Electronic Supplementary Material (ESI) for Analyst.
This journal is © The Royal Society of Chemistry 2021

Expanding Current Applications and Permitting the Analysis of Larger Intact Samples by means of a 7 mm CMP-NMR Probe

Paris Ning,[a] Daniel Lane,[a] Rajshree Ghosh Biswas,[a] Amy Jenne,[a] Monica Bastawrous,[a] Ronald Soong,[a] Daniel Schmidig,[b] Thomas Frei,[b] Peter De Castro,[b] Ivan Kovacevic,[b] Stephan Graf,[b] Sebastian Wegner,[c] Wolfgang Bermel,[c] Falko Busse,[c] Till Kuehn,[b] Rainer Kuemmerle,[b] Jochem Struppe,[d] Michael Fey,[d] Henry J. Stronks,[e] Martine Monette,[e] Myrna J. Simpson,[a] and André J. Simpson*[a]

[a]*Environmental NMR Centre, University of Toronto, Toronto, ON, Canada, M1C 1A4*
[b]*Bruker BioSpin AG, Industriestrasse 26, 8117 Fällanden, Switzerland*
[c]*Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany*
[d]*Bruker BioSpin Corp., 15 Fortune Drive, Billerica, Massachusetts, USA, 01821-3991*
[e]*Bruker Ltd., 2800 High Point Drive, Milton, ON, Canada, L9T 6P4*
***Corresponding Author:** André J. Simpson (andre.simpson@utoronto.ca)

Supporting Information

Number of Pages: 8

Number of Figures: 1

Materials and Sample Preparation

Materials

^{13}C enriched (99%) *C. vulgaris* (a green algae) was purchased from Silantes GmbH (Munich, Germany). D_2O (99.96%) was purchased from Cambridge Isotopes Laboratories, Inc. (Tewksbury, MA). Hydroxyzine hydrochloride (97%) was purchased from Toronto Research Chemicals (Toronto, ON). *Hyalella azteca* was cultured as previously described.[1,2] Chicken heart and pomegranate fruit were purchased from a local supermarket. Hydroxyzine softgel capsule was obtained from a local pharmacy.

7 mm Rotor Seals

7 mm MAS zirconia rotors and Kel-F caps were purchased from Cortecnet Corp. (Brooklyn, NY). To prevent leakage of the multiphase samples, the 7 mm rotor uses a plug seal system similar to that of the 4 mm. However, the seal and sealing screw are not available commercially and were made in-house from a Kel-F rod (see Results and Discussion).

Sample Preparation

C. vulgaris (lyophilized powder) was packed dry into a 4 mm rotor (~ 25mg) and a 7 mm rotor (~ 80mg) for signal comparison (Figure 1b). ~ 50mg of hydroxyzine hydrochloride standard was packed in a 7 mm MAS rotor for cross polarization (CP) MAS experiment (Figure 5, red trace). For the softgel capsule sample, the hydroxyzine softgel was placed into the 7 mm rotor and sealed with insert and plugscrew to prevent accidental breakage or leakage of the excipient (filler). In the space between the plugscrew and the cap, a small Teflon capillary filled with D_2O was used as lock.[3] The *ex vivo H. azteca* rotor was prepared with ~ 50 mg of freeze-dried freshwater shrimps. 50 µl of D_2O was added to swell the sample and to act as the lock. The rotor was thoroughly mixed and stored in a fridge overnight to allow thorough swelling. Chicken heart tissue was cut and packed into a 7 mm rotor directly after purchase from a local supermarket. A 50 µl aliquot of D_2O was added to the rotor and the excess was pushed out when the rotor

was sealed with the plugscrew. The addition of excess lock solvent ensured the elimination of air pockets inside the rotor. For the pomegranate berry rotor, one single seed berry (aril) was placed into the rotor and a 50 µl aliquot of D_2O was added to serve as the lock. The rotor then was sealed with insert and plugscrew. For *ex vivo* shrimp, pomegranate, and chicken heart tissue, a small amount of 0.1% w/v sodium azide (Millipore Sigma, St. Louis, MO) solution was added as a preservative to prevent bacterial growth.[4]

Experimental Details

Spectral Editing

To differentiate components in different phases (solid, gel, and solution), spectral editing was used as described previously [5–7] with key parameter summarized below.

1D ^{1}H Detection (liquids, gels, and semi-solids)

For ^{1}H 1D experiments, water suppression was achieved via presaturation, and a recycle delay between scans of 5.27s was used. All ^{1}H 1D spectra were recorded with 65536 time-domain points, a spectral width of 20 ppm, and 128 scans. All spectra were processed with an exponential function corresponding to 1 Hz (liquid phase) and 5Hz (gel-phase/semi solids) in the transformed spectrum along with a zero-filling factor of 2. For diffusion-based editing, solution/gel-phase spectra were acquired with a bipolar pulse pair longitudinal encode-decode (BPPLED) sequence.[8] Encoding/decoding gradients were typically set at 1.6 ms at ~ 50 G cm^{-1} with a diffusion time of 80 ms. Complete details regarding spectral editing are provided in previous studies[6,7,9] and the procedures for generating the various spectra are described in **Results and Discussion (main paper)**.

^{13}C Detection of the Solids

^{1}H to ^{13}C CP-MAS was used to select the solid phase (mobile solids and crystalline solids).[5] For ^{13}C CP-MAS experiment, spectra were obtained with a spectral width of 400 ppm, 2048 time-domain points, and 16384 scans, and time between scans was 2 seconds. The contact time was set at 1 ms and ramped from 90%-100% in a linear ramp and total suppression of spinning sidebands (TOSS) was used to suppress spinning sidebands.[10] ^{13}C CP-MAS spectra were multiplied by an exponential function corresponding to a broadening of 25 Hz in the transformed spectrum. To further discriminate signals from the crystalline/rigid solid in samples, a short Carr−Purcell− Meiboom−Gill relaxation filter (2 echoes of 15 μs each) on ^{1}H prior to CP was deployed.[7,11] ^{1}H coupling to ^{13}C was removed using *SPINAL64* decoupling. Procedures for generating the various spectra are described in **Results and Discussion** and in previous studies.[6,7,9]

2D NMR

For 2D analysis, ^{1}H-^{13}C heteronuclear single quantum coherence spectroscopy (HSQC) and ^{13}C-^{1}H heteronuclear correlation spectroscopy (HETCOR) were performed. ^{1}H-^{13}C HSQC spectra were obtained in phase sensitive mode, with Echo/Antiecho encoding and gradients for coherence selection and sensitivity improvement. Inversion was achieved using an adiabatic chirp pulse (Crp60,0.5,20.1) synchronized to 2 rotor periods (400 μs) and a refocusing Crp60comp.4 pulse synchronized to 8 rotor periods (1600 μs). Decoupling was achieved using *GARP-4*. 256 scans were acquired for each of the 128 increments in the F1 dimension. In the F2 dimension, 2048 time-domain points were obtained, with a ^{1}J coupling (^{1}H - ^{13}C) of 145 Hz. The F1 dimension was processed with an exponential function (line broadening of 8 Hz) and the F2 dimension was processed using a sine-square function with a phase shift of $\pi/2$.

^{13}C-^{1}H HETCOR experiments were performed using a ^{1}H-^{13}C coupling constant of 145 Hz and a set of broadband inversion pulses (166 μs Bip720,100,10.1) for all 180° rotations on the ^{13}C spins. 256 scans were acquired for each of the 128 increments in the F1 dimension. In the F2 dimension, 2048 time-domain points were collected. The F1 dimension was processed with an exponential function (line broadening of 25 Hz) and the F2 dimension was processed using a sine-square function with a phase shift of π/2. The displayed HETCOR spectra have their X and Y axes transposed to allow direct comparison to their HSQC counterparts.

Quantitative Considerations

CMP-NMR can be used to estimate the quantities in each phase[7] and even provide absolute quantification.[12] For phase specific quantification, readers are referred to recent work by Ning et al. that includes a detailed description of how to quantify the relative contribution from each phase.[7] Once each sub-phase is quantified, then the relative contribution of peaks within each sub-phase would also be quantitative (assuming 5 x T_1 recycle) in all spectra. The exception is the CP-MAS spectra (semi-solids and solids), which are only "semi-quantitative" in terms of peak area. This is because in cross polarization, magnetization is transferred from dipole coupled protons, onto the carbons, which is not efficient in proton deficient regions/components of a sample (for example, condensed aromatics that have no protons in proximity). However, if absolute quantification is needed across all phases, then the approach of stepped decoupling[12] can be used, that uses a single pulse to excite all components (solids, gels, liquids) with high power decoupling (decouples all phases) until the solids relax, followed by low power decoupling (continues to decouple the gels and liquids). The switch to low power is required as NMR probes (including dedicated solids probes) can only maintain high power decoupling generally <50ms without hardware damage.

When applied quantitively, phase separation is relatively robust, and the weighted summation of the sub-phases has been shown to match the quantitative reference (all phases observed equally) within ~2%.[7] However, sometimes the goal is to cleanly discriminate how the liquid and gel fractions differ

qualitatively rather than quantitatively. For example, if the liquid fraction is 97% carbohydrate, and 3% protein, and the gel-faction is dominated by proteins, then the gel fraction can be subtracted from the liquid fraction to give purely carbohydrates by difference. Such an approach can be used to give cleaner differentiation of phases if qualitative changes within the sub-phases take precedent over the exact quantification, dependant on the goals of the study.

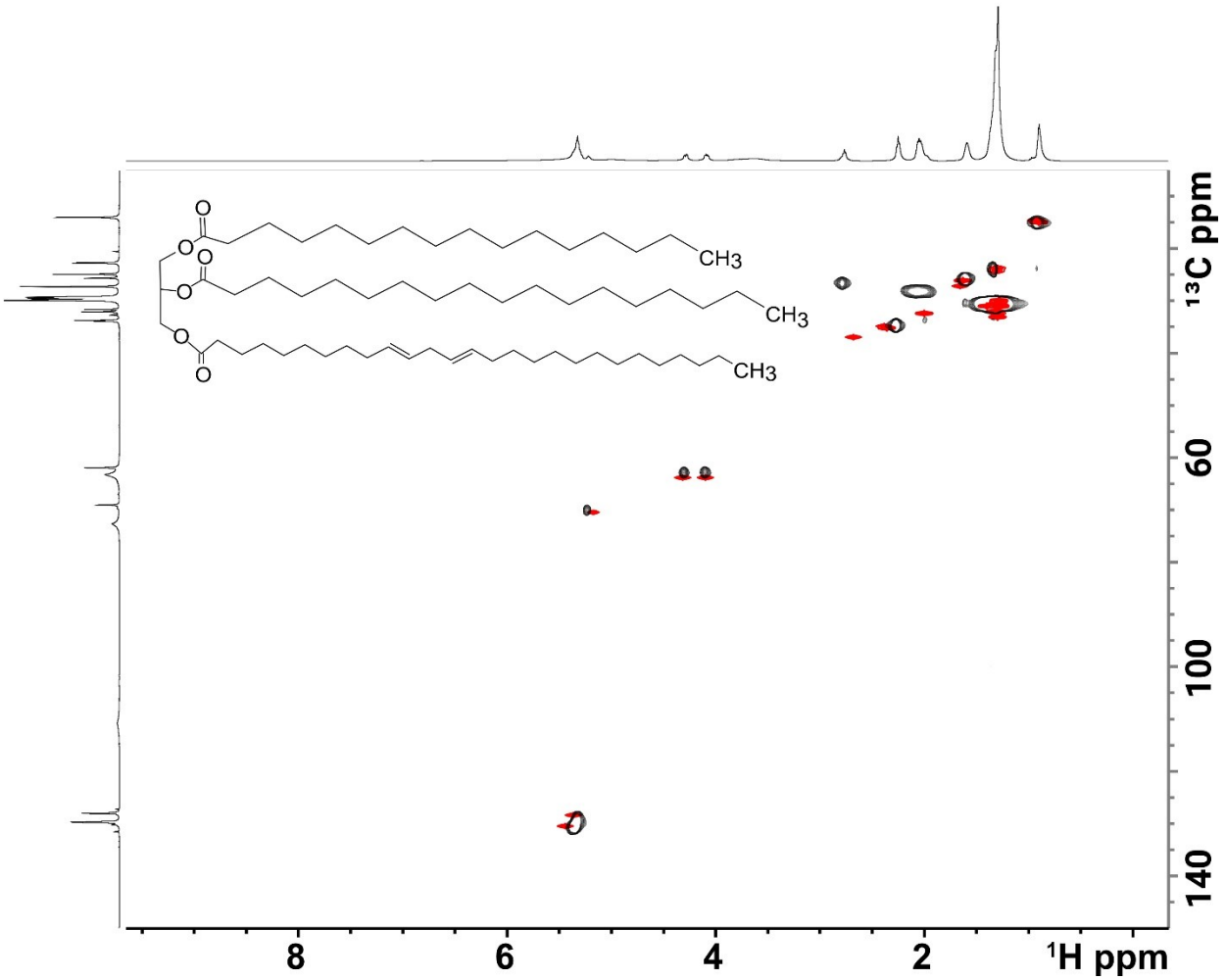

Figure S1 Figure S1 ^{1}H-^{13}C HSQC spectrum of the softgel capsule (black) overlaid with Triacylglyceride (TAG) 2D NMR prediction by ACD/Labs (red). The peaks match well, indicating the majority of the material in the solution phase (excipient) is indeed TAG.

References

(1) Mobarhan, Y. L.; Fortier-McGill, B.; Soong, R.; Maas, W. E.; Fey, M.; Monette, M.; Stronks, H. J.; Schmidt, S.; Heumann, H.; Norwood, W.; Simpson, A. J. *Chem. Sci.* **2016**, *7* (8), 4856–4866. https://doi.org/10.1039/C6SC00329J.

(2) Liaghati Mobarhan, Y.; Soong, R.; Lane, D.; Simpson, A. J. *Magn. Reson. Chem.* **2019**, No. November 2018, 1–18. https://doi.org/10.1002/mrc.4832.

(3) Masoom, H.; Adamo, A.; Simpson, A. J. *Environ. Chem.* **2016**, *13* (4), 767–775. https://doi.org/10.1071/EN15139.

(4) Nagato, E. G.; D'eon, J. C.; Lankadurai, B. P.; Poirier, D. G.; Reiner, E. J.; Simpson, A. J.; Simpson, M. J. *Chemosphere* **2013**, *93* (2), 331–337. https://doi.org/10.1016/j.chemosphere.2013.04.085.

(5) Courtier-Murias, D.; Farooq, H.; Masoom, H.; Botana, A.; Soong, R.; Longstaffe, J. G.; Simpson, M. J.; Maas, W. E.; Fey, M.; Andrew, B.; Struppe, J.; Hutchins, H.; Krishnamurthy, S.; Kumar, R.; Monette, M.; Stronks, H. J.; Hume, A.; Simpson, A. J. *J. Magn. Reson.* **2012**, *217*, 61–76. https://doi.org/10.1016/j.jmr.2012.02.009.

(6) Ghosh Biswas, R.; Fortier-McGill, B.; Akhter, M.; Soong, R.; Ning, P.; Bastawrous, M.; Jenne, A.; Schmidig, D.; De Castro, P.; Graf, S.; Kuehn, T.; Busse, F.; Struppe, J.; Fey, M.; Heumann, H.; Boenisch, H.; Gundy, M.; Simpson, M. J.; Simpson, A. J. *Anal. Chim. Acta X* **2020**, *6*. https://doi.org/10.1016/j.acax.2020.100051.

(7) Ning, P.; Lane, D.; Soong, R.; Schmidig, D.; Frei, T.; De Castro, P.; Kovacevic, I.; Graf, S.; Wegner, S.; Busse, F.; Struppe, J.; Fey, M.; Stronks, H.; Monette, M.; Simpson, M. J.; Simpson, A. J. *ACS Sustain. Chem. Eng.* **2020**, *8* (47), 17551–17564. https://doi.org/10.1021/acssuschemeng.0c07205.

(8) Wu, D. H.; Chen, A. D.; Johnson, C. S. *J. Magn. Reson. Ser. A* **1995**, *115* (2), 260–264. https://doi.org/10.1006/jmra.1995.1176.

(9) Masoom, H.; Courtier-Murias, D.; Soong, R.; Maas, W. E.; Fey, M.; Kumar, R.; Monette, M.; Stronks, H. J.; Simpson, M. J.; Simpson, A. J. *Environ. Sci. Technol.* **2015**, *49* (24), 13983–13991. https://doi.org/10.1021/acs.est.5b03251.

(10) Mobarhan, Y. L.; Struppe, J.; Fortier-McGill, B.; Simpson, A. J. *Anal. Bioanal. Chem.* **2017**, *409* (21), 5043–5055. https://doi.org/10.1007/s00216-017-0450-3.

(11) Masoom, H.; Courtier-Murias, D.; Farooq, H.; Soong, R.; Kelleher, B. P.; Zhang, C.; Maas, W. E.; Fey, M.; Kumar, R.; Monette, M.; Stronks, H. J.; Simpson, M. J.; Simpson, A. J. *Environ. Sci. Technol.* **2016**, *50* (4), 1670–1680. https://doi.org/10.1021/acs.est.5b03410.

(12) Ning, P.; Soong, R.; Bermel, W.; Lane, D.; Simpson, M. J.; Simpson, A. J. *Anal. Bioanal. Chem.* **2018**, *410* (27), 7055–7065. https://doi.org/10.1007/s00216-018-1306-1.