Fall 2018

CapPack devices for the evaluation of parameters and pulse performances in liquid and solid-state NMR spectroscopy

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CAPPACK DEVICES FOR THE EVALUATION OF PARAMETERS AND PULSE PERFORMANCES IN LIQUID AND SOLID-STATE NMR SPECTROSCOPY

by

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A DISSERTATION

Presented to the Faculty of the Graduate School of the

MISSOURI UNIVERSITY OF SCIENCE AND TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

In

CHEMISTRY

2018

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PUBLICATION DISSERTION OPTION

This dissertation consists of the following three papers, which either have been published as full research articles in peer-reviewed journals or issued as a U.S. patent:

Paper I (Pages 7-22): "Exponentially Converging Eradication Pulse Train (EXCEPT) for solvent signal suppression in investigations with variable T_1 times," by Emmalou T. Satterfield, Annalise R. Pfaff, Wenjia Zhang, Lingyu Chi, Rex E. Gerald II, and Klaus Woelk has been published in the Journal of Magnetic Resonance (*J. Magn. Reson.* **2016**, *268*, 68–72).

Paper II (Pages 23-48): "CapPack devices for the quantitative performance evaluation of NMR Spectrometers and pulse sequences," by Lingyu Chi, Ming Huang, Annalise R. Pfaff, Jie Huang, Rex E. Gerald II, and Klaus Woelk has been accepted for publication in Review of Scientific Instrument (*Rev. Sci. Instrum.*).

Paper III (Pages 49-116): "Solid state NMR spectroscopy in situ measuring devices and methods for calibration and determining one or more quantitative properties of a target sample," by inventers Lingyu Chi, Ming Huang, Rex E. Gerald II, and Klaus Woelk has been issued the U.S. Patent 10,067,079 B2 on September 4, 2018 by the United States Patent and Trademark Office (USPTO).

ABSTRACT

NMR spectroscopy is a powerful analytical tool to obtain explicit information about molecular structures, conformations, dynamics, and functions. To extract the desired information most accurately, NMR reference standards and devices are needed for calibrating the spectrometer hardware, generating reference data for the NMR software, and testing the applicability of pulse sequences. In this dissertation, the CapPack (**Cap**illary-tube **Pack**age) platform is introduced which provides well-defined external reference and calibration standards for liquid and solid-state NMR applications. CapPacks consist of one or more permanently sealed capillary tubes that, depending on the intended application, come arranged in different geometries such as side-by-side or clustered. They are used to generate performance measures for novel pulse sequences and spectrometer hardware, as well as to calibrate intensities and chemical shift axes of NMR spectra. The spectral information recorded with CapPack devices can also be used to provide independent, external measures for temperature, pH, pressure, or concentration of samples under investigation. When the information is collected in situ together with the sample, the CapPack's NMR signals are included into the recorded NMR spectra creating an inseparable spectral imprimatur. Three CapPack devices and representative sample applications are discussed in detail: (1) A Gradient CapPack device made from 10 side-by-side capillary tubes and used to determine the suppression profile, (2) a T_1 CapPack device made from seven clustered capillary tubes and used to evaluate the T_1 robustness of the EXCEPT-12 pulse sequence, and (3) a patented small, single-capillary CapPack thermometer for monitoring the temperature inside a MAS rotor.

ACKNOWLEDGMENTS

I would like to thank all who helped me with the successful completion of my doctoral degree work. First of all, I would like to express my gratitude to my advisor Dr. Klaus Woelk, without whom this work couldn't be considered complete. I deeply thank him for guiding me through the process of obtaining a graduate degree and for his more than five years of academic support, research advice, and personal friendship. More importantly, his continuous encouragement and great patience had a great impact on me.

I would like to thank NMR specialist and inventor Dr. Rex E. Gerald II who instructed me to the fundamental concepts and experimentation strategies of NMR, magic angle spinning (MAS), and magnetic resonance imaging (MRI). With his help, I was able to complete the documents leading up to two full utility patent filings by the university, one of which has been issued a US patent by the USPTO, and one is still pending.

I am grateful to Drs. Yinfa Ma, Paul Nam, V. Prakash Reddy, and Douglas K. Ludlow for their interest in my work and for serving on my committee.

I also would like to thank the members of Dr. Woelk's research group for their support and cooperation on many occasions. I am particularly grateful to Dr. Annalise R. Pfaff, Emmalou T. Schmittzehe (Satterfield), and Ming Huang.

Finally, I would like to thank my parents for everything they have done for me, and my husband Jie Huang for his love and support in my life. Jie's assistance was crucial to the completion of many CapPack devices. He introduced the arc-fusion splicing technique to my research work, which helped me develop the microscale glass-sealing technique used to permanently seal the CapPack's capillary tubes.

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1. INTRODUCTION

Nuclear Magnetic Resonance (NMR) spectroscopy and Magnetic Resonance Imaging (MRI) are well-established, powerful analytical techniques in chemistry and other areas of science. Users are able to analyze chemicals, polymers, biomolecules, human tissues, as well as human body parts non-invasively and non-destructively. To extract the most unambiguous information about structures, conformations, dynamics, and functions of chemical compounds, materials, and human tissues, the NMR instrumentation need specialized devices to calibrate the spectrometer hardware and software. In this dissertation, a newly developed calibration-device platform, i.e., the CapPack (**Cap**illary-tube **Pack**ages) platform, will be introduced as well as several applications in liquid and solid-state NMR spectroscopy. A CapPack consists of one or more permanently glass-sealed capillary tubes filled with the same, or sometimes different, reference materials or solutions. CapPacks can be arranged in different geometries such as side-by-side, clustered, coaxial, or off-centered parallel to the sample axis. They are intended to generate unique NMR signals or profiles that allow the user to corroborate specific sample conditions, and understand how NMR probes, hardware, software, and novel pulse sequences work. Through the use of CapPack devices users can conveniently assess the performance of pulse sequences, optimize pulse parameters, explain artifacts that may occur in spectra, reveal spectroscopic results that deviate from theoretical predictions, or monitor sample conditions such as temperature, pressure, or pH. To successfully design and manufacture CapPack devices, several fundamental aspects of nuclear spins in strong magnetic fields need to be considered.

1.1. QUANTITATIVE NMR DETERMINATION OF SMALL MOLECULES

Quantitative NMR (qNMR) refers to the use of NMR for determining the concentration of one or more chemical species in solution. Conducting qNMR properly and to a very high degree of precision takes some special consideration [1]. For example, in one-dimensional experiments without hyperpolarization or polarization transfer from other nuclei, the area under an NMR signal is directly proportional to the number of nuclear spins in the sample. Thus, the concentration of a sample can easily be deduced by comparison of the sample's NMR signals to signals from compounds of know concentrations. This direct comparison is possible because the NMR "response" is the same for all nuclear spins [2] excited by the spectrometer's radiofrequency field. More and more analytical, pharmaceutical, and clinical applications use qNMR as a method of choice for determining absolute sample amounts and sample concentrations, and for measuring the amount of impurities quantitatively [3-5]. To render NMR experiments quantitative, an external signal-intensity reference with a well-defined number of nuclear spins is needed. For hydrogen-containing compounds of low molecular weight, a 99% precision and accuracy can be obtained when the external reference is placed in the magnetic field together with the sample but in a separate, isolated volume. The CapPack platform can provide such external standard by utilizing permanently sealed, small capillary tubes that contain reference samples of known concentrations.

1.2. SOLVENT-SIGNAL SUPPRESSION AND SHAPED PULSE

In many NMR investigations, the solvent of a sample generates the dominating NMR signal. Because strong solvent signals, such as the water signal that typically occurs between 4.6 and 4.8 ppm, can obstruct the quantitative determination of dissolved

compounds, deuterated solvents are commonly used in NMR investigations. To investigate samples in which the solvent cannot be substituted by its deuterated equivalent, solvent-signal suppression sequences were developed to quench strong solvent signal but preserve the quantitative information of the other signals. Common solvent-signal suppression sequences include continuous-wave (CW) pre-saturation, Excitation Sculpting, WATERGATE (WATER suppression by GrAdient-Tailored Excitation), WET (Water suppression Enhanced through T_1 effects), and PURGE (Presaturation Utilizing Relaxation Gradients and Echos) [6-9]. Before the development of CapPack devices, which is reported in this dissertation, several solvent-signal suppression sequences were tested for their applicability with in-situ NMR investigations of hydrothermal biomass-to-fuel (BTF) conversions. It was notized that the ^{1}H spin-lattice relaxation times for the hydrogen atoms in water, i.e., the solvent of the BTF conversions, changed substantially during the reactions. As a consequence, the common solvent-suppression sequences could not provide consistent signal suppression, and quantitative results were not obtained for the reactants and products of the BTF conversion.

As reported in Paper I, a solvent-signal suppression sequence was developed, which was designed to suppress solvent signals that may vary within one order of magnitude in T_1 relaxation times without adjusting pulse or delay parameters between experiments, and without applying pulsed field gradients in the sequence. The new sequence was termed EXCEPT (EXponentially Converging Eradication Pulse Train) [10] and was able to function properly when investigating hydrothermal BTF reactions with in situ NMR spectroscopy. EXCEPT provides a viable alternative to standard solvent-signal

suppression sequences particularly when relaxation times change during the course of an investigation. To illustrate the solvent-signal suppression features in more detail, the newly developed EXCEPT sequence was used as an example for performance tests with side-by-side Gradient CapPack devices and with clustered T_1 CapPack devices. The two different CapPack devices were used to assess different aspects (spectral bandwidth and T_1 insensitivity) of the same pulse sequence EXCEPT-12, where the number 12 in the acronym EXCEPT-12 indicates the number of progressively converging interpulse delays applied in the sequence.

1.3. B_0 MAGNETIC FIELD GRADIENT

To evaluate the suppression bandwidth of a solvent-signal suppression sequence, it is customary to either (a) apply a static magnetic field gradient across a homogeneous NMR sample and record a suppression profile [11] or (b) conduct multiple independent NMR measurements with the resonance of a single NMR signal varied across the expected bandwidth [12]. The suppression factor is then calculated as the ratio of NMR intensities with and without signal suppression. If a standard solvent-containing NMR tube and a magnetic *z*-gradient is used in a suppression bandwidth experiment according to strategy (a), the suppression profile is observed as a suppressed area in a signal profile that ideally is rectangular. However, it is often difficult to quantify the suppression profile from such measurement. An experiment according to strategy (b) usually requires many independent NMR experiments in which the resonance offset of the NMR signal or the resonance offset of the suppression sequence is varied [13]. Such a series of NMR experiments, however, can take a long time to complete.

A Gradient CapPack device is designed to combine features of both strategies (a) and (b) by subdividing the sample volume into multiple small and equal compartments. With the application of a magnetic field gradient similar to strategy (a), multiple equally spaced NMR signals of equal intensity are yielded, which can then be used to record spectra similar to those of strategy (b) but within a single NMR experiment.

1.4. SPIN-LATTICE RELAXATION TIME (T_1)

Nuclear spin-lattice relaxation describes the process of a nuclear-spin ensemble returning from excitation to its thermodynamic equilibrium. According to the solution of the Bloch equations, spin-lattice relaxation is mathematically described by an exponential rise-to-maximum functionality and characterized by the spin-lattice relaxation time T_1 [14]. If inversion-recovery experiments are used to measure spin-lattice relaxation times, the following well-known equation applies:

$$M_z = M_0 [1 - 2 \exp (- t / T_1)] \quad (1)$$

where M_z is the current longitudinal magnetization, M_0 is the magnetization at thermodynamic equilibrium, and t is the time provided for free relaxation after the inversion. The time constant T_1 can be greatly affected by conditions that surround the nuclear spins, i.e., by the lattice. One such condition is the concentration of paramagnetic ions, where a higher concentration typically increases relaxation and shortens the time constant T_1 [14]. The primary design feature of T_1 CapPack devices is to combine samples of different, well-defined relaxation times in one device. By filling small capillary tubes with samples that contain different amounts of paramagnetic ions, different relaxation times are achieved for the samples in each capillary tube. Combining

capillary tubes with samples of different T_1 times in one NMR device (T_1 CapPack device) generates NMR signals of different relaxation times in a single NMR spectrum.

1.5. PARAMAGNETIC IONS AND ISOTROPIC SHIFT

When the relaxation time of a dissolved sample compound is altered by the addition of paramagnetic ions such as Cu^{2+} in $CuSO_4$, the chemical shifts of the dissolved sample compound can also change [15]. The unpaired d-electrons in the paramagnetic Cu^{2+} ions, for example, modify the local magnetic field which induces an isotropic chemical shift in the ^1H resonance frequencies of the surrounding molecules. The magnitude of the additional field, and thus the extent of the chemical-shift change, depends on the paramagnetism, the geometry, and the concentration of the Cu^{2+} ions [16]. Because paramagnetic shifts coincide with a change in T_1 relaxation time, the NMR signals of T_1 CapPack capillary tubes are automatically separated on the chemical-shift axis in the NMR spectra, and no added magnetic field gradient, such as applied in the Gradient CapPack experiments, is needed to separate the signals.

In summary, the CapPack platform provides NMR devices that can be used to create performance measures for spectrometer hardware and software, evaluate novel pulse sequences, monitor sample conditions such as temperature, pressure, or pH, and calibrate the chemical-shift and intensity axes of NMR spectra. The overarching goal of evaluating NMR spectrometer and probe performance as well as calibrating NMR spectra with CapPack devices is to improve the accuracy and precision of qNMR measurements so that the remaining uncertainties and errors are smaller than 1%.

PAPER

I. EXPONENTIALLY CONVERGING ERADICATION PULSE TRAIN (EXCEPT) FOR SOLVENT-SIGNAL SUPPRESSION IN INVESTIGATIONS WITH VARIABLE T_1 TIMES

ABSTRACT

Selective presaturation is a common technique for suppressing excessive solvent signals during proton NMR analysis of dilute samples in protic solvents. When the solvent T_1 relaxation time constant varies within a series of samples, parameters for the presaturation sequence must often be re-adjusted for each sample. The EXCEPT (EXponentially Converging Eradication Pulse Train) presaturation pulse sequence was developed to eliminate time consuming pulse-parameter re-optimization as long as the variation in the solvent's T_1 remains within an order of magnitude. EXCEPT consists of frequency-selective inversion pulses with progressively decreasing interpulse delays. The interpulse delays were optimized to encompass T_1 relaxation times ranging from 1 to 10 seconds, but they can be easily adjusted by a single factor for other ranges that fall within an order of magnitude with respect to T_1. Sequences with different numbers of inversion pulses were tested to maximize suppression while minimizing the number of pulses and thus the total time needed for suppression. The EXCEPT-16 experiment, where 16 denotes the number of inversion pulses, was found satisfactory for many standard applications. Experimental results demonstrate that EXCEPT provides effective T_1-insensitive solvent suppression as predicted by the theory. The robustness of EXCEPT with respect to changes in solvent T_1 allows NMR investigations to be carried out for a series of samples without the need for pulse-parameter re-optimization for each sample.

1. INTRODUCTION

For our research in hydrothermal biomass-to-fuel (BTF) reactions of lignocellulosic biomass, quantitative proton NMR has proven to be a more expedient and accurate method for analysis of kinetics and mechanisms than the more commonly used HPLC technique. As with most large biomolecules, BTF compounds are often studied in aqueous solution. However, the overwhelming 110 M ^{1}H solvent signal can impede these investigations particularly when quantitative information is desired [1, 2, 3, and 4]. If the receiver gain is set low enough to avoid analog-to-digital converter (ADC) overflow by the solvent magnetization then the dynamic range available to resolve the solute signals is limited. Even if the solute signals can be adequately resolved, the magnitude of the solvent signal may still obscure solute signals close to the solvent resonance. A common solution to the limitations imposed by a protic solvent is the substitution with a deuterated solvent. However, this is impractical for substances that are naturally dissolved in water or react to produce water as a by-product, such as with biomolecules and BTF compounds. In addition, accelerated proton-deuteron exchange occurs during hydrothermal BTF conversions at elevated temperatures due to multiple keto-enol tautomerisms which also obstruct quantitative NMR analysis.

There is already a wide variety of solvent suppression techniques available [2]. Nonetheless, the challenge here is not simply to suppress a large solvent signal, but to do so for a series of samples that vary in T_1. During hydrothermal BTF reactions the pH of the solution fluctuates significantly as a result of acidic and basic by-products. Additives such as inorganic salts and mineral acids that are used to optimize the desired product yields cause additional variations in the pH of the solution. These factors significantly

alter the longitudinal relaxation rates of the solvent protons over the course of the reaction, which can affect the ability of an NMR sequence to suppress the solvent signal. An ideal suppression sequence would require minimal re-optimization of pulse-sequence parameters between samples, exhibiting robustness with respect to large variations in the longitudinal relaxation rates for the solvent protons.

Some of the most common solvent suppression sequences – CW presaturation, WATERGATE (WATER suppression by GrAdient-Tailored Excitation), WET (Water suppression Enhanced through T_1 effects), and PURGE (Presaturation Utilizing Relaxation Gradients and Echos) – were tested considered to find a sequence that fulfills the demands of hydrothermal BTF analysis. CW presaturation is one of the most straightforward methods of solvent suppression, however, this method is subject to baseline distortions and the soft-pulse frequency requires re-adjustment when the solvent signal's resonance frequency changes for different samples. WATERGATE is said to provide "pure phase" spectra without baseline distortions [5] and can be tailored to a narrow suppression region to reduce its effect on surrounding peaks [6]. However, it is still too sensitive with respect to the T_1 fluctuations present in BTF investigations. WET is reportedly less B_1- and T_1-sensitive [7, 8], but its tolerance of T_1 variations is still too narrow for fast and convenient NMR investigations of BTF samples. PURGE is reported to result in highly selective suppression with flat baselines and excellent phase properties [9]; however, solvent suppression pulse sequences that use CW soft pulses suffer from a loss of quantitative signal information if solute protons are in exchange with solvent protons. The EXCEPT (EXponentially Converging Eradication Pulse Train) sequence introduced here provides a viable alternative for solvent suppression, tolerating at least an

order of magnitude variation in solvent T_1's while maintaining quantitative signal information. It uses only delays and selective inversion pulses without the need for multiple channels, gradients, or adjusting power levels.

2. THEORY OF EXCEPT

EXCEPT is a pulse train of selective inversion pulses, such as adiabatic hyperbolic secant pulses (sech pulses) [10], with progressively decreasing interpulse delays. It follows the concept of multiple inversion-recovery nulling [11, 12] and was inspired by earlier approaches utilizing aperiodic pulse trains for saturation of magnetization [13, 14]. Figure 1 shows the timeline of EXCEPT-16, which is an EXCEPT sequence of 16 pulses and 16 interpulse delays.

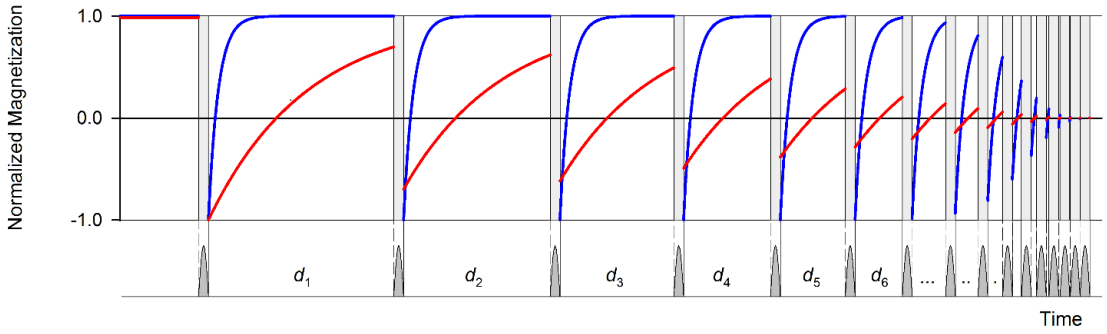


Figure 1. Timing of the EXCEPT-16 selective inversion pulses and interpulse delays (lower part) for effective suppression of longitudinal magnetization. Two sample magnetizations (upper part) with T_1 time constants that differ by a factor of five are equally suppressed to zero by the end of the pulse sequence.

Also shown in Figure 1 are two longitudinal magnetization recovery curves (depicted in red and blue) of equal initial magnitude as they evolve through the inversion and recovery periods in the sequence. Although the T_1 time constants differ by a factor of

five the sample magnetizations are similarly suppressed at the conclusion of the sequence.

The interpulse periods in Figure 1 are adjusted to suppress magnetization within an order of magnitude in T_1. This renders EXCEPT extremely robust for signal suppression with a high tolerance for variations in T_1. The progressively decreasing delays d_i, where i denotes the delay following the i^{th} pulse (Figure 1), were calculated according to the recursive formula

$$d_i = d_n\,(n\text{-}i\text{+}1) \qquad (1)$$

where x is the exponent of convergence and n is the number of inversion pulses in the sequence, i.e., n = 16 for EXCEPT-16. In an alternating least-squares procedure [15], dn and x were iteratively optimized to cover T_1 times from 1 – 10 s (Table 1). The optimized exponents of convergence were found to be x = 2.95, 3.65, 3.89, and 4.01 for EXCEPT-12, EXCEPT-16, EXCEPT-20, and EXCEPT-24, respectively, resulting in least-squares deviations from complete signal suppression of $\chi^2 = 1.4 \times 10^{-5}$, 2.4×10^{-7}, 3.5×10^{-9}, and 4.9×10^{-11}, respectively.

Because solvent suppression does not need to be executed on fully relaxed magnetization, EXCEPT can utilize the relaxation delay between consecutive scans by beginning to suppress solvent signals immediately after data acquisition. For example, if the solvent T_1 is suspected to be between 1 and 10 s then a relaxation delay of 50 s ($5\times T_1$) would normally be implemented. EXCEPT-16 for this T_1 range requires 75 s to be fully executed, thus extending the relaxation delay by 50 percent.

To further illustrate how EXCEPT acts on magnetizations with different relaxation time constants, Figure 2 shows a linear plot (Figure 2a) and a semi-log plot

Table 1. Interpulse delays optimized for the suppression of longitudinal magnetization with relaxation time constants, T_1, within the range of 1 – 10 s.

	Interpulse delays (s)			
Delay	EXCEPT-12	EXCEPT-16	EXCEPT-20	EXCEPT-24
d_i	x = 2.95	x = 3.65	x = 3.89	x = 4.01
d_1	17.12268	18.88342	20.47159	21.28725
d_2	13.24374	14.92077	16.76581	17.94438
d_3	9.995602	11.59956	13.58337	15.01201
d_4	7.323546	8.850851	10.87336	12.45500
d_5	5.172570	6.608794	8.587350	10.23979
d_6	3.487383	4.810769	6.679394	8.334415
d_7	2.212361	3.397455	5.106032	6.708471
d_8	1.291496	2.312937	3.826320	5.333143
d_9	0.668329	1.504821	2.801848	4.181196
d_{10}	0.285851	0.92437	1.996757	3.226971
d_{11}	0.086352	0.526655	1.377762	2.446384
d_{12}	0.011157	0.270743	0.914176	1.816926
d_{13}	---	0.119919	0.577939	1.317663
d_{14}	---	0.041969	0.343642	0.929231
d_{15}	---	0.009556	0.188568	0.633835
d_{16}	---	0.000762	0.092725	0.415249
d_{17}	---	---	0.038896	0.258813
d_{18}	---	---	0.012691	0.151429
d_{19}	---	---	0.002618	0.081562
d_{20}	---	---	0.000176	0.039234
d_{21}	---	---	---	0.01602
d_{22}	---	---	---	0.005049
d_{23}	---	---	---	0.000992
d_{24}	---	---	---	0.000061

(Figure 2b) of the longitudinal magnetization that remains at the end of each interpulse delay di of EXCEPT-16 as a function of T_1. A salient feature of Figure 2a is the change in curvature of the magnetization traces as the sequence progresses. With each additional

delay from d_1 to d_7, the point of inflection in the traces shifts to lower T_1 relaxation time constants. The magnetization inverts from negative to completely positive curvature after d7 which was found to be essential for the sequence to be successful.

While EXCEPT was originally optimized to suppress solvent signals with relaxation time constants within the range 1 – 10 s, it may easily be adjusted to cover different ranges of T_1. A factor for delay adjustment (delay adjustment factor, f_{da}) is used to adjust each interpulse delay d_i such that Eq. (1) changes to

$$d_i = f_{da} [d_n (n\text{-}i\text{+}1)x] \quad (2)$$

This adjustment changes the T_1 range of optimal signal suppression from 1 – 10 s to the range f_{da} ×1 s to f_{da} ×10 s. For instance, if the solvent's T_1 value is expected to be within the range 0.5 – 5 s, it is recommended to set f_{da} = 0.5 cutting all interpulse delays listed in Table 1 in half. Figure 3 provides a useful tool for estimating the value of the delay adjustment factor, where the grey-shaded area corresponds to the optimized range of solvent signal suppression. For example, f_{da} = 0.28 can be used to suppress solvent signals in routine ^{1}H-NMR investigations of small molecules in aqueous solutions where the solvent's longitudinal relaxation times are typically within the range 0.28 – 2.8 s. Other ranges include f_{da} = 1 for samples of small molecules dissolved in degassed organic solvents, f_{da} = 0.2 for most biological samples from adipose tissue ($T_1 \approx$ 240 ms) to blood ($T_1 \approx$ 1350 ms), and f_{da} = 0.015 for larger biomolecules or small soluble polymers in acidic solutions where T_1 is typically within 15 - 150 ms.

The calculations indicate that an increased number of inversion pulses and interpulse delays in the EXCEPT sequence (i.e., changing the presaturation sequence successively from EXCEPT-12 to EXCEPT-16, EXCEPT-20, and EXCEPT-24) leads to

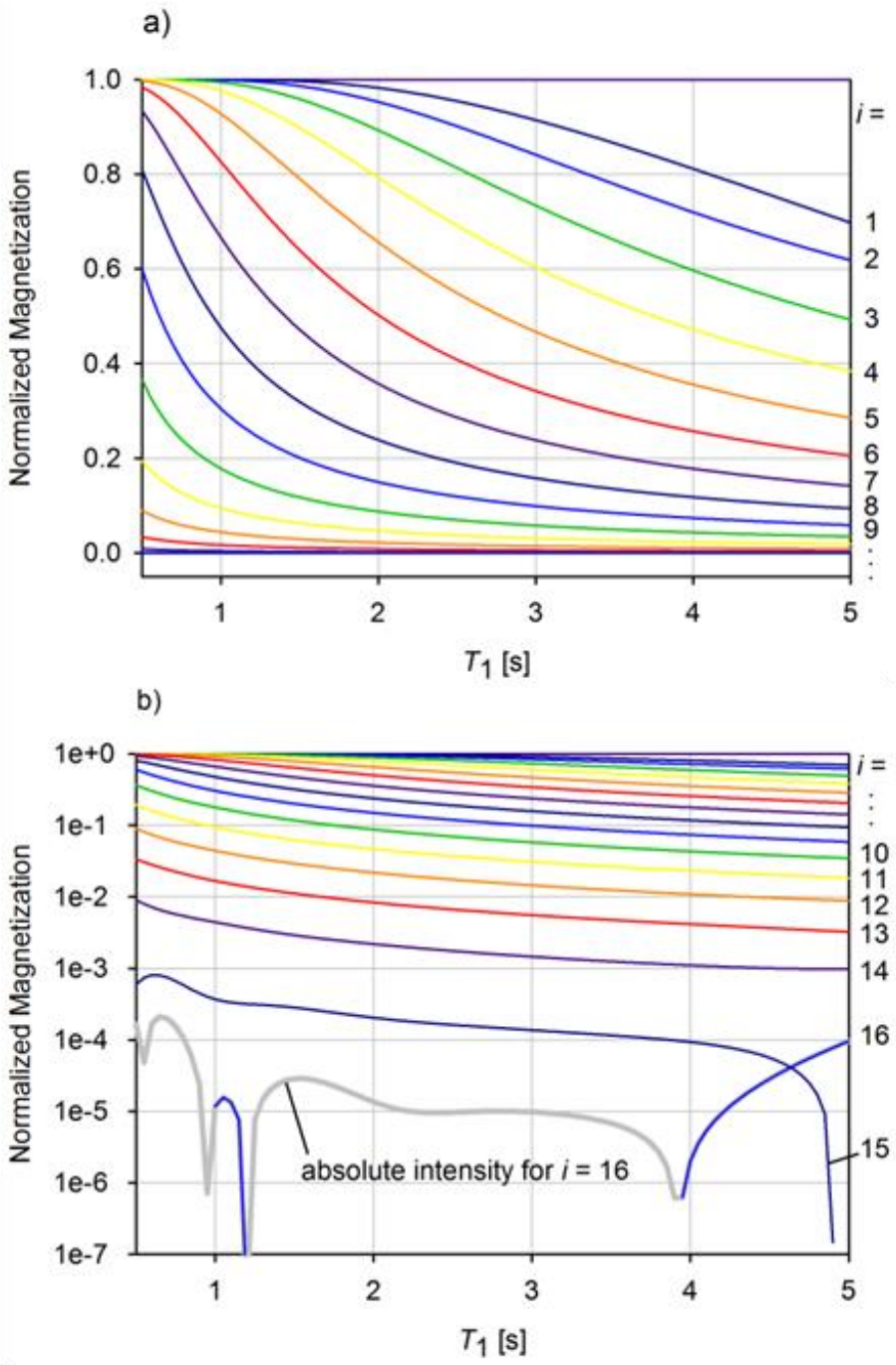


Figure 2. Residual longitudinal magnetization after each interpulse delay d_i of EXCEPT-16 as a function of T_1. The T_1 axis covers the range for which suppression of magnetization was optimized: a) linear plot showing the effects of the early delays, b) semi-log plot showing effects of the final delays. The grey portion of the trace for i = 16 indicates where the final signal intensity is negative but is displayed as a log of the absolute intensity.

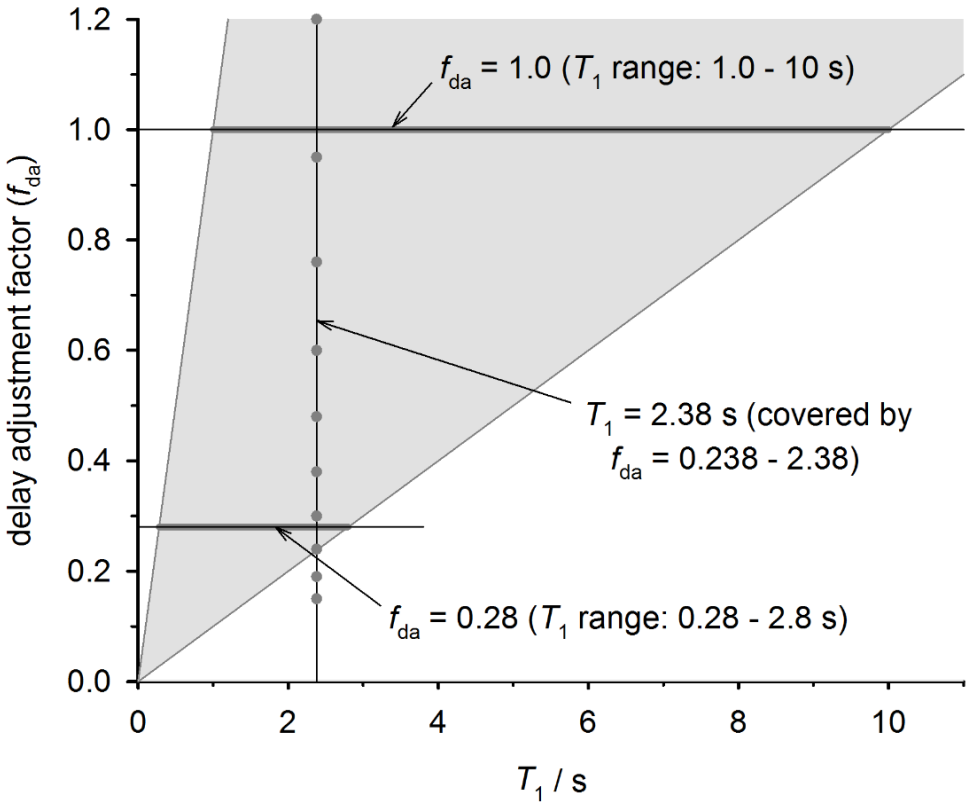


Figure 3**.** Ranges of T_1 for which EXCEPT successfully suppresses solvent signals (grey-shaded area) as a function of the delay adjustment factor (f_{da}). The T_1 range can easily be adjusted by changing the f_{da}. While f_{da} = 1 covers T_1 range = 1 – 10 s, f_{da} = 0.28 should be used if the solvent's T_1 falls within the range 0.28 – 2.8 s (horizontal lines). On the contrary, a solvent relaxation time of T_1 = 2.38 is sufficiently supressed by any f_{da} value that lies between 0.238 and 2.38 (vertical line).

better solvent suppression in the final spectrum. However, due to the very short durations of the later delays in EXCEPT-20 and EXCEPT-24 (i.e., interpulse delays that are shorter than 1 ms, while the selective inversion pulse might last longer than 100 ms), neglecting relaxation during inversion pulses in the calculations might no longer produce accurate results. Furthermore, instrumental limitations may make it difficult to manifest the full efficacy of EXCEPT in an NMR experiment where short delays are combined with long selective inversion pulses.

3. RESULT AND DISCUSSION

All NMR experiments were carried out at room temperature with a 400-MHz Varian Inova spectrometer employing a standard 5-mm broad-band probe. No post-acquisition treatment was applied to any of the data shown.

The suppression range of EXCEPT was tested using EXCEPT-16 with a delay adjustment factor f_{da} = 0.22, which is optimized for suppression of signals with T_1 = 0.22 s to T_1 = 2.2 s. Spectra of the residual HDO resonance in $CuSO_4/D_2O$ solutions were recorded from samples with T_1 ranging from 0.121 s to 3.17 s. As can be seen in Figure 4 the EXCEPT sequence is very robust with respect to solvent relaxation time constants that differ by more than an order of magnitude; samples with T_1 from 0.235 s to 2.39 s were suppressed by amounts greater than 99% in signal magnitude. Even solvent magnetizations with relaxation time constants outside the optimized range were reduced by 93% or more by the sequence.

The T_1 suppression range of EXCEPT-16 was also tested by adjusting the f_{da}, thus shifting the relaxation time suppression range, while analyzing a single sample with a solvent relaxation time of 2.98 s. The f_{da} value was increased from 0.19 to 3.01, placing the actual T_1 value of the HDO protons at different positions within the optimized range of EXCEPT-16. The smallest f_{da} value used in this series was 0.15 optimizing EXCEPT-16 suppression for solvent protons with longitudinal relaxation times from 0.15 s to 1.5 s, which is well below the T_1 of the tested sample. Similarly, the longest f_{da} tested was 3.01, which results in an optimal suppression range of 3.01 s to 30.1 s.

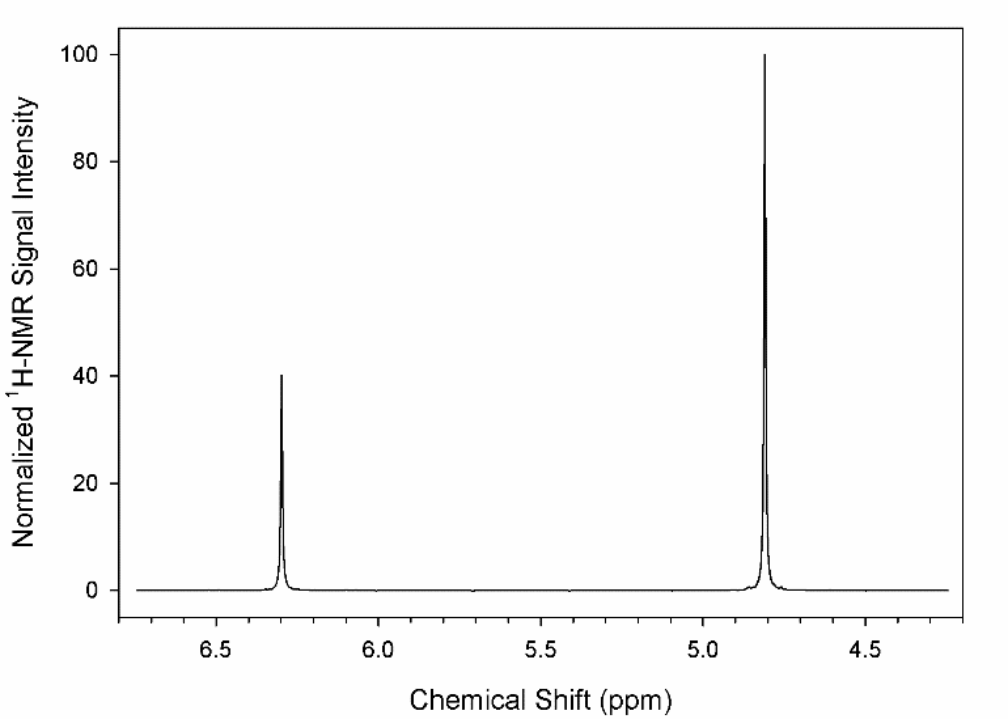
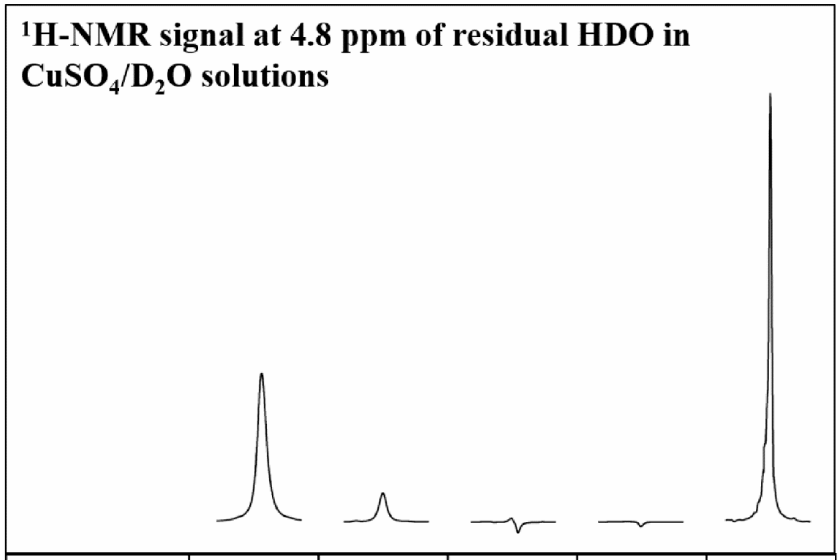


Figure 4. Residual HDO peaks versus T_1 following application of EXCEPT-16 solvent suppression pulse sequence.

Figure 5 shows a typical NMR spectrum of a sample containing maleic acid with an unsuppressed residual HDO solvent signal at 4.81 ppm. Figure 6 displays the experimental results for the HDO solvent signal suppression for various f_{da} values with the maleic acid proton signal unaffected. For f_{da}= 0.30 the residual water signal demonstrated greater than 97% reduction, and for f_{da} = 2.39 the residual water signal was suppressed greater than 99%. This shows effective solvent suppression by EXCEPT-16 for at least an order of magnitude variation in T_1, i.e., from f_{da} to 10 × f_{da}.

A BTF sample with an HDO proton T_1 of 2.38 s was used in order to demonstrate EXCEPT-16 suppression on a sample for which the solvent suppression pulse sequence was originally devised. As shown in Figure 7a, without solvent suppression the water



$CuSO_4$/M	0.0125	0.00625	0.00156	0	0 (deoxygenated)
T_1/s	0.121	0.235	0.805	2.39	3.17
% Suppression	97.57	99.53	99.83	99.93	93.00

Figure 5. ^{1}H NMR spectrum of 0.5 M maleic acid in 99.5% D_2O.

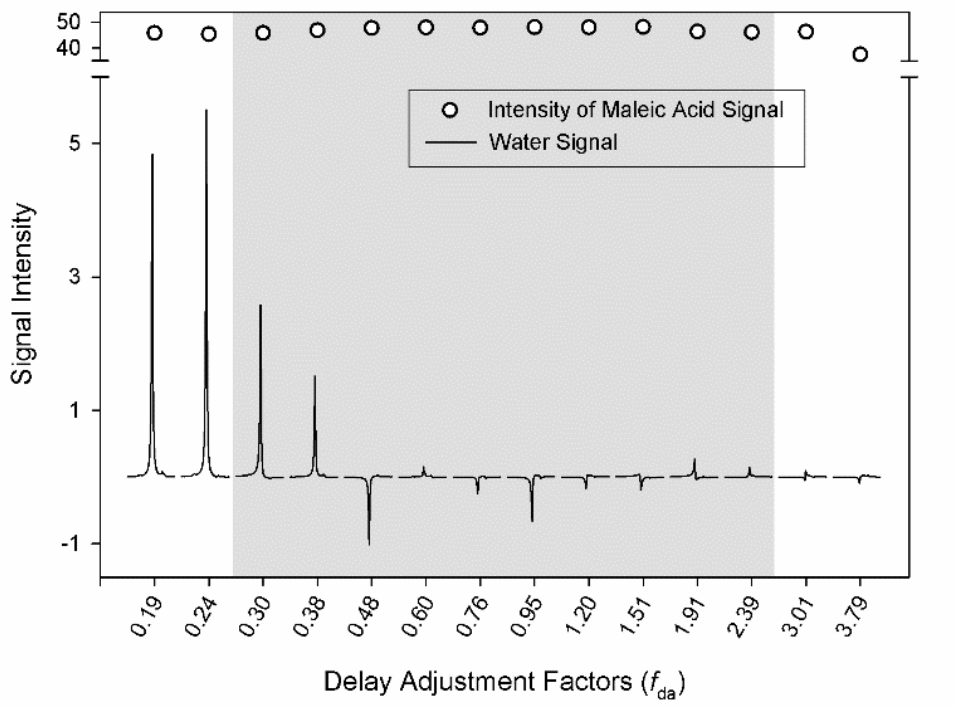


Figure 6. Residual water signal versus delay adjustment factor using EXCEPT-16 solvent suppression on 0.5 M maleic acid sample in 99.5% D_2O. The shaded area corresponds to f_{da} values for which the T_1 falls within the optimized range.

signal impedes the analysis of the dilute BTF products; the analog-to-digital conversion cannot accurately represent the weak signals which are substantially distorted in the baseline as well as lost in the noise. Figure 7b shows the outstanding suppression along with the excellent baseline and phase properties that can be achieved with EXCEPT-16. For this sample, a sech pulse duration of 100 ms was chosen leading to the suppression bandwidth of 200 Hz. Comparison of the spectra in Figure 7, specifically the peak at 5.05 ppm and the cluster of peaks at 4.50 ppm, reveals the potential of the EXCEPT-16 pulse sequence to reveal solute peaks near or under the wings of the solvent peak

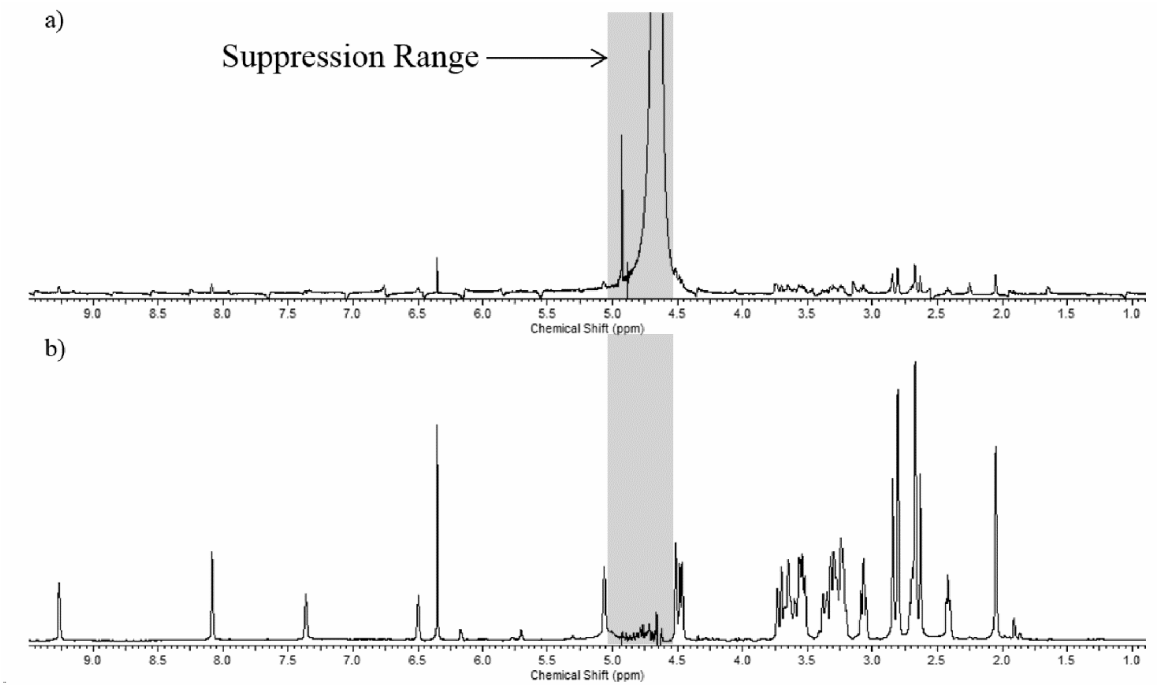


Figure 7. ^{1}H-NMR spectra from sample consisting of 600 µL of room temperature solution taken from the reaction of 0.2 M D-glucose in citric acid buffer in a standard glass pressure vessel for 9 hours at 150°C. 150 µL of 99.5% D_2O was added for a field-frequency lock. a) Spectrum obtained with 90° pulse (11 µs) and 16 scans. b) Spectrum obtained with EXCEPT-16 solvent suppression pulse sequence under an identical set of acquisition parameters.

The EXCEPT-16 sequence resulted in an approximate 3000-fold reduction of the water signal intensity from Figure 7a to Figure 7b. Suppression factors were calculated using an external standard of 0.5 M maleic acid (1.0 M ^{1}H)/99.9% D_2O in a 1 mm capillary NMR tube as a basis for comparison between water signal magnitudes with and without the application of EXCEPT-16 suppression. These results were confirmed by directly comparing the absolute magnitude of the full water signal to that of the EXCEPT-16-suppressed signal. The receiver sensitivity was increased by a factor of 100 for the EXCEPT-16-suppressed spectrum (Figure 7b), which was taken into account when comparing the magnitudes of the full and residual water signals.

4. CONCLUSION

The superior stability of EXCEPT suppression with respect to changes in solvent longitudinal relaxation time makes it possible to conduct ^{1}H-NMR analysis on dilute samples with wide variation in T_1 without the need for re-adjustment of pulse sequence parameters between samples. Changing the f_{da} parameter modifies the delay periods in the sequence, making it applicable for suppression of any desired range within an order of magnitude in T_1. Suggested values are $f_{da} = 0.28$ for routine investigations of small molecules in aqueous solutions, $f_{da} = 1$ for samples of small molecules dissolved in degassed organic solvents, $f_{da} = 0.2$ for most biological samples from adipose tissue to blood, and $f_{da} = 0.015$ for larger biomolecules or small soluble polymers in acidic solutions. EXCEPT may also be used as a general saturation sequence by replacing the frequency-selective soft pulses with hard pulses.

EXCEPT-16. It was found that the maximum suppression predicted by the theory was not achieved, which was likely due to the long soft pulses versus the short delays at the end of the sequence. The pulse times were not accounted for in the optimization of the sequence even though they may allow for non-negligible relaxation of the solvent signal. The issue created by short delays coupled with long pulses becomes more pronounced as the delay adjustment factor becomes smaller which may result in less effective solvent signal suppression. Alternatively, a shorter duration for the soft pulse could be chosen leading to a wider than necessary suppression bandwidth.

Both simulated results and experimental observations demonstrate that EXCEPT's selective suppression is extremely tolerant of changes in the longitudinal relaxation time of solvent protons, with minimal adverse effects on the signals of interest. A suppression of 97% or more can be achieved over at least one order of magnitude of T_1 relaxation time constants regardless of where the actual value falls within the optimized T_1 range, while suppressing greater than 99% is accomplished over the majority of the T_1 suppression range. Greater than 94% suppression is still achieved when the actual T_1 value lies just above the optimized suppression range as well as greater than 99% suppression when the actual T_1 value falls somewhat below the optimized suppression range.

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II. CAPPACK DEVICES FOR THE QUANTITATIVE PERFORMANCE EVALUATION OF NMR SPECCTROMETERS AND PULSE SEQUENCES

ABSTRACT

With the increased sensitivity of modern nuclear magnetic resonance (NMR) spectrometers, the minimum amount needed for chemical-shift referencing of NMR spectra has decreased to a point where a few microliters can be sufficient to observe a reference signal. The reduction in the amount of required reference material is the basis for the NMR CapPack platform that utilizes capillary tubes with inner diameters smaller than 150 μm as NMR-tube inserts for external reference standards. It is shown how commercially available electrophoresis capillary tubes with outer diameters of 360 μm are filled with reference liquids or solutions and then permanently sealed by the arc discharge plasma of a commercially available fusion splicer normally employed for joining optical fibers. The permanently sealed capillaries can be used as external references for chemical-shift, signal-to-noise, resolution, and concentration calibration. Combining a number of permanently sealed capillaries to form CapPack devices leads to additional applications such as performance evaluation of NMR spectrometers and NMR pulse sequences. A 10-capillary-tube side-by-side Gradient CapPack device is used in combination with one or two constant gradients, produced by room-temperature shim-coils, to monitor excitation profiles of shaped pulses. One example illustrates the performance of hyperbolic secant (sech) pulses in the solvent suppression sequence EXCEPT. The excitation profile of the pulse sequence is obtained in a single gradient NMR experiment and is practically free from the effects of molecular diffusion. A clustered T_1 CapPack device is introduced consisting of a coaxial NMR-tube insert that

holds seven capillary tubes filled with aqueous solutions of different concentrations of the paramagnetic relaxation agent copper (II) sulfate ($CuSO_4$). The different $CuSO_4$ concentrations lead to spin-lattice relaxation times in the seven capillary tubes that cover a range which extends to more than an order of magnitude. Clustered T_1 CapPack devices are best suited to quantify the effects relaxation has on magnetizations and coherences during the execution of NMR experiments, which is demonstrated for the order-of-magnitude T_1 insensitivity of signal suppression with EXCEPT.

1. INTRODUCTION

The frequencies of nuclear-spin resonances depend on the strength of the external magnetic field, B_0, which varies from one nuclear magnetic resonance (NMR) spectrometer to another. Because of this variability, the chemical-shift axes of NMR spectra must be referenced to the resonances of known materials or internal/external standards [1, 2]. Resonances of known materials may include solvent signals, signals from previously recorded spectra, or ^{2}H resonances of deuterated solvents locked in with the spectrometer's field-frequency lock [3]. The most commonly used internal standard is perhaps tetramethylsilane (TMS) which, by convention, defines the zero-point of reference for the chemical-shift ppm axes of ^{1}H, ^{13}C, and ^{29}Si spectra [4]. However, if a material under investigation is suspected to interfere with or alter the resonances of a reference standard, external standards must be used [5]. External standards can be the same materials as internal standards, but they are typically sequestered in coaxial NMR-tube inserts mounted concentrically with spacers inside standard 5-mm or 10-mm NMR tubes [6]. With the increased sensitivity of modern NMR instrumentation, capillary-tube

inserts with I.D. < 150 μm may already be large enough to hold a sufficient amount of referencing material. For several years, we have successfully used capillary tubes with an O.D. of 360 μm and an I.D. as small as 20 μm to provide enough NMR-sensitive reference materials for standard NMR measurements.

In this article, we report the expansion of the capillary-tube reference standard concept to a versatile testing platform which we termed “CapPack” as an abbreviated form of Capillary-tube Packages. The CapPack platform allows users to conveniently assess the performance of pulse sequences, optimize pulse parameters, explain artifacts that may occur in spectra, and reveal spectroscopic results that deviate from theoretical predictions. A CapPack consists of one or more glass-sealed capillary tubes containing different, or sometimes the same, reference standards or solutions. CapPacks are designed to generate unique NMR signals or profiles that allow the user to verify that, and understand how, NMR probes, hard- and software, and pulse programs work. CapPacks can be arranged in different geometries such as side-by-side (see Section 2.2) or clustered (see Section 2.3). For example, Figure 1 shows a CapPack consisting of 10 permanently sealed water-filled capillary tubes assembled in an in-plane side-by-side fashion. Inserted into a 5-mm NMR tube, the side-by-side CapPack forms a Gradient CapPack device that is ready to be used in pulse-sequence evaluations.

In the following, we introduce the essential techniques to fill and permanently seal commercially available electrophoresis capillary tubes (O.D. = 360 μm, I.D. = 20 – 125 μm). Electrophoresis capillary tubes are typically manufactured to a high degree of concentricity for the inner cylindrical volume and with very narrow tolerances for O.D.

and I.D. [7]. When aligned parallel with the main magnetic field, the narrow tolerances of electrophoresis capillary tubes easily meet the O.D., I.D., and concentricity specifications

Figure 1. Photograph of a 10-capillary Gradient CapPack contained in a 5-mm NMR sample tube.

for high-resolution, high-field NMR spectroscopy [8]. Two different CapPack devices, the 10-capillary-tube side-by-side Gradient CapPack device (Figure 1) and a seven-capillary-tube clustered T_1 CapPack device are used to exemplify two different applications for CapPacks in assessing the performance of pulse sequences.

In particular, the side-by-side Gradient CapPack device is used to illustrate the performance (bandwidth and symmetry) of the newly developed solvent-suppression sequence EXCEPT [9]. The EXCEPT sequence utilizes low-power, frequency-selective adiabatic hyperbolic-secant pulses (sech pulses) [10] under conditions where the pulse

width is of the order of the sample's spin-lattice relaxation time [11, 12]. To show limitations in the applicability of EXCEPT, the experiments were conducted with an older 200-MHz NMR spectrometer, where soft-pulse amplitude and phase adjustments require more time (~ 500 ns) compared with newer NMR instrumentation (< 20 ns). Furthermore, the EXCEPT solvent-suppression sequence was developed to avoid parameter adjustments even if the solvent's T_1 time changes up to an order of magnitude during a series of experiments (e.g., during the in situ NMR process monitoring of a chemical reaction). The clustered T_1 CapPack device is used to show that resonances with a wide range of spin-lattice relaxation times (T_1) are sufficiently suppressed by the EXCEPT sequence without changing the time frame of the inversion-recovery-nulling interpulse delays [9, 13].

2. DESIGN AND FABRICATION OF CAPPACK DEVICES

To assemble CapPacks we chose 25-cm or longer portions of commercially available hollow fused-silica capillary tubing typically sold for applications in capillary electrophoresis by the manufacturer [Molex, Polymicro Technologies™, Lisle, IL]. The O.D. of the capillary tubing's glass portion is 320 μm while the I.D. can be any value between 20 and 125 μm depending on the intended use of the CapPack, the sensitivity of the NMR nuclide, and the concentration of the reference material. It is noted that the concentration of some reference materials may be limited by the solubility in desired solvents. The capillary tubing comes with an added 20-μm thick polyimide protective coating, which gives the fused-silica material strength and flexibility. To fill a capillary tube with the reference material, one end of the tube is inserted into a syringe needle and

affixed with epoxy glue. The NMR-sensitive reference material is then injected into the open-ended capillary tube, and the distal end sealed using an optical-fiber fusion splicer (see Section 2.1.). Fusion splicers are commonly used to join optical fibers designed for the transmission of digital information. In a similar way, the other end of the capillary tube is cut and separated from the needle, and then sealed. The result of this procedure are 5 to 20 cm-long capillary tubes that contain, depending on the I.D. and length of the capillary tube, between 2 and 40 μL of isolated, NMR-sensitive reference material.

2.1 MICRO-SCALE GLASS SEALING TECHNIQUE

The CapPack technology provides an NMR platform that isolates the capillary volumes from each other and from the remaining volume of the NMR tube. The reference materials inside the capillary tubes are intended to be isolated so that composition, amount, concentration, and spin relaxation are constant, and that consistent, reliable, and quantitative results are obtained. We found that traditional techniques such as sealing the ends of a capillary tube with epoxy glue are not chemically stable, especially when organic solvents are used in the NMR tube. Also, it is quite challenging to flame-seal the electrophoresis capillary tubes without losing most of the enclosed reference material when it is volatile. Ultimately, we developed a different process to reliably seal filled capillary tubes at both ends. The method utilizes an electric optical fiber arc fusion splicer [Type-36 SM MM, Sumitomo Electric Industries, Ltd. Osaka, Japan]. In this sealing method, the open end of the filled capillary tube is inserted into the alignment portion of the fusion splicer while it is still connected to the syringe that is filled with the reference sample. Each open end of the capillary tube is stripped of the polyimide coating a distance of about 1 mm from the end before sealing, which protects the fusion splicer

components (e.g., electrodes, camera lenses) from charred polymer. The arc-discharge plasma of the fusion splicer is applied for 20 s to melt and seal the end of the capillary tube in a process known as melt-back [14, 15]. The capillary tube is then cut to size, which separates it from the sample-filled syringe. The sealing procedure is repeated at the cut end and the capillary tube ends examined under an optical microscope to ensure a complete and strong seal. A small head-space volume of gas will typically remain at the sealed ends of the capillary.

Figure 2 shows pictures obtained with a near-field optical scanning microscope [WiTec Instruments Corp., Knoxville, TN] from the end of a capillary tube filled with a reference solution. The four major areas identified in the pictures are (from left to right):

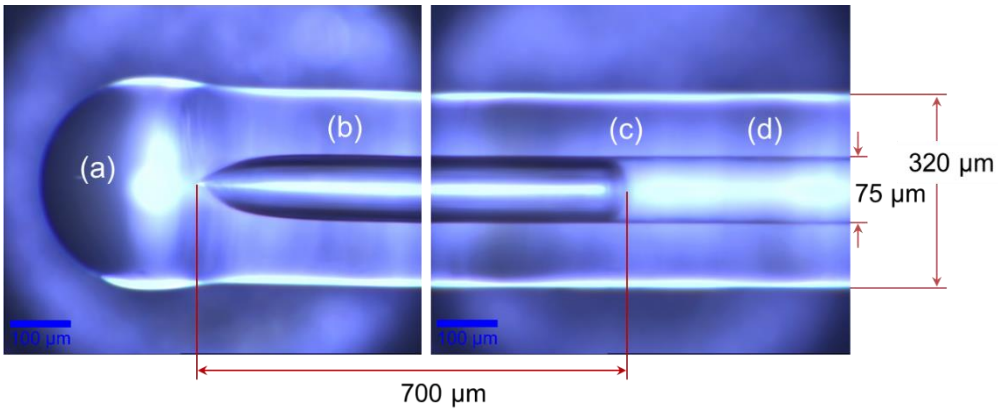

Figure 2. Photograph of a 320-µm O.D. fused-silica capillary tube sealed with the melt-back method of a commercial arc fusion splicer. The polyimide coating of the capillary was removed about 1 mm from the end of the capillary before applying the arc.(a) glass-sealed end, (b) head-space gas volume, (c) gas-liquid interface, (d) liquid-filled part of the capillary. The scale bar in each image represents 100 µm.

(a) the glass-sealed end, (b) a head-space volume of gas in the capillary tube, (c) the gas-liquid interface, and (d) the liquid-filled volume in the capillary tube. In this example, the length of the gas head space at the end of the capillary tube is 700 µm. Air bubbles were not observed in the liquid-filled volume. The glass-sealed capillary tube can withstand

large temperature and pressure variations and is stable in many chemical environments [16]. With the use of a fusion splicer, capillary tubes originally intended for capillary electrophoresis can be permanently sealed. Even though the sealing process involves high heat that can decompose the enclosed reference materials, we have not seen NMR evidence of decomposition impurities in the sealed capillary tubes.

2.2 GRADIENT CAPPACK DEVICE

A Gradient CapPack consists of multiple side-by-side mounted glass-sealed capillary tubes filled with the same NMR-sensitive reference material. Without the application of a magnetic field gradient, the NMR signals from the capillary tubes overlap in one combined resonance. When a field gradient is applied across the side-by-side coordinate and perpendicular to the long axes of the capillary tubes, the combined signal from the capillary tubes will separate into individual signals on the chemical-shift axis depending on the gradient field strength. Figure 3 shows schematics of a 10-capillary-tube Gradient CapPack designed to fit into a standard 5-mm sample tube. The capillary tubing's O.D. of 360 μm determines the maximum number of capillary tubes an (10) that can be mounted side-by-side and inserted into a standard 5-mm NMR tube with I.D. of 4.2 mm. A strip of sealing film (e.g., Parafilm®) is typically used at both ends of the CapPack to hold the capillaries together side by side in a plane. In some instances, a butane micro torch was used to carefully soften the polyimide coatings of the side-by-side capillary tubes so that they can bond together.

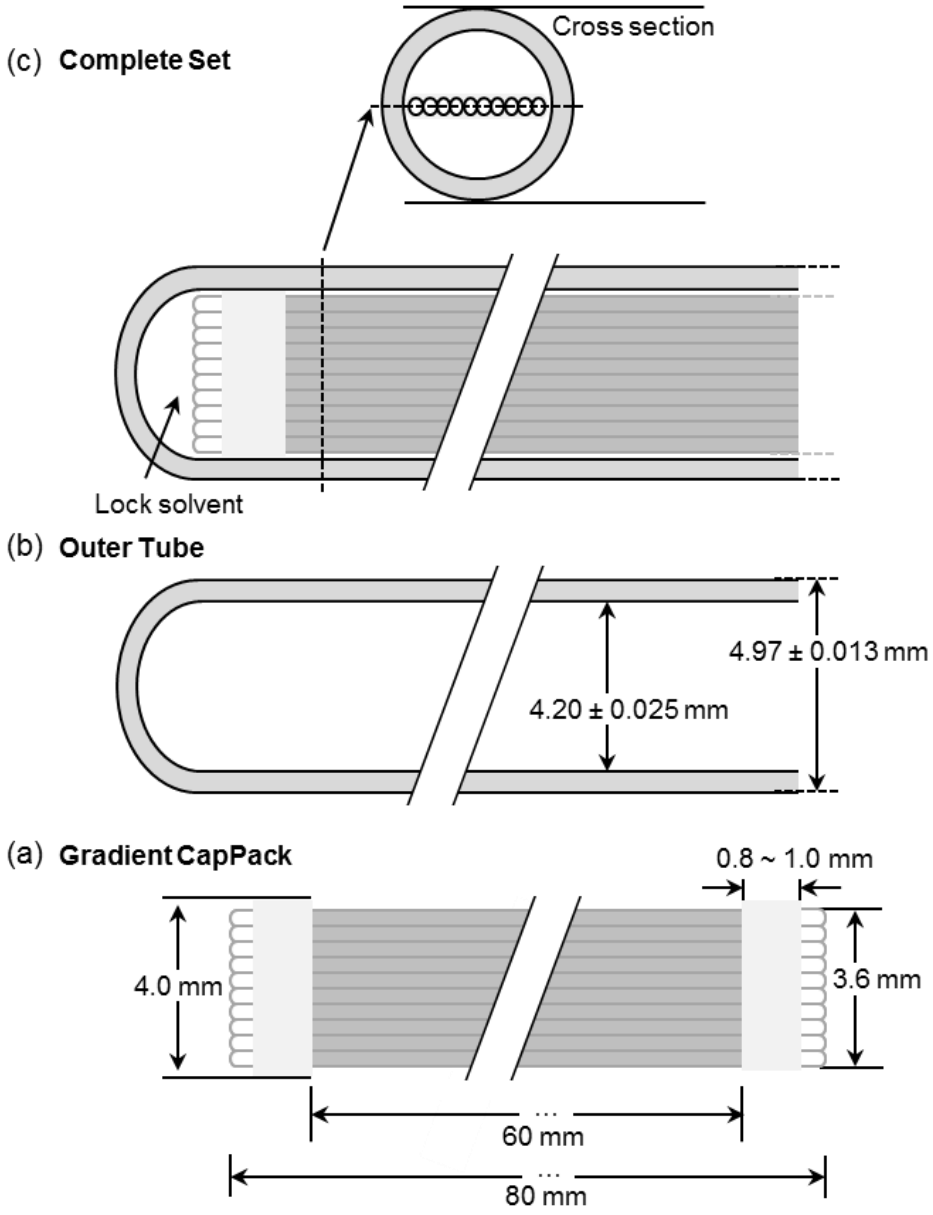

Figure 3. Schematic drawings of a 10-capillary-tube Gradient CapPack: (a) side-by-side assembly of 10 glass-sealed capillary tubes fixed with Parafilm® on both ends, (b) dimensions of a typical 5-mm NMR tube, (c) Gradient CapPack mounted inside the 5-mm sample tube for NMR performance measurements and experimentation. A lock solvent may be added to the 5-mm NMR sample tube to allow for field-frequency lock and shimming.

Figure 4 shows a computer simulation of a spectrum recorded from a 10-capillary-tube Gradient CapPack device with a single 90° hard pulse in a constant field gradient that is aligned across the capillary tubes and perpendicular to the long axes of the capillary tubes. Without the gradient, the material inside the capillary tubes would show a superposition of singlet signals at the chemical shift referenced with $\delta = 0$ Hz in the spectrum of Figure 4 (a). Because the NMR-sensitive material is the same in all capillary tubes, the constant gradient splits the original signal into 10 evenly spaced singlets, each arising from just one of 10 capillary tubes. The spacing of the signals depends on the gradient strength which, in this case, was adjusted to 0.81 mT m^{-1}. Figure 4 (b) shows the cross section of the Gradient CapPack aligned with the 10 signals recorded in the gradient field. The experiment is presumed to be conducted in a standard superconducting NMR magnet, where the long axis of the NMR sample tube coincides with the *z* coordinate of the laboratory frame of reference. In this example, a gradient that linearly modifies the main magnetic field (i.e., a constant gradient) is applied along the *y* direction of the laboratory frame as indicated by the arrow. The capillary tubes' ratio of O.D. to I.D. is pictured in the enlargement of Figure 4 (c). It was chosen to be large enough to completely resolve the capillary tube NMR signals in the applied gradient. In the case presented here, the O.D. (including the polymer coating) is 360 μm and the I.D., which defines the volume available for the NMR-sensitive reference material, is 25 μm. These values lead to a diameter ratio of $r_d = 14.4$, which is sufficiently large for most applications. The degree of separation between the individual capillary-tube signals, however, does not only depend on the ratio r_d and the gradient field strength but also on the linewidths of the NMR signals, and thus on the T_2 relaxation time of the interrogated

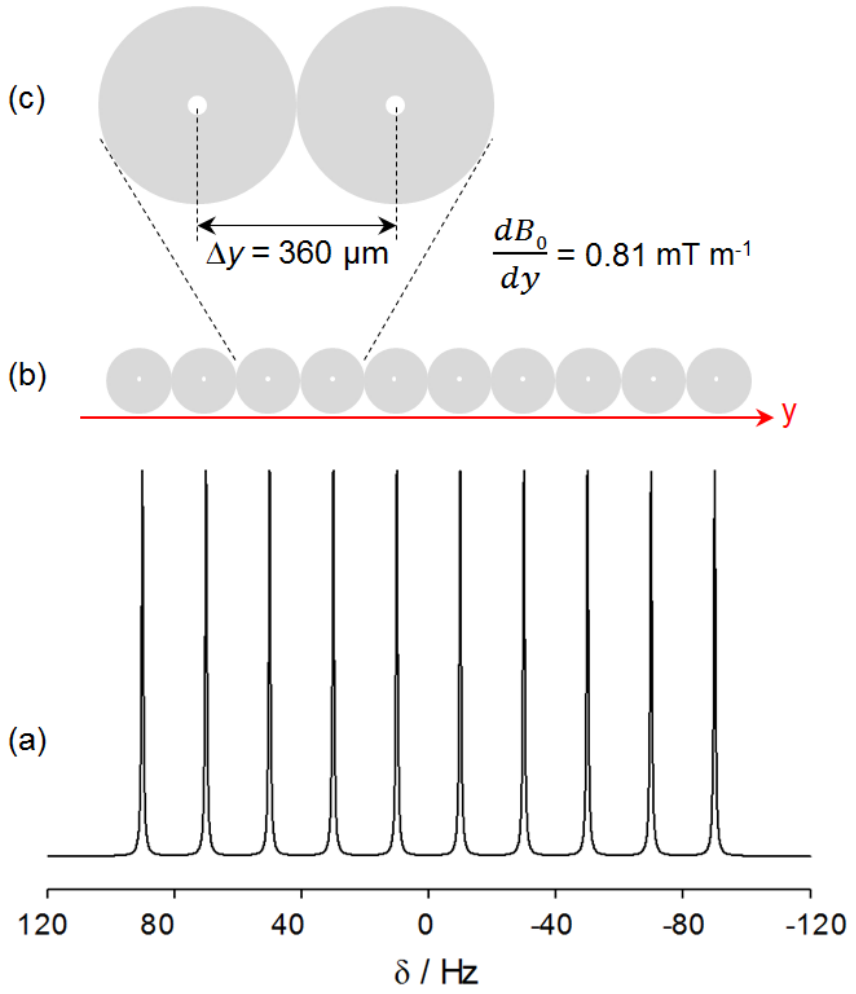

Figure 4. Schematic drawing and predicted spectrum of an in-plane side-by-side Gradient CapPack in a magnetic field gradient: (a) computer simulation of a spectrum of a 10-capillary-tube Gradient CapPack device in a constant magnetic field gradient obtained with a single hard pulse. The field gradient is aligned across the array of side-by-side capillary tubes; (b) cross section of the Gradient CapPack along the gradient y direction; (c) enlargement of the cross section of two side-by-side capillary tubes to visualize the capillary tubes' O.D. to I.D ratio (r_d).

spins in the reference material. In addition, it must be realized that the signals of Figure 4 (a), even though they appear to be Lorentzian lines, are a convolution of the signal's natural linewidth with the capillary tubes' cross-section geometry and the functional form of the gradient in the direction of the *y*-coordinate of the laboratory frame. The spectrum

reflects the geometry of the sample-filled capillary tubes projected onto the gradient axis (*y* axis) convoluted with the natural linewidth of the interrogated spins in the reference material.

In summary, the separation of Gradient CapPack signals into multiple, evenly spaced resonances across the NMR spectrum make it possible to accurately and conveniently evaluate on- and off-resonance performance of NMR pulse sequences. Particularly for frequency-selective pulses, the Gradient CapPack offers a fast and convenient method to determine irradiation profiles. In the experimental section, the Gradient CapPack shown in Figure 1 is used to observe the saturation profile of the solvent-suppression sequence EXCEPT [9] and identify deviations from theoretical predictions. The EXCEPT sequence utilizes long-lasting (> 200 ms), frequency-selective (< 200 Hz) inversion pulses such as the adiabatic hyperbolic secant pulse HS1 [10].

2.3 T_1 CAPPACK DEVICE

A T_1 CapPack device is designed to evaluate the effect spin-lattice relaxation has on magnetizations and coherences during the execution of NMR experiments. If water is used as the reference material, paramagnetic relaxation agents such as Cu^{2+} salts can be added to adjust the spin-lattice relaxation time of the ^1H resonances in the capillary tubes [17]. If the relaxation agent also causes a concentration-dependent paramagnetic shift ($\Delta\delta$) that is sufficient to separate the signal from one capillary tube on the chemical-shift axis completely from the signals of the other capillary tubes, no external magnetic field gradient is needed [18]. Figure 5 shows a computer simulation of a spectrum expected from seven capillary tubes each filled with water and different amounts of dissolved $CuSO_4$. With increasing amounts of the dissolved $CuSO_4$, the water resonances not only

shift toward higher chemical-shift values but also show substantially broader linewidths caused by shorter T_2 relaxation times. The separation of signals is sufficient to determine the relaxation times of water in each capillary tube by a standard inversion-recovery experiment. However, T_1 CapPacks are best suited to explore and quantify the effects spin relaxation has on magnetizations and coherences during the execution of NMR pulse

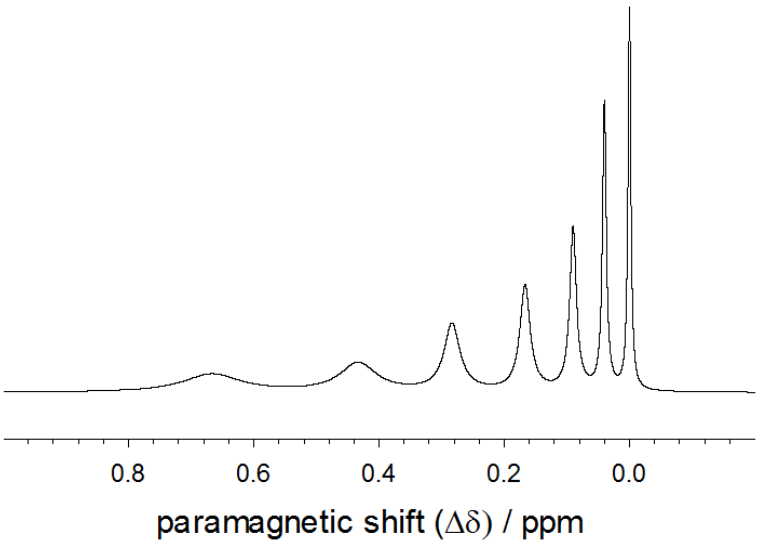

Figure 5. Computer simulation of a spectrum recorded with seven water capillaries containing different amounts of the paramagnetic relaxation agent $CuSO_4$. The concentration-dependent change in relaxation time coincides with a concentration-dependent paramagnetic shift of the solvent signals. Consequently, the chemical shift axis can be viewed also as a T_1 relaxation axis across the T_1 CapPack signals, and no magnetic field gradient is needed to separate the capillary-tube signals.

sequences. Because no gradient is needed to separate the signals on the chemical-shift axis, the capillary tubes can be assembled in a clustered CapPack device as shown in Figure 6. A clustered arrangement is less demanding and easier to assemble compared with the side-by-side arrangement of a Gradient CapPack. In addition, capillary tubes in a clustered CapPack device are easily exchanged without completely disassembling the CapPack. For convenient coaxial alignment and mounting of the CapPack in a

commercial NMR tube, the seven capillaries are inserted into a larger capillary tube micro-capillary with an O.D. between 1.5 mm and 1.8 mm) and aligned with a commercially available Teflon spacer [Kimble Glass, Inc., Vineland, NJ]. The Teflon spacer was originally designed to hold a 1-mm external-standard NMR-tube insert. To hold the larger micro-capillary, we carefully enlarged the central hole of the Teflon spacer.

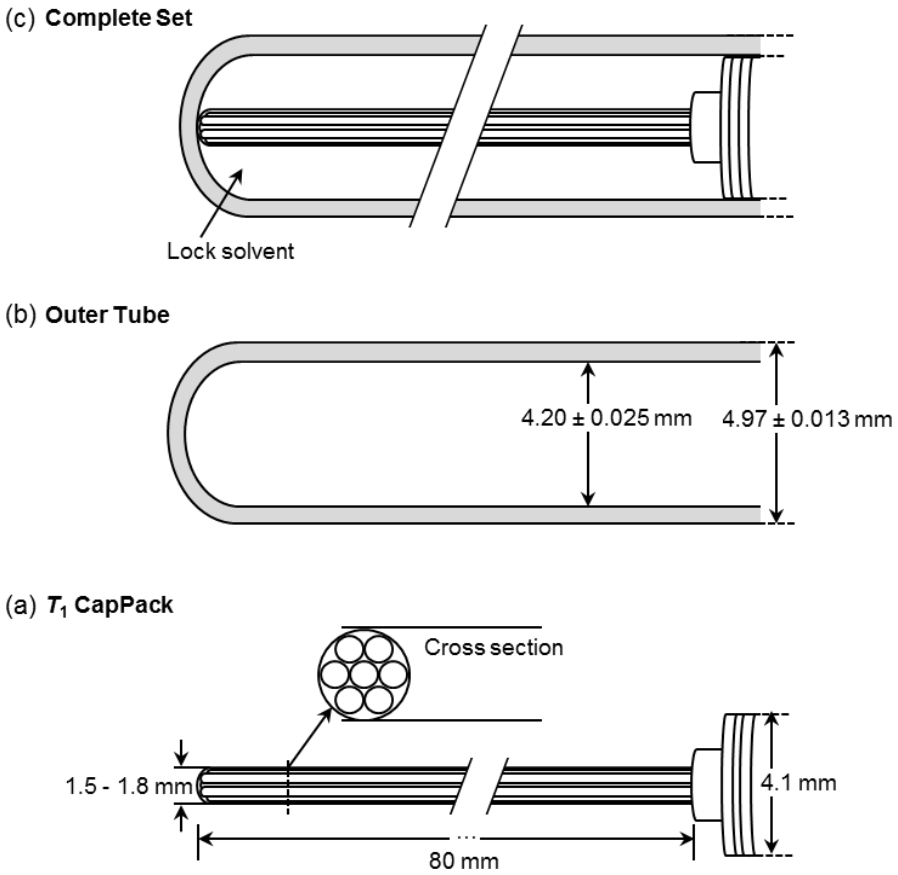

Figure 6. Schematic drawing of a seven-capillary-tube T_1 CapPack: (a) clustered assembly of seven glass-sealed capillary tubes inside a larger capillary tube (micro-capillary with O.D. between 1.5 and 1.8 mm). The cross section insert shows the hexagonal setting of the seven capillary tubes in the larger capillary tube, (b) dimensions of a typical 5-mm NMR tube, (c) T_1 CapPack mounted inside the 5-mm sample tube for NMR performance measurements and experimentation. A lock solvent may be added to the 5-mm NMR sample tube to allow for field frequency lock and shimming.

In summary, the singlet signals from H_2O in aqueous solutions with different amounts of dissolved $CuSO_4$ make it possible to obtain side-by-side NMR signals with different relaxation times without the application of a magnetic field gradient. Up to seven 360-μm capillary tubes can be assembled and conveniently packed into a commercially available micro-capillary and mounted coaxially inside a standard 5-mm NMR tube with a modified commercially available Teflon spacer. The amount of dissolved $CuSO_4$ in the capillary tubes and the combined volume of the T_1 CapPack capilary tubes are both small enough that no dielectric effect on probe tuning and matching is observed. In the experimental section, the T_1 CapPack is used to demonstrate the effectiveness for suppressing signals with different spin-lattice relaxation times (T_1). It is also shown how the range of T_1 that can be suppressed effectively by the EXCEPT sequence changes with the adjustment of a single parameter in the EXCEPT pulse sequence (i.e., the delay adjustment factor: f_{da} [9]).

3. RESULT AND DISCUSSION

All NMR experiments were carried out at room temperature, under deuterium field-frequency lock conditions, and without sample spinning using a 5-mm broadband probe inside a 200WB Bruker AVANCE DRX spectrometer. No post-acquisition treatment other than matched line broadening, fast Fourier transformation, and automated phase correction was applied.

3.1 GRADIENT CAPPACK EXPERIMENTS

The 10-capillary-tube Gradient CapPack device (Figure 1) was used to test the performance of low-power, frequency-selective adiabatic hyperbolic secant pulses (HS1) [10] in the EXCEPT-12 (Exponentially Converging Eradication Pulse Train) solvent-suppression sequence. The number 12 in the acronym EXCEPT-12 indicates the number of progressively converging interpulse delays applied in the sequence [9]. Figure 7 (a) shows a ^{1}H NMR spectrum of the Gradient CapPack surrounded by acetone-d6 (99.8%) in a 5-mm sample tube recorded using a standard 90° hard pulse (64 scans). The proton signals from the water samples inside the 10 capillary tubes overlap in the combined resonance visible at 3.78 ppm. Other resonances in the spectrum are assigned to acetone-d5 (2.04 ppm), dissolved water in the deuterated solvent (1.27 ppm), and other unidentified impurity signals marked by asterisks. Figure 7 (b) shows the spectrum of the same sample recorded with the same hard-pulse experiment but with a 0.81 mT m^{-1} gradient applied using the spectrometer's *y*-axis room-temperature shim coil. The orientation of the side-by-side capillary tubes along the *y*-coordinate of the laboratory frame, i.e., the direction in which the *y*-axis shim coil applies its constant gradient, was achieved using several short air bursts through the NMR magnet's upper spinner stack, thus rotating the sample successively by a few degrees. Alternatively, a suitable gradient across the side-by-side coordinate of the capillaries could also be achieved with an appropriate combination of *x*-axis and *y*-axis shim-coil gradients. As predicted in Section 2.1, the spectrum shows the water signals of the 10 capillary tubes spread out evenly and with equal intensity (1/10 of the total intensity) over a range of approximately 1 ppm (200 Hz). For better visualization, the spectrums in Figure 7 (b) as well as the spectra of

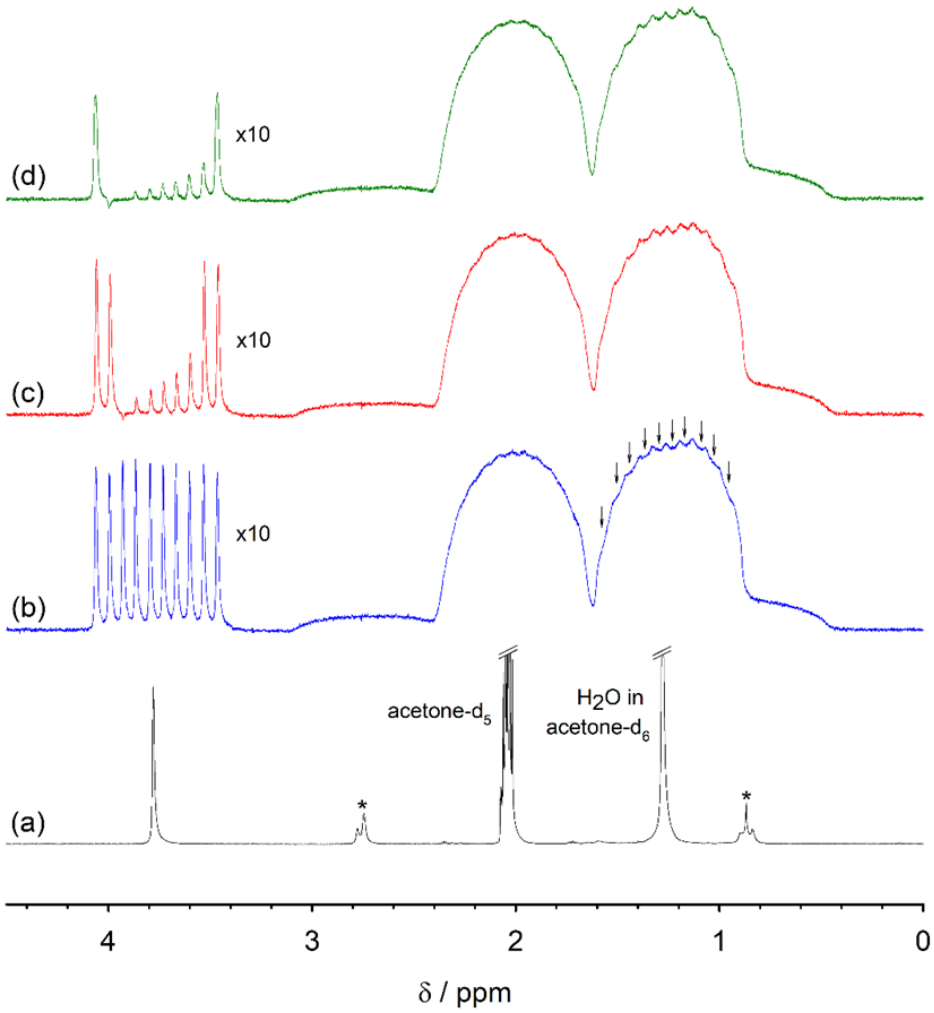

Figure 7. : ^{1}H NMR spectra recorded from a 10-capillary-tube Gradient CapPack contained in a 5-mm sample tube using acetone-d_6 (99.8%) as the surrounding solvent: (a) with a standard 90° observe pulse, (b) with a the 90° observe pulse while a 0.81 Gauss cm^{-1} gradient is applied across the 10 side-by-side capillary tubes, (c) with observe pulse following the application of a series of 400-ms HS1 pulses in an EXCEPT-12 experiment, (d) following the application of 300-ms HS1 pulses in an EXCEPT-12 experiment. To better exemplify the suppression performance, the signal-intensity axes of spectra (b), (c), and (d) are amplified by a factor of 10. Signals from resonances other than the CapPack are broadened by the field gradient across the sample, representing a one-dimensional profile of the NMR tube's circular cross section. The arrows in spectrum (b) point to voids in the circular profile of the H_2O in acetone-d_6 signal that result from sample displacements by the 10 capillary tubes of the Gradient CapPack.

Figure 7 (c) and (d) are displayed with a 10-fold magnification in the spectral intensity.

While the capillary signals become separated into individual signals, the signals from the

surrounding solvent at 2.04 ppm, 1.27 ppm, and the unidentified impurities broaden, resulting in a projection of the 5-mm sample tube's circular cross section onto the spectral dimension. The arrows in Figure 7 (b) point to small circular voids in one of the projection profiles which occur as a result of sample displacements from the side-by-side capillary tubes. Spectra (c) and (d) show in Figure 7 after the application of EXCEPT-12 with the HS1 pulse frequency set at the center of the CapPack signals (i.e., at 3.78 ppm). The pulse duration (pulse width) of HS1 in the EXCEPT sequence was adjusted to 400 ms in the experiment of Figure 7 (c) and 300 ms in the experiment of Figure 7 (d) leading to suppression bandwidths of 90 Hz and 120 Hz, respectively. As seen in the spectrum of Figure 7 (c), the signals from the two outer capillary tubes on each side of the Gradient CapPack are undisturbed by the EXCEPT sequence confirming that HS1 pulses affect only resonances within a very limited bandwidth. However, within the desired frequency range, signal suppression is asymmetrical and incomplete, which shows that the application of EXCEPT-12 with an older DRX spectrometer, where phase and amplitude adjustments during the application of a shaped pulse require more time (~ 500 ns) compared with modern NMR instrumentation (< 20 ns), may lead to suppression results that are less effective than predicted by the corresponding theory. Comparisons between Figure 7 (c) and (d) also reveal that the asymmetry and insufficient suppression increases with decreasing HS1 bandwidth, i.e., with longer HS1 pulses. The results of the EXCEPT-12 sequence experiments using adiabatic pulses shown in Figure 7 demonstrate that incomplete solvent signal suppression may be observed because of hardware limitations. These limitations can result in suppression factors significantly lower than predicted by the EXCEPT theory [9]. In the supplementary material to this article

additional evidence is provided showing that the cause for the asymmetric suppression profile originates in the DRX spectrometer hardware and not, for example, in the design of the Gradient CapPack.

3.2 T_1 CAPPACK EXPERIMENTS

The T_1 CapPack described in Section 2.3 was used to demonstrate the T_1 insensitivity of signal suppression using EXCEPT-12 over at least one order of magnitude in spin-lattice relaxation times. The seven capillary tubes were filled with aqueous solutions of different concentrations of $CuSO_4$. The concentrations of the paramagnetic relaxation agent $CuSO_4$ and the resulting T_1 relaxation times for the water signals are shown in Table 1.

Table 1. T_1 relaxation times and concentrations of relaxation agent $CuSO_4$ in the T_1 capillary tubes

capillary labeling used in Fig 8	I	II	III	IV	V	VI	VII
[$CuSO_4$] / mM	100	75.0	42.5	25.0	5.60	3.13	0.450
T_1 / ms	13.7	17.9	24.2	47.5	163	490	2880

Figure 8 (a) shows the ^1H spectrum of the T_1 CapPack's water signals recorded with a standard 90° observe pulse (64 scans). Because of concentration-dependent Cu^{2+} paramagnetic shifts, the capillary-tube water signals are separated from each other and spread out over a chemical-shift range of about 1 ppm without the application of an external magnetic field gradient. Across the seven T_1 CapPack signals, the chemical shift axis can now also be viewed as a T_1 relaxation axis. To avoid the insufficient and asymmetric suppression profiles observed earlier (see Section 3.1) with the frequency-selective HS1 pulses in the DRX spectrometer, the T_1 CapPack EXCEPT-12 experiments

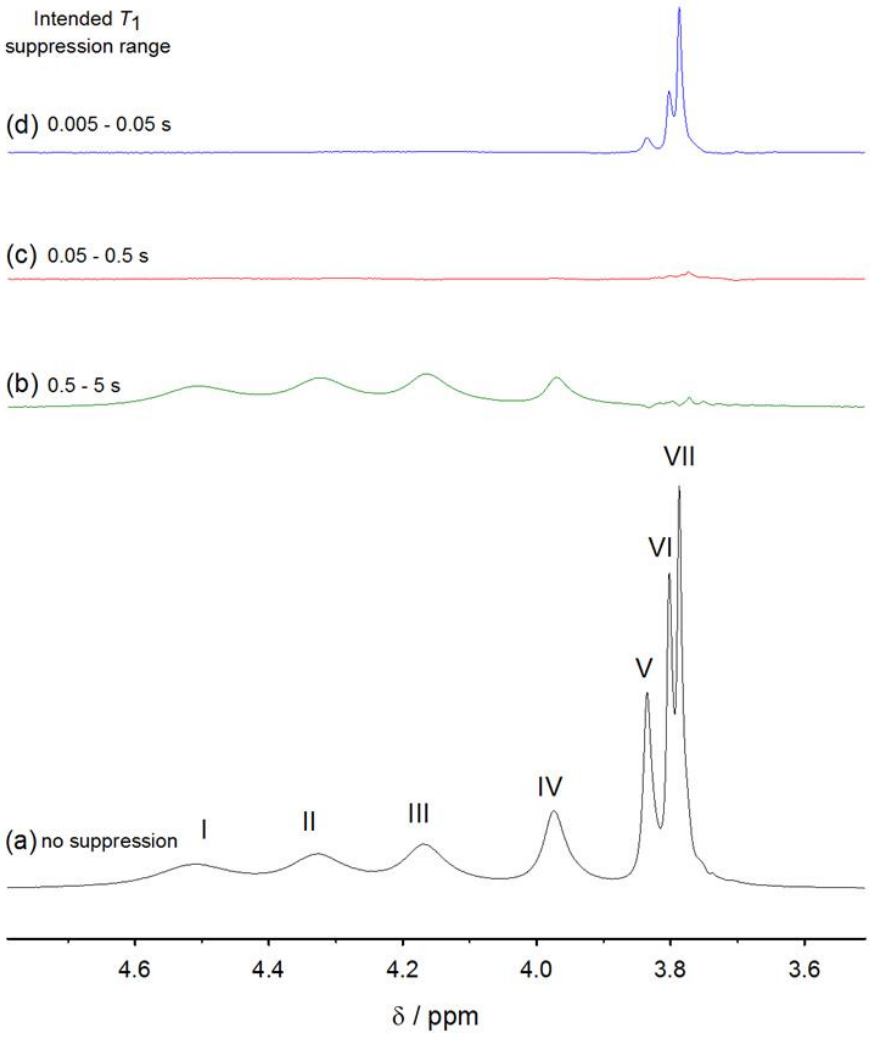

Figure 8. ^{1}H NMR spectra from a seven-capillary-tube T_1 CapPack device: (a) obtained with a standard observe pulse; labels in the spectrum refer to the $CuSO_4$ concentrations and T_1 relaxation times reported in Table 1, (b) obtained after the application of EXCEPT-12 adjusted for long T_1 times from 0.5 to 5 s, (c) after applying EXCEPT-12 adjusted for T_1 times from 0.05 to 0.5 s, and (d) obtained after applying EXCEPT-12 adjusted for very short T_1 times from 0.005 to 0.05 s. All spectra are displayed with the same signal-intensity magnification.

were first executed with rectangular hard pulses. Consequently, the spectral bandwidth of suppression covers the entire range of the ^{1}H spectrum. Spectra (b), (c), and (d) show Figure 8 recorded with different delay adjustment factors f_{da} in the EXCEPT sequence (i.e., f_{da} = 0.5 s, 0.05 s and 0.005s, respectively) [9]. The delay adjustment factor is a

multiplication factor in the EXCEPT pulse program applied to all interpulse delays which shifts the suppression range from 1-10 s in T_1 relaxation time to the range of $f_{da} \times 1$ s to $f_{da} \times 10$ s. The spectra (b), (c), and (d) in Figure 8 demonstrate how adjusting f_{da} changes the T_1 suppression range, leading to the suppression of most capillary-tube signals or, if set correctly, to the suppression of all capillary-tube signals. The spectrum in Figure 8 (c) also shows that even signals outside the intended suppression range of $T_1 = 0.05$ s to $T_1 = 0.5$ s are sufficiently suppressed, and that EXCEPT-12 typically exceeds the desired range of suppression in spin-lattice relaxation times (one order of magnitude) when hard pulses are used in the EXCEPT-12 sequence.

When frequency-selective adiabatic pulses with pulse widths larger than 100 ms are used in EXCEPT, relaxation during the pulses can no longer be neglected [11]. As a consequence, the use of EXCEPT-12 for suppressing fast relaxing signals ($T_1 < 50$ ms) may be limited. Figure 9 shows ^{1}H NMR spectra recorded with a series of experiments that is similar to the series exemplified in Figure 8 but with an EXCEPT-12 sequence that uses adiabatic HS1 pulses instead of rectangular hard pulses. The adiabatic pulse width was adjusted to 125 ms resulting in a suppression bandwidth of 240 Hz. The HS1 pulses were applied at 4.2 ppm so that the suppression bandwidth (shaded area from 3.6 ppm to 4.8 ppm in Figure 9) covers all seven T_1 CapPack resonances. Spectra (b), (c), and (d) in Figure 9 reveal that none of the delay adjustment factors (i.e., $f_{da} = 0.5$ s, 0.05 s, and 0.005s) led to a suppression of signals with relaxation times below 50 ms (i.e., sample capillaries I, II, III, and IV in Table 1). The T_1 CapPack experiments that led to the spectra in Figure 8 and Figure 9 provide good examples for testing the performance of a novel pulse sequence. For example, in this case the CapPack test results illustrate that

EXCEPT-12 performs exceptionally well with rectangular hard pulses but might not succeed in suppressing signals with short T_1 relaxation times when frequency-selective adiabatic pulses are used.

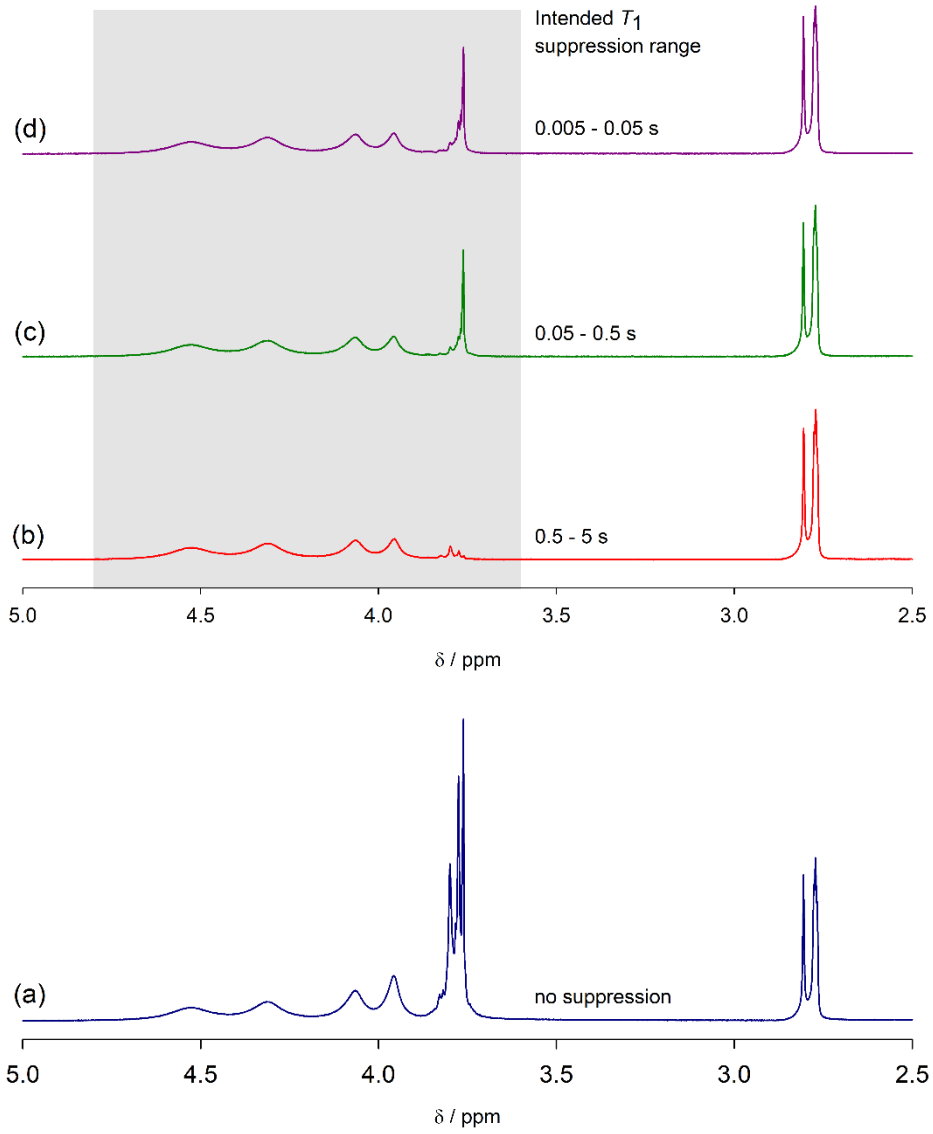

Figure 9. ^{1}H NMR spectra from a T_1 CapPack device: Spectrum (a) was recorded with a standard 90° observe-pulse experiment. Spectra (b) - (d) were recorded from EXCEPT-12 experiments using 125-ms adiabatic HS1 pulses resulting in a suppression bandwidth of 240 Hz applied at 4.2 ppm (shaded area, 3.6 – 4.8 ppm). The interpulse delays used in EXCEPT-12 were adjusted to suppress various ranges of T_1 times: (b) 0.5 to 5 s, (c) 0.05 to 0.5 s, and (d) 0.005 to 0.05 s. All spectra are displayed with the same signal-intensity magnification.

4. CONCLUSION

CapPack devices are unique NMR tools for the evaluation, calibration, and optimization of NMR parameters, pulse sequences, and probes, as well as NMR spectrometer hardware and software. They are fabricated from sealed electrophoresis capillary tubes that have been filled with specific NMR-sensitive reference materials. It is shown for the first time how electrophoresis capillary tubes are filled with NMR-sensitive liquids or solutions and then permanently sealed by the arc discharge plasma of a commercially available fusion splicer. The sealed capillary tubes are inserted into NMR sample tubes as external standards without reacting or chemically interfering with the samples under investigation. They are also used to generate unique NMR signals or profiles that allow the user to assess pulse sequences, optimize pulse parameters, identify artifacts, and investigate spectroscopic results that deviate from theoretical predictions. The information recorded with CapPack devices is inherently quantitative when CapPack volumes are filled with reference materials of known concentrations, and the capillary-tube volumes are determined from the manufacturers' specifications.

The CapPack testing platform covers devices of different geometries, which may be intended for a variety of calibration and performance-evaluation experiments. A 10-capillary-tube side-by-side Gradient CapPack and a seven-capillary-tube clustered T_1 CapPack were used to assess different aspects of the same pulse sequence EXCEPT-12. The EXCEPT sequence was originally developed to suppress solvent signals that may vary within one order of magnitude in T_1 relaxation times without adjusting pulse or delay parameters between experiments, and without applying pulsed field gradients in the sequence. Each performance metric, i.e., spectral bandwidth and T_1 insensitivity, could be

determined with a minimal amount of experimental time, and the desired information was typically gathered in a single experiment.

Molecules in liquids or solutions change location during an NMR experiment due to Brownian motion, i.e., molecular diffusion. While this usually isn't a problem for experiments conducted in the homogeneous magnetic fields of high-resolution NMR spectrometers, it can lead to artifacts, loss of coherence, and added signal decay in experiments that utilize magnetic field gradients. The capillary tubes of a Gradient CapPack confine the molecules of the reference standards to the I.D. of the capillary tubes (25 μm). This spatial restriction can be useful to minimize the effects of diffusion during the evaluation of metrics for NMR pulses, probes, and console hardware. Further investigations are needed to determine how the effects of restricted diffusion can be utilized in future experiments with CapPacks devices.

ACKNOWLEDGEMENTS

This work was supported by the Institute for Applied Chemistry and Nuclear Magnetic Resonance at the Missouri S&T Department of Chemistry. The authors like to thank Brewer Science Inc. (Rolla, MO) for materials and supplies used in this study. The engagement of Missouri S&T undergraduate students, who conducted experiments with CapPack devices as part of their Accelerated Physical Chemistry Laboratory course (CHEM 3459) is greatly appreciated.

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III. SOLID STATE NMR SPECTROSCOPY IN SITU MEASURING DEVICES AND METHODS FOR CALIBRATION AND DETERMINING ONE OR MORE QUANTITATIVE PROPERTIES OF A TARGET SAMPLE

ABSTRACT

In situ measuring devices, methods of making the same, and methods of using the same are provided herein. The in situ measuring devices can include a capillary tube having a reference material sealed inside the capillary tube, where the capillary tube is positioned inside of a solid state or MAS NMR rotor. A target sample can also be positioned in the interior of the solid state or MAS NMR rotor but is seques- tered from the reference material by a capillary tube wall. The in situ measuring devices can be used in solid state MAS NMR spectroscopy to quantify one or more parameters of a target sample, such as the quantity of a sample, chemical identity of a sample, or temperature of a sample.

1. FIELD OF THE INVENTION

The present disclosure relates to the field of NMR spectroscopy/imaging, and more specifically, to in situ measuring devices for calibration and determining one or more quantitative properties of a target sample.

2. BACKGROUND

Certain conventional electronic devices are commercially available for monitoring intensive properties of NMR samples, such as an electronic pH meter that measures pH values for NMR samples by installing a sensor at the tip of a long,

small-diameter rod and positioning such sensor inside an NMR tube, e.g., a 5 mm NMR tube. However, such a conventional device and technique requires the removal of the NMR tube from the NMR probe in order to measure the pH value of the sample. Removing an NMR tube for this purpose is inconvenient when monitoring chemical reactions by in situ NMR spectroscopy, especially when the pH changes unexpectedly and rapidly throughout the course of the reaction. Further, other conventional devices for monitoring NMR samples, such as an NMR temperature probe require placing the device in the probe, making a series of NMR measurements at different probe temperature settings, making a series of corresponding probe temperature measurements with an independent thermocouple or other electronic temperature sensor, removing the device from the probe, and creating a calibration curve. The NMR sample to be analyzed is then placed in the probe, the probe temperature setting is adjusted to a desired value, the NMR sample is allowed to equilibrate to the probe temperature, and the 60 calibration curve is used to predict the temperature of the NMR sample. The explicit assumption is that the calibration curve provides an accurate prediction of the temperature of the NMR sample. It is often the case that the assumption is invalid and that the predicted temperature of the NMR 65 sample is erroneous. Additionally, the conventional device is costly and the procedure for measuring and assigning the temperature of the NMR sample is extensive, tedious, time consuming, and inherently prone to operator error. Furthermore, the numerical value of temperature that is assigned to the corresponding recorded NMR spectrum lacks incipient integrity and, therefore, can be called into question in legal proceedings.

3. SUMMARY OF THE INVENTION

A high-level overview of various aspects of the invention is provided here for that reason, to provide an overview of the disclosure and to introduce a selection of concepts that are further described below in the detailed description section below. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in isolation to determine the scope of the claimed subject matter.

In an exemplary aspect, a device is provided for in situ pH monitoring of a sample using NMR spectroscopy. The device comprises a sample housing member configure d to house a target sample, at least one pH sensor configure d to exhibit an NMR spectral change due to a change in pH value of the target sample, and a pH sensor containment member configure d to house the at least one pH sensor. The pH sensor containment member is positioned inside at least a portion of the sample housing member. Further, at least a portion of the pH sensor containment member comprises one or more pores, which are configure d to allow diffusion of hydronium cations and hydroxide anions.

In another exemplary aspect, a method is provided for measuring a pH of a sample in situ using NMR spectroscopy. The method comprises providing an in situ NMR pH measurement device. The device includes a sample housing member configure d to house a target sample, at least one pH sensor configure d to exhibit an NMR spectral change due to a change in pH value of the target sample, and a pH sensor containment member configure d to house the at least one pH sensor. The method further comprises adding the target sample to the sample housing member

obtaining one or more NMR spectra, and determining the pH of at least a portion of the target sample.

In another exemplary aspect, a device is provided for monitoring a temperature of a sample in situ using NMR spectroscopy. The device comprises an NMR sample tube and at least one capillary tube positioned inside the NMR sample tube. The at least one capillary tube is configure d to house a reference material. Further, the device comprises a glass seal at a first end and at a second end of the at least one capillary tube that seals the first end and second end after the reference material has been added.

In another exemplary aspect, a method is provided for measuring a temperature of a sample in situ using NMR spectroscopy. The method comprises providing an in situ NMR temperature measurement device. The device includes an NMR sample tube and at least one capillary tube positioned inside the NMR sample tube. The at least one capillary tube is configure d to house a reference material and be sealed once the reference material has been added. The method further comprises adding the target sample to the NMR sample tube, obtaining one or more NMR spectra, and determining the temperature of at least a portion of the target sample based on at least one NMR spectrum of the reference material.

Still yet, in another exemplary aspect, a method is pro- vided for forming a seal at one or both ends of a capillary tube used in an in situ NMR temperature measurement device. The method comprises providing at least one capillary tube used in the in situ NMR temperature measurement device, adding a reference

material to at least a portion of the at least one capillary tube, and using an Optical Fiber Arc Fusion Splicer to seal a first end of the at least one capillary tube.

In a further exemplary aspect there is provided a method for performing one or more quantitative measurements of a target sample using solid state MAS NMR spectroscopy. The method includes providing an in situ measuring device, the in situ measuring device including a solid state MAS NMR rotor and at least one sealed capillary tube positioned inside the solid state MAS NMR rotor. The at least one sealed capillary tube having a reference material sealed inside the at least one capillary tube. A target sample is positioned on the inside of the solid state MAS NMR rotor. The method further includes obtaining MAS NMR spectra of the target sample and the reference material; and deter- mining one or more quantitative properties of the target sample. The one or more quantitative properties include one or more of a quantity of the target sample, a chemical identity of the target sample, or a temperature of the target sample.

In yet another exemplary aspect there is provided a method for performing one or more quantitative measurements of a target sample using solid state MAS NMR spectroscopy. The method includes providing an in situ measuring device, the in situ measuring device including a solid state MAS NMR rotor and at least one sealed capillary tube positioned inside the solid state MAS NMR rotor, the at least one sealed capillary tube having a reference material sealed inside the at least one capillary tube. A target sample is positioned on the inside of the solid state MAS NMR rotor. The method also includes inserting the in situ measuring device inside a probe of a solid-state MAS NMR instrument. In addition, the method includes, while

the in situ measuring device is positioned inside a probe of a solid state MAS NMR instrument, obtaining MAS NMR spectra of the target sample and the reference material, where at least one MAS NMR spectral peak associated with the reference material is spaced apart from at least one MAS 40 NMR spectral peak associated with the target material. The method also includes determining one or more of a quantity of the target sample or a chemical identity of the target sample based on the MAS NMR spectra of the target sample and the reference material.

In another exemplary aspect there is provided a method for forming an in situ measuring device for solid state MAS NMR spectroscopy. The method includes providing at least one capillary tube and adding at least one reference material to an inside cavity of the at least one capillary tube. The method also includes sealing one or more ends of the at least one capillary tube to form at least one sealed capillary tube having the at least one reference material inside and pro- viding a solid state MAS NMR rotor, the solid state MAS NMR rotor configure d to house a target sample. In addition, the method includes positioning the at least one sealed capillary tube inside the solid-state MAS NMR rotor.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Illustrative embodiments of the present invention are described in detail below with reference to the attached drawing Figures, and wherein:

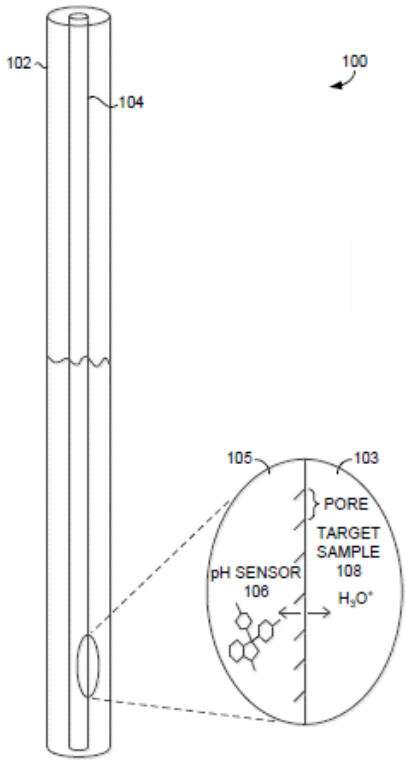

Figure l. An in situ pH measuring device.

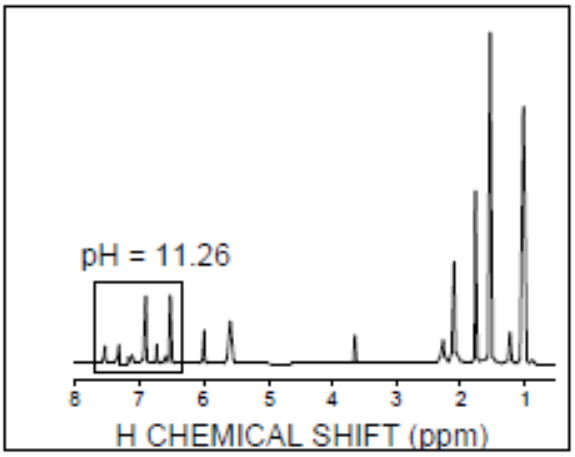

Figure 2. An example NMR spectrum that includes the spectral imprint of the in situ pH sensor in the same raw data output NMR spectra of a target sample.

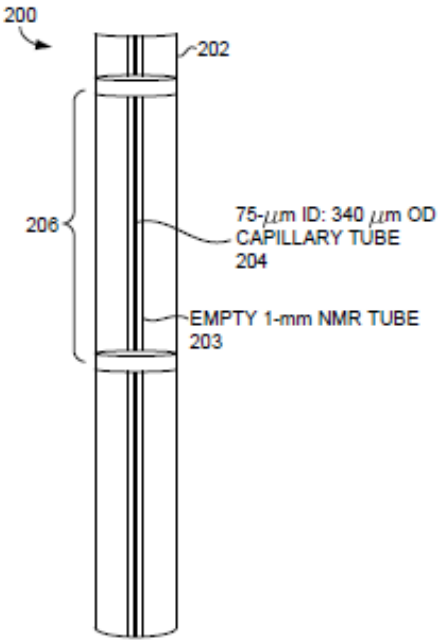

Figure 3. An in situ NMR thermometer.

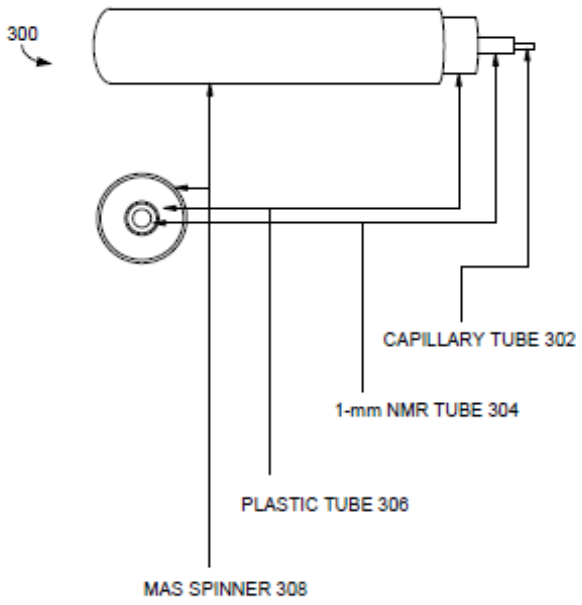

Figure 4. Another in situ NMR thermometer.

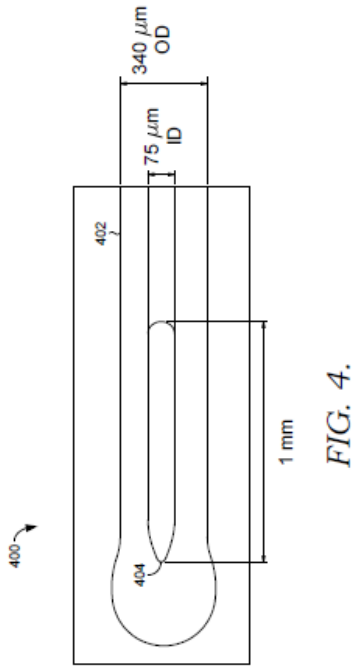

FIG. 4.

Figure 5. A schematic view of a sealed capillary tube end.

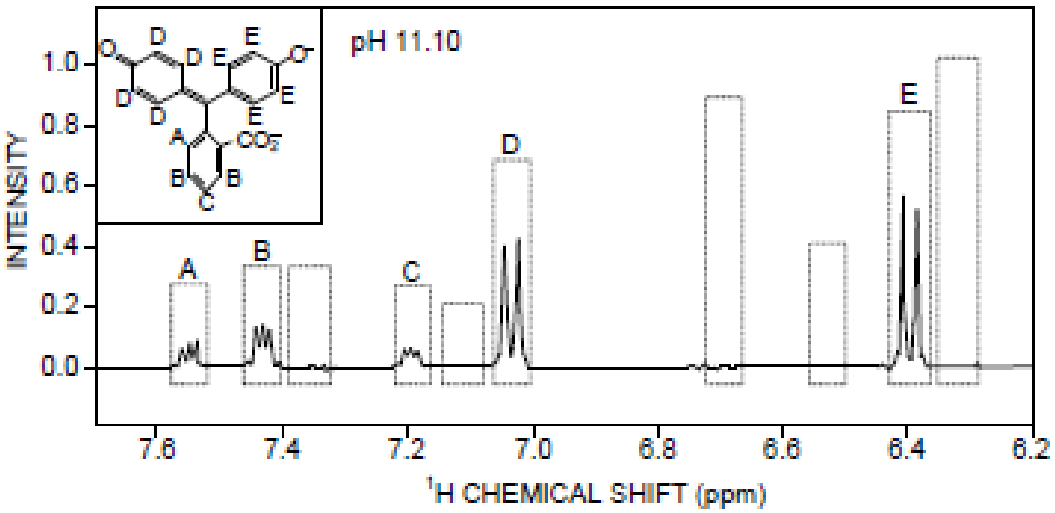

Figure 6. A graph of proton NMR peaks of phenolphthalein at a pH of 11.1 as described in Example 1.

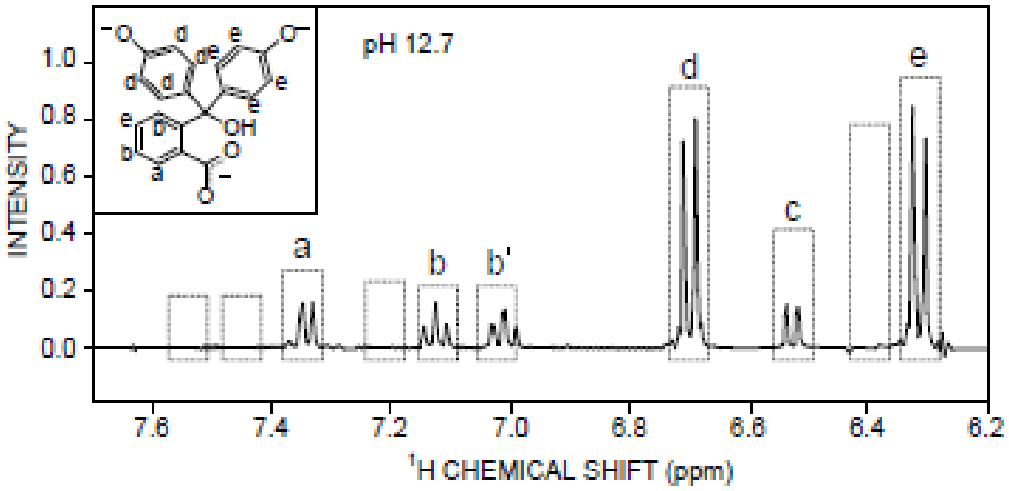

Figure 7. A graph of proton NMR peaks of phenolphthalein at a pH of 12.7 as described in Example 1.

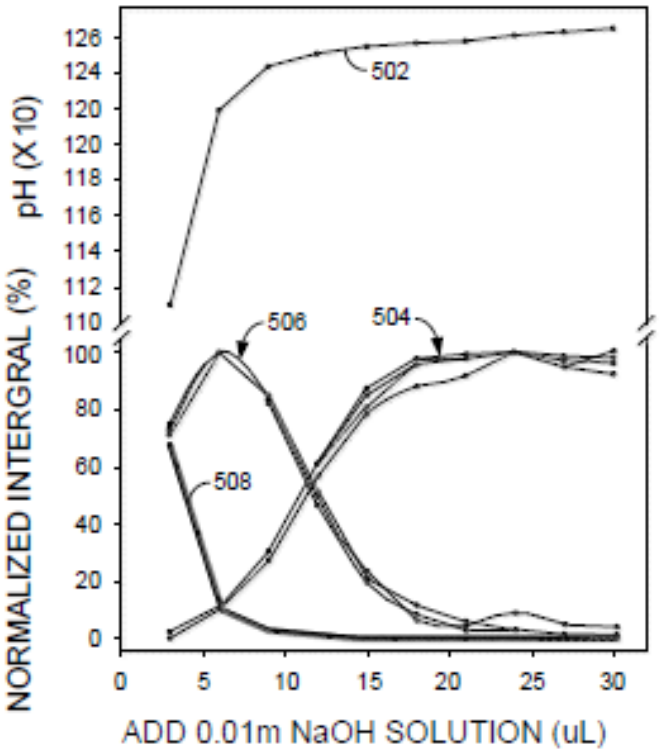

Figure 8. A graph containing multiple plots of peak integrals, ratios of peak integrals and pH values for a phenolphthalein solution as described in Example 1.

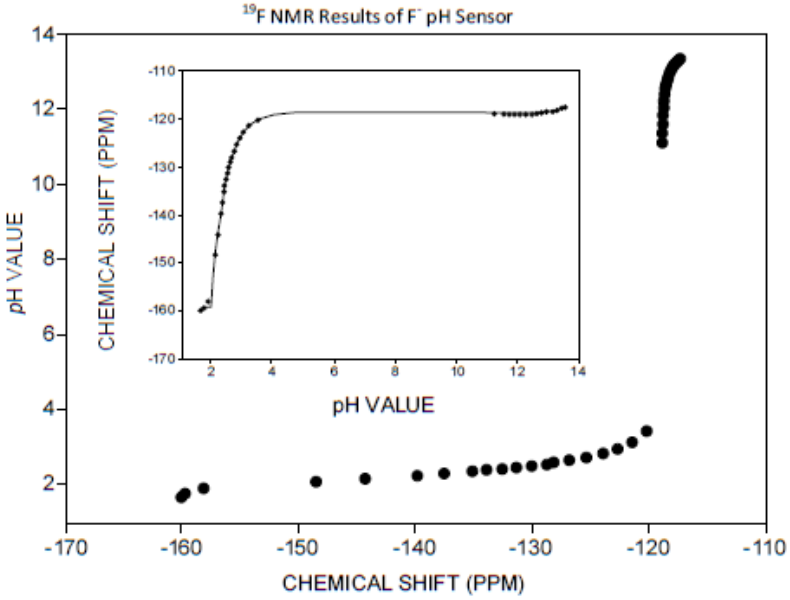

Figure 9. A plot of ^{19}F pH as a function of chemical shift of a solution of NaF in D_2O as described in Example 2.

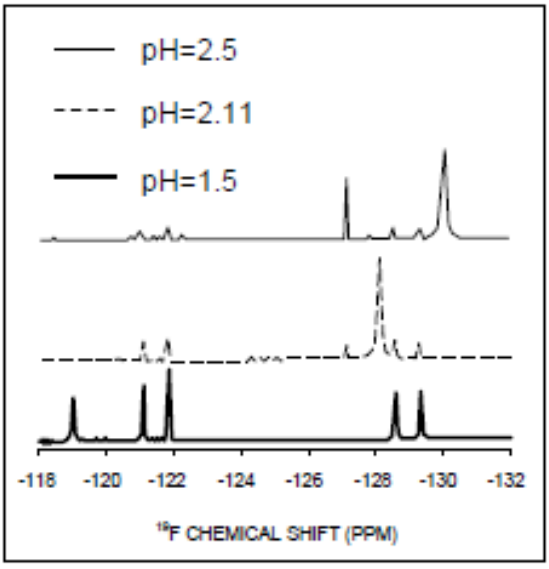

Figure 10. ^{19}F NMR spectra of pH test molecule 1, 1, 1, 2, 2-pentafluorododecan-3-ol with NMR pH sensor NaF for a range of pH as described in Example 2.

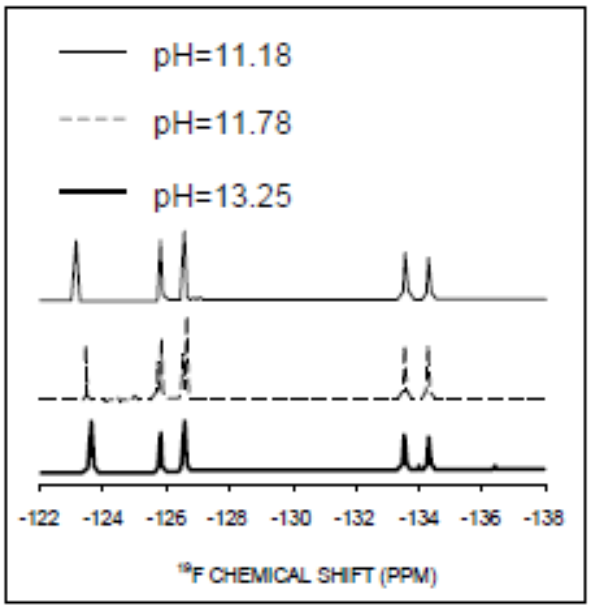

Figure 11. ^{19}F NMR spectra of pH test molecule 1, 1, 1, 2, 2-pentafluorododecan-3-ol with NMR pH sensor NaF for another range of pH as described in Example 2.

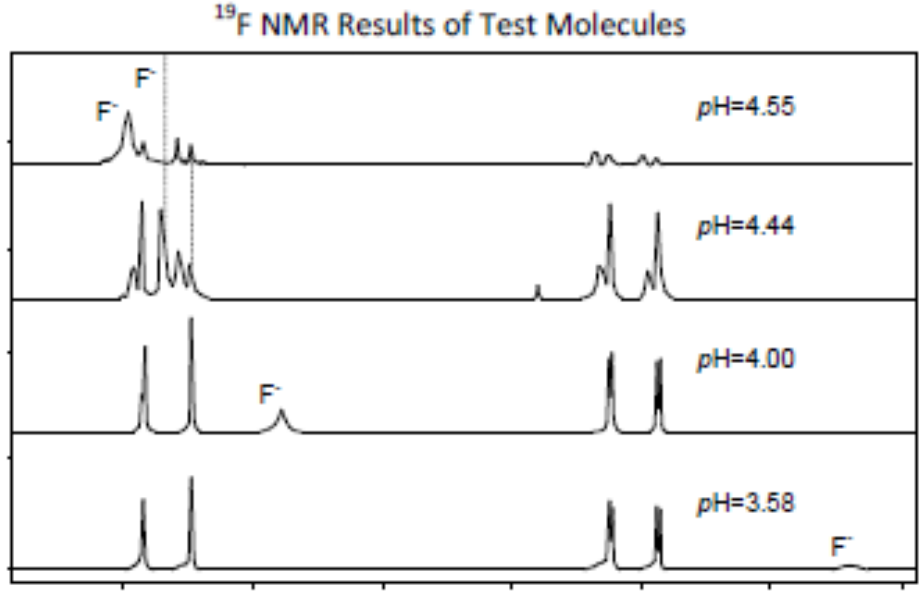

Figure 12. ^{19}F NMR spectra of pH test molecule 1, 1, 1, 2, 2-pentafluorododecan-3-ol at various pHs as described in Example 2.

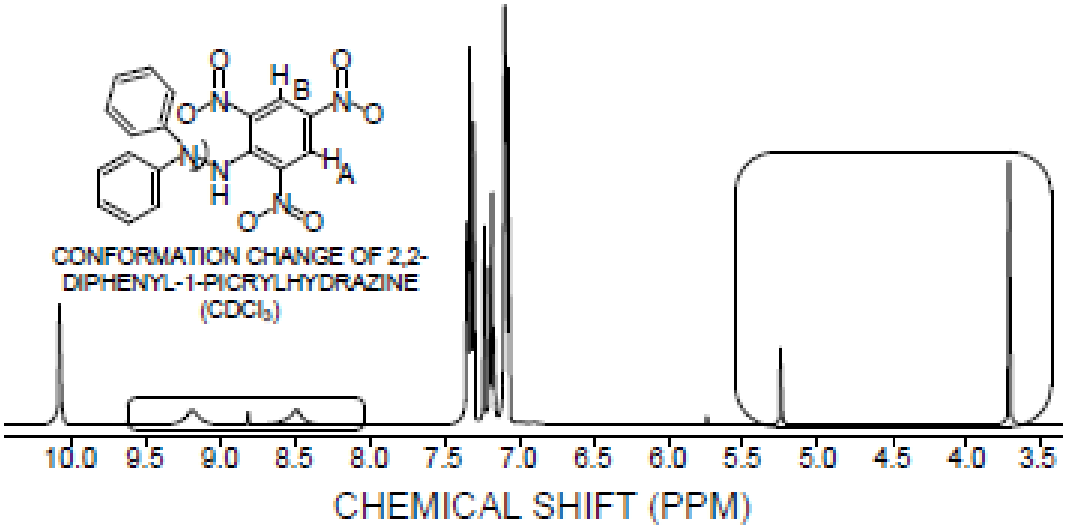

Figure 13. The NMR spectrum of 2, 2-diphenyl-1-pic-rylhydrazine at various temperatures using an in situ temperature sensor as described in Example 3.

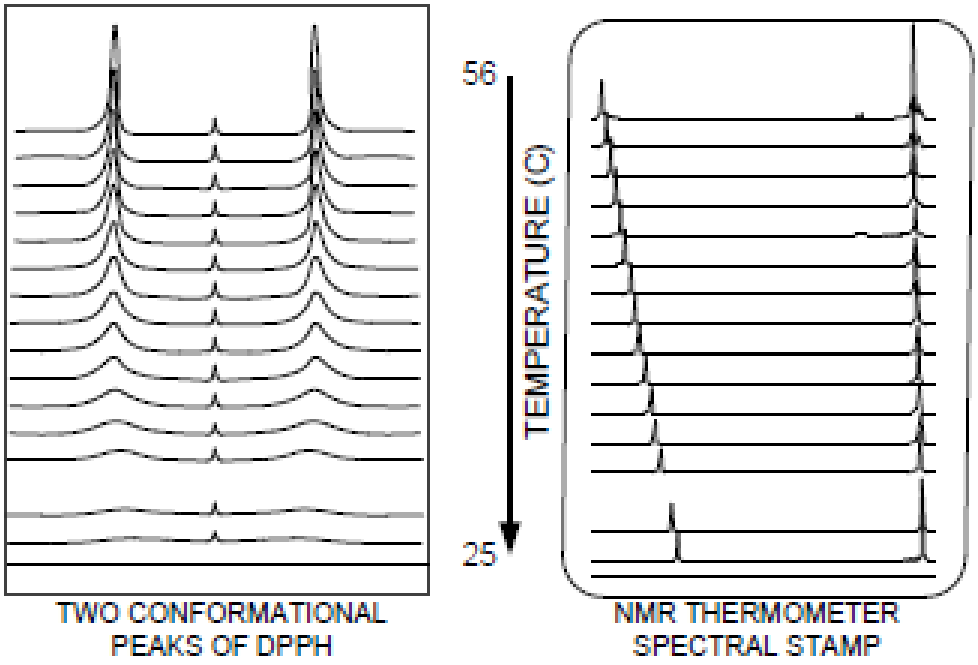

Figure 14. The NMR spectra of 2, 2-diphenyl-1-pic-rylhydrazine at various temperatures using and the NMR spectra of in situ temperature sensor as described in Example 3.

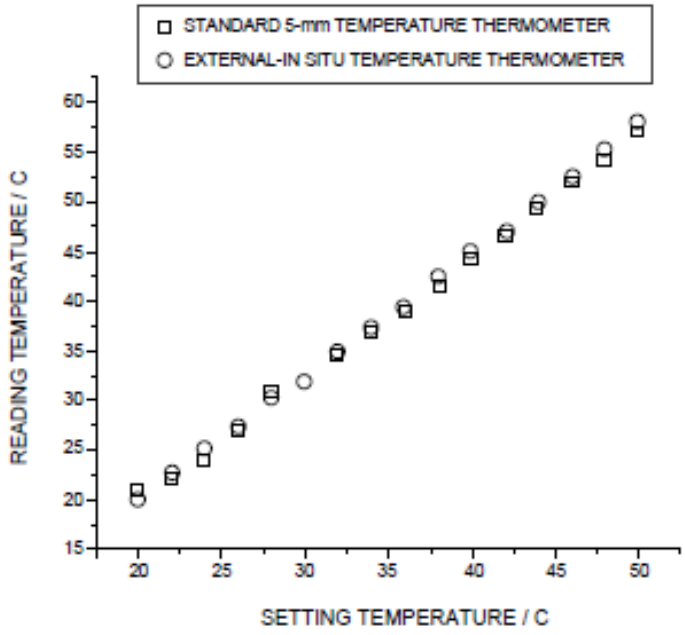

Figure 15. A plot of the setting and reading temperatures of ethylene glycol using a commercially available temperature probe and the in situ temperature sensor as described in Example 3.

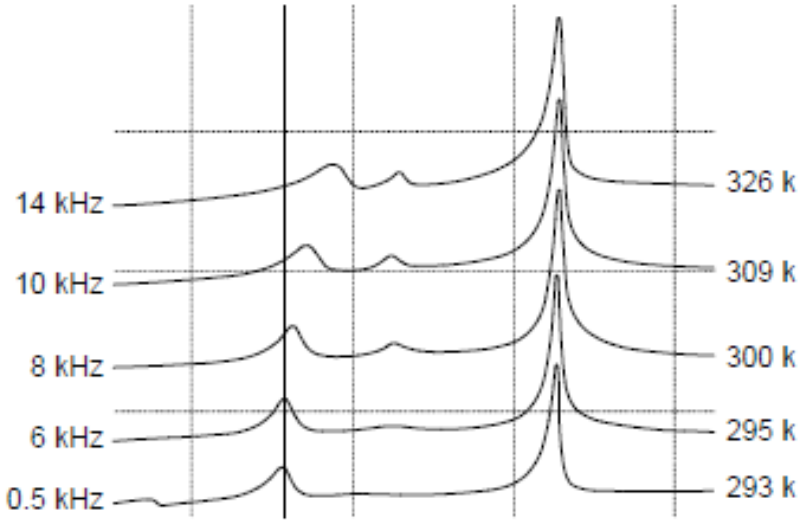

Figure 16. NMR spectra of a solid sample using an in situ temperature sensor as described in Example 4.

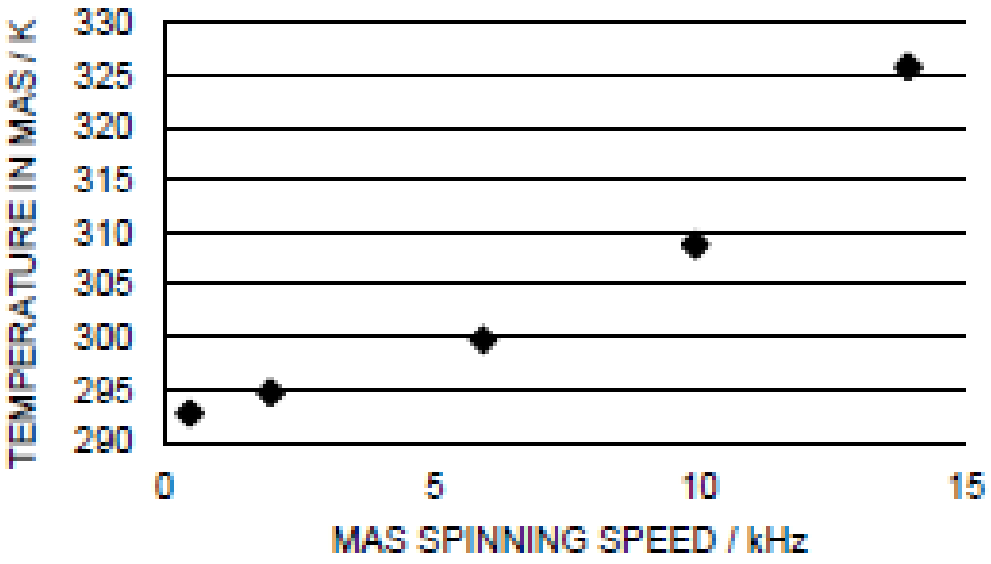

Figure 17. A plot of the spinning speed of the MAS rotor and the temperature of the reference material, as described in Example 4.

SPINNING SPEED	DISTANCE	TEMPERATURE
14 kHz	1.39 ppm	325.78 K
10 kHz	1.56 ppm	308.72 K
6 kHz	1.65 ppm	299.69 K
2 kHz	1.7 ppm	294.67 K
0.5 kHz	1.72 ppm	292.67 K

Figure 18. A numerical table is provided for the graph above, as described in Example 4.

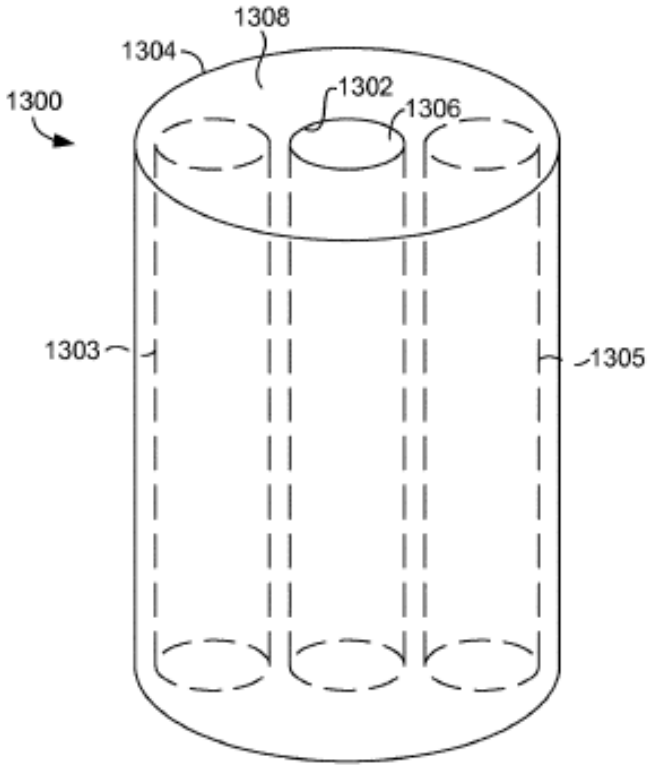

Figure 19. A schematic side perspective view of an in situ measuring device, particularly showing a capillary tube positioned inside of a solid state NMR or MAS rotor.

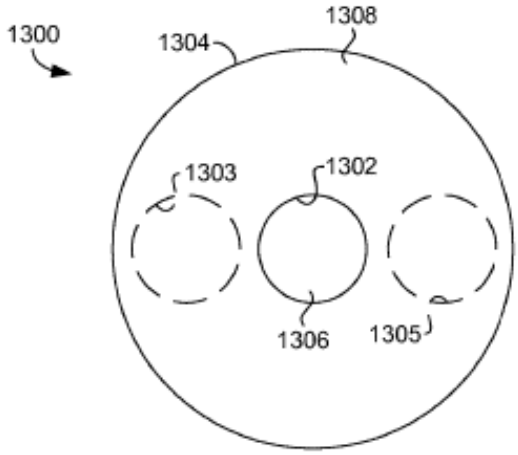

Figure 20. A top view of the in situ measuring device of Figure 19.

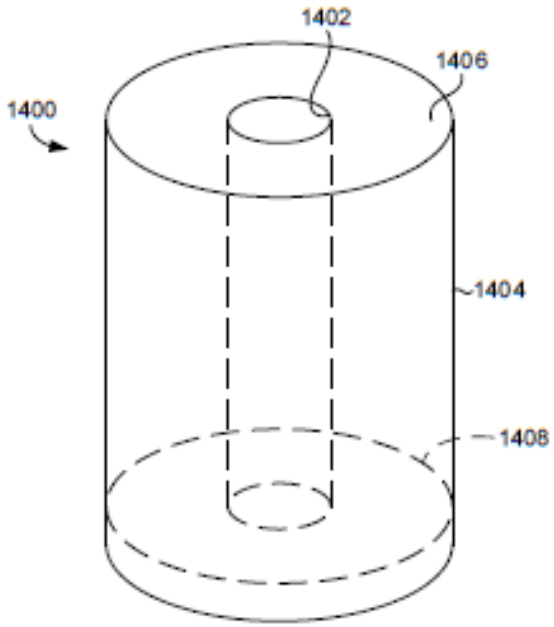

Figure 21. A schematic side perspective view of an in situ measuring device, particularly showing a capillary tube coupled to a foundation member.

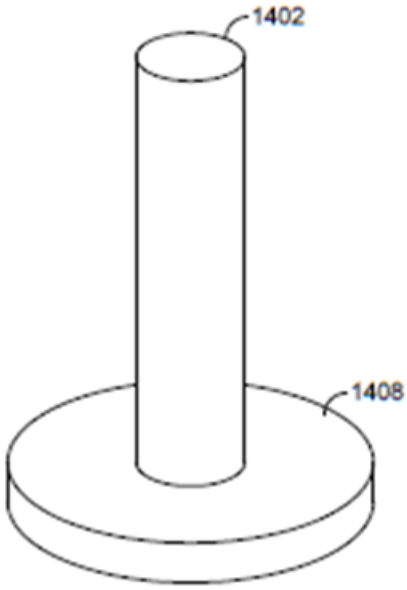

Figure 22. The capillary tube and foundation member of the in situ measuring device of Figure 21 positioned outside of the solid state NMR or MAS rotor.

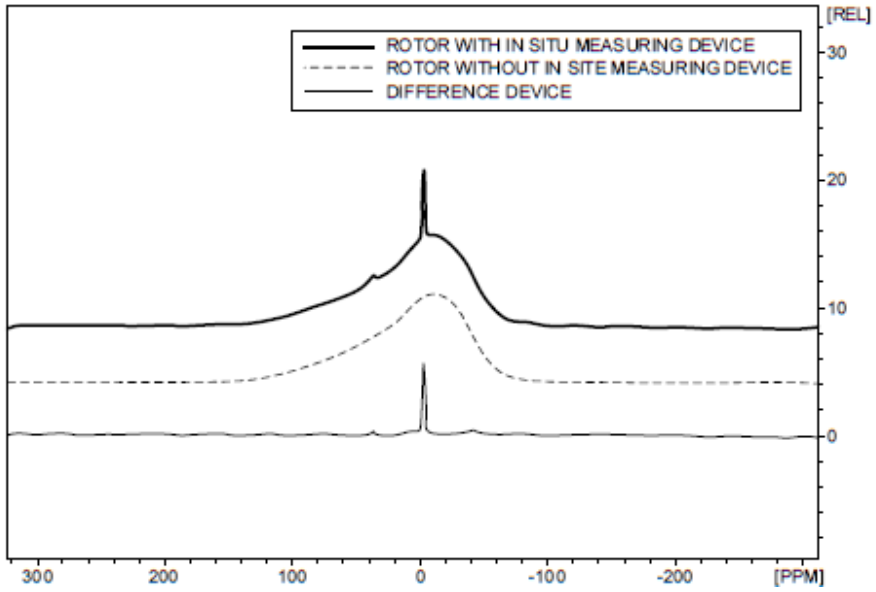

Figure 23. A plot of the ^{11}B solid state NMR spectra of the in situ measuring device described in Example 5 with and without an empty capillary tube.

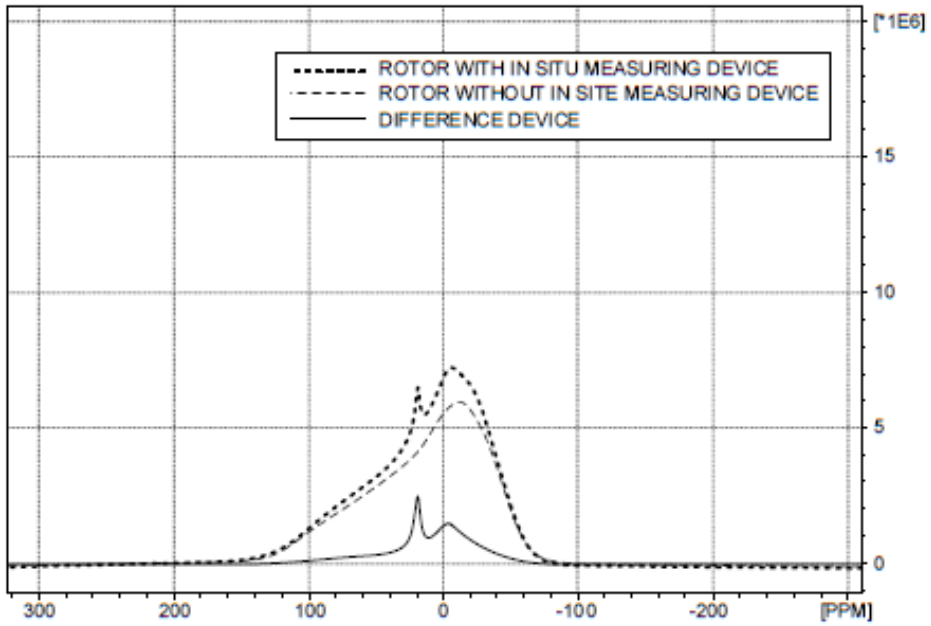

Figure 24. A plot of the ^{11}B solid state NMR spectra of the in situ measuring device described in Example 5 with and without an empty capillary tube filled with a boric acid/DMF solution, and further shows the difference spectrum.

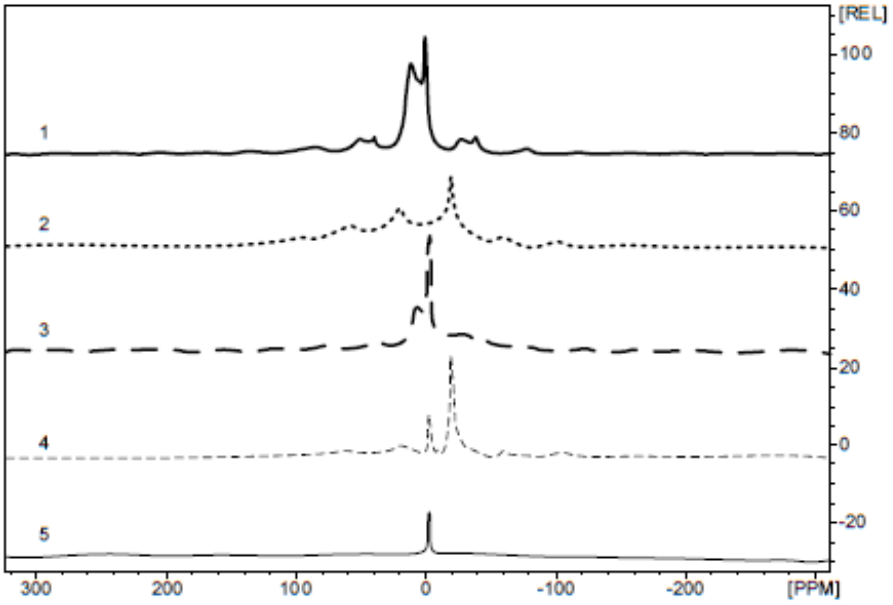

Figure 25. A plot of ^{11}B solid state NMR spectra of the in situ measuring device described in Example 5, where the 5 capillary tube and/or MAS rotor are filled with various materials or empty as described in Table 1.

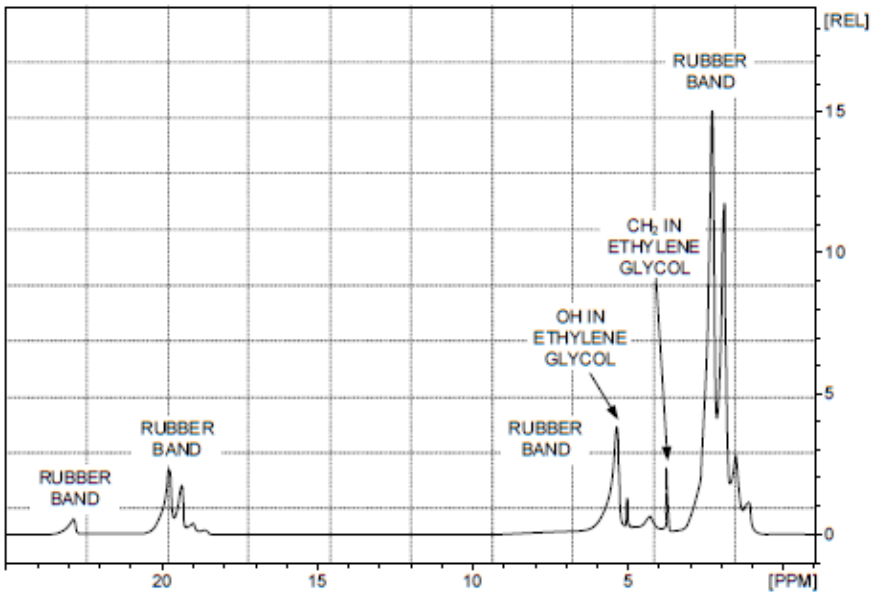

Figure 26. A plot of the proton solid state NMR spectrum the in situ measuring device described in Example 6.

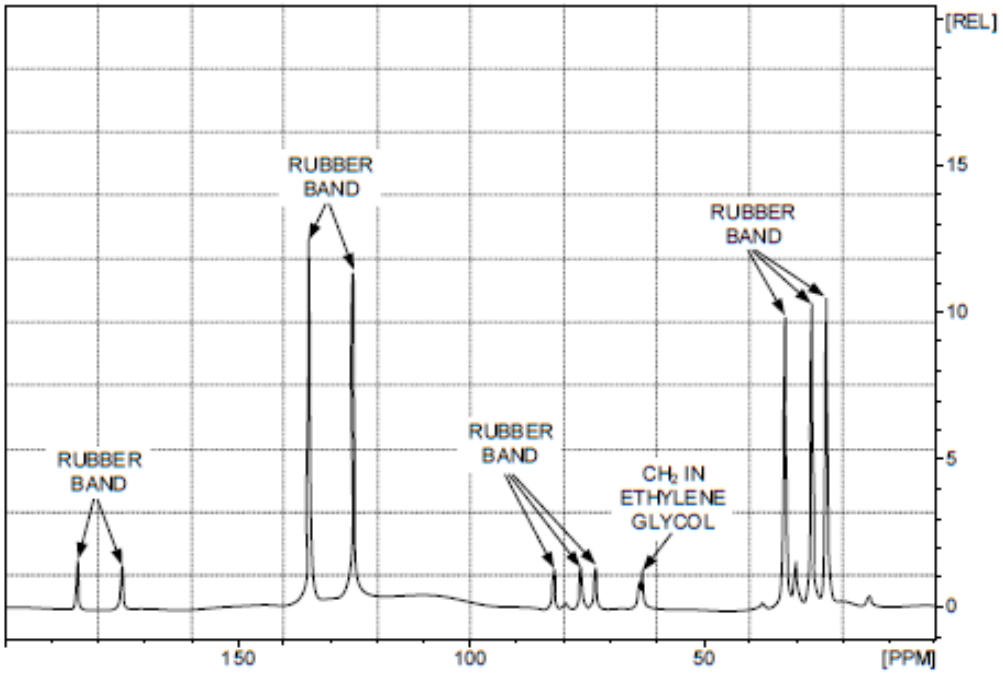

Figure 27. A plot of the carbon solid state NMR spectrum of the in situ measuring device described in Example 6 and 7.

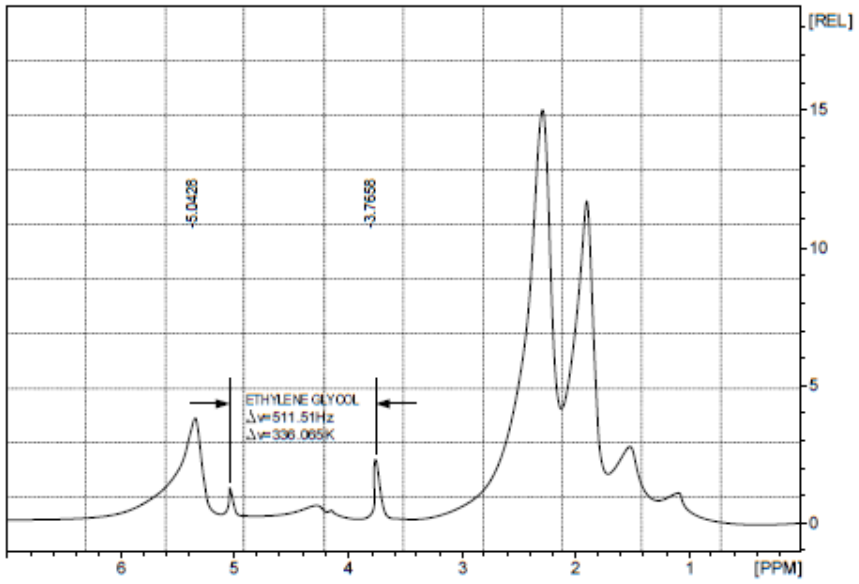

Figure 28. A plot of the proton solid state NMR spectrum of the in situ measuring device described in Example 6 and 8.

5. DETAILED DESCRIPTION

The subject matter of select embodiments of the present invention is described with specificity herein to meet statutory requirements. However, the description itself is not intended to define what we regard as our invention, which is what the claims do.

5.1 OVERVIEW

Various embodiments described herein include systems and methods for the in situ monitoring of one or more intensive properties of an NMR sample.

5.2 IN SITU PH SENSOR

In one or more embodiments, an in situ pH measuring 30 device can be utilized to measure pH of a sample, or sample environment, in a continuous fashion while observing and/or measuring the NMR spectrum of that sample. The in situ pH measuring device is capable of measuring the pH of an NMR sample in situ that is simple to implement and that encodes 35 and affixes an imprimatur of the measured value of the pH in the NMR spectrum, affording inseparability of the pH and the NMR data and incipient integrity of same.

One embodiment of an in situ pH measuring device 100 is depicted in Figure l. In embodiments, the in situ pH 40 measuring device 100 can determine pH values of a target sample from a peak or peaks of a spectral pH imprimatur (NMR peaks of a pH sensor molecule which are "embedded" within the NMR spectrum of the sample solution/ environment). For example, an example NMR spectra shown in

Figure 2 shows the spectral imprint from the pH sensor molecule (in the box) that is in the same output raw data spectra from the NMR sample.

Unlike a conventional NMR pH meter requiring a user to take out the sample to measure the pH value of a solution contained in a 5-mm NMR tube, the in situ pH measuring device described herein can monitor the pH of a solution while the sample is inside the NMR magnet. Thus, the in situ pH measuring device described herein can be employed to monitor the pH values of a sample solution during the course of a reaction.

The device 100 of Figure 1 may include a sample housing member 102 and a pH sensor containment member 104 positioned inside of the sample housing member 102. The containment member 104 can be positioned inside of the 60 sample housing member 102 using any techniques known to one skilled in the art, such as one or more annular spacers.

The sample housing member 102 may be any structure suitable for use in NMR and/or MRI that can accommodate a pH sensor containment member. In one embodiment, the sample housing member 102 may be a conventional, commercially available NMR tube, such as a 5 mm outer diameter NMR borosilicate glass tube having a length of about 17 cm. In one or more embodiments, the sample housing member 102 can have an outer diameter of at least about 1 mm, about 2 mm, or about 4 mm, and/or less than about 20 mm, about 15 mm, or about 10 mm.

In embodiments, the sample housing member 102 may define a volume such that a target sample 108 in a preselected sample solution, volume, and/or environment can be positioned in the interior 103 of the sample housing member

102. In the same or alternative embodiments, the pH sensor containment member 104 may define a volume such that a pH sensor 106 can be positioned in the interior 105 of the containment member 104.

In various embodiments, the pH sensor containment member 104 may include various structures and/or materials to provide an interface allowing for the interaction of the pH sensor 106 with hydronium cations and/or hydroxide anions present in the target sample 108 or in the sample housing member 102, but precluding physicochemical interactions between the pH sensor 106 and the target sample 108, e.g., by physically sequestering the pH sensor 106 from direct interaction with the target sample 108. In certain embodiments, the containment member 104 may be a capillary tube with porous walls, e.g., nano-porous walls, having a desired porosity, or a capillary tube with microscopic cracks or fissures. In such embodiments, the desired porosity or the microscopic size or location of the cracks/fissures of the containment member 104 can allow for the bidirectional passage of only small molecules, such as the hydronium cations and/or hydroxide anions. For example, in certain embodiments, the containment member 104 may include pores sized to allow hydronium cations and/or hydroxide anions to diffuse from the sample housing member 102 to the pH sensor containment member 104.

In one or more embodiments, the maximum opening of one or more pores, cracks, and/or fissures present on at least a portion of the containment member 104 to allow for the bidirectional passage of hydronium cations and/or hydroxide anions may be at least about 0.2 Angstroms, at least about 0.3 Angstroms, at least about 0.5 Angstroms, or at least about 1 Angstroms; and/or not more than about 5 Angstroms,

not more than about 4 Angstroms, not more than about 3 Angstroms, or not more than about 2 Angstroms. One non-limiting example of a containment member 104 may be a porous VYCOR® capillary tube.

In the one or more embodiments, the containment member 104 may include an interface material, such as any high surface area fiber or thin rod that can tether or entrap pH sensor molecules thereto. In such embodiments, a tethered pH sensor molecule may not be free to diffuse and mix with molecules in the target sample 108 because it is tethered to a fiber; however, such a tethered pH sensor molecule should be chemically inert or innocuous towards the target sample 108, as it may contact the tethered pH sensor molecules.

In certain embodiments, the pH sensor containment member 104 may include a pH sensor 106. In one or more embodiments, the pH sensor 106 exhibits one or more of the following properties: the pH sensor is larger than hydronium ions and/or hydroxide ions so that it may be trapped in a pH sensor containment member 104 while such ions could freely diffuse in and out of the containment member 104; the pH sensor changes structure with a change in pH; the pH sensor can produce NMR signals; the pH sensor can produce NMR signals from nuclei other than the nuclei that produce the NMR spectrum of the target sample; and the pH sensor incorporates nuclei (e.g., ^{2}D, ^{12}C, ^{19}F, ^{14}N) into their architecture to make them invisible in the NMR spectrum of the target sample. This last property means that the pH sensor molecule can substitute some of these "dark" nuclei for protons in the pH sensor molecule architecture so that a proton NMR spectrum of the sample under investigation will not include proton signals from the pH sensor molecule.

The pH sensor 106 may include any molecule or ion entity that exhibits a change in particular (or predetermined) concentration as a well-defined function of pH. For example, a change in molecule or ion entity concentration may produce, in direct or other proportion, an NMR and/or MRI detectable change in signal intensity and integral.

In various embodiments, the pH sensor 106 may be of any molecule or ion entity that exhibits a change in the peak volume and/or peak height, or change in chemical shift of one or more nuclear constituents of these entities as a well-defined function of pH. For example, a change in electronic structure of a molecule or ion entity may produce a single-valued NMR and/or MRI detectable change in proton signal chemical shift. In one embodiment, the pH sensor 106 may be unreactive to the target sample 108. In alternative embodiments, the pH sensor may be reactive with the target sample 108.

In one or more embodiments, various molecular and/or ion entities that exhibit an NMR spectral change due to a change in pH may be employed as the pH sensor 106. The changes induced by pH may be in terms of spectral peak volume or chemical shift, whereas the changes may be well-defined/well-calibrated and not interfering with the spectral information of the target sample. In certain embodiments, multiple pH sensors may be employed simultaneously to monitor the pH changes during the course of a reaction. A non-limiting list of commercially available pH sensors with chemical shifts inducible by pH over a certain pH range includes: thymol blue (4-[9-(4-hydroxy-2-methyl-5-propan-2-yl-phenyl)-7,7-dioxo-8-oxa-7A6-thiabicyclo [4.3.0] nona-1,3,5-trien-9-yl]-5-methyl-2-propan-2-yl-phe-

nol) (pH range of 1.2-2.8); methyl orange (Sodium 4-[(4 imethy amino) phenyldiazenyl] benzenesulfonate) (pH range of 3.1-4.4); methyl red (2-(N,N-dimethyl-4-amino- phenyl)azo benzene carboxylic acid) (pH range of 4.4-6.2); lithmus (pH range of 5-8); bromothymol blue (4,4'-(1,1- dioxido-3H-2,l -benzoxathiole-3,3-diyI)bis(2-bromo-6-iso- propyl-3-methylphenol) (pH range of 6-7.6); BCECF Acid (2',7'-Bis-(2-Carboxyethy1)-5-(and-6)-Carboxy fluorescein) (pH range 6.1-8); thymol phthalein (3,3-bis(4-hydroxy-2-methyl-5-propan-2-ylphenyl)-2-benzofuran-1-one) (pH range of 9.3-10.5); and 4-mercaptobenzoic acid (pH range of 2.5-11). It is appreciated that one skilled in the art would understand how to choose a particular pH sensor and how to prepare it (e.g., prepare a solution comprising a specific concentration of the pH sensor that is applicable to measuring the pH of a sample of interest).

In operation, in certain embodiments, a solution comprising a pH sensor 106 can be placed inside of the pH sensor containment member 104 and placed inside the sample housing member 102, e.g., by using one or more annular spacers. Further, in such embodiments, a target sample can be added to the inside of the annular volume between the outside wall of the pH sensor containment member 104 and the inside wall of the sample housing member 102. In such embodiments, standard NMR and/or MRI analyses known by those skilled in the art may be performed on this double tube assembly. In embodiments, when NMR analyses are applied, the resulting proton NMR signals that emanated from the target sample 108 and the pH sensor 106 may be recorded simultaneously and synchronously by the NMR spectrometer and can be inextricably comingled in the raw data structure, the free

induction decay (FID). In such embodiments, a fast Fourier transform (FFT) protocol may be applied to the FID to generate a proton NMR spectrum of the target sample 108 and the pH sensor 106.

In embodiments, in order to generate pH information, a calibration curve can be utilized. In such embodiments, the calibration curve can be composed of a plot of independently, electronically measured pH versus the corresponding NMR parameter (e.g., peak intensity, peak integral, peak chemical shift, spin-lattice relation, spin-spin relaxation). Further in such embodiments, a mathematically-defined curve specific to each sensor molecule is constructed. For example, for a peak intensity NMR parameter measurement, one could select a peak in the ^{1}H NMR spectrum of the pH-active molecule, measure and map the peak intensity as a function of independently, electronically-measured pH, use a mathematical function to fit the data, and use the mathematical correlation function to calculate the pH from the ^{1}H NMR peak intensity of the pH sensor molecule, and correct pH for temperature variation.

In embodiments utilizing a chemical shift NMR parameter measurement, one could select a peak in the ^{1}H NMR spectrum of the pH-active molecule, select another peak from an additionally incorporated nucleus (e.g., ^{2}D, ^{13}C, ^{19}F, ^{15}N) as a chemical shift reference observed in a second NMR probe channel, measure and map the relative peak chemical shift, which corresponds to the chemical shift difference from an NMR peak observed in the second NMR probe channel (used a chemical shift reference), as a function of independently, electronically-measured pH, use a mathematical function to fit the data, and use the mathematical correlation

function to calculate the pH from the ^{1}H NMR peak chemical shift of the pH sensor molecule, and correct pH for temperature variation.

In embodiments utilizing a spin-spin NMR parameter measurement, one could select a peak in the ^{1}H NMR spectrum of the pH-active molecule, measure and map spin-spin relaxation rate or time constant as a function of independently, electronically-measured pH, use a mathematical function to fit the data, and use the mathematical correlation function to calculate the pH from the ^{1}H NMR peak spin-spin relaxation time constant of the pH sensor molecule, and correct pH for temperature variation.

5.3 IN SITU NMR THERMOMETER

As discussed above, various embodiments herein describe an in situ thermometer that can be used in an NMR and/or MRI machine. In one or more embodiments, a device for monitoring actual temperature of a sample in situ with a temperature imprimatur encoded onto the NMR spectrum for accurately determining the thermal properties of the target sample is described. The in situ NMR thermometer described herein is capable of measuring the temperature of an NMR sample in situ and is simple to implement and that encodes and affixes an imprimatur of the measured value of the temperature in the NMR spectrum, affording inseparability of the temperature and the NMR data and incipient integrity of same.

In certain embodiments, the temperature measuring device may include one or more capillary tubes containing a reference material, where such tube(s) is/are centrally or spatially arranged in a sample tube (for solution sample) or a rotor (for solid sample). Any or all tubes may be sealed or unsealed. In embodiments, as

discussed below, one or more of the capillary tubes may be sealed using a flame or plasma arc.

Figure 2 shows one embodiment of a temperature measuring device 200. The temperature measuring device 200 can include an NMR tube 202, e.g., a 5 mm coaxial sample 400 MHz J-Young tube, with a smaller inner NMR tube 203, e.g., a conventional 1 mm NMR tube positioned inside of the NMR tube 202. In the temperature measuring device 200 of Figure 3, a capillary tube 204 can be positioned inside the smaller inner NMR tube 203. The smaller inner NMR tube 203 and/or the capillary tube 204 can be secured inside the NMR tube 202, e.g., by annular spacers 206. In embodiments, the smaller inner NMR tube 203 is empty besides the inserted capillary tube 204, while the NMR tube 202 contains the target sample.

The capillary tube 204 may be configure d to house a reference material for the measurement of temperature. In embodiments, the capillary tube 204 can be about 152 mm in length. In one embodiment, the capillary tube 204 can have an outer diameter of at least about 100 micrometers, about 200 micrometers, or about 300 micrometers, and/or an outer diameter of less than about 600 micrometers, 500 micrometers, or 400 micrometers. In certain embodiments, the capillary tube 204 can have an outer diameter of about 340 micrometers. In the same or alternative embodiments, the capillary tube 704 can have an internal diameter of at least about 2 micrometers, about 5 micrometers, about 10 micrometers, about 20 micrometers, about 30 micrometers, 40 micrometers, or 50 micrometers, and/or an internal diameter of less than about 150 micrometers, 125 micrometers, or 100 micrometers.

In one embodiment, the capillary tube 204 can have an internal diameter of about 75 micrometers.

In various embodiments, the capillary tube 204 can have an outer diameter that is at least about 2 times larger than the internal diameter, 3 times larger, or 4 times larger. In the same or alternative embodiments, the capillary tube can have an internal diameter that is less than about 75%, about 50%, about 30%, about 20% about 10%, about 5%, or 1% of the outer diameter of the capillary tube 704.

In various embodiments, the smaller inner NMR tube 202 can be about 203 mm in length and about a 0.8 mm internal diameter. In the same or alternative embodiments, the NMR tube 202 can be about 178 mm in length and about a 4.2 mm internal diameter.

As discussed below, in certain embodiments, it may be beneficial to seal the capillary tube 204 so that the reference sample is sealed off from the target sample in the NMR tube 202.

As seen in Figure 4, the temperature sensor device 200 only includes one reference capillary tube 204, and in this embodiment the capillary tube 204 is centrally located. In alternative embodiments, when more than one reference capillary tube is utilized in the NMR tube 202, these capillary tubes may be spatially arranged, e.g., to measure temperature gradients that may span the target sample.

In various embodiments, a temperature sensor device for use with a solid sample is disclosed. In such embodiments, an external in situ capillary NMR thermometer device for a solid sample can include a capillary tube of a desired length with the reference material (such as ethylene glycol) centrally embedded in the

sample contained in a Magic Angle Spinning (MAS) rotor. Such a device is described further below with respect to Figure 4.

In one or more embodiments, an external in situ capillary NMR thermometer device for a solid sample may include multiple capillary tubes of a desired length with the reference material. In such embodiments, the capillary tubes may be spatially disposed within the sample to monitor spatial variations in sample characteristics, such as the variation of temperature at various locations in the sample contained in the cylindrical rotor.

Figure 4 depicts a temperature sensor device 300 for in situ temperature measuring for solid state NMR. As shown in Figure 4, the temperature sensor device 300 can include a sealed capillary tube 302 with a reference material centrally placed in the sample (e.g., a pressed powder) contained in the Magic Angle Spinning (MAS) rotor 308. In the embodiment depicted in Figure 5, the capillary tube 302 is positioned inside of a 1 mm NMR tube 304, which is positioned inside of a plastic tube 306, which is positioned inside of the MAS rotor. In embodiments, the 1 mm NMR tube 304 and the plastic tube 306 are used to center with capillary tube 302 when no target sample or a minimal amount of target sample used in the MAS rotor.

In embodiments, that do not require a NMR tube 304 and/or a plastic tube 306, the temperature sensor device 300 may only include the MAS rotor 308 and the capillary tube 302. In such embodiments, a powder sample can be packed inside the MAS rotor 308. Further, in such embodiments, a drill bit or similar device can be used to bore a hole in the sample centrally located in the MAS rotor 308. In addition, in such embodiments, a sealed capillary tube 302 with reference material is placed in

the bored hole. Further, a packing tool having an end in the shape of a tube is used to pack the powder sample tightly around the sealed capillary tube 302.

As discussed above, a capillary tube, e.g., the capillary tube 204 and/or the capillary tube 302, can include a reference material for the in situ measurement of pH. The reference material may be one or more of compounds that have one or more NMR peaks that change chemical shift as a function of temperature. The reference material may be a liquid, solid, and/or gaseous material. In one or more embodiments, a liquid reference material may include one or more of ethylene glycol, methanol, and ethanol, NaF in D_2O, alcohols, glycols, and polyethylene glycols. In various embodiments, solid reference materials may include one or more of lead nitrate, cobalt complexes, etc. In certain embodiments, gaseous reference materials include one or more of methane, xenon, mixture of xenon with oxygen, CF_4, CFi, F_2, CF_3CF_3, CF_2 CF_2.

The capillary tubes 204 and 302, may in various embodiments, may include one or more materials that include glass, quartz, Peek, Torian, Teflon, Arum, and other polymers and ceramic materials. In one embodiment, the capillary tubes 204 and 302 do not include a metal material.

5.4 SYSTEMS AND METHODS FOR SEALING A CAPILLARY TUBE FOR AN IN SITU NMR THERMOMETER

As discussed above, one or more capillary tubes that house the temperature reference material for an in situ NMR thermometer may be sealed. It is appreciated that the sealing system and methods described herein can be useful for use with other devices, in addition to an in situ NMR thermometer, such as devices requiring sealed capillaries to survive at high temperatures and pressures in harsh environments.

In embodiments, a glass seal may be used at one or both ends of the capillary tube. In certain embodiments, an Optical Fiber Arc Fusion Splicer may be used to generate such a glass seal.

In certain embodiments, a method generally comprises the steps of i) selecting a suitable capillary tube with desired length, internal diameter, and outer diameter for a particular application, ii) filling said capillary tube with preselected (solid, liquid, or gas) reference material, iii) sealing a first end of such capillary tube, and iv) sealing a second end of capillary tube, whereas sealing a capillary tube may involve means of glue, epoxy, plugs, etc., or an electric arc fusion approach utilizing an Optical Fiber Arc Fusion Splicer.

According to an exemplary embodiment of the invention, the inventive method for sealing a capillary tube comprises the following steps:

(1) Use epoxy to seal a needle to one end of a desired capillary tube.

(2) Use a knife to scrape off the coating (about 1 cm long) from the other end of the capillary tube.

(3) Fill a sample solution into a syringe.

(4) Connect the needle to the syringe.

(5) Push the solution though the capillary tube until 2 to 3 drops comes out of the open end of the capillary tube.

(6) Wipe the end of the capillary tube.

(7) Place the open end of the capillary tube in the arc fusion splicer.

(8) Check the fusion splicer display screen to find the gas/liquid interface.

(9) Push the syringe plunger in order to keep the gas liquid 20 interface about 0.1 mm from the open end of capillary tube.

(10) Apply the arc with a constant 0.05N force on the syringe plunger.

(11) Take the sealed capillary tube out of the arc fusion splicer.

(12) Choose the length of the capillary tube that you want, and cut off the portion that is affixed to the syringe needle.

(13) Use a knife to scrape off the coating (about 1 cm long) from the open end of the capillary tube.

(14) Place the open end of the capillary tube in the arc fusion splicer, and then apply the arc.

(15) Use a microscope to check the sealed ends of the capillary tube.

A schematic representation 400 of a sealed capillary tube 402 is depicted in Figure 6. The capillary tube 402 includes an end 404 that is sealed by glass or other material from the capillary tube 402. In embodiments, when using an Optical Fiber Arc Fusion Splicer to seal the end 404 of a capillary tube 402, it may be beneficial to maintain a liquid, gas, or solid reference material inside the capillary tube 402 at least about 1 mm away from the end 404 of the tube so as to not inadvertently heat up the reference material. In various embodiments, the reference material should be kept at least about 0.75 mm away from the end 404 being sealed by the Optical Fiber Arc Fusion Splicer, or at least about 0.5 mm, or at least about 0.3 mm, or at least about 0.1 mm.

5.5 SOLID STATE IN SITU MEASURING DEVICES AND METHODS FOR QUANTIFYING ONE OR MORE PROPERTIES OF A TARGET SAMPLE

As discussed above, embodiments disclosed herein concern in situ measuring devices, methods of making in situ measuring devices, and methods for utilizing in situ measuring devices in solid-state NMR for the quantitative determination of various properties of a target sample, such as chemical shifts, amounts of a target sample material, and temperature of the target sample material in an unknown sample. Generally, in embodiments, an in situ measuring device can include one or more reference materials sealed in a capillary tube, where the sealed capillary tube is positioned inside of a solid state NMR MAS rotor amongst a target sample. In such embodiments, the in situ measuring devices described herein can provide a single device having a reference material for calibration or other reference measurements that is physically separated from a target sample, where both the reference material and the target sample are in one single solid state NMR MAS rotor.

The in situ measuring devices described herein, e.g., the in situ measuring devices described below with reference to Figure 19, 20, and 21, can allow for the simultaneous, or near simultaneous recording of NMR spectra of the reference sample and of the target sample material in the MAS or solid state NMR rotor. This simultaneous or near simultaneous recording of NMR spectra of both a calibration or reference material and a target sample creates a single set of NMR spectra imprinted with calibration or reference devices disclosed herein, such as the in situ measuring devices 1300 and 1400 discussed below, one can obtain NMR spectra of the target

sample material that also includes the NMR spectra of the reference or calibration material.

Such target sample data imprinted with the calibration or reference data is critical in solid state NMR measurements, as the NMR spectra of the calibration or reference material is collected in the same or substantially the same environment and at the same time as the NMR spectra of the target sample material.

This simultaneous or near simultaneous recording of the NMR spectra of the reference material and the target sample material avoids problems associated with the conventional solid state NMR calibration techniques, which can include the sequential measurement of a reference material and a target sample. For example, in such a conventional technique, when one is calibrating the NMR spectra of a target sample using a reference material NMR spectra, one relies on the assumption that the environment of the reference sample in the NMR instrument when the reference NMR spectra are obtained is identical to the environment of the target sample when the target sample NMR spectra are obtained, even though such NMR spectra were obtained at different times. The in situ measuring devices described herein allow one to eliminate such an assumption since the reference sample and target sample are both in the NMR probe at the same time, thereby allowing for simultaneous or near simultaneous recording of NMR spectra.

In certain embodiments, such an in situ measuring device can include a sealed capillary tube having a reference material inside the capillary tube, and the sealed capillary tube can be positioned inside of a MAS or solid state NMR rotor. In such embodiments, the sealed capillary tube may be positioned within a target sample of

interest that is inside the MAS rotor. In one or more embodiments, an in situ measuring device can include a plurality of sealed capillary tubes, each potentially containing a reference material therein, where the plurality of sealed glass capillary tubes are centrally or spatially arranged in a rotor, e.g., among a solid sample.

An embodiment of the in situ measuring device 1300 is depicted in Figure 19 and 20. The in situ measuring device 1300 can include a capillary tube 1302 positioned inside of a MAS rotor 1304. In certain embodiments, a plurality of capillary tubes can be present in the in situ measuring device. For example, as depicted in Figure 19 and 20, in addition to the capillary tube 1302, capillary tubes 1303 and 1305 (shown in phantom lines) may also be present. While only three capillary tubes are depicted, it is appreciated that any number of capillary tubes may be utilized in the in situ measuring devices described herein. It is also appreciated that one skilled in the art would under- stand that when using a plurality of capillary tubes in the in situ measuring device 1300, it can beneficial to arrange them such that the in situ measuring device is balanced for spinning in an NMR instrument. While, herein, properties and processes are described primarily with respect to the capillary tube 1302, it should be understood that such descriptions equally apply to all or any of the plurality of capillary tubes in the in situ measuring device 1300 (or in the in situ measuring device 1400 discussed below with reference to Figure 21).

In embodiments, the sealed capillary tube 1302 can be made from any type of common material used to make capillary tubes, as long as such material is capable of withstanding the forces in the spinning MAS rotor in an MAS-NMR experiment. In one or more embodiments, the sealed capillary tube 1302 can be made from

borosilicate glass and/or one or more polymeric materials, such as polyether ether ketone.

In certain embodiments, the sealed capillary tube 1302 can be any size that is capable of fitting inside of a conventional, commercially available MAS rotor. In certain embodiments, the sealed capillary tube 1302 can have a length of at least about 10 millimeters, or at least about 20 millimeters, or at least about 40 millimeters, or at least about 50 millimeters. In embodiments, the sealed capillary tube 20 1302 can have an internal diameter of at least about 50 micrometers, or at least about 100 micrometers, or at least about 200 micrometers, or at least about 500 micrometers, or at least about 1 millimeter, or at least about 1.2 millimeters; and/or an outer diameter of at least about 250 micrometers, or at least about 350 micrometers, or at least about 500 micrometers, or at least about 700 micrometers, or at least about 1 millimeter, or at least about 1.4 millimeters. In certain embodiments, the sealed capillary tube 1302 can have an internal diameter of about 150 micrometers, an outer diameter of about 365 micrometers, and a length of about 13 millimeter. In alternative embodiments, the sealed capillary tube 1302 can have an internal diameter of about 1.2 millimeters, an outer diameter of about 1.4 millimeters, and a length of about 13 millimeters.

As discussed above, the sealed capillary tube 1302 can include a reference material positioned in the interior 1306 of the sealed capillary tube 1302. The reference material can be any material that could be used for calibration and/or as a reference for NMR measurements, such as solid state NMR measurements. In various embodiments, the reference material can be one or more compounds that

have one or more sharp NMR peaks spaced apart from the NMR peaks of the target sample. In one or more embodiments, the reference material can include ethylene glycol, ethanol, methanol, water, and/or mixtures thereof.

In one or more embodiments, the reference material can be inserted into the interior 1306 of the capillary tube 1302 using any technique known to one skilled in the art. An exemplary method for adding a reference material into the interior 1306 of the capillary tube 1302 can include the use of a syringe needle. For example, in such embodiments, the capillary tube 1302 can be inserted into the needle of a syringe and sealed at the end of the needle with epoxy glue or any other appropriate glue that creates an air-tight seal and bond between the capillary tube 1302 and the needle. Further, in such embodiments, the syringe (filled with the reference material) can be used to fill the capillary tube 1302 by the application of moderate pressure put on the syringe's piston until the capillary tube 1302 is completely filled with the reference material. In addition, in such embodiments, it may be beneficial to visually inspect, e.g., under a lens or optical microscope, to ensure that no air bubbles remain inside the capillary tube 1302.

In embodiments, the capillary tube 1302 can be sealed using any technique that is capable of sealing one or more ends of the capillary tube 1302. In one or more embodiments it may be advantageous to seal the capillary tube 1302 in such a manner that the capillary tube 1302, or the in situ measuring device 1300, would not be unbalanced for spinning in an MAS or solid state NMR experiment. In certain embodiments, the capillary tube 1302 can be sealed with Teflon® tape. In alternative embodiments, the capillary tube 1302 may be sealed by the arc of a glass fiber fusion

splicer (OPTICAL FIBER FUSION SPLICER, MODEL: TYPE-36) or any other appropriate fusion splicer. In embodiments, where the capillary tube 1302 was filled with the reference material via a syringe such that one end of the capillary tube 1302 is bonded to the syringe, as discussed above, the open end of the capillary tube 1302 opposite to the end bonded to the syringe needle may be the end that is sealed first. Then, the end of the capillary tube 1302 bonded to the syringe needle is cut to a desired length and also sealed by a desired method.

In one or more embodiments, the capillary tube 1302 can be flame sealed, e.g., via the use of a commercially available micro torch. In certain embodiments, an exemplary method for sealing the capillary tube 1302 using a micro torch can include concentrically mounting the capillary tube 1302 at the top of the spindle of a commercially available stepper motor. In such embodiments, a thin metal plate can be prepared with a hole 1.6 millimeter in diameter and can be positioned above a conventional stepper motor such that the capillary reference tube passes through the hole in the metal plate and extends above the metal plate by about 1 millimeter.

Further, in such embodiments, the stepper motor can be activated, thereby causing the capillary tube 1302 to rotate at about 2-4 revolutions per second. The flame of the micro torch, burning a combustible gas, may be aimed at the 1 millimeter portion of the glass reference capillary tube that extends above the metal plate. In embodiments, the metal plate is utilized to prevent the flame and heat from impacting the portion of the reference capillary tube 1302 that may contain the reference material, which may be positioned below the metal plate. Further, a stream of very cold nitrogen gas may be directed at the portion of the glass capillary

reference sample tube that is located below the metal plate for the purpose of cooling the capillary tube 1302 and the reference material. The purpose of cooling the reference material is to prevent evaporation and degradation caused by the high temperatures from this glass-sealing process. Cooling of the capillary tube 1302 can be performed to maintain the reference material at temperatures above, below or at room temperature. It may be advantageous to maintain the temperature of the reference material below its freezing point so that very little volatility occurs. The cooling gas may be applied to the bottom of the capillary tube 1302 after the flame is applied to the top of the tube. Using this sequence, the mixture of reference sample vapor and air (located above the liquid reference sample), which may contain water vapor and other gaseous impurities, may be caused to be excluded from the capillary tube 1302 before it was completely sealed. Continuous or intermittent applications of the flame to the top, open portion of the capillary tube 1302, which may be rotated by the stepper motor, can cause the glass or other material of which the capillary tube 1302 is comprised to melt and close the tube opening. At the moment that the seal is fully formed, the flame may be turned away from the capillary tube 1302. The soft molten glass or other material may form a small expanded bubble that may be approximately 2 millimeters in diameter, which may be treated by cooling to room temperature and applying intermittent heat for purposes of annealing such a glass bubble.

As discussed above, the in situ measuring device 1300 can include one or more sealed capillary tubes, e.g., the sealed 5 capillary tube 1302, positioned in the interior 1308 of the MAS or solid state rotor 1304. One exemplary method for

positioning the sealed capillary tube 1302 in the interior 1308 of the MAS rotor can include first completely filling an open end of the MAS rotor 1304 with a solid substance under investigation (e.g., the target sample of interest), which could be crystalline, amorphous, gel-like or in any state that stays in its shape even after machining with tools. In such embodiments, the target sample can be compacted to a desired density, as done by one skilled in the art in conventional solid-state NMR experimentation. Further, in such embodiments, a small hole of a diameter that matches the outside diameter of the capillary tube 1302 can be drilled precisely along the long axis of the MAS rotor 1304 into the compacted target sample material, which can allow the sealed capillary tube 1302 to be inserted snuggly into this drilled hole. In alternative embodiments, this hole may be fabricated by other hole-generating tools and/or the hole could have been aligned parallel to the long axis of the MAS rotor but off its center. In addition, in such embodiments, the sealed capillary tube 1302 can be inserted into the prepared hole in the target sample material, and the target sample material around the capillary tube 1302 may be compacted again, as needed. Finally, in such embodiments, the MAS rotor 1304 can be closed and/or sealed with the standard rotor lid and prepared to be inserted into the solid-state NMR probe for the recording of NMR spectra.

Figure 21 depicts another embodiment of an in situ measuring device 1400. The in situ measuring device 1400 includes a sealed capillary tube 1402 positioned in the interior 1406 of MAS rotor 1404. The in situ measuring device 1400 depicted in Figure 21 also includes a foundation 1408 fused to the sealed capillary tube 1402. In embodiments, the foundation 1408 fused to the sealed capillary tube 1402 allows for

an alternative method for forming an in situ measuring device, e.g., the in situ measuring device 1400, compared to method of forming the in situ measuring device 1300 discussed above with reference to Figure 19. That is, by having a foundation 1408 fused to the sealed capillary tube 1402 (as depicted in Figure 22, the capillary tube may be 45 inserted into the MAS rotor 1404 prior to adding the target sample material, as opposed to adding the capillary tube 1302 to target sample-filled MAS rotor 1300, as discussed above with reference to Figure 19.

In embodiments, one method for forming the in situ measuring device 1400 of Figure 21 can include fusing, flame-sealing, or gluing the capillary tube 1402 to the foundation 1408 such that the sealed capillary tube 1402 is perpendicular to the foundation 1408, as depicted in Figure 22. The sealed glass capillary tube can be placed in the center or at any other location on the foundation 1408. The foundation 1408 can be comprised of a glass, ceramic, and/or a polymeric material. The sealed capillary tube 1402 can be coupled to the outer surface of the foundation 1408 or can be coupled to a hole in the foundation 1408 designed to fit the sealed capillary tube 1402. In embodiments, once the sealed capillary tube 1402 is coupled to the foundation 1408, it can be inserted into an NMR solid-state sample rotor, e.g., the MAS rotor 1404. In such embodiments, after the capillary tube 1402-foundation 1408 assembly is placed in the MAS rotor 1404, the MAS rotor 1404 can be filled with the target sample of interest, and can be compacted, as needed. Further, a MAS rotor lid can then be placed on the top of the MAS rotor 1404 to seal the MAS rotor 1404, and the in situ measuring device 1400 can be inserted into the NMR probe for recording NMR spectra.

The in situ measuring devices 1300 and 1400 discussed above with reference to Figure 19, 20, and 21, allow for the simultaneous recording of NMR spectra of the reference sample inside the capillary tube, e.g., the capillary tubes 1302 and/or 1402, and of the target sample material in the MAS rotor. This simultaneous recording of NMR spectra of a calibration or reference material and a target sample creates a single set of NMR spectra imprinted with calibration or reference material data. That is, utilizing the in situ measuring devices disclosed herein, such as the in situ measuring devices 1300 and 1400 discussed above with reference to Figure 19, 20, and 21, can provide NMR spectra of the target sample material that also includes the NMR spectra of the reference or calibration material. Such imprinted calibration or reference data is critical in solid state NMR measurements, as the NMR spectra of the calibration or reference material is collected in the same exact environment and same time as the NMR spectra of the target sample material.

As discussed above, in certain embodiments, the in situ measuring devices disclosed herein, such as the in situ measuring devices 1300 and 1400 discussed above with reference to Figure 19, 20, and 21, can be utilized to determine the quantity, identity, and/or the temperature of a target sample material in an unknown, or known, sample. For example, in one or more embodiments, one can deter- mine the number of protons in a target sample material of an unknown sample, by adding a measured amount (e.g., weighed amount) of a known reference material inside a capillary tube, e.g., the capillary tube 1302 of the in situ measuring device 1300 discussed above with reference to Figure 19 and Figure 20. In such embodiments, one can then add an unknown material to the MAS rotor, e.g., the MAS rotor 1304 of

the in situ measuring device 1300 discussed above with reference to Figure 19 and Figure 20 and record proton NMR spectra. In one or more embodiments, one can record the proton solid state NMR spectra using the Bloch Decay Pulse Sequence experiment and then perform a Fourier Transform of the proton MAS spectra using commercial NMR software. In such embodiments, the spectra can be analyzed using standard baseline correction and integration procedures. In such embodiments, one can then identify a particular proton peak, e.g., the CH_2 peak of ethylene glycol, in the NMR spectra of the reference material using known methods, and integrate such a peak using known methods, to obtain a ratio of and compare by ratio to the integration of the proton peaks of the unknown material to obtain the number of protons in the unknown sample. Example 6 below, describes an embodiment of this process further detail.

In certain embodiments, one can determine the chemical identity of a target sample material of an unknown sample, by utilizing the in situ measuring devices described herein. For example, one may utilize a known reference material inside a capillary tube, e.g., the capillary tube 1302 of the in situ measuring device 1300 discussed above with reference to Figure 19 and Figure 20 to obtain a Carbon Bloch Decay Magic Angle Spinning NMR spectrum of the reference material and of the unknown. In such embodiments, one can then calibrate the chemical shift axis of this spectrum using a particular carbon in the known reference material (e.g., the methylene carbon peak of ethylene glycol), and then one can identify the chemical shifts of carbon peaks of the unknown sample and then compare those to the chemical shifts of carbon peaks known in the field, to obtain information on all types

of chemical carbons in the unknown sample. Example 7 below describes an embodiment of this process further detail.

In one or more embodiments, one can determine the temperature of a target sample material of an unknown sample, by utilizing the in situ measuring devices described herein. For example, one may utilize a known reference material inside a capillary tube, e.g., the capillary tube 1302 of the in situ measuring device 1300 discussed above with reference to Figure 19 to obtain proton Magic Angle Spinning NMR spectra of the reference material and the unknown sample. In such embodiments, the reference sample may reveals at least two peaks for particular types of protons, e.g., the CH_2 and OH groups of ethylene glycol. Further in such embodiments, the difference between the resonance frequencies of these two peaks can be used as a numerical input to calculate the absolute temperature of the unknown sample using a conventional equation known to one skilled in the art (e.g., J. Magn. Reson. 1982, 46, 319-321, which is incorporated by reference herein). It is well known in the art that the chemical shifts and widths for peaks in the proton NMR spectra of materials change as a function of temperature and in doing so reveal the specific molecular dynamics that the materials undergo. Molecular dynamics information may be useful for characterizing the dynamic architecture of materials and is used to explain the physical properties (e.g., glass transition temperature) of various materials, including polymeric materials.

Many different arrangements of the various components depicted, as well as components not shown, are possible without departing from the scope of the claims below. Embodiments of our technology have been described with the intent to be

illustrative rather than restrictive. Alternative embodiments will become apparent to readers of this disclosure after and because of reading it. Alternative means of implementing the aforementioned can be completed without departing from the scope of the claims below. Certain features and sub-combinations are of utility and may be employed without reference to other features and sub-combinations and are contemplated within the scope of the claims.

6. EXAMPLES

The concepts discussed herein will be further described in the following examples, which do not limit the scope of various embodiments described in the claims.

6.1 EXAMPLE 1: PHENOLPHTHALEIN AS A PH SENSOR FOR IN SITU PH MEASUREMENTS

In this example, the in situ pH measuring device included a standard NMR tube as the sample housing member where a sample (in the sample solution) was contained and a central capillary tube made of VYCOR® glass as the containment member was used where the pH sensor was contained. In this example, phenolphthalein was utilized as the pH sensor.

Specifically, the exemplary embodiment comprised a commercial 5 mm outer diameter, 17 cm long borosilicate glass NMR tube as the sample housing member and a 1 mm outer diameter, 17 cm long VYCOR® porous capillary tube as the containment member for the pH sensor molecule phenolphthalein. The size of a sequestered phenolphthalein molecule is approximately seven Angstroms; the

VYCOR® porous capillary pH sensor tube is selected for pore sizes that are smaller than the phenolphthalein molecule, but large enough (about two Angstroms in diameter) to allow unobstructed passage of hydronium and hydroxide ions. During the NMR testing, the VYCOR® porous capillary tube was filled to a height of approximately 7 cm from the bottom with a 0.001 molar aqueous solution of phenolphthalein, and placed approximately concentrically within the 5 mm glass NMR tube. The target sample, an aqueous acid solution, analyzed by NMR or MRI methods was placed inside the annular volume between the outside wall of the containment tube and the inside wall of the 5 mm glass NMR tube and filled to a level of approximately 7 cm from the bottom of both tubes. Standard NMR and MRI analyses were performed on the entire concentric tube assembly. The resulting proton NMR signals that emanated from the target sample solution and the phenolphthalein pH sensor molecule were recorded simultaneously and synchronously by the NMR spectrometer and were inextricably comingled in the raw data structure, also known as the free induction decay (FID). A fast Fourier transform (FFT) was applied to the FID data to generate a proton NMR spectrum of the target sample solution and the phenolphthalein pH sensor molecule.

To generate the NMR spectra, 3 microliters of a 0.01 molar NaOH solution was added to the NMR tube and the proton NMR spectrum was recorded. This was repeated (ten times) until a total of 30 microliters had been added. Additionally, an electronic pH meter was utilized to test the pH of each different target sample solution that was in the NMR tube.

The phenolphthalein molecule is known to exist exists in two different structural forms depending on the pH of the solution. Figure 6 and 7 illustrate the pH induced spectral changes of the exemplary pH sensor (phenolphthalein) at two different pH values. One form of the pH sensor probe molecule is shown in Figure 6 for pH 11.1; a different structural form of the pH sensor probe molecule is shown in and Figure 7 for pH 12.7. A comparison of Figure 6 and Figure 7 reveals two sets of proton NMR peaks for the pH sensor probe molecule phenolphthalein when it exists in two different forms, under conditions of different pH. The proton resonances for each group of chemically equivalent protons are contained in eleven distinct boxes and labeled with capital letters (Figure 6) or lower case letters (Figure 7) according to each of the respective structures depicted in Figure 6 and 7. At pH 11.1 only one form of phenolphthalein molecule is present, while at pH 12.7 only the other form is present. These changes in peak intensities of the ^{1}H NMR spectrum of the phenolphthalein pH sensor were correlated with the electronically-measured pH values that ranged from pH 11.1 to 12.7.

Measurements of the intensities from both sets of proton NMR peaks from Figure 6 and 7 are plotted in Figure 8, and were used in conjunction with a pH calibration curve shown in the top portion of Figure 8 to determine the pH of the target sample solution. Figure 8 is a graph containing multiple plots of peak integrals and pH values for a solution of 7.7 mg of phenolphthalein in 600 microliters of D_2O as a function of 3 microliter additions of 0.01 molar NaOH. The group of lines 506 are plots of normalized proton NMR peak integrals for the phenolphthalein structure shown in Figure 6 as a function of 3 microliter additions of0.01 molar NaOH. The

group of lines 504 are plots of normalized proton NMR peak integrals for the phenolphthalein structure shown in Figure 7 as a function of 3 microliter additions of 0.01 molar NaOH. The line 508 is a plot of the ratio of intensities of corresponding selected peaks from the proton NMR spectra from Figure 6 and 7. The line 502 is a plot of the measured pH using an electronic pH meter. The proton NMR spectra for phenolphthalein solutions at pH values between 11.1 and 12.7 revealed the proton resonances for both structures in varying proportions.

The absolute intensity of each peak in the spectrum was measured by integration over the signal. Further, a mathematical correlation function was used to calculate the pH from an ^{1}H NMR peak intensity of phenolphthalein pH sensor molecule. The pH was corrected for temperature variation by repeating the previous steps for each temperature setting for a series of temperatures that covered the temperature range of interest for investigations of the target sample solution. This example provides the paradigm for in situ monitoring of pH by ^{1}H NMR spectroscopy via peak intensities with the advantage of a spectral pH imprimatur.

6.2 EXAMPLE 2: NAF AS A PH SENSOR FOR IN SITU PH MEASUREMENTS

In this example, the pH-induced spectral changes of another exemplary pH sensor, sodium fluoride (NaF), was categorized by Fluorine-19 NMR chemical shift. The in situ pH measuring device utilized in this example includes a commercial 5 mm outer diameter, 17 cm long borosilicate glass NMR tube as the sample housing member and a 1 mm outer diameter, 17 cm long cracked-tip capillary pH sensor tube as the containment member for the pH sensor molecule, NaF. The cracked-tip

capillary tube was prepared by heating the bottom (closed end) of the tube and rapidly quenching the hot glass in cold water, causing a crack in the glass. The size of a sequestered solvated NaF molecule is approximately three Angstroms, and thus, the cracked-tip capillary pH sensor tube was selected for crack sizes that are smaller than the solvated NaF molecule, but large enough (about two Angstroms in diameter) to allow unobstructed passage of hydronium and hydroxide ions. The cracked-tip capillary tube was filled to a height of approximately 7 cm from the bottom with a 0.001 molar aqueous solution of NaF, and placed approximately concentrically within the 5 mm glass NMR tube.

The target sample material analyte molecule (1, 1, 1, 2, 2-pentafluorododecan-3-ol) was analyzed by NMR or MRI methods by placing an aqueous solution of it inside the annular volume between the outside wall of the cracked-tip pH sensor tube and the inside wall of the 5 mm glass NMR tube, filled to a level of approximately 7 cm from the bottom of both tubes. Standard 19F NMR and MRI analyses were performed on the concentric tube assembly. The resulting fluorine-19 NMR signals that emanated from the sample material (1,1,1,2,2-pentafluorododecan-3-ol) and the NaF pH sensor probe molecule were recorded simultaneously and synchronously by the NMR spectrometer and were inextricably comingled in the raw data, also known as the free induction decay (FID). A fast Fourier transform (FFT) was applied to the FID to generate a ^{19}F NMR spectrum of the target sample material analyte molecule (1, 1, 1, 2, 2- pentafluorododecan-3-ol) and the sodium fluoride pH sensor probe molecule.

Figure 9 depicts a plot of pH as a function of the ^{19}F chemical shift for an acidic and basic solution of 7 milligrams of NaF in 600 microliters of D_20. The acidic solution was made using 2.5 microliter additions of0.001 molar HCl from pH 1.7 to pH 3.5. The basic solution was made using 2.5 microliter additions of 0.005 molar NaOH from pH 11.0 to pH 13.5. The inset on the plot shows ^{19}F chemical shift as a function of pH and the equation that was used to fit the data points. The change of NaF chemical shifts from pH 1.7 to pH 4.5 varied greatly. In that range, a change in one or more decimal positions for the pH value was observed, e.g., a chemical shift of NaF between pH 4.40 and 4.41 was observed, as opposed to 4.4 to 4.5.

NMR spectra for the NaF pH sensor and a target molecule were obtained by preparing the NMR tube using 600 microliters of D_2 0.7 mg of NaF, and 3 microliters of the target molecule, 1,1,1,2,2-pentafluorododecan-3-ol. 2.5 microliters of 0.001 molar HCl solution was added to the NMR tube and the ^{19}F NMR spectrum was recorded. This was repeated until a total of 42.5 microliters of HCl had been added to the tube, then two additions of 10 microliters of the HCl solution were performed, followed by three 50 microliter additions of the HCl solution until a total of 212.5 microliters of the HCl solution had been added. After each HCl addition, the target sample in the NMR tube was measured with an electronic pH meter.

Another NMR tube was prepared using 600 microliters of D_2O, 7 mg of NaF, and 3 microliters of 1, 1, 1, 2, 2-pentafluo- rododecan-3-ol. 2.5 microliters of a 0.005 molar NaOH solution was added to the NMR tube and the ^{19}F NMR spectrum was recorded. This was repeated until a total of 20 microliters was added, then 10 microliter portions of the NaOH solution was added until a total of 80 microliters of

NaOH had been added to the NMR tube, followed by addition of 100 microliter portions of the NaOH solution until a total of 1080 microliters of the NaOH solution had been added to the NMR tube. After each NaOH addition, the target sample in the NMR tube was measured with an electronic pH meter.

Figure 10 depicts the 19F NMR spectrum of NaF for a multitude of different structural forms depending on the pH of the acidic target solution. For example, one form of the pH sensor probe molecule is represented by an NMR peak at approximately -119 ppm for pH 1.5; a different structural form of the pH sensor molecule is depicted by an NMR peak at approximately-128 ppm for pH 2.11. Figure 10 also shows the 19F NMR spectrum of the pH sensor probe molecule at approximately -130.5 ppm for pH 2.5.

Figure 11 depicts the ^{19}F NMR spectrum of NaF for a multitude of different structural forms depending on the pH of the basic target solution. For example, one form of the pH sensor probe molecule is represented by an NMR peak at approximately -123 ppm for pH 11.18; a different structural form of the pH sensor molecule is depicted by an NMR peak at approximately -123.5 ppm for pH 11.78. Figure 11 also shows the fluorine NMR spectrum of the pH sensor probe molecule at approximately -123.75 ppm for pH=13.25.

Figure 10 and 11 also include the ^{19}F NMR spectra of the target sample material analyte molecule (1, 1, 1, 2, 2-penta- fluorododecan-3-ol) for multiple pH environments. The 19F NMR spectrum of the target sample material analyte in Figure 10 is revealed by a set of five NMR peaks at approximately -121, -122, -127, -128.5, and -129.5 ppm. The ^{19}F chemical shifts have shifted to the right for

different pH values and the target sample peaks have changed. The ^{19}F NMR spectrum of the target sample material analyte molecule (1, 1, 1, 2, 2-pentafluorododecan-3-ol) in Figure 11 is revealed by a set of four NMR peaks at approximately -126, -126.5, -133.5, and -134.5 ppm. The ^{19}F chemical shifts have shifted to the left for different pH values and the target sample peaks have not changed.

Figure 12 depicts the ^{19}F NMR spectra of the target sample material analyte molecule (1, 1, 1, 2, 2-pentafluorododecan-3-ol). Peaks changed with NMR pH sensor peak (NaF) for four different pH values. When the pH was 3.58 and 4, the peaks for the target sample analyte did not change much. When the pH was 4.44 and 4.55 the target sample analyte peak changed to indicate two separate molecular species.

Measurements of the ^{19}F chemical shifts for NaF can be used in conjunction with a calibration curve shown in Figure 9 to determine the pH of the solutions of target sample material analyte molecules. A mathematical correlation function was used to calculate pH from a ^{19}F peak chemical shifts of the NaF pH sensor molecule. The pH was corrected 10 for temperature variation. This example provides the paradigm for in situ monitoring of pH by ^{19}F NMR spectroscopy via chemical shifts with the advantage of a spectral pH imprimatur.

6.3 EXAMPLE 3: USING AN IN SITU TEMPERATURE SENSOR TO MEASURE THE ACTIVATION ENERGY OF THE CONFORMATION CHANGE OF DPPH

External in situ temperature monitoring during NMR test of 2, 2-diphenyl-1-picrylhydrazine (DPPH) in $CDC1_3$. The external in situ temperature sensor device

was made of a sealed capillary tube (75 um internal diameter, 364 um outer diameter, and 6 cm length). The capillary tube was filled with 100% ethylene glycol. The external in situ temperature sensor device was assembled similar to that depicted in Figure 3. For example, the external in situ temperature sensor device was inserted into a 5-mm NMR tube, which was filled with DPPH solution. The external in situ temperature sensor device monitored the actual temperature of the DPPH sample in situ, and provided a temperature imprimatur. That is, the raw data/NMR spectra of the DPPH also includes the data of the ethylene glycol external in situ temperature sensor.

In this example, NMR spectra were recorded for DPPH sample (and the ethylene glycol external in situ temperature sensor) at temperatures from 25-56° Celsius in 2° Celsius increments. Figure 14 shows a full exemplary spectrum with the box on the left highlighting to the two conformational peaks of DPPH and the box on the right highlighting the in situ thermometer spectral stamp (of ethylene glycol). Below the full spectrum is the individual spectrum at various temperatures in 2° increments (between 25-56° Celsius). By conducting a one pulse experiment, two peaks were successfully observed to reveal the conformational exchange of DPPH, and the two peaks of ethylene glycol that provided the temperature measurement. This data shows how the conformational change of DPPH correlates with various temperatures of the DPPH, as evidenced by the spreading out of the two prominent spectral peaks in the in situ thermometer containing ethylene glycol.

The proton NMR spectrum of ethylene glycol reference material can be used to determine the actual temperature of the ethylene glycol reference material, and of

the DPPH, as they are in the same thermal environment of the in situ thermometer device. For example, a proton NMR spectrum of the ethylene glycol reference material contained in the capillary tube produces two sharp peaks. The separation of the two peaks measured in frequency units of Hz is entered into a standard published temperature calibration formula specific to ethylene glycol. The formula generates a numerical output that is the temperature of the ethylene glycol, capillary tube and surrounding sample.

Figure 15 depicts a graph comparing the temperature determined for the in situ temperature sensor used in this5 Example 3 with the temperature determined using a commercial NMR thermometer device. As can be seen in Figure 15, the in situ temperature sensor used in this Example 3 produces similar temperature results as that using a commercial NMR thermometer.

6.4 EXAMPLE 4: IN SITU TEMPERATURE MONITORING DURING NMR TEST OF A SOLID SAMPLE

External in situ temperature monitoring during NMR test of a solid sample. The in situ temperature monitoring device was made of a sealed capillary tube (75 micrometer internal diameter, 364 micrometer outer diameter, 1 centimeter length). The capillary tube was filled with 100% ethylene glycol.

The MAS rotor and in situ temperature monitoring device were assembled similar to that described with respect to Figure 4. For example, the MAS rotor contained a powder target sample illustrated. The plastic tube can be made of a polymer that is the target sample material that is to be analyzed for composition of plasticizer, for example. The 1 mm NMR tube is made of glass and is used as an

element to keep the target sample and the NMR thermometer sensor centered so that the rotor will maintain balance during high speed rotation. Any cylindrical element made of a material that does not produce an NMR signal that will interfere with the NMR signals from the target sample is suitable (glass, ceramic tubes, etc.) can be used for this purpose. The MAS rotor itself is made of a ceramic (zirconia) and it does not produce NMR signals that interfere with the NMR signals produced by the target sample material. The arrangement of the target sample material, the cylindrical spacer element, the NMR capillary thermometer should be arranged so as to produce a rotationally-balanced system within the rotor, so cylindrical symmetry is not absolutely necessary. This embodiment has cylindrical symmetry. A cap is placed on the rotor to seal in the contents. The rotor is placed in the MAS probe.

The in situ temperature monitoring device monitored the actual temperature of the sample in situ, and provided a temperature imprimatur. Air jets were used to cause the rotor to spin at from 1-14 kHz in the. A one-pulse experiment during MAS was conducted.

Figure 16 shows a set of stacked spectra that reveal the temperature as metered by the thermometer and indicated by the difference between left most peak (with the vertical line) and the right most sharper peak as measured in Hz or PPM. This difference is entered into a well-known equation that outputs the temperature at the location of the NMR thermometer sensor. The temperature is indicated at the right. As the rotation speed of the rotor increases, the two peaks come closer together. The equation takes in the separation of the two peaks in Hz or PPM and outputs the temperature of the NMR temperature sensor in Kelvins. The bottom axis

of the NMR plots is Hz or PPM. The temperature of the rotor increases with spinning speed as shown in Figure 17. Also Figure 18 shows a plot of the temperatures of the NMR thermometer sensor molecule as a function of spinning speed.

6.5 EXAMPLE 5: IDENTIFICATION OF A ^{11}B INTEGRATED REFERENCE SAMPLE IN AN IN SITU MEASURING DEVICE FOR AN MAS ROTOR

The in situ measuring device used in this example included a sealed capillary tube positioned inside a solid state NMR rotor, such as that depicted in Figure 19, 20, and 21. Specifically, as described in this Example 5 further below, the sealed capillary tube having an internal diameter of 1.2 millimeter contained a 1.0 M boric acid solution (anhydrous boric acid dissolved in DMF) or was left empty, and the top of the capillary tube was sealed with Teflon tape. The sealed capillary tube was placed in a solid state NMR rotor to form an in situ measuring device.

The in situ measuring device was then inserted in an MAS rotor and ^{11}B NMR spectra were recorded. Specifically, the in situ measuring device and MAS rotor were positioned and spun at the magic angle, 8m (54.74°), inside the static magnetic field with respect to the direction of B_0, which narrows the NMR peaks. In order to compensate for the lack of molecular motion in solid samples, MAS was developed to remove the anisotropic interactions. MAS is the rapid mechanical rotation of the whole sample at the "magic angle," with respect to the external magnetic field. MAS decouples CSA and weak homo-nuclear and/or heteronuclear dipolar coupling to provide a well resolved spectrum; spinning sidebands are reduced in intensity with increasing spinning speed. However, the strong centrifugal force caused by magic angle spinning in MAS rotors makes it impossible to have a fluid reference material

in an external integration standard for quantitative MAS-NMR determination because the fluid is made to have high pressure and flow out of the MAS rotor.

Figure 23 depicts the ^{11}B solid state NMR spectra of the MAS rotor with and without the capillary tube, which is empty. Further, Figure 23 illustrates the difference spectrum of the two aforementioned spectra. By comparing these three NMR spectra, one can discern that the difference spectrum represents the ^{11}B spectrum of the empty capillary tube, which can be used as an integration and/or chemical shift reference sample peak.

Figure 24 depicts the ^{11}B solid state NMR spectra of the MAS rotor with and without the capillary tube, where the capillary tube is filled with a boric acid/DMF solution, as described above. The difference spectrum shown in Figure 24 represents the ^{11}B spectrum of the substance that composes the capillary tube, as well as, boric acid inside the capillary tube. Comparing the difference spectrum from Figure 23 and 24, which shows the spectrum of the capillary tube without and with the boric acid/DMF solution, respectively, one can discern that the sharp signal on the left shoulder of the difference spectrum for the capillary tube with the boric acid/DMF solution depicted in Figure 24 is indicative of the empty capillary tube. The broad peak on the right of the sharp peak results from a target sample material in the unknown sample material that simultaneously occupies the MAS rotor.

In addition, the in situ measuring device was prepared as described above with varying materials in the sealed capillary tube (or nothing) and with varying materials or nothing in the rotor. Table 1 below shows the five particular in situ measuring devices examined.

Table 1. In situ measuring devices for ^{11}B NMR spectra in Figure 25

Spectrum	Sample in capillary tube	Sample in MAS rotor
1	Empty (capillary tube)	Boron Amide
2	Dodecaborane	Empty (rotor)
3	Ground glass	Empty (rotor)
4	Boric Acid/DMF	Boric Acid Anhydrous
5	Empty (capillary tube)	Empty (rotor)

As can be observed from the ^{11}B NMR spectra in Figure 25 that of the five in situ measuring devices tested, the best ^{11}B NMR spectra in situ reference material is an empty capillary tube (single, sharp, easily discernable peak). However, such an in situ reference standard cannot be utilized, because the sharp ^{11}B signal is present at the same location that many other ^{11}B signals are observed, as can be seen in Figure 25**.** NMR spectra overlapping ^{11}B signals (or NMR signals in general) cannot be easily used for quantitative determination. Yet, these experiments in Example 5 do demonstrate that by using an empty capillary tube in an in situ measuring device, and running MAS-NMR experiment with signal subtraction, a single narrow ^{11}B peak as successfully observed and is useful for many samples that do not show overlapping peaks in their corresponding NMR spectra.

6.6 EXAMPLE 6: USING IN SITU MEASURING DEVICE TO DETERMINE THE QUANTITY OF A TARGET SAMPLE

In this Example 6, an in situ measuring device was used to measure the number of protons in a target sample (rubber band in this case). Generally, in this Example 6, the in situ measuring device included a sealed capillary tube with a known amount of a reference material positioned inside a MAS rotor, with the target sample positioned inside the MAS rotor, around the capillary tube. The in situ measuring device was substantially similar to that depicted in Figure 19 and 20, and discussed in detail above.

Specifically, the in situ measuring device was fabricated by cleaning a closed-ended glass capillary reference tube with dimensions 1 millimeter internal diameter, 1.5 millimeter outer diameter, and 13 millimeter length using acetone, followed by air drying. The glass capillary reference tube was weighed five times to determine its mass and mass uncertainty as 6.100+/-0.002 mg. Commercially avail- able high-purity ethylene glycol was placed inside the glass capillary reference tube to fill the bottom half portion of the container.

The glass capillary reference tube was then flame sealed using a commercially available micro torch. Particularly, the open end of the glass capillary reference tube was concentrically mounted at the top of the spindle of a commercially available stepper motor. A thin metal plate was prepared with a hole 1.6 millimeter in diameter and positioned above the stepper motor such that the glass capillary reference tube passed through the hole in the metal plate and extended above the metal plate by 1 millimeter.

The stepper motor was activated and caused the glass reference capillary tube to rotate at 2-4 revolutions per second. The intense blue flame of the micro torch burning a combustible gas was aimed at the 1 millimeter portion of the glass reference capillary tube that extended above the metal plate. The metal plate prevented the flame and heat from impacting the portion of the glass reference capillary tube that contained the ethylene glycol and that was positioned below the metal plate. A stream of very cold nitrogen gas was directed at the portion of the glass capillary reference sample tube that is located below the metal plate for the purpose of cooling the glass capillary reference sample tube and the ethylene glycol. The purpose of cooling the reference sample was to prevent evaporation and degradation caused by the high temperatures from the glass-sealing process. Cooling of the glass capillary reference sample tube can be performed to maintain the reference material at temperatures above, below or at room temperature. It may be advantageous to maintain the temperature of the reference material below its freezing point so that very little volatility occurs. The cooling gas was applied to the bottom of the glass reference capillary tube after the flame was applied to the top of the tube. Using this sequence, the mixture of reference sample vapor and air (located above the liquid reference sample), which may contain water vapor and other gaseous impurities, were caused to be excluded from the glass capillary reference sample tube before it was completely sealed. Continuous or intermittent applications of the flame to the top, open portion of the glass reference capillary tube, that was made to rotate by the stepper motor, caused the glass to melt and close the tube opening. At the moment that the seal was fully formed, the flame was turned away from the glass

reference tube. The soft molten glass formed a small expanded bubble that was approximately 2 millimeters in diameter. The bubble was formed by the residual gases that were trapped and heated inside the top of the reference capillary tube. The glass capillary reference sample tube was allowed to cool to room temperature with intermittent applications of heat for purposes of annealing the glass bubble.

The half-filled and sealed glass capillary reference sample tube was then weighed and its mass was 13.785+/-0.002 milligrams. The mass difference between the empty capillary reference sample tube and the capillary reference sample tube half-filled with high-purity ethylene glycol was, therefore, the mass of the ethylene glycol reference material, 7.685+/-0.002 milligrams. As can be seen in Table 2, the number of corresponding moles of ethylene glycol was 0.000124+/-0.000002 moles, and the corresponding moles of methylene protons measured for the ethylene glycol was four times larger, 0.000495312+/-0.000002 moles.

Table 2. Ethylene Glycol Amounts and Properties

Molar mass ($g \cdot mol^{-1}$)	Mass (mg)	Mass(g)
62.07	7.686+/- 0.002	0.007686
Number of moles in CH_2 group	Number of mole of protons in CH_2 group	Moles of Ethylene Glycol
0.000248	0.000495312	0.000124

The number of moles of methylene protons in the glass capillary reference sample tube containing ethylene glycol was used to directly calculate the number of protons of all chemical types in the target sample material of the unknown sample, in this case, a rubber band. Because the glass capillary reference sample tube was

sealed, it can be used repeatedly for determining the total number of all types of chemical protons in a target sample material of an unknown sample.

The procedure used to measure the amount of protons of all chemical types contained in the target sample material of a rubber band sample (that includes isoprene, ethanol, and other compounds) included the steps of weighing the rubber band sample, placing the rubber band in the commercial MAS rotor container, arranging and packing the rubber band symmetrically around the glass capillary reference sample tube, and covering the commercial MAS rotor with a rotor cap. These steps were followed to symmetrically and evenly dispose the rubber band in the commercial MAS rotor with the glass capillary reference sample tube disposed in the 60 center and along the center axis of the commercial MAS rotor.

An innocuous, non-protonic solid fine powder material, B_2O_3, was used to fill all empty space in the commercial MAS rotor. The B_2O_3 fine powder was symmetrically pressed into the commercial MAS rotor. A small piece of Kimwipe paper in the shape of a circle (with diameter of the commercial MAS rotor) was placed on top of the B20 3 to prevent shifting of the fine powder, and then the rotor cap was secured in place to contain all of the components and to prevent any materials from shifting positions inside the commercial MAS rotor during high speed rotation. The capped commercial MAS rotor was placed in the commercial MAS probe and rotated at 7000+/-l Hz and the temperature of the commercial MAS probe was set and regulated at a probe temperature reading of 340.0+/-0.2K. Various multinuclear solid-state carbon and proton MAS NMR experiments were conducted

on the sample to deter- mine the identity, quantity, and temperature of a target sample material in the unknown sample.

In this Example 6, to determine the quantity of all types of chemical protons contained in the target material of the rubber band, a proton solid-state MAS spectrum was recorded using a Bloch Decay pulse sequence experiment with a recycle delay of 10 seconds, a pulse width of 4 microseconds, and a total accumulation of 128 scans. The spectrum is depicted in Figure 26. A Fourier Transform of the proton MAS NMR spectrum was performed by the commercial NMR spectrometer computer software and analyzed by performing standard baseline correction and integration procedures. The relative integration of the reference peak for CH_2 in ethylene glycol is 4.00. The total integration of all other peaks except OH peak of ethylene glycol was 202.6643. Given that the gravimetrically-determined absolute amount of CH_2 protons in the reference material inside the capillary tube was 0.0003329 moles, the total number of protons in the sample target materials contained in the rubber band sample was calculated to be 0.01687 moles. The explicit calculation was 0.0003329 mol *202.6643/4.00 = 0.01687 mol, as shown in Table 3. Furthermore, the reference sample chemical shift secondary proton standard for CH_2 in ethylene glycol is 3.765 ppm. Measuring the chemical shift of the peaks for the target materials in the unknown rubber band sample, it was possible to obtain information of all the types of chemical protons present in the unknown rubber band sample. For example, a proton peak observed around 5.0-6.5 ppm is known in the art to correspond to a CH=C type proton. Therefore, since in the spectrum of the unknown rubber band sample one peak was observed at 5.40 ppm, it was deduced

that a CH=C group is a constituent chemical group in one or more of the target sample materials in the rubber band sample. In fact, the CH=C chemical group is known in the prior art to be found in isoprene.

Table 3. Determination of the Number of Protons in the Rubber Band Sample

Ratio of Ethylene Glycol (CH_2) with Rubber band: 4.000:202.6643	
Rubber Band Mass (mg): 57.696 +/- 0.001	Number of moles of proton in rubber band: 0.01687

6.7 EXAMPLE 7: USING IN SITU MEASURING DEVICE TO DETERMINE THE CHEMICAL IDENTITY OF A TARGET SAMPLE

In this Example 7, the in situ measuring device of Example 6 was used to determine the identity of chemicals contained in the target material of the rubber band target unknown sample. Specifically, a carbon solid-state MAS spectrum of the in situ measuring device of Example 6 was recorded using the Bloch Decay pulse sequence experiment with a recycle delay of 10 seconds, a pulse width of 4 microseconds, and a total accumulation of 1024 scans. The spectrum is depicted in Figure 27. A Fourier Transform of the carbon MAS NMR spectrum was performed by commercial NMR spectrometer computer software and analyzed by performing standard baseline correction. The chemical shift 5 axis of the carbon MAS NMR spectrum was calibrated to 63.1724 ppm by using the carbon resonance of the methylene group of the ethylene glycol reference standard in the capillary reference sample tube. According to the chemical shifts of carbon peaks known in the art, information of all types of chemical carbons in the rubber band sample were

obtained. For example, the carbon types $\underline{C}H_3$, H_2-$\underline{C}$H=C, $\underline{C}$H=C-CH_2, H$\underline{C}$=C and HC=$\underline{C}$ peaks were identified for the rubber band sample by observing the corresponding carbon chemical shifts at 23.28, 26.41, 32.15, 125.04 and 134.58 ppm, respectively. Alternative chemical shift assignments may also be possible according to what is known in the prior art. These peaks and their chemical shifts constitute unique and specific data that was used to characterize and define the target sample material of the rubber band sample.

6.8 EXAMPLE 8: USING IN SITU MEASURING DEVICE TO DETERMINE THE TEMPERATURE OF A TARGET SAMPLE

In this Example 8, the in situ measuring device of Example 6 was used to determine the temperature of the target material of the rubber band target sample for the purposes of studying molecular dynamics, for example. Specifically, a proton solid-state MAS spectrum of the in situ measuring device of Example 6 was recorded using the Bloch Decay pulse sequence experiment with a recycle delay of 10 seconds, a pulse width of 4 microseconds, a total accumulation of 128 scans, and a rotor rotation speed of 7 kHz. The spectrum, expanded from 0 ppm - 7 ppm, is depicted in Figure 28. A Fourier Transform of the proton MAS NMR spectrum was performed by commercial NMR spectrometer computer software and analyzed by performing a standard baseline correction procedure and by providing frequency assignments of the two sharp peaks for the ethylene glycol reference standard at 5.01 ppm (hydroxyl group) and 3.73 ppm (methylene group). The difference between the two frequencies ($\Delta\upsilon$ = 511.51 Hz, as shown in Figure 28) was used as a numerical input to calculate, using an equation known in the art (e.g., J. Magn.

Reson. 1982, 46, 319-321, incorporated by reference herein), the absolute temperature in Kelvins of the target sample material of the rubber band sample. As can be seen in Figure 28, the absolute temperature of the rubber band sample was 336.065K. The absolute temperature and proton MAS NMR spectral features (peak positions, shapes, etc.) of the target sample material in the unknown sample constitutes unique and specific data that was used to characterize and define the target sample material in the unknown sample. It is well known in the art that the chemical shifts and widths for peaks in the proton NMR spectra of materials change as a function of temperature and in doing so reveal the specific molecular dynamics that the materials undergo. Molecular dynamics information is useful for characterizing the dynamic architecture of materials and is used to explain the physical properties of polymer materials.

SECTION

2. CONCLUSION

NMR spectroscopy and magnetic resonance imaging is used as a standard analytical and diagnostic tool in many academic institutions, industries, and hospitals around the world. Diverse strategies and methodologies are used in NMR to extract most unambiguous information about molecular structures, conformations, dynamics, and functions. To improve the accuracy and precision of NMR experiments and investigations, tools are needed that help the user to evaluate the spectrometer hardware, software, and pulse-sequence performance, to measure sample conditions such as temperature, pressure, or pH in situ, and to calibrate the chemical-shift and intensity axes of the recorded NMR spectra. This dissertation has introduced a new calibration and performance-evaluation platform, i.e., the NMR CapPack (**Cap**illary-tube **Pack**ages) platform, as well as several applications for liquid-state and solid-state NMR investigations. A Gradient CapPack device can be used to conveniently assess the performance of pulse sequences, optimize pulse parameters, or explain artifacts that may occur in the recorded spectra. A T_1 CapPack device can be used to test T_1 insensitivity and robustness of novel pulse sequences, and to test the effect relaxation may have on the performance of NMR experiments. CapPacks are also useful to determine sample conditions, which has led to a collection of patented techniques including the measurement of temperatures inside magic-angle spinning rotors for solid-state NMR measurements. Most importantly, the information acquired with CapPack devices is collected either together with the sample under investigation or in calibration experiments that take only a minimal amount of experimental time.

CapPack devices are designed to generate unique NMR signals or profiles that allow the user to corroborate the magnetic conditions and understand how NMR probes, hardware, software, and pulse sequences work. In this dissertation, a Gradient CapPack device was used to evaluate the solvent-signal suppression profile of the newly developed pulse sequence EXCEPT on an older 200 MHz Bruker DRX spectrometer. It was shown that the older hardware was not able to provide an even suppression profile as predicted by the theory. In a further investigation, a T_1 CapPack device demonstrated the T_1 robustness of the EXCEPT sequence when hard pulse are used. However, it also showed that EXCEPT may fail when long-lasting, frequency-selective pulses, such as hyperbolic secant (sech) pulses are used with samples of fast spin-lattice relaxation, i.e., short T_1 relaxation times. An in situ NMR CapPack thermometer was used in solid-state NMR spectroscopy for generating a temperature imprimatur in the recorded spectra. It demonstrated that the sample temperature increases with the magic-angle spinning speed of the sample rotor even if the temperature setting in the spectrometer software remains unaltered.

APPENDIX A.

SUPPLEMENTARY MATERIAL TO PAPER II

Figure 1 shows spectra recorded with opposite constant magnetic field gradients applied with the y-axis shim coil in a 200 MHz Avance DRX spectrometer. The asymmetry in the suppression profile does not change when the gradient is inverted from 0.81 mT m^{-1} to -0.81 mT m^{-1}. It indicates that the asymmetric suppression profile is not the result of an irregularity in the manufacturing of the Gradient CapPack, nor is it the result of the of the shim-coil gradient application.

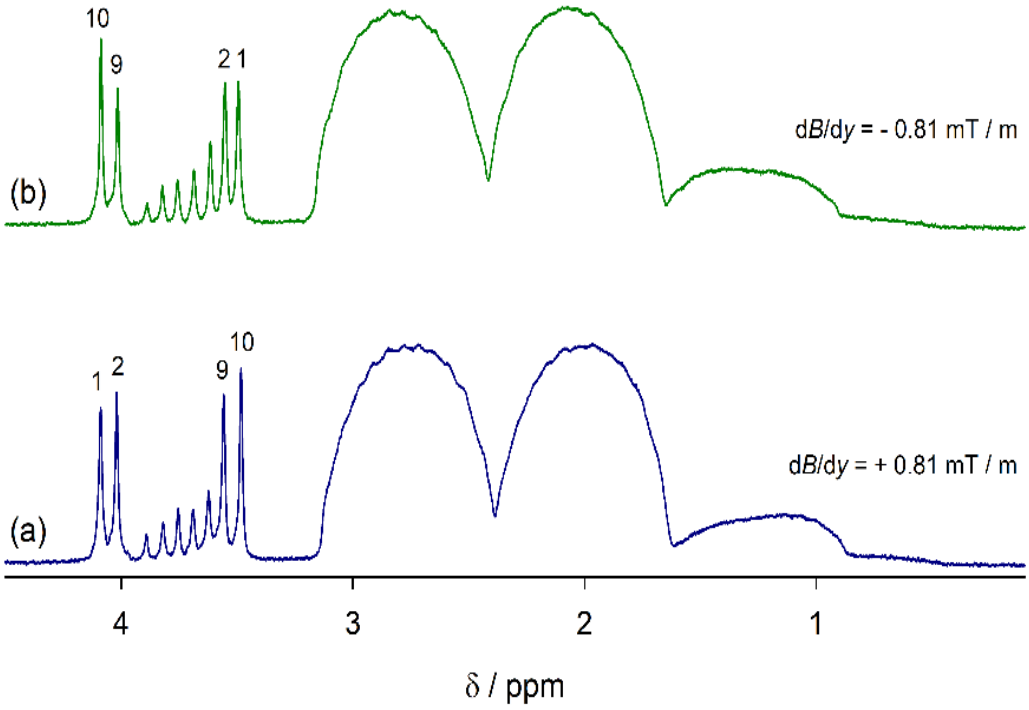

Figure 1: ^{1}H NMR spectra recorded from a 10-capillary-tube Gradient CapPack contained in a 5-mm sample tube using acetone-d6 (99.8%) as the surrounding solvent. (a) A y-axis shim-coil gradient (dB/dy = 0.81 mT m^{-1}) was applied during an EXCEPT-12 experiment in which an observe pulse followed a series of twelve 400-ms HS1 pulses with progressively decreasing interpulse delays. (b) The same experiment was conducted with an inverted y-axis shim-coil gradient (dB/dy = – 0.81 mT m^{-1}). Numbers above the Gradient CapPack signals are capillary-tube labels demonstrating that the gradient was reversed. Consequently, the signals from capillary 3 in (a) and capillary 8 in (b) were completely suppressed.

Figure 2 shows spectra recorded from a series of experiments in which the transmitter frequency was set to three different values. The center of the Gradient CapPack signals was placed at a positive resonance-frequency offset with respect to the incident transmitter frequency (Figure 2 (a)), on resonance (Figure 2 (b)), or at negative resonance-frequency offset (Figure 2 (c)). The suppression profile does not change, providing evidence that the profile's asymmetry is not caused by a resonance-frequency offset.

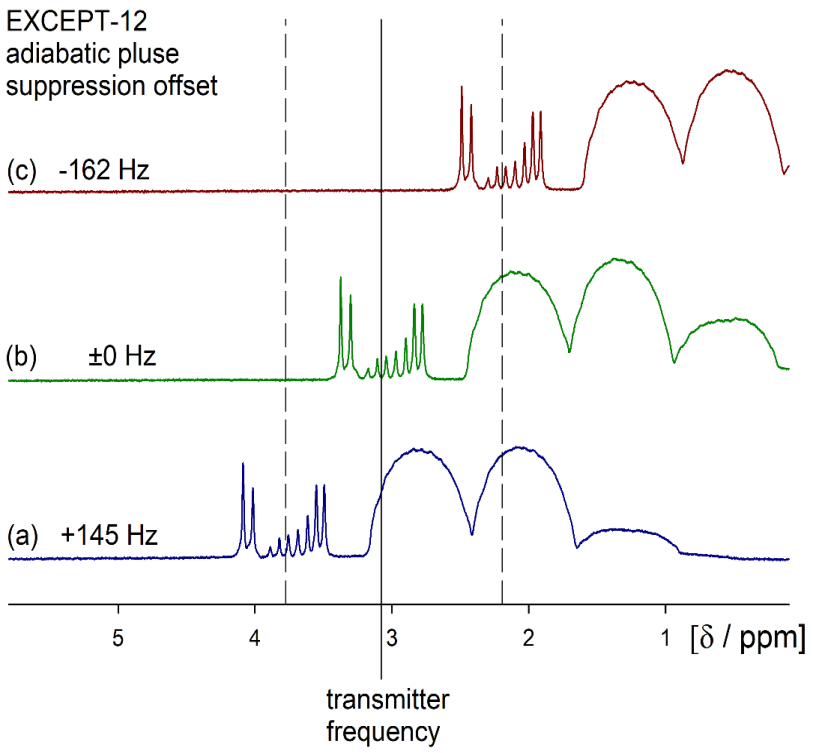

Figure 2: ^{1}H NMR spectra recorded from a 10-capillary-tube Gradient CapPack contained in a 5-mm sample tube using acetone-d6 (99.8%) as the surrounding solvent. A y-axis shim-coil gradient (dB/dy = + 0.81 mT m^{-1}) was applied during an EXCEPT-12 experiment, in which an observe pulse followed a series of twelve 400-ms HS1 pulses with progressively decreasing interpulse delays. The center of the Gradient CapPack signals was placed (a) at +145 Hz resonance-frequency offset, (b) on resonance, and (c) at -162 Hz resonance-frequency offset with respect to the transmitter frequency. The adiabatic HS1 pulses were always applied at the center frequency of the Gradient CapPack signals.

APPENDIX B.

CLAIMS OF U.S. PATENT 10,067,079 B2: "SOLID STATE NMR SPECTROSCOPY IN SITU MEASURING DEVICES AND METHODS FOR CALIBRATION AND DETERMINING ONE OR MORE QUANTITATIVE PROPERTIES OF A TARGET SAMPLE" (ISSUED SEPTEMBER 4, 2018)

What is claimed is:

1. A method for performing one or more quantitative measurements of a target sample using solid state Magic 65 Angle Spinning (MAS) Nuclear Magnetic Resonance (NMR) spectroscopy, the method comprising: providing an in situ measuring device, the in situ measuring device comprising a solid state MAS NMR rotor and at least one sealed capillary tube positioned inside the solid state MAS NMR rotor, the at least one sealed capillary tube having a reference material sealed inside the at least one capillary tube between first and second fused glass ends of the at least one sealed capillary tube, wherein a target sample is positioned on the inside of the solid state MAS NMR rotor, and wherein a first portion of the target sample is in contact with an inner surface of the solid state MAS NMR rotor and a second portion of the target sample is in contact with an outer surface of the at least one sealed capillary tube; obtaining MAS NMR spectra of the target sample and the reference material; and determining one or more quantitative properties of the target sample, the one or more quantitative properties comprising one or more of a quantity of the target sample, a chemical identity of the target sample, or a temperature of the target sample.
2. The method of claim 1, wherein the reference material comprises one or more of ethylene glycol, methanol, ethanol, water, or mixtures thereof.
3. The method of claim 1, wherein the at least one sealed capillary tube comprises a plurality of sealed capillary tubes, wherein each of the

plurality of sealed capillary tubes are positioned inside the solid state MAS NMR rotor.

4. The method of claim 3, wherein each of the plurality of sealed capillary tubes has the same reference material sealed inside.
5. The method of claim 1, wherein the obtaining MAS NMR spectra of the target sample and the reference material comprises simultaneously obtaining the MAS NMR spectra of the target sample and the reference material.
6. The method of claim 1, wherein the one or more quantitative properties comprises the chemical identity of the target sample.
7. The method of claim 1, wherein the one or more quantitative properties comprises the quantity of the target samples.
8. The method of claim 1, wherein the one or more quantitative properties comprises the temperature of the target sample.
9. The method of claim 1, wherein the at least one sealed capillary tube has an outer diameter less than 600 micrometers.
10. A method for performing one or more quantitative measurements of a target sample using solid state Magic Angle Spinning (MAS) Nuclear Magnetic Resonance (NMR) spectroscopy, the method comprising: providing an in situ measuring device, the in situ measuring device comprising a solid state MAS NMR rotor and at least one sealed capillary tube positioned inside the solid state MAS NMR rotor, the at least one sealed capillary tube having a reference material sealed inside the at least

one capillary tube between first and second fused glass ends of the at least one sealed capillary tube, wherein a target sample is positioned on the inside of the solid state MAS NMR rotor, and wherein a first portion of the target sample is in contact with an inner surface of the solid state MAS NMR rotor and a second portion of the target sample is in contact with an outer surface of the at least one sealed capillary tube;

inserting the in situ measuring device inside a probe of a solid state MAS NMR instrument, while the in situ measuring device is positioned inside a probe of a solid state MAS NMR instrument, obtaining MAS NMR spectra of the target sample and the reference material, wherein at least one MAS NMR spectra peak associated with the reference material is spaced apart from at least one MAS NMR spectra peak associated with the target material; and

determining one or more of a quantity of the target sample or a chemical identity of the target sample based on the MAS NMR spectra of the target sample and the reference material.

11. The method of claim 10, wherein the reference material comprises one or more of ethylene glycol, methanol, ethanol, water, or mixtures thereof.
12. The method of claim 10, wherein the obtaining MASNMR spectra of the target sample and the reference material comprises recording the solid state MAS NMR spectra using a Bloch decay pulse sequence.
13. The method of claim 10, wherein the at least one sealed capillary tube has an outer diameter less than 600 micrometers.

14. A method for forming an in situ measuring device for solid state Magic Angle Spinning (MAS) Nuclear Magnetic Resonance (NMR) spectroscopy, the method comprising:

providing at least one capillary tube;

adding at least one reference material to an inside cavity of the at least one capillary tube;

sealing the at least one capillary tube by fusing glass at a first end and a second end to form at least one sealed capillary tube having the at least one reference material sealed inside;

providing a solid state MAS NMR rotor, the solid state MAS NMR rotor configure d to house a target sample;

positioning the at least one sealed capillary tube inside the solid state MAS NMR rotor; and

adding at least one target material to the interior of the solid state MAS NMR rotor such that at least a portion of the target material is in contact with an inner surface of the solid state MAS NMR rotor and at least another portion of the target material is in contact with an outer surface of the at least one sealed capillary tube.

15. The method of claim 14, wherein the sealing the at least one capillary tube is performed using a micro torch.

16. The method of claim 14, wherein the at least one capillary tube is coupled to a foundation member at one end of the at least capillary tube.

17. The method of claim 16, wherein the positioning the at least one sealed capillary tube inside the solid state MAS NMR rotor comprises positioning the foundation member adjacent to a bottom inner surface of the solid state MAS NMR rotor.
18. The method of claim 14, wherein the at least one sealed capillary tube is positioned in the center of the solid state MAS NMR rotor.
19. The method of claim 14, wherein the at least one sealed capillary tube comprises a plurality of sealed capillary tubes.
20. The method of claim 14, wherein the at least one sealed capillary tube has an outer diameter that is less than 600 micrometers.

APPENDIX C.

ACCOMPLISHMENTS

PUBLICATIONS (4)

1. Lingyu Chi, Ming Huang, Annalise R. Pfaff, Jie Huang, Rex E. Gerald II, and Klaus Woelk, **CapPack devices for the quantitative performance evaluation of NMR Spectrometers and pulse sequences**, *Rev. Sci. Instrum.* (accepted for publication).

2. Lingyu Chi, Ming Huang, Rex E. Gerald II, and Klaus Woelk, **Solid State NMR Spectroscopy/Imaging in situ Measuring Devices and Methods for Calibration and Determining one or more Quantitative Properties of a Target Sample**, U.S. Patent 10,067,079 B2 (issued Sep. 4th, 2018).

3. Emmalou T. Satterfield, Annalise R. Pfaff, Wenjia Zhang, Lingyu Chi, Rex E. Gerald II, and Klaus Woelk, **Exponentially Converging Eradication Pulse Train (EXCEPT) for Solvent-Signal Suppression in Investigations with Variable T_1 Times** [J]. ISSN: 0884-2914, *J. Magn. Reson.* 2016, 268, 68-72.

4. Vivek Bagchi, Patrina Paraskevopoulou, Purak Das, Lingyu Chi, Qiuwen Wang, Amitava Choudhury, Jennifer S. Mathieson, Leroy Cronin, Daniel B. Pardue, Tomas R. Cundari, George Mitrikas, Yiannis Sanakis, and Pericles Stavropoulos, **A Versatile Tripodal Cu(I) Reagent for C–N Bond Construction via Nitrene-Transfer Chemistry: Catalytic and Mechanistic Insights on C–H Aminations/Amidinations and Olefin Aziridinations** [J]. ISSN: 0002-7863, *JACS, J. Am. Chem. Soc.* 2014, 136, 11362 - 11381

ORAL PRESENTATIONS (17)

Primary Oral Presentations (8)

1. Lingyu Chi, **New High-field Toroid Cavity NMR Methodology to Analyze the Pore-size Distribution in Monolithic Gas-shale Materials**, Comprehensive Exam, December 10th, 2015, Faculty conference room, Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri, USA

2. Lingyu Chi, Rex E. Gerald II, and Klaus Woelk, **Inspire and Empower Students at Missouri S&T With New NMR Tools**, Miner Talk of Innovation Proposals at Missouri S&T, October 15th, 2015, Carver Turner Room, Havener Center, Missouri University of Science and Technology, Rolla, Missouri, USA

3. Lingyu Chi, **CapPack Devices for Enhanced qNMR Measurements in ^{1}H NMR Spectroscopy**, Master's Thesis Defense, July 7th, 2015, Faculty conference room, Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri, USA

4. Lingyu Chi, Jie Huang, Ming Huang, Rex E. Gerald II, Klaus Woelk and Eric Anderson, **CapPack Devices**, MISSOURI TECH EXPO 2014, October 16th, 2014, Christopher S. Bond Life Sciences Center, Columbia, Missouri, USA *(invited)*

5. Lingyu Chi, Rex E. Gerald II, and Klaus Woelk, **New CapPack Devices for NMR and MRI Markets**, Ignite Rolla Meeting, February 26th, 2014, St. Pat's Ballroom C, Havener Center, Missouri University of Science and Technology, Rolla, Missouri, USA *(invited)*

6. Lingyu Chi, Rex E. Gerald II, and Klaus Woelk, **Toroid Cavity Detectors and Imagers**, Branson NMR Meeting, February 23rd, 2014, The Suites at Fall Creek Resort, Branson, Missouri, USA *(invited)*

7. Lingyu Chi, Klaus Woelk, Rex E. Gerald II, Annalise R. Pfaff, Ming Huang, Jie Huang, and Emmalou T. Satterfield, **New Quantitative NMR Techniques for Determining the Product Yield of Hydrothermal Biomass to Fuel Reaction**, Chemistry Seminar, December 2nd, 2013, Rm 126, Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri, USA

8. Lingyu Chi, Klaus Woelk, Rex E. Gerald II, Robert J. Klingler, Perry Novak, Annalise R. Pfaff, Ming Huang, Jie Huang, Emmalou T. Satterfield, and Ariel Mollhagen, **Cap-Pack Devices for Quantitative NMR/MRI Investigation**, 2013 Chicago Area Discussion Group, November 9th, 2013, TCS Conference Center, Argonne National Lab, Chicago, Illinois, USA *(invited)*

Co-authored Oral Presentations (9)

1. Rex E. Gerald II, Klaus Woelk, Jie Huang, Ming Huang, and Lingyu Chi, **Advanced Applications of Quantitative NMR Using Toroid Cavity Detectors**, April 26th 2018, Department of Chemistry, Clemson University, Clemson, SC.

2. Klaus Woelk, Robert J. Klingler, Rex E. Gerald II, Lingyu Chi, and Ming Huang**, From NMR Relaxation Experiments to High-resolution NMR Relaxometry**, Organic Chemistry Seminar Series, December 26th, 2015, Department of Chemistry, University of Missouri, Columbia, MO.

3. Klaus Woelk, Robert J. Klingler, Rex E. Gerald II, Lingyu Chi, and Ming Huang, **From Inversion Recovery and CPMG to High-resolution NMR Relaxometry**, 2015 American Chemical Society (ACS) Saint Louis Award Symposium and Banquet, December 16th, 2015, Washington University, St. Louis, MO.

4. Klaus Woelk, Robert J. Klingler, Rex E. Gerald II, Lingyu Chi, and Ming Huang, **From Standard NMR Relaxation Experiments to High-resolution Relaxometry**, Chemistry Department Seminar, September 28th 2015, Rm 126, Schrenk Hall, Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri, USA

5. Klaus Woelk, Robert J. Klingler, Rex E. Gerald II, Lingyu Chi, and Ming Huang, **A Revised NNLS Approach to High-Resolution NMR Relaxometry**, 56th Experimental NMR Conference (ENC), April 23rd, 2015, Pacific Grove, CA.

6. Pericles Stavropoulos, Vivek Bagchi, Patrina Paraskevopoulou, Purak Das, Lingyu Chi, Qiuwen Wang, Richard Dawes, and Rex E. Gerald II, **Versatile Catalytic Systems for C–N Bond Construction via Nitrene-Transfer Chemistry,** Chemistry Department Seminars, April 21st, 2014, Rm 126, Schrenk Hall, Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri, USA

7. Rex E. Gerald II, Klaus Woelk, Baojun Bai, Lingyu Chi, Ming Huang, and Annalise R. Pfaff, **Subdivision of Zeeman NMR Fields by Static and Dynamic Magnetic Field Distributions and Capillary Tube Compartments: Applications of Quantitative NMR/MRI Employing Cap-Pack Devices**, CM/Bio/ECE Seminars, Oct. 30th 2013, Rm 233A, Physics Bldg., Department of Physics and Astronomy, University of Missouri – Columbia, Columbia, Missouri, USA (*invited*)

8. Rex E. Gerald II, Klaus Woelk, Baojun Bai, Lingyu Chi, Ming Huang, and Annalise R. Pfaff, **Applications of Quantitative NMR/MRI Employing Cap-Pack Devices**, MIT *NewNMRGroup*, October 15th, 2013, Department of Chemistry Instrumentation Facility, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA (*invited*)

9. Rex E. Gerald II, Klaus Woelk, Baojun Bai, Lingyu Chi, and Hao Zhang, **Topic in Nuclear Magnetic Resonance Spectroscopy and Imaging**, Chemistry Department Seminars, February 21st, 2011, G3, Schrenk Hall, Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri, USA

POSTER PRESENTATIONS (19)

International Conference Poster Presentations (11)

1. Rex E. Gerald II, Jie Huang, Klaus Woelk, William Stocker, Lingyu Chi, Ming Huang, and Sean Cartwright, **Acupuncture-MRI Skin Cancer Probe**, 59th EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, April 30th – May 5th, 2018, Hyatt Regency Grand Cypress, Orlando, Florida, USA

2. Lingyu Chi, Ming Huang, Annalise R. Pfaff, Rex E. Gerald II, Jie Huang, and Klaus Woelk, **Optimizing NMR hardware and pulse sequences with CapPack devices**, 59th EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, April 30th – May 5th, 2018, Hyatt Regency Grand Cypress, Orlando, Florida, USA

3. Robert J. Klingler, Lingyu Chi Rex E. Gerald II, and Klaus Woelk, **A Revised NNLS Approach to High-Resolution NMR Relaxometry**, 56th EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, April 19th – 24th, 2015, Asilomar Conference Grounds, Pacific Grove, California, USA

4. Lingyu Chi, Robert Block, Sierra Herndon, Rex E. Gerald II, and Klaus Woelk, **Integration Standard for MAS-NMR Investigations**, 56th EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, April 19th – 24th, 2015, Asilomar Conference Grounds, Pacific Grove, California, USA

5. Lingyu Chi, Jie Huang, Ming Huang, Rex E. Gerald II, and Klaus Woelk, **Two CapPack Devices for Solution and Solid State NMR Applications**, 55th EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, March 23rd – 28th, 2014, Boston, Massachusetts, USA

6. Ming Huang, Lingyu Chi, Rex E. Gerald II, and Klaus Woelk, **Development of *In Situ* NMR pH Meter**, 55th EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, March 23rd – 28th, 2014, Boston, Massachusetts, USA

7. Lingyu Chi, Rex E. Gerald II, and Klaus Woelk, **Quantitative NMR and MRI using Toroid Cavity Detectors and Imagers**, 2nd PRACTICAL APPLICATIONS OF NMR IN INDUSTRY CONFERENCE, February 3rd – 5th, 2014, Charlotte, North Carolina, USA

8. Lingyu Chi, Annalise Pfaff, Rex E Gerald II, and Klaus Woelk, **Absolute qHNMR Calibration and the Use of Cap-Packs to Assess the Effectiveness of EXCEPT-20 Water-Signal Suppression**, 54th EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, April 14th – 19th, 2013, Asilomar Conference Grounds, Pacific Grove, California, USA

9. Lingyu Chi, Klaus Woelk, and Rex E Gerald II, **Quantitative HNMR Tests for Determining the Mass percentage of Small Molecules in Biomass Conversion Reactions**, 1st PRACTICAL APPLICATIONS OF NMR IN INDUSTRY CONFERENCE, October 15th – 17th, 2012, Schaumburg, Illinois, USA

10. Lingyu Chi, Justin A. Cobb, Ariel Mollhagen, Douglas K. Ludlow, Klaus Woelk, and Rex E Gerald II, **Quantitative NMR to Determine the Kinetics of the Hydrothermal Degradation of D-glucose**, 53rd EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, April 15th – 20th, 2012, Miami, Florida, USA

11. Rex E Gerald II, Lingyu Chi, and Klaus Woelk, **Spin-Lattice Relaxation Time-Constant Distributions of Heterogeneous Samples Used as Models for Shale**, 53rd EXPERIMENTAL NUCLEAR MAGNETIC RESONANCE CONFERENCE, April 15th – 20th, 2012, Miami, Florida, USA

Regional Conference Poster Presentations (7)

1. Lingyu Chi, Ming Huang, Annalise R. Pfaff, Rex E. Gerald II, Jie Huang, and Klaus Woelk, **Optimizing NMR hardware and pulse sequences with CapPack devices**, 2018 *Council of Graduate Students* (CGS) Graduate Research Showcase, April 25th, 2018

2. Lingyu Chi, Rex E. Gerald II, and Klaus Woelk, **Fundamentals of Rotating Frame Imaging in Toroid Cavity Detectors**, 2014 Midwest Regional Meeting of the American Chemistry Society, November 12th –15th, 2014, Columbia, Missouri, USA

3. Lingyu Chi, Ming Huang, Rex E. Gerald II, and Klaus Woelk, **Calibration CapPack Devices for Magic Angle Spinning (MAS) NMR Measurements**, 2014 Midwest Regional Meeting of the American Chemistry Society, November 12th –15th, 2014, Columbia, Missouri, USA

4. Lingyu Chi, Rex E. Gerald II and Klaus Woelk, **Advanced CapPack Devices: Multiple Functions for Quantitative NMR Applications**, 2014 *Council of Graduate Students* (CGS) Graduate Research Showcase, April 10th 2014, Havener Center Atrium, Missouri University of Science and Technology, Rolla, Missouri, USA

5. Lingyu Chi, Justin A Cobb, Rex E. Gerald II and Klaus Woelk, **Two Applications of NMR Spectroscopy for Future Fuels**, 2012 *Council of Graduate Students* (CGS) Graduate Research Showcase, April 2012, Havener Center, Missouri University of Science and Technology, Rolla, Missouri, USA

6. Justin A. Cobb, Lingyu Chi, Rex E Gerald II, Klaus Woelk, and Douglas K. Ludlow, **Recent Improvements in Hydrothermal Biomass Conversions to Obtain Liquid Fuel Precursors**, 2012 *Council of Graduate Students* (CGS) Graduate Research Showcase, April 2012, Havener Center, Missouri University of Science and Technology, Rolla, Missouri, USA

7. Lingyu Chi, Rex E Gerald II and Klaus Woelk, **Low- Field NMR Spin-Lattice Relaxation Time-Constant Distributions of Shale**, 46th Midwest 139th Great Lakes Joint Regional Meeting of the American Chemistry Society, October 19th – 22nd, 2011, Saint Louis, Missouri, USA

<u>HONORS & ACTIVITES</u> (Professional)

- 2014 MISSOURI S&T Women Student of the Year Gold Award
  Oct. 13th 2014
- *Council of Graduate Students* (CGS) Travel Grant Jan. – June 2014
- 2nd PANIC Conference Student Travel Grant Feb. 2nd – 5th 2014
- Chemistry Department Outstanding Graduate Teaching Assistant Award
  Jan. 2013 – Jan. 2014
- 54th ENC Conference Student Travel Recipient Apr.14th– 19th 2013
- 1st PANIC Conference Student Travel Grant Oct. 15th – 17th 2012
- Chemistry Department Outstanding Graduate Teaching Assistant Award
  Jan. 2011 – Jan. 2012

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VITA

Lingyu Chi was born in Tianjin, China. In June 2009, she obtained a bachelor's degree in the Department of Biological Engineering, College of Bio-technology and Food Science from Tianjin University of Commerce, Tianjin, P.R. China.

In January 2011, she enrolled at Missouri University of Science and Technology (Missouri S&T) to pursue a doctoral degree of Physical Chemistry under the guidance Dr. Klaus Woelk. Her research interest is in magnetic resonance spectroscopy/imaging research and design.

In January 2012 and January 2014, she won the chemistry department outstanding graduate teaching assistant award in the department of Chemistry from Missouri S&T; In October 2014, she won the 2014 MISSOURI S&T Women Student of the Year Gold award.

In August 2015, she obtained a master degree with thesis "CapPack devices for enhanced qNMR measurements in ^{1}H NMR spectroscopy" in Chemistry from Missouri S&T.

During the seven academic years, from 2011 to 2018, at Missouri S&T, she published three peer reviewed scientific papers, one issued US patent; accomplished 17 oral presentations, and eight of those were presented as primary presenter; she also accomplished 19 international and regional conference poster presentations, and 11 of those were presented as first author.

In December 2018, she received her PhD degree in Chemistry from Missouri S&T.