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A new signal processing method to observe weak ^{31}P and ^{17}O NMR peaks

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Abstract

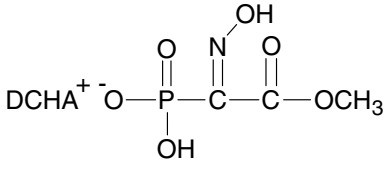
In NMR spectroscopy, situations may arise where sample concentrations are below the threshold for FT NMR detection, or sample lability constrains allowable acquisition times. In ^{31}P NMR, for example, observation of ^{13}C satellites may not be practical under given conditions. For ^{17}O NMR, which is useful to characterize ^{17}O-labeled phosphate derivatives, similar considerations may apply, and added factors are the cost of isotopically enriched samples and the requirement to obtain spectra at relatively high temperatures if narrow spectral peak line widths are desired. We report here application of a new signal processing method [S.D. Kunikeev, H.S. Taylor, J. Phys. Chem. A 108, 2004 743] to observation of weak ^{31}P and ^{17}O NMR peaks.

Keywords: Phosphorus-31 NMR; Oxygen-17 NMR; NMR sensitivity enhancement

1. Introduction

In FT NMR spectroscopy, paucity of sample may sometimes lead to marginal or unobservable resonances under practical conditions of spectrometer usage. Another limiting situation occurs with samples that are labile to the extent that an insufficient number of transients can be acquired, even for a concentrated solution.

Recently, a new harmonic inversion (HI) signal processing method [1] was proposed for weak NMR signals at or below the threshold of detecting conventional FFT approaches. In this paper, we explore applications of the method to several model problems in NMR detection. The first is detecting the weak (~0.5% of parent peak) ^{31}P NMR ^{13}C satellites. In this case the parent ^{31}P peak may be readily observed, while the small J_{PC} satellites are lost in the noise. The magnitude of J_{PC} conveys useful structural information, and in the case of α-imino- and related phosphonocarboxylates, was shown to provide an empirical basis to determine *E* vs. *Z* geometric isomerism [2]. Thus, we have chosen as our ^{31}P example the *E* isomer of methyl α-(hydroxyimino)phosphonoacetic acid, dicyclohexylammonium (DCHA) salt (**I**, methyl ester of *E* -Troika Acid, DCHA salt) [3].



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^{17}O NMR has been applied in both chemical [4] and biochemical studies [5], as a unique spectroscopic probe for reaction mechanisms [6,7], dynamic behavior of water in different organic environments [8,9], conformational and stereochemical characterization of organic molecules [10–13], determination of oxygenated additives in gasoline [14] and estimation of buried water in proteins [15–17]. Our own interest has stemmed from a reinvestigation [18] of the reported CD properties of [^{17}O, ^{18}O]-labeled phosphate derivatives. An advantage of ^{17}O NMR is its wide range of signals (~600 ppm). As ^{17}O NMR is very sensitive to the local environment, it can provide useful information about oxygen environments, indicating protonation state or H-bonding [19,20]. However, ^{17}O NMR suffers from several significant limitations. Firstly, the natural abundance of the isotopic spectrophore is quite low (0.037%); and ^{17}O-enriched compounds are expensive. Secondly, its quadrupolar nature ($I = 5/2$) tends to broaden the signals and high temperatures (e.g., ~95 °C) are needed to reduce resonance line widths [19]. Phosphorus chemists will not be indifferent to the fact that ^{17}O directly bonded to P can broaden the ^{31}P resonance to such an extent that it merges with baseline noise [21], a potential problem for highly ^{17}O-enriched samples. The spin–lattice relaxation times (T_1) of the ^{17}O nucleus are rather short (ms), requiring rapid data acquisition. For our ^{17}O NMR example, we have selected naturally abundant $H_2^{17}O$ dissolved in pyridine and analyzed at 92 °C (365 K).

2. Results and discussion

2.1. Description of the methodology

In reference [1] (where the mathematics supporting this section is given), a signal processing scheme aimed at increasing the sensitivity of signal detection was introduced. Here, it is used to process weak ^{31}P and ^{17}O signals. The scheme, which is an alternative to FFT for converting a time dependent FID into a frequency domain spectrum, first processes the signal in time space to achieve a noise reduced (NR) "clean" signal. This signal can then be subjected to FFT transformation but is most profitably transformed using the HI method [22,23]. HI is less generic than Fourier analyses in that it assumes the spectrum will be composed of Lorentzians, corresponding to a FID signal which is modeled by a sum of damped harmonics (complex exponentials). This limits the method to solution phase NMR. The method then in a minimal error sense fits the Lorentzian parameters which appear in the complex exponentials to the FID data. There are many ways to carry out this fit and these appear under such names as the linear predictor method, the Pade method, the decimated diagonalization method, etc. [22,23]. Two advantages of the HI method, given a signal measured out to a particular time, are: (a) it achieves a higher resolution spectrum than FFT; and, (b) it produces the Lorentzian parameters (frequency, area and width) simultaneously with the spectrum.

When HI is fed a "clean" signal, a general rule of thumb is that if scanning had been carried out until a local (as defined to mean "near possible features of interest") S/N ratio of 1.5 can be observed (spectral features barely discerned and hard to distinguish from the noise), the HINR method can then, without further scanning, produce a higher resolution spectrum of a quality usually associated with a FFT spectrum scanned to a S/N ratio of roughly eight. Thus, the HINR method can detect signals that normally would require much more scanning. Experience indicates that reductions in the number of transients needed of two to sixtyfold are obtainable, depending upon the situation.

Clearly, the method can be used either to conserve spectrometer time or to reveal spectral features when signals are too weak to detect conventionally, due to low concentration.

To avoid large matrices and/or large numbers (roughly half the signal length) of coupled linear equations which can lead to numerically unstable results, we employ a windowing technique [22]. Three hundred Fourier grid point sections of the presumably unsatisfactory FFT spectra are used to construct, as input to the HINR, a new FID of many fewer entrees valid only for the window. Our examples should avoid the tedium (no general software is yet developed) of analyzing the entire Fourier bandwidth using multiple overlapping windows (since edge effect errors can creep into the method). We here focus on examples where the target peaks are expected to be found within some general spectral region but cannot be seen for reasons mentioned above.

A feature of the NR method that may be unfamiliar to chemists but is pervasive in signal processing theory is the use of singular value decomposition to create a "clean" signal. Using the sample data available in the FID, a Hermitian correlation matrix is created [1]. The all-positive eigenvalues of this matrix are called the singular values. When these singular values are arranged in decreasing order, for measured signals that are sums of true and random noise signals (as assumed in NMR), if the S/N ratio is high enough (here roughly above 1.5), the singular values and their associated eigenvectors can be divided into a high singular value signal-containing group, and a low noise-containing group made of values near the σ^2 of the noise. Hence a "gap" containing no singular values should appear in the graphical representation of all singular values. If noise overwhelms the signal, no gap appears. We collect transients

until the gap appears, which experience says occurs when the signal and noise are locally on a par; the 1.5 *S*/*N* ratio mentioned above. When the gap appears, the signal eigenvectors can be used to project the noise out of the measured signal leaving a "cleaned" signal. Increased scanning always gives better defined gaps and in turn more accurate Lorentzian parameters and spectra. The Lorentzian spectral parameters converge to a given accuracy with increased scanning.

The HINR spectra, a sum of Lorentzians, appear strangely noiseless; the error due to noise is in the parameter values and not in the spectral fine structures. Frequency converges fastest; area soon follows but line widths, which in one dimensional NMR are spectrometer-determined, are less accurately obtained. Since line heights are area divided by width, when a calculated incorrect width is near zero, the Lorentzian line can appear too intense and too narrow even though the sought after frequency and area are correct and known independently of the appearance of the spectra. The noiselessness and occasional peak narrowness will surprise the observer who is used only to FFT spectra.

In summary, once a stable gap appears, a much more sensitive spectrum can be obtained than Fourier methods could have achieved with the same number of scans. Historically, the failure to keep scanning until a stable gap (and spectrum) is reached plus the numerical instabilities encountered when windows are not used have lead to some true lines being missed in the spectra and false ones to appear; all to the discredit of the HI method.

A procedure used here for very low concentration samples that speeds up the appearance of a converged gap, at the expense of some accuracy and an associated loss of resolution, is to damp the signal by multiplying the data points with a damped exponential. The damping rate is chosen to maximize the gap.

Does automated software exist even for working in a window? Not yet. Determining optimal window size and searching for the emergence of a gap as a function of scans is still a manual process [1]. Fortunately, the process can be learned in a few days without a deep understanding of the mathematics. It should be stressed that for HINR processing, signal phasing is not needed.

We report here results obtained for model ^{31}P and ^{17}O analyses chosen to demonstrate the advantage of our new NMR signal processing method.

2.2. First case: ^{31}P NMR

The first analysis, shown in Fig. 1, presents the 161.983 MHz ^{31}P NMR of a "saturated", 67 mM solution of **I**. The sample solution concentration was not limiting in this experiment for detection of the main ^{31}P signal, rather we sought to detect the $^{1}J_{PC}$ and $^{2}J_{PC}$ satellites (each ~0.5% of the unsplit ^{31}P signal)

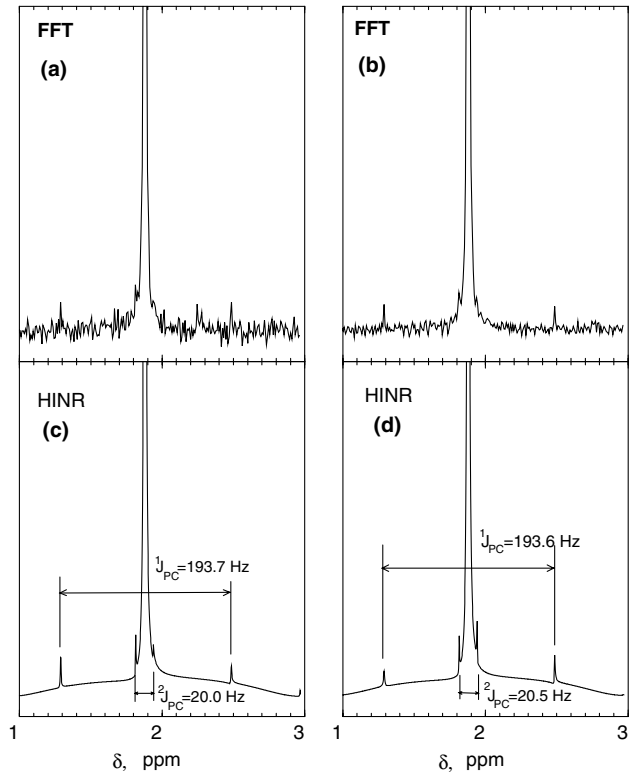

Fig. 1. ^{31}P NMR spectra of **I**, 67 mM in methanol-d_4/methanol (1/4). (a) FFT, after 0.5 h. (b) FFT, after 2 h. (c) HINR, after 0.5 h. (d) HINR, after 2 h.

using the HINR method, noting that FFT broadened the main peak, obscuring the tiny $^{2}J_{PC}$ satellite doublet. As shown in Fig. 1, within 30 min not only does HINR reveal clearly both sets of satellites, which are difficult to distinguish from the noise using FFT, but also produces a clearer spectrum and the same $^{1}J_{PC}$ value as obtained by two hour of scanning followed by FFT analysis. The two hours HINR spectrum is included to confirm convergence. Increasing the signal length to extend Fourier resolution from the 16,384 data points used in this experiment produces more spurious noise features and makes the FFT spectrum less informative.

In Fig. 2, the analyses are repeated for a sample concentration of 8.4 mM (i.e., 8× diluted). One would expect to need 64×2 h = 128 h of scanning to match by Fourier analysis the two hour FFT spectrum in Fig. 1. Fig. 2(a) shows the FFT spectrum after twenty hours of scanning (40,000 transients). Clearly the $^{1}J_{PC}$ value cannot be measured and damping the signal did not improve the situation. Twenty hours was arbitrarily deemed a practical limit for multiuser spectrometer time. HINR gave no gap with this undamped signal: the concentration was too low. Fig. 2(b) shows the HINR result using a damping constant of 1.25 Hz. As a minimal value, this was considered optimal to

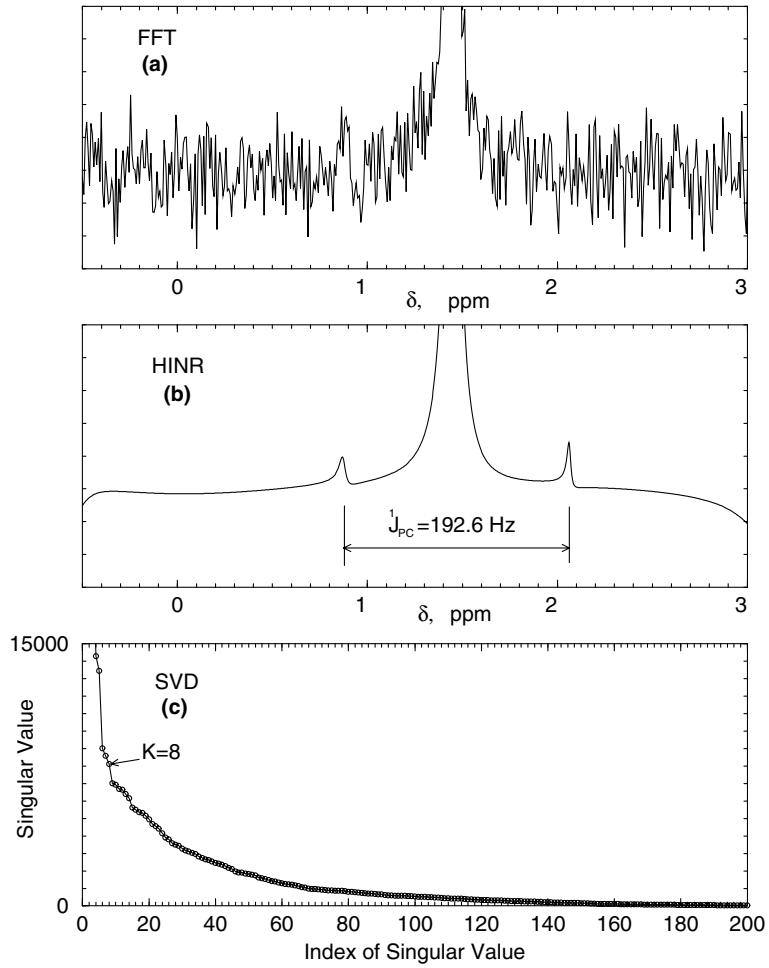

Fig. 2. ^{31}P NMR spectra of **I**, 8.4 mM in methanol-d$_4$/methanol (1/4). N_{tr} = 40,000 (20 h). (a) FFT without damping. The one-bond ^{13}C P-C satellites are difficult to distinguish from random noise peaks with (not shown) or without damping, as predicted from the results in Fig. 1 by the formula, $S/N \propto C\sqrt{N_{tr}}$ (S/N, signal to noise ratio; C, concentration; N_{tr}, number of transients). (b) HINR-derived spectrum. (c) Singular value curve corresponding to (b). The 8th singular value is indicated ($K = 8$); the top three SV's are above 15,000.

maintain resolution and accuracy, and gave a gap at the 8th singular value as shown in Fig. 2(c). The $^1J_{PC}$ value obtained is 192.2 Hz; <1% different from the value obtained conventionally from a concentrated sample ($^2J_{PC}$ is obscured by main peak broadening). Larger damping constants enhanced the appearance of the gap but produced a less accurate spectrum. Increased damping can be used to confirm the number of signal singular values if very high resolution is not an issue. The "dips" at the edge of the window in Fig. 2(c) can be ignored as "edge effects".

Fig. 3 shows results for **I** at the lowest concentration used, 0.058 mM, at which the main ^{31}P peak could still be detected by HI after twenty hours (40,000 scans). The conditions were chosen by starting with a 58 mM result with the S/N ratio given, using the formula $S/N \propto C\sqrt{N_{tr}}$ to estimate the scan time needed after successive dilution factors of 10. As also shown in the lower panel, conventional FFT with the same damped signal gave an uninterpretable spectrum under these conditions.

2.3. Second case: ^{17}O NMR

Our objective was to demonstrate that within acceptable spectrometer scanning periods, the naturally abundant ^{17}O in a sample too dilute for conventional ^{17}O NMR analysis could be detected by HINR. Figs. 4 and 5 show results for pyridine solutions containing 170 mM (63 μM of ^{17}O) and 68 mM (25.2 μM of ^{17}O) water, respectively. The NMR spectrometer (Bruker AMX-500) probe was kept at 92 °C in order to achieve a narrower line width [19]. Roughly nine times as many scans were needed to maintain a $S/N \sim 1.5$ in going from the higher to the lower concentration. In Fig. 4, we see that after 2.6 h (8000 transients), the FFT spectrum shows nothing and is unphasable. In contrast, application of the HINR method to the same data set shows a singular value in the signal space, giving a spectrum that reveals the ^{17}O resonance with a weight (which can be used to calculate area) of 31. The FFT and HINR spectra of the more diluted (68 mM) solution

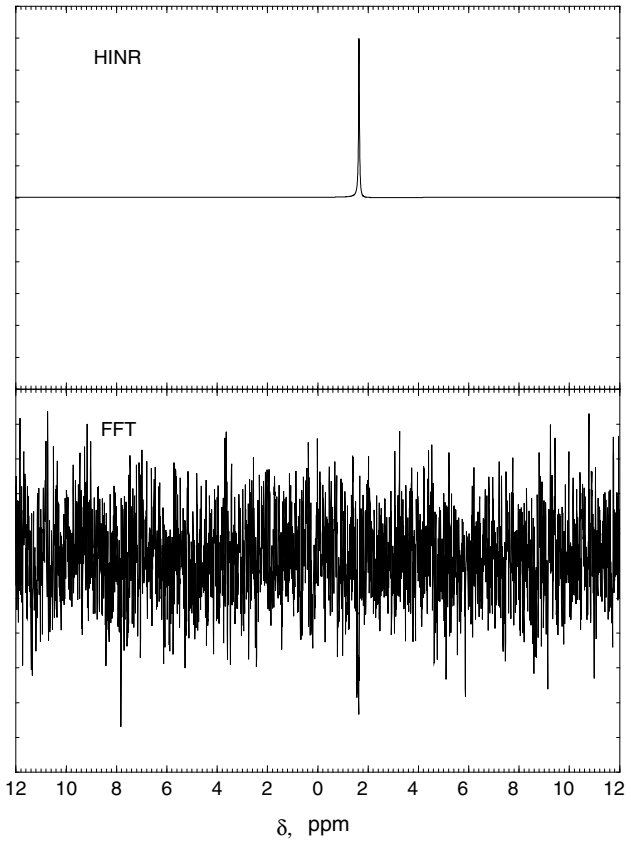

Fig. 3. ^{31}P NMR spectra of **I**, 0.058 mM in methanol-d_4/methanol (1/4). $N_{tr} = 40{,}000$ (20 h). FFT spectrum (lower); HINR-derived spectrum (upper).

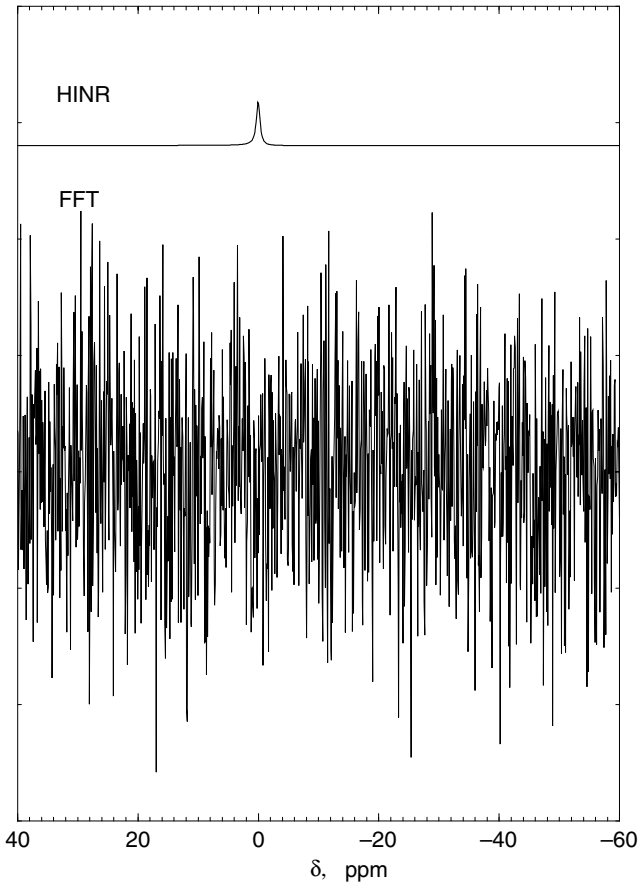

Fig. 4. ^{17}O NMR spectra of H_2O (natural abundance, 0.037%) in pyridine, concentration 170 mM (63 μM of ^{17}O) at 92 °C. HINR spectrum (upper); FFT spectrum (lower). The chemical shift of the observed HINR peak was arbitrarily set = 0.00 ppm, see Fig. 5 for comparisons to FFT peak chemical shifts using the same offset.

both show nothing after 58,000 transients (18.3 h) which was deemed a realistic maximal scanning time. Fig. 5 shows the FFT and HINR results obtained with a damped signal (damping constant $G_f = 10^{-3}$). Higher damping gives a clear signal singular value separation. Lower dampings suggest one singular value but do not unequivocally demonstrate it. The FFT for the damped cases in the region where the HINR yields a definitive result gives a feature that can not confidently be differentiated from a noise artifact. Note that here the spectra are unphased. As mentioned above, we need not phase to read the frequency (f_r) and weight ($|d_k|$) as we obtain the Lorentzian parameters simultaneously with the spectra. f_r is given to one part in three thousand and the weight may be erroneous by up to 10%. The lower damping result which introduces less error should yield the most reliable value.

3. Conclusion

We have shown that the HINR method can be used to increase sensitivity in ^{31}P and ^{17}O NMR experiments. Increased sensitivity implies that lower concentrations can be observed and/or that observation time (e.g., for labile samples) can be decreased. As more experience is gained with the method [25], commercial or library software to facilitate the analyses is likely to follow.

4. Experimental

4.1. Sample preparation

Compound **I** was kindly provided by Dr. B.A. Kashemirov. Specified concentrations of (**I**) in methanol instead of water (1:4 = methanol-d_4:methanol) were prepared to abate the pH effects on the chemical shift and line width. Solutions at 68 mM (25 μM of ^{17}O) and 170 mM (63 μM of ^{17}O) of H_2O were prepared by dissolving HPLC water (Burdick & Johnson) in dry pyridine (anhydrous 99.8%, Aldrich).

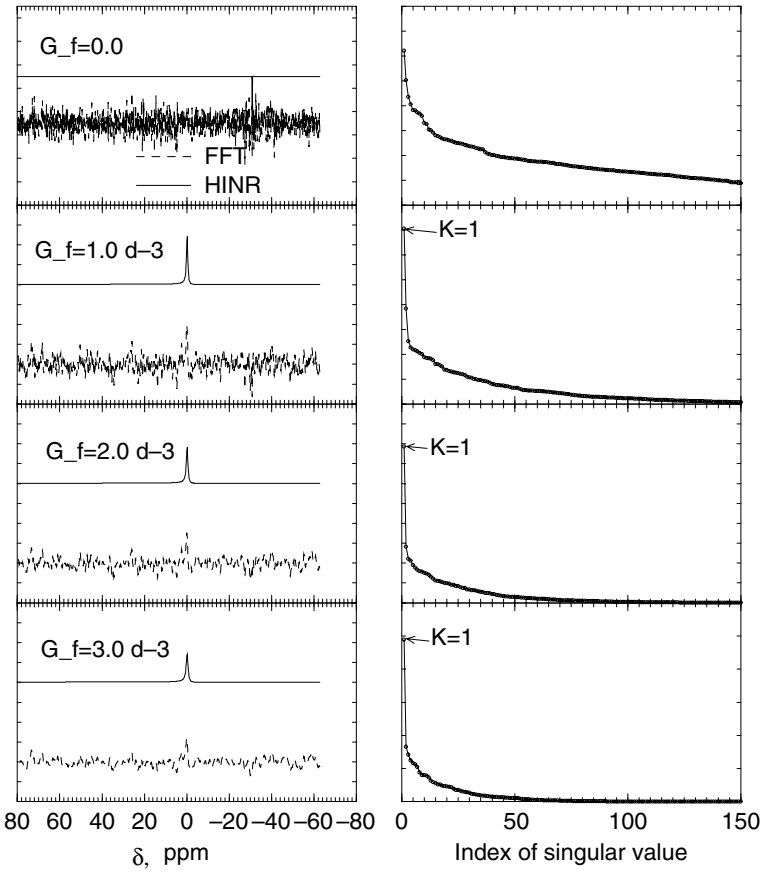

Fig. 5. Comparison of FFT (lower, broken trace) and HINR (upper, solid trace) ^{17}O NMR spectra (left) and singular value plots (right) of H_2O (natural abundance, 0.037%) in pyridine, concentration 68 mM (25 μM of ^{17}O) at 92 °C. N_{tr} = 58,000 (18.3 h). Damping constants (G_f) applied are indicated in the spectra. In the ^{17}O NMR spectra displaying signals not attributed to noise (lower four panels), the deviation of the chemical shift values derived from HINR relative to those obtained conventionally (FFT, set as reference, δ_O = 0.00 ppm) were not significant (<0.02 ppm). The ^{17}O NMR chemical shift of H_2 ^{17}O in pyridine at 25 °C is −7.5 ppm relative to natural abundance ^{17}O water [24]. At this temperature, the observed linewidth is much broader.

4.2. NMR spectroscopy

^{31}P NMR was recorded at 161.983 MHz on a Varian Mercury 400 MHz NMR spectrometer, using a 5 mm probe. Default ^{31}P acquisition parameters and pulse-sequence routine were used. FID data were saved accumulatively at every 64 transients until manually stopped at the required acquisition time.

^{17}O NMR was recorded at 67.804 MHz on a Bruker AMX-500 NMR spectrometer, using a 5 mm probe. In order to minimize the line width due to the quadrupole nature of ^{17}O nucleus, experiments were run at 365 K. For the 68 mM sample, FID data were saved at every increment of 80 into 690 (No. 1–690) data sets, making a total of 55,200 transients. For the 170 mM sample, transients were collected in a different way: the first 1040 transients were saved at increments of 104 into 10 (No. 1–10) data sets, and after that another 54 (No. 11–64) data sets were saved at every increment of 1000, making a total of 55,040 scans. All transients were collected using 8192 data points with a spectral width (swh) of 50,000 Hz, a flip angle of ∼90° ($p1$ = 8 μs), and an acquisition time of 0.16389 s. A relaxation delay (d1) of 1 s was added between pulses resulting in a 1.163898 s recycle time.

Acknowledgments

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[25] Interested potential users are invited to contact H.S.T. for practical details on implementation.