# Letters to *Analytical Chemistry*

# Heat-Associated Field Distortion in Electro-Migration Techniques

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**Electro-migration techniques, such as electrophoresis, are widely utilized in analytical sciences. If a single electrolyte is used, the field strength is typically assumed to be well-defined. Heat-associated field distortion (HAFD) has been suggested as a result of the nonuniform heat dissipation throughout the electrolyte; however, it has never been experimentally studied. Here, we experimentally demonstrated HAFD for the first time. We used capillary electrophoresis (CE) with a capillary having parts with different heat dissipation efficiencies. Our experiments showed a difference in field strength of approximately 1.5 times between the different parts of the capillary for a typical CE electrolyte. This result suggests that HAFD is a well pronounced phenomenon that can be a potential source of errors and instabilities in electro-migration experiments.**

Electro-migration techniques, in which charged particles (e.g., molecules, organelles, cells, etc.) are moved by an electric field in an electrolyte, are widely used in analytical sciences.[1] Electrophoresis, which can be run in different formats, is the most prominent example of such techniques.[2] The velocities of particles in electro-migration are dependent on their charge, size, and the electric field strength. Thus, any uncertainty or instability in the field strength can translate into uncertainty and instability of the quantitative results obtained from electro-migration experiments. The electrical field strength, $E$, is accurately determined from the voltage, $V$, applied to the electrolyte of length $L$: $E = V/L$. Voltage is a parameter that can be kept very stable, and because of this, the field strength is typically assumed to be stable and well-defined for a single electrolyte.[3,4]

This work was motivated by understanding, recently described by Xuan and Li,[5] that the assumption about a well-defined and stable electric field does not hold if the efficiency of heat removal is not uniform throughout the electrolyte. Our work differs from that of Xuan and Li, in that instead of using assumed values of
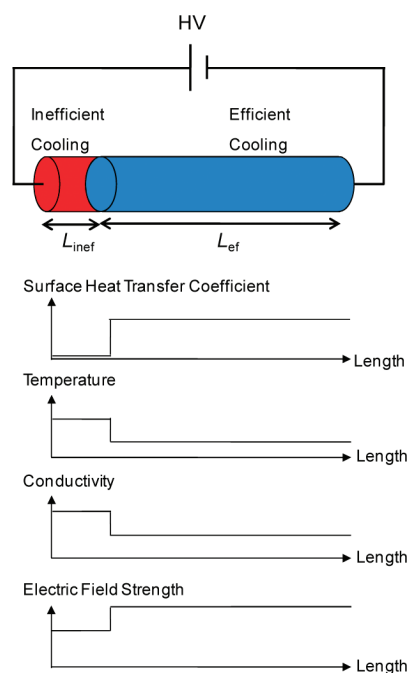


**Figure 1.** Schematic diagram depicting a one-dimensional electro-migration medium (electrolyte) to which a high voltage is applied. Differences in the efficiency of heat dissipation in different regions of the medium lead to differences in the temperature, electric conductivity, and electric field strength experienced in these regions once thermal equilibrium is achieved.

the surface heat transfer coefficient to calculate the change of temperature in each section of the capillary, we determine them experimentally indirectly from the voltage applied to the capillary and the current it causes. We introduce a term of heat-associated field distortion (HAFD) to describe the nonuniformity of the electric field caused by the nonuniformity of heat dissipation. (It is important to emphasize that nonuniform heat dissipation along the electrolyte surface is not an abstraction but a reality of electro-migration techniques.[3]) Figure 1 uses a one-dimensional electro-migration medium to illustrate the following straightforward logic that calls for the existence of HAFD. A voltage, $V$, applied to an electrolyte creates an electric current, $I$, and inevitably generates heat at a rate, $P = VI$. Heat is dissipated radially through the electrolyte and the surrounding walls. The efficiency of heat

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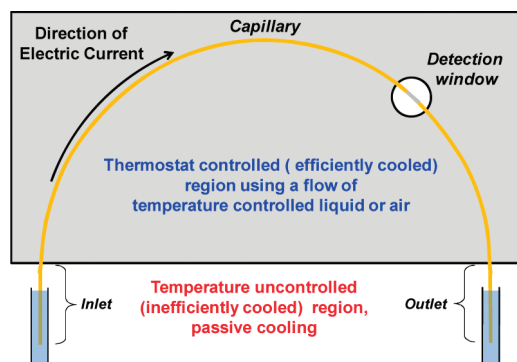
**Figure 2.** Schematic representation of a capillary cartridge assembly of a standard commercial CE instrument. The major part of the capillary is efficiently cooled by a thermostat-controlled flow of air or liquid. The inlet and outlet regions of the capillary and the detection window are either inserted in the running buffer vials, hanging in the air, or in contact with the rubber or plastic instrument interface; these regions are not subjected to any form of temperature control.

dissipation depends on the temperatures and thermal conductivities of the electrolyte and its surroundings.[4] It is characterized by a parameter called the surface heat-transfer coefficient, $h_S$.[2,3] Unless the heat transfer coefficient is uniform throughout the electrolyte, the temperatures of the electrolyte will be nonuniform. The electrolyte's electrical conductivity, $\kappa$, is a function of its temperature and, thus, will also be nonuniform.[5,6] Finally, the electric field strength, $E$, which depends on the electric current and the electrolyte's electrical conductivity, will also be nonuniform, concluding the chain of dependencies that should lead to HAFD. The above logic is not counterintuitive; therefore, it is rather surprising that HAFD has not been previously experimentally quantified.

Here we tested HAFD experimentally using capillary electrophoresis (CE) as an example electro-migration technique. To make the CE example fully practical, we used a commercial CE instrument, Beckman Coulter MDQ P/ACE, (Beckman Coulter, Oakville, Canada) and a typical set of CE conditions. Most of the capillary was "efficiently" cooled by contact with a liquid heat exchanger, but three short segments, the inlet, outlet, and detection window, were not in contact with the heat exchanger and were thus cooled "inefficiently" (Figure 2). Experimentally testing HAFD is challenging as there are no handy electric field probes that could help us to accurately determine field strengths in different parts of the capillary. To resolve this challenge, we invented a method that determines the field strengths in efficiently- and inefficiently cooled parts of the capillary based on measurements of the voltage applied to the capillary and the electric current it causes (these parameters can be measured very accurately). By applying this method, we showed that the difference in field strengths between the efficiently- and inefficiently cooled parts of the electrolyte grows with the voltage applied and can reach approximately 1.5 times for a typical CE electrolyte. Thus, HAFD is a significant phenomenon, at least in CE. HAFD in chips may be even greater dues to higher field strengths and poorer heat removal. Our results call for a deeper study of HAFD and its practical implication in electro-migration techniques.

## RESULTS AND DISCUSSION

We first explain the essence of our method for the determination of field strengths in the efficiently- and inefficiently cooled parts of the capillary, $E_{ef}$ and $E_{inef}$. Subscripts "ef" and "inef" refer to efficiently- and inefficiently cooled parts of the electrolyte, respectively. The total heating power produced in the electrolyte, $P$, is comprised of heating powers produced in the efficiently- and inefficiently cooled parts of the capillary. If the these heating powers are known, the total heating power can be calculated:

$$P_{calculated} = P_{eff} + P_{inef} \quad (1)$$

The total power can be accurately determined through measuring the voltage and current directly:

$$P_{measured} = VI \quad (2)$$

The values of $P_{eff}$ and $P_{inef}$ cannot be determined directly through measured parameters; however, they can be expressed through electric field strengths, $E$, electrolyte lengths, $L$, and the electric current, $I$:

$$P_{ef} = E_{ef}L_{ef}I$$
$$P_{inef} = E_{inef}L_{inef}I \quad (3)$$

Using eqs 1 and 3, we can write an equation that links the total power with the field strengths in the two electrolyte parts:

$$P_{calculated} = E_{ef}L_{ef}I + E_{inef}L_{inef}I \quad (4)$$

The electric field strengths in each electrolyte part cannot be determined directly either but depend on the electrical conductivities of the electrolytes in both parts.

$$E_{ef} = \frac{\kappa_{inef}V}{\kappa_{inef}L_{ef} + \kappa_{ef}L_{inef}}$$
$$E_{inef} = \frac{\kappa_{ef}V}{\kappa_{inef}L_{ef} + \kappa_{ef}L_{inef}} \quad (5)$$

Equation 5 also contains two unknown parameters, namely, the electrical conductivities which depend linearly on the temperature within each part of the electrolyte:

$$\kappa = \kappa_0(1 + \alpha\Delta T) \quad (6)$$

where $\kappa_0$ is the electrical conductivity at the ambient temperature, $\alpha$ is the temperature coefficient of electrical conductivity, and $\Delta T$ is the increase in temperature of the electrolyte relative to the ambient temperature.[7] Both $\kappa_0$ and $\alpha$ can be determined experimentally from voltage and current data collected directly from the instrument using the method of Hruska et al.[8] $\Delta T$ in each section can be determined using the well-established theory of heat dissipation from electrically heated cylindrical objects, if the following parameters are known: the thermal

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conductivities of each of the materials, the surface heat transfer coefficients for the external surfaces of the inefficiently- and efficiently cooled regions of the object, $h_{S\ inef}$ and $h_{S\ ef}$, respectively, and the power per unit length $P/L$ in each region.[4,9,10] The surface heat transfer coefficient for the liquid-cooled portion of the capillary was supplied by the instrument manufacturer, $h_{S\ ef} = 1136$ W m$^{-2}$ K$^{-1}$.[9] Estimates of $h_{S\ inef}$ from the literature vary from 50 to 100 W m$^{-2}$ K$^{-1}$.[11-13] By circumventing the capillary cooling system, we determined $h_{S\ inef}$ for our instrument using the technique of Hruska et al. and obtained $h_{S\ inef} = 75 \pm 10$ W m$^{-2}$ K$^{-1}$.[8]

According to heat dissipation theory, the increases in temperature of the electrolyte in the inefficiently- and efficiently cooled regions of the capillary, $\Delta T_{inef}$ and $\Delta T_{ef}$, varies linearly with the power (rate of heat generation) per unit length, $P/L$:[14]

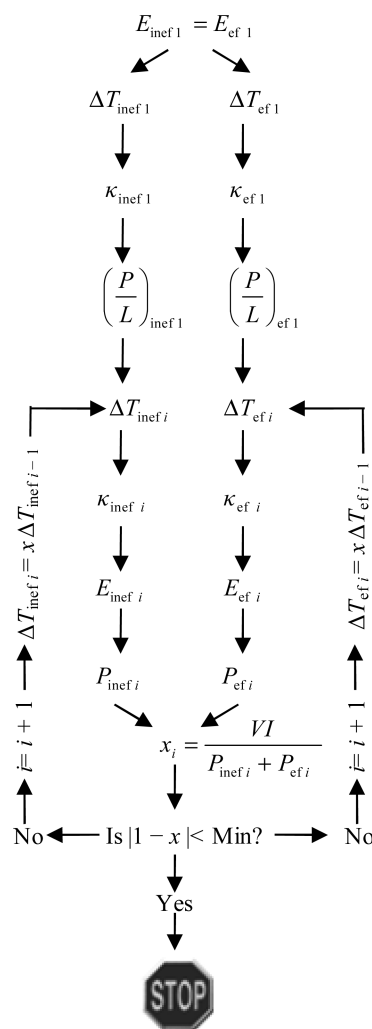$$\Delta T_{inef} = p_{L\ inef}\left(\frac{P}{L}\right)_{inef} = p_{L\ inef}E_{inef}I$$

$$\Delta T_{ef} = p_{L\ ef}\left(\frac{P}{L}\right)_{ef} = p_{L\ ef}E_{ef}I \tag{7}$$

where $p_L$ is a coefficient measured in Kelvin meter per watt that varies inversely with the surface heat transfer coefficient.

$$p_{L\ ef} = \frac{1}{2\pi}\left[\frac{1}{4\lambda_{H_2O}} + \frac{1}{\lambda_{FS}}\ln\left(\frac{d_{FS}}{d_i}\right) + \frac{1}{\lambda_{PI}}\ln\left(\frac{d_o}{d_{FS}}\right)\right] + \frac{1}{\pi d_o h_{S\ ef}}$$

$$p_{L\ inef} = \frac{1}{2\pi}\left[\frac{1}{4\lambda_{H_2O}} + \frac{1}{\lambda_{FS}}\ln\left(\frac{d_{FS}}{d_i}\right) + \frac{1}{\lambda_{PI}}\ln\left(\frac{d_o}{d_{FS}}\right)\right] + \frac{1}{\pi d_o h_{S\ inef}} \tag{8}$$

where $\lambda$ is the thermal conductivity, $d$ is the diameter, and the subscripts FS, PI, i, and o refer to fused silica, poly(imide), inner, and outer, respectively. Using the dimensions and thermal characteristics published for our capillary[8,15] and the values $h_{S\ ef} = 1136$ W m$^{-2}$ K$^{-1}$ and $h_{S\ inef} = 75$ W m$^{-2}$ K$^{-1}$, we calculated $p_{L\ ef} = 1.127$ K m W$^{-1}$ and $p_{L\ inef} = 12.15$ K m W$^{-1}$.

The problem of quantifying the electric field strength in each part of the electrolyte looks like a "Catch 22"; to determine the electrical field strength in each section, the temperatures are required and the determination of the temperatures requires the electric field strengths. Figure 3 shows our algorithm for overcoming this problem by using an iterative process. As a first approximation, we assume that $E$ is uniform throughout the capillary ($E_{ef} = E_{inef}$). Equation 7 was used to estimate the temperature increase in each region as a result of Joule heating. These temperature increases were used to predict the electrical conductivities using eq 6, which in turn were used to find more accurate estimates (the second approximation) of the electric field strengths and the power per unit length in each region.

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**Figure 3.** Flowchart showing the iterative approach to find the electric field strength, $E$, in each section of the electrolyte. $P/L$ is the power per unit length, $\Delta T$ is the increase in temperature of the electrolyte relative to the set temperature, $\kappa$ is the conductivity of the electrolyte, inef and ef refer to the inefficiently- and efficiently cooled sections of the electrolyte, $i$ is the iteration number, and $x$ is a factor that compares the average power per unit length calculated from the original voltage and current data with $P/L_{Average}$ calculated after $i$ iterations. Min is a number chosen according to the precision required.
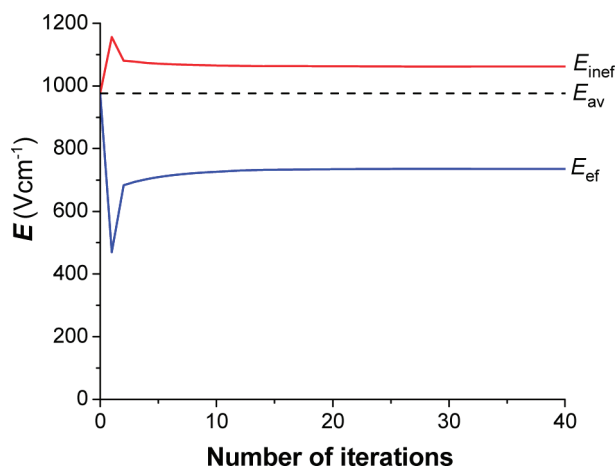
We define a factor, $x$, which indicates the goodness of the fit; it compares the values of the total power obtained using eqs 2 and 4.

$$x = \frac{P_{measured}}{P_{calculated}} \tag{9}$$

At the end of an iteration, "$x$" is used to adjust the estimates of the $\Delta T$ for the next iteration where $\kappa$, $E$, and $P/L$ are recalculated in the two regions until $x$ converges to 1. Complete derivations of the equations used and a more detailed flowchart showing the iterative process may be found in the Supporting Information. Computer programs can be downloaded from the Research section of the following Web site: http://www.chem.yorku.ca/profs/krylov/.

To demonstrate HAFD, we investigated a 30.7 cm-long fused-silica capillary with an inner diameter of 75.5 $\mu$m containing 50

**Figure 4.** Convergence of electric fields with increasing iteration number. The conditions used were $L_{inef}$ = 8.0 cm; $L_{ef}$ = 22.7 cm; i.d. = 75.5 $\mu$m; electrolyte 50 mM TRIS + 25 mM CH$_3$COOH, pH 8.3; $V$ = 30 kV.

mM TRIS and 25 mM CH$_3$COOH (a standard electrolyte used for CE). A voltage of 30.0 kV or an average electrical field strength $E_{average}$ = 977 V cm$^{-1}$ was used; in the first approximation, we assume that $E_{inef\ 1}$ = $E_{ef\ 1}$ = $E_{average}$. The resulting electric current was $I$ = 77.45 $\mu$A. On the basis of eqs 2 and 7, $P_{calculated}/L \approx 7.57$ W m$^{-1}$ and produced the first approximation of temperature increases: $\Delta T_{inef\ 1}$ = 92.0 °C and $\Delta T_{ef\ 1}$ = 8.53 °C, resulting in the first approximations of increases in the conductivity, $\kappa$, of the electrolyte in these regions from 0.141 S m$^{-1}$ at 20 °C to values of $\kappa_{inef\ 1}$ = 0.405 S m$^{-1}$ and $\kappa_{ef\ 1}$ = 0.166 S m$^{-1}$. This resulted in the first approximations of electric field strengths of $E_{inef\ 1}$ = 473 V cm$^{-1}$ and $E_{ef\ 1}$ = 1155 V cm$^{-1}$. Clearly a significant difference in electric field strengths for the two regions is indicated; however, these first approximations are inaccurate due to the initial assumption that $E_{inef\ 1}$ = $E_{ef\ 1}$, which is equivalent to $(P/L)_{inef\ 1}$ = $(P/L)_{ef\ 1}$. More accurate estimates of $E$ in each region can be obtained using these first approximation values of $E$ to recalculate the temperature increases, conductivities, and electrical field strengths in each region by iteration. Correct solutions for $E_{inef}$ and $E_{ef}$ were obtained when the total power calculated using eq 4 agreed with the value obtained from eq 2. Figure 4 shows the convergence of the calculated electric field strengths in the inefficiently- and efficiently cooled regions with increasing numbers of iterations. The initial estimates of $E_{inef}$ and $E_{ef}$ exaggerated the
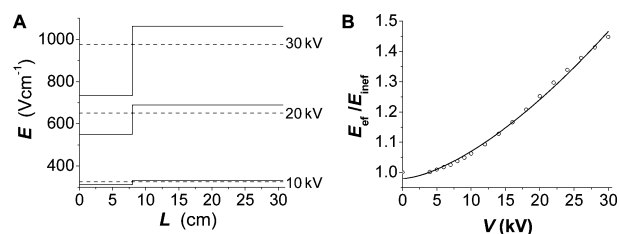


**Figure 5.** (A) Electric field strength in cooled and uncooled sections of a 30.7 cm capillary. The lengths of the inefficiently- and efficiently cooled regions were $L_{inef}$ = 8.0 and $L_{ef}$ = 22.7 cm, respectively. The dashed line indicates the average electric field strength for the corresponding voltage. (B) Ratio of the electrical field strength in the efficiently cooled region to that in the inefficiently cooled region as a function of the total voltage applied. The electrolyte was 50 mM TRIS, 25 mM CH$_3$COOH, pH 8.3.

difference between them, but in less than 40 iterations their values converged to 736 and 1062 V cm$^{-1}$, respectively, a difference of more than 1.4 times.

We further used the iterative procedure in order to determine the values of $E$ in the inefficiently- and efficiently cooled regions of the capillary for different voltages (Figure 5). At low voltages, when heating is negligible, $E$ is essentially uniform throughout the capillary, $E_{ef} \approx E_{inef}$, but at high voltages heating is appreciable and differences in cooling efficiency lead to differences in conductivity that are reflected in increased values of $E_{ef}$ and a substantial reduction in $E_{inef}$.

To conclude, we have shown conclusively that the electric field is nonuniform in a conducting medium unless it is cooled uniformly over its entire length. This has implications for the precision of measurements made in all electrophoretic techniques including slab gels, free flow electrophoresis, capillary electrophoresis, and microfluidic devices that employ intense electric fields. The greater the heating, the greater will be the variation in the electrical field strength due to unequal cooling efficiencies.

# Supporting Information

# Heat-Associated Field Distortion (HAFD) in Electro-Migration Techniques

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## Experimental Section

**Chemicals and Reagents**

*Tris*(hydroxymethyl)aminomethane (TRIS), NaOH(s), 35% w/w HCl(aq)  and glacial acetic acid were purchased from Sigma Aldrich (Oakville, Canada). The electrolyte was prepared by dissolving TRIS and acetic acid in 18.3 MΩcm distilled water obtained using a Millipore (Bedford, MA, USA) Milli-Q water purification system to produce a solution containing 50.0 mM TRIS and 25.0 mM $CH_3COOH$ with a pH of 8.30. Approximately 0.1 M solutions of NaOH(aq) and HCl(aq) were prepared by dissolving the reagents in 18.3 MΩcm distilled $H_2O$(l).

**Apparatus**

All experiments were conducted on a Beckman Coulter MDQ P/ACE (Beckman Coulter, Oakville, Canada) instrument equipped with liquid cooling. Fused silica capillary with an internal diameter of 75.5 μm, fused silica diameter of 321.0 μm and an external diameter of 359.5 μm was purchased from Polymicro Industries (Phoenix, AZ). A 30.7cm long capillary in which 8.0 cm had inefficient cooling and 22.7 cm was efficiently cooled by a flow of thermostat-controlled liquid coolant over the capillary, was used to examine the effect of differences in cooling efficiency on the electrical field strength in a commercial CE instrument. According to the instrument manufacturer, the cooling efficiency for the actively-cooled section (measured by the surface heat transfer coefficient, $h_S$) is $h_S$ =1136 $Wm^{-2}K^{-1}$.[1]

**Electrophoretic procedures**

Before use capillaries, were rinsed by applying a pressure of 100 kPa for enough time to introduce 10 capillary volumes of 0.1 M NaOH(aq), 0.1 M HCl(aq), 18.3 MΩcm distilled $H_2O$(l) and the electrolyte in that order. Conductance measurements were made by applying increments of 1 kV from 1 kV- 30 kV for periods of 1 minute at each voltage at a set temperature of 20°C. Current and voltage data were collected at a frequency of 4 Hz. The temperature coefficient of conductance, $\gamma$, was determined by repeating these measurements at set temperatures of 15.0, 18.0, 20.0, 21.0, 24.0, 27.0, and 30.0°C.

To evaluate the heat transfer coefficient for un-cooled sections of the capillary, the liquid cooling system of the instrument was circumvented for capillary lengths of 50, 100 and 150 cm and conductance measurements made at ambient temperature ~20°C.

**Data processing and analysis**

The average values of the electric current and voltage were obtained by averaging the data from the last 100 s at each voltage and used to calculate the conductance $G_{exp}$. Plots of $G_{exp}$ *vs* $V$ were fitted using the approach of Hruska *et al.*[2] to determine the offset error in the electric current and the conductance free of Joule heating effects at each set temperature of $G_0(T)$ for temperatures of 15 – 30°C.

The variation of conductance with temperature was determined by plotting $G_0(T)$ *vs* $T$ and interpolating to find $G_0(22.5°C)$. The value of $\gamma$ was found by calculating the slope of a plot $G_0(T)/G_0(22.5°C)$ *vs* $T$.

## Theoretical Section

**Determining the conductivity of the electrolyte at ambient temperature, $\kappa_0$ and its temperature coefficient, $\gamma$**

In order to quantitate HAFD, it is essential to know the conductivity of the electrolyte in the absence of Joule heating effects, $\kappa_0$ and to determine its increase with temperature (see Equation 6 in the main manuscript). Conductivity at the ambient temperature, $\kappa_0$, can be determined from the conductance at ambient temperature, $G_0$. Conductance, $G$, is the reciprocal of resistance:

$$G = \frac{I}{V} \qquad (S1)$$

where $I$ is the electric current that flows in an electrolyte when a voltage, $V$, is applied across it.

$$\kappa_0 = \frac{G_0 L}{A} = \frac{4 G_0 L}{\pi d_i^2}$$
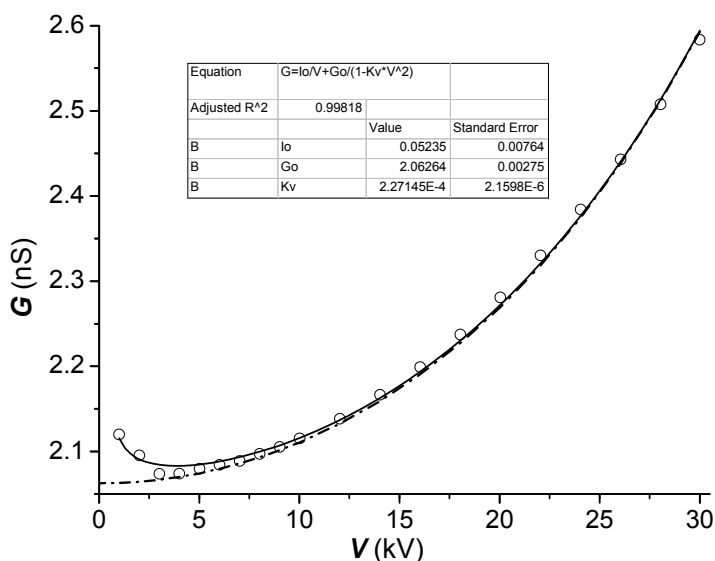
(S2)

In Equation (S2), $A$ is the internal cross-sectional area of the electrolyte, $L$ is the length of the capillary and $d_i$ is its internal diameter.

***Determining the conductance free of Joule heating, $G_0$.*** For a perfectly functioning instrument with no systematic error in measurement of the current and a uniform cooling efficiency for the entire capillary, the conductance of the electrolyte within the capillary is expected to increase with the applied voltage as shown below:[2]

$$G(V) = \frac{G_0}{1 - K_v V^2}$$

(S3)

where $G(V)$ is the conductance of the solution as a function of the voltage, $V$, $G_0$ is the conductance in the absence of an electrical field, and $K_v$ is the autothermal parameter ($V^{-2}$). **Figure S1** shows a typical plot of experimentally determined conductance versus voltage and a plot of the theoretical conductance versus voltage based on Equation (S3). In commercial CE instruments, a discrepancy between the theoretical and experimental plots occurs due to a systematic error in measurement of the electric current; sometimes referred to as an offset error, $I_0$.

$$I_{exp} = I_0 + I$$

(S4)



| Equation | G=Io/V+Go/(1-Kv*V^2) | |
|---|---|---|
| Adjusted R^2 | 0.99818 | |
| | Value | Standard Error |
| B    Io | 0.05235 | 0.00764 |
| B    Go | 2.06264 | 0.00275 |
| B    Kv | 2.27145E-4 | 2.1598E-6 |

**Figure S1.** Plots of conductance, *G versus* voltage, V for a 30.7 cm capillary containing 50 mM TRIS and 25 mM acetic acid, pH = 8.3. Open circles are experimental values, the solid line shows the curve of best fit with parameters: $G_0$ = 1.5934 nS, $I_0$ = 0.05235 µA and $K_v$ = 2.27146 $\times$ $10^{-10}$ $V^{-2}$. $R^2$ = 0.99818 for the fitting. The dashed line shows the actual conductance obtained by substituting the same parameters into Equation (S3).

where $I_{exp}$ is the current measured by the instrument and $I$ is the actual current. It follows that any measurement of conductance is influenced by this systematic error in the electric current.

$$G_{exp}(V) = \frac{I_0}{V} + \frac{G_0}{1 - K_V V^2}$$ (S5)

where $G_{exp}(V)$ is the experimentally determined conductance. Figure S1 shows that the influence of the offset error is the greatest at low voltages. This is to be expected as the error in the conductance, $I_0/V$ increases as $V \rightarrow 0$. The parameters $I_0$, $G_0$ and $K_V$ can be determined by curve fitting of Equation (S5) using suitable software.

***Finding the temperature coefficient of conductivity, γ***. As $G_0$ is the conductance free from Joule heating effects, by collecting $G_0$ data for a range of set temperatures, it is possible to determine the temperature coefficient of electrical conductivity, $\gamma$.

$$\gamma = \frac{G - G_0}{G_0 \Delta T} = \frac{\kappa - \kappa_0}{\kappa_0 \Delta T}$$ (S6)

**Figure S2** shows how the quotient $G_0/G_0(22.5°C)$ varies with temperature. The temperature coefficient of conductivity was determined from the slope of this graph; $\gamma = 0.0204 \pm 0.0002$ K$^{-1}$.
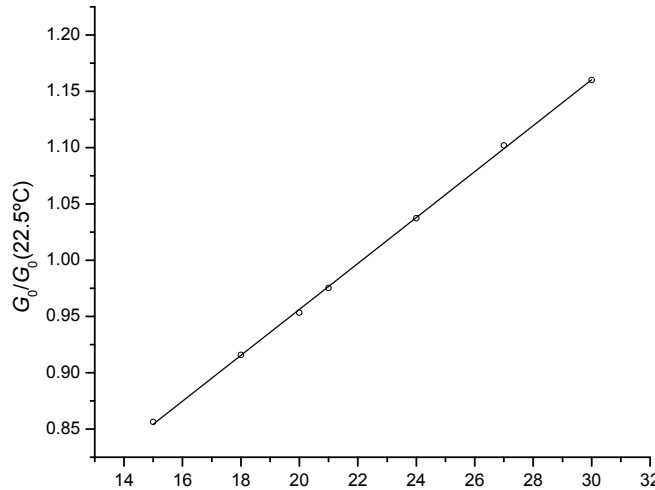


**Figure S2.** Variation of quotient of conductance and conductance at 22.5°C with temperature. The fitted line had the equation: $G_0(T) / G_0(22.5°C) = 0.0204\ T + 0.5484$, R$^2$ = 0.9996.

**Calculating the temperature increase of the electrolyte**

The increase in temperature of the electrolyte can be found from the power dissipation per unit length using Eq S7.[2,3]

$$\Delta T_{\text{Mean}} = p_{\text{L}} \frac{P}{L} \tag{S7}$$

where $p_{\text{L}}$ is a constant measured in K/Wm$^{-1}$, $P$ is the rate at which heat energy is generated in watts and $L$ is the capillary length in metres. The constant, $p_{\text{L}}$, varies inversely with the heat transfer coefficient, $h_{\text{S}}$, which measures the rate of heat transfer across a unit of area for a unit temperature difference in units of Wm$^{-2}$K$^{-1}$. In this study, we will use $h_{\text{S}}$ as a measure of the cooling efficiency at the external surface of the capillary. In fused silica capillaries, $p_{\text{L}}$ can be calculated using the expression:[4]

$$p_{\text{L}} = \frac{1}{2\pi}\left[\frac{1}{4\lambda_{\text{H}_2\text{O}}} + \frac{1}{\lambda_{\text{FS}}}\ln\left(\frac{d_{\text{FS}}}{d_{\text{i}}}\right) + \frac{1}{\lambda_{\text{PI}}}\ln\left(\frac{d_{\text{o}}}{d_{\text{FS}}}\right)\right] + \frac{1}{\pi d_{\text{o}} h_{\text{S}}} \tag{S8}$$

where $\lambda$ is the thermal conductivity and $d$ refers to the diameter. The subscripts: FS, i, PI, and o refer to fused silica, internal, poly(imide) and outer, respectively. Differences in the value of $p_{\text{L}}$ for the cooled and un-cooled sections are solely due to variations in $h_{\text{S}}$ as all the other terms in Eq S7 are constants. The value of $h_{\text{S}}$ for the cooled section of an air-cooled system that uses an air velocity of 10.0 ms$^{-1}$ is $h_{\text{S}} = 566$ Wm$^{-2}$K$^{-1}$ whereas for liquid cooling a value of $h_{\text{S}} = 1136$ Wm$^{-2}$K$^{-1}$ was supplied by the manufacturer of the instrument. [1,5] Estimates for $h_{\text{S}}$ for passively-cooled sections range from about 50-100 Wm$^{-2}$K$^{-1}$.[6-8]

To a good approximation, for typical capillaries used for capillary electrophoresis with $d_{\text{o}} = 360$ μm, Eq S8 simplifies to:

$$p_{\text{L}} = \frac{885}{h_{\text{S}}} + 0.347 \text{ K / Wm}^{-1} \tag{S9}$$

For the particular capillary used for the study, $d_{\text{i}} = 75.5$ μm, $d_{\text{FS}} = 321.0$ μm and $d_{\text{o}} = 359.5$ μm.

At 20ºC, the values of the constants are $\lambda_{\text{H}_2\text{O}} = 0.5984$ Wm$^{-1}$K$^{-1}$, $\lambda_{\text{FS}} = 1.40$ Wm$^{-1}$K$^{-1}$, and $\lambda_{\text{PI}} = 0.155$ Wm$^{-1}$K$^{-1}$.[4]

$$\frac{1}{\pi d_{\text{o}} h_{\text{S}}} = \frac{1}{\pi \times 359.5 \times 10^{-6}\text{m} \times h_{\text{S}}} = \frac{885.4 \text{ m}^{-1}}{h_{\text{S}}}$$

$$\frac{1}{2\pi}\left[\frac{1}{4\lambda_{\text{H}_2\text{O}}} + \frac{1}{\lambda_{\text{FS}}}\ln\left(\frac{d_{\text{FS}}}{d_{\text{i}}}\right) + \frac{1}{\lambda_{\text{PI}}}\ln\left(\frac{d_{\text{o}}}{d_{\text{FS}}}\right)\right] = \frac{1}{2\pi}\left[\frac{1}{4 \times 0.5984} + \frac{1}{1.40}\ln\left(\frac{321.0}{75.5}\right) + \frac{1}{0.155}\ln\left(\frac{359.5}{321.0}\right)\right]$$

$$= \frac{1}{2\pi}[0.4178 + 1.0337 + 0.7307]$$

$$= 0.3473 \text{ K/Wm}^{-1}$$

$$\therefore p_{\text{L}} = \frac{885.4 \text{ m}^{-1}}{h_{\text{S}}} + 0.3473 \text{ K/Wm}^{-1} \tag{S10}$$

***Finding the temperature increases of the electrolyte in the efficiently- and inefficiently cooled parts of the capillary.*** Using $h_{\text{S}} = 1136$ Wm$^{-2}$K$^{-1}$ for the efficiently cooled (liquid-cooled) section of the capillary, Equation (S10) predicts that $p_{\text{L ef}} = 1.127$ K/Wm$^{-1}$. As the surface heat transfer coefficient for the inefficiently cooled section of the

capillary is known less accurately, it was determined experimentally. Using the approach of Hruska *et al.* [2], curve fitting of experimental conductance *vs* voltage data using Equation (S5) was used to determine $G_0$ and $K_V$ for un-cooled capillaries. The temperature increase when the power per unit length, $P/L = 1$ Wm$^{-1}$, $p_L$ was calculated using Equation (S11):

$$p_L = \frac{LK_V}{\gamma G_0} \qquad (S11)$$

where $L$ is the length of the capillary (m), $K_V$ is the autothermal parameter (V$^{-2}$), $\gamma$ is the temperature coefficient of conductance (K$^{-1}$), and $G_0$ is the conductance free of Joule heating (S = AV$^{-1}$) Plots of experimental conductance versus voltage are shown for 50, 100, and 150 cm capillaries in **Figure S3**. Only the longest capillary behaved in an approximately Ohmic fashion; $G$ increased by ~ 6% when the voltage was increased from 1 to 30 kV. The corresponding variations were almost 18% for the 100 cm capillary and exceeded 120% for the 50 cm capillary. Using Equation (S11), the values of $p_{L\,inef}$ for the 50, 100, and 150 cm capillaries were: $11.7 \pm 0.1$, $13.2 \pm 0.1$ and $11.8 \pm 0.1$ K/Wm$^{-1}$, respectively. The surface heat transfer coefficients for the inefficiently-cooled capillaries were found by rearranging Equation (S10). The values of $h_S$ for the same capillary lengths were: $77.6 \pm 0.7$, $68.7 \pm 0.7$, and $77.2 \pm 0.7$ Wm$^{-2}$K$^{-1}$, respectively. For our calculations, we used $h_{S\,inef} = 75.0$ Wm$^{-2}$K$^{-1}$. Applying Equation (S10), $h_{S\,inef} = 75.0$ Wm$^{-2}$K$^{-1}$ corresponds to $p_{L\,inef} = 12.15$ K/Wm$^{-1}$.
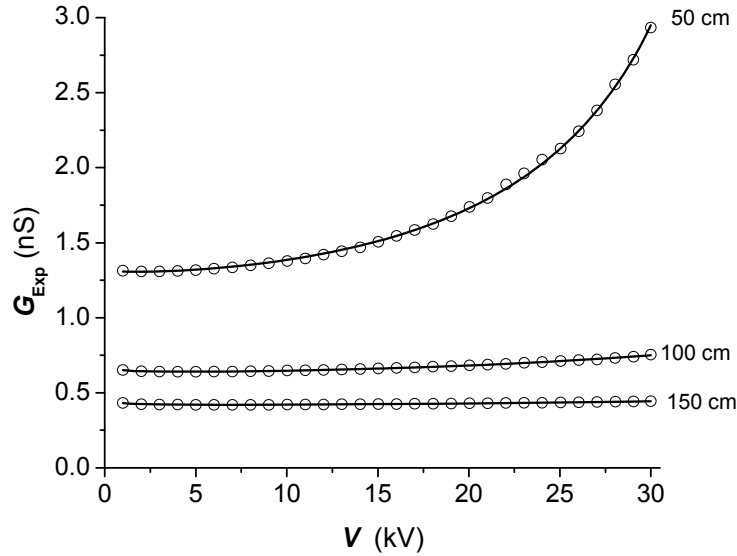


**Figure S3.** Plots of experimental conductance, $G_{exp}$ *vs* voltage, $V$ for capillaries without active cooling. The solid lines show the curves of best fit using Equation (S5). The fitting parameters for the three curves are:
50 cm: $R^2 = 0.9994$, $I_0 = 0.00846 \pm 0.01095$ µA, $G_0 = 1.298 \pm 0.003$ nS, and $K_v = 6.22 \pm 0.02 \times 10^{-10}$ V$^{-2}$
100 cm: $R^2 = 0.9987$, $I_0 = 0.01724 \pm 0.00131$ µA, $G_0 = 0.6343 \pm 0.0004$ nS, and $K_v = 1.71 \pm 0.01 \times 10^{-10}$ V$^{-2}$
150 cm - $R^2 = 0.9995$, $I_0 = 0.01446 \pm 0.00019$ µA, $G_0 = 0.41649 \pm 0.00006$ nS, and $K_v = 6.68 \pm 0.03 \times 10^{-11}$ V$^{-2}$

**Calculating the conductivity of the electrolyte, $\kappa$**

Now that we have a means of calculating the temperature increase in each section of the capillary and we know the temperature coefficient for the electrical conductivity of the electrolyte, it is possible to calculate the conductivity of the electrolyte in each section of the capillary. In the absence of Joule heating, the conductivity at the set temperature can be found using Equation S2. In the presence of Joule heating, conductivity is estimated from the temperature changes in the inefficiently- and efficiently-cooled sections of the capillary.

$$\kappa_{ef} = \kappa_0(1 + \gamma\Delta T_{ef})$$
$$\kappa_{inef} = \kappa_0(1 + \gamma\Delta T_{inef})$$

(S12)

**Calculating the electrical field strength in each section (full derivation)**

By Kirchhoff's current law, the currents in each section of the capillary must be equal.

$$I = I_{inef} = I_{ef}$$

$$\therefore I = GV = \frac{\overline{\kappa}AV}{L_{tot}} = \frac{\kappa_{inef}AV_{inef}}{L_{inef}} = \frac{\kappa_{ef}AV_{ef}}{L_{ef}}$$

$$\therefore \frac{\overline{\kappa}V}{L_{tot}} = \frac{\kappa_{inef}V_{inef}}{L_{inef}} = \frac{\kappa_{ef}V_{ef}}{L_{ef}}$$

$$\therefore V_{inef} = \frac{\kappa_{ef}L_{inef}V_{ef}}{\kappa_{inef}L_{ef}}$$

$$V = V_{inef} + V_{ef} \text{ (by Kirchhoff's Voltage Law)}$$

$$\therefore V_{ef} + \frac{\kappa_{ef}L_{inef}V_{ef}}{\kappa_{inef}L_{ef}} = V$$

$$\therefore V_{ef}\left(1 + \frac{\kappa_{ef}L_{inef}}{\kappa_{inef}L_{ef}}\right) = V$$

$$\therefore V_{ef} = \frac{V}{\left(\frac{\kappa_{inef}L_{ef} + \kappa_{ef}L_{inef}}{\kappa_{inef}L_{ef}}\right)}$$

$$\therefore V_{ef} = \frac{\kappa_{inef}L_{ef}V}{\kappa_{inef}L_{ef} + \kappa_{ef}L_{inef}}$$

$$V_{inef} = V - V_{ef}$$

$$\therefore V_{inef} = V\left(1 - \frac{\kappa_{inef}L_{ef}}{\kappa_{inef}L_{ef} + \kappa_{ef}L_{inef}}\right)$$

$$\therefore V_{\text{inef}} = \frac{\kappa_{\text{ef}} L_{\text{inef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$\therefore E_{\text{ef}} = \frac{V_{\text{ef}}}{L_{\text{ef}}} = \frac{\kappa_{\text{inef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}} \quad \text{and} \quad E_{\text{inef}} = \frac{V_{\text{inef}}}{L_{\text{inef}}} = \frac{\kappa_{\text{ef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}} \tag{S13}$$

It follows that as $\kappa_{\text{inef}} > \kappa_{\text{ef}}$, $E_{\text{ef}} > E_{\text{inef}}$ so that any changes in temperature of the medium lead to heterogeneity in the electrical field.

**Calculating the power per unit length in each section (full derivation)**

$$I_{\text{inef}} = \frac{\kappa_{\text{inef}} A V_{\text{inef}}}{L_{\text{inef}}}$$

$$V_{\text{inef}} = \frac{\kappa_{\text{ef}} L_{\text{inef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$\therefore I_{\text{inef}} = \frac{\kappa_{\text{inef}} A}{L_{\text{inef}}} \times \frac{\kappa_{\text{ef}} L_{\text{inef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$= \frac{\kappa_{\text{inef}} \kappa_{\text{ef}} V A}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$\therefore \left( \frac{P}{L} \right)_{\text{inef}} = \frac{V_{\text{inef}} I_{\text{inef}}}{L_{\text{inef}}}$$

$$= \frac{\kappa_{\text{ef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}} \times \frac{\kappa_{\text{inef}} \kappa_{\text{ef}} V A}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$= \frac{\kappa_{\text{inef}} \kappa_{\text{ef}}^2 V^2 A}{\left( \kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}} \right)^2}$$

Similarly

$$I_{\text{ef}} = \frac{\kappa_{\text{ef}} A V_{\text{ef}}}{L_{\text{ef}}}$$

$$V_{\text{ef}} = \frac{\kappa_{\text{inef}} L_{\text{ef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$\therefore I_{\text{ef}} = \frac{\kappa_{\text{ef}} A}{L_{\text{ef}}} \times \frac{\kappa_{\text{inef}} L_{\text{ef}} V}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$= \frac{\kappa_{\text{inef}} \kappa_{\text{ef}} V A}{\kappa_{\text{inef}} L_{\text{ef}} + \kappa_{\text{ef}} L_{\text{inef}}}$$

$$\left( \frac{P}{L} \right)_{\text{ef}} = \frac{V_{\text{ef}} I_{\text{ef}}}{L_{\text{ef}}}$$

$$\therefore \left(\frac{P}{L}\right)_{\text{ef}} = \frac{\kappa_{\text{inef}}V}{\kappa_{\text{inef}}L_{\text{ef}} + \kappa_{\text{ef}}L_{\text{inef}}} \times \frac{\kappa_{\text{inef}}\kappa_{\text{ef}}VA}{\kappa_{\text{inef}}L_{\text{ef}} + \kappa_{\text{ef}}L_{\text{inef}}}$$

$$= \frac{\kappa_{\text{inef}}^{2}\kappa_{\text{ef}}V^{2}A}{\left(\kappa_{\text{inef}}L_{\text{ef}} + \kappa_{\text{ef}}L_{\text{inef}}\right)^{2}}$$

It follows that as $\kappa_{\text{inef}} > \kappa_{\text{ef}}$, $\left(\frac{P}{L}\right)_{\text{ef}} > \left(\frac{P}{L}\right)_{\text{inef}}$.

**Finding a stable solution for the temperature, conductivity and electrical field strength in each part of the capillary.**

The process of finding $E_{\text{c}}$ and $E_{\text{u}}$ is complicated by the differing temperatures and electrical conductivities that exist in the efficiently cooled and inefficiently-cooled sections of the capillary. **Figure S4** shows the detailed iterative process used to determine stable solutions for the electrical field in each part of the capillary. Initially the raw voltage and current data is used to generate a plot of the experimental conductance versus the voltage, $G_{\text{exp}}$ *vs* $V$. Curve fitting of this plot based on Eq S5 is used to determine $I_0$, $G_0$, and $K_V$. The electric current data is corrected by subtracting $I_0$ from each of the currents. The iterative process starts with the corrected electric currents being used to calculate $P/L_{\text{Measured}}$ for each of the applied voltages; these in turn are used to estimate the temperature increases $\Delta T_{\text{ef 1}}$ and $\Delta T_{\text{inef 1}}$ in the efficiently- and inefficiently-cooled sections of the capillary using $p_{\text{Lc}} = 1.127$ and $p_{\text{Lu}} = 12.15$ K/Wm$^{-1}$ respectively. $\Delta T_{\text{ef 1}}$ and $\Delta T_{\text{inef 1}}$ are used to find the first order approximations for the electrical conductivities, $\kappa_{\text{ef 1}}$ and $\kappa_{\text{inef 1}}$, and the electrical field strengths, $E_{\text{ef 1}}$ and $E_{\text{nef 1}}$. These in turn are used to determine the power per unit length in each section of the capillary, $(P/L)_{\text{ef 1}}$ and $(P/L)_{\text{inef 1}}$ and the total power dissipated in the capillary as a whole,
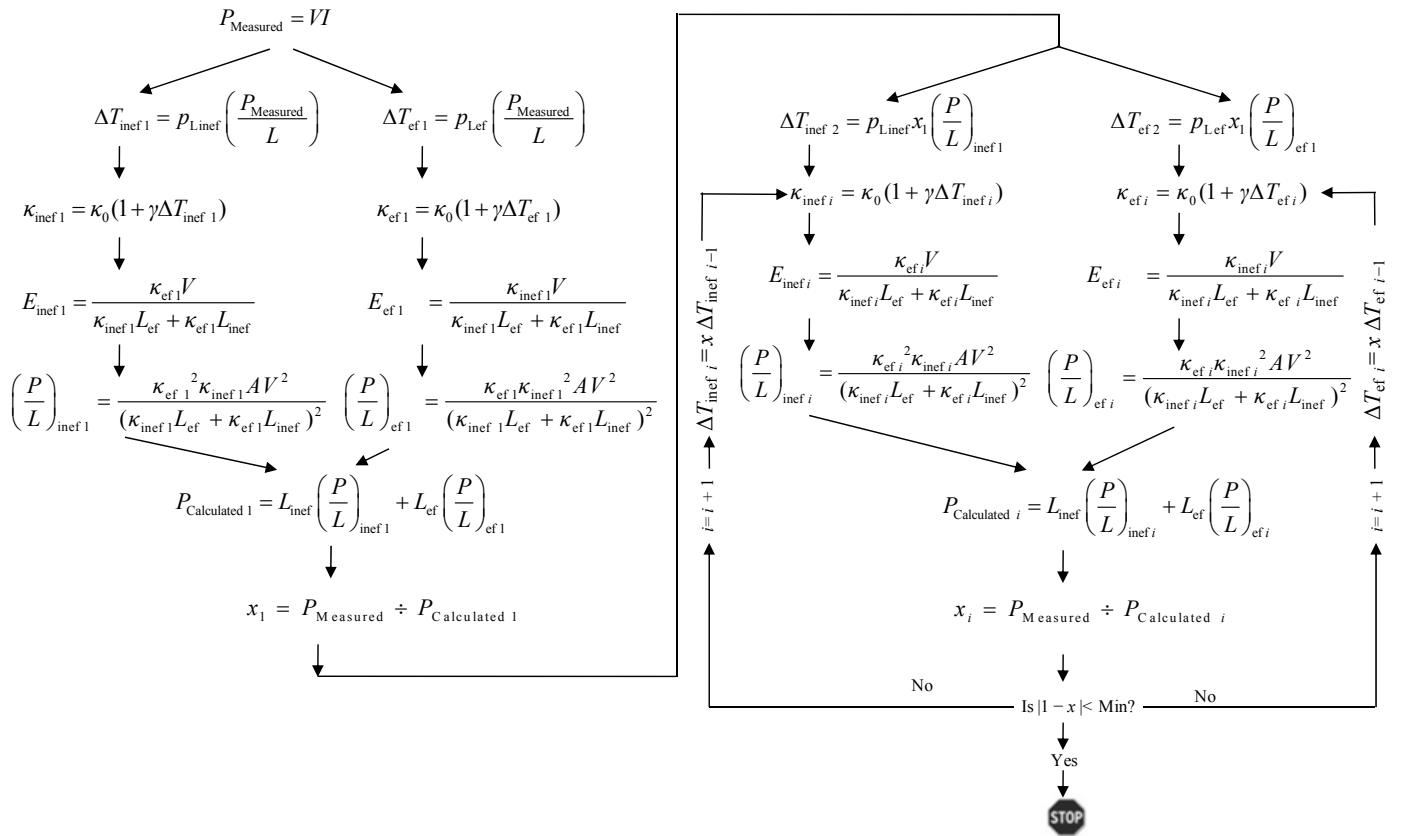


**Figure S4.** Flowchart illustrating the iterative process used to find the electrical field in each part of the capillary.

$P_{\text{Calcualted 1}}$. The accuracy of the first order approximation for $E_{\text{ef 1}}$ and $E_{\text{nef 1}}$ is checked by comparing the total power calculated with the actual power dissipated, $P_{\text{Measured}}$. This first step of the iteration is shown in the left hand side of the flowchart. The next step is to adjust the calculated temperature increases by including the influence of unequal heat dissipation which led to differing values of $P/L$. The new calculated temperature increases are used to adjust the estimates of the conductivity, electrical field strength and power per unit length in each section and to determine the accuracy by calculating $x_2$. In the third and all subsequent iterations the calculated parameters are fine-tuned by adjusting the calculated temperatures and recalculating. The process is deemed to be complete when the factor $x_i$ is sufficiently close to 1. The number of iterations, $i$, required for convergence depends on the setting of the parameter "Min". The smaller the value of "Min", the more iterations that are needed. If for example, "Min" is set to $1 \times 10^{-4}$, the value of the variable being calculated will only vary in its $5^{\text{th}}$ significant figure once $|1\text{-}x| < \text{Min}$ and this is typically accomplished in less than 100 iterations. Once a stable solution is found, the electric field strength in each section can be determined. In practice, $x$ converges to 1 for most voltages. At very low voltages, the influence of minor errors in $I_0$ may prevent this.

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