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Discussion

Recalculation of the temperature inside capillaries using high buffer concentrations

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In a recent contribution to the *Journal of Chromatography A* [1] by Cross and Cao, the internal temperature of a capillary column during capillary electrophoresis was determined experimentally. However, there was much difference between the results calculated by different theories. According to Cross and Cao's method [1], the temperature inside the capillary was about 37°C, while it was about 59.5°C using Knox and McCormack's method [2] and 66.97°C by Liu et al.'s method [3].

To evaluate the rationality of the two results, our strong preference was to use a theoretically based model or one of the generally accepted empirical methods [4–6]. However, to date we have been unsuccessful in adapting any of the accepted methods to recalculate the temperature inside the capillary on the basis of the data provided in Cross and Cao's paper. The methods of Bello et al. [5] and Burgi et al. [6] have the feasibility to estimate the buffer temperature, but they impose some undesirable limitations. Accordingly, we describe here an extremely useful method that satisfies the need for recalculating temperature inside the capillary on the basis of the Cross and Cao original data.

The migration time for an analyte depends on the temperature according to the following relation [7,8]:

$$\log t = \log \frac{Al}{\epsilon E\left[\zeta_c + \frac{2\zeta_a}{3}\cdot f(\kappa\alpha)\right]} + \frac{B}{T} \quad (1)$$

in which t is migration time, l is the effective length of the capillary, ϵ is the dielectric constant, η is the viscosity of the buffer medium, ζ_c and ζ_a are the zeta potentials of the inner wall and the analyte, respectively, $f(\kappa\alpha)$ is a function dependent upon the shape, κ is the reciprocal of the double layer thickness, α is the radius of the analyte, T is the temperature inside the capillary, A and B are constants related to the properties of the buffer. According to elementary theory the electroosmotic velocity ν_{eo} can be expressed as:

$$\nu_{eo} = \frac{\epsilon\zeta_c E}{\eta} \quad (2)$$

Now, the following relation holds between electric current I and electric strength E [9]:

$$I = \pi r^2 k_e E \quad (3)$$

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where k_e and r denote specific conductance and radius, respectively.

Combining Eqs. (2) and (3):

$$I = k_e \eta \frac{\pi r^2}{\epsilon \zeta_c} \cdot \nu_{eo} \quad (4)$$

Knox and McCormack's experimental results have demonstrated that ν_{eo} increases linearly with electric current [2]. Since the conductivity of buffer solution, k_{eo}, varies inversely with viscosity (Walden rule), the linearity of I with ν_{eo} suggests that the product of ϵ and ζ_c is constant. The experiment conducted by Watanabe et al. [10] showed that the product of electrophoretic mobility and viscosity is fairly constant with temperature. This suggests that the product of ϵ and ζ_a is also temperature independent. As $\epsilon\zeta_c$, $\epsilon\zeta_a$ and $f(\kappa\alpha)$ are all relatively insensitive to the temperature change [11–13] the first term on the right hand side of Eq. (4) may be regarded as a constant when the same electrolyte solution and capillary are used. On this assumption, the migration time for a specific analyte is only dependent on the temperature inside capillary.

We assume that the ideal migration time of an analyte (n) is $t_n(I)$ under no Joule heating in capillary zone electrophoresis (CZE), and its corresponding temperature inside the capillary is $T(I)$, which is equal to the set temperature. Similarly, the real experimentally obtained migration time is $t_n(R)$, and its corresponding temperature is $T(R)$. ''R'' and ''I'' in parentheses denote real and ideal conditions, respectively.

Thus, Eq. (1) yields:

$$T(R) = \frac{B}{\log \frac{t_n(R)}{t_n(I)} + \frac{B}{T(I)}} \quad (5)$$

Once the values of B, $t_n(R)$ and $t_n(I)$ are known, $T(R)$ can be calculated from Eq. (5). In order to obtain the value of B, we have to make the assumption that the change of viscosity of the buffer used for capillary electrophoresis with temperature is identical to that of water. Eq. (60 relates the viscosity of the buffer to the temperature as [14]:

$$\log \eta = \log A + \frac{B}{T} \quad (6)$$

where A is a constant when the buffer is fixed. One hundred sets of the viscosities for water from 0 to 100°C were obtained from the literature [15]. By plotting a line of the logarithm of the viscosity to the reciprocal of the absolute temperature, the value of B is 797.5.

According to the theory of CZE, as described by Jorgenson [16], the following relationship exists:

$$t = \frac{lL}{\mu_e + \mu_{eo}} \cdot \frac{1}{V} \quad (7)$$

where L is the total length, V is the applied voltage, μ_e is the electrophoretic mobility and μ_{eo} is the electroosmotic mobility. In the case of the neglect of the heating generated during a run, the first part of the right-hand-side in Eq. (7) is a constant. Thus, there exists a linear relationship between t and $1/V$, and this line should pass through the point (0,0). This suggests $t_n(I)V$ is constant under this circumstance.

Similarly, we have found [17] that there also exists a linear relationship between t and $1/V$ under real electrophoretic conditions. It should be pointed out that the latter linear relationship has a negative intercept, that is:

$$t = \frac{b}{V} - a \quad (8)$$

For a specific analyte and the fixed electrophoretic buffer, a and b are positive constants. This suggests that $t_n(R)V$ decreases linearly with V in this circumstance. Thus the $t_n(I)V$ value can be obtained from the intercept of the plot of $t_n(R)V$ against V. Using this method the ratio of $t_n(R)$ to $t_n(I)$ in Eq. (5) at any voltage can be calculated.

Next we use our method to estimate the temperature inside the capillary under Cross and Cao's experimental conditions. Variation in electroosmotic mobilities with applied voltage for 210 mM sodium phosphate buffer was shown in their Table 2. Because the total length and the effective length of the capillary were provided in their paper, the corresponding migration time can be easily calculated. The variation in the calculated migration time with applied voltage is shown in Table 1.

Regression analyses of $t_n(R)$ (listed in Table 1) against $1/V$ indicated migration time and reciprocal

Table 1
The migration times t_n(R) and the calculated temperatures inside the capillary T(R) at various applied voltages

V (kV)	t_n(R) (min)	T(R) (°C)
6.0	34.51	39.14
8.0	23.55	42.73
10.0	17.96	46.67
12.0	14.04	51.02
14.0	11.12	55.88
16.0	8.62	61.35
18.0	6.87	67.59

voltage is in a good linear relationship with a correlation coefficient of 0.99962. The regression equation is as follows:

$$t_n(\mathrm{R}) = -6.65737 + 245.7116 \cdot \frac{1}{V} \quad (9)$$

Thus:

$$t_n(\mathrm{R})V = -6.65737V + 245.7116 \quad (10)$$

For the ideal electrophoretic conditions, there exists the following relationship:

$$t_n(\mathrm{I})V = 245.7116 \quad (11)$$

The change of tV with V under ideal and real electrophoretic conditions are shown in Fig. 1. The temperatures inside the capillary at various voltages are presented in Table 1.

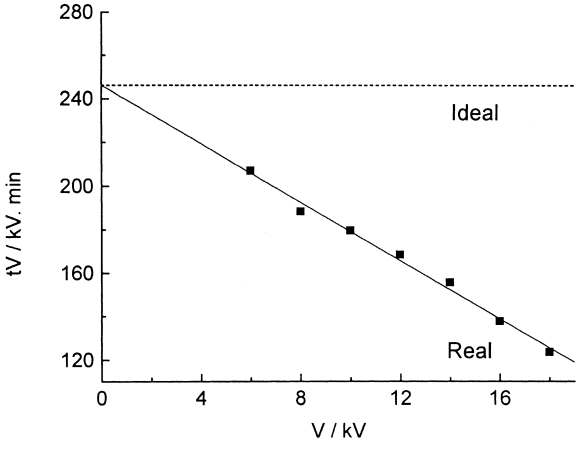

Fig. 1. The dependence of tV on V under ideal (where the Joule heating is negligible) and real electrophoretic conditions.

Fortunately, Cross and Cao provided enough information to verify our method. Firstly, our calculated result can be compared with that of Liu et al.'s method [3]. Liu et al. determined the temperature inside capillary by Raman spectroscopy. Cross and Cao [1] pointed out that the 18°C rise for Liu et al.'s experiment in internal temperature observed with the application of 1.7 W m^{-1} would be equivalent to 15.8°C for their system. At the first line of the right side on page 170 in Ref. [1], Cross and Cao gave the following information for 210 mM: V=18 kV, L=67.3 cm, E=26.7 kV m^{-1}, I=149 μA, EI=1.49 W m^{-1}. There is no doubt that the value of EI that they gave is incorrect. The correct value of EI should be 3.978 W m^{-1}. Thus, there is:

$$\frac{1.7}{15.8} = \frac{3.978}{\Delta T} \quad (12)$$

where ΔT is the temperature rise. As their all experiments were performed at the thermostatted temperature of 30°C, that is to say that T(I) is 30°C, $T(\mathrm{R}) = T(\mathrm{I}) + \Delta T = 30 + 36.97 = 66.97$°C. This value is in good agreement with that from our method. Secondly, our value is also close to the temperature calculated from the Knox and McCormack method, 59.7°C (this value was given in Cross and Cao's paper).

From the above analysis and calculation, we have reason to believe that the temperature calculated by Cross and Cao is incorrect. Perhaps some factors influencing the temperature inside the capillary have been overlooked in their calculating process.

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