INTERNATIONAL STANDARD

ISO 13319-2

First edition
2023-09

Determination of particle size distribution — Electrical sensing zone method —

Part 2: Tunable resistive pulse sensing method

Détermination de la distribution granulométrique — Méthode de détection de zones électrosensibles —

Partie 2: Méthode par détection d'impulsions résistives accordable (TRPS)

Reference number
ISO 13319-2:2023(E)

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

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 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 and other fine particles. Over the last decades, the application range has expanded to nanoparticles, such as liposomes, exosomes, and nano- and micro-bubbles, 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 to 100 µm, using tunable resistive pulse sensing (TRPS). This document provides a comprehensive overview of 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

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 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
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]

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]

3.9
electrophoretic mobility
electrophoretic velocity per unit electric field 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 "μ" 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 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]

4 Symbols

For the purpose of this document the following symbols apply.

A_i	pulse height of particle *i*
C	particle number concentration
C_5	particle concentration at which coincidence probability is 5 %
D	aperture diameter
d	particle diameter
E	electric field
f_{CM}	Clausius-Mossotti factor
F_{dep}	dielectrophoretic force

f_p	pulse frequency
K_C	calibration constant of concentration
K_d	calibration constant of diameter
L	aperture length
N	true count of particles
n	observed count of particles
P	pressure
S	applied stretch
U	voltage
V_m	analysis volume
V_{sens}	sensing volume
$\epsilon_0\epsilon_{fl}$	absolute permittivity of the fluid

5 Principles

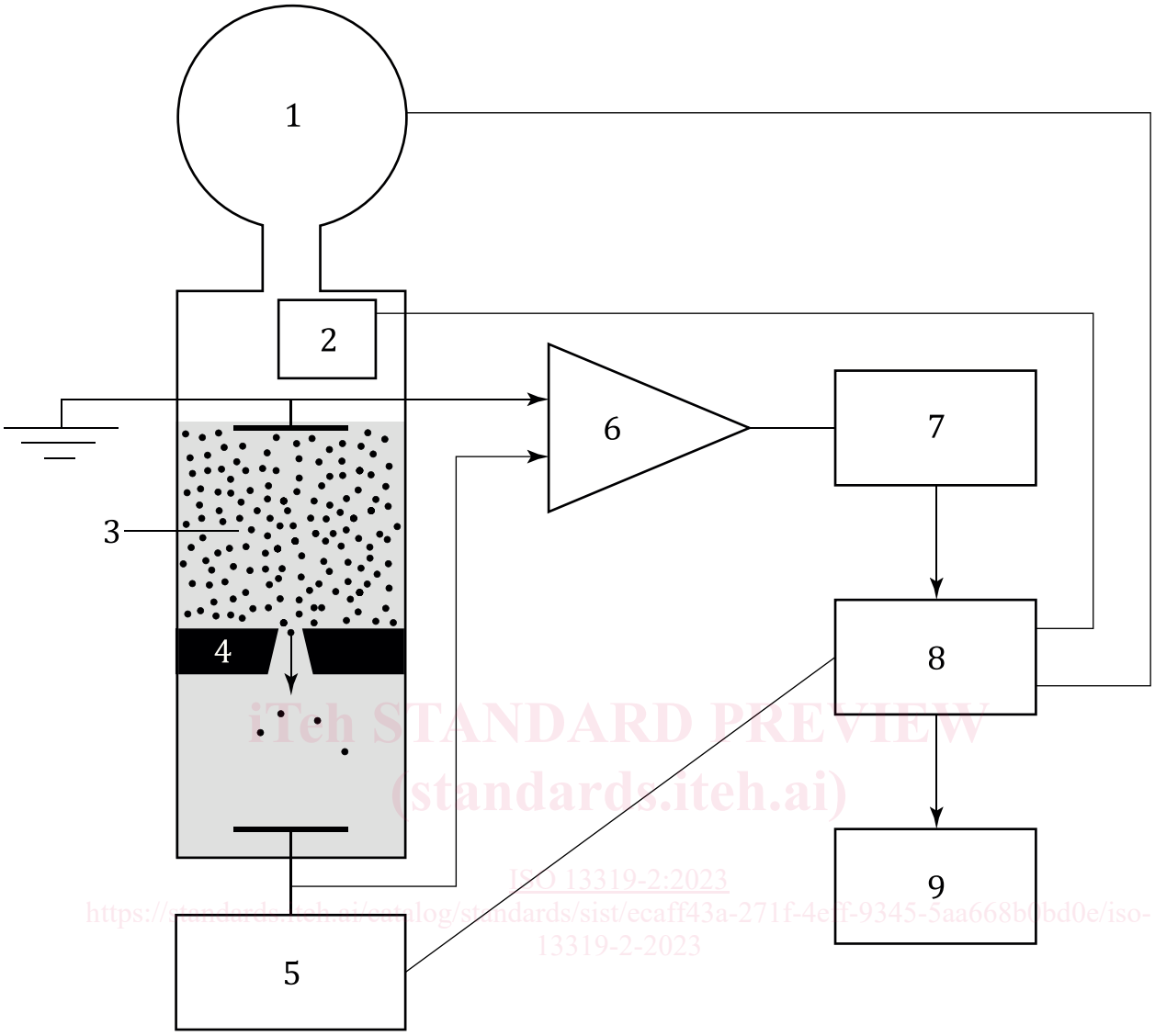
TRPS is an electrical sensing zone technique that can be used for characterization of the particle size distribution, concentration and zeta potential of synthetic (e.g. metallic, polymeric or ceramic particles), biological particles [e.g. nano-pharmaceuticals or extracellular vesicles (EVs)] and naturally occurring organic and inorganic nano- and 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 analogue to digital converter
8 computer
9 output device

Figure 1 — Schematic representation of TRPS instrument

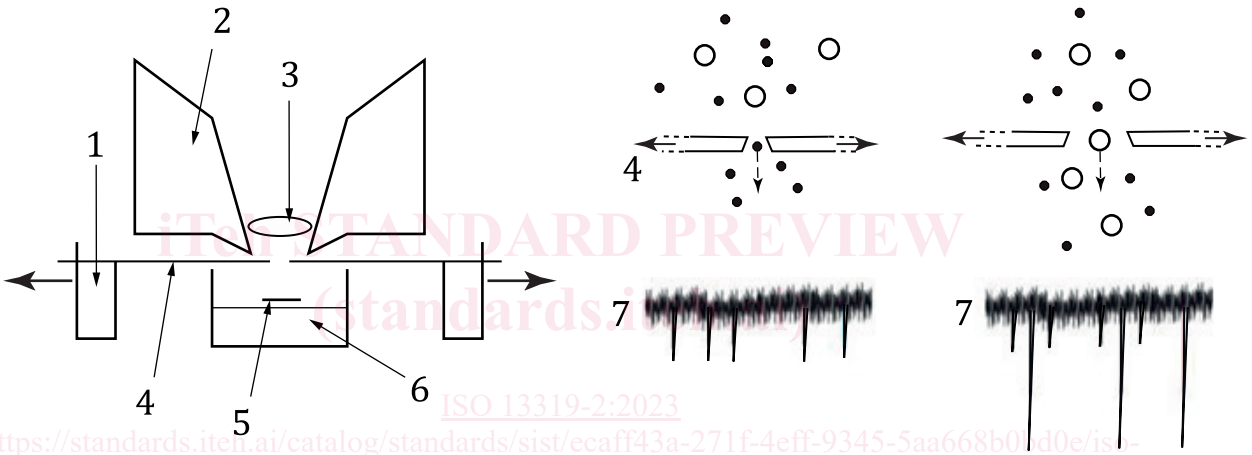
There are three main differences between conventional electrical sensing zone and TRPS equipment. Firstly, calibration standards are typically used to calibrate the aperture and provide traceable and accurate TRPS measurements. However, measurements can also be done without the use of calibration standards, 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. 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 can be required for the full measurement of the sample size distribution and concentration (see example in Figure C.1).

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



a) TRPS with a tunable TPU aperture **b) Detail of a TPU aperture**

Key

1 stretching device
2 top fluid cell
3 ground electrode
4 tunable aperture
5 signal electrode
6 bottom fluid cell
7 current

Figure 2 — Schematic representation of TRPS with a tunable TPU aperture

6 General operation

6.1 Determination of particle size

As in the conventional electrical sensing zone technique, a pressure drop over the aperture causes the suspension to flow through it and the pulse height resulting from particle passage is regarded directly proportional to particle volume (see ISO 13319-1). TRPS is a particle by particle, as opposed to an averaging ensemble measurement technique, with each pulse corresponding to a single particle. Calibration, if required, is executed through the application of certified reference materials with

traceable certified values[3]. The calibration factor relates the height of the measured pulses to volume-equivalent diameters, as shown in Formula (1).

$$d_i = \mathrm{K}_d \sqrt[3]{A_i} \tag{1}$$

where

d_i is the size of particle i (volume-equivalent diameter);

K_d is the calibration constant;

A_i is the pulse height of particle i.

6.2 Determination of particle concentration

At sufficient pressure drop over the aperture, convection is the dominant transport mechanism. Then, the stochastic pulse frequency (f_p), which is equal to the particle count rate at 0 coincidence probability, is proportional to the product of the particle concentration (C) and the fluid flow rate (Q)[4]. Since the fluid flow rate is proportional to the applied pressure drop (P), the particle concentration can be calculated as the slope of the linear relationship of pulse frequency versus applied pressure, with K_C being a calibration constant [see Formula (2)]. Formula (2) also applies to scenarios where convection is not the dominant transport mechanism, with the slope (equal to $\frac{\Delta f_p}{\Delta P}$) of the linear f_p versus P relationship determining the particle concentration.

$$C = \mathrm{K}_C \left(\frac{\Delta f_p}{\Delta P} \right) \tag{2}$$

The use of a calibration standard of known concentration allows the determination of the concentration of a sample at a single pressure, if convection is the dominant transport mechanism. This is typically the case for larger particles and larger apertures. Particle concentration and size can be determined simultaneously.

However, concentration measurements over a defined particle size range are typically obtained with a multi-pressure calibration procedure, where particle rates for sample and calibration are analysed at two or more pressures[5],[6]. Particle concentrations are calculated from the slope of the linear particle rate versus pressure dependence, while particle sizes are determined individually from the respective resistive pulse heights, which are linearly related to particle volumes [see Formula (1)]. This includes information about the size distribution, i.e. the number concentration of each size population within a sample, given by the number of particles per ml and per nm (bin-size). An example of a typical TRPS concentration measurement is shown in Figure C.1.

A summary of recommended setting for TRPS size distribution and concentration measurements using TRPS instrumentation with a tunable TPU aperture is shown in Table 1, and reference guidance to aperture selection can be found in Table 2.

Table 1 — Typical settings for various TRPS measurements using TRPS instrumentation and TPU apertures

Parameter				
Applied voltage	**Applied pressure**	**Sample size range**	**Sample concentration**	**Reference**
≤5 V	≤2,5 kPa	40 nm to 100 µm	Size dependent	[3] and [7]

6.3 Calibration

TRPS instruments are preferably calibrated with monodisperse polystyrene particles, whose certified mean diameter is traceable to the International System of Units (SI), however that does not exclude