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# Application of a high-resolution superconducting NMR probe in natural product structure determination

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ABSTRACT: The r.f. properties of a high-resolution NMR probehead constructed of epitaxial thin films of high-temperature superconducting ceramics are presented. Operating the probe transmit/receive elements at 25 K significantly decreases the thermal noise of the r.f. coils and increases the probe quality factor ($Q$), resulting in a substantial increase in the signal-to-noise ratio. The lineshape and r.f. transmission characteristics of this probe were investigated. The utility of this probe for natural product structure determination was demonstrated using selective 1D NOE difference spectra. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: NMR; $^1$H NMR, sensitivity; high-temperature superconducting probe; natural products

## INTRODUCTION

The exquisite sensitivity of the NMR frequency to the local chemical environment, combined with favorable relaxation properties and tractable quantum mechanical descriptions, have made NMR the premier method for characterizing the structures and dynamics of molecules in solution. There is a bewildering array of pulse experiments for measuring scalar and dipolar couplings between nuclei and, more recently, to the external magnetic field, and the theory for relating these parameters to molecular structure is well known. However, there is an Achilles heel in the NMR arsenal, namely the inherently low sensitivity of the NMR experiment, which is a consequence of the small energy gap between the ground and excited states (*ca* $10^{-25}$ J/spin). Steady improvements in magnetic field strength and probe technology over many years have made dramatic strides in the battle to improve sensitivity, but these changes are only incremental, representing only a few per cent increase per year. Substantial increases in sensitivity should lead to significant advances in the way NMR is performed and in the types of applications that are amenable to NMR characterization.

The signal strength in an NMR experiment depends on several factors, as described by Hoult and Richards:[1]

$$\text{signal} \approx (N\omega_0^2)\left(\frac{GQ\omega_0}{c_2 T_c}\right)^{1/2} \qquad (1)$$

The first term on the right-hand side in Eqn (1) reflects sample-related factors such as concentration (or number density, $N$) and Boltzmann factors (or spin polarization, $\omega_0$). The second term contains factors relating to the probe design ($G$) and quality factor ($Q$), the design of the preamplifier and its noise factor ($c_2$) and the operating temperature of the r.f. coil ($T_c$). Sensitivity increases are traditionally pursued by optimizing the sample volume for the specific availability or solubility of a given sample, increasing the external magnetic field strength and optimizing the probe $Q$ and r.f. coil geometry. Operating the r.f. coils at cryogenic temperatures is another route to improving the signal-to-noise ratio (SNR), as first demonstrated by Styles and co-workers.[2,3] Because the probe $Q$ is expected to rise as the coil resistance decreases at low temperatures, this approach can lead to dramatic increases in SNR.[2,3] Substantial increases in sensitivity have been documented for NMR surface coils constructed of high-temperature superconducting (HTS) ceramics operating at cryogenic temperatures.[4–7] The use of HTS materials for constructing high-resolution NMR probes has been demonstrated,[8,9] but the r.f. properties and range of applications of these probes have not been thoroughly evaluated. Here, we report substantial increases in SNR obtained from a high-resolution probe operating at 25 K that uses HTS ceramics for the r.f. transmit/receive circuitry. We evaluated some of the r.f. properties of this probe and demonstrated its utility in natural product structure determination.

## RESULTS AND DISCUSSION

The HTS probe operates at a $^1$H frequency of 400 MHz. A detailed description of the design and construction of the

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probe is available elsewhere[8,9] and will not be repeated here. Briefly, the HTS probe is cast as two epitaxial thin-film ceramics, disposed on either side of a 5 mm NMR sample tube, which are inductively coupled to r.f. input and tuning capacitors. The coils are doubly tuned to $^{1}H$ and $^{2}H$ to provide a dedicated, $^{1}H$-only probe with an internal lock channel. There are no pulsed field gradients or low-$\gamma$ heteronuclear r.f. coils.

## SNR and lineshape

SNR was measured using a standard ethylbenzene sample, following standard procedures. As shown in Fig. 1(A) an SNR of 1691 : 1 was obtained on a 0.1% ethylbenzene standard sample, which represents a substantial improvement over the *ca* 450 : 1 SNR obtained on the same instrument using a standard probe. Following the discussion by Hill,[9] an estimated SNR enhancement for the HTS probe of *ca* 22-fold is obtained using a loaded probe $Q$ of *ca* 10 000 at $T_c = 25$ K. A predicted enhancement of *ca* 4.5-fold is obtained when the geometrical filling factor of 0.2 for this probe[9] relative to a standard probe is included in this calculation. We observed only a 3.5-fold signal enhancement. Nevertheless, the HTS probe increases the sensitivity of the 400 MHz instrument to that of a typical 750 or 800 MHz system. This increased sensitivity can be translated directly into a decreased amount of signal averaging to obtain a desired SNR on a real sample, or into smaller amounts of material used to generate interpretable NMR spectra. Figure 1(B) shows an expansion of the 1D spectrum of $CHCl_3$, showing the lineshape obtained on this probe with the 26-channel shim set supplied by the instrument vendor. The linewidth measured at 50% maximum intensity was 0.88 Hz and the lineshape (peak width) at 0.55 and 0.11% intensity was 7.26 and 14.14 Hz, respectively. These values are slightly larger than those typically obtained using conventional probes, which may complicate spectral analysis in some samples. In the examples shown below, this lineshape did not impede spectral interpretation.

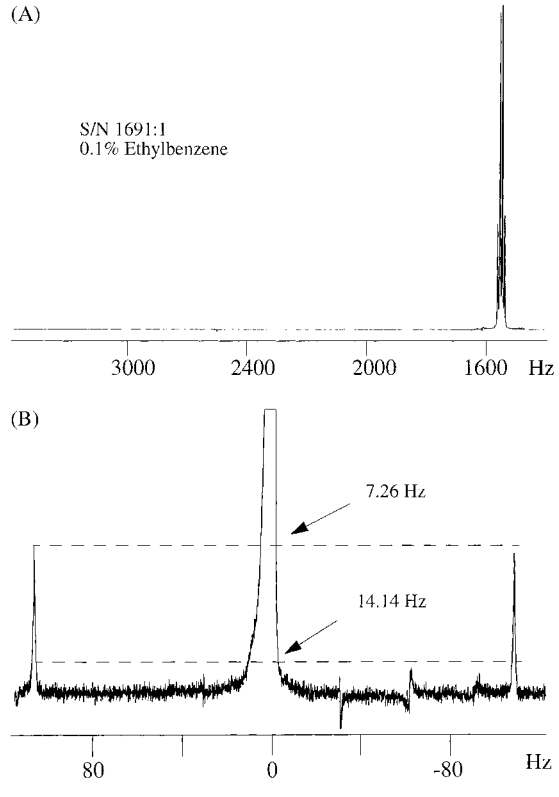


**Figure 1.** 1D spectra indicating the SNR obtained on 0.1% ethylbenzene (top) and lineshape measurements using $CHCl_3$ (bottom). The lines in the bottom spectrum indicate the points at which the lineshape is measured.

## R.f. transmission characteristics

The 90° pulse was 16 μs measured using 9 dB attenuation of the 50 W power on a $CHCl_3$ sample in acetone-$d_6$ solvent. The value is reproducible over time and between chargings of the probe. The r.f. homogeneity, measured as the ratio of the 450°/90° pulse widths, was 65%, which is comparable to that observed on standard probes. The

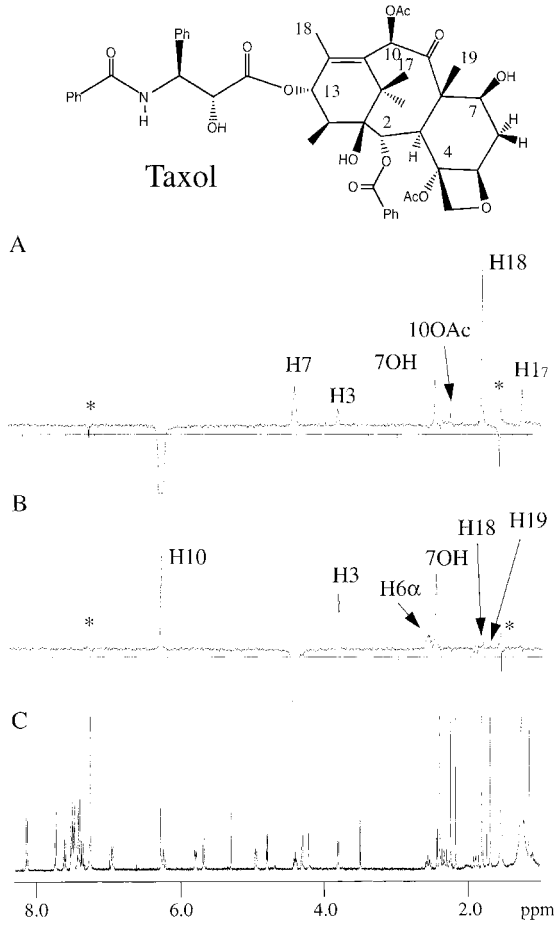


**Figure 2.** NOE difference spectra collected on taxol using the HTS probe. (A) NOE difference spectrum resulting from selective saturation of H-10; (B) NOE difference spectrum resulting from saturation of H-7; (C) a high-resolution 1D spectrum of the same sample collected using a standard, $^{1}H$-only probe. The structure of taxol is shown at the top, with the numbering system indicated. Assignments for the NOE enhancements are provided in the text.

linearity between r.f. power levels was poor, meaning that decreasing the r.f. amplitude by half did not lead to a doubling of the 90° pulse length. Specifically, values of 18 μs (38 μs) and 30 μs (68 μs) were obtained for the 90° (180°) pulse lengths on the chloroform sample when the r.f. amplitude was decreased by 6 and 12 dB, respectively. Although this is not an impediment to using the probe, the operator needs to be aware of this limitation and accurately calibrate pulses at all power levels in each experiment.

## Application to natural product characterization

The 1D NOE difference experiment provides critical spatial relationships used when determining the structures of organic molecules and natural products, and places stringent requirements on instrument and probe stability.[10] We typically perform this experiment in the steady-state mode by saturating a single spin multiplet with low-power selective irradiation. The resulting NOE enhancement in dipolar-coupled spins is measured as the difference in resonance intensity of 1D spectra collected with the irradiation on-resonance and far off-resonance. Figure 2 shows representative NOE difference spectra collected on taxol, a natural product of considerable interest owing to its anticancer properties. Figure 2(C) shows the 1D spectrum collected on a traditional, dedicated $^1$H probe at 400 MHz, and the top two spectra represent 1D NOE difference spectra collected following irradiation of (A) H-10 and (B) H-7. These spectra were collected on *ca* 1 mg of taxol in 650 μl of $CDCl_3$ (*ca* 2 mM solution) in 64 scans with a 5 s preirradiation pulse using the Varian 'cyclenoe' experiment. This experiment interleaves the on- and off-resonance irradiations from a single transmitter on alternate scans, and collects a 'difference' spectrum. The quality of NOE difference spectra collected using the HTS probe is fairly high, with flat baselines and NOE enhancements devoid of subtraction errors, indicating high stability of the irradiation pulse and receiver over the course of the experiment. Specifically, saturation of H-10 generates NOEs to H-7 (4.4 ppm), H-3 (3.8 ppm), 7-OH (2.43 ppm), the methyl on the C-10 acetate (2.15 ppm) and the H-18 methyl (1.8 ppm). The H-10 singlet at 6.25 ppm is selectively saturated with only minimal saturation of the adjacent triplet, H-13; the enhancement at *ca* 1.4 ppm is the H-17 methyl group, which arises from a direct NOE from H-13, showing that the r.f. selectivity achieved can be fairly high. Higher selectivity was achieved by further reduction of the saturation power (not shown). Saturation of H-7 shows strong NOEs to H-10 (6.2 ppm), H-3 (3.8 ppm) and the vicinal hydroxyl proton (2.4 ppm) and weak NOEs to the H-18 and H-19 methyl groups. The poorer lineshape associated with the HTS probe is apparent on comparing the NOE difference spectra with the 1D reference spectrum collected using a traditional probe. For example, the splitting of the H-7 resonance (4.44 ppm) due to scalar coupling is resolved to the baseline using a traditional probe, but is barely perceptible in the spectrum collected with the HTS probe. However, the decreased lineshape did not impede interpretation of the NOE difference spectra.

A more striking comparison of the sensitivity advantage afforded by the HTS probe is presented in Fig. 3, which

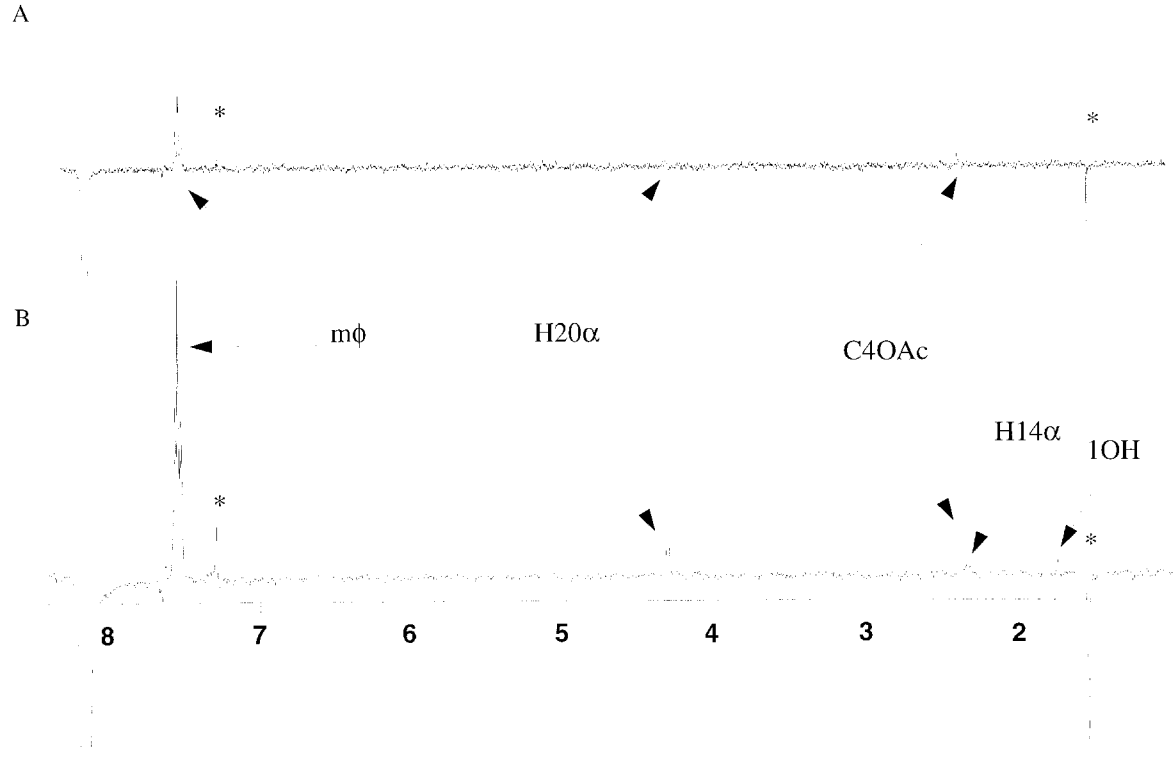


**Figure 3.** Comparison of SNR enhancement afforded by the HTS probe over a conventional probe. NOE difference spectra collected on a 250 μM sample of taxol, under identical conditions using either (A) a conventional probe or (B) the HTS probe.

 

directly compares NOE difference spectra collected under identical conditions on the HTS probe and a traditional $^1$H-only probe. For this comparison, the taxol sample was diluted to 250 μM and the signal was averaged over 1024 scans. The spectrum collected on the traditional probe [Fig. 3(A)] contains a strong NOE from the C-2 *ortho* benzoate proton (8.2 ppm) to the *meta* proton (m$\phi$; 7.5 ppm), with weak NOEs observed at 4.3 (H20$\alpha$) and 2.45 ppm (acetate methyl on C-4; labeled C4OAc). The SNR on these latter NOEs was low and they could easily be missed with only slightly degraded instrument performance. The difference spectrum collected using the HTS probe [Fig. 3(B)] shows the NOEs to the *meta* proton, H20$\alpha$, and also C4OAc, but with dramatically increased sensitivity. The NOEs observed at 2.4 (H14$\alpha$), and 1.8 (1OH) ppm in the difference spectra collected using the HTS probe were not observed using standard probes (even at higher taxol concentrations), and are consistent with the known structure of taxol.

## CONCLUSION

The HTS probe provides a significant increase in sensitivity that is directly translatable into decreased data collection times and/or decreased amounts of sample required. The increased SNR comes from a large increase in the probe $Q$ and a decrease in the thermal noise of the probe circuit. The increased $Q$ generates some non-linearity in power transmission, requiring careful calibration of pulses applied at different power levels, although once the pulses have been calibrated they are stable and reproducible. Eliminating the coil resistance means that the total resistance of the r.f. circuit is dominated by the sample.[11] Hence the probe is extremely sensitive to changes in sample conductivity and radiation damping, and is best used with organic solvents, although we have used samples in $D_2O$ with presaturation of the residual water resonance. The current probe is not equipped with heteronuclear r.f. coils or pulsed-field gradient coils, which severely limits its utility in natural product and structural biology applications. For example, the 90% $H_2O$–10% $D_2O$ solutions commonly used in structure and dynamics studies of biological macromolecules should not be used. Nevertheless, the increased sensitivity afforded by the HTS probe is substantial and will facilitate structure elucidation of natural products, especially when the sample is sparingly soluble or is mass limited. For the latter case, probes with limited sample volumes have been designed that optimize sensitivity and lineshape characteristics.[12,13] For low-solubility samples, large-diameter probes are available, but these probes are often impractical. The HTS probe, which uses standard 5 mm sample tubes, is expected to be of particular use with samples that are sparingly soluble.

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