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A ^{1}H/^{19}F minicoil NMR probe for solid-state NMR: Application to 5-fluoroindoles

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Abstract

We show that it is feasible to use a minicoil for solid-state ^{19}F ^{1}H NMR experiments that has short pulse widths, good RF homogeneity, and excellent signal-to-noise for small samples while using low power amplifiers typical to liquid-state NMR. The closely spaced resonant frequencies of ^{1}H and ^{19}F and the ubiquitous use of fluorine in modern plastics and electronic components present two major challenges in the design of a high-sensitivity, high-field ^{1}H/^{19}F probe. Through the selection of specific components, circuit design, and pulse sequence, we were able to build a probe that has low ^{19}F background and excellent separation of ^{1}H and ^{19}F signals. We determine the principle components of the chemical shift anisotropy tensor of 5-fluoroindole-3-acetic acid (5FIAA) and 5-fluorotryptophan. We also solve the crystal structure of 5FIAA, determine the orientation dependence of the chemical shift of a single crystal of 5FIAA, and predict the ^{19}F chemical shift based on the orientation of the fluorine in the crystal. The results show that this ^{1}H/^{19}F probe is suitable for solid-state NMR experiments with low amounts of biological molecules that have been labeled with ^{19}F.

Keywords: Fluorine; Crystal structure; Minicoil; Probe design; Solid-state

1. Introduction

Biomolecular NMR has always evolved to tackle problems that had been considered “too difficult” by developing new methodologies. Recent examples include TROSY for large proteins [1], cold probes [2,3], and microcoils [4] for low concentration samples, and solid-state NMR for membrane proteins [5,6]. A promising application is the use of ^{19}F as a reporter group in biological molecules. Fluorine NMR has a number of advantages, including a nuclear spin of 1/2, a chemical shift anisotropy (CSA) on the order of at least 100 ppm and high sensitivity due to a high-magnetogyric ratio [7]. ^{19}F is also 100% naturally abundant, and the biological background is minimal since only six distinct natural compounds containing fluorine have been described [8]. The stereochemical similarity of ^{19}F to both H and OH groups allows it to be readily incorporated into amino acids, nucleic acids, and drugs, where fluorine can be used to study their binding properties, structure, and orientation in a magnetic field. For a recent review on the use of fluorine in biomolecular NMR, see Ulrich [9].

There are, however, a number of difficulties in the use of ^{19}F NMR. The γ_F and γ_H differ by ~6%, necessitating the use of elaborate filters and circuit design to prevent radio frequency (RF) resonant signals at one frequency interfering with that at the other in multipulse and broadband decoupling experiments. In a typical ^{19}F NMR experiment, microwatt signals from ^{19}F need to be measured while ^{1}H irradiation is occurring at hundreds of watts. Additionally, the >100 ppm CSA results in rapidly decaying fids and requires the collection of large sweepwidths, which can tax high-field spectrometers. Strong ^{1}H/^{19}F and ^{19}F/^{19}F

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dipole–dipole interactions also place demands on the pulse sequences and complicate spectral analysis. Suppression of the $^1H/^{19}F$ dipolar interactions is necessary if weak $^{19}F/^{19}F$ dipolar interactions are to be detected. While not abundant in natural compounds, fluorine is ubiquitously used in plastics and especially in materials used in electronic circuits, which results in high-background signal in ^{19}F NMR experiments using commercially available ^1H NMR probes. As such, components and materials must be judiciously selected for their use in a ^{19}F NMR probe.

We present here the design and implementation of a $^1H/^{19}F$ single input, double-tuned circuit, narrow bore NMR probe using a minicoil for solid-state experiments on biological samples. This probe provides low ^{19}F background, short pulses, good RF homogeneity, and high signal-to-noise (S/N) for small samples using low power amplifiers and components. We also demonstrate the use of an NMR minicoil probe with solid samples, determine the magnitude of the ^{19}F CSA for two fluoroindoles, and determine the orientation of fluorine atoms in a fluoroindole crystal.

2. Coil design

A 1.7-mm capillary design was chosen for the probe coil, since it offers an increased S/N for small samples compared to the 5-mm coils typically used in liquid-state NMR. The coils were constructed from 18 AWG (1.024-mm diameter) oxygen-free copper wire (Fig. 1A) (MWS Wire Industries, Westlake Village, CA). Coils were formed by winding the wire around the sample capillary to ensure a maximum filling factor. The five-turn sample coil measured 6.25-mm long with a 2-mm outer diameter. The sample capillary (melting-point capillary, Kimax–Kontes Vineland, NJ) was chosen with an OD ≅ 1.7 mm. The volume of sample in the capillary was approximately 10 μL. It has been shown that at a fixed length to diameter ratio of a coil, the S/N ratio increases as the diameter is decreased [10]. This is advantageous with biological samples where a limited amount of material may be available. Microcoils have an additional advantage that lower power (or a shorter pulse width at the same power) is required to implement NMR experiments, thereby reducing the potential for arcing in the probe and allowing amplifiers used in liquid-state NMR to be used for solid-state experiments.

3. Probe and circuit components

One important aspect in the selection of electronic components is that they do not contain fluorine, which is often present in the component's insulation, grease, and dielectrics. The presence of polytetrafluoroethylene (PTFE) in the trimmer capacitors used in this probe required a compromise to be made; the lengths of the leads of the sample coil in the circuit needed to be long enough so that the PTFE dielectric is outside the effective B_1 field (to keep background low), yet short enough so as not to increase unwanted stray inductance and capacitance in the circuit. The result is a distance of 5 cm between the tops of the capacitors and the bottom of the ^{19}F minicoil. The remaining background was reduced by the use of the DEPTH pulse sequence [11], which selects for homogeneous regions of the RF field.

4. Probe design

The probe body was constructed from a 600 MHz Varian triple-resonance liquid-state probe with a 38-mm outer

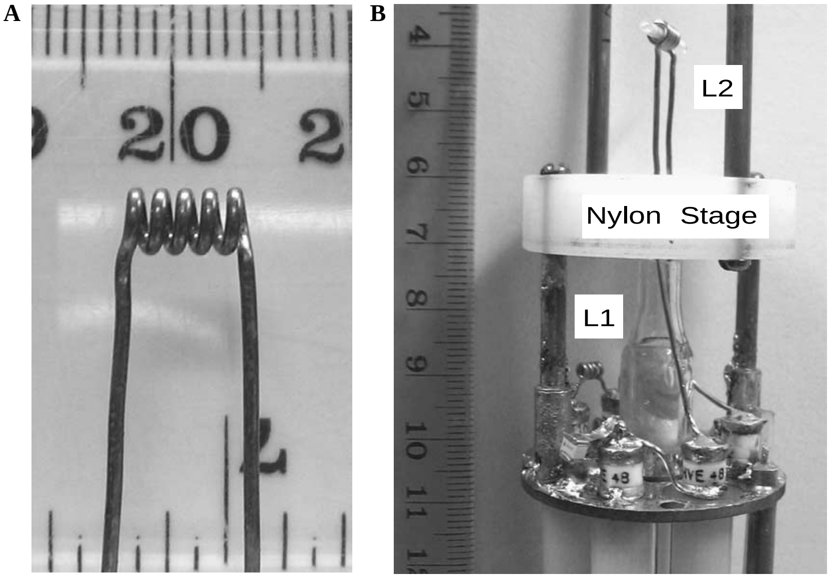

Fig. 1. (A) View of the sample minicoil. (B) Circuit design and layout showing a view of the components within the probe. All ruler divisions are in millimeter.