

## X. Solvent Suppression

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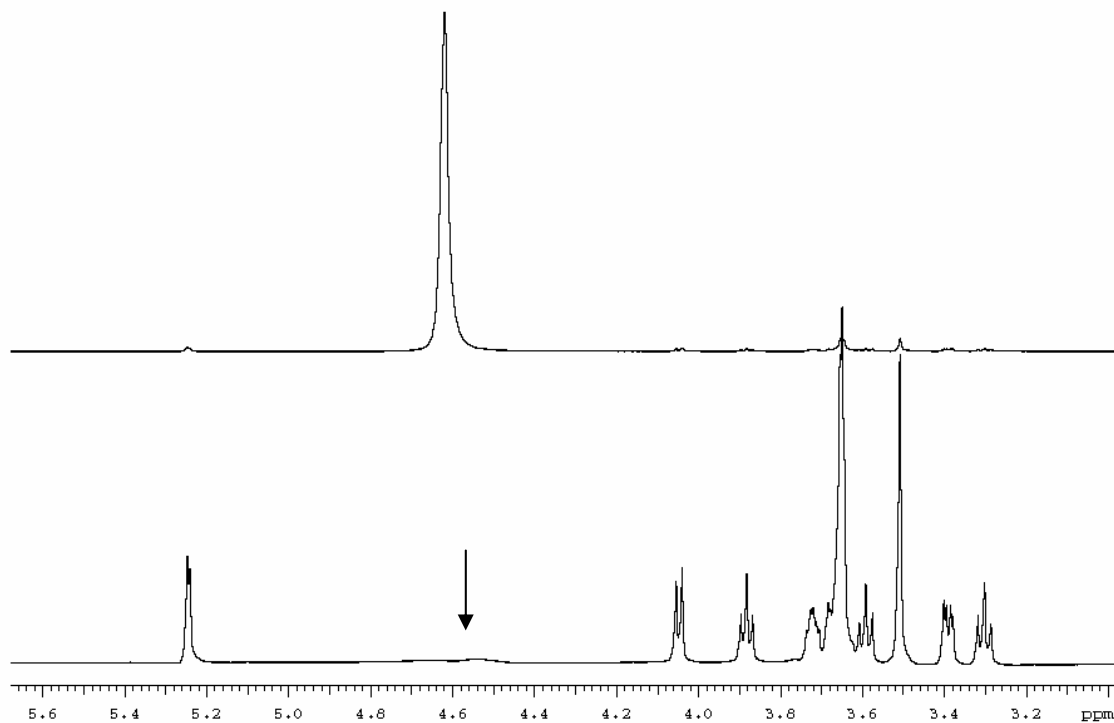
The first rule of any solvent suppression is usually a well-shimmed sample - the narrower the solvent peak, the better the suppression. Also, any sample containing 90% water or 90% non-deuterated solvent, should be gradient shimmed for best results.

### Presaturation

Presaturation is a selective pulse on the solvent peak using the transmitter to saturate the resonance. Presaturation will adversely affect signals from protons exchanging with the water. Presaturation is the easiest option for suppression of one residual solvent peak.

1. Shim the sample well (use proton gradient shimming if possible). Collect a 1-D spectrum. Find the 90-degree pulse width. Set the transmitter offset (**tof**) on top of the solvent peak (**nl movetof**).
2. Set up the experiment **>presat**. Use **>dps** to take a look at the pulse sequence. The new parameters are:  
**satdly** - saturation delay, how long is the selective pulse; common value: 1.5sec  
**satfrq** - saturation frequency, set equal to the **tof**  
**satmode** - saturation mode set to **'ynn'**  
**sspul** - set to **'n'** (**sspul** is an extra pulse to remove residual X-Y signals. Set this to **'y'** if desired.)  
**satpwr** - saturation power, this is dependent on sample; common range: 2-10 dB (less for residual water versus 90% water)
3. Do not exceed 10 dB on the saturation power without asking for assistance. If that much power is required, something may be wrong.
4. Set the **d1=0**. Collect 1 transient to test.
5. Optimize the parameters for best suppression. Parameters that may be altered to improve the saturation include checking the saturation frequency (**satfrq**). This parameter can be arrayed around the starting value to find an optimum. Also, increasing **satpwr** may help. Be sure to increase the gain, use steady state scans (**ss=32** optimum) and collect at least 8 transients for the final spectrum. A well-suppressed sample can often achieve a **gain=30,40** or 50 without a receiver overflow.

<p><b>NOTE:</b> If suppressing a solvent other than water, a different <b>tof</b> and <b>satfrq</b> can be used to avoid having to center the spectrum on a solvent far from the normal center.</p>
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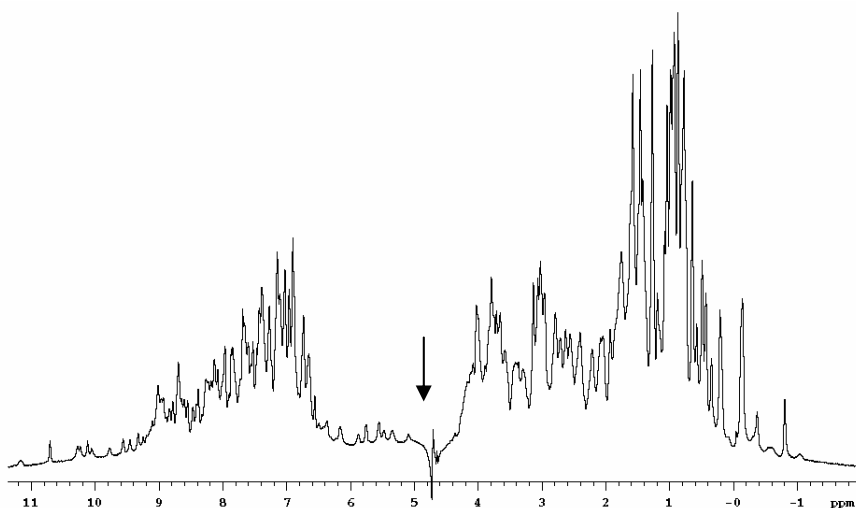


**Figure 23:** A residual solvent peak can be suppressed easily using a presaturation technique. In this example, the spectrum without presaturation (top) is dominated by the solvent peak and the sample peaks are weak. Suppression of the residual solvent (bottom) allows the increase of the receiver **gain** that greatly enhances the signal-to-noise in the sample peaks with the same number of scans.

## Autowatergate 1-D

Watergate is not necessary for samples that require residual water suppression and is best utilized on 90% water samples. The macro described here runs in an account *that does not* have BioPack activated.

1. Acquire a 1-D spectrum and shim (proton gradient shimming if possible). Find the 90-degree pulse width. Set **pw90=pw**. This parameter is used by the macro.
2. In the command line, **>autowatergate**  
The macro will optimize the watergate parameters and then collect 16 scans using a wide sweep width. This macro uses a pulse sequence called 'gwatergate.c' that may need to be compiled first.
3. To run the spectrum again with a different spectral window, set the **sw** using **sw=#** (don't use **>movesw** or the **tof** will move and the water suppression will not work!). Also change the number of scans, **gain**, etc. and just re-acquire with **>ga**
4. If only part of the spectrum appears instead of the full spectrum during the optimization arrays, type **>f full**  
to look at the whole spectrum and prevent wrong answers. Be sure to use steady state scans (**ss**) and increase the gain.



**Figure 24:** A lysozyme protein sample where a 90% water signal is suppressed using a Watergate sequence allows more optimal observation of the exchangeable protons.

## Water suppression in BioPack

Set up **tof**, **sw**, and **pw**. Then run auto-calib. by **[SetUp] [Water] [AutoCalibration] [Watergates][3-9-19]**.

Respond to questions and use the calibrated value of **pw90** instead of the standard parameters. For the H<sub>2</sub>O selective pulse time (msec) use a value 1-2 msec.

BioPack uses a pulse sequence called “water.c” for basic 1-D water suppression. The autowatergate version above using a pulse sequence called “gwatergate.c” and is independent of BioPack. The water pulse sequence in BioPack has many flags that allow the same pulse sequence to be used for many types of water suppression including preset, watergate, shaped, jump-return, and wet.