**ISO/FDIS 13319-~~-~~2:~~2022~~2023(E)**

**ISO/TC 24/SC 4/WG 5**

**Secretariat: BSI**

# Determination of particle size distribution — Electrical sensing zone method — Part 2: ~~Tunable~~Tuneable resistive pulse sensing method

*Détermination de la distribution granulométrique — Méthode de détection de zones électrosensibles — Partie 2: Méthode par détection d'impulsions résistives accordable*

**First edition**

**Date: ~~2022-11-25~~2023-03-01**

# Contents Page


Foreword ..... v

Introduction ..... vi

1 Scope ..... 1

2 Normative references ..... 1

3 Terms and definitions ..... 1

4 Symbols ..... 2

5 Principles ..... 2

6 General operation ..... 4
6.1 Determination of particle size ..... 4
6.2 Determination of particle concentration ..... 5
6.3 Calibration ..... 6
6.4 Dynamic range ..... 6
6.5 Coincidence events ..... 7
6.6 Off-axis particle transport ..... 7
6.7 Polarisation ..... 7
6.8 Dielectrophoresis ..... 8
6.9 Drag ..... 9

7 Operational procedure ..... 10
7.1 Instrumental components ..... 10
7.2 System set-up and optimisation ..... 10
7.2.1 Preparing fluid cell and stretching the TPU aperture ..... 10
7.2.2 Wetting the TPU aperture ..... 11
7.2.3 Establishing stable baseline current and estimating the TPU aperture size ..... 11
7.2.4 Coating the TPU aperture ..... 11
7.2.5 Optimising measurement parameters and running calibration ..... 12
7.2.6 Adjusting conditions for the sample and recording data ..... 12
7.2.7 Re-calibrating to ensure system stability ..... 13
7.3 Sample preparation ..... 14
7.3.1 General ..... 14
7.3.2 TRPS suspension requirements ..... 15
7.3.3 Excluding contaminants ..... 15
7.3.4 Removal of proteins and solutes ..... 15
7.3.5 Maintaining sample integrity ..... 15
7.3.6 Enhancing suspension stablility ..... 15

Annex A (informative) Best practice ..... 17

Annex B (informative) Troubleshooting ..... 18

Annex C (informative) Size distribution and concentration measurements of phospholipid nano/micro-bubbles ..... 19

Annex D (informative) Method development ..... 20

Bibliography ..... 21

# 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

A list of all parts in the ISO 13319 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

Monitoring particle size distributions and particle concentrations are required in various fields, where particle dispersions in liquid play a role. The electrical sensing zone technique has, since its discovery by W.H. Coulter around 1950, been widely employed for size and count analysis of (blood) cells, bacteria, viruses and other fine particles. Over the last decades, the application range has expanded to nanoparticles, such as liposomes, exosomes and microbubbles, as a result of improved electronics and aperture fabrication. The tunable electrical sensing zone technique is useful for the determination of the size distribution, concentration and zeta potential of micro- and nanoparticles suspended in a liquid. The purpose of this document is to provide the background and procedures for application of tunable electrical sensing zone equipment for particle size distribution and concentration measurements, so as to improve the reproducibility and the accuracy of the acquired results.

# Determination of particle size distribution — Electrical sensing zone method — Part 2: Tunable resistive pulse sensing method

## 1 Scope

This document specifies the measurements of particle size distribution and concentration of suspended particles, ranging from 40 nm ~~up~~ to 100 µm, using tunable resistive pulse sensing (TRPS). This document provides a comprehensive overview as to the methodologies that are applied to achieve reproducible and accurate TRPS measurement results. This document also includes best practice considerations, possible pitfalls and information on how to alleviate or avoid these pitfalls.

## 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.~~



~~13319-1 *Determination of particle size distribution — Electrical sensing zone method — Part 1: Aperture/orifice tube method*~~

~~13099-1 *Colloidal systems – Methods for zeta potential determination. Part 1: Electroacoustic and electrokinetic phenomena*~~



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 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**
**aperture**
small diameter hole through with suspension is drawn

[SOURCE: ISO 13319-1:2021, 3.2]

**3.2**
**sensing zone**
volume of electrolyte within, and around, the aperture in which a particle is detected

[SOURCE: ISO 13319-1:2021, 3.3]

**3.3**
**pulse frequency**

number of pulses ~~perduration,~~ per duration

**3.4**
**detection range**
size range between the smallest and largest detectable particle diameter

**3.5**
**dynamic range**
ratio between the largest and smallest detectable particle diameter

**3.6**
**electrokinetics**
~~describes~~ phenomena that are associated with the tangential liquid motion in respect to a charged surface

[SOURCE: ISO 26824:2022, 3.17.16]

**3.7**
**electrophoresis**
movement of charged colloidal particles or polyelectrolytes, immersed in a liquid, under the influence of an external electric field

[SOURCE: ISO 13099-1:2012, 2.2.4~~; ISO 13099-3:2014, 3.2.4; ISO 26824:2022, 3.17.20]3.8~~]



**3.8**
**electroosmosis**
motion of liquid through or past a charged surface, e.g. an immobilized set of particles, a porous plug, a capillary or a membrane, in response to an applied electric field, which is the result of the force exerted by the applied field on the countercharge ions in the liquid



[SOURCE: ISO 13099-1:2012, 2.2.1~~; ISO 13099-2:2012, 3.1.5; ISO 13099-3:2014, 3.2.1 ISO 26824:2022, 3.17.17~~]

**3.9**
**electrophoretic mobility**
electrophoretic velocity per unit electric field ~~strengthNote~~strength

Note 1 to entry: Electrophoretic mobility is expressed in metres squared per volt second.

[SOURCE: ISO 13099-3:2014, 3.2.5~~]~~, modified — the symbol “$\mu$” and the former Note 1 to entry have been deleted.]

**3.10**
**zeta potential**
difference in electric potential between that at the slipping plane and that of the bulk ~~liquidNote~~liquid

Note 1 to entry: Slipping plane is the abstract plane in the vicinity of the liquid/solid interface where liquid starts to slide relative to the surface under influence of a shear stress.

Note 2 to entry: The zeta potential is expressed in volts.

[SOURCE: ISO 13099-1:2012, 2.1.8~~; ISO 13099-2:2012, 3.1.4; ISO 13099-3:2014, 3.1.8~~]

## 4 Symbols

For the purpose of this document the following symbols apply.

| | |
|---|---|
| ~~$U$~~$A_i$ | ~~voltage~~pulse height of particle $i$ |
| ~~$P$~~$C$ | ~~pressure~~particle number concentration |
| $C_5$ | particle concentration at which coincidence probability is 5 % |
| $D$ | aperture diameter |
| $d$ | particle diameter |
| ~~$L$~~$E$ | ~~aperture length~~electric field |
| ~~$K_d$~~$f_{CM}$ | ~~calibration constant of diameter~~Clausius-Mossotti factor |
| $F_{dep}$ | dielectrophoretic force |
| $f_p$ | pulse frequency |
| $K_C$ | calibration constant of concentration |
| ~~$C$~~$K_d$ | ~~particle number concentration~~ calibration constant of diameter |
| ~~$f_p$~~$L$ | ~~pulse frequency~~aperture length |
| $N$ | true count of particles |
| $n$ | observed count of particles |
| $P$ | pressure |
| $S$ | applied stretch |
| $U$ | voltage |
| $V_m$ | analysis volume |
| ~~$C_5$~~$V_{sens}$ | ~~particle concentration at which coincidence probability is 5 %~~ sensing volume |
| ~~$\epsilon_0$~~$\epsilon_{fl}$ | absolute permittivity of the fluid |



## 5 Principles

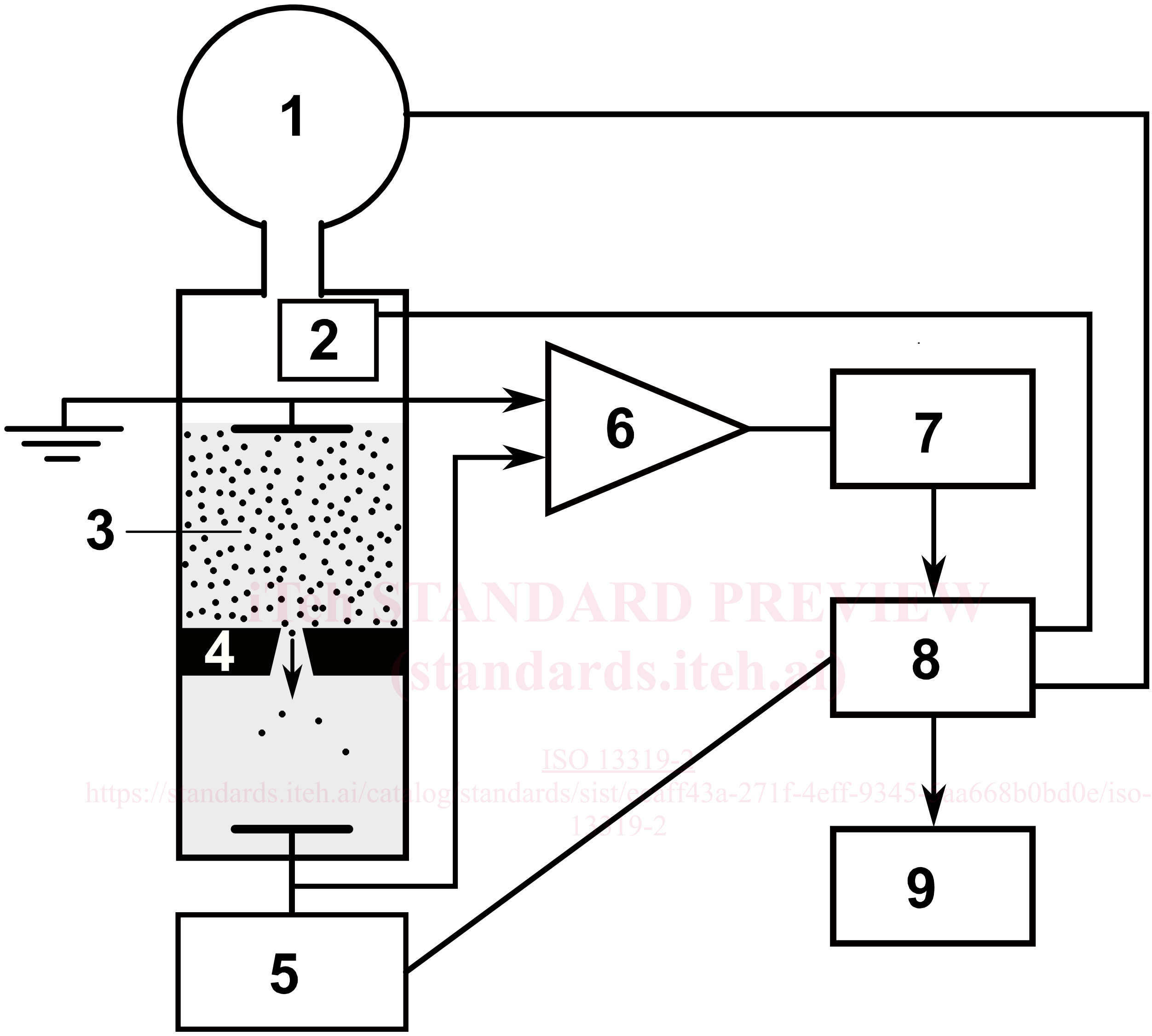
TRPS is an electrical sensing zone technique that can be used for ~~characterisation~~characterization of the particle size distribution, concentration~~,~~ and zeta potential of synthetic (e.g. metallic, polymeric or ceramic particles), biological particles (e.g. nano-pharmaceuticals or extracellular vesicles) and naturally occurring organic and inorganic nano/microparticles suspended in liquids. A dilute suspension of particles in an electrolyte passes through an aperture in a membrane. There is an Ag/AgCl electrode on both sides of the membrane, between which an electric potential is applied, which causes a stable ionic current passing through the aperture. When a particle translocates the aperture, it causes a resistive pulse due to the replacement of conductive electrolyte solution by a non-conductive solid particle~~.~~.[1] The height, width and frequency of these pulses provide all the information required to determine particle size, concentration and zeta potential~~.~~.[2] Particle passage through the aperture is caused by:

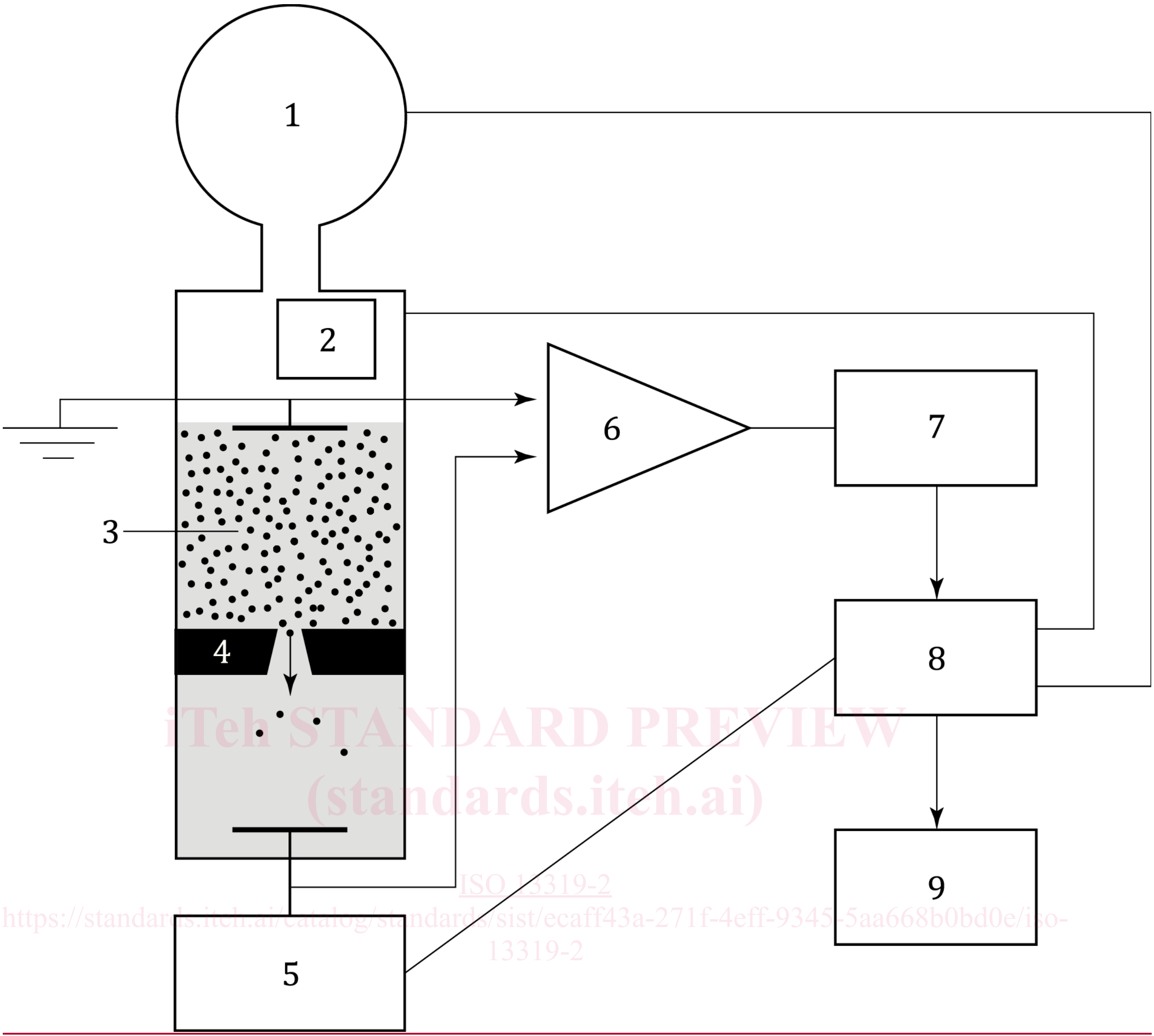
— a pressure difference across the aperture for particle size determination and concentration;

— a voltage difference between the two electrodes across the aperture for zeta potential measurement;

— both a voltage and a small pressure difference between the two electrodes across the aperture for simultaneous measurement of particle size and zeta potential.

More background and a schematic of the instrumentation is given in ISO 13319-1 and Figure 1. Pressure can be monitored directly via a pressure sensor as shown in Figure 1 or indirectly via a flow rate meter.








**Key**

~~1 pressure module~~
~~2 pressure sensor~~
~~3 nanoparticle/microparticle suspension~~
~~4 aperture~~
~~5 voltage source~~
~~6 amplifier~~
~~7 analog to digital converter~~
~~8 computer~~
~~9 output device~~

1 pressure module
2 pressure sensor
3 nanoparticle/microparticle suspension
4 aperture
5 voltage source
6 amplifier
7 analogue to digital converter
8 computer
9 output device

**Figure 1 — Schematic representation of TRPS instrument**

There are three main differences between conventional electrical sensing zone and TRPS equipment. Firstly, calibration standards are typically used to calibrate the aperture and provide traceable and accurate TRPS measurements. However, measurements can also be done without the use of calibration standards, in particular when fixed aperture geometries are applied.

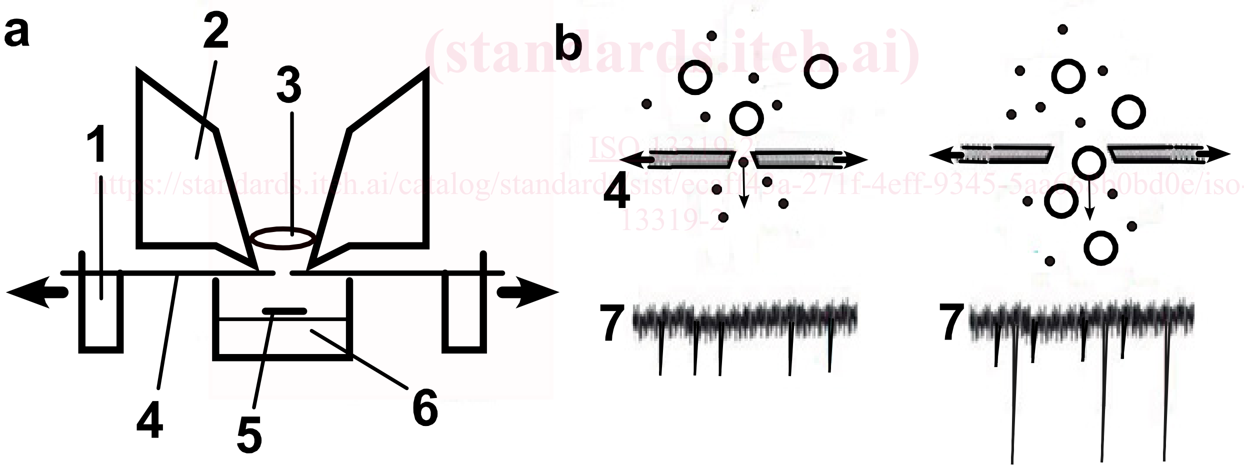
The second difference is that pressure (pressure module) and voltage (voltage source) are tunable to allow for full control of convective and electrokinetic velocity contributions of single particles translocating the aperture, a prerequisite for measuring particle size, concentration and zeta potential.

The third difference is the use of both fixed and tunable apertures for TRPS application. ~~Whilst~~While there are several chip and aperture providers using 3D printed microfluidic and glass-based fixed geometry apertures that can be used, there are also tunable apertures, for example, made in an elastic thermoplastic polyurethane (TPU) membrane. Despite having several aperture options, the focus is on tunable TPU aperture based TRPS operation.

TPU apertures are formed by generating a micron-sized hole into an elastic TPU membrane, which can be stretched mechanically to the desired size for measurement. Thus, the aperture can be tuned to the optimum size for the particles at hand. A schematic of the setup is given in Figure 2. For very polydisperse samples, a range of apertures ~~might~~can be required for the full measurement of the sample size distribution and concentration (see example in ~~Annex C1~~Figure C.1).

NOTE In ~~this figure~~Figure 2, jaws are used for clamping and stretching/relaxing the membrane.






~~a TRPS with tunable TPU aperture~~
~~b detail of TPU aperture~~
~~1 stretching device~~
~~2 top fluid cell~~
~~3 ground electrode~~
~~4 tunable aperture~~
~~5 signal electrode~~
~~6 bottom fluid cell~~
~~7 current~~