EPR Sample Preparation

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Credits to Sarah Cady at Iowa State University

Safety First:

Wear gloves and goggles when you are handing chemicals. Lab coat is also highly recommended. Wear cryogen gloves and goggles when you are handing cryogens.

Cryogens are used for cooling samples via the cryostat. Liquid nitrogen is extremely cold and should not come in contact with bare skin. Liquid helium is even colder, and one should take care not to come into contact with cold helium gas, as even the cold gas can cause cryogenic skin burns.

Solvent Selection

For solution EPR samples to be measured at room temperature, select a low dielectric solvent. High dielectric solvents will "spoil the Q" and lower the sensitivity of the resonator, or possibly eliminate signal completely.

Solvent	Chemical Formula	Boiling Point	Dielectric Constant	Density	Dipole Moment
Pentane	CH_3-CH_2-CH_2-CH_2-CH_3	36 °C	1.84	0.626 g/ml	0.00 D
Cyclopentane	C_5H_{10}	40 °C	1.97	0.751 g/ml	0.00 D
Hexane	CH_3-CH_2-CH_2-CH_2-CH_2-CH_3	69 °C	1.88	0.655 g/ml	0.00 D
Cyclohexane	C_6H_{12}	81 °C	2.02	0.779 g/ml	0.00 D
Benzene	C_6H_6	80 °C	2.30	0.879 g/ml	0.00 D
Toluene	C_6H_5-CH_3	111 °C	2.38	0.867 g/ml	0.36 D
1,4-Dioxane	/-CH_2-CH_2-O-CH_2-CH_2-O-\	101 °C	2.30	1.033 g/ml	0.45 D
Chloroform	$CHCl_3$	61 °C	4.81	1.498 g/ml	1.04 D
Diethyl Ether	CH3-CH2-O-CH2-CH3	35 °C	4.30	0.713 g/ml	1.15 D
~~Water~~	H_2O	100 °C	~~80.1~~	~~1.000 g/ml~~	~~1.85 D~~

NOT Good for room temperature EPR

For liquid samples that will be frozen, samples should be prepared in a solvent that will "glass" upon freezing with liquid nitrogen. Consult the table below for a selection of suitable solvents/ratios:

Glassing Agents

Pure Substance

3-methylpentane	sulfuric acid	sugar (.4 M sucrose)
methylcyclopentane	phosphoric acid	triethanolamine
paraffin oil (Nujol)	ethanol	2-methyltetrahydrofuan
isopentane	isopropanol	di-n-propyl ether
methylcyclohexane	1-propanol	decalin
isooctane	1-butanol	triacetin
boric acid	glycerol	toluene

Mixtures

Components	*Ratio A:B:C...*
hydrocarbon	
3-methylpentane/isopentane	1:1
isopentane/methylcyclohexane	1:6
methylcyclopentane/methylcyclohexane	1:1
3-methylpentane/isopentane	1:2
alcohol	
ethanol/methanol	4:1, 5:2, 1:9
isopropanol/isopentane	3:7
ethanol/ispopentane/diethyl ether	2:5:5
isopentane/n-butanol	7:3
isopentane/isopropanol	8:2
diethyl ether/isooctane/isopropanol (or ethanol)	3:3:1
diethyl ether/isopropanol (or ethanol)	3:1
diethyl ether/toluene/ethanol	2:1:1
butanol/diethyl ether	2:5
aromatic	
toluene/methylene chloride	1:1 or excess toluene
toluene/acetone	1:1 or excess toluene
toluene/EtOH or MeOH	1:1 or excess toluene
toluene/acetonitrile	1:1 or excess toluene
toluene/chloroform	1:1 or excess toluene
water	
water/propylene glycol	1:1
water/glycerol	4:1 to 1:4
water/(poly)ethylene glycol	4:1 to 1:4

*Adapted from: Drago, R. S. Physical methods for chemists; 2nd ed.; Saunders College Pub: Ft. Worth, 1992.

Freezing Samples

The amount of sample/solvent in the EPR tube is important, especially for samples that will be frozen.

Preferred Sample Height: X-band, 35 mm
Minimum Sample Height: X-band, 6 mm

Samples must be frozen in liquid nitrogen before insertion into the cryostat. Start by placing the bottom tip of the tube in liquid nitrogen until it starts to fizzle, and then slowly lower the tube into the liquid nitrogen at a rate of about 1 mm/sec. Gradual freezing allows for sample expansion upwards during freezing, preventing the tube from cracking. Tube caps should be removed because they will admit liquid nitrogen, resulting in tube explosion and injury upon removal from storage. After freezing is complete, check the tube to make sure that it's not cracked. A damaged tube may burst or fall apart upon slight warming or bumping, and can damage equipment or injure EPR users. We have found that small rubber septa caps sealed with parafilm are good for air-sensitive samples that must be prepared in a glove box prior to data acquisition.

Solid Samples

For solid powder samples in EPR tubes, the tubes must be flushed with gaseous helium before insertion into the cryostat. This is to ensure there is no formation of air ice surrounding the sample, since all other atmospheric gasses will freeze at liquid helium temperatures, and many gasses will also freeze at liquid nitrogen temperatures. Air ice can insulate the sample, causing the internal sample temperature to be higher than the temperature inside the cryostat. To flush with helium, attach a glass Pasteur pipette to the end of some tygon tubing connected to a helium cylinder. Hold the sample at a slight angle such that the tube opening is angled down, but the solid sample does not shift in the tube. Flush the tube for a few seconds with a light flow of helium gas, and cap the sample with an EPR tube cap or septa. Wrap the cap with parafilm to slightly seal the tube. The seal is not airtight, so flushing the sample just prior to cryostat insertion is suggested.

Tube Cleaning for Biological Samples

1. Clean any residual sample out of tube using solvent in which previous sample was dissolved.
2. Fill and soak tubes for 12-24 hours in 1M KOH/NaOH to remove protein residue.
3. Repeat filling and soaking with 1M nitric acid overnight.
4. Rinse tubes inside and out with ddH2O, then fill and soak with 4mM EDTA overnight to remove possible metal contamination.
5. Rinse the tubes with distilled/deionized H2O, then rinse inside and out with acetone.
6. Dry the tubes in a drying oven for a minimum of 1 hour. Check tubes to make sure there is no visible residue. If there is still material evident, repeat entire process from step 1.