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Communication

Reduced data acquisition time in multi-dimensional NMR spectroscopy using multiple-coil probes

Han Wang[a,b], Luisa Ciobanu[b], Andrew Webb[a,b,*]

[a] Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[b] Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

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Abstract

A new hardware-based approach is presented to reduce data acquisition times in multi-dimensional NMR spectroscopy using a multiple-coil probe. Using a four-coil setup, two-dimensional COSY and TOCSY spectra were acquired in one-quarter the time of conventional spectra by simultaneous acquisition of different effective t_1 evolution times for each coil. Data processing consists of simple phase-shifting and intensity normalization of the individual data sets, and results in spectra almost identical to those acquired in a conventional manner. This method can potentially be integrated with other new data acquisition and processing schemes for further increases in data acquisition speed.

Keywords: Multi-dimensional NMR; Multiple coils; Rapid data acquisition

1. Introduction

A number of new data acquisition and processing methods have been developed recently in order to reduce data acquisition times in multi-dimensional NMR spectroscopy [1–27]: a detailed discussion of the technical aspects of each method can be found in [27]. These methods are, in general, extremely useful in cases where the limiting factor in data acquisition time is not the signal-to-noise (S/N) but the time required for adequate resolution in the N-dimensional spectral domains. In the case of N-dimensional spectroscopy, filter diagonalization [1–7], Hadamard encoding [11–14], and single-shot acquisition [15–22] can be used for $N = 2$ and higher, and G-matrix Fourier transform [8–10] and projection reconstruction methods [23–26] for $N = 3$ and higher. The majority of the methods effectively result in a reduction in the number of time increments, t_1, t_3, ..., t_N required to generate the N-dimensional spectrum, although the single-shot acquisition method is based on a fundamentally different principle of using magnetic field gradients to refocus chemical shift evolution.

The purpose of this study was to explore an alternative, hardware-based method in order to reduce the acquisition time in multi-dimensional NMR spectroscopy: this approach uses probes containing multiple coils. Such probes have been used previously for increasing NMR throughput [28,29], to implement novel methods of solvent suppression [30], to facilitate hyphenation with electrophoretic microseparations [31], and to follow reaction kinetics [32]. The basis of the proposed method is the physical "splitting" of a sample into M separate fractions placed in M coils which, with the appropriate pulse sequence, allows each fraction to experience a different t_1 increment, in the case of 2D spectroscopy, thus reducing the data acquisition time by a factor of M. In this study a four-coil probehead was designed to interface with a commercial spectrometer with four receiver channels. As proof-of-principle, two-dimensional COSY

* Corresponding author. Fax: +1 217 244 0105.
E-mail address: agwebb@uiuc.edu (A. Webb).

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and TOCSY datasets have been collected with the t_1 dimension split into four sections: the spectra obtained were virtually identical to those obtained with a single coil, but were acquired four times faster.

2. Experimental setup

The construction of the four-coil probehead was very similar to previous descriptions [33]. Four separate solenoid coils were fabricated using 7 turns of 75-μm-diameter copper wire with a 6-μm-thick polyurethane coating, wrapped around a 600-μm-outer diameter and 450-μm-inner diameter polyimide-coated fused-silica capillary: the coil length was 1 mm which results in an observation volume of ~160 nL. This coil size was chosen so that spectra of reasonable concentrations of small molecules (5–10 mM) could be acquired in a single scan. Impedance matching to 50 Ω at 600 MHz was performed using variable and fixed capacitors. The coils were mounted one above another with a vertical spacing of approximately 5 mm and alternate coils were rotated 90° with respect to each other to minimize the coupling: for all coils the isolation was better than 20 dB. The coils were surrounded by an 18 mm inner-diameter container filled with Fluorinert to minimize susceptibility-mismatch distortions [34]. Teflon flow tubes were attached to both ends of the capillary for sample loading. The sample was split into four equal parts and each sub-sample injected into each of the four capillaries via the separate Teflon flow tubes.

3. Methods

Two implementations of the generalized pulse sequence used are shown in Fig. 1. In Fig. 1A, a fixed delay equal to one-quarter of $t_{1,\max}$ is inserted into the successive t_1 evolution times experienced by successive coils. In comparison to an experiment using a single coil to acquire ni increments in the t_1 dimension, ni/4 increments are acquired on each coil: the increment Δt_1 is given by $1/\mathrm{sw}_1$, where sw_1 is the spectral width in the f_1 dimension, as normal. In the second possible implementation, shown in Fig. 1B, a delay of Δt_1 is used between excitation of successive coils. In the vast majority of practical situations the two approaches are essentially identical. In both approaches the data were acquired simultaneously on four separate receiver channels of the Inova system (Varian NMR instruments, Palo Alto, CA). Each receiver channel has an independent preamplifier, transmit/receive switch and analog-to-digital converter. Four single-pole-two-throw RF switches (Mini-circuits, ZSDR-230, switching time ~15 ns) were used to control which coil was connected to the transmitter at a particular time. The position of the switch is controlled within the pulse program via one of the TTL outputs from the Varian Inova console. During the relaxation delay all switches are turned off. Then the first switch is turned on to allow transmission to the first coil only: the switch is turned off after the 90° pulse. After the requisite time delay the second switch is turned on to produce a 90° pulse on the second coil and then turned off. This routine repeats until the fourth switch is turned on in order to transmit on the fourth coil. Subsequently, all switches are turned on for the rest of the pulse sequence. For the COSY sequence, the remainder of the pulse sequence consists of a second 90° pulse followed by data acquisition. For the TOCSY sequence, trim and spin lock pulses were applied to all coils.

The data set from each coil is an $\mathrm{np} \times \mathrm{ni}/4$ data matrix, where np is the number of complex data points acquired in the t_2 dimension, and ni the total number of t_1 increments from all four coils. To reconstruct the complete two-dimensional spectrum from the four individual data sets one needs to correct for frequency and intensity differences between the signals acquired from

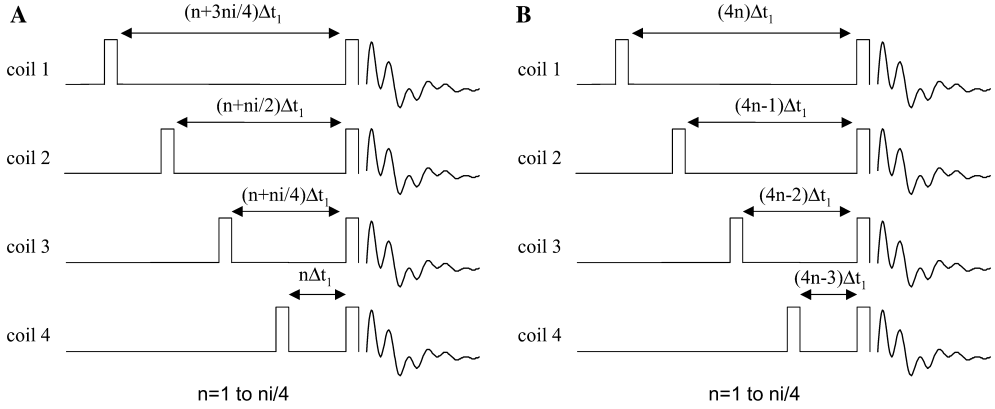

Fig. 1. (A and B) Two pulse sequence timing diagrams for the four-coil COSY experiment; the timing is identical for the TOCSY experiment with the final 90° pulses replaced by "trim" and spin-locking pulses. Values of n range from 1 to ni/4, where ni is the total number of increments in the t_1 dimension for all of the coils, with the value of n incremented after each set of four simultaneous data acquisitions. Pulses were sent to individual or all coils using TTL inputs from the spectrometer to the four switches (see text for details, the typical switching time is ~15 ns). Data were acquired on all four coils simultaneously using the four receiver channels of the spectrometer.

the four coils. The former effects are due to slightly different local B_0 fields within the four coils arising from the currents in the shim coils, in particular the z_1 coil which imparts a linear magnetic field gradient in the z direction. The current in this shim coil thus corrects local existing z gradients, but also results in the resonant frequencies in the individual coils being slightly different. Intensity differences can arise from small differences in coil performance as well as minor variations in the gain stages of the four separate receivers. A simple pulse-acquire spectrum was acquired from all four coils simultaneously, and the frequencies and signal intensities of the largest peak measured. Compensation for the differences in the local B_0 field can be performed either in the frequency- or time-domain. For "magnitude-mode" experiments such as COSY, reconstruction in the frequency domain is simplest: the number of points shifted is given by $\Delta f \times \mathrm{np/sw}$, where Δf represents the frequency difference between the sample in different coils. The spectra are normalized via the measured values, inverse Fourier transformed into the time-domain, interleaved and finally forward Fourier transformed to give the "combined" COSY spectrum. For "phase-sensitive" experiments such as TOCSY, the normalization steps are most easily performed by phase-shifting the data in the time-domain by an amount $\Delta\phi = 2\pi\Delta f/\mathrm{sw}$, and applying intensity normalization. Data were processed with the Varian VNMR 6.1C software package and Matlab 6.5 Release 13 (The Mathworks, Natick, NJ).

4. Results

Fig. 2 shows COSY spectra acquired from the chloroquine sample (Sigma Chemicals, St. Louis, MO, 10 mM in D_2O) in a single coil in the probe with 1024 increments in the t_1 dimension, shown in Fig. 2A compared to a COSY spectrum reconstructed from all four coils, each coil acquiring data at ni/4 increments. The measured intensity normalization was less than 5% between all the coils, primarily due to slightly different gains of the four receiver channels. Almost identical S/N values (measured as the intensity of the largest peak of each spectrum divided by the standard deviation of the noise) for the two spectra were found, showing that the data processing routine, as expected, incurs negligible intrinsic loss in S/N. Of course, were the total sample masses in the two experiments to be made equal by, for example, increasing the concentration in the single coil to 40 mM, the S/N of the spectrum from the proposed technique would be one-quarter that of the single coil. Thus, as with other rapid spectroscopic techniques, one has a S/N vs. time trade-off. Although chloroquine is a good model compound in terms of widely dispersed chemical shifts over ~8 ppm, it does not contain significant scalar coupled fine-structure. In order to compare

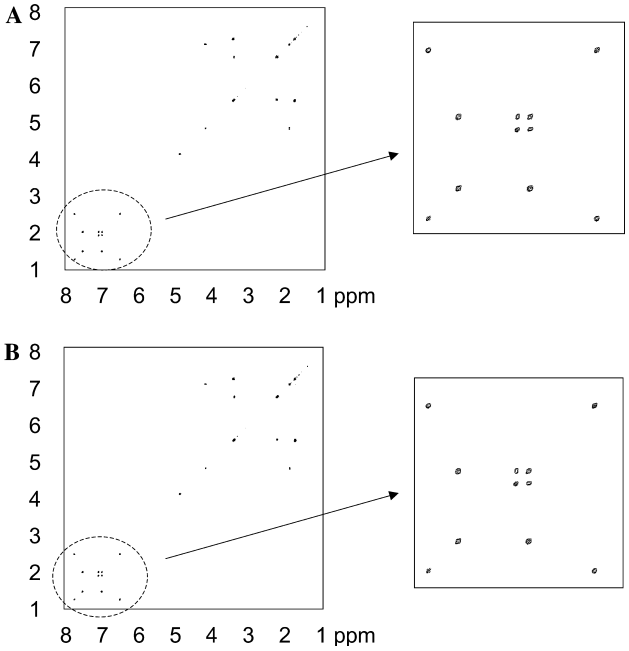

Fig. 2. Comparison of COSY data collected using a single coil and the four-coil probe on a solution of 10 mM chloroquine in D_2O. (A) COSY spectrum of chloroquine acquired with a single coil, data matrix 1024×1024, nt = 2, sw = 5000 Hz, sw_1 = 5000 Hz, relaxation delay 2 s, data acquisition time 68 min, (B) reconstructed COSY spectrum of chloroquine acquired with the four-coil probe, data matrix $1024 \times 256 \times 4$, data acquisition time 17 min. The data were processed by zero-filling in t_1 to 2048 data points with shifted sine-bell apodization in both dimensions, symmetrized and displayed in magnitude mode.

the new method on a more challenging sample, COSY spectra were also acquired on a sample of fructose (10 mM in D_2O). The single coil spectrum comprised of 512 t_1 increments, with a corresponding number of 128 increments per coil for the four-coil experiment. As can be seen in Figs. 3A and B, as well as the associated traces, the spectra are very similar. A "difference spectrum," Fig. 3C, shows that there are small signal variations between the two sets of data, but that no "extra" peaks are introduced and none are missing, and that spectral interpretation would be the same from either data set.

Fig. 4 shows conventional phase-sensitive TOCSY spectra and reconstructed TOCSY spectra of chloroquine using the same data acquisition approach. Again, both conventional and four-coil acquisition schemes display very similar spectral information. The traces also show that this new approach does not present any additional problems in terms of phasing datasets acquired in phase-sensitive mode. Fig. 5 shows corresponding TOCSY data from fructose.

5. Discussion and conclusions

This paper has presented a simple method to reduce the data acquisition time in multi-dimensional NMR

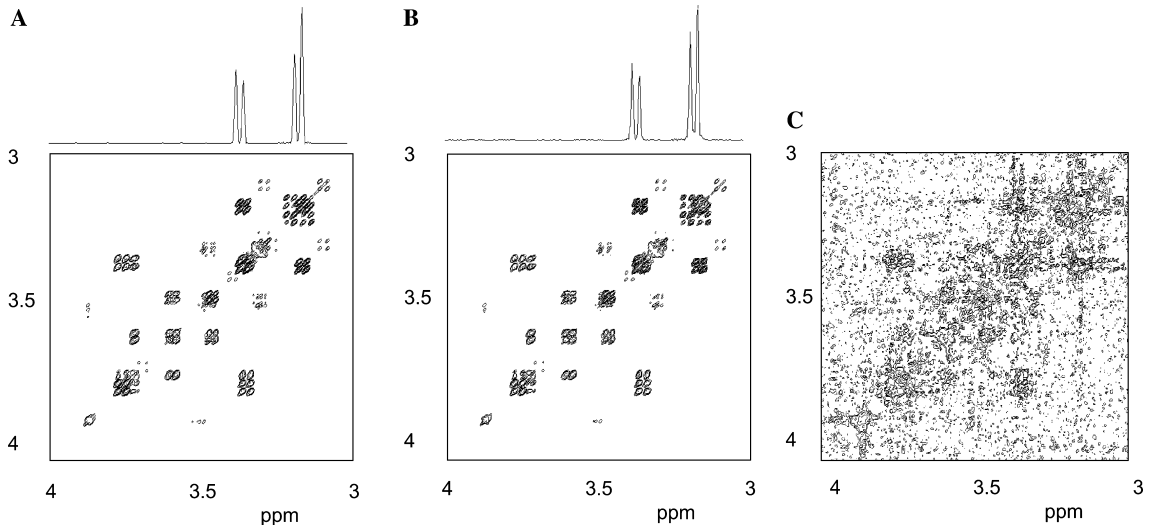

Fig. 3. Comparison of COSY data collected using a single coil and the four-coil probe on a solution of 10 mM fructose in D_2O. (A) Zoomed region to show the fine structure acquired with a single coil, data matrix 512×512, nt = 2, sw = 1250 Hz, $sw_1 = 1250$ Hz, relaxation delay 2 s, data acquisition time 34 min, (B) reconstructed COSY spectrum of fructose acquired with the four-coil probe, data matrix $512 \times 128 \times 4$, data acquisition time 8 min. The traces displayed are at 3.43 ppm. The data were processed by zero-filling in t_1 to 2048 data points with shifted sine-bell apodization in both dimensions, the data were then symmetrized and displayed in magnitude mode. (C) A difference spectrum between the data displayed in (A) and (B).

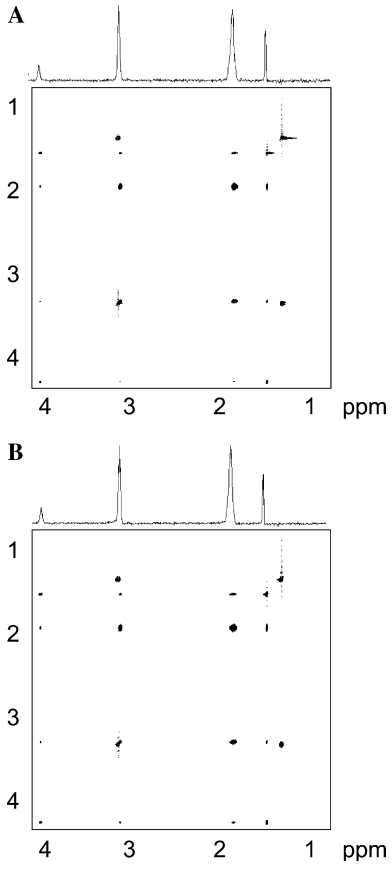

Fig. 4. As for Fig. 2 but using a phase-sensitive TOCSY sequence with States acquisition, four signal averages, and mixing times of 80 ms. The data were zero-filled in the t_1 dimension to 1024 points, apodized with a gaussian window function applied in both dimensions, and then phased. (A) Single coil and (B) reconstructed four-coil data. A trace at 1.7 ppm in f_1 is shown.

spectroscopy using multiple coils. The scheme requires minimal hardware additions, software modifications, and data processing algorithms. The sample can either be split into different fractions in small capillary tubes, or flow-through sample loading [28] can be used. Clearly, for practical rapid data acquisition the number of t_1 increments might be much lower than for the spectra acquired in this paper. Further reductions in data acquisition time by a factor of 2 could also be realized by using gradient versions of the COSY and TOCSY sequences. As with all multi-dimensional NMR experiments, drifts in the main magnetic field or changes in signal intensity could potentially cause artifacts, i.e., extra peaks, in the reconstructed spectra. Although a lock-channel was not incorporated into the current probe design, since the measured field drift is well below 5 Hz per hour, this is very simple to implement and has been shown in many previous microcoil-based probes. Measurements of the time-dependent intensities from the four coils showed less than a 1% variation over the course of the experiment.

These initial experiments used a four-coil probe, which matches the number of available receiver channels on the spectrometer: however, the experiments could equally well be carried out with a single receiver channel and "staggered" data acquisition [33]. Although the use of multiple receiver channels is not yet routine in high-resolution NMR spectrometers, all major manufacturers now offer at least four channels. This development parallels that in magnetic resonance imaging (MRI) systems, in which systems of 32 channels are now available commercially, primarily for integration with parallel imaging techniques for rapid data acquisition [35–38]. Expansion

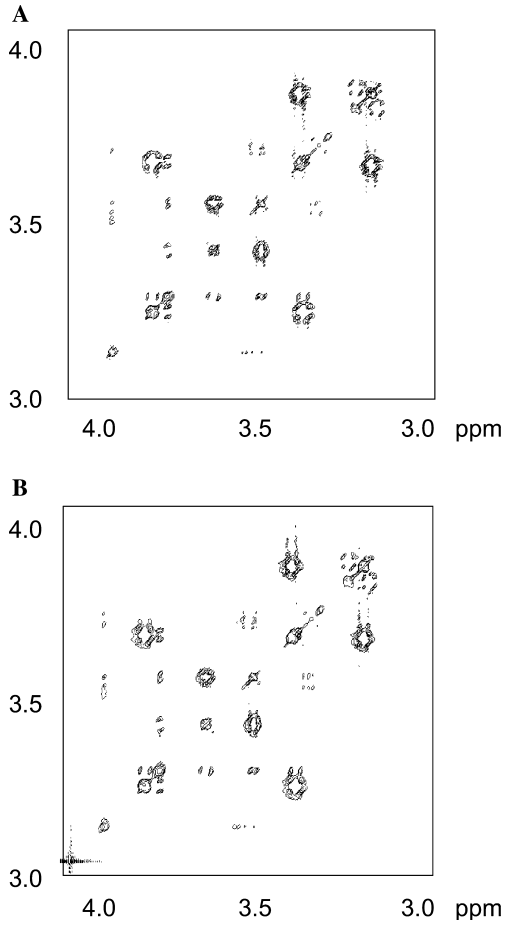

Fig. 5. As for Fig. 3 but using a phase-sensitive TOCSY sequence with States acquisition, four signal averages, and mixing times of 50 ms. The data were zero-filled in the t_1 dimension to 1024 points, apodized with a gaussian window function applied in both dimensions, and then phased. (A) Single coil and (B) reconstructed four-coil data for fructose.

of high-resolution multiple-coil probes to 16 or more coils may be feasible, possibly through the integration of microfabrication techniques [39–41] which are ideally suited to issues of sample handling.

Extensions to three- and four-dimensional homonuclear and heteronuclear spectroscopy are also possible, based on existing designs for small triple-resonance and multi-frequency multiple-coil designs. The proposed technique can also be integrated with many of the new data acquisition techniques proposed recently. Integration with FDM, single-shot GFT and projection reconstruction would be transparent, although multiple transmitter channels would be required for Hadamard encoding.

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