Determination of trace elements in nanoparticle form in food and biological matrices

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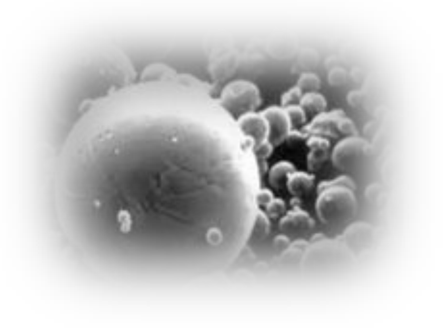
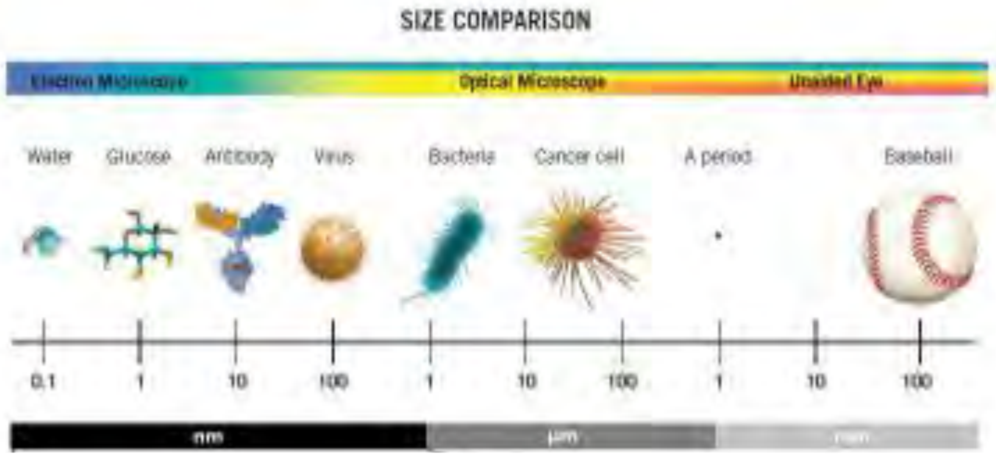


Workshop ‘Trace elements in food: application with ICP-MS and ICP-MS/MS’
Piacenza 21 June 2019

Outline

- **Introduction to nanotechnologies**
- **Metrological issues**
- **ICP-MS-based techniques for NMs detection and characterization**
  - ✓ Single particle ICP-MS (sp ICP-MS)
  - ✓ Asymmetric flow field flow fractionation-ICP-MS (AF4-ICP-MS)
- **Detection of NMs in food and biological matrices**
- **Take home messages**

What is nanotechnology?



Nanotechnology applications in the food sector

Nanotechnology	Application	Function
Nanosensors	Nanosprays Hand-held devices	Binding and coloring micro-organisms; Detection of contaminants, mycotoxins and microorganism
Nano-sized additives	Organic or inorganic nano-sized additives for feed applications, inclusing removal of toxins in feed	Various (including nutritional additives) with lesser amounts needed for a specific function
Nanopesticides	Nano-emulsions, encapsulates Triggered release nano-encapsulates	Increased efficacy and water solubility Triggered (local) release
Other nanosized agrochemicals	Nano-sized fertilisers, biocides, veterinary medicines	Improved delivery of agrochemicals in the field, better efficacy, better control of application/dose, less use of solvents in agricultural spraying

Agricultural production

Nanotechnology applications in the food sector

Nanotechnology | Application | Function

Nanotechnology	Application	Function
Processed nano-structured or nano-textured food products	Nanoemulsions, micelles	Use of less fat and emulsifiers, stable emulsions, better tasting food products
Nano-sized additives	Organic or inorganic nano-sized additives for food and health-food applications	Various, but lesser amounts would be needed for a function or a taste attribute, better dispersability may also occur
Nanoencapsulates	Nano-carrier systems in the form of liposomes or biopolymer-based nano-encapsulated substances	Providing protective barriers, flavour and taste masking, controlled release, and better dispersability for water-insoluble food ingredients and additives
Nutritional supplements and nutraceuticals	Nano-ingredients and additives Nano-carrier systems for delivery of nutrients	Enhanced absorption and bioavailability of nano-sized ingredients in the body
Nanofilters	Nanofiltration (e.g. porous silica, regenerated cellulose membranes)	Filtration of water and removal of some undesired components in food, such as bitter taste in some plant extracts

Food processing

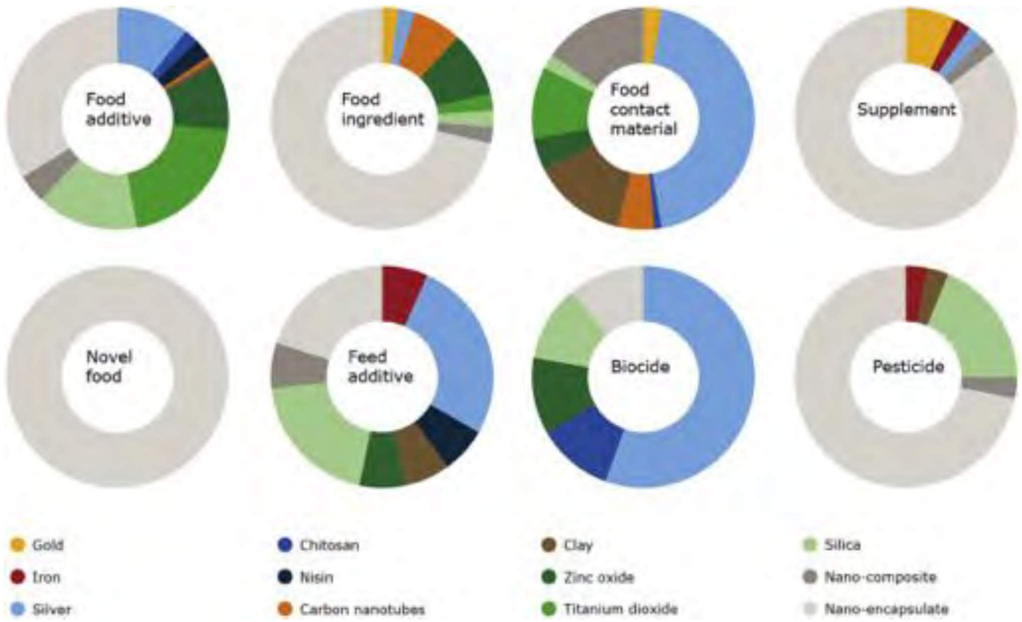
Nanotechnology applications in the food sector

Nanotechnology | Application | Function
Nanotechnology | Application | Function

Nanotechnology	Application	Function
Nanocomposites	Incorporating nanoparticles into a polymer to form a composite	Improving strength of materials, durability, barrier properties, biodegradation
Nano-coatings	Incorporating nanomaterials onto the packaging surface	Improving barrier properties
"Active" FCMs	Incorporating active nanoparticles with intentional release into- and consequent effect on the packaged food	Oxygen scavenging, prevention of growth of pathogens
"Intelligent" packaging materials	Incorporating nanosensors for food labelling	Detection of food deterioration, monitoring storage conditions

Packaging & storage

Nanotechnology in the agri-food industry



Ag
Au
Al_2O_3
$CaCO_3$
Se
SiO_2
MgO
TiO_2
TiN
ZnO
Fe_xO_y
nanoclays

- ❑ Sistematic literature review listed the most frequently used types of NM in applications in agri/feed/food

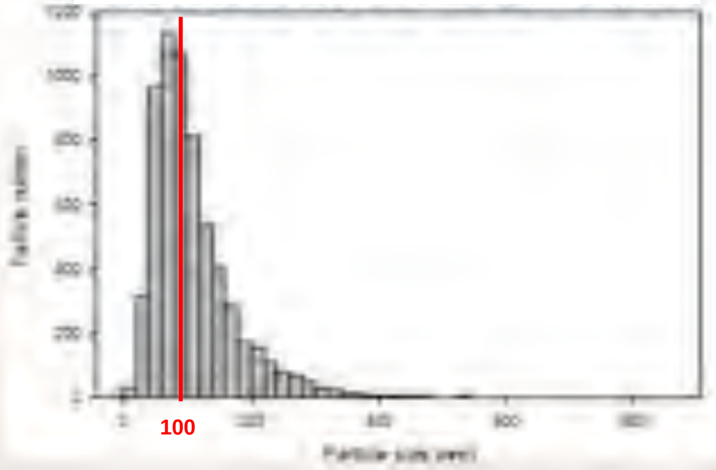
RIKILT and JRC, 2014. EFSA Supporting Publication 2014; 11(7):EN-621, 125 pp.

Terminology: ENMs, MNMs, assemblies of particles

Useful ISO terms

The International Organisation for Standardization ha defined the term ‘‘nano-object’’ as the generic term fc all discrete nanoscale objects (material with one, two c three external dimensions in the nanoscale)

Particle size distribution



Overarching European Commission’s definition of nanomaterial for legislative and policy purposes (Recommendation 2011/696/EU)

“a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

Percentage of particles with one or more external dimensions is in the size range 1-100 nm

THE NEW ONE CAN APPEAR AT ANY MOMENT

Metrological issues

The term “**identity**” consists of two parts, namely **size** and **chemical composition**

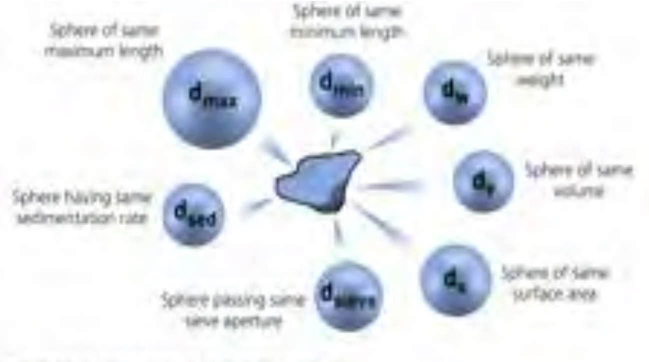


Illustration of the concept of equivalent spheres.

PSD relevant metric for regulatory purposes (legally binding)

diameters are either average, median, or modal values obtained from the number-based size distribution)

DLS: *hydrodynamic diameter* of particles (expressesd as diameter of a spherical particle that has the same Brownian motion behaviour in suspension)

CLS: *sedimentation behaviour* of a particle and expresses size as diameter of a sphere of equal sedimentation properties

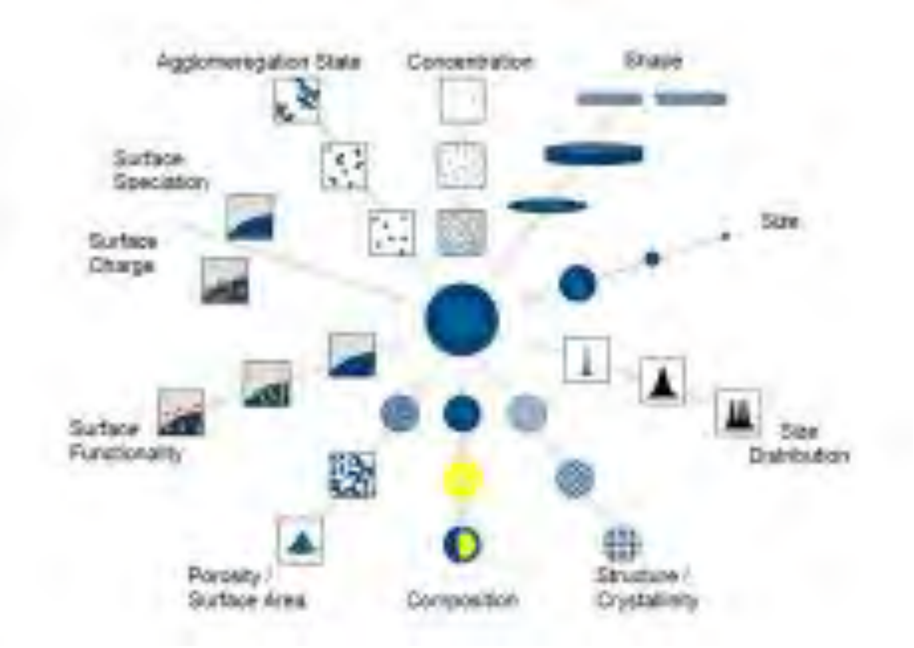
Linsinger TPJ et al. (2013) Food Chem 138, 1959

Physicochemical characterization of nanomaterials

- **Many parameters** play a major role in NM properties and **characterization of nanomaterials**, even in their pristine state, **is not trivial**

- **Electron microscopy** (e.g. TEM, SEM) is essential for NMs imaging (*e.g.* investigation of morphology) but **is costly and hardly provides quantitative data in complex samples**

- A **multi-method approach** is preferred and the use of **robust techniques more readily available** in most laboratories is targeted nowadays



Focusing on key techniques

Techniques for measuring size distributions of inorganic NMs

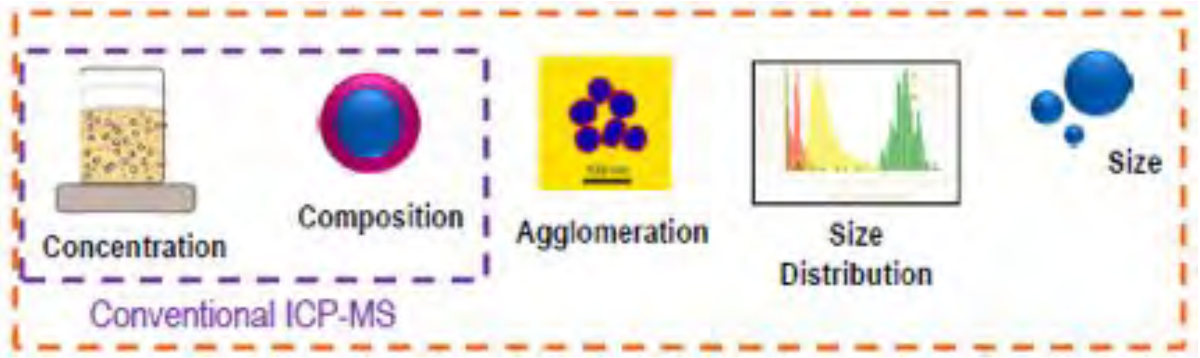
- **Centrifugation-based techniques**
  - Centrifugal particle sedimentation (CPS)
  - Analytical ultracentrifugation (AUC)
- **Laser light-scattering techniques**
  - DLS (*Dynamic light scattering*)
- **Electron microscopy**
  - SEM ~~(*Scanning electron microscopy*)~~
  - TEM (*Transmission electron microscopy*)
- **Hyphenated ICP-MS based methods**
  - HDC-ICP-MS (*Hydrodynamic chromatography* ICP-MS) **Also chemical identitiy**
- **FFF-ICP-MS** (*Field Flow Fractionation* ICP-MS) **Also chemical identitiy**
- ***single particle* ICP-MS Also chemical identitiy**

There is **no single technique** that is able to satisfactorily and routinely measure the number particle size distribution of objects in the 1–100 nm size range

ICP-MS: an essential tool for characterization of inorganic NMs

- Inductively coupled plasma mass spectrometry **(ICP-MS)-based** techniques are becoming **essential tools** to cope with the **quantitative characterization** of inorganic NMs (iNMs)

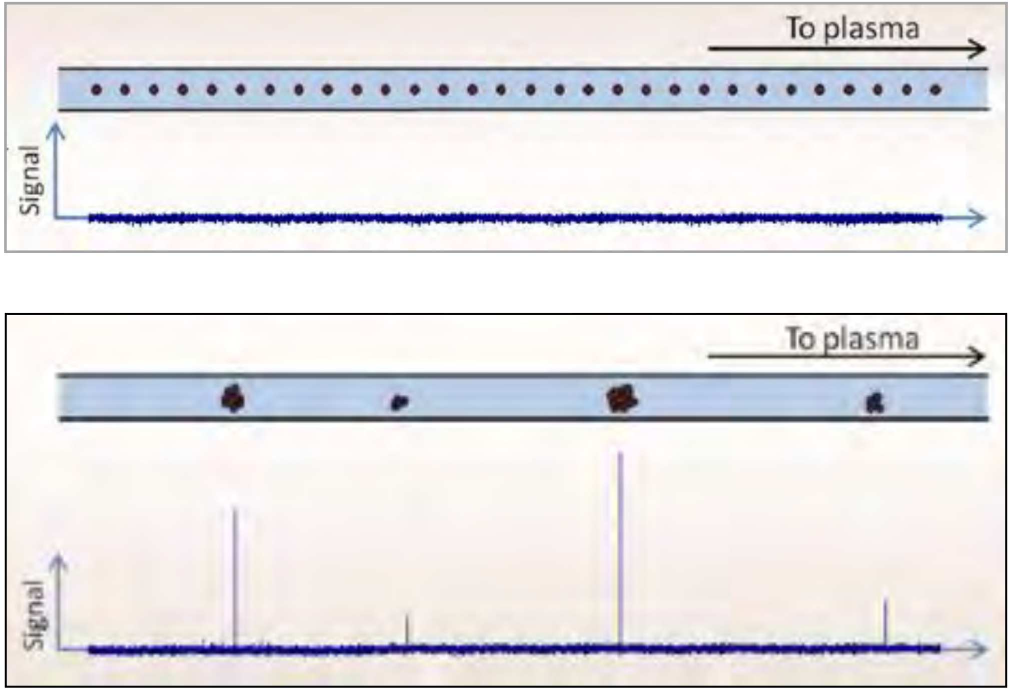
- ICP-MS-based techniques are attractive since they are able not only to measure **size and size distributions**, but also to determine the **chemical identity** of the particles at the same time
  - ✓ **Single particle ICP-MS (sp ICP-MS)**
  - ✓ **Asymmetric flow field flow fractionation-ICP-MS (AF4-ICP-MS)**



Single-particle ICP-MS and FFF-ICP-MS

Single particle ICP-MS

Time resolved analysis of very diluted NP dispersions using short dwell times (≤10 ms)

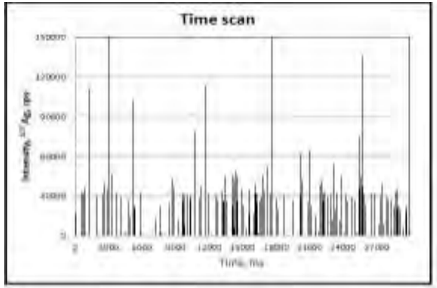


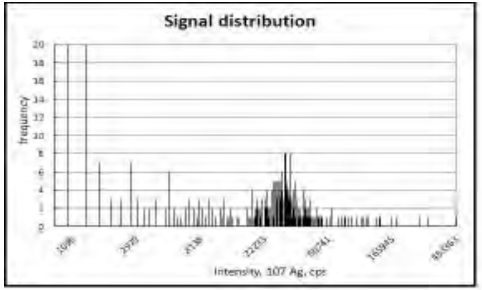
- ❑ **sp ICP-MS: element-specific method** for counting particles

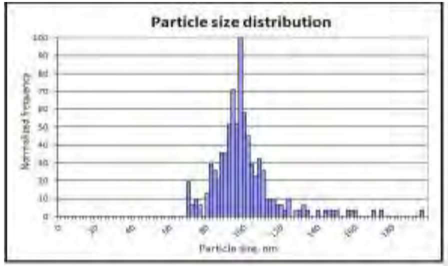
- ❑ **Accurate size determination is possible (spherical assumption)**

- ❑ The particle concentration determines the frequency of the signal peaks

Single particle ICP-MS: metrics





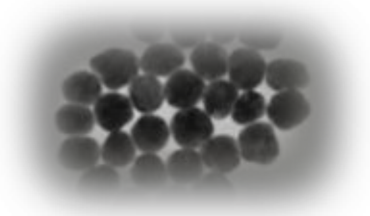


$i_{NP}=Kd^3$

- ✓ **NP average size**
- ✓ **NP size distribution**
- ✓ **NP number concentration**
- ✓ **NP mass concentration**
- ✓ Ability to distinguish between **dissolved ions** and **particulate matter**

EU

Interlaboratory studies on single particle-ICP-MS determination of Ag NPs in: (1) aqueous or ethanol dispersions and (2) in chicken meat



Use of μs dwell time and Sampling multiple data points per particle

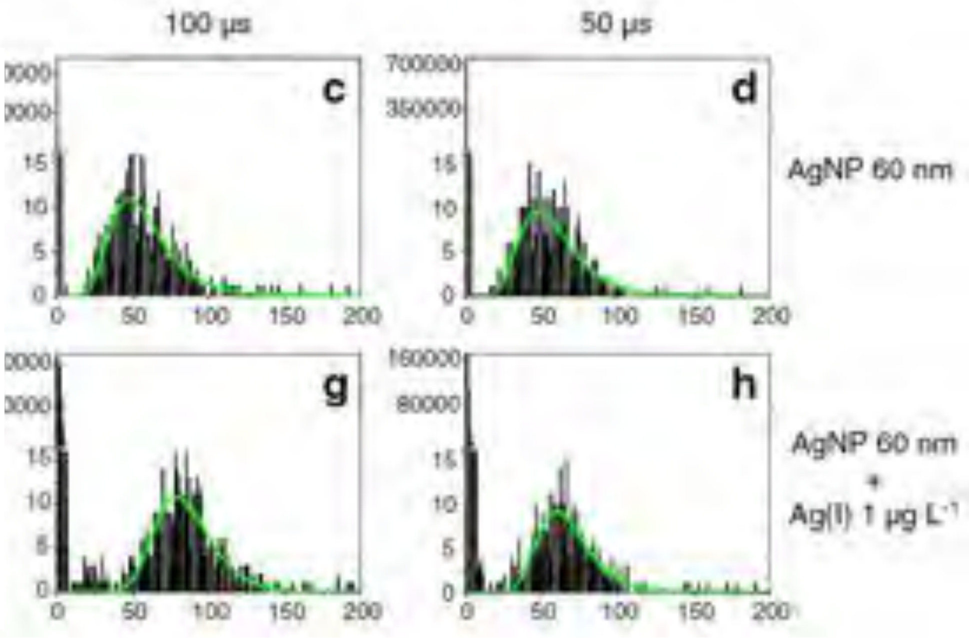
In sp ICP-MS, transient data acquisition speed consists of two parameters:

- **dwell time** (reading time)
- **settling time** (overhead and processing time)

Dwell time and the effect of the dissolved species

10 ms 5 ms

Dwell time	LOD_{size} (nm) Ultrapure water	LOD_{size} (nm) 1 µg L^{-1} Ag(I)
10 ms	17	34
5 ms	16	30
100 µs	14	17
50 µs	14	16



Size limits of detection for silver nanoparticles decreased in the presence of different concentrations of dissolved silver (I)

Anal Bioanal Chem (2016) 408:5089

Size characterisation of particulate food additives and nutrient sources

Materials studied:

Anatase and rutile TiO_2 (food colours, E171)
Yellow iron oxide (FeO(OH)) (food colour, E172)
Red iron oxide (Fe_2O_3) (food colour, E172)
Zinc oxide (a source of zinc in food supplements)

Aim:

Size characterization of these real-world particulate materials **as such (i.e. as pristine materials) and in food**, focusing on the **nanosized fractions** (% of particles <100 and <200 nm)

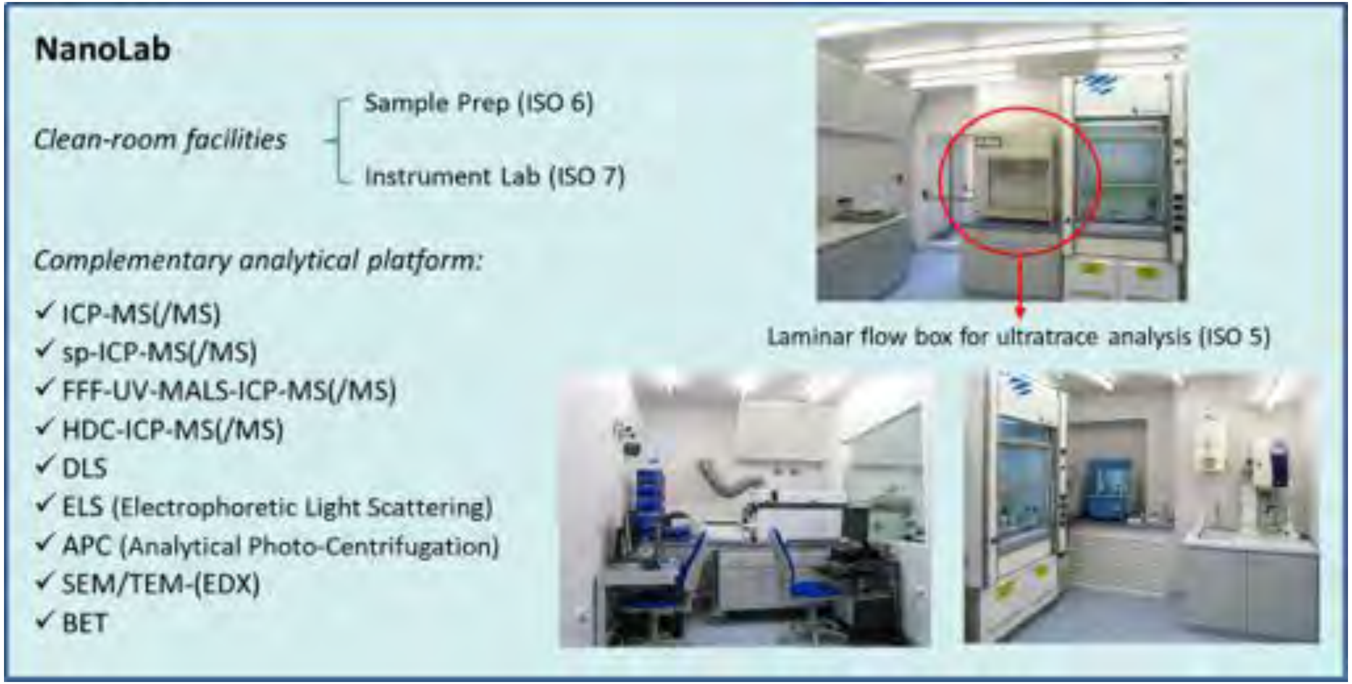
Instrumentation and analytical conditions:

Analyses performed with a PerkinElmer Nexion 350D, equipped with a Nanomodule, using dwell times ≤ 100 µs

The pristine materials were studied after dispersion with a standardized protocol (Nanogenotox)

Nanomaterial characterization facility @ ISS

To deal with the multifaceted challenges posed by the **characterization of nanomaterials** and their **analytical determination in complex matrixes** such as food and biological samples



Reproductive/endocrine effects on low dose, short-term exposure to TiO_2

Single particle ICP-MS used to assess deposition in particulate form

Successful detection of the tiny deposition in spleen of TiO_2 NP agglomerates (130 nm average size) after short-term oral exposure to low doses of TiO_2 NPs

Nanotoxicology, 2013; Early Online, 1–9
© 2013 Informa UK, Ltd.
ISSN: 1743-5390 print / 1743-5404 online
DOI: 10.3109/17435390.2013.822114
informa healthcare

Oral, short-term exposure to titanium dioxide nanoparticles in Sprague-Dawley rat: focus on reproductive and endocrine systems and spleen

Roberta Tassinari[1], Francesco Cubadda[1], Gabriele Moracci[1], Federica Aureli[1], Marilena D'Amato[1], Mauro Valeri[1], Barbara De Berardis[1], Andrea Raggi[1], Alberto Mantovani[1], Daniele Passeri[2], Marco Rossi[2,3]

Agreement with SEM/EDX, but sp ICP-MS provided quantitative data

SEM/EDX

Reproductive/endocrine effects on low dose, short-term exposure to TiO_2

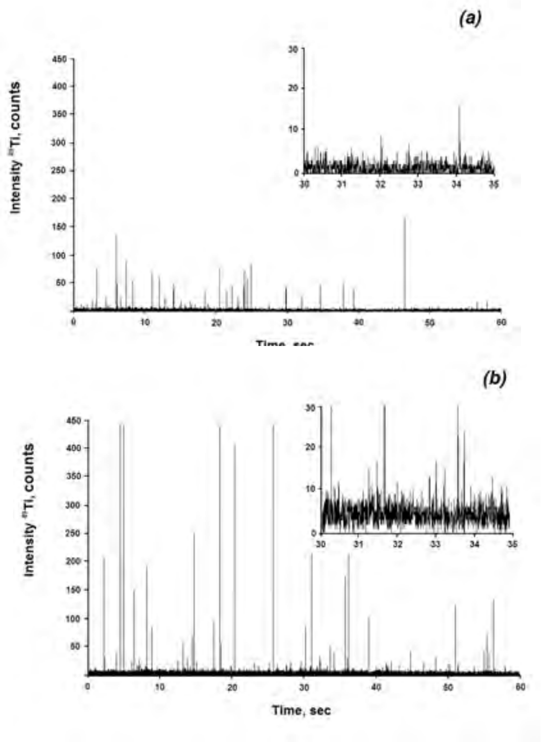
Continued: Single particle-ICP-MS on enzymatic extracts of spleen tissue

In treated animals, average **size** of the detected particles ca. **130 nm**

Mass concentration, control-corrected and expressed as Ti:

0.007 ± 0.002 µg/g spleen tissue

in accordance with the results of total Ti in digested tissues



Inhalation exposure of pregnant mice to Ag NPs: effects on foetuses

Demonstration that inhalation exposure of pregnant mice to Ag NPs results in particles reaching the placenta and thereby in exposure of embryos


NANOTOXICOLOGY, 2017
VOL. 11, NO. 5, 687–698
https://doi.org/10.1080/17435390.2017.1343875
Taylor & Francis
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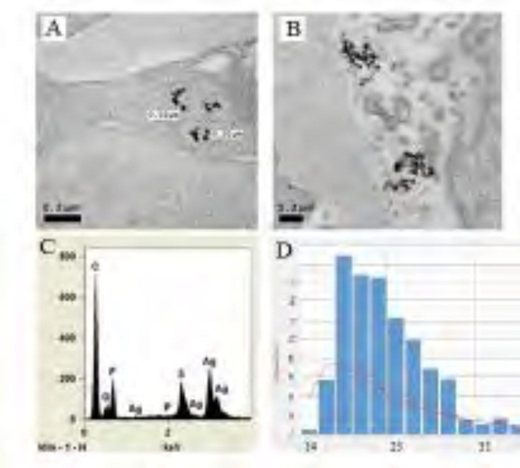
ORIGINAL ARTICLE
Check for updates
Silver nanoparticles inhaled during pregnancy reach and affect the placenta and the foetus



A & B: TEM images;

C: EDX spectrum;

D: spICP-MS size distribution (TMAH extracts)

Ag nanoparticles in mouse placenta after inhaltion exposure (*nose-only*) during the first 15 days of gestation

Agreement with TEM, but sp ICP-MS provided quantitative data

SAMPLE PREPARATION IS CRITICAL
Need to extract particles from biological tissues, ideally without altering their state

Field flow fractionation

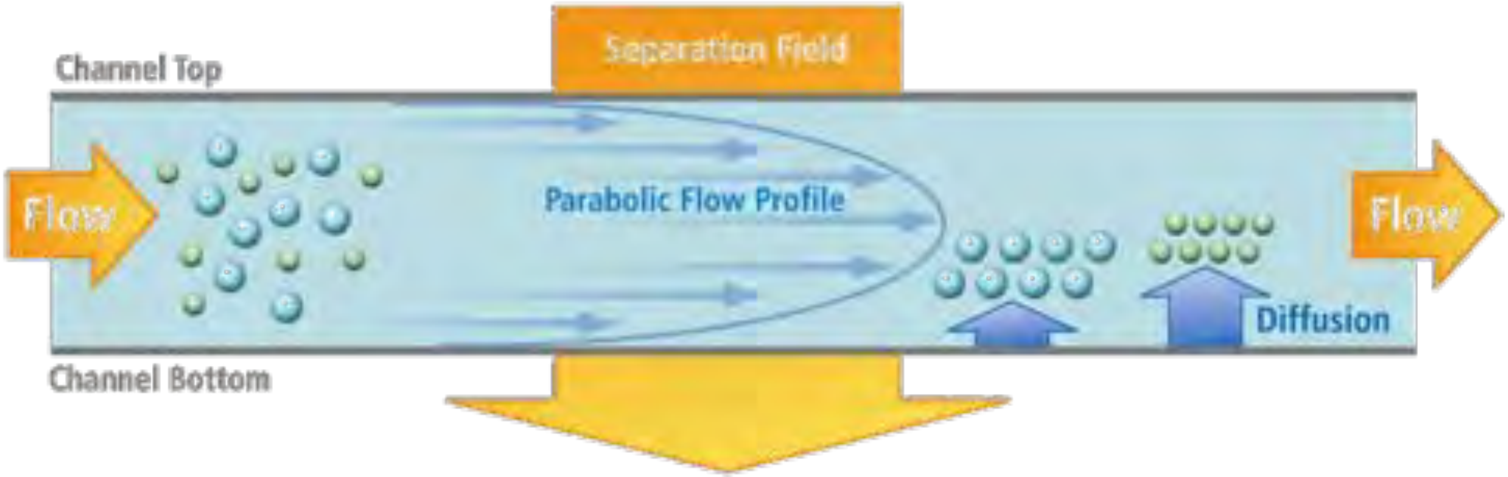
Separation range

- ❑ Thermal Field
- ❑ Sedimentation Field
- ❑ Gravitational Field
- ❑ Flow Field

Courtesy of Postnova Analytics

Asymmetric flow field flow fractionation

Separation Mechanism

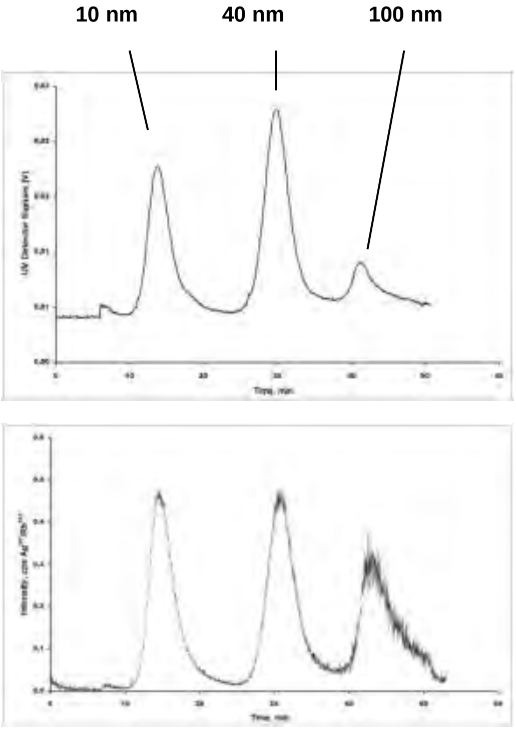


- ❑ Gentle separation, no interaction with a stationary phase (important since NMs are reactive and stick to surfaces): **fractionation of particles according to their size**

- ❑ **Enormous potential** due to the possibility for **on-line combination** with **optical** detectors (size determination by MALS, DLS, UV abs.) and **ICP-MS** (elemental detection and quantification)

First interlaboratory study on AF4-ICP-MS 'Determination of Ag particles'

Organized by European Commission (JRC-Ispra) in 2013

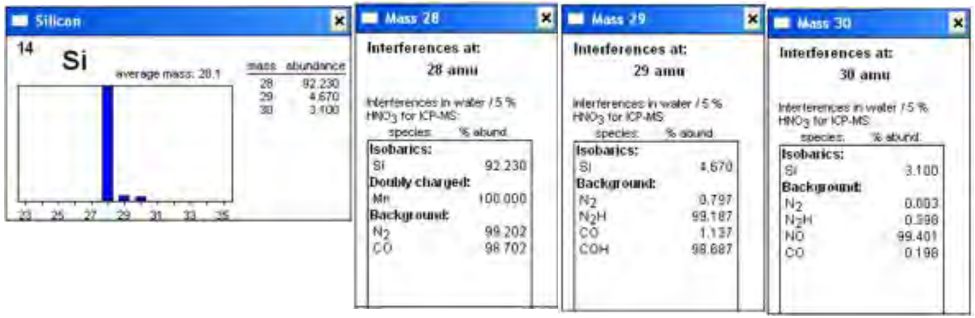


Standard bimodal and trimodal mixtures

First phase completed- method optimization

Challenges of the determination of nanosized oxides such as SiO_2

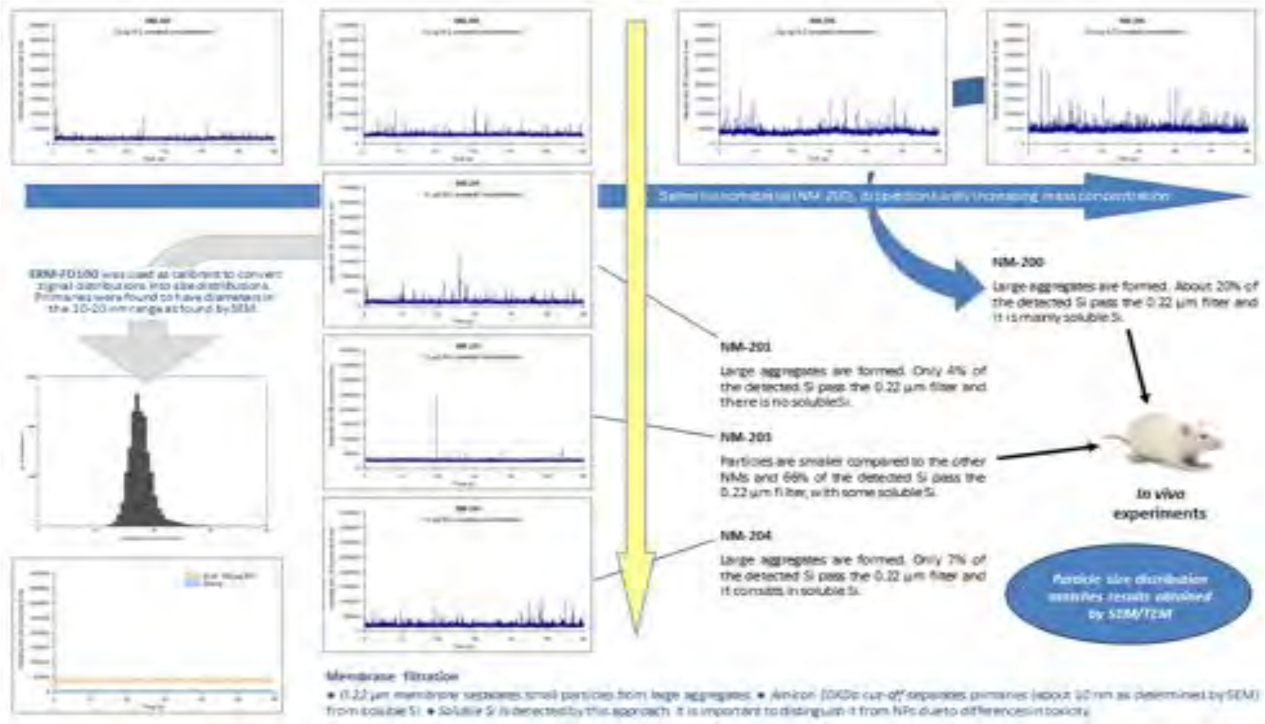
- A limitation of ICP-MS-based techniques is represented by the determination of certain oxides such as SiO_2 and TiO_2 that is hampered by significant analytical challenges
- Sensitive and accurate silicon determination in standard, i.e. single quadrupole, mode is precluded owing to polyatomic interferences affecting the three naturally occurring isotopes



- The use of ICP-MS systems equipped with dynamic reaction cell (ICP-DRC-MS) or tandem mass spectrometry (ICP-MS/MS) does represent a viable solutions for the interference-free quantitative determination of SiO_2 in different sample types

Time resolved analysis as characterization tool

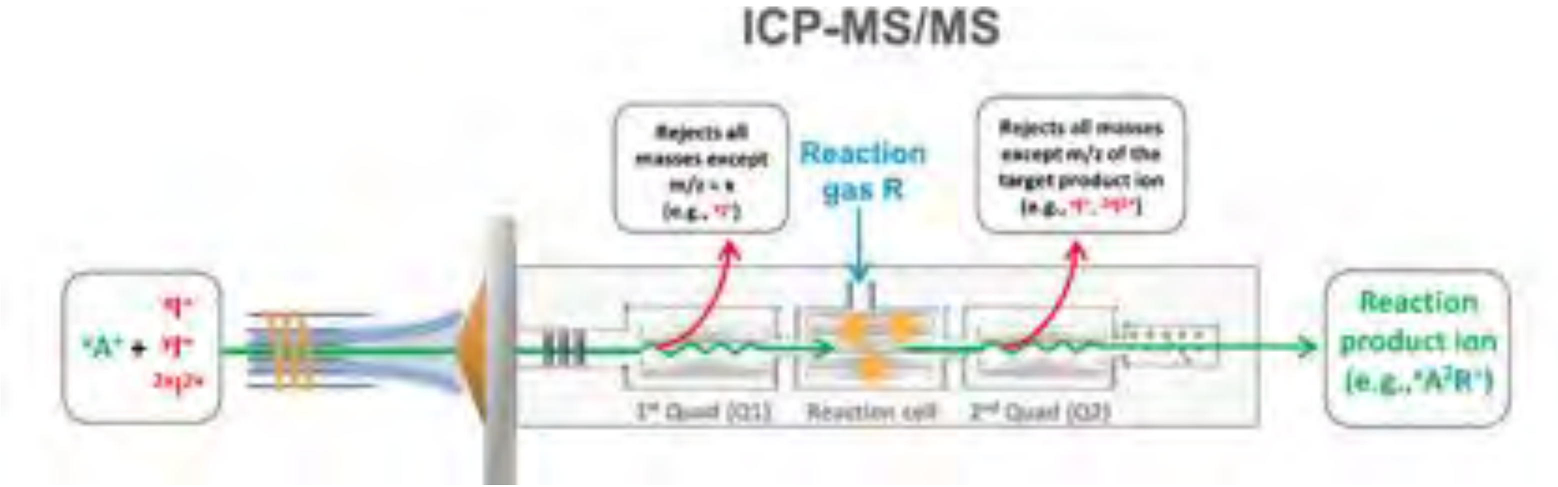
Investigating agglomeration and dissolution of silica nanoparticles in aqueous suspensions



Aureli et al. (2012) J Anal At Spectrom 27, 1540

Removal of interferences affecting Si detection by ICP-MS/MS

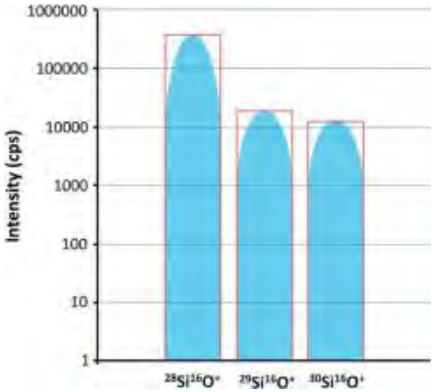
- The ICP-MS/MS can be used in mass-shift mode to selectively shift the target analyte ion away from intense spectral interferences at the analyte ion's original native mass



Interferences affecting Si detection removed by ICP-MS/MS

- O_2 was added as a reactive gas into the octopole cell to convert the Si^+ ions (Q1) into SiO^+ ions, which were measured at the corresponding m/z ratios of 44, 45 and 46 (Q2)

- In the MS/MS mass-shift mode, after optimization of the O_2 flow rate the signal intensities obtained at m/z ratios of 44, 45 and 46 followed the natural isotopic pattern of Si



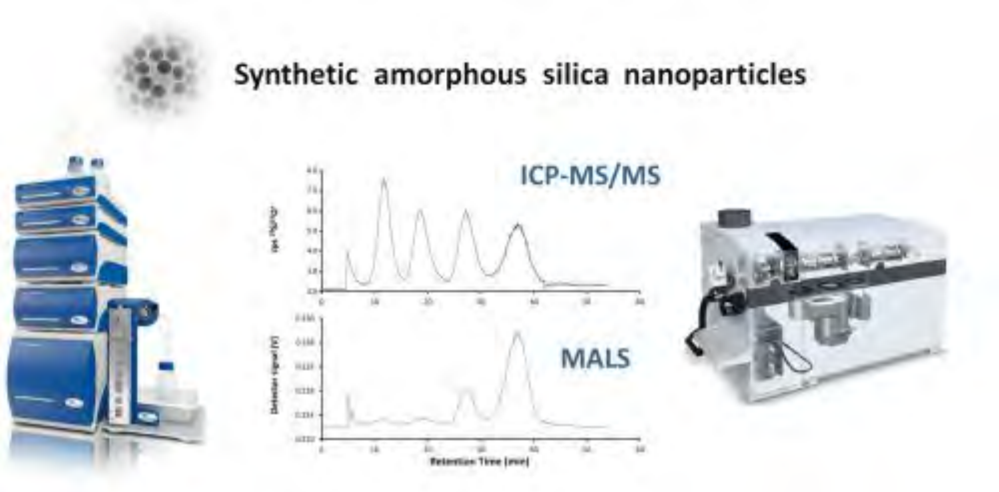
Obtained (blue peaks)[a] and theoretical (red boxes) silicon isotopic pattern.
[a]Corrected to account for the oxygen isotopic composition and the contribution of $^{28}Si^{17}O^+$ at m/z =45 and of $^{28}Si^{18}O^+$ at m/z=46

Precursor and Product		LoD	BEC µg/L
[illegible]	[illegible]	[illegible]	2.8
[illegible]	[illegible]	[illegible]	3.0
Si → Si O	0.9994	0.50	6.1

Satisfactorly applied to complex matrices, *i.e.* bovine liver spiked with soluble silicon

Aureli et al J Anal At Spectrom 30, 1266 (2015)

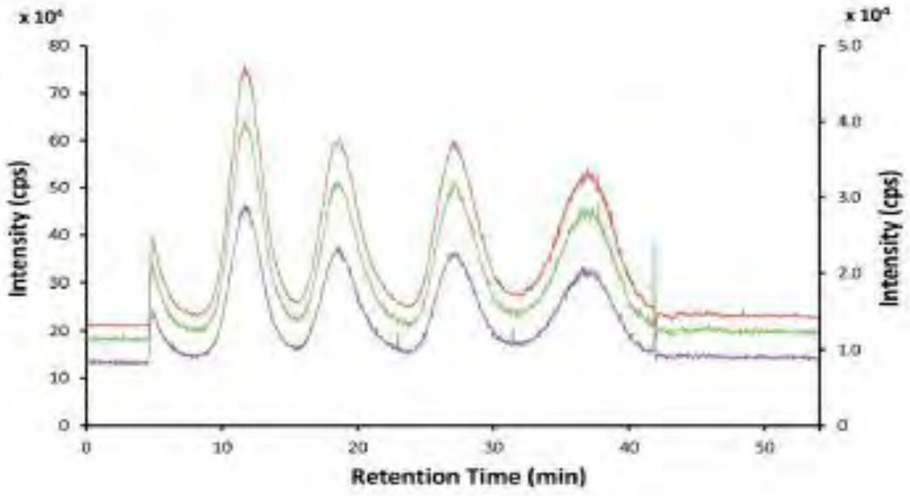
Asymmetric flow field flow fractionation-ICP-MS/MS



Novel method for the simultaneous determination of **particle size** and **mass concentration** of silica nanoparticles by asymmetric flow field flow fractionation coupled with **online** (i) **multiangle light scattering (MALS)** and (ii) **ICP-MS/MS** detection

Aureli et al. J Anal At Spectrom 30, 1266 (2015)

Asymmetric flow field flow fractionation-ICP-MS/MS



The method opens the way to studies involving the use of isotopically enriched silica NPs

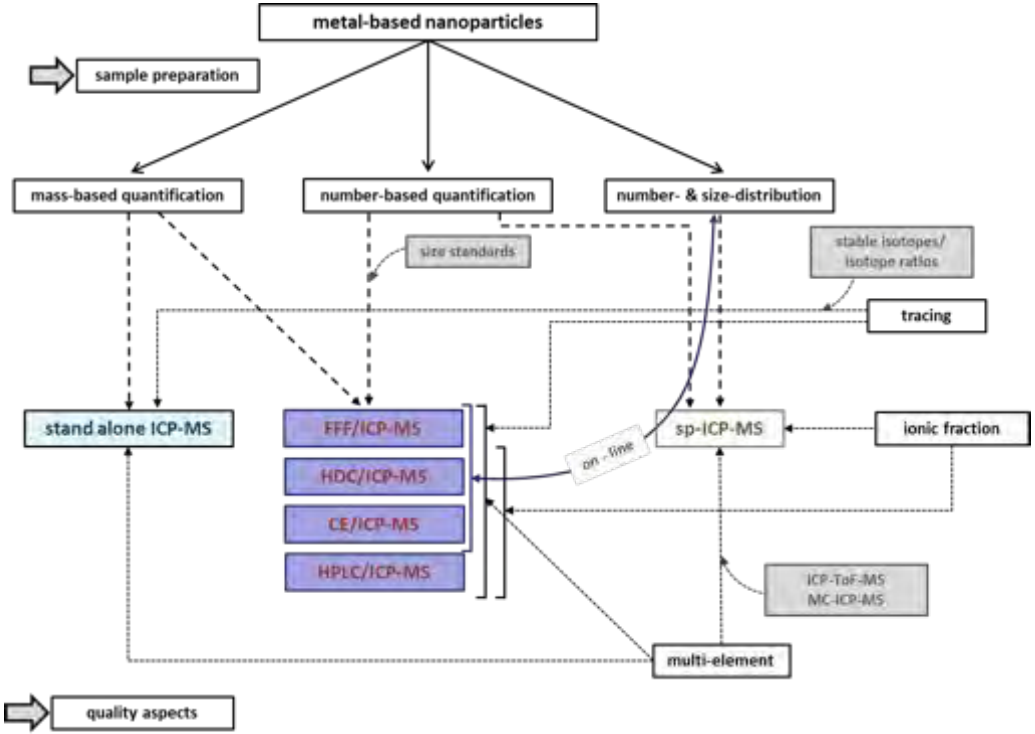
FFF-ICP-MS/MS of a quadrimodal mixutre of silica NP's obtained by recording the signals of $^{28}Si^{16}O^+$ (red, left axis) , $^{29}Si^{16}O^+$ (green, right axis), and $^{30}Si^{16}O^+$ (blue purple, right axis)

- ❑ Element-specific detection by ICP-MS/MS using all the three silicon isotopes

Aureli et al. J Anal At Spectrom 30, 1266 (2015)

ICP-MS-based techniques

- Decision flow-chart for ICP-MS based techniques for nanoparticle analysis



Modified from Meerman et al. J Anal At Spectrom 33, 1452 (2018)

Take home messages

- ICP-MS based techniques are the most promising tools for routine characterisation and detection of inorganic NMs, also in complex matrices after proper sample preparation
- In single particle ICP-MS, use of µs dwell times is key to resolve particle signal from dissolved/background signal and improve size LoDs
- Use of µs dwell times may have to be combined with the use of ion–molecule chemistry for resolution of spectral interferences
- Combination of AF4 with a variety of detectors (UV, DLS, MALS) offers a powerful characterization tool also for organic NMs; ICP-MS is the ideal detector for inorganic ones
- Development of multi-technique approaches and combination of separation and sizing techniques appears to be the way forward