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Experimental studies, analysis and comparison of EPR spectroscopy for DPPH, Myoglobin and Manganese Chloride

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ABSTRACT

Electron Paramagnetic Resonance (EPR) Spectroscopy is a spectroscopic technique that can be used to study complexes containing unpaired electrons, due to a phenomenon known as the Zeeman effect. This paper will aim at performing an experiment to retrieve and analyse Electron Paramagnetic Resonance (EPR) Spectra. Samples of 2,2-diphenyl-1-picrylhydrazyl (DPPH), Myoglobin and Manganese Chloride were analysed through room temperature and low temperature EPR runs, using liquid nitrogen as the cooling agent. The spectra obtained were then used to calculate quantitative values and the values were analysed and interpreted to provide the nature of the unpaired electrons in the complex. Lastly, the results were compared with literature values and future opportunities for research about EPR in biochemistry and geology were also discussed.

Keywords: *EPR Spectroscopy, ESR Spectroscopy, DPPH, Myoglobin, Manganese Chloride*

1. INTRODUCTION

Through retrieving the Electron Paramagnetic Resonance (EPR) Spectra of various samples like DPPH, Myoglobin and Manganese Chloride, the spectral patterns will be analysed and discussed in detail. The paper will begin by explaining Electron Paramagnetic Resonance (EPR) Spectroscopy and will provide a brief description of the physical concepts behind this phenomenon. It will then discuss the experimental design by exploring the mechanism and functioning of the instrumentation used, i.e, an EPR spectrometer. It will also describe the experimental process and the steps involved in preparing the sample, preparing the instrument, and collecting data in detail. After a detailed description of the experimental design, the resulting spectra will be presented along with their analysis and discussion. Lastly, the paper will also compare the results obtained with those of existing theories and experiments and will also explore future avenues of research opened up by EPR spectroscopy.

EPR spectroscopy is important to study as it has several applications ranging from simple results and interpretations to very complex analyses. Reading an EPR spectra can reveal important details like the coordination environment or the oxidation state of the transition metal ion. An EPR spectra, however, can provide not just qualitative information like the one mentioned above but also quantitative data, thereby, greatly increasing its relevance in biological and biochemical contexts. The concentration of a metal can be devised from an EPR spectra which can then be compared to the concentration of the metalloprotein tested as a sample to find out the metal/protein ratio. Other observations from the spectra can also help researchers identify ligands in the metal coordination, which have greatly beneficial applications in enzymology. Clusters of metal ions, for example, iron-sulphur clusters, can also be identified using EPR experiments. Other calculations involving quantitative interpretation in terms of accurate distance and mutual orientation are also possible if the data is collected at several microwave frequencies. The numerous applications of EPR spectra in biology and biochemistry are, thus, the motivation behind conducting the experiments described in this paper.

2. THEORETICAL BACKGROUND

Electronic Paramagnetic Resonance, or EPR, is a spectroscopic technique that can be used to study systems or complexes with unpaired electrons. As mentioned above, an EPR spectra can provide researchers with a lot of information about the complex being studied. This includes the identity, oxidation state and spin state of the paramagnetic metal ion, the nature of the ligands surrounding the metal ion, and the interaction of the metal ion with the surrounding lattice structure.

2.1 Mechanism behind EPR Spectroscopy

The EPR technique is based on the Zeeman effect, a phenomenon that occurs when the spin magnetic moments of the unpaired electrons interact with the surrounding external magnetic field. Due to this interaction, the degenerate m_s spin energy levels split into two (Figure 1). An electron present in the lower energy level can absorb and gain energy in the form of electromagnetic radiation from its surroundings and transfer itself to the higher energy level, known as the excited state. This event gives rise to the EPR spectrum.

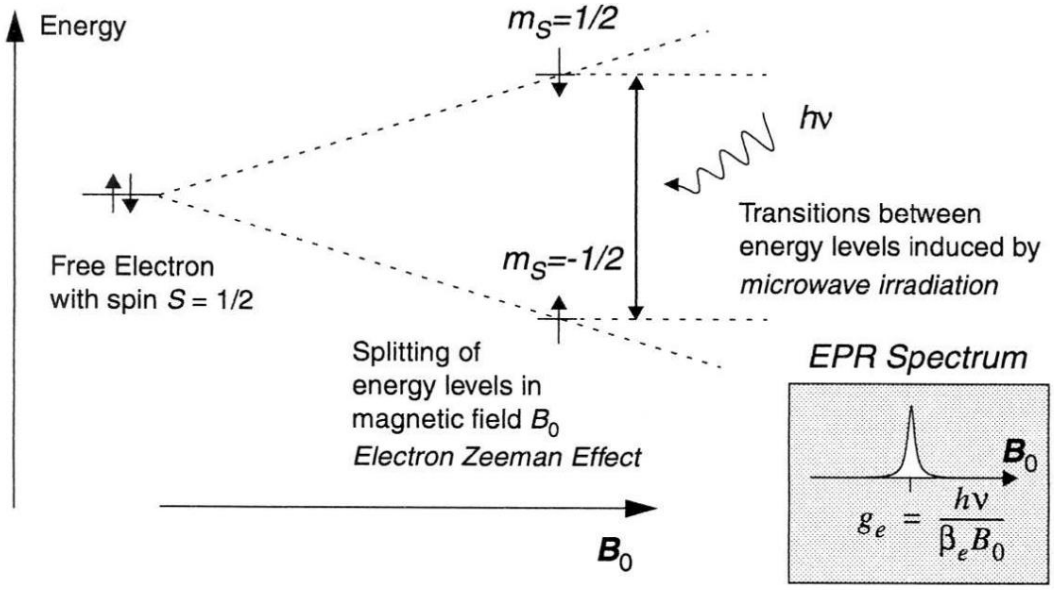

Figure 1: Illustration of the Zeeman splitting for a S = 1/2 system with one unpaired electron in an external magnetic field B_0, along with the EPR absorption line

The EPR resonance condition refers to the condition that is required to be fulfilled in order to complete an electron's transition from the lower energy level to the higher energy level.

$$h\nu = \Delta E = g\mu_B B$$

In this formula, v refers to the frequency of the electromagnetic radiation, g refers to the electronic g-factor, μ_B refers to the Bohr magneton constant and B refers to the applied magnetic field.

2.2 A Typical EPR Spectrum

An EPR experiment involves a device called the klystron which produces microwave radiation at a constant frequency. The magnetic field is then swept through a range that usually covers a few kilogauss (kG). When the magnetic field reaches a value that satisfies the aforementioned resonance condition, microwave energy is absorbed and this absorption appears as a bell-shaped curve in the microwave power spectrum. This curve is called the EPR resonance line and the EPR spectrometer depicts the first derivative of this line.

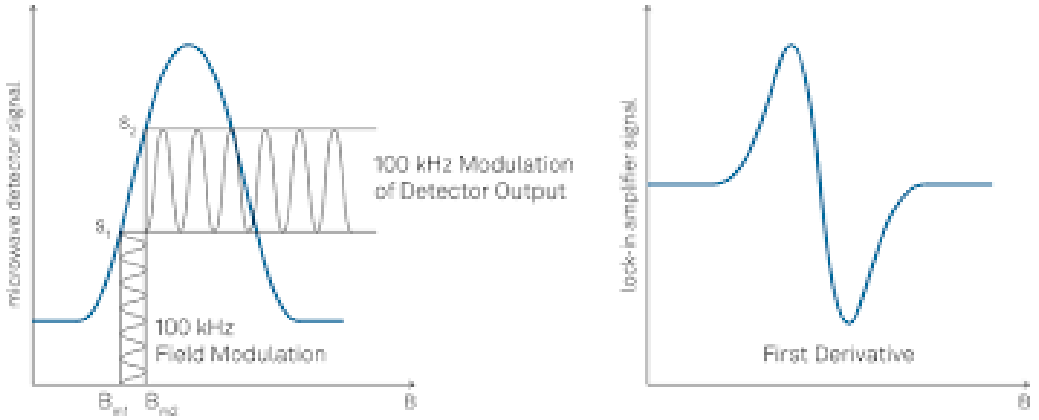

Figure 2: The left panel shows a typical signal detected with the microwave detector, while the right panel shows the first derivative of the absorption observed in the EPR spectrometer.

EPR spectra, as the one shown in Figure 2, can help determine the g-value of the spin system. This g-value, in turn, provides a lot of information about the interactions among the spin system like spin-orbit coupling, hyperfine interactions, ligand symmetry and interaction with ligands.

Figure 2 shows an isotropic EPR spectrum, i.e, the symmetry produced by atoms in the lattice at the location of the paramagnetic ion belongs to the cubic system. All directions around the paramagnetic ion are equal to each other and to the overall g-value. Other types of symmetry include axial and rhombic symmetry. Axial symmetry includes two axes with the same g-value and one with a different g-value (typically the z-axis); this type of system has two different g-values. Rhombic symmetry is one in which each axis has a different g-value, resulting in three unique g-values.

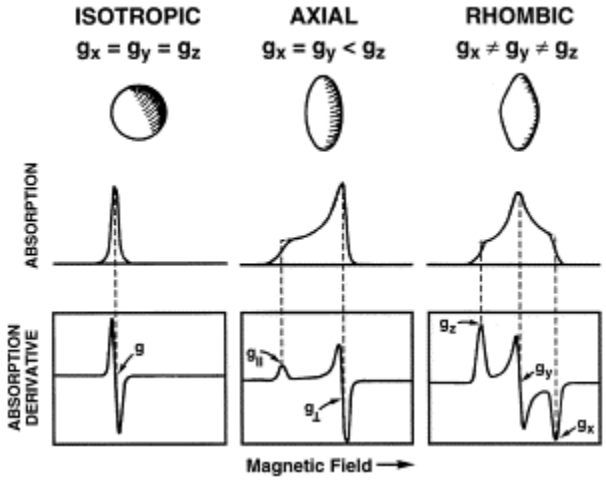

Figure 3: Schematic presentation of the relationship between g values and the EPR spectral line shapes.

3. Experimental Procedure

3.1 Instrumentation

The instrument used for the experiments is a Varian E-3 X-band spectrometer (Figure 4) with a liquid nitrogen flow cryostat. The spectrometer operates in the frequency range of 8.5-12 GHz and a temperature range of 80K- room temperature.

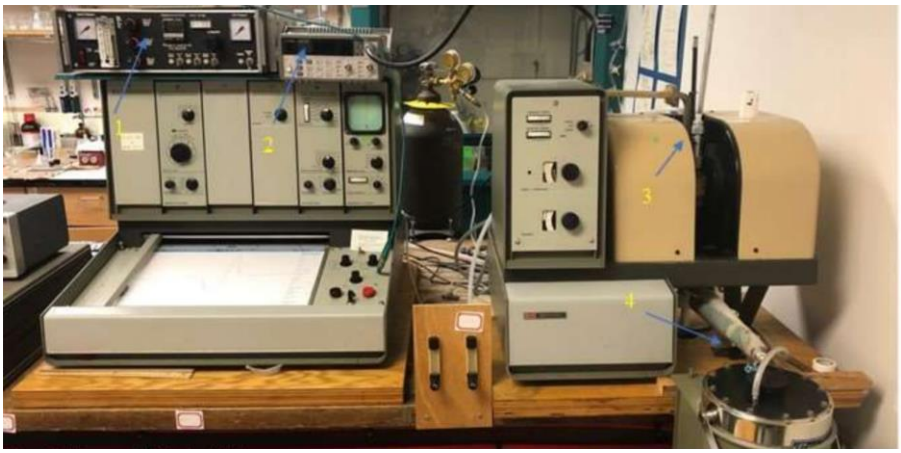

Figure 4: Setup of the Varian E-3 X-band spectrometer used for the experiment.

The arrow labelled '1' indicates the temperature controller, i.e, a device that allows the constant monitoring and regulation of temperature throughout the experiment. The arrow '2' points at the universal counter, which displays the frequency throughout the duration of the experiment. '3' represents the cavity in which samples are placed in order to be examined. '4' indicates the dewar, where the liquid nitrogen is placed in order to cool the sample. (Liquid nitrogen was used in this experiment, however, liquid helium can also be used.)

3.2 Steps for conducting a room temperature scan

The first step before conducting the experiment is to turn on the coolant water, which prevents overheating of the magnet attached in the instrument. During a room temperature scan, the spectrometer and universal counter is turned on next; the temperature controller does not need to be turned on for this type of experiment. The frequency channel is then set to channel three to get an accurate reading. The intensity of the oscilloscope is turned completely to the right and the sample is placed in the cavity as soon as a signal is visible. The mode knob is then turned to tune and a dip can be observed on the oscilloscope. The horizontal position of the dip can be regulated by the frequency knob and the vertical position can be regulated using the power attenuation knob. The horizontal position of the dip must be aligned with the black line on the oscilloscope. The dip is then bottomed out using the Teflon rod, which is located behind the cavity and controls the coupling iris. Once the dip has been adjusted, the mode knob is turned and the recorder switch is switched on. The frequency and power attenuation knobs are then adjusted to ensure the frequency error and detector current are in the proper positions. The magnetic field mid-range and scan range are then set up and the scan button is pressed to begin the EPR scan.

3.3 Additional steps for conducting a low temperature scan

In order to conduct a low temperature scan, certain additional steps need to be taken before conducting the scan. Firstly, the temperature controller is turned on after the coolant water. Next, the main valve on the liquid nitrogen tank is opened to ensure a steady flow of nitrogen at a pressure of around 25 to 30 psi. Some more detailed steps required for the operation of the temperature controller are conducted before the liquid nitrogen is inserted into the dewar. Once inserted, the sample is slowly lowered into another dewar filled with liquid nitrogen. Lowering the sample at a slow rate is crucial in this step as it prevents the sample tube from breaking. After the sample has been cooled, it is placed into the cavity and the remaining steps are followed as described in 4.2.

3.4 Instrumental parameters used in this experiment

- DPPH: The DPPH sample is prepared by placing a small amount of grease on the inside of a sample tube and then placing a sample of DPPH on the grease spot such that it aligns with the cavity region.
- Myoglobin: The Myoglobin (Mb) sample is prepared by suspending dry Mb crystals in an empty tube, a tube containing water (H_2O) and a tube containing glycerol.
- $MnCl_2$: The $MnCl_2/H_2O$ sample is prepared by adding Manganese Chloride to water and transferring it to an EPR tube using a pipet.

Table 1: The instrument parameters used to conduct the experiment

Sample	Microwave frequency	Microwave power	Center field	Sweep range	Modulation field	Modulation frequency	Scan time	Gain	Temperature
DPPH	9.139 GHz	1.25 mW	3.3kG	±500G	20G	100kHz	4 mins	5 x 10^2	room temp
Dry Myoglobin	9.146 GHz	5 mW	3 kG	±2500 G	20 G	100 kHz	4 mins	2.5×10^5	room temp
Myoglobin in H2O	9.120 GHz	25 mW	3 kG	±2500 G	40 G	100 kHz	2 mins	8×10^4	117 K
Myoglobin in glycerol	9.122 GHz	25 mW	3 kG	±2500 G	40 G	100 kHz	2 mins	2.5×10^3	110 K
Manganese chloride in water	9.144 GHz	5 mW	3 kG	±2500 G	40 G	100 kHz	2 mins	5×10^3	104 K

4. RESULTS AND DISCUSSION

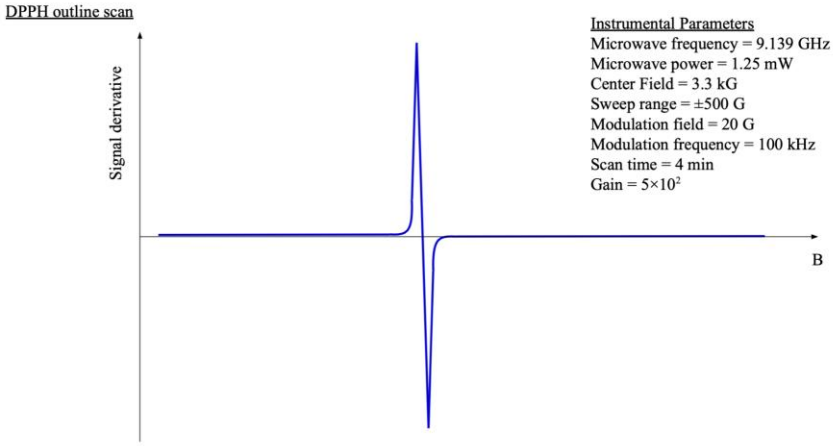

Figure 5: The EPR spectrum for DPPH. The instrumental parameters mentioned in the Figure are given in Table 1.

DPPH: The DPPH EPR spectrum obtained is shown in Figure 5. Using the centre field and field sweep values, it can be deduced that the magnetic field at the beginning of the scan was 2.8kG, and at the end of the scan, it was 3.8kG. The width of the spectrum on the computer screen is approximately 14.9cm, and the point at which the curve crosses the baseline is approximately at 6.45cm, making the ratio of B_0 to the total sweep field approximately 0.433.

B_0= 2800+(0.433 x 500)

= 3016.5 G = 3.0165 kG

Using the formula

$$g = 0.71449\frac{\nu(GHz)}{B_0(kG)}$$

g= 0.71449 x (9.139 GHz⁄3.0165 kG)

g= 2.16467

Since the g value is approximately equal to 2, this shows that the DPPH sample is a free radical. The literature value for DPPH is considered to be g = 2.0037 which is very close to the value obtained experimentally.

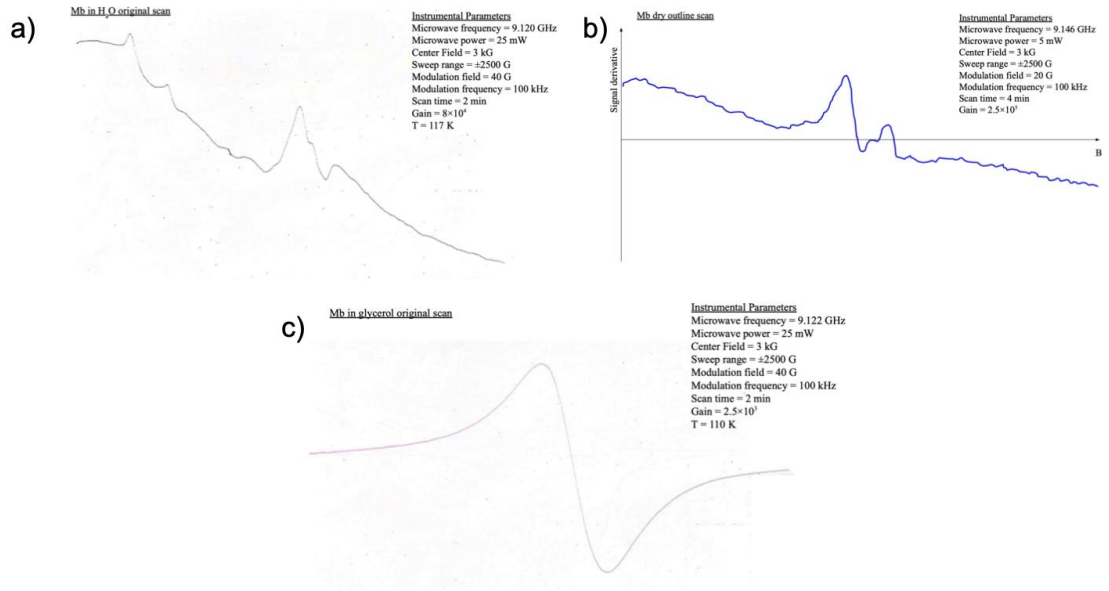

Figure 6: a) The EPR spectrum of Mb in water. b) The EPR spectrum of dry Mb. c) The EPR spectrum of Mb in glycerol. The instrument parameters mentioned along with the spectra are listed in Table 1.

Myoglobin (Mb): The Mb EPR spectra are shown in Figure 6. By performing the same calculations as those performed for DPPH, the magnetic field is deduced to be 500G at the beginning of the scan and 5500G at the end of the scan for all three samples. The B_0 and g-factor values have also been calculated for each of the samples using the same procedure as DPPH.

Table 2: The B_0 and g-factor values for Myoglobin samples

Sample	B_0	g-factor
Dry Mb	1.73311kG	3.770
Mb in water	1.920kG	3.394
Mb in glycerol	1.87405kG	3.4778

Since the g-factors for all three samples are much greater than 2, this indicates the electron is bound to an atom and spin-orbit coupling is present. There are slight changes in the EPR spectra for all 3 samples due to a change in solvent. While the overall spectrum and B_0 and g-factor values are similar, there are certain fluctuations. These fluctuations can be attributed to a redistribution of the pi-electron spin density due to the interactions between the molecules of the sample and the solvent.

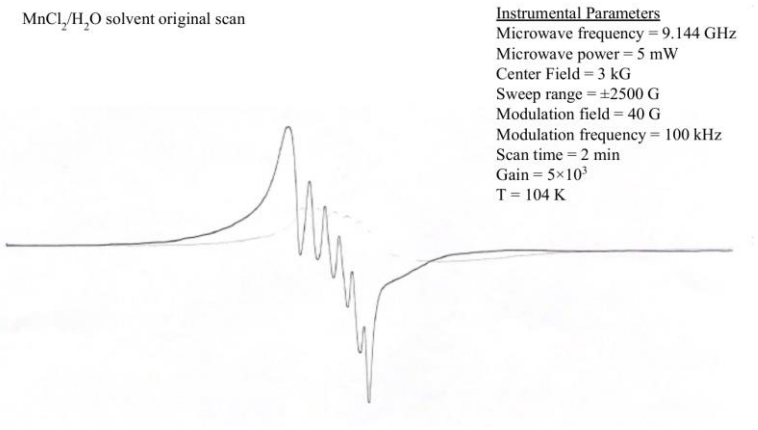

Figure 7: The EPR spectrum of $MnCl_2/H_2O$

$MnCl_2/H_2O$: The $MnCl_2/H_2O$ EPR spectrum is shown in Figure 7. 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 1.99978 or 2. 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.

5. CONCLUSION

Electronic Paramagnetic Resonance, or EPR, is a spectroscopic technique that can be used to study systems or complexes with unpaired electrons. The EPR technique is based on the Zeeman effect, a phenomenon that occurs when the spin magnetic moments

of the unpaired electrons interact with the surrounding external magnetic field. The experiment described in this paper aimed at retrieving EPR spectra for 5 samples, namely DPPH, dry Myoglobin, Myoglobin in water, Myoglobin in glycerol and Manganese Chloride in water. The steps to perform the experiment, along with the instrument parameters, are mentioned in section 4 of the paper. The spectra obtained are shown in Figures 5, 6a-c and 7. These spectra have then been analysed and the calculations of the magnetic field range, B_0 value and g-factors have been conducted. The implication of these values has also been described along with further discussion on what affects the shape of the spectra. The values obtained comply with literature values and align with the theories presented by previous studies conducted on similar samples.

EPR spectroscopy has numerous applications and great scope for future research. The applications of EPR spectroscopy range from geological and archaeological dating to radiation dosimetry and microscopic magnetic resonance imaging (MRI). EPR spectroscopy (also known as ESR spectroscopy in biochemical literature) also has several applications in biochemistry and plays a crucial role in investigating free radicals and metalloproteins. Further research into compounds like metalloproteins, metalloenzymes, reactive oxygen species (ROS), and reactive nitrogen species (RNS) could provide insight on disorders and diseases associated with these biomolecules and open new avenues for their treatments.

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