
**Fine bubble technology —
Characterization of microbubbles —
Part 1:
Off-line evaluation of size index**

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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*.

A list of all parts in the ISO 21910 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

Recent development in the fine bubble technology expands its market, such as cleaning, water treatment, agriculture and aquaculture as well as biomedical. Above all, the application of microbubble technology accelerates the market penetration.

Many measurement technologies have been historically developed to assess the characteristics of microbubbles and are now used in various application fields. However, the dynamic nature of microbubbles makes it hard for the users to report their measurement results with confidence. The low stability of microbubbles that includes shrinking, deformation, coalescence and dissolution of individual microbubble can require a specific sampling procedure and short measurement time.

This document is intended to specify an evaluation method for size index of microbubbles in water to be used in a measurement laboratory. The application of the document to measurement system will yield comparable results over an application field, as far as the specified types of measuring instruments are equipped and the specified sampling procedures are met. Since the comparability relevance depends on the sampling procedures and the measurement environments, each measurement can require relevant descriptions. The specifications of the measuring instruments are described in other standards or the individual operation instruction manuals.

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Fine bubble technology — Characterization of microbubbles —

Part 1: Off-line evaluation of size index

1 Scope

This document specifies the evaluation method for the size index of microbubbles in microbubble dispersion. It is only applicable to microbubbles with or without shell in water within the range from 1 μm to 100 μm . It describes the sampling methods from the point generating or dispersing microbubbles in the retention container to the detecting point of the measuring instruments.

2 Normative references

There are no normative references in this document.

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:

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

3.1

measurement time

period for a sequence of measuring process, whereas the size index and/or the number concentration index of the microbubbles can be assumed stable all through the period and reproducible over the periods with similar measurement condition

Note 1 to entry: Measurement time is described by the starting time and the ending time, or by either of them and the duration.

3.2

water diluent

homogeneous water which is used for dilution without causing any deleterious effects and whose number concentration of ultrafine bubbles is known

Note 1 to entry: Water diluent is used to decrease the number concentration of ultrafine bubbles in a dispersion without changing their total number, state of aggregation with particles, size or surface chemistry.

Note 2 to entry: Water diluent is called blank water when its number concentration of ultrafine bubbles is known to be zero and when it is used for the evaluation of ultrafine bubbles.

[SOURCE: ISO 20298-1:2018, 3.2]

3.3

retention time

period from the point generating or dispersing microbubbles in the retention container to the detecting point of the measuring instruments

3.4

method repeatability

closeness of agreement between multiple measurement results of a given property in different aliquots of a sample, executed by the same operator in the same instrument under identical conditions within a short period of time

Note 1 to entry: The variability includes those uncertainties due to operator sub sampling technique, any changes in the sampled material together instrument variations.

[SOURCE: ISO 13320:2020, 3.22]

4 Requirements

4.1 Requirements on the sample

Microbubbles should be present in pure water or can be in tap water if the numbers of contaminant particles are much lower than those of microbubbles. The microbubbles should contain air, nitrogen and oxygen. In cases where they are surrounded with a coating, e.g. a lipid, other gas can be applied.

The size range of microbubbles that can be measured depends on the specification of the measuring instrument to be used as is the number or volume concentration range of microbubbles. The reliability of the results shall be confirmed for each measuring instrument.

Microbubbles which diameter is equal or larger than 10 μm should be measured promptly (see 7.6.1). Microbubbles less than 10 μm and microbubbles with shell may not need to be measured quickly. This classification is referred to in ISO 20480-2.

4.2 Requirements on the sample transfer and measurement system

An appropriate sample transfer and measurement system which connects a microbubble generating system and a measuring instrument enable to characterize unstable microbubble dispersion in water as a size index. Because of the low bubble stability of microbubbles without shell and the decrease in dispersibility of microbubbles with shell, it is recommended to have the measuring instrument in the vicinity of either the generating system or the dispersing system.

The size index of microbubbles should be determined just after the sample transfer. Signal acquisition time of measuring instrument should be set at a minimum interval which is necessary for the detection of sufficient signals from the microbubbles to determine its size index with good reproducibility. Before loading the sample into a measuring instrument, clean the inside of a generating system by rinsing it several times with water diluent to remove contaminants.

Attention should be paid to avoid microbubbles to adhere to the inside of the tube which is used for loading to the measuring instrument.

Before each measurement, it should be confirmed if microbubbles are dispersed homogeneously or saturated in water at a trial run.

Microbubbles with shell are inherently stable; however, they may settle down to the bottom of the container. The procedure to measure microbubbles without shell can be applicable to measure microbubbles with shell when they are dispersed uniformly during the measurement.

Storage or transportation of microbubbles without shell in microbubble dispersion is almost impossible due to the low bubble stability.

5 Measuring instruments

Measuring instruments based on the following measurement techniques should be used to determine the size index of microbubbles.

- Dynamic image analysis methods (see ISO 13322-2).
- Laser diffraction methods (see ISO 13320).
- Light extinction liquid-borne particle counter (see ISO 21501-3).

The reference of the method used shall be reported.

Refer to [Annex E](#) for an example of comparison among three measurement techniques.

6 Environment

The classification of air cleanliness should be applied for the measurement to prevent the contamination of impurities.

Ambient temperature and atmospheric pressure should be stable to maintain the stability of microbubble size.

7 Sample transfer and measurement system

7.1 General

The dynamic changes of microbubbles without shell may make it difficult to measure the microbubble size. To obtain reproducible results with off-line measurement, the appropriate way to load microbubble dispersion is the key technology to measure them promptly before they disappear.

For this purpose, the sample transfer and measurement system as shown in [Figure 1](#) shall be used.

Essential information to define a sample transfer and measurement system is given in [7.2](#) to [7.7](#).

7.2 System structure

Microbubbles to be measured shall be generated or discharged in a retention container. The container may work as a buffer to circulate microbubbles dispersion. The samples shall be sucked into a flow cell using loading tube and loading pump set at the back of a flow cell.

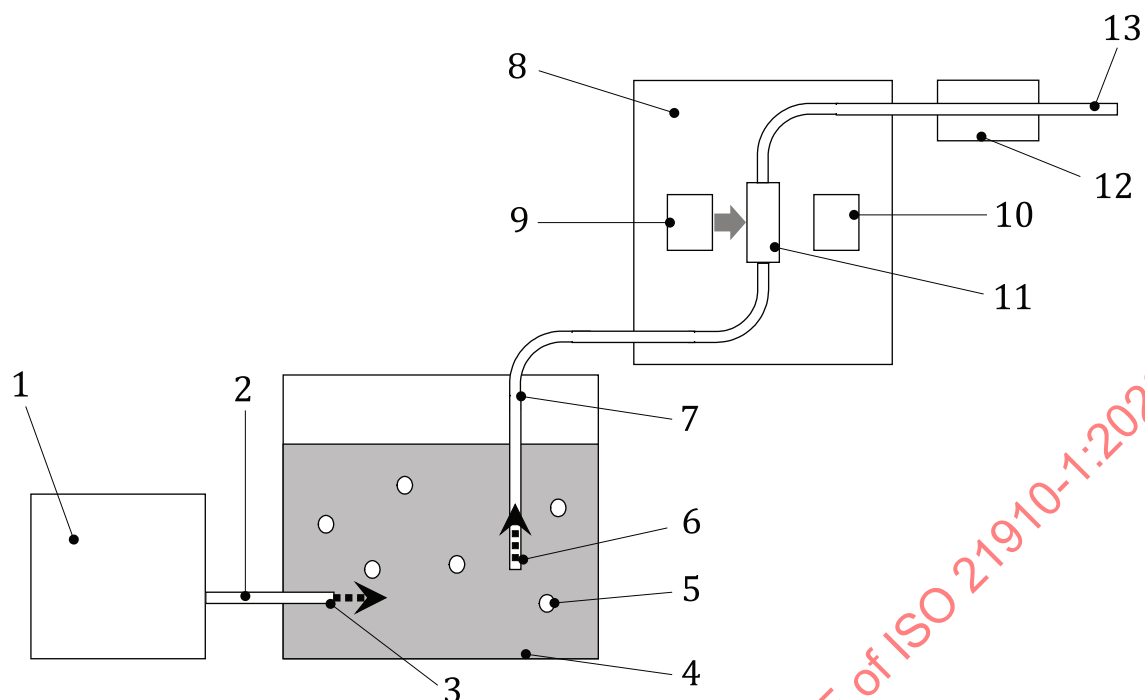
7.3 Arrangement of components

7.3.1 Position of the inlet tube mouth

The position of the inlet tube mouth and the direction of it should be optimized for the spatial stability at a trial run, or microbubbles should be measured at several positions to be averaged in consideration to the velocity distribution in the retention container. If the position of the inlet tube mouth is set close to the container wall and the surface of microbubbles dispersion, results may show low concentration. If the position of the inlet tube mouth is set in front of outlet from a generating system, results may show high concentration.

7.3.2 Position of the measuring instrument

The position of the measuring instrument should be close to a retention container to minimize the tube length.



Key

- | | |
|---|------------------------|
| 1 generating system | 8 measuring instrument |
| 2 outlet tube for generating system | 9 light source |
| 3 outlet tube mouth for generating system | 10 detector |
| 4 retention container | 11 flow cell |
| 5 microbubble | 12 loading pump |
| 6 inlet tube mouth for measuring instrument | 13 drainage tube |
| 7 loading tube | |

Figure 1 — Sample transfer and measurement system (side view)

7.4 Retention container

7.4.1 General

A retention container should be defined depending on the purpose of the measurement, e.g. to evaluate the specification for a generating system under the recommended condition or to understand the dispersed situation for each actual usage.

7.4.2 Configuration requirements for retention container

The retention container size should be defined after testing different sized containers at a trial run. In a large container, microbubbles may disperse quickly leading to a low concentration.

The retention container structure should not be cylindrical shape in which microbubbles may rotate inhomogeneously. It also should not be shallow shape in which microbubbles may float quickly to the surface.

7.5 Loading tube

7.5.1 Loading tube inner diameter

Inner diameter shall be equal or larger than at least 2 mm. Small inner diameter can affect microbubbles characteristics due to interaction of the loading tube inner surface (see [B.2](#)).

7.5.2 Loading tube length

Loading tube length should be short and may be defined by the length from the inlet tube mouth for the measuring instrument to the detecting point of the measuring instrument. Long tube length may cause microbubbles extinction due to interaction of the tube inner surface. It may be defined by the retention time, flow rate and tube inner diameter. It is recommended to consider the volume inside a measuring instrument, that is the hidden tube and the flow cell volume up to the detecting point (see [B.2](#)).

7.5.3 Curvature of the loading tube

Curvature of the loading tube should be larger than 100 mm. Small curvature may cause an inner vortex and affect microbubbles characteristics.

7.5.4 Surface roughness

As surface roughness of loading tube, the arithmetical mean deviation of the assessed profile, Ra ^[1], is available. Surface roughness should be less than 0,4 μm . High surface roughness may cause microbubbles adhesion and extinction due to interaction of the loading tube inner surface.

7.5.5 Loading tube materials

Loading tube materials shall have hydrophobic characteristic and shall not be eluted into microbubble dispersion. They shall not be soft materials like natural rubber to prevent vibration. If microbubbles are negatively charged as microbubbles without shell, the tube materials shall not be positively charged materials like polyamide to prevent adhesion. They should be negatively charged materials like PTFE (polytetrafluoroethylene) and PFA (perfluoroalkoxy alkane). (See [B.3](#)).

7.5.6 Suppression of loading tube sway

Sway or vibration of the loading tube may damage a measuring instrument and may interfere with accurate measurements.

To minimize the sway or vibration of the loading tube, the tube should be fixed at appropriate position.

7.6 Loading pump

7.6.1 General

The samples shall be sucked from the back of the detecting point using a loading pump. The loading pump should be adjustable of the flow rate. In addition, it should be sucked stably and be drained under the atmospheric pressure. The pulsation created by a loading pump should be minimized to prevent from producing large uncertainties in the results. The loading pump can include devices to minimize pulsation like a dumper, an air chamber and longer drainage tube.

7.6.2 Flow rate (Flow velocity)

Flow rate should be slow to avoid deformation and coalescence due to rapid fluid flow. Reynolds number calculated from flow rate shall be less than 2 300 at which the transition from laminar to turbulent flow occurs. It should preferably be less than 1 150.

Calculate the Reynolds number using [Formula \(1\)](#):

$$Re = \frac{V \times d}{\nu} \quad (1)$$

where

Re is Reynolds number;

V is the flow velocity;

d is the inner diameter of the loading tube;

ν is the kinetic viscosity.

The flow rate should be greater than 25 ml/min for prompt measurement, although the lower limit of flow rate may be automatically defined by the loading tube inner diameter and length. (See [B.1](#)).

7.7 Retention time

The retention time can be calculated using [Formula \(2\)](#):

$$\text{retention time} = \left(\pi \left(\frac{d}{2} \right)^2 \times l + V_c \right) / q \quad (2)$$

where

d is the inner diameter of loading tube;

l is the loading tube length including inside tube in the measuring instrument;

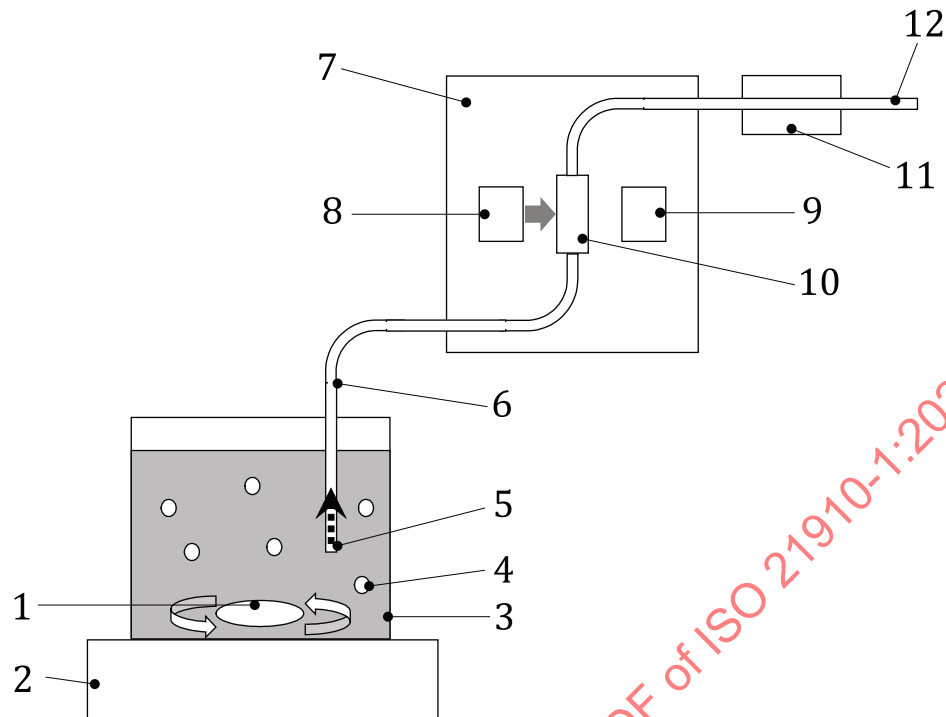
V_c is the cell volume to reach the detecting point;

q is the flow rate from the point generating or dispersing microbubbles in the retention container to the detecting point of the measuring instruments.

The retention time is the most important parameter to measure microbubbles without shell with diameter equal or larger than 10 μm . For prompt measurement, it should be equal or less than 5 s. It can be set at several timing to reveal the behaviour of microbubbles, like 5 s, 10 s and 15 s. (See [Annex A](#)).

7.8 Dispersion before/during sampling for microbubbles with shell

Microbubbles with shell shall be dispersed homogeneously before/during the measurement. A magnetic stirrer can be used to disperse homogeneously. See [Figure 2](#).



Key

- | | |
|---|------------------------|
| 1 stirrer | 7 measuring instrument |
| 2 magnetic stirrer | 8 light source |
| 3 retention container | 9 detector |
| 4 microbubbles with shell | 10 flow cell |
| 5 inlet tube mouth for measuring instrument | 11 loading pump |
| 6 loading tube | 12 drainage tube |

Figure 2 — Dispersion before/during sampling system (side view)

8 Procedure

Measurements shall be carried out in accordance with the following procedure.

- Generate microbubbles without shell or disperse microbubbles with shell into a retention container until they are homogenized.
- Suck microbubbles dispersion with the inlet tube to load into the flow cell.
- Adjust the retention time referring to 7.7.
- Measure microbubbles five times or more during generating.
- Measure the blank water.

9 Data acquisition

9.1 General

All data should be accumulated and summed statistically.

9.2 Measurement time

Measurement time should be set as to minimize the variability. It shall be defined at a trial run.

9.3 Number of measurements

Data should be acquired 5 times or more repeatedly. The sequential data can be used to sum the average of the size indices and uncertainty evaluation. (See [Annex D](#)).

9.4 Size category

The size category of microbubbles is defined as follows in accordance with fine bubble index (see ISO 20480-2).

1 μm to 10 μm , 10 μm to 100 μm

NOTE Some measuring instruments do not include the size range from 1 μm to 10 μm . In all cases, the reliability and the uncertainty of the instruments need to be checked.

9.5 Results

The average size and/or the modal size of microbubbles can be reported as the representative value. The size range should be categorized as specified in [9.4](#).

9.6 Calibration and traceability

The measuring instruments shall be calibrated in accordance with the standard used, e.g. ISO 13322-2, ISO 13320, or ISO 21501-3.

9.7 Uncertainty evaluation

The uncertainty of measurements should be composed of repeated measurement uncertainty, calibration uncertainty and others uncertainties (e.g. operators, flow rate and temperature). (See ISO/IEC Guide 98-1^[2], ISO/TS 21749^[3]).

10 Correction

10.1 General

Other corrections as well as calibrations especially important for microbubbles are specified as follows and shall be applied.

10.2 Reserved water used for blank preparation

The micro-scale objects are all picked up by the measuring instrument no matter if they are microbubbles or not, and may be wrongly identified as microbubbles. In order to correct the reading of the measuring instrument, one can assume that microbubbles without shell with low bubble stability differ from those with high bubble stability, other micro-scale objects such as solid particles. Practically, after the measurement on sample water, the sample water is kept for considerably longer time than the bubble stability of microbubbles. The latter status of the sample water is used as the blank, whose measurement result is to be subtracted from that of previous status of the sample water, yielding the number distribution of the microbubbles. The size index is then calculated by taking statistics on the number distribution. (See [Annex C](#)).

The preparation of the blank is one of the essential process of the measurement and is conducted typically by leaving the sample water for a day in a given environment. The optimized environment

and procedure should be established by using reference sample water with used liquid and standard materials such as poly-styrene latex.

11 Report

The following information should be reported based on the testing results:

- a) size index of microbubbles with measurement uncertainty;
- b) the measurement time;
- c) the retention time from the generating or dispersing point to the detecting point;
- d) gas medium, liquid medium;
- e) history of microbubbles, guaranteeing the reproducibility of the measurement results;
- f) environmental condition of microbubble dispersion, temperature, relative humidity, pressure;
- g) date, time of measurement;
- h) identification of measuring instrument;
- i) identification of generating system;
- i) identification of measurement officer;
- k) identification of location of the measurement;
- l) declaration statement on the reliability of the measurement result.

Annex A **(informative)**

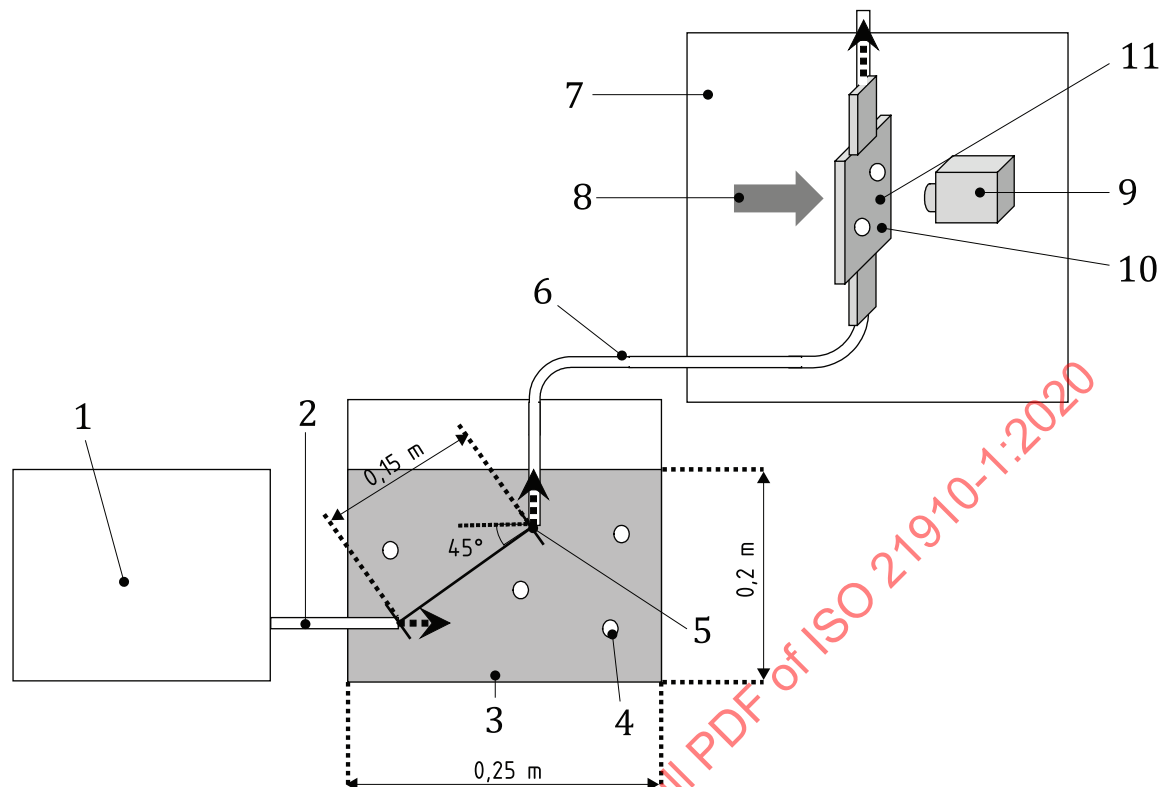
Example of microbubbles without shell measurement using dynamic image analysis

A.1 Sample transfer and measurement system

This sample transfer and measurement system contains the retention container generated microbubbles and the measuring instrument whose principle is dynamic image analysis methods with flow cell. See [Figure A.1](#).

It can be set a given container for actual usage. The position of inlet tube mouth for measuring instrument can be optimized to meet the purpose, or microbubbles can be measured at several positions. The water, after microbubbles disappear, works as diluent in a large retention container. In this case, the concentration may decrease.

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Key

- | | |
|---|------------------------|
| 1 generating system | 7 measuring instrument |
| 2 outlet tube for generating system | 8 light source |
| 3 retention container | 9 detector |
| 4 microbubble | 10 flow cell |
| 5 inlet tube mouth for measuring instrument | 11 detecting point |
| 6 loading tube | |

NOTE The size of the retention container is 0,25 m in width, 0,25 m in depth, and 0,25 m in height.

Figure A.1 — Example of sample transfer and measurement system

A.2 Procedure

Generate microbubbles into a retention container until they are homogenized.

Suck microbubbles dispersion with the inlet tube to load into the flow cell.

Adjust the retention time as to set 5 s, 10 s and 15 s.

Measure them five times or more during generating.

Leave the sample water for a day to prepare the blank water of microbubbles without shell.

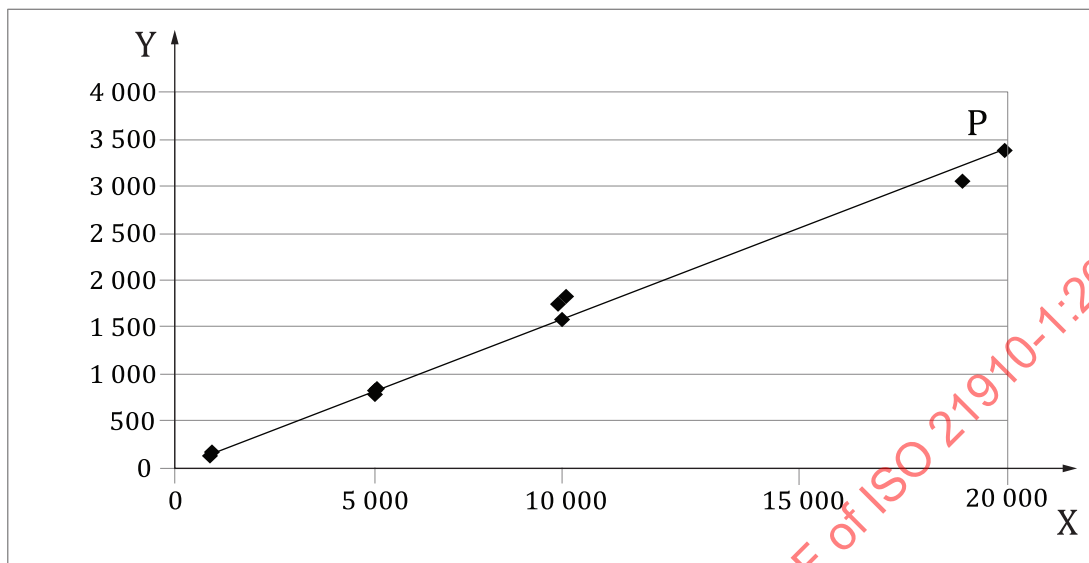
Measure the blank water in the same way as microbubbles dispersion.

A.3 Number concentration

Measure the number concentration of the standard particle diluted water prepared as several kinds of the concentration under the same condition as microbubble measurements.

Draw a calibration curve with the known number concentration and the output data.

Calculate the number concentration of microbubble from the output data using a calibration curve. See [Figure A.2](#).



Key

X number concentration of standard particle dilute sample (ml)

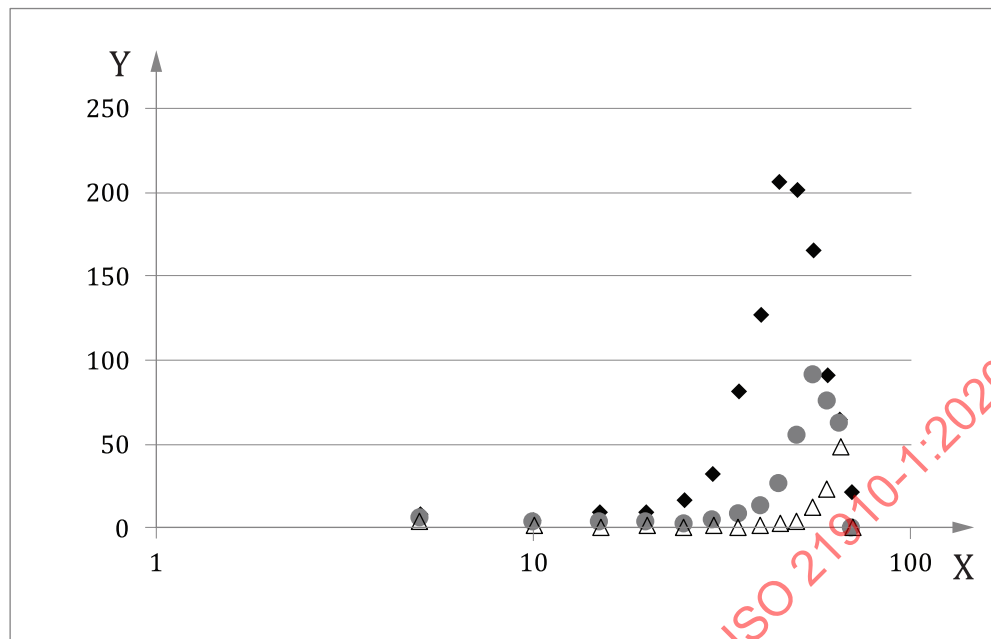
Y value of measuring instrument (counts/10 min)

P regression line by the least squares method

Figure A.2 — Example of calibration curve

A.4 Example of measurement results

[Figure A.3](#) shows the relationship between size distribution and retention time, and [Table A.1](#) shows the relationship between mean or modal size and retention time within the size range of 10 μm to 100 μm .

**Key**X size (μm)Y number concentration($/\text{ml}$)

◆ size distribution at 5 s of retention time

● size distribution at 10 s as the retention time

△ size distribution at 15 s as the retention time

Figure A.3 — Relationship between size distribution and retention time**Table A.1 — Relationship between mean or modal size and retention time within the size range of 10 μm to 100 μm**

Retention time	5 s	10 s	15 s
Mean size (μm)	50,2	56,1	61,4
Modal size (μm)	47,5	61,4	69,8

Annex B (informative)

Effect of setting arrangement

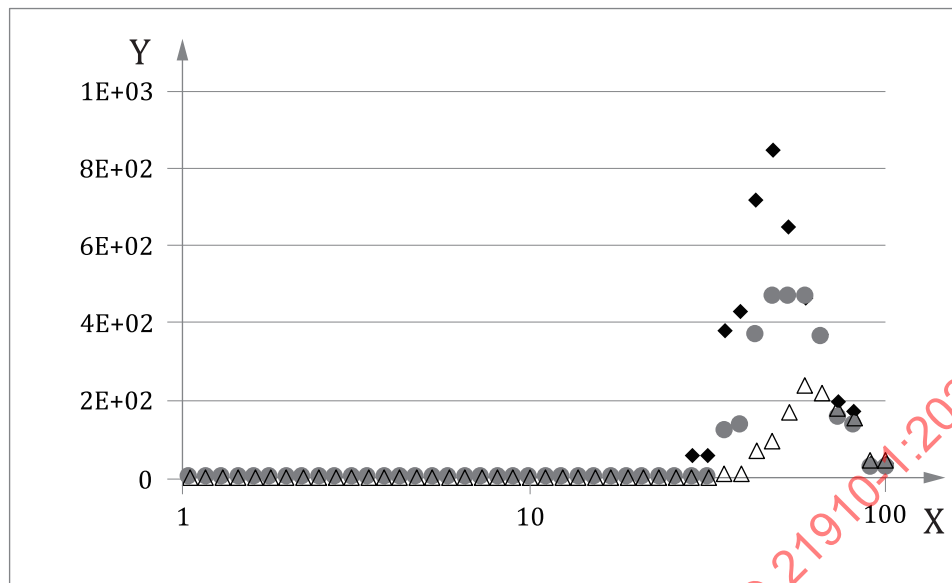
B.1 Flow rate and tube length

This is the example of comparisons among different flow rates using laser diffraction methods with flow cell.

Flow rate and tube length are adjusted to keep the identical retention time. Higher flow rate and longer tube length cause decrease the number of microbubbles and enlarge the size of them. See [Table B.1](#) and [Figure B.1](#).

Table B.1 — Example of the setting comparing among the flow rate

		Condition 1	Condition 2	Condition 3
q	Volume flow rate (ml/min)	166	211	362
Re	Reynolds number ^a	986	1 254	2 151
V	Velocity (mm/sec) ^b	220	280	480
l	Tube length (m) ^c	0,7	1,0	2,0
d	Tube diameter (mm)	4		
v	Kinetic viscosity (cSt{mm ² /s})	0,893		
RT	Retention time (second)	5		
V _c	Cell volume (ml)	5		
^a Calculated by Formula (1) (see 7.5.1).				
^b Calculated using volume flow rate and tube diameter.				
^c Adjusted to keep the identical retention time.				



Key

- X size (μm)
- Y number concentration (/ml)
- ◆ size distribution under condition 1
- size distribution under condition 2
- △ size distribution under condition 3

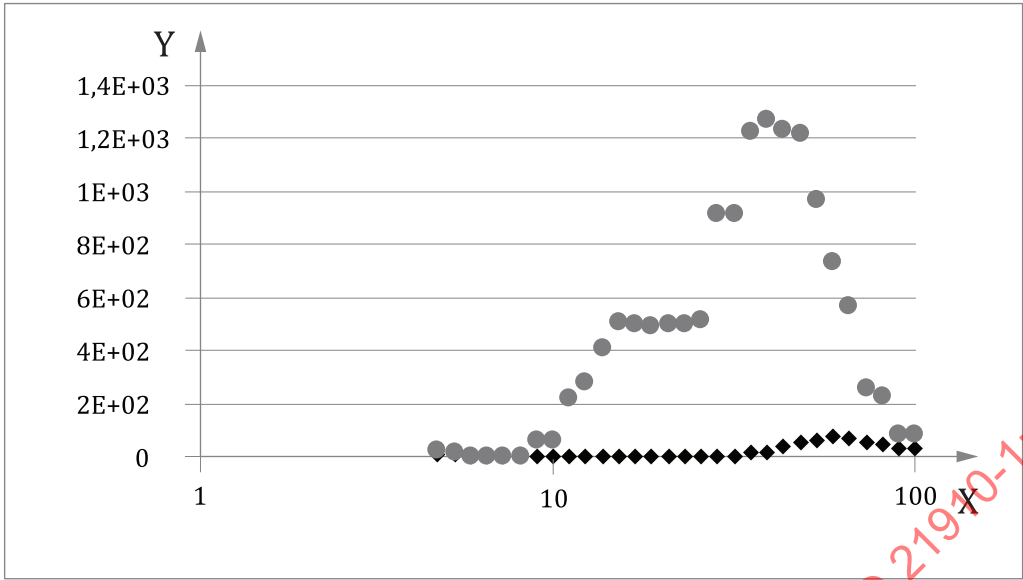
Figure B.1 — Relationship between size distribution and flow rate

B.2 Tube diameter and length

This is the example of comparisons between different tube diameters. Tube diameter and length are adjusted to keep the identical retention time and the nearly identical flow rate. Smaller tube diameter and long tube length cause decrease the number of microbubbles. See [Table B.2](#) and [Figure B.2](#).

Table B.2 — Example of the setting comparing among the tube diameter and length

		Condition 4	Condition 5
d	Tube diameter (mm)	2	4
l	Tube length (m) ^a	2,5	0,7
Re	Reynolds number ^b	1 854	986
V	Velocity (mm/sec)	828	220
q	Volume flow rate (ml/min)	156	166
ν	Kinetic viscosity [cSt{mm ² /s}]	0,893	
RT	Retention time (second)	5	
V _c	Cell volume (ml)	5	
^a Adjusted to keep volume flow rate the same level.			
^b Calculated by Formula (1) (see 7.5.1).			



- Key**
- X size (μm)
 - Y number concentration ($/\text{ml}$)
 - ◆ size distribution under condition 4
 - size distribution under condition 5

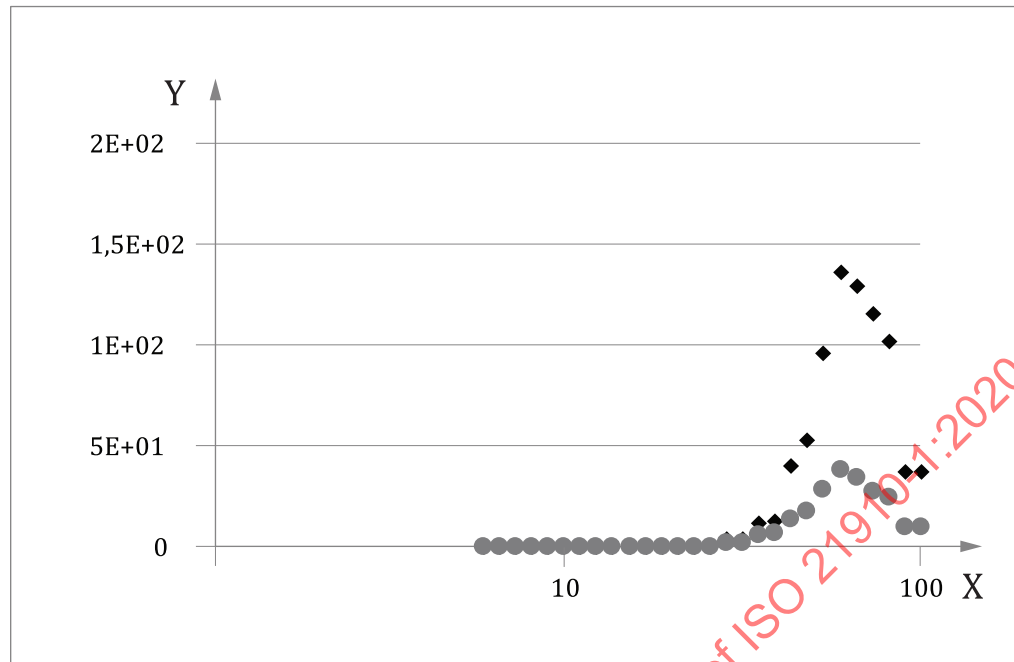
Figure B.2 — Example of size distribution

B.3 Tube materials

This is the example of comparisons between different tube materials. One is PFA, negatively charged as microbubbles without shell. Another is polyamide, positively charged materials. Latter causes decrease the number of microbubbles. See [Table B.3](#) and [Figure B.3](#).

Table B.3 — Example of the setting comparing among the tube materials

	Condition 6	Condition 7
Material	PFA	Polyamide
Charge	Negative	Positive
Surface roughness Ra	0,19	0,08

**Key**X size (μm)Y number concentration($/\text{ml}$)

◆ size distribution under condition 6

● size distribution under condition 7

Figure B.3 — Example of size distribution comparing among the tube materials

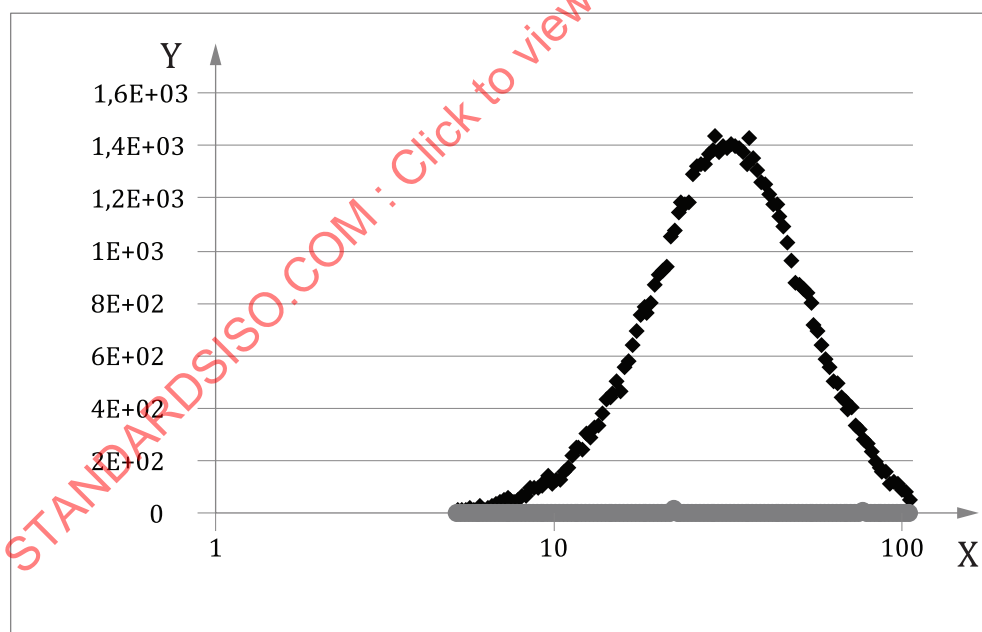
Annex C (informative)

Example of corrected data by subtraction of blank water

This is the example of subtraction of blank water using dynamic image analysis methods with flow cell. Microbubble dispersion A shows the result measuring microbubble dispersion, which may include some solid particles as contaminants. Blank water B shows the result measuring blank water, which is prepared by leaving the microbubble dispersion for a day under a given environment. The corrected data are subtracted value of Blank water B from value of microbubble dispersion A. See [Table C.1](#) and [Figure C.1](#).

Table C.1 — Example of the measurement results (Size range: 10 μm to 100 μm)

Sample description	Microbubble dispersion A	Blank water B	A - B
Number concentration (/ml)	68 639	18	68 621
Mean size(μm)	32,7	37,5	32,7
Modal size(μm)	27,0	20,5	27,0
NOTE1 This is an example of the measurement results under the condition of 5 s' retention time.			
NOTE2 Number concentration is calculated using the calibration curve made for each measurement.			



Key

- X size (μm)
- Y number concentration(/ml)
- ◆ size distribution of microbubble dispersion
- size distribution of blank water

Figure C.1 — Example of size distribution comparing among microbubble dispersion and blank water