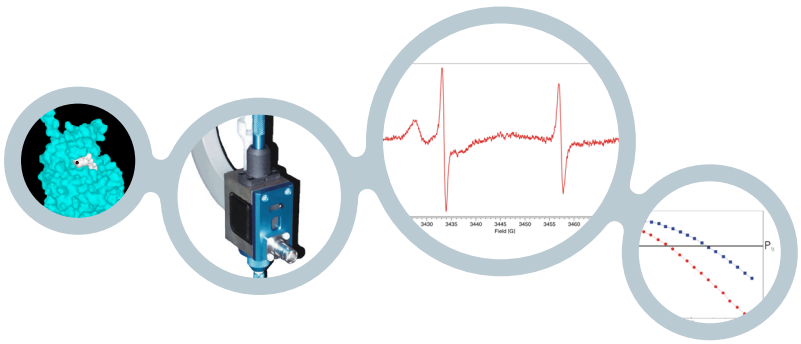
Bruker BioSpin



A Quick Start Guide for the ER 4123D CW-Resonator

The ER 4123D with the TPX system was designed for measurements of relaxation effects using CW EPR. These measurements are often performed on aqueous samples with low concentrations. A result of these two characteristics is the requirement of a high B_1 and a high Q-factor for the resonator. In addition to these requirements, one current method for measuring the relaxation effects involves the use of gas exchange to study T_1 and T_2. The material which has the desired properties is TPX or polymethylpentene (PMP). To aide in these measurements the TPX system for sample placement and gas exchange is included with the ER 4123D probe.

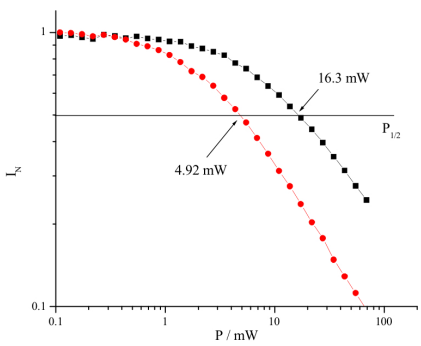
Power Saturation Measurements

The power saturation behavior of an EPR species depends on several factors two of which are the relaxation times, T_1 and T_2. Thus, a simple method to obtain information about the relaxation times is to measure the power saturation curve, signal intensity vs. microwave power. The saturation behavior is often characterized by the power at half saturation, $P_{1/2}$. $P_{1/2}$ is the power where the signal intensity would be half of the signal intensity in the absence of saturation. This is easily obtained by measuring the CW EPR spectrum as a function of the microwave power then plotting the integrated signal intensity versus microwave power. If the signal intensity is normalized, $P_{1/2}$ can be read directly from figure 1 (note both axes are log scaled).

Applications

The information about the system available from the determination of a single $P_{1/2}$ is limited because $P_{1/2}$ depends on the product of the relaxation times. This prevents the determination of either T_1 or T_2 by measurements of $P_{1/2}$. The usefulness of these measurements is not diminished since any process which affects either T_2 or T_1 will be reflected

Figure 1



10 µM TEMPOL in Water. Saturation curves in air (black) and nitrogen (red).

in a change in $P_{1/2}$. Some process which influence T_1 and T_2 are: collisions with other molecules (T_1), molecular vibrations (T_1), the interaction of one unpaired spin with another (T_2), and rotations within the molecule (T_2). The most useful of these processes is collisions with other molecules, where the collisions result in an decrease in T_1 and/or T_2 which is observed as an increase in $P_{1/2}$.

While collisional relaxation measurements can be used in many different fields of study, the main field where these studies are used is in biology. Methods for the attachment of spin labels to specific locations in proteins and DNA have allowed EPR to play a larger role in structure studies. One application of these methods is in studying the overall structure of proteins, by attaching a spin label to a portion of the protein and then looking for changes in $P_{1/2}$ with the addition of different relaxation enhancing species. Using a water soluble species would allow the determination of locations accessible to the solvent and the use of lipid soluble species allows the determination of areas within lipid membranes. An example of a water soluble species is chromium oxalate while a more lipid soluble species would be oxygen. A typical measurement to determine the accessibility of an EPR spin label could be to first measure $P_{1/2}$ in a normal EPR tube with air present, then determine $P_{1/2}$ after removing any oxygen in the sample, and finally determine $P_{1/2}$ after the addition of chromium oxalate and removing oxygen from the sample.

Standard Operation

The ER 4123D probe includes 5 sample tube supports intended for use with quartz EPR tubes. The sample tube supports function based upon a two point compression to hold the tube in place and align the tube vertically in the resonator. Over tightening of the sample tube support will result in breaking of the sample tube.

One method for use of the sample tube supports is:

1. Insert sample tube support into place in probe.
2. Insure sample tube support is inserted through both o-rings and can proceed no further.
3. Loosen the sample tube support.
4. Insert sample tube until it rest on gas flow guide (indicated in Figure 4).
5. Tighten sample tube support.
6.

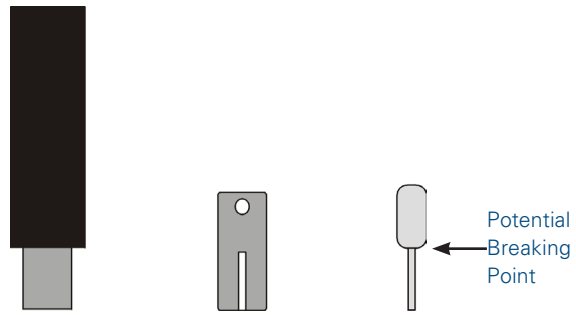
The above method assumes the sample is at the bottom of the sample tube and a sufficient volume is present to fill the resonator.

For smaller sample volumes, the sample tube should be raised to the center of the resonator (26 mm from bottom of sample tube support). It may be necessary to remove the gas flow guide for correct alignment of the sample within the resonator.

For precise sample alignment in the resonator:

1. Remove gas flow guide from the bottom of the probe.
2. Loosen sample tube support and insert sample tube.
3. Tighten sample tube support slightly and position sample center 26 mm from the bottom of the sample tube support.
4. Tighten sample tube support to firmly hold the sample tube.
5. Carefully insert the sample tube support into the top of the probe.
6. Firmly push sample tube support through both o-rings until it can proceed no further.

Figure 2



TPX holder components

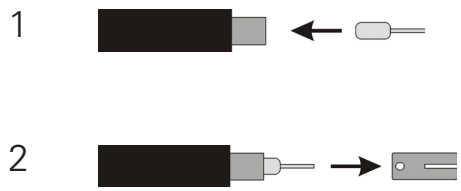
TPX System

The TPX system is composed of three pieces: the TPX support, the TPX capillary, and the TPX holder. The support is for mounting and stabilizing the capillary in the probe. The holder is for protection of the fragile TPX capillary and for directing the gas flow over the capillary. The capillary is for sample containment and gas exchange.

Assembly

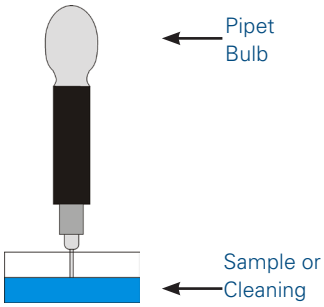
The TPX capillary is first gently attached to the support. For reproducible sample positioning, the capillary should be screwed in until it comes to a stop. The holder is then attached to the support/capillary for protection and direction of the gas flow. While inserting the capillary into the holder, care must be taken due to the potential for an off angle insertion. This can place stress on the capillary and result in breakage. The capillary is fragile and can break easily at the point were the capillary tube meets the threaded screw (indicated in Figure 2). Further, due to the machining process, the capillary may initially be difficult to screw into the holder or the support the first few times. Caution should be exercised the first few times a new TPX capillary is used with the support and holder.

Figure 3



TPX holder assembly

Figure 4



TPX holder loading

Sample Loading

Filling of the TPX capillary with an aqueous sample is best achieved after first attaching the capillary to the support (Figure 3 step 1). A pipet bulb is then attached to the top of the support. By carefully lowering the capillary tip into the sample solution and aspirating with the pipet bulb, more than enough sample can be loaded into the capillary tube. The aspiration of excess sample also serves to flush potential contaminates from the TPX capillary. After the desired amount of sample is loaded, carefully remove the pipet bulb. The risk of aspirating air or expelling the sample exists when removing the pipet bulb. The capillary can be gently wiped to remove excess sample and then inserted into the holder as noted above.

It is typically not necessary to seal either end of the TPX capillary prior to use. Capillary action is often sufficient to maintain the sample in the correct position during measurements. If desired however, a small amount of sealing wax may be used to seal either the tip of the TPX capillary or the base of the capillary which is attached to the support. The use of wax complicates the cleaning process since the wax may not be readily dislodged by an air or water flow. The wax must then be removed by other methods. The risk of breaking the TPX capillary is high when trying to dislodge the wax, so caution is warranted. After loading the TPX capillary, the holder is attached for protection.

Cleaning

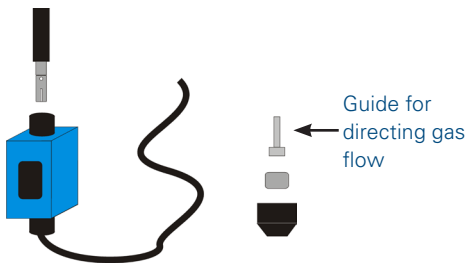
Cleaning the capillary is achieved in a similar way as to loading the capillary. After use, the holder is removed from the TPX capillary. The capillary is attached to the support and a suitable pipet bulb is attached to the top of the support. The sample can be expelled by simply forcing air through the TPX capillary. A thorough cleaning is effected by aspirating several mL of water through the capillary. The capillary and support can then be left at room temperature for drying. Elevated temperatures should be avoided to reduce the risk of deformation of the components.

Purging with Gas Flow

The TPX assembly is inserted into the top of the probe until no further movement is possible. The support is held tightly into position by two o-rings, which can be felt during insertion. The port for gas inlet is located at the bottom of the probe. Two additional components are located within the housing of the gas inlet, an o-ring seal and a gas flow guide. Before attaching the gas flow line and starting the gas flow it is advised to check for the presence of these components.

It is necessary to set the gas flow prior to attaching the gas flow line to the gas inlet port. If a high flow rate is used, the sample may be forced out of the capillary tube. A low gas flow rate is sufficient to effect complete gas exchange within a few minutes. Considering the low volume of sample (~4 µL), the high concentration of flow gas, and the high permeability of TPX, a flow rate of 25 - 50 mL/min is reasonable. Higher flow rates may lead to migration of the sample up the capillary tube. One must experiment to find the appropriate flow rate which will not result in significant sample migration. With this range of flow rates using nitrogen gas, a time of 15 min was sufficient to deoxygenate a water sample.

Figure 5



Left: ER 4123D with TPX assembly and gas flow attached. Right: Diagram of components of the bottom portion of the probe.

Properties of TPX

TPX is the trademark name for polymethylpenetene (PMP) from Mitsui Petrochemicals Ltd. with outstanding properties for power saturation studies. Because oxygen is an efficient collisional relaxation enhancer and is abundant, a power saturation curve generated in the presence of oxygen and another generated in the absence of oxygen can show different saturation curves. Experimentally, the difficulty lies in the removing of the oxygen from the sample. For small volume samples, this is more difficult and time consuming using traditional methods. This is where the properties of TPX have proven beneficial. TPX is highly permeable to oxygen (relaxing) and nitrogen (non-relaxing).

TPX or PMP has a gas permeability to oxygen of 270 (kg m / (s m^2 Pa) [the amount of gas which passes through a 1 m thick 1 m^2 piece of the material in one second under a pressure of 1 Pa]. For comparison, low density polyethylene (LDPE) has a oxygen permeability of 60. Similarly, TPX is very permeable to nitrogen, 65, while LDPE is not, 20.

Gas Permeability
(kg m / s m^2 Pa)

Material	Oxygen Permeability	Nitrogen Permeability
TPX	270	65
Polyethylene (PE)	60-10	20-3
Polypropylene (PP)	28	4.4
Polyvinyl chloride (PVC)	1.2	0.4

TPX does posses two properties which necessitate special handling. TPX is a rigid polymer therefore care in handling is necessary to avoid inadvertently breaking the capillary.

TPX has poor chemical resistance to many organic solvents therefore use of the solvents for cleaning or as a sample solvent should be strictly avoided to prevent damage and contamination of the TPX capillary.

TPX Chemical Resistance

Solvent	Resistance	Solvent	Resistance
Acid Dilute	E	Aldehydes	G
Acid Concen-trated	E	Esters	G
Alcohols	E	Hydrocarbons Aliphatic	G
Bases	E	Hydrocarbons Aromatic	F
Mineral Oils	E	Ketones	F
Oxidizing Agents	F	Hydrocarbons Halogenated	P
E = Excellent	G = Good	F = Fair	P = Poor

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