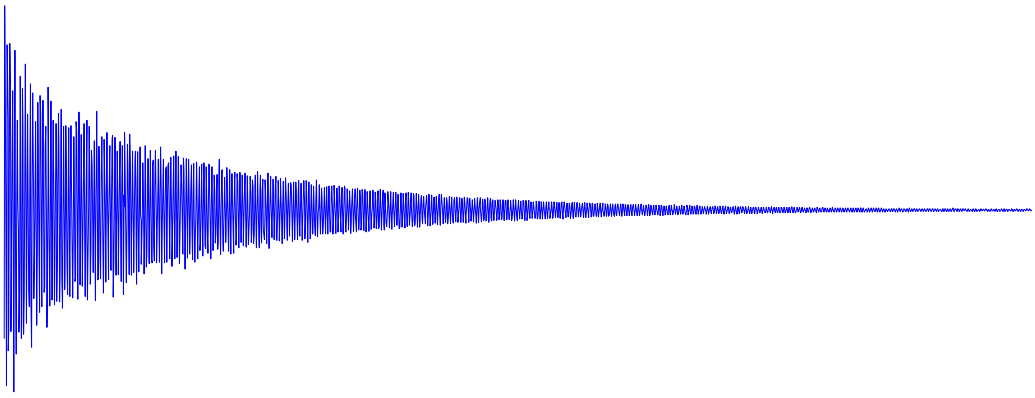
Tips for Better Solvent Suppression by Presaturation

These notes present some points that should help you get better ^1H solvent suppression for weak samples in D_2O. They do not describe the solvent presaturation procedure from scratch, but aim to help those already familiar with the basics.

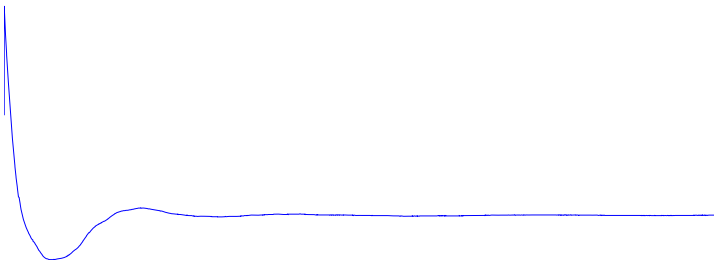
1. Sample Preparation: Before you even get to the spectrometer you can help yourself by preparing your sample correctly. This will save a great deal of time when you come to acquire your spectra. The object is to reduce the proton content in your sample solution as much as possible. This is best achieved by dissolving your sample in D_2O, then freeze drying it, then dissolving it in D_2O again to acquire the spectrum. Freeze drying from D_2O NOT H_2O is essential for good results. You should also consider using high grade D_2O ("100%" D) for the NMR sample, such as that in sealed 0.5 ml ampoules. D_2O from large bottles is invariably quite wet and can contribute a large residual HDO signal.

2. Temperature Stability: As the lock solvent you use is that of a system undergoing chemical exchange, the lock signal is subject to drift with changes in temperature as is the HDO signal you wish to suppress. This will result in poor suppression of the solvent and to a loss of resolution in your spectrum. Therefore you must take the following precautions. Firstly, use the variable temperature unit to stabilise the probe temperature at all times. This needs to be a few degrees above the ambient probe temperature to be effective. Secondly, let your sample come to equilibrium in the probe for a few minutes before doing anything as it is pointless to try and shim your sample when the lock is drifting because of temperature changes. An alternative approach which may prove beneficial especially for longer-term experiments is to add 5-10% of deuterated acetone-d_6 to your sample and lock on this (since the acetone resonance is not (as) temperature sensitive).

3. Shimming: For good solvent suppression it is necessary to have good line shape. To achieve this you will need a well shimmed sample and this process can be helped by using a good quality tube. Shimming itself is not discussed here, although some useful points follow. When shimming your sample it is useful to use both the lock level and the FID display to assess your progress (it is also useful to monitor the line shape directly, which is possible on more modern instruments). Do not suppress the HDO resonance at this point. The FID should be a uniform exponential decay, such as below.

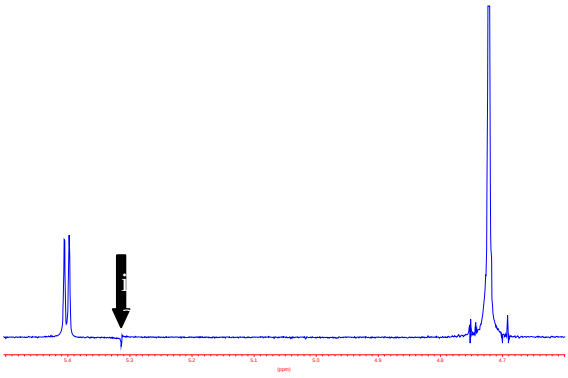


To achieve this type of display, it may be necessary to ensure that the transmitter frequency (O1) is *different* from that of the water resonance (ca 0.2 ppm or more away). If they are the same, and you have a very large HDO resonance, the FID may well look distorted, as below, and be unsuitable for shimming.



A distorted FID arising from the transmitter frequency coinciding with that of the intense HDO resonance

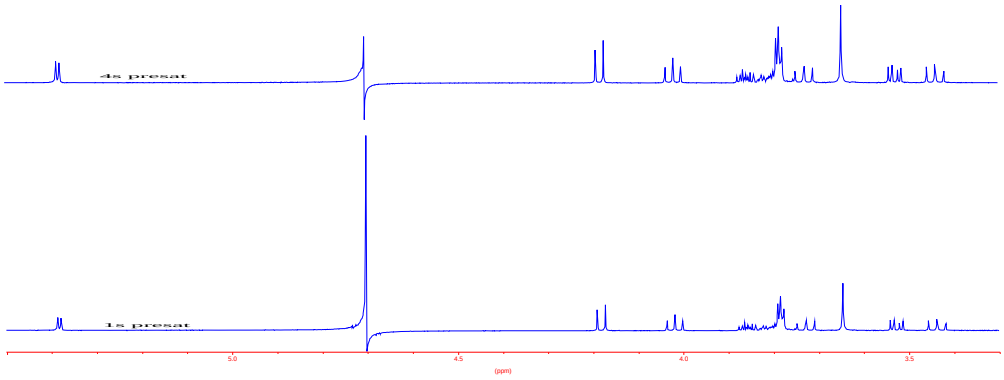
It is useful to check the line shape every so often by transforming the FID (NB: on older spectrometers and with only a small number of scans a quadrature image may appear in the spectrum, as below. This is simply an image of the large solvent resonance about the central (transmitter) frequency and should not be mistaken for a genuine resonance).



4. Acquisition: When you come to acquire your solvent suppressed spectrum, it is wise to set the transmitter O1 frequency on the solvent resonance (make it the same as the decoupler O2 frequency) as this eliminates the quad image discussed above and places other possible undesirable artefacts in an unimportant spectral region. Assuming you have the basic solvent suppression scheme set-up, here are some tips for improving the results. You should aim to reduce the solvent resonance to a level similar to the other resonances, although this may not actually be possible.

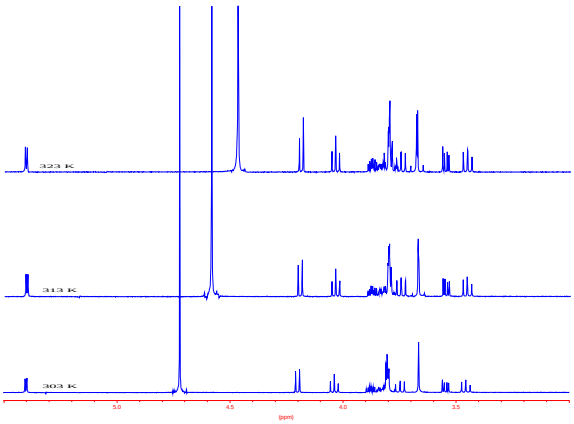
   - Try making changes in the saturation power you are using. Too much may cause some degree of saturation to the resonances close to the HDO, resulting in reduced integrals for these peaks. Too little may still leave a considerable solvent peak. The greater the power you use, the wider the window of saturation gets; selectivity is inversely proportional to power.

   - Increase the length of the presaturation delay. This is especially helpful if you wish to use a low decoupler power to avoid saturating nearby resonances of interest (see the above point). A longer presaturation period should attenuate the solvent more. The two spectra below illustrate this point; both were acquired with one scan and the same decoupler power, the only difference was the presaturation delay, (1s and 4s).



- Change the irradiation frequency. Small changes in the irradiation frequency (<2 Hz) can improve the suppression achieved, especially if the line shape was not quite symmetrical when you started. This is a case of trial and error.

- A common problem with suppressing the solvent resonance is that you may also attenuate the resonances of interest close to the water. Just how much these are attenuated, and how close resonances have to be before they are effected, depends largely on the decoupler power in use. These problems can, in many cases, be overcome quite readily. The most effective way for samples in water is to alter the sample temperature (the shift of the water resonance has already been alluded to when discussing the need for temperature stability above). The spectra below illustrate this. Clearly, the water resonance undergoes a large shift, whereas the other resonances are affected little as they are not undergoing chemical exchange.



Similarly, changes in pH will shift the water resonance, which may also be used to advantage in some circumstances.

5. Referencing: Because of the large variations in chemical shift of the water resonance with changes in temperature and pH, it should never be used as a reference for chemical shifts. You should not expect chemical shifts referenced in this way to be very reproducible, unless a conscious effort is made to make them so. Instead, you should use an internal reference, such as TSP or DSS (at 0.00 ppm) or dioxan (at 3.75 ppm) and quote all shifts relative to these; *never* quote shifts referenced to HDO in publications, unless you can justify this.