Thermostating in Capillary Electrophoresis

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Capillary electrophoresis
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Summary

The use of high voltages across a electrophoresis capillary will increase the temperature of the buffer due to Joule heating. As a result temperature control in CE is rather important since variations in the buffer temperature will result in changes in the pH of the buffer, peak shape, migration time, reproducibility, efficiency, 3-D structure of macromolecular analytes, etc. Six different thermostating systems have been evaluated: (i) natural convection, (ii) fan, (iii) home-made and (iv and v) two commercially available high-speed air and a (vi) liquid thermostated device. In all cases the temperature of the buffer in the capillary is calculated according to the temperature-conductivity relationship. For this purpose two parameters are introduced describing temperature control: the temperature onset (δT) and the temperature rise factor (α). From these results, it can be concluded that high speed air thermostating can be as efficient as liquid thermostating.

Introduction

During the past five years the number of commercially available capillary electrophoresis (CE) systems has increased rapidly. Comparison of these systems shows important differences such as automation potential, maximum number of samples that can be analyzed, method of thermostating the capillary, thermostating range and detection unit. One of the most important features, with respect to reproducibility, is temperature control of the capillary.

In CE a relatively high power (up to 9 W) is produced in a rather small volume (about 1–10 µl), which corresponds to a generated heat of up to 900 J/cm^3/s. Therefore, accurate control of the buffer temperature will avoid excessive temperature effects. An increase of the temperature (which can be over 70 °C [1]) in the capillary can have the following effects: (i) a change in the pH of the buffer because dissociation constants strongly depend on the temperature [2], a part of the sample can be purged out of the capillary due to buffer expansion when a voltage is applied across the capillary resulting in sample losses[3], (iii) the radial temperature gradient of the buffer in the capillary is proportional to the increase in temperature of the buffer compared with the temperature of the surrounding medium. The viscosity is also temperature-dependent. This results in a radial dependence of the ion mobility, which affects band broadening [4, 5], (iv) when the voltage is increased the system reproducibility is decreased in the order of migration time > peak height > peak area [6], (v) as a result of the current and voltage limitations of the power supply. When the current increases faster than expected according to Ohm's law – due to Joule heating – the current limitation may be reached at lower voltages resulting in longer analysis times, (vi) the concentration profile can change on boundary surfaces due to axial temperature fluctuations [7], (vii) because of the temperature-dependence of the refractive index in the capillary, the fluctuations of the baseline can be increased. Limitation of buffer temperature changes, will stabilize fluctuations of the baseline [8] (viii) the mobility of (analyte) ions can be changed by using higher electric fields [9, 10] and the peak shapes can deteriorate because the equilibrium constants of micellar processes, protein orientations and chiral processes are changing with temperature [11].

Thermostating can be performed in two ways: by using either convection or thermal conductivity. In the latter approach, designated solid-state thermostating, the capillary is placed in a medium with high thermal conductivity (i.e. aluminium). This possibility is not frequently used in CE, although it is rather efficient [12].

With respect to convection thermostating, two modes can be distinguished: natural and forced convection. Natural, or non-controlled, convection implies cooling of the capillary by heat transfer to a stationary medium. When a voltage is applied across the capillary, the buffer and capillary temperature will increase, leading to an increase in the temperature of the surrounding medium and to a decrease of its density. Therefore, the surrounding medium will start moving by convection. On the other hand, forced convection implies that the outside wall of the capillary is heated/cooled by a moving thermostating medium. The magnitude of at least two parameters seems to be relevant to ensure efficient thermostating: the heat capacity and the velocity of the surrounding medium. Two media are generally used in CE: liquids (i.e. halogenated alkanes) or air. Liquids have the advantage of possessing a larger heat absorption capacity [1], but air has the advantages of being safe (in case of a broken capillary), easy-to-use and cheap. In natural convection the velocity of the medium is related to the temperature difference between the medium and the capillary; with forced convection this difference is related to the velocity of the medium. Air cooling is generally performed either by means of a fan (typical air velocities are 1.5 m/s [1]) or by using a high-speed gas flow (> 10 m/s). In the latter approach air thermostating can be as effective as liquid thermostating [13].

Because the influence of temperature and thermostating on a CE separation has been discussed in detail [2–11], the emphasis in this study is on the thermostating process itself. To quantify this effect, first of all, the theoretical background will be outlined.

In CE the resistance (expressed in Ω/m) is constant for all voltages during increase of the electric field, E (in V/m) across the capillary, providied that all heat generated in the buffer is transported out of the capillary. The electric current, i (in A) is directly proportional to the electric field.

When the transport of heat out of the capillary is not sufficient, the temperature will increase and the resistance will decrease. The effect of this is an increase in current above that expected on the basis of Ohm's law. This information can be used to quantify the thermostating behaviour of CE systems by measuring the current during a stepwise increase of the voltage and plotting the current versus the applied field [14]. The lower the resistance of the buffer, the more critical is the thermostating of the system. However, it should be emphasized that even in a properly thermostated capillary, a temperature increase of the buffer can appear if a low resistance is combined with a high E and a large I.D. of the capillary [1]. For a properly thermostated capillary with an I.D. of 100 μm the radial difference in temperature between the buffer in the capillary, and the capillary wall, is about 1 °C when a field of 7 W/m is applied [15].

Various researchers have addressed the problem of measuring the temperature inside the capillary and several approaches have been followed:

Wätzig added thermochromic molecules to the buffer [16]. The absorbance of these molecules is a function of the temperature. By measuring the increase of the background absorption, caused by these molecules, the temperature of the buffer can be calculated. The disadvantage of this method is that solutes have to be added to the buffer which can interfere with the analysis. Another disadvantage is that the thermochromic behaviour of the added solutes depends on the pH which requires calibration for each individual pH.

Burgi et al. used the interrelation between the electro-osmotic flow and temperature [17]. This approach has the disadvantage that a quantitative relation has to be determined for each individual pH and each ionic strength. Another disadvantage is that in the same CE system, using the same buffer and capillary, a highly fluctuating electric-osmotic flow can be found [18].

Terabe et al. followed a straightforward approach, i.e. measurement of the temperature with a thermocouple [19]. Unfortunately, the exact temperature in the capillary is difficult to measure because of a relatively large surface of the thermocouple compared with the O.D. of the capillary, resulting in disturbance of the temperature measurement by the surrounding medium (i.e. air).

Hjertén calculated the buffer temperature for a non-thermostated as well as a thermostated system [20]. For the first system this is based on the increase of the current immediately after applying the voltage compared with the end of the run. In this approach a higher current is correlated with an increase of the temperature of the buffer. This method can only be applied when the voltage is applied instantaneously. This is in contrary to the normally used procedure, where the voltage is applied rather gradually (in at least a few seconds). For the second system the temperature inside the capillary is calculated from the geometry of the capillary and the thermal conductivities of quartz and the polyimide coating. In this case the velocity and the type of the surrounding medium are not taken into consideration. However the limitation of both procedures is that they are not based on experimental data, but use only theoretically derived equations

Bello et al. correlated the change of the electric conductivity of the buffer (κ in 1/Ω cm) with the temperature (T in °C) using experimentally determined data, as described below [10, 21].

Only by using this method, can the temperature of the buffer be determined accurately during the analysis without adding solutes to the buffer or modifying the CE system. The method introduced by Bello et al. will be used in this paper.

The temperature(T)-conductivity(κ) relation of a buffer can be written as:

$$T = c + d\kappa + e\kappa^2 \qquad (1)$$

where c, d and e are empirical parameters. If the values of these parameters are determined for a buffer (using a thermostated box and conductivity meter), the temperature in the capillary (T_{cal}) during an electrophoretic process can be calculated from the current, length of the capillary (L in m) and cross-sectional area (A in m^2) of the capillary [22]. The values of T_{cal} obtained at different fields can be plotted as a function of the power (P in W/m). According to the literature T_{cal} is linear with the generated power [10, 15, 17, 18]:

$$T_{cal} = T_{0\,Watt} + \alpha P \qquad (2)$$

In this equation $T_{0\,Watt}$ is the temperature of the buffer in the capillary (in °C), when no power is generated. The temperature rise factor (in °C m/W) is called α.

The difference between $T_{0\,Watt}$ and the temperature set (T_{set} in °C) is called the temperature onset (δT in °C). Incorporation of this term into Eq. (2) results in:

$$T_{cal} = Tset + \delta T + \alpha P \qquad (3)$$

Equation (3) indicates that the thermostating quality of a CE system can be characterized by the parameters α and δT, which can be determined by plotting the temperature versus the generated power. The parameter δT provides information on the systematic bias in the temperature control of the system due to incomplete thermostating of the capillary. The second parameter α reflects the ability of the CE system to remove the generated heat. In an ideal situation the temperature is independent of the generated power ($\alpha = 0$) and the complete capillary is thermostated ($\delta T = 0$). Only under these conditions, is the temperature of the buffer really independent of the generated power ($T_{cal} = T_{set}$).

The thermostating behaviour of CE systems can be characterized by the following procedure: (i) a test buffer, with high conductivity, is selected, (ii) the temperature-conductivity relation and the parameters of Eq. (1) are determined from the test buffer, (iii) the current is measured in different CE systems, by using a stepwise increase of the field across capillaries filled with the test buffer, and at different temperatures, (iv) the temperatures at different field strengths are calculated from Eq. (1) and finally, (v) the CE systems are characterized by calculating δT and α from Eq. (2).

It should be kept in mind that, during the use of the procedure described above, the radial temperature profile is neglected and the temperature in the whole capillary is assumed to be constant. Therefore, the calculated temperature is not the local temperature of the buffer in the capillary but a length- and radial-averaged temperature.

Experimental

Materials

Sodium chloride, pro analyse, was purchased from Riedel-de Haën (Seelze, Germany), boric acid, pro analyse, and sodium hydroxide came from J.T. Baker (Deventer, The Netherlands). Water was demineralized and distilled before use.

Capillary Electrophoresis

The six CE configurations used to compare the thermostating capabilities were:

(i) The natural thermostated configuration:
    The system consisted of a PrinCE unit (Lauer Labs, Emmen, The Netherlands) and was equipped with an UV detector, model 759A (Applied Biosystems, Foster City, CA). The UV detector was set at 210 nm. The device consisted of three compartments: the sample/injection, the UV-detector and the thermostating compartment. In this set-up the fan was in the off position resulting in a non thermostated capillary.

(ii) The fan thermostated configuration:
    The CE was the same as in (i) but in this set-up the fan was switched on. Only 25 % of the capillary was thermostated.

(iii)The home-made high-speed thermostated configuration:
    The system set-up used was the same as in (i) except that the capillary was placed for 70 % in a tube with an I.D. of 6 mm through which temperature controlled air was blown (35 m/s). The air was thermostated in a copper tube, situated in a thermostated bath, and transported via an insulated tube with an I.D. of 8 mm. The bath consisted of a cryostat and a temperature controlled heater with a water jet.

(iv)The commercial high-speed thermostated configuration A:
    The CE system was an HPCE 3D system (Hewlett Packard, Waldbronn, Germany). The capillary was placed in a cassette, in which a fast, temperature controlled flow of air (10 m/s) is used to thermostat the capillary. About 78 % of the capillary was temperature-controlled. The UV/VIS detector was switched on, to include heating of the capillary by the lamp. The sample tray temperature was 25 °C.

(v)The commercial high-speed thermostated configuration B:
    A SpectroPhoresis 2000 system (TSP, San Jose, CA) was used. The capillary was placed in a cassette through which a thermostated gas was blown at high speed. In this way 77 % of the capillary was thermostated. The UV lamp was switched on.
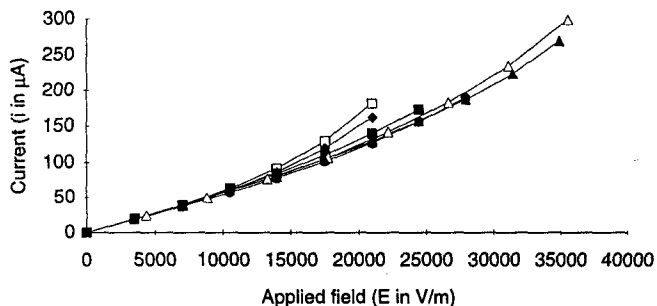
Figure 1

Ohm's plot for the Configurations i (o), ii (u), iii (n), iv (l), v (Δ) and vi (s). The temperature of Configuration i was 21 °C, of Configuration iii 33.1 °C and of Configuration ii and iv–vi 30.0 °C.

Figure 2

Plot of the temperature versus the specific conductivity measured with the conductivity electrode.

(vi)The liquid thermostated configuration:

The BioFocus 3000 (Bio-Rad, Hercules, CA) was used. The capillary was placed in a tube, 1.5 mm I.D., through which thermostated liquid flows (0.29 m/s). The tube was placed in a cassette and 87 % of the capillary was temperature controlled. Again in this set-up the UV/VIS-detector was switched on. The sample tray temperature was 20 °C.

All CE experiments were performed with the same fused silica capillary of 75 μm I.D. and 375 μm O.D. The length of the capillary was 860 mm in the experiments with Configurations i to iv, 669 mm in Configuration v and 858 mm in Configuration vi.

The capillary was obtained from LC-Service (Emmen, The Netherlands). No window was burned off because only the electric current was measured. The capillary was conditioned by flushing with 1 M NaOH (5 min), water (15 min) and finally the test buffer (30 min) using a pressure of 2 bar. After the experiments the capillary was washed with water (5 min).

The test buffer (100 mM sodium chloride, 40 mM boric acid, pH = 8.0) was filtered through a 0.2 μm filter, purchased from Schleicher & Schuell (Dassel, Germany).

The voltage was increased every 2 min by 3 kV starting from 0 kV until the voltage was 30 kV. During the run the current was measured.
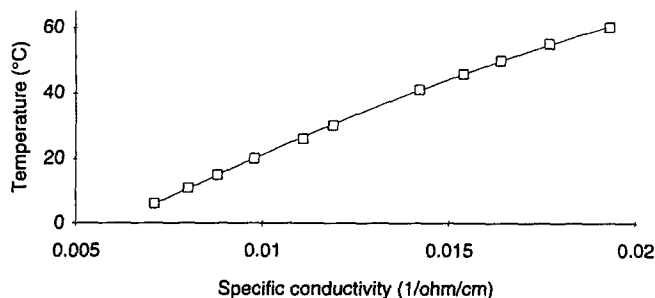
Conductivity Measurements

A conductivity meter, model PW 9501/01, equipped with a conductivity electrode, type PW 9550/60 (both Philips, Eindhoven, The Netherlands) was used. The samples were thermostated as described above.

Results and Discussion

Ohms Law Plots

Values of the electric current against the applied electric field in the different set-ups using temperatures of about 30 °C (except for the experiments performed at room temperature) are given in Figure 1. It can be seen that the current depends on the set-up employed. For

fields below 10 kV/m the differences are rather small. This is in contrast to the experiments performed at higher fields, where it significantly depends on the set-up used.

The highest currents are found for the natural convection device, despite the fact that for this system the T_{set} value is the lowest. With the fan temperature controlled capillary a smaller current is measured; significantly lower currents are found for the high speed air and the liquid thermostated devices which indicates better thermostating capabilities.

Temperature-Conductivity Relationship

The relationship observed between the measured temperature and conductivity of the test buffer, is shown in Figure 2 .

The parameters of Eq. (1), calculated in the temperature range of 6–60 °C, were $c = -37.834$, $d = 6777$ and $e = -87480$. The maximum error between the calculated and measured temperatures is ± 0.4 °C in this range.
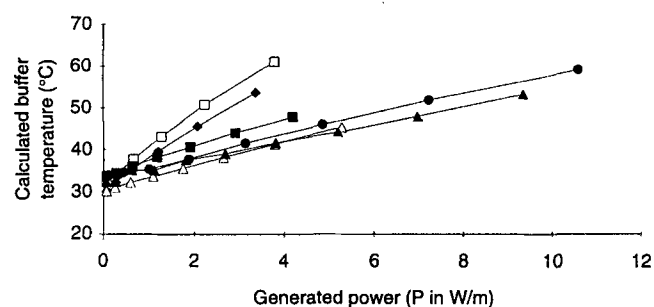
Calculated Temperatures

As anticipated, the buffer temperatures calculated on the basis of Ohm's law (Figure 1), strongly depend on the generated power. This is shown in Figure 2. These temperatures change significantly with the applied field. This is rather pronounced for Configuration i where the temperature was set on 21 °C and the actual temperature inside the capillary was about 60 °C when 4 W/m was generated. The buffer temperature of the fan thermostated device increased rather rapidly with the field. The other four devices behaved quite similarly. The temperature was set on 33.1 °C for Configuration iii and 30 °C for Configurations iv–vi.

The temperature can also be calculated according to Hjertén [21]. In order to do this the current should be measured immediately after applying the voltage and after equilibration of the system. The current just after the voltage has been applied is not known in this study, because the voltage is applied stepwise. This value can be estimated assuming that at a low voltage (i.e. 3 kV)

Table I. Comparison of the calculated temperatures according to the conductivity method and the method according to Hjertén (for details see text).

V	Current	Tcal (°C)	
(kV)	(mA)	Conductivity method	Hjertén method
0	0		
3	19	32.3	
6	39.1	34.1	33.4
9	62.4	37.7	35.8
12	91.1	43.2	39.7
15	128.5	50.9	45.4
18	180.6	61.1	53.9

Figure 3

Calculated buffer temperatures in the capillary versus the generated power with symbols and temperatures as in Figure 1.
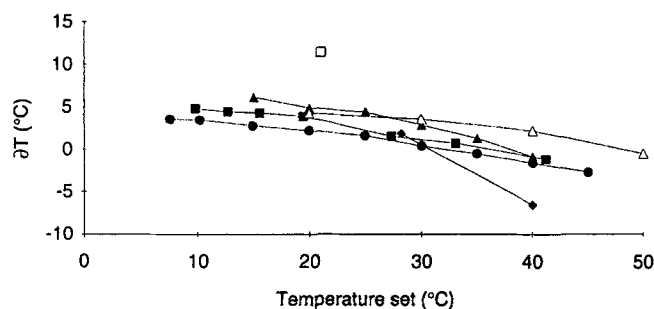
Figure 4

Temperature rise factors plotted against the temperature set with symbols as in Figure 1.
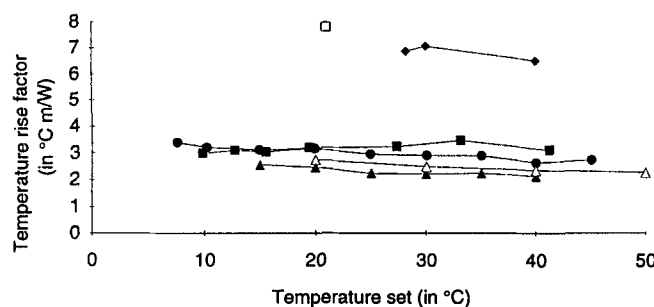
Figure 5

The temperature onset plotted against the temperature set. Symbols as in Figure 1.

Joule heating is minimal and extrapolation to a higher voltage, according to Ohm's law, is allowed. In Table I the two calculated temperatures are compared . As can be seen the temperature calculated with the procedure used in this study is higher than calculated with the method proposed by Hjertén. This difference will be taken in account when comparing α values.

Temperature Rise Factor and Temperature Onset

The parameters δT and α can be derived from the data given in Figure 3 as already described.

For the six devices compared, δT as a function of T_{set} is depicted in Figure 4. Similarly, Figure 5 shows a as a function of T_{set}.

The δT values mainly depend on T_{set}. The smaller the difference between T_{set} and room temperature the smaller δT. A value of T_{set} below the temperature in the CE capillary (which is somewhat higher than room temperature) results in a positive δT; the capillary is heated. A value of T_{set} higher than the temperature in the CE capillary results in a negative δT; the capillary is cooled by the surrounding medium.

The smallest deviations of zero for δT are found for the commercial high-speed air thermostated configuration A as can be seen in Figure 4 (Configuration iv).

For the α values the following results are found: natural convection has the highest value, the values for fan thermostating systems are slightly smaller and much lower values are found with forced air thermostated devices and the liquid thermostated device. The difference between the three high speed gas thermostating systems is larger than the difference between the high speed gas thermostating systems (iii–v) and the liquid thermostating device (vi). Because of this, it can be concluded that the α value is about the same for liquid and high-speed thermostating methods with a small benefit for the liquid thermostating method. Although liquid thermostating will become more efficient using a power higher than 6 W/m, this is not realistic because this will have a negative influence on the peak widths in the electropherogram.

The thermostating potential is characterized by the combination of α and δT. When these two parameters are taken into account it is difficult to decide which one (liquid or high speed gas thermostating) is better. However, both are significantly better than the free convection and the fan thermostated devices.

Evaluation of the Results

CE systems can be compared by using the temperature rise factors as shown in Table II. The results are calculated with data that can be found in the literature [8, 12, 16, 17]. The results of the present study are in agreement with those found in the literature, except for two α values which are greater than previously presented [15]. Except these two, there is a constant factor (ca. 1.5–2) between the literature data and the values presented

Table II. Comparison of the determined temperature rise factors with those calculated from the literature.

Thermostating type	Source	Average α ($^{\circ}$C m/W)	Temperature ($^{\circ}$C)
Natural convection	PS	7.8	21
	[17]	11.1	25
	[17]	12.5	25
	[17]	11	25
	[12]	12.4	?
	[21]/PS	5.8	21
Fan thermostating	PS	6.8	28–40
	[8]	10	27
Forced air thermostating	PS	3.2	10–41
	PS	3.0	8–45
	PS	2.5	20–50
	[17]	5	25
	[17]	6	25
	[12]	5	?
Liquid thermostating	[15]	0.35	25
	PS	2.3	15–40
Solid state thermostating	[12]	0.6	?

PS = present study.

above. The α value for the liquid thermostated system is a factor 7 higher than found in the literature [16]. The latter value is rather small, and implies an increase of only 6 °C when a power of 10 W/m is applied. This is unlikely given the data shown in Figure 1.

The value for the non-thermostated device calculated according to Hjertén is smaller than the values found in this study and given in the literature. The most important conclusion is that accurate calculation of α and δT values, from existing data in the literature, is rather difficult because in most studies quite a number of assumptions have been made. This probably explains the differences found between the present and former studies.

Conclusions

The present method can be used to determine the buffer temperature in the capillary, even for experiments which have been performed in the past.

By using the temperature rise factor and temperature onset the thermostating quality of a CE device can be characterized in a simple way. From the results presented it can concluded that high speed air thermostating can be as efficient as liquid thermostating .

In addition it is shown that using the PrinCE with a high speed air thermostating unit significantly improves its thermostating quality.

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