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# NMR Detection with Multiple Solenoidal Microcoils for Continuous-Flow Capillary Electrophoresis

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**Nuclear magnetic resonance (NMR) spectroscopy represents a promising on-line detector for capillary electrophoresis (CE). The inherent poor sensitivity of NMR mandates the use of NMR probes with the highest mass sensitivity, such as those containing solenoidal microcoils, for CE/NMR hyphenation. However, electrophoretic current degrades the resolution of NMR spectra obtained from solenoidal coils. A new method to avoid microcoil NMR spectral degradation during continuous-flow CE is demonstrated using a unique multiple solenoidal coil NMR probe. The electrophoretic flow from a single separation capillary is split into multiple outlets, each possessing its own NMR detection coil. While the CE electrophoretic flow is directed through one outlet, stopped-flow, high-resolution NMR spectra are obtained from the coil at the other outlet. The electrophoretic flow and NMR measurements are cycled between the outlets to allow a continuous CE separation with "stopped-flow" detection. As a new approach for improving multiple coil probe performance, the magnetic field homogeneity is automatically adjusted (via the shim coils of the magnet) for the active coil. The multiple microcoil CE/NMR coupling has been used to analyze a < 3 nmole mixture of amines while obtaining between 1 and 2 Hz line width, demonstrating the ability to avoid electrophoretic current-induced line broadening.**

Since early demonstrations two decades ago,[1] capillary electrophoresis (CE) has become a widely employed and powerful separation technique, as demonstrated by the success of the human genome project using this technology.[2,3] Based upon the separation of analytes by their electrophoretic mobility ($\mu_e$), electrophoresis operates well at the capillary scale, because efficient Joule heat dissipation enables higher electric fields. However, as the separation channel diameter becomes smaller, the sample loading capacity also decreases, thereby necessitating more sensitive detection. A diverse set of detectors has been adapted for CE, including ultraviolet/visible (UV/vis) absorption, laser-induced fluorescence, electrochemical, and radionuclide. Although well suited for marking migration times, these detection methods typically do not provide information on the structure of unknown compounds.

Nuclear magnetic resonance (NMR) provides critical structural information unattainable by other methods, particularly for those of intermediate molecular weight, such as potential pharmacological agents actively under investigation in the pharmaceutical industry. In normal operation, NMR requires relatively pure samples. For mixtures exposed to a transient electric field, NMR can assign individual spectra to analytes with different electrophoretic mobilities, despite the absence of an actual physical separation, by utilizing specially designed pulse sequences to discern between different degrees of coherent motion.[4−12] However, in part because of the low sensitivity of NMR as compared to other detection techniques,[13] this method currently requires relatively large sample quantities.

As an alternative approach for mixture analysis, NMR can be coupled on-line to separation modes, such as high performance liquid chromatography (HPLC),[14,15] to isolate the individual components prior to detection. Since NMR mass sensitivity (S/N per sample quantity) is, to a first approximation, inversely proportional to NMR coil diameter,[16,17] reduced diameter coil probes have enabled better observation of microscale and capillary-scale separations.[13,18,19] Further improvements in sensitivity by a factor of 2−3 can be gained by using a solenoidal coil rather than a Helmholtz coil.[17] Microcoils, solenoidal NMR coils with a

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diameter of 1 mm or less, have achieved 40-fold enhancement in mass sensitivity over conventional 5-mm NMR probes.[13,20] By wrapping the microcoil directly around fused-silica capillary[21] or polyimide tubing,[22] they can be easily integrated with microscale and capillary-scale separations.

With their high mass sensitivity, microcoil probes enabled the first on-line coupling of CE to $^1$H NMR.[23,24] However, electric field application across the separation capillary adversely affects microcoil NMR spectra. Specifically, electrophoretic current causes a broadening of NMR signals, resulting in a loss of scalar coupling information and a decrease in S/N. This effect arises from the electrophoretic current inducing a magnetic field gradient orthogonal to its current flow direction. Modeling the capillary filled with electrolyte as a uniform cylindrical conductor, the following relationship between the current-induced magnetic field, $B_I$, at a radial distance, $r$, from the center of the capillary to the current, $i$, can be derived from Ampere's Law,

$$B_I = (\mu_0 ir)/(2\pi R^2)$$

where $\mu_0$ is the permeability constant of free space, and $R$ is the internal radius of the capillary.[25] To detect the NMR signal optimally, solenoidal coils must be aligned perpendicular to the static magnetic field. Consequently, electrophoretic current through the capillary within the microcoil creates an undesired magnetic field gradient along the static magnetic field, thereby broadening the NMR signal.

To circumvent this problem, stopped-flow microcoil NMR spectra can be acquired during periodic interruptions of the applied electric field, albeit at the cost of longer CE analysis times.[25] Alternatively, CE/NMR can be conducted on-flow with Helmholtz coils, within which the separation capillary is aligned parallel to the static magnetic field, since the NMR spectra do not suffer from electrophoretic current-induced broadening.[18,26−30] However, as mentioned previously, for a given coil diameter, Helmholtz coils are 2−3 times less sensitive than solenoidal coils.[17] Moreover, Helmholtz coils are more difficult to fabricate than solenoidal coils at small dimensions (diameters of 1 mm or less), so that coils larger than the ideal size tend to be used, further reducing probe sensitivity.

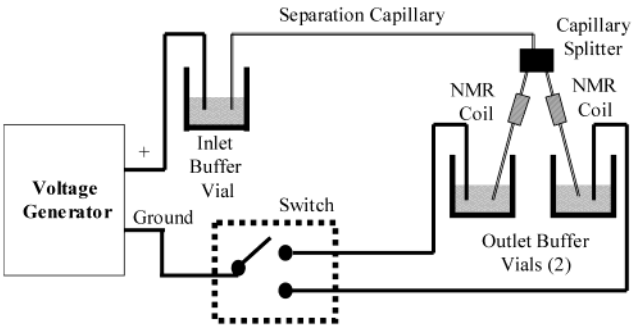


Figure 1. Schematic representation of the CE/NMR system highlighting the arrangement of the separation capillary, the two outlet capillaries and the two NMR detection coils.

Using recently developed multiple coil NMR technology,[31−37] a new method for obtaining stopped-flow microcoil $^1$H NMR spectra from a continuous CE separation is demonstrated. Figure 1 illustrates the instrumental arrangement. The electrophoretic flow from a separation capillary is sequentially directed toward different outlet capillaries, each with its own microcoil, by alternating the ground point of the circuit between different outlet buffer vials. By cycling the electrophoretic flow between the two outlets, stopped-flow NMR spectra are acquired from one coil while the other one is being filled with new electrolyte solution from the CE separation. Using relatively simple programming, the NMR spectrometer controls the timing of the electrophoretic field cycling as well as NMR data acquisition.

Each individual NMR coil is located in a physically different position inside the magnet and, consequently, requires slightly different shim settings to optimize the magnetic field homogeneity over its detection volume, which is necessary to obtain the highest spectral resolution. To acquire ideal spectra from both NMR coils, the magnetic field homogeneity is automatically optimized for the active coil throughout the CE separation. In other words, when the electrophoretic flow path is switched, the shim settings are set to the predetermined optimum values for the coil that does not have current passing through it. Such NMR "active" probes with multiple coils, the use of dynamic shim settings, and electrophoretic control of analyte flow through connectors offers the possibility of trapping individual analyte peaks isolated from a mixture in separate coils for extended stopped-flow NMR analysis, thereby greatly increasing the flexibility afforded by hyphenated instrumentation.

## EXPERIMENTAL SECTION

**Chemicals.** All chemicals were used as purchased from the manufacturer without further purification. Boric acid, sodium


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tetraborate decahydrate, and tetraethylammonium (TEA) bromide were obtained from Sigma Chemical Company (St. Louis, MO). Tetramethylammonium (TMA) acetate was purchased from Aldrich Chemical Co. (Milwaukee, WI). Sodium hydroxide was acquired from EM Science (Gibbstown, NJ). Triethylamine was obtained from Fisher Scientific (Fair Lawn, NJ). $D_2O$ (D, 99.9%) was purchased from Cambridge Isotope Laboratories (Andover, MA). $H_2O$ was dispensed from a Milli-Q water purification system (Millipore; Bedford, MA). For $D_2O$ solutions, after measuring pH with a standard commercial meter (Ag/AgCl electrode; model 98-26; Thermo Orion; Beverly, MA), pD was determined from the equation $pD = pH' + 0.40$, where $pH'$ is the meter reading.[38]

**Microcoil NMR.** The multiple microcoil $^1H$ NMR probe employed in this experiment follows the basic design described previously.[31,34] For this particular probe, sleeve microcoils were used to allow easy fused-silica capillary exchange.[22,31,37] The microcoils were wrapped around individual segments of 370-$\mu$m-i.d./420-$\mu$m-o.d. polyimide tubing, through which 360-$\mu$m-o.d. capillaries could be inserted. Each microcoil consisted of 15 turns of 50-$\mu$m-diam round copper wire with polyurethane coating (63-$\mu$m diameter with coating) (California Fine Wire Company; Grover Beach, CA), resulting in a coil length of ~1 mm.

Because of the flexible nature of thin polyimide tubing, excessive strain on the separation capillary housed within the sleeve microcoil probe can substantially degrade NMR spectral resolution.[37] To provide better rigidity to the sleeve probe, short segments of 530-/700-$\mu$m i.d./o.d. fused-silica capillary held within 700-/850-$\mu$m i.d./o.d. fused-silica capillary were glued onto both ends of the microcoil polyimide tubing. Each microcoil polyimide sleeve was then affixed by epoxy at the reinforced segments to its own printed circuit board.

After stripping the polyurethane coating, the coil leads were soldered onto standard impedance-matching networks in a balanced configuration for tuning up to $^1H$ observation at 500 MHz. To attain high-resolution NMR spectra from copper wire microcoils, the surrounding environment must possess a magnetic susceptibility similar to that of copper. Consequently, a plastic bottle was constructed around both microcoils and was subsequently filled with MF-1 (Magnetic Resonance Microsensors Corporation; Savoy, IL), a perfluorinated organic liquid possessing a volume magnetic susceptibility within 3% of the susceptibility of copper.[37] Each coil can attain 1−2 Hz full-width at half-maximum (fwhm) line width when the shims are optimized for that particular coil.

Coupling between different coils and their impedance matching networks is a significant concern in multiple coil probes.[31,34,36,37] This leads to signal bleedthrough from samples present in neighboring coils and, in severe cases, problems tuning the coils to the same frequency. To minimize interference, the two microcoils were arranged 1 cm apart in the vertical direction (along the static NMR magnetic field) with the impedance-matching networks situated at ends that were opposite to each other. A network analyzer (HP 8751A network analyzer; Hewlett-Packard; Palo Alto, CA) was used to measure the electrical characteristics of the multiple microcoil sleeve probe. When both coils are tuned to 500 MHz, the electrical coupling between circuits is −28 dB (4.0% voltage loss). For this probe, tuning one coil had no measurable effect on the resonance of the other coil.

To quantify NMR signal bleedthrough, the peak S/N was measured in spectra obtained by (a) transmitting and receiving on the coil containing the sample, (b) transmitting and receiving on the neighboring coil, (c) transmitting on the coil containing the sample and receiving on the neighboring coil, and (d) transmitting on the neighboring coil and receiving on the coil containing the sample. By normalizing with respect to (a), the NMR signal bleedthrough fraction was calculated. For the test, 180- /340-$\mu$m i.d./o.d. fused-silica capillary was inserted into each coil, creating an NMR observe volume of ~25 nL. The same sample, 5% $H_2O$/95% $D_2O$, was injected into both coils. Since the two coils were in slightly different magnetic fields, the HOD peaks from the two coils appeared at different frequencies in NMR spectra. Aside from the number of acquisitions (NA) and relaxation delay (RD), the same standard data acquisition parameters were used for each spectrum. A long RD was employed to ensure full longitudinal relaxation in spectra with NA > 1. To accurately compare spectra, S/N was normalized by dividing by $(NA)^{1/2}$. No line broadening (LB) was used for S/N measurements.

During on-line CE/NMR detection, only one coil of the multiple microcoil probe was active. Consequently, no additional transmit/receive channels were needed in addition to the one normally present in the spectrometer. An electromagnetic radio frequency relay switch (model 3506-16-7000; MBF Microwave, Inc.; Henderson, NV) determined which coil was connected to the spectrometer's transmit/receive channel. Controlled by TTL pulses from the spectrometer, the low-loss switch enabled automatic spectra acquisition from the active coil. Specific details about the switch have been previously reported.[31] For a given transmission power, the two microcoils had similar 90° pulse widths. Comparing the 90° pulse widths of the microcoils with and without the switch, a 5% loss in S/N occurred when the switch was used.

**CE/NMR.** All CE/NMR experiments were conducted on a Varian 500 MHz spectrometer with a wide-bore (89-mm diameter) magnet. Figure 1 illustrates the instrumental schematic setup for coupling multiple microcoil NMR detection to CE. For this series of experiments, a 75-/360-$\mu$m i.d./o.d. capillary served as the inlet separation channel, which leads into the central port of a 3-way capillary junction (29-nL dead volume) (PEEK MicroTee, P ≅ 775; Upchurch Scientific; Oak Harbor, WA). Two 180-/340-$\mu$m i.d./o.d. capillaries were connected to the capillary junction as outlet channels. Each outlet capillary was threaded through a different microcoil sleeve, creating an NMR observe volume of ~25 nL. For both paths, the length of the outlet capillary from the junction to the microcoil was ~12 cm. Once the probe was inserted into the magnet bore, all CE operations were performed externally.

For this study, CE was conducted with the inlet buffer vial at positive potential (anode) and the outlet buffer vial at ground (cathode). The electrophoretic flow was directed through one of the outlet capillaries by grounding its outlet buffer vial (closed circuit) while leaving the other one floating (open circuit). In these experiments, a high voltage power supply (Series 230; Bertan Associates; Hicksville, NY) delivered a potential up to 9.0 kV. Platinum electrodes were used at buffer reservoirs to avoid unwanted electrochemical reactions. Digital multimeters monitored the current through both outlet channels.

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The NMR spectrometer was also used to alternate the electrophoretic flow between the two outlet capillaries. TTL lines from the spectrometer provided the requisite input for a Labview program (National Instruments; Austin, TX) on a separate computer that operates two high voltage relays (Series 3000; MBF Microwave, Inc.; Henderson, NV), each connected to a different outlet channel circuit. The shim settings are automatically adjusted to the optimum values for the active microcoil.

Before performing CE, the capillary system was flushed first with 100 mM NaOH, then with 5% $H_2O$/95% $D_2O$, and finally, the run buffer. The coils were shimmed while the 5% $H_2O$/95% $D_2O$ solution was in the capillary system. To ascertain if the CE/NMR instrumental coupling avoided spectral degradation, spectra were acquired during electrophoretic flow switching from (a) only one microcoil (NMR observation switch bypassed; shims optimized for active coil throughout run) or (b) alternating microcoils (NMR observation switch in-line; shims changed to optimal values for active coil). For this test, the run buffer consisted of 1 M borate with 200 mM triethylamine, which served as a marker for spectral resolution, in $D_2O$ (pD = 8.51). The inlet separation capillary was 100 cm long, and both outlet capillaries were 110 cm in length. By applying a potential of 8.0 kV, the electric field strengths across the separation capillary and the two outlet capillaries were 67 and 12 V/cm, respectively. A total of four spectra were acquired. The NMR spectral parameters were as follows: spectral width (SW) = 5500.6 Hz, number of data points (NP) = 22 002, acquisition time (AT) = 2.00 s, RD = 0 s, pulse width (PW) = 50°, NA = 4, dummy scans (DS) = 2/spectra.

For the CE/NMR separation, a mixture of two amines was analyzed. The run buffer consisted of 800 mM borate in $D_2O$ (pD = 8.36). The sample, 20 mM of both TMA acetate and TEA bromide, was dissolved in run buffer. The inlet separation capillary was 90 cm long, and both outlet capillaries were 100 cm in length. Electrokinetic injection at 8.0 kV for 2.0 min loaded ~3.0 nmoles TMA (3.4-cm plug length) and ~2.6 nmoles TEA (2.9-cm plug length) into the inlet capillary. By applying a potential of 8.0 kV, the electric field strengths across the separation capillary and the two outlet capillaries were 75 and 13 V/cm, respectively. A total of 720 spectra were acquired (360 from each coil). The NMR spectral parameters were as follows: SW = 3000 Hz, NP = 8192, AT = 1.365 s, RD = 0.635 s, PW = 60°, NA = 8, dummy scans (DS) = 2/spectra. The 20 s allotted for electrophoretic flow through one outlet channel before switching to the other was based upon the expected migration times of the analytes through the NMR observe volume. With the current program to change shim settings between spectra, the data cannot be processed during collection.

**Data Processing.** All NMR spectra were processed with NUTS (2D-version 19990629; Acorn NMR Inc.; Fremont, CA).

## RESULTS AND DISCUSSION

**Multiple Microcoil NMR Probe Performance.** For multiple coil NMR probes with separate impedance matching networks for each coil, electrical interference between different coils and their circuits leads to deleterious effects, such as bleedthrough peaks (or unwanted excitation) from samples present in neighboring coils. Because standard NMR spectrometers possess one transmit/receive channel for a given frequency range, multiple coil probes currently operate with only one coil active at a time.

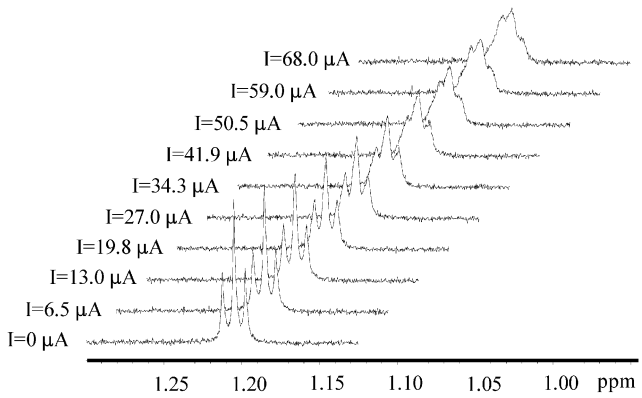


Figure 2. Microcoil CE/NMR spectra of triethylamine methyl peak acquired as the applied voltage is increased from 0.0 to 9.0 kV by increments of 1.0 kV. Spectral parameters: NA = 1, AT = 2 s, LB = 0.

In this mode of operation, excitation must pass from the active coil to the inactive one and then back again in order to observe a bleedthrough peak. The NMR signal bleedthrough has been measured when (a) transmitting and receiving on the neighboring coil, (b) transmitting on the coil containing the sample and receiving on the neighboring coil, and (c) transmitting on the neighboring coil and receiving on the coil containing the sample. Following expectations, the signal bleedthrough is 4−6% for cases (b) and (c), but only 0.5−0.7% for case (a). The NMR signal bleedthrough correlates well with the measured voltage bleedthrough of 4%.

For multiple coil probes operating with only one coil active at a given time, further improvements in NMR signal isolation can be achieved by detuning the inactive coils, which reduces the electrical coupling between them.[37] Moreover, to further reduce unwanted excitation of neighboring samples, spatially selective excitation pulses can be employed.[32] As the shim coils are automatically adjusted to the optimal values for the active coil during CE/NMR, the spectral resolution in the neighboring coil is not a concern, in contrast to other multiple coil experiments.[31−36] For this particular probe with active shimming (only one coil shimmed at a time), the inactive coil typically has a fwhm of ~15 Hz, thereby further reducing peak bleedthrough. Consequently, the probe performance is high enough that signal bleedthrough during CE/NMR is not significant.

**NMR Spectral Resolution during Electrophoretic Flow.** Before performing a CE separation, the ability of the multiple microcoil probe to obtain stopped-flow NMR spectra during continuous electrophoretic flow in the separation capillary was investigated. Figure 2 shows the effect of electrophoretic current on microcoil NMR spectral resolution when the capillary system contains 200 mM triethylamine (in 1 M borate buffer in $D_2O$). As the voltage applied across the outlet capillary is increased, the triethylamine peaks are broadened, resulting in a loss of scalar coupling information and a decrease in S/N.

With the multiple microcoil approach illustrated in Figure 1, no electrophoretic current-induced spectral degradation was observed. Figure 3 depicts NMR spectra acquired with the electrophoretic flow alternating between the two outlets: (A) exclusively from the upper coil (NMR observation switch bypassed, shims optimized for upper coil), (B) exclusively from the

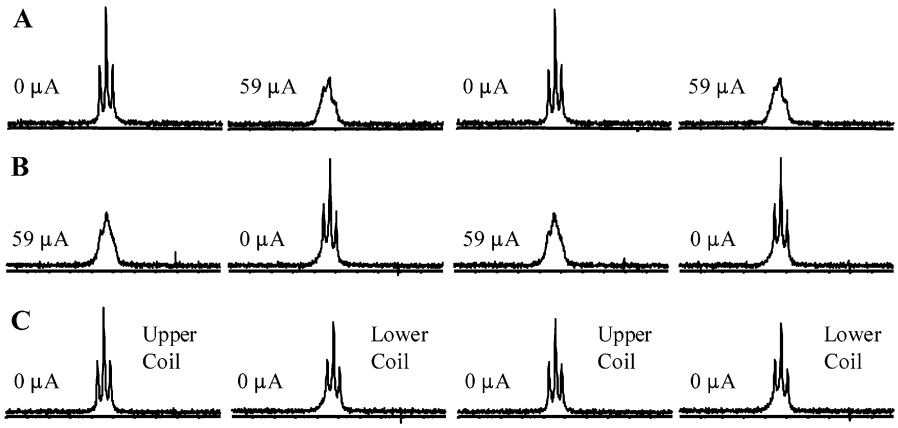


Figure 3. Multiple microcoil CE/NMR spectra of the triethylamine methyl peak acquired during the alternation of electrophoretic flow between the two outlet capillaries: (A) spectra acquired from the upper coil (shim settings optimized for the upper coil and the NMR observation switch bypassed); (B) spectra acquired from the lower coil (shim settings optimized for the lower coil and the NMR observation switch bypassed); (C) spectra acquired from microcoil on the outlet capillary without electrophoretic flow (shim settings optimized for the active coil and the NMR observation switch in-line).

lower coil (NMR observation switch bypassed, shims optimized for lower coil), and (C) alternating between coils in the appropriate manner (NMR observation switch in-line, shims optimized for active coil). The electrophoretic flow was initially directed through the lower coil at the start of the sequence. As observed in Figure 3A,B, alternating NMR spectra suffer from spectral degradation as the electrophoretic flow passes through the active coil outlet capillary. Comparing Figure 3A to 3B, one of the two data sets affords a high-resolution spectrum at each slice of the sequence. By alternating data acquisition to the coil where there is no electrophoretic current, NMR spectral degradation can be avoided despite continuous electrophoretic flow in the separation capillary, as observed in Figure 3C.

With regard to switching shims and electrophoretic flow between coils, a delay time is needed before acquiring high-resolution NMR spectra. In the absence of electrophoretic flow, ~1 s is needed for the shims to settle after switching to obtain spectra with optimal resolution. Because of the diminutive size of the microcoils, optimal resolution can be achieved by adjusting several low-order shims (Z1, X, and Y), which typically settle faster than higher-order shims (such as Z2). However, when an electrophoretic current (~60 $\mu$A) is passing through the capillary, a delay of 8 s is required to obtain optimal high-resolution spectra after switching. A delay of 4 s gives only a slight increase (0.2−0.4 Hz at fwhm) in NMR spectral resolution.

**Multiple Microcoil Detection for CE/NMR.** Having validated the multiple-microcoil approach for avoiding spectral degradation during continuous electrophoresis, a CE/NMR experiment was performed. The sample, ~3.0 nmoles TMA acetate (3.4 cm plug length) and ~2.6 nmoles TEA acetate (2.9 cm plug length), was injected into the inlet capillary, and an 8.0 kV potential (current of 26 $\mu$A) was applied. For this buffer and capillary system, higher potentials cause deleterious Joule heating effects. Figure 4 shows an NMR electropherogram obtained from the upper coil. The two analyte peaks are well separated, and their identities can easily be established from the chemical shift data. The lower coil gave similar results. The two data sets can be co-added to improve S/N; however, since the two microcoils are in slightly different magnetic fields, the spectra from the two coils have to be shifted relative to each other to properly align the peaks.

The electroosmotic flow is low at this high buffer concentration, and the transfer volume from the capillary junction to the microcoils is large (~3 $\mu$L); consequently, the migration times of the analyte peaks are long. TMA reaches the upper coil at 130 min, corresponding to a velocity of 77 nL/min. At this velocity, TMA has a residence time of 19 s within the coils, which is close to the electrophoretic flow switch time of 20 s. TMA has a peak half-width ($W_{1/2}$) of 5.0 min, with an efficiency of ~4000 theoretical plates. The low separation efficiency partly arises from the large CE sample injection plug, which is needed for NMR analysis. At peak maximum, the TMA concentration is ~6 mM. In comparison, TEA first appears in the upper coil at 160 min. With a velocity of 63 nL/min, TEA migrates through the microcoils in 24 s. TEA has a $W_{1/2}$ of 5.7 min and an efficiency of ~4700 theoretical plates. At peak maximum, the TEA concentration is also ~6 mM.

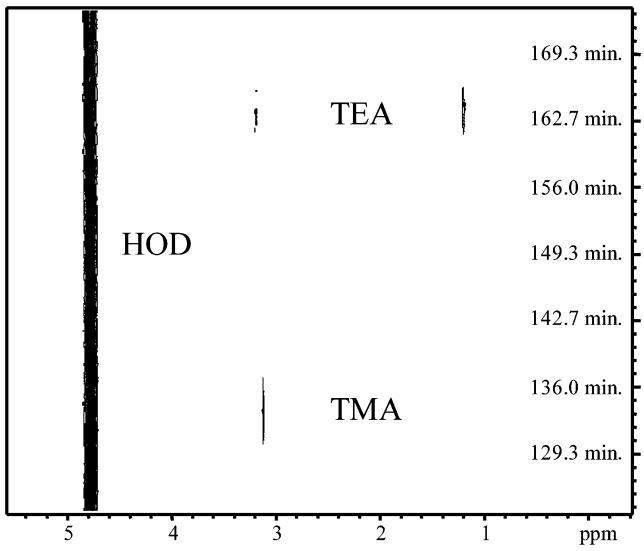


Figure 4. Two-dimensional CE/NMR electropherogram (chemical shift vs migration time) obtained from upper coil showing separation of 20 mM TMA and 20 mM TEA in 800 mM borate buffer.

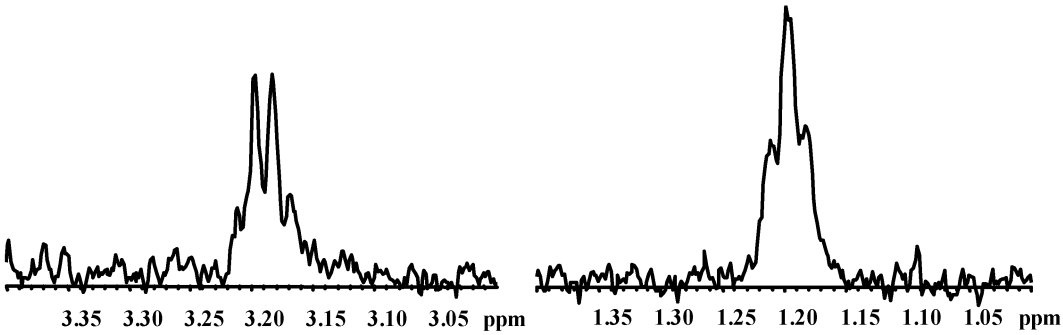


Figure 5. One-dimensional CE/NMR spectrum of TEA obtained from the electropherogram in Figure 4 obtained by co-adding nine spectra (NMR acquisition time of 3.0 min; data acquired over 5.7 min, LB 1.2). The methylene quartet (downfield) and methyl triplet (upfield) are observed.

Even though the S/N of the NMR spectra is low, by co-adding neighboring slices, scalar couplings can be observed. Figure 5 shows an NMR spectrum from the upper coil created by co-adding nine slices (NMR acquisition time of 3.0 min; data acquired over 5.7 min) in which the TEA concentration was between 3 and 6 mM. The TEA peaks can be properly identified as a quartet (downfield) and a triplet (upfield) despite the low S/N. If multiple microcoil detection is not used, the migrating analyte peaks would not be observed during continuous-flow CE/NMR as a result of the loss in S/N.

**Future Directions.** An "active" CE/NMR probe with multiple coils has been demonstrated that uses electrophoretic control of analyte movement through multiple NMR detection points and optimizes individual coil performance using dynamic NMR shim settings. This combination blurs the line between continuous and stopped-flow data acquisition and greatly increases the flexibility afforded by hyphenated instrumentation.

Further improvements in microfluidics, hardware, and programming can further enhance CE/NMR performance. Experimental time can be reduced substantially by minimizing the transfer volume from the capillary junction to the NMR coils. This can be accomplished by using bubble cells, which have previously been employed in CE/NMR,[18,26−30] rather than uniform-bore-size capillaries. By connecting a large-internal-diameter capillary to each bubble cell via a capillary union after the detector, the majority of the electric field can be dropped across the separation capillary. To further improve S/N, larger detection volumes, bigger sample injection volumes, and higher magnetic field strengths can be employed. Moreover, the larger detection volumes will create longer residence times for extended observation. By selectively detuning the inactive coil,[37] interference and concurrent S/N loss can be minimized. In addition, a preamplifier can be positioned prior to the radio frequency switch to avoid sensitivity loss. Programming advancements to enable NMR data processing during the CE run will facilitate operation. Moreover, the time allotted for coil observation can be increased as the run progresses to match the increasing sample residence times caused by slower analyte velocities, as has been done for other CE detection modes.[39] Because both coils obtain similar data sets, the spectra from the two coils can be co-added to improve S/N (the transmitter frequency can be shifted between the coil observations so that the NMR peaks overlap in the frequency spectra). Overall, an estimated 10-fold gain in sensitivity can be achieved through these enhancements.

With the current fluidic arrangement of a single separation channel split into multiple outlets, each with its own detector, the time required for intensive NMR analysis of numerous components present in a separated mixture can be reduced by on-line peak trapping. By adding a sensitive on-line detector (such as UV/vis absorption) before the capillary junction, migrating analyte peaks can be detected and then directed to separate NMR coils for time-consuming stopped-flow experiments, such as multidimensional NMR. Not confined to CE/NMR, this fluidic concept can be applied to the integration of NMR to any separation mode, including capillary electrochromatography (CEC) and HPLC (both of whose higher sample loading capacities are beneficial to NMR). For multiple coil peak trapping in HPLC and pressurized CEC, valves can be positioned at capillary junctions to direct the flow from the separation channel to the selected NMR coil. This multiple coil approach will greatly improve the throughput and flexibility of hyphenating NMR to separations.

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