

Extremely Low-Frequency Spectroscopy in Low-Field Nuclear Magnetic Resonance

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We demonstrate a new phenomenon in nuclear magnetic resonance spectroscopy, in which nuclear spin transitions are induced by radio frequency irradiation at extremely low frequencies (of the order of a few Hz). Slow Rabi oscillations are observed between spin states of different exchange symmetry. These “forbidden” transitions are rendered weakly allowed by differential electronic shielding effects on the radio frequency field. We generate coherence between the singlet and triplet states of ^{15}N -labeled nitrous oxide in solution, and estimate the scalar coupling between the two ^{15}N nuclei with a precision of a few mHz.

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In nuclear magnetic resonance, radio frequency irradiation resonant with the nuclear Zeeman splitting is used to induce nuclear spin transitions. In conventional high-field NMR, the resonant frequency is typically hundreds of MHz, while in NMR at very low field, such as NMR in Earth’s field [1,2], the resonant frequency may be a few kHz. Here, we demonstrate resonant nuclear spin effects at still lower frequencies. We show that electromagnetic irradiation in the extremely low-frequency (ELF) region of the radio frequency spectrum, of the order of a few Hz, can induce nuclear spin transitions between states of different spin exchange symmetry. The transitions are weakly allowed because of differential chemical shielding of the ELF field.

Our demonstration system is doubly ^{15}N -labeled nitrous oxide ($^{15}\text{N}_2\text{O}$, dinitrogen monoxide), which supports a low-field nuclear singlet state with a lifetime of tens of minutes in solution [3]. Nitrous oxide is nontoxic, dissolves in many important solvents such as water and oil, and is widely used in medicine [4] and food processing. The extraordinarily long singlet lifetime of $^{15}\text{N}_2\text{O}$ suggests applications to the characterization of slow diffusion and flow in medical and industrial materials [3]. The ELF spectroscopy of $^{15}\text{N}_2\text{O}$ creates further possibilities for the scientific applications of this substance.

The two ^{15}N nuclei of $^{15}\text{N}_2\text{O}$ are in chemically inequivalent sites, with a chemical shift difference of $\Delta\delta = 82.3$ ppm and a scalar spin-spin coupling $J_{NN} \approx 8$ Hz [3]. In zero magnetic field, the pair of ^{15}N nuclei become magnetically equivalent, with nuclear eigenstates given by the singlet state and the three triplet states, defined as follows:

$$\begin{aligned} |S_0\rangle &= \frac{1}{\sqrt{2}}(|\alpha\beta\rangle - |\beta\alpha\rangle) & |T_1\rangle &= |\alpha\alpha\rangle \\ |T_0\rangle &= \frac{1}{\sqrt{2}}(|\alpha\beta\rangle + |\beta\alpha\rangle) & |T_{-1}\rangle &= |\beta\beta\rangle. \end{aligned} \quad (1)$$

Spin-1/2 states with angular momentum $\pm(1/2)\hbar$ along

the z axis of the (arbitrarily defined) laboratory reference frame are denoted α and β , respectively. In zero magnetic field, the three degenerate triplet states are higher in energy than the singlet state by the singlet-triplet splitting $2\pi\hbar J_{NN}$. Perturbation of the singlet-triplet splitting is very small if the low-field chemical shift difference (in Hz) is less than the scalar spin-spin coupling, as is the case for the experiments described below.

The three triplet populations mutually interconvert with the conventional spin-lattice relaxation time constant T_1 . The singlet-triplet interconversion, on the other hand, is an order of magnitude slower, since many common relaxation mechanisms are ineffective [3,5–16]. In the case of $^{15}\text{N}_2\text{O}$, the time constant T_S for singlet-triplet interconversion may be as long as 26 minutes [3].

The singlet state is antisymmetric, and the triplet states are symmetric, with respect to exchange of the two nuclei. Singlet-triplet transitions are therefore symmetry-forbidden under any exchange-symmetric external perturbation. Conventional spin-dynamical theory assumes that the interaction of a spin system with an applied radio frequency field is exchange-symmetric, i.e., exactly the same for all resonant spins [17,18]. This assumption is plausible since each molecule is much smaller than the length scale of radio frequency field variations. However, this assumption neglects the shielding effects of the local molecular electrons on the applied radio frequency field. Chemical shift effects are very well known for the strong static magnetic fields used in conventional high-field NMR. However, the influence of electronic shielding on the applied radio frequency field has been consistently neglected. This is reasonable, since such effects are extremely small, as discussed below. Nevertheless, in the context of low-field NMR, the minute radio frequency chemical shift effects render singlet-triplet transitions weakly allowed.

Consider, for example, a weak magnetic field B_z along the z axis of the laboratory reference frame. The nuclear spin Hamiltonian describing the interaction of the 2-spin

system with the magnetic field is given by

$$H_z = -\gamma B_z \{ (1 + \delta_1) I_{1z} + (1 + \delta_2) I_{2z} \} \quad (2)$$

where the spin angular momentum operators are denoted I_1 and I_2 , the chemical shifts are δ_1 and δ_2 , and γ is the nuclear magnetogyric ratio. The off-diagonal matrix element connecting the singlet state and the central triplet state is given by

$$\langle S_0 | H_z | T_0 \rangle = -\frac{1}{2} \gamma \Delta \delta B_z \quad (3)$$

where $\Delta \delta = \delta_1 - \delta_2$. If the field B_z is periodically modulated so that one of its Fourier components is resonant with the singlet-triplet splitting, Rabi oscillations between the states $|S_0\rangle$ and $|T_0\rangle$ are induced. If the modulated magnetic field has the form $B_z(t) = B_{\text{ELF}} \cos(\omega_{\text{ELF}} t)$ where B_{ELF} is the peak ELF field amplitude, and the modulation frequency ω_{ELF} exactly matches the singlet-triplet splitting, the Rabi frequency for singlet-triplet nutation is given by

$$\omega_{\text{Rabi}}^{\text{ST}} = \left| \frac{1}{2} \gamma B_{\text{ELF}} \Delta \delta \right|. \quad (4)$$

This corresponds to the Larmor frequency in the ELF field, scaled by the minuscule chemical shift difference $\Delta \delta = 82.3$ ppm. For practical ELF fields, the singlet-triplet Rabi frequency is only a fraction of 1 Hz.

The ELF resonance condition is extremely narrow. Nevertheless, the resonance is readily observed since ordinary laboratory oscillators provide mHz frequency stability, and the energy difference between the singlet state $|S_0\rangle$ and the central triplet state $|T_0\rangle$ is highly insensitive to magnetic field variations.

The apparatus used to detect ELF singlet-triplet transitions is shown in Fig. 1. This is based on a conventional high-field NMR magnet ($B^0 = 7.05$ T) equipped with conventional radio frequency electronics, but with the facility of transporting the sample in and out of the magnet using a stepper motor attached to a piece of string, passing over a

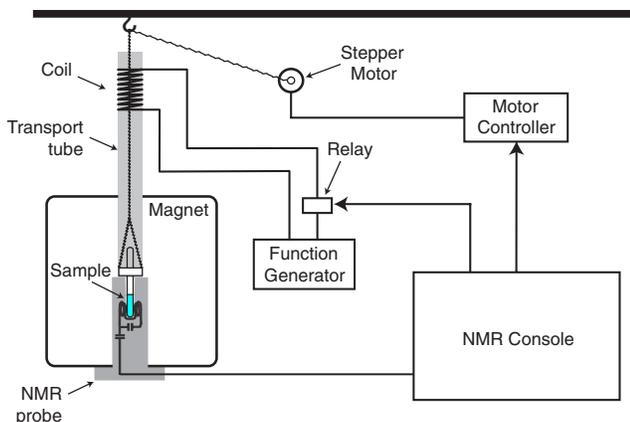


FIG. 1 (color online). Equipment used to perform ELF NMR experiments.

hook in the laboratory ceiling. At the top end of its trajectory, the sample enters a 300-turn copper solenoid, located about 1 m above the top of the NMR magnet, and with its axis parallel to the fringe field of the magnet. The peak field generated by the ELF coil was $B_{\text{ELF}} \approx 0.6$ mT. The ELF pulses were generated by gating the sinusoidal output of a commercial signal generator using a mechanical relay, controlled by the spectrometer software.

The sample used for all experiments consisted of $^{15}\text{N}_2\text{O}$ dissolved in locally produced Calabrian olive oil at a pressure of ~ 3 bar.

The timing sequences used for ELF experiments are shown in Fig. 2. Frames (a) and (b) indicate the timing sequences for a measurement of singlet relaxation time T_S , as described in Ref. [3]. The sample is allowed to equilibrate in high magnetic field for 200 seconds before a sequence of two 90° radio frequency pulses, resonant with the ^{15}N Larmor frequency, is applied. The pulse sequence timings and relative phases are chosen so as to invert the magnetization of ^{15}N nuclei at only one of the two chemically distinct sites. The sample is then winched out of the NMR magnet. As described in Ref. [3], this

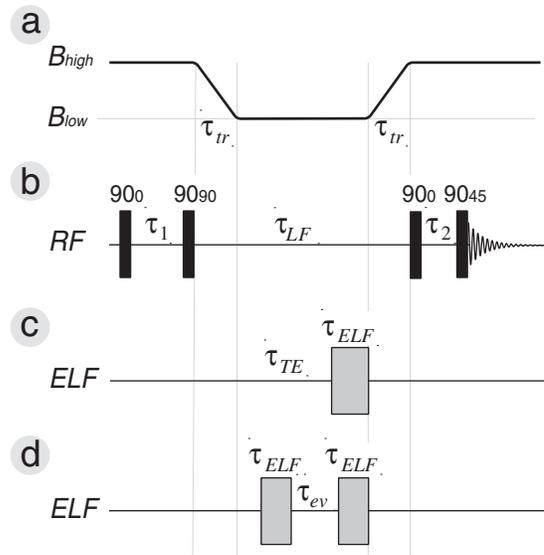


FIG. 2. Experimental timing sequences. (a) Magnetic fields, involving transport of the sample from the high magnetic field B_{high} to the low magnetic field B_{low} , and back again. The transport time is τ_{tr} . (b) Radio frequency fields, resonant with the ^{15}N nuclei in B_{high} . The pulse flip angles and rf phases are given in degrees. The time spent in B_{low} is τ_{LF} . (c) ELF pulse sequence for observing singlet-triplet nutation. After a time τ_{TE} to allow triplet equilibration, a resonant ELF pulse is applied of duration τ_{ELF} . (d) ELF pulse sequence for observing the precession of singlet-triplet coherence. Two ELF pulses, with the same phase, are separated by a variable evolution interval τ_{ev} . The ELF sequences are executed in parallel with sequences (a) and (b). The timing sequences are not to scale: the low-field pulses are about 4 orders of magnitude longer than the high-field ones.

causes a singlet-triplet population difference to be generated by adiabatic transport of the sample out of the magnetic field. The sample is left in low magnetic field for an interval τ_{LF} before the sample is let down into the magnet, a further two-pulse rf sequence is applied, and the NMR signal is detected. In the current case, measurement of the NMR signals for a set of different low-field intervals τ_{LF} provided the following estimation of the singlet relaxation time: $T_S = 1585 \pm 57$ s, consistent with earlier experiments [3].

The timing sequence in Fig. 2(c) demonstrates the phenomenon of ELF-induced singlet-triplet nutation. The sample is left in the low-field region for a “triplet equilibration interval” $\tau_{TE} = 60$ seconds. This allows the populations to redistribute within the triplet manifold, while maintaining the singlet-triplet population difference [3]. This protocol generates a more reproducible starting point for the singlet-triplet nutation procedure, as will be described in detail elsewhere.

Singlet-triplet nutation is observed by applying an ELF pulse of duration τ_{ELF} , with a frequency matching the singlet-triplet splitting. The sample is transported back into the high-field region, and the NMR signal generated by a further sequence of two 90° pulses. Fourier transformation of the signal generates the NMR spectrum. The experiment is repeated for a set of different pulse durations τ_{ELF} . The amplitudes of two high-field NMR peaks are plotted as a function of the ELF pulse duration τ_{ELF} in Fig. 3(a).

This plot shows clear Rabi oscillations indicating the coherent driving of the singlet-triplet transitions by the extremely low-frequency field. The Rabi oscillations induced by the ELF pulse are extraordinarily slow: The singlet-triplet Rabi frequency is of the order of $\omega_{Rabi}^{ST}/2\pi = 116$ mHz, corresponding to a 90° singlet-triplet pulse of duration 2.15 seconds. This is consistent with the known chemical shift difference $\Delta\delta = 82.3$ ppm and a peak ELF field of 0.65 mT. The asymmetric pattern of peak amplitudes is consistent with spin-dynamical theory, as will be described elsewhere.

In principle, singlet-triplet nutation could also be induced by an unmodulated magnetic field pulse, or a sudden magnetic field step [19]. However, the field changes required in those methods are much stronger and require specialized hardware.

A resonant ELF pulse induces a rotation in the singlet-triplet subspace through the singlet-triplet flip angle defined $\beta_{ST} = \omega_{Rabi}^{ST}\tau_{ELF}$. For example, a 180° ELF pulse, for which $\beta_{ST} = \pi$, interchanges the populations of $|S_0\rangle$ and $|T_0\rangle$. A 90° ELF pulse, for which $\beta_{ST} = \pi/2$, transforms the singlet state $|S_0\rangle$ into a superposition of $|S_0\rangle$ and $|T_0\rangle$. If a 90° ELF pulse is applied to a spin ensemble displaying a singlet-triplet population difference, a coherent superposition of singlet and triplet states is induced. This is called *singlet-triplet coherence*.

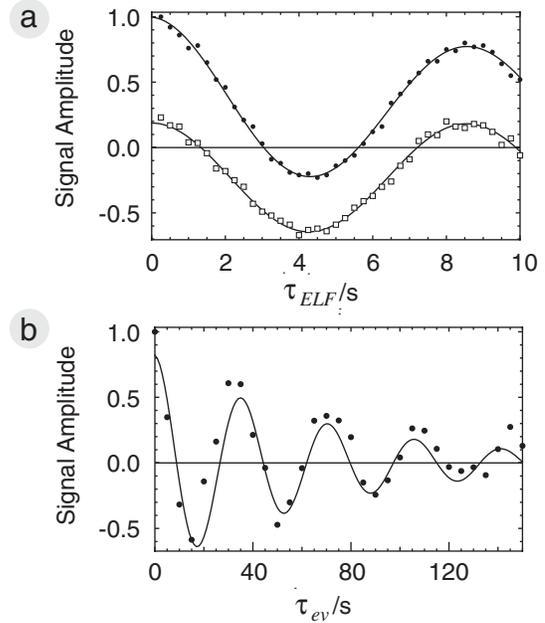


FIG. 3. Experimental peak amplitudes plotted against the timing intervals of ELF pulse experiments. (a) Singlet-triplet nutation experiment, varying the duration τ_{ELF} of the ELF pulse in Fig. 2(c). (b) Singlet-triplet coherence evolution experiment, varying the duration τ_{ev} of the evolution interval in Fig. 2(d), with the two ELF pulse durations fixed to $\tau_{ELF} = 2.15$ s. Black circles and white boxes refer to the amplitudes of the two components of the high-field doublet in the $^{15}\text{N}_2\text{O}$ spectrum. The solid lines are best fits to exponentially decaying cosine functions with an added offset from zero. Experiments were performed on a sample of $^{15}\text{N}_2\text{O}$ dissolved in locally produced Calabrian olive oil using the following timings: $\tau_1 = 198 \mu\text{s}$, $\tau_2 = 99 \mu\text{s}$, $\tau_{tr} = 10$ s, and $\tau_{TE} = 60$ s. The ELF irradiation frequency was $\omega_{ELF}/2\pi = 8.693$ Hz in all cases.

Figure 2(d) shows the procedure for studying the free evolution of singlet-triplet coherence. Two 90° ELF pulses are separated by a variable evolution interval τ_{ev} . Incrementation of the evolution interval τ_{ev} generates prominent oscillations in the NMR signal, as shown in Fig. 3(b). These oscillations may be fitted to a damped sinusoidal oscillation with frequency 50.8 ± 0.3 mHz.

Since the ELF pulses are phase-coherent at the ELF carrier frequency, the observed oscillation frequency is given by $|\omega_{ST} - \omega_{ELF}|$, where ω_{ST} is the singlet-triplet splitting in angular frequency units, and ω_{ELF} is the angular frequency of the ELF oscillator. A second experiment, with a slightly different ELF frequency, was performed in order to determine whether the singlet-triplet frequency was larger or smaller than the ELF carrier frequency. In this way, it was possible to determine the singlet-triplet splitting to be $\omega_{ST}/2\pi = 8.695 \pm 0.001$ Hz in angular frequency units. The decay time constant for the singlet-triplet coherence was determined to be $T_2^{ST} = 77 \pm 10$ s.

In zero magnetic field, the singlet-triplet splitting, expressed in frequency units, corresponds exactly to the spin-

spin coupling, i.e., $\omega_{ST} = 2\pi J_{NN}$. However, in our experiments, the fringe field of the NMR magnet at the center of the ELF coil was estimated to be $B_{low} \approx 2.0 \pm 0.1$ mT by using a Hall effect sensor. This finite ambient field leads to small second-order chemical shift effects. The singlet-triplet splitting in this regime is given by

$$\omega_{ST} \approx 2\pi J_{NN} + \frac{(\gamma B_{low} \Delta \delta)^2}{4\pi J_{NN}}. \quad (5)$$

When this small shift is taken into account, the J coupling between the two ^{15}N nuclei was determined to be $J_{NN} = 8.665 \pm 0.004$ Hz, where the error margin takes into account the uncertainty in the chemical shift values, the magnetic field inside the ELF coil, and the measured singlet-triplet splitting.

The determination of a nuclear spin-spin coupling with such accuracy is possible because of the following factors: (1) Magnetic field inhomogeneity, which plays a strong role in conventional high-field NMR, has almost no effect on the singlet-triplet splitting; (2) The decay of the singlet-triplet coherence is very slow, in part because of the long singlet lifetime; (3) Even the diffusion of the molecules into sample regions that experience different magnetic fields has a negligible effect.

We have used singlet-triplet coherence to investigate whether J_{NN} depends on the solvent in which $^{15}\text{N}_2\text{O}$ is dissolved. Important components of olive oil include the triglyceride esters of oleic acid (9Z-octadecenoic acid) and linoleic acid (9Z, 12Z-octadecadienoic acid). We dissolved $^{15}\text{N}_2\text{O}$ in separate samples of oleic and linoleic acid and measured the singlet-triplet oscillation frequency in both cases. The J_{NN} couplings between the ^{15}N nuclei were found to be 8.727 ± 0.004 Hz and 8.698 ± 0.004 Hz for oleic acid and linoleic acid solutions, respectively. These small but significant differences suggest that the accurate measurement of J couplings in low magnetic field can convey information on the chemical environment of the nitrous oxide.

The extremely long-lived singlet of $^{15}\text{N}_2\text{O}$ should allow hyperpolarized nuclear spin order, such as that generated by dynamic nuclear polarization (DNP) [20], to be transported through the sample with relatively little loss of order. The experiments described here shows that this use of the singlet may be combined with singlet-triplet interconversion by applying resonant ELF fields of relatively low amplitude. Since the triplet states are magnetic, they respond to applied magnetic field gradients, and may therefore be used for spatial characterization.

In summary, we have demonstrated a new form of nuclear magnetic resonance spectroscopy, involving irradiation in the extremely low-frequency (ELF) region of the electromagnetic spectrum. The ELF pulses induce extremely slow Rabi oscillations between states of different spin exchange symmetry. In the case of ^{15}N -labeled nitrous oxide, the ELF pulses induce singlet-triplet population

exchange, and excite singlet-triplet spin coherence, which oscillates at a characteristic and extremely well-defined frequency. Furthermore, the ELF pulses may be used to exchange spin order at will between magnetic triplet states with conventional relaxation properties, and nonmagnetic singlet states with exceptionally long lifetimes. The phenomenon is not restricted to ^{15}N -labeled nitrous oxide and should be observable for many coupled nuclear spin species in low magnetic field.

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