

Rapid Two-Dimensional Inverse Detected Heteronuclear Correlation Experiments with <100 nmol Samples with Solenoidal Microcoil NMR Probes

Raju Subramanian,[†] Jonathan V. Sweedler,^{†,‡} and Andrew G. Webb^{*,†,§}

Department of Chemistry, Department of Electrical and Computer Engineering, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, 405 North Mathews Avenue Urbana, Illinois 61801

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Nuclear magnetic resonance (NMR) is a powerful analytical tool capable of determining three-dimensional structures for molecules as complicated as large proteins. It suffers, however, from an inherently low sensitivity compared to almost all other analytical methods. Studies of mass-limited samples such as minor components of drug metabolites, microseparation eluates, and pheromone isolates are extremely difficult with current NMR detectors. A number of approaches have been taken to improve the intrinsic sensitivity of an NMR experiment. The primary solution has been to perform the experiment at a higher static magnetic field (B_0), but there is an exponential increase in cost with field strength. A second, highly novel approach is to transfer polarization from optically pumped nuclei (e.g. ^{129}Xe) via spin polarization induced nuclear Overhauser enhancement (SPINOE).¹ Signal enhancements of ~50-fold for ^1H and over 70-fold for ^{13}C have been realized for model compounds soluble in liquid xenon. A third method has been to limit the receiver coil noise and increase the coil quality factor by using either high-temperature superconducting materials² or cryogenically cooled coils.³ Sensitivity improvements of a factor of 4–5 over a conventional 5 mm high-resolution probe have been reported. In a final approach, we have used submillimeter sized solenoidal NMR coils with diameters as small as 150 μm , a method that has proved highly effective since the detector sensitivity is inversely proportional to its diameter. Given a limited amount of sample, the signal received is maximized when the sample is concentrated in the minimum solvent volume possible, and the smallest RF coil is constructed to enclose this sample volume. In conjunction with a new method of magnetic susceptibility matching, high-resolution spectra have been reported with improvements of 2 orders of magnitude in mass sensitivity over conventional probes.⁴ Here, we show results from a new inverse detection probe that is capable of obtaining full two-dimensional heteronuclear single quantum coherence (HSQC) and hetero-

nuclear multiple quantum coherence (HMQC) spectra at 500 MHz in a few hours from considerably less than 100 nmol of material. While other NMR microprobes have been designed for mass-limited samples,⁵ the much smaller size of the solenoids, plus their higher intrinsic sensitivity than the saddle coil design, results in over an order of magnitude reduction in data acquisition time.

The new inverse detection microprobe, specifically designed for flow experiments, consists of a close-wound solenoidal microcoil and surface coil as the ^1H and ^{13}C channels, respectively. The 1.9 mm long solenoid, 9 turns of 50 $\mu\text{m} \times 175 \mu\text{m}$ polyurethane coated flat copper wire, is wound on an 850 μm o.d./700 μm i.d. polyimide coated fused silica capillary and encloses an observe volume (V_{obs}) of 745 nL. The surface coil consists of a single circular loop, 7 mm in diameter, and is mounted approximately 1 mm from the edge of the microcoil. Although surface coils have a high sensitivity and are simple to construct, they are traditionally not used in high-resolution NMR due to a spatially inhomogeneous magnetic field profile.⁶ However, since the volume enclosed by our solenoidal microcoils is so small, they prove to be highly efficient both for pulsing and decoupling. Probe assembly⁷ and electrical characterization at the ^1H and ^{13}C frequencies are accomplished as detailed previously.⁸

Figure 1 displays the first HSQC spectrum obtained from a solenoidal microcoil. The sample is chloroquine diphosphate (MW 515.9; 53.5 mM in D_2O), a first line antimalarial drug: the V_{obs} of the coil contains 40 nmol (13 μg) of chloroquine.⁹ The 1024 \times 128 ($\times 2$, hypercomplex) dataset consists of 32 transients per slice and is acquired in 3.6 h: all twelve ^1H – ^{13}C cross-peaks are clearly visible. The sample is introduced into the probe via Teflon tubing attached to the capillary, allowing several samples to be run sequentially with either perfluorocarbon or air as inter-sample spacers.¹⁰ The high sensitivity of this new probe also opens up the possibility of performing inverse detection experiments, for the first time, on eluates from microbore liquid chromatography or capillary electrophoresis¹¹ in a stopped flow mode, thereby improving dramatically the capabilities of these hyphenated methods.

Inverse detection experiments may also be performed on a single, sealed sample. A member of the steroid class of medicinal

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(7) A chip capacitor is placed across the coil leads as close to the loop as possible. The surface coil and solenoidal microcoil are housed in a Teflon bottle containing the FC-43 susceptibility matching fluid. All the circuit elements, including the coils, are positioned on a 3 cm \times 5 cm double sided printed circuit board. When impedance matched, the S_{11} parameters for ^1H and ^{13}C channels are each less than –30 dB and the S_{12} parameter, a direct measure of the isolation between the ^1H and ^{13}C channels, is <–25 dB at both frequencies.

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(9) NMR experiments are carried out on a Varian UNITY-500 spectrometer with an 11.7 T, 51 mm bore magnet. Data are processed on a PC platform with use of the NUTS software package (Acorn NMR, Inc., Fremont, CA). The typical ^1H and ^{13}C 90° pulse widths are 1.7 and 11.5 μs with 8 and 0 dB transmitter attenuations (amplifier rated outputs were 50 and 300 W, respectively). Full ^{13}C decoupling is achieved by using the WALTZ-16 scheme with 14 dB decoupler attenuation (γB_1 equals 6630 Hz).

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[†] Department of Chemistry.

[‡] Beckman Institute for Advanced Science and Technology.

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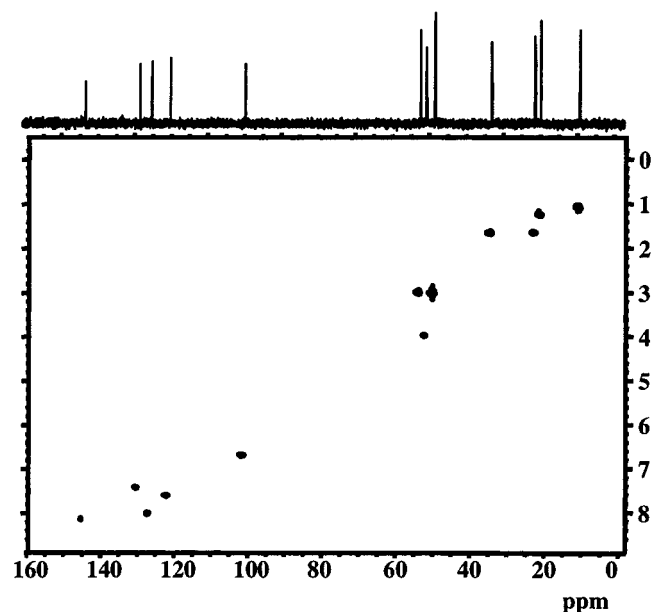


Figure 1. A ^{13}C decoupled HSQC spectrum of 53.5 mM chloroquine diphosphate in D_2O acquired with the flow probe: 40 nmol ($13\ \mu\text{g}$) of chloroquin is in the 745 nL V_{obs} . The data, 32 transients per slice, 1024×128 ($\times 2$, hypercomplex) points, are acquired in 3.6 h. The data are zero-filled to 256 points in the ^{13}C dimension and Gaussian multiplication is applied prior to Fourier transform in both dimensions. Other data parameters: 4.6 kHz ^1H spectral width; 24 kHz ^{13}C spectral width; 1.6 s prepulse delay; Gaussian line broadening, 4 Hz in ^1H and 12 Hz in the ^{13}C dimension. A DEPT ($\theta = 45^\circ$) ^{13}C spectrum acquired with use of a standard 5 mm probe is shown on top of the 2D plot.

compounds is used to demonstrate the sample-limited case. Figure 2 shows an HMQC spectrum of progesterone (MW 314.4; 31.1 mM in toluene- d_8).¹² The total amount of sample in the capillary is 85 nmol ($27\ \mu\text{g}$) with 37 nmol ($11.4\ \mu\text{g}$) of the steroid enclosed by the V_{obs} of the coil. The 16 transients per slice, 1024×128 ($\times 2$, hypercomplex) data are acquired in 1.9 h. The ^1H – ^{13}C cross-peaks arising from the directly protonated carbons agree exactly with an HMQC spectrum acquired with use of a 5 mm commercial probe.

(12) A 7 mm long sample plug of progesterone (31.1 mM in toluene- d_8) is sealed in the center of a 3 cm long $850\ \mu\text{m}$ o.d./ $700\ \mu\text{m}$ i.d. polyimide coated fused silica capillary. A 3 mm long solenoidal microcoil (consisting of 11 turns of $50\ \mu\text{m} \times 225\ \mu\text{m}$ polyimide coated copper wire) encloses the restricted sample and has a V_{obs} of 1.2 μL . The restricted sample has perfluorocarbon (FC-43) fluid plugs at either end of the sample.

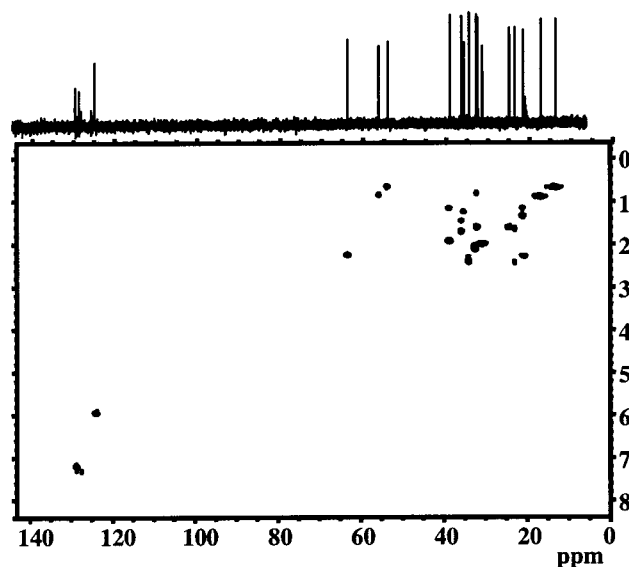


Figure 2. ^{13}C decoupled HMQC spectrum of 31.1 mM progesterone in toluene- d_8 acquired with the restricted sample probe. 85 nmol ($27\ \mu\text{g}$) total sample is in the probe, with 37 nmol ($11.4\ \mu\text{g}$) in the V_{obs} of the coil. The 16 transients per slice, 1024×128 ($\times 2$, hypercomplex) data, are acquired in 1.9 h. Other data parameters: 20 kHz ^{13}C spectral width, rest same as in Figure 1. A DEPT ($\theta = 45^\circ$) ^{13}C spectrum acquired with use of a standard 5 mm probe is shown on top of the 2D plot.

In conclusion, the new inverse detection microcoil probes make possible acquisition of two-dimensional ^1H – ^{13}C correlation spectra for samples with masses as low as tens of micrograms with data acquisition times of ~ 2 h. The combination of total mass, data matrix size, and data acquisition time represents an order of magnitude improvement compared to a state-of-the-art commercial NMR probe.

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