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Nanotechnologies — Testing the photocatalytic activity of nanoparticles for NADH oxidation

Nanotechnologies — Test de l'activité photocatalytique des nanoparticules pour l'oxydation du NADH

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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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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.

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Introduction

Photocatalytic activity (PCA) is the measure of capacity of a material to promote a specific photochemical reaction under defined conditions (as defined in ISO 20507:2014, 2.3.31). With the expanding use of nanomaterials in various industries, the possible impacts on human health and the environment due to the enhancement of detrimental chemical reactions in the presence of light (both natural and artificial) is an ongoing concern. The absorption of a photon with sufficient energy generates an electron-hole pair that can migrate to the nanoparticle (NP) surface and react with water and oxygen, thus forming extremely reactive radicals and reactive oxygen species (ROS). Generation of the ROS by some wide-bandgap materials, such as TiO_2, ZnO, WO_3, CeO_2, carbon nanotubes, quantum dots and some metal NPs when illuminated by UV-VIS light, can cause oxidative stress, resulting in toxic effects in living organisms[5]. Therefore, measuring the nanomaterial PCA under physiological conditions allows for an assessment of its photo-toxicity potency.

Existing standard test methods for particle and surface PCA measurement (see ISO 10676 and ISO 10678) are not directly applicable to determine nanomaterial PCA leading to photo-toxicity, as they require a large test volume and/or long measurement duration, while utilizing organic dyes as indicators that are not biocompatible.

The in vitro NP PCA test for NADH oxidation is intended to evaluate the nanomaterial photo-toxicity potency when exposed to an ultraviolet (UV) light.

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Nanotechnologies — Testing the photocatalytic activity of nanoparticles for NADH oxidation

1 Scope

This document specifies a method for the measurement of the photocatalytic activity (PCA) of nanoparticles (NPs), suspended in an aqueous environment in physiologically relevant conditions, by measuring the ultraviolet (UV)-induced nicotine adenine dinucleotide hydrate (NADH) oxidation.

The measurement is intended to assess the potential for the photo-toxicity of nanomaterials. The method is also applicable to NP aggregates and agglomerates.

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

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

3 Terms, definitions, symbols and abbreviated terms

For the purposes of this document, the terms and definitions given in 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 Terms and definitions

3.1.1
actinometry
method to measure the number of photons integrally or per unit of time

3.1.2
catalytic activity
property of a component corresponding to the catalysed substance rate of conversion of a specified chemical reaction, in a specified measurement system

[SOURCE: ISO 18153:2003, 3.2, modified — The notes have been deleted.]

3.1.3
oxidation
chemical reaction accompanying a gain of oxygen, loss of hydrogen of an organic substrate or loss of one or more electrons from a molecular entity

3.1.4
NADH equivalent specific PCA
PCA measured as the NADH *photo-oxidation* (3.1.5) rate per unit weight of nanoparticles

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3.1.5
photo-oxidation
oxidation reactions induced by light

3.2 Symbols and abbreviated terms

DIW	deionized water with ≥ 18 MΩ·cm resistivity
NADH	nicotine adenine dinucleotide hydrate
NaOH	sodium hydroxide
2NB	2-nitrobenzaldehyde
NP	nanoparticle
PB	phosphate buffer
PCA	photocatalytic activity
ROS	reactive oxygen species
TI	trans-illuminator
TiO_2	titanium dioxide
UV	ultraviolet
UV-Vis	ultraviolet and visible
$A_c(i,j)$	phenolphthalein absorbance before exposure to trans-illuminator UV irradiation in each well (i = B, C, D, E, F, G; j = 2, 3, 4, …, 10, 11)
$A_e(i,j)$	phenolphthalein absorbance after exposure to trans-illuminator UV irradiation in each well (i = B, C, D, E, F, G; j = 2, 3, 4, …, 10, 11)
$\Delta A(i,j)$	change in phenolphthalein absorbance after exposure to trans-illuminator UV irradiation in each well (i = B, C, D, E, F, G; j = 2, 3, 4, …, 10, 11)
ΔA_a	average change of phenolphthalein absorbance over all wells before and after UV irradiation by using a UV trans-illuminator
C_0	starting concentration of the NP suspension for a dilution series of test solutions; the suspension absorbance at 310 nm or 365 nm (depending on the used UV trans-illuminator) is $1{,}4 < A < 1{,}6$
$C(i,j)$	light intensity correction factor of each well, which accounts for the UV irradiation intensity variation of the UV trans-illuminator at the location of each well (i = B, C, D, E, F, G; j = 2, 3, 4, …, 8, 9)
$I_{F,0}(i,j)$	NADH fluorescence intensity measured before UV irradiation in each well (i = B, C, D, E, F, G; j = 2, 3, 4, …, 8, 9)
$I_{F,t}(i,j)$	NADH fluorescence intensity measured following the UV irradiation of t duration by using a UV trans-illuminator in each well (i = B, C, D, E, F, G; j = 2, 3, 4, …, 8, 9)
$k_{app}(i,j)$	apparent NADH photo-oxidation rate in each well, expressed in µmol/min (i = B, C, D, E, F, G; j = 2, 3, 4, …, 8, 9)
λ_{exc}	excitation wavelength used to record fluorescence in multiple well plate readers
λ_{ems}	emission wavelength used to record fluorescence in multiple well plate readers
$A\,\lambda$(max)	maximum absorbance of NP suspension in a wavelength range from 300 nm to 800 nm
λ(max,TI)	wavelength at which a UV trans-illuminator provides the maximum intensity of light
$S(i,j)$	slope of the NADH fluorescence intensity versus the UV irradiation time in each well (i = B, C, D, E, F, G; j = 2, 3, 4, …, 8, 9)
$S_c(i,j)$	$S(i,j)$ corrected for the trans-illuminator light intensity variation at each well
b	slope of k_{app} versus NP concentration in the linear range, expressed in units of mmol/min·g

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