Operation Hints

for the

MiniScope MS100-MS300

Magnettech GmbH
Louis – Bleriot – Straße 5
D-12487 Berlin
phone: +49 / 30 / 6780 2526
fax: +49 / 30 / 6322 4101
e-mail: info@magnettech.de
homepage: http://www.magnettech.de

1. Switch on the spectrometer

After each switch on of the spectrometer and start of the control program the parameters have to be transmitted immediately from the computer to the spectrometer! In this manner the MiniScope MSx00 will be initialized. Subsequently a first adjust has to be carried out.

(1) Switch on the PC
(2) Switch on the ESR spectrometer MiniScope MSx00
(3) Start the control program MiniScope Control 6.xx.xxxx
(4) After starting the control program click on the button „Apply params“ to transfer the default parameters from the PC to the spectrometer.
(5) Click on the button „MW adjust“ and perform a first adjust of the microwave bridge.
(6) The adjust is finished when the green LED at the front of the spectrometer is shining. Simultaneously “(ready)” appears on the button „MW adjust“.

Measurements for qualitative analysis of radicals are possible from immediately. In the warm up period of the spectrometer the pointer for the "AFC level" often moves out of the green area. Therefore an adjust has to be carried out before each measurement.
For measurements that are supposed to be carried out for quantitative analysis or with multiple accumulations, we recommend to warm up the spectrometer for at least 30 minutes.

2. Adjustment of the measurement parameters

After starting the control program it is opened with a set of default parameters. Now you have the chance to change the following parameters:

- B_0-field
- Sweep
- Sweep time
- Smooth
- Steps
- Number (pass)
- Modulation
- MW atten
- Gain
- Phase 180

If you are ready with your changes click on “apply params” so that the parameters that you have adjusted are transferred to the spectrometer. Afterwards you can tune the microwave bridge by clicking on “MW adjust”. When the adjust is finished (green LED is shining) you can start the measurement. If you want to save the parameters you adjusted for future measurement, please generate a folder for parameter files and save the spectrum you have measured under a name of your choice in this folder. The parameters are saved together with the spectrum in your file. Under “file” “open” you can reload this file and the saved parameters will appear together with the spectrum. Afterwards you just have to click on “apply params” and the reloaded parameters are transferred to the spectrometer. With the further steps you are already familiar.

2.1. Parameter B_0-field

The absolute position of the ESR signal is characterized by the g-value (Landé factor). For the absorbed energy the following equation is valid:

$\Delta E = h \times \nu = g \times \mu_B \times B_0$

h = Planck's constant (constant)
ν = frequency
g = g-value (Landé factor) (constant for a paramagnetic species in a certain environment)
μ_B = Bohr's magneton (constant)
B_0 = magnetic momentum ≈ magnetic field strength

The g-value is a constant for a paramagnetic species in a certain environment. Therefore resonance frequency decides on the magnetic field respectively the position of a signal in the spectrum. The construction of the resonator fixes the resonance frequency; for the MiniScope about 9.43 GHz. In dependence of the glassware that is used as a sample vessel the resonance frequency can vary between 3340 and 3380 G. The parameter B_0-field stipulates the middle of the sweep. The value has to be adjusted in Gauss (G). As Tesla (T) is the SI-Unit 10 G equals 1 mT (milliTesla). The parameter B_0-field should be adjusted between 500 and 4500 G. In combination with the adjusted sweep this magnetic field range shouldn't be exceeded. If you exceed any parameter the program will ask you to adjust the correct one.

Example: A sweep of 1000 G allows a B_0-field between 1000 and 4000 G.
Hint: Most of the ESR signals have a g-value of about 2 and appear at about 3353 G.

2.2. Sweep

During a scan the magnetic field is run from „B_0-field – sweep/2" until „B_0-field + sweep/2". The value has to be adjusted in Gauss (G) and can be varied between 0 and 4000 G.

Example: A sweep of 4000 G allows a B_0-field of 2500 G so that the limits of the magnetic field range are not exceeded.
Hint: For spin trap reaction products a sweep between 60 G and 200 G is common.

2.3. Sweep time

The sweep time is the period in which one scan passes through. It has to be adjusted in seconds and can be varied between 12 and 2047 s. A scan velocity (sweep/sweep time) between 0.5 and 2.5 G/s is optimal. If you measure with a scan velocity between 5 and 8 G/s you loose between 15 and 30 % intensity of the ESR signal.

Example: ESR signal intensity of a 10µM TEMPOL solution depending on the scan time respectively the scan velocity

Scan time [s]	Scan velocity [G/s]	Intensity [a.u.]	Deviation in %
12	8.3	35.58	32.13
20	5.0	43.48	17.05
40	2.5	49.21	6.12
60	1.67	50.65	3.38
120	0.83	51.94	0.92
180	0.56	52.42	-------

Hint: A prolongation of the sweep time leads to an improvement of the signal to noise ratio (decrease of the noise while the signal intensity remains the same).

2.3. Smooth

If you like to perform a smoothing of your spectrum during the measurement, adjust a time in seconds being different from zero. The higher the adjusted value of the time constant the stronger is the smoothing. In practice the value that makes sense to adjust is influenced by the ratio of sweep to line width of the ESR signal. The smaller this ratio is the higher is the value you can adjust for the filtering. The time constant is a very simple smoothing algorithm being equivalent to the hardware filters in former spectrometer. Smoothing by using the time constant decreases the signal amplitude and shifts the spectrum towards higher field. Today's modern calculation software possesses much more effective Fourier transformation based filters. Therefore we recommend to adjust zero and to use the band pass filter in the calculation software analysis.

2.4. Steps

Steps gives the number of data points that are saved in your spectrum file. You can adjust 4096, 2048, 1024 or 512. The physical measurement is not changed by a smaller number. The spectrometer always records 4096 data points. If you have adjusted a lower number the 4096 data points will be reduced by taking only every second, fourth or eighth data point for the spectrum. Another consequence of measuring always 4096 data points is that a reduction of the number of data points will not lead to an increased scan velocity. The maximum scan velocity is always the same. Therefore we recommend to us always 4096 data points because otherwise you may loose some information.

2.5 Number (pass)

„Number (pass) is the number of sweeps being measured. If you adjust a number higher than 1 the sweeps are added up and divided by the number of steps. The second scan or further scans being displayed on the screen are always the result of the accumulation at that step. None of the single scans is saved. You can only save the result of your accumulation after the end of your last scan. The classical accumulation is just an addition of the spectra. The averaging over the number of spectra has the advantage that you can compare the accumulated spectrum with a single scan too. As the noise is statistical, but the signal appears always at the same place, you can reduce the noise accumulating the spectra. A 2^2 = 4fold accumulation decreases the noise to ½. A 2^3 = 8fold accumulation will decrease the noise to ⅓. A 2^4 = 4fold accumulation will decrease the noise to ¼ and so on. Theoretically 1 scan with a sweep time of 240 s should "give the same result as 4 accumulations with a sweep time of 60 s. Practically the result of the accumulations is better, because every spectrometer has a certain hardware filter, which is applied 4 times in the case of the accumulations but only one time for the single scan. This makes clear, why the result of the accumulations practically gives the better result. Furthermore the baseline itself is also averaged by the accumulations, leading to a better baseline itself.

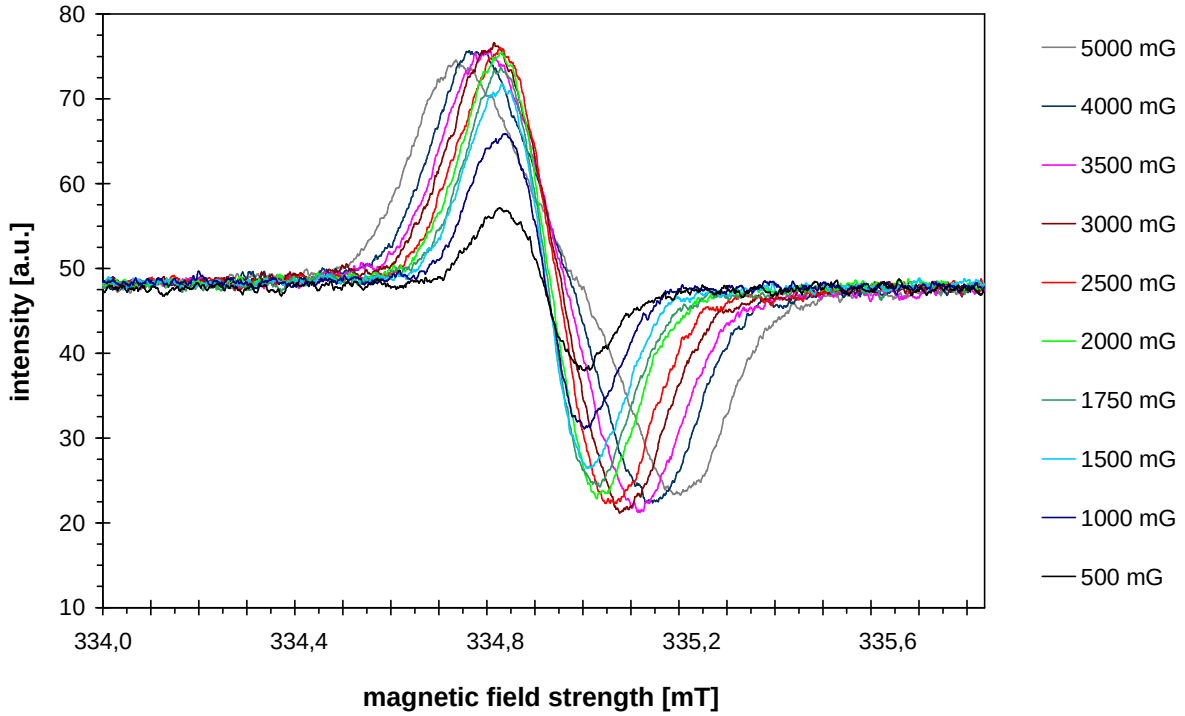
Hint: Accumulations only make sense, if the half lifetime of your radical species is 5 to 10 fold higher then your overall sweep time.

2.6. Modulation

The magnetic field is not swept linearly, because the change in intensity from one field point to the next would be too small to be detectable. Therefore the magnetic field is modulated with 100 kHz inside the resonator. The field amplitude of the modulation is adjustable

between 50 mG and 7000 mG. The intensity is measured versus the magnetic field strength as Δintensity/$\Delta B_{\text{modulation amplitude}}$. The practically performed differential scan is the reason why the EPR spectrum is the first derivative of the absorption spectrum of the intensity of the microwave radiation. The line width of a signal is defined as the magnetic field difference between the maximum and the minimum of the EPR signal. Every paramagnetic species in a certain environment has a natural line width. This natural line width can be determined by recording a spectrum with low modulation amplitude of e. g. 200 mG. Afterwards the line width of the signal is estimated. If the line width of the signal is significantly higher than the adjusted modulation amplitude you have estimated the natural line width of your signal. If the line width of the signal is equal to the adjusted modulation amplitude you have to adjust the lowest possible modulation amplitude of 50 mG. If this will not lead to a line width being significantly higher then the adjusted modulation amplitude you have to accept that the natural line with of you signal is below 50 mG. The estimated natural line width helps you to adjust the right modulation amplitude. If you use the natural line width as modulation amplitude you achieve the highest resolution of the signal. If you adjust double of the natural line width this leads to the highest intensity of the signal. In the case you adjust more than the natural line width the expert calls it “overmodulation”. The twofold overmodulation of the signal for getting the highest intensity makes only sense, if you have very small signals working close to the detection limit of the spectrometer. At modulation amplitudes lower than the natural line width, the line width of the signal is independent from the modulation amplitude and remains equal. The signal intensity increases with increasing modulation amplitude. In the case of overmodulation the line width of the signal is about the same value you adjusted as the modulation amplitude. The higher the modulation amplitude the broader is the signal. Until a twofold overmodulation the signal intensity increases. Afterwards the signal intensity decreases.
The following table and figure show the results of experiments with a 10 µM solution of TEMPOL leading to a natural line width of 1700 mG (G = Gauss) which is equivalent to 0.17 mT (T = Tesla).

Intensity and line shape in dependence on the modulation amplitude



Line width and relative intensity in dependence on the modulation amplitude.

modulation amplitude [mG]	line width [mG]	relative intensity [a. u.]
500	1706	18.22
1000	1663	33.47
1500	1807	43.79
1750	1981	47.94
2000	2053	50.96
2500	2299	52.55
3000	2675	53.97
3500	3123	53.21
4000	3658	52.54
5000	4540	50.20

Hint: As the line width depends on the oxygen content of your solution and therefore on the environmental conditions, the natural line width you estimated is only valid for the paramagnetic species under the measurement conditions.

2.7. MW atten

„MW atten“ is the microwave attenuation. The microwave is emitting with a certain microwave power. Directly behind the microwave source being a gunn-oscillator the microwave power is attenuated by an attenuation unit. The microwave attenuation is calibrated between 0 and 30 db which corresponds to 100 to 0.1 mW. The following equation gives the connection between attenuation and microwave power:

$$\text{attenuation}\,[\text{dB}] = 10 * \log\left(\frac{100 mW}{microwave\,power\,[mW]}\right).$$

To make things more easy have a look at the following table to find the microwave power corresponding to your adjusted microwave attenuation.

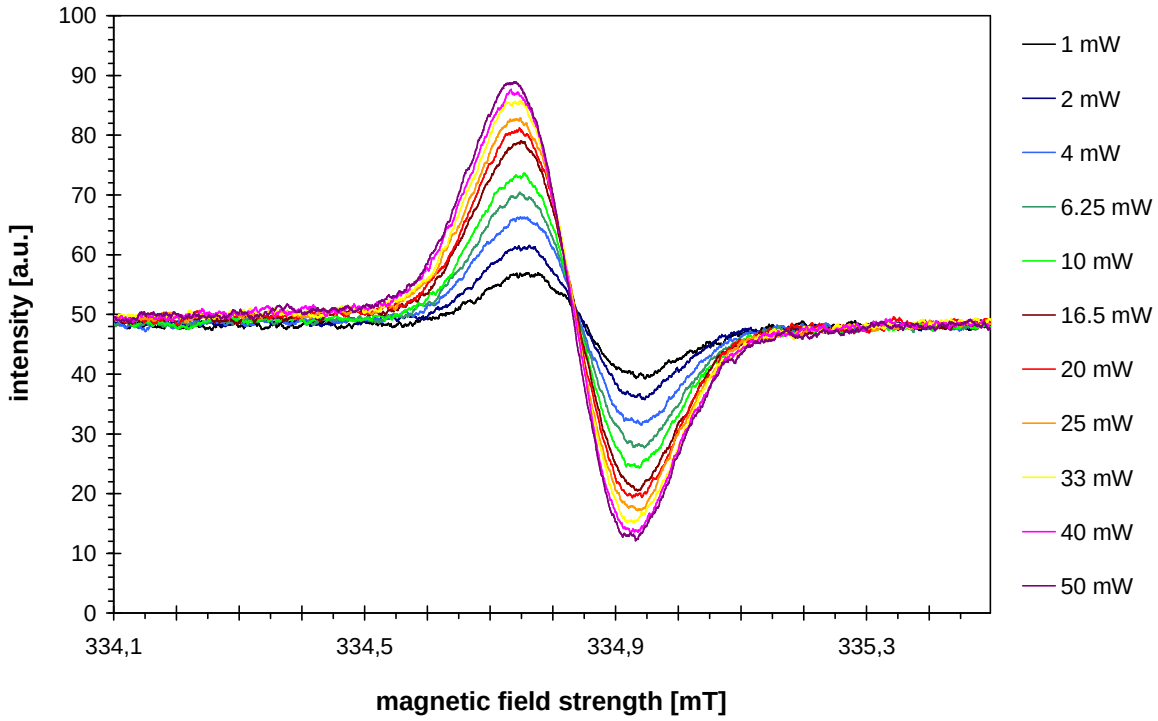
Microwave attenuation [db]	Microwave power [mW]
0	100.0
1	79.43
2	63.10
3	50.12
4	39.81
5	31.62
6	25.12
7	19.95
8	15.85
9	12.59
10	10.00
11	7,943
12	6.310

13	5.012
14	3.981
15	3.162
16	2.512
17	1.995
18	1.585
19	1.259
20	1.000
21	0.7943
22	0.6310
23	0.5012
24	0.3981
25	0.3162
26	0.2512
27	0.1995
28	0.1585
29	0.1259
30	0.1000

The number of electrons being shifted from the energetic level ‘with the field’ (basic level) to the one ‘against the field’ (excited level) depends on the power of the microwave radiation. The electrons in the excited state will go back to the basic energy level by spontaneous relaxation. Starting at low microwave power the absorption of the microwave radiation depends linearly on the microwave power (occupation of the basic level > occupation of the excited level). Afterwards an area follows, where the electrons are distributed nearly equal between both levels. This is the area of so called ‘spin saturation’. By a further increase of the microwave power all electrons which show a spontaneous spin relaxation will be excited again immediately (occupation of the basic level < occupation of the excited level). This means there is a reduced number of electrons being able to absorb the microwave energy and the signal intensity decreases. The optimal choice regarding the microwave power is a value at the transition between the linear increase and the spin saturation. Working at the spin saturation area can not be recommended in the case of analytical purpose because secondary phenomena like spin exchange and spin transitions are observed.

Experiments with a 10 µM solution of TEMPOL lead to an optimal microwave power of 7 mW. Maximum usable microwave power is 15 mW before saturation is achieved. This means that attenuation has to be adjusted between 8 and 11 db.

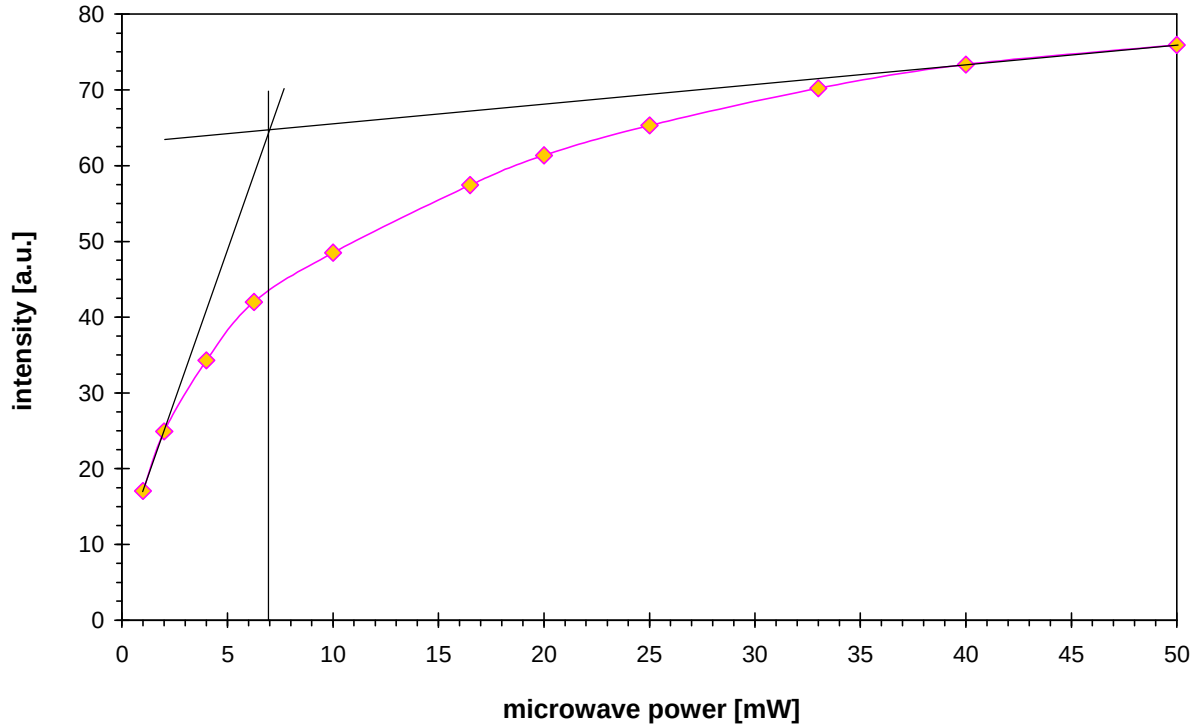
Spin saturation in dependence on the microwave power.



Relative intensity (amplitude) depending on the microwave power

microwave attenuation [db]	microwave power [mW]	relative intensity (amplitude)
20	1	17.06
17	2	24.92
14	4	34.28
12	6.25	42.00
10	10	48.52
8	16	57.43
7	20	61.36
6	25	65.31
5	33	70.22
4	40	73.35
3	50	75.94

Spin saturation of a 10 µM aqueous TEMPOL sample



2.8. Gain

The amplification of the EPR signal is adjusted by the parameter gain. The format of the number is a scientific one:

“value” “E order”

“Value” stands for the factor and “E order” for 10 to the power of “order”. Therefore the gain results in the following way:

Range: value: 1 - 9
order: 0 - 2

Example: value = 2, order = 1 --> Gain = 20
value = 5, order = 2 --> Gain = 500

Hint: The gain can only amplify the spectrum the way that during the measurement the signal will be right visible on the screen and that it will be resolved high enough for the later calculation. In general the increase of the gain leads to an increase of both signal and noise in the same manner.

An increase of the signal in comparison to the noise can only be achieved by an increase of the modulation amplitude or the microwave power and not by an increase of the gain!

2.9. Phase

The amplifier works after the principle of phase sensitive rectification. The “phase” can be adjusted at 180° by activating the switch panel or at 0° if the switch field is not activated. An adjust „out of phase“ at 90° or 270° is not possible. A switch between phase 0° and 180° and vice versa will result in an inversion of the signal. Usually the phase is adjusted the way that during the scan the signal first reaches the maximum. The reason for this is that after the first integration of the signal you will get a positive integral of the signal.

3. The sample preparation

3.1. Sample positioning

The position of the sample inside the resonator has a big influence on the measured signal intensity and the properties of the resonator. Therefore you have to tune the microwave bridge by „MW adjust“ after every exchange of the sample. The sample has to be positioned in the centre of the resonator to achieve the maximal signal intensity. The distance from the upper edge of the white sample tube holder to the middle of the resonator is 94 mm (see Figure 2).

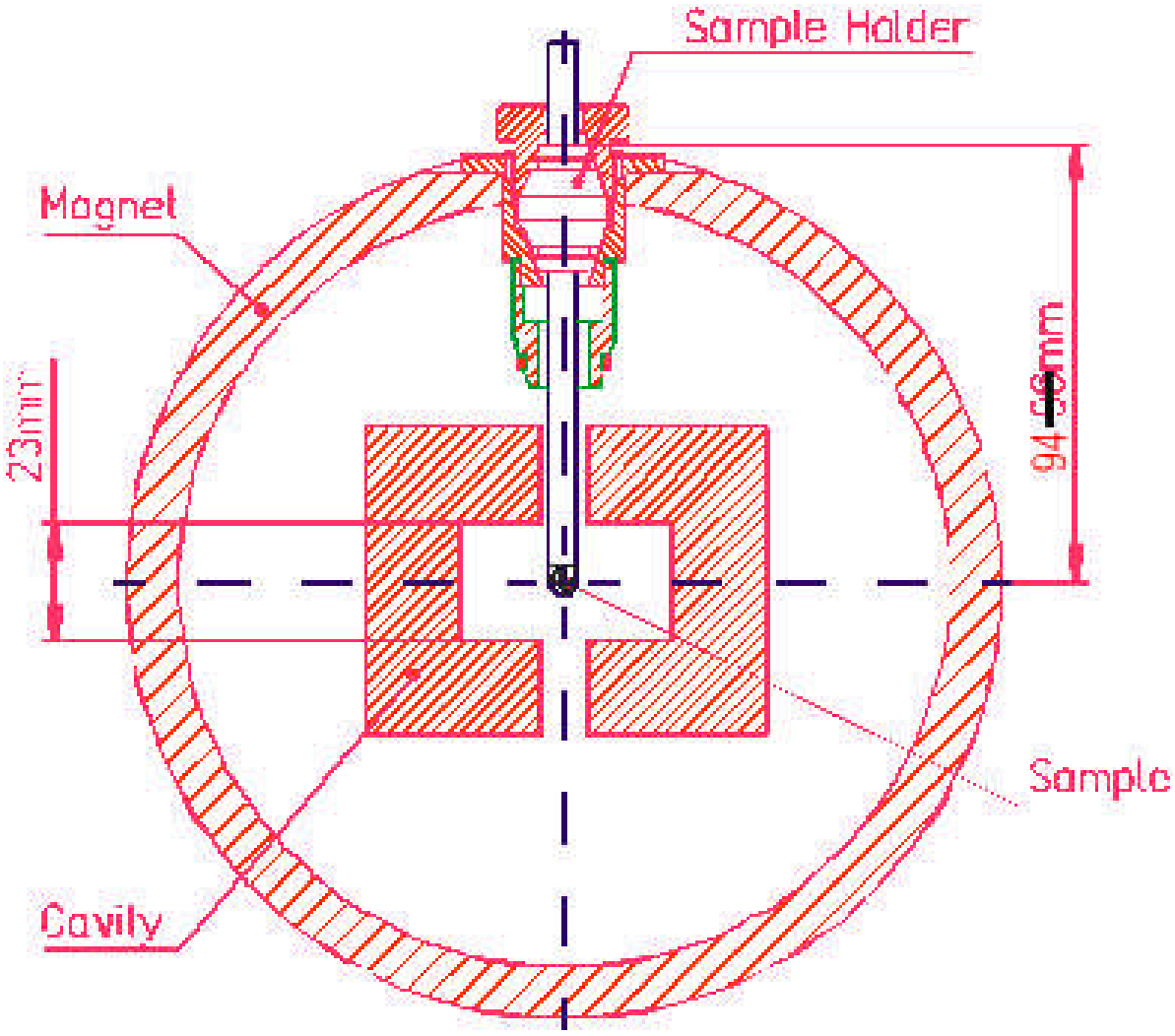


Figure 2

Measurements using sample tubes:
If the sample tube is filled with a small amount of the sample (filling level < 1 mm) the distance between the bottom of the sample tube and the upper edge of the white sample tube holder has to be 94 mm. In the case of a higher filling level the distance between the middle of the sample filling and the upper edge of the sample tube holder has to be 94 mm (Figure 3). The active measurement area of the resonator in vertical direction is 23 mm. Over this 23 mm the signal intensity follows a Gauss distribution with the maximum signal intensity in the centre of the resonator. Only the sample compound positioned in the active measurement area of the resonator contributes to the EPR signal. For reproducible measurements it is recommended to fill the sample tube over the whole active measurement area homogenously and to use sample tubes with equal dimensions.

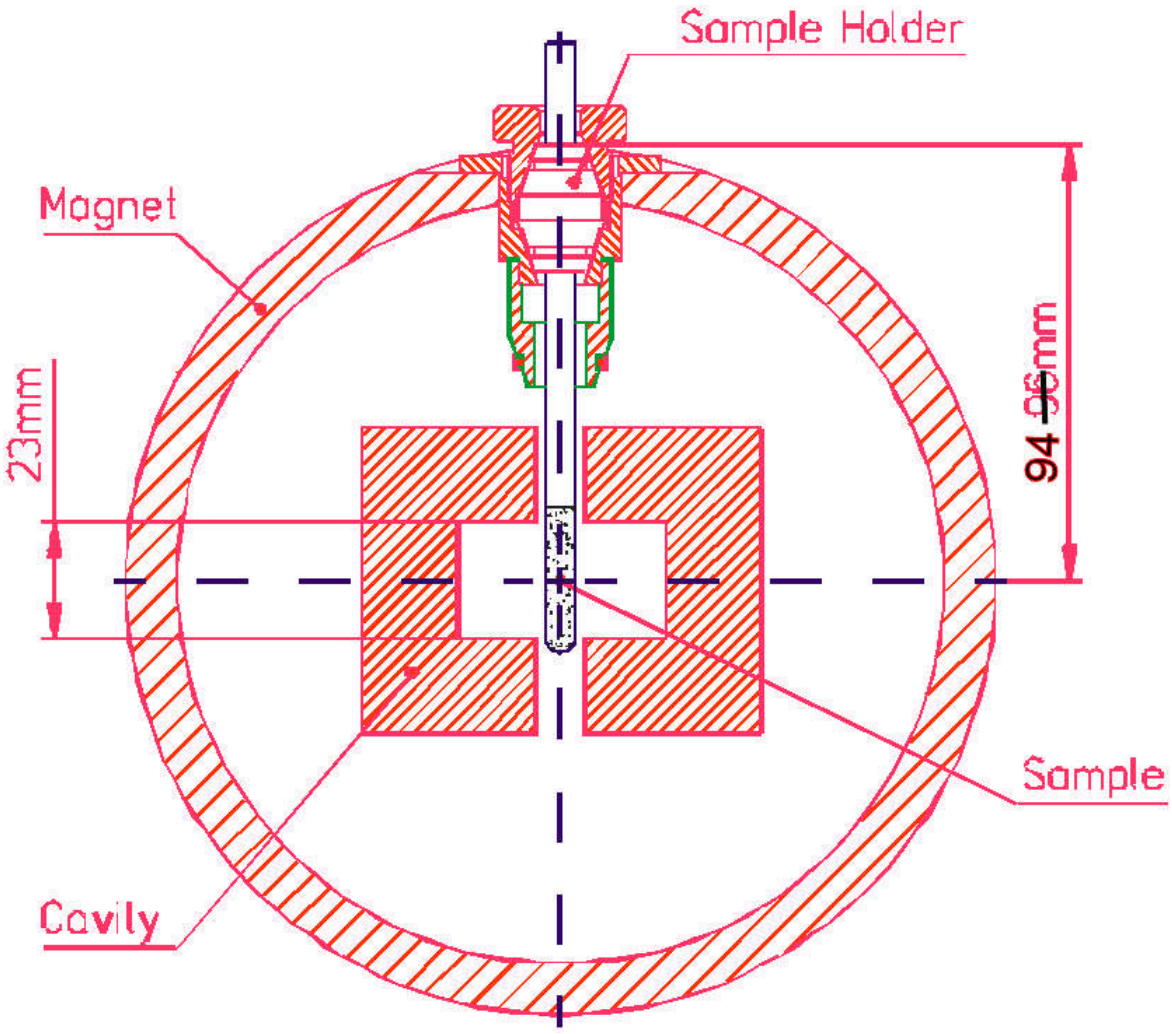


Figure 3

Measurements using capillaries:
The capillary is to thin for a direct sample holder. Therefore the capillary is inserted into a guidance tube of 120 mm length (see under glassware in our catalogue). The crucial point for the selection of the guidance tube is that the inner diameter is not too big to achieve that the capillary is sitting always at the same position inside the resonator. For aqueous solutions the maximum volume of the used capillaries must be 50 µl. About 50 mm of the capillary should be filled and positioned in the middle of the vertical active measurement area and in the middle of the resonator area.

Measurements using flat cells:
The easiest way to assure a perfect positioning of the flat cell is to purchase our special holder SH-P and to use the fixed holder with fitting with the flat cell of any kind (see under positioning systems in our catalogue). The position of the flat area has to be perpendicular to the front (see Figure 4). As the flat cells are hand made they are not always absolutely straight. Therefore a turn of 180° can lead to a different signal intensity of the same sample. So please mark the side of the flat cell that has to be positioned closest to the front. Beside the position perpendicular to the front a parallel position to the front is possible too. This is used in the case of measuring samples that strongly attenuate the resonator or if this positions leads to higher intensity in comparison to the perpendicular one. In the case you position the flat cell by hand using the standard holder small changes of the absolute position in the resonator will have a big effect on the signal intensity respectively the reproducibility of the measure-ments. Therefore we recommend the use of the positioning system special holder SH-P.

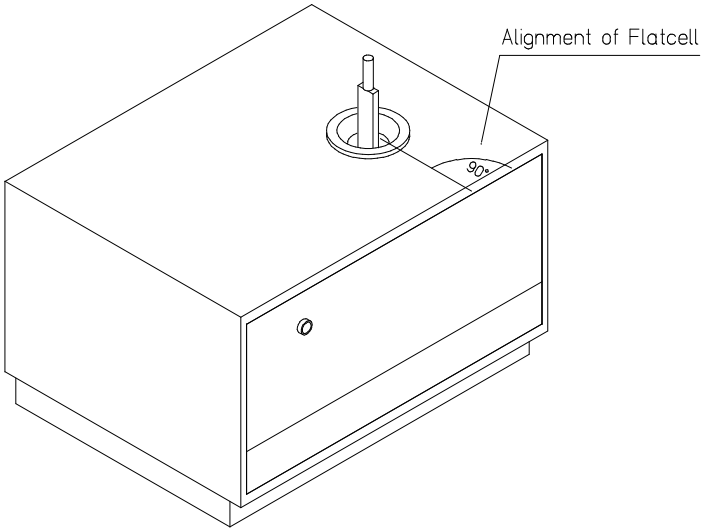


Figure 4

3.2. Sample features, selection of the right sample vessel

For measurement of samples in the EPR spectrometer you have the choice between the following sample vessels:

- **Capillary**
- **Sample tube**
- **Flat cell**
- **Tissue cell**
- **Special cuvette**
- **Fixed temperature dewar**

Some general remarks

The active volume in the vertical direction of the MiniScope rectangular resonator is 23 mm. Over this 23 mm you have a Gauss distribution of the intensity. This means in the centre you measure 100 % intensity. 1.65 mm top and bottom of the centre the intensity is 95 %. 2.5 mm top and bottom of the centre the intensity is 90 %. This means you have two possibilities to get reproducible and comparable measurements:

1. You fill the sample vessel over the whole active volume of the resonator.
2. You fill 5 mm of your sample vessel. In this case you have to make sure that the sample is positioned absolutely in the centre of the resonator. A further requirement is that in the case you are using different sample vessels of the same kind, that they are selected for the same intensity or that you have compared them using a standard solution introducing correction factors.

In the case you are using sample vessels with a flat section you can orientate the flat section parallel or perpendicular to the front panel of your spectrometer. Please try, which orientation gives the higher intensity.

Capillaries are standardized calibrated sample vessels. They are cheap one way items and allow an easy sample exchange. Usually capillaries are used together with a matching guidance tube.
For aqueous solutions the maximum volume of capillaries is 50 µl. In the case you use organic solvents with a lower dielectric constant you can use capillaries with a higher volume or sample tubes.

Sample tubes are used for liquid and solid samples. We offer sample tubes of different diameters and length. The dielectric constant of the solvent of your liquid sample lays down the diameter of the sample tube. For example radicals in hydrocarbons can be measures using a 4 mm or 5 mm sample tube. Concerning the solid samples the humidity of the sample restricts the diameter of the sample tube. The length 135 mm is used in general. The length 240 mm is adapted to the finger dewars we offer. In the case a sample is not tuneable you can either choose a sample tube with a smaller diameter or you reduce the amount of sample inserted into the resonator.

Flat cells are used for liquid samples in solvents with a high dielectric constant. The ideal sample distribution in the resonator leads to a 2 – 2.5 fold higher intensity compared to 50 µl capillaries. The crucial point, when using a flat cell is the orientation of the flat section, the vertical position and the filled volume of the flat cell.
Therefore we recommend using the special holder SH-P as the system to achieve always the same sample positioning.

Tissue cells can be used to measure skin sections, paste and emulsions. The same requirements have to be fulfilled that are valid for flat cells.

A **special cuvette** we offer for alanine dosimetry of thin film test strips. This cuvette has a flat section too. Furthermore we offer special tubes for alanine tablet dosimeters.

Fixed temperature dewars are used to measure samples at liquid nitrogen temperature of 77K. You can either drop the sample into the liquid nitrogen and the frozen drops are collected in the finger for measurement. A further possibility is to freeze a cylinder of 4 mm diameter matching to the size of the inner finger of the dewar. In the case you like to measure solid samples you can use sample tubes of 3 or 4 mm outer diameter and 240 mm length.

3.3. Effect of the filling factor

One of the Parameters that influence the intensity of the EPR signal is the filling factor. This means that the intensity of the signal increases with increased sample volume inside the resonator. Please pay attention concerning this effect especially when you perform quantitative measurements. The effect of the filling factor can be eliminated by filling the sample tube over the whole active measurement area of the resonator (23 mm). Capillaries are volume calibrated and therefore especially suitable for quantitative measurement.

Hint: 1. Quartz sample tubes are hand made and therefore may differ in volume among each other. For quantitative measurements the sample tubes have to be selected on the base of measurements with a 100 µM TEMPOL solution.

2. For sample that strongly attenuate the resonator like e.g. aqueous solutions the linear connection between the increase of the sample volume e.g. through the use of a flat cell with increased volume und the resulting increase of the signal intensity is not absolutely valid. The reason is that by an increase volume of a strongly attenuating sample the quality of the resonator will be decreased. This will result in lower signal intensity then expected only based on the filling factor.

3.4. Sample tube features

Glass contains Fe^{3+} cations which lead to an EPR signal that might disturb the measurement. For this reason synthetic quartz glass, e.g. Suprasil® or SQ2®, is used for the sample tubes. Synthetic quartz glass compared with natural quartz glass has the advantage that it develops no signal while being irradiated. The commercially available capillaries (green mark) are made of ordinary glass but the effect on most of the measurements is negligible.
A further property of the sample tubes is the influence on the resonance frequency of the resonator. An increase of the amount of quartz glass in the resonator leads to a decrease of the resonance frequency. The use of thick-walled sample tubes may lead to shift in the resonance frequency which may not be compensated by the microwave bridge. The typical wall width for sample tubes is 0.5 mm.

Protocol of the sample preparation using the method of A. Kleschyov

Preparation of vessels:

The vessel should be prepared very clean. Outside all fat should be removed, because otherwise the penetration of the spin trap is hindered. 50 – 100 mm^2 of the vessel are needed. For different species respective pieces of Aorta or Vena Cava have to be prepared:
- rabbit vessel ring of 5 mm width – can be incubated in one piece.
- rat vessel length 1 – 1,2 cm – cut it into three or better four rings before incubation
- mouse vessel length 1,5 cm – cut it into four or five rings before incubation

The vessels have to be prepared as fresh as possible and kept ready in 250 µl KrebsHEPES buffer in a 24 well plate on ice for incubation with the spin trap. In the case of L-arginine depleted vessels please add 1 mM L-arginine. According to you experimental design add stimulator, inhibitor or radical scavenger.

Preparation of the spin trap

Bubble argon into 2 times 10 ml of KrebsHEPES buffer each in a vessel (50 ml) on ice for 30 minutes.
Weigh in 3,6 mg sodium DETC and 2,25 mg $Fe(II)SO_4 \cdot 7\ H_2O$.
Dissolve DETC and Fe(II)sulfate each in its 10 ml KrebsHEPES buffer while bubbling (about 30 seconds).
Clean the syringe of the one channel dispenser with argon.
The two solutions are mixed and immediately sucked into a one channel dispenser being able to aliquot 250 µl.
Press out the bubble that has been generated by suck in the solution and reject 1 ml of the spin trap solution. Afterwards immediately pipette 250 µl of the spin trap solution to each well with vessels.

Incubate the vessels at 37 °C for 1 h in an incubator.

Take the plate with the vessels out of the incubator and put it on ice.

Chemicals:

KrebsHEPES buffer, 50 mM, pH 7.4
L-Arginine
Diethyldithiocarbamate
Fe(II)sulfate heptahydrate
Argon (cylinder)
Liquid nitrogen

Material:

24 well plate
1 ml syringe
dispenser with the option to aliquot 250 µl

Exchange of the hosts:

Before you start with preparing the samples you have to remove the host of the holder with the guidance tube for the capillaries. You do this by removing the three screws. Pay attention, that the screws will not fall into the resonator. You can avoid this by putting the holder with the guidance tube into the host. Now remove the complete holder with the protection tube. When you have done that and the resonator is empty, it is not allowed to make a tuning, because the spectrometer will not find a resonance frequency and the engines of the adjust will run all the time and the spectrometer may get damaged. Now put in the host for the finger dewar and fix it with the three screws. Again please pay attention, that the screws will not fall into the resonator. Adjust the big screw for the finger dewar that way that the it is screwed in completely. Tune or adjust the spectrometer only in the case the finger dewar is in the cavity.

Preparation of the sample:

Cut off the tip of 1 ml syringes using a scissors and cut it even with a scalpel. Estimate the distance between the cotton at the bottom of the inner finger of the finger dewar and the middle of the resonator (the screw of the holder has to be screwed in completely). The syringes have to be marked according to the number of samples you have. Adjust the plug of the syringe that way, that the distance between the front tip and the plug is equal to the distance between the Cotton and the middle of the resonator + 2,5 mm. The distance is not all allowed to higher. Adjust the vessel or the vessel rings directly on the plug of the syringe together with a little bit of buffer. Pay attention that no bubbles are visible. To get a good stability of the frozen cylinder, it is recommended to degas the buffer you are using to freeze it. Freeze the syringe with the plug directed to the bottom of the big dewar (not the finger dewar) with liquid nitrogen. Freeze further samples in the same way. Take the syringe out of the dewar with liquid nitrogen using a tweezers and warm it up at the tip by hand. Fill the syringe up till the front tip with Krebs HEPES buffer and freeze it with the plug directed to the bottom of the dewar with liquid nitrogen. Take the syringe out of the liquid nitrogen put off frozen solution that exceeds the tip of the syringe. Warm the syringe up by hand at the place where the plug is located until the plug can be moved. Move the plug to the rear end of the syringe warm up the position of the plug again and remove it completely. Fill the Syringe from the rear end with 400 µl buffer (no bubbles) and freeze the syringe with the tip directed

to the bottom of the finger dewar. Fill the finger dewar by 2/3 with liquid nitrogen and bring it into a position with an angle of 20° to the table (use corrugated board or other material to achieve this). Take the syringe out of the liquid nitrogen and turn it in between your hands to achieve a even warm up. Put in the plug again and push the sample cylinder rapidly into the liquid nitrogen of the finger dewar and in one piece if possible. Let the sample slide into the finger of the dewar and use some cotton on a wooden stick to „seal“ the finger dewar above the sample. Wait until the liquid nitrogen in the environment of the sample cylinder has evaporated, put the finger dewar immediately into the cavity, tune the spectrometer and start the measurement.

Remarks concerning ROS trapping

Starting with Chelex treatment of the buffer. Afterwards:

Fe chelator = Desferal 25 µM not affecting the cells

Cu chelator (Cu water pipes) = 5 µM diethyldithiocarbamate (DETC)

Measurement of the autoxidation with and without chelators as a control

Stock solution in 20 mM phosphate buffer pH 7.4, dilution of the stock with phosphate buffer assures the pH remains constant in the case of hydrochloride spin traps too. The trapping activity of CM-H, CP-H and other hydroxylamines is markedly decreased at pH < 6.

For trapping radicals generated by vessels it is important to wash them carefully, because there is always a thin layer of erythrocytes at the inner surface.

For dismutation of superoxide in tissue use PEG(polyethyleneglycol)-SOD which penetrates into the cells.

To increase the trappable superoxide concentration inhibit NO synthases.

Chemicals

Deferoxamine (Desferal)
Diethyldithiocarbamate
20 mM phosphate buffer, pH 7.4
CM-H or CP-H
PEG-SOD
NOS inhibitors