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Magnetization Transfer in a Partly Deuterated Lyotropic Liquid Crystal Crystal by Single- and Dual-Frequency RF Irradiations

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Abstract

The mechanism of magnetization transfer (MT) in a lyotropic liquid crystal made of sodium dodecyl sulfate, decanol, and water molecules is investigated by using deuterated molecules and single- and dual-frequency RF irradiations. The resulting Z-spectra suggest that the decanol molecules are mainly responsible for the MT effects in this system, through proton exchange to water. This is further confirmed by monitoring the relaxation of dipolar order, which allows one to estimate the transfer rate of magnetization from decanol to water. The potential benefits of using dual-frequency RF irradiation for inducing MT effects are explored through numerical solutions to a MT model based on Provotorov's partial saturation theory.

Graphical abstract

Keywords

Magnetization transfer; Uniform saturation; Provotorov's partial saturation theory; Dipolar order; Lyotropic liquid crystal

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INTRODUCTION

The so-called magnetization transfer (MT) [1] is a popular contrast mechanism used in magnetic resonance imaging (MRI). Like other saturation transfer (ST) methods, it measures the effect on the water signal of a saturating RF irradiation that is applied at a frequency offset far from the water resonance [2–5]. Since the MT effects are observed for a broad range of the frequency offsets, it is believed that they probe solid-like macromolecules in tissues and organs, which may manifest broad proton nuclear magnetic resonance (NMR) spectra due to slow motion and residual dipolar couplings.

When the system causing MT effects, called a MT pool, is subject to spectral broadening due to residual dipolar couplings, its behavior under a RF irradiation may be understood through the framework of the spin temperature [6–8]. The nuclear polarization established by the external field at the thermal equilibrium, called the Zeeman order, is not simply saturated but converted into another state wherein nuclear spins are aligned with the local dipolar fields, called dipolar order. Recently, it has been shown that the addition of the second RF irradiation at a distinct frequency offset can achieve unequivocal saturation, in which both the Zeeman and dipolar orders vanish, regardless of the frequency positions of two simultaneously applied RF irradiations [9,10].

The efficient saturation by two simultaneous RF irradiations has been demonstrated to be useful for enhancing MT contrast [11] and isolating chemical exchange saturation transfer (CEST) contrast from the MT effects asymmetric with respect to the water frequency [11–13]. In addition, there have been other works using dual-frequency RF irradiation named ‘inhomogeneous MT’ [14–16]. The term appears ill-chosen since the effects may not require any inhomogeneous nature of the resonance involved [17].

In this work, we attempted to identify the dynamic processes involved in MT effects by controlling the residual dipolar interactions. The constituents of a well-known lyotropic liquid crystal, which exhibits a molecular arrangement commonly found in some biological structures such as cell membranes and myelin sheaths, were replaced one by one with the corresponding deuterated molecules, after which MT effects and the relaxation of dipolar order were studied. The study not only revealed the passage of the MT effects in this system but also uncovered how the internal state of the MT pool is affected by the MT effects, which may be generalized to MT phenomena arising from a cluster of dipolar coupled spins. We also numerically examined the difference in MT effects between single- and dual-frequency RF irradiations for the varying strengths of dipolar interactions, which shows the potential benefits of using the dual-frequency RF irradiation for ST applications.

Theoretical Background

A system composed of N coupled nuclear spins-1/2 can have 2^N energy levels, and the number of single-quantum transitions between them, i.e., the number of peaks on a NMR spectrum, can be $\binom{2N}{N+1} \sim 2^{2N}$ maximum [18]. The number of peaks can be far less than 2^{2N} if the system is subject to fast isotropic translation and rotational motions so only scalar couplings between a few neighboring spins are effective. When the motions of the system

are anisotropic or restricted, like in liquid crystals and solids, residual dipolar couplings between nuclear spins may cause a rich number of peaks. In such systems, individual peaks usually become unresolvable when N is, roughly speaking, larger than 10.

While it is possible to coherently manipulate the dynamics of a system with an unresolved spectrum [19], a thermodynamic approach based on the concept of spin temperature may suffice for the description of its saturation [8], which states that a weak RF irradiation causes the thermal mixing of Zeeman and dipolar orders. A set of the kinetic equations can be derived by following Provotorov's thermodynamic theory [8,20], which treats the weak RF irradiation as a perturbation and solves the master equation by iteration, under the assumption that the state of the system is described by a quasi-equilibrium form.

The following summarize the expressions and results from Provotorov's theory [8]. The spin Hamiltonian is written as

$$\mathcal{H} = -\omega_0 S_z + \mathcal{H}_d + 2\omega_1 S_x \cos \omega t \quad (1)$$

in the laboratory frame, where S_x and S_z are respectively the x and z components of the total spin angular momentum operator, $\mathcal{H}_d$ is the dipolar Hamiltonian, ω_0 is the resonance frequency, and ω_1 and ω are respectively the amplitude and frequency of a RF irradiation. The term $2\omega_1 S_x \cos \omega t$ is assumed to be small and regarded as a perturbation. The density operator of the system is assumed to be in the quasi-equilibrium form:

$$\rho(t) = \frac{1}{2^N} [\mathbf{1} + \beta_s(t) \omega_0 S_z - \beta_d(t) \mathcal{H}_d], \quad (2)$$

where N is the number of spins, $\mathbf{1}$ is the identity operator, and β_s and β_d are respectively the inverse spin temperatures for Zeeman and dipolar orders. By introducing the spin polarization

$$P_s = \frac{2}{N_s} \text{tr}\{\rho S_z\} \quad (3)$$

and dipolar polarization

$$P_d = \frac{2}{N_s} \frac{\text{tr}\{\rho \mathcal{H}_d\}}{\omega_{\text{loc}}}, \quad (4)$$

where $\omega_{\text{loc}}^2 = \text{tr}\{\mathcal{H}_d^2\} / \text{tr}\{S_z^2\}$, the kinetic equations can be written as

$$\frac{dP_s(t)}{dt} = -W \left[P_s(t) - \frac{\Delta}{\omega_{\text{loc}}} P_d(t) \right] - \frac{P_s(t) - P_{s,0}}{T_{1,S}} \quad (5)$$

and

$$\frac{dP_d(t)}{dt} = -W \frac{\Delta}{\omega_{loc}} \left[P_S(t) - \frac{\Delta}{\omega_{loc}} P_d(t) \right] - \frac{P_d(t)}{T_d}, \quad (6)$$

where $W = \pi \omega_1^2 g(\Delta)$, $\omega_0 = \omega$, $T_{1,S}$ and T_d are respectively the relaxation times for the Zeeman and dipolar polarizations, and $P_{S,0}$ is the Zeeman polarization at the thermal equilibrium. The function $g(\Delta)$ is the normalized absorption line shape:

$$g(\Delta) \equiv \frac{1}{2\pi \text{tr}\{S_x^2\}} \int_{-\infty}^{+\infty} \text{tr}\{S_x S_x(\tau)\} e^{i\Delta\tau} d\tau, \quad (7)$$

where

$$S_x(\tau) = e^{+i\mathcal{H}_d\tau} S_x e^{-i\mathcal{H}_d\tau}. \quad (8)$$

Ignoring the relaxation terms in Eqs. (5) and (6), the steady state solution is given by

$$P_S(\infty) = \frac{\Delta}{\omega_{loc}} P_d(\infty) = P_{S,0} \frac{\Delta^2}{\Delta^2 + \omega_{loc}^2}, \quad (9)$$

and the other non-stationary solution decays at the rate

$W \left[1 + \left(\frac{\Delta}{\omega_{loc}} \right)^2 \right] = \pi \omega_1^2 \left[1 + \left(\frac{\Delta}{\omega_{loc}} \right)^2 \right] g(\Delta)$, which is the rate of the thermal mixing. Note that this rate would decrease as $|\Delta|$ increases since $g(\Delta)$ gets smaller.

Recently, Provotorov's theory has found two new applications [10,21]. One of them is uniform saturation under simultaneous two-frequency RF irradiation [10]. With two RF irradiations applied at the frequencies ω and ω' , the spin Hamiltonian can be written as

$$\mathcal{H} = -\omega_0 S_z + \mathcal{H}_d + 2\omega_1 S_x \cos \omega t + 2\omega'_1 S_x \cos \omega' t. \quad (10)$$

It is straightforward to follow the steps of Provotorov's theory with the second RF term $2\omega'_1 S_x \cos \omega' t$, which makes its own contribution to the kinetic equations like the original RF term $2\omega_1 S_x \cos \omega t$ does and causes an additional cross effect proportional to the time integral $\iint d\tau d\tau' \cos \omega \tau \cos \omega' \tau'$. Unless the frequency distance between two RF irradiations $|\omega - \omega'|$ is comparable to ω_1 or ω'_1 , this time integral will be negligible. With the cross effect being ignored, the kinetic equations are given as

$$\frac{dP_S(t)}{dt} = -W \left[P_S(t) - \frac{\Delta}{\omega_{10c}} P_d(t) \right] - W' \left[P_S(t) - \frac{\Delta'}{\omega_{10c}} P_d(t) \right] - \frac{P_S(t) - P_{S,0}}{T_{1,S}} \quad (11)$$

$$\frac{dP_d(t)}{dt} = W \frac{\Delta}{\omega_{10c}} \left[P_S(t) - \frac{\Delta}{\omega_{10c}} P_d(t) \right] + W' \frac{\Delta'}{\omega_{10c}} \left[P_S(t) - \frac{\Delta'}{\omega_{10c}} P_d(t) \right] - \frac{P_d(t)}{T_d}, \quad (12)$$

where $W' = \pi \omega_1'^2 g(\Delta')$ and $\omega' = \omega_0 - \omega$. Note that Eqs. (11) and (12) are reduced to Eqs. (5) and (6) if either of W or W' is set to be zero. More RF irradiations may be added in the same way as far as the cross effects among them can be ignored, i.e., if they are distinct from one another in the frequency domain.

Without the relaxation terms, the solutions of Eqs. (11) and (12) have the form of $Ae^{\lambda_+ t} + Be^{\lambda_- t}$, and it was shown that the characteristic constants $\lambda_{\pm}$ are always real and negative [10]. Therefore, $P_S(\infty) = P_d(\infty) = 0$, i.e., both the Zeeman and dipolar polarizations vanish. The state corresponds to the maximally mixed state $\frac{1}{2N} \mathbf{1}$, which means that all the energy eigenstates are equally populated. This result is independent of the amplitudes and frequency offsets of the RF irradiation, so the scheme has been named as *uniform saturation*.

Through uniform saturation, dual-frequency RF irradiation is expected to induce more MT effects than single-frequency RF irradiation, if a MT pool is made of dipolar-coupled nuclear spins and if $|\lambda_+|^{-1}$ and $|\lambda_-|^{-1}$ are much shorter than the timescale for the transfer of magnetization vectors. As the longer relaxation time for the water is favorable to accumulate MT effects, the longer relaxation times for the MT pool would be advantageous to keeping the Zeeman and dipolar polarizations saturated. While MT effects through uniform saturation are supposed to be less sensitive to the frequency positions of RF irradiation, as long as they are within the spectral range of the MT pool, the characteristic constants $\lambda_{\pm}$ depend on W , W' , $(\Delta/\omega_{10c})^2$, and $(\Delta'/\omega_{10c})^2$ [10], and hence the saturation of a MT pool as well as the ensuing MT effects should be a function of the frequency positions, with relaxation being an additional factor.

Methods

Sample preparation

Sodium dodecyl sulfate [$\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$] and decanol [$\text{CH}_3(\text{CH}_2)_9\text{OH}$] were purchased from Sigma-Aldrich, and deuterated sodium dodecyl sulfate [$\text{CD}_3(\text{CD}_2)_{11}\text{OSO}_3\text{Na}$, 98%] and decanol [$\text{CD}_3(\text{CD}_2)_9\text{OH}$, 98%] from Cambridge Isotope Laboratories. The first liquid-crystal sample was made of protonated sodium dodecyl sulfate, protonated decanol, and H_2O with their relative weights 9.5%, 25.5%, and 65.0%, respectively. This corresponds to 0.025 SDS molecules and 0.016 decanol molecules per H_2O molecule. The same ratios were maintained for the other liquid-crystal samples including deuterated species. When D_2O was used, the ratios were evaluated with respect to the number of D_2O molecules. The samples were centrifuged to be made homogeneous and transferred to 5 mm o.d. NMR tubes.

NMR experiments

All NMR experiments were performed using a Bruker Avance 500 MHz NMR spectrometer equipped with a broadband observe (BBO) probe at the room temperature (298 K). The deuterium lock was not used. The duration of the 90° pulse was 12~13 μs, based on which the setting for the RF power of saturating RF irradiation was evaluated. The relaxation delay was 30 s, which was longer than five times the longest T_1 relaxation time (= 2.7 s), and the free induction decays (FIDs) were recorded for 300 ms with the spectral width of 200 kHz. While the receiver gain was set as high as possible for each sample, other acquisition parameters were not optimized.

To obtain Z-spectra [22], off-resonance saturating RF irradiation was applied for 1 s following the relaxation delay. The frequency offsets of the saturating RF irradiation were from -10 kHz to +10 kHz with respect to the water resonance, and the RF amplitudes ($\lambda B_1/2\pi$) were $100\sqrt{2}$, $200\sqrt{2}$, and $400\sqrt{2}$ Hz. The residual proton polarization of H₂O was flipped by a 90° or 5° pulse to produce an FID.

An additional set of experiments were performed by replacing the saturating RF irradiation with cosine-shaped RF pulses, which were applied at the water resonance. The number of steps in these shaped pulses were 100,000, and the modulation frequencies were between 1 and 10 kHz. Since

$$2A\cos 2\pi ft = Ae^{+2\pi ft} + Ae^{-2\pi ft}, \quad (13)$$

a cosine-shaped pulse delivers its RF power to two frequency offsets $\pm f$, where f is the modulation frequency. In this work, the RF strength of a cosine-shaped pulse will be denoted as $A \times 2$, where A is the RF amplitude of each frequency component. The RF strengths used to obtain Z-spectra were $100 \text{ Hz} \times 2$, $200 \text{ Hz} \times 2$, and $400 \text{ Hz} \times 2$. The root-mean-square (r.m.s.) power of the function in Eq. (13), $2A^2$, is equal to the r.m.s. power of a single-frequency RF irradiation with the RF amplitude $\sqrt{2}A$. Hence, we would call the pair 'power-matched'. For example, a cosine-shaped RF pulse with the RF strength $100 \text{ Hz} \times 2$ and a single-frequency RF irradiation with the RF amplitude $100\sqrt{2} \text{ Hz}$ are power-matched.

The measurements of the T_1 and T_2 relaxation times were performed by the inversion recovery and spin echo sequences. The intensities for individual delay times were estimated by integrating the spectra from -15 kHz to 10 kHz and fitted to bi-exponential decaying curves. If the bi-exponential fitting could not catch two distinct decay times, for example when the amplitude of one component was extremely small, the results were fitted to mono-exponential decaying curves.

The relaxation of dipolar order was observed by two methods. One was the adiabatic demagnetization in the rotating frame (ADRF) followed by the adiabatic remagnetization in the rotating frame (ARRF) [23,24], and the other was the Jeener-Broekaert (JB) sequence [25]. These pulse sequences are presented in Fig. 1.

For the ADRF-ARRF sequence, ADRF and ARRF were performed by linearly decreasing and increasing ramps for 4 ms, respectively. The delay between the two ramps was varied from 100 μ s to 16 s to observe the relaxation of dipolar order. The phase cycling consisted of 4 steps, which was performed once for the samples containing D₂O and was repeated four times for those containing H₂O. The relaxation delay was 30 s.

For the JB sequence, the duration of the 45° pulses was set to be half of the duration of the 90° pulse, and the delay between the first and second pulses, τ , was set to be 50 μ s, which maximized the signal intensity when the delay between the second and third pulse, t , was 0 s. The delay t was varied from 100 μ s to 16 s to allow dipolar order to relax. The phase cycling consisted of 8 steps, which was performed once for the samples containing D₂O and was repeated twice for those containing H₂O. The relaxation delay was 30 s.

Numerical studies

The MT effects under single- and dual-frequency RF irradiations were numerically investigated by connecting Eqs. (5) and (6) or Eqs. (11) and (12), through a simple exchange model [26,27], to an equation for the polarization of an abundant pool [12]

$$\frac{dP_I(t)}{dt} = -W_I P_I(t) - \frac{P_I(t) - P_{I,0}}{T_{1,I}}, \quad (14)$$

where $W_I = \pi \omega_1^2 g_I(\Delta)$ and the line shape $g_I(\Delta)$ was assumed to be Lorentzian: $g_I(\Delta) = (T_{2,I}/\pi)[1 + (\Delta/T_{2,I})^2]^{-1}$. The combined equations were solved with using the parameter values as follows: $T_{1,I} = T_{1,S} = 3$ s, $T_{2,I} = 0.06$ s, $T_d = 0.3$ s and 3 ms, the exchange rate = 30 s⁻¹, the duration of RF irradiation = 1 s, and $\Delta = -50$ kHz $\sim$ +50 kHz with the step size of 12.5 Hz. For the single-frequency RF irradiation, $\omega_1/2\pi = 100 \sqrt{2}$ Hz. For the dual-frequency RF irradiation, $\omega_1/2\pi = \omega'_1/2\pi = 100$ Hz, and $\Delta' = -$. The line shape of the MT pool was assumed to be Gaussian: $g_S(\Delta) = (\sqrt{6\pi}\omega_{loc})^{-1} \exp[-\Delta^2/(6\omega_{loc}^2)]$. No chemical shifts between the abundant and MT pools were assumed. First, the Z-spectra for the abundant and MT pools were obtained while $\omega_{loc}/2\pi$ was varied from 1 kHz to 5 kHz with a step size of 1 kHz. Second, the Z-spectra were calculated when the line shape of a MT pool is given by the average of two Gaussian line shapes with $\omega_{loc}/2\pi = 0.5$ kHz and 1.0 kHz and when there were two independent MT pools sharing a common abundant pool.

Results

The Z-spectra from the liquid-crystal samples are presented in Fig. 2. The upward triangles connected by a dashed line represent half of a whole Z-spectrum along the positive frequency offsets. The other half along the negative frequency offsets is plotted against the absolute value of the frequency offset, displayed as the downward triangles connected by a thin solid line. The circles connected by a thick solid line show the results obtained with the cosine-shaped pulses under the ‘power-matched’ condition, which are plotted against the modulation frequency of the cosine function.

The difference between the positive and negative halves of the Z-spectra, i.e., the asymmetry of the MT effects, as well as the difference between the single- and dual-frequency RF irradiations was reduced when decanol molecules were deuterated, when comparing Fig. 2(a) with Fig. 2(b) or when comparing Fig. 2(c) with Fig. 2(d). At the same time, the overall MT effects were reduced.

With SDS molecules deuterated, the MT effects and their spectral range increased, as seen between Fig. 2(a) and Fig. 2(c) or between Fig. 2(b) and Fig. 2(d). With the presence of protonated decanol molecules, the deuteration of SDS molecules made the asymmetry of the MT effects more pronounced, when comparing Fig. 2(a) with Fig. 2(c).

Except for the sample with both SDS and decanol molecules deuterated, the Z-spectra acquired with using the dual-frequency RF irradiation display increased MT effects and their broader spectral range, as seen in Figs. 2(a-c).

Proton NMR spectra from the sample containing only the protonated molecules are presented in Fig. 3, zoomed in for a clear display of the broad spectrum at the bottom of the water resonance. Since the proton spectrum of the liquid crystal spans a relatively small range of frequency offsets (~15 kHz), it is possible to observe how the system behaves under single- and dual-frequency RF irradiations despite the strong and sharp spectrum of water protons.

In Fig. 3, the black lines present the spectra recorded without applying any saturating RF irradiation, which show that the center of the underlying spectrum is located at about -3.5 ppm or -1750 Hz from the water resonance. The red and blue lines display those acquired following single-frequency RF irradiation, the frequency offsets of which are indicated by the corresponding colored arrows. The green lines exhibit those recorded following dual-frequency RF irradiation, which were irradiated simultaneously at the frequency positions pointed by the red and blue arrows. The single- and dual-frequency RF irradiations were power-matched.

When FID's were excited by a 90° pulse, the corresponding spectra, presented in the left column of Fig. 3, show that the single- and dual-frequency RF irradiations reduced the overall spectral intensities. The signal intensities were the smallest with the dual-frequency RF irradiations and, as for the single-frequency RF irradiation, negative frequency offsets caused more reduction than the corresponding positive frequency offsets.

The T_1 and T_2 relaxation times are presented in Table 1. While the deuteration of decanol molecules elongated T_1 and shortened T_2 , the deuteration of SDS molecules made T_2 longer but had little effects on T_1 . Note that the residual H₂O may interfere with the relaxation measurements for the samples containing D₂O.

In addition, the characteristic decay times estimated from the ADRF-ARRF experiments are presented as T_d 's in Table 1. Since the ADRF-ARRF sequence cannot filter out the signal growing due to the T_1 relaxation during the delay times, the longer T_d 's are related to the T_1 relaxation. Hence the shorter T_d 's should estimate the lifetimes of dipolar orders. Between the samples containing H₂O and D₂O, the shorter values of T_d 's were comparable in

magnitude (320 ms vs. 310 ms) for the samples containing deuterated decanol molecules, which became quite different (35 ms vs. 350 ms) for the samples containing deuterated SDS molecules.

For the samples where either SDS or decanol was deuterated, the spectra recorded from the ADRF-ARRF experiments are displayed in Fig. 4. With decanol molecules deuterated, the underlying spectra, which originate from protonated SDS molecules, exhibited similar decaying trends between the samples containing H₂O and D₂O, as shown in Fig. 4(a). When SDS molecules were deuterated, in contrast, the sample containing H₂O showed much faster decay than that containing D₂O. The Jeener-Broekaert sequence also confirmed the above observations, as seen in Fig. 5.

The simulated Z-spectra of abundant and MT pools are presented in Fig. 6. When $T_d = 300$ ms, the saturation by single-frequency RF irradiation grows incrementally with increasing the width of the Gaussian line shape, as depicted by the dashed lines in Fig. 6(a). On the other hand, the saturation by dual-frequency RF irradiation displays crossovers between Z-spectra, as represented by the solid lines in Fig. 6(a). Around the zero frequency offset, MT pools with smaller linewidths are more saturated and induce larger MT effects. As the frequency offset increases, the saturations become inefficient, starting from the MT pool with the narrowest linewidth.

When $T_d = 3$ ms, the difference between the simulated Z-spectra with single- and dual-frequency RF irradiations was reduced, as seen in Fig. 6(b). When compared to Fig. 6(a), the shortened T_d affected mostly the saturation by single-frequency RF irradiation.

When a line shape for a MT pool is given as the average of two Gaussian line shapes with different linewidths, the simulated Z-spectra manifest a clear distinction between the three cases, as depicted by the solid and dotted lines in Fig. 6(c). The numerical results even suggest that the MT pool with the averaged line shape could be distinguished from the case when two separate MT pools share a common abundant pool, the Z-spectra of which are shown as the dashed line in Fig. 6(c).

Discussion

The results of this work reconfirm decanol molecules as the major origin of the MT effects in this liquid-crystal system [28]. A decanol molecule has a labile proton in its hydroxyl group, which facilitates the transfer of spin polarization between decanol and water. The labile proton would be responsible for the MT effects even when both SDS and decanol molecules were deuterated, as seen in Fig. 2(d). When D₂O is substituted for H₂O, i.e., when the labile proton of a decanol molecule is replaced with a deuterium, the converse is evidenced by the elongated lifetime of the dipolar order established among the proton spins in a decanol molecule, as seen in Figs. 4(b) and 5(b).

As seen in Figs 4(a) and 5(a), the replacement of H₂O by D₂O hardly affects the lifetime of the dipolar order built in a SDS molecule, suggesting that the proton spins in a SDS molecule are rather isolated from water. On the other hand, the deuteration of SDS molecules seem to broaden the dip of the Z-spectrum, as seen between Figs. 2(a) and 2(c)

and between Figs. 2(b) and 2(d). One possible explanation for this apparent increase in MT effects may be the weakened intermolecular dipolar couplings caused by the deuteration of SDS molecules, which should make the relaxation times of decanol and water protons longer. While most noticeable with the T_2 relaxation times, as seen in Table 1, the longer relaxation times would help MT effects to be accumulated and kept intact. In other words, the proton spins in SDS molecules have an influence on the MT process between decanol and water molecules through cross-relaxation. Besides, SDS molecules may have another contribution to the MT effects in this liquid crystal system: It has been known that the sodium ions from SDS molecules modulate the exchange process between decanol and water molecules [28,29].

With protonated decanol molecules, the MT effects were stronger when the frequency offset was set to be negative, as seen in Figs. 2(a) and 2 (c). The reasons why the MT effects is asymmetric are because the proton NMR spectrum of decanol molecules is centered around about 3.5 ppm upfield from the resonance frequency of water protons and because a Z-spectrum is presented against the frequency offset from the water resonance. Due to the direct saturation of water protons, which is always centered around the water resonance, shifting the Z-spectrum along the frequency offset might not produce a symmetric one.

In Fig. 3, we presented proton NMR spectra recorded with a 5° pulse as well as a 90° pulse. Regardless of the flip angle of the pulse applied following the saturating RF irradiation, the normalized area of a spectrum gives the same information, which is the total z polarization or $\text{tr}\{\rho S_z\}$ right before the pulses. The use of a small flip angle is known to be useful for revealing the population differences between energy eigenstates [9,30–35]. For example, the spectra recorded with a 5° pulse following single-frequency RF irradiation cross zero at the frequency offset where the RF irradiation was applied, which can be clearly seen in Fig. 3(a), because the corresponding single-quantum transitions are saturated. The negative intensities indicate the population inversions between the eigenstates connected by the single-quantum transitions at the corresponding frequencies. Sometimes, the spectral intensity is observed to be higher than that from the thermal equilibrium state, which is due to the increased population differences resulting from a population redistribution during a RF irradiation. However, the total z polarization should always be reduced. As the flip angle used to record a spectrum increases, the population of a single eigenstate may be distributed over several eigenstates connected by successive single-quantum transitions. When the flip angle reaches 90° , the above-mentioned features disappear and the spectrum reflects only the total z polarization. Note that there have been misunderstandings regarding the effect of a flip angle on the spectrum of a spin cluster [16,17].

By comparing the relaxation of dipolar order in decanol between samples containing H_2O and D_2O , the transfer rate of the magnetization vector from the MT pool to the abundant pool may be estimated, which is $(35 \text{ ms})^{-1} - (350 \text{ ms})^{-1} \sim 26 \text{ s}^{-1}$. Note that the estimation of T_d for the proton spins in a decanol molecule was possible because of the selective deuteration of SDS molecules. Such separate measurements might not be likely to happen in general.

The kinetic equations of Provotorov's theory, Eqs. (5) and (6), deal with a 'homogeneous' system in the sense that a single Hamiltonian is assumed. It means that the parameters in the Hamiltonian of Eq. (1), such as the resonance frequency, RF frequency, and dipolar coupling constants, are uniquely given. In other words, the normalized absorption line shape $g(\Delta)$ in Eq. (7) originates from a system described by a single Hamiltonian. For example, the so-called super-Lorentzian line shape [36–38], which is given as the integral of a Gaussian function,

$$g_{\text{super-Lorentzian}}(\Delta) = \int_0^{\pi/2} d\theta \sin\theta \sqrt{\frac{2}{\pi}} \frac{T_2}{|3\cos^2\theta - 1|} \exp \left[-2 \left(\frac{\Delta T_2}{|3\cos^2\theta - 1|} \right)^2 \right], \quad (15)$$

presumes a homogeneously broadened spectrum if it is used as the normalized absorption line shape for solving the kinetic equations (*cf.* Ref. [15]).

There may be at least two strategies to cope with non-homogeneous cases. First, one may investigate how saturation and MT effects depend on the parameters in the Hamiltonian of Eq. (1). This approach would be suitable when parameters vary spatially or with respect to the orientation of a given system, so each location or orientation can be treated as an independent homogeneous system. For example, the strength of the dipole-dipole interaction depends on the direction connecting two interacting spins with respect to the external magnetic field. For a spin cluster, the overall strength of dipole-dipole interactions between nuclear spins is parameterized as ω_{loc} . So, the simulated Z-spectra presented in Figs. 6(a) and 6(b) may illustrate the dependences of saturation and MT effects on the orientation of a given MT pool, which may spatially vary.

When spatial or orientational variations cannot be separated, for example due to insufficient resolution, one may build a model with multiple MT pools, each of which is described by its own line shape. This is merely a heterogeneous situation with the assumption that there may not be any direct connections between individual components except through water. The simulated Z-spectra shown in Figs. 6(c) demonstrate that dual-frequency RF irradiation could have potential for distinguishing between different situations, which is mainly because the uniform saturation by dual-frequency RF irradiation is sensitive to the spectral range of a given system. Unless both frequency components touch the spectrum, the saturation would become less efficient, which leads to decreased MT effects.

It has been noted that the difference in the MT effects between single- and dual-frequency RF irradiations may depend on T_d [15,16] or WT_d [17]. As seen in Figs. 6(a) and 6(b), the MT effects with single-frequency RF irradiation increased as T_d decreased until they became similar to those with the power-matched dual-frequency RF irradiations. The discussion of a dipolar order would be meaningful if a given system reaches the quasi-equilibrium state described by Eq. (2) in a time scale faster than that given by T_d . For MT effects, however, the transfer rates of the magnetization vectors between abundant and MT pools would also be important. When the transfer rate is faster than the rate of dipolar relaxation, the difference in the saturation of the MT pool between single- and dual-frequency RF irradiations would be reflected in the MT effects, which is what was observed with the

liquid-crystal samples. As T_d decreases, the dipolar order would end up closer to its original state, which is zero. Note that the dipolar order is kept zero under dual-frequency RF irradiations. Therefore, the conditions for MT effects become more alike between single- and dual-frequency RF irradiations.

Conclusion

The difference in MT effects between single- and dual-frequency RF irradiations has been investigated on a lyotropic liquid crystal with a help of deuteration. Under the ‘power-matched’ condition, dual-frequency RF irradiation induced more MT effects due to more efficient saturation of a MT pool. This may happen because the MT pool consists of a cluster of strongly coupled nuclear spins, and thus dipolar order can be established within the spin cluster and can continue to exist while the magnetization of the MT pool is transferred to the abundant pool, i.e., the water protons. In addition, the MT effects induced by dual-frequency RF irradiation could be more sensitive to the spectral features of MT pools. As the lyotropic liquid crystal used in this work manifests a molecular arrangement common for some biological structures such as cell membranes and myelin sheaths, the results can be useful for interpreting MT effects in tissues containing such components, for example white matter in the brain and spinal cord. Whether used alone or together with single-frequency RF irradiation, dual-frequency RF irradiation would be beneficial to the spectroscopic and imaging applications that use saturation transfer techniques.

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Highlights

- Dual-frequency saturation enhances MT effects from a dipolar-coupled spin cluster.
- Such MT effects can be more sensitive to the spectral features of a MT pool.
- With the help of deuteration and T_{1d} measurements, the exchange rate was estimated.

Figure 1.

NMR pulse sequences for T_d measurements. (a) The ADRF/ARRF sequence. The black rectangle represents a 90° pulse, and the gray triangles decreasing and increasing linear ramps. The delay t was varied to measure T_d . (b) The JB sequence. The wide and narrow black rectangles represent 90° and 45° pulses, respectively. The delay τ was fixed to be 50 μ s, and the delay t was varied to measure T_d . Under the individual pulses, their phases used in the phase cycling are displayed.

Figure 2.

Z-spectra of water following single- and dual-frequency presaturations. The r.m.s. powers were matched between the single- and dual-frequency RF irradiations. For single-frequency RF irradiation, the upward (downward) triangles connected by a dashed (solid) line represent the part of the Z-spectrum along the positive (negative) frequency offsets. The circles connected by a thick solid line comprise the Z-spectrum with the RF irradiation applied simultaneously at both the positive and negative frequency offsets. The liquid-crystal systems consist of (a) protonated SDS and protonated decanol, (b) protonated SDS and deuterated decanol, (c) deuterated SDS and protonated decanol, and (d) deuterated SDS and deuterated decanol.

Figure 3.

Saturation of ^1H NMR spectrum by single- and dual-frequency RF irradiations. The vertical scales were adjusted to reveal the spectrum belonging to SDS and decanol molecules. The black lines represent the spectra recorded without any saturating RF irradiation. The red and blue lines display the spectra recorded following single-frequency RF irradiation

($\gamma B_1/2\pi = 100\sqrt{2}$ Hz) respectively at the positive and negative frequency offsets, the positions of which are indicated by the colored arrows. The green lines exhibit the spectra acquired with the dual-frequency RF irradiation (RF strength = $100\text{ Hz} \times 2$), which were applied simultaneously at the frequency offsets indicated by the colored arrows. The spectra in the left and right columns were recorded by using the excitation pulses with the flip angles of 90° and 5° , respectively. The absolute values of the frequency offsets are (a) 3 kHz, (b) 6 kHz, and (c) 9 kHz.

Figure 4.

The relaxation of dipolar order as a function of the delay t of the pulse sequence shown in Fig. 1(a). The liquid-crystal samples were prepared with H_2O (upper row) and D_2O (lower row), and comprised of (a) protonated SDS and deuterated decanol and (b) deuterated SDS and protonated decanol.

Figure 5.

The relaxation of dipolar order as a function of the delay t of the pulse sequence shown in Fig 1(b). The liquid-crystal samples were prepared with H_2O (upper row) and D_2O (lower row), and comprised of (a) protonated SDS and deuterated decanol and (b) deuterated SDS and protonated decanol.

Figure 6.

Simulated Z-spectra with varying the spectral width of a MT pool. (a,b) Z-spectra of abundant and MT pools for $\omega_{loc}/2\pi = 1$ kHz, 2 kHz, 3 kHz, 4 kHz, and 5 kHz and T_d (a) 300 ms and (b) 3 ms. Solid and dashed lines display those under dual- and single-frequency RF irradiations, respectively. (c) Z-spectra of abundant and MT pools for $\omega_{loc}/2\pi = 0.5$ kHz and 1.0 kHz (dotted lines), the averaged spectral line (solid lines), and two separate MT pools (dashed lines).

Relaxation times measured from the liquid crystal samples made of different combinations of protonated and deuterated molecules. T_1 and T_2 are respectively the longitudinal and transverse relaxation times. T_d is the characteristic decay time from the ADRF-ARRF experiment.

Table 1

	Protonated SDS		Protonated SDS Deuterated Decanol		Deuterated SDS Protonated Decanol	
	H ₂ O	D ₂ O	H ₂ O	D ₂ O	H ₂ O	D ₂ O
T_1	2.1 s	0.8 s	2.9 s	0.9 s	2.1 s	2.7 s
	0.8 s		1.0 s			1.0 s
T_2	2.4 ms	29 ms	1.6 ms	9.2 ms	16 ms	72 ms
	38 μ s	31 μ s		21 μ s	450 μ s	140 μ s
T_d	1.9 s	1.1 s	2.4 s	4.1 s	2.2 s	2.4 s
	140 ms	240 ms	320 ms	310 ms	35 ms	350 ms