Electron paramagnetic resonance

EPR everywhere

Electron paramagnetic resonance (EPR) measures the signal from unpaired electrons and is a technique already widely used in the bioscience and pharmaceutical industries. Dr Joshua R. Biller of TDA Research, Inc, USA is championing the novel use of EPR to answer research questions. He explains that rapid innovations in EPR will allow a wider group of researchers to image tumours, learn about the structure and function of proteins, and observe the mechanisms used by enzymes, amongst many other possibilities.

Spectroscopy is the study of the interaction between a substance and electromagnetic radiation. The output is called a spectrum and can be viewed as the signature of the sample. Whilst spectroscopy is vital to progressing knowledge about the structure of atoms and molecules, and in analysing materials with unknown chemical composition, it also has practical applications. Spectroscopy can be used to improve the structure of drugs, to search for biomarkers of disease, and is the basis of medical imaging.

A well-known form of spectroscopy is nuclear magnetic resonance (NMR) spectroscopy. NMR is a technique used to explore magnetic fields around the very centre of atoms, an area called the nucleus. An atom is the smallest particle of a chemical element that can exist, and it is made up of protons, electrons, and neutrons. NMR is most often used in chemistry to identify different molecules, as each molecule has its own distinct spectrum. NMR is part of a broader category of spectroscopic techniques based on the magnetic properties of an atom. A second type of magnetic spectroscopy which measures information about unpaired electrons is called electron paramagnetic resonance (EPR).

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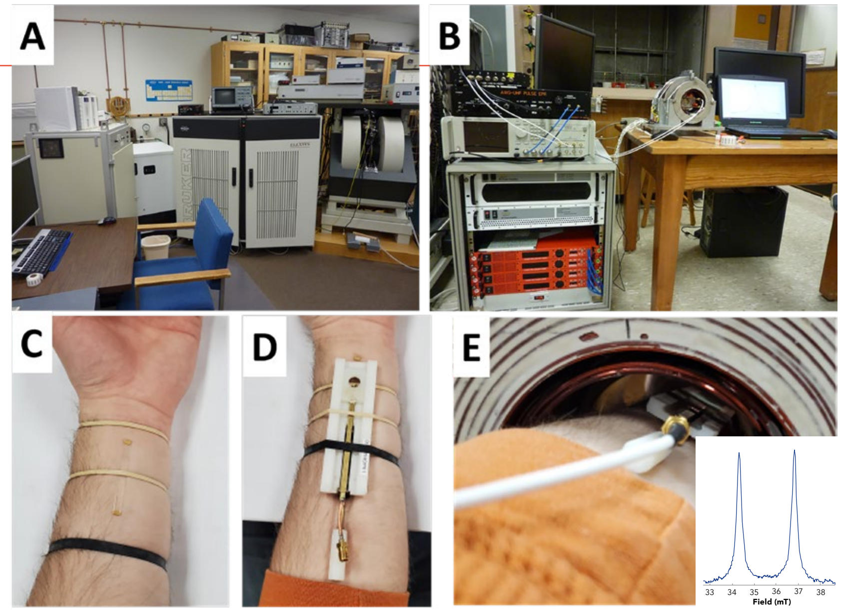
THE MAGNETIC PROPERTIES OF ELECTRONS

Usually, electrons exist in pairs as this is the preferred lowest energy state. The magnetic characteristic of an electron is cancelled out when it is paired, and compounds with all paired electrons are termed diamagnetic. However, many compounds exist with unpaired electrons which can interact with an external magnetic field, and this is called paramagnetism.

Electron paramagnetic resonance (EPR) spectrometry is the study of molecules or atoms with unpaired electrons. It was first discovered by Yevgeny Zavoisky in 1944 in Kazan, Russia. The technique characterises the paramagnetic material by placing it in a magnetic field and applying microwave radiation. The EPR spectrum provides information on the type of chemical environment that the unpaired electron resides in.

In EPR, the environment surrounding an unpaired electron can be carefully measured and analysed. This information can be used to learn more about materials, including metals like iron, nickel, or chromium, as well as a variety of organic radicals.

Historically EPR instruments have been developed to operate at increasingly higher magnetic field strengths, since the sensitivity of the experiment increases with the applied magnetic field. However, advances in computer power and improvement in the quality of electronics now allow quality EPR experiments in magnetic fields with low strength. This leads to more opportunities to use the technique outside a laboratory setting, as has been done with NMR. One benefit of low field EPR is sample preparation, which is very different from the traditional high field approaches. For example, low field EPR can be used to analyse much larger sample sizes.



Credit: Gareth and Sandra Eaton/University of Denver

Figure 1. (A) Traditional, laboratory sized EPR spectrometer operating at 9.5 GHz. **(B)** Next generation complete EPR imaging system at 1 GHz. All waveform generators, power amplifiers and gradient amplifiers sit in a 3 ft x 3ft box. The electromagnet is about the size of the laptop used to run the instrument. The larger analysis space in **(B)** permits use of a surface coil resonator as demonstrated in **C-E**. **(C)** a small flat vial is laid down on the forearm containing 15N, d13-Tempol (9.4 mM) **(D)** a low-profile surface coil resonator is attached over the arm and sample **(E)** The EPR spectrum (inset) is recorded with good signal strength in about 60 s.

Over the years, there have also been changes in how data are collected from EPR. Rapid-scan allows the magnetic field to be repeatedly scanned much quicker than conventional EPR, leading to an increase in signal acquired per unit time. Pulsed EPR allows different types of interactions between the unpaired electron and surrounding nuclei to be separated more efficiently.

> **Electron paramagnetic resonance (EPR) spectrometry is the study of molecules or atoms with unpaired electrons.**

EPR was once considered inflexible, expensive, and only useful in a laboratory setting. However, advancements in technology mean that this impression no longer rings true and the EPR community are keen to show others the benefits of the technology. One example of this outreach is the NSF-funded SHARED-EPR network (sharedepr.org). The website is a portal to the EPR community designed to bring together scientists from the fields of chemistry, biochemistry, physics, materials science, medicine, and biology to disseminate and advance the field of EPR spectroscopy.

Dr Joshua Biller, of TDA Research, Inc, USA, claims that EPR is often under-recognised and under-appreciated, and his aim is to change this.

HOW DOES EPR WORK?

The EPR sample is held in the magnetic field in a structure called a resonator. This is where the microwaves interact with the sample and a signal is detected. Newer resonators have a much wider range of geometries than in older spectrometers which permits analysis of a much wider range of samples, and also a wider range of sample sizes. This is one limitation to wider application of EPR which has now been overcome.

Dr Biller explains that another previous limitation of EPR was a lack of computer power; the hardware did not have a large enough capacity to analyse the data. Due to a rapid progression of technologies, it is now possible to use miniaturised electronic devices with sufficient processing power, to analyse results. One of the main advantages of this improvement in computing power and resonator geometries is that EPR hardware can now be configured to analyse samples in situ (in their natural environment).

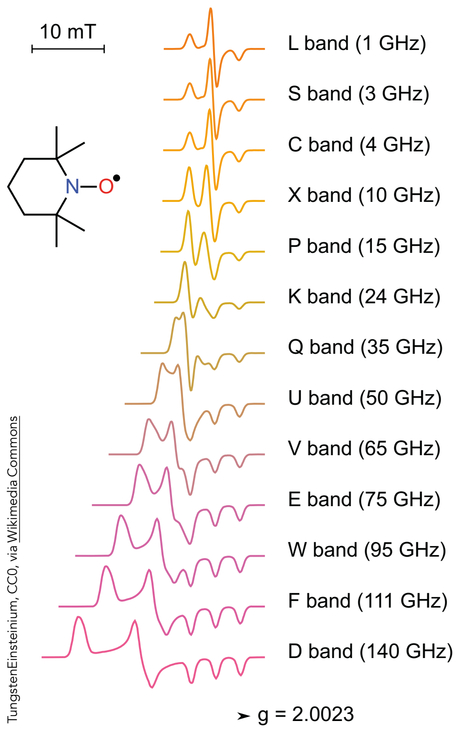
The study of molecules or atoms with unpaired electrons: electron paramagnetic resonance (EPR) was first discovered in 1944 by Yevgeny Zavoisky in Kazan, Russia.

USING EPR TO ANSWER RESEARCH QUESTIONS

Dr Biller highlights several novel uses for EPR, in treating cancer and learning more about neurodegenerative diseases.

Low oxygen levels are seen in tumours with uncontrollable growth and proliferation, as the formation of abnormal blood vessels can lead to reduced transport of oxygen and nutrients to the tumour. Tumour hypoxia has shown to be linked to a poor prognosis and increased resistance to treatment, therefore the ability to modify treatment based on oxygen levels may help improve survival. One major limitation has been the inability to identify patients for whom this approach may be helpful.

In pre-clinical imaging, EPR is used to image tumour pO_2 to help guide radiotherapy treatment. This is done through oxygen-sensitive spectroscopy and imaging, which is used to work



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Variation in the EPR spectrum of a nitroxide radical as the energy of excitation changes.

out the quantitative 3D distribution of oxygen in a solid tumour. Knowledge of this oxygen distribution can improve how well radiotherapy works, and minimise collateral damage to surrounding healthy tissue. There is currently a push to translate this approach into more clinical settings.

Dr Biller currently leads the development of an injectable nano-sized encapsulated EPR imaging agent designed to be used for clinical tumour imaging. Bridging the gap between pre-clinical animal imaging and clinical imaging in humans is a major effort of the community involving collaborators in government, academia, and industry. A parallel effort to move EPR into clinical use is being led by Periannan Kuppusamy at Dartmouth College in the USA. Dr Kuppusamy's implantable EPR oxygen sensor (OxyChip) is undergoing clinical trials for use in humans and has so far shown no adverse clinical effects.

Clinical imaging is a developing contribution of EPR to biomedical work, but site-directed-spin-labelling (SDSL) has been used routinely to gather information about proteins which play important roles in neurodegenerative conditions such as Parkinson's and Alzheimer's disease. Spin labels are molecules that have an unpaired electron, and are able to bind to another molecule, such as amino acids which are the building blocks of proteins. In SDSL at least two EPR active molecules are attached to different amino acids on a protein. The interaction between the two spin labels can be measured, and used to monitor conformational changes in proteins under certain conditions. In this way EPR and SDSL can be used to investigate the structure of proteins in their native state, and determine how structural changes influence disease state.

Outside the realm of biomedical use, new forms of EPR are finding use for samples which are too large to fit in the spectrometer, and too valuable to be destroyed. An EPR mobile universal explorer (EPR-MOUSE) enables in situ analysis of challenging samples. Samples, such as culturally significant paintings, can be placed directly on top of the instrument and analysed for the presence of different pigments containing EPR detectable transition metals. Novel EPR spectrometers have been developed based on voltage-controlled-oscillators (VCOs) which allow the entire EPR spectrometer to be miniaturised – down to only a few square millimetres in size. At such a size the EPR device can be used to monitor control processes in manufacturing. In situ EPR applications can help expand the field into new applied research directions, while also identifying new and exciting questions for basic research to solve.

'SPINS' ARE EVERYWHERE

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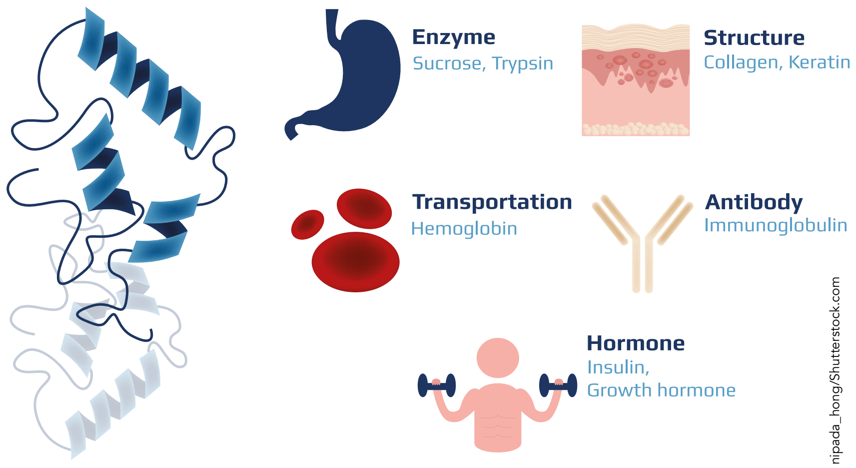
EPR can be applied to many research problems, including neurodegenerative diseases, and may play a vital role in uncovering new ways to combat these conditions. The technology can also be used in the pharmaceutical industry to monitor product stability and shelf life. The same types of information that are important to medical researchers, like redox state, oxygen concentration, and pH, are also of interest to researchers looking at technologically advanced materials like carbon fibre and those interested in corrosion research. In situ materials research represents an untapped potential for future EPR applications.

Since its discovery in the 1940s, EPR has become an increasingly elegant and accessible technology, and with a focus on securing funding to continue to explore the abilities of EPR, its research potential is boundless.



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Site-directed-spin-labelling (SDSL) EPR can gather information about proteins which play important roles in neurodegenerative conditions such as Parkinson's and Alzheimer's disease.

Behind the Research

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Research Objectives

Dr Joshua R. Biller utilises advances in EPR to answer research questions with a broad range of vital applications.

Detail

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Bio
Joshua R. Biller completed his Chemistry degree (BS) at Edgewood College in 2005 and then began work as a Clinical Chemist at the Mayo Clinic in Minnesota. In 2009 he joined the group of Gareth and Sandra Eaton at the University of Denver. He completed his thesis "Nitroxide Radicals for Low Frequency Electron Paramagnetic Resonance Imaging (EPRI)" in 2014. From 2015–2018 he was a NRC Post Doctoral scholar at the National Institute of Standards and Technology (NIST) in Boulder, CO. In 2018 he joined TDA Research, Inc. in nearby Golden, CO where he is the primary investigator on a NCI project to develop nanoscale EPR imaging agents for clinical use.

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Personal Response

How do you think EPR will continue to develop over the next ten years?

" The first challenge of executing a successful EPR experiment, assembling the right hardware for any application, has been overcome. The second challenge is interpreting the acquired spectrum quickly and efficiently. Decreasing data analysis time allows us to talk about high-throughput EPR just like any other analytical method. The knowledge base for this has already been generated by decades of hard work from EPR researchers across the world. We now need fast, efficient ways for any user to query that knowledge of the EPR signature to quickly identify what is important. Fast analysis and spectrometers which function where the problems are can create a great expansion of EPR spectroscopy applications. "

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