# Direct $^1$H NMR spectroscopy of dissolved organic matter in natural waters†

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Nuclear magnetic resonance (NMR) spectroscopy arguably provides the greatest insight into the overall chemical composition of dissolved organic matter (DOM). However, in a standard 5 mm NMR probe, a sample of sea water at natural abundance only contains *ca.* 500–600 ng of organic matter, distributed among the heterogeneous components of DOM. Additionally, the intensity of the water signal, which may be many orders of magnitude greater than the signals from DOM, makes the detection and analysis of DOM at natural abundance extremely demanding. Here, we demonstrate, that although challenging, the application of an improved water suppression technique allows NMR spectra of DOM to be obtained directly (*i.e* without pre-concentration) for major bodies of water, including rivers, lakes and the ocean. The technique described here provides a compositional overview of an intact sample, permitting researchers to investigate and assess the impact of concentration, isolation and extraction procedures that are employed routinely. Also the technique permits NMR to be performed on 'precious' samples for which traditional isolations are not possible, for example, water from ice cores and pore water, which are key in hydrology and for paleoclimatic reconstruction.

## Introduction

Dissolved organic matter (DOM) is ubiquitous in all natural waters and is known to play important roles in the global carbon and nitrogen cycles,[1–3] the fate, transport and transformation of contaminants and nutrients,[4–7] and the health and biodiversity of aquatic species.[8–11] Dissolved organic matter in the oceans represents the largest pool of *active* carbon on Earth. If this dissolved organic carbon were to experience a net annual decomposition of 1%, it would create a $CO_2$ flux larger than that created by human fossil fuel usage.[12] Thus, there is a great scientific need to further understand the composition, variability and reactivity of dissolved organic matter in the environment, and how this pool will change with respect to varying agricultural practices and land use, as well as future climatic shifts. Of all the analytical approaches employed to study DOM, NMR spectroscopy has arguably provided the greatest insights into its general composition.[2,13–17] However, NMR studies often require a considerable amount of isolated DOM (milligram quantities) and are adversely influenced by high salt and/or metal content, which can result from sample concentration. Therefore, much emphasis has been placed on the development of methods for the isolation of aquatic organic matter[18–23] which is essential before comprehensive analysis. Despite this, there is still concern that DOM can be altered during isolation to varying extents and may not be completely representative of the material in its natural state.[20,24,25] While spectroscopic approaches (mainly fluorescence, due to its excellent sensitivity) have been employed successfully on unaltered natural water samples,[25–28] the structural and compositional information that can be extracted is very limited.

In this manuscript, we demonstrate that, although challenging, it is possible to obtain direct $^1$H NMR spectra of organic matter, chemically unaltered from its natural environment, for a number of major bodies of water, including water from lakes, rivers, and the ocean. This approach allows direct and detailed compositional information of the organic constituents in water without pre-treatment. For this challenging application, an improved water suppression approach was required. Water suppression is essential as the large water signal can swamp the NMR receiver, preventing digitization of the solute signals and producing large baseline distortions. In general, a suppression factor of $10^3$–$10^5$ for solutes of *ca.* 1 mM is recommended.[29] However, in the case of dissolved organic matter, assuming a molecular weight of *ca.* 2000 Daltons,[30,31] the solute present is in the nanomolar range (ocean water) and its heterogeneity leads to broad signals which are very challenging to detect. Thus, detecting dissolved organic matter in field samples at natural abundance truly challenges the limits of NMR. Numerous approaches to water suppression have been proposed.[29] The simplest employ pre-irradiation of the water signal which reduces the water signal due to 'saturation' of the water protons. Pre-saturation is easy to implement, but reduces the signals of exchangeable groups (for example, OH and NH groups) that exchange with the water signal during pre-irradiation.[32] Improvements to the basic pre-saturation sequence have been numerous, including the use of composite pulses,[33] the use of low flip angles,[34] and phase-modulated irradiation.[35] Other methods rely on pulse field

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gradients to suppress water. Most commonly, a selective pulse is used to flip the water resonance followed by a pulse field gradient to dephase the water signal. In solution NMR this is the basic premise of the WET[36] approach which uses a series of four radio frequency pulses and gradients to suppress the water. The simpler CHESS[37] sequence [90° (selective)-gradient] can also be used but has been shown to be less effective in solution NMR[36] but is commonplace in imaging applications.[37] Recently, the use of gradients and pre-saturation have been combined to create a simple, yet efficient, approach to water suppression, named PURGE.[38] However, arguably the most effective water suppression method to date was introduced by Hwang and Shaka.[39] Termed 'excitation sculpting', the method employs two gradient spin echoes sandwiched around a frequency-selective element. Most implementations use a water-selective pulse to manipulate the water such that it is effectively dephased by the two gradient echoes. However, in 1998 Liu *et al.* used the same principle but incorporated an improved W5 DANTE element, which completely inverts all signals *except* the water which is then dephased by gradients. While this sequence is based upon the principle of excitation sculpting, it is more commonly known in the literature as W5-WATERGATE.[40]

In this study a range of sequences were employed to test their applicability for the study of dissolved organic matter in natural waters. Ultimately, it was found that W5-WATERGATE used in combination with a train of water-selective shaped irradiation pulses provided unsurpassed suppression, produced clean baselines, and was compatible with all natural water samples tested. While not particularly exotic or elaborate, the technique described here produces unsurpassed water suppression compared with conventional techniques currently employed. The experiment may find far-reaching application in any system where trace components need to be detected in the presence of an extremely large water signal. The authors are happy to supply the Bruker version of the SPR-W5-WATERGATE sequence, and interested parties are encouraged to e-mail the corresponding author directly.

## Materials and methods

### Samples

Surface water was collected from four different locations between May and June of 2006: (1) the Pacific Ocean, near Merville, British Columbia, Canada (49°46′ N, 124°58′ W); (2) Tombigbee River, Columbus, Mississippi, USA (33°22′ N, 88°23′ W); (3) Lynde Shores Conservation Area, Ajax, Ontario, Canada (43°49′ N, 78°59′ W), and (4) Lake Ontario, at Pickering Beach, Ajax, Ontario, Canada (43°49′ N, 79°58′ W). Surface water was sampled at *ca.* 20 cm depth and collected directly into 20 mL glass vials (in the case of Lynde shores a second 10 L sample was collected for lypholization). All glassware was pre-cleaned prior to sampling in the field with a minimum of ten volumes of distilled water. Further pre-cleaning was carried out on-site by rinsing with an additional ten volumes (minimum) of the water sample to be collected. Samples were syringe-filtered using a 5 $cm^3$ luer lock, Micro-Mate glass syringe (Fisher Scientific, Oakville), and filtered through a 0.45 μm luer lock Cameo Teflon filter (Fisher Scientific, Oakville). Note: syringes were pre-cleaned with distilled water (ten volumes) in a similar fashion as stated above, with at least one volume of sample passed through the syringe filter prior to collection for sample analysis. 5% $D_2O$ (v/v) was added to the sample (to act as a spectrometer lock) and transferred to a 5 mm Royal Imperial Grade NMR tube (Wilmad, New Jersey). NMR tubes and all transfer glassware (*i.e.* pipettes) were pre-cleaned with ten volumes of HPLC grade water, followed by washing with three volumes of the sample to be analyzed. Samples were analyzed as soon as possible after collection from the field (NMR analysis was started within one hour), with the exception of the water samples from Mississippi and the Pacific Ocean which were shipped on ice and analyzed four days after collection. The Suwannee River reverse osmosis natural organic matter (NOM) standard was purchased directly from the International Humic Substances Society, re-dissolved in $D_2O$ and adjusted to pH 7 (NaOD/DCl) for analysis. Readers should be aware that the direct NMR analysis using 65 536 scans takes over two days to complete per sample.

Note: direct NMR was attempted on some samples before and after filtering. In most samples it was possible to collect spectra using either approach. However, in some cases, especially for the deeper groundwater, a very fine suspension of 'clay-like' particles made shimming extremely challenging. Furthermore, in the samples that were not filtered, biological changes were noted over time. Interested readers should refer to the supporting information for example spectra and further details.†

### TOC measurements

Total Organic Carbon (TOC) measurements were performed on a Shimadzu TOC-V Series Analyzer. Filtered samples were acidified to pH 2 followed by sparging to remove dissolved inorganic carbon. The sample aliquots were then subjected to high-temperature catalytic oxidation to form $CO_2$, and subsequent $CO_2$ concentrations measured with a non-dispersive infrared detector.[41]

### NMR experiments

All NMR experiments were carried out on a Bruker Avance 500 MHz equipped with a 5 mm $^1$H-BB-$^{13}$C TBI probe with an actively shielded Z-gradient. Important experimental parameters are supplied in the appropriate figure captions. 1D solution state $^1$H NMR experiments were acquired with a recycle delay of 2 s, and 32 768 time domain points. Spectra were apodized by multiplication with an exponential decay producing a 10 Hz [1 Hz for sucrose (see Fig. 1)] line broadening in the transformed spectrum, and a zero-filling factor of 2. Where appropriate, pre-saturation was applied on resonance generated by a 60 W amplifier attenuated at 50 dB during the relaxation delay. Direct $^1$H NMR was performed using WATER suppression by GrAdient-Tailored Excitation (WATERGATE) and was carried out using a W5 train and a 125 μs binomial delay[40] such that the 'sidebands' occurred at *ca.* 12 ppm and −2 ppm and were outside the spectral window. W5-WATERGATE was preceded by a train of selective pulses: 2000, 2 ms, calibrated π (180°) pulses were used, each separated by a 4 μs delay. Each selective pulse had a theoretical

 

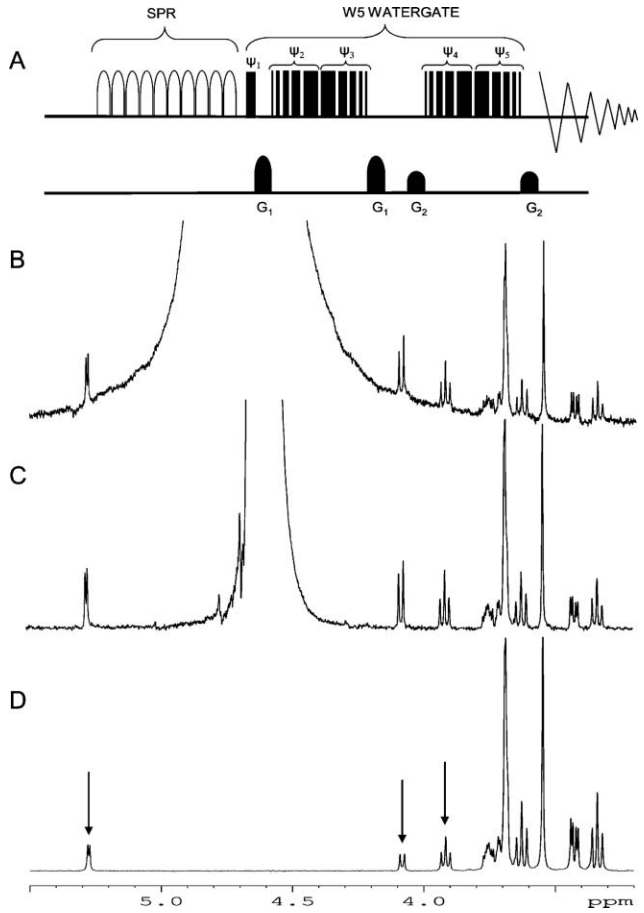


**Fig. 1** **A**) depicts the SPR-W5-WATERGATE sequence. Selective pulses are depicted by an open 'shape', whereas hard pulses are indicated by solid blocks. Selective π pulses were applied for 2 ms using a rectangular profile defined by 1000 points, and truncated to 100%. 2000 pulses were applied each along *x*, with a 4 μs delay separating each pulse. *G* represents gradient pulses. Full details of the W5-WATERGATE approach can be found in Liu *et al.* (1998).[40] Basic phase, gradient and pulse parameters are provided here simply to assist the reader. $\psi_1 = x,-x$; $\psi_2 = x,x,y,y,-x,-x,-y,-y$; $\psi_3 = -x,-x,-y,-y,x,x,y,y$; $\psi_4 = x,x,x,x,x,x,x,x,\ y,y,y,y,y,y,y,y,\ -x,-x,-x,-x,-x,-x,-x,-x,\ -y,-y,-y,-y,-y,-y,-y,-y$; $\psi_5 = -x,-x,-x,-x,-x,-x,-x,-x,\ -y,-y,-y,-y,-y,-y,-y,-y,\ x,x,x,x,x,x,x,x,\ y,y,y,y,y,y,y,y$, receiver = $x,-x,-x,x,x,-x,-x,x,\ -x,x,x,-x,-x,x,x,-x$. Pulse lengths during the W5 block are 7.83, 18.54, 37.17, 70.2, 134.19, 134.19, 70.2, 37.17, 18.54, and 7.83° (the block is repeated twice) with each pulse separated by a 125 μs binomial delay, such that the side bands appear at *ca.* 12 and −2 ppm (outside the spectral window). Gradients are applied at $G_1$ = 34% and $G_2$ = 22%. **B**) shows the water suppression standard without any solvent suppression. **C**) using a basic pre-saturation as a comparison, and **D**) using the SPR-W5-WATERGATE sequence with the parameters optimized for natural water samples. All spectra were collected using eight scans, the increase in S/N in spectrum **D** results from the maximal use of the receiver gain. Signals up to 1.1 ppm on each side of the water signal are slightly attenuated (the most affected signals are those highlighted with arrows), those up to 0.4 ppm on either side of the water resonance are completely destroyed. This wide suppression profile is necessary to handle the extremely broad and intense water signal in natural samples.

inversion profile of *ca.* 0.4 ppm (calculated using Bruker Biospin's, shape tool in Topspin™ 1.3) and is similar to that employed by Hwang and Shaka.[39] In practice, the combination of the W5 sequence and the train of selective pulses resulted in signals up to 1.1 ppm on each side of the water signal being slightly attenuated at natural abundance, while those signals <0.4 ppm from the water resonance were completely destroyed. This was estimated by comparing the profile of concentrated DOM samples using pre-saturation to that of the SPR-W5-WATERGATE at natural abundance (see Fig. 2, for an example).

## Results and discussion

In ocean water, Total Organic Carbon (TOC) is present at only 1.1 mg $L^{-1}$. However, using an approximate conversion factor of 1.7 [commonly used in geochemistry to convert organic carbon to organic matter (OM)[42]], the concentration of OM present can be approximated at *ca.* 1.9 mg $L^{-1}$. Thus, in a standard 5 mm NMR probe (assuming *ca.* 300 μL volume inside the coil), only *ca.* 500–600 ng of DOM are available for analysis. Furthermore, considering that the intensity of the water signal is many orders of magnitude greater than the weak signals from DOM (itself a heterogeneous mixture), it becomes clear that fingerprinting of DOM at natural abundance is not trivial by any standards, and truly pushes the boundaries of modern NMR spectroscopy.

Many common water suppression approaches [namely, pre-saturation,[32] composite pulse pre-saturation,[33] WATER suppression by GrAdient-Tailored Excitation (WATERGATE),[43] Pre-saturation Utilizing Relaxation Gradients and Echoes (PURGE),[38] Water Suppression enhanced through T1 effects (WET),[36] and Excitation Sculpting[39]] were found to be unsuitable for this application. No single experiment could be found which permitted the NMR receiver to be set with the maximum gain such that the minute signals from the DOM could be detected at full sensitivity. Details of the approaches tested and a summary of the results are provided in Table 1. Furthermore, under these challenging conditions, many approaches produced baseline distortions (that in most applications would be negligible or not apparent), and created significant artifacts that often masked part, or all, of the very weak signals in the natural water samples.

In an effort to overcome these problems, various water suppression techniques were combined and ultimately it was found that WATERGATE (using optimized W5 pulse trains[40]), within a double pulse field gradient spin echo,[39] preceded by a train of shaped 180° pulses [the latter element simply shaped pre-saturation (SPR)] proved to be robust and produce excellent suppression for a range of natural samples with waters of varying line widths. Essentially, the SPR-W5-WATERGATE sequence involves two major components that act synergistically, the W5-WATERGATE and SPR (see Fig. 1A). The W5 pulse train's aim is to invert the sample signals, while leaving the water unperturbed. The water is then dephased by the gradients and the signals from the sample refocused. However, in natural samples with extremely large, and sometimes very broad, water signals, W5-WATERGATE alone proved insufficient for complete suppression, often

**Table 1** Water suppression techniques tested for the observation of DOM at 'natural abundance'

| Solvent suppression technique | Comment | OH/NH[a] | Results |
|---|---|---|---|
| Pre-saturation[32] | Water reduced by saturation, up to 48 dB[b] tested. | Attenuated | Incomplete suppression made detection of DOM signals impossible. |
| CPPR[33] | Water reduced by saturation, up to 48 dB[b] tested. | Attenuated | Incomplete suppression made detection of DOM signals impossible. |
| PURGE[38] | Water reduced by saturation and dephasing by gradients, up to 48 dB[b] tested. | Attenuated | Suppression poor. At very high pre-saturation power, <50 dB,[b] phase twisting of the baseline was observed. In higher concentration samples DOM signals could be observed. |
| WET[36] | Water flipped by a selective pulse and dephased by a gradient. Sinc, square, and Gauss shapes tested. | Full | Incomplete suppression made detection of DOM signals impossible. |
| Excitation Sculpting (ES)[39] | Water flipped by two selective pulses and dephased by two pulse field gradient spin echoes. 1–2 ms water-selective square-shaped pulses used. | Full | Better than all above. Showed promising results in select samples. Residual water *ca.* 10–20 times larger than DOM signals. Distortions in baseline of up to 4 ppm around the water. For optimal suppression, pulse length needed to be varied per sample, which complicated direct comparison of different samples. Extensive calibration per sample required. |
| SPR-Excitation Sculpting | As above with shaped pre-irradiation of the water using 1000, 2 ms, π pulses. | Attenuated | Much better than ES alone. Distortions in baseline reduced, and residual water similar to the DOM signals in higher concentration samples. See ESI, Fig. S2, for examples. |
| W5-WATERGATE[40] | All signals, *except the water*, flipped by two W5-DANTE trains. Water dephased by pair of gradient pulses. | Full | Best 'single' technique. DANTE blocks require no additional calibration and are relatively robust working with samples containing water signals of varying line widths. Least baseline distortions, residual water *ca.* 10 times larger than the DOM signals. Baseline distortions present in samples with very wide (>2 ppm at base) water signals. |
| SPR-W5-WATERGATE | As above with shaped pre-irradiation of the water using 1000, 2 ms, π pulses. | Attenuated | As above but worked with all samples. Residual water not apparent even in very dilute samples (ocean water). Compatible with all samples tested. Main drawback: wide region around the water attenuated (see main text). |

[a] OH/NH indicates whether exchangeable protons should be observable at full sensitivity or partially attenuated by the water suppression sequence. [b] Corresponds to an attenuation on a 60 W amplifier.

producing broad 'lobes' resulting from partial inversion of the water signal, masking real signals from the sample. For full details of W5-WATERGATE, readers should refer to Liu *et al.* (1998).[40] By replacing the conventional relaxation delay with a train of on-resonance, shaped pulses, the water signal (both height and width) can be effectively reduced to a point that it is unaffected by the subsequent W5 pulse trains and thus completely 'gated' from the final spectrum. It is possible to adjust both the length of the selective pulses in the pre-irradiation period and the binomial delay in the W5 trains to effectively tailor the spectral bandwidth that is suppressed in the final spectrum. However, when working with natural samples that contain varying concentrations of DOM and inhomogeneities, it is critical to create a method that is highly robust and can be used with all natural samples and not just a select few. Ultimately, the conditions that proved to be the best for a range of natural samples including rainwater, groundwater, snow, river, lake and ocean water (note: data for all these samples were collected but not all are explicitly considered in this paper) were an 'aggressive' binomial delay of 125 μs and a shaped pulse length of 2 ms ($B_{eff}$ ≈ inversion profile of *ca.* 400 Hz [calculated using Bruker Biospin's, shape tool in Topspin™ 1.3)]. Unfortunately, while these parameters produce outstanding and robust suppression that is effective in all natural samples, they result in signals up to 1.1 ppm on each side of the water signal being slightly attenuated, and those up to 0.4 ppm from the water resonance being completely destroyed. It should be pointed out that in some samples more selective irradiation fields and inversion profiles produced reasonable results, but were ultimately found unsuitable for more challenging samples. Thus, while the parameters employed here are relatively 'aggressive' they are robust and work for all natural samples tried, which was not the case with other sequences or when more selective water suppression was attempted.

Fig. 1 highlights the SPR-W5-WATERGATE sequence used in this paper (Fig. 1A) and demonstrates its effectiveness on the common water suppression standard (2 mM sucrose solution in 10% : 90% $D_2O$–$H_2O$, Fig. 1B). Fig. 1C shows the suppression that can be achieved using conventional pre-saturation as a comparison. From Fig. 1D, it is clear that outstanding suppression can be achieved using the parameters optimized here for the observation of DOM in natural samples, but at a cost of attenuation of the signals close to the water.

 

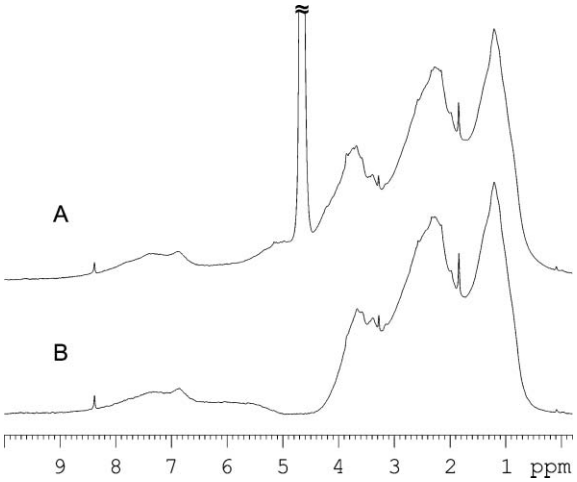


**Fig. 2** $^{1}$H NMR of the Suwannee River reverse osmosis standard dissolved at 5000 mg $L^{-1}$: **A**) acquired using conventional pre-saturation; **B**) acquired using the SPR-W5-SEQUENCE parameters optimized for natural abundance detection (see Experimental). Both spectra were acquired using 128 scans.

Fig. 2 demonstrates the application of the optimized SPR-W5-WATERGATE to the Suwannee River reverse osmosis NOM standard. This sample is dissolved at 5000 mg $L^{-1}$ and is not at natural abundance; it is included here so that readers can evaluate the information lost when the SPR-W5-WATERGATE sequence is used. The signals close to the water [*ca.* 0.4 ppm each side (water resonance is at 4.65 ppm in this sample)] are completely suppressed, while signals up to *ca.* 1.1 ppm are also attenuated. In the downfield attenuated region (*ca.* 5.15–5.75 ppm), signals mainly from double bonds and anomeric protons in carbohydrates will be suppressed, while in the upfield region (*ca.* 3.55–4.25 ppm), signals mainly from carbohydrates and methoxyl in lignin will be attenuated.[44–47] Integration indicates that *ca.* 30% of the intensity of the total 'carbohydrate region' from 4.25–3.2 ppm is lost when the optimized SPR-W5-WATERGATE approach is applied to the Suwannee River NOM control sample. However, it is important to note that this does not necessarily reflect the absolute attenuation that will be observed in samples at natural abundance, and this aspect is considered later in this manuscript.

Fig. 3 compares the natural abundance $^{1}$H NMR spectrum from a wetland [11 mg $L^{-1}$ DOM, or *ca.* 3–4 μg in the coil region (assuming 300 μL coil volume)] with the same sample after lypholization and re-dissolution (*ca.* 20 000 mg $L^{-1}$). Fig. 3A clearly shows that it is possible to collect NMR spectra of DOM at natural abundance. Furthermore, it demonstrates that the approach can be applied to reconstituted samples, which for the first time, permits scientists to use NMR to evaluate compositional changes that may occur during isolation, preparation, and/or pre-concentration procedures. This is essential, as nearly all detailed structural studies of DOM first require its isolation/concentration. The extent to which samples are changed during isolation is an area of scientific debate and concern.[18–20] Arguably, lypholization is one of the least destructive concentration procedures, although with lypholization, salts are also concentrated along with the

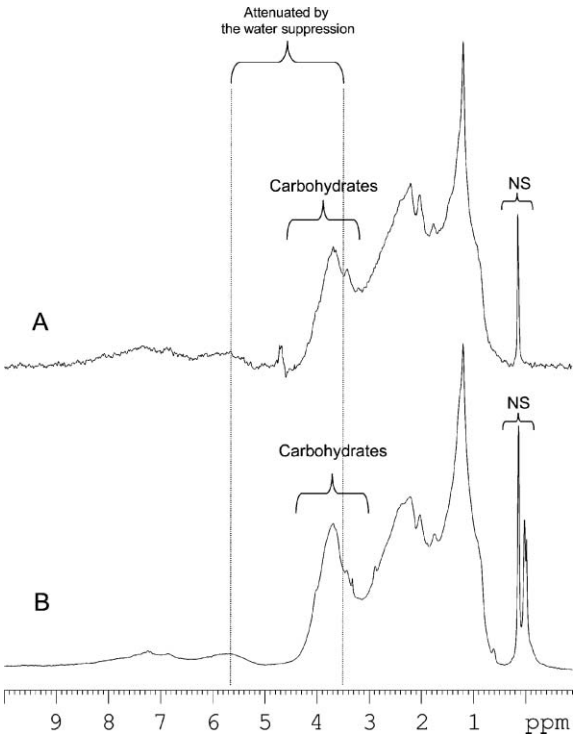


**Fig. 3** $^{1}$H NMR acquired using the optimized SPR-W5-WATERGATE experiment for **A**) Lynde Shores Conservation area (natural abundance *ca.* 11 mg $L^{-1}$), **B**) same sample at 20 000 mg $L^{-1}$ (concentrated by lypholization). **A** was acquired with 65 536 scans, **B** with 128. NS = natural silicates.

organic matter, making the material unsuitable for many types of analysis (including NMR if salt concentrations are very high). The NMR spectra in Fig. 3 demonstrate that lypholization has little effect on the overall profile of the NMR spectrum (note that this is a freshwater sample with relatively low salt contributions), with the exception of the region labeled NS, which most likely results from the concentration of natural silicate species during freeze drying. There are, however, some considerable differences in the central region of the NMR spectrum that result not from sample treatment, but from the water suppression method itself.

Both spectra in Fig. 3 have been recorded with identical parameters. However, at natural abundance, owing to the extremely weak nature of the DOM signals, even the smallest radio frequency field (resulting from the water suppression method) leads to significant suppression of peaks in proximity to the water, while at very high concentration the same weak radio frequency field has a smaller or negligible effect. Thus the carbohydrate region especially is attenuated in the natural abundance spectrum when compared to the spectrum collected for a very concentrated sample, as the attenuation caused by the water suppression has a larger relative effect. However, it should be stressed that the freeze-dried sample contains >1800 times the material compared to the natural abundance sample. At natural abundance, all DOM signals will be extremely weak and this concentration-dependent effect will likely be negligible as long as samples do not vary drastically in concentration. Indeed, this is partially demonstrated in Fig. 4

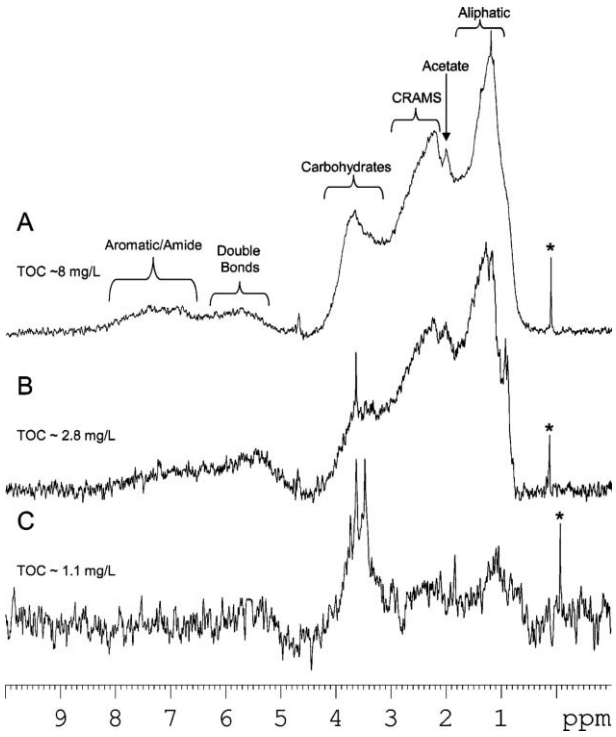


**Fig. 4** $^{1}$H NMR of natural waters using SPR-W5-WATERGATE. **A**) Tombigbee River (33°22′ N, 88°23′ W), **B**) Lake Ontario (43°49′ N, 79°58′ W), and **C**) Pacific Ocean (49°46′ N, 124°58′ W). * is a naturally occurring species (silicate) and not an internal reference standard. 65 536 scans were accumulated for each sample. CRAMS = carboxyl-rich alicyclic molecules.[46]

which compares natural abundance NMR spectra for the Pacific Ocean, Lake Ontario, and a river system in Mississippi.

The Pacific Ocean sample (Fig. 4C) results in an NMR spectrum with minimal signal-to-noise due to the extremely low concentration of the sample. Yet even at this low concentration the major signals from carbohydrates (*ca.* 3–4 ppm) can be discerned. This is consistent with other work that identifies carbohydrates as major constituents in dissolved organic matter (DOM) from sea water isolated by ultra-filtration.[48,49] This clearly demonstrates that while the carbohydrate signal will be partially attenuated by the water suppression, essentially negating absolute quantification for this region, it is still possible to obtain semi-quantitative information for the carbohydrate region when comparison is carried out between natural abundance samples acquired with identical NMR parameters.

While it is clear that the oceanic sample is mainly dominated by carbohydrate resonances, both the river and lake samples demonstrate a natural abundance signature similar to that for the wetland sample (from Fig. 3A). The general assignments offered are consistent with those determined from 2D NMR,[45,46,50] indicating that once detailed assignments have been performed, even the relatively broad 1D profile of DOM at natural abundance can provide excellent compositional information of unaltered DOM in its natural state. In particular, it is interesting to note that in a recent publication, Hertkorn *et al.*[46] demonstrated, using 2D NMR, that acetate in dissolved organic matter most likely originates from O and N acetylated carbohydrates that are abundant in water.[46] However, the authors also added the caveat that the acetate potentially could also result from reaction with the sodium hydroxide used to dissolve the DOM for NMR analysis. From the results presented here, an acetate signal can be observed in a chemically unaltered DOM sample, strongly supporting the main hypothesis of Hertkorn *et al.* that acetate in DOM is in fact from natural origins.[46] This is just one example as to the role that direct NMR spectroscopy of natural waters can play in unravelling the complexities of aquatic chemistry in the environment. Not only can the approach be potentially used to assess the alterations during sample preparation and concentration, but it may also be used to study very 'precious' volume/mass-limited samples, such as pore water or ice cores, in a non-destructive manner. Finally, even from basic 1D lineshape analysis, natural abundance data may also prove key in evaluating the aggregation state of DOM in natural water samples, which, in turn, is essential for understanding contaminant transport and flocculation processes in the environment. Thus far, NMR spectroscopy has proved extremely useful in monitoring and understanding aggregation processes;[30] however, it is not clear how the unusually high concentrations required for detailed NMR spectroscopy influence these processes. Interestingly, at natural abundance most samples from larger water bodies (see Fig. 3) show a very broad lineshape. This suggests that the broad lineshape seen in DOM after pre-concentration[45,46,48–50] is not simply due to aggregation from the high concentrations required for most NMR experiments. At this point, it is not clear whether the lineshape results from broadening due to interactions with paramagnetic metals, the heterogeneity/complexity of the mixtures or a combination of both. Ultimately, however, direct NMR provides the basis through which detailed molecular knowledge of DOM obtained at high concentration can be related to samples as they exist in their unperturbed natural environment and should prove useful for answering this and other environmentally significant questions.

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