TECHNICAL ISO/TS

First edition
2017-06

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

Reference number
ISO/TS 18827:2017(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

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).

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

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 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.