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Certification of Standard Reference Material® 1992 / ERM®-FD305
Zeta Potential – Colloidal Silica
(Nominal Mass Fraction 0.15 %)

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NIST

**National Institute of
Standards and Technology**

U.S. Department of Commerce

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Abstract

This report describes the production of SRM 1992/ERM-FD305, silica particles suspended in borate buffer, certified for electrophoretic mobility and zeta potential by electrophoretic light scattering (ELS). This material was produced following ISO 17034:2016 and is certified in accordance with ISO Guide 35:2017. The certified reference material (CRM) was jointly produced by the Directorate F - Health, Consumers and Reference Materials of the European Commission's Joint Research Centre (JRC) in Geel (Belgium) and the US National Institute of Standards and Technology (NIST), Gaithersburg (USA). The CRM was produced from a buffer-modified and diluted commercial colloidal silica slurry. Between-ampoule homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017. The minimum sample intake for the ELS method was determined from the results and information provided by the laboratories that participated in the interlaboratory comparison (ILC) exercise. The material was characterized by an interlaboratory comparison between laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated solely on statistical grounds. Uncertainties of the certified values were calculated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, instability and characterization. The material is intended for quality control and assessment of method performance. The CRM is available in 5 mL pre-scored amber glass ampoules each containing approximately 5 mL of suspension.

Key words

electrokinetic potential; electrophoretic mobility; light scattering; silica; surface charge; zeta potential.

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Glossary

a	Sphere radius
ANOVA	Analysis of variance
APD	Avalanche photodiode detector
b	Slope of regression line in stability study
CCL	Consultative Committee for Length
CIPM	Comité International des Poids et Mesures (International Committee of Weights and Measures)
CRM	Certified reference material
DLS	Dynamic light scattering
E	Electric field strength
EDL	Electrical double layer
ELS	Electrophoretic light scattering
EM	Electron microscopy
ERM®	Trademark of the European Reference Materials
EU	European Union
GUM	Guide to the Expression of Uncertainty in Measurement
IEC	International Electrotechnical Commission
ILC	Interlaboratory comparison
ISO	International Organization for Standardization
ISO/TC24/SC4	ISO/technical committee 24/subcommittee 4
JRC	Joint Research Centre of the European Commission
k	Coverage factor
$MS_{between}$	Mean of squares between-ampoule from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Refractive index of the medium
n.a.	Not applicable (or not available)
NIST	National Institute of Standards and Technology
p	Number of technically valid datasets
PALS	Phase analysis light scattering
PMT	Photomultiplier tube
PSD	Particle size distribution

PTA	Particle tracking analysis
QC	Quality control
rel	Relative value
RI	Refractive index
RM	Reference material
RSD	Relative standard deviation
s	Standard deviation
s_{bb}	Between-ampoule standard deviation; an additional index "rel" is added when appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA
SEM	Scanning electron microscopy
SI	International System of Units
SRM®	Trademark of the National Institute of Standards and Technology
s_{within}	Standard deviation within groups as obtained from ANOVA
s_{wb}	Within-unit standard deviation; an additional index "rel" is added when appropriate
$\bar{t}$	Mean of all t_i
t_i	Time elapsed at time point *i*
t_{sl}	Shelf life
t_{tt}	Transport time
TEM	Transmission electron microscopy
TFF	Tangential flow filtration
TSEM	Transmission-mode scanning electron microscopy
U	Expanded uncertainty; an additional index "rel" is added when appropriate
u	Standard uncertainty; an additional index "rel" is added when appropriate
u^{*}_{bb}	Standard uncertainty related to a maximum between-ampoule inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added when appropriate
u_{bb}	Standard uncertainty related to a possible between-ampoule inhomogeneity; an additional index "rel" is added when appropriate
u_c	Combined uncertainty
u_{char}	Standard uncertainty of the material characterization; an additional index "rel" is added when appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added when appropriate

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U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added when appropriate
u_Δ	Combined standard uncertainty of measurement result and certified value
u_{deg}	Standard uncertainty corresponding with a potential degradation observed in the stability study
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added when appropriate
u_{rec}	Uncertainty estimated from a rectangular distribution
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added when appropriate
$\bar{y}$	Arithmetic mean
Z-PTA	Zeta potential measurement by PTA
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\Delta\omega$	Doppler frequency shift
ε	Dielectric permittivity of the medium
ζ	Zeta potential
η	Dynamic viscosity
θ	Angle between the incident light and the scattered light
θ'	Angle between the two beams
κ	Reciprocal of the Debye double layer
λ_0	Laser wavelength in the vacuum
μ	Electrophoretic mobility
ν_{eff}	Effective degrees of freedom
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}
ξ	Angle between the scattered light and the orientation of the electric field

Summary

This report describes the production of SRM 1992/ERM-FD305, silica particles suspended in a borate buffer, certified for electrophoretic mobility and zeta potential by electrophoretic light scattering (ELS). This material was produced following ISO 17034:2016 [1] and is certified in accordance with ISO Guide 35:2017 [2].

The certified reference material (CRM) was jointly produced by the Directorate F - Health, Consumers and Reference Materials of the European Commission's Joint Research Centre (JRC) in Geel (Belgium) and the US National Institute of Standards and Technology (NIST), Gaithersburg (USA). The CRM was produced from a buffer-modified and diluted commercial colloidal silica slurry.

Between-ampoule homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017 [2]. The minimum sample intake for the ELS method was determined from the results and information provided by the laboratories that participated in the interlaboratory comparison (ILC) exercise.

The material was characterized by an interlaboratory comparison between laboratories of demonstrated competence and adhering to ISO/IEC 17025 [3]. Technically invalid results were removed but no outlier was eliminated solely on statistical grounds.

Uncertainties of the certified values were calculated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) [4] and include uncertainties related to possible inhomogeneity, instability and characterization.

The material is intended for quality control and assessment of method performance. The CRM is available in 5 mL pre-scored amber glass ampoules each containing approximately 5 mL of suspension.

The following certified values were assigned:

	Silica particles in aqueous solution	
	Certified value [3]	Uncertainty [4]
Mean electrophoretic mobility [1] $(10^{-8} \, m^2 \, V^{-1} \cdot s^{-1})$	-4.5	0.4
Mean zeta potential (mV) [2]	-58	5

[1] As obtained with electrophoretic light scattering (ELS) at a sample temperature of 25 °C and by applying ISO 13099-2:2012

[2] As calculated from the certified electrophoretic mobility value using the Smoluchowski approximation $f(\kappa a)$ = 1.5

[3] Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data; each set being obtained in a different laboratory or with a different method of determination. The certified value and its uncertainty are traceable to the International System of units (SI).

[4] The uncertainty is the expanded uncertainty of the certified value with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO 17034:2016 and ISO Guide 35:2017.

1. Introduction

1.1. Background

Zeta potential is a parameter that can be used to predict the long-term stability of suspensions and emulsions and to study surface morphology and adsorption onto particles and other surfaces in contact with a liquid. Zeta potential is not a directly measurable parameter. It can be determined using appropriate theoretical models from experimentally determined parameters, such as electrophoretic mobility. Traditionally, zeta potential is defined as the electric potential (relative to the bulk medium) at or near the shear (slipping) plane, an imaginary short distance displaced from the particle surface. Its value is related to, but differs from, the surface potential.

The electrophoretic mobility can be measured by acoustic methods using the electroacoustic phenomena described in the documentary standards ISO 13099, Part 1 [5] and Part 3 [6], and by optical methods, especially electrophoretic light scattering (ELS), as described in ISO 13099 Part 1 [5] and Part 2 [7])

The project to develop a common CRM for both electroacoustic and optical techniques was born out of contacts between members of ISO/TC24/SC4 from NIST and JRC, with the critical support of industry members. The CRM shall serve two purposes:

- It may be used as quality control material for electrophoretic methods. Electrophoretic methods are based on first principles and therefore do not require a calibrant.

- It may serve as calibration material for acoustic methods. Unlike electrophoretic methods, electroacoustic methods require calibration. This is currently accomplished using Ludox TM (commercial colloidal silica) and using a value for its zeta potential published in the 1960s, or using a potassium silicotungstate (KSiW) electrolyte solution with a known dynamic mobility.

However, the typical working concentration ranges for the acoustic and optical based methods are generally at opposite ends of the spectrum. Indeed, optical methods (electrophoretic) require an optically dilute sample, whereas electroacoustic methods are generally applied to concentrated optically opaque suspensions. Despite some overlap (material dependent) of the upper limit for optical methods and the lower limit for acoustic methods, reference materials should be produced at the most relevant particle mass concentrations:

- one dilute material (1.5 g kg^{-1}), SRM 1992/ERM-FD305 suitable for optical methods; the production of which is described in this report.

- one concentrated material (22 g kg^{-1}), ERM-FD306/SRM 1993, suitable for acoustic methods, the production of which is described elsewhere [8],

The same materials are released and co-branded by the JRC and NIST under their own CRM codes (SRM 1992/ERM-FD305 and ERM-FD306/SRM 1993, respectively). This report will therefore refer to both codes for the same CRM. The basis of the project is that it is possible to dilute suspensions without changing the zeta potential, as long as the original suspending medium is used for dilution [5]. This provides the possibility to certify the zeta potential of a concentrated suspension using electrophoretic methods.

For this study, the zeta potential value is assigned indirectly from the measurement of electrophoretic mobility by applying the Henry equation (Equation 1) [5] and using the thin electric double layer (EDL) assumption (i.e., the Smoluchowski approximation)

$$\mu = \frac{2\varepsilon\zeta}{3\eta_0} \cdot f(\kappa a)$$ **Eq. 1**

where

μ ....electrophoretic mobility ($m^2 \cdot V^{-1} \cdot s^{-1}$ or $A \cdot s^2 \cdot kg^{-1}$)
ζ ....potential (V or $kg \cdot m^2 \cdot s^{-3} \cdot A^{-1}$)
η_0 ....dynamic viscosity of the medium ($Pa \cdot s$ or $kg \cdot m^{-1} \cdot s^{-1}$)
ε ....dielectric permittivity of the medium ($F \cdot m^{-1}$ or $s^4 \cdot A^2 \cdot m^{-3} \cdot kg^{-1}$)
a ....sphere equivalent radius (m)
κ ....reciprocal Debye double layer thickness (m^{-1})
$f(\kappa a)$ ....Henry's function (unitless)

The ratio of the particle radius to the EDL thickness is given by the dimensionless parameter κa, which varies from 0 to ∞. The monotonic Henry function $f(\kappa a)$ approaches unity for the Hückel model (thick EDL, $\kappa a << 1$) and 1.5 for the Smoluchowski approximation (thin EDL, $\kappa a >> 1$).

Therefore, in the Smoluchowski limit, the zeta potential is calculated as follows (Equation 2).

$$\zeta = \frac{\eta_0 \mu}{\varepsilon}$$ **Eq. 2**

Electrophoretic mobility (velocity per unit field strength) is commonly expressed in so-called "standard mobility units", ($\mu m \cdot cm \cdot V^{-1} \cdot s^{-1}$) which are equivalent to ($10^{-8} m^2 \cdot V^{-1} \cdot s^{-1}$). Zeta potential is commonly expressed in mV. This report follows these established practises in order to avoid very small numerical values.

The techniques used in the study on the dilute version of the material, SRM 1992/ERM-FD305, are summarised below.

Electrophoretic light scattering (ELS)

In traditional electrophoretic light scattering (ELS), the electrophoretic mobility of particles suspended in a solution is measured via the Doppler shift of the scattered light. Similar to dynamic light scattering (DLS) experiments, a monochromatic coherent incident light beam illuminates suspended particles. Whereas in DLS the suspended particles are moving due to Brownian motion, in ELS the particles are also moving due to an applied electric field, if they have a net charge. Particles will move towards either the anode or the cathode, depending on the sign of their net charge. Because of this motion, the frequency and phase of the scattered light will be different from that of the incident light. This phenomenon is referred to as the Doppler effect.

The relation between the Doppler frequency shift of scattered light and particle electrophoretic mobility, μ, depends on the optical arrangement of the instrumentation [6].

For reference beam optics:

$$\mu = \frac{\Delta\omega\lambda_0}{4\pi E n \sin\left(\theta/2\right)\sin\left(\theta/2 + \xi\right)}$$ **Eq. 3**

For cross-beam optics:

$$\mu = \frac{\Delta\omega\lambda_0}{4\pi E n \sin\left(\theta'/_2\right)}$$

Eq. 4

where

$\Delta\omega$ .... Doppler frequency shift (s^{-1})
λ_0 .... laser wavelength in vacuum (m)
E .... electric field strength ($V \cdot m^{-1}$ or $kg \cdot m\ A^{-1} \cdot s^{-3}$)
n .... refractive index of the medium
θ .... angle between the incident light and the scattered light
ξ .... angle between the scattered light and the orientation of the electric field
θ' .... angle between the two beams.

Many commercial systems now use phase analysis light scattering (PALS), which measures the phase shift between light scattered from the sample and a reference beam. The rate of change of phase shift between the two signals is proportional to the velocity of the particles. PALS is much more sensitive than Doppler-based techniques and allows measurement at higher salt concentrations where the high electric fields required for Doppler analysis can be detrimental to the sample and generate resistive heating.

In closed cells, electroosmotic motion of the ion-containing solution occurs concurrently with the electrophoretic motion of the particles when an electric field is applied. The fluid motion biases the apparent particle motion. The traditional approach to address this issue required measuring the particle velocity at the so-called stationary point, a geometrically defined distance from the side walls of a capillary cell, where electroosmotic motion is zero. Today, most ELS instruments avoid this problem entirely by using "dip cells", in which the electrodes are immersed in a cuvette, or by using a method known as fast field reversal. In both cases, electro-osmosis is eliminated and the unbiased electrophoretic mobility can be measured at any location in the cell between the anode and cathode.

Zeta potential by particle tracking analysis (Z-PTA)

Z-PTA combines laser light scattering with a digital video camera. The camera records the light scattered by the particles that are moving under the electric field. The electric field causes motion of the sample particles (i.e. electrophoresis), and of the aqueous medium (i.e. electro-osmosis). The PTA software records the total apparent drift velocity, which is a superposition of these two motions, for each tracked particle. By observing the total velocity at different depths within the closed sample chamber, and assuming a zero net flow summed over the entire chamber depth, it is possible to separate these two components. The electrophoretic mobility (due to the electric force on the particles) can therefore be measured for every particle tracked by the instrument software.

1.2. Selection of the material

Given its industrial relevance and the ability to remain colloidally stable on a timescale of years, silica particles were selected as a candidate material. SRM 1992/ERM-FD305 was produced from a commercially available suspension that consisted of silica particles suspended in an undefined aqueous solution of electrolyte. The dispersing solution was exchanged by a tangential flow filtration process to a borate buffer at pH 9 with a defined composition. SRM 1992/ERM-FD305 was prepared at a ready-to-use concentration of 1.5 g kg^{-1} for ELS measurements.

1.3. Design of the CRM project

A material was selected for which the Smoluchowski approximation applies. This is not strictly necessary, as the measured value is the electrophoretic mobility, but is useful as most users report zeta potential.

The stability and the homogeneity of the material were evaluated through measurements of electrophoretic mobility using ELS. The certified and additional material information values were established by a comparison between different laboratories with different measurement methods.

The certified zeta potential value and its uncertainty are calculated using the Smoluchowski relation (Eq. 2) and the electrophoretic mobility values reported by the laboratories. The zeta potential values reported directly by the laboratories are provided in Annex E for comparison.

2. Participants

2.1. Project management and evaluation

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE
(accredited to ISO 17034 for production of certified reference materials, BELAC No. 268-RM)

National Institute of Standards and Technology (NIST), Gaithersburg, USA

2.2. Processing

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE
(accredited to ISO 17034 for production of certified reference materials, BELAC No. 268-RM)

2.3. Homogeneity study

National Institute of Standards and Technology (NIST), Gaithersburg, USA

2.4. Stability study

National Institute of Standards and Technology (NIST), Gaithersburg, USA

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE
(measurements under the scope of ISO/IEC 17025 accreditation BELAC No. 268-TEST)

2.5. Characterization

The participants in the interlaboratory comparison study were (in alphabetical order):

Anton Paar GmbH, Graz, AT

European Commission, Joint Research Centre, Directorate F – Health, Consumers and Reference Materials, Geel, BE
(Measurements under the scope of ISO/IEC 17025 accreditation BELAC No. 268-TEST)

Fraunhofer Institut für Keramische Technologie and Systeme (IKTS), Dresden DE
(Measurements under the scope of ISO/IEC 17025 accreditation; The Deutsche Akkreditierungsstelle GmbH N°. D-PL-11140-15-00)

Horiba, Palaiseau, FR

LGC Ltd, Teddington, UK

Malvern Panalytical Ltd, Malvern, UK

Malvern Panalytical Inc., Westborough, USA

Microtrac Inc., Montgomeryville, USA

Moscow Institute of Physics and Technology, Moscow, RU

National Institute of Standards and Technology (NIST), Gaithersburg, USA

National Measurement Institute Australia (NMIA), West Lindfield, AU

National Metrology Institute of Japan (NMIJ), Tsukuba, JPN

National Physical Laboratory (NPL), Teddington, UK

Otsuka Electronics, Osaka, JPN

Postnova Analytics GmbH, Landsberg, DE

Wyatt Technology, Santa Barbara, USA

3. Material processing and process control

3.1. Origin of the starting material and available information

The colloidal silica starting material Acesol WP4 was supplied by Ace Nanochem (Republic of Korea). Material specifications for the starting material as provided by the manufacturer are listed in Table 1.

In preliminary studies prior to certification, a number of material properties were assessed:

- The nominal SiO_2 (dry) mass fraction was determined by in-house measurements at 180 g kg^{-1}.

- The scattered light intensity-weighted arithmetic mean hydrodynamic particle diameter was confirmed to be 140 nm by in-house DLS measurements (cumulants method) on a sample that was diluted 1000-fold with 10 mmol·L^{-1} NaCl (Fig 1).
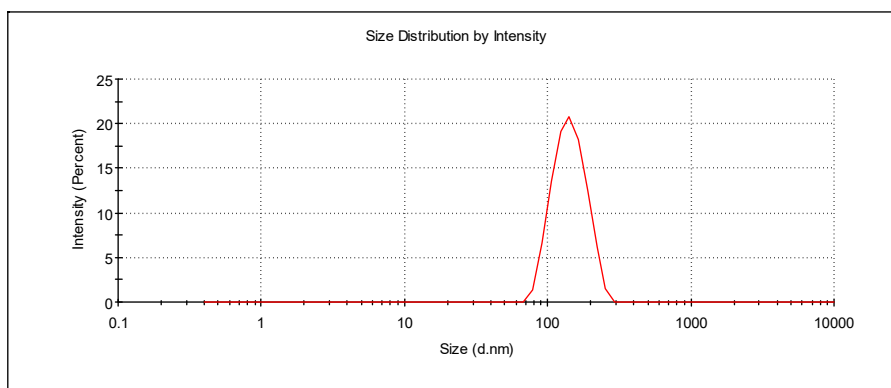
Fig 1: Scattered light intensity-based particle size distribution of Acesol WP4 obtained by DLS

- Particle size measurements on a sample from a previous batch of Acesol WP4 were performed by an independent laboratory (MVA Scientific Consultants, Duluth, USA) using transmission electron microscopy (TEM). TEM grids were dipped in a sample which had been diluted 2-fold with deionised water. The prepared test specimens were imaged in a Philips CM120 transmission electron microscope operated at an acceleration voltage of 100 kV. A typical TEM micrograph is shown in Fig. 2. Two distinct particle populations can be distinguished: One population is centred around 120 nm while the second one is around 30 nm. The particle aspect ratio, defined as the ratio of the major diameter (length) to the minor diameter (width) of a fitted ellipse, is close to one indicating a near-spherical morphology.
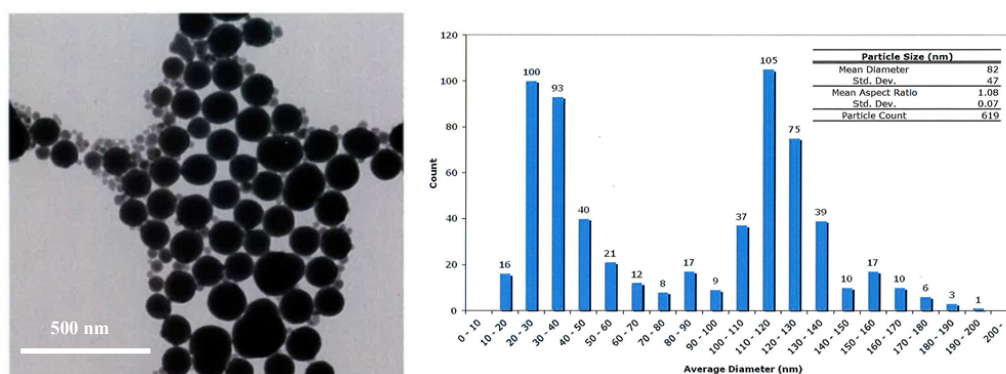
Fig 2: TEM micrograph and number-based area-equivalent particle size distribution of Acesol WP4 silica particles (MVA Scientific Consultants, Duluth, USA)

Despite the presence of a small-size particle population, the material was selected for the production of the CRM material. Indeed, as value assignment is by ELS, the number of small particles is too low to affect the light scattered by the larger size population (intensity based signal).

In-house characterization was performed on the new batch of Acesol WP4 received for the production of the candidate material (1:1000 dilution with purified water) with a field emission scanning electron microscope (SEM) JEOL JSM–7800F, operated in both conventional SEM mode and transmission (TSEM) mode. The SEM and TSEM micrographs (Fig. 3) show, as with the previous sample batch, a population of particles with diameters above 120 nm and a second population around 30 nm.

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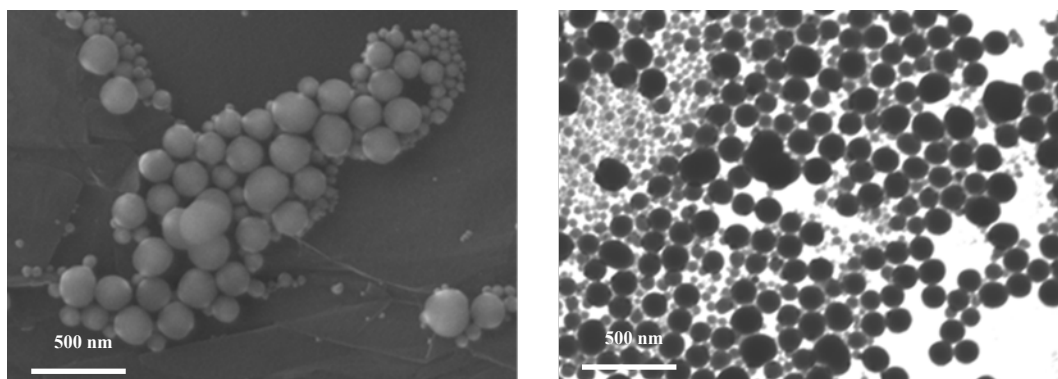
Fig 3: SEM image (left) and TSEM image (right) on Acesol WP4 (JRC, Geel, BE)

Table 1: Information on Acesol WP4 starting material provided by the manufacturer

Property	Specifications/Observations
Batch identification	Sample 2015-12-29
Appearance	Milky white
Nominal particle diameter (hydrodynamic diameter)	140 nm
Nominal SiO_2 concentration	200 g·kg^{-1}
pH (20°C)	10-12
Suspension density (20°C)	1.11-1.13

3.2. Processing

Acesol WP4 is a commercial colloidal silica slurry. As the composition of the dispersant is unknown, the initial dispersant was exchanged with a borate buffer solution having a defined composition (see Annex) at pH 9 using tangential flow filtration (TFF) in the first step. The process is described briefly below:

- Acesol WP4 (180 g kg^{-1}) was diluted with 10 mmol·L^{-1} NaCl to a concentration of 10 g·kg^{-1}. The obtained solution was filtered through a mixed cellulose ester hollow fibre module (ME, cut-off 0.1 μm) with a KrosFlo Research IIi TFF system (Spectrumlabs, USA) operated in the concentration mode to the target concentration of 25 g·kg^{-1}. The retentate was then re-diluted to the initial concentration with 10 mmol·L^{-1} NaCl and the permeate discarded. The filtration/re-dilution process was repeated a second time.
- The buffer exchange was then performed by repeating four cycles of the same filtration/re-dilution procedure using the borate buffer in place of NaCl.
- At the end of the TFF process, a colloidal silica suspension at the target concentration of 25 g·kg^{-1} in the borate buffer solution was obtained.

Due to the capacity of the TFF system set-up, the material was produced in batches of approximately 5 kg of colloidal silica. 15 batches (total mass of 74.2 kg), which had been tested for zeta potential, particle size (by DLS) and conductivity, were selected for the next step of the production.

In the second step, the 25 g·kg^{-1} colloidal silica batches were again filtered by TFF, now on a polyethersulfone hollow fibre module (PES, cut-off 0.2 μm) still in concentration mode in order to remove potential bacteria or spores. This time, the permeate was kept and the retentate was discarded. This process yielded 66 kg of a 0.2 μm filtered colloidal solution at a concentration of 28 g·kg^{-1}. The different batches were stored in sterile cans for the production of the final CRM.

SRM 1992/ERM-FD305 was then prepared by gravimetrically diluting the previously described 28 g·kg^{-1} colloidal solution with the sterile borate buffer solution to produce a final concentration of 1.5 g·kg^{-1}. To do this, 4 kg of the colloidal silica suspension were diluted with

59 kg of sterile borate buffer solution under semi-sterile conditions under a movable clean cell (Terranova, Fullerton, USA).

Pre-scored 5 mL amber glass ampoules (Nederlandse Ampullenfabriek B.V., Nijmegen, NL) were chosen to provide a rugged and gas tight containment for the colloidal silica samples. The ampoules were loaded to a Rota ampouling machine R 910 PA (Rota, Wehr, DE). Every ampoule was flushed with Argon gas immediately before filling with approximately 5 mL of suspension. The suspension in the supply bottle was continuously mixed by circulation of the suspension in sterile tubing with a peristaltic pump during the process of filling the ampoules. Immediately after filling, the ampoules were again flushed with Ar and closed. In total 11040 ampoules were produced. The Batch was split into two lots with half of the ampoules being retained for distribution by the JRC and half of them for distribution by NIST.

3.3. Process control

Before ampouling, an aliquot was sampled and analysed by DLS for particle size, and by ELS for zeta potential and electrophoretic mobility. The conductivity was measured by the ELS instrument. In addition, the pH was measured potentiometrically. Results are summarized in Table 2.

Table 2 Process control parameters on SRM 1992/ERM-FD305

Measurement method	Information value
Electrophoretic mobility (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$) [a]	-4.4
Zeta potential (mV) [a]	-56
Conductivity ($mS \cdot cm^{-1}$) [b]	0.38
pH [c]	8.9
Hydrodynamic diameter (nm) [d]	140
Concentration ($g \cdot kg^{-1}$) [e]	1.49

[a] As obtained by ELS at (25 ± 1) °C

[b] As measured with the ELS instrument

[c] As determined by potentiometric method at (21 ± 1) °C

[d] As obtained by DLS using the cumulants method (scattered light intensity-weighted harmonic mean diameter) at (25 ± 1) °C

[e] Concentration of SRM 1992/ERM-FD305 calculated based on the gravimetric preparation

4. Homogeneity

A key requirement for any reference material aliquoted into units is equivalence between those units. In this respect, it is relevant whether the variation (heterogeneity) between units is significant compared to the uncertainty of the certified value, but it is not relevant if this variation between units is significant compared to the analytical variation. Consequently, ISO 17034 [1] requires RM producers to quantify the between unit variation. This aspect is covered in between-ampoule homogeneity studies.

The within-unit heterogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit heterogeneity is therefore necessary to determine the minimum sample intake.

4.1. Between-ampoule homogeneity

The between-ampoule homogeneity was evaluated to ensure that the certified values of the CRM are valid for all units of the material, within the stated uncertainties.

The number of selected units corresponds to approximately the cube root of the total number of produced units. Thus, 22 units, selected using a random stratified sampling scheme covering the whole batch, were analysed to test the between-ampoule homogeneity. From each of the 22 units, two independent subsamples (aliquots) were taken, and analysed in-house for electrophoretic mobility by ELS. Due to the number of samples, the separate aliquots of each sample were measured on two consecutive days and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. For 7 units, a problem due to the mixing procedure (generating small bubbles at the electrode surface) was spotted. On at least one of the aliquots, this led to a low conductivity and a higher electrophoretic mobility value. It was decided to eliminate all data coming from the affected ampoules based on technical reasons and to perform the homogeneity data evaluation on the 15 remaining ampoules (still covering the entire batch).

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trend in the filling sequence was observed at a 95 % confidence level. The first four measurements presented a value relatively high compared to the 26 following measurements, leading to a trend in the analytical sequence (significant at a 95 % confidence level). As the origin of the trend is unknown and there is no technical reason to remove the data, the dataset was not corrected.

The dataset was assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the unit means. No outlying unit mean was detected. Two outlying individual results were detected. Since no technical reason for the outliers could be identified, all the data were retained for statistical analysis.

Quantification of between-ampoule heterogeneity was undertaken by analysis of variance (ANOVA), which separates the between-ampoule variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples are representative of the whole unit.

Evaluation by ANOVA requires mean values per unit, which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per unit was visually tested using histograms and normal probability plots. Too few data are available for the unit means to make a clear statement of the distribution. Therefore, it was checked visually whether all individual data follow a unimodal distribution using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-ampoule standard deviations. The results of all statistical evaluations are given in Table 3.

Table 3: Results of the statistical evaluation of the homogeneity studies

Measurand	Trends (before correction)*		Outliers**		Distribution	
	Analytical sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
Electrophoretic mobility (10^{-8} m$^2\cdot$V^{-1}s^{-1})	yes	No	2	none	Bimodal (-4.6;-4.2)	Bimodal (-4.4;-4.2)

* 95 % confidence level

** 99 % confidence level

The data does not follow a unimodal distribution for the electrophoretic mobility. Therefore, u_{rec} was estimated using a rectangular distribution between the highest and lowest unit mean [9]. The uncertainty in those cases is given in:

$$u_{rec} = \frac{|highest\ result - lowest\ result|}{2\cdot\sqrt{3}\cdot\overline{y}}$$ **Eq. 5**

where

$\overline{y}$ ....mean of all results of the homogeneity study

The result of the evaluation of the between-ampoule variation is summarised in Table 4. The resulting value from the above equation is converted into relative uncertainty.

Table 4: Results of the homogeneity study

Measurand	$u_{bb,rel}$ (%)
Electrophoretic mobility	3.4

The homogeneity study showed no outlying unit means or trends in the filling sequence. However, the first four measurements present a value relatively high comparing to the other measurements resulting in a higher mean for the affected unit and a bimodal distribution of the data. However, taking these extreme values into account the heterogeneity, quantified as u_{rec}, is still sufficiently small as to allow the material to be considered useful. Therefore, u_{rec} was used as an estimate of u_{bb}.

4.2. Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus should be used in an analysis. Using sample sizes equal to or above the minimum sample intake guarantees the certified value within its stated uncertainty.

The minimum sample intake, in terms of volume taken from the as-received sample unit, was determined from the results of the characterization study, using the method information supplied by the participants. The smallest sample intake that yielded results with acceptable

accuracy to be included in the respective studies was taken as minimum sample intake. Using the data from Annex D, the minimum sample intake has been determined to be 0.4 mL.

5. Stability

Time and temperature were regarded as the most relevant factors having an influence on the stability of the material. The influence of ultraviolet or visible light was minimised by storing the material in amber glass containers which reduce light exposure. In addition, materials are stored in the dark and dispatched in boxes, thus removing any possibility of degradation by light.

Stability testing is necessary to establish the conditions for storage (long-term stability) as well as the conditions for dispatch of the materials to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C can be reached and stability under these conditions must be demonstrated if the samples are to be transported without any additional cooling. Additionally, exposure to temperatures typical for a refrigerator or that might be expected during cold weather transport, must also be demonstrated.

The stability studies were carried out using an isochronous design [10]. In this approach, samples are stored for a particular length of time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples were analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

5.1.   Short-term stability study

For the short-term stability study, samples were stored at 4 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to 18 °C. Four units per storage time were selected using a random stratified sampling scheme. From each unit, three aliquots were measured for electrophoretic mobility by ELS. Each aliquot was measured three times in a consecutive manner. The measurements were performed under repeatability conditions and a randomised sequence was used to differentiate any potential analytical drift from a trend over storage time.

The data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. No outliers were detected.

In addition, the data were evaluated against storage time, and regression lines of electrophoretic mobility versus time were calculated. The slope of the regression was then tested for statistical significance (indicative of a decrease/increase due to the shipping). The results of the measurements are shown in Annex B. The results of the statistical evaluation of the short-term stability are summarised in Table 5.

Table 5: Results of the short-term stability tests

Measurand	Number of individual outlying results*		Significance of the trend **	
	4 ºC	60 ºC	4 ºC	60 ºC
Electrophoretic mobility	none	none	no	yes

* 99 % confidence level

** 95 % confidence level

No statistical outliers were detected for the electrophoretic mobility. All data were retained for the estimation of u_{sts}. For the electrophoretic mobility set of results at 60 °C storage temperature, although the slope of the regression line was found to be statistically significantly different from zero at a confidence level of 95 %, it is technically negligible due to the low value of the slope (0.02 10^{-8} m^2 V^{-1}·s^{-1} per week) and the value obtained for U_{CRM}.

During the production of a similar colloidal silica CRM, ERM-FD304 [11], it was observed that freezing of the suspension led to irreversible agglomeration of the particles. Therefore, SRM 1992/ERM-FD305 must be protected against freezing as also recommended by the manufacturer of the source material.

Supported by the experimental data and taking into account a maximum dispatch period of one week, it is concluded that the material can be safely shipped under ambient conditions, so long as ambient conditions do not subject the material to freezing temperatures. The uncertainty due to potential degradation is included in the uncertainty of the certified values.

5.2. Long-term stability study

For the long-term stability study, samples were stored at 18 °C for 0, 8, 16 and 24 months. The reference temperature was set to 4 °C. Four samples per storage time were selected using a random stratified sampling scheme. From each unit, three aliquots were measured for electrophoretic mobility by ELS. The measurements were performed under repeatability conditions, in a randomised sequence in order to be able to separate any potential analytical drift from a trend over storage time.

The long-term stability data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. No outlier was detected.

In addition, the data were plotted against storage time and linear regression line of electrophoretic mobility versus time was calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to storage). No significant trend was detected at a 95 % confidence level.

The results of the long-term stability measurements are shown in Annex C. The result of the statistical evaluation of the long-term stability study is summarised in Table 6.

Table 6: Results of the long-term stability tests

Measurand	Number of individual outlying results*	Significance of the trend**
Electrophoretic mobility	none	no

* 99 % confidence level

** 95 % confidence level

No technically unexplained outliers were observed and the trend was not statistically significant at a 95 % confidence level for any temperature tested. The material can therefore be stored at 18 °C.

5.3. Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can entirely rule out degradation of materials, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be that there is no detectable degradation within an uncertainty to be estimated.

The uncertainties of stability during dispatch and storage were estimated, as described in [12] for each analyte. In this approach, the uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{s_{rel}}{\sqrt{\sum\left(t_i - \bar{t}\right)^2}} \cdot t_{tt} \qquad \textbf{Eq. 6}$$

$$u_{lts,rel} = \frac{s_{rel}}{\sqrt{\sum\left(t_i - \bar{t}\right)^2}} \cdot t_{sl} \qquad \textbf{Eq. 7}$$

where

s_{rel} ....relative standard deviation of all results of the stability study
t_i..............time elapsed at time point i
$\bar{t}$ ....mean of all t_i
t_{tt}.............chosen transport time (1 week at 60 ºC)
t_{sl}.............chosen shelf life (24 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 60 ºC studies. The uncertainty describes the possible change during a dispatch at 60 ºC lasting for one week.

- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the 18 °C study. The uncertainty contribution describes the possible degradation during 24 months storage at 18 °C. The results of these evaluations are summarised in Table 7.

Table 7: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of 60 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of 18 °C and 24 months

	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)
Electrophoretic mobility	0.42	1.80

After the certification study, the released CRM will be included in the JRC's regular stability monitoring programme, to control its further stability.

6. Characterization

The material characterization is the process of determining the property values of a reference material. This process was based on an interlaboratory comparison (ILC) of expert laboratories, i.e. the zeta potential and the electrophoretic mobility of the material were determined in different laboratories that applied different measurement procedures to demonstrate the absence of a measurement bias. Crucial in this characterization approach is that the measurements must be performed under intermediate precision (i.e. on different days) and reproducibility conditions and that the results of the different laboratories are independent. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

The material characterization was based on a primary method of measurement, confirmed by an independent method. A primary method of measurement (also called "primary reference method" in the International Vocabulary of Metrology (VIM) [13]) is a method that does not require calibration with a standard of the same measurand and does not depend on a chemical reaction. Such methods are of highest metrological order and often yield results with low uncertainties. However, it is nevertheless prudent to demonstrate absence of bias or gross errors by use of an independent method of lower metrological order.

Material characterization was based on ELS and the result was confirmed by an independent method (Z-PTA).

6.1. Selection of participants

Sixteen laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the field of zeta potential/ electrophoretic mobility measurements. Having a formal accreditation was not mandatory but meeting the requirements of ISO/IEC 17025 was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.2. Study setup

Each laboratory received three units of the candidate CRM together with a detailed measurement protocol and was requested to provide 6 independent results (2 aliquots per unit) for ELS and 9 independent results (3 aliquots per unit) for Z-PTA. The units for material characterization were selected using a random stratified sampling scheme and covered the whole batch. The measurements were spread over three days to ensure intermediate precision conditions.

Each ILC participant received a sample of reference material Malvern DTS 1235 (Malvern Panalytical Ltd, UK), whose assigned values are metrologically traceable to NIST SRM 1980, as a blinded quality control (QC) sample for the ELS and Z-PTA measurements. The results for the QC sample were used to support the evaluation of the characterization results.

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the replicate measurement results. No approach for the estimation was prescribed, i.e. top-down and bottom-up were regarded as equally valid procedures.

6.3. Methods used

6.3.1. Electrophoretic light scattering (ELS)

The characterization of SRM 1992/ERM-FD305 was performed by ELS in terms of zeta potential and electrophoretic mobility.

Laboratories were asked to perform sample handling, preparation and measurements according to the documentary standard ISO 13099. Laboratories were free to choose the type of the measurement cell (e.g., dip cell, folded capillary cell, quartz capillary, etc.) appropriate for their instrument. The type of the cell used along with its optical path length had to be reported. All ELS measurements were performed on samples in the as-received state (i.e. without dilution). Furthermore, the protocol required measurements to be performed at 25 °C, an equilibration time of 120 s, and a dynamic viscosity and refractive index of the dispersing medium (both at 25 °C) of 0.8872 mPa·s and 1.330, respectively. From each ampoule, two aliquots had to be taken and each aliquot had to be measured five times under repeatability conditions.

The laboratories were instructed to report results for the zeta potential, the electrophoretic mobility, the applied electric field and the conductivity of the sample.

6.3.2. Zeta potential by particle tracking analysis (Z-PTA)

For the as-received SRM 1992/ERM-FD305, the intensity of the light scattered by the particles is too high for Z-PTA instruments, resulting in the impossibility of tracking individual particles to determine their zeta potentials. Depending on the optical system of the specific instrument, laboratories had to dilute the as-received silica suspension to the optimal concentration for the measurement using the provided buffer. In addition, also the QC sample needed to be diluted with buffer obtained by passing 5 mL of the suspension through a filter (provided with the sample) with nominal pore size of 0.1 µm. Neither the as-received, nor the diluted silica suspension were treated by filtration, centrifugation or sonication.

6.4. Evaluation of results

The characterization campaign resulted in a total of 19 independent datasets. Sixteen datasets were received for the ELS method and three for the Z-PTA method. All individual results of the participants, grouped per technique/method are displayed in tabular and graphical form in Annex E.

6.4.1. Technical evaluation

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- appropriate validation of the measurement procedure

- compliance with the provided measurement protocol: sample preparations and measurements performed over three days,

- method performance, i.e. agreement of the measurement results with the assigned value of the QC sample following the procedure described in ERM Application Note 1 [14]

Based on the above criteria, the following datasets were rejected as not technically valid (Table 8).

Table 8: Datasets that showed non-compliances with the analysis protocol and technical specifications, and actions taken

Method	Lab code	Description of problem	Action taken
ELS	L0	Result for QC sample did not agree with the assigned value within the reported uncertainty	Data retained based on low within-lab *RSD* value on QC sample, and the data obtained on SRM 1992/ERM-FD305 agreed with other laboratories.
ELS	L6	Laboratory indicated that the concentration of the QC sample is too low for the optics set up of their instruments. No results were provided for the QC sample for technical reason.	Data retained based on good agreement with other laboratories.
ELS	L7	The laboratory did not provide measurement uncertainty. *RSD* of 23% for results of QC sample	Data not used for evaluation
Z-PTA	L15	Result for QC sample did not agree with the assigned value within the reported uncertainty	Data not used for evaluation

Electrophoretic light scattering: Thirteen laboratories participated in the ILC study. One laboratory submitted four datasets (two instruments and four different cells). One laboratory (L0) failed on the measurement of the QC sample (with low *RSD*), but taking into account that the QC sample is not a certified reference material, that there were no indications of technical problems, and because the results obtained on SRM 1992/ERM-FD305 agreed with the data from other laboratories, the dataset was retained. One laboratory (L6) could not measure the QC sample as its particle mass concentration was too low for the optical set up of the instrument. As the concentration of SRM 1992/ERM-FD305 did not cause any compatibility issues and as there were no indications of technical problems during measurement, and also as the results obtained on SRM 1992/ERM-FD305 agreed with the other laboratories, the dataset was retained. One laboratory (L7) did not provide a measurement uncertainty and as the *RSD* calculated from the results obtained on the QC sample was about 23 %, the data was not used for evaluation. Ultimately, 15 datasets were accepted for statistical evaluation.

The results of the ELS technical evaluation are summarised in Annex E.

Zeta potential by particle tracking analysis: Three laboratories participated in the ILC study. One laboratory (L15) failed on the measurement of the QC sample. Two datasets were accepted. As insufficient datasets are available for the statistical evaluation, Z-PTA measurements are provided as additional material information.

The results of the Z-PTA technical evaluation are summarised in Annex E.

6.4.2. Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations, (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 9 .

Table 9: Statistical evaluation of the technically accepted datasets for SRM 1992/ERM-FD305, where p is the number of technically valid datasets

	p	Outliers		Normally distributed	Statistical parameters (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)			
		Means	Variances		Mean	s	$s_{between}$	s_{within}
Electrophoretic mobility	15	none	L9	yes	-4.54	0.25	0.24	0.10

Electrophoretic mobility: The statistical evaluation of the SRM 1992/ERM-FD305 datasets flagged the variance of laboratory L9 as outlier for the electrophoretic mobility. In essence, outlier of variance shows that the repeatability varies between laboratories. The heterogeneity of variances prevents pooling of all individual results, so the evaluation is based on the mean of laboratory means instead. In conclusion, an outlying variance is not a reason for exclusion of data.

The uncertainty related to the characterization (u_{char}) is estimated as the standard error of the mean of laboratory means (Table 10), i.e. $s/\sqrt{p}$ with s and p taken from Table 9.

Table 10: Uncertainty of characterization results for SRM 1992/ERM-FD305, where *p* is the number of technically valid datasets

	p	Mean (10^{-8} m²·V⁻¹·s⁻¹)	*s* (10^{-8} m²·V⁻¹·s⁻¹)	u_{char} (10^{-8} m²·V⁻¹·s⁻¹)	u_{char} (%)
Electrophoretic mobility	15	-4.54	0.24	0.06	1.39

7. Value Assignment

Certified and informative values were assigned.

<u>Certified values</u> are values that fulfil the highest standards of accuracy. Procedures at the JRC, Directorate F require generally pooling of not less than six datasets to assign certified values. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [4] were established.

<u>Additional material information</u> refers to values that were obtained in the course of the study. For example, results reported from only one or two laboratories or in cases where individual measurement uncertainty is high, would fall under this category.

7.1.   Certified values and their uncertainties

The unweighted mean of the means of the accepted electrophoretic mobility datasets as shown in Table 11 was assigned as certified value for each parameter.

The assigned uncertainty consists of components relating to characterization, u_{char} (Section 6), potential between-ampoule heterogeneity, u_{bb} (Section 4.1), and potential degradation during transport, u_{sts}, and long-term storage, u_{lts} (Section 5.3).

These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM,rel}$) with a coverage factor *k* given as follow:

$$U_{CRM,rel} = k \cdot \sqrt{u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2 + u_{char,rel}^2} \qquad \textbf{Eq. 8}$$

- u_{char} was estimated as described in Section 6

- u_{bb} was estimated as described in Section 4.1.

- u_{sts} and u_{lts} were estimated as described in section 5.3

Because of the sufficient numbers for the degrees of freedom of the different uncertainty contributions, a coverage factor *k* = 2 was applied to obtain the expanded uncertainties representing an approximately 95 % uncertainty interval.

The certified values and their uncertainties are summarised in Table 11.

An alternative analysis of the certified value and uncertainty was performed as follows to confirm the statistical approach. The individual replicates in Table E1.1 were modelled as observations of Gaussian random variables y_{ij} with expected values $\mu + \delta_i$ and standard deviations σ_i. The δ_i are the laboratory effects which account for any between-laboratory variability (dark uncertainty). These were modelled as Gaussian random variables with mean 0 and standard deviation τ. Using a Bayesian hierarchical model gave an estimate of -4.5 (10⁻

^{8} m^2·V^{-1}·s^{-1}) for the measurand μ and 0.067 (10^{-8} m^2·V^{-1}·s^{-1}) for the standard uncertainty. Table 11 describes four different sources of uncertainty. These are uncertainties due to characterization u_{char} (the τ in this alternative analysis), potential between-ampoule heterogeneity u_{bb} (possibly in σ_i), potential degradation during transport u_{sts}, and long-term storage u_{lts}. This analysis quantifies uncertainty due to characterization, but it is possible that the size of the between-ampoule heterogeneity is not fully captured by the characterization dataset as a result of its smaller data set size. Thus, to be conservative, the u_{bb}, the u_{sts} and the u_{lts} of Table 11 were added in quadrature to the 0.067 to obtain a standard uncertainty of 0.19 (10^{-8} m^2·V^{-1}·s^{-1}), which matches the result in Table 11, thus confirming the analysis described above.

Table 11: Electrophoretic mobility certified value and their uncertainties for SRM 1992/ERM-FD305

	Certified value (10^{-8} m^2·V^{-1}·s^{-1})	$u_{char, rel}$ (%)	$u_{bb,rel}$ (%)	$u_{sts,rel}$ (%)	$u_{lts,rel}$ (%)	$U_{CRM, rel}$ (%)	U_{CRM} [a] (10^{-8} m^2·V^{-1}·s^{-1})
Electrophoretic mobility	-4.5	1.39	3.40	0.42	1.80	8.3	0.4

[a] Expanded (k = 2) and rounded uncertainty

The certified zeta potential value (Table 12) is calculated from the electrophoretic mobility certified value using Equation 2 for a temperature of 25 °C with a rounded value of 0.89 mPa·s for the dynamic viscosity [15], [16] and a value of 78.4 for the dielectric constant of water [17], [18].

Considering a temperature of (25 ± 0.2) °C, the uncertainty component due to the viscosity becomes negligible, therefore the relative expanded uncertainty U_{CRM} (k = 2) of the electrophoretic mobility is used as relative expanded uncertainty U_{CRM} (k = 2) of the zeta potential.

Table 12: Zeta potential certified value and its uncertainty for SRM 1992/ERM-FD305

	Certified value (mV)	U_{CRM} [a] (mV)
Zeta potential	-58	5

[a] Expanded (k = 2) and rounded uncertainty

7.2. Additional material information

The data provided in this section regarding the general composition of the material should be regarded as informative only and cannot, under any circumstances, be used as certified or indicative values

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7.2.1. Zeta potential by particle tracking analysis

As reported before, only 2 datasets were accepted for the Z-PTA. The zeta potential average value of both laboratories is provided as an information value in Table 13. No electrophoretic mobility values were reported (not provided by the instrument software).

Table 13: Additional material information value obtained by Z-PTA

	Mean (mV)
Zeta potential (at 25 °C)	-55

7.2.2. Conductivity

Most ELS instruments also measure the electrolyte conductivity of samples. The average and the standard deviation of the mean laboratory results are reported as additional material information in Table 14.

Table 14: Additional material information value obtained by conductivity measurement

	Mean (mS·cm^{-1})	s (mS·cm^{-1})
Conductivity (at 25 °C)	0.39	0.04

7.2.3. Electrical asymmetrical-flow field-flow fractionation

One laboratory analysed SRM 1992/ERM-FD305 using an electrical asymmetrical-flow field-flow fractionation (EAF4) instrument hyphenated to a multiple-angle light scattering detector. Instrument details are listed in Annex Table D3.

The drift velocity induced by different electric field strengths is calculated. By applying a linear regression to the different obtained data points, the electrophoretic mobility is determined as the slope of the regression analysis. Zeta potential was calculated from the determined electrophoretic mobility using the Smoluchowski approximation.

The laboratory analysed two units of SRM 1992/ERM-FD305 in triplicate. The average value is reported in Table 15 for zeta potential, electrophoretic mobility and conductivity.

Table 15: Additional material value obtained by EAF4

Measurand	Information value (at 25 °C)
Zeta potential	-65 mV
Electrophoretic mobility	-5.1 x 10^{-8} m^2·V^{-1}·s^{-1}
Conductivity	0.391 mS·cm^{-1}

8. Metrological traceability and commutability

8.1. Metrological traceability

Identity

Electrophoretic mobility is the electrophoretic velocity per unit electric field strength.

Zeta potential is the difference in electric potential between that at the slipping plane and that of the bulk liquid. It is calculated from the electrophoretic mobility measurement results according to the Smoluchowski limit of the Henry equation, using a viscosity of 0.89 mPa·s (25 °C) and a dielectric constant for water of 78.4 (25 °C).

SRM 1992/ERM-FD305 has been characterized using the ELS method and the assigned properties values are intrinsically linked to their corresponding operationally-defined measurand. The certified values can be regarded as reliable estimates of the 'true' electrophoretic mobility and zeta potential values, as underpinned by the agreement of the laboratory results with the assigned value on Malvern DTS 1235 that was used as a QC sample.

Quantity value

Since the ELS method is intrinsically first principle in nature, there is no need for instrument response calibration or for the introduction of corrective terms. Traceability of the measured electrophoretic mobility values depends on the traceability of the values corresponding with the parameters occurring in Equation 3 or 4.

Measured decay rate: the traceability of the measured decay rates depends on the accurately known constant resonant frequency of quartz crystal oscillators that are integrated in programmable logic devices such as a field-programmable gate array (FPGA).

Detector angle: the angles at which the detectors were fixed had been geometrically determined as they depend on the mechanical design of the ELS systems. The accuracy of the angle is assured by respecting the applied mechanical tolerances.

Refractive index and viscosity of the dispersant: refractive index and viscosity values were obtained from tables in the literature reporting traceably measured values [12] [13] [15].

Laser wavelength: traceability of the wavelength value to the SI was assured by using helium-neon lasers with a nominal wavelength of 633 nm.

In the ILC study, the majority of the instruments used a He-Ne laser as laser source. Unstabilised He-Ne lasers of 633 nm are used in most laser interferometers and many instruments used for length measurements. These instruments, including ELS instruments, are very often used at uncertainty levels that are large compared to the possible variation of the He-Ne laser vacuum wavelength. Based on these considerations, the International Committee of Weights and Measures (CIPM) recognised the need for providing documentary evidence regarding the value of the vacuum wavelength and its uncertainty that can be expected in the absence of calibration. During its 96th meeting, the CIPM adopted a wavelength of 632.9908 nm with a relative standard uncertainty of 1.5×10^{-6} [19]. Following thorough evaluation of the Consultative Committee for Length (CCL) of the CIPM, the CCL recommended including unstabilised red He-Ne lasers, operating on the 633 ($3s2 \rightarrow 2p4$) neon transitions, in the new list of standard frequencies, "Recommended values of standard frequencies for applications including the practical realization of the metre and secondary

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representations of the second". This list replaces the Mise en Pratique for the definition of the metre.

For the electric field strength:

- The distance to the electrodes is set by the cell used for the measurements.
- The voltage is factory and SI-traceably calibrated by the manufacturer

Temperature: the sample temperatures have been measured by sensors which had either been accurately calibrated by their manufacturer or which had been verified following alpha testing using Pt100 sensors.

Because of the calibration or traceable values of these input parameters, the certified value and uncertainty of the electrophoretic mobility and the zeta potential obtained with ELS are traceable to the SI.

8.2. Commutability

Many measurement procedures include one or more steps which select specific (or specific groups of) analytes from the sample for the subsequent whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions that define this concept. For instance, the CLSI Guideline C53-A [20] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and is therefore a crucial characteristic when applying different measurement methods. When the commutability of a CRM is not established, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

SRM 1992/ERM-FD305 has been characterized by ELS and PTA methods. Although two PTA datasets correlated with the result obtained by ELS, due to the limited data and the fact that PTA measurement requires a substantial dilution of the native sample (below the LOD for ELS), the commutability for PTA could not be assessed.

9. Instructions for use

9.1. Safety information

The material should be handled with care. The material contains a fraction of nanoparticles (size range of 1 nm to 100 nm) that could have an impact on environment and human health. Any spilling of the suspension should be handled according to the standard laboratory safety precautions.

For further details refer to the safety data sheet.

9.2. Storage conditions

The materials should be stored at ambient temperature (20 ± 5) °C. Ampoules must not be allowed to freeze, as this will irreversibly compromise the integrity of the material.

Please note that neither the European Commission nor the National Institute of Standards and Technology can be held responsible for changes that happen during storage of the material at the customer's premises, especially for opened ampoules.

9.3. Preparation and use of the material

Before opening, the ampoule should be gently inverted several times to ensure the homogeneity of the suspension and to re-suspend any settled particles. Remove any suspension that remains in the upper part (conical top) of the ampoule by gently flicking the conical part with the forefinger while tilting the ampoule. The ampoule is pre-scored and can be opened by applying gentle pressure with one's thumb to snap off the conical part. The content of the ampoule should be used the same day as opened and should be gently homogenised (shaking or vortexing) before every measurement without introducing air bubbles.

ELS method: Aliquot of SRM 1992/ERM-FD305 shall be measured as received, i.e. without dilution. The measurement temperature shall be 25 °C ± 0.2 °C. Values to be used for the viscosity and refractive index of the dispersing medium (water) at 25 °C are 0.89 mPa·s and 1.332, respectively. The value of the viscosity must be adjusted when tests are not performed at 25 °C.

9.4. Minimum sample intake

The minimum sample intake representative for ELS measurement is 400 µL.

9.5. Use of the certified value

The main purpose of this material is to assess performance of instruments and/or methods that are used for measuring zeta potential and electrophoretic mobility. As with any reference material, it can be used for establishing control charts or validation studies.

Use as a calibrant

It is not recommended to use this material as calibrant. If used nevertheless, the uncertainty of the certified value must be taken into account in the estimation of the measurement uncertainty.

Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, https://crm.jrc.ec.europa.eu/e/132/User-support-Application-Notes [21].

When assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is summarised here:

-   Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).

-   Combine the measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_\Delta = \sqrt{u_{meas}^2 + u_{CRM}^2}$

- Calculate the expanded uncertainty (U_Δ) from the combined uncertainty (u_Δ,) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %

- If $\Delta_{meas} \leq U_\Delta$ then no significant difference exists between the measurement result and the certified value, at a confidence level of approximately 95 %.

<u>Use in quality control charts</u>

The materials can be used for quality control charts. Using CRMs for quality control charts has the added value that a trueness assessment is built into the chart.

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Annexes

Annex A: Results of homogeneity measurements

Fig. A shows the averages of the mean electrophoretic mobility results obtained by ELS for different replicates per ampoule and their 95 % confidence intervals (error bars). These confidence intervals are based on the relative expanded measurement uncertainties ($k = 2$) that are relevant for zeta potential measurement results.
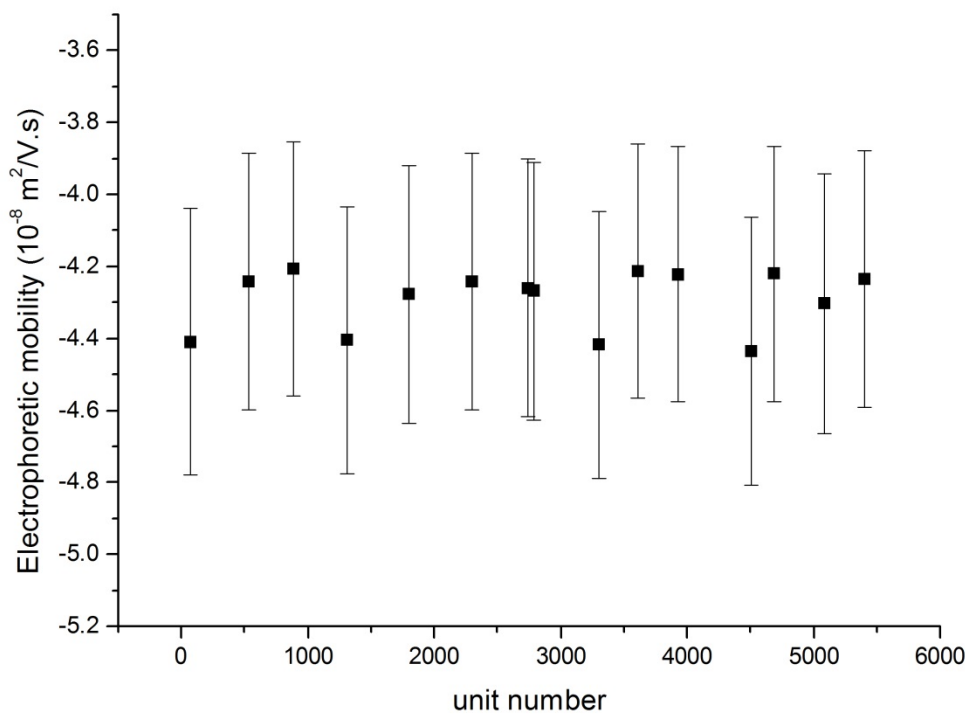
Fig. A Homogeneity data (averages of duplicate results) of SRM 1992/ERM-FD305; electrophoretic mobility by ELS; error bars correspond to the expanded measurement uncertainties ($k = 2$) for use of the method in repeatability conditions.

Annex B: Results of short-term stability measurements

Graphs depicted in Fig. B show the short-term stability data (average electrophoretic mobility) as obtained by ELS. Error bars are omitted in the graphs for clarity.

Fig. B Short-term stability data (averages of triplicate results) of SRM 1992/ERM-FD305; electrophoretic mobility by ELS, when stored for several weeks at 4 °C (a) and 60 °C (b). Results at time point 0 weeks correspond to units that were stored at the reference temperature of 18 °C.

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Annex C: Results of long-term stability measurements

Fig. C shows the long-term stability data (average electrophoretic mobility) obtained by ELS. Error bars are omitted in the graphs for clarity.

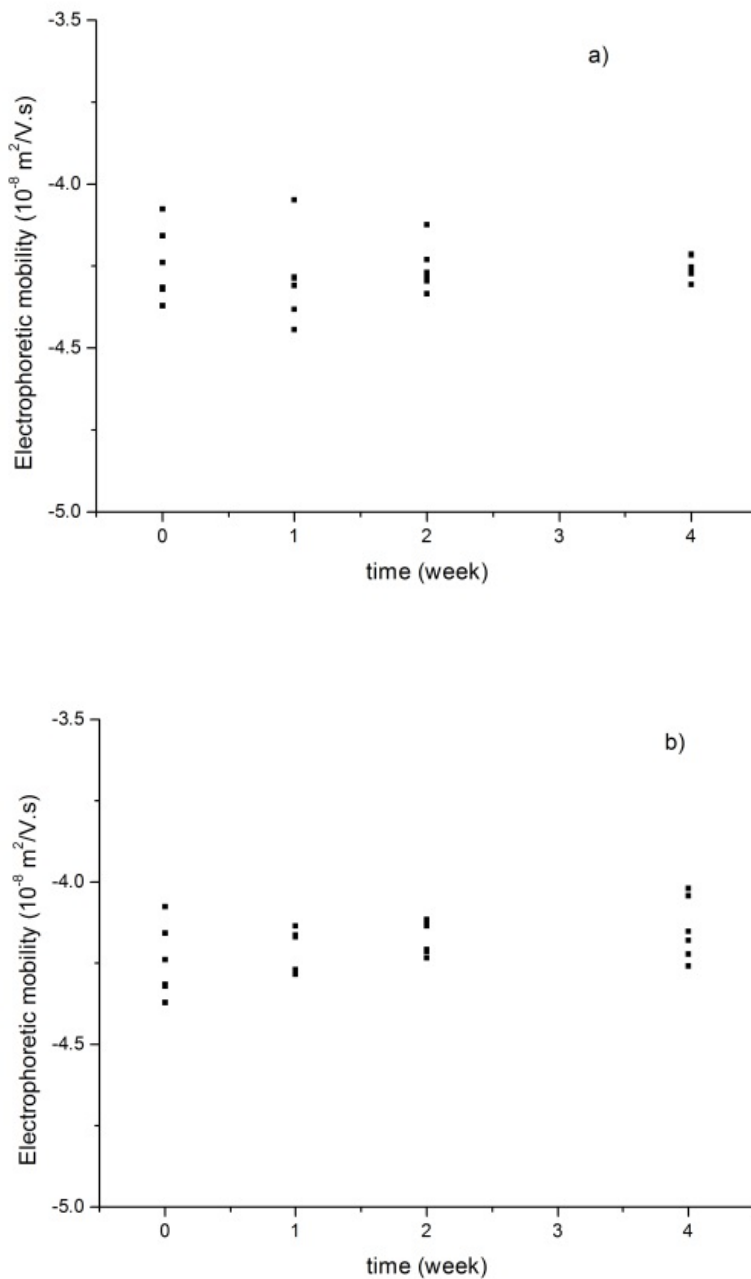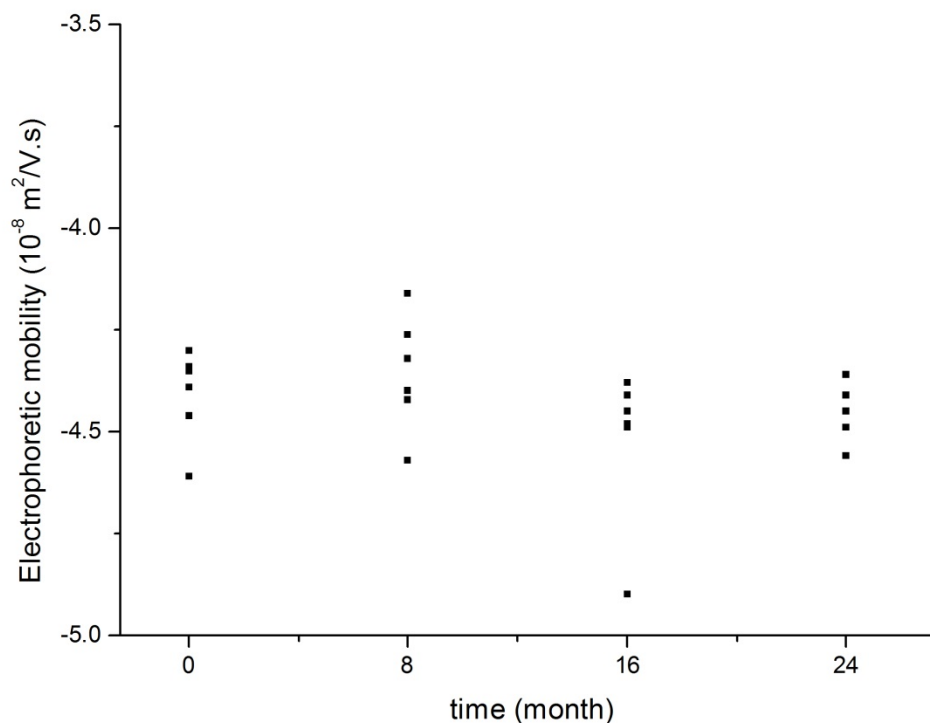**Fig. C** Long-term stability data (averages of triplicate results) of SRM 1992/ERM-FD305; electrophoretic mobility by ELS, when stored for several months at 18 °C. Results at time point 0 months correspond to the units that were stored at the reference temperature of 4 °C.

Annex D: Summary of methods used in the characterization study

Table D1 Electrophoretic light scattering (ELS): relevant instrumental and method details (as reported by the participants)

Lab Code	Instrument manufacturer	Instrument	Software version	Laser source (type, wavelength, power)	Photodetector detection angle	Type of measurement cell	Optical path length cell (mm)	Sample intake (mL)
0	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 7.11	He-Ne 632.8 nm 4 mW	APD 17°	dip cell with PMMA cuvette	10	1
1	Wyatt Technology	Mobius	Dynamics 7.8.0.26	532 nm 50 mW	APD 163.5°	flow cell: PEEK, platinum, fused silica	12	2
2	Otsuka Electronics	ELSZ-2000ZS	vers. 7.11	Laser diode 660 nm 30 mW	APD 15°	flow cell: quartz	5	1
3	Anton Paar	Litesizer™ 500	Kalliope™ version 2.0.2	Laser diode 658 nm 40 mW	APD 15°	folded capillary cell (Polycarbonate)	2	0.4
4	Malvern Panalytical	Zetasizer Nano	Zetasizer 7.11	He-Ne 633 nm 4 mW	APD 17°	folded capillary cell (Malvern DTS 1070)	2	1
5	Malvern Panalytical	ZEN3600	Zetasizer 7.03	He-Ne 633 nm 4 mW	APD 13°	folded capillary cell (Malvern DTS 1070)	4	0.75
6	Microtrac	Nanotrac Wave II	Flex 11.1.0.5	Laser diode 780 nm 3 mW	Silicon Photodiode 180°	n.a.	0.05	n.a.

Lab Code	Instrument manufacturer	Instrument	Software version	Laser source (type, wavelength, power)	Photodetector detection angle	Type of measurement cell	Optical path length cell (mm)	Sample intake (mL)
7	HORIBA	SZ100Z	2.2	Laser diode 532 nm 10 mW	PMT 13°	folded capillary cell	n.a.	0.75
8a	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 7.12	He-Ne 632.8 nm 4 mW	APD 13°	folded capillary cell (Malvern DTS 1070)	4	0.75
9	Malvern Panalytical	Zetasizer Nano ZS	Zetasizer 7.11	He-Ne 632.8 nm < 2 mW	APD 173 °	folded Capillary cell (Malvern DTS 1070)	54	0.8
10	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 6.34	He-Ne 632.8 nm 4 mW	APD 12.8°	dip cell (ZEN1002)	10	1
11a	Malvern Panalytical	Zetasizer Ultra	1.00.436	He-Ne 633 nm 10 mW	APD 13°	folded capillary cell (Malvern DTS 1070)	4	0.8
11b	Malvern Panalytical	Zetasizer Pro	1.00.436	He-Ne 633 nm 10 mW	APD 13°	quartz capillary cell (ZEN1010)	1.5	0.5
11c	Malvern Panalytical	Zetasizer Pro	1.00.436	He-Ne 633 nm 10 mW	APD 13°	dip cell with plastic cuvette (ZEN1002)	10	0.5
11d	Malvern Panalytical	Zetasizer Ultra	1.00.436	He-Ne 633 nm 10 mW	APD 13°	folded capillary cell (Malvern DTS 1080)	4	0.8

Lab Code	Instrument manufacturer	Instrument	Software version	Laser source (type, wavelength, power)	Photodetector detection angle	Type of measurement cell	Optical path length cell (mm)	Sample intake (mL)
12	Malvern Panalytical	ZEN3600	Zetasizer 7.03	He-Ne 633 nm 4 mW	APD 17°	folded capillary cell (Malvern DTS 1070)	4	1.1

n.a. = not available or reported by participant, APD: Avalanche photodiode detector; PMT: photomultiplier tube

Table D2 Particle tracking analysis: relevant instrumental and method details (as reported by the participants)

Lab code	Instrument type/ make	Dilution factor	Laser			Camera type	Aliquot volume (mL)	Measurement duration (s)	Analysis software	PQ
			Source	Wave-length (nm)	Power (mW)					
L8	NanoSight NS500	5000	Diode	405	60	sCMOS	0.1	90	NTA 3.3	Malvern DTS 1235
L14	NanoSight NS500	7500	Diode	405	< 60	EMCCD	n.a.	60	NTA 3.2	Malvern NTA4096
L15	Zetaview PMX100	100	Laser diode	650	5	CCD	n.a.	120	ZetaView 8.02.6	Malvern DTS 1235

n.a. = not available or reported by participant, sCMOSE: scientific complementary metal–oxide–semiconductor; EMCCD: electron multiplying charge-coupled device; CCD: charge-coupled device

Table D3 Electrical Asymmetrical-Flow Field-Flow Fractionation (EAF): relevant instrumental and method details (as reported by the participants)

Lab code	Instrument type/make	Aliquot preparation	Measurements parameters
Lab17	Postnova AF2000 MF separation system – aqueous solvents Electrical-Analytical Standard Channel PN3621 MALS Detector PN2410 Electrical FFF Module	Sample was diluted with the provided borate buffer (pH = 9.0) to 0.15 g/kg	Injection volume: 10 µL Total injected mass : 1.5 µg Eluent: borate buffer, pH=9.0 Membrane type: amphiphilic regenerated cellulose membrane, cut-off = 10 kDa Focusing flow rate: 0.2 mL/min Detector flow rate: 0.5 mL/min Cross flow rate: 0.5 mL/min Cross flow profile: constant Injection + focusing time: 5 min Elution time: 47 min Rinse step: 13 min, for removing potential aggregates/agglomerates and flushing the system Applied electric field: from 4.0 V/m to 9.5 V/m

ANNEX E: Results of characterization measurements

Annex E1: Results of characterization measurements – ELS

Table E1.1 Electrophoretic mobility results obtained by ELS as reported by the laboratories

Lab Code	Replicate results (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)						Mean (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)	s [a] (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)
	1	2	3	4	5	6		
L0	-4.47	-4.49	-4.39	-4.38	-4.57	-4.47	-4.46	0.07
L1	-4.77	-4.82	-4.86	-4.87	-4.77	-4.84	-4.82	0.04
L2	-4.23	-4.37	-4.37	-4.31	-4.27	-4.44	-4.33	0.08
L3	-4.48	-4.48	-4.45	-4.41	-4.48	-4.39	-4.45	0.04
L4	-4.51	-4.43	-4.41	-4.60	-4.35	-4.58	-4.48	0.10
L5	-4.87	-4.96	-4.96	-4.64	-4.83	-4.63	-4.82	0.15
L6	-4.06	-4.03	-4.11	-4.07	-4.12	-3.92	-4.05	0.07
L8	-4.50	-4.28	-4.40	-4.34	-4.44	-4.28	-4.37	0.09
L9	-4.43	-4.28	-4.57	-4.23	-4.42	-3.97	-4.31	0.21
L10	-4.50	-4.36	-4.54	-4.52	-4.56	-4.58	-4.51	0.08
L11a	-4.88	-4.97	-4.76	-4.88	-4.86	-4.93	-4.88	0.07
L11b	-4.73	-4.61	-4.65	-4.71	-4.68	-4.80	-4.70	0.07
L11c	-4.30	-4.22	-4.37	-4.48	-4.55	-4.29	-4.37	0.13
L11d	-4.69	-4.79	-4.95	-4.93	-5.15	-4.93	-4.91	0.16
L12	-4.56	-4.59	-4.54	-4.59	-4.50	-4.58	-4.56	0.03
Results not used for data evaluation								
L7	-4.54	-4.22	-4.53	-4.96	-4.85	-4.71	-4.64	0.27

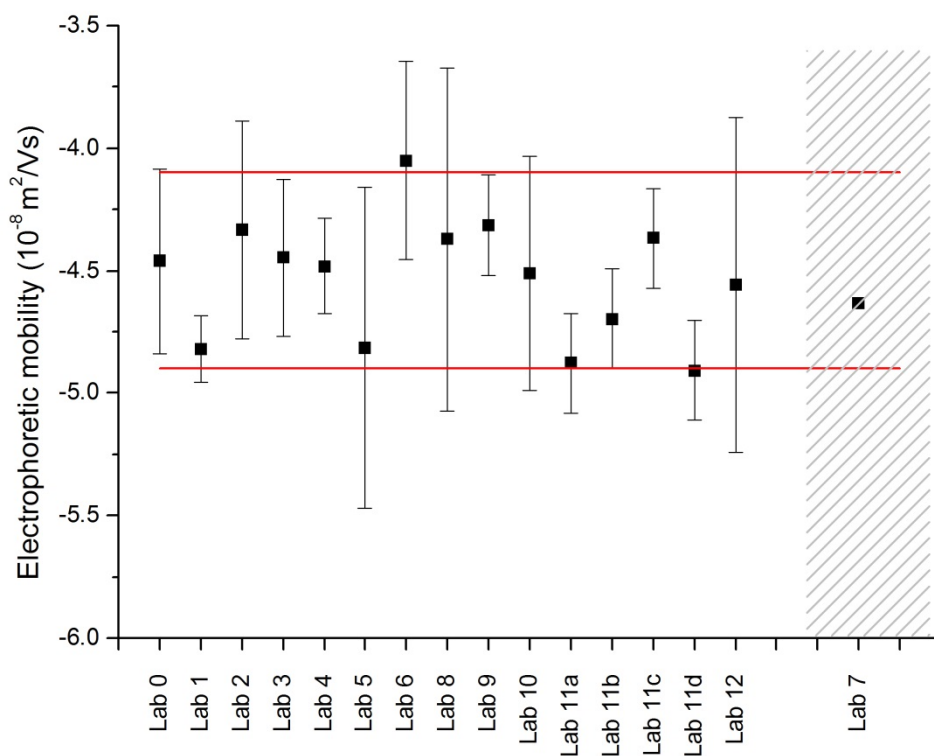[a] Standard deviation of the mean aliquot results

Fig. E1.1 Laboratory mean values (used for certification) of the electrophoretic mobility as obtained by 12 laboratories (15 datasets); error bars indicate the expanded (k = 2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range. Technically invalid results are indicated in the hatched region.

Table E1.2 Zeta potential results obtained by ELS as reported by the laboratories

Lab Code	Replicate mean results (mV)						Mean (mV)	s [a] (mV)
	1	2	3	4	5	6		
0	-57.1	-57.3	-56.0	-56.0	-58.2	-57.1	-56.9	0.9
1	-59.8	-60.4	-60.9	-61.0	-59.7	-60.6	-60.4	0.6
2	-54.2	-56.0	-56.0	-55.3	-54.7	-57.0	-55.5	1.0
3	-57.3	-57.3	-56.9	-56.4	-57.2	-56.2	-56.9	0.5
4	-57.5	-56.6	-56.3	-58.6	-55.5	-58.4	-57.2	1.2
5	-62.1	-63.3	-63.3	-59.2	-61.6	-59.1	-61.4	1.9
6	-51.9	-51.6	-52.5	-52.1	-52.8	-50.1	-51.8	0.9
8	-57.4	-54.6	-56.2	-55.3	-56.6	-54.7	-55.8	1.1
9	-56.5	-54.6	-58.2	-53.9	-56.4	-50.6	-55.0	2.7
10	-57.5	-55.6	-57.9	-57.6	-58.1	-58.4	-57.5	1.0
11a	-62.3	-63.4	-60.7	-62.3	-62.0	-63.0	-62.3	0.9
11b	-60.4	-58.8	-59.4	-60.1	-59.7	-61.3	-60.0	0.8
11c	-54.9	-53.9	-55.7	-57.2	-58.1	-54.7	-55.8	1.6
11d	-59.9	-61.1	-63.2	-62.9	-65.8	-62.9	-62.6	2.0
12	-58.1	-58.6	-58.0	-58.5	-57.4	-58.4	-58.2	0.4
Results not used for data evaluation								
7	-58.7	-54.4	-58.5	-64.1	-62.6	-60.9	-59.9	3.4

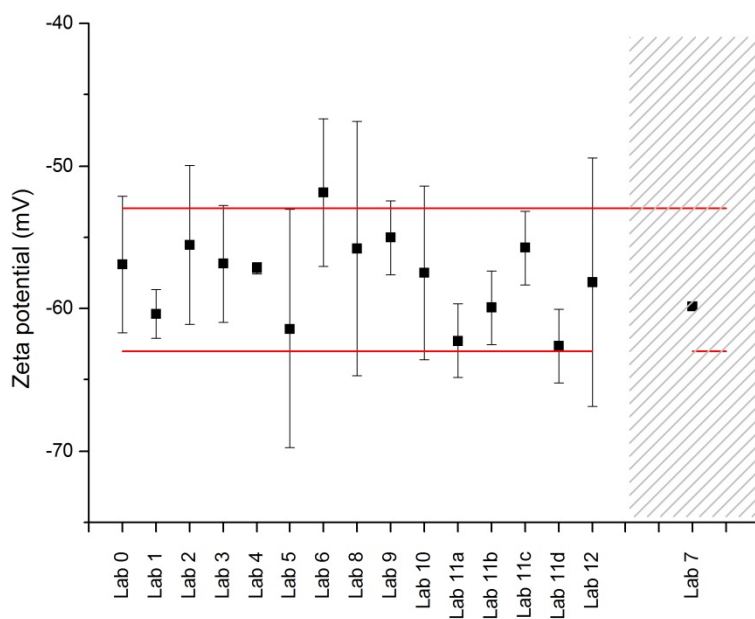[a] Standard deviation of the mean aliquot results

Fig. E1.2 Laboratory mean values (used for certification) of the zeta potential as reported by 12 laboratories (15 datasets); error bars indicate the expanded *(k* = 2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range. Technically invalid results are indicated in the hatched region.

Table E1.3 Zeta potential results recalculated from the electrophoretic mobility results (mean of replicate means) as reported by the laboratories

Lab Code	Mean of Replicates mean (10^{-8} m²·V^{-1}·s^{-1})	Mean recalculated ζ (mV)	Mean reported ζ (mV)
L0	-4.46	-57.2	-56.9
L1	-4.82	-61.8	-60.4
L2	-4.33	-55.6	-55.5
L3	-4.45	-57.0	-56.9
L4	-4.48	-57.5	-57.2
L5	-4.82	-61.7	-61.4
L6	-4.05	-51.9	-51.8
L8	-4.37	-56.1	-55.8
L9	-4.31	-55.3	-55.0
L10	-4.51	-57.8	-57.5
L11a	-4.88	-62.5	-62.3
L11b	-4.70	-60.2	-60.0
L11c	-4.37	-56.0	-55.8
L11d	-4.91	-62.9	-62.6
L12	-4.56	-58.5	-58.2
Results not used for evaluation			
L7	-4.64	-59.4	-59.9

Annex E2: Results of the characterization measurements Z-PTA

Table E2: Zeta potential results obtained by Z-PTA

Lab Code	Replicate results (mV)									Mean (mV)	s [a] (mV)
	1	2	3	4	5	6	7	8	9		
8	-52.6	-52.7	-53.1	-56.3	-51.8	-52.1	-56.9	-56.7	-56.7	-54.3	2.2
14	-55.4	-59.5	-53.8	-52.8	-55.1	-57.7	-53.4	-55.2	-61.1	-56.0	2.8
Invalid data due to fail on QC sample											
15	-34.1	-36.6	-36.0	-37.7	-40.3	-40.6	-41.5	-39.5	-41.3	-38.6	2.6

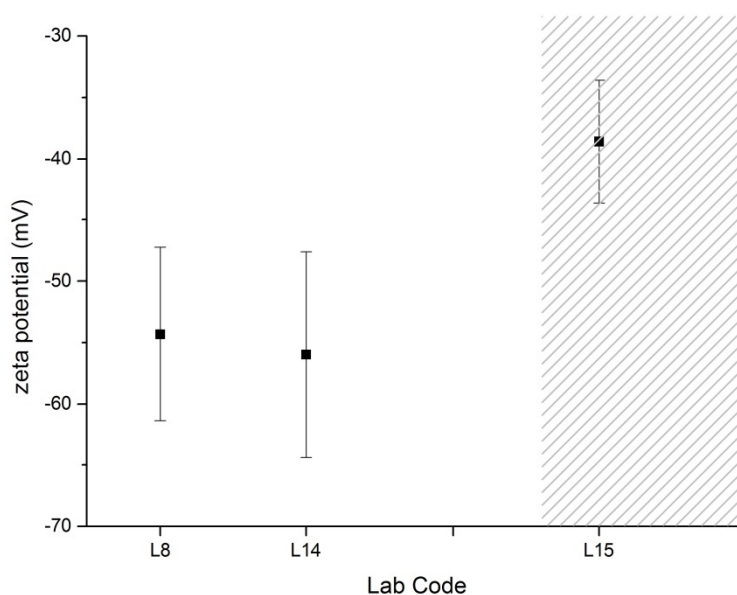[a] Standard deviation of the mean aliquot results

Fig. E2 Laboratory mean values (used for additional information) of the zeta potential as obtained by three laboratories; error bars indicate the expanded *(k* = 2) measurement uncertainties as reported by the participants.

Annex F: Buffer composition

BORATE BUFFER (pH 9)

Materials : sodium tetraborate ($Na_2B_4O_7$), boric acid (H_3BO_3), NaOH solutions (0.1 mol·L^{-1} and 0.01 mol·L^{-1})

- Weigh 0.77 g of sodium tetraborate, dissolve in 200 mL ultrapure water (heating at 60 °C will help to dissolve the crystalline powder)
- Weigh 0.25 g of boric acid, dissolve in 200 mL ultrapure water
- Combine the two solutions in a 1 L volumetric flask
- Make up volume to 1 L with ultrapure water
- Measure the pH. The pH should be 9.0 (±0.1). pH can be adjusted with NaOH solution if necessary. Conductivity of the solution should be between (0.36 and 0.4) mS·cm^{-1}.