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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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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](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

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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](http://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](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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](http://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](http://www.iso.org/members.html).

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## 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<sup>[22][25][26]</sup>, carbon nanotubes<sup>[24]</sup>, CuS<sub>2</sub> nanorods<sup>[37]</sup> and silver<sup>[38]</sup> or palladium nanoplates<sup>[23]</sup>.

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.

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# 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, expiry 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 <sup>a</sup>
Surface chemical composition	Yes	Yes	Yes	<sup>b</sup>	See 2.3 in <a href="#">Table B.2</a>
Mean size <sup>c</sup> and size distribution <sup>d</sup>	Yes	Yes	Yes	nm	For nanoparticles, see 1.1 in <a href="#">Table B.1</a> and 2.1 in <a href="#">Table B.2</a>  For nanofibres and nanoplates, see 2.4 in <a href="#">Table B.2</a>
Mean primary crystallite size	Yes (if crystalline)	Not applicable	Yes (if crystalline)	nm	See 1.2 in <a href="#">Table B.1</a> and 2.2 in <a href="#">Table B.2</a>
Mean length and length distribution <sup>d</sup>	Not applicable	Yes <sup>e</sup>	Not applicable	nm	See 2.5 in <a href="#">Table B.2</a>
Number of walls, i.e. single, double or multi-walled	Not applicable	Yes (if nanotubes)	Not applicable	N/A	See 2.6 in <a href="#">Table B.2</a>
Surface functional group <sup>f</sup>	Yes	Yes	Yes	N/A	See 2.7 in <a href="#">Table B.2</a>
<sup>a</sup> The measurement is conducted on the powder sample or the suspension sample depending on the characteristics. <sup>b</sup> Surface chemical composition is the elemental composition and usually expressed as atomic percent. <sup>c</sup> Mean size is mean particle size for nanoparticles or mean diameter for nanofibres and nanoplates. <sup>d</sup> Size distribution is provided in histogram, percentile plot or standard deviation. <sup>e</sup> This characteristic is exceptionally not applicable to any bundled, branched and/or entangled nanofibres <sup>f</sup> 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 <sup>2</sup>	Calculation (if drop casted, see <a href="#">Annex C</a> ) Weighing (for other deposition methods)
Root mean square height	µm	See 2.8 in <a href="#">Table B.2</a>
Specific electrochemically active surface area (ECSA)	cm <sup>2</sup> /g	Cyclic voltammetry See <a href="#">Annex D</a>

#### 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:

- weighing the test specimen before and after dissolving the assembled layer and taking the difference;
- dissolving the substrate and weighing the assembled layer directly.