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Determination of particle size distribution — Electrical sensing zone method —

Part 2: 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 (TRPS)

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The committee responsible for this document is ISO/TC 24/SC 4 *Particle characterization*.

ISO 13319 *Determination of particle size distribution — Electrical sensing zone method* consists of the following parts:

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— *Part 1: Aperture/orifice tube method*

— *Part 2: Tunable resistive pulse sensing method*

— *Part 3: Nano-constriction method*

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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 part of ISO 13319-3 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.

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Determination of particle size distribution — Electrical sensing zone method —

Part 2:
Tuneable resistive pulse sensing method

1 Scope

This guide details the measurements of particle size distribution and concentration of suspended particles, ranging from 20 nm up to 10 µm, using tunable resistive pulse sensing (TRPS). It provides a comprehensive overview as to the methodologies that should be applied to achieve reproducible and accurate TRPS measurement results. It also includes best practice considerations, possible pitfalls, and how to alleviate/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.

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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*

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3 Terms and definitions

3.1
aperture
small diameter hole through with suspension is drawn

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

3.3
pulse FWHM
pulse duration of full width at half of maximum height

3.4
pulse frequency
number of pulses/sec = particle count rate

3.5
detection range
size range between the smallest and largest detectable particle diameter

3.6
dynamic range
ratio between the largest and smallest detectable particle diameter

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3.7
electrokinetics
comprising electrophoresis, dielectrophoresis and electroosmosis

3.8
electrophoresis
describes movement of charged particles in electric field

3.9
electroosmosis
describes the movement of dispersion medium due to aperture zeta potential

3.10
electrophoretic mobility
velocity of particles per unit of electric field

3.11
zeta potential
electrical potential at the slip plane of a particle

4 Symbols

For the purpose of this document the following symbols apply.

U	voltage
P	pressure
D	aperture diameter
d	particle diameter
L	aperture length
K_d	calibration constant of diameter
K_C	calibration constant of concentration
C	particle concentration [/ml]
p/min	particles per minute
V_m	analysis volume
N_{c5}	count for 5 % coincidence

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5 Principles

TRPS is an electrical sensing zone technique that can be used for characterisation 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,

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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.

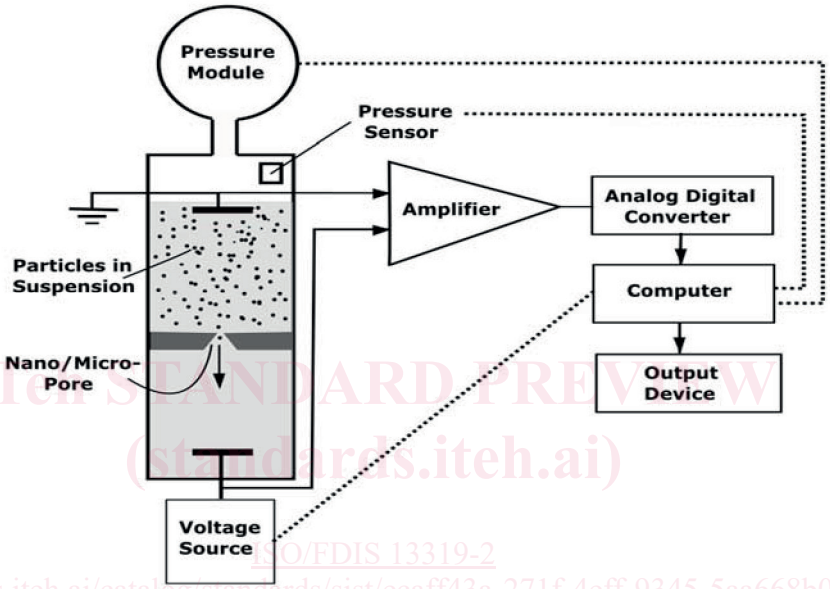

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Figure 1 — Schematic of TRPS instrument.

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 pore 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 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, e.g. 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 be required for the full measurement of the sample size distribution and concentration (see example in Annex C1).

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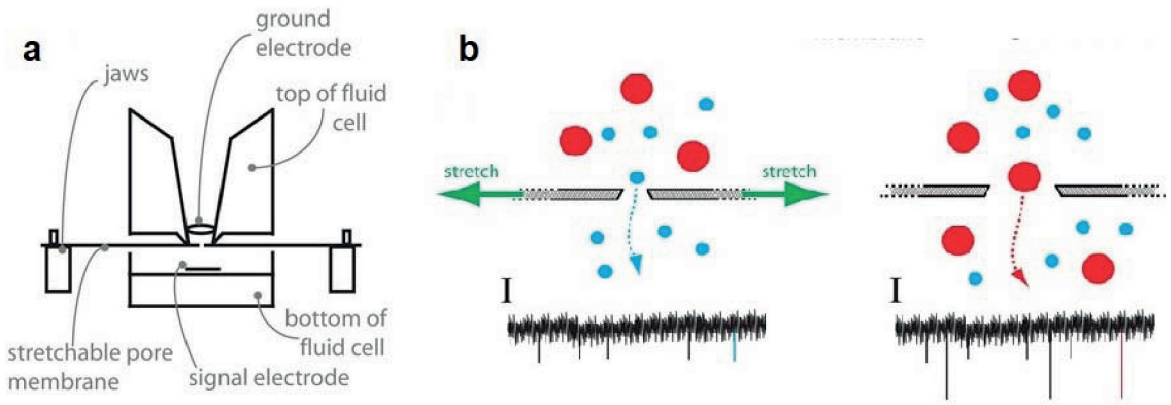

Figure 2 — Schematic of TRPS (a) with a tunable TPU aperture (b). In this example jaws are used for clamping and stretching/relaxing the membrane.

6 General operation

6.1 Determination of particle size

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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 (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 application of certified reference materials with traceable certified values.[3] The calibration factor relates the height of the measured pulses to volume-equivalent diameters:

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$$d_i = K_d \sqrt[3]{A_i} \tag{1}$$

where:

d_i = size of particle i (volume-equivalent diameter)

K_d = calibration constant

A_i = 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 pulse frequency (PF), i.e. the particle count rate, 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:

$$C = K_C \left(\frac{\Delta PF}{\Delta P} \right) \tag{2}$$

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 pores. Particle concentration and size can be determined simultaneously.

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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 vs pressure dependence, whilst particle sizes are determined individually from the respective resistive pulse heights, which are linearly related to particle volumes (see equation 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 Annex 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 a reference guide to aperture selection can be found in Table 2.

Table 1 — Typical settings for various TRPS measurements using TRPS instrumentation and TPU apertures[9]

Settings	Size and Concentration
Applied voltage	≤ 1,6 V
Applied pressure	≤ 2,5 kPa
Sample size range	40 nm–100 µm
Sample concentration	size dependent
References	[3]

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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 the use of standards such as silica particles or liposomes, depending on the application at hand. For particle TRPS concentration measurements, using elastic TPU pores, the knowledge of the pore characteristics is generally unavailable. It is therefore required to use a calibration standard with known particle size and number concentration to obtain the concentration information of the analytes. However, it is possible to calculate particle concentration by predicting the size and geometry of the pore through the measured background current at a given voltage. Nevertheless, calibration increases measurement reproducibility and traceability and hence it is predominantly used.[6] For TRPS concentration measurements calibration and sample are typically measured in alternation in order to virtually eliminate the impact of any change in pore geometry occurring during the measurement process.

The concentration of the standards is determined gravimetrically (mass-fraction of solids) with the knowledge of the mean particle diameter and particle density. Concentration standards are typically bare polystyrene particles, whose certified mean diameter is traceable to the internation system of Units (SI) or alternatively carboxylated polystyrene standards. Ideally, the standards should have concentration values that are traceable to SI, but unfortunately such standards are not available yet. The linearity of the counting system can be tested by obtaining three repeat measurements of the total counts (across all channels) at an arbitrary concentration. The concentration is then reduced and three further repeat total counts obtained (ISO13319-1).

Table 2 shows a reference guide to TPU aperture selection, target calibrant and sample particle concentrations. The size range shown can be detected across the standard stretch of 3 mm-7 mm under optimal setting conditions. Note that target concentrations lie well below N5 % for respective apertures.

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