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## Technical Notes

# On-Line Temperature Monitoring in a Capillary Electrochromatography Frit Using Microcoil NMR

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**A new nuclear magnetic resonance (NMR) spectroscopy probe has been designed to measure the temperature of the water inside a capillary. The probe provides the ability to measure the temperature in a several hundred micrometer long capillary section, corresponding to liquid volumes in the picoliter to nanoliter range with a temperature monitoring accuracy of 0.2 °C. The NMR probe is based on a novel two-turn vertical solenoidal design, and its performance for capillary-scale temperature measurements is characterized. The temperature rise in a chromatographic frit of the type used in capillary electrochromatography is measured as a function of applied power, and temperature rises of more than 50 °C are observed. The temperature of the electrolyte cools rapidly after exiting the frit and can be followed as a function of distance from the frit. The ability to accurately monitor the temperature of water as it moves through porous materials such as packed chromatographic beds and frits is important to allow the effects of temperature on CEC separation performance to be determined.**

While temperature is an important factor in all electroseparations, thermal considerations become particularly significant with the packed or partially packed columns and frits used in capillary electrochromatography (CEC). The Joule heat dissipated in electroseparations can influence a myriad of phenomena such as dispersion, flow profiles, and partitioning of analytes between the mobile and stationary phase. In addition, viscosity, diffusion coefficients, and even buffer pH all depend on temperature.<sup>1–3</sup> As such, the thermal characteristics of electroseparations offer a fertile source of information about the fundamental mechanisms that lead to optimized performance. In fact, control of the temperature of a CEC column can be used to improve the resolution and speed of a separation.<sup>4,5</sup> However, since the applied

voltage directly impacts the internal temperature through Joule heating, a means to monitor accurately the temperature in real time is essential to the success of attempts to improve performance by manipulating temperature.

As a hybrid of capillary electrophoresis and liquid chromatography (LC), CEC has found its way into an increasing number of applications during the past decade.<sup>6,7</sup> The much lower pressures involved with CEC (compared to LC) allow the use of smaller particle diameters for chromatographic packing material and thus offer the prospect of superior performance. However, while certain reports of CEC have demonstrated higher separation efficiencies than in LC, the experimentally measured plate heights for submicrometer particles remain lower than predicted by theory.<sup>2</sup> The difference between theory and experiment has been attributed to either the higher Joule heating or packing irregularities in the CEC column. While CEC continues to demonstrate its capabilities in various environments, bubble formation at the frits that form the interface between the packed beds and the open tubular sections has proven a liability. Local temperature rises in the frit regions could explain the tendency for bubble formation at the frits. The capability to accurately measure the column temperature in packed chromatographic beds and frits can provide important feedback for column design.

A variety of experimental approaches has been reported to measure or deduce the temperature of columns during electroseparations in open tubes. For example, miniature thermocouples can accurately measure the external capillary temperature, a technique that has been demonstrated for micellar electrokinetic chromatography<sup>8</sup> and capillary zone electrophoresis.<sup>9,10</sup> In addition to measurements of capillary surface temperature during electrophoresis, theoretical internal temperatures also have been calculated from observed changes in micellar capacity factors,<sup>8</sup> electroosmotic mobilities,<sup>11,12</sup> and buffer conductivities.<sup>9,11,12</sup> Several

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optical strategies have probed the local temperature within CE capillaries.<sup>13–15</sup> In one example, the temperature dependence of the water O–H stretch equilibrium between weakly bent and strongly bent hydrogen-bonded species was monitored by Raman spectroscopy during CE.<sup>15</sup> The steady-state and transient intracapillary temperature gradients were monitored during electrophoresis.<sup>13</sup> In another case, changes in the absorption spectrum of cobalt(II) chloride were correlated with intracapillary temperature.<sup>14</sup> While optical techniques have demonstrated the capability to measure temperature in open capillaries, packed chromatographic beds and frits have proven a difficult challenge due to light scatter and opacity.

NMR thermometry is frequently implemented by monitoring the proton resonance frequency of the water signal in magnetic resonance imaging.<sup>16</sup> As the <sup>1</sup>H NMR water resonance shows a strong linear dependence on temperature<sup>17</sup> and because many CE separations are conducted in aqueous media, NMR is well suited to measure the temperature within capillaries during electroseparations. We recently described the use of microcoils to measure the temperature of the fluid inside a fused-silica capillary filled with a conductive buffer solution in an apparatus used for capillary zone electrophoresis.<sup>17</sup> Subsecond temporal resolution and high accuracy were reported. Here, we demonstrate a novel NMR microprobe with greatly improved spatial resolution for temperature measurements and illustrate its capability to measure temperature in and just outside of the chromatographic frits that are used in CEC as the boundary between the packing material and the open section of a column.

## EXPERIMENTAL SECTION

**Reagents.** Sodium borate decahydrate and boric acid were from Sigma (St. Louis, MO). The D<sub>2</sub>O (99.9% D) was obtained from Cambridge Isotope Laboratories (Andover, MA). All reagents were used as received. H<sub>2</sub>O was dispensed from a Milli-Q water purification system (Millipore, Bedford, MA). Buffer solutions were sonicated and filtered prior to use.

**NMR Probes.** The capillary NMR experiments employed a Varian Inova 500-MHz (11.7 T) spectrometer with an 89-mm bore. Two custom probes (designated as probes A and B) were used to conduct the NMR measurements described herein. In contrast to the typical horizontal orientation of a solenoid, the coil and capillary in probes A and B were rotated by 90° so that the longitudinal axis of the sample was parallel to the external magnetic field ( $B_0$ ). The two vertical solenoids were constructed using a procedure similar to that reported previously<sup>17</sup> except that they operated at 500 MHz and were wrapped around thin polyimide sleeves to facilitate capillary positioning. This vertically oriented solenoid geometry eliminated the degradation due to a current-induced magnetic field gradient that arises during the application of the CE voltage.<sup>17</sup> Probe A consisted of 15 turns of 50- $\mu$ m-diameter Cu wire wrapped around a 370- $\mu$ m-i.d., 420- $\mu$ m-

o.d. polyimide sheath (MicroLumen, Tampa, FL), while probe B had only two turns. The rf coils were surrounded by a perfluorinated liquid (FC-43, 3M, St. Paul, MN), which served as a magnetic susceptibility-matching medium and a liquid-cooling reservoir (for the CE–NMR thermometry experiments). A printed circuit board was mounted on a Delran probe head surrounded by a cylindrical Delran cap to provide sufficient electrical isolation between the NMR magnet and the high voltages used in the CE experiments.

**Characterization of Vertical Solenoids.** To compare the performance of the 15-turn vertical solenoid (probe A) versus its 2-turn counterpart (probe B), 100- $\mu$ m-i.d., 360- $\mu$ m-i.d. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) were passed through the polyimide sheaths of both probes and filled with 100% H<sub>2</sub>O. After vertically positioning each probe at the center of  $B_0$ , <sup>1</sup>H NMR spectra were collected for a series of Z1 shim settings (with all other shims set to zero). The Z1 shim produces a linear magnetic field gradient along the axis of the magnet (the Z direction), coincident with the main magnetic field. The optimal Z1 shim value was determined empirically based upon the setting that produced the highest spectral resolution. After acquiring an NMR spectrum at the optimal Z1 value and referencing the water signal to 0 Hz, the Z1 shim setting was changed in 5000-unit increments in both the positive and negative directions. For each Z1 setting, an NMR spectrum was acquired and the peak position for the water signal was recorded.

**Temperature Calibration.** As reported previously,<sup>17</sup> a commercial, 5-mm probe in a 500-MHz spectrometer with variable-temperature (VT) control was used to calibrate the shift of the proton resonance frequency of the water signal as a function of temperature. The NMR probe temperature was set to values between 25 and 75 °C in increments of 10 °C. For each temperature setting, the sample equilibrated in the magnet bore for 10 min after the resonance frequency ceased to shift by more than 1 Hz. The peak position was recorded and averaged ( $n = 10$ ) at each temperature. Previous results demonstrated that pH, ionic strength, and solvent deuteration have a negligible effect on the calibration.<sup>17</sup> To examine the accuracy of the VT controller, an ethylene glycol temperature standard<sup>18</sup> showed agreement to within 2%, with a measurement precision of better than 0.2 °C.

**CE–NMR Apparatus.** For the CE–NMR thermometry experiments, a 0–30-kV high-voltage power supply (Series 230; Bertan Associates, Hicksville, NY) was located outside of the NMR magnet. The exposed portion of the platinum electrodes placed in the buffer vials were covered with electrical tape (3M) and heat-shrink PTFE/FEP tubing (Small Parts, Inc., Miami Lakes, FL) to prevent arcing to the grounded impedance matching circuit for the rf coil. Liquid levels in all vials were maintained at equivalent heights to minimize gravimetric flow. The electrical current in the capillary and the applied voltage were recorded with a data acquisition card (Lab-PC+, National Instruments, Austin, TX) in a personal computer. The current was also monitored via a resistor that was placed in series with the high-voltage power line. For thermal measurements made during electroseparation conditions, probes A and B were used with positive high voltage on the inlet side and ground on the outlet side.

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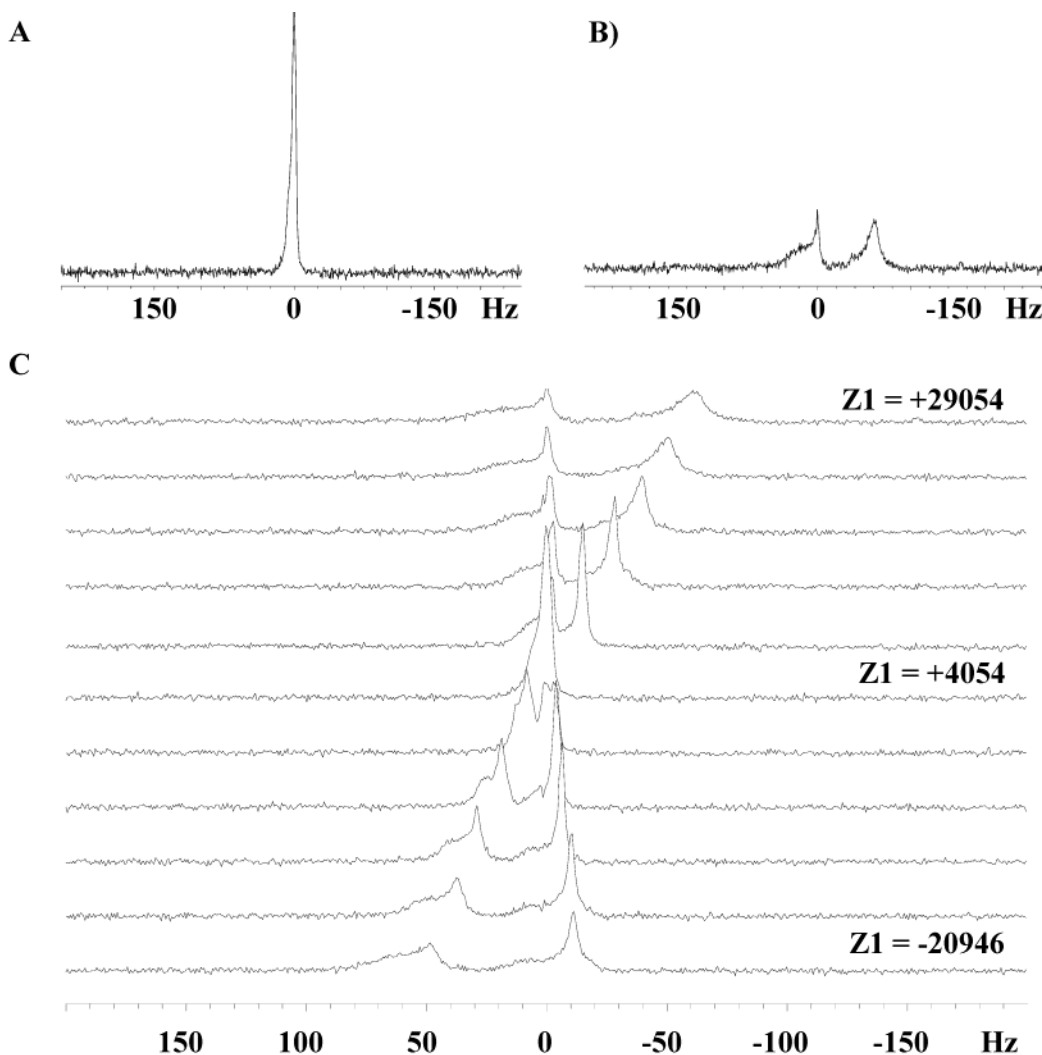


Figure 1. Effect of Z1 shim on NMR line shape of 100% H<sub>2</sub>O using a 15-turn vertical solenoid tuned to 500 MHz: (A) optimal Z1 setting (+4054); (B) peak splitting observed for Z1 = +29054; (C) series of NMR spectra for Z1 values that each differ by 5000 units.

**Thermal Measurements in and around Chromatographic Frits.** To measure the temperature in and around a chromatographic frit, an open-tubular 100- $\mu$ m-i.d., 360- $\mu$ m-o.d. fused-silica capillary of 14.5 cm in length was connected to a 23.5-cm-long PTFE tube. The unpacked fused-silica capillary had a commercially manufactured  $\sim$ 1.5-mm-long frit designed for CEC (UnimicroTechnologies, Pleasanton, CA) located  $\sim$ 5 cm from one end. The capillary was passed through the polyimide sheath of probe B and filled with 50 mM borate buffer in 100% H<sub>2</sub>O. Prior to each CE-NMR thermometry experiment, the temperature within the bore of the magnet was measured with a thermocouple. After allowing the probe to equilibrate thermally with the environment within the magnet bore, the resonance frequency of the water signal with no applied voltage was referenced to 0 Hz. Since the radio frequency coil was not physically attached to the capillary, the coil could be positioned in a controllable manner with respect to the frit. The applied potential was stepped to a given value, and NMR spectra were recorded 5 min after each voltage change. With the microcoil positioned before, in, and after the frit, NMR spectra were collected for a series of applied potentials. Spectral acquisition conditions: AT = 3.0 s, D1 = 2.0 s, SW = 1000 Hz, and NA = 1.

## RESULTS AND DISCUSSION

In capillary zone electrophoresis, sharp spatial demarcations in temperature are not expected because of the relatively constant conductivity of the solution throughout the capillary. However, in CEC with packed columns, the boundary between the open capillary and the chromatographic frit and between the frit and the packed column are predicted to have sharp temperature gradients. In addition, these boundaries will have differing magnetic susceptibilities, possibly impacting the obtainable NMR line width, which may also affect the ability to monitor the temperature. In this proof-of-concept study, we investigate the temperature rise in a 1.5-mm-long frit. Ideally, the active NMR observed length should be shorter than the frit length and so our NMR probe should have an active probe area smaller than the frit. Thus, we needed to determine whether the coils could be fabricated with fewer turns while retaining sufficient sensitivity.

A vertical solenoid can be modeled electromagnetically as a series of stacked, current-carrying circular loops. At the center of each loop, the associated magnetic field is oriented along the direction of  $B_0$ , the main magnetic field, and therefore, no NMR signal is detected from this region. Since the magnetic field lines must form a closed loop, the form of the magnetic field within

and outside the solenoid gives a component in the transverse plane. The signal from a particular location depends on the magnitude of the radio frequency ( $B_1$ ) field, its transverse component, and the number of protons at that location. This signal is typically largest just outside the ends of the coil. In fact, due to the natural pitch of the coil, there will be a small signal component from sample in the coil center. Thus, for a one- or two-turn solenoid with sample found only along the center axis (constrained by the lumen of the capillary), most of the signal is expected to be from just outside the ends of the coil.

To verify that the signal from a vertical solenoid actually originates from the two edges of the coil and that the sample within the interior does not make a significant contribution, a series of NMR spectra was acquired with different Z1 shim values. Since the Z1 shim generates a magnetic field in the Z direction (i.e., parallel to  $B_0$ ), the resonance signal frequency as a function of Z1 can be used to ascertain the vertical profile of an rf coil's observed volume. As shown in Figure 1, the resonance signal from water began to split and separate as the Z1 shim was changed from its optimal value. Furthermore, the separation of the peaks plotted against the Z1 setting yielded a straight line.

To further test this hypothesis, a two-turn solenoid (probe B) was constructed. A comparison of integrated signal intensity between the 15-turn vertical solenoid and the 2-turn probe showed that the sensitivity of two coils was comparable. Of course, the sensitivity of both coils is greatly inferior to a horizontally oriented solenoid. Nevertheless, the performance is sufficient to detect the water signal for the thermometry experiments described. Based upon the values of the shim settings, the linear dimensions probed by the 15-turn and the 2-turn vertical solenoids were determined to be approximately 1 mm and 400  $\mu\text{m}$ , respectively. The two-turn solenoidal coil is the smallest solenoidal coil that we have constructed to date and probes a volume between 800 pL and 30 nL for 50–300- $\mu\text{m}$ -i.d., 360- $\mu\text{m}$ -o.d. capillaries inserted through the sleeve.

Using the improved effective spatial resolution of the two-turn solenoid, temperature measurements were made in and around a chromatographic frit similar to those that are used in CEC to form the interface between the packed bed and the open-tubular section of the separation path. By using a sleeve probe, the position of the rf coil with respect to the frit can be easily manipulated. NMR spectra were collected for a series of applied voltages with the rf coil observe volume located before, within, and after the frit (Figure 2A). The recorded temperatures as a function of the average electrical power dissipated throughout the capillary are plotted in Figure 2B. Fits to linear least-squares regression are listed in Table 1 and indicate that the temperature was lowest prior to the frit, highest within the frit, and at an intermediate value following the frit.

The data can be explained by the fact that the frit acts as a region of increased resistance. Since the particles that constitute the frit occluded the total volume available for the conductive buffer, more power was dissipated in this region per unit length. Therefore, the temperature of the solution reached a maximum in the frit. The liquid began to cool as it exited the frit but was still warmer than before passing through this interface. Two sets of data are reported for the interfrit and postfrit measurements because the water signal was split into two peaks under these

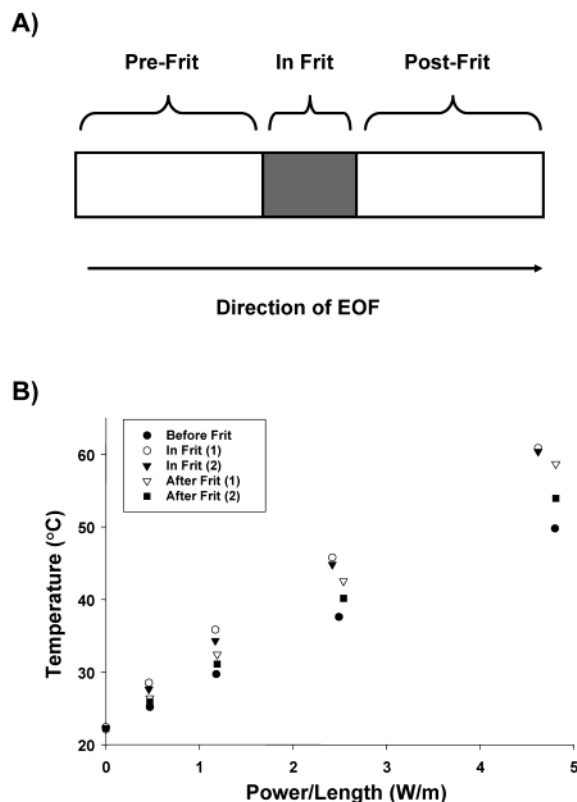


Figure 2. (A) Schematic of a chromatographic frit placed within a fused-silica capillary. (B) Plot of temperature in and around a chromatographic frit as a function of electrical power dissipated in a 100- $\mu\text{m}$ -i.d., 360- $\mu\text{m}$ -o.d. capillary.

Table 1. Linear Least-Squares Fits of Temperature Measured by CEC NMR Thermometry in and around a Chromatographic Frit

location	slope <sup>a</sup>	intercept	r <sup>2</sup>
before frit	5.7	22.6	0.998
in frit (1)	8.1	24.6	0.987
in frit (2)	8.1	23.8	0.993
after frit (1)	7.5	22.8	0.998
after frit (2)	6.6	22.8	0.998

<sup>a</sup> The regression is of the following form: temperature = (slope) × power/length + intercept, where temperature is in units of °C and power/length is in W/m.

conditions. Each peak was treated as a distinct source of data that originated from slightly different parts of the capillary. The temperature region probed by each end of the two-turn solenoid is ~100  $\mu\text{m}$ , demonstrating the ability to acquire temperature readings from spatially well-defined locations separated by 300  $\mu\text{m}$ . While the plots of the two measurements within the frit are nearly identical, the postfrit region shows a significant difference in the two measurement locations. This result can be explained by the fact that the solution is cooling very rapidly as it exits the frit but is relatively thermally homogeneous within the frit. One postfrit set of data likely corresponds to the end of the microcoil closer to the frit (and hence is warmer) than the other. The data lend credence to the theory of a thermal origin of bubble formation in CEC frits.

How precise and accurate is on-line NMR thermometry? The temperature accuracy of the measurement depends on the



accuracy of the calibration thermocouple in the NMR spectrometer (0.2 °C in this case) and has precision of better than 0.2 °C. Determinations of temperature from the difference in the frequencies of two signals would obviate the need for a thermocouple to give an absolute temperature rather than a temperature change. Using this technique, subsecond temporal resolution is easily obtainable, even with the two-turn solenoidal coil, yielding 400- $\mu$ m spatial resolution for accurate localized measurements of intracapillary temperature. If desired, each end of the solenoid can be used as a separate temperature monitoring position, providing the temperature from an  $\sim$ 100- $\mu$ m region separated by 300  $\mu$ m.

In CEC, electrophoretic mobility and solute partitioning are temperature-dependent processes, and so efforts have focused on the control of the temperature (exterior to the capillary) to optimize the separations. However, there has been a lack of a capability to actually monitor the temperature inside the capillary. Future work may include the measurement of temperatures within

the packed beds of CEC columns. Such studies would provide insight into the distinct performance characteristics of packing materials of different sizes, packing uniformity, and compositions. Pulsed-field gradient NMR has been used to probe the fluid dynamics in open capillaries and in packed CEC columns.<sup>19</sup> By combining pulsed-field gradient NMR with these spatially defined temperature measurements, even greater information on fluid flow in packed beds and through chromatographic frits is possible.

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