Analyzing bio-chemical samples using EPR Spectroscopy

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Date: August 2021

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Summary

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.

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.

Theory

Electron Paramagnetic Resonance(EPR) is a spectroscopic technique which helps us make observations about systems with unpaired electrons .It tells us a great deal of information about the paramagnetic ions in a sample and is useful in in the following ways:

- for the detection and identification of free radicals in the solid, liquid, or gaseous state, and in paramagnetic centers such as F-centers.
- understanding the identity, oxidation, and spin state of the paramagnetic ion(s).
- useful tool to investigate their electronic structures, which is fundamental to understand their reactivity.

Electron Paramagnetic Resonance can be used to analyze a number of compounds such as Free Radicals, Transition metal compounds and is even useful to understand the kind of interactions that occur between the paramagnetic ion(s) and the lattice.

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.

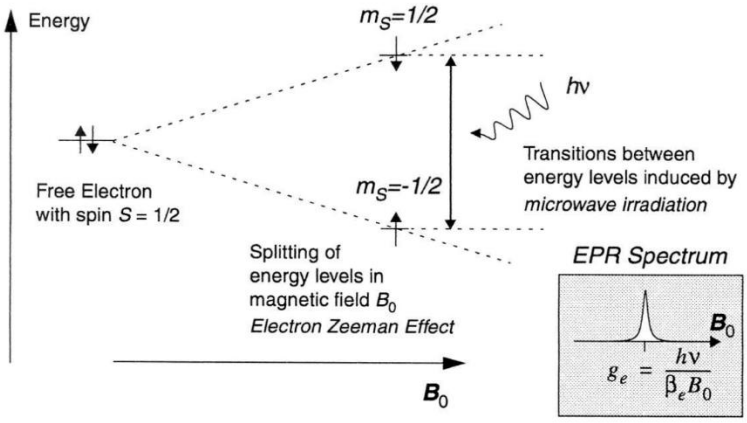

Figure 1:Zeeman splitting of the degenerate electronic spin states for an S=1/2 system. Transition between energy levels induced by absorption of microwave radiation.

Benitez J 2012, ETH Zurich, digital image, ALIEM, accessed 2 August 2015, < https://epr.ethz.ch/>

Condition for electronic transition

$hv = \triangle E = gu_B B$

where v is the frequency of the electromagnetic radiation, g is the electronic g-factor, u_B is the Bohr Magneton and B is the applied magnetic field. This expression is called the EPR resonance condition .

Hyperfine Interactions

Another very important factor in EPR is hyperfine interactions. Besides the applied magnetic field B_0, the compound containing the unpaired electrons are sensitive to their local "micro" environment. Additional information can be obtained from the so-called hyperfine interaction. The nuclei of the atoms in a molecule or complex usually have their own fine magnetic moments. Such magnetic moments occurrence can produce a local magnetic field intense enough to affect the electron. Such interaction between the electron and the nuclei produces a local magnetic field which gives rise to the *hyperfine interaction*. Then the energy level of the electron can be expressed as:

$$\boldsymbol{E = gm_B B_0 M_S + aM_s m_I}$$

In which a is the hyperfine coupling constant, m_I is the nuclear spin quantum number. Hyperfine interactions can be used to provide a wealth of information about the sample such as the number

and identity of atoms in a molecule or compound, as well as their distance from the unpaired electron.

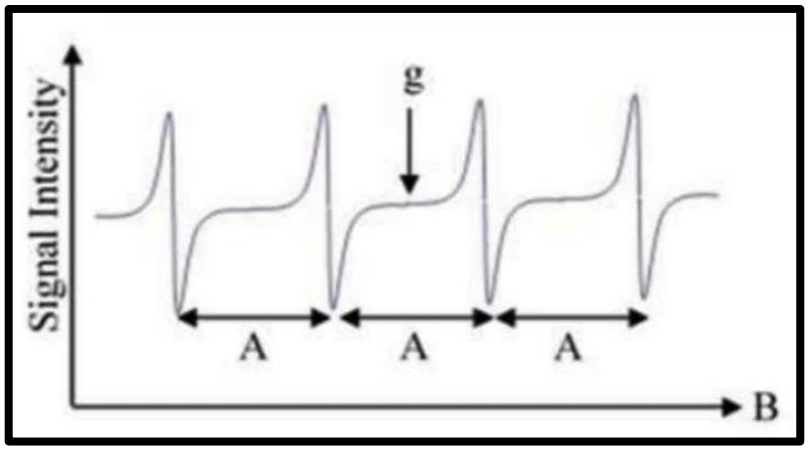

Figure 2: A typical hyperfine spectrum of a species with I=3/2

D.Petasis, EPR Handout, Allegheny College, Accessed July 24,2021

Ligand Interactions

The interaction of the paramagnetic ions with the various ligands in the lattice is due to the interaction of the d-orbitals of the metal ion with the electrostatic field created by the ligands. The nature and strength of the interactions are dependent on the geometry of the ligand. The most common symmetry is octahedral and moreover, the octahedral ligands interact more strongly with the e_g orbitals than with the t_{2g} orbitals resulting in a splitting of the degenerate orbital states.

Experimental Design and Procedure

The instrument that I have used in the experiment is a Varian E-3 X-band EPR spectrometer with a liquid nitrogen flow cryostat. This spectrometer operates in the frequency range 8.5-12 GHz and in the temperature range from about 80 K to room temperature. It utilizes a microwave bypass arm to bias a diode detector for increased sensitivity as shown in Fig. 4. The signal from the detector is processed by an electronics console and is plotted on an X-Y plotter. We make use of the Scanner and the computer to digitize the spectra and convert them into electronic copies.

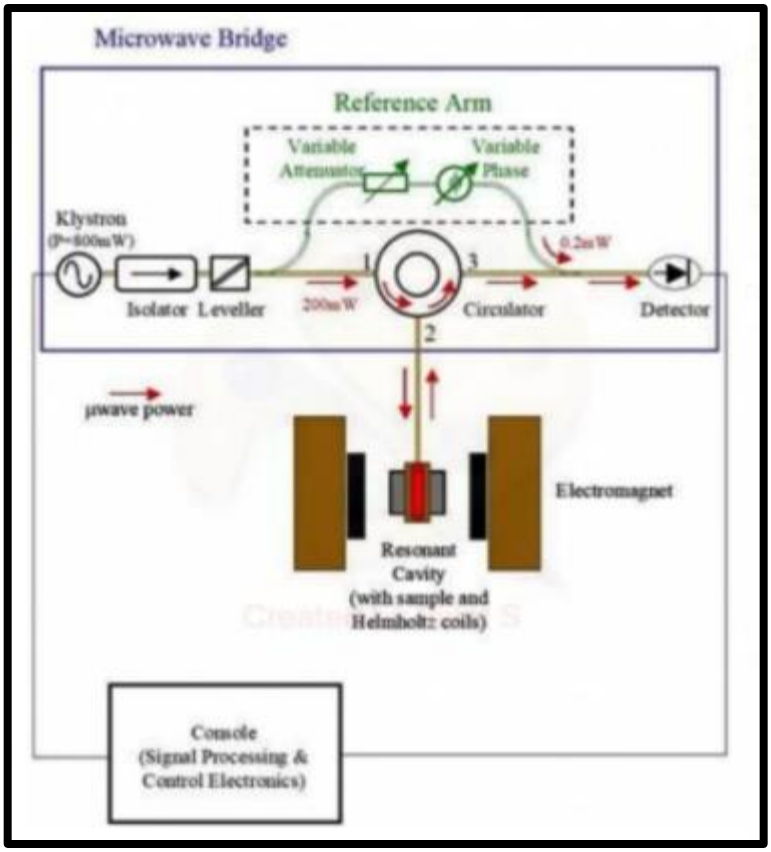

Fig.3 Schematic Diagram of the ESR Spectrometer

D.Petasis, EPR Handout, Allegheny College, Accessed July 20,2021

Klystron: Klystron tube acts as the source of radiation. It is stabilized against temperature fluctuation by immersion in an oil bath or by forced air cooling. The frequency of the monochromatic radiation is determined by the voltage applied to klystron. It is kept at a fixed frequency by an automatic control circuit and provides a power output of about 300 milli watts.

In a nutshell, EPR spectrometers work by generating microwaves from a source (typically a klystron), sending them through an attenuator, and passing them on to the sample, which is located in a microwave cavity.

Microwaves reflected back from the cavity are routed to the detector diode, and the signal comes out as a decrease in current at the detector analogous to absorption of microwaves by the sample.

The samples used in the experiment were 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 measurements were carried out at 300K with a Varian E-3 X band spectrometer.

Myoglobin - Three samples of Myoglobin with different solvents were taken in the experiment:

1. **Dry myoglobin** – A sample of dry 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.
3. **Myoglobin in glycerol –** A sample of myoglobin with glycerol as a solvent was placed in an EPR tube and measurements were carried out at a temperature of 110K.

MnCl 2 in H_2O – A sample of MnCl2 was taken in an EPR tube and measurements were carried out at 104K.

The first thing to do when operating the Varian E-3 Spectrometer is to turn on the coolant water which is located under the fume hood. This ensures that the magnet does not overheat. When carrying out a room temperature scan, the spectrometer and the universal counter should be turned on. The frequency channel must be set to channel three to get an accurate reading. Next, the intensity of the oscilloscope must be turned all the way to the right. Once a signal is seen, the sample is then placed within the cavity .At this point, the mode knob must be turned to tune. A dip will then be displayed on the oscilloscope .We then adjust the horizontal position of the dip until it lines up with the black line present on the oscilloscope. Once the dip is in the proper position, the mode knob can be turned to operate. At this point, the recorder switch can be turned on. Next, the magnetic field mid-range and scan range is set up, and the scan button is then pushed to the right to begin a room temperature EPR run.

An important point to note is that while conducting a low temperature EPR run, the temperature controller needs to be turned on after coolant water is turned on. Next, the main valve on the nitrogen tank, located behind the spectrometer, needs to be opened. The operations of the temperature controller need to be completed before liquid nitrogen can be inserted into the dewar. Once liquid nitrogen is in the dewar , the sample being tested needs to be slowly lowered into a separate dewar which contains liquid nitrogen. It is imperative to go slow, lest the EPR tube will break. Once the sample is cooled, it can then be placed into the cavity. Then, the same steps can be carried out as one does in a room temperature EPR run.

RESULTS AND DISCUSSION

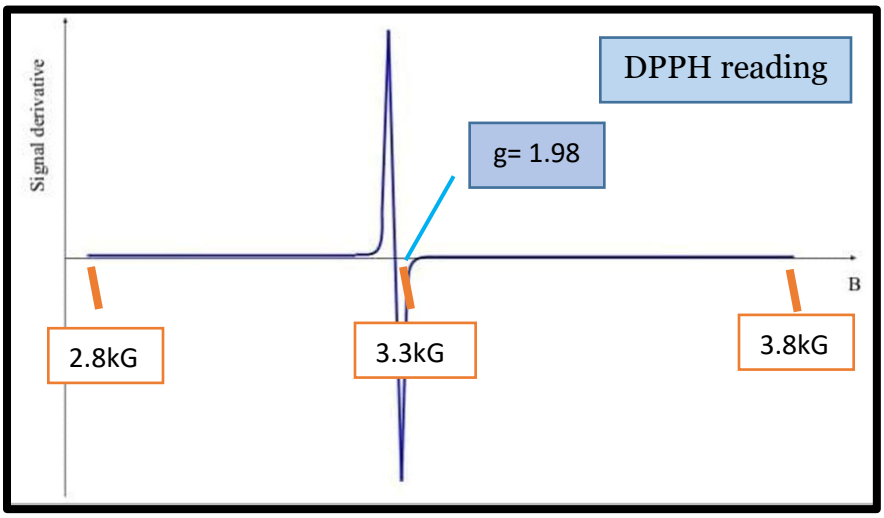

Figure 4: EPR absorption spectrum of DPPH. Experimental parameters are as follows: Temp.-300K, Modulation frequency-100kHz, Microwave frequency-9.139 GHz, Microwave power-1.25mW

DPPH: Upon calculation, we find that the g-value for the above spectrum is **g=1.98** when measured at a temperature of 300K.We know that the theoretical/ideal g-value of DPPH is **2.00**.Hence, it can be concluded that there is a presence of instrumental error within the experimentation. The error percent is calculated to be approximately 1%. As the experimental error is small, we can conclude that **DPPH** is a free radical.

Myoglobin: 3 samples of myoglobin in 3 different solvents were tested under EPR spectroscopy. The samples of dry Mb and Mb in glycerol revealed similar g values of **2.17** thus revealing that Mb isn't a free radical. In case of Mb in glycerol, more broad signals were obtained with almost no noise.

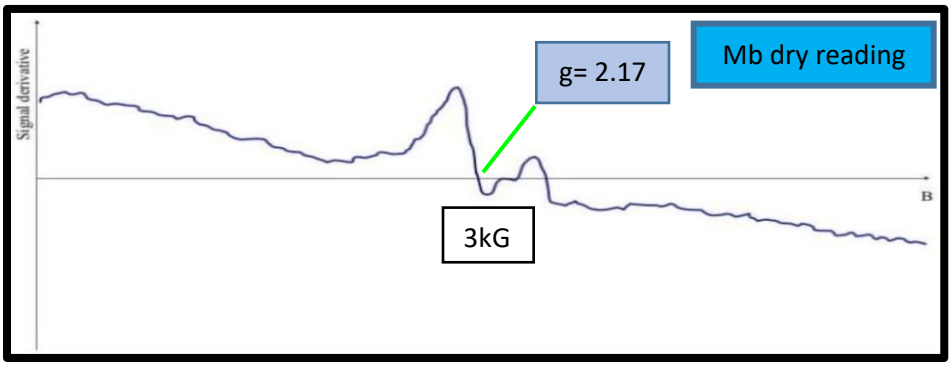

Figure 5: EPR spectrum of dry Mb. Experimental parameters are as follows: Microwave frequency-9.146GHz,Modulation field-20G,Modulation frequency-100kHz,Microwave power-5mW

But the g-value of Mb in H_2O upon calculation comes out to be **2.017**.The difference in g-value between the dry myoglobin sample and the myoglobin in water sample is due to the difference in characteristics of the two solvents.

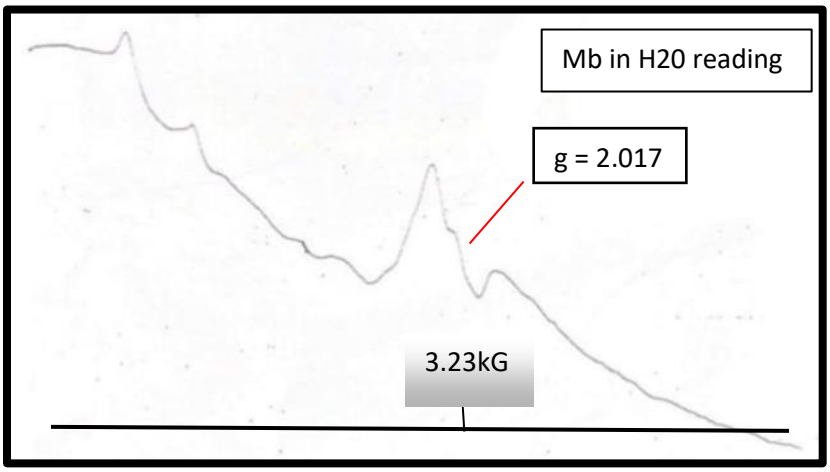

Figure 6: EPR spectrum of Mb in H_2O .Experimental parameters: Temp- 117K, Microwave frequency-9.12 GHz, Microwave power- 25mW, Modulation field- 40G , Modulation frequency-100kHz

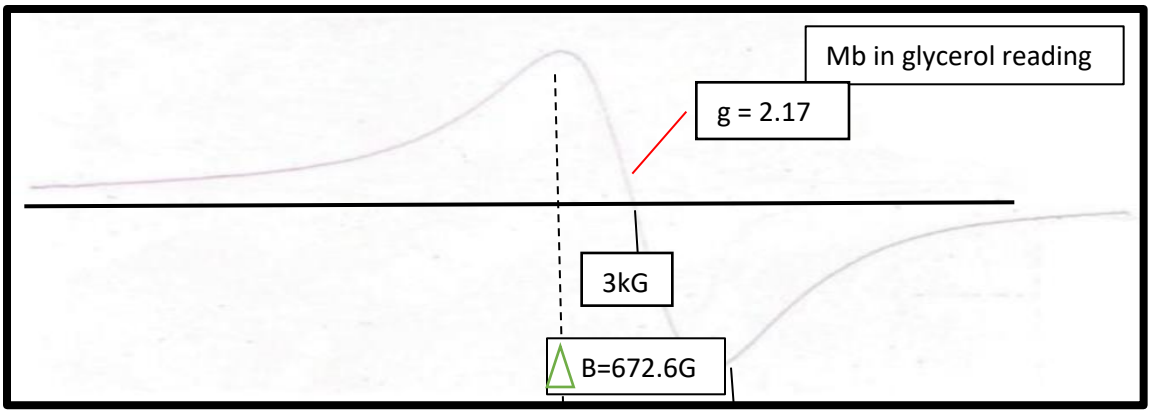

Figure 7: EPR spectrum of Mb in glycerol. Experimental parameters: Temp.-110K, Microwave frequency - 9.122GHz, Microwave power-25mW, Modulation field- 40G, Modulation frequency - 100kHz

Reason for difference in g-values: H_2O has a high specific heat than glycerol due to which a change in g-value is observed. Moreover, due to this high specific heat of H_2O, the sample under water cools slower than glycerol due to which there is a thermal energy difference .Based on the thermal energy, Mb in H_2O has a higher thermal energy and thus has a g-value close to 2.01.Hence, due to a difference in the external conditions under different samples, a difference in

g-factor is obtained. Moreover, I believe it is also due to the polar nature of water as a solvent. The Mb/H2O sample exhibits both high and low spin Fe(III) signals which means that there are different geometries of the ligands around the Fe(III) ion. It looks like the glycerol solvent causes broadening of all of the signals that causes them to merge and produce a single line with a huge width.

$MnCl_2$ in H_2O: The EPR spectrum yields a g-value of **2.92** thus, providing us with enough evidence that the unpaired electrons of the free radical Mn2+ is in a bounded state within the compound $MnCl_2$.The ESR spectra also has a hyperfine structure with a multiplicity level of **6**.As a result of six hyperfine structures, it can be easily shown using the **(2I+1)** relation that **Mn2+** has a nuclear spin of **5/2**.

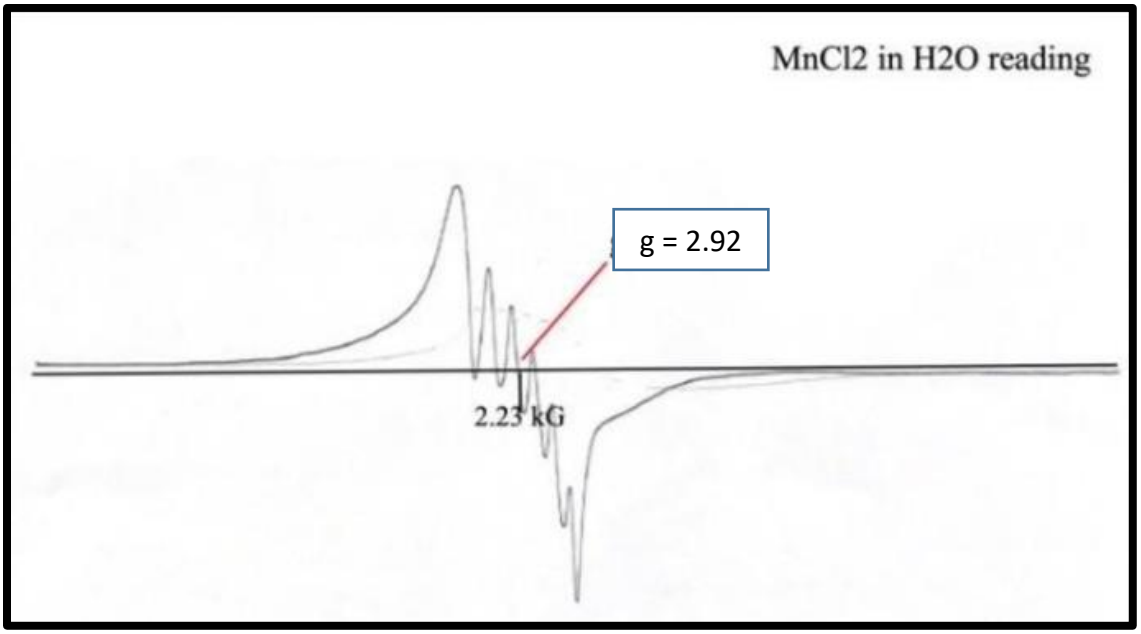

Figure 8: EPR absorption spectrum of $MnCl_2$ in H2O. Experimental parameters are as follows: Temp.-104K, Microwave power- 5mW, Microwave frequency-9.144 GHz, Modulation frequency- 100kHz, Modulation field- 40G

CONCLUSION

In the future, as more stable heavy main-group radicals are isolated, both EPR and its advanced techniques will continue to shed light on the electronic structure of the radical center. The techniques' ability to probe both the electronic and magnetic structure through the g-tensor and hyperfine interactions, respectively, yields valuable insight for the electronic and geometric

structure of the radical center. New approaches in synthesis will allow for continued tuning of the electronic structure of these radicals and influence their reactivity, all to be probed by EPR spectroscopy. Such experiments should be conducted to get a fair idea of EPR as well as to get a wealth of information about the electronic structure of the system.

DPPH as expected displayed the characteristics of a free radical.

In **Myoglobin**, we observed how solvents could play an effective role on the EPR signal of the sample and the g-values.

Meanwhile, **MnCl2** displayed a hyperfine structure of level 6, as a result of its nuclear spin. The nuclear spin was later found to be I = **5/2.** ▭

Sample	***g- value***
DPPH	**g=1.98**
Dry Mb	**g = 2.17**
Mb in H_2O	**g = 2.017**
Mb in glycerol	**g = 2.17**
MnCl2 in H_2O.	**g = 2.92**

Table 1: An overview of the g-values of the various samples under study

References

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