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Nanotechnologies — Electron spin resonance (ESR) as a method for measuring reactive oxygen species (ROS) generated by metal oxide nanomaterials

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Nanotechnologies — Electron spin resonance (ESR) as a method for measuring reactive oxygen species (ROS) generated by metal oxide nanomaterials

Nanotechnologies — Résonance paramagnétique électronique (RPE) pour la mesure des espèces réactives de l'oxygène (ROS) générées par des nanomatériaux sous forme d'oxyde métallique

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Foreword

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This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

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Introduction

Recently, the use of metal or metal oxide-based nanomaterials has dramatically increased in biomedical and industrial applications. However, the scientific basis for the cytotoxicity and genotoxicity of most manufactured nanomaterials are not fully understood. An important mechanism of nanotoxicity is the generation of reactive oxygen species (ROS). The study on the hazardous effects of metal oxide nanomaterials is still in its initial stage. The ability to generate ROS is one main source of toxicity of metal oxide nanomaterials. Overproduction of ROS can induce oxidative stress, resulting in cells failing to maintain normal physiological redox-regulated functions. This in turn may lead to DNA damage, unregulated cell signalling, change in cell motility, cytotoxicity, apoptosis and cancer initiation. There are critical determinants that can affect the generation of ROS. The critical determinants include size, shape, particle surface, surface positive charges, surface-containing groups, particle dissolution, metal ion release from nanometals and nanometal oxides, UV light activation, aggregation, mode of interaction with cells, inflammation and pH of the medium[1]. Thus, to detect and quantify ROS formation on the surface of metal oxide nanomaterials, this document suggests the electron-spin-resonance (ESR) method.

Amongst ROS, the most biologically relevant and widely studied are hydroxyl radical (OH), superoxide anion radical (O_2^-), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2).

However, direct detection of some free radicals (e.g. superoxide anion and hydroxyl radical) is very difficult or impossible[2] in solution at room temperature. ESR spin trapping is a valuable tool in the study of transient free radicals[3]. Spin trapping is a technique, developed in the late 1960s, where a nitrone or nitroso compound (a spin trap) reacts with a target free radical to form a stable and distinguishable free radical (spin adducts) to be detected by ESR spectroscopy.

Spin adducts can be observed directly by ESR spectroscopy. The ESR spectra of these spin adducts are unique and provide a fingerprint for the presence of ROS.

This document specifies methods of detection by ESR of 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) hydroxyl adduct, 5-tert-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide (BMPO) superoxide adduct and 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide (TPC) singlet oxygen adduct formation from metal oxide nanomaterials. This document provides a method to assess ROS generation on the metal oxide nanomaterials in a cell free condition. This method may provide valuable information for the prediction of ROS-mediated cytotoxicity without cytotoxicity assay at physico-chemical evaluation phase.

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Nanotechnologies — Electron spin resonance (ESR) as a method for measuring reactive oxygen species (ROS) generated by metal oxide nanomaterials

1 Scope

This document provides a procedure for the detection of ROS (OH, O_2^-, $^1O_2$) generated by metal oxide nanomaterials in aqueous solution with a reactive oxygen species-specific spin trapping agent using ESR, but excludes ESR procedures that do not use a spin trapping agent.

2 Normative references

There are no normative references in this document.

3 Terms, definitions and abbreviations

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1 Terms and definitions

3.1.1
nanomaterial
material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

Note 1 to entry: This generic term is inclusive of nano-object and nanostructured material.

Note 2 to entry: See also ISO/TS 80004-1:2015, 2.8 to 2.10.

[SOURCE: ISO/TS 80004-1:2015, 2.4]

3.1.2
test sample
material, device, device portion, component, extract or portion thereof that is subjected to biological or chemical testing or evaluation

[SOURCE: ISO/TS 10993-5:2009, 3.5]

3.1.3
zero baseline control
equivalent of the positive control where no radicals are detected

Note 1 to entry: For example, zero baseline control for the positive control of fenton reaction will be H_2O_2 and DMPO in the absence of iron; for hypoxanthine–xanthine oxidase (HX-XO) system, it will be hypoxanthine and BMPO in the absence of HX-XO; for rose bengal photosensitization, it will be rose bengal and TPC in the absence of light.

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3.1.4
positive control
well-characterized material or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response in the test system

[SOURCE: ISO/TS 10993-12:2012, 3.12]

3.2 Abbreviations

ROS	reactive oxygen species
ESR	electron spin resonance
DMPO	5,5-dimethyl-1-pyrroline-N-oxide
BMPO	5-tert-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide
TPC	2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide
DTPA	diethylenetriaminepentaacetic acid
OH	hydroxyl radical
OH-	hydroxide ion
O_2	superoxide anion radical
$^1O_2$	singlet oxygen
TEMPOL	4-hydroxyl-2,2,6,6-tetramethylpiperidine-1-oxyl

4 Principle

4.1 General

In most atoms and molecules, electrons are paired. The paired electrons do not give an ESR signal while atoms and molecules with unpaired electrons give an ESR signal. When an atom or molecule with an unpaired electron is placed in a magnetic field, the spin of the unpaired electron can align either in the same direction or in the opposite direction as the field. These two alignments of electron spin have different energies. The application of a magnetic field to an unpaired electron lifts the degeneracy of its spin states. ESR spectroscopy measures the absorption of microwave radiation associated with the transition between these non-degenerate spin states[4].

4.2 Spin trapping method

4.2.1 General

Spin trapping is used in ESR spectroscopy for detection and identification of short-lived free radicals. Ideally, the adduct formed between a spin trapping agent and a free radical has an ESR spectrum characteristic and specific to that free radical. Advanced ESR studies employing spin-trap agents were adopted to distinguish the different types of ROS.

4.2.2 DMPO

DMPO has significant advantages over other nitrone spin traps. It is particularly useful for identifying oxygen-centred radicals, e.g. superoxide anion and hydroxyl radicals. The spin adduct formed between

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