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Sequential Inversion Recovery with RIDE—Simultaneous Suppression of Two Solvent Signals in ^{17}O NMR Spectroscopy

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Well-resolved ^{17}O NMR spectra of *D*-mannopyranose in a mixture of solvents were obtained. The ^{17}O NMR signals of DMSO and water have been successfully suppressed by incorporating two sequential inversion recovery steps, tuned to the relaxation of the two solvent signals, into the RIDE pulse sequence. The heights of the solvent signals are decreased by a factor greater than 1000.

Key Words: **^{17}O NMR; *D*-mannopyranose; solvent suppression; inversion recovery; RIDE.**

In ^{17}O NMR spectroscopy at natural abundance one frequently encounters the problem that one or more solvent signals cover or at least obscure signals in the region of interest (*1, 2*). Since these signals are usually several orders of magnitudes larger than the solute signals the dynamic range of the receiver is limited by their intensities (*2*).

This is particularly bothersome during the observation of compounds, which carry hydroxyl and ether functions (*3*). For solubility reasons water and dimethyl sulfoxide (DMSO) are often the only choices as solvents and a combination of both is frequently used to study the hydration and hydrogen bonding of small organic molecules (*4*). With the ^{17}O NMR signals of neat water at 0 ppm and neat DMSO at 12.5 ppm the resonances for OH and ether oxygen atoms in the region between -30 and $+100$ ppm are difficult to observe. In particular, the signals of the non-anomeric hydroxyl groups of sugar molecules (-10 to $+20$ ppm) are covered or distorted by the overwhelming solvent signals. All these factors might prevent detection or lead to a misassignment of ^{17}O NMR resonances.

Solvent suppression techniques that rely on avoiding the excitation of a frequency region around the solvent signal (*5–7*) are frequently used in ^{1}H NMR spectroscopy, because they have well-defined excitation maxima and minima. However, they are unsuitable for ^{17}O NMR studies of carbohydrates in aqueous solutions, because they attenuate and eliminate all signals close to the solvent signal (*2*).

More promising is the application of techniques, which take advantage of the different T_1 relaxation times of solvent and solute molecules. Inversion recovery (WEFT) (*8*) and its application under the condition of truncated relaxation (*9*) have proven very efficient for water signal suppression. In previous ^{17}O NMR investigations a combination of inversion recovery with the RIDE sequence (*10*) for the elimination of the acoustic ringing response (*11*) facilitated an easy observation of the signals of the substrate, even when the resonances were close to the solvent signal (*2, 12*).

If the suppression of more than one signal is desired, however, a single inversion recovery sequence will not completely eliminate all signals due to differences in their T_1 relaxation times. This incomplete cancellation may be tolerable (*13*), when the relaxation times differ by less than 50%, but it will be insufficient for the ^{17}O nucleus, since the quadrupolar relaxation of the water molecule is several times slower than that of most organic solvents (*14*). In studies of stationary and flowing samples, i.e., in LC-NMR or in *in vivo* NMR, the problem of multiple solvent or background signals has been solved by the combination of frequency selective pulses on the solvent resonances followed by dephasing gradient pulses (CHESS, WET) (*15, 16*) and by multiple inversion recovery (*17, 18*). The first two techniques, however, require gradient pulses and therefore equipment that may not be available on all spectrometers, whereas the latter methods do not require special hardware.

This Communication presents an extension of the original inversion recovery/RIDE combination (*12*) by a second inversion recovery step. This technique is intended to simultaneously reduce the size of the two ^{17}O solvent signals of a 0.25 *M D*-mannopyranose solution in 50 vol.% DMSO and 50 vol.% water and also to minimize the effects of acoustic ringing. The ^{17}O T_1 relaxation times in this solution are approximately 6 ms for H_2O, 2.5 ms for DMSO, and less than 1 ms for the oxygen atoms in the monosaccharide molecule.

In the pulse sequence

$$T_d\text{–}180^\circ\text{–}\tau_w\text{–}\tau_D\text{–}180^\circ\text{–}\tau_D\text{–}90^\circ_x\text{–}AQ(+)$$

$$T_d\text{–}180^\circ\text{–}\tau_w\text{–}\tau_D\text{–}180^\circ\text{–}\tau_D\text{–}180^\circ\text{–}\tau_A\text{–}90^\circ_x\text{–}AQ(-)$$

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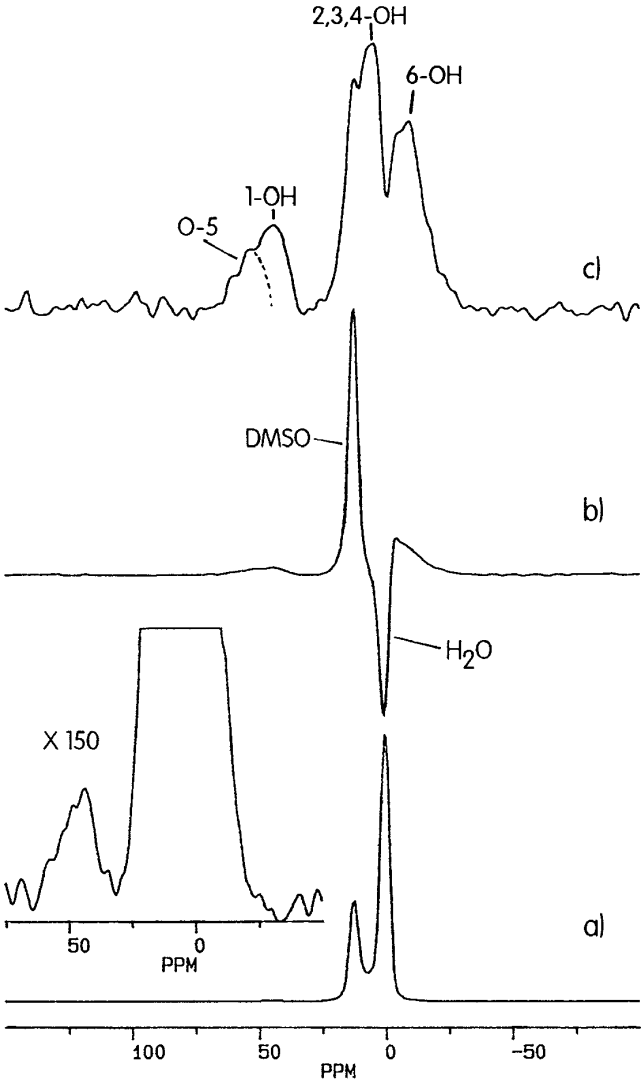

FIG. 1. 40.6 MHz natural abundance ^{17}O NMR spectra of a 0.25 *M* solution of *D*-mannopyranose in a 1:1 mixture of DMSO and water at 80°C, obtained with a 10-mm broadband probe on a Bruker AC300 NMR spectrometer. (a) Without solvent suppression (RIDE, $\tau_A = 20$ μs). (b) Solvent suppression with inversion recovery/RIDE; the parameters were optimized for minimum solvent signals ($\tau_W = 3.3$ ms, $\tau_A = 20$ μs). (c) Double solvent suppression (sequential inversion recovery/RIDE, $\tau_W = 4.6$ ms, $\tau_D = 1.6$ ms, $\tau_A = 20$ μs.) All spectra were recorded with $T_d = 1$ ms, $AQ = 8.192$ ms, 90° pulse = 22 μs. No deuterium lock was used. 10^6 transitions with 1K data points were averaged, followed by apodization with a shifted sine bell function (SSB = 3) and zero filling to 8K prior to the Fourier transformation. The chemical shifts are referenced against the internal water signal.

two consecutive 180°–τ segments are used to eliminate two signals with different relaxation times. τ_W, τ_D, and τ_A have to be optimized to suppress the water signal, the DMSO signal, and the acoustic ringing response, respectively. The recovery delays have to be applied in decreasing length. T_d and AQ need only be long enough to satisfy the condition $5 \cdot T_1$ for the substrate molecule. The relaxation of the solvent nuclei can be truncated (*9*) and driven into a steady state with 8 dummy scans.

Experimentally it is advantageous to first optimize the recovery delay for the faster relaxing species (τ_D), followed by the delay for the slower relaxing species.

Figure 1 shows ^{17}O NMR spectra of *D*-mannose, obtained with three different techniques. To give a visual comparison of the efficiency of the solvent suppression techniques all spectra were printed with identical intensities of the biggest signals. Spectrum 1a shows the result, when no solvent suppression is used. An intensity ratio of 300:1 (H_2O:1-OH) makes it difficult to distinguish the substrate signals from the baseline and the expanded insert shows that the solvent signals cover some of the substrate signals. The application of the previously published inversion recovery/RIDE combination improves the spectrum (Fig. 1b), however, it is apparently insufficient to completely suppress the NMR signals of both solvents. The best result is achieved when both solvent signals have equal size, but opposite signs.

The introduction of a second inversion recovery step into the pulse sequence allows a nearly complete suppression of both the water and the DMSO signals (Fig. 1c), and even the signals of the primary alcohol functions 2-, 3-, and 4-OH and the secondary 6-OH of the mannopyranose molecule, which are hidden underneath the solvent signals in the other spectra, can be easily assigned.

It was found that τ_w in the new pulse sequence needs to be longer than the recovery delay in the original inversion recovery/RIDE sequence (τ_W^0). This can be explained with the exponential behavior of the T_1 relaxation. Since the decay of negative longitudinal magnetization ($-Mz \rightarrow 0$) happens faster than the build-up of the same amount of positive magnetization ($0 \rightarrow +Mz$), the delay between the first two 180° pulses must be extended in order to satisfy the condition

$$Mz(\tau_W + \tau_D) = -Mz(\tau_W^0 - \tau_D), \qquad [1]$$

which describes the magnetization of the solvent signal of the slower relaxing species (H_2O) prior to and immediately after the second 180° pulse. Using a formula (*19*) describing the build-up of magnetization after 180° inversion under conditions of truncation of the relaxation period this equation can be rewritten as

$$1 - a \times \exp[-(\tau_W + \tau_D)/T_1] = a \times \exp[-(\tau_W^0 - \tau_D)/T_1] - 1, \qquad [2]$$

with $a = 2 - \exp(-(AQ + T_d)/T_1)$, and T_1 as the relaxation time for the slower relaxing species. This allows us to calculate τ_W as

$$\tau_W = -\tau_D - T_1 \ln((2/a) - \exp(-(\tau_W^0 - \tau_D)/T_1)). \qquad [3]$$

Using a T_1 of 6 ms for the water molecule and the experimental parameters in Fig. 1, τ_W becomes 4.4 ms. This is in agreement with the experimentally determined value of 4.6 ms. The small difference may be attributed to the fact that the relaxation of the DMSO oxygen atom is also truncated ($AQ + T_d < 5 \cdot T_1$) and therefore τ_D is determined too short.

Solvent suppression factors (SSF) were calculated for both solvent suppression techniques. By comparing the intensity ratios between the strongest solvent signal and an undisturbed substrate signal (1-OH at 42.8 ppm) from Fig. 1a with the ratios from Fig. 1b or 1c, SSFs of approximately 10 for the inversion recovery/RIDE combination and >1000 for the newly proposed pulse sequence were found.

It can be concluded that the sequential inversion recovery/RIDE combination is perfectly suited for ^{17}O NMR investigations of samples in solvent mixtures. Investigations of hydration and hydrogen bonding of monosaccharides will likely benefit from this procedure. The proposed experiment is tailored for a system with two solvent signals, but it could easily be extended to suppress more than two signals by inserting another τ–180°–τ segment.

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