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Research Article

Simplified universal method for determining electrolyte temperatures in a capillary electrophoresis instrument with forced-air cooling

Temperature increase due to resistive electrical heating is an inherent limitation of capillary electrophoresis (CE). Active cooling systems are used to decrease the temperature of the capillary, but their capacity is limited; and in addition, they leave "hot spots" at the detection interface and at the capillary ends. Until recently, the matter was complicated by the lack of a fast and generic method for temperature determination in efficiently and inefficiently cooled regions of the capillary. Our group recently introduced such a method, termed "Universal Method for determining Electrolyte Temperatures" (UMET). UMET is a probe-less approach that requires only measuring current versus voltage for different voltages and processing the data using an iterative algorithm. Here, we apply UMET to develop a Simplified Universal Method of Temperature Determination (SUMET) for a CE instrument with a forced-air cooling system using an Agilent 7100 CE instrument (Agilent Technologies, Saint Laurent, Quebec, Canada) as an example. We collected a wide set of empirical voltage–current data for a variety of buffers and capillary diameters. We further constructed empirical equations for temperature calculation in efficiently and inefficiently cooled parts of the capillary that require only the data from a single 1-min voltage–current measurement. The equations are specific for the Agilent 7100 CE instrument (Agilent Technologies) but can be applied to all kinds of capillaries and buffers. Similar SUMET approaches can be developed for other CE instruments with forced-air cooling using our approach.

1 Introduction

The principle of Joule heating, heat dissipation, and variation of conductance with temperature and their effects on capillary electrophoresis (CE)-based affinity separations of temperature-sensitive samples (such as DNA and proteins) is being researched extensively. It is important to consider Joule heating in electromigration techniques as it stimulates temperature changes in the electrolyte, thereby resulting in imprecise migration times and peak areas [1–3]. Approaches to eliminating this problem are instrument specific and involve the use of built in Peltier cooling devices, forced air cooling, or using a flow of liquid coolant. However, as shown in previous studies, there are some regions of capillary such as the inlet, outlet, and the detection window that are not actively cooled and sitting in ambient air [3, 4]. This difference in heat dissipation in efficiently cooled and inefficiently cooled regions of capillaries causes axial temperature differences in the electrolyte. The latter, in turn, lead to thermal peak broadening due to different electrophoretic mobilities at the axis and near the inner wall of the capillary and a loss of plate numbers [3–6].

Despite the acknowledgment of the importance of temperature in CE, this temperature difference between efficiently cooled and inefficiently cooled parts of capillaries had not been determined until very recently and its effect on the quality of CE-based analyses has largely been ignored. This problem is successfully solved by a universal method for determining electrolyte temperatures (UMET) that was recently

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developed in our lab [4]. UMET is solely based on measuring the electric current, I, versus voltage, V, for different values of V. Having gathered the voltage and current data, they are inputted into an iterative algorithm that allows the determination of the temperature increases, ΔT_{ef} and ΔT_{inef}, of the electrolyte in the efficiently cooled and inefficiently cooled regions [4]. UMET also uses such parameters as (i) geometrical characteristics of the capillary (the inner diameter, d_i; the thickness of fused silica, d_{FS}; the outer diameter, d_o; the total length, L; and the lengths, L_{ef} and L_{inef}, of efficiently cooled and inefficiently cooled parts of the capillary, respectively); (ii) the thermal conductivities, λ_{FS} and λ_{PI}, of the fused silica wall of the capillary and the poly(imide) coating, respectively; (iii) the surface heat transfer coefficients, $h_{S,ef}$ and $h_{S,inef}$, for the efficiently cooled and inefficiently cooled parts of the capillary, respectively; and (iv) the electrolyte conductivity at the ambient temperature, κ_0, and the temperature coefficient of electrolyte conductivity, α. The latter two parameters are obtained from analysis of variation of the measured conductance $G_{exp} = I/V$ with the voltage [3, 4, 7]. In previous studies, UMET was applied for a wide range of capillary inner diameters and diverse electrolytes in a commercial CE instrument, the Beckman MDQ (Beckman Coulter, Oakville, Canada), in which efficient cooling was supplied by a continuous stream of liquid coolant pumped in the capillary cartridge [4]. Data on ΔT_{ef} and ΔT_{inef} obtained by UMET allowed simplified universal method for temperature determination (SUMET) to be formulated for the Beckman MDQ CE instrument [4]. SUMET does not use an iterative procedure and, thus, is much simpler than UMET. However, SUMET has to be specifically formulated for each CE instrument with typical electrolytes used whereas UMET can be applied directly to any of them.

In this study, we demonstrate the use of UMET for a commercial CE instrument, Agilent 7100 (Agilent Technologies, Saint Laurent, Quebec, Canada), in which the capillary is effectively cooled by a stream of air traveling through the capillary cassette at a speed of approximately 12 m/s. The temperature is controlled using a built-in Peltier device. We determined the temperature increase of the electrolyte in the air-cooled section of the capillary and in the passively cooled parts of the capillary as a function of the electric field strength for a variety of electrolytes and inner capillary bores in the Agilent 7100 CE instrument (Agilent Technologies). The data obtained for Agilent 7100 (Agilent Technologies) by UMET were used to establish all the instrument-specific parameters that should be used in SUMET formulated for Agilent 7100 (Agilent Technologies). These results allow an operator to accurately predict the temperature increases for the electrolyte in use in only a few minutes.

2 Materials and methods

2.1 Chemicals and reagents

Analytical reagent grade sodium tetraborate, potassium chloride, magnesium chloride, sodium acetate, sodium hydroxide, 35% w/w hydrochloric acid, glacial acetic acid, high-performance liquid chromatography grade methanol, *Tris*(hydroxymethyl)aminomethane (TRIS), and N-*Tris*(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) were purchased from Sigma Aldrich (Oakville, ON, Canada). All solutions were made using the Milli-Q-quality deionized water and filtered through a 0.22 μm filter (Millipore, Nepean, ON, Canada) system. The TRIS acetate buffer was prepared by dissolving acetic acid and TRIS in distilled water to produce a solution containing 50.0 mM TRIS and 25.0 mM CH_3COOH with pH 8.30. A similar approach was used to produce the same buffer to which KCl or $MgCl_2$ was added quantitatively to produce TRIS acetate buffers containing from 50 to 400 mM KCl or 2.5 mM $MgCl_2$. Sodium tetraborate buffer with a pH of 9.20 was prepared by dissolving the salt in distilled water to make a 25.0 mM solution. TES buffer was prepared by dissolving the acid in distilled water and titrating it to a pH 7.5 using a concentrated solution of NaOH(aq). Approximately 0.1 M solutions of NaOH(aq) and HCl(aq) were prepared by dissolving the reagents in distilled $H_2O(l)$.

2.2 Apparatus

All experiments were conducted using an Agilent CE 7100 (Agilent Technologies) instrument equipped with air-cooling system. Fused silica capillaries with inner diameters of 20, 50, 75, 100, 150, and 200 μm were purchased from Polymicro Industries (Phoenix, AZ, USA). To control the temperature of the electrolyte in the vials, the vial tray was cooled continuously using a flow of temperature-controlled water from a refrigerating circulator (Thermo Neslab Inc., Newington, NH, USA).

2.3 Electrophoretic procedures

Before use, capillaries were rinsed by applying a pressure of 150 kPa for enough time to introduce 10 capillary volumes of methanol, 0.1 M HCl(aq), 0.1 M NaOH(aq), distilled $H_2O(l)$, and the electrolyte in that order. Conductance measurements were made for each of the electrolytes by applying increments of 1 kV from 1 kV to 10 kV followed by increments of 2 kV from 10 kV to 30 kV for periods of 1 min at each voltage at a set temperature of 20°C for capillary lengths of 50 cm and inner diameters ranging from approximately 20 to 200 μm. Current and voltage data were collected at a frequency of 10 Hz.

2.4 Data processing and analysis

Representative values of the voltage and electric current were obtained by averaging the data from the last 50 s at each applied voltage. These values were used to calculate the experimental conductance for each voltage, $G_{exp} = I/V$, where I and V are the electric current and voltage recorded by the

instrument. Plots of G_{Exp} versus V were fitted with the first relation (13) using Origin 8.0® software (OriginLab Corporation, Northampton, MA, USA). As a result, the conductance at the set temperature, G_0, the offset error in the electric current, I_0, and the autothermal parameter, K_v, were determined for each capillary length. For each electrolyte, data were collected at set temperatures of 15°C, 18°C, 20°C, 21°C, 24°C, 27°C, and 30°C. The electrolyte conductivity at the ambient temperature, κ_0, was calculated from the third relation (13) at G_0 corresponding to $T = 20°C$. The temperature coefficient of electrical conductivity, α, was found by calculating the slope of a plot of $G_0(T)/G_0(20°C)$ versus $(T - 20°C)$ (Supporting Information Table S1). The temperature increases, ΔT_{ef} and ΔT_{inef}, at each applied voltage were determined by using the iterative procedure described previously [4].

3 Results and discussion

3.1 General theory

To calculate the rise in temperatures, ΔT_{ef} and ΔT_{inef}, of the electrolyte in the efficiently and inefficiently cooled sections of the capillary UMET employs the following expressions [4]:

$$\Delta T_{ef} = p_{L,ef} \left(\frac{P}{L} \right)_{ef}, \quad \Delta T_{inef} = p_{L,inef} \left(\frac{P}{L} \right)_{inef} \quad (1)$$

Coefficients $p_{L,ef}$ and $p_{L,inef}$ are calculated using classical theory for energy dissipation from a heated cylinder [8–11]

$$p_{L,ef} = \frac{1}{2\pi} \left[\frac{1}{4\lambda} + \frac{1}{\lambda_{FS}} \ln \frac{d_{FS}}{d_i} + \frac{1}{\lambda_{PI}} \ln \frac{d_0}{d_{FS}} + \frac{2}{d_0 h_{S,ef}} \right] \quad (2)$$

$$p_{L,inef} = \frac{1}{2\pi} \left[\frac{1}{4\lambda} + \frac{1}{\lambda_{FS}} \ln \frac{d_{FS}}{d_i} + \frac{1}{\lambda_{PI}} \ln \frac{d_0}{d_{FS}} + \frac{2}{d_0 h_{S,inef}} \right] \quad (3)$$

where λ is the thermal conductivity of the coolant. Ratios $(P/L)_{ef}$ and $(P/L)_{inef}$ are values of the electric power per unit length in the efficiently and inefficiently cooled sections, respectively. They are determined by relations [4]

$$\left(\frac{P}{L} \right)_{ef} = \frac{\kappa_{ef} \kappa_{inef}^2 A V^2}{(\kappa_{ef} L_{inef} + \kappa_{inef} L_{ef})^2} \quad (4)$$

$$\left(\frac{P}{L} \right)_{inef} = \frac{\kappa_{inef} \kappa_{ef}^2 A V^2}{(\kappa_{ef} L_{inef} + \kappa_{inef} L_{ef})^2} \quad (5)$$

Here, κ_{ef} and κ_{inef} are the electrical conductivities of the electrolyte in the efficiently and inefficiently cooled sections, respectively. They depend linearly on ΔT_{ef} and ΔT_{inef} [3, 4, 7]

$$\kappa_{ef} = \kappa_0 (1 + \alpha \Delta T_{ef}), \quad \kappa_{inef} = \kappa_0 (1 + \alpha \Delta T_{inef}) \quad (6)$$

UMET employs an iterative process that allow finding ΔT_{ef} and ΔT_{inef} from Eqs. (1)–(6) provided that all geometrical and physical characteristics of the capillary are known. In

particular, $h_{S,ef}$ is determined by the method of forced convective cooling used in the efficiently cooled section whereas $h_{S,inef}$ is usually determined by free convection that present in the inefficiently cooled section. UMET was originally applied to a Beckman MDQ CE instrument equipped with the liquid cooling system [4].

Here, we consider the air-cooling system employed in an Agilent 7100 CE instrument (Agilent Technologies). It uses forced air convection in the efficiently cooled section and, therefore, we have to find corresponding new values of $h_{S,ef}$ and $h_{S,inef}$. In the efficiently cooled section, we use Newton's law of cooling [2]

$$\Delta T_{air} = \frac{1}{\pi d_0 h_{S,ef}} \left(\frac{P}{L} \right)_{ef} \quad (7)$$

and an empirical expression for the Nusselt number, Nu_{ef}, corresponding to the forced air convection [2, 12]

$$Nu_{ef} \equiv \frac{h_{S,ef} d_0}{\lambda} = a Re^m, \quad Re \equiv \frac{v d_0}{\eta} \quad (8)$$

$$a = 0.615, \quad m = 0.466, \quad 40 < Re < 4000 \quad (9)$$

Here, ΔT_{air} is the temperature difference across the air layer surrounding the capillary, Re is the Reynolds number, v is the airflow speed in the efficiently cooled section, and η is the kinematic viscosity of air. Equations (7) and (8) allow one to determine ΔT_{air} and $h_{S,ef}$, given the experimental linear dependency of combination $\lambda \Delta T_{air}/\eta^m$ on ΔT_{air} at $T_{air} \approx 20°C$ [2]. As a result, we have [2]

$$\Delta T_{air} = \frac{0.1127}{(v d_0)^{0.466}} \left(\frac{P}{L} \right)_{ef}, \quad 40 < \frac{v d_0}{\eta} < 4000 \quad (10)$$

A typical speed v for the airflow in an efficiently cooled CE system ranges from 10 to 12 m/s [3, 4]. If air is cooled to 20°C with a kinematic viscosity of 1.5×10^{-5} m²/s and blown against a capillary with an outer diameter $d_0 = 3.65 \times 10^{-4}$ m, we have Re ≈ 242 to 290. Thus, inequalities in 9 and 10 are satisfied. Substitution of (10) into (7) gives $h_{S,ef}$ in the range from 566 to 616 W/Km². For this work, we will assume an intermediate value, $h_{S,ef} = 591$ W/Km², as a basis for UMET calculations.

Similarly, $h_{S,inef}$ can also be found from an empirical expression for the corresponding Nusselt number, Nu_{inef} [2, 13]

$$Nu_{inef} \equiv \frac{h_{S,inef} d_0}{\lambda} = b Ra^n, \quad Ra \equiv \frac{g_E \beta d_0^3 \Delta T_{air}}{\eta \chi} \quad (11)$$

$$b = 1.02, \quad n = 0.148, \quad 0.01 < Ra < 100 \quad (12)$$

Here, Ra is the Rayleigh number, describing a free convection in the inefficiently cooled section, g_E is the acceleration due to gravity, β is the thermal expansivity, and χ is the thermal diffusivity. Let us assume that the capillary is only surrounded by air and is not in contact with parts of the instrument or liquid in the vials. Then, relations (11) and (12)

allow one to calculate the Nusselt number and, therefore, to estimate $h_{S,inef}$ (see Supporting Information). This leads to a value of approximately $h_{S,inef} \sim 50$ W/Km^2 in reasonable agreement but smaller than values published in the literature [1–4].

By circumventing the instrument's cooling system altogether, one can also determine $h_{S,inef}$ using the approach introduced by Hruska et al. [4, 7]. It is based on theoretical dependences of the electrolyte conductance G on the voltage V

$$G = \frac{I_0}{V} + \frac{G_0}{1 - K_V V^2}, \quad K_V = \frac{\alpha p_{L,inef} G_0}{L}, \quad \kappa_0 = \frac{4 G_0 L}{d_i} \quad (13)$$

where I_0 is the offset error in the electrical current, G_0 is the conductance free of Joule heating effects at the ambient temperature, and K_V is the autothermal parameter. In this approach, values of I_0, G_0, and K_V are found by fitting the first relation from (13) into the experimental dependence $G_{exp}(V)$ measured at various temperatures of the ambient temperature. Then, values of κ_o and α are determined using the third relation from (13) and expression (6) for κ_{ef}. After that, the second relation from (13) gives $p_{L,inef}$. Substituting it into (3) and solving the equation obtained with respect to $h_{S,inef}$, we finally have

$$h_{S,inef}$$
$$= \left(\frac{\pi d_o K_V L}{\alpha G_0} - \frac{d_o}{2} \left[\frac{1}{4\lambda} + \frac{1}{\lambda_{FS}} \ln\left(\frac{d_{FS}}{d_i}\right) + \frac{1}{\lambda_{PI}} \ln\left(\frac{d_o}{d_{FS}}\right) \right] \right)^{-1}$$
$$(14)$$

Relation (14) gives values of $h_{S,inef}$ in the range from 50 to 100 W/Km^2 in the case of commercial instrument for capillaries with $d_i = 75$ µm and lengths ranging from 50 to 150 cm [4]. This result is consistent with an estimate $h_{S,inef} \sim 50$ W/Km^2 derived from (11) and (12). We will assume that an intermediate value of $h_{S,inef} \sim 75$ W/Km^2 is appropriate for an air-cooled instrument studied in this work.

3.2 Application of UMET

The determination of temperature increases of the background electrolyte (BGE) in Agilent CE 7100 (Agilent Technologies) was performed by UMET. Two upper panels in Fig. 1 show the calculated temperature increases versus the electric field strength for an electrolyte over a range of inner capillary diameters. It was noticed that temperature increase of the BGE in the inefficiently cooled part of the capillary was approximately 2–4 times greater than in the efficiently cooled section. It is generally accepted that both ΔT_{ef} and ΔT_{inef} increase with the inner diameter of the capillary if the voltage remains constant. Indeed, the square of a radius and current have a proportional relationship and, thus, so does P, the rate of heat production. However, in practice, the maximum electric field strength is restricted by the maximum current that is allowed by the instrument to flow, which would be the case here for diameters greater than 150 µm. In such cases,

increasing the inner diameter of the capillary will require decreasing the voltage to keep the current from exceeding its maximum. This, in turn, may lead to a decrease in ΔT_{ef} and ΔT_{inef} [4]. However, it would occur only under a special condition of the constant current (when the latter reached its maximum) rather than under more a common condition of the constant voltage. Another important observation, which agrees with the heat-associated field distortion in CE [3], concerns the shape of the fitted curves in Fig. 1. In previous studies on thermal effects in CE, the influence of electrical field strength on the temperature increase was modeled by the power function [3, 4, 14]. Based on this information, for each inner diameter, the temperature increases were fitted to power functions of the form

$$\Delta T_{ef} = A_{ef} (E_{Average})^{B_{ef}}, \quad \Delta T_{inef} = A_{inef} (E_{Average})^{B_{inef}} \quad (15)$$

The observations show that $B_{ef} > 2$ and $B_{inef} < 2$. Values of B_{ef} and B_{inef} increase with the electrical field strength due to the positive feedback between conductance and heat production. As heat is produced, the temperature and therefore the conductance of the electrolyte increase leading to even greater heat production. This process continues until a steady state is reached at which the rate of heat removal is equal to its rate of production. The results are also consistent with the electrical field strength being stronger in the efficiently cooled section and weaker in the inefficiently cooled section.

UMET was also applied to investigate the influence of electrical conductivity, κ_0, on temperature increases in the efficiently and inefficiently cooled regions. Lower panels in Fig. 1 show the influence of ionic strength on the temperature increase of electrolyte versus electric field strength for a fixed inner diameter, $d_i = 75$ µm, in the Agilent instrument (Agilent Technologies). As expected with increasing conductivity, the temperature increase in the efficiently and inefficiently cooled regions of the capillary became greater for particular electric field strength. The greatest temperature increases occurred for the capillary containing an electrolyte of low conductivity, 50 mM TRIS, 25 mM acetic acid, and 50 mM KCl ($\kappa_0 \sim 0.678$ S/m at 20°C). In the efficiently cooled region, the temperature increase ΔT_{ef} is 35°C, and in the inefficiently cooled region, the ΔT_{inef} is 87°C at the maximum electric field allowed by the instrument. Again, the greatest temperature increases occur at the lowest conductivity in Fig. 1 due to restrictions on voltage resulted from the maximum current that is allowed to flow by the instrument.

It should be emphasized that temperature increase depends on the product of voltage and current, for which maximum values are limited by the instrument. As a result, there are two possible scenarios for how ΔT_{ef} and ΔT_{inef} depend on conductivity. When the instrument is functioning at its maximum voltage and the electric current is below its maximum value, ΔT_{ef} and ΔT_{inef} increase with the electrical conductivity of the electrolyte. However, if the instrument is operating at its maximum electric current, increasing κ_0 leads to a decrease in ΔT_{ef} and ΔT_{inef} because the voltage corresponding
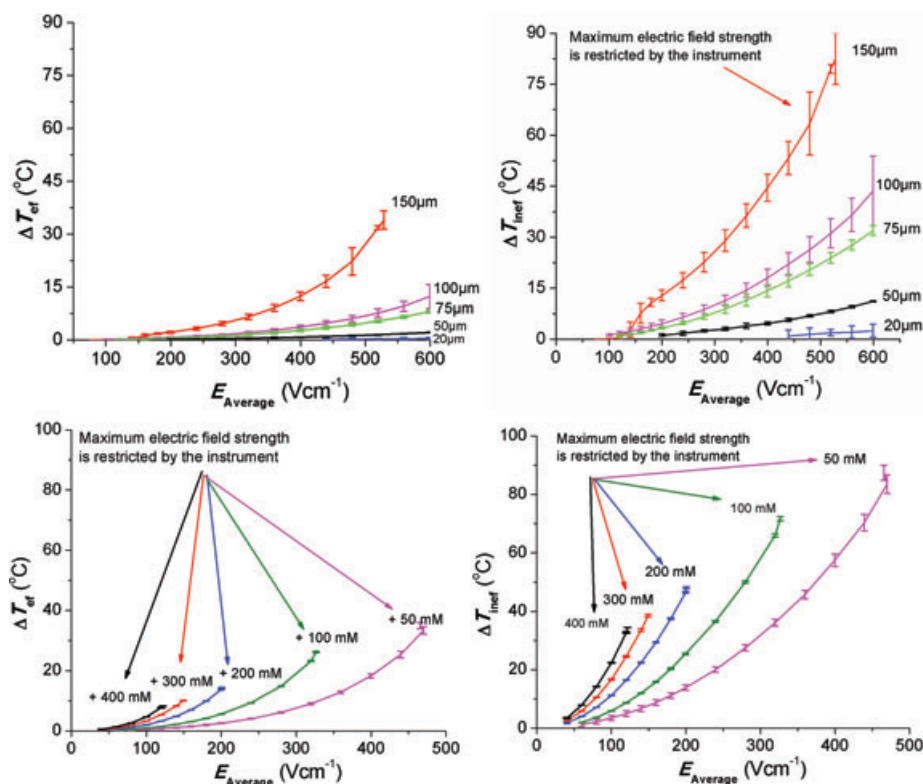
Figure 1. UMET-determined variation of temperature increase for the electrolyte in the efficiently cooled (left panels) and inefficiently cooled (right panels) sections of the capillary versus electric field strength for a range of inner diameters (top panels) and a range of ionic strengths (bottom panels). Error bars show 2 standard deviations ($n = 3$). The total length of each capillary was 50.0 cm and the electrolyte was 50 mM TRIS + 25 mM CH_3COOH at pH 8.3. For the bottom panels, the inner diameter of the capillary was 75 μm and varying concentrations of KCl were added; KCl concentrations are shown in the panels. Buffer vials were kept at the same temperature as the set temperature of the capillary coolant before electrophoresis.

to the maximum current decreases and consequently heat energy is produced at a lower rate. We found that values of A_{ef} and A_{inef} increased with the capillary diameter as expected. It should be emphasized that coefficients A_{ef} and A_{inef} and powers B_{ef} and B_{inef} are not universal but depend on the conductivity of the electrolyte used. In practice, when carrying out CE-based separation, one might be tempted to assume that remarkable temperature rises can be avoided by using capillaries with inner diameters of 50 μm or less. It is true if buffers with the same conductivities are used in different capillaries. However, narrower capillaries allow one to use very high conductive buffers that are not applicable in wider capillaries due to restrictions on the maximum current. As a result, even in 20 μm capillaries, ΔT_{ef} and ΔT_{inef} may exceed 30°C and 70°C, respectively (Supporting Information Fig. S1), if $E_{Average} = 600$ V/cm and the electrolyte conductivity is sufficiently high ($\kappa_0 > 5$ S/m). The variations of ΔT_{ef} and ΔT_{inef} with the average electric field strength, $E_{Average}$, were also determined over a range of capillary inner diameters for various electrolyte conductivities (Figs. S1–S5 in the Supporting Information). The temperature increased linearly for smaller diameter capillaries and exponentially for capillaries with larger diameters in both the efficiently and inefficiently cooled regions.

From the above analysis, one can assume that the rate of heat generation can be minimized by lowering the conductivity of the electrolyte and the voltage used, by decreasing the inner diameter of the capillary and increasing the length of the capillary. Unfortunately, these measures may not be in the

interests of separation efficiency and separation times. Separation efficiency and separation time are both dependent on the electric field strength applied. Conducting experiments at low voltage leads to a lower electric field strength and lower velocity of the ions in the electrolyte and, thus, increased sample diffusion and increased separation time.

It would be instructive to compare temperature increases in an Agilent 7100 CE instrument (Agilent Technologies) to those in a Beckman MDQ CE instrument (for capillary lengths of 50.0 cm). The analysis of data shown in Fig. 1 and similar data for Beckman MDQ [4] leads to the following conclusions. Dependencies of the temperature increase on the electric field strength $E_{Average}$ calculated at various values of the internal diameter d_i and the electrolyte conductivity κ_0 look similar in both instruments. However, the temperature increase is more prominent in Agilent 7100 (Agilent Technologies) than that in Beckman MDQ in both efficiently cooled and inefficiently cooled sections (at the same values of $E_{Average}$, d_i, and κ_0). It is consistent with the fact that cooling by a liquid flow is typically more efficient than that by air flow.

3.3 Derivation of SUMET for Agilent 7100

UMET predicts temperature for a CE instrument and an electrolyte based on data collected for that pair. As a result, UMET is a fairly accurate method of temperature determination. However, it is experimentally challenging, and moderately
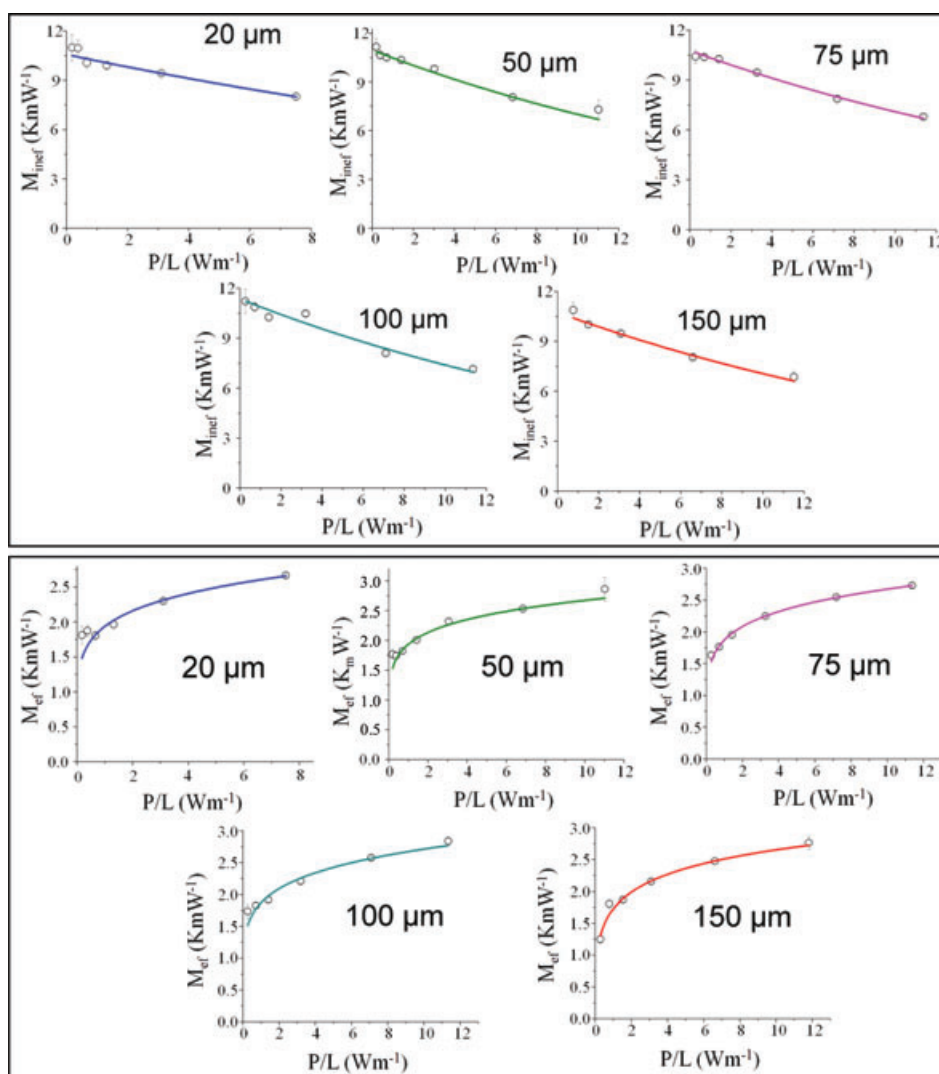
Figure 2. Universal multipliers for the determination of the temperature rise in capillaries of different inner diameters. The top panel shows how the multipliers vary with the power per unit length in the inefficiently cooled section of the capillary and the bottom panel shows how they vary in the efficiently cooled sections. Error bars show ±2 standard deviations.

time consuming to implement. The method requires measuring values of current at different voltages applied. The procedure is then repeated for a few temperatures and the collected data are used to determine the temperature coefficient of electrical conductivity for each new electrolyte. This obstacle is overcome in SUMET developed and implemented for the Beckman Coulter MDQ instrument [4]. SUMET is based on previously formulated hypothesis that the temperature increase in both efficiently and inefficiently cooled sections of the capillary should depend on the power per unit length averaged over the whole capillary [4]

$$\Delta T_{\text{ef}} = M_{\text{ef}}(V \times I)/L, \quad \Delta T_{\text{inef}} = M_{\text{inef}}(V \times I)/L \quad (16)$$

where M_{ef} and M_{inef} are multipliers with units of Km/W. They depend on the power per unit length, P/L, and have to be found specifically for each CE instrument with typical electrolytes used. To design SUMET for the Agilent 7100 CE instrument (Agilent Technologies), we carried out UMET approach to collect the required data set of ΔT_{ef} and ΔT_{inef} (Fig. 1 and Figs. S1–S5 in the Supporting Information). Then, the

entire data set was used to determine dependences of M_{ef} and M_{inef} on P/L (Fig. 2). Each of the plots of M_{ef} versus P/L was sigmoidal in shape and the plots of M_{inef} versus P/L resembled exponential decay curves. Since analytical solutions for these dependences do not exist, we trialed a number of fitting functions using Origin® software. The curves of M_{ef} and M_{inef} versus P/L were best fitted to the following equations:

$$M_{\text{ef}} = c\left(\frac{P}{L}\right)^{n}\left(g + \left(\frac{P}{L}\right)^{n}\right)^{-1}, \quad M_{\text{inef}} = ka^{\left(\frac{P}{L}\right)} \quad (17)$$

Here, c, g, n, k, and a are instrument-specific and capillary-dependent empirical parameters shown in Tables 1 and 2. These tables were obtained for 50 cm length capillaries of various inner diameters using UMET calculations. Tables 1 and 2 will change slightly for instruments with different capillary lengths as the key parameter for determining ΔT_{ef} and ΔT_{inef} is the average power per unit length for the whole capillary, which can be determined for any length of capillary.

Table 1. Constants for calculating M_{inef} for capillaries of varying internal diameters for an Agilent 7100 instrument (Agilent Technologies)

d_i (μm)	k	a	R^2
20	10.566	0.964	0.994
50	10.950	0.956	0.991
75	10.771	0.959	0.992
100	11.361	0.958	0.917
150	10.741	0.958	0.979

Table 2. Constants for calculating M_{ef} for capillaries of varying internal diameters for an Agilent 7100 instrument (Agilent Technologies)

d_i (μm)	c	g	n	R^2
20	14 229.121	7348.067	0.157	0.962
50	13 314.457	6881.039	0.140	0.979
75	12 075.791	6445.318	0.154	0.992
100	22 892.817	12 237.401	0.161	0.949
150	5.404	2.137	0.313	0.968

It should be noted that the first equation from (17) gives $M_{ef} = 0$ at $P/L = 0$ whereas the linear extrapolation of data in Fig. 2 to $P/L = 0$ gives values of M_{ef} around 1.5 Km/W. This discrepancy can suggest that the corresponding experimental curves undergo very fast decline when P/L approaches zero and, therefore, such simple extrapolation does not work in this range of P/L. On the other hand, if the linear extrapolation is legitimate, then the fitting Eq. (17) for M_{ef} itself does not work for very small values of P/L. Anyway, the latter are out of the range of experimental data shown in Fig. 2 for which both fitting equations 17 give satisfactory results. Equations (17) allow one to predict ΔT_{ef} and ΔT_{inef} for any experiment conducted using an Agilent 7100 instrument (Agilent Technologies) using only the experimental values of the currents and voltages recorded by the instrument. First, parameters c, g, n, k, and a (corresponding to the internal diameter d_i of the capillary used in experiments) should be found from Tables 1 and 2. Various values of d_i are shown in the first columns of Tables 1 and 2. Then, parameters c, g, n, k, and a as well as the measured power per unit length, P/L, should be substituted in Eqs. (17). They give values of coefficients M_{ef} and M_{inef} present in Eqs. (16). Finally, theses equations allow one to calculate the temperature increases ΔT_{ef} and ΔT_{inef}.

4 Concluding remarks

In this work, we applied UMET approach to the Agilent 7100 CE instrument (Agilent Technologies) to determine the temperature increases in the efficiently and inefficiently cooled

sections for various capillaries and electrolytes. Based on this entire data set, we designed simplified UMET (SUMET) and determined all specific parameters (Tables 1 and 2) required for its application. SUMET is an instrument-specific approach that is much simpler and less time consuming. It requires only one current–voltage measurement that can be performed in a minute and a simple algebraic calculation using the derived instrument-specific empirical Eq. (17) with parameters determined by Tables 1 and 2. These results allow an operator to accurately and easily predict the temperature increases in the Agilent 7100 CE instrument (Agilent Technologies).

The authors have declared no conflict of interest.

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