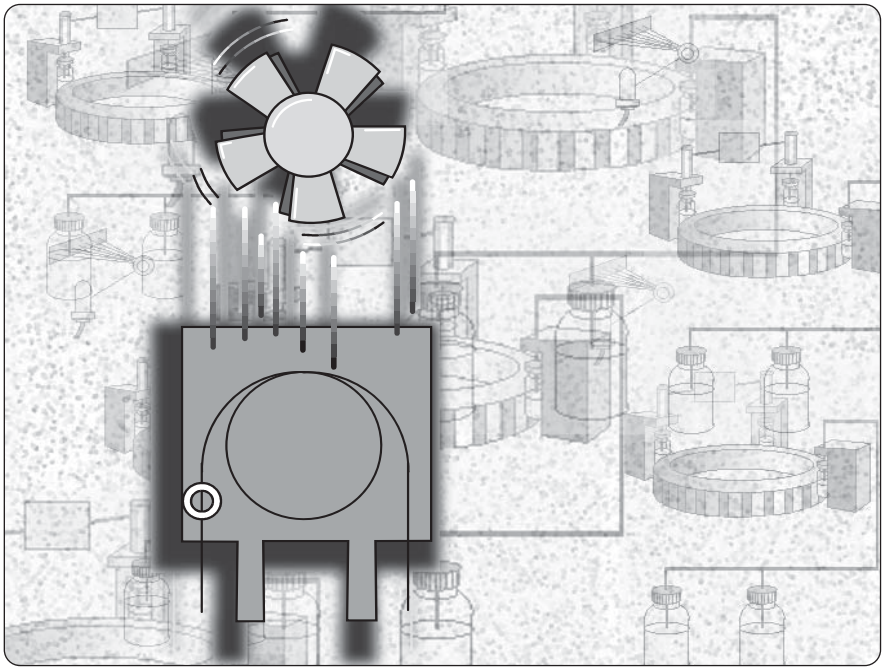
Capillary thermostatting in capillary electrophoresis

Technical Note



Introduction

Forced air cooling in the Agilent Capillary Electrophoresis system ensures:

- temperature consistency,
- reproducible migration times,
- column flexibility,
- no maintenance, and
- lower cost of ownership

without compromising the separation.

This Technical Note focuses on a variety of applications and demonstrates the reproducibility, ease of use, sensitivity, and resolution obtained using the Agilent CE system.

Capillary thermostatting is fundamental to CE instrumentation. The thermostatting system serves three distinct purposes. It

- ensures high reproducibility by isolating the capillary from changes in ambient temperature,
- removes Joule heat generated within the capillary, and
- provides a wide experimental and stable temperature range.

Agilent Technologies

Reasons for temperature control in CE

1. High reproducibility
Buffer viscosity and therefore migration time are highly dependent on capillary temperature. It has been reported that migration time varies 2 to 3 % per 1 °C temperature change.[1] Laboratory temperature fluctuations of 5 °C, for example, alter migration time 10 to 15 % in a non-thermostatted system.

Isolation of the capillary from changes in ambient temperature is therefore necessary to obtain high reproducibility. Since ambient temperature can increase or decrease, a system that can cool and heat to maintain constancy is beneficial compared with one that functions simply as an oven. The thermostatting system used in this study has this capability and maintains the set-point temperature to within ± 0.1 °C.

2. Control of Joule heating
In CE power and heat are generated upon the application of voltage (where power is defined by the product of voltage and current). Generation of heat within the capillary causes radial temperature gradients, with a higher temperature in the center of the capillary. These gradients can produce parabolic flow profiles, zone broadening, and loss of resolution.

Further, high temperature can cause degradation of thermally labile sample components. The effects of heating can be greatly limited by thermostatting the capillary to remove heat. Even with efficient thermostatting, however, it is much more beneficial to limit heat generation rather than remove it afterwards.

Heating can be reduced by the expedient choice of buffers and buffer concentration. Even more beneficial is the use of narrow internal diameter capillaries since highly conductive buffers and high electric fields can be used. Current generation in capillaries of 25 µm id, for example, will be four times lower than that in 50-µm id capillaries and 16 times lower than that in 100-µm id capillaries.

3. Wide range of temperature control
Active control of capillary temperature can be a useful parameter in optimizing separations. Further, lowering the temperature increases buffer viscosity and decreases current. This approach can be particularly useful in high current situations.

Finally, temperature extremes can be used to affect selectivity in separations based on interactions of analytes with buffer additives, such as for MEKC and chiral separations. With the Agilent CE system, temperature can be controlled from 10 °C below ambient to 60 °C.

Thermostatting systems

The overall design of the capillary electrophoresis system and the efficiency with which it can dissipate heat is as important as the temperature control mechanism itself. While thermostatting with liquid is theoretically more efficient than with air, the high-velocity, forced air thermostatting design used in the Agilent CE has been proven to be as efficient as liquid cooling.

In addition to excellent reproducibility, there are a number of other advantages with high-velocity forced air thermostatting:

- simplification of the capillary cartridge so that no gaskets or screws are required,
- quick capillary change,
- fast equilibration of set-point temperature (including temperature gradients during an analysis),
- no expensive, volatile cooling fluids, and
- no leakage of coolant fluids. The following sections demonstrate the efficiency and flexibility of the high-velocity forced air thermostatting in the Agilent CE system.

Experimental conditions

Capillary electrophoresis experiments were performed on an Agilent CE system with built-in diode-array detector and ChemStation software. The capillary thermostatting system employed high-velocity forced air, circulating at 10 m/sec. A Peltier device was used to control the temperature between 10 °C below ambient and 60 °C, with a precision of 0.1 °C.

High reproducibility

Heating within the capillary is often presented in the form of an Ohm's Law plot, which shows current as a function of voltage as demonstrated in figure 1. Excess heat is indicated by non-linearity of this plot. While sometimes used to "illustrate" the superior efficiency of one type of thermostatting system over another, in practice curvature of the Ohm's law plot can be irrelevant to the separation.

The real issues for the experimenter are whether the required resolution is obtained and if the electropherograms are reproducible under the conditions employed. In this section, examples of separations obtained under high power conditions will be presented to demonstrate the efficiency of the thermostatting used in the Agilent CE system.

High buffer concentrations are often used to limit solute-wall interactions, especially for peptides and proteins. When combined with wide-bore capillaries (75- to 100-µm id) for micropreparative use, high currents are generated and efficient heat removal is required to maintain high resolution and reproducible separations.

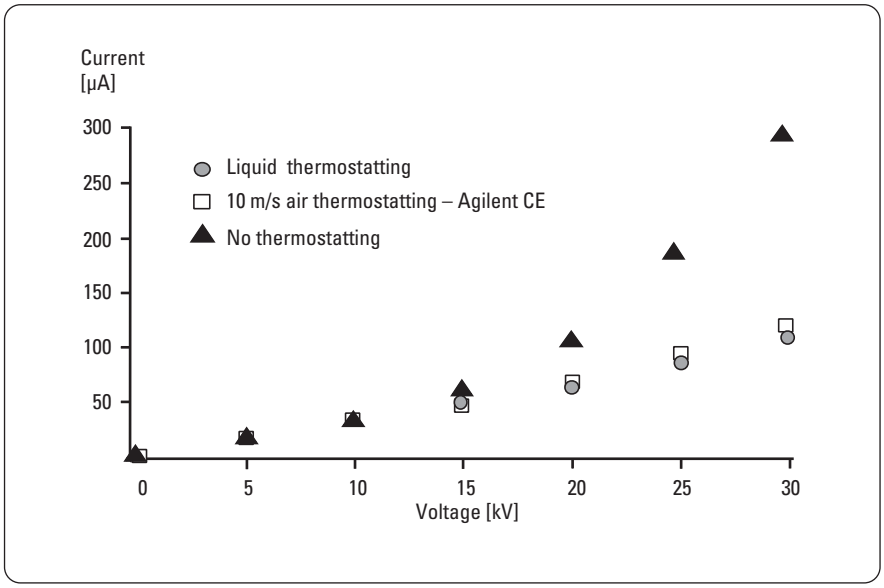


Figure 1
Ohm's plot demonstrating high-velocity forced air thermostatting.

Figure 2 shows the separation of a tryptic digest of human growth hormone (hGH) using 105 mM phosphate buffer in a 75-µm id capillary. Upon application of 25 kV, a current of 130 µA developed, yielding a power generation of 4.0 W/m. As can be seen in the figure, even under such excessive heating conditions excellent separation and reproducibility were obtained. In fact, the reproducibility was sufficient to obtain multiple fraction collections of each peptide peak for further analysis by sequencing and mass spectrometry.

Another example of the efficiency and stability of the thermostatting system is shown in figure 3 which illustrates the use of 100-µm id capillaries with a highly conductive phosphate buffer. While current generation in such wide bore capillaries is often excessive, their use is desirable since single-run fraction collection can often contain sufficient sample for further analysis. Again, excellent separation was obtained and each peak indicated in the electropherogram was collected and identified.

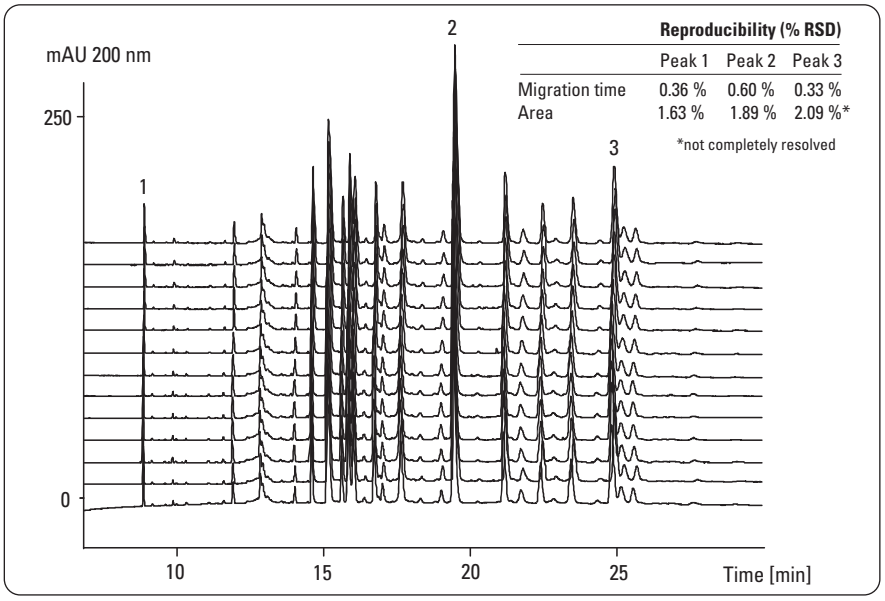


Figure 2
Demonstration of capillary thermostatting efficiency and system stability under high power conditions (Voltage=25 kV, Current=130 µA, Power=4.0 W/m).

Chromatographic conditions

Sample:	hGH tryptic digest (2 mg/mL)
Running buffer:	105 mM phosphate, pH 2.0
Effective/total length:	72/80.5 cm
Internal diameter:	75 µm BF 3
Injection:	150 mbar x s
Electric field:	310 V/cm
Current:	130 µA
Detection wavelength:	200/20 nm
Temperature:	35 °C

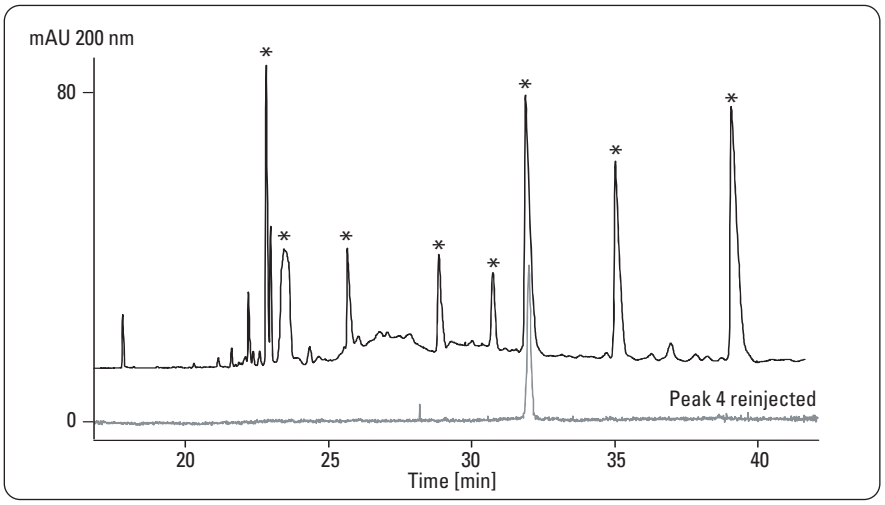


Figure 3
Use of 100 µm id capillaries for micro-preparative separation and sub-ambient temperature was used to limit heating. Peaks indicated (*) were successfully collected and sequenced.

Chromatographic conditions

Sample:	Tryptic digest of bacterial chaperonin GroES Protein (7 mg/mL)
Running buffer:	105 mM phosphate, pH 2.0
Effective/total length:	87.5/96 cm
Internal diameter:	100 µm
Injection:	150 mbar x s
Electric field:	Gradient to 250 V/cm
Current:	155 µA
Detection wavelength:	200/20 nm

Control of Joule heating

Applications where extremely high ionic strength buffers are used can present problems for any thermostatting system. By using a very narrow, 25-µm id capillary, the current generated can be restricted. The sensitivity of this narrow bore capillary can be improved five-fold over standard 25-µm id capillaries by using an extended light path capillary ("bubble cell") which has a 125 mm pathlength, bubble factor BF5. This combination of narrow bore capillary with extended detection light path is ideal when using extremely high buffer concentrations.

Figure 4 shows the use of 150 mM phosphate buffer with 200 mM ammonium sulfate, pH 7.0 to separate proteins while minimizing their interaction with the capillary wall. The rapid separation required the use of a high electric field (400V/cm). Under these extreme conditions, current generation was limited to 138 µA by use of 25-µm id capillaries and capillary thermostatting at 15 °C. These conditions could not be replicated with 50-µm or even 75-µm id capillaries since current generation would exceed 500 and 1200 µA respectively.

Figure 5 shows the use of 25 µm BF5 capillaries when using highly sulphonated cyclodextrins. These chiral selectors have associated high currents in solution, due to the high number of substituted sulphonic acid groups and the associated sodium counter-ions. The excellent control of joule heating is demonstrated by the Ohm's Law plot (V vs I) linearity. This conforms almost perfectly to theoretical expectations and the stability of thermostatting is further demonstrated by the reproducibility of the peak migration.

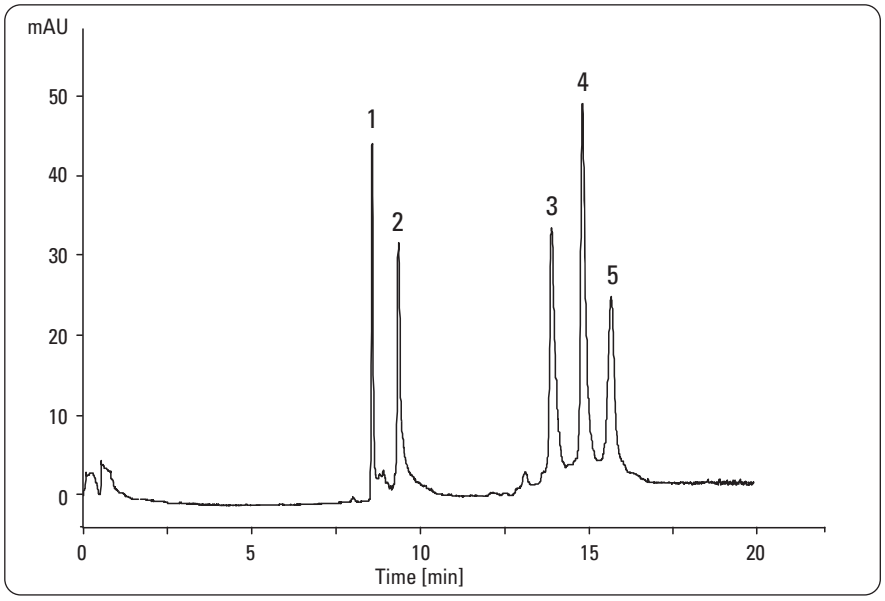


Figure 4
Use of 25 µm id capillaries to limit heating with highly conductive buffers.

Chromatographic conditions

Sample Protein mixture:	1 trypsin inhibitor, 2 bovine milk ß-lactoglobulin B, 3 bovine milk ß-lactoglobulin A, 4 horse skeletal muscle myoglobin 5 bovine pancreatic ribonuclease A, (approximately 1 mg/mL, each
Running buffer:	150 mM phosphate, 200 mM ammonium sulfate, pH 7.0
Effective/total length:	41/49.5 cm
Internal diameter:	25 µm BF 5
Injection:	250 mbar x s
Electric field:	400 V/cm
Current:	138 µA
Detection wavelength:	210/16 nm
Temperature:	15 °C

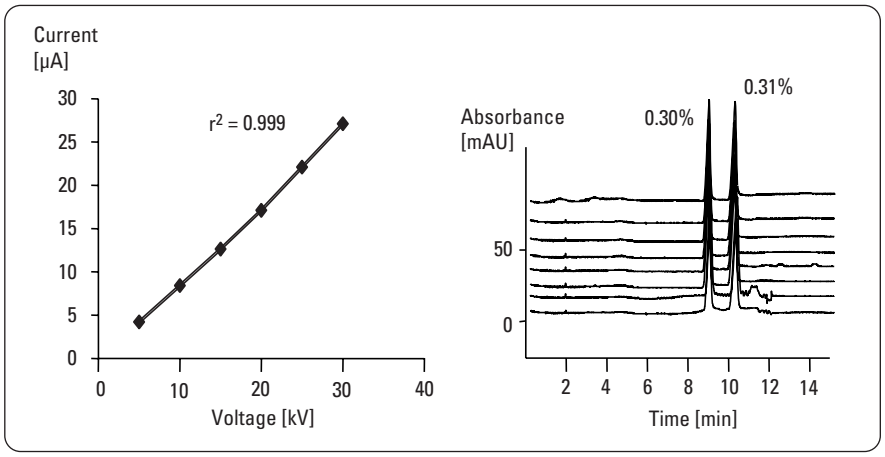


Figure 5
Ohm's law plot for highly sulphonated cyclodextrins, 25-µm id BF 5 capillary.

Chromatographic conditions

Buffer:	25 mM phosphate/TEA, pH 3.3, 5 % 9 W/v), heptakis-6 sulfato-cyclodextrin,
Effective/total length:	40/48.5 cm
Internal diameter:	25 µm BF 5
Injection:	3000 mbar x s
Voltage:	30 kV
Temperature:	20 °C

Wide range of temperature control

Decreasing the capillary temperature is another effective way of minimizing the effects of heating. Since buffer viscosity increases as temperature is reduced, current and therefore heating are also reduced. In addition, this increases the time the solutes remain in the capillary, having a similar benefit to increasing the capillary length while holding the electric field constant. The benefit of this approach is shown in the chiral separation in figure 6, where decreasing the temperature from 35 °C to 15 °C improved the separation. In the case of chiral separations, the lower temperature may also increase the difference in complexation of one of the enantiomers with the chiral selector.

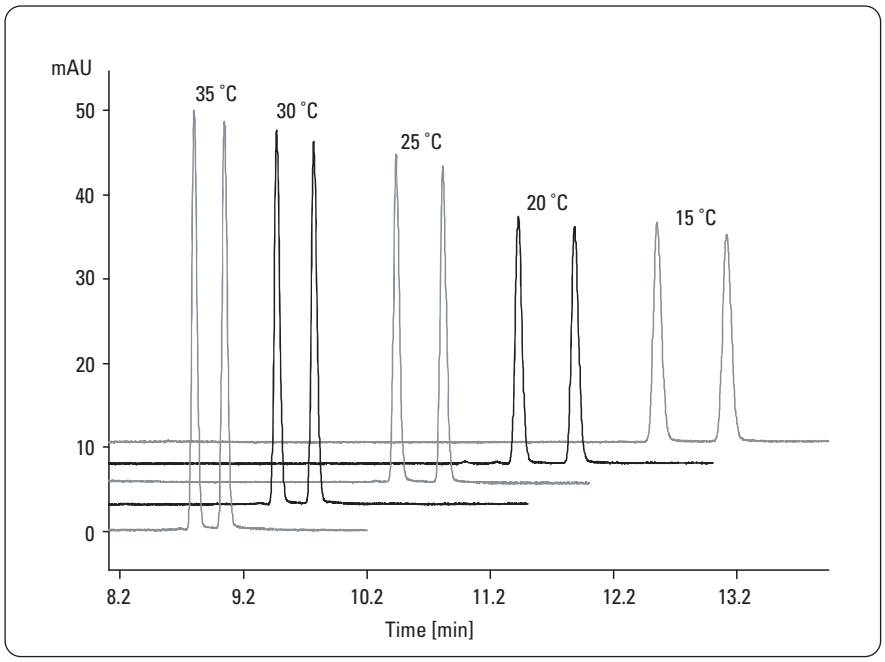


Figure 6
Optimization of capillary temperature for chiral analysis.

Chromatographic conditions

Sample:	Epinephrine (10 µg/mL)
Running buffer:	50 mM phosphate-Tris, pH 2.4, 20 mM dimethyl-bcyclodextrin
Effective/total length:	56/64.5 cm
Internal diameter:	50 µm
Injection:	100 mbar x s
Electric field:	465 V/cm
Detection wavelength:	214/10 nm

Conclusions

The efficiency, reproducibility and stability of the high velocity forced air thermostatting used in the Agilent CE system was demonstrated. High resolution and reproducible separations were shown using capillaries from 25-µm id to 75- and 100-µm id capillaries with highly conductive buffers. The benefit of sub-ambient capillary temperatures was also shown to improve chiral separations. Despite excellent thermostatting efficiency, it is still a well known fact that current and heat generation should always be minimized. The prudent selection of buffers and the use of narrow bore capillaries should always be the goal.

References

1.
S.Hjertén, "Zone Broadening in Electrophoresis with Special Reference to High Performance Electrophoresis in Capillaries: An Interplay Between Theory and Practice," *Electrophoresis 11, 665-690,* **1990.**

2.
R.Grimm, and M.Herold, "Micropreparative Single Run Fraction Collection of Peptides Separated by CZE for Protein Sequencing," *J. Cap. Elec., 1, 79-82,* **1994.**

3.
"Diode-array Detection in Capillary electrophoresis using Agilent Extended Light Path Capillaries", *Agilent Technologies Technical Note, Publication Number 5990-3410EN,* **2009.**

www.agilent.com/chem/ce

Published March 1, 2009
Publication Number 5990-3407EN

Agilent Technologies