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Monitoring the Electroosmotic Flow in Capillary Electrophoresis Using Contactless Conductivity Detection and Thermal Marks

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The fundamental aspects and the capillary electrophoresis usage of thermal marks are presented. The so-called thermal mark is a perturbation of the electrolyte concentration generated by a punctual heating of the capillary while the separation electric field is maintained. The heating pulse is obtained by powering tungsten filaments or surface mount device resistors with 5 V during a few tens to hundreds of milliseconds. In the proposed model, the variation of the transport numbers with the rising temperature leads to the formation of low- and high-concentration regions during the heating. After cooling down, the initial mobilities of the species are restored and these regions (the thermal mark) migrate chiefly due to the electroosmotic flow (EOF). The mark may be recorded with a conductivity detector as part of a usual electropherogram and be used to index the analyte peaks and thus compensate for variations of the EOF. In a favorable case, 10 mmol/L KCl solution, the theory suggests that the error in the measurement of EOF mobility by this mean is only -6.5×10^{-7} cm^2 V^{-1} s^{-1}. The method was applied to the analysis of alkaline ions in egg white, and the relative standard deviations of the corrected mobilities of these ions were smaller than 1%. This is a challenging matrix, because albumin reduces the EOF to 20% of its initial value after 11 runs. The combination of thermal mark, electrolysis separated, and contactless conductivity detection allowed the measurement of the EOF of a silica capillary with unbuffered KCl solution with constant ionic strength. The overall approach is advantageous, because one can easily control the chemical composition of the solution in contact with the inner surface of the capillary.

A keystone of any analytical technique is the reproducibility. In capillary electrophoresis (CE), this basically means the maintenance of the peak area or height and the migration time when the same solution of an analyte is introduced. There are several sources of imperfections that account for the actual quality of the analysis, among them, fluctuations in the electroosmotic flow (EOF), which contributes to the effective mobilities of all the analytes and, therefore, to their migration times. For example, changes of pH or ionic strength cause changes of EOF. However, a chief problem in stabilization of the EOF is the adsorption of macromolecules or highly charged species, because they are often present in the sample. Thus, a run-to-run variation in migration time is a very common problem that may lead to misidentification of the peaks, especially in complex matrices. Besides rinsing procedures to remove adsorbed species, the addition of an internal standard is a common practice to help overcome this problem.

In fact, EOF plays a central role in CE and microchip systems, which goes far beyond the cited problem. For example, it affects both efficiency and selectivity of separation and is essential for the electrokinetic separation techniques as well as in most mobilization approaches in a microchip. This ubiquitous phenomenon has received the attention of several groups concerned with its practical and theoretical characterization as well as with the implementation of methods for its determination.[1−11] Among these methods, two works are highlighted, because they are similar in some respect to the present proposal.

StClaire and Hayes proposed a method based on heating and subsequent laser-induced interferometric backscatter (LIB) detection.[9] A small portion of the capillary before the detection point is electrically heated with a Nichrome coil, and the variation of the refractive index of the hot solution is further detected by LIB. The flow rate is then calculated based on the distance between the coil and the detector and the time interval. Although this method can potentially be used in CE, it was applied only to flow generated by pressure difference in 184-μm-i.d. capillaries. Markov

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and co-workers have also proposed LIB as a flow rate quantification in microfluidic devices.[12,13]

Gilman and co-workers proposed the EOF monitoring by photobleaching of a neutral fluorophore that is incorporated in the running buffer.[10,11] Similarly to the previous work, the idea is to induce a transformation of the electrolyte inside a small portion of the capillary that is moved by the flow to the detector. In this case, such a transformation is obtained by photodegradation of rhodamine B 10^{-7}–10^{-8} mol/L. Because the EOF monitoring device is positioned after the UV/visible detection point, it is possible to continuously monitor the flow during an electrophoretic run.

In the present paper, we propose the use of controlled heating of a small portion of the column during the electrophoretic run to produce a permanent change of composition in the electrolyte that can be recorded and used as an internal standard. This disturbance—called here a thermal mark—is a convenient way to generate such a standard, because no substance is added to the sample and it may be freely positioned within the electropherogram. Although, in such an application, the internal standard does not necessarily need to have the same mobility as the EOF, we have observed that thermal marks can be used for measurements of EOF mobility as well. The conditions and limitations are discussed.

EXPERIMENTAL SECTION

Reagents and Solutions. Dimethyl sulfoxide (DMSO), dibenzo-18-crown-6 (DB18C6), histidine (His), and 2-morpholinoethanesulfonic acid (MES) were purchased from Sigma (St. Louis, MO). Sodium acetate trihydrate, sodium tetraborate, and cetyltrimethylammonium bromide (CTAB) were purchased from Merck (Rio de Janeiro, Brazil). Sodium dodecyl sulfate (SDS) was purchased from Synth (Diadema, São Paulo, Brazil). All reagents were of analytical grade and were used without additional treatments. Deionized water (Nanopure-UV, Barnsted, Dubuque, IA) was used to prepare the solutions.

Instrumentation. Electrophoresis experiments were carried out in equipment with additional compartments for external electrolysis[14] and capacitively coupled contactless conductivity detection (C^4D).[15,16] Unless otherwise stated, the detector was operated at 550 kHz and 2.0 $V_{peak-to-peak}$.

The simplest version of the thermal marker was implemented with a 10-W, 12-V automotive lamp. The glass bulb was carefully removed to allow the tungsten filament to be placed in contact with the silica capillary (Figure 1). After the bulb is removed, the lamp cannot be powered by 12 V, because the filament would be burned. Thus, in all the experiments, only 5-V voltage was applied. The actuation of this voltage was controlled by software to improve synchronization with the electrophoresis and reproducibility of the pulse width.

The thermal marker was also implemented with a surface mount device (SMD) resistor. A 15-Ω SMD resistor (chip size 0402) was soldered on a printed circuit board as shown in Figure 1. In both cases, the heater touches only a small portion of the capillary (~0.5 mm).

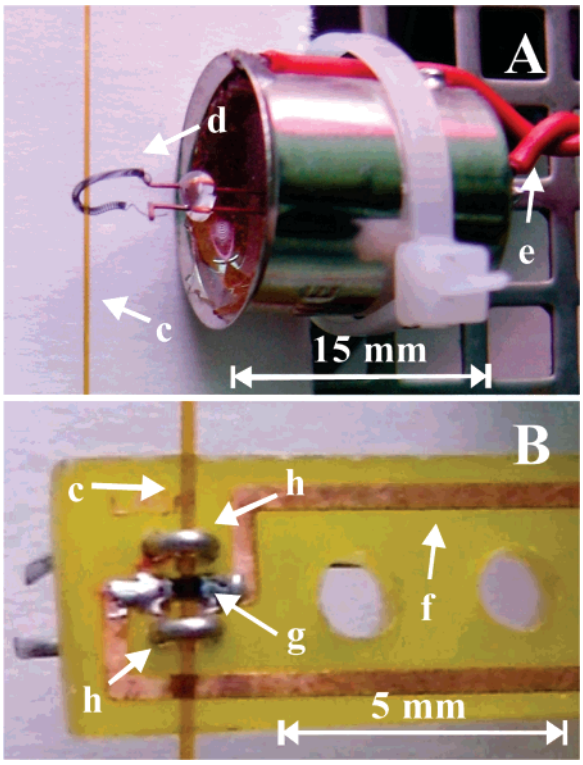

Figure 1. Thermal markers based on (A) tungsten lamp and (B) SMD resistor: (c) silica capillary, (d) tungsten filament, (e) electrical connections, (f) copper track on the printed circuit board for electrical connection, (g) SMD resistor chip size 0402 ($L \times W \times H = 1.0 \times 0.5 \times 0.3$ mm), and (h) capillary holder.

Capillary electrophoresis equipment operates with high-voltage power sources. Thus, the user should be aware of the risk of electrical shock. Although extensive tests were carried out, there is the risk of electrical discharges through the silica wall of a broken capillary to the metallic rings of the detector or the thermal markers, which may cause damage to the equipment or injury to the operator.

RESULTS AND DISCUSSION

The initial tests were carried out with the tungsten filament described in the Experimental Section. The contact between the tungsten filament and the polyimide coat of the silica capillary allows the transference of the heat generated by the electrical current flowing through the filament to the solution inside the capillary. Of course, the actual amount of heat transferred to a specific portion of solution—and consequently the temperature—depends on the effectiveness of the thermal contact as well as on the chosen lamp (manufacturer, length and shape of the filament, and so on) and operational conditions such as air flow and presence of electroosmotic flow inside the capillary. Thus, it is not practicable to predict the temperature inside the capillary, and the best condition should be empirically obtained. However, in spite of the simplicity of the device, reproducible results were obtained after the filament was positioned on the capillary. Usually, pulses ranging from 50 to 300 ms give observable marks in several kinds of running electrolytes.

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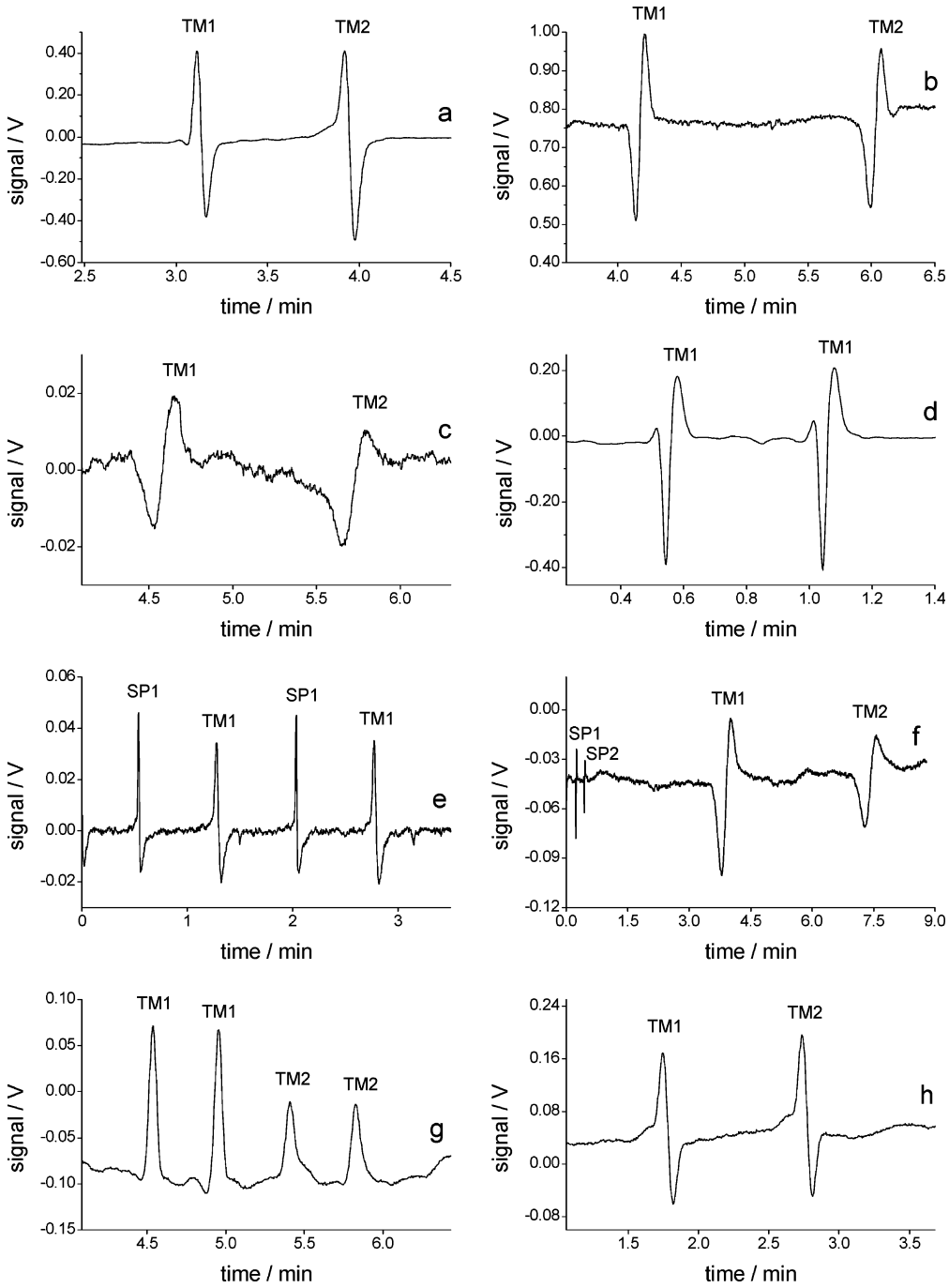

Figure 2. Usage examples of thermal marks in real electrolytes: (a) 10 mmol/L NaCl, (b) 10 mmol/L KCl, (c) 20 mmol/L MES/His (pH 6.0), (d) 10 mmol/L Borax buffer (pH 9.2), (e) 20 mmol/L MES/His with 0.2 mmol/L CTAB, (f) 10 mmol/L KCl (pH 4.0), (g) 25 mmol/L sodium phosphate buffer (pH 7.0) with 100 mmol/L SDS, and (h) 10 mmol/L KCl in DMSO with 4.0 mmol/L DB18C6. Electropherograms a and b were obtained with double filament marker fired once. Electropherograms c, f, and h were obtained with double SMD marker fired once. Electropherograms d and e were obtained with one filament fired twice. Electropherogram g was obtained with double filament marker fired twice.

Figure 2 shows examples of electropherograms obtained for different running electrolytes and conditions. The intensity and the shape of the marks depend on the heating and the electrolyte composition. However, in all studied cases, a perturbation of the baseline (the so-called thermal marks) appeared and migrated toward the detector with velocity close to the electroosmotic flow, which was estimated by monitoring the water peak migration time.

The experiments shown in Figure 2 will be analyzed in detail after a discussion about the nature of the phenomenon.

Nature of the Thermal Mark. Before applications are presented, it is important to discuss the phenomena involved and how the marks are formed.

The first hypothesis is that the change of conductivity due to the rising temperature is observable at the detection point. To test this hypothesis, the thermal marker was placed at different positions along the capillary (Figure 3). The temperature of the solution at the detection point depends on the distance traveled since the heating point. Thus, the farthest heating point should

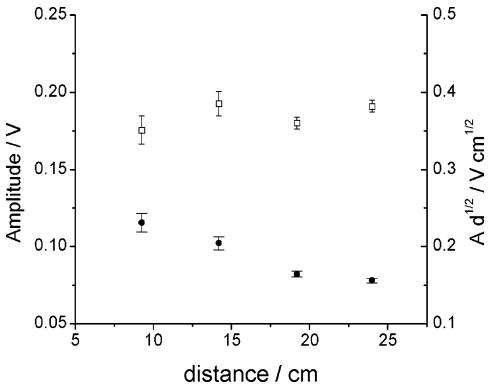

Figure 3. Amplitude (●) and corrected amplitude (□) of the thermal mark as a function of the distance from the marker until the detector. Each point is the average and its standard deviation of 6 replicates. Electrolyte, KCl 10 mmol/L; capillary, 75-μm i.d. and 57.3 cm long; voltage, 10 kV; width of the thermal marks, 200 ms.

give the smallest thermal mark, because the temperature would approach that one along the capillary and only a very small difference in conductivity would be observed. Figure 3 shows that the amplitude of the thermal mark falls as a function of the distance. However, one should consider that diffusion also contributes to the reduction of the thermal mark amplitude.

It is straightforward to demonstrate that the amplitude of a peak under diffusion (Fick's second law) varies with one over the square root of the time. In the present case, the peaks migrate with constant velocity. Thus, one can conclude that the amplitude depends on one over the square root of the distance between the position of the thermal marker and the detector. To compensate this natural reduction of the amplitude, Figure 3 also shows the amplitude multiplied by the square root of the distance as a function of the distance. The results of this experiment show that longitudinal diffusion is sufficient to account for the observed peak attenuation.

Thus, thinking of thermal mark as the result of a conductivity measurement of a hot region implies that the region heated by a few milliseconds stays nearly at the same temperature for several minutes, which is not likely to happen. The findings of Waldron and Dovichi,[17] according to whom the thermal relaxation time of a laser-heated solution inside the capillary is on the order of 1 ms, also support our conclusion.

Another strong argument against this first hypothesis is that, most of the time, thermal marks are symmetrical; i.e., there is a positive half-cycle (increasing of conductivity) and a negative half-cycle (reduction of conductivity). Heating can explain the positive half-cycle, but not the negative one.

The monitoring of a hot region of the solution was explored by StClaire and Hayes in their method based on heat indexing and LIB detection.[9] In that case, however, the detection point was only 9 mm far from the heating point. Thus, the difference in refractive index caused by heating could be detected, because the solution is still hot when it reaches the detector. In the present case, the detector is far from the heated region, and the evidence shows that the solution is cool.

The second hypothesis is that new species are formed during the heating which migrate to the detector. In fact, the heating can cause chemical reactions and therefore new species could arise or the heating can even shift some chemical equilibrium, increasing or decreasing for a moment the concentration of species in the running electrolyte. However, the experiments with simple electrolytes such as NaCl and KCl demonstrated that this is not the bottom line, because aqueous solutions of these salts heated below the ebullition point certainly do not undergo chemical transformations. Of course, heat-induced chemical transformations can occur in some cases and account for some anomalies and additional peaks. However, it is not the fundamental phenomenon behind the thermal marks.

The third hypothesis is that the temperature rising changes the viscosity of the solution and consequently the mobilities of the species. Thus, the hot zone of the solution has a different composition for a few moments. In that case, the hot zone would behave like a sample plug. The species would migrate with different velocities, but when temperature falls, the viscosity would become homogeneous again, as well as the behavior of the species. The result would be zones of different concentrations of electrolytes and therefore different conductivities. These regions would be moved by the electroosmotic flow toward the detector. This hypothesis may be evaluated as follows.

The electric field inside the capillary is given by $E = \mathrm{d}V/\mathrm{d}x$, where V is the potential and x is the position along the capillary. From Ohm's law and considering that the current remains constant, the electric field becomes

$$E = I\frac{\mathrm{d}R}{\mathrm{d}x} = \frac{I}{\kappa a} = \frac{J}{\kappa} \tag{1}$$

where I is the current, R and κ are, respectively, the resistance and conductivity of the solution, a is the inner sectional area of the capillary, and J is the current density.

The conductivity is the sum of the contribution of all charge carriers:

$$\kappa = \sum_j \lambda_j c_j \tag{2}$$

where c_j is the concentration of the species j and λ_j is its molar conductivity given by

$$\lambda_j = z_j \mu_j F \tag{3}$$

where F is the Faraday constant and z_j and μ_j are the charge and mobility, respectively, of the species j. The mobility of a spherical species j of radius r_j, greater than the molecules of the solvent, in a solvent of viscosity η may be obtained from Stokes' relation:

$$\mu_j = \frac{e}{6\pi} \cdot \frac{1}{\eta} \cdot \frac{z_j}{r_j} \tag{4}$$

where e is the elementary charge. By substituting eqs 2−4 in 1

$$E = \eta J \frac{6\pi}{eF}\left(\sum_j \frac{z_j^2 \cdot c_j}{r_j}\right)^{-1} \tag{5}$$

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one can note that electric field in a specific position along the capillary depends on the viscosity and consequently on the temperature. However, taking into account that the velocity of a species *s* is given by the product of the electric field (eq 5) by its mobility (eq 4), the species will migrate with the velocity given by

$$v_s = \frac{Jz_s}{Fr_s}\left(\sum_j \frac{z_j^2 c_j}{r_j}\right)^{-1} \tag{6}$$

From this equation, one can note that the velocity does not depend on the viscosity of the solution, because the mobility rises and the electric field falls to the same extent. This equation shows that, although the dependence of the viscosity on temperature is by far the most important effect on the mobilities of the species at the heated region, it does not explain the thermal marks.

However, eq 6 allows one to propose a new hypothesis similar to the previous one, but considering the effect of the temperature on the radius of the species (r_s). If the hydrodynamic radius varies differently for each species, then their velocities in the heated region are different. This region would behave as a plug of a solution of different composition. One more time, after the solution is cooled, the mobilities would be restored, but a permanent mark (fluctuation in the electrolyte concentration) would arise.

Another form of eq 6 may be obtained from the previous ones:

$$v_s = \frac{J}{F}\frac{\mu_s}{\sum_j z_j c_j \mu_j} \tag{7}$$

For an electrolyte composed of one univalent cation and one univalent anion, this equation becomes

$$v_s = \frac{J}{Fc}\frac{\mu_s}{\sum_j \mu_j} = \frac{J}{Fc}\frac{\lambda_s}{\sum_j \lambda_j} = \frac{J}{Fc}t_s \tag{8}$$

where c is the concentration of the electrolyte and t_s is the transport number of the species *s*.

The transport number may be calculated from molar conductance data from literature.[18] Figure 4 shows the transport number for Na^+ and K^+ in 10 mmol/L NaCl and KCl solutions as a function of the temperature. In fact, these curves suggest that the mobilities of the hot ions vary differently with the temperature. In addition, they suggest that hot sodium (named here $^*Na^+$) becomes relatively faster than hot chloride ($^*Cl^-$), while the contrary occurs with hot potassium ($^*K^+$). If this is true, $^*Na^+$ would migrate faster during the heating process. After cooling down, the global effect would be a concentrated region followed by a diluted one, which would be detected as a signal with the shape of a peak−valley sequence. For a KCl solution, a valley−peak shape would be expected. (One can get an insight about the phenomenon by numerical simulation of the proposed condition using software such as Simul program from Gaš's group available at http://www.natur.cuni.cz/~gas/ (accessed February 1, 2006). Both predictions are supported by the results shown in Figures 2 and 4 for NaCl and KCl solution, which endorse the hypothesis.

(18) Conway, B. E. *Electrochemical Data*; Elsevier: Dordrecht, The Netherlands; 1952; pp 139−146.

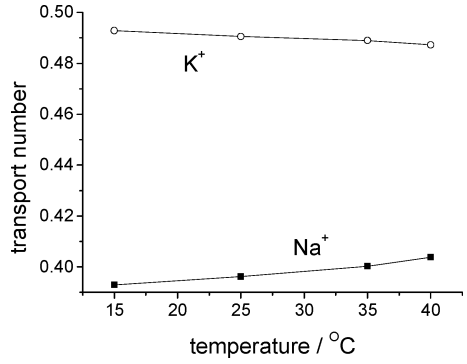

Figure 4. Transport number for Na^+ and K^+ in 10 mmol/L NaCl and KCl solutions, respectively, as a function of the temperature (calculated based on data from ref 18). The smooth curves are drawn as a guide to the eye.

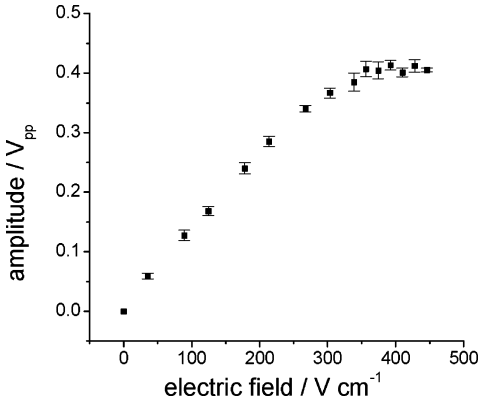

Figure 5. Amplitude of the thermal mark as a function of the electric field applied during the heating.

Another feature of this model (eqs 6 and 8) is that it predicts the dependence of the amplitude of the thermal mark on the current and thus on the electric field applied during heating. This dependence arises from the fact that the greater the relative difference of the $^*Na^+$ velocity over Na^+, the greater accumulation of salt at the front side of the region. For example, an extreme condition is the heating while a null electric field is applied. Equations 6 and 8 suggest that no thermal mark would be generated in that case.

A set of electropherograms were obtained after a previous period in which thermal marks were formed at different electric fields. In these experiments, a specific electric field was applied during 15 s while a 100-ms-width heating pulse was generated. The thermal mark was generated after an initial period of 5 s to ensure the stabilization of the column. The final 10-s period ensures complete cooling down before the acquisition of the electropherograms, which were obtained always at the same electric field (178 V/cm).

The results shown in Figure 5 indicate that in fact the amplitude of the thermal mark depends on the electric field during heating. The curve shows two distinct regions: one for low electric field that matches the models and a second one that suggests saturation. This second portion of the curve was not yet extensively investigated.

Mobility of the Thermal Mark. It is clear that, for simple electrolytes, such as those ones considered in the previous section, thermal marks result in disturbances in the concentration of the electrolyte, which remains, almost all the time, with the same chemical composition along the capillary.

At first glance, one can suppose that this disturbance does not migrate by itself and that it has an effective mobility equal to the EOF. This would be a desirable feature, because the marks could be used as EOF markers. However, the inhomogeneity generated by the thermal mark, as well as the one generated by any kind of sample injection, will produce one or more zones that propagate through the capillary. Gaš and Kenndler reviewed the work dealing with these system zones[19] and also demonstrated that the difference between the migration of a neutral marker and one from the so-called water zone may be experimentally observable.[20]

In fact, the movement of a concentration boundary is an old issue. According to Stockmayer, the following relation must hold for the steady movement of a boundary between two solutions of different compositions submitted to an electric field:[21]

$$v_b = \frac{J}{F}\frac{(t_1 - t_2)}{(c_1 - c_2)} \tag{9}$$

where t is the transport number and the indexes 1 and 2 refer to the two different zones faced to the boundary. Equation 9 must hold for the transport numbers and concentrations of all chemical species present in the two solutions. It shows that, during an electrophoretic run, the velocity of the boundary would be zero only if t_1 and t_2 were equal. However, the transport number depends on the concentration in a complex way.[20]

For our purposes, a particularly interesting case of eq 9 is the limiting velocity of a boundary for very small concentration differences, because the experimental evidence suggests that the variations of concentration induced by the heating are small.

For the case of monovalent ions, the transport number may be related to the concentration through the molar conductance (Kohlrausch's law):

$$\lambda = \lambda^o - \alpha\sqrt{c} \tag{10}$$

where λ^o is the limiting conductance and α is a coefficient that depends on the species, temperature, and solvent properties. Thus, the limiting velocity of a boundary becomes

$$\lim_{\Delta c \to 0} v_b = v_0 = \frac{J}{F}\frac{\mathrm{d}t}{\mathrm{d}c} = \frac{J}{2F}\frac{\lambda^o{}_+\alpha_- - \lambda^o{}_-\alpha_+}{(\lambda^o{}_+ + \lambda^o{}_- - (\alpha_+ + \alpha_-)\sqrt{c})^2\sqrt{c}} \tag{11}$$

where + and − signal stand for the cation and anion properties, respectively. By substituting the value of the electric field given by eq 1 into eq 11, one can obtain a more convenient form of this equation:

$$v_0 = \frac{E}{2F}\frac{(\lambda^o{}_+\alpha_- - \lambda^o{}_-\alpha_+)\sqrt{c}}{(\lambda^o{}_+ + \lambda^o{}_- - (\alpha_+ + \alpha_-)\sqrt{c})} \tag{12}$$

Equation 12 allows one to define the mobility of the boundary by

$$\mu_0 = \frac{(\lambda^o{}_+\alpha_- - \lambda^o{}_-\alpha_+)\sqrt{c}}{2F(\lambda^o{}_+ + \lambda^o{}_- - (\alpha_+ + \alpha_-)\sqrt{c})} \tag{13}$$

This mobility should be added to the EOF to describe the migration of a boundary between zones of similar concentrations. For a proper measurement of the EOF, μ_0 should be as small as possible. There are two possible situations where μ_0 would be zero: (1) when the concentration is zero and (2) when the difference in the numerator is zero. Of course, only the second condition is of practical use. In fact, the coefficient α is also a function of the limiting conductance according to Onsager equation.[18] Thus, the mobility will be zero when

$$\frac{A + B\lambda^o{}_+}{\lambda^o{}_+} = \frac{A + B\lambda^o{}_-}{\lambda^o{}_-} \tag{14}$$

where A and B are coefficients that do not depend on the species and the numerators of this equation stand for the α of each ion. It is clear that the above equality is attained when both molar conductances are equal.

This assertion means that the migration of a concentration boundary becomes a reliable marker for the EOF when the zones have similar concentrations and the ions have similar mobilities. It is also worthwhile to note that, although the derivative was considered in eq 11, the conclusions can also be applicable when transport number varies linearly in the range delimited by the concentration of the zones. As stated before, the dependency is not linear, but the conclusions hold for small differences. For example, if one considers that the potassium transport number varies linearly with concentration within the range from 10 to 11 mmol/L in KCl solutions, he/she is committing a relative error for the calculated transport number smaller than 1/10 000. By calibrating the C^{4}D detector with KCl 10.0 and 10.1 mmol/L solutions, it was possible to determine that the amplitude of the thermal mark is smaller than 0.4 mmol/L, which means that the previous approximation is valid. In addition, the calculated mobility of the thermal mark (eq 13) is -6.5×10^{-7} cm^2 V^{-1} s^{-1}, which introduces a systematic error smaller than −0.7% for EOF greater than 10^{-4} cm^2 V^{-1} s^{-1}. Although these conditions and results cannot be generalized, they show that it is possible to obtain good EOF measurement with reasonable experimental conditions.

Double Thermal Marker. It is straightforward to obtain the mobility of the thermal mark starting from its migration time, the electric field, and the distance from the marker to the detector. However, this last parameter is not easily obtained, because sometimes the exact point where detection occurs cannot be precisely identified. To overcome this problem, a double thermal

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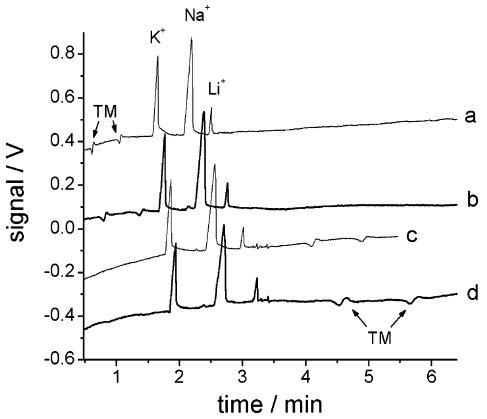

Figure 6. Electropherograms of chicken egg white. (a–d) are, respectively, the second, fifth, seventh, and ninth consecutive runs after flushing with 1 mol/L NaOH. Capillary: 75-μm i.d., 55.3-cm total length, 46.2 cm effective. Thermal markers positioned at 4.2 and 7.2 cm from detector. Running electrolyte, MES/His 20 mmol/L (pH 6.0); running potential, 25.22 kV; sample, chicken egg white diluted 30 times with 333 μmol/L $LiNO_3$ as internal standard; injection, hydrodynamic for 3 s and 10 cm of H_2O.

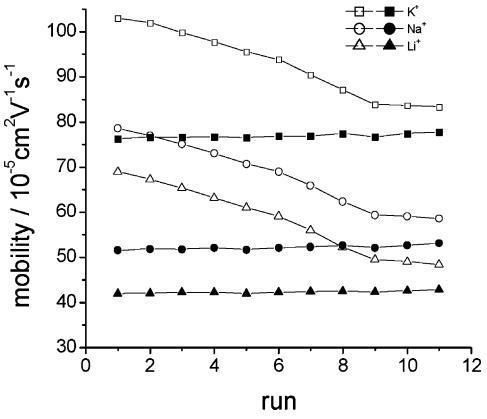

Figure 7. Evolution of the apparent (open symbols) and corrected (filled symbols) mobilities of K^+, Na^+, and Li^+ in consecutive electropherograms of chicken egg white (details in Figure 6). The smooth curves are drawn as a guide to the eye.

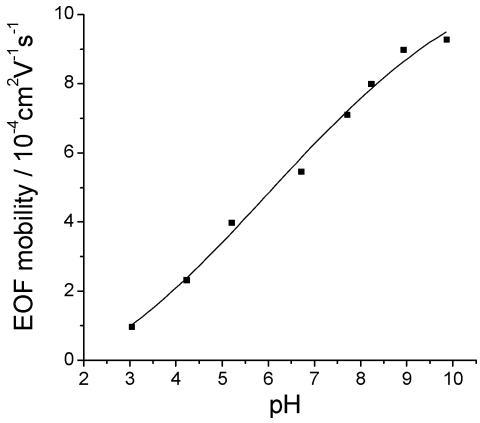

Figure 8. EOF measurement using thermal mark. The different pH values were obtained mixing KOH and HCl solutions and KCl was used to correct the ionic strength to 10 mmol/L. Capillary: 75-μm i.d., 59.5-cm total length. Thermal markers positioned at 5.6 and 10.6 cm from detector. Temperature, 32 °C. The smooth curve is drawn as a guide to the eye.

marker was developed. In this implementation, two filaments or SMD resistors are positioned along the capillary and they are simultaneously heated. Thus, two subsequent marks are observed in the electropherogram. Now, the migration time may be calculated from the distance between the markers and the difference of migration time of the two marks in the electropherogram. The time difference should be considered between the same relative portion of the mark, e.g., from top to top or from bottom to bottom. Of course, the final precision depends on the relative precision of the measurement of the distance between the markers. Thus, in general, the greater the distance between the markers, the greater will be the precision.

The electropherograms a–c and f–h in Figure 2 were obtained using the double thermal marker. The differences in thermal coupling between the capillary and the markers are evident, because the amplitudes are different, especially in the case of electropherogram g. One should also take into account that the marks are generated in different places, and thus, the diffusion partially accounts for the flatness of the farthest mark from the detector. However, we have observed that the thermal coupling is the most important factor to determine the relative intensities of the marks. For example, the second mark in electropherogram a is greater than the first one.

Applications of Thermal Marks. Figure 6 shows a usage example of a thermal mark in the analysis of Na^+ and K^+ in egg white using Li^+ as internal standard. This example was selected because the albumin present in egg white easily adsorbs on the inner surface of the capillary and changes the EOF significantly in a few runs. For the freshly cleaned capillary (flushing with 1 mol/L NaOH), the EOF was 27×10^{-5} cm^2 V^{-1} s^{-1} and it decayed to 5.5×10^{-5} cm^2 V^{-1} s^{-1} after 11 runs; i.e., the final value was only 20% of the initial value.

A double thermal marker was positioned near the detector (4.2 and 7.2 cm). For the first six runs, the EOF was high enough to allow the detection of the marks before the fastest analyte ion (K^+) had reached the detector—the sample was injected at 46.2 cm from the detection point. However, after this run, the adsorbed protein reduces the EOF to an extent enough to provoke the overlapping of the mark and the analyte peaks. The solution to this problem is to delay the heating pulse by 180 s. Thus, the analyte peaks are recorded before the thermal marks as shown in Figure 6 (runs 7 and 9). Figure 7 shows the evolution of the mobilities of K^+, Na^+, and Li^+ for several runs, as well as their corrected values taking into account the thermal marks. The low relative standard deviations of the corrected mobilities (smaller than 1%) suggest that the identification of the analyte peaks across several electropherograms may be done with confidence.

This example also illustrates the possibility of using the thermal marks not necessarily at the beginning of the run but at any time after it to prevent overlapping of the marks and other peaks in the electropherogram.

Figure 8 shows the usage of thermal marks for measurement of EOF mobility. The use of equipment with electrolysis separated configuration[14] allowed the use of nonbuffered solutions prepared with different proportions of KOH, HCl, and KCl to obtain different pH values, keeping constant the ionic strength. The use of nonbuffered solutions is an interesting feature of the system, because it allows an easy control of the chemical composition of the solution, preventing adsorbing species, for example.

Figure 2 shows that thermal marks can be obtained in different media and electrolytes. Although the shape of the mark depends on the nature of the running electrolyte, it may be classified basically into two classes: peak−valley and valley−peak shapes. Two representatives of these classes are the aqueous solutions NaCl an KCl, respectively.

Other electrolytes behave in similar fashion, and they were not included in Figure 2 for the sake of saving space. The aqueous solutions of NaI, KNO_3, tetrabutylammonium bromide, and tetramethylammonium bromide and a DMSO solution of KNO_3 are also in the class of peak−valley shapes. On the other hand, aqueous solutions of KBr, KI, sodium phosphate buffer (pH 7.0), and sodium acetate buffer (pH 4.7) may be included in the valley−peak class.

Sodium borate and SDS/sodium phosphate buffers are examples of media that render asymmetrical marks (Figure 3). However, there is no evidence of disagreement between the velocities of the water peak and the thermal mark.

Although we are focusing on the marks that migrate with the EOF, some electrolytes render additional marks that migrate with different velocities. This occurs when complex electrolytes are used. For example, MES/His buffer with CTAB (Figure 2e), which is useful for anion analysis, presents a faster peak that is related to the presence of a second anion in small concentration, i.e., bromide. The same occurs even in simpler solutions. For example, an electrolyte prepared with KOH, KCl, and HCl to obtain 10 mmol/L KCl (pH 4.0) presents a very fast peak (Figure 2f), which is related to H^+. In neutral KCl solutions, this peak is not present. On the other hand, a peak with negative mobility would be observable in alkaline solution due to the OH^- ions. This phenomenon may be understood as system peaks or eigenpeaks[19,22] and should be considered in practical use of thermal marks to prevent artifacts. On the other hand, this phenomenon opens the possibility of using punctual heating as a sample injection approach, similarly to the optically gated vacancy one,[23,24] and using the two-marker method for correction of variations in both electroosmotic and electrophoretic mobilities.[25]

Special demonstration of the generality of the approach is the presence of thermal marks even in micellar and nonaqueous media. It is worthwhile to note that the shape for the KCl solution changes from valley−peak to peak−valley if water is substituted by DMSO. Most probably, not only the solvent makes a difference in this case, because DB18C6—added to augment the solubility of the salt in DMSO—affects the potassium or chloride mobilities as well as their thermal behavior.

It is worthwhile to compare the proposed method with the simpler one based on monitoring the so-called water plug. In fact, most probably, all kinds of detectors that are able to record a thermal mark are also able to record the water plug, because in both cases, a variation of concentration of the electrolyte is monitored. However, the thermal mark approach has two advantages over the water plug: (1) the unpredictable size of the water peak when a real sample is injected and (2) the possibility of generating several thermal marks without any mechanical procedure as occur in sample injection. The first point is well known by the CE-C^4D users. The size of the water peak depends on the composition of the sample. It is possible to obtain negative or positive peaks or even no peak. Also the peak may be so huge and sided by overshoot peaks that it becomes unmanageable. On the other hand, intensity and position of the thermal marks may be easily managed. The second point is a very interesting feature, because it allows, for example, the measurement of the EOF at virtually any moment while the electropherogram is being obtained or the continuous monitoring of EOF during capillary conditioning.

It is worthwhile to note that, although a thermal mark is advantageous compared to a water-peak approach, the resulting disturbance of concentration has the same behavior in both cases. This means that when an electrolyte does not render a system peak that migrates with the velocity of the EOF, neither water peak nor thermal mark will be useful to measure the EOF. For example, Jaros and co-workers demonstrated that oxalic acid/imidazole solution with pH 3.70 is a particular case where two system peaks are observed in the C^4D, but one has significantly positive velocity while the other one has negative mobility.[26] Of course, these peaks are still useful as an index to help with the identification of the peaks in a electropherogram.

The same considerations applied to the comparison between neutral marker and water peak also apply to thermal marks, because of their similar nature. Basically, a neutral marker is advantageous, because its mobility is zero, while a concentration disturbance may have an effective mobility (eq 13). However, high concentrations of a neutral marker may significantly change the medium, which leads to concentration boundaries.[7] Thermal marks are advantageous, because they may be continuously generated to monitor EOF and C^4D does not detect neutral markers.

CONCLUSIONS

Thanks to its simplicity, thermal marker may be easily implemented and used as a standard device for indexing of electropherograms. Of course, such application demands normalization about how to index that is out of the scope of the present paper.

The final perturbation provoked by the heating on the electrolyte concentration is very small, but it could be easily recorded with a C^4D. Other detectors that are sensitive to such a change in the running electrolyte can potentially be used, e.g., indirect UV detection. Direct detection based on UV or fluorescence, for example, is not a candidate to be used as a monitor for thermal marks. On the other hand, systems with these kinds of detector could be complemented with thermal markers and C^4D without problems of overlap of the marks with the analyte peaks.

Likewise other methods based on some kind of perturbation that propagates until the detection point; thermal mark mobility does not perfectly match the EOF one. This shortcoming is the same observed with the use of the water plug method:[20,22] C^4D detects system zones that, only in favorable conditions, migrate

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with almost the same velocity as the EOF. However, taking into account the several factors that do eventually contribute to EOF in actual CE systems—and that are often neglected—one can conclude that thermal mark is a very suitable approach, especially for monitoring of capillaries under a conditioning procedure.

Of course, it is not possible to generalize the good agreement between mark migration time and EOF, as demonstrated in the previous section for the case of KCl solution. However, even in those cases where μ_0 is considerably different from zero, the mark can be used as an internal reference. In this manner, one can compare and identify peaks among several electropherograms.

An interesting feature of the system including electrolysis separated is the possibility of dismissing the use of a buffer solution. This simplification eases the control of the ionic strength and of adsorbing species in the test solution while the EOF measurement is done.

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