Electron paramagnetic resonance (EPR) spectroscopy analysis using the spin probe CPH

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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 probe 1-hydroxy-3-carboxy-pyrrolidine (CPH) to detect a “surface reactivity” caused by particle surface bounded compounds and/or the physical-chemical characteristics (e. g. electron transfer).

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

2 Basics

The potential ROS (reactive oxygen species) activity of new materials depending e. g. on the physical-chemical properties like chemical identity, surface area or shape is forwarded nowadays as a possible additional characterisation metric for novel materials. Due to their properties, especially their surface area as the interface between the material and the environmental media, the materials might induce electron transfers or generation of oxidants e. g. superoxide ($O_2^{\cdot}$), possibly is revealing a “surface reactivity”.
One promising method for the detection of a “surface reactivity” is the electron paramagnetic resonance (EPR) spectroscopy using the spin probe 1-hydroxy-3-carboxy-pyrrolidine (CPH). This method is mainly sensitive for the detection of superoxide ($O_2^{\cdot}$) and induced electron transfers, both leading to a CPH transformation into the paramagnetic CP·.
Described briefly, the method consists in a particle suspension being mixed with the CPH and incubated for 10 minutes at 37 °C in a 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).
- 1 mM 1-hydroxy-3-carboxy-pyrrolidine (CPH, provider Enzo Life Science) in PBS
- desferoxamine (DFO)
- 1.5 ml centrifuge vials
- 50 µl EPR capillaries/micropipettes
- Haematocrit sealing compound

- Cleaning wipes
- 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

- 30 µl of the particle suspension for investigation and 30 µl of a DFO (final concentration 10 µM) and CPH (final concentration 0.5 mM) mixture are filled in an appropriate vial (e. g. 1.5 ml centrifuge vial) by pipetting.
  Note: The volume is variable whilst the ratio of 1/1 is kept constant.
- The suspension is vortexed for 30 seconds
- The vial is placed in a heated shaking water bath (60-100 movements/min) and incubated for 10 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 seconds, number of scans: 3, modulation amplitude: 2 G, receiver gain: adapted to signal intensity (10 - 900); settings at room temperature.

4.3 Quantification

- The quantification is carried out by Analysis Software (2.0 or higher from Magnettech GmbH, Berlin) on the first derivation of EPR-signals of the $CP^{\cdot}$ triplet as the average of

total amplitudes and is expressed in arbitrary units (a.u.).

- The detected values are expressed as ratio to the time-dependent blank (dH_2O). Due to the method and used spin probe the blank (dH_2O) EPR signal is increasing over time caused by an auto-oxidation of the spin probe. Consequently for each measuring time point a slightly different blank signal exist and is calculated via regression analysis including at least four measuring time points of a blank. The results of the blank (dH_2O) measurement revealed always a highly linear correlation/increase ($R^2 > 0.95$) of the signal over time. The calculated regression was used for correcting the sample values (adjusted to time).
- As blank a mixture of dH_2O and CPH 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 NM300K (Nanosilver, JRC) showing a reproducibility (CV ≤ 20%).

  Note: For use of 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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Responsible for the implementation of this SOP: Measurement personal

Previous changes: Primary version

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