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Analyzing bio-chemical samples using EPR spectroscopy

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1 Abstract

EPR is a "something for everyone" spectroscopy: 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 spectroscopy, provides detailed information about the electronic structure of metal centers with unpaired electrons and interactions with neighboring nuclear or electron spins. Samples may be in fluid solution or solid state.

The research paper focuses on examining and analyzing 5 different bio-chemical samples, thereby making observations about it and its electronic structure with the help of EPR signals and their respective g-values. The g-values help us predict the structure of the compound as well as the nature of the unpaired electron .In case of hyper- fine structures, nuclear spins are also calculated for the samples. In essence, EPR is a very important technique giving us a great deal of information about the spin state of ions, the nature of ligands that surround the chemical sample, the interaction of the ions with the lattice, among others.

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 centers in the active site of proteins.

The purpose of this experiment is to analyze a variety of paramagnetic samples using EPR spectroscopy. Upon observing the EPR signals, we analyze it and calculate the g-factor of the unpaired electrons present in the paramagnetic sample using the resonance condition. The g-factor EPR is often used to investigate systems in which electrons have both orbital and spin angular momentum, which necessitates the use of a scaling factor to account for the coupling between the two momenta. This factor is the g-factor, and it is roughly equivalent in utility how chemical shift is used in NMR. The value of the g-factor varies according to the orientations of the molecule in an external magnetic field.

The g-value is very vital to understand the properties and characteristics of the system with the unpaired electrons and hence values are carefully calculated to understand the electronic structure of the given paramagnetic ion sample.

Given that EPR has such a huge variety of applications in various fields, it is imperative to understand in detail about its apparatus, working and key observations which help us deduce important facts.

3. Background EPR 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.

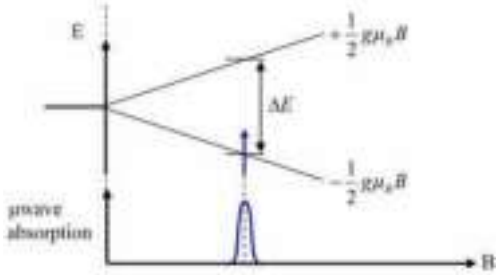


Figure 1: The Zeeman effect,where in the presence of magnetic field a split in energy level is observed

As an when an external magnetic field is applied, there is a split in the de generate 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, represents the Bohr magneton and B represents the magnetic field.

Meanwhile as the magnetic field(B) is increased (Magnetic field in this case normally lies between 0 to 1T), microwave frequencies of certain energies(hv) 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:

$$hv = \delta E = g\mu B$$

Where in h is the Planck's constant, v is the frequency of the electromag netic 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 fre quencies (in GHz). Upon absorbing the radiation at the resonance condition, a bell-shaped curve is observed as the absorption spectrum(Figure 2), repre senting the frequency interval at which energy is absorbed and released.

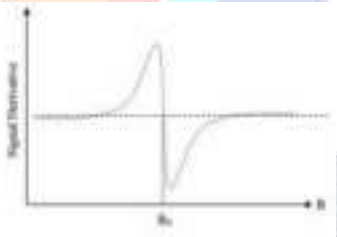


Figure 2: An ESR absorption spectrum with spin $+\frac{1}{2}$system

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

$$g = 0.71449\frac{v(GHz)}{B(kG)} \quad (1)$$

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 in a bounded to an atom.

Often in many cases, transition series ions have nuclei with spin which in teracts with the unpaired electron and produce hyper-fine splitting in the EPR spectra. This kind of interactions cause hyper-fine structures. 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,

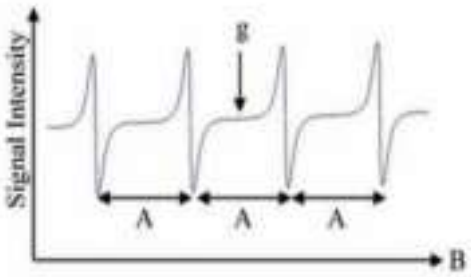


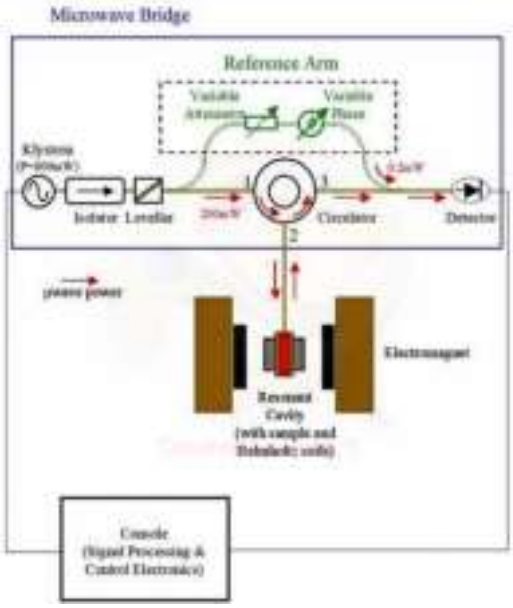
Figure 3: A typical hyperfine spectrum of a species with $I = \frac{3}{2}$ based on which the nuclear spin(I) can be calculated to be $I = \frac{3}{2}$.

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 Fig.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

Figure 4: Schematic representation of the ESR spectrometer 4

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 analyzed.

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.

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. EPR measure ments were carried out at 300K with a Varian E-3 X band spectrometer

Myoglobin- 3 samples of Myoglobin with different solvents were taken in the course of the following experiments.These are as follows :

1. Dry myoglobin- A dry sample of myoglobin was taken in an EPR tube to be tested under room temperature conditions.
2. Myoglobin in H_2O - A sample of myoglobin with water as a solvent was taken in an EPR tube. EPR measurements were carried out at a temperature of 117K.
3. Myoglobin in glycerol - A sample of myoglobin with glycerol as a sol vent was placed in an EPR tube and measurements were carried out at a temperature of 110K

Manganese Chloride in H_2O - A sample of MnCl 2 was taken in an EPR tube and measurements were carried out at 104K.

5 Results and discussions

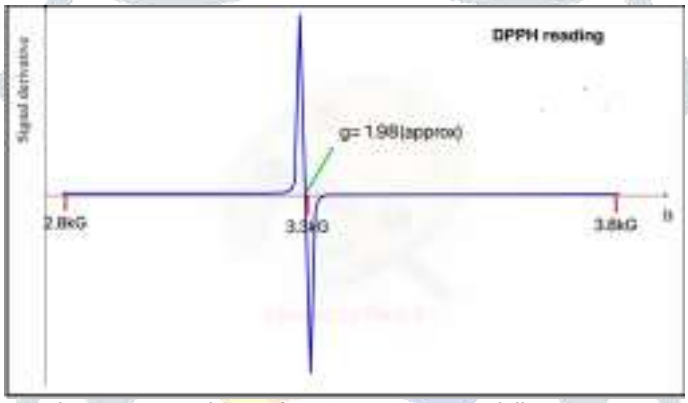


Figure 5: EPR absorption spectrum of DPPH. Experimental param eters are as follows: Temp-300K, Microwave frequency –9.139 GHz, Microwave power – 1.25mW, Modulation frequency - 100kHz

DPPH- The obtained EPR spectrum of DPPH as shown in Figure 5, pre dicts that g = 1.98 in the case of DPPH when measured at a temperature of 300K . However, the theoretical value of DPPH is calculated to be 2.00. There fore, 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 experimen tal error percent is small, therefore we can conclude that DPPH is a free radical.

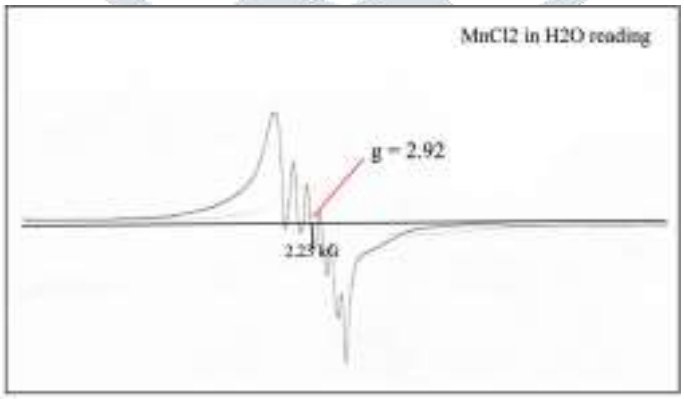


Figure 6: EPR absorption spectrum of MnCl 2 in H2O. Experimental parameters are as follows: T-104K, Microwave power- 5mW, Mi crowave frequency-9.144 GHz, Modulation frequency- 100kHz, Mod ulation field- 40G

Manganese Chloride in H_2O- The EPR spectrum obtained from the sample of $MnCl_2$ predicts a g-value of 2.92 thus, proving that the unpaired electrons of the free radical $(Mn)^{2+}$ is in a bounded state within the compound $MnCl_2$. The ESR spectra also reveals a hyper-fine structure with a multiplic ity level of 6 as observed in Figure 6. As a result of 6 hyper-fine structures present within the spectrum, it can be predicted using the $(2I + 1)$ relation that $(Mn)^{2+}$ has a nuclear spin(I) of 2.5.

Myoglobin - As mentioned previously, 3 samples of myoglobin under dif ferent solvents were tested under EPR spectroscopy. The samples of both dry Mb and Mb in water revealed multiple g values in the system. In all the three samples the value of g never equates to g = 2.00 thus revealing that myoglobin isn't a free radical but instead it has a metal ion at it's base.

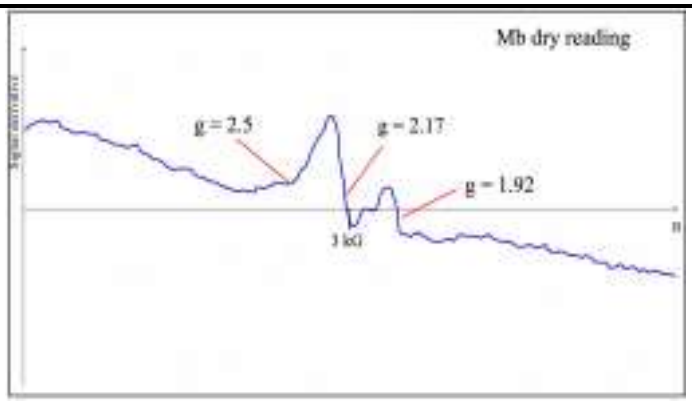


Figure 7: EPR spectrum of dry Mb. Experimental parameters are as follows: Microwave power- 5mW, Microwave frequency- 9.146 GHz, Modulation field- 20G, Modulation frequency-100kHz.

In the case of dry Mb three different signals were observed(as shown in Fig ure 7. The *g* values which were observed are g_1 = 2.5, g_2 = 2.17 and g_3 = 1.92. Based on the observations it can be predicted that these multiple *g* values arise from the low spin *Fe*(*III*) ion present in the active site of the dry myoglobin sample.

In the case Mb in glycerol a simple bell shaped absorption graph was observed and a *g*- value of 2.17 was recorded as shown in figure 8.

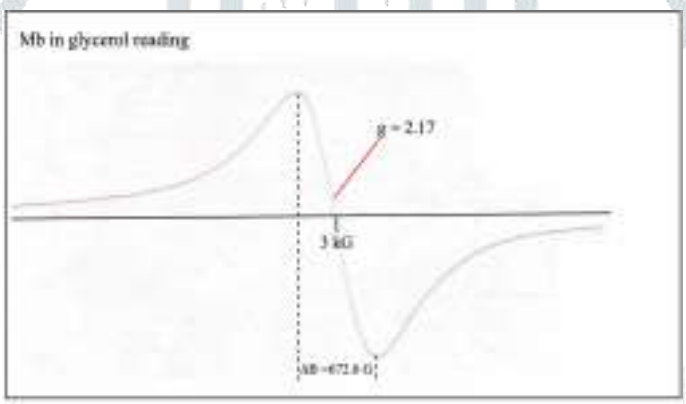


Figure 8: EPR spectrum of Mb of glycerol. Experimental parameters: T-110K, Microwave frequency - 9.122GHz, Microwave power-25mW, Modulation field- 40G, Modulation frequency - 100kHz

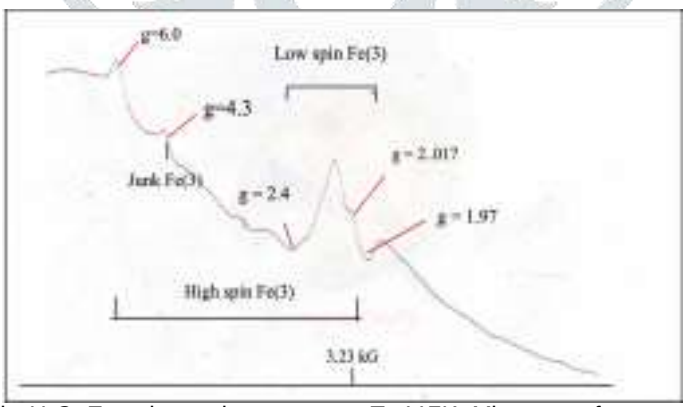


Figure 9: EPR spectrum of Mb in H_2O, Experimental parameters: T- 117K, Microwave frequency-9.12 GHz, Microwave power-25mW, Modulation field- 40G , Modulation frequency-100kHz

Meanwhile in the case of Mb in H_2O, multiple EPR signals were recorded. Upon analyzing the signals, a pair of high spin Fe(III) along with low spin Fe(III) signals were observed(the *g* values for which are summarized in Figure 9 and Table 1)

Upon comparing the readings of Mb sample under different solvents, it can be concurred that the solvent plays a crucial role in altering the readings of the sample. This shift in readings of the EPR signals and the *g* values can be explained by two major chemical phenomena.

The major reason behind this is due to the nature of the two solvents. Both the solvents under low temperatures cool in a different manner. As, both the samples freeze in different ways, the geometry of the ligand around the Fe(III) ion gets affected , due to which there is a change in the signal observed. As a result of this (as shown in Figure 8) in the glycerol samples , the EPR signals broaden up to form a broad EPR line.

Another possible explanation for the change in readings can explained with context to the specific heat capacities of both the solvents. H_2O has a higher specific heat than glycerol due to which a change in absorption spectrum and further a change of g value is observed. Due to this high specific heat of H_2O, the sample under water cools slower than glycerol due to which there exists a thermal energy difference. Based on the thermal energy difference, a change in reading is also observed.

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 (as shown in Table1) .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 $I = 2.5$

In the case of Myoglobin, three different spectra were obtained for different solvent conditions. For dry Myoglobin, three g values were observed a result of the Fe(III) ion present at the active site of Myoglobin.

Further the change in readings in the case of Mb under different solvents was predicted to be due to the nature of cooling of the solvents , which further affects ligand geometry.

Sample	Spectrum details
DPPH	$g = 2.17$
$MnCl_2$	h.s: 6 splittings spaced at, A=109.65 G, centred on g=2.92
Dry Mb	l.s axial:$g_x = 2.5, g_y = 2.17, g_z = 1.92$
Mb in water	h.s axial: $g_1 = 6.00, g_2 = 2.01$ and l.s:$g_3 = 2.4, g_4 = 1.97$
Mb in glycerol	g=2.17 and δB = 672.6G

Table 1: Summary of obtained EPR results. All spectroscopic measure ments of samples were recorded on a Varian E-3 X-band EPR spectrometer equipped with liquid nitrogen cryostat. High spin and low spin have been abbre viated to h.s and l.s respectively. A represents the hyper fine coupling constant measured for Mn(II) ion.

7. References

1. D. T. Petasis and M. P. Hendrich, "Quantitative Interpretation of Mul tifrequency Multimode EPR Spectra of Metal Containing Proteins, En zymes, and Biomimetic Complexes," in *Methods in Enzymology (vol. 563)*, Amsterdam, Elsevier, 2015, pp. 171-208.
2. Petasis, D. T., *EPR handout 1,* LS 190: Introduction to College Research in Physics, Allegheny College, Summer 2020
3. Petasis, D. T., *Lecture 10*, 22/7/2021
4. Petasis, D. T., *Lecture 11*, 27/7/2021
5. Petasis, D. T., *Lecture 12*, 29/7/2021
6. W.R. Hagen, "Practical Approaches to Biological Inorganic Chemistry", Second Edition, 2020
7. Daniella Goldfarb, Stefan Stoll, *EPR SPECTROSCOPY FUNDAMENTALS AND METHODS,* New York, *Wiley, 2018*