Electron paramagnetic resonance (EPR) spectroscopy analysis using the spin trap DMPO for OH· detection

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Version
1.0 English

Contents

1 Scope

This Standard Operating Procedure (SOP) describes the experimental procedure and settings of the electron paramagnetic resonance (EPR) spectroscopy analysis using the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) to detect particle elicit hydroxyl radical (OH·) generation potency.

Note: The SOP is specific for the use of EPR instrument MS-300 or higher (Magnettech GmbH, Berlin).

2 Basics

The oxidant generation capacity of particles has been forwarded as a possible additional characterisation metric to predict potential hazard of novel materials. One promising method for the detection of oxidant generation capacity is the EPR spectroscopy using the spin trap DMPO to detect such particle elicit hydroxyl radical generation in presence of hydrogen peroxide (H_2O_2). This method is mainly sensitive to metal mediated reactive oxygen species, especially OH·, by Fenton-type-reactions.
Described briefly, the method consists in a particle suspension being mixed with the chemical ingredients, followed by incubation for 15 minutes at 37 °C in a heated shaking water bath prior to EPR analysis. For particle suspension preparation prior EPR analysis see SOP – Dispersion protocoll_sonication_cup horn_1.1.

3 Materials & Instruments

3.1 Materials

The following materials and chemicals are required:

- Particle suspension for investigation
- Dulbecco´s Phosphate Buffered Saline (PBS) without calcium chloride and magnesium chloride (provider Sigma Aldrich)
- 0.05 M 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) (provider Enzo Life Science) in PBS buffer
- 1.5 ml centrifuge vials
- 0.5 M hydrogen peroxide (H_2O_2 p. A.) in PBS buffer 50 µl EPR capillaries/micropipettes
- Haematocrit sealing compound
- Cleaning wipes
- Pipette
- Ethanol (for hydrophobic materials; for dispersion a mixture of dH2O with Ethanol 0.5 vol% is used).

3.2 Instruments

The following instruments are required:

- Electron paramagnetic resonance (EPR) spectrometer (MS-300 or higher, Magnettech GmbH, Berlin)
- Heated, shaking water bath
- Vortexer (≥ 2800 rpm)

Note: Only the settings for the EPR instrument but not the usage and maintenance of the instruments will be described in this SOP. Please refer to the appropriate manuals.

4 Experimental procedure

4.1 Preparation and measurement

- 25 µl of the particle suspension for investigation, 50 µl DMPO (0.05 M) and 25 µl H_2O_2 (0.5 M) are filled in an appropriate vial (e. g. 1.5 ml centrifuge vial) by pipette.
  Note: The volume is variable whilst the ratio of 1/2/1 is kept.
- The suspension is mixed by vortexing for 30 seconds
- The vial is placed in a heated shaking water bath (60-100 movements/min) and incubated for 15 minutes at 37 °C in the dark
- The suspension is mixed by vortexing for additional 10 seconds
- The suspension is transferred to the EPR capillary, the capillary is sealed at the bottom side by Haematocrit sealing compound and cleaned with cleaning wipe
- The capillary is placed in the EPR cavity and the EPR signal is measured for the following settings

4.2 EPR settings

- Microwave frequency: 9.39 GHz, Magnetic field: 3.365 G, sweep width: 100 G, scan time: 30 s, number of scans: 3, modulation amplitude: 2 G, receiver gain: adapted to signal intensity (10-900); settings at room temperature.

4.3 Quantification

- Quantification is carried out with the Analysis Software (2.0 or higher, Magnettech GmbH, Berlin) on first derivation of EPR signals of DMPO–OH quartet as the average of total amplitudes and expressed in arbitrary units (a.u.).
- As blank a mixture of dH_2O, H_2O_2 and DMPO (ratio 1/1/2) were used. For the hydrophobic materials a mixture of dH_2O with Ethanol (0.5 vol%) was additionally measured. Additionally analysis was checked by an internal positive control of $CuSO_4$ (2.5 mM) showing a good reproducibility (CV ≤ 10%).

Note: For using the Analysis software please refer to the manual.

5 Safety precautions

For all working steps protective clothing, safety goggles and gloves have to be worn.

6 Waste disposal

Sample and chemical vials, as well as EPR capillaries have to be collected and disposed off separately.

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