Rapid Estimation of Nuclear Magnetic Resonance Experiment Time in Low-Concentration Environmental Samples

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Abstract—Nuclear magnetic resonance (NMR) spectroscopy is an essential tool for studying environmental samples but is often hindered by low sensitivity, especially for the direct detection of nuclei such as $^{13}$C. In very heterogeneous samples with NMR nuclei at low abundance, such as soils, sediments, and air particulates, it can take days to acquire a conventional $^{13}$C spectrum. The present study describes a prescreening method that permits the rapid prediction of experimental run time in natural samples. The approach focuses the NMR chemical shift dispersion into a single spike, and, even in samples with extremely low carbon content, the spike can be observed in two to three minutes, or less. The intensity of the spike is directly proportional to the total concentration of nuclei of interest in the sample. Consequently, the spike intensity can be used as a powerful prescreening method that answers two key questions: (1) Will this sample produce a conventional NMR spectrum? (2) How much instrument time is required to record a spectrum with a specific signal-to-noise (S/N) ratio? The approach identifies samples to avoid (or pretreat) and permits additional NMR experiments to be performed on samples producing high-quality NMR data. Applications in solid- and liquid-state $^{13}$C NMR are demonstrated, and it is shown that the technique is applicable to a range of nuclei. Environ. Toxicol. Chem. 2013;32:129–136. © 2012 SETAC

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INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is arguably the most powerful tool for the study of organic structures and has been central in understanding a broad range of environmental systems, including carbon cycling in the arctic [1–3], the effects of global warming on soil [4], unraveling the structure and interaction of humic substances [5–9], and elucidating organic components in air particles [10,11]. In particular, cross-polarization magic angle spinning (CP/MAS) experiments have been used extensively because of the qualitative structural information that is gained and because of its non-destructive nature [12]. Cross-polarization magic angle spinning is now routinely used in environmental studies on a variety of samples, including natural organic matter, wood, sediments, and black carbon [13–16]. In most cases, $^{13}$C is the nucleus of interest, which yields an overall profile of the organic composition of the sample; however, studies involving CP/MAS of other nuclei such as $^{15}$N and $^{31}$P also have wide environmental application [17,18].

Most environmental studies have to deal with rather limited amounts of sample. In some cases, obtaining enough sample in the first place is the limiting factor, for example, with studies that isolate dilute dissolved organics from water or air particulates from the atmosphere or projects dealing with samples from remote locations (i.e., deep sediment cores, the Arctic or Antarctic), from which it is logistically difficult to return with large sample volumes. In other cases, samples become precious and limited due to extensive pretreatment or fractionation that may be required prior to NMR analysis [19]. Consider, for example, that a sediment low in organic carbon may require treatment by hydrofluoric acid many times to dissolve minerals, reduce paramagnetics, and concentrate the organic material [20]. Such treatments can take months and, when the organic carbon content is low, can consume a large amount of the original sample to produce the 100 mg required for common solid-state NMR analysis. Carbon combustion analysis may be performed to determine sufficient carbon content in a sample and helps determine whether $^{13}$C NMR analysis is feasible. However, in many cases, there is simply not enough material to permit accurate carbon analysis by combustion, which requires approximately 5 g per measurement (for samples <10% organic carbon) and should be performed in triplicate, which would require 15 g total [21]. As such, it is often the case that an environmental researcher desperately needs high-quality NMR data and has spent months (possibly years) collecting and preparing the samples but cannot accurately gauge the feasibility of, or the time involved in, acquiring the NMR data. Additionally, most NMR centers have many users, and the analysis of low-carbon organic samples (especially by solids NMR) can routinely take 1 to 4 d per sample, making the spectrometer demands for large projects considerable. In cases in which no carbon data are available, a great deal of NMR time can be wasted on running a precious sample that produces no signal.

It would therefore be a considerable step forward for the field if it were possible to prescreen natural samples in a nominal time frame (2–3 min) and determine if a sample will produce an acceptable NMR spectrum and how much time is required to generate a desired signal-to-noise ratio (S/N). This would identify samples to avoid and permit additional NMR experiments to be performed on samples producing high-quality...
NMR data. Consequently, this approach greatly increases the amount of NMR information that can be obtained from natural samples in a set amount of time. Such an approach is introduced in the present study. The conventional NMR signal for a typical environmental sample is very weak (and could take many days to observe), so the prediction method is not based on the conventional NMR signal but instead is based on a “spikelet” that represents the summation of all the nuclei in the sample in one extremely narrow and easily observed spike. The approach is based on manipulation of the Carr–Purcell Meiboom–Gill (CPMG) pulse sequence.

The CPMG sequence (Fig. 1A and B) is commonly used in environmental studies for T2 relaxation measurements to quantify contaminant binding in environmental media and in water suppression techniques as a relaxation filter [22–24]. It uses a series of spin echoes separated by a delay period (τ). If this delay is shortened to the point where the echoes are applied at a faster rate than the chemical shift can evolve, the result is that the chemical shift envelope is reduced to a single spike within the recorded bandwidth at the transmitter frequency [25]. In addition, to concentrating the signal into a single spike, the spin lock also perturbs T2 relaxation and removes the effect of nonhomogeneous broadening, which can be significant in complex, heterogeneous samples [26–28]. Thus, signal persists for much longer, permitting increased sampling, which leads to increased S/N (see Fig. 1C and D for an example with hemoglobin). The signal intensity of the CPMG spike is proportional to the total carbon signal from the sample. Thus, it can be used not only to indicate the existence of observable nuclei but also to predict the time required to attain a particular S/N in a conventional NMR spectrum. The use of CPMG is well documented in many NMR applications, and various articles describe the pulse sequence in greater detail [28–32].

The single-spike technique has been previously demonstrated by Morris and colleagues [33], who explain it as a method to improve sensitivity by reducing and controlling chemical shift scaling. Lim et al. [25] use the single-spike technique to enhance multiple quantum NMR experiments. In the present study, the use of the CPMG single spike (CPMG-SS) spectroscopy to predict whether a sample will yield an adequate spectrum is demonstrated, and how the intensity of the CPMG spike correlates with the time required to obtain a full 13C NMR spectrum of a particular S/N is evaluated. To illustrate the approach, the present study analyzes several environmental samples in solid- and liquid-state NMR, and its use in 13C and 31P NMR is demonstrated.

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** (A) Illustration of the solid-state Carr–Purcell Meiboom–Gill (CPMG) pulse sequence with cross-polarization. An 18-μs echo delay period (τ) between each 180° pulse is used to avoid chemical shift evolution and is repeated 280 times (n). High-power composite pulse decoupling (Spinal-64) is used during acquisition. (B) The liquid-state CPMG experiment consists of a 90° on the X nucleus with Waltz-16 proton decoupling during acquisition. An 18-μs echo delay period (τ) between each 90° pulse is used to avoid chemical shift evolution and is repeated 2,000 times (n). One hundred eighty degree pulses were not used in the liquid-state experiments because only one-quarter of the power (essential on a solution-state probe) is required for the corresponding 90° pulse and produced essentially the same result as a 180° pulse at a fast repetition rate. See Materials and Methods and Supplemental Data for further details. The 90° flip angle could also be used for solid experiments if required. (C) Free induction decay (FID) from a standard cross-polarization–magic angle spinning (CP-MAS) experiment for hemoglobin. The FID is fully relaxed in approximately 4 ms. (D) FID from the CPMG-SS experiment for the same sample. The FID extends well beyond 80 ms. Recording a solid-state CPMG-SS spectrum for an acquisition this long is pushing the limits of the probe and was produced only once to demonstrate the concept. Readers should not try to reproduce this and are strongly encouraged to read the Supplemental Data for procedures on safe CPMG-SS optimization. If optimized as outlined in the Supplemental Data, the CPMG-based solids experiment will actually use much less power than a conventional CP experiment and if applied appropriately can be carried out without stress to the spectrometer.
**MATERIALS AND METHODS**

**Sample preparation**

Crystalline hemoglobin from bovine blood was purchased from Sigma Aldrich and was used as is. Concentrations were varied by mixing with powered sodium chloride purchased from Fisher Scientific. An earthworm was selected from a healthy population of *Eisenia fetida* maintained within our laboratory since 2006, as described by Brown et al. [34]. The original progenitor earthworms were purchased from The Worm Factory. The worm was flash frozen with liquid nitrogen, freeze dried, and stored at −20°C until required. Each solid sample was homogenized using a Wig-L-Bug with a steel ball and pestle for approximately 5 min, and a full rotor was packed each time. Suwannee River dissolved organic matter (DOM) purchased from the International Humic Substances Society was dissolved in 600 μL D$_2$O (99.9%) and set to a pH greater than 12 using sodium deuterioxide (NaOD 99.5% D, 30% in D$_2$O) for liquid-state studies. The D$_2$O and NaOD were purchased from Cambridge Isotope Laboratories.

**NMR spectroscopy**

Nuclear magnetic resonance experiments were performed on a Bruker Avance III 500 MHz spectrometer. Cross-polarization (CP) and CPMG-SS experiments on hemoglobin and worm were performed on a 4-mm broad-banded solid-state probe. Cross-polarization experiments were performed at a spinning speed 13 kHz and a ramp of 80 to 100% with a CP contact time of 2 ms. A total of 3,072 points were used in the time domain, with an acquisition time of 40 ms and high-power composite pulse decoupling (Spinal-64). Spectra were zero filled by a factor of 2 and processed using an exponential function corresponding to a line broadening of 30 Hz in the final spectrum.

Carr–Purcell Meiboom–Gill single-spike parameters were identical to the CP parameters with the exceptions that the sample was static and that the decoupling power was attenuated by 3 dB (see Supplemental Data). Although it is possible to run the CPMG-SS with magic angle spinning (MAS), rotor synchronization is required to ensure proper echo formation [35]. It was found that a static sample produced an adequate S/N in the CPMG-SS spectrum and indeed more than that observed in a nonsynchronized experiment under MAS. Given that the approach here is to prescreen samples, the use of a static sample is a considerable advantage in that the rotor does not have to be carefully balanced to ensure spinning, and time is not wasted in achieving a set spinning speed. As such, all the solid-state CPMG-SS experiments were acquired without MAS. A 15-μs 18-W pulse (corresponding to a low-power 180°) was used for echo formation with 790 loops and a delay of 18 μs. Spectra were zero filled by a factor of 2 and processed with an exponential function corresponding to a line broadening of 30 Hz in the final spectrum. Once the CPMG echoes are optimized for a specific probe, recalibration on a per-sample basis (even between very different samples) provided little to no improvement in the CPMG signal. Readers are strongly advised to read the Supplemental Data on safely implementing this approach and how to safely calibrate the CPMG pulse.

Soils were run on a second 4-mm broad-banded solid-state probe. The acquisition time was 14 ms, with 1,024 time domain points. The rotor spinning speed was 13 kHz. Cross-polarization experiments were performed with a ramp of 80 to 100%, a CP contact time of 2 ms, and high-power composite pulse decoupling (Spinal-64). Carr–Purcell Meiboom–Gill single-spake parameters were identical to those of the CP experiments except that they were acquired without spinning. A 12-μs 12-W pulse (corresponding to a low-power 180°) for echo formation was found to be optimal for the second probe, and 280 loops were used with an 18-μs delay.

Liquid-state experiments on DOM were conducted on a 5-mm broad-banded probe. Carbon experiments were acquired with 16,384 points, an acquisition time of 275 ms, and low-power Waltz-16 proton decoupling during acquisition. Spectra were zero filled by a factor of 2 and processed using an exponential function corresponding to a line broadening of 50 Hz in the final spectrum. Carr–Purcell Meiboom–Gill single-spake experiments in the liquid state were acquired with 16,384 points, a 131-ms acquisition time, and low-power Waltz-16 proton decoupling (same power level as in the conventional experiments). The CPMG pulse was calibrated to 18 μs at 20 W for the solution-state broadband probe. This actually corresponds to a low-power 90° pulse, because a 180° pulse on this specific probe required considerably more power. The 180° pulse was tested, but only slightly enhanced S/N was obtained, so the lower power 90° pulse was chosen to reduce stress on the solution-state probe. However readers should note that, because the delay between each pulse is so short, a spin lock condition is still satisfied, and a single spike is still achieved as discussed in detail by de Andrade et al. [36]. Delay in the CPMG sequence was set to 18 μs with 2,000 loops. Spectra were zero filled by a factor of 2 and processed using an exponential function corresponding to a line broadening of 50 Hz in the final spectrum. See the Supplemental Data for the technique used to optimize the CPMG-SS sequence as well as some practical considerations.

**RESULTS AND DISCUSSION**

Carr–Purcell Meiboom–Gill single-spake focuses all signals from all nuclei in a sample into one spike in the recorded bandwidth. The result is a complete loss of chemical shift information but an intense spike that is directly proportional to the concentration of NMR-active nuclei in the sample. The intensity of this spike can be used to estimate the amount of NMR time required to record a conventional spectrum. However, before the technique can be applied directly to environmental samples, it is important discuss some of the fundamentals behind the approach and demonstrate proof of principle. The present study demonstrates the concept and proof of principle on hemoglobin. Hemoglobin was chosen because it has a heterogeneous structure, contains paramagnetic centers, and exhibits fast relaxation, giving rise to broad NMR signals. As such it is difficult to work with in NMR spectroscopy (as are most environmental matrices) and represents a readily available and challenging surrogate for demonstration purposes. The approach is then applied to various soils (including a very-low-organic-carbon soil from the Arctic), dissolved organic matter from Lake Ontario, and a freeze-dried worm. The latter represents whole-tissue samples that are now being studied extensively by NMR to understand in situ environmental stress, metabolism and toxicity [37].

**Proof of principle**

A relationship between the S/N of the CPMG spike (S/N$_{SS}$) and the number of scans required in a conventional $^{13}$C CP experiment to achieve a specific S/N (NS$_{CP}$) can be determined. In any conventional NMR experiment, the S/N is directly proportional to the square root of the number of scans, the amount of sample (n), and various other spectrometer and
environmental conditions, which are generally kept constant \( (k) \), as shown in Equation 1 [38].

\[
S/N = k_n \sqrt{N_S/S}
\]

When implementing the CPMG-SS experiment, if the number of scans is kept constant, the spike intensity can be used as a comparison between samples. Equation 1 then reduces to

\[
S/N_{SS} = k_1 n \
\]

where \( N_{SS} \) is the number of scans of the CPMG-SS experiment and \( k_1 \) is equal to \( k \sqrt{N_{SS}} \). If the goal is to acquire a CP experiment until a target \( S/N \) (\( S/N_{CP} \)) is achieved, then \( S/N_{CP} \) is kept constant, and Equation 1 can again be rewritten for a CP experiment as

\[
S/N_{CP} = k_n \sqrt{N_{SS}}
\]

where \( k_2 \) is equal to \( S/N_{CP} \) divided by \( k \). Finally, to relate \( S/N_{SS} \) and \( S/N_{CP} \), it is assumed that both experiments (CP and CPMG-SS) are run on the same sample, and therefore, \( n \) is equal. By solving for \( n \) in Equations 2 and 3 and equating them, the final expression relating \( S/N_{SS} \) to the \( S/N_{CP} \) is found

\[
S/N_{SS} = k \sqrt{N_{CP}^2}
\]

Equation 4 very well \( (r^2 > 0.98) \), as shown in Figure 2. In addition to confirming the relationship between the \( S/N_{SS} \) and \( S/N_{CP} \), the plot is also useful as a calibration curve from which the \( S/N \) in a conventional \(^{13}\)C CP-MAS (\( S/N_{CP} \)) can be predicted from the \( S/N \) in the CPMG-SS experiment (\( S/N_{SS} \)) for a hemoglobin sample of unknown concentration.

To validate the predictive ability of the standard curve shown in Figure 2, the \( S/N \) that would be obtained after a given experiment run time for hemoglobin samples with unknown concentration was predicted. Three samples of differing concentration were used, and the \( S/N_{SS} \) was determined for each. Depending on whether the intersection between \( S/N_{SS} \) and experimental run time lies in the light gray, dark gray, or white region in Figure 3, the corresponding \( S/N_{CP} \) can be estimated to be under 10, between 10 and 20, or over 20, respectively.

For example, from the \( S/N_{SS} \) value attained in the CPMG-SS experiment after 64 scans and a given experiment time of 4 h, it can be predicted that the highest concentration sample (red lines in Fig. 3) should have an \( S/N_{CP} > 20 \). Experimentally, an \( S/N_{CP} \) of 25.50 was obtained, which agrees with the prediction. The next sample (blue lines) was to be run overnight for 16 h and falls into the dark gray region between 10 and 20 \( S/N_{CP} \) After the overnight CP experiment, the actual \( S/N_{CP} \) was 16.27. Finally, the last sample produced a very low \( S/N_{SS} \) (green lines) of only 5.26 and was to be a 16-h experiment as well. Based on the standard curve, it was estimated that an \( S/N_{CP} < 10 \) would be produced, and an experimental \( S/N_{CP} \) of 2.94 was obtained. This last example is a situation in which running the CPMG-SS experiment prior to running the CP would have been enough to conclude that an adequate \( S/N \) would not be obtained overnight and that more scans would be required to achieve a discernible spectrum. Each CPMG-SS spectrum took 2 min to run, so determining whether an adequate \( S/N \) would be obtained for each sample took only a few minutes to calculate. In a real-life scenario, the spectrometer time for the last overnight run would have been saved if the CPMG-SS experiment was used to determine that an acceptable spectrum would not have been obtained in the allocated amount of spectrometer time.

\[\text{Estimating experimental runtime in environmental samples}\]

The standard curve developed for hemoglobin cannot be used for samples that are significantly dissimilar in chemical composition because of the differences in heterogeneity. Other proteins could possibly use the standard curve developed in Figure 2, but samples with very different compositions will require recalibration. Here, the approach is tested using real environmental soil samples. A different standard curve for calibration is required because the composition of soil significantly differs from that of hemoglobin.

In this section, a single-point calibration curve is used in which only a single point (one sample from the study) along with the origin from the coordinate plane is plotted and used to predict experimental run time. This is much faster and requires the acquisition of only one conventional CP experiment from which the experiment time and \( S/N \) for the other samples in a series can be predicted from the CPMG-SS (2–3 min per sample). A single point calibration is adequate, because the technique is most useful for estimation purposes and is helpful in answering the common questions, “Will my sample give an acceptable spectrum?” and “How much spectrometer time do I need?”

To demonstrate the concept, a series of three forest-soil samples was used. The \( N_{SS} \) and the CPMG spike intensity
Estimation of NMR experiment time for environmental samples

Environ. Toxicol. Chem. 32, 2013 133

Very-low-abundance samples

Soil and sediment carbon contents can vary drastically, so obtaining total organic carbon measurements can be very beneficial. In some cases, however, the amount of sample required for this procedure may exceed the amount of sample available, and the carbon content remains unknown. Samples with high amounts of carbon can produce an acceptable NMR spectrum in minutes to hours, whereas the signals from samples with low carbon may require 12 h or longer. These are samples that are most problematic and would benefit from a technique such as the CPMG-SS, which allows for rapid detection and experiment time estimation.

To really push the limits of detection, a sample with extremely low amounts of carbon nuclei was employed, and the S/NSS was observed using 128 scans. The sample used was an Arctic watershed soil sample that had previously been reported to have a carbon content of approximately 1% [2]. Here CPMG-SS was used in its most basic form, simply attempting to ascertain whether this single sample will ever give rise to an acceptable NMR spectrum, the logic being that, if no CPMG-SS signal is seen after 128 scans, then acquiring a conventional spectrum is unlikely. However, if even a small CPMG-SS can be recorded, then there is a chance to obtain a reasonable conventional NMR spectrum over an extended run.

The spike obtained for this soil is very small (Fig. 4A) compared with the other forest soils described above, as expected because of its very low carbon content. Because of this, that the 13C CP experiment returned a barely discernible spectral profile overnight, as illustrated in Figure 4B, was not a surprise. In this case, the low CPMG-SS S/N is an indicator that considerably more experiment time is required to obtain a useful spectrum or that pretreatment to concentrate this sample should be considered. Figure 4C shows that, after hydrofluoric acid treatment, an Arctic soil sample that had previously been reported to have a carbon content of approximately 1% [2]. Here CPMG-SS was used in its most basic form, simply attempting to ascertain whether this single sample will ever give rise to an acceptable NMR spectrum, the logic being that, if no CPMG-SS signal is seen after 128 scans, then acquiring a conventional spectrum is unlikely. However, if even a small CPMG-SS can be recorded, then there is a chance to obtain a reasonable conventional NMR spectrum over an extended run.

The variability mainly arises because of the chemical differences in the samples. Although they are all forest soils, they are not chemically identical and tend to vary significantly with soil type, location, drainage, vegetation cover, microbial populations, and the ecosystem that they support as a whole. As a result, the S/N is calculated slightly differently for each of the conventional carbon CP spectra. Despite this, it is clear that CPMG-SS spectroscopy is very powerful for estimation of runtime and S/N in unknown samples and can save spectrometer time if used efficiently.

It is important to stress that a calibration should be performed for each particular series of samples to be studied. In this example, all three soils were forest soils and thus had broadly similar carbon chemical shift dispersion. However, it is unlikely that a single calibration curve will hold for all soils because soils can drastically vary in chemical composition and heterogeneity. Similarly, in a biological setting, the calibration curve for hemoglobin is unlikely to hold for human blood because blood contains many components other than hemoglobin, but, once constructed from a single point, a human blood calibration curve should be fairly accurate for all human blood samples.

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See...
proving the applicability of this technique to spin half nuclei other than $^{13}$C. S/N ratio was measured from noise to signal for the concentrated sample, and the NSCP was then calculated for the dilute sample. It was found that the calculated NSCP was roughly 1% different from the actual value, suggesting that a pretreatment technique, if applicable, should be used to concentrate the organic component, or the experiment should be run for a longer time. In this case, hydrofluoric acid treatment was used to concentrate the sample and a resolvable spectrum (black) was observed. Running the CPMG-SS experiment allows the spectroscopist to discern a resolvable spectrum that cannot be obtained in a reasonable amount of time and may not be achievable at all in some cases, hydrofluoric acid treatment was used to concentrate the sample and a resolvable spectrum (gray) was then produced with the same number of scans. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

very unlikely that a $^{13}$C conventional NMR spectrum can ever be recorded; and (2) if a CPMG-SS can be discerned in 128 scans but has an S/N of 10 or less, the sample likely will require an extended run (a weekend, at least). If the S/N in the CPMG-SS is greater than 10, an adequate spectrum likely can be acquired overnight.

Other nuclei

A range of other spin-half NMR nuclei is of interest for environmental research from the cycling of $^{31}$P and $^{15}$N [17,18], to the study of metal contaminants such as $^{199}$Hg, $^{113}$Cd, and $^{207}$Pb [24,39,40]. Recording NMR spectra is often challenging in part because the nuclei are relatively insensitive and in part because of the wide range of chemical environments present in heterogeneous environmental samples. To demonstrate that the CPMG-SS approach is not limited to $^{13}$C, $^{31}$P NMR was performed on a freeze-dried worm.

Figure 5A (left) displays the CPMG-SS spike and corresponding $^{31}$P CP experiment for a freeze-dried worm. Cross-polarization was optimized for a $^{31}$P spectrum, and the number of echoes and $\tau$ in the CPMG-SS experiments were kept the same as in the $^{13}$C experiments because they met the requirements for a single spike within the bandwidth of the $^{31}$P spectrum. The CP spectrum shows side bands at 13 kHz, which is equivalent to the spinning speed, and the spectrum itself is a broad peak characteristic of the inhomogeneity in the sample. As illustrated in the graph in Figure 5, a calibration curve similar to the one constructed above for hemoglobin and soil can be constructed for $^{31}$P NMR using worm tissue (circle in Fig. 5). To test the predictability of the calibration, a second worm sample was diluted 7.75 times with an inert filler. From the constant $K$ (from the slope of the calibration) and Equation 4, the number of scans required to achieve an S/N of 15 is predicted to be 27,667 scans. When running the sample, the actual NSCP to achieve an S/N of 15 was 27,950 and is represented by the square in the graph, a difference of 1% compared with the estimated value. Thus, experimental time can also be estimated for spin half nuclei other than $^{13}$C using CPMG-SS spectroscopy.

Liquid-state experiments

The aqueous phase is important to environmental NMR studies, including those involving soils, all bodies of water, plants, and metabolomics [1,6,37]. The use of CPMG-SS to estimate experimental runtime can also be applied to solution-state NMR, as demonstrated in Figure 6. Here, liquid state...
13C CPMG-SS spectra (left panel) of riverine dissolved organic matter (DOM) were obtained at various concentrations, and the corresponding conventional 13C experiments (top right) are shown in the corresponding color. The graph at bottom right is a correlation between the S/NSS and the S/N in the conventional 13C experiments (S/NCC) of each of the three spectra and demonstrates that the CPMG-SS method has predictive capabilities in the solution state.

The sensitivity of the CPMG-SS technique is illustrated once again with the green spectra in Figure 6. Readers should note that, in solution-state NMR, the 128-scan CPMG-SS takes 3 min versus 2 min in solid-state NMR. This is due to the acquisition time being shortened in solid-state NMR to permit high-power decoupling, which, if applied too long, can damage the NMR probe. In 3 min, a CPMG-SS spectrum is obtained for the lowest concentration, whereas the features are still indistinguishable from the noise in the conventional 13C spectrum after 16 h. This is consistent with solid-state results indicating that a sample with a CPMG-SS S/N of less than 10 in 128 scans will require much longer than overnight to record a reasonable spectrum. The approach provides the knowledge needed by the spectroscopist to pretreat and concentrate the sample if necessary, or in some cases simply make the experimental run time long enough to achieve an acceptable spectrum. In addition, it provides a concrete method to identify samples that are simply too dilute for conventional NMR and avoids wasting instrument time.

### CONCLUSION

By using the predictive single-spike technique described here, it is relatively easy to determine whether a sample with unknown carbon content will produce an acceptable NMR spectrum in a given amount of time. If the time required to obtain an adequate conventional spectrum is prohibitive, then the sample can be concentrated and/or pretreated and wasted NMR time is avoided. The CPMG-SS method is very rapid (2–3 min) and does not require MAS in solids, resulting in a rapid and simple prescreening approach. To predict the runtime in a series of similar samples (note that they have to be only roughly similar in terms of chemical composition and can vary greatly in concentration), all that is required is one conventional NMR spectrum along with a CPMG-SS from any sample in the series. The intensity from the CPMG-SS experiment is then used to predict the experimental runtime for other samples in the series. This approach yields enough accuracy (≤±20% of the true S/N) for estimation purposes. If only a crude estimate is required, for example, to answer the question, “Will a specific sample produce an acceptable NMR spectrum at all?”, an approximation can be made from the CPMG-SS S/N alone, as was demonstrated with an Arctic soil. Finally, the CPMG-SS pulse program can be applied to other nuclei, as demonstrated by the 31P experiments. The present study demonstrates a novel approach to estimate experimental time required for the NMR analysis of various environmental samples and has the potential to save spectrometer time and associated instrument costs.

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