IV. Primer for Obtaining Accurate/Consistent Proton Integrations in DISNMR

by cg fry: revised 28-Oct-98

Accurate integrations in NMR is a non-trival task. In all cases where quantitation is required, care should be used. I highly recommend “playing around” with the software, i.e., experimenting with various options in the software, and document how the integrals are changed. Be especially careful of baseline correction. I have seen instances where > 100% differences in integrations seemed quite easy to achieve. These have always been cases with a narrow large peak compared to a broader smaller peak. A lot of care must be exercised to get reasonably accurate integrations (and I wouldn't be over optimistic about how good you can get; i.e. assign reasonable error bars, reflecting your experience with the software). The best method of obtaining accurate intensities (actually areas), not discussed here (see the NUTS or VNMR help), is to use deconvolution methods in NUTS or VNMR.

Through trial and error, I have yet to see a way to use any automation sequence or macros to get consistently good integrations for quantitative work. These comments apply when small and/or broad lines exist in the spectra; automatic procedures seem to work well for "routine" spectra where only semi-quantitative integrations are sufficient. I recommend the following procedure for performing integrations within DISNMR (and I currently prefer NUTS much more than *PCNMR4Windows*; advanced users are fine with VNMR, but be careful(!) with baseline corrections):

1. Obtain the data with a large sweep width (e.g. 20ppm) to help reduce baseline rolling in the data region. Make absolutely certain that no signal clipping is occurring (reduce RG by a factor of 2 if uncertain!). Take one data set with a relaxation delay 5 times longer to make certain T_1 effects are not affecting the results.
2. Take all data if possible at one time using identical (RG, RD, etc.) settings. If this can be done, then PK will work well and phasing should not be adjusted differently for different spectra. Use an automation routine to EM;FT;PK and write out spectra (see FT.AU on the spectrometers). If data is taken at different times, phasing should be performed manually, with care taken to try to get the baseline to have an identical slope over the region of interest (use some defined criteria to keep from becoming overly subjective).
3. You can use a matched Lorenztian line broadening for simplicity, but for best results use Gerald Pearson’s Gaussian apodization (not the same as DISNMR’s GM!!); see the next primer for more information.
4. Baseline corrections always have to be performed, and I have never gotten consistent autobaseline routines to work sufficiently well. For a lot of spectra, performing the manual baseline corrections is the most tedious and subjective part of the process. Do _not_ rely on slope adjustments to correct a poor baseline!!
5. I have found that with good baseline correction, the slope adjustment is straightforward and only has to be done once. The integrals are not nearly as sensitive to slope as they are to phasing and baseline correction (at least in my experience).

For small numbers of spectra, DISNMR works quite well, albeit a bit tediously.