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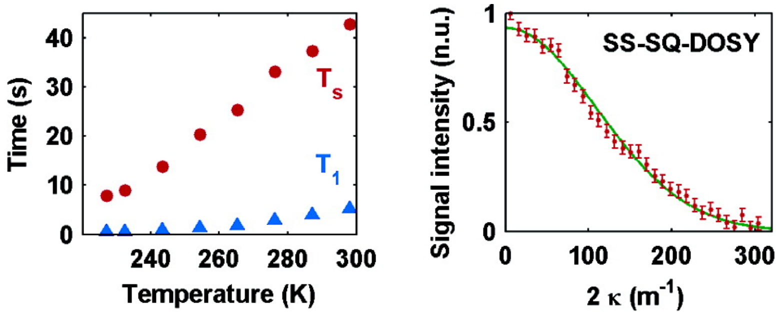

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Slow Diffusion by Singlet State NMR Spectroscopy

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Abstract: Small diffusion coefficients can be measured by using populations of singlet states that have a relaxation time constant, T_s, which can be much longer than the longitudinal relaxation time, T_1. Spatial information can be encoded with pulsed field gradients in the manner of stimulated echo sequences. Singlet states can be excited via double-quantum coherences to enhance the efficiency of phase encoding and decoding.

Introduction

Nuclear magnetic resonance (NMR) has long been known as a flexible tool to study transport phenomena, such as diffusion, flow, convection, or electrophoretic mobility.[1−3] To this effect, information about the localization of molecules can be encoded and decoded by pulsed field gradients (PFG) before and after a delay where translational motions occur. A PFG can be characterized by a product $\kappa = \gamma p s G_{\max}\delta$, where γ is the gyromagnetic ratio, p the coherence order, $G_{\max}$ the peak intensity of the gradient, and δ its duration. The dimensionless shape factor $0 < s \leq 1$ is defined by $s = \int_0^\delta G(t)\mathrm{d}t/(G_{\max}\delta)$. When the signal S is observed as a function of the gradient strength and compared to a signal S_0 obtained with very weak gradients, with all other parameters remaining the same, the decay of the ratio obeys a Gaussian function:

$$S/S_0 = \exp(-D\kappa^2\Delta) \tag{1}$$

where D is the diffusion coefficient and Δ the effective interval between encoding and decoding by PFGs. The analysis of the Gaussian decays (for example, by a Laplace transformation) allows one to determine the diffusion coefficients D, and when different species in solution with different isotropic chemical shifts are separated by Fourier transformation of the signals, the resulting two-dimensional pictures are referred to as "diffusion ordered NMR spectroscopy" (DOSY).[3]

Depending on the details of the pulse sequences, one must take into account attenuation factors that depend on various delays where transverse and longitudinal magnetization components suffer from T_2 or T_1 relaxation. In the most elementary spin−echo experiments, T_2 relaxation limits the interval where diffusion can be observed to $\Delta \approx T_2$. In stimulated echo experiments, such as in Figure 1b, where $\Delta = T + 2\tau_1 - 2\delta/3$, the information is stored in the form of longitudinal magnetization, so that the window can be extended to $T \approx T_1$, which allows one to probe slower transport processes since $T_1 \geq T_2$. Usually, PFG experiments are applied to protons ($I = {}^1\mathrm{H}$) because of their favorable gyromagnetic ratio γ. With so-called "heteronuclear stimulated echo" (X-STE) sequences, the spatial information can be "stored" in the form of longitudinal magnetization of nuclei with low γ and long T_1.[4−6] For $I = {}^1\mathrm{H}$ and $S = {}^{15}\mathrm{N}$ in biomolecules, one may typically have $T_1({}^{15}\mathrm{N}) \approx 1$ s $> T_1({}^1\mathrm{H}) \approx 100$ ms, thus making it possible to determine 10-fold smaller diffusion coefficients D with the same gradient strength $G_{\max}$.

Recently, Carravetta et al.[7,8] have shown that nuclear spin order can be stored as *singlet states* which relax with a time constant, T_s, that can be much longer than T_1. Singlet states can be generated in scalar-coupled two-spin systems. After exciting a zero-quantum coherence, ZQC_y, and allowing it to evolve under the effects of the chemical shifts into $\mathrm{ZQC}_x = |\alpha\beta\rangle\langle\beta\alpha| + |\beta\alpha\rangle\langle\alpha\beta|$, radio frequency (RF) irradiation leads to the suppression of the chemical shifts, while the J_{IS} interaction remains. As a result, the IS system is converted into an I_2 system, where the two nuclei have, in effect, become magnetically equivalent. In the process, the two eigenstates, $|\alpha\beta\rangle$ and $|\beta\alpha\rangle$, become degenerate and can be expressed as a superposition of a triplet state $|T_0\rangle = 2^{-1/2}\{|\alpha\beta\rangle + |\beta\alpha\rangle\}$ and a singlet state $|S_0\rangle = 2^{-1/2}\{|\alpha\beta\rangle - |\beta\alpha\rangle\}$, so that the zero-quantum coherence

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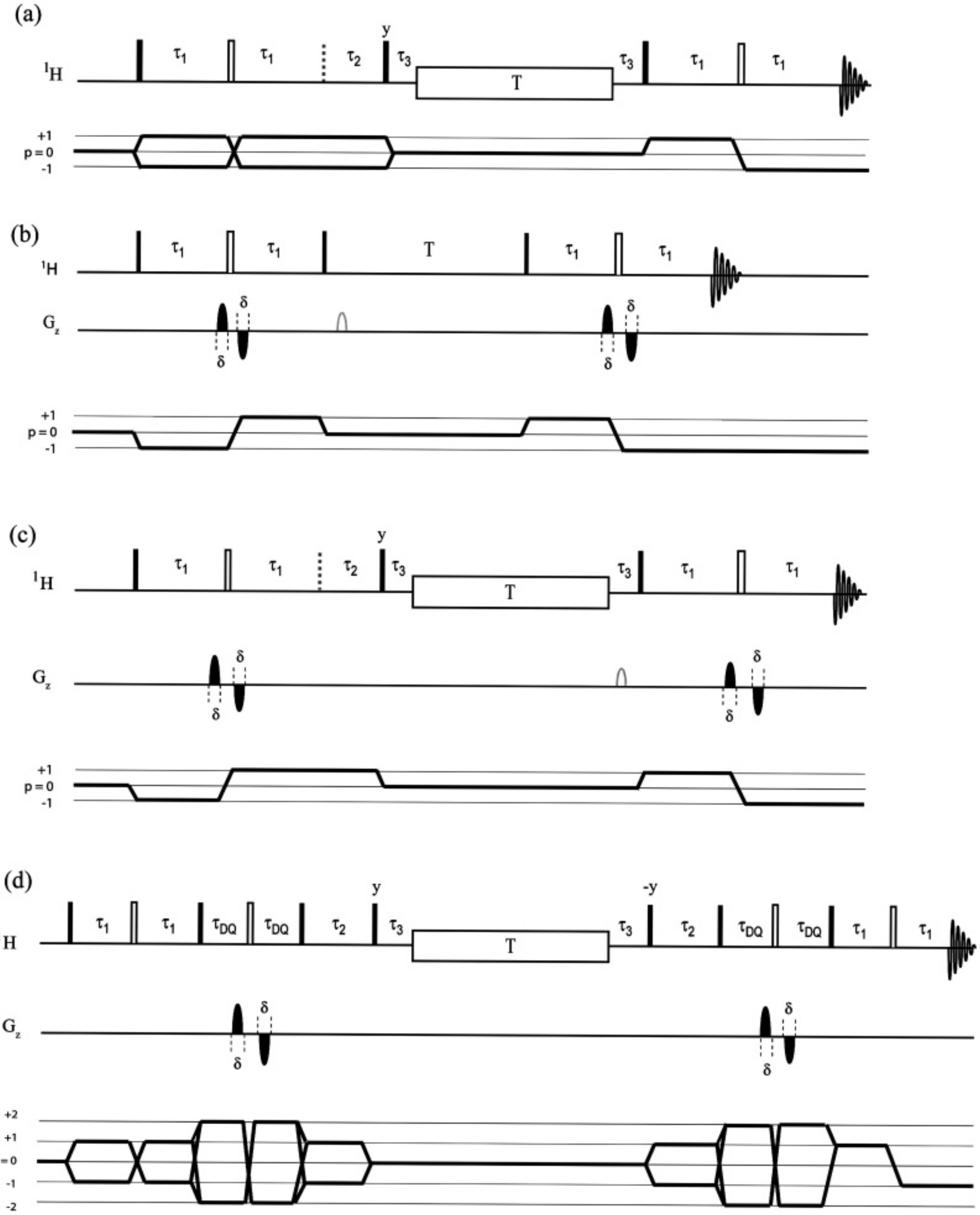

Figure 1. Pulse sequences and coherence transfer pathways. (a) Method of Carravetta et al.[8] for the excitation and detection of long-lived singlet states in scalar-coupled two-spin systems with a coupling J_{IS} and chemical shifts ω_I and ω_S. The optional sandwich $[\tau_1 - \pi - \tau_1]$ at the end allows one to convert anti-phase into in-phase magnetization. The intervals must be $\tau_1 = 1/(4J_{IS})$, $\tau_2 = \pi/|\omega_I - \omega_S|$, and $\tau_3 = \tau_2/2$. Filled and open rectangles represent $\pi/2$ and π pulses, respectively. Pulsed field gradients (PFGs) have a sinusoidal shape. Filled symbols refer to PFGs for encoding and decoding; open symbols represent "purge" pulses used to destroy transverse magnetization. (b) Standard stimulated echo sequence with bipolar pulse pairs (BPPSTE), often used for "diffusion-ordered spectroscopy" (DOSY). (c) "Singlet-state-single-quantum-DOSY" (SS-SQ-DOSY), which combines the key features of (a) and (b), the information about spatial localization being stored in the form of singlet state populations with a relaxation time T_s in the interval *T*. The intervals τ_1, τ_2, and τ_3 are defined as in sequence (a). (d) Singlet-state-double-quantum-DOSY (SS-DQ-DOSY), where the bipolar PFGs are applied to double-quantum coherences so that slower diffusion can be studied, or so that the gradient intensities or durations can be attenuated by a factor 2. The intervals τ_1, τ_2, and τ_3 must be like those in sequence (a). The sandwich $[\tau_1 - \pi - \tau_1]$ is again optional. All pulses are applied along the *x*-axis unless specified otherwise. Phase cycles can be obtained from the authors upon request.

can be written as $ZQC_x = {}^1/_2\{|T_0\rangle\langle T_0| - |S_0\rangle\langle S_0|\}$. The expectation values of the operators $|T_0\rangle\langle T_0|$ and $|S_0\rangle\langle S_0|$ amount to *populations* of the central triplet and singlet states. The former have a lifetime of the order of T_1, while the latter have a longer singlet state lifetime T_s. The long lifetimes of singlet states make them attractive for the study of slow transport processes.

Theory

Figure 1a shows the sequence proposed by Carravetta et al.,[8] which has been designed for molecules containing weakly coupled homonuclear *IS* spin systems. The RF carrier is positioned in the middle between the two doublets at $\omega_0 = (\omega_I + \omega_S)/2$. After the initial preparation "sandwich" $[(\pi/2)_x - \tau_1 - (\pi)_x - \tau_1]$ with $\tau_1 = 1/(4J_{IS})$, one has a density operator $\sigma = -2I_xS_z - 2I_zS_x$. In the subsequent interval $\tau_2 = \pi/(2|\omega_I - \omega_0|) = \pi/(2|\omega_S - \omega_0|) = \pi/|\omega_I - \omega_S|$, one obtains $\sigma = 2I_yS_z - 2I_zS_y$ since the transverse components undergo a precession in opposite directions through one-quarter of a full cycle. The $(\pi/2)_y$ pulse at the end of the τ_2 interval converts this into $\sigma = 2I_yS_x - 2I_xS_y = -i(I_+S_- - I_-S_+) = 2ZQC_y$. During the interval $\tau_3 = \tau_2/2 = \pi/(2|\omega_I - \omega_S|)$, this is transformed under the effect of the chemical shift of the zero-quantum coherence $\omega_{ZQC} = \omega_I - \omega_S$ into $\sigma = 2I_xS_x + 2I_yS_y = I_+S_- + I_-S_+ = 2ZQC_x$. Finally, at the onset of RF irradiation, $2ZQC_x$ is expressed as a superposition $\sigma = |T_0\rangle\langle T_0|$ and $|S_0\rangle\langle S_0|$. The triplet term $|T_0\rangle\langle T_0|$ does not survive the protracted interval T since it relaxes on a time scale of T_1, while the singlet term $|S_0\rangle\langle S_0|$ decays much more slowly following a monoexponential function $\exp\{-T/T_s\}$. At the end of the interval T, the remaining singlet term is converted into $\sigma = {}^1/_2(2ZQC_x)$, plus other terms that cannot contribute to the signals. Note the factor ${}^1/_2$ which is due to the dissipation of the triplet term $|T_0\rangle\langle T_0|$. During the second interval $\tau_3 = \pi/(2|\omega_I - \omega_S|)$, the density operator is converted back into $\sigma = ZQC_y = {}^1/_2(2I_yS_x - 2I_xS_y)$. The final $(\pi/2)_x$ pulse generates observable anti-phase single-quantum coherence $\sigma = {}^1/_2(2I_zS_x - 2I_xS_z)$. Note that this corresponds to an up−down doublet for I, and a down−up doublet for S. If a $[\tau_1 - (\pi)_x - \tau_1]$ sequence is appended as shown in Figure 1a, one obtains $\sigma = -{}^1/_2(I_y - S_y)$, i.e., two in-phase doublets with opposite signs. A further delay $\tau_2 = \pi/|\omega_I - \omega_S|$ can be added to obtain $\sigma = -{}^1/_2(I_x + S_x)$, i.e., two in-phase doublets with the same signs. Whether the reconversion from anti-phase into in-phase coherences is worth doing depends on the coupling constant, J_{IS}, on the inhomogeneous line-width, which is proportional to $1/T_2^*$, and on the spin−echo decay rate $1/T_2$.

Figure 1b shows a conventional stimulated echo sequence[1,9−13] that is widely used to measure diffusion and other transport phenomena. This sequence is normally applied to spins without scalar couplings, most often to protons because of their favorable gyromagnetic ratio. To measure very small diffusion coefficients, one can use special probes equipped with heavy-duty coils capable of generating very intense gradients, albeit at the expense of spectral resolution. The initial sandwich $[(\pi/2)_x - \tau_1 - (\pi)_x - \tau_1 - (\pi/2)_x]$ generates longitudinal magnetization, which is modulated along the z-axis of the sample[14] because of the two bipolar PFGs with $G_z = dB/dz$. Eddy currents induced by the bipolar pairs of field gradients with opposite signs partly cancel each other. The refocusing pulses in the preparation and detection sandwiches inter alea eliminate artifacts due to chemical shifts.[13] In systems with homonuclear scalar couplings, one may use $\tau_1 = 1/(2J_{IS})$ to have in-phase magnetization at the end of the second τ_1 interval, if transverse relaxation is not too fast. The state of the system at the beginning of the T interval can be described by $\sigma = I_z \cos(2\kappa z) + I_x \sin(2\kappa z)$, where the factor 2 reflects the fact that two pulsed field gradients are applied.[6b] The I_x term can be eliminated by phase-cycling or by applying a spoiling gradient. At the end of the interval T, the cosine modulation of the longitudinal magnetization across the sample will be attenuated by diffusion and longitudinal relaxation. The remaining order $\sigma(z) = I_z \cos(2\kappa z) \exp\{-D4\kappa^2\Delta\} \exp\{-2\tau_1/T_2\} \exp\{-T/T_1\}$ will be transformed by another sandwich $[(\pi/2)_x - \tau_1 - (\pi)_x - \tau_1]$, again combined with two bipolar pulsed field gradients, which lead to spatial "decoding", that is, to a multiplication with another factor $\cos(2\kappa z)$ or a global weighting with $\cos^2(2\kappa z)$. The receiver detects the *average* magnetization over the sample, that is, $\langle\cos^2(2\kappa z)\rangle = 1/2$, so that the net effect can be described by $\sigma = (1/2)I_z \exp\{-D4\kappa^2\Delta\} \exp\{-2\tau_1/T_2\} \exp\{-T/T_1\}$.

The key idea of this paper is shown in Figure 1c, which results from a simple combination of a and b in Figure 1. Like the experiment of Carravetta et al., a singlet state is excited at the beginning of the diffusion interval T in Figure 1c. Similar to the conventional stimulated spin−echo sequence, spatial information is encoded and later decoded by a bipolar pair of PGFs. The phase-decoding occurs during the reconversion sandwich $[\tau_1 - (\pi)_x - \tau_1]$, where $\tau_1 = 1/(4J_{IS})$ as in Figure 1a. The signals are attenuated in proportion to $\exp\{-D4\kappa^2\Delta\} \exp\{-2\tau_1/T_2\} \exp\{-T/T_s\}$, where $\Delta = T + 2\tau_1 + \tau_2 + 2\tau_3 - 2\delta/3$. Transverse relaxation in the short delays τ_2 and τ_3 can be neglected. Thus the main difference lies in the fact that T_1 is replaced by the much longer singlet state relaxation time, T_s.

Figure 1d shows a variant where the anti-phase single-quantum coherences generated at the end of the second τ_1 interval, $\sigma = 2I_xS_z + 2I_zS_x$, are temporarily converted into double-quantum coherences $\sigma = -(2I_xS_y + 2I_yS_x) = i(I_+S_+ - I_-S_-) = -2DQC_y$, with coherence orders $p = \pm 2$. In the two intervals τ_{DQ}, these coherences can be spatially encoded with greater efficiency than their single-quantum counterparts since the effect of the bipolar PGFs is proportional to the coherence order p. Thus, compared to Figure 1c, it is possible to obtain the same effect with field gradients that are half as strong or half as long. Alternatively, with a given gradient strength and duration, it is possible to determine diffusion constants that are 4 times smaller. Both $p = \pm 2$ pathways can be selected in the τ_{DQ} intervals. After spatial encoding, the DQC terms are converted back into anti-phase SQC, allowed to undergo precession for $\tau_2 = \pi/|\omega_I - \omega_S|$ to turn $\sigma = 2I_xS_z + 2I_zS_x$ into $\sigma = -2I_yS_z + 2I_zS_y$, transformed into ZQC, and from there into singlet state populations $|S_0\rangle\langle S_0|$. The final reconversion sandwich $[\tau_1 - (\pi)_x - \tau_1]$, which leads to in-phase doublets, is optional.

We like to refer to the method of Figure 1c as "singlet-state-single-quantum-diffusion-ordered spectroscopy" or SS-SQ-DOSY. The method of Figure 1d can be dubbed "singlet-state-double-quantum-DOSY" or SS-DQ-DOSY.

In principle, singlet states can be excited for any pair of homonuclear coupled spins, and their lifetimes can be attractive provided that they are sufficiently isolated from magnetic interactions that break their symmetry. Relaxation of the singlet state is very slow if there are no mechanisms other than the intramolecular dipole−dipole coupling between the two nuclei. However, intramolecular couplings to remote protons within the same molecule, CSA interactions, and intermolecular couplings, including protons on solvent molecules, can contribute to the relaxation of singlet states. Partial deuteration offers a possibility of reasonably isolated subsystems comprising only two coupled protons, as may occur in CH_2 groups in nonsymmetrical environments. Large molecules with long correlation times should behave like small molecules in viscous solvents with respect to their rotational diffusion properties.

Results and Discussion

We have used 2-chloroacrylonitrile (i.e., $H_IH_SC{=}CRR'$ with $R = Cl$ and $R' = CN$), as suggested by Carravetta et al.[8] Clearly, many other substituents can be considered, including systems

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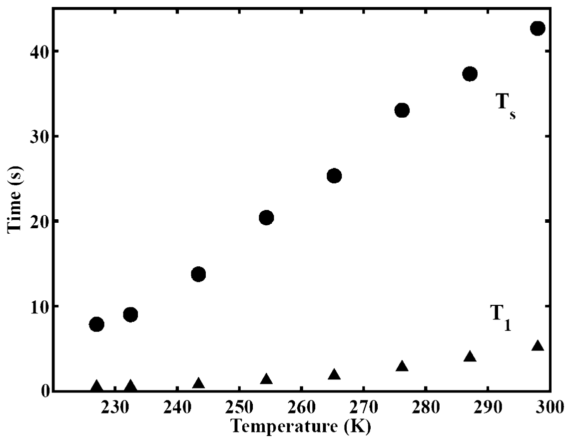

Figure 2. Temperature dependence in the range from −46 to +25 °C (227 to 298 K) of the spin−lattice relaxation time T_1 and the singlet state lifetime T_s of the protons H_I and H_S in 10 mM 2-chloroacrylonitrile $H_IH_SC{=}CClCN$, dissolved in a mixture of deuterated DMSO-d_6/D_2O with a 1:3 ratio, measured at 300 MHz with a Bruker spectrometer equipped with a triple-resonance triple-gradient probe. The T_1 relaxation times were measured with the standard inversion−recovery method; the T_s lifetimes were determined by incrementing the interval T in the sequence of Figure 1a.

where R and/or R′ denote some bulky molecular fragments. To study the dependence of the relaxation rates and diffusion coefficients on viscosity, we dissolved 10 mM 2-chloroacrylonitrile in a mixture of deuterated DMSO-d_6/D_2O with a 1:3 molar ratio. In a temperature range from −46 to +25 °C, the viscosity[15] varies from 150 to 2.3 cP, so that a wide range of rotational correlation times, τ_c, could be investigated.

Figure 2 shows the temperature dependence at 300 MHz of the ordinary spin−lattice relaxation time T_1 of the protons I and S (measured by the standard inversion−recovery method) and the lifetime T_s of the singlet state (measured by incrementing the interval T in the sequence of Figure 1a). In this sample, the gain is about 1 order of magnitude over the whole temperature range.

The Gaussian decays of the signal intensities in Figure 3a, obtained with the conventional stimulated echo sequence of Figure 1b, can be compared with the signal intensities in Figure 3b obtained with the singlet-state sequence of Figure 1c, both recorded at −18.8 °C (254 K) and 300 MHz. Since the lifetime T_s of the singlet state is about 20 s at 254 K, it was possible to set $T = 19.3$ s ($\Delta = 19.5$ s) in the SS-SQ-DOSY sequence, compared to $\Delta = 0.21$ s in the conventional stimulated echo sequence, and hence to reduce κ by a factor of 13. Using the same gradient strength, $G_{\max}$, the length δ of the gradients could thus be reduced from 2000 to 155 μs.

Figure 4 shows diffusion coefficients of 2-chloroacrylonitrile in 1:3 DMSO-d_6/D_2O measured with the singlet state method as a function of temperature. At −46 °C, we found that $D = (1.5 \pm 0.1) \times 10^{-11}$ m^2 s^{-1}. The duration of the diffusion interval can be extended by a factor 10 compared to the conventional stimulated echo method since $T_s \approx 10\ T_1$ in the sample under investigation.

Conclusions

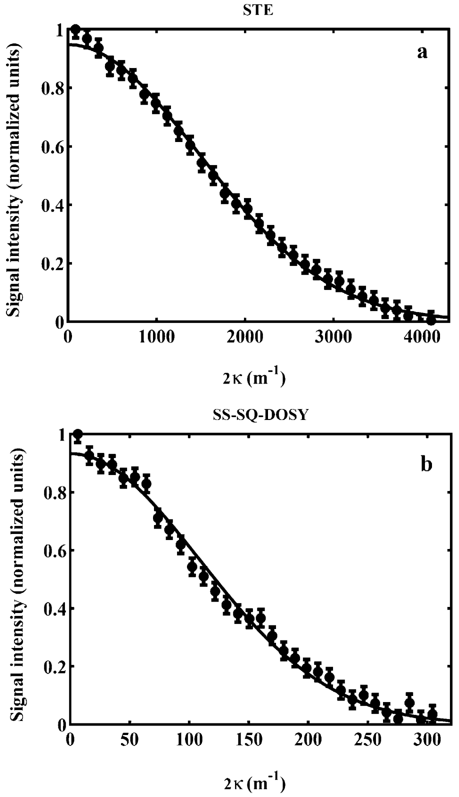

Figure 3. Gaussian decays of proton signal intensities of 10 mM 2-chloroacrylonitrile in DMSO-d_6/D_2O = 1:3, measured at 300 MHz and $T = -18.8$ °C (254 K) obtained (a) with the conventional stimulated echo sequence of Figure 1b with $\Delta = 0.21$ s, $\delta = 2$ ms, and (b) with the single-quantum singlet-state sequence of Figure 1c with $\Delta = 19.5$ s, $\delta = 155\ \mu$s, $\tau_1 = 80$ ms, $\tau_2 = 10$ ms, $\tau_3 = 5$ ms. The parameter κ is defined by $\kappa = \gamma p s G_{\max}\delta$. Note the scales of the horizontal axes.

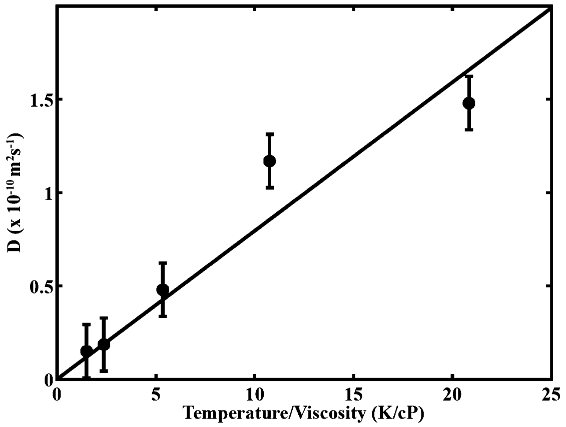

Figure 4. Diffusion coefficients of 2-chloroacrylonitrile in 1:3 DMSO-d_6/D_2O measured with the singlet state method of Figure 1c as a function of the ratio of the temperature over the viscosity.

It has been shown that slow transport phenomena can be characterized by combining established methods for the measurement of diffusion coefficients with the excitation and detection of singlet states. The transient excitation of double-quantum coherences allows one to use shorter gradients. In 2-chloroacrylonitrile, the lifetimes T_s of the singlet states were found to be about 1 order of magnitude longer than the longitudinal relaxation times T_1 over a wide range of temperatures.

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