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Nanotechnologies — Guidance on the measurement of nanoparticle number concentration

Nanotechnologies — Conseils pour la mesure de la concentration en nombre de nanoparticules

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Foreword

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This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

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Introduction

Nanoparticle number concentration refers to the number of nanoparticles per unit of volume or mass in a sample. It is an important measurand when analysing dispersions containing nanoparticles. Nanoparticle number concentration is also considered a useful metric for supporting materials toxicological assessments. Furthermore, the capability to accurately measure nanoparticle number concentration can help industry to increase product manufacturing quality control and implement quality assurance. Currently, in most applications, nanoparticle number concentration is estimated from indirect mass-balance considerations and validated direct techniques for this measurand are required.

This document provides an overview of commonly used methods for the measurement of nanoparticle number concentration. These are the ensemble measurement techniques of differential centrifugal sedimentation (DCS) (line start incremental disc-type centrifugal liquid sedimentation), multi-angle dynamic light scattering (MDLS), small-angle X-ray scattering (SAXS) and ultraviolet-visible spectroscopy (UV-vis) and the particle counting techniques of particle tracking analysis (PTA), resistive pulse sensing (RPS), single particle inductively coupled plasma mass spectrometry (spICP-MS), condensation particle counter (CPC), and differential mobility analysing system (DMAS).

This document focuses on the analysis of nanoparticles in suspensions (liquid dispersions) but also addresses aerosols measured using a CPC or a DMAS. Particles on surfaces or incapsulated in solid materials are not covered in this document. Nanoparticles rather than nano-objects are discussed as most techniques use the spherical approximation model to measure particle diameter which is more applicable to nanoparticles as opposed to nanofibres and nanoplates. Most of the techniques discussed can also analyse particles of size greater than the nanoscale.

This document provides guidance to help users to select the most appropriate techniques for nanoparticle number concentration measurements suitable for their applications.

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Nanotechnologies — Guidance on the measurement of nanoparticle number concentration

1 Scope

This document provides an overview of the methods used to determine the nanoparticle number concentration in liquid dispersions and aerosols. The methods described are the ensemble measurement techniques of differential centrifugal sedimentation (DCS), multi-angle dynamic light scattering (MDLS), small-angle X-ray scattering (SAXS) and ultraviolet-visible spectroscopy (UV-vis) and the particle counting methods of particle tracking analysis (PTA), resistive pulse sensing (RPS), single particle inductively coupled plasma mass spectrometry (spICP-MS), condensation particle counter (CPC), and differential mobility analysing system (DMAS). This document provides information on the use of each technique, along with considerations on sample preparation, advantages and limitations.

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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ISO 80004-1, *Nanotechnologies – Vocabulary — Part 1: Core vocabulary*

ISO/TS 80004-6, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

ISO/TS 80004-8, *Nanotechnologies — Vocabulary — Part 8: Nanomanufacturing processes*

3 Terms and definitions

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For the purposes of this document, the terms and definitions given in ISO 80004-1, ISO/TS 80004-6, ISO/TS 80004-8 and the following 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
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.

[SOURCE: ISO 26824:2022, 3.1]

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3.2
nanoparticle
nano-object with all external dimensions in the nanoscale

Note 1 to entry: If the dimensions differ significantly (typically by more than three times), terms such as nanofibre or nanoplate are preferred to the term nanoparticle.

[SOURCE: ISO 80004-1:2023, 3.3.4]

3.3
primary particle
original source *particle* (3.1) of *agglomerates* (3.4) or *aggregates* (3.5), or mixtures of the two

Note 1 to entry: Constituent particles of agglomerates or aggregates at a certain actual state may be primary particles, but often the constituents are aggregates.

Note 2 to entry: Agglomerates and aggregates are also termed secondary particles.

[SOURCE: ISO 26824:2022, 3.1.4]

3.4
agglomerate
collection of weakly or medium strongly bound *particles* (3.1) 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* (3.3).

[SOURCE: ISO 80004-1:2023, 3.2.4]

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3.5
aggregate
particle (3.1) comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components

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Note 1 to entry: The forces holding an aggregate together are strong forces, for example, covalent or ionic 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 80004-1:2023, 3.2.5]

3.6
differential centrifugal sedimentation
DCS
analytical centrifugation in which the sample is introduced at a defined position in a rotating disc partially filled with a fluid

Note 1 to entry: Normally the fluid has a density gradient to ensure uniform sedimentation.

Note 2 to entry: Normally there is one detector at a pre-determined position and the times taken for the *particles* (3.1) to reach this detector are recorded.

Note 3 to entry: Depending on the effective density of the particles, the technique can measure particle size and particle size distribution between 2 nm and 10 µm, and can resolve particles differing in size by less than 2 %.

[SOURCE: ISO/TS 80004-6:2021, 4.4.5, modified — the term “line-start incremental disc-type centrifugal liquid sedimentation” has been removed.]

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3.7
condensation particle counter
CPC
instrument that measures the *particle* (3.1) number concentration of an aerosol using a condensation effect to increase the size of the aerosolized particles

Note 1 to entry: The sizes of particles detected are usually smaller than several hundred nanometres and larger than a few nanometres.

Note 2 to entry: A CPC is one possible detector suitable for use with a differential electrical mobility classifier (DEMC).

Note 3 to entry: In some cases, a condensation particle counter may be called a "condensation nucleus counter (CNC)".

[SOURCE: ISO/TS 80004-6:2021, 4.3.1]

3.8
differential mobility analysing system
DMAS
system to measure the size distribution of sub-micrometre aerosol *particles* (3.1) consisting of a differential electrical mobility classifier (DEMC), flow meters, a particle detector, interconnecting plumbing, a computer and suitable software

[SOURCE: ISO/TS 80004-6:2021, 4.3.3]

3.9
dynamic light scattering
DLS
method in which *particles* (3.1) in a liquid suspension are illuminated by a laser and the time dependant change in intensity of the scattered light due to Brownian motion is used to determine particle size

Note 1 to entry: Analysis of the time-dependent intensity of the scattered light can yield the translational diffusion coefficient and hence the particle size as the hydrodynamic diameter via the Stokes–Einstein relationship.

Note 2 to entry: The analysis is applicable to *nanoparticles* (3.2) as the size of particles detected is typically in the range 1 nm to 6 000 nm. The upper limit is due to limited Brownian motion and sedimentation.

Note 3 to entry: DLS is typically used in dilute suspensions where the particles do not interact amongst themselves.

[SOURCE: ISO/TS 80004-6:2021, 4.2.7, modified — the term "photon correlation spectroscopy" has been removed.]

3.10
nanoparticle tracking analysis
NTA
particle tracking analysis
PTA
method in which *particles* (3.1) undergoing Brownian and/or gravitational motion in a 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 the translational diffusion coefficient and hence the particle size as the hydrodynamic diameter using the Stokes-Einstein relationship.

Note 2 to entry: The analysis is applicable to *nanoparticles* (3.2) as the size of particles detected is typically in the range 10 nm to 2 000 nm. The lower limit requires particles with high refractive index and the upper limit is due to limited Brownian motion and sedimentation.

Note 3 to entry: NTA is often used to describe PTA. NTA is a subset of PTA since PTA covers larger range of particle sizes than nanoscale.

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[SOURCE: ISO/TS 80004-6:2021, 4.2.8]

3.11
resistive pulse sensing
RPS
method for counting and size measurement of *particles* (3.1) in electrolytes by measuring a drop in electrical current or voltage as a particle passes through an aperture between two chambers

Note 1 to entry: The drop in current or voltage is proportional to the particle volume (Coulter principle).

Note 2 to entry: The particles are driven through the aperture by pressure or an electric field.

Note 3 to entry: The aperture can be nanoscale in size allowing the size measurement of individual nano-objects.

[SOURCE: ISO/TS 80004-6:2021, 4.4.7, modified — the terms "electrical sensing zone method" and "Coulter counter" have been removed.]

3.12
single particle inductively coupled plasma mass spectrometry
spICP-MS
method using inductively coupled plasma mass spectrometry whereby a dilute suspension of nano-objects is analysed and the ICP-MS signals collected at high time resolution, allowing particle-by-particle detection at specific mass peaks and number concentration, size and size distribution to be determined

[SOURCE: ISO/TS 80004-6:2021, 4.4.8]

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3.13
small-angle X-ray scattering
SAXS
method in which the elastically scattered intensity of X-rays is measured for small-angle deflections

Note 1 to entry: The scattering is typically measured in the angular range up to 5°. This provides structural information about inhomogeneities in materials with characteristic lengths typically ranging from 1 nm to 100 nm. Under certain conditions the limit of 100 nm can be significantly extended.

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[SOURCE: ISO/TS 80004-6:2021, 4.24, modified — Note 1 to entry has been replaced.]

3.14
ultraviolet-visible spectroscopy
UV-Vis spectroscopy
spectroscopy of radiation that consists of electromagnetic radiation with wavelengths in the ultraviolet and/or visible regions

[SOURCE: ISO/TS 80004-6:2021, 5.6]

4 Abbreviated terms

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

BIPM-CCQM	bureau international des poids et mesures consultative committee for amount of substance: metrology in chemistry and biology
CLS	centrifugal liquid sedimentation
CPC	condensation particle counter
DCS	differential centrifugal sedimentation
DLS	dynamic light scattering

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DMA	differential mobility analyser
DMAS	differential mobility analysing system
ES	electrospray
MDLS	multi-angle dynamic light scattering
PTA	particle tracking analysis
RPS	resistive pulse sensing
SAXS	small-angle X-ray scattering
spICP-MS	single particle inductively coupled plasma mass spectrometry
TRPS	tunable resistive pulse sensing
UV-vis	ultraviolet-visible spectroscopy
VAMAS	Versailles project on advanced materials and standards

5 Overview

5.1 General

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The number concentration of nanoparticles can be measured by techniques that average the number of particles measured over a specific sample volume (henceforth referred to as “ensemble techniques”) or count individual nanoparticles (henceforth referred to as “particle counting” or “particle-by-particle techniques”). The ensemble techniques described in this document are DCS, MDLS, SAXS and UV-vis spectroscopy. In these ensemble techniques, the measured sample volume can have some fractionation, for example in the case of DCS, but an ensemble of particles rather than individual particles are measured at the detector. The particle counting methods described are PTA, RPS, spICP-MS, CPC and DMAS. All the techniques discussed in this document are used for measuring nanoparticles in suspensions except for CPC and for DMAS, which are used to determine the particle number concentration in aerosols, which includes aerosolised suspensions.

The selection of the method of choice is ultimately dictated by the nature of the sample. The measurement of the number concentration of a particle population intrinsically depends on the limits of detection, sensitivity and resolution of the applied technique in terms of particle size. Depending on particle size, some techniques are capable of measuring the relative concentration of particle populations within the same sample. Some techniques measure aggregates or agglomerates as one particle, giving no information on primary particles unless separated by other means. Ensemble techniques generally require the knowledge of other particle characteristics, such as size and refractive index, in order to measure the number concentration.

A summary of VAMAS and BIPM-CCQM P194 international interlaboratory studies on the measurement of the number concentration of colloidal gold nanoparticles with selected techniques is described in Annex A and a guide on sample preparation for nanoparticles in suspension is described in Annex B.

5.2 Comparison of different techniques

The techniques described in this document are outlined in Table 1. This is not an exhaustive list of methods to measure nanoparticle number concentration measurements; other methods include electron microscopy and asymmetrical flow-field flow fractionation (AF4) coupled to PTA or ICP-MS, but are not discussed in this document. The techniques in Table 1 and Clauses 6 and 7 are grouped by ensemble and particle counting, and then listed in alphabetical order. Methods for particles in suspensions (liquid dispersions) are listed first followed by those for aerosols (i.e. CPC and DMAS). Here, instrument footprint refers to the area that the instrument takes up in the laboratory.

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Table 1 — Comparison of techniques for measuring nanoparticle number concentration in suspensions (ensemble and particle counting) and airborne

	Technique	Particle type	Critical input parameters	Advantages	Limitations
Ensemble	DCS	Organic and inorganic materials which absorb and/or scatter light or X-rays	Effective density and complex refractive index of the particles, refractive index, average density and viscosity of the density gradient (medium), and some instrument-related parameters. The viscosity of the gradient, as well as the instrument-related parameters, can be replaced by a single method constant based on calibration with spherical reference particles of known effective density and size.	Multiple information (e.g. size and concentration). High resolution of the size distribution. Concentration per size population. Minimal sample preparation.	Longer sedimentation times for smaller nanoparticles or lower density materials. Calibration of particle losses required. Spherical model assumption applied. Spherical calibrant of known size and density required. Data post processing required.
	MDLS	Organic and inorganic materials which scatter light	Complex refractive index and temperature of the medium and the particles, viscosity of the medium	Multiple information (e.g. size and concentration). Rapid measurements. [a] Concentration per size population. Minimal sample preparation.	Spherical model assumption applied. Lower performance for heterogeneous samples.
	SAXS	Organic and inorganic materials which scatter X-rays	Density of the materials (more specifically: effective electron density)	Multiple information (e.g. size, internal structure and concentration). Minimal sample preparation.	Spherical model assumption applied.
	UV-vis	Organic and inorganic material which absorb and/or scatter light	Average particle size and extinction cross-section	Widely available. Rapid measurements. [a] Minimal sample preparation.	Material dependent, particle extinction cross-section is not known for many materials and is also size dependent which limits its applicability.
Particle counting	PTA	Organic and inorganic materials, which scatter light	Effective sensing volume of the instrument	Multiple information (e.g. size and concentration). Concentration per size population. High resolution of the size distribution. Rapid measurements. [a] Low analyte volume.	Sample dilution to optimal concentration. Expert setting of signal thresholds. Calibration of sampling volume required. Dependencies on tracking algorithms.
	RPS	Organic and inorganic materials	Size of the aperture selected	Multiple information (e.g. size and concentration). High resolution of the size distribution. Concentration per size population. Rapid measurements. [a] Low analyte volume.	Concentration calibrant can be required. Stable analyte dispersion in electrolyte solution required. Sample dilution to optimal concentration. Expert setting of signal thresholds.
	spICP-MS	Particles with an element/ tag suitable for ICP-MS detection	Transport efficiency of particles	Rapid measurement. [a] Multiple information [e.g. element mass per particle (from which size can be calculated by taking into account density and shape) and number concentration]. Low analyte volume. Very diluted matrix thus minimizing matrix effects. Minimal sample preparation. Information on the dissolved and nanoparticulate fractions simultaneously.	Expert selection of optimal particle concentration Expert setting of signal thresholds. Calculation of transport efficiency required. Limits of detection for sizing limited by procedural blanks, instrumental background and contribution of dissolved fraction.

[a] Rapid measurements refer to those that take approximately 60 s or less per measurement.

[b] Liquid dispersion.

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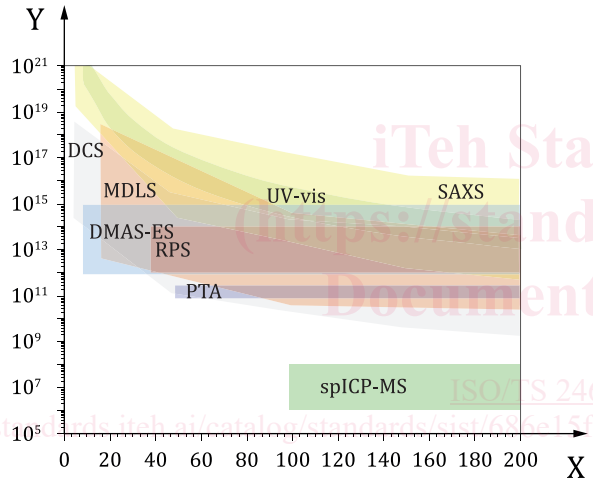
Table 1 *(continued)*

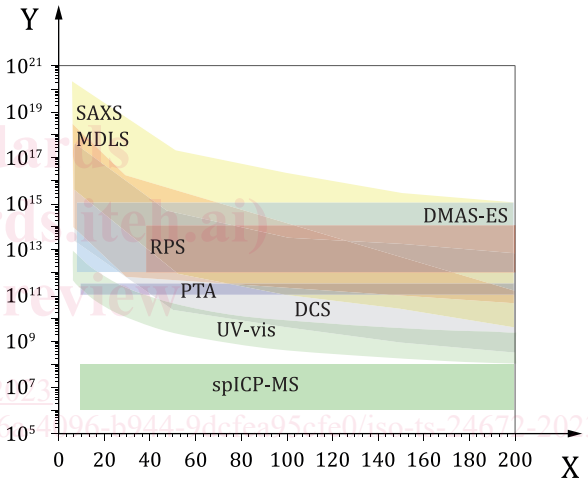
	Technique	Particle type	Critical input parameters	Advantages	Limitations
Airborne particles	CPC	Airborne particles, including aerosolised particles from a suspension[b]	Flow rates of air or gas mixture (aerosol flow and sheath flow)	Rapid measurement (one second resolution). [a]	Sample dilution to optimal concentration. Calibration of transport efficiency required. For dispersions: artefacts derived from solutes in liquid dispersions (electrospray or nebulisation).
	DMAS	Airborne particles, including aerosolised particles from a suspension[b]	The flow rates of air or gas mixture (aerosol flow and sheath flow). Voltage for DMA size discrimination. Efficiency and size distribution preservation of the aerosolization method.	Multiple information (e.g. size distribution and concentration) High resolution of the size distribution. Rapid measurement. [a]	Sample dilution to optimal concentration. Calibration of transport efficiency required. Less direct than CPC for aerosols. For dispersions: artefacts derived from solutes in liquid dispersions (electrospray or nebulisation).

[a] Rapid measurements refer to those that take approximately 60 s or less per measurement.

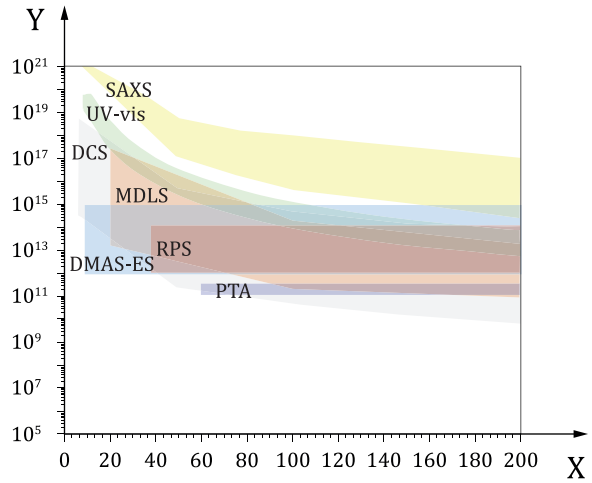
[b] Liquid dispersion.



a) Silica



b) Gold



c) Polystyrene

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Key

X particle diameter (nm)

Y particle number concentration (kg^{-1})

Figure 1 — Comparison of techniques and estimates of related number concentration measurement ranges as a function of particle diameter for various materials types in suspension

Figure 1 shows a summary of the estimated silica, gold and polystyrene particle diameter and number concentration ranges for the different techniques that analyse samples in suspensions and outlined in this document. This includes DMAS with an electrospray (DMAS-ES) for analysis of suspensions. The ranges are different for different types of materials. The technical notes given in Table 2 provide a general description on how the estimated values for various techniques were obtained. For the measurement of aerosol samples using CPC or DMAS, the concentration range for the CPC or DMAS is detailed in 7.4.2.

Table 2 — Technical notes on the calculation of Figure 1

Techniques	Technical notes
DCS	The data was obtained from unpublished experimental work on gold nanoparticles and inferred for the other materials and sizes based on mass equivalence considerations.
MDLS	The data are based on literature values[2].
PTA	The data are based on literature values[1],[3].
RPS	The data are based on unpublished experimental work using typical TRPS measurement parameters.
SAXS	The data are based on experimental measurements. The size range depends on the available q-range and thus the used instrument. For high density particles like gold, large particles will tend to sediment and would need a constant flow-through or a vertical setup for accurate measurement. For low density particles like polystyrene, the concentration determination can be facilitated by increasing the contrast between particles and suspending medium, e.g. by dilution 1:5 in ethanol.
spICP-MS	The data are based on the literature values.[4],[5],[6] The stated diameter and particle-concentration ranges reflect currently available commercial state-of-the art instrumentation (e.g. instruments with microsecond dwell times).
UV-vis	The data are based on the calculations using 405 nm wavelength, Mie theory for extinction cross-sections and an absorbance within the range 0,1 to 2.
DMAS - ES	The data are for DMAS with electrospray analysis of suspensions and is based on literature values[7] and unpublished data for gold nanoparticles. There can be variations as the size becomes very small or very large, but in the current absence of information a flat response as a function of size range is given.

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5.3 Considerations when selecting a technique

5.3.1 General

The selection of a suitable technique for the measurement of the number concentration of a nanoparticle sample requires users to consider a number of factors, some of which are discussed in 5.3.2 to 5.3.5.

5.3.2 Type of particles

All the techniques can be applied to a large variety of particle types, including organic, inorganic and biological materials, as summarized in Table 1. Among these, CPC and DMAS are the only techniques that can measure the number concentration of airborne particles whereas the others are suitable for nanoparticle analysis in liquid dispersions (suspensions).

Several of the techniques that operate in suspension (i.e. DCS, MDLS, SAXS, UV-vis and PTA) detect light extinction and thereby require the particles to absorb or scatter light at the wavelength of the light source applied. The analysis of particles and suspending medium with relatively low differential scattering cross-section can be better analysed with other techniques. An example here is measuring

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