

Communications

Heteronuclear Electronic Reference NMR Method for the Measurement of Concentration

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Received April 12, 2007

Key Words : Heteronuclear electronic reference, NMR reference, Quantitative sodium analysis

Quantitative analysis is one of the most important and fundamental subjects in all the field of science. Among analytical instruments, NMR was distinctly unpopular for the quantitative analysis until the appearance of high field magnet because of the many inferior intrinsic aspects, particularly its sensitivity. In spite of those defects, NMR has been continuously used for the quantitative analysis since the first appearance of NMR instruments because of the direct proportionality of the peak intensities to the concentration of the individual compounds – a feature not to be found for other instrumental methods.¹ Although the significant development of NMR technology enables the NMR method to be used more easily for the quantitative analysis, its applications are generally limited to the use of hydrogen nucleus with several experimental difficulties.²

Recently, Akoka and *et al.* have reported that the ERETIC (Electronic REference To access *in vivo* Concentrations) method, based on a proton reference signal synthesized on a carbon coil by an electronic device, avoids all the drawbacks associated with the use of chemical references to measure concentration.^{3,4} This method used the proton pseudo-FID produced on the carbon coil which was connected directly to proton frequency unit and showed excellent accuracy and precision for the measurement of concentrations.^{5,6}

We now report that the proton coil can be used as a broad band antenna to detect the heteronuclear pseudo-FID generated by multiplication of an exponential function and heteronuclear frequency and this easily extends the method to the measurement of heteronuclear species concentrations; again, without the difficulties associated with chemical references.

The reference signal obtained by the Heteronuclear Electronic Reference To access *in vivo* Concentration (HERETIC) method is a pseudo-FID produced on a proton coil of a broad band probe by multiplication of an exponential wave function as a low frequency element and heteronuclear frequency generated from frequency control unit (FCU) in Bruker AVANCE-700 spectrometer. The high frequency component in reference signal was provided during the acquisition time of observing channel by the second channel of the spectrometer using “eretic” pulse program of Bruker. It was derived from the FCU and

modulated with low frequency element as a shape pulse which was sent directly to the proton coil of broad band probe without amplification. Therefore, the phase of reference signal is independent of the transmitter pulse and its magnitude and frequency can be freely determined as decoupler parameters in pulse program by the operator. The broad band channel was used for transmitting corresponding rf hard pulses and receiving the real and pseudo-FID signal (Figure 1).

HERETIC pseudo-FID signals defined by frequency and amplitude modulated by several different powers were acquired with the real FID signals derived from the real samples contained in 5 mm NMR tube and showed in Figure 2. The heteronucleus used for this experiment was sodium-23 observed on Bruker AVANCE-700 spectrometer at

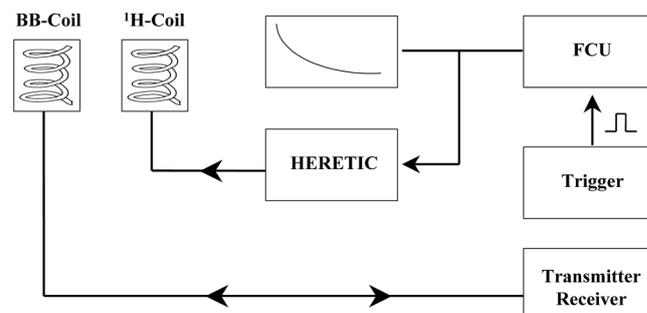


Figure 1. Block diagram for the HERETIC Method.

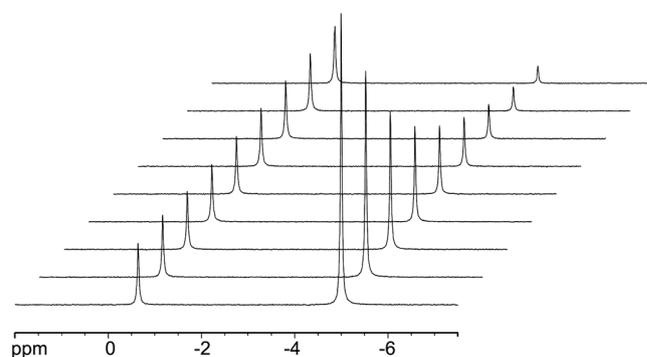


Figure 2. HERETIC signals at -5 ppm modified by the power of the shape pulse relative to the 100 $\mu\text{g/mL}$ sodium solution.

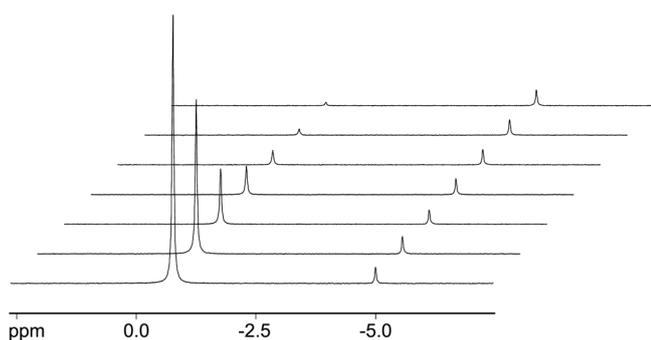


Figure 3. Sodium-23 NMR spectra (500, 250, 100, 50, 25, 10, 5 $\mu\text{g}/\text{mL}$) with the HERETIC signals at -5 ppm calibrated as 25 $\mu\text{g}/\text{mL}$ sodium.

185.24 MHz. The power of the shape pulse was attenuated from 46 dB to 70 dB by 3 dB units and the frequency of HERETIC signal was placed at -5 ppm. The signal intensities obtained from the 100 $\mu\text{g}/\text{mL}$ of sodium solution are constant while the intensities of HERETIC signal vary depending on the power of shape pulse. This HERETIC signal could have been observed even at 68.64 MHz, the chlorine-35 resonance position, which was about 10 times lower than the tuned frequency.

To validate the applicability of this method, the Fourier transformed reference signal calibrated against the known standard solution was used for the quantitative analysis of sodium in solution which has been a system of great interest.^{7,8} Using the power of shape pulse, the intensity and line width of the HERETIC signal was adjusted to have similar shape as that of standard solution to reduce the error of integration. Figure 3 shows representative sodium-23 NMR spectra obtained from the different concentrations of known samples, 5, 10, 25, 50, 100, 250, and 500 $\mu\text{g}/\text{mL}$ sodium solution, respectively, each compared with the HERETIC signal calibrated as 25 $\mu\text{g}/\text{mL}$ of sodium. The spectra were collected with fast pulsing due to the quadrupole relaxation of sodium and the HERETIC signal which was free from the relaxation delay: 0.1 s relaxation delay, 0.55 s acquisition time, and 8 K data points over a 7440 Hz spectral width using 30° pulse. 5 minutes of acquisition (NS=512) were enough to give reasonable signal-to-noise ratio even for 5 $\mu\text{g}/\text{mL}$ of sodium solution. An exponential multiplication (LB=1) was applied to the FID prior to FT and 5th order polynomial baseline corrections were employed.

The area ratio of HERETIC and sample peaks obtained from the standard samples was used to measure the concentrations of different sample directly because of good stability

of HERETIC peak. The area deviation of HERETIC peak relative to the known sodium concentration (100 $\mu\text{g}/\text{mL}$) was less than 1% for 1 week with the same probe tuning and power level.

The use of HERETIC peak corresponding 25 $\mu\text{g}/\text{mL}$ of sodium calibration showed less than 3% standard deviations over the entire range of concentrations determined in this experiment. With the exception of the 5 $\mu\text{g}/\text{mL}$ of sodium solution (>3%), the application of a 100 $\mu\text{g}/\text{mL}$ of sodium calibration gave comparable results as that of 25 $\mu\text{g}/\text{mL}$ of sodium calibration.

The chemical reference method for the measurement of sodium concentration as well as the other heteronuclei has the intrinsic drawbacks generally associated with external references including the need for chemical shift reagent to separate the resonances of sample and reference.^{8,9} However, the HERETIC method used here avoids not only the difficulty of chemical shift reagent but also the cumbersome exploitation of an external reference. In conclusion, HERETIC is an attractive alternative to existing standard methods for measuring heteronuclear concentrations. In addition to the description of exact procedure, it will be interesting to see what types of limitation are for the other heteronuclei using this method. These investigations are underway in my laboratory and will be reported in due course.

Acknowledgements. This work was partially supported by Korea Research Institute of Chemical Technology, grant no. BSK-0701. The author would like to thank Dr. Ruediger Weisemann of Bruker for helpful discussions.

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