

NMR Probe for the Simultaneous Acquisition of Multiple Samples

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A dual channel probe for the simultaneous acquisition of NMR data from multiple samples has been developed. This multiplex probe consists of two noninteracting sample coils that are each capable of detecting NMR signals at the same resonant frequency with good sensitivity and resolution. ¹³C free induction decays for the two samples, methanol (¹³C, 99%) and carbon tetrachloride (¹³C, 99%), were acquired simultaneously at 75.44 MHz using a single transmitter pulse and separate NMR receivers. S/N measurements are comparable to those observed using single coils. No evidence of cross talk is evident in the spectra even after considerable signal averaging. The probe demonstrates the feasibility of significant parallelism in NMR, which will be of interest in situations where high throughput analysis is desired. © 1999 Academic Press

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Parallel detection methods are advantageous in situations where multiple measurements are required, and such methods are routinely applied to a large number of analytical techniques to improve sensitivity, precision, and throughput. For example, analytical techniques based on Fourier transform methods or alternatively employing imaging detectors allow multiplex sampling of entire spectral regions. Emerging areas such as combinatorial chemistry (1–3) and high throughput screening (4), which rely on the rapid analysis of large numbers of samples, benefit greatly from the development of such methods. Parallel detection methods, as applied to multiple samples, are beginning to be implemented in a number of different analytical methods (5, 6). To date, however, such methods have not been developed for NMR spectroscopy, particularly for the observation of the same nuclei in different samples. Previously, separate lock samples were often incorporated into electromagnet-based spectrometers. More recently, the observation of three samples (one liquid and two solids) using separate probes tuned to observe different nuclei in the same high-field magnet was reported (7). NMR measurements, though invaluable for structural analysis, are normally made on a single sample at a time.

We describe below a new type of NMR probe that employs parallel, noninteracting sample coils to allow for simultaneous

detection of multiple samples. This approach has the potential to increase significantly the throughput of NMR measurements which have until now been limited to serial data acquisition. This multiplex probe is designed to interface to a normal NMR spectrometer, with minor modifications to the hardware. These modifications, as well as data acquisition procedures for our prototype two-coil probe, are discussed below.

EXPERIMENTAL

A schematic diagram of the multiplex probe is shown in Fig. 1 for a dual channel configuration used in the initial experiments. The resonant circuit for each channel is based on a transmission line design (8, 9), which conserves space and reduces complexity in the sample region of the probe. LC tank circuits located near the sample region were tuned closely to the transmitter frequency using a 4 turn, 4 mm inductor of solenoid geometry wrapped from 20 gauge insulated magnet wire and fixed 11 pf tuning capacitors (ATC Corp.). The inductors were attached to a 30 mm long glass tube measuring 4 mm o.d., and 2 mm i.d. using epoxy cement. Sealed glass sample tubes (2 mm o.d. and 6 mm long) were placed within these glass tubes. The inductors were electrically isolated from one another by a horizontal piece of copper-plated circuit board that was grounded to the probe body. In this manner, cross talk was all but eliminated between the two circuits. A fixed 32 pf matching capacitor was added between the end of the transmission line and the tank circuit. Variable tuning (3–11 pf) and matching capacitors (Voltronics Corp.) for each of the tank circuits were placed in a single aluminum box located just below the NMR magnet to allow for easy tuning and matching of the circuits. The regions containing the variable capacitors were electronically isolated from one another by an aluminum plate. As a result, the two coils are independently tunable, and do not exhibit any coupling due to mutual inductance when they are tuned and matched to the same resonant frequency.

The data were acquired at 7.4 Tesla (corresponding to 75.440 MHz for ¹³C) using a Varian Unity Plus spectrometer in conjunction with a separate, home-built receiver in the manner illustrated in Fig. 2. The home-built NMR receiver is based on

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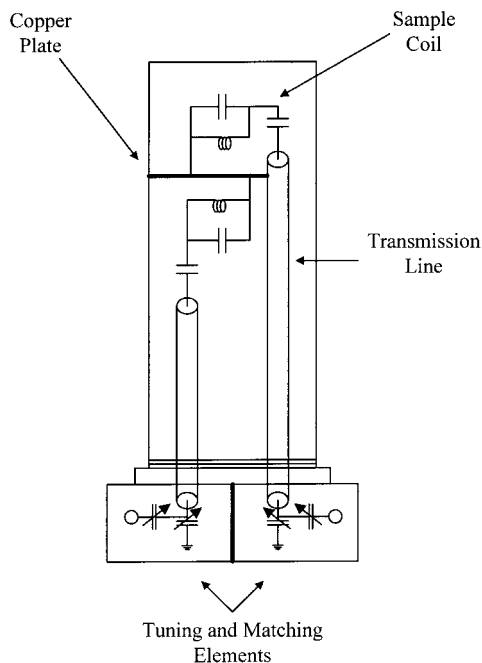


FIG. 1. Schematic diagram of the multiplex dual channel probe and parallel RF circuits. See text for a detailed description.

a super-heterodyne configuration, and is built around a duplexer, mixer/preamp module, phase sensitive detector and RF spectrum analyzer. This receiver and its use as part of a NQR spectrometer will be described in more detail in a subsequent publication (10). The transmitted RF excitation pulse from a single power amplifier was split through a power splitter and the two outputs were routed through crossed diodes and subsequent, independent duplexer/preamp stages connected to each of the NMR receivers. Besides reducing amplifier noise, the crossed diodes act to isolate the NMR signals from interfering with one another during data acquisition. The local (85.94 MHz) and intermediate (10.5 MHz) oscillator frequencies for the home-built receiver were supplied by two PTS 160 synthesizers. These synthesizers were phase locked to the commercial transmitter with a 10 MHz reference signal. Simultaneous data acquisition was accomplished by using the A/D card in the commercial system and a separate 400 MHz digital scope (Tektronix), which was part of the home-built receiver. The trigger pulse for initiating data acquisition and for blanking the preamplifier on the home-built receiver was supplied through an external logic line provided by the Varian spectrometer and controlled by the pulse program. All experiments were performed using a standard 90° pulse without proton decoupling.

Methanol (^{13}C , 99%) and carbon tetrachloride (^{13}C , 99%) were purchased from Cambridge Isotope Laboratories and used without further purification. The two NMR samples were prepared by freezing approximately $4\ \mu\text{L}$ of each liquid into 2 mm

o.d. Pyrex glass tubes using a liquid nitrogen Dewar. The ends of the sample tubes were then sealed with a small torch.

RESULTS AND DISCUSSION

Representative data taken with the multiplex dual channel probe are shown in Fig. 3. ^{13}C samples were used to demonstrate the capabilities of the probe primarily due to the home-built receiver's limited frequency range. Each spectrum is the result of a single acquisition using a $10\ \mu\text{s}$ 90° pulse and a transmitter power of 3 Watts measured at the output of the transmitter amplifier. Figure 3a shows the proton spin-coupled ^{13}C -methanol quartet centered at 50.3 ppm relative to TMS acquired using the commercial spectrometer. The frequency domain spectrum from this system is a result of a complex Fourier transform of 32,768 real and imaginary points. The J -coupling is 141 Hz and the linewidth is approximately 9.2 Hz (FWHM). Figure 3b shows a ^{13}C singlet from carbon tetrachloride at 96 ppm relative to TMS acquired using the home-built spectrometer. The data from the home-built system was obtained on a single channel (i.e., not in quadrature); consequently, only 15,000 real data points were acquired. These data were then processed using a cosine (real) Fourier transform. The linewidth is approximately 10.7 Hz (FWHM). No evidence of cross talk was observed in the spectra, even after considerable signal averaging was performed (100 averages, not shown), to better than 1% of the signal intensity.

Signal to noise measurements showed that the probe had a sensitivity that compares favorably with that of commercially available liquids probes, in agreement with known results observed for mass-limited samples in small NMR coils (11–13). Despite the relatively poor fill factor of the 2 mm o.d. samples inside the 4 mm o.d. sample coils used in these experiments, we found the integrated, mass-limited sensitivity of this probe to be roughly twice that of our 5 mm high-resolution probe due to its smaller coil diameter. This calculation takes into account the total integrated signal intensity from an uncoupled ASTM ^{13}C sensitivity sample (0.6 ml 40% 1,4-dioxane in benzene- d_6) and the differences in linewidth of this sample (1 Hz) as compared to the methanol sample used in our experiments.

Although the achievement of very high spectral resolution was not the primary aim of this work, the resolution demonstrated thus far is obviously a current limitation. Our experiments show that the spectral resolution in the two coils is primarily limited by magnetic susceptibility mismatches between the coils, the glass sample holders, and the samples, in agreement with previous work (11, 13). Initial attempts to use larger fill factors resulted in somewhat larger linewidths. Remedies to this situation include the use of smaller samples and coils or by the incorporation of a susceptibility matching fluid around the sample region and coils (13). A limitation to the former approach will be the ultimate LOD that can be obtained in non- ^{13}C -enriched samples as the sample sizes become

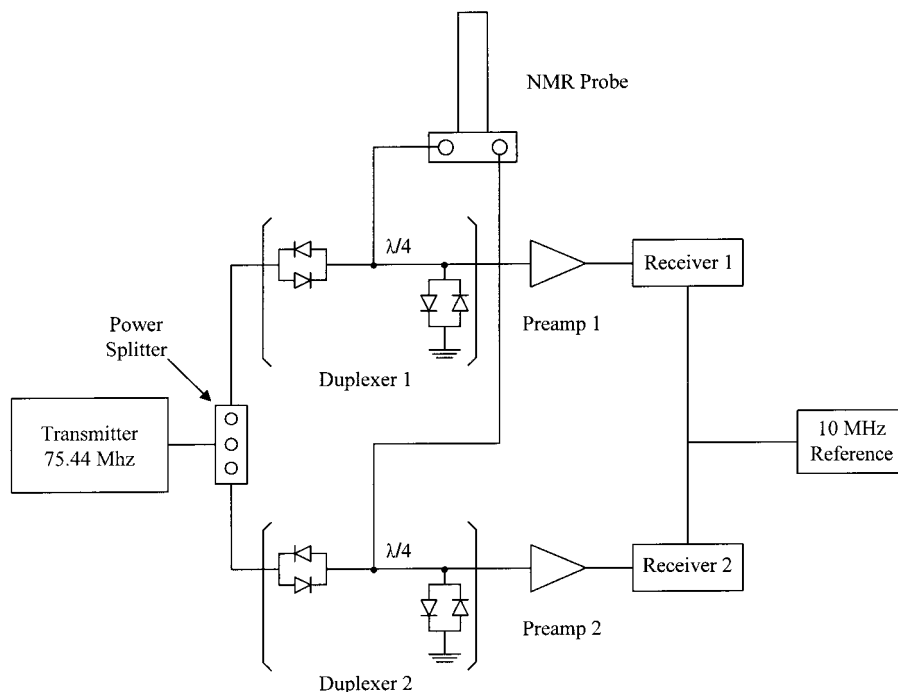


FIG. 2. Schematic diagram of the spectrometer system used to acquire the data including RF connections to the probe. One sample (methanol) is detected by a commercial NMR spectrometer (Varian Unity Plus) operating at 75.44 MHz for ^{13}C , while the other sample (carbon tetrachloride) is detected by a home-built receiver operating at the same frequency and phase locked (referenced) to the main oscillator of the Varian spectrometer.

smaller. Another possibility would be the use of zero magnetic susceptibility magnet wire (14). We are pursuing these avenues to explore the trade-offs between high spectral resolution and large numbers of sample coils in the probe.

There are a number of refinements that can be envisioned to improve the utility of the multiplex probe. Currently, the need for multiple receivers is one factor complicating the design of more parallel NMR detection schemes. Nevertheless, such receiver systems have been proposed and implemented in magnetic resonance imaging (15). One approach would be to construct multiple, phase-locked receivers on a single board whose outputs are digitized by a multiplexed A/D card. The primary limiting factors in such experiments would be the number of isolated sample coils which could fit into the homogenous region of the magnet, and the number of inputs on the A/D card being used, which is determined by the speed of the digitizer and the necessary spectral width. Another possible scheme to increase the parallelism of NMR detection is to use an imaging approach whereby gradient pulses would be used to frequency label the different samples depending on their position in space. Only a single NMR receiver is needed in this case, and the requirement on gradient strength is modest. The spectral resolution will again be a limiting factor, but by combining this technology with NMR micro-coils (11, 13) a substantial number of samples could be simultaneously analyzed. Work on implementing this imaging methodology is in progress at this time (16).

In summary, we have shown that the development of parallel

methods for the detection of multiple NMR samples at the same resonant frequency appears viable, and has sufficient sensitivity and resolution to be of use in a variety of applications. For example, there is a growing need for the rapid analysis of large numbers of compounds in the pharmaceutical industry to identify potential drug candidates. Parallel NMR

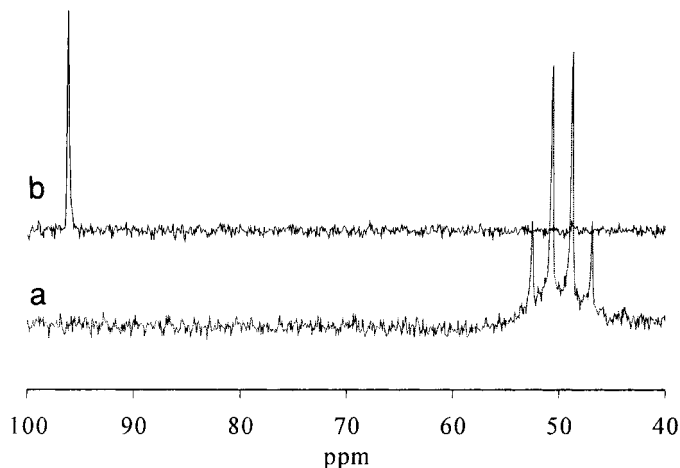


FIG. 3. Proton coupled ^{13}C NMR spectra acquired simultaneously using the multiplex probe. Both spectra were acquired using a single 90° pulse and the same 50 kHz spectral widths: (a) $4\ \mu\text{l}$ of ^{13}C enriched methanol detected using the commercial spectrometer; (b) $4\ \mu\text{l}$ of carbon tetrachloride detected using the home-built receiver. J coupling evident in the methanol spectrum is 141 Hz. No evidence of cross talk was observed in the spectra.

detection methods will be advantageous in applications such as combinatorial chemistry (17) where the demand for significantly increased throughput is increasing. Additionally, there are a growing number of applications where NMR detection methods are used in conjunction with liquid chromatography (18–21). Implementation of a multiplex NMR probe with existing LC-NMR techniques would allow further development of this approach.

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