

Mass Spectrometry & Spectroscopy

Using Electron Paramagnetic Resonance (EPR) to Track Free Radicals in the Environment

Kalina Ranguelova, PhD, EPR Applications Scientist at Bruker BioSpin Corp

A number of chemicals exist within food, medicines and the environment that have the ability to form free radicals (single atoms with unpaired electrons). For example, oxidative stress occurs when an oxygen molecule splits into free radicals, which may persist in the environment and enter biological systems. Inside the body, free radicals can attack biomolecules, damaging cells, proteins and DNA and potentially leading to disease.

Monitoring free radicals and other species with unpaired electrons (such as some transition metal ions and defects in materials) in the environment is therefore of critical importance. Free radicals are usually short-lived, and play important roles in processes such as photosynthesis, oxidation, catalysis and polymerisation. However, long-lived species, known as environmentally persistent free radicals (EPFRs), can remain in the environment almost indefinitely, especially when associated with the surfaces of fine particles [1].

Electron paramagnetic resonance (EPR) spectroscopy is the only analytical technique capable of detecting species with unpaired electrons, in a direct and non-invasive manner. It can be used to detect, quantify and monitor the intrinsic generation of short-lived radicals, such as hydroxyl radicals, as well as EPFRs. EPR is therefore extremely useful for tracking free radicals in the environment, in addition to detecting toxic metal ions in the air, groundwater and soil, and monitoring their uptake by plants. By furthering this knowledge, researchers can also increase their understanding of the behaviour of free radicals in the body and their impacts on human disease.

Analysing different substrates

Pressure on global governments and companies to curb environmental pollution has been mounting over recent years, so the requirement for advanced analytical equipment to reliably monitor toxins in the air, water and soil is growing. Quantitative EPR data on the identity of a species can be collected in minutes, making this technology ideally suited to environmental analysis.

Soil analysis

Soil pollution can arise from a number of sources, and impacts on a range of environmental and agricultural processes. Toxins can be assimilated by plants, and leach into groundwater and distribute across landscapes, potentially entering food systems. Common soil pollutants originate from industrial waste and heavy metal by-products, and from agricultural practices such as pesticide, insecticide, herbicide and fertiliser use.

One example of the use of EPR spectroscopy to understand the generation and properties of free radicals is in the analysis of biochar – a substance often added to soil to improve fertility and increase carbon sequestration. However, it is thought that the process of biochar production (heating biomass at high temperatures) can generate free radicals and potentially limit plant growth, although there are a limited number of studies which have assessed this. Liao et al. conducted experiments on three different types of biochar derived from corn stalk, rice, and wheat stalk biomass, and observed EPR signals in the biochar but not in the original material, therefore concluding that free radicals exist in the biochar (Figure 1) [2].

The free radicals detected in this experiment were found to be persistent, as the EPR signals were stable with approximately less than a 10% signal decrease after one month. This could be due to the generation of free radicals near to the solid particle surface, therefore causing strong interactions.

Understanding how EPFRs form in contaminated soils and sediments is extremely important. Transition metals, such as Fe, Cu, Mn, Cr, V, Co, Mo, W, can act as catalysts for EPFR production, as well as short-lived reactive oxygen species (ROS) such as hydroxyl radical ($\cdot\text{OH}$), and are often toxic themselves.

Air pollution

Contaminate particles in the air perhaps receive the most widespread media attention as a key environmental hazard. Many epidemiological and toxicological studies have drawn a link between the inhalation of ambient particulate matter (PM) and a number of health conditions, such as cardiovascular and pulmonary disease, and cancer. Transition metals have been identified as crucial PM components, triggering $\cdot\text{OH}$ generation.

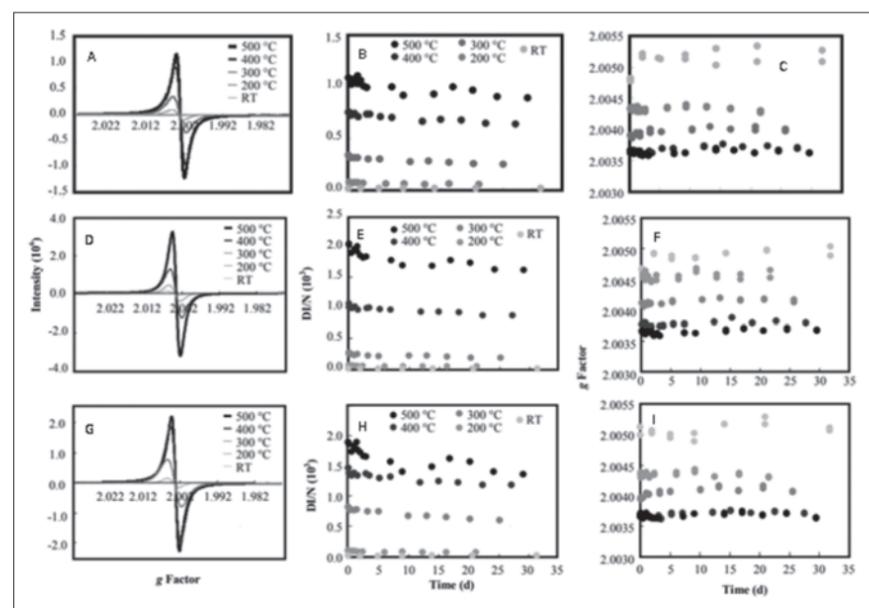


Figure 1: EPR signals detected in the biochars produced from rice straw (A, B, and C), wheat stalk (D, E and F) and corn stalk (G, H and I). The EPR signal intensities (A, D, and G), that normalised the intensities (DI/IN) (B, E, and H) and g factors (C, F, and I) are presented. Both the DI/IN and g factors indicated that these free radicals were stable for over one month. (Figure reproduced from reference [2]).

This occurs via Fenton-like reactions, which results in the formation of $\cdot\text{OH}$ radicals from hydrogen peroxide and an iron catalyst. Short-lived ROS and reactive nitrogen species (RNS) are produced from polycyclic aromatic hydrocarbons (PAHs), which are frequently found in ambient PM and are known to have toxic and mutagenic effects in the body, as do their oxygenated derivatives [3]. EPFRs have also been observed in PM and have the ability to generate ROS in the body.

A study to quantify the traffic-related oxidative potential (OP), or $\cdot\text{OH}$ generation potency, assessed samples of ambient PM alongside a motorway in Germany during a one year period. Upwind and downwind sample sites were included and measurements analysed hydrogen peroxide-dependent oxidant generation by EPR spectroscopy [4].

Previous studies have demonstrated a relationship between intrinsic (non-cellular) PM-induced generation of $\cdot\text{OH}$, and biological adverse health effects. Therefore, the intrinsic particle-induced ROS generation potency could be a promising additional metric linking environmental monitoring and adverse health effects. Transition metals, soot or quinones are assumed to trigger ROS generation and as transition metals and soot are crucial parts of traffic emissions. The study found that transition metals were the major compounds of PM triggering $\cdot\text{OH}$ generation near the motorway.

Water pollution

Hazardous organic waste is widespread in water systems across the globe by industrial, military and domestic sources, and is a persistent and universal pollution issue. Advanced Oxidation Processes (AOPs) are efficient methods to remove these contaminants and are based on the chemistry of $\cdot\text{OH}$ radicals, which are non-selective ROS, being able to oxidise water pollutants into inactivated end-products, yielding carbon dioxide and salt. AOPs decontaminate water through the destruction of organic pollutants, the neutralisation of transition metals, and the inactivation of bacteria and viruses.

By using a EPR spin-trapping technique, the intrinsic generation of short-lived radicals such as $\cdot\text{OH}$ produced during AOPs can be identified, quantified and monitored. In spin-trapping experiments, unstable radicals are converted to stable radical adducts by reactions with spin-trapping agents and are detected by EPR.

A recent study on DDT (Dichlorodiphenyltrichloroethane), one of the most persistent pesticides with a half-life of 2-15 years, used EPR to directly detect the reactive species involved in DDT degradation [5]. Two radicals were identified: the sulphate anion radical ($\text{SO}_4^{\cdot-}$) and hydroxyl radical ($\cdot\text{OH}$), using 5,5-dimethyl-pyrroline N-oxide (DMPO) – one of the most commonly-used spin traps. In this study, a Co^{2+} ions-activated peroxymonosulphate (PMS) system was used to degrade DDT in aqueous solutions. Figure 2a shows that $\text{SO}_4^{\cdot-}$ and $\cdot\text{OH}$ were produced in the PMS/ Co(II) system, and Figure 2b shows that the addition of ethanol inhibits the degradation of DDT.

The study also found that the degradation of DDT was facilitated by increasing temperature and reducing solution pH, while higher pH lowered the degradation efficiency of DDT. These findings allow researchers to develop useful remediation strategies for DDT-contaminated groundwater, and therefore minimise risk to environmental and human health.

The future of EPR

There is no escaping the environmental pollution issues of the 21st century. According to a World Health Organisation (WHO) study, nine out of every 10 people on the planet breathe highly polluted air, which kills seven million people each year [6].

Since its commercialisation in the 1950s, EPR has become an increasingly valuable method in detecting free radicals, in material research and structural biology, as well as environmental contamination. Bench-top EPR systems have greatly enhanced the ease-of-use, reduced cost of ownership, and advanced capabilities while minimising footprint.

EPR can now 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 continuously evolving.

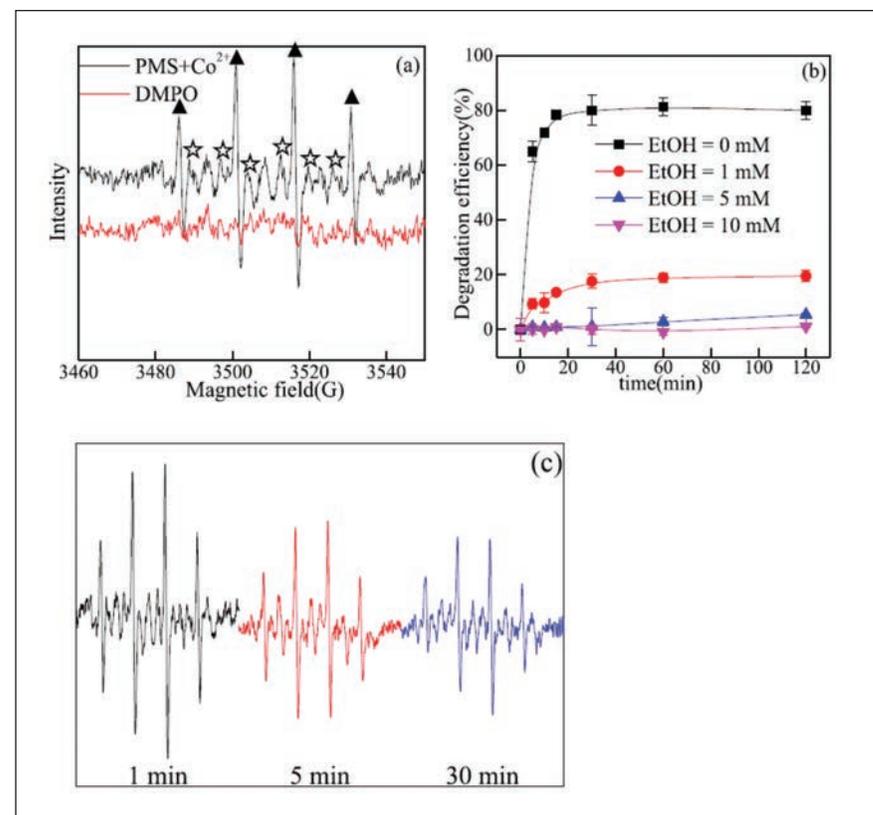


Figure 2: EPR spectra of 5,5-Dimethyl-1-pyrroline N-oxide (DMPO)-OH and DMPO-SO₄ adducts in peroxymonosulphate (PMS)/cobalt (Co(II)) system (a); Effect of ethanol (EtOH) on the degradation of DDT in PMS/Co(II) system (b); EPR spectra of DMPO-OH and DMPO-SO₄ adducts as a function of reaction time (c). Conditions: [PMS]₀ = 1.0 mM, [DDT]₀ = 1.0 mg/L, [Co(II)]₀ = 20 μM, [EtOH]₀ = 0-10 mM, [DMPO]₀ = 0.1 M, T = 30°C, SO₄^{•-} adduct, $\cdot\text{OH}$ adduct (Figure used with permission from reference [5]).

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