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Capillary-tube package devices for the quantitative performance evaluation of nuclear magnetic resonance spectrometers and pulse sequences

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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 Capillary-tube Package (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 the excitation profiles of shaped pulses. One example illustrates the performance of hyperbolic secant (sech) pulses in the EXponentially Converging Eradication Pulse Train (EXCEPT) solvent suppression sequence. The excitation profile of the pulse sequence is obtained in a single gradient NMR experiment. 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 that 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. *Published by AIP Publishing.* https://doi.org/10.1063/1.5052374

I. 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 reference 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 **Cap**illary-tube **Pack**ages. 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

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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 and understand how NMR probes, hardware, software, and pulse programs work. CapPacks can be arranged in different geometries such as side-by-side (see Sec. II B) or clustered (see Sec. II C). For example, Fig. 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 can be used for 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 and 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 for high-resolution, high-field NMR spectroscopy.[8] Two different CapPack devices, the 10-capillary-tube side-by-side Gradient CapPack device (Fig. 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 demonstrate 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 instruments (<20 ns). Furthermore, the EXCEPT solvent-suppression sequence was developed to avoid pulse-sequence parameter adjustments even if the solvent's T_1 time changes by 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]

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

II. 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 is sealed using an optical-fiber fusion splicer (see Sec. II A). 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 5–20 cm-long capillary tubes that contain, depending on the I.D. and length of the capillary tube, between 2 and 40 μl of the isolated, NMR-sensitive reference material.

A. 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. In addition, 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 guide of the fusion splicer while it is still connected to the syringe that is filled with the reference sample. Each open

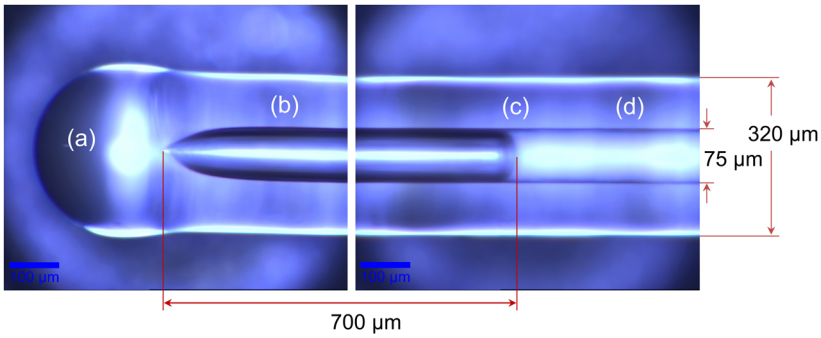

FIG. 2. Photograph of a 320-μm O.D. fused-silica capillary tube sealed using the melt-back method by using a commercial arc fusion splicer. The polyimide coating of the capillary was removed about 1 mm from the end of the capillary before applying the arc. (a) Glass-sealed end, (b) head-space gas volume,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

end of the capillary tube is stripped off of the polyimide coating a distance of about 1 mm from the end before sealing, which protects the fusion splicer components (e.g., electrodes and 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 the pictures obtained with a near-field optical scanning microscope (WiTec Instruments Corp., Knoxville, TN) of the end of a capillary tube filled with a reference solution. The four major areas identified in the pictures are (from left to right) (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.

B. 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 the 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 (10) that can be mounted side-by-side and inserted into a standard 5-mm NMR tube with an 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 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

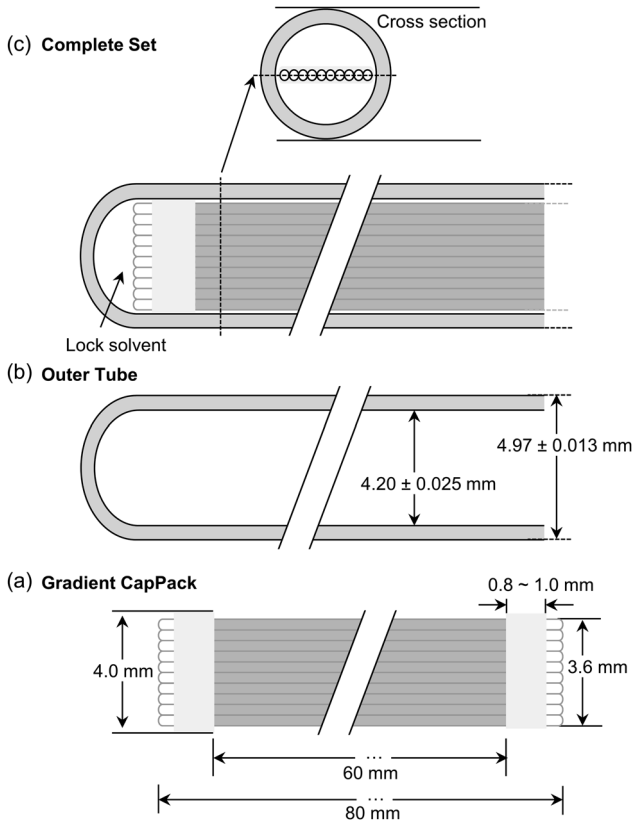

FIG. 3. Schematic drawings of a 10-capillary-tube Gradient CapPack: (a) side-by-side assembly of 10 glass-sealed capillary tubes fixed with Parafilm on both ends, (b) dimensions of a typical 5-mm NMR tube, and (c) a Gradient CapPack mounted inside the 5-mm sample tube for NMR performance measurements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

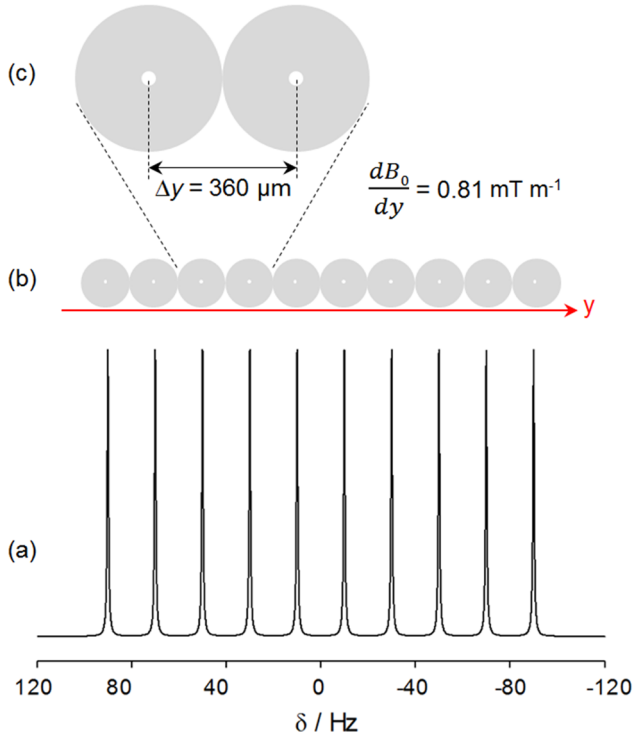

FIG. 4. Schematic drawing and predicted spectrum of an in-plane side-by-side Gradient CapPack in a magnetic field gradient: (a) computer simulation of a spectrum of a 10-capillary-tube Gradient CapPack device in a constant magnetic field gradient obtained with a single hard pulse. The field gradient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

the spectrum of Fig. 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 shown in the zoomed-in view of Fig. 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, not only depends 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 spins in the reference material. In addition, it must be realized that the signals of Fig. 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 makes it possible to accurately and conveniently evaluate the 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 Sec. III A, the Gradient CapPack shown in Fig. 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 (>200 ms), frequency-selective (<200 Hz) inversion pulses such as the adiabatic hyperbolic secant pulse HS1.[10]

C. T_1 CapPack device

A T_1 CapPack device is designed to evaluate the effects that 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 ^{1}H 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

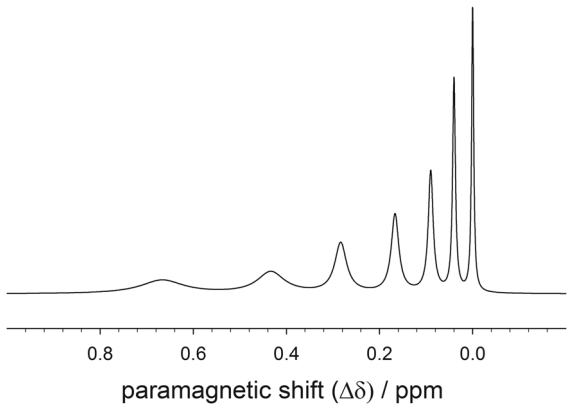

FIG. 5. Computer simulation of a spectrum recorded with seven capillary tubes containing aqueous solutions with different amounts of the paramagnetic relaxation agent $CuSO_4$. The concentration-dependent change in relaxation time coincides with a concentration-dependent paramagnetic shift of the solvent signals. Consequently, the chemical shift axis can also be viewed 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.

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. T_1 CapPacks are best suited to explore and quantify the effects that spin relaxation has on magnetizations and coherences during the execution of NMR pulse 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 Fig. 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), which was 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.

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 capillary 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]).

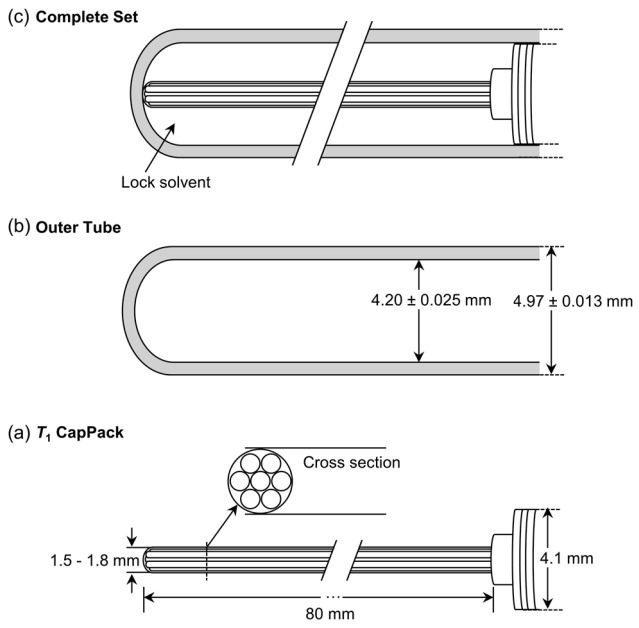

FIG. 6. Schematic drawing of a seven-capillary-tube T_1 CapPack: (a) clustered assembly of seven glass-sealed capillary tubes inside a larger capillary tube (micro-capillary with O.D. between 1.5 and 1.8 mm). The cross section of the insert shows the hexagonal setting of the seven capillary tubes in the larger capillary tube, (b) dimensions of a typical 5-mm NMR tube, and (c) a 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.

III. RESULTS 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.

A. Gradient CapPack experiments

The 10-capillary-tube Gradient CapPack device (Fig. 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-d_6 (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-d_5 (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 Sec. II B, the experimental 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 spectrum in Fig. 7(b) and the spectra of Figs. 7(c) and 7(d) are displayed with a 10-fold magnification of the spectral intensity. While the capillary signals become separated into individual

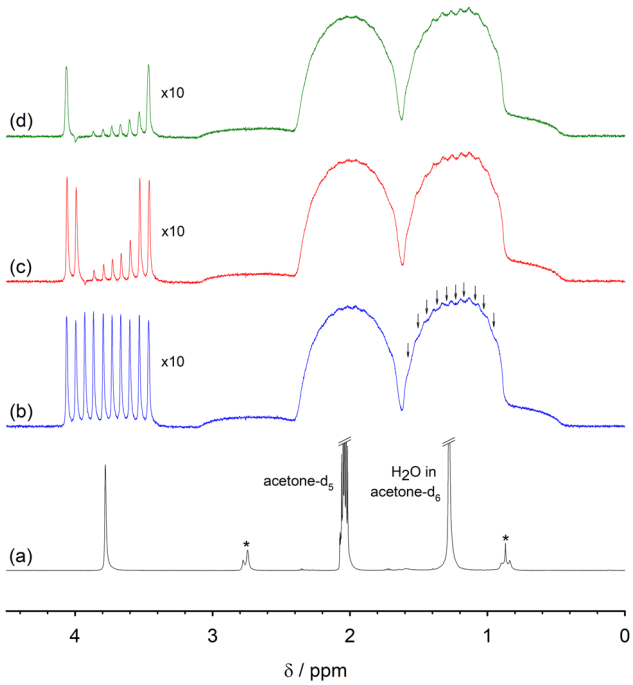

FIG. 7. ^{1}H NMR spectra recorded from a 10-capillary-tube Gradient CapPack contained in a 5-mm sample tube using acetone-d_6 (99.8%) as the surrounding solvent: (a) with a standard 90° observe pulse, (b) with the 90° observe pulse while a 0.81 G cm^{-1} gradient is applied across the 10 side-by-side capillary tubes, (c) with observe pulse following the application of a series of 400-ms HS1 pulses in an EXCEPT-12 experiment, and (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)–(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 results from sample displacements by the 10 capillary tubes of the Gradient CapPack.

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 Fig. 7(b) point to small semi-circular voids in one of the projection profiles which occur as a result of sample displacements from the side-by-side capillary tubes. Figures 7(c) and 7(d) show gradient spectra 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 Fig. 7(c) and 300 ms in the experiment of Fig. 7(d) leading to suppression bandwidths of 90 Hz and 120 Hz, respectively. As seen in the spectrum of Fig. 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 instruments (<20 ns), may lead to suppression results that are less effective than predicted by the corresponding theory. Comparisons between Figs. 7(c) and 7(d) also reveal that the asymmetry and insufficient suppression increase with decreasing HS1 bandwidth, i.e., with longer HS1 pulses. The results of the EXCEPT-12 sequence experiments using adiabatic pulses shown in Fig. 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.

B. T_1 CapPack experiments

The T_1 CapPack device described in Sec. II C 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 I.

Figure 8(a) shows the ^{1}H 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 Sec. III A) with the frequency-selective HS1 pulses in the DRX spectrometer, the T_1 CapPack EXCEPT-12 experiments were first executed with rectangular hard pulses. Consequently, the spectral bandwidth of suppression covers the entire range of the ^{1}H spectrum. Figures 8(b)–8(d) show spectra recorded with different delay adjustment factors f_{da} in the EXCEPT sequence (i.e., f_{da} = 0.5, 0.05, and 0.005, 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 to 10 s in T_1 relaxation time to the range of $f_{da} \times 1$ s to $f_{da} \times 10$ s. The spectra in Figs. 8(b)–8(d) demonstrate how adjusting f_{da} changes the nominal T_1 suppression range, leading to the suppression of most capillary-tube signals or, if set correctly, to the nearly complete suppression of all capillary-tube signals. The spectrum in Fig. 8(c) also shows that even signals outside the intended

TABLE I. Concentrations of the paramagnetic relaxation agent $CuSO_4$ and the independently measured T_1 relaxation times of water 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

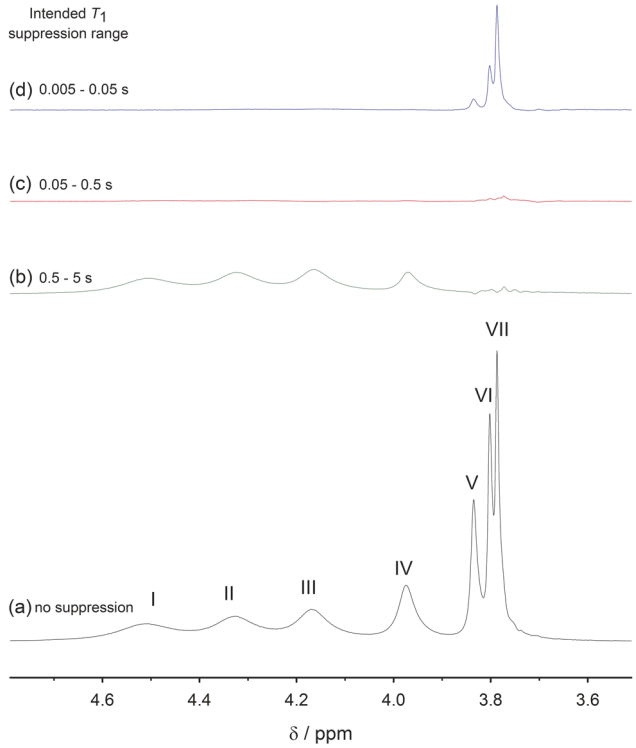

FIG. 8. ^{1}H NMR spectra from a seven-capillary-tube T_1 CapPack device: (a) obtained with a standard observe pulse; labels in the spectrum refer to the $CuSO_4$ concentrations and T_1 relaxation times reported in Table I, (b) obtained after the application of EXCEPT-12 adjusted for long T_1 times from 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.

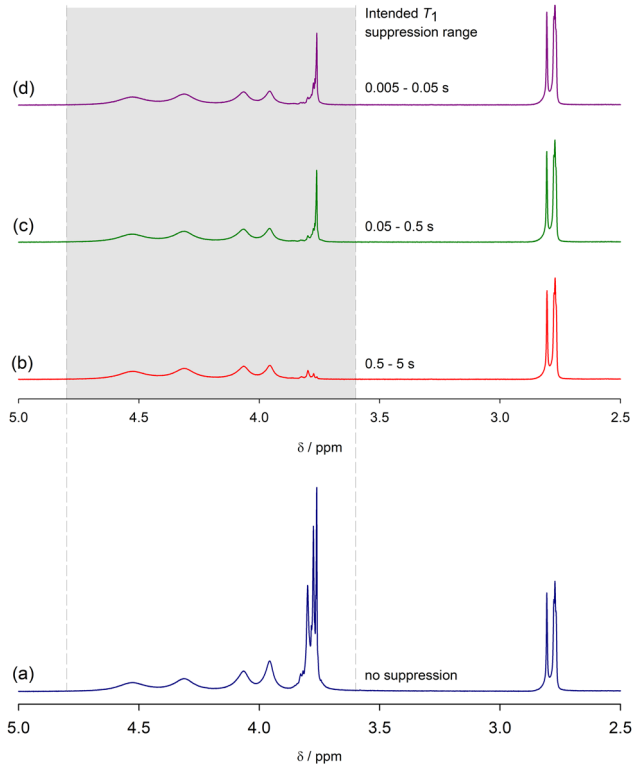

FIG. 9. ^{1}H NMR spectra recorded from a T_1 CapPack device: Spectrum (a) was recorded with a standard 90° observe-pulse experiment. Spectra (b)–(d) were recorded from EXCEPT-12 experiments using 125-ms adiabatic HS1 pulses resulting in a suppression bandwidth of 240 Hz applied at 4.2 ppm (shaded area,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

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 Fig. 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 Fig. 9) covers all seven T_1 CapPack resonances. Spectra (b)–(d) in Fig. 9 reveal that none of the delay adjustment factors (i.e., $f_{da} = 0.5$, 0.05, and 0.005) led to a suppression of signals with relaxation times below 50 ms (i.e., sample capillaries I–IV in Table I). The T_1 CapPack experiments that led to the spectra in Figs. 8 and 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.

IV. 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 device and a seven-capillary-tube clustered T_1 CapPack device 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, was 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 their location during an NMR experiment due to Brownian motion, i.e., molecular diffusion. While this is not usually 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 CapPack devices.

SUPPLEMENTARY MATERIAL

See supplementary material for additional evidence that the asymmetrical suppression profiles reported in Sec. III A (Gradient CapPack Experiments) and shown in Fig. 7 are not the result of an irregularity in the manufacturing of the Gradient CapPack, nor are they the result of the shim-coil gradient application or the resonance offset at which the shaped pulses were applied.

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