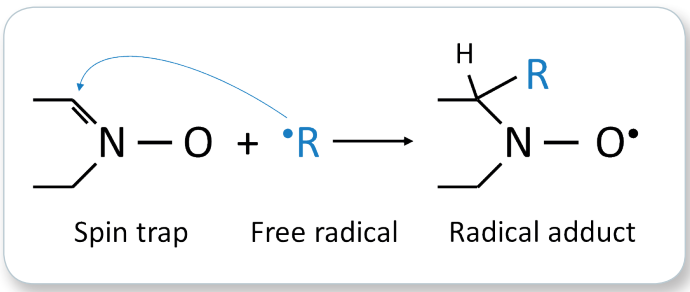
Biological ROS and RNS Detection
Part I. EPR Spin Trapping

Oxidative stress and damage in cells is associated with the development of cancer, Alzheimer's disease, atherosclerosis, autism, infections and Parkinson's disease. Reactive Oxygen Species (ROSs) are the main cause of oxidative stress and damage in cells, causing damage to proteins, lipids and DNA. Two leading ROS are radicals such as the superoxide radical ($O_2^{\cdot -}$) and the hydroxyl radical (·HO) as shown here in the Xanthine/Xanthine oxidase system where their generation and decomposition can be accurately followed with the EMXnano.

Introduction

The interest in free-radical processes in living systems has increased exponentially during the last decade. The huge complexity of the evolved processes makes necessary the analysis of the problem from a fundamental point of view. Radicals are intermediates in a variety of biochemical reactions. Some of the most abundant radicals produced in natural biochemical reactions are Reactive Oxygen Species (ROS) such as hydroxyl, hydroperoxyl and superoxide radicals, and Reactive Nitrogen Species (RNS), such as nitrogen monoxide and peroxynitrite.



Challenge

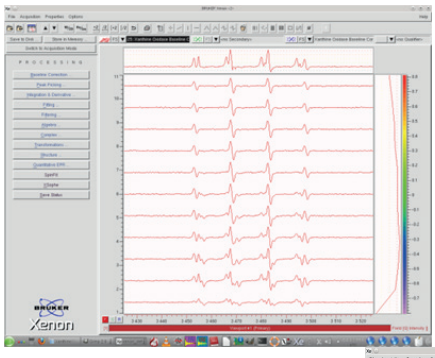
Direct detection of ROS and RNS is very difficult or impossible in solution at room temperature due to their very short half-life.

Solution

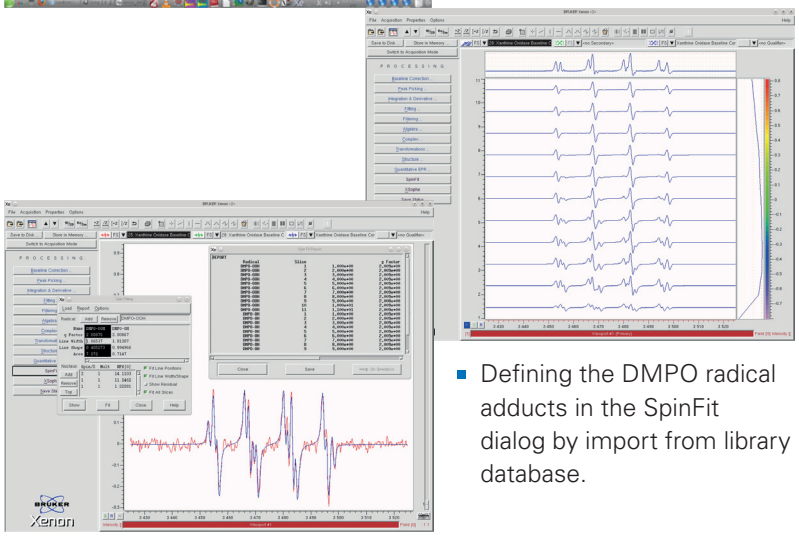
EPR spin trapping is a technique developed in the late 1960s where a nitrone or nitroso compound reacts with a target free radical to form a stable and distinguishable free radical that is detected by EPR spectroscopy. The spin trapping reaction involves the addition of the reactive free radical to the double bond of a diamagnetic "spin trap" to form a much more stable free radical, which can then be examined with EPR. This "radical adduct" has spectral features that allow easy identification of the reactive radical originally generated.

Equipment

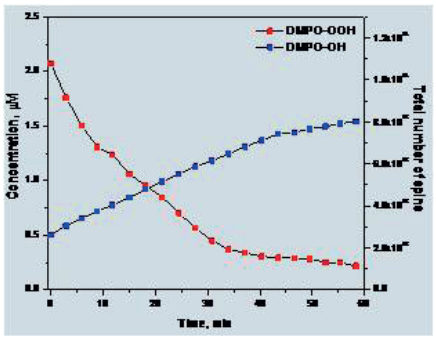
Built with a new generation magnet system and a highly efficient microwave resonator, EMXnano gives consistently accurate results and superior sensitivity. Enhance your research, teaching or process applications with the power of Electron Paramagnetic Resonance spin trapping spectroscopy. The EMXnano spectrometer enables researchers and students with limited EPR experience to use the power of EPR spin trapping spectroscopy to identify and quantify free radicals in biological systems (proteins, blood, tissues, etc).



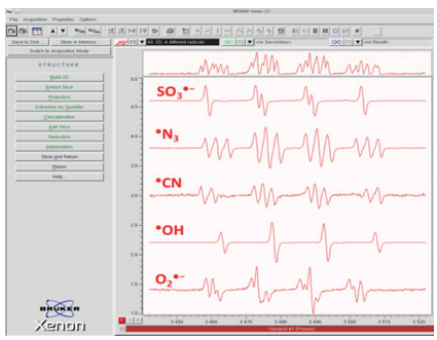
- Experimental data (in red) and SpinFit simulations (in blue) of DMPO radical (superoxide and hydroxyl) adducts in Xanthine/ Xanthine oxidase at a given time in the 2D Field versus Time experiment.



- Defining the DMPO radical adducts in the SpinFit dialog by import from library database.



- SpinCount provides a report showing the time evolution of the concentration of the radical adducts.
- The report can be saved as an ASCII file for further evaluation.



- A variety of radicals can be identified and studied with the user friendly EMXnano software.
- DMPO is a specific spin trap which identifies the radicals formed via the spectrum of the spin-adduct.

Key Features include:

- Easy-to-use software for free radical detection
- 2D EPR spin trapping experiments showing the formation of the radical adducts
- Spin trap library – a wide database collection of previously simulated spectra for many radical adducts
- SpinFit module to simulate and identify multiple radicals in the sample using the spin trap library spectra
- SpinCount module to quantify the total number of spins and to determine the radical concentration

References for further reading

1. Zielonka J., Cheng G., Zielonka M., Ganesh T., Sun A., Joseph J., Michalski R., O'Brien W. J., Lambeth J.D., Kalyanaraman, B. High-throughput assays for superoxide and hydrogen peroxide: design of a screening workflow to identify inhibitors of NADPH oxidases, J. Biol. Chem. (2014) 289(23) 16176

2. Abbas K., Hardy M., Poulhes F., Karoui H., Tordo P., Ouari O., Peyrot F. Detection of superoxide production in stimulated and unstimulated living cells using new cyclic nitrone spin traps, Free Rad. Biol. Med. (2014) 71 281

3. Das A., Gopalakrishnan B., Druhan L.J., Wang T., De Pascali F., Rockenbauer A., Racoma I., Varadharaj S., Zweier J.L., Cardounel A.J. and Villamena F.A. Reversal of SIN-1-induced eNOS dysfunction by the spin trap, DMPO, in bovine aortic endothelial cells via eNOS phosphorylation, Br. J. Pharmacol. (2014) 171(9) 2321

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