JOURNAL OF MAGNETIC RESONANCE **133,** 227–231 (1998)
ARTICLE NO. MN981450

RF Microcoil Design for Practical NMR of Mass-Limited Samples

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Received September 5, 1997; revised March 18, 1998

This paper addresses practical issues involved in obtaining high resolution ^{1}H NMR spectra from samples containing less than 10 nmol. Solenoidal microcoils have been constructed to: (a) assess the effects of magnetic susceptibility mismatches at 500 MHz, (b) increase the concentration sensitivity of microcoil probes, (c) incorporate a lock channel for 2D experiments and long 1D acquisitions, and (d) assess the total amount of the sample required (with respect to the coil length) to avoid line broadening due to edge effects. Compared to previously published microcoil results, sample volumes have been increased by a factor of 20 with a concomitant decrease in the required concentration (5–20 mM). Perfluorocarbon susceptibility matching remained effective at 500 MHz, allowing acquisition of high resolution NMR spectra. A lock channel has also been successfully incorporated in microcoil probes. The limits of detection for sucrose with a 10 min acquisition time were found to be 17.8 and 34.1 pmol for the single and double resonance coils, respectively. A sample length of approximately 10 times than that of the coil was required to avoid magnetic susceptibility artifacts.

Key Words: **microcoils; nanovolume NMR; mass-limited samples; solenoids; magnetic susceptibility.**

It has long been recognized that the mass sensitivity of NMR detection coils improves as the coil diameter is reduced (*1, 2*). However, practical implementation of high resolution NMR using coils in the submillimeter range requires some form of magnetic susceptibility matching to obtain narrow linewidths. By immersing the coil in a perfluorocarbon Olson *et al.* (*3*) were able to obtain a linewidth of 0.6 Hz for neat ethyl benzene using a solenoidal coil 357 μm in diameter with an observe volume (V_{obs}, the volume enclosed by the microcoil) of 5 nl. Operating at 300 MHz, they showed that the limits of detection (LOD) for sucrose in this coil were 18.8 pmol for a 10 min acquisition time. This represented a mean mass sensitivity enhancement of ~130 relative to a conventional 5-mm (~275 μl) NMR probe. Despite these impressive results and subsequent improvements in microcoil filling factor (*4*), a number of issues remain to be addressed for practical experiments on mass-limited samples. First, the solenoidal coils constructed so far have used a long tube to fill the observe volume. This creates a very low observe factor (defined as the ratio of coil observe volume to the total volume of loaded sample) since most of the loaded solution remains in the tube. Reducing the effective sample length to match the coil dimensions raises the observe factor but can lead to susceptibility artifacts due to sample/air interfaces. Realistic sample volumes that can be handled practically are typically greater than 200 nl, and the size of the coil should be chosen to reflect this value. An advantage in increasing the V_{obs} is that it allows more realistic solute concentrations in the 1–20 mM range to be used rather than hundreds of mM reported in previous microcoil studies. Second, to perform 2D NMR experiments or 1D experiments with long acquisition times, a lock channel should be incorporated and the efficiencies of both the ^{1}H and ^{2}H channels optimized. Finally, it is desirable to operate at high frequencies to take advantage of the gain in sensitivity and the increased chemical shift dispersion. The potential barrier to using a higher field is an increase in magnetic susceptibility effects that could lead to increased linewidths. These three issues are addressed in this paper.

Three different types of microcoil probes were constructed. Probe A is a single resonance flowthrough probe tuned to 499.89 MHz and built as described previously (*3, 4*). The sample is injection loaded from outside the magnet. Probe B is a double resonance flow probe with the solenoid tuned to both ^{1}H and ^{2}H (76.7 MHz) frequencies. Probe C is a single resonance probe with a limited amount of analyte (typically 300 nl–2 μl) which is preloaded into the capillary and sealed before the microcoil is wound. The probe schematics are shown in Fig. 1 and the descriptions are summarized in Table 1.

A detailed description of the fabrication of solenoidal microcoils has been previously published (*3, 4*) and is touched upon only briefly in this section. The coils were wound directly on a 700-μm outer diameter (O.D.)/530-μm inner diameter (I.D.) polyimide coated silica capillary (Polymicro Technologies, Phoenix, AZ). For the single resonance probes, there are 12 closely wound turns of 50-μm-diameter H-poly red coated copper wire (California Fine Wire Co., Grover Beach, CA), creating an observe volume of 131 nl. The double resonance probe consisted of 8 closely wound turns of 50-μm-diameter wire, giving a volume of 88 ml. A 5-ml polyethylene bottle was glued around the microcoil to house the FC-43 susceptibility matching liquid (3M Corporation, St. Paul, MN). Unlike pre-

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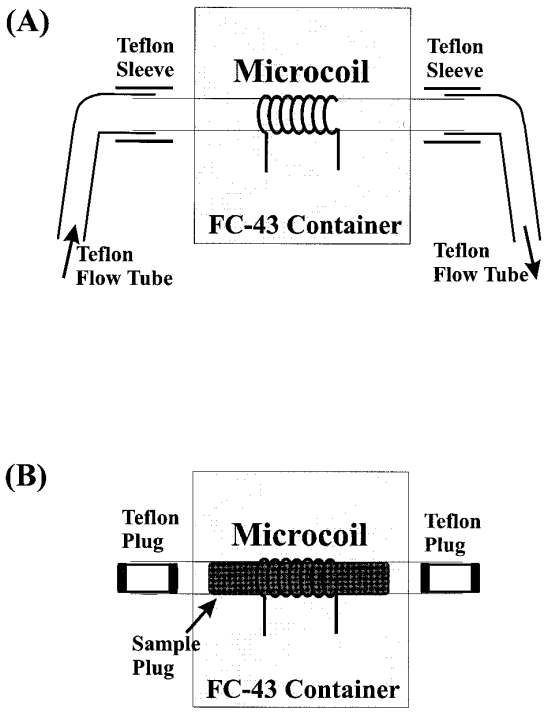

FIG. 1. Schematic of the different probes. (A) Flow probe configuration used in Probes A (V_{obs} = 131 nl) and B (V_{obs} = 88 nl). (B) Limited volume configuration used in Probe C. The volume of the sample plug is 0.4–1.4 μl and the V_{obs} is 131 nl. Teflon plug ends were sealed with epoxy. For all three probes, the microcoil is surrounded by the susceptibility matching liquid FC-43.

viously, no rigid coaxial cable was used as a physical spacer between the microcoil and the rest of the tuning elements in the circuit. This removes extra inductance (~10 nH), and no effect was seen in terms of linewidth between its presence or absence.

In the single resonance circuit, two balanced ceramic chip capacitors (American Technical Ceramics, Huntington, NY) are placed in series with the microcoil (one on either side) before a parallel tuning capacitor and a balanced matching network. Nonmagnetic Gigatrim capacitors (0.4–3.5 pF, Johanson Mfg. Co., Boonton, NJ) were used as the variable caps in the circuit because of their small size.

Incorporating a capacitor in series with the coil in the single resonance circuit described above reduces the impedance of the microcoil by $(\omega^2 C)^{-1}$, where ω is the frequency of operation in rad/sec, and C is the added series capacitance. Two 2.2 pF were placed on either side of the microcoil. Although using lower values of capacitance would decrease the impedance further, these have been found to cause an unstable circuit. The self-resonance frequencies (F_{self}) of microcoils wound on a 700-μm O.D./530-μm I.D. with different number of closely wound turns are listed in Table 2. In order to impedance match the circuit to 50 Ω at 499.89 MHz, the desired frequency of operation should be well removed from the self-resonant frequency of the coil. For this reason, the 12-turn coil was selected.

TABLE 2

Variation of the Self-Resonance Frequency (F_{self}) with Respect to the Number of Closely Spaced Turns of Insulated Copper Wire (50 μm diameter) on a 700-μm-O.D./530-μm-I.D. Polyimide Coated Fused Silica Capillary

No. of turns	F_{self} (MHz)
12	832
16	679
20	566

The double resonance circuit is shown in Fig. 2. All the circuit elements including the solenoid were housed on a 3-cm × 9-cm printed circuit board. The circuit was printed on a single-sided ''S'' photosensitized copper clad board (Kepro Circuit Systems, Inc., Fenton, MO) and processed according to the manufacturer's instructions. The inductors for the proton (L_1) and deuterium frequency (L_2) trap circuit consist of 6 and 9 turns, respectively, of 30 AWG magnet wire wound on a 0.15″ screw. The inductor (L_3) that connects the proton frequency circuit to the deuterium frequency arm is 2 to 3 turns of 30 AWG wire. Gigatrim variable capacitors and ATC chip capacitors were used to complete the circuit. The proton and deuterium traps were tuned independently before being soldered onto the PC board. Electrical characterization was performed using a two-port 1–500 MHz network analyzer (8751 A with Integrated Scattering (*S*) parameter test set; Hewlett–Packard, Palo Alto, CA). The network analyzer was first calibrated to remove the effects of connecting cables. The proton and deuterium sides of the circuit were connected to ports 1 and 2, respectively. When tuned and matched, the measured

TABLE 1

Summary of the Different Probes Demonstrated in This Paper

Probe	O.D. (μm)	I.D. (μm)	*n*	V_{obs} (nl)	Description
A	700	530	12	131	^{1}H flow probe
B	700	530	8	88	Double-tuned ^{1}H/^{2}H flow probe
C	700	530	12	131	^{1}H limited volume probe

Note. The O.D. and I.D. are outer and inner dimensions, respectively, of the capillary that houses the microcoil; *n* is the number of turns in the solenoid; V_{obs} is the coil observed volume in nanoliters.

S_{11} were less than −25 and −18 dB, respectively. This means that the reflected power is less than 2% and therefore the circuit is well matched at both frequencies. The S_{12} and S_{21} parameters, which are a direct measure of the isolation of the proton from the deuterium frequency (and vice versa) were measured to be less than −30 and −32 dB, respectively.

All 500-MHz experiments were carried out on a Unity-500 spectrometer (Varian NMR Instruments, Palo Alto, CA); 11.7 T, 500 MHz, 51 mm narrow bore magnet (Oxford Instruments, Oxford, England). Thirteen decibel attentuation was added to the output of the 50-W amplifier due to the low power requirements of these microcoils. Typical ^{1}H 90° pulse lengths were 1 and 2 μs for the single and double resonance probes, respectively. This means that the ^{1}H channel is only 50% efficient in the double-tuned probe compared to the single resonance. This is in part because, with the low volumes used, the lock channel efficiency cannot be compromised as in conventional NMR probes where the proton channel is typically optimized at the expense of the lock channel. Losses are also incurred in the extra components in the double resonance coil design probes, and schemes for reducing these losses are currently being investigated. The 250-MHz experiment was performed using a 89-mm-wide bore magnet (Oxford Instruments, Oxford, England) and a Libra console (Tecmag, Inc., Houston, TX). Typical 90° pulse lengths were 1 μs from a similarly rated amplifier with 13 dB transmitter attenuation. Data were processed using the NUTS software package (Acorn NMR, Inc., Fremont, CA).

Chemicals were used without further purification. Sucrose and (±) chloroquin diphosphate were purchased from SAF chemicals (St. Louis, MO), and deuterium oxide was purchased from Cambridge Isotope Labs (Andover, MA).

The first experiment was designed to investigate the effect of field strength on magnetic susceptibility induced line broadening. Figure 3A displays the ^{1}H spectrum of chloroquin diphosphate at 500 MHz obtained with Probe A. The coil was preshimmed on the water resonance of a 10% H_2O/90% D_2O volume (v/v) mixture, and a 1.8 Hz linewidth was achieved. An 18.3 mM solution of chloroquin diphosphate in D_2O was then injected into the probe and the spectrum shown is obtained by coaddition of 3200 scans acquired with a recycle delay of 2 s. The observe volume of the coil was 131 nl, corresponding to 1.24 μg (2.4 nmol) of sample in the detection cell. A spectrum of 50 mM chloroquine diphosphate solution acquired with the same coil retuned to 250 MHz is shown in Fig. 3B. The linewidth obtained on a 10% H_2O/90% D_2O sample was 2 Hz. By comparing the two spectra it is evident that the perfluorocarbon susceptibility matching remains effective when the operating frequency is doubled from 250 to 500 MHz. Linewidths are larger than those reported using smaller fill factors (*3, 4*), as would be expected.

The next experiment was performed to evaluate the lock channel in the double resonance probe (Probe B). The coil was shimmed on a 10% H_2O/90% D_2O (v/v) mixture to a linewidth of 1.8 Hz. Following the shimming, the H_2O/D_2O sample was re-

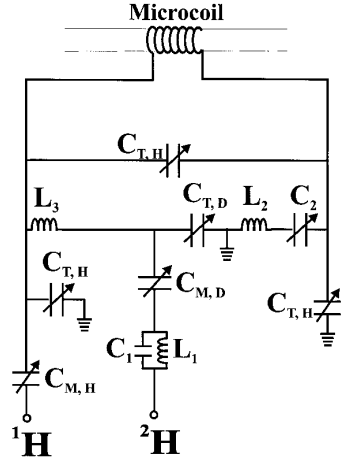

FIG. 2. Circuit design of double resonance Probe B. L_1, C_1 and L_2, C_2 form the traps for the proton and deuterium frequencies, respectively. $C_{T,H}$, $C_{M,H}$ are tuning and matching capacitors for the proton frequency; $C_{T,D}$, $C_{M,D}$ are tuning and matching capacitors for the deuterium frequency. The L_3 helps tune the low frequency arm of the circuit.

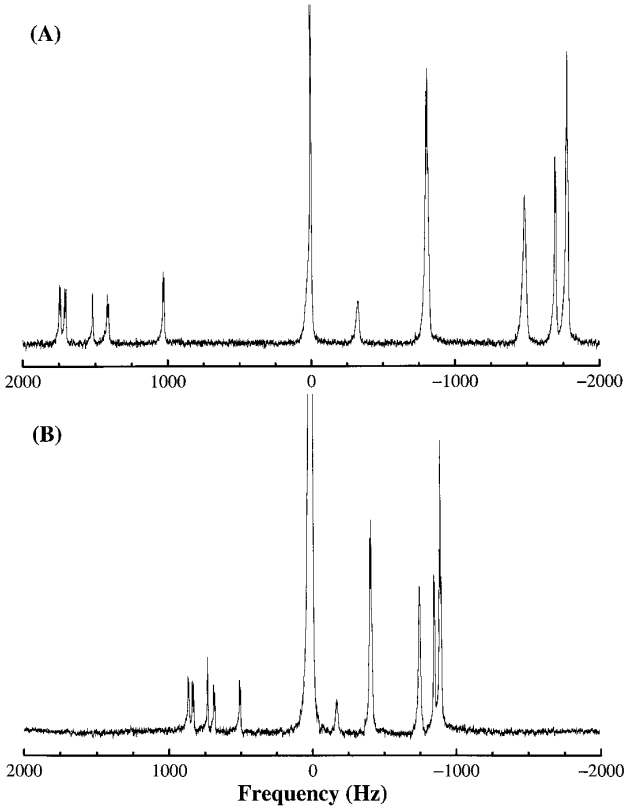

FIG. 3. Chloroquine diphosphate spectrum obtained with Probe A. (A) 500-MHz spectrum of 18.3 mM solution in D_2O. The linewidth of the solvent resonance was 1.8 Hz. Data parameters: 3200 scans; 2-s recycle delay; 4300-Hz spectral width; 4096 points. (B) 250-MHz spectrum of 50 mM solution in D_2O.Data parameters: 2-Hz solvent linewidth; 500 scans; 2-s recycle delay; 4000-Hz spectral width; 8192 points.

placed with a 5 mM solution of sucrose in D_2O, giving 0.15 μg (0.44 nmol) of sample in the observe volume of the coil. Figure 4 displays the sucrose spectrum with 6300 coadded scans collected over a period of 3.5 h. The efficacy of locking is shown in the narrow linewidths obtained over this long acquisition time.

The LODs for a 10-min acquisition of sucrose with probe A (spectrum not shown) and probe B were calculated to be 17.6 and 34.1 pmol, respectively. The first number is comparable to the LOD of 18.8 pmol obtained in an earlier study using a 357-μm-O.D. coil with 5 nl observe volume (*3*). The increase in the static field strength compensates for the reduction in the sensitivity which should result from use of the larger, less sensitive coil (*5*) and the broader linewidths obtained here compared to the previous microcoil work (*3*). The factor of ~2 increase in the LODs from the single to the double resonance coil correlates well with the differences in the respective 90° pulse widths as expected. We are currently testing more efficient double resonance probe designs.

To compare the linewidth that could be obtained from a limited sample with that from an ''infinite sample'' (as in Probes A and B), 1.4 μl of 5 mM sucrose in D_2O was loaded into a 700-μm (O.D.)/530-μm (I.D.) capillary and epoxy sealed with Teflon plugs, and a coil wound directly onto the capillary (Probe C). Shimming was performed on the proton resonance of the residual water in the solvent and a linewidth of 2.4 Hz was achieved. The ^1H spectrum is shown in Fig. 5. There is 2.4 μg (7.0 nmol) of sucrose in the observe volume, and the figure represents a spectrum of 1024 scans acquired with a recycle delay of 1 s.

With an observe volume of 131 nl, Probe C yields an observe factor of 9.4%. In contrast, the flow probes (Probes A and B) have an observe factor of less than 0.2%, although they have the advantage of allowing sample loading from outside the magnet. In theory, the sample needs to extend well beyond either side of the cylinder enclosed by the microcoil to avoid susceptibility-induced artifacts (*6*). Two more probes (of type C) were constructed with total sample volumes of 400 nl and 1 μl of 5 mM sucrose in D_2O. The respective linewidths that could be obtained were 4.5 and 3.3 Hz for the residual water resonance. While still allowing larger scalar couplings to be resolved, these linewidths significantly degrade the information content of the spectra. In terms of practical sample handling, 200 nl volumes are possible when the solvents are water, dimethyl sulfoxide, and their deuterated counterparts which possess a high viscosity and/or a high surface tension. However, with organic solvents like methylene chloride and acetone a more practical volume to handle is 1–2 μl due to their low surface tension and/or viscosity.

It should be noted that the subject of high resolution NMR of mass-limited samples has also been approached by Barbara (*6*) and the Varian Nano.nmr probe (*7*). A 5-mm-diameter solenoidal coil oriented at the magic angle is used and is made with zero-susceptibility wire. The samples are spun at 2 kHz or higher during the NMR experiment. The observe volume in a Nano.nmr probe is 40 μl, although the coil volume need not be filled completely without compromising the linewidths, and the entire volume is contained within the coil. The Nano.nmr probe is capable of narrow linewidths and may be a suitable approach to any mass-limited sample with solid or gel-type properties. For

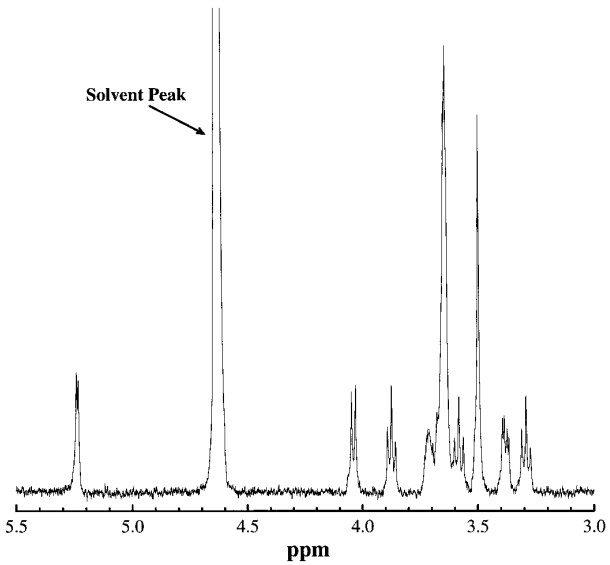

FIG. 4. Sucrose (5 mM in D_2O) spectrum obtained with Probe B. The microcoil probe has ^1H and ^2H-lock channels. The SNR of the largest non-solvent peak is 181:1. The linewidth of the solvent resonance was 1.8 Hz, and 40% anomeric peak splitting was observed. Data parameters: 6300 scans; 2-s recycle delay; 1480-Hz spectral width; 4096 points.

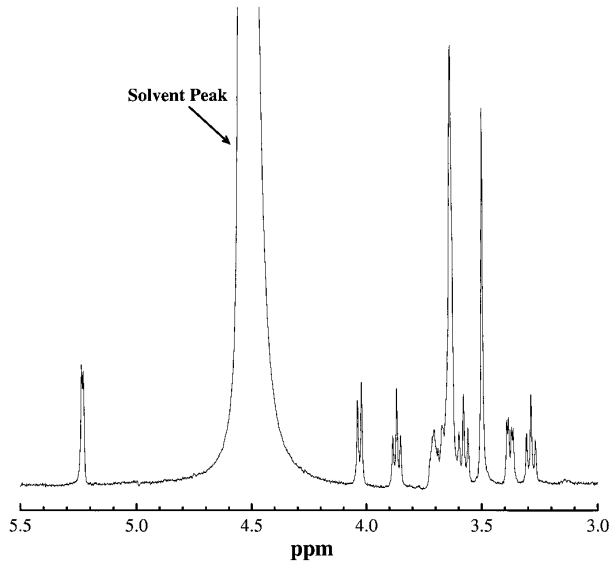

FIG. 5. Sucrose spectrum obtained with Probe C. The total sample volume in the microcoil was 1.4 μl of 5 mM sucrose in D_2O, and the V_{obs} for the coil was 131 nl. The observe factor, defined as the ratio of V_{obs} to the total sample volume, is 9.4%. The linewidth of the solvent resonance was 2.4 Hz, and 20% anomeric peak splitting was observed. Data parameters: 1024 scans; 1-s recycle delay; 2000-Hz spectral width; 4096 points.

conventional high resolution NMR, the major potential disadvantage in its use is sideband aliasing in 2D applications since the sample *must* be spun to obtain narrow linewidths.

In conclusion, application of solenoidal microcoil NMR has been successfully extended for ^{1}H NMR of mass-limited samples at 500 MHz. Concentration sensitivities have been improved to 5–20 mM. The linewidths achieved are comparable to those obtained at 250 MHz since perfluorocarbon susceptibility matching remains effective at 500 MHz. A lock channel has been successfully incorporated into RF microprobes by double tuning the solenoidal coil. Empirically, the total sample volume should be at least 10 times larger than the coil volume to obtain high resolution spectra comparable to that from an ''infinite'' sample. As a result of these improvements in microcoil probes, acquisition of ^{1}H NMR spectra with less than 10 nmol of analyte and at concentrations of $\leq$10 mM in a reasonable time ($\sim$1 h) is now relatively simple.

ACKNOWLEDGMENTS

This work was supported by grants from Smith–Kline Beecham and the National Institutes of Health (PHS 1 R01 GM53030-01). Spectra were obtained using facilities provided by a Shared Instrumentation Grant (1S10RR06243), and the Biomedical Research Technology Grant (PHS5 P41 RR05964) at the Biomedical Magnetic Resonance Laboratory, University of Illinois at Urbana-Champaign. We acknowledge the assistance and technical support of Billy McNeill, Samuel Grant, Babak Behnia (Magnetic Resonance Engineering Laboratory), and Drs. Tim Peck and James Norcross (Magnetic Resonance Microsensors Corp.).

Note added in proof: The performance of Varian Nano·nmr probe has been compared to the microcoil probe for the same limited mass of menthol by Dean L. Olson, Michael E. Lacey, and Jonathan V. Sweedler in "High-Resolution Microcoil NMR for Analysis of Mass-Limited, Nanoliter Samples," *Anal. Chem.* **70,** 645 (1998). In addition, they also report improved concentration LODs for microcoils with a V_{obs} of 31 nL.

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