

Chemical Analysis of an Isotopically Labeled Molecule Using Two-Dimensional NMR Spectroscopy at 34 μ T

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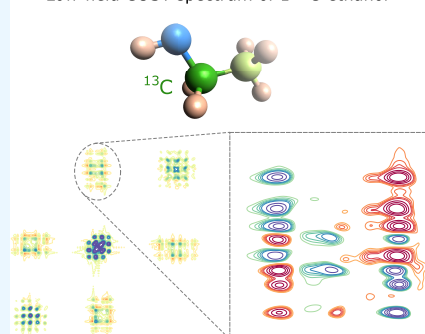
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ABSTRACT: Low-field nuclear magnetic resonance (NMR) spectroscopy, conducted at or below a few millitesla, provides only limited spectral information due to its inability to resolve chemical shifts. Thus, chemical analysis based on this technique remains challenging. One potential solution to overcome this limitation is the use of isotopically labeled molecules. However, such compounds, particularly their use in two-dimensional (2D) NMR techniques, have rarely been studied. This study presents the results of both experimental and simulated correlation spectroscopy (COSY) on 1- ^{13}C -ethanol at 34.38 μT . The strong heteronuclear coupling in this molecule breaks the magnetic equivalence, causing all J -couplings, including homonuclear coupling, to split the ^1H spectrum. The obtained COSY spectrum clearly shows the spectral details. Furthermore, we observed that homonuclear coupling between ^1H spins generated cross-peaks only when the associated ^1H spins were coupled to identical ^{13}C spin states. Our findings demonstrate that a low-field 2D spectrum, even with a moderate spectral line width, can reveal the J -coupling networks of isotopically labeled molecules.

Low-field COSY spectrum of 1- ^{13}C -ethanol



INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is a useful analytical tool in several areas of science and medicine.¹ High-field NMR spectroscopy can determine the structure of molecules, even at atomic resolution, with high sensitivity and spectral resolution.^{2–4} Establishing a framework of electron-mediated indirect nuclear spin–spin interaction (J -coupling) is essential, and a variety of two-dimensional (2D) spectroscopies can be readily conducted to this end.^{5,6}

Low-field NMR spectroscopy using a static magnetic field at or below a few millitesla is considered an alternative to low-cost and/or portable solutions.^{7–9} With a resonance frequency below tens of kilohertz, it is insensitive to inhomogeneity in magnetic susceptibility.¹⁰ In addition, the heteronuclear J -coupling constant can be measured with high precision,^{11,12} and efficient hyperpolarization using breakthrough techniques is feasible, resulting in significant enhancement of sensitivity.^{13–19} Despite such benefits, however, fundamental limitations remain: a lack of chemical shift resolution and the invisibility of homonuclear J -coupling have been major hurdles to the widespread use of low-field NMR spectroscopy for chemical analysis. One solution, although limited, is the use of isotopically labeled molecules such as 1- ^{13}C -ethanol ($\text{CH}_3^{13}\text{CH}_2\text{OH}$), as shown in Figure 1a.^{12,20–22} The labeled nuclear spin lifts the degeneracy in the resonance frequency through a strong J -coupling effect. The one-dimensional (1D) NMR spectrum, then, becomes highly complicated but reflects all J -couplings, including homonuclear coupling, which is

otherwise invisible in low magnetic fields.^{11,12,20,23–25} Given the position of the labeled nucleus within the molecule, structural analysis using low-field NMR spectroscopy can be feasible. There is no doubt that, in high fields, 2D spectroscopy provides more informative results by efficiently visualizing spin networks through better-resolved signals. However, in zero to ultralow fields (ZULF), which is typically below microtesla fields, 2D spectroscopy can reveal cross-peaks only between the transitions belonging to the same spin-manifold.^{26–28} Although the ZULF 2D spectrum can serve as a fingerprint to discriminate molecules, extracting structural information from it requires peak-assignment processes involving a heavy load of numerical iterations. We conducted experiments on $^{13}\text{CH}_3\text{OH}$ at 5 μT .²⁶ At this magnetic field, the spin system is situated within the strongly coupled regime, where the resonance frequency is comparable to the strength of the J -coupling. Consequently, 2D spectroscopy at 5 μT leads to a completely nontrivial pattern with $^{13}\text{CH}_3\text{OH}$. On the other hand, in magnetic fields ranging from the Earth's magnetic field (~ 50 μT) to a few millitesla, different nuclei can be discriminated by corresponding Larmor frequencies, and the frequency difference

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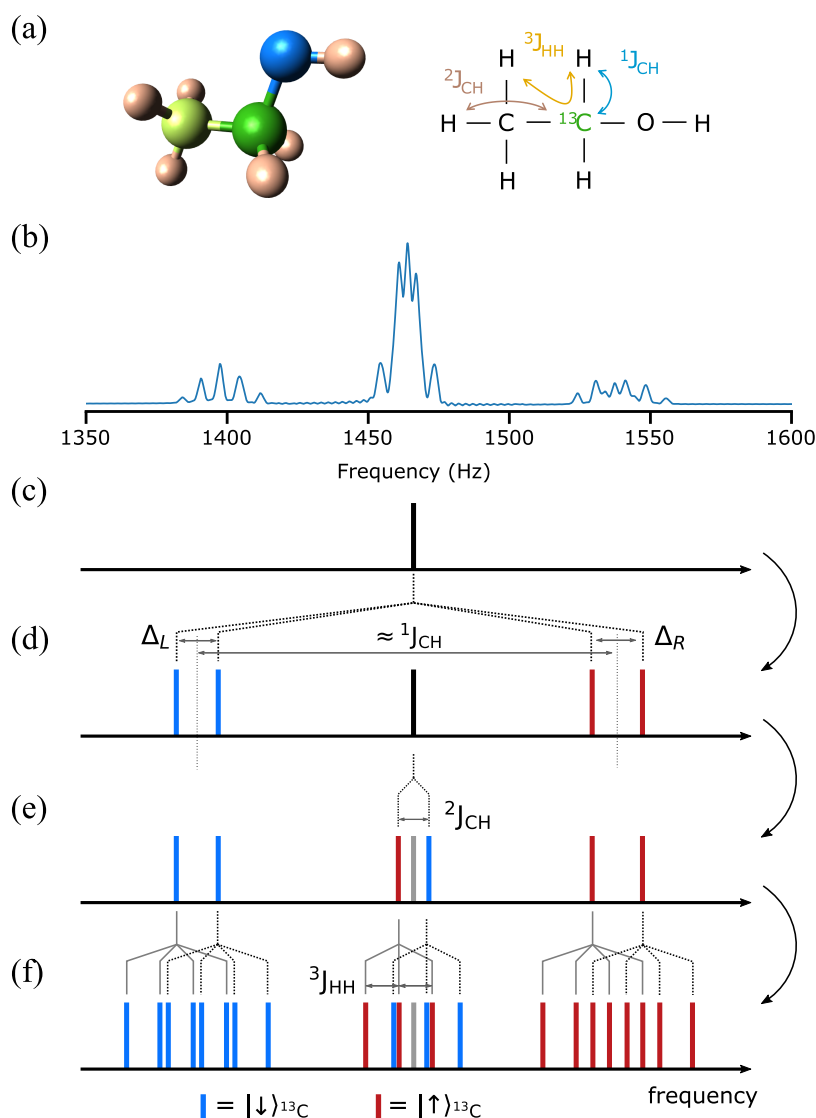


Figure 1. (a) Molecular and J -coupling structures of $\text{CH}_3^{13}\text{CH}_2\text{OH}$. (b) Simulated ^1H NMR spectrum at $34.38\ \mu\text{T}$. J -coupling splitting pattern with (c) no J -couplings, (d) only $^1J_{\text{CH}}$, (e) $^1J_{\text{CH}}$ and $^2J_{\text{CH}}$, and (f) $^1J_{\text{CH}}$, $^2J_{\text{CH}}$, and $^3J_{\text{HH}}$. The red and blue colors represent the associated ^{13}C nuclear up- and down-spin states, respectively. The gray lines represent the uncoupled ^1H spins of the OH group (the peak intensities are normalized, not to scale).

is larger than the J -coupling constants. This property may facilitate chemical analysis analogous to that in high fields. While a number of low-field correlation spectroscopy (COSY) studies have been reported, they have primarily focused on investigating the effect of heteronuclear coupling.^{29–31} A comprehensive analysis of the low-field 2D spectra, encompassing hetero- and homonuclear coupling effects, has yet to be conducted.

In this study, we demonstrate the 1D and 2D ^1H NMR spectroscopic analyses of $\text{CH}_3^{13}\text{CH}_2\text{OH}$ at a magnetic field of $34.38\ \mu\text{T}$, close to the Earth's magnetic field, using an NMR system based on a superconducting quantum interference device (SQUID) for detection.^{10,11,15–17,23,24,26} To the best of our knowledge, this is the first study in a low magnetic field to demonstrate that the ^1H COSY spectrum clearly reveals a network of J -coupling through cross-peak multiplets. $\text{CH}_3^{13}\text{CH}_2\text{OH}$ exhibits not only strong and weak heteronuclear couplings but also a homonuclear coupling among the ^1H spin, as illustrated in Figure 1a, and hence is suitable for

investigation using the 2D NMR technique. Our results indicate that low-field multidimensional NMR spectroscopy can identify isotopically labeled compounds at the atomic scale using J -coupling frameworks.

RESULTS AND DISCUSSION

Low-Field 1D Spectrum of $\text{CH}_3^{13}\text{CH}_2\text{OH}$. Before analyzing the 2D spectrum, we interpreted the 1D spectrum of $\text{CH}_3^{13}\text{CH}_2\text{OH}$ using spectral simulation. Figure 1b displays the simulated spectrum at $34.38\ \mu\text{T}$, based on the known parameters ($^1J_{\text{CH}} = 141.35\ \text{Hz}$, $^2J_{\text{CH}} = -4.7\ \text{Hz}$, and $^3J_{\text{HH}} = 7\ \text{Hz}$ ³²). The line width (Δf) was set to approximately 2 Hz, similar to that observed experimentally (see the Supporting Information for details of the numerical simulation). The proton-exchange ^1H of $\text{CH}_3^{13}\text{CH}_2\text{OH}$ (i.e., that of OH) exhibits the strongest peak at $1464\ \text{Hz}$ (Figure 1b).

Given the ^1H and ^{13}C resonance frequencies at $1464\ \text{Hz}$ ($=f_{\text{H}}$) and $388\ \text{Hz}$ ($=f_{\text{C}}$), respectively, the heteronuclear coupling $^1J_{\text{CH}}$ corresponds to a strongly coupled regime of 0.01

$< {}^1J_{\text{CH}}/(f_{\text{H}} - f_{\text{C}}) < 1$,³³ while ${}^2J_{\text{CH}}$ corresponds to a weakly coupled regime. ${}^3J_{\text{HH}}$ can be considered strong because $0.01 < {}^3J_{\text{HH}}/({}^1J_{\text{CH}} - {}^2J_{\text{CH}}) < 1$. Strong J -coupling contributes to a higher-order expansion of the transition (resonance) frequency, resulting in additional peak splits in the spectrum. However, this higher-order effect becomes invisible if the spectral line width is broader than this. In the present experiment, the typical line width was about $\Delta f = 2$ Hz. The second-order expansion for ${}^3J_{\text{HH}}$ is given by ${}^3J_{\text{HH}}^2/({}^1J_{\text{CH}} - {}^2J_{\text{CH}}) = 0.3$ (Hz), which is much lower than Δf . Thus, the homonuclear coupling ${}^3J_{\text{HH}}$ virtually functions like a weak coupling.

Figure 1c shows the spectral pattern of $\text{CH}_3^{13}\text{CH}_2\text{OH}$ with none of the J -couplings. When only ${}^1J_{\text{CH}}$ is introduced, the ${}^1\text{H}$ lines of the methylene group (${}^{13}\text{CH}_2$) are separated, as shown in Figure 1d. The left-shifted lines (blue) correspond to the ${}^{13}\text{C}$ spin-down state ($|\downarrow\rangle$) because ${}^1J_{\text{CH}}$ is positive. The right-shifted lines (red) are associated with the ${}^{13}\text{C}$ spin-up state ($|\uparrow\rangle$). The strong coupling between the ${}^{13}\text{C}$ and ${}^1\text{H}$ spins induces additional lines, whose number is determined by the number of ${}^1\text{H}$ spins.³³ For ${}^{13}\text{CH}_2$, two lines appear on both the left (blue) and right (red) sides with the splittings of Δ_{L} and Δ_{R} , respectively. In general, Δ_{L} and Δ_{R} are not identical, and their values can be precisely estimated with an expression up to third-order expansion (see below for more details). The mean distance between the red and blue lines is approximately ${}^1J_{\text{CH}}$. When ${}^2J_{\text{CH}}$ is turned on, the ${}^1\text{H}$ lines of the methyl group (CH_3) split due to the weakly coupled ${}^{13}\text{C}$ spin, as shown in Figure 1e. The red and blue sides are reversed because ${}^2J_{\text{CH}}$ is negative. Lastly, in Figure 1f, the homonuclear coupling ${}^3J_{\text{HH}}$ was added. The ${}^{13}\text{C}$ nuclear spin breaks the magnetic equivalence between the ${}^1\text{H}$ spins. Then, ${}^3J_{\text{HH}}$ produced multiple peaks. In Figure 1f, the individual outer (2 each at the left and right) lines of the ${}^{13}\text{CH}_2$ split into quartets because of the ${}^1\text{H}$ spins of the CH_3 group. The total spin number (F_{I}) of the ${}^1\text{H}$ spins of the CH_3 group is $3/2$, and its quantum number m_{I} (H_3) is associated with $-F_{\text{I}} \leq m_{\text{I}} (\text{H}_3) \leq F_{\text{I}}$. Similarly, each of the two central lines (red and blue) of the CH_3 split into triplets because of the ${}^1\text{H}$ spins of the ${}^{13}\text{CH}_2$ group ($F_{\text{I}} = 1$).

The number of individual lines in Figure 1f is different from what the simulation predicted (Figure 1b). This is because, by coincidence, the two lines, which are close to each other, appear to merge into single lines, resulting in five lines for the left multiplet and four lines for the central multiplet. For verification, we calculated the Δ_{L} and Δ_{R} values and compared them with the ${}^3J_{\text{HH}}$ value. The experimentally obtained values are $\Delta_{\text{L}} (= f_2 - f_1) = 7.4$ Hz and $\Delta_{\text{R}} (= f_4 - f_3) = 10.9$ Hz. The four resonance (transition) frequencies (f_i) of the ${}^1\text{H}$ spins of the ${}^{13}\text{CH}_2$ group can be calculated using a second-order Taylor expansion.²⁰ However, we found that the calculation cannot explain the experimental values. Therefore, a third-order expansion was carried out (see the Supporting Information for the derivation),

$$\begin{aligned} f_{1,3} &= f_{\text{I}} \mp \frac{J_{\text{CH}}}{2} \pm \frac{J_{\text{CH}}^3}{2(f_{\text{I}} - f_{\text{S}})^2}, \\ f_{2,4} &= f_{\text{I}} \mp \frac{J_{\text{CH}}}{2} + \frac{J_{\text{CH}}^2}{2(f_{\text{I}} - f_{\text{S}})} \mp \frac{J_{\text{CH}}^3}{4(f_{\text{I}} - f_{\text{S}})^2}, \end{aligned} \quad (1)$$

where f_{I} and f_{S} are the resonance frequencies of the I and S spins, respectively, in a given magnetic field. Δ_{L} and Δ_{R} are

calculated to be 7.4 and 10.9 Hz, respectively. The difference between Δ_{L} and ${}^3J_{\text{HH}}$ is almost negligible. This explains the merging of the lines in the left multiplet in Figure 1f. In contrast, the difference between Δ_{R} and ${}^3J_{\text{HH}}$ is comparable to the line width. Thus, the eight lines on the right multiplet are barely resolvable.

Low-Field 2D Spectrum of $\text{CH}_3^{13}\text{CH}_2\text{OH}$. Figure 2a displays the experimentally obtained COSY spectrum (see the

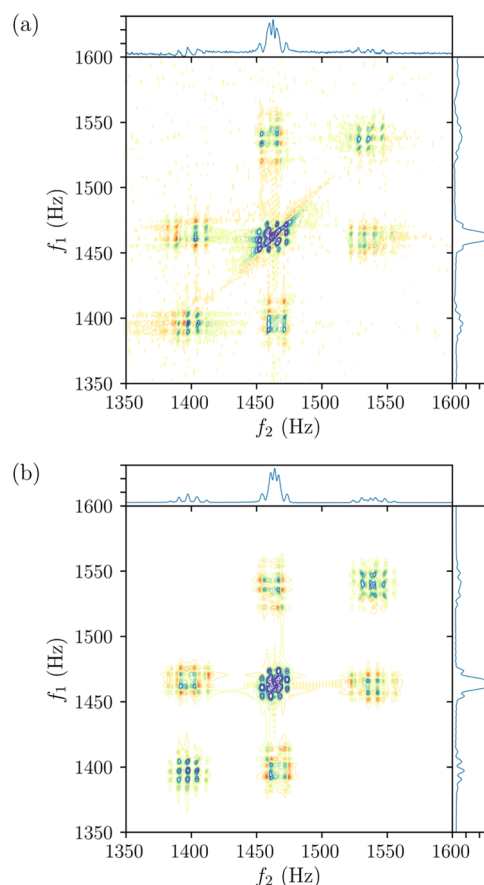


Figure 2. (a) COSY spectrum of $\text{CH}_3^{13}\text{CH}_2\text{OH}$ obtained at 34.38 μT . (b) Numerically simulated COSY spectrum with the parameters (line width and time increment) resembling the experimental conditions. The projected f_1 and f_2 domain J -spectra are displayed to the right and above the COSY spectrum. The blue and red colors correspond to positive and negative values, respectively.

Supporting Information for experimental details). It is in good agreement with the numerical simulation shown in Figure 2b. In both the experiment and the simulation, two features are notable. First, there are no cross-peaks between the left and right multiplets. Second, the lines in the central multiplet show correlations with either the left or the right multiplets (note that the proton-exchange ${}^1\text{H}$ spins of the OH groups have no cross-peaks). Considering the expected lines in Figure 1f, one can find that a cross-peak occurs between the two lines within the same color (red or blue). In other words, for each cross-peak, the associated ${}^1\text{H}$ lines share an identical ${}^{13}\text{C}$ spin state. The pulse sequence used to obtain the COSY spectrum provides a physical explanation of this fact. During 2D NMR experiments, the applied pulses selectively excite ${}^1\text{H}$ spins but do not affect ${}^{13}\text{C}$ spins. Consequently, the coherence causing a cross-peak cannot be created between two ${}^1\text{H}$ states with different ${}^{13}\text{C}$ spin states.

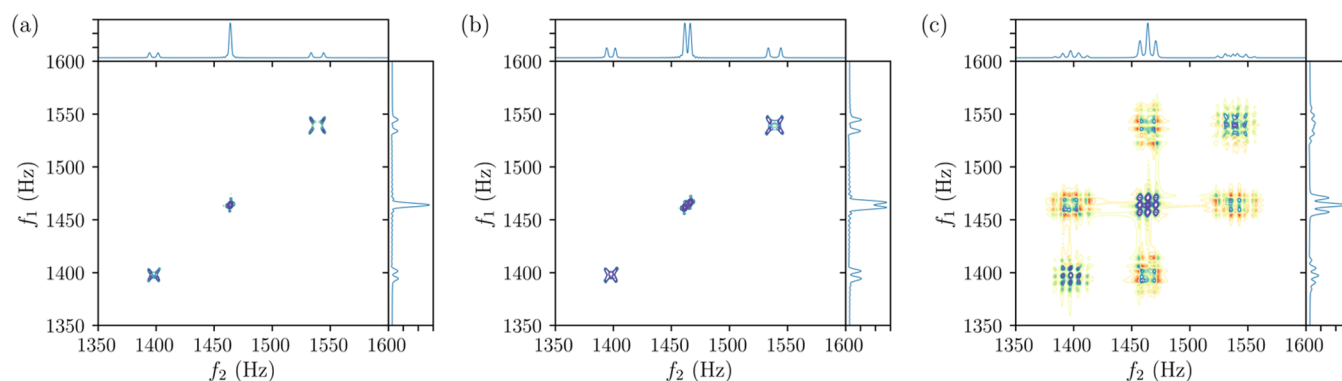


Figure 3. The COSY spectrum of $\text{CH}_3^{13}\text{CH}_2\text{OH}$ is numerically simulated for three different J -coupling values as (a) only $^1J_{\text{CH}}$, (b) $^1J_{\text{CH}}$ and $^2J_{\text{CH}}$, and (c) $^1J_{\text{CH}}$ and $^3J_{\text{HH}}$. For simplicity, the simulations were performed without the uncoupled ^1H spins, considering only the ^1H spins of the CH_3 and $^{13}\text{CH}_2$ groups. The projected f_1 and f_2 domain J -spectra are displayed to the right and above the COSY spectrum.

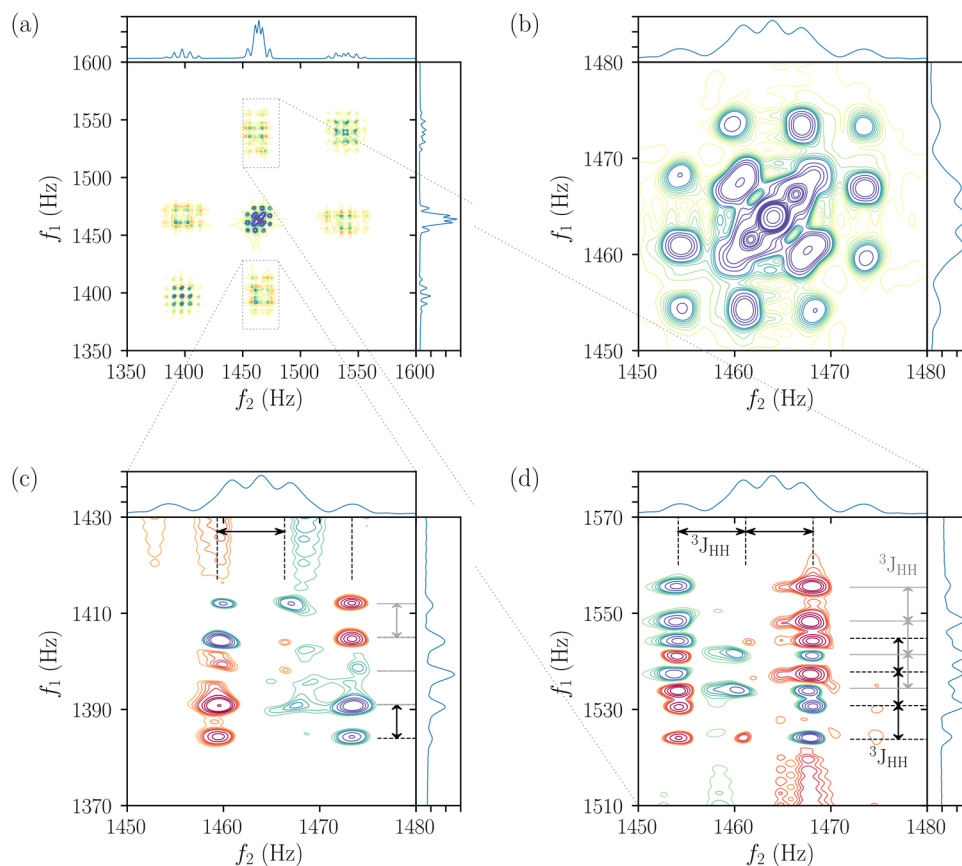


Figure 4. (a) Numerically simulated COSY spectrum for $\text{CH}_3^{13}\text{CH}_2\text{OH}$ with all J -couplings and the six ^1H and one ^{13}C spins (i.e., including the uncoupled ^1H spins). To improve the resolution of the projected f_1 domain J -spectrum, the t_1 time was increased up to 400 ms with the same time increment. (b) Magnified view of the central diagonal multiplet. (c, d) Magnified view of the spectrum for the bottom-middle and top-middle cross-peak multiplets, respectively. Phase corrections were performed to make the cross-peaks appear in real phases. The blue and red colors correspond to positive and negative values, respectively.

The numerical simulations shown in Figure 3 provide further insights into the low-field 2D spectrum. Figure 3a includes only $^1J_{\text{CH}}$. The 1D spectrum in Figure 3a is analogous to that in Figure 1d. Cross-peaks appear only between peaks with the same ^{13}C state. When $^2J_{\text{CH}}$ is added, the central peak at 1464 Hz is split, as shown in Figure 3b; however, only diagonal peaks appear in the COSY spectrum. Thus, we can conclude that heteronuclear couplings in the strong regime can generate cross-peaks. Besides, the requirement of sharing an

identical ^{13}C spin state still needs to be fulfilled to produce cross-peaks.

To visualize the effects of homonuclear coupling, we included only $^3J_{\text{HH}}$ and $^1J_{\text{CH}}$ in Figure 3c. Because of the absence of $^2J_{\text{CH}}$, the central multiplet appears as a triplet in the 1D spectrum. The homonuclear coupling $^3J_{\text{HH}}$ induces correlations between the ^1H spins when exposed to excitation pulses. Accordingly, cross-peaks emerge at all available positions in the COSY spectrum as long as the ^1H spins share an identical ^{13}C spin state.

Figure 4a depicts a simulation of the ^1H COSY spectrum with improved spectral resolution of the projected f_1 domain. The t_1 time was increased up to 400 ms using the same time increment (see the Supporting Information for the definition of t_1 and the related pulse sequence). Here, all J -couplings ($^1J_{\text{CH}}$, $^2J_{\text{CH}}$, and $^3J_{\text{HH}}$) and uncoupled ^1H spins are included. Figure 4b displays a magnified view of the central region and shows the following pattern: two of the nine (3×3) peaks that appear at the center of Figure 3c overlap and are slid equally by the $^2J_{\text{CH}}$ value along the f_1 and f_2 axes. Subsequently, a strong peak corresponding to the uncoupled ^1H spins is observed at the center ($f_1 = f_2 = 1464$ Hz), and some of the peaks partially overlap diagonally.

Magnified views of the cross-peak multiplets between the ^1H spins of the CH_3 and $^{13}\text{CH}_2$ groups are shown in Figure 4c,d, respectively. To improve the accuracy of the distances between the peak positions, phase correction was performed to make the cross-peaks appear in real phases. The spectra in Figure 4c,d correspond to the ^{13}C down- and up-spin states, respectively. Figure 4d exhibits a more complicated pattern, mainly consisting of eight peaks along the f_1 axis. These eight peaks are divided into two parts (marked by the gray and black lines). In each part, the peaks are separated by $^3J_{\text{HH}}$. The whole cross-peak multiplet in Figure 4d can be deconvoluted into two patterns, each of which exhibits a typical COSY spectrum between the triplet ($F_1 = 1$) of $^{13}\text{CH}_2$ and the quartet ($F_1 = 3/2$) of CH_3 . The signs of the cross-peaks are determined by multiplying m_z ($F_1 = 3/2$) and m_z ($F_1 = 1$). In Figure 4c, the same two patterns emerge, but they overlap because $\Delta_L \approx ^3J_{\text{HH}}$, as illustrated in Figure 1f. When the signs mismatch, the overlapped peaks cancel each other, leading to collapsed lines at the center (1398 Hz) of the f_1 axis.

Figure 4c,d show that the cross-peak multiplets in the COSY spectrum provide improved spectral details, resolving the peaks of the broad multiplets in the 1D spectrum. Moreover, the information that can be obtained from the cross-peak multiplets includes not only the strength of $^3J_{\text{HH}}$ but also the relative signs of $^1J_{\text{CH}}$ and $^2J_{\text{CH}}$. This can be confirmed by comparing the former with the inverted sign of $^2J_{\text{CH}}$. The colors of the peaks in the central multiplet in Figure 1f are exchanged from blue to red, leading to exchanged peak positions on the f_2 axes of Figure 4c,d. This indicates that in low fields, the relative signs of the heteronuclear coupling constants can be obtained without using double-resonance techniques such as heteronuclear correlation (HETCOR), which are commonly used in high-field NMR spectroscopy.^{5,6}

Finally, two limitations of the analytical method presented in this study must be noted. First, isotope labeling is not a general solution for complete chemical analyses based on the J -coupling framework. As emphasized above, the strong heteronuclear coupling introduced by isotopically labeled nuclear spins at appropriate molecular sites is crucial.^{20,33} ^{13}C -enriched acetic acid ($\text{CH}_3^{13}\text{COOH}$) is a counterexample (see the Supporting Information for its COSY spectrum). Because weak heteronuclear coupling cannot break the magnetic equivalence between the ^1H spins in the CH_3 group, neither the 1D nor the 2D spectra allow the identification of the CH_3 group in $\text{CH}_3^{13}\text{COOH}$. Second, the explanations for the peak splittings shown in Figure 1d–1f are inconsistent with the peak intensities in Figure 1b. To assess the multiplets of the 1D spectrum, we treat the homonuclear coupling as if it belonged to a weak coupling regime. This approximation successfully explains the spectral

lines shown in Figure 1f, which is also consistent with the results of the COSY spectrum. However, it does not describe the peak intensities in Figure 1b. The peak intensities can be explained by considering the number of actual spectral lines contained within the line width of a peak.³³ Therefore, numerically simulated NMR spectra from the spin Hamiltonian are necessary to understand the peak splitting and intensities correctly. This has been demonstrated in this work with the simulated spectra shown in Figures 1b, 2b, 3, and 4.

Nevertheless, the weak-coupling approximation of homonuclear coupling can be usefully applied in many cases. Typically, the strengths of the (strong) hetero- (J_{het}) and homonuclear (J_{hom}) couplings are on the order of 100 and 10 Hz, respectively. The line width, then, is expected to be less than 1 Hz ($\approx J_{\text{hom}}^2/J_{\text{het}}$) in order to observe a strong coupling effect of J_{hom} . However, it is challenging to meet this spectral resolution even in low or ultralow magnetic fields. In a previous study,¹⁷ we achieved a line width of approximately 0.5 Hz by significantly restricting even the use of metallic components. The current line width of 2 Hz can thus be attributed to magnetic inhomogeneity, which possibly is associated with the use of magnetizable materials in building the electromagnet for prepolarization. Improving the uniformity of the magnetic field through the improvement of electromagnet fabrication could lead to line width reduction. This, consequently, would enhance the spectral resolution, which would enable more accurate resolution of the strongly coupling effects in both 1D and 2D spectra.

CONCLUSIONS

In this study, we successfully obtained the J -coupled COSY spectrum of $\text{CH}_3^{13}\text{CH}_2\text{OH}$ at 34.38 μT . This is in good agreement with our numerical simulation. In low magnetic fields, isotopic labeling generates magnetically inequivalent ^1H spins via heteronuclear coupling. Homonuclear coupling then results in additional splitting of the ^1H peaks, which is analogous to the high-field NMR spectra. However, moderate line widths on the order of 1 Hz make it difficult to deduce the J -coupling network directly from the 1D spectrum. In contrast, 2D spectroscopy resolves spectral peaks by displaying cross-peaks induced by homonuclear coupling. To the best of our knowledge, this is the first report on low-field 2D NMR spectroscopy that successfully demonstrates the effects of both hetero- and homonuclear J -couplings and interprets them clearly. We find that for a correlation peak in the ^1H COSY spectrum, the associated ^1H peaks must share the same ^{13}C spin state. This work is expected to inspire future research aimed at low-cost or miniaturized implementations operating in low magnetic fields by exploring the feasibility of chemical analysis using low-field NMR spectroscopy. While the SQUID-based NMR system currently possesses a large volume due to its cryogenic components, this volume can be substantially minimized by adopting atomic magnetometers for detection.¹⁴ When combined with the benefits of low magnetic fields, its application could potentially be extended to the in situ analysis of reactions in fuel cells and batteries by identifying organic molecules.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c05128>.

Experimental details of the low-field NMR experiment; details of the numerical simulation; 1D spectrum of CH₃CH₂OH; third-order expansion of the resonance frequencies in the strong coupling regime; COSY spectrum of CH₃¹³COOH (PDF)

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Author Contributions

J.H.S. and O.H.H. conceived the project. S.-J.L. conducted the COSY experiment with the help of K.K.Y. S.-J.L. and J.H.S. conducted the computer programming and simulation, analyzed the COSY results, and mainly prepared the manuscript after discussing the results with other authors.

Notes

The authors declare no competing financial interest.

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