European Metrology Programme for Innovation and Research

Delivering Impact

EURAMET

Bi-pyramidal TiO_2 reference nanoparticles generated in nPSize

A new ISO standard on the identification of nanoparticles

Nanoparticles (NPs) are tiny – around 1 to 100 billionths of a metre – and can have different chemistries and behaviours than the same material of larger size. This property has led to advances in a wide range of industries, but it can also confer toxicity. Size measurements are the main way NPs are identified but a lack of standardised methods for identifying ones with complex shapes has hindered evaluation of their potential harm.

Europe's National Measurement Institutes working together

The European Metrology Programme for Innovation and Research (EMPIR) has been developed as part of Horizon 2020, the EU Framework Programme for Research and Innovation. EMPIR funding is drawn from 28 participating EURAMET member states to support collaborative research between Measurement Institutes, academia and industry both within and outside Europe to address key metrology challenges and ensure that measurement science meets the future.

Challenge

Nanoparticles (NP), defined as having one or more dimension of 1-100 nm, are used in a range of modern products, including cosmetics, sunscreen, coatings for solar cells, and materials for water treatment. A factor for their extensive use is the greater chemical activity imparted by their large surface area relative to their size. As they are small enough to enter the body through the lung, skin and bypass the blood brain barrier, they are also finding use in medical applications.

However the properties of increased activity and small size can have adverse consequences. Nanoplastics can cause environmental damage and some NPs, such as metal oxides and carbon nanotubes, have the potential to cause permanent cell or organ damage.

The recommended way to identify NPs is based on their size, and the 'gold' standard for assessing this is electron microscopy (EM) which measures this particle-by-particle from an image. However, a lack of both traceable NP reference materials and metrologically valid methods to identify particles with non-uniform sizes has hindered this approach.

In response, in 2010 the European Commission (EC) published Mandate M/461 calling on standardisation bodies to develop improved measurement procedures for size-identification of real-world nanoparticles as a prerequisite for the reliable evaluation of their potential toxicity to the environment and human health.

Solution

The nPSize project developed 8 different types of complex nanomaterials composed of silicon dioxide (SiO_2) or titanium dioxide (TiO_2) with various geometries, and gold nanoparticles with rod or cube shapes.

To assess the best measurement method these were characterised using 5 different techniques, 3 types of electron microscopy (SEM, TEM and TSEM), atomic force microscopy (AFM) and small-angle X-ray scattering (SAXS). This included instrument calibration, sample preparation, measurement and data analysis conditions.

Parameters ranged from ideal NPs (monodisperse spheres) to complex particles, polydisperse in size with non-spherical shapes and different chemical compositions.

Images obtained from SEM, TEM and TSEM were then manually annotated to develop a neural network for automatic particle analysis.

Traceable data was determined on size, size distribution, shape and concentration, and results submitted to ISO/TC 229 'Nanotechnologies', who develop standards for test methodologies and specifications for NP reference materials. Knowledge has also been included in the OECD (Organisation for Economic Cooperation and Development) Working Party on Manufactured Nanomaterials (WPMN), responsible for NP toxicity screening.

Impact

In 2020, ISO/TC 229 published the international ISO 21363 standard, "Measurements of particle size and shape distributions by transmission electron microscopy" - the very first full ISO standard developed under this committee. This contained 6 pages of nPSize data on the use of TEM to characterise the project's bipyramidal shaped TiO_2 as one of the most challenging model NP to measure.

In 2021 ISO 19749 was published, incorporating project knowledge on the use of SEM for NP measurement using the TiO_2 and SiO_2 materials. Then, in 2023, the OECD ***Test Guideline 125 Nanomaterial Particle Size Distribution and Size Distribution of Nanomaterials*** was updated to include best practice for measuring NPs using AFM, SAXS, TEM and SEM. To accompany this, the consortium published a dedicated scientific paper on the use of EM for counting NPs.

Towards the end of nPSize, two Versailles Project on Advanced Materials and Standards (VAMAS) interlaboratory comparisons were launched on the measurement of two project materials, TiO_2 nano bipyramids and bimodal SiO_2, chosen due to their complex-shape. These results are anticipated to be incorporated into further revisions of ISO 21363 and ISO 19749 in the future.

Validated methodologies for identifying NPs, such as those developed, are an essential step in regulating these substances - ensuring that the economic and technical benefits they bring won't come at a cost to human health or the environment.

Improving the methods to identify nanoparticle populations

The nPSize project produced three classes of NPs to address the lack of complex reference materials: ones with well-defined non-spherical shape, ones with a relatively high size polydispersity, and ones with well-defined number concentrations. These were validated using TSEM, SEM, AFM and SAXS.

A hybrid approach was developed incorporating AFM and SEM along with a new method, Transmission Kikuchi Diffraction.

Advanced data processing using machine learning was utilised to allow, for the first time, automated data processing for NP analysis.

Results were presented to ISO/TC 229, CEN/TC 352, ISO/TC 24/SC 4, ISO/TC 201 and the OECD WPMN and significant contributions made to international standards ISO 21363, ISO 19749 and into OECD TG 125.

Two VAMAS interlaboratory comparisons were launched using project NPs with results anticipated to be incorporated into future revisions of the ISO standards.

The work has provided a more reliable basis for the characterisation of NPs, essential for assessing their potential toxic effects.

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http://www.euramet.org/project-17NRM04

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