Long-Lived Heteronuclear Spin-Singlet States in Liquids at a Zero Magnetic field

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We report an observation of long-lived spin-singlet states in a ^{13}C-^{1}H spin pair in a zero magnetic field. In ^{13}C-labeled formic acid, we observe spin-singlet lifetimes as long as 37 s, about a factor of 3 longer than the T_1 lifetime of dipole polarization in the triplet state. In contrast to common high-field experiments, the observed coherence is a singlet-triplet coherence with a lifetime T_2 longer than the T_1 lifetime of dipole polarization in the triplet manifold. Moreover, we demonstrate that heteronuclear singlet states formed between a ^{1}H and a ^{13}C nucleus can exhibit longer lifetimes than the respective triplet states even in the presence of additional spins that couple to the spin pair of interest. Although long-lived homonuclear spin-singlet states have been extensively studied, this is the first experimental observation of analogous singlet states in heteronuclear spin pairs.

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Long-lived spin-singlet states have recently attracted considerable attention both experimentally [1–11] and theoretically [12–16]. Such states are of practical interest for their ability to store hyperpolarization for more than an order of magnitude longer than T_1 [9,10] and for their utility in tracking slow chemical and biological processes [3,5]. Long-lived spin states offer a wide range of applications in medicine, materials science, physiology, and chemistry, such as targeting molecules relevant to neuroscience [17], monitoring of protein conformation changes [18], and probing of slow diffusion of biomolecules [19]. For recent reviews of long-lived states in nuclear magnetic resonance (NMR), see Refs. [16,20].

In the case where two coupled spins are magnetically equivalent, the singlet state is protected from intramolecular dipole-dipole relaxation, often the dominant source of relaxation in NMR. This is because the dipole-dipole interaction is symmetric with respect to the exchange of particles and therefore cannot produce transitions between the antisymmetric singlet and symmetric triplet states. Other sources of relaxation, such as chemical-shift anisotropy, spin rotation, and effects from paramagnetic impurities are also often suppressed for the singlet compared to the triplet states. As a result, singlet lifetimes can significantly exceed triplet lifetimes. The most dramatic case of a long-lived nuclear spin-singlet state is a parahydrogen, which has a lifetime of weeks, compared to the T_1 lifetime of an orthohydrogen, which is on the order of seconds under typical experimental conditions (6 bar hydrogen pressure at room temperature in the absence of paramagnetic impurities).

In high-field NMR such singlet states must be composed of same-species (homonuclear) spin pairs, since the magnetic field breaks the equivalence of heteronuclear spin pairs. Here we demonstrate the existence of heteronuclear spin singlets at a zero magnetic field; the spin singlet in question is formed by a strongly coupled ^{13}C-^{1}H pair in ^{13}C-labeled formic acid (^{13}CHOOH). Note, the hydroxyl proton can be ignored because two- and three-bond couplings between the ^{13}C-^{1}H system and the acidic proton can be neglected due to rapid exchange in protic solvents. We show that the lifetime of this spin singlet, T_s, can exceed the relaxation time of the triplet-state dipole moment, T_1, by a factor of 3. Furthermore, the transverse relaxation time of the singlet-triplet coherence, T_2, exceeds the longitudinal relaxation lifetime, an unusual situation in NMR. Zero-field NMR experiments detect transitions between a triplet state and a singlet state that is protected from relaxation mechanisms, whereas in high-field NMR the observed transitions generally are between two states equally exposed to all relaxation mechanisms. We also show that the extended singlet-state lifetimes are not limited to isolated heteronuclear pairs. In singly labeled benzene ^{13}C$_1$, with a strongly coupled heteronuclear spin pair weakly coupled to several distant spins, we show that the lifetime of manifolds where the strongly coupled spins are in the singlet state exceeds the lifetime of manifolds where the strongly coupled spins are in the triplet states by about a factor of 1.5. An appealing application for long-lived heteronuclear spin singlets may be in metabolic studies, along the lines of research employing hyperpolarized ^{13}C-labeled pyruvate [21].

In the present experiments, the samples are polarized in a permanent magnet and then pneumatically shuttled to the zero field immediately next to the detection cell. dc magnetic-field pulses are used to manipulate the polarization in the singlet and triplet manifolds. This is in contrast to the case of low-field homonuclear singlet spin pairs [2], which require more elaborate multipulse sequences for the interconversion of singlet and triplet states. In the experiments we present here, the phase of the resulting signals can be used to identify the type of nuclear spin polarization from which the signal arises. The use of a sensitive alkali-vapor magnetometer as a detector allows us to directly probe the resulting spin state.

In liquids, as examined here, inter- and intramolecular dipolar interactions average to zero due to fast Brownian motion; hence, the Hamiltonian for two spins, $\mathbf{I}$ and $\mathbf{S}$, in the presence of the Zeeman interaction and scalar coupling is well approximated by

$$H = J\mathbf{I}\cdot\mathbf{S} + (\gamma_I\mathbf{I} + \gamma_S\mathbf{S})\cdot\mathbf{B}. \quad (1)$$

Here, γ_I and γ_S are the gyromagnetic ratios of the respective spins, and J is the scalar coupling between them. In the high-field limit, the eigenstates are those of I_z and S_z, $|M_I M_S\rangle$. In the zero field, the eigenstates are those of F^2 and F_z, where $\mathbf{F} = \mathbf{I} + \mathbf{S}$ is the total angular momentum. We denote the triplet states with $F = 1$ as $|T_{+1,0,-1}\rangle$, and the singlet state with $F = 0$ as $|S_0\rangle$, where the subscript indicates the magnetic quantum number, M_F. The manifolds are separated in energy by J. In the zero field, the triplets are symmetric and the singlet is antisymmetric with respect to the interchange of the spin labels. Therefore, transitions between the two manifolds due to the symmetric dipole-dipole interaction are forbidden. As the magnetic field is increased, the $|T_0\rangle$ and $|S_0\rangle$ levels are mixed and dipole-dipole relaxation is gradually turned on.

The energy levels of this two-spin system are shown as a function of the magnetic field in Fig. 1. Polarization of the zero-field levels is achieved in our experiment by transferring the sample from thermal equilibrium in a prepolarizing magnet (20 kG) through a guiding field (a solenoid generating a magnetic field of 10 mG) into the shielded magnetometer. The rate of transit (occurring over 1.5 s) is slow compared to J; hence, populations of the thermally polarized high-field states are adiabatically transferred to those of the low-field states. This results in an excess population of the $|S_0\rangle$ state over the $|T_0\rangle$ state, and an excess of the $|T_{+1}\rangle$ state over $|T_{-1}\rangle$. The latter corresponds to the dipole moment of the triplet state oriented in the direction of the quantization axis (z). The observable is the magnetization along z, which is also the direction from the detector to the sample. The axis of the adiabatic-transfer solenoid is also collinear with z. In order to avoid evolution in unknown residual fields, the guiding field stayed switched on during storage, producing a field of ~1 mG

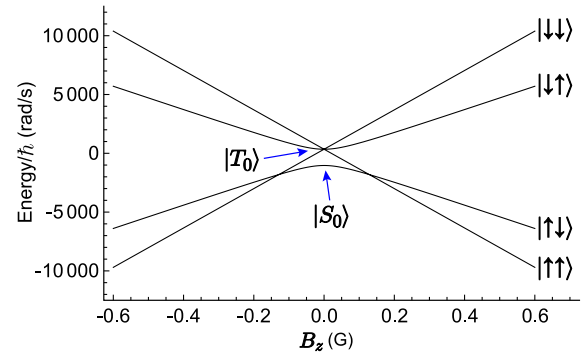

FIG. 1 (color online). Energy levels for two spins, $\mathbf{I}$ and $\mathbf{S}$, $I = S = 1/2$, in the presence of a scalar J coupling as a function of magnetic field B_z. Here, $J = 220$ Hz, and gyromagnetic ratios correspond to carbon-13 and hydrogen. High-field eigenstates are those of the uncoupled basis, as indicated by the kets on the right. At zero-field eigenstates are the singlet and triplet states, as indicated in the center.

at the sample. At this field, the difference of spin-precession frequencies of ^{1}H and ^{13}C nuclei is 3.2 Hz. This is sufficiently small compared to the ^{1}H-^{13}C J coupling of 219.3 Hz that the singlet state remains very close to an eigenstate of the Hamiltonian and does not evolve into states unprotected from relaxation. Simultaneously, this residual field prevents uncontrolled precession of the stored triplet polarization. Both the singlet lifetime T_s as well as the dipole lifetime T_1 stay unaffected, as was verified experimentally by taking data at different values of the guiding field. After the adiabatic transfer from the polarizing field to this very low field, a certain storage time τ elapses to allow for relaxation. Subsequently, the guiding field is turned off and the states are probed. More details of polarization via adiabatic transfer can be found in the Supplemental Material [22].

Once the guiding field is turned off, dc magnetic-field pulses are used to excite a coherence between zero-field substates and to manipulate the polarization in the singlet and triplet manifolds. The use of a sensitive alkali-vapor magnetometer (described in [23]) allows us to directly probe the resulting spin state. We observe the z component of the nuclear magnetization, proportional to the trace of $\rho(\gamma_I I_z + \gamma_S S_z)$. Since the term in parentheses is a vector operator with a magnetic quantum number equal to zero, observable transitions are those with $\Delta F = \pm 1$ and $\Delta M_F = 0$. These are transitions between the $|T_0\rangle$ and $|S_0\rangle$ states, which produce magnetization oscillating in the z direction with frequency J.

Coherences between $|T_0\rangle$ and $|S_0\rangle$ can be established from the excess population in the singlet state by the application of a dc pulse of the magnetic field in the z direction, resulting in z magnetization proportional to $\sin(2\pi J t)$. This corresponds to a dispersive peak in the real part of the Fourier transform of the magnetometer signal. The amplitude of the signal in this case is

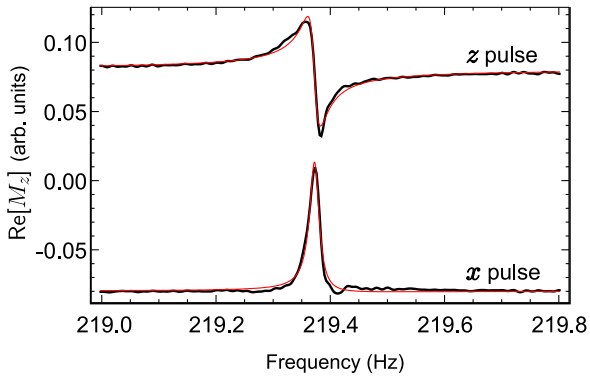

FIG. 2 (color online). Zero-field NMR signals of formic acid (sample FAIII). The real part of the signal following a pulse in the z direction originates from an initial population imbalance between the $|S_0\rangle$ and $|T_0\rangle$ states and is dispersive. The real part of the signal due to a pulse in the x direction originates from a dipole moment in the triplet manifold (excess of $|T_{+1}\rangle$ over $|T_{-1}\rangle$ states), and is absorptive. The curves have been vertically offset by $+0.075$ (z pulse) and -0.075 (x pulse) to avoid overlap.

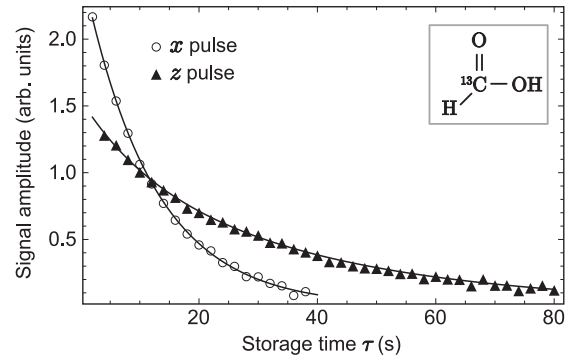

FIG. 3. Decay of signal amplitude as a function of storage time for magnetic-field pulses applied in the indicated directions using sample FAIII. The solid lines overlaying the data are fits to single (double) exponentials for x (z) magnetic-field pulses.

proportional to the difference in population between the $|T_0\rangle$ and $|S_0\rangle$ states. Coherences between $|T_0\rangle$ and $|S_0\rangle$ can also be established from dipole polarization in the triplet state by the application of a pulse of the magnetic field in the x direction, resulting in z magnetization proportional to $\cos(2\pi Jt)$, corresponding to an absorptive peak in the real part of the Fourier transform. In this case, the amplitude is proportional to the difference in population between the $|T_{+1}\rangle$ and $|T_{-1}\rangle$ states. Note that a y pulse would have the same effect as an x pulse; they are both transverse to the privileged z direction. Details of how coherences are produced from the different polarization moments and the resulting signals are presented in the Supplemental Material [22].

Figure 2 shows the real part of the Fourier transform of the magnetometer signal following adiabatic transition of the formic acid sample (FAIII) to zero field and subsequent application of magnetic-field pulse in either the x or the z directions. The phase is in agreement with the discussion above, with absorptive and dispersive line shapes for x and z pulses, respectively. Overlying each trace is the real part of a fit to a complex Lorentzian, with HWHM linewidth equal to about 10 mHz, corresponding to coherence lifetime $T_2 = 16$ s. In both cases there is a small deviation from Lorentzian line shape. We attribute this to variations in J across the sample due to temperature gradients, as discussed further in the Supplemental Material [22].

The amplitude of the zero-field NMR signal as a function of the storage time τ for the application of magnetic-field pulses in the x or z directions is shown in Fig. 3 for sample FAIII. The decay of the signal amplitude obtained by the application of x pulses is well described by a single decaying exponential, with time constant $T_1 = 11.8(1)$ s. We note that our definition of T_1 deviates slightly from the high-field case, which usually corresponds to the lifetime of the population difference between single-particle Zeeman eigenstates. Here, T_1 is the lifetime of the difference in population between the triplet states $|T_{+1}\rangle$ and $|T_{-1}\rangle$ in the coupled system. The singlet lifetime T_s was determined by fitting the decay curve (Fig. 3) of the signal amplitude after the application of a z pulse. The decay is well described by a double exponential with fast and slow time constants $T_f = 10(3)$ and $T_s = 37(2)$ s. The slow decay is due to transitions between the singlet and triplet states and reflects the lifetime of the singlet state T_s. The fast decay is a result of equilibration within the triplet states, which reduces the population imbalance between the $|T_0\rangle$ and $|S_0\rangle$ states. Single and biexponential decays for the relaxation of dipole and singlet polarization are predicted by a phenomenological rate-equation model, described in the Supplemental Material [22].

Formic acid samples were mixtures of ^{13}C-labeled formic acid, acetonitrile, and H_2O. The samples were flame sealed in 5-mm NMR tubes after four or five cycles of freezing and thawing under vacuum in order to remove dissolved gases, in particular oxygen, which can cause additional relaxation.

The main results for three different samples (containing formic acid, acetonitrile, and water) are presented in Table I. Dilution of formic acid with acetonitrile and water mixtures was performed to reduce intermolecular interactions and to promote fast exchange of the hydroxyl proton.

As can be seen in Table I, an increased fractional content of acetonitrile increases the singlet lifetime, likely due to a reduction in viscosity and decreased correlation times. It is interesting to note that the lifetime of the singlet-triplet coherence is longer than the T_1 relaxation time for all samples listed in Table I. This is a situation rarely encountered in high-field NMR because T_1 relaxation in high field corresponds to an exchange of populations between two Zeeman eigenstates, and T_2 relaxation corresponds to the dephasing of a coherence between the

TABLE I. Summary of contents (in $\mu\mathbf{L}$) and decay times T_1, T_f, T_s, and T_2 for several samples. FA = formic acid, A = acetonitrile.

Sample and content (FA, A, H_2O)	T_1(s)	T_f(s)	T_s(s)	T_2(s)
FAI (100,0,10)	5.8(1)	2.4(3)	18.4(3)	8.0(5)
FAII (50,100,10)	8.0(5)	8.84(7)	26.5(5)	10.3(3)
FAIII (5,50,5)	11.8(1)	10(3)	37(2)	16.2(4)

Zeeman eigenstates. In the zero-field case at hand, the T_1 relaxation of the dipole moment corresponds to the relaxation of population differences between the states $|T_{\pm 1}\rangle$, whereas the observed singlet-triplet coherence involves the states $|T_0\rangle$ and $|S_0\rangle$.

The theory of nuclear spin relaxation in two-spin systems has been studied extensively for the case of homonuclear spins in low magnetic fields [12–14,16] and can be found in Ref. [12]. These formulas can be adapted to heteronuclear systems. Rate expressions for relaxation due to dipole-dipole interactions or external fluctuating fields can be found in Ref. [12]. In the case of dipole-dipole relaxation, the ratio of T_2 to T_1 should be $T_2/T_1 = 9/5$. If externally fluctuating fields due to paramagnetic impurities or other nuclei (for example, the hydroxyl proton) limit the lifetime, we expect a ratio $T_2/T_1 = 2$, assuming that the fields are uncorrelated at the two nuclei. We measure T_2/T_1 to be 1.28–1.37, which indicates that T_2 may be limited by temperature or magnetic-field inhomogeneities.

We suspect that the exchange of the hydroxyl proton, or possibly some paramagnetic impurity, ultimately limits the lifetime of the singlet state. This is supported by the observation that all lifetimes have a roughly linear dependence on temperature. In sample FAII, T_s decreases by about 10% as the temperature is increased in the range of 20–60°C, while T_f, the fast component of the relaxation, increases by a similar fractional amount. We observe that T_1 increases by about 10% over this range, consistent with the expectation that dipole-dipole relaxation is suppressed by improved motional narrowing at elevated temperatures.

The increased lifetime of the singlet state is not strictly limited to the case of two-spin systems [15,24,25]. In systems where there are two strongly coupled spins and a series of weaker couplings, we also observe an extension in the lifetime of the manifolds where the strongly coupled system is in the spin-singlet state. Singly labeled ^{13}C benzene is one such example, in which there is a strongly coupled ^{1}H-^{13}C system, weakly coupled to a set of distant spins, resulting in the splitting of the single zero-field NMR line. A portion of the zero-field spectrum of singly labeled ^{13}C benzene in the neighborhood of the one-bond J-coupling frequency, acquired after the application of an x directed pulse, is shown in the inset in Fig. 4. A

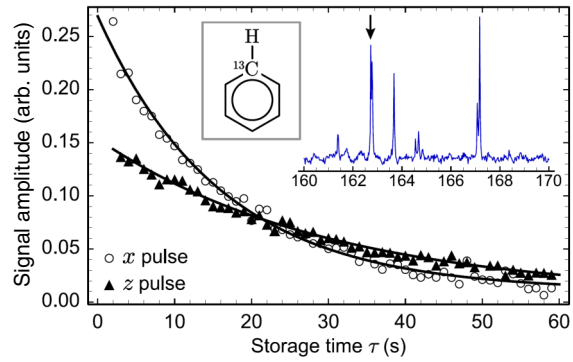

FIG. 4 (color online). Decay of signal amplitude in benzene $^{13}C_1$ for magnetic-field pulses applied in the x (circles) and z (triangles) directions (this spectrum is the result of averaging five transients). The solid lines overlaying the data are fits to single decaying exponentials. The inset shows a portion of the zero-field spectrum, and the arrow indicates the peak from which these decay curves are extracted.

complete discussion of the zero-field spectrum resulting from ^{13}C-labeled benzene is presented in Ref. [26]. Individual lines are typically about 10 mHz (HWHM). The signal is absorptive as expected from dipole polarization of the triplet manifolds. Application of a pulse in the z direction produces a signal with the same frequencies and with dispersive line shape, as expected from polarization of the singlet state. The decay of the signal amplitude (for the peak indicated by the arrow in the inset) is given in the main panel for x pulses (corresponding to triplet polarization) and z pulses (corresponding to singlet polarization). While multiple decay rates are likely, our signal-to-noise ratio is not sufficient to cleanly differentiate between them, so we fit these data to single decaying exponentials, as indicated by the solid lines. The decay times extracted from the fit are $T_1 = 19(1)$ s and $T_s = 30(2)$ s, for pulses applied in the x and z directions, respectively. Decay curves for the other peaks yield similar lifetimes. These results confirm that the long lifetime of heteronuclear spin-singlet states is not limited to the case of two isolated heteronuclear spins, but can be extended to systems consisting of two strongly coupled heteronuclear spins with weakly coupled distant spins.

In conclusion, we have shown that the lifetime of heteronuclear spin-singlet states in a zero magnetic field can be substantially longer than that of the dipole moment in the triplet state. In ^{13}C-labeled formic acid, we find that the singlet lifetime is a factor of 3 longer than the lifetime of the triplet dipole moment. The extended lifetime of singlet states in homonuclear spin pairs has been known for some time, but the extended lifetime of heteronuclear spin singlets has not been observed before. One advantage of working with heteronuclear spin singlets is that they can be manipulated by dc magnetic-field pulses to produce observable magnetization. The use of sensitive atomic magnetometers, as employed here, enables direct observation of this

magnetization in zero field. Typically, singlet states are not eigenstates of the high-field Zeeman Hamiltonian, and if a high degree of symmetry is not present in the molecule of choice, RF spin locking has to be implemented in order to remove chemical shifts. At a zero magnetic field, heteronuclear as well as homonuclear spin systems are naturally coupled into long-lived eigenstates. This vastly expands the range of chemical systems which can exhibit long-lived spin order, motivating further research in zero-field NMR as a valuable spectroscopic tool.

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