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# Thermally induced fluctuations of the electric current and baseline in capillary electrophoresis

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## ABSTRACT

Fluctuations of the electric current and baseline that appear in air-cooled capillaries when a high voltage is applied were studied both theoretically and experimentally. The theoretical part relates the amplitudes of the baseline and electric current fluctuations with the fluctuations of the buffer temperature originating from the thermal fluctuations at the outer surface of the capillary. It is shown theoretically that an increase in the outer diameter of the capillary decreases the amplitudes of the electric current and the baseline fluctuations and does not lead to a substantial elevation of the buffer temperature. Experiments confirmed the mutual correlation of the baseline and current fluctuations as predicted by the theory. Jacketing of the outer surface of the capillary with a polymer tubing is shown to be useful for reducing the fluctuations in capillary electrophoresis units lacking liquid cooling.

## INTRODUCTION

It is observed, we believe, by almost every user of a capillary electrophoresis (CE) unit lacking liquid cooling that, when a relatively high voltage is used for an analysis, substantial fluctuations of the electric current and UV detector baseline occur.

The electric current stability in CE having cooling provided by natural air convection, forced air convection and solid thermostat cooling has been studied by Nelson *et al.* [1], who showed that the highest current fluctuations occur for a capillary cooled by natural air convection, which is the least effective means of cooling. When a capillary was subjected to forced air cooling or to solid-state thermoelectric cooling, the amplitude of the fluctuations decreased significantly for the case of forced air cooling and vanished for thermoelectric cooling. It is clear that the amplitude of the electric current fluctuations depends on the effectiveness of the heat removal from the capillary. However, the origin of the fluctuations has not been investigated.

As many of the commercially available and laboratory-made CE units lack liquid or solid thermostat cooling (which appear to be the best means of avoiding difficulties with internal overheating and current fluctuations), the problem of suppressing the electric current and baseline fluctuations remains important. Our preliminary experiments and the results presented by Nelson *et al.* [1] show that the relative amplitude of the electric current fluctuations increases with the applied voltage much faster than the square root of the relative absolute buffer temperature as one might expect according to general thermodynamic reasoning.

This paper investigates the nature and mechanism of the current and baseline fluctuations in capillary electrophoresis. The paper consists of theoretical and experimental parts. The theoretical part relates the amplitudes of the electric

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current and baseline fluctuations with the parameters of the capillary, cooling system and operating conditions. The theory predicts that an increase in the capillary outer diameter (O.D.) reduces the fluctuations whereas the buffer temperature in the capillary lumen does not increase significantly. The experimental part confirms the main results of the theory and gives examples of the reduced current and baseline fluctuations in the air-cooled capillary. A decrease in the fluctuations is obtained by jacketing the capillary with a polymer tubing.

## THEORY

The thermal theory of capillary electrophoresis [2–8] deals with steady-state [2–6] and unsteady, *i.e.*, varying with time [7,8], distributions of the buffer temperature within the capillary. In the following section we present the basic theory necessary for further considerations.

### *General*

Let us assume the buffer temperature within the capillary to be uniform along the capillary axis and the capillary to be air-cooled. The first condition together with the suggestion that the electric conductivity is linearly dependent on the buffer temperature gives for the electric current

$$I = I_0[1 + \alpha(T - T_0)] \tag{1}$$

$$I_0 = V/r_0 \tag{2}$$

where $T_0$ denotes a reference temperature, $T$ is the buffer temperature averaged over the cross-section of the capillary, $I$ is the electric current in the capillary, $\alpha$ is the thermal coefficient of the electric conductivity, $I_0$ is the value of the current that would be in the capillary if the temperature of the buffer is equal to the reference temperature, $V$ is the applied voltage and $r_0$ is the capillary electrical resistance at reference temperature. Eqn. 1 represents the linear dependence of the electric current on the buffer temperature and eqn. 2 is Ohm's law.

The second condition implies that the temperature profile within the capillary lumen is flat and the inside wall temperature is approximately equal to the buffer average temperature [7,8]. The average buffer temperature at steady state may be expressed as

$$T_S = T_C + \frac{\Delta T_{ref}[1 + \alpha(T_C - T_0)]}{2Bi_{OA} - \alpha\,\Delta T_{ref}} \tag{3}$$

$$\Delta T_{ref} = \frac{VI_0}{\pi L \chi_L} \equiv \frac{V^2}{\pi L \chi_L r_0} \equiv \frac{E^2 \sigma_0 D_L^2}{4\chi_L} \tag{4}$$

where $T_S$ is the steady-state average temperature of the buffer, $Bi_{OA}$ is the overall Biot number representing an integral relative thermal conductivity of the capillary and the cooling system, $\Delta T_{ref}$ is the characteristic temperature of the Joule heating, $T_C$ is the temperature of the coolant, $V$ is the applied voltage, $\chi_L$ is the buffer thermal conductivity, $E$ is the intensity of the electric field, $\sigma_0$ is the buffer conductivity at the reference temperature, $D_L$ is the capillary inner diameter (I.D.) and $L$ is the capillary length. The equation for the overall Biot number is given in the Appendix.

Eqn. 3 is derived by rearranging terms in eqn. 17a in ref. 7 and is valid (as the original equation is) for relatively small values of $Bi_{OA}$, when terms having the order of $Bi_{OA}$ are negligible in comparison with unity. This is usually the case with the air-cooled capillaries being considered here. Eqn. 3 agrees with the approximate eqn. 12 of Gobie and Ivory [6] for $Bi_{OA} \ll 1$ and if $T_C$ is set equal to $T_0$ in eqn. 3. When comparing these two equations, note that $Bi_{OA}$ as defined in ref. 6 is twice as large as ours. Eqn. 4 gives three equivalent equations for the reference temperature.

It is useful to introduce a dimensionless function which would show the influence of the Joule heating on the electric current:

$$f = \left(\frac{2Bi_{OA}}{\alpha\,\Delta T_{ref}} - 1\right)^{-1} \tag{5}$$

The dimensionless function $f$ will be called below the "function of the electric current non-linearity". It can be expressed as follows:

$$f = \frac{I}{I_C} - 1$$
$$I_C = I_0[1 + \alpha(T_C - T_0)] \tag{6}$$

where $I_C$ is the value of the electric current that would flow through the capillary in the absence of the Joule heating at a temperature equal to that of the coolant.

Eqn. 3 for the buffer steady-state temperature may be rewritten as

$$T_S = T_C + f\left(\frac{1}{\alpha} + T_C - T_0\right) \tag{7}$$

For low applied voltages and good cooling conditions, $f$ is negligibly small whereas if $\alpha\,\Delta T_{ref}$ approaches $2Bi_{OA}$, values of $f$ and, thus, the buffer temperature, electric current and transient time grow infinitely [6,8]. This effect has been called "autothermal runaway" [6].

In order to analyse the influence of variations of the coolant temperature on the buffer temperature and the electric current, we consider first relatively slow variations of the coolant temperature. A fluctuation of the coolant temperature is regarded as either fast or slow by comparing its characteristic time (the time it takes the fluctuation to occur) with the characteristic transient time of the capillary (the time that is necessary to reach steady-state current and temperature after application of a voltage) [7,8]. The latter depends on the outer and inner capillary diameters, applied voltage and cooling conditions. If the fluctuation time is much longer than the characteristic transient time we call the fluctuation "slow" and, *vice versa*, if it is shorter we call it "fast".

*Slow fluctuations of the coolant temperature*

A characteristic transient time $\tau$ for the commonly used air-cooled capillary of *ca.* 360–385 $\mu$m O.D. is about 1 s [8]. If a fluctuation of coolant temperature occurs within 10 s or longer it may be considered as quasi-static. This means that the buffer temperature and electric current fluctuations follow in time the coolant temperature. Thus, the buffer temperature and the electric current are given by eqns. 1 and 2.

Assume that the coolant temperature fluctuates during a relatively long time (in the sense discussed above) from a certain value $T_C$ to $T_C + |\delta T_C|$. By differentiating eqns. 1 and 7 and taking into account eqns. 5 and 6, one obtains the following expressions:

$$|\delta T| = (f+1)|\delta T_C| \tag{8a}$$

$$\frac{|\delta I|}{I_0} = \alpha(f+1)|\delta T_C| \tag{8b}$$

where $|\delta T_C|$ is the amplitude of the fluctuation of the coolant temperature, $|\delta T|$ and $|\delta I|$ are the amplitudes of the fluctuations of the buffer temperature and the electric current, respectively, and $f+1$ is the derivative of the buffer temperature with respect to the coolant temperature.

It is seen from eqn. 8a that $(f+1)$ is a magnification factor for the buffer temperature which is never less than unity. For example, for an air-cooled (forced) capillary $Bi_{OA}$ is approximately equal to 0.05. Assume that a buffer solution has $\alpha \approx 0.02$ ($K^{-1}$), $\chi_L \approx 0.6$ (W/mK) and $\sigma_0 = 6$ (mS/cm). A capillary of 75 $\mu$m I.D. and 50 cm long filled with this buffer has at 25°C a resistance $r_0 = 1.89 \cdot 10^8$ Ω. For $V = 20$ kV, eqn. 4 gives $\Delta T_{ref} = 2.21$°C and for $f$ from eqn. 5 it follows $f \approx 0.8$. Then eqn. 8a shows that an increase of the coolant temperature of 1°C leads to an increase of 1.8°C in the buffer temperature and eqn. 8b gives 3.6% for the relative amplitude of the electric current fluctuation. This effect is relevant in the vicinity to the critical point of the autothermal runaway. If 10 kV are applied, the magnification factor $(f+1)$ is only 1.01. For large $Bi_{OA}$ corresponding to very good cooling conditions or low applied voltages, $f$ approaches zero and $f+1$ approaches unity.

*Fast fluctuations of the coolant temperature*

The case of fast fluctuations is not so straightforward as that of slow fluctuations, as the steady-state eqns. 1 and 2 are no longer applicable. According to our previous results [7], time evolution of the average buffer temperature is approximately governed by an ordinary differential equation:

$$\frac{dT}{dt} = -\frac{1}{\tau_1}(T - T_S) \tag{9a}$$

with the initial condition

$$T(0) = T_C^0 \tag{9b}$$

where $t$ is the time, $T_C^0$ is the value of the coolant temperature at the moment when the voltage has been applied and $\tau_1$ is the characteristic transient time. In ref. 8 the transient time $\tau_{tr}$ was also introduced as the time necessary to reach the steady state and is equal to the characteristic transient time $\tau_1$ multiplied by a factor of 4. The characteristic time $\tau_1$ may be calculated by a procedure described in ref. 7. Additionally, an approximate equation for a capillary having a thick polymer coating will be derived below. The temperature $T_S$ in eqn. 9a now depends on time as it includes the time-dependent coolant temperature.

Assume the coolant temperature to be the sum of the constant value $T_C^0$ and a relatively small fluctuation $\delta T_C$:

$$T_C = T_C^0 + \delta T_C \tag{10}$$

In order to estimate the influence of the coolant temperature fluctuations on the buffer temperature, we represent the temperature fluctuation of the coolant as a harmonically oscillating function:

$$\delta T_C = |\delta T_C| \cos \omega t \tag{11}$$

where $\omega$ is the circular frequency of the fluctuation.

The solution to eqns. 9a and 9b with $T_C$ given by eqns. 10 and 11 is

$$T = T_C^0 \exp(-t/\tau_1) + T_S^0[1 - \exp(-t/\tau_1)] - \frac{|\delta T_C|(f+1)\exp(-t/\tau_1)}{1+\omega^2\tau_1^2} + \frac{|\delta T_C|(f+1)\cos(\omega t - \varphi)}{\sqrt{1+\omega^2\tau_1^2}} \tag{12}$$

where $\varphi = \arctan(\omega\tau_1)$.

It is seen from eqn. 12 that an oscillating part of the buffer temperature representing the fluctuation is given by

$$\delta T = |\delta T| \cos(\omega t - \varphi) \tag{13a}$$

$$|\delta T| = \frac{(f+1)|\delta T_C|}{\sqrt{1+\omega^2\tau_1^2}} \tag{13b}$$

Obviously, eqn 13b reduces to eqn. 8a for a limiting case of slow fluctuation ($\omega\tau_1 \ll 1$).

An important result can be drawn from an analysis of eqn. 13b: one concludes that the amplitude of fluctuations decreases with increasing characteristic transient time $\tau_1$ and/or increasing fluctuation frequency. In other words, high-frequency fluctuations are filtered if the characteristic time $\tau_1$ is large.

A reader familiar with electronics has probably found an analogy between the reaction of the buffer temperature to the external temperature fluctuations with the reaction of an RC integrating circuit to the electric current oscillations.

*Fluctuations of the coolant temperature*

Above we considered the coolant temperature fluctuations to originate from an external source. Most probably, these fluctuations originate from the temperature pulsations in the turbulent air boundary layer flowing around the capillary. The air flow produced by a fan is turbulent, which means that the air velocity pulsates in time. There are also vortices of different scale depending on the fan power and geometry of the box containing the capillary. We believe that these vortices and pulsations produce temperature fluctuations near the external surface of the capillary which has a temperature higher than that of cooling air. If so, then the amplitude of the temperature fluctuations must be proportional to the temperature difference between the temperature of the air far from the capillary and the temperature of the external surface of the capillary:

$$|\delta T_C| = \gamma(T_{EX} - T_C) \tag{14}$$

where $T_{EX}$ is the temperature of the external surface of the capillary and $\gamma$ is the coefficient of proportionality reflecting aerodynamic properties of the system.

In order to find $T_{EX}$ we use eqns. A1.3–A1.5a from ref. 7 and substitute the exact solution involving Bessel functions by the approximate eqn. 3 and after some algebra (see the Appendix for the definitions of $R_P$ and $h$) finally derive the following expression:

$$T_{EX} - T_C = (T_S - T_C)\frac{\chi_L Bi_{OA}}{hR_P} \tag{15}$$

and by using eqn. 14 we find for the amplitude of coolant temperature fluctuations

$$|\delta T_C| = \gamma(T_S - T_C)\frac{\chi_L Bi_{OA}}{hR_P} \tag{16}$$

It is seen from this equation that the higher the buffer temperature the higher is the amplitude of fluctuations of the coolant temperature at the external surface of the capillary.

*Baseline and electric current fluctuations*

It is reasonable to suppose that fluctuations of the buffer temperature are responsible for both the electric current and the baseline fluctuations. The latter is the consequence of the temperature dependence of the refractive index of water. Therefore, when reducing the amplitude of the fluctuations of the buffer temperature, one reduces the amplitude of the baseline and electric current fluctuations.

By substituting eqn. 14 into eqn. 13b, the following expression for the amplitude of the buffer temperature fluctuations is derived:

$$|\delta T| = \frac{(f+1)\gamma(T_{EX} - T_C)}{\sqrt{1+\omega^2\tau_1^2}} \tag{17}$$

where the temperature of the capillary external surface is given by eqn. 15.

As a result of eqn. 16, the following equation for the relative amplitude of the electric current fluctuations can be derived from eqns. 5, 18b, 15 and 17:

$$\frac{|\delta I|}{I} = f\gamma \cdot \frac{\chi_L Bi_{OA}}{hR_p\sqrt{1+\omega^2\tau_1^2}} \tag{18}$$

Eqn. 18 predicts direct proportionality of the relative amplitude of the current fluctuations to the function of electric current non-linearity $f$. The latter, as is seen from eqn. 5, can be measured experimentally. Therefore, if the validity of eqn. 18 is proved experimentally, it justifies the theory developed above.

It follows directly from eqn. 17 that the amplitude of the buffer temperature fluctuations may be decreased by decreasing the difference between the surface temperature of the capillary and that of the coolant and by increasing the characteristic transient time. The next section shows that these goals may be reached simply by increasing the thickness of the capillary polymer coating.

*Increasing the polymer coating thickness*

At first glance, increasing of the coating thickness (*i.e.*, the capillary O.D.) seems to cause a significant elevation of the buffer temperature owing to some prevention of the heat removal. However, a variation of the radius of the polymer coating affects simultaneously the overall Biot number, the heat transfer coefficient and the characteristic transient time. The first two parameters determine the increase in the buffer temperature provided that other parameters are fixed. Fig. 1 shows the dependences of the buffer temperature and the temperature of the external surface of the capillary on the radius of the polyimide coating. The buffer temperature was calculated by using eqn. 3. Eqn. 15 was used for the temperature of the external surface.

The capillary and buffer parameters were the same as those which were used in the example following eqn. 8b. Additionally, we specified the radius of the fused-silica wall $R_W = 170$ $\mu$m,

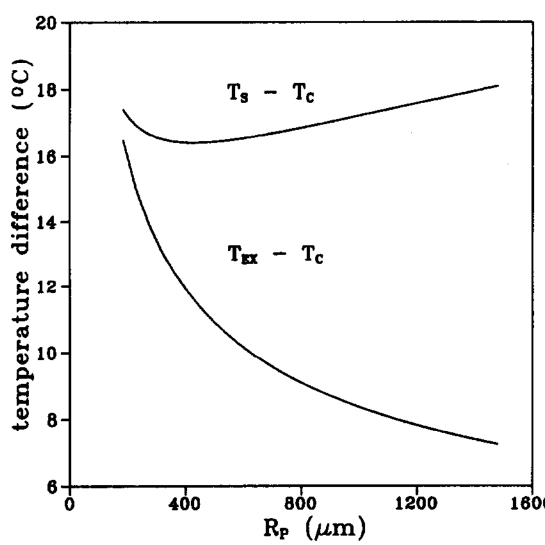


Fig. 1. Dependences of the buffer temperature (upper curve) and the temperature of the external surface (lower curve) on the radius of the polyimide coating (simulation). The temperature difference scale on the ordinate represents the temperature above that of the coolant. The capillary and the buffer parameters are given in the text.

thermal conductivity of the wall $\chi_W = 1.5$ W/mK, thermal conductivity of the polyimide coating $\chi_P = 0.15$ W/mK and heat transfer coefficient $h = 170$ W/m$^2$K, $V = 15$ kV, O.D. = 370 $\mu$m and $Bi_{OA} = 0.05$. The value of the heat transfer coefficient $h$ is in agreement with that found in ref. 1. The constant $A$ in eqn. A.2 (see Appendix) is approximately equal to unity if $R_P$ is measured in metres. It is seen from Fig. 1 that an increase in the polyimide coating thickness leads to a significant decrease in the external surface temperature whereas the buffer temperature has a shallow minimum and then increases slightly. Therefore, we expect a decrease in the amplitude of the coolant temperature fluctuations (eqn. 14), lowering the buffer temperature fluctuations (eqns. 8a and 13b) and, hence, a decrease in the electric current and the baseline fluctuations when the thickness of the polyimide coating increases. The decrease in the external surface temperature with increase in the capillary O.D. follows from the facts that the area of the capillary external surface increases proportionally to the capillary O.D. whereas the heat transfer coefficient decreases more slowly (see eqn. A.2), and that the heat flux remains approximately the same.

Another effect of the large O.D. is an increase in the characteristic transient time. In order to derive an estimate for the characteristic transient time for the capillaries having a thick coating and a low coefficient of heat transfer, we assume the electric conductivity to be independent of temperature and the capillary to be uniform and to have thermal properties equal to those of polyimide. The characteristic transient time for this system is determined as [7,9]

$$\tau_1 = \frac{R_P^2}{\kappa_P} \cdot \lambda_1^{-2} \tag{19}$$

where $\kappa_P$ is the thermal diffusivity of the polyimide and $\lambda_1$ is the first root of the following equation:

$$J_0(\lambda) - \left(\frac{\chi_P}{hR_P}\right)\lambda J_1(\lambda) = 0 \tag{20}$$

where $J_0$ and $J_1$ are Bessel functions of the first kind. The first root of eqn. 20 can be found in ref. 9.

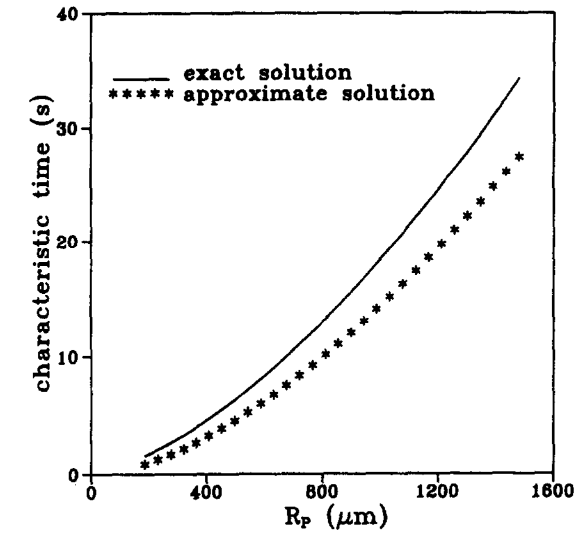


Fig. 2. Characteristic transient time as a function of the radius of the polyimide coating (simulation). Parameters of the buffer, capillary and applied voltage are the same as in Fig. 1. The solid line is the exact solution [7] and the asterisks are the estimates given by eqns. 19 and 20.

The characteristic transient time $\tau_1$ as a function of the polyimide radius is shown in Fig. 2. The solid line is the exact solution obtained by the procedure described in detail in ref. 7 and the asterisks represent the estimated values according to eqns. 19 and 20. Fig. 2 illustrates the growth of transient time with increase in polyimide thickness for the same parameters as in Fig. 1. It is also seen that the approximate eqns. 19 and 20 may be used as an estimate of the characteristic transient time.

The large values of the characteristic transient time shown in Fig. 2 have the positive effect of filtering and smoothing fluctuations of the coolant temperature at the external surface of the capillary, according to eqn. 17. Hence, the theory predicts that an increase in polyimide thickness reduces the amplitude of the fluctuations of the buffer temperature because of the decrease in the temperature of the external surface of the capillary and the increase in the characteristic transient time.

## EXPERIMENTAL

This section presents experimental proof of the basic assumptions and results derived in the theoretical section. It also gives an example of separations with a decreased amplitude of the baseline noise.

The main assumptions and results to be checked experimentally are the following: (1) the baseline signal depends on the buffer temperature and the baseline noise at high voltages is thermally induced; (2) fluctuations of the electric current are also caused by the fluctuations of the buffer temperature; (3) the amplitude of fluctuations of the coolant temperature at the external surface is proportional to the temperature difference between the surface and the coolant (eqn. 14); and (4) an increase in thickness of the polyimide coating decreases the surface temperature of the capillary and the characteristic transient time and thus decreases the amplitude of the baseline and electric current fluctuations.

*Materials and methods*

The following experimental procedure was utilized. During a run, progressively increasing voltages $V_1, V_2, \ldots, V_j, \ldots, V_n$ were applied, for certain time intervals $\Delta t$, to the capillary filled with the buffer solution. The electric current and UV detector signal were monitored simultaneously at a rate of four points per second and stored on the hard disk of a computer. Mean values of the electric current and the baseline were calculated for the time intervals $\overline{\Delta t} = \Delta t - \tau_{tr}$, where $\tau_{tr}$ is the transient time necessary to obtain a steady-state buffer temperature [8]. In order to eliminate the influence of slow baseline and current drifts, regression lines were calculated for each of the time intervals $\overline{\Delta t}$ and the amplitudes of the fluctuations were obtained as square roots of the mean squares about the regression.

Mean values of the electric current $I_j$ corresponding to applied voltages $V_j$ were used to find the I.D. of the capillary and calculate its overall Biot number and the temperature of the buffer, $T_j$ [10,11]. In order to simulate a capillary with a very thick polyimide coating an ordinary capillary was covered with polymer tubing.

A Waters (Millipore, Milford, MA, USA) Quanta 4000 unit having fan cooling and a UV detector set at 254 nm was used. The unit was connected to a NEC APC 4 computer and the data were stored by using BASELINE 810 software. The voltages were turned on and off manually during the run. The data were translated into ASCII files and statistically processed by laboratory-written software. Calculation of the buffer temperature and of the I.D. were performed by using the CZEA software package developed in our laboratory [11]. A fused-silica capillary of 75 $\mu$m I.D., total length 50 cm, obtained from Polymicro Technologies (Phoenix, AZ, USA) was used. The buffer solution was 50 m$M$ $Na_2HPO_4$ titrated to pH 6 with orthophosphoric acid. The specific conductivity of the solutions at 25°C was $\sigma_0 = 6.59 \pm 0.02$ mS/cm and its thermal coefficient was $\alpha = 0.0221 \pm 0.0003$ $K^{-1}$. (It was found that both the specific conductivity and thermal coefficient of the buffer solution depend on the degree of degassing. Thus, the specific conductivity and thermal coefficient of the same buffer stirred for 30 min without degassing were $\sigma_0 = 6.43 \pm 0.04$ mS/cm and $\alpha = 0.0233 \pm 0.0003$ $K^{-1}$, respectively. These values are different from those of the degassed buffer given above.) Deionized, distilled water was utilized. Before the experiments the buffers were degassed for 30 min with water pumps, under stirring. The tubings used for covering the capillaries were made of polyethylene (PE) with I.D. 1 mm and O.D. 2 mm, obtained from LKB, and PVC with I.D. 0.8 mm and O.D. 2.4 mm (Isoflex Kartell). Immobilines were obtained from Pharmacia-LKB (Bromma, Sweden). The length of the covered part of the capillary was 33 cm.

## RESULTS AND DISCUSSION

Synchronized time behaviours of the electric current and the baseline UV signal obtained as described in the previous section are shown in Fig. 3a and b. The time intervals $\Delta t$ during which a constant voltage was applied were 1.2 min, the idle time intervals were 0.6 min each and the time intervals used for data processing were $\overline{\Delta t}$ = 0.7 min. The progression of the applied voltages was from 2 to 18 kV in steps of 2 kV. Voltages are shown by the italic numbers in Fig. 3. The capillary was an ordinary one, without a cover. The actual I.D. (A.I.D.) and the overall

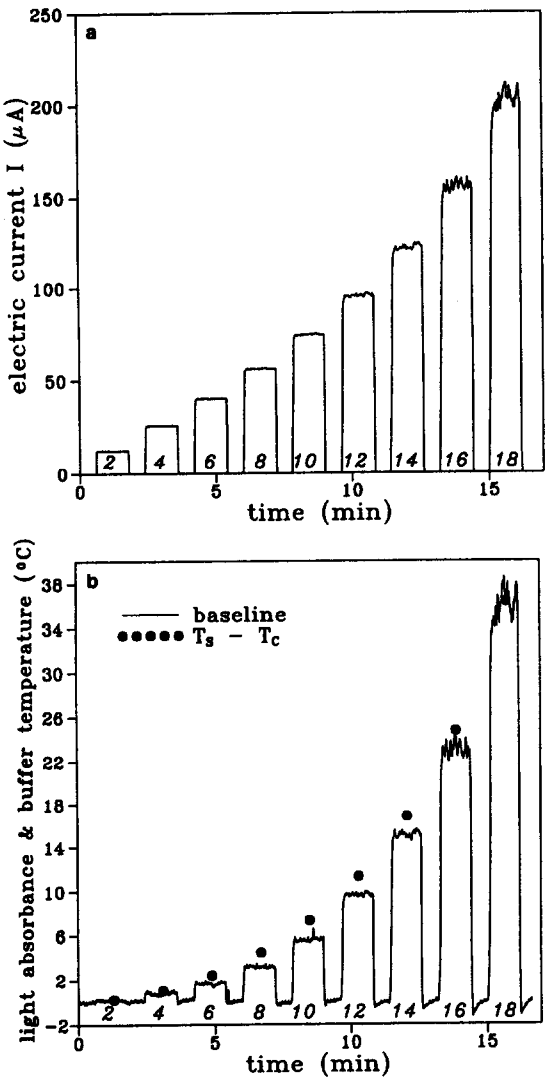


Fig. 3. (a) Electric current in the capillary for progressively increasing voltages in steps of 2 kV from 2 kV to 18 kV (italic numbers). $T_C = 27°C$. Parameters of the capillary and the buffer are given in the text. (b) Baseline for progressively increasing voltages (italic numbers, kV). The baseline is plotted in arbitrary units. Solid circles represent the elevation of the buffer temperature above that of the coolant. All parameters as in (a).

Biot number were found by statistical processing of the mean values of the electric current [11]: A.I.D. = 77 ± 1 μm, $Bi_{OA} = 0.053 \pm 0.002$. The electrical resistance of the capillary at 25°C was $r_0 = (0.163 \pm 0.001) \times 10^9$ Ω.

It can be seen from Fig. 3a and b that starting from 12 kV both the electric current and baseline show fluctuations with an amplitude increasing with increments of the applied voltage. An analysis of the time dependences of the electric current and the baseline showed that the fluctuations of both values are correlated if voltage is applied. The highest correlation coefficient $\rho$ between the baseline and electric current is $\rho = 0.88$ in the last time interval when a voltage of 18 kV is applied. (It is worth remembering that the correlation coefficient of two variables is equal to unity if these variables are linearly dependent and approaches zero for two stochastic independent variables. The correlation coefficient should not be confused with the regression coefficient, which is the slope of the regression line and can have any value for linearly dependent variables.) For lower voltages (*i.e.*, lower heat dissipation) the correlation coefficient becomes smaller and for 2 kV applied it is 0.07. These results show unambiguously that fluctuations of the baseline and the electric current occurring at high voltage originate from the same source.

Elevations of the buffer temperature for each time interval are shown as solid circles in Fig. 3b. We stress that these temperatures were calculated by using only mean values of the electric current. It can be seen that the baseline level

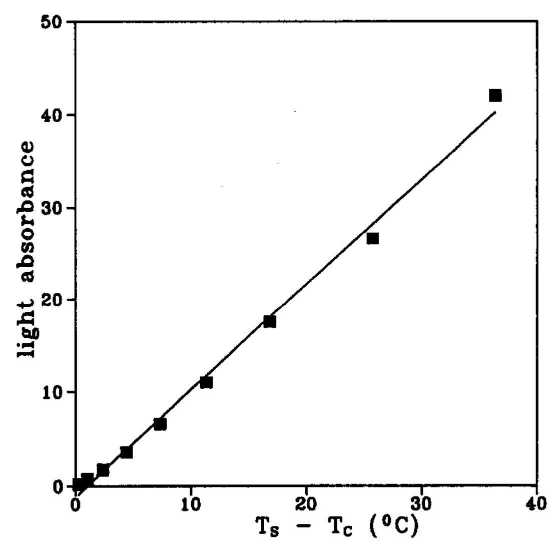


Fig. 4. Time-average values of the baseline (in arbitrary units) *versus* elevation of the buffer temperature. The solid squares are experimental points and the straight line is the regression line. Correlation coefficient $\rho = 0.997$.

follows the buffer temperature. Fig. 4 shows the mean values of the baseline signal *versus* the buffer temperature. The correlation coefficient between the buffer temperature and the mean baseline is $\rho = 0.996$. This value is very close to 1 and it justifies our assumption that the baseline signal is directly proportional to the temperature of the buffer.

Fig. 5 shows the relative amplitude of the fluctuations of the electric current *versus* the function of the current non-linearity. It can be seen that these variables are directly proportional to each other, as it is predicted by eqn. 18 (the correlation coefficient is 0.997). This result proves that fluctuations of the electric current are thermally induced. The same is valid for the baseline fluctuations, as they are correlated with the fluctuations of the electric current, as has been shown above. Additionally, this result proves our assumption in eqn. 16 and, therefore, the theoretical conclusions that an efficient means of suppressing fluctuations is to increase the thickness of the polymer coating.

An example of the time behaviour of the electric current in an ordinary capillary and in a capillary covered with polyethylene tubing is shown in Fig. 6. It can be seen that the am-

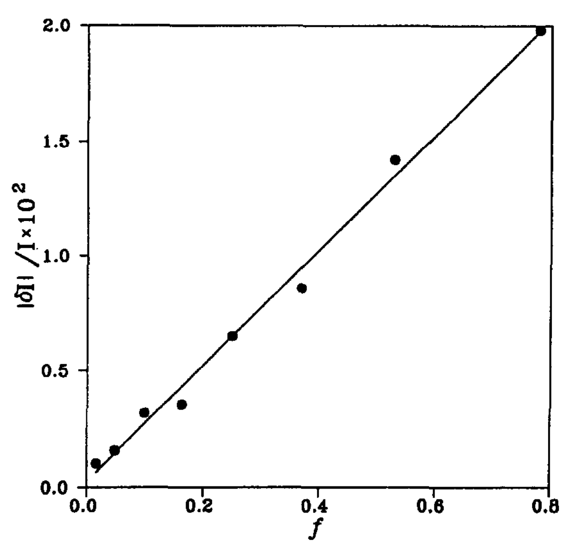


Fig. 5. Relative amplitude of the electric current fluctuations *versus* function of the current non-linearity. The circles are experimental points and the solid line is the regression line. Correlation coefficient $\rho = 0.996$.

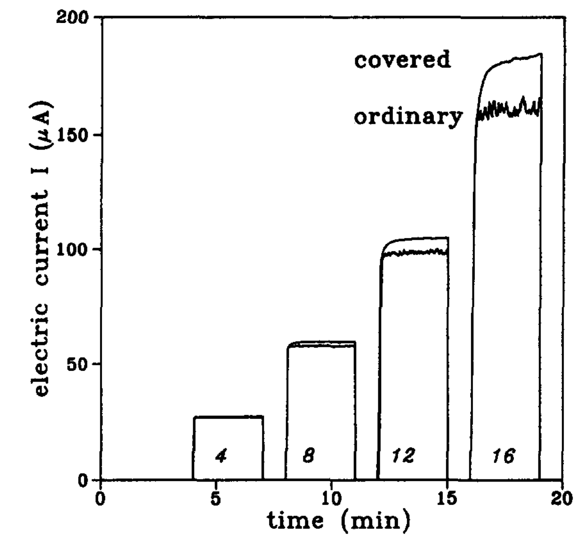


Fig. 6. Electric current in ordinary and covered capillaries for progressively increasing voltages. $T_C = 28.5$°C for the ordinary capillary and 29.4°C for the covered one.

plitude of the current fluctuations for the jacketed capillary is significantly smaller than that for the ordinary capillary. The mean values of the electric current are higher for the covered capillary. This indicates that the buffer temperature in the covered capillary is higher than that in the ordinary capillary. The buffer temperature differences between the covered and the ordinary capillaries were found to be 1.1, 2, 4.2 and 10.2°C for 4, 8, 12 and 16 kV, respectively. The difference of 1.1°C for 4 kV is equal to the difference between the temperatures of the coolant corresponding to the two runs. The increase in buffer temperature for high voltages in the jacketed capillary relative to the ordinary capillary is due to additional thermal insulation caused by a layer of air between the capillary and the tubing covering the capillary. This effect was not taken into account by the theory and appears to be not very significant. Fig. 6 demonstrates also an increase in the transient time for a covered capillary in comparison with that of the ordinary capillary. Experiments with the capillary covered with PVC tubing showed that the buffer temperature remains approximately the same as in the capillary covered with the PE tubing whereas the transient time grows.

Fig. 7 compares the separation of a mixture of

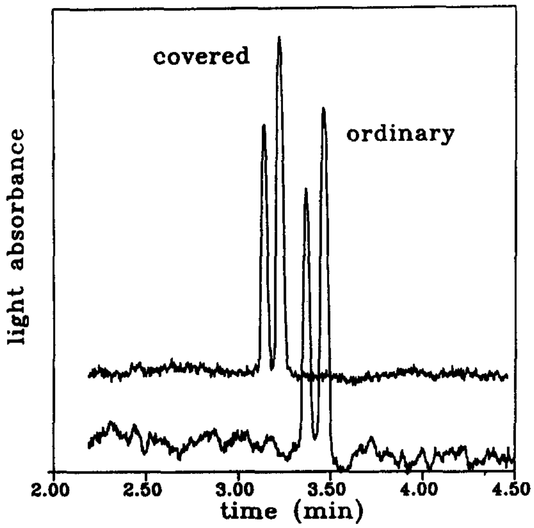


Fig. 7. Separation of a mixture of two Immobilines, p$K$ = 8.5 and 9.3, in ordinary and covered capillaries. $V$ = 14 kV.

two Immobilines of 0.4 m$M$ concentration in the ordinary capillary and in that covered with PE tubing. It illustrates a large decrease in the baseline noise with the covered capillary. The migration time of the Immobilines in the covered capillary is less than that in the ordinary capillary because of an increase in buffer temperature by *ca.* 4°C.

CONCLUSIONS

The baseline and electric current fluctuations have been shown to originate from one source, namely fluctuations of the buffer temperature. The latter fluctuations are produced by the temperature fluctuations at the outer surface of the capillary, which increase with elevation of the buffer temperature. Theoretical solutions for the amplitudes of the baseline and electric current fluctuations predict a large increase near the point of autothermal runaway. It follows from the theory presented here that an increase in the capillary O.D. decreases the amplitudes of both the baseline and current fluctuations and does not lead to a substantial increase in the buffer temperature.

Experiments have shown that the elevation of the baseline that occurs when the applied voltage increases is directly proportional to the increase in buffer temperature. Theoretically predicted proportionality of the relative amplitude of the electric current fluctuations to the function of the current non-linearity has been confirmed experimentally. Jacketing of the capillary with polymer tubing is useful for decreasing baseline and electric current fluctuations.

SYMBOLS

| | |
|---|---|
| $Bi_{OA}$ | overall Biot number |
| $E$ | electric field strength |
| $f$ | function of electric current non-linearity |
| $h$ | heat transfer coefficient |
| $I$ | electric current |
| $L$ | length of the capillary |
| $R$ | radius |
| $r$ | electric resistance of the capillary |
| $T$ | temperature |
| $t$ | time |
| $V$ | voltage |

*Greek letters*

| | |
|---|---|
| $\alpha$ | temperature coefficient of electric conductivity of the buffer |
| $\chi$ | thermal conductivity |
| $\kappa$ | thermal diffusivity |
| $\sigma$ | specific conductivity |
| $\tau$ | transient time |
| $\rho$ | correlation coefficient |
| $\omega$ | circular frequency of the temperature fluctuation |

*Subscripts*

| | |
|---|---|
| L, W, P | lumen, capillary wall and polyimide coating, respectively |
| 0, C | at reference temperature and at the temperature of the coolant, respectively |

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APPENDIX

For a capillary consisting of a fused-silica wall and coated with a layer of a polymer (polyimide), the reciprocal value of the Biot number is given by [1,4,6,7]

$$Bi_{OA}^{-1} = \chi_L[\ln(R_W/R_L)/\chi_W + \ln(R_P/R_W)/\chi_P + (R_P h)^{-1}] \quad (A.1)$$

where $\chi_W$ and $\chi_P$ are the thermal conductivities of the capillary wall and polymer coating, $R_L$, $R_W$ and $R_P$ are the radii of the capillary lumen, wall and the coating, respectively, and $h$ is the heat transfer coefficient. $R_P$ is equal to the capillary O.D.

For a forced air-cooled capillary the heat transfer coefficient is given by (see ref. 8 and references cited therein)

$$h = AR_P^{-0.6} \quad (A.2)$$

where $A$ is a constant for a given coolant and fan rate.

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