

# Computer-assisted determination of the inner temperature and peak correction for capillary electrophoresis

Michael S. Bello<sup>☆</sup>, Eugene I. Levin<sup>☆☆</sup> and Pier Giorgio Righetti<sup>\*</sup>

*Faculty of Pharmacy and Department of Biomedical Sciences and Technologies, University of Milan, Via Celoria 2, Milan 20133 (Italy)*

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## ABSTRACT

A statistical algorithm, software package and experimental validation are presented for the buffer temperature and electric current predictions and recalculations of peak migration times. The statistical procedure allows the calculation of the confidence intervals for the buffer temperature and electric current and shows the necessity for an accurate determination of the thermal coefficient of the buffer electric conductivity. The software package implements predictions of the buffer temperature, electric current and migration times for any given voltage and coolant temperature.

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## INTRODUCTION

The temperature dependence of the analyte migration velocity in capillary electrophoresis (CE) weakens the reproducibility of this powerful technique because the buffer temperature varies with the variations of the coolant temperature and with dissipation of the Joule heat. Even when thermostating a capillary within a CE unit, one can guarantee only a constant coolant temperature, whereas the inner temperature of the buffer may vary from run to run owing to the Joule heating if the buffer conductivity and/or voltage changes. In principle, direct measurements of the buffer temperature are possible [1] and, provided that the temperature dependence of migration velocities is known, the correct migration times can be derived. However, direct measurements need special equipment and can

hardly be recommended as a tool for routine analysis. Temperature measurements by means of thermochromic solutions [2] are useful for obtaining the buffer temperature without a sample inside but the possibility of using them during an analytical run is not clear.

Another way to obtain the buffer temperature has been proposed recently [3]. The procedure of capillary calibration presented there consists of two steps: (1) at low voltage, when thermal effects are negligible, one determines the capillary resistance by measuring the electric current at this voltage; (2) then, also by measuring the electric current at a certain voltage, which must be high enough to produce thermal effects, one finds the overall Biot number, which is a thermal characteristic of a given capillary in a given cooling system.

These data are sufficient to predict the temperature and electric current within the capillary for any given voltage, provided that the buffer electric conductivity and its thermal coefficient are known. The usefulness of this procedure is that, after some algebraic transformations, it gives the buffer temperature and electric current not only for the calibrated capillary–buffer pair

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\* Corresponding author.

<sup>☆</sup> Permanent address: Institute of Macromolecular Compounds, Bolshoi 31, St. Petersburg 199004, Russian Federation.

<sup>☆☆</sup> Permanent address: A.F. Ioffe Physico-Technical Institute, St. Petersburg 194021, Russian Federation.

but also for a capillary once calibrated and filled with another buffer of known properties. A shortcoming of the calibration procedure [3] is that it relies on intuitive definitions such as “low voltage” and “high voltage” and does not give a quantitative criterion for the accuracy of the predicted values.

It was verified by Hjertén [4] that the migration velocity of a species at constant current is almost independent of temperature. Later, many workers showed that migration velocities are directly proportional to the electric current even when the temperature elevation is significant (see references cited in ref. 5). These results had been summarized and extended by Lee and Yeung [5], who introduced a migration index as a quantity proportional to the integral of electric current over time. Therefore, an ability to predict buffer temperature, which is important in itself, allows the prediction of electric current and migration time for a given voltage on condition that a migration time for a certain voltage is known.

The first aim of this paper is to report a method for the calculation of confidence intervals for the temperature and electric current predicted on the basis of the calibration measurements. Confidence intervals reflect the accuracy of predictions and indicate if the calibration has been made properly, *i.e.*, the electric current has been measured at both low and high voltage.

We present here a software package for an IBM-compatible PC implementing the calibration procedure, statistical algorithm, temperature and current prediction and peak correction due to the temperature changes. The package works under Microsoft Windows version 3.0 or later and does not require any preliminary knowledge of the thermal theory of CE.

A typical example of calibration, temperature and electric current predictions and comparison of the calculated peaks with the experimental peaks is given.

## THEORY

### Statistical algorithm

The purpose of this statistical algorithm is to process the data obtained as a result of a calibra-

tion procedure and to give estimates of the electric current and buffer temperature and their confidence intervals. This paper calls “calibration” a series of measurements of electric current  $I$  and the coolant temperature  $T_C$  corresponding to the applied voltage  $V$ .

We shall base the following treatment on the relationships from ref. 6:

$$I\left(r_0 - \frac{\alpha V^2}{2Bi_{OA}\pi\chi L}\right) = [1 + \alpha(T_C - T_0)]V \quad (1)$$

$$T = T_C + \frac{V^2[1 + \alpha(T_C - T_0)]}{2Bi_{OA}\pi\chi L\left(r_0 - \frac{\alpha V^2}{2Bi_{OA}\pi\chi L}\right)} \quad (2)$$

$$r_0 = \frac{4L}{\kappa_0\pi d_L^2} \quad (3)$$

where  $I$  is the electric current,  $V$  the voltage,  $T$  the buffer temperature,  $\kappa_0$  the specific conductivity of the buffer at the reference temperature  $T_0$ ,  $\alpha$  the temperature coefficient of electric conductivity,  $\chi$  the thermal conductivity of the buffer,  $T_C$  the temperature of the coolant,  $r_0$  the capillary resistance at reference temperature and in the absence of the Joule heating,  $d_L$  the capillary inner diameter,  $L$  the capillary length and  $Bi_{OA}$  is the overall Biot number.

For the  $i$ th measurement eqn. 1 can be rewritten in the following form:

$$r_i = a_i r_0 + b_i x, \quad i = 1, n \quad (4)$$

where  $r_i = V_i/I_i$ ,  $x = \alpha/Bi_{OA}\chi$ ,  $a_i = 1/[1 + \alpha(T_{Ci} - T_0)]$ ,  $b_i = -a_i(V_i^2/2\pi L)$ ,  $n$  is the number of measurements,  $r_i$  is the resistance of the capillary at the voltage  $V_i$  and coolant temperature  $T_{Ci}$  and  $a_i$  and  $b_i$  are coefficients. We are interested in estimates of unknown parameters  $r_0$  and  $x$  and their covariation matrix. These values are necessary for finding the estimates and confidence intervals for the temperature and/or electric current.

In order to find estimate values of  $r_0$  and  $x$ , which we denote by  $\bar{r}_0$  and  $\bar{x}$ , the least-squares (LS) method is applied [7]. Normal LS equations are to be solved [7]:

$$S\begin{pmatrix} \bar{r}_0 \\ \bar{x} \end{pmatrix} = Q \quad (5)$$

where  $S$  is the matrix of the range two with elements given by

$$s_{11} = \sum_{i=1}^n \frac{a_i a_i}{\sigma_i^2}, \quad s_{12} = s_{21} = \sum_{i=1}^n \frac{a_i b_i}{\sigma_i^2},$$

$$s_{22} = \sum_{i=1}^n \frac{b_i b_i}{\sigma_i^2}$$

$Q$  is a vector with two components:

$$q_1 = \sum_{i=1}^n \frac{a_i r_i}{\sigma_i^2}, \quad q_2 = \sum_{i=1}^n \frac{b_i r_i}{\sigma_i^2}$$

and  $\sigma_i^2$  are the variances for each measurement.

Absolute values of  $\sigma_i$  are not important for solving eqn. 5, but rather their ratios to each other. Assuming that the variance of  $r_i$  in eqn. 5 is determined by the variance of the electric current  $I_i$ , and that the experimental error in the current measurements is the same for all measured currents, we assume  $\sigma_i \approx 1/I_i$  in our software and in this paper.

The covariation matrix  $C$  for estimates of  $r_0$  and  $x$  is given by

$$C = \frac{1}{n-2} \sum_{i=1}^n \sigma_i^{-2} (r_i - a_i \bar{r}_0 - b_i \bar{x})^2 S^{-1} \quad (6)$$

where  $S^{-1}$  is the inverse matrix to the matrix  $S$ .

Variances for  $r_0$  and  $x$  are the diagonal elements of the matrix  $C$ :

$$\sigma_r^2 = c_{11}, \quad \sigma_x^2 = c_{22} \quad (7)$$

When estimate values  $\bar{r}_0$  and  $\bar{x}$  are obtained, one can find an estimate of the electric current in the mutually calibrated capillary–buffer pair for any voltage by substituting  $\bar{r}_0$  and  $\bar{x}$  into eqn. 1:

$$\bar{I} = \frac{[1 + \alpha(T_C - T_0)]V}{\bar{r}_0 - \frac{\bar{x}V^2}{2\pi L}} \quad (8)$$

The electric current variance is found by the equation [7]

$$\sigma_I^2 = \left(\frac{\partial \bar{I}}{\partial \bar{x}}\right)^2 c_{22} + \left(\frac{\partial \bar{I}}{\partial \bar{r}}\right)^2 c_{11} + 2 \cdot \frac{\partial \bar{I}}{\partial \bar{r}} \cdot \frac{\partial \bar{I}}{\partial \bar{x}} \cdot c_{12}$$

$$+ \left(\frac{\partial \bar{I}}{\partial \alpha}\right)^2 \sigma^2(\alpha) \quad (9)$$

which gives, after substituting eqn. 8 into eqn. 9,

$$\sigma_I^2 = \left(\frac{\bar{I}}{\bar{r}_0 - \frac{\bar{x}V^2}{2\pi L}}\right)^2 \left[ \sigma_r^2 + \frac{V^2}{\pi L} \cdot c_{12} + \left(\frac{V^2}{2\pi L}\right)^2 \sigma_x^2 \right]$$

$$+ \left[ \frac{\bar{I}(T_C - T_0)}{1 + \alpha(T_C - T_0)} \right]^2 \sigma_\alpha^2 \quad (10)$$

The relative standard deviation of the electric current caused by the uncertainty in the thermal coefficient is

$$\left(\frac{\sigma_I}{\bar{I}}\right)_\alpha = \frac{\sigma_\alpha(T_C - T_0)}{1 + \alpha(T_C - T_0)} \quad (11)$$

if we assume  $\alpha = 0.021 \text{ K}^{-1}$ ,  $\sigma_\alpha = 0.002 \text{ K}^{-1}$  and  $T_C - T_0 = 5 \text{ K}$ , then we have a relative standard deviation of only 1%.

Therefore, the only information needed for eqn. 8, apart from the calibration points, is the capillary length. Having this and, say, ten calibration points, one is able to predict the electric current and, thus, the shift in migration time with reasonable accuracy. To obtain higher accuracy a more precise value for  $\alpha$  is necessary.

For acquiring the estimate of the actual inner diameter of the capillary one needs the value of the electric conductivity at a reference temperature  $T_0$  [3] and eqn. 3 is used. The corresponding variance is given by

$$\sigma_d^2 = \frac{d_L^2}{4} \left( \frac{\sigma_r^2}{r_0^2} + \frac{\sigma_\kappa^2}{\kappa_0^2} \right) \quad (12)$$

where  $\sigma_\kappa^2$  is the variance of the electric conductivity at the reference temperature. Our experiments usually give  $\sigma_d \approx 0.5 \mu\text{m}$  for the standard deviation of the capillary diameter.

For the temperature estimate one obtains from eqns. 2 and 4

$$\bar{T} = T_C + \frac{V^2[1 + \alpha(T_C - T_0)]\bar{x}}{2\pi L\alpha\left(\bar{r}_0 - \frac{\bar{x}V^2}{2\pi L}\right)} \quad (13)$$

The temperature variance can be found by using eqn. 9 in analogy with that of the electric current. The important difference is that the part of the relative standard deviation associated with the error in the determination of  $\alpha$  is

$$\left(\frac{\sigma_T}{\bar{T} - T_C}\right)_\alpha \approx \frac{\sigma_\alpha}{\alpha} \quad (14)$$

If  $\sigma_\alpha$  and  $\alpha$  have the same values as given in an example following eqn. 9, the temperature relative standard deviation is about 10%. In order to obtain better accuracy one is expected to find  $\alpha$  with higher accuracy.

Now let us consider the case when the capillary has been calibrated with some buffer and then filled with another buffer with different electric conductivity  $\kappa_{02}$  and thermal coefficient of electric conductivity  $\alpha_2$  but the same heat conductivity. It was shown previously [3] that even in this instance one can expect a reasonable accuracy of the electric current prediction.

For a calibrated capillary its inner diameter estimate and corresponding standard deviation are  $\bar{d}_L$  and  $\sigma_d$ , where  $\bar{d}_L$  is obtained from eqn. 3 ( $r_0$  is replaced by its estimate  $\bar{r}_0$ ) and  $\sigma_d$  is given by eqn. 12. The Biot number variance, as follows from eqn. 4, is given by

$$\sigma_{Bi}^2 = Bi_{OA}^2 \left[ \left(\frac{\sigma_x}{\bar{x}}\right)^2 + \left(\frac{\sigma_\alpha}{\alpha}\right)^2 \right] \quad (15)$$

Thus, new values of  $\bar{x}_2$  and  $\bar{r}_{02}$  and their variances are given by

$$\bar{x}_2 = \frac{\alpha_2}{Bi_{OA}\chi}, \quad \bar{r}_{02} = \frac{4L}{\kappa_{02}\pi\bar{d}_L^2} \quad (16)$$

$$\sigma_{x2}^2 = \bar{x}_2^2 \left( \frac{\sigma_x^2}{\bar{x}^2} + \frac{\sigma_\alpha^2}{\alpha^2} + \frac{\sigma_{\alpha2}^2}{\alpha_2^2} \right),$$

$$\sigma_{r2}^2 = \bar{r}_{02}^2 \left( 4 \cdot \frac{\sigma_d^2}{\bar{d}_L^2} + \frac{\sigma_{\kappa2}^2}{\kappa_{02}^2} \right) \quad (17)$$

It is seen from eqn. 15 that  $\sigma_{x2}^2$  and, hence, the accuracy of the electric current and temperature predictions depends strongly on the precision of the thermal coefficient  $\alpha$ . Nevertheless, if the precision is high enough, the predictions come out in good agreement with experimental measurements.

When one is able to calculate and predict electric current and buffer temperature for a given voltage, one has a possibility of predicting the transformation of the migration time and the dispersion of the experimentally obtained peak.

### Peak transformations

We assume, according to previous findings [4,5] that the migration velocity of the analyte varies according to the following expression for migration time:

$$t_m = t_m^* \cdot \frac{I^*}{I} \quad (18)$$

where  $t_m^*$  is the experimentally observed migration time and  $I^*$  is the experimental electric current, both corresponding to the applied voltage  $V^*$  and coolant temperature  $T_C^*$ ,  $t_m$  is the predicted migration time and  $I$  is the predicted current, both corresponding to voltage  $V$  and coolant temperature  $T_C$ .

Transformation of the peak dispersion is much more complex. Diverse origins of the peak broadening in CZE have been extensively discussed in a number of papers [8–16]. They include initial zone broadening, diffusion, convection, Joule heat, electroosmosis, adsorption, conductivity and pH differences between the analyte zone and the buffer and broadening due to the size of the detection zone. All broadening mechanisms are divided by us into two classes: those leading to symmetrical peaks and those causing skewed peaks. This division is correct only if the compositions of the analyte and the buffer are kept the same from run to run. For instance adsorption can lead to either symmetrical or skewed peaks depending on the concentrations of the analyte and adsorbing sites.

For the first class of symmetrical peaks an efficient diffusion coefficient  $D_{\text{eff}}$  can be introduced as has been done in chromatography. The peak shape is assumed to be Gaussian:

$$p_G(t) = \frac{A(t_m)^{1/2}}{\sigma_G(2\pi t)^{1/2}} \cdot \exp\left[-\frac{(t-t_m)^2 t_m}{2\sigma_G^2 t}\right] \quad (19)$$

$$\sigma_G = (2D_{\text{eff}} t_m)^{1/2} / v_m \quad (20)$$

$$A = \int_0^\infty p_G dt \quad (21)$$

where  $p_G$  is the peak indicated by a detector,  $t$  is the time,  $\sigma_G$  is the standard deviation,  $A$  is the peak area found by peak integration over time and  $v_m$  is the migration velocity.

At this stage we assume the efficient diffusion coefficient to be independent of the buffer temperature:

$$D_{\text{eff}} = D_{\text{eff}}^* \quad (22)$$

where  $D_{\text{eff}}^*$  is the experimentally found efficient diffusion coefficient, corresponding to the applied voltage  $V^*$  and the coolant temperature  $T_c^*$ . This assumption appears to be restrictive; however, experimental results presented by Huang *et al.* [16] show that the efficient diffusion coefficients may be twenty times higher than the molecular diffusion coefficients. Our experiments confirmed these observations. These high values cannot be explained either by a viscosity decrease due to the temperature rise or by additional broadening caused by the buffer temperature profile. Therefore, additional efforts should be applied to understanding peak broadening in CZE and our assumption is not worse than any other previously presented.

The transformation rule for the peak standard deviation follows from eqns. 18 and 20:

$$\sigma_G = \sigma_G^* \left( \frac{I^*}{I} \right)^{3/2} \quad (23)$$

where  $\sigma_G^*$  is the experimentally obtained standard deviation.

The peak area transforms as follows [16,17]:

$$A = A^* v_m^* / v_m = A^* t_m / t_m^* = A^* I^* / I \quad (24)$$

where  $A^*$  is the experimentally obtained peak area.

It is worth noting that the product  $A v_m$  is proportional to the analyte mass divided by the capillary cross-sectional area and is, therefore, a constant provided that the same amount of analyte is loaded for every run.

For the second class of skewed peaks we assume the shape of the peak to be exponential:

$$p_s(t) = \begin{cases} h \exp[(t_m - t)/\sigma_s], & t_m > t \\ 0, & t < t_m \end{cases} \quad (25)$$

where  $h$  is the peak height and  $\sigma_s$  is a parameter responsible for the peak width.

It is assumed for the skewed peaks that the height of the peak remains independent of the

buffer temperature:  $h = h^*$ . This assumption, together with eqn. 24, gives for  $\sigma_s$

$$\sigma_s = \sigma_s^* I^* / I \quad (26)$$

#### Software package

All algorithms and ideas described above have been implemented in a software package called "Capillary Zone Electrophoresis Assistant" (CZEA). We shall describe its main features briefly. The package runs under Microsoft Windows and does not require any preliminary knowledge of the CE thermal theory. The following input information should be provided: the capillary length, its name and the inner diameter given by a manufacturer (this value is assumed to be corrected by CZEA), the specific conductivity of the buffer at 25°C and the thermal coefficient of conductivity. To perform calibration and to predict buffer temperature, CZEA needs experimental points of electric current for incremented voltage. The coolant temperature should be also given for every point.

A user can choose one of the two models for the peak shape, either "GAUSSIAN" or "SKEWED". Depending on this choice, the peak is modelled either by eqn. 19 or by eqn. 25. Additional characteristics of the peak are required: the migration time, the peak area and the peak height. Two peaks can be displayed simultaneously and their transformation caused by the voltage and/or coolant temperature can be performed according to eqns. 18–26.

#### EXPERIMENTAL

A fused-silica capillary of 75  $\mu\text{m}$  I.D., total length 50 cm and distance from the injection to detection point 42.5 cm was obtained from Polymicro Technologies (Phoenix, AZ, USA). The temperature of the air surrounding the capillary was measured by a thermosensor positioned 10 cm from the capillary. The thermosensor was a Digiterm Quartz 1505 (Hanhart, Schwenningen, Germany). The electric current and voltage were read from the Waters (Milford, MA, USA) Quanta 4000 displays. Immobilines of pK 8.5 and 9.3 were obtained from Pharmacia-LKB (Uppsala, Sweden).

## RESULTS AND DISCUSSION

An example of CZEA calibration is presented below. It consists of twelve readings of the electric current and the ambient temperature for incremented voltage. Two series of readings each of six points were made with a time interval of 3 days. The capillary was of 75  $\mu\text{m}$  nominal I.D. and length 50 cm. The buffer used for calibration was 100 mM phosphate buffer, pH = 7.0,  $\kappa_0 = 9.64$  mS/cm (at  $T_0 = 25^\circ\text{C}$ ),  $\sigma_\kappa = 0.1$  mS/cm,  $\alpha = 0.021$  K $^{-1}$  and  $\sigma_\alpha = 0.0003$  K $^{-1}$ . Results of the calibration given by CZEA are that the actual I.D. is  $79.3 \pm 1.4$   $\mu\text{m}$  and the overall Biot number  $Bi_{0A} = 0.0544 \pm 0.006$ . Fig. 1, which is the image of the computer screen, shows two graphs, one for the electric current and the other for the buffer temperature dependences on the applied voltage. Solid lines represent predicted values and dashed lines show confidence intervals. Both graphs were computed for  $T_c = 27.3^\circ\text{C}$ . This value is displayed in the upper box and may be changed by the user (Fig. 1), in which event new graphs for the electric current and buffer temperature will be drawn. Values for the electric current and buffer temperature corresponding to the particular voltage of 12 kV are shown with their confidence intervals. It is seen that the buffer temperature is  $19^\circ\text{C}$  higher than the coolant temperature and is  $46^\circ\text{C}$ .

In order to check the ability of CZEA to predict migration times, electric current and

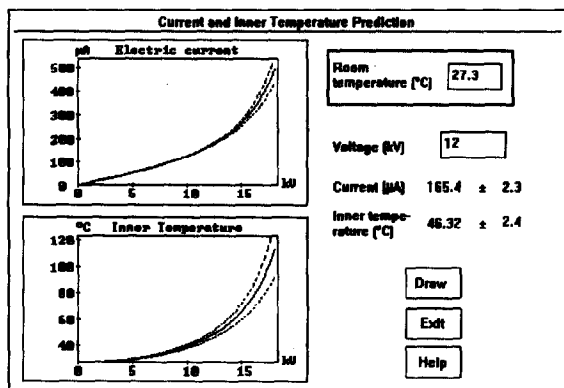


Fig. 1. Computer screen image of electric current and temperature as functions of applied voltage. The solid curves are the mean values and the dashed lines represent 97% confidence intervals.

temperature if the voltage or coolant temperature change, we used the capillary calibration reported above and filled it with another buffer, namely 50 mM phosphate buffer, pH = 7.7,  $\kappa_0 = 5.88$  mS/cm. A 2.5 mM model mixture of two Immobilines of pK 8.5 and 9.3 diluted in the buffer was separated at  $V = 12$  kV. The temperature of the cooling air during the run was  $T_c = 27.3^\circ\text{C}$ . The electric current fluctuated within the range 87.6–88  $\mu\text{A}$ . Fig. 2 shows two peaks, both skewed. The migration times are  $t_{m1} = 3.68$  min and  $t_{m2} = 3.83$  min. The ratio of the peak heights is 1:0.64. In order to simulate transformations of the peaks when the voltage changes one has to classify the peak shapes. The shape and transformation model for the first peak was chosen with certainty as "SKEWED". The second peak (Fig. 2) appears to be subject to diffusional broadening and we tentatively classified it as "GAUSSIAN". This means that eqns. 19–24 are used for the peak transformations and quantitative prediction for the peak broadening is not expected. All these data were entered into CZEA. The predicted electric current and temperature were  $I = 87.6$   $\mu\text{A}$  and  $T = 37.4^\circ\text{C}$ , respectively. The first value is in excellent agreement with the experimental value. Note that the

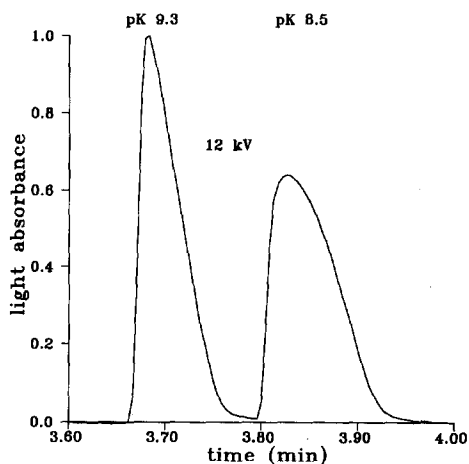


Fig. 2. Experimental electropherogram of a 2.5 mM mixture of two Immobilines having pK values of 8.5 and 9.3 and diluted in 50 mM, pH = 7.7,  $\kappa_0 = 5.88$  mS/cm phosphate buffer. The sample was loaded hydrostatically for 10 s with a height difference of 9.8 cm. The applied voltage was  $V = 12$  kV. The temperature of the cooling air during the run was  $T_c = 27.3^\circ\text{C}$ .

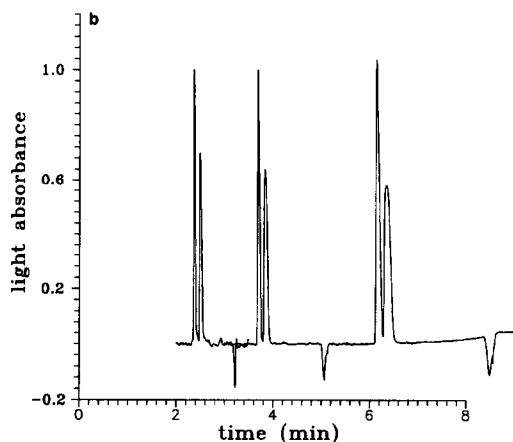
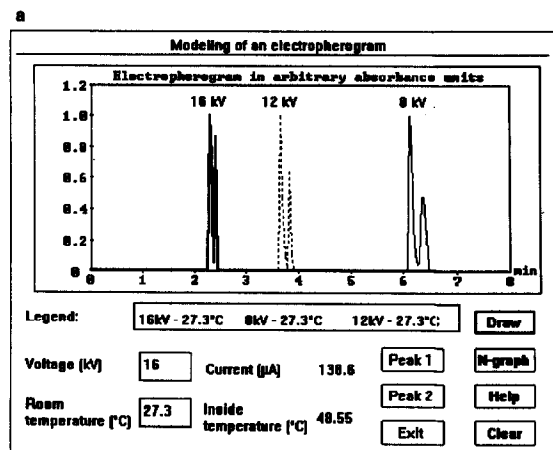


Fig. 3. Separation of the model mixture at  $V=8, 12$  and  $16$  kV. (a) image of the computer screen; (b) experimental. Peaks obtained at  $V=12$  kV were used as a template for the prediction of peaks at  $16$  and  $8$  kV. All experimental conditions except voltages as in Fig. 2.

capillary had been calibrated with a buffer different to that used for the separation.

Fig. 3 shows predicted and experimentally obtained results for the separation of the same mixture at  $12, 16$  and  $8$  kV. The pair of peaks corresponding to  $V=12$  kV was used as a template for CZEA. The two other pairs corresponding to  $V=16$  and  $8$  kV in Fig. 3a are the results of a calculation performed by CZEA. The buffer temperatures calculated by CZEA were  $48.6$  and  $33.3^\circ\text{C}$  at  $16$  and  $8$  kV, respectively. Comparison of Fig. 3a and b shows very good agreement between predicted and experimental peaks, especially for the skewed peaks. The peak classified as "GAUSSIAN" is predicted to be broader than it is experimentally. That means that the dispersion of the second peak is not fully diffusional but is determined also by electrochemical and wall interactions.

Experimental and predicted values of the electric current and migration times for two positive and one negative peaks are shown in Table I. The agreement between the predicted and experimental values is within 2%. The best agreement is obtained for the capillary filled with the same buffer as was used for its calibration. It was observed also that the discrepancy between the predicted and experimental retention times was a minimum for negative peaks (less than 1%) which represent the initial zone moving without interactions with the capillary walls. On the other hand, when a sample was diluted in water, the relative difference between the predicted and experimental migration times was maximum (up to 6%). Therefore, a significant

TABLE I

COMPARISON OF EXPERIMENTAL AND PREDICTED MIGRATION TIMES FOR TWO POSITIVE ( $t_{m1}$  AND  $t_{m2}$ ) AND ONE NEGATIVE ( $t_{m3}$ ) PEAKS OBTAINED AT  $V=16$  AND  $8$  kV

Parameter	$V=16$ kV				$V=8$ kV			
	$I$ ( $\mu\text{A}$ )	$t_{m1}$ (min)	$t_{m2}$ (min)	$t_{m3}$ (min)	$I$ ( $\mu\text{A}$ )	$t_{m1}$ (min)	$t_{m2}$ (min)	$t_{m3}$ (min)
Experimental	140	2.36	2.39	3.22	51.8	6.13	6.344	8.48
Predicted	139	2.33	2.42	3.20	52.5	6.14	6.39	8.45
Error	0.7%	1.3%	1.3%	0.6%	1.3%	0.2%	0.73%	0.35%

difference between predicted and experimental values indicates that effects additional to the temperature dependence of viscosity are involved in the migration of the analyte.

#### CONCLUSIONS

The statistical procedure described in this paper allows one to calculate the buffer temperature and the electric current in the capillary and confidence intervals for both values. The latter make it possible to separate the shift in migration times caused by the temperature dependence of the buffer viscosity from other temperature-sensitive phenomena such as changes in the buffer pH, pK values of the analyte and degree of ionization of the silanol groups. The accuracy of predicted values for the buffer temperature, electric current and migration times depends strongly on the accuracy of the thermal coefficient of electric conductivity.

The CZE software package allows easy calibration of the capillary and prediction of the temperature and electric current in the capillary, and also recalculation of the experimental electropherograms to different voltages and different temperatures of the ambient air.

The best agreement between the predicted and experimental migration times was obtained for the capillary filled with the same buffer as was used for its calibration. It was observed that the discrepancy between predicted and experimental migration times was a minimum for negative peaks (less than 1%) representing the initial zone moving without interactions with the capillary walls.

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