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Nanotechnologies — Nano-object-assembled layers for electrochemical bio-sensing applications — Specification of characteristics and measurement methods

Nanotechnologies — Couches nanostructurées pour des applications de biodétection électrochimique — 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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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

Electrochemical electrodes can exhibit nano-enhanced performance after the deposition of nano-objects on the electrode surface. The increased surface area, orientation, the assembled density and ability to control the bio-receptor of the nano-object layer improves the performance of nano-biosensors. Nano-biosensor sensitivity, selectivity and reliability can be enhanced with specific nano-objects, e.g. gold nanoparticles[22][25][26], carbon nanotubes[24], CuS_2 nanorods[37] and silver[38] or palladium nanoplates[23].

Currently, most of the nano-enhanced electrochemical electrodes are fabricated by researchers in order to achieve predictable performance in their own programs without mass-production. However, the technology is maturing into a commercial phase. Fabricators are offering nano-enhanced electrodes to instrument manufacturers as a platform to add additional coatings for specific sensing applications. This document supports the development of material specifications for the transaction between electrode fabrications and instrument manufacturers to allow the purchase of electrodes with predictable performance.

This document is intended to help address this issue. It is also relevant to the process of qualification, specification and use of nano-object-modified electrodes. The standardization of protocols to specify various types of nano-object-modified electrodes related to electrochemical detection will be used by most manufacturers or business owners of electrochemical electrodes products. This document focuses on the nano-object-assembled layer on electrodes by means of a solution process for electrochemical applications.

In this document, the specifications for a nano-object constituting an assembled layer are provided, based on ISO/TS 12805, which describes the characteristics of manufactured nano-objects and their measurement methods (see Annex A). In addition, the characteristics of nano-object-assembled layer for enhanced electrochemical bio-sensing applications and their measurement methods are provided in detail.

Nanotechnologies — Nano-object-assembled layers for electrochemical bio-sensing applications — Specification of characteristics and measurement methods

1 Scope

This document specifies the characteristics to be measured of nano-object-assembled layers on electrodes by means of a solution process and of nano-objects constituting the layers for electrochemical applications such as nano-biosensor or diagnosis applications. It also provides measurement methods for determining the characteristics.

It does not apply to:

— the requirements of nanostructures by top-down nanomanufacturing;

— the subsequent coating of materials such as biomaterials onto nano-object-assembled layers;

— specific health and safety requirements during manufacturing;

— the experimental conditions of electrochemical sensing;

— the packaging, labelling, expiratory dates and transport of nano-object-enhanced electrochemical electrodes.

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.

ISO/TS 80004-2:2015, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-4:2011, *Nanotechnologies — Vocabulary — Part 4: Nanostructured materials*

ISO/TS 80004-8:2013, *Nanotechnologies — Vocabulary — Part 8: Nanomanufacturing processes*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-2, ISO/TS 80004-4, ISO/TS 80004-8 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
nanoscale
size range from approximately 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.

Note 2 to entry: The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as *nano-objects* (3.2) or elements of nanostructures, which might be implied by the absence of a lower limit.

[SOURCE: ISO/TS 80004-4:2011, 2.1]

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

Note 1 to entry: Generic term for all discrete nanoscale objects.

[SOURCE: ISO/TS 80004-4:2011, 2.2]

3.3
particle
minute piece of matter with defined physical boundaries

[SOURCE: ISO/TS 80004-2:2015, 3.1, modified — The notes to entry have been deleted.]

3.4
agglomerate
collection of weakly bound *particles* (3.3), *aggregates* (3.5) or a mixture 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 80004-2:2015, 3.4, modified — "weakly bound particles, aggregates or a mixture of the two" has replaced "weakly or medium strongly bound particles".]

3.5
aggregate
particle (3.3) 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 or ionic 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.

[SOURCE: ISO/TS 80004-2:2015, 3.5]

3.6
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* (3.7) or *nanoplate* (3.8) are intended to be used instead of the term nanoparticle.

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

3.7
nanofibre
nano-object (3.2) with two similar external dimensions in the *nanoscale* (3.1) and the third dimension significantly larger

Note 1 to entry: A nanofibre can be flexible or rigid.

Note 2 to entry: The two similar external dimensions are considered to differ in size by less than three times and the significantly larger external dimension is considered to differ from the other two by more than three times.

Note 3 to entry: The largest external dimension is not necessarily in the nanoscale.

[SOURCE: ISO/TS 80004-4:2011, 2.5, modified — The original note has been deleted and Notes 1, 2 and 3 to entry have been added.]

3.8
nanoplate
nano-object (3.2) with one external dimension in the *nanoscale* (3.1) and the two other dimensions significantly larger

Note 1 to entry: The smallest external dimension is the thickness of the nanoplate.

Note 2 to entry: The two significantly larger dimensions are considered to differ from the nanoscale dimension by more than three times.

Note 3 to entry: The larger external dimensions are not necessarily in the nanoscale.

[SOURCE: ISO/TS 80004-4:2011, 2.6]

3.9
top-down nanomanufacturing
processes that create structures at the *nanoscale* (3.1) from macroscopic objects

[SOURCE: ISO/TS 80004-8:2013, 3.13]

4 Characteristics and measurement methods

4.1 General

This clause describes the characteristics of nano-object-assembled layers and constituting nano-objects on flat substrate electrodes for electrochemical application. Because electrochemical biosensing requires an efficient electron transfer and a stable immobilization of biomolecules retaining their bioactivity, the characteristics given in 4.2 are selected for constituting nano-objects if they logically and/or experimentally affect the high electron conducting pathway, high surface energy, high binding-site density and high functioning ability of the nano-object[25] to [30]. From the perspective of the assembled layer, nano-objects may not be evenly assembled on a flat substrate electrode but produce surface topography throughout the whole area of the substrate. Because electrochemical biosensing requires an electrochemically active surface and a robust conducting layer, the characteristics in 4.3, such as mass per unit area and root mean square height, are selected to describe how thick and evenly the assembled layer is formed.

4.2 Characteristics of constituting nano-objects

The characteristics given in Table 1 shall be measured to describe the raw materials of nano-objects constituting the nano-object-assembled layer. Measurement methods for the individual characteristics in Table 1 are described in Annex B. The measured value of the characteristics in Table 1 may be adopted from the material specifications by the provider of the nano-object in suspension form if the intrinsic dimensional characteristics are unchanged after assembling.

Table 1 — Characteristics required to describe constituting nano-objects

Characteristic	Nanoparticles	Nanofibres	Nanoplates	Units	Measurement method[a]
Surface chemical composition	Yes	Yes	Yes	[b]	See 2.3 in Table B.2
Mean size[c] and size distribution[d]	Yes	Yes	Yes	nm	For nanoparticles, see 1.1 in Table B.1 and 2.1 in Table B.2 For nanofibres and nanoplates, see 2.4 in Table B.2
Mean primary crystallite size	Yes (if crystalline)	Not applicable	Yes (if crystalline)	nm	See 1.2 in Table B.1 and 2.2 in Table B.2
Mean length and length distribution[d]	Not applicable	Yes[e]	Not applicable	nm	See 2.5 in Table B.2
Number of walls, i.e. single, double or multi-walled	Not applicable	Yes (if nanotubes)	Not applicable	N/A	See 2.6 in Table B.2
Surface functional group[f]	Yes	Yes	Yes	N/A	See 2.7 in Table B.2

[a] The measurement is conducted on the powder sample or the suspension sample depending on the characteristics.

[b] Surface chemical composition is the elemental composition and usually expressed as atomic percent.

[c] Mean size is mean particle size for nanoparticles or mean diameter for nanofibres and nanoplates.

[d] Size distribution is provided in histogram, percentile plot or standard deviation.

[e] This characteristic is exceptionally not applicable to any bundled, branched and/or entangled nanofibres

[f] The type of this characteristic is required while the content is not required.

4.3 Characteristics of nano-object-assembled layer

4.3.1 General

The characteristics given in Table 2 shall be measured to describe a nano-object-assembled layer for electrochemical application. Measurement methods for the individual characteristics in Table 2 are described in Annexes B, C and D.

Table 2 — Characteristics required to describe a nano-object-assembled layer

Characteristic	Units	Measurement method
Mass per unit area	mg/cm^2	Calculation (if drop casted, see Annex C) Weighing (for other deposition methods)
Root mean square height	µm	See 2.8 in Table B.2
Specific electrochemically active surface area (ECSA)	cm^2/g	Cyclic voltammetry See Annex D

4.3.2 Mass per unit area

Generally, the mass of the coated or adhered layer can be determined by one of two methods if the layer is thick enough:

a) weighing the test specimen before and after dissolving the assembled layer and taking the difference;

b) dissolving the substrate and weighing the assembled layer directly.

NOTE Guidance on the terms, definitions and determination of mass per unit area is given in ISO 10111.

However, in case of a nano-object-assembled layer where the amount of adhered nano-objects on flat substrate by solution-based deposition process is too small, determining the mass per unit area by using the weighing methods described above may be not appropriate because the weighing error becomes similar to the mass of the layer itself. To address this problem, the calculation of the mass per unit area by the drop-casting method has been widely used as an alternative method. In this calculation method, the mass per unit area of nano-object-assembled layer can be determined by dividing the loaded mass of nano-objects on the electrode by the area size of the substrate electrode. The detailed calculation procedure of mass per unit area is described in Annex C.

As many nano-objects are reactive, their chemical properties can be affected by the sampling point and their storage environment. However, their physical properties, such as mass, are very unlikely to vary during the solution-based deposition process, thus this theoretical approach using the drop-casting method is applicable and advantageous to avoid troublesome weighing.

4.3.3 Root mean square height

Nano-object-assembled layers by the solution-based deposition process may not yield a flat topographic surface in macroscopic millimetre range, even if the nano-object-assembled layer still looks flat in the microscopic range. In this case, the uneven surface of the assembled layer can affect the electrochemical performance, especially near the edge of the electrode. The major cause of uneven topography is the strong surface tension of solvent, which drags and piles nano-objects during drying. As a result, although the nano-object-assembled layers have the same mass per unit area, their electrochemical performance can be very different from one another if the loaded nano-objects are assembled on a partial part of electrode; in the worst cases, revealing the substrate electrode.

Unevenness of the nano-object-assembled layer can be assessed using surface roughness characteristics such as root mean square height (S_q), maximum height of surface (S_z) and arithmetical mean height (S_a). Among these parameters, root mean square height is the most widely used in various industries. Therefore, low root mean square height can convince users to consider that the nano-objects are evenly assembled layer on the entire surface area of electrode.

NOTE Guidance on the terms, definitions and root mean square height is given in ISO 25178-2.

The measurement of the root mean square height using an atomic force microscope is not adequate for an uneven surface area of the nano-object-assembled layer because the maximum scan area and the Z range of the piezo-scanner do not exceed the scale of uneven topography. Surface area metrologies such as a contact profiler and optical profiler should be used to measure the root mean square height of the nano-object-assembled layer. If a contact profiler is selected, care should be taken to put an adequate load so as not to damage the assembled layer on the electrode surface in order to guarantee the integrity of the electrode surface.

4.3.4 Specific electrochemically active surface area (ECSA)

ECSA specifies the order of electrochemical enhancement by nano-object-modified electrochemical electrodes. Electrochemical enhancement is generally accomplished by an enlarged surface area of electrodes. Unlike a flat surface area of pristine electrodes, however, a whole enlarged surface area of the nano-object-assembled layer does not point to electrochemical enhancement because some of the enlarged surface area is not active for electrochemical reaction. Therefore, electrochemical enhancement cannot be specified by a specific surface area, but it can be assessed by ECSA, which is widely used in the electrochemical industry[31] to [36].

Electrochemical methods for determining ECSA fall into two general categories:

— the first type uses a surface-limited adsorption of gas;

— the second type uses a well-characterized and reversible redox reaction such as the reduction of ferricyanide $\left[Fe(CN)_6\right]^{3-}$ to ferrocyanide $\left[Fe(CN)_6\right]^{4-}$.

As the method using surface-limited adsorption is not suitable for small mass of nano-object on electrode, ECSA should be measured by the cyclic voltammetry method using a redox reaction to get a precise and accurate measured value. In the redox reaction, ECSA is determined by the calculation of Randles-Sevcik equation after obtaining a cyclic voltammogram using a redox couple in aqueous solution. The measurement procedure of a specific ECSA of a nano-object-assembled layer is described in Annex D.

5 Test report

5.1 General

A user prefers specific synthetic properties of nano-object-assembled layer according to their bio-sensing application and experimental conditions. To meet the preference of users, besides the characteristics of the nano-object-assembled layer, additional specifications are required to provide the information about the synthetic procedure of the nano-object-assembled layer such as substrate type and deposition process. All the characteristics and relevant information of the nano-object-assembled layer shall be concisely arranged for effective delivery to stakeholders. This clause describes the formation and the order of specifications. An example of a test report is given in Annex F. The test report does not require to use the tables but may list the items in three groups.

5.2 General information on nano-object-modified electrode

General information on the nano-object-modified electrode given in Table 3 shall be provided to describe the synthetic procedure of the nano-object-assembled layer in the first of all tables. This is because the characteristics of the nano-object-assembled layer could become meaningless when they are not matched to the preferences of the user's application.

Table 3 — General information on nano-object-modified electrode

Item	Information
Name of substrate material of electrochemical electrode	See Table E.1
Material name and type of constituting nano-objects	Nominal description (e.g. gold nanoparticles)
Name of deposition process	See Table E.1
Name of adhesion method	See Table E.1

5.3 Measurement results of characteristics

In accordance with Clause 4, relevant characteristics are categorized into two tables. The characteristics of nano-objects given in Table 4 shall be listed if the characteristics are applicable, next to Table 3. Measurement methods for the individual characteristics in Table 4 are described in Annex B.

Characteristics of the nano-object-assembled layer given in Table 5 shall be provided, next to Table 4. Measurement methods for the individual characteristics in Table 5 are described in Annexes B, C and D.

Table 4 — Measurement results of characteristics for constituting nano-objects

Characteristic	Measurement method	Measurement result[a]
Surface chemical composition	Method name	Quantity value (graphical data may be added)
Mean size and size distribution	Method name	Quantity value (graphical data may be added)
Mean primary crystalline particle size	Method name	Quantity value
Mean length and length distribution	Method name	Quantity value
Number of walls, i.e. single, double or multi-walled	Method name	Number
Surface functional group	Method name	Nominal functional type

[a] The measurement result shall be given with the uncertainty of quantity value, the date of the test and name of the testing laboratory.

Table 5 — Measurement results of characteristics for nano-object-assembled layer

Characteristic	Measurement method	Measurement result[a]
Mass per unit area	Method name	Quantity value
Root mean square height	Method name	Quantity value
ECSA	Method name	Quantity value

[a] The measurement result shall be given with the uncertainty of quantity value, the date of the test and name of testing laboratory.

Annex A
(informative)

Value chain of nano-object-modified electrochemical electrode

In the value chain of a nano-object-modified electrochemical electrode, the buyers are the instrument manufacturers and researchers who use the electrode for nano-enhanced electrochemical sensing of biomolecules, and the sellers are the manufacturers who produce nano-object-modified electrochemical electrodes. Currently, most of the nano-enhanced electrochemical electrodes are fabricated by researchers in order to achieve predictable performance in their own programs without mass-production. However, the technology is maturing into a commercial phase. To manufacture nano-object-assembled layers on flat electrodes by means of solution processes, the sellers purchase nano-objects as raw materials from a nano-object provider and measure the characteristics of nano-objects given in Table 1. After the assembling of a constituting nano-object, the characteristics of the nano-object-assembled layer given in Table 2 are measured for quality control. The sellers report the measurement results in accordance with Clause 5 and provide a specification sheet as a test report with the products to the buyers.

This sequence is represented in the flow diagram given in Figure A.1.

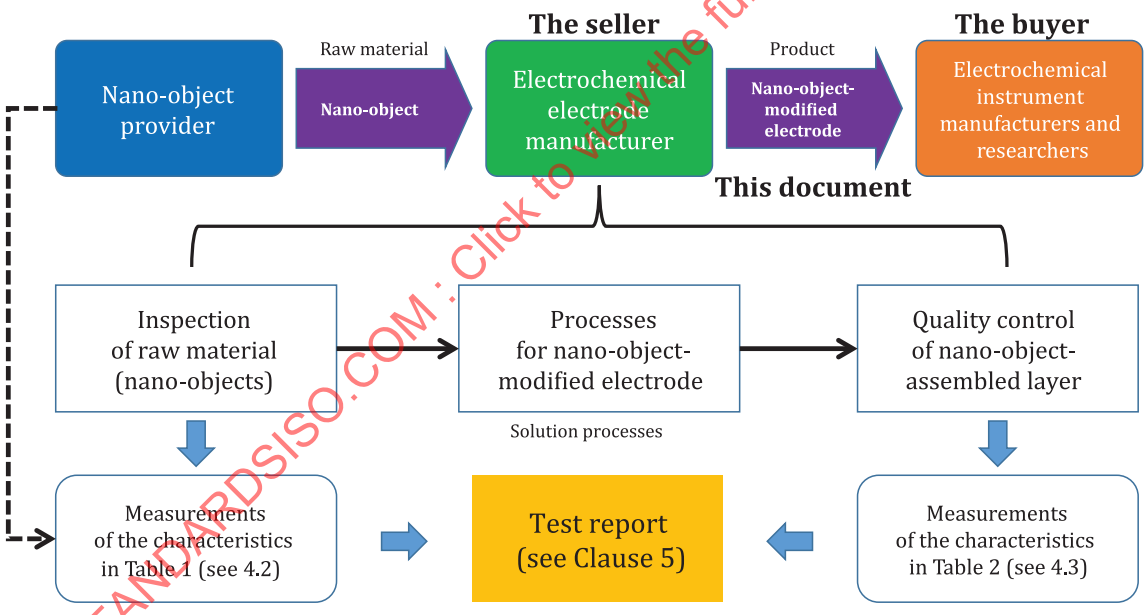

Figure A.1 — Value chain of a nano-object-modified electrochemical electrode and the test report processes

Annex B
(informative)

Measurement methods for characteristics

Table B.1 — Measurement methods for use in routine quality control in an industrial environment

No.	Characteristic	Method	Guidance
1.1	Mean size and size distribution	—	For general guidance, see ISO/TS 12805.
		Dynamic light scattering (DLS)	For a concise description of this method, see ISO/TS 12805. Calibration of the equipment and measurement procedure should be in accordance with ISO 22412.
		Laser diffraction spectroscopy	For a concise description of this method, see ISO/TS 12805. The lateral diameter of nanoplates are usually measured by laser diffraction method because it can be larger than 100 nm and normally micrometre order.
		Electrokinetic sonic amplitude testing	For a concise description of this method, see ISO/TS 12805. Guidance can be found in ISO 13099-1.
1.2	Mean primary crystallite size	X-ray diffraction line broadening (XRDLB)	For a concise description of this method, see ISO/TS 12805. Guidance can be found in EN 13925-1, EN 13925-2 and EN 13925-3.

Table B.2 — Measurement methods for additional assessment purposes

No.	Characteristic	Method	Guidance
2.1	Mean size and size distribution (for nanoparticles)	—	For general guidance, see ISO/TS 12805.
		Condensation particle counter	For a concise description of this method, see ISO/TS 12805.
		Scanning mobility particle sizer	For a concise description of this method, see ISO/TS 12805.
		Electron microscopy and image analysis	This technique can be used in dried form of nano-objects, which are likely to agglomerate, resulting in difficulty to measure the particle size. Guidelines for the calibration of image magnification can be found in ISO 16700. Guidance on static image analysis methods is available in ISO 13322-1.
2.2	Mean primary crystallite size	Electron backscatter diffraction (EBSD)	For a concise description of this method, see ISO/TS 12805.
		Transmission electron microscope diffraction	For a concise description of this method, see ISO/TS 12805.

Table B.2 *(continued)*

No.	Characteristic	Method	Guidance
2.3	Surface chemical composition	—	For general guidance, see ISO/TS 12805.
		Auger electron spectroscopy (AES) and scanning auger microscopy (SAM)	For a concise description of this method, see ISO/TS 12805. Guidance on AES can be found in ISO/TR 14187 and ISO 20903.
		Electron energy loss spectroscopy (EELS)	For a concise description of this method, see ISO/TS 12805.
		Ion beam analysis (IBA)	For a concise description of this method, see ISO/TS 12805.
		Secondary ion mass spectrometry (SIMS)	A method in which a mass spectrometer is used to measure the mass-to-charge ratio and abundance of secondary ions emitted from a sample as a result of sputtering by energetic ions. Guidance on the method can be found in ISO/TR 14187.
		Dynamic secondary ion mass spectrometry (D-SIMS)	For a concise description of this method, see ISO/TS 12805.
		Ultraviolet photoelectron spectroscopy (UPS)	For a concise description of this method, see ISO/TS 12805.
		X-ray photoelectron spectroscopy (XPS)	For a concise description of this method, see ISO/TS 12805. Guidance on the method can be found in ISO 20903.
2.4	Mean size and size distribution (for nanofibres and nanoplates)	Scanning electron microscopy	This technique can be used to measure the diameter of individual nanofibres or nanoplates.
		Raman spectroscopy (for nanotubes)	For a concise description of this method, see ISO/TS 12805.
2.5	Mean length and length distribution	Scanning electron microscopy	For a concise description of this method, see ISO/TS 12805. Guidelines for the calibration of image magnification can be found in ISO 16700.
2.6	Number of walls	Transmission electron microscopy	Number of walls can be measured by imaging cross sections of nanotubes.
2.7	Surface functional group	—	Surface functional group is the group of atoms in a compound, such as the hydroxyl group in an alcohol, which determines the chemical behaviour of the compound. Surface functional group of nano-objects determines how to interact with biomaterials in bio-sensing processes.
		UV-visible spectroscopy	Absorption of this relatively high-energy light causes electronic excitation. The easily accessible part of this region (wavelengths of 200 nm to 800 nm) shows absorption only if conjugated pi-electron systems are present.
		Nuclear magnetic resonance (NMR)	Absorption in the low-energy radio-frequency part of the spectrum causes excitation of nuclear spin states. NMR spectrometers are tuned to certain nuclei (e.g. ^{1}H, ^{13}C, ^{19}F and ^{31}P). For a given type of nucleus, high-resolution spectroscopy distinguishes and counts atoms in different locations in the molecule.

Table B.2 *(continued)*

No.	Characteristic	Method	Guidance
		Fourier transform infrared spectroscopy (FT-IR)	Absorption of this lower energy radiation causes vibrational and rotational excitation of groups of atoms within the molecule. Because of their characteristic absorptions identification of functional groups is easily accomplished. 4 000 cm^{-1} to 1 500 cm^{-1}: Peaks in this region are characteristic of specific kinds of bonds, and therefore can be used to identify whether a specific functional group is present. 1 500 cm^{-1} to 400 cm^{-1}: Peaks in this region arise from complex deformations of the molecule. They may be characteristic of molecular symmetry, or combination bands arising from multiple bonds deforming simultaneously. FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. The term "Fourier transform infrared spectroscopy" originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum.
2.8	Root mean square height	Contact (stylus) profiler	This method is the most common surface measurement technique. The advantages are that it is a cheap instrument and has higher lateral resolution than optical techniques, depending on the stylus tip radius chosen. However, the disadvantages are that the stylus tip has to be in physical contact with the surface, which may alter the surface and/or stylus and cause contamination. Furthermore, due to the mechanical interaction, the scan speeds are significantly slower than with optical methods. Guidance on contact profilers is given in ISO 25178-601.
		Confocal chromatic probe	This method has the advantage of measuring certain height ranges without a vertical scan, can measure very rough surfaces with ease, and smooth surfaces down to the single nm range. The fact that these sensors have no moving parts allows for very high scan speeds and makes them very repeatable. Guidance on confocal chromatic probe is given in ISO 25178-602.
		Phase-shifting interferometric microscopy	This method is for areal surface characterization that relies on digitisation of interference data acquired during a controlled phase shift, most often introduced by controlled mechanical oscillation of an interference objective. Guidance on focus variation microscopy is given in ISO 25178-603.
		Coherence scanning interferometric microscopy	This method refers to a class of optical surface measurement methods wherein the localization of interference fringes during a scan of optical path length provides a means to determine surface characteristics such as topography, transparent film structure and optical properties. This method is currently the most common interference microscopy technique for areal surface topography measurement. Guidance on coherence scanning interferometry is given in ISO 25178-604.
		Point autofocus probe	This method is a non-contact surface texture measuring that consists of an autofocus microscope and a high precision x-y scanning stage. Guidance on point autofocus profiling is given in ISO 25178-605.

Table B.2 *(continued)*

No.	Characteristic	Method	Guidance
		Focus variation microscopy	This method delivers colour information, can measure on steep flanks and can measure on very rough surfaces. The disadvantage is that this method cannot measure on surfaces with a very smooth surface roughness like a silicon wafer. The main application is metal (machined parts and tools), plastic or paper samples. Guidance on focus variation microscopy is given in ISO 25178-606.
		Confocal microscopy	A confocal microscope system provides measurement of the variation in height of the sample (z-axis) as it is moved across the detection plane using a xy translation stage. Such a system uses point illumination and a pinhole in an optically conjugate plane in front of the detector to eliminate out-of-focus information. The resolution of the measurements is < 1 µm in the x- and y-axes and < 10 nm in the z-axis. Hence, microfeatures and topographic variations can be monitored in detail. Sample sizes from a few mm^2 to 100 mm × 100 mm can be measured. Guidance on confocal microscopy is given in ISO 25178-607.

Annex C
(informative)

Calculation of mass per unit area using drop-casting method

C.1 Preparation of nano-object suspension and substrate electrode

Many nano-object preparations require the dispersion of powders in liquids. Sample preparation involving dispersion in liquids should be carried out following the guidance given in ISO 14887. Surface cleaning of substrate electrode should be also carried out following the guidance given in ISO 27831-2.

C.2 Preparation of drop-casting test

First, a purified powder of the nano-object is well dispersed in deionized water or proper volatile solvent with additives. Some of the suspension is dropped on the whole surface of the pre-cleaned substrate electrode in a well-defined disk shape, but does not exceed over exposed surface. The adhesion step is carried out by drying, resulting in the assembling of the nano-object on the substrate electrode.

C.3 Determination of mass per unit area of nano-object-assembled layer

The mass of dispersed nano-objects per volume of suspension, m_v, is calculated using Formula (C.1):

$$m_v = \frac{m_d}{V_t} \tag{C.1}$$

where

m_d is the mass of dispersed nano-objects in suspension, in mg;

V_t is the total volume of suspension, in cm^3.

Calculate the mass per unit area of assembled layer, m_c, using Formula (C.2):

$$m_c = \frac{m_v \times V_d}{A} \tag{C.2}$$

where

m_c is the mass per unit area, in mg/cm^2;

m_v is the mass of dispersed nano-objects per volume of suspension, in mg/cm^3;

V_d is the dropped volume of suspension, in cm^3;

A is the surface area of substrate electrode, in cm^2.

Annex D
(informative)

Measurement of specific ECSA of nano-object-assembled layer

D.1 Preparation of test specimen

The test specimen is the nano-object-assembled layer on a substrate electrode. The ferrocyanide/ferricyanide redox couple in aqueous solution is prepared by dissolving 1 mM potassium ferrocyanide $K_4\left[Fe(CN)_6\right]$ and potassium ferricyanide $K_3\left[Fe(CN)_6\right]$ in 0,1 M KCl solution.

D.2 Procedure of electrochemical test

The electrochemical test to determine the ECSA is carried out in cyclic voltammetry using a potentiostat with a cell fitted with three electrodes in aqueous ferrocyanide/ferricyanide redox solution. The reference electrode is a silver chloride (Ag/AgCl) electrode, the counter electrode is a platinum wire, and the working electrode is the prepared specimen. The potential of working electrode ramps linearly versus time in cyclical phases from 0 V to 1 V. The rate of voltage change over time during each of these phases is known as the scan rate (V/s), which is strongly recommended to be set less than 0,1 V/s. Data acquisition for the calculation of ECSA should be taken after obtaining a robust voltammogram. The mass of the nano-object is calculated by multiplying mass per unit area from Annex C by known size of electrode.

D.3 Determination of ECSA

Calculate the specific EASA (A_{ec}) by using the Randles-Sevcik equation[31] as shown by Formula (D.1):

$$A_{ec} = \frac{I_p}{m \times 0,446\,3nFC\left(\frac{nF\upsilon D}{RT}\right)^{\frac{1}{2}}} \tag{D.1}$$

where

A_{ec} is the specific EASA, in cm^2/g;

I_p is the peak current from cyclic voltammogram, in C/s;

m is the mass of nano-object on the known-size of electrode, in g;

n is the number of electrons transferred in the redox event (1 for ferrocene/ferrocenium redox couple);

F is the Faraday constant, in C/mol;

C is the bulk concentration of ferrocene/ferrocenium couple, in mol/cm^3;

υ is the scan rate of cyclic voltammetry, in V/s;

D is the diffusion constant of ferrocene/ferrocenium redox coupl,e in cm^2/s;

R is the gas constant, in $JK^{-1}mol^{-1}$;

T is the temperature, in K.

Annex E
(informative)

General information on nano-object-modified electrode

Table E.1 — General information on nano-object-modified electrode

Subclause	Item	Information	Guidance
3.1	Substrate of electrochemical electrode	—	Any conducting materials can be used for substrates of electrochemical electrodes. However, the widely used types of substrate in electrochemical industries are selectively listed.
		Glass-like carbon electrode	Glass-like carbon is a non-graphitizing, or non-graphitizable, carbon that combines glassy and ceramic properties with those of graphite. The most important properties are high temperature resistance, hardness, low density, low electrical resistance, low friction, low thermal resistance, extreme resistance to chemical attack and impermeability to gases and liquids. Glassy carbon is widely used as an electrode material in electrochemistry.
		Noble metal electrode	Noble metals are metals that are resistant to corrosion and oxidation in moist air. The short list of chemically noble metals comprises ruthenium (Ru), rhodium (Rh), palladium (Pd), silver (Ag), osmium (Os), iridium (Ir), platinum (Pt) and gold (Au). Platinum and gold are the most widely used substrates in bio-sensing applications.
		Indium-tin oxide (ITO) electrodes	Indium tin oxide (ITO) is a ternary composition of indium, tin and oxygen in varying proportions. Depending on the oxygen content, it can either be described as a ceramic or alloy. ITO is one of the most widely used transparent conducting oxides because of its two main properties: its electrical conductivity and optical transparency, as well as the ease with which it can be deposited as a thin film.
3.2	Deposition process	—	There are plentiful methods to synthesize a nano-object-assembled layer, However, the solution-based methods that have been widely used in the modification of electrochemical electrodes are selectively listed. Classification of the nanomanufacturing process is given in ISO/TS 80004-8.

Table E.1 *(continued)*

Subclause	Item	Information	Guidance
		Drop-casting	For small substrates (~1 cm^2), an easy and tunable deposition method is drop-casting: spreading a nano-object dispersion over a substrate and allowing it to dry under controlled conditions, i.e. pressure and temperature. In principle, film thickness depends on the volume of dispersion used and the nano-object concentration, both of which can be easily varied. There are also other variables that affect the assembled layer structure, such as how well the solvent wets the substrate, evaporation rate, capillary forces associated with drying, etc. Generally, it is desirable to use solvents that are volatile, wet the substrate and are not susceptible to thin film instabilities (de-wetting). Water tends to be a poor solvent for drop-casting due to the low vapor pressure and large surface tension. In some cases, alcohols can be in place of water, while organic solvents (such as hexane, toluene or halogenated solvents) are often very good choices for nanoparticles with hydrophobic capping ligands. One drawback of drop-casting is that even under near ideal conditions, differences in evaporation rates across the substrate or concentration fluctuations can lead to variations in film thickness or internal structure. However, drop-casting does serve a quick and accessible method to generating thin films on relatively small substrates.
		Spin-coating	Spin-coating often provides more uniform layer thicknesses across the substrate compared with drop-casting, and with the right equipment can accommodate much larger substrates. In this technique, a substrate is spun at high RPM and a volume of material with known nano-object concentration is introduced into the centre. Centrifugal force leads to uniform spreading of the dispersion across the substrate, followed by evaporation of solvent to yield a nano-object-assembled layer. Layer thickness depends on the dispersion concentration, volume, and the rotational velocity. As with drop-casting, solvents other than water are favoured.
		Dip-coating	Slowly withdrawing a substrate from a nano-object dispersion causes nano-objects to be drawn into the meniscus and deposited as the thin liquid layer dries. This technique has been used to produce very uniform, close-packed particle films, but does have a number of interrelated variables to be tuned to produce good films. The substrate pull rate, nano-object concentration in the solution, and surface tension between substrate and solution are all important. The literature details specific methods for the fabrication of monolayer and multilayer films, as well as more complex geometries such as uniformly spaced stripes of nano-objects on a substrate.