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Particle size analysis — Particle tracking analysis (PTA) method

Analyse granulométrique — Méthode d'analyse de suivi de particule (PTA)

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Foreword

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The committee responsible for this document is Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

Introduction

Regulatory, scientific and commercial requirements for nanomaterial characterization or characterization of particulate suspensions where particle sizing and counting is required provide a strong case for further development of techniques such as Particle Tracking Analysis (PTA), also known as Nanoparticle Tracking Analysis (NTA) [14]. Due to the fact that the term PTA covers a larger size range and is more generic[1)], the term PTA is used throughout this document to refer to NTA and PTA. For all aims and purposes, the term PTA also means NTA in this document.

PTA is based on measuring the diffusion movement of particles in a suspension by means of laser illumination, imaging of scattered light, particle identification and localization, and individual particle tracking[2)]. In this case, suspension is an even dispersion of particles, gas bubbles or other liquid droplets. The hydrodynamic diameter of the individual particles, droplets or bubbles is related to Brownian motion parameters via the Stokes–Einstein equation.

In recent years the academic community working in fields such as liposomes and other drug delivery vehicles, nanotoxicology, viruses, exosomes, protein aggregation, inkjet inks, pigment particles, cosmetics, foodstuffs, fuel additives and fine bubbles began using the PTA technology for characterization. An ASTM standard guide (E2834–12) [10] was developed to give guidance to the measurement of particle size distribution by means of Nanoparticle Tracking Analysis. The present document aims to broaden the scope of the specification and to introduce system tests for PTA operation.

This document outlines the theory and basic principles of the particle tracking analysis method along with its limitations and advantages. It also describes commonly used instrument configurations and measurement procedures as well as system qualifications and data reporting. One of the key aspects is the meaning of the data and its interpretation. It should be noted that the key measurand obtained from PTA measurement is the number-based particle size distribution where the size is taken to mean the hydrodynamic diameter (3.11) of the particles in the sample. This size can be different from other sizes obtained with different techniques such as dynamic light scattering [6] or electron microscopy [4].

1) NTA is the most recognised abbreviation for the technique described in this document. However the Particle Tracking Analysis (PTA) includes NTA in its size range of measurements.

2) For the purpose of this document “tracking” will mean “following in terms of particle x and y position” and the “track” will mean “the path of that particle defined by such x and y coordinates of each step”

Particle size analysis — Particle tracking analysis (PTA) method

1 Scope

This document describes the evaluation of the number–based particle size distribution in liquid dispersions (solid, liquid or gaseous particles suspended in liquids) using the particle tracking analysis method for diffusion velocity measurements.

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 http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
nanoscale
length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from larger sizes are predominantly exhibited in this length range.

[SOURCE: ISO/TS 80004-1:2015, 2.1]

3.2
nano-object
material with one, two or three external dimensions in the *nanoscale* (3.1)

Note 1 to entry: The second and third external dimensions are orthogonal to the first dimension and to each other.

[SOURCE: ISO/TS 80004-1:2015, 2.5]

3.3
nanoparticle
nano-object (3.2) with all three external dimensions in the *nanoscale* (3.1)

Note 1 to entry: If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than three times), the terms *nanofibre* or *nanoplate* are intended to be used instead of the term nanoparticle.

[SOURCE: ISO/TS 80004-4:2011, 2.4]

3.4
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

Note 3 to entry: This general particle definition applies to *nano-objects* (3.2).

[SOURCE: ISO/TS 80004-6:2013, 2.9]

3.5
agglomerate
collection of weakly bound particles or *aggregates* or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

Note 1 to entry: The forces holding an agglomerate together are weak forces, for example van der Waals forces, or simple physical entanglement.

Note 2 to entry: Agglomerates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-4:2011, 2.8]

3.6
aggregate
particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components

Note 1 to entry: The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed primary particles.

[SOURCE: ISO/TS 80004-4:2011, 2.7]

3.7
particle size
linear dimension of a *particle* (3.4) determined by a specified measurement method and under specified measurement conditions

Note 1 to entry: Different methods of analysis are based on the measurement of different physical properties. Independent of the particle property actually measured, the particle size can be reported as a linear dimension, e.g. as an equivalent spherical diameter.

[SOURCE: ISO/TS 80004-6:2013, 3.1.1]

3.8
particle size distribution
distribution of *particles* (3.4) as a function of *particle size* (3.7)

Note 1 to entry: Particle size distribution may be expressed as cumulative distribution or a distribution density (distribution of the fraction of material in a size class, divided by the width of that class).

[SOURCE: ISO/TS 80004-6:2013, 3.1.2]

3.9
equivalent diameter
diameter of a sphere that produces a response by a given particle-sizing method, that is equivalent to the response produced by the particle being measured

Note 1 to entry: The physical property to which the equivalent diameter refers is indicated using a suitable subscript [ISO 9276-1:1998].

Note 2 to entry: For discrete-particle-counting, light-scattering instruments, an equivalent optical diameter is used.

Note 3 to entry: Other material constants like density of the particle are used for the calculation of the equivalent diameter like Stokes diameter or sedimentation equivalent diameter. The material constants, used for the calculation, should be reported additionally.

Note 4 to entry: For inertial instruments, the aerodynamic diameter is used. Aerodynamic diameter is the diameter of a sphere of density 1 000 kg m^{-3} that has the same settling velocity as the irregular particle.

[SOURCE: ISO/TS 80004-6:2013, 3.1.5]

3.10
light scattering
change in propagation of light at the interface of two media having different optical properties

[SOURCE: ISO 13320:2009, 3.1.17]

3.11
hydrodynamic diameter
equivalent spherical diameter of a particle in a liquid having the same diffusion coefficient as the real particle in that liquid

[SOURCE: ISO/TS 80004-6:2013, 3.2.6]

3.12
particle tracking analysis
PTA
method where particles undergoing Brownian motion in a liquid suspension are illuminated by a laser and the change in position of individual particles is used to determine particle size

Note 1 to entry: Analysis of the time-dependent particle position yields translational diffusion coefficient and hence the particle size as hydrodynamic diameter using the Stokes-Einstein relationship.

Note 2 to entry: Nanoparticle Tracking Analysis (NTA) is often used to describe PTA. NTA is a subset of PTA since PTA covers larger range of particle sizes than *nanoscale* (3.1).

[SOURCE: ISO/TS 80004-6:2013, 3.2.8, modified — Nanoparticle tracking analysis has been removed from the term, and Notes 1 and 2 have been modified.]

3.13
nanomaterial
material with any external dimension in the *nanoscale* (3.1) or having internal structure or surface structure in the nanoscale

[SOURCE: ISO/TS 80004-1:2015, 2.4]

3.14
diluent
non-volatile homogeneous liquid which is used to decrease the number concentration of *particles* (3.4) in a suspension without any deleterious effects such as changing particle total number, state of aggregation, *particle size* (3.7) or surface chemistry

3.15
viscosity
measure of the resistance to flow or deformation of a liquid

[SOURCE: ISO 3104:1994]

3.16
percentile
value of a variable below which a certain percentage of observations fall

[SOURCE: ISO 11064-4:2013, 3.7]

4 Symbols and abbreviated terms

For the purposes of this document, the following symbols and abbreviated terms apply.

CCD	Charge Coupled Device		
CMOS	Complementary Metal Oxide Semiconductor		
CV	Coefficient of Variation (standard deviation divided by arithmetic average)(ISO 27448:2009, 3.11)		
CCD	Charge Coupled Device		
d	hydrodynamic diameter	metre	m
D_x	translational diffusion coefficient in 1 dimension		m^2/s
D_{xy}	translational diffusion coefficient in 2 dimensions		m^2/s
D_{xyz}	translational diffusion coefficient in 3 dimensions		m^2/s
η	viscosity of the suspension medium	pascal second	Pa·s
k_B	Boltzmann's constant		$m^2\ kg\ s^{-2}\ K^{-1}$
RSD	Relative Standard Deviation (ISO/TR 13843:2000, 2.34)		%
T	absolute temperature	kelvin	°K
t	time	second	s
$\overline{(x)^2}$	mean square displacement in 1 dimension	metre squared	m^2
$\overline{(x,y)^2}$	mean square displacement in 2 dimensions	metre squared	m^2
$\overline{(x,y,z)^2}$	mean square displacement in 3 dimensions	metre squared	m^2

5 Principles

5.1 General

Determination of particle size distribution by PTA makes use of the Brownian motion and light scattering properties of particles suspended in liquids. Irradiation of the sample (typically by means of a laser beam of wavelength in the visible region) leads to light scattering by objects with a refractive index that is different from that of the surrounding medium. Light scattered from each particle is collected by magnifying optics and visualized by way of a suitable detector, such as a Charge Coupled Device (CCD) or Complementary Metal Oxide Semiconductor (CMOS) camera. By recording a series of sequential images, the instrument's software tracks positions of particles as a function of time, allowing analysis of their movement.

By tracking individual particles, undergoing random Brownian motion [6] [13], from frame[3)] to frame, the average spatial displacement of the particles per unit time can be calculated, and this displacement

3) For the purpose of this document, "frame" will mean a still image obtained from video capturing of the moving objects in PTA measurement equipment".

can be related to the hydrodynamic diameter of the particles through the Stokes-Einstein equation[13]. Although translational Brownian motion is a three-dimensional process, it is possible to use a one-, two-, or three-dimensional diffusion coefficient to determine particle hydrodynamic diameter. The relevant formulae are derived in Annex A and can be summarized with three formulae below:

$$\overline{\left(x\right)^2} = D_x t = \frac{2k_B Tt}{3\pi\eta d} \tag{1}$$

$$\overline{\left(x, y\right)^2} = D_{xy} t = \frac{4k_B Tt}{3\pi\eta d} \tag{2}$$

$$\overline{\left(x, y, z\right)^2} = D_{xyz} t = \frac{2k_B Tt}{\pi\eta d} \tag{3}$$

Mean square displacement $\overline{\left(x\right)^2}$ can be measured in x and y directions independently to give two independent values for particle size [Formula (1)]. In most PTA instruments, $\overline{\left(x, y\right)^2}$ is evaluated as shown in Formula (2). It should be noted that in all three cases there is no assumption of two dimensional movement of particles. All particles are assumed to be moving freely in all three dimensions while the measurement is sampling the projection of each x, y and z component of that movement onto the xy observation plane. As described in Annex A, these components (observables) are independent variables.

5.2 Key physical parameters

Formulae (1) to (3) show that as well as the diffusion coefficient, the temperature and the viscosity of the sample shall be known in order to calculate the hydrodynamic diameter.

5.3 Detection limits

Like any measurement technique, PTA has detection limits in terms of the particle size and the particle number concentration. These limits are heavily dependent on the particle material, diluent and polydispersity of the sample.

Depending on the physical properties of the particles, the typical working range of the PTA can be from about 10 nm to about 2 µm in diameter.

5.3.1 Lower size limit

The lower limit of detection in terms of the particle hydrodynamic diameter is determined (apart from sensitivity and dynamic range of the camera) by the light scattering from the particles. It is the combination of refractive indexes of the particle material and the diluent that affect the amount of light scattering the detection and tracking system. A large difference in refractive indexes results in higher scattering and therefore lower detection limit for all other parameters being the same.

Better tracking of highly scattering particles results in preferential counting of particles. The accuracy of counting is covered in 5.4.4.

Sample polydispersity affects the ability to track and therefore analyse different size fractions in the particle number-size distribution. The underlying effect is linked to the dynamic range of the video capture and image analysis. In a polydisperse sample large particles scatter a lot more than small particles making it difficult to detect or track small size particles. All the values in Table 1 are given for monodisperse samples. In the case of a monodisperse gold spheres in suspension, the lower limit of detection is typically 15 nm but can range from approximately 10 nm to 20 nm.

Below is the table of detection limits for commonly used dispersions (particle-diluent combinations).

Table 1 — Lower limit of detection for monodisperse suspensions of nanoparticles

Particle material	Approximate lower detection limit (Hydrodynamic diameter in nm)
Gold	15
Polystyrene	45
Silica	75
Biological materials	60
Other metals or metal oxides	25

General effects of samples and measurement parameters on the detection limits are described in the subclauses below. The typical values quoted for room temperature water dispersion are provided in Table 1. These values are approximate and could vary (as much as 30 %, for example leading to low detection limit for gold ranging from approximately 10 nm to 20 nm) depending on factors such as porosity of silica or the type of biological material.

5.3.2 Upper size limit

The upper particle size limit is limited by slowing Brownian motion at larger particle sizes. The motion of such particles is very slow and long observation periods may be required. Very large particles can also produce so much scattering that the detection system may not track much smaller particles in the same polydisperse sample.

In the limit of very large particles (or gas bubbles) the sample may separate with heavy particles sedimenting (or large bubble creaming). These effects shall be considered at all times for PTA measurement.

5.3.3 Sample and sampling volume

In a number of applications the knowledge of sample volume and sampling volume involved in a PTA measurement can be important. Typically used equipment requires approximately 1 ml of sample to be used for measurement.

The subsampling methods can vary between manufacturers, yet the sampling volume of liquid that is being investigated within the PTA microscope field of view[4)] is often limited to a range of approximately 0,1 nl to 1 nl volume. The sampling volume, for the PTA measurement is limited laterally by the optical field of view of the system to (typically of the order of) 100 µm by 100 µm area. The particles in that area are tracked using imaging power of the optics with an approximate focus depth of (the order of) 10 µm which is taken as the sampling volume depth. This results in a sampling volume of 0,1 nl. Larger sampling volumes may be obtained for optical systems with larger field of view or lower magnification.

In order to obtain a representative measurement of a sample (especially for low particle number concentrations), the sampling volume should be increased. This is often achieved by sampling multiple parts of the sample and performing a new measurement as described in Clause 7.

5.3.4 Maximum particle number concentration

When preparing samples or evaluating the applicability of PTA to existing samples, the limits of particle number concentration shall be considered. This subclause addresses the limitations of the PTA method in terms of the particle number concentration. All references in this document to concentration are made to particle number concentration and not molar concentration or mass concentration due to the nature of the measurement. Appropriate conversions can be made from (for example) mass concentration to particle number concentrations at sample preparation stages. PTA requires highly diluted samples and the optimal particle concentration is sample-dependent. The optimal particle concentration should be

4) As defined in ISO 10360-7:2011, 3.3, “field of view” is the area viewed by the imaging probing system.