

# Prediction of current–voltage dependence and electrochemical calibration for capillary zone electrophoresis

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## ABSTRACT

An experimental procedure is described for predicting the current–voltage dependence and temperature rise due to Joule heating in capillary zone electrophoresis. An equation is given for calculating the Biot number from known values of capillary electric resistance in the absence of thermal effects ( $R_0$ ) and the new value of the resistance when thermal effects become pronounced. Once the  $R_0$  and Biot number have been obtained for a given capillary, one can easily predict the electric current and temperature inside it. Current–voltage plots theoretically derived for phosphate (pH 6.87) and acetate (pH 4.7) buffers and for two capillary diameters (75 and 100  $\mu\text{m}$  I.D.) were found to coincide accurately with experimentally measured data. An equation is also given allowing the determination of a capillary inner diameter from current readings in low-voltage runs (where thermal effects would be negligible) in buffers of known conductivity. A reasonable agreement was found between “electrochemical” and “optical” (or electron microscope) measurements of a capillary lumen. The equations presented allow the prediction of the maximum voltage gradient compatible with minimum band spreading from a single preliminary run in buffers of known conductivity.

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## INTRODUCTION

Reproducibility of results is essential for any separation method and all factors that influence the separation process have to be accounted for in order to secure it. In capillary zone electrophoresis (CZE), it was understood that reproducibility is related to a great extent to temperature effects and cooling conditions of a capillary [1–5].

Electric current within a capillary generates inter-

nal heating of the buffer solution. This heating elevates the average temperature of the buffer and produces a radial temperature gradient in the capillary. It is very important that the electric conductivity of the buffer increases with increasing temperature. The temperature dependence of the electric conductivity has been shown by Gobie and Ivory [2] to be a cause of non-Ohmic behaviour of a current–voltage characteristic of the capillary. This means that, *e.g.*, a twofold increase in an applied voltage does not lead to a twofold increase in the electric current. Moreover, if the voltage exceeds a certain value the electric current and temperature rise dramatically. This phenomenon has been studied and called “autothermal runaway” [2]. Heating of the buffer and deviations from Ohm’s law strongly

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depend on the cooling conditions of the capillary [1].

By assuming that longitudinal diffusion is the major source of zone broadening (in the absence of interactions of the analyte with the capillary wall) [6], it follows that high applied voltages are the most direct way to achieve high separation efficiencies. However, this relationship breaks down at very high voltages (e.g., > 500 V/cm) because the high heat generated promotes strong dispersion of the analyte.

Issaq *et al.* [3] considered the beginning of the deviation of the current–voltage dependence from a straight line to be the upper limit for the voltage applied to the capillary. We assume, however, that this deviation, indicating of course a reasonable temperature rise of the buffer within the capillary, has no adverse effect on the separation (provided that the sample is thermostable enough) and reproducibility.

If a high voltage is used for rapid separations, it is necessary to have a reliable method allowing an experimenter to predict the current–voltage dependence and the buffer temperature rise in the capillary. However, the autothermal theory of Gobie and Ivory [2], which could in principle be applied to this problem, gives only qualitatively correct predictions. In addition, this theory needs a precise knowledge of the diameters of a capillary lumen, fused-silica wall and polyimide coating, the exact determination of which would require considerable efforts.

Although the dimensions of a capillary (inner diameter, diameter of the fused-silica wall and diameter of the polyimide coating) are usually given by the producer, the tolerance intervals are too large for use in quantitative predictions. Thus, Gobie and Ivory [2] reported considerable deviations (from the point of view of quantitative predictions) of capillary dimensions from their nominal values.

In order to predict the current–voltage dependence and temperature rise within a capillary, we suggest a procedure of electrochemical calibration of a capillary based on the approximate thermal theory developed by Bello and Righetti [4,5]. This procedure allows one, after measurements of current at low and high voltages, to calculate current–voltage and temperature–voltage dependences. In addition, the capillary inner diameter can be calculated if the specific conductivity of the buffer solution is known.

The aim of this paper is to propose and verify

experimentally the procedure of electrochemical calibration and to compare the approximate thermal theory with experimental results. This paper consists of a theoretical section, with all relevant equations and a description of the calibration procedure, followed by an experimental section that includes measurements of buffer solution conductivities at different temperatures, measurements of the capillary inner diameters by using electron and optical microscopes, a comparison of the theoretical and experimental results and a discussion with practical guidelines.

## THEORY

### *Determination of the capillary resistance and its inner diameter*

According to Ohm's law, the relationship between current and voltage drop for a conductor of cylindrical cross-section is given by

$$RI = V \quad (1a)$$

$$R = \frac{4L}{\sigma\pi d^2} \quad (1b)$$

where  $I$  is the electric current,  $V$  the voltage applied,  $R$  the electrical resistance of the capillary filled with the buffer solution,  $\sigma$  the buffer electric conductivity,  $d$  the capillary inner diameter and  $L$  its length.

We use eqn. 1a and b for the determination of the capillary diameter by applying them when the voltage is relatively low and thermal effects are negligible:

$$d = \sqrt{\frac{4IL}{\sigma_0\pi V}} \quad (2)$$

where  $\sigma_0$  is the buffer conductivity at the reference temperature  $T_0 = 25^\circ\text{C}$ .

We identify the voltage applied to electrodes with the voltage applied to the ends of the capillary and neglect the voltage drop at the electrode compartment (note also that in most instruments the capillary extremities and the platinum wires are only a few millimetres apart).

### *Prediction of the temperature and current and determination of the Biot number*

The temperature elevation of the buffer inside the capillary depends on the cooling conditions of the

capillary. These conditions can be classified as lack of forced cooling (natural cooling), forced air cooling and liquid cooling. Deviations from Ohm's law have been shown to be significant for the cases of natural cooling and forced air cooling [1]. These conditions determine the heat removal from the external surface of the capillary. At the steady state, heat exchange between the heated buffer and the coolant surrounding the capillary is characterized by the overall Biot number. The latter is the coefficient of proportionality between dimensionless heat flux and dimensionless difference of the inner surface temperature of the capillary and the temperature of the coolant in the bulk. It is assumed that the law of proportionality is valid, which is, in fact, a reasonable approximation. If the Biot number, capillary inner diameter, its length and the properties of the buffer are known then it is possible to calculate the temperature inside the capillary and electric current for any given voltage [2,4]. Direct calculation of the Biot number is possible for some particular cases [1,2] but in general it is a complex problem. We propose not to calculate the Biot number but to obtain it from fitting experimental points on a current-voltage plot with the curves given by the approximate thermal theory [4]. This theory is assumed to be valid for the natural and forced air cooled capillaries. In these cases the temperature profile within the capillary is flat and can be replaced by its average value. It simplifies significantly solutions of equations of heat transfer and leads to simple equations for the electric current and average temperature [5].

All thermal theories assume the buffer conductivity to be linearly dependent on temperature:

$$\sigma = \sigma_0[1 + \alpha(T - T_0)] \tag{3a}$$

where  $T$  is the buffer temperature within the capillary and  $\alpha$  is the temperature coefficient of conductivity.

The capillary resistance, according to eqns. 1b and 3a, is given by the following relationship:

$$R = R_0/[1 + \alpha(T - T_0)] \tag{3b}$$

where  $R_0$  is the reference resistance at 25°C.

Experimentally,  $R_0$  can easily be evaluated from eqns. 1a and 3b applied for low voltage conditions. As elsewhere [2,4,5], we introduce a characteristic temperature difference as

$$\Delta T_{ref} = \sigma_0 E^2 d^2 / 4\chi \tag{4a}$$

where  $E$  is the electric field intensity and  $\chi$  the thermal conductivity of the buffer solution set equal to the water thermal conductivity ( $\chi = 0.61 \text{ W m}^{-1} \text{ K}^{-1}$ ). The characteristic temperature difference can be also expressed as

$$\Delta T_{ref} = \frac{V^2}{\pi\chi LR_0} \tag{4b}$$

The electric current and average buffer temperature within the capillary are given by [4]

$$T = \frac{2Bi_{0A}(T_C - T_0) + \Delta T_{ref}}{2Bi_{0A} - k^2} + T_0 \tag{5}$$

$$I = \frac{V}{R_0} \left\{ \frac{2Bi_{0A}[1 + \alpha(T_C - T_0)]}{2Bi_{0A} - k^2} \right\} \tag{6}$$

where  $k^2 = \alpha\Delta T_{ref}$  is the autothermal parameter,  $T_C$  is the temperature of the surrounding medium and  $Bi_{0A}$  is the Biot number.

When  $k^2 \ll 2Bi_{0A}$  (low voltage, small capillary diameter, low conductivity and liquid cooling), eqn. 6 reflects currents variations due to the coolant temperature changes. In order to separate these changes of current from the changes caused by internal heating, we denote by  $I_{25}$  the value of current corresponding to a given voltage if the temperature of the coolant were equal to the reference temperature of 25°C. Then eqn. 6 can be represented in the following form:

$$I = I_{25}[1 + \alpha(T_C - T_0)] \tag{7a}$$

$$I_{25} = \frac{V}{R_0} \left( \frac{2Bi_{0A}}{2Bi_{0A} - k^2} \right) \tag{7b}$$

Eqns. 7 allow one to evaluate the Biot number by measuring the current  $I$  for a given voltage, applying eqn. 7a to calculate  $I_{25}$  and using the following equation, derived from eqn. 7b:

$$Bi_{0A} = \frac{I_{25}k^2}{2(I_{25} - V/R_0)} \tag{8a}$$

Eqn. 8a can be also rewritten as follows:

$$Bi_{0A} = \frac{k^2}{2(1 - R_{25}/R_0)} \tag{8b}$$

where  $R_{25} = V/I_{25}$  is the capillary electric resistance at a given voltage provided that the coolant temperature is equal to 25°C.

### Calibration of capillary

The procedure for electrochemical calibration of the capillary is as follows. At low voltage, when thermal effects are negligible, one determines the capillary resistance  $R_0$  by using eqn. 1a. The capillary diameter can be calculated from eqn. 1b. By measuring the electric current  $I$  at a given voltage  $V$ , which should be high enough to produce a deviation from Ohm's law, and knowing the coolant temperature  $T_C$  and thermal coefficient of conductivity  $\alpha$ , one finds the value of  $I_{25}$  or  $R_{25}$  (the values of current and electric resistance which would be generated if the temperature of the coolant or surrounding medium is equal to the reference temperature). The values of  $R_0$  and  $I_{25}$  thus obtained are then used for calculating  $\Delta T_{ref}$  (eqn. 4b),  $k^2$  and, finally,  $Bi_{0A}$  (eqn. 8a or 8b). Knowing both  $R_0$  and  $Bi_{0A}$ , one can easily predict the electric current and temperature within a capillary. Note that  $Bi_{0A}$  is a characteristic of the given capillary in the given system and does not vary when the buffer solution is changed.

### EXPERIMENTAL

All chemicals were purchased from Merck (Darmstadt, Germany).

Two buffer solutions were used, phosphate and acetate. The phosphate buffer was 10 mM  $\text{Na}_2\text{HPO}_4$ –10 mM  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ –50 mM KCl (pH 6.87) and the acetate buffer was 50 mM  $\text{CH}_3\text{COOH}$ –25 mM NaOH (pH 4.7). The specific conductivity of buffer solutions was measured in the temperature range 25–45°C with an Orion Research conductimeter at a frequency of 1 kHz. The temperature was stabilized with a thermostat and controlled by a Digiterm Quartz 1505 thermocouple (Hanhart, Schwennigen, Germany). Before measurements, the conductimeter was calibrated by using standard solutions of KCl and the temperature dependence of the specific conductivity of the standard solution was measured and shown to be in agreement with the reference values [7]. Capillaries of length 50 cm and with nominal inner diameters of 50, 75 and 100  $\mu\text{m}$  were obtained from Polymicro Technologies (Phoenix, AZ, USA). All measurements of current–voltage dependence were performed on a Waters Quanta 4000 capillary electrophoresis system (Millipore, Milford, MA, USA). The current was measured from the

standard output of the Waters Quanta 4000 by a Kipp and Zonen voltage recorder.

### Electron and visible microscopy

All samples were covered with a thin layer of gold in an Edwards 306 metallizer (Edwards High Vacuum, Crawley, UK). The capillaries were then viewed in a Stereoscan 250 scanning electron microscope (Cambridge Scientific Instruments, Cambridge, UK) operated at 20 kV. Photographs were taken with Kodak Tri-X Pan 120 film. The capillaries were also examined under an Orthoplan visible microscope (Leitz, Wetzlar, Germany) at 900 $\times$  magnification.

### RESULTS AND DISCUSSION

As the calibration procedure needs an accurate value of thermal coefficients of buffer electric conductivity, we suggest standardizing for calibration two buffer solutions with relatively high and low electric conductivities.

The linear temperature dependence of the phosphate buffer solution has already been reported [2]. However, the value of  $\alpha$  given in Table III in ref. 2 (denoted there by  $k_1$ ) is  $0.046^\circ\text{C}^{-1}$ , whereas the value of  $\alpha$  evaluated from Fig. 5 in the same paper, with reference to the temperature  $T_0 = 25^\circ\text{C}$ , is

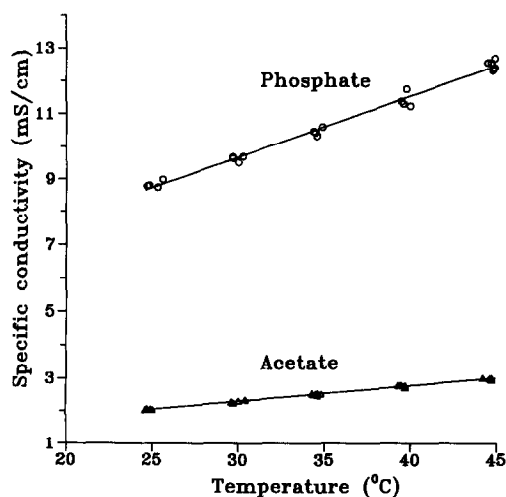


Fig. 1. Temperature dependences of specific conductivities for (○) phosphate and (△) acetate buffers. The measurements were taken at 5°C increments in the range 25–45°C with an Orion Model 101 conductivity meter with a 1-cm path cell. Each measurement was repeated four times. Note the apparent difference in slopes between the two buffers.

TABLE I  
CAPILLARY DIAMETERS MEASURED ELECTROCHEMICALLY AND OPTICALLY

Nominal diameter ( $\mu\text{m}$ )	Electrochemical calibration ( $\mu\text{m}$ )	Optical microscope ( $\mu\text{m}$ )	Electron microscope ( $\mu\text{m}$ )
50	48.4	47.55	50.3
75	74.3	74.2	78.7
100	104	103.6	109.5

approximately equal to  $0.02^\circ\text{C}^{-1}$ . Therefore, we could not use the previously measured data for calibration and measured the temperature dependences of the specific electric conductivities for both buffers.

The temperature dependences of the specific electric conductivity for the phosphate and acetate buffers are shown in Fig. 1. Four runs were performed for each solution and the data were fitted by straight lines by using the least-squares method. It can be seen from Fig. 1 that linear dependences of the electric conductivity of the buffers on temperature in the range  $20\text{--}50^\circ\text{C}$  are good approximations for both phosphate and acetate buffers. The coefficients for linear representation (eqn. 3) of the electric conductivities of the buffers are found to be

$$\sigma_0 = 8.72 \pm 0.14 \text{ (mS cm}^{-1}\text{)},$$

$$\alpha = 0.021 \pm 0.001 \text{ (}^\circ\text{C}^{-1}\text{)} \quad (9a)$$

for phosphate buffer and

$$\sigma_0 = 2.04 \pm 0.03 \text{ (mS cm}^{-1}\text{)},$$

$$\alpha = 0.023 \pm 0.001 \text{ (}^\circ\text{C}^{-1}\text{)} \quad (9b)$$

for acetate buffer. The reference temperature  $T_0$  is  $25^\circ\text{C}$ .

According to the calibration procedure described above, we measured the current and voltage for capillaries with nominal diameters of 50, 75 and  $100 \mu\text{m}$  filled with either phosphate or acetate buffer. The inner diameters calculated according to eqn. 2 for both buffers agree well with each other and with those measured using the optical microscope. Measurements made by using the electron microscope gave different results, probably owing to

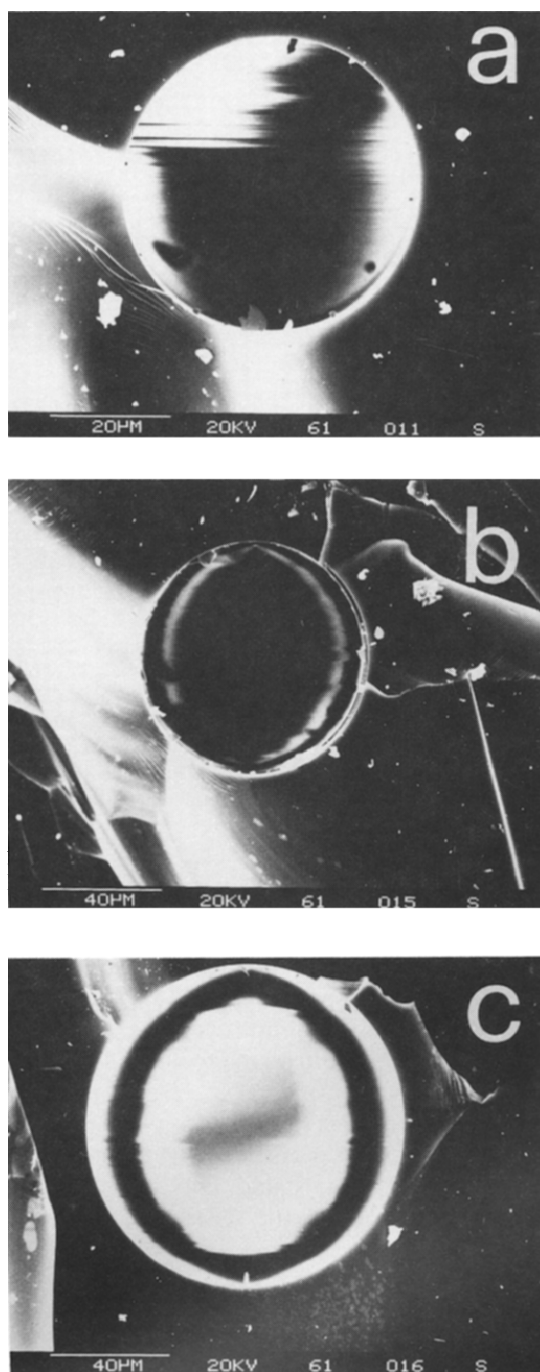


Fig. 2. Scanning electron micrographs of capillaries of different diameters: (a)  $50 \mu\text{m}$ ; (b)  $75 \mu\text{m}$ ; (c)  $100 \mu\text{m}$ . Note that the round object is the lumen of the capillary. At these magnifications, only a small portion of the fused-silica wall can be seen around the lumen. The white, star-like objects are minute chips of fused-silica as a result of cutting a sharp edge.

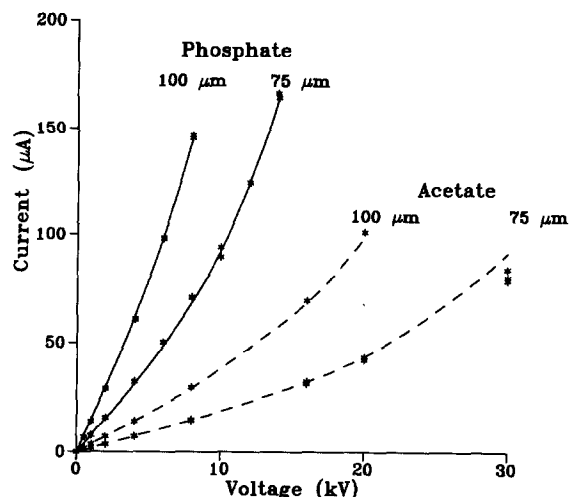


Fig. 3. Current–voltage plots for phosphate (solid lines) and acetate (dashed lines) buffers. The experiments were performed in two sets of capillaries of nominal inner diameter 75 and 100  $\mu\text{m}$ . Note how, owing to the much higher conductivity of phosphate buffer, much lower voltage gradients can be applied as compared with an acetate buffer. Note also the excellent agreement between the experimental points (stars) and the theoretically calculated lines (the latter were calculated from eqn. 7b and do not represent the lines of best fit joining the experimental points!). Note also that each star represents four different experiments (not the average values, the data simply overlap completely on the target, except for a few points!).

the insufficient statistics for electron microscope measurements. Results of the determination of capillary diameters are presented in Table I. Fig. 2 shows a series of photographs of the capillary lumen, made with the electron microscope, for three sizes (50, 75 and 100  $\mu\text{m}$ ).

Series of measurements of current at progressively increasing voltage were performed for four combinations of two capillaries and two buffers. The temperature of the air in the CZE unit was also measured and the values of the current were reduced to the reference temperature of 25°C according to eqn. 7a.

The capillary inner diameters found by electrochemical calibration were used for obtaining Biot numbers for the 75- and 100- $\mu\text{m}$  capillaries from measurements for phosphate buffer at voltages of 14 and 8 kV, respectively. The values of the Biot numbers calculated from eqn. 8a are  $Bi_{0A}^{75} = 0.0455$  and  $Bi_{0A}^{100} = 0.061$  for the 75- and 100- $\mu\text{m}$  diameters,

respectively. These values were used to calculate current–voltage dependences over the whole range of voltages for both phosphate and acetate buffers by using eqn. 7b.

The theoretical curves are compared with the experimental points in Fig. 3. It can be seen that there is very good agreement between the theoretical and experimental data. Note that the Biot number obtained with the single value for a relatively high current for a capillary filled with the phosphate buffer was also suitable for the same capillary filled with the acetate buffer.

The Biot numbers were applied to calculate the buffer temperature according to eqn. 5. The dependences of the buffer temperature are shown in Fig. 4. These curves show that our measurements of  $R_0$  were correct in the sense that the voltages used did not produce a significant temperature rise able to affect the capillary resistance. Together with the current–voltage curves the temperature–voltage curves appear to be useful for setting the correct voltage for an analysis.

Fig. 4 also illustrates the effect of the buffer electric conductivity on the average temperature of the buffer. It can be seen by comparing curves that higher voltages are applicable for the low-conductivity acetate buffer. As a general rule, buffers of low

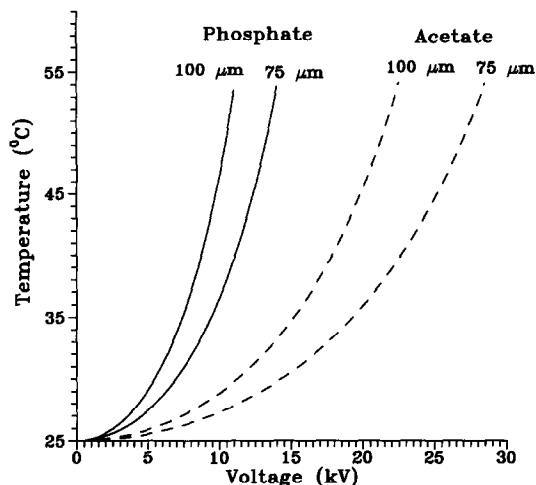


Fig. 4. Temperature–voltage plots for phosphate (solid lines) and acetate (dashed lines) buffers. Note how, owing to the much lower conductivity of the acetate ion, much higher voltage gradients can be applied for a given value of temperature rise as compared with a phosphate buffer.

conductivity should be preferred, as they allow high voltages (and thus rapid separations) with minimum temperature rises inside the capillary. Zwitterionic (such as Good's) buffers should therefore be selected. A recent study [8] has shown that, e.g., a histidine buffer (with a conductivity of only 123  $\mu\text{mho}$ , 0.1 M, pH 7.81) is vastly superior to a Tris-phosphate buffer (0.1 M, pH 7.74) with a conductivity of 2060  $\mu\text{mho}$ .

An important consequence of our conductivity measurements is discussed further. It is seen from eqn. 9 that the thermal coefficients of both buffers are close to each other. The reason for this is that the ion mobilities and, thus, electric conductivities of different electrolyte solutions depend on the viscosity of the solution in approximately the same way. With dilute solutions the electric conductivity is inversely proportional to the viscosity. We approximated the reciprocal of the water viscosity (taken from ref. 9) by a straight line and plotted it together with the relative conductivities of phosphate and acetate buffers in Fig. 5. It can be seen that these lines are close to each other. It is well known that the electrophoretic mobility of a charged particle and the electroosmotic mobility, which determine the

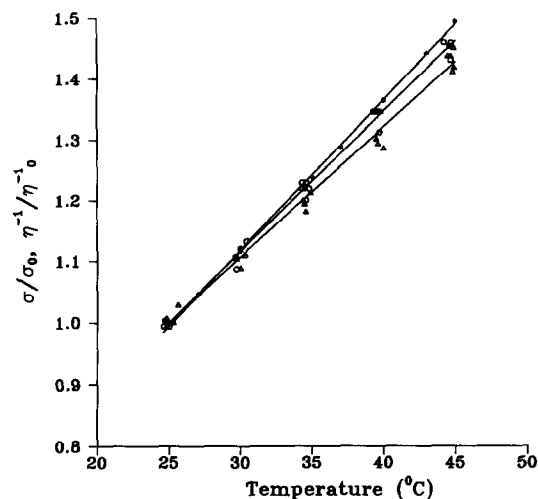


Fig. 5. Plot of relative electric conductivities for ( $\Delta$ ) phosphate and ( $\circ$ ) acetate buffers and ( $*$ ) relative reciprocal water viscosity vs. temperature. Note how, on such plots, the slopes are the same in the three cases (in contrast to Fig. 1), suggesting that the conductivity increments of the buffers follow closely (in fact are due to) the reciprocal viscosity increments of the solvent.

retention time, are inversely proportional to the viscosity of the buffer. Therefore, these mobilities are very likely to depend on temperature just as do the buffer conductivity and the electric current. Thus, electrophoretic and electroosmotic mobilities are supposedly given by

$$U_{ep} = U_{ep}^0 [1 + \alpha(T - T_C)] \quad (10a)$$

$$U_{os} = U_{os}^0 [1 + \alpha(T - T_C)] \quad (10b)$$

where  $U_{ep}^0$  and  $U_{os}^0$  are the electrophoretic and electroosmotic mobilities, respectively, at the reference temperature.

Retention time is given by the following expression:

$$t_R = \frac{L^2}{(U_{ep} + U_{os})V} \quad (11)$$

which can be reduced algebraically to

$$t_R = L^2 / [(U_{ep}^0 + U_{os}^0)IR_0] \quad (12)$$

One concludes from eqn. 12 that retention time is inversely proportional to the electric current, as the other variables in eqn. 12 do not depend on temperature and voltage. It is also seen from eqn. 12 that the ratio of the retention times of a substance at different voltages is equal not to the reciprocal of the ratio of voltages but to the reciprocal of the ratio of currents:

$$t_{R1}/t_{R2} = I_2/I_1 \quad (13)$$

whereas at low applied voltage the ratio of voltages or of currents can be used interchangeably (see also ref. 10).

## CONCLUSIONS

Most workers perform a series of measurements at progressively increasing voltage gradients in order to obtain the point of deviation from Ohm's law. In our case, two current-voltage measurements (a first one at low-voltage, in the absence of thermal effects, and a second one at high voltage) allow the derivation of the overall Biot number and easy prediction of electric current and temperature within a capillary. The calibration procedure proposed here appears to be correct and useful for air-cooled CZE data interpretation and for setting parameters of an analytical run.

The present data experimentally justify the approximate thermal theory [5] for air-cooled capillaries in that the theoretical current–voltage curves fit the experimental points well.

We propose eqn. 12, which states that at high voltages the retention time is inversely proportional not to applied voltage but to the electric current. Although this equation has not been directly proved, we believe it to be accurate (see ref. 10).

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