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Communication

An eight-coil high-frequency probehead design for high-throughput nuclear magnetic resonance spectroscopy

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Abstract

In order to increase the throughput of high-resolution nuclear magnetic resonance spectroscopy a multiple-coil probe, which enables the simultaneous analysis of eight different samples, was designed. The probe, consisting of eight identical solenoidal coils, was constructed for operation at 600 MHz. By using four receivers and radiofrequency switches, spectra from eight different chemical solutions were acquired in the time normally required for one. Two-dimensional COSY, gradient COSY, and TOCSY data have been acquired. Intercoil electrical isolation was between 25 and 45 dB, with signal cross-talk between ~1 and 5% measured by NMR. The spectral linewidths for the eight coils were between 3 and 6 Hz for a single optimized shim setting.

Keywords: High-throughput; NMR; Probe design; Multiple coils; Parallel data acquisition

1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy provides a versatile and powerful analytical tool for structure determination and elucidation of intermolecular interactions. While NMR spectroscopy is potentially well suited to applications in which a large number of samples must be analyzed, such as process monitoring or the screening of combinatorial libraries, the throughput of NMR is limited. Recent advances in drug discovery processes have made the development of high-throughput NMR analysis methods highly desirable. Current approaches to improve the throughput of NMR include the use of automatic sample changers or employ "flow-through" NMR, which uses pumps to push successive samples, separated by inert gas spacers, through the coil. An alternative method to increase throughput, which can also be used to enhance flow-through systems, is to design a probehead containing more than one radiofrequency (RF) coil [1–8]. Since these coils must all fit within the homogeneous region of the magnet, the coils are relatively small, usually of a solenoidal geometry. Most previous studies have acquired one- and two-dimensional proton spectra from small molecular weight compounds, although ^{1}H–^{15}N heteronuclear single quantum coherence spectra from two different proteins have also been collected [7]. Decoupled resonant circuits [1,2,5,7], and selective sample excitation and phase encoding methods [4] have all been used to acquire data from such probeheads. Recently Macnaughtan et al. [8] have successfully integrated a four-coil probe with an automatic flow-injection system.

In this paper, we describe several advances in the design of multiple-coil probeheads. The major one is to increase the number of coils to eight. Second, these coils have been interfaced to a commercial spectrometer which has four, separate receiver channels. Finally, the

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probehead is designed to operate at 600 MHz, improving sensitivity over previous probes at 300 and 250 MHz.

2. Materials and experimental methodology

2.1. Coil design

Shown in Fig. 1 (top) is a photograph of the eight-coil probehead, constructed for operation at 600 MHz. Each solenoidal coil was fabricated using 10 turns of 50-μm-diameter copper wire (California Fine Wire, Grover Beach, CA) with a 6-μm thick polyurethane coating, wrapped around a 350-μm-outer diameter, 250-μm-inner diameter polyimide-coated fused-silica capillary (Polymicro Technologies, Phoenix, AZ). The length of each coil is 0.7 mm, which results in an observation volume of ~35 nL. Teflon flow tubes were attached to both ends of the capillary for sample loading. The coils shown in Fig. 1 (top) were mounted on printed circuit boards, and impedance matching capacitors were added in a balanced configuration. Fig. 1 (bottom) also shows the circuit diagram for each matching network. Two fixed non-magnetic capacitors (700A series, American Technical Ceramics, Huntingdon Station, NY) and two variable non-magnetic capacitors (Gigatrim, 0.6–4.5 pF, Johanson, Boonton, NJ) were used for the network. The "tuning capacitors" were a 4.3 pF fixed and a 0.4–4.5 pF variable capacitor and the "matching capacitors" were a 0.8 pF fixed and a 0.4–4.5 pF variable capacitor. The coils were mounted one above the other with a vertical spacing of approximately 3 mm and alternate coils were rotated 90° with respect to each other to minimize the coupling, as in a previous design [2]. Using a smaller vertical separation than 3 mm between the coils was found to give substantial distortions of the local static magnetic field. The coils were surrounded by an 18-mm-inner diameter container filled with FC-43, a non-conducting perfluorinated fluid that has a magnetic susceptibility very similar to that of copper, in order to minimize susceptibility–mismatch distortions [9]. The outer-diameter of the entire assembly was ~42 mm.

2.2. Hardware modification

The hardware additions to the standard spectrometer, shown in Fig. 2 (top), consist of a four-way power splitter (Mini-circuits, 15542 ZB4CS-440-12W) which is placed between the transmitter and the coils, and four stand-alone radio frequency switches used to control the particular coil connected to the receiver. The switches used are single-pole-two-throw (SP2T) coaxial devices

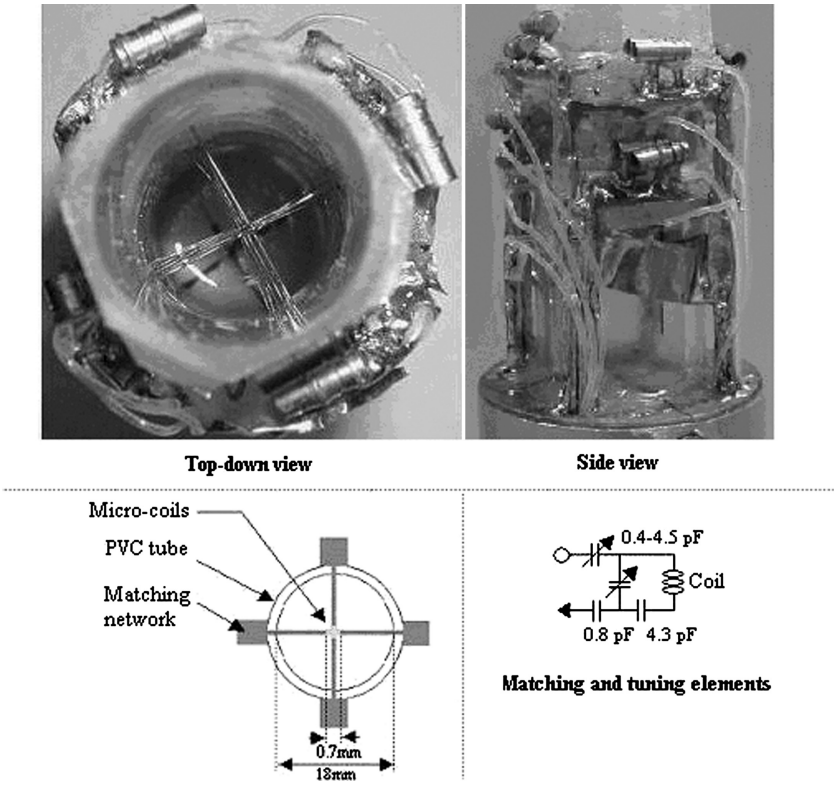

Fig. 1. (Top) Photograph of the eight-coil probehead showing the configuration of the microcoils and matching networks, and side view of the probehead. (Bottom) Schematic of the coil arrangement from top-to-bottom, and the circuit diagram for each matching network. See text for a detailed description.

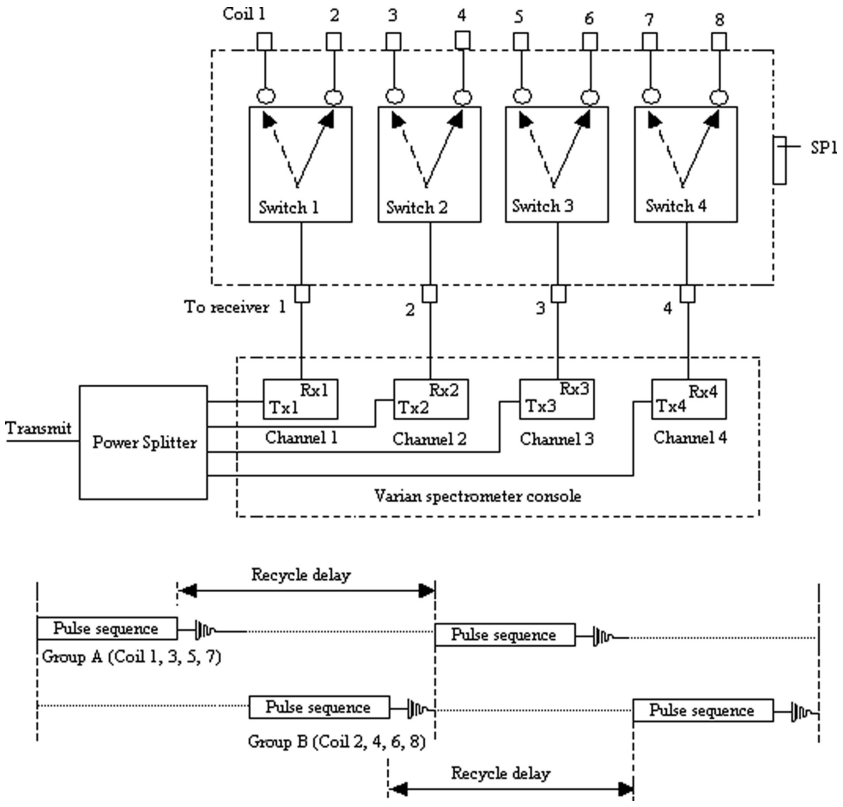

Fig. 2. (Top) Schematic showing the full transmit and receive scheme. The four switching networks were controlled by TTL signals from the Varian Unity console. The eight coils are divided into two groups, A (coils 1, 3, 5, and 7) and B (coils 2, 4, 6, and 8). (Bottom) Timing diagram showing the pulse sequence and data acquisition scheme used.

(Model 8761A, Agilent Technologies, Palo Alto, CA) with excellent electrical and mechanical characteristics for 50 Ω transmission systems. The switch operates from DC to 18 GHz, with a measured insertion loss of 0.03 dB at 600 MHz. Since we use four stand-alone switches there is no additional coupling between the four channels. The position of the switch is controlled by one of the five TTL outputs from the Varian Inova console, which can be set to high (+5 V) or low (0 V) from within the pulse program. The TTL signal is fed into the drive circuit for the switch, which includes an inverter (DM7404, National Semiconductor), integrated circuit (ULN2003AN, National Semiconductor) and driver integrated circuit (DS0026, National Semiconductor). The common connection of the switches goes to a power supply operating at +24 V. The four outputs of the switches are connected to the four receivers.

2.3. NMR spectroscopy

NMR spectroscopy was carried out on a Unity Inova spectrometer (Varian NMR Instruments, Palo Alto, CA) with a 600 MHz, wide-bore (89 mm) magnet (Oxford Instruments, Oxford, England). Magnetic field gradients external to the probe reduce the available diameter to approximately 45 mm. This system has four independent receiver channels, with more than 20 dB (measured) isolation between the channels. Each receiver channel contains an independent preamplifier, a transmit/receive switch and an analog-to-digital converter. The receiver gains can be set independently for the individual receiver channels, but the spectral widths used must be the same value. Data were processed with the Varian VNMR 6.1C software package.

The timing diagram describing the pulse sequence and data acquisition scheme is shown in Fig. 2 (bottom). Since the spectrometer has only four receiver channels, the eight coils are divided into two groups, each containing four coils. The RF pulse sequence was transmitted to, and data acquired from, one coil group at a time. While for a simple one dimensional pulse-acquire experiment the acquisition time is effectively equal to the recycle delay (meaning that a throughput increase of only four could be achieved), for typical two dimensional experiments the acquisition time is typically much smaller than the recycle delay due to the reduced number of data points acquired in the t_2 dimension compared to the one dimensional experiment. With a relaxation delay set to $\sim 1.3\,T_1$, less than 10% of the relaxation delay is used for acquisition. Therefore, the strategy we employ is to use the remaining time to acquire data from the second coil group. As a result, a full

factor of eight improvement in throughput was achieved.

2.4. Chemicals

Sucrose, galactose, arginine, chloroquine, cysteine, caffeine, fructose, and glycine were all purchased from Sigma Chemicals (St. Louis, MO). D_2O was obtained from Cambridge Isotope Laboratories (Andover, MA). Fluorinert (FC-43) was obtained from 3M (St. Paul, MN). All chemicals were used without further purification.

3. Results

3.1. Electrical Characterization and measured NMR intercoil coupling

One major challenge in building multiple-coil probes is the limited space within the bore of the magnet and the electrical decoupling of a large number of coils. To be able to perform high-resolution NMR experiments line widths of a few Hertz must be obtained for each coil. We achieved the desired spectral resolution by placing the impedance matching circuits 9 mm away from the coils, which leaves only 8 mm for all the impedance matching elements. The relatively long leads are undesirable in that they reduce the efficiency of the RF coils, but some compromise between optimal filling factor and B_0 homogeneity is necessary. A thin copper shield was placed between the impedance matching elements of each coil to minimize the coupling. Effective grounding is extremely important in this multiple-coil design, and an external shield surrounding the entire assembly was used as a common ground.

Interactions between different coils were measured both electrically and by NMR. Table 1 lists S_{21} parameters for the eight-coil configuration using a network analyzer (HP 8761A Hewlett–Packard, Palo Alto, CA). The S_{21} measures the ratio of the output voltage as a function of the input voltage with a 50 Ω impedance match. In the ideal case the value of S_{21} approaches minus infinity for zero coupling: realistic values are in the range between −20 dB (1% power coupling) and −40 dB (0.01% power coupling). For this eight-coil configuration the highest value of S_{21} at 600 MHz is −25 dB. The cross-talk between coils can also be measured by NMR. Table 2 contains the NMR measurements of signal cross-talk within each coil group. The result shows the small degree of NMR cross-talk between the coils, in general agreement with the electrical measurements.

Table 1
S_{21} parameter (in dB) for intercoil coupling

Coil	1	2	3	4	5	6	7	8
1	—							
2	−30	—						
3	−29	−31	—					
4	−35	−27	−30	—				
5	−26	−40	−33	−25	—			
6	−42	−25	−39	−31	−33	—		
7	−45	−38	−42	−37	−28	−27	—	
8	−48	−36	−42	−32	−45	−38	−30	—

Table 2
Measured NMR signal bleedthrough between coils

Group A coils	1	3	5	7
1	—			
3	0.034	—		
5	0.063	0.021	—	
7	0.010	0.013	0.045	—
Group B coils	**2**	**4**	**6**	**8**
2	—			
4	0.065	—		
6	0.042	0.028	—	
8	0.027	0.021	0.016	—

3.2. NMR results

The eight coils were initially filled with a 10% H_2O in D_2O mixture for shimming at 600 MHz. Linewidths between 2 and 4 Hz were achieved for each of the eight coils. Since the coils are arranged one above the other along the z axis with a spacing of approximately 3 mm and alternate coils were rotated 90° with respect to each other, the ideal shim currents for optimal linewidth varied from coil to coil; the largest current variation being encountered for the z_1, z_2, x, and y shim values. It should be noted that ideally the values of all shim currents would be switched to the respective optimum values for each coil group within the data acquisition period. However, this is not practically possible due to software limitations. Thus, a "compromise" value of the shim settings was used for all eight coils. The linewidths for the eight coils were between 3 and 6 Hz. Two-dimensional COSY, TOCSY, and gradient COSY experiments were run on the eight different samples (sucrose, galactose, arginine, chloroquine, cysteine, caffeine, fructose, and glycine). The results are shown in Figs. 3–5, respectively. The pulse programs for COSY, TOCSY, and gCOSY were adapted from the standard Varian sequence by repeating the same sequence after switching to the second coil group. For the data shown in Figs. 3–5 the signal contamination between the coils is below the noise level.

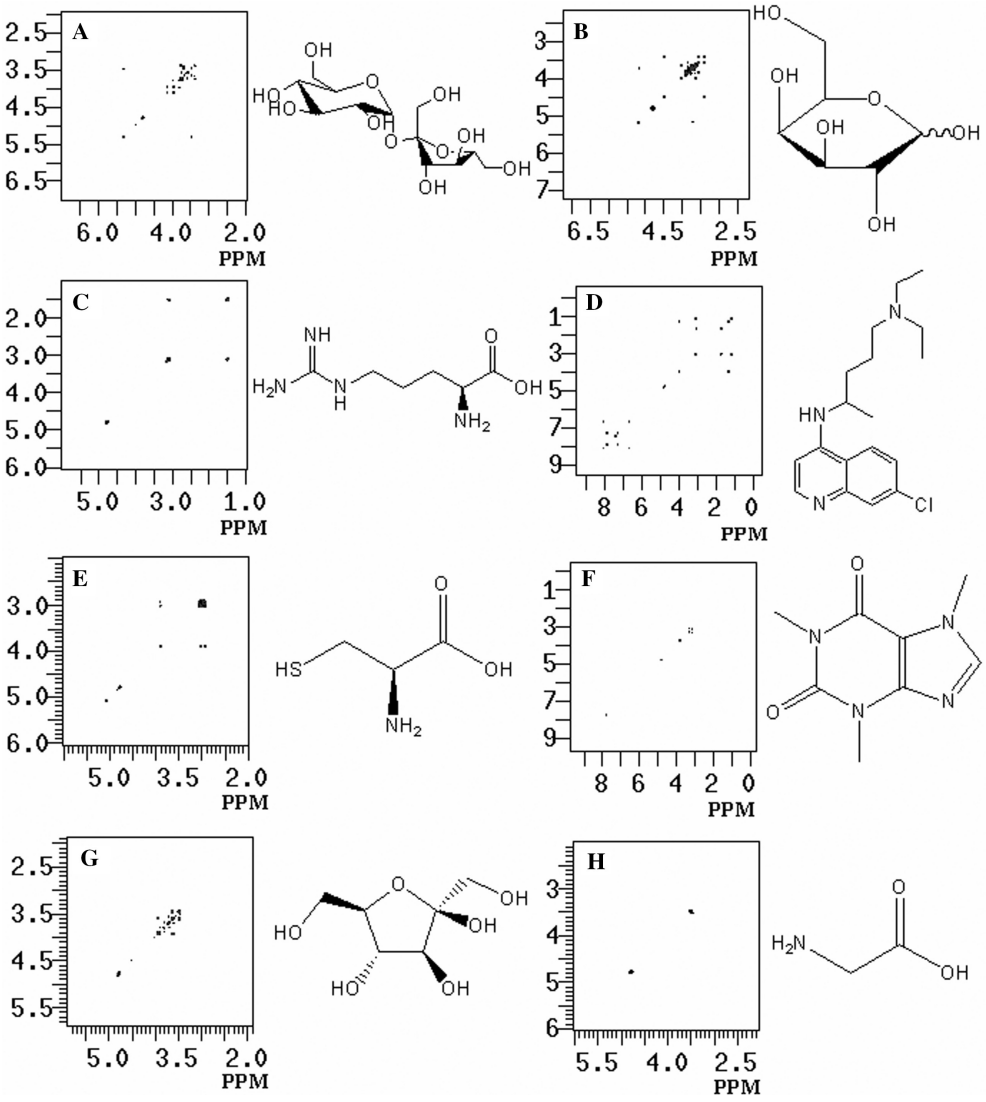

Fig. 3. COSY spectra acquired with the 8-coil probe and the chemical structures of the compounds used. Each sample (10 mM solution in D_2O) was loaded into the coil via the attached teflon tubes. (A) sucrose, (B) galactose, (C) arginine, (D) chloroquine, (E) cysteine, (F) caffeine, (G) fructose, and (H) glycine. Data acquisition parameters: data matrix 2048×256, 8 scans, $sw = 6000$ Hz, $sw_1 = 6000$ Hz. Data were zero filled in t_1 to 2048 points, processed with shifted sine-bell window functions applied in both dimensions, symmetrized, and displayed in magnitude mode.

4. Discussion and conclusions

The application of small-coil NMR spectroscopy has widened considerably with the commercialization of probes with sample volumes of $\sim 1\,\mu L$ and very high mass sensitivity [10–15]. Although NMR spectroscopy is well suited for the analysis of a large number of samples, the throughput of NMR is limited. Thus, methods of increasing throughput could have a large impact. While the construction and use of an eight-coil probe have been demonstrated here, further receivers or time-domain multiplexing into individual receivers would enable the addition of more coils to be added, thus further improving the throughput. Three major considerations for this approach should be noted. First, with more coils, the electrical decoupling between coils would be more complicated. Second, increasing the number of coils will make their placement in the homogeneous region of the magnet more difficult. Third, the effect of multiple coils on the sensitivity of coils should be considered. When RF coils are placed in very close proximity, the B_1 fields can be perturbed significantly. Thus, the trade-off between placing coils close together for better B_0 homogeneity, and perturbations in the magnetic field from the proximity of multiple conductor elements is an important design issue.

In summary, using solenoidal micro-coils, we have designed and built an eight-coil probe capable of ac-

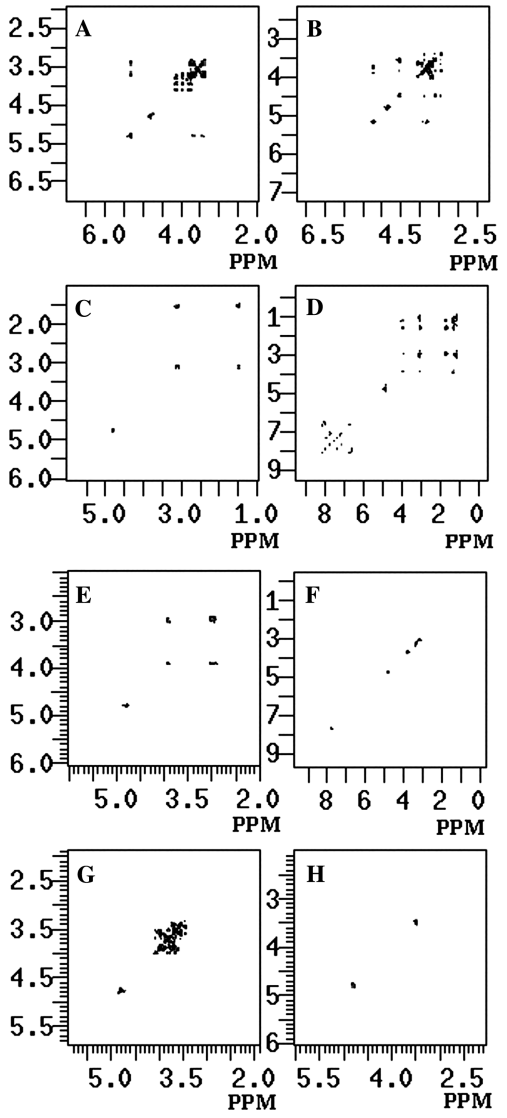

Fig. 4. Phase sensitive TOCSY spectra acquired with the 8-coil probe. Sample details as for Fig. 3. Data acquisition parameters: data matrix 2048×256, 8 scans, $sw = 6000$ Hz, mixing time = 30 ms. Data were zero filled in the t_1 dimension to 1024 points and processed with a gaussian window function applied in both directions.

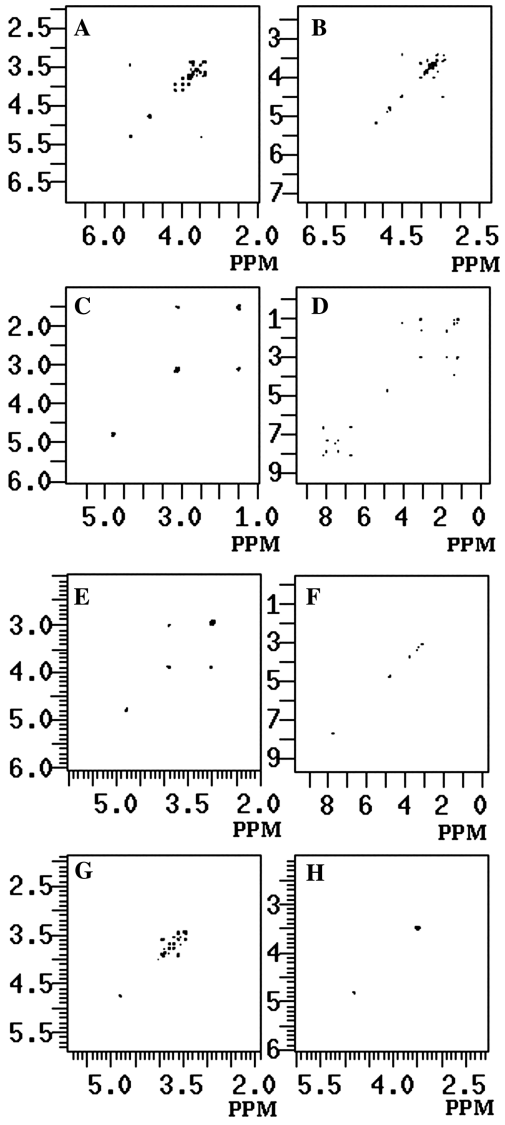

Fig. 5. gCOSY spectra acquired with the 8-coil probe. Sample details as for Fig. 3. Data acquisition parameters: data matrix 2048×256, 8 scans, $sw = 6000$ Hz, $sw_1 = 6000$ Hz, gradient duration 1.9 ms, gradient strength 10 gauss/cm. Data were zero filled in t_1 to 2048 points, processed with a shifted sine-bell window function applied in both dimensions, symmetrized, and displayed in magnitude mode.

quiring multidimensional high-resolution NMR spectra from eight samples in the same time that it takes to acquire a single spectrum. This scheme requires minimal modifications to the spectrometer hardware. By adding more coils, the throughput could be improved further. As modern high-field NMR spectrometers require considerable financial investment, a method of increasing the throughput of such systems can have a large impact. We believe that the multiple coil approach can provide a significant advantage in applications where large numbers of samples must be analyzed rapidly, such as the screening of combinatorial libraries.

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