

SUPPORTING INFORMATION

Advanced solid-state NMR characterization of marine dissolved organic matter isolated using the coupled reverse osmosis/electrodialysis method

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NMR Spectroscopy

Quantitative ^{13}C DP/MAS NMR. Quantitative ^{13}C direct polarization/magic angle spinning (DP/MAS) spectra were obtained at a spinning speed of 14 kHz. The 90° ^{13}C pulse-length was 4 μs . Recycle delays were tested by the cross polarization/spin-lattice relaxation time/total sideband suppression (CP/T₁-TOSS) technique to make sure that all carbon sites were >95% relaxed.¹ Recycle delays were 80 s, and 1024 transients were averaged. In order to obtain quantitative information on the nonprotonated aromatic carbon fraction, DP/MAS ^{13}C NMR with recoupled dipolar dephasing was used.² The dipolar dephasing time was 68 μs .

^{13}C CP/TOSS and ^{13}C CP/TOSS plus dipolar dephasing. Qualitative compositional information was obtained with good sensitivity by ^{13}C cross polarization/total sideband suppression (CP/TOSS) NMR experiments at a spinning speed of 6.5 kHz and a CP time of 1 ms, with a ^1H 90° pulse-length of 4 μs . Four-pulse total suppression of sidebands (TOSS)³ was employed before detection, and two-pulse phase-modulated (TPPM) decoupling was applied for optimum resolution. The corresponding sub-spectrum with signals of nonprotonated carbons and mobile groups like rotating CH_3 was obtained by ^{13}C CP/TOSS combined with 40- μs dipolar dephasing. The number of scans was 30720.

^{13}C chemical-shift-anisotropy filter. Because both anomeric carbons (O-C-O, e.g. in carbohydrate rings) and aromatic carbons may resonate between 120 and 90 ppm, the aromatic carbon signals were selectively suppressed by a five-pulse ^{13}C chemical-shift-anisotropy (CSA) filter with a CSA-filter time of 35 μs to clarify whether there are carbon signals of anomeric overlapping with those of aromatics. The number of transients averaged for Coastal DOM was 6144, and more than 20,000 for Marine DOM. The details of this technique have been described elsewhere.¹

CH spectral editing. For CH (methine) selection, the dipolar distortionless enhancement by polarization transfer (DEPT) method was used at a spinning speed of 4 kHz.⁴ A 0.5 s ¹³C T₁-filter was applied to reduce residual CH₃ signals. The number of transients averaged was 71,680, with a recycle delay of 0.5 s. The details of this technique, which also suppresses mobile methylene signals, have been described elsewhere.⁴

CH₂ Spectral Editing. Spectral editing of CH₂ signals was achieved by selection of the three-spin coherence of CH₂ groups, using a ¹³C 90° pulse and ¹H 0°/180° pulses applied after the first quarter of one rotation period with MREV-8 decoupling.⁵ The spinning speed was 5.787 kHz. The number of transients averaged was 73,728, with a recycle delay of 0.5 s.

Spectral editing of immobile CH₂ + CH. The combined spectrum of these functional groups was obtained with good sensitivity in a simple spectral-editing experiment. First, a ¹³C CP/TOSS spectrum is recorded using a short CP of 50 μs. It shows predominantly protonated carbons in immobile segments, but residual peaks of quaternary carbons result from two-bond magnetization transfer. Second, a ¹³C CP/TOSS spectrum is recorded using a short CP of 50 μs and 40 μs dipolar dephasing. It contains only the residual signals of quaternary carbons or mobile segments (including CH₃ groups with > 50% efficiency). The difference of the two spectra is the spectrum of immobile CH₂ and CH carbons, with a small CH₃ contribution.⁶

¹H-¹³C two-dimensional heteronuclear correlation (2D HETCOR) NMR and 2D HETCOR with dipolar dephasing. ¹H-¹³C 2D HETCOR NMR experiments were performed at a spinning speed of 14 kHz. Lee-Goldburg cross polarization (LGCP) of 0.5 ms was used to suppress ¹H-¹H spin diffusion during polarization transfer, and the spectra show mostly one- and two-bond ¹H-¹³C connectivities.⁷ We used 128 t₁ increments of 5 μs. In addition, 40 μs dipolar

dephasing was inserted in the LGCP HETCOR to reveal multi-bond C-H connectivities for nonprotonated carbons. The number of scans was 131,072.

¹⁵N CP/MAS. ¹⁵N CP/MAS NMR was conducted with a contact time of 1 ms, a recycle delay of 0.5 s, and at a spinning speed of 6.5 kHz. The number of transients averaged was 227,328 for Coastal DOM, and 460,800 for Marine DOM. The Hahn echo was employed before detection in order to refocus magnetization and avoid baseline distortion.⁸

Note that all ¹³C spectra were referenced to external glycine COO signal at 176.49 ppm and the ¹⁵N chemical shifts were referred to NH₃ using the signal of N-acetyl-valine at 122 ppm as a secondary reference. In addition, Gaussian broadening and only constant phase correction were applied so that no baseline correction was necessary.

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