

Direct Carbon Detection Using an Enhanced 5 μL and a Prototype 3 μL CapNMR™ Flow Probe

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In the pharmaceutical industry, we are often asked to unambiguously determine the structures of unknown analytes available only in limited quantities. Obtaining reasonable NMR data on these limited quantities is essential in the success of drug discovery and development. We are constantly looking for ways to improve signal to noise (S/N) and allow us to further reduce sample amount. With that in mind, utilizing probe technology that provides for the rapid analysis of mass-limited samples is critical. We have selected the Protasis/MRM CapNMR™ capillary flow probes to meet those needs. Our lab has two of these probes at 600 MHz: an enhanced 5 μL probe, (~2.5 μL active; standard E5 version) and a 3 μL prototype (~2 μL active).

In our hands, these probes have shown a 5-6 fold improvement in ^1H S/N as compared to a standard 5 mm Triple Resonance probe. We also see performance that is reasonably equivalent to that obtained using a 3 mm tube in a 5 mm cryogenically cooled probe. While most structures can be determined by using 1D and 2D homo- and heteronuclear experiments, this is not possible for those compounds containing a significant number of heteronuclei. In those cases, the use of direct ^{13}C detection is crucial for structure determination.

Since the CapNMR™ probe contains a single transceiver coil, one does not see the filling factor loss typically found for ID probes with the outer ^{13}C coil. We have determined that the ^{13}C sensitivity of the CapNMR™ probe is quite high even relative to a room temperature, 5 mm, direct ^{13}C detect probe with the inner ^{13}C coil. To illustrate, Figure 1 shows the single scan carbon spectra of a standard 60% Benzene- d_6 solution flushed through the enhanced 3 μL (top) and 5 μL (bottom) probes. The S/N is 10.4:1 and 20.0:1 for the 3 μL and E5 probes, respectively. If the S/N is corrected for the flow cell volume (3 or 5 μL), then they perform 5.7 and 6.6 times better than a 5 mm direct detect carbon probe (S/N of 356:1 from vendor specs).

For more realistic samples, hundreds to thousands of scans are necessary to obtain sufficient S/N. After a large number of scans, the probe material begins to create a significant background signal. This background is illustrated in the top spectrum of Figure 2. The middle spectrum in Figure 2 is for 125 μg (500 nmol) of pindolol on the enhanced 5 μL taken with 4096 scans, and requiring approximately 4 hours. One resonance at 108 ppm overlaps with the background signal, but since it is much narrower than any background feature, it is easily observed. Nonetheless, one may want to remove the background signal for clarity. This is easily performed via background subtraction, as shown in the bottom spectrum in Figure 2. The subtraction does not interfere with the carbon resonance at 108 ppm. Figure 3 further illustrates direct carbon detection of 206 μg (1 μmole) of ibuprofen using 4096 scans and background subtraction for both probes. Each probe contained the specified amount of sample in the flow cell (3 or 5 μL). We estimate that the limit of detection is ~25 μg for an overnight experiment.

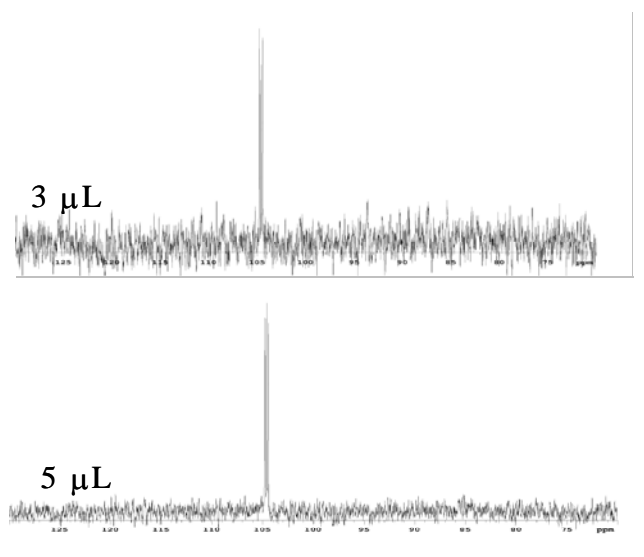


Figure 1. Single scan 600 MHz ^{13}C spectrum of 60% Benzene- d_6 flushed through the 3 μL (top) and enhanced 5 μL probe (bottom). S/N is 10.4:1(top) and 20.0:1 (bottom).

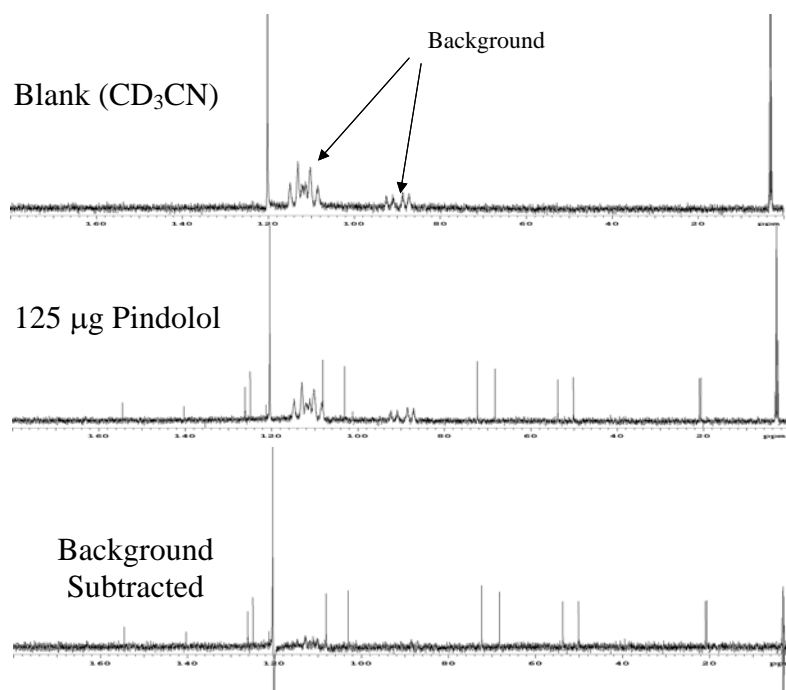


Figure 2. 600 MHz ^{13}C spectra using the enhanced 5 μL probe. $\text{ACN-}d_3$ background (top), 125 μg pindolol (middle), background subtracted (bottom). 4096 scans.

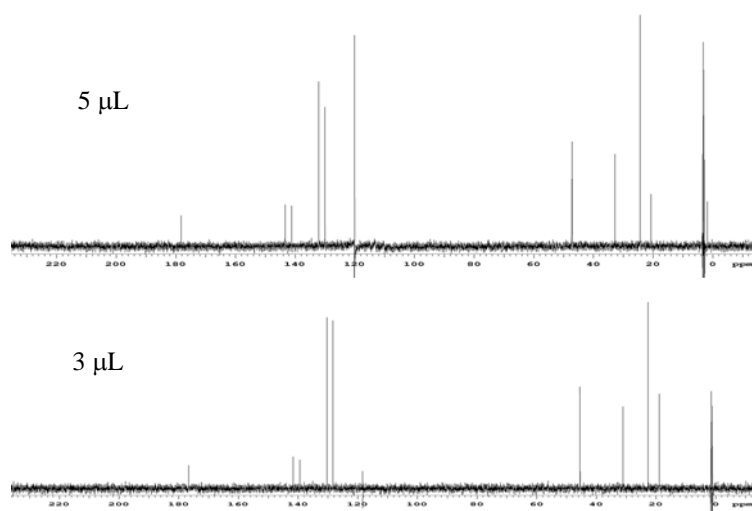


Figure 3. 600 MHz ^{13}C background subtracted spectra of 206 μg (1 μmole) ibuprofen using the enhanced 5 μL (top) and 3 μL (bottom) probes. 4096 scans.