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Optimization of Diffusion-Filtered NMR Experiments for Selective Suppression of Residual Nondeuterated Solvent and Water Signals from ^{1}H NMR Spectra of Organic Compounds

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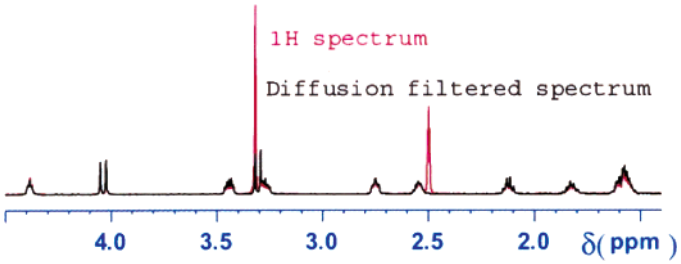

Interpretation of ^{1}H NMR spectra of organic compounds is sometimes hampered by the presence of strong peaks arising from residual nondeuterated solvent and water that obscure compound signals. Classical solvent suppression techniques such as presaturation or those based on pulsed field gradients are not effective in this regard because they also remove the compound resonances that overlap with the solvent signal being suppressed. Here, we propose an alternative scheme by using an optimized NMR diffusion filter that eliminates the nondesired peaks while retaining the signals of interest. This strategy has proved to be useful in three common deuterated solvents, namely, $CDCl_3$, DMSO-d_6, and CD_3OD, resulting in clean spectra with no interference from solvent or water peaks.

Introduction

The acquisition of a ^{1}H NMR spectrum is a necessary step for the structural characterization of new synthetic organic compounds. Although the solvents used in organic NMR spectroscopy are readily available in the deuterated form, the recorded spectrum often shows signals corresponding to the residual nondeuterated solvent and water. These nondesired peaks cause dynamic range problems and complicate the interpretation of the spectrum, especially when the intrusive peaks obscure compound resonances. Moreover, additional signals corresponding to solvent not removed during the purification process may appear in the spectrum, further complicating the analysis.

A number of solvent suppression techniques have been developed to remove, or at least reduce, the intensity of solvent signals, which can be classified into three areas:[1] (a) sequences that presaturate the solvent resonance through the application of continuous, weak radio frequency irradiation; (b) sequences that produce no net excitation of the solvent resonances, like the "jump and return" method;[2] and (c) sequences that destroy the solvent magnetization by pulsed field gradients such as WATERGATE[3] or WET.[4] The major limitation of these approaches is their lack of selectivity and, therefore, any compound resonances that overlap with the solvent peaks are also suppressed.

In the past few years, diffusion-ordered spectroscopy[5] has emerged as a powerful tool to study the molecular diffusion of compounds in solution, providing information on their molecular sizes and aggregation states.[6] This methodology has been used

(1) Claridge, T. D. W. High-Resolution NMR Techniques in Organic Chemistry. *Tetrahedron Organic Chemistry Series*; Elsevier Science: Amsterdam, The Netherlands, 1999; Vol. 19, p 359.

(2) Plateau, P.; Guéron, M. *J. Am. Chem. Soc.* **1982**, *104*, 7310.

(3) Piotto, M.; Saudek, V.; Sklenár, V. *J. Biomol. NMR* **1992**, *2*, 661.

(4) Ogg, R. J.; Kingsley, P. B.; Taylor, J. S. *J. Magn. Reson., Ser. B* **1994**, *104*, 1.

(5) (a) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288. (b) Tanner, J. E. *J. Chem. Phys.* **1970**, *52*, 2523. (c) Morris, K. F.; Johnson, C. S., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 4291. (d) Keeler, J.; Clowes, R. T.; Davis, A. L.; Laue, E. D. *Methods Enzymol.* **1994**, *239*, 145. (e) Price, W. S. *Concepts Magn. Reson.* **1997**, *9*, 299.

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to analyze mixtures of compounds by separating the signals of the compounds according to their diffusion coefficients,[7] characterize dendrimers and metallodendrimers,[8] monitor aggregation,[9] determine the molecular weight distributions for polymers,[10] characterize organic supramolecular assemblies,[11] and detect ligand binding to a biological receptor.[12] Also, techniques have been developed that take advantage of the significant translational diffusional differences between the solvent and the solute (a large biomolecule or a compound attached to a solid support) to remove the solvent signals completely; such experiments have been used for proteins in aqueous solution[13] and for resin-bound molecules.[14] Diffusion-ordered spectroscopy has also been applied recently as a solvent signal filter in neat ionic liquids.[15] In this case, the signals belonging to the slower moving species are filtered out, allowing reaction monitoring in ionic liquids.

However, the application of diffusion-ordered spectroscopy for the suppression of the residual nondeuterated solvent and water signals from the NMR spectra of organic compounds in deuterated solvents has not been exploited. Given the small differences between the molecular sizes of an organic compound and the solvent, the application of diffusion NMR pulse sequences as solvent filters is not straightforward because the nonnegligible diffusion of the solute would lead to a severe loss in the intensities of the compound signals. In the present work, we investigate whether, for a certain amount of an organic compound in three common deuterated solvents ($CDCl_3$, DMSO-d_6, and CD_3OD), the use of diffusion filters would result in spectra in which the solvent and water signals are significantly reduced, facilitating their interpretation.

Results and Discussion

The bipolar gradient pulse pairs longitudinal-eddy-current delay (BPPLED) pulse sequence,[16] a widely accepted scheme for diffusion-ordered spectroscopy that markedly reduces eddy-current effects, is shown in Figure 1. Because the magnetization is confined to the *z*-axis during the diffusion delay, relaxation

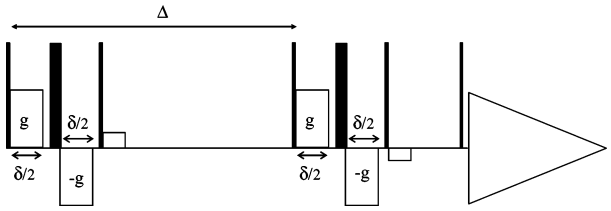


FIGURE 1. BPPLED pulse sequence. Narrow and wide black rectangles represent 90° and 180° pulses, respectively. The open rectangles indicate the bipolar gradients and the purge gradients. The parameters g and δ represent the strength and length of the bipolar gradients, respectively, and Δ represents the length of the diffusion delay. The phase cycling for the different pulses is given in ref 16.

losses are dictated mainly by longitudinal (T_1) relaxation rates. If the diffusion period is short relative to T_1 values, relaxation losses can be neglected and the signal intensity, Ac, for a proton of a certain organic compound is given by eq 1:

$$\mathrm{Ac} = \mathrm{A_o c}\,\exp(-\gamma^2 g^2 \delta^2 \mathrm{Dc}(\Delta - \delta/3)) \qquad (1)$$

where A_oc is the signal intensity in the absence of gradients, γ is the magnetogyric ratio, g is the strength of the gradient, δ is the length of the bipolar gradient, Dc is the translational self-diffusion coefficient of the compound, and Δ is the diffusion period, that is, the delay between the leading edges of the two bipolar gradients. Analogously, eq 2 governs the intensity of the residual nondeuterated solvent signal, As:

$$\mathrm{As} = \mathrm{A_o s}\,\exp(-\gamma^2 g^2 \delta^2 \mathrm{Ds}(\Delta - \delta/3)) \qquad (2)$$

where A_os is the signal intensity in the absence of gradients of the nondeuterated solvent and Ds its translational self-diffusion coefficient.

In this work, our goal is to optimize the parameters of the BPPLED sequence to remove the residual nondeuterated signal from the ^{1}H spectrum of an organic compound. For this purpose, we consider that this signal is removed when its intensity is equal to or less than 5% of that of one proton of the compound of interest. This condition can be expressed mathematically as As = 0.05Ac, leading to eq 3:

$$\gamma^2 g^2 \delta^2 (\Delta - \delta/3)(\mathrm{Ds} - \mathrm{Dc}) = \ln(\mathrm{A_o s}/0.05\mathrm{A_o c}) \qquad (3)$$

Because A_oc and A_os are proportional to the concentration of the compound and the residual nondeuterated solvent, eq 3 can be rewritten as follows:

$$\gamma^2 g^2 \delta^2 (\Delta - \delta/3)(\mathrm{Ds} - \mathrm{Dc}) = \ln(SM_{\mathrm{w}}V/0.05m) \qquad (4)$$

where S is the concentration of the nondeuterated solvent, m is the amount of the organic compound, M_w is the molecular weight of the compound and V is the volume of the deuterated solvent. The concentrations of the nondeuterated solvents were determined by comparing the intensity of the residual solvent peak with that of the methyl protons of 1,4-bis(trimethylsilyl)-benzene that was added as internal standard,[17] and their diffusion coefficients were measured using the BPPLED sequence, yielding values similar to those reported for the pure solvents (Table 1).[18]

(6) Brand, T.; Cabrita, E. J.; Berger, S. *Prog. NMR Spectrosc.* **2005**, *46*, 159.
(7) (a) Johnson C. S., Jr. *Prog. NMR Spectrosc.* **1999**, *34*, 203. (b) Antalek, B. *Concepts Magn. Reson.* **2002**, *14*, 225. (c) Rajagopalan, S.; Chow, C.; Raghunathan, V.; Fry, C. G.; Cavagnero, S. *J. Biomol. NMR* **2004**, *29*, 505. (d) Kellenbach, E.; Burgering, M.; Kaspersen, F. *Org. Process Res. Dev.* **1999**, *3*, 141.
(8) (a) Young, J. K.; Baker, G. R.; Newkome, G. R.; Morris, K. F.; Johnson, C. S., Jr. *Macromolecules* **1994**, *27*, 3464. (b) Valentini, M.; Ruegger, H.; Pregosin, P. S. *Helv. Chim. Acta* **2001**, *84*, 2833.
(9) Burini, A.; Fackler, J. P., Jr.; Galassi, R.; Macchioni, A.; Omary, M. A.; Rawashdeh-Omary, M. A.; Pietroni, B. R.; Sabatini, S.; Zuccaccia, C. *J. Am. Chem. Soc.* **2002**, *124*, 4570.
(10) Chen, A.; Wu, D.; Johnson, C. S., Jr. *J. Am. Chem. Soc.* **1995**, *117*, 7965.
(11) (a) Cameron, K. S.; Fielding, L. *J. Org. Chem.* **2001**, *66*, 6891. (b) Frish, L.; Sansone, F.; Casnati, A.; Ungaro, R.; Cohen, Y. *J. Org. Chem.* **2000**, *65*, 5026.
(12) (a) Lucas, L. H.; Yan, J.; Larive, C. K.; Zartler, E. R.; Shapiro, M. J. *Anal. Chem.* **2003**, *75*, 627. (b) Lin, M.; Shapiro, M. J.; Wareing, J. R. *J. Am. Chem. Soc.* **1997**, *119*, 5249.
(13) van Zijl, P. C. M.; Moonen, C. T. W. *J. Magn. Reson.* **1990**, 87, 18.
(14) (a) Warrass, R.; Wieruszeski, J.-M.; Lippens, G. *J. Am. Chem. Soc.* **1999**, *121*, 3787. (b) Chin, J.; Chen, A.; Shapiro, M. J. *Magn. Reson. Chem.* **2000**, *38*, 782. (c) Chin, J. A.; Chen, A.; Shapiro, M. J. *J. Comb. Chem.* **2000**, *2*, 293.
(15) Giernoth, R.; Bankmann, D. *Eur. J. Org. Chem.* **2005**, 4529.
(16) Wu, D.; Chen, A.; Johnson, C. S., Jr. *J. Magn. Reson., Ser. A* **1995**, *115*, 260.
(17) Pinciroli, V.; Biancardi, R.; Colombo, N.; Colombo, M.; Rizzo, V. *J. Comb. Chem.* **2001**, *3*, 434.
(18) Holz, M.; Sacco, A. In *Bruker Biospin Almanac* 2005; Bruker Biospin: Karlsruhe, Germany, 2005, p 78.

TABLE 1. Concentrations and Self-Diffusion Coefficients of the Residual Nondeuterated Solvents in the Deuterated Solvents under Analysis

solvent	concentration[a] (mM)	D (10^{-9} m^2 s^{-1})
$CDCl_3$	21.2 (2.3)	2.44
CD_3OD	114.7 (30.9)	2.19
DMSO-d_6	134.2 (22.4)	0.70

[a] Different solvent lots were used. The mean value is provided together with the standard deviation (in parentheses) as an index of variability.

For the determination of the self-diffusion coefficient of the organic compound under analysis, an alternative strategy is proposed. Instead of performing an accurate measurement of this parameter, which would imply a significant amount of experimental work prior to the utilization of the BPPLED sequence as a diffusion filter, we propose to determine it from the molecular weight of the compound. The relationship between the size of a molecule and its diffusion coefficient, D, is given by the Stokes-Einsten equation:

$$D = kT/6\pi\eta R \tag{5}$$

where k is the Boltzmann constant, T is the temperature, η is the viscosity of the solvent, and R is the effective hydrodynamic radius of the molecule, a parameter that is inversely related to the cube root of molecular mass.[19] A Stokes−Einstein relationship between D and M_w has already been established, among others, for proteins,[20] oligosaccharides,[21] and steroid−cyclodextrin inclusion complexes,[22] and we have explored whether a similar expression can be employed to estimate the diffusion coefficient of small organic molecules in organic solvents.

To that end, we have measured the translational self-diffusion coefficients of a training set of 25 structurally diverse organic compounds (the list of compounds can be found in the Supporting Information) with molecular weights ranging from 150 g/mol to 550 g/mol in DMSO-d_6, $CDCl_3$, and CD_3OD, using the BPPLED sequence, and then plotted against the reciprocal of the cube root of their molecular weights. A fairly good fit was observed for the three solvents, given the assumptions of the Stokes−Einstein equation that hold only for spherical molecules (correlation coefficients of 0.89, 0.82, and 0.84 for $CDCl_3$, DMSO-d_6, and CD_3OD, respectively). Figure 2 shows the plot obtained in $CDCl_3$, along with the derived equation (the plots in DMSO-d_6 and CD_3OD are shown in the Supporting Information). The diffusion coefficients of a testing set of 20 organic compounds were measured and compared with those calculated with the empirical equations, and a reasonably good agreement between both sets of data was obtained (data not shown).[23]

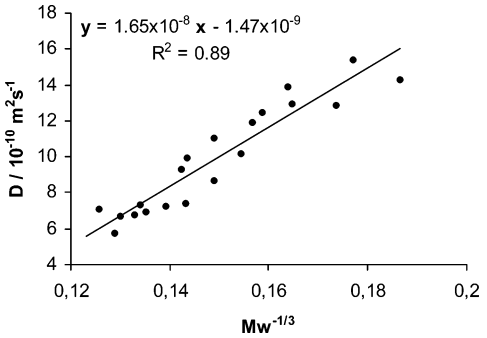


FIGURE 2. Linear relationship between the self-diffusion coefficient, D, and the reciprocal of the cube root of molecular weight for the training set of organic compounds in $CDCl_3$.

If the strength of the gradient, g, is set to the maximum power, the diffusion period, Δ, and the length of the gradient, δ, are the parameters that need to be adjusted to fulfill the condition expressed by eq 4. A long diffusion period would question the neglecting of relaxation losses that has led to this equation, and, in addition, the relative intensities of the compound signals would be altered, owing to the T_1 differences between the protons. By contrast, a very short value for the diffusion period will demand long gradient pulses, the maximum length being limited by the duty cycle of the gradient coil. At this point, we set the diffusion delay to 50 ms as a compromise between both extremes, leaving the length of the gradients as the only unknown in eq 4. For simplicity, this expression can be approximated to eq 6, because the gradient length is of the order of a few milliseconds and $\delta/3$ is, therefore, much less than the 50 ms diffusion period:

$$\gamma^2 g^2 \delta^2 \Delta(\mathrm{Ds} - \mathrm{Dc}) = \ln(SM_wV/0.05m) \tag{6}$$

Then eq 6 allows us to determine the gradient length that needs to be used in the BPPLED sequence to remove the solvent signal from the ^{1}H spectrum of an organic compound. A longer gradient would degrade the sensitivity of the experiment without a significant improvement in its performance, and a shorter gradient length will leave an appreciable magnitude of solvent signal in the spectrum. In addition, as a consequence of the extremely rapid diffusion of the water molecule, the water peak is significantly reduced or even completely removed from the ^{1}H spectrum.

As a proof of concept, we acquired diffusion-filtered experiments of a solution of 3 mg of strychnine (**1**) in 600 μL of $CDCl_3$ using the BPPLED sequence with different lengths of gradients. The concentration and the self-diffusion coefficient of $CHCl_3$ in $CDCl_3$ was taken from Table 1; the self-diffusion coefficient of strychnine was estimated to be 9.01×10^{-10} m^2 s^{-1} using the empirical relationship derived in $CDCl_3$, and the diffusion period was set to 50 ms, as discussed above. Thus, the gradient length to be applied in the BPPLED experiment was determined directly from eq 6, yielding a value of 1.42 ms. Figure 3 shows the spectra recorded using different lengths of gradient along with the conventional ^{1}H spectrum. It can be observed that the intended selective attenuation of the $CHCl_3$ signal at 7.26 ppm is achieved when the calculated length is applied, whereas the application of a shorter gradient results in

(19) Price, W. S. *Annu. Rep. Prog. Chem., Sect. C: Phys. Chem.* **2000**, *96*, 3.

(20) (a) Wilkins, D. K.; Grimshaw, S. B.; Receveur, V.; Dobson, C. M.; Jones, J. A.; Smith, L. J. *Biochemistry* **1999**, *38*, 16424. (b) Nesmelova, I. V.; Skirda, V. D.; Fedotov, V. D. *Biopolymers* **2002**, *63*, 132.

(21) Groves, P.; Rasmussen, M. O.; Molero, M. D.; Samain, E.; Cañada, F. J.; Driguez, H.; Jiménez-Barbero, J. *Glycobiology* **2004**, *14*, 451.

(22) Cameron, K. S.; Fielding, L. *Magn. Reson. Chem.* **2002**, *40*, S106.

(23) The errors of the predicted diffusion coefficients for the molecules of the testing set were less than 20% in all cases and less than 10% in most cases. Further experimentation revealed that the prediction works fairly well, given the assumptions of the approach, for molecules containing, exclusively, atoms of C, N, O, H, and F. However, the predicted coefficient deviates from the experimental value when the compound bears heavy atoms such as Br or I.

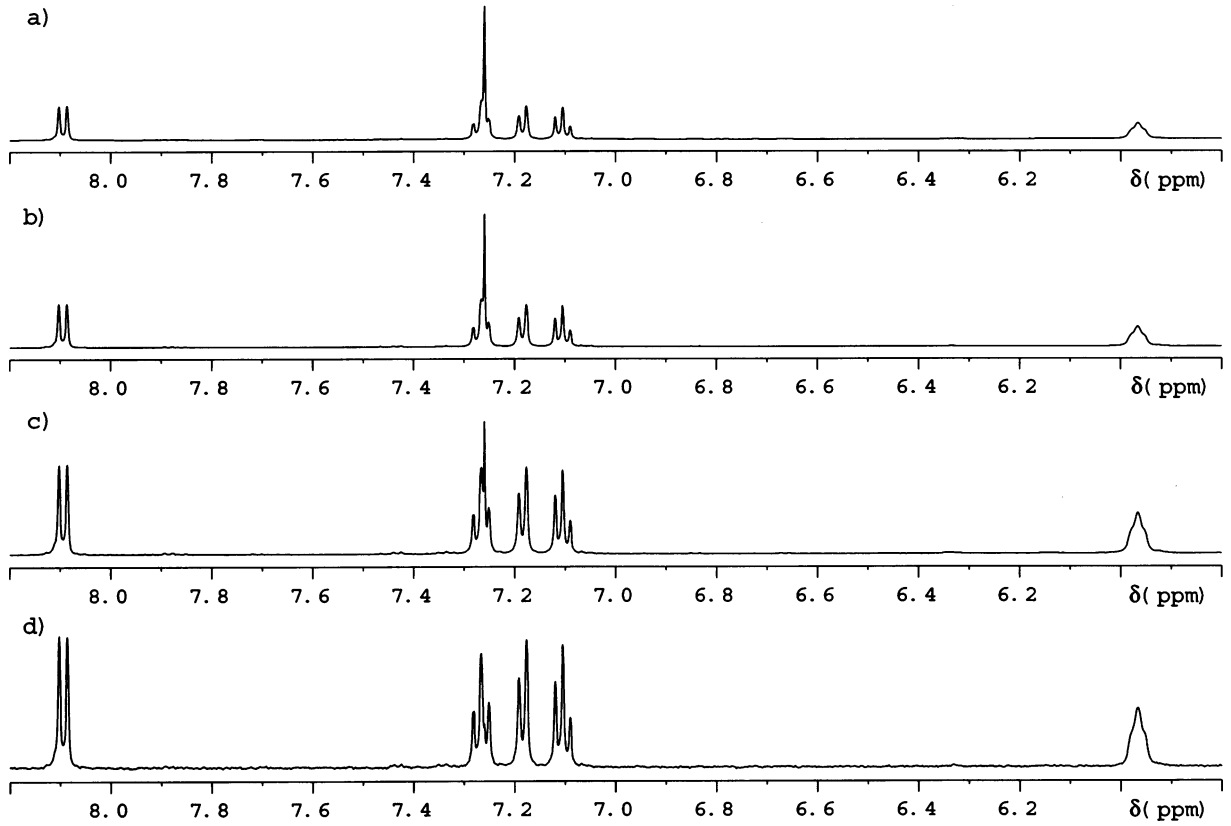
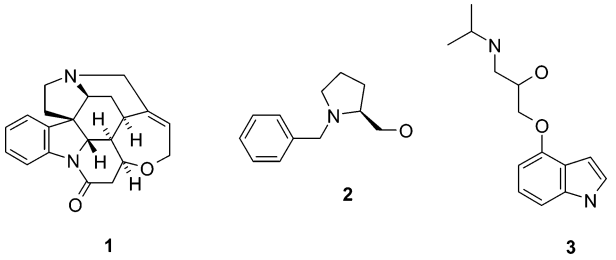


FIGURE 3. Expansion of the aromatic region of the 500-MHz ^{1}H spectra of **1** in $CDCl_3$. (a) Conventional; (b)−(d) diffusion-filtered using the BPPLED sequence with a gradient length of (b) 0.40 ms, (c) 0.80 ms, and (d) 1.42 ms. In (d) the area of the solvent signal is about 5% of that of one proton of the organic molecule, as intended. All the spectra were recorded with 16 transients. The signal-to-noise ratios are (a) 2816, (b) 1057, (c) 578, and (d) 154.

lower attenuation of the solvent signals relative to the compound signals.



The nonnegligible translational diffusion of the organic molecule obviously implies a significant attenuation of the compound signals that can be calculated with eq 1. Besides, only one-half of the magnetization is refocused in the BPPLED sequence, further reducing the intensity by a factor of 2.[7b] Figure 4 shows the intensity of the signals in the diffusion-filtered experiment relative to those in the ^{1}H spectrum as a function of the amount and molecular weight of the organic compound (see the Supporting Information for the plots in DMSO-d_6 and CD_3OD). For instance, the intensity of the signals in the optimized diffusion-filtered spectrum of the sample containing 3 mg of **1** in $CDCl_3$ (Figure 3d) is predicted to be 7.2% of that of the ^{1}H spectrum. The comparison of both spectra reveals that the intensities in the diffusion-filtered experiment are, on average, 5.5% of those of the ^{1}H experiment, slightly lower than the calculated value. This difference could arise from pulse miscalibrations, imperfect matching of bipolar gradient pulses,

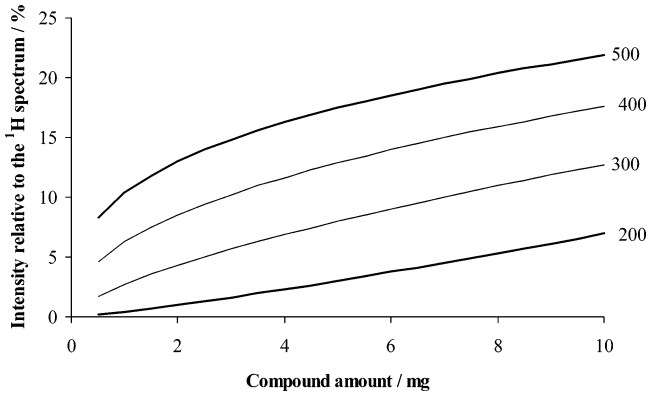


FIGURE 4. Intensity of the signals of the optimized diffusion-filtered spectrum relative to that of the ^{1}H spectrum in $CDCl_3$ as a function of the amount of compound for different molecular weights.

or relaxation losses occurring during the pulse sequence. This loss of sensitivity precludes the application of the proposed methodology when only a small amount of compound is available, especially for molecules of low molecular weight, as very long acquisition times would be necessary to obtain acceptable signal-to-noise ratios. Nevertheless, good results are often obtained for samples containing as little as 1 mg of compound on spectrometers operating at 500 MHz with a reasonable number of transients. For instance, Figure 5 shows the comparison of the diffusion-filtered and ^{1}H experiments acquired for a sample containing only 1 mg of **1** in DMSO-d_6. A good-quality diffusion-filtered spectrum in which the DMSO

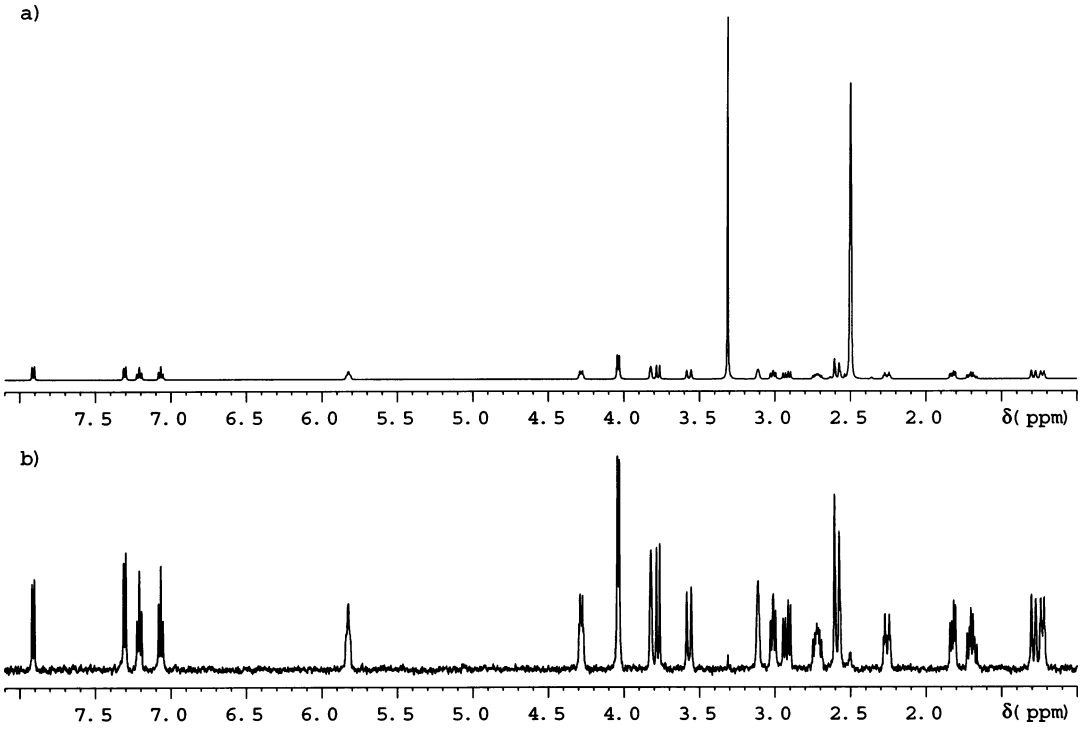


FIGURE 5. The 500-MHz ^{1}H spectra of 1 mg of **1** in DMSO-d_6. (a) Conventional spectrum acquired with 16 transients and (b) diffusion-filtered using the BPPLED sequence with a gradient length of 3.61 ms acquired with 512 transients. The signal-to-noise ratios are (a) 2183 and (b) 25.

and water peaks have been removed almost completely is obtained after 512 transients. The intensity of the compound signals in the diffusion-filtered experiment is 1.1% of that of the ^{1}H spectrum (1.4% was predicted).

In another example, we compared the results provided by the optimized diffusion filter with those obtained with double presaturation, the most widely used technique for solvent suppression, and with WET, one of the most effective approaches to date. The ^{1}H spectrum acquired for a solution of 5 mg of *N*-benzyl-l-prolinol (**2**) in 600 μL of DMSO-d_6 shows the residual DMSO and water signals at 2.50 and 3.31 ppm, respectively, the latter peak presenting partial overlapping with a compound signal, a doublet at 3.30 ppm (Figure 6a). The standard presaturation experiment suited for simultaneous suppression of both the DMSO and the water signals removes this doublet completely and strongly attenuates the resonances at 2.55 and 3.27 ppm, close to the solvent frequencies (Figure 6b). The signal of the hydroxyl proton at 4.39 ppm also experiences some loss in intensity as a consequence of the saturation transfer from water to the exchangeable proton. The use of weaker irradiation reduces signal losses but at the expense of incomplete saturation of the solvent. The use of the WET sequence results in effective solvent suppression with improved selectivity, but leads also to the almost complete suppression of the compound doublet and to the attenuation of the hydroxyl proton (Figure 6c). The spectrum obtained using the optimized BPPLED experiment is shown in Figure 6d for comparison. The length of the gradient (3.44 ms) was determined in a manner similar to that described above, once the diffusion coefficient of **2** had been estimated (3.8×10^{-10} m^2 s^{-1}) from the calibration curve in DMSO-d_6. In contrast to the spectra obtained using standard solvent suppression schemes, the doublet at 3.30 ppm and the hydroxyl proton appeared in the spectrum with a correct integral value, while the solvent and water peaks were removed efficiently and selectively. It should be noted that the signals of protons with very short T_1 relaxation times or were involved in rapid exchange phenomena may present lower intensities due to the magnetization losses experienced by these resonances during the diffusion period.

In addition to the residual nondeuterated solvent and the water peaks, the signals of other organic solvents that were incompletely eliminated during the reaction workup can occasionally appear in the ^{1}H spectrum, further complicating the analysis. The chemical shifts and multiplicities of the resonances of the most common contaminants in the three deuterated solvents under analysis have been reported.[24] A JAVA tool to facilitate their identification in the ^{1}H spectrum has been described.[25] Because these "extra" peaks arise from low molecular weight solvent molecules, they could also be eliminated by the diffusion filter, resulting in clean proton spectra that can be interpreted without the assistance of NMR tables. We have measured the self-diffusion coefficients of some of the solvents that appear most frequently in the ^{1}H spectra of organic compounds (acetone, acetonitrile, chloroform, DMSO, dioxane, ethanol, diethy ether, ethyl acetate, methanol, and hexane) in the three deuterated solvents (Table 2), with the goal of estimating the concentration of these solvent impurities that are also removed by the diffusion filter for a certain length of gradient.

For instance, Figure 7a shows the proton spectrum of 5 mg of pindolol (**3**) in 600 μL of CD_3OD upon the addition of 45 mg of ethyl acetate and 3 mg of diethyl ether to simulate the typical situation encountered when dealing with NMR spectra of synthetic compounds. After a calculation of the gradient length for suppression of residual nondeuterated methanol, the resulting value (1.97 ms) was applied in eq 6 to yield 2.9 and 5.0 mg of ethyl acetate and diethyl ether, respectively, which

(24) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512.

(25) Jones, I. C.; Sharman, G. J.; Pidgeon, J. *Magn. Reson. Chem.* **2005**, *43*, 497.

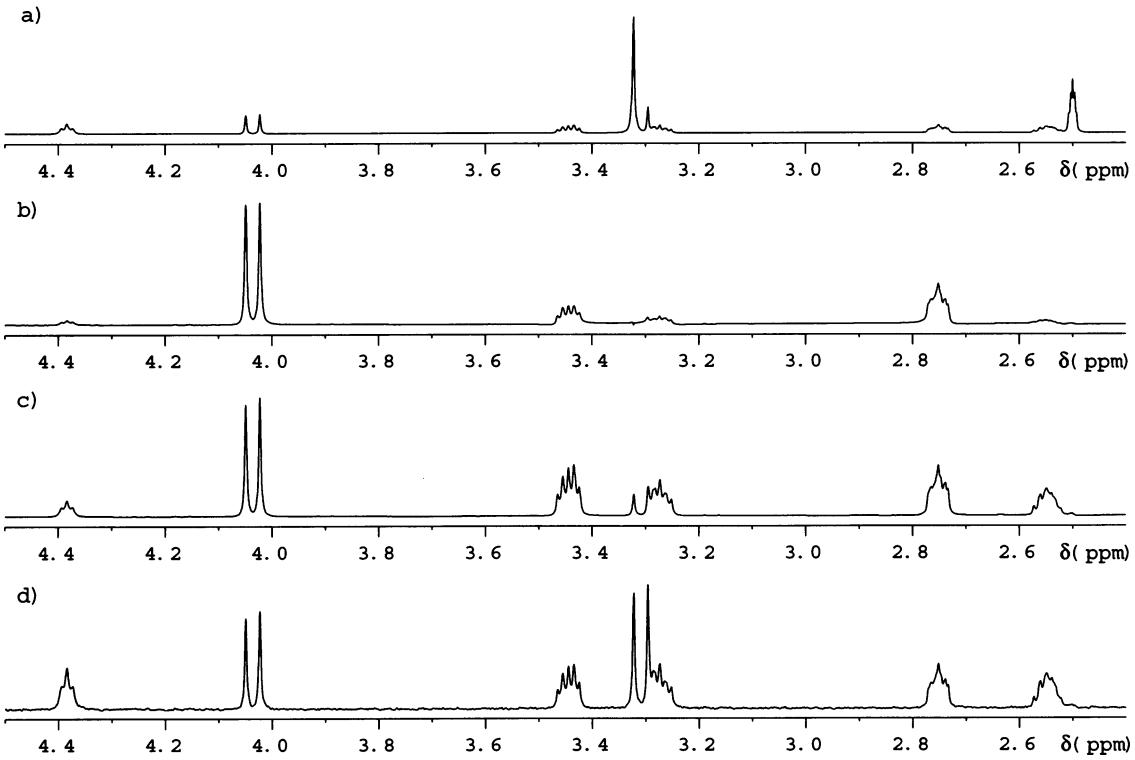


FIGURE 6. Expansion of the aliphatic region (2.4−4.5 ppm) of the 500-MHz ^{1}H spectra of **2** in DMSO-d_6. (a) Unsuppressed; (b) and (c) suppression of the DMSO and water signals with (b) conventional double presaturation and (c) WET; (d) diffusion-filtered experiment using the optimized BPPLED sequence with a gradient length of 3.44 ms.

TABLE 2. Self-Diffusion Coefficients of Some Common Organic Solvents in the Three Deuterated Solvents under Analysis[a]

		diffusion coefficient, D (10^{-9} m^2 s^{-1})		
solvent	M_w (g mol^{-1})	DMSO-d_6	$CDCl_3$	CD_3OD
acetone	58.1	0.94	2.25	2.60
acetonitrile	41.5	0.95	2.39	3.03
chloroform	119.4	0.71		2.15
diethyl ether	74.1	0.86	2.09	2.19
DMSO	78.1		1.72	1.67
dioxane	88.1	0.65	1.76	1.97
ethanol	46.1	0.69	2.36	1.84
ethyl acetate	88.1	0.69	1.82	2.01
methanol	32.0	0.80	2.66	
hexane	86.2	0.74	2.09	2.19

[a] The measurements were performed using samples that contained 10 μl of two or three organic solvents in 600 μl of each deuterated solvent.

represent the amount of the "extra" solvents that are also removed by the diffusion filter. As the added amount of diethyl ether is less than the calculated value, its signals are eliminated by the diffusion filter (Figure 7b). In contrast, as the added amount of ethyl acetate is greater than the calculated value, its signals, although reduced significantly relative to compound signals, are not removed completely. In this latter case, the diffusion-filtered spectrum is still useful in identifying the peaks that belong to the solvents after comparison with the ^{1}H spectrum. It is worth noting that eq 6 assumes that the delay between acquisitions is long enough that all resonances can return to their equilibrium before starting a new transient. Nevertheless, the use of a short delay between acquisitions would be beneficial, because the signals of the solvent protons, which usually have longer T_1 values, would be reduced even more with respect to the compound signals than those predicted on the basis of the differences in self-diffusion coefficients. Obviously, if a sample contains an impurity with a molecular weight greater than that of the compound of interest, the intensity of its signals would be increased. As the diffusion-filtered spectrum is not an accurate representation of the purity of the compound and the molecular details of the impurity, which dictate whether its signals are enhanced or reduced with respect to the compound signals, are not always known, the proposed experiment should always be used in conjunction with the standard ^{1}H spectrum for a correct interpretation of the NMR data.

Conclusions

We have shown that a judicious selection of the parameters of the BPPLED pulse sequence enables this experiment to filter out the residual nondeuterated solvent and water peaks that often complicate the interpretation of the proton spectra of organic compounds. The usefulness of the proposed approach has been verified in three common deuterated solvents, namely, $CDCl_3$, DMSO-d_6, and CD_3OD. The optimum length of the gradients in the BPPLED pulse sequence is determined on the basis of the concentration and the self-diffusion coefficient of the organic compound in a given deuterated solvent, the latter parameter being estimated from the molecular weight of the compound using predetermined calibration curves. The additional peaks of other organic solvents that may be present in the sample are also reduced or removed, depending on their concentrations and diffusion coefficients. This methodology constitutes an alternative to standard solvent suppression methods such as presaturation, "jump and return", or other sequences based on pulse field gradients that involve selective frequency excitations and whose main drawback is the nondesired removal of compound

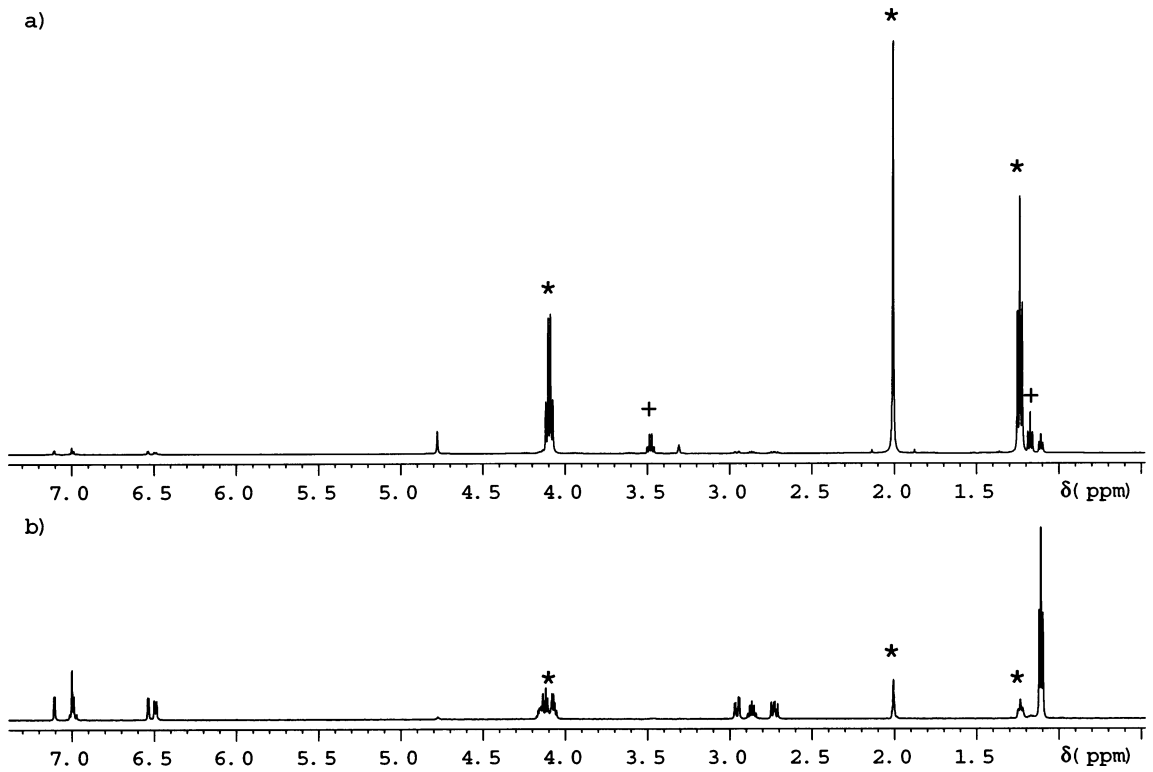


FIGURE 7. The 500-MHz ^{1}H spectra of 5 mg of **3** unpurified with 45 mg of ethyl acetate (*) and 3 mg of diethyl ether (+) in 600 μL of CD_3OD. (a) Conventional and (b) diffusion-filtered optimized for the elimination of the residual nondeuterated methanol using a gradient length of 1.97 ms. The signal-to-noise ratios are (a) 2409 and (b) 220.

signals that overlap with the unwanted solvent peak. Thus, the lower sensitivity of the diffusion-filtered experiment relative to the proton spectrum is compensated by the selective elimination of the residual nondeuterated solvent and water peaks even in the presence of strong resonance overlapping, a result that cannot be achieved by other solvent suppression methods. We think that the optimized diffusion-filtered experiment, in combination with the standard ^{1}H spectrum, is a very promising tool in organic NMR spectroscopy.

Experimental Section

Materials. The deuterated solvents used in this study were purchased from a commercial supplier at a deuteration degree of >99.8% and with a water content less than 0.01% for $CDCl_3$ and less than 0.03% for DMSO-d_6 and CD_3OD. The organic compounds and the nondeuterated organic solvents were purchased from commercial suppliers and used without further purification. The samples were prepared by dissolving 1−5 mg of the organic compound in 600 μL of the appropriate deuterated solvent. The silane standard, 1,4-bis(trimethylsilyl)benzene was purified through sublimation in a coldfinger apparatus at 80 °C under reduced pressure, affording a crystalline product of high purity with a melting point of 95 °C. A 1.48 mM DMSO-d_6 solution was prepared, and 300 μL of this solution was mixed with 300 μL of the deuterated solvent for quantification of the concentration of the residual nondeuterated solvent.

Nuclear Magnetic Resonance Spectroscopy. All NMR experiments were acquired at 25 °C on a 500-MHz spectrometer equipped with a 5-mm, inverse, broadband probe head and a *z*-gradient coil. The temperature was stabilized into a range of ±0.1 K using an air flow-rate of 400 L/h and calibrated using a standard methanol sample. The ^{1}H spectra were acquired using a 90° pulse and referenced to the residual solvent signal at 7.26, 2.50, and 3.31 ppm for $CDCl_3$, DMSO-d_6, and CD_3OD, respectively. The diffusion-filtered spectra were referenced from the ^{1}H spectra. The standard BPPLED sequence with rectangular pulse field gradients was used. A recovery delay of 100 μs and an LED delay of 5 ms were employed to reduce the effects of eddy currents. In some cases, the sample was spun at 20 Hz to minimize convection effects, as described.[26] The gradient strength was calibrated by measuring the self-diffusion coefficient of the residual HDO signal in D_2O (1.90×10^{-9} m^2 s^{-1}).[27] The gradient strength was set to the maximum power (55 G cm^{-1}), the diffusion period was 50 ms, and the gradient length was adjusted for each organic compound according to eq 6, as described in the text. The self-diffusion coefficients of the compounds of the training set and solvents were measured by monitoring the intensity decay of the signals as a function of the gradient strength. The diffusion period and the gradient length were optimized in each sample, and the gradient strength was incremented from 2 to 95% of the maximum strength in eight equally spaced steps. Double presaturation experiments involved the application of continuous, weak, radio-frequency irradiation through the transmitter and decoupler channels (power level corresponding to 54 Hz) before excitation and acquisition with the offset adjusted on the residual solvent and water frequencies. The WET technique used a series of 80-ms double-selective Gaussian pulses (81.4, 101.4, 69.3, and 161.0°), where each selective pulse is followed by a dephasing field gradient pulse (gradient strength ratio, 80:40:20:10).

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(26) Esturau, N.; Sanchez-Ferrando, F.; Gavin, J. A.; Roumestand, C.; Delsuc, M.-A.; Parella, T. *J. Magn. Reson.* **2001**, *153*, 48.
(27) Mills, R. *J. Phys. Chem.* **1973**, *77*, 685.

Supporting Information Available: Calibration curves that relate self-diffusion coefficients with the reciprocal of the cube root of molecular mass in DMSO-d_6 and CD_3OD, list of compounds used to build such curves, and intensities of the optimized diffusion-filtered spectra relative to that of the ^1H spectra in DMSO-d_6 and CD_3OD as a function of the amount of compound for different molecular weights. This material is available free of charge via the Internet at http://pubs.acs.org.

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