Using EPR spectroscopy to study biological samples

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Summary:

EPR spectroscopy is short for Electron paramagnetic resonance a technique used for finding out information about materials with unpaired electrons, EPR was first discovered in the mid 1900s by Soviet physicist Yevgeny Zavoisky. The theory behind EPR is that every electron moves in two different manners, one orbital movement which is basically spinning around its nucleus this sort of movement gives orbital magnetic moment and the second is movement along its own axis this movement gives spin magnetic moment. In a molecule with paired electrons the magnetic moments of the two electrons are opposite to each other hence they cancel each other out this is why unpaired electrons play a huge role in affecting the magnetic moment of a molecule. The total spin angular moment is given by the equation

Introduction:

$$M_S=((S(S+1))^{1/2})\ h/2\pi$$

Where h is the Planck's constant and S is the spin quantum number
a magnetic moment and a spin quantum number in the presence of an increasing magnetic field this increasing magnetic field interacts with the unpaired electron's magnetic moment to split the electron into two different energy levels: +½ state and –½ state. When there is no magnetic field being applied on the system then there is no splitting taking place, hence the electron is in the same energy state also called the degenerate state.

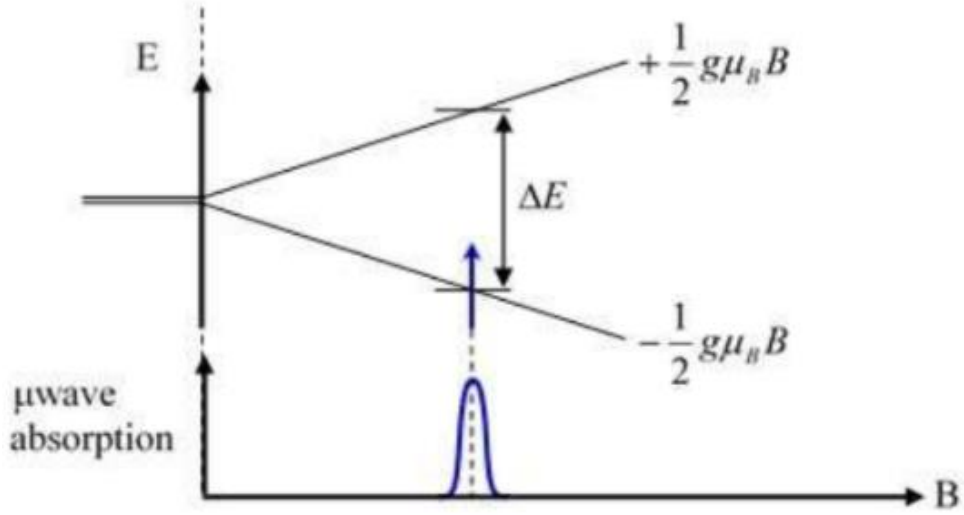


Figure 1: The Zeeman effect

Due to this split the electrons' energy levels get split into two: +1/2gμB and –1/2gμB (as shown in figure 1) with g as the g-factor, μ as the Bohr magneton (a physical constant) and B as the magnetic field.

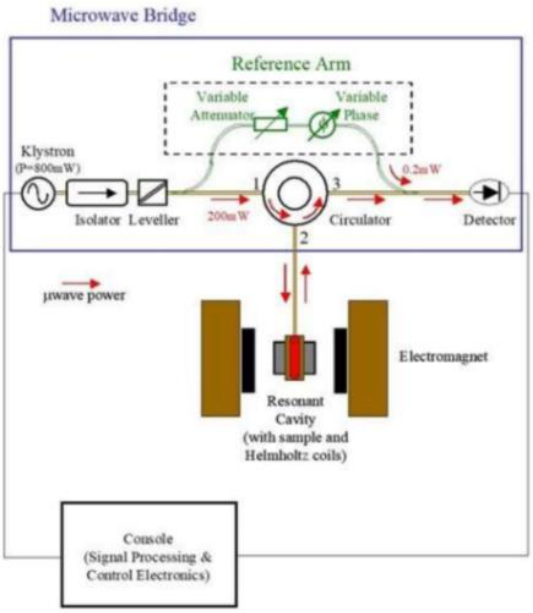
In the graph there can also be seen a small parabola emerging from the x axis, this parabola represents the resonance occurring in the system, this occurs when the difference between the two energy levels becomes so high so as to that it matches the energy of the microwave radiation it is being subjected to. This situation is called resonance and is given by:

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

Where **hν** represents the energy level of the radiation (ν- frequency of Electromagnetic wave, **h**-Planck's constant), **ΔE** is the energy difference between the two electron states which is also given as **g μB**.

An EPR spectrometer was used to perform the experiment, the diagrammatic description of EPR spectrometer ig given below.

Sometimes the absorption spectrum can look a little different and there can be multiple absorptions taking place one after the other, such scenarios are represented by hyperfine structures, this occurs when a nuclei of the atom interacts with the unpaired electron (such interaction is only possible when the nuclei have a spin).

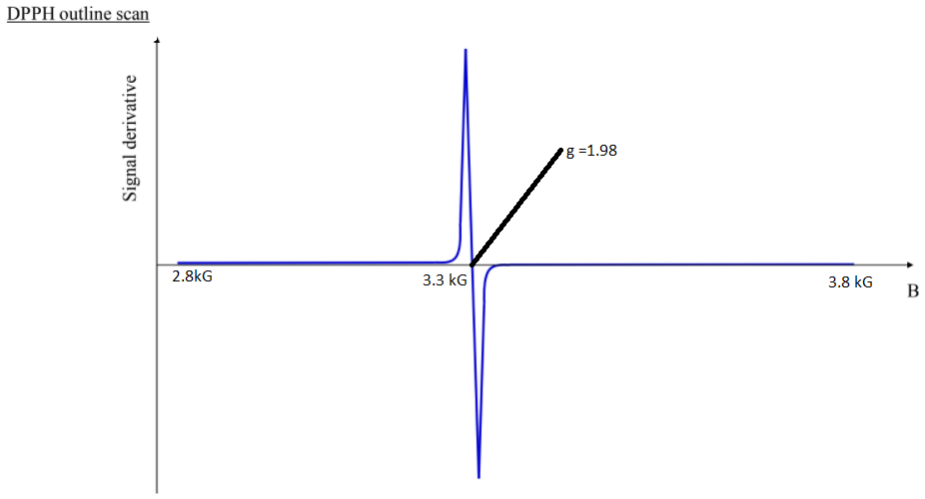


1. The purpose of each equipment is described in brief below.
2. The klystron provides the system with the microwave radiation of constant frequency
3. the leveller, isolator and reference arm play a role in guiding the wave towards the resonant cavity.
4. The sample is kept inside the resonant cavity which detects the absorption signal
5. The electromagnet provides the applied magnetic field
6. A liquid nitrogen flow crystal surrounds the resonant cavity, to cool down the sample and conduct low temperature spectroscopy.

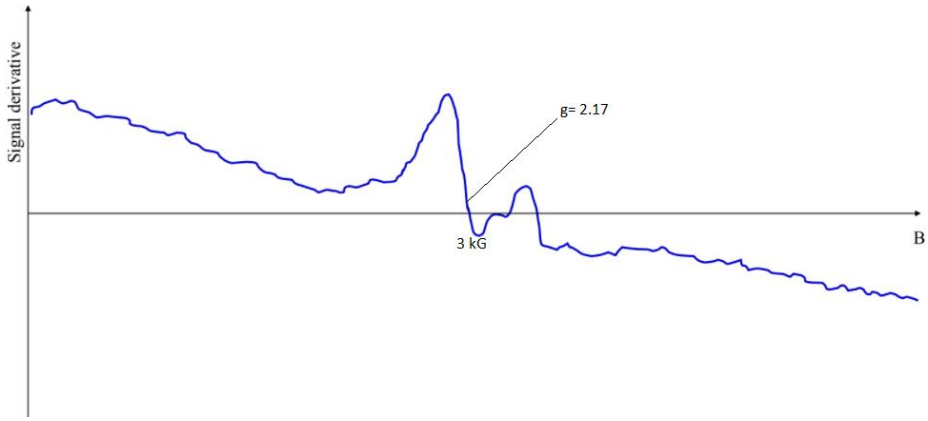
The samples used for the experiment were DPPH, myoglobin in different solutes namely, myoglobin dry, myoglobin in water (H2O), myoglobin in glycerol and MnCl2.]

The following readings were recorded Temp, Microwave power, Microwave Frequency, Modulation frequency, Modulation field

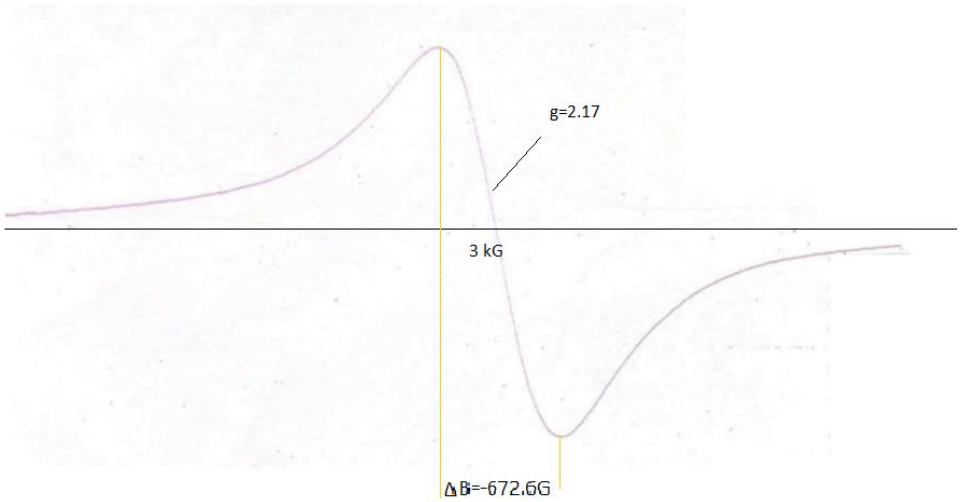
The following graphs were obtained:



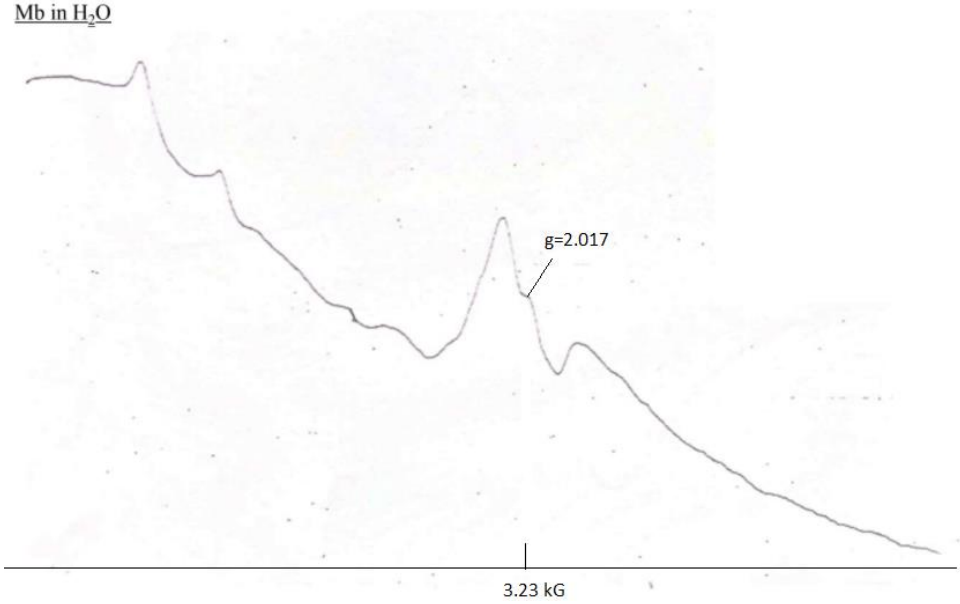
Mb dry outline scan



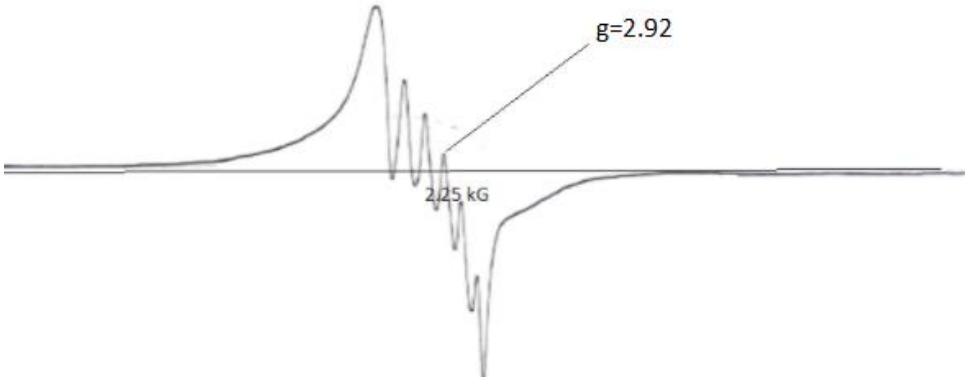
Mb in glycerol



Mb in H_2O



$MnCl_2/H_2O$



The data and its analyses led to the following conclusion

2,2-diphenyl-1-picrylhydrazyl (DPPH) was proved to be a free radical because of an appropriate g value.

Manganese (II) chloride (MnCl2) because of its nuclear spin gave a hyperfine structure. The nuclear spin was later calculated to be I = 2.5

Three different spectra of myoglobin were analyzed for different solvent conditions, results showed that Myoglobin in water had a different g value compared to dry Mb and Mb in glycerol.

Sample	**g value**
Myoglobin	2.17
Myoglobin in H2O	2.01
Myoglobin in Glycerol	2.17
DPPH	1.97
Manganese (II) chloride	2.92

References:

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