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Determination of particle size distribution by centrifugal liquid sedimentation methods —

Part 1:
General principles, requirements and guidance

Détermination de la distribution granulométrique par les méthodes de sédimentation centrifuge dans un liquide —

Partie 1: Principes généraux, exigences et orientation

Reference number
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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

This second edition cancels and replaces the first edition (ISO 13318-1:2001), which has been technically revised.

The main changes are as follows:

- revision of core terms (3.3, 3.5, 3.10, 3.11, 3.16, 3.14) and inclusion of further terms;
- revised and expanded explanation of measurement principle and techniques (Clauses 5 and 6);
- inclusion of the terminal sedimentation velocity as a measurand;
- introduction of informative annexes that provide:
  - an overview of particle sizing techniques based on centrifugal liquid sedimentation (Annex A);
  - remarks on particle density (Annex B);
  - information regarding sedimentation beyond the validity of Stokes' law (Annex C);
  - trueness, reproducibility and uncertainty determination for velocity and particle size (Annex D);
  - an explanation on the multiwavelength approach (Annex E);
  - a description of the spatial distribution of particles in centrifugal fields (Annex F);
  - the use of CLS for particle characterization beyond the particle size and sedimentation velocity (Annex G).

A list of all parts in the ISO 13318 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Particle size analysis by centrifugal liquid sedimentation (CLS) methods has been performed for several decades, and there are numerous national and international standards employed in various academic and industrial applications. Along with the development of manifold new measurement techniques during the last two decades, sedimentation methods are currently enjoying a renaissance due to technical progress (e.g. multiwavelength features) and the fact that most sedimentation techniques are based on the first-principle measurement of the directed motion (migration) of particles in gravitational or centrifugal fields. The rate of this motion is the particle sedimentation velocity, its acceleration-specific value is called "sedimentation coefficient". Both are individual particle properties, which are related to the particles' external dimensions, and can be considered as primary measurands of liquid sedimentation methods.

Analytical centrifuges determine distributions of these primary measurands from the variation of concentration-dependent signals over time and/or along the radial coordinate of the centrifugal field. This step does not require any pre-knowledge of the dispersed or liquid phase. Further, data processing allows for particle size distributions to be derived from sedimentation velocity and sedimentation coefficient. For this purpose, spherical particles and the applicability of Stokes' law are assumed; the equivalent particle diameter is called the Stokes diameter. The conversion requires knowledge of the relevant properties of the particles and liquids (e.g. particle shape, density or refractive index). In this regard, CLS resembles gravitational liquid sedimentation (see ISO 13317-1 for further information).

The ISO 13318 series covers methods for characterizing dispersed materials in liquids by centrifugation with respect to the particle size distribution and the related distributions of sedimentation velocity and sedimentation coefficient. Their common principle is allocating a particle quantity to the rate of migratory motion in the centrifugal field. They differ with respect to particle quantification, mode of operation and data analysis.

The measurement techniques described in the ISO 13318 series are applicable to liquid dispersions, like suspensions and emulsions, with the continuous phase being a Newtonian liquid. Particles and liquid should not undergo any interactions, which cause significant changes of the dispersed phase in the course of the measurement, such as swelling, shrinking, and dissolution. For some instrumentation, the density difference (also called density contrast) between the dispersed and continuous phase should be limited to positive values.

The measurable particle size range depends on the properties of the materials and typically reaches from a few nm to 100 µm for aqueous samples, whereas the migration velocity can be quantified for the range of 10 nm/s to 1 mm/s. Sedimentation analysis is conducted for low particle concentrations. The permissible range of concentration depends on the measurement technique and theory of analysis; with no correction, it is typically no higher than 0,5 vol%.

As a fractionating technique, sedimentation analysis allows for distinguishing between particle fractions of close sedimentation velocity and the corresponding equivalent Stokes diameter. Accordingly, particle size distributions can be finely resolved, which is an advantage compared to spectroscopic ensemble techniques.

Finally, CLS techniques principally offer the chance to characterize liquid dispersions beyond sedimentational particle properties (see Annex G). For instance, some CLS techniques facilitate the quantification of the total and fractional particle concentration. Moreover, if particles are very fine, i.e. in the case of nanoparticles such as protein aggregates or quantum dots, the diffusional flux of particles can be in the order of the sedimentation flux. Such a situation allows a multidimensional characterization of particle systems, i.e. the simultaneous determination of more than one distributed quantity (e.g. particle size and density or shape factor).

Determination of particle size distribution by centrifugal liquid sedimentation methods —

Part 1:
General principles, requirements and guidance

1 Scope

This document specifies the principles of particle size analysis by centrifugal liquid sedimentation (CLS). It also:

— defines the relevant terms;

— describes the various measurement techniques;

— gives guidance for sample preparation, conducting measurements as well as data analysis;

— establishes rules for method validation, determination of the uncertainty budget as well as representation of results.

An important part of this document deals with the derivation of particle size distributions from CLS data, including discussions on:

— the impact of Brownian motion;

— the parallel determination of particle concentrations;

— the working range with respect to size and concentration;

—the handling of non-spherical and porous particles.

This document applies to samples composed of dispersions of low particle concentration, so that the particles' motion can be considered as that of fully isolated particles. This document does not cover particle migration by gravity, electric or magnetic forces. It also does not cover deriving particle properties other than size, sedimentation velocity and sedimentation coefficient.

NOTE This document can involve hazardous materials, operations and equipment. It does not purport to address all the safety problems associated with its use. Regulations regarding explosion-proof analysers can apply when examining volatile liquids with a low flash point. It is the responsibility of the user of this document to establish appropriate safety and health practices and to determine the applicability of the regulatory limitations prior to its use.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 9276-1, *Representation of results of particle size analysis — Part 1: Graphical representation*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

3.1
sedimentation
directional motion of particles (3.7) in a viscous liquid under the action of gravity or centrifugal fields

Note 1 to entry: For a positive *density contrast* (3.18), sedimentation occurs in the direction of centrifugal acceleration; it is counter directed to this acceleration for a negative density contrast.

Note 2 to entry: An outward motion due to centrifugal force is also called "settling" or "falling".

Note 3 to entry: An inward motion due to centrifugal force is also called "creaming" (e.g. droplets) or, more generally, "rising" and "floating".

[SOURCE: ISO 13317-1:2024, 3.1, modified — notes are adapted for centrifugal liquid sedimentation]

3.2
migration
directional motion of particles in a viscous liquid under the action of a force field

Note 1 to entry: Migration in gravitational or centrifugal fields is called *sedimentation* (3.1).

[SOURCE: ISO 13317-1:2024, 3.2]

3.3
terminal sedimentation velocity
sedimentation velocity in the case that gravity or the centrifugal force is completely balanced by buoyancy and the drag force

[SOURCE: ISO 13317-1:2024, 3.3]

3.4
sedimentation coefficient
velocity of sedimentation divided by the acceleration of the force field (gravitation or centrifugation)

[SOURCE: IUPAC Gold book[1]]

3.5
Stokes diameter
equivalent diameter of a sphere that has the same *buoyant density* (3.17) and *terminal sedimentation velocity* (3.3) as the real particle in the same liquid under *creeping flow* (3.20) conditions

Note 1 to entry: The general rule that the *buoyant density* is used for calculating the Stokes diameter also applies to coated particles or multiconstituent particles (such as droplets in multiple emulsions). The *buoyant density* can be approximated with the *skeleton density* (3.15) for monoconstituent particles.

Note 2 to entry: For porous particles, it is common use to compute particle size based on the *apparent particle density* (3.16). This approach considers the stagnant liquid in the *open pores* (3.10) as intrinsic constituent of the dispersed phase. Thus, the obtained size values are hydrodynamic equivalent diameters.

Note 3 to entry: For close-packed agglomerates or aggregates, the *buoyant density* can be replaced by the *apparent particle density*, with "particle" referring to the agglomerate or aggregate, in order to get the hydrodynamic equivalent diameter.

[SOURCE: ISO 13317-1:2024, 3.4]

3.6

shape correction factor

ratio of the sedimentation velocity of a non-spherical particle to the one of a spherical particle of the same volume and *apparent particle density* (3.16)

[SOURCE: ISO 13317-1:2024, 3.5]

3.7

hindrance function

ratio of the *terminal sedimentation velocity* (3.3) of a particle placed in well-mixed dispersion to its sedimentation velocity in an infinite vessel in the absence of other particles

[SOURCE: ISO 13317-1:2024, 3.6, modified "divided by" changed to "to" and "for" changed to "in".]

3.8

particle

minute piece of matter with defined physical boundaries

[SOURCE: ISO 26824:2022, 3.1.1, modified — Notes 1, 2 and 3 to entry have been deleted.]

3.9

agglomerate

collection of weakly or medium-strongly bound *particles* (3.8) where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces such as, for example, van der Waals forces or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO 26824:2022, 3.1.2]

3.10

open pore

pore that is not totally enclosed by its walls and is open to the particle surface, either directly or by interconnecting with other pores, and is therefore accessible to liquid

[SOURCE: ISO 15901-1:2016, 3.11, modified — the word "fluid" is replaced by "liquid" and "surface" is changed to "particle surface".]

3.11

closed pore

pore that is totally enclosed by its walls and, hence not interconnecting with other pores and not accessible by liquids

[SOURCE: ISO 15901-1:2016, 3.10, modified— the word "fluids" is replaced by "by liquids".]

3.12

dynamic viscosity

measure of flow resistance for Newtonian liquids, calculated as the ratio of the shear stress to the rate of shear for laminar flow exposed to a pre-set shear stress or strain

[SOURCE: ISO 13317-1:2024, 3.11]

3.13

apparent viscosity

measure of flow resistance for non-Newtonian liquids at a defined shear stress or strain, calculated as the ratio of the shear stress to the shear rate

[SOURCE: ISO 13317-1:2024, 3.12]

3.14
true density of the dispersed phase

ratio of mass to volume for a body solely consisting of the dispersed phase without pores, voids, inclusions or surface fissures

[SOURCE: ISO 13317-1:2024, 3.13]

3.15
skeleton density

ratio of the sample mass to the volume of the sample, including the volume of *closed pores* (3.11) (if present) but excluding the volume of *open pores* (3.10)

Note 1 to entry: The skeleton density refers to solid particles and is determined for samples of dry powder.

[SOURCE: ISO 12154:2014, 3.3, modified — reference to the volume of void spaces between particles within the bulk sample is removed and Note 1 is added]

3.16
apparent particle density

effective particle density

ratio of mass to volume for a particle including particulate inclusions, entrapped stagnant liquid and gas in pores, voids and surface fissures as well as surface layers and coatings

Note 1 to entry: The apparent particle density is the density of a migrating entity and is calculated as the weighted average of its constituents.

Note 2 to entry: The apparent particle density depends on the wettability of *open pores* (3.10) and the kinetics of wetting or the replacement of pore liquid. Therefore, it is affected by sample preparation.

Note 3 to entry: The apparent particle density is not identical with the *buoyant density* (3.17). They deviate from each other for porous particles and particle agglomerates (3.8) in particular.

[SOURCE: ISO 13317-1:2024, 3.15]

3.17
buoyant density

ratio of mass to volume for a particle (3.7) including particulate inclusions, liquid and gas in *closed pores* (3.11) and voids, as well as surface layers and coatings, but excluding the liquid continuous phase that penetrates *open pores* (3.10)

Note 1 to entry: The buoyant density equals the (hypothetical) density of the continuous phase for which the gravitational force acting on the immersed particle is counterbalanced by buoyancy.

Note 2 to entry: The buoyant density of a particle can be experimentally determined. See ISO 18747-1 and ISO 18747-2 for further information.

Note 3 to entry: The buoyant density of monoconstituent particles can be approximated with their *skeleton density* (3.15).

Note 4 to entry: The buoyant density of multiconstituent particles (e.g. coated pigments and droplets of multiple emulsions) can be approximated with the averaged densities of the single constituents.

Note 5 to entry: The buoyant density is affected by the adsorption of dissolved species at the particle surface and, therefore, depends on the solvent and its composition.

Note 6 to entry: The buoyant density is not identical with the *apparent particle density* (3.16), particularly for porous particles and particle agglomerates.

[SOURCE: ISO 13317-1:2024, 3.16]

3.18
density contrast

difference between the particle density and the density of the continuous phase

Note 1 to entry: For quantifying the density contrast, the *buoyant* (particle) *density* (3.17) is used, but for porous particles the *apparent particle density* (3.16) is more appropriate.

[SOURCE: ISO 13317-1:2024, 3.17]

3.19
particle Reynolds number

dimensionless parameter expressing the ratio of inertial to viscous forces within a fluid flowing past a particle

Note 1 to entry: The particle Reynolds number is based on the volume equivalent diameter.

Note 2 to entry: In other contexts, the definition of the particle Reynolds number can refer to different equivalent diameters or to the equivalent radii.

Note 3 to entry: The particle Reynolds number is a characteristic of the flow field and mobility of the particle.

[SOURCE: ISO 13317-1:2024, 3.18]

3.20
creeping flow

type of flow solely governed by viscous forces and not affected by inertial forces

Note 1 to entry: For moving particles or for the flow past a particle, the creeping flow condition applies if the *particle Reynolds number* (3.19) is well below 0,25.

[SOURCE: ISO 13317-1:2024, 3.19]

3.21
Brownian motion

random motion of particles caused by collisions with the molecules or atoms of the surrounding continuous phase

Note 1 to entry: The trajectory of Brownian motion is not differentiable.

Note 2 to entry: Brownian motion results on a macroscopic level in mass transport of the dispersed phase, e. g., in case of diffusion, thermophoresis or photophoresis.

[SOURCE: ISO 13317-1:2024, 3.20]

3.22
lower size limit

size of the smallest particles that are detectable and with a diffusional particle flux that is negligible compared to the sedimentational one

Note 1 to entry: The ratio of sedimentational flux to diffusional flux (also called Péclet number, Pe) should be > 1.

[SOURCE: ISO 13317-1:2024, 3.21]

3.23
upper size limit

size of the largest particle that satisfies the condition of *creeping flow* (3.20) and of which the *terminal sedimentation velocity* (3.3) is detectable

[SOURCE: ISO 13317-1:2024, 3.22]

3.24

type of quantity

specification of the physical property employed to quantify the individual particle fractions

Note 1 to entry: The type of quantity is a cumulable property of single particles or disperse systems, such as number, mass, intensity of scattered light (within the single scattering limit), light extinction (within the Lambert-Beer-limit), refractive index increment or X-ray attenuation.

Note 2 to entry: The type of quantity is indicated by a numerical or character subscript when symbolising the density and cumulative function of a size distribution. Moreover, the subscript also specifies distribution parameters such as median, mean and modal values or any quantiles.

Note 3 to entry: The following conventions apply for the subscript of geometric or gravimetric properties:

number: subscript $r = 0$

length: subscript $r = 1$

area: subscript $r = 2$

volume or mass: subscript $r = 3$

Note 4 to entry: The following conventions apply for the subscript of physical properties:

light extinction: subscript toq = “ext”

light intensity: subscript toq = “int”

[SOURCE: ISO 9276-1:1998, 4.3, modified — the definition and notes are expanded to include frequently used, non-geometrical quantities.]

3.25

sensitivity

change of instrument response with respect to changes in concentration or absolute quantity of particles in a specified size class

Note 1 to entry: Concentration or quantity can be given in relative or absolute values depending on the detection aim.

Note 2 to entry: Sensitivity depends on the *type of quantity* (3.24).

Note 3 to entry: Sensitivity is a function of size.

[SOURCE: ISO 13317-1:2024, 3.24]

3.26

limit of quantity detection

smallest quantity of specified particle size class for which the instrument response can be distinguished from the background

Note 1 to entry: The limit of quantity detection depends on factors such as the size range, precision, noise level, smoothing algorithms, etc.

Note 2 to entry: The limit of quantity detection affects the *lower* (3.22) and *upper size limit* (3.23).

[SOURCE: ISO 13317-1:2024, 3.25]

3.27

measurement uncertainty

uncertainty of measurement

parameter associated with the result of a measurement that characterizes the dispersion of the values that can reasonably be attributed to the measurand

[SOURCE: ISO/IEC Guide 98-3:2008, 2.2.3, modified — the term "measurement uncertainty" has been added]

3.28
homogeneous-start method
HSM
method characterized by measurement cell(s) filled with a homogeneously mixed dispersion

3.29
line-start method
LSM
method characterized by measurement cell(s) filled with a thin layer of the sample either on top or below a particle free (spin) liquid depending on the density contrast of the dispersed and continuous phase

4 Symbols and abbreviated terms

For the purposes of this document, the following symbols apply. In some cases, an alternative SI unit is given in parentheses in the right column.

Ar	Archimedes number	1
a_c	centrifugal acceleration	$\mathrm{m\cdot s^{-2}}$ ($\mathrm{cm\cdot s^{-2}}$)
b	systematic deviation of measured value from true value	varying
C_D	drag coefficient	1
C_{rel}	relative centrifugal acceleration	1
C_{toq}	concentration density of particle size being indicated by the index "toq"	varying
$\tilde{C}_{toq}$	concentration density of the sedimentation coefficient with respect to the type of quantity being indicated by an index	varying
D_p	particle diffusion coefficient	$\mathrm{m^2\cdot s^{-1}}$ ($\mathrm{\mu m^2\cdot s^{-1}}$)
c_{toq}	particle concentration with respect to a specified index "toq"	varying
F_D	drag force (also known as "hydrodynamic resistance")	N
g	gravitational acceleration	$\mathrm{m\cdot s^{-2}}$ ($\mathrm{cm\cdot s^{-2}}$)
H	hindrance function	1
K_c	auxiliary coefficient of centrifugation	$\mathrm{m^{-2}\cdot s^{-1}}$
k_B	Boltzmann's constant, $k_B = 1{,}380\,649\times10^{-23}\ \mathrm{J\cdot K^{-1}}$	$\mathrm{J\cdot K^{-1}}$
k_{shape}	shape correction factor	1
Lj	Ljaščenko number	1
M_{disp}	is the amount of dispersed phase quantified by a specified property	varying
Pe	Péclet number	1
Q_{toq}	cumulative function of distributed quantity, index “toq” indicates which of the fractions are weighted	1
q_{toq}	density function of particle size, index “toq” indicates which of the fractions are weighted	$\mathrm{m^{-1}}$

$\tilde{q}_{\mathrm{toq}}$	density function of sedimentation coefficient, index “toq” indicates which of the fractions are weighted	s^{-1}
Re_{p}	particle Reynolds number	1
r	radial distance from rotor axis, radial coordinate	m
S	signal of CLS measurement, being proportional to particle concentration	varying
T	absolute temperature	K
t	time	
t_{sed}	sedimentation time	s
U	expanded uncertainty of a measurand	varying
u	uncertainty of a measurand	varying
v_{sed}	terminal sedimentation velocity	$\mathrm{m \cdot s^{-1}}$ ($\mathrm{\mu m \cdot s^{-1}}$)
v_{Stokes}	terminal sedimentation velocity for creeping flow (Stokes regime)	$\mathrm{m \cdot s^{-1}}$ ($\mathrm{\mu m \cdot s^{-1}}$)
x	particle size	m (µm, nm)
x_{Stokes}	Stokes diameter	m (µm, nm)
Δr	radial sedimentation distance	m
$\Delta \rho$	density contrast between the dispersed and continuous phase	
ε	porosity	1
η_{c}	viscosity of the continuous phase	$\mathrm{N \cdot s/m^2}$ ($\mathrm{Pa \cdot s}$)
θ	volume fraction of open pores that is filled with liquid	1
$\tilde{\kappa}, \kappa$	kernel functions of Formulae (22) and (23), respectively	1
ρ_{c}	density of the continuous phase	$\mathrm{kg \cdot m^{-3}}$ ($\mathrm{g \cdot cm^{-3}}$)
ρ_{p}	particle density	$\mathrm{kg \cdot m^{-3}}$ ($\mathrm{g \cdot cm^{-3}}$)
τ_{sed}	sedimentation coefficient	s
φ_V	volume fraction of the dispersed phase	1
Ψ	sphericity	1
ω	angular frequency	$\mathrm{rad \cdot s^{-1}}$

For the purposes of this document, the following subscripts apply.

0	initial state, start position
aggl	agglomerate, aggregate
app	apparent
bias	bias, deviation from true value
bouy	bouyancy
c	continuous phase
cr	critical
d	dispersed phase
incl	inclusions
max	maximum
meas	measurement position
min	minimum
occl	occluded voids
ref	reference
rel	relative
rep	repeatability
RMS	root mean square, quadratic mean
Rw	reproducibility
sed	sedimentation
sk	skeleton
toq	type of quantity
tot	total

For the purposes of this document, the following abbreviated terms apply.

CLS	centrifugal liquid sedimentation
CRM	certified reference material
DBM	direct boundary method
HSM	homogeneous-start method
LSM	line-start method
QCM	quality control material
RCA	relative centrifugal acceleration
RM	reference material

5 Measurement principle and technical realisations

5.1 General measurement principle

Centrifugal liquid sedimentation (CLS) techniques[1] quantify the separation of particles (dispersed phase) from a liquid (continuous phase) under the presence of a centrifugal force. This phase separation relies on the directional, migratory motion of each particle, which this document refers to as "sedimentation". Its rate is called the "sedimentation velocity", while the acceleration-specific rate is named the "sedimentation coefficient". Both depend on the particle size and, thus, offer a chance for the granulometric characterization of particle systems. Additional factors are particle shape and the particle density.

NOTE 1 CLS techniques are generally called “sedimentation techniques” in this document, irrespective of whether the particles move along or against a centrifugal force.

CLS occurs for any particle dispersed in a quiescent viscous liquid and exposed to a centrifugal field, as long as a density contrast exists. It is driven by centrifugal forces that act on the particle (weight) and the displaced liquid (buoyancy). The resulting net force (excess force) causes a migratory directed particle motion, which is retarded by a frictional force (also called the drag force). The drag force increases linearly with the migration velocity. If the excess force is positive (i.e. weight > buoyancy), the particle motion is called falling or settling. In the opposite case, it is called rising, creaming or floating. In contrast to gravitational sedimentation (see ISO 13317-1), particle motion never reaches a true steady state as the centrifugal force depends on the radial position (in relation to the centre of rotation) and thus changes in the course of sedimentation (see 6.1). Particles settling outwards are steadily accelerated whereas creaming, i.e. inwards moving, particles experience a gradual deceleration. Nevertheless, the time scale of this alteration is much larger than the time scale of viscous relaxation. Hence, the particles are considered to be in a quasi-steady state, which means that at each radial position, they move with the terminal sedimentation velocity [see Formula (4)] they would achieve if the local force field prevailed at all radial positions.

The main advantage of centrifugal versus gravitational liquid sedimentation is in the considerably higher and more variable driving force, which allows the characterization of nanosized particles and facilitates a broadening of the measurement range. Particle size analysis for CLS is typically conducted at centrifugal accelerations of 10 × *g* to about 25 000 × *g*. Yet some instruments (analytical ultracentrifugation) can even realise rotation speeds of up to 60 000 rpm corresponding to centrifugal accelerations considerably above 100 000 × *g*, which facilitates an expanded characterization to very fine objects, such as macromolecules or quantum dots, and may include shape and material analyses.[2] It is common to calculate the relative centrifugal acceleration (RCA) as a ratio of the centrifugal to gravitational acceleration. This dimensionless parameter relates the time scales of sedimentation phenomena under gravity to those in centrifugal fields.

Sedimentation results in a size-dependent separation of dispersed particles from the liquid, which coincides with classification in the case of polydisperse materials. These effects can be employed to measure the particle size distribution. In CLS there are two principal approaches (see Figure 1).

- One approach is a gradual demixing of a homogenously mixed dispersion [i.e. the homogeneous-start method (HSM)], which consists of the following:
  - an initially homogenous sample undergoes a gradual depletion of the dispersed phase which leads to the formation of a particle layer at the outermost or innermost cell position for settling and creaming, respectively;
  - in HSM, depletion occurs as successive separation of the respective largest particles at a given position and time;

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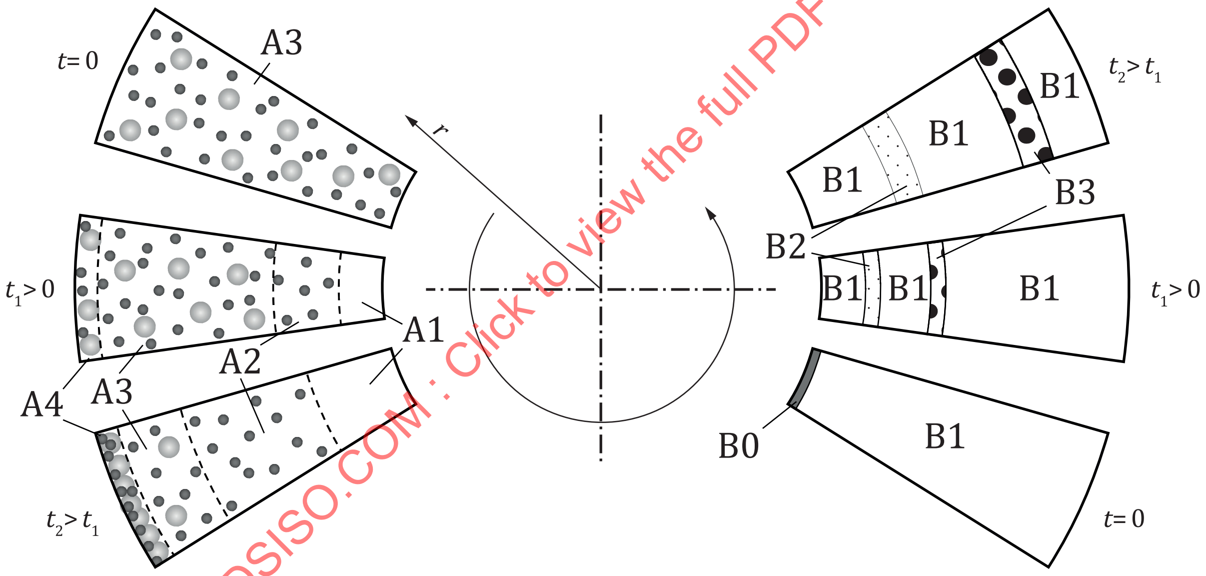
[1] CLS techniques are also referred to as analytical centrifugation. Instruments are called analytical centrifuges.

  - within the dispersed phase, a concentration gradient is observed, which is directed to the aforementioned particle layer.
- The other approach is a chromatography-like separation in a particle-free medium (i.e LSM), which consists of the following:
  - a thin layer of the particle sample is placed on the top of a particle-free liquid (supernatant or spin liquid);
  - particles move through this “stationary phase” according to their size and density;
  - polydisperse materials are split into separate bands, which contain all particles of a certain size and density fraction;
  - this kind of separation requires density gradients within the supernatant or spin liquid in order to avoid instabilities and attain a high size resolution.

Particle motion in centrifugal fields occurs along radial trajectories at a steadily increasing or decreasing velocity resulting in a change of the particle concentration, which is independent from the separation between fine and coarse particles. A radial dilution is observed for settling particles (i.e. for positive density contrast), which is due to the divergence of the trajectories and to the acceleration along the sedimentation path. The effect is particularly comprehensible for the LSM. Radial acceleration broadens the chromatographic band, while radial divergence widens its perimeter. As the total amount of particles within the band remains constant, their concentration becomes reduced.



Key

r	radial coordinate, starting at the axis of rotation
t	sedimentation time
$t_{1/2}$	two successive time points
A1	particle-free supernatant
A2	depleted dispersion phase (due to loss of coarse particles)
A3	original dispersion phase
A4	sediment
B0	initial layer with the bi-disperse particle sample

B1 particle-free zones

B2 band with fine particle fraction

B3 band with coarse particle fraction

NOTE This figure shows spatial distribution of fine and coarse particles at the start (t=0) and at two later instances (t2 > t1 >0 for HSM on the left and LSM on the right) .

Figure 1 — Illustration of phase separation due to sedimentation in a centrifugal field for a bimodal sample with a positive density contrast

The principal effects of CLS are illustrated in Figure 1, which depicts the spatial distribution of settling particles for a bi-disperse system at the start of centrifugation and two subsequent instances. The illustration refers to both modes of operation, the HSM and the LSM. In either case, a classification of the particle system is observed, as coarse particles move faster in the centrifugal field than fine ones.

The formation of four zones along the radial coordinate is observed in case of the HSM (see Figure 1). The innermost layer, the supernatant (zone A1), has become free of all particles. In the subsequent layer (zone A2), there are only particles from the fine fraction, whereas the next layer (zone A3) contains particles of both fine and coarse size fractions. The particles from zones A2 and A3 migrate outwards due to a centrifugal force and eventually deposit as a sediment (zone A4). As a result, a step-like concentration profile is seen at each instance. However, while the particle concentrations in zones A2 and A3 are invariant with respect to space, they steadily decrease with time as the zones move to the outermost part of the cell. This is due to the increasing centrifugal acceleration along the radial coordinate and, on the other hand, the divergence of particle trajectories (that is, radial dilution). The effect also concerns the composition of zone A3 because coarse particles leave zone A3 faster than fine ones. Consequently, the size distribution of zone A3 becomes gradually dominated by fine particles.

NOTE 2 In the general case of polydisperse materials, zone A3 is composed of all the size fractions that were originally present and experiences a gradual shift in size distribution towards the fine edge. Zone A2 can be considered as a blurred sedimentation front, which is partly depleted of coarse particles (with the coarsest particles at the border to A3, and all but the finest ones at the border to A2).

NOTE 3 In systems with a negative density contrast, i.e. for floating particles, the order of the four zones along the radial coordinate is reversed. In addition, the effect of radial dilution causes thickening and coarsening of the size distribution in zone A3.

When LSM is applied to materials with a positive density contrast (see Figure 1), a very thin layer (zone B0) of the original sample is, at first, placed “on the top” (i.e. close to the rotor) of a particle free spin liquid (zone B1). During centrifugation, all particles of a given size fraction move together at the same velocity at a given radial position through the spin liquid to “the bottom” (i.e. the outermost wall of the cell). A polydisperse system is thus split into size-specific yet generally coherent bands, with the coarsest, i.e. fastest settling, particles leading the particle sedimentation front and the finest, i.e. slowest settling, particles following behind. For a bi-disperse system as shown in Figure 1, two distinct bands are observed for each size fraction. The separation of the bands and their broadening increases with centrifugation time.

The rate of phase separation for CLS, e.g. quantified by the sedimentation velocity or sedimentation coefficient, depends on the properties of the continuous phase (such as density and rheological behaviour) and on characteristics of the particles (i.e. size, density and shape) in HSM as well as in LSM. This allows its conversion into particle size based on Stokes’ law and its corresponding presumptions (see 6.1). This (indirect) quantification yields an equivalent diameter with respect to sedimentation velocity, called the Stokes diameter (x_{Stokes}). It reflects the weight or volume of the particles, as well as their mobility or drag coefficient. It is typically one of the smallest equivalent diameters for a given particle. According to Leschonski,[3] Formula (1) applies:

$$x_{\mathrm{Stokes}} < x_V < x_{\mathrm{S}} = x_{\mathrm{proj,m}} < x_{\mathrm{proj,st}}, \tag{1}$$

where

x_V is the volume equivalent diameter;

x_S is the surface equivalent diameter;

$x_{\text{proj,m}}$ is the projected area equivalent diameter for mean orientation;

$x_{\text{proj,st}}$ is the projected area equivalent diameter for stable orientation.

The difference between these equivalent diameters is particularly pronounced for particle aggregates and agglomerates, where the following relationship applies[4]:

$$x_{\text{Stokes}} \ll x_{\text{hd}} < x_{\text{gyr}} < x_{\text{Feret,max}} \quad (2)$$

where

x_{hd}	hydrodynamic equivalent diameter
x_{gyr}	diameter of gyration
$x_{\text{Feret,max}}$	maximum Feret diameter

NOTE 4 The Stokes diameter is not identical to the hydrodynamic or mobility equivalent diameter, which solely refers to the particles' drag coefficient. Colloid scientists often use the radius of gyration (i.e. 0,5 × x_{gyr}).

Particle size analysis by means of CLS relies on the assumption that centrifugal sedimentation completely explains the observed phase separation. This holds true for the vast majority of practical applications. However, in the event of extremely low sedimentation velocities, e.g. for fine nanoparticles (< 100 nm), sedimentation can be superposed by the diffusion fluxes due to Brownian motion.[5],[6] Diffusion causes a smoothing of concentration gradients, thus broadening the measured distribution function. This effect is well-understood and described by quantitative models, which can be used to correct the impact of broadening due to diffusion.[2],[6],[7] A more sophisticated method (Lamm equation) is described in 6.3.7.[8]

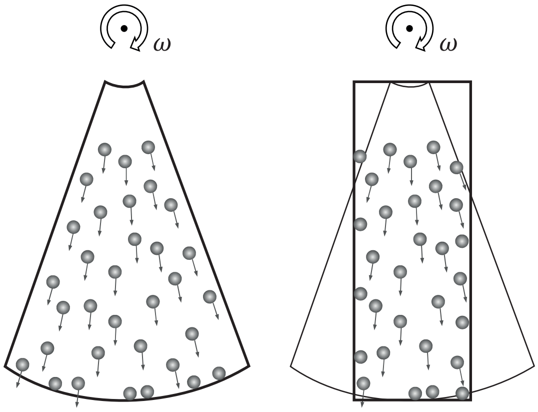
An essential feature of each sedimentation technique is the way of quantifying the particles and thus the particle size fractions. It occurs separately from the physical process of particle classification via sedimentation and can employ very different measurands (e.g. local mass concentration, local X-ray attenuation or light obscuration, hydrostatic pressure, sediment height). The quantification defines the intrinsic type of quantity, by which the individual size fractions are weighted (see 5.2 and Annex A). The conversion of these quantities to fundamental ones (i.e. volume or number) can either rely on an unbiased or biased linear scaling or involve parametric models (e.g. for light obscuration).

5.2 Technical realisation of sedimentation-based centrifugal measurement techniques

There is considerable variety in analytical instrumentation for particle characterization based on CLS (refer to Tables A.1 and A.2). All types of CLS instruments probe the Stokes diameter of dispersed particles by either monitoring the depletion of the dispersion phase or observing the growth of the deposited particle layer (sediment or cream). However, these techniques differ in several aspects which can significantly affect data interpretation and comparability.[9],[10]

Geometry of the measurement cell:

The most popular criteria to distinguish an analytical centrifuge refers to the cell-type, in which the dispersion samples are exposed to a centrifugal field. This cell can be either a cuvette, or a hollow, cylindrical disc. Accordingly, the instruments are called disc-type centrifuge or cuvette-type centrifuge.



Key

ω angular frequency

NOTE This figure shows a sector-shaped cuvette which mimics disc cells on the left, and a rectangular cuvette on the right.

Figure 2 — Schematic representation of CLS in regards to the sample cell geometry

Cuvettes for CLS have either a sector-like or rectangular shape in the centrifugal plane (Figure 2). The former can be considered as a representative part of a disc-cell, in which particle trajectories cannot meet the sidewalls, which ensures a uniform particle concentration along the tangential coordinate. In contrast, rectangular cells inevitably cause the deposition or an increase of concentration of particles at the sidewalls, which can promote convective flow due to temperature gradients or density gradients.[11] However, rectangular cells are acceptable for most applications if the ratio of the distance between rotational centre and position of the particle sensor to the sedimentation path is larger than 5 (based on observations documented in Reference [12]). Disc-like measurement cells possess the advantage of a uniform particle distribution along the tangential coordinate and, additionally, facilitate the operation with the LSM (see 5.1).

While the difference between disc-type and cuvette-type centrifugation is probably the most evident one in centrifugal instrumentation, it is not the most fundamental one.

Detection and quantification of particles:

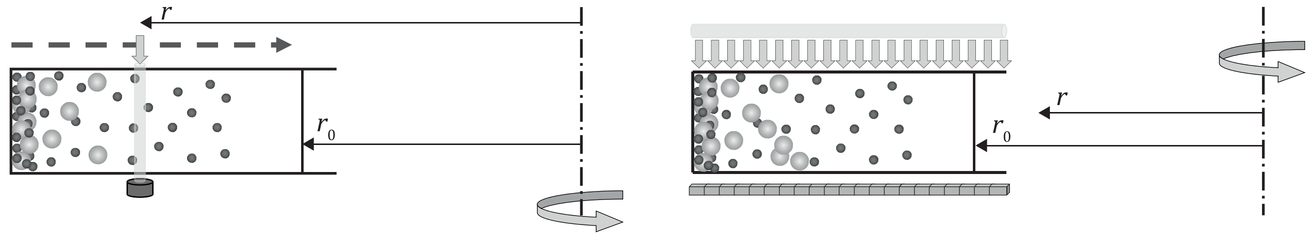
A further distinction refers to the detection of temporal and/or spatial variation in the particle concentration within the centrifugal field. While centrifugation time and radial position determine the sedimentation velocity and thus the particle size, further techniques for detecting concentration shifts and/or gradients as prerequisite must be used for weighing the individual size fractions of polydisperse systems. Techniques can:

— probe integral properties of segregated particles (e.g. sediment mass);

— address the integral (cumulated) quantity of dispersed particles (e.g. via hydrostatic pressure);

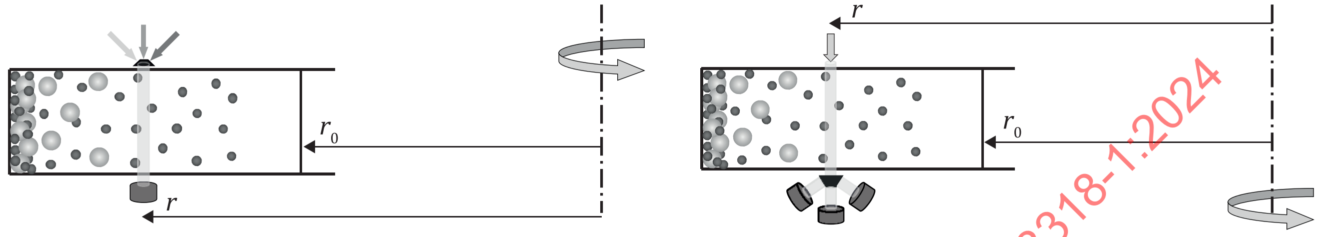
— measure the local concentration of the dispersed phase (e.g. via X-ray attenuation, light extinction, interpherometric or Schlieren optics).

With regard to data analysis, the first two types of detection techniques are named integral or cumulative techniques, whereas the third type is an incremental technique. In addition, there are even different modes of detection at fixed position or scanning over a radial range, observing the evolution of complete concentration profiles (Figure 3). The detection technique affects the working range of analytical centrifuges and determines the intrinsic type of quantity, in which the measured distribution functions are weighted (see Tables A.1 and A.2).



a) radiative monitoring of the local concentration at a fixed or scanning position

b) radiative monitoring of the local concentration at multiple radial positions



c) radiative monitoring of the local concentration with multiple wavelengths

d) radiative monitoring of the local concentration with multiple detectors

Key

r	radial coordinate
r_0	radial coordinate of the meniscus

Figure 3 — Important measurement principles of CLS techniques

Figure 3 indicates the principles of common measuring techniques using CLS analysis, however there are other techniques, mainly realised in historical instrumentation or for academic purposes;[9],[10] an overview is provided in Annex A.

6 Measurement data and basic rules of data evaluation

6.1 Primary and derived measurands in centrifugal liquid sedimentation

The primary measurand of CLS is the sedimentation velocity of the particles. It can be derived either from changes in the local or integral concentration or from the formation of a sediment or cream layer. Sedimentation velocity is the rate of particle displacement in the centrifugal field. The driving force of this migratory motion, the centrifugal excess force, changes along with the particles' radial trajectories (see 5.1), for which reason the sedimentation velocity v_{sed} is also a function of the radial coordinate r as shown in Formula (3):

$$v_{\text{sed}} = \frac{\partial r}{\partial t} = \text{f}(r) \tag{3}$$

This is a principal difference to gravitational liquid sedimentation (ISO 13317-1) and affects the evolution of particle concentration (e.g. Figure 1) as well as the analysis of measurement results. Indeed, the functional relationship between the sedimentation velocity and the radial position is not conventionally derived from the measurement, rather it is supposed to obey the law of creeping flow (Stokes regime) which leads to Formula (4):

$$v_{\text{sed}}(r) = v_{\text{Stokes}}(r) = \frac{a_{\text{c}}(r)\Delta\rho}{18\eta_{\text{c}}} x_{\text{Stokes}}^2 = a_{\text{c}}(r) \cdot \tau_{\text{Sed}}\text{, with } \Delta\rho = \rho_{\text{p}} - \rho_{\text{c}} \tag{4}$$

$$\text{and } a_{\text{c}}(r) = \omega^2 r = C_{\text{rel}}(r) \cdot g \tag{5}$$

where

a_c	centrifugal acceleration
C_{rel}	relative centrifugal acceleration (RCA)
g	gravitational acceleration
r	radial coordinate
v_{Stokes}	terminal sedimentation velocity for small particle Reynolds numbers (Stokes regime)
$\Delta\rho$	difference between particle density (ρ_p) and density of the continuous phase (ρ_c)
η_c	dynamic viscosity of the continuous phase
ω	angular frequency
τ_{sed}	sedimentation coefficient

In practice, the sedimentation velocity is compared to a certain reference point (r_{ref}) such as meniscus position or outermost cell wall:

$$v_{sed,ref} = \frac{r_{ref}}{r} \cdot \frac{\partial r}{\partial t} \neq \text{funct.}(r) \tag{6}$$

This quantity should be always reported together with the centrifugal acceleration at the reference point. It facilitates the comparison among different samples and measurements and can be easily transformed to gravitational sedimentation velocity. Alternatively, the sedimentation coefficient τ_{sed} can be used as the primary measurand.[6],[7]

$$\tau_{sed} = \frac{1}{\omega^2 r} \cdot \frac{\partial r}{\partial t} \tag{7}$$

It can be considered as an acceleration-specific sedimentation velocity or as a characteristic time, which refers to the particles' viscous relaxation. Unlike the sedimentation velocity, the sedimentation coefficient can facilitate a direct comparison of results from gravitational and centrifugal sedimentation velocity. In addition, the parameter remains constant in speed ramp experiments (see F.4), whereas the velocity does not. However, for many (non-academic) users, it is less comprehensible than a velocity value.

Both the sedimentation velocity and sedimentation coefficient depend on the physical properties of the continuous phase (density and viscosity). This dependency is irrelevant for particulate objects whose morphology and density are bound to a specific liquid (e.g. micelles or proteins). However, in many situations a pure particle property representing the sedimentation behaviour is the Stokes diameter, which is specified by Formula (8):

$$x_{Stokes} = \sqrt{\frac{18\eta_c}{\Delta\rho} \cdot \frac{v_{sed,ref}}{\omega^2 r_{ref}}} = \sqrt{\frac{18\eta_c}{\Delta\rho} \cdot \tau_{Sed}} \tag{8}$$

The Stokes diameter is the equivalent diameter for particle migration in gravitational or centrifugal fields. It is affected by the particle's inertia and drag coefficient under creeping flow conditions. Within this document, this is the measurand of the greatest interest.

Under certain conditions, additional measurands can be obtained from CLS that are not related to sedimentation. A first group of such measurands quantify the particle concentration in the original sample (see 6.3.6). The signals of particle detection systems must reflect the total amount of particles in the detection zone. This applies, for instance, to the attenuation or phase shift of an electromagnetic radiation. The detection technique determines the CLS-type of quantity of concentration (e.g. volume for X-ray attenuation), which can require elaborate models for conversion in number or volume concentration (e.g. Mie theory for light extinction by spherical particles). Analytical sedimentation also offers an opportunity to measure particle density (see the ISO 18747 series for methods on how to measure particle density). Another group of supplementary measurands can be derived for fine nanoparticles (< 0,1 µm) if Brownian motion significantly influences the spatial distribution of particles in the centrifugal field (see 6.3.7). In this event, the particle diffusion coefficient can be computed and so too the hydrodynamic or mobility equivalent particle diameter, which further offers a chance to extract particle density and shape parameters.[8],[15],[16] The shell-thickness of core-shell particles is reported based on this approach.[11]

6.2 Spatial distribution of particles in centrifugal fields

The characterization of a material's dispersity by CLS relies on the evaluation of time curves for the local or cumulated particle concentration, concentration profiles for defined sedimentation times or the evolution of these profiles over time. It thus relies on a clear understanding of the spatial distribution of particles in the centrifugal field, which is governed by phase separation and radial dilution (see 5.1).

Annex F provides a detailed derivation of the evolution of concentration profiles in centrifugal experiments. The solution depends on the initial conditions, i.e. whether the measurements were performed with homogeneous-start method (HSM) or line-start method (LSM). In the former case, the spatial and temporal distribution of particle concentration, c, can be expressed with Formula (9):

$$c_{\mathrm{toq}}(r,t)=c_{\mathrm{toq}}^{0}\int_{0}^{x_{\mathrm{cr}}(r,t)} q_{\mathrm{toq}}(x)\cdot\left(\frac{r}{r_0}\right)^{-2x^2/x_{\mathrm{cr}}^2}\mathrm{d}x \tag{9}$$

$$\text{with } x_{\mathrm{cr}}(r,t)=\sqrt{\frac{\ln(r/r_0)}{K_{\mathrm{c}}\cdot t}} \tag{10}$$

$$\text{and } K_{\mathrm{c}}=\frac{\omega^2\Delta\rho}{18\eta_{\mathrm{c}}} \tag{11}$$

where

c_{toq}	particle concentration with respect to the type of quantity defined by index "toq"
c_{toq}^{0}	initial, uniformly distributed particle concentration with respect to the type of quantity defined by index "toq"
K_{c}	auxiliary variable
$q_{\mathrm{toq}}(x)$	density function of particle size; the type of quantity being indicated by toq
r	radial coordinate
r_0	radial coordinate of meniscus ($\Delta\rho > 0$) or bottom ($\Delta\rho < 0$)
t	time
x	particle size (more precisely, the Stokes diameter)
x_{cr}	critical particle size
$\Delta\rho$	density contrast
η_{c}	dynamic viscosity of the continuous phase
ω	angular frequency

In case of the LSMs, the sample is uniformly distributed on an extremely thin layer at position r_0 over a particle-free spin liquid. Centrifugation causes a separation of all particle fractions from each other, so that at a certain radial position, r, infinitesimal narrow particle fractions can be observed passing by consecutively, with the fastest particles appearing first. In practice, the observation is performed over a small radial distance, δr, which means that a measurement at instance t captures all particles in the size range $x_{\mathrm{cr}} \ldots x_{\mathrm{cr}}+\delta x_{\mathrm{cr}}$. The observed local concentration then reads:

$$\delta c_{\mathrm{toq}}(r,t)=c_{\mathrm{toq}}^{0}\cdot\left(\frac{r_0}{r}\right)^2\frac{\delta r}{2r\ln(r/r_0)}\cdot x_{\mathrm{cr}}q_{\mathrm{toq}}(x_{\mathrm{cr}})\text{ with } x\cdot\tilde{q}_{\mathrm{toq}}(x)=\tilde{q}_{\mathrm{toq}}^{*}(x) \tag{12}$$

where $q_{\mathrm{toq}}^{*}(x)$ is the transformed density function of the particle size.

Hence, the time curves at certain measurement positions can be considered as scaled, transformed density functions for the distribution of sedimentation coefficient and particle size.

6.3 Determination of distribution functions

6.3.1 General remarks

Within the context of this document, there are three interrelated characteristics of a particle's sedimentation behaviour:

$$\text{sedimentation velocity: } v_{\mathrm{sed,ref}}=\frac{r_{\mathrm{ref}}}{r}\cdot\frac{\partial r}{\partial t} \tag{13}$$

$$\text{sedimentation coefficient: } \tau_{\mathrm{sed}}=\frac{1}{\omega^2 r}\cdot\frac{\partial r}{\partial t} \tag{14}$$

$$\text{Stokes diameter: } x_{\mathrm{Stokes}}=\sqrt{\frac{18\eta_{\mathrm{c}}}{\omega^2 r\Delta\rho}\cdot\frac{\partial r}{\partial t}} \tag{15}$$

Formula (4) reveals the interrelation between these characteristics. Although the definition of sedimentation velocity and sedimentation coefficient does not rely on a specific model for particle sedimentation, data analysis assumes sedimentation under creeping flow conditions when computing distribution functions. This is in order to ease the correction for radial dilution, which is virtually always met by experimental conditions in CLS.

In real particle systems, these quantities are distributed and it is therefore insufficient to describe the sedimentation behaviour with just one value. Instead, distribution functions are required. They are typically defined for a property z as:

$$\text{cumulative distribution function: } Q_{\mathrm{toq}}(Z)=\frac{M_{\mathrm{disp}}(z\le Z)}{M_{\mathrm{disp,tot}}}=\int_0^Z q_{\mathrm{toq}}(z)\,\mathrm{d}z \tag{16}$$

$$\text{distribution density function: } q_{\mathrm{toq}}(Z)=\left.\frac{\mathrm{d}Q_{\mathrm{toq}}(z)}{\mathrm{d}z}\right|_Z \tag{17}$$

$$\text{transformed density function: } q_{\mathrm{toq}}^{*}(Z)=\left.\frac{\mathrm{d}Q_{\mathrm{toq}}(z)}{\mathrm{d}z/z}\right|_Z \tag{18}$$

where

M_{disp} is the amount of dispersed phase with a specified property, e.g. $z \le Z$

$M_{\mathrm{disp,tot}}$ is the total amount of dispersed phase

and where index "toq" denotes the type of quantity, in which the "amount of dispersed phase" is quantified.

Sedimentation velocity and sedimentation coefficient possess negative signs for negative density contrast, as their motion is then counter-directed to the centrifugal acceleration. In this event, the definitions of cumulative function and density function refer to the absolute values of the two quantities, for pragmatic convenience. However, if creaming and settling occurs simultaneously, Formulae (16) and (17) need to be replaced by the more general Formulae (19) and (20), respectively:

$$Q_{\text{toq}}(v) = \frac{M_{\text{disp}}(v_{\text{sed}} \leq v)}{M_{\text{disp,tot}}} = \int_{-\infty}^{v} q_{\text{toq}}(v_{\text{sed}}) \mathrm{d}v_{\text{sed}} \tag{19}$$

$$q_{\text{toq}}(v) = \left. \frac{\mathrm{d}Q_{\text{toq}}(v_{\text{sed}})}{\mathrm{d}v_{\text{sed}}} \right|_{v} \tag{20}$$

Most analytical centrifuges now employ an incremental type of quantification (see 5.2). This means that the distributions of dispersity characteristics are calculated from either the spatial or local variation, or both, of particle concentration (beyond sediment or cream layer), that is:

— as a function of time, for a fixed position or for a steady change of position (scan);

— as a function of position (profile), for a given sedimentation time;

— as a function of position and time (profile evolution).

Each sedimentation technique can be attributed an intrinsic type of quantity, in which the separate particle fractions contribute to the measured signal. These contributions are considered to superpose linearly so that the measurement signal $S_{\text{toq}}(x)$ can be related to the local concentration of the different particle fractions:

$$S_{\text{toq}}(r,t) \propto c_{\text{toq}}(r,t) \text{ or } S_{\text{toq}}(r,t) \propto \delta c_{\text{toq}}(r,t) \tag{21}$$

The two relationships constitute the basis for computing particle size distributions from the measured signals.

6.3.2 Distribution analysis for incremental techniques —homogeneous-start method (HSM)

Formulae (12) and (14) describe the dependence of concentration on time and radial position for the HSM. Elapsed time and measurement position define the critical sedimentation properties, which refer to the progressing sedimentation front [see Formulae (13) and (15), respectively]. A reformulation of the two equations yields:

$$c_{\text{toq}}(\tau_{\text{cr}}) / c_{\text{toq}}^{0} = \int_{0}^{\tau_{\text{cr}}} \tilde{\kappa}(\tau_{\text{cr}}, \tau) \tilde{q}_{\text{toq}}(\tau) \mathrm{d}\tau \text{ or} \tag{22}$$

$$c_{\text{toq}}(x_{\text{cr}}) / c_{\text{toq}}^{0} = \int_{0}^{x_{\text{cr}}} \kappa(x_{\text{cr}}, x) q_{\text{toq}}(x) \mathrm{d}x \tag{23}$$

where $\tilde{q}_{\text{toq}}(\tau)$ and $q_{\text{toq}}(x)$ represent the target of analytical investigation. As concentration profiles are measured at discrete positions and time-curves are recorded at defined time intervals, these equations are commonly discretised for computing the distribution functions:

$$C_{\text{rel},k} = \sum_{\ell=1}^{k} \kappa_{k,\ell} \, \Delta Q_{\text{toq},\ell} \text{ or } \mathbf{C}_{\text{rel}} = \mathbf{K} \cdot \mathbf{\Delta Q}_{\text{tot}} \tag{24}$$

$$\text{with: } \kappa_{k,\ell} \approx \kappa(x_k, x_\ell) \approx \tilde{\kappa}(\tau_k, \tau_\ell) \tag{25}$$

The coefficients $\kappa_{k,\ell}$ vanish for $k < \ell$, for which reason the mathematical solution is a well-posed problem and the distribution function (ΔQ_{toq}) can be directly calculated from Formula (24) without the need for regularisation, e.g. by Reference [17].

If the measurement yields only one profile or one time-curve, the elements $C_{\text{rel},k}$ should monotonically increase or decrease for an ideal measurement. In practice, however, noisy signals are observed. The noise sets a limit for the achievable resolution of the distribution function. In order to avoid noisy distribution functions, the original data can be smoothed (e.g. by averaging over positions or time) or the resolution of the distribution function reduced (i.e. the number of elements in ΔQ_{toq}).

If the measurements provide a number of individual profiles (for different instances) or time-curves (for different positions) for the very same phase separation, noise can be handled in different ways, such as:

- averaging the individual distribution functions;
- merging all individual measurement data in a combined data set, which can be evaluated by numerically solving Formula (24).[19]-[21]

The former approach effectively suppresses the impact of fine noise on measurement results. The variation among the results can be also used to evaluate their consistency. For instance, systematic differences between individual results typically indicate that the computation relies on a wrong value of the meniscus position. The latter approach is a powerful tool to obtain finely resolved distribution functions from noisy data but requires the handling of big data sets. Therefore, it has to employ regularisation for solving the matrix equation (24).

6.3.3 Distribution analysis for incremental techniques — line-start method (LSM)

The LSM principally facilitates a relatively simple data analysis because the signals $S_{toq}(r,t)$ measured at radial position, r, and time, t, concentrations are proportional to:

- the transformed density functions ($q^*_{toq}(x)$ or $\tilde{q}^*_{toq}(\tau)$);
- an invariant geometric factor;
- and the original sample concentration [see Formulae (12), (21) and (F.12)].

The measurement thus directly yields the distribution density functions of the dispersity characteristic:

$$\tau_{sed}\tilde{q}_{toq}(\tau_{sed}) \propto \delta c_{toq}(r,t) \propto S_{toq}(r,t) \tag{26}$$

$$\text{with } \tau_{sed} = \frac{\ln(r/r_0)}{\omega^2 \cdot t}\text{, and} \tag{27}$$

$$x \cdot q_{toq}(x) \propto \delta c_{toq}(r,t) \propto S_{toq}(r,t) \tag{28}$$

$$\text{with } x = \sqrt{\frac{18\eta_c \cdot \ln(r/r_0)}{\omega^2 t \cdot \Delta\rho}} \tag{29}$$

This principal scheme of data analysis needs to be modified in analytical practice, primarily because the LSM is most often performed with a density gradient in the liquid phase. Consequently, the liquid properties (density and viscosity) cannot be considered as constant for the sedimentation process. In addition, the established density profile changes with time, which coincides with changes in the refractive index profile and, thus, affects the detection of photometric signals. That is, the signal $S_{toq}(r,t)$ experiences a systematic alteration even for the absence of dispersed particles (baseline shift). In order to cope with these implications, the data analysis is typically modified, wherein:

- the actual density profile is indirectly quantified by the measurement of reference particles (calibration), and the material properties in Formula (29) can thus be replaced by a calibration coefficient;
- the measured signal $S_{toq}(r,t)$ is corrected for the baseline shift before applying Formulae (26) or (28).

Apart from the presence of a density gradient, signal processing must also consider the finite thickness of the initial sample layer, the radial width of the measurement zone, possible temperature drifts and, for some techniques, the spectral composition of the illuminating radiation. Hence, details of the implemented algorithms for data analysis can differ from the principal scheme described above. They are explained in literature for specific centrifugation techniques[22] and typically provided by the manufacturer.

6.3.4 Distribution analysis for integral techniques

Integral centrifugation techniques, such as manometric centrifugation[23] or centrifugal sedimentation balances[10] are irrelevant for particle characterization.

6.3.5 Conversion with respect to the type of quantity2)

The technique-specific principle of quantifying the amount of particles for each size fraction defines the type of quantity in which the distribution of the sedimentation coefficient or Stokes diameter is intrinsically described. These distribution functions possibly necessitate a conversion with respect to the type of quantity before further interpretation. For spherical particles, the conversion is conducted by means of

$$q_B(x) = \frac{f_B(x)}{f_A(x)} q_A(x) \Big/ \int \frac{f_B(x)}{f_A(x)} \mathrm{d}Q_A \tag{30}$$

where A and B are size dependent quantities:

$$A = f_A(x), \text{ and } B = f_B(x). \tag{31}$$

EXAMPLE Photocentrifuges quantify the local particle concentration via the attenuation of a transmitted light beam (see ISO 13318-2). The transmitted signal is transformed into an extinction value, E, which is proportional to the product of number concentration c_N and the particles' extinction cross section C_{ext}. When converting an intrinsically measured extinction-weighted particle size distribution into a volume-weighted one, the extinction cross section $C_{\mathrm{ext}}(x)$ has to be employed as quantity function $f_A(x)$ and x^3 as quantity function $f_B(x)$ in Formula (30).

The quantity functions generally obey the power laws of particle size, for instance:

- X-ray attenuation of dispersed particles: $f_{\mathrm{Xray}}(x) \propto x^3 \rightarrow q_{\mathrm{Xray}}(x) = q_3(x)$
- light extinction of opaque micrometre particles: $f_{\mathrm{ext}}(x) \propto x^2 \rightarrow q_{\mathrm{ext}}(x) = q_2(x)$
- light extinction of opaque nanoparticles: $f_{\mathrm{ext}}(x) \propto x^3 \rightarrow q_{\mathrm{ext}}(x) = q_3(x)$
- light extinction of transparent nanoparticles: $f_{\mathrm{ext}}(x) \propto x^6 \rightarrow q_{\mathrm{ext}}(x) = q_6(x)$
- refractive index increment of nanoparticles: $f_{\mathrm{RI}}(x) \propto x^3 \rightarrow q_{\mathrm{RI}}(x) = q_3(x)$

The last examples indicate that optical sample properties can scale to a power of particle size if the size range is rather restricted. In general, such a simplification is not possible and sophisticated optical models, such as Mie's solution for electromagnetic scattering from spheres, need to be employed.[24]

Formula (30) defines the general way of converting with respect to the type of quantity, but it does not mean that this conversion is only performed at the computed distribution functions from centrifugal particle characterization. In fact, this conversion can be easily implemented in the numerical schemes for deriving distribution functions from measured signals. For size analysis with HSM, conversion is achieved with Formula (32)

$$S_B(r,t) \propto c_B(x_{\mathrm{cr}})/c_B^0 \propto \int_0^{x_{\mathrm{cr}}} b(x_{\mathrm{cr}},\tau) \cdot \frac{f_B(x)}{f_A(x)} q_A(x)\,\mathrm{d}x \tag{32}$$

while for LSM, Formula (33) applies:

$$S_B(r,t) \propto \delta c_B(r,t)/c_B^0 \propto x \cdot \frac{f_B(x)}{f_A(x)} q_A(x) \tag{33}$$

The proportionality coefficient in both formulae is chosen in such a way that the normalisation criterion of the density function ($\int q(x)\,\mathrm{d}x = 1$) is fulfilled.

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2) The following parts of Clause 6 are focused on the determination of particle size distributions.

Whichever approach is chosen, any conversion increases the uncertainties of the size distribution, in particular at the fringes of the distribution function. The integral effect of this error propagation can be calculated from the original measurement data and helps to qualify the meaningfulness of the conversion.[25] In addition, non-spherical particles require different quantity functions [$f_A(x)$ and $f_B(x)$] to spheres. In practice however, the quantity functions for spheres are employed in each situation. This introduces an error which is the more pronounced the larger the deviation is from a spherical shape.

6.3.6 Total quantity of dispersed phase and non-normalised particle size distributions

Particle size distributions are commonly presented by normalised functions, i.e. the total integral of the density function $q_{\mathrm{toq}}(x)$ amounts to 1 and the sum function $Q_{\mathrm{toq}}(x)$ varies between 0 and 1. That is because the detected signals cannot always be transformed into absolute quantities, such as mass concentration, and because normalisation ensures the comparability of measured data.

However, most centrifugation techniques facilitate the direct measurement of particle concentrations in the measurement zone or, at least, their calculation from measured data. Hence, they offer a chance to derive a non-normalised size distribution, which quantifies the particle concentration of the different size fractions:

$$\text{fractional concentration: } c_{\mathrm{toq},i} = c_{\mathrm{toq,tot}} \cdot \Delta Q_{\mathrm{toq},i} \tag{34}$$

$$\text{concentration density: } C_{\mathrm{toq}}(x) = c_{\mathrm{toq,tot}} \cdot q_{\mathrm{toq}}(x) \tag{35}$$

where

$C_{\mathrm{toq}}(x)$	concentration density function with respect to the quantity indicated by the index "toq"
$c_{\mathrm{toq},i}$	particle concentration of the *i*-th size class, the type of quantity is indicated by index "toq"
$c_{\mathrm{toq,tot}}$	total particle concentration, the type of quantity being indicated by the index "toq"
$q_{\mathrm{toq}}(x)$	density function of particle size distribution with respect to the quantity "toq"

In practice, non-normalised particle size distributions only refer to the number, volume or mass of the quantities.

NOTE Non-normalised particle size distributions may also be expressed with extensive quantities (e.g. total mass of original sample below 100 nm) rather than by concentrations.

Prerequisites for the derivation of non-normalised size distributions include the following:

— particles contribute independently from each other to the evaluated signal (i.e. light extinction, refractive index increment or X-ray attenuation);

— particle contributions superpose linearly.

Moreover, the size dependency of the signals, i.e. the quantity function (see Formula (31) and 6.3.5), needs to be known. For centrifugation analyses operated in HSM, it is sufficient to use the signals of the initially well-mixed sample and to combine them with the measured, normalised particle size distribution in order to derive the total particle concentration in the measurement cell, as shown in Formula (36):

$$S_{\mathrm{toq},t=0} \propto c_{N,\mathrm{tot}} \cdot \int f_{\mathrm{toq}}(x) q_0(x) \mathrm{d}x \propto c_{V,\mathrm{tot}} \cdot \int f_{\mathrm{toq}}(x) \big/ x^3 \, q_3(x) \mathrm{d}x \tag{36}$$

where the proportionality coefficients depend on the instrumentation and are well-specified.

When the LSM is employed, the signals for each instant reflect the concentration density of the passing size fraction, which has already experienced radial dilution or thickening for settling or rising particles, respectively (see Formula (18) and Reference [26]). Therefore, the total number, volume or mass concentration of the original sample is obtained by summation of the measured signal over the complete period of centrifugal phase separation:

$$c^0_{\mathrm{toq}} \propto \sum \delta c_{\mathrm{toq}}(r,t_k) \propto \sum S_{\mathrm{toq}}(r,t_k)\ \delta t_k = \frac{1}{K_{\mathrm{c}} x^2_{\mathrm{cr}}(r,t_k)} \cdot \frac{\delta r}{r} \tag{37}$$

where

$c^0{}_{\mathrm{toq}}$	original particle concentration with respect to the specified type of quantity
r	radial position
S_{toq}	measured signal, the type of quantity is indicated by index "toq"
t_k	time step
δc_{toq}	increment in particle concentration related to specified time step

As the instantaneous signal refers to a certain size range [see Formula (F.11)], time steps have to be chosen adequately.

For instance, number and volume concentration are derived from Formulae (38) and (39) respectively:

$$c^0_N \propto \sum \delta c_N(r,t_k) = \sum \frac{\delta c_{\mathrm{toq}}(r,t_k)}{f_{\mathrm{toq}}(x_{\mathrm{cr}}(r,t_k))} \propto \sum \frac{S_{\mathrm{toq}}(r,t_k)}{f_{\mathrm{toq}}(x_{\mathrm{cr}}(r,t_k))} \tag{38}$$

$$c^0_V \propto \sum \delta c_V(r,t_k) = \sum \frac{x^3_{\mathrm{cr}}(r,t_k) \cdot \delta c_{\mathrm{toq}}(r,t_k)}{f_{\mathrm{toq}}(x_{\mathrm{cr}}(r,t_k))} \propto \sum \frac{x^3_{\mathrm{cr}}(r,t_k) \cdot S_{\mathrm{toq}}(r,t_k)}{f_{\mathrm{toq}}(x_{\mathrm{cr}}(r,t_k))} \tag{39}$$

where the proportionality coefficients depend on the instrumentation yet are well-specified.

Computations based on Formulae (36) to (39) refer to the quantity of particles in the analysed sample. This quantity is typically adjusted to match the working range of given instrument. Hence, comparison among different samples should account for preparative steps, such as sample dilution.

The determination of absolute particle quantities and non-normalised particle size distributions constitutes a bonus feature of some commercial instruments but cannot be considered as highly reliable for arbitrary types of particle systems, i.e. without restriction to particle shape, polydispersity, size range or interfacial aspects (see Reference [26]).

6.3.7 Measurement analysis with respect to sedimentation and diffusion

Particles are subject to a stochastic, non-directed Brownian motion, which can become relevant for centrifugation analysis if particles are very fine ($x < 100$ nm). Brownian motion results in a smearing of concentration gradients and, thus, artificially broadens the measured distributions of sedimentation coefficient or particle size, when the data analysis of the previous subclauses is applied. The relevance of Brownian motion depends on the dimensionless Péclet number, which can be regarded as the ratio of the sedimentational to diffusional flux:[27]

$$Pe = \frac{v_{\mathrm{sed}} \Delta r}{D_{\mathrm{p}}} = \frac{a_{\mathrm{c}} \tau_{\mathrm{sed}} \Delta r}{D_{\mathrm{p}}} = \frac{9\pi a_{\mathrm{c}} \Delta r}{\mathrm{k_B} T} \sqrt{\frac{\eta^3 \tau^3_{\mathrm{sed}}}{\Delta\rho}} = \frac{\pi a_{\mathrm{c}} \Delta\rho x^3 \Delta r}{6 \mathrm{k_B} T} \tag{40}$$

where

a_c	centrifugal acceleration
D_p	particle diffusion coefficient
$\mathrm{k_B}$	Boltzmann's constant, $\mathrm{k_B} \approx 1{,}381 \times 10^{-23}$ J/K
T	temperature
v_sed	terminal sedimentation velocity
x	particle diameter
Δr	radial sedimentation distance
$\Delta \rho$	density contrast between the dispersed and continuous phase
τ_sed	sedimentation coefficient

A different definition for the Péclet number exists referring to a factor of two.[11] According to Formula (40), the ratio of the sedimentation to the diffusion flux depends on particle parameters (size and density) and on characteristic centrifugal parameters (acceleration and radial sedimentation distance). As a rule of thumb, the impact of Brownian motion can be ignored for Pe > 200.[5]

EXAMPLE Consider the centrifugation of colloidal silica in an aqueous medium $\Delta\rho$ = 1,2 g/cm^3), the centrifugal acceleration is to be 1 000 × g and the measurement position is 10 mm from the start position. According to the criterion of Reference [5] (Pe_cr = 200), it is permissible to ignore the impact of Brownian motion for particles larger than 21 nm. This limit would rise for a lower density contrast (e.g. to 54 nm for $\Delta\rho$ = 0,1 g/cm^3) or a lower centrifugal acceleration (e.g. to 76 nm for a_c = 30 × g).

Formula (40) indirectly defines a lower size limit for a dominant sedimentation flux. For finer particles, Brownian motion significantly affects the evolution of the concentration profiles in the measurement cell and, as a consequence, the simplified transport equation [Formula (10)] fails to quantify this impact. Instead, it needs to be expanded to also account for diffusion fluxes (such as the Lamm equation[28]):

$$\frac{\partial c}{\partial t} = -\frac{\omega^2 \tau_\mathrm{sed}}{r} \frac{\partial}{\partial r}\left(r^2 c\right) + D_\mathrm{p} \cdot \frac{1}{r} \frac{\partial}{\partial r}\left(r \frac{\partial c}{\partial t}\right) \qquad (41)$$

The solution of this equation and its application to derive distribution functions requires numerical tools.[29-31] Such solutions do not only provide the distribution of sedimentation coefficient or particle size, but also the distribution of the particle diffusion coefficient, which can be further transformed into a hydrodynamic equivalent diameter, a shape factor,[32] the molar mass[29] or particle density.[15] Indeed, the complete result of a centrifugation analysis in the Brownian regime is a two-dimensional distribution of two independent dispersity characteristics.[8],[16] In practice, such a multi-dimensional particle characterization is restricted to small Péclet numbers (1,4 < Pe < 60[11]), which typically corresponds to nanoparticles (< 100 nm), vesicles (e.g. liposomes), and micelles or macromolecules (e.g. RNA, proteins).

A special feature of centrifugation within the Brownian regime refers to the process of phase separation. Unlike with coarse particles (i.e. large Péclet numbers), this process does not end with the finest particles arriving in the sediment or cream layer. Instead, a concentration equilibrium of particles evolve between sedimentational deposition and the diffusion-driven counter-flux. The concentration profile in this layer is a function of Péclet number and can be also used for the determination of additional particle parameters.[33]-[35],[95],[96]

6.4 Deviations from Stokes-based analysis

6.4.1 General

The calculation of particle size from the sedimentation velocity according to Formula (8) relies on the following assumptions:

- Particle motion:
  always in steady state; balance among gravity, buoyancy and hydrodynamic drag; under creeping flow; no viscous interaction with neighbouring particles and walls.
- Particle properties:
  spherical shape; smooth and rigid surface; homogenous density; inert with respect to liquid.
- Liquid properties:
  incompressible, Newtonian.

The individual effects are discussed partly in Annex C and in ISO 13317-1, which covers gravitational sedimentation analysis.

Some of these assumptions can be addressed by proper sample preparation (see 7.2 and 7.3). For instance, liquids should show Newtonian behaviour, but shall not promote dissolution or swelling and may contain dispersing agents that impede particle agglomeration.[10]

Other assumptions set limits on the working range, with regard to particle size and concentration, or they must be addressed by data analysis.

6.4.2 Upper limit for sedimentation velocity and particle size

The assumption of creeping flow (see 6.1) imposes general upper limits for particle size and sedimentation velocity, because it only applies for small particle Reynolds numbers, which is specified by Formula (42):

$$Re_\mathrm{p} = \frac{\rho_\mathrm{c} v_\mathrm{sed} x}{\eta_\mathrm{c}} = \frac{\rho_\mathrm{c} a_\mathrm{c} \tau_\mathrm{sed} x}{\eta_\mathrm{c}} \tag{42}$$

where

a_c	centrifugal acceleration
v_Sed	terminal sedimentation velocity
x	particle size
η_c	dynamic viscosity of the continuous phase
ρ_c	density of the continuous phase
τ_sed	sedimentation coefficient

Conventionally, a threshold value of $Re_\mathrm{p} = 0{,}25$ is used.

It is often more convenient to calculate the upper limits of size and sedimentation velocity from the corresponding thresholds of the dimensionless Archimedes and Ljaščenko numbers (see C.1). These equations show to which degree the upper limits of particle size and sedimentation velocity can be shifted to values above 100 µm and 1 mm/s without violating the creeping flow condition, but only by adjusting the viscosity and the density of the continuous phase.

Apart from the creeping flow condition, there are further instrument-specific restrictions on operations conditions that directly or indirectly affect the maximum particle size or sedimentation velocity.

- The impact of gravity on phase separation should be minimised.
  If the centrifugal rotor is horizontally aligned, gravitational sedimentation occurs perpendicular to the centrifugal particle motion., causing deposition of particles at the lower cell wall. Deposited layers can induce convective flow due to density gradients. It is recommended to use a minimum RCA of 30, which sets a limit for maximum particle size at which the creeping-flow condition is fulfilled.

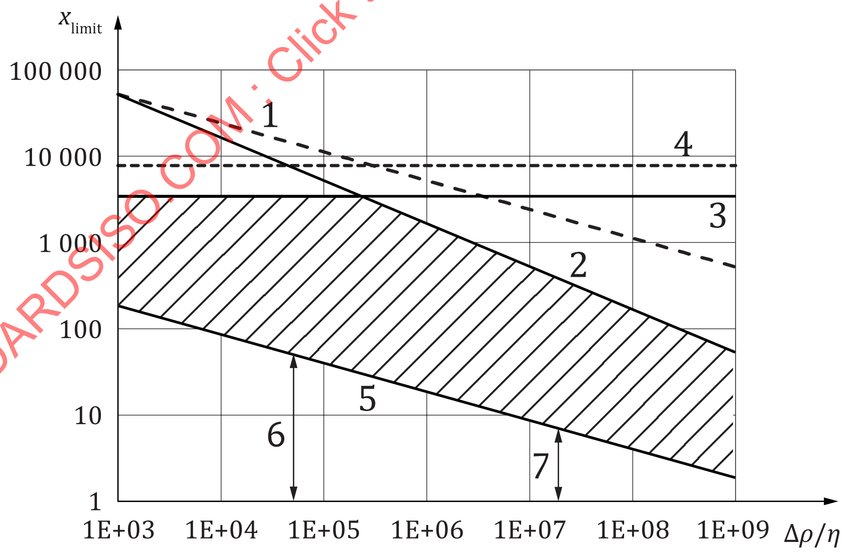
- The sedimentation of the fastest settling particles should be detectable.
  For this purpose, the time resolution (Δt_{min}) of the measurement should be adjusted in such a manner that the first signals represent the fastest sedimenting particles. The finest available time resolution defines the upper limits for velocity ($v_{\text{sed,max}}$) and thus size ($x_{\text{Stokes,max}}$):

  $$v_{\text{sed,max}} \le 0{,}5 \Delta r / \Delta t_{\text{min}} \tag{43}$$

- The primary measurement signals are not markedly affected by stochastic number fluctuations. Incremental centrifugation techniques probe the particle quantity in a measurement zone of defined volume, which decreases monotonically during the centrifugation process due to radial dilution and phase separation. The average number, *N*, of particles in the measurement zone obeys the Poissons statistics, which means that signals are principally affected by stochastic number fluctuation of particles in the measurement zone. If the impact is kept below 2 %, the particle number needs to exceed 4 000.

6.4.3 Lower limits for particle size

Brownian motion results in a diffusional nondirectional flux which superposes the migration in the centrifugal field and essentially broadens the sedimentation front. The average Brownian displacement is proportional to the square root of time, unlike a migratory displacement which grows linearly with time. Hence, the impact of Brownian motion on the results of a sedimentation analysis becomes lower with progressing phase separation and increasing sedimentation distance. The relevance of this adverse effect depends on the Péclet number, as discussed in 6.3.7. It is important to avoid temperature gradients perpendicular to the migration direction.



Key

$\Delta\rho$	density contrast, in kg/m^3
η	dynamic viscosity, in Pa·s
x_{limit}	limits for particle size, in nm
1	upper limit due to creeping flow condition
2	upper limit due to a time resolution of 1 s
3	upper limit due to number fluctuation, for a volume fraction of 0,01 %

4	upper limit due to number fluctuation, for a volume fraction of 0,1 %
5	lower limit due to Brownian motion
6	horizontal position for polystyrene particles in water
7	horizontal position for gold particles in water

NOTE This figure shows a measurement range of CLS for RCA of 3 000, which is valid for aqueous dispersions.

Figure 4 — Measurement range of CLS for RCA of 3 000

6.4.4 Limits for particle concentration

Stokes Formula (4) was developed for the unhindered settling of a single particle in an infinite liquid. This assumption is only fulfilled for dilute (polydisperse) particle systems of volume fractions of the dispersed phase below 0,5 %. Under these conditions, individual particles settle sufficiently independently from each other.

The lower concentration limit essentially results from the limit of quantity detection. This means that it is defined by the need for sufficiently high signals for the quantification of particles. It may be partially overcome by increasing the measuring volume by increasing the optical path length by using adequate optical cells. Hence, these are the specific limits for the various centrifugation techniques and can depend on the material (X-ray centrifuges) and even particle size (e.g. photocentrifuges). They are discussed in the other parts of the ISO 13318 series.

6.4.5 Handling of porous and heterogeneous particles

Transformation of sedimentation velocity into particle size [see Formula (8)] relies on the material properties, in particular the particle density. For homogeneous liquid and solid particles, data is commonly available in textbooks or in material data sheets (e.g Reference [36] and [37]). However, quite a few particulate materials consist of inhomogeneous particles. That is, particles can:

— have open or closed pores;

— be coated;

— be composed of multiple constituents.

Since particle size analysis typically aims at the outer dimensions of moving entities, the apparent particle density is employed for calculating particle size. The apparent particle density can be considered as the average density of the settling entity. It is identical to the buoyant density in the absence of open pores (for details, see Annex B). This facilitates the experimental determination of the relevant particle density within the framework of sedimentation (see the ISO 18747 series). Pore issues regarding sedimentation techniques are discussed in ISO 13317-1.

6.4.6 Handling of non-spherical particles and particle agglomerates

In contrast to the assumption behind Formula (4), the majority of particle systems that are characterized by analytical sedimentation consist of non-spherical and evenly agglomerated or aggregated particles. Moreover, particle shape and agglomerate structure are typically distributed, unknown quantities. For the sake of feasibility, all specific effects of non-sphericity [e.g. deviation of drag force as per Formula (C.1) and possible orientation distribution] are ignored and an equivalent diameter, the Stokes diameter, is computed from Formula (8). This calculation uses the buoyant density of the particles and yields size values, which are smaller than e.g, the hydrodynamic or volume equivalent diameters. For close-packed agglomerates, which are typically isometric and with uniform porosity, the buoyant density can be replaced by the apparent density, which refers to the constituent particles and the stagnant liquid in the voids. In this case, the calculated size is a hydrodynamic equivalent diameter. This approach fails for fractal-like agglomerates with size-dependent, rather high porosity, and only buoyant density should be employed.

7 Performing size analyses

7.1 General

The granulometric characterization of particle systems with regard to particle size distribution comprises several steps, all of which affect the outcome of the analysis. Hence, they need to be thoroughly planned and organised in advance. A starting point for each analysis is the definition of its objectives, which specify the relevant state of dispersion, the suitable measurands and their acceptable uncertainty. Measurands in the context of sedimentation analysis:

— can refer to the distribution of sedimentation velocity or the one of the Stokes diameter;

— can consist of selected distribution parameters, e.g. median, arithmetic mean or modal sizes;

— are specified with regard to the type of quantity.

In addition, all data that ensure an adequate analysis of measurement, such as material properties and applicable analysis algorithms, should be identified. After all pre-considerations are complete, the granulometric analysis can start. Typical steps are described in the subclauses 7.2 to 7.8.

The quality of an analysis depends on whether all of these steps are conducted in an adequate and careful manner. In addition, the quality is influenced by sample properties (e.g. polydispersity, optical properties of particle and its concentration, density contrast), instrumentation (e.g. type of sedimentation technique and corresponding details, such as the wavelength of radiation) and measurement conditions (e.g. temperature, temporal resolution).

7.2 Sampling

A representative sample is taken from a powder or liquid dispersion by using adequate techniques for sampling and sample splitting (see ISO 14488) and obeying constraints on sample mass or volume resulting from the demands of the measurement technique and the requirements for statistical uncertainty.

7.3 Continuous phase and primary sample preparation

Sample preparation constitutes a crucial step in granulometric analyses because it defines, or at least affects, the state of dispersion and thus the measured quantity. Errors and uncertainty in particle size analysis are commonly caused by improper sample preparation.

The purpose of primary sample preparation consists of producing stock suspensions or emulsions which have experienced a specified dispersion process and are thus defined with respect to their state of dispersion. Hence, primary sample preparation typically modifies the original sample in order to ensure a reproducible granulometric state. For sedimentation analysis, it yields a master sample which can be further divided to conduct multiple measurements with one instrument or comparative analyses with different granulometric techniques.

The primary sample preparation consists of various steps, which depend on the objectives of analysis and the type of the original material. Powders shall be suspended in an appropriate liquid (i.e. a continuous phase, dispersion liquid) at first and then dispersed in it, which includes both distributive and disruptive processes (see ISO/TS 22107). The dispersion process can be supported by wetting agents and the liquid may contain dispersing agents, which help to maintain colloidal stability. The liquid shall ensure the chemical stability of the dispersed phase (i.e. neither dissolution, nor chemical reaction) and its sedimentation (i.e. the density contrast must not vanish). In addition, the liquid should be chemically compatible with the material of the measurement cell. Reference [38] lists liquids and dispersing agents which have proven successful for a couple of solid materials.

If the original material is a liquid dispersion, the original continuous phase (dispersion medium) is typically used, although a different liquid is possible. In some situations, such as trying to retard the sedimentation process, it can be helpful or necessary to adjust the density contrast by adding solutes to the original continuous phase.

Independent from the initial state of the material, the dispersion process constitutes a central element of sample preparation and decides on the size of the particulate entities that are probed by sedimentation analysis. The dispersion process shall be specified, i.e. the dispersion technique, the intensity of treatment and the dispersion energy. These parameters should be selected as to best match the state of the particulate material at the relevant process, product application or test scenario.

The primary sample preparation can also include the defined removal of oversize particles. Its purpose is to ensure that all particles fall within the acceptable limits of the size range (see 6.4.1). Upper size limits of analytical centrifuges are typically much larger than 1 µm. For that size range, wet sieving (see ISO 2591-1) with test sieves of electroformed sheets (ISO 3310-3.) is the preferred method of coarse particle removal because it provides the sharpest separation and allows a gravimetric quantification of the coarse particle content.[39]

The following shall be specified when conducting reproducible sample preparation for sedimentation analysis:

- the continuous phase and possible additives (substances and concentration);
- particle concentration;
- technique(s) employed for dispersing the particles;
- if applicable, settings that correlate with the local stress intensity (e.g. rotational speed for given geometry, or power densities);
- the energy density (e.g. as determined by calorimetric analyses, or calculated from pressure drop and flow rate);
- further conditions of the dispersion process (e.g. temperature, cooling systems);
- if necessary, relevant data on the vessel (beaker) containing the sample during dispersion (e.g. for indirect ultrasonication with ultrasonic baths or inverted cup-horns)

See ISO 14887 and ISO 20247 regarding dispersion procedures for powders in liquids.

7.4 Secondary sample preparation (sample conditioning)

The master samples of the defined state of dispersion, which were produced at the primary sample preparation, typically require further processing before the measurement can take place. The purpose is to adapt the sample to the measurement conditions without affecting the particle size distribution. Secondary sample preparation can involve:

- sample splitting (i.e. producing aliquots, see ISO 14488 for further information);
- dilution;
- adjustment of viscosity or density;
- thermal equilibration.

It is mainly driven by specific requirements of the instrumentation (e.g. sample volume, working range with respect to particle concentration) and the analytical objectives (e.g. evaluation of the temperature sensitivity).

A typical task is the adjustment of particle concentration via dilution, for avoiding mutual sedimentation hindrance and for adapting to the linear range of sensing techniques (see 6.4.2 and 6.4.3). Dilution of fine particle dispersions can severely affect their stability against agglomeration (e.g. due to changes in pH) or promote dissolution of the dispersed phase. Hence, the impact of dilution on the state of dispersion should be checked and the granulometric state should be maintained (see ISO 14887).

Adjustment of the continuous phase' viscosity and density can be meaningful for the analysis of heterogeneous dispersions with multiple dispersed phases.

7.5 Instrument preparation

Before an analysis, the instrument needs to be set up for operation. Procedures for instrument preparation depend on the sedimentation technique and instrumentation used. The instrument manufacturer's instructions should be followed. Instructions can include:

- the formation of a density gradient (LSM);
- a thermal equilibration of the measurement zone for the intended measurement temperature;
- the specification of measurement parameters in the instrument software (e.g. temperature, wavelength, temporal resolution of signals);
- the selection of measurement cells (disc or cuvettes), which can differ with regards to volume, pathlength of measurement zone or resistance to liquids or which can be specifically designed for settling or creaming particles.

Thermal equilibrium is achieved by running the instrument at operation conditions without samples as the internal heat production depends on rotor speed. This step is especially important when the specified measurement temperature deviates from room temperature. Typically, such procedures are described in detail in the manuals of the specific centrifuges. Optimum measurement conditions depend on both instrumentation and sample, for which reason their determination can require preliminary experiments.

7.6 Measurement

7.6.1 General procedures for CLS

After instrument operational readiness (see 7.4), general procedures for the measurement of sedimentation velocity, sedimentation coefficient and particle size and their distributions shall conform to the following:

- specify the measurement condition according to the SOP and the name of sample(s);
- any prepared master sample (see 7.2 and 7.3) shall be homogeneously mixed and thermally equilibrated to the measurement temperature by appropriate means;
- if possible, maintain a constant temperature of the sample and perform continuous recording of sample temperature during the measurement;
- keep the time between master sample preparation and filling of the test sample into the measuring cell as short as possible;
- time-resolved, original instrument readings, including temperature and rotor speed, should be saved together with the instrument settings by software.

Repeated measurements should be performed for instrument repeatability, including sample preparation (see Clause 8 for more details). Repeated analyses cannot replace tests of signal quality and plausibility checks. Such tests depend on the respective measurement technique, and can refer to:

- the position of the liquid-air interface:
  it should be correctly detected by software (if applicable) and any temporal changes (e.g. due to evaporation, repeated measurements with the same filling of spin fluid) should stay within certain limits;
- initial and final signals:
  these are values indicating the transmission (attenuation) of radiation through the sample, usually the plausibility can be tested at the start of the measurement (e.g. expected attenuation based on mass concentration of master sample) and the finished sedimentation process (zero concentration of dispersed phase); additionally, requirements on the range of signal values in order to ensure a linear instrument response (physical model, sensor characteristics) can apply;
- shape of time curves or concentration profiles:
  profiles facilitate a discrimination between settling or creaming — in the context of particle size analysis, this should agree with expectation based on material properties; moreover, concentration profiles are

monotonic for all monoconstituent disperse systems and for multi-constituent samples, if the sign of the density contrast agrees for all dispersed constituents; time curves of transmission or attenuation should be monotonic for all types of material.

To a large degree, the specific measurement procedures depend on the initial distribution of the dispersed phase in the continuous phase, i.e. on whether the measurements are conducted with HSM or LSM (see Figure 1 and subclauses 6.3.2 and 6.3.3). The operational procedures for the two methods can be completely or partly controlled by instrument software, but they can also be left to the responsibility of the operator.

7.6.2 Procedures for homogeneous-start method (HSM)

To operate analytical centrifuges with HSM necessitates ensuring that homogeneously mixed samples are at the requested temperature in the measurement cells for the beginning of centrifugation. However, the time needed for thermal equilibration and phase separation under gravity poses a challenge. Appropriate measurement procedures, such as the following, are necessary for initial sample homogeneity and to avoid temperature drifts.

- Allow the instrument to reach the programmed temperature of the rotor, set via SOP. This is especially important if the set measurement temperature is below or above room temperature (if applicable). Check the temperature recording, as it can oscillate. Add the equilibrated samples (see below) only after oscillations have fallen below the temperature set precision.
- Place the optical fillers or filters on the rotor during temperature equilibration (applies for multi-channel instruments) if they are used to check performance and collect corresponding data for each sample run.
- Fill the measurement cell with the sample as gently as possible, avoid high shear stress and the creation of air bubbles. Follow the recommended sample volume (use pipettes or syringes or a balance). Close the cell.
- Equilibrate the cells in a thermostat. If the master batch was thermally equilibrated outside, fill the sample into the cells (see above) afterwards.
- Open the thermally equilibrated centrifuge and place the filled cell(s) into the instrument immediately. This is especially important in the case of fast sedimenting particles.
- Start the centrifuge and data acquisition.
- After stopping the centrifuge, remove the cell(s).
- Analyse the saved instrument readings by instrument software or specified algorithms. Result changing settings, like smoothing, etc. have to be registered.

For more information see 7.6.1.

7.6.3 Procedures for line-start method (LSM)

The LSM is mainly applied to disc-type centrifuges with photometric or interferometric detectors. It requires the implementation of a density gradient in the spin liquid phase and a subsequent calibration with reference particles. Repeated calibration helps to cope with degradation of the gradient and with any possible temperature drifts. The LSM procedure is as follows.

- Verify the cleanliness of the disc and, if needed, clean the outer and inner walls of the disc according to the instrument manufacturer's instructions.
- Inject the corresponding liquids to create the density gradient followed by some organic solvent to prevent evaporation from cooling into the rotating disc. Follow manufacturers' instructions.
- Allow time for the creation and stabilisation of the density gradient within the spin fluid. Follow the manufacturer's instructions.
- Allow the instrument to reach a constant extinction reading and, if applicable, a constant temperature of the spin disc. Avoid changes in room temperature if the instrument does not have temperature control.

- Calibration measurements shall be performed before each sample injection by means of reference materials, in order to correct for changes in the gradient during the measurement session. The sample that is to be analysed may be spiked with the reference particles where this operation is compatible with the analytical software of the instrument. Inject the sample into the disc as gently as possible, avoiding high shear stress and the creation of air bubbles. Start data acquisition immediately after injecting the sample into the spinning disc.

- If the absorption reading is back to baseline, a new sample may be injected.

- Analyse the saved instrument readings by instrument-specific software or special algorithms. Result changing settings, like smoothing etc., shall be registered.

See 7.6.1 for general recommendations.

7.6.4 Criteria for adjusting the rotational speed

Unlike gravitational sedimentation, operators can influence the driving force of the migratory motion by adjusting the rotational speed of the centrifuge. This adjustment should provide an optimal resolution for the size distribution of the respective material. The following constraints should be taken into account:

- gravitational sedimentation of coarse particles to the lower wall of the rotor or measurement cells; the significance of this effect is inversely related to centrifugal speed and sedimentation distance;

- time resolution of the measurement, which defines the upper limit of sedimentation velocity size and thus particle size (see 6.4.1); the higher the centrifugal speed, the finer the coarsest particle that can be detected in the centrifugal experiment;

- longest acceptable duration of the centrifugal experiment; operation time can be restricted by laboratory rules or by physical constraints (e.g. degradation of a density gradient) which limits the smallest particle size to be measurable for a given centrifugal speed or, vice versa, defines the minimum speed required to measure particles of a given size;

- diffusional flux, which counteracts the sedimentational flux and affects the measured particle size distribution; in order to suppress the impact of particle diffusion, the centrifugal speed should be sufficiently high (see 6.4.2);

- stratification effects by convective instabilities due to coupling between a horizontal thermal gradient and a vertical gradient in the concentration of the dispersed fine particles[97] in rectangular cells; the onset of such instabilities can be suppressed or retarded at low RPM and low temperature;[100] they can be suppressed by using sector-shaped cells.[11],[98]

7.7 Data analysis

Centrifugal liquid sedimentation (CLS) techniques generally monitor the sedimentation-induced phase separation in an initially homogeneous liquid dispersion by measuring indicative properties of the dispersed phase or the deposited particle layer, i.e. the sediment or cream layer. Correspondingly, each data set of the measurement comprises at least 3 parameters.

- Time elapsed after having stopped mixing.

- Position of the measurement zone.

- Value of the observed quantity (e.g. sediment mass or X-ray attenuation).

The analysis of these data can be considered as a tiered process:

a) Calculation of sedimentation velocities:
from the time and position of the data sets based on the definition of velocity. It does not require model parameters, yet assumes that the value of the observed quantity is attributed to a time point and space point (i.e. no smearing).

b) Calculation of particle sizes:
from the sedimentation velocity. Typically, the calculation is conducted by assuming spherical particles (i.e. the analysis yields the distribution of the Stokes diameter), Newtonian liquid, creeping flow condition and the absence of concentration effects. This transformation requires knowledge of the dynamic viscosity (η_c), density contrast ($\Delta\rho$) and centrifugal acceleration (a_c). See 6.1 and 6.3.1 for further information.

c) Computation of the distribution function with respect to the intrinsic type of quantity:
from the observed quantity based on Formulae (22) and (23) (integral techniques) or (26) and (28) (incremental techniques). It does not require model parameters.

d) Computation of the distribution function with respect to another type of quantity:
from the distribution function with respect to the intrinsic type of quantity and particle size based on a model that correlates particle size with the observed quantity [see Formulae (30) and (31)]. This computation frequently requires further model parameters, e.g. wavelength of radiation and refractive index (see 6.3.5 and Annex E).

The distribution of sedimentation velocity (weighted by the intrinsic type of quantity) can be derived from the measured data without additional model parameters. In contrast, the computation of particle distributions always requires information on the measurement conditions and the material properties. There are also several books with tabulated values of material properties (e.g References [36] and [37]). Additionally, parameters like particle density, liquid density, liquid viscosity or refractive index can be measured by means of established and standardised techniques (e.g. ISO 18747 series, ISO 15212-1, ISO 2555, ISO 3219 series and ISO 280). Data regarding temperature, centrifugal speed, the baseline data of sensors, as well as date and measurement time shall, at a minimum, be recorded for any measurement. Data should be saved by the instrument software together with a record of the time course of transmission or attenuation.

7.8 Reporting

Good laboratory practice requires documentation of all steps and results. Measurement reports shall:

— allow a reproduction of the analysis;

— visually emphasize the most important results;

— observe the international conventions in particle characterization, including ISO 9276-1.

The test report should include the following details:

a) File name of measurement/report.

b) Name and contact information of the customer.

c) Name and address of testing laboratory.

d) Operator.

e) Date of testing.

f) File name of report and link to where the experimental data and unique report identifier (where applicable) are stored.

g) Sample name (identifier) and type of sample (powder, suspension, emulsion) quantity.

h) Additional information about sample, e.g. particle shape, agglomeration state, density contrast of particles and liquid, viscosity and type of continuous phase, volume concentration, optical properties if necessary, and safety information.

i) ISO-standard applied for testing, and any other operation not specified in this or other applied parts of the ISO 13318 series, which can influence the result.

j) Instrument type and identifier used, software version, measurement mode HSM or LSM (see 3.28 and 3.29) respectively and date of last performance test.

k) Sampling place and conditions (if applicable).

l) Sample preparation:

  - Continuous phase and/or dilutant: composition (solvent as well as wetting and dispersing agents), and viscosity and refractive index, as well as relevant properties of dispersed phase (e.g. density, refractive index).
  - Dispersion and/or homogenisation process and instrumentation.
  - Condition, such as temperature, energy, time, volume, etc. (if applicable).

m) Characteristic parameters for the average particle size, including its unambiguous specification (type of location parameter, e.g. median, mode, arithmetic mean or harmonic mean, and indication of the type of quantity – e.g. weighted by extinction, X-ray attenuation, intensity, volume, mass, particle number) and details of measurement uncertainty (e.g. number of replicate analyses, standard deviation for the in-group-variation for a given preparation and the among group variation for individual sample preparations).

n) Information about polymodality, e.g. bimodal, central size of modes, and polydispersity, e.g. index of polydispersity. Graphical representation, if available.

o) If size quantities depend on particle concentration, size values have to be indicated for the lowest concentration or the value extrapolated to infinite dilution.

p) Principle of used algorithms (including references) for calculation of the distribution function.

q) Conditions of measurement:

  - relative centrifugal acceleration (rotor speed), Speed ramping protocol;
  - particle concentration, if known;
  - sedimentation time;
  - temperature of sample during measurement;
  - type of measurement cells and sample volume;
  - details on the mode of measurement:
    - LSM: disc, gradient, liquid to prevent evaporation, and sample volume.
    - HSM: type of cells (material, optical path), and sample volume.

r) Date of report.

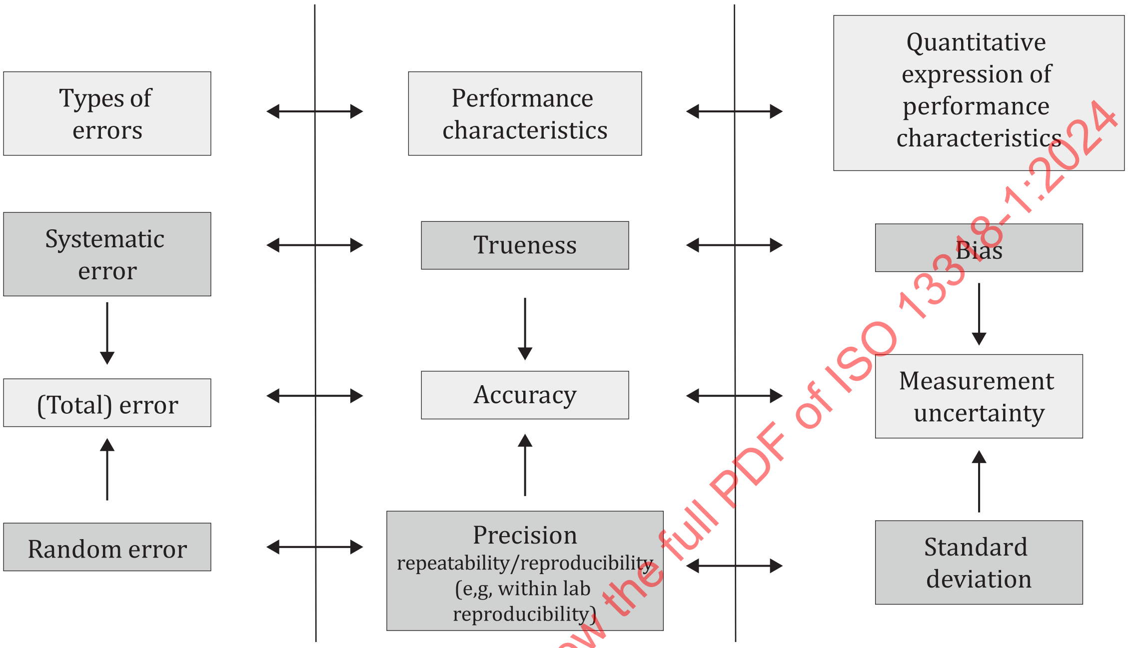
s) Name and position of the person releasing the report.

8 System qualification and quality control

8.1 General remarks

The measurement of the particle velocity by means of sedimentation using the homogeneous-start method (HSM) is based on first principles and does not require calibration by the user with a reference material (RM) of specified velocity or size. However, line-start method (LSM) does require calibration in regards to the signal response. The obtained sedimentation velocity can be transformed into particle size according to 6.1, by relying on the input quantities centrifugal acceleration (g), density contrast ($\Delta\rho$) and liquid viscosity (η_c).

Performance validation is determined by comparing measured values of the particle velocity and its deviation from the true value due to errors that are related to sample preparation (see 7.1 to 7.3), instrument design, measurement procedure, operator skill and environmental conditions. Sources of the measurement uncertainty can be random or systematic in nature (see Figure 5) corresponding to a reduction in precision and trueness. Trueness is estimated via the systematic deviations (bias) of measured size values from the true ones (e.g. approximately known for CRM), whereas precision may be quantified by the standard deviation, which is the variation among repeated measurements of a sufficiently stable quality control material (QCM) (i.e. RMs with proven homogeneity and stability can be a CRM). Trueness and precision determine the accuracy of a method, which is expressed quantitatively as the measurement uncertainty.



NOTE This figure shows interrelations between error types, the performance characteristics used to estimate them and expressions of quantitative estimates, adapted from Reference [40].

Figure 5 — Error types

8.2 Reference materials

Reference materials (RMs) serve different purposes (e.g. calibration or performance qualification) and, as such, they need to fulfil different requirements (see ISO TS 4807). However, RMs for sedimentation-based particle sizing shall consist of non-porous particles of homogeneous composition and uniform particle density. Spherical particles and narrow size distribution facilitate the comparison among different sedimentation techniques but are not a prerequisite. Polydisperse RMs are particularly advantageous for the evaluation of sedimentation techniques with regard to the sensitivity for fine and coarse particle detection. RM size distribution shall ensure the negligibility of Brownian motion (see 6.4.2), as well as hydrodynamic wall effects and wall adhesion (see 8.4). In case of CRMs, the certificate should:

a) refer to the distribution of Stokes diameters and clearly indicate this;

b) specify the kind of distribution parameter used and the type of quantity (e.g. median of volume-weighted distribution or modal size of extinction-weighted distribution);

c) include an uncertainty and coverage factor;

d) indicate the material properties used for data analysis (e.g. particle density);

e) reveal the type of sedimentation technique used in the certification process;

f) specify the procedures of sample preparation.

The temperature of use and continuous phases shall be specified for RMs. The continuous phase should ensure a fast wetting and easy dispersion of the particles (in the case of powder). The state of dispersion shall be preserved at the time of use, i.e. no swelling, shrinking, dissolution, flocculation or agglomeration (see ISO 14887). Some RMs are available as ready-to-use suspensions which only require a slight re-homogenization of deposited particles before being analysed. They avoid the risk of destabilisation by diluting or segregation due to sampling. If RMs are employed to test the instrument performance for specific materials, they should match the expected size range and resemble the desired material's properties (i.e. particle density and, if relevant, also refractive index or X-ray attenuation).

8.3 Installation, operational and performance qualifications

RMs are required for calibration (see 7.6.3), installation qualification, operational qualification and performance qualification. Installation qualifications indicate whether an instrument conforms to the manufacturer's specifications for installation. Generally, it is in the responsibility of the manufacturer to ensure correct installation. Installation qualification can be coupled with an operational qualification, which demonstrates that an instrument is able to produce correct results and for which purpose CRMs are used.

While operational qualification indicates the capability of deriving correct results, performance qualification tests if a method or an instrument performs consistently over time. Performance qualification shall be conducted at least once a year and after each major change of the instrument (such as repairs, change of location, etc.), as well as in case of any doubt about the validity of measurement results. It shall be performed in accordance with the manufacturer's instructions.

NOTE Local regulations can apply regarding quality. Testing organisation can also specify quality requirements.

Beside a general careful visual inspection, performance qualification should cover the full range of equipment capabilities, such as the following for example:

- the positioning of the sedimentation cell;
- constancy of the distance between the sensor system and cell bottom;
- precision of the clock and temperature measurement;
- the sensor system to quantify the concentration changes during sedimentation or the growth of the sediment.

Results shall be documented. One method to graphically record the results of regular performance tests, for example, is through the use of control charts (see Reference [99]) performed as stipulated by the organisation. This allows for the easy identification of performance changes over time. These performance tests serve to estimate the intra-laboratory reproducibility (NT TR 537) under conditions such as changing environmental conditions, preparation of primary and secondary samples, batches of chemicals, pipette tips, cells, room temperature fluctuations, skill of the operator, etc. For details, see ISO 13317-1.

8.4 Sources of measurement uncertainty

A major source for the variation among results of particle size analysis, in general, is inappropriate or insufficiently specified sample preparation, including sample splitting. This also applies to centrifugal liquid sedimentation (CLS). The importance of this general issue is addressed in Clause 7. The discussion in 8.4 assumes that samples have been prepared according to a well-defined procedure, which ensures a reproducible and stable granulometric state during the measurement and proper adaption to the measurement conditions, especially with regard to temperature and particle concentration. Given this, different sources for the measurement uncertainty in CLS can be discerned.

- A non-homogeneous mixing state at the start of sedimentation:
  Demixing can become relevant for samples containing particles with gravitational sedimentation velocity of 10 µm/s or above (i.e. commonly particles ≥1 µm). The time between placing samples into the centrifuge and starting the measurement should be kept short.

- Incorrect values for the radial sedimentation distance:
  Time and radial distance are the basic input parameters for calculating for sedimentation velocity and, thus, particle size and sedimentation coefficient (6.1). The sedimentation distance is the radial distance between the measurement position and the most inward or outward particle position (start position) for positive or negative density contrast respectively. For HSM, the start position is the meniscus position; for LSM, it is the position of interface between sample and spin liquid. In principle, the start position can be determined from cell geometry and liquid volume. Alternatively, the start position can be derived from photometric measurements. For volatile liquids and/or long centrifugation times, it can be necessary to cover the liquid surface in order to reduce evaporation.

- Width of the detection zone:
  As particle quantification by X-ray attenuation, light extinction etc. requires a sufficiently high signal-to-noise ratio, the measurement zone requires some radial extension. Therefore, signals refer to a range of radial distances, i.e. a range of sedimentation coefficients or particle sizes. This mainly causes a smoothing of measured size distributions, but can also affect the mean particle size.

- Incorrect values for sedimentation time:
  Recorded time signals are typically highly accurate. However, particle displacement during the initial phase of rotor acceleration is clearly shorter than in the same period of time during a later phase of centrifugation. If data analysis does not account for the initial low rotor speeds, this is equivalent to an overestimating bias in effective sedimentation time. This source of uncertainty is avoided for disc-type centrifuges operated with LSM.

- Non-intended variation of the rotor speed:
  The rotor speed is the decisive parameter for adjusting the centrifugal acceleration. It is frequently set to a fixed value. Speed ramps, that gradually or step-wise increase the rotor speed, are also used. The rotor speed fluctuates with low amplitude around the requested value, which shifts the average RCA above the nominal value. Yet, the effect is negligible in practice. More important is the deviation between nominal and real rotor speed during the initial phase of rotor acceleration. The time period depends on the desired speed and can amount to several seconds. The impact on measurands sedimentation coefficient and particle size can be neutralised if the varying speed is recorded and considered in data analysis. Otherwise, both measurands are underestimated, in particular for small sedimentation distances or short measurement times.

- Temperature variations in the measurement cell:
  Temperature fluctuations (related to temperature control) or temperature shifts (in the absence of an effective temperature control) can occur and be less than 1,0 K. Temperature gradients perpendicular to the path of sedimenting particles should be kept at a low level (< 0,1 K/mm) in order to prevent convective flow. In contrast, a small negative gradient along the radial position can prevent temperature driven convection.

- Uncertainty with respect to the viscosity and density of the liquid phase:
  These values are required for the computation of particle size (Stokes diameter). For LSM, these values are (implicitly) derived from the gradient calibration with reference particle systems with their own uncertainty range. Gradient calibration addresses the combined effect of density and viscosity gradient in the stratified spin liquid, which results in an effective deceleration along the sedimentation path. Multiple calibrations during one run of the centrifuge compensate for the steady degradation of the gradient and possible temperature shifts. Temperature changes can be also responsible for uncertain density and viscosity values in HSM, particularly when temperature shifts occur as the sample temperature lags behind the instrument temperature in an undefined manner.

- Uncertainties with regard to the quantification of particles:
  Such uncertainties can be related to sensor characteristics (e.g. stability of radiation intensity, signal noise, linearity, sensitivity) or to the applicability of Lambert-Beer law (i.e. linear concentration dependence of signals). They affect the minimum and maximum detectable particle fraction regarding velocity and size.

- Adhesion of particles at wall:
  Particles adhering at the walls of a measurement cell affect the quantification of dispersed particles.

This effect can be checked via visual inspection after the sedimentation process. Wall adhesion depends on particle properties, the wall material and the continuous phase.

- Uncertainty of the certified values of calibration materials:
  When data analysis of CLS relies on calibration with CRMs (see 7.6.3), the uncertainty of the certified values contributes to overall measurement uncertainty.

8.5 Accuracy and measurement of uncertainty of particle velocity and particle size

Precision (short-term repeatability, between-run variation or reproducibility) and trueness are the factors determining accuracy (Figure 5); they are discussed in detail in Annex D, ISO 13317-1:2024 as well as in Reference [99]. Precision is quantified by the corresponding mean values and standard deviations and can be determined both on the level of sedimentation velocity as well as particle size. Calculating particle size requires the knowledge of the viscosity of the continuous phases as well as the density contrast of both phases, see Formula (4) and (8).

Regarding the current state-of-the-art instrumentation for centrifugal liquid sedimentation (CLS) techniques, it is recommended to perform either of the following:

- ≥ 6 replicate measurements for 1 master sample to assess the repeatability of a measurement;
- ≥ 3 replicate measurements for each of ≥ 3 independent master samples to assess the intermediate precision of the analysis, including sample preparation.

Such repeated analyses yield statistical measures for the uncertainty due to stochastic variations of the measurement conditions. They cannot replace tests of signal quality and plausibility checks. For more details, see ISO 13317-1.

Trueness determination demands a CRM for the measurand velocity v or size x. Due to the lack of CRM for velocity, trueness estimations are mostly only performed for the particle size despite the fact that it can be transformed back based on Stokes' law.

8.6 Combined and expanded uncertainty of particle velocity and particle size measurement (Stokes diameter)

The basic uncertainty estimation is given in detail in ISO/IEC Guide 98-3. Several documents build on the ISO/IEC Guide 98-3 with the aim to simplify the calculations by not quantifying all uncertainty components separately. This is achieved by using long-term reproducibility measurements to estimate combined uncertainties (see Reference [99] or ISO 21748). The approach specified in NT TR 537 is used in this subclause, but other approaches, such as that specified in ISO 21748, are also valid. The uncertainty of a measurement indicates the possible extent of the measurement error. It quantifies an interval associated with the measured value that characterizes the deviation from the true value and where the true value lies with some high probability (Figure D.1). In general, uncertainty comprises many components. Some can be evaluated from the statistical distribution of the results of series of measurements (experimental uncertainty, often called standard deviation) and the other components (often called systematic effects), which can also be characterized by standard uncertainties (see Figure 5), are evaluated from assumed probability distributions based on experience or other information.

According to NT TR 537, the full uncertainty budget can be experimentally determined based on the concept of reproducibility (intermediate precision) measurement (estimated as the measurement standard deviation) and bias quantification of a specific sedimentation method. Bias can be estimated from repeated measurements of one or several CRM samples. The number of measurements performed on the CRM needs to be sufficiently high in order to ensure that the observed bias is not dominated by random uncertainties

(u_{rep}). Sources of bias uncertainty (u_{bias}) are the average root mean square bias of reproducibility results (b_{RMS}) obtained for the CRMs and the average uncertainty of the reference values u_{ref}:

$$u_{bias} = \sqrt{b_{RMS}^2 + u_{ref}^2} \tag{44}$$

where b_{RMS} is the average bias of the different CRMs and is calculated as the RMS of the bias, as follows:

$$b_{RMS} = \sqrt{\frac{1}{m}\sum_{i}^{m}\left(v_{lab,i} - v_{ref,i}\right)^2} \tag{45}$$

where

b_{RMS}	average bias for the all bias determinations with m CRMs
m	number of bias determinations carried out for different CRMs
$v_{lab,i}$	measured value of v for the i-th bias determination; it is the average of all determinations for a given CRM
$v_{ref,i}$	reference value of v for the i-th bias determination.

The average standard uncertainty of the reference values of the reference samples (u_{ref}) is the RMS of given value uncertainty.

In the context of a performance evaluation with respect to sedimentation velocity, one CRM shall be used with certified velocity values in the centre of the specified velocity range and another two CRMs with average sedimentation velocities that are twice as high as the lower velocity limit and about half the upper velocity limit, respectively. These velocity recommendations should be evaluated depending on the real-world particle properties, as detectable velocity ranges can be smaller under practical circumstances. For instance, the working range of photosedimentometers depends on the optical properties of the particles.

The combined standard measurement uncertainty, taking into account the reproducibility determination and the possible bias, is given in Formula (46).

$$u_c = \sqrt{u_{Rw}^2 + u_{bias}^2} \tag{46}$$

The calculated combined uncertainty (u_c) spans a range around the arithmetic mean, which covers 68,3 % of all measurement results if normally distributed (see Figure D.1). To increase the probability, a numerical factor, k (coverage factor) as a multiplier of u_c, is used to estimate the expanded combined uncertainty U_c [see Formula (47)].

$$U_c = k \cdot u_c = k \cdot \sqrt{u_{Rw}^2 + u_{bias}^2} \tag{47}$$

For the sedimentation velocity, usually a coverage factor ($k = 2$) can be proposed (with 95 % probability and normal distribution) to calculate the expanded uncertainty (U_c).

The expanded uncertainty describes the half-width of the uncertainty range of a measured sedimentation velocity $v_{sed,meas}$. The true sedimentation velocity v_{sed} lies within the range of $v_{sed,meas} \times (1 \pm U_{rel,c})$ with a 95 % probability (see Figure D.1). If the number of performed measurements is below 30, the Student's t-distribution should be assumed, resulting in a reduction of the probability. If the values of expanded combined uncertainty are communicated, coverage factor or assumed distribution should be indicated.

If corresponding CRM samples are not available, inter-laboratory comparison measurements can be used to estimate the combined uncertainty. A practical way is to calculate the uncertainty of the mean (standard deviation) of all participants of the interlaboratory test. In general, this approach leads to an overestimation of u_{bias}.

Annex D describes an exemplary procedure for the application of the uncertainty calculations described in this clause.

The full uncertainty budget is underestimated within Formula (47) because sampling and sample preparation of real-world materials can affect analytical results more strongly than in experiments with a QCM or CRM.

Annex A
(informative)

Particle sizing techniques based on centrifugal liquid sedimentation (CLS)

Table A.1 and Table A.2 describe the most common types of analytical centrifuges by a set of general measurement attributes. They also list relevant standards and instructive references.

Table A.1 — Characteristics of analytical centrifuges (AC) based on optical detection units

Category	cuvette-type, turbidity sensor (cuvAC-turb)	cuvette-type, interferometric (cuvAC-RI)	disc-type, turbidity sensor (discAC-turb)	cuvette-type, analytical ultracentrifuge (AUC)	capillary-type
Standards	ISO 13318-2, ASTM C1182-09	Non-existent	ISO 13318-2, ISO 15825, ASTM C1182-09	Non-existent	Non-existent
References	[41] and [42]	[43] and [44]	[45] and [46]	[2], [54] and [55]	Reference [53]
detected quantity:	optical particle concentration at defined position(s)	particle concentration at defined position(s)	optical particle concentration at defined position(s)	particle concentration at defined position(s)	profile of particle concentration within a capillary
detection method:	incremental	incremental	incremental	incremental	incremental
detection modes:	fixed position, scanning position, complete profile	fixed position, scanning position, complete profile	fixed position, scanning position	fixed position, scanning position, complete profile	
sample supply:	homogeneous start	homogeneous start	homogeneous start or line-start	homogeneous start, line-start	line-start
quantification:	photometric (extinction)	refractive-index increment	photometric (extinction)	photometric extinction, refractive index increment, fluorescence	photometric extinction or refractive index increment
intrinsic result	$Q_{\text{ext}}(x_{\text{Stokes}})$	$Q_{\text{RI}}(x_{\text{Stokes}})$ $(\approx Q_3(x_{\text{Stokes}})$ esp. for $x \ll 1$ µm)	$Q_{\text{ext}}(x_{\text{Stokes}})$	$Q_{\text{ext}}(x_{\text{Stokes}})$ or $Q_{\text{RI}}(x_{\text{Stokes}})$ or $Q_3(x_{\text{Stokes}})$	$Q_{\text{ext}}(x_{\text{Stokes}})$ or $Q_{\text{RI}}(x_{\text{Stokes}})$
special features	multiple wavelengths or spectral optics	estimates for particle concentration	estimates for particle concentration	extremely high centrifugal acceleration; spectral optics	historic use

Table A.2 — Characteristics of analytical centrifuges (AC) based on non-optical detectors

Category	disc-type, X-ray sensor (discAC-X)	pipette-type, (AC-pipette)	cuvette-type, manometric (AC-mano)	analytical centrifugation balance
standards	ISO 13318-3	Non-existent	Non-existent	Non-existent
references	[47]	[48], [49], and [50]	Reference [51]	Reference [52]
detected quantity:	X-ray attenuation at defined position(s)	particle concentration at defined position(s)	density of dispersion phase	mass of sediment
detection method:	incremental	incremental	integral	integral
detection modes:	fixed position, scanning position	fixed position	fixed position	fixed position
sample supply:	homogeneous start or line-start	homogeneous start	homogeneous start	homogeneous start
quantification:	X-ray extinction	gravimetric (on dried samples	manometric	force transducer
intrinsic result	$Q_X(x_{Stokes})$ (= $Q_3(x_{Stokes})$)	$Q_3(x_{Stokes})$	$Q_3(x_{Stokes})$	$Q_3(x_{Stokes})$
special features		historic use	historic use	historic use

Annex B
(informative)

Remarks on particle density

B.1 Particle density and particle size in sedimentation analysis

The transformation of sedimentation velocity into a particle size (Stokes diameter) according to Formula (8) requires solid knowledge of the density contrast between the particle and continuous phase. In general, particle density is a well-defined quantity, computed as ratio of particle mass to particle volume. However, in practical applications there can be some confusion when particles are inhomogeneous, that is, when they are composed of a different internal and external phase. This confusion mainly results from the question of which volume should be attributed to the particle.

From the perspective of an observer of sedimentation experiments, the question seems to have a simple answer at first. The complete migrating entity is considered as a particle. This entity has a sedimentation velocity (the measurand of analysis) and contributes to the observed quantity (e.g. sediment weight or light extinction). The density of this entity is a weighted average of all its constituents. This average is commonly called "apparent particle density" or "effective particle density" and can be calculated if structural details, such as the thickness of a coating or the specific pore volume, are known. Frequently, this density is identical to the buoyant density, which can be experimentally determined via sedimentation (see the ISO 18747 series) or pycnometry (see ISO 787-10 and ISO 8130-2). However, some types of particulate materials should be further considered.

- Porous particles: Open pores, which are filled with continuous phase in sedimentation or pycnometry, do not contribute to buoyancy; yet pore liquid is stagnant during sedimentation and is thus part of the migrating entity.
- Agglomerates and aggregates: Interstitial voids are filled with the continuous phase and do not contribute to the buoyancy; the void liquid is not necessarily stagnant. In addition, the specific void volume is frequently size-dependent; for small numbers of constituent particles or fractal dimensions below 2, it is not meaningful to define a void volume.
- Particles with adsorbed layers of surfactants, polyelectrolytes, polymers etc.: These layers are added to the original material in order to tune product functionality, ensure wettability, dispersibility, stability during application or just during measurement; though moving with the migrating particle, they are commonly not considered as part of the particle. A solvate shell, especially in case of nanoparticles, should be considered as well.

B.2 describes cases of experimental practice. It includes information on the buoyant density and apparent particle density.

NOTE The buoyant density can be understood as the (hypothetical) density of a continuous phase for which the gravitational force acting on the immersed particle is counterbalanced by buoyancy. Whereas the apparent particle density is the mass-to-volume ratio for the migrating particle, including all entrapped stagnant liquid and gas.

B.2 Selected cases

Table B.1 shows particle structures with corresponding remarks on the buoyant density and the apparent particle density.

Table B.1 — Selected cases of particle structure

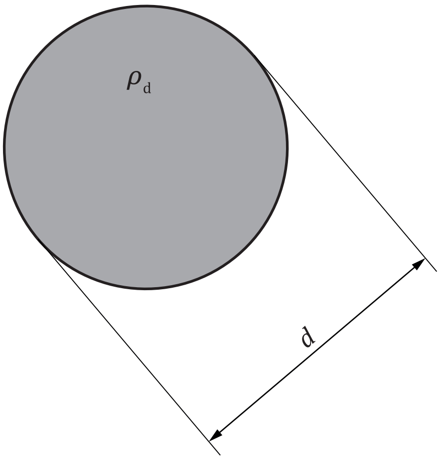
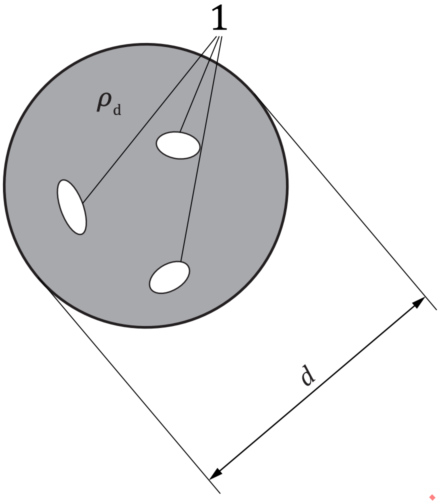
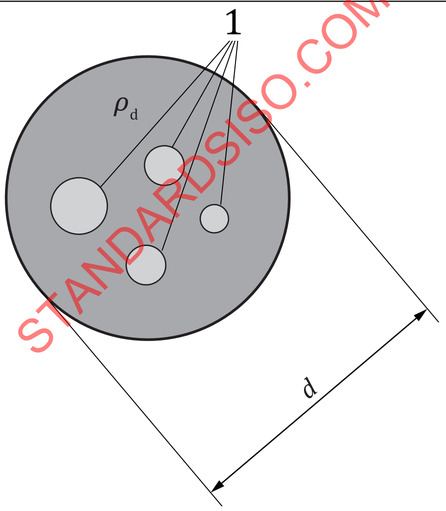
Configuration	Size and density
	Homogeneous particle without inclusions and pores; buoyant density: $\rho_{p,buoy} = \rho_d$ apparent particle density: $\rho_{p,app} = \rho_d$ where ρ_d denotes the true density of the dispersed phase, which is also known as true particle density (see ISO 18747-1:2018, 3.4) size: $d^2 = x^2_{Stokes} = \dfrac{18\eta_c v_{sed}}{g(\rho_d - \rho_c)}$
 **Key** 1 occluded voids (ρ=0)	Particle with occluded voids; buoyant density: $\rho_{p,buoy} = \rho_{p,sk} = (1 - \varepsilon_{occl}) \cdot \rho_d$ apparent particle density: $\rho_{p,app} = \rho_{p,buoy}$ where $\rho_{p,sk}$ denotes the skeleton density, which is also known as skeletal density (see ASTM D3766) size: $d^2 = x^2_{Stokes} = \dfrac{18\eta_c v_{sed}}{g(\rho_{p,sk} - \rho_c)}$
 **Key** 1 particulate inclusions (ρ_{incl}) e.g. droplets of a W/O/W-emulsion, debris particles from a composite material or coating.	Particle with particulate inclusions; buoyant density: $\rho_{p,buoy} = (1 - \varphi_{V,incl}) \cdot \rho_d + \varphi_{V,incl} \cdot \rho_{incl}$ apparent particle density: $\rho_{p,app} = \rho_{p,buoy}$ size: $d^2 = x^2_{Stokes} = \dfrac{18\eta_c v_{sed}}{g(\rho_{p,bouy} - \rho_c)}$

Table B.1 *(continued)*

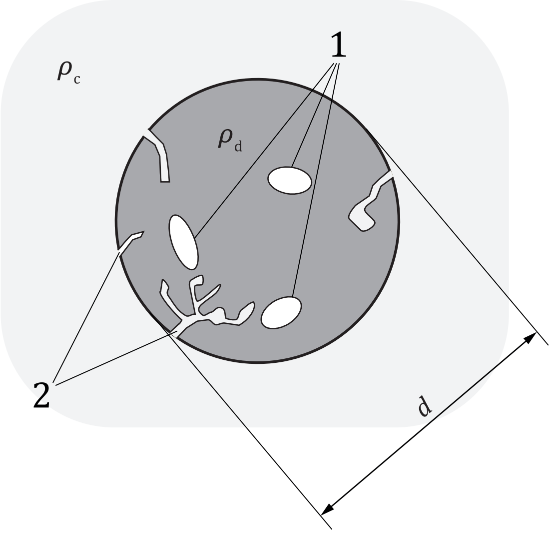
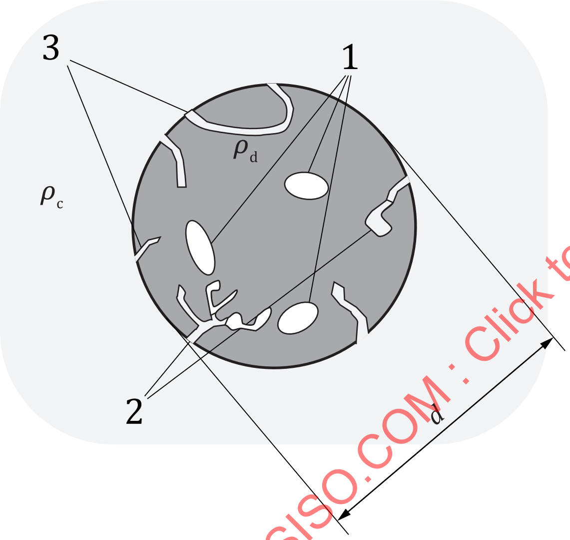
Configuration	Size and density
 **Key** 1 occluded voids (ρ=0) 2 open pores and surface fissures (ρ_c)	Particle with occluded voids and open, completely filled pores; buoyant density: $\rho_{p,buoy} = \rho_{p,sk} = (1-\varepsilon_{occl})\cdot\rho_d$ apparent particle density: $\rho_{p,app} = (1-\varepsilon_{tot})\cdot\rho_d + \varepsilon_{open}\cdot\rho_c$ size: $d^2 = x_{Stokes}^2 = \dfrac{18\eta_c v_{sed}}{g(\rho_{p,app}-\rho_c)}$
 **Key** 1 occluded voids (ρ=0) 2 partly filled pores ($\rho < \rho_c$) 3 open pores and surface fissures (ρ_c)	Particle with occluded voids as well as open and partly filled pores; buoyant density: $\rho_{p,buoy} = \left(1-\varepsilon_{tot}+\theta\cdot\varepsilon_{open}\right)\cdot\rho_d$ apparent particle density: $\rho_{p,app} = (1-\varepsilon_{total})\cdot\rho_d + \theta\cdot\varepsilon_{open\ pores}\cdot\rho_c$ where θ denotes the volume fraction of liquid in the open pores size: $d^2 = x_{Stokes}^2 = \dfrac{18\eta_c v_{sed}}{g(\rho_{p,app}-\rho_c)}$

Table B.1 *(continued)*

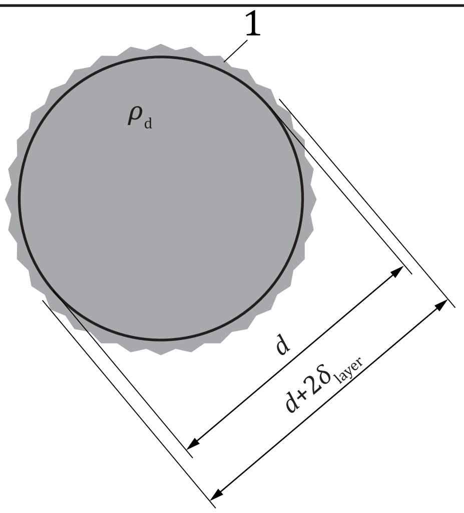
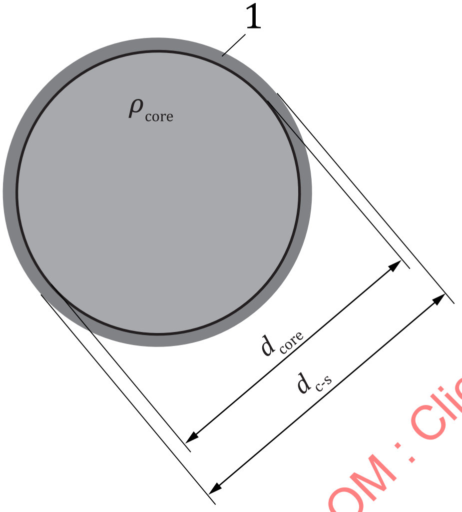
Configuration	Size and density
 **Key** 1 adsorbed layer (ρ_{layer})	Homogeneous particle with an adsorbed layer; buoyant density: $\rho_{p,buoy} = \left(1+2\frac{\delta_{layer}}{x_V}\right)^{-3} \cdot \left(\rho_d - \rho_{layer}\right) + \rho_{layer}$ $\rho_{p,buoy} = \left(1+2\frac{\delta_{layer}}{d}\right)^{-3} \cdot \left(\rho_d - \rho_{layer}\right) + \rho_{layer}$ apparent particle density: $\rho_{p,app} = \rho_{p,buoy}$ size: $\left(d+2\delta_{layer}\right)^2 = \frac{18\eta_c v_{sed}}{g\left(\rho_{p,buoy} - \rho_c\right)}$ sedimentation only yields outer dimension, i.e. $d+2\delta_{layer}$.
 **Key** 1 fixed coating/shell (ρ_{shell})	Homogeneous particle with a fixed shell or coating; buoyant density: $\rho_{p,buoy} = \left(1+2\frac{\delta_{shell}}{d_{core}}\right)^{-3} \cdot \left(\rho_d - \rho_{shell}\right) + \rho_{shell}$ apparent particle density: $\rho_{p,app} = \rho_{p,buoy}$ size: $d_{c\text{-}s}^2 = \frac{18\eta_c v_{sed}}{g\left(\rho_{p,buoy} - \rho_c\right)}$ sedimentation only yields outer dimension, i.e. $d_{c\text{-}s}$

Table B.1 *(continued)*

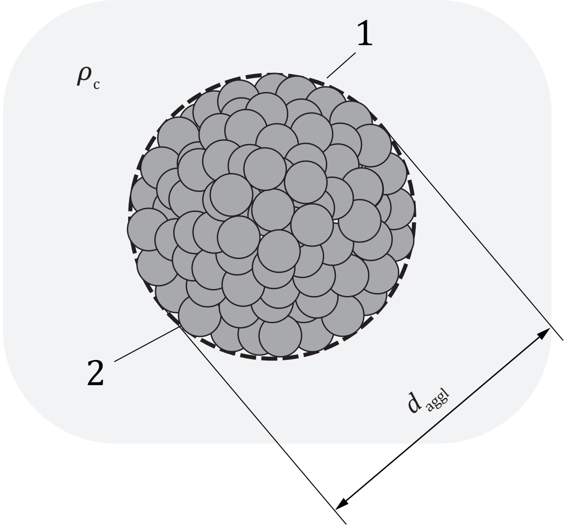
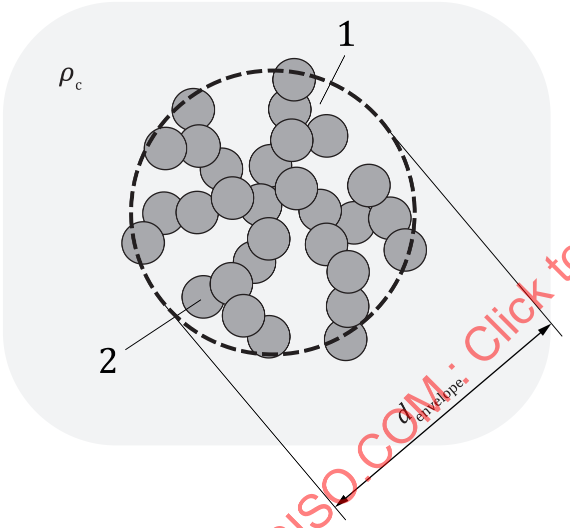
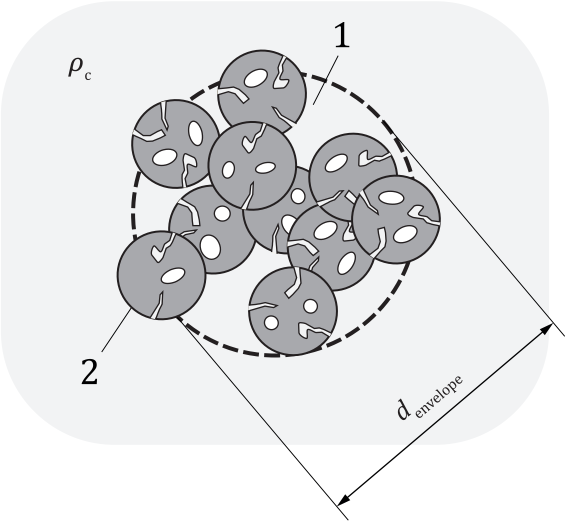
Configuration	Size and density
 **Key** 1 agglomerate ($\varepsilon_{\text{aggl}}$) 2 constituent particles (ρ_{d})	Close-packed agglomerate of homogeneous particles; buoyant density: $\rho_{\text{aggl,buoy}} = \rho_{\text{d}}$ apparent agglomerate density: $\rho_{\text{aggl,app}} = \left(1-\varepsilon_{\text{aggl}}\right)\cdot\rho_{\text{d}} + \varepsilon_{\text{aggl}}\cdot\rho_{\text{c}}$ size: i) if $\varepsilon_{\text{aggl}}$ is known, the approximate diameter of the convex hull can be calculated, which is very close to the hydrodynamic diameter: with: $x_{\text{env}}^2 \approx x_{\text{hd}}^2 \approx \frac{18\eta_{\text{c}} v_{\text{sed}}}{g\left(\rho_{\text{aggl,app}}-\rho_{\text{c}}\right)}$ ii) typically, $\varepsilon_{\text{aggl}}$ is not known, hence ρ_{d} is used: $x_{\text{Stokes}}^2 = \frac{18\eta_{\text{c}} v_{\text{sed}}}{g(\rho_{\text{d}}-\rho_{\text{c}})} < x_{\text{env}}^2$
 **Key** 1 fractal agglomerate (D_{fractal}) 2 constituent particles (ρ_{d})	Fractal-like agglomerate of homogeneous particles; buoyant density: $\rho_{\text{aggl,buoy}} = \rho_{\text{d}}$ apparent agglomerate density: not meaningful, fractal aggregates defy any attempt to define and measure $\varepsilon_{\text{aggl}}$ size: $x_{\text{Stokes}}^2 = \frac{18\eta_{\text{c}} v_{\text{sed}}}{g(\rho_{\text{d}}-\rho_{\text{c}})} < x_{\text{hd}}^2 < x_{\text{env}}^2$

Table B.1 *(continued)*

Configuration	Size and density
 **Key** 1 fractal agglomerate (D_{fractal}) 2 constituent particles ($\rho_{\text{p,sk}}$)	Fractal-like agglomerate of porous particles; buoyant density: $\rho_{\text{aggl,buoy}} = \rho_{\text{sk}}$ $\rho_{\text{aggl,buoy}} = \rho_{\text{p,sk}}$ apparent agglomerate density: not meaningful, fractal aggregates defy any attempt to define and measure $\varepsilon_{\text{aggl}}$ for porous constituents, size can be calculated based on: i) the skeleton density, ρ_{sk} $\rho_{\text{p,sk}}$: $x_{\text{Stokes}}^2 = \frac{18\eta_c v_{\text{sed}}}{g(\rho_{\text{sk}} - \rho_c)}$ $x_{\text{Stokes}}^2 = \frac{18\eta_c v_{\text{sed}}}{g(\rho_{\text{p,sk}} - \rho_c)}$ ii) or the apparent density of the constituent particles, $\rho_{\text{p,app}}$: $\tilde{x}_{\text{Stokes}}^2 = \frac{18\eta_c v_{\text{sed}}}{g(\rho_{\text{p,app}} - \rho_c)}$ The second approach requires knowledge of the specific pore volume of the constituent particles; in practice, the first approach is more often applied.
SOURCE: Reproduced with the permission of Frank Babick.	

The buoyant density can be measured within the framework of sedimentation, for which reason it can be considered as the most appropriate particle density for computing the Stokes diameter. Only particles with open porosity need to be replaced with the apparent particle density.

Moreover, the buoyant density can be approximated by the skeleton density, which is frequently reported on data sheets. Such an approximation can be critical for the presence of adsorbed layers of molecules and ions from the continuous phase. Typically, there is a lack of relevant data (layer thickness and composition), in particular because the structures of adsorbate layers depend on the environment.

Annex C
(informative)

Sedimentation beyond the validity of Stokes' law

C.1 Settling beyond creeping flow

Stokes' law of flow resistance [Formula (C.1)] was derived for single spherical objects in an unconfined Newtonian fluid (see C.7) under the condition of creeping flow. For particle sedimentation in quiescent liquids, it reads:

$$F_D = 3\pi\eta_c x v_{sed} \text{ or } C_D = \frac{F_D}{\frac{1}{2}\rho_c v_{sed}^2} = \frac{24 \cdot \eta_c}{\rho_c x v_{sed}} \tag{C.1}$$

where

C_D	drag coefficient
F_D	drag force
v_{sed}	terminal sedimentation velocity
x	particle diameter
η_c	dynamic viscosity of the continuous phase
ρ_c	density of the continuous phase

The equations are valid when inertia effects of the liquid flow field are negligible, which fine particles and low sedimentation velocities. An appropriate criterion is the particle Reynolds number, Re_p, for sedimenting particles [see Formula (42)].

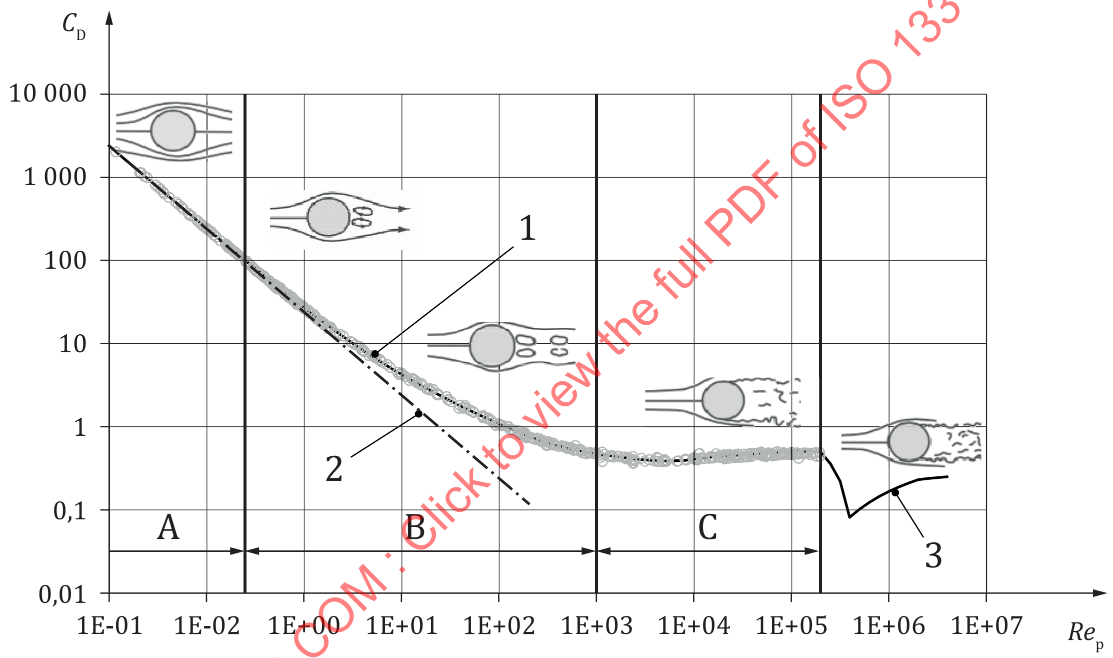
Stokes' law applies to creeping laminar flow, where $Re_p < 0{,}25$ and the drag force is entirely due to viscous friction within the liquid (Stokes regime). The flow field is axisymmetric and the drag coefficient inversely proportional to the particle Reynolds number ($C_D = 24/Re_p$). As the Reynolds number increases, the flow field becomes steadily deformed and eventually loses its axial symmetry due to flow separation and eddy formation (Figure C.1). At high Reynolds numbers, the flow field in the wake region becomes turbulent and the drag coefficient is approximately constant (Newton regime).

As the definition of Re_p is based on size and sedimentation velocity, it is more convenient to calculate the upper limits of size and sedimentation velocity from the corresponding thresholds of the dimensionless Archimedes and Ljaščenko numbers:

$$Ar_{cr} = \frac{a_c \Delta\rho \rho_c x_{max}^3}{\eta_c^2} = 4{,}68 \text{ and } Lj_{cr} = \frac{\rho_c^2 v_{max}^3}{a_c \Delta\rho \eta_c} = 0{,}003\,34 \tag{C.2}$$

Beyond the Stokes regime, Formula (C.1) underestimates the hydrodynamic drag of spherical particles. Accordingly, a calculation of Stokes diameter from measured velocity based on Formula (8) would underestimate the particle size (see Figure C.2).

EXAMPLE For a particle Reynolds number of Re_p = 0,25, Stokes law for the drag force fails by 3,9 % and the attendant error in calculating particle size from a measured sedimentation velocity [see Formula (8)] amounts to 2,0 %. The critical Reynolds number Re_p of 0,25 defines upper limits for the sedimentation velocity and particle size, which depend on the examined materials and the applied centrifugal force field. The minimum centrifugal acceleration required for ignoring effects of gravity amounts to 30 × g. Hence, the upper size limit for the creeping flow condition are 11,3 mm/s and 19,7 µm for aqueous dispersions at 25 °C and particles with a density contrast $\Delta\rho$ of 1,65 g/cm^3 (e.g. quartz).



Key

C_D drag coefficient, dimensionless

Re_p particle Reynolds number, dimensionless

A Stokes regime

B intermediate regime

C newton regime

1 experimental data, collected and revised by Reference [56]

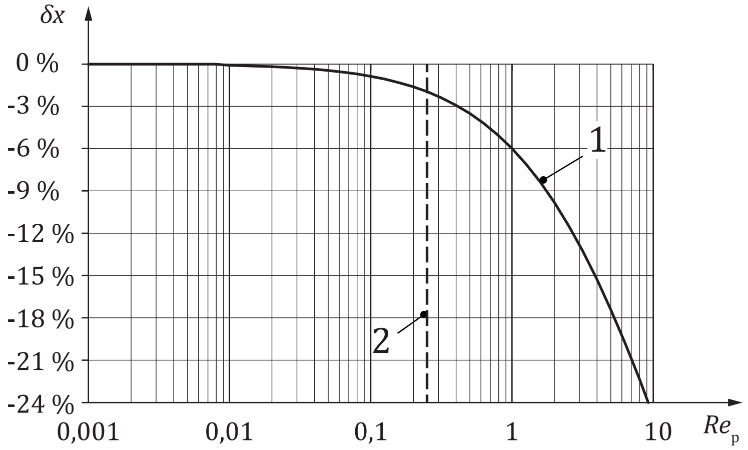
2 solution for Stokes regime, see Formula (C.1)

3 regression according to Reference [57]

NOTE This figure shows the drag coefficient of spheres, experimental data,[56] corresponding regression,[57] and Stokes solution [Formula (C.1)].

Figure C.1 — Streamline images for flow regimes

In order to avoid errors in particle size determination for large Reynolds numbers, i.e. rather coarse particles, two different strategies can be adopted. One consists of the application of correction functions based on established models for the hydrodynamic drag at moderate and high Reynolds numbers. The other is recommended by this document and consists of the modification of sedimentation conditions, so that the creeping flow condition ($Re_p \leq 0{,}25$) is satisfied for the largest particles of the sample.



Key

Re_p	particle Reynolds number, dimensionless
δx	relative error of calculated particle size
1	relative error
2	conventional threshold for the applicability of Formula (8), i.e. $Re_p = 0{,}25$

NOTE This figure shows an underestimation of the size of spherical particles when derived from sedimentation velocity with Formula (8), the transition from Stokes regime to intermediate flow regime is marked by the dashed line (at Rep = 0,25).

Figure C.2 — Underestimation of the size of spherical particles depending on the Reynolds number

The drag of either non-isotropic or non-spherical particles, or both, is not only a function of the Reynolds number, but also depends on the particles' alignment in the flow field. Similarly, their sedimentation velocity is affected by the particle orientation with regard to the gravity vector.[58,59]

C.2 Superposition with Brownian motion

Sedimentation-based measurement techniques derive the distribution of particle size or sedimentation velocity by either of the following:

- monitoring the concentration changes in the dispersion phase;
- monitoring the growth of the sediment or cream layer.

Both methods rely on the assumption that these changes are solely due to sedimentation, i.e. the steady migratory motion induced by gravity and retarded by buoyancy and viscous drag. However, particles also experience undirected Brownian motion, which results in a diffusive flux, smearing the concentration steps and gradients that are related to sedimentation. Consequently, measured distribution functions appear broader and smoother than real particle size distribution.[6],[19] In practice, this effect is only relevant for fine particles as the particle diffusion coefficient is inversely proportional to particle size. The dimensionless Péclet number [see Formula (40)], which expresses the ratio between migratory and diffusional particle flux, helps to estimate the additional effect of Brownian diffusion. The number should be larger than unity. For gravitational sedimentation, this criterion is typically met for micrometre particles. However, Brownian motion can significantly affect the sedimentation of submicrometre particles.

The overall impact of Brownian motion on the measured particle size distributions depends on the following:

- the particle system (average particle size, degree of polydispersity, and density contrast);

—the measurement set-up (sedimentation distance, fixed or scanning measurement position, and incremental or integral sedimentation technique).

This is particularly pronounced for the fine fractions of a particle system, materials with low density contrast and very small sedimentation distances. The effect can be reduced by lowering the temperature and by conducting the measurements at large sedimentation distances or long sedimentation times. Brownian motion is one criterion for the lower size limit of gravitational sedimentation techniques. From experimental data, Koglin et al.[5] derived that for Péclet numbers above 200 [see Formula (C.3)], Brownian motion is irrelevant for gravitational sedimentation. More elaborate estimations by Reference [27] indicated that its impact is negligible when the Péclet number of the volume-weighted median ($x_{50,3}$) exceeds 100. Reference [93] concludes that the impact of Brownian motion “becomes critical at particle sizes under 0,1 µm in the gravity field”.

The importance of Brownian diffusion is much higher for centrifugal, than for gravitational, sedimentation techniques.

C.3 Non-Newtonian continuous phase

Particle size analysis by means of analytical centrifugation relies on a Newtonian flow behaviour of the continuous phase (see 6.4). Similarly, spin liquids required for LSM shall also be Newtonian. However, there can be samples that are composed of particles dispersed in non-Newtonian liquid that cannot be diluted or that defy an exchange of liquid for the purpose of measurement. In this event, data analysis must be considerably modified in order to derive a particle size from phase separation in centrifugal fields (see ISO 13317-1). A pragmatic approach is therefore to replace the dynamic viscosity, η_c, in Formula (C.1) by the apparent viscosity, η_{app}, which corresponds to the average shear rate at the particle surface. Estimates of the maximum shear rate in the vicinity of a migrating particle under creeping-flow condition are discussed in Reference [145].

C.4 Effects of hydrodynamic particle interaction, hindrance

Formula (C.1) was developed for an unhindered single particle settling in an infinite liquid. This assumption of an unhindered settling [see 6.1 and Formula (4)] is practically only fulfilled for dilute particle systems of volume fractions of the dispersed phase below 0,5 %. Under these conditions, individual particles settle sufficiently independently from each other.

If more particles are dispersed in a liquid, different particle-particle interactions take place due to the finite separation distances. Such interactions can lead to structured arrangements of particles having their origin in steric and osmotic or long-ranging and short-ranging non-viscous forces. All effects related to increased particle concentration affect the viscous interaction among particles (e.g. via structure). They create an additional hydrodynamic hindrance and thus reduce the sedimentation velocity. Particle sizes which are derived by referring to Formula (8) are underestimated. In addition, hindrance depends on particle size distribution at a given volume concentration because the mean distance between two particles narrows with decreasing size of the dispersed phase. The lessening of the sedimentation velocity can be quantified by the so-called hindrance function, $H(\varphi_V)$, which is defined as the ratio of sedimentation velocity at a given volume fraction, $v_{\text{sed}}(\varphi_V)$, to the Stokes velocity of an isolated particle ($v_{\text{sed},0}$) under otherwise identical settling conditions.[60][61] The hindrance function has been investigated in experimental studies[62] as well as in theoretical analyses.[63]-[65] A generic hindrance function, $H(\varphi_V)$, is frequently used as proposed by Kynch: [66]

$$H(\varphi_V)=\frac{v_{\text{sed}}(\varphi_V)}{v_{\text{sed},0}}=\frac{(1-\varphi_V)^2}{\eta_{\text{rel}}(\varphi_V)} \tag{C.3}$$

where

φ_V volume fraction of the dispersed phase

η_{rel} relative apparent viscosity of the liquid dispersion

Numerous empirical, semi-empirical and analytical one-to-multiparameter models have been proposed since the pioneering work of Kynch. Approaches allow taking into account any viscosity-concentration relationship $\eta_{\mathrm{rel}}(\varphi_V)$ for dispersions.[67]-[69] Hindrance function depends also on particle shape 61]

There are numerous articles, giving evidence that concentration effects can be accounted for volume concentrations of up to about 5 % to 10 %.[61] For a polydisperse particle system, concentration effects are more complex. As swarm sedimentation passes over into zone sedimentation, all particles separate with the same velocity independent of the particle size. At this point, determination of particle size distribution is not possible anymore.

It should be noted that beside the phenomena described above, multiple particles in the measuring volume can also affect the concentration determination by the measuring techniques (e.g. light intensity) due to obscuration, interference of scattering of different particles or structured systems.

As a practical approach to determine the necessary dilution of a dispersion, experiments with decreasing volume concentrations can be performed. If sedimentation velocity does not increase further, hydrodynamic hindrance can practically be neglected.

C.5 Non-spherical particles and particle agglomerates

The geometry of particles has relevance for particle motion in two regards in the context of this document. The first one is spatial alignment of non-spherical particles and agglomerates, the second one is hydrodynamic friction due to gravity settling.

The sedimentation velocity of non-isotropic, non-spherical particles depends on their orientation with regard to the gravity vector.[9],[58],[59] Most literature assumes that, in the Stokes regime, symmetrical particles are randomly oriented and they migrate due to gravity in any (stable) orientation. Orientation effects due to migration develop only for moderate and high Reynolds numbers Re_{p} particles. Nonetheless, monodisperse, non-isotropic shaped homogeneous particles of the same mass, exhibit different sedimentation velocities according to their orientation. In general, the velocity scatter for non-spherical particles with aspect ratios smaller than 10 is rather small. Experimentally, it manifests itself in a larger standard uncertainty of the average size.

As any non-spherical shaped particle has a larger surface area than a sphere with the same volume, frictional force [see Formula (C.1)] will consequently differ along with its terminal sedimentation velocity. This effect can be taken into account by a so-called sphericity, Ψ, which is defined as the ratio between the surface area of a sphere having the same volume or mass and the one of the non-spherical particles.[9],[70] By definition, it is smaller than one. If particles are randomly aligned, the measured velocity based on local concentration change during sedimentation will be an average of the sedimentation velocities of the differently oriented particles and smaller than the sedimentation velocity of volume equivalent spherical particles. The ratio between the two velocities is called the shape correction factor and is tabulated for various shapes (see ISO 13317-1). For the Stokes regime, it can be approximated:[71]

$$k_{\mathrm{shape}} = 0{,}843 \lg \frac{\Psi}{0{,}065} \tag{C.4}$$

If the sedimentation velocity was measured for single particles, it would be distributed due to the varying orientation and thus mimic an artificial polydispersity. The shape of particles can also affect the signals used for the quantification of size fractions, which becomes relevant when the measured quantities are converted in volume or number. This is particularly relevant for photosedimentation. For sedimentation techniques based on a mass-proportional detection (e.g. X-ray attenuation), it only becomes important for samples of fractal-like agglomerates.

Agglomerates and fractals can be characterized based on a Stokes velocity for an equivalent spherical diameter. To obtain a size estimate, the apparent density of these objects need to be available. In some cases, like close-packed agglomerates, they can be treated like porous particles (see C.6).

C.6 Density of porous particles or particles with any kind of internal structure

The calculation of particle size from the sedimentation velocity [see Formula (8)] relies on knowledge of the density contrast between particles and continuous phase. The density of most common liquids is temperature-dependent and well-tabulated in several handbooks. Such data are also available for crystalline or amorphous solid particles without any internal structure. However, there are many particulate materials that are composed of porous particles, coated particles or agglomerated particles. Moreover, when dispersed in liquids, the dispersing, emulsifying or stabilising agents used (e. g. polyelectrolytes, polymers and surfactants) can possibly adhere as a fixed layer on the particle surface.

Hence, migrating entities of sedimentation experiments are frequently not monoconstituent. One can encounter:

— porous particles with stagnant liquid in the open pores;

—emulsion droplets with a stabilising polymer film or particle layer on their surface;

— pigments with an inorganic coating or plastic particles with included pigments.

Their density differs from the one of the respective main constituents, e.g. solid skeleton, droplet liquid, pigment core, or pure polymer (see Annex B).

For an isolated particle without porosity or pore sizes very much smaller than the particle size, the outer dimensions of the moving entity can be derived by employing the apparent particle density for calculating particle size. The apparent density can be calculated if the internal composition and the density values of all constituents are known. In the absence of open pores, it can be identified with the buoyant density, which is accessible by experiment (see the ISO 18747 series).

The situation is different for aggregates and agglomerates for several reasons:

a) the interstitial distance is in the order of the constituent particles and included liquid is not necessarily stagnant;

b) the surface that separates the continuous phase from the moving entity is ill-defined;

c) the structure can be fractal-like, i.e. the porosity depends on agglomerate size;

d) relevant details on the morphology are typically not known.

Hence, the apparent particle density is typically meaningless for such particles and defies its computation, except for in close-packings with a large number of constituent particles. Therefore, sedimentational size determination of aggregates and agglomerates relies on the buoyant density.

In conclusion, the buoyant density is the appropriate density value in sedimentational size analysis for most types of materials and frequently is equal to and resembles the apparent particle density. Only in particles with fine open pores, the buoyant density is replaced by the apparent particle density.

C.7 Miscellaneous effects

Some effects can principally impair the signal quality or interfere with the conventional data analysis, but are not relevant for most situations in analytical practice. However, user should be aware of their possible existence.

— Hydrodynamic retardation in the vicinity of a wall:
The effect is virtually negligible if the wall distance is much larger than particle size ($x_p/L_{min} < 0{,}01$) and the measurement position is at least 50 particle diameters apart from the inner and outer interface. These conditions are typically met in analytical centrifugation.

— Transient phase of particle motion in the centrifugal field:
The acceleration of particles from rest to terminal sedimentation velocity takes a certain period of time, which depends on the particle size and the liquid properties. It is vanishingly small under creeping flow condition.

- Inward motion in case of a negative density contrast:
  Analytical centrifugation can, in principle, be applied to particles with negative or positive density contrast to the continuous phase. However, particles with negative density contrast (e.g. droplets of vegetable oils in water) can require different procedures for sample supply (in LSM). In addition, the relevant sedimentation distance needs to be referred to the outer interface (bottom) of the sample. If the primary measurement signals consist of radial profiles of the particle concentration, the direction of particle motion can be concluded. In contrast, time curves do not provide such an indication.

- Retarded motion in case of charged particles:
  Electrical double layers, which form around charged particles, lower the sedimentation velocity due to the sedimentation-induced polarisation of the double layer (Dorn-effect[72]). The retarding Dorn effect is a second order effect, which is only relevant for very high zeta-potentials and very thick double layers. It can become relevant for organic liquids, for aqueous solutions with low salt content or very fine particles. If there are doubts regarding the relevance of the effect, the size analysis should be repeated for samples of varying ionic strength.

Annex D
(informative)

Trueness, reproducibility and uncertainty determination for velocity and particle size

D.1 General

This annex describes an exemplary procedure for computing the full combined uncertainty of results from centrifugal liquid sedimentation (CLS) techniques.[99] It discusses the uncertainty in the following measurands:

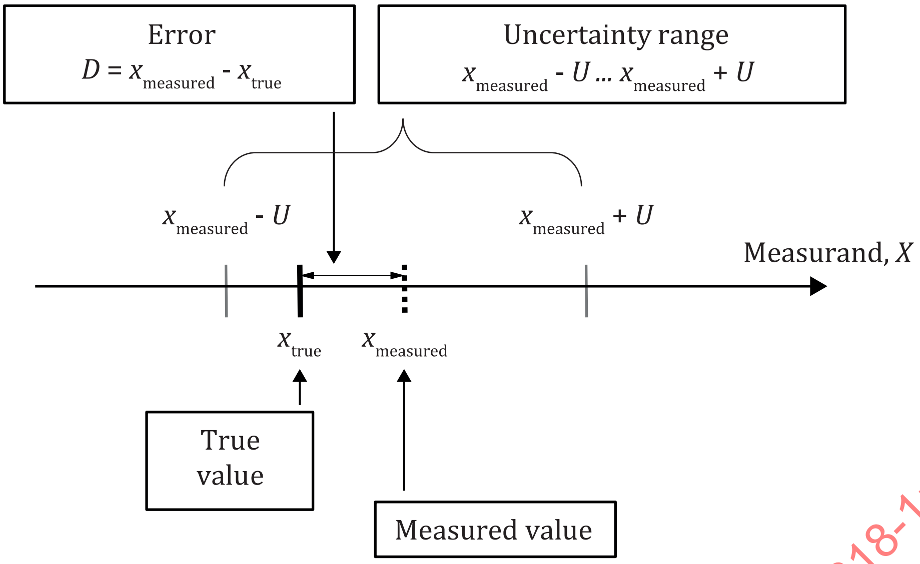
- sedimentation velocity;
- particle size.

The presented data refers to measurements made with centrifugal photosedimentation using the HSM, for which reason the details of the procedure possibly need to be modified when another measurement technique, such as X-ray centrifuges (see ISO 13318-3), or the LSM are employed. The description is restricted to the uncertainty of mean values in velocity or size; uncertainty with respect to the measured quantities of each particle fraction is not considered.

D.2 Preliminary remarks on uncertainty in sedimentation experiments

D.2.1 Relationships between different terms describing the uncertainty of a measurement

Figure D.1 provides an overview of the different terms used to describe the relation between the true value and the measured value of a measurand as well as the equations for the measurement error and uncertainty range, e.g. for particle sizing.[73] Figure D.1 presents the notation regarding different types of error, performance and expression of performance.



Key

x_{true}	true value of the measurand		U	uncertainty
$x_{measured}$	measured value of the measurand		X	measurand
D	measurement error			

SOURCE Reference [73], reproduced with the permission of the authors.

Figure D.1 — Measured value, true value, error and uncertainty range

D.2.2 Relationship between uncertainties in sedimentation velocity and particle size

The direct results of sedimentation analysis are the relative quantities of particles in narrow fractions of sedimentation velocity or its corresponding particle size (Stokes diameter). The latter is computed from the former by assuming the validity of Formulae (4) and (8) and that the model parameters used are correct. This assumption introduces additional uncertainty to the measured size value, which is not present for sedimentation velocity, because the uncertainties in sedimentation distance and time are small.

D.3 Experiments for deriving precision, trueness and reproducibility

D.3.1 General

Clause D.3 describes the determination of performance characteristics and quantitative expressions of uncertainty for centrifugal photosedimentation. This includes the quantification of precision, trueness, reproducibility and combined uncertainty (see 8.5 and 8.6 and Figure 5) of measured sedimentation particle size (median values). The analyses were based on a CRM with a certified value for the Stokes diameter of 490,0 ± 20,0 nm.

The measured data are interpreted through the framework shown in Figure D.1 and the Nordtest approach (NT TR 537).

D.3.2 Determination of method short-term precision and trueness

Short-term precision under repeatability conditions of a measurement system refers to the closeness of results from replicate measurement, i.e. those which are repeated under the same conditions. It is usually just referred to as the "repeatability". In this example, the same operator determined the sedimentation velocity and particle size for ten consecutive measurements (i.e. without significant time delays between measurements) with the same photocentrifuge device. All the measurements were conducted at 25,0 °C with uniform settings for wavelength (470 nm) and measurement cell properties (2 mm polycarbonate).

Calculation of Stokes diameters from measured sedimentation velocities employed the following values for the model parameters:

r	measurement position = 125 mm
n	rotor speed = 2 000 rpm
η_c	dynamic viscosity of the continuous phase = 0,891 mPa·s
ρ_c	density of the continuous phase = 997,3 kg·m^3
ρ_p	buoyant density of the particle = 2 000 kg·m^{-3}
φ_m	silica (SiO_2) weight fraction of 0,1 %

The density and viscosity of the continuous phase were obtained from literature at a temperature of 25 °C ± 1 °C, held constant in the centrifuge.[113] A complete detail of the measurement parameters, procedure, and individual values of the measurements are described in Reference [96]. The mean values of the measurements velocity and size and their (relative) standard deviations were 74,08 µm/s (1,46 %) and 483,0 nm (0,73 %). The difference between the mean experimental values and the certified values of the reference particle (CRM) are the measurement errors due to bias (see Figure 5 and Figure D.1). The smaller these differences are, the better the instrument (method) trueness. Taking the certified value for Stokes diameter as 490 nm, the bias is calculated as -7,0 nm, which is within the uncertainty of the CRM value of ±20 nm which means that it is not significant.

D.3.3 Reproducibility of the particle velocity methods

Reproducibility is another aspect of the precision of a measurement or test method, also referred to as intermediate precision. Similar to repeatability, reproducibility is quantified and reported as a standard deviation (standard uncertainty). This kind of uncertainty covers deviations with broader factors influencing the measurement results compared to the repeatability, short-term precision. Experiments are intentionally performed over a period of 6 months to a year. Reproducibility is sometimes called the "intermediate precision" or "within-lab precision". The measurement uncertainty includes both random errors and systematic errors.

Measurements were taken over a period of six months with conditions referring to several operators, and different photosedimentometers, which were operated at different wavelengths (470 nm and 870 nm) and different cell types (optical pathlength).

It additionally reflects unintended variations in sample preparation (e.g. induced by different batches of chemicals). The measurement cell itself was thermostatically controlled at 25,0 °C, but temperature in the laboratory varied between 22,0 °C and 28,0 °C. Overall, the observed variation in measured values reflects typical day-to-day variations in the corresponding laboratory.

The average values of sedimentation velocity and particle size amount to 73,6 µm/s (relative standard deviation of 3,03 %) and 481,3 nm (relative standard deviation of 1,53 %), respectively. The uncertainty with respect to the calculated mean gradually decreases with the number of measurements conducted. For example, with 40 measurements, the uncertainty of the average values is reduced by a factor of $\sqrt{40} = 6{,}3$ when compared to the indicated values, (i.e. the uncertainty of averages amounts to 0,48 and 0,24 %). Regarding the uncertainty of the measurements, a higher standard deviation is clearly observed under reproducibility conditions than under repeatability ones.

NOTE Since the measurement values refer to only one measurement in each case, the standard deviation for reproducibility or intermediate precision can be identified with the standard deviation for the listed values. If the listed data were average values of n individual runs at each day, less variation would be seen among the listed data and, therefore, the average standard deviation of repeatability had to be included in the calculation of the reproducibility (intermediate precision) ($s_{\mathrm{Rw}}^2 = (n-1)/n \cdot s_{\mathrm{rep}}^2 + s_{\mathrm{groups}}^2$, e.g. see References [74] and [76]).

D.4 Quantification of the combined uncertainty

D.4.1 General

The various contributions to the overall uncertainty have to be considered for a full uncertainty calculation (budget) for the sedimentation data obtained in a laboratory. These include the following:

- the systematic deviations (*bias*), which can be intrinsically related to the analytical method or to a specific instrument or laboratory;
- random deviation related to any kind of stochastic variation (*reproducibility*), e.g. with respect to measurement conditions or sample preparation;
- uncertainty introduced in the signal processing due to a lack of knowledge about the exact values of model parameters relevant for the calculation of particle size [Formula (8)].

The combined uncertainty is calculated according to Formula (46).

Since the experimental data refer to only one CRM, the dimensional uncertainty descriptors of 8.6 can be replaced by relative standard uncertainties, which are associated to the certified values of Stokes diameter. This common procedure partly simplifies the uncertainty calculation and eases comparison and evaluation of uncertainty data.

D.4.2 Trueness, measurement error and bias of particle size

D.4.2.1 General

As indicated above, the CRM used in the experiments discussed in D.3.2 and D.3.3 had certified values of 490 nm ± 20 nm for the Stokes diameter. The average values obtained within the repeatability test and reproducibility study differed from the certified values, differ by 1,8 % from the certified value, indicating a lack of trueness of the results, however, which were determined to be not significant (see Reference [99]). The quantitative expression of the trueness performance is the bias. It is calculated based on reproducibility data according to Formula (D.1)

$$b_x = \bar{x}_{\mathrm{lab}} - x_{\mathrm{ref}} = 481{,}3\,\mathrm{nm} - 490\,\mathrm{nm} = -8{,}7\,\mathrm{nm} \quad (\mathrm{D.1})$$

where

b_x bias in particle size, x, from bias determination with a CRM

$\bar{x}_{\mathrm{lab}}$ measured value of particle size, x; average of all bias determinations in the laboratory

x_{ref} reference value of particle size, x

In accordance, a relative bias then reads

$$b_{x,\mathrm{rel}} = \frac{\overline{x}_{\mathrm{lab}} - x_{\mathrm{ref}}}{x_{\mathrm{ref}}} = \frac{481{,}3\,\mathrm{nm} - 490\,\mathrm{nm}}{490\,\mathrm{nm}} = -1{,}78\,\% \qquad \text{(D.2)}$$

In order to estimate the complete bias uncertainty, one needs to include the uncertainty of the reference values. The relative bias uncertainty ($u_{\mathrm{bias,rel}}$) is derived from the relative bias and the relative standard uncertainty of the reference values ($u_{\mathrm{ref,rel}}$) with the following formula:

$$u_{\mathrm{ref,rel}} = \frac{u_{\mathrm{ref}}}{v_{\mathrm{ref}}} = \frac{\frac{U_{\mathrm{ref}}}{k}}{v_{\mathrm{ref}}} \qquad \text{(D.3)}$$

The corresponding values for the reproducibility bias and for the CRM (see above) are obtained with the following:

$$u_{\mathrm{bias,rel}}\left(x_{\mathrm{Stokes}}\right) = \sqrt{\left(\frac{-8{,}7}{490{,}0}\right)^2 + \left(\frac{\frac{1}{2}\cdot 20{,}0}{490{,}0}\right)^2} = \sqrt{(-1{,}78\,\%)^2 + (2{,}04\,\%)^2} = 2{,}71\,\% \qquad \text{(D.4)}$$

D.4.2.2 Combined uncertainty for Stokes diameter from particle size data

The combined uncertainty for the Stokes diameter can be determined from the bias uncertainty and the relative standard deviation due to the reproducibility experiments ($u_{\mathrm{Rw,rel}}$) in accordance with Formula (D.5):

$$u_{\mathrm{c,rel}}\left(x_{\mathrm{Stokes}}\right) = \sqrt{u^2{}_{\mathrm{bias,rel}}\left(x_{\mathrm{Stokes}}\right) + u^2{}_{\mathrm{Rw,rel}}\left(x_{\mathrm{Stokes}}\right)} = \sqrt{(2{,}71\,\%)^2 + (1{,}53\,\%)^2} = 3{,}11\ \% \qquad \text{(D.5)}$$

D.4.3 Expanded combined uncertainty

The combined standard uncertainty derived for the Stokes diameter (3,11 %) quantifies the uncertainty of a measurement result with a level of confidence of approximately 68 % for a normal distribution. In order to cover a higher level of confidence, the uncertainty range needs to be expanded, which is commonly achieved by multiplying the standard uncertainty values by a coverage factor k. The value of k depends on the number of measurements and the aspired confidence level. Its values equal the quantiles $t_{n\text{-}1}$ of the Student's t-distribution (ISO/IEC Guide 98-3). Frequently, a confidence level of 95 % is considered sufficient, which corresponds to a coverage factor $k = 2$ if the number of measurements exceeds 30. Hence, the expanded combined uncertainty is:

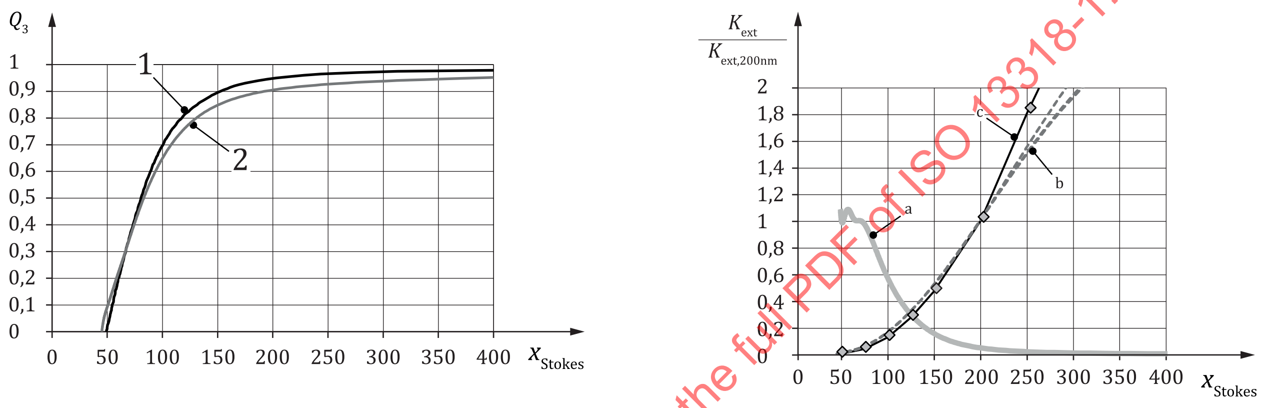
$$U_{\mathrm{c,rel}}\left(x_{\mathrm{Stokes}}\right) = k \cdot u_{\mathrm{c,rel}}\left(x_{\mathrm{Stokes}}\right) = 6{,}22\,\% \quad U_{\mathrm{c}}\left(x_{\mathrm{Stokes}}\right) = k \cdot u_{\mathrm{c}}\left(x_{\mathrm{Stokes}}\right) = 30{,}5\,nm \qquad \text{(D.6)}$$

which means that the true Stokes diameter of an analysed particle system lies within a range of 94 % to 106 % of the measured value (when aiming for 95 % confidence).

Annex E
(informative)

Multiwavelength approach

The extinction coefficient of particles at visible light depends on size and optical properties (refractive index difference with respect to the continuous phase and particle shape) at a given wavelength. If the refractive index is known, the scatter and absorption of light by spherical particles of different sizes can be taken into account by the Mie theory.[24] Conversion of extinction-based size distribution to volume-based size distribution, as described in 6.3.5, requires knowledge of the complex refractive index of the particles. Problems arise if these optical properties are not available.



a) Measured particle size distribution

b) Relative extinction efficiency according to Mie theory and derived from CLS measurement

Key

K_{ext}	extinction efficiency
$K_{\text{ext,200 nm}}$	extinction efficiency for particle size of 200 nm
Q_3	sum function of the volume-weighted particle size distribution, dimensionless
x_{Stokes}	particle size (Stokes diameter), in nm
1	classical data processing based on Mie scattering and refractive index value
2	multiwavelength analysis without referring to the particles' refractive index
a	Scaled density function of the volume-weighted particle size distribution, arbitrary units.
b	Experimental values for the relative extinction efficiency.
c	Relative extinction efficiency for Mie scattering at alumina spheres (RI = 1,778).

SOURCE Reference [77]. Reproduced with permission of the authors.

NOTE Figure E.1 shows a comparison of classic data analysis in CLS (based on Mie scattering) and the multiwavelength approach for polydisperse colloidal Al_2O_3 measured with an analytical photocentrifuge operated at wavelengths of 410 nm, 455 nm and 470 nm.

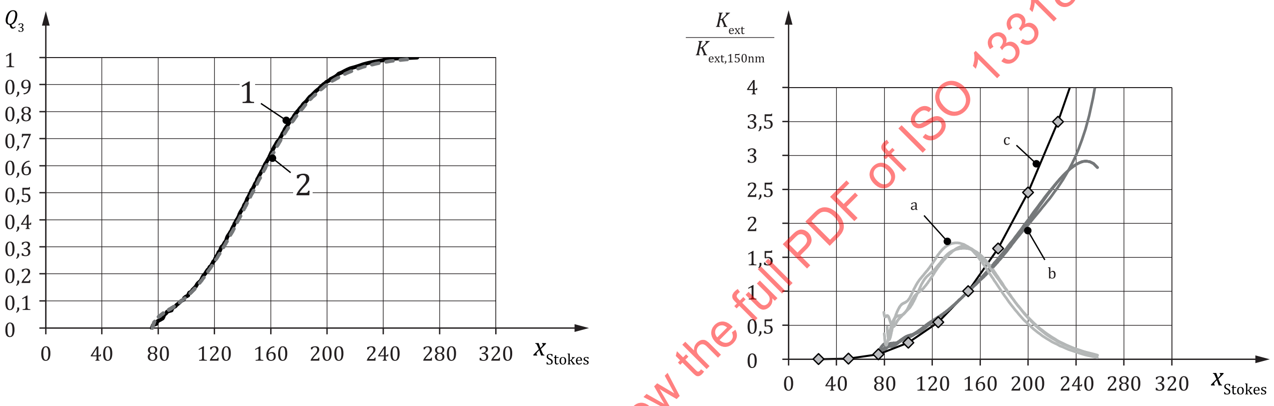
Figure E.1 — Comparison of classic data analysis in CLS (based on Mie scattering) and the multiwavelength approach for polydisperse colloidal Al_2O_3

The way to circumvent the necessity of quantitative optical models consists in determining extinction-based particle size distributions at different wavelengths, as proposed by Weichert.[75] The wavelength dependency of turbidity for defined particle sizes is approximately equivalent to the size dependency of turbidity at fixed wavelengths. Hence, the multiwavelength detection itself yields the required optical model. Extinction-weighted size distributions can be transferred into a volume-weighted distribution with no assumption regarding material parameter and particle shape. It relies on the following assumptions:

a) that the extinction coefficient of a particle is a function of the dimensionless size x/λ;

b) that the extinction-weighted size distributions of different wavelengths give the same volume-weighted distribution.

Figure E.1 shows the results for colloidal Al_2O_3 particles.[77] With regard to the volume-weighted size distribution, they reveal conformity between the classic Mie approach and the newly developed analysis algorithm based on multiwavelength data. The multiwavelength analysis also provides the size dependency of the relative extinction efficiency, which is found to agree fairly well with the theoretical curve from Mie scattering at alumina spheres. Figure E.2 depicts analogous results for a suspension of pyrogenic silica that is composed of polydisperse, fractal-like aggregates in the micrometre range. The Stokes diameters of such materials are significantly smaller than the outer dimensions of the aggregates and their light scattering behaviour obeys Rayleigh-Debye-Gans, scattering for aggregates, rather than Mie, scattering for homogeneous spheres.



a) Measured particle size distribution

b) Relative extinction efficiency according to Mie theory and derived from CLS measurement

Key

$K_{ext,150\ nm}$ extinction efficiency for particle size of 150 nm

c relative extinction efficiency for Rayleigh-Debye-Gans scattering at silica aggregates (RI = 1,464)

SOURCE Reference [77]. Reproduced with permission of the authors.

NOTE Figure E.2 shows a comparison of classic data analysis in CLS (based on Mie scattering) and the multiwavelength approach for pyrogenic SiO_2 aggregates measured with an analytical photocentrifuge operated at wavelengths 410 nm, 455 nm and 470 nm.[77]

Figure E.2 — Comparison of classic data analysis in CLS (based on Mie scattering) and the multiwavelength approach for pyrogenic SiO_2 aggregates

Annex F
(informative)

Spatial distribution of particles in centrifugal fields

F.1 Evolution of concentration profiles in centrifugal experiments

The characterization of a material's dispersity by centrifugal liquid sedimentation (CLS) rests on the evaluation of time curves for the local or cumulative particle concentration, concentration profiles for defined sedimentation times or the evolution of these profiles over time. It thus relies on a clear understanding of the spatial distribution of particles in the centrifugal field, which is governed by phase separation and radial dilution (see 5.1).

Phase separation means the formation of a liquid zone which is depleted of particles larger than a specified size (see Figure 1). This critical size (or its corresponding sedimentation coefficient) is described by the time elapsed, t_{sed}, to proceed from the meniscus (in the case of a positive density contrast, $\Delta\rho$) or from the cell bottom (if $\Delta\rho < 0$) to the measurement position, r_{meas} [see Formula (F.1)]:

$$\ln\frac{r_{\text{meas}}}{r_0} = \frac{v_{\text{Stokes,ref}}}{r_{\text{ref}}}\cdot t_{\text{sed}} = \omega^2 \tau_{\text{sed}} \cdot t_{\text{sed}} \qquad \text{(F.1)}$$

where

r_0	radial coordinate of meniscus ($\Delta\rho > 0$) or bottom ($\Delta\rho < 0$)
r_{meas}	radial coordinate of measurement position
r_{ref}	radial coordinate of reference position
t_{sed}	time period to migrate from r_0 to r_{meas}
$v_{\text{Stokes,ref}}$	terminal sedimentation velocity for reference position
τ_{sed}	sedimentation coefficient
ω	angular frequency

In turn, Formula (F.1) allows for the computation of the radial positions of a particle having started at r_0 over time.

Considering a monodisperse sample prepared for the homogeneous start method (HSM), a jump-like increase of concentration can be expected at position r_{meas} after a sedimentation time t_{sed}. The concentration, c, is zero between the start and the measurement (i.e. r_0 and r_{meas}, respectively) and at a maximum beyond r_{meas}. The value of this maximum changes with progressing sedimentation due to radial dilution. In order to