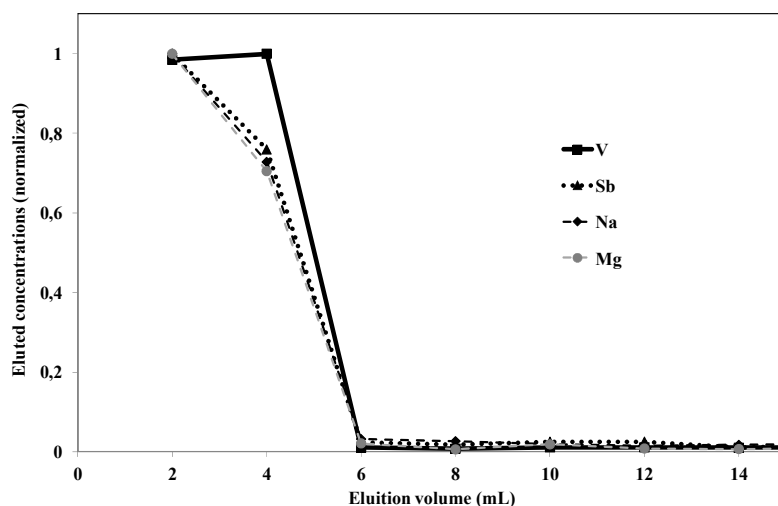


Fig. 5. Normalized concentration of Sb, V, Mg, Na in the eluate from the chromatographic pre-column as a function of time.

ammonium tartrate) and carried out the ICP-MS analysis of the eluted solutions. For all the elements and all the experimental conditions the concentration in the eluate was below the detection limits. Solid NPs in the eluted fraction were undetectable also to both SEM and DLS analyses.

As a result of this further optimization phase, in all subsequent experiments the elution of the soluble species was performed with deionized water and the collected volume was set at 6 mL.

A scheme of the optimized analytical method is shown in Fig. 6.

APPLICATION TO URBAN DUST SAMPLES

Standard Reference Material NIST 1649a

The optimized procedure was applied to NIST 1649a. The recovery percentage of the total concentration for all certificates elements (As, Ba, Mg, Mn, Pb, Rb, Sb, Sn, Sr, Tl, V), calculated as sum of the residual and the suspended fraction, was higher than 90%.

Fig. 7 reports the distribution of elements between the residual and the suspended fraction; the latter includes suspended NPs (retained fraction) and soluble species (eluted fraction). The results show a variable apportionment of the considered elements among the different fractions. Some elements (Ba, Li, Pb, Rb, Sb and Sn) are mainly in the non-extractable form (> 80%) and thus associated to large and insoluble particles. For the other elements, Fig. 7 shows a major contribution of the suspended fraction, divided into the eluted and retained fractions. As previously discussed, this latter fraction should be almost completely due to solid NPs. For some elements (Ba, K, Li, Mg, Mn, Na, Rb, Sr and Tl) the retained fraction is negligible, and the suspended fraction includes then only soluble species. The retained fraction is very relevant, instead, for some other elements of significant environmental interest (As, Cd, Pb, Sb and V) whose main atmospheric source, such as waste incineration and fossil fuel combustion, are assumed to emit NPs (Lin *et al.*, 2005; Buonanno *et al.*, 2011; Waheed *et al.*, 2011).

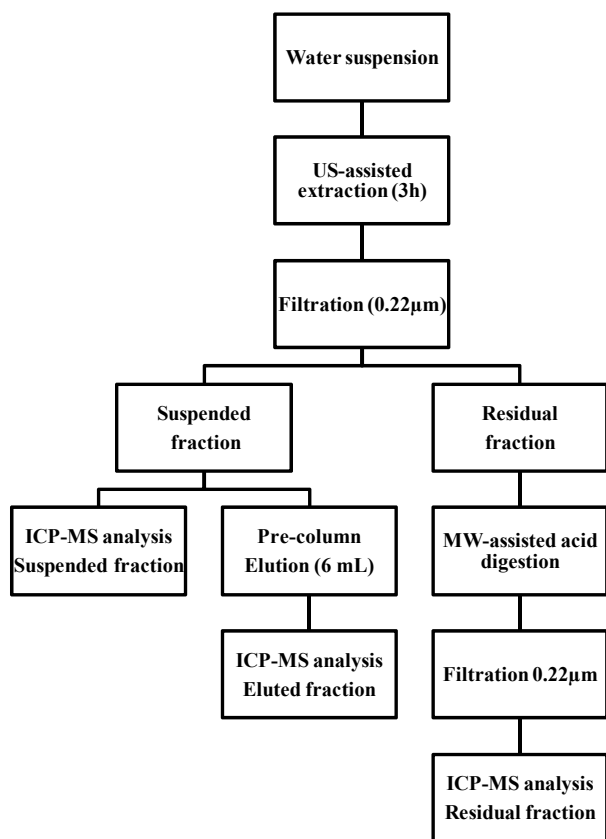


Fig. 6. Scheme of the analytical method.

Size-Segregated Field Samples

The method was applied to field environmental samples obtained from a 13-stage impactor; the collection of size-segregated samples allows to perform the separation of coarse and fine particles in the sampling phase. Fine particles are mainly emitted from combustion sources and may contain NP aggregates, while coarse particles are generally produced by mechanical processes.

The total elemental concentrations (sum of suspended and

residual fractions) measured on each stage of the impactor are reported in Table 1. The dimensional distribution is in a good agreement with the results previously obtained at the same site (Canepari *et al.*, 2008; 2010b) and reflects the relative weight of combustive and/or mechanical sources for each element. Elements that are mainly released into the atmosphere by natural sources or by re-suspension processes (Ba, Li, Mg, Mn, Na, Sn, Sr) are mostly present in coarse particles, while elements having significant contribution from combustive sources (As, Cd, K, Pb, Rb, Sb, Tl, V) show a bimodal distribution, with substantial contributes also from fine particles.

The dimensional distributions of the suspended and eluted fraction are of particular interest, as they allow the evaluation, by difference, of the size ranges showing the highest contribution from NPs. Fig. 8 reports the size distribution of the suspended and eluted fractions of some elements (Na, Li, Rb, Tl, Sb and Cd) that are illustrative of three different behaviors. Some elements (Na and Li, shown in the first row of Fig. 8, but also Mg, Ba, Mn and Sr) show a dimensional distribution almost completely in the coarse stages of the impactor. For all these elements the ICP results obtained before and after passing the chromatographic pre-column agreed well, indicating that the suspended fraction contained only soluble species able to be eluted from the pre-column. It is worth noting that the recovery after the elution was always very good, confirming the results obtained for NIST 1649a.

The size distribution of a second group of elements (Rb and Tl, shown in the second row of Fig. 8, but also K) was mainly confined in the fine fraction; again, the ICP results obtained before and after the elution were in quite good agreement, as for the previous group. These elements were thus contained in fine particles as soluble species, not retained by the chromatographic pre-column.

A third group of elements (Sb and Cd, shown in the third row of Fig. 8, but also V, As, Sn and Pb) also exhibited a significant fine contribution, but the values obtained after the elution were markedly lower than those obtained from

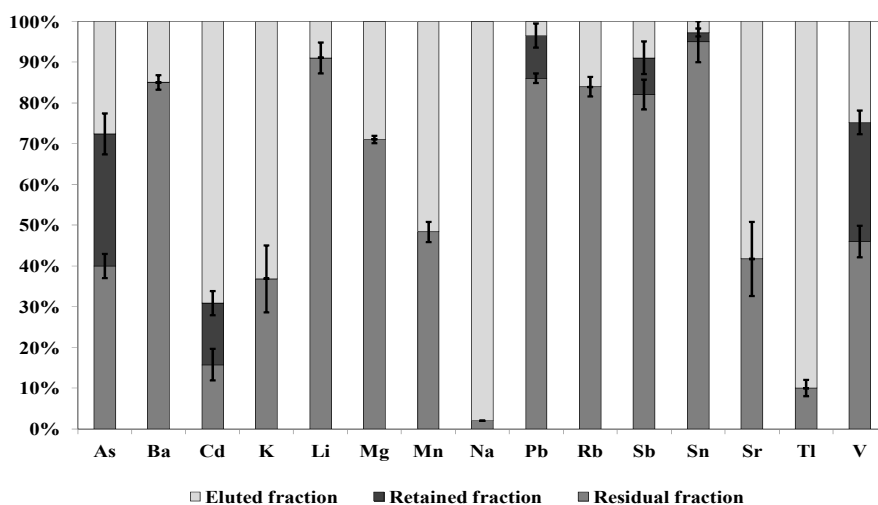


Fig. 7. Distribution of elements in NIST 1649a between the residual, retained and eluted fractions (6 replicate measurements; standard deviation in error bars). The sum of the retained and eluted fractions constitute the suspended fraction.

Table 1. Total elemental concentrations (sum of suspended and residual fractions) in the size-segregated PM samples.

stage μm	As ng/Nm^3	Ba ng/Nm^3	Cd ng/Nm^3	K ng/Nm^3	Li ng/Nm^3	Mg ng/Nm^3	Mn ng/Nm^3	Na ng/Nm^3	Pb ng/Nm^3	Rb ng/Nm^3	Sb ng/Nm^3	Sn ng/Nm^3	Sr ng/Nm^3	Tl ng/Nm^3	V ng/Nm^3
0.03–0.06	0,01	0,04	0,004	11	0,007	7,3	0,25	18	0,17	7,3	0,04	0,08	0,03	0,001	0,01
0.06–0.108	0,01	0,05	0,014	18	0,005	4,3	0,15	16	0,21	18	0,14	0,05	0,05	0,006	0,02
0.108–0.17	0,07	0,04	0,033	45	0,014	3,9	0,14	30	0,44	50	0,36	0,07	0,04	0,011	0,05
0.17–0.26	0,14	0,05	0,044	53	0,015	4,6	0,16	15	0,69	80	0,59	0,07	0,05	0,015	0,09
0.26–0.4	0,28	0,06	0,057	69	0,028	3,4	0,19	19	1,7	100	0,98	0,08	0,04	0,016	0,24
0.4–0.65	0,25	0,17	0,077	83	0,026	13	0,71	32	3,0	116	1,5	0,19	0,10	0,020	0,54
0.65–1	0,26	0,41	0,080	56	0,029	9,2	0,91	78	2,9	83	1,6	0,23	0,12	0,014	0,48
1–1.6	0,40	1,3	0,025	29	0,029	30	1,1	230	1,8	71	0,74	0,33	0,27	0,003	0,23
1.6–2.5	0,15	2,4	0,011	53	0,067	63	1,3	691	0,80	78	0,69	0,52	0,60	0,003	0,12
2.5–4.4	0,02	4,3	0,008	80	0,099	92	2,2	1136	0,90	121	1,0	1,1	1,1	0,002	0,17
4.4–6.8	0,29	4,6	0,007	103	0,13	111	2,5	1401	1,0	165	0,93	1,0	1,7	0,002	0,21
6.8–10	0,10	4,7	0,007	92	0,13	105	2,7	1033	1,3	177	0,68	0,77	2,2	0,001	0,26
> 10	0,23	3,2	0,008	66	0,096	106	1,8	682	1,3	138	0,25	0,44	2,0	0,001	0,17

the direct analysis. All these elements are likely contained in a NP fraction that is analyzed by ICP-MS, as well as the soluble fraction, but is retained by the chromatographic pre-column. Most of these elements, which are conceivably emitted in NPs (Tolocka *et al.*, 2004; Lin *et al.*, 2005; Lough *et al.*, 2005; Waheed *et al.*, 2011), showed a similar behavior also in the Certified Material (Fig. 7).

Fig. 9 reports the distribution of each element among the eluted, retained and residual fractions in fine (upper panel) and coarse (lower panel) particles. Almost all elements show different behavior in the two size fractions. The elemental concentrations in fine particles, obtained as total amount in the impactor stages up to $1 \mu\text{m}$ (PM_{10}), show a significant contribution of the suspended fraction. As shown also in Fig. 8, some elements (Ba, K, Li, Mg, Mn, Na, Rb, Sr, Tl) are present in the suspended fraction only as soluble species (eluted fraction), while for V, Sn, As, Sb and Pb the retained fraction constitutes more than 50% of the suspended fraction. For Cd this percentage reduces to less than 15%.

In the impactor stages from $1 \mu\text{m}$ to $10 \mu\text{m}$ (coarse particles - PM_{10-1}), the elemental concentrations of As, Cd, Li, Rb, Sb, Sn, Tl, V show a contribute from the residual fraction much higher than in the case of fine particles. This result is in agreement with the low solubility of the chemical species from soil and traffic-related non-exhaust emission (abrasion of brake pads and mechanical parts), which are the main sources of the coarse fraction in atmospheric PM at urban sites (Canepari *et al.*, 2008). Retained fraction was not detectable for all these elements. This result is not surprising, as NP clusters are expected to be almost completely contained in the fine fraction of PM (US EPA, 2004).

It is worth noting that most studies regarding the bio-availability of toxic metals in PM are performed by US-assisted water extraction followed by filtration and ICP analysis (Mukhtar and Limbeck, 2013), a procedure that is very similar to the method used in this work to obtain the suspended fraction. It follows that the bio-available fraction considered in those studies likely includes suspended solid NPs, whose effect on human health needs to be further investigated and separated by the effects due to soluble forms.

CONCLUSIONS

This study describes how the exposure of a water-suspension of PM to ultrasounds is able to produce a suspension of hydrophilic NPs that can be separated from coarser particles by centrifugation or filtration. The contribution of suspended NPs to the elemental concentration in PM can be differentiated from the contribution of soluble species by passing the suspension through a chromatographic pre-column able to retain NPs and to elute soluble forms. NP contribution can be then estimated as the difference between the elemental concentration determined by ICP-MS in the solution before and after passing the chromatographic column. The method offers thus a viable solution to detecting aggregated NPs in atmospheric PM samples.

The application of the proposed method to the certified material NIST 1649a and to field PM samples collected by

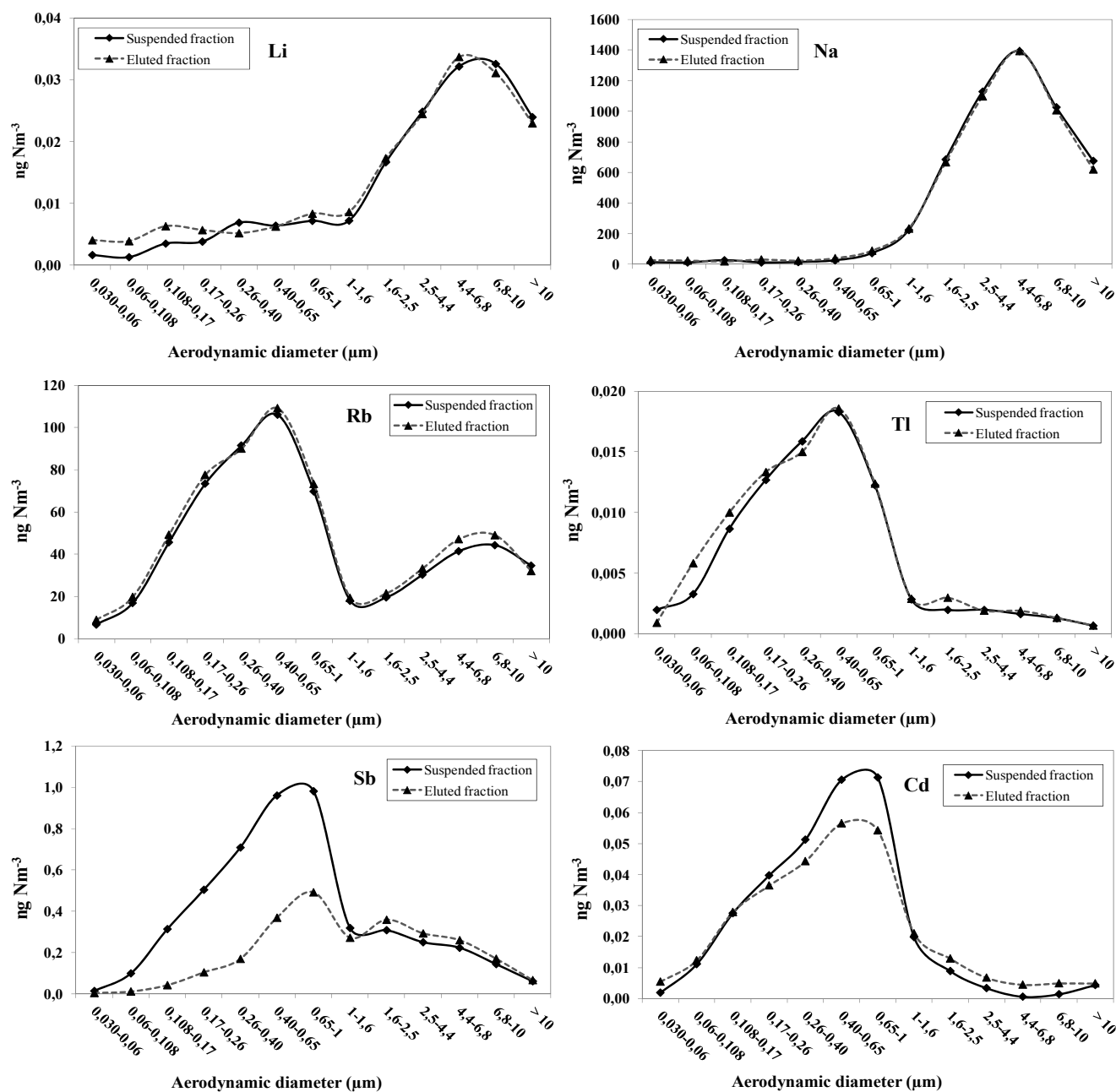


Fig. 8. Size distribution of the suspended and eluted fractions of Na, Li, Rb, Tl, Sb and Cd.

a 13-stage impactor has shown that some elements, mainly alkali metals and alkaline earth metals, are found in the suspension only in soluble form, also when they are contained in the fine fraction. A different behavior was detected for most of the elements typically emitted from combustion sources, which are considered as the main responsible for NP emission in the atmosphere. We showed that a relevant part of the concentration of these elements in the fine fraction of PM is due to insoluble NPs that can be easily dispersed in water under US supply.

Although the proposed method needs a wider field application, these results are of environmental and toxicological relevance because the health and environmental effects of the elements identified in the NP fraction (As,

Cd, Sb, V, Pb) could be considerably different from the effects caused by the same elements in soluble forms or contained in coarser particles.

Furthermore, this fractionation scheme may allow an increase of the selectivity of elements as tracers of PM sources responsible for the emission of inorganic NPs (e.g., waste incineration).

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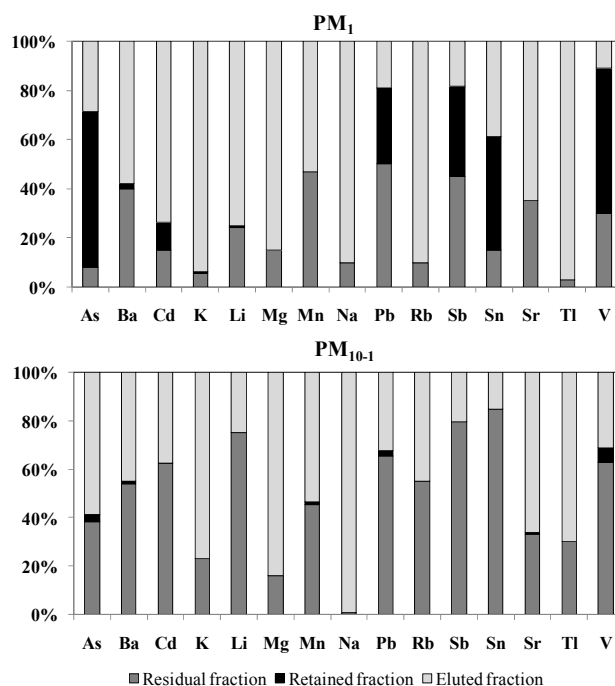


Fig. 9. Distribution of elements in PM₁ (upper panel; sum of the air concentrations on the impactor stages up to 1 μm) and PM₁₀₋₁ (lower panel; sum of the air concentrations on the impactor stages from 1 to 10 μm) among the residual, retained and eluted fractions. The sum of the retained and eluted fractions constitute the suspended fraction.

SUPPLEMENTARY MATERIALS

Supplementary data associated with this article can be found in the online version at <http://www.aaqr.org>.

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