Susceptibility-matched plugs for microcoil NMR probes

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Abstract

For mass-limited samples, the residual sample volume outside the detection coil is an important concern, as is good baseline resolution. Here, we present the construction and evaluation of magnetic susceptibility-matched plugs for microcoil NMR sample cells which address these issues. Mixed-epoxy glue and ultem tube plugs that have susceptibility values close to those of perfluorocarbon FC-43 (fluorinert) and copper were used in small volume (0.5–2 μL) and larger volume (15–20 μL) thin glass capillary sample cells. Using these plugs, the sample volume efficiency (i.e. ratio of active volume to total sample volume in the microcoil NMR cell) was improved by 6–12-fold without sensitivity and resolution trade-offs. Comparison with laser etched or heat etched microcoil sample cells is provided. The approaches described are potentially useful in metabolomics for biomarkers detection in mass limited biological samples.

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1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a premier analytical method for molecular structure determination, and is widely used in chemical and pharmaceutical research. As such, NMR is increasingly used for the analysis of complex samples such as bio-fluids [1]. Yet the sensitivity of NMR is sometimes limiting, and therefore a variety of sensitivity enhancement techniques [2–6] have been introduced. Among these methods, microcoil NMR has been shown to improve mass-sensitivity, 5m (SNR per micro-mole) dramatically, which is achieved by the use of smaller diameter detection coils [7].

Microcoils that utilize a solenoidal geometry with multiple coil-turns enhance the coil sensitivity compared to the standard Helmholz coil geometry [8] used in NMR probes. However, several factors affect the spectral resolution that is dependent on the static magnetic field inhomogeneity inside the coil volume. Field inhomogeneity can arise from magnetic susceptibility differences between the coil and surrounding air, coil winding pitch and coil turns, sample-length and shape, the ratio of coil-length to its diameter and even the thickness of the sample cell holder [9–14]. The susceptibility mismatch between the copper coil and the sample can be dramatically reduced by immersing the solenoidal coil/sample system in a perfluorocarbon FC-43 (fluorinert) fluid that has a close susceptibility match to copper [7]. Alternatively, zero-susceptibility wire can be used to avoid the usage of susceptibility matching fluid [14–16]. While a variable pitch [11] solenoidal coil is preferred, a shorter solenoid with a greater number of turns [13] is best suited for obtaining a homogenous RF magnetic field. On the other hand, the thickness of the sample cell holder needs to be optimized to obtain optimum sensitivity by increasing the fill factor [17,18] while avoiding resolution degradation due to the proximity of the sample and coil in thinner sample holder walls [19].

With all the above criteria fulfilled, good resolution in microcoil NMR can be achieved (below 1 Hz at FWHM, full width at half-maximum) [7,17,18,20]. Additionally, the baseline resolution has to be equally considered if microcoil probes are to be utilized for analyzing complex bio-samples. Fuku et al. [10] elucidated that, in order to reduce end-effects from the perturbing magnetic field and thereby achieve a nearly constant field at the center of the tube, the lengths of the coil and glass tube should be at least 1–2-fold larger than their diameters. For longer microcoil glass tubes, the baseline resolution at 0.55%/0.11% peak height can be reduced below 20/30 Hz. This constraint on the sample tube typically leads to the requirement for larger sample volumes needed to fill not only long residual sample tube volume across each end of the small coil, but also input and output flow transfer lines. Thus, the volume efficiency defined as the ratio of active volume to total sample volume degrades. For example, if the detection volume is 1 μL, the total sample volume might be 50–60 μL or even 100–150 μL for a 20 μL detection volume, depending on the inner diameter and length of sample tube and transfer lines [17,18,21]. Further, the diameter of the transfer lines has to be optimized to provide easy flow of viscous samples. Larger volume requirements often result in the dilution of mass-limited samples, and thus reduced sample sensitivity. Etching techniques [17,18,22,23] can be used to create...
a “bubble” sample cell that increases the fill factor and lowers the sample volume in the regions outside the active detection volume. However, this approach often leads to thicker glass wall regions at each end of the coil, which changes the volume susceptibility difference between the sample and the glass-wall [17,18] and creates wider baselines. Alternatively, the sample can be sandwiched between plugs of immiscible fluorocarbon fluid FC-43 [21,24,25] which reduces the residual sample volumes inside the probe without degrading baseline resolution. However, miscibility of the analytes in the FC-43 fluid must be considered before using a sandwiching fluid with the sample. Also, if sample recovery (such as for MS analysis) is required, this is more difficult.

Another approach, which we introduce below, is to incorporate solvent susceptibility-matched solid plugs into the horizontal microcoil sample flow-tube. Solvent-susceptibility-matched plugs are often used in conjunction with standard NMR tubes and are inserted in the sample tube to remove susceptibility differences at the air-sample or glass-sample interfaces that are not parallel to B₀. Susceptibility plugs also reduce the required sample amount for NMR analysis by up to 80% (Doty Scientific, Columbia, SC). Using a similar approach, we demonstrate the use of solid ultem and dried epoxy-glue plugs in larger (greater than 15–20 µl or 2 mm ID sample cell tube) and small volume (less than a few µl or 1 mm ID sample cell tube) microcoil cells, respectively. The plugs have close magnetic-susceptibility values to surrounding fluorinert FC-43 and copper in the coil. The technique is simple and easy to implement, thus allowing the designer to adjust the plug length to optimize baseline resolution. This approach provides sample volume reductions by 6-12-fold without compromising resolution or sensitivity as compared to the microcoil cells without plugs. Performance of the proposed technique is shown in terms of volume efficiency, resolution, line shape and sensitivity, as well as high resolution 1D and 2D spectra. The resolution performance using susceptibility-matched plugs is also compared to our previous probes [17,18] that used etching techniques to reduce sample volume and to improve fill factor. Potential applications and on-going work in the study and structural identification of metabolites in human plasma/serum and urine samples using this technique are discussed.

2. Methods

2.1. Sample cell construction

Plug types and the corresponding sample tube sizes were chosen based on ease of implementation, commercial availability and practicality. For example, ultem plugs were used in larger volume capillary glass cells (larger ID ≥ 2 mm), while dried epoxy-glue plugs were used for smaller volume capillary glass cells (smaller ID ≤ 1 mm ID) since it was much easier to make a plug by flowing/drying glue in small ID tubes than to custom machine solid ultem plugs with diameters below 1 mm. Ultem and mixed-epoxy were used as plugs for their volume magnetic-susceptibility values (χv, cgs) that are close to those for susceptibility matching fluid fluorinert FC-43 and RF coil copper wire (χv = −0.71 × 10⁻⁶ for ultem,= −0.699 × 10⁻⁶ for mixed epoxy; FC-43 = −0.70 × 10⁻⁶, and Cu wire = −0.78 × 10⁻⁶) [26–29].

As shown in the schematic drawing in Fig. 1A, for the larger volume cell, two 1 cm long thick wall tubes (2.36 mm OD, −0.8 mm ID; CPI Intl., Santa Rosa, CA) were cut. Two pieces of 1 cm long flexible Teflon tubing (0.76 mm OD, 0.300 mm ID; Cole-Parmer, Vernon Hills, IL) were inserted and glued into each ultem plug with a little epoxy (Devcon, Danvers, MA). Flexible Teflon tubing was used to make a joint between the ultem plug and input/output transfer lines. Each 55 cm long fused-silica transfer line (360 µm OD, 70 µm ID; Polymicro Technologies, Phoenix, AZ) was glued to a 1 cm piece of Teflon tubing using epoxy. Finally, the open end of each ultem plug (with the glued Teflon tubing and fused silica capillary at its other end) was inserted and epoxied into a 2 cm long thin glass capillary (2.8 mm OD, 2.423 mm ID; Friedrich & Dimmock, Millville, NJ), leaving a 5 mm long detection region at the center. A variable pitch RF coil was manually wound with four turns of round copper wire (150 µm OD, polyimide-coated; California Fine Wire, Grover Beach, CA) at the center of the above sample glass capillary to produce a 4.3 mm long coil that covered the 20 µL detection volume with less than 4 µL residual volume outside the coil ends.

Smaller sample cells were constructed from 1 mm OD and 1.43 mm OD thin glass capillaries (Friedrich & Dimmock, Millville, NJ), resulting in 500 nL and 2 µL detection volumes, respectively. As shown in the schematic drawing in Fig. 2, two 1 cm long fused silica capillaries (360 µm OD, 70 µm ID; Polymicro Technologies, Phoenix, AZ) were inserted and glued to each end of the thin glass capillaries using epoxy. To control the length of the glue plug inside the cells precisely, partially air-filled syringes were connected to each end of the fused silica capillaries (Fig. 2A). The syringes were pulled in or out either to flow the glue into the cells or to prevent the overflow of the glue into the detection volume. Plug lengths were adjusted by leaving 3 or 4 mm central detection regions for the 1 mm OD and 1.43 mm OD cells, respectively. A variable pitch RF coil was manually wound with five turns of 150 µm

Fig. 1. (A) Procedure for creating 20-µL detection sample cell with susceptibility-matching ultem plugs. Each insert consisted of (a) 1 cm long, 300-µm ID Teflon tubing; (b) 1 cm long, 2.36 mm OD, −0.8 mm ID ultem plug; (c) 2.8 mm OD, 2.42 mm ID, 2 cm long glass capillary; and (d) 55 cm long, 360 µm OD, 70 µm ID fused-silica transfer line. The length of the ultem plugs inside a glass capillary is adjusted to cover slightly more than a desired active volume defined by a 4 turn copper wire (150 µm OD) coil (e). Total sample volumes of the cell and transfer lines are 24 µL and 4.5 µL (each), respectively, compared to a 140 µL total volume without the ultem plugs. (B) Actual sample cell with ultem plugs and Teflon transfer line inserts.
matched to a 1H frequency of 300 MHz with two variable width capillaries connected to 1 cm long flexible Teflon tubing which was again connected to 55 cm fused-silica transfer lines (not shown in Fig. 2).

Prepared samples were injected into the probes using a 300 MHz NMR spectrometer installed with VNMR 6.1 processing software. Prepared samples were injected into the probes using a 600 μL Hamilton syringe (Hamilton, Reno, NV) and syringe adapter (VICI Valco, Houston, TX).

Each probe was manually shimmed to obtain the best line width and line shape. Raw signal to noise ratios (SNR) were calculated without any processing parameters such as zero-filling and apodization. For small diameters coils, the 1H 90° pulse length was measured to be 2.4 μs at 40 dB. For the larger diameter coil, the 1H 90° pulse length was 6.2 μs at 40 dB. A recycle delay time of 1.5 s was used for all the NMR experiments. A constant concentration sample of 1% v/v H2O/D2O was used to assess the resolution, line shape and SNR for the microcoil probes with susceptibility-matched plugs. The performance of the coils was further tested by running 1D 1H NMR and 2D COSY experiments for the standard glucose and threonine mixture. In addition, the applicability of the coils to analyze complex biological mixtures was assessed using 1D 1H NMR of treated human plasma samples.

3. Results

For the most part, 1H resolution and line shape results (see Fig. 3 and Table 1) were obtained with better than 1 Hz at FW/HM and less than 30/40 Hz at 0.55%/0.11% peak heights. Fig. 3 also includes the spectrum acquired in a small diameter (1.0 mm OD) capillary tube cell that was manually customized by grinding a 2.36 mm OD, ~0.8 mm ID ultem tube (CPI Intl., Santa Rosa, CA) with a Dremel® (Racine, WI) hand tool. The ultem cell was used to investigate if better resolution and line shape could be obtained by lowering the susceptibility differences around the sample region. Theoretically, the susceptibility difference values between ultem/mixed-epoxy is smaller than glass/mixed-epoxy. Although similar resolution was obtained, the line shape was better using the ultem cell with epoxy plugs.

Table 1 shows a comparison of the results for different diameter coils with and without susceptibility-matched plugs in terms of their volume efficiency, resolution and SNR. Using a susceptibility-matched plug, a nearly 6–12-fold improvement in volume efficiency can be achieved without significant loss in resolution and sensitivity for both the smaller and larger volume microcoils. The 20-μL volume cell has similar resolution to that for the smaller coils, but has more than 5-fold better SNR for the constant concentration sample of H2O/D2O. Additionally, in comparison to a standard 5 mm probe that requires 600 μL total sample volume, the 20 μL cell probe has a 30 μL total sample volume and therefore offers a volume efficiency enhancement of 20-fold (of course, the use of Doty susceptibility plugs and Shigemi tubes would reduce this enhancement factor).

Fig. 4 shows highly resolved 1D 1H spectra (36 scans) and 2D COSY (8 scans and 128 increments) of the two standard compound mixture – glucose and threonine, acquired in 20-μL ultem plug cell (2.8 mm OD). The figure also shows the spectrum for the deproteinated human plasma sample acquired with 36 scans in the same microcoil susceptibility plug probe (lower) in comparison to the commercial 5 mm probe (upper). Lower inset shows a resolved peak...
(~5.2 ppm) for the anomeric proton from α-glucose in plasma at the base of the solvent (~4.7 ppm). The raw SNR for this proton was 28.5:1, which is 9.5-fold higher than that for 5-mm probe for the same mass of analyte. Similar resolution and SNR enhancement (figure not included) was obtained in smaller coils with epoxy plugs.

4. Discussion

We demonstrate here that the use of small epoxy and ultem plugs can be of high utility to improve the volume efficiency of microcoil probes. Volume efficiency is often a major concern in the analysis of mass-limited samples. In this study, high volume efficiency without trade-offs in sensitivity and resolution was achieved by introducing susceptibility-matched plugs into the microcoil sample cells. Our previous probes that employed etching techniques to create ellipsoidal bubble detection cells [17,18] yielded FWHM line widths of less than 1 Hz; however, the baseline resolution was poorer, with 0.55%/0.11% peak values of 80/120 Hz. Imprecise etching often resulted in relatively large bubble cells that increased the residual volume and lowered the volume efficiency of the analyte.
efficiency. Incorporation of the susceptibility-matched plugs greatly alleviates these problems.

The flexibility of the proposed approach allows one to adjust the plug to increase or decrease the desired detection volume. Application of such probes can be envisioned in hyphenation with HPLC in which a number of potential issues, such as system dead volume, the imperfect match between HPLC elution and NMR detection volumes, sharp concentration gradients near the NMR coil, the equilibration time in moving the analyte from HPLC to NMR coil, and the sample diffusion from the flow cell can be minimized by eliminating residual volumes as much as possible [21]. Another major application of the plugged microcoil probes is targeted towards analyzing mass limited metabolic samples and bio-fluids in which one can optimize the detection limit by taking maximum advantage of the available sample. Better sample volume efficiency provides the option of using increased sample concentration, and thus, decreased experiment time.

The raw SNR for the 20-μL solenoidal microcoil probe is approximately 5-fold less than observed in the 5 mm commercial probe. This value is less than what would be expected by simply comparing active volumes because the loss is mitigated by the solenoid geometry. When the sample can be concentrated, we find that the improved volume efficiency and other positive attributes of the plugged-microcoil approach yield an overall 8–10-fold enhancement in SNR based on the improved mass sensitivity. The experimental results (shown in Fig. 4C) yield values close to this rough calculation. While the ultem or epoxy plugs are not compatible with all organic solvents, this approach is highly useful for bio-fluid or other aqueous samples. Along with high mass-sensitivity, the enhanced SNR due to effective volume management of the susceptibility plug microcoil probe may provide a practical solution for the NMR analysis of a variety of precious samples. Currently, we are using susceptibility plug microcoil probes for the analysis and structural identification of low concentration metabolites in human plasma/serum and urine samples.

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References


