
**Fine bubble technology —
Measurement technique matrix for
the characterization of fine bubbles**

*Technologie des fines bulles — Matrice de méthodes de mesure pour
la caractérisation des fines bulles*

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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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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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 281, *Fine bubble technology*.

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

Fine bubble technology has numerous applications across industries such as cleaning, transport, maintenance, agriculture, aquaculture, food and drink, cosmetics as well as biomedical. The detection, characterization and quantification of properties of fine bubble mixtures are central to the development of this horizontal general purpose technology.

A number of techniques used for particle detection and characterization may be applicable to the characterization of fine bubble mixtures in liquids. Some techniques may have a number of special sample handling, sample preparation or equipment settings to yield quantifiable and reliable results.

This document lists a number of techniques and discusses their applicability for the characterization of fine bubble mixtures as well as their limitations. Fine bubbles are able to exist in opaque liquids or liquids of high viscosity. Some fine bubble samples are turbid due to a large number of bubbles. All fine bubble samples are dynamic in nature and their properties change with time. For this reason, the acquisition time of each technique is of great relevance. Most fine bubble samples contain particles as well as fine bubbles. Distinguishing particles and bubbles and then additionally characterizing them by size and number or vice-versa may not be possible with all particle characterization equipment.

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Fine bubble technology — Measurement technique matrix for the characterization of fine bubbles

1 Scope

This document focuses on listing most commonly used preparation and characterization techniques for fine bubbles and their interpretation. The merits and limitations of each of the techniques are outlined.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

4 Abbreviated terms

CCD	Charge coupled device
DLS	Dynamic light scattering
EZ	Electrical sensing zone method
LD	Laser diffraction methods
PSD	Particle size distribution
PTA	Particle tracking analysis method
RMM	Resonance mass measurement
SPOS	Single particle light interaction methods
SMLS	Static multiple light scattering
USS	Ultrasonic attenuation spectroscopy
ZP	Methods for Zeta-potential determination

5 Fine bubble characterization

5.1 General

A number of general particle counting and sizing techniques are available commercially. Some of them are applicable for the characterization of fine bubble dispersions and ultrafine bubble dispersions. Such dispersions may be in liquid of any kind. Some liquids may not be transparent (e.g. printer ink) or stable

(e.g. flammable fuel). This document refers to a selection of commercially available techniques and evaluates their applicability and their limitations.

The parameters of interest are as follows.

- Fine bubble size – This usually refers to the equivalent hydrodynamic diameter but could be different depending on the techniques.
- Fine bubble size distribution – For the purpose of this document, this is the number-size (or equivalent) distribution.
- Number concentration – The total number (or equivalent) of bubbles per unit volume.
- Measurement time – The time to complete data acquisition.

5.2 Comparison of size and concentration indices from different sources

Consideration should be given when different techniques are being compared, that each technique measures a different physical property of the sample. In deriving size and/or concentration indices from different techniques, it should be anticipated that results will demonstrate differences in value but they will likely show trend and/or correlate.

Care should be taken when comparing size and concentration indices. Even if the same technique is used, the method from example two laser diffraction machines will need to be checked to verify parameters such as measurement time, analysis models and pump rate. Table 1 provides a quick reference for the typical size and concentration indices of different techniques in the measurement of bubbles.

Table 1 — Quick-use matrix

Techniques		International Standard	Size	Size distribution	Number concentration (bubbles per ml)	Measurement time
Dynamic light scattering	DLS	ISO 22412	5 nm - 10 µm	Intensity-based	$> 10^9$	Typical 5 min
Methods for Zeta-potential determination	ZP	ISO 13099-2				< 5 min
Particle tracking analysis method ^b	PTA	ISO 19430	50 nm - 1 000 nm	Number-based	$10^7 - 10^9$	~5 min
Laser diffraction methods	LD	ISO 13320	100 nm - 3 mm	Volume-based	0,000 1 %	ms - 10 s
Resonance mass measurement ^a	RMM	Not available	120 nm - 1 000 nm	Number-based	$10^7 - 10^9$ micro-sensor ^a ; 9×10^6 nano-sensor ^a ; 2×10^8	0,2 nl/s ~15 min
Electrical sensing zone method	EZ	ISO 13319	50 nm- 8 000 nm	Number-based	1×10^8	10 min
Ultrasonic attenuation spectroscopy	USS	ISO 20998-1	100 nm - 1 mm	Volume-based	$> 1 \times 10^6$ For ultra-fine $> 1 \times 10^8$	5 min

^a Technique may require a special procedure or detector for obtaining appropriate results.

^b Nanoparticle tracking analysis (NTA) is often used to describe PTA. NTA is a subset of PTA since PTA covers larger range of particle sizes than nanoscale.

Table 1 (continued)

Techniques		International Standard	Size	Bubble measurands		
				Size distribution	Number concentration (bubbles per ml)	Measurement time
Single particle light interaction methods ^a	SPOS	ISO 21501-2 ISO 21501-3	0,1 µm - 100 µm	Number-based	< 10 ⁸	30 s - 5 min
Static image analysis methods	—	ISO 13322-1	0,5 µm - > 1 000 µm	Number-based	> 10 ⁸	Typical 15 min
Dynamic image analysis methods ^a	—	ISO 13322-2	0,5 µm - > 1 000 µm	Number-based	> 10 ⁸	> 5 min
Static multiple light scattering	SMLS	Under development	10 nm - 100 µm	No	10 ⁸ - 10 ¹²	~10 s
^a Technique may require a special procedure or detector for obtaining appropriate results.						
^b Nanoparticle tracking analysis (NTA) is often used to describe PTA. NTA is a subset of PTA since PTA covers larger range of particle sizes than nanoscale.						

6 Characterization techniques

This clause deals with individual techniques and how they are applied to characterizing fine bubble samples. Most samples are assumed to be in a transparent liquid, but some reference to opaque samples may be found for techniques that allow their treatment.

6.1 Dynamic light scattering

6.1.1 General

Dynamic light scattering (DLS) is also known as photon correlation spectroscopy (PCS). It measures the hydrodynamic particle size by measuring the Brownian motion of the fine bubbles in a sample.

The technique uses a laser which is passed through a sample and the light scattering measured on a detector. The detector(s) could be at a variety of angles. The intensity of light is measured over a rapid timescale. The change in scattering intensity over time as particles diffuse in and out of the measurement zone is related to their size, small particles diffuse rapidly and large particles slowly. The translational diffusion coefficient is measured which can be turned into the hydrodynamic diameter by the Stokes Einstein equation [see ISO 22412:2017, Formula (A.5)].

This intensity change is expressed as a correlation function which examines the signal change over time. From this information on the size and polydispersity of the sample can be obtained. A size distribution is derived by applying an appropriate algorithm to the correlation function. It is an intensity-based technique.

6.1.2 Reference standard

ISO 22412, *Particle size analysis — Dynamic light scattering (DLS)*

NOTE Originally, there were two International Standards relating to this technique ISO 13321¹⁾ and ISO 22412 now merged into one single document, ISO 22412.

6.1.3 Size

The lowest size in DLS will depend on the system sensitivity, and for most systems will be sufficient for sizing fine bubbles. The upper size range will depend on when the bubbles no longer behaving as

1) Withdrawn.

objects undergoing Brownian motion. In the case of particles this is normally sedimentation, but for bubbles, rising is more likely.

6.1.4 Size distribution

Dynamic light scattering measures an intensity-based distribution. This leads to sensitivity on the large particle end of the distribution as they scatter light with more intensity. In size area of interest, there is a 10^6 dependence of scattering intensity with size. This may lead to issues obtaining accurate data if large particles or contaminants are present. For a clean sample, the technique is able to provide a distribution for fine bubbles in the size area of interest.

6.1.5 Concentration

Most dynamic light scattering instruments are set up to auto adjust for concentrations ranging from low to high. Number concentration of bubbles required for appropriate measurements is generally higher than that given for particles. A concentration of (or over) 10^9 bubbles/ml is normal.

6.1.6 Measurement time

As for PTA (see 6.3), theoretical derivation of Stokes-Einstein equation [see ISO 22412:2017, Formula (A.5)] assumes no change in particle population, their size or number on the time scale of the measurement. This may not be true for dynamically changing fine bubble dispersion where all these parameters may change. The measurement time is therefore very important. An appropriate technique needs to be selected in order to ensure appropriate sampling and data acquisition.

6.2 Methods for Zeta potential determination (electrophoretic mobility)

6.2.1 General

Zeta potential is the electrical potential at the slipping plane (hydrodynamic plane of shear). It is a measure of the electrical charge on a particle and hence the stability of a system (Zeta potentials < -30 mV and > 30 mV denote a stable system. The Zeta potential is derived from the electrophoretic mobility (the movement of a particle under an applied electric field). The technique of laser Doppler electrophoresis is used.

6.2.2 Reference standard

ISO 13099-2, *Methods for zeta potential determination — Part 2: Optical methods*

6.2.3 Charge

A mean Zeta potential is normally reported which is an indicator to the stability of the system.

6.2.4 Zeta distribution

A distribution is recorded, but the mean is normally used rather than the distribution.

6.2.5 Concentration

Dilution is complex as dilution can change the particle charge, so media with exactly the same pH, ionic strength, and stabilizer concentration should be used.

6.2.6 Measurement time

Less than 5 min.

6.3 Particle tracking analysis method

6.3.1 General

Particle tracking analysis (PTA) also known as nanoparticle tracking analysis (NTA) is a method where particles undergoing Brownian motion in a liquid suspension are illuminated by a laser and the change in position of individual particles is used to determine particle size. Analysis of the time-dependent particle position yields translational diffusion coefficient. The particle size (hydrodynamic diameter) is determined from the diffusion coefficient using the Stokes-Einstein relationship (see ISO 19430).

This technique uses an optical microscope fitted with a laser beam. The scattered light of each particle is detected by a CCD camera. The motion of each particle is tracked from frame to frame by image analysis software. The Brownian motion of each particle (fundamental measurand), related to its hydrodynamic diameter, is recorded. A number-based PSD is therefore obtained.

Like in DLS, the viscosity of the dispersing medium needs to be known. The refractive index does not need to be known.

This technique applies to rather dilute specimens in the range from 20 nm to 1 000 nm. For polydisperse samples, bigger counting statistics needs to be performed to reduce uncertainties on the PSD.

6.3.2 Reference standards

ISO 19430, *Particle size analysis — Particle Tracking Analysis (PTA) Method*

ASTM E2834, *Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Nanoparticle Tracking Analysis (NTA)*

NOTE Some of the procedures described in the above standards can be slightly different for fine bubbles.

6.3.3 Size

The size range in PTA/NTA measurement is determined by various factors. The lower size range is limited by the ability of the fine bubble to scatter light and to be detected and tracked by the imaging system. Smaller bubbles scatter much less light and are therefore not detected.

The lowest limit of detection is also dependent on the bubble dispersion. If very small and very large (1 μm in diameter) bubbles are present in the same dispersion, the optical particle tracking system would be “blinded” by the bright scattering from the larger bubbles and would fail to track very small and faint bubbles.

The larger the bubbles get, the slower their Brownian motion is. This would therefore require a long period of sampling and a high stability of bubble suspension to characterize very large bubbles with diameters of the order of 1 μm .

6.3.4 Size distribution

The PTA/NTA technique is able to produce a number-based bubble size distribution. Depending on the experimental settings, this measurement can be performed relatively fast and the size distribution of bubbles in the sampling volume can be monitored as a function of time.

However, the change of bubble population in the sampling volume should not be faster than the measurement itself. Therefore, the changes within the sampling volume needs to be characterized by a much faster process.

6.3.5 Concentration

Following the discussions in ISO 19430, the number-based concentration of fine bubbles can be obtained in a measurement. However, since the measurement is performed on a very small fraction of the sample volume and not all bubbles are counted, the sub-sampling error in concentration may arise.

This does not depend on the technique used but rather on the sample preparation (sample uniformity) and sampling (how and where the suspension is taken from).

6.3.6 Measurement time

Theoretical derivation of Stokes-Einstein equation [see ISO 22412:2017, Formula (A.5)] assumes no change in particle population, their size or number on the time scale of the measurement. This may not be true for dynamically changing fine bubble dispersion where all these parameters may change. The measurement time is therefore very important. An appropriate technique needs to be selected in order to ensure appropriate sampling and data acquisition.

6.4 Laser diffraction methods

6.4.1 General

A size distribution is reported, where the predicted scattering pattern for the volumetric sum of spherical particles of known optical properties, matches the measured scattering pattern. A mathematical procedure is employed to convert the measured scattering pattern into a volumetric size distribution. A constrained mathematical procedure is used to ensure non-negative values, which limits the resolution.

6.4.2 Reference standard

ISO 13320, *Particle size analysis — Laser diffraction methods*

Laser diffraction is the first principle measurement method verified using spherical reference materials.

6.4.3 Size

Bubble sizes ranging from approximately 0,1 μm to 3 mm. With special instrumentation and conditions, the applicable size range can be extended above 3 mm and below 0,1 μm .

6.4.4 Concentration

For accurate results, single scattering limits should be observed. Randomly positioned particles should not be closer to each other than about 3 particle diameters. The number density of the smallest size of particles usually set the concentration limit which is typically $> 0,1$ volume fraction. Concentration limit is dependent upon particle size and optical properties.

6.4.5 Measurement time

Milliseconds to seconds: the measurement time depends upon the averaging period needed to ensure that a fully representative selection of particles sizes has been presented in the measurement zone.

6.5 Resonant mass measurement

6.5.1 General

Resonant mass measurement (RMM) detects and counts a particle in the size range from 50 nm to 5 μm , and measures their buoyant mass, dry mass and size. RMM uses a microfluidic channel embedded in a resonating cantilever to detect, count and measure the buoyant mass of the particles in the liquid passing through the channel. The buoyant mass of the particle or bubble changes the resonant frequency of the cantilever. Such a change is directly related to the buoyant mass of the particle. The technique also allows the accurate counting of particles or fine bubbles in the sample. RMM is applicable to characterization of protein aggregates in formulation or buffer. RMM is also able to distinguish between fine bubbles and solid contaminants by means of comparing their buoyancy relative to the liquid. Using RMM for ultra-fine bubble characterization enables quantitative assessment of bubble size distribution

and count, but critically, also provides differentiation by buoyant mass to discriminate other particles in the sample.

6.5.2 Reference standard

There is no standard available for the use of this technique for fine bubbles or particles in general.

6.5.3 Size

The size range of the equipment is determined by several factors. The largest detectable particle size limit is determined by the width of the microfluidic channel. The lower mass limit is a function of the system noise floor as well as sample and fluid densities. Smaller resonators are more sensitive to smaller bubbles but may be blocked by larger particulates in the mixture.

6.5.4 Size distribution

The effective measurands for this method is the buoyant mass of the bubble. When this is converted to size, a spherical model is assumed.

NOTE To convert the buoyant mass to size, the value of the density is required.

6.5.5 Concentration

This technique allows direct count of bubbles in a given volume of liquid. It should be noted that a significant sub-sampling occurs.

6.5.6 Measurement time

Typical measurement throughput for RMM is 0,2 nl/s. For a 10 min measurement, this equates to around 120 nl.

6.6 Electrical sensing zone method

6.6.1 General

A Coulter counter is an apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more micro channels that separate two chambers containing electrolyte solutions. As fluid containing particles or bubbles is drawn through each microchannel, each particle causes a brief change to the electrical resistance of the liquid. The counter detects these changes in electrical resistance.

NOTE Coulter counter requires mixing fine bubble samples into electrolyte solutions. However, electrolyte solutions can affect the characterization of fine bubbles.

6.6.2 Reference standard

ISO 13319, *Determination of particle size distributions — Electrical sensing zone method*

6.6.3 Size

0,2 μm to 1 600 μm .

6.6.4 Size distribution

The effective measurands for this method is the electric pulse generated by the passing of a particle through an aperture. It is assumed to be proportional to the volume of the particle passing through.

6.6.5 Concentration

The frequency of the electric pulses provides a direct number-based count of the bubbles. Too high a concentration can lead to coincidence (multiple particles passing through together) which should be avoided.

6.6.6 Measurement time

3 min to 5 min.

6.7 Ultrasonic attenuation spectroscopy

6.7.1 General

An ultrasound spectrometer is an apparatus which can be used to measure particle size distribution of concentrated particulate carrying fluids; particle types include solids, liquids and gases. Broadband ultrasound signals are transmitted through a volume containing the homogenous sample of dispersion of fine bubbles to be analysed. The received signal is detected at a known distance from the receiver. This received signal has been attenuated by the presence of the bubbles. The specific attenuation is due to a number of different physical processes and is a frequency dependant function of the bubble size distribution. The received signal or signals are used to create a frequency spectrum of the sample and as described in ISO 20998-1, the attenuation spectrum is fitted to a model of the physical processes to yield a particle size distribution.

In the case of bubbles larger than 5 μm , it is possible to detect individual bubbles using alternative detection methods.

6.7.2 Reference standard

ISO 20998-1, *Measurement and characterization of particles by acoustic methods — Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy*

6.7.3 Size

100 nm to 1 mm.

6.7.4 Size distribution

Volume-based, log-normal 2 or 3 parameter fit.

6.7.5 Concentration

From 10^6 to 10^8 (concentration cannot be given as a number-based range if the size distribution is volume-based) typical concentration range is 0,1 v% to 10 v%.

6.7.6 Measurement time

Signal generation and detection method dependant, from 1 s to approximately 5 min.

6.8 Single particle light interaction methods

6.8.1 General

This method is based on the transit of bubbles through a measurement zone or flow cell. A light source, usually a laser, is focused at the particles in the flow path and the result detected with a solid-state detector at a known angle.

Two methods are used with differing size ranges for smaller sized bubbles light scattering and the resulting scattering pattern are used to determine the particle size, while for larger particle extinction and hence light intensity can be used to determine size.

For bubbles sizes less than 1 μm .

6.8.2 Reference standards

ISO 21501-2, *Determination of particle size distribution — Single particle light interaction methods — Part 2: Light scattering liquid-borne particle counter*

ISO 21501-3, *Determination of particle size distribution — Single particle light interaction methods — Part 3: Light extinction liquid-borne particle counter*

6.8.3 Size

Light extinction, 700 nm to 100 μm .

Light scattering, 100 nm to 10 μm .

6.8.4 Size distribution

Number-based.

6.8.5 Concentration

Instrument specific however typically for size ranges $< 1 \mu\text{m}$, maximum concentration 10^7 ; $> 1 \mu\text{m}$ 10^4 . Limited by coincidence loss which is defined by optical and cell geometry a coincidence loss $\leq 10 \%$, typically.

6.8.6 Measurement time

30 s to 5 min depending on the concentration.

6.9 Static image analysis method

6.9.1 General

This method is based on the image analysis of relatively large bubbles. The sample is translated in the X-Y plane underneath a digital camera mounted behind a magnifying lens. Images are captured that contain many particles and algorithms are used to identify the particle perimeters, then from that, to calculate a variety of size and shape parameters. After analysing thousands of particles, distributions can be determined. Because of the power of modern desktop computers, the analysis of a dozen size and shape parameters for thousands of bubbles can be performed within minutes.

6.9.2 Reference standard

ISO 13322-1, *Particle size analysis — Image analysis methods — Part 1: Static image analysis methods*

6.9.3 Size

0,5 μm to $> 1\,000 \mu\text{m}$.

6.9.4 Size distribution

Number-based particle size distribution.

6.9.5 Concentration

It is difficult to state an absolute upper limit, as specialist software algorithms can provide good estimates of overlapping or occluded particles, so it is possible to get a particle size distribution from a high-quality image of foam. However, unless specialist algorithms are used, the preferred concentration is such that the number of touching particles is minimised and edge killing algorithms can be used to sharpen images.

Smaller particles can be measured at higher concentrations than larger particles.

If a polydisperse sample is measured, care needs to be taken in regard to the depth of field as bubbles of different sizes may present their widest diameter at different planes of focus.

Cover slips are not recommended due to potential to deform bubbles.

6.9.6 Measurement time

> 15 min depending on number of particles measured.

6.10 Dynamic image analysis methods

6.10.1 Reference standard

ISO 13322-2, *Particle size analysis — Image analysis methods — Part 2: Dynamic image analysis methods*

6.10.2 Size

0,5 µm to > 1 000 µm.

6.10.3 Size distribution

Number-based.

6.10.4 Concentration

Algorithms will be needed to cope with reductions in concentration due to edge killing effects.

With specialist algorithms, high concentrations can be measured. Without these algorithms, particles should not be touching.

6.10.5 Measurement time

> 5 min.

6.11 Static multiple light scattering (SMLS)

6.11.1 General

This method is based on the measurement of light intensity after multiple scattering from a randomly dispersed sample, in a given geometry. Multiple scattering consists of a successive scattering of radiation within the scattering medium.

NOTE In multiple scattering samples, incident light is scattered successively a number of times, thereby rapidly losing the memory of the incident direction.

Static multiple light scattering is based on static light scattering principle applied to concentrated or turbid bubble media. This method relies on successive scattering of the radiation within the scattering medium. In concentrated samples, the incoming light is scattered successively numerous times, thereby rapidly losing the memory of the incident direction. The intensity of the multi-scattered light depends