
**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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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 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.

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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	Dynamic 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	Transmission 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	nm	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	Colloidal	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 electrophoretic 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.

Annex A (informative)

Measurement methods for antibacterial silver nanoparticles

[Table A.1](#) gives measurement methods for essential characteristics.

Table A.1 — Measurement methods for essential characteristics

	Characteristic	Method	Guidance
1.1	Average size of primary particle and size distribution	TEM	Transmission electron microscopy (TEM) is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick or a suspension on a grid. An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a charge-coupled device. Which coupled with EDS widely used for elemental analysis and chemical analysis. Guidelines on the application of the method and the sample preparation process can be found in ISO/TS 10797. [Source: ISO/TS 12805, modified]
		SEM	Scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition. The electron beam is scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometre. Specimens can be observed in high vacuum in conventional SEM, or in low vacuum or wet conditions in variable pressure or environmental SEM, and at a wide range of cryogenic or elevated temperatures with specialized instruments. Guidelines on the application of the method and the sample preparation process can be found in ISO 16700 and 10798. [Source: ISO 16700, modified]
1.2	Zeta potential	ELS	Zeta potential is the electrostatic potential at the slipping plane (which marks the region where the liquid molecules surrounding the particle first begin to move with respect to the surface) relative to the potential in the bulk solution. ISO 13099-2 provide methods for measuring electrophoretic mobility using optical means and for calculating zeta potential. [Source: ISO/TS 12805, modified]
1.3	Specific surface area	BET analysis	A technique based on the model developed by Brunauer, Emmet and Teller that allows the surface area of powders to be estimated by the amount of gas that is adsorbed. Typically, nitrogen or carbon dioxide is used, but gases such as krypton or argon may be used for low surface area materials because of their sensitivity (mass gain per unit area). The specific surface area is the ratio of surface area to mass. Guidance on this method can be found in ISO 9277 and ISO 18757. [Source: ISO/TS 12805, modified]

Table A.1 (continued)

	Characteristic	Method	Guidance
1.4	Total silver content	ICP-MS	ICP-MS uses an inductively coupled plasma source to ionize sample materials for analysis by mass spectrometer. ICP-MS provides accurate and quantitative determinations of elemental impurities using ICP-MS. Guidance on ultrasonic spectroscopy methods is available in ISO 17294-1 and ISO 17294-2.
		ICP-OES	ICP-OES is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. It is a flame technique with a flame temperature in a range from 6 000 to 10 000 K. The intensity of this emission is indicative of the concentration of the element within the sample. Guidance on ICP-OES is available in ISO 11885. [Source: ISO 11885, modified]
		AAS	Metals in solution may be readily determined by flame (direct aspiration) atomic absorption spectrophotometry. Guidance on this method can be found in ISO 26845.

Table A.2 gives measurement methods for additional characteristics.

Table A.2 — Measurement methods for additional characteristics

	Characteristic	Method	Guidance
2.1	Hydrodynamic size	DLS	This method measures hydrodynamic diameter from Brownian motion. It is applicable to the measurement of particle diameters greater than 3 nm, depending on the test material. Guidelines on the application of the method can be found in ISO 22412. [Source: ISO/TS 12805, modified]
		PTA	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. The hydrodynamic diameter of the individual particles, droplets or bubbles is related to Brownian motion parameters via the Stokes–Einstein equation. Guideline on the application of the method can be found in ISO 19430. [Source: ISO 19430]
2.2	Silver nanoparticle number concentration	spICP-MS	spICP-MS is a method capable of detecting single nanoparticles at very low concentrations. spICP-MS determines the size of AgNPs in aqueous suspensions. Particle number concentrations that can be determined in aqueous suspensions range from 106 particles/L to 109 particles/L which corresponds to mass concentrations in the range of approximately 1 ng/l to 1 000 ng/l. Actual numbers depend on the type of mass spectrometer used and the type of nanoparticle analysed. In addition to the particle concentrations, ionic concentrations in the suspension can also be determined. Guideline on the application of the method can be found in ISO/TS 19590. [Source: ISO/TS 19590, modified]
		SAXS	SAXS is a method capable of determining NP size distributions, resolving the size and shape of monodisperse NP. Silver nanoparticle number concentration can be determined by SAXS.

Annex B (informative)

Relationship between silver nanoparticle characteristics and antibacterial performance

B.1 General

Silver nanoparticles (AgNPs) are well known for their interesting properties and have become one of the most widely utilized nanomaterials in consumer products to control the growth of microorganisms on the surfaces or interiors of products, owing to their antibacterial properties[22]. They are employed in catalysis, photonics, medical applications, or even energy storage and conversion[1]. The successful utilization and manufacture of AgNPs with improved performances have opened up a world of possibilities in a variety of industries and scientific endeavours. In recent years, AgNPs and other metal nanoparticles have been widely investigated, owing to their unique size, shape, composition, crystallinity, and structure-dependent physicochemical properties[1]. This document provides recommendations concerning the essential and additional characteristics of antibacterial silver nanoparticles to be included in material specifications. The scientific evidence underlying the selection of the aforementioned characteristics is presented in this section.

B.2 Average size and size distribution of primary particle, and specific surface area

In general, size is an important factor that determines the biological effects of nanoparticles, such as cellular uptake, cellular activation, and intercellular distribution[23]-[28]. The antibacterial results for spherical AgNP with sizes of 7, 29, and 89 nm were observed by Martinez-Castanon et al.[29]. The author found that the antibacterial effect of AgNPs decreased with an increasing particle size. The effect of the AgNP size on the antibacterial activity has been shown comprise an increase in the reactivity with a decreasing particle size and increased surface area to volume ratio[30]-[33]. Admirable efforts also have been made to explore this property using electron microscopy, which has revealed the size-dependent interaction of AgNPs with bacteria. Khaydarov et al. reported the enhancement of antibacterial efficacy with smaller AgNPs pertaining to a larger contact surface[34][35]. Monteiro-Riviere et al. reported that the increased agglomeration of carbon-coated AgNPs could also contribute to their decreased antibacterial efficacy compared to washed AgNPs[36].

B.3 Zeta potential

A direct method of specifying the surface charge is through the zeta potential. The zeta potential value can be influenced by the measurement conditions such as the temperature, pH level, sample concentration, and viscosity. Badawy et al. revealed that the difference in the measured zeta potential as a function of the pH for various AgNPs illustrates the importance of capping agents when evaluating the surface charging properties of NPs[37]. The decrease in the magnitude of the zeta potential for uncoated AgNPs with a decreasing pH is most likely the result of the decreasing OH⁻ concentration and not necessarily protonation of the NP surface. Silver atoms at the surface of the NP are coordinately unsaturated[38], so that a nucleophilic molecule (OH⁻ and H₂O) can donate a pair of electrons to these. Therefore, as the pH level increases from 2 to 10 the concentration of OH⁻ increases, thus allowing the OH⁻ to more effectively compete for surface sites. This generates a negative surface charge in alkaline pH environments.

The type of stabilizing mechanism has a profound effect on the aggregation potential of AgNPs. Capping agents are chemicals that are utilized in the synthesis of AgNPs to prevent their aggregation through electrostatic repulsion, steric repulsion, or both. In the case of AgNP, the most prevalent capping agents are citrate and polyvinylpyrrolidone[39]. However, the mechanism and functional groups involved

in colloid stabilization differ between capping agents, which may lead to varying particle size and stability. Capping agents such as ionic liquids, which are normally used to alter the surface charges of nanoparticles, can also influence the bioactivity of AgNPs [40]–[42]. Gholami et al., Chang et al., and Salvioni et al. have reported that AgNPs with positively or negatively charged surfaces kill bacteria at very low concentrations [43]–[45]. Both gram-positive and gram-negative bacterial cell walls have a net negative charge. In gram-positive bacteria, the negative charge is provided by teichoic acids, which are linked to either the peptidoglycan or the underlying plasma membrane. These teichoic acids are anionic, owing to the presence of phosphates within their structures. Gram-negative bacteria have an outer covering consisting of phospholipids and lipopolysaccharides. These lipopolysaccharides confer a strong negative charge onto the surfaces of gram-negative bacteria cells [46]. Recently, the antibacterial activity of various positively or negatively charged AgNPs has been extensively evaluated [47], and the results indicate that positively charged AgNP have higher bactericidal activity against all the tested microorganisms than negatively charged or neutral AgNPs [48]–[49]. This phenomenon is mainly a result of the nonspecific electrostatic interaction between positive and negative charges, which accelerates the antibacterial effect.

B.4 Total silver content and silver nanoparticle number concentration

It is well known that the dissolution rate of silver, as well as the formation of insoluble silver chloride, will affect the antimicrobial properties. Therefore, measurements of the amount of dissolved vs. particulate silver need to be reported to end-users. One technique capable of distinguishing between dissolved and particulate silver is spICP-MS. ICP-MS, OES, and AAS can be utilized to quantify the total amount of silver in a sample.

Sotiriou et al. have argued that silver particles release silver ions faster, leading to a higher toxicity resulting from a higher effective total silver concentration [50]. They observed an antibacterial effect at a silver concentration between 1 and 30 ppm, depending on the AgNP silver content and size. When silver nanoparticles interact with microorganisms silver ions are released, and these ions may affect and damage the microorganisms in various ways [1]. Silver ions released by AgNPs are likely to interact with chloride, which is often present in bacterial growth media and exhibits a strong affinity for oxidized silver [51]. High concentrations of chloride ions in routinely used media can cause the precipitation of Ag ions as AgCl, thus masking the contribution of dissolved silver to the antibacterial effect of AgNPs. In a biological system, the mechanism of the antibacterial effect is not well understood. However, there have been several possible mechanisms proposed in scientific references: 1) silver ions with positive electricity released from silver nanoparticles are able to rapidly bind to sulfhydryl groups on the surface of bacteria, which leads to the structures of bacteria becoming changed and damaged, 2) the uptake of silver ions or small nanoparticles disrupts adenosine tri-phosphate (ATP) production and deoxyribonucleic acid (DNA) replication, and 3) silver nanoparticles and ions generate reactive oxygen species (ROS), resulting in a series of oxidative damages, and so on [2]–[4].

B.5 Hydrodynamic size

Multiangle DLS is measurement method for characterizing the hydrodynamic radii of particles, as well as aggregation and particle–particle interactions. These ions and other associated molecules make a particle appear larger to the instrument in comparison to TEM. Hence, the hydrodynamic diameter is always greater than the size estimated by TEM. Nevertheless, many studies have emphasized importance of the hydrodynamic diameter for understanding and optimizing the sizes of nanoparticles and their performance in biological assays.

Annex C (informative)

Antibacterial performance test

A number of approaches may be utilized to determine the antibacterial activity of silver nanoparticle formulations. In particular, the requirements outlined by national pharmacopeia or regulatory bodies are consulted and followed if appropriate. In particular, jurisdictions where the material may ultimately be sold should be considered.

The antibacterial performance of silver nanoparticles has not been standardized. Moreover, performance tests are not within the scope of this document. Relevant antibacterial performance tests include ISO/TS 16550 and performance standards for antimicrobial disk and dilution susceptibility test: M2-A9 from the Clinical and Laboratory Standards Institute^[53].

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