



BSI Standards Publication

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

National foreword

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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

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 on 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 the following URL: www.iso.org/iso/foreword.html.

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

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 nitron 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.

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^- , $^1\text{O}_2$) 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.