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Nanotechnologies — Antibacterial silver nanoparticles — Specification of characteristics and measurement methods

Nanotechnologies — Nanoparticules d'argent antibactériennes — Spécification des caractéristiques et des méthodes de mesure

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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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).

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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 229, *Nanotechnologies*.

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

Silver nanoparticles have become one of the most widely utilized nanomaterials in consumer products for their antibacterial properties. The application of silver nanoparticles is increasingly being adopted in consumer products to control the growth of microorganisms on the surfaces or interiors of products. When silver nanoparticles interact with microorganisms silver ions are released, and these ions may affect and damage microorganisms in different ways. However, the mechanism behind the bactericidal effect is not well known[1]. There have been several possible mechanisms proposed in the scientific literature: 1) silver ions with positive electricity released from silver nanoparticles are able to rapidly bind to sulfhydryl groups on the surfaces of bacteria, which leads the structures of bacteria to change and become damaged, 2) the uptake of silver ions or small nanoparticles disrupts adenosine triphosphate production and DNA replication, and 3) silver nanoparticles and ions generate reactive oxygen species resulting in oxidative damage[2]-[4]. Other scientific evidence of the antibacterial performance of silver nanoparticle is listed in Annex B. The antibacterial properties of silver nanoparticles are related to their physicochemical characteristics.

Although antibacterial products that utilize silver nanoparticle are widely distributed in the market, most of these products are sold without providing information on the physicochemical and corresponding antibacterial characteristics of nanoparticles. Currently, most manufacturers provide specifications based on their own practices.

This document provides guidance for the specification of characteristics and relevant recommended measurement methods, referenced from other standards for silver nanoparticles in powder and colloidal forms that are intended for antibacterial applications in nanotechnology. The major measurement methods available to industry for the determination of parameters specified in this document are of course recommended in the specification. This document reviews selected measurement methods that are commonly used at present, and therefore will require updating on a regular basis.

Nanotechnologies — Antibacterial silver nanoparticles — Specification of characteristics and measurement methods

1 Scope

This document provides guidance for the specification of characteristics and relevant measurement methods for silver nanoparticles in powder or colloidal forms that are intended for antibacterial applications in nanotechnology.

This document is intended to aid the producer in providing the physicochemical characteristics of silver nanoparticles that have an antibacterial effect to the buyer.

This document does not cover considerations specific to health and safety issues either during manufacturing or use.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the cited edition applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 26824, *Particle characterization of particulate systems — Vocabulary*

ISO/TS 80004-1, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 26824, ISO/TS 80004-1, ISO/TS 80004-2 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

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

3.1
silver nanoparticle
nanoparticle composed of silver with all three external dimensions in the nanoscale

[SOURCE: modified from ISO/TS 80004-2, 4.1, modified]

3.2
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* (3.3) 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, 1.4]

3.3
nanoscale
size range from approximately 1 nm to 100 nm

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

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

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 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* (3.2).

[SOURCE: ISO 26824, 1.2]

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

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, or otherwise combined former primary particles.

Note 2 to entry: Aggregates are also termed secondary particles and the original source particles are termed *primary particles* (3.2).

[SOURCE: ISO 26824, 1.3]

3.6
antibacterial activity
property of substances or phenomena that kills (bactericidal) or slow down (bacteriostatic) the growth of bacteria

4 Symbols and abbreviations

For the purposes of this document, the following symbols and abbreviations apply:

Abbreviation	Meaning
AAS	Atomic absorption spectrometry
AgNP	Silver nanoparticle
BET	Brunauer-Emmett-Teller
DLS	Dymanic light scattering
ELS	Electrophoretic light scattering
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
NP	Nanoparticle
PTA	Particle tracking analysis
SAXS	Small angle X-ray scattering

SEM	Scanning electron microscopy
spICP-MS	Single particle inductively coupled plasma mass spectrometry
TEM	Tranmission electron microscopy

5 Characteristics and measurement methods

5.1 General

The specification of characteristics and measurement methods for antibacterial silver nanoparticles is separated into two categories: those that are essential are listed in Table 1 and those that are additional are listed in Table 2. A producer of antibacterial silver nanoparticles shall measure the characteristics in Table 1 and should also measure the characteristics in Table 2 and report the results to the buyer of AgNPs. In Tables 1 and 2, guidance for the measurement methods is listed as information. The listed ISO standards have been written generically, and measurement methods can be added as technology advances. Adopting the relevant document be agreed upon by the buyer and producer of AgNPs. The measurement results for characteristics shall be expressed in the units listed in Tables 1 and 2. See the informative Annex A describing measurement methods for the individual characteristics listed in Tables 1 and 2. See the informative Annex B for descriptions of the relationships between silver nanoparticle characteristics and antibacterial performance. See Annex C for description of the antibacterial performance test of AgNPs.

Material properties are either intrinsic to the material, or defined by the measurement method. The values of method-defined properties cannot be directly compared with those obtained using a different method. In addition, methods for assessing intrinsic properties may be biased, and lead to results that are different from other methods assessing the same property. Consequently, the results from one measurement method may not be directly comparable with results from a second measurement method.

Table 1 — Essential characteristics to be measured

Characteristics	Units	Measurement method	Application form	Relevant documents
1) Average size and distribution of primary particle	M	SEM	Powder or Colloidal	ISO 16700
		TEM	Powder[a] or Colloidal	ISO 10797
2) Zeta potential	V	ELS	Colloidal	ISO 13099-2
3) Specific surface area	m^2/kg	BET	Powder	ISO 9277, ISO 18757
4) Total silver content	kg/kg or mol/mol	ICP-MS	Powder[a] or Colloidal	ISO 17294-1, ISO 17294-2
		ICP-OES	Powder[a] or Colloidal	ISO 11885
		AAS	Powder[a] or Colloidal	ISO 26845

[a] Powder form has to be dispersed in solvent for measurement. Colloidal form can be directly measured.

Table 2 — Additional characteristics to be measured

Characteristics	Units	Measurement method	Application form	Relevant documents
1) Hydrodynamic size	m	DLS	Collodial	ISO 22412
		PTA	Colloidal	ISO 19430
2) Silver nanoparticle number concentration	kg^−1	spICP-MS	Powder[a] or Colloidal	ISO/TS 19590
		SAXS	Powder or Colloidal	Pauw et. al.[17]
[a] Powder form has to be dispersed in solvent for measurement. Colloidal form can be directly measured.				

5.2 Average size and size distribution of primary particles

SEM or TEM shall be used to measure the average size of the primary particle. The reference ISO 16700 and ISO 10797 may be useful concerning the measurement of the average size of the primary AgNP. The primary particles are identified by image processing. Their size may be estimated as an equivalent spherical diameter or as one or a combination of the Feret diameters of the nanoparticles on SEM and TEM images. The average primary particle size and its standard deviation shall be calculated from the distribution of the chosen diameters obtained over the sample.

5.3 Zeta potential

The surface charge of a nanomaterial is one of the key factors determining its stability in a suspension, and is itself a function of the pH and ionic strength of the AgNP solution[18][19]. Depending on the solution's ionic strength, multiple measurements need to be performed to calculate the zeta potential. Ideal samples for zeta potential analysis are monodispersed in size, have sufficiently high concentration to effectively scatter, have low salt concentrations (<1 ms/cm), and are suspended in particulate free media. The surface charge shall be measured using the electrophoreic method, and the pH value shall be reported along with the surface charge. Guidance concerning this method can be found in ISO 13099-2.

5.4 Specific surface area

The surface area shall be measured using the gas adsorption method. A technique based on the model developed by Brunauer, Emmett, and Teller (BET) allows the surface area of a powder to be estimated by measuring the amount of gas that is adsorbed. The BET analysis is the standard method for determining the surface area from nitrogen adsorption isotherms, and was originally derived for multilayer gas adsorption onto flat surfaces. ISO 9277 applies to the measurement of the specific surface area[12]. This standard specifies the measurement procedures for the overall specific external and internal surface areas (diameter > 2 nm) of disperse or porous solids by measuring the amount of physically adsorbed gas according to the BET method. ISO 18757 provides some useful detailed information concerning specific materials. Measurement instruments for the BET method are commercially available. Metrological traceability should be maintained. Reference materials are available for the application of the BET method to nanoparticles in powder form.

5.5 Total silver content

The total silver content is defined as the ratio of the mass of the total silver content to that of the mass of the AgNP product. As standard techniques in elemental analysis, ICP-MS, ICP-OES, or AAS have been utilized to measure the total concentration of dissolved and particulate silver. ICP-MS, ICP-OES, and AAS offer quantitative capabilities owing to the high degree of ionization for most elements. Guidance concerning related methods can be found in ISO 17294-1, ISO 17294-2, ISO 11885, and ISO 26845. The amount and type of acid used to decompose the AgNPs and the conditions of microwave digestion can be modified if necessary.

5.6 Hydrodynamic size

Unlike NPs in powder form, the hydrodynamic size is the characteristic used to determine the particle size for NPs in an aqueous solution. The hydrodynamic size is larger than the core diameter in general, because the hydrodynamic diameter of the particles includes the hydration layer, polymer shells, or other possible stabilizers. The result for the hydrodynamic size is normally larger than the size result determined by TEM by an offset that is a function of the capping agent. The hydrodynamic size of particles shall be measured using DLS or PTA. DLS will give reliable result with a constituent monomer for a non-agglomerated sample. Guidance concerning this method can be found in ISO 22412 and 19430.

5.7 Silver nanoparticle number concentration

spICP-MS is a technique that is able to generate the number-based particle size distribution of nanoparticles and quantify the dissolved fraction of the AgNP suspension. Guidance concerning this method can be found in ISO/TS 19590. SAXS is also applied to measure the AgNP number concentration[17].

6 Sampling

A sample subjected to measurements shall be chosen to be representative of the parent population of the nanoparticles in powder or suspended form. Sampling and dispersion in liquids of powders should be carried out in accordance with ISO 14488 and ISO 14887, respectively.

As many nano-objects are reactive, their physical and chemical properties can be affected by the sampling point and storage environment. Consequently, the producer and end user should agree on the sampling point and storage of samples for the comparability of results.

7 Test report

The test report shall contain at least the following information:

a) all details generally necessary to identify the product tested (product name, chemical name);

b) a reference to this document;

c) sample description;

d) the relationship between sample applied to the measurements and product tested, to which characteristics are assigned;

e) the date of test, name of testing laboratory, and statement on the quality system of testing laboratory;

f) measurement results for the characteristics, with their name and measurement methods as in Table 1 and, if applicable Table 2;

g) any special information supporting the reliability of measurement results.

Report the results of any antibacterial performance testing with documented test procedure, if it is available.