CERTIFICATION REPORT

The certification of electrophoretic mobility/zeta potential of silica particles in aqueous solution: ERM®-FD306/SRM 1993

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Abstract

This report describes the production of ERM-FD306/SRM 1993, 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 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 characterised 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 characterisation.
The material is intended for quality control and assessment of method performance. The method-defined certified values are regarded as reliable estimates of the true values and ERM-FD306/SRM 1993 can therefore be used for calibration purposes. The CRM is available in 25 mL pre-scored amber glass ampoules each containing approximately 25 mL of suspension.

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Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM-FD306/SRM 1993, 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 characterised 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 characterisation.

The material is intended for quality control and assessment of method performance. The method-defined certified values are regarded as reliable estimates of the true values and ERM-FD306/SRM 1993 can therefore be used for calibration purposes. The CRM is available in 25 mL pre-scored amber glass ampoules each containing approximately 25 mL of suspension.

The following values were assigned:

	Silica particles in aqueous solution	
	Certified value[3)]	Uncertainty[4)]
Mean electrophoretic mobility[1)] (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)	-4.3	0.3
Mean zeta potential (mV) [2)]	-56	4

[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(κa) = 1.5 (for a temperature of 25 °C, a dynamic viscosity of 0.89 mPa.s and a value of 78.4 for the dielectric constant of water).

[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 of the certified value is the expanded uncertainty 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.

Table of contents

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
CVI	Colloid vibration current
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
ESA	Electrokinetic sonic amplitude
EU	European Union
FPGA	Field programmable gate array
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_{char}	Standard uncertainty of the material characterisation; 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
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_{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

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, a 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 International Standards ISO 13099, Part 1 [5] and Part 3 [6]) and also by optical methods using electrophoretic phenomena, especially electrophoretic light scattering (ELS) (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 industry input and cooperation. 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. Neither option is very satisfactory and industry requested a CRM with traceable values to calibrate the instruments.

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 $g \cdot kg^{-1}$), ERM-FD305/SRM 1992, suitable for most optical methods, the production of which is described elsewhere [8];
- one concentrated material (22 $g \cdot kg^{-1}$), ERM-FD306/SRM 1993, suitable for acoustic methods but still in the limit of quantification of the ELS method; the production of which is described in this report.

The same materials are released and co-branded by the JRC and NIST under their own CRM codes (ERM-FD305/SRM 1992 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. 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)

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

μ .....electrophoretic mobility ($m^2 \cdot V^{-1} \cdot s^{-1}$or $A \cdot s^2 \cdot kg^{-1}$)
ζ .....zeta potential (V or $kg \cdot m^2 \cdot s^{-3} \cdot A$)
η_0 .....viscosity of the medium (Pa·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 radius (m)
κ .....reciprocal of the 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)

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

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 high concentration version of the material, ERM-FD306/SRM 1993, 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 [7].

For reference beam optics:

$$\mu = \frac{\Delta\omega\lambda_0}{4\pi E n s \left(\theta/_2\right)\sin\left(\theta/_2+\xi\right)}$$ **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 $kg \cdot m \cdot A^{-1} \cdot s^{-3}$)
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 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.

Electroacoustic method

Electroacoustic phenomena arise from the coupling between the acoustic field and electric field in liquid containing charged colloids or ions.

When a high frequency electric field is applied to the dispersion, dynamic motion of charged particles is induced. If a density difference exists between the particles and the suspending liquid, this motion will generate a sound wave in the ultrasonic range whose amplitude can be measured. This signal is referred to as the electrokinetic sonic amplitude (ESA).

Conversely, when an ultrasonic wave is applied to a dispersion of charged particles, any differences in the effective mass or friction coefficient between anions and cations surrounding these particles result in different displacement amplitudes. This difference in displacement creates alternating electric dipoles that in turn produce a measurable electric field. The colloid vibration current (CVI) associated with this field is the principal measurand.

From the magnitude and phase of the electroacoustic signal (CVI or ESA), the dynamic electrophoretic mobility can be obtained from which the zeta potential can be calculated

using an appropriate model. It should be noted that dynamic mobility is also particle size-dependent due to inertial effects on the induced motion; this dependence decreases with decreasing particle size, and is insignificant for particles smaller than approximately 100 nm in diameter.

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. Moreover, colloidal silica has a long history of use as a calibrant in electroacoustics.

ERM-FD306/SRM 1993 was produced from a commercially available suspension that consisted of silica particles suspended in an undefined aqueous solution of electrolytes. The dispersing solution was exchanged by a tangential flow filtration process to a borate buffer at pH 9 with a defined composition. ERM-FD306/SRM 1993 was produced at a concentration suitable (22 $g \cdot kg^{-1}$) for both electroacoustic measurement and some ELS measurements (without dilution), where the ranges of these methods can overlap.

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 principle of the value assignment and the intended use is as follows:

1. While modern ELS instruments can measure suspensions of a mass fraction as the one present in ERM/FD306/SRM 1993 directly, it is important to rule out concentration effects, i.e. to show that the electrophoretic mobility at lower mass fractions is the same as at higher ones. This can be done as it has been shown that it is possible to dilute suspensions without changing the zeta potential, as long as the original suspending liquid is used for dilution [5]. This provides the possibility to certify the zeta potential of a concentrated suspension using ELS.
2. SI traceable values for electrophoretic mobility are assigned using ELS. This is possible as electrophoretic light scattering does not require calibration with particles and the values are based on first principles and as the value is not concentration dependent.
3. Electroacoustic measurements are subsequently performed to confirm the suitability and commutability of the material for electroacoustic methods and to allow a plausibility check: while none of the current calibration methods for electroacoustic methods ensures satisfactory traceability, experience with the method shows that it is unlikely that the bias is very large. Agreement of electroacoustic measurements with the ELS-certified value therefore shows that the value is also applicable to electroacoustic methods.
4. As the material is shown to be commutable for electroacoustic measurements, the ELS-certified value can be used to calibrate electroacoustic methods. Strictly speaking, electroacoustic methods thus calibrated are traceable to the SI-traceable results of electrophoretic light scattering measurements.

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) from 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

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)

2.4 Stability study

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 Characterisation

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

Anton Paar GmbH, Graz, AT

Colloidal Dynamics LLC, Ponte Vedra Beach, USA

Dispersion Technology Inc., Bedford Hills, 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)

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

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 Metrology Institute of Japan (NMIJ), Tsukuba, JPN
National Physical Laboratory (NPL), Teddington, UK
Otsuka Electronics, Osaka, JPN
Takeda Colloid Techno-Consulting Co Ltd., Osaka, JPN
Wyatt Technology, Santa Barbara, USA
3P instruments GmbH & Co.KG, Odelzhausen, DE

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 $g \cdot kg^{-1}$.
- 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 $mmol \cdot L^{-1}$ NaCl (Fig 1).

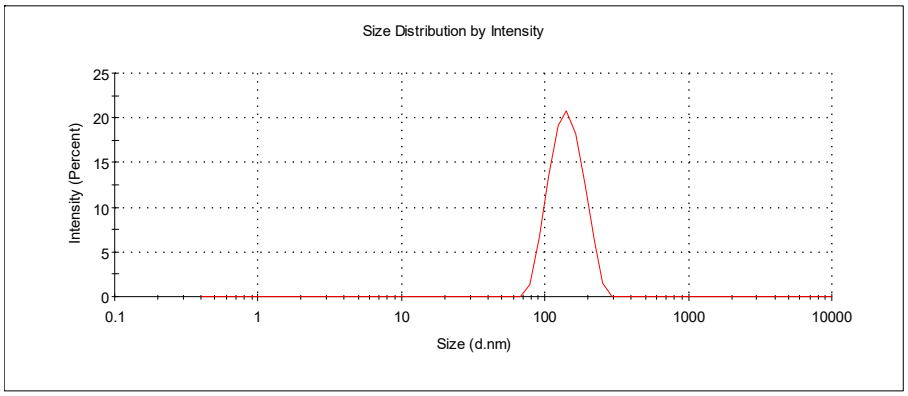

Fig 1: Scattered light intensity-based particle size distribution of Acesol WP4 obtained by DLS

- 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.

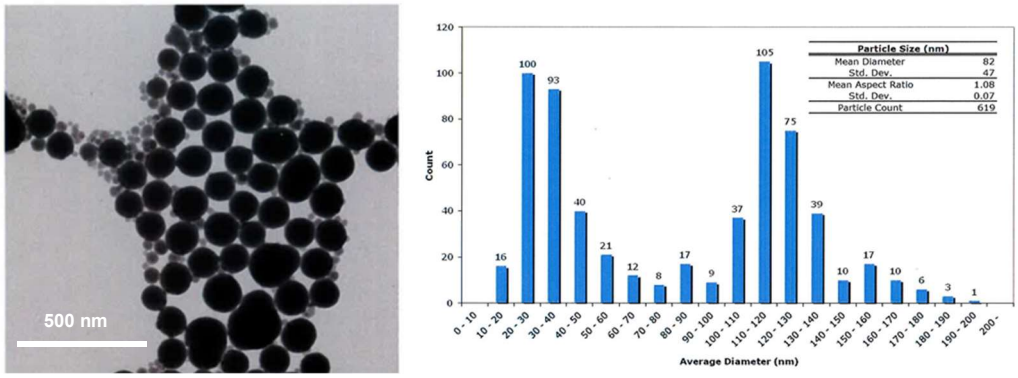

Fig 2: TEM micrograph and number-based area-equivalent particle size distribution of Acesol WP4 silica particles (MVA Scientific Consultants, Duluth, USA)

Despite the presence of a small size particle population, the material was selected for the production of the CRM material. Indeed, as the value assignment is done 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 characterisation 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 the conventional SEM mode and in the 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.

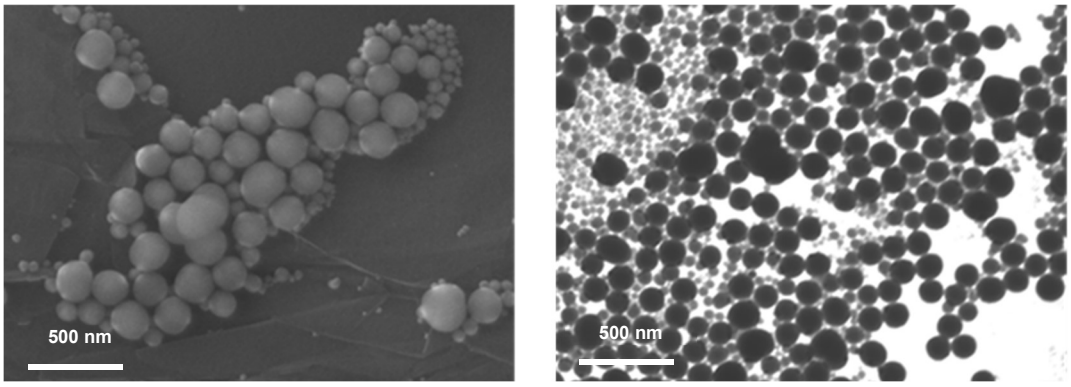

Fig 3: SEM image (left) and TSEM image (right) on Acesol WP4 (JRC, Geel, BE)

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 $g \cdot kg^{-1}$
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 $g \cdot kg^{-1}$) was diluted with 10 $mmol \cdot L^{-1}$ NaCl to a concentration of 10 $g \cdot kg^{-1}$. 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 $g \cdot kg^{-1}$. The retentate was then re-diluted to the initial concentration with 10 $mmol \cdot L^{-1}$ 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 $g \cdot kg^{-1}$ 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 $g \cdot kg^{-1}$ 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 $g \cdot kg^{-1}$. The different batches were stored in sterile cans for the production of the final CRM.

ERM-FD306/SRM 1993 was then produced by dilution with sterile borate buffer (obtained by filtration with 0.2 µm cut-off hollow fiber module) of this material to a mass fraction of 22.0 $g \cdot kg^{-1}$ under a movable clean cell (Terranova, Fullerton, USA).

Pre-scored 25 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 an ampouling machine (R 910 PA, Rota, Wehr, DE). Every ampoule was flushed with argon (Ar) gas immediately before filling with about 25 mL of suspension. The suspension in the supply bottle was continuously mixed by circulation of the suspension in sterile tubing with a peristatic pump during the process of filling the ampoules. Immediately after filling, the ampoules were again flushed with Ar and closed. In total 2100 ampoules were produced. The batch was split into 2 lots. The odd numbers were reserved for JRC and the even numbers reserved for NIST. All produced ampoules are packed in 60 mL polypropylene transparent tubes with a white screw cap.

3.3 Process control

Before ampouling, an aliquot was sampled and analysed by DLS for particle size, 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 ERM-FD306/SRM 1993

Measurement method	Information value
Electrophoretic mobility (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$) [a)]	-4.3
Zeta potential (mV)[a)]	-55
Conductivity $mS \cdot cm^{-1}$ [b)]	0.42
pH[c)]	8.9
Hydrodynamic diameter (nm)[d)]	140
Concentration ($g \cdot kg^{-1}$)[e)]	22.0

[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 harmonic mean) at (25 ± 1) °C

[e)] As obtained by dry mass determination

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-ampoule 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. Thirteen units, selected using a random stratified sampling scheme covering the whole batch, were analysed to test the between-ampoule homogeneity. From each of the 13 units, three independent subsamples (aliquots) were taken and analysed in-house for electrophoretic mobility by ELS. The measurements were performed under

repeatability conditions and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence.

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. A significant trend (95 % confidence level) is visible in the analytical sequence. However due to the low value of the slope (0.04 mV/measurement), no correction was applied to the data.

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. One outlying unit mean was detected. Since no technical reason for the outlier could be determined, this data was 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 unit means 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 unit mean was visually tested 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	yes	No	none	1	unimodal	normal

* 95 % confidence level

** 99 % confidence level

As a single outlying unit mean was detected for the electrophoretic mobility, the between-ampoule heterogeneity was modelled as a rectangular distribution limited by the largest outlying unit mean, and the rectangular standard uncertainty of homogeneity was estimated by:

$$u_{rec} = \frac{\left| outlier - \overline{y} \right|}{\sqrt{3} \cdot \overline{y}} \qquad \textbf{Eq. 5}$$

where

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

The homogeneity study showed no trend in the filling sequence.

The heterogeneity as quantified as u_{rec} is sufficiently small to not affect the intended purpose, ensuring the material is useful. Therefore, u_{rec} was used as an estimate of u_{bb}.

The results of the evaluation of the between-ampoule variation are summarised in Table 4.

Table 4: Results of the homogeneity studies

Measurand	$u_{bb,rel}$ (%)
Electrophoretic mobility	2.7

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 can be used in an analysis. Using sample sizes equal 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 characterisation 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 0.2 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. 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 [9]. 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 are 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 tests 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 slopes of the regression lines were 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

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

* 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}. No trends were statistically significant at a 95 % confidence level for any of the temperatures.

During the production of a similar colloidal silica CRM, ERM-FD304 [10], it was observed that freezing of the suspension led to irreversible agglomeration of the particles. Therefore, ERM-FD306/SRM 1993 must be protected against freezing as recommended also 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 as 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 random sequence 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 tests at a confidence level of 99 %. No outlying individual results were 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 results of the statistical evaluation of the long-term stability study are summarised in Table 6.

Table 6: Results of the long-term stability tests

	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 no trends were statistically significant at a 95 % confidence level for any of the temperatures. 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 [11] for each measurand. 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 °C studies. The uncertainty describes the possible change during a dispatch at 60 °C 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	0.36	1.44

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

6. Characterisation

The material characterisation 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/electrophoretic mobility of the material was determined in different laboratories that applied different measurement procedures to demonstrate the absence of a measurement bias. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

The material characterisation 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) [12]) 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 characterisation was based on ELS and the plausibility of the result was confirmed by an independent method (electroacoustics).

6.1 Selection of participants

Eighteen 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 nine independent results (three replicates per unit). The units for material characterisation were selected using a random stratified sampling scheme and covered the entire batch. The measurements were spread over at least three days to ensure intermediate precision conditions.

Each participant received a sample of Malvern DTS 1235, Zeta potential transfer standard traceable to NIST SRM 1980 (Malvern Panalytical, UK), as a blinded quality control (QC) sample for the ELS. The results for this sample were used to support the evaluation of the characterisation results.

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the replicate 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

The characterisation of ERM-FD306/SRM 1993 was performed by ELS in terms of zeta potential and electrophoretic mobility.

Laboratories were asked to perform sample handling, preparation and measurements according to ISO 13099. Laboratories could choose the type of measurement cell (e.g., dip cell, folded capillary cell, quartz capillary, etc.) appropriate for the instrument used. The cell type and optical path length were reported. All measurements were performed on samples in the as-received state. Furthermore, the protocol required measurements to be performed at 25 °C, with an equilibration time of 120 s, using a viscosity for the dispersing medium of 0.8872 mPa·s and a refractive index (at 25 °C) of the dispersing medium of 1.330. Moreover, with each unit of ERM-FD306/SRM 1993, the laboratories received a borate buffer and were asked to prepare 2,10 and 20-fold dilutions (see table 8). An equilibration time of 1 hour was required before measuring the diluted samples.

Table 8: Dilution protocol of ERM-FD306/SRM 1993 with borate buffer

Dilution factor	ERM-FD306/SRM 1993 (mL)	Buffer (mL)
2	2.5	2.5
10	0.5	4.5
20	0.5	9.5

From each ampoule and each diluted sample, two aliquots were taken and each aliquot was measured five times under repeatability conditions.

The laboratories reported the zeta potential, the electrophoretic mobility, the applied electric field and the conductivity of the sample.

6.3.2 Electroacoustic measurements

The characterisation of ERM-FD306/SRM 1993 was performed by electroacoustics in terms of zeta potential and electrophoretic mobility on the as-received, undiluted, sample.

Laboratories were asked to perform sample handling, preparation and measurements according to ISO 13099. Depending on the setup of their instruments (volume of the measurement cell), some laboratories had to combine two or more ampoules for the measurements.

From each ampoule two aliquots had to be taken and each aliquot had to be measured five times under repeatability conditions, except where ampoules were combined.

The laboratories reported the zeta potential, the dynamic electrophoretic mobility, the applied electric field and the electrolyte conductivity of the sample.

6.4 Evaluation of results

The characterisation campaign resulted in a total of 26 independent datasets. Twenty datasets were received from the ELS method and six from the electroacoustic 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 on three different 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 [13]

Based on the above criteria, the following datasets were rejected as not technically valid.

Table 9: Datasets that showed non-compliances with the analysis protocol and technical specifications, and action taken

Method	Lab-method code	Description of problem	Action taken
ELS	L0a	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 ERM-FD306/SRM 1993 agreed with other laboratories.
ELS	L0b, L8b	No uncertainty provided because the measurement cell used is out of the scope of the laboratory method validation	Data not used for the evaluation
ELS	L1	Laboratory indicated that the sample was too concentrated for the optical set-up of the instrument	Data not used for the evaluation
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 on ERM-FD306/SRM 1993 retained based on good agreement with other laboratories
ELS	L7	The laboratory did not provide measurement uncertainty. RSD of 23 % on QC sample.	Data not used for evaluation
ELS	L11d	The results do not agree with the certified value, within the reported uncertainty	Data retained due to an underestimation of the reported uncertainty

Electrophoretic light scattering: Thirteen laboratories participated in the ILC study. One laboratory submitted four datasets (two instruments and four different cells). Twenty datasets were received for the as-received ERM-FD306/SRM 1993 and for the three dilutions.

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 ERM-FD306/SRM 1993 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 ERM-FD306/SRM 1993 did not cause any compatibility issues and as there were no indications of technical problems during measurement, and also as the results obtained on ERM-FD306/SRM 1993 agreed with the other laboratories, the dataset was retained.

One laboratory (L7) did not provide measurement uncertainty and as the RSD on the QCM was about 23 %, the data was not used for evaluation.

Two laboratories (L0b, L8b) reported results using a measurement cell that was not included in the scope of their method validation; the data were not retained.

The results of one laboratory (L11d) do not agree with the certified value within the reported uncertainty. The data was retained due to the underestimation of their reported uncertainty.

On the as received ERM-FD306/SRM 1993/SRM 1993, only one laboratory (L1) reported that the concentration of the as received material is too high for the setup of its instrument. Therefore only 16 datasets data were retained at this concentration.

For each of the 2-, 10- and 20-fold dilutions, 17 data sets were retained for evaluation. Dilution series measurements confirmed the results obtained on the as-received samples (see Annex Fig E1.9). As a result, the value assignment was performed on the as-received, undiluted, ERM-FD306/SRM 1993.

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

Electroacoustic methods

Five laboratories participated in the study. A total of six data sets were received (one laboratory used two different instruments). Five datasets were obtained from instruments using Ludox TM as calibrant and applying the CVI mode to obtain the dynamic electrophoretic mobility. One dataset was obtained from an instrument using a KSiW electrolyte solution as calibrant and using the ESA mode to obtain the dynamic electrophoretic mobility.

Four laboratories reported the standard deviation as an estimation of the measurement uncertainty.

One laboratory did not report the dynamic electrophoretic mobility (five datasets received). The five datasets were in good agreement, showing that the material is suitable for electroacoustic methods.

Six datasets were received for zeta potential. Although the dynamic electrophoretic measurements were in agreement, the zeta potential values reported by the participants showed a discrepancy due to the various models used by the instrument manufacturers.

Data obtained by electroacoustic methods is reported as additional value.

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 10.

Table 10: Statistical evaluation of the technically accepted datasets for ERM-FD306/SRM 1993, 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	16	none	L9	yes	-4.34	0.25	0.24	0.12

Electrophoretic mobility: Statistical evaluation of the ERM-FD306/SRM 1993 datasets flagged the variance of laboratory L9 as an outlier for 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, outlying variance is not a reason for exclusion of data.

The uncertainty related to the characterisation *(u_{char})* is estimated as the standard error of the mean of laboratory means, i.e. $s/\sqrt{p}$ with s and p taken from Table 11.

Table 11: Uncertainty of characterisation for ERM-FD306/SRM 1993, where *p* is the number of technically valid datasets

	p	Mean (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)	*s* (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)	u_{char} (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)
Electrophoretic mobility	16	-4.34	0.25	0.06

7. Value Assignment

Certified and informative values were assigned.

Certified values 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.

Additional material information 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 7 was assigned as certified value.

The assigned uncertainty consists of uncertainties relating to characterisation, u_{char} (Section 6), potential between-ampoule inhomogeneity, u_{bb} (Section 4.1), and potential degradation during transport, u_{sts}, and long-term storage, u_{lts} (Section 5).

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:

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

- 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 of the degrees of freedom of the different uncertainty contributions, a coverage factor k of 2 (approximating a 95 % confidence interval) was applied, to obtain the expanded uncertainties.

The certified values and their uncertainties are summarised in Table 12.

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 -4.34 (10^{-8} $m^2 \cdot V^{-1} s^{-1}$) for the measurand μ and 0.066 (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$) for the standard uncertainty. Table 12 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 data set 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 standard uncertainty of 0.147 (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$), which matches the result in Table 12, thus confirming the analysis above.

Table 12: Certified values and their uncertainties for ERM-FD306/SRM 1993

[1)] Expanded (k = 2) and rounded uncertainty.

	Certified value (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)	u_{char} (%)	u_{bb} (%)	u_{sts} (%)	$u_{lts,}$ (%)	$U_{CRM,rel}$ (%)	U_{CRM} [a)] (mV)
Electrophoretic mobility	-4.3	1.42	2.69	0.36	1.44	7.0	0.3

a) Expanded (k = 2) and rounded uncertainty

The certified zeta potential value is calculated from the electrophoretic mobility certified value using Equation 2 for a temperature of 25 °C with a rounded value of a 0.89 mPa.s for the viscosity [14, 15], and a value of 78.4 for the dielectric constant of water [16, 17].

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 13: Zeta potential certified value and its uncertainty for ERM-FD306/ SRM 1993

	Certified value (mV)	U_{CRM} a) (mV)
Zeta potential	-56	4

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.

7.2.1 Electroacoustic method

As reported before, 6 data sets were received for the electroacoustic methods; 6 for zeta potential and 5 for dynamic electrophoretic mobility. The dynamic electrophoretic mobility average value and the zeta potential average value are provided as an additional information value in Table 14.

Table 14: Additional material information value obtained by electroacoustic method

	Mean (mV)	s (mV)
Zeta potential	-54.7	8.4

	Mean (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)	s (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)
Dynamic electrophoretic mobility	-5.4	0.3

7.2.2 Conductivity

Most ELS instruments also measured the conductivity of the samples. The measured conductivity values were in the range (0.32-0.48) $mS \cdot cm^{-1}$. The average of the mean laboratory results is reported as additional material information.

Table 14: Additional material information value obtained by conductivity measurement

	Mean ($mS \cdot cm^{-1}$)	s ($mS \cdot cm^{-1}$)
Conductivity (at 25°C)	0.42	0.03

8. Metrological traceability and commutability

8.1 Metrological traceability

Identity

Electrophoretic mobility is the electrophoretic mobility per electric field strength.

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

ERM-FD306/SRM 1993 has been characterised using 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 electrophoretic mobility and zeta potential, are underpinned by the agreement of the laboratories' results with the assigned value on Malvern DTS 1235 that was used as QC samples.

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 sample/particle: 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 x 10^{-6} [18]. 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→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 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 [19] 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 confirm that a bias does not exist in calibration, nor can the CRM be used as a calibrant.

ERM FD306/SRM 1993 has been characterised by ELS and electroacoustic methods. Although the electroacoustic method is dependent on the calibrant and also for the zeta potential measurement on the model used for the calculations, the electroacoustic results correlated with the ELS results. Therefore ERM-FD306/SRM 1993 is commutable for ELS and electroacoustics.

9. Instructions for use

9.1 Safety information

The material should be handled with care. The material contained nanoparticles that could have an impact on environment and human health. Any spillage 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 (20 ± 5) °C. Ampoules must not be allowed to freeze, as this will irreversibly compromise the integrity of the material.

Please note that the 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 before every measurement without introducing air bubbles.

ELS and electroacoustic method: Aliquot of ERM-FD306/SRM 1993 shall be measured as received, i.e. without dilution. The measurement temperature shall be (25 ± 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 at 25 °C, 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 200 µL.

9.5 Use of the certified value

The main purpose of this material is to calibrate electroacoustic instruments that are used for measuring zeta potential and 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

The material can be used as a calibrant. The uncertainty of the certified value shall 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 [20].

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 ($u_{\Delta,}$) 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 %.

Use in quality control charts

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.

10. Acknowledgments

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12. Annexes

Annexes

Annex A: Results of the 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.

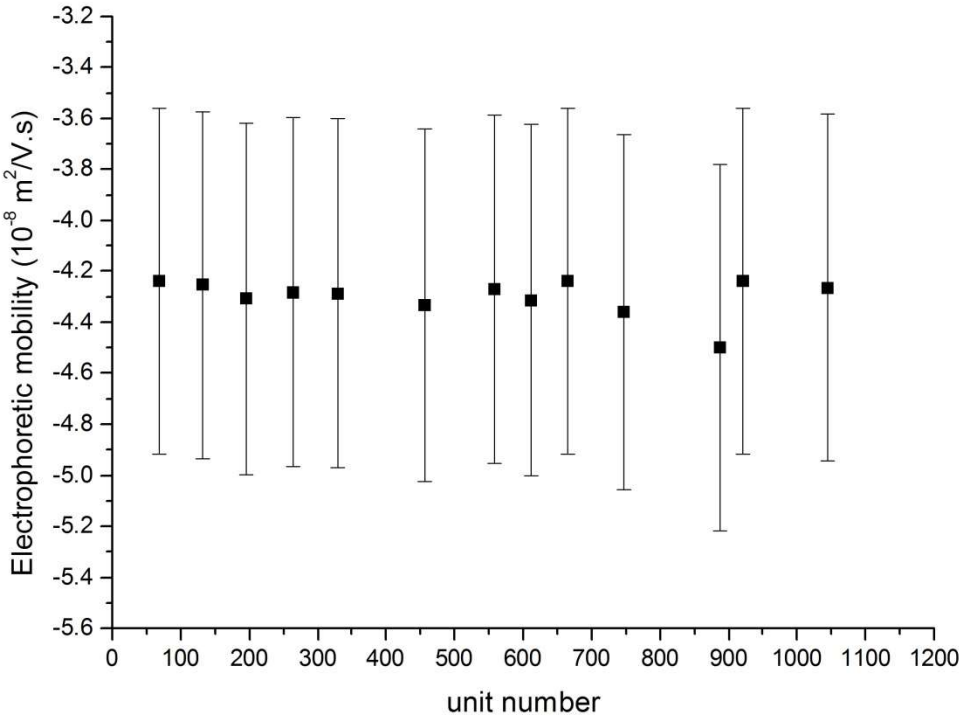

Fig. A Homogeneity data (average results of two replicates) of ERM-FD306/SRM 1993; electrophoretic mobility by ELS; error bars correspond to the expanded measurements uncertainties (k = 2) for use of the method in repeatability conditions.

Annex B: Results of the 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. Absolute values do not necessarily agree with the certified values due to potential laboratory bias, but this is irrelevant for the evaluation of stability.

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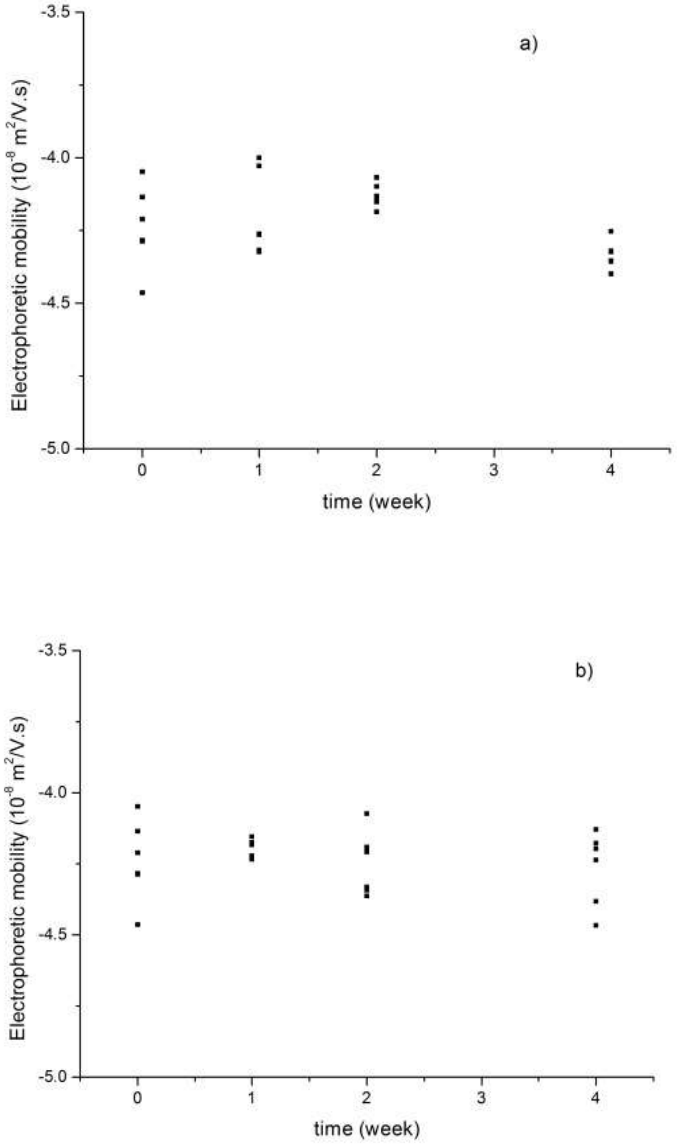

Fig. B Short term stability data (results of individual replicates) of ERM-FD306/SRM1993; electrophoretic mobility results by ELS, when stored several weeks at 4 °C (a) and 60 °C (b). Results at time point 0 week correspond to units that were stored at the reference temperature of 18 °C.

Annex C: Results of long-term stability measurements

Fig. C shows the long-term stability data obtained by ELS for the electrophoretic mobility results. Absolute values do not necessarily agree with the certified value due to the potential laboratory bias, but this is irrelevant for the evaluation of the stability.

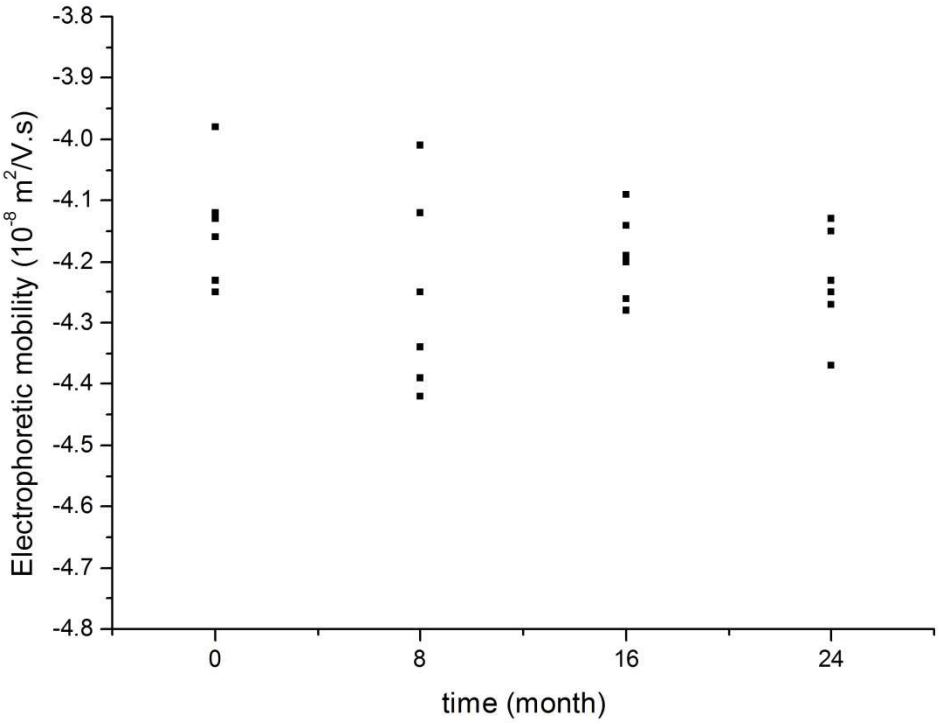

Fig. C Long term stability data (results of individual replicates) of ERM-FD306/SRM 1993; electrophoretic mobility results by ELS, when stored several months at 18 °C. Results at time 0 months correspond to the units that were stored at the reference temperature of 4 °C.

Annex D: Summary of methods used in the characterisation study

Table D1 Electrophoretic light scattering: 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 cells	optical path length cell (mm)	Sample intake (mL)
0a	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 7.11	He-Ne 632.8 nm 4 mW	APD 17°	dip cell with PMMA cuvette	10	1
0b	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 7.11	He-Ne 632.8 nm 4 mW	APD 17°	high concentration cell	2	0.2
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

Lab Code	Instrument Manufacturer	Instrument	Software version	Laser source (type, wavelength, power)	Photodetector Detection angle	Type of measurement cells	optical path length cell (mm)	Sample intake (mL)
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.
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
8b	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 7.12	He-Ne 632.8 nm 4 mW	APD 13°	high concentration cell	2	0.2
8c	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 7.12	He-Ne 632.8 nm 4 mW	APD 13°	dip cell with PS cuvette	10	1
8d	Malvern Panalytical	ZetaSizer Nano ZS	Zetasizer 7.12	He-Ne 632.8 nm 4 mW	APD 13°	dip cell with PS cuvette	10	1
9	Malvern Panalytical	Zetasizer Nano ZS	Zetasizer 7.11	He-Ne 632.8 nm < 2 mW	APD 173 °	folded capillary cell (Malvern DTS 1070)	4	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

Lab Code	Instrument Manufacturer	Instrument	Software version	Laser source (type, wavelength, power)	Photodetector Detection angle	Type of measurement cells	optical path length cell (mm)	Sample intake (mL)
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
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 not reported by participant, APD: Avalanche photodiode detector; PMT: photomultiplier tube

Table D2 Electroacoustic measurement: relevant instrumental and method details (as reported by the participants)

Lab Code	Instrument manufacturer	Instrument	Measurement	Calibrant	Measurement Frequency (MHz)
10	Dispersion technology	DT1202	CVI	Ludox TM	3.3
13a	Dispersion technology	DT1200	CVI	Ludox TM	3.3
13b	Dispersion technology	DT300	CVI	Ludox TM	3.3
16	Dispersion technology	DT1202	CVI	Ludox TM	3.3 -3.4
18	Dispersion technology	DT1202	CVI	Ludox TM	3.3
19	Colloidal Dynamics	AcoustoSizer IIx	ESA	Colloidal Dynamics' Potassium Silico Tungstate ("KSiW") electrolyte solution	3.3

ANNEX E: Results of characterisation measurements

Annex E1: Results of characterisation measurements – ELS

Table E1.1 Electrophoretic mobility obtained by ELS on the as received samples

Lab Code	Replicate mean 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		
0a	-4.01	-4.15	-4.12	-4.13	-4.17	-4.20	-4.13	0.06
2	-4.25	-4.36	-4.21	-4.42	-4.35	-4.28	-4.31	0.08
3	-4.46	-4.61	-4.52	-4.53	-4.40	-4.48	-4.50	0.07
4	-4.30	-4.45	-4.26	-4.46	-4.23	-4.47	-4.36	0.11
5	-4.68	-4.60	-4.54	-4.48	-4.60	-4.38	-4.55	0.10
6	-4.01	-4.04	-4.05	-4.01	-4.01	-3.77	-3.98	0.11
8a	-4.25	-4.14	-4.30	-4.47	-4.08	-4.14	-4.23	0.14
8c	-4.03	-4.22	-4.27	-4.18	-4.24	-4.17	-4.18	0.08
8 d	-3.91	-4.10	-3.95	-4.14	-3.97	-3.93	-4.00	0.10
9	-4.23	-4.29	-4.23	-3.63	-3.99	-4.26	-4.10	0.26
10	-4.34	-4.33	-4.38	-4.23	-4.28	-4.61	-4.36	0.13
11a	-4.57	-4.90	-4.39	-4.63	-4.51	-4.46	-4.58	0.18
11b	-4.56	-4.70	-4.59	-4.69	-4.57	-4.59	-4.62	0.06
11c	-4.37	-4.23	-4.34	-4.20	-4.19	-3.96	-4.21	0.14
11d	-4.98	-4.99	-5.03	-4.84	-4.78	-4.77	-4.90	0.11
12	-4.40	-4.42	-4.37	-4.43	-4.40	-4.48	-4.42	0.04
Results not used for data evaluation								
1	-2.79	-2.77	-2.27	-2.52	-2.47	-2.47	-2.55	0.20
0b	-3.78	-3.85	-3.91	-3.88	-3.77	-3.74	-3.82	0.07
7	-4.85	-5.00	-4.99	-4.90	-4.90	-4.75	-4.90	0.09
8b	-4.06	-4.03	-4.22	-4.40	-4.22	-4.23	-4.19	0.14

[a] Standard deviation of the mean aliquot results

n.a.= not available or not reported by participant

Table E1.2 Zeta potential obtained by ELS on the as received samples

Lab Code	Replicate mean results (mV)						Mean (mV)	s [a)] (mV)
	1	2	3	4	5	6		
0a	-51.1	-53.0	-52.6	-52.7	-53.2	-53.5	-52.7	0.8
2	-54.4	-55.9	-54.0	-56.6	-55.7	-54.9	-55.2	1.0
3	-57.0	-58.9	-57.2	-57.7	-56.3	-57.3	-57.4	0.9
4	-54.8	-56.7	-54.3	-56.9	-54.0	-57.0	-55.6	1.4
5	-59.7	-58.7	-58.0	-57.2	-58.6	-55.9	-58.0	1.3
6	-51.4	-51.8	-51.8	-51.3	-51.4	-48.3	-51.0	1.4
8a	-54.2	-52.8	-54.9	-57.0	-52.1	-52.8	-54.0	1.8
8c	-53.6	-53.9	-54.4	-53.3	-54.1	-53.2	-53.7	0.5
8 d	-49.9	-52.4	-50.4	-52.8	-50.6	-50.1	-51.0	1.2
9	-53.9	-54.8	-53.9	-46.3	-50.9	-54.3	-52.3	3.3
10	-55.4	-55.2	-55.9	-54.0	-54.6	-56.0	-55.2	0.8
11a	-58.3	-62.6	-56.0	-59.1	-57.5	-56.9	-58.4	2.3
11b	-58.2	-59.9	-58.6	-59.9	-58.4	-58.6	-58.9	0.8
11c	-55.8	-53.9	-55.4	-53.6	-53.5	-50.6	-53.8	1.8
11d	-63.5	-63.7	-64.2	-61.8	-61.0	-60.9	-62.5	1.4
12	-56.2	-56.3	-55.8	-56.5	-56.1	-57.1	-56.3	0.5
Results not used for data evaluation								
1	-35.0	-34.8	-28.4	-31.5	-31.0	-30.9	-31.9	2.5
0b	-48.3	-49.2	-49.9	-49.5	-48.1	-47.7	-48.8	0.9
7	-62.7	-64.5	-64.5	-63.4	-63.4	-61.3	-63.3	1.2
8b	-51.8	-51.4	-53.8	-56.2	-53.8	-54.0	-53.5	1.7

[a)]Standard deviation of the mean aliquot results

n.a.= not available or not reported by participant

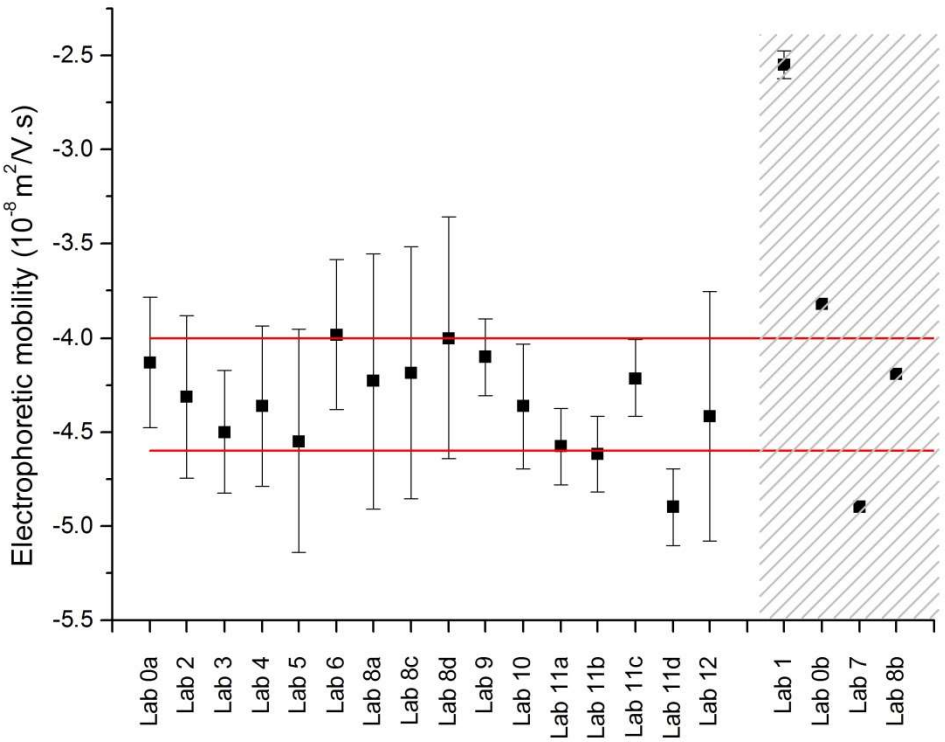

Fig.E1.1 Laboratory mean values (used for certification) of the electrophoretic mobility as obtained by 11 laboratories (16 datasets); error bars indicate the expanded *(k=2)* measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range. Technically invalid results are indicated in the hatched region.

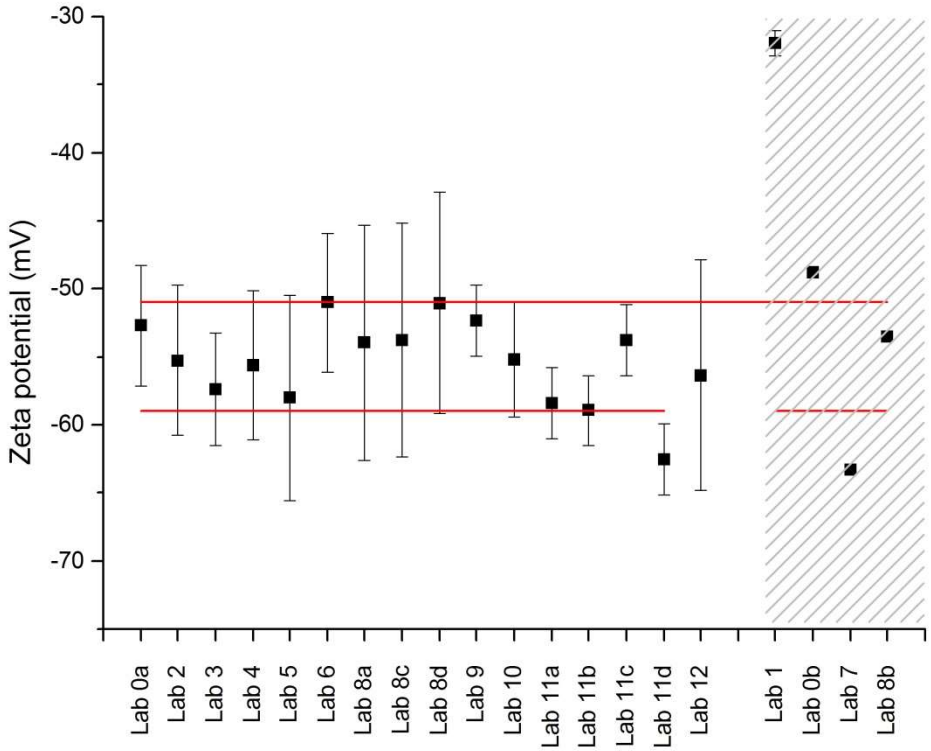

Fig.E1.2 Laboratory mean values of the zeta potential as reported by 11 laboratories (16 datasets); error bars indicate the expanded *(k*=2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range. Technically invalid results are indicated in the hatched region.

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

Lab Code	Mean of replicate means (10^{-8} m/V·s)	Mean calculated ζ (mV)	Mean reported ζ (mV)
0a	-4.13	-53.0	-52.7
2	-4.31	-55.3	-55.2
3	-4.50	-57.7	-57.4
4	-4.36	-55.9	-55.6
5	-4.55	-58.3	-58.0
6	-3.98	-51.1	-51.0
8a	-4.23	-54.2	-54.0
8c	-4.18	-53.7	-53.7
8 d	-4.00	-51.3	-51.0
9	-4.10	-52.6	-52.3
10	-4.36	-55.9	-55.2
11a	-4.58	-58.7	-58.4
11b	-4.62	-59.2	-58.9
11c	-4.21	-54.0	-53.8
11d	-4.90	-62.8	-62.5
12	-4.42	-56.6	-56.3
Results not used for evaluation			
1	-2.55	-32.7	-31.9
0b	-3.82	-49.0	-48.8
7	-4.90	-62.8	-63.3
8b	-4.19	-53.7	-53.5

Table E1.4 Electrophoretic mobility obtained by ELS on the x2 diluted ERM-FD306 sample

Lab Code	Replicate results (10^{-8} $m^2/V \cdot s$)						Mean (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)	s (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)
	1	2	3	4	5	6		
0a	-4.36	-4.02	-4.38	-4.32	-4.42	-4.38	-4.31	0.15
1	-4.91	-4.87	-4.79	-4.87	-4.91	-4.86	-4.87	0.04
2	-4.34	-4.42	-4.40	-4.34	-4.38	-4.40	-4.38	0.03
3	-4.47	-4.52	-4.53	-4.52	-4.49	-4.52	-4.51	0.02
4	-4.62	-4.63	-4.53	-4.44	-4.53	-4.54	-4.55	0.07
5	-4.71	-4.61	-4.49	-4.54	-4.68	-4.70	-4.62	0.09
6	4.12	4.07	4.12	4.06	4.12	3.78	-4.04	0.13
8a	-4.21	-4.39	-4.19	-4.33	-4.39	-4.40	-4.32	0.10
8c	-4.47	-4.53	-4.45	-4.09	-4.44	-4.47	-4.41	0.16
8 d	-4.13	-4.17	-4.20	-4.06	-3.86	-3.61	-4.01	0.23
9	-3.89	-4.22	-4.50	-3.77	-4.52	-4.58	-4.25	0.35
10	-4.65	-4.63	-4.60	-4.54	-4.56	-4.61	-4.60	0.04
11a	-4.57	-4.72	-4.61	-4.80	-4.51	-4.74	-4.66	0.11
11b	-4.59	-4.77	-4.54	-4.56	-4.74	-4.63	-4.64	0.10
11c	-4.39	-4.34	-4.32	-4.36	-4.04	-4.03	-4.25	0.17
11d	-5.10	-4.95	-4.99	-4.90	-4.47	-4.75	-4.86	0.22
12	-4.54	-4.61	-4.60	-4.59	-4.54	-4.55	-4.57	0.03
Results not used for data evaluation								
0b	-3.93	-4.05	-4.28	-4.12	-4.10	-4.12	-4.10	
7	-4.42	-4.76	-4.40	-4.79	-4.87	-4.76	-4.67	
8b	-4.31	-4.26	-4.15	-4.37	-4.34	-4.24	-4.28	

Table E1.5 Zeta potential obtained by ELS on the x2 diluted ERM-FD306 sample

Lab Code	Replicate mean results (mV)						Mean (mV)	s (mV)
	1	2	3	4	5	6		
0a	-55.6	-51.3	-55.8	-55.1	-56.5	-55.9	-55.0	1.9
1	-61.5	-61.0	-60.0	-61.0	-61.5	-60.9	-61.0	0.6
2	-55.7	-56.7	-56.4	-55.7	-56.2	-56.3	-56.2	0.4
3	-57.1	-57.7	-57.6	-57.9	-57.4	-58.0	-57.6	0.3
4	-58.9	-59.0	-57.8	-56.6	-57.7	-58.0	-58.0	0.9
5	-60.0	-58.9	-57.3	-57.9	-59.7	-59.9	-59.0	1.1
6	-52.7	-52.0	-52.7	-51.9	-52.7	-48.4	-51.8	1.7
8a	-53.7	-56.1	-53.4	-55.3	-56.0	-56.1	-55.1	1.2
8c	-57.0	-57.8	-56.8	-52.2	-56.7	-57.1	-56.3	2.0
8 d	-52.7	-53.3	-53.6	-51.8	-49.2	-46.0	-51.1	2.9
9	-49.6	-53.8	-57.5	-48.1	-57.6	-58.4	-54.2	4.4
10	-59.3	-59.1	-58.7	-57.9	-58.2	-58.8	-58.7	0.5
11a	-58.4	-60.3	-58.9	-61.2	-57.6	-60.5	-59.5	1.4
11b	-58.6	-60.9	-58.0	-58.3	-60.5	-59.1	-59.2	1.2
11c	-56.1	-55.5	-55.1	-55.6	-51.6	-51.4	-54.2	2.1
11d	-65.1	-63.2	-63.7	-62.6	-57.1	-60.7	-62.0	2.8
12	-57.9	-58.8	-58.7	-58.5	-57.9	-58.1	-58.3	0.4
Results not used for data evaluation								
0b	-50.1	-51.7	-54.6	-52.6	-52.3	-52.6	-52.3	1.5
7	-57.1	-61.5	-56.9	-61.8	-63.0	-61.5	-60.3	2.6
8b	-54.9	-54.3	-53.0	-55.8	-55.3	-54.2	-54.6	1.0

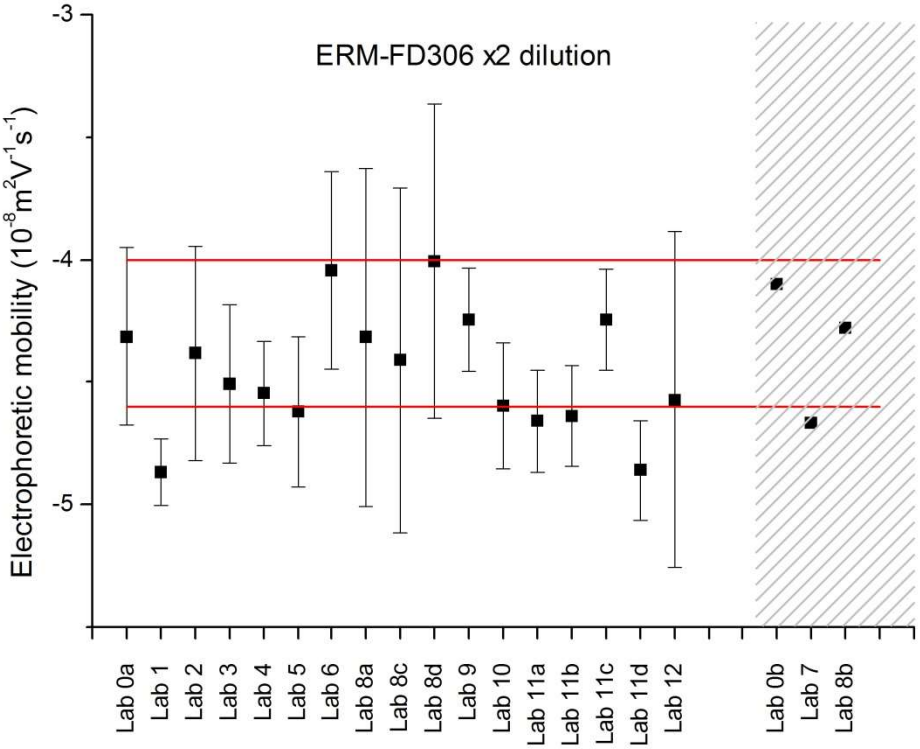

Fig.E1.3 Laboratory mean values of the electrophoretic mobility as reported by 12 laboratories (17 datasets); error bars indicate the expanded (*k*=2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range of the as received ERM-FD306. Technically invalid results are indicated in the hatched region.

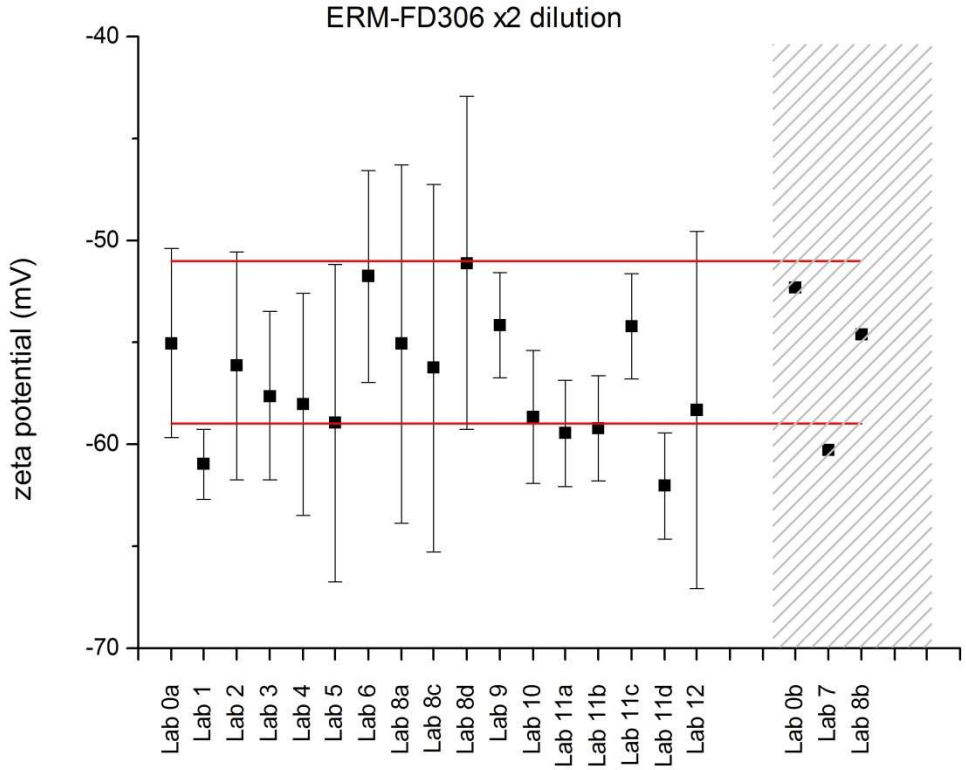

Fig.E1.4 Laboratory mean values of the zeta potential as reported by 12 laboratories (17 datasets); error bars indicate the expanded *(k*=2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range of the as received ERM-FD306. Technically invalid results are indicated in the hatched region.

Table E1.6 Electrophoretic mobility obtained by ELS on the x10 diluted ERM-FD306 sample

Lab Code	Replicate results (10^{-8} $m^2/V{\cdot}s$)						Mean (10^{-8} $m^2{\cdot}V^{-1}{\cdot}s^{-1}$)	s (10^{-8} $m^2{\cdot}V^{-1}{\cdot}s^{-1}$)
	1	2	3	4	5	6		
0a	-4.47	-4.50	-4.58	-4.52	-4.51	-4.50	-4.51	0.03
1	-4.95	-4.98	-4.89	-4.96	-5.00	-4.98	-4.96	0.04
2	-4.43	-4.48	-4.45	-4.46	-4.46	-4.47	-4.46	0.02
3	-4.53	-4.55	-4.51	-4.54	-4.52	-4.54	-4.53	0.01
4	-4.57	-4.55	-4.65	-4.58	-4.63	-4.68	-4.61	0.05
5	-4.76	-4.79	-4.65	-4.77	-4.67	-4.59	-4.70	0.08
6	4.08	4.10	4.11	4.10	4.08	3.98	-4.07	0.05
8a	-4.39	-4.46	-4.47	-4.23	-4.26	-4.45	-4.37	0.10
8c	-4.61	-4.63	-4.69	-4.11	-4.42	-4.41	-4.48	0.22
8 d	-4.26	-4.28	-4.30	-4.30	-4.26	-4.04	-4.24	0.10
9	-4.29	-4.57	-4.62	-4.44	-4.59	-4.62	-4.52	0.13
10	-4.38	-4.54	-4.56	-4.58	-4.59	-4.58	-4.54	0.08
11a	-4.72	-4.87	-4.79	-4.99	-4.83	-4.85	-4.84	0.09
11b	-4.77	-4.66	-4.63	-4.74	-4.74	-4.80	-4.72	0.07
11c	-4.46	-4.44	-4.45	-4.43	-4.22	-4.53	-4.42	0.11
11d	-5.10	-4.98	-4.85	-4.93	-4.65	-4.81	-4.89	0.16
12	-4.63	-4.64	-4.72	-4.69	-4.65	-4.64	-4.66	0.03
Results not used for data evaluation								
0b	-4.50	-4.51	-4.45	-4.46	-4.42	-4.35	-4.45	
7	-4.30	-4.41	-4.77	-4.65	-4.70	-4.47	-4.55	
8b	-4.08	-4.27	-4.38	-4.40	-4.40	-4.38	-4.32	

Table E1.7 Zeta potential obtained by ELS on the x10 diluted ERM-FD306 sample

Lab Code	Replicate results (mV)						Mean (mV)	s (mV)
	1	2	3	4	5	6		
0a	-57.1	-57.5	-58.4	-57.6	-57.5	-57.5	-57.6	0.4
1	-62.0	-62.4	-61.3	-62.1	-62.6	-62.4	-62.1	0.5
2	-56.7	-57.4	-57.0	-57.1	-57.1	-57.2	-57.1	0.2
3	-58.0	-58.2	-57.8	-57.8	-57.8	-58.0	-57.9	0.2
4	-58.3	-58.0	-59.3	-58.4	-59.1	-59.7	-58.8	0.7
5	-60.8	-61.0	-59.3	-60.9	-59.6	-58.5	-60.0	1.0
6	-52.2	-52.4	-52.7	-52.5	-52.2	-50.9	-52.2	0.6
8a	-55.9	-56.9	-57.0	-53.9	-54.4	-56.7	-55.8	1.3
8c	-58.8	-59.1	-59.9	-52.4	-56.4	-56.3	-57.1	2.8
8 d	-54.4	-54.6	-54.9	-54.9	-54.4	-51.5	-54.1	1.3
9	-54.8	-58.3	-58.9	-56.6	-58.6	-59.0	-57.7	1.7
10	-55.9	-57.9	-58.2	-58.4	-59.3	-58.4	-58.0	1.1
11a	-60.3	-62.2	-61.1	-63.7	-61.6	-61.9	-61.8	1.2
11b	-60.9	-59.4	-59.1	-60.5	-60.5	-61.2	-60.3	0.8
11c	-57.0	-56.6	-56.8	-56.6	-53.8	-57.8	-56.4	1.4
11d	-65.1	-63.6	-61.9	-62.9	-59.3	-61.5	-62.4	2.0
12	-59.0	-59.2	-60.2	-59.8	-59.3	-59.2	-58.6	0.4
Results not used for data evaluation								
0b	-57.4	-57.6	-56.8	-56.9	-56.3	-55.5	-56.8	
7	-55.6	-57.0	-61.6	-60.1	-60.7	-57.7	-58.8	
8b	-52.1	-54.4	-55.9	-56.1	-56.1	-55.8	-55.1	

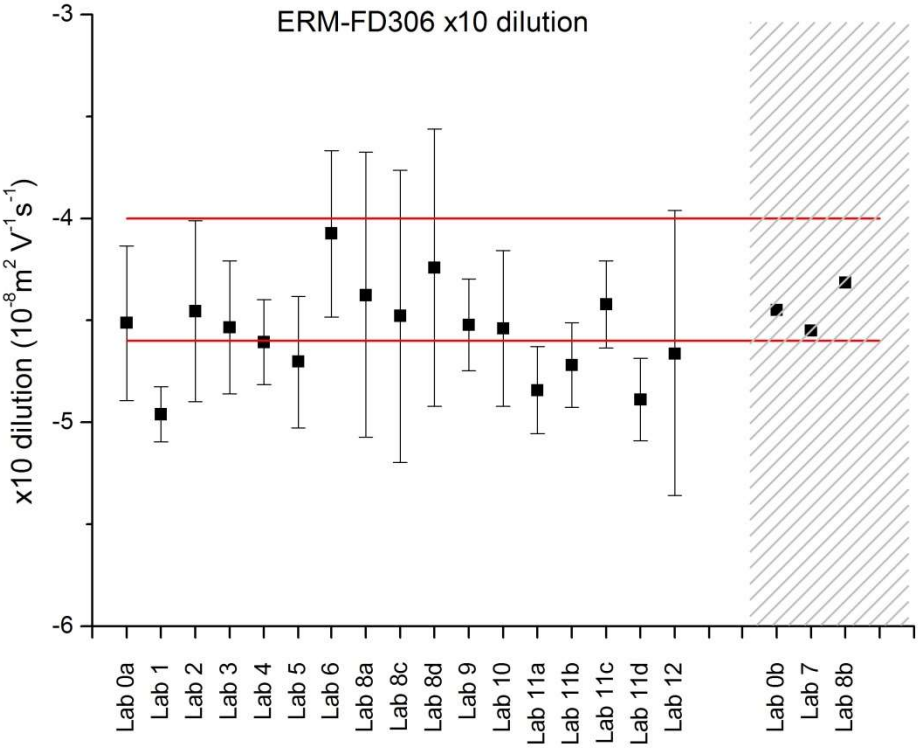

Fig.E1.5 Laboratory mean values of the electrophoretic mobility as reported by 12 laboratories (17 datasets); error bars indicate the expanded (*k*=2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range of the as received ERM-FD306. Technically invalid results are indicated in the hatched region

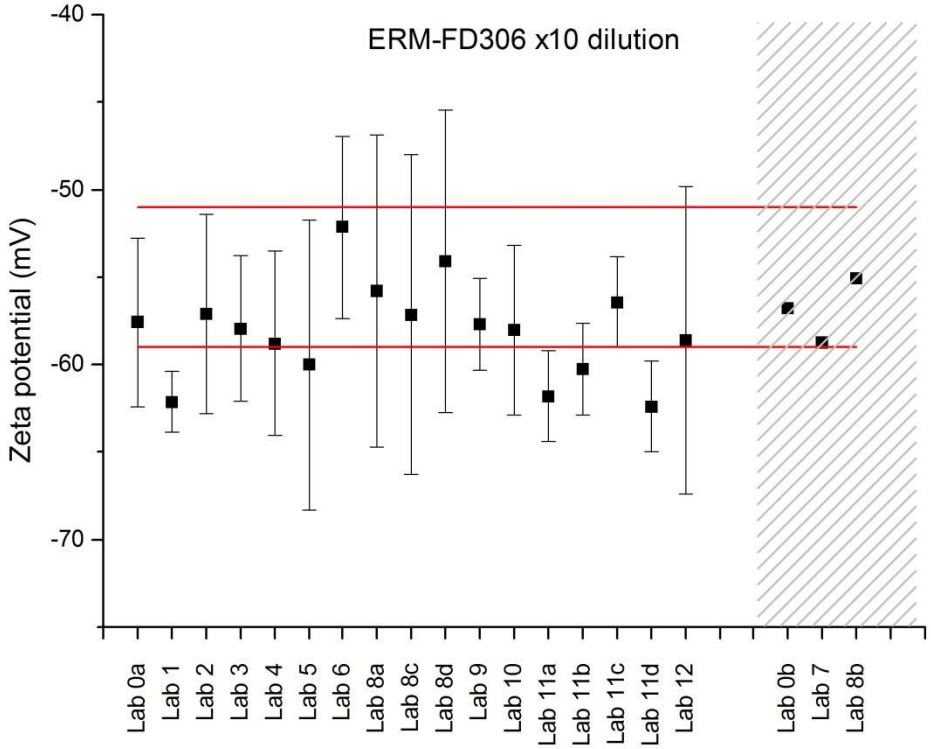

Fig.E1.6 Laboratory mean values of the zeta potential as reported by 12 laboratories (17 datasets); error bars indicate the expanded (*k*=2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range of the as received ERM-FD306. Technically invalid results are indicated in the hatched region.

Table E1.8 Electrophoretic mobility obtained by ELS on the x20 diluted ERM-FD306 sample

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 (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)
	1	2	3	4	5	6		
0a	-4.57	-4.53	-4.54	-4.65	-4.60	-4.47	-4.56	0.06
1	-5.00	-4.96	-4.87	-4.86	-4.94	-4.94	-4.93	0.05
2	-4.43	-4.39	-4.48	-4.53	-4.34	-4.40	-4.43	0.07
3	-4.47	-4.44	-4.46	-4.52	-4.46	-4.46	-4.47	0.03
4	-4.73	-4.60	-4.66	-4.58	-4.65	-4.77	-4.66	0.07
5	-4.76	-4.90	-4.86	-4.86	-4.61	-4.69	-4.78	0.11
6	4.14	4.05	4.10	4.10	4.14	4.13	-4.11	0.03
8a	-4.42	-4.38	-4.37	-4.50	-4.49	-4.47	-4.44	0.06
8c	-4.70	-4.74	-4.73	-4.70	-4.66	-3.95	-4.58	0.31
8 d	-4.21	-4.23	-4.38	-4.47	-4.10	-4.39	-4.30	0.14
9	-4.43	-4.03	-4.29	-4.61	-4.62	-4.53	-4.42	0.23
10	-4.60	-4.58	-4.23	-4.61	-4.39	-4.61	-4.51	0.16
11a	-4.65	-4.84	-4.95	-5.06	-4.85	-4.88	-4.87	0.14
11b	-4.73	-4.81	-4.71	-4.64	-4.78	-4.62	-4.71	0.08
11c	-4.55	-4.53	-4.53	-4.53	-4.11	-4.55	-4.47	0.18
11d	-4.90	-4.89	-5.09	-5.23	-4.83	-4.67	-4.93	0.20
12	-4.60	-4.66	-4.67	-4.71	-4.60	-4.70	-4.66	0.05
Results not used for data evaluation								
0b	-4.50	-4.46	-4.50	-4.50	-4.19	-4.35	-4.42	0.12
7	-3.65	-4.06	-4.58	-4.03	-4.65	-4.56	-4.25	0.40
8b	-4.34	-4.20	-4.49	-4.31	-4.51	-4.42	-4.38	0.12

Table E1.9 Zeta potential obtained by ELS on the x20 diluted ERM-FD306 sample

Lab Code	Replicate results (mV)						Mean (mV)	s (mV)
	1	2	3	4	5	6		
0a	-58.3	-57.8	-57.9	-59.3	-58.7	-57.1	-58.2	0.8
1	-62.7	-62.1	-61.0	-60.9	-61.9	-61.8	-61.7	0.7
2	-56.8	-56.2	-57.3	-58.1	-55.5	-56.4	-56.7	0.9
3	-57.2	-56.7	-57.7	-57.8	-57.0	-57.0	-57.2	0.4
4	-60.4	-58.7	-59.4	-58.4	-59.3	-60.9	-59.5	1.0
5	-60.8	-62.5	-62.0	-60.5	-58.8	-59.9	-60.7	1.4
6	-53.0	-51.9	-52.5	-52.5	-53.0	-52.9	-52.6	0.4
8a	-56.4	-55.8	-55.8	-57.4	-57.3	-57.0	-56.6	0.7
8c	-59.9	-60.4	-60.4	-59.9	-59.4	-50.4	-58.4	3.9
8 d	-53.7	-54.0	-55.9	-57.0	-52.3	-56.0	-54.8	1.8
9	-56.5	-51.4	-54.7	-58.8	-59.0	-57.8	-56.4	2.9
10	-58.7	-58.4	-54.0	-58.9	-58.6	-58.8	-57.9	1.9
11a	-59.4	-61.7	-63.2	-64.6	-61.9	-62.3	-62.2	1.7
11b	-60.4	-61.4	-60.2	-59.2	-61.0	-58.9	-60.2	1.0
11c	-58.1	-57.8	-57.8	-57.8	-52.4	-58.1	-57.0	2.2
11d	-62.5	-62.4	-65.0	-66.7	-61.6	-59.6	-63.0	2.5
12	-58.6	-59.5	-59.6	-60.1	-58.6	-59.9	-59.4	0.6
Results not used for data evaluation								
0b	-57.4	-56.9	-57.4	-57.4	-53.5	-55.5	-56.4	
7	-47.2	-52.4	-59.2	-52.1	-60.0	-58.9	-55.0	
8b	-55.4	-53.6	-57.3	-55.0	-57.5	-56.4	-55.9	

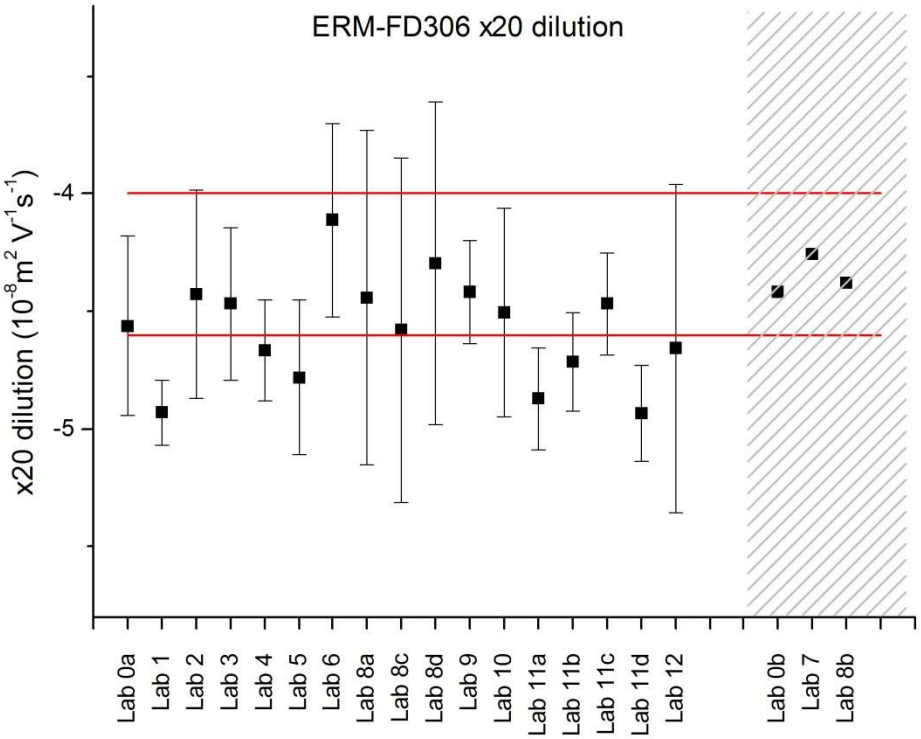

Fig.E1.7 Laboratory mean values (used for certification) of the electrophoretic mobility as obtained by 12 laboratories (17 datasets); error bars indicate the expanded *(k*=2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range of the as received ERM-FD306. Technically invalid results are indicated in the hatched region.

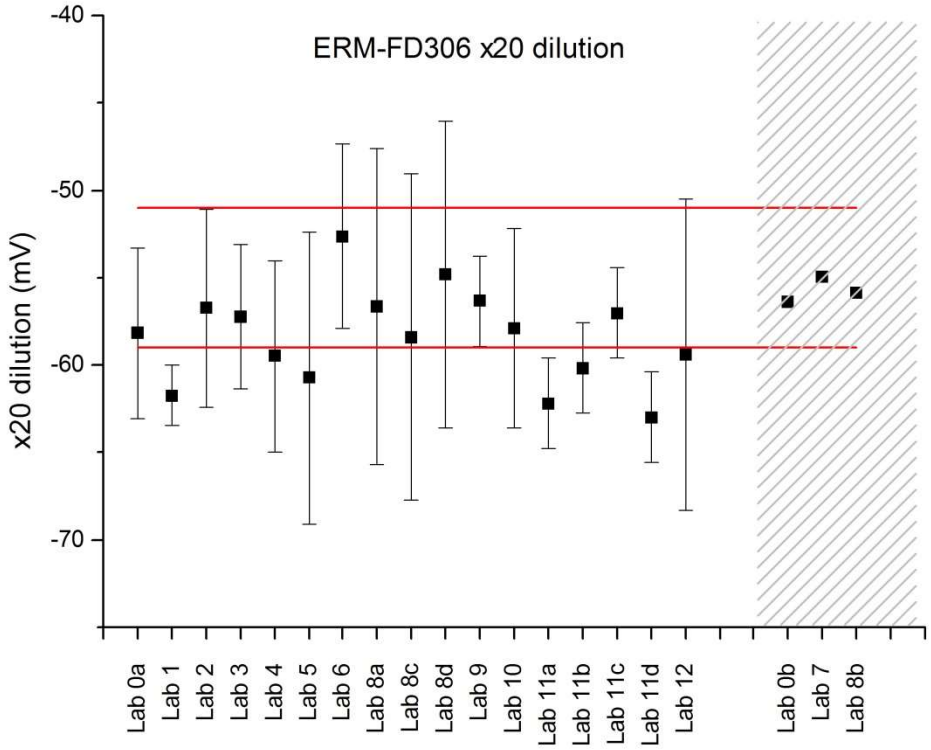

Fig.E1.8 Laboratory mean values of the zeta potential as reported by 12 laboratories (17 datasets); error bars indicate the expanded *(k*=2) measurement uncertainties as reported by the participants. The two horizontal lines reflect the certified range of the as received ERM-FD306. Technically invalid results are indicated in the hatched region.

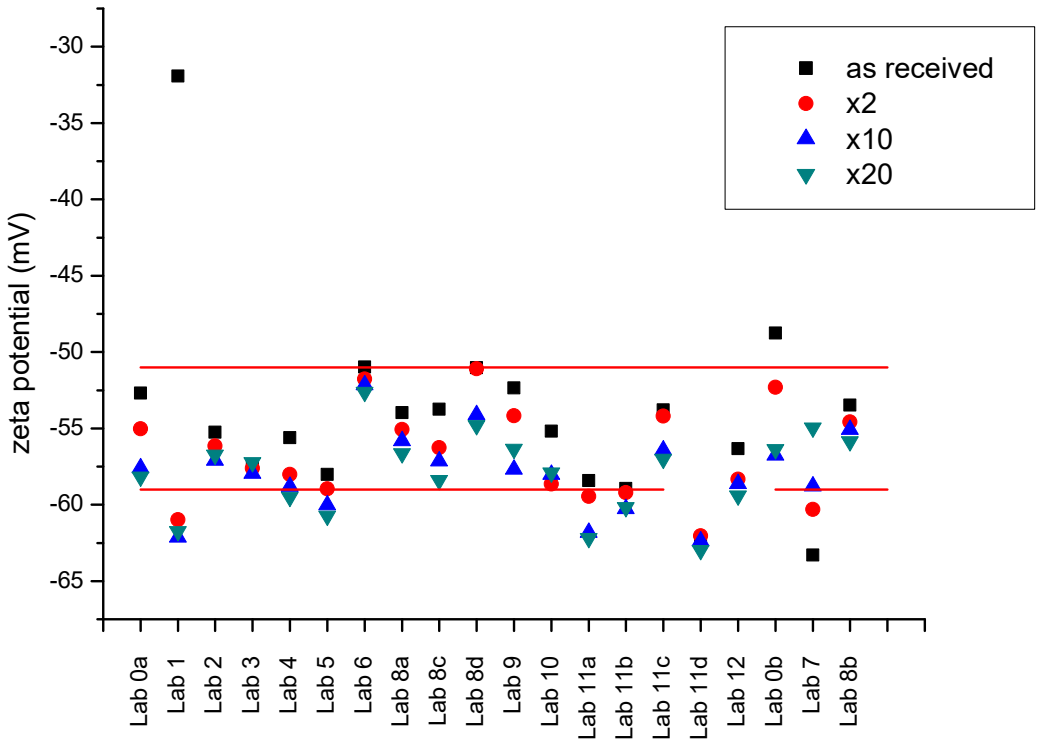

Fig.E1.9 Laboratory mean values of zeta potential in the dilution series as obtained by the laboratories.

Annex E2: Results of electroacoustic measurements

Table E2.1: Zeta potential obtained by electroacoustic measurements

Lab Code	Replicate results (mV)									Mean (mV)	s (mV)
	1	2	3	4	5	6	7	8	9		
Lab 10	-49.3	-49.0	-52.5	-52.7	-50.3	-48.3	n.a.	n.a.	n.a.	-50.4	1.8
Lab 13 a	-48.7	-48.8	-47.4	-47.3	-47.6	-47.6	n.a.	n.a.	n.a.	-47.9	0.7
Lab 13 b	-51.8	-52.2	-49.7	-49.8	-50.2	-49.6	n.a.	n.a.	n.a.	-50.6	1.2
Lab 16	-50.1	-50.0	-49.5	-49.7	-49.6	-49.7	n.a.	n.a.	n.a.	-49.8	0.2
Lab 18	-60.0	-60.2	-60.2	-53.5	-62.4	-62.7	-64.5	-53.4	-54.2	-59.8	3.3
lab 19	-71.3	-72.4	-68.2	-67.7	-68.4	-70.0	n.a.	n.a.	n.a.	-69.7	1.9

n.a.= not available or not reported by participant

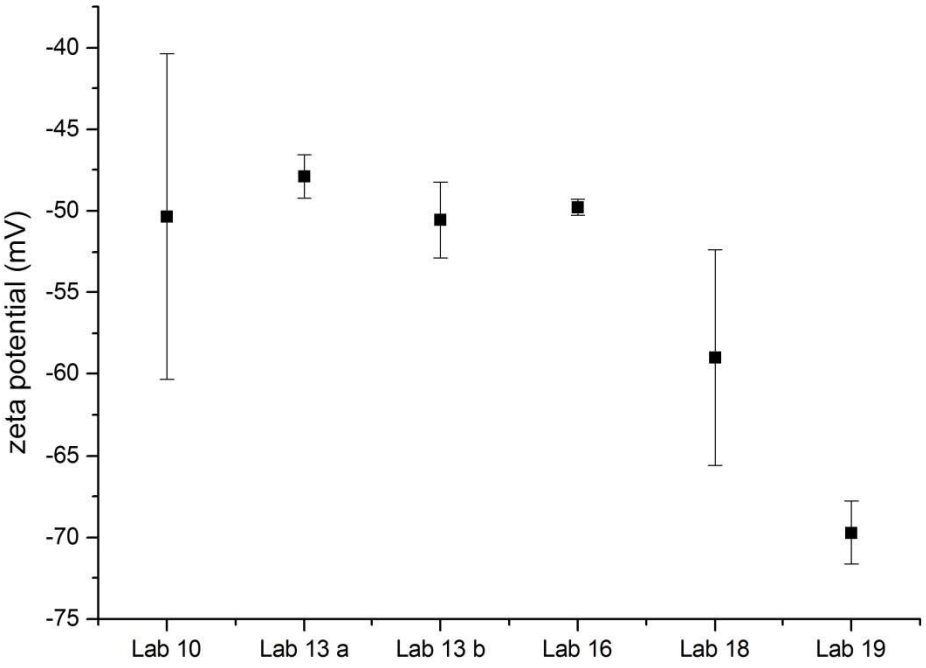

Fig.E2.1 Laboratory mean values (used for additional information) of the zeta potential as obtained by 5 laboratories (6 datasets); error bars indicate the expanded *(k*=2) measurement uncertainties as reported by the participants.

Table E2.2: Dynamic electrophoretic mobility obtained by electroacoustic measurements

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 (10^{-8} $m^2 \cdot V^{-1} \cdot s^{-1}$)
	1	2	3	4	5	6		
Lab 10	-4.90	-4.90	-4.95	-4.93	-4.91	-4.87	-4.91	0.03
Lab 13 a	-5.46	-5.46	-5.39	-5.38	-5.43	-5.43	-5.43	0.03
Lab 13 b	-5.80	-5.84	-5.71	-5.71	-5.79	-5.73	-5.76	0.05
Lab 16	-5.68	-5.65	-5.55	-5.59	-5.54	-5.57	-5.60	0.06
Lab 18	n.a							
Lab 19	-5.56	-5.64	-5.31	-5.27	-5.33	-5.46	-5.43	0.15

n.a.= not available or not reported by participant

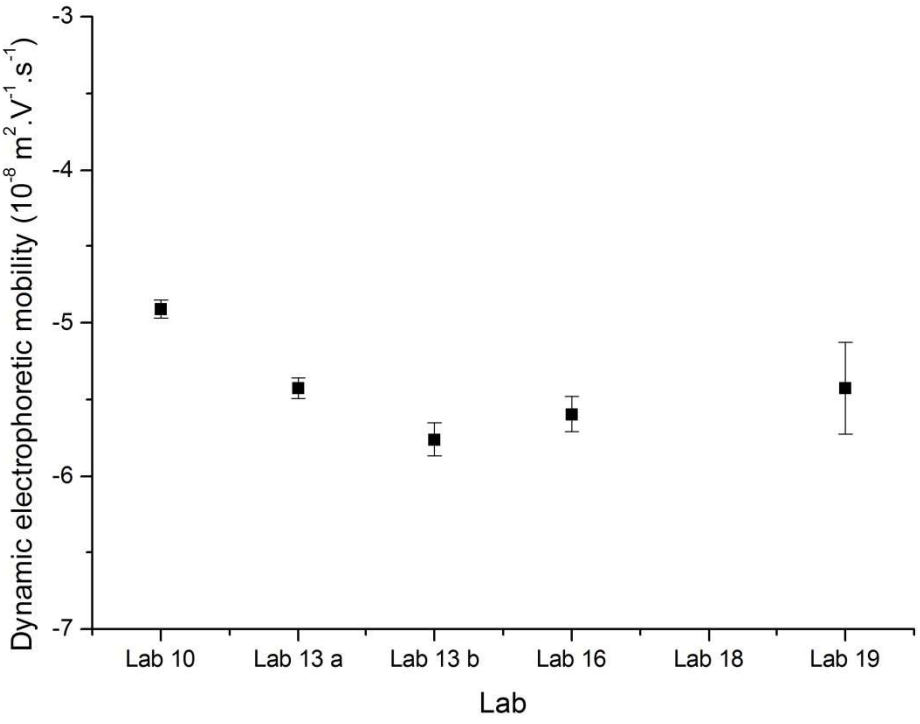

Fig.E2.2 Laboratory mean values (used for additional information) of the dynamic electrophoretic mobility as obtained by 4 laboratories (5 datasets); error bars indicate the expanded *(k* = 2) measurement uncertainties as reported by the participants.

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}.

European Commission

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Title: **The certification of electrophoretic mobility/zeta potential of silica particles in aqueous solution: ERM®-FD306/SRM 1993**

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