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A High-Pressure, High-Resolution NMR Probe for Experiments at 500 MHz

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A novel high-pressure, high-resolution NMR probe is described which operates at a frequency of 500 MHz. The design features an alternative RF coil (8 mm sample tube) for high frequency, sensitivity, probe power, and resolution (< 3.0×10^{-9}). The probe is capable of pressures to at least 5 kbar over a temperature range of −30 to 80°C, and has a double-tuned $^1H/^2H$ circuit which can tune at ^1H frequencies of either 300 or 500 MHz. The sensitivity of the 300-MHz circuit is over twice that of previous 10-mm high-pressure NMR probe designs, while at 500 MHz the sensitivity is nearly five times that of previous 300-MHz pressure probes. Potential biochemical applications are demonstrated by 2D NOESY spectra of a Troponin C mutant.

Key Words: **high pressure; NMR; probe design.**

INTRODUCTION

The combination of advanced high-resolution NMR and high-pressure capabilities is very valuable in that it can provide information on dynamic biochemical problems of considerable interest, including pathways of pressure-assisted protein unfolding (*1–3*). In addition to its fundamental thermodynamic value, pressure can also be used as a tool which allows one to cool aqueous solutions well below the atmospheric freezing point of water and thereby study such interesting biochemical phenomena as cold denaturation (*3*). We have recently reviewed our own progress in developing high-pressure NMR instrumentation and have described several applications of the technique to biochemistry (*3–5*).

A continuing goal of our instrumentation efforts has been performance improvements in our high-pressure NMR probes. For example, previously (*4*) we have detailed a probe which extended our working pressure range to around 9 kbar (900 MPa) on a 300-MHz spectrometer with 1 Hz resolution (3×10^{-9}). As we explained, a pressure range of at least 5 kbar is often a necessity in protein unfolding studies. The only reasonably safe method for achieving these pressures with high resolution, however, is the autoclave-style approach, in which both the sample and the RF coil are located inside a high-strength pressure vessel. The signal is transmitted through the vessel by a pressure feedthrough to an external tuning network. With such an arrangement, the feedthrough becomes part of the circuit and behaves as a transmission line, imposing limitations on both the sensitivity and the tuning range of the NMR probe. The problem is compounded by corrosion concerns in vessels similar to ours which use a carbon disulfide (CS_2) pressurizing medium. Inclusion of a porcelain chip capacitor inside the pressure vessel with a long-lasting, high-strength Berylco feedthrough helped alleviate concerns about durability, but at best could only offer a sensitivity comparable to our previous 300-MHz NMR pressure probes. Excluding our own probes, the highest reported frequency for an autoclave-style pressure vessel has been 400 MHz, although with limited pressure ranges of only ~2 kbar (*6, 7*).

Since NMR itself is inherently a low-sensitivity technique, one can readily foresee the additional problems facing our high-pressure NMR probes as we routinely work with dilute biochemical samples. Apart from increasing the concentration, methods available for improving sensitivity include improving the electronic circuit, increasing the sample coil filling factor, and increasing the spectrometer frequency (*8*). To meet this challenge, we have been focusing our efforts on an alternative RF coil design which has increased the sensitivity, power, and tuning range of our double-tuned ($^1H/^2H$) NMR probes. The pressure vessel, pressure feedthroughs, and internal capacitor concepts described earlier (*4*) are all incorporated. This communication describes the RF coil and details the improved performance features of the versatile new probe. A more complete description of the probe, including detailed drawings, has been published separately (*9*).

This project has more than doubled the sensitivity and power of our 300-MHz probes (over our previous 10-mm probes), while also using a smaller 8-mm sample tube. The same 5-kbar probe also tunes at 500 MHz where we have achieved a sensitivity nearly five times that of previous 300-MHz probes (the change to higher frequency requires a minor adjustment to the match capacitor of the deuterium lock channel). In addition, the combination of high resolution with improved sensitivity has made the 500-MHz probe quite suitable for 2D NMR studies of biochemical systems.

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HIGH-PRESSURE NMR SYSTEM AND PROBE

Spectrometer, Pressure Generation System, and High-Pressure NMR Vessel

As was previously described (*4*), our system is composed of a commercial NMR spectrometer (former GE system with a Tecmag interface) and an Oxford wide-bore superconducting magnet (ϕ = 89 mm, 7.04 T) operating at a proton Larmor frequency of 300 MHz. In addition, we have recently obtained access to a commercial 500-MHz system composed of an Oxford wide-bore superconducting magnet (ϕ = 89 mm, 11.7 T) and a Varian UNITY INOVA spectrometer (Varian-Oxford Instruments Center for Excellence NMR Laboratory, School of Chemical Sciences, University of Illinois).

The 5-kbar pressure generation system used for the new system is similar to our earlier systems (*4, 5*), with CS_2 as the pressure transmitting fluid. Since the probe circuit can tune at either 300 or 500 MHz, two different NMR pressure vessels are being used. The 300-MHz vessel has been described previously (*5*), while an entirely new pressure vessel has been built for the 500-MHz system. The new vessel was constructed of the high-strength beta alloy of titanium, 3A1–8V–6Cr–4Zr–4Mo [RMI Titanium, 180 kpsi yield strength (YS)]. The vessel design is similar to that of the 9-kbar vessel described previously (*4*), although with a larger inner diameter (1.9 cm) to accommodate NMR tubes up to 10 mm. The vessel has a calculated ultimate burst pressure $>$ 12 kbar at the seal flange and has a temperature range of -30 to 80°C. Complete drawings of the vessel and its associated components, as well as a more detailed description, have been published separately (*9*).

Feedthroughs, RF Coil, NMR Probe, and Samples Tubes

The new NMR pressure probe uses our recently developed high-pressure feedthrough (*4*), consisting of a short Berylco 25 feedthrough seated in a matching Vespel SP-1 (DuPont) polyimide plastic insulator. The feedthrough design has been tested to pressures of 10 kbar.

The primary unique feature of the new probe is its RF sample coil. In previous designs we opted for a two-turn saddle coil (20 AWG Teflon-coated, silver-plated wire). It is well known, though, that such a coil will limit probe performance due to its number of turns (more turns means higher inductance and lower attainable frequencies) as well as its limited surface area (which increases the effective resistance at high frequencies due to ''skin effects''). It is for this latter reason that most commercial probes use a copper or gold foil in probe construction, rather than a wire. Our earlier wire-coil designs, however, were motivated by concerns arising from the corrosive nature of our carbon disulfide pressurizing fluid.

For the current design, we have machined a cylindrical single-turn saddle coil (Fig. 1) from low magnetic susceptibility oxygen-free copper (Wesgo, Inc., Belmont, CA). The coil is incorporated into the RF circuits described previously (*4, 9*).

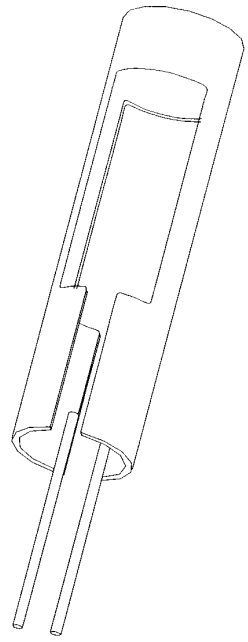

FIG. 1. Schematic design of the new RF coil design. The single-turn coil is machined from low magnetic susceptibility oxygen-free copper, with a total length of 34.3 mm. For more details, consult the text and Ref. (*9*).

Porcelain chip capacitors are soldered directly onto the coil, and the coil is used ''directly'' without any coating. The inner diameter (8.1 mm) is just slightly larger than an 8-mm NMR tube to promote a high filling factor, while the walls of the coil were made relatively thick (0.5 mm) both to provide mechanical strength and to help extend the lifetime of the coil in corrosive carbon disulfide. Standard tests, described in the next section, show that the coil exhibits no degradation in performance over several months of use, despite the onset of marked discoloration. As was noted in the Introduction, the circuit tunes at both 300 and 500 MHz, with the double-tuned circuit requiring only a minor change in size of the deuterium channel's match capacitor (see Ref. (*9*)). The probe is used with our ''typical'' 8 mm piston-assembly NMR pressure tubes (*4, 9*).

PERFORMANCE CHARACTERISTICS AND APPLICATIONS

We routinely perform standard tests to assess our NMR pressure probes. This includes a resolution test (1% chloroform in either acetone-*d*6 or chloroform-*d*) and a sensitivity test (0.1% ethylbenzene and 0.2% TMS in chloroform-*d*). We note that there are some differences for these measurements between Tecmag MacNMR and Varian VNMR software, but in general, the magnitude of the results should be comparable. Figure 2 contains examples of standard spectra obtained for the new coil at 300 and 500 MHz, clearly demonstrating the excellent resolution attainable without sample spinning—0.7 Hz at 300 MHz (2.3×10^{-9}) and 1.5 Hz at 500 MHz ($3.0 \times$

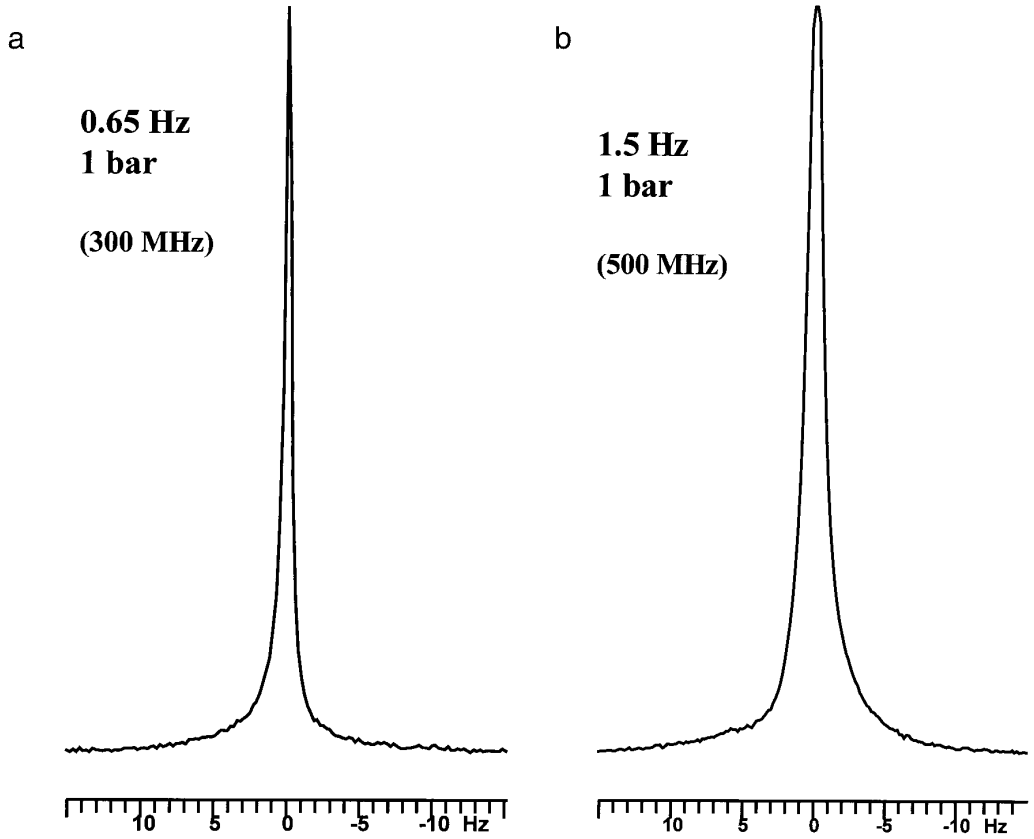

FIG. 2. $CHCl_3$ peak of the lineshape sample (1% v/v $CHCl_3$ in acetone-*d*6, 300 MHz; 1% v/v $CHCl_3$ in chloroform-*d*, 500 MHz) measured at 1 bar in an 8-mm tube using the new RF coil probe at 300 MHz (a) and 500 MHz (b).

10^{-9}). The data in Fig. 2 are best-case spectra, with more typical values of 1.5 and 2.5 Hz for the 300- and 500-MHz systems, respectively. Table 1 demonstrates the dramatic increases in both sensitivity and probe power we have observed as a result of the improved coil design.

While one can readily appreciate the advantages of higher sensitivity and probe power on dilute biochemical studies using one-pulse methods, we feel that an even more important extension of this work is in the field of high-pressure 2D NMR. As an example of this capability, we include Fig. 3 which

TABLE 1
Comparison of Pulse Widths and Sensitivity (S/N) of Various High Pressure NMR Probes

	^{1}H Freq. (MHz) [Max. Press.-bars]	Sample O.D. (mm)	Pulse width (μs)[a]	S/N[b]	Ref.
Commercial HB[c]	300 [—]	5	18	81	This work
Two-turn wire coil, no internal capacitor	300 [5000]	10	55	50	(*5*)
Two-turn wire coil, with internal capacitor	300 [9000]	8	77	34	(*4*)
Two-turn wire coil, with internal capacitor	300 [5000]	10	57	62	(*9*)
One-turn machined coil, 300 MHz	300 [5000]	8	20.25	131	This work
One-turn machined coil, 500 MHz	500 [5000]	8	14.5	260	This work

[a] Value of the 90° pulse width used for the S/N determination. This value is not necessarily typical, as the ionic strength of the sample will in some cases significantly increase the pulse widths. All of these values, with the exception of the 500-MHz probe value, were obtained on the same spectrometer.

[b] All reported values (sensitivity is based on the height of the quartet region) represent the average of multiple tests, and error is estimated to be within 10%. A homemade 0.1% ethylbenzene/0.2% TMS/chloroform-*d* sample was used for all determinations. Within experimental error, the value for this homemade sample agreed with a similar determination on the commercial probe using a standard 0.1% ethylbenzene/0.1% TMS/chloroform-*d* sample (Wilmad). No attempt has been made at scaling these values to equal volumes—for more details on volume scaling, see Ref. (*9*).

[c] GE300WB Commercial High Band Liquids Probe, 10 mm coil diameter, using a 5 mm sample tube.

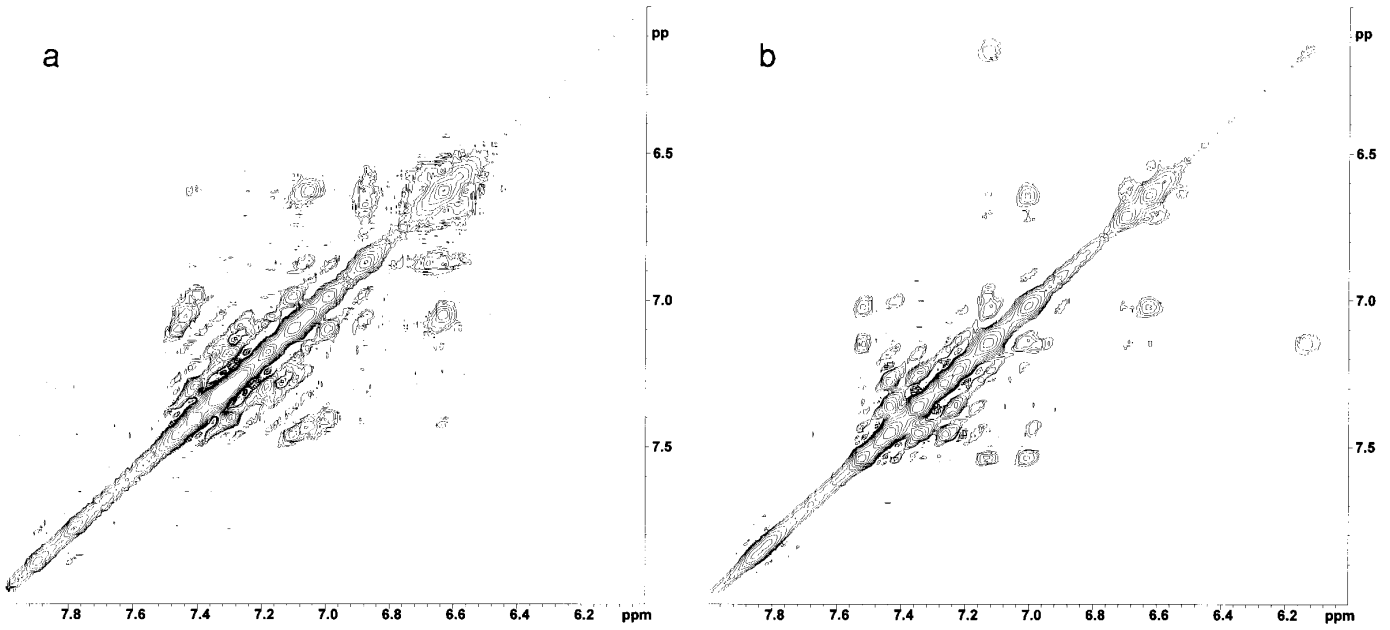

FIG. 3. Demonstration of the 2D capabilities of the new probe design at 500 MHz with the calcium-saturated F29W fragment of Troponin C. The phase-sensitive 2D NOESY spectra of the aromatic region at 1 bar (a) and 5 kbar (b) were acquired with ~0.6 mM protein (20 mM TRIS-*d*11 buffer, 100 mM KCl, 2 mM EGTA, 5 mM DTT, 8 mM $CaCl_2$) at pH 7.0 and 25°C using 48 scans and 1K × 128 (×2) complex data points with a mixing time of 0.15 s. Data processing, performed with NUTS software (AcornNMR), included zero-filling to 2K × 2K data points, multiplication by a Gaussian weighting function, and symmetrization. Full spectral analysis is in progress and will be published at a later time.

contains the aromatic region of a 2D NOESY spectrum for the calcium-saturated F29W N-domain fragment of Troponin C (detailed analysis is in progress and will be published at a later time) at both ambient pressure and 5 kbar. In this example, one clearly observes that pressure-induced spectral changes have occurred. An important aspect to mention is that the spectra in Fig. 3 were obtained for a ~0.6 mM solution within ~12 h.

In summary, we feel that this work demonstrates a significant advance for our high-pressure NMR capabilities. By using the new sample coil design with its lower inductance and larger surface area, we have developed the first 500-MHz autoclave-style probe (5 kbar pressure range) and have observed major increases in both sensitivity and probe power. These advances have been demonstrated with 2D NMR applications to a biochemical system. Our current objective is to capitalize on this work by incorporating the coil design into triple-tuned NMR probes for applications such as high-pressure studies of isotope-labeled proteins (*10*).

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