analytical chemistry

Letter

pubs.acs.org/ac

NPK NMR Sensor: Online Monitoring of Nitrogen, Phosphorus, and Potassium in Animal Slurry

Morten K. Sørensen,*[,†] Ole Jensen,[‡] Oleg N. Bakharev,[†] Tavs Nyord,[§] and Niels Chr. Nielsen[†]

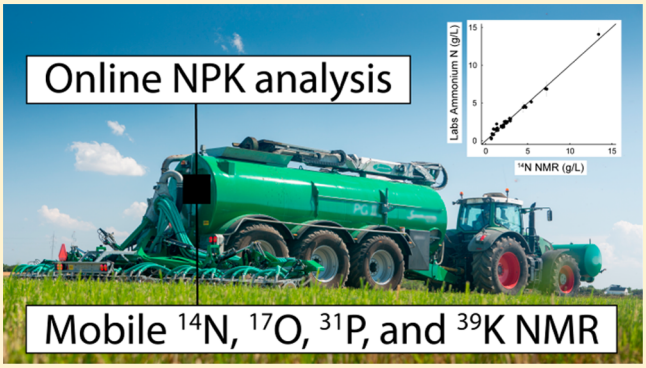
[†]Center for Insoluble Protein Structures (inSPIN), Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, Aarhus University, Gustav Wieds Vej 14, Aarhus C, DK-8000, Denmark

[‡]NanoNord A/S, Skjernvej 4A, Aalborg Ø, DK-9220, Denmark

[§]Department of Engineering, Aarhus University, Hangøvej 2, Aarhus N, DK-8200, Denmark

Supporting Information

ABSTRACT: Knowledge of the actual content of nitrogen, phosphorus, and potassium (NPK) in animal slurry is highly important to optimize crop production and avoid environmental pollution when slurry is spread on agricultural fields. Here, we present a mobile, low-field nuclear magnetic resonance (NMR) sensor suitable for online monitoring of the NPK content in animal slurry as an alternative to crude estimates or tedious nonspecific, off-site laboratory analysis. The sensor is based on ^{14}N, ^{17}O, ^{31}P, and ^{39}K NMR in a digital NMR instrument equipped with a 1.5 T Halbach magnet for direct detection of ammonium N, total P, and K and indirect evaluation of the organic N content, covering all practical components of NPK in animal slurry. In correlation studies, the obtained NMR measurements show good agreement with reference measurements from commercial laboratories.



Huge amounts of animal slurry (liquid manure) are produced from intensive livestock farming every year. Animal slurry is an inhomogeneous mixture composed of feces, urine, straw, and similar substances, with large contents of nitrogen, phosphorus, and potassium (NPK). In modern farming, it is a highly important resource of essential plant nutrients in crop production.[1] Generally, animal slurry is applied as fertilizer to agricultural fields, which implies a possible source of pollution to the environment if overdosed. In order to optimize plant yields and minimize environmental effects, it is of immense importance to know the actual composition of the slurry and thus be able to apply the right amounts of nutrients on the fields. Some of the most important parameters are the contents of ammonium N, total N, total P, and K. Roughly seen, ammonium N represents all dissolved N and 40−70% of the total N content (the remaining fraction considered as organic N). The total P content usually covers both a large fraction of phosphorus in solid forms (like phosphate salts and organic phosphorus) and dissolved phosphate, whereas all potassium is considered dissolved as potassium ions.[1] In general, the content of NPK in animal slurry is highly variable depending on animal type, feeding strategy, season, storage conditions, and sedimentation in storage tanks which imply highly inhomogeneous distributions even in the same tank of slurry. Chemical characterization using standard laboratory methods at best only provides averaged information masking intrinsic big variations, and they are time-consuming, costly, and impractical and in particular measurements of total N and total P contents are demanding due to essential predegradation of the solid fractions.[2] Therefore, the amount of NPK nutrients delivered to the fields through spreading of animal slurry is today mainly determined on the basis of standard values based on general information on animal and housing type, without accurate knowledge of the potential fertilizer value or environmental impact.

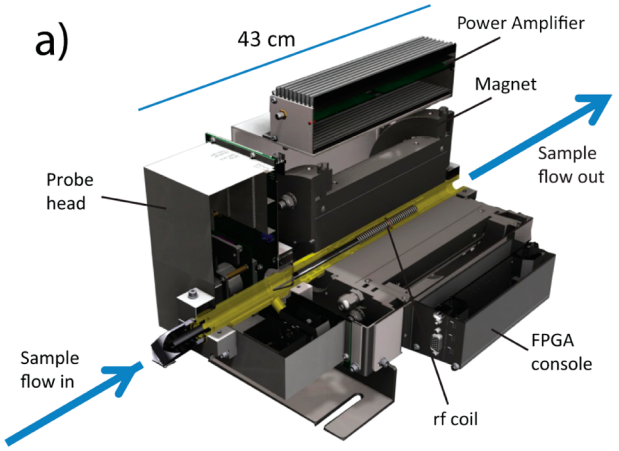
Addressing these challenges, we here propose a nuclear magnetic resonance (NMR) sensor suitable for online monitoring of NPK in animal slurry directly at, for example, slurry spreaders, slurry trucks during transportation, or storage tanks. This sensor has been seen in the context of previous extensive research in rapid monitoring of nutrient values which mainly has led to methods relying on measurements of electric conductivity[3−5] or near-infrared spectroscopy.[6−8] Opposed to these techniques, NMR offers direct measurements of NPK in all types of animal slurries without any sensitive parts in contact with the slurry media and without calibration requirements on the user side. The proposed NMR sensor originates in the recently presented ^{27}Al NMR sensor for detection of aluminosilicate particles (so-called catfines) in marine fuel oil onboard ships,[9] recently extended also for ^{17}O NMR

Received: March 6, 2015
Accepted: May 28, 2015

 © XXXX American Chemical Society

DOI: 10.1021/acs.analchem.5b01924
Anal. Chem. XXXX, XXX, XXX−XXX

applications.[10] Aimed at direct detection of dissolved N (mainly ammonium N), total P, and K, the sensor has been modified to multinuclear operation tuned for ^{14}N, ^{31}P, and ^{39}K detection, whereas ^{17}O detection is applied for determination of the organic N (and dry matter) fraction. The essential parts of the NMR sensor for NPK monitoring is shown in Figure 1a, and



c)

	Spin	Abundance	Sensitivity	Frequency
^{14}N	1	99.6%	1.01	4.6 MHz
^{17}O	5/2	0.037%	0.011	8.7 MHz
^{31}P	1/2	100%	66.3	26.0 MHz
^{39}K	3/2	93.1%	0.473	3.0 MHz

Figure 1. (a) Illustration of the NMR sensor with the probe bore and NMR coil in the center of a compact setup with magnet, probe, FPGA, and power amplifier shown around it. (b) Three examples of the sample tubes with animal slurry (samples 1, 8, and 9 defined in the Supporting Information) used for demonstration. (c) Table of characteristics for the four relevant isotopes, including nuclear spins, natural abundances, NMR sensitivities relative to 1000 for ^{1}H, and the resonance frequencies at 1.5 T.

they consist of a cylindrical Halbach magnet with a static magnetic field of 1.5 T, a digital field-programmable gate array (FPGA) console, a 400 W power amplifier, and a shielded probe with a 10 cm (4 cm for ^{31}P experiments) long radio frequency (rf) coil with an inner diameter of 9.2 mm. To minimize drift of the permanent magnetic field, the hardware is in the laboratory prototype assembled in a temperature-controlled cabinet at either 25 ± 0.1 or 32 ± 0.1 °C like the sensor in ref 9. While the setup is suitable for measurements with the sample flowing directly in the probe bore, for demonstration purposes, we used test samples in tubes of perfluoroalkoxy alkane (PFA) with an outer/inner diameter of 9/8 mm and a sample length of 120 mm as shown in Figure 1b. For the applied sample volume for demonstration, the magnetic field inhomogeneity is approximately 2500 ppm given as full-width at half-maximum of the field distribution.

Several issues have to be addressed for the NMR-based NPK sensor to be functional for detection of highly challenging quadrupolar nuclei such as ^{14}N, ^{17}O, and ^{39}K characterized by large quadrupole moments and low NMR frequencies as given in Figure 1c. These characteristics induce severe challenges in regard to sensitivity, probe ringing, differential decaying signals, and the potential of very broad resonances. Adapting protocols similar to those presented for our previous catfine NMR sensor,[9] we have systematically optimized sensitivity through the use of large sample volumes and detection of resonances using multiple-echo QCPMG[11] experiments also coping with the challenges imposed by the present inhomogeneous magnet field conditions. We should here note that the low natural abundance of the detected isotopes is partly compensated by their presence in high concentrations in animal slurry samples.

It is well-known that the chosen ^{14}N, ^{17}O, and ^{39}K nuclei all possess challenges over the more commonly studied spin-1/2 nuclei in terms of strong quadrupole coupling interactions which may induce very fast relaxation for molecules in solution and broad spectral lineshapes from multiple transitions in the solid state. Nonetheless, for symmetry and fast internal motion reasons, ammonium and potassium ions do not show fast quadrupole relaxation, and dissolved ammonium (and ammonia) may be readily observed by ^{14}N NMR as was also studied early in NMR history.[12−14] However, the ^{14}N resonances from covalently bonded, nonflexible nitrogen are usually very broad (e.g., quadrupolar coupling constants exceeding 3 MHz for peptides and proteins[15,16]) and will not contribute significantly to the ^{14}N NMR signal of animal slurry when taken over a narrow spectral range. Hereby, ^{14}N NMR analysis provides a selective measure of nitrogen in dissolved small molecules (ammonium in animal slurry), while the potassium ions are similarly detected by ^{39}K NMR. To unravel the organic fraction of nitrogen, we apply ^{17}O NMR. We assume that the ^{17}O NMR intensity is correlated to the content of water and thus dry matter, and on the basis of laboratory analysis of different animal slurries, the content of organic N correlates well to the dry matter content as shown in the Supporting Information. Following this, we propose that the content of organic N may be determined from the ^{17}O NMR signal. The spin-1/2 ^{31}P isotope is highly abundant and readily detectable with a relative high sensitivity (see Figure 1c). This renders ^{31}P NMR suitable for detection of both dissolved and solid phosphorus constituents and thereby monitoring of the total P content in animal slurry. We note that potential solid-state broadening effects from dipole−dipole couplings and ^{31}P chemical shift anisotropies are negligible relative to the inhomogeneity of the static magnetic field. In all cases, for quantitative measurements, it is important to consider the relaxation times of the compounds of interest, and in general, care must be taken in interpretation of results in cases of short T_2 or long T_1 relaxation times relative to the echo delay and recycle delay, respectively. Therefore, care has been taken in the interpretation of the echo trains T_2, and we have generally acquired NMR data using 3−4 different recycle delays to ensure T_1 stability, in a setup which for automated monitoring purposes may be arranged for progressive changes of the applied recycle delay.

To demonstrate the performance of the proposed NMR sensor for measurements of NPK in animal slurry, we performed ^{14}N, ^{17}O, ^{31}P, and ^{39}K NMR measurements of 40 test samples, and corresponding commercial NPK analyses were obtained from five external laboratories for comparison

DOI: 10.1021/acs.analchem.5b01924
Anal. Chem. XXXX, XXX, XXX−XXX

with current common practice. Samples of 16 animal slurries were collected from Danish farms and biogas plants and included five slurries from cattle, five from pigs, three from mink, and three anaerobically codigested slurries from biogas plants. Care was taken to find different slurries to ensure a good representation of such slurries in general. Duplicate samples were made for all 16 slurries to give 32 slurry samples. In addition, eight NPK solutions of NH_4Cl, K_2HPO_4, KH_2PO_4, and KCl dissolved in water were included to yield a total of 40 samples (see details and an overview of all samples in the Supporting Information). Aliquots of each sample were packed in PFA tubes (inner diameter of 8 mm and sample length of 120 mm) for NMR experiments and in 500−1000 mL containers for external laboratory analysis. All samples were kept cold (2−5 °C), and the sample containers for the laboratories were sent with cooling items in insulated boxes. Laboratory results were obtained for dry matter, ammonium N, total N, total P, K, and pH. Half of the samples (with randomized sample numbers or duplicates) were sent for laboratory analysis 3 weeks after analysis of the first samples, and the results show no significant differences between duplicates on the mean values from the five laboratories. However, considerable uncertainties are observed on the individual laboratory results (see the Supporting Information). On the basis of the results for all samples, standard deviations between individual measurements and the mean of the measurements for each sample are 0.25 g/L for ammonium N, 0.19 g/L for organic N (without the eight samples of NPK solutions), 0.18 g/L for total P, and 0.16 g/L for K; see the Supporting Information for plots of the individual laboratory results. Large deviations are particularly observed for organic N and total P in the slurries. This may be related to large solid fractions implying inhomogeneous distributions of the nutrients and crucial challenging subsampling at the laboratories. In turn, this may represent a major advantage of analyzing large total volumes by an online NMR sensor.

While ^{17}O and ^{31}P NMR experiments were conducted at one sensor each, ^{14}N and ^{39}K NMR measurements were both conducted at six identical sensors to demonstrate the reproducibility. At each sensor, every sample was analyzed for approximately 1 h using a QCPMG (CPMG for ^{31}P) echo-train pulse sequence.[11,17] The ^{14}N NMR experiments were conducted with pulse lengths of 40 μs for both $\pi/2$ and π pulses (rf field strengths of 6.25 and 12.5 kHz, respectively), acquisition of 50 echoes separated by 400 μs, and four different recycle delays of 250−1500 ms. The ^{17}O NMR experiments were performed with 25 μs pulses, 24 echoes separated by 180 μs, and recycle delays of 50−100 ms, whereas the ^{31}P NMR experiments were performed with 8 μs pulses, 15 echoes separated by 100 μs, and recycle delays of 300−900 ms (for the eight samples of NPK solutions, 40 mM $CuSO_4$ was added prior to ^{31}P NMR experiments to have similar T_1 relaxation times as for the animal slurries); the ^{39}K NMR experiments were performed with 50 μs pulses, acquisition of 30 echoes separated by 440 μs, and recycle delays of 30−120 ms. Detailed descriptions of all experiments are given in the Supporting Information.

Figure 2 shows examples of the ^{14}N, ^{17}O, ^{31}P, and ^{39}K echo trains obtained with the parameters given above. Minor modulations of the echo intensities (potentially due to imperfect rf pulses[18]) may be observed in the echo trains which are, however, not crucial for the present application. From the echo-train measurements, the NMR intensities were

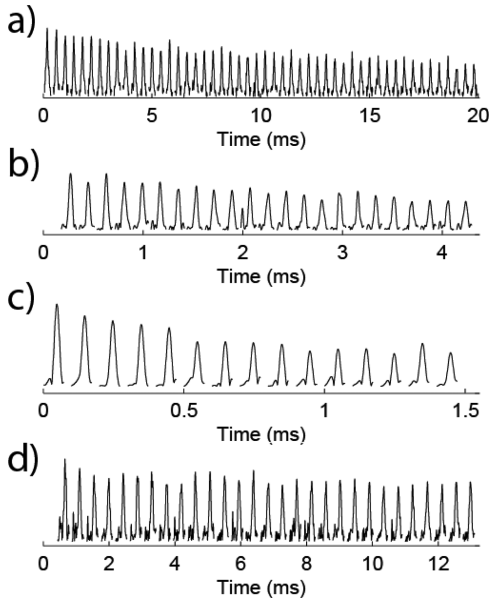


Figure 2. Examples of ^{14}N (a), ^{17}O (b), ^{31}P (c), and ^{39}K (d) echo trains acquired using the proposed NPK sensor with parameters as described in the text and data accumulated over approximately 1 h for each echo train. (a−d) Signals acquired at resonance frequencies of 4.6, 8.7, 26.0, and 3.0 MHz and of samples with about 4.4 g/L ammonium N, 95.7% water, 1.8 g/L P, and 8.9 g/L K, respectively. The first echo is not shown for ^{17}O (b) and ^{39}K (d). See the Supporting Information for further details.

obtained by multiexponential fits of the integrated echo intensities based on the data accumulated independently of the different recycle delays. To ensure stability, the fits were restricted to correspond to T_2 relaxation times of at least 5 ms for ^{14}N, 4 ms for ^{17}O, 1 ms for ^{31}P, and 10 ms for ^{39}K. For ^{14}N, ^{17}O, and ^{39}K, the first echo of the echo train was discarded from the analysis (and not shown for ^{17}O and ^{39}K in Figure 2) due to artifacts related to probe ringing. On the basis of the reference measurements, the NMR intensities are calibrated to provide actual NPK concentrations.

Figure 3 provides statistical evidence for the correctness of the measurements in terms of calibrated ^{14}N, ^{17}O (used as a probe for organic N), ^{31}P, and ^{39}K NMR intensities correlated to the mean laboratory results for ammonium N, organic N, total P, and K, respectively. The results show good correlation between laboratory results and the NMR intensities. For the complete set of samples, the standard deviation between the shown NMR and laboratory results is 0.28 g/L for ammonium N, 0.26 g/L for organic N (NPK solutions not included), 0.20 g/L for total P, and 0.51 g/L for K. For the ^{17}O NMR measurements, it should be emphasized that the observed intensity also correlates directly to the dry matter contents ranging from 2 to 94 g/L with a calibrated standard deviation of 11 g/L (not shown).

Improved accuracy may be obtained using more elaborate, although straightforward, data analysis on the same data sets. For the ^{14}N measurements (cf. Figure 3a) including a fit of the T_1 relaxation based on the experiments with four different recycle delays (250, 500, 1000, and 1500 ms), along with T_2 fits, a standard deviation of 0.21 g/L is obtained between NMR and laboratory results for ammonium N. This improvement is due to different ^{14}N relaxation times for the samples with observed T_1's from below 80 ms to about 280 ms for the animal

DOI: 10.1021/acs.analchem.5b01924
Anal. Chem. XXXX, XXX, XXX−XXX

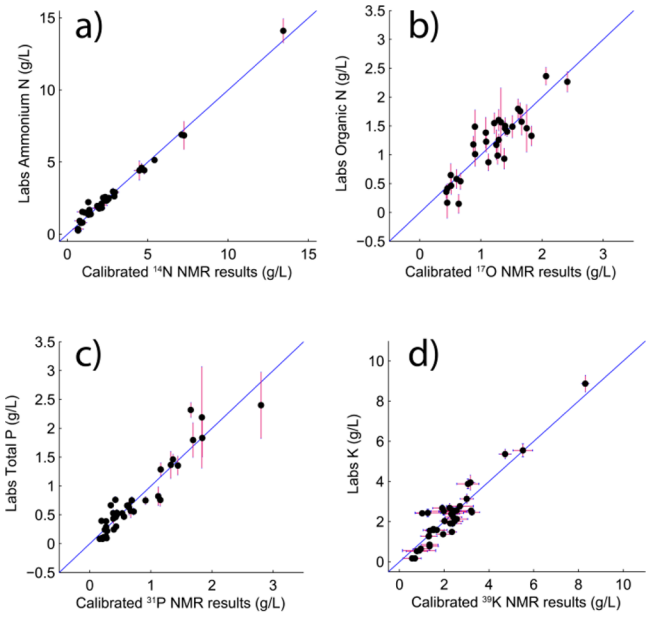


Figure 3. Calibrated NMR intensities (horizonthal) correlated to reference measurements from commercial laboratories (vertical). (a) ^{14}N NMR versus ammonium N, (b) ^{17}O NMR versus organic N obtained as total N minus ammonium N, (c) ^{31}P NMR versus total P, and (d) ^{39}K NMR versus K content. Vertical error bars show the obtained standard deviations on the laboratory analysis, whereas the horizontal error bars plotted for (a) and (d) show the standard deviations obtained for measurements at five and six NMR sensors, respectively. Vertical (a–d) and horizontal (a, d) error bars are plotted for all data points although some are to small to be visible. Experimental parameters are given in the text.

slurries and of about 300 ms for most of the NPK solutions. However, the algorithm applied for analysis with combined T_2 and T_1 fits reduces the stability of particular short-term measurements (*vide infra*). Also for K, the relaxation times seem to be a source to variation, but it appears for K that slightly outlying results are mainly observed for the group of cattle slurries with higher viscosities and thereby shorter T_2 relaxation times. If all cattle slurries are ignored, the standard deviation between NMR and laboratory K measurements is 0.33 g/L (0.22 g/L for the NPK solutions solely). Whereas duplicate samples are generally in good agreement with each other for ammonium-N and K, significant variations are observed between some of the duplicate samples for both laboratory and NMR measurements of organic N and in particular P. As mentioned for the laboratory measurements, this is most likely due to challenges with subsampling of small volumes for analysis, which illustrates the need for evaluation of large total volumes as is feasible by online NPK measurements.

For the ^{14}N and ^{39}K NMR evaluations, measurements at multiple sensors are in good agreement with each other with standard deviations of 0.13 g/L for ^{14}N and 0.36 g/L for ^{39}K (see the Supporting Information). Additionally, faster measurements are possible for particular analysis of nitrogen and phosphorus. For online measurements where the full sample volume is utilized, we find estimated standard deviations of 0.23 g/L in 3.7 min to be effective for measuring ammonium N, 0.27 g/L in 3.5 min for organic N, 0.20 g/L in 3.3 min for P, and 0.76 g/L in 4.4 min for K (see the Supporting Information). For ammonium N, organic N, and P, these deviations in the NMR results are similar to the deviations in the laboratory results.

In conclusion, we have presented a multinuclear NMR sensor suitable for online analysis of NPK in animal slurry. Measurements of the ammonium N, organic N, total P, and K contents have been demonstrated to be in good agreement with external laboratory analysis.

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01924.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +45 2182 6697. E-mail: moks@inano.au.dk.

Notes

The authors declare the following competing financial interest(s): An associated patent is filed.

■ ACKNOWLEDGMENTS

In particular, we thank Christina Ø. Pedersen and Peter S. Nielsen for help on the sample processing, and we acknowledge financial support from the Danish Ministry of Food, Agriculture and Fisheries (GUDP research funding), the Lauritzen Foundation, the Danish Advanced Technology Foundation, and the Danish National Research Foundation (DNRF59). Additionally, we thank Flemming Nielsen and Samson Agro for the background image used in the TOC and abstract graphics.

■ REFERENCES

(1) Sommer, S. G.; Christensen, M. L.; Schmidt, T.; Jensen, L. S. *Animal Manure Recycling: Treatment and Management*; John Wiley & Sons Ltd.: Chichester, West Sussex, 2013.
(2) Peters, J.; Combs, S. M.; Hoskins, B.; Jarman, J.; Kovar, J. L.; Watso, M. E.; Wolf, A. M.; Wolf, N. *Recommended Methods of Manure Analysis*; Cooperative Extension Publishing: Madison, Wisconsin, USA, 2003.
(3) Yagüe, M. R.; Quílez, D. *J. Environ. Qual.* **2012**, *41*, 893–900.
(4) Provolo, G.; Martínez-Suller, L. *Bioresour. Technol.* **2007**, *98*, 3235–3242.
(5) Moral, R.; Perez-Murcia, M. D.; Perez-Espinosa, A.; Moreno-Caselles, J.; Paredes, C. *Waste Manage.* **2005**, *25*, 719–725.
(6) Reeves, J. B.; Van Kessel, J. S. *J. Dairy Sci.* **2000**, *83*, 1829–1836.
(7) Sørensen, L. K.; Sørensen, P.; Birkmose, T. S. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1398–1405.
(8) Chen, L.; Xing, L.; Han, L. *J. Environ. Qual.* **2013**, *42*, 1015–1028.
(9) Sørensen, M. K.; Vinding, M. S.; Bakharev, O. N.; Nesgaard, T.; Jensen, O.; Nielsen, N. C. *Anal. Chem.* **2014**, *86*, 7205–7208.
(10) Sørensen, M. K.; Bakharev, O. N.; Jensen, O.; Nielsen, N. C. *Magn. Reson. Chem.* **2015**, in press. DOI: 10.1002/mrc.4207.
(11) Larsen, F. H.; Jakobsen, H. J.; Ellis, P. D.; Nielsen, N. C. *J. Phys. Chem. A* **1997**, *101*, 8597–8606.
(12) Ogg, R. A.; Ray, J. D. *J. Chem. Phys.* **1957**, *26*, 1339–1340.
(13) Schmidt, B. M.; Carlton Brown, L.; Williams, D. *J. Mol. Spectrosc.* **1958**, *2*, 539–550.
(14) Harris, R. K.; Mann, B. E. *NMR and the periodic table*; Academic Press: London, 1978.
(15) Tycko, R.; Opella, S. J. *J. Am. Chem. Soc.* **1986**, *108*, 3531–3532.
(16) Basse, K.; Jain, S. K.; Bakharev, O.; Nielsen, N. C. *J. Magn. Reson.* **2014**, *244*, 85–89.
(17) Meiboom, S.; Gill, D. *Rev. Sci. Instrum.* **1958**, *29*, 688–691.

DOI: 10.1021/acs.analchem.5b01924
Anal. Chem. XXXX, XXX, XXX–XXX

(18) Mitchell, J.; Gladden, L. F.; Chandrasekera, T. C.; Fordham, E. J. *Prog. Nucl. Magn. Reson. Spectrosc.* **2014**, *76*, 1−60.

DOI: 10.1021/acs.analchem.5b01924
Anal. Chem. XXXX, XXX, XXX−XXX