Dr. Mike Lumsden
Coordinator, ARMRC
November 22, 2007

ATLANTIC REGION MAGNETIC RESONANCE CENTRE

Quantitative NMR Without Internal Standards

Introducing the Eretic Experiment

NMR spectroscopy is a powerful tool for quantifying the amount of material present in a sample, since the area under a given resonance signal is proportional to the number of moles of nuclei responsible for that signal. Although this fact alone is the reason we can accurately integrate ^{1}H spectra and obtain relative signal areas, to quantify areas and relate these to concentrations requires additional effort. Most commonly, the approach taken has involved spiking the sample with a known amount of some internal calibration standard. Such an approach has found widespread use is various areas of chemistry, biology, and medicine. Although this approach can yield very good accuracy, it has several drawbacks. First, the sample is contaminated and so sample recovery, if desired, can become problematic. Second, great care must be exercised in preparing the sample and third, the T_1's of the reference compound must be known, sometimes requiring initial T_1 measurements. Lastly, and perhaps most significantly, a number of restrictions are placed on the choice of a suitable reference compound. For example, the compound chosen must (a) be soluble, (b) be chemically inert in the sample, (c) preferably have a shorter T_1 relative to the analyte(s) being studied, (d) be stable over time, and finally (e) have minimal resonance overlap with both the analyte(s) as well as water if water suppression is to be performed.

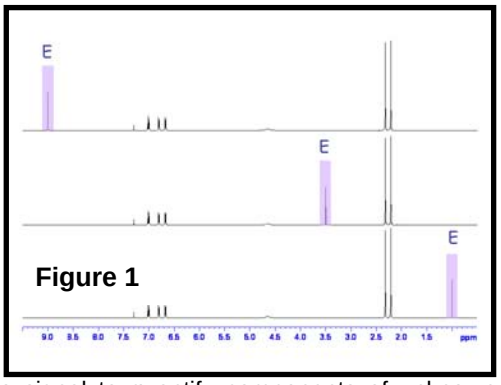

Figure 1

An alternative technique, published in 1999 by the group of Serge Akoka, is called ERETIC (**E**lectronic **RE**ference **T**o access **I**n vivo **C**oncentrations). Rather than spike the sample with a known amount of a standard compound, in eretic the spectrum is "spiked" with a new resonance using a signal generated on a free channel of the NMR spectrometer. Since all modern spectrometers can generate shaped rf pulses, eretic uses an exponential decay shape to spike the spectrum. This signal makes its way to the receiver for detection along with the fid either by sending it to an unused coil in the probe to radiate (normal eretic) or alternatively, to route the signal directly to the receiver and combine it with the fid there (inverse eretic). Regardless, the NMR signal entering the receiver now has a new frequency component added and a subsequent Fourier transform will yield a spectrum with a "synthetic" peak added in. An initial calibration of the area of this eretic signal against a standard of known concentration then permits the use of this signal to quantify components of unknown concentration.

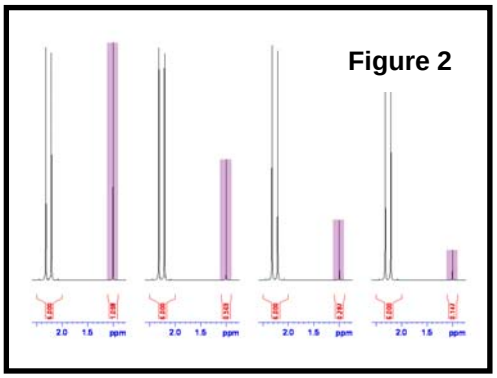
Figure 2

As compared to adding an internal standard, one can see some immediate advantages to eretic. No longer is anything added to the sample under study and so sample integrity is not an issue. The experiment is fast and there is no longer any dependence on relaxation times of the reference signal. The approach need not be restricted to ^{1}H observe but can be easily extended to the observation of other nuclei. Furthermore, it is extremely easy to move the position of the eretic peak to a favourable position in the spectrum, away from analyte signals of interest (equivalent to finding a different reference compound when using internal standards). **Figure 1** shows three different eretic spectra obtained for a standard sample of 2,3-dimethylphenol. In each spectrum, the position of the eretic signal (labelled 'E') is modified, an adjustment easily controlled by a single parameter in the spectrometer software. Lastly, the area of the eretic peak is also easily scalable using the power control settings in your spectrometer software (equivalent to preparing internal standards of different concentrations). **Figure 2** shows 4 different eretic spectra with the eretic peak doubled in intensity each time (corresponding to a 6 dB gain). Although it would be best to recalibrate the eretic peak after rescaling, the superb linearity of modern NMR spectrometers makes this step unessential, provided one can live with a slight decrease in accuracy.

All users of the Bruker AV-500 at ARMRC now have access to the eretic experiment in their ICON-NMR experiment list. Eretic has been implemented using the inverse approach mentioned above; the eretic signal never goes to the probe but rather is routed directly from the transmitter into the receiver. The one drawback of this approach is related to lossy samples. With such samples, the NMR signal is attenuated whereas the eretic signal intensity will be unaffected, causing errors in the calibration. To compensate for this, each eretic experiment in automation is preceded by a single scan ninety degree pulse calibration, the result of which can be used to generate a correction factor. Please speak to me regarding the details surrounding this issue.

In closing, it is my hope that those of you interested in quantitative NMR will give this new experiment a try. I would like to thank Stan Woodman of Bruker BioSpin Canada, Amy Freund of Bruker BioSpin USA and David VanderVelde, the Director of the NMR facility at the University of Kansas, for their technical assistance in helping me implement this experiment.