

Analysis Of Chemical substances using Electron Spin Resonance (ESR) Spectroscopy

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Summary:

1 Abstract

EPR spectroscopy is a very powerful bio-physical technique: practical and useful EPR applications on biomolecules and models can range from very simple to very involved experiments and analyses. Electron paramagnetic resonance spectroscopy, also known as electron spin resonance, provides detailed information about the electronic structure of metal centres with unpaired electrons and interactions with neighbouring nuclear or electron spins. It has been used by scientists over the years to look for free radicals within compounds. These free radicals take part in various organic and inorganic reactions and detecting them are crucial in a wide variety of research applications. Therefore, this paper serves as an experimental and theoretical introduction to the field of EPR spectroscopy.

Over the course of this paper, various chemical samples were tested on a Varian X-Band spectrometer, with the purpose of predicting the g-values of these compounds which help us detect not only the free radicals present, but also, to decode the chemical structure of the given sample.

2 Introduction

ESR is a branch of absorption spectroscopy in which radiation having frequency in the microwave region is absorbed by paramagnetic substance to induce transition between magnetic energy level of electron with unpaired spin. Therefore, EPR has a very wide scale importance in various branches of sciences such as enzymology, where in EPR signal readings are used to study the metal centres in the active site of proteins.

EPR turns out to be a very important technique because it discloses a lot of information regarding the paramagnetic ions in the sample such as:

1. The spin state of ions.
2. The nature of ligands that surround the chemical sample.
3. The interactions of the ions with the lattice.
4. Identity of metal ion
5. Oxidation state

The structure, dynamics, and interactions of inherently disordered proteins like alpha-synuclein can be studied using electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed spin labelling. Electron spins pervade research and have an impact on a variety of chemical processes: they underpin transition metal chemistry and biochemistry, mediate photosynthesis and photovoltaics, and are crucial in quantum information, to mention a few.

In this experimental procedure, a variety of paramagnetic samples were tested using EPR spectroscopy. The EPR readings observed are then used to analyse information and calculate the spin states and the g-factor (Lande's g-factor) of the unpaired electrons present in the paramagnetic sample. The g-factor is essentially the ratio of the electron's magnetic dipole moment to its angular momentum. The value of the g-factor varies according to the orientations of the molecule in an external magnetic field.

3 Background ESR Theory

The ESR technique is essentially based on the interaction between the external magnetic field (B) and the spin magnetic moment (μ) of the unpaired electrons present in the sample. In the absence of any magnetic field, all the electrons have the same energy state and therefore, are in their degenerate state.

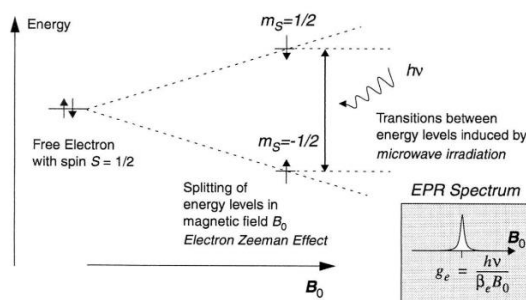


Figure 1: The Zeeman effect, where in the presence of magnetic field a split in energy level is observed[10]

When an external magnetic field is applied, there is a split in the degenerate energy levels (Zeeman effect) due to the interaction of magnetic field with the magnetic moment. Due to this split the electrons take two separate energy levels i.e., $+\frac{1}{2}g\mu B$ and $-\frac{1}{2}g\mu B$ where g represents the g-factor, μ represents the Bohr magneton and B represents the magnetic field[1]. Meanwhile as the magnetic field (B) is increased (Magnetic field in this case normally lies between 0 to 1T), microwave frequencies of certain energies ($h\nu$) are constantly

being emitted. But the electron can only absorb the energy and go to the excited spin state only when the resonance condition is fulfilled.

The resonance condition is as followed:

$$h\nu = \delta E = g\mu B$$

where h is the Planck's constant, ν is the frequency of the electromagnetic radiation emitted. Only when the above condition is agreed the electron can absorb the energy from the radiation and go to a higher energy spin state as shown in **Figure 1**.

In a typical EPR setup, a klystron is used to produce the microwave frequencies (in GHz). Upon absorbing the radiation at the resonance condition, a bell-shaped curve is observed as the absorption spectrum (**Figure 2**), representing the frequency interval at which energy is absorbed and released.

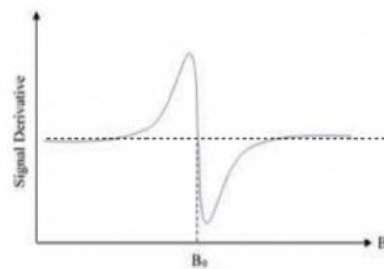


Figure 2: An ESR absorption spectrum with spin +1/2 system [4]

Based on the given reading the g -factor as previously discussed can be calculated using the following relation:

$$g = 0.71449 \frac{\nu(\text{GHz})}{B(\text{kG})}$$

For a free radical the g value lies approximately around 2.002. If the g value deviates from the given value, it's concluded that the electron is bounded to an atom.

Often in many cases, transition series ions have nuclei with spin which interact with the unpaired electron and produce hyper-fine splitting in the EPR spectra. This kind of interactions causes hyper-fine structures. If a nucleus with spin I which interacts with an electron of spin S , the multiplicity for the hyper fine splitting is given as $(2I + 1)$.

The hyper-fine splitting as discussed above is represented in Figure 3, where in A represents the hyper-fine constant and it's the characteristic of a particular ion. In the figure a sample with a hyperfine multiplicity of 3 can be observed, based on which the nuclear spin(I) can be calculated to be $I = 3/2$.

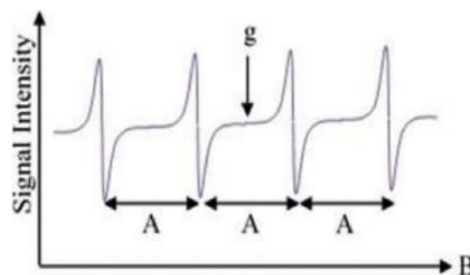


Figure 3: A typical hyper-fine spectrum of a species with $I = 3/2$

Zeeman Effect: EPR Spectroscopy is due to the interaction of an external magnetic field with the spin magnetic moments of unpaired electrons. This effect is known as the Zeeman Effect. This interaction causes splitting of the degenerate m_s spin energy levels as shown in **Figure**

1. An electron in its lower energy state can absorb electromagnetic radiation and jump to its excited state giving rise to the EPR phenomenon.

4 Experimental Procedure

For the set of experiments done for the purpose of this paper, a Varian E-3 X-band spectrometer with a liquid nitrogen flow cryostat was used. The frequency range of the spectrometer lies from 8.5-12 GHz and the temperature range lies from 80K to room temperature.

As portrayed in **Figure 4**, the spectrometer uses a Klystron to provide the microwave energy to be absorbed by the electron. The sample to be observed is placed inside the resonant cavity area. The resonant cavity is placed inside an electromagnet. As the instrumentation is switched to 'OPERATE' mode, microwave power of constant frequency is produced by the Klystron and it travels down to the resonant cavity through a series of wave guides. Initially no microwave is absorbed but upon sweeping the magnetic field through the resonance, energy is absorbed by the sample in cavity.

Upon resonance the absorption signal is reflected from the resonance cavity to the detector and further to the electronics console for processing. This signal will then be plotted on X-Y plotter and analysed.

In the given spectrometer, a liquid nitrogen flow cryostat is also attached to the resonant cavity, to cool down the sample and conduct low temperature EPR spectroscopy.

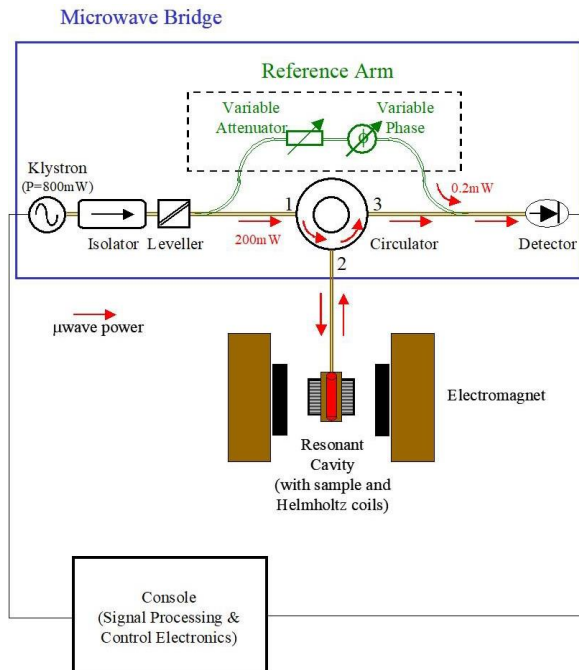


Figure 4: Schematic representation of the ESR spectrometer

The samples used for experimentation are as follows:

DPPH- A small amount of grease was taken and placed inside a clean EPR tube and a sample of DPPH was placed inside the grease spot such that it aligns with the cavity region. EPR measurements were carried out with a Varian E-3 X-band spectrometer.

Manganese Chloride in H_2O - A sample of $MnCl_2$ in H_2O is prepared by adding Manganese Chloride to water and transferring it to an EPR tube using a pipet.

5 Results and Discussions

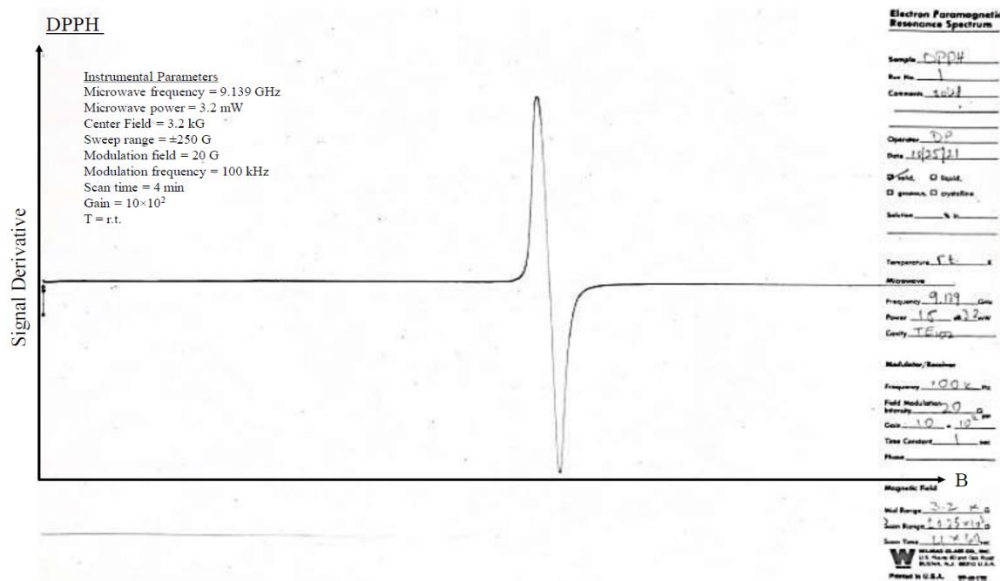


Figure 5: EPR absorption spectrum of DPPH. Experimental parameters are as follows: Microwave frequency - 9.139 GHz, Microwave power – 3.2mW, Modulation frequency - 100kHz, Centre Field – 3.2G

DPPH- The obtained EPR spectrum of DPPH as shown in **Figure 5**, predicts that $g = 1.98$ in the case of DPPH. However, the theoretical g -value of DPPH is calculated to be 2.00. Therefore, it can be concluded that there is a presence of instrumental error within the experimentation. The error percent is calculated to be 1%. As the experimental error percent is small, therefore we can conclude that DPPH is a free radical.

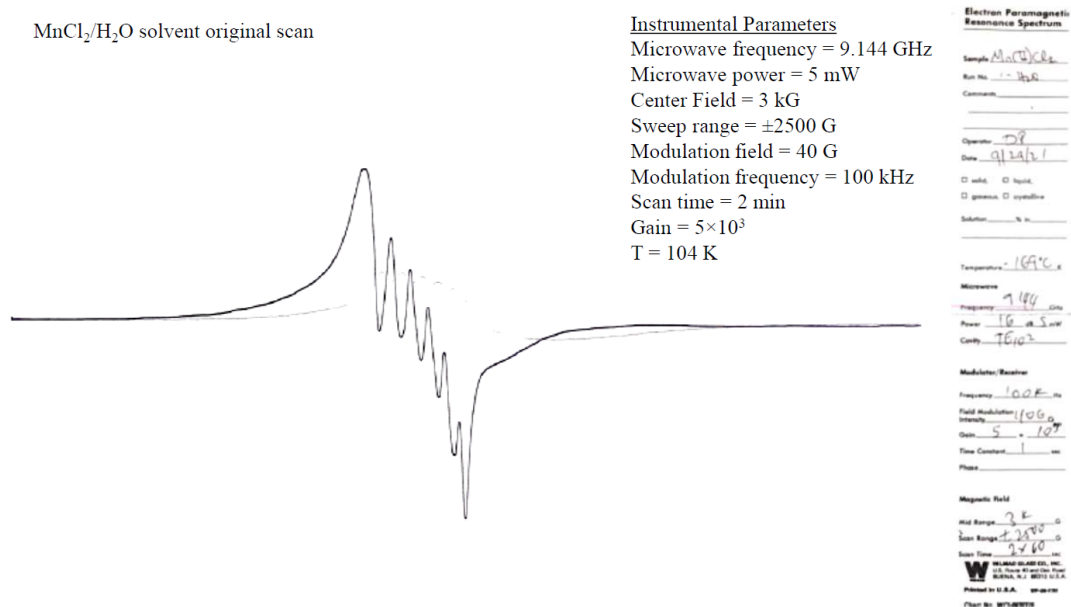


Figure 6: EPR absorption spectrum of $MnCl_2$ in H_2O . Experimental parameters are as follows: T-104K, Microwave power-5mW, Microwave frequency-9.144 GHz, Modulation frequency- 100kHz, Modulation field- 40G, Centre field-3kG

Manganese Chloride in H_2O - The $MnCl_2/H_2O$ EPR spectrum is shown in **Figure 6**. The magnetic field is deduced to be 500G at the beginning of the scan and 5500G at the end of the scan. The spectrum has several peaks, each of which will give rise to a different B_0 value. If calculated from the centre peak, the B_0 value is approximately 3.267 kG. Consequently, the g -factor comes out to be approximately 2.17. Since the g -factor is approximately 2, this indicates that the electron is a free radical and spin-orbit coupling is absent. The Mn^{+2} ion leads to hyperfine splitting and produces 6 peaks.

6 Conclusion

Data collected from various EPR spectra were used to study various bio-chemical samples and the effect that solvent has on the EPR spectra of a compound. The g value for each sample was calculated. DPPH as expected resembled the characteristics of a free radical.

Meanwhile $MnCl_2$ displayed a hyper-fine structure of level 6, as a result of its nuclear spin. The nuclear spin was later calculated to obtain a value of $I = 2.5$.

Therefore, over the given set of results and observations, it can be concluded that EPR spectroscopy is a very powerful experimental technique, offering us a great deal of insight into any kind of paramagnetic sample using the calculated g-factor of the sample.

7 Acknowledgements

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References:

- [1] Petasis, D. T., *EPR handout 1*, LS 102 – 07/08: Introduction to College Research in Physics, Allegheny College, Summer 2022.
- [2] Petasis, D. T., *EPR handout 2*, LS 102 – 07/08: Introduction to College Research in Physics, Allegheny College, Summer 2022.
- [3] W. Hagen, “EPR spectroscopy,” *Practical Approaches to Biological Inorganic Chemistry*, pp. 121–154, 2020.
- [4] Z. Instruments, “Electron Paramagnetic Resonance (EPR),” *Electron Paramagnetic Resonance (EPR) / Zurich Instruments*.
- [5] E. Duin, “Electron Paramagnetic Resonance Theory.
- [6] Tomoko Ohnishi, Iron–sulfur clusters/semiquinones in Complex I, *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, Volume 1364, Issue 2, 1998, Pages 186-206
- [7] L. von. Alvensleben, *Laboratory experiments: physics*. Göttingen: PHYWE SYSTEME, 2001.
- [8] M. Ikeya, M. R. Zimmerman, and N. Whitehead, *New applications of electron spin resonance: dating, dosimetry and microscopy*. Singapore: World Scientific, 2007.
- [9] Libretexts, “EPR - Interpretation,” *Chemistry LibreTexts*, 21-Aug-2020. [Online].
- [10] J. Gendell, J. H. Freed, and G. K. Fraenkel, “Solvent Effects in Electron Spin Resonance Spectra,” *The Journal of Chemical Physics*, vol. 37, no. 12, pp. 2832–2841, 1962.