

2

Practical
Aspects

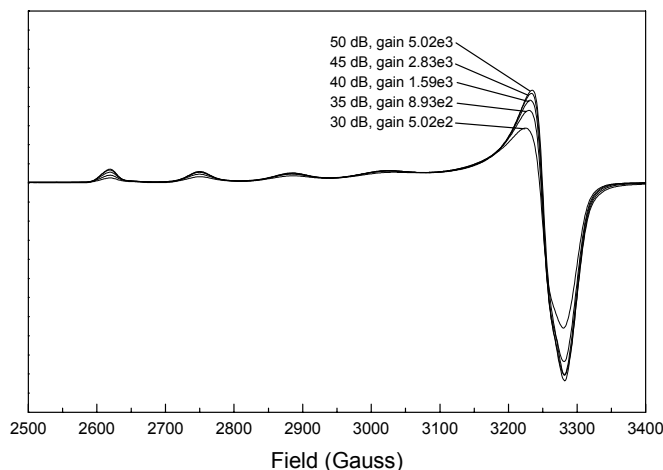
2.1 Proper measurement conditions for your sample, using a Copper Standard as an example

In the two chapters on EPR measurements with liquid nitrogen and liquid helium, an overview is given, under the heading 'Acquiring spectra', of the different parameters you need to set to obtain a spectrum. The effect of some of these is described there. Of course with a completely new sample you will not know all these conditions. First of all you are not sure if you have a signal at all. So one of the first things you have to find out, are the optimal measuring temperatures of possible paramagnetic species in your sample. For this you have to scan the sample at different temperatures, for example 4.5 K, 20 K and 50 K. At every temperature you make a broad scan in the hope you will see a signal. Preferably, you do this at different powers, 40 dB, 20 dB and 0 dB. If you detect a signal, you can zoom in and measure the signal again with a smaller sweep to obtain more detail.

To obtain useful data you have to make sure that you measure under non-saturating conditions. There is a very easy check to do this. The next formula explains the relationship of the amplitude, gain and the power in dB:

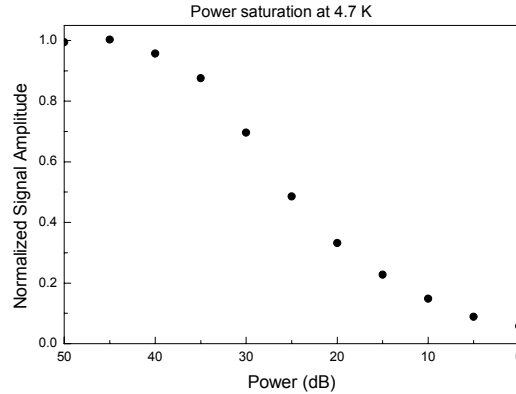
$$\left(\frac{\text{amplitude}}{\text{gain}} \right) \cdot 10^{-\text{dB}/20} = \text{constant}$$

The spectrometer is designed such, that a non-saturating signal remains constant in amplitude when each change of 1 dB in the microwave power is compensated by 1 step in the gain. (In the older software, OS 9 based, the steps in gain are bigger and then the relationship is 2 dB for every step in gain.) Note that a lower amount of dB means a higher amount of power in mW(atts). If you increase the power you have to decrease the gain. This 'design' also explains why the gain cannot be changed to any value we want but can only have certain values. What this practically means is that we can measure a spectrum, for example at 20 dB. Then we can change the power to 15 dB, additionally decrease the gain five steps and remeasure the spectrum again. If the signal is not saturating both spectra should have exactly the same amplitude. As an example, I measured the Copper Standard at 4.7 K at different powers:

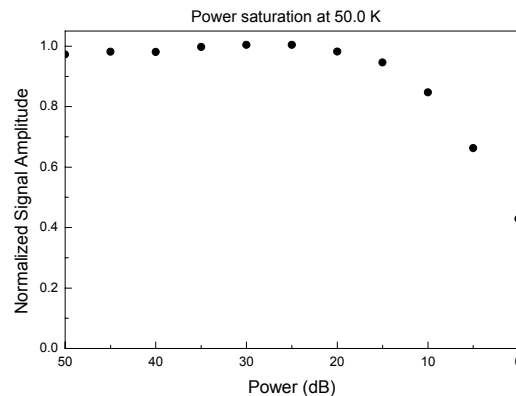
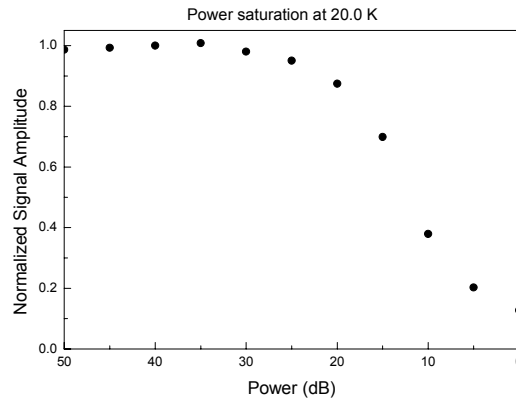


What can be seen from this plot is that from 50 dB to 45 dB the signal amplitude hardly changes, but that the signal amplitude clearly starts to decrease at 40 dB and lower. So if we want to measure the Copper Standard at 4.7 K we should measure with a power of 50 dB.

When we measure the copper spectra at different powers going from 50 dB to 0 dB we can make a so-called power plot:



At the lowest powers (higher dB) you can see there is an area where the curve is horizontal, where the Copper Standard can be measured without saturating the signal. Then starting at 40 dB the signal starts to saturate, the signal broadens and loses amplitude. The Copper Standard has also been measured at 20 K and 50 K:



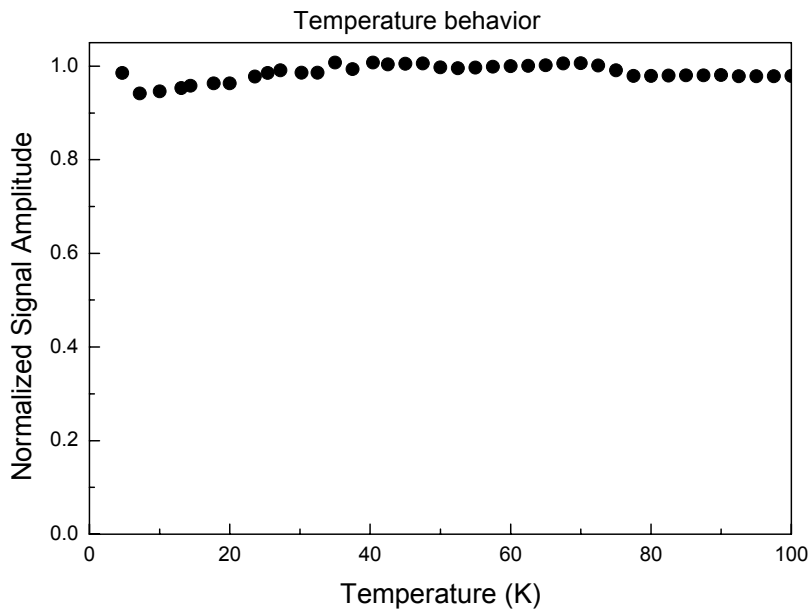
The power plots show that at higher temperatures there is a larger power range where the signal can be measured without saturating. At 50 K we can even measure at 20 dB.

It is very important to know the temperature and power behavior of your signal. The lower the temperature you can measure and the higher the power you can use the better the signal-to-noise ratio. The Copper Standard is used because it can be measured at all temperatures. Normally, however, there is only a certain temperature range where your signal can be measured. To get to know this you have to make a so-called Curie plot. Here we plot again the signal amplitude, but now against the temperature. Since the observed signal intensity or amplitude (I_0) will decrease going up in temperature, we will use a normalized intensity or amplitude (I_n), according to the formula:

$$I_n = \frac{(I_0 \cdot T \cdot 10^{\text{dB}/20})}{(\text{gain})}$$

- I_n normalized value for the intensity (normalized double integral)
- I_0 observed intensity
- T absolute temperature in K
- dB reading of the attenuator
- gain gain

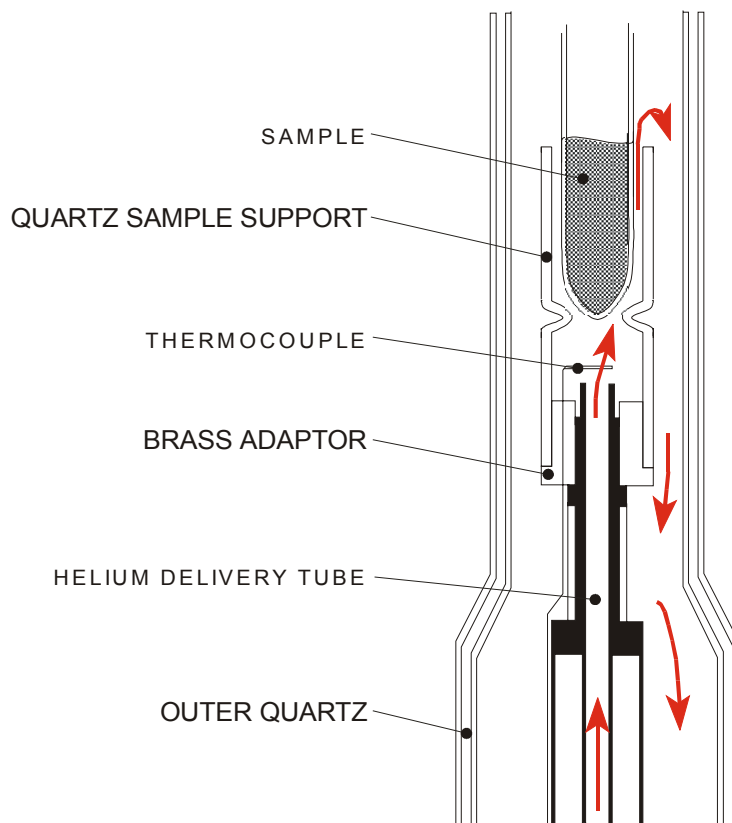
If we do this for the Copper Standard we get the following figure:



In principle we should get a straight line. Small deviations can be expected dependent on the accuracy and calibration of the heater system.

2.2 Quick temperature test for the helium-flow system

If you are using the liquid nitrogen finger dewar the whole sample is immersed. That means that the whole sample has the same temperature, 77 K. With the helium-flow-system you cannot always be sure about the temperature of your sample. This is due to the design of the helium cryostat:



This is a small cross section of the part of the cryostat that is situated in the EPR cavity. The helium is pumped out of the big 100 l dewar through the transfer line, through the cryostat, through the outer tube of the transfer line and then via the GAST® pump into the air. In the cryostat the helium flows through a small delivery tube. (The helium flow is indicated with red arrows.) Just above the outlet of this tube the thermocouple is placed that measures the temperature. The EPR sample is placed about 5 mm above the thermocouple. **This means that not the temperature of the EPR sample is measured but that of the helium flow just below the sample.** As a result it is possible that we measure at the wrong temperature without knowing it.

Of course this system is designed that if it is used properly we get accurate temperature readings. First of all we have to make sure the helium flow is always high enough. To get to a temperature between 4.5 K and 8 K we can change the helium flow with the needle valve (see section 2.5 of the chapter on measuring with liquid helium). Decreasing the flow to get to higher temperatures than 8 K will lead to a temperature difference between the thermocouple and the sample and a temperature gradient in the sample. At this point we cannot trust the temperature readings anymore. This is why we leave the flow at a basic

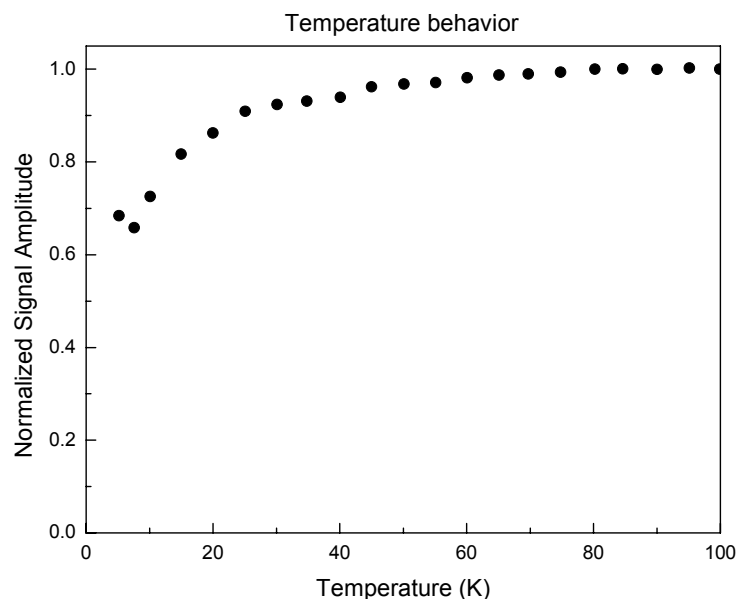
value, giving a temperature of 8 K, and use the heater to increase the temperature of the helium flow.

Still there are several things that can go wrong:

- 1) The quartz sample support is damaged. The little quartz tube has three little knobs that support the sample. This quartz is very thin. **If for some reason you would push to hard on your EPR sample tube when putting it in the sample compartment you will damage the quartz support.** As a result the helium flow will go through the hole in the damaged support tube and will not cool the EPR sample properly.
- 2) The quartz sample support is not properly attached to the brass adapter. This is done with high-vacuum crease. If the crease is not equally divided between the brass and the quartz there might be a small leakage, which will again interfere with the helium flow.
- 3) There is a small blockage in the helium transfer line. This might result in a too low flow in the whole system.
- 4) After changing the sample a little air came into the sample compartment and was frozen when the helium flow was started again. Now the frozen air partially blocks the helium flow.

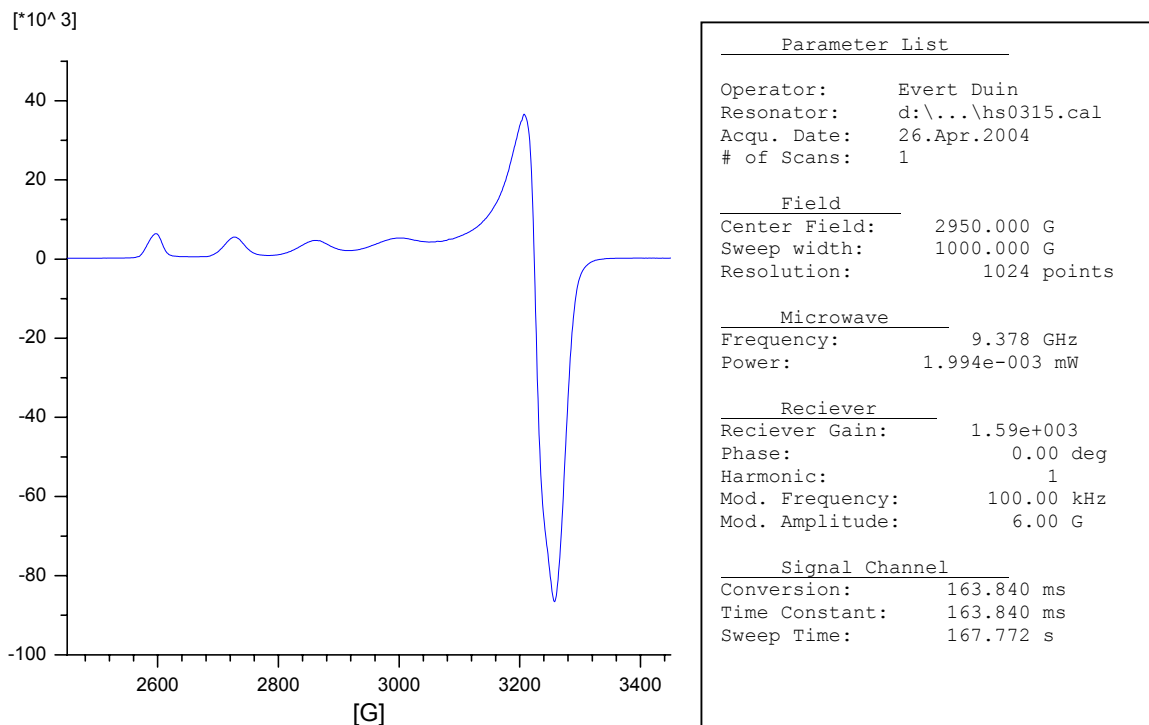
Big blockages are easily to detect: The Flow Controller/Meter indicates a low flow or no flow at all although the needle valve is completely open. Some times, however, the blockage is very small, but just big enough to interfere with the measurements. Also a damaged quartz sample support is difficult to detect when you are running a sample. The quartz support (and also the cryostat and the transfer line) is checked before every run, but it can easily have been damaged during a run.

There is one simple way to detect whether for some reason the temperature reading you get is not the same as the actual temperature of your sample. Again we can use a Copper Standard to do this:



This is an example of a Curie Plot measured with a Copper Standard when the quartz sample support was not correctly positioned. Now the curve is not linear but shows a large deviation at lower temperatures. This behavior can easily be explained by the fact that the actual temperature of the sample was higher than the value obtained from the Temperature Controller.

To make a whole temperature plot is quite some work. Most of the time it is sufficient to measure a Copper Standard at 4.5 K. Since you have to cool down the cryostat to this temperature anyway, it is not much extra work to do this:



This is a copy of the print out of the copper spectrum measured at 4.9 K. This spectrum can be used as a quick reference. If you would measure a Copper Standard (10 mM) under the same conditions (power, gain, etc.) it should have the same signal amplitude. If this is not the case you should ask for assistance.

2.3 Integration of signals and determination of the intensity of a signal with a Copper Standard

The recorded EPR spectra are *first derivatives* of the normal absorption spectra. The area under the *absorption spectrum* is, just as in optical spectroscopy, a direct measure for the concentration of unpaired electrons. In EPR spectroscopy, however, there is no 'absorption coefficient'. All S=1/2 systems absorb equally well. To correlate the intensity of the EPR signal with a concentration, a standard is needed. Different standards can be used. Here we will use a copper standard, a 10 mM Cu(II) solution. Made in the proper way (see below) all the copper is 2+ and contributes to the EPR spectrum. So the 'spin concentration' of this standard is also 10 mM. By comparing the spin concentration of the copper standard with the spin concentration of the signal of interest the concentration of that signal can be determined.

Since an EPR spectrum is a first derivative, we have to integrate twice to obtain the intensity (I_0) (= area under the absorption spectrum). In addition, corrections are needed for a number of parameters, to 'normalize' the spectra. Only then a direct comparison of double integral values of standard and unknown is possible:

$$I_n = \frac{(I_0 \cdot d^2 \cdot T \cdot 10^{\text{dB}/20})}{(g_p^{\text{av}} \cdot f \cdot a)}$$

where

- I_n normalized value for the intensity (normalized double integral)
- I_0 observed intensity
- d distance between the starting and ending points (in Gauss)
- T absolute temperature in K
- dB reading of the attenuator
- f tube calibration factor
- a gain

and

$$g_p^{\text{av}} = \frac{2}{3} \sqrt{\frac{(g_x^2 + g_y^2 + g_z^2)}{3}} + \frac{(g_x + g_y + g_z)}{9}$$

NOTE: The sample of interest and the copper standard should be measured under exactly the same conditions. At lower temperatures, however, the copper standard will be saturated at higher powers. Therefore the copper standard might have to be measured at a lower power than that used for the sample of interest. To calculate the spin concentration an extra correction for the power is needed using the above formula.

Using the SAE02 program for double integration

Start the SAE02 program and read in the spectrum you want to integrate. (The spectrum has to be of the #####.E6 type.) The program will then ask for the temperature in K, the power in dB and the gain (written as for example 1.25e4). It will also ask for the tube factor. This is important when you use tubes, which differ in size and thickness of the quartz wall. Here in Marburg we normally use tubes from one batch that differ maximally 10% in the filling factor. So normally for the tubefactor we can fill in 1.

The program will give you different options. It is possible to only integrate one peak for example. This can be handy if you have a lot of overlap between different signals. Normally we want to integrate a whole spectrum (select 1).

The next screen will show the spectrum again and the first integral. Now you can select the area you want to integrate by selecting the start and end point with the ←, ↑, ↓, and → arrows. Confirm with Return. The next screen will now show the first integral again an also a corrected integral. The correction exists out of setting the start and the end Y-value of the integral at 0. In the best case these two curves are exactly the same. It is possible, however, that you have some underlying broad signal or a sloping baseline. In those cases the two curves might differ greatly and you will get a warning that the value of the double integral might be off by more than 10%. In those cases it is recommended to first do a baseline correction and/or a subtraction of underlying disturbing signals (This can be done with the programs SAE03 or WINEPR).

The next screen allows you to indicate the g values of the signal by simply using the ←, ↑, ↓, and → arrows and again (confirm with Return). The last screen will then give you all the values you have entered. Unfortunately we are not able to print these values, select n for printed copy (y/n)?. You can write down these values, but the only important value is the Normalized double integral value or I_n .

By determining the I_n for both your sample and the copper standard you can now determine the concentration of your detectable signal.

The concentration of the unknown (C_u) is now:

$$C_u = \frac{I_{n(u)} \cdot C_{st}}{I_{n(st)}}$$

where

- C_u spin concentration of unknown
- C_{st} spin concentration of standard
- $I_{n(u)}$ normalized intensity of unknown
- $I_{n(st)}$ normalized intensity of standard

IMPORTANT: this comparison only holds if two S=1/2 spectra are compared. For unequal spin systems the term $1/[S(S+1)]$ has to be added to the formula of I_n (Weil et al. (1994) pp 497-498).

Using the WINEPR program for integration

Load the file you want to integrate. Under WINEPR System select 1D-processing. Then under 1D-processing select Integrate Region or Baseline Correction.

Under Baseline Correction select Define Region. Now you can select an area on the left side of the signal and an area on the right side of the signal by using the left mouse button to select two points for every area and the right mouse button to confirm your selection. Now under Functions you can select a function that best fits the form of the baseline. Normally a linear function gives a good result. Now select Subtract. Select Return to go back to 1D-processing.

Select Integrate Region. Select Integration, Define Integrals to select the start and the end of the area on the spectrum you want to integrate. Use the left and right button on the mouse again. Now you will see the first integral. A good integral will have the Y-values of the start and the end at the same position. Select Integration, Integral type, Double. You have the option to change the slope and the bias of the double integral. These might give you a better-looking curve, but doesn't add to the reliability of your integral. By now selecting Integration, Report you get a new screen which shows the start position of the integral, the end position of the integral, the double integral (DI) and the normalized double integral (DI/N). This last value is the value you need to write down. Select Integration, Return for the next spectrum.

2.4 Preparation of the Copper Perchlorate Standard

The copper standard contains:

- 10 mM CuSO₄
- 2 M NaClO₄
- 10 mM HCl