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Communication

Two-dimensional NMR spectroscopy in Earth's magnetic field

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Abstract

We demonstrate the first two-dimensional correlation NMR (COSY) spectra obtained at ultra low frequencies (ULF) using the Earth's magnetic field. Using a specially developed spectrometer with multiple audio-frequency pulses under controlled pulse phase, we observe magnetisation transfer arising from heteronuclear *J*-couplings in trifluoroethanol and *para*-difluorobenzene. The 2D COSY spectra exhibit all diagonal and off-diagonal multiplets consistent with known *J*-couplings in these molecules.

Keywords: Earth field; COSY; Heteronuclear; Difluorobenzene

Nuclear magnetic resonance spectroscopy is powerful precisely because it allows a multi-dimensional representation of various interactions between different atomic sites in molecules [1]. Indeed, the shift from one-dimensional methodology to two and more dimensions [2] represents the single most important transformation in the development of modern NMR. We here demonstrate this multi-dimensional transition in an emerging branch of magnetic resonance spectroscopy, Earth's field NMR (EFNMR) [3–5]. This is of especial interest because EFNMR has been recently shown to provide exceptional resolution [6], largely because the magnetic field provided by the Earth is so uniform. EFNMR is of further interest both because of its portability, the apparatus requiring no magnet, and because all of nature's organisms are immersed in the Earth field, and potentially subject to its influences.

Since 1954, Earth's field NMR (EFNMR) [3] has been something of a curiosity, used primarily for geophysical magnetometry, or as a simple low cost (<\$5k) demonstration of magnetic resonance principles. However, as early as 1971, G. Bene showed that it is possible to measure heteronuclear *J*-couplings in Earth field NMR [7], while in 1990, Stepisnik et al. [8], used Earth's field NMR to carry out an imaging experiment. Here we show, for the first time, that EFNMR, despite operating at audio-frequencies in the ultra low frequency (ULF) band, surprisingly manifests the power of modern (>\$500k) multi-dimensional high field NMR spectroscopy. This development is of particular interest, in the light of recent demonstrations that the method might, in principle, be used in molecular spectroscopy [6,9] or imaging [8,10]. While the low value of the Earth magnetic field (around 60 μT) causes homo-nuclear chemical shifts to disappear, hetero-nuclear resonances, and their corresponding frequency differences, may be observed very easily [7,9]. Using this phenomenon, Traebesinger et al. [9] and Appelt et al. [6] have demonstrated that electron-mediated heteronuclear *J*-couplings (spin–spin couplings), which are field independent, may be clearly observed at low field. Indeed it appears that enhanced resolution is possible due to the long spin relaxation times and high field homogeneity which prevail in EFNMR [6]. Until now, these low field spectroscopic approaches (as opposed to

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the imaging experiments) have been one-dimensional, restricting the method to pre-1970's NMR techniques. Further, the EFNMR experiment performed by Appelt et al. required the apparatus to be situated outdoors, with manual transport of the sample from polarising magnet to the receiver apparatus, making the experiment inherently "single-shot". This latter feature, in particular, limits the method to the one-dimensional domain.

Modern multi-dimensional NMR [1], allows nuclear spin ensembles to evolve under local interactions, as determined by trains of radiofrequency pulses, whose duration and phases guide the quantum coherence pathways through which the spin states are directed. The evolution times may be stepped independently of the signal acquisition process so as to provide additional temporal dimensions, each of which may be Fourier transformed to an independent (multiplexed) frequency domain. The suggestion by Jeener [2], that the method could be extended to two or more dimensions, led to an explosion of methodology [1]. First, the ability to spread spectral information through more than one-dimension allowed spectroscopists to tackle the more complicated spectra from much larger molecules. Second, the correlations between different parts of the spectra, manifest in the off-diagonal peak structure, allowed electron-mediated spin–spin couplings to be used to more precisely determine molecular structure, and through-space spin–spin dipolar interactions to be used to ascertain molecular distance geometry, leading eventually to protein structure determination. Finally exchange of spectral properties through the multi-dimensional domains could be used to reveal molecular dynamics.

The shift from 1-D NMR to modern multi-dimensional NMR is made possible by sophisticated computer control of the NMR phase and timing parameters, and by the use of multi-dimensional Fourier transformation. We have made this shift for EFNMR, by means of a number of significant technical improvements, including a specially developed spectrometer with the necessary digital pulse sequence control. Our EFNMR system incorporates strong pre-polarising fields, electromagnetic screening, precise phase control, and signal-averaging, thereby enhancing signal-to-noise ratios such that automated experiments may be carried out indoors in a conventional laboratory setting. The spectrometer is sufficiently flexible to allow control of magnetisation evolution pathways to a level typical of modern high field NMR spectrometers.

Here we demonstrate the classic 2D correlation spectroscopy (COSY) experiment [1] for two different molecules, trifluoroethanol and *para*-difluorobenzene, in both cases taking advantage of the heteronuclear ^{19}F and ^{1}H spin systems which are coupled by the intramolecular electron orbitals. In each experiment two resonant audiofrequency pulses of precisely controlled amplitude and phase, were applied to the sample following a static field pre-polarising pulse, as shown in Fig. 1. The pre-polarising pulse is applied using a copper coil which surrounded the sample and which, by being shorted during the NMR experiment, additionally provides substantial electromagnetic screening of any unwanted ULF interference. It is this feature that has allowed us to move the EFNMR apparatus indoors.

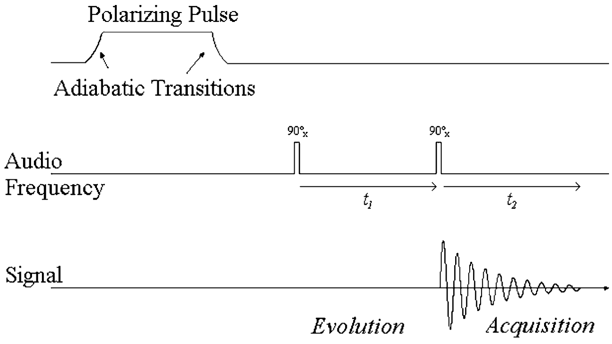

Fig. 1. Timing sequence showing the pre-polarising pulse (duration around 6 s), and the two phase-locked audiofrequency excitation pulses. The evolution period is t_1 and the acquisition time domain is t_2.

In the evolution domain the duration, t_1 is incremented in 512 steps of 1.82 ms intervals. Because the evolution domain bandwidth is much less than the resonant frequency, we are effectively under-sampling, while retaining a digital frequency resolution of around

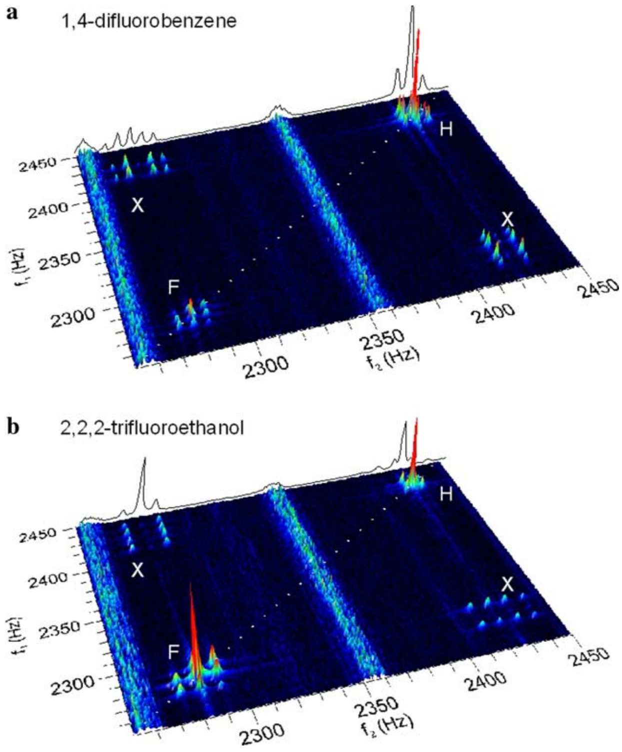

Fig. 2. Experimental 2D COSY NMR spectra for (a) difluorobenzene and (b) trifluoroethanol. Both were obtained at audiofrequencies 2.28 kHz (^{19}F) labelled F, and 2.43 kHz (^{1}H) labelled H. X represents the cross-peaks). The streaks along f_1 are from low frequency interference.

1 Hz. However, we have chosen the under-sampling domain so that no spectral distortion or complexity results as a consequence of Nyquist fold-back. In the acquisition domain we used direct digitisation of the ~2.4 kHz Free Induction Decay signals using 16 k points and a bandwidth of 5.56 kHz, and hence an acquisition domain frequency resolution of 0.68 Hz. The total experiment time was 16 h and 500 ml sample volumes were used. 1,4-difluorobenzene and 2,2,2-trifluorethanol were obtained from Sigma–Aldrich (Castle Hill, NSW, Australia).

Because only real data were detected, the 2D Fourier Transformations produced reflected quadrants, each one of which yields a magnitude COSY spectrum. A single

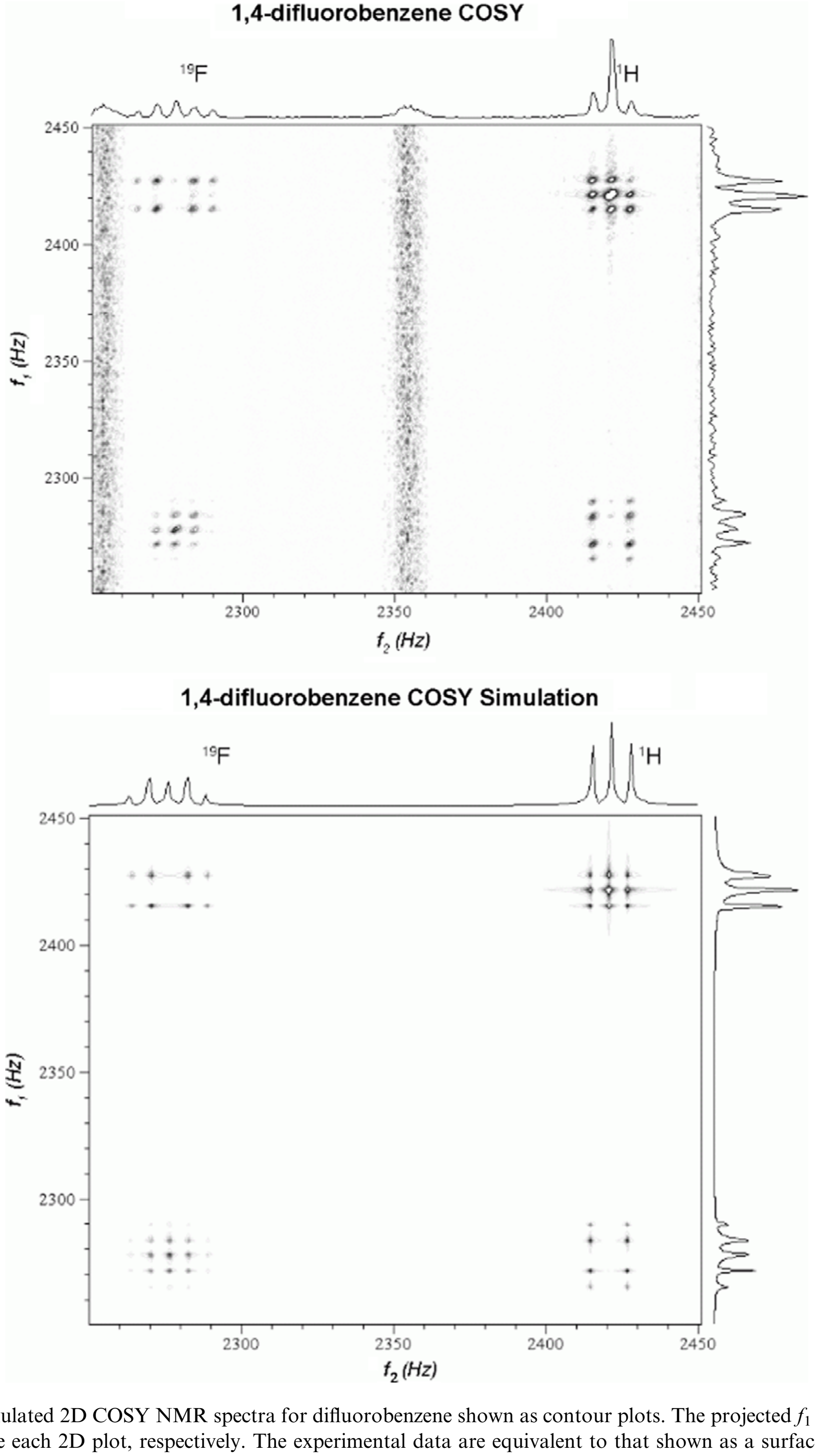

Fig. 3. Experimental and simulated 2D COSY NMR spectra for difluorobenzene shown as contour plots. The projected f_1 and f_2 domain 1-D spectra are shown to the right and above each 2D plot, respectively. The experimental data are equivalent to that shown as a surface plot in Fig. 2.

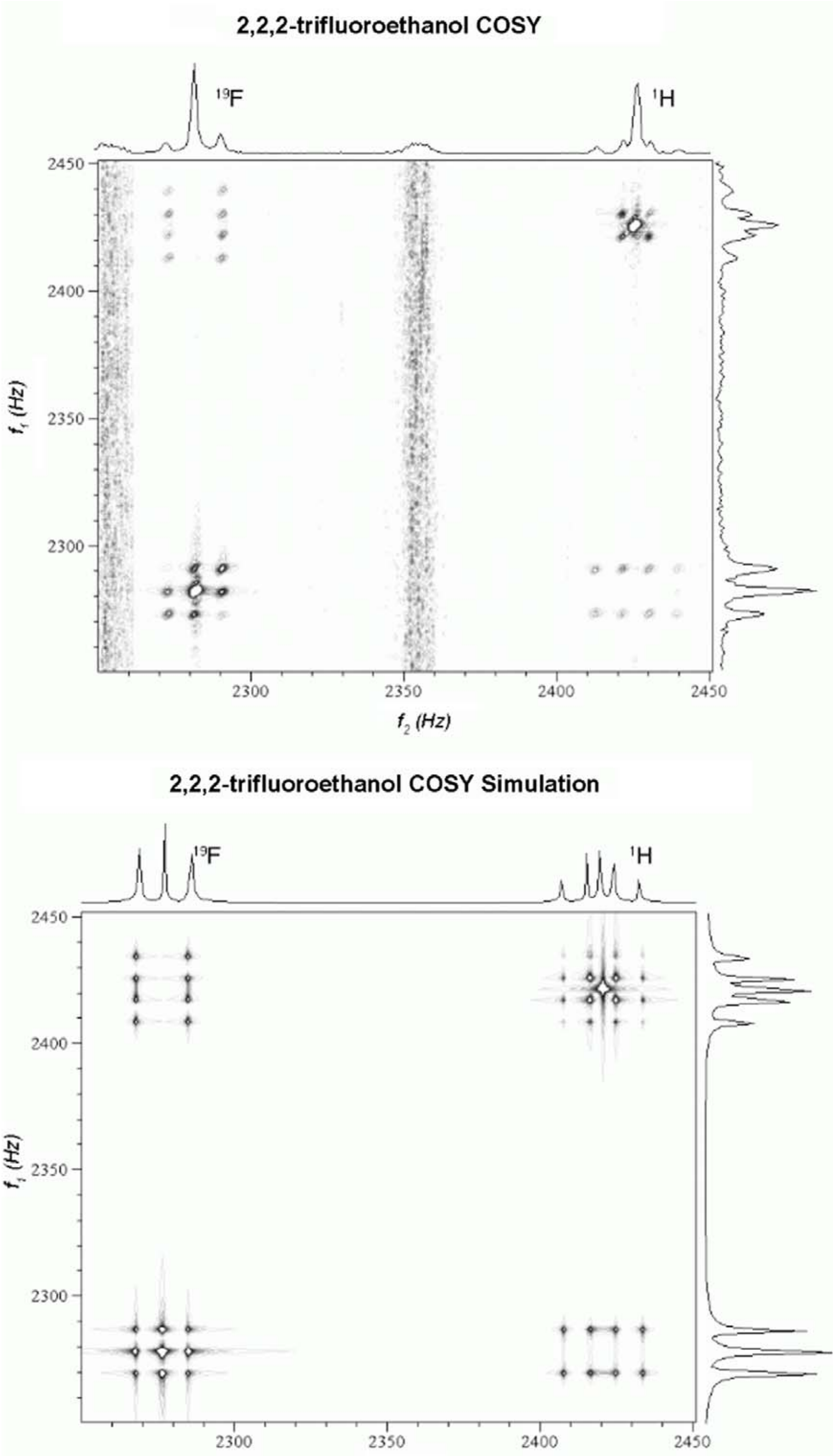

Fig. 4. Experimental and simulated 2D COSY NMR spectra for trifluoroethanol shown as contour plots. The projected f_1 and f_2 domain 1-D spectra are shown to the right and above each 2D plot, respectively. The experimental data are equivalent to that shown as a surface plot in Fig. 2.

quadrant, representing the complete 2D spectrum, is shown for each molecule as a surface plot in Fig. 2. The noise along the f_1 direction is residual electromagnetic interference, its 50 Hz harmonic spacing suggesting that it is largely caused by due to mains frequency interference. In the present instance, this noise is positioned so that it does not interfere with the spectra.

Figs. 3 and 4 shows more traditional contour plots of the experimental and simulated 2D COSY spectra for each molecule, as in Fig. 2, the higher frequency resonances arising from ^{1}H resonances and the lower from ^{19}F. The 1-D NMR spectrum is seen in the projections, while the off-diagonal features reveal which nuclei experience electron mediated *J*-couplings, a measure of molecular orbital connectedness and atomic proximity. In each case there is good agreement with the experimental and simulated spectra and the multiplet separations agree well with known values. Note in *para*-difluorobenzene the classic binomial 1:2:1 ^{1}H NMR multiplet and the 1:4:6:4:1 ^{19}F NMR multiplet, are seen, indicating a curious magnetic equivalence of protons, first observed in early high field NMR [11].

The quality of the COSY spectra obtained in these ~kHz experiments is comparable with those found in superconducting magnet NMR experiments at 10^{5} times higher frequencies (~100 MHz). Given the superior spectral resolution possible [6] in EFNMR experiments, where the magnetic field may be extraordinarily homogeneous, the facility for such multi-dimensional NMR spectroscopy potentially allows new insight regarding heteronuclear spin-coupling in organic- and bio-molecules, although we note the many practical limitations of working at such low fields. These include, *inter alia*, the need for large sample volumes and long measurement times. We do believe however, that with improved pre-polarisation methods, and thereby enhanced sensitivity, further improvements may be possible. Finally, we note, such "natural NMR" processes, including the remarkable coherence transfer phenomena observed here, can happen in oblivious organisms subject to environmental ULF pulses, for example from lightning "whistler modes".

Acknowledgment

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