NMR Spectroscopy

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NMR Spectroscopy: Pushing the Limits of Sensitivity

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Nuclear magnetic resonance (NMR) spectroscopy is an indispensable tool in physics, chemistry, biology, and medicine. Its applications go far beyond routine chemical analysis and include structure determination of biomacromolecules in solution,[1] and the structure and dynamics of polymers and supramolecular systems that are not crystalline in the traditional sense.[2] NMR spectroscopy can be carried out at very high magnetic fields, with a ^{1}H NMR frequency of up to 2.4 GHz now possible.[3] Despite the success of NMR spectroscopy, which results from its extreme site selectivity, the inherently low signal intensity remains a serious drawback which limits its application. In contrast, single-molecule detection is well established in scanning probe microscopy[4] and in optics, in which single electron spin resonance signals and couplings between these spins and a single nuclear spin can be observed.[5] To meet the ever-increasing demands of miniaturization, the sensitivity of NMR spectroscopy has to be increased substantially. Fortunately, several new approaches for improving signal intensity in NMR spectroscopy have recently been reported and should be brought to the attention of the scientific community. They include completely new detection methods and extensions of established techniques. Needless to say, in view of the innumerable problems that NMR spectroscopy can tackle, the signal intensity issue cannot be solved by a single technique. Therefore, this article briefly describes several of the new approaches and puts them in perspective.

One technique which is already well advanced involves increasing the nuclear polarization by laser-polarized noble gases. In fact, these hyperpolarized gases can themselves be put to use, for example, in lung imaging by magnetic resonance tomography (MRT).[6] To increase site selectivity in high-resolution NMR spectroscopy of liquids and in medical imaging, concepts from supramolecular chemistry have been used to construct a dendrimer-cage biosensor for hyperpolarized ^{129}Xe.[7] An important question, however, is how to dissolve ^{129}Xe in a controlled fashion in aqueous solutions. To this end, a membrane-based continuous flow system has been developed,[8] which should considerably simplify applying these methods in biomolecular NMR spectroscopy.

The sensitivity of NMR spectroscopy can also be increased through transfer of electron spin polarization to the nuclei (dynamic nuclear polarization, DNP). After a long period of development, enhancement factors of around 150 are now routinely achieved and make it possible to compare the packing of prion proteins in amyloid fibers and nanocrystals of the same material by solid state NMR spectroscopy.[9]

Another approach to miniaturization is to use the cantilever of a force microscope for mechanical detection of magnetic resonance signals (magnetic resonance force microscopy, MRFM). In recent work,[10] signals from a volume of less than 650 zeptoliters were detected, which is 60000 times smaller than the previous smallest volume for nuclear magnetic resonance microscopy. The basic setup and the microfabricated components of the MRFM experiment are shown in Figure 1. The sensitivity achieved corresponds to a spatial resolution of better than 100 nm and demonstrates the feasibility of pushing magnetic resonance imaging into the nanoscale regime.

Particularly promising are approaches which utilize established techniques, and in particular allow the use of sophisticated pulse sequences provided by commercial NMR spectrometers and used in all fields of NMR spectroscopy today. The development of several new probe designs can dramatically improve the sensitivity of conventional NMR spectroscopy, which usually employs radio frequency (RF) coils as detectors. The coil sensitivity can be increased substantially by a reduction in size and by cooling (micro- and cryoprobes). However, other methods can be used to detect the RF flux generated by the nuclei in the sample. Recently, planar microslot waveguide NMR probes, shown in Figure 2, have been designed with an induction element that was fabricated with sizes ranging over a large range, from centimeter to nanometer scale. This allows analysis of biomolecules in nano- or picomole quantities, reducing the required amount of material by several orders of magnitude.[11] Indeed, two-dimensional NMR spectra of 1.57 nmol of ribonuclease A were successfully recorded with this new device, requiring about 3300 times less sample than in a conventional 5-mm NMR probe. Beyond the applications in biomolecular NMR spectroscopy, this integrated geometry can be packed in parallel arrays and combined with microfluidic systems for combinatorial processes and in "lab-on-a-chip" devices, for example in metabolomics. A related

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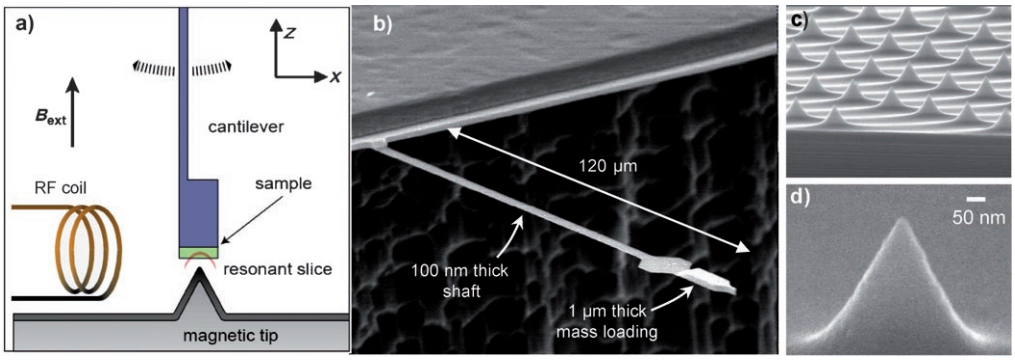

Figure 1. Basic setup and components of the MRFM experiment. a) A cantilever with a thin-film sample at the end is oriented perpendicular to a substrate supporting a conical magnetic tip. Nuclei that are within the resonant slice region near the tip can undergo magnetic resonance. b) Single-crystal silicon cantilever of the type used in the experiment. c) Scanning electron micrograph of an array of etched silicon tips used to form magnetic tips. d) Close-up of an individual magnetic tip after coating. Reproduced from reference [10].

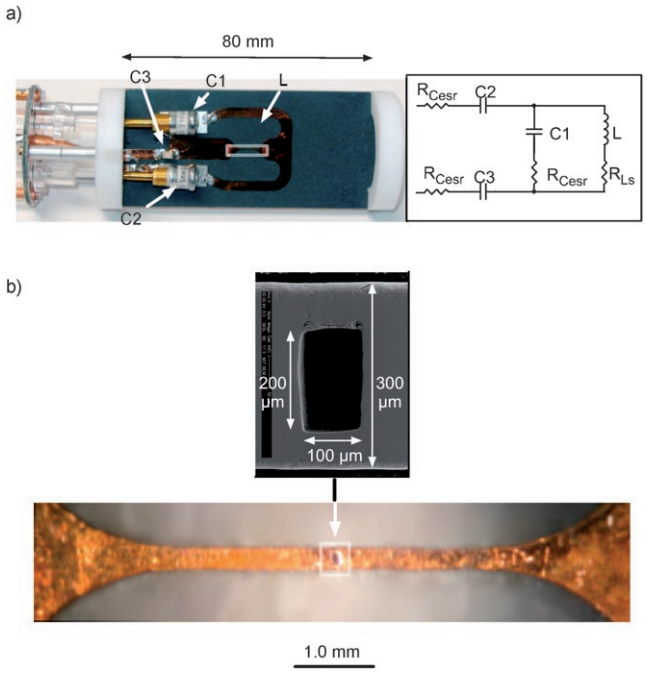

Figure 2. Microslot waveguide probe. a) Probe with housing removed. The probe circuit is manufactured on a planar substrate, with the detector area denoted by the white rectangle. Schematic circuit diagram is shown on right (C capacitor, L coil, R resistance). b) Microslot fabricated by using a 248-nm excimer laser, immediately after laser exposure. Reproduced from reference [11].

approach with great promise is based on stripline probes, which are easy to produce by lithographic methods and may eventually lead to NMR probes that are cheap disposable consumables.[12]

In solid-state NMR spectroscopy, the lack of spectral resolution arising from anisotropic interactions, such as dipole–dipole coupling, anisotropic chemical shift, and quadrupole coupling,[13] means additional procedures are required to record high-resolution spectra. Most are based on sample rotation at the so-called magic angle of 54.7° (MAS-NMR)[14] combined with high-power decoupling. In addition, spinning frequencies of up to 70 kHz can be achieved[15] for optimum removal of anisotropic interactions. As in solution NMR spectroscopy, cryoprobes are being designed to further increase sensitivity. MAS-NMR spectroscopy combined with double-quantum techniques provides unique information about structure and dynamics in supramolecular systems, probing hydrogen bonds, π–π interactions, shape persistence, and attachment to surfaces[2] in as-synthesized samples. Moreover, MAS-NMR spectroscopy has been successfully applied to study secondary structure, dynamics, and membrane topology of an entire seven-helix receptor, uniformly labeled with ^{13}C and ^{15}N, in a native membrane environment.[16] In both types of applications, however, the use of NMR spectroscopy is limited by the amount of available material. This not only means samples for which absolute quantity is restricted, but also a fraction of the material which occurs in a complex molecular arrangement such as fibrous proteins (e.g., silks or amyloid proteins) or self-assembled biomimetic materials.

In such cases, the use of microcoils provides a conceptually rather straightforward, yet highly promising, approach.[17] It paves the way for NMR studies of solid samples with nanoliter volumes. Furthermore, the very strong RF fields that can be generated by microcoils facilitate a much broader excitation bandwidth and/or decoupling efficiency, which is crucial for many solid-state NMR spectroscopy applications. Spinning a rotor with submillimeter dimensions at kHz frequencies seems a formidable task. However, Figure 3 shows that there is a much simpler approach. The microrotor can simply be "piggy-backed" onto a conventional rotor. In this way, conventional stators can be used and the larger 4-mm rotor assures stability of sample rotation, including that of the microsample container. These containers can have dimensions down to 170/125 μm outer/inner diameter, which corresponds to a 10-nanoliter sample volume. A critical step in the probe assembly is the alignment of the microcoil circuit with respect to the rotor axis, but spinning frequencies up to 15 kHz have indeed been achieved with a conventional 4 mm rotor. The feasibility of this new approach has been convincingly demonstrated with uniformly labeled polypeptides in antiparallel β-sheet packing and in ordered fibers of silk.[17]

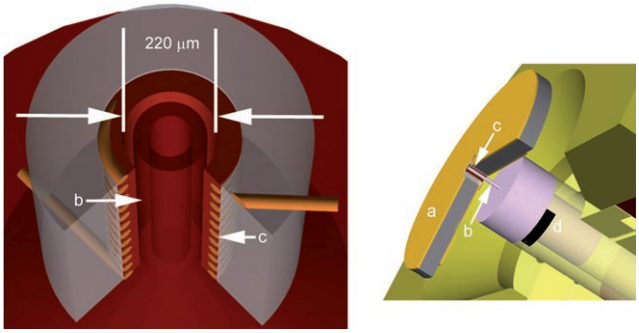

Figure 3. Schematic view of the microMAS circuit consisting of a capacitor (a) with embedded microcoil (c) and rotor (b) piggy-backed onto a 4-mm MAS stator (d). View at left shows assembled microcoil and rotor. Reproduced from reference [17].

It can be expected, therefore, that the use of microcoils will be the method of choice in many applications of solid-state NMR spectroscopy in biophysics and materials science with mass-limited samples. Problems will arise, however, if the samples to be studied need to be confined inside multiple safety barriers, such as highly toxic or radioactive materials. With such cases in mind, yet another detection method that makes use of conventional NMR spectrometers has recently been proposed.[18] It makes use of inductive coupling between a conventional static large coil and a rotating microcoil that spins together with the sample, as shown in Figure 4. In this

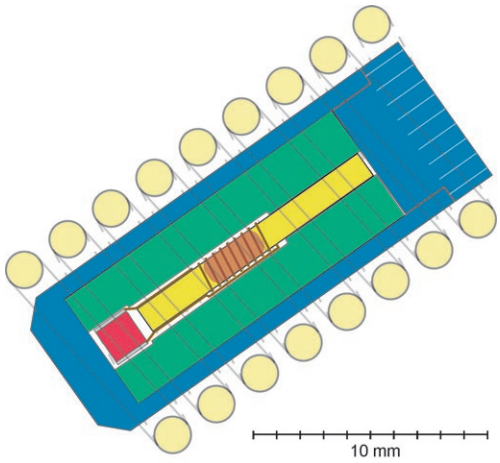

Figure 4. Schematic diagram of a magic-angle coil insert for high-sensitivity signal detection by inductive coupling of a coil that rotates with the sample. The sample is placed inside a glass capillary (dark yellow) with a tuned microcoil tightly wound around it. A cylindrical ceramic insert (green) is used to keep the capillary and the tuning capacitor (red) centered while spinning. Reproduced from reference [18].

way, wireless transmission of RF pulses and reception of NMR signals with fast spinning are achieved. As with the use of static microcoils, this approach provides not only high sensitivity in the nanoliter range, but also high RF fields that facilitate high power decoupling.

Although these two new MAS-NMR spectroscopic techniques are still at an early stage of development, they promise to provide new ways for obtaining highly informative spectra from samples in nanoliter or even picoliter quantities. They utilize the fact that miniaturization is advantageous for stable spinning and for amplification of RF field and thus sensitivity and decoupling. Moreover, MAS with comparatively low rotation frequencies is also applied to remove residual anisotropic couplings and susceptibility broadening in the signals of highly mobile systems, such as gels or biological tissue samples. In microcoils, the centrifugation effects commonly present under MAS are expected to be minimal owing to the small capillary diameter of the microsized sample container. Thus, the high sensitivity offered by these methods could potentially reduce the need for large biopsy samples, and MAS of a few cells should become possible. Furthermore, metabolomics studies on frozen systems are possible, because freezing minimizes sample degradation and because sample preparation does not require extraction and homogenization. Cryogenic design could, in principle, be combined with these approaches to further enhance the sensitivity of these techniques. High-amplitude RF fields of the order of MHz could change the landscape of modern solid-state NMR spectroscopy in areas such as decoupling of dipolar interactions, excitation of nuclei in paramagnetic systems, or spectroscopy of quadrupolar nuclei, such as ^{14}N.

In conclusion, several new approaches aimed at substantially increasing the sensitivity of NMR spectroscopy for gases, liquids, and solids have been developed in recent years. Some involve completely new detection methods (Figure 1), whereas others improve sample irradiation and signal detection in commercial spectrometers (Figures 2–4). Such developments, which are far from complete, will assure that NMR spectroscopy remains a major tool for many fields of science in general and chemistry in particular for years to come.

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