Get More Information for Your Efforts with 2D

by Paul Bowyer on 29 August, 2012

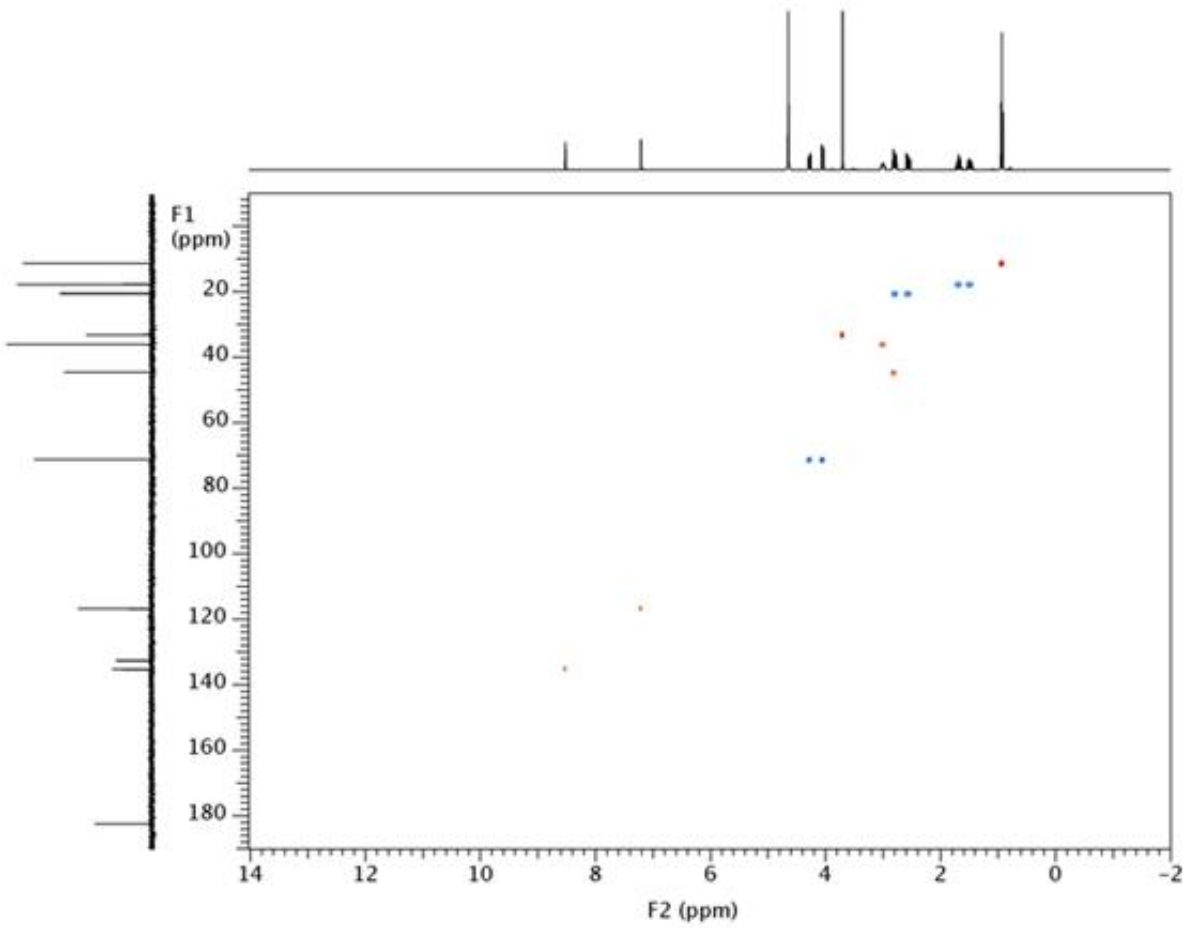

Figure 1. Multiplicity-edited HSQC spectrum of pilocarpine in D2O. The corresponding 1D (^{1}H and ^{13}C) spectra are shown along the axes of the 2D spectrum.

Some of the comments I occasionally hear from customers are things like “we don’t need 2D experiments,” or “2D NMR is complicated.” In years gone by these statements might have held some truth, but with a modern NMR system, attitudes like these cause people to miss out on powerful and efficient experiments that will save spectrometer time and provide more useful NMR data.

Consider the Distortionless Enhancement by Polarization Transfer, or DEPT, experiment. It is a popular 1D carbon experiment and it has been around for a long time. DEPT provides information about how many protons are attached to each carbon in your molecule of interest. That’s a very useful bit of information. A lot of customers are very comfortable with DEPT, and think of it like an old friend. And it’s a very nice experiment, but these days, under a lot of conditions, you can get the

same information and more—faster—from a proton-detected heteronuclear shift correlation 2D experiment.

One of the most widely-used 2D techniques is the HSQC (heteronuclear single quantum correlation) experiment. It has become the cornerstone experiment for structure elucidation in most modern labs. HSQC is used to correlate proton and carbon resonances from atoms that are directly attached to each other. The data basically say, "this hydrogen atom is attached to this carbon atom." In addition to correlating spins over one chemical bond, however, HSQC can also provide multiplicity information. A common implementation of the HSQC sequence yields a 2D spectrum in which CH and CH3 signals point one way (say, "up"), while CH2s point the other way ("down")—exactly as in a DEPT-135 spectrum. So in the 2D spectrum you might have your CH and CH3 resonances denoted by a certain color and your CH2 resonances denoted by a different color. With the HSQC experiment you can get the same information obtained by the DEPT experiment, but you also get the correlations between the chemical shifts.

The other advantage: you can often get this information in significantly less time than it takes to collect a carbon-detected experiment such as DEPT. The difference will depend on a number of factors, such as the type of probe you're using, the sample concentration, and so on, but with a relatively modest sample concentration on a modern spectrometer you can typically collect an HSQC data set in less than 10 minutes. Because DEPT is a carbon-detected experiment, it often takes significantly longer to get acceptable signal-to-noise . And if you've got a probe that's optimized for proton detection, the advantages of doing an HSQC over a carbon-detect experiment like DEPT are even greater.

To illustrate the point, Figure 1 at the top left shows a multiplicity-edited HSQC spectrum of a sample of ca. 100 mM pilocarpine in D2O, recorded on an Agilent 400MR system equipped with a OneNMR Probe. The total measurement time for the HSQC experiment was 10 minutes and 25 seconds. In the 2D spectrum the red peaks denote CH3 or CH signals, while the blue peaks denote CH2 signals. Using the proton integrals and chemical shifts it is straightforward to differentiate between CH3 and CH—there is simply no need to run a DEPT experiment here.

DEPT can still sometimes be a very useful experiment, particularly when you've got a crowded carbon spectrum. With a conventional HSQC experiment the resolution in the carbon dimension is significantly lower than you would get in a carbon 1D data set. If you really need that extra resolution in the carbon dimension, then running DEPT can be useful. Even then, though, the resolution issue in 2D can often be overcome by using a band-selective experiment, such as bs(g)HSQCAD. By "concentrating" the same number of t1 increments into a smaller 13C chemical shift range, the resolution in the F1 dimension of the 2D spectrum can be significantly increased without paying the penalty of increased experiment time. There's a plethora of variants on the HSQC experiment for various circumstances; it depends how far you want to go with these things. But that is a topic for another day!

The "take-home" message is this: you can often get more structural information in much less time by including a 2D experiment as a routine part of your NMR analyses.