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Review Article

Electron spin resonance spectroscopy for the study of nanomaterial-mediated generation of reactive oxygen species☆

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ABSTRACT

Many of the biological applications and effects of nanomaterials are attributed to their ability to facilitate the generation of reactive oxygen species (ROS). Electron spin resonance (ESR) spectroscopy is a direct and reliable method to identify and quantify free radicals in both chemical and biological environments. In this review, we discuss the use of ESR spectroscopy to study ROS generation mediated by nanomaterials, which have various applications in biological, chemical, and materials science. In addition to introducing the theory of ESR, we present some modifications of the method such as spin trapping and spin labeling, which ultimately aid in the detection of short-lived free radicals. The capability of metal nanoparticles in mediating ROS generation and the related mechanisms are also presented.

1. Introduction

Rapid development of the nanoscience and technology has produced numerous nanomaterials that offer revolutionary benefits in electronics, energy, medical, and health applications, but unfortunately also lead to environmental, health, and safety concerns [1]. For example, Au nanoparticles (NPs) have been explored as nanopharmaceuticals for the treatment of cancer [2], and Ag NPs have been established as superior antibacterial materials [3]. However, the wide use of nanomaterials has raised concerns regarding their potentially hazardous effects on biological systems, and the associated short- and long-term risks are not well understood. A variety of nanomaterials can generate reactive oxygen species (ROS) under certain experimental conditions [4–9]. Among various toxic responses, nanomaterial-induced oxidative stress mediated by ROS has been studied most extensively [10–12].

ROS, e.g., superoxide, hydroxyl radical, singlet oxygen, and hydrogen peroxide, are powerful oxidants that can damage cellular targets nonselectively. Free radicals, including ROS, are short lived and represent a broad range of chemically distinct entities; consequently, these species are difficult to

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detect in dynamic environments such as biological systems. The use of fluorescent probes (e.g., dichlorodihydrofluorescein, hydroethidine, and dihydrorhodamine) and chemiluminescent assays is a simple and easy way of detecting free radicals and ROS in cellular systems, but there are inherent limitations and many sources of artifacts [13,14]. Electron spin resonance (ESR) spectroscopy has become a powerful and direct method to detect free radicals generated chemically or formed in biological systems. We have a long-standing interest in employing ESR techniques to identify and quantify free radicals in biological systems, and study the mechanisms of interactions between biologically relevant systems and nanomaterials, metal ions, and organic molecules [4,5,7,9,15–43]. We have also published several book chapters on this subject [44–46]. In this special issue, we demonstrate that ESR spectroscopy is a powerful tool for exploring the capability of NPs to generate ROS. The ESR spin-trapping techniques used to detect ROS (including hydroxyl radicals, superoxide radical anion, and singlet oxygen) and the ESR oximetry methodology employed for monitoring oxygen and the formation of lipid peroxidation are also discussed briefly.

2. ESR spectroscopy

2.1. *Principle of ESR spectroscopy*

ESR, also called electron paramagnetic resonance, is a powerful technique for studying chemical species or materials that have one or more unpaired electrons. The basic physical concepts of ESR are analogous to those of nuclear magnetic resonance, except that in ESR electron spins are excited instead of atomic nuclei. ESR has been studied for several decades since it was first observed by Y. Zavoisky in 1944 [47]. A number of review articles and books are available that provide a useful introduction to the basic concepts of ESR and its applications [47–49]. An electron has a spin quantum number $s = 1/2$ with magnetic components $m_s = +1/2$ and $-1/2$. In an external magnetic field, free electrons align with their spin parallel (low energy) or perpendicular (high energy) to the magnetic field (Fig. 1). A transition between low- and high-energy states can occur when sufficient energy is absorbed. This energy lies within the microwave frequencies of the electromagnetic spectrum. The energy ($h\upsilon$) required for this transition is given by the following equation:

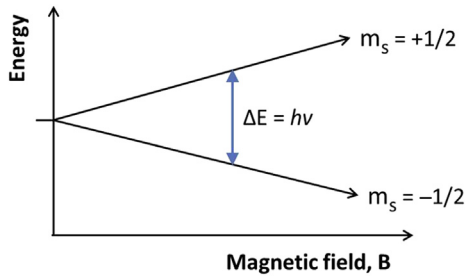

Fig. 1 – Energy diagram showing the origin of an electron spin resonance signal.

$$h\upsilon = g_e \mu_B B_0$$

where μ_B is the Bohr magneton, B_0 is the magnetic field strength, and g_e is the Landé g-factor (2.0023 for free electron). An ESR spectrum is usually obtained by varying the magnetic field strength at a fixed microwave frequency. Magnetic field strengths at which the microwave frequency is absorbed are recorded in the ESR spectrum. A typical continuous wave X-band (9.5 GHz) ESR instrument, as shown in Fig. 2, includes the following major components: (1) a magnet that generates and modulates a magnetic field; (2) a microwave supply system that includes an electromagnetic radiation source and a detector to control the microwave power; (3) a sample cavity to which microwave energies are directed and in which samples are placed; and (4) a data processing and display system. Under certain conditions, each free radical exhibits a specific ESR spectrum, and the intensity of an ESR signal is proportional to the concentration of free radicals; therefore, qualitative identification of free radical species along with their quantitative measurements can be performed.

Spin trapping and spin labeling are the two principal ESR techniques used for the detection and identification of free radicals [9,44,50]. ROS are usually very reactive and present in low concentrations, which is a major limitation to the detection of ROS. However, the instability of free radicals is largely solved by the use of either spin trapping or spin labeling. The ESR spin-trapping technique uses chemical species called spin traps, which react with short-lived free radicals to form relatively stable adducts having a half-life long enough for ESR measurement [50]. The ESR spin-labeling technique uses a stable paramagnetic spin label agent to interact with the target chemical, e.g., the oxygen molecule or electrons, and is a powerful tool for probing structural and/or dynamic changes in complex chemical or biological systems [51,52]. Here, our discussion focuses on oxygen-centered free radicals, particularly ROS. In this review, carbon- and sulfur-centered free radicals have not been included.

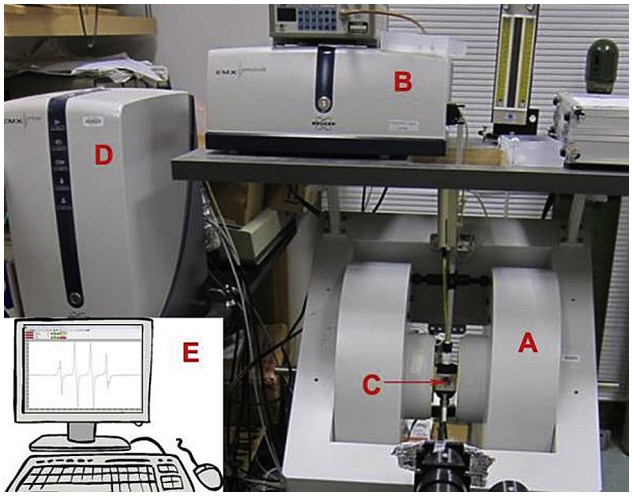

Fig. 2 – Photograph of a typical Bruker EMX continuous-wave electron spin resonance instrument. The components A, B, C, D, and E represent magnet, microwave supply and control system, sample cavity, data processing, and display system, respectively.