

COMMUNICATIONS

A Probe Design for the Acquisition of Homonuclear, Heteronuclear, and Inverse Detected NMR Spectra from Multiple Samples

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A new probe design is presented for obtaining homonuclear, heteronuclear, and inverse detected NMR spectra from more than one sample in the same total data acquisition time as for a single sample, thus increasing data acquisition efficiency. Specifically, a two-coil system, with each solenoidal coil impedance matched to 50 Ω at both proton and nitrogen frequencies, has been designed for operation at 11.7 T with an observe volume of 15 μL for each coil. Isolation between the two frequencies for each individual coil, and at each frequency between coils, was greater than 30 dB. Two-dimensional COSY and HMQC spectra were obtained with negligible NMR cross-talk between the two coils. © 2001 Elsevier Science

Key Words: solenoidal coils; parallel data acquisition; high throughput; HMQC spectroscopy; electrical decoupling.

INTRODUCTION

High-resolution nuclear magnetic resonance (NMR) spectroscopy is typically a single input, single output system in which either a set of time-sequential experiments is run on an individual sample or a single experiment is performed on samples introduced successively into the magnet. Until recently there had been no demonstration of parallelism in high-resolution NMR data acquisition. The major reason for this has been the requirement for multiple transmit/receive coils to be placed within a homogeneous region of the magnet. Oldfield (1) acquired solid-state spectra from three nuclei (^2H , ^{27}Al , and ^{17}O) from three different samples placed in the same magnet, with spectral linewidths of approximately 1 ppm. For high-resolution liquid state spectroscopy, the magnetic field homogeneity over each sample must be improved to obtain linewidths of a few hertz or less, limiting the physical dimensions of the active region of the detectors to approximately 2 cm in length and 5–10 mm in width for most magnets. Since conventional saddle or Helmholtz coils are already close to these dimensions it is necessary to use much smaller coils for incorporation of multiple detectors, for example, solenoidal “microcoils” which have been used for studies

of mass limited samples (2). Using such coils, Fisher *et al.* (3) have designed a two-coil probe, with each coil electrically isolated from the other using a copper ground plane, and the impedance matching networks isolated similarly. Using two independent duplexer/preamplifier stages and two receivers, simultaneous acquisition of two ^{13}C spectra was demonstrated. In later publications, the same research group introduced a method termed “multiplex NMR” (4–6) in which four coils were arranged vertically and connected in parallel. Application of a magnetic field gradient in the vertical direction during data acquisition, and subsequent deconvolution of the spatially dependent frequency shifts in successively acquired spectra, yielded spectra corresponding to each of the four compounds. In an alternative approach, Li *et al.* (7) showed that both one- and two-dimensional proton spectra could be obtained from separate samples contained in four electrically isolated solenoidal coils using a radiofrequency switch to separate the data from each coil. The data showed minimal loss in sensitivity (<5%) compared to spectra obtained from a single coil, resulting in an increase in throughput effectively equal to the number of coils.

An important next step in the continued development of multiple-coil probes is the ability to acquire heteronuclear data sets, either via direct detection or, more importantly, using indirect detection techniques such as heteronuclear multiple quantum coherence (HMQC), heteronuclear single quantum coherence (HSQC), or heteronuclear multiple bond coherence (HMBC). In the case of two coils, each double-tuned to proton and nitrogen frequencies, for example, the design of such a probe must provide not only efficient electrical decoupling between the proton and nitrogen frequencies for each individual coil, but must also provide a high degree of decoupling between coils at each frequency. In this paper, one such design is presented and used to acquire two-dimensional COSY and HMQC spectra from two compounds in the same time that a single-coil probe can acquire data from one sample.

MATERIALS AND METHODS

Coil design. The two-coil probe was designed to operate in a wide-bore (89 mm) 500-MHz magnet and to allow sealed sample tubes to be placed in each coil. Each solenoid was fabricated identically using 5 turns of 24 AWG circular cross-section copper wire (California Fine Wire Co., Grover Beach, CA), optimally spaced, wrapped around a 2.67-mm outer diameter (o.d.), 2.57-mm inner diameter (i.d.) 3-cm length of polyimide tubing (Polymicro Technologies, Phoenix, AZ). The two solenoids were mounted at the same height with a spacing of 6 mm between coils: a vertical, grounded copper sheet (Sheldahl, G230004, 15 μm copper thickness) was placed symmetrically between the coils. Impedance-matching for both proton and nitrogen channels was accomplished using the circuit shown in Fig. 1. The impedance-matching networks for the two coils were housed in shielded copper boxes and were placed at 90° to each other to reduce coupling. The coils were surrounded by a container filled with FC-43 (3M Corporation, St. Paul, MN), a fluid which has a magnetic susceptibility very similar to that of the copper coil. The outer diameter of the entire assembly was 3.4 cm. Sample tubes (2.5-mm o.d., 2.2-mm i.d., Wilmad WG-1364-25A) can be slid in and out of the polyimide tubing.

Electrical characterization. The quality (Q) factor of each of the solenoids was measured to be ~ 150 , with less than 2% difference between the coils. Interactions between the two coils were measured using a network analyzer (HP 8751A Hewlett-Packard, Palo Alto, CA) via the S_{21} parameter. This parameter can be used to measure the isolation between the two operational frequencies of a single coil, or the isolation between frequency channels in different coils. The S_{21} parameter measures the ratio of the output voltage as a function of the input voltage, with the condition that the input is impedance-matched to 50 Ω . For coil 1, the S_{21} between the proton and nitrogen channels was measured as -60 dB at 500 MHz and -35 dB at 50 MHz. For coil 2 these values were -50 dB at 500 MHz and -35 dB at 50 MHz. Equally important is the value of the

S_{21} parameter between the two coils. At 500 MHz the value of S_{21} between the proton channels of the two coils is -65 dB showing excellent isolation. At 50 MHz there is greater than 40 dB of isolation between the two nitrogen channels. The S_{21} between the proton channel of coil 1 and the nitrogen channel of coil 2 was also measured: at 500 MHz the coupling is in the noise floor at below -80 dB, and at 50 MHz the value is approximately -65 dB. Similar numbers are recorded for the S_{21} between the proton channel of coil 2 and the nitrogen channel of coil 1.

Hardware modifications. The only hardware addition to the standard spectrometer setup is two stand-alone radiofrequency switches used to control which coil is connected to the transmitter and receiver chains at any one time. The switch used is a single-pole-five-throw (SP5T) device (Model 3506-16-7000, MBF Microwave, Inc.). This operates from DC to 18 GHz, with a measured insertion loss of 0.1 dB at 500 and 50 MHz. The isolation between channels is greater than 60 dB, with a maximum switching time of 15 ms. The position of the switch is controlled by TTL outputs from the Varian Inova console. The console has five spare lines (SP1–SP5) which can be set to high (+5 V) or low (0 V) from within the pulse program. The switch for the proton channels is controlled by lines SP1 and SP2, and the switch for the nitrogen channels by lines SP3 and SP4, as shown in Fig. 2. The TTL signal is fed into an integrated circuit (ULN2003AN), which is an array of open-collector Darlington transistors. Five of the open-collectors are connected to the five inputs. The common connection of the switch goes to a power supply operating at +28 V. It should be noted that this low-loss switch obviates the need for multiple preamplifiers as described in a previous paper (7).

NMR spectroscopy. NMR spectroscopy was performed on an Inova spectrometer (Varian NMR Instruments, Palo Alto, CA) with a 500-MHz, wide-bore (89 mm) magnet (Oxford Instruments, Oxford, England). The two coils were initially filled with a 10% $\text{H}_2\text{O}/90\%$ D_2O mixture for shimming. The probe height was adjusted so that the two coils were at the center of

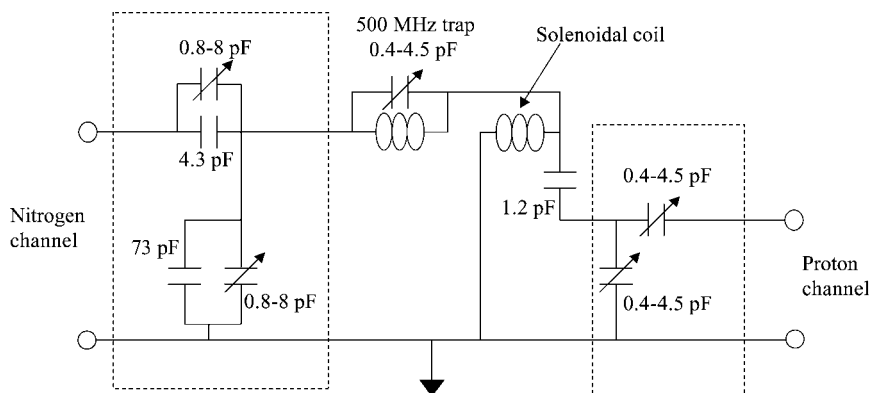


FIG. 1. Circuit diagram for each coil in the two-coil probehead, with each coil impedance matched to 50 Ω at 500 and 50 MHz for proton and nitrogen frequencies, respectively. Copper shielding is indicated by the broken lines.

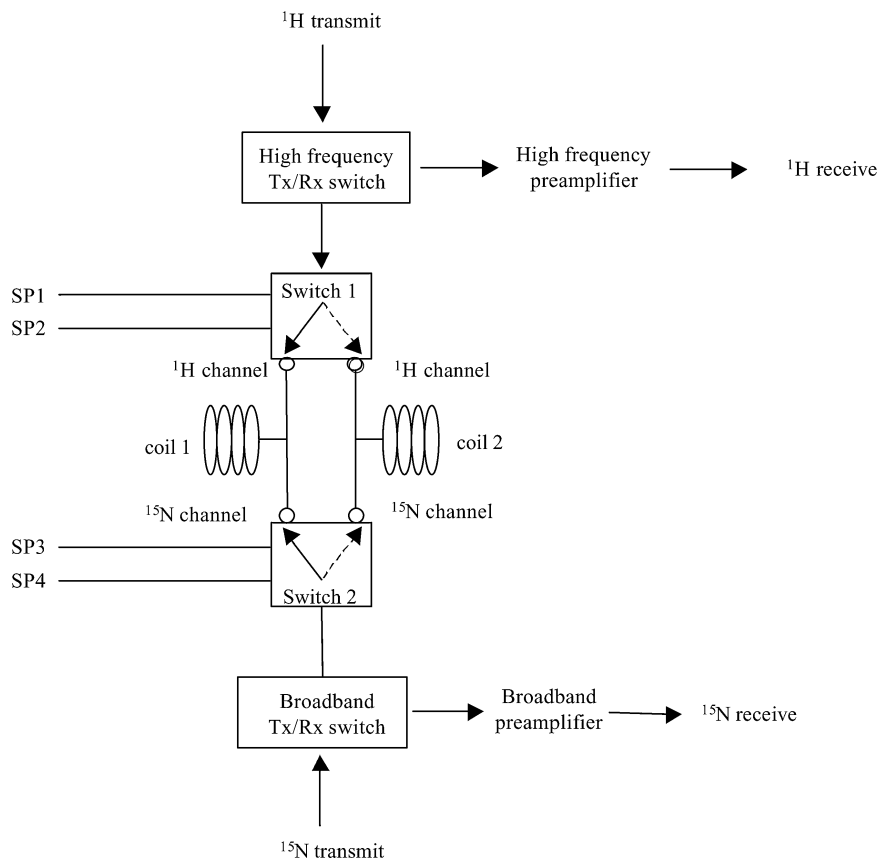


FIG. 2. Schematic showing the two switching networks controlled by the TTL signals from four of the spare lines (SP1–SP4) of the Varian Unity console.

the magnet by minimizing the frequency shifts produced from changes in the Z_1 shim.

Chemicals. Sucrose, adenosine triphosphate (ATP), and formamide were purchased from Sigma Chemicals (St. Louis, MO), ^{15}N -labeled ammonium chloride was from Aldrich Chemicals (Milwaukee, WI), and D_2O was from Cambridge Isotope Laboratories (Andover, MA).

RESULTS

The linewidths for the two coils, when optimized separately using an automatic shimming routine with all higher-order shims, were 1.5 Hz for coil 1 and 1.8 Hz for coil 2 for the $\text{H}_2\text{O}/\text{D}_2\text{O}$ solution. Since the coils were arranged with the long axes aligned as closely as possible to the x axis of the shims, the shim currents for optimal linewidth varied mainly in the current through the y , xy , and x^2-y^2 shim coils. The coil sensitivity was measured using a solution of 0.1% ethylbenzene in deuterated chloroform, giving a signal-to-noise of 98:1 for a one-pulse experiment, with 1 Hz line broadening applied.

Practical implementation of multiple-coil spectroscopy with a single receiver involves signal acquisition from one coil during the relaxation delay for the second coil. Ideally, the values of all of the shim currents would be switched to the respective

optimum values between the two data acquisition periods. In practice, this is not possible due to the long settling times of the currents in the shim coils, particularly those in the higher-order shims. Therefore, in order to make experiments relevant to practical implementation, a “compromise” value of the shim settings was chosen for experiments utilizing both coils. In this case the linewidths increased to 5 and 6 Hz, respectively, for the two coils. In part, these relatively large linewidths were due to the inability, through software control, to change any of the values of the shim currents between successive acquisitions. If this restriction were to be lifted, or pulsed-field gradients used as supplementary x , y , and z shims, the linewidths could probably be reduced considerably. These linewidths are certainly larger than ideal for small-molecule NMR work, but nevertheless reasonable for heteronuclear studies of larger molecules.

The test solutions used were 20 mM sucrose and 20 mM ATP, both in D_2O , in coils 1 and 2, respectively. Figures 3a and 3b show two COSY spectra acquired with sequential switching between coils 1 and 2. As only a small proportion of the relaxation time in multidimensional NMR is used for data acquisition, sequential sampling of the signal from coil 1 and coil 2 leads to improvements in the efficiency of data acquisition. For a typical COSY experiment, the relaxation delay might be set to 2 s. In the experiments shown in Fig. 3, the time between beginning

data acquisition on coil 1 and the initial 90° pulse of the COSY sequence applied to coil 2 (and vice versa) was 1 s, and so the effective relaxation time for each sample is 2 s. Since spectra from both samples have been acquired, the NMR sample throughput has doubled. For the data shown in Fig. 3 the signal contamination between coils is below the noise level and cannot be measured. The pulse program is adapted from the standard Varian COSY sequence by the simple addition of lines in the software code turning the spare lines on and off at appropriate times: no hardware modifications are needed to the spectrometer.

For inverse detection experiments, samples of ^{15}N -labeled ammonium chloride at a concentration of 1 M in D_2O and unlabeled neat formamide were placed in coils 1 and 2, respectively. For both coils, the 90° pulse width on the proton channel was $8\ \mu\text{s}$ at a power level of $\sim 20\ \text{W}$, and the nitrogen 90° pulse width was $16\ \mu\text{s}$ at a power level of $\sim 25\ \text{W}$. The standard Varian HMQC pulse sequence was adapted as described previously for the COSY sequence. Four scans with appropriate phase cycling

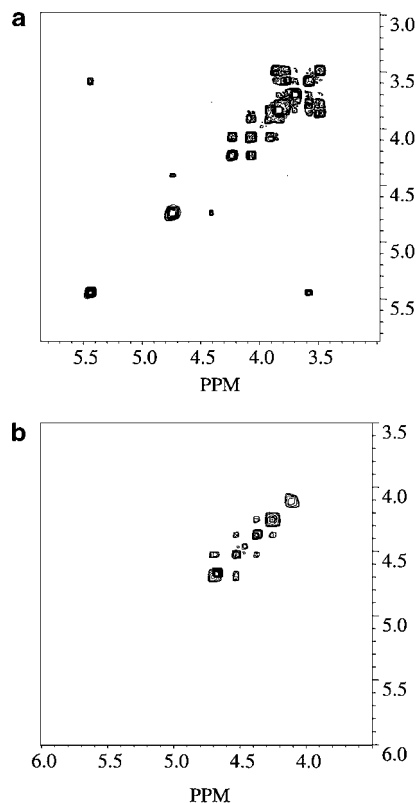


FIG. 3. COSY spectra acquired serially from the two coils. (a) Coil 1 contains 20 mM sucrose in D_2O with 512×128 complex data points zero-filled to 512×512 , an effective TR of 2 s, 4 transients, data processed with shifted sine-bell weighting in both dimensions, symmetrized and displayed in magnitude mode. (b) Coil 2 contains 20 mM adenosine triphosphate in D_2O , with data acquisition and processing parameters identical to those in (a). The spectrum is presented as an expanded region from within the full spectrum to show more clearly the isolation between the proton channels.

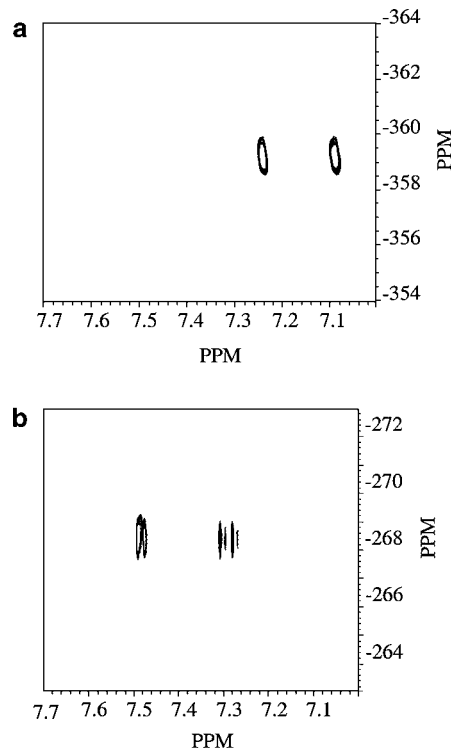


FIG. 4. HMQC spectra acquired serially from the two coils. (a) Coil 1 contains 1 M ^{15}N -labeled (50%-labeled) ammonium chloride in D_2O with $512 \times 128 \times 2$ (States) complex data points, effective TR 2 s, direct dimension spectral width 5000 Hz, indirect dimension 1000 Hz. The data were zero-filled to 512×512 and processed using shifted sine-bell squared weighting in both dimensions. The ^{15}N chemical shifts were referenced to CH_3NO_2 . (b) Coil 2 contains unlabeled formamide with identical data acquisition and processing parameters.

gave excellent suppression of the proton signals not attached to ^{15}N in the two-dimensional HMQC spectra shown in Fig. 4.

DISCUSSION

As modern high field NMR spectrometers require considerable financial investment, any method of increasing the number of samples that can be studied by each spectrometer has great significance. By designing a probehead containing two double-tuned solenoidal coils, we have demonstrated the acquisition of multidimensional high-resolution NMR spectra from two samples in the same time that it takes to acquire a single spectrum with a standard probehead. Homonuclear and inverse-detection spectra have been shown, as these types of experiments constitute the vast majority of NMR experiments. Direct detected two-dimensional heteronuclear experiments have also been carried out (data not shown). In the current configuration, where spectra are obtained serially, simpler probe designs incorporating active detuning of the “passive” coil can certainly be envisioned. However, the ultimate aim of this work is true simultaneous data acquisition from multiple samples. This relies on the availability of

NMR spectrometers with multiple receive channels, and these have become commercially available only very recently. Although this work has shown results from only two samples, preliminary data show that it should be possible to increase this to at least four, while still maintaining adequate spectral resolution and good electrical isolation. For the full range of NMR experiments to be performed on samples from small organics to proteins, it will be necessary to incorporate further frequency channels and to integrate pulsed field magnetic field gradients into the multiple-coil design. The optimal method of adding such functionality is currently under investigation. The possibility of using multiple coils with “saddle” geometries is also being studied.

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