



Publishable Summary for 17NRM04 nPSize Improved traceability chain of nanoparticle size measurements

Overview

Nanomaterials and nanotechnology are widely used today, but their use may expose humans and the environment to new health and sustainability risks. To control and minimise these risks, the European Commission has mandated European standardisation bodies to develop standardised methods that can reliably characterise manufactured nanomaterials. This project has developed reference materials: i) TiO₂ and Au nanoparticles with well-defined non-spherical shape, ii) SiO₂ nanoparticles with relatively high polydispersity index, and iii) Au and SiO₂ nanoparticles with accurate particle concentrations. Based on these new reference nanoparticles, measurement procedures for improved traceable measurement of the size of non-spherical and non-monodisperse nanoparticles have been also developed and published. Finally, full algorithm sequences for nanoparticle detection and size measurement as developed on both a physical basis and by machine learning have been developed. All newly developed reference nanoparticles, measurement procedures and modelling algorithms contribute significantly to the improvement of the traceability chain, comparability and compatibility of nanoparticle size measurements to support standardisation within the framework of CEN/TC 352 “Nanotechnologies”, ISO/TC 229 ‘Nanotechnologies’, ISO/TC 24/SC 4 ‘Particle characterization’ and ISO/TC 201/SC 9 ‘Scanning probe microscopy’.

Need

The global nanotechnology market is predicted to reach 110 Billion US\$ by 2022. Further, nanotechnology has been identified by the European Commission (EC) as one of its 5 key technologies. New and emerging uses of nanomaterials include medical and pharmaceutical applications as well as use in conductive inks, optical sensing and power delivery. However, nanotechnologies and nanoparticles may expose humans and the environment to new health and sustainability risks, which need to be reliably linked to their size, shape, concentration and chemical properties.

In order to support the increasing and safe use of nanomaterials it is essential that robust normative standards are introduced. NMIs have a key role to play in this by developing comparable and traceable measurements and instrument calibrations for real-world nanoparticles. The EC has recognised this need and therefore mandated CEN, CENELEC and ETSI (EC Mandate M/461) to develop standardisation activities regarding nanotechnologies and nanomaterials as one of the building blocks for the “safe, integrated and responsible” use of nanomaterials as outlined in the EC European Strategy for Nanotechnologies. A significant part of this strategy relates to the need for improved, traceable measurement procedures for size of real-world nanoparticles as a prerequisite for the reliable evaluation of their potential toxicity to the environment.

Three different ISO technical committees offer suitable standardisation platforms for projects on the accurate measurement of nanoparticle size and shape distribution: ISO/TC 229 for electron microscopy, ISO/TC 201/SC 9 for atomic force microscopy (AFM) and ISO/TC 24/SC 4/WG 10 for small angle X-ray scattering (SAXS). Three projects on the measurement of nanoparticle size and shape distribution using scanning electron microscopy (SEM), scanning electron microscopy in transmission mode (TSEM) and transmission electron microscopy (TEM) have been developed under ISO/TC 229, at all of them the present project have provided significant contributions. Alignment of European standardisation activities within CEN/TC 352 to the ongoing ISO standardisation projects is further necessary. The physical modelling of the output signals in electron microscopy and new reference nanoparticles with more complex shape and size distribution were also required. This is also the case both for future AFM standards on the measurement of nanoparticle size and shape distribution, which would be developed under ISO/TC 201/SC 9, and for the SAXS activities on nanoparticle size distribution in progress under ISO/TC 24/SC 4/WG 10.

The preselection by the present project of only traceable sizing methods was of special relevance. For the first time, both critical aspects, namely the lack of reference nanoparticles and signal modelling were systematically considered. The advanced data analysis software developed by the present project together with a public database with representative documented data include a robust determination of the measurement

uncertainty. Last, but not least, hybrid measurement approaches contribute to a more accurate final result by data fusion, particularly for non-spherical nanoparticles.

Objectives

The overall objective was to improve the traceability chain, comparability and compatibility for nanoparticle size measurements to support standardisation and to ensure that these developments are fed into the standards development process within CEN/TC 352 and ISO/TC 229 and related groups. The specific objectives were:

1. To assess the performance and establish the traceability of existing nanoparticle size and characterisation methods, such as SEM, TSEM, TEM, AFM and SAXS, in terms of their sensitivity to material, shape and quantity (number, volume or mass) for representative nanoparticulate materials (i.e. metals, oxides and polymers), including analysis of the effect of material and shape parameters on size and size distribution measurements, as well as the effect of conversion of the measured signal on the particle size distribution.
2. To develop validated nanoparticle reference materials with (i) non-spherical shapes, (ii) non-monodisperse size distributions and (iii) accurate concentrations. In addition, to use such nanoparticle reference materials to evaluate measurement uncertainties for nanoparticle quantity determination (expressed as number, volume, mass or intensity) and to establish their dependence on particle size.
3. To develop improved physical models of the output signals from nanoparticle size measurement systems, that accurately account for nanoparticle material, shape and quantity. The physical models will include nanoparticle material type, shape and quantity parameters such as number, volume, and mass. The goal of the models is to improve the evaluation of nanoparticle measurement uncertainty and comparability between results of different methods.
4. To use the new physical models (from objective 3) to develop validated and traceable methods for the transfer of nanoparticle size from (certified) reference nanoparticles of spherical shape and monodisperse size distribution to other types of nanoparticles. This will include different nanoparticle shapes (such as elongated nanoparticles and platelets) as well as nanoparticles with non-monodisperse size distribution.
5. To contribute significantly to the standards development work of the technical committees CEN/TC 352 Nanotechnologies and ISO/TC 229 Nanotechnologies ensuring that the outputs of the project are aligned with their needs, communicated quickly to those developing the standards and to those who will use them, and in a form, that can be incorporated into the standards at the earliest opportunity.

Progress beyond the state of the art

Performance and traceability of Characterisation Methods:

Most previous studies on nanoparticle size measurement focus on suspensions of spherical and monodisperse nanoparticles and are limited to 'simple' systems such as gold, silica or polystyrene particles. Little or no research has been carried out on nanoparticles deviating from this idealised case. To address this, this project has investigated the sensitivity of traceable nanoparticle sizing techniques SEM, TEM, TSEM, AFM and SAXS to the type of nanoparticle material, shape, polydispersity and number concentration.

Nanoparticle Reference Materials preparation and characterisation:

There is still a lack of non-spherical nanoparticle reference materials or materials certified for particle concentration available on the market. Therefore, three classes of nanoparticle-reference materials (RMs) as a unique set have been developed and were validated in the project: particles with i) well-defined non-spherical shape, ii) relatively high polydispersity index, and iii) well-defined concentrations.

Modelling and development of measurement procedures:

The project aimed to improve the performance and traceability of current characterisation methods with advanced data processing. Different approaches have been considered including physical modelling of nanoparticle shape and simulations to evaluate the signals produced by input models for TSEM, SEM, AFM and SAXS. Modern modelling based on machine learning have been used with a-priori information from the measured data. For the first time, the project has made contributions to implementing physical modelling into machine learning algorithms.

Standardisation as knowledge transfer to end users:

Project partners (BAM, PTB, LGC) have been actively contributing to the development of international Standards and Guidelines in collaboration with ISO/TC 229 'Nanotechnologies', CEN/TC 352 'Nanotechnologies', ISO/TC 24/SC 4 'Particle characterization', ISO/TC 201 'Surface analysis'/SC 9 'Scanning probe microscopy' and OECD Working Party on Manufactured Nanomaterials (WPMN) based on the new technical knowledge gained within this project. A particular attention has been paid to the contributions to VAMAS/TWA 34 Nanoparticles Population, which is definitely the best international platform suited to launch projects on new reference measurement procedures of more complex nanoparticles. The cooperation with different technical committees has focused on the improvement of the traceability of the nanoparticle size measurements and quantity error evaluation, including method combination. All these aspects are currently addressed more and more within the respective standardisation committees, also including significant ongoing contributions from this project. In addition, the project will facilitate in the near future uptake of the improved procedures, the unique RM candidates and models developed within this project by accredited laboratories, instrument manufacturers and reference material providers.

Results

1. Towards assessment of performance and establishment of the traceability of existing nanoparticle sizing methods, such as SEM, TSEM, TEM, AFM and SAXS:

The performance of the characterisation methods SEM, TEM, TSEM, AFM and SAXS for traceable measurement of nanoparticle size, size distribution, shape and concentration in suspensions have been evaluated systematically by LNE, PTB, BAM, CEA, VSL and SMD regarding parameters including calibration, measurement uncertainties and limitations. From these results, the key steps for measuring processes, i.e. sample preparation, data acquisition, metrological qualification and data analysis were elucidated for each method. It was found that sample preparation using non-agglomerated nanoparticle deposition on solid supports for EM and AFM methods is crucial for optimising performance regarding particle size distribution and shape analysis. As such, these methods are ideal candidates to accurately characterise particle shapes and sizes even within broad size distribution. However, EM and AFM methods have limited performance for analysing particle concentration, size ratios and agglomeration states in dispersion in comparison to SAXS. SAXS has good performance in analysing particle sizes and agglomeration state in dispersion but is not in favour to get accurate particle shape information for broad size distribution. Hence, EM/AFM and SAXS represent a perfectly complementary set of techniques in order to accurately determine all relevant parameters regarding nanoparticle size (distribution) and shape. For the subgroups of methods selected in the project, a matrix with corresponding performance parameters (size, shape, size distribution, concentration) has been filled according to the current state after the end of the project. The objective has been successfully achieved.

2. Towards development of validated nanoparticle reference materials with non-spherical shapes, non-monodisperse size distributions and accurate concentrations:

To identify the future needs for novel reference nanomaterial a public workshop with stakeholders from academia, industry and standardisation committees was organized by PTB and BAM and held at the beginning of the project. Based on the information gathered from stakeholders, eleven RM candidates were chosen and synthesised ranging from nanoparticles of titania, silica and gold that possess (i) non-spherical shapes, e.g. cubic, bipyramidal, acicular, flaky and/or (ii) defined concentration or (iii) polydisperse or bimodal size distributions. The following candidates were successfully synthesised: (i) titania nanoplatelets, (ii) titania bipyramids, (iii) titania acicular particles, (iv) gold nanocubes, (v) gold nanorods, and (vi) spherical silica particles with various size polydispersities. Spherical gold nanoparticles with accurate concentration were sourced and mixed accurately to nominal NP number concentrations. All synthesised nanoparticle candidates have undergone characterisation at LNE, LGC, CEA, PTB, BAM, VSL and SMD with respect to their homogeneity and stability, using the methods from objective 1. Protocols for the deposition of the nanoparticles on silicon wafers, TEM grids and mica have been developed and optimized for all the candidate materials. This was a key prerequisite for the application of automatic data analysis, physical modelling and machine learning in objective 3 for EM and AFM methods. The bipyramidal titania as well as the two different bimodal silica nanoparticles have been chosen as the most promising candidate reference materials. Two inter-laboratory comparisons with these three types of materials have been started under the VAMAS framework in November 2020. The objective has been successfully achieved.

3. *Towards development of improved physical models of the output signals from nanoparticle size measurement systems, that accurately account for nanoparticle material, shape and quantity, with the final aim to improve the evaluation of nanoparticle measurement uncertainty and comparability between results of different methods.*

The project has used for the first time physical modelling of the signals and advanced data processing to extend the capabilities of methods for nanoparticle sizing and inter-method comparison. More specifically, the project has used at LNE and PTB simulations of elastic and inelastic scattering in electron microscopy. A complete algorithm has been implemented to generate simulated TSEM micrographs. For SEM, the program JMONSEL was used together with a second algorithm to properly determine nanoparticle dimensional properties. For AFM, a model for probe shape reconstruction has been established by VSL and SMD and for SAXS, a software tool for scattering simulation and shape reconstruction was created by PTB, BAM and CEA. In addition, measurement results obtained with the methods from objective 1 have been processed with the Platypus™ software. In order to automatically detect advanced nanoparticle shapes and extract features, up-to-date machine learning methods were implemented. A pre-trained deep learning algorithm was proposed due to easier usability if compared to the training with user data. The detection of objects at different scales with different sizes were performed successfully. The objective has been successfully achieved.

4. *Towards the use of the new physical models to develop validated and traceable methods for the transfer of nanoparticle size from (certified) reference nanoparticles of spherical shape and monodisperse size distribution to other types of nanoparticles.*

The newly developed physical models and machine learning algorithms from objective 3 have been tested and validated using the reference nanoparticles developed in objective 2. A total of 12 particle families have been provided along the project by the different partners, who annotated manually images to feed the project public database. The annotations have been made with help of the Pollen Platypus™ tool. A unique “particle detector” has been trained. Even if the detection scores of Deep-Learning are high, it is important to mention that not all the detected particles match the expectation. Besides, it remains difficult for the model to separate particles from agglomerates/aggregates. Artificial Intelligence methodology was identified by Pollen for SEM, TSEM and AFM. Instead of very resource-consuming training with the data of the user, a method has been considered that relies on deep learning features already trained on larger features. The first implementation has showed very satisfying results. The robust particle detection by Deep Learning is followed by measurement of critical dimensions, then followed by specific corrections. The accuracy and traceability of the size results obtained by standard techniques and data fusion techniques have been benchmarked under the lead of LNE: each technique has been analysed and compared with each other. Compared to individual laboratory/microscope standard statistics calculations, the data fusion provides better confidence interval width: i) statistical methods: gain of 50%, and ii) non-colocalized methods: gain of 60%, over the different particle families. The objective has been successfully achieved.

Impact

The project website has been developed by BAM and is available to stakeholders at: <https://www.bam.de/Content/DE/Projekte/laufend/nPSize/npsize.html>. Four public workshops covering the topics ‘current situation and future needs for reference nanomaterials’, ‘production and certification of reference nanomaterials’, ‘metrology for measurement of nanoparticle size by electron microscopy and atomic force microscopy’ and ‘Traceable characterisation of NPs (size, size distribution, concentration determination) by SAXS’ were organised. A total of about 250 people from public institutes, academia, and industry, the JRC, DIN, ISO, CEN, EU and the Chief Stakeholder have participated. A new nPSize YouTube channel has been created by CEA where 12 videos mainly on measurement of NP size by SAXS have been uploaded: <https://www.youtube.com/channel/UC6kdn4epvHF4OZM7T-mLXJQ/videos>. 24 subscribers and about 1500 views of the published videos have been attained at the end of the project (Dec 2021). The project results were presented at international conferences including posters and talks (25 presentations). The project technical results are available in 14 peer reviewed publications. One textbook on the characterisation of nanoparticles was published. The topics cover the synthesis and characterisation of non-spherical nanoparticles, machine learning approaches to predict final size and shape parameters of synthesized nanomaterials, accurate analysis of particle size distribution for non-spherically shaped nanoparticles by new hybrid approaches, etc.

Impact on industrial and other user communities

Representatives of manufacturers of nanomaterials attended the project’s public Workshop on Reference Nanomaterials on May 14-15, 2018 organized by PTB and BAM. At the workshop the VCI (German Chemistry Industry Association) Chairman presented and discussed the needs expressed by this large industry

association in terms of regulatory framework, development of measurement strategy at nanoscale, and European definition of nanomaterials. Stakeholder requirements were also systematically collected for use in the project. The consortium has links with instrument manufacturers such as Zeiss and Hitachi (manufacturers of electron microscopes) and Xenocs (a manufacturer of X-ray based devices for nanomaterial characterisation), which will support prompt uptake of the project results by industry. In addition, the project has developed under the lead of Pollen a nPSize 'community' on the Zenodo platform for a public database containing tagged electron microscopy (SEM, TEM and TSEM) images, AFM and SAXS measurement data from nanoparticle characterisation methods: <https://zenodo.org/communities/17nrm04-npsize/?page=1&size=20>. A Training course on Reference nanoparticles Production and Certification has taken place successfully at LGC (https://twitter.com/NML_ChemBioGC/status/1207582000313442304). Further, a fruitful virtual training course on the Metrology for Measurement of Nanoparticle Size by Imaging (EM and AFM) was organised online by VSL (<https://www.vsl.nl/en/about-vsl/news/virtual-training-course-metrology-measurement-nanoparticle-size>). At the Training Course 100 participants have attended from academia (many PhD students and postdocs) and industry.

Impact on the metrology and scientific communities

The project will have direct impact on several metrology committees, especially the EURAMET Technical Committee for Length (TC-L), and the CIPM Consultative Committee for Length (CCL) Working Group on Nanometrology (WG-N). The in-depth evaluation of the performance of the traceable nanoparticle sizing techniques selected in this project enhances the understanding of the physical processes involved in the signal generation and their dependence on shape and material variations. For the first time in a large research project both critical aspects, namely the lack of reference nanoparticles and signal modelling are systematically considered. Furthermore, the data fusion for hybrid methods facilitates better knowledge of nanoparticle 3D size measurements.

The project partners have disseminated the results to the scientific nanoparticle characterisation community at international conferences such as NANOSAFE 2018, Microscopy & Microanalysis 2019, Nanoscale 2019, ECASIA 2019, Microscopy & Microanalysis 2020, NANOSAFE 2020, E-MRS Spring 2021, Microscopy & Microanalysis 2021, and E-MRS Fall 2021. Following recommendation by the Chief Stakeholder, the project has been presented at the 2019 International Congress of Metrology.

Impact on relevant standards

The project has the following links between project partners and national standardisation bodies; ISO/TC 229 (BAM, DIN, LGC), CEN/TC 352 (DIN, BAM, LGC) and ISO/TC 202 (BAM, PTB). The project has been presented to DIN NA 062-08-17 AA Nanotechnologies. A case study on the analysis of the size and shape distribution of TiO₂ bipyramidal NPs by TEM under the lead of BAM, with support from UNITO and PTB has been included in the standard ISO 21363 Nanotechnologies — Measurements of particle size and shape distributions by transmission electron microscopy, recently published (August 2020). This documentary standard is of special importance as the very first full ISO standard developed under ISO/TC 229 Nanotechnologies. With support from DIN, a project liaison between the present project with BAM as a coordinator and CEN/TC 352 has been successfully contracted. The project has also provided input to OECD (Organisation for Economic Cooperation and Development) Test 110 Guideline on Particle Size and Particle Size Distribution of Manufactured Nanomaterials. The specification DIN SPEC 52407 was adapted, and the proposal was prepared by PTB with contributions from BAM to be submitted to ISO/TC 229 Nanotechnologies JWG 2 as a new work item. A contribution to ISO/TC 24 SC 4 ISO 19430 was made by PTB by drafting parts of the revised document.

Of particular impact for the standardisation nanoparticle community, two new VAMAS projects consisting of two inter-laboratory comparisons have been approved and started in November 2021 under VAMAS/TWA 34 'Nanoparticle population' having BAM as lead of both projects. Within these projects, TiO₂ nano bipyramids and two bimodal SiO₂ materials were selected as materials of choice for the measurement of complex shape nanoparticles as well as the measurement of the number concentration of bimodal nanoparticles. Sample preparation, measurement and data analysis protocols developed in the present project are offered to all participants. If successful, the results of the ongoing VAMAS inter-laboratory studies will be published and used to extend case studies in the available standard ISO 21363 under the lead of BAM.

Longer-term economic, social and environmental impacts

It is intended that the measurement capabilities for accurate size distribution of nanoparticles developed by this project will be further transferred via normative documents, dedicated workshops and new reference

nanoparticles to European large-scale manufacturers of nanoparticles, to service laboratories, and measurement instrument manufacturers. The new reference nanoparticles, improved signal modelling and data fusion offered by the project, will also contribute to metrological measurement capabilities to address the question of how to implement the EC definition of a nanomaterial. Furthermore, the project will contribute to the accurate identification of nanomaterials and nano-products and thus will provide a more reliable link to characterisation of potential toxic effect, environmental protection and safety will be improved.

List of publications

1. Crouzier, L., Feltin, N., Delvallée, A., Pellegrino, F., Maurino, V., Cios, G., Tokarski, T., Salzmann, C., Deumer, J., Gollwitzer, C., Hodoroaba, V.-D. Correlative Analysis of the Dimensional Properties of Bipyramidal Titania Nanoparticles by Complementing Electron Microscopy with Other Methods. *Nanomaterials* 11 (2021), pp. 1-18. DOI: <https://doi.org/10.3390/nano11123359>
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10. Hodoroaba, V.-D., Cios, G., Tokarski, T., Mansfeld, U., Ortel, E., Mielke, J., Pellegrino, F., Maurino, V. Towards 3D Understanding of Non-spherical Nanoparticles by Transmission Kikuchi Diffraction (TKD) for Improved Particle Size Distribution by Electron Microscopy. *Microscopy and Microanalysis Proceedings* (2020), 1-3. DOI: <https://doi.org/10.1017/S1431927620013999>

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This list is also available here: <https://www.euramet.org/repository/research-publications-repository-link/>

Project start date and duration:		01 May 2018, 42 months
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Project website address: https://www.bam.de/Content/DE/Projekte/laufend/nPSize/npsize.html		
Chief Stakeholder Organisation: CEN/TC 352, WG1		Chief Stakeholder Contact: Dr. Emeric Frejafon
Internal Funded Partners:	External Funded Partners:	Unfunded Partners:
1 BAM, Germany	7 CEA, France	-
2 LGC, United Kingdom	8 DIN, Germany	
3 LNE, France	9 POLLEN, France	
4 PTB, Germany	10 UNITO, Italy	
5 SMD, Belgium		
6 VSL, Netherlands		
RMG: -		