Biomonitoring and biomarkers to assess metal-based nanomaterial exposure of consumers, workers and the general population

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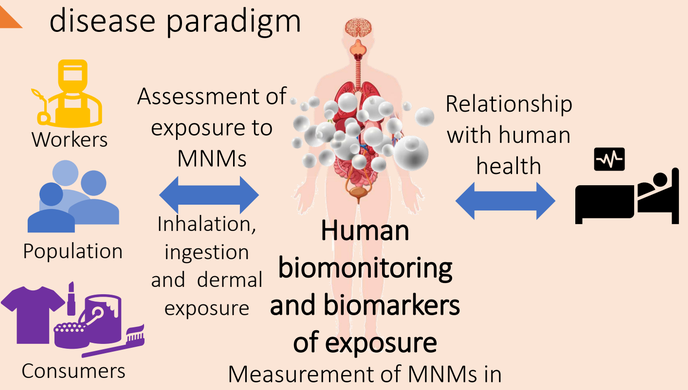
Introduction

Human biomonitoring and biomarkers of exposure are central for measuring metal-based nanomaterials (MNMs) internal dose in human biological matrices, understanding the progression from MNMs exposure to effects and for health surveillance and evaluation of public interventions (Figure 1). There is a strong need to develop and harmonize MNMs detection methods because methods are limited and expensive, MNMs concentrations are low and many parameters have to be estimated.

Figure 1. Biomonitoring in the exposure-disease paradigm



Aim of the study

A Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) method capable of determining size, particle size distribution, composition and concentration of several MNMs was in-house validated in human blood and urine to be used in routine biomonitoring campaigns. Method performances as limit of detection (LoD), linearity working range, accuracy and repeatability were evaluated according to ISO/IEC 17025:2017 and others ISO on nanomaterials.

The process of MNMs detection

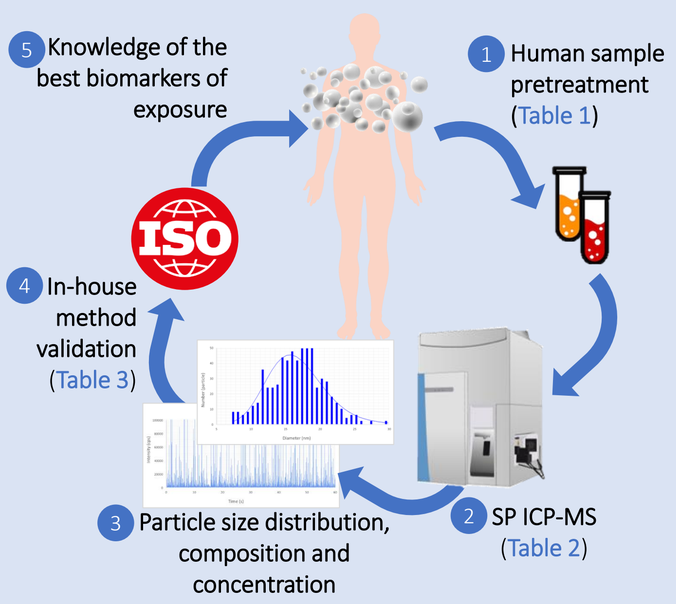


Table 1. Sample pre-treatment and standards

Urine	1:10 v/v water dilution, sonication 10 min.
Blood	Alkaline extraction with 25% v/v TMAH, sonication 10 min and 24 hours at room temperature. Then, dilution with 0.1% v/v Triton-X.
Certified reference standards	Al_2O_3 (30 nm), Co_3O_4 (15 nm), Cr_2O_3 (60 nm), CuO (25-55 nm), Ir_2O_3 (20-70 nm), Ir (15 nm), Mn_3O_4 (30 nm), NiO (18 nm), Pd (15 nm), TiO_2 (< 100 nm), and ZnO (30-40 nm), CeO_2 (30-50 nm), Pt (15 nm) and ZrO_2 (30-40 nm) spiked in matrix.

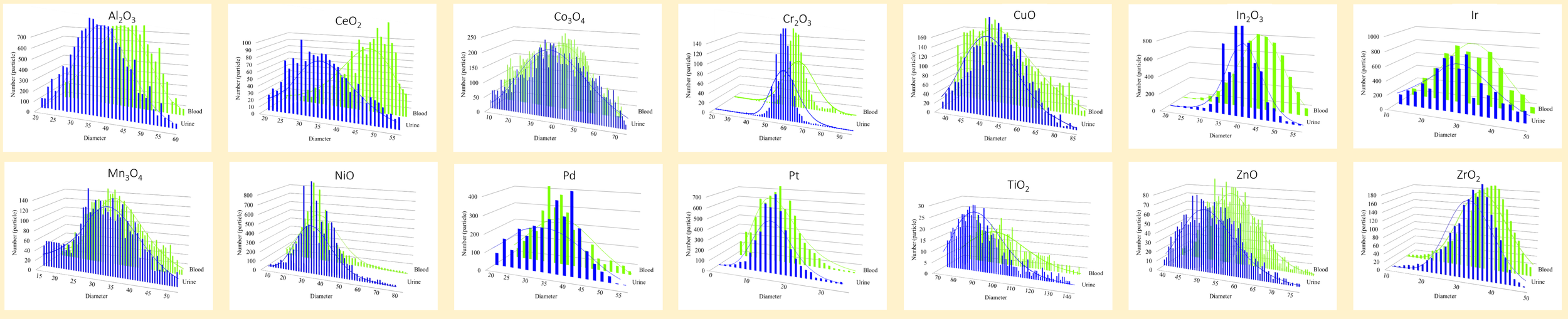
Table 2. Instrumental parameters for SP-ICP-MS data acquisition

Parameter	Value
Instrument	iCAP-Q (Thermo Fisher)
Nebulizer; flow	Concentric cyclonic; 0.33 mL/min
Torch and Injector	Quartz torch and Quartz 2.0 mm injector
RF Power	1450 W
Dwell time	5 msec
Acquisition mode	Q-Cell in KED (4.8 mL/min He), 60 s
Analytes	^{27}Al, ^{140}Ce, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{115}In, ^{192}Ir, ^{55}Mn, ^{60}Ni, ^{106}Pd, ^{195}Pt, ^{47}Ti, ^{66}Zn, ^{91}Zr
Density (g/cm^3)	Al_2O_3, 3.95; CeO_2, 7.22; Co_3O_4, 6.11; Cr_2O_3, 5.22; CuO, 6.31; In_2O_3, 7.18; Ir, 22.14; Mn_3O_4, 4.86; NiO, 6.67; Pd, 12.0; Pt, 21.4; TiO_2, 4.23; ZnO, 5.61; ZrO2, 5.68

Table 3. In-house validation parameters

Parameter	Value
LoD	in diameter (nm) was automatically generated by the software in concentration (particles/mL) was calculated with the 3σ threshold criteria on 5 different measurements of matrix.
Accuracy	on size (%) was assessed by the ratio between the size certified by supplier and that found by the SP-ICP-MS analysis.
Repeatability	on diameter (nm) and number of particles/mL was calculated as the relative standard deviations (RSDs%) on 5 replicated measurements in matrix
Linearity range	in terms of R^2 of the calibration curve between number of particles/mL and the increasing of concentration.

Particle size distribution in urine and blood



In-house validation results

Figure 2. LoD in size and concentration

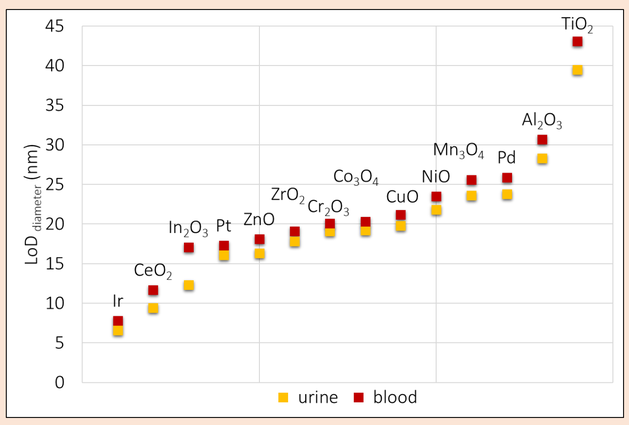


Figure 3. Measured diameter of MNMs respect to certified diameter

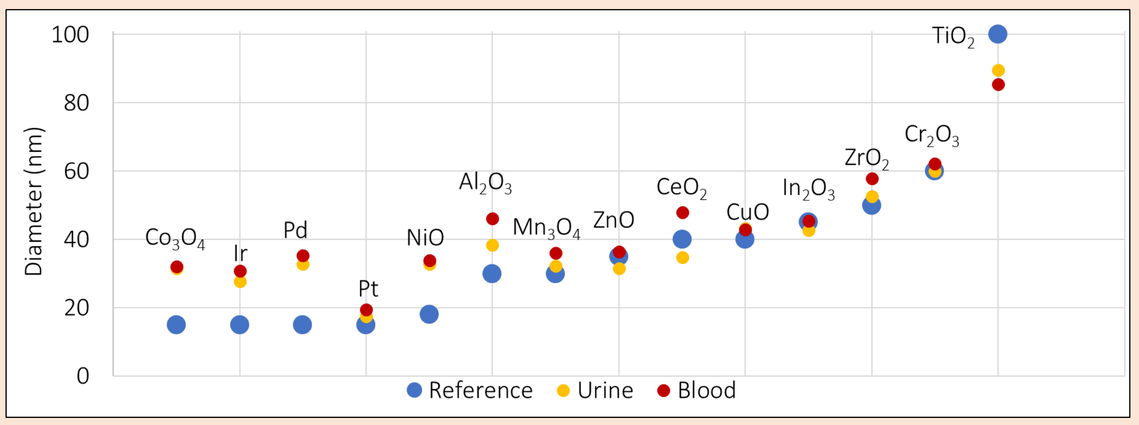
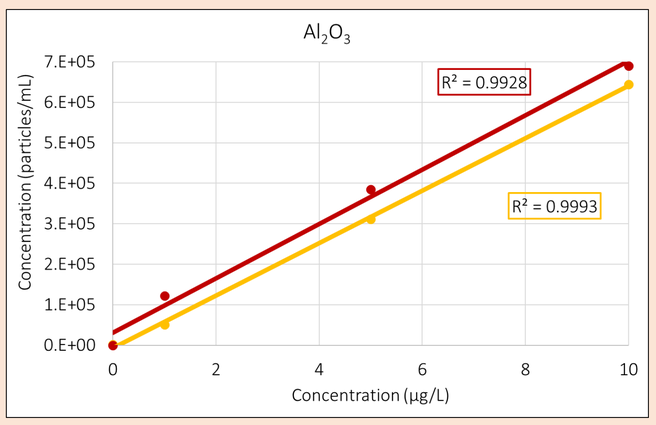
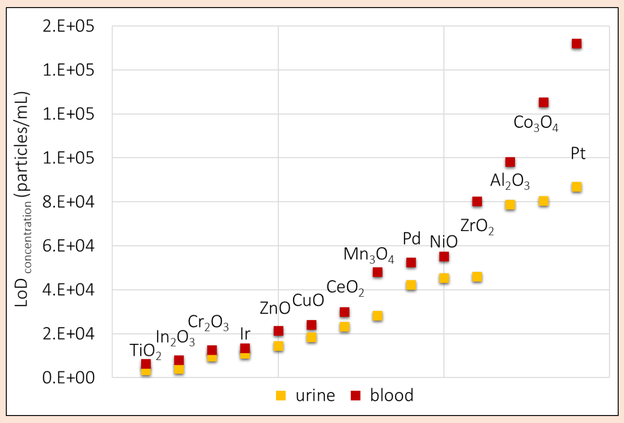


Figure 4. Example of linearity working range





- Size LoD (Figure 2) were low (6.6 nm) for some elements (Ir in urine) and higher (43.1 nm) for others (TiO_2 in blood)
- Particle count LoD (Figure 2) were low, e.g., ca. 3500 particles/mL in urine and 6000 particles/mL in blood for TiO_2
- Accuracy (Figure 3) was <±20% for CeO_2, Cr_2O_3, CuO, In_2O_3, Mn_3O_4, Pt, TiO_2, ZnO and ZrO_2; accuracy for Al_2O_3, Co_3O_4, Ir, NiO, Pd was inappropriate due to certified size too close to method LoD or uncontrolled formation of aggregates/agglomerates
- Repeatability in diameter (RSD<8.7%) and particle counting (RSD<9.8%) were obtained in urine and blood for all MNMs
- Particle number increased linearly (Figure 4) in the range 0.10-10.0 µg/L with the R^2 values between 0.990 and 0.999

Conclusions

The success of the SP-ICP-MS method validated in this study lies on the following aspects:

- ❑ simultaneous determination of composition, concentration (particles/mL) and size (nm)
- ❑ sample preparation simple without pre-concentration steps
- ❑ speed of analysis (60 sec) for routine measurements
- ❑ high instrumental sensitivity, accuracy and repeatability

Overall, this work identifies the MNMs (no. 14) to which the validated SP ICP-MS approach can be applied, in order to enable quantification of very small MNMs at low concentrations in human biomonitoring matrices

The method validated can be applied for the assessment of human exposure to MNMs both for the general population, consumers and workers

Acknowledgement

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References

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