

# D.5.47 Annex 1

Inter-laboratory comparison on measurand Particle size/Particle size distribution

## Report of the Results

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## Abstract

An inter-laboratory comparison on the particle size, expressed as mean diameter  $d$ , of nanoscaled SiO<sub>2</sub> (#14 BAM Silica (see D.5.41/5.42)) has been performed. The majority of participants used Dynamic Light Scattering (DLS). A few used Electron Microscopy as method. Following methods had been applied by only one partner, respectively: Small Angle X-ray Scattering, Analytical Ultracentrifugation, Atomic Force Microscopy and Atomizer with electric mobility spectrometer.

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## 1 Introduction

The Task 5.4 of NanoValid is designed to test, compare and validate current methods to measure and characterize physicochemical properties of selected engineered nanoparticles. This will be achieved by inter-laboratory comparisons. The measurand of one of these round robins is *Particle size/Particle size distribution*. The measurements are to be accompanied by estimates of the uncertainties at a confidence level of 95%, deduced from the standard uncertainties. Therefore an uncertainty budget comprising statistical (Type A) and systematic (Type B) errors has to be established and delivered for each measurand. The inter-laboratory comparison protocol comprises two Annexes addressing the establishment of uncertainty budgets following GUM. The final goal of the comparison is to identify those methods of measurement which have potential as reference methods in pc characterization of nanoparticles for the determination of a given measurand (Task 5.4 of the NanoValid Project).

## 2 Inter-laboratory Comparison particle size

### 2.1 Objective

The objective of the Inter-laboratory Comparison was to determine the average particle size of nanoscaled SiO<sub>2</sub> (#14 BAM Silica (see D.5.41/5.42)), expressed in nanometer. The participants were free to choose the analytical method, provided it was fit for purpose.

### 2.2 Participation

In the beginning of the NanoValid project partners took part in a survey (Deliverable D5.46) asking for devices and ability to participate in a round robin with measurand size. Members and other interested parties were informed about the organisation of the inter-laboratory comparison. Individual invitation e-mails were sent to those potential participants together with a standardized round robin protocol in April 2013 after announcing it at the regular project meeting in November 2012.

Finally, 11 organizations and companies participated in the inter-laboratory comparison. There were two Non-EU partners.

### 3 The test material

#### 3.1 Production

The test material stems from a batch made for the inter-laboratory comparison. Batch was produced by BAM in Germany in form of a dispersion followed by dilution to 0.05 wt% and distribution in bottles á 5 ml sample amount. Sufficient material was available that all participants received material not previously used for any measurements except as in 3.2.

#### 3.2 Initial characterisation of the test material

Material which has been measured is BAM-silica. It has been preliminary tested regarding potential to be developed as a certified reference material by (T)-SEM, TEM and XPS (Deliverable 5.40, Task 5.1). With electron microscopes operated in transmission mode (T-SEM) of two laboratories area-equivalent mean diameters of 14 nm (standard deviation 3 nm) and of 17 nm (standard deviation 2 nm) were determined, respectively. Round robin batch has been characterized with SAXS, (T)-SEM, DLS and zeta-potential. In Deliverable 5.46 suitable methods are documented. For SAXS, curve fitting was performed with a sphere model and assuming a Schulz-distribution and bimodal Schulz-distribution, respectively. Results indicate two size classes present in the dispersion. The first size class comprises particles with a mean diameter of 14.6 nm and polydispersity 0.13. Particles of the second size class exhibit a negligible polydispersity of 0.003 with a mean diameter of 40.6 nm. Particles can be seen in the SEM and T-SEM image, which are displayed in Fig. 1.

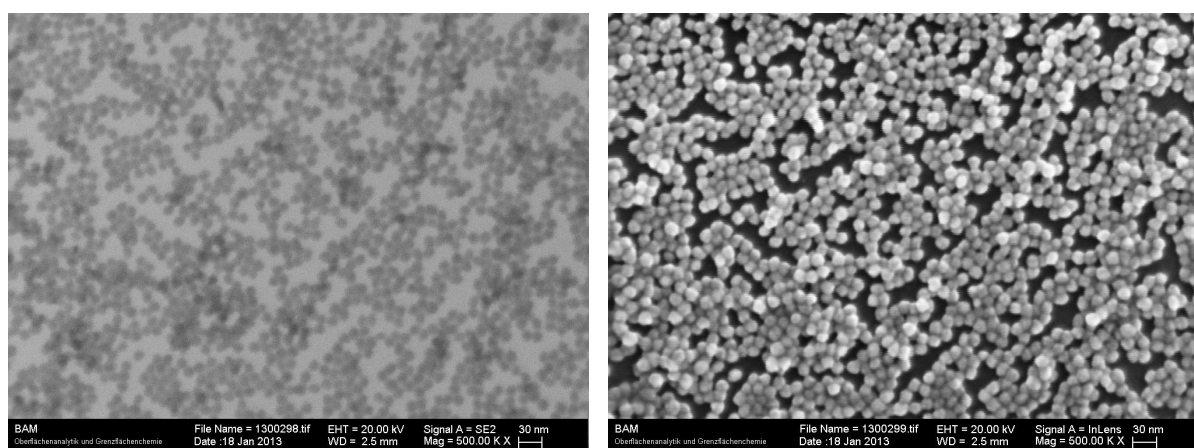


Fig. 1: T-SEM image left and SEM (In Lens) image right and of round robin batch BAM-silica

### **3.3 Sample distribution and deadline for reporting results**

Discussions were held with participants concerning their requirements for sample numbers and sizes. Standard sets comprised 1, 2 or even more sample bottles in dependence of type and number of size and zeta potential method(s) chosen by the participants, as the size inter-laboratory comparison was combined with the zeta-potential round robin for efficiency reasons in order to save money and time in terms of one combined round robin protocol and usage of the same test material. Sample bottles together with cover letter were dispatched to laboratories in April 2013 accompanied by the round robin protocol via email. One participant did not receive his sample and others requested more amount of sample, therefore dispatchment had to be repeated in several cases.

The deadline was set to 15 June 2013 initially, which was extended subsequently as due to different reasons several participants delivered their results not before end of 2014.

### **3.4 Instructions to the participants**

The shipped package contained

- a 1 page covering letter
- the bottled samples.

The protocol was sent in parallel via email and contained

- a statement of the objectives
- the specific serial code for each sample
- the timetable
- details of the material, sample handling and safety information
- details of issues to report including the uncertainty calculation for 95% confidences
- a table to report the size and uncertainties.
- annexes describing sources of uncertainties for several methods from literature

## **4 The participants' measurement procedures**

All participants have submitted results. To establish a full uncertainty budget, a number of participating labs also calculated or estimated systematic uncertainty contributions. They stated a functional relationship and described the various uncertainty contributions to the uncertainty budget.

In the following paragraphs, information as derived from the questionnaire is summarized.

#### **4.1 Methods, instrumentation and brief experimental details**

Lab1 used SAXS with a SAXSess camera (Anton Paar, Austria). This camera is attached to a laboratory X-ray generator (PW3830, PANalytical)

Lab2 used AUC with a Beckman instrument (detector type XL-I/XL-A, rotor type An-50 Ti).

Lab3 used AFM with a JPK Nanowizard with capacitive position sensors

Lab4 used SEM with a FEI FIB/SEM Helios NanoLab 650,

Lab5 used TEM with a Tecnai G2 Spirit (FEI Company),

Lab6 used SEM in transmission mode with a LEO (Zeiss) Supra 35 VP.

Lab7 used SEM in transmission mode with a Zeiss Supra 40 and a Zeiss single unit transmission set-up.

Lab8 used DLS with a Zetasizer Nano ZS (Malvern instruments) and He-Ne laser (632.8 nm), detector angle 173°.

Lab9 used DLS with a Zetasizer Nano ZS (Malvern Instruments) with 173 ° detector angle and He-Ne laser (633 nm).

Lab10 used DLS with a Beckman Coulter Delsa Nano C with 165 ° detector angle and He-Ne laser (633 nm).

Lab11 used DLS with a Mobius (Wyatt), 170 ° detector angle and DPSS laser (532 nm, 52 mV),

Lab12 used DLS with a Zetasizer Nano ZS (Malvern Instruments) with 173 ° detector angle and He-Ne laser (633 nm).

Lab13 used DLS with a Zetasizer Nano ZS (Malvern Instruments) with 173 ° detector angle and He-Ne laser (633 nm, max. 4 mW).

Lab14 used DLS with a Zetasizer Nano ZS (Malver instruments), detector angle 173 ° and a He-Ne-Gas laser (633 nm, 4 mW)

Lab15 used DLS with a Zeta PALS (Brookaven) with 15 ° and 90 ° detector angles and laser wavelength of 658 nm (solid state complies with BRH 21CFR1040:10, 15mW).

Lab16 used Atomizer with electric mobility spectrometer with a two-component atomizer (Topaz ATM 220, operated at 1.6 bar) to form airborne primary droplets. Primary droplets were dried in a diffusion dryer (GRIMM 7814) and measured with a Scanning Mobility Particle Sizer (SMPS).

## **5 Results and discussions**

### **5.1 Results**

The values of the particle size expressed as equivalent spherical particle diameter, which has been determined by the participating laboratories and methods as well as their associated uncertainties at a 95% level of confidence are presented in Table 1 for the inter-laboratory comparison. Figures 2 and 3 display the results from Table 1 in graphical form, which means y-axes show the mean particle diameter and x-axes the name of participant in Fig. 2 and participant plus method in Fig. 3, respectively.

From the submitted results the DLS measurements are considered separately from the others delivered by AFM, T-SEM, SEM, TEM, SAXS, SMPS and AUC measurements.



Table 1: Participants' results for the particle size of BAM-silica #14 and measurement uncertainties delivered by the laboratories

BAM-silica dispersion										
Participant	Sample preparation	Measurement and data analysis method	Value of Measurand $d$ / nm	Measurand (equivalent spherical particle diameter "Size") calculated as	Uncertainties in units of measurand					
					A-type (Standard uncertainty of the mean of measurand), $u_s$	B-type 1: Standard uncertainty $u_1$	B-type 2: Standard uncertainty $u_2$	...more B-type errors	Combined standard uncertainty $u_c^*$	Expanded uncertainty, $U = k u_c$ (Coverage factor for 95 % confidence level, $k=2$ )
Lab1	Syringe filtered (PES)	SAXS	15.8	Number-weighted, Mean	0.07*	0.132 (bias)	0.085 (q-Vector)	0	0.235	0.469
Lab2 <sup>a</sup>	As received	AUC	14.1	Hydrodynamic diameter (Stokes diameter), Mean	0,78*	0	0	0	0	0
Lab3	Diluted	AFM	13.12	Number-based, Mean	0.03	0,60	0	0	0	1.20
Lab4	Diluted	SEM	15.30	Number-based, Mean	0.06	0.2 (image calibration)	0.4 (image processing)	0	0	0.8
Lab5	As received	TEM	14.2	Number-based, Mean	0.04	0.2 (image calibration)	0.6 (image correction)	0	0	1.2
Lab6	As received	T-SEM	18.5	Number-based, Mean	0.03	0.01 (Pixel calibration x-direction)	0.07 (Pixel calibration y-direction)	See Table	0.81	1.6
Lab7	Diluted	T-SEM	14.5	Number-based, Modal	0.04	0.658998	0	0	0.660455	1.32

Lab8 <sup>d</sup>	Syringe filtered (Polyether-sulfone, PES)	DLS, NNLS	41.7	Hydrodynamic diam., Intensity-weighted, Mean	0.7943 *	0,3008* ( <i>u</i> <sub>bias</sub> )	0	0	0,8493*	1,7*
Lab9 <sup>a,b</sup>	Syringe filtered (Cellulose acetate, CA)	DLS, Cumulant analysis	25.17	Hydrodynamic diam., Intensity-weighted, Mean	0.93	0	0	0	0	0
Lab10 <sup>b,c,e</sup>	As received	DLS Cumulant analysis	26.10	Hydrodynamic diam., Intensity-weighted, Mean	0.36*	0	0	0	0	0
Lab11 <sup>a,b</sup>	As received	DLS Cumulant analysis	28.2	Hydrodynamic diam., Intensity-weighted, Mean	0.5*	0	0	0	0	0
Lab12 <sup>a,b</sup>	As received	DLS Cumulant analysis	28.25	Hydrodynamic diam., Intensity-weighted, Mean	1.11*	0	0	0	0	0
Lab13 <sup>a,b,d,f</sup>	As received	DLS Cumulant analysis	25.12	Hydrodynamic diam., Intensity-weighted, Mean	0.25*	0	0	0	0	0
Lab14 <sup>b</sup>	As received	DLS Cumulant	28.74	Hydrodynamic diam., Intensity-weighted, Mean	0.439*	0*	0*	0*	0	0*
Lab15	Diluted	DLS Cumulant analysis	34.08	Hydrodynamic diam., Intensity-weighted, Mean	0.368	6.08	0	0	0	12.18*
Lab16 <sup>c</sup>	Diluted	Atomizer+SMPS	37	Modal	0	0	0	0	0	0

<sup>a</sup> No uncertainties of type B were provided

<sup>b</sup> expert-based estimated B-type uncertainties introduced in tables 3 and 4

<sup>c</sup> No uncertainties (type A and B) were provided

<sup>d</sup> BAM1.3: varied further parameters in additional tests as material of syringe filters and dilution receiving following values for mean of intensity-weighted hydrodynamic diameter and

A-type standard uncertainties: 34.84 nm ± 1.95 nm (RC), 33.0 nm ± 0.3 nm (CME) and 82.4 nm ± 1.1 nm (PES and diluted) calculated with NNLS; Lab13: reported additional measurements of diluted sample (30.2 nm mean of intensity-weighted hydrodynamic diameter and 1.6 nm standard deviation)

<sup>e</sup> 5 instead of 6 replicates

<sup>f</sup> Lab10 noted a size dependency on time of their measurements of 6 replicates, hence reported mean of 3 replicates

NNLS – Non Negative Least Squares Algorithm

\* values recalculated by BAM

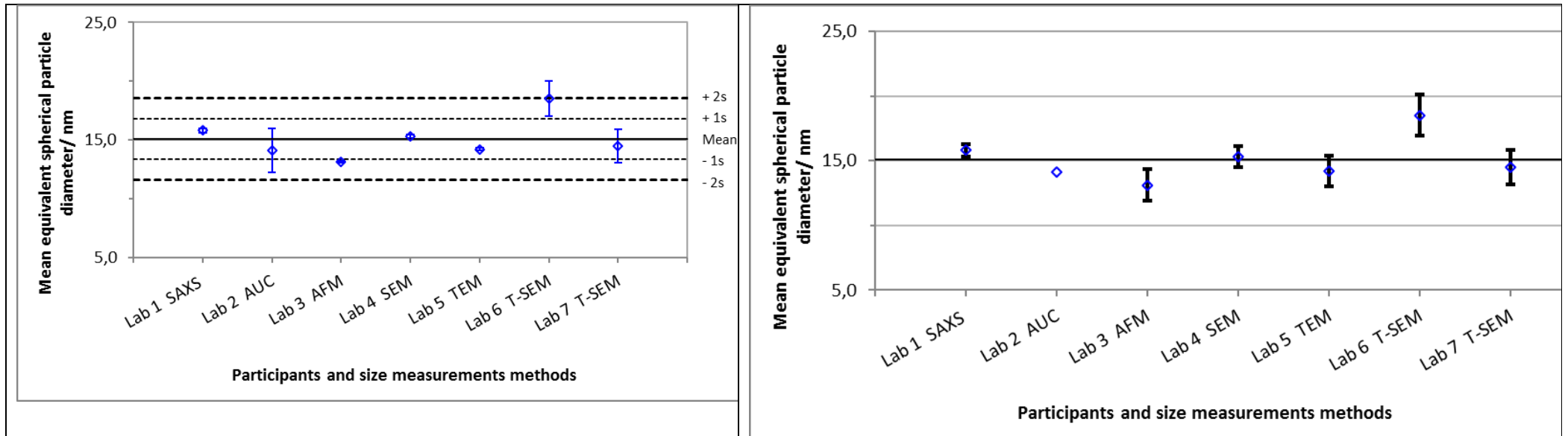


Fig. 2 Mean of equivalent spherical particle diameter versus participating laboratory and sizing method, left: with error bars indicating standard deviation (A-type) and right: with error bars indicating combined uncertainty at 95 % confidence level (AUC delivers Stokes diameter, SMPS data are removed as outliers).

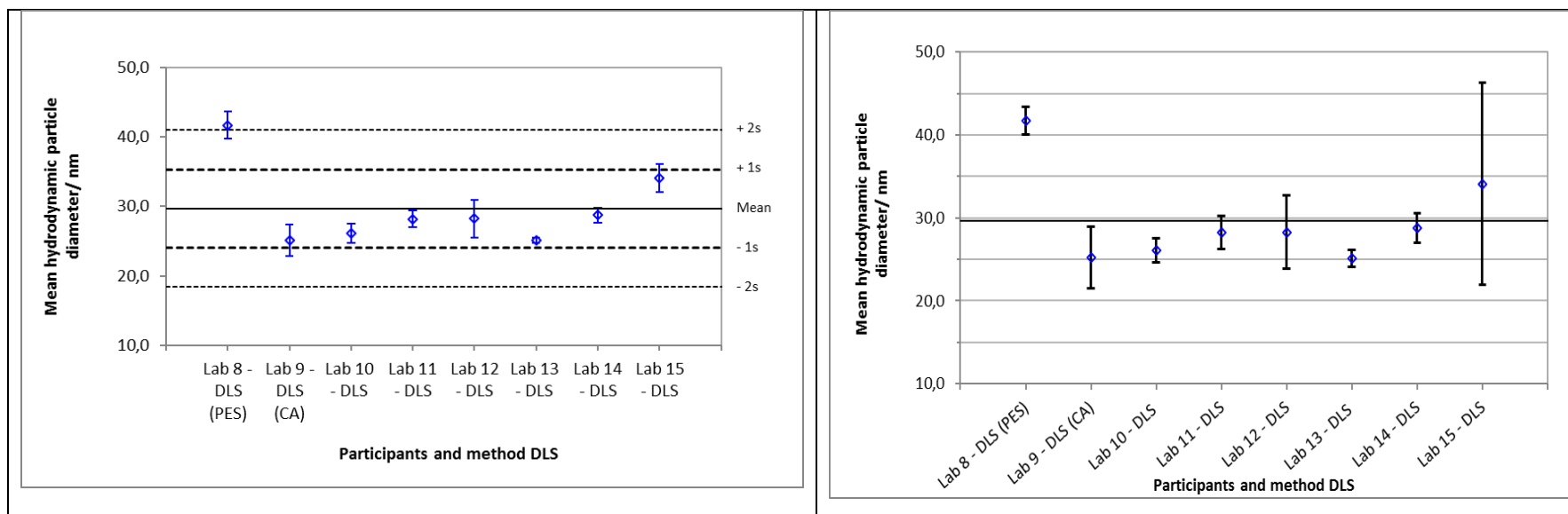


Fig. 3 Mean of hydrodynamic particle diameter (as measured by DLS) versus participating laboratory, left: with error bars indicating standard deviation; and right: with error bars indicating combined uncertainty at 95 % confidence level

### 5.1.1 Method T-SEM – Uncertainty budget

Participant Lab6 delivered a full uncertainty budget, whose contributions are detailed in Table 2. There, Lab6 applied the bottom-up approach. In contrast, Lab7 chose to follow the bottom-down approach by measuring a reference material certified by size (dispersed silica nanoparticles, ERM-FD 100, IRMM, Belgium).

**Table 2 Method T-SEM - Detailed uncertainty budget of established by participant Lab6**

Uncertainty contribution	Value/ nm (k=1)
Pixel calibration X-direction	0,01
Pixel calibration Y-direction	0,07
Threshold	0,43
Grey value, middle of particle	0,06
Grey value, outside of particle	0,17
Statistical contribution	0,03
Selection of particles	0,60
Pixelation error	0,02
Influences of contaminations	0,20
Deviation from circular shape	0,20
Uncertainty (k=1)	0,81
Expanded uncertainty (k=2)	1,6
Only uncertainty contributions > 0.01 nm are considered, uncertainty contribution of sample preparation is not included	

### 5.1.2 Method DLS – Uncertainty budget

Most participants did not deliver a full uncertainty budget comprising statistical as well as systematic uncertainties. Nevertheless, a full uncertainty budget has been established for respective participants after receiving of all data in terms of an expert-based estimation of the

systematic uncertainty. Estimation of uncertainty refers to the work of Takahashi et al. [1], who determined uncertainty of DLS measurements in accordance to the Guide to the Expression of Uncertainty in Measurement (GUM). Table 3 shows parameters which influence the hydrodynamic particle diameter together with their identified uncertainties. Uncertainty component of **Boltzman constant**,  $u_k(k_B)$ , was taken from literature [2].

A fluctuation of  $\pm 0.1^\circ$  of component **temperature** is based on Malvern Zetasizer software note (Dispersion Technology Software, version 6.01) which states temperature is stable within  $0.1^\circ$  after it has been reached equilibrium of the pre-set temperature. The temperature fluctuation can be assumed to be a uniform *a priori* probability distribution according to ISO Guide GUM [8] with a half-width of  $0.1^\circ$ , thus standard uncertainty is:  $u_T = \frac{0.1^\circ}{\sqrt{3}}$ . Furthermore, Malvern Technischer Bericht [3] provides a temperature precision of the instrument of  $0.1^\circ\text{C}$  and an accuracy of  $0.2^\circ\text{C}$ . Thus, the combined uncertainty of both is  $u_T = \sqrt{\left(\frac{0.1^\circ}{\sqrt{3}}\right)^2 + \left(\frac{0.2^\circ}{\sqrt{3}}\right)^2}$ .

**Viscosity**,  $\eta$ , of dispersion medium is adopted from literature, ISO 3666 [4] of pure water with a value of  $\eta = 1.0016 \text{ mPa s}$  ( $20^\circ\text{C}$ ) and a standard uncertainty,  $u_\eta$ , of  $0.00085 \text{ mPa s}$ . A second contribution of viscosity depends on experimental temperature and its variation and is calculated as follows [5]:  $\eta = A \exp \frac{1+BT}{CT+DT^2}$  with  $A = 0.1257187 \times 10^{-1} \text{ mPa s}$ ,  $B = -0.5806436 \times 10^{-2} \text{ K}^{-1}$ ,  $C = 0.1130911 \times 10^{-2} \text{ K}^{-1}$ ,  $D = -0.5723952 \times 10^{-5} \text{ K}^{-2}$ . It gives a viscosity of  $0.890214270 \text{ mPa s}$  at  $25^\circ\text{C}$  and of  $0.954702029 \text{ mPa s}$  at  $22^\circ\text{C}$ . Considering a stability of temperature within  $\pm 0.1^\circ$  as mentioned above viscosity of solvent ranges between  $0.888186939 \text{ mPa s} \leq \eta \leq 0.892249108 \text{ mPa s}$  for  $25^\circ\text{C}$  and between  $0.952432076 \leq \eta \leq 0.956980733$  for  $22^\circ\text{C}$ . Both contributions result under the assumption of a symmetric and rectangular probability distribution in a combined standard uncertainty of:  $u_\eta = \sqrt{0.00085^2 + \left(\frac{0.002031085}{\sqrt{3}}\right)^2}$  at  $25^\circ\text{C}$  and  $u_\eta = \sqrt{0.00085^2 + \left(\frac{0.002274328}{\sqrt{3}}\right)^2}$  at  $22^\circ\text{C}$ .

For Malvern Zetasizer Nano ZS instrument any uncertainty of the **scattering angle**,  $\theta$ , is stated in the Technischer Bericht from Malvern Instruments) [3] as becoming insignificant. Thus, determination of standard uncertainty of scattering angle could be discarded. In lieu of discarding it as mentioned by the manufacturer, value of uncertainty has been copied from Meli et al. [6] as they determined it from the geometry of goniometer system of the same instrument used in this round robin (by participants except IUTA, INMETRO and MCGU) and for the same scattering angle of  $173^\circ$ :  $u_\theta = 2.52 \times 10^{-4} \text{ rad}$  (rectangular distribution).

The standard uncertainty of laser wavelength comprises standard deviation of **wavelength**,  $\lambda$ , of He-Ne gas laser ( $632.816 \text{ nm}$ ) and DPSS laser ( $532 \text{ nm}$ ), respectively. For the former, Meli et al. [6] published a fluctuation of wavelength of  $\pm 0.001 \text{ nm}$  due to thermal expansion and

contraction of the cavity. A normal probability distribution is assumed for the standard uncertainty of wavelength [1]:  $u_\lambda = \frac{3 \cdot 0.001 \text{ nm}}{3} = 0.001 \text{ nm}$ . The change of **refractive index**,  $n$ , of solvent water in dependence of temperature between 20 °C – 30 °C and 532 nm wavelength of laser according to [1] is within the range 1.330 to 1.340 [10]. A triangular distribution is assumed [1]:  $u_n = \frac{0.005 \text{ nm}}{\sqrt{6}} = 0.0020$ . Analogously, participants with device' laser wavelength of 633 nm a range from 1.33211 to 1.33105 of  $n$  is taken from literature [10] and  $u_n$  calculated as 0.00022. Among the contributions to the uncertainty budget the uncertainty of the decay rate has the strongest impact on the budget [1,7]. Takahashi et al. [1] and Kwon et al. [7] had determined the uncertainty contribution of decay rate experimentally. As this experimental approach could not been realized here, a contribution of decay rate of 70 % of the total uncertainty which was taken from literature [1] was transferred to the results here and uncertainty contribution calculated accordingly.

Finally, all hydrodynamic diameters include a **hydration layer** on the particles [1].

**Table 3: DLS measurement components and their uncertainties\***

Component, symbol	Value of component	Standard uncertainty of component	Sensitivity coefficient
Boltzmann constant, $k_B$	$1.3806503 \times 10^{-23} \text{ J K}^{-1}$	$u_k(k_B) = 2.3 \times 10^{-29} \text{ J K}^{-1}$	$R / k_B$
Absolute temperature, $T$	298.15 K (25 °C) – Labs 9, 10, 11, 12, 14; 295.15 K (22 °C) – 13	$u_T(T) = 1.29099 \times 10^{-1} \text{ K}$	$R / T$
Solvent viscosity, $\eta$	0.8902141264 mPa s (at 25 °C); 0.954702019 mPa s (at 22 °C)	$u_\eta(\eta) = 0.001448310 \text{ mPa s}$ $u_\eta(\eta) = 0.001564190 \text{ mPa s}$	$R / \eta$
Scattering angle, $\theta$	173 ° – Labs 9, 12, 13, 14;  165 ° – Lab10; 170 ° – Lab11;	Insignificant [3] or $u_\theta = 2.52 \times 10^{-4} \text{ rad}$ [6]	$R / \tan(\theta/2)$
Wavelength of laser, $\lambda$	He-Ne laser (633 nm) – Labs 9, 10, 12, 13, 14; DPSS laser (532 nm) – Lab11	$u_\lambda(\lambda) = 1.0 \times 10^{-12} \text{ m}$	$2R / \lambda$
Refractive index of solvent, $n$	1.33 - Labs 9, 10, 12; 1.330 - 13; 14 1.333 - Lab11;	$u_n(n) = 0.00022$ (for 633 nm – Labs 9, 10, 12, 13, 14) $u_n(n) = 0.0020$ (for 532 nm – Lab11)	$2R / n$
Decay rate of correlation function, $\Gamma$	Not available	Taken from literature [1] with 70 % contribution of total uncertainty	$2R / \Gamma$

\*based on model approach of Takahashi et al. [1] and Meli [6] et al.

**Table 4: Estimated uncertainty budgets of DLS results (from cumulant analysis) of participants including systematic uncertainties derived from table 3 (all units in nanometer)**



Participant	Sample preparation	Hydro-dyn. diameter $d$ / nm	Contributions to uncertainty budget of either A-type (A) oder B-type (B)								Combined uncertainty $u_c$ / nm	Expanded uncertainty $U$ ( $k=2$ ) / nm
			A Standard uncertainty $u(s)$ / nm	B Boltzmann constant $R/k_B \cdot u_k(k_B)$ / m	B Absolute temperature $R/T \cdot u_T(T)$ / m	B Solvent viscosity $R/\eta \cdot u_\eta(\eta)$ / m	B Scattering angle $R/\tan(\theta/2) \cdot u_\theta(\theta)$ / m	B Wave-length $2R/\lambda \cdot u_\lambda(\lambda)$ / m	B Refractive index $2R/n \cdot u_n(n)$ / m	B Decay Rate $e$ $2R/\Gamma \cdot u_\Gamma(\Gamma)$ / m		
Lab9 <sup>a,b</sup>	Syringe filtered (CA)	25.17	0.93	$2.097 \cdot 10^{-14}$	$5.449 \cdot 10^{-12}$	$4.843 \cdot 10^{-10}$	$1.940 \cdot 10^{-13}$	$3.977 \cdot 10^{-14}$	$4,090 \cdot 10^{-12}$	$3.296 \cdot 10^{-11}$	1.86	3.73
Lab10 <sup>b,c,d</sup>	As received	26.10	0.361*	$2.174 \cdot 10^{-14}$	$5.651 \cdot 10^{-12}$	$5.021 \cdot 10^{-10}$	n.a.	$4.124 \cdot 10^{-14}$	$4.241 \cdot 10^{-12}$	$3.418 \cdot 10^{-11}$	0.73	1.46
Labs11 <sup>b,d</sup>	As received	28.2	0.5 *	$2.349 \cdot 10^{-14}$	$6.105 \cdot 10^{-12}$	$5.426 \cdot 10^{-10}$	n.a.	n.a.	$4.225 \cdot 10^{-11}$	$7.402 \cdot 10^{-11}$	1,00	2.00
Lab12 <sup>a,b</sup>	As received	28.25	1.11 *	$2.353 \cdot 10^{-14}$	$6.116 \cdot 10^{-12}$	$5.435 \cdot 10^{-10}$	$2.177 \cdot 10^{-13}$	$4.464 \cdot 10^{-14}$	$4.590 \cdot 10^{-12}$	$3.700 \cdot 10^{-11}$	2.22	4.43
Lab13 <sup>a,b</sup>	As received	25.12	0.25	$2.091 \cdot 10^{-14}$	$5.489 \cdot 10^{-12}$	$2.056 \cdot 10^{-11}$	$1.935 \cdot 10^{-13}$	$3.966 \cdot 10^{-14}$	$4.079 \cdot 10^{-12}$	$3.310 \cdot 10^{-11}$	0.51	1.02
Lab14 <sup>b</sup>	As received	28.74	0.439 *	$2.394 \cdot 10^{-14}$	$6.222 \cdot 10^{-12}$	$5.529 \cdot 10^{-10}$	$2.215 \cdot 10^{-13}$	$4.542 \cdot 10^{-14}$	$4.670 \cdot 10^{-12}$	$3.764 \cdot 10^{-11}$	0.88	1.77

<sup>a</sup> No uncertainties of type B were provided

<sup>b</sup> Expert-based estimated B-type uncertainties

<sup>c</sup> No uncertainties (type A and B) were provided

<sup>d</sup> Uncertainty of temperature, which was calculated for Malvern Zetasizer instrument was adopted for IUTA and MCGU though they used another device due to lack of specification of temperature fluctuation;

<sup>e</sup> Contribution of uncertainty of decay rate adopted from [1] as 70% of total uncertainty

\*values recalculated by BAM

### 5.1.3 Method AUC – Uncertainty budget

Lab2 did not deliver an Uncertainty budget for their AUC data measured with a Beckman instrument.

## 5.2 Discussion

Two laboratories, Lab13 and Lab8, performed analysis of both, as-received as well as of diluted sample with method DLS. Hydrodynamic diameter changed of about 5 nm and of even 40 nm up to bigger sizes, respectively. In the case of Lab8, a syringe filtration step was applied additionally to the diluted as well as to the undiluted sample. The third laboratory, Lab15, which measured the sample only in a diluted state, received size values in the range as Lab13 did which is close to the hydrodynamic diameters of those participants who measured the dispersion in an as-received state. Although the size difference of 40 nm in case of Lab8 laboratory is very high, an influence of dilution cannot be inferred in general as the Z-averages of Lab15 and Lab13 show. Besides dilution another factor may influence the size distribution, which is the sample pre-treatment step syringe filtration. Participant Lab8 performed three analyses with different syringe filter material each and participant Lab9 one analysis with a fourth kind of filter material. With filter material PES Lab8 gained bigger hydrodynamic sizes than with the other filter materials CME, RC and CA (the latter material used by participant Lab9). As these sample pre-treatments have not been evaluated systematically no final conclusion can be drawn. Moreover, for the filter materials there are neither standards, certificates nor is there any reference available. Finally, Lab13 reported a time dependence of the particle size, more precisely, an increase of about 5 nm in diameter (“Z-average”) about half an hour after the measurement started.

Applied particle sizing methods can be divided into two groups, *in-situ* (SAXS, DLS, AUC) and *ex-situ* methods (AFM, TEM, SEM, T-SEM). Here, an *in-situ* method refers to particles analyzed in a dispersed state, whereby an *ex-situ* method refers to particles measured in a dried state. With the so-called *ex-situ* methods AFM, TEM, SEM and T-SEM single particles can be measured in contrast to the *in-situ* methods (SAXS, DLS and AUC) which are restricted to the analysis of ensembles of particles. On the other hand, from the results of Table 1 it can be seen that the mean particle diameter in dependence of the method differs that it is > 25.0 nm for DLS and Atomizer+SMPS method and < 20.0 nm for all other sizing methods. Therefore, graphical

display of results from Table 1 has been split up into two figures (Fig. 2 and 3). It is clear from Fig. 2 and 3, that there is a different capability (expressed as different measurands) of the applied methods to measure primary particles, which have, according to Fig. 2, an estimated mean size of about 15 nm.

Results of method Atomizer+SMPS could not be evaluated further. One initial drawback was that the provided amount of sample liquid was too low to be analyzed undiluted. Thus, several measurements with different dilution degrees of the sample had been made. It revealed a dependence of decreasing size on increasing dilution. The smallest size, 37 nm, was achieved with the highest dilution ratio of 1:1000 and is in the size range of those diameters which have been measured with DLS. Unfortunately, as the blank test gave a similar signal compared to the signal from 1:1000 diluted sample, thus, the result cannot be considered for further evaluation. Furthermore, it is assumed that several particles may stick together within the droplet when nebulized and after the water has been evaporated an agglomerate/aggregate is left for detection. Hence, the size of the primary particle cannot be inferred from these measurements.

Besides DLS size the polydispersity index, PI, had to be reported. In general, PI was found to be between values of 0.22 and 0.35, of the participating laboratories with one exception. These PI values resemble a non-monodisperse material according to ISO 22412:2008 (PI typically  $\geq 0.1$ ) [8]. The PI values are well below 0.5; if they would be above 0.5 then it is not recommended to use the Z-average mean but rather to determine the peak positions via distribution analysis [9]. A high value of PI indicates the presence of agglomerates and/ or of bigger particles besides smaller ones and/or contaminations in terms of dust [9]. The presence of bigger particles can be neglected as it has been found out by imaging with electron microscopy. Instead, T-SEM images revealed a big portion of agglomerated particles besides individual particles distributed on the TEM-grid. As the T-SEM measurements are performed in dry state this result cannot necessarily be assigned one-by-one to the dispersed state. Nevertheless, the finding of agglomerates and lack of bigger particles in T-SEM images support the indication that agglomerates are causing the measured bigger "DLS sizes" in comparison to particle sizes determined with all other methods. For comparative purposes the "DLS sizes" which are intensity-weighted have been transformed into volume- and number-weighted particle sizes by using the Mie theory and by applying a refractive index of silica of 1.46 and an absorption of 0.01. These calculated sizes, not to be considered as absolute values, are displayed in table 5. Particle sizes in table 5 decline according to the row  $D(i) < D(v) < D(n)$ . This can be explained in case of the intensity distribution

that agglomerates/aggregates or larger particles are more emphasized than smaller ones reversely to the volume distribution and number distributions where smaller particles are more emphasized. This relies on the fact that volume and number of the smaller particles are counted with the third and sixth power relatively compared to intensity. According to table 4, both, volume- and number-based sizes are now settled in the range of sizes which have been determined with all other methods than with DLS. Thus, agglomerates seem to be present either in terms of two or more particles forming respective agglomerates.

**Table 4: DLS particle sizes weighted by intensity, volume or number and polydispersity indices**

Participant and note of sample preparation	Intensity-weighted hydrodynamic mean diameter (Standard deviation)	Polydispersity index (Standard deviation)	Volume-weighted hydrodynamic mean diameter	Number-weighted hydrodynamic mean diameter	Method of analysis
Lab8 PES	41.9 (3.5) <sup>1</sup>	0.5580 (n.a.) <sup>1,2</sup>	16.6 (0.9)	11.4 (1.1)	NNLS (General Purpose)
Lab8 RC	33.4 (0.4) <sup>1</sup>	0.2154 (n.a.) <sup>1,2</sup>	19.8 (0.5)	14.8 (0.7)	NNLS (General Purpose)
Lab8 CME	33.2 (0.5) <sup>1</sup>	0.25 (n.a.) <sup>1,2</sup>	18.4 (0.5)	13.2 (0.8)	NNLS (General Purpose)
Lab8 PES, diluted 1:10	82.4(2.8) <sup>1</sup>	0.2368 (n.a.) <sup>1,2</sup>	49.9 (2.5)	35.0 (2.8)	NNLS (General Purpose)
Lab9 CA	25.17 (n.a.) <sup>3</sup>	0.232 (n.a.)	–	–	Cumulants
Lab10	26.1 (1.39) <sup>* 3</sup>	0.291(0.028) <sup>*</sup>	–	–	Cumulants
Lab11	28.2 (1.2) <sup>3</sup>	0.23 (0.04)	–	–	Cumulants

	35.9(2.8)	0.213 (0.065) <sup>2</sup>	17.8(2.0)	12.2(5.4)	Regularization
Lab12	28.25 (2.71) * <sup>3</sup>	0.33 (0.06) *	–	–	Cumulants
	?	?	11.8 (1.53)	?	?
Lab13	25.1 (0.4) <sup>3</sup>	0.35 (0.01)	–	–	Cumulants
	34.83(24.83) <sup>5</sup> (97.0 Int. %)	?	12.59(7.394) <sup>5</sup> (100.0 Vol. %)	8.968(3.251) <sup>5</sup> (100.0 Num. %)	NNLS
Lab13 Diluted 1:1	30.2 (1.6)	0.35 (0.005)	–	–	Cumulants
Lab14	28.74 <sup>3</sup> (1.075)	0.304 (0.031)	–	–	Cumulants
	33.85 <sup>5</sup> (18.93) (93.7 Int. %)	–	16.58 <sup>5</sup> (8.367) (95.6 Vol. %)	11.54 <sup>5</sup> (4.02) (94.5 Num. %)	NNLS (General Purpose)
Lab15 Diluted	34.08 <sup>3</sup> (2,02) * <sup>6</sup>	0.2434* (0.0396) * <sup>6</sup>	–	–	Cumulants

Abbreviations of syringe filter materials: PES – Polyethersulfone, RC – Renegerated cellulose, CME – Cellulose mixed esters, CA – Cellulose acetate;

**1** Values differ in comparison to values in table 2 because of removal of single values here due to transformation into volume-weighted and number-weighted mean diameters

**2** Calculation: Square of quotient of peak width and peak mean

**3** Z-average (= Cumulants mean)

**4** Values differ in comparison to values in table 2 because they are here based on regularization analysis in contrast to result in table 2 which is based on cumulant approach

**5** Mean of strongest peak

**6** Mean of 30 single values (6 replicates á 5 measurements) instead of the mean of 6 replicates

\*Values recalculated by BAM

## 6 Conclusion and Outlook

From DLS method it can be concluded that direct inference from raw intensity-weighted data to the primary particle size was hindered, as there is some agglomeration in dilution present which overcounts the bigger size of these agglomerates in competition to primary particles (independently of the analysis method, i.e. cumulant or regularization). As from the other methods it shows to be a rather monodisperse material DLS is contradictory in this point. Relatively small uncertainty budgets are gained with more than one method, though the lowest value of  $U$  at a 95 % confidence level was gained with SAXS. Thus, this method is considered a suitable “sizing” method for the nanomaterial which has been investigated here.

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