Journal of Magnetic Resonance 197 (2009) 186–192

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Design and construction of a versatile dual volume heteronuclear double resonance microcoil NMR probe

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ARTICLE INFO

Article history:
Received 13 September 2008
Revised 16 December 2008
Available online 25 December 2008

Keywords:
^{1}H NMR
^{13}C NMR
Microcoil
Dual volume probe
High throughput
Double resonance
HETCOR
Metabolite-profiling

ABSTRACT

Improved NMR detection of mass limited samples can be obtained by taking advantage of the mass sensitivity of microcoil NMR, while throughput issues can be addressed using multiple, parallel sample detection coils. We present the design and construction of a double resonance 300-MHz dual volume microcoil NMR probe with thermally etched 440-nL detection volumes and fused silica transfer lines for high-throughput stopped-flow or flow-through sample analysis. Two orthogonal solenoidal detection coils and the novel use of shielded inductors allowed the construction of a probe with negligible radio-frequency cross talk. The probe was resonated at ^{1}H–^{2}D (upper coil) and ^{1}H–^{13}C (lower coil) frequencies such that it could perform 1D and 2D experiments with active locking frequency. The coils exhibited line widths of 0.8–1.1 Hz with good mass sensitivity for both ^{1}H and ^{13}C NMR detection. ^{13}C-directly detected ^{2}D HETCOR spectra of 5% v/v ^{13}C labeled acetic acid were obtained in less than 5 min. Demonstration of the probe characteristics as well as applications of the versatile two-coil double resonance probe are discussed.

1. Introduction

Microcoil NMR [1,2] probes address the need to increase the intrinsic sensitivity of NMR for small samples. Smaller diameter detection coils increase the signal-to-noise ratio (SNR) for mass limited samples, thus improving the mass sensitivity [3–7]. The use of microcoils with solenoidal geometry results in an additional increase in sensitivity because they capture more magnetic flux than Helmholtz geometry coils. The construction of solenoidal microcoil NMR probes with flow-through [4] or sealed-sample [8,9] designs provides improved sensitivity and good resolution for small and low concentrated samples as little as nanoliters to several microliters [10]. With improved sensitivity, microcoil flow-NMR can be used to advantage in conjunction with a variety of chromatographic techniques [11–16]. In particular, the use of microcoil NMR probes provides a better match to the sample volumes that elute off chromatographic columns, resulting in a significant improvement in hyphenated NMR techniques.

^{1}H NMR has been main focus of the initial microcoil NMR work, and single resonance circuits with single or multiple sample microcoils have been employed to acquire 1D and 2D homonuclear data with improved sensitivity and good resolution [17–20]. The development of probes that incorporate multiple microcoils, such as Multiplex NMR [17,19,20] and two to eight-coil systems [18,21,22] has been driven by a desire to achieve higher sample throughput. Multiply tuned resonance circuits employing single sample coils that are based on a variety of circuit designs [23,24] have also been used in microcoil probes to acquire 1D and 2D homonuclear experiments [16,25]. However, little work has been done to date to employ multiple resonance circuits in conjunction with multiple sample microcoils. One example is the work by Zhang et al. [9], who designed a 15 μL observe-volume two-coil probe that operated at ^{15}N and ^{1}H frequencies. Both sample coils were mounted at the same height in this probe. The authors used the probe to demonstrate the acquisition of COSY and HMQC spectra at 500-MHz from two sealed-samples in the same time that it takes to acquire a single spectrum with a standard probe-head. There are a variety of ways to expand on this multiple coil, multiple frequency microcoil NMR idea, such as different observe-volumes (smaller for mass limited samples, potentially larger for concentration limited samples); different frequencies between the multiple coils for variety of experiments; inclusion of a lock frequency for maintaining the line shapes for longer acquisitions; and addition of sample transfer lines for both analysis of flow-samples and easier hyphenation of NMR with other analytical tools.

With these incentives, we report the construction and characterization of a versatile double resonance 300-MHz dual volume double resonance microcoil NMR probe. The upper coil is tuned to ^{1}H/^{2}D frequencies and the lower coil to ^{1}H/^{13}C frequencies. The observe-volumes of both the coils are 440 nL. The oval sample cell has been designed to improve the fill factor using a quick-thermal-etching technique that requires only 10 min to etch a single cell. The detection coils with different multiple resonances allow

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doi:10.1016/j.jmr.2008.12.020

for homonuclear and heteronuclear 1D or 2D NMR experiments for structural analysis. A ^{2}D lock channel has been incorporated into the resonant circuit of the upper coil to improve the line shape for longer 2D acquisitions on either sample coil. A variety of experiments can be performed by using one or both of the ^{1}H/^{13}C frequencies, and a combination of experiments can be run with or without the lock.

2. Methods

2.1. Detection cell fabrication

Detection cells for the probe were created using a thermal HF etching technique that is similar in principle to one described in previous work [26,27]. However, a quick-etch technique very similar to that described in our previous publication [28] was applied and is summarized in Fig. 1. Briefly, 0.5 cm of the middle portion of a 7–8 cm section of 1.8 mm OD, 127 μm ID fused silica glass tubing (Polymicro, Phoenix, AZ) was wound with 30 AWG Nichrome wire (10 cm long, six turns). Thermal etching was performed by passing a 2 A current from a voltage source (Beckman Industrial, Taiwan) and simultaneously flowing 48% HF (Mallinckrodt AR, Phillipsburg, NJ) through the glass tubing using a Harvard syringe pump (Harvard Apparatus, Holliston, MA). The pump was programmed for a 2 min infuse and 2 min refill, and then three alternating cycles of 1 min infuse and 1 min refill, thus resulting a total etch time of 10 min. Each end of the 0.5 cm center-etched section of the glass tubing was cooled with chilled nitrogen gas resulting in an enlarged etched volume of approximately 1 μL. The etched tube was bent into a U-shape with a torch. At this point, the tip of a 65–70 cm length of fused silica capillary tubing (360 μm OD, 70 μm ID) was inserted into each end of the sample holder and glued using polyimide sealing resin (Supelco, Bellefonte, PA). There was no need to etch the two ends of the sample tube with above protocol to fit the transfer line since the ends were being etched sufficiently from the infuse/refill flow of heated HF during the etching period. The second detection cell was etched, bent and glued with transfer lines similarly.

2.2. Probe construction

A standard bore (40 mm OD) probe body was constructed in a similar fashion to our previous work [28]. For each detection cell, two and one-half turns using two parallel wires (~40 nH with 2 cm leads), were manually wound with 150 μm OD round copper wires (California Fine Wire, Grover Beach, CA) and affixed to the sample cell using Quicktite© superglue (Henkel Loctite, Rocky Hill, CT). The coil length of 0.8 mm created an active sample volume of 440 nL. The two sample cells were then placed one cm apart and oriented orthogonally on a specially designed U-shaped Ultem plastic support (Fig. 2). AutoDesk Inventor Professional 2008 software was used to design the spatial layout of the rf coils, sample container and structural elements of the probe.

The upper and lower coils were tuned and matched using the double resonance RF design illustrated in Fig. 3, which features LC trap and pass elements tuned for use at 7 T. A 5 cm, 0.181 in OD, 50 Ω semi-rigid coaxial cable (Haverhill Manufacturing, Haverhill, MA) was used to connect the coils to the rest of the resonant circuit, and with this transmission line in place, the final coil/lead inductance was measured to be ~100 nH. Fixed value capacitors (ATC, Huntington Station, NY) and shielded 5 mm tunable inductors (Coilcraft, Cary, IL) were used in the trap and pass circuit elements, whereas fixed and tunable capacitors (0.1–9 pF, Voltronics, Denville, NJ) were used to provide tuning and matching. A single coil double resonance circuit tuned and matched to ^{1}H/^{2}D frequencies (300 and 46.05 MHz) is shown in Fig. 3. The circuit is based on previous NMR resonant circuit designs [16,23,25] and incorporates a high frequency trap (10 pF, 28 nH), low band pass filter (66 pF, 79 nH), and a variable length transmission line [28,29] that is used both to tune the circuit and to connect the sample coil leads to the other circuit components. A similar circuit design (not shown) with different capacitor and inductor values was used for tuning and matching the ^{1}H/^{13}C (75.44 MHz) frequencies used for the lower detection coil. Each of the coil components was placed at the same level and connected to their respective detection coil with 5 cm transmission lines. Tunable non-magnetic shielded inductors (Coilcraft, Cary, IL) were used both to provide flexibility in tuning the trap and pass components to their respective frequencies, and to minimize the rf cross talk of the components. Shielded inductors have been widely used commercially, mainly in microwave and tele-communication circuit designs to configure traps, as tuning elements, and to prevent magnetic coupling and rf interferences that is especially important in densely packed circuit boards [30–32]. It proved advantageous to incorporate this concept while putting together all the tuning, matching, trapping and passing elements for the four resonances in a single probe circuit board of 38 mm circumference. In addition, a grounded copper sheet was placed between the reactive components of the two circuits to minimize the rf cross talk. As a result of this shield and the use of shielded inductors, it was relatively straightforward to keep the proton resonances from interacting. Finally, the detection cells and their transmission lines were

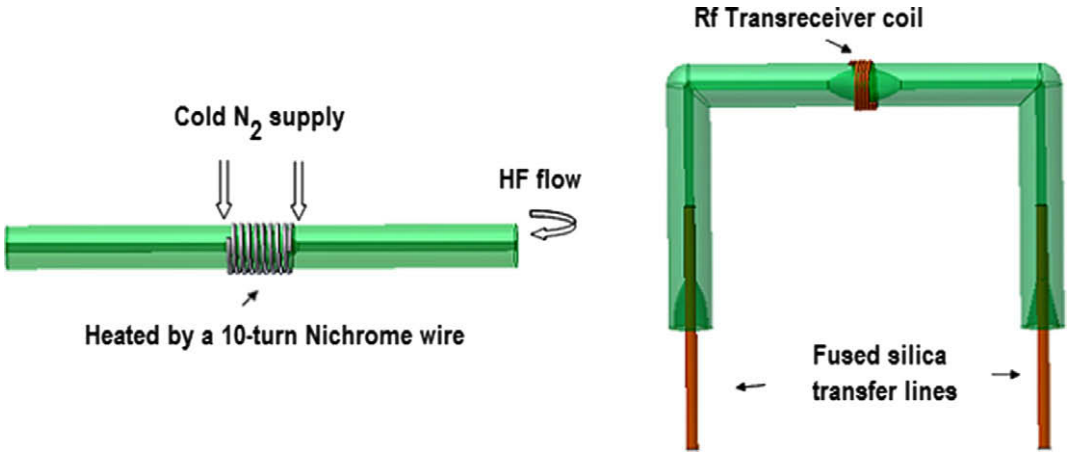

Fig. 1. Quick-thermal etching procedure for creating capillaries showing the main components used to create the sample detection region. A detailed description of the procedure is provided in the text. The result is an enlarged oval-shaped detection volume within the probe coil.