



BSI Standards Publication

Fine bubble technology — Evaluation method for determining the reactive oxygen species in ultrafine bubble dispersions

Part 1: Probe based kinetic model

National foreword

This British Standard is the UK implementation of ISO 24758-1:2025.

The UK participation in its preparation was entrusted to Technical Committee LBI/50, Fine Bubble Technology (FBT).

A list of organizations represented on this committee can be obtained on request to its committee manager.

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient's own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

© The British Standards Institution 2025
Published by BSI Standards Limited 2025

ISBN 978 0 539 32994 0

ICS 07.030

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 December 2025.

Amendments/corrigenda issued since publication

Date	Text affected
------	---------------



International
Standard

ISO 24758-1

**Fine bubble technology —
Evaluation method for determining
the reactive oxygen species in
ultrafine bubble dispersions —**

**Part 1:
Probe based kinetic model**

*Technologie des fines bulles — Méthode d'évaluation pour
déterminer les espèces réactives de l'oxygène dans les dispersions
de bulles ultrafines —*

Partie 1: Modèle cinétique basé sur les sondes

**First edition
2025-12**

Reference number
ISO 24758-1:2025(en)

Full document details: BS ISO 24758-1:2025

© ISO 2025  **Documents**
Document Sharing Hub



COPYRIGHT PROTECTED DOCUMENT

© ISO 2025

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
3.1 Terms	2
3.2 Abbreviations and chemical formulas	2
4 Principle	3
5 Apparatus and materials	3
5.1 Gas chromatography with electron capture detector	3
5.1.1 Headspace vial and clamping machine	3
5.1.2 Carrier gases and column	3
5.1.3 Gas chromatograph	3
5.2 High-performance liquid chromatography–mass spectrometry	3
5.2.1 Vial	3
5.2.2 Carrier gases and column	3
5.2.3 Liquid chromatograph	4
5.3 Reagents	4
5.3.1 Probes	4
5.3.2 Other chemicals	4
6 Requirements	4
6.1 Sample	4
6.2 Evaluation system	4
6.3 Environment	5
7 Procedure	5
7.1 General	5
7.2 Introduce probe into the FB system and sample over time	5
7.3 Measurement of the probe compound concentration	6
7.4 Calculation of the probe kinetics model	6
8 Results and calculation	6
8.1 Commonly used probe for selection	6
8.2 Calibration curve for the probe-concentration measurement	6
8.2.1 High-performance liquid chromatography–mass spectrometry	6
8.2.2 Gas chromatography with ECD	9
8.3 Analysis of the probe with reaction time	10
8.4 Model development	11
8.5 Model verification	12
9 Report	13
9.1 Report of the testing results	13
9.2 Report of the testing conditions	13
Annex A (Informative) Commonly used probes for selection	14
Annex B (Informative) R code used for solving systems of Formulae	18
Annex C (Informative) Example of test results obtained with ozone FB treatment	20
Annex D (Informative) Example of test results obtained with FB combined plasma treatment	26
Annex E (Informative) Example of test results for detecting ROS in quiescent ozone MB water after H₂O₂ addition over storage time	31
Bibliography	34

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 document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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 (FB) technology holds considerable promise for application in water- and wastewater-treatment processes. Over the past several decades, numerous studies have demonstrated that microbubble (MB) and ultrafine bubble (UFB) dispersions can accelerate various advanced oxidation processes (AOPs), such as those using ozone, plasma, ultraviolet (UV), and electrochemical processes, thereby enhancing the efficiency of pollutant treatment. Although the treatment effects of fine-bubble processes are promising, their underlying mechanisms have not been fully understood. Understanding the generation mechanism of reactive species is imperative. Consequently, it is essential to clarify the role of reactive oxygen species (ROS) in pollutant abatement via MB and UFB treatments.

There are many techniques for detecting ROS in water. Electron paramagnetic resonance (EPR) spectroscopy stands out as a proficient tool for the direct detection of ROS, specifically those possessing unpaired electrons, even at concentrations as low as 1 μM . However, its application is limited as EPR falls short in detecting ROS, such as ozone and hydrogen peroxide (H_2O_2), which are devoid of unpaired electrons. Moreover, challenges persist in the detection of the superoxide anion radical ($\text{O}_2^{\cdot-}$) in water. The $\text{O}_2^{\cdot-}$ radical can stably exist in alcohols, such as methanol, where they are readily trapped by 5,5-dimethyl-1-pyrroline N-oxide (DMPO). In aqueous solutions, however, the addition rate of $\text{O}_2^{\cdot-}$ to DMPO is comparatively low, whereas the decomposition of its DMPO-adduct into DMPO-OH occurs at an exceedingly high rate, rendering effective detection impracticable. Additionally, the EPR technique is predominantly employed for the qualitative or semi-quantitative analysis of ROS. Determining the cumulative and real-time concentrations of ROS with precision remains a complex task.

Probe-based kinetic models are useful for optimizing water-treatment processes for efficient pollutant abatement and elucidating the ROS reaction mechanism driven by fine-bubble technology. By employing low concentrations (μM scales) of ROS probes, which will not significantly affect the reaction system, we can more realistically elucidate the reaction mechanisms occurring in water- and wastewater-treatment processes. With probe-based kinetic models, it is possible to measure the accumulated concentration of these ROS over time and their real-time concentrations at each treatment interval. However, caution is advised in the utilization of this method to measure the concentration of H_2O_2 within systems, as the relatively low oxidizing capacity of H_2O_2 compared to other ROS may lead to inaccuracies due to significant differences in reaction rate constants, potentially introducing significant errors in the analytical evaluations.

Fluorescence spectroscopy, in which suitable probes (e.g., 3'-(p-aminophenyl) fluorescein (APF)) are employed, is an excellent technique for detecting ROS because of its high sensitivity, simplicity in data collection, and high spatial imaging resolution. The fluorescent response of the APF probe to different ROS varies significantly. Notably, fluorescence spectroscopy is not a direct measurement method. When multiple ROS are present in a system simultaneously, it becomes challenging to distinguish them.

This document specifies detection methods for ROS in MB and UFB dispersions. In ISO 24758-1, the application of probe-based kinetic models for measuring the cumulative concentrations of various ROS over time, as well as their real-time concentrations at each treatment interval, is described. In ISO 24758-2, the application of APF to complement the probe kinetic model, which can detect H_2O_2 in MB and UFB dispersions, is described. The establishment of this document will provide substantial value for various industries. Manufacturers of MB and UFB equipment for environmental remediation can adopt this document to assess their equipment and wastewater-treatment processes. Companies with wastewater-treatment needs can use this document as a basis for selecting appropriate processes and instrumentation. This document will play a proactive role in evaluating the application and research of MBs in fields including wastewater treatment and disinfection etc.

Fine bubble technology — Evaluation method for determining the reactive oxygen species in ultrafine bubble dispersions —

Part 1: Probe based kinetic model

1 Scope

This document specifies evaluation methods for determining the content of reactive oxygen species (ROS) used in the FB-facilitated advanced oxidation for pollution abatement in the wastewater treatment process.

The probe based kinetic model is applicable to systems that generate ROS in substantial quantities, rather than at physiological concentrations, and is applicable to short-lived ROS. However, it is not applicable to long-lived ROS, such as ozone (O_3) and hydrogen peroxide (H_2O_2).

The probe based kinetic model method specifies:

- cumulative concentration of different types of ROS during the reaction process;
- concentration of different types of ROS at each time point during the reaction process.

This method does not define the mechanisms of ROS generation, nor the correlation between bubble size and ROS production.

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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 14644-1, *Cleanrooms and associated controlled environments — Part 1: Classification of air cleanliness by particle concentration*

ISO 20480-1:2017, *Fine bubble technology — General principles for usage and measurement of fine bubbles — Part 1: Terminology*

ISO 23015, *Fine bubble technology — Measurement technique matrix for the characterization of fine bubbles*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23015 and ISO ISO 20480-1 apply.

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

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

3.1 Terms

3.1.1

reactive oxygen species

highly reactive chemicals formed from diatomic oxygen (O_2), water, and hydrogen peroxide

Note 1 to entry: Some prominent ROS include ozone (O_3), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($\cdot OH$), and singlet oxygen ($^1O_2$).

3.1.2

probe

series of specialized chemical agents designed specifically for detecting and quantifying ROS

Note 1 to entry: These probes consist of small organic molecules, and their reactions with ROS are the elementary processes.

3.2 Abbreviations and chemical formulas

AOPs	advanced oxidation processes
FBD	fine bubble dispersion
ROS	reactive oxygen species
$\cdot OH$	hydroxyl radical
$O_2^{\cdot-}$	superoxide anion radical
$^1O_2$	singlet oxygen
H_2O_2	hydrogen peroxide
O_3	ozone
ECD	electron capture detector
PTFE	polytetrafluoroethylene
ESI	electrospray ionization
r_{RMSE}	root mean square error
p_{RPD}	ratio of performance to deviation
r	correlation coefficient
$[P]$	concentration of probe
$[R]$	concentration of ROS
$k_{R,P}$	rate constants between ROS and probe
$\int_{t_0}^t [R] dt$	ROS exposures concentration
$-\frac{d[P]}{dt}$	the negative rate of change of the probe concentration

4 Principle

Probe-based kinetic models are developed based on the assumption that pollutants are abated from the aqueous phase through simultaneous bulk reactions with ozone and various ROS. This approach involves the use of internal or spiked probe compounds to determine the exposures of the main reactive species in AOPs systems. Based on the measured exposures, the abatement efficiency of the pollutant can be calculated if the rate constants for the reaction of the pollutant with the ROS are known. The radicals that can be measured by this method include the hydroxyl ($\cdot\text{OH}$), singlet oxygen ($^1\text{O}_2$), and superoxide radicals ($\text{O}_2^{\bullet-}$).

During FB-facilitated advanced oxidation, a probe (P) is eliminated by its reaction with a ROS. The elimination of P in terms of the logarithmic relative residual concentration of P can be calculated if the ROS rate constants (k_{ROS}) and the ROS exposures ($\int_{t_0}^t [R] dt$) are known.

5 Apparatus and materials

5.1 Gas chromatography with electron capture detector

5.1.1 Headspace vial and clamping machine

- Vial: a 20 mL headspace vial made of glass.
- Cap: made of aluminium.
- Septum: made of silicone or polytetrafluorethylene (PTFE).
- Clamping machine for a flip top cap.

5.1.2 Carrier gases and column

- Carrier gas: nitrogen (N_2).
- Gastight plunger syringe: 1 mL in volume.
- Chromatographic column: fused silica capillary column (Ultra Inert Columns, 30 m $\times$ 0,32 mm, 1,8 μm film thickness) or any other column with comparable characteristics.

5.1.3 Gas chromatograph

The gas chromatograph is equipped with an electron capture detector (ECD) for the detection of probes in the headspace vial.

5.2 High-performance liquid chromatography–mass spectrometry

5.2.1 Vial

- Vial: a 1,5–2 mL vial made of glass or plastic material.
- Cap: a screw-cap or snap-cap type.
- Septum: made of silicone or PTFE for multiple penetrations by the autosampler needle.

5.2.2 Carrier gases and column

- Carrier gas: nitrogen (N_2).
- Chromatographic column: C18 column (4,6 $\times$ 250 mm, 5 μm) or any other column with comparable characteristics.

5.2.3 Liquid chromatograph

The system comprises a liquid chromatograph (for separating mixtures of the sample) and a mass spectrometer (for identifying and quantifying the separated entities).

5.3 Reagents

5.3.1 Probes

The selection of a probe should adhere to the following principles and procedures:

- the first step should involve theorizing based on literature to identify the likely ROS present in the reaction system and those that contribute most considerably to the oxidation reaction;
- the reaction rate constants of the probe with various types of ROS are known;
- the choice of the probe should be predicated on the principal ROS within the system. For instance, if $\cdot\text{OH}$ radicals are among the primary ROS, the selected probe shall exhibit a pronounced selectivity toward $\cdot\text{OH}$ radicals. This is evident in the reaction rate constant of the probe, which should be substantially higher for $\cdot\text{OH}$ radicals compared to other ROS, such as nitrobenzene. Similarly, if superoxide anions are the primary ROS, at least one chosen probe should have a reaction rate constant with a strong oxidizing ROS, such as $\cdot\text{OH}$ radicals, that is comparable to its reaction rate constant with superoxide anions, exemplified by compounds such as carbon tetrachloride (CCl_4) and trichloromethane (CHCl_3);
- each type of ROS should react with at least one probe. Commonly used probes for selection are presented in [Annex A](#); these probes have been previously used in previous studies for ROS measurement;
- the number of probes should exceed the number of ROS being measured in the system. Consequently, some probes are utilized to detect the accumulative and real-time concentrations of various ROS within the reaction system, while others are employed to validate the accuracy of the measurement outcomes;
- volatile probes are known for their high sensitivity in gas chromatography, which is essential for accurately detecting trace amounts of ROS. This exceptional sensitivity, coupled with considerably affordable equipment cost, makes volatile probes a highly practical and cost-effective option for ROS measurement. However, for aerated systems, preference should be given to non-volatile probes. Volatile probes are suitable for detecting ROS in non-aerated systems, such as quiescent ozone MB water.

5.3.2 Other chemicals

- Sodium thiosulfate: $\text{Na}_2\text{S}_2\text{O}_3$ (MW: 158,10 g/mol).

6 Requirements

6.1 Sample

In scenarios involving ROS determination, two main applications shall be considered: treating wastewater with MB and UFB and cleaning with water containing MB and UFB. In these scenarios, water purity varies depending on the specific application. For instance, in wastewater treatment applications, water quality requirements are less stringent, focusing mainly on identifying ROS types and accumulated concentrations in the system. However, purified water can be necessary for cleaning processes. When purified water is used, the water shall conform to ISO Grade 1 purity (see ISO 3696) and the air shall conform to ISO Class 5 cleanliness (see ISO 14644-1) for generating fine bubble dispersion (FBD).

6.2 Evaluation system

In ROS determination systems, probes are present at extremely low concentrations. Consequently, high cleanliness levels shall be maintained within the testing environment to avoid contamination from external pollutants that can could adversely affect the precision of sample measurements. For example, using disposable sample tubes for introducing samples in experiments ensures a contamination-free process.

Moreover, using tubing resistant to oxidation in the experimental setup enhances the overall reliability and integrity of experimental results.

6.3 Environment

The air cleanliness should be considered during measurement to prevent impurity introduction.

Air cleanliness, ambient temperature, and atmospheric pressure depend on the local environment and may vary. However, as they are important settings and can influence the evaluation process, they should be recorded before any evaluation takes place.

7 Procedure

7.1 General

The comprehensive protocol encompasses a 3-step methodology:

- step 1, introduce the probe into the FB system and sample over time;
- step 2, probe the compound-concentration measurement;
- step 3, probe the kinetics-model calculation.

A schematic representation of the ROS-detection apparatus is depicted in [Figure 1](#). The detailed processes are as follows.

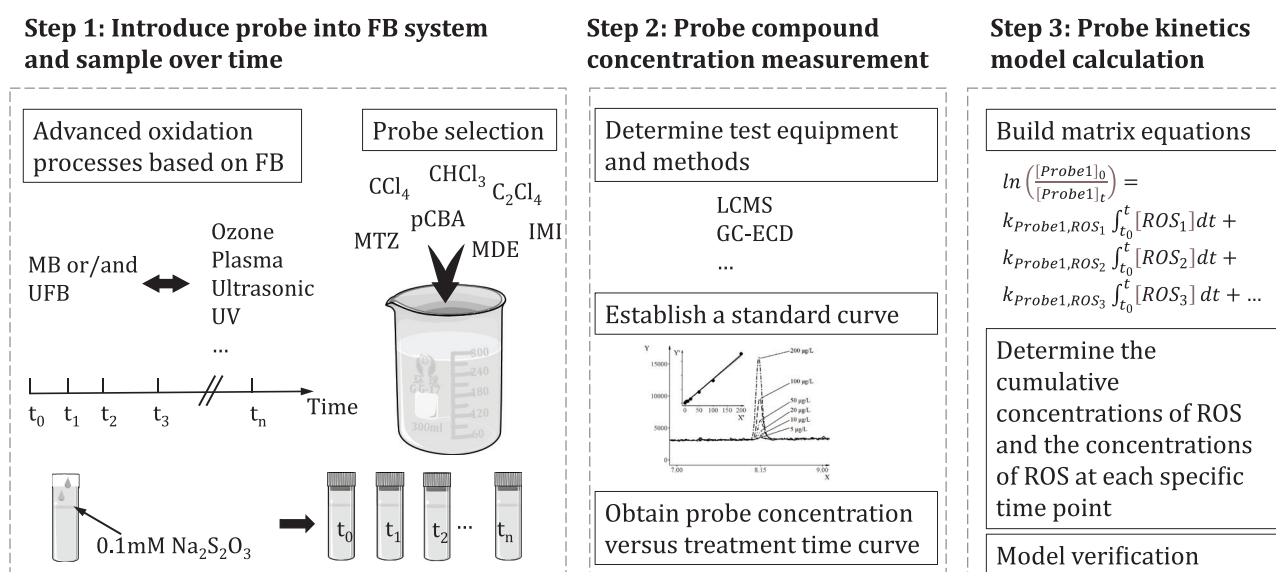


Figure 1 — General view of the ROS-determination system

7.2 Introduce probe into the FB system and sample over time

Select a specified number of probes according to the methods and principles described in Clause 4 and 5.3.1. Typically, the concentration of pollutants in wastewater ranges from tens to hundreds or even thousands of ppm. Introduce the probes at extremely low concentrations compared with the pollutant concentrations, thereby ensuring that the presence of the probes does not alter the degradation pathway of the pollutants within the MB-facilitated advanced oxidation system. Nonetheless, overly low probe concentrations may induce substantial deviations in measurement accuracy. The probe concentration should be 1–2 orders of magnitude lower than the pollutant concentration, with a suggested range from 200 $\mu\text{g/L}$ to 1 mg/L . After probe addition, sampling should be implemented at various time points as the treatment progresses. The timing of these samplings should be determined based on the reaction rate, with a minimum of 6–8

temporal points recommended to bolster the precision of ensuing model constructions. If substances that continuously produce ROS, such as ozone, are present in the sample, an excess amount of sodium thiosulfate solution should be added immediately after sampling to quench the oxidizing processes generating ROS, thereby halting further ROS production.

In cases where cavitation occurs during the generation of MBs or UFBs, the high temperatures associated with this process may trigger probe decomposition. To mitigate this risk, conducting a control experiment can prove beneficial. For instance, employing a nitrogen (N_2) UFB generation system, which experiences cavitation with a relatively small amount of ROS production, allows us to assess whether probes decompose over time with a relatively small amount of ROS. Comparing these baseline findings with results from the FB system enhances the ability to accurately determine the extent of probe decomposition attributed solely to ROS.

7.3 Measurement of the probe compound concentration

Volatile probes, such as $CHCl_3$, CCl_4 , and C_2Cl_4 , shall be analysed using a gas chromatograph equipped with an ECD. Non-volatile probes shall be analysed using high-performance liquid chromatography-mass spectrometry.

7.4 Calculation of the probe kinetics model

By analysing the changes in the probe concentrations over time and the reaction rate constants between various probes and different ROS, a system of equations for the accumulated concentrations of different ROS is established. Subsequently, this system shall be solved in a software environment such as R (a programming language and software environment for statistical computing and graphics). The equations for the time-dependent change curves of different ROS shall be further simulated. The resulting functions shall be differentiated to obtain the real-time concentrations of the ROS. Additional probes shall be used to validate the calculated ROS concentrations; the results shall be accepted as valid when the difference between the measured and simulated values lies within the 95 % confidence interval.

8 Results and calculation

8.1 Commonly used probe for selection

[Table A.1](#) in [Annex A](#) lists some commonly used probes for selection. The rate constant can be obtained from the NDRL/NIST Solution Kinetic Database (<https://kinetics.nist.gov/solution/>).

Researchers may opt to utilize either known probes or organic small molecules that have not been previously employed in research works as probes. Experimental determinations shall be conducted of the reaction order and rate constants between the new probe and various ROS before measuring the cumulative concentrations of the ROS.

8.2 Calibration curve for the probe-concentration measurement

8.2.1 High-performance liquid chromatography-mass spectrometry

Prior to each injection, allow the system to equilibrate for 8-10 min to stabilize the gradient elution's impact on the baseline. Throughout the analysis, the pump pressure shall be maintained consistently below 20 MPa and ensure no liquid leakage occurred from the liquid injection pump. The probe-compound concentrations were analysed using the built-in analysis tools in LabSolutions.

The recommended run conditions used are as follows:

- injection volume: 20 μ L;
- column: C18 column (4,6 mm $\times$ 250 mm, 5 μ m, GL Sciences).
- mobile phase: solvent A = 10 mM ammonium acetate in water and solvent B = 100 % acetonitrile, delivered as a binary gradient at a flow rate of 0,8 mL/min.