



Technical Bulletin F0200 – CapNMR Troubleshooting and Blockage Maintenance

1) Identify Where the Blockage is Located

The very first action item should be to disconnect the filter from the probe and test just the tubing and filter. (Do not inject any into the CapNMR probe without a filter inline.)

- Inject some solvent through the capillary and filter and if you feel resistance, take the filter off the capillary/tubing.
 - If you have acceptable flow through just the injection port and tubing, then the filter is blocked and simply change the filter.
 - Be sure to rinse the new filter with solvent before connecting to the probe
 - If you still have resistance through just the tubing, then there is a blockage in the tubing before the filter. Change the capillary/tubing with a new segment. Be sure to rinse the tubing with fresh solvent to flush out any debris in the tubing before connecting to the filter and probe.
 - In order to keep push volume / delivery volumes the same, measure the old piece and cut a new piece the same length.
 - If flow is still acceptable through the tubing and filter, check the tips of your tubing/capillary for smudged or pinched tubing tips or crushed fused silica tips.
 - Check all your tips, rinse all the tubing well, re-attach the filter, and rinse the entire flowpath with fresh solvent.
- If your tubing is well flushed, have a new filter inline, and only feel back pressure when connecting back up to the probe, back flush the probe.
 - Back flush the probe by flushing fresh solvent through the other leg of the probe.
 - If you have a BioFlow FEP tubing probe, simply unscrew the fitting from the base of the probe and switch it to the other union on the base of the probe.
 - If you have a fused silica capillary probe, take the filter off your current leg and attach the filter to the other capillary leg. (Always inject through your filter.)
 - If you are able to flush solvent through the probe, this may or may not have cleared the blockage so continue to flush 100-150 uL of solvent through the probe.
 - Switch back to your original configuration to confirm that the blockage has been cleared. If there is still high back pressure, continue onto section 2 and 3 for rinsing and cleaning the probe.

2) Clearing a Blockage from within the Probe: Rinse the Probe with Solvent & Heat the Flowcell

This section is primarily for blockages that have occurred inside the probe. DON'T PANIC! Generally, blockages within the probe are samples that have precipitated due to high concentration, poor solubility, or thermal instability. Whatever the cause, the sample has precipitated and practically all users are able to recover from such an event.

Whenever rinsing the probe during any blockage maintenance, make sure you use the syringe vise from the installation kit. This spring loaded syringe vise allows you to use a larger syringe and apply pressure over time. For greater detail on using the syringe vise and connecting capillary/tubing to the 250 uL syringe, please see the tech support website (www.microNMR.com) for: Technical Bulletin G0112 - Fluidic Connections (for Fused Silica Capillary and Syringe Vise) Technical Bulletin G0310 - FEP Tubing Fluidic Tutorial (for FEP Tubing and Syringe Vise)



- ❑ Start by heating the flowcell with the standard variable temperature (VT) air supply and heater. The CapNMR thermal limits are 0°C to 50°C so set VT air to 40°C to heat the flowcell to see if it will redissolve into solution without flushing.
- ❑ Fill a 250 uL Gastight syringe with either a solvent the known precipitated compound is most soluble in OR with a mixture of solvents (30% DMSO, 30% Acetone, 40% D2O) for an unknown precipitate.
 - Connect the syringe to the tubing/capillary that is connected to the probe through the filter and turn the syringe vise shaft multiple times to ramp up the pressure on the syringe vise. (Caution: If you turn the syringe vise shaft too many times, the luer lock fitting, a low pressure fitting, will pop off the syringe!) You will feel a slight resistance as the spring makes contact with the syringe plunger. Keep turning in order to compress the spring and apply pressure the flowpath.
 - Be sure to use this pressure technique on both sides / capillary legs of the probe.
 - *This may take overnight.* Many users come back the next morning to find the probe blown out with solvent and after a little more rinsing, are ready for use.
 - If after several hours (>8hr), the probe has still not been blown out, try back flushing the probe in the same manner and then continue onto section 3 for cleaning the probe.

3) Rinse the probe with cleaning solution

If the precipitated sample isn't redissolving in regular solvent, *using the same equipment as section 2* (syringe vise, 250 uL syringe, etc.) use these cleaning solutions to see if the compound can be cleared out.

- Urine, Plasma, or any kind of biological work
 - 10% H₂O₂ (Hydrogen Peroxide) in H₂O
 - 70% formic acid / 30% Acetonitrile (ACN)
 - 2% Micro-90 (Cleaning Solution by Cole-Parmer)

- Small Molecule
 - o 0.1 M HCl in 50% H₂O / 50% ACN
 - o Bleach (full strength - 6% Sodium Hypochlorite)
- Protein and peptide samples
 - o 70% formic acid / 30% Acetonitrile (ACN)
 - o 10% H₂O₂ (Hydrogen Peroxide) in H₂O

Other categories of samples require a judgement call about what kind of cleaning solution is most likely to break up a precipitated chunk of the sample.

- Fill a 250 uL Gastight syringe with the desired cleaning solution.
 - Connect the syringe to the tubing/capillary that is connected to the probe through the filter and turn the syringe vise shaft multiple times to ramp up the pressure on the syringe vise. (Caution: If you turn the syringe vise shaft too many times, the luer lock fitting, a low pressure fitting, will pop off the syringe!) You will feel a slight resistance as the spring makes contact with the syringe plunger. Keep turning in order to compress the spring and apply pressure the flowpath.
 - Be sure to use this pressure technique on both sides / capillary legs of the probe.
 - o *This may take overnight.* Many users come back the next morning to find the probe blown out and, after more rinsing with an appropriate solvent, is ready for use.

If this process has not cleared the blockage, then the flowpath may need to be changed which would require the probe be returned to the factory. Contact Protasis Sales at (508) 481-4163 for a quote. This is not that costly of a repair and should only take a couple of weeks as the probe is fully tested and run through QC before it is returned to ensure your success. Be sure to run through Section 4 to exhaust your options for in-house fixes.

4) Checking the Probe for Poor Lineshape and/or After a Blockage

There are very few reasons why you would want to take the probe actually out of the bore of the magnet during troubleshooting. However, if you still have poor lineshape, taking the probe out is a 'wild-card' option. There are a few external reasons why you are not able to shim a probe (especially after you've flushed through excess amounts of solvent and cleaning solution).

- At the top of the CapNMR probe, there is a red O-ring that is meant to restrict the top of the probe from moving and prevent any kind of vibrations. Since the O-ring does make contact with the inside of the bore, the O-ring tends to collect all the metal shards and flakes as it is taken in and out of the magnet. This metallic debris in such close proximity to the coil has been found to make shimming very challenging.
 - Take a cloth or chemwip that has been wetted with isopropanol or ethanol and simply wipe around the o-ring to remove the metallic debris from the o-ring. This has been found to drastically improve shimming on heavily contaminated magnets.
- A final check to ensure the flowcell is clean is to tilt the probe on its side to orientate the flowcell in a vertical position and flush through more solvent.

- Place the probe length-wise on its side so the BNC cable connectors are facing up towards the ceiling. Connect the 250 uL syringe with solvent higher fitting (should be the 'B' side) so you are flowing vertically down the flowcell. This has been found to help probes that may have had metallic debris from unwashed filters getting metallic remnants from its manufacturing washed into the flowcell and get trapped by the magnetic field. This is an important reason to wash each filter before connecting to anything else!

If you have any questions about these procedures, please feel free to call CapNMR Technical Support at (217) 351-4359 or email techinfo@microNMR.com.

Good luck!