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Determination of particle density by sedimentation methods — Part 2: Multi-velocity approach

Détermination de la densité de particules par méthodes de sédimentation — Partie 2: Approche à multi vitesses

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Foreword

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

A list of all parts in the ISO 18747 series can be found on the ISO website.

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Introduction

Dispersions are widely used in industry and everyday life. There is a need to understand the density of dispersed particles or droplets, e.g. for physico-chemical calculations such as kinematic viscosity of dispersions[1], determination of particle size distribution by sedimentation[2][3] or acoustic techniques[4], particle characterization by field-flow approaches[5], optimization of dispersion long-term stability by density matching[6] as well as, more generally, characterization of particles (e.g. composition, internal phase content of double emulsions or homogeneity of hollow capsules) in manifold academic and industrial areas. Nowadays there is an increasing interest in using particle density to estimate the mass transfer of nanoparticles atop cell layers by sedimentation (dosage calculation for in vitro nanotoxicity assessment[7][8][9]).

The density of a body is defined as its mass divided by its volume. This calculation is straightforward for a large uniform body or particle. However, determination of the volume of a macroscopic body is difficult. The geometrical volume (defined by length, width and thickness) and the volume relevant for the determination of density may differ due to surface irregularities, fractures, fissures and pores or the measuring techniques employed.

Density determination of micro-particles, especially nanoparticles dispersed in a liquid, is difficult not only due to the determination of mass and volume for small particles, but also due to the fuzzy boundary between the liquid and the particle, which is often described in terms of a corona[10]. Liquid and solute molecules in the continuous phase are partially immobilized at the surface. Physico-chemical properties (e.g. viscosity, ion composition, solute concentration) in the fuzzy coat differ from the bulk. This effect is especially important for small microparticles and nanoparticles that are dispersed in a polymer or biological media[11]. The so-called corona may be interpreted as an integral part of the particle and increases the effective/apparent volume compared to the space occupied by the dry particle. The thickness of this layer ranges between a few to tens of nanometres. The effective/apparent volume deviates increasingly from the “geometrical” volume of dry particles as the particles become smaller. Correspondingly, density determination by traditional methods is affected. These concerns hold also for particle size, which may refer to different geometrical and physical properties. In the context of this document, the Stokes diameter and diameter of the enveloping sphere/hull are particularly relevant.

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Determination of particle density by sedimentation methods — Part 2: Multi-velocity approach

1 Scope

This document specifies an in situ method for the determination of the density of solid particles or liquid droplets (herein referred to as “particle”) dispersed in liquid continuous phase. The method is based on direct experimental determination of particle velocity in these liquids or media in gravitational or centrifugal fields based on Stokes law. The particle density is calculated from experimentally determined particle velocities in different liquids or media, taking into account their dynamic viscosities and densities, respectively. The approach does not require the knowledge of particle size distribution but assumes that sedimentation relevant characteristics (e.g. volume, shape, agglomeration state) do not change. This document does not consider polydispersity with regard to particle density, i.e. all particles are assumed to be of the same material composition.

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 14887, *Sample preparation — Dispersing procedures for powders in liquids*

3 Terms and definitions

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

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

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

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3.1
buoyant density
ratio of particle mass to particle volume including filled or closed pores as well as adjacent layers of liquid or other coating materials

3.2
dynamic viscosity
measure of the resistance of a fluid which is being deformed by shear stress

Note 1 to entry: Dynamic viscosity is calculated by shear stress divided by shear rate and determines the dynamics of an incompressible Newtonian fluid.

3.3
migration
directed particle movement (sedimentation or creaming/flotation) due to acting gravitational or centrifugal fields

Note 1 to entry: Sedimentation occurs when the density of droplets/particles is larger than that of the liquid. Creaming/flotation occurs when the density of droplets/particles is smaller than that of the liquid. In these two processes, particles move in opposite directions.

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3.4
migration velocity

absolute value of sedimentation or creaming/flotation terminal velocity

Note 1 to entry: Velocity of creaming/flotation is indicated by a negative sign.

3.5
shape factor

ratio of the sedimentation velocity of a non-spherical particle to the one of a spherical particle of the same volume and density

4 Symbols

Quantity	Symbol	Unit	Derivative unit
Acceleration	a	m/s²	
Angular velocity	ω	rad/s	
Coverage factor	k	—	
Dynamic viscosity	η	Pa·s	mPa·s
Expanded uncertainty for density	U	kg/m³	
Liquid density	ρ_L	kg/m³	
Maximum density	ρ_{max}	kg/m³	
Minimum density	ρ_{min}	kg/m³	
Particle density	ρ_P	kg/m³	
Radius	r	m	mm
Relative centrifugal acceleration	RCA	—	
Standard acceleration due to gravity	g	m/s²	
Temperature	ϑ	°C	
Time	t	s	
Velocity	v	m/s	
Wavelength	λ	m	nm

5 Basic principle of the method

Density is the mass of a body divided by its volume. In case of fine particles, microscopic surface and internal structure have to be taken into account to define the true particle volume of a dry particle. The true volume can be defined as the volume of the particle envelope minus the volume of external and internal voids as depicted in Figure 1 a) and Figure 1 b). Voids may also be pores [see Figure 1 d)]. The measured "volume" depends on the applied determination technique (ideally 3D) and conditions of measurement. When determining the envelope volume, adequate resolution is crucial for detecting external voids due to surface irregularities, small fractures, fissures etc. Often the only information available is from image analysis[13][14], and the volume is extrapolated based on geometric assumptions. True particle density according to Reference [15] is defined as the ratio of particle mass to its volume, excluding open and closed pores.

If a particle is dispersed into a liquid continuous phase, additional uncertainties emerge[9], due to the creation of a heterogeneous system. Liquid molecules, solutes etc. interact with the particle surface, and an "unstirred" or adsorption layer forms, becoming an integral part of the particle and consequently of its volume [see Figure 1 c)], similar to a soft core-shell particle. The thickness of such a layer is fuzzy

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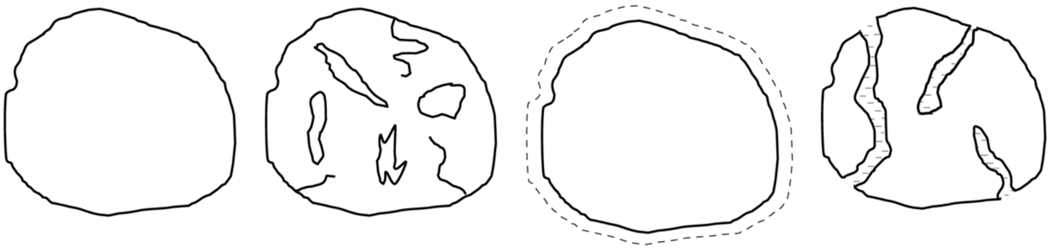
and the term corona was introduced to emphasize that fact. In general, the density and physico-chemical parameters in the corona are not constant, and gradients with respect to the distance from the "real" dry particle surface exist. The "soft" structure of this layer may be influenced by ions, surface active molecules, macromolecules or polymers of the continuous phase as well as by particle kinematics. This peculiarity is especially distinct in the case of surface-functionalized particles, such as polymer brush-grafted particles (brushed particles). The influence on "apparent particle volume" increases with decreasing particle size. Finally, filled or incompletely filled pores affect the values of these quantities [see Figure 1 d)]. Especially for dead end pores, wettability is crucial. In the case of Figure 1 d), the widely-used term "skeletal density" is defined as the ratio of the mass of the discrete particle of solid material to the sum of the volumes of the solid material in the particle and closed (or blind) pores within the solid particle[16].



a) Macroscopic solid particle

b) Particle with closed pores

c) Particle with an adjacent/immobilised layer to its surface

d) Particle with open pores

NOTE Bold lines indicate obtained solid volume relevant envelope by applied measurement technique (reproduced with permission from Reference [17]).

Figure 1 — Schematic structures of particles (cross section) with regard to the measurand particle density

Sedimentation techniques allow, principally, in situ density determination, ρ_P, of particles dispersed in liquid continuous phases. In any case, density of the liquid ρ_L has to be known and in most cases also liquid viscosity η. Four experimental approaches have been used for decades.

- Density calculation from experimentally determined velocity based on Stokes law [see Formula (1)], if shape and particle size are known.

- Measurement of migration direction of particles dispersed in a series of continuous liquid phases with different densities. Liquids densities are required to be lower and higher than that of particles to be analysed ($\rho_{L,i} < \rho_P < \rho_{L,(i+1)}$). Particle density is obtained interpolating quantitative data reflecting the reversal of migration direction to isopycnic liquid density (zero velocity). Shape does not matter, but the particles should not shrink or swell in used liquids. For details refer to ISO 18747-1.

- Buoyant density centrifugation or isopycnic gradient centrifugation. This approach is predominantly employed for preparative particle separation but was adopted for particle density determination. Density gradient centrifugation separates particles solely based on their density in contrast to migration velocity (see Annex A).

- Density determination based on precise measurement of migration velocity of particles dispersed into at least two continuous phases exhibiting different densities, driven by gravitational or centrifugal fields.

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This document deals with the latter approach. It was first applied by the analytical ultracentrifugation (AUC) community[18][19] and is based on Stokes law [see Formulae (1) and (2)]. Migration velocity v for a given particle of size x and shape factor w depends on the density contrast between liquid ρ_L and particle ρ_p and on the liquid viscosity η, respectively. If sedimentation or creaming velocity of the same particle in at least two different liquids is experimentally determined, the two velocities v_1 and v_2 are connected with particle density via Stokes law as given in Formula (1) and Formula (2).

$$v_1 = \frac{\left(\rho_P - \rho_{L,1}\right) \cdot w \cdot x^2 \cdot a}{18 \cdot \eta_1} \quad (1)$$

$$v_2 = \frac{\left(\rho_P - \rho_{L,2}\right) \cdot w \cdot x^2 \cdot a}{18 \cdot \eta_2} \quad (2)$$

where

a corresponds to the standard acceleration due to gravity g or centrifugal acceleration $a = \omega^2 \cdot r$;

v is the migration velocity;

w is the shape factor;

x is the apparent spherical particle size;

ρ_L is the density of the liquid;

ρ_P is the density of the particle;

η is the liquid viscosity;

1 and 2 are indices corresponding to the two liquids.

This approach does not require the knowledge of particle size distribution. It assumes that sedimentation related particle characteristics do not change being dispersed into the different liquids or during the measurement, i.e. the shape-size value $w \cdot x^2$ does not alter. With that assumption, Formulae (1) and (2) can be rearranged with regard to $(w \cdot x)^2$ and equated. The result in Formula (3) calculates particle density based on velocity determination of particles dispersed in liquid 1 and liquid 2.

$$\rho_P = \frac{v_1 \cdot \eta_1 \cdot \rho_{L,2} - v_2 \cdot \eta_2 \cdot \rho_{L,1}}{v_1 \cdot \eta_1 - v_2 \cdot \eta_2} \quad (3)$$

To calculate particle density ρ_P, the viscosities η_1 and η_2 as well as the fluid densities $\rho_{L,1}$ and $\rho_{L,2}$ need to be known at the measurement temperature. Uncertainty is reduced if i samples ($i > 2$) are used and is calculated for all possible pairs ρ_P (multivelocity approach).

Size and shape are not included in Formula (3) but equalization of Formulae (1) and (2) presupposes that migrating particles shall not be altered by the chosen liquids.

6 Measuring techniques to determine sedimentation and creaming/flotation velocity of dispersed particles

Determination of the particle density according to Formula (3) requires an accurate measurement of the particle velocity in chosen liquids. Any method is appropriate that allows quantitative measurement of particle migration velocity (e.g. sedimentation or creaming/flotation[2][3]). Particle migration may be driven by gravity or, especially for nanoparticles, by enhanced gravity (centrifugal field). Basic measurement principles of standard techniques which each generates information about an aspect of the sample are described in detail in References [20] and [21]. In recent years, space-resolving techniques with high resolution have become available[22][23][24].

Velocity determination should be in accordance with the Stokes law. It is obtained from direct observation of distance of particle travel over a period of time. The uncertainty of velocity measurement depends mainly on the time resolution of the measuring system and the resolution of position. A precise determination of the starting (reference) point, typically the meniscus, is important. Care shall be taken to meet the required conditions for the Stokes equation to be valid. These conditions include Reynolds number, particle concentration, wall interaction, particle-particle interaction and rheological behaviour of the continuous phase[25].

Gravitational migration velocity may be very slow in the case of particles of low density contrast or submicron particles. In such cases, analytical centrifugation is advantageous to accelerate the evaluation of particle migration (see ISO/TR 13097[26] and ISO 13318-2[27]). Multichannel instruments increase the throughput and allow for increased similarity in measurement conditions.

Care should be taken to maintain temperature stability, since liquid density and viscosity are sensitive to temperature. Therefore, instruments shall provide temperature control. Measurements for different samples shall be performed at the same temperature. Multichannel instruments are advantageous since they increase the sample throughput, and samples are measured under similar experimental conditions. Analytical cuvette centrifugation is especially appropriate for nanoparticles and continuous phases of high viscosity.

7 Preparation of samples

7.1 Continuous phase liquids

Very often water of different hydrogen isotope composition are used as liquids: normal (H_2O) and heavy (D_2O) water. Viscosity and density values with small uncertainties are tabulated for these waters in Reference [28] Both liquids interact with the particles in the same way chemically; therefore, the shape and size of the particles are expected to be the same in both liquids, and the values $w \cdot x^2$ in Formulae (1) and (2) are identical.

It may also be appropriate to prepare solutions which differ in density due to different solute concentration. It is convenient to start with a concentrated solution $\rho_{L,1}$ and dilute with the pure solvent to obtain density difference for the second one, $\rho_{L,2}$. In contrast to ISO 18747-1, densities of both liquids can be below or over particle density ρ_p.

Another approach consists of mixing two liquids of different density. Typical examples may be water-ethanol-mixtures or water-glycerol mixtures. Both of them are well characterized[29][30]. Densities of these mixtures range from 789,7 kg/m³ to 998,2 kg/m³ and 998,2 kg/m³ to 1 263,9 kg/m³ at $\vartheta = 20$ °C, respectively.

Numerical values of density as well as dynamic viscosity are functions of temperature. If the density and viscosity values are not known for a specific temperature, they shall be determined experimentally (see ISO 3105[1] for viscosity and ISO 2811-3[31] for density) and meet corresponding uncertainty requirements for particle density [see Formula (4), 9.2].

CAUTION — Liquids shall be chosen so that the particles, especially of organic or hydrocolloid matter, do not alter in shape nor swell or shrink due to, e.g. effects of solvation, osmotic pressure or ionic strength. In the case of particles with open pores, the pores shall be fully filled with the liquid; therefore, liquids with contact angle > 90° shall be avoided. Furthermore, the selected liquid should not allow gelation or particle network formation.

7.2 Dispersing procedure

Powders shall be dispersed in test liquids in accordance with the procedures specified in ISO 14887 or appropriate for particles to be analysed. Any agglomerates or flocs shall be avoided since such suspensions exhibit a wider size distribution, and the state of agglomeration or flocculation may alter during the experiment and affect the velocity distribution. All particles shall be wetted thoroughly to