Deuterium Nuclear Magnetic Resonance Spin–Lattice Relaxation of Analytically Relevant Solvent Systems

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We previously reported that the nuclear magnetic resonance (NMR) ^{14}N spin–lattice relaxation times (T_1) of CH_3CN in CH_3CN–H_2O mixtures directly correlate with the solution viscosity when scaled for temperature (η/T) in this common chromatographic mobile phase system.[1] Here, we demonstrate that the ^{2}H T_1 relaxation times also correlate with viscosity, contrary to a previous report.[2] This establishes ^{2}H T_1 relaxation times as a useful means of measuring changes in solution viscosity in CH_3CN–H_2O mixtures. We show thermal convection to result in grossly decreased, apparent T_1's, by as much as ~40%, in nonspinning samples. This effect can be eliminated by moderate sample rotation or confinement of the sample to within the rf-irradiated region. The problem of thermal convection is revealed in systems having long T_1's and has implications in T_1 experiments employing nonspinning samples at elevated temperatures, including inherently nonspinning systems, such as those used in high-pressure studies.

Many analytical systems involve species in confined environments or near an interface, making it difficult to measure macroscopic properties that can be easily measured in bulk systems. Our interest is in the determination of viscosity changes in analytical systems in which species are in contact with or affected by an interface through the measurement of changes in a microscopic quantity, namely, NMR T_1 relaxation times of quadrupolar nuclei. Quadrupolar relaxation resulting from the interaction between the quadrupole moment and the local electric field gradient generally dominates over other relaxation mechanisms, and measured T_1 values can be directly related to molecular reorientation through the reorientational correlation time (τ_c),[3]

$$\frac{1}{T_1} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \left(1 + \frac{\eta_q^2}{3}\right)\left(\frac{eQq}{h}\right)\tau_c(\theta) = \frac{3\pi^2}{2}\chi^2\tau_c(\theta) \quad (1)$$

where θ is the angle between the vector containing the pertinent

relaxation interaction (along the bond axis of the quadrupolar nucleus of interest) and the symmetry axis of the molecule, I is the spin quantum number, η_q is the asymmetry parameter, e is the fundamental unit of electrical charge, Q is the quadrupole moment, q is the local electric field gradient, and h is Planck's constant. For ^{2}H and ^{14}N, $I = 1$, and $\eta_q = 0$. Consolidation of the terms related to the quadrupole interaction into the quadrupolar coupling constant, $\chi = eQq/h$, results in the simplified expression shown on the right-hand side of eq 1. This relation holds within the motionally narrowed regime ($\tau_c\omega \ll 1$, where ω is the Larmor frequency) and under the condition that the spin–lattice relaxation time is equal to the spin–spin relaxation time ($T_1 \approx T_2$). We have used $\chi = 1.65$ kHz for ^{2}H and 3.74 MHz for ^{14}N in our determination of τ_c from the experimental T_1 values.[4] This τ_c can be related to two rotational diffusion coefficients, one perpendicular and one parallel to the molecular axis of symmetry. For CH_3CN, ^{14}N T_1 measurements probe only the perpendicular rotational diffusion, because the nitrogen atom is coincident with the molecular symmetry axis, whereas ^{2}H T_1 measurements probe a mixture of the two rotations due to the angle the deuterium atom makes with respect to the symmetry axis of the molecule.

We previously measured the T_1 relaxation times of CH_3CN–H_2O mixtures as a function of temperature and composition through ^{14}N measurements of CH_3CN.[1] T_1 linearly increases with temperature, and τ_c is directly correlated with the bulk viscosity of the solution when scaled for temperature (η/T) over the entire binary composition range, as predicted by traditional hydrodynamic theory. We note that the η/T correlations have varying slopes as a function of composition, showing a deviation from traditional hydrodynamic theory that can be attributed to solute–solvent interactions and the molecularity of the system. These results indicate that measurements of quadrupolar T_1 relaxation times provide a reliable measure of *changes* in viscosity as a function of thermodynamic state for a given composition. Again, we believe the validity of this relationship to be important in systems not amenable to macroscopic viscosity measurements and have demonstrated its use for determining viscosity changes of CH_3CN–H_2O in contact with a C_{18}-bonded stationary phase.[1]

Previous ^{2}H T_1 relaxation studies conducted examining common chromatographic solvent mixtures, such as CH_3CN–H_2O and CH_3OH–H_2O, both in the bulk solvent and in contact with

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stationary phases, led us to initially choose ^2H for our investigations.[2,5-9] We wanted to examine the ^2H T_1 relaxation times of CH_3CN in CH_3CN-H_2O as a function of temperature and composition in an effort to validate the quadrupolar relaxation measurements as a means of determining changes in the solution viscosity. Our initial experiments indicated that there was not a simple relationship between the measured T_1 and η/T. Additionally, previous ^2H work by others on the CH_3CN-H_2O system indicated no apparent relation to solution viscosity,[2] and we were led to conclude that NMR relaxation could not be used as a probe of the solution viscosity in this system.

In an effort to understand the nature of the ^2H relaxation in CH_3CN and the nonmonotonic temperature response present in the initial measurements, we reinvestigated the ^2H NMR of this solvent system. The present studies show that systematic error arises in the measurements as a result of the effects of thermal convection, but that the elimination of convective flow by moderate sample rotation provides T_1's that increase monotonically with temperature. We also note significant differences between our ^2H T_1 results in spinning samples at 30.0°C and those presented in an earlier study by Bliesner and Sentell[2] that cannot be contributed to convective flow. Finally, we show through comparison of ^2H and ^{14}N τ_c values that the ^2H T_1 measurements do correlate with the solution viscosity of CH_3CN-H_2O when scaled for temperature (η/T), allowing the determination of viscosity changes in this system through a measurable microscopic quantity.

MATERIALS AND METHODS

Sample Preparation. H_2O (Fisher, HPLC Grade) was used as received. CH_3CN (Burdick & Jackson, 99.9+%) was dried with molecular sieves (Fisher, type 4 Å) and used as received. Acetonitrile-d_3 (Cambridge Isotopes, 99.96%) and deuterium oxide (Aldrich, 99.9%) were both used as received. For ^2H experiments, 20% deuterated solvents were used. CH_3CN-H_2O mixtures were prepared at various compositions in small glass vials using calibrated volumetric pipets, and mixed well before transferring samples to clean, dry 5-mm NMR tubes (Wilmad). Subjecting the samples to multiple freeze−pump−thaw cycles to remove dissolved oxygen had no effect on the T_1 values outside the nominal error associated with the T_1 measurement. Subsequently, no degassing step was employed in sample preparation.

NMR Measurements. Measurements were made on a Bruker 400 MHz spectrometer, operating at 55.5 MHz for ^2H and 28.5 MHz for ^{14}N, employing a Bruker 5-mm broadband probe. The 10-mm experiment was performed on a Bruker 500 MHz spectrometer operating at 76.8 MHz for ^2H and using a Bruker 10-mm broadband probe. Sample temperature was calibrated using a type J-K-T thermocouple (Omega Engineering, model HH21) and controlled to within ±0.1 °C using the Eurotherm BVT3000 temperature controller supplied with the spectrometer. Temperature was allowed to equilibrate between experiments.

The standard inversion recovery sequence (180°−τ−90°− acquire) was employed using nine delay times. All spectra with the exception of those presented in Figure 1A were acquired with

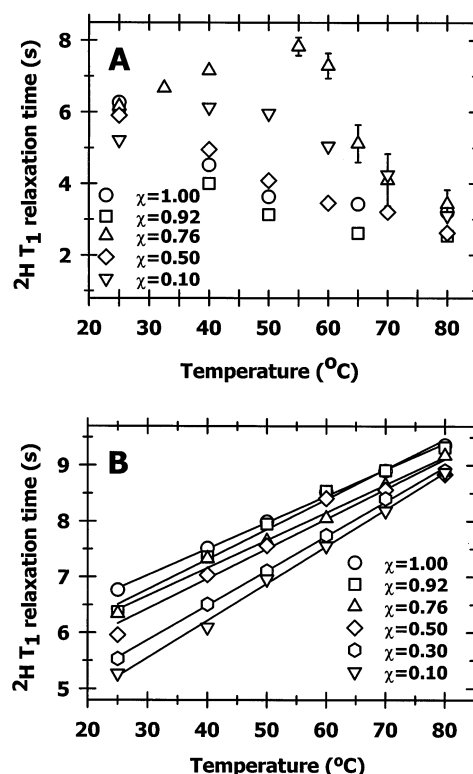(5) Marshall, D. B.; McKenna, W. P. *Anal. Chem.* **1984**, *56*, 2090−2093.

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Figure 1. ^2H T_1 relaxation times for CH_3CN in CH_3CN-H_2O mixtures as a function of temperature for various mole fractions (χ) of CH_3CN. (A) Nonspinning samples. Error bars shown for $\chi = 0.76$ are ±1 standard deviation of triplicate measurements. (B) Spin rate = 20 Hz.

a sample spin rate of 20 Hz. Spectra were acquired unlocked, and the preacquisition delay was always >$5T_1$. The null point (τ_{null}) was determined for each sample to ensure that appropriate delay times were chosen. The shortest delay time used was 0.3 μs, followed by τ_{null}, $0.5\tau_{null}$, $0.25\tau_{null}$, $0.75\tau_{null}$, $1.5\tau_{null}$, $2\tau_{null}$, $2.75\tau_{null}$, and $5T_1$. Experiments at different delay times were collected nonsequentially to ensure that systematic error was not introduced. T_1 values were determined from the data in several ways. First, T_1 values were calculated by fitting both the intensity and integrated area of the peak as a function of delay time to a 3-parameter single-exponential function within the Bruker software. T_1 values were also calculated by plotting $\ln[(I_\infty - I_t)/(I_\infty - I_0)]$, where I_∞, I_0, and I_t are the measured intensities or areas at the longest delay, shortest delay, and as a function of delay time, and extracting T_1 from the slope of the line (slope = $-1/T_1$). It has been shown that an accurate I_∞ value is necessary when determining the T_1 data from a log−linear plot, because an inappropriate value can cause increased error at long delay times.[10] We have found using $I_\infty = 5T_1$ to introduce no deviations from linearity at long times, and all linear fits have correlation coefficients (R^2) ≥ 0.9915.

RESULTS AND DISCUSSION

The ^2H T_1 relaxation times for CH_3CN in CH_3CN-H_2O mixtures as a function of temperature in nonspinning samples are shown in Figure 1A. Studies have shown that the ^2H T_1 relaxation times in neat CH_3CN show a linear increase as a function of

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temperature,[11-14] and so it was expected that our initial ^2H T_1 experiments would provide similar results. However, Figure 1A shows nonmonotonic changes in the T_1 times with increasing temperature, with the T_1 values at 80.0 °C being well below those at room temperature. This behavior was reproducible, with the error generally increasing as the temperature is increased. It is important to mention that the intensity vs delay time data in these experiments still fit well to both the exponential and linear functions. A number of possible explanations for this nonmonotonic temperature dependence were initially discussed. The possibility of dissolved oxygen was eliminated by performing several freeze−pump−thaw cycles prior to data collection, and the results agreed with those for a nondegassed sample to within the uncertainty of the T_1 measurement. A phase change or instability within the sample as a function of increasing temperature seemed an unlikely explanation considering the phase behavior of the mixture.[15] Additionally, we considered the idea of discrete changes in solvent structure and postulated mechanisms based on the recent structural calculations and experiments of Reimers and Hall.[16] However, an extensive effort in this regard led to no consistent physicochemical arguments that could explain the nonmonotonic behavior. The experimental results seemed to indicate a reproducible, systematic error that changed as a function of temperature and composition. Since the overall goal was to develop NMR relaxation as a technique to quantitatively examine viscosity changes, this unexpected ^2H relaxation data prompted us to investigate the ^{14}N nucleus in this solvent system, as previously mentioned.

It is known that convection introduced by the sample temperature regulation system can cause distortions in the measurement of self-diffusion coefficients[17-20] and T_1 relaxation times[10,21-26] by NMR. When measuring T_1 relaxation times that are rather long (seconds), or when thermal gradients are particularly high within the sample, the measured T_1 would appear to be shorter than the actual T_1 of the sample because of the movement of nuclei in and out of the coil region. Lounila et al.[25] have discussed the theory of the onset of thermal convection for ^{129}Xe and ^{19}F T_1 relaxation. Their results demonstrate that employing moderate sample spinning rates of $\geq$10 Hz during the acquisition counteracts these

effects on long T_1 measurements. In experiments conducted in a 5-mm NMR tube, we have observed thermal convection to result in a decrease of the measured T_1 for CH_3CN−H_2O samples at temperatures as low as 40.0 °C and for T_1's as short as $\sim$1.5 s, with an increase in the difference between the spinning and nonspinning T_1 as temperature is further increased. No difference is found between spinning and nonspinning T_1 times at room temperature. These convective effects were not seen in our previous ^{14}N T_1 measurements in this solvent mixture, because the ^{14}N T_1 relaxation time is shorter (several ms) than the transport time scale that would significantly interfere with the measured T_1. We reinvestigated the ^2H T_1 experiments, with the only change being that a spin rate of 20 Hz was employed. These results are presented in Figure 1B and show a linear increase in the T_1 with temperature for all compositions investigated. There are ^2H T_1 studies of similar systems in the analytical literature,[2,7-9] but systematic errors or the effects of thermal convection on T_1 measurements are not discussed in these reports.

It can be determined if thermal convection is the cause of these differences in measured T_1 values by confining the entire sample to the rf-irradiated region for the duration of the experiment. This experiment was performed using a 5-mm NMR tube with a glass plug at the base (Shigemi, Inc.). A nonspinning sample of χ_{CH_3CN} = 0.257 at 60.0 °C in a normal 5-mm NMR tube resulted in a ^2H T_1 of 4.23 $\pm$ 0.12 s, whereas the confined sample gave a T_1 of 7.09 $\pm$ 0.23 s, a difference of 42%. For comparison, T_1 = 7.27 $\pm$ 0.05 s in a conventional 5-mm NMR tube at a spin rate of 20 Hz. The T_1 value with spinning is statistically identical to the Shigemi tube result at the 99.9% confidence level. Errors reported are $\pm$1 standard deviation for triplicate measurements. This result has important implications for similar investigations employing nonspinning samples at elevated temperatures, including investigations utilizing high-pressure NMR cells, such as our recently developed polymeric NMR cell.[27] The present results regarding the importance of convection cannot explain the previous results of Bliesner and Sentell[2] at 30.0 °C, which indicate no definitive trend in the ^2H T_1 values with varying composition of the mixture, although the viscosity is clearly changing.

Figure 2 shows a comparison of ^2H T_1 times for CH_3CN in CH_3CN−H_2O at 30.0 °C reported by Bliesner and Sentell[2] and the same data collected in the present study using linear fits to the integrated areas of the peaks to determine T_1 values. Error bars are reported as $\pm$1 standard deviation for both data sets. In their investigation of the bulk CH_3CN−H_2O solvent mixture, they report the T_1 values to be "essentially constant" over the entire binary composition range, with an approximate T_1 value of 6 s. This claim seems reasonable in light of the error bars reported for these measurements. However, the present study shows a very obvious trend in the ^2H T_1 times, with the largest error bar representing only $\pm$1.3% relative standard deviation. In addition, our previous studies of ^{14}N T_1 times in this mixture give a similar trend in T_1 times with changing composition and an excellent correlation with the bulk solution viscosity.[1] One would expect the T_1 times of Bliesner and Sentell to be consistently shorter than those in the present study if thermal convection were problematic. Since their study employed 10-mm NMR tubes, we

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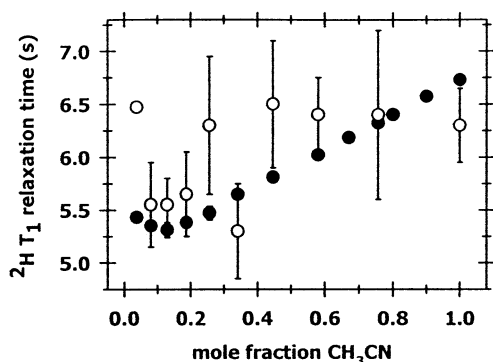(27) Wallen, S. L.; Schoenbachler, L. K.; Dawson, E. D.; Blatchford, M. A. *Anal. Chem.* **2000**, *72*, 4230−4234.

Figure 2. ^2H T_1 relaxation times for CH_3CN in CH_3CN-H_2O samples at 30.0 °C. Filled circles are data from the present study, and open symbols are data of Bliesner and Sentell.[2] Error bars shown are ±1 standard deviation of triplicate measurements for both studies, and are smaller than the symbol size for most compositions in the present study.

have measured the ^2H T_1 for $\chi_{CH_3CN} = 0.257$ at 30.0 °C in a 10-mm tube to determine if this causes the onset of convection at a lower temperature. We have found the 5- and 10-mm samples to give the same T_1 value (5.53 ± 0.03 s and 5.47 ± 0.07 s, respectively), and so we cannot attribute the differences in Figure 2 to convective effects. It was initially thought that different sets of delay times used in the two studies might be the cause of the differences. An experiment was conducted in which two different sets of delay times were chosen: one as mentioned in the Methods and Materials section, and one to mimic the experiment of Bliesner and Sentell (ranging from 25 ms to 24 s). Bain has shown that the results of an inversion recovery experiment do not strongly depend on the choice of delay times,[24] and indeed, the T_1 values calculated from a 3-parameter exponential fit were only 2% different. Other processing or acquisition parameters may have played a role in the error associated with the measurements of Bliesner and Sentell, but we do not wish to present a full analysis of sources of the differences between the two studies. However, it is important to note that Figure 2 indicates a clear trend in the ^2H T_1 data for this system, showing a linear decrease in the T_1 as the CH_3CN content is decreased to $\chi_{CH_3CN} \sim 0.15$ that then increases slightly at even lower CH_3CN concentrations.

We previously reported that the ^{14}N reorientational correlation times, τ_c, of CH_3CN are linearly proportional to η/T for all compositions with H_2O at temperatures ranging from 25.0 to 80.0 °C.[1] This result indicated that ^{14}N spin−lattice relaxation measurements are an excellent probe to follow changes in solution viscosity for this mixture.[1] This linear dependence between ^{14}N τ_c and η/T can be used to illustrate that the present ^2H measurements also correlate with η/T. In Figure 3, we plot the ^2H τ_c determined through eq 1 versus the ^{14}N τ_c at 30.0 °C. Error bars shown in each direction are ±1 standard deviation of triplicate measurements, and the R^2 for the linear regression is 0.994. These results represent 13 mole fractions of CH_3CN ranging from 0.04 to 1.00. It is interesting that both the ^2H and ^{14}N T_1 measurements correlate with η/T, since they represent reorientational molecular motions about different axes.

Although our initial attempts to use ^2H T_1 values to monitor changes in viscosity in the CH_3CN-H_2O system were unsuccessful, the present results show that a linear dependence of the ^2H T_1 times and η/T exists. As indicated, however, one must be
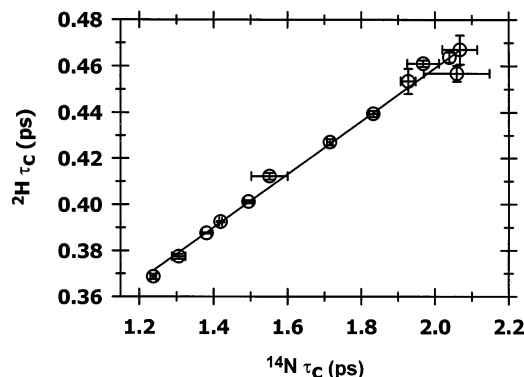
Figure 3. Plot of ^2H versus ^{14}N reorientational correlation times (τ_c) at 30.0 °C.

cautious in the interpretation of ^2H T_1 data for systems such as CH_3CN-H_2O. The effects of thermal convection on long T_1 measurements are quite severe and may go undetected if one is measuring a single relaxation time at an elevated temperature due to the fact that the data still fits well to both exponential and linear functions in the presence of convection. It is important to address the issue of convective flow for experiments involving temperatures as low as 40.0 °C and in systems with T_1 times as short as 1 s, regardless of the nucleus being observed. These effects of thermal convection can be eliminated simply by employing sample rotation or confining the sample to within the rf coil volume, but this may introduce difficulty for T_1 experiments that require the use of nonspinning samples. Though systematic errors in T_1 measurements due to convection have been previously realized, we believe that this effect should be further highlighted in light of the present results.

CONCLUSIONS

We have shown that ^2H T_1 measurements in CH_3CN-H_2O mixtures can be used to reliably measure changes in viscosity of this common chromatographic mobile phase system. Systematic error in the observed T_1 values at elevated temperatures were found to result from thermal convection for temperatures as low as 40.0 °C and for T_1 times as short as 1.5 s. Thermal convective effects can be eliminated by employing sample spinning or confining the entire sample volume to within the rf-irradiated region. We have repeated the experiments of an earlier investigation of the ^2H T_1 times in this mixture that did not scale with viscosity[2] and have shown that the ^2H T_1 times do correlate to the viscosity of the mixture. In conclusion, ^{14}N and ^2H T_1 relaxation measurements are excellent probes of the solution viscosity in this RPLC solvent system over the entire binary composition range.