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An NMR probe with a high-pressure chamber made from composite materials

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ABSTRACT: The development of an NMR probe operating at high pressure is described. It can operate up to 1.5 kbar, with a burst pressure of 2 kbar. The pressure chamber consists of a simple cylinder built from fibre-glass–epoxy resin composite material. Its internal diameter is 6 mm. The main ^{1}H coil is buried in the composite material. Simple O-ring seals are used, mounted on a conical housing. This set-up allows the mounting of an additional coil at ambient pressure, acting through the composite material, with little loss of efficiency compared with a regular NMR probe, thus allowing the acquisition of heteronuclear NMR experiments. The sensitivity and resolution permit 1D and 2D studies of aqueous solutions of large biomolecules such as proteins. © John Wiley & Sons, Ltd.

KEYWORDS: NMR; high-pressure NMR probe; composite material; apomyoglobin

INTRODUCTION

Pressure is an important thermodynamic parameter which determines chemical equilibria and chemical reactions as much as temperature does. In the field of NMR studies of biological molecules, pressure permits the induction of protein denaturation under mild and reversible conditions, the displacement of equilibria revealing the ΔV of the reactions, the observation of cold denaturation, due to the anti-freeze properties of pressure, and the study of molecules from lifeforms coming from deep-sea/high-pressure ecosystems.[1,2]

Varying the pressure conditions permits the exploration of important aspects of solutions, and an NMR probe operating at varying high pressures, while maintaining good spectroscopic qualities, is a very useful piece of equipment.

Several workers have already shown elegant designs for high-pressure NMR probes and very high hydrostatic pressures have been demonstrated.[3–8] The general set-up is the same for all the probes which have been presented so far.[9] A pressure chamber is built in a non-magnetic metallic material such as titanium or beryllium–copper alloy. The external dimensions of this chamber are chosen to fit exactly the internal dimensions of the NMR magnet bore. Within this chamber, an NMR measurement system is adapted, comprising a coil and a sample chamber.

Very high pressures can be obtained with this set-up. Careful design permits pressures to be achieved which are nearly equivalent to that attainable with the best steels. Another advantage is that the design is traditional, and techniques can be adapted from other high-pressure devices. However, this approach suffers from the difficulties one finds in designing a good NMR system in such a chamber. Several problems arise from this set-up. First, the need of r.f. lead-throughs and the problem of electrical coupling of the coil with the chamber walls jeopardizes the efficiency of the coil, resulting in low sensitivity and long 90° pulses. Second, serious difficulties have arisen in fitting two independent coils into such a chamber, thus making multichannel NMR spectroscopy very difficult.[10]

The approach we propose here is completely different to those developed so far, and consists in using a composite material of fibre-glass and epoxy resin for the pressure chamber. The main advantage of this novel approach is that, since this material is a pure insulator, the wall can be put as close to the r.f. coil as needed. In fact, we chose to have a coil located inside the wall itself, in order to minimize the connection problems. This approach completely obviates the need for high-pressure r.f. lead-throughs, with the associated tuning problems. With this set-up, the pressure piping is also highly simplified, and simple O-ring seals can be used. The design of a reverse multichannel probe is possible.

This paper describes the development of an NMR probe built with this design. This probe can operate up to 1.5 kbar, with a burst pressure of 2 kbar. The sensitivity obtained with this system is nearly equivalent to that of commercial high resolution probes. The resolution, although not optimum, is certainly good enough to study aqueous solutions of large biomolecules such as proteins and oligonucleotides.

The probe presented here operates at 360 MHz for the proton frequency, and fits into a regular 73 mm (wide-bore) system. However, this design can certainly be easily extended to higher field strengths and narrower accesses.

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EXPERIMENTAL

The proposed system consists of a simple composite material tube with an internal diameter equal to the sample diameter and with the ^1H detection coil buried into the wall of the pressure chamber. This tube is directly connected to the high-pressure system on one side, and a stopper is located on the other side (Fig. 1).

The pressure chamber is made from a fibre-glass–epoxy resin composite material. Fibre-glass (E-type) was chosen because it has the best tensile strength of all the insulating fibre materials, and also because it does not show any background NMR signal for usual high-resolution observation. Carbon fibres could not be chosen here because the electrical conductivity is too high.

The pressure chamber was built from simple fibres that were wound in a cylindrical shape with a constant angle of the fibre with the cylinder axis. The epoxy resin was deposited on the fibre during the winding process by two nozzles, spraying each resin component on to the fibre. Care was taken to ensure that the ^1H background NMR signal of this material was small and wide enough to allow observation of high-resolution spectra. Fibre-glass and epoxy resin were purchased from Hexcel (St-Ouen l'Aumône, France).

The winding of the fibre was performed at a constant angle of 20° and at a constant angular velocity of 1 cm s^{-1}. The tension of the fibre was kept constant with a simple brake system. The final diameter of the cylinder was 13 mm external and 6 mm internal. To ensure a perfect cylindrical geometry of the tube, and thus minimize field inhomogeneity problems, the pressure tube was machined to a smooth surface on a lathe.

After trial, we found that such a design does not ensure good water tightness for pressures above 500 bar, because water can percolate through the composite material. For this reason, the inner surface of the pressure chamber was coated with a thin poly(ethyleneterephthalate) (PET) film.

The ^1H coil was positioned during the winding process, on the fibre cylinder after a thickness of about 0.5 mm of material had been reached. The fibre was then wound to the final diameter of 13 mm. Several coil designs were explored, and we finally chose a classical saddle coil design.

Two seals are located at the top and bottom of the pressure cylinder. Because of their simplicity of use, we chose to use O-ring seals. The chosen seals were made of Viton, with external and internal diameters of 6.3 and 4 mm, respectively. The seals are mounted in a non-classical geometry, in which the housing of the seal is opened on one side, and presents a conical shape. The seals cannot bear directly on the PET which coats the internal side of the pressure chamber because the PET material would puncture. To ensure proper contact for

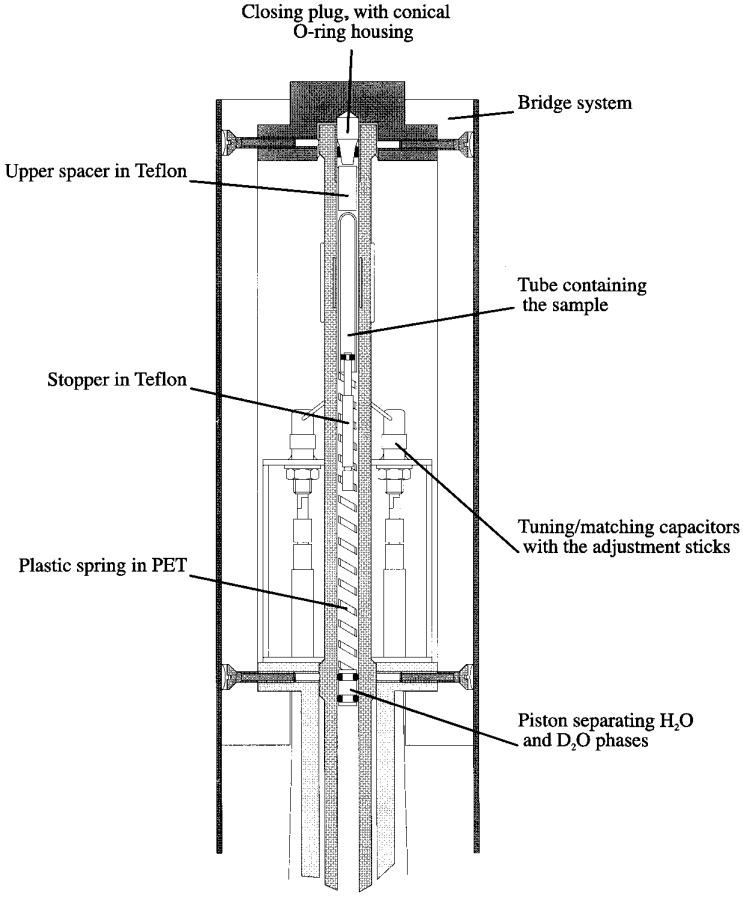

Figure 1. Schematic diagram of the probe design.

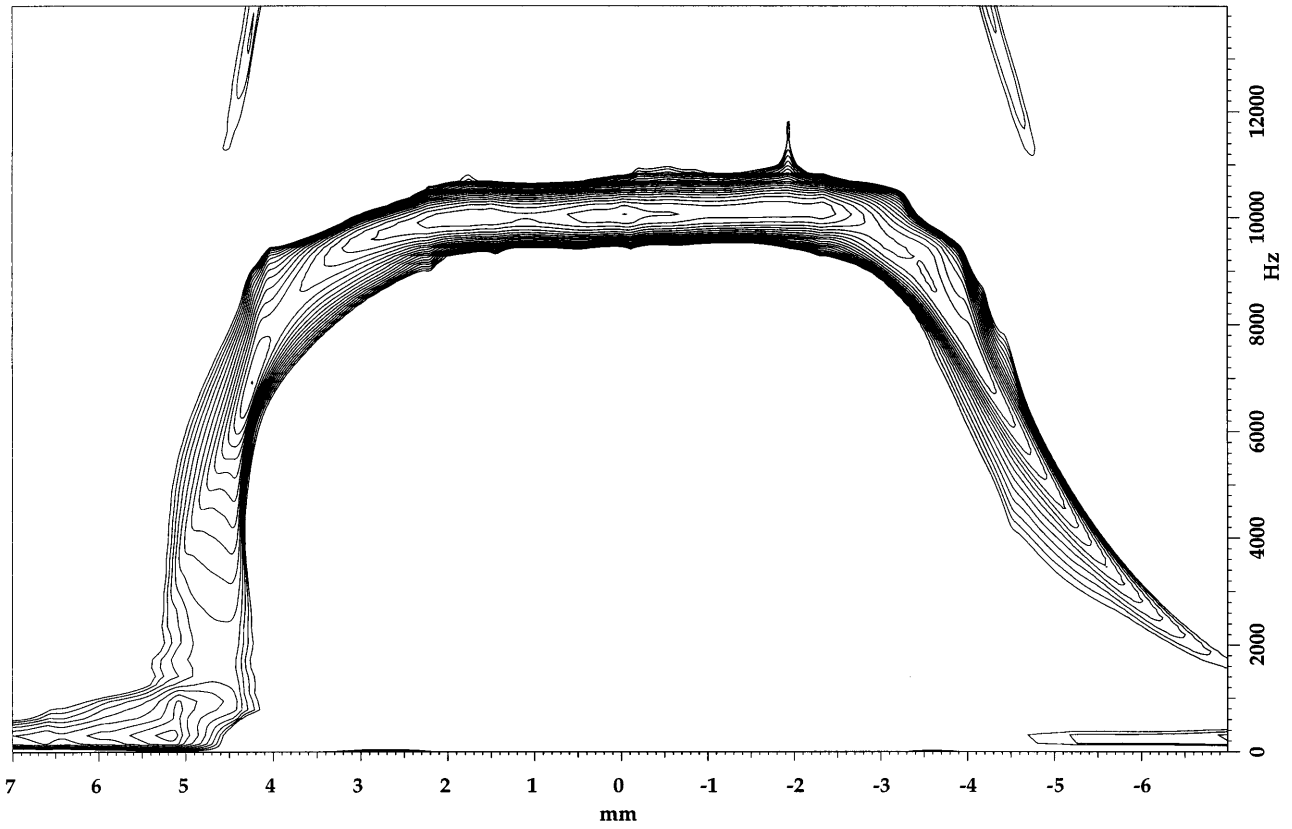

Figure 2. The B_1 profile of the probe. The experiment was performed on water by acquiring a single pulse sequence, in the presence of a moderate linear B_0 z-gradient; 512 experiments were performed by incrementing the pulse flip angle and a real Fourier transform was applied along the F_1 axis.

the seal to work on, two brass rings were added to the pressure chamber at the location of the seals. These rings were glued in place by the epoxy resin, and tightness between the PET coating and the brass ring was ensured by using a glue made with an epoxy polymer with a default of cross-linker. This set-up ensured sufficient water tightness for pressures up to 1.5 kbar.

The upper part of the pressure tube has to be closed in a manner that can be easily opened in order to introduce the sample. This is done with a plug which penetrates into the pressure tube, and carries the axial force of pressure on an external bridge system. The 12 mm long rod was made from molybdenum, a strictly non-magnetic material with a very good Rockwell hardness.

The axial forces of pressure are applied to the plug on the top of the system, and to the entering piping, in the bottom. These forces are transmitted through the body of the probe and through a bridge from one side to the other. The bridge and the probe body are made from large-section aluminium. The lower part of the probe body was adapted from an old Bruker probe.

The probe is connected to the pressure pump system with standard piping purchased from NovaSwiss (Cesson, France). The pressure fluid is H_2O except in the upper part of the probe where D_2O is used to minimize NMR artefacts. A simple piston made of Teflon is used to separate the two phases. The solution under study is placed in a 5 mm glass tube, obtained by sectioning a standard 5 mm NMR tube to a length of 45 mm. The sample tube is sealed with a small Teflon stopper and located at the top of the pressure chamber secured by a Teflon spacer on one side and a plastic spring on the other.

The design of the electronics of the probe is very classical. All the coupling electronics are located on the outside of the pressure chamber, a few centimetres below the central magnetic point. The inner coil is matched and tuned to the ^1H channel of the spectrometer (here 50 Ω, 360 MHz). This coil is equipped with a double tuning system which permits the detection of a deuterium signal with a sensitivity sufficient to lock on a 10% D_2O sample.

RESULTS AND DISCUSSION

The chosen design permits the minimization of the forces that are applied to the system for a given pressure. The applied forces can be decomposed into two components, one axial and the other radial. The axial force is completely borne by the bridge system which transmits the forces applied to the stopper to the probe body. Similarly, the forces applied to the piping are transmitted to the probe body by two large brass screws. Owing to the small internal diameter of the pressure chamber, the applied forces are relatively small (e.g. 2.7 kN at 1 kbar). Radial forces are applied only to the chamber walls. The pressure thus applied is believed

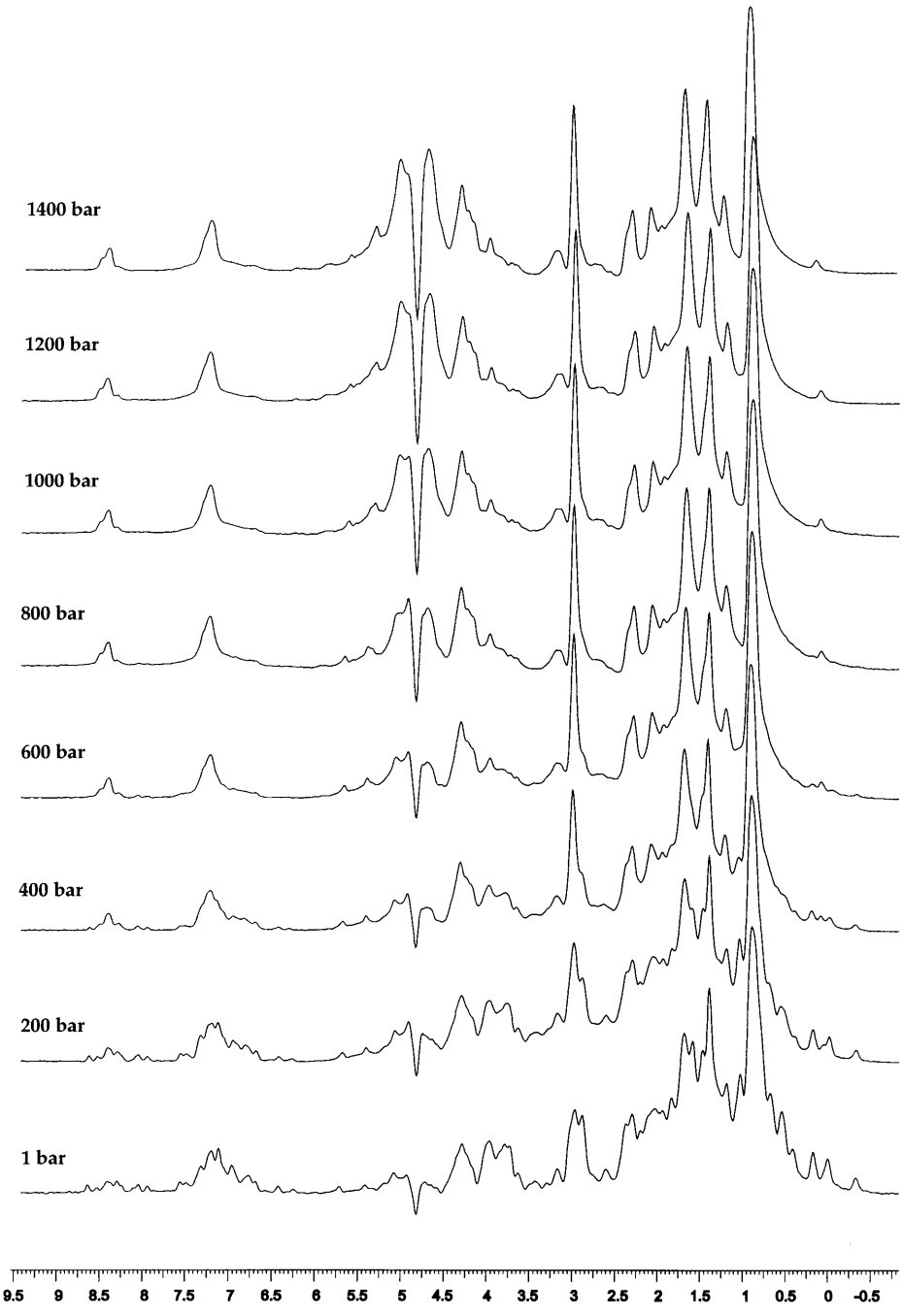

Figure 3. 1D spectra of 2.8 mM apomyoglobin in D_2O for pressures ranging from 1 to 1400 bar. Spectra were acquired in 128 scans.

to be well below the rupture pressure of the fibre-glass material itself. Additionally, the tension applied to the fibre during the winding probably strengthens the structure slightly. The burst pressure of this design is assumed to be at least 2 kbar.

The O-ring seal non-classical mounting was observed to present a very high resistance to pressure. In one test case, we observed such simple O-ring seals supporting 5 kbar of pressure without failing. On the other hand, the contact point between the PET coating and the brass rings appeared to be the weak point of this design, and a lot of care had to be taken in the design of this part.

In some cases, leaks up to 1 bar min^{-1} were detected during the pressure runs. This kind of leak does not hamper in any manner normal 1D operation. Short 2D experiments could be easily performed by manually correcting the pressure during the run; 2D acquisitions of several hours could thus be performed. However, overnight 2D acquisitions would certainly require automatic pressure control, available from NovaSwiss but not tested here.

The ^1H 90° degree pulses were measured at 12 μs on a water sample using the standard Bruker 35 W pulse amplifier. The B_1 profile was measured by the using a

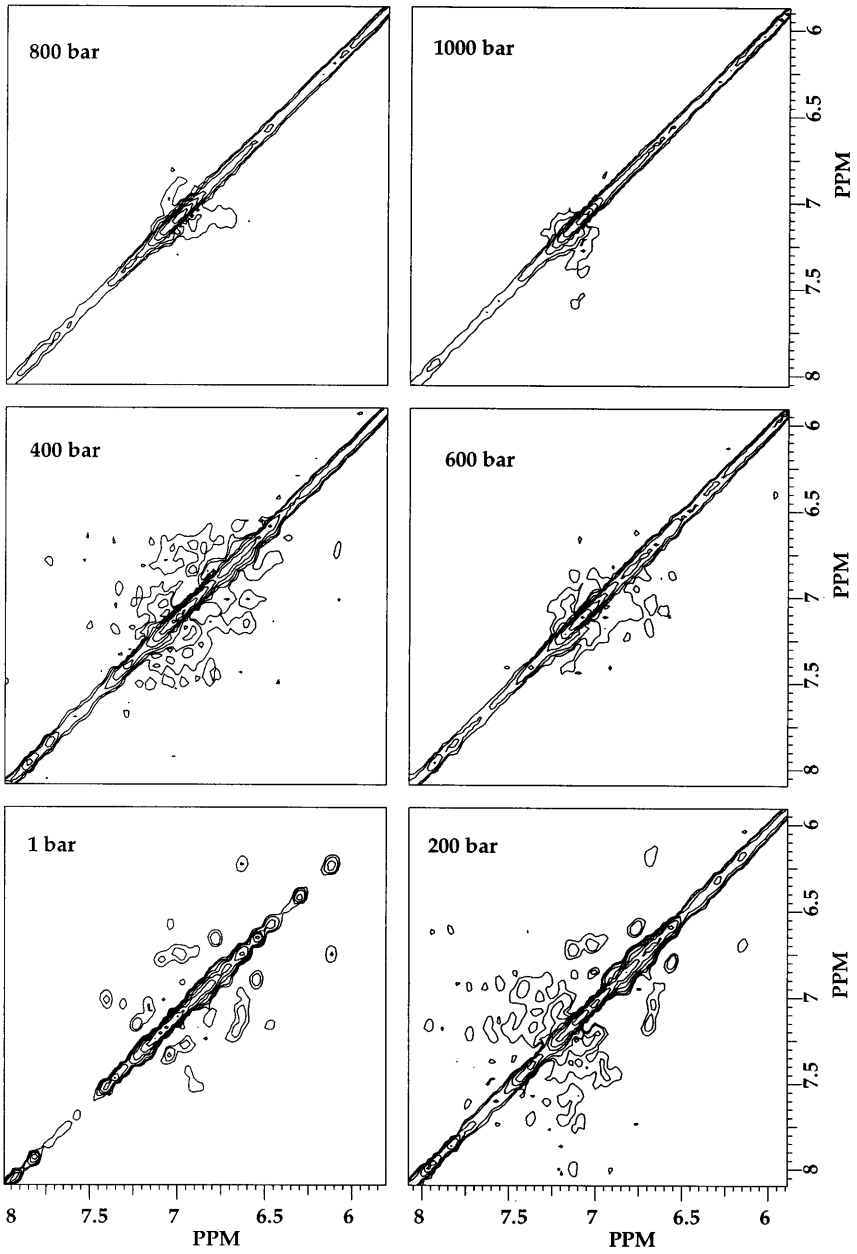

Figure 4. The aromatic region of the 2D NOESY spectra of 2.8 mM apomyoglobin in D_2O for pressures ranging from 1 to 1000 bar. 2D NOESY were acquired in 270 experiments of 16 scans, the mixing time was 100 ms and each 2D acquisition took 95 min.

z-gradient technique[11] and very good B_1 field homogeneity was observed with this design (Fig. 2).

With this system, the sensitivity appeared to be adequate; the ASTM sensitivity was measured as 70, compared with the value of 195 obtained with the standard Bruker probe on the same spectrometer. On biological samples dissolved in water or heavy water, the high-pressure probe proved to be about half as sensitive as the standard Bruker probe. The sensitivity is probably limited here by the poor dielectric properties of the epoxy resin used in the probe construction. This effect probably accounts for the sub-optimal Q factor (*ca.* 120) observed with this probe.

The maximum resolution achieved is 5 Hz at mid-width on a 500 µl sample of ethanol in a 5 mm diameter tube. This poor resolution is interpreted as being due to a mismatch in the susceptibility of the copper coil with the bulk susceptibility of composite material in which it is buried. This resolution, however, appears to be perfectly suitable for the study of biological samples in water, using either presaturation or jump-and-return water suppression techniques.

As an example, the 1D and 2D spectra of apomyoglobin obtained at various pressures are presented in Figs 3 and 4. The protein was obtained from Sigma, the heme was removed with acetone and the protein dissolved in D_2O, and the pH was adjusted to 5.5. The pressure was gradually raised from ambient to 1.4 kbar in 200 bar steps, and at each step a 1D and a 2D NOESY were acquired. The temperature was 27 °C.

Progressive denaturation of the protein could be observed for pressures ranging between 400 and 800 bar.

CONCLUSION

We have developed a high-pressure NMR probe, with a pressure chamber built from fibre-glass–epoxy resin composite material. This design is original compared with other reported high-pressure NMR probes. With the present set-up, we were able to reach a pressure of 1.5 kbar. This moderately high pressure is intermediate between pressures reported for metallic probes and pressures attainable with capillaries or sapphire tubes. Such a pressure is certainly sufficient for the study of many chemical and biological systems. This was exemplified here by the preliminary study of the pressure denaturation of apomyoglobin, and several examples can be found in the recent literature[12–17] which show the interest in such intermediate pressures.

The use of insulating materials allows much more freedom in the design of the r.f. part of the probe, giving access to sensitivity and a pulse width nearly equivalent to that of a standard high-resolution probe. We have demonstrated high-quality 1D and 2D NMR spectra of proteins. However, there is still room for improvement of this system. First, the epoxy resin should be more carefully chosen in order to optimize the sensitivity of the probe. The static magnetic field homogeneity should also be optimized, probably by finding a coil wire the magnetic susceptibility of which more closely matches that of the composite. Finally, temperature control has yet to be adapted to the pressure chamber. This can be done in a classical manner by fitting around the pressure chamber a jacket with circulation of a temperature-regulated fluid, such as can be found in most metallic probe designs. Of course, the composite material provides a much higher thermal insulation than a metal, but the reduced thickness of the chamber walls (here 7 mm) should still permit good temperature control.

A multichannel probe could also easily be obtained by adding an external coil, glued on the external surface of the tube. This second coil could be used to pulse on heteronuclear spins during indirect detection, and a triple-channel probe can certainly easily be built, with double tuning of this external coil. Additionally, a gradient unit could certainly be fitted on the outside of the pressure chamber, further extending the capabilities of this probe.

The weakest part in the present design of the pressure chamber is certainly the junction of the PET coating with the brass rings. This point has been a constant problem in the design of the probe, but we think that we have now reached a satisfactory solution, as demonstrated here.

Finally, it should be pointed out that the present probe is quite cheap to produce, since all the starting materials are of extremely low cost and the manufacturing remains simple.

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