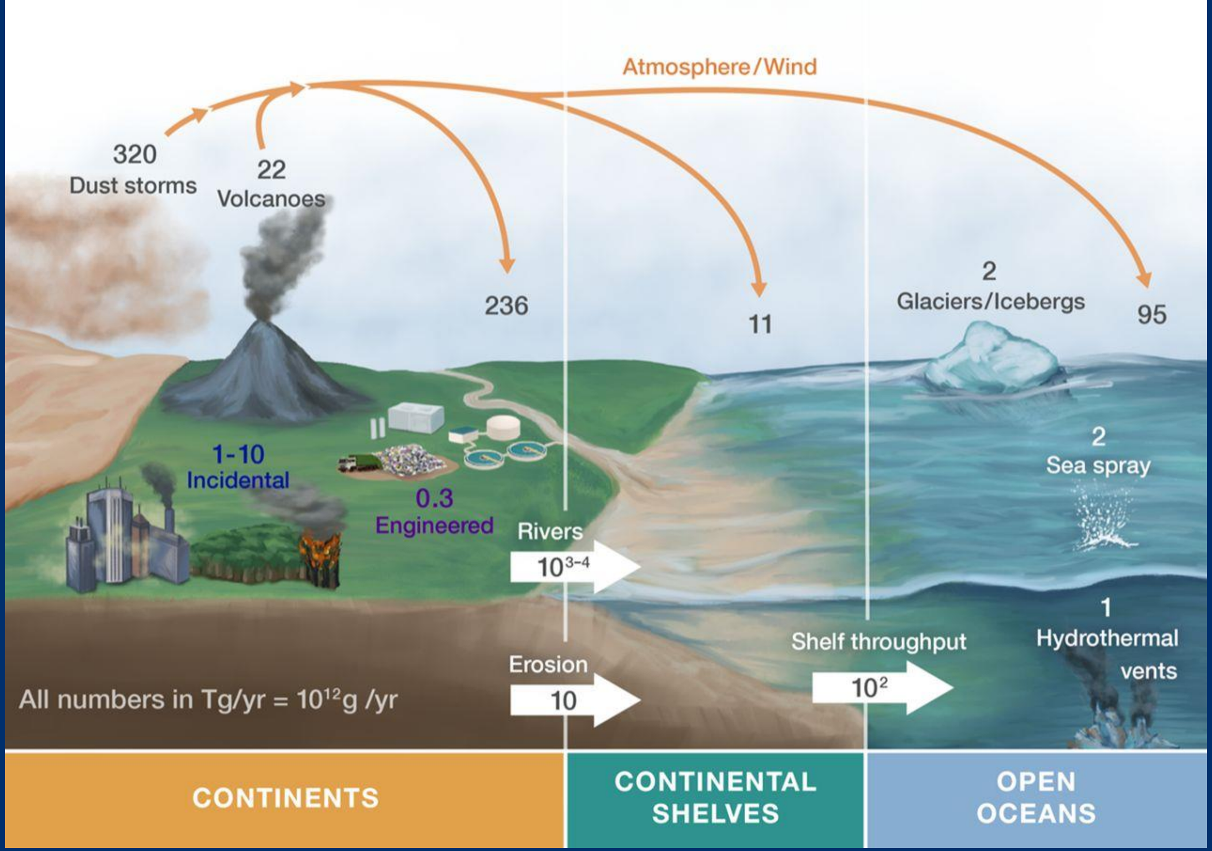
MACRAME Webinar 1: Pathways to Validation and Standardisation

Part B: Landscape and opportunities

Caterina Minelli

Natural, incidental, and engineered nanomaterials



M.F. Ochella et al., Science 363 (2019)

- natural => are not created directly through human actions;
- incidental => form unintentionally during human activities;
- engineered => created for specific applications

- Nanotechnology platforms offer unique opportunities for global challenges and their **performance** needs to be documented.
- Despite being a very small fraction of the global nanomaterial population, engineered nanoparticles are concentrated around humans and their **safety** needs to be understood.

Landscape

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Product quality and safety:

TSCA

Chemistry, food, drugs and medical devices regulation

Quality

Guidelines

Measurement methods for critical quality attributes:

Method development: BIPM, NMIs, academia, instrument manufacturers

Method validation/verification

Method standardisation

Nanotechnology -based product

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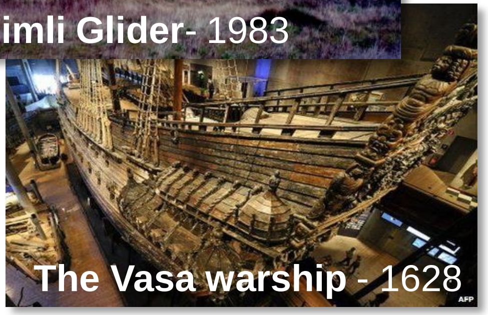
BIPM and CCQM

NPL

Notable accidents due to lack of agreement on units…

The Mars Orbiter - 1999

Gimli Glider- 1983



The Vasa warship - 1628

THE METRE CONVENTION
International convention established in 1875 with 51 member states in 2008.

CGPM CONFÉRENCE GÉNÉRALE DES POIDS ET MESURES
Committee with representatives from the Metre Convention member states. First conference held in 1889 and meets every 4th year. Approves and updates the SI-system with results from fundamental metrological research.

CIPM COMITÉ INTERNATIONALE DES POIDS ET MESURES
Committee with up to 18 representatives from CGPM. Supervises BIPM and supplies chairmen for the Consultative Committees. Co-operates with other international metrological organisations.

- CEN*
- IEC*
- ISO*
- Others

BIPM BUREAU INTERNATIONAL DES POIDS ET MESURES
International research in physical units and standards. Administration of interlaboratory comparisons of the national metrology institutes and designated laboratories.

CONSULTATIVE COMMITTEES

CCAUV	CC for Acoustics, Ultrasound and Vibrations
CCEM	CC for Electricity and Magnetism
CCL	CC for Length
CCM	CC for Mass and related quantities
CCPR	CC for Photometry and Radiometry
CCQM	CC for Amount of Substance
CCRI	CC for Ionising Radiation
CCT	CC for Thermometry
CCTF	CC for Time and Frequency
CCU	CC for Units

NMI
NATIONAL MEASUREMENT INSTITUTES

The missions of the **CCQM** are:

- to contribute to the resolution of **global challenges**;
- to promote the **uptake of metrologically traceable** chemical and biological measurements;
- to **progress the state of the art** of chemical and biological measurement science;
- to improve efficiency and efficacy of the **global system of comparisons** for chemical and biological measurement standards it conducts;
- to **meet stakeholders needs**

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Task Group on Particle Metrology

NPL

CCQM

Working Groups

Select

View

Joint CCQM-IAWG/SAWG Task Group on Particle Metrology (CCQM-IAWG-SAWG-TG-PARTICLE)

Chair

Dr Caterina Minelli
National Physical Laboratory
United Kingdom

Terms of Reference

To identify activities that the IAWG and SAWG should undertake with respect to particle metrology over the next ten years, including pilot studies, key comparisons, and cooperative research projects. To accomplish this, the TG will:

- Examine the outcomes of the CCQM Workshop on Particle Metrology held 25-27 October 2022
- **Liaise with external stakeholders** to understand better the important needs and gaps in particle metrology that can be addressed by the IAWG and SAWG
- **Liaise with the CCL** WG-N to leverage knowledge and identify opportunities for cooperation between (nano)dimensional, chemical and biological activities with respect to particle metrology.

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Current standardisation efforts

- ISO/TC 229 – Nanotechnologies → *Liposome terminology, Doxyl loading, concentration*
- ISO/TC 24/SC4 - Particle characterization → *PTA, DLS, SAXS, analytical centrifugation*
- ISO/TC 201 - Surface chemical analysis → *Chemical analysis of nanoparticles*
- ISO/TC 276 – Biotechnology → *Nucleic acid synthesis, cellular analysis*

INTERNATIONAL
Standards Worldwide

- ASTM E56.08 – Nano-enabled medical products → *LNP lipid composition, PEG quantification, LNP characterisation*

- CEN/TC 352 - Nanotechnologies → *Nanoparticle agglomeration/aggregation*

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MACRAMÉ
Advanced Characterisation Methodologies to assess and predict the Health and Environmental Risks of Advanced Materials

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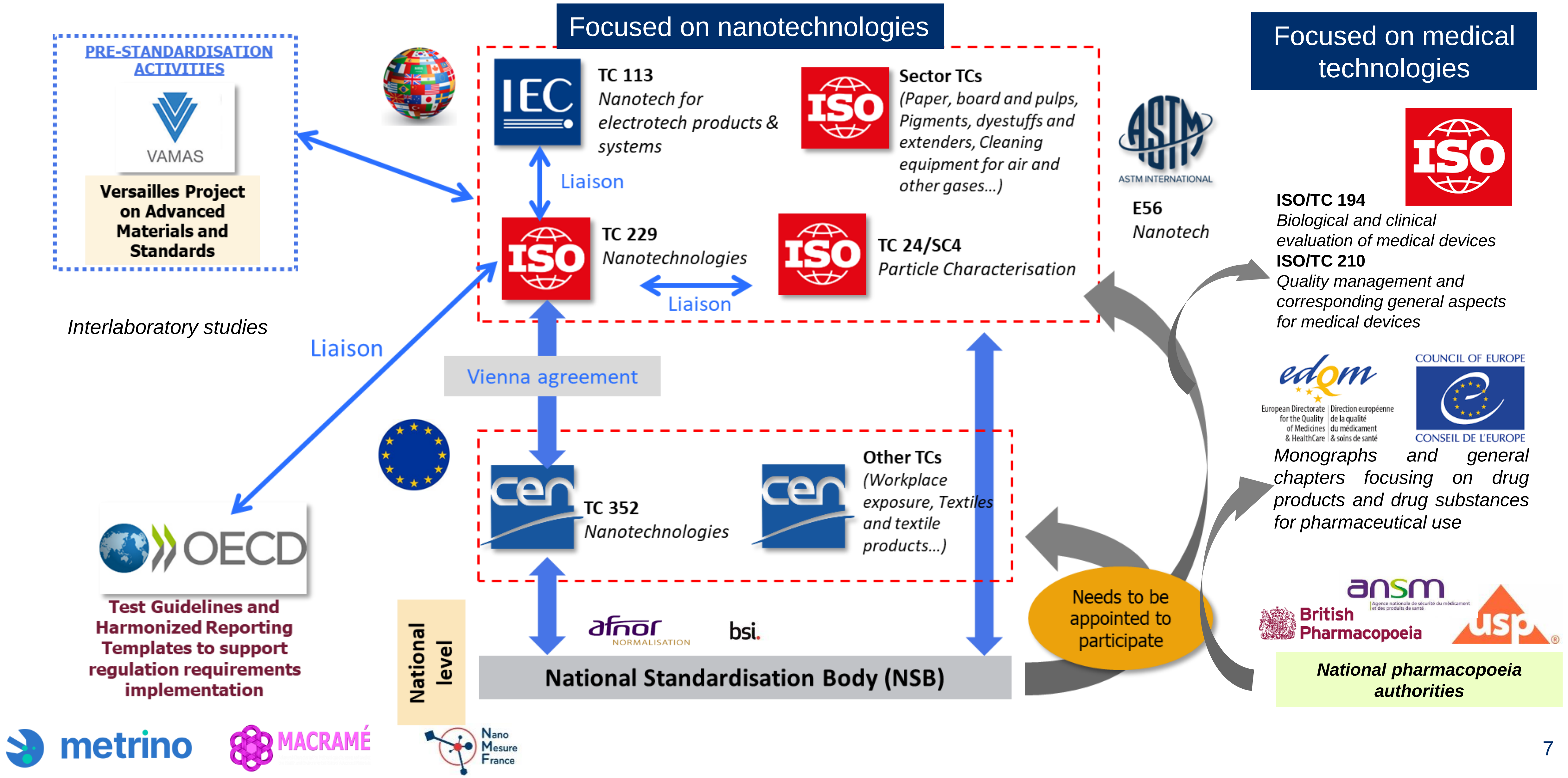
Advanced Materials and Nanomedicine landscape



VAMAS

Versailles Project on Advanced Materials and Standards: To promote world trade by innovation and adoption of advanced materials through international collaborations that provide the technical basis for harmonization of measurement methods, leading to **best practice, reference materials and standards**

1982 8th G7 Economic Summit

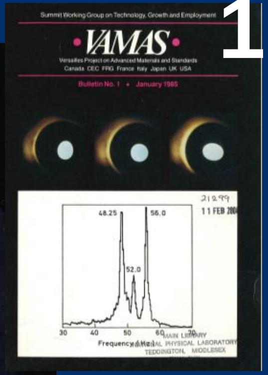
Canada . France . Germany . Italy . Japan . UK . USA . EC . Brazil . Mexico . Chinese Taipei . South Africa . Australia . Korea . India . China

1982 1983 2007 2008 2013

1983

NPL

1985



1987:

Results reported from first round-robin test (Wear test methods)

…celebrating 40+ years

First VAMAS technical groups:

- Wear Test Methods
- Surface Chemical Analysis
- Polymer Blends
- Ceramics

Currently active Technical Work Areas (TWA)

Surface Chemical Analysis	Quantitative Microstructural Analysis
Polymer Composites	Solid Sorbents
Superconducting Materials	**Synthetic Biomaterials**
Properties of Electroceramics	**Graphene and Related 2D Materials**
Creep, Crack and Fatigue Growth in Weldments	**Raman Spectroscopy and Microscopy**
Polymer Nanocomposites	Thermal Properties
Nanoparticle Populations	Self Healing Ceramics
Printed, flexible and stretchable electronics	**Micro and Nano Plastics in the Environment**

Benefits to stakeholders

- ❑ Insights into new standards for materials
- ❑ Insights into novel materials technologies
- ❑ Access to a global network of experts.

- ❑ Opportunity to define and learn best practice
- ❑ Develops skilled workforce and benchmark capability
- ❑ International agreement on testing and characterisation before standards are available

- ❑ Reduces risks of adopting advanced materials
- ❑ Accelerates the standardisation process
- ❑ Facilitates world trade in materials

VAMAS

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EURAMET

NPL

EMRP European Metrology Research Programme
Programme of EURAMET
The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

EMPIR EURAMET
The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

METROLOGY PARTNERSHIP EURAMET

EPM

236 projects
2014 up to 2020

7 **EMPIR** calls
EU contribution 300 M€

finalize projects

119 projects
2009-2013

5 **EMRP** calls
EU contribution 200 M€

finalize projects

2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 202

FP7 | Horizon 2020 | Horizon Europe

EURAMET coordinates the cooperation of National Metrology Institutes in Europe in research in metrology, traceability of measurements to the SI units, international recognition of national measurement standards and related Calibration and Measurement Capabilities (CMC).

Improving measurement science to drive innovation and competitiveness and to support societal challenges and regulation.

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Take-home message

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Landscape is complex but there are multiple opportunities for participation into standardisation.

Connection to EMP projects like Metrino (e.g. through ETPN) is an effective way to keep up-to-date with latest development and best practice in nanomedicine.

Acknowledgements

MACRAMÉ
Advanced Characterisation Methodologies to assess and predict the Health and Environmental Risks of Advanced Materials

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EUROPEAN PARTNERSHIP
Co-funded by the European Union
METROLOGY PARTNERSHIP
EURAMET

Department for Science, Innovation & Technology

National Physical Laboratory

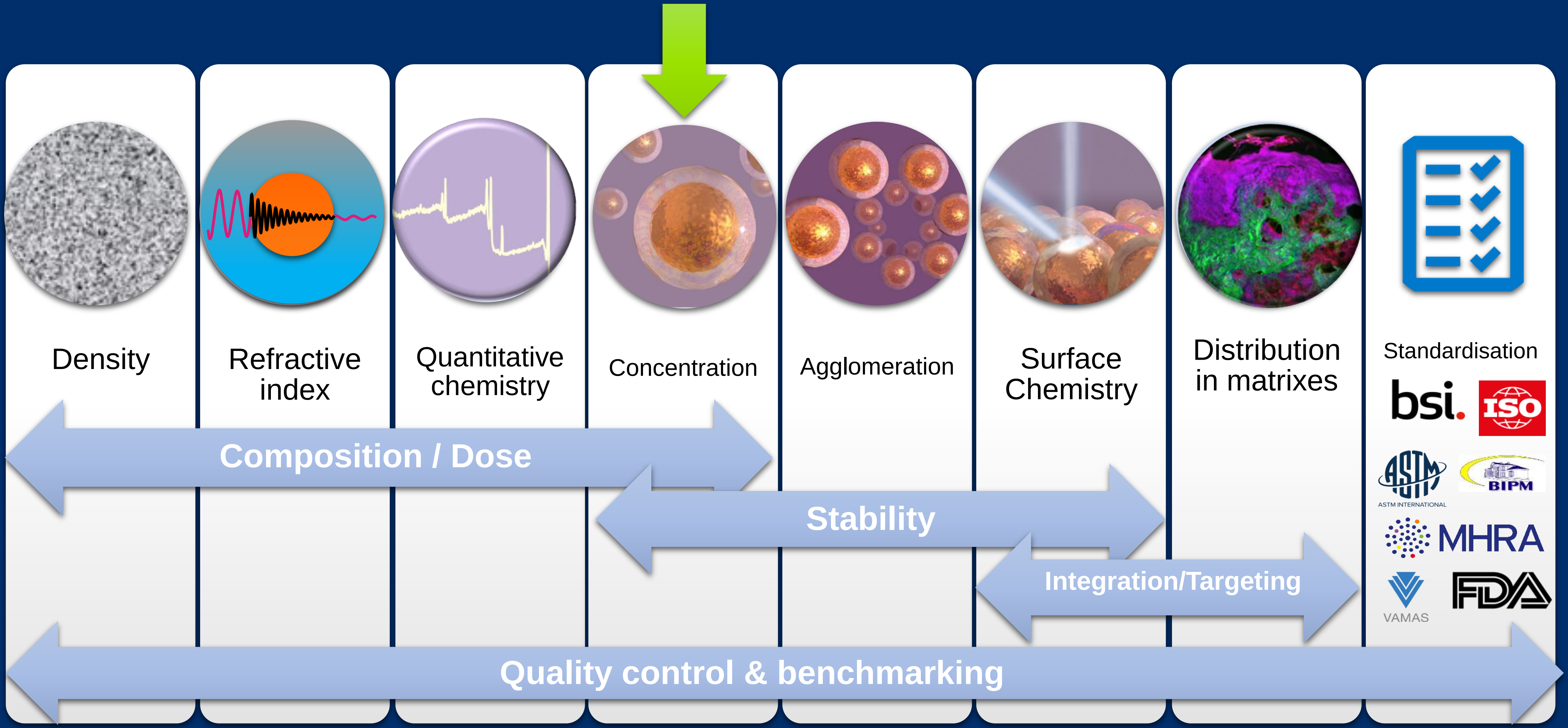
npl.co.uk

MACRAME Webinar 1: Pathways to Validation and Standardisation

Part C: An exemplar journey from need to standard

Caterina Minelli

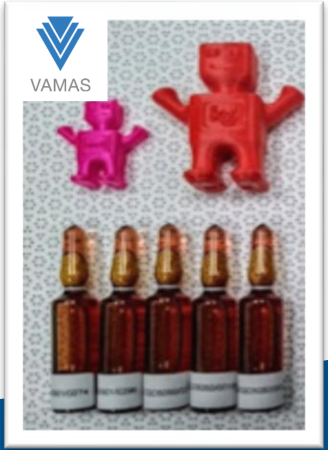
Measurands & Performance



Methods for colloidal concentration timeline

NPL National Physical Laboratory

2013: call particle manufacturer and start of stakeholder consultation



2020: publication with Malvern Panalytical on new instrument Zetasizer ultra

2019: TR work item proposed at ISO TC229

Cells, viruses LNPs, etc.

BIPM

First BIPM key comparison

BIPM pilot study

VAMAS TWA34 P10

Industrial projects

2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024

INNANOPART

Development of ISO standards

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2015: International workshop at Peckforton Castle.

2014-2017: NPL leads EMPIR Innanopart project for development of measurement capability

NPL National Physical Laboratory ROYAL SOCIETY OF CHEMISTRY

2018: NPL international symposium on concentration measurements

2019: LGC commercialise the world-first particle test materials with known number concentration.

2023: publication of ISO/TS 24672:2023 “Guidance on the measurement of nanoparticle number concentration”.

Publications

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Innanopart project:
establishing a framework to measure number concentration

Primary traceable methods
(SAXS and spICPMS)

Reference particles with known number concentration and uncertainty
(gold, silica, polystyrene)

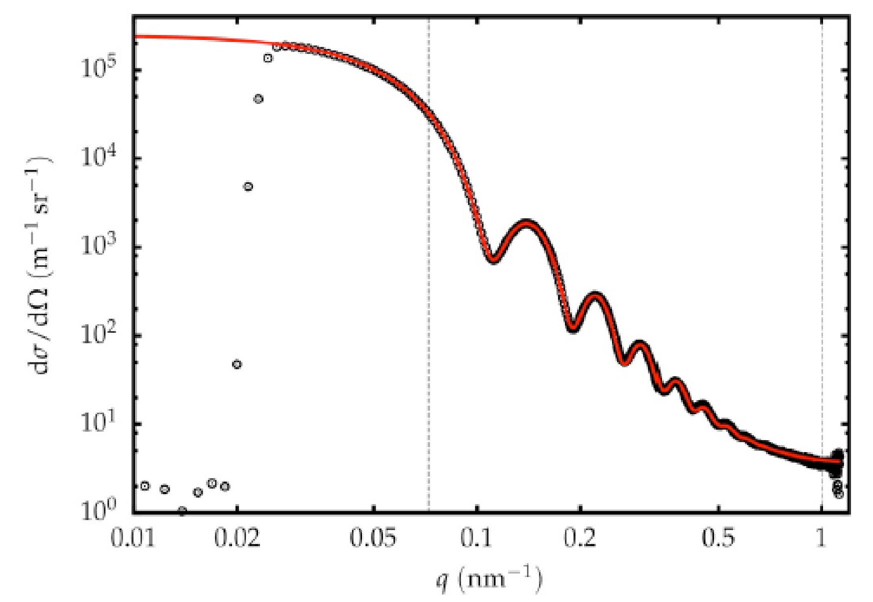
Verification/validation of laboratory methods
(PTA, DLS, TRPS, UV-Vis, etc)

The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

Traceable SAXS

Measured scattering, Incident flux, Sample thickness, Sample transmittance, Number concentration, Electron density contrast, Electron radius, Form factor

$$\frac{d\Phi}{d\Omega} = \Phi_0 \cdot t \cdot T \cdot \frac{N}{V} \cdot (\rho_p - \rho_s)^2 \cdot r_e^2 \cdot F(q, R)$$



Largest sources of uncertainty

Number Concentration of Gold Nanoparticles in Suspension: SAXS and spICPMS as Traceable Methods Compared to Laboratory Methods
A. Schavkan, et al., Nanomaterials 9 (2019) 502

Validation of laboratory methods

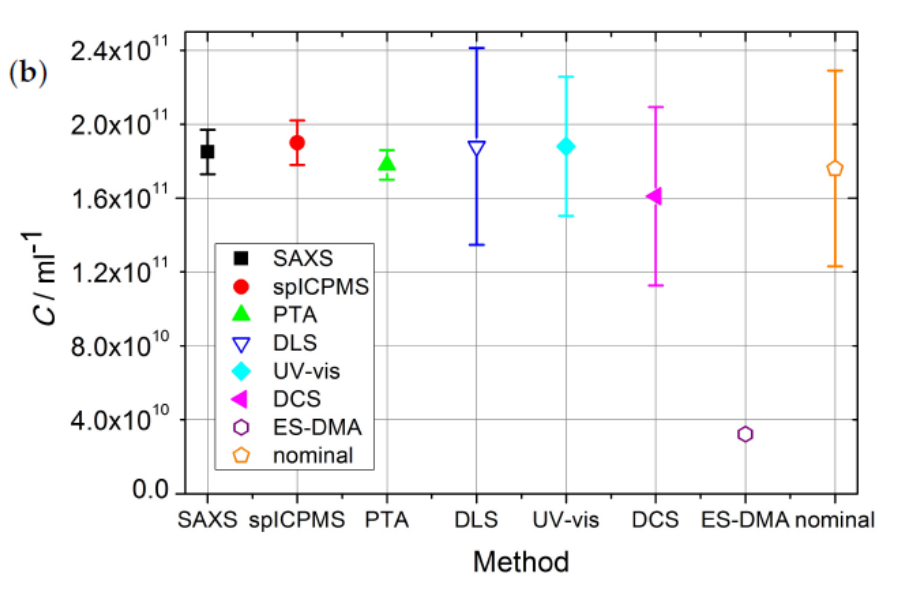
NPL

- SAXS and spICP-MS delivered traceable measurements of measured nanoparticle number concentration with well-defined uncertainty budget.
- This dataset was used to verify and validate a range of laboratory methods.
- We investigated gold, silica and polystyrene spherical particles.

Table 2. Number concentration of the gold suspensions. The tag "(ref)" denotes reference methods.

Method	C_{Au10}/mL^{-1}	C_{Au30}/mL^{-1}	C_{Au100}/mL^{-1}
SAXS (ref)	$(7.08 \pm 1.13) \cdot 10^{12}$	$(1.85 \pm 0.13) \cdot 10^{11}$	-
spICPMS (ref)	-	$(1.80 \pm 0.14) \cdot 10^{11}$	$(4.10 \pm 0.26) \cdot 10^{9}$
PTA	-	$(1.78 \pm 0.08) \cdot 10^{11}$	$(4.31 \pm 0.24) \cdot 10^{9}$
DLS	$(5.17 \pm 2.96) \cdot 10^{12}$	$(1.88 \pm 0.61) \cdot 10^{11}$	$(8.47 \pm 2.18) \cdot 10^{9}$
UV-vis	$(7.64 \pm 1.53) \cdot 10^{12}$	$(1.88 \pm 0.38) \cdot 10^{11}$	$(4.27 \pm 0.85) \cdot 10^{9}$
DCS/DLS	$(8.42 \pm 2.53) \cdot 10^{12}$	$(1.61 \pm 0.48) \cdot 10^{11}$	$(2.08 \pm 0.62) \cdot 10^{9}$
ES-DMA-CPC	$(9.03 \pm 0.32) \cdot 10^{11}$	$(3.22 \pm 0.12) \cdot 10^{10}$	–
Nominal	$(4.84 \pm 1.45) \cdot 10^{12}$	$(1.76 \pm 0.53) \cdot 10^{11}$	$(4.16 \pm 1.25) \cdot 10^{9}$

Number Concentration of Gold Nanoparticles in Suspension: SAXS and spICPMS as Traceable Methods Compared to Laboratory Methods
A. Schavkan, et al., Nanomaterials 9 (2019) 502



30 nm Au

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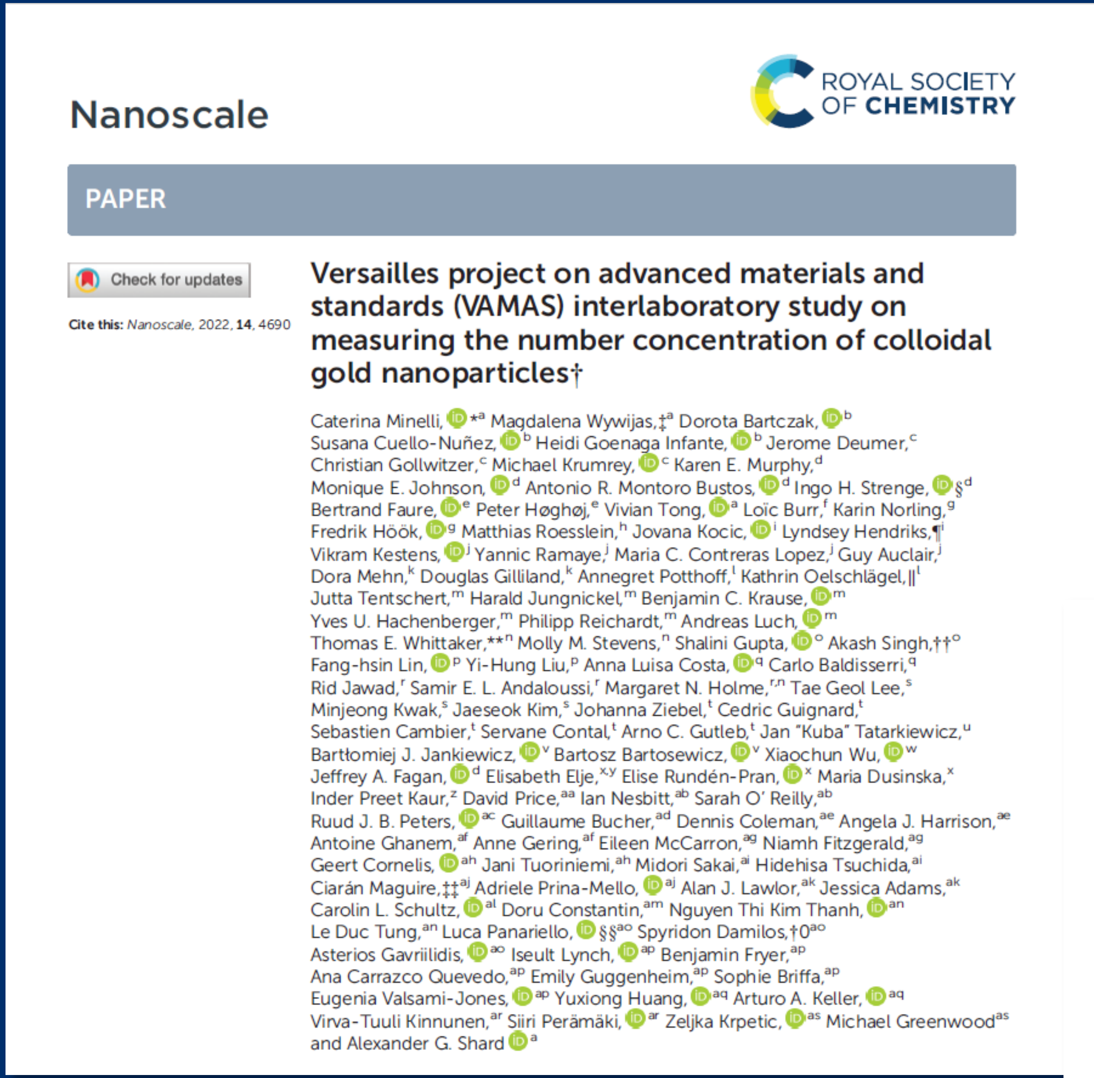
VAMAS TWA34 Project 10



Nanoscale

PAPER

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Cite this: *Nanoscale*, 2022, **14**, 4690

Versailles project on advanced materials and standards (VAMAS) interlaboratory study on measuring the number concentration of colloidal gold nanoparticles†

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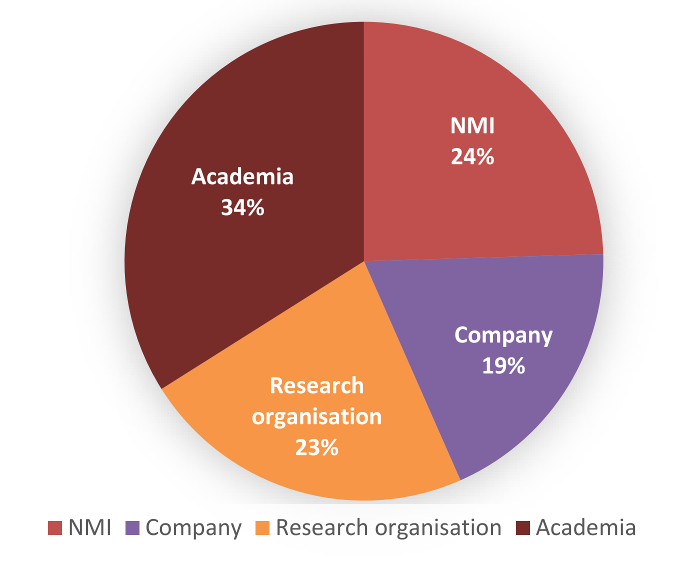

TWA 34

Minelli et al. Nanoscale 14 (2022) 4690

Participant pack:

Buffer salts to prepare dispersant

Filters, to filter dispersant

Empty vials, to prepare sample dilutions

Weights to test variability of scale for mass measurements.

Experimental protocol for the measurements.

5 vials of test material LGCQCC5050, consisting of 30 nm gold nanoparticles.

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Interlaboratory study results

This video is a courtesy of James Burgon and the training team at NPL.
This material as part of a training course on method validation now available on the NPL e-learning portal.

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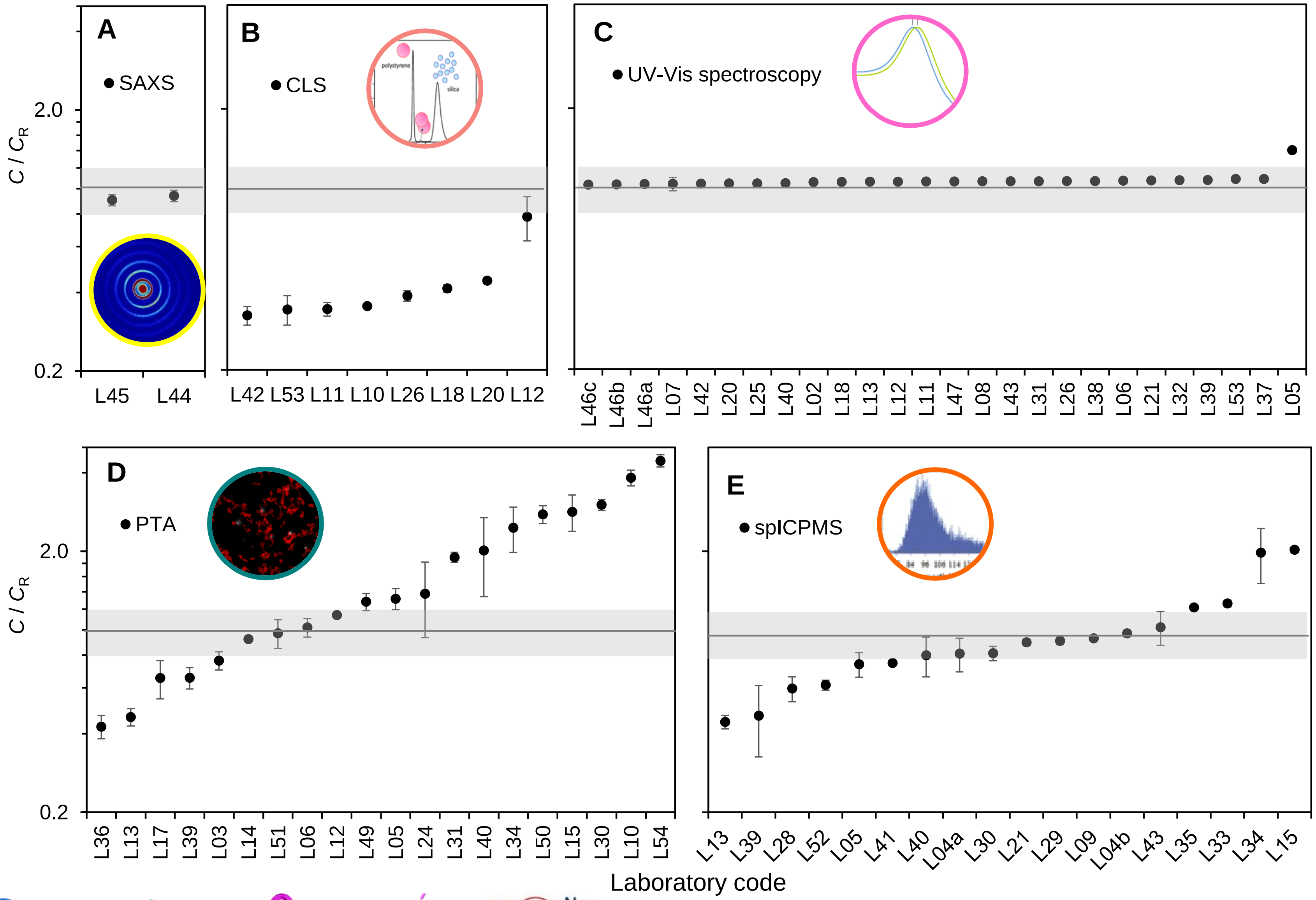
Interlaboratory study results



Population-average methods:

- Concentration value is typically derived from a mathematical formula.
- High precision.
- Requires knowledge of other experimental values and materials properties.
- Can be calibrated for sample losses in the system.

Particle-counting methods:

- Concentration value is typically derived from “counting” the signal from individual particles.
- Significant variability.
- Samples typically require high dilutions, which may cause sample instabilities.
- Software typically requires manual setting of signal thresholds.

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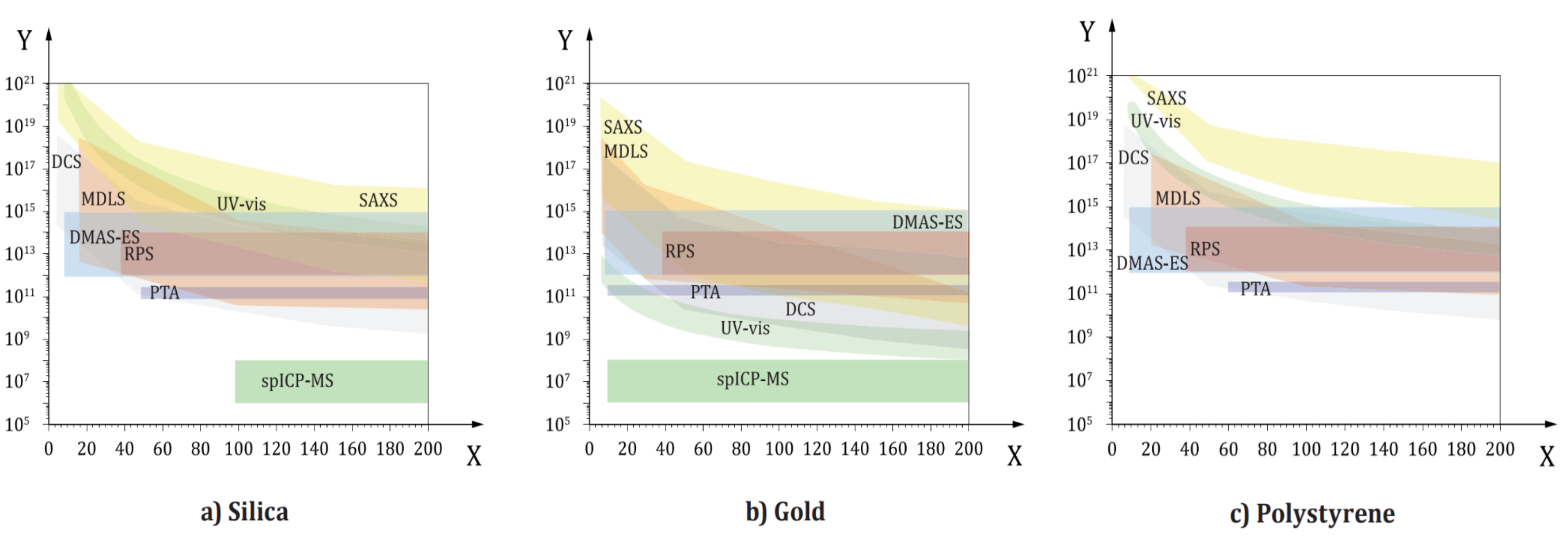
ISO technical documents

ISO/TC 24672



TECHNICAL SPECIFICATION

ISO/TS 24672

First edition 2023-11

Nanotechnologies — Guidance on the measurement of nanoparticle number concentration

Nanotechnologies — Conseils pour la mesure de la concentration en nombre de nanoparticules

Also relevant:

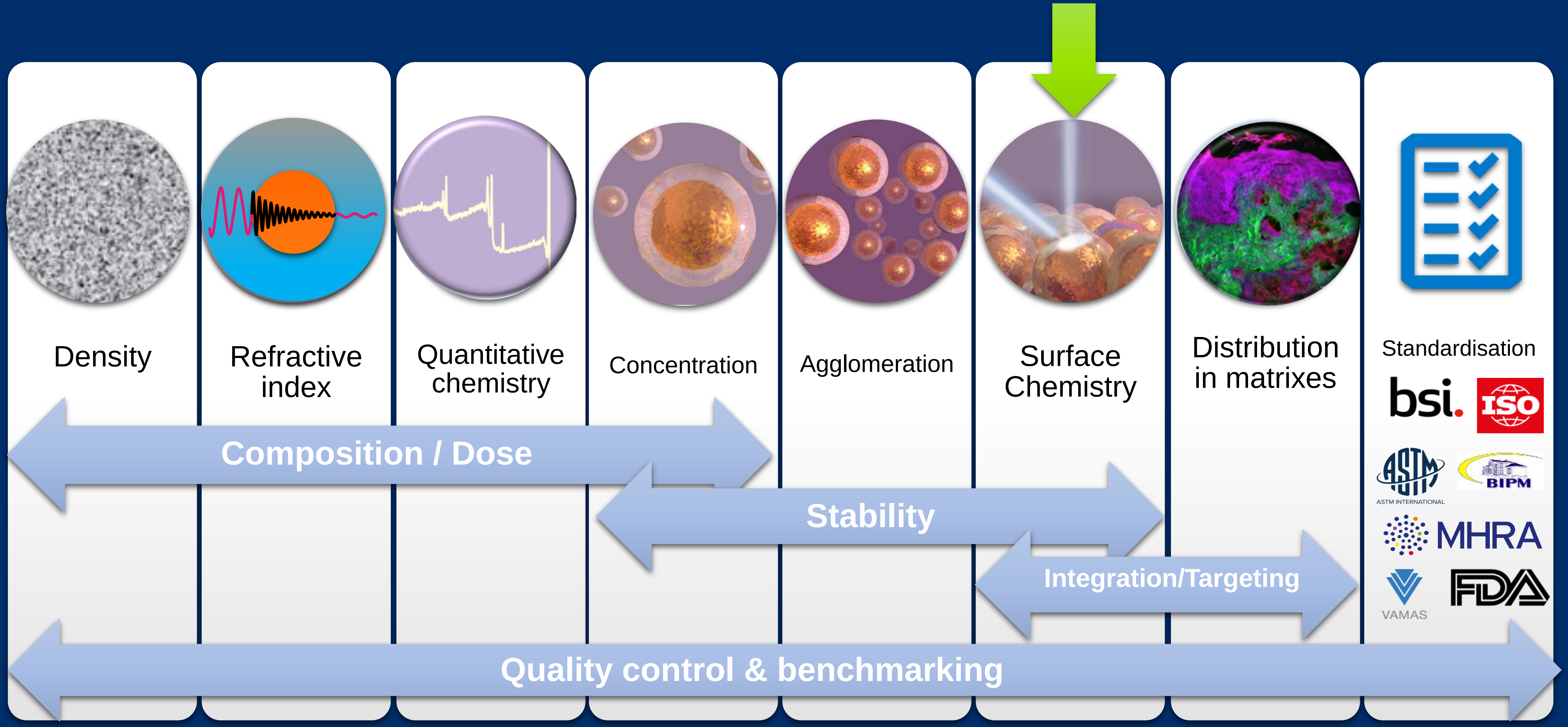
- ISO 23484:2023 – SAXS
- ISO/TS 19590:2017 – spICP-MS
- ISO/DIS 19430 – PTA – *in preparation for publication*

Long term impact

- LGC, who was partner in the EU project, has commercialised the test material, which is now available to the community.
- A BIPM interlaboratory comparison took place to certify the number concentration of the test material.
- Anyone can purchase the material, run the measurements and compare the results to those of the VAMAS studies to validate and benchmark their measurement capabilities.
- We translated the knowledge developed within these studies into three ISO documentary standards.
- We applied the methods in industrial collaborative R&D.

LGCQC5050

Measurands & Performance



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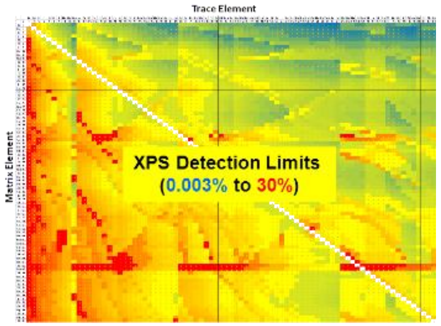
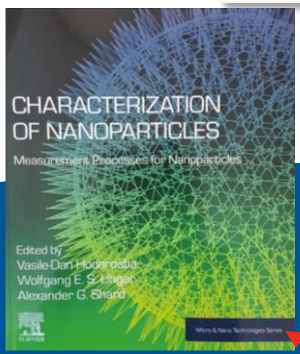
XPS methods for nanoparticle timeline



2015: VAMAS lead, Composition measuremen in organic depth profile VAMAS

2016: VAMAS lead, Nanoparticle coating Thickness and composition VAMAS



2019: first calibration of a HAXPES instrument with Kratos Analytical and IUK

2020: first demonstration of 200 nm information depth HAXPES instrument with Royce Institute HENRY ROYCE INSTITUTE

2020: VAMAS lead, XPS intensity calibration using polyethylene VAMAS

2021: Application of HAXPES to nanomedicines AstraZeneca

2022: PoC of CryoXPS for nanomedicines U.PORTO SINTEF

BIPM P190

BIPM K157

2013 2014 2015 2016 2017 2018 2019 2020 2021 2022

EMRP Biosurf project

EMPIR Innanopart project

20+ industrial projects

2015: International workshop at Peckforton Castle.

2014-2017: NPL leads EMPIR Innanopart project on nanoparticle coatings

2017: ISO 19668, XPS detection limits ISO

2019: ISO 22415, Ar cluster yield volume ISO

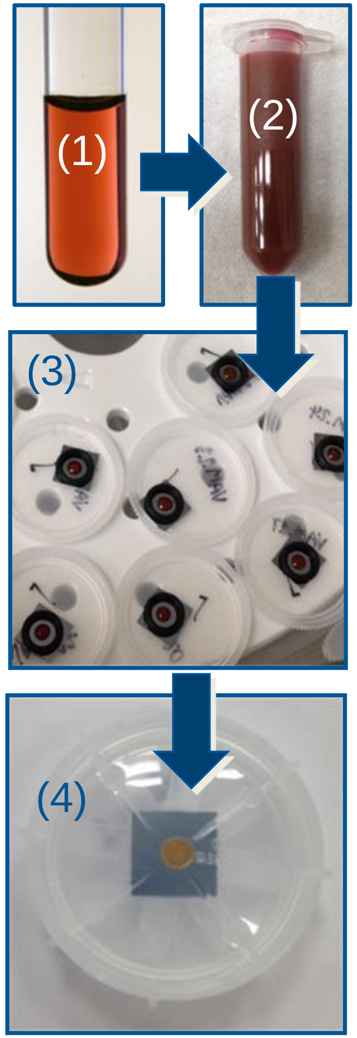
2020: RSC Industrial Analytical Chemistry Award ROYAL SOCIETY OF CHEMISTRY

2021: ISO 23173, Nanoparticle coatings ISO

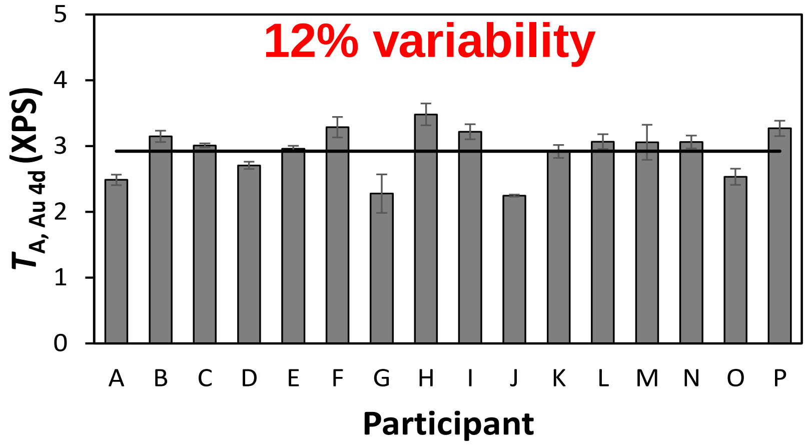
2020: Addressing Reproducibility Crisis In JVSTA AVS

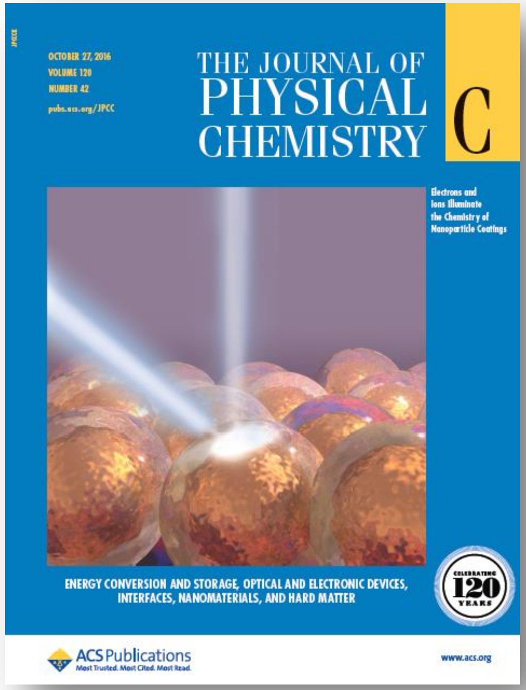
Publications

VAMAS inter-laboratory study



Measurement of chemistry and thickness of coatings





NPL National Physical Laboratory

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Belsey, et al. *J Phys Chem C* 120 (**2016**) 24070

Challenge with soft materials

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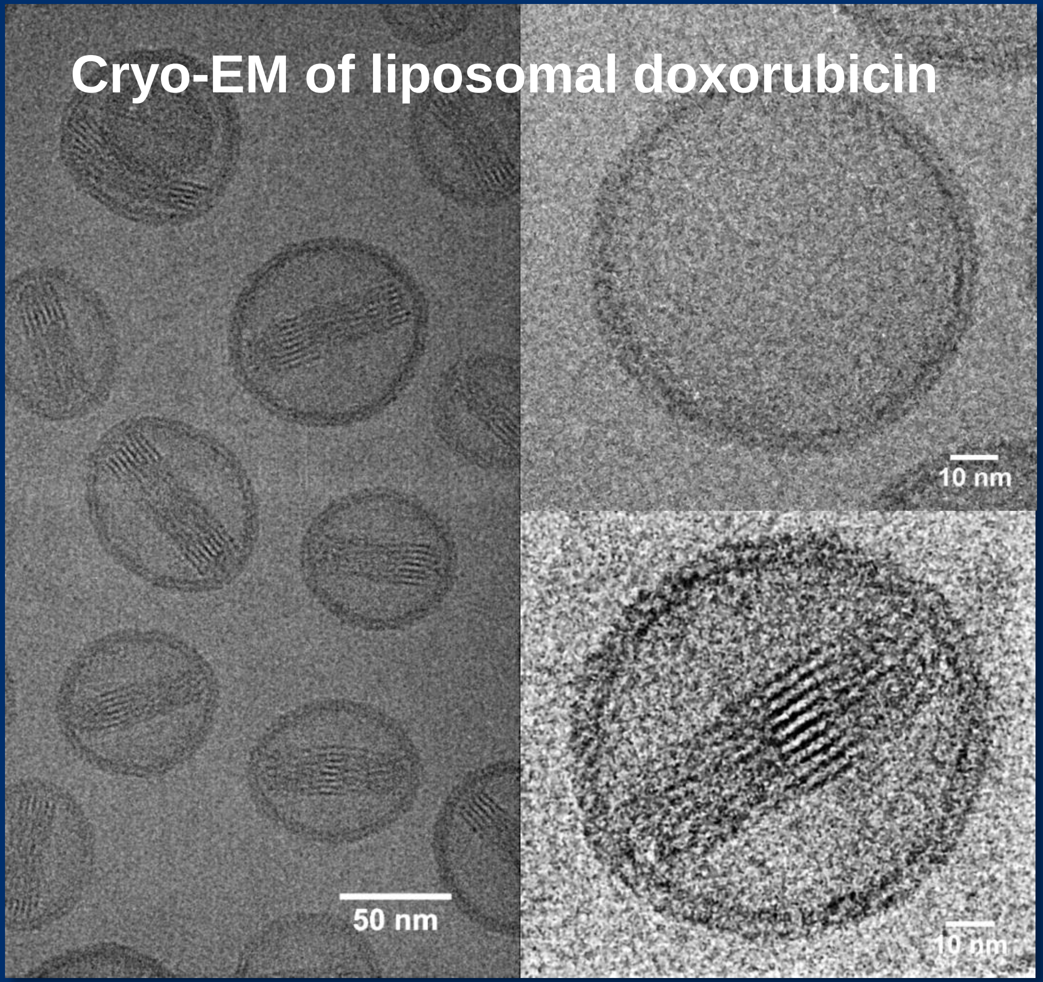
- Introducing soft materials such as LNPs in vacuum is a challenge as structure is generally disrupted.
- However, vacuum methods holds the potential for robust chemical analysis for
  - Functional surfaces
  - Quality of encapsulation and drug distribution
  - Chemical composition and drug loading

- Potential approaches to the preparation of materials include:
  - Lyophilisation
  - Cryogenic preparation



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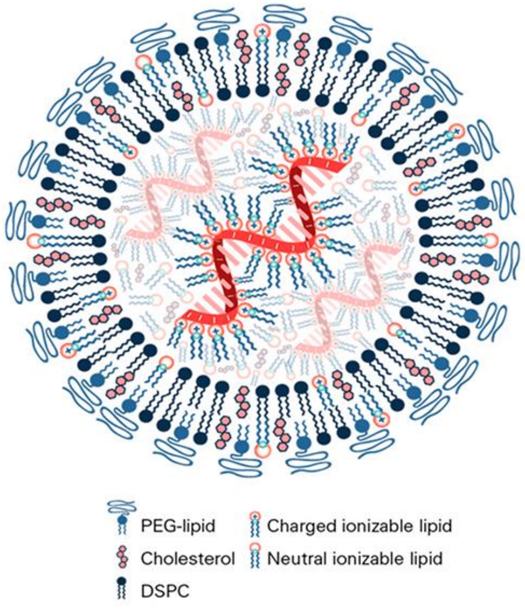
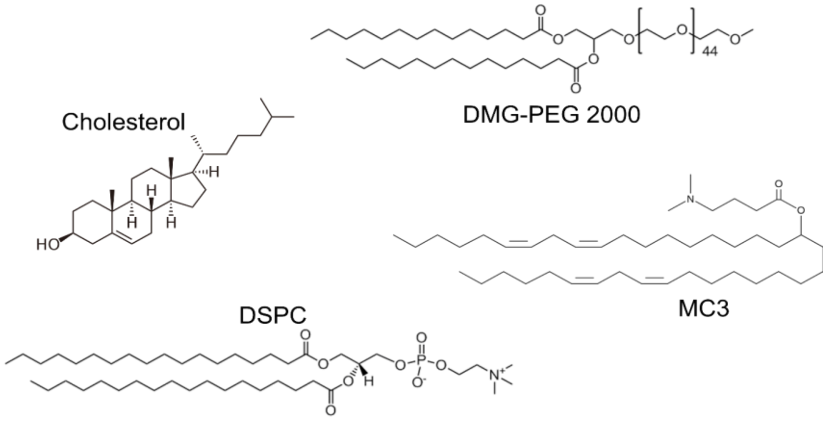
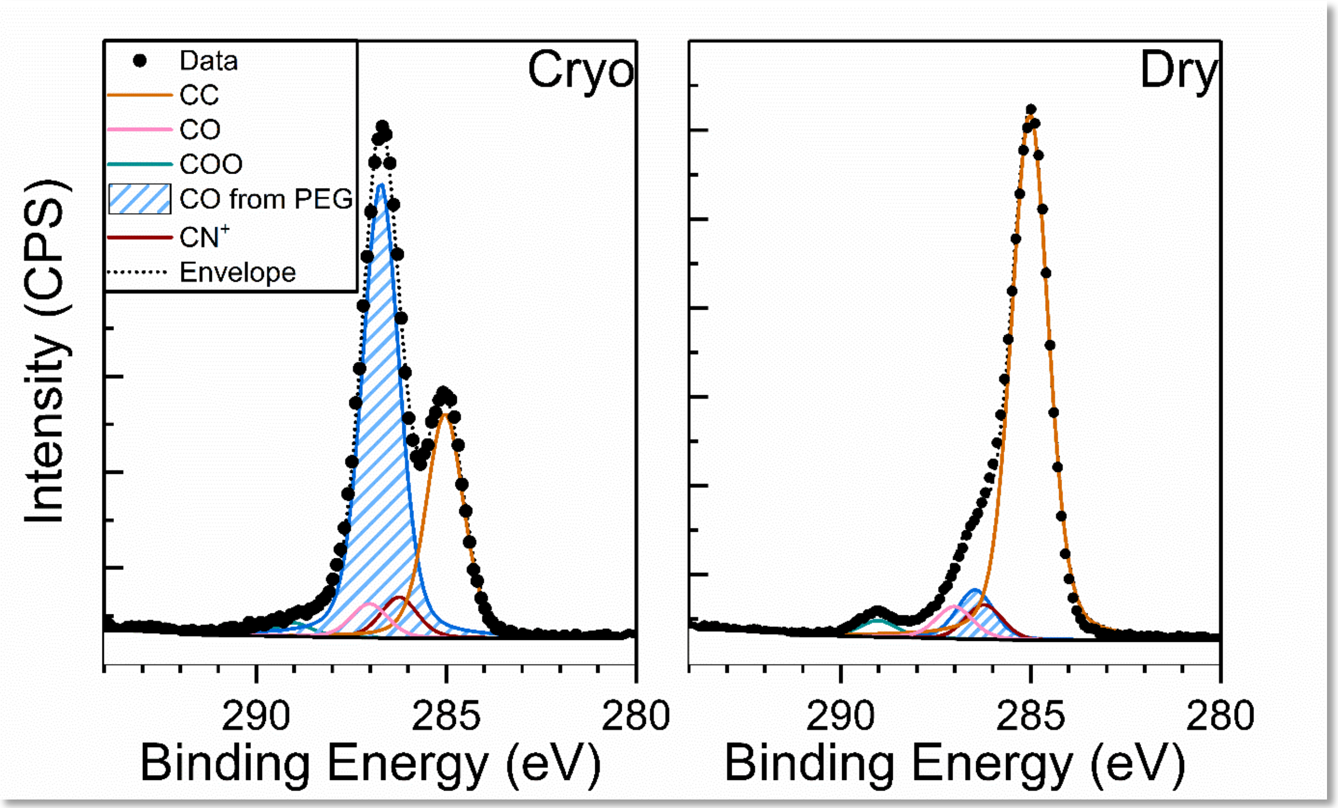
CryoXPS of RNA loaded LNPs







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Cant, et al. *J. Phys. Chem. A 127 (2023) 8220–8227*

Metrology for precision delivery

NPL
National Physical Laboratory

Bureau International des Poids et Mesures

ISO

ASTM INTERNATIONAL

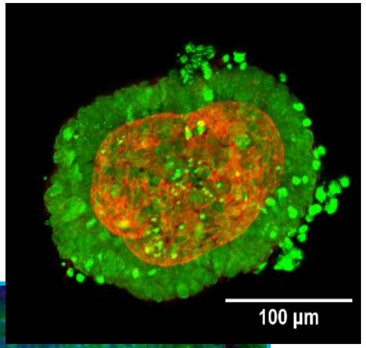
cen

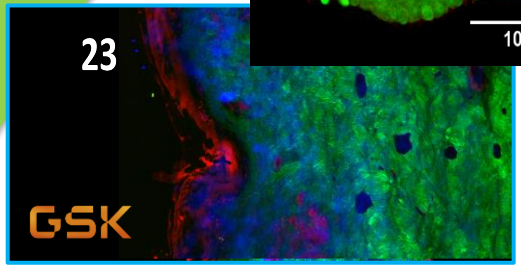
edqm

FDA

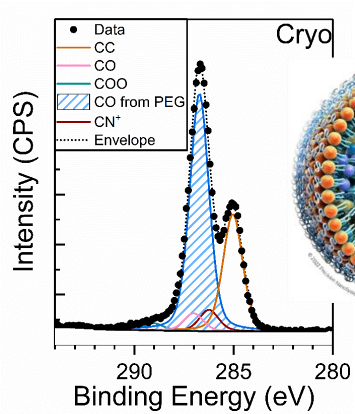
complexity

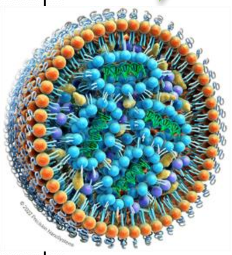
Potency, therapeutic index



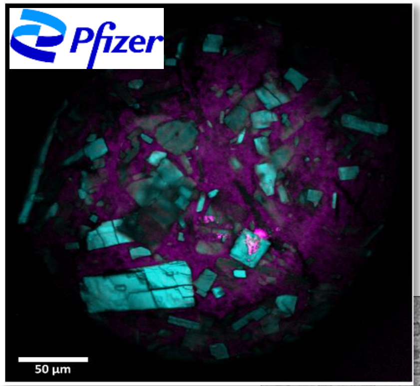


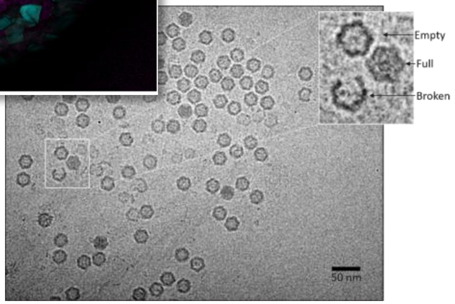
Localisation, identification and quantification in biological matrices



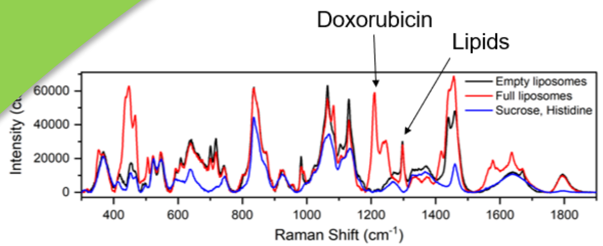


Surface functionalities





Formulations



In-line spectroscopy

2023 Workshop on the international standardisation roadmap for nanomedicine (Paris)

Speakers from the EC/Joint Research Center, SINTEF Industry, international National Metrology, PHOENIX and SAFE-n-MEDTECH Open Innovation Test Beds, EDQM, CEN, ISO, ASTM and VAMAS.

Platform to gain insights into ongoing initiatives and to advocate for a more active role of the European Metrology community in fostering connections among the diverse stakeholders involved

The development of standard test methods and reference materials are key priorities for the European Commission, international metrology institutes, industrial stakeholders and regulators.

However, the standardisation framework is very complex with multiple independent bodies.

Recommendation:

- **create a European interest group on standardisation focusing on nanomedicine**
  - A specific Working Group "*Nanomedecine*" under CEN/TC 352 *Nanotechologies* could be created aiming at mapping the ongoing and existing standardisation efforts developed by ISO, ASTM, CEN, and to propose a standardisation nanomedicine roadmap.
- strengthen interactions between standardisation committees and dedicated organisations specifically associated to the quality assessment of pharmaceutical products such as the ICH and the EDQM.
- create a dedicated European Metrology Network (EMN).
  - In the meanwhile, **the newly formed CCQM Task Group on *Particle Metrology*** in collaboration with relevant Joint Research Projects such as MetrINo and/or specific associations of stakeholders (e.g. NanoMesureFrance, or the ETPN- Nanomedicine European Technology Platform) could initiate specific networking actions.

Take-home message

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- Interaction with stakeholders is critical at identifying metrology gaps.
- Standardisation is a long process.
- Stakeholder participation to the process is important to ensure best practice is fit-for-purpose and adopted.

Acknowledgements

MACRAMÉ
Advanced Characterisation Methodologies to assess and predict the Health and Environmental Risks of Advanced Materials

Nano Mesure France

metrino

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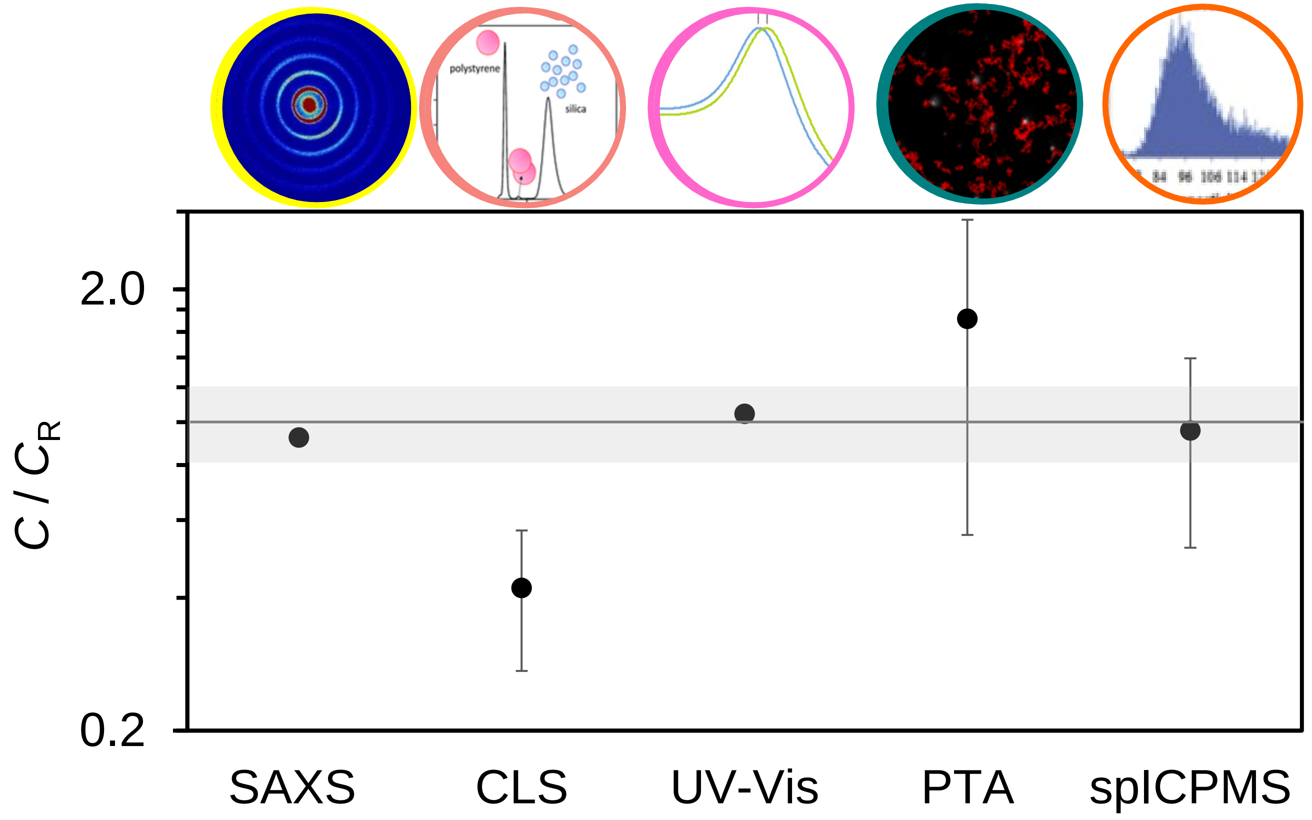
Measurement reproducibility



	SAXS	CLS	UV-Vis	PTA	spICPMS
within-lab variability	5 %	5 %	0.5 %	11.3 %	8.9 %
between-lab variability	3 %	11 %	1.2 %	68 %	45 %

National Physical Laboratory

VAMAS

metrino MACRAMÉ Nano Mesure France

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Analytical challenges for nanoparticles

NPL

- Variety of nanoparticles – one size does not fit all.
- Complexity – What critical quality attributes? full characterisation can be cost prohibitive.
- Heterogeneity – but what tolerance? What analytical specifications?
- Generics/biosimilars, scale up manufacturing – but how much similar?
- Measurements at relevant concentration and in relevant matrixes.
- Some technologies are advancing fast, outpacing the analytical capability.
- New manufacturing paradigms, such as continuous manufacturing, require a shift from quality control of products to quality assurance of methods.
- Digital tweens and other digital tools require quality data for training and prediction. What specifications?