



APPLICATIONS OF ELECTRON PARAMAGNETIC RESONANCE (EPR) IN MEDICAL SCIENCE: FROM FREE-RADICAL DETECTION TO ADVANCED DIAGNOSIS AND THERAPEUTIC MONITORING

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Abstract: Electron Paramagnetic Resonance (EPR), also known as Electron Spin Resonance (ESR), is a powerful spectroscopic technique for detecting and characterising species containing unpaired electrons. Unlike many conventional analytical techniques, EPR can provide direct information about free radicals, reactive oxygen species, nitric oxide, transition-metal ions, and other paramagnetic centres. These species are closely associated with oxidative stress, inflammation, cancer, neurodegenerative disorders, cardiovascular disease, tissue hypoxia, drug toxicity, and radiation injury.

The application of EPR in medical science has expanded from basic free-radical chemistry to tissue oximetry, cancer research, drug development, structural biology, pharmaceutical analysis, radiation biodosimetry, and experimental clinical diagnosis. EPR oximetry is particularly important because it can provide repeated measurements of tissue oxygen concentration and has potential applications in tumour characterisation, wound healing, diabetic complications, and peripheral vascular disease. EPR spectroscopy can also investigate the mechanisms of drugs and antioxidants by directly monitoring radical formation and disappearance. Furthermore, EPR imaging and spin-labelling techniques provide information about molecular structure, membrane dynamics, protein conformational changes, and drug-delivery systems.

This paper reviews the major medical applications of EPR and discusses its advantages, limitations, emerging technologies, and future clinical potential. Recent advances in miniaturized spectrometers, pulsed EPR, high-sensitivity resonators, and EPR imaging are expected to improve the accessibility and biomedical utility of the technique. Overall, EPR represents an important bridge between physical science and medicine, particularly for studying biological processes that involve paramagnetic species.

Keywords: Electron Paramagnetic Resonance, EPR, ESR, free radicals, reactive oxygen species, oxidative stress, cancer, tissue oxygenation, EPR oximetry, drug delivery, medical diagnosis, radiation biodosimetry.

I. INTRODUCTION

EPR spectrum can be observed when a RF magnetic field induced transition between Zeeman energy levels of unpaired electron system are available in solid, liquid and gas. The separation of the Zeeman levels of magnetic ion in a system is a function of not only the applied external magnetic field but also of the ligand field. In systems where nuclear spin is non zero, the interaction of the unpaired electrons with the nuclear Spin is manifested in the form of Hyperfine structure. In magnetically concentrated system, it is difficult to obtain well resolved spectrum exhibiting various interactions clearly because of line broadening due to dipole-dipole interaction or exchange interaction.

Hence to get valuable information about the molecular structure, suitable paramagnetic centers are doped in diamagnetic lattice of interest. EPR technique has proved to be analytical tool in extracting significant molecular structure information from the systems with known crystal structure. Along with the study of magnetic behaviour of paramagnetic substances, EPR has emerged as one of the most powerful tools for studying wide varieties of physical, chemical and biological effects. Today EPR find its application in widely diversified fields like biomedical sciences, radiation biology, photosynthetic process etc.

EPR is especially crucial in biomedical contexts because free radicals play pivotal roles in both healthy physiology and disease mechanisms. It directly identifies paramagnetic molecules without depending on indirect biochemical markers; it remains the gold-standard method for analyzing free radicals in living systems.

More recent developments have extended EPR toward tissue oxygen measurements, imaging, pharmaceutical research, drug-delivery systems, cancer research, and experimental human applications. EPR has therefore been described as a method of choice for studying free radicals and other paramagnetic species in biomedical systems. [1]

II. PRINCIPLE

EPR spectroscopy is based on the magnetic properties of unpaired electrons. An electron has an intrinsic spin angular momentum and an associated magnetic moment. In an external magnetic field, the energy levels of the electron spin separate. Because different paramagnetic species have different electronic environments, their EPR spectra contain characteristic information that can be used for identification and analysis.

If a static magnetic field is applied to system of free electrons ($L=0$, $S=1/2$), the electron align themselves either parallel or anti parallel to the applied magnetic field. On applying electromagnetic field of frequency ν in the direction perpendicular to a DC magnetic field, the transition of electrons from lower level to upper level according to the selection rule $\Delta m_s = \pm 1$ take a place and a sharp absorption of power occurs provided the relation

$$h \nu = g \beta H \quad (1)$$

is satisfied. Mostly the population ratio of the two energy states is given by the Maxwell- Boltzmann distribution.

$$n_1 / n_2 = e^{-E/kT} \quad (2)$$

Where n_1 and n_2 are the numbers of free electrons in the upper and lower state respectively and E is the energy separation between two states.

The hyperfine splitting of an electron paramagnetic resonance line is obtained when the interaction between unpaired electron and the magnetic nucleus takes place within the molecule. It is of extreme importance in determining the distribution of unpaired electron density and leads us to reach the valuable information regarding the intra and inter molecular interactions. The total Hamiltonian of the system inclusive of EPR and Hyperfine process is given by

$$\mathbf{H} = g \beta \bar{H} \cdot \bar{S}_z + A \bar{S} \cdot \bar{I} \quad (3)$$

assuming the ion to be free.

III. DETECTION AND DIAGNOSIS USING EPR TECHNIQUE IN MEDICAL SCIENCE

One of the most important medical applications of EPR is the investigation of free radicals. Reactive oxygen species are continuously produced during normal cellular metabolism. At controlled concentrations, they participate in cell signalling and physiological processes. Excessive production, however, can result in oxidative stress and cellular damage.

EPR can detect radicals either directly, when their lifetime and concentration are sufficient, or indirectly through **spin-trapping techniques**. A spin trap reacts with a short-lived radical and forms a more stable radical adduct that can be detected by EPR. In this paper we have used EPR technique in studying Superoxide radicals, lipid radical, oxidative stress and radical-mediated cellular damage which contributes to research on aging, inflammation, cancer, cardiovascular disease, neurodegeneration, and toxicology.

3.1 EPR IN CANCER RESEARCH

Cancer is one of the most promising areas for biomedical EPR. Tumors frequently exhibit abnormal oxygen metabolism and regions of low oxygen concentration, known as **hypoxia**. Tumor hypoxia can influence tumor progression and may reduce the effectiveness of some cancer treatments.

EPR oximetry provides a method for repeatedly measuring tissue oxygen concentration. Research has demonstrated the potential of EPR measurements for understanding tumor oxygenation and its relationship to cancer treatment. [2]. In oncology Measurement of tumor oxygen concentration, Evaluation of changes in oxygenation during treatment, Study of free radicals associated with cancer is particularly valuable because tissue oxygenation can change during disease progression and treatment.

3.2 EPR OXIMETRY AND TISSUE OXYGENATION

EPR oximetry is one of the most significant medical applications of EPR. A paramagnetic probe is introduced into or localized within biological tissue. Its EPR spectral properties respond to the local oxygen concentration. By measuring the changes in the EPR signal, oxygen concentration can be estimated. Unlike a single-point biochemical measurement, EPR oximetry has the potential to monitor tissue oxygen repeatedly over time.

Clinical reviews have identified tissue oximetry as one of the most promising areas such as Cancer, Chronic wounds, Diabetic complications etc. because oxygen measurements through EPR can potentially influence diagnosis and treatment decisions. [3,4]

3.3. EPR IN NEUROLOGICAL AND NEURODEGENERATIVE DISEASES

Free radicals and transition-metal ions have important roles in several neurodegenerative disorders. Metals such as iron and copper can participate in redox reactions and contribute to oxidative damage. EPR can directly investigate paramagnetic metal centres and radical species. In neurodegenerative disease research, EPR has been used to investigate Metal-ion interactions, Protein aggregation, Oxidative stress, Free-radical formation.

For example, EPR-based investigations have contributed to understanding how copper ions may participate in amyloid or amyloid-like fibril formation. [1]. EPR spin-labelling can also be used to study protein structure and dynamics, making it valuable for understanding molecular mechanisms associated with neurological diseases.

3.4 EPR IN DRUG DISCOVERY AND PHARMACEUTICAL RESEARCH

The pharmaceutical industry represents another rapidly developing application of EPR. Many drugs undergo redox reactions or produce reactive intermediates. EPR can detect radical intermediates and provide information about their mechanisms of formation. A recent review of pharmaceutical applications highlighted EPR's potential for studying antioxidant activity, drug mechanisms, drug-induced toxicity, drug-membrane interactions, drug delivery, metallodrugs, and pharmaceutical quality control. [5,6]

3.5. EPR IN RADIATION BIODOSIMETRY

Another important medical application is the assessment of radiation exposure. Exposure to ionizing radiation can generate stable paramagnetic changes in certain biological materials. EPR can detect these radiation-induced signals and provide information about radiation dose. Its applications include Accidental radiation exposure, Nuclear emergencies, Radiation-therapy research, Large-scale radiation incidents

In vivo EPR has been investigated as a method for measuring clinically significant radiation exposure after the event, potentially assisting medical triage and treatment decisions. [4]. This is particularly significant because conventional biological measurements may require considerable time, whereas EPR-based biodosimetry can potentially provide useful information about radiation exposure more rapidly.

3.6 EPR IMAGING

Conventional EPR spectroscopy provides information about paramagnetic species within a sample. **EPR imaging (EPRI)** extends this concept by providing spatial information.

EPR imaging may be used to investigate Oxygen distribution, Free-radical distribution, Tumor microenvironment, Tissue oxygenation, Distribution of spin probes, Drug-delivery systems. Combining EPR spectroscopy with imaging provides both chemical and spatial information, creating an approach that is complementary to other medical imaging technologies.

3.7 EPR IN CARDIOVASCULAR RESEARCH

Oxidative stress and nitric oxide signalling are important in cardiovascular physiology and disease. EPR can investigate nitric oxide and other paramagnetic species associated with vascular biology. Potential applications include studying nitric oxide signalling, investigating oxidative stress, examining vascular dysfunction, studying ischemia and reperfusion injury, and investigating oxidative damage to cardiovascular tissues. These applications remain largely research-oriented but demonstrate the potential of EPR to investigate molecular mechanisms that are difficult to measure using conventional imaging alone.

IV. RECENT DEVELOPMENTS AND FUTURE DIRECTIONS

The future of medical EPR is closely connected with improvements in sensitivity, portability, imaging, and computational analysis.

Recent developments include:

- Miniaturized EPR spectrometers.
- High-sensitivity resonators.
- Pulsed EPR.
- Advanced spin-labeling techniques.
- Improved oxygen-sensitive probes.
- Automated spectral analysis.
- Integration with other biomedical imaging methods.

Miniaturization is particularly important because conventional EPR systems are often large and laboratory-based. Smaller instruments could make EPR more accessible for pharmaceutical manufacturing, biomedical laboratories, and eventually selected clinical applications. Recent work on superconducting resonators has demonstrated improved sensitivity and high-bandwidth pulsed EPR measurements of biologically relevant spin-labeled samples, illustrating the direction of current technological development. [7]

V. CONCLUSION

Electron Paramagnetic Resonance has evolved from a fundamental spectroscopy technique into a valuable biomedical research tool. Its unique ability to detect unpaired electrons makes it especially suitable for investigating free radicals, reactive oxygen species, nitric oxide, transition-metal ions, and paramagnetic probes. [8,9,10]

Among its medical applications, EPR oximetry, cancer research, oxidative-stress analysis, drug development, drug-delivery research, structural biology, wound healing, neurodegenerative disease research, and radiation biodosimetry are particularly important. The major challenge is the transition from specialized laboratory instrumentation to routine clinical technology. Improvements in sensitivity, miniaturization, imaging, probe design, and automated spectral analysis may help overcome these limitations. EPR is therefore unlikely to

replace established medical imaging methods such as MRI, CT, or ultrasound. Instead, its greatest future value may come from providing molecular and functional information that conventional anatomical imaging cannot directly provide.

The continuing development of EPR technology suggests that the technique could become increasingly important in precision medicine, particularly where monitoring tissue oxygenation, oxidative stress, free radicals, drug action, or radiation exposure is clinically relevant.

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