
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

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 documents 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*.

A list of all parts in the ISO 18747 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

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>

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.

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

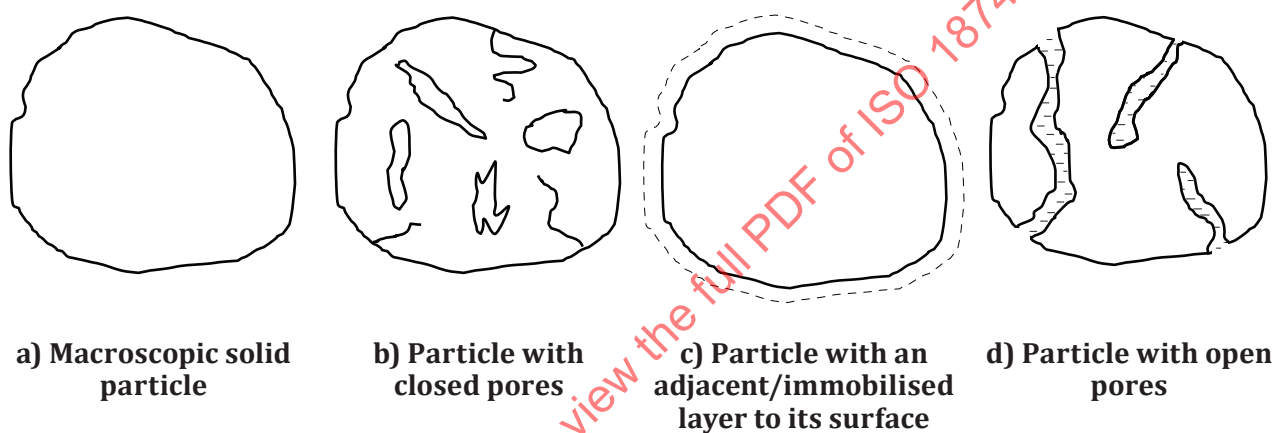
4 Symbols

Quantity	Symbol	Unit	Derivative unit
Acceleration	a	m/s ²	
Angular velocity	ω	rad/s	
Coverage factor	k	—	
Dynamic viscosity	η	Pa·s	mPa·s
Expanded uncertainty for density	U	kg/m ³	
Liquid density	ρ_L	kg/m ³	
Maximum density	$\rho_{\max}$	kg/m ³	
Minimum density	$\rho_{\min}$	kg/m ³	
Particle density	ρ_P	kg/m ³	
Radius	r	m	mm
Relative centrifugal acceleration	RCA	—	
Standard acceleration due to gravity	g	m/s ²	
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 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].



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.

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 \(2\)](#).

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

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

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 (multivelocit 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 (H₂O) and heavy (D₂O) 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 $\vartheta = 20^\circ\text{C}$, 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 $> 90^\circ$ 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 avoid density underestimation due to adhering gas bubbles to the particle surface. The continuous phase should be free of gas bubbles to avoid interference with particle migration.

The volume concentration of all samples should be the same and in accordance with the requirements of the measuring technique. In general, the volume fraction of particles shall be below 0,005, to avoid corrections for hindered settling that may differ due to different hydrodynamic particle interactions in the two continuous phases^{[32][33]}.

If the original samples are provided as dispersions, a high volume concentration is desirable. The concentrated samples should be centrifuged; the supernatant discharged and replaced by the corresponding test liquid. This procedure should be repeated until the continuous phase of original dispersion is ultimately exchanged. Any alteration of particle size distribution or shape shall be avoided.

If the density and mass of original continuous phase of test sample are known, the liquid density can also be adjusted by adding defined amounts of liquids with different density or soluble substances.

One should avoid creating bubbles in the sample. Attached to particles, bubbles can give false low-density values, and in test liquids, they can interfere with particle migration.

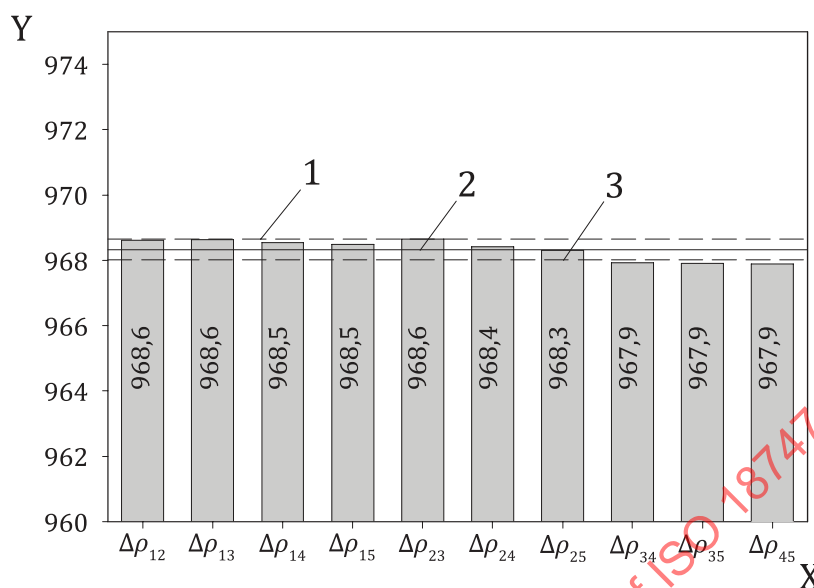
The particle density measured for a batch of material is valid only if the test sample taken is representative for that batch.

8 Measurements and data analysis

The sedimentation velocities of particles dispersed into at least two liquids differing in density shall be determined according to [Clause 6](#). It is recommended to use in addition 2 or 3 mixtures of these two liquids and to perform duplicate measurements for each liquid pair. [Table 1](#) shows example data obtained for an emulsion. The continuous phase density was tuned by mixtures of H₂O/D₂O (first column of [Table 1](#)). Density and viscosity data of the corresponding continuous phases are summarized in [Table 1](#). The mean velocities were calculated according to Reference [\[33\]](#) and are given in [Table 1](#) (fourth column). Based on the five experimentally obtained velocities, 10 pairwise velocity combinations were sorted and for each pair the droplet density was calculated according to [Formula \(3\)](#) and was displayed in [Figure 2](#). The mean particle density and its standard uncertainty are calculated from each pair result. For details refer to [B.1](#). The use of additional dispersions based on the mixtures of the two different liquids improves the standard uncertainty of the density result. It allows also to check, whether the chosen different continuous phases have an influence on density relevant particle properties. For details refer to [B.1](#).

Table 1 — Stock emulsion diluted with mixtures of H₂O and D₂O of different fractions, density and dynamic viscosity of continuous phase (tuned by normal and heavy water mixtures) and harmonic mean separation velocity of dispersed oil droplets calculated from velocity distributions (see [Figure B.1](#))

Fraction H ₂ O:D ₂ O	Liquid density ρ_L kg/m ³	Viscosity η mPa·s	Mean velocity v µm/s
1:0	997	0,891	4,96
1:3	1 021	0,941	8,67
1:1	1 047	0,991	12,44
3:1	1 072	1,038	15,62
0:1	1 104	1,094	19,26

**Key**

X	density difference $\Delta\rho$	1	standard uncertainty
Y	particle density ρ_p in kg/m³	2	mean value
		3	standard uncertainty

NOTE Density of continuous phase was tuned by mixtures of different fractions of H₂O and D₂O.

Figure 2 — Experimental determined droplet density of polydimethylsiloxane emulsion

Particle density determination can also be carried out utilizing liquids with densities lower and higher than the particle density, similar to the isopycnic interpolation approach (see ISO 18747-1 [12]). Examples are described in B.2.

Above, the mean particle velocity was employed. If the measurement technique provides particle velocity distributions [33], percentiles of the velocity may also be used. This allows getting information about the density distribution of the dispersed particles [19] [34].

The report of the analysis shall at a minimum contain the following information:

- test liquids (quality, supplier or how prepared);
- sample preparation;
- density and viscosity values (reference to literature or by which method determined);
- method and analysis used to determine particle velocity;
- temperature of sample during measurement.

9 Reference materials and measurement uncertainty

9.1 Reference materials

Particles of any shape may serve as reference material if density and its uncertainty are known or can be determined by an independent technique. Certified reference particles are preferred. Density uncertainty of the reference material shall be smaller than the accuracy needed by the application.

All reference particles shall have the same density, i.e. there shall be no density distribution due to particle composition or structure. Reference particles shall be specified regarding useable test fluids and temperature of use. They should be easy to disperse into test liquids, but they shall be inert against those test liquids (no swelling, shrinking or dissolution; no change of shape). Reference particles shall be stabilized to avoid any flocculation/agglomeration or Ostwald ripening being dispersed in the corresponding test liquids. Reference particles without pores are preferred.

9.2 Measurement uncertainty

Uncertainty of a velocity measurement depends mainly on the time resolution of the measuring system and on the resolution of position. A precise determination of the starting (reference) point to determine the sedimentation distance, typically the meniscus, is important. The influence on the result will be more pronounced if the sedimentation distance is short with respect to the uncertainty of meniscus position. For example, a 100 µm meniscus error would result in a contribution of 2 % of velocity uncertainty for a sedimentation distance of 5 mm neglecting the time uncertainty. It decreases with longer migration distances. The precision and accuracy evaluation of analytical instruments shall be performed in accordance with operational and performance validation recommended by the manufacturer. In general, laboratory repeatability shall be < 2,5 %.

The uncertainty of ρ_p [35] depends on the uncertainty of velocity determination and the accuracy of liquid density and viscosity data. The contribution of the temperature dependence on liquid viscosity and density, respectively, shall be considered.

Particle density is estimated according to [Formula \(3\)](#). Based on uncertainty propagation rules, the uncertainty of particle density can be calculated based on the uncertainties of experimental quantities on the right side of [Formula \(4\)](#) [35].

The propagation of combined uncertainty according to [Formula \(3\)](#) reads:

$$\Delta\rho_p = \sqrt{\left(\frac{v_2 \cdot \eta_1 \cdot \eta_2 \cdot (\rho_{L,1} - \rho_{L,2})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2}\right)^2 \cdot \Delta v_1^2 + \left(\frac{v_1 \cdot \eta_1 \cdot \eta_2 \cdot (\rho_{L,2} - \rho_{L,1})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2}\right)^2 \cdot \Delta v_2^2 + \left(\frac{v_1 \cdot v_2 \cdot \eta_2 \cdot (\rho_{L,1} - \rho_{L,2})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2}\right)^2 \cdot \Delta \eta_1^2 + \left(\frac{v_1 \cdot v_2 \cdot \eta_1 \cdot (\rho_{L,2} - \rho_{L,1})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2}\right)^2 \cdot \Delta \eta_2^2 + \left(\frac{-v_2 \cdot \eta_2}{v_1 \cdot \eta_1 - v_2 \cdot \eta_2}\right)^2 \cdot \Delta \rho_{L,1}^2 + \left(\frac{v_1 \cdot \eta_1}{v_1 \cdot \eta_1 - v_2 \cdot \eta_2}\right)^2 \cdot \Delta \rho_{L,2}^2} \quad (4)$$

where

- $\Delta\rho_p$ is the uncertainty of the particle density;
- ρ_L is the density of the liquid;
- ρ_p is the density of the particle;
- v is the migration velocity;
- η is the liquid viscosity;
- 1 and 2 are indices corresponding to the two liquids.

The quantity $\Delta\rho_p$ corresponds to the combined absolute uncertainty of the particle density [35]. Taking a coverage factor $k = 2$ into account, the expanded uncertainty reads $U = k \Delta\rho_p$ defining an interval about the result of a density measurement that may be expected to encompass a large fraction of measured

values within the confidence interval. The derivation of uncertainty propagation and some example calculation are given in [Annex C](#).

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Annex A (informative)

Isopycnic density gradient (buoyant density) centrifugation

This annex is adapted from ISO 18747-1:2018, Annex D.

The isopycnic density gradient technique is predominantly used for preparative particle separation, especially for biological objects, e.g. subcellular organelles or macromolecules or cancer cells^[36]. However, it can also be used for separation of minerals and for analytical purposes^[37].

Basically, the first step consists in creating a density gradient in a column or cell, using a solution with a changing concentration over the column height obeying $\rho_{\min} < \rho_p < \rho_{\max}$. This gradient can be achieved by layering several solutions with decreasing density or by building up the gradient by centrifugation of a salt, sucrose or polymer solution^{[38][39]}.

Next, particles to be analysed are carefully delivered to the bottom or top layer of the column, while preventing any mixing, and allowed to settle or float/cream, respectively. They will migrate until they reach the column position (isopycnic zone) where the column liquid density corresponds to the particles' buoyant density. Finally, the zone of particle accumulation is detected (e.g. by light, X-ray absorption or fluorescence analysis); the particle density is equal to that of the identified zone.

The steepness of the density gradient within the column and the accuracy with which it is possible to detect the position of particle accumulation determine the density resolution. The gradient may be calibrated by reference particles of known density. Shape and size do not play a role (see ISO 18747-1). Temperature shall be kept constant during gradient formation and particle migration. Care shall be taken that physico-chemical properties of the gradient column continuous phase do not influence the particles (e.g. through dissolution, swelling or shrinking).

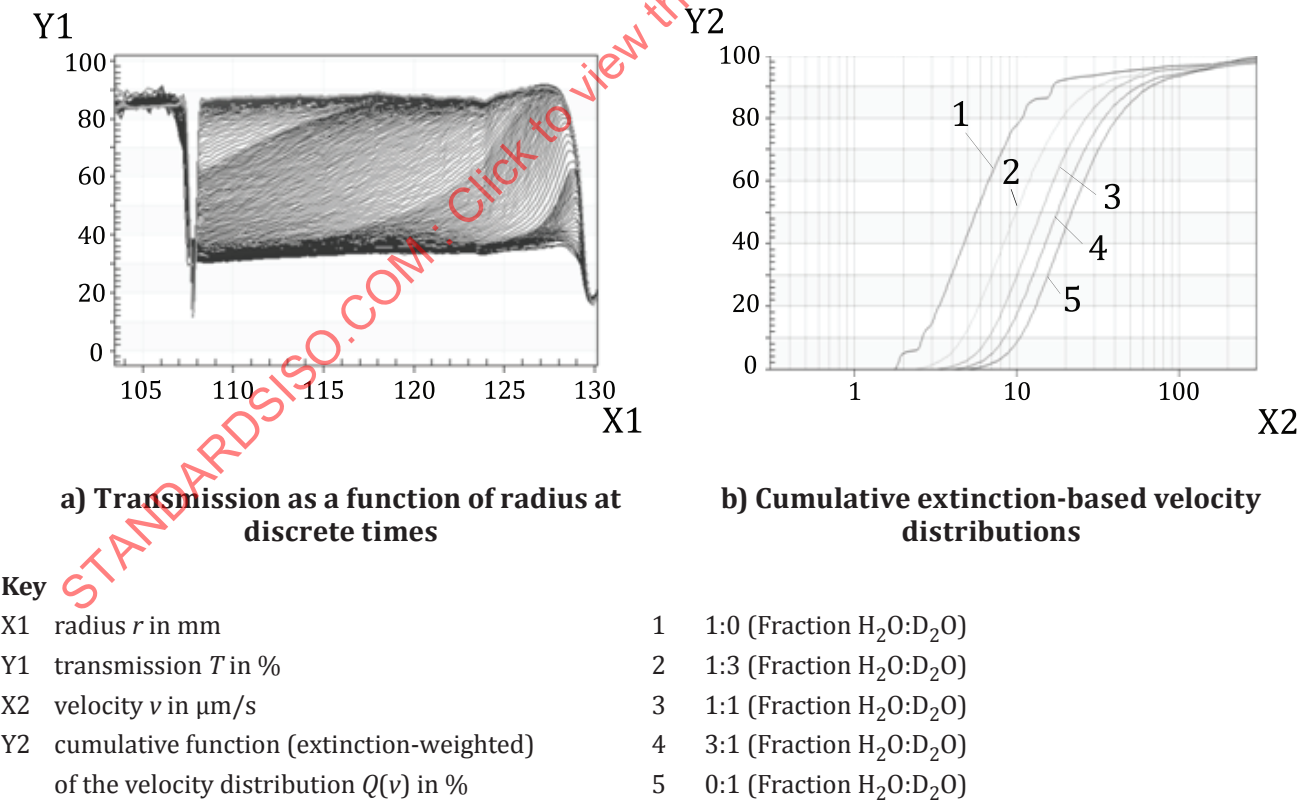
Annex B
(informative)

Examples of measurements and data analysis to determine
particle density by multi-velocity approach

B.1 Density determination of liquid particles (droplets) of polydimethylsiloxane emulsion

The continuous phase density was tuned by diluting the purchased stock of oil and water emulsion with mixtures of H₂O/D₂O. with fractions of both types of water amount of 1:0, 1:3, 1:1, 3:1 and 0:1 (see Reference [34]). Velocity of oil droplets was determined by analytical photocentrifugation[33].

Figure B.1 displays a typical fingerprint of oil droplet separation in pure heavy water (D₂O). Insertion at about 108 mm indicates the meniscus (filling height of the sample cell). The cell bottom is marked by the low transmission (129 mm). The first profile at the centrifugation start has nearly uniform transmission level (approximately 35 %), independent of radius. Due to the negative density difference ($\rho_p - \rho_L$), particles cream up, and the transmission gradually increases starting at the bottom, progressing towards the meniscus. Finally, the emulsion is separated into a small creaming layer on top of a transparent continuous phase (D₂O).



NOTE Continuous phase D₂O; analytical photocentrifuge, $\vartheta = 25\text{ }^{\circ}\text{C}$, $RCA = 2\ 300$, $\lambda = 865\text{ nm}$, 2 mm PC-cells, time interval between profiles increased in 4 steps.

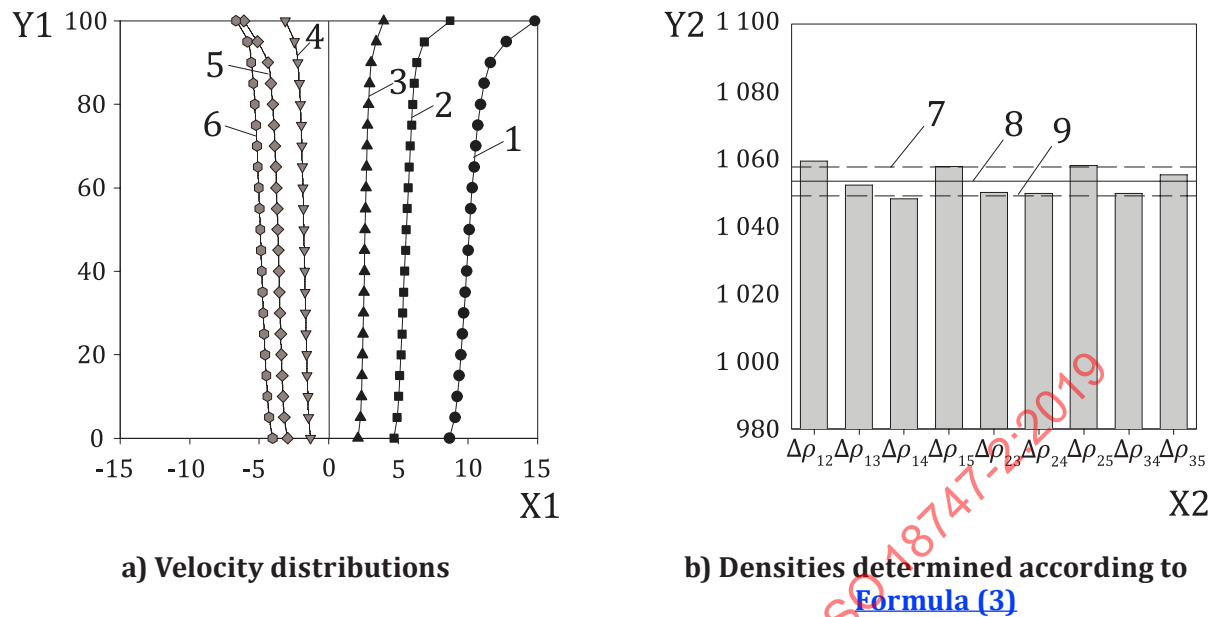
Figure B.1 — Creaming of dispersed phase (oil droplets) during centrifugation

Cumulative, extinction-weighted velocity distribution was calculated in accordance with ISO 13318-2 and displayed for all five continuous phases in [Figure B.1](#). The mean velocities as well as density and viscosity data of the continuous phases are summarized in [Table 1](#) [34]. Based on the five experimentally obtained velocities, 10 pairwise combinations were sorted. For each pair the droplet density was calculated according to [Formula \(3\)](#). Results are displayed in [Figure 2](#).

The estimated densities range from 968,6 kg/m³ to 967,9 kg/m³ at 25 °C, with mean value 968,3 kg/m³ and standard uncertainty 0,34 kg/m³ (0,31 %). For comparison, Reference [40] lists a polydimethylsiloxane density of 970 kg/m³ (no temperature given). The very minor drift towards liquid pairs with higher D₂O ratio (see [Figure 2](#)) may reflect differences in “water” adsorption for H₂O and D₂O to the droplet surface.

B.2 Density determination of spherical monodisperse polystyrene (PS) particles

PS particles (diameter 1,1 µm) from the same batch were dispersed in water or sucrose solution, with sucrose/water mass fractions of 0 %, 4,1 %, 8,3 %, 16,5 %, 20,6 % and 28,9 % [34]. Migration velocity was recorded in situ by an analytical photocentrifuge [33]. Cumulative velocity distributions of the six suspensions are shown in [Figure B.2 a\)](#). Positive velocities correspond to sedimentation and negative velocities to creaming, respectively. According to [Formula \(3\)](#), particle density is calculated based on pairs of different sucrose solutions. As [Figure B.2 b\)](#) reveals, very similar densities were obtained for all nine possible combinations (subscript 1 stands for water, 2 for 4,1 % and so on). The mean value is 1 053,4 kg/m³ [with a standard uncertainty of 4,2 kg/m³ (0,4 %)], which is the known density of polystyrene (1 055 kg/m³). Since under the above experimental conditions both creaming and sedimentation were observed, the density was also determined according to ISO 18747-1 “Isopycnic interpolation approach”. This approach reveals a particle density of 1 053,2 kg/m³. Values obtained with both approaches are in good agreement.



Key

X1	velocity v in $\mu\text{m/s}$	X2	density difference $\Delta\rho$
Y1	cumulative function (extinction-weighted) of the velocity distribution $Q(v)$ in %	Y2	particle density ρ_p in kg/m^3
1	water	7	positive standard uncertainty
2	4,1 % sucrose/water mass fraction	8	mean value
3	8,3 % sucrose/water mass fraction	9	negative standard uncertainty
4	16,5 % sucrose/water mass fraction		
5	20,6 % sucrose/water mass fraction		
6	28,9 % sucrose/water mass fraction		

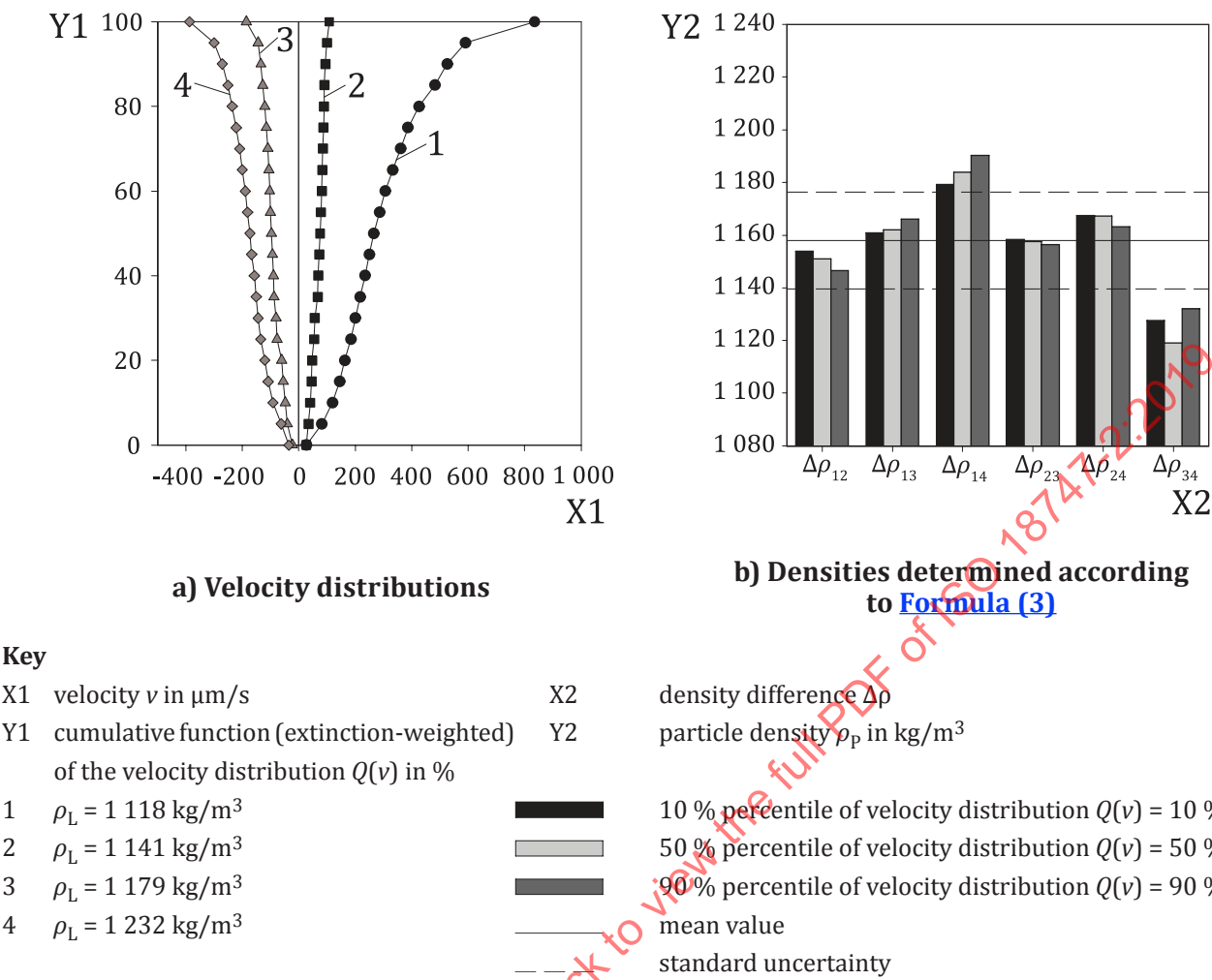
NOTE Data recorded in situ by an analytical photocentrifuge with $RCA = 480$, $\vartheta = 4^\circ\text{C}$, 2 mm PC-cells, ROI 0,5 mm width at 20 mm (sedimentation), and 2 mm and 5 mm (creaming) above bottom. Adapted and reproduced with permission from Reference [34].

Figure B.2 — Cumulative velocity distributions and densities of monodisperse polystyrene particles ($x = 1.1 \mu\text{m}$) dispersed in water and in five different concentrated sucrose solutions

B.3 Density determination of non-spherical reference particles produced from pine pollen

See Reference [41].

These very isomorphic prolate spheroidal particles have apparent spherical particle size of about $31 \mu\text{m}$ and an aspect ratio of 0,53 (standard uncertainty = $\pm 8,5 \%$). Dry pollen particles were dispersed in four differently concentrated sucrose solutions with densities of $1\,118 \text{ kg/m}^3$ to $1\,232 \text{ kg/m}^3$, and the migration velocity [see Figure B.3 a)] was obtained by an analytical photocentrifuge ($RCA = 480$, $\vartheta = 4^\circ\text{C}$, 2 mm PC-cells). Based on the four different velocity distributions, six pairwise velocity combinations can be used to calculate the particle density according to Formula (3). In this example, 10 %, 50 % and 90 % velocity percentiles were used to calculate density [see Figure B.3 b)]. The data reveal that the density values calculated from different percentiles do not systematically differ from each other indicating similar densities of analysed percentiles. The mean density value is $1\,157,9 \text{ kg/m}^3$ with a relative standard uncertainty of 18 kg/m^3 (1,6 %).



Annex C (informative)

Uncertainty derivation of particle density based on uncertainty propagation rules

Particle density measurement is based on the experimentally determined migration velocity of particles dispersed in at least two different liquids (continuous phases). According to [Formula \(3\)](#) (see [Clause 5](#)),

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

the particle density is calculated from experimentally obtained particle velocities v_1 and v_2 in liquids having densities $\rho_{L,1} \neq \rho_{L,2}$ and dynamic viscosities η_1 and η_2 respectively. Assuming that the absolute uncertainties of measured particle velocities in continuous phase 1 and 2 are Δv_1 and Δv_2 , the uncertainties of continuous phase densities are $\Delta \rho_{L,1}$ and $\Delta \rho_{L,2}$ and the uncertainties of dynamic viscosity values are $\Delta \eta_1$ and $\Delta \eta_2$, then the absolute uncertainty $\Delta \rho_P$ of the particle density ρ_P is determined by [Formula \(C.1\)](#):

$$\Delta \rho_P = \sqrt{\left(\frac{\partial \rho_P}{\partial v_1} \cdot \Delta v_1\right)^2 + \left(\frac{\partial \rho_P}{\partial v_2} \cdot \Delta v_2\right)^2 + \left(\frac{\partial \rho_P}{\partial \eta_1} \cdot \Delta \eta_1\right)^2 + \left(\frac{\partial \rho_P}{\partial \eta_2} \cdot \Delta \eta_2\right)^2 + \left(\frac{\partial \rho_P}{\partial \rho_{L,1}} \cdot \Delta \rho_{L,1}\right)^2 + \left(\frac{\partial \rho_P}{\partial \rho_{L,2}} \cdot \Delta \rho_{L,2}\right)^2} \quad (C.1)$$

$$\begin{aligned} \frac{\partial \rho_P}{\partial v_1} &= \frac{(\eta_1 \cdot \rho_{L,2} - 0) \cdot (v_1 \cdot \eta_1 - v_2 \cdot \eta_2) - (v_1 \cdot \eta_1 \cdot \rho_{L,2} - v_2 \cdot \eta_2 \cdot \rho_{L,1}) \cdot (\eta_1 - 0)}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\ &= \frac{v_1 \cdot \eta_1^2 \cdot \rho_{L,2} - v_2 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,2} - v_1 \cdot \eta_1^2 \cdot \rho_{L,2} + v_2 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,1}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\ &= \frac{v_2 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,1} - v_2 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,2}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\ &= \frac{v_2 \cdot \eta_1 \cdot \eta_2 \cdot (\rho_{L,1} - \rho_{L,2})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \end{aligned} \quad (C.2)$$

$$\begin{aligned}
 \frac{\partial \rho_P}{\partial v_2} &= \frac{(0 - \eta_2 \cdot \rho_{L,1}) \cdot (v_1 \cdot \eta_1 - v_2 \cdot \eta_2) - (v_1 \cdot \eta_1 \cdot \rho_{L,2} - v_2 \cdot \eta_2 \cdot \rho_{L,1}) \cdot (0 - \eta_2)}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{-v_1 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,1} + v_2 \cdot \eta_2^2 \cdot \rho_{L,1} + v_1 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,2} - v_2 \cdot \eta_2^2 \cdot \rho_{L,1}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,2} - v_1 \cdot \eta_1 \cdot \eta_2 \cdot \rho_{L,1}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot \eta_1 \cdot \eta_2 \cdot (\rho_{L,2} - \rho_{L,1})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot \eta_1 \cdot \eta_2 \cdot (\rho_{L,2} - \rho_{L,1})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2}
 \end{aligned} \tag{C.3}$$

$$\begin{aligned}
 \frac{\partial \rho_P}{\partial \eta_1} &= \frac{(v_1 \cdot \rho_{L,2} - 0) \cdot (v_1 \cdot \eta_1 - v_2 \cdot \eta_2) - (v_1 \cdot \eta_1 \cdot \rho_{L,2} - v_2 \cdot \eta_2 \cdot \rho_{L,1}) \cdot (v_1 - 0)}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1^2 \cdot \eta_1 \cdot \rho_{L,2} - v_1 \cdot v_2 \cdot \eta_2 \cdot \rho_{L,2} - v_1^2 \cdot \eta_1 \cdot \rho_{L,2} + v_1 \cdot v_2 \cdot \eta_2 \cdot \rho_{L,1}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot v_2 \cdot \eta_2 \cdot \rho_{L,1} - v_1 \cdot v_2 \cdot \eta_2 \cdot \rho_{L,2}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot v_2 \cdot \eta_2 \cdot (\rho_{L,1} - \rho_{L,2})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot v_2 \cdot \eta_2 \cdot (\rho_{L,1} - \rho_{L,2})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2}
 \end{aligned} \tag{C.4}$$

$$\begin{aligned}
 \frac{\partial \rho_P}{\partial \eta_2} &= \frac{(0 - v_2 \cdot \rho_{L,1}) \cdot (v_1 \cdot \eta_1 - v_2 \cdot \eta_2) - (v_1 \cdot \eta_1 \cdot \rho_{L,2} - v_2 \cdot \eta_2 \cdot \rho_{L,1}) \cdot (0 - v_2)}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{-v_1 \cdot v_2 \cdot \eta_1 \cdot \rho_{L,1} + v_2^2 \cdot \eta_2 \cdot \rho_{L,1} + v_1 \cdot v_2 \cdot \eta_1 \cdot \rho_{L,2} - v_2^2 \cdot \eta_2 \cdot \rho_{L,1}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot v_2 \cdot \eta_1 \cdot \rho_{L,2} - v_1 \cdot v_2 \cdot \eta_1 \cdot \rho_{L,1}}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot v_2 \cdot \eta_1 \cdot (\rho_{L,2} - \rho_{L,1})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2} \\
 &= \frac{v_1 \cdot v_2 \cdot \eta_1 \cdot (\rho_{L,2} - \rho_{L,1})}{(v_1 \cdot \eta_1 - v_2 \cdot \eta_2)^2}
 \end{aligned} \tag{C.5}$$