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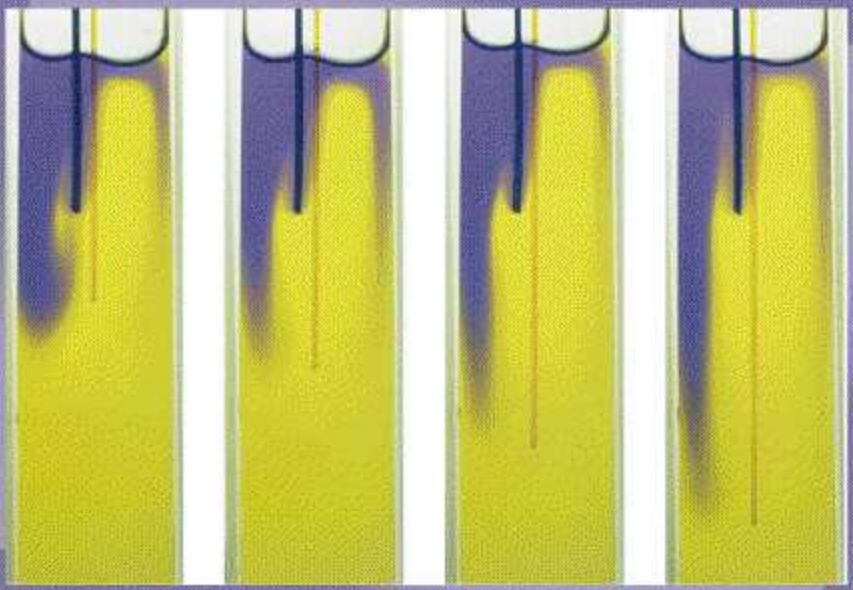

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Changes in Electrolyte pH Due to Electrolysis during Capillary Zone Electrophoresis

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The positioning of the capillary relative to the electrode in the electrolyte vial and buffering of the background electrolyte (BGE) in capillary zone electrophoresis have been found to have a crucial influence on the magnitude of pH changes of the BGE in the separation capillary. On-capillary measurement of the pH changes was accomplished by adding a suitable pH indicator dye to the BGE and employing absorbance detection in the visible region. This method has been demonstrated using xylenol blue in a chromate BGE commonly used for anion analysis (pH 8.0) and bromocresol green in an acetate BGE (pH 4.8). Using the chromate BGE, high pH changes of more than 2.5 pH units were observed when the capillary end was positioned close to the electrode surface in the inlet electrolyte vial, but changes of less than ±0.3 pH unit resulted if the capillary end was displaced at least 1 mm laterally from the electrode and/or at least 1 mm vertically below the electrode end. These effects were explained by direct observations of pH changes around a platinum electrode placed in a photometric cell serving as a BGE reservoir. These observations showed a zone of altered pH being generated close to the electrode and spreading over time through the surrounding BGE by thermal convection. Further, a simple way of buffering the chromate electrolyte with a suitable cation (Tris) is presented, with the buffered electrolyte showing pH fluctuations in the capillary of less than ±0.2 pH unit. Practical implications of these findings on the practice of capillary zone electrophoresis are discussed.

It is well known that electrolysis of the background electrolyte (BGE) caused by the applied separation voltage is an accompanying phenomenon in electrophoresis.[1] For most aqueous BGEs, this electrolysis results in a decrease in pH at the anode, where hydrogen ions are formed, and an increase in pH at the cathode, where hydroxyl ions are formed. In capillary zone electrophoresis (CZE), the pH of the BGE is a major parameter influencing the electrophoretic mobilities of the analytes and thus the separation selectivity: even a pH change as small as only 0.03 pH unit can significantly change the selectivity and deteriorate the resolution dramatically.[2] The pH also influences electroosmotic flow (EOF), and, therefore, the migration times of all analytes. In addition, detection properties of analytes may change with pH.

While controlled, reproducible pH changes in the BGE can serve as a useful separation tool in CZE,[3] pH changes resulting from electrolysis by the separation voltage are usually irreproducible and can be considered to be detrimental. Despite the fact that electrolysis is an inevitable accompanying phenomenon in electrophoresis, surprisingly few authors have associated electrolysis-induced pH changes with problems such as poor precision of migration time (e.g., Vinther and Soeberg[4]), injection (e.g., Zhang and Thormann,[5] who have attributed poor reproducibility of injections for a head-column field-amplified sample stacking system to electrolysis), and detection (e.g., Carson et al.,[6] who have reported electrolysis as a factor for increased baseline noise in fluorescence detection).

There are relatively few studies which contribute to a better understanding of the nature of pH changes and their consequences for CZE, particularly when these pH changes are studied directly in the capillary rather than in the BGE vials. Zhu et al.[7] observed faster pH changes in the BGE reservoirs for BGEs with lower buffering capacity. Strege and Lagu[8] reported poor migration time reproducibility due to electrolysis induced by pH changes in the BGE reservoirs and pointed out the necessity of BGE replenishment. Corstjens et al.[9] and, recently, Bello[10] found experimentally measured pH shifts in the BGE reservoirs to be in good agreement with values predicted by theoretical considerations. The time at which the BGE had to be replenished was expressed as a function of the electric field strength, mobilities of the BGE components, capillary cross section, and volume of the BGE reservoir.[10] Electrolysis-induced pH changes of the BGE inside the capillary have recently been reported in two studies. Corstjens et al.[9] used the change in effective mobility as well as the spectral change in the visible region of an injected pH indicator dye for in situ measurements of pH. However, this approach did

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not allow for continuous monitoring of the BGE pH in the capillary, which was developed by Timperman et al.[11] This work utilized a wavelength-resolved fluorescence detector and a fluorescent pH indicator limited to a pH between 6 and 9. Although this report shows many interesting phenomena associated with pH changes in the capillary, the outlet vial was of very small volume (2 μL or less), which is of limited value for practical CZE.

In studies on applications of CZE to the determination of inorganic ions using direct or indirect absorbance detection with poorly buffered BGEs, we have often encountered problems with poor precision and baseline stability. We have noticed that moving the capillary away from the electrode or simply swirling the inlet or outlet vials solved these problems temporarily. In the present work, we describe how the position of the capillary relative to the electrode and the level of buffering of the BGE affect the electrolysis-induced pH change of the zone that enters the capillary. Consequently, an approach for on-capillary pH monitoring over a wide pH range utilizing pH indicator dyes and absorbance detection in the visible region has been developed, together with a photographic setup providing visualization of the pH changes around the electrode.

EXPERIMENTAL SECTION

Reagents. Xylenol blue (XB, *p*-xylenolsulfonephthalein), bromocresol green (BCG, 3′,3″,5′,5″-tetrabromo-*m*-cresolsulfonephthalein), cetyltrimethylammonium bromide (CTAB), and Tris (tris(hydroxymethyl)aminomethane) were purchased from Aldrich (Milwaukee, WI). Chromium trioxide was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade. Water was treated with a Millipore (Bedford, MA) Milli-Q water purification apparatus.

The sodium chromate−CTAB background electrolyte was prepared as described previously,[12] and xylenol blue was added to give a final composition of 0.5 mM XB, 2 mM CTAB, and 5 mM sodium chromate, pH 8.0. The Tris-buffered chromate electrolyte was prepared from chromium trioxide to give 5 mM chromate and titrated with Tris to pH 8.0 (∼15 mM Tris), and then CTAB and XB were added, and the solution was ultrasonicated until a clear solution was obtained. The BGEs were degassed by vacuum and filtered with a Millex-HA 0.45-μm disk filter (Millipore). The acetate BGE contained 0.5 mM BCG in 20 mM acetic acid and 10 mM Tris, pH 4.8.

Instrumentation and Procedures. The CZE instrument used was a capillary ion analyzer (Waters Corp., Milford, MA) interfaced to a Millenium data station (Waters Corp.). In addition to the original mercury lamp (254 nm), a light-emitting diode (LED) light source (maximum emission at 614 nm) was used as described earlier.[13] The LED used here was RS 578−200 (RS Components Pty Ltd., Tullamarine, VIC, Australia). The EOF was determined from the water peak by detection at 254 nm. Injection of water was performed hydrostatically by elevating the sample at 100 mm for 5 s. At both the injection and detection sides, BGE reservoirs of 18-mL volume were used. The running voltage was −20 kV for the chromate-based BGEs or +30 kV for the acetate BGE.

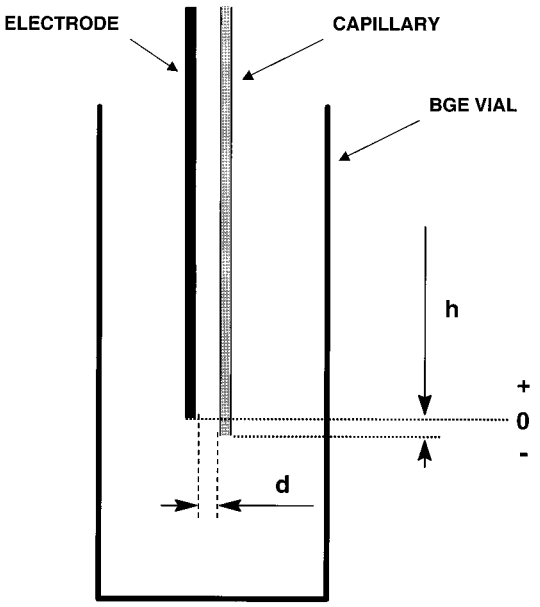

Figure 1. Schematic representation of the positioning of the electrode and capillary.

Table 1. Configurations of Electrode and Capillary Used in This Study[a]

	configuration											
	A	B	C	D	E	F	G	H	I	J	K	L
distance (*d*, mm)	0	0	0	0	0	1	1	1	1	3	3	3
height (*h*, mm)	+5	+1	0	−1	−5	+1	0	−1	−5	+1	−1	−5

[a] For an explanation of the parameters *d* and *h*, see Figure 1.

A Polymicro Technologies Inc. (Phoenix, AZ) fused-silica capillary, 75-μm i.d., 360-μm o.d., 0.500-m length and 0.420 m to detector or 0.600-m length and 0.520 m to detector, was used. The capillary was flushed initially with 1 M NaOH (1 h) and then with water and BGE each for 10 min. The position of the capillary relative to the electrode at the injection side was defined by two parameters, as shown in Figure 1, and the positions were adjusted by bending the platinum electrode and by pulling the capillary up or down. The parameters for the electrode and capillary positions used in our experiments are summarized in Table 1.

In the direct observation and photographic experiments, a platinum wire (0.5-mm diameter) was used as the grounded electrode and positioned vertically in the center of a 2-mm photometric glass cell (10-mm width, 40-mm height) filled with BGE up to ∼35 mm (1.75 mL). The electrode was immersed in the BGE solution to a depth of ∼10 mm. The cell was fixed in a plastic holder at the same level as a BGE vial, with the active electrode placed in a cabinet equipped with a safety switch on the door. A Spellman high-voltage source (High Voltage Electronics Corp., Plainview, NY) was used (i) for the chromate BGE with the polarity indicator set to positive, i.e., the observed electrode in the photometric cell was negative, or (ii) for the acetate BGE with either polarity (see text). A matte white background behind the cell was illuminated with a lamp for the time of film exposure.

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(12) Jandik, P.; Jones, W. R. *J. Chromatogr.* **1991**, *546*, 431.
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RESULTS AND DISCUSSION

Choice of BGE. The magnitude of any pH change of an electrolyte will depend on its buffering capacity, so the criteria for the choice of electrolytes to be investigated in this study were first to include a BGE having relatively poor buffering capacity but at the same time substantial conductivity, and second to include a BGE having relatively good buffering capacity at relatively low conductivity. Consequently, chromate and acetate BGEs were chosen as models in this work.

The chromate electrolyte has been commonly used as BGE for anion separations since its introduction in 1990 by Jones and Jandik.[14] Its advantages include fast coelectroosmotic separations in fused-silica capillaries with reversal of EOF using quaternary alkylammonium additives, and universal indirect photometric detection provided by the chromate acting as a UV-absorbing probe co-ion.[12,15] In the present study, the chromate BGE has been used without the addition of buffers (which is consistent with the routine use of this BGE in most publications) or after buffering with Tris in order to demonstrate the effect of buffering. Tris was added to the BGE as the countercation (instead of sodium) by titrating a solution of chromic trioxide with Tris to pH 8.0 (see Experimental Section).

Acetate is another commonly used BGE and was employed in this study to demonstrate the versatility of the developed techniques of direct and continuous on-capillary pH measurement and direct observation in a photometric cell. As high ionic strength BGEs are frequently used for protein separations, the acetate buffer was also used with addition of sodium sulfate to illustrate the effects of increased current at the same buffering capacity.

Effect of the Relative Positions of the Electrode and the Capillary. In previous studies on CZE separations in poorly buffered BGEs, we have encountered problems with reproducibility of migration times and peak areas, and poor baseline stability. These problems were found to be dependent on the relative positions in the inlet electrolyte vial of the electrode and the end of capillary. This situation is illustrated in Figure 2 for a separation of five anions using the sodium chromate BGE.[12] In each experiment, the electrode versus capillary position was changed in such a way that the capillary and the electrode were always held parallel (Figure 1), and only the distance between the electrode and the capillary (*d*, mm) and the height of the capillary end relative to the electrode end (*h*, mm) were changed. The actual positions used are shown in Table 1. It should be noted that, for all experiments, the capillary end at the detection side was held 10 mm below the electrode end and displaced laterally by 3 mm, so that any interference by pH changes from electrolysis at the anode is unlikely.

Three consecutive electropherograms (Figure 2a−c) showing good reproducibility and baseline stability were obtained when the capillary end was positioned 5 mm below the electrode end and displaced laterally by 1 mm from the electrode (configuration I in Table 1). However, a completely different result occurred when the capillary end was moved closer to the electrode. The consecutive electropherograms shown in Figure 2d−f correspond to the situation when the capillary was touching the electrode and both had the same length (configuration C in Table 1), while Figure 2g−i shows consecutive electropherograms obtained for the capillary and electrode again touching but with the capillary end 1 mm higher than the electrode end (configuration B in Table 1). For the latter two series, poor reproducibility and baseline instability are evident.

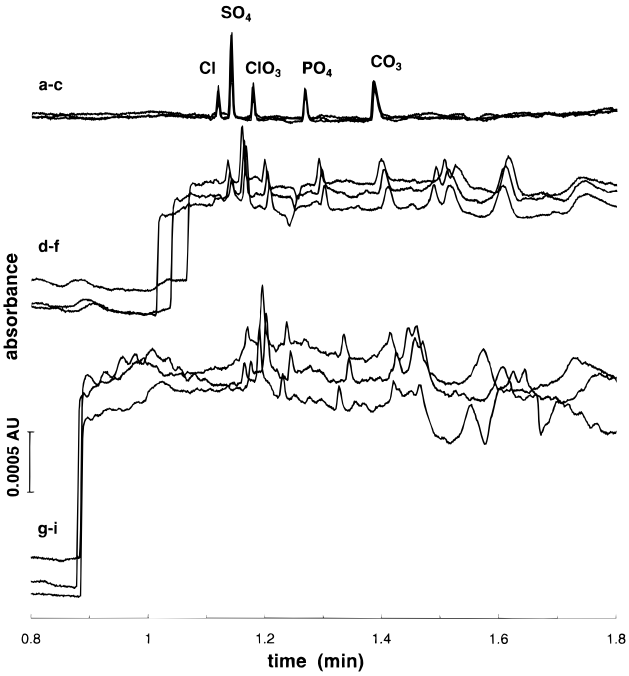

Figure 2. Electropherograms recorded in the unbuffered chromate BGE for different capillary−electrode configurations (see Table 1): (a−c) configuration I, (d−f) configuration C, and (g−i) configuration B. Capillary: fused silica, 73-μm i.d., 0.500-m total length, 0.425 m to detector; BGE, 0.5 mM CTAB, 5 mM sodium chromate, pH 8.1; separation voltage, −30 kV; current, 39 (a−c), 39−44 (d−f), and 39−49 μA (g−i); temperature, 25 °C; detection, 254 nm; injection, standard mixture of chloride, sulfate, chlorate, phosphate, and carbonate, 0.1 mM each, for 5 s hydrostatically (100 mm).

A general trend can be followed in observations made for various combinations of the parameters *d* and *h* (see Table 1): when the electrode and capillary are touching ($d = 0$), the relative capillary end height (*h*) is very critical, and reproducible results were obtained only when the capillary end was below the electrode end ($h < 0$). With increasing lateral distance between the electrode and capillary ($d = 1$ and $d = 3$), the parameter *h* becomes less important. These observations were consistent with the occurrence of pH changes of the BGE inside the separation capillary, and these effects were, therefore, investigated further.

Measurement of pH Changes inside the Capillary. Several methods can be used for continuous on-capillary monitoring of pH in CZE. Sustacek et al.[16] have employed conductivity detection to follow a pH gradient, but this detector responds to all changes in conductivity, not only those caused by pH, and is also strongly temperature dependent. Timperman et al.[11] applied wavelength-resolved fluorescence using a fluorescent pH indicator for the pH range 6−9, but this detector is not available on most commercial CZE instruments. Direct and continuous pH measurement by

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Table 2. Properties of the pH Indicators Used in This Study[a]

indicator	formula (A)	FW (A)	charge[17] A	charge[17] B	λ_{max} (nm) A	λ_{max} (nm) B	pK_a
xylenol blue (XB)	$C_{23}H_{22}O_5S$	410.50	−1	−2	424[24]	596[b]	9.6[c]
bromocresol green (BCG)	$C_{21}H_{14}Br_4O_5S$	698.04	−1	−2	423[24]	612[24]	4.9[20]

[a] A denotes the acidic form of the indicator and B the alkaline form, FW denotes the formula weight, and λ_{max} is the wavelength of maximum absorption. [b] Determined from a spectrum obtained for 0.05 mM XB in 10 mM borate buffer, pH 9.5. [c] Evaluated from the sigmoidal absorbance−pH calibration for the chromate BGE solution.

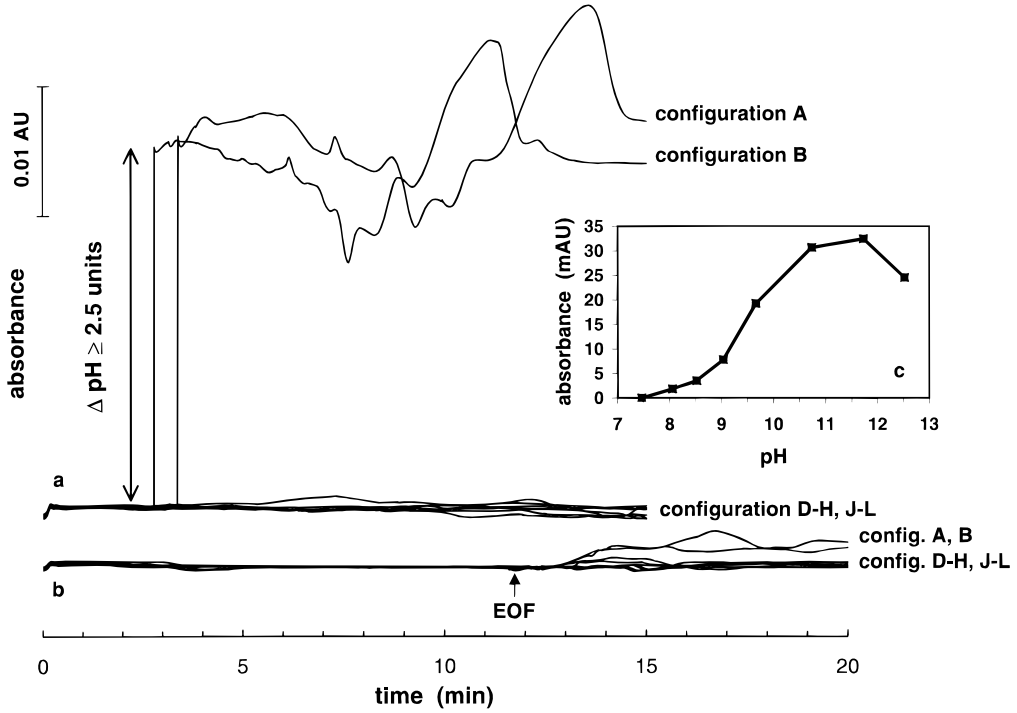

Figure 3. Electropherograms showing pH changes for a BGE containing xylenol blue recorded for the unbuffered chromate BGE (a) and the Tris-chromate buffered BGE (b) for different capillary−electrode configurations. The absorbance versus pH plot used for pH calibration is shown in the inset (c). Capillary: fused silica, 75-mm i.d., 0.600-m total length, 0.520 m to detector, BGE, 0.5 mM XB in 2 mM CTAB, 5 mM sodium chromate, pH 8.0 (a), 0.5 mM XB in 2 mM CTAB, 5 mM Tris−chromate, pH 8.0 (b).

photometry using additions of pH indicator dyes[17] has not been used in CZE, although Slais and Friedl[18] have used ampholytic pH indicators to determine local pH in an isoelectric focusing capillary.

In this study, the addition of a pH indicator to the BGE was used for direct and continuous monitoring of the electrolyte pH using on-capillary absorbance detection in the visible range. The observed changes in absorbance were expressed as pH changes using an absorbance versus pH calibration curve. Table 2 lists the characteristics of the indicators used. Calibration for the xylenol blue (XB) dye at different pH values in a 5 mM chromate electrolyte (see Figure 3c) was used to measure pH between 7.5 and 11, while bromocresol green (BCG) was used to monitor the pH of the acetate BGE in the range 4−6. As expected, the measurement range using a single dye is limited, but considering the large number of pH indicator dyes that can be used with this approach, a mixture of dyes could be used in a similar way as for mixed or universal pH indicators if an extended range of pH measurement is required.

The pH changes inside the capillary during a CZE run were monitored while varying the position of the capillary relative to the electrode in a manner similar to that used in Figure 2. Electropherograms were obtained using both the unbuffered chromate BGE and the new Tris-buffered chromate BGE for each of the nine capillary and electrode configurations shown in Table 1. For the unbuffered chromate BGE (Figure 3a), configurations A and B showed large pH changes (>2.5 pH units—the exact pH increase could not be determined because the pH range of the indicator was exceeded) at about 3 min, whereas configurations D−H and J−L exhibited only relatively small pH changes (not exceeding ±0.3 pH unit). In the case of the buffered chromate BGE (Figure 3b), changes of pH were again observed for configurations A and B only, but these were of much smaller magnitude (0.4−0.7 pH unit), showing the positive effect of the BGE buffering. The pH changes also occurred much later in the electropherogram (12 min). It is noteworthy that the two configurations, A and B, were those in which the capillary and

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(18) Slais, K.; Friedl, Z. *J. Chromatogr. A* **1995**, *695*, 113.

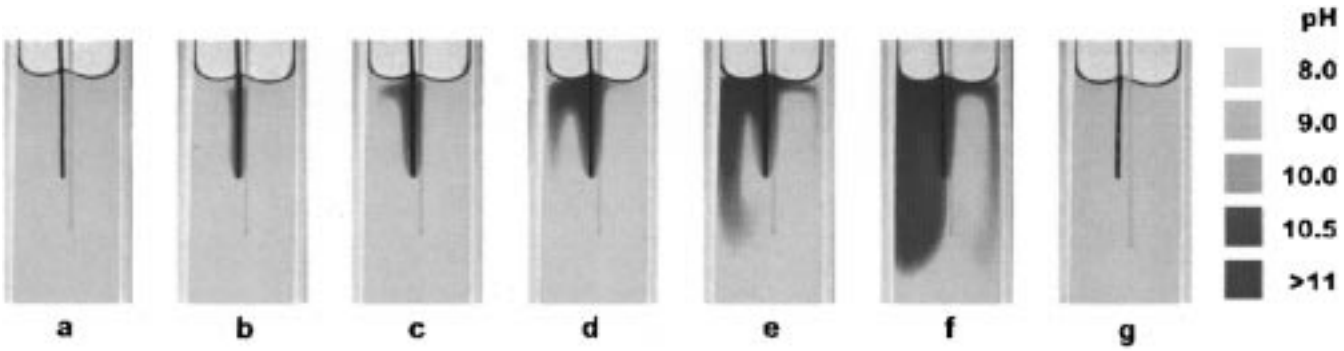

Figure 4. Photographs showing pH changes in the unbuffered chromate BGE to 0 (a), 1 (b), 2 (c), 4 (d), 8 (e), and 16 min (f) and in the Tris−chromate-buffered BGE at 16 min (g). Configuration H in Table 1 was used for the electrode and capillary; other conditions as in Figure 3.

electrode were touching and the capillary inlet was above the end of the electrode. This suggests that mobile hydroxyl ions generated at the cathode could gain easy access to the capillary with these configurations.

In practice, the rate at which the pH change moves through the capillary will govern the likelihood of interference with the electrophoretic separation. This rate depends on the separation voltage, and an electrophoretic mobility can be ascribed to the onset of the sharp pH change. The electrophoretic mobility calculated for this pH change in the unbuffered chromate BGE (which varied from 2.5 to 3.4 min) was in the range (-82 to -54) $\times 10^{-9}$ m^2 V^{-1} s^{-1}. In the sodium chromate electrolyte, the pH change will be carried by ions exhibiting pH equilibria: that is, hydroxyl ions ($\mu = -205 \times 10^{-9}$ m^2 V^{-1} s^{-1} [19]), the anionic indicator XB itself, and carbonate present as impurity from air ($\mu = -72 \times 10^{-9}$ m^2 V^{-1} s^{-1} [20]). The observed mobility of the pH change will result from equilibria between these species according to their concentrations and mobilities, as well as a certain time delay necessary to change the pH around the electrode where the capillary inlet is positioned. The pH change shown for the buffered chromate BGE in Figure 3b corresponds to an electrophoretic mobility of only $+1.4 \times 10^{-9}$ m^2 V^{-1} s^{-1}. In this BGE, the main pH-determining species is Tris (for TrisH$^+$, $\mu = +29.5 \times 10^{-9}$ m^2 V^{-1} s^{-1} [19]), which can explain the positive electrophoretic mobility of the pH change.

It should be noted that, for each of the configurations D−H and J−L, the pH was more stable in the buffered BGE, with fluctuations being less than ±0.2 pH unit. The applicability of this approach to buffering of electrolytes for indirect absorbance detection and analytical performance characteristics of buffered chromate electrolytes for analysis of anions are reported separately.[21,22] To confirm that the above observations were not in any way limited to the chromate-based BGEs, a 20 mM acetate BGE containing bromocresol green as the pH indicator was investigated using a positive voltage on the injection side. While very similar observations were made, the largest pH changes (i.e., those for configurations A and B) were only 0.04 pH unit, which can be explained by the good buffering capacity of the acetate.

Observation of pH Changes in the BGE Vial. To explain the differences observed in Figure 3 for the various capillary and electrode configurations, the pH changes occurring around the electrode were observed using the same BGE but with a photometric cell being employed as the BGE reservoir. It was anticipated that pH changes in the BGE could be observed directly as they occurred at the electrode and that their movement toward the capillary inlet could be monitored. The possible mechanisms of the spreading of the zone of changed pH through the BGE include (i) diffusion, (ii) migration of pH-determining ions under the influence of the electric field, and (iii) convection. Diffusion could be expected to play a minor role under the conditions used, while migration of ions in the electric field was also likely to make only a small contribution due to the reduced field strength in the bulk electrolyte. However, it was difficult to make predictions about the significance of convection, which might be caused by factors such as temperature and density changes.

Figure 4 shows photographs taken at various times (1−16 min) for the unbuffered chromate electrolyte with the electrode and capillary placed 0.5 mm apart and with the capillary end 5 mm below the electrode. For comparison, the Tris-buffered chromate electrolyte is shown after electrolysis for 16 min (Figure 4g). The pH change is illustrated by the blue coloration due to the change of the indicator, with the maximum color change corresponding to an increase of >2.5 pH units. This pH change is in agreement with the results of the on-capillary pH measurements presented in Figure 3. The area of changed pH after 16 min (Figure 4f) occupies quite a large portion of the solution in the cell. Although the volume of the electrolyte with the changed pH can only be estimated, the theoretical pH change in that estimated volume can be calculated from the current passed through the electrolyte.[9] Thus, for a current of 22 μA applied for 16 min, the amount of generated OH$^-$ is 0.219 μmol, which in a volume of approximately one-fifth of the electrolyte volume in the photometric cell (0.35 mL) would bring an additional concentration of OH$^-$ ions of 0.63 mmol/L, thereby raising the pH of the unbuffered BGE to 10.8. This simple calculation shows that the observed pH changes are possible from the viewpoint of the total charge passed through the electrolyte. In contrast to the large pH changes observed in the unbuffered chromate BGE, Figure 4g illustrates that, after 16 min, hardly any visible changes of pH for Tris-buffered chromate

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(21) Doble, P.; Macka, M.; Andersson, P.; Haddad, P. R. Presented at International Ion Chromatography Symposium '96, Reading, UK, September 1996; Paper 115.
(22) Doble, P.; Macka, M.; Andersson, P.; Haddad, P. R. *Anal. Commun.* **1997**, *34*, 351.

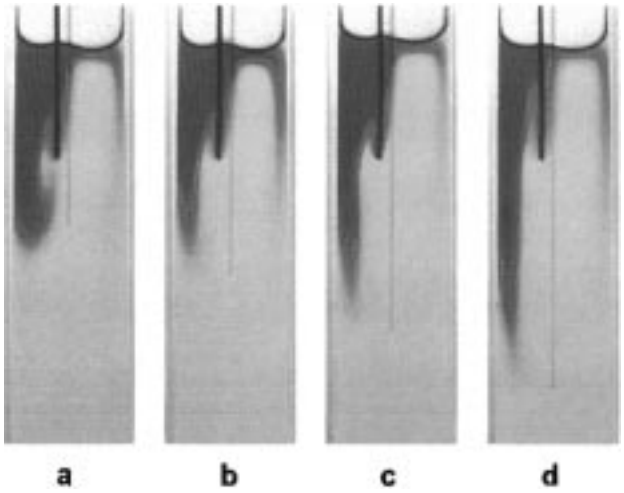

Figure 5. Photographs showing pH changes in the unbuffered chromate BGE at 16 min for $d = 1$ mm and $h = -5$ (a), -10 (b), -15 (c), and -20 mm (d). Other conditions as in Figure 4.

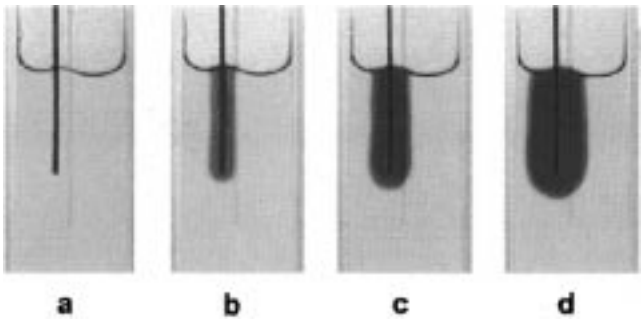

Figure 6. Photographs showing pH changes in the unbuffered chromate BGE using agarose to form a gel, taken at 0 (a), 4 (b), 8 (c), and 16 min (d). Other conditions as in Figure 4.

electrolye were evident. A further aspect revealed by the patterns shown in Figure 4 is that, even after 1 min, the zone of changed pH extends sufficiently far from the electrode that it would enter the end of a capillary placed at the same level or above the end of the electrode. This supports the observations made in Figures 2 and 3.

Another important feature of the experiments shown in Figure 4 was the patterns of spreading of the areas of increased pH. These patterns were generally reproducible, and the zone of altered pH tended to reach the depth of the capillary end after 16 min. To examine this effect further, the experiment was repeated for a range of vertical displacements between the capillary and the electrode. The results are shown in Figure 5, which shows that similar behavior was evident, regardless of the vertical displacement, suggesting that a convection current is generated in the solution. To verify this, an experiment similar to that in Figure 4 was undertaken using an unbuffered chromate electrolyte set to a gel by the addition of 0.2% agarose (Figure 6). Convection is not possible in such a gel; therefore, the pattern of spreading of the pH change was symmetrical around the electrode. Finally, a series of experiments in the unbuffered chromate BGE similar to those shown in Figures 4 and 5 was conducted, but with the whole setup (cell, capillary, electrode) inverted. A PTFE lid was used to prevent leakage of the BGE from the cell. In all observations made (results not shown), the zone of changed pH streamed

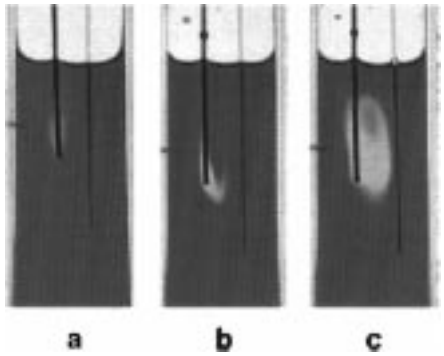

Figure 7. Photographs showing pH changes in the acetate BGE after 9 min (a) and in acetate BGE with added 5 mM sodium sulfate at 2.5 (b) and 9 min (c). BGE: 0.5 mM XB in either (a) 20 mM acetate/Tris buffer at pH 5.80 or (b) 5 mM Na_2SO_4 in 5 mM acetate/Tris buffer at pH 5.80. Separation voltage, +22 kV; current, 18 (a) or 45 μA (b); temperature, 25 °C. Electrode−capillary configuration was $d = 3-4$ mm, $h = -7$ mm.

upwards (i.e., away from the end of the electrode), indicating that the observed pattern is most likely a thermal convection.

The observed circular patterns of spreading of the pH changes are in agreement with the expectation that the highest current density and, therefore, the most intense heating will be in the electrolyte inside the capillary, thereby heating the liquid on the outside of the capillary, causing it to move upward. Cooler portions of the electrolyte (particularly that adjacent to the cell walls) will move downward, causing a circular motion of electrolyte typical of thermal convection. From this, it can be expected that the predominant circular convection pattern will be established only in that portion of the electrolyte extending from the surface down to the end of the capillary. An important conclusion for the practice of CZE can be drawn, namely that both the capillary and the electrode should be immersed in the electrolyte vial so that they extend through the major part of the solution. This will prevent the establishment of a localized convection in a limited volume at the top of the BGE reservoir, which could cause rapid introduction of pH changes into the capillary.

The versatility of the demonstrated approach to on-capillary pH measurement and observation of pH changes is illustrated in Figure 7 using an acetate BGE with added bromocresol green at a pH close to its pK_a. The indicator appears green (color transition yellow to blue, see indicator properties listed in Table 2). A positive voltage was used, so the zone with changed pH (yellow) represents the expected decrease due to the production of hydronium ions at the anode. Reversal of the electrode polarity gave a blue-coloured zone of BGE with increased pH spreading around the electrode. The acetate BGE exhibits good buffering capacity (similar to that of the Tris−chromate), which is reflected in the slow pace of the observed changes of pH (Figure 7a). Addition of a neutral (nonbuffering) salt, such as sodium sulfate, which contributes to an increase in the total current, can be expected to result in a faster pace of the pH change. This can be seen from Figure 7b, where a larger pH change has occurred after only 2.5 min, compared to 9 min in Figure 7a without the salt addition. After 9 min (Figure 7c), the BGE with added salt shows an even more significant pH change. This can have an implication for high-conductivity BGEs, such as the use of salt additions as high as 0.25 M K_2SO_4 in separations of proteins.[23] It

should be also noticed that the pattern of spreading of the pH change in the acetate BGE without salt addition (Figure 7a) is diffuse and does not exhibit the characteristic circular convection pattern. This can be explained by the much lower current (18 μA compared to 45 μA in parts b and c of Figure 7), so that the dissipated heat was obviously insufficient to cause convection.

Practical Implications. The above results have several direct implications on the practice of CZE. With regard to the positioning of the electrode and capillary in the BGE vials, the stability of the pH in the capillary is significantly better when the capillary inlet is positioned away from the electrode surface in order to prevent the zone of changed pH from quickly reaching the capillary inlet. This can be achieved by leaving a horizontal gap between the electrode and the capillary and/or by positioning the capillary end below the electrode end. It is also important to position the capillary and the electrode in the electrolyte so that they protrude down through a major part of the solution to avoid rapid, localized exhaustion of the BGE buffering capacity in a limited portion of the BGE.

In separation systems with significant electroosmotic flow, correct positioning of the capillary in the electrolyte vial where the EOF enters the capillary (usually the injection side) can be expected to be more significant than capillary positioning in the other electrolyte vial. However, due to the very high mobility of a pH change when carried by H_3O^+ or OH^- ions in poorly buffered BGEs, positioning of both capillary ends relative to the respective electrodes is important.

Finally, suppression of pH fluctuations in the separation capillary by using buffered BGEs can result in better reproducibility of the separations (migration times, peak areas/heights) and improved baseline stability, leading to lower limits of detection.

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(24) *Aldrich Catalogue Handbook of fine chemicals*; Aldrich Chemical Co., Inc.: Castle Hill, 1995; p 1788.

CONCLUSIONS

This study has shown that the addition of a suitable pH indicator dye to the BGE is a simple way for on-capillary measurement of pH. Use of this technique has established that the positioning of the electrode and capillary is crucial for preventing zones of changed pH generated by electrolysis reactions from entering the capillary when the electrolyte is unbuffered. It is necessary that the capillary ends in both reservoirs be displaced both laterally and vertically from the electrodes. It has been demonstrated that mixing by convection usually takes place in the electrolyte reservoir, probably driven by the heat dissipating from the capillary. The existence of convection in the BGE reservoirs should be taken into account in situations where it might affect CZE processes, such as electromigration injection.

It should be emphasized that correct positioning of the electrodes should not be viewed as a substitute for buffering of the electrolyte. Buffering substantially reduces the magnitude of the pH changes and influences their onset time. Suppression of pH fluctuations in the capillary can have a significant beneficial effect on the analytical method parameters. Buffering counterion(s) can be used where additional buffering co-ions cannot be introduced into the BGE, such as when applying indirect detection.

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