Tracking Free Radicals in the Environment with Electron Paramagnetic Resonance (EPR)

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Electron paramagnetic resonance (EPR) spectroscopy is a technique that detects species that have unpaired electrons. A large number of materials have unpaired electrons, including free radicals, many transition metal ions, and defects in materials. Free radicals are often short-lived, but play crucial roles in significant processes such as photosynthesis, oxidation, catalysis, and polymerization. Consequently, the application of EPR spans one of the widest ranges of any major analytical technique, from molecular research to quality control in fields as diverse as: chemistry, quantum physics, structural biology, materials science, medical research and many more.

As EPR data can be collected in seconds, and the analysis of the data delivers not only the identity, but also quantitative information about the species being measured, it provides a useful tool for environmental analysis. EPR spectroscopy is a valuable tool for environmental research as it is not only used to track free radicals, but can also detect toxic metal ions in air, groundwater and soil, and follow their uptake by plants, as outlined in this paper.

EPR for air pollution, soil analysis and water pollution

I. EPR for air pollution

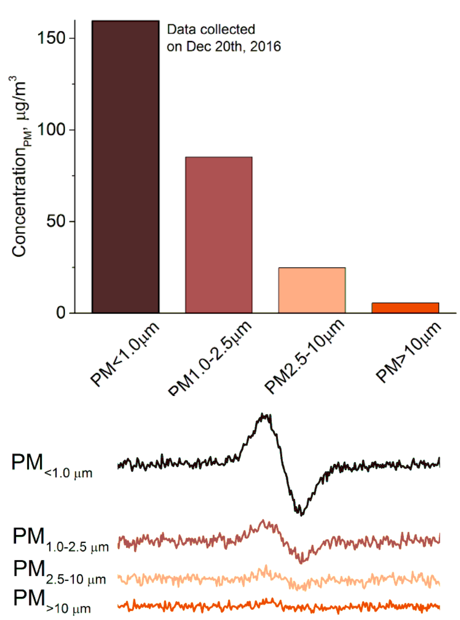
Outdoor air pollution is a major environmental hazard that affects human health worldwide. The link between inhalation of ambient particulate matter (PM) and various adverse health effects is documented extensively by epidemiological and toxicological studies. Below are some selected transition metals and free radicals:

- Transition metals (Fe, Cu, Mn, Cr, V, Co, Mo, W) are identified as crucial PM components triggering hydroxyl radical (•OH) generation via Fenton-like reactions. This shows that the oxidative potential of PM is an important health-relevant metric.
- Reactive oxygen and reactive nitrogen species (ROS and RNS) are produced from polycyclic aromatic hydrocarbons (PAHs) and redox cycling quinoids that are part of PM. These short-lived toxic radicals have tremendous potential for harmful oxidative effects in pulmonary tissue.

- Long-lived radicals, called environmentally persistent free radicals (EPFRs), are also part of ambient PM. They are typically oxygen-centered semiquinone or carbon-centered PAH radicals that promote the generation of ROS. Their half-life varies from several days to several months and even indefinitely on the internal surface of fine particles.

Identifying and monitoring generation of free radicals from ambient particulate matter and determining their oxidative potential is of great concern due to adverse effects on human health. Figure 1 is an EPR study on airborne PM in Beijing during haze events (1).

Figure 1



EPR study on airborne PM in Beijing during haze events

The results show that EPR detects EPFRs identified as semiquinone radicals in PM with different particle size and that EPFRs are mainly persistent in the PM fraction of $d_{ae} < 1$ µm which are the most hazardous. The daily monitoring of the EPFRs (spins/g) shows environmental changes that impact long-term effects on human health. Such monitoring can be used to enact counter measures to reduce health risks to the public.

II. EPR for soil analysis

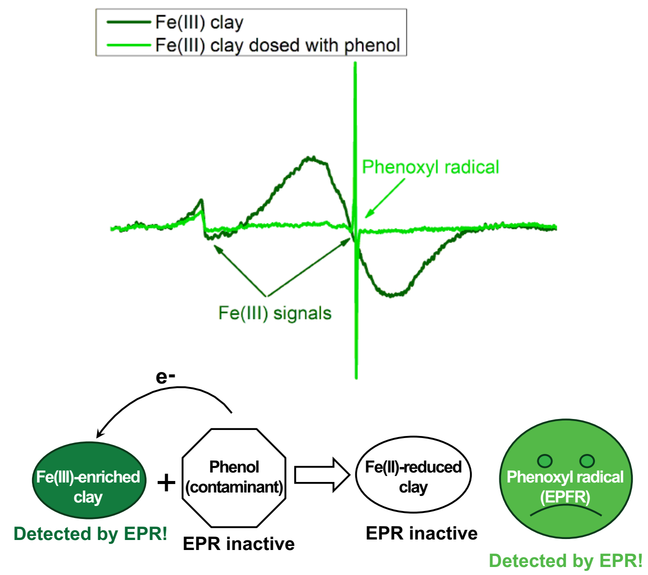
Soil pollution is a direct result of industrial, agricultural, and societal growth. Many of the common pollutants can be detected with EPR leading to measures to control their distribution and aid in clean-up strategies.

Common soil pollutants are a result of industrial waste, including poisonous gases, cytotoxic chemicals, radioactive materials and cancer causing agents. They can also be a result of industrial heavy metal by-products such as cadmium, chromium, lead and mercury, or agricultural burdens including pesticides, insecticides, herbicides and fertilizers.

All these pollutants are toxic and often participate in processes resulting in the formation of surface-stabilized EPFRs. EPFRs play a role in the further generation of toxic compounds and are additionally involved in radical processes that impact the formation of humic substances, and carbon sequestration. It has also been found that EPFR-containing particles can generate ROS that induce oxidative stress.

Metal toxicity studies using EPR have been conducted on plants ranging from lichens to lupins. EPR has also been used to examine the mobility and availability of transition metal ions such as manganese (II) in soils.

Figure 2



Free radicals in Fe(III)-enriched clay

Detailed research is required to understand the impact of pollution from industrial and agricultural sources on the soil environment. Understanding the mechanisms and roles of the inorganic, organic, and biological components of soil leads to effective strategies to neutralize toxic compounds. EPR works well in soil analysis as scientists can identify, quantify and monitor long-lived ERFRs in soil organic matter, short-lived ROS's and paramagnetic heavy metals by analyzing an EPR signal.

Figure 2 details research on free radicals in Fe(III)-enriched clay (2). Clay minerals act as a potential reservoir of transition metals and toxic organic pollutants.

In this example, EPR demonstrates the catalytic role of transition metal centers (Fe^{3+}) in phenol contaminated clay minerals in the formation of EPFRs. EPR monitors and quantifies the production of EPFRs via oxidation-reduction mechanism.

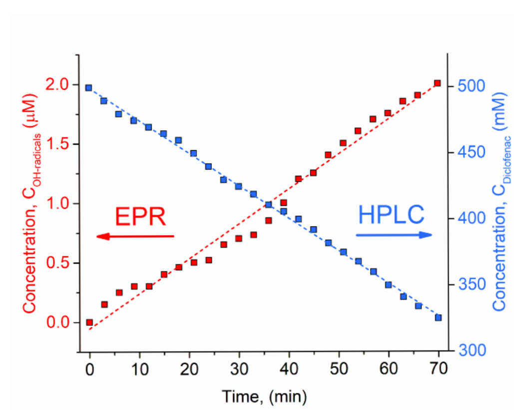
III. EPR for water pollution

Hazardous organic waste that is widely spread in water by industrial, military and domestic sources, is a current and universal pollution issue. Advanced Oxidation Processes (AOPs) are efficient methods to remove these contaminants that are not biologically degradable. AOPs are based on the chemistry of hydroxyl radicals (•OH), which are non-selective ROS, able to oxidize water pollutants into inactivated end-products, yielding carbon dioxide and salt.

The design and optimization of AOPs depends on several parameters, including reagent dosage, additional reactants, and reaction time. The optimal conditions must be determined in order to achieve the most effective treatment and reduce operating costs.

The hydroxyl radical is the most reactive species in AOPs and its interaction with the pollutants determines the efficiency of the oxidation process. Therefore, it is very important to increase the yield of hydroxyl radicals generated during AOPs. Figure 3 shows an EPR study on pharmaceutical residues (3).

Figure 3



AOP – pulsed corona plasma - EPR study on pharmaceutical residues

The research had seven resistant pharmaceutical agents (Diclofenac, Ibuprofen, Diazepam, etc.) decomposed by pulsed corona plasma generated in water. The degradation of Diclofenac measured by HPLC is directly correlated to the increase in hydroxyl radical concentration over time and found that hydroxyl radicals detected by EPR are responsible for the decomposition of pharmaceutical compounds.

By using EPR spin-trapping technique, the user can identify, quantify and monitor intrinsic generation of short-lived radicals such as hydroxyl radicals produced during AOPs. In spin trapping experiments unstable radicals are converted to stable radicals by reactions with spin-trapping agents and are detected by EPR.

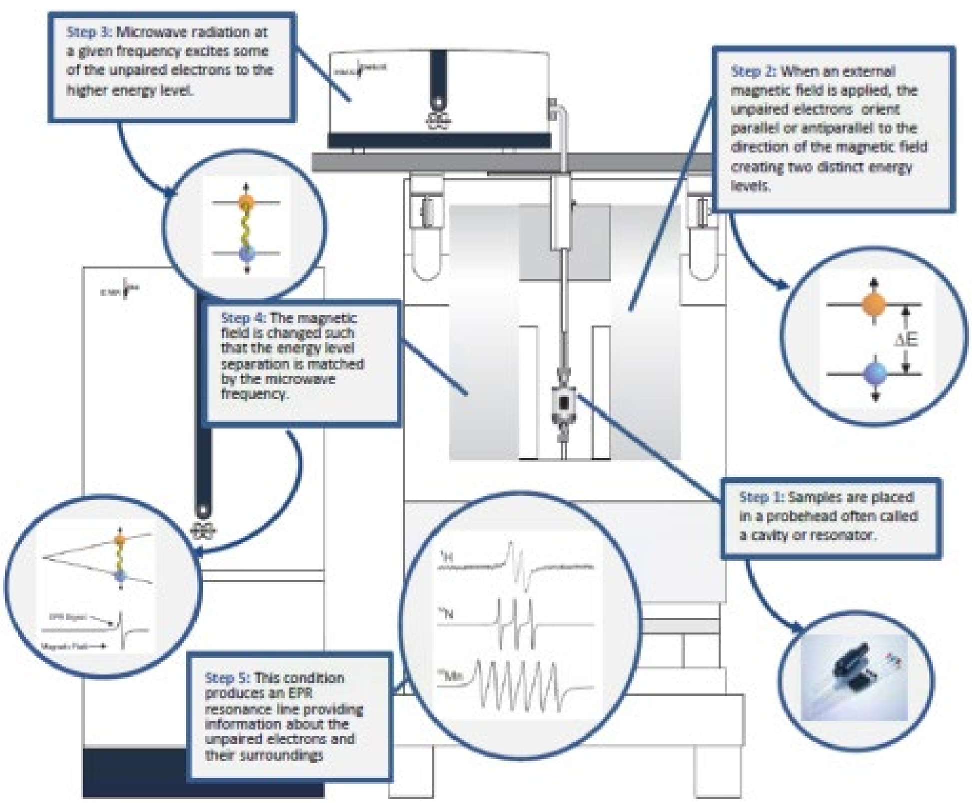
CASE STUDY: *EPR 101*

EPR is a magnetic resonance technique very similar to NMR (Nuclear Magnetic Resonance). However, instead of measuring the nuclear transitions in a sample, the transitions of unpaired electrons in an applied magnetic field is detected. Like a proton, the electron has "spin", which gives it a magnetic property known as a magnetic moment. The magnetic moment makes the electron behave like a tiny bar magnet. When an external magnetic field is applied, the unpaired electrons can either orient in a direction parallel to or antiparallel to the direction of the magnetic field. This creates two distinct energy levels for the unpaired electrons and allows the measurement of them as they are driven between the two levels.

Initially, there will be more electrons in the lower energy level (i.e., parallel to the field) than in the upper level (anti-parallel). A fixed frequency of microwave irradiation is used to excite some of the electrons in the lower energy level to the upper energy level. For the transition to occur the external magnetic field must be at a specific strength, such that the energy level separation between the lower and upper states is exactly matched by the microwave frequency. To achieve this condition, the external magnet's field is 'swept' while exposing the sample to a fixed frequency of microwave irradiation. The condition where the magnetic field and the microwave frequency are "just right" to produce an EPR resonance (or absorption) is known as the resonance condition.

Below is a diagram of a typical EPR spectrometer showing the 5-steps in a standard measurement.

Figure 4



Typical EPR spectrometer

EPR in environmental use – working with customers

The application of EPR, such as those listed above, has also been driven by developments in instrumentation. The fundamentals of sensitivity, resolution and stability are all related to the microwave or magnet technology in the spectrometer. Users and instrument manufacturers, like Bruker, have worked in close partnership to push the boundaries of application and create increasingly powerful, stable and flexible EPR spectrometers.

Decades of experience at the forefront of EPR research have made Bruker's benchtop offerings suitable for a wide-range of laboratory types. The desktop research quality EMXnano, the quality control e-scan, and the compact microESR all require minimal infrastructure and low cost of ownership for customers.

Customers around the globe are utilizing the latest in EPR technology for their environmental applications. One example is the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences that is conducting two projects funded by National Nature Science Foundation of China (NSFC) and Ministry of Science and Technology. Guorui Liu, associate professor position in the State Key Laboratory of Environmental Chemistry and Eco-toxicity (SKLECE) at Research Center for Eco-Environmental Sciences (RCEES), explains the aims for these projects:

"One of the important aims of these two projects is clarifying the free radical mechanisms during formations of dioxins and other unintentional persistent organic pollutants (POPs). The samples we tested using EPR include airborne particles, fly ash from industrial plants and contaminated soils. We also performing the in-situ monitoring of free radicals formed during designed thermochemical reactions of organic chemicals."

The SKLECE has worked with Bruker for over 10 years with 3 years of communication in the area of free radicals monitored by EPR. Guorui Liu and her team purchased the EMX-plus X-band EPR and FT-MS from Bruker, helping the team achieve progress in clarification of EPFR contamination, formation and control.

Guorui Liu describes the methods in use for environmental research and the scope for further development with Bruker for methods in complex environmental samples:

"We previously used GC/MS or GC/HRMS for monitoring POPs contaminations and we use EPR for detection of free radicals or EPFRs in environmental samples. However, the accurate attribution of the free radicals in complex environmental samples can still prove a challenge by EPR alone. We see scope to collaborate with Bruker to develop methods for the structure identification of EPFRs in complex environmental samples."

EPR in the future

The 21st century environmental pollution issues are clear for all to see. According to a World Health Organization (WHO) study, nine out of every 10 people on the planet breathe air that contains high levels of pollutants, killing seven million people each year (4). People across Asia and Africa face the biggest issues with more than 90% of air pollution-related deaths. Additionally, cities across the Americas, Europe and the Eastern Mediterranean also have air pollution levels that are beyond WHO health limits.

Since its commercialization in the 1950s, EPR has become an increasingly valuable method in detecting free radicals. Benchtop EPR systems, have made the technology even more accessible, offering greatly enhanced ease-of-use, reduced cost of ownership, and advanced capabilities in a minimal footprint.

As scientists get to grips with EPR, the possibilities of the method have grown across a number of areas in addition to environmental, such as health and food. For example, EPR can be used to detect the carbon based radicals derived from roasting coffee beans or toasting bread, radicals from drug degradation, and even to detect antioxidants' radicals in vitamins. The importance of EPR as a detection tool is one that continues to evolve.

For information about Bruker EPR instrumentation, and to access live and on-demand webinars and other training materials please visit:
https://www.bruker.com/products/mr/epr.html

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