Methods For Calculating the Internal Temperature of Capillary Columns During Capillary Electrophoresis

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METHODS FOR CALCULATING THE INTERNAL TEMPERATURE OF CAPILLARY COLUMNS DURING CAPILLARY ELECTROPHORESIS

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Abstract

The internal temperature, the buffer viscosity and the efficiency of heat removal from a silica capillary can be calculated by measuring the differential electroosmostic mobility at a low voltage and a high voltage. The calculated temperature is plotted vs the power generated by the electrophoretic instrument and yields a linear relationship when the power is below 3.0 W. The temperature can also be calculated using the conductivity of the solution. The two methods provide the same temperature, which compares well with literature values. A rule of thumb for a quick calculation of internal temperatures is that a power of 0.1 W

increases the internal temperature by 1.1 C° for natural convection cooling and 0.6 C° for forced air cooling. The efficiency of heat removal, indicated by the slope of the line, is dictated by the heat transfer between the outer wall of the column and the surrounding environment, and not by the inside diameter of the column. The proper choice of buffer and the counter cation will lead to a lower internal temperature. The type of cation used in the buffer will influence the electroosmotic mobility; greater mobilities are observed in sodium buffers compared to potassium buffers.

METHODS FOR CALCULATING THE INTERNAL TEMPERATURE OF CAPILLARY COLUMNS DURING CAPILLARY ELECTROPHORESIS

In electrophoresis, the migration time is inversely proportional to the applied voltage; a higher voltage will provide a shorter analysis time. A high voltage placed across a conductive medium, however, generates heat which can cause perturbations in the sample zone during the separation process. Thus for most electrophoresis, the applied voltage has to be kept low (100-300 V). In 1981, Jorgenson was able to perform zone electrophoresis in a thin walled, fused silica capillary column using a very high applied voltage (~30 kV) because the columns were effective at dissipating the generated heat.[1] Since then, there have been many studies, both experimental and theoretical, to determine the effects that the generation of heat in the capillary columns has on the electrophoretic separation process.[2-11]

An understanding of the effect of Joule heating in the capillary column is important. Joule heating causes loss of resolution because the sample zones are broadened due to increased thermal diffusion. Boiling of the support solution can occur when the voltage is increased beyond the point of efficient heat removal. Finally, because of the high ionic strength solutions used in biological separations, the large amount of heat generated by Joule heating can cause the biological material to denature or otherwise lose its biological activity.

In order to control Joule heating and its undesirable effects, it is necessary to know the temperature inside the capillary column. As a first approximation Terabe measured the outside temperature of the column with a micro thermocouple and found the temperature to be 70 C° at 25 kV for a 200 μm I.D. column.[2]. Incomplete contact with the column due to size and curvature of the column, however, made exact measurements difficult. Nelson *etal.* derived the internal temperature of the column based on the radius of the column, the thermal properties of the column and the support solution, and from inferred thermal transfer coefficients from Ohm's law plots.[11] In addition, they also used the thermal transfer coefficients to show the efficiency of heat removal for a natural convection cooled system, a forced-air cooled system, and a solid-state thermoelectric cooled system.

We propose two simple empirical methods for calculating the temperature inside the column and showing the efficiency of heat removal of a capillary column. One method uses the measurement of

the electroosmotic mobility at different voltages and the second method uses the measurement of the conductivity of the support buffer at different voltages. When the calculated temperature of the column is plotted vs. the power generated by the electrophoresis instrument, a linear relationship is obtained. Therefore, one can predict the temperature inside the capillary column from the measured power of the CE instrument. These methods are simple and extend over wide linear dynamic ranges of temperatures.

EXPERIMENTAL

The CE instrument was an in-house constructed instrument similar to that described by Jorgenson[1]. A cooling fan was added for the efficiency study. The capillary columns were fused silica (PolyMicro, AZ) with inside diameters of 50 μm, 75 μm, and 100 μm, and an outside diameter of 365 μm. The columns were one meter long. The detector window was located 75 cm from the injection end. Injection was done by gravity at 15 cm above the reservoir for 10 sec.

Voltage was adjusted with a -30 kV power supply (Glassman, NJ) and the output voltage was measured by monitoring a 10 V controller used to select the operating voltages. Current was measured across a 10 kΩ resistor in series with the grounded end of the system.

The studies were done using reagent grade potassium phosphate and sodium phosphate buffer at pH 8.30 and 7.00 (1.0 M Na_2HPO_4, NaH_2PO_4, K_2HPO_4, and KH_2PO_4 (Aldrich, WI)). Concentrations

Table I.   Currents measured for different buffers and counter ions..

Buffer	Counter ion	Concentration of counter ion (mM)	Voltage (kV)	Current (µA
MES	Li	40	25	11.5
	Na	40	25	14.5
	K	40	25	16.3
	Rb	40	25	19.8
	histidine	100	30	10.4
Phosphate	Na	50	30	50.0
	K	50	30	76.7
CAPSO	Na	50	30	15.0
Citrate	Na	20	30	15.0
Tricine	K	10	30	9.3

ranged from 200 mM to 12.5 mM.   The neutral marker was acetone
(Aldrich, WI) detected at 265 nm.

The buffers in Table I were prepared as follows:
The 2-N-(morpholino)ethanesulfonic acid (MES) buffer (Sigma, MO)
was adjusted to pH 6.05 using any of the following bases: histidine
(Sigma, MO); lithium hydroxide (Alfa, MA); sodium hydroxide (Baker,
PA); potassium hydroxide (Baker, PA); rubidium hydroxide (Alfa,
MA).   RbOH dilutions were prepared under an argon atmosphere.
The 3-(cyclohexylamino)-2-hydroxy-1-propane-sulfonic acid
(CAPSO) buffer (Sigma, MO) was brought to a pH of 9.55 with NaOH.
The sodium citrate buffer (Applied BioSystems, CA) was purchased at
a pH of 2.50.   Tricine (Sigma, MO) was brought to a pH of 8.2 with
KOH, after 20 mM KCl was added.

Nordoxepin (Sigma, MO) was prepared in a 50% water-methanol solution (1 mM) and then diluted 10-fold with the sodium phosphate buffer. The compound was detected at 210 nm with methanol (Aldrich, WI) in the sample serving as the neutral marker.

The water used for the buffers and dilutions was HPLC grade distilled water (Aldrich, WI).

RESULTS AND DISCUSION

Electroosmotic mobility method

The effect of Joule heating on the electroosmotic mobility, μ_{eo} is shown by plotting μ_{eo} verses voltage for different buffer concentrations (Fig. 1). A definite increase in μ_{eo} is seen at higher voltages and the effect is greater at higher buffer concentrations. Eq. 1 relates μ_{eo} in the column to the viscosity of the support buffer (η).

$$\mu_{eo} = \frac{\varepsilon \zeta}{4 \pi \eta} \qquad \text{Eq. 1}$$

where ε is the dielectric constant, ζ is the zeta potential. Under ideal conditions, μ_{eo} is constant with voltage; however, heating will cause the viscosity to decrease and result in an increase in μ_{eo}.

To ascertain whether the increase in μ_{eo} is, in fact, related to the viscosity, the electrophoretic mobility, μ_{ep}, of a charged molecule, nordoxepin, is monitored as the voltage is increased (Fig. 2). If the viscosity is changing due to heating, μ_{ep} of nordoxepin will also be changed. The electrophoretic mobility of a molecule is described by the following equation:

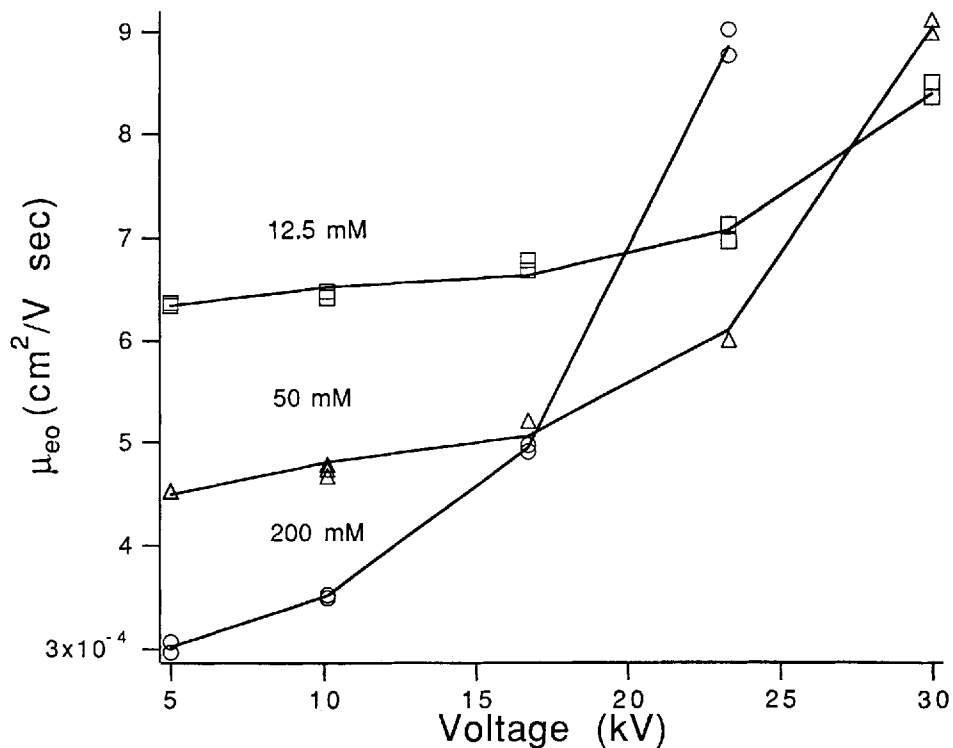$$\mu_{ep} = \frac{q}{6 \pi a \eta} \qquad \text{Eq. 2}$$

Fig. 1   Electroosmotic flow vs. voltage.   The electroosmotic mobility is measured using acetone as a neutral maker at 265 nm. The column is a 75 μm I.D., 100 cm long with the detector window at 75 cm.   The buffer is sodium phosphate at a pH of 8.3.   Injection is gravity at 15 cm for 10 sec.   Squares are 12.5 mM, triangles are 50 mM, and circles are 200 mM buffer concentrations
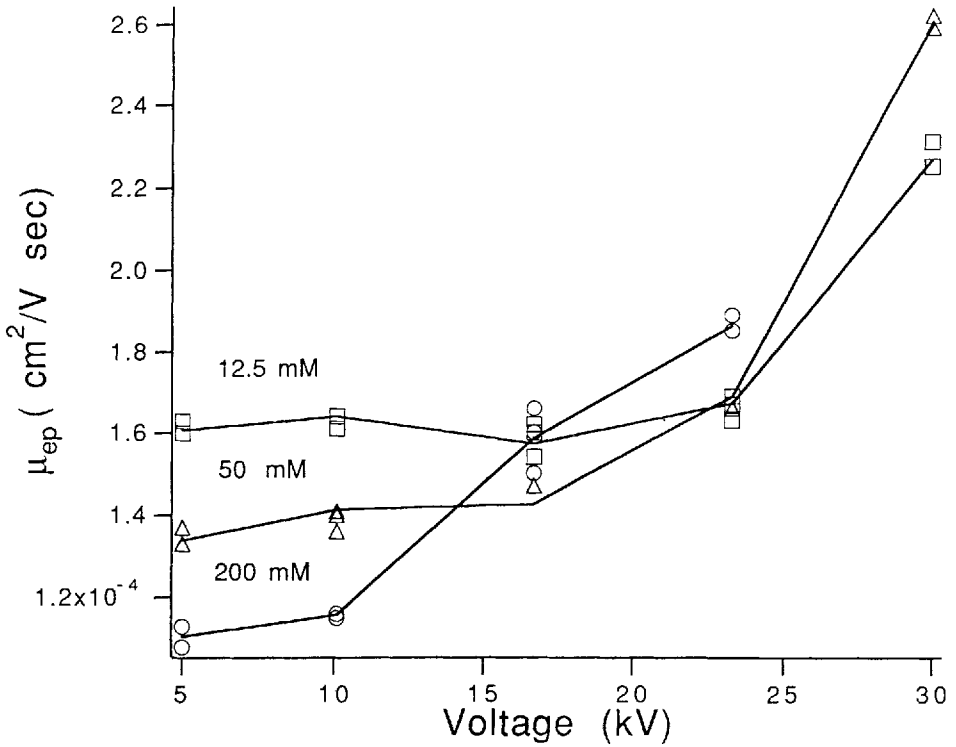
Fig. 2   The ion mobility of nordoxepin vs. voltage.   The
wavelength of detection is 210 nm.   Methanol is the neutral marker.
The mobility is measured using the same conditions as Fig. 1.

where **q** is the charge on the molecule and **a** is the effective radius.
As indicated in Fig. 2, μ_{ep} of nordoxepin exhibits the same increase
in mobility at higher voltages as μ_{eo}.

The temperature is related to viscosity by,[12]

$$\frac{1}{\eta} = A e^{-\frac{B}{T}}$$ Eq. 3

thus, the ratio of electroosmotic mobilities is related to temperature as follows:

$$\frac{\mu_{eo1}}{\mu_{eo2}} = e^{-\left(\frac{B}{T_1} - \frac{B}{T_2}\right)}$$

Eq. 4

where subscript 1 refers to a measurement at a low voltage (5 kV), subscript 2 denotes measurements at a higher voltage (>5 kV), and B is a constant. It is assumed that ε and ζ are constant because measurements are made at constant pH and buffer concentration.

To determine B, a plot of Eq. 3, using literature values[13] for water ranging from 293 $^\circ$K to 353 $^\circ$K, is shown in Fig. 3. From the slope of the line, a value of 1820 $^\circ$K is obtained for B. This value of B is different than the 2400 $^\circ$K reported in the CE literature[6,10]. Assuming T_1 is ambient temperature (298 $^\circ$K), the temperature in the capillary column can be calculated by rearranging Eq. 4.

$$T_2 = \frac{1820\ ^\circ K}{\ln(\mu_{eo1}) - \ln(\mu_{eo2}) + 6.11}$$

Eq. 5

Therefore, one can use Eq. 5 to calculate the temperature inside the capillary column by measuring μ_{eo} at a low voltage where one assumes there is no heating and then at the high voltage where one wishes to know the temperature.
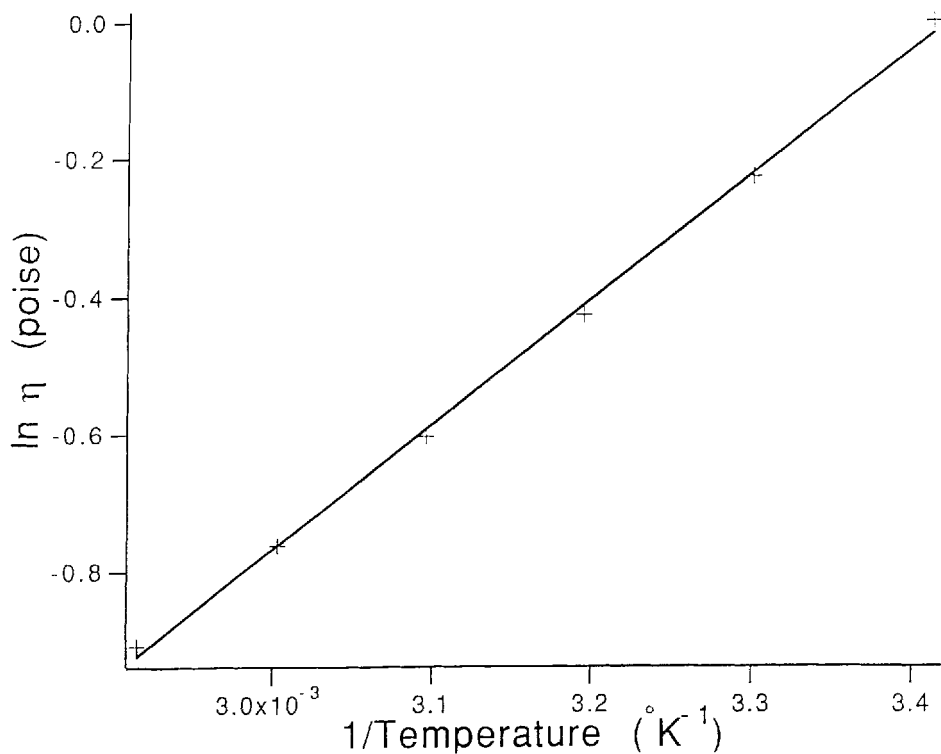
Fig. 3   A plot of the viscosity of water as a function of
temperature using literatures values.

A plot of the temperature calculated using from Eq. 5 versus
the power of the system measured at different buffer concentrations
is shown in Fig. 4.   A linear relationship is observed for powers less
than 3.0 W for all concentrations.   Below 3.0 W, one can calculate an
internal temperature from the power generated by the system using
the following equation which is a linear fit of the data in Fig. 4.

$$T = 11.5(\text{Power (W)}) + 297.9 \, ^{\circ}K$$ Eq. 6
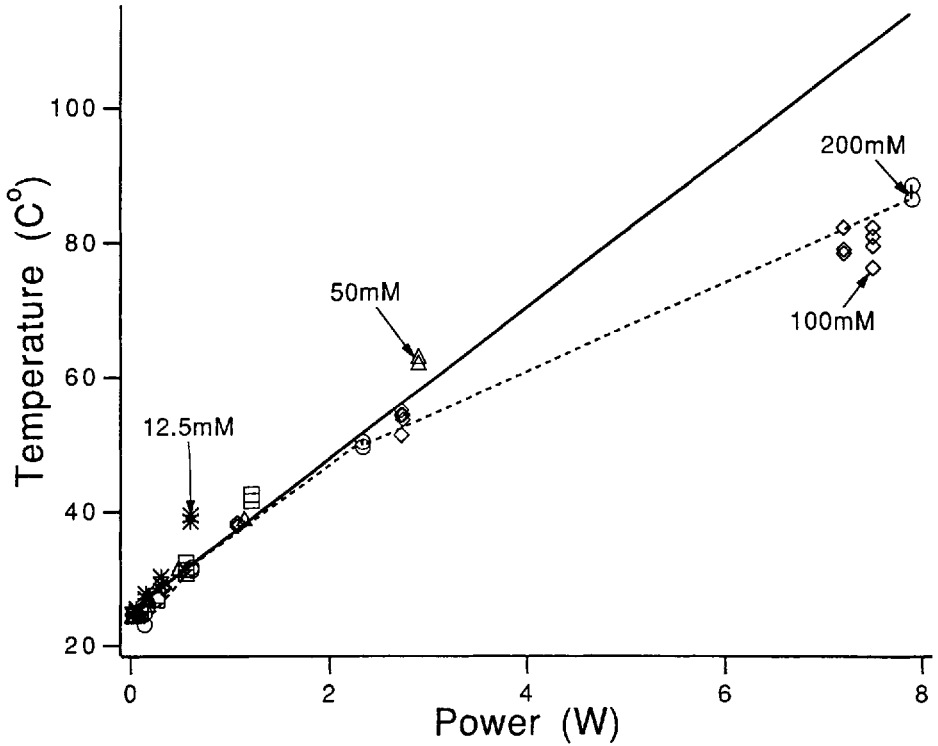
Fig. 4   Calculated internal temperatures of a 75 μm I.D. column
vs. power used by the CE instrument.   Column is cooled by natural
convection.   The solid line is the linear regression of data less than
100 mM buffer.   Stars are 12.5mM, squares are 25mM, triangles are
50mM, diamonds are 100mM, and circles are 200mM buffer
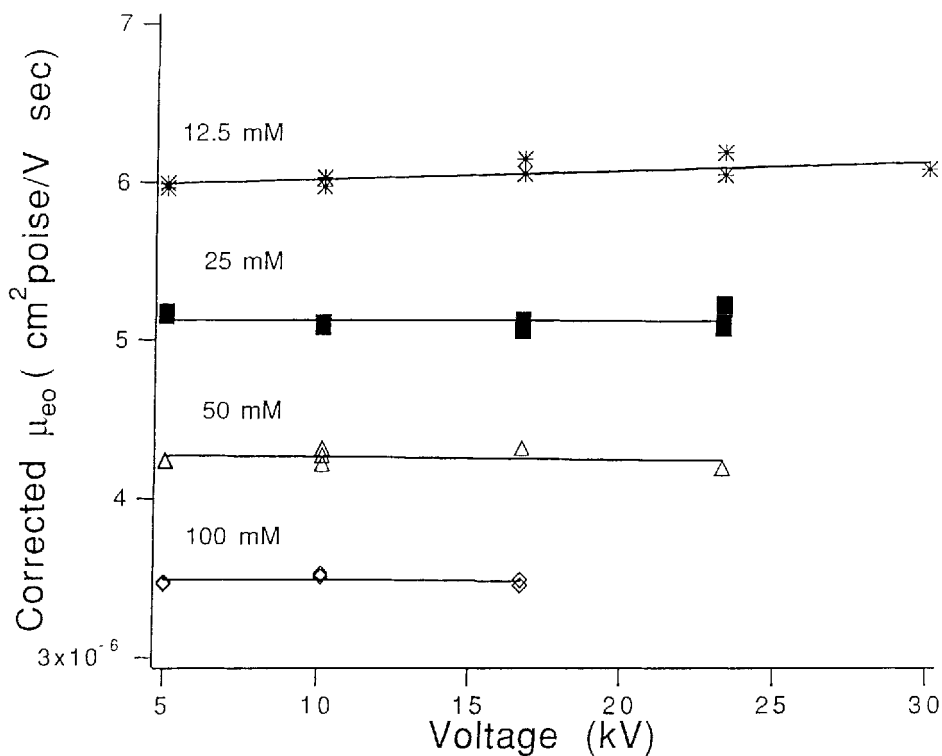concentrations.

Fig. 5   The corrected electroosmotic mobility vs. voltage.   The viscosity is calculated using temperatures from Fig. 4.

This equation is valid for a natural convection cooled column with an outside diameter of 365 μm.   It should be emphasized that the temperature does not vary greatly from ambient temperature until power levels reach above 0.3 W.

Since μ_{eo} is a function of viscosity which in turn is a function of temperature, a value of μ_{eo} measured at high power levels can now be corrected by the calculated viscosity.   The viscosity is determined from Eq. 3 using the temperature obtained from Eq. 6.   The

corrected μ_{eo} is now independent of voltage in the regions where the temperature is linear with power as seen in Fig. 5.

Deviations from the calculated line which occur at higher power levels in Fig. 4 might be due to a variation of the dielectric constant and the zeta potential with temperature and their product in Eq. 1 are not essentially independent of temperature [15]. Another possible explanation is that the viscosity and the dielectric constant in the double layer change at high electric fields.[16]

We are interested in the response of the system to cooling with a fan. The temperatures calculated for a column cooled by natural convection and forced air are shown in Fig. 6. The slope of the line is a measure of the effective heat removal of the column and the intercept is the ambient temperature of the surrounding environment. The slopes and intercept are in excellent agreement with values calculated from Nelson's data.[11] A rule of thumb for a quick calculation of internal temperatures is that an increase in power of 0.1 W increases the internal temperature by 1.1 C° for natural convection cooling and 0.6 C° for forced air cooling.

Conductivity method

The internal temperature can also be calculated using the measured conductivity of the solution. The conductivity of the solution, σ, is calculated from the measured current, I, at a given voltage, V.

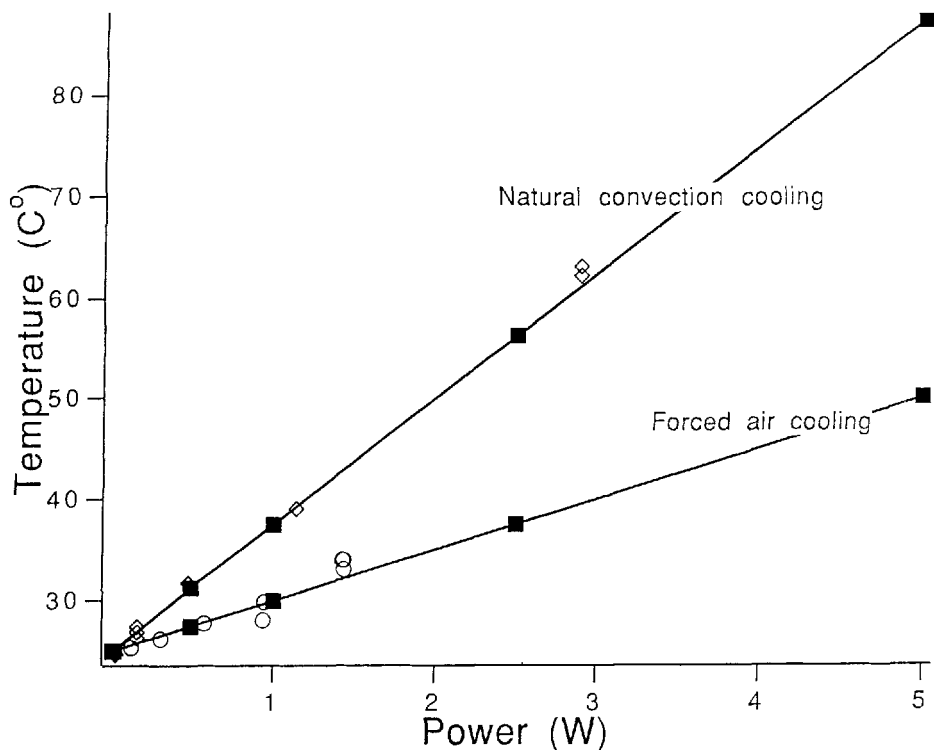$$\sigma = \frac{IA}{V\ell}$$

Eq. 7

Fig. 6   A plot of the internal temperaures as a function of cooling
techniques.   The slope of the lines are the efficiency of heat removal
The dark squares are data for a 100 μm I.D. column from Nelson's
work in reference 11.

where A is the cross-section of the column, and ℓ is the length of the
column.   Conductivity is inversely proportional to the resistivity of
the solution.[14]   An increase in the viscosity will yield a decrease in
the velocity of the conducting ions; thus, the resistance of the
solution increases.   Therefore the conductivity of the solution is

directly related to the viscosity of the solution. Rearranging Eq. 3 reveals the change in temperature as a function of viscosity.

$$\frac{\Delta \eta}{\eta} = \frac{1820}{298^2} \Delta T \qquad\qquad \text{Eq. 8}$$

From Eq. 8 it is determined that viscosity and thus conductivity changes by 2.05% per degree which is in agreement with other work.[6,7,14]

The internal temperature of the column can be then calculated from the following equation.[14]

$$T_2 = \left(\frac{\dfrac{\sigma_2}{\sigma_1} - 1}{0.0205} \right) + 25\,^{\circ}C \qquad\qquad \text{Eq. 9}$$

Again, subscript 1 is the conductivity of the solution at a low voltage (5 kV) and subscript 2 is the conductivity of the solution at a higher voltage (>5 kV). The assumption is that at low voltages the internal temperature of the column is at room temperature (25°C). A plot of the calculated temperature versus power using both conductivity and the electroosmotic mobility methods is shown in Fig. 7. As seen the two methods are in good agreement.
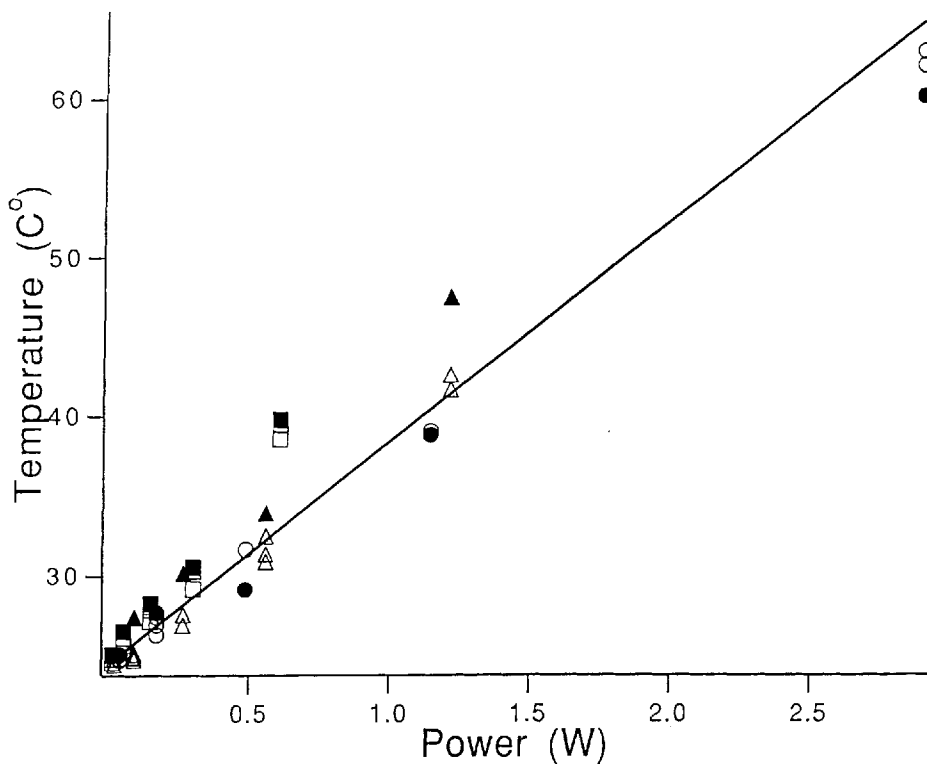
Fig 7.   A comparison between temperatures calculated using the electroosmotic method and the conductivity method.   The dark symbols are the conductivity calculations.   The data is for a natural convection cooled column.   The circles are 50 mM, the triangles are 25 mM, and the squares are 12.5 mM buffer concentrations.   The run conditions are the same as in Fig. 1.

Efficiency Study

A trend in CE research is toward the utilization of smaller I.D. columns. An advantage is that at a given voltage the current is lower; thus, less heat is generated. A drawback, however, is a degradation in detection limits resulting from the use of the smaller I.D. columns.

We are interested in determining if enhanced cooling occurs because of a smaller I.D. column. Calculation of the internal temperature using the electroosmotic mobility method at several different powers for 100 μm, 75 μm, and 50 μm I.D. capillary columns is shown in Fig. 8. The internal temperatures of all three columns lie on the same line; thus, the efficiency of heat removal is unchanged. In other words, the 50 μm column is equivalently efficient at removing heat as the 100 μm column. The heat transfer between the outside wall and the surrounding environment appears to be the dominant factor for heat removal.

Another way of controlling the Joule heating in the column is to use different cations with the buffer. It can lead to a reduction in the observed currents; the use of potassium ions generates more power at a fixed voltage than sodium ions.[17] A short list of buffers with different counterions and the currents they generate is given in Table I.

From Eq. 5 and Eq. 6 it can be seen that a relationship should exist between μ_{eo} and the power of the system. Fig. 9 is a plot of μ_{eo} versus power. Note that the potassium data are offset from the sodium data. From Fig. 9 and Table I, the use of potassium ions
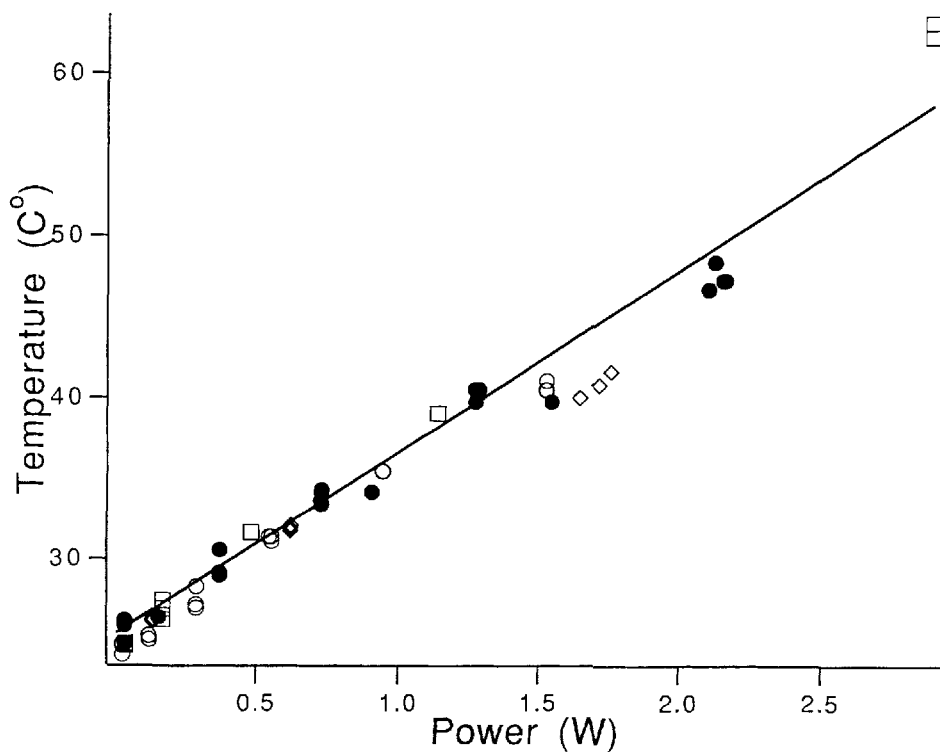
Fig. 8.   A comparison between temperatures calculated for
different internal diameters; 100, 75, and 50 μm for sodium and
potassium phosphate buffer.   The diamonds are 100 μm I.D., the
squares are 75 μm I.D., and the circles are 50 μm I.D. columns.   The
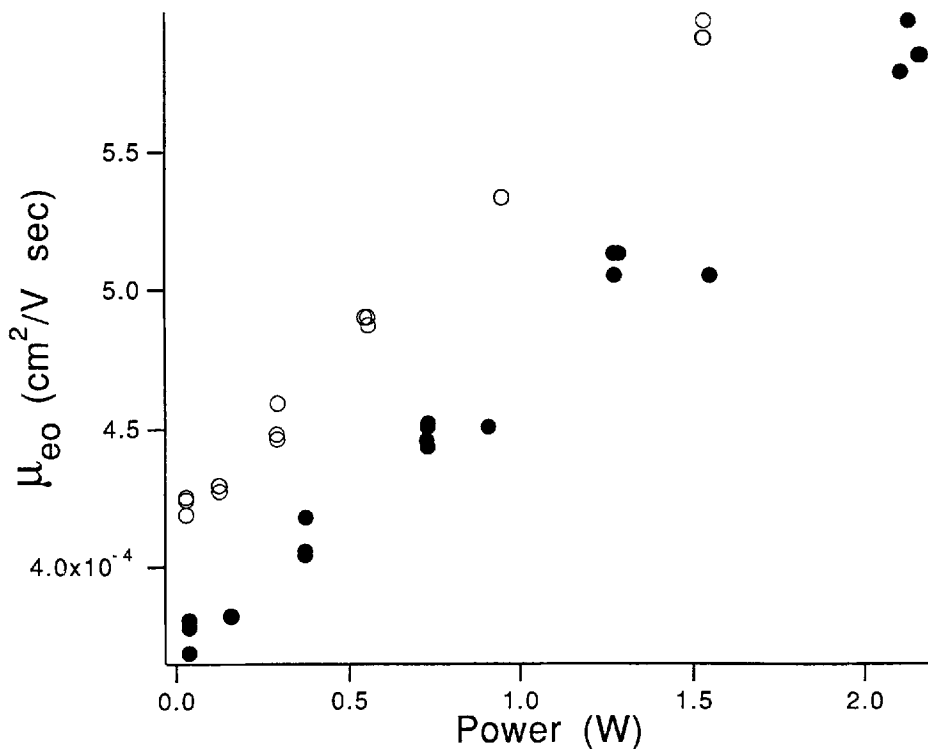dark symbols are potassium data.

Fig. 9.   Plot of μ_{eo} vs power for sodium and potassium counter
ions.   The dark symbols are the potassium data.   The inside diameter
of the column is $50 \, \mu m$ The run conditions are the same as in Fig. 1.

gives a slower μ_{eo} but generates more current than the sodium ion.
Thus the offset is not due to a thermal effect because the viscosity
will decrease, therefore the μ_{eo} will decrease, with the higher
currents.   The offset may be an indication that the size and/or type
of counter ion is influencing the zeta potential on the wall of the
column.   Investigation into this area is ongoing.

CONCLUSION

The internal temperature, the buffer viscosity and the efficiency of heat removal from a silica capillary can be calculated by measuring the electroosmostic mobility at a low voltage and then at a higher voltage. The calculated temperature is plotted vs the power consumed by the electrophoretic instrument and yields a linear relationship when the power is below 3.0 W. The temperature can also be calculated by measuring the current used by the system and calculating the conductivity of the solution. The two methods provide the same temperatures, which compare well with literature values. A rule of thumb for a quick calculation of internal temperatures is that a power of 0.1 W increases the internal temperature by 1.1 C° for natural convection cooling and 0.6 C° for forced air cooling. The efficiency of heat removal, reflected in the slope of the line, is dictated by the heat transfer between the outer wall of the column and the surrounding environment and not by the inside diameter of the column. The proper choice of buffer and the countercation will lead to a lower internal temperature. It may be possible to avoid the loss of detection limits in going from a 75 μm column to a 50 μm column by a choice of buffer and counterion, which consumes less powers in a 75 μm column. The type of cation used in the buffer will influence the electroosmotic mobility, with greater mobilities observed in NaOH buffers compared to KOII buffers.

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