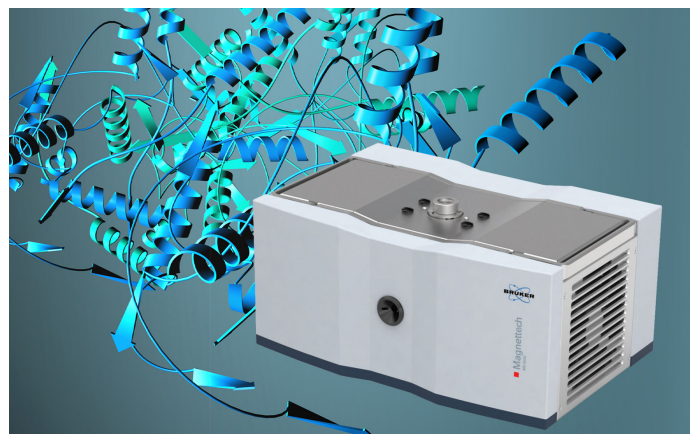




MagneTech ESR5000: Bench-top EPR Instrument for Life Science

Introduction

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. The major reason to measure ROS and RNS in biological systems is to determine whether they play a role in physiological or pathophysiological processes. Detection of radicals in biological materials is the sole method for their characterization done using EPR (electron paramagnetic resonance) methodology.

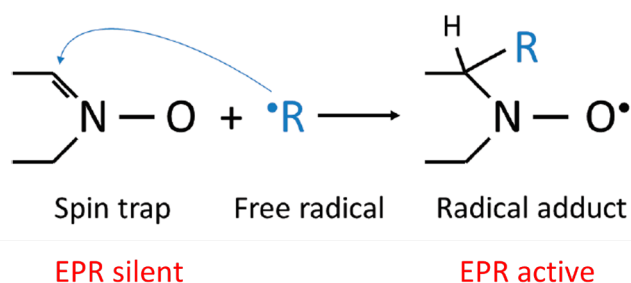
Reactive oxygen and nitrogen species are involved in a vast range of major diseases including cardiovascular disease, Alzheimer's, Parkinson's, cancer, multiple sclerosis, autism, and infections. These free radicals have an unpaired electron, creating a highly reactive molecule. In the cells and tissues of the human body this can result in major damage to lipids, proteins and DNA, ultimately affecting the function of cells, tissues and organs.

The interest in free-radical processes in living systems has increased exponentially during the last decade. The huge complexity of the evolved processes necessitates the analysis of the problem from a fundamental point of view. Radicals are intermediates in a variety of biochemical reactions. The rapid

and rigorous detection and quantification of free radicals will lead to a better understanding of chemical biology and will also help in the discovery of specific inhibitors. Identifying sources of excessive, defective, or unbalanced ROS and RNS levels and developing alternative strategies to regulate their respective levels should help in designing novel rational therapies.

Most of the biologically relevant radicals are very short lived and, therefore, impossible to detect in biological samples. For this reason, compounds (spin traps and spin probes) have been used that form stable adducts with radicals (Figure 1):

Figure 1



1. ROS Detection Using Spin Traps and Quantitative Analysis

ROS are the main cause of oxidative stress and damage in cells. Two leading ROS are radicals such as the superoxide radical ($O_2^{\bullet-}$) and the hydroxyl radical ($^{\bullet}OH$) as shown here in the xanthine/xanthine oxidase system (Figure 2) where their generation and decomposition can be accurately followed with quantitative EPR.

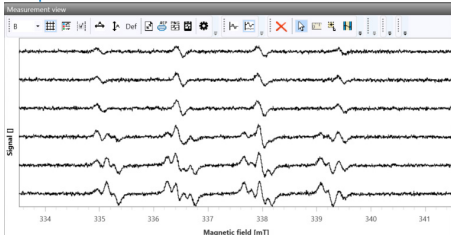
EPR spin trapping is a technique developed where a nitron or nitroso compound reacts with a target free radical to form a stable and distinguishable free radical that is detected by EPR

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.

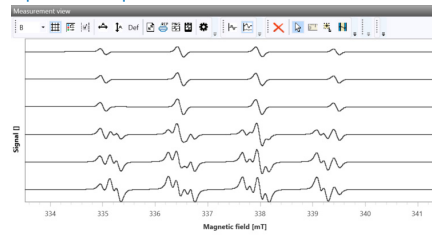
With superior sensitivity and stability, the Bruker Magnetech ESR5000 spectrometer delivers accurate results without the need of prior experience in EPR spectroscopy. The Magnetech ESR5000 spectrometer enables researchers to use the power of EPR spin trapping spectroscopy to identify and quantify free radicals in biological systems (proteins, blood tissues, etc.)

Figure 2

Experimental data:



SpinFit Liquids simulated data:

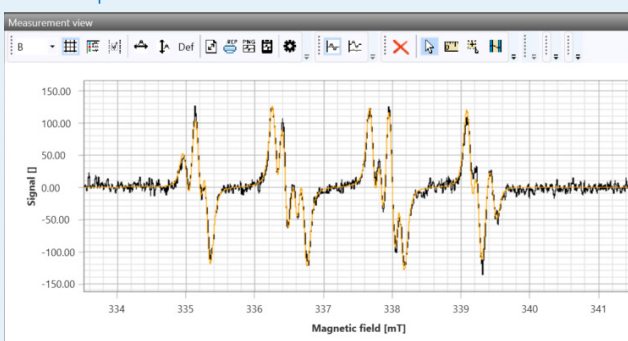


Experimental data and SpinFit Liquids simulations at a given time in kinetics experiment.

Quantitative procedure

Load simulation parameters from the library

Fit all experimental data



Get a SpinCount report

Data name	Time [s]	Height [mm]	Diameter [mm]	Volume [ml]	Spins	Spin conc. [M]
XOX_kinetics_full_01_001_fit DMPO-OH	0.000	23	0.94	0.016 2.041e+013	2.123e-006	
XOX_kinetics_full_01_001_fit DMPO-OH	0.000	23	0.94	0.016 1.152e+014	1.199e-005	
XOX_kinetics_full_01_002_fit DMPO-OH	60.005	23	0.94	0.016 1.839e+013	1.914e-006	
XOX_kinetics_full_01_002_fit DMPO-OH	60.005	23	0.94	0.016 3.549e+013	3.692e-006	
XOX_kinetics_full_01_003_fit DMPO-OH	120.014	23	0.94	0.016 1.247e+013	1.297e-006	
XOX_kinetics_full_01_003_fit DMPO-OH	120.014	23	0.94	0.016 1.310e+013	1.363e-006	
XOX_kinetics_full_01_004_fit DMPO-OH	180.013	23	0.94	0.016 8.392e+012	8.730e-007	
XOX_kinetics_full_01_004_fit DMPO-OH	180.013	23	0.94	0.016 1.399e+014	1.456e-005	
XOX_kinetics_full_01_005_fit DMPO-OH	240.013	23	0.94	0.016 7.138e+012	7.426e-007	
XOX_kinetics_full_01_005_fit DMPO-OH	240.013	23	0.94	0.016 1.044e+012	1.086e-007	
XOX_kinetics_full_01_006_fit DMPO-OH	300.002	23	0.94	0.016 3.480e+012	3.701e-007	
XOX_kinetics_full_01_006_fit DMPO-OH	300.002	23	0.94	0.016 1.350e+017	1.405e-002	
XOX_kinetics_full_01_007_fit DMPO-OH	360.005	23	0.94	0.016 4.196e+012	4.366e-007	
XOX_kinetics_full_01_007_fit DMPO-OH	360.005	23	0.94	0.016 5.171e+011	5.379e-008	
XOX_kinetics_full_01_008_fit DMPO-OH	420.007	23	0.94	0.016 4.514e+012	4.696e-007	
XOX_kinetics_full_01_008_fit DMPO-OH	420.007	23	0.94	0.016 0.000e+000	0.000e+000	
XOX_kinetics_full_01_009_fit DMPO-OH	480.013	23	0.94	0.016 2.545e+012	2.648e-007	
XOX_kinetics_full_01_009_fit DMPO-OH	480.013	23	0.94	0.016 8.807e+011	9.162e-008	
XOX_kinetics_full_01_010_fit DMPO-OH	540.000	23	0.94	0.016 2.742e+012	2.852e-007	
XOX_kinetics_full_01_010_fit DMPO-OH	540.000	23	0.94	0.016 0.000e+000	0.000e+000	
XOX_kinetics_full_01_011_fit DMPO-OH	600.000	23	0.94	0.016 2.423e+012	2.521e-007	
XOX_kinetics_full_01_011_fit DMPO-OH	600.000	23	0.94	0.016 0.000e+000	0.000e+000	
XOX_kinetics_full_01_012_fit DMPO-OH	660.005	23	0.94	0.016 1.789e+012	1.861e-007	
XOX_kinetics_full_01_012_fit DMPO-OH	660.005	23	0.94	0.016 2.505e+012	2.609e-007	
XOX_kinetics_full_01_013_fit DMPO-OH	720.006	23	0.94	0.016 1.561e+012	1.624e-007	
XOX_kinetics_full_01_013_fit DMPO-OH	720.006	23	0.94	0.016 4.126e+017	4.293e-002	

Calculate

Provide sample dimensions

Defining the DMPO radical adducts in the SpinFit Liquids dialog can be done by importing from library databases or by manual entry.

- SpinCount provides a report showing the time evolution of the concentration of the radical adduct concentration.
- The report can be saved as a *.csv file for further evaluation.
- A variety of radicals can be identified and studied with the user friendly ESRStudio software.
- DMPO is a specific spin trap which identifies the radicals formed via the spectrum of the spin-adduct.

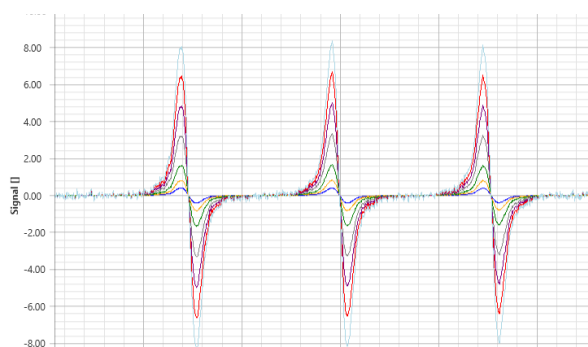
2. ROS Detection Using Spin Probes and Quantitative Analysis

In vascular cells, increased generation of superoxide has been suggested to occur in hypertension, diabetes, and heart failure. Thus, the accurate detection and ability to quantify superoxide are critically important in understanding the pathogenesis of these various cardiovascular disorders and other non-cardiovascular diseases.

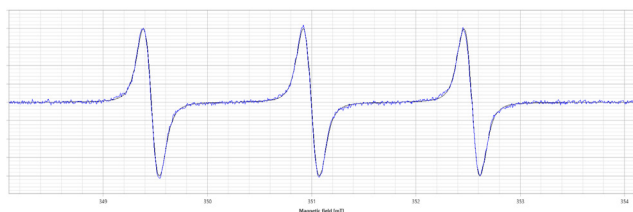
Spin probes are not spin traps, in that they do not “trap” radicals, but they are oxidized by free radicals to form nitroxides with a half-life of several hours, which can readily be detected by EPR. For example, the cyclic hydroxylamine 1-hydroxy- 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH) can provide quantitative measurements of superoxide ($O_2^{\bullet-}$) with high sensitivity and it has been used for detection of intracellular $O_2^{\bullet-}$ in cultured cells and tissue samples. As shown on the right, the generation of superoxide over time can be easily monitored with the Magnettech ESR5000.

Bench-top EPR spectrometers can be used effectively for mechanistic studies and kinetic analysis of multiple radicals (ROS and RNS) generated in enzyme reactions. Properly controlled spin probe experiments can verify that the formation of radical adducts is due to free radical production in the reaction system being studied.

Figure 3

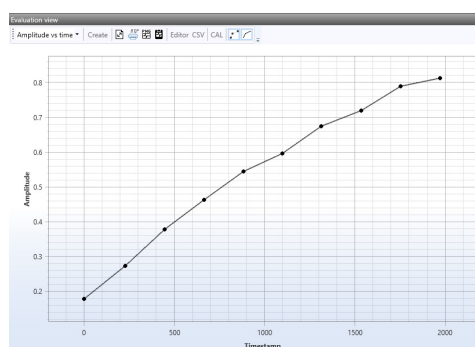


- Addition of xanthine and xanthine oxidase to CMH leads to a steady rise in EPR peak-to-peak amplitude of the nitroxide radical $CM^{\bullet}$
- Formation of $CM^{\bullet}$ nitroxide is due to the reaction: $CMH + O_2^{\bullet-} \rightarrow CM^{\bullet} + H_2O_2$



SpinCount report

Data name	Time [s]	Height [mm]	Diameter [mm]	Volume [ml]	X start [mT]	X end [mT]	Spins	Spin conc. [M]
CMH_001	0.000	23	0.94	0.016	333.360	341.200	5.194e+012	5.404e-007
CMH_002	60.003	23	0.94	0.016	333.360	341.200	2.538e+014	2.640e-005
CMH_003	120.004	23	0.94	0.016	333.360	341.200	2.535e+014	2.637e-005
CMH_004	180.014	23	0.94	0.016	333.360	341.200	3.482e+014	3.622e-005
CMH_005	240.013	23	0.94	0.016	333.360	341.200	2.684e+014	2.792e-005
CMH_006	300.013	23	0.94	0.016	333.360	341.200	3.260e+014	3.392e-005
CMH_007	360.005	23	0.94	0.016	333.360	341.200	3.585e+014	3.730e-005
CMH_008	419.999	23	0.94	0.016	333.360	341.200	3.130e+014	3.256e-005
CMH_009	480.011	23	0.94	0.016	333.360	341.200	2.603e+014	2.708e-005
CMH_010	540.004	23	0.94	0.016	333.360	341.200	2.033e+014	2.115e-005



- The Evaluation View in ESRStudio software is useful for monitoring the kinetics of $CM^{\bullet}$ formation via changes in peak-to-peak amplitude or double integral over time
- The rate of superoxide formation by xanthine oxidase can be obtained from a linear fit of the kinetic trace and presented as $\mu M O_2^{\bullet-} / \text{min}$

SpinFit - Liquids

Load Report Options

Species: Add Remove CMH

Lorentzian Width [G] 1.18152 τ_c [ns] 0 Regime auto

Gaussian Width [G] 1.34329 Area 102.208

Isotropic Axial Rhombic

g_{iso} 2.0064

Nuclei: Add Remove Unit Gauss

Selected Nucleus ^{14}N n 1

Isotropic Axial Rhombic

A_{iso} 15.3865

Fit

☒ Line Positions ☒ Line Widths ☐ Correl. Times ☒ All Slices Advanced

Show Fit Close Help

- SpinFit Liquids and SpinCount modules provide ROS concentration for quantitative EPR analysis at any given time point during the reaction

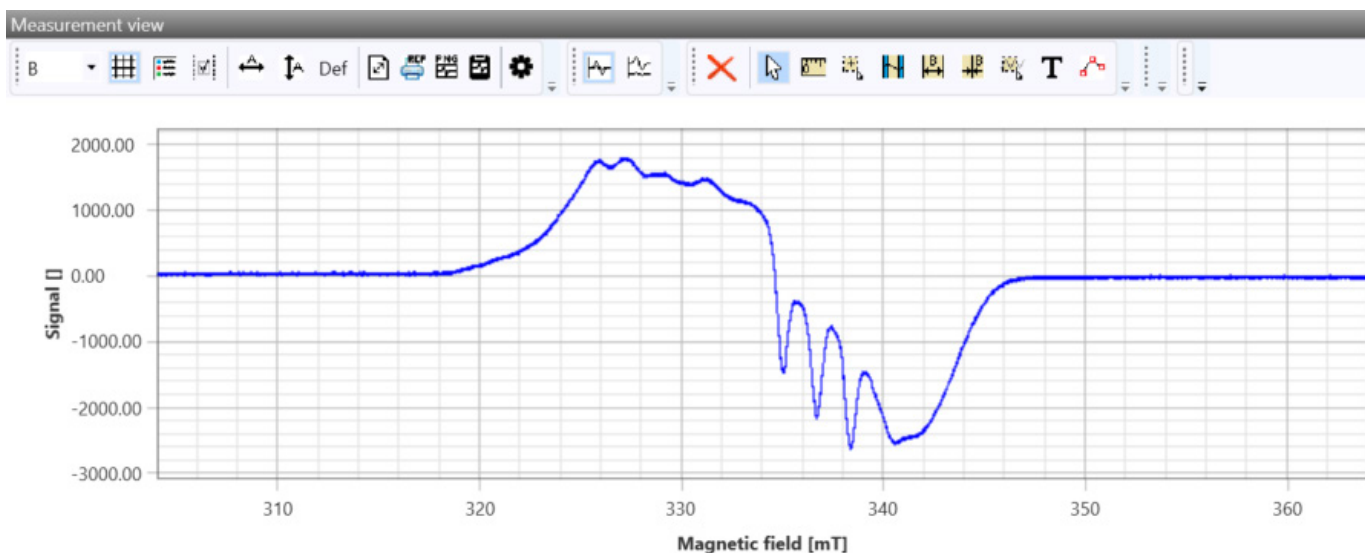
3. Nitric Oxide Detection

Nitric Oxide (NO) is a highly reactive regulatory molecule which has many important physiological roles, such as a neurotransmitter in the central nervous system, a regulator of vasomotor tone in the cardiovascular system, and a cytotoxic mediator of the immune system. NO is a free radical and its short half-life (< 30 sec), has rendered direct measurement difficult. The instability of NO can be overcome by using a NO-trapping technique, in which a more stable complex is formed and subsequently detected by EPR. N-methyl-D-glucamine dithiocarbamate (MGD)-ferrous complex is a commonly used spin trap for NO, resulting in the formation of the (MGD)₂-Fe(II)-NO complex, which is water-soluble and forms a characteristic triplet EPR signal.

Oxidation of nitric oxide (NO) to nitrate by oxyhemoglobin (oxyHb) is a fundamental reaction in NO biology and binding of NO to the heme can be characterized by Magnettech ESR5000 spectrometer (Figure 4).

EPR provides the means to detect NO by both room temperature and low temperature techniques with high sensitivity. With accessories for room temperature and low temperature measurements, detection of NO with MGD as a spin trap or HbNO complex is easy to achieve.

Figure 4



- Nitric oxide (NO) plays a central role in a multitude of diseases such as intravascular hemolysis and hemolytic anemias. The reaction of NO scavenging by Hb is recognized to play a major role in several of these diseases
- Trapping of the HbNO complex with the finger dewar or VT accessories permit the direct detection of NO-hemoglobin complex

References

- [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 nitron 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
- [4] Gielis J.F., Boulet G.A., Briedé J.J., Horemans T., Debergh T., Kussé M., Cos P., and Van Schil P.E.Y. Longitudinal quantification of radical bursts during pulmonary ischaemia and reperfusion, *Eur. J. Cardiothorac. Surg.* (2015), in press.
- [5] Mrakic-Spota S., Gussoni M., Montorsi M., Porcelli S., Vezzoli A. Assessment of a standardized ROS production profile in humans by Electron Paramagnetic Resonance, *Oxid Med Cell Longev.* (2012) Article ID 973927
- [6] Xiao D., Powolny A.A., Moura M.B., Kelley E.E., Bommarreddy A., Kim S., Hahm E., Normolle D., Van Houten B., and Singh S.V. Phenethyl isothiocyanate inhibits oxidative phosphorylation to trigger Reactive Oxygen Species-mediated death of human prostate cancer cells, *J. Biol. Chem.* (2010) 285(30) 26558
- [7] Opländer C., Deck A., Volkmar C.M., Kirsch M., Liebmann J., Born M., van Abeelen F., van Faassen E.E., Kröncke K., Windolf J., Suschek C.V. Mechanism and biological relevance of blue-light (420-453 nm)-induced nonenzymatic nitric oxide generation from photolabile nitric oxide derivatives in human skin in vitro and in vivo, *Free Rad. Biol. Med.* (2013) 65 1363
- [8] Hawkins C.L., Davies M.J. Detection and characterisation of radicals in biological materials using EPR methodology, *Biochim. Biophys. Acta* (2014) 1840(2) 708
- [9] Li H., Hemann C., Abdelghany T.M., El-Mahdy M.A., and Zweier J.L. Characterization of the mechanism and magnitude of cytoglobin-mediated nitrite reduction and nitric oxide generation under anaerobic conditions, *J. Biol. Chem.* (2012) 287(43) 36623