Supplementary Materials

Establishing SI-Traceability of Nanoparticle Size Values Measured with Line-Start Incremental Centrifugal Liquid Sedimentation

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1. **Sedimentation in a linear gradient of density and viscosity**

The following equation for velocity is assumed at any point in the gradient,

$$\frac{dR}{dt} = \frac{\omega^2(\rho_\mathrm{p} - \rho_\mathrm{R})d_\mathrm{St,p}^2 R}{18\eta_\mathrm{R}} \tag{S1}$$

where ω is the angular frequency of the disc, ρ_p is the effective particle density, ρ_R and η_R are the density and viscosity of the fluid at position R. This requires integration between S and M in R. If ρ_R and η_R are constant, then Equation (1) in the main paper is the result. If a linear gradient in viscosity and density exists, then the equivalent result is given in Equation (S2),

$$d_\mathrm{St,p} = \sqrt{\frac{18}{\omega^2 t_\mathrm{p}}\left(\frac{\eta_0}{\rho_\mathrm{p} - \rho_0}\ln\left(\frac{M}{S}\right) + \left(\frac{\eta_0}{\rho_\mathrm{p} - \rho_0} + \frac{\eta'}{\rho'}\right)\ln\left(\frac{\rho_\mathrm{p} - \rho_\mathrm{S}}{\rho_\mathrm{p} - \rho_\mathrm{M}}\right)\right)} \tag{S2}$$

$$\rho' = \frac{\rho_M - \rho_S}{R_M - R_S} \tag{S3}$$

$$\eta' = \frac{\eta_M - \eta_S}{R_M - R_S} \tag{S4}$$

where $d_\mathrm{St,p}$ is the Stokes diameter of a particle detected after a transit time t_p, M and S are, respectively, the ending (detection) and starting (inner liquid surface) radii of rotation, ρ' and η' are, respectively, the fluid density and viscosity terms, ρ_S and ρ_M are, respectively, the fluid density of the gradient at the radial positions M and S, and ρ_0 and η_0 are the extrapolated density and viscosity values at the center of rotation. Note that this extrapolation can result in negative values for ρ_0 and almost always results in a negative value for η_0. These extrapolated values are unphysical, but are used for mathematical convenience. Because the integration is performed over a range in R where the density and viscosity values have physical meaning, the final result is valid. Despite the very different form of Equation S2 to that of Equation (1) in the main paper, the values returned are closely identical, provided that the particles are significantly more dense than the fluid density at M and that the radius-averaged mean values of density and viscosity are employed.

2. **Alternative traceability network**

To establish SI-traceability of particle size results obtained with the calibrated disc-CLS method, one must demonstrate that both the assigned size and density values of the calibration particles are traceable to the relevant SI units. Figure S1 shows an elaborated traceability network which includes the original traceability network (Figure 1 in the main paper) complemented with the traceability

networks of the applied transmission electron microscopy (TEM) method and the isopycnic velocity interpolation sedimentation method used for measuring the particle size and the effective particle density, respectively [1].

Although, TEM is a widely used and recognized technique for size measurements of nanoparticles [2], one can justifiably question whether the use of TEM in the given measurement scheme compromises the integrity of the traceability network. Indeed, disc-CLS and electron microscopy based methods apply distinctly different measurement principles. The disc-CLS method measures light-extinction weighted Stokes diameters while particle size results from electron microscopy are number-weighted 2D projection-based equivalent diameters. These are examples of particle size as method-defined measurand [3]. While the between-method variability for monodisperse hard spheres may be acceptable for some applications, significant differences are expected for industrially-relevant materials. Also, PVC particles that are often used as calibrants in disc-CLS measurements are rather soft and it has been reported that their size can be altered upon exposure to the electron beam and high vacuum, as applied in electron microscopes [4]. Considering the method-defined nature of particle size measurands, and the evidence that PVC particles can be subject to morphological changes when exposed to electron microscopy conditions, one can argue that the use of TEM for establishing SI-traceability of particle size results from disc-CLS is, from a metrological point of view, is not the preferred approach.

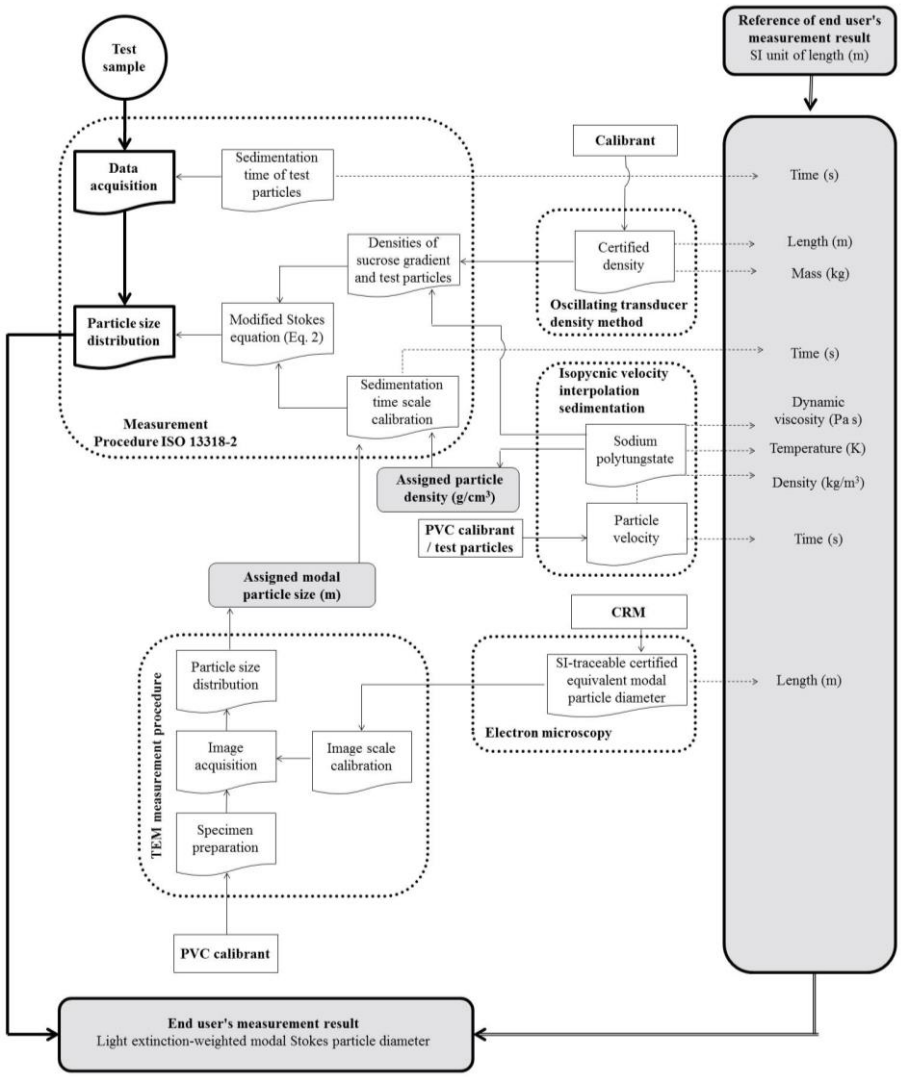

Figure S1. Metrological traceability network attempting to link disc-CLS particle size measurement results to the SI unit of length through PVC calibration particles characterized by transmission electron microscopy and isopycnic velocity interpolation sedimentation.

3. **Density and viscosity of sucrose solutions**

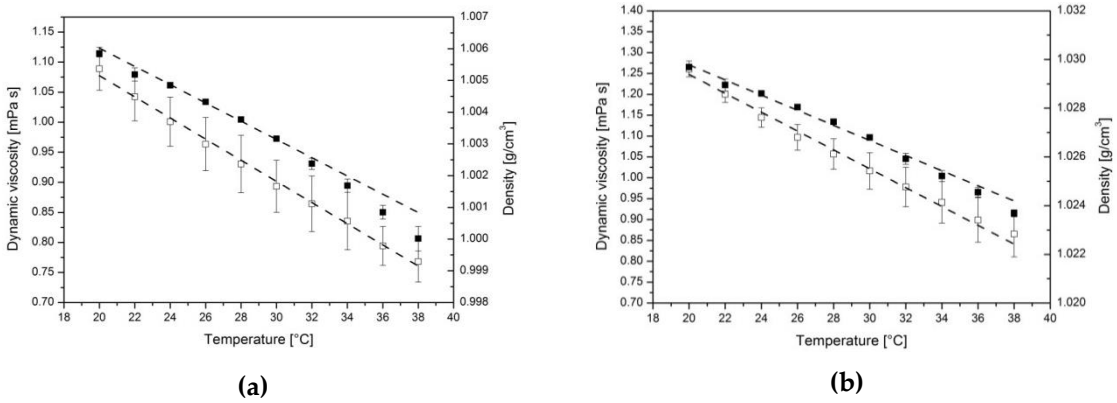

Figure S2. Temperature dependence of dynamic viscosity (open squares) and density (solid squares) of 20 g/kg (a) and 80 g/kg (b) sucrose solutions. The error bars correspond to the standard deviation, dashed lines represent linear fits.

4. **Particle size analysis by disc-CLS**

Disc-CLS measurements were performed using a disc centrifuge DC20000 model (CPS Instruments, Inc., Prairieville, USA). After stabilization, the carrier fluid prepared from sucrose solutions with mass fractions of (20.0 ± 0.1) g/kg and (80.0 ± 0.1) g/kg, comprised a radial density and viscosity profile with an inner liquid surface at a distance S_0 from the center of the disc (Figure S3). With every sample injected, a ring of water-based dispersant (light blue ring) is formed on top of the density/viscosity gradient (dark blue ring), causing the inner liquid surface to gradually shift toward the center of the disc and thereby reducing the distance to the center (e.g. S_1 and S_2). For each new position of the inner liquid surface, the average density of the carrier fluid is estimated via linear extrapolation (Equation S5),

$$\langle \rho_{(M-S_1)} \rangle = \langle \rho_{(M-S_0)} \rangle \frac{(M - S_0)}{(M - S_1)} + \rho_{(\text{water})} \frac{(S_0 - S_1)}{(M - S_1)} \quad \text{(S5)}$$

where, $\langle \rho_{(M\text{-}S1)} \rangle$ is the average density of the carrier fluid (sucrose density gradient + accumulated water layer) from the detector's position M to the inner liquid surface S_1, $\langle \rho_{(M\text{-}S0)} \rangle$ is the average density of the carrier fluid (sucrose density gradient) from the detector's position M to the inner liquid surface of the gradient S_0, $\langle \rho_{(\text{water})} \rangle$ is the density of water (dispersant of ERM-FD102 and ERM-FD304) and was taken from ISO/TR 3666:1998 [5].

The average viscosity of the carrier fluid at each new position of the inner liquid surface was assessed using a similar linear extrapolation approach.

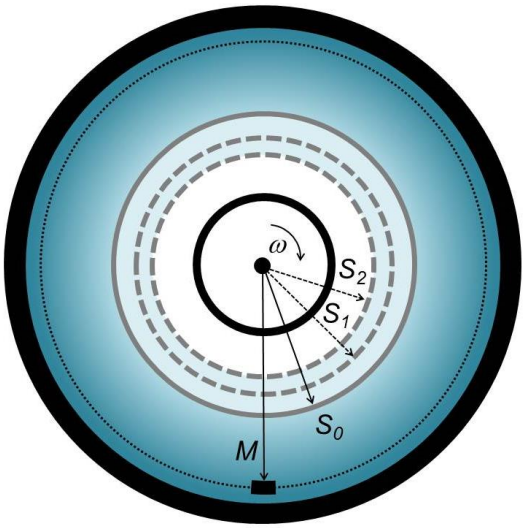

Figure S3. Simplified schematic of the functioning hollow disc (front view) of a disc-CLS instrument. The disc is partly filled with a carrier fluid which comprises a radial gradient in density and viscosity after stabilization. The dilute sample is injected at the center of the disc (solid black circle). Particles travel radially outward through the density/viscosity gradient (dark blue ring) and sedimentation times are recorded by the photodetector (solid black square) whose radial position is given by the dotted black circle. A ring of water-based dispersant liquid (light blue ring) is formed on top of the density/viscosity gradient.

Table S1. Overview of key parameters (all replicates) for the direct calculation of Stokes diameter results using the reference disc-CLS method.

CRM	$d_{st,p}$ [1] [nm]	$\langle\eta\rangle$ [2] [Pa s]	$\langle\rho_f\rangle$ [2] [g/cm³]	ρ_p [3] [g/cm³]	M [cm]	S [cm]	ω [rad/s]	t_p [s]
ERM-FD102								
Population 1								
Replicate 1	22.5	0.0091	1.0070	2.0	4.25	3.87	2094	688
Replicate 2	22.6	0.0090	1.0066	2.0	4.25	3.86	2094	713
Replicate 3	23.0	0.0090	1.0061	2.0	4.25	3.84	2094	711
Replicate 4	23.4	0.0090	1.0057	2.0	4.25	3.82	2094	713
Replicate 5	23.3	0.0089	1.0054	2.0	4.25	3.81	2094	745
Replicate 6	23.5	0.0089	1.0050	2.0	4.25	3.79	2094	755
Population 2								
Replicate 1	83.7	0.0091	1.0070	2.0	4.25	3.87	2094	50
Replicate 2	83.3	0.0090	1.0066	2.0	4.25	3.86	2094	52
Replicate 3	84.0	0.0090	1.0061	2.0	4.25	3.84	2094	53
Replicate 4	84.5	0.0090	1.0057	2.0	4.25	3.82	2094	55
Replicate 5	84.7	0.0089	1.0054	2.0	4.25	3.81	2094	56
Replicate 6	84.7	0.0089	1.0050	2.0	4.25	3.79	2094	58
ERM-FD304								
Replicate 1	31.0	0.0095	1.0079	2.3	4.25	3.88	2094	285
Replicate 2	30.8	0.0094	1.0074	2.3	4.25	3.86	2094	300
Replicate 3	31.1	0.0094	1.0069	2.3	4.25	3.85	2094	305
Replicate 4	31.2	0.0093	1.0064	2.3	4.25	3.83	2094	314
Replicate 5	31.6	0.0093	1.0060	2.3	4.25	3.81	2094	317
Replicate 6	30.9	0.0092	1.0057	2.3	4.25	3.80	2094	343

[1] Light extinction-weighted modal Stokes diameter. [2] At a temperature of 30.0 °C (ERM-FD102) and 27.8 °C (ERM-FD304). [3] As stated on the CRM certificates.

5. **Uncertainty budget ERM-FD304**

Table S2. Uncertainty budget for a single disc-CLS measurement (reference method) of ERM-FD304.

Quantity, x_i [unit]	**Quantity value**	**Standard uncertainty, $u(x_i)$**	**Distribution type** [1]	**Contribution $\partial f/\partial x_i \cdot u(x_i)$ [nm]**
Angular frequency, ω [rad/s]	2094	9	N	-0.12
Average viscosity of the carrier fluid between M and S, η [Pa s]	0.0095	0.0004	N & R	0.71
Average density of the carrier fluid between M and S, ρ_f [g/cm^3]	1.0079	0.0001	N & R	< -0.01
Radial position photodetector, M [cm]	4.25	0.05	N	0.52
Radial position of inner liquid surface, S [cm]	3.88	0.14	T	-1.40
Density silica, ρ_p [g/cm^3]	2.3	0.058	N	-0.7
Sedimentation time silica, t_p [s]	285	3.3	T	-0.18
Stokes diameter, $d_{St,p}$ [nm]	31.0	Combined measurement uncertainty, $u_c(d_{St,p})$ [nm]		1.8
		Expanded (k = 2) measurement uncertainty, U [nm]		3.6

[1] N, normal; R, rectangular; T, triangular.

Abbreviations and symbols

CRM	**certified reference material**
$d_{St,p}$	Stokes diameter of test particles
Disc-CLS	disc centrifugal liquid sedimentation
ISO	International Organization for Standardization
k	coverage factor
M	radial position of the detector
PVC	polyvinyl chloride
R	random position in sucrose gradient
R	rectangular distribution
S	radius of the inner liquid surface; an additional index "0, 1 or 2" is added to indicate the change due to injected samples
SI	International System of Units
t_p	sedimentation time of the test particles
TEM	transmission electron microscopy
u	standard uncertainty (confidence level 68 %)
u_c	combined uncertainty (confidence level 68 %)
U	expanded uncertainty (confidence level 95 %)
x_i	quantity
η'	fluid viscosity term
η	average dynamic viscosity between M and S
η_0	extrapolated viscosity of the fluid at the center of rotation
η_R	viscosity of the fluid at position R
ρ'	fluid density term

ρ_0	extrapolated density of the fluid at the center of rotation
ρ_f	average density of the carrier fluid between *M* and *S*
ρ_M	density of the fluid at position M
ρ_S	density of the fluid at position S
ρ_P	effective particle density
ρ_R	density of the fluid at position *R*
ω	angular frequency of the disc

Reference

1 Lerche, D.; Sobisch, T. Consolidation of concentrated dispersions of nano- and microparticles determined by analytical centrifugation. *Powder Technol.* **2007**, *174*, 46–49.

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4 McDonald, S.A.; Daniels, C.A.; Davidson, J.A. Determination of the true size of poly(vinyl chloride) latices by electron microscopy using a vertical shadowing technique. *J. Colloid Interface Sci.* **1977**, *59*, 342–349.

5 International Organization for Standardization (ISO). *Viscosity of Water*; ISO/TR 3666; ISO, Geneva, Switzerland, 1998.