Nanoliter Volume Sample Cells for ^1H NMR: Application to On-Line Detection in Capillary Electrophoresis[1]

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Capillary electrophoresis (CE) is a powerful method capable of efficient separations of extremely small volume samples.[2-4] Along with the ability to inject and separate nanoliter volumes, the availability of a detection scheme capable of working with such small volume samples is important.[5] NMR, one of the most molecular information rich detection schemes, has not been reported previously as a CE detector. This is primarily due to the inherently low sensitivity of NMR, although NMR microprobes have been developed for samples as small as 0.4 μg and 1–10 μL volumes.[6,7] NMR has been used as an on-line detector for HPLC,[8-11] but the detector cells have been in the 25–200 μL volume range, orders of magnitude larger than acceptable in CE. Here we report the design of an rf microcoil wrapped directly around a fused silica separation capillary that provides a detection cell of ~5 nL and limits of detection (LODs) in the nanogram range for short (<1 min) NMR acquisitions. Because of the nearly 3 order of magnitude decrease in sample volume, a host of new applications become amenable to NMR analysis, including the analysis of microgram samples and on-line NMR detection for microseparations. Although there are many applications for such NMR microcells, we have chosen to emphasize the combination of CE and NMR. The combination of NMR and electrophoresis has been demonstrated previously.[12-14] In the work of Johnson and He,[13] a large (several hundred microliters) U-tube elecrophoresis system was constructed that allowed the electrophoretic mobilities and diffusion coefficients to be determined for each line in an NMR spectrum (although no separations were performed). However, they had to limit their maximum applied voltage because of joule heating. By scaling down the sample cell volume 3 orders of magnitude, the heating effects are reduced to the point that much higher field strengths can be used.[2,3,15]

The experiment described here involves wrapping Cu wire around a 75 μm i.d. fused silica capillary to form a miniature rf coil (typically with 17 turns for a total 1.1 mm detection length).[16] The capillary and microcoil are mounted in an NMR probe so that the capillary and coil are perpendicular to the static magnetic field over the detection region.[17] Wrapping a 17 turn microcoil directly around the separation capillary has the advantage of providing a good filling factor for detection in the 5 nL cell. However, one of the disadvantages of placing the coil in such close proximity to the sample is that significant susceptibility induced line broadening occurs. Figure 1 shows the NMR spectrum collected over a 4000 Hz spectral width for a 0.8 M sample of arginine (~5 nL in detection cell). In Figure 1a, a thin-walled capillary (145 μm o.d.) is used, and the resulting line width is over 200 Hz, while in Figure 1b, a thick-walled capillary (350 μm o.d.) is used, and the line width is narrowed to 11 Hz. The narrowest line widths observed for these hand-wrapped coils are 7 Hz at 7.05 T (300 MHz ^1H NMR). As the wrapping uniformity of the microcoil and the relative configuration of the coil, capillary, and impedance matching circuitry to the sample greatly affect the observed line widths, line width reduction results from the use of a highly uniform coil and positioning the capillary/coil far from other nonunity susceptibility materials. Because of the small sample size, shimming the static magnetic field using standard NMR shim coils has little effect on the observed line width; we are investigating the use of customized miniature shim coils located in close proximity to the detection cell to further reduce the line width.[10]

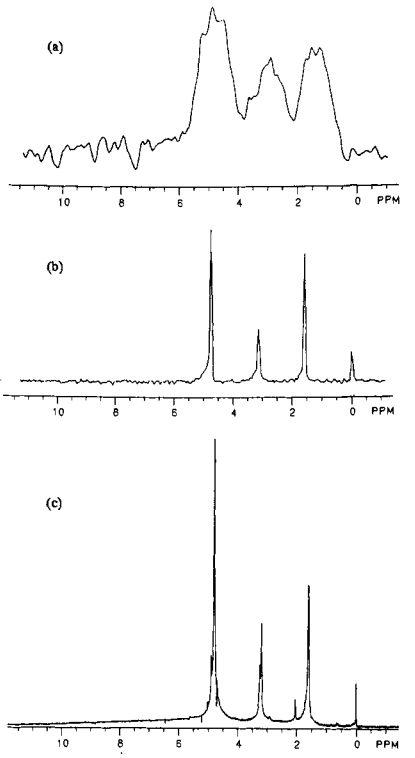

Figure 1. NMR spectra of arginine (0.8 M) in a 75 μm i.d. capillary: (a) 145 μm o.d. capillary with a 1.2 mm microcoil made from 25 μm Au wire (128 scans); (b) 350 μm o.d. capillary with a 1.1 mm microcoil made from 63 μm Cu wire (32 scans); and (c) reference NMR spectrum of 35 mM arginine from a GE GN-300 NMR (four scans, approximately 10^5 times larger volume and 23 times lower concentration).

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(16) The spectra were recorded with a General Electric GN-300 (7.05 T) 300 MHz NMR spectrometer. The microcoils are hand-wrapped using either 42 ga (63.1 μm) insulated copper magnet wire or 50 ga. (25 μm) insulated gold wire. Closely matching the impedance of the capillary/coil to the rf transmiter using capacitors located in close proximity to the microcoil is important. The 0-30 kV CE power supply is located outside the superconducting magnet, and the separation capillary and the inlet and outlet CE buffer reservoirs are located in a custom NMR probe. In order to insure against damaging the rf amplifier by the high voltages used in CE, the separation voltage is kept well below the calculated breakdown voltage of the fused silica capillary at the coil position (≤12.5 kV for the thick-walled, 48 cm long capillary with the detection coil 8 cm from the grounded outlet end).

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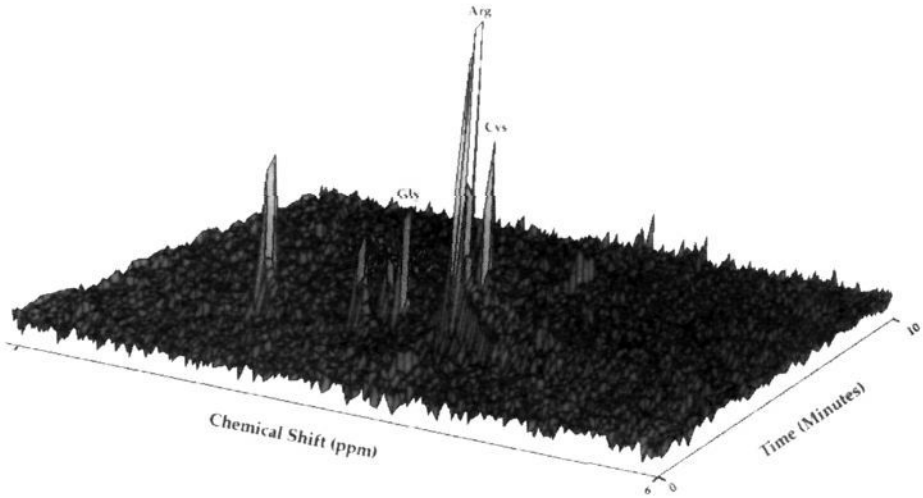

Figure 2. On-line NMR detection of the capillary electrophoresis separation of 0.8 M arginine, 0.7 M glycine, and 0.7 M cysteine. Each NMR spectrum is eight coadded scans of a 512 point, 4000 Hz spectral width NMR acquisition (one complete NMR spectrum is acquired ~16 s).

As shown in Figure 1b, a signal to noise ratio (SNR) of 65 is obtained on the 4 nmol arginine sample. This yields a LOD (SNR = 3) of less than 0.2 nmol of 35 ng for arginine for 32 scans and a 64-s spectral acquisition time. One of the most significant aspects of this technology is the ability to dissolve ~10 μg of a soluble compound in a small amount of the appropriate solvent and obtain stereochemically rich structural information and physical properties such as diffusion coefficients from such mass-limited samples.

Figure 2 shows a two-dimensional NMR electropherogram of a three amino acid mixture.[18] The injection is performed with the probe outside the magnet and involves placing the inlet end of the capillary in the sample vial and raising the vial 15 cm over the outlet end for 30 s, therefore injecting approximately 20 nL of sample. The relatively large sample injection reduces the separation efficiency but allows us to acquire multiple NMR spectra on every analyte band (eight scans at a 2-s repetition rate). As can be seen in Figure 2, the observed separation efficiency is ~5000 theoretical plates for cysteine, poor for CE but comparable to the best reported LC-NMR results. The separation represents more than a 2 order of magnitude decrease in sample volume for a flowing NMR system;[8,9,19] the concentration LOD of ~35 mM is close to previously reported LC-NMR results, but the mass LODs represent more than a 2 order of magnitude improvement.

There is a complex dependence of flow rate, electric field, and current on the observed NMR band intensities.[13,20-22] High flow rates reduce the effective T_1, allowing faster pulse repetition and improved sensitivity by replacing the sample volume for each acquisition so that the signal is not saturated. (This can also increase the observed line widths.) In addition, the electrophoretic current induces an internal magnetic field, and the resulting field gradient may perturb the NMR measurement. As additional complications, the high electric field can partially align large molecules, and joule heating affects the diffusion rates and electrophoretic mobilities. The complex dependence on NMR band intensities on experimental parameters in the capillary electrophoresis separation is explored in much greater detail elsewhere.[23]

In order to improve the utility of this approach further, the areas needing further refinements are the poor concentration sensitivity (resulting in a limited dynamic range) and the broad line widths. We are confident that with optimized coil geometry and the use of uniformly wound microcoils, the line broadening due to susceptibility variations in the local region of the sample may be minimized. However, due to the limited residence time of a nucleus in the NMR microcoil, spin–spin relaxation, T_2, is reduced, and the minimum line width is increased to just over 1 Hz under typical electrophoretic flow conditions.[8,11,20] One of the obstacles to improving the sensitivity of the NMR detection is the limited time we can observe the sample; with a typical <10-s sample residence time, we only can obtain several scans due to the long T_1s of the sample. By decreasing the applied voltage as the analyte migrates across the observation window, the analyte residence can be increased.[10,24] A number of on-line CE injection methods have been demonstrated that concentrate the sample as it is introduced to the capillary,[25-27] although these methods do not improve the mass LODs of the detection method, they extend the useful sample concentration range. As another approach to improving the concentration LOD, the noise introduced by the rf coil can be greatly reduced by using lower resistance coil materials; we expect more than a 2 order of magnitude increase in sensitivity by using superconducting microcoils.[28] These refinements are expected to decrease the LOD of the CE-NMR technique to below the 1 mg/mL concentration for a nanoliter volume of analyte. The 5 nL detection cell represents nearly a 3 order of magnitude decrease in cell size compared to previous work; while such a small volume is required for on-line CE, an intermediate size (e.g. 50–500 nL) offers improved concentration sensitivity and so is more appropriate for mass-limited static measurements and for micro-LC-NMR. We are currently investigating 50 and 200 nL volume NMR cells for such applications.

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(18) The separation was carried out in a 48 cm long (40 cm to the detector), 75 μm i.d./350 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ). The samples were dissolved in the running buffer, 20 mM Na_3PO_4 dissolved in D_2O, and pD was adjusted with DCl to 7.

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