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Development of a Micro Flow-Through Cell for High Field NMR Spectroscopy

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Abstract

A highly transportable micro flow-through detection cell for nuclear magnetic resonance (NMR) spectroscopy has been designed, fabricated and tested. This flow-through cell allows for the direct coupling between liquid chromatography (LC) and gel permeation chromatography (GPC) resulting in the possibility of hyphenated LC-NMR and GPC-NMR. The advantage of the present flow cell design is that it is independent and unconnected to the detection probe electronics, is compatible with existing commercial high resolution NMR probes, and as such can be easily implemented at any NMR facility. Two different volumes were fabricated corresponding to between ~3.8 and 10 μL detection volume. Examples of the performance of the cell on different NMR instruments, and using different NMR detection probes were demonstrated.

Acknowledgements

The authors acknowledge support of the Sandia RTFB program, and Rand Garfield for machining of selected parts.

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Nomenclature

DOE	Department of Energy
SNL	Sandia National Laboratories
NMR	Nuclear Magnetic Resonance
GPC	Gel Permeation Chromatography
NW	Nuclear Weapons

Development of a Micro Flow-Through Cell for High Field NMR Spectroscopy

Executive Summary

This report describes the initial efforts at coupling gel permeation chromatography with nuclear magnetic resonance (GPC-NMR) [1-4] to study polymer production and aging effects under the RTFB program. A micro flow-through cell has been developed that allows very small volumes to be detected by NMR along with the possibility of monitoring of a continuous flow. One of the goals of this effort was the design of a flow-through NMR cell that could be easily transported and utilized in different facilities, including any NMR laboratory in the NW complex regardless of the field strength or equipment manufacturer or vintage. This requirement is realized using the novel micro flow-through NMR cell described here.

1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy continues to be a powerful analytical tool in the identification and characterization of chemical species. Recent advances in hyphenated NMR techniques including combination with gel permeation chromatography (GPC-NMR), liquid chromatography (LC-NMR), supercritical fluid chromatography (SFC-NMR), solid phase extraction (SPE-LC-NMR), flow injection analysis (FIA-NMR), high pressure liquid chromatography (HPLC-NMR), capillary liquid chromatorgraphy (capLC-NMR) and liquid chromatography mass spec (LC-MS-NMR) now allow very complex problems to be explored.[5-17] For hyphenated NMR techniques, either a flow-through cell or a specialized NMR probe is utilized that allows the effluent stream from the LC or GPC to flow directly through the detection coil of the NMR spectrometer.

Initial micro NMR flow cells ranged from 60 to 220 μL in volume and commonly required the modification of probes to allow the transport of solutions in and out of the active volume.[4-5, 18-19] while later dedicated micro probes have reduced this volume to tens of μL amounts.[17, 20-21] More recently a NMR microchip “MICCS” (Micro Channeled Cell for

Synthesis monitoring) probe has been reported that allowed for the real time monitoring of chemical reactions.[22] This MICCS probe uses a microchip in which channels have been fabricated, but has the distinct advantage of being directly used in a standard 5 mm solution probes without any modifications. We have borrowed on this concept to develop the micro flow-cell described here, but have elected to use easily obtainable commercial micro-capillaries in the cell design.

2. Experimental

2.1. NMR Spectroscopy

The high resolution ^{1}H NMR experiments were performed using a 5mm broadband (BB) and a 5mm inverse triple resonance (TXI) probe on a Bruker DRX 400 operating at 399.87 MHz, or on a Bruker Avance 600, using a 5mm broadband (BB) probe, and operating at 600.14 MHz. The high resolution ^{13}C NMR spectra were obtained on the higher field instrument at 150.92 MHz, using standard single pulse Bloch decay with WALTZ-16 ^{1}H decoupling. For the ^{1}H NMR spectra at both fields solvent presaturation was obtained using a frequency modulated low power pulse sequence with GARP ^{13}C decoupling.[23] The ^{1}H DOSY experiments were performed on the Avance 600 using a stimulated echo with a 1 ms gradient pulse on a DIFF30 diffusion probe with 1000 G/cm gradients. Standard acquisition conditions for ^{1}H were typically: 4 s recycle delay, 16 to 64 scan averages, 64K complex points, zero-filled to 64K points; solvent presaturation experiments employed a 100 ms shaped presaturation pulse. The ^{13}C spectra were acquired with a 10 s recycle delay and 5k scan averages.

2.2. Flow Cell Construction

The flow cells (see Appendix 1 for technical drawings) consisted of a 1.5 or 2.0 mm outer diameter (OD) split-theta capillary glass (Harvard Apparatus, Holliston, MA) closed on the lower end with a polyetheretherketone (PEEK) cap machined internally to allow flow from one side of the split capillary to the other. The outside diameter of the PEEK cap is machined to fit snugly in a standard 5mm NMR tube. A piece of 360μm OD (150μm ID) PEEK tubing (IDEX/Upchurch, Oak Harbor, WA) was glued using Norland Optical Adhesive 76 (Norland, Cranbury, NJ) into separate channels of the theta-capillary allowing for sample transport in and

out. This adhesive was cured at 50°C for approximately 12 hours after initial curing in a UV reactor. Other epoxies were explored, but all showed considerable loss of function with exposure to tetrahydrofuran (THF) carrier solvent. A second piece of machined PEEK was glued slightly below the attachment point of the 360µm tubing to the capillary to further support the flow cell when placed in a 5mm NMR tube. This PEEK support, as well as the lower PEEK cap, was grooved to allow solvent flow around it. In this manner it was possible to place deuterated solvent around the inner capillary to assist in shimming if required. Figure 1 shows a schematic of this cell design, while Figure 2 shows the constructed cell inside and outside the outer NMR tube.

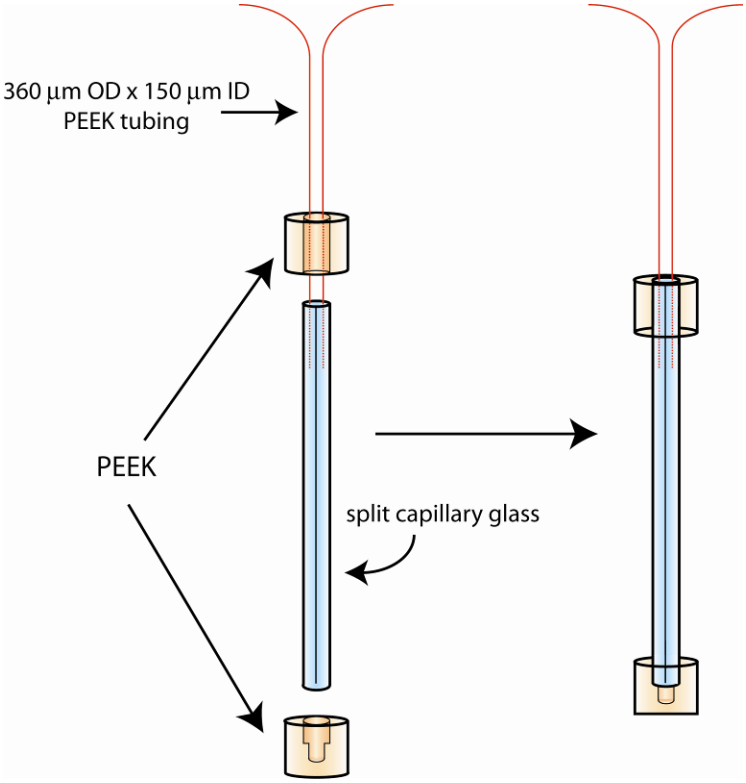

Figure 1: Drawing showing the construction and layout of the theta-capillary micro flow cell.

This constructed flow cell was then carefully slid into a standard 5 mm NMR tube, inserted into a spinner assembly and lowered into the NMR magnet with the transport PEEK tubing exiting through the upper end of the transport shuttle/shim tube. All experiments were performed in a non-spinning mode due to the presence of the PEEK tubing.

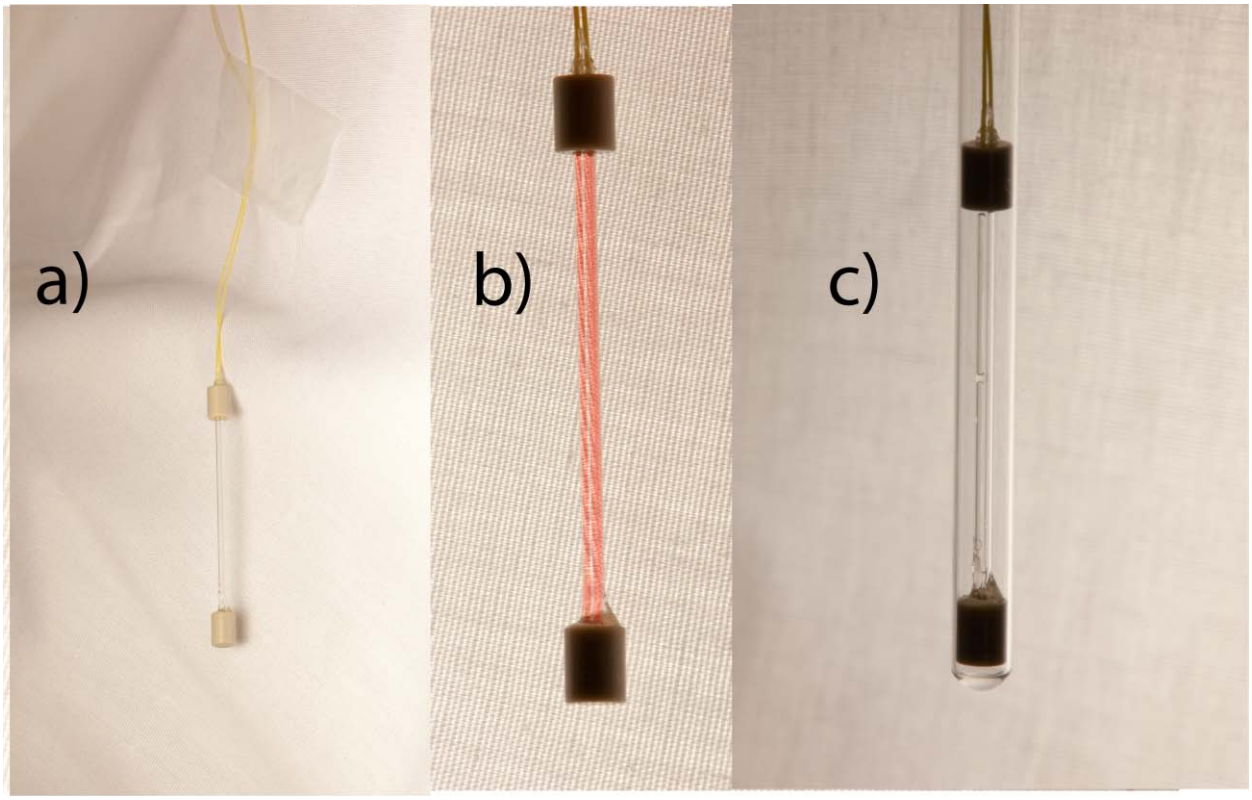

Figure 2: Photos of 1.5mm OD micro flow cell. a) unfilled flow cell. b) filled flow cell to show split capillary. c) flow cell situated inside a standard 5mm NMR tube.

To introduce sample flow to the micro NMR cell, a 1/16" to 360μm adapter was attached to one end of the flow cell 360μm tubing. The 1/16" end of the adapter was attached to a Hamilton Gastight syringe modified with a Hamilton compression fitting to accept 1/16" tubing in the needle port. The total volume of the flow cell fabricated with 1.5 mm OD capillary glass was ~24 μL, corresponding to a detection volume of ~ 10 μL. The total volume of the cell fabricated with 2.0 mm OD capillary glass was ~40 μL, with a detection volume of ~ 20 μL. For the NMR results presented here, the micro flow cell was filled with 100 mM glucose in D_2O (containing 1% w/w DSS, 4,4-dimethyl-4-silapentane-1-sulfonic acid). The area inside the NMR tube surrounding the cell was filled with D_2O (neat) unless otherwise noted.

3. Results and Discussion

3.1. Flow Cell Detection Volume Determination

While the total volume of the 1.5 mm and 2.0 mm OD capillary glass flow cells were 24 μL and 40 μL, respectively, the actual detection volume for these cell is determined by the length of the particular coil in the probe utilized. The calculated detection volumes for the 1.5 mm and 2.0 mm micro flow cells with the four different NMR probes employed in this work are summarized in Table 1.

Table 1. Detection volumes (μL) by probe (percentage of the detection volume for a 5mm standard tube).

Flow cell OD (mm)	400 MHz BB	400 MHz TXI	600 MHz BB	600 MHz diffusion
1.5	3.8 μL (16.0%)	3.8 μL (15.7%)	3.8 μL (16.0%)	3.8 μL (16.0%)
2.0	10.7 μL (26.7%)	10.7 μL (26.3%)	10.7 μL (26.7%)	10.7 μL (26.7%)

3.2. ^{1}H NMR Spectra of Glucose/D_2O Solution

To address and compare the performance of these micro flow cells in different probe configurations, ^{1}H NMR spectra of 100mM glucose in D_2O (containing 1% w/w DSS) were obtained from each flow cell under a variety of instrumental configurations. In particular the impact of possible susceptibility effects on shimming performance were to be addressed Spectra from the 1.5mm flow cell were acquired on 400 and 600 MHz NMR instruments, using a 5mm broadband probe for experiments at 400 MHz, and 5mm broadband and diffusion probes for experiments at 600 MHz. Spectra from the 2.0 mm flow cell were acquired at 400 MHz with 5mm broadband and inverse probes. The spectra collected in these experiments are summarized in Figure 3. These results show that similar performance was obtained using the different commercial NMR probes, with the predicted change in sensitivity and resolution performance resulting from changing magnetic field strength dominating the differences.

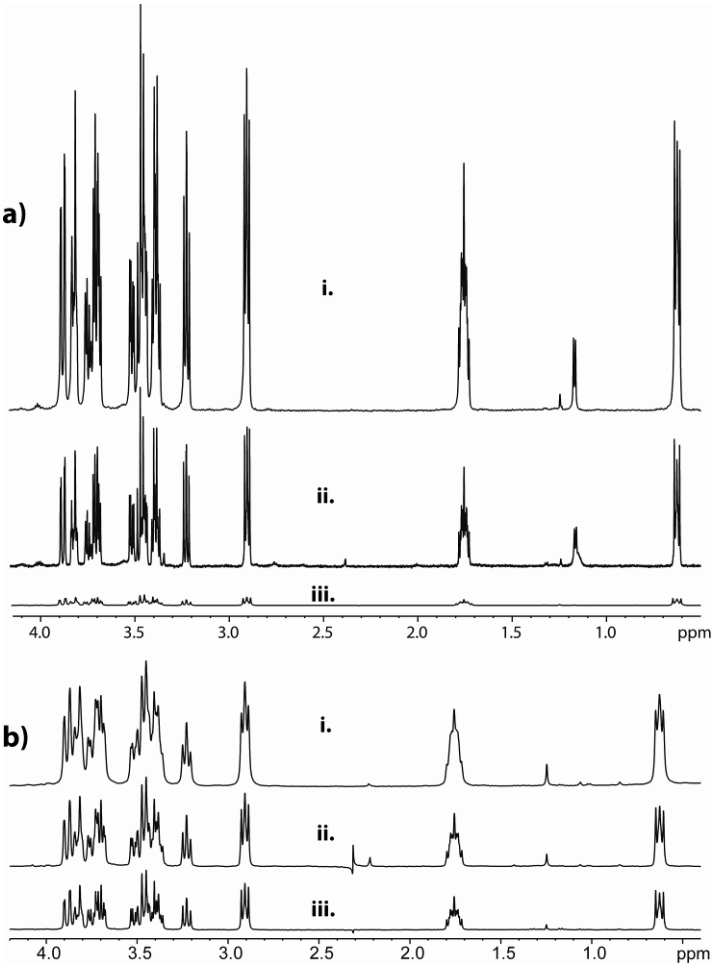

Figure 3: Summary of ^{1}H NMR data collected from 1.5 mm OD and 2.0 mm OD flow cells; a) comparison of data collected from 1.5 mm OD cell: i. 600 MHz broadband probe, ii. 600 MHz diffusion probe, iii. 400 MHz broadband probe; b) comparison of data collected from 2.0 mm OD and 1.5 mm OD flow cells: i., 2.0 mm OD cell, 400 MHz TXI probe, ii., 2.0 mm OD flow cell, 400 MHz broadband probe, iii., 1.5 mm OD cell, 400 MHz broadband probe.

3.3. Solvent Presaturation

For flow application in hyphenated techniques it is necessary to remove the dominant carrier solvent resonance, even in cases using deuterated solvents. To test the performance of solvent presaturation using these micro flow cells, ^{1}H NMR spectra of 100 mM glucose were acquired, applying selective saturation to the dominant H_2O/HDO resonance. Comparison of presaturated and standard spectra from each flow cell (Figure 4), clearly demonstrate that the flow cell approach to NMR analysis of low-concentration, small-volume samples in protonated solvents (or very exchangeable solvents, like D_2O) is feasible.

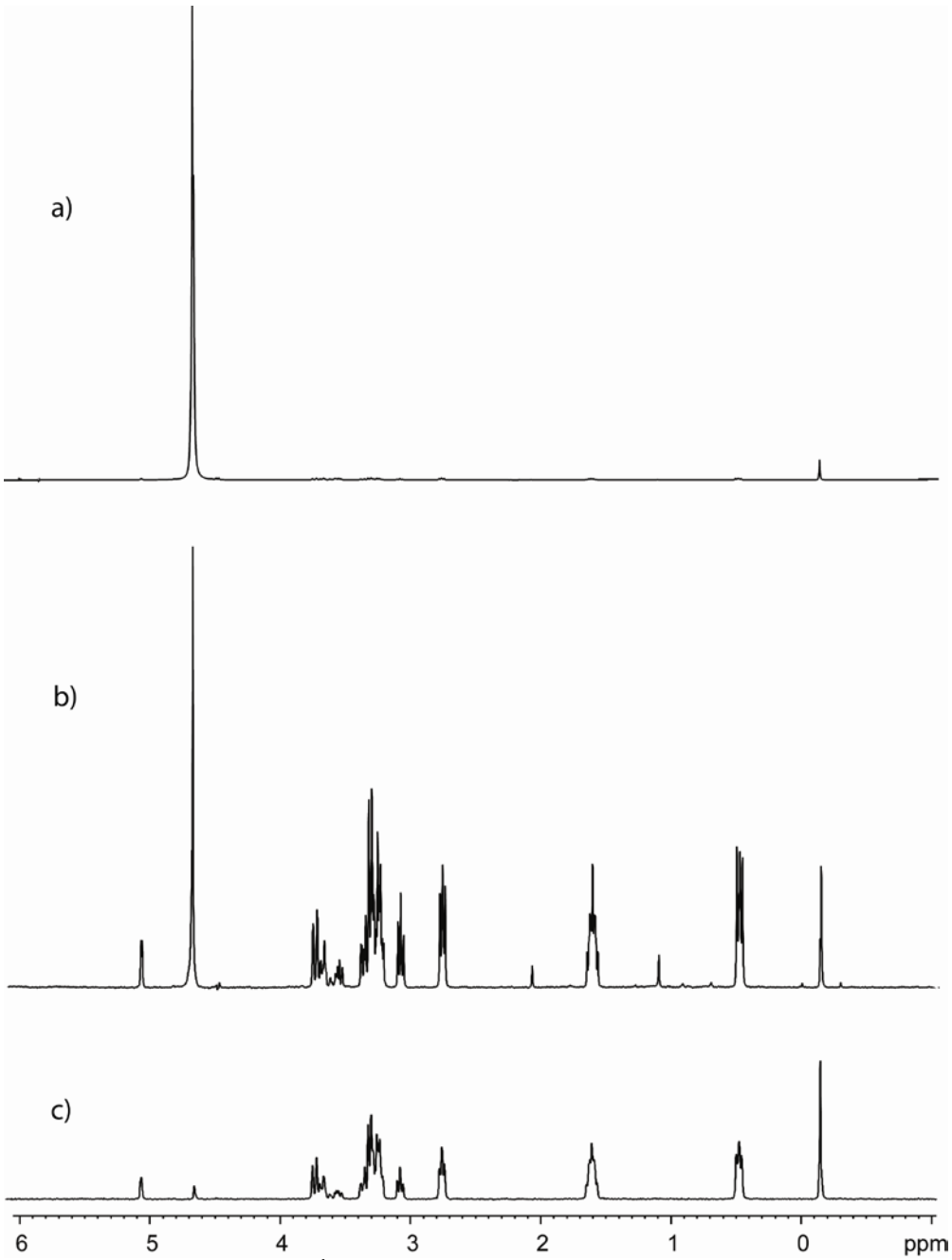

Figure 4: Comparison of ^{1}H NMR spectra acquired a) without presaturation in 2.0 mm OD flow cell, b) with presaturation in 2.0 mm OD flow cell, c) with presaturation in 1.5 mm OD flow cell.

3.4. ^{13}C NMR

One of the advantages of this micro flow cell design is the ability to utilize any probe configuration such that different types of NMR experiments could be performed. As an example, the solution ^{13}C NMR spectrum of 100 mM glucose/D_2O is shown in Figure 5. The small

detection volume requires extensive signal averaging (~14 hours), but does demonstrate that heteronuclear NMR can be performed on mass limited sample volumes.

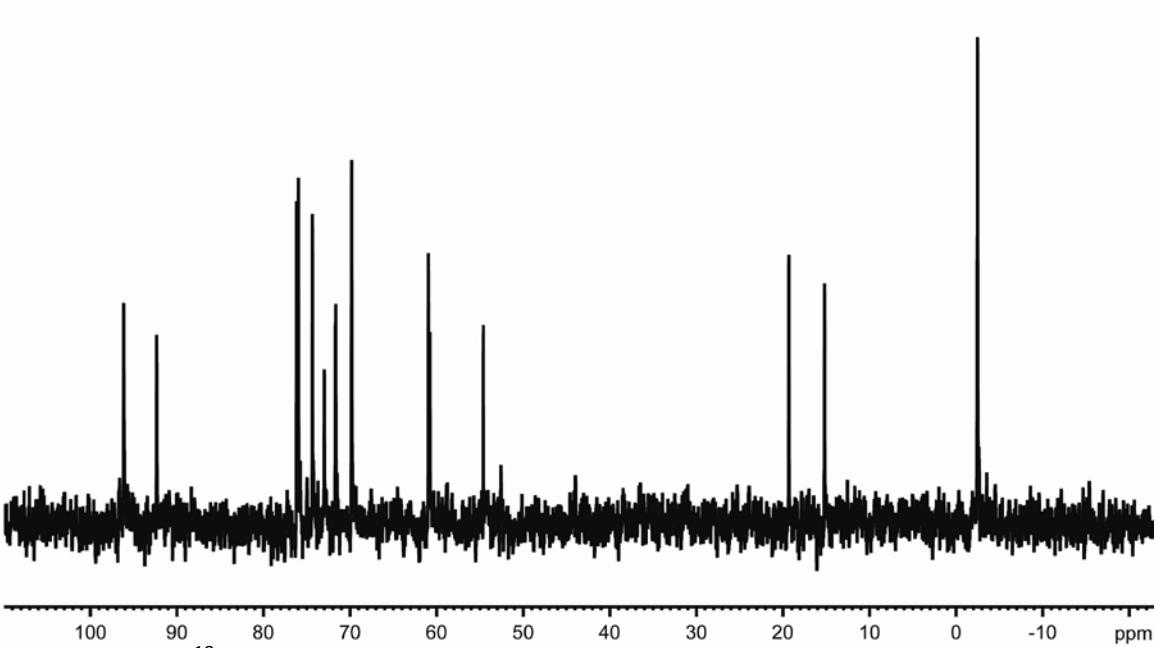

Figure 5: The ^{13}C NMR spectra of 100 mM glucose at 150 MHz using a 1.5 mm OD micro flow cell (3.8 µL) on a 5 mm broadband probe.

3.5. ^{1}H Diffusion Experiments

The utility of diffusion-ordered spectroscopy (DOSY) NMR has been demonstrated for a variety of different applications, ranging from characterization of water diffusion in proton exchange membranes to identification of biological metabolites in complex mixtures by elucidation of their diffusion coefficients. Diffusion based techniques could enhance NMR analysis of mixed-analyte samples with limited sample amounts. To show that the micro flow cell developed here is applicable to such experiments spectra as a function of gradient strength for the 100 mM glucose sample (acquired at 600 MHz) is shown in Figure 6. Even for these 3.8 µL µl detect volume, the self-diffusion coefficient could be easily measured.

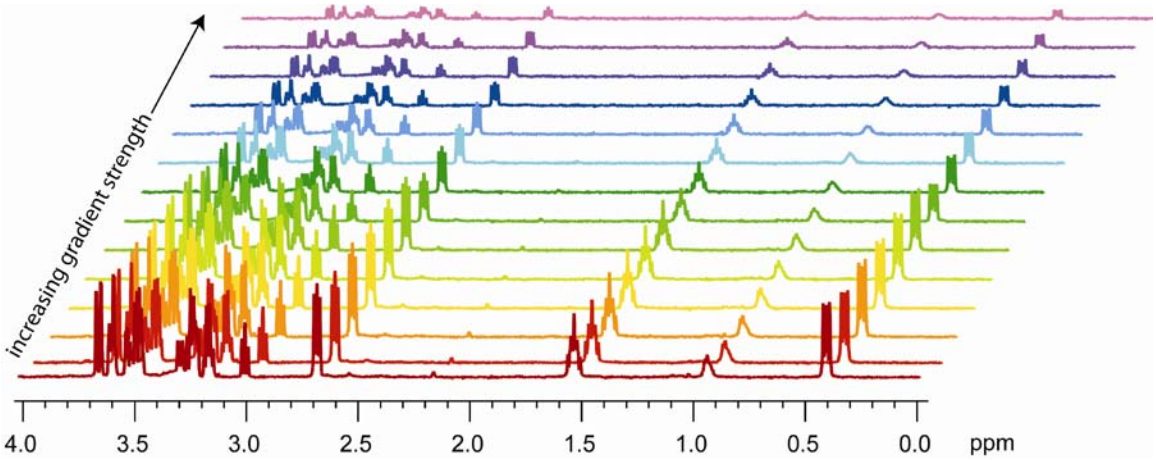

Figure 6: ^{1}H NMR 600 MHz spectra as a function of gradient strength obtained for the 100 mM glucose sample using a 1.5 mm OD flow cell on the DIFF30 5 mm diffusion probe.

4. Conclusions

The fabrication and testing of micro flow cells for NMR spectroscopy based on a split theta-capillary design have been described. This design allows different commercial probes and instrumental configurations to be easily used as demonstrated by the ^{1}H, ^{13}C and diffusion-based NMR experiments obtained. This flow cell design is low cost and can easily be implemented in any NMR facility with standard commercial probes.

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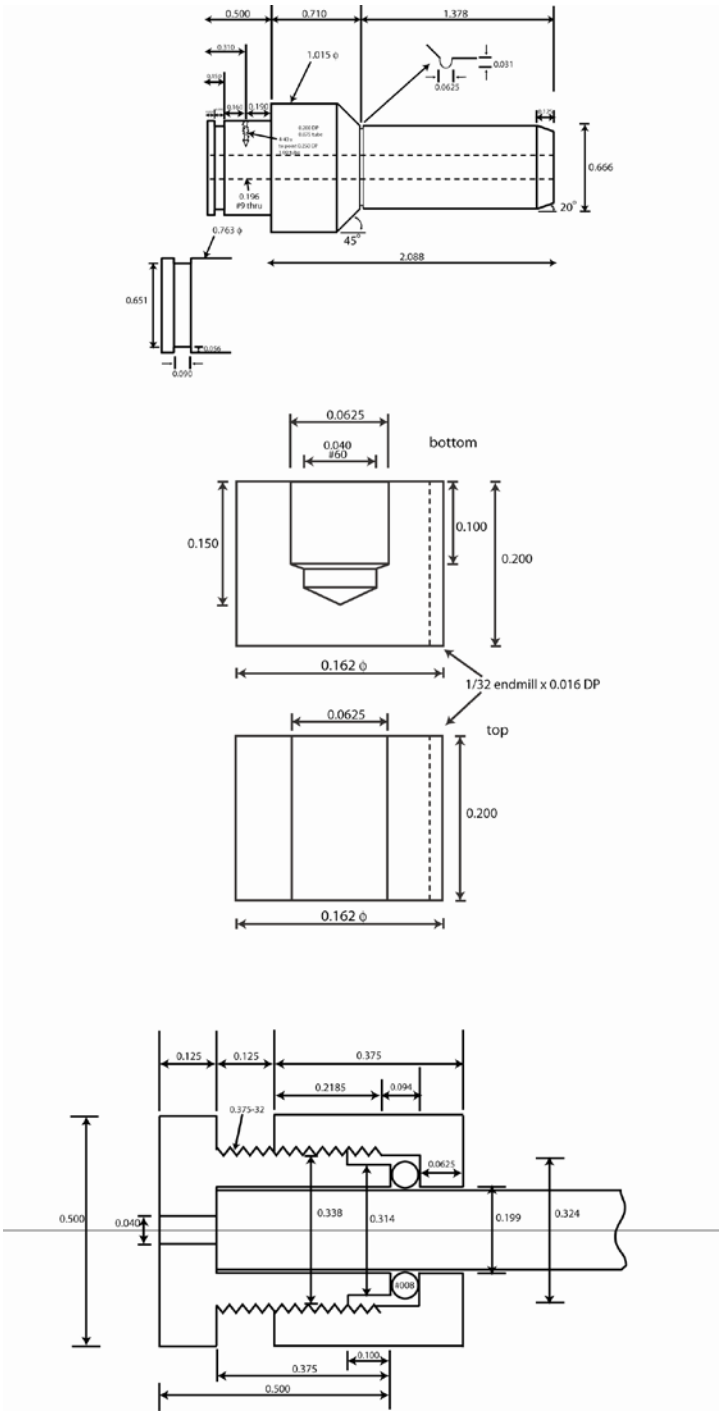
6. APPENDIX 1

Fabrication Drawings for Micro Flow-Through Cell



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